Small Molecule Inhibitors of Stat3 Protein as Cancer Therapeutic Agents

by

Brent D. G. Page

A thesis submitted in conformity with the requirements for the degree of Doctorate of Philosophy
Department of Chemistry
University of Toronto

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Abstract

Advances in anti-cancer drug development have vastly improved cancer treatment strategies over the past few decades. Chemotherapeutic agents are now being replaced with targeted therapies that have much greater potency and far fewer unpleasant side effects. At the center of this, cell signaling pathways have been targeted as they moderate gene expression, control proliferation and are often dysregulated in cancer.

The signal transducer and activator of transcription (STAT) proteins represent a family of cytoplasmic transcription factors that regulate a pleiotropic range of biological processes in response to extracellular signals. Of the seven mammalian members described to date, Stat3 has received particular attention, as it regulates the expression of genes involved in a variety of malignant processes including proliferation, survival, migration and drug resistance. Aberrant Stat3 activation has been observed in a number of human cancers, and its inhibition has shown promising anti-tumour activity in cancer cells with elevated Stat3 activity.
Thus, Stat3 has emerged as a promising target for the development of cancer therapeutics. While Stat3 signaling can be inhibited by targeting upstream regulators of Stat3 activation (such as Janus kinase 2), direct inhibition of Stat3 protein may offer improved response, larger therapeutic windows for treatment and fewer side effects.

The work presented within this thesis is focused on optimizing known Stat3 inhibitor S3I-201, a small molecule Stat3 SH2 domain binder that was discovered in 2007. We have performed an extensive structure activity relationship study that has produced some of the most potent Stat3 inhibitors in the scientific literature. These compounds showed high-affinity binding to Stat3’s SH2 domain, inhibited intracellular Stat3 phosphorylation and selectively induced apoptosis in a number of cancer cell lines. Lead agents further inhibited tumour growth in xenograft models of human malignancies and had favourable pharmacokinetic and toxicity profiles.
Acknowledgments

I would like to dedicate this work to my grandparents, George and Velma Page, who have both had their lives disrupted by battles with cancer. Thank you to my family: Dad, Marybeth, Tracey, Steve, Ryan and Nicole. A very special thank you to Nicole Kraumanis; your unwavering love, support and encouragement have been an essential part of this work; I love you.

To my supervisor, Patrick Gunning: your hard work and dedication to the success and expansion of this research program has been inspiring. Thank you for all the guidance and opportunities to grow as a researcher. Further thanks to Steven Fletcher, who played an instrumental role in the early stages of this research project; working out synthetic strategies and providing extensive training in the art of organic chemistry. I would like to thank all members of the Gunning group, past and present, for useful discussions, assistance and support.

To our many collaborators: the work presented herein is a compilation of great work performed by many people. It is with the collective intellect of all of those involved that we have made such progress. Thank you for your dedication towards the success of this project.
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<tbody>
<tr>
<td>Å</td>
<td>Angstrom</td>
</tr>
<tr>
<td>AcOH</td>
<td>Acetic acid</td>
</tr>
<tr>
<td>Arg</td>
<td>Arginine</td>
</tr>
<tr>
<td>AML</td>
<td>Acute myeloid leukemia</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>Bn</td>
<td>Benzyl</td>
</tr>
<tr>
<td>BnBr</td>
<td>Benzyl bromide</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
</tr>
<tr>
<td>Boc</td>
<td>tert-butyl carbonate</td>
</tr>
<tr>
<td>Boc₂O</td>
<td>di-tert-butyl dicarbonate</td>
</tr>
<tr>
<td>°C</td>
<td>Degrees Celcius</td>
</tr>
<tr>
<td>Cacld</td>
<td>Calculated</td>
</tr>
<tr>
<td>CCD</td>
<td>STAT coiled coil domain</td>
</tr>
<tr>
<td>CCyR</td>
<td>Complete cytogenetic response</td>
</tr>
<tr>
<td>cf</td>
<td>confer - the imperative of confer (compared to)</td>
</tr>
<tr>
<td>CH₂Cl₂</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>CH₃CN</td>
<td>Acetonitrile</td>
</tr>
<tr>
<td>CHCl₃</td>
<td>Chloroform</td>
</tr>
<tr>
<td>CM</td>
<td>Conditioned media</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
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</tr>
<tr>
<td>CML</td>
<td>Chronic myeloid leukemia</td>
</tr>
<tr>
<td>CML-CP</td>
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</tr>
<tr>
<td>CMV</td>
<td>CytoMegalovirus</td>
</tr>
<tr>
<td>cPARP</td>
<td>Cleaved Poly ADP ribose polymerase</td>
</tr>
<tr>
<td>Da</td>
<td>Dalton</td>
</tr>
<tr>
<td>DART</td>
<td>Direct Analysis in Real Time</td>
</tr>
<tr>
<td>DBD</td>
<td>STAT DNA binding domain</td>
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<tr>
<td>DIPEA</td>
<td>N,N-diisopropylethylamine</td>
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<tr>
<td>DMAP</td>
<td>4-(Dimethylamino)pyridine</td>
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<td>DMF</td>
<td>N,N-dimethylformamide</td>
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<td>DMSO</td>
<td>Dimethylsulfoxide</td>
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<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EC&lt;sub&gt;50&lt;/sub&gt;</td>
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<td>ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme linked immuno sorbent assay</td>
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<td>Electrophoretic mobility shift assay</td>
</tr>
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<td>FAM</td>
<td>Aminofluorocein</td>
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<td>Description</td>
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<td>-------------</td>
</tr>
<tr>
<td>FBS</td>
<td>Fetal bovine serum</td>
</tr>
<tr>
<td>Fmoc</td>
<td>Fluorenlymethyloxycarbonate</td>
</tr>
<tr>
<td>FP</td>
<td>Fluorescence polarization</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Glyceraldehyde 3-phosphate dehydrogenase</td>
</tr>
<tr>
<td>Glu</td>
<td>Glutamic acid</td>
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<tr>
<td>GM-CSF</td>
<td>Granulocyte-macrophage colony-stimulating factor</td>
</tr>
<tr>
<td>GOLD</td>
<td>Genetic optimization for ligand docking</td>
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<tr>
<td>HBTU</td>
<td>$O$-(Benzotriazol-1-yl)-$N,N,N',N'$-tetramethyluronium hexafluorophosphate</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>HRMS</td>
<td>High resolution mass spectrometry</td>
</tr>
<tr>
<td>IC$_{50}$</td>
<td>Half maximal inhibitory concentration</td>
</tr>
<tr>
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<td>Interferon gamma</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>Ile</td>
<td>Isoleucine</td>
</tr>
<tr>
<td>i.v.</td>
<td>Intra venous</td>
</tr>
<tr>
<td>JAK</td>
<td>Janus kinase family</td>
</tr>
<tr>
<td>Jak</td>
<td>A specific JAK isoform</td>
</tr>
<tr>
<td>JH</td>
<td>JAK homology</td>
</tr>
<tr>
<td>LRMS</td>
<td>Low resolution mass spectrometry</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>------------------------------------</td>
</tr>
<tr>
<td>Lys</td>
<td>Lysine</td>
</tr>
<tr>
<td>MeI</td>
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</tr>
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<td>M</td>
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</tr>
<tr>
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<td>Mega Hertz</td>
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<td>MLM</td>
<td>Mouse liver microsome</td>
</tr>
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<td>MM</td>
<td>Multiple myeloma</td>
</tr>
<tr>
<td>MNC</td>
<td>Mononuclear cells</td>
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<td>3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium</td>
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<td>ND</td>
<td>STAT N-terminal domain</td>
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<td>NADPH-regeneration solution</td>
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<td>Poly ADP ribose polymerase</td>
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<td>PB</td>
<td>Peripheral blood</td>
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<tr>
<td>PDB</td>
<td>Protein data bank</td>
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<td>Acronym</td>
<td>Description</td>
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<td>PDGFR</td>
<td>Platelet derived growth factor receptor</td>
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<td>Ph</td>
<td>Phenyl</td>
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<tr>
<td>PI</td>
<td>Propidium iodide</td>
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<td>PIAS</td>
<td>Protein inhibitors of activated STATs</td>
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<td>Protein tyrosine kinase</td>
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<td>RM</td>
<td>Regular media</td>
</tr>
<tr>
<td>rp</td>
<td>Reversed phase</td>
</tr>
<tr>
<td>rpm</td>
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<tr>
<td>SCF</td>
<td>Stem cell factor</td>
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<tr>
<td>SDS-PAGE</td>
<td>sodium dodecyl-polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>Ser</td>
<td>Serine</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
</tr>
<tr>
<td>SH2</td>
<td>Src Homology 2</td>
</tr>
<tr>
<td>SIE</td>
<td>Sis inducible element</td>
</tr>
<tr>
<td>SOCS</td>
<td>Supressors of cytokine signaling</td>
</tr>
<tr>
<td>SPR</td>
<td>Surface plasmon resonance</td>
</tr>
<tr>
<td>STAT</td>
<td>Signal transducers and activators of transcription family of proteins</td>
</tr>
<tr>
<td>Stat</td>
<td>A specific STAT isoform</td>
</tr>
<tr>
<td>$t$-butyl</td>
<td>tert-butyl</td>
</tr>
<tr>
<td>TBST</td>
<td>Tris-buffered Saline with 0.01% Tween-20</td>
</tr>
<tr>
<td>TAD</td>
<td>STAT transcriptional activation domain</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>Thr</td>
<td>Threonine</td>
</tr>
<tr>
<td>TKI</td>
<td>Tyrosine kinase inhibitor</td>
</tr>
<tr>
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<td>transcriptional response element</td>
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<td>Tryptophan</td>
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<tr>
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<td>Tyrosine</td>
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<tr>
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<td>Ultra-performance liquid chromatography-mass spectrometry</td>
</tr>
<tr>
<td>Val</td>
<td>Valine</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
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Chapter 1
Stat3 Protein as a Target for Anti-cancer Drug Development

1 Introduction

Despite far reaching advances in anti-cancer drug development, cancer remains the leading cause of death in Canada, killing an estimated 75,000 Canadians annually.\(^1\) Cancer is a disease that is caused by misfiring biological signals. This can be due to a number of different factors; however, the key player in cancer development is our genetic material, our DNA. Damage to our DNA can result in the production of mutated proteins that have abnormal function. Of particular importance are proteins that mediate cell signaling pathways and relay information from the cell surface to the nucleus to initiate cell division processes. When these signaling pathways become dysregulated, cells may be instructed to multiply in the absence of external stimuli, which can lead to the development of cancer.

One way in which cells relay signals is through phosphorylation of proteins. Phosphorylation of particular amino acids of specific proteins will activate them to perform their cellular function. Likewise, removing the phosphate group will deactivate the protein and stop it from functioning. Kinases are enzymes that transfer phosphate groups from nucleoside triphosphate (such as ATP) to a target residue on a protein. Phosphatases are enzymes which remove phosphate groups from proteins. Phosphorylation most commonly occurs on serine (Ser), threonine (Thr) and tyrosine (Tyr) residues.\(^2\) In cancer research there has been a lot of focus on phosphotyrosine (pTyr) signaling cascades as these are found to be dysfunctional in a wide variety of cancers.\(^2\)

Protein tyrosine kinases (PTKs) activate signaling cascades by phosphorylating specific tyrosine residues on a target protein. Highly regulated tyrosine phosphorylation events are critically important for cell growth and development.\(^3\)\(^-\)\(^5\) Mutations in PTKs can disrupt signal transduction pathways and can cause a number of different disease states such as immunodeficiency, neurological disorders, inflammatory disorders and cancer.\(^6\) In this light, a number of PTKs are considered oncogenic and have been the target of novel cancer therapeutic agents.\(^7\)\^-\(^12\)
Over the past two decades, anti-cancer drug discovery has been focused on selectively targeting proteins that are critical for cancer cell survival. However, targeting specific proteins has only become feasible as molecular biology techniques have allowed for the rapid discovery, validation and characterization of novel protein targets. Targeted therapeutics have allowed researchers to perform more informed drug design which has resulted in more effective cancer therapies with fewer side effects. Molecular biology has identified a number of protein families that appear to be good candidates for molecular targeted therapies, one of which is the PTKs.

Anti-cancer drug development has been revolutionized by Tyr kinase inhibitors (TKIs) which are now used as the first line of defense against a number of malignancies. These inhibitors represent some of the first “targeted therapies” that were designed to inhibit specific proteins within a particular signaling cascade. Although these compounds can effectively knock out signaling pathways that are critical for cancer cell survival, there are still several drawbacks to kinase inhibitor therapy.

Because most TKIs target the highly conserved ATP binding site, it is difficult to achieve selectivity between kinases. Furthermore, there are redundancies within many signaling cascades where a number of kinases can activate a particular downstream target. This is the case with the JAK-STAT (JAK = Janus kinase, STAT = signal transducer and activator of transcription) pathway, as STAT proteins can be activated by other kinases (such as Src). Additionally, kinases are also prone to mutations in the kinase domain which can prevent inhibitors from binding. Finally, because specific kinases can activate a number of downstream targets, inhibiting a single kinase can have far reaching effects within the cellular environment.

Indeed, TKIs generally suffer from poor selectivity, considerable side effects and narrow therapeutic windows for treatment. Thus, there is a great need for inhibitors of signal transduction pathways that target molecules other than kinases.

2 JAK-STAT Signaling

There are four members of the Janus kinase (JAK) family of proteins; Jak1, Jak2, Jak3 and Tyk2, and seven members of the STAT family of proteins; Stat1, Stat2, Stat3, Stat4, Stat5a, Stat5b and Stat6. Together these proteins form the JAK-STAT signaling pathway. The JAK-
STAT signal cascade is involved in many cellular processes such as replication, inflammation, apoptosis, and even drug resistance. In particular, Jak2 and downstream STATs, Stat3 and Stat5, have important roles in tumourigenesis and in the maintenance of the cancer phenotype. Therefore, these proteins represent promising targets for the development of therapeutic agents.\textsuperscript{24-29} The work presented in this thesis is focused on the development of small molecule inhibitors of Stat3 protein and their application as cancer therapeutic agents.

The Jak2-Stat3 pathway is a surprisingly uncomplicated signaling cascade that begins at the cell surface and ends with the expression of specific gene profiles. There are several recent review papers that highlight the intricacies of the JAK-STAT signaling cascade which is briefly described below.\textsuperscript{16, 18, 19}

Prior to activation, Jak2 is bound to transmembrane cytokine receptors and unphosphorylated Stat3 is found throughout the cytoplasm (it is now widely accepted that unphosphorylated Stat3 is predominantly found as the anti-parallel Stat3:Stat3 dimer). Extracellualr cytokine stimulation induces a conformational change in the cytoplasmic domain of the cytokine receptors. This activates the receptor associated Jak2, which phosphorylates the receptor at specific Tyr residues. The phosphorylated receptor then recruits Stat3 which binds to the pTyr motif via its Src Homology 2 (SH2) domain. Once bound to the receptor, Stat3 is phosphorylated on a Tyr residue near the c-terminus (Tyr705). The activated Stat3 protein (pStat3) then dissociates from the receptor and forms transcriptionally active pStat3:pStat3 homodimers. Heterodimerization has also been reported, primarily between pStat3 and pStat1.\textsuperscript{30} However, pStat3:pStat3 homodimerization is most commonly observed.\textsuperscript{30} Dimerization of pStat3 is mediated by a reciprocal interaction between the pTyr of one pStat3 monomer and the SH2 domain of another. After forming the pStat3:pStat3 dimer, the complex translocates to the nucleus where it binds to specific DNA response elements.\textsuperscript{31} Once bound to DNA, pStat3 interacts with other transcription factors and promotes the expression of specific gene profiles.\textsuperscript{20, 22, 32} The downstream genetic targets of the Jak2-Stat3 pathway encode for anti-apoptotic proteins, such as survivin, Mcl-1 and Bcl-xL, cell cycle regulators, such as cyclin D1 and c-Myc, and growth factors such as VEGF.\textsuperscript{20, 22, 32}
Figure 1.1. The Jak2-Stat3 Signaling pathway A) At rest, unphosphorylated Stat3 (red) is found in an anti-parallel dimer mediated via interactions between the N-terminal domains of the monomers. B) Upon cytokine binding (purple, denoted CYT), conformational change in the receptor activates Jak2, which phosphorylates the receptor and promotes Stat3 binding. C) After Stat3 is phosphorylated by Jak2, the activated pStat3 dissociates from the receptor and forms the transcriptionally active, parallel pStat3 dimer. D) The activated dimer translocates to the nucleus where it binds to DNA and promotes the expression of its target genes which include VEGF, survivin, c-Myc and Bcl-xL.

In healthy cells, Jak2-Stat3 signaling is tightly controlled by several negative regulatory proteins such as: suppressors of cytokine signaling (SOCS), protein inhibitors of activated STATs (PIAS), nuclear and cytoplasmic phosphatases and proteosomal degradation via the ubiquitin proteosome pathway. These deactivation mechanisms ensure that the Jak2-Stat3 signaling pathway is transiently activated, and only remains active for 20 minutes to a few hours. This effectivley moderates the production of anti-apoptotic factors and cell cycle regulators. In cancer cells, this pathway is often critically dysregulated, resulting in constitutively activated Jak2-Stat3 signaling. This leads to the over-production and accumulation of anti-apoptotic proteins and makes cancer cells resistant to apoptotic cues from their environment. Importantly, cancers that possess constitutively active Jak2-Stat3 signaling become reliant on high levels of anti-apoptotic...
proteins and therefore sensitive to Jak2 or Stat3 inhibition. Thus, both Jak2 and Stat3 have emerged as promising targets for the development of targeted anti-cancer agents.

3 Inhibiting Jak2-Stat3 Signaling

3.1 Structural Domains of Jak2 and Stat3

Since its discovery in 1992, Jak2 has emerged as a key mediator of cell signaling and has been shown to have an important role in malignant transformation. With seven distinct structural domains (named Janus Homology (JH) domains 1-7), the JAKs possess a complex molecular structure that is atypical of most PTKs. The four N-terminal domains (JH4 to JH7) make up a FERM domain that is involved in binding to the cytoplasmic tail of a transmembrane receptor. The functions of the JH3 domain and JH2 pseudokinase domain are not well characterized, although it is proposed that these domains are involved in regulation of kinase activity. The pseudokinase domain is also home to Val617, which is often found to be mutated to Phe in Jak2 driven cancers (the infamous Jak2 V617F mutation). The JH1 domain is a highly conserved tyrosine kinase domain which is responsible for phosphorylating the associated receptor and downstream signaling molecules such as STATs. Although the precise methods of Jak2 activation are not currently well characterized, Jak2 tyrosine kinase activity is dependent on phosphorylation of two Tyr residues in the JH1 activation loop (Tyr1007 and Tyr1008). Jak2 inhibitors typically target the JH1 domain to halt aberrant kinase activity.
STAT proteins are structurally divided into six domains. The N-terminal domain (ND) is involved in regulatory protein-protein interactions (PPIs) and mediates the binding of inactive Stat3 dimers, it stabilizes pStat3:pStat3 DNA binding and can interact with other transcription factors and regulatory proteins. The coiled-coil domain (CCD) is comprised of four α-helices and plays an important role in nuclear translocation. The DNA-binding domain (DBD) consists of an eight-stranded β-barrel and is responsible for binding to a 9-base-pair consensus sequence, TTCCGGGAA. The linker domain acts as a bridge between the DBD and the SH2 domain but also contributes to DNA binding. The highly conserved SH2 domain is critical for receptor recruitment and for the formation of the transcriptionally active pStat3:pStat3 homodimer. Finally, the C-terminal transcriptional activation domain (TAD) is responsible for binding to nuclear transcription factors and initiating transcription of Stat3 target genes. Most inhibitors of Stat3 bind to the SH2 domain, however, the ND and DBD have also been targeted.

4 Inhibiting Jak2

The most advanced of Jak2 inhibitors, ruxolitinib (marketed by Novartis as Jakafi) is a Jak1 and Jak2 inhibitor that received clinical approval in 2011 for the treatment of intermediate and high-risk myelofibrosis. Myelofibrosis is a myeloproliferative neoplasm which usually presents with the Jak2 V617F activating mutation (a mutation in the pseudokinase domain that causes constitutive Jak2 activation). Despite its potency against Jak2 and key Jak2 mutants, ruxolitinib does not reduce disease burden or improve patient outcomes. Thus, ruxolitinib is only used to treat disease related symptoms and to improve the quality of life for patients with myelofibrosis. Therefore there is a great interest in developing novel inhibitors of Jak2 and of target molecules that lie downstream of Jak2, namely Stat3 protein. There are several recently published reviews highlighting the latest developments in Jak2 inhibition, including several promising inhibitors that are in various stages in clinical trials. Despite their initial promise, many patients do not respond to Jak2 inhibitor therapy and others develop secondary resistance. Thus, inhibitors of STAT proteins, or other effectors of the JAK-STAT signaling pathway, are of great interest for the development of myelofibrosis therapeutics.
5 Inhibiting Stat3

Once thought to be “undruggable”, the interfaces for PPIs are now a promising avenue for developing novel cancer therapeutic agents. Characterized by large, flat and hydrophobic surfaces, PPI interfaces represent a substantial challenge for the development of small molecule inhibitors. Because PPIs occur between two macro-molecular structures, key binding points may be located at distal regions of the PPI interface. This means that PPI inhibitors may have to be much larger than inhibitors of classical enzyme pockets. As such, traditional medicinal chemistry guidelines, such as Lapinski’s rules, will not likely apply to inhibitors of PPIs.49,50

The Stat3 SH2 domain is a PPI interface that is essential for Jak2-Stat3 signaling.16 It is not only critical for recruiting Stat3 to activated cytokine receptors, but it also mediates the formation of the transcriptionally active pStat3:pStat3 homodimer. Thus, the Stat3 SH2 domain is a PPI interface that represents an attractive target for the development of Stat3 inhibitors.51

This section will highlight work that has been done to target Stat3’s SH2 domain with organic small molecules prior to 2008, the year when our group entered this field of research. This section is primarily taken from our 2009 review in Biochemistry and Cell Biology.52 In accordance with the copyright transfer agreement, a link to the web version of this publication can be found at the end of this thesis.

Peptidic and peptidomimetic inhibitors of Stat3 protein laid much of the ground work to validate Stat3 as a target for therapeutic intervention.23,52 However, when this project began, there were just a few examples of small organic molecules that had shown activity as direct Stat3 inhibitors. Indirect inhibitors of Stat3 signaling or molecules that bind to Stat3 at interfaces other than the SH2 domain have not been included in this brief review.

The small molecule inhibitors of Stat3 described below can be separated into three major categories, those found through high-throughput in vitro screening assays, those found by in silico screening assays, and those found through peptidomimetic-inspired rational design.
5.1 *In vitro* screening

**Figure 1.3. Small molecule Stat3 inhibitors discovered from *in vitro* screening techniques**

In 2006, Schust et al. reported Stattic (1.1), a small molecule, identified through *in vitro* screening of more than 17 000 compounds using a competitive fluorescence polarization (FP) assay. As one of the first non-peptidic small molecule Stat3 inhibitors, Stattic was found to prevent Stat3 phosphorylation, and inhibit dimerization and nuclear translocation of activated Stat3. Stattic showed selectivity for the SH2 domain of Stat3 over other closely related proteins and STAT isoforms. Most significantly, the authors observed that Stattic effectively and selectively disrupted Stat3-dependent signaling in cancer cells, and induced apoptosis in Stat3-dependant breast cancer cell lines at 10 µM.

Although the exact nature of inhibition remains to be clearly demonstrated, it was postulated that the inhibitory activity of Stattic might arise from covalent modification of the Stat3-SH2 domain. This was suspected as analogues of Stattic that had reduced capacity to behave as a Michael acceptor had weaker Stat3 inhibition. This was explored by changing the electronic nature of the benzene ring; changing the electron withdrawing nitro-group to an electron donating amine group led to loss of activity. Saturation of the five membered ring also led to loss of function.

Upon screening a 10 000 member library primarily of natural product extracts, Maloney et al. identified the natural product phaeosphaeride A (1.2) as a moderate inhibitor of Stat3. In the primary ELISA-based screen, phaeosphaeride A inhibited Stat3 with an IC$_{50}$ of 0.61 mM; however, its diastereomer, phaeosphaeride B, showed no activity against Stat3. Significantly, phaeosphaeride A displayed selectivity for Stat3 over Stat5 and Stat1. The authors also observed that the inhibitory activity of phaeosphaeride A was found to be around 100-fold more potent in cells, inhibiting the growth of Stat3-dependent U266 multiple myeloma cells with an IC$_{50}$ of 6.7
μM. This result suggested that phaeosphaeride A may have additional cellular targets, which is further supported by the fact that phaeosphaeride A exhibited low μM activity against Stat3-independent K562 cells.55

5.2 In silico screening

Figure 1.4. Small molecule Stat3 binders discovered using in silico screening techniques

With the publication of the Stat3 crystal structure (pdb 1BG1) in 1998 by Becker et al.37 a number of research groups have used in silico docking assays to screen for molecules that might bind to Stat3’s SH2 domain. In 2005, Song et al. conducted an in silico virtual screen of 429 000 energy-minimized structures from the National Cancer Institute (NCI), Merck, Sigma-Aldrich, and Ryan databases.56 These compounds were docked to the Stat3 SH2 domain using the molecular docking program DOCK (4.0).56, 57 In silico screening identified approximately 100 prospective compounds, which were then evaluated in a Stat3-dependant luciferase assay in carcinoma cells containing constitutively activated Stat3. STA-21 (1.3), a deoxytetramycin natural product, was identified as the most promising compound, inhibiting Stat3 dimerization and nuclear translocation, as well as suppressing Stat3-dependant gene expression. Significantly, compound 1.3 was found to greatly inhibit the growth and survival of Stat3-dependant breast carcinoma cell lines at low μM concentrations.

More recently, to facilitate an SAR study, the structurally complex benzo[a]anthracene-1,7,12-trione moiety of STA-21 (1.3) was simplified by Bhasin et al. to produce a small library of structurally similar anthraquinone analogues.58 Compound 1.4 showed similar computer-simulated (Autodock v 4.0) docking patterns to STA-21 and exhibited comparable low μM whole-cell activity against the prostate cancer cell lines DU145, PC3, and LNCaP, and the breast cancer cell line MCF-7 as measured by colourometric MTS assays.
Similarly, Hao et al. conducted an extensive virtual screen of Wyeth’s proprietary small molecule library and identified 1000 compounds that exhibited favourable Stat3-SH2 domain binding activity. These hit compounds were then subjected to in vitro screening with a Stat3-DNA binding assay (ELISA). Of the 56 compounds exhibiting SH2 domain binding activity in vitro, it was noted that many contained a catechol moiety. Subsequent screening of other catechol-containing compounds identified inhibitor 1.5 with an IC$_{50}$ value of 106 µM. Docking studies suggested that the catechol moiety functions as a pTyr mimic, forming hydrogen bonds with Arg609 and Glu612. Importantly, due to their lack of charge, catechol structures may serve as effective bioisosteres of the pTyr moiety, as they might be more cell permeable than anionic pTyr mimetics.

In a similar manner, Siddiquee et al. identified S3I-201 (1.6) from the NCI chemical libraries. The salicylic acid component of S3I-201 (1.6) is a known pTyr mimic and utilizing docking studies with GLIDE (Grid-based Ligand Docking from Energetics) software, the authors reported that the salicylic acid unit docks in the pTyr binding region of the SH2 domain. Further experiments using an electrophoretic mobility shift assay (EMSA) supported that S3I-201 does indeed bind Stat3 with an IC$_{50}$ value of 86 µM. Moreover, good isoform selectivity was observed for the inhibition of Stat3 over other STAT isoforms (Stat1: IC$_{50}$ > 300 µM; Stat5: IC$_{50}$ = 166 µM). Compound 1.6 (30 µM) inhibited proliferation of cancer cell lines that contained constitutive Stat3 activation without effecting non-transformed cells at concentrations of 100 µM. Western blot analysis confirmed that 1.6 inhibited cellular pStat3 and decreased the expression of Stat3 gene targets cyclin D1, Bcl-xL and survivin. Finally, 1.6 inhibited MDA-MB-231 tumour growth mouse xenografts upon intravenous (i.v.) treatment with just 5 mg/kg every two to three days.
5.3 Rationally designed small molecule Stat3 Inhibitors

Figure 1.5. Rationally designed small molecule Stat3 inhibitors

In 2007, Siddiquee and colleagues published a focused set of oxazole-based small molecule mimetics of ISS610, a peptidomimetic compound derived from the Stat3 pTyr705 binding sequence. Synthetic studies identified S3I-M2001 (1.7), a hetero-trisubstituted oxazole, which was evaluated for biochemical and biological activity in Stat3 dependent cancer cell lines. Structurally, compound 1.7 is composed of an oxazole core projecting two hydrophobic substituents (1-naphthyl and n-hexyl) and a p-benzylphosphate group as a pTyr mimetic. Oxazole 1.7 was shown to suppress Stat3 dimerization (IC$_{50}$ = 79 µM, EMSA) and displayed a two-fold selectivity for Stat3 over Stat1. Additionally, mouse xenograft models of human breast tumours treated with oxazole 1.7 (5-20 mg/kg) were strongly inhibited and resulted in tumour growth suppression. Moreover, compound 1.7 inhibited Stat3 activation in whole cells at 100 µM, which was a marked improvement over its peptidomimetic predecessor, ISS610, which required a 10-fold higher concentration to inhibit intracellular Stat3 activity.

Given its potency, the Hamilton lab at Yale University conducted an SAR of 1.7 by preparing a family of diversely substituted 1.7 analogues. Several compounds from this series displayed modest improvements in Stat3-Stat3 dimer disruption (EMSA). Variation of the hydrophobic substituents, as well as modulation of the heterocyclic core, yielded several molecules with improved potency. The two most notable compounds identified from the SAR study were thiazole 1.8 and oxazole 1.9.
GOLD docking studies showed that both compounds 1.8 and 1.9 adopted similar docking poses to lead inhibitor 1.7. Increased hydrophobic contacts were believed to confer increased potency against Stat3 dimerization. Lead agent 1.9 showed selective inhibition of transformed MDA-MB-231 breast cancer cells (EC$_{50}$ = 180 µM) and NIH3T3/v-Src cells (EC$_{50}$ = 120 µM) containing aberrant Stat3 activity. Non-transformed cells were unaffected by treatment with inhibitors (EC$_{50}$ > 1000 µM).

6 Discussion

For medicinal chemists, molecular disruption of aberrant Stat3 activity in human cancers is a daunting and challenging goal. Stat3’s SH2 domain is a particularly difficult target as it is primarily hydrophobic, but possess a pocket that is designed to bind to a pTyr residue. Many of the compounds shown in this chapter are amphipathic in nature possessing a very polar pTyr mimic to bind the pTyr binding region and hydrophobic moieties to interact with proximal hydrophobic areas. As such, these compounds are plagued with solubility issues, poor cell permeability and lack of metabolic stability.

Despite significant advances in the last decade, molecules with clinical therapeutic potential against Stat3 have yet to be discovered. Encouragingly, the diversity of agents currently undergoing investigation by numerous groups is successfully uncovering ever more potent inhibitors of the Stat3 protein. Despite the well-documented drawbacks associated with targeting PPIs, Stat3 dimer disruption has emerged as a valid and feasible target for small molecule inhibitors. A growing body of evidence suggests that successful disruption of Stat3 activity could furnish a novel targeted chemotherapeutic.
Chapter 2
Antagonism of the Stat3-Stat3 Protein Dimer with Salicylic Acid-Based Small Molecules

1 Introduction

This chapter is primarily taken from our 2011 article in ChemMedChem and our 2010 article in Biochemical Pharmacology. In accordance with the copyright transfer agreements, links to the web versions of these publications can be found at the end of this thesis.⁶⁴,⁶⁵ For this chapter, chemical syntheses, in silico docking studies and FP assays were completed in the laboratory of Prof. Patrick T. Gunning with the assistance of Dr. Steven Fletcher. MTS assays were conducted in the laboratory of Prof. Aaron D. Schimmer at Princess Margaret Hospital with the assistance of Sumaiya Sharmeen. EMSA and advanced biological assays with 2.18h were performed by members of Prof. James Turkson’s research group at the University of Central Florida.

As discussed in Chapter 1, inhibition of aberrant Stat3 activity is a promising avenue for the development of cancer therapeutic agents. In particular, small molecules that bind to the SH2 domain of Stat3 hold considerable value as they have many drug-like attributes. This research project centers around S3I-201 (1.6) and a thorough SAR study focused on transforming this in silico “hit” into one of the most potent and promising small molecule Stat3 inhibitors in the literature.

S3I-201 (1.6) was identified as a Stat3 inhibitor by conducting an in silico structure-based virtual screen of the National Cancer Institute chemical libraries as described in Chapter 1 (Figure 2.1A; 1.6: IC₅₀ = 86 µM, as determined by an EMSA).⁶⁰ Through these studies and our own docking experiments we have identified key areas on S3I-201 that would be amenable to functionalization in order to optimize inhibitor binding.⁶⁶

Broadly speaking, the Stat3 SH2 domain is composed of three main sub-pockets: a hydrophilic domain bounded by Lys591, Arg609, Ser611 and Ser613, and two relatively hydrophobic domains, the first comprising Ile634 and the hydrocarbon portions of the side chains of Lys591
and Arg595, and the second comprising Trp623, Val637, Ile659 and Phe716. There may also be a fourth subdomain that could be accessed consisting of residues Cys712, Val713, Thr714, and Tyr640.

Since 1.6 possesses only two binding appendages off the main scaffold, it can only occupy two of these three sub-pockets as depicted in Figure 2.1B. The salicylic acid moiety of 1.6 is a known pTyr mimic and has been shown to dock into the pTyr binding region of Stat3’s SH2 domain. Likewise, we found that the O-tosyl moiety docks in the Arg595 / Ile634 sub-pocket, which leaves the Trp623 / Phe716 sub-pocket unoccupied. We therefore proposed that functionalizing 1.6 to access this third pocket would allow for improved inhibitory activity.

Analyzing the docking experiments, we notice that the secondary amide of 1.6 docks very close to this third hydrophobic pocket, and could serve as a handle to attach hydrophobic appendages. Thus, we sought to make functionalized derivatives of 1.6 that possessed hydrophobic moieties stemming from this secondary amide position.

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**Figure 2.1.** A) S3I-201 (1.6) B) Low energy GOLD docking conformation of 1.6 bound to Stat3’s SH2 domain (pdb 1BG1) coloured by residue type; hydrophobic residues in pink, hydrophilic residues in blue.

Although 1.6 had shown promising anti-cancer activity, the tosylate group is an excellent leaving group, thus 1.6 could feasibly react with a variety of cellular nucleophiles. This may cause non-specific effects that would complicate a SAR study using 1.6. Therefore, prior to our full scale
SAR study, we sought to replace the O-tosyl moiety with bioisosteres that would prevent non-specific alkylation.

These initial synthetic modulations to 1.6 abrogated inhibitory activity (IC$_{50}$ > 300 µM) for NH (2.1), NCH$_3$ (2.2) and NBoc (2.3) analogues. This drop in activity lends support to the claim that 1.6 might operate, at least in part, as a covalent inhibitor. Nevertheless, when we functionalized these derivatives with hydrophobic substituents off the amide nitrogen, (i.e. R$^1$ = Bn (2.4-2.7)), we observed a slight recovery in inhibitor activity (2.6 IC$_{50}$ = 292 µM). This is important as the inhibitory effect of 2.6 can no longer be attributed to covalent inhibition and is likely due to enhanced noncovalent interactions with the protein surface.

We therefore proceeded to make a series of hydrophobic N-functionalized derivatives of 1.6 utilizing the N(CH$_3$)toluenesulfonamide moiety in place of the O-tosyl moiety.
Table 2.1. EMSA inhibition data for the disruption of the Stat3-Stat3-DNA complex *in vitro* by a focused set of S3I-201 (1.6) analogues.\(^{66}\)

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<tbody>
<tr>
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<td>H</td>
<td>O</td>
<td>86 ± 33</td>
</tr>
<tr>
<td>2.1</td>
<td>H</td>
<td>NH</td>
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</tr>
<tr>
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<td>H</td>
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</tr>
<tr>
<td>2.3</td>
<td>H</td>
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<td>292 ± 35</td>
</tr>
<tr>
<td>2.7</td>
<td></td>
<td>NBoc</td>
<td>&gt; 300</td>
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</table>

2 Synthesis

2.1 Functionalized Amide Derivatives

Analogs of **1.6** depicted in Table 2.2, were synthesized using the synthetic routes shown in Schemes 2.1-2.6. Inhibitors were synthesized from 4-aminosalicylic acid, which was doubly O-benzyl protected to afford aniline **2.8**. Compound **2.8** was then subjected to reductive amination with a variety of hydrophobic aldehydes to furnish secondary anilines **2.9** and **2.10a-j** in very good yields. Sulfonylation of glycine methyl ester (**2.11**) with para-toluenesulfonyl chloride (**p-TsCl**) furnished sulfonamide **2.12**, which was subsequently methylated using methyl iodide.
Saponification of \textbf{2.13} using lithium hydroxide gave carboxylic acid \textbf{2.14} which was then coupled to primary aniline \textbf{2.8} and secondary anilines \textbf{2.9} and \textbf{2.10a-j} to give \textbf{2.15, 2.16} and \textbf{2.17a-ja}. Peptide coupling reactions were performed using the peptide coupling agent, dichlorotriphenylphosphorane (PPh$_3$Cl$_2$). Prior to deprotection, intermediate molecules could be further functionalized to introduce diverse functional groups. This was accomplished through the use of peptide couplings, sulfonamide couplings, nucleophilic aromatic substitutions, and Suzuki cross couplings. Finally, a global debenzylation of compounds with H$_2$ over 10 \% Pd/C yielded a series of \textbf{1.6} analogues shown in Table 2.2. Since the reduction of aryl-bromides with H$_2$ and Pd/C catalyst proceeds quickly, hydrogenolysis of the benzyl protecting groups in intermediates \textbf{2.7a} and \textbf{2.7b} was not attempted. Instead, we employed two-step protocol where we first hydrolyzed the benzyl ester using LiOH, and then removed the benzyl ether under acidic conditions with trifluoroacetic acid (TFA).\textsuperscript{67}
Scheme 2. Preparation of compounds 2.2, 2.6 and 2.18a-ja. a) BnBr (2eq), KOtBu, DMF, 0 °C-rt, 16 h, 73 %; b) 1. RCHO, AcOH, 4Å MS, MeOH, 45 °C, 3 h; 2. NaCNBH₃, rt, 12 h, 75-96 %; c) p-TsCl, DIPEA, CH₃CN, 0 °C-rt, 1 h, 93 %; d) MeI, Cs₂CO₃, DMF, rt, 16 h, 85 %; e) LiOH.H₂O, THF/H₂O, 3:1, rt, 1 h, 95 %; f) PPh₃Cl₂, CHCl₃, 60 °C, 12 h, 89-95 %; g) H₂, 10 % Pd/C, THF/MeOH, 1:1, rt, 1-16 h, 85-100 %; or for 2.18a and 2.18b: h) LiOH.H₂O, THF/H₂O, 3:1, rt, 24 h, 76-86 %; i) TFA/toluene, 1:2, rt, 16 h, 85-93 %.

2.2 N-Substituted Piperidinylmethyl Derivatives

To further explore this hydrophobic pocket, we synthesized a variety of N-substituted piperidinylmethyl derivatives that would allow for facile extension into the proposed sub-pocket. To this end, compound 2.17j was synthesized as described in Scheme 2.1, where the RCHO aldehyde was N-Boc-piperidinylformaldehyde (the Boc group was inadvertently removed during the peptide coupling step with PPh₃Cl₂). The piperidine nitrogen was then functionalized with a variety of hydrophobic groups as shown in Scheme 2.2. Because we are targeting a hydrophobic
pocket, \( \text{R}^2 \) groups were chosen such that they would decrease the basicity of the piperidine nitrogen.

![Chemical structure](image)

**Scheme 2.2. Functionalization of N-substituted piperidinylmethyl derivatives 2.18ja-jd a)**

\( \text{R}^2 = \text{Boc}: \text{Boc}_2\text{O}, \text{cat. DMAP, CH}_2\text{Cl}_2, \text{rt, 1 h, 95 \%}; \text{R}^2 = \text{aryl}: \text{R}^2\text{F or R}^2\text{Cl, DIPEA, DMSO, 120 °C, 16 h, 76-96 \%}; \) b) \( \text{H}_2, 10 \% \text{ Pd/C, THF/MeOH, 1:1, rt, 1-16 h, 85-100 \%}. \)

### 2.3 N-Substituted 4-(Piperidinyl)benzyl Derivatives

In a similar manner, we synthesized a variety of N-substituted piperidinylbenzyl derivatives to extend into the hydrophobic pocket and improve inhibitor activity. These were prepared from the aldehyde 4-[N-trifluoroacetyl-(piperidin-4-yl)]-benzaldehyde (2.23) as illustrated in Scheme 2.3. Briefly, protection of the piperidine nitrogen of 4-phenylpiperidine (2.20) was accomplished as its acid-stable trifluoroacetamide 2.21. Subsequently, regioselective para-chlorocarbonylation of 2.21 was effected under Friedel-Crafts conditions,\(^{68}\) and then the crude acid chloride 2.22 was reduced to the target aldehyde 2.23 in a modification of the Rosenmund reaction. The aldehyde was then coupled to aniline 2.8 under reductive amination conditions and then coupled to functionalized acid 2.14 to furnish intermediate 2.17k. Next, as shown in Scheme 2.4, the trifluoroacetyl group of 2.17k was cleaved in excellent yield by brief treatment with LiOH to reveal the piperidine nitrogen in 2.24. Subsequent functionalization of this nitrogen was accomplished with a variety of reagents to furnish compounds 2.25c-h. Compounds 2.17k, 2.24 and 2.25c-h were deprotected to give compounds 2.18ka-kg depicted in Table 2.2. As in the case of the N-piperidinylmethyl series, we elected to functionalize the piperidine nitrogen in 2.24 with functionalities that would reduce its basicity through withdrawal of its lone pair of electrons into aryl systems, and acyl and sulfonyl groups.
Scheme 2.3. a) (CF₃CO)₂O, DIPEA, CH₂Cl₂, 0 °C → rt, 3 h, 93 %; b) (COCl)₂, AlCl₃, CH₂Cl₂, 0 °C, 1 h; c) H₂, 10 % Pd/C, DIPEA, EtOAc, rt, 2 h, 63 % (2 steps).

Scheme 2.4. a) LiOH·H₂O, THF/H₂O, 3:1, rt, 10 min, 98 %; b) R³ = Boc: Boc₂O, cat. DMAP, CH₂Cl₂, rt, 1 h, 95 %; R³ = aryl: R³F or R³Cl, DIPEA, DMSO, 120 °C, 16 h, 80-99 %; R³ = p-CNC₆H₄SO₂: p-CNC₆H₄SO₂Cl, DIPEA, rt, 16 h, 99 %; R³ = p-CNC₆H₄CO₂: p-CNC₆H₄CO₂H, HBTU, DIPEA, DMF, rt, 16 h, 89 %; c) H₂, 10 % Pd/C, THF/MeOH, 1:1, rt, 1-16 h, 85-100 %.

Inhibitors 2.18kh and 2.18ki were prepared as shown in Scheme 2.5. Specifically, deprotection of the tert-butyl ester of 2.25h with TFA also led to the concomitant removal of the benzyl ether, as reported previously, to deliver mono-benzyl protected compound 2.26. HBTU was utilized to couple carboxylic acid, 2.26 with NH₄Cl, to generate carboxamide 2.27 in excellent yield.
Deprotection of the benzyl esters of 2.26 and 2.27 under hydrogenolysis conditions furnished the corresponding inhibitors 2.18kh and 2.18ki.

Since the N-(piperidin-4-yl)benzyl moiety was predicted to bind in a hydrophobic sub-pocket, we anticipated that the polar acid- and carboxamide-containing inhibitors might demonstrate poor activity against Stat3.

Scheme 2.5. a) TFA/toluene, 1:1, rt, 4 h, 95 \% ; b) NH₄Cl, DIPEA, HBTU, DMF, rt, 16 h, 99 \%; c) H₂, 10 \% Pd/C, THF/MeOH, 1:1, rt, 1-16 h, 85-100 \%. 

\[ \text{Scheme 2.5. a) TFA/toluene, 1:1, rt, 4 h, 95 \%; b) NH}_4\text{Cl, DIPEA, HBTU, DMF, rt, 16 h, 99 \%; c) H}_2, 10 \% \text{ Pd/C, THF/MeOH, 1:1, rt, 1-16 h, 85-100 \%.} \]
2.4 Biphenyl and Terphenyl Derivatives

Scheme 2.6. a) R₄B(OH)$_2$, Pd(PPh$_3$)$_4$, K$_2$CO$_3$, DMF, 100 °C, 24 h, 16-73 %; b) H$_2$, 10 % Pd/C, THF/MeOH, 1:1, rt, 1-16 h, 85-00 %.

To further probe the Trp623 / Phe716 hydrophobic sub-pocket, we then chose to make functionalized biphenyl derivatives. The biphenyl moiety should project hydrophobic character in a rigid fashion. The aryl bromide moiety in 2.17a provides an excellent handle for diversification via Suzuki chemistry, allowing for the rapid construction the desired biphenyl analogues. To this end, and as described in Scheme 2.6, 2.17a was treated with a variety of aryl boronic acids in the presence of catalytic Pd(PPh$_3$)$_4$ to furnish a series of meta- and para-substituted biphenyl compounds, which were deprotected to give final molecules 2.18la-lh shown in Table 2.2. Likewise, the corresponding 4-(4-bromophenyl)-benzyl derivative 2.17m furnished the terphenyl-based inhibitors 2.18na-nh.
3 Results and Discussion

3.1 EMSA Activity

Table 2.2. EMSA data showing the disruption of the Stat3-Stat3-DNA complex formation in vitro.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R1</th>
<th>IC50 (uM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2</td>
<td>H</td>
<td>&gt; 300</td>
</tr>
<tr>
<td>2.6</td>
<td>292 ± 35</td>
<td></td>
</tr>
<tr>
<td>2.18a</td>
<td>290</td>
<td></td>
</tr>
<tr>
<td>2.18b</td>
<td>Br</td>
<td>&gt; 300</td>
</tr>
<tr>
<td>2.18c</td>
<td>NC</td>
<td>280 ± 47</td>
</tr>
<tr>
<td>2.18d</td>
<td>NC</td>
<td>285 ± 11</td>
</tr>
<tr>
<td>2.18e</td>
<td>300</td>
<td></td>
</tr>
<tr>
<td>2.18f</td>
<td>104 ± 47</td>
<td></td>
</tr>
<tr>
<td>2.18g</td>
<td>115 ± 60</td>
<td></td>
</tr>
<tr>
<td>2.18h</td>
<td>35 ± 9</td>
<td></td>
</tr>
<tr>
<td>2.18i</td>
<td>280 ± 15</td>
<td></td>
</tr>
<tr>
<td>2.18j</td>
<td>H</td>
<td>&gt; 300</td>
</tr>
<tr>
<td>2.18b</td>
<td>O</td>
<td>&gt; 300</td>
</tr>
<tr>
<td>2.18c</td>
<td>NC</td>
<td>&gt; 300</td>
</tr>
<tr>
<td>2.18d</td>
<td>N</td>
<td>&gt; 300</td>
</tr>
<tr>
<td>2.18e</td>
<td>H</td>
<td>&gt; 300</td>
</tr>
<tr>
<td>2.18f</td>
<td>MeO</td>
<td>191 ± 8</td>
</tr>
<tr>
<td>2.18a</td>
<td>191 ± 8</td>
<td></td>
</tr>
<tr>
<td>2.18b</td>
<td>NC</td>
<td>215 ± 23</td>
</tr>
<tr>
<td>2.18c</td>
<td>H2N</td>
<td>43 ± 1.3</td>
</tr>
</tbody>
</table>

R1 indicates the chemical structure as shown in the diagram.
Compounds were evaluated using EMSA as previously reported. Briefly, nuclear extracts of NIH3T3/vSrc transformed cells were treated with varying concentration of inhibitors for 30 minutes prior to the addition of a 32P-labelled oligonucleotide probe (high affinity sis-inducible element (SIE) from the c-fos gene, m67 variant, 5’-AGCTTCATTTCCGTTAACCCCTA) which binds to dimerized Stat3. After 30 minutes, the treated extracts were then run on polyacrylamide gel and the resultant bands corresponding to the Stat3-Stat3-DNA complex were quantified using ImageQuant biomolecular imaging unit. Experimental points were plotted as percent of control and IC\textsubscript{50} values were determined by fitting to a dose response curve. Compounds and their corresponding IC\textsubscript{50} values are plotted in Table 2.2 and are discussed below.

In general, it appears that inhibitor activity improved with the increasing size of hydrophobic R\textsuperscript{1} group. As an example, 4-(tert-butyl)benzylated agent, 2.18f, showed a marked improvement in activity over compound 2.6 (R\textsuperscript{1} = benzyl), while replacement of the tert-butyl group with a phenyl ring to give the large biphenyl-based inhibitor 2.18g (R\textsuperscript{1} = 4-phenylbenzyl) led to a nearly two-fold increase in potency (2.18g, IC\textsubscript{50} = 115 µM cf. IC\textsubscript{50} = 194 µM for 2.18f). Furthermore, the especially hydrophobic 4-cyclohexylbenzyl group in the R\textsuperscript{1} position provided optimal potency and which was more than two-fold better than parent compound 1.6 (IC\textsubscript{50} = 35 µM for 2.18h cf. IC\textsubscript{50} = 86 µM for S3I-201 (1.6)).

Despite similar structure, and considerable hydrophobicity, none of the N-substituted piperidinylmethyl derivatives (2.18ja-jd) were active, all exhibiting EMSA IC\textsubscript{50} values greater than 300 µM. However, the N-substituted piperidinylbenzyl derivatives (2.18ka-ki) demonstrated good activity especially compounds 2.18kd and 2.18kg with IC\textsubscript{50} values of 45 µM and 50 µM, respectively.

Furthermore, none of the biphenyl-based inhibitors 2.18la-lh offered any improvement in Stat3 inhibitory activity relative to the parent biphenyl inhibitor 2.18g (IC\textsubscript{50} = 115 µM). However, excluding the carboxylic acid-substituted compounds 2.18nb and 2.18nf, the terphenyl-based inhibitors 2.18na-nh showed promising activity, with the most active compound 2.18nh disrupting the Stat3-Stat3-DNA complex with an IC\textsubscript{50} value of 43 µM.
The improved activity of the terphenyl-based inhibitors over their biphenyl-based counterparts is likely due, at least in part, to enhanced hydrophobic interaction between the larger terphenyl moieties and the protein surface, possibly in the proposed Trp623/Phe716 sub-pocket.

3.2 SAR of the Sulfonyl Group.

Of all the compounds that were tested, the most potent inhibitor was 2.18h which possessed the hydrophobic cyclohexylbenzyl moiety. Thus, to extend our research program, we modified the \( X = \text{NCH}_3 \) component (\( X = \text{O} \) in 1.6) while utilizing the cyclohexylbenzyl moiety in the \( R^1 \) position to help identify even more potent Stat3 inhibitors. The more hydrophobic NBoc group, the more polar NH group and oxygen substitutions gave the small library shown in Table 2. 3. The syntheses of these target molecules are depicted in Scheme 2.7. Briefly, secondary aniline 2.10h was coupled to TsN(Boc)CH\(_2\)CO\(_2\)H using PPh\(_3\)Cl\(_2\), which, due to the generation of HCl \textit{in situ}, led to the inadvertent loss of the Boc group to furnish 2.30. Standard hydrogenolysis of 2.30 gave the NH derivative 2.31. Alternatively, the NH of 2.30 was re-tert-butoxycarbonylated and then debenzylated as usual to deliver 2.33. In order to synthesize the labile O-tosyl analogue, first compound 2.10h was coupled to 2-acetoxyacetyl chloride to produce 2.34. Hydrolysis of the acetate group proceeded cleanly, without appreciable cleavage of the benzyl ester. Tosylation of the resultant primary alcohol was non-trivial and required the use of 20 equivalents of \( p\)-TsCl in order to suppress symmetrical ether formation through the reaction of the starting alcohol with the product tosylate. Debenzylation of 2.35 was achieved through standard hydrogenolysis conditions to give 2.36 in good yield.
Scheme 2.7. a) TsN(Boc)CH₂CO₂H, PPh₃Cl₂, CHCl₃, 60 °C, 12 h, 48 %; b) AcOCH₂COCl, DIPEA, CH₂Cl₂, rt, 4 h, 64 %; c) (Boc)₂O, cat. DMAP, THF, 12 h, 81 %; d) H₂, 10 % Pd/C, THF/MeOH, 1:1, rt, 1-16 h, 85-94 %; e) LiOH.H₂O, THF/H₂O 3:1; 89 % f) p-TsCl, DIPEA, CH₂Cl₂, rt, 3 h, 85 %.

Table 2.3. EMSA inhibition data for the disruption of the Stat3-Stat3-DNA complex in vitro by a series of X-substituted analogues of inhibitor 2.18h.
The EMSA data for compounds 2.31 and 2.33 in Table 2.3 indicate that changing the X = NCH$_3$ group in compound 2.18h to NH or NBoc, respectively, had a detrimental effect on Stat3 inhibitory activity, reducing the IC$_{50}$ value from 35 µM to around 100 µM. However, more interestingly, the O-tosyl analogue 2.36 was equipotent with the parent inhibitor 2.18h, within experimental error. Compound 2.36 carrying the labile O-tosyl group has the capacity to function as an irreversible inhibitor, while 2.18h with the non-labile N(CH$_3$)$_2$-tosyl moiety possesses no such potential. This suggests that the inhibitory activity of 2.36 likely arises from non-covalent interactions with the Stat3-SH2 domain. At the same time, it is interesting to note that the similar activities of 2.36 and 2.18h are in stark contrast to the very different activities of the analogous R$^1$ = H derivatives 1.6 and 2.2, where replacement of the X = O atom with NCH$_3$ abolished Stat3 inhibitory activity (IC$_{50}$ > 300 µM). Taken together, these results suggest that the R$^1$ = 4-(cyclohexyl)benzyl moiety in 2.18h and 2.36 contributes to the inhibition of Stat3. Furthermore, it is evident that the nature of the X group in scaffold of 1.6 plays a considerable role in the subsequent Stat3 inhibitory activity.

3.3 Fluorescence Polarization Assay

Table 2.4. EMSA and FP assay data of selected inhibitors for the disruption of the Stat3-Stat3-DNA complex and Stat3-gp130 sequence complex in vitro.
We selected several of our analogues to be further evaluated in vitro using an FP assay. This assay measures the polarized fluorescence of a high affinity fluorescent peptide probe that binds to Stat3’s SH2 domain (5-FAM-GpYLPQTV-NH\(_2\)). In this assay, varying concentrations of inhibitor were incubated with Stat3 protein and fluorescent probe for 30 minutes, then polarized fluorescence was measured. Generally, the FP assay data (Table 2.4) corroborates with the EMSA data; potent activity in one assay is reflected by potent activity in the other assay. This lends support to our hypothesis that analogues 1.6 cause disruption of the ternary Stat3-Stat3-DNA complex, through the direct inhibition of the Stat3 SH2 domain.
3.4 STAT Isoform Selectivity

Using a similar Stat1 SH2 domain FP-based binding assay, we also investigated the isoform-selectivity of some of our most potent Stat3 inhibitors by evaluating their inhibitory activities against Stat1, which is similar in structure to Stat3. Lead compound 2.18h exhibited a greater than three-fold selectivity for Stat3 (Stat3: IC$_{50}$ = 30 µM; Stat1 IC$_{50}$ > 100 µM). However, the 4-cyanobenzenesulfonyl-based compound 2.18kg showed only limited isoform specificity (Stat3: IC$_{50}$ = 42 µM; Stat1: IC$_{50}$ = 56 µM), which, given the structural similarities of these two compounds, suggests that the 4-cyclohexylbenzyl group at the R$_1$ position is also a source of Stat3-isoform specificity.

Table 2.5. Comparative STAT isoform selectivity assessed by a Stat3 and Stat1 FP assay.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$ µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stat3</td>
<td>Stat1</td>
</tr>
<tr>
<td>2.18h</td>
<td>31 ± 9</td>
</tr>
<tr>
<td>2.18kd</td>
<td>37 ± 3</td>
</tr>
<tr>
<td>2.18kg</td>
<td>43 ± 4</td>
</tr>
<tr>
<td>2.18 nh</td>
<td>&gt; 100</td>
</tr>
</tbody>
</table>

3.5 Cancer Cell Activity

Table 2.6. EC$_{50}$ values for selected R$_1$-substituted analogues in cell viability studies.
Inhibitors were then assessed for anti-cancer activity by screening inhibitors at a concentration of 100 µM across a range of human tumour cell lines (MDA-468 breast cancer, DU145 prostate cancer and OCI-AML2 acute myeloid leukemia) which all harbour constitutively active Stat3. Compounds that demonstrated activity at 100 µM were then tested at lower concentrations and IC<sub>50</sub> values were determined. As expected, treatment of cells with compounds 2.2, 2.6, 2.18a-e, 2.18i, 2.18ja-jd, 2.18ld, 2.18lh, and 2.18nd had no effect on cell growth reflecting their poor IC<sub>50</sub> values in the EMSA assay (data not shown). However, compounds that showed good activity in vitro generally showed good anti-cancer activity against MDA-468, OCI-AML2 and DU145 cell lines. In particular, lead compound 2.18h demonstrated most potent activity against these cancer cell lines.
with IC$_{50}$ values of 17 µM, 37.2 µM and 35.9 µM against MDA-468, DU145, and OCI-AML2 cells respectively.

Table 2.7. IC$_{50}$ values for para-toluenesulfonyl X analogues of inhibitor 2.18h in cell viability studies.

<table>
<thead>
<tr>
<th>Compound</th>
<th>X</th>
<th>EC$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MDA-468</td>
<td>OCI AML2</td>
</tr>
<tr>
<td>2.18h</td>
<td>NCH$_3$</td>
<td>17 ± 4</td>
</tr>
<tr>
<td>2.31</td>
<td>NH</td>
<td>21 ± 6</td>
</tr>
<tr>
<td>2.33</td>
<td>NBoc</td>
<td>10 ± 9</td>
</tr>
<tr>
<td>2.36</td>
<td>O</td>
<td>43 ± 6</td>
</tr>
</tbody>
</table>

As detailed in Table 2.7, analogues carrying the optimized R$_1^1$ = 4-cyclohexylbenzyl group all exhibited sub-100 µM activities in the three Stat3-dependent tumour cell lines. Compounds 2.18h (X = NCH$_3$) and 2.31 (X = NH) were approximately equipotent. The most potent compound of this series in the Stat3-dependent cell lines was compound 2.33, the X = NBoc analogue of 2.18h, inhibiting MDA-468 cell growth with an IC$_{50}$ of 10.5 µM. The improved whole cell activities of 2.33 relative to 2.18h is possibly due to the greater hydrophobicity of the NBoc group relative to the NCH$_3$ group, which might facilitate cellular entry. Encouragingly, when assessed by FP for Stat3 binding potency, compound 2.33 was shown to have an IC$_{50}$ = 15 ± 0.2 µM. The O-tosyl derivative 2.36, a potentially irreversible inhibitor, demonstrated relatively poor activity in these cell based assays. Further optimization of the sulfonamide nitrogen substituent will be addressed in Chapter 4. We therefore selected compound 2.18h as a lead compound and subjected this inhibitor to further biological analysis.

3.6 Activity of Lead Compound 2.18h
Figure 2.2. A) Western blot analysis showing inhibition of Stat3 phosphorylation (pYStat3) and the repression of Stat3-regulated genes, Bcl-xL and Survivin in human breast (MDA-468) and multiple myeloma (JJN-3) cells upon 24 h treatment with 2.18h. B) EMSA showing recombinant SH2 domain can abrogate inhibitory effects on Stat3-Stat3-DNA complex formation. C) Compound 2.18h inhibits proliferation in cancerous cell lines NIH3T3/v-Src, Panc-1 and MDA-MB-231, however exhibits no affect against non-transformed cell lines HPDEC, TE-71 and NIH3T3. D) Inhibition of tumour growth in MDA-MB-231 xenograft models, dosing at 3 mg/kg (i.v. injection) every 2-3 days, significant reduction of tumour growth is shown compared to vehicle control (p < 0.05).

To further investigate cellular activity we conducted Western blot assays to probe for inhibition of Stat3 phosphorylation in a cellular environment. Consistent with the effects on viability, 2.18h strongly inhibited Stat3 phosphorylation in breast cancer (MDA-468) and multiple myeloma (JJN-3) cell lines (Figure 2.2A, upper panel). Furthermore, treatment with 2.18h inhibited the expression of Bcl-xL and survivin, which are known Stat3 regulated genes (Figure 2.2A, lower panel). These findings suggest that the modulation of aberrant Stat3 signaling in MDA-468 and JJN-3 cells leads to the suppression of Stat3-mediated gene regulation.
Based on our preliminary docking studies, we proposed that we were exhibiting these cellular effects through binding of Stat3’s SH2 domain. In this light, we sought to gather support for this claim by invoking a competitive EMSA with recombinant Stat3 SH2 domain. If we were binding to the SH2 domain, we would expect that the recombinant SH2 domain would compete with native Stat3 to bind our inhibitor. However, because the recombinant SH2 domain does not have a DNA binding domain, it will not bind to the radiolabelled DNA probe. Thus, if our inhibitors were binding to Stat3’s SH2 domain, we should observe recovery of our Stat3-Stat3-DNA complex with increasing concentration of recombinant SH2 domain. Indeed, as depicted in Figure 2.2B, with addition of recombinant SH2 domain, we get recovery of our Stat3-Stat3-DNA spot on our EMSA gel, which further suggests that 2.18h binds to Stat3’s SH2 domain.

To be considered for in vivo testing, it was important to ensure that 2.18h was non-toxic to healthy cell lines that do not rely on elevated Stat3 activity. Figure 2.4C shows that treatment of Stat3 independent cells HPDEC, TE-71 and NIH3T3 with 2.18h had no effect on proliferation up to 100 µM. However, when cancerous cell lines Panc-1, NIH3T3/v-Src and MDA-468 were treated with 2.18, potent and dose dependent inhibition of proliferation was observed. Thus, as 2.18h demonstrated a favourable cell selectivity profile, we performed in vivo experiments using MDA-MB-231 xenografted mice.

MDA-MB-231 tumour bearing mice were treated with 3 mg/kg (i.v. injection) or vehicle control every two to three days for 17 days. Compared to control animals, 2.18h significantly inhibited tumour growth (p < 0.05) without obvious signs of toxicity. Following the 17 day study, tumours were excised and analyzed for their gene expression profiles. Most excitingly, mice treated with 2.18h had decreased pStat3, and downregulated levels of Stat3 gene targets (Bcl-xL, c-Myc and survivin) compared to vehicle treated controls (Figure 2.3).
Figure 2.3. Post treatment gene expression profile of MDA-MB-231 tumour bearing mice as measured using Western blot. Stat3 phosphorylation and expression of downstream target genes are inhibited.

4 Conclusion

We have conducted an SAR study centered on the previously identified Stat3 inhibitor S3I-201 (1.6) to derive analogues with improved Stat3 inhibitory activity. Novel agents possess an additional appendage that allows for more complete binding to Stat3’s SH2 domain. Furthermore, by incorporating bioisosteres of the O-tosyl moiety, these new inhibitors are no longer able to function as covalent inhibitors and should be less likely to react non-specifically with cellular nucleophiles. New lead compound 2.18h shows significantly improved in vitro activity, with an IC_{50} value of 35 µM in EMSA, and shows very promising activity against several cancer cell lines and in mouse xenografts of MDA-MB-231 breast cancer.
Chapter 3
Identification of a Non-phosphorylated, Cell Permeable, Small Molecule Ligand for the Stat3 SH2 Domain

This chapter is primarily taken from our 2011 article in *Bioorg. Med. Chem. Lett.* and our 2012 article in *Proc. Nat. Acad. Sci.* In accordance with the copyright transfer agreements, links to the web versions of these publications can be found at the end of this thesis. For this chapter, chemical syntheses, *in silico* docking studies and FP assays were performed in the laboratory of Prof. Patrick T. Gunning with the assistance of Dr. Steven Fletcher. MTS cell viability assays were performed in the laboratory of Prof. Aaron D. Schimmer at Princess Margaret Hospital with the assistance of Sumaiya Sharmeen. Western blots and cell viability experiments in multiple myeloma cells were conducted by members of Prof. Suzanne Trudel’s research group at Princess Margaret Hospital. EMSA and advanced biological assays with 3.7o were conducted by members of Prof. James Turkson’s research group at the University of Central Florida.

1 Introduction

In Chapter 2, we identified a potent salicylic acid-based Stat3 inhibitor, 2.18h after a structure activity relationship (SAR) study of compound 1.6 (S3I-201, Figure 2.1). Inhibitor 2.18h showed promising anti-Stat3 activity *in vitro*, disrupting Stat3-Stat3-DNA and Stat3-phoshopeptide interactions and elicited *in vivo* suppression of breast tumour xenografts. Moreover, FP binding experiments showed that 2.18h is selective for Stat3’s SH2 domain *cf.* Stat5 and Stat1 isoforms (Stat3 IC₅₀ = 31 μM; Stat5, IC₅₀ > 100 μM; Stat1, IC₅₀ > 100 μM). Encouragingly, 2.18h showed negligible effects against cells lacking Stat3 activity and selectively killed cancer cells harbouring aberrant Stat3 activity. GOLD docking studies revealed that compound 2.18h binds to the pTyr-binding portion of the Stat3 SH2 domain, with the salicylic acid making interactions with Lys591, Glu594 and Arg609. In addition, the hydrophobic cyclohexylbenzyl appendage forms Van der Waal’s interactions with a series of predominantly hydrophobic residues including Ile659 and Val637 (Figure 3.1).
In this chapter we will investigate the binding significance of the sulfonamide-S substituent to Stat3 SH2 domain recognition. We herein report an SAR of the sulfonamide portion of compound 2.18h, and present novel analogues, including 3.7o, which exhibited improved inhibition of Stat3 function both in vitro and in cell based assays. Furthermore, 3.7o shows potent inhibition of breast cancer xenografts at oral dosing of just 3 mg/kg.

2 Materials and Methods

2.1 MTS Assay

Human cell lines, DU145, OCI-AML2 and JJN3 were prepared in 96 well plates and treated with varying concentration of inhibitor. After 72 hours, cell growth and viability was measured with the CellTiter96 aqueous nonradioactive (MTS) assay according to the manufacturer’s instructions (Promega, Madison, WI) and as described previously. Relative viability was plotted versus concentration and EC50 values were determined by fitting to a standard dose-response curve.

2.2 Immunoblotting

Cells were lysed in lysis buffer (50 mM Tris–HCl, 1 mM EDTA, 1 % NP-40, 150 mM NaCl) for 30 minutes on ice, then freeze/thaw once at -80 °C and clarified by centrifugation at 12 000 g for 15 minutes. Proteins were separated by 6.5 % to 15 % sodium dodecyl-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotted with the specified antibody. Protein bands were
visualized using secondary antibodies coupled to horseradish peroxidase and the Chemiluminescence Reagent Plus (from Perkin Elmer Life Sciences) according to the manufacturer's instructions. Anti-cMyc was purchased from Santa Cruz, anti-survivin was purchased from NOVUS Biologicals, anti-Mcl-1, and anti-Bcl-xL from BD Biosciences, (Mississauga, ON), anti-phospho STAT3, anti-STAT3 and anti-PARP are from Cell Signaling Technology, (Pickering, ON).

2.3 CyQUANT Cell Proliferation Assay

These studies were performed as previously reported. Briefly, proliferating cells in 6- or 96-well plates were treated once with 0-30 μM 3.7o for 24 h or with 10 μM 3.7o for up to 96 h. Viable cells were counted by trypan blue exclusion/phase-contrast microscopy or assessed by a CyQUANT Cell Proliferation Kit, according to the manufacturer’s (Invitrogen) instructions.

2.4 Mice and In Vivo Tumour Studies

Six-week-old female athymic nude mice were purchased from Harlan and maintained in institutional animal facilities approved by the American Association for Accreditation of Laboratory Animal Care. All mice studies were performed under an Institutional Animal Care and Use Committee (IACUC)-approved protocol. Athymic nude mice were injected subcutaneously in the left flank area with 1 × 10⁶ human breast cancer MDA-MB-231 cells in 100 μL PBS. After 5-10 days, tumours of a 30-100 mm³ volume were established. Animals with established tumours were grouped so that the mean tumour sizes in all groups were nearly identical and then given 3.7o (in 0.05 % DMSO in water) at 1 or 3 mg/kg (i.v.) every 2 or every 3 days or 3 mg/kg (oral gavage, 100 μL) every day for 15 or 20 days. Animals were monitored every day, and tumour sizes were measured with calipers and body weights were taken every 2 or 3 days. Tumour volumes were calculated according to the formula \( V = 0.52 \times a^2 \times b \), where \( a \) is the smallest superficial diameter and \( b \) is the largest superficial diameter. For each treatment group, the tumour volumes for each set of measurements were statistically analyzed in comparison with the control (untreated) group.
2.5 Plasma and Tumour Tissue Analysis

Compound 3.7o concentrations in mouse plasma and tumour tissue lysates were assayed using a validated analytical procedure via UHPLC (Prominence UHPLC; Shimadzu Scientific Instruments) and LC/MS/MS (API 4000 linear ion trap mass spectrometer; MDS Sciex). The mass spectrometer was operated in a product ion-scanning mode. 3.7o diluted in methanol was infused directly into the MS source at a flow rate of 10 μL/min. Tuning was evaluated in both positive and negative MS modes using both turbo ion spray and atmospheric pressure chemical ionization sources. The chromatography used a Phenomenex Kinetex C18 2.1 × 50 mm, 1.7 μUHPLC column, with a flow rate of 0.300 mL/min using 5 mM ammonium acetate (in water) and 5 mM ammonium acetate (in acetonitrile) as mobile phases A and B, respectively.

3 Results

A family of 16 novel sulfonamide analogues of 2.18h were prepared as outlined in Scheme 3.1. Briefly, we TFA protected the amino group of sarcosine tert-butyl ester (3.1) to furnish 3.2, and then removed the tert-butyl ester under acidic conditions (TFA/CH₂Cl₂) to yield the carboxylic acid, 3.3. We also prepared secondary aniline 2.10h as described in Chapter 2. Condensation of 3.3 with secondary aniline 2.10h furnished tertiary amide, 3.4. The TFA protecting group was then removed by LiOH mediated hydrolysis revealing secondary amine, 3.5. In the penultimate step we coupled a diverse variety of sulfonyl chlorides to 3.5, yielding compounds, 3.6a-o. Finally, hydrogenolysis conditions (H₂, 10 % Pd/C) were employed to debenzylate the salicylic acid moiety, exposing final compounds 3.7a-o. Of note, in cases where hydrogenolysis conditions were incompatible with the sulfonyl substituent (3.7f, 3.7j, 3.7k and 3.7n), we employed a step-wise, hydrolysis of the benzyl ester followed by TFA mediated debenzylation of the benzyl ether (Scheme 3.1, steps i, j).67
Scheme 3.1. a) BnBr (2eq), KOTBu, DMF, 0 °C-rt, 16 h, 73 %; b) 4-cyclohexylbenzaldehyde, AcOH, NaCNBH$_3$, rt, 16 h, 79 %; c) (CF$_3$CO)$_2$O, DIPEA, CH$_2$Cl$_2$, rt, 3 h, 96 %; d) TFA/CH$_2$Cl$_2$, 1:1, rt, 5 h, 100 %; e) 2.10h, PPh$_3$Cl$_2$, CHCl$_3$, 60 °C, 12 h, 97 %; f) LiOH. H$_2$O, THF/H$_2$O, 3:1, rt, 10 min, 98 %; g) RSO$_2$Cl, DIPEA, CH$_2$Cl$_2$, rt, 16 h, 78-98 %; h) H$_2$, 10 % Pd/C, THF/MeOH, 1:1, rt, 1-16 h, 85-100 %; or for 3.7j, 3.7k and 3.7n: i) LiOH·H$_2$O, THF/H$_2$O, 3:1, rt, 24 h, 73-89 %; j) TFA/CH$_2$Cl$_2$, 1:2, rt, 16 h, 65-92 %.

Table 3.1. EMSA inhibition data for the disruption of the Stat3-Stat3-DNA complex by sulfonamide analogues 2.18h and 3.7a-o.
We first assessed for inhibitor induced Stat3-Stat3 dimer disruption using EMSA, which measures dimer disruption through inhibition of DNA binding. As illustrated in Table 3.1, varying the sulfonamide substituent resulted in varying degrees of inhibitor potency. We incorporated a range of appendages to cater for the relatively hydrophobic pocket composed of residues Ile634, Ser636, Glu594 and the hydrophobic chain of Lys591. In general, hydrophobic R groups afforded the most potent inhibitors. The polar 3.7h, incorporating a 1-methyl-1H-imidazole group, lost all inhibitory potency (IC₅₀ > 300 µM). Interestingly, employing the meta-
tolyl isomer 3.7a significantly reduced activity, (3.7a (meta-) IC$_{50}$ = 118.8 µM cf. 2.18h (para-) IC$_{50}$ = 35 µM)). The bulkier 2,4,6-tri-methylphenyl substituted inhibitor, 3.7b exhibited weaker activity than the parent compound 2.18h, with an IC$_{50}$ = 51.9 µM. The larger biphenyl sulfonamide, 3.7c, was a modest inhibitor of Stat3 dimerization (IC$_{50}$ = 65.4 µM), as was the 2-naphthyl derivative, 3.7d (IC$_{50}$ = 79.2 µM). Notably, bis-aryl sulfonyl derivatives substituted at the 1-position, including, 3.7e (R = 1-naphthyl, IC$_{50}$ = 28.8 µM), 3.7f (R = 8-quinolinyl, IC$_{50}$ = 25 µM) and 3.7g (R = dansyl, IC$_{50}$ = 29 µM) proved to be active Stat3 inhibitors. In general, replacement of the methyl group in the para-tolyl moiety of 2.18h with different isosteres (F, Br, Cl, OMe, NO$_2$) led to a reduction in Stat3 inhibitory activity. However, 3.7o, incorporating a pentafluorophenyl sulfonamide substituent, proved to be the most active of the phenyl sulfonamide series. Indeed, 3.7o was approximately two-fold more potent as the parent compound 2.18h (3.7o, IC$_{50}$ = 20 µM cf. 2.18h, IC$_{50}$ = 35 µM).

Table 3.2. FP assay binding data for selected sulfonamide derivatives
Next, we investigated the binding potency of select agents against Stat3’s SH2 domain by employing a routinely used FP assay, the results of which are shown in Table 3.2. Encouragingly, compounds 3.7b (IC₅₀ = 16 µM), 3.7c (IC₅₀ = 12 µM), 3.7g (IC₅₀ = 26 µM), 3.7k (IC₅₀ = 22 µM) and 3.7o (IC₅₀ = 26 µM) exhibited improved activity compared to compound 2.18h. Although similar trends were observed in the EMSA and FP data, there are some notable deviations between the two sets of data. For example, compound 3.7b, IC₅₀ = 51.9 µM in EMSA is much more potent in the FP assay (IC₅₀ = 16 µM). As previously reported, this anomaly between EMSA and the FP assay is likely due to the presence of other STAT isoforms and nuclear proteins found in the nuclear extracts used in EMSA. Taken together, the EMSA and FP results suggest that we are able to disrupt Stat3-phosphopeptide and Stat3-Stat3-DNA complexation events by effectively blocking the Stat3 SH2 domain.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>IC₅₀ (µM)</th>
<th>Compound</th>
<th>R</th>
<th>IC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.18h</td>
<td></td>
<td>31 ± 9</td>
<td>3.7g</td>
<td></td>
<td>26 ± 1</td>
</tr>
<tr>
<td>3.7b</td>
<td></td>
<td>16 ± 5</td>
<td>3.7h</td>
<td></td>
<td>&gt; 100</td>
</tr>
<tr>
<td>3.7c</td>
<td></td>
<td>12 ± 4</td>
<td>3.7i</td>
<td></td>
<td>61 ± 22</td>
</tr>
<tr>
<td>3.7e</td>
<td></td>
<td>53 ± 1</td>
<td>3.7k</td>
<td></td>
<td>22 ± 1</td>
</tr>
<tr>
<td>3.7f</td>
<td></td>
<td>82 ± 2</td>
<td>3.7o</td>
<td></td>
<td>26 ± 1</td>
</tr>
</tbody>
</table>
Table 3.3. Effects on cancer cell viability as determined by an MTS assay. Cells were treated with varying concentrations of inhibitors for 72 hours.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>EC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MDA-468</td>
</tr>
<tr>
<td>2.18h</td>
<td></td>
<td>17.0 ± 4.4</td>
</tr>
<tr>
<td>3.7e</td>
<td></td>
<td>46.5 ± 12.4</td>
</tr>
<tr>
<td>3.7o</td>
<td></td>
<td>10.9 ± 3.0</td>
</tr>
</tbody>
</table>

Since blockage of Stat3 signaling in cancer cell lines leads to induced apoptosis,\(^75\) we reasoned that our most potent inhibitors would kill cells harbouring constitutively activated Stat3. Thus, we employed a 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay to assess cellular potency of select inhibitors including, 2.18h, 3.7e and 3.7o, which showed promising activity in both EMSA and FP assays.\(^76,\)\(^77\) DU145 (prostate), MDA-468 (breast) and JNJ-3 (multiple myeloma) cancer cells were incubated for 72 hours with varying concentrations of inhibitors and relative viability assessed colorometrically after treatment with MTS for 3 hrs. Notably, compound 3.7o displayed an approximately two-fold increase in potency over 2.18h, with IC₅₀ values of 10.9, 22.7 and 16.7 µM in breast, prostate and MM cells, respectively. Compound 3.7e showed less activity in cells than both 2.18h and 3.7o, possibly a result of increased lipophilicity and poorer water solubility. We noted that 3.7o exhibited much improved water solubility over both 2.18h and 3.7e. While the \textit{in vitro} activities of 3.7o and 2.18h are comparable, we postulated that the resultant increase in cellular activity may be, in part, a result of greater cell permeability and reduced aggregation/precipitation.
Figure 3.2. SDS-Page and Western blotting analysis of whole cell lysates prepared from MDA-468 human breast cancer and multiple myeloma JJN-3 cells, untreated (DMSO, control) or treated with 3.7o (15 µM), 3.7e (125 or 150 µM), and 2.18h (100 or 125 µM) for 24 h and subjected to immunoblotting analysis for pYStat3, Stat3, c-Myc, Bcl-xL, Mcl-1 and survivin.

Due to the promising activity observed in tumour cells, 3.7o was assayed for inhibition of Stat3 phosphorylation in both MDA-468 and JJN-3 cell lines harbouring activated Stat3. As a control, Western blot analysis showed that control inhibitor, 2.18h effectively knocked down Stat3 phosphorylation at approximately 100 µM in both MDA-468 and JJN-3 cancer cells. Most encouragingly, 3.7o inhibited Stat3 phosphorylation at much lower concentrations (15 µM). Furthermore, immunoblotting analysis of the same cell lines revealed that 3.7o effectively reduced levels of Stat3 downstream targets, including, c-Myc, Bcl-xL and survivin. The data showed that 3.7o is a more potent inhibitor of Stat3 function in cells than previous lead compound, 2.18h, presumably due to improved solubility and cell permeability.
Figure 3.3. A) Cultured MDA-MB-231 (231 and 231/St3C), A549, DU145, Panc-1, and NIH3T3/vSrc (v-Src) cells harbouring aberrantly active Stat3 and NIH3T3 (3T3), NIH3T3/vRas (v-Ras), TE-71 mouse thymus stromal epithelial cells, Stat3−/− MEFs Stat3-null mouse embryonic fibroblasts (-/-MEFs), A2780S cisplatin-sensitive ovarian cancer cells that do not have constitutively active Stat3, were treated once with 0-30 μM 3.7o and subjected to CyQUANT cell proliferation assay. B) Human breast tumour xenografts and the antitumour effects of 3.7o. Mice bearing MDA-MB-231 tumours were administered 3.7o via i.v., 1 or 3 mg/kg or vehicle (0.05 % DMSO in PBS) every 2 or 3 days. Tumour sizes, measured every 2 or 3 days, were converted to tumour volumes and plotted against days of treatment. C) Western blot analysis of excised tumour cell lysates post treatment.

To assess suitability for in vivo study, 3.7o was screened against a panel of healthy cell lines and against transformed cell lines that possessed constitutively activated Stat3. Consistent with parent compound 2.18h, treatment with 3.7o had no effect on cell lines that did not possess constitutive Stat3 activation. However, cancer cell lines that possessed constitutively activated Stat3 were sensitive to treatment with 3.7o in a dose dependent manner.

As depicted in Figure 3.3B, significant delay of tumour growth was observed with dosing of 1 mg/kg, and suppression of tumour growth was observed at 2 mg/kg (by tail vein injection). After
completion of the 15 day study, tumours were excised and analyzed for their gene expression profile. Western blot analysis of tumour cell lysates revealed inhibition of Stat3 activity and down regulation of the expression of Stat3 target genes (c-Myc, cyclin D1, Bcl-xL survivin and VEGF) in a dose-dependent manner. Similarly, oral dosing at 3 mg/kg daily induced potent suppression of tumour growth over the course of the 20 day study (Figure 3.4A). Analysis of tumour cell lysates post treatment also showed potent inhibition of Stat3 phosphorylation and Stat3 gene targets (Figure 3.4B). Importantly, no significant changes in body weight or obvious signs of toxicity, such as loss of appetite, decreased activity, or lethargy were observed with either dosing strategy.

_In vivo_ pharmacokinetic profiling of plasma samples post-i.v. or post-oral treatment (3 mg/kg) with a single dose of 3.7o are displayed in Figure 3.4, C and D. Both dosing strategies resulted in high levels of 3.7o being detected in the plasma (concentrations of 35 μM and 30 μM with i.v. and oral dosing, respectively). Furthermore, both dosing methods resulted in detectable levels of 3.7o in the plasma up to 6 hours post treatment. Taken together, this data confirmed that 3.7o had good oral bioavailability and was present at levels sufficient to inhibit aberrantly active Stat3 function and inhibit tumour growth.
Figure 3.4. Human breast tumour xenografts and the antitumour effects and in vivo pharmacokinetic properties of 3.7o. Mice bearing MDA-MB-231 tumours were administered 3.7o (3 mg/kg) or vehicle (0.05 % DMSO) every day. Tumour sizes, measured every 2 or 3 days, were converted to tumour volumes and plotted against days of treatment. B) Post treatment, tumours were extracted and inhibition of Stat3 phosphorylation and expression of genetic targets was assessed using Western blot. C and D) Graphical representations of 3.7o levels in plasma samples collected from mice 15-360 minutes post-single dosing of 3 mg/kg via i.v. (C) or oral gavage (D).

4 Discussion and Conclusions

We have presented the design and synthesis of a novel family of Stat3 inhibitors that exhibit promising in vitro binding potency for the Stat3 SH2 domain, as well as improved tumour cell activity. Most notably, hit compound, 3.7o, showed an approximately 2- to 4-fold increase in in vitro activity compared to previous lead agent, 2.18h, and nearly 6-fold higher potency in cell based assays. Compound 3.7o was non-toxic to a panel of cell lines that did not have
constitutively active Stat3. Furthermore, agent 3.7o effectively inhibited tumour growth of MDA-MB-231 tumours in mouse xenograft models with treatment of just 1 to 3 mg/kg orally or intravenously. Finally, 3.7o was detectable at µM concentrations up to 6 hours post treatment. Taken together, these findings indicated that 3.7o is a potent inhibitor of Stat3 protein and has potent anti-cancer activity. Currently, 3.7o (also known as BP-1-102) is being pursued as a candidate for advanced preclinical trials as a cancer therapeutic agent.
Chapter 4  
Inhibiting Activated Stat3 Proteins with Tetrapodal Small Molecule SH2 Domain Binders: Promising Agents Against Multiple Myeloma

This chapter is primarily taken from our 2013 article in *J. Med. Chem.* In accordance with the copyright transfer agreements, a link to the web version of this publication can be found at the end of this thesis. For this chapter, chemical syntheses, *in silico* docking studies and FP assays were performed in the laboratory of Prof. Patrick T. Gunning. MTS cell viability assays were conducted in the laboratory of Prof. Aaron D. Schimmer at Princess Margaret Hospital with the assistance of Sumaiya Sharmeen and Dr. Paul Spagnuolo. MTT cell viability assays, Western blots, Annexin V apoptosis assays, luciferase assays and phosphoflow cytometry were performed by members of Prof. Suzanne Trudel’s research group at Princess Margaret Hospital.

1 Introduction

Multiple myeloma (MM) is the second most common hematologic malignancy and is responsible for approximately 13% of blood cancers and 1% of all cancers.\(^{80}\) Although MM is generally regarded as incurable, traditional high-dose chemotherapeutics and currently available targeted therapies can improve the prognosis of MM patients when used as part of an aggressive treatment regimen.\(^{80-83}\) Despite this, the median survival time after conventional treatment remains disappointingly low (3–4 years).\(^{80}\) In the search for novel molecular targets in MM, Stat3 has emerged as a driving force behind the maintenance and progression of the disease and it is anticipated that Stat3 inhibitors will provide a novel and effective weapon in the fight against MM.\(^{24, 84, 85}\)

In previous work, we discovered several tripodal inhibitors of Stat3 that have demonstrated promising anti-cancer activity.\(^{65, 71, 72, 86}\) Two lead compounds, 2.18h and 3.7o, have demonstrated potent Stat3 SH2 domain binding, improved inhibition of cellular Stat3 activity, and promising activity against cancer cell lines and xenograft models.\(^{72, 86}\) In Chapter 2, we identified lead compound 2.18h and briefly explored modifying the substituent on the sulfonamide nitrogen, where the NCH\(_3\) group of 2.18h was replaced with an oxygen atom, an
NH or an NBoc group (compounds 2.36, 2.31 and 2.33 respectively, shown in Table 4.1). The subsequent changes in inhibitor activity prompted further investigation into the sulphonamide nitrogen position.

**Table 4.1. Preliminary SAR leading to the development of lead compounds 2.18h and 3.7o.**

Compound activity was assessed using EMSA as previously reported.\(^{66, 86}\)

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>X</th>
<th>R’</th>
<th>IC(_{50}) (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S31-201 (1.6)</td>
<td>H</td>
<td>O</td>
<td>p-Tolyl</td>
<td>84 ± 33</td>
</tr>
<tr>
<td>2.1</td>
<td>H</td>
<td>NH</td>
<td>p-Tolyl</td>
<td>&gt; 300</td>
</tr>
<tr>
<td>2.2</td>
<td>H</td>
<td>NCH(_3)</td>
<td>p-Tolyl</td>
<td>&gt; 300</td>
</tr>
<tr>
<td>2.3</td>
<td>H</td>
<td>NBoc</td>
<td>p-Tolyl</td>
<td>&gt; 300</td>
</tr>
<tr>
<td>2.36</td>
<td></td>
<td>O</td>
<td>p-Tolyl</td>
<td>43 ± 13</td>
</tr>
<tr>
<td>2.31</td>
<td></td>
<td>NH</td>
<td>p-Tolyl</td>
<td>95 ± 35</td>
</tr>
<tr>
<td>2.18h</td>
<td></td>
<td>NCH(_3)</td>
<td>p-Tolyl</td>
<td>35 ± 9</td>
</tr>
<tr>
<td>2.33</td>
<td></td>
<td>NBoc</td>
<td>p-Tolyl</td>
<td>115 ± 35</td>
</tr>
<tr>
<td>3.7o</td>
<td></td>
<td>NCH(_3)</td>
<td>C(_6)F(_5)</td>
<td>19.7 ± 5.8</td>
</tr>
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</table>
2 Materials and Methods

2.1 GOLD Docking Simulations

Inhibitors were docked using GOLD docking software to Stat3 crystal structure, pdb 1BG1. Compounds were first optimized into a low energy geometry. The compound binding site was set to an area with a 12 Å radius surrounding Ser636. Best solutions were visualized using Pymol, which was utilized to create the images shown in Figures 4.2-4.4.

2.2 Fluorescence Polarization Assay

The fluorescence polarization assay was performed as previously reported. Briefly, fluorescently labelled peptide probe (5-FAM-GpYLPQTV-NH₂) was incubated with Stat3 protein, and inhibitor for 30 minutes then analyzed on a Tecan M1000 fluorimeter. Polarized fluorescence was plotted against concentration of inhibitor and IC₅₀ values were determined by fitting to a dose response curve. Representative curves of the top compounds are shown in the Experimental section.

2.3 Cell Viability Assays

The MTS and MTT assays were used to measure cellular metabolic activity, which reflects the number of viable cells. Cells were seeded in 96 well plates at 1-3 × 10⁴ cells/well in 90 μL of fresh culture medium. Prior to the addition of cell suspensions, 10 μL of test compound (or vehicle control) was added to wells in triplicate. Cultures were then incubated for 72 hours at 37 °C, 5 % CO₂. Following treatment, cell viability was assessed by MTS assay (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium for AML2, DU145 and MDA468 cell lines, or MTT assay (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide for MM cell lines. Relative cell viability (to DMSO control) was determined colourometrically and EC₅₀ values determined by fitting to a standard dose response curve when applicable.

2.4 Immunoblotting

Following treatment, target cells were harvested and washed twice in ice cold PBS. The resulting cell pellets were lysed in lysis buffer ((50mM Tris-HCl pH 7.4, 150 mM NaCl, 1 mM...
ethylenediaminetetraacetic acid (EDTA), and 1% NP-40) supplemented with 1 mM phenylmethanesulfonyl fluoride (PMSF), 2 mM sodium vanadate (Na$_3$VO$_4$) and protease inhibitor cocktail (Roche Applied Science) for 30 minutes on ice. Protein lysates were collected by centrifugation at 14,000 g for 15 minutes. Protein concentration was determined by Bradford Assay (Thermo Scientific, Rockford, IL) and normalized with lysis buffer before the addition of β-Mercaptoethanol-supplemented Lamelli sample buffer (Bio-Rad Laboratories, Hercules, CA). Proteins (10-30 µg) were resolved by SDS-PAGE using 7-10% gels, and transferred to polyvinylidene fluoride (PVDF) membranes using wet transfer at 70 V for 1 hour. Membranes were rinsed in Tris-buffered Saline with 0.01% Tween-20 (TBST) and blocked for 1 hour at room temperature in TBST containing 5% bovine serum albumin (BSA) powder, followed by overnight incubation with primary antibodies at 4 °C. Primary antibodies against the indicated proteins were diluted in TBST with either 5% BSA or 5% milk, as specified by manufacturer. Following three 15-minute washes in TBST, membranes were incubated with horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse secondary antibodies (Thermo Scientific Pierce, Rockford, IL) diluted 1:4000 in TBST for 1 hour at room temperature. Membranes were developed using the enhanced chemiluminescence kit (Perkin Elmer, Waitham, MA) according to the manufacturer’s instructions and visualized by autoradiography. Resulting autoradiographs were analyzed by densitometry using the Gel Doc XR station and Quantity One Software (Bio-Rad, Hercules, CA).

### 2.5 Luciferase Reporter Assay

Target cells were transduced with replication incompetent, VSV-g pseudotyped lentiviral particles containing the Stat3-driven Firefly luciferase reporter constructs (pCignal Lenti-Stat3$_{TRE}$-FLuc). Transductions were performed with polybrene (8 µg/µl) in accordance with the Cignal Lenti Reporter Assay Kit (SA Biosciences, Frederick, MD). The pCignal Lenti-Stat3$_{TRE}$-FLuc reporter construct is under the control of a basal promoter element (TATA box) joined to tandem repeats of a specific Stat3 transcriptional response element (TRE), and regulates the expression of the mammalian codon-optimized, non-secreted form of the Firefly luciferase gene. Stably transduced cells were selected using puromycin (2 µg/µl) for 2 weeks. As an internal control, Stat3FLuc-expressing cells were stably transfected with the pCignal Lenti-CMV-RLuc reporter construct, which contains a CMV immediately early enhancer/promoter that
constitutively drives *Renilla* luciferase expression. Transductions were performed as previously described and stable cells selected with hygromycin (50 µg/µl) for 2 weeks. *In vitro* reporter construct activity of drug treated cells was measured using the Stop & Glo® Dual Luciferase Assay System (Promega, Madison, WI), with data presented as relative luciferase units (RLU = *Firefly* luciferase/*Renilla* luciferase).

3 Results

Figure 4.1. GOLD\textsuperscript{73,87} docking images of compounds bound to Stat3’s SH2 domain (Stat3 pdb 1BG1\textsuperscript{37}). A) S3I-201 (1.6); B) 2.18h; C) 3.7o; D) 2.33.

We first utilized docking simulations to explore potential binding interactions of 2.33 which possesses the hydrophobic Boc group appended to the sulfonamide nitrogen. Comparing 2.33 to parent compounds 1.6, 2.18h and 3.7o, we observed interesting differences. Compounds 1.6, 2.18h and 3.7o were found to dock to the SH2 domain of Stat3 with similar conformations as previously reported\textsuperscript{60,65,71,88} Again, we presume the salicylic acid group mimics the pTyr motif and facilitates docking with the polar, phosphate binding region. The N-cyclohexylbenzyl substituent, common to 2.18h, 3.7o and 2.33 was found to interact *in silico* with the hydrophobic residues, Val637 and Trp623. The sulphonamide moieties interacted with the amphipathic region which contained Ile634 and Glu594 as well as the hydrophobic side-chain of Lys591.
When the NBoc derivative (2.33) was docked in silico, it was found to orient similarly to 2.18h and 3.7o. However, the bulky, hydrophobic t-butyl group was found to disfavourably orientate away from the protein surface (Figure 4.2, A and B). However, this seemingly unfavourable docking position is not reflected in the in vitro: by EMSA, only a slight decrease in potency was observed,26 and an improved binding affinity was observed by FP assay (Compound 2.33 IC₅₀ = 15.8 ± 0.2 µM cf. compound 2.18h IC₅₀ = 31.0 ± 9.4 µM).

Further docking studies revealed an alternative binding mode for 2.33 where the Boc group contributed to protein surface binding (Figure 4.2, C and D). However, unlike previous studies, the N-cyclohexylbenzyl moiety was positioned within the amphipathic binding pocket containing residues Ile634, Glu594 and the side chain of Lys591. As a result, the substituted sulphonamide group projects into the hydrophobic cleft composed of residues including, Trp623 and Val637. The substituent on the sulphonamide nitrogen then interacts with Trp623 and Phe716 and places the sulfonamide S-substituent in closer proximity to Cys712. This orientation allows for improved interaction between the protein and larger hydrophobic substituents appended to the sulphonamide nitrogen.
Figure 4.2. Popular binding modalities of 2.33 bound to Stat3’s SH2 domain (pdb 1BG137). Images A and B show the previously predicted docking poses of 2.33. Images C and D show an alternative binding mode, where the NBoc group contributes to protein surface binding.

To further explore the in vitro Stat3 binding potency and whole cell biological effects of N-alkylated analogs of 2.18h. Inhibitors were first functionalized with a small set of simple alkyl and benzyl groups stemming from the sulfonamide nitrogen to furnish a preliminary series of tolyl-N-alkyl derivatives (compounds 4.2a to 4.2k). An initial FP assay, suggested that functionalized N-benzyl compounds may confer optimal binding, thus, a further series of N-benzylated analogs were synthesized (compounds 4.2l-4.2ap).
Scheme 4.1. Synthesis of the tolyl-N-alkyl derivatives, 2.30 was prepared as described in Scheme 2.7; a) RBr or RCl, Cs₂CO₃, DMF, rt, 1 h, 43-100 %; b) H₂, Pd/C, THF/MeOH, 1:1, rt, 6 h, or i. LiOH·H₂O, H₂O/THF, 1:3, rt, 16 h; ii. TFA/toluene, 1:1, rt, 1 h, 61-94 %.

The tolyl-N-alkyl derivatives were prepared from sulfonamide 2.30 (prepared as described in Scheme 2.7) which was then functionalized with a variety of alkyl bromides, alkyl chlorides or Boc₂O and then deprotected using hydrogenolysis or a step-wise saponification of the benzyl ester followed by treatment with TFA to cleave the benzyl ether. This protocol was used to produce a library of 45 compounds depicted in Table 4.2.

A similar strategy was used to synthesize a library of prefluorobenzene-N-alkyl derivatives, where top N-alkyl groups were appended to the prefluorobenzene-N-alkyl scaffold. To make the prefluorobenzene-N-alkyl derivatives, Fmoc-glycine was coupled to secondary aniline, 2.33, using PPh₃Cl₂. The Fmoc group was removed using piperidine in DMF to afford the free amine. Sulfonamide 4.5 was prepared by treating amine 4.4 with pentafluorobenzenesulfonyl chloride. A variety of different alkyl bromides (or Boc₂O) were then used to furnish the sulphonamide nitrogen then treatment with hydrogen and 10 % Pd/C gave the deprotected final molecules. Of note, a step-wise deprotection procedure could not be used for the synthesis of the perfluorobenzene-N-alkyl derivatives as treatment with LiOH led to nucleophilic aromatic substitution of the perfluorobenzene ring, placing a hydroxyl group para- to the sulphonamide.
Scheme 4.2. Synthesis of perfluorobenzene-N-alkyl derivatives; a) PPh$_3$Cl$_2$, Fmoc-Gly-OH, CHCl$_3$, 0.5 h, 110 °C, microwave, 76 %; b: DMF/piperidine, 9:1, rt, 0.5 h, 62 %; c: C$_6$F$_5$SO$_2$Cl, K$_2$CO$_3$, MeCN, 4 Å MS, 0 °C-rt, 4 h, 64 %; d: RBr, Cs$_2$CO$_3$, DMF, rt, 1 h, 64-100 % or Boc$_2$O, DMAP, CH$_2$Cl$_2$, rt, 0.5 h, 99 %; e: H$_2$, Pd/C, THF/MeOH, 1:1, rt, 1-6 h, 72-100 %.
Table 4.2. Tolyl-N-alkyl Derivatives IC₅₀ values reported for FP assay.

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Table 4.3. Perfluorobenzene-N-alkyl derivatives IC<sub>50</sub> values reported for FP assay.

![Perfluorobenzene-N-alkyl derivatives](image)

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To assess inhibitor binding we tested all compounds an FP assay as previously reported. The FP assay measures the disruption of a high affinity phosphopeptide probe from Stat3’s SH2 domain. Relative to parent compounds, 2.18h and 3.7o, many of the N-alkyl derivatives showed improved activity. Moderate improvements were observed through addition of simple alkyl groups however, it appears that substituted benzyl moieties provided the greatest enhancement of inhibitory activity. Substitution with the polar pyridine or aminobenzyl appendages led to a marked loss in protein binding affinity.

All compounds were then subjected to an MTS assay to assess the in vitro anti-tumour activity of compounds. Most promisingly, a number of these compounds potently inhibited the viability of a range of cancer cell lines including prostate cancer (DU145), breast cancer (MDA-468) and leukemia (AML2). EC<sub>50</sub> values and corresponding structures for the top five compounds from each family and their corresponding parent structures are summarized in Figure 4.3. Representative dose response curves can be found in Appendix 3.
Figure 4.3. EC\textsubscript{50} values for the top five tolyl-N-alkyl and perfluoro-N-alkyl compounds as determined by MTS assay.

Based on protein binding affinity and inhibition of cell proliferation, lead compounds 4.2i and 4.7h demonstrated very promising activity within their respective libraries and were selected as promising new lead molecules.

Similar to compound 2.33 these new lead compounds dock in a different orientation, compared to their respective parent compounds. Figure 4.4 shows compounds 4.2i and 4.7h docked to the SH2 domain of Stat3, where the N-alkyl group contributes to binding at the protein surface. We propose that this favourable interaction helps to explain the increased activity of these inhibitors relative to parent compounds 2.18h and 3.7o.
Figure 4.4. GOLD docking images of lead compounds 4.7h (A and B) 4.2i (C and D). Compounds were found to optimally bind in a similar orientation to compound 2.33. The N-alkyl group improves binding potency due to a more complete occupation of the Stat3 SH2 domain.

To further evaluate our new lead compounds, we tested their ability to inhibit MM proliferation in an MTT assay. We reasoned that these new lead compounds should demonstrate more activity against MM cell lines that harbour high levels of pStat3 as they would presumably be more reliant on aberrant Stat3 activity. Therefore, prior to testing, baseline Stat3 activation was first examined in a panel of genetically heterogeneous MM cell lines using Tyr705 phosphorylation as a surrogate marker of Stat3 activation. Whole cell lysates prepared from MM cell lines in logarithmic growth conditions were subject to immunoblot analysis and probed with antibodies
against pStat3 (Tyr705) and total Stat3 protein. Although Stat3 protein was expressed in all MM cell lines, albeit to varying degrees, constitutive pStat3 was only detected in 6 of 8 MM cell lines, with two cell lines, MM1.S and SKMM2, lacking detectable pStat3 (Figure 4.4A). Densitometric analysis of pStat3 and total Stat3 protein confirmed variability in baseline pStat3 levels. In the interests of examining the effects of our Stat3 inhibitors, we selected MM cell lines possessing a variety of pStat3 levels for screening lead compounds, predicting that cell lines with high pStat3 should be more sensitive to Stat3 inhibition.

As shown in Figure 4.5B, both 4.2i and 4.7h demonstrated dose-dependent inhibition of MM cell viability after 72 hours of treatment. Compared to baseline pStat3 levels in tested MM cell lines, we noted that 4.7h, the perfluorobenzenesulfonamide analogue, showed activity against non-pStat3 containing MM cell lines SKMM2 and MM1.S, whereas 4.2i exhibited lower biological activity against these cell lines. Moreover, JJN3, which contains high constitutive Stat3 activation, was more resistant to 4.7h than 4.2i. Notably, these new lead compounds were generally around two-fold more potent than parent compounds 2.18h and 3.7o (data not shown). Taken together, these findings suggest that the potent anti-MM activity of 4.7h may be, at least in part, a result of off-target effects, whereas 4.2i delivered a more desirable activity profile. Alternatively, the broad activity of 4.7h against this panel of MM cell lines, regardless of baseline Stat3 phosphorylation status, may reflect a universal dependence of MM tumour cells on non-canonical Stat3 signaling pathways that are dependent on a functional SH2 domain, but not Stat3 phosphorylation.
Figure 4.5. A) Western blot analysis of basal pStat3 activation in a panel of human MM cell lines. Quantitative analysis by densitometry shown below reveals relative levels of pStat3 to total Stat3 protein, and relative total Stat3 protein to GAPDH; B) MTT cell viability assay with 4.2i (upper) and 4.7h (lower) against panel of MM cell lines.
To further characterize the cellular activity of **4.2i** and **4.7h**, and the mechanisms of their effect on MM cell viability, we evaluated whether treatment with **4.2i** and **4.7h** induced apoptosis using flow cytometric analysis of Annexin V and PI staining. As indicated in Figure 4.6A, both **4.2i** and **4.7h** induced apoptosis dose- and time-dependently in 8226 cells as represented by a shift of cells from the lower left quadrant (viable cells), to the lower right quadrant (early apoptotic cells) at 24 hours, and migration to the upper right quadrant (late apoptosis) at 48 hours. Most promisingly, analysis of apoptosis in MM cell lines with varying degrees of sensitivity to **4.2i** and **4.7h** revealed similar results to those observed in the MTT assay, with a greater induction of apoptosis in 8226 cells compared to XG6 and JJN3 cells (Appendix 3).

To further confirm the induction of apoptosis following treatment with **4.2i** and **4.7h**, whole cell lysates drug treated JJN3 cells were collected and subject to immunoblot analysis for the detection of an additional marker of apoptosis, cleaved Poly ADP-ribose polymerase (cPARP). During the process of programmed cell death, PARP proteins are cleaved by proteases such as caspase-3, with the resulting cleavage fragment facilitating cellular disassembly, and thus serving as a marker of cells undergoing apoptosis. As shown in Figure 4.6B, both **4.2i** and **4.7h** induced cleavage of PARP, results which are consistent with flow cytometry experiments and the induction of the apoptotic program.
Figure 4.6. A) Flow cytometric analysis of 4.2i- and 4.7h-mediated apoptosis as measured by Annexin V and PI staining. Representative scatterplots for 8226 cells showing increased population of cells in the lower right quadrant after 24 hours of treatment, which migrate to the upper right quadrant after 48 hours. This pattern is indicative of time-dependent apoptotic responses, and consistent with cells respectively undergoing the early and late stages of apoptosis. B) Whole cell lysates from 4.2i- or 4.7h-treated JJN3 cells were subject to immunoblot analysis, and consistently, results reveal a dose-dependent increase in the apoptotic marker, cPARP.

Given the activity profiles of 4.2i and 4.7h in MM cell lines, we next evaluated the ability of these compounds to inhibit Stat3 phosphorylation. Exposure to 4.2i and 4.7h for 6 hours lead to dose-dependent inhibition of pStat3, and as expected, no inhibition of total Stat3 protein levels (Figure 4.7A). As Stat3 is a master transcriptional regulator, we also employed a Stat3-driven luciferase reporter construct to evaluate Stat3 transcriptional activity. In agreement with inhibition of Stat3 phosphorylation, treatment with 4.2i and 4.7h potently inhibited the transcriptional activity of Stat3 in 8226 and XG6 cell lines, with reductions in relative luciferase
ranging from approximately 50-80% after 6 hours (Figure 4.7B). For 4.2i, inhibition of transcriptional activity correlated well our initial MTT results. Conversely, treatment with 7.5 µM of 4.7h had little effect on luciferase production, however this concentration had drastic effects in our MTT assay. These results suggest that while both 4.2i and 4.7h inhibit Stat3 phosphorylation and transcriptional activity, it is proposed that some of the increased cellular activity of 4.7h may be due to off-target effects.

To evaluate whether 4.2i-4.7h-mediated inhibition of Stat3 phosphorylation and transcriptional activity was sufficient to abrogate downstream Stat3-induced gene expression, we evaluated the effect of these compounds on a known Stat3 target gene, c-Myc. Since this particular protein is known to have a very short half-life (20-30 minutes),89 we evaluated the resulting effects of drug treatment on c-Myc protein expression after 6 hours using immunoblot analysis. Consistent with the previously observed decrease in Stat3 transcriptional activity, both 4.2i and 4.7h dose-dependently reduced c-Myc protein expression (Figure 4.7C), however, in a separate analysis, negligible decreases were observed in other known Stat3 targets such as Bcl-xL and survivin (data not shown), which we speculate to be a result of differences in protein-specific kinetics.
Figure 4.7. Analysis of 4.2i and 4.7h targeted inhibition of Stat3 signaling. A) Western blot analysis of 4.2i- and 4.7h- mediated effects on pStat3 inhibition in JJN3 tumour cells, revealing dose-dependent inhibition of Stat3 phosphorylation. B) Luciferase assay demonstrating that after 6 hours, both 4.2i and 4.7h dose dependently inhibit Stat3-driven
luciferase expression, with consistent reduction in protein expression of Stat3 target gene c-Myc, as assessed by immunoblot analysis (C).

To address the selectivity of these compounds for inhibiting Stat3 over other Stat proteins, we performed FP assays to look at compound binding to Stat1 and Stat5. In this assay we found that both 4.2i and 4.7h show little selectivity for the Stat3 isoform over Stat1 and Stat5 (4.2i, Stat1 IC$_{50}$ = 5.8 ± 0.6 µM, Stat5 IC$_{50}$ = 8.5 ± 1.1 µM cf. Stat3 IC$_{50}$ = 2.8 ± 4.3 µM; 4.7h Stat1 IC$_{50}$ = 10.9 ± 0.8 µM, Stat5 IC$_{50}$ = 13.3 ± 0.9 µM cf. Stat3 IC$_{50}$ = 12.8 ± 2.8 µM, Appendix 3). This was also reflected using phospho-flow cytometry to investigate the effects of these compounds on cytokine-induced Stat1/3/5 phosphorylation. Although both 4.2i and 4.7h were shown to inhibit IL-6-induced Stat3 phosphorylation in these experiments, similar levels of inhibition were also observed for GM-CSF-induced Stat5 phosphorylation and IFNγ-induced Stat1 phosphorylation (Appendix 3). Thus, improving STAT isoform selectivity remains a goal for future compound libraries.

Nonetheless, given the preferred activity profile observed of 4.2i in the initial MTT assay, we continued analysis of this compound in primary MM patient samples. Most promisingly, 4.2i demonstrated activity against malignant plasma cells (CD138+) from primary MM patient samples (Figure 4.8A), with 20 µM treatment reducing MM tumour cell viability by over 50% in 3 patient samples. Most importantly, at doses exceeding 20µM, 4.2i demonstrated little activity against non-MM (CD138-) cells (Figure 4.8B). Furthermore, at doses of 30 µM, 4.2i had little effect on hematopoietic progenitor colony formation, suggesting that this compound does not inhibit the ability of normal hematopoietic progenitors to proliferate or form distinct colonies (Figure 4.8C). Taken together, our analysis of 4.2i in the context of primary MM patient samples has revealed that there is indeed a therapeutic window for this compound, which ultimately contributes its therapeutic validity, and that of other small molecule Stat3 inhibitors in at least a subset of MM tumours. Furthermore, although our data suggests that 4.7h may be a less selective inhibitor compared to 4.2i, it remains an intriguing anti-cancer compound, displaying potent in vitro cytotoxic effects in MM cell lines at low µM concentrations.
Figure 4.8. Activity of 4.2i against primary MM patient samples. Mononuclear cells (MNC) from MM patients were obtained by Ficoll-Paque separation of 6 patient derived bone marrow aspirates. Samples were cultured and treated with 4.2i followed by staining with antibodies against CD138 (MM cell surface marker) or Annexin V (apoptosis). Results are presented as the decrease in CD138+ cell population, representing MM cells (A), and decrease in CD138- cell population, representing non-MM cells (B left). Alternatively, isolated MNCs were cultured in MethoCult (StemCell Technologies), and treated with 4.2i to evaluate the activity of this agent on healthy hematopoietic progenitor colony formation (B right).

4 Discussion and Conclusions

We have presented a novel library of salicylic acid-based small molecule Stat3 inhibitors that offer promising Stat3 SH2 domain binding affinity and potent anti-MM activity. Lead
compounds, 4.2i and 4.7h, offer improved in vitro binding activity over precursors, 2.18h and 3.7o, respectively, and improved anti-cancer activity. We have presented in silico binding evidence that suggests that these compounds offer improved binding potency by docking in a different orientation than parent compounds. While we have yet to verify this through NMR or X-ray structural data, what we can deduce from the SAR presented is that Stat3’s SH2 domain can accommodate larger, tetrapodal analogs of the 2.18h and 3.7o scaffolds.

Both 4.2i and 4.7h are among the most potent small molecule Stat3 inhibitors to emerge from our laboratory. Although 4.7h was among the more potent inhibitor, further evaluation of its potential off-target effects are needed, and are currently underway. Both 4.2i and 4.7h were shown to disrupt phosphopeptide-Stat3 protein complexes, inhibit Stat3 phosphorylation and Stat3 transcriptional activity, with concurrent downregulation of Stat3 target genes. Moreover, both compounds have significant anti-MM activity, potently reducing MM cell line viability and promoting the induction of apoptosis. Although not as potent, 4.2i is predicted to have a more favourable selectivity profile, with limited cytotoxicity in healthy hematopoietic cells or in MM cell lines that harbour minimal pStat3. Current experiments aim to further characterize lead compounds 4.2i and 4.7h by analyzing their efficacy in vivo. It is hoped that these studies will help identify a Stat3 inhibitor that is suitable for advanced preclinical trials against MM.
Chapter 5
Targeting Stat3 in Therapy-resistant Chronic Myeloid Leukemia: a Novel Therapeutic Approach

For this chapter, chemical syntheses, in silico docking studies and FP assays were performed in the laboratory of Prof. Patrick T. Gunning. Luciferase assays and colony assays were performed by members of Prof. Michael W. Deininger’s research group at the Huntsman Cancer Institute. Mouse liver microsome metabolism studies were performed at the Ontario Institute for Cancer Research by Dr. Carly Griffin and under the supervision of Dr. Ahmed Aman and Dr. Rima Alwar.

1 Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm characterized by the presence of the BCR-ABL oncoprotein, a constitutively active tyrosine kinase responsible for malignant transformation.90,91 The standard therapy for CML is treatment with a tyrosine kinase inhibitor (TKI) such as imatinib (Gleevec™), a small molecule inhibitor of the ABL, c-Kit, and PDGFR tyrosine kinases.92 CML treatment with TKI therapy is very effective; 87 % of newly diagnosed CML patients treated with imatinib experience complete cytogenetic response (CCyR; a normal karyotype in 20 metaphase spreads).10 TKI therapy has revolutionized CML treatment, decreasing CML progenitor cell populations to undetectable levels. However, because CML stem cells are not sensitive to BCR-ABL inhibition, patients must remain on TKI therapy indefinitely to avoid disease relapse.10,14,93,94

Despite impressive results, some patients do not respond to TKI treatment (primary resistance) and others lose their response (acquired resistance), with an estimated failure rate of 20-30 %.10 95 The best characterized mechanism of CML drug resistance involves point mutations in the BCR-ABL kinase domain that prevent TKI binding.96,97 However, mutation-based resistance only accounts for a small cohort of TKI-resistant patients.95 In the majority of non-responding and resistant patients, no mutations in BCR-ABL are detected.95 Thus, kinase-independent resistance mechanisms warrant further investigation.
The evolution of second and third generation TKIs (dasatinib, nilotinib, ponatinib) have improved survival rates of CML patients. However, these new compounds are still incapable of managing many BCR-ABL mutations, and they do not address kinase-independent resistance mechanisms.\textsuperscript{11, 12, 14, 95, 98}

Stat3 protein was recently linked to kinase-independent resistance mechanisms in CML. In non-resistant CML, where TKIs are effective, there is minimal Stat3 activation.\textsuperscript{99, 100} However, Stat3 was found to be constitutively phosphorylated in models of TKI resistant CML.\textsuperscript{94, 99} Therefore, targeting Stat3 in combination with BCR-ABL may induce synthetic lethality and may help overcome kinase-independent resistance mechanisms in CML.\textsuperscript{99, 101} Furthermore, as Stat3 is absolutely required for malignant transformation in CML,\textsuperscript{100} it is proposed that by inhibiting Stat3 we may be able to target the CML stem cells and bring about a possible cure for CML.

This chapter will discuss our most recent work where we have used the framework of CML to screen our latest libraries of Stat3 inhibitors and have identified new lead compounds with promising activity against TKI resistant CML.

2 Materials and Methods

2.1 Cell Cultures and Primary Cells

K562, K562\textsuperscript{R}, AR230, AR230\textsuperscript{R}, HS-5 cells and derivative lines were maintained in RPMI1640 supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin-streptomycin, and 2 mM L-glutamine (GIBCO). K562\textsuperscript{R} and AR230\textsuperscript{R} cells that are resistant to imatinib were produced by long-term culture in the presence of low-dose imatinib, followed by incremental increases in the dose of drug (0.1-1.0 µM imatinib), and maintenance in the continual exposure of 1.0 µM imatinib. K562 and AR230 derivative lines were generated by retroviral or lentiviral infection followed by antibiotics-mediated selection or by sorting of green fluorescent protein-positive (GFP\textsuperscript{+}) cells. Mononuclear cells MNCs from peripheral blood (PB) of patients with chronic phase CML (CML-CP) or normal donors were Ficoll-separated and used for automated isolation of the CD34\textsuperscript{+} fraction using an autoMACS Pro (Miltenyi Biotech). Purity was determined to be >90% by fluorescence activated cell sorting (FACS). Prior to use in assays, CD34\textsuperscript{+} progenitors from CML patients were kept overnight in RPMI containing 20% FBS, 2 mM L-glutamine,
rhIL-3 (20 ng/mL), rhIL-6 (20 ng/mL), rhFlt-3 ligand (100 ng/mL), and rhSCF (100 ng/mL) (Stem Cell Technologies). Where indicated, 96-hour CML CD34+ cell assays were performed in the absence of cytokines. All patients gave their informed consent in accordance with the Declaration of Helsinki, and all studies with human specimens were approved by The University of Utah Institutional Review Board.

2.2 Clonogenic Assays

Methylcellulose colony assays were performed by plating in 0.9 % MethoCult (H4230; Stem Cell Technologies) in the presence or absence of imatinib (1.0 µM) and/or the indicated Stat3 inhibitors. For cell lines, $10^3$ cells were plated in the absence of cytokines. For CML CD34+ cells, $10^3$ cells were plated in the presence of rhIL-3 (20 ng/mL), rhIL-6 (20 ng/mL), rhFlt-3 ligand (100 ng/mL), and rhSCF (100 ng/mL).

2.3 HS-5 Conditioned Media Protection Assays

For protection assays using HS-5 conditioned medium (CM), HS-5 cells were grown to 80 % confluency overnight in regular medium (RM). The media was removed the next day, and cells were cultured for another 24 hours in fresh media. Following 24 hours, the resulting CM was harvested, centrifuged at 1200 rpm for 5 minutes to remove contaminating cells, and stored at -80 °C. K562 ($10^5$ cells/mL) or CML CD34+ ($10^6$ cells/mL) cells were cultured in either RM or CM with for 24-96 hours, followed by plating of $10^3$ cells in methylcellulose assays as described above. Viability was assayed using the Guava ViaCount Reagent (Millipore). Apoptosis was assayed using the Guava Nexin Annexin V Binding Assay (Millipore) or by staining with anti-Annexin antibodies and 7AAD.

2.4 Immunoblot Analysis

For CML cell lines, equal number cells were incubated in equal volume RM or CM and treated with TKIs for 24 hours. For CML CD34+ cells, assays were performed with $10^6$ cells/mL in (RPMI1640 plus 10 % BIT9500) RM or CM and treated TKIs for 24 hours. Following TKI exposure, cells either lysed (0 °C; 30 min.) in 30 µL RIPA buffer (150 mM NaCl, 1 % NP40, 0.1 % SDS, 1 M Tris [pH 8.0]) containing protease (Complete Mini, Roche) and phosphatase (PhosStop, Roche) inhibitors, or were used for direct lysis in 20 µL Laemmli buffer. Samples
were denatured (100 °C; 10 min) prior to SDS-PAGE and transfer to nitrocellulose. Antibodies used were as follows: rabbit anti-Stat3, rabbit anti-pStat3Y705, rabbit anti-pStat3S727, rabbit anti-Stat5, rabbit anti-pStat5Y694, anti-Abl, anti-pTyr (Cell Signaling Technologies); mouse anti-tubulin (Sigma).

2.5 Fluorescence Polarization Assay.

To assess Stat3 SH2 domain binding, a fluorescence polarization assay was used as previously reported. Briefly, Stat3 protein (150 nM), fluorescently labeled peptide probe (5-FAM-GpYLQTV-NH₂, 10 nM) and varying concentration of potential inhibitors were combined in a black, flat bottom 384 well plates. After a 30 minute incubation period, polarized fluorescence was measured using a Tecan M1000 fluorimeter. Polarized fluorescence was plotted as a function of concentration and IC₅₀ values were determined by fitting to a standard dose response curve.

2.6 Luciferase Inhibitor Screen.

To detect endogenous pStat3Y705 activity, we transduced AR230R cells with the pGreenFire Lenti-Reporter system (pGF1; System Biosciences) harbouring either sequential Stat3 sis-inducible elements (SIE) or negative control (NEG) sequences. The oligonucleotide sequence used to clone the SIE was 5’-
GTCGACATTTCGGTAAATCGTGAGTTCGACTTTCCCCTTAAATCGTCG3’; the sequence used for the NEG was 5’-
GTCGACATTACCGGTCGTATCTCCTGATCTCGACATTACCGGTCGTATCTCCTGTCG3’. AR230R (3x10⁵ cells) expressing either pGF1-SIE (AR230R-SIE) or pGF1-NEG (AR230R-NEG) were treated with 20 % HS-5 CM and exposed to imatinib (1.0 µM) and/or Stat3 inhibitors (5-10 µM) for 6 hours, followed by detection of luciferase reporter activity using the OneGlo luciferase kit (Promega). The relative ratio of AR230R-SIE to AR230R-NEG vs. untreated control was used to rank compounds.

2.7 Mouse Liver Microsome Metabolism Assay

For Phase I analysis, compounds were incubated at a final concentration of 1 µM. CD-1 mouse liver microsomes were utilized at a final concentration of 0.5 mg/ml. Duplicate wells were used
for each time point (0 and 30 minutes). Reactions were carried out at 37 °C in a shaking water bath. The final volume for each reaction was 200 µL, which includes the addition of an NADPH-Regeneration Solution (NRS) mix. The NRS mix was comprised of glucose 6-phosphate dehydrogenase, NADP+, MgCl₂, and glucose 6-phosphate. Upon completion of the 30 minute time point, reactions were terminated by the addition of 400 µL of ice-cold, acetonitrile. Samples were then centrifuged at 14 000 rpm for 10 minutes to remove debris and precipitated protein. 100 µL of supernatant was subsequently transferred to a new sample vial for UPLC-MS analysis.

3 Results

In the previous chapter, we identified several promising Stat3 inhibitors that potently disrupted Stat3-phosphopeptide binding, and inhibited Stat3 activity in MM cell lines and patient samples. Of the two families of compounds presented in Chapter 4, the perfluorobenzenesulfonamide derivatives demonstrated more potent anti-cancer activity. However, the cellular activity of these compounds could not be fully explained by Stat3 inhibition, implying a lack of target specificity. While the toluenesulfonamide derivatives demonstrated better selectivity profiles, they were not as potent as analogous compounds from the perfluorobenzenesulfonamide series. Thus, we designed a series of sulfonamide-S derivatives to incorporate the potency of the perfluorobenzenesulfonamide family while maintaining the selectivity profile of the toluenesulfonamide derivatives. We chose to functionalize the sulfonamide nitrogen with a 2,3,4,5,6-pentafluorobenzyl group as this had shown promising activity in preliminary work.
Scheme 5.1. a) 2,3,4,5,6-pentafluorobenzyl bromide, Cs₂CO₃, DMF, rt, 16 h, 87 %; b) RSO₂Cl, DIPEA, CH₂Cl₂, rt, 16 h, 48-100 %; c) H₂, 10 % Pd/C, THF/MeOH 1:1, rt, 1-6 h, 82-100 %.

A library of 24 derivatives was synthesized according to Scheme 5.1. These compounds were evaluated for Stat3 binding using a fluorescence polarization assay and are displayed in Table 5.1.54

Table 5.1. Library of N-2,3,4,5,6-pentafluorobenzylsulfonamide derivatives evaluated in the FP assay.
Of the compounds tested, a number of them showed good activity in the FP assay. To further expand this library, we selected two early leads, 5.3c and 5.3n and functionalized these derivatives with other top N-alkyl groups that were identified in Chapter 4. This was done as 5.3c and 5.3n had potent activity in the FP assay and exhibited close structural homology to previous lead compounds 4.2i and 4.7h. Thus, we synthesized a focused series of 4-trifluoromethylbenzenesulfonamide and 4-fluorobenzenesulfonamide N-alkyl derivatives (Schemes 5.2 and 5.3).
Scheme 5.2. Synthesis of N-CH₃-4-trifluoromethylbenzenesulfonamide analogue; a) 4-CF₃PhSO₂Cl, DIPEA, CH₂Cl₂, rt, 16 h, 84 %; b) LiOH·H₂O, THF/H₂O, 3:1, rt, 0.5 h, 96 %; c) 2.10h, PPh₃Cl₂, CHCl₃, 110 °C, 0.5 h, microwave, 42 %; f) H₂, 10 % Pd/C, THF/MeOH 1:1, rt, 6 h, 94 %.
Scheme 5.3. Synthesis of top N-Alkyl compounds; a) $R^1\text{SO}_2\text{Cl}$, K$_2$CO$_3$, MeCN, 0 °C-rt, 16 h, 48-83 %; b) $R^2\text{CH}_2\text{Br}$, Cs$_2$CO$_3$, DMF, rt, 1 h, 68-93 %; c) H$_2$, 10 % Pd/C, THF/MeOH, 1:1, rt, 1-6 h, 82-100 %.
Figure 5.1. Activity of top compounds by fluorescence polarization assay, IC\textsubscript{50} values (µM) are reported below the compound number

Compounds were screened in the FP assay (IC\textsubscript{50} values are reported in Figure 5.1) to identify further leads. The N-methyl functional group may not be optimal for Stat3 inhibitor binding, as all four of the N-methyl derivatives show IC\textsubscript{50} values greater than 20 µM (Figure 5.1, row A). However, potent disruption of Stat3-phosphopeptide interaction is observed with the three different N-benzyl groups in combination with any of the sulfonamide groups that were tested.

To confirm that these inhibitors are applicable to therapy-resistant CML, we designed a luciferase reporter assay to monitor endogenous Stat3 activity in TKI resistant AR230\textsuperscript{R} cells that express high levels of pStat3\textsuperscript{Y705}. AR230\textsuperscript{R}-SIE and AR230\textsuperscript{R}-NEG cells were treated with 5 µM of candidate Stat3 inhibitors to investigate inhibitor effects in a cellular environment. The resulting scatter plots of inhibitor activity are shown in Figure 5.2.
The pattern of response was variable, however several compounds offered improved potency and selectivity compared to lead compounds from Chapter 4.
Figure 5.2. Scatter plot of Stat3 inhibitor performance in the luciferase assay. AR230\textsuperscript{R}-NEG and AR230\textsuperscript{R}-SIE cells were incubated treated with 5 µM of inhibitor. The bottom plot displays the area highlighted by the blue square in the top plot.
Figure 5.3. Luciferase assay activity of lead compounds. Colour coding is representative of the difference in activity of SIE versus NEG luciferase constructs upon treatment with 5 µM of inhibitor.

Two of these compounds, 3.7o and 4.7z, demonstrated a significant effect (p<0.05) against the AR230R-NEG cells. Further, dose response experiments imply that this was not a concentration dependent effect suggesting that these compounds may inhibit other signaling pathways. This supports the theory that the perfluorobenzenesulfonamide group may contribute to inhibitor promiscuity. Other potent inhibitors from this series include 4.2aj, 5.10c and 5.10d.

Around this time, we had previous lead compounds (from Chapters 2 and 3) tested for metabolic stability using mouse liver microsome (MLM) assay. In this assay, compounds were exposed to
MLMs for 30 minutes and then examined by UPLC-MS to identify potential metabolites. Expected metabolites of 2.18h, 3.7o and 5.7 for comparison, were found and quantified to identify potential metabolic “soft spots”. Because of their structural similarity, we proposed that variations in metabolic stability could be attributed to the sulfonamide-S substitutent.

Looking at overall stability, we found that 3.7o is much more stable than both 2.18h and 5.7. All three of these compounds were susceptible to N-demethylation, especially compound 5.7: N-demethylation attributed to 17 % of degradation for 5.7 versus only 4.2 % and 3.9 % for 2.18h and 3.7o, respectively. This may help to explain the decreased activity observed for 5.7 in the luciferase assay. We proposed that N-benzylated derivatives such as those highlighted in Chapter 4, should be less prone metabolism via N-de-alkylation.

The major metabolite for 2.18h is the mono-hydroxylated product, which likely occurs on the activated benzylic position of the 4-tolyl substituent. Importantly, replacing this group with the trifluoromethylbenzene- or pentafluorobenzenesulfonamide group negates this mode of metabolic breakdown.
The major metabolites of 3.7o include substitution of a fluorine atom with a hydroxyl group or glutathione. We propose that this was result of nucleophilic aromatic substitution of the pentafluorobenzene ring. While this does not have a major role in metabolic stability (as overall stability is nearly 80 %), it may help to explain why we are seeing off-target effects with the perfluorobenzenesulfonamide derivatives. The perfluorobenzenesulfonamide moiety may undergo non-specific nucleophilic aromatic substitution.

Based on these data, we chose compound 5.10d as our new lead inhibitor. This molecule was a potent inhibitor of Stat3-phosphopeptide interactions (IC₅₀ = 5.6 ± 1.1 µM), performed well in the luciferase assay (41 % inhibition of Stat3 dependent luciferase activity without significant affect on the negative reporter) and possesses the 4-trifluoromethylbenzenesulfonamide moiety, which confers moderate metabolic stability. Thus, compound 5.10d was subjected to further biological evaluation in the context of TKI-resistant CML.
We first performed a colony growth assay to assess the ability of 5.10d to inhibit clonogenic potential of CML\(^{CD34+}\) progenitor cells from newly diagnosed CML patients. In combination with 2.5 µM imatinib, 5 µM 5.10d impaired clonogenic potential of cells grown in HS-5 conditioned media by 66.4 % following 96-hours in culture with drug (Figure 5.5). Similarly, 5.10d in combination with imatinib also reduced the clonogenic potential of CML\(^{CD34+}\) cells from TKI-resistant patients at both 5 µM and 1 µM when grown in either RM or HS-5 CM, confirming the potency of this novel Stat3 inhibitor (Figure 5.6). Furthermore, 5.10d had no effect on clonogenicity of MNCs from normal individuals.

Figure 5.5. Treatment of CML CD34+ progenitor cells from newly diagnosed patients with 5.10d (5 µM) and imatinib (2.5 µM) can overcome stromal mediated TKI resistance.
Figure 5.6. Treatment with 5.10d sensitizes TKI resistant CML patient samples to imatinib treatment in regular and conditioned media.

4 Discussion and Conclusions

Compound 5.10d had the desired balance of potency and specificity that remained a challenge with earlier lead Stat3 inhibitors. Using a TKI-resistant CML cell line we have developed a luciferase assay and screened inhibitor libraries to reliably evaluate potency and selectivity in a cellular environment. Furthermore, we have demonstrated that lead agent 5.10d can restore imatinib sensitivity to TKI resistant CML patient samples. Current work is focused on evaluating the in vivo efficacy of lead compound 5.10d, which holds great promise as an agent to overcome TKI resistance in CML.
Chapter 6
State-of-the-Art Stat3 Inhibitors

1 Introduction

In the previous chapters several libraries of Stat3 inhibitors have been presented that are now among the most potent in the literature. Given the data, and the resultant response from members of the medical and pharmaceutical communities, it is hoped that lead compounds will advance to clinical trials within the next 2-3 years. As we move towards more advanced preclinical trials, these compounds have continued to show great promise as cancer therapeutic agents. This final chapter will highlight the latest progress in the field of Stat3 inhibitor development and offer perspective of where this field is headed.

This work is taken, in part, from our 2011 paper in Expert Opinion on Therapeutic Patents. In accordance with the copyright transfer agreement a link to the full text has been provided at the end of this thesis.21

2 State of the field
2.1 Stat3 Inhibitors since 2008

Over the past 5 years, Stat3 inhibitor development has grown from a high-risk academic adventure, to now be a promising avenue for therapeutic development.16, 102, 103 Greater knowledge of Stat3’s role in biological systems has prompted further efforts to identify inhibitory molecules. As such, there have been a number of promising small molecule inhibitors of Stat3 that have emerged in the literature.16, 102

While most groups have developed inhibitors that block the Stat3 SH2 domain, others have targeted the DNA binding domain and N-terminal domain to inhibit aberrant Stat3 function.104-106 Thus, there is an excitingly large diversity of Stat3 inhibitor scaffolds. This chapter will focus on small molecule inhibitors of Stat3’s SH2 domain that have been discovered since 2008. Inhibitors that block upstream effectors of Stat3 activation, such as kinases, or molecules that bind other domains of Stat3 have not been included in this chapter.
As described in Chapter 1, there were just a few examples of small molecule Stat3 inhibitors prior to 2008. However, these compounds laid the ground work for the development of further generations of inhibitory molecules. Advances in high throughput screening have facilitated the rapid discovery of Stat3 inhibitors which can then be optimized using medicinal chemistry techniques. While some of these early leads have been further improved, others are no longer being pursued as therapeutic candidates.

In 2009, Tweardy and coworkers\textsuperscript{107, 108} identified a promising group of Stat3 inhibitors that emerged from an \textit{in silico} screen of 920,000 compounds (from Chembridge, Asinex, ChemDiv, Enamine, KeyOrganics and LifeChemicals). Promising compounds were then purchased from
their respective suppliers. Complementary screens against the Stat1 SH2 domain were performed to identify isoform specific compounds. Hit molecules were screened in vitro using high-throughput Surface Plasmon Resonance (SPR) binding experiments. SPR confirmed that compound C188 (6.1), a functionalized naphthylene sulfamoyl benzoic acid, bound to Stat3 with an IC\textsubscript{50} value of 20 µM. Encouragingly, inhibitor 6.1, displayed potent cellular activity with an IC\textsubscript{50} value of 0.73 µM in Stat3-dependent cells, and a 10-fold lower activity in cells lacking aberrant Stat3 activity.\textsuperscript{108} Furthermore, in combination with known chemotherapeutic agent docetaxel, 6.1 inhibited tumour growth in chemotherapy resistant BCM2665 breast cancer xenograft models (12.5 mg/kg).\textsuperscript{109} In further work, Tweardy and coworkers identified compound C188-9 (6.2) as a more potent derivative of 6.1 that inhibited Stat3 phosphorylation, decreased expression of Stat3 target genes and induced apoptosis in AML cell lines and patient samples at 10 µM.\textsuperscript{110}

![Image of compounds C188 (6.1) and C188-9 (6.2)]

**Figure 6.2.** Small molecule inhibitors of Stat3 discovered by in silico screens by Tweardy and coworkers.\textsuperscript{108-110}

Although its complex structure had complicated the synthesis of new analogues of 1.3, Lin and coworkers, have recently published novel analogues of compounds 1.3 and 1.4.\textsuperscript{85,111} Compound LLL-12 (6.3) was identified from a small library of compounds and was shown to inhibit Stat3 phosphorylation and induce apoptosis in a number of cancer cell lines at treatments of just 5 and 10 µM.\textsuperscript{85,112} Compound 6.3 inhibited MDA-231 breast cancer and U87 glioblastoma xenograft models at dosings of 2.5 and 5 mg/kg.\textsuperscript{111} Compound 6.3 has been tested in a variety of different cancers and continues to demonstrate potent anti-Stat3 and anti-cancer activity in vitro and in vivo.\textsuperscript{85,113-116}
In an effort to find novel drug-like inhibitors of the Jak2-Stat3 pathway, Frank and coworkers screened the Prestwick Chemical Library of 1120 bioactive compounds using a luciferase assay in U3A fibrosarcoma cells. This approach has yielded some very promising inhibitors of Stat3 signaling, however it was difficult to identify the molecular target of some of the hit compounds. Although both pyrimethamine (6.4) and nifuroxazide (6.5) effectively inhibit Stat3 phosphorylation at low micromolar concentrations, it appears that 6.5 inhibits Stat3 phosphorylation by targeting Jak2. Nonetheless, these two drug-like compounds show promising anti-cancer activity and represent a promising new avenue for the discovery of novel Stat3 inhibitors.

In 2010, Asai and coworkers published a promising quinoline carboxamide-based Stat3 inhibitor. In silico docking of $3.6 \times 10^5$ structures identified 136 hit compounds that were evaluated in vitro using a Stat3-dependant luciferase assay. Compound STX-0119 (6.6) emerged as a Stat3 inhibitor, with luciferase activity completely inhibited upon treatment with 100 µM of 6.6. Western blot analysis of MDA-MB-468 cell lysates treated with 6.6 (10-50 µM) showed reduced levels of c-Myc, cyclin D-1 and survivin expression. However, levels of pStat3 were surprisingly unaffected. ELISA STAT-DNA binding experiments in MDA-468 cells showed
selective inhibition of Stat3-DNA complexation at 50 μM of 6.6, and minimal reduction in both Stat1 and Stat5 isoform binding.

A preliminary SAR study was performed, however, structural modifications were poorly tolerated and 6.6 was taken forward into preclinical trials. Mice bearing SCC-3 human lymphoma tumours were treated orally with 6.6 (160 mg/kg) over a 4-day period. Compound 6.6 inhibited tumour growth relative to untreated control subjects. Pharmacokinetic analysis showed 6.6 to have good bioavailability, with plasma levels exceeding 260 μM. Thus, compound 6.6 is an orally bioavailable small molecule Stat3 inhibitor.

Figure 6.5. Compound 6.6 (STX-0119); the first reported bioavailable Stat3 inhibitor reported.

Curcumin (6.7, the major component of the spice turmeric) was one of the first inhibitors of the Jak2-Stat3 signaling pathway to be identified. Further work with 6.7 identified that this compound could interact with a number of molecular targets. Thus it was difficult to claim that the anti-cancer activity of 6.7 was due solely to its activity against Stat3.

In 2010, Bill and colleagues developed novel analogues of curcumin which were designed to overcome non-specific behavior by rigidifying the inhibitor scaffold. This led to the discovery of compound FLLL32 (6.8) which potently inhibited Stat3 activation in melanoma cell lines upon treatment with just 2 μM. Compound 6.8 did not effect upstream kinases, such as Jak2, nor did it effect the activity of other prominent cellular kinaes. Recent efforts with 6.8 have focused on the in vivo efficacy of this compound. At a dosing of 50 mg/kg, 6.8 significantly suppressed tumour growth in OS-33 osteosarcoma xenografts over a 15 day study.
In 2011, Li and coworkers utilized a fragment based multiple ligand docking *in silico* screen to identify new scaffolds for Stat3 inhibitor development.\(^{124}\) Using a variety of hydrophobic and hydrophilic moieties that were characteristic of known Stat3 inhibitors, they performed multiple ligand fragment based docking experiments. Docked fragments were then linked using a variety of core scaffolds and the resultant structures were then subjected to a similarity search with FDA approved drugs. The authors found three lead candidates (celecoxib (6.9), 6.10 and 6.11, shown in Figure 6.7) that were then evaluated *in vitro*. These three hit compounds inhibited cell viability and Stat3 phosphorylation at 10-50 µM concentrations in pancreatic and colon cancer cell lines.\(^{124}\)
Finally, in 2013, a group of researchers from the Moffit Cancer Institute identified other derivatives of S3I-201 (1.6) that demonstrate moderate activity against Stat3 protein. Similar to our work, their lead compounds utilize the 4-cyclohexylbenzyl moiety appended to a salicylic acid group to obtain inhibitor potency. Although their lead compound, 6.12, showed reasonable activity in the FP assay (IC$_{50}$ ~ 10 µM), concentrations in excess of 50 µM were required to see an effect in a cellular environment. Thus, it appears that our latest compounds based on S3I-201 (1.6) are on the order of 10-100 times more potent as the ones arising from this new study.

2.2 Jak2 Inhibition

Several major pharmaceutical companies have invested in Jak2 inhibitor development programs. Some promising Jak2 inhibitors include OPB-31121 from Tekada (currently in phase II), Jakarta from Sanofi (SAR302503, currently in phase III) and Jakafi (ruxolitinib, clinically approved) from Incyte and Novartis. These compounds are being used for treating myelofibrosis, an incurable hematologic malignancy that is driven by abnormal Jak2 activation.

As discussed in Chapter 1, ruxolitinib effectively inhibits Jak2 and key Jak2 mutants, however, it does not improve survival times of myelofibrosis patients and is used only to reduce pain and disease related symptoms. At this time, it is unclear whether new Jak2 inhibitors will be able to more effectively treat myelofibrosis or, if these new agents will only be able to treat symptoms of the disease as well. It is also unclear whether direct inhibition of Stat3 protein will be
able to better treat this disease. Because Stat3 is downstream of Jak2, it is expected that a potent and selective Stat3 inhibitor would allow for a more focused cellular effect than Jak2 inhibition.

3 Discussion

While targeting Stat3 protein still remains a ‘high-risk’ endeavor for much of the pharmaceutical industry, a better understanding of Stat3’s role in biological processes has undoubtedly played a major role in promoting Stat3 as a target for therapeutic intervention. The recent discovery of several bioavailable Stat3 inhibitors with potent activity in vivo has sparked interest from the pharmaceutical industry. Indeed, the growing number of successful preclinical in vivo studies in numerous cancer types has further validated Stat3’s therapeutic potential.

Given the complex nature of PPIs, there is little question that Stat3 inhibitor development still faces many challenges, however, there is little doubt that a potent Stat3-targeted drug would be a most valued weapon in the fight against cancer. We have positioned our research program at the leading edge of this field. The Stat3 inhibitors presented in this thesis represent some of the most potent and promising small molecule Stat3 inhibitors in the literature. Lead agents 2.18, 3.7, 4.2, 4.7 and 5.10 are among the strongest binders of Stat3 protein; they have potent anti-cancer activity in vitro and in vivo; and have favourable pharmacokinetic profiles. Current work on this project and future directions are summarized below.

4 State of the Project

The work highlighted in this thesis focuses on SAR studies of three of the four arms of our tetrapodal Stat3 inhibitor scaffold (summarized in Figure 6.1). In Chapter 2, modifications to the amide nitrogen substituent improved contact with a hydrophobic region within Stat3’s SH2 domain. In Chapter 3, the toluene sulfonamide moiety was altered and a more potent derivative, 3.7 was discovered. Guided by some interesting preliminary results, functionalizing the sulfonamide nitrogen gave compounds 4.2 and 4.7 which more completely occupied the Stat3 SH2 domain. Finally Chapter 5 focused on balancing selectivity and lack of cellular potency by further optimizing the sulfonamide-S substituent which resulted in the discovery of new lead compound 5.10.
Figure 6.9. Summary of SAR presented in this work

The final appendage, the salicylic acid group, was subjected to SAR by Sina Haftchenary, a graduate student in the Gunning laboratory. In this study, 40 analogues based on the structures of 2.18h and 3.7o were prepared possessing salicylic acid bioisosteres and prodrugs. These compounds were evaluated within the framework of glioblastoma, a deadly brain cancer where Stat3 plays a master regulatory role. It was proposed that modifications to the salicylic acid moiety would further optimize inhibitor binding and might improve the pharmacokinetic profile of our inhibitor scaffold. Additionally, more hydrophobic salicylic acid mimetics may be better suited to pass the hydrophobic blood brain barrier and effectively treat glioblastoma.

Of the compounds tested, two lead compounds were discovered and are displayed in Figure 6.10. Compounds SH-04-54 and SH-05-07 were the most potent inhibitors from this library, possessing the p-benzoic acid and p-hydroxamic acid moieties, respectively. These two lead compounds offered comparable binding affinity to 3.7o by SPR (3.7o $K_D = 504$ nM, SH-04-54 $K_D = 300$ nM and SH-05-07 $K_D = 612$ nM) and also killed a panel of brain cancer cells and brain
cancer stem cells with greater potency than parent compound 3.7o (EC₅₀ values below 1 µM compared to 3.7o, EC₅₀ ~ 1.5 µM).

Figure 6.10. Lead compounds from SAR of the salicylic acid moiety. SH-04-54 and SH-05-07 offer improved cellular activity compared to previous lead 3.7o.

Thus, to continue this research project, key elements from SH-04-54 and SH-05-07 will be combined with potent N-alkyl moieties discussed in Chapters 4 and 5 to further optimize inhibitor activity. Potential inhibitors are displayed below in Figure 6.11.

Figure 6.11. Potential Stat3 inhibitors. Proposed lead compounds would combine key elements of lead inhibitor 5.10d and salicylic acid derivatives.

4.1 Future Direction

Having modified all four appendages of our inhibitor scaffold, the task is now to optimize the core of the structure. Previous lead inhibitors all rely on the highly flexible glycine core. While a number of very promising inhibitors have been prepared using this scaffold, rigidifying the core
will reduce the entropic cost of binding and potentially lock the drug in its active conformation. We have proposed that less flexible amino acids, cyclic or heterocyclic cores will improve inhibitor activity and provide a better pharmaceutical candidate. Figure 6.12 illustrates some of the proposed core derivatives.

![Proposed core derivatives](image)

**Figure 6.12. Proposed small molecule core derivatives for optimizing inhibitor activity**

A variety of cores will be utilized to fully explore conformations that could be accessed by our previous lead compounds. Thus, several libraries of inhibitors are proposed, including benzene cores, truncated cores and less flexible amino acids. Once the optimum size and orientation of the core is determined, we will then further substitute lead cores with heterocycles that will help prevent metabolic breakdown and reduce hydrophobicity.

## 5 Concluding Remarks

Throughout this work we have focused on designing inhibitors for a protein that was once thought to be “undruggable”. A growing body of evidence suggests that these agents will be
valuable tools in the fight against cancer. Despite the recent breakthroughs with Jak2 inhibitors and other TKIs, it remains unclear whether these drugs will effectively treat Jak2 driven malignancies. Additionally, it is not known whether targeting Stat3 will be able to overcome the shortcomings of Jak2 inhibitor therapy. Further biological testing and in-human trials are needed before this can be determined.

While there are many molecules that inhibit Stat3 function, there are relatively few compounds that have the potential to become Stat3 targetted drugs. Many reported Stat3 inhibitors lack cellular activity however, our inhibitors are cell-permeable, have potent cellular activity, and selectively inhibit Stat3 function within cells. Furthermore, lead compounds inhibit Stat3 without inhibiting JAKs or other upstream kinases.

Stat3’s SH2 domain is a complex molecular target. It is primarily hydrophobic, yet contains a hydrophilic sub-pocket that binds to pTyr motifs. Furthermore, the SH2 domain binds to pTyr peptide sequences in a linear fashion, which allows for distal contributions to binding epitopes.

Thus, targeting the pTyr binding site of Stat3 SH2 domain is a very complex task. SH2 domain binders should possess a highly polar or negatively charged pTyr mimetic, while also incorporating hydrophobic moieties to make favourable hydrophobic contacts. These appendages should be orientated in such a way that will mimic the natural binding sequences, and should also occupy a large area on the protein surface. While many of the Stat3 SH2 binders in the literature utilize docking simulations to support their claims of binding to the SH2 domain, many of these inhibitors bear limited structural similarity to the natural binding partners of the Stat3 SH2 domain.

Throughout our work we have gone to great lengths to ensure our Stat3 inhibitors are in fact binding to the Stat3 SH2 domain. Docking simulations, FP assays and competitive EMSA have all supported the claim that we are inhibiting Stat3 by disrupting SH2 domain function. Although many other research groups have claimed that their molecules bind the Stat3 SH2 domain, few have gathered as much support for this claim as we have.

We have created several compounds that effectively disrupt JAK-STAT signaling by targeting the Stat3 SH2 domain. Lead agents potently bind to Stat3’s SH2 domain and halt aberrant Stat3
function in cancer cells. Promising activity has been demonstrated against a broad spectrum of cancers including: acute and chronic myeloid leukemia, multiple myeloma, glioblastoma and cancers of the prostate and breast. Additionally, lead agents have been tested against pancreatic cancer, myelofibrosis, acute lymphoblastic leukemia, lung cancer and other diseases.

While current lead compounds (4.2i, 4.7h and 5.10d) are being tested in vivo, previous leads 2.18h and 3.7o have demonstrated very promising activity in mouse xenograft models. In particular 3.7o is one of the most potent and effective direct Stat3 inhibitors that has been reported in the scientific literature. By further optimizing the core of our inhibitor scaffold, we hope to further improve our Stat3 inhibitors with the goal of entering clinical trials in the next 2-3 years.

Unlike many drug discovery programs, this research project has not been funded with the support of major pharmaceutical companies. These molecules have been the product of public funds issued through government granting agencies, academic institutions, charitable organizations and private donations. This work is a direct reflection of the public’s interest in finding novel treatments for cancer. The emergence of academic drug discovery programs, like this one, present an appealing avenue for the development of novel pharmaceutical candidates. Academic laboratories are able to pursue higher-risk targets (such as Stat3) that may produce innovative treatment options in the fight against cancer. While targeted therapies have revolutionized the pharmaceutical landscape over the past few decades, it will be interesting to see how academic drug discovery efforts will influence the future advancement of cancer therapeutic agents.
References


27. Ferbeyre, G.; Moriggl, R. The role of Stat5 transcription factors as tumor suppressors or oncogenes. *Biochimica et Biophysica Acta - Reviews on Cancer* 2011, 1815, 104-114.


Appendix 1: Chapter 2 Experimental

1 Experimental

1.1 Chemical Methods

Anhydrous solvents methanol, DMSO, CH₂Cl₂, THF and DMF were purchased from Sigma Aldrich and used directly from Sure-Seal bottles. Molecular sieves were activated by heating under vacuum. All reactions were performed under an atmosphere of dry nitrogen and were monitored for completeness by thin-layer chromatography (TLC) using silica gel (visualized by UV light, or developed by treatment with KMnO₄ stain). ¹H and ¹³C NMR spectra were recorded on Bruker 400 MHz spectrometers in either CDCl₃, CD₃OD or d₆-DMSO. Chemical shifts (δ) are reported in parts per million after calibration to residual isotopic solvent. Coupling constants (J) are reported in Hz. Before biological testing, inhibitor purity was evaluated by reversed-phase HPLC (rpHPLC). Analysis by rpHPLC was performed using a Microsorb-MV 300 Å C18 250 mm x 4.6 mm column run at 1 mL/min, and using gradient mixtures. The linear gradient consisted of a changing solvent composition of either (I) 100 % H₂O with 0.1 % TFA for two minutes to 100 % MeCN with 10 % H₂O and 0.1 % TFA (v/v) at 22 minutes and UV detection at 254nm or (II) 100 % H₂O with 0.1 % TFA for 2 minutes to 100 % MeCN with 10 % H₂O and 0.1 % TFA (v/v) at 62 minutes and UV detection at 214nm or (III) 100 % H₂O (0.01 M NH₄OAc) for 2 minutes to 100 % MeOH at 22 minutes and UV detection at 254nm or (IV) 100 % H₂O (0.01 M NH₄OAc) for 2 minutes to 100 % MeOH at 62 minutes and UV detection at 254nm or (V) 100 % H₂O (0.01 M NH₄OAc) for 2 minutes to 100 % MeOH at 25 minutes and UV detection at 254nm or (VI) 100 % H₂O (0.01 M NH₄OAc) for 2 minutes to 100 % MeOH at 62 minutes and UV detection at 254nm, each ending with 5 mins of 100 % B. For reporting HPLC data, percentage purity is given in parentheses after the retention time for each condition. All biologically evaluated compounds are > 95 % chemical purity as measured by HPLC. The HPLC traces for all tested compounds are provided in supporting information.

1.2 Electrophoretic Mobility Shift Assay

Nuclear extract preparations and EMSA were carried out as previously described.³ The ³²P-labeled oligonucleotide probes used were hSIE (high affinity sis-inducible element from the c-
fos gene, m67 variant, 5’-AGCTTCATTTCCGTAAATCCCTA) that binds Stat1 and Stat3. Except where indicated, nuclear extracts were pre-incubated with compound for 30 min at room temperature prior to incubation with the radiolabeled probe for 30 min at 30 °C before subjecting to EMSA analysis. Bands corresponding to DNA-binding activities were scanned and quantified for each concentration of compound using ImageQuant and plotted as percent of control (vehicle) against concentration of compound, from which the IC50 values were derived.

1.3 Stat3 and Stat1 Fluorescence Polarization Assay

Fluorescence polarization experiments were performed on an Infinite M1000 (Tecan, Crailsheim, Germany) using black 384-flat bottom well plates (Corning), and buffer containing 50 mM NaCl, 10 mM Hepes, pH 7.5, 1 mM EDTA, and 2 mM dithiothreitol and a final concentration of 5 % DMSO. Stat1/Stat3 solutions (120 nM and 150 nM for Stat1 and Stat3, respectively) were treated with varying concentrations of inhibitor compounds (200 to 12.5 µM final concentrations). The fluorescent probe was added at a final concentration of 10 nM. Protein, inhibitor and probe were combined and incubated for 15 minutes prior to analysis. Polarized fluorescence was plotted against concentration and fitted using a standard dose response curve.
Figure A1.1. Competitive binding of 2.18h measured by Stat3 fluorescence polarization assay, with a calculated IC$_{50}$ = 31 ± 9 µM. Curve fitted using ORIGIN software.
Figure A1.2. Competitive binding of 2.18kd measured by Stat3 fluorescence polarization assay, with a calculated IC\textsubscript{50} = 37 ± 3 µM. Curve fitted using ORIGIN software.
Figure A1.3. Competitive binding of 2.18kg measured by Stat3 fluorescence polarization assay, with a calculated IC\textsubscript{50} = 43 ± 4 μM. Curve fitted using ORIGIN software.

![Graph](image)

Figure A1.4. Competitive binding of 2.18ng measured by Stat3 fluorescence polarization assay, with a calculated IC\textsubscript{50} = 43 ± 2 μM. Curve fitted using ORIGIN software.

1.4 Cell Viability Studies

MDA-468 (breast), DU145 (prostate) and OCI-AML2 (Leukemia) cells were loaded in 96 well plates at 10 000 cells per well and incubated with various concentration of inhibitor for 72 hours followed by 3 hour incubation with MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium). Relative cell viability (to DMSO control) was determined colourometrically and IC\textsubscript{50} values determined by fitting to a standard dose response curve.

1.5 Immunoblotting analysis

Cells were lysed in lysis buffer (50 mM Tris-HCl, 1 mM EDTA, 1 % NP-40, 150 mM NaCl) for 30 minutes on ice, then freeze/thaw once at -80°C and clarified by centrifugation at 12000g for
15 minutes. Proteins were separated by 6.5 % to 15 % SDS-PAGE and immunoblotted with the specified antibody. Protein bands were visualized using secondary antibodies coupled to horseradish peroxidase and the Chemiluminescence Reagent Plus (from Perkin Elmer Life Sciences) according to the manufacturer's instructions. Anti cMyc is from Santa Cruz, anti survivin is from NOVUS Biologica,l Anti-Mcl-1, and anti-Bcl-x from BD Biosciences, (Mississauga, ON), anti-phospho Stat3 and Stat3, anti PARP are from Cell Signaling Technology, (Pickering, ON).

1.6 MDA-MB-231 Mouse Xenograft Studies

Six-week-old female athymic nude mice were purchased from Harlan and maintained in the institutional animal facilities approved by the American Association for Accreditation of Laboratory Animal Care. Athymic nude mice were injected subcutaneously in the left flank area with 5 × 10^6 human breast cancer MDA-MB-231 cells in 100 mL of PBS. After 5-10 days, tumours of a diameter of 3 mm were established. Animals were grouped so that the mean tumour sizes in all groups were nearly identical, then given 2.18h, i.v. at 3 mg/kg every 2 or 3 days for 17 days and monitored every 2 or 3 days. Tumour sizes were measured with calipers and volume, V, was calculated according to the formula V = 0.52 x a^2 x b, where a, smallest superficial diameter, b, largest superficial diameter. For each treatment group, the tumour volumes for each set of measurements were statistically analyzed in comparison to the control (non-treated) group. Upon completion of the study, tumours were extracted and tumour tissue lysates were prepared for immunoblotting and gel shift analyses.

1.7 General Reaction Procedures

**General Procedure A (Reductive amination of amino salicylic acid).** Reaction of R^1 aldehydes with benzyl protected 4-aminosalicylic acid. To a solution of amine (1.0 equiv) and acetic acid (1.5 equiv) stirred in anhydrous MeOH (0.1 M) with 4 Å mol. sieves was added 1.0 equiv of aldehyde. The solution was then heated to 45 °C for 3 hr and then allowed to cool to rt. Next, NaCNBH₃ (1.3 equiv) was added portion-wise and the reaction allowed to stir at rt overnight. When TLC indicated the reaction was complete, the reaction was diluted with CH₂Cl₂, filtered and concentrated *in vacuo.*
**General Procedure B (PPh₃Cl₂ mediated Amide Coupling)**-Reaction of secondary anilines with carboxylic acids. To a stirred solution of the secondary aniline (1.0 equiv) and carboxylic acid (1.0 equiv) in CHCl₃ (0.1 M) was added PPh₃Cl₂ (2.5 equiv). The reaction was then heated to 60 °C and stirred overnight. The reaction was allowed to cool and the solvents removed under reduced pressure. The concentrate was absorbed directly onto silica for column chromatography purification.

**General Procedure C (Boc Protection).** To a stirred solution of the appropriate secondary amine (1.0 equiv) and DIPEA (2.0 equiv) in CHCl₃ (0.1 M), was added Boc₂O (1.1 equiv) and left to stir overnight at rt. The reaction was then diluted with CH₂Cl₂, washed with H₂O, brine and dried over Na₂SO₄, filtered and concentrated under reduced pressure.

**General Procedure D (Nucleophilic aromatic substitution).** The desired secondary amine (1.0 equiv) and arylfluoride substrate (1.5 equiv) were dissolved in anhydrous DMSO (0.1 M) followed by the addition of DIPEA (3.0 equiv). The reaction was heated to 120 °C and allowed to stir overnight. The reaction was quenched with H₂O and the aqueous layer extracted repeatedly into EtOAc. The combined organic layers were then washed with brine, dried over anhydrous Na₂SO₄ and the solvent removed under reduced pressure.

**General Procedure E (TFA deprotection with K₂CO₃ and MeOH).** K₂CO₃ (3.0 equiv) was added to a stirred solution of TFA-protected compound (1 equiv) in MeOH (0.1 mol). The reaction was allowed to stir at room temperature for 1.5 hrs before quenching with saturated NaHCO₃ solution. The aqueous layer was then repeatedly extracted with EtOAc. The organic layers were then combined, washed with saturated NaCO₃ and dried over anhydrous Na₂SO₄, filtered and the solvent removed under reduced pressure.

**General Procedure F (HBTU mediated condensation reactions).** The required carboxylic acid (1 equiv) was added in one portion to a solution of HBTU (1.1 equiv) and DIPEA (3.0 equiv) in DMF (0.1 M), and the resulting solution stirred at room temperature for 10 minutes. The required amine was then dissolved in a solution of DIPEA (2.0 equiv) in DMF (0.1 M) and added to the activated acid in one portion. The resulting solution was stirred for 4 hours, then diluted with EtOAc (0.1 M) and washed successively with equal volumes of: 2M HCl, saturated bicarbonate and brine. The organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated.
**General Procedure G (Sulfonylation of secondary amines).** To a stirred solution of amine (1.0 equiv) dissolved in CH$_2$Cl$_2$ (0.1 M) was added DIPEA (1.1 equiv) and the appropriate sulfonyl chloride (1.1 equiv). After 1 hr, the reaction was diluted with CH$_2$Cl$_2$, washed with water, followed by a brine wash and dried over Na$_2$SO$_4$. The organic layer was then concentrated under reduced pressure and purified by silica gel column chromatography to yield product.

**General Procedure H (Suzuki Cross Coupling).** A mixture of arylbromide (1.0 equiv), boronic acid (1.1 equiv), K$_2$CO$_3$ (2.5 equiv) and Pd(PPh$_3$)$_4$ (0.03 equiv) was suspended in DMF (0.1 M) in a sealed tube vessel and irradiated in a Biotage Initiator microwave reactor (17 mins, 170 °C). After cooling to rt, the reaction was diluted with water and repeatedly extracted with CH$_2$Cl$_2$. The combined organic extracts were washed with brine, dried over anhydrous Na$_2$SO$_4$, filtered and concentrated under reduced pressure.

**General Procedure I (Hydrogenolysis of benzyl ether and benzyl ester).** Global deprotection of benzylated salicylic acid. The benzyl protected salicylic acid (1 equiv) was dissolved in a stirred solution of THF/MeOH (1:1) (0.1 M). The solution was degassed thoroughly before careful addition of 10 % Pd/C (10 mg/mmol). H$_2$ gas was bubbled through the solvent for 5 mins before the solution was put under an atmosphere of H$_2$ gas and stirred continuously for 3 hrs. The H$_2$ gas was evacuated and the reaction filtered (to remove the Pd catalyst) and concentrated under reduced pressure.

**General Procedure J (TFA deprotection of benzyl ether).** The benzyl protected compound (1 equiv) was dissolved in a 1:1 mixture of TFA:toluene (0.1 M) at rt for 5 minutes, then all solvents were evaporated under reduced pressure.

### 1.8 Intermediate characterization data

**2.8, Benzyl 4-amino-2-(benzyloxy)benzoate.** To a stirred solution of 4-aminosalicylic acid (3.00 g, 19.6 mmol) in DMF (0.1 M) at 0 °C, was added KOtBu (2.42 g, 21.6 mmol). After 15
mins, benzyl bromide (2.57 mL, 21.6 mmol) was added drop-wise. The suspension was allowed to stir at rt for a further 4 hrs before the reaction vessel was again cooled to 0 °C. A further 1.1 equivs of KtOBu (2.42 g, 21.6 mmol) were added prior to the drop-wise addition of benzyl bromide (2.57 mL, 21.6 mmol). The reaction was then stirred overnight before quenching with H₂O. The solution was then repeatedly extracted with ethyl acetate and the organics combined. The organics were then washed with H₂O and brine then concentrated, dried over Na₂SO₄ and concentrated in vacuo (3.40 g, 74 %): δH (400 MHz, d₆-DMSO) 5.07 (s, 2H, CH₂), 5.21 (s, 2H, CH₂), 5.99 (s, br), 2H, NH₂), 6.18 (d of d, J = 8.6 and 1.8 Hz, 1H, CH), 6.32 (d, J = 1.7 Hz, 1H, CH), 7.28-7.38 (8H, m, CH), 7.47 (d, J = 7.2 Hz, 2H, CH), 7.60 (d, J = 8.6 Hz, 1H, CH); δC (100 MHz, d-CDCl₃) 65.8, 70.2, 99.1, 106.7, 109.0, 126.3, 126.8, 127.5, 127.7, 127.9, 128.1, 128.3, 128.4, 134.3, 136.6, 136.7, 152.2, 160.7, 165.7; LRMS (ES+) Calcd for [C₂₁H₁₉NO₃ + H] 334.14 found 334.17.

2.9, Benzyl 4-(benzylamino)-2-(benzyloxy)benzoate. Primary aniline 2.8 was coupled to benzaldehyde on a 0.3 mmol scale via General Procedure A to furnish 2.9 (109 mg, 86 %): δH (400 MHz, d-CDCl₃) 4.21 (s, 2H, NH₂CH₂), 4.95 (s, 2H, CH₂), 5.20 (s, 2H, CH₂), 6.03 (d, J = 2.0 Hz, 1H, CH), 6.09 (d of d, J = 8.6 and 2.1 Hz, 1H, CH), 7.15-7.34 (m, 15H, CH), 7.73 (d, J = 8.6 Hz, 1H, CH); δC (100 MHz, d-CDCl₃) 47.5, 66.7, 70.3, 97.2, 104.8, 108.3, 126.8, 127.2, 127.4, 127.5, 127.6, 127.9, 128.2, 128.3, 128.6, 134.2, 136.6, 136.7, 138.1, 152.8, 160.8, 165.6; LRMS (ES+) Calcd for [C₂₈H₂₅NO₃ + H] 424.19 found 424.22.
2.10a, Benzyl 2-(benzyloxy)-4-(4-bromobenzylamino)benzoate. Primary aniline 2.8 was coupled to 4-bromobenzaldehyde on a 0.7 mmol scale via General Procedure A to furnish 2.10a (274 mg, 78 %): $\delta_H$ (400 MHz, d-CDCl$_3$) 4.12 (s, 2H, CH$_2$), 4.50 (s (br), 1H, NH), 4.92 (s, 2H, CH$_2$, CH$_2$), 5.18 (s, 2H, CH$_2$, CH$_2$), 5.98 (d, $J = 1.8$ Hz, 1H, CH), 6.04 (d of d, $J = 8.6$ and 1.8 Hz, 1H, CH), 7.02 (d, $J = 8.2$ Hz, 2H, 2 CH), 7.11-7.34 (m, 12H, 12 CH), 7.70 (d, $J = 8.6$ Hz, 1H, CH); $\delta_C$ (100 MHz, d-CDCl$_3$) 46.5, 65.4, 69.9, 97.0, 104.5, 108.2, 120.7, 126.4, 127.2, 127.4, 127.6, 128.0, 128.1, 128.4, 131.4, 133.9, 136.3, 137.0, 152.3, 160.4, 165.3; LRMS (ES+) Calcd for [C$_{28}$H$_{24}$BrNO$_3$ + H] 502.10, found 502.06.

2.10b, Benzyl 2-(benzyloxy)-4-(3-bromobenzylamino)benzoate. Primary aniline 2.8 was coupled to 3-bromobenzaldehyde on a 0.7 mmol scale via General Procedure A to furnish 2.10b (267 mg, 89 %): $\delta_H$ (400 MHz, d-CDCl$_3$) 4.27 (s, 2H, CH$_2$), 4.65 (s (br), 1H, NH), 4.93 (s, 2H, CH$_2$), 5.19 (s, 2H, CH$_2$), 5.98 (d, $J = 2.1$ Hz, 1H, CH), 6.04 (d of d, $J = 8.6$ and 2.1 Hz, 1H, CH), 7.16-7.30 (m, 12H, 12 CH), 7.47 (d, $J = 8.3$ Hz, 2H, CH), 7.70 (d, $J = 8.6$ Hz, 1H, CH); $\delta_C$ (100 MHz, d-CDCl$_3$) 46.9, 65.8, 70.3, 97.4, 104.9, 108.7, 122.7, 125.6, 126.8, 127.5, 127.7, 127.9, 128.3, 128.4, 130.1, 130.2, 130.5, 134.3, 136.7, 140.8, 152.5, 160.8, 165.6; LRMS (ES+) Calcd for [C$_{28}$H$_{24}$BrNO$_3$ + H] 502.10 found 502.00.
2.10c, Benzyl 2-(benzyloxy)-4-(4-cyanobenzylamino)benzoate. Primary aniline 2.8 was coupled to 4-formylbenzonitrile on a 0.7 mmol scale via General Procedure A to furnish 2.10c (211 mg, 79 %): $\delta_H$ (400 MHz, $d$-CDCl$_3$) 4.27 (s, 2H, CH$_2$), 4.65 (s (br),1H, NH), 4.93 (s, 2H, CH$_2$), 5.19 (s, 2H, CH$_2$), 5.98 (d, $J = 2.1$ Hz, 1H, CH), 6.04 (d of d, $J = 8.6$ and 2.1 Hz, 1H, CH), 7.16-7.30 (m, 12H, 12 CH), 7.47 (d, $J = 8.3$ Hz, 2H, CH), 7.70 (d, $J = 8.6$ Hz, 1H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 46.6, 65.5, 70.0, 97.2, 104.5, 108.7, 110.7, 118.3, 126.4, 127.1, 127.3, 127.4, 127.6, 128.0, 128.1, 132.1, 134.0, 136.2, 143.7, 151.9, 160.4, 165.2; LRMS (ES+) Calcd for [C$_{29}$H$_{24}$N$_2$O$_4$ + H] 449.19 found 449.15.

2.10d, Benzyl 2-(benzyloxy)-4-(3-cyanobenzylamino)benzoate. Primary aniline 2.8 was coupled to 3-formylbenzonitrile on a 0.7 mmol scale via General Procedure A to furnish 2.10d (251 mg, 94 %): $\delta_H$ (400 MHz, $d$-CDCl$_3$) 4.19 (s, 2H, CH$_2$), 4.89 (s, 2H, CH$_2$), 5.17 (s, 2H, CH$_2$), 5.97 (d, $J = 2.0$ Hz, 1H, CH), 6.02 (d of d, $J = 8.6$ and 2.0 Hz, 1H, CH), 7.13-7.42 (m, 14H, 14 CH), 7.69 (d, $J = 8.6$ Hz, 1H, CH); LRMS (ES+) Calcd for [C$_{29}$H$_{24}$N$_2$O$_4$ + H] 449.19 found 449.15.
2.10e, benzyl 2-(benzyloxy)-4-((cyclohexylmethyl)amino)benzoate. Primary aniline 2.8 was coupled to cyclohexanecarboxaldehyde on a 0.6 mmol scale via General Procedure A to furnish 2.10e (184 mg, 72 %): $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.15-1.30 (m, 5H, CH$_2$), 1.45-1.55 (m, 1H, CH), 1.65-1.81 (m, 5H, CH$_2$), 2.94 (d, $J = 6.4$ Hz, 2H, CH$_2$), 5.14 (s, 2H, CH$_2$), 5.32 (s, 2H, CH$_2$), 6.11 (d, $J = 2.0$ Hz, 1H, CH), 6.16 (d of d, $J = 8.8$ and 2.0 Hz, 1H, CH), 7.29-7.36 (m, 10H, CH), 7.41 (d, $J = 8.0$ Hz, 2H, CH), 7.48 (d, $J = 8.0$ Hz, 2H, CH), 7.85 (d, $J = 8.8$ Hz, 1H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 25.7, 26.3, 31.0, 37.5, 49.8, 65.6, 70.3, 96.8, 104.6, 107.5, 126.8, 127.5, 127.6, 127.9, 128.3, 128.4, 134.2, 136.8, 136.9, 153.4, 161.0, 165.7, 171.0; LRMS (ES+) Calcd for [C$_{28}$H$_{31}$NO$_3$ + H] 430.24 found 430.20.

2.10f, Benzyl 2-(benzyloxy)-4-(4-tert-butylbenzylamino)benzoate. Primary aniline 2.8 was coupled to 4-tert-butylbenzaldehyde on a 0.7 mmol scale via General Procedure A to furnish 2.10f (276 mg, 96 %): $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.19 (s, 9H, 3 CH$_3$), 4.13 (s, 2H, CH$_2$), 4.40 (s (br),1H, NH), 4.92 (s, 2H, CH$_2$), 5.17 (s, 2H, CH$_2$), 6.02 (d, $J = 2.0$ Hz, 1H, CH), 6.06 (d of d, $J = 8.6$ and 2.0 Hz, 1H, CH), 7.08-7.32 (m, 14H, 14 CH), 7.71 (d, $J = 8.6$ Hz, 1H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 31.0, 34.2, 47.0, 65.5, 70.1, 96.9, 104.6, 107.9, 125.3, 126.7, 126.9, 127.3,
2.10g, Benzyl 2-(benzyloxy)-4-(biphenyl-4-ylmethylamino)benzoate. Primary aniline 2.8 was coupled to biphenyl-4-carbaldehyde on a 0.7 mmol scale via General Procedure A to furnish 2.10g (235 mg, 79 %): δ_H (400 MHz, d-CDCl_3) 4.20 (s, 2H, NH_2CH_2), 4.48 (s (br), 1H, NH), 4.94 (s, 2H, CH_2), 5.18 (s, 2H, CH_2), 6.03 (d, J = 1.8 Hz, 1H, CH), 6.09 (d of d, J = 8.6 and 1.8 Hz, 1H, CH), 7.13-7.34 (m, 15H, CH), 7.43 (d, J = 8.2 Hz, 2H, 2 CH), 7.46 (d, J = 7.4 Hz, 2H, CH), 7.73 (d, J = 8.6 Hz, 1H, CH); δ_C (100 MHz, d-CDCl_3) 46.6, 65.2, 69.7, 96.7, 104.3, 107.7, 126.3, 126.4, 126.7, 126.8, 126.9, 127.1, 127.4, 127.7, 127.8, 128.1, 133.7, 136.1, 136.2, 136.7, 139.7, 139.9, 152.3, 160.3, 165.1; LRMS (ES+) Calcd for [C_{34}H_{29}NO_3 + H] 500.22 found 500.17.

2.10h, Benzyl 2-(benzyloxy)-4-(4-cyclohexylbenzylamino)benzoate. Primary aniline 2.8 was coupled to 4-cyclohexylbenzaldehyde on a 0.6 mmol scale via General Procedure A to furnish 2.10h (250 mg, 83 %): δ_H (400 MHz, d-CDCl_3) 1.25-1.48 (m, 6H, CH_2CH_2), 1.74 -1.95 (m, 4H,
CH₂CH₂), 2.48-2.52 (m, 1H, CH), 4.28 (s, 2H, NH₂CH₂), 4.49 (s (br), 1H, NH), 5.08 (s, 2H, CH₂), 5.32 (s, 2H, CH₂), 6.17 (d, J = 2.0 Hz, 1H, CH), 6.21 (d of d, J = 8.6 and 2.0 Hz, 1H, CH), 7.19-7.27 (m, 4H, 4 CH), 7.28-7.37 (m, 6H, 6 CH), 7.40-7.49 (m, 4H, 4 CH), 7.85 (d, J = 8.6 Hz, 1H, CH); δC (100 MHz, d-CDCl₃) 26.0, 26.7, 34.3, 44.1, 47.3, 65.7, 70.3, 97.1, 104.8, 108.2, 126.8, 127.0, 127.4, 127.5, 127.6, 127.9, 128.2, 128.3, 134.2, 135.4, 136.7, 136.8, 147.4, 152.9, 160.8, 165.8; LRMS (ES+) Calcd for [C₃₄H₃₅NO₃ + H] 506.27 found 506.22.

2.10i, Benzyl 2-(benzyloxy)-4-(naphthalen-2-ylmethylamino)benzoate. Primary aniline 2.8 was coupled to 2-naphthaldehyde on a 0.6 mmol scale via General Procedure A to furnish 2.10i (223 mg, 88 %): δH (400 MHz, d-CDCl₃) 4.35 (s, 2H, NH₂CH₂), 4.52 (s (br), 1H, NH), 4.92 (s, 2H, CH₂), 5.19 (s, 2H, CH₂), 6.70 (d, J = 2.0 Hz, 1H, CH), 6.12 (d of d, J = 8.6 and 2.0 Hz, 1H, CH), 7.11-7.22 (m, 6H, CH), 7.25-7.30 (m, 4H, 4 CH), 7.34-7.39 (m, 2H, 2 CH), 7.61-7.75 (m, 5H, CH); δC (100 MHz, d-CDCl₃) 47.1, 65.2, 69.7, 96.7, 104.4, 107.9, 124.7, 125.2, 125.3, 125.7, 126.2, 126.9, 127.0, 127.1, 127.3, 127.7, 127.8, 127.9, 132.1, 132.7, 133.7, 135.1, 136.1, 152.3, 160.3, 165.1; LRMS (ES+) Calcd for [C₃₂H₂₇NO₃ + H] 474.21, found 474.16.

2.13, methyl 2-(N,4-dimethylphenylsulfonamido)acetate. To a stirred solution of methyl 2-(4-methylphenylsulfonamido)acetate (3.10 g, 12.8 mmol) and Cs₂CO₃ (8.31 g, 25.5 mmol) in DMF (0.1 M) was added MeI (877 µL, 14.1 mmol). The reaction was allowed to stir overnight at rt. The reaction was then diluted with water and repeatedly extracted with CH₂Cl₂. The combined
organic extracts were washed with brine, dried over anhydrous Na$_2$SO$_4$, filtered and concentrated under reduced pressure to furnish 2.13 (2.80 g, 85 %): $\delta$H (400 MHz, d$_2$CDCl$_3$) 2.42 (s, 3H, CH$_3$), 2.87 (s, 3H, CH$_3$), 3.66 (s, 3H, CH$_3$), 3.97 (s, 2H, CH$_2$), 7.31 (d, $J$ = 8.4 Hz, 2H, CH), 7.69 (d, $J$ = 8.4 Hz, 2H, CH); LRMS (ES+) Calcd for [C$_{11}$H$_{15}$NO$_4$S + H] 258.08, found 258.06 [M+H].

2.14, 2-(N,4-dimethylphenylsulfonamido)acetic acid. Methyl ester 2.13 (2.60 g, 10.1 mmol) was dissolved in a 3:1 mixture of THF/H$_2$O. LiOH.H$_2$O (0.53 g, 12.6 mmol) was added at room temperature and the reaction allowed to stir for 3 hrs. All solvents were evaporated, apart from water. The remaining aqueous solvent was diluted further and thoroughly washed with ethyl acetate. The aqueous basic aqueous layer was then acidified to pH 2 with 1 M HCl and the product extracted with ethyl acetate. The organic layers were then combined and dried over Na$_2$SO$_4$, filtered and concentrated. (2.33 g, 95 %): $\delta$H (400 MHz, d$_2$CDCl$_3$) 2.43 (s, 3H, CH$_3$), 2.87 (s, 3H, CH$_3$), 3.99 (s, 2H, CH$_2$), 7.32 (d, $J$ = 8.0 Hz, 2H, CH), 7.69 (d, $J$ = 8.0 Hz, 2H, CH); $\delta$C (100 MHz, d$_2$CDCl$_3$) 21.4, 35.7, 50.6, 127.3, 129.6, 134.8, 143.7, 173.5; LRMS (ES+) Calcd for [C$_{10}$H$_{13}$NO$_4$S + H] 244.06, found 244.07 [M+H].

2.15, benzyl 2-(benzylxy)-4-(2-(N,4-dimethylphenylsulfonamido)-acetamido)benzoate. Primary aniline 2.8 was coupled to 2.14 on a 1.3 mmol scale via General Procedure B to furnish 2.15 (650 mg, 92 %): $\delta$H (400 MHz, d$_2$CDCl$_3$) 2.38 (s, 3H, CH$_3$), 2.83 (s, 3H, CH$_3$), 3.75 (s, 2H, CH$_2$), 5.08 (s, 2H, CH$_2$), 5.33 (s, 2H, CH$_2$), 7.10 (d, $J$ = 8.4 Hz, 1H, CH), 7.25-7.34 (m, 8H, CH), 7.36-7.40 (m, 2H, CH), 7.43 (d, $J$ = 7.2 Hz, 2H, CH), 7.64 (s, 1H, CH), 7.67 (d, $J$ = 8.0 Hz, 2H, CH), 7.89 (d, $J$ = 8.0 Hz, 1H, CH); $\delta$C (100 MHz, d$_2$CDCl$_3$) 21.4, 37.1, 54.7, 66.4, 70.4, 104.6,
2.16, benzyl 4-(N-benzyl-2-(N,4-dimethylphenylsulfonamido)acetamido)-2-(benzyloxy) benzoate. Secondary aniline 2.9 was coupled to 2.14 on a 0.18 mmol scale via General Procedure B to furnish 2.16 (76 mg, 70 %): LRMS (ES+) Calcd for [C_{31}H_{30}N_{2}O_{6}S + H] 559.19, found 559.19.

2.17a, Benzyl 2-(benzyloxy)-4-(N-(4-bromobenzyl)-2-(N,4-dimethylphenylsulfonamido)acetamido) benzoate. Secondary aniline 2.10a was coupled to carboxylic acid 2.14 on a 0.2 mmol scale via General Procedure B to furnish 2.17a (167 mg, 90 %): $\delta_H$ (400 MHz, d-CDCl$_3$) 2.33 (s, 3H, CH$_3$), 2.73 (s, 3H, CH$_3$), 3.54 (s, 2H, CH$_2$CO), 4.64 (s, 2H, CH$_2$), 4.99 (s, 2H, CH$_2$), 5.28 (s, 2H, CH$_2$), 6.50 (s (br),1H, CH), 6.55 (d of d, $J = 8.3$ and 1.8 Hz, 1H, CH), 6.88 (d, $J = 8.3$ Hz, 2H, CH), 7.16-7.34 (m, 14H, CH), 7.51 (d, $J = 8.3$ Hz, 2H, CH), 7.75 (d, $J = 8.3$ Hz, 1H,
CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 21.4, 29.6, 36.0, 51.2, 52.4, 53.3, 66.9, 70.7, 113.9, 119.8, 120.9, 121.7, 126.9, 127.3, 127.9, 128.2, 128.5, 129.4, 130.5, 131.6, 133.1, 135.2, 135.5, 135.6, 135.7, 143.3, 144.7, 158.7, 165.2, 167.0; LRMS (ES+) Calcd for [C$_{38}$H$_{35}$BrN$_2$O$_6$S + H] 727.15 found 726.83.

2.17b, Benzyl 2-(benzylxy)-4-(N-(3-bromobenzyl)-2-(N,4-dimethylphenyl-sulfonamido) acetamido) benzoate. Secondary aniline 2.10b was coupled to carboxylic acid 2.14 on a 0.25 mmol scale via General Procedure B to furnish 2.17b (149 mg, 82 %): LRMS (ES+) Calcd for [C$_{38}$H$_{35}$BrN$_2$O$_6$S + H] 727.15 found 726.89. “Salicyclic acid derivatives as potent inhibitors of transcriptionally active Stat3 dimers.” ChemBioChem 2009, 10, 1959-1964.

2.17c, Benzyl 2-(benzylxy)-4-(N-(4-cyanobenzyl)-2-(N,4-dimethylphenyl-sulfonamido) acetamido) benzoate. Secondary aniline 2.10c was coupled to carboxylic acid 2.14 on a 0.2 mmol scale via General Procedure B to furnish 2.17c (115 mg, 83 %): $\delta_H$ (400 MHz, $d$-CDCl$_3$) 2.40 (s, 3H, CH$_3$), 2.81 (s, 3H, CH$_3$), 3.67 (s, 2H, CH$_2$), 4.79 (s, 2H, CH$_2$), 5.11 (s, 2H, CH$_2$), 5.36 (s, 2H, CH$_2$), 6.63 (d, $J = 8.0$ Hz, 1H, CH), 6.67 (s, 1H, CH), 7.20 (d, $J = 8.4$ Hz, 2H, CH), 7.24 (d, $J = 8.4$ Hz, 2H, CH) 7.29-7.36 (m 7H, CH), 7.38-7.42 (m, 2H, CH), 7.51 (d, $J = 8.4$ Hz,
2H, CH), 7.58 (d, J = 8.4 Hz, 2H, CH), 7.83 (d, J = 8.0 Hz, 1H, CH); δC (100 MHz, d-CDCl₃) 21.4, 36.1, 51.2, 52.8, 67.0, 70.6, 111.5, 113.7, 118.4, 119.6, 121.1, 126.8, 127.3, 128.0, 128.2, 128.5, 128.6, 129.2, 129.5, 132.2, 133.2, 135.0, 135.5, 135.6, 141.7, 143.4, 144.6, 158.8, 165.1, 167.3; LRMS (ES+) Calcd for [C₃₉H₃₅N₃O₆S + Na] 696.21 found 696.17.

2.17d, Benzyl 2-(benzyloxy)-4-(N-(3-cyanobenzyl)-2-(N,4-dimethylphenylsulfonamido)acetamido)benzoate. Secondary aniline 2.10d was coupled to carboxylic acid 2.14 on a 0.30 mmol scale via General Procedure B to furnish 2.17d (159 mg, 76%): δH (400 MHz, d-CDCl₃) 2.39 (s, 3H, CH₃), 2.80 (s, 3H, CH₃), 3.67 (s, 2H, CH₂), 4.82 (s, 2H, CH₂), 5.11 (s, 2H, CH₂), 5.35 (s, 2H, CH₂), 6.65 (d, J = 8.0 Hz, 1H, CH), 6.66 (s, 1H, CH), 7.27 (d, J = 8.0 Hz, 2H, CH), 7.29-7.44 (m 13H, CH), 7.52-7.56 (m, 1H, CH), 7.59 (d, J = 8.4 Hz, 2H, CH), 7.84 (d, J = 8.0 Hz, 1H, CH); δC (100 MHz, d-CDCl₃) 21.6, 36.3, 51.5, 52.6, 67.2, 70.9, 112.8, 113.9, 118.6, 119.9, 121.4, 127.1, 127.5, 128.3, 128.4, 128.7, 128.8, 129.6, 129.7, 131.6, 132.3, 133.3, 133.5, 135.3, 135.8, 135.8, 138.2, 143.7, 144.7, 159.1, 165.3, 167.5; LRMS (ES+) Calcd for [C₃₉H₃₅N₃O₆S + Na] 696.21 found 696.29 [M+H].

2.17e, benzyl 2-(benzyloxy)-4-(N-(cyclohexylmethyl)-2-(N,4-dimethylphenyl-sulfonamido)acetamido)benzoate. Secondary aniline 2.10e was coupled to carboxylic acid 2.14 on a 0.2
mmol scale via General Procedure B to furnish 2.17e (92 mg, 68 %): $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.04-1.17 (m, 3H, CH$_2$), 1.25-1.41 (m, 3H, CH$_2$ and CH), 1.50-1.71 (m, 5H, CH$_2$), 2.39 (s, 3H, CH$_3$), 2.86 (s, 3H, CH$_3$), 3.47 (d, $J = 8.4$ Hz, 2H, CH$_2$), 3.67 (s, 2H, CH$_2$), 5.22 (s, 2H, CH$_2$), 5.38 (s, 2H, CH$_2$), 6.79-6.84 (m, 2H, CH), 7.25 (d, $J = 8.4$ Hz, 2H, CH), 7.28-7.39 (m, 6H, CH), 7.40-7.47 (m, 4H, CH), 7.61 (d, $J = 8.4$ Hz, 2H, CH), 7.91 (d, $J = 8.4$ Hz, 1H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 21.5, 25.6, 26.2, 30.6, 35.9, 51.3, 55.4, 67.0, 70.8, 113.8, 119.8, 120.6, 127.0, 127.4, 128.0, 128.1, 128.2, 128.5, 128.6, 129.4, 133.1, 135.7, 135.9, 143.2, 145.9, 158.9, 165.3, 167.0; LRMS (ES+) Calcd for [C$_38$H$_{42}$N$_2$O$_6$S + Na] 677.27 found 677.36.

2.17f, Benzy 2-(benzyl)oxy)-4-((N-(4-tert-butylbenzyl)-2-(N,4-dimethylphenylsulfonamido)acetamido)benzoate. Secondary aniline 2.10f was coupled to carboxylic acid 2.14 on a 0.30 mmol scale via General Procedure B to furnish 2.17f (146 mg, 76 %): $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.27 (s, 9H, 3 CH$_3$), 2.38 (s, 3H, CH$_3$), 2.73 (s, 3H, CH$_3$), 3.57 (s, 2H, CH$_2$), 4.67 (s, 2H, CH$_2$), 4.87 (s, 2H, CH$_2$), 5.26 (s, 2H, CH$_2$), 6.46 (s, 1H, CH), 6.60 (d, $J = 8.2$ Hz, 1H, CH), 6.95 (d, $J = 8.2$ Hz, 2H, CH), 7.14-7.33 (m, 14H, CH), 7.52 (d, $J = 8.2$ Hz, 2H, CH), 7.75 (d, $J = 8.2$ Hz, 1H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 21.3, 31.1, 34.3, 35.7, 51.2, 52.5, 66.8, 70.5, 114.0, 119.8, 120.4, 125.2, 126.9, 127.3, 127.8, 128.0, 128.1, 128.3, 129.3, 132.9, 133.4, 135.2, 135.5, 135.6, 143.1, 150.5, 158.5, 165.2, 166.6; LRMS (ES+) Calcd for [C$_{42}$H$_{44}$N$_2$O$_6$S + H] 705.30 found 705.04.
2.17g, Benzyl 2-(benzyloxy)-4-(N-(biphenyl-4-ylmethyl)-2-(N,4-dimethylphenylsulfonamido) acet-amido)benzoate. Secondary aniline 2.10g was coupled to carboxylic acid 2.14 on a 0.2 mmol scale via General Procedure B to furnish 2.17g (138 mg, 86%): δ_H (400 MHz, d-CDCl_3) 2.30 (s, 3H, CH_3), 2.74 (s, 3H, CH_3), 3.59 (s, 2H, CH_2CO), 4.74 (s, 2H, CH_2), 4.92 (s, 2H, CH_2), 5.26 (s, 2H, CH_2), 6.53 (s, 1H, CH), 6.60 (d of d, J = 8.2 and 1.7 Hz, 1H, CH), 7.08 (d, J = 8.2 Hz, 2H, CH), 7.14-7.37 (m, 14H, CH), 7.40 (d, J = 8.2 Hz, 2H, CH), 7.46-7.49 (m, 2H, 2 CH), 7.52 (d, J = 8.2 Hz, 2H, CH), 7.75 (d, J = 8.2 Hz, 1H, CH); δ_C (100 MHz, d-CDCl_3) 21.4, 35.9, 51.3, 52.7, 66.9, 70.6, 114.0, 119.9, 120.7, 126.9, 126.9, 127.1, 127.4, 127.9, 128.1, 128.2, 128.4, 128.5, 128.7, 129.2, 129.4, 133.1, 135.5, 135.6, 135.7, 140.3, 140.5, 143.3, 158.7, 165.3, 166.9; LRMS (ES+) Calcd for [C_{44}H_{40}N_2O_6S + Na] 747.25 found 747.26.

2.17h, Benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(N,4-dimethylphenyl-sulfonamido) acetamido) benzoate. Secondary aniline 2.10h was coupled to carboxylic acid 2.14 on a 0.2
mmol scale via General Procedure B to furnish 2.17h (145 mg, 86 %): δH (400 MHz, d-CDCl₃) 1.15-1.35 (m, 6H, CH₂), 1.61-1.80 (m, 4H, CH₂), 2.31 (s, 3H, CH₃), 2.37-2.38 (m, 1H, CH), 2.73 (s, 3H, CH₃), 3.57 (s, 2H, CH₂), 4.67 (s, 2H, CH₂), 4.86 (s, 2H, CH₂), 5.26 (s, 2H, CH₂), 6.43 (s, 1H, CH), 6.59 (d of d, J = 8.2 and 1.5 Hz, 1H, CH), 6.93 (d, J = 8.0 Hz, 2H, CH), 7.02 (d, J = 8.0 Hz, 2H, CH), 7.14-7.35 (m, 12H, CH), 7.52 (d, J = 8.2 Hz, 2H, CH), 7.75 (d, J = 8.2 Hz, 2H, CH); δC (400 MHz, d-CDCl₃) 21.4, 25.9, 26.6, 34.3, 35.8, 44.0, 51.2, 52.7, 66.8, 70.5, 114.1, 119.9, 120.5, 126.8, 126.9, 127.3, 127.8, 128.0, 128.1, 128.4, 128.5, 128.7, 129.3, 133.0, 133.8, 135.2, 135.6, 135.7, 143.1, 144.9, 147.6, 158.6, 165.2, 166.6; LRMS (ES+) Calcd for \([C_{44}H_{46}N_2O_6S + H]\) 731.32 found 731.28.

2.17i, Benzyl 2-(benzyloxy)-4-(2-(N,4-dimethylphenylsulfonamido)-N-(naphthalen-2-ylmethyl) acetamido)benzoate. Secondary aniline 2.10i was coupled to carboxylic acid 2.14 on a 0.2 mmol scale via General Procedure B to furnish 2.17i (127 mg, 84 %): δH (400 MHz, d-CDCl₃) 2.30 (s, 3H, CH₃), 2.75 (s, 3H, CH₃), 3.60 (s, 2H, CH₂), 4.82 (s, 2H, CH₂), 4.87 (s, 2H, CH₂), 5.25 (s, 2H, CH₂), 6.47 (s, 1H, CH), 6.58 (d of d, J = 8.2 and 1.4 Hz, 1H, CH), 7.12-7.32 (m, 13H, CH), 7.36-7.44 (m, 3H, CH), 7.53 (d, J = 8.2 Hz, 2H, CH), 7.63-7.75 (m, 4H, CH); δC (100 MHz, d-CDCl₃) 21.3, 35.9, 51.3, 53.1, 66.8, 70.5, 114.0, 119.9, 120.7, 126.0, 126.1, 126.4, 126.8, 127.3, 127.5, 127.7, 127.8, 128.0, 128.1, 128.3, 128.4, 129.3, 132.6, 133.0, 133.9, 135.6, 143.2, 158.6, 165.2, 166.9; LRMS (ES+) Calcd for \([C_{42}H_{38}N_2O_6S + H]\) 699.25 found 699.23 [M+H].
2.17j, Benzyl 2-(benzyloxy)-4-(2-(N,4-dimethylphenylsulfonamido)-N-(piperidin-4-ylmethyl) acetamido) benzoate. Secondary aniline 2.10j tert-butyl 4-(((3-(benzyloxy)-4-((benzyloxy)carbonyl)phenyl)amino)methyl)piperidine-1-carboxylate was coupled to carboxylic acid 2.14 on a 2.8 mmol scale via General Procedure B to furnish 2.17j (1.50 g, 67 %): δH (400 MHz, d-CDCl3) 1.40-1.86 (m, 4H, CH₂), 2.38 (s, 3H, CH₃), 2.64-2.93 (m, 5H, CH and CH₂), 2.72 (s, 3H, NCH₃), 3.28-3.70 (m, 4H, CH₂), 5.24 (s, 2H, CH₂), 5.38 (s, 2H, CH₂), 6.85 (d of d, J = 8.2 and 1.7 Hz, 1H, CH), 6.89 (d, J = 1.7 Hz, 1H, CH), 7.20-7.43 (m, 1H, CH), 7.58 (d, J = 8.2 Hz, 1H, CH), 7.90 (d, J = 8.2 Hz, 1H, CH); δC (100 MHz, d-CDCl₃) 21.4, 27.4, 29.7, 33.2, 36.1, 44.1, 51.5, 54.3, 67.0, 70.7, 113.7, 119.5, 120.9, 127.0, 127.3, 127.4, 128.0, 128.2, 128.5, 128.6, 129.4, 129.5, 133.3, 135.0, 135.7, 135.9, 143.5, 145.5, 158.9, 165.3, 167.1, 167.6; LRMS (ES+) Calcd for [C₃₇H₄₁N₃O₆S + H] 656.28 found 656.44.

2.19b, tert-butyl 4-((N-(3-(benzyloxy)-4-(benzyloxy carbonyl)phenyl)-2-(N,4-dimethylphenyl-sulfonamido) acetamido)methyl)piperidine-1-carboxylate. Compound 2.17j was Boc protected with (Boc)₂ via General Procedure C on a 0.15 mmol scale to furnish 2.19b
(99 mg, 86 %): $\delta_H$ (400 MHz, $d$-CDCl$_3$) 0.80-1.02 (m, 3H, CH$_2$), 1.10-1.25 (m, 2H, CH$_2$), 1.36 (s, 9H, CH$_3$), 2.31 (s, 3H, CH$_3$), 2.45-2.55 (m, 2H, CH$_2$), 2.72 (s, 3H, CH$_3$), 3.42 (s (br), 2H, CH$_2$), 3.58 (s, 2H, CH$_2$), 3.93 (s, 2H, CH$_2$), 5.16 (s, 2H, CH$_2$), 5.30 (s, 2H, CH$_2$), 6.71-6.75 (m, 2H, 2CH), 7.15-7.38 (m, 12H, CH), 7.52 (d, $J$ = 8.2 Hz, 2H, CH), 7.83 (d, $J$ = 8.2 Hz, 1H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 21.4, 28.3, 29.6, 34.5, 36.0, 36.5, 51.3, 54.7, 66.9, 70.7, 79.3, 113.7, 119.5, 120.7, 126.9, 127.3, 128.0, 128.1, 128.5, 128.6, 129.4, 133.2, 135.6, 135.8, 143.3, 145.6. 154.5, 158.9, 165.2, 167.2.

![Chemical structure](image)

2.19c, benzyl 2-(benzylxy)-4-(N-((1-(4-cyanophenyl)piperidin-4-yl)methyl)-2-(N,4-dimethylphenylsulfon amido)acetamido)benzoate. Nucleophilic aromatic substitution of 2.17j with 4-fluorobenzonitrile on a 0.2 mmol scale via General Procedure D furnished 2.19c (87 mg, 76 %): $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.20-1.31 (m, 3H, CH$_2$), 1.60-1.68 (m, 2H, CH$_2$), 2.38 (s, 3H, CH$_3$), 2.72 (t, $J$ = 12.0 Hz, 2H, CH$_2$), 2.78 (s, 3H, CH$_3$), 3.55 (d, $J$ = 6.8 Hz, 2H, CH$_2$), 3.66 (s, 2H, CH$_2$), 3.74 (d, $J$ = 13.0 Hz, 2H, CH$_2$), 5.24 (s, 2H, CH$_2$), 5.38 (s, 2H, CH$_2$), 6.77-6.84 (m, 4H, CH), 7.21-7.47 (m, 14H, CH), 7.59 (d, $J$ = 8.2 Hz, 2H, CH), 7.91 (d, $J$ = 8.2 Hz, 1H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 21.4, 28.9, 34.3, 36.1, 47.1, 51.3, 54.5, 67.0, 70.7, 99.2, 113.7, 114.1, 119.5, 120.1, 120.8, 126.9, 127.3, 128.0, 128.2, 128.5, 128.6, 129.4, 133.2, 133.4, 135.6, 135.8, 143.3, 145.6, 152.9, 158.8, 165.2, 167.3.
2.19d, benzyl 2-(benzox)-4-(2-N,4-dimethylphenylsulfonamido)-N-(1-(pyrimidin-2-yl)piperidin-4-yl)methylacetamido)benzoate. Nucleophilic aromatic substitution of 2.17j with 2-chloropyrimidine on a 0.2 mmol scale via General Procedure D furnished 2.19d (108 mg, 96 %): \( \delta \)H (400 MHz, d-CDCl\(_3\)) 1.15-1.40 (m, 2H, CH\(_2\)), 1.54-1.75 (m, 3H, CH\(_2\) and CH), 2.38 (s, 3H, CH\(_3\)), 2.74 (t, \( J = 10.4 \) Hz, 2H, CH\(_2\)), 2.80 (s, 3H, CH\(_3\)), 3.52 (d, \( J = 7.2 \) Hz, 2H, CH\(_2\)), 3.68 (s, 2H, CH\(_2\)), 4.64 (d, \( J = 13.2 \) Hz, 2H, CH\(_2\)), 5.23 (s, 2H, CH\(_2\)), 5.37 (s, 2H, CH\(_2\)), 6.42 (t, \( J = 4.8 \) Hz, 1H, CH), 6.80-6.85 (m, 2H, CH), 7.23-7.37 (m, 8H, CH), 7.38-7.45 (m, 4H, CH), 7.60 (d, \( J = 8.0 \) Hz, 2H, CH), 7.91 (d, \( J = 8.8 \) Hz, 1H, CH), 8.27 (d, \( J = 4.8 \) Hz, 2H, CH); \( \delta \)C (100 MHz, d-CDCl\(_3\)) 21.4, 29.4, 34.8, 35.9, 36.5, 43.4, 51.3, 54.8, 66.9, 70.7, 109.3, 113.7, 119.5, 120.7, 126.9, 127.3, 128.0, 128.1, 128.4, 128.6, 129.4, 133.2, 135.2, 135.6, 135.8, 143.3, 145.7, 157.5, 158.9, 161.2, 165.2, 167.1.

\[ \text{O=C} \quad \text{N} \quad \text{O=C} \quad \text{CF}_3 \]
\[ \text{N} \quad \text{O=C} \quad \text{CF}_3 \]
\[ \text{N} \quad \text{O=C} \quad \text{CF}_3 \]

2.23, 4-(1-(2,2,2-trifluoroacetyl)piperidin-4-yl)benzaldehyde (2 step procedure): (a) To a flask containing AlCl\(_3\) (534 mg, 4.0 mmol) under an N\(_2\) atmosphere was added anhydrous CH\(_2\)Cl\(_2\) (0.1 M), and the drop wise addition of oxalyl chloride (523 \( \mu\)L, 6.0 mmol) over a 20 min period at 15 °C. Next, a solution of 2.21 (2.0 mmol) in anhydrous CH\(_2\)Cl\(_2\) (0.1 M) was added
drop-wise to the initial solution over a 45 min period at 15 °C. When the reaction was complete as judged by TLC, ice was added to the solution in addition to CaCl$_2$ (1.70 g). The product was extract into CH$_2$Cl$_2$, washed with brine and dried over anhydrous Na$_2$SO$_4$ before concentrating under reduced pressure to yield crude 2.22. (b) To a stirred solution of 2.22 (2.0 mmol) and DIPEA (697 µL, 4.0 mmol) in EtOAc (0.1 M) was added 10 % Pd/C. The flask was then evacuated and filled with H$_2$ gas and allowed to stir for 30 mins. After which time the reaction contents were filtered and concentrated under reduced pressure to give crude product which was purified by silica gel column chromatography (hexanes:EtOAc, 2:1) to furnish 2.23 (320 mg, 59 % (yield over 2 steps)) $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.69-1.81 (m, 2H, CH$_2$), 1.98-2.06 (m, 2H, CH$_2$), 2.83-2.97 (m, 2H, CH$_2$), 3.27 (td, $J = 12.8$ and 2.4 Hz, 1H, CH), 4.13-4.21 (m, 1H, CH), 4.70-4.76 (m, 1H, CH), 7.37 (d, $J = 8.4$ Hz, 2H, CH), 7.85 (d, $J = 8.4$ Hz, 2H, CH), 9.99 (s, 1H, CHO); LRMS (ES+) Calcd for [C$_{14}$H$_{14}$F$_3$NO$_2$ + Na] 308.09 found 308.19 [M+Na].

![Chemical Structure](image)

2.24, benzyl 2-(benzyloxy)-4-(2-(N,4-dimethylphenylsulfonamido)-N-(4-(piperidin-4-yl)benzyl)acetamido)benzoate. Compound 2.17k was TFA-deprotected on a 0.04 mmol scale via General Procedure E to furnish 2.24 (0.89 g, 81 %): $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.68-1.82 (m, 2H, CH$_2$), 1.95-2.05 (m, 2H, CH$_2$) 2.41 (s, 3H, CH$_3$), 2.55-2.64 (m, 1H, CH), 2.69-2.78 (m, 2H, CH$_2$), 2.80 (s, 3H, CH$_3$), 3.17-3.21 (m, 2H, CH$_2$), 4.70 (s, 2H, CH$_2$), 4.75 (s, 2H, CH$_2$), 4.99 (s, 2H, CH$_2$), 5.30 (s, 2H, CH$_2$), 6.53 (s, 1H, CH), 6.66 (d, $J = 8.4$ Hz, 1H, CH), 7.03 (d, $J = 8.0$ Hz, 2H, CH), 7.11 (d, $J = 8.0$ Hz, 2H, CH), 7.22-7.40 (m, 12H, CH), 7.60 (d, $J = 8.4$ Hz, 2H, CH), 7.80 (d, $J = 8.4$ Hz, 1H, CH); LRMS (ES+) Calcd for [C$_{43}$H$_{46}$N$_3$O$_6$S + H] 732.31 found 732.40.
2.25c, tert-butyl 4-(4-((N-(3-benzyloxy)-4-((benzyloxy)carbonyl)phenyl)-2-(N,4-dimethylphenylsulfonamido)acetamido)methyl)phenyl)piperidine-1-carboxylate. Compound 2.24 was Boc protected with (Boc)$_2$ via General Procedure C on a 0.10 mmol scale to furnish 2.25c (83 mg, 99 %): $\delta_H$ (400 MHz, $d$-$CDCl_3$) 1.47 (s, 9H, CH$_3$), 1.53-1.64 (m, 2H, CH$_2$), 1.72-1.80 (m, 2H, CH$_2$), 2.39 (s, 3H, CH$_3$), 2.54-2.64 (m, 2H, CH$_2$), 2.70-2.82 (m, 4H, CH$_3$ and CH), 3.64 (s, 2H, CH$_2$), 4.75 (s, 2H, CH$_2$), 4.97 (s, 2H, CH$_2$), 5.34 (s, 2H, CH$_2$), 6.56 (s, 1H, CH), 6.65 (d of d, $J = 8.0$ and 1.6 Hz, 1H, CH), 7.03 (d, $J = 8.0$ Hz, 2H, CH), 7.09 (d, $J = 8.0$ Hz, 2H, CH), 7.24 (d, $J = 8.0$ Hz, 2H, CH), 7.28-7.40 (m, 10H, CH), 7.59 (d, $J = 8.0$ Hz, 2H, CH), 7.81 (d, $J = 8.4$ Hz, 1H, CH); $\delta_C$ (100 MHz, $d$-$CDCl_3$) 21.4, 28.3, 33.0, 35.9, 40.8, 42.2, 51.3, 52.7, 66.9, 70.6, 79.3, 114.0, 119.9, 120.6, 126.8, 127.0, 127.3, 127.9, 128.1, 128.2, 128.4, 128.5, 128.9, 129.4, 133.0, 134.5, 135.2, 135.6, 135.7, 143.2, 144.9, 145.3, 154.7, 158.6, 165.2, 166.7; LRMS (ES+) Calcd for [C$_{48}$H$_{53}$N$_3$O$_8$S + Na] 854.35 found 854.62 [M+Na].
2.25d, benzyl 2-(benzyloxy)-4-(N-(4-(1-(4-cyanophenyl)piperidin-4-yl)benzyl)-2-(N,4-dimethylphenylsulfonamido)acetamido)benzoate. Nucleophilic aromatic substitution of 2.24 with 4-fluorobenzonitrile on a 0.1 mmol scale via General Procedure D furnished 2.25d (87 mg, 95 %); $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.68-1.80 (m, 2H, CH$_2$), 1.85-1.94 (m, 2H, CH$_2$), 2.39 (s, 3H, CH$_3$), 2.66-2.76 (m, 1H, CH), 2.80 (s, 3H, CH$_3$), 2.90-3.00 (m, 2H, CH$_2$), 3.65 (s, 2H, CH$_2$), 3.91-3.98 (m, 2H, CH$_2$), 4.77 (s, 2H, CH$_2$), 4.97 (s, 2H, CH$_2$), 5.34 (s, 2H, CH$_2$), 6.58 (s, 1H, CH), 6.66 (d of d, $J = 8.0$ Hz and 1.6 Hz, 1H, CH), 6.87 (d, $J = 8.8$ Hz, 2H, CH), 7.06 (d, $J = 8.0$ Hz, 2H, CH), 7.11 (d, $J = 8.0$ Hz, 2H, CH), 7.24 (d, $J = 8.0$ Hz, 2H, CH), 7.28-7.41 (m, 10H, CH), 7.48 (d, $J = 8.8$ Hz, 2H, CH), 7.59 (d, $J = 8.4$ Hz, 2H, CH), 7.82 (d, $J = 8.4$ Hz, 1H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 21.4, 32.5, 35.9, 41.9, 48.1, 51.3, 52.7, 66.9, 70.6, 99.4, 114.0, 114.2, 120.0, 120.1, 150.6, 126.8, 127.0, 127.3, 128.1, 128.2, 128.4, 128.5, 129.0, 129.4, 133.0, 133.4, 134.6, 135.1, 135.6, 135.7, 143.3, 144.8, 144.9, 153.1, 158.6, 165.3, 166.8; LRMS (ES+) Calcd for [C$_{50}$H$_{48}$N$_{4}$O$_{6}$S + Na] 855.32 found 855.34.
2.25e, benzyl 2-(benzoyloxy)-4-(2-(N,4-dimethylphenylsulfonamido)-N-(4-(1-(pyrimidin-2-yl)piperidin-4-yl)benzyl)acetamido)benzoate. Nucleophilic aromatic substitution of 2.24 with 2-chloropyrimidine on a 0.1 mmol scale via General Procedure D furnished 2.25e (70 mg, 80 %): $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.59-1.72 (m, 2H, CH$_2$), 1.83-1.94 (m, 2H, CH$_2$), 2.39 (s, 3H, CH$_3$), 2.71-2.83 (m, 4H, CH$_3$ and CH), 2.88-2.99 (m, 2H, CH$_2$), 3.65 (s, 2H, CH$_2$), 4.75 (s, 2H, CH$_2$), 4.86-4.94 (m, 2H, CH$_2$), 4.97 (s, 2H, CH$_2$), 5.34 (s, 2H, CH$_2$), 6.46 (t, $J = 8.4$ Hz, 1H, CH), 6.56 (s, 1H, CH), 6.65 (d of d, $J = 8.0$ and 2.0 Hz, 1H, CH), 7.03 (d, $J = 8.0$ Hz, 2H, CH), 7.11 (d, $J = 8.0$ Hz, 2H, CH), 7.24 (d, $J = 8.4$ Hz, 2H, CH), 7.27-7.41 (m, 10H, CH), 7.59 (d, $J = 8.4$ Hz, 2H, CH), 7.82 (d, $J = 8.4$ Hz, 1H, CH), 8.30 (d, $J = 8.8$ Hz, 2H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 21.4, 32.9, 35.8, 41.9, 48.1, 51.3, 52.7, 66.9, 70.6, 109.4, 113.9, 119.9, 120.6, 126.9, 127.0, 127.4, 127.9, 128.1, 128.2, 128.4, 128.5, 128.9, 129.4, 133.0, 134.4, 135.2, 135.6, 135.7, 143.2, 144.9, 145.5, 157.6, 158.6, 161.4, 165.2, 166.7; LRMS (ES+) Calcd for [C$_{47}$H$_{47}$N$_5$O$_6$S + H] 810.33 found 810.44.
2.25f, benzyl 2-(benzyloxy)-4-(N-(1-(4-cyanobenzoyl)piperidin-4-yl)benzyl)-2-(N,4-dimethylphenylsulfonamido)acetamido)benzoate. Condensation of 2.24 with 4-cyanobenzoic acid on a 0.10 mmol scale via General Procedure F furnished 2.25f (63 mg, 89 %): δ$_H$ (400 MHz, d-$CDCl_3$) 1.59-1.72 (m, 4H, CH$_2$), 1.83-1.94 (m, 2H, CH$_2$), 2.39 (s, 3H, CH$_3$), 2.79-3.21 (m, 8H, CH$_3$, CH and CH$_2$), 3.65 (s, 2H, CH$_2$), 4.77 (s, 2H, CH$_2$), 4.95 (s, 2H, CH$_2$), 4.97 (s, 2H, CH$_2$), 5.34 (s, 2H, CH$_2$), 6.60 (t, $J = 8.4$ Hz, 1H, CH), 6.69 (d of d, $J = 8.0$ and 1.6 Hz, 1H, CH), 7.03-7.12 (m, 4H, 4 CH), 7.21-7.82 (m, 12H, 12 CH); δ$_C$ (100 MHz, d-$CDCl_3$) 21.4, 29.5, 35.9, 42.0, 48.1, 51.3, 52.7, 66.9, 70.6, 113.3, 113.9, 119.9, 120.7, 126.7, 126.9, 127.3, 127.4, 127.9, 128.1 (2), 128.4, 128.5, 129.0, 129.4, 132.3, 132.9, 134.8, 135.0, 135.5, 135.6, 140.0, 143.3, 144.0, 144.8, 158.6, 165.2, 167.0, 168.3; LRMS (ES+) Calcd for [C$_{51}$H$_{48}$N$_4$O$_7$S + H] 861.33 found 861.38.
2.25g, benzyl 2-(benzyloxy)-4-(N-(4-(1-((4-cyanophenyl)sulfonyl)piperidin-4-yl)benzyl)-2-(N,4-dimethylphenylsulfonamido)acetamido)benzoate. Sulfonation of secondary amine 2.24 with 4-cyano-benzene-1-sulfonyl chloride on a 0.08 mmol scale via General Procedure F furnished 2.25g (77 mgs, 99 %): $\delta_{H}$ (400 MHz, $d$-CDCl$_3$) 1.72-1.91 (m, 4H, CH$_2$), 2.31-2.45 (m, 7H, CH$_3$ and 2 CH$_2$), 2.80 (s, 3H, CH$_3$), 3.62 (s, 2H, CH$_2$), 3.89-3.99 (m, 2H, CH$_2$), 4.74 (s, 2H, CH$_2$), 5.01 (s, 2H, CH$_2$), 5.34 (s, 2H, CH$_2$), 6.60 (t, $J = 8.4$ Hz, 1H, CH), 6.69 (d of d, $J = 8.0$ and 1.6 Hz, 1H, CH), 6.99-7.09 (m, 4H, 4 CH), 7.21-7.91 (m, 12H, 12 CH); $\delta_{C}$ (100 MHz, $d$-CDCl$_3$) 21.3, 29.5, 32.3, 35.9, 40.8, 41.1, 46.5, 51.3, 52.6, 66.9, 70.6, 113.9, 116.3, 117.1 119.9, 120.6, 126.6, 126.9, 127.3, 127.9, 128.0, 128.1 (2), 128.4, 128.5, 129.0, 129.3, 132.7, 132.9, 134.9, 135.1, 135.5, 135.6, 140.7, 143.2, 143.8, 144.9, 158.6, 165.2, 166.8; LRMS (ES+) Calcd for [C$_{50}$H$_{48}$N$_{4}$O$_{8}$S$_{2}$ + Na] 919.28 found 919.40.
2.25h, benzyl 2-(benzyl-4-(N-(4-(1-(4-(tert-butoxycarbonyl)phenyl)piperidin-4-yl)benzyl)-2-(N,4-dimethylphenylsulfonylamido)acetamido)benzoate. Nucleophilic aromatic substitution of 2.24 with tert-butyl 4-(chlorosulfonyl)benzoate on a 0.11 mmol scale via General Procedure D furnished 2.25h (113 mg, 98 %): $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.57 (s, 9H, 3CH$_3$), 1.72-1.84 (m, 2H, CH$_2$), 1.86-1.96 (m, 2H, CH$_2$), 2.39 (s, 3H, CH$_3$), 2.64-2.75 (m, 1H, CH), 2.81 (s, 3H, CH$_3$), 2.91 (t, $J = 10.4$ Hz, 2H, CH$_2$), 3.66 (s, 2H, CH$_2$), 3.93 (d, $J = 12.8$ Hz, 2H, CH$_2$), 4.77 (s, 2H, CH$_2$), 4.98 (s, 2H, CH$_2$), 5.35 (s, 2H, CH$_2$), 6.57 (s, 1H, CH), 6.67 (d, $J = 8.0$ Hz, 1H, CH), 6.88 (d, $J = 8.8$ Hz, 2H, CH), 7.06 (d, $J = 8.0$ Hz, 2H, CH), 7.12 (d, $J = 8.0$ Hz, 2H, CH), 7.24 (d, $J = 7.6$ Hz, 2H, CH), 7.29-7.39 (m, 10H, CH), 7.60 (d, $J = 8.0$ Hz, 2H, CH), 7.83 (d, $J = 8.4$ Hz, 1H, CH), 7.88 (d, $J = 8.8$ Hz, 2H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 21.4, 28.2, 32.6, 35.9, 42.1, 48.7, 51.3, 52.7, 66.9, 70.6, 79.9, 113.8, 114.0, 119.8, 120.3, 121.2, 126.8, 127.0, 127.4, 128.0, 128.1, 128.2, 128.4, 128.5, 129.0, 129.4, 130.9, 133.0, 134.5, 135.3, 135.6, 135.7, 143.2, 144.8, 145.2, 153.8, 158.6, 165.3, 165.9, 166.8.
2.26, 4-(4-(4-((N-(4-((benzyloxy)carbonyl)-3-hydroxyphenyl)-2-(N,4-dimethylphenylsulfon-amido)acetamido)methyl)phenyl)piperidin-1-yl)benzoic acid.  t-Butyl ester 2.25h (0.14 mmol) was dissolved in a 1:1 mixture of TFA:toluene (2.8 ml) and stirred at rt for 4 hrs. All solvents were subsequently evaporated, and the crude product passed through a short pad of silica gel (CH₂Cl₂) to furnish 2.26 (130 mg, 95 %):  δH (400 MHz, d-CDCl₃) 1.73-1.85 (m, 2H, CH₂), 1.90-1.98 (m, 2H, CH₂), 2.40 (s, 3H, CH₃), 2.68-2.78 (m, 1H, CH), 2.86 (s, 3H, CH₃), 2.98 (t, J = 12.0 Hz, 2H, CH₂), 3.82(s, 2H, CH₂), 4.02 (d, J = 12.8 Hz, 2H, CH₂), 4.80 (s, 2H, CH₂), 5.28 (s, 2H, CH₂), 5.39 (s, 2H, CH₂), 6.56 (d of d, J = 8.4 and 1.6 Hz, 1H, CH), 6.67 (d, J = 1.6 Hz, 1H, CH), 6.91 (d, J = 8.8 Hz, 2H, CH), 7.08 (d, J = 8.0 Hz, 2H, CH), 7.12 (d, J = 8.0 Hz, 2H, CH), 7.26 (d, J = 7.6 Hz, 2H, CH), 7.34-7.48 (m, 5H, CH), 7.64 (d, J = 8.0 Hz, 2H, CH), 7.87 (d, J = 8.4 Hz, 1H, CH), 7.98 (d, J = 8.8 Hz, 2H, CH);  δC (100 MHz, d-CDCl₃) 21.4, 32.5, 35.7, 42.0, 48.3, 51.4, 52.7, 53.3, 67.3, 112.2, 113.5, 116.8, 117.9, 119.0, 126.8, 127.4, 128.3, 128.6 (br), 129.4, 131.4, 131.9, 134.4, 134.8, 135.2, 143.3, 144.9, 147.2, 154.4, 162.4, 166.7, 169.1, 171.9.
2.27, 4-(N-(4-(1-(4-carbamoylphenyl)piperidin-4-yl)benzyl)-2-(N,4-dimethylphenyl-sulfonamido)acetamido)-2-hydroxybenzoate. Condensation of 2.26 with NH₄Cl on a 0.10 mmol scale via General Procedure F furnished 2.27 (58 mg, 98 %): δ_H (400 MHz, d-CDCl₃) 1.70-1.96 (m, 4H, CH₂), 2.39 (s, 3H, CH₃), 2.63-2.73 (m, 1H, CH), 2.85 (s, 3H, CH₃), 2.86-2.9 (m, 2H, CH₂), 3.80 (s, 2H, CH₂), 3.92-3.97 (m, 2H, CH₂), 4.79 (s, 2H, CH₂), 5.38 (s, 2H, CH₂), 5.86 (d of d, J = 8.4 and 2.0 Hz, 1H, CH), 6.67 (d, J = 2.0 Hz, 1H, CH), 6.91 (d, J = 8.4 Hz, 2H, CH), 7.03-7.14 (m, 4H, CH), 7.35-7.43 (m, 5H, CH), 7.63 (d, J = 8.4 Hz, 2H, CH), 7.73 (d, J = 7.6 Hz, 2H, CH), 7.86 (d, J = 8.4 Hz, 1H, CH); δ_C (100 MHz, d-CDCl₃) 21.4, 32.6, 35.7, 41.9, 48.7, 51.4, 52.7, 67.3, 112.2, 114.2, 116.8, 117.9, 118.9, 122.2, 126.8, 127.4, 128.3, 128.5, 128.6, 128.9, 129.4, 131.4, 134.4, 134.8, 135.2, 143.2, 145.0, 147.2, 153.6, 162.4, 166.6, 169.0; LRMS (ES+) Calcd for [C₄₃H₄₄N₄O₇S + Na] 783.28 found 783.34.
2.28a, Methyl 4'-(N-(3-(benzyloxy)-4-(benzyloxy carbonyl)phenyl)-2-(N,4-dimethylphenylsulfonamido)acetamido)methyl)biphenyl-3-carboxylate. Aryl halide 2.17a was coupled to 3-(methoxycarbonyl)phenyl boronic acid to give 2.28a on a 0.1 mmol scale via General Procedure H (65 mg, 62 %): \(\delta_H\) (400 MHz, \(d\)-CDCl\(_3\)) 2.40 (s, 3H, CH\(_3\)), 2.83 (s, 3H, CH\(_3\)), 3.68 (s, 2H, CH\(_2\)), 3.94 (s, 3H, CH\(_3\)), 4.84 (s, 2H, CH\(_2\)), 5.03 (s, 2H, CH\(_2\)), 5.35 (s, 2H, CH\(_2\)), 6.62 (s, 1H, CH), 6.68 (d of d, \(J = 8.0\) and 1.6 Hz, 1H, CH), 7.19 (d, \(J = 8.0\) Hz, 2H, CH), 7.30-7.42 (m, 10H, CH), 7.52 (d, \(J = 8.4\) Hz, 2H, CH), 7.60-7.64 (m, 4H, CH), 7.78 (d, \(J = 7.6\) Hz, 2H, CH), 7.84 (d, 1H, \(J = 8.0\) Hz, CH), 8.02 (dt, \(J = 8.0\) and 1.2 Hz, 1H, CH), 8.25 (t, \(J = 2.0\) Hz, 1H, CH); \(\delta_C\) (100 MHz, \(d\)-CDCl\(_3\)) 21.5, 36.0, 52.2, 52.8, 56.0, 67.5, 70.7, 117.2, 117.5, 120.1, 125.2, 127.0, 127.2, 127.4, 127.5, 128.0, 128.1, 128.1, 128.2, 128.2, 128.5, 128.6, 128.9, 129.5, 130.7, 131.3, 133.2, 135.7, 135.7, 136.1, 139.5, 141.2, 142.4, 158.8, 161.7, 166.9, 167.0, 168.4; LRMS (ES+) Calcd for [C\(_{46}\)H\(_{42}\)N\(_2\)O\(_8\)S + H] 783.27 found 783.26.
2.28b, benzyl 2-(benzoyloxy)-4-(N'-(3'-cyanobiphenyl-4-yl)methyl)-2-(N,4-dimethylphenylsulfon-amido)acetamido)benzoate. Aryl halide 2.17a was coupled to 3-cyanophenylboronic acid to give 2.28b on a 0.1 mmol scale via General Procedure H (58 mg, 60 %): \(\delta_H\) (400 MHz, \(d\)-CDCl\(_3\)) 2.33 (s, 3H, CH\(_3\)), 2.76 (s, 3H, CH\(_3\)), 3.62 (s, 2H, CH\(_2\)), 4.78 (s, 2H, CH\(_2\)), 4.97 (s, 2H, CH\(_2\)), 5.28 (s, 2H, CH\(_2\)), 6.60-6.62 (m, 2H, CH), 7.14-7.28 (m, 12H, CH), 7.25-7.28 (m, 2H, CH), 7.38 (d, \(J = 8.0 \text{ Hz}, 2H, \text{CH}\)), 7.44-7.49 (m, 1H, CH), 7.54-7.57 (m, 3H, CH), 7.71 (dt, \(J = 7.6 \text{ and } 1.2 \text{ Hz}, 1H, \text{CH}\)), 7.76 (s, 1H, CH), 7.78 (s, 1H, CH); \(\delta_C\) (100 MHz, \(d\)-CDCl\(_3\)) 21.4, 36.0, 51.4, 52.7, 67.0, 70.7, 112.9, 114.0, 118.6, 120.0, 120.9, 127.0, 127.1, 127.4, 128.0, 128.2, 128.2, 128.5, 128.6, 128.7, 129.5, 129.5, 129.6, 130.5, 130.8, 131.2, 133.1, 135.6, 135.7, 136.8, 138.2, 141.7, 143.3, 158.8, 165.2, 167.1, 167.7; LRMS (ES+) Calcd for \([C\(_{45}\)H\(_{39}\)N\(_3\)O\(_6\)S + H]\) 750.26 found 750.26.

2.28c, benzyl 2-(benzoyloxy)-4-(N'-(3'-carbamoylbiphenyl-4-yl)methyl)-2-(N,4-dimethylphenylsulfonamido)acetamido)benzoate. Aryl halide 2.17a was coupled to 3-cyanophenylboronic acid to give 2.28c on a 0.1 mmol scale via General Procedure H (57 mg, 52 %): \(\delta_H\) (400 MHz, \(d\)-CDCl\(_3\)) 2.38 (s, 3H, CH\(_3\)), 2.81 (s, 3H, CH\(_3\)), 3.68 (s, 2H, CH\(_2\)), 4.83 (s, 2H, CH\(_2\)), 5.02 (s, 2H, CH\(_2\)), 5.34 (s, 2H, CH\(_2\)), 5.93 (s, 1H, NH\(_2\)), 6.33 (s, 2H, NH\(_2\)), 6.64 (s, 1H, CH), 6.67 (d of d, \(J = 6.0 \text{ and } 1.2 \text{ Hz}, 1H, \text{CH}\)), 7.18 (d, \(J = 6.0 \text{ Hz}, 2H, \text{CH}\)), 7.31-7.39 (m, 13H, CH), 7.50 (d, \(J = 6.0 \text{ Hz}, 2H, \text{CH}\)), 7.60 (d, \(J = 6.3 \text{ Hz}, 2H, \text{CH}\)), 7.70 (d, \(J = 5.7 \text{ Hz}, 1H, \text{CH}\)), 7.77 (d, \(J = 5.7 \text{ Hz}, 1H, \text{CH}\)), 7.83 (d, \(J = 6.0 \text{ Hz}, 1H, \text{CH}\)), 8.03 (s, 1H, CH); \(\delta_C\) (100 MHz, \(d\)-CDCl\(_3\)) 21.3, 35.9, 51.3, 52.7, 66.9, 70.6, 113.9, 119.2, 119.9, 126.0, 126.9, 127.0, 127.2, 127.3, 127.9, 128.1, 128.4, 128.4, 128.7, 128.9, 129.2, 129.4, 130.3, 132.3, 133.0, 133.8,
2.28d, Methyl 4′-((N-(3-(benzyloxy)-4-(benzyloxycarbonyl)phenyl)-2-(N,4-dimethylphenylsulfonamido)acetamido)methyl)biphenyl-4-carboxylate. Aryl halide 2.17a was coupled to 4-(methoxycarbonyl)phenylboronic acid to give 2.28d on a 0.1 mmol scale via General Procedure H (53 mg, 52 %): \( \delta_H \) (400 MHz, \( d-\text{CDCl}_3 \)) 2.39 (s, 3H, \( CH_3 \)), 2.82 (s, 3H, \( CH_3 \)), 3.68 (s, 2H, \( CH_2 \)), 3.93 (s, 3H, \( CH_3 \)), 4.84 (s, 2H, \( CH_2 \)), 5.03 (s, 2H, \( CH_2 \)), 5.34 (s, 2H, \( CH_2 \)), 6.64 (s, 1H, CH), 6.68 (d of d, \( J = 8.4 \text{ Hz} \) and 1.2 Hz, 1H, CH), 7.19 (d, \( J = 8.0 \text{ Hz} \), 2H, CH), 7.23-7.34 (m, 8H, CH), 7.37-7.40 (m, 2H, CH), 7.50-7.54 (m, 3H, CH), 7.60 (d, \( J = 8.0 \text{ Hz} \), 2H, CH), 7.22 (d, \( J = 8.4 \text{ Hz} \), 2H, CH), 7.70 (d of d, \( J = 5.6 \text{ and 3.2 Hz} \), 1H, CH), 7.83 (d, \( J = 8.0 \text{ Hz} \), 1H, CH) 8.09 (d, 2H, \( J = 8.4 \text{ Hz} \), CH); \( \delta_C \) (100 MHz, \( d-\text{CDCl}_3 \)) 21.4, 36.0, 51.4, 52.1, 52.8, 67.0, 70.7, 114.1, 120.0, 120.9, 126.8, 126.9, 127.3, 127.4, 128.0, 128.2, 128.2, 128.5, 128.6, 128.7, 129.0, 129.4, 129.5, 130.1, 132.4, 133.1, 135.3, 135.7, 135.7, 136.5, 139.3, 143.3, 144.8, 144.9, 158.8, 166.8, 167.0, 167.7; LRMS (ES+) Calcd for [C_{46}H_{42}N_{2}O_{8}S + H] 783.27 found 783.26.
2.28e, Methyl 4’-((N-(3-(benzyloxy)-4-(benzyloxy carbonyl)phenyl)-2-(N,4-dimethylphenyl sulfon-amido)acetamido)methyl)biphenyl-4-carboxylate. Aryl halide 2.17a was coupled to 4-carboxyphenylboronic acid to give 37e on a 0.1 mmol scale via General Procedure H. 2.28e was not purified at this stage and was deprotected without purification.

2.28f, Benzyl 2-(benzyloxy)-4-((4’-cyanobiphenyl-4-yl)methyl)-2-(N,4-dimethylphenyl-sulfon-amido)acetamido)benzoate. Aryl halide 2.17a was coupled to 4-cyanophenylboronic acid to give 2.28f on a 0.1 mmol scale via General Procedure H (73 mg, 71 %): $\delta_H$ (400 MHz, $d$-CDCl$_3$) 2.39 (s, 3H, CH$_3$), 2.82 (s, 3H, CH$_3$), 3.68 (s, 2H, CH$_2$), 4.85 (s, 2H, CH$_2$), 5.05 (s, 2H, CH$_2$), 5.35 (s, 2H, CH$_2$), 6.67 (s, 1H, CH), 6.69 (d, $J = 1.2$ Hz, 1H, CH), 7.22-7.34 (m, 13H,
CH), 7.38−7.40 (m, 2H, CH), 7.61 (d, J = 8.0 Hz, 2H, CH), 7.64 (d, J = 8.4 Hz, 2H, CH), 7.70−7.72 (m, 3H, CH), 7.84 (d, J = 8.4 Hz, 1H, CH); δC (100 MHz, d-CDCl3) 21.4, 36.0, 51.4, 52.7, 67.0, 70.7, 111.0, 114.0, 118.7, 119.9, 120.8, 126.9, 127.3, 127.4, 127.5, 128.0, 128.2, 128.4, 128.5, 128.7, 129.5, 130.8, 132.4, 132.5, 133.1, 135.6, 135.7, 137.1, 138.4, 143.3, 144.8, 158.8, 165.2, 167.1, 167.6; LRMS (ES+) Calcd for [C_{45}H_{39}N_{3}O_{6}S + H] 750.26 found 750.26.

2.28g, Benzyl 2-(benzyloxy)-4-(N-((4'-carbamoylbiphenyl-4-yl)methyl)-2-(N,4-dimethyl phenylsulfonamido)acetamido)benzoate. Aryl halide 2.17a was coupled to 4-carbamoylphenylboronic acid to give 2.28g on a 0.1 mmol scale via General Procedure E (62 mg, 49 %): δH (400 MHz, d-CDCl3) 2.38 (s, 3H, CH3), 2.82 (s, 3H, CH3), 3.68 (s, 2H, CH2), 4.39 (s, 2H, CH2), 5.03 (s, 2H, CH2), 5.34 (s, 2H, CH2), 5.91 (s, 1H, NH2), 6.44 (s, 1H, NH), 6.65 (s, 1H, CH2), 6.68 (d, 1H, CH), 7.19 (d, J = 6.0 Hz, 2H, CH), 7.24-7.35 (m, 4H, CH), 7.38-7.41 (m, 2H, CH), 7.44-7.50 (m, 6H, CH), 7.53 (d, J = 4.8 Hz, 1H, CH), 7.59 (s, 2H, CH), 7.61 (s, 2H, CH), 7.64 (d, J = 5.4 Hz, 2H, CH), 7.68 (d, J = 5.4 Hz, 2H, CH), 7.83 (d, J = 6.3 Hz, 1H, CH), 7.89 (d, J = 6.0 Hz, 2H, CH); δC (100 MHz, d-CDCl3) 21.3, 35.9, 51.3, 52.7, 66.9, 70.6, 71.9, 119.9, 120.8, 126.8, 126.9, 127.1, 127.3, 127.9, 128.1, 128.1, 128.3, 128.4, 128.4, 129.4, 131.8, 131.8, 131.8, 131.9, 132.0, 132.8, 133.0, 135.1, 135.5, 135.6, 136.3, 139.1, 143.2, 143.8, 144.8, 158.7, 165.2, 167.0, 168.8; LRMS (ES+) Calcd for [C_{45}H_{41}N_{3}O_{7}S + H] 768.27 found 768.27.
2.29a, methyl 4’-((N-(3-(benzyloxy)-4-(benzyloxycarbonyl)phenyl)-2-(N,4-dimethylphenylsulfonamido)acetamido)methyl)terphenyl-3-carboxylate. Aryl halide 2.17m was coupled to 3-(methoxycarbonyl)phenylboronic acid on a 0.1 mmol scale via General Procedure H to yield 2.29a (39 mg, 38 %): δ_H (400 MHz, d-CDCl_3) 2.40 (s, 3H, CH_3), 2.83 (s, 3H, CH_3), 3.68 (s, 2H, CH_2), 3.96 (s, 3H, CH_3), 4.84 (s, 2H, CH_2), 5.02 (s, 2H, CH_2), 5.35 (s, 2H, CH_2), 6.62 (s, 1H, CH), 6.70 (d, J = 8.0 Hz, 1H, CH) 7.19 (d, J = 8.0 Hz, 2H, CH), 7.26-7.34 (m, 10H, CH), 7.38-7.41 (m, 3H, CH), 7.54 (d, J = 8.0 Hz, 2H, CH), 7.62 (d, J = 8.4 Hz, 2H, CH), 7.68 (q, J = 8.0 Hz, 4H, CH), 7.84 (t, J = 8.0 Hz, 2H, CH), 8.03 (d, J = 8.0 Hz, 1H, CH), 8.32 (s, 1H, CH); LRMS (ES+) Calcd for [C_{52}H_{46}N_{2}O_{8}S + H] 859.31 found 859.25.
2.29b, 4’-((N-(3-(benzyloxy)-4-(benzyloxy carbonyl)phenyl)-2-(N,4-dimethylphenylsulfonamido)acetamido)methyl)terphenyl-3-carboxylic acid. Aryl halide 2.17m was coupled to 3-carboxyphenylboronic acid on a 0.1 mmol scale via General Procedure H to yield 2.29b (76 mg, 64 %): δH (400 MHz, d-CDCl3) 2.33 (s, 3H, CH3), 2.78 (s, 3H, CH3), 3.65 (s, 2H, CH2), 4.80 (s, 2H, CH2), 4.80 (s, 2H, CH2), 5.30 (s, 2H, CH2), 6.60 (s, 1H, CH), 6.66 (d, J = 8.4 Hz, 2H, CH), 7.15 (d, J = 8.0 Hz, 2H, CH), 7.13-7.28 (m, 12H, CH), 7.30-7.38 (m, 3H, CH), 7.50 (d, J = 7.2 Hz, 2H, CH), 7.47-7.63 (m, 6H, CH), 7.90 (d, J = 8.0 Hz, 2H, CH); LRMS (ES+) Calcd for [C51H44N2O8S + H] 845.29 found 845.15.
2.29c, benzyl 2-(benzyloxy)-4-(N-((3′-Cyanoterphenyl-4-yl)methyl)-2-(N,4-dimethylphenyl sulfonamido)acetamido)benzoate. Aryl halide 2.17m was coupled to 3-cyanophenylboronic acid on a 0.1 mmol scale via General Procedure H to yield 2.29c (59 mg, 56 %): δ_H (400 MHz, d-CDCl_3) 2.38 (s, 3H, CH_3), 2.83 (s, 3H, CH_3), 3.69 (s, 2H, CH_2), 4.85 (s, 2H, CH_2), 5.03 (s, 2H, CH_2), 5.34 (s, 2H, CH_2), 6.65 (s, 1H, CH), 6.70 (d, J = 8.0 Hz, 1H, CH), 7.20 (d, J = 8.0 Hz, 2H, CH), 7.23 (t, J = 8.4 Hz, 4H, CH), 7.30-7.34 (m, 6H, CH), 7.38-7.40 (m, 3H, CH), 7.50 (d, J = 8.4 Hz, 2H, CH), 7.60-7.63 (m, 5H, CH), 7.67 (d, J = 8.4 Hz, 2H, CH), 7.81 (d, J = 8.0 Hz, 2H, CH), 7.86 (s, 1H, CH). LRMS (ES+) Calcd for [C_51H_43N_3O_6S + H] 848.28 found 848.45.

2.29d, 4-(N-((3′-carbamoylbiphenyl-4-yl)methyl)-2-(N,4-dimethylphenylsulfon-amido)acetamido)-2-hydroxybenzoic acid. Aryl halide 2.17m was coupled to 3-carbamoylphenylboronic acid on a 0.1 mmol scale via General Procedure E to give 2.29d (38 mg, 32 %): δ_H (400 MHz, d-CDCl_3) 2.39 (s, 3H, CH_3), 2.83 (s, 3H, CH_3), 3.69 (s, 2H, CH_2), 4.84 (s, 2H, CH_2), 5.03 (s, 2H, CH_2), 5.35 (s, 2H, CH_2), 6.63 (s, 1H, CH), 6.70 (d, J = 8.8 Hz, 1H, CH), 7.19 (d, J = 8.0 Hz, 2H, CH), 7.30-7.36 (m, 10H, CH), 7.38-7.41 (m, 3H, CH), 7.54 (d, J = 8.0 Hz, 2H, CH), 7.62 (d, J = 8.0 Hz, 2H, CH), 7.67 (q, J = 7.2 Hz, 4H, CH), 7.79 (t, 2H, J = 7.6 Hz, CH), 7.85 (d, J = 8.0 Hz, 1H, CH), 8.12 (s, 1H, CH). LRMS (ES+) Calcd for [C_51H_48N_3O_7S + Na] 866.29 found 866.51.
2.29e, methyl 4'-(N-(3-(benzyloxy)-4-(benzyloxy carbonyl)phenyl)-2-(N,4-dimethyl phenylsulfonamido)acetamido)methyl)terphenyl-4-carboxylate. Aryl halide 2.17m was coupled to 4-(methoxycarbonyl)phenylboronic acid to give 2.29e on a 0.1 mmol scale via General Procedure H (59 mg, 47 %): $\delta_H$ (400 MHz, $d$-CDCl$_3$) 2.38 (s, 3H, CH$_3$), 2.83 (s, 3H, CH$_3$), 3.69 (s, 2H, CH$_2$), 3.93 (s, 3H, CH$_3$), 4.84 (s, 2H, CH$_2$), 5.02 (s, 2H, CH$_2$), 5.35 (s, 2H, CH$_2$), 6.64 (s, 1H, CH), 6.70 (d of d, $J = 8.4$ and 1.6 Hz, 1H, CH), 7.20 (d, $J = 8.0$ Hz, 2H, CH), 7.24-7.28 (m, 6H, CH), 7.30-7.33 (m, 5H, CH), 7.36-7.41 (m, 3H, CH), 7.54 (d, $J = 8.4$ Hz, 2H, CH), 7.61 (d, $J = 8.4$ Hz, 2H, CH), 7.64-7.73 (m, 6H, CH), 7.85 (d, $J = 8.4$ Hz, 1H, CH). LRMS (ES+) Calcd for [C$_{52}$H$_{46}$N$_2$O$_8$S + H] 881.29, found 881.39.
2.29f, 4’-((N-(3-(benzyloxy)-4-(benzyloxycarbonyl)phenyl)-2-(N,4-dimethylphenyl-sulfonamido)acetamido)methyl)terphenyl-4-carboxylic acid. Aryl halide 2.17m was coupled to 4-carboxyphenylboronic acid to give 2.29f on a 0.1 mmol scale via General Procedure H (47 mg, 47 %): $\delta_H$ (400 MHz, $d$-CDCl$_3$) 2.40 (s, 3H, CH$_3$), 2.84 (s, 2H, CH$_2$), 3.70 (s, 2H, CH$_2$), 4.86 (s, 2H, CH$_2$), 5.04 (s, 2H, CH$_2$), 5.36 (s, 2H, CH$_2$), 6.65 (s, 1H, CH), 6.71 (d, $J = 8.4$ Hz, 1H, CH), 7.21 (d, $J = 8.0$ Hz, 2H, CH), 7.27-7.43 (m, 12H, CH), 7.39-7.41 (m, 3H, CH), 7.55 (d, $J = 8.4$ Hz, 2H, CH), 7.63 (d, $J = 8.0$ Hz, 2H, CH), 7.67-7.75 (m, 4H, CH), 7.86 (d, $J = 8.0$ Hz, 1H, CH), 8.20 (d, $J = 7.6$ Hz, 1H, CH); LRMS (ES+) Calcd for [C$_{51}$H$_{44}$N$_2$O$_8$S + H] 845.29 found 845.35.
2.29g, Benzyl 2-(benzyloxy)-4-(N-((4'-Cyanoterphenyl-4-yl)methyl)-2-(N,4-dimethylphenyl sulfonamido)acetamido)benzoate. Aryl halide 2.17m was coupled to 4-cyanophenylboronic acid to give 2.29h on a 0.1 mmol scale via General Procedure H (32 mg, 30%): δH (400 MHz, d-CDCl₃) 2.36 (s, 3H, CH₃), 2.79 (s, 3H, CH₃), 3.65 (s, 2H, CH₂), 4.81 (s, 2H, CH₂), 6.00 (s, 2H, CH₂), 5.31 (s, 2H, CH₂), 6.61 (s, 1H, CH), 6.66 (d, J = 8.4 Hz, 1H, CH), 7.17 (d, J = 8.4 Hz, 2H, CH), 7.23 (t, J = 8.0 Hz, 4H, CH), 7.30-7.33 (m, 5H, CH), 7.36-7.40 (m, 3H, CH), 7.70 (d, J = 8.0 Hz, 2H, CH), 7.58 (d, J = 8.0 Hz, 2H, CH), 7.66-7.73 (m, 8H, CH), 7.81 (d, J = 8.4 Hz, 1H, CH). LRMS (ES+) Calcd for [C₅₁H₄₄N₃O₆S + H] 848.28 found 848.35.
**2.29h, benzyl 2-(benzyloxy)-4-(N-((4'-carbamoylterphenyl-4-yl)methyl)-2-(N,4-dimethylphenylsulfonamido)acetamido)benzoate.** Aryl halide 2.17m was coupled to 4-carbamoylphenylboronic acid to give 2.29h on a 0.1 mmol scale via General Procedure H (31 mg, 28 %): $\delta_H$ (400 MHz, $d$-CDCl$_3$) 2.40 (s, 3H, CH$_3$), 2.83 (s, 3H, CH$_3$), 3.69 (s, 2H, CH$_2$), 4.58 (s, 2H, CH$_2$), 5.04 (s, 2H, CH$_2$), 5.36 (s, 2H, CH$_2$), 6.64 (s, 1H, CH), 6.70 (d, $J = 7.6$ Hz, 1H, CH), 7.21 (d, $J = 8.0$ Hz, CH), 7.24-7.29 (m, 5H, CH), 7.31-7.36 (m, 5H, CH), 7.38-7.42 (m, 2H, CH), 7.54 (d, $J = 8.4$ Hz, 2H, CH), 7.62 (d, $J = 8.4$ Hz, 2H, CH), 7.65-7.76 (m, 8H, CH), 7.85 (d, $J = 8.0$ Hz, 1H, CH). LRMS (ES+) Calcd for [C$_{51}$H$_{45}$N$_3$O$_7$S + Na] 866.29 found 866.32.
2.30, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(4-methylphenylsulfonamido)acetamido)benzoate

Secondary aniline 2.10h was coupled to 2-(N-(tert-butoxycarbonyl)-4-methylphenylsulfonamido)acetic acid on a 0.2 mmol scale using General Procedure B to give 13a (90 mg, 83%); \(\delta_H\) (400 MHz, \(d\)-CDCl\(_3\)) 1.27-1.42 (m, 5H, CH\(_2\)), 1.69-1.87 (m, 5H, CH\(_2\)), 2.36-2.51 (m, 1H, CH), 2.42 (s, 3H, CH\(_3\)), 3.38 (d, \(J = 3.7\) Hz, 2H, CH\(_2\)), 4.68 (s, 2H, CH\(_2\)), 4.86 (s, 2H, CH\(_2\)), 5.34 (s, 2H, CH\(_2\)), 5.59 (t, \(J = 4.6\) Hz, 1H, NH), 6.34 (s, 1H, CH), 6.48 (d, \(J = 8.0\) Hz, 1H, CH), 6.91 (d, \(J = 8.0\) Hz, 2H, CH), 7.07 (d, \(J = 8.0\) Hz, 2H, CH), 7.26 (d, \(J = 8.0\) Hz, 2H, CH), 7.30-7.42 (m, 10, CH), 7.66 (d, \(J = 8.4\) Hz, 2H, CH), 7.80 (d, \(J = 8.2\) Hz, 1H, CH); \(\delta_C\) (100 MHz, \(d\)-CDCl\(_3\)) 21.5, 25.9, 26.7, 34.3, 44.0, 44.1, 52.9, 67.0, 70.6, 113.7, 119.8, 121.2, 126.9, 126.9, 127.2, 128.0, 128.1, 128.2, 128.4, 128.5, 128.8, 129.5, 133.2, 133.2, 135.4, 135.5, 136.0, 143.4, 143.7, 147.8, 158.7, 165.2, 166.7.

2.32, benzyl 2-(benzyloxy)-4-(2-(N-(tert-butoxycarbonyl)-4-methylphenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)benzoate. Compound 2.30 was Boc protected with (Boc)$_2$ via General Procedure C on a 0.10 mmol scale to furnish 2.32 (71 mgs, 89%); \(\delta_H\) (400 MHz, \(d\)-CDCl\(_3\)) 1.30 (s, 9H, 3CH\(_3\)), 1.31-1.43 (m, 5H, CH\(_2\)), 1.68-1.86 (m, 5H, CH\(_2\)), 2.42-2.52 (m, 4H, CH\(_3\) and CH), 4.33 (s, 2H, CH\(_2\)), 4.85 (s, 2H, CH\(_2\)), 4.93 (s, 2H, CH\(_2\)), 5.34 (s, 2H, CH\(_2\)), 6.66 (s, 1H, CH), 6.78 (d, \(J = 8.8\) Hz, 1H, CH), 7.07-7.13 (m, 4H, CH), 7.29-7.40 (m, 12H, CH), 7.84 (d, \(J = 8.8\) Hz, 1H, CH), 8.02 (d, \(J = 8.4\) Hz, 2H, CH); \(\delta_C\) (100 MHz, \(d\)-CDCl\(_3\)) 21.5, 26.0, 26.7, 27.6, 34.3, 44.1, 47.2, 52.8, 66.9, 70.6, 84.4, 114.0, 120.1, 120.7, 126.8, 127.1, 127.9, 128.0,
128.1, 128.4, 128.5, 128.6, 128.7 (br), 128.9, 133.0, 133.9, 135.5, 135.6, 136.8, 144.0, 145.0, 147.5, 150.5, 158.6, 165.3, 166.5.

2.34, benzyl 4-(2-acetoxy-N-(4-cyclohexylbenzyl)acetamido)-2-(benzyloxy)benzoate.

Secondary aniline 2.10h was coupled to 2-chloro-2-oxoethyl acetate on a 0.19 mmol scale via General Procedure B to furnish 2.34 (184 mg, 72 %): \( \delta_H(400 \text{ MHz}, d\text{-CDCl}_3) \) 1.31-1.42 (m, 5H, CH\(_2\)), 1.68-1.84 (m, 5H, CH\(_2\)), 2.40-2.50 (m, 1H, CH), 4.28 (s, 2H, CH\(_2\)), 4.79 (s, 2H, CH\(_2\)), 4.91 (s, 2H, CH\(_2\)), 5.27 (s, 3H, CH\(_3\)), 5.32 (s, 2H, CH\(_2\)), 6.56 (s, 1H, CH), 6.71 (d, \( J = 8.4 \text{ Hz}, 1H, \text{CH} \)), 7.05 (d, \( J = 8.0 \text{ Hz}, 2H, \text{CH} \)), 7.10 (d, \( J = 8.0 \text{ Hz}, 2H, \text{CH} \)), 7.28-7.39 (m, 10H, CH), 7.81 (d, \( J = 8.4 \text{ Hz}, 1H, \text{CH} \)); LRMS Calcd for C\(_{38}\)H\(_{39}\)NO\(_6\) + H = 606.29, found 606.56.
2.35, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(tosyloxy)acetamido)-benzoate. To a stirred solution of 2.37 (54 mgs, 0.09 mmol) and DIPEA (25 µL, 1.44 mmol), was added TsCl (20 mgs, 0.10 mmol) and allowed to stir overnight at rt. The reaction was diluted with CH$_2$Cl$_2$, washed with 0.1 M HCl, water, brine and dried over Na$_2$SO$_4$, filtered and concentrated under reduced pressure. The crude product was purified using silica gel chromatography (hexanes:EtOAc, 1:1) to yield pure 52 (52 mg, 76 %): $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.30-1.41 (m, 5H, CH$_2$), 1.70-1.86 (m, 5H, CH$_2$), 2.39 (s, 3H, CH$_3$), 2.40-2.50 (m, 1H, CH), 4.33 (s, 2H, CH$_2$), 4.76 (s, 2H, CH$_2$), 4.89 (s, 2H, CH$_2$), 5.34 (s, 2H, CH$_2$), 6.46 (s, 1H, CH), 6.61 (d, $J = 8.0$ Hz, 1H, CH), 7.01 (d, $J = 8.0$ Hz, 2H, CH), 7.09 (d, $J = 8.0$ Hz, 2H, CH), 7.27-7.43 (m, 12H, CH), 7.73 (d, $J = 8.0$ Hz, 2H, CH), 7.79 (d, $J = 8.0$ Hz, 1H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 21.5, 25.9, 26.7, 34.3, 44.1, 52.8, 65.3, 66.9, 70.6, 113.8, 119.7, 120.9, 126.9, 127.0, 127.9, 128.0, 128.1, 128.2, 128.4, 128.5, 128.9, 129.6, 132.6, 133.1, 133.3, 135.5, 135.6, 144.0, 144.9, 147.8, 158.6, 163.9, 165.2

\[
\begin{align*}
\text{2.35, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(tosyloxy)acetamido)-benzoate.}
\end{align*}
\]

Compound 2.34 (0.19 mmol) was dissolved in a THF:H$_2$O (3:1) solution and treated with LiOH.H$_2$O (17 mg, 0.39 mmol). After 30 mins the reaction was completed and diluted with H$_2$O. The product was extracted into EtOAc and the combined extracts washed with 50 % sat. NaHCO$_3$, water, brine and dried over Na$_2$SO$_4$ and concentrated under reduced pressure to yield 2.37 (60 mg, 55 %): $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.33-1.47 (m, 5H, CH$_2$), 1.59-1.87 (m, 5H, CH$_2$), 2.42-2.52 (m, 1H, CH), 3.71 (s, 2H, CH$_2$), 4.82 (s, 2H, CH$_2$), 4.90 (s, 2H, CH$_2$), 5.33 (s, 2H, CH$_2$), 6.43 (s, 1H, CH), 6.64 (d, $J = 7.6$ Hz, 1H, CH), 7.05 (d, $J = 8.0$ Hz, 2H, CH), 7.11 (d, $J =
8.0 Hz, 2H, CH), 7.27-7.40 (m, 10H, CH), 7.81 (d, J = 7.6 Hz, 1H, CH); δC (100 MHz, d-CDCl3) 25.9, 26.7, 34.3, 44.0, 52.9, 60.3, 66.9, 70.6, 113.9, 119.9, 121.0, 126.9, 128.0, 128.1, 128.2, 128.4, 128.5, 128.9, 133.0, 133.4, 135.4, 135.5, 143.5, 147.8, 158.5, 165.2, 171.3.

1.9 Characterization of Final Compounds

![Structure image]

1.6, 2-Hydroxy-4-(2-(tosyloxy)acetamido)benzoic acid (S31-201). δH (400 MHz, d6-DMSO) 2.39 (s, 2H, CH3), 4.70 (s, 2H, COCH2), 6.96 (d of d, J = 8.6 and 2.0 Hz, 1H, CH), 7.21 (d, J = 2.0 Hz, 1H, CH), 7.47 (d, J = 8.0 Hz, 2H, 2 CH), 7.71 (d, J = 8.6 Hz, 1H, CH), 7.83 (d, J = 8.3 Hz, 2H, 2 CH), 10.35 (s (br), 1H, OH); δC (100 MHz, d6-DMSO) 21.0, 67.2, 106.2, 108.6, 110.1, 127.7, 130.1, 131.0, 131.9, 144.0, 145.2, 161.9, 163.7, 171.4; HRMS (ES+) calcd for [C16H16NO7S + H] 366.0660, found 366.0642; HPLC (I) tR = 19.09 min (98.94 %), (II) tR = 36.92 min (98.98 %).

![Structure image]

2.1, 2-hydroxy-4-(2-(4-methylphenylsulfonylamido)acetamido)benzoic acid δH (400 MHz, d6-DMSO) 2.33 (s, 3H, CH3), 4.03 (d, J = 6.0 Hz, 2H, CH2), 7.04(d of d, J = 8.4 and 2.4 Hz, 1H, CH), 7.26 (d, J = 8.0 Hz, 2H, CH2), 7.32 (d, J = 2.4 Hz, 1H, CH), 7.69 (d, J = 8.4 Hz, 1H, CH), 7.77 (d, J = 8.0 Hz, 2H, CH), 8.76 (t, J = 6.0 Hz, 1H, NH); HRMS (ES+) calcd for [C17H16N2O6S + H] 365.0807, found 365.0811; HPLC (I) tR = 16.84 min (100.0 %), (II) tR = 30.53 min (100.0 %).
2.2, 4-(2-(N,4-Dimethylphenylsulfonamido)acetamido)-2-hydroxy-benzoic acid. $\delta_{\text{H}}$ (400 MHz, d6-DMSO) 2.40 (s, 3H, CH$_3$), 2.79 (s, 3H, CH$_3$), 3.92 (s, 2H, CH$_2$CO), 7.00 (d of d, $J = 8.7$ and 2.0 Hz, 1H, CH), 7.27 (d, $J = 2.0$ Hz, 1H, CH), 7.43 (d, $J = 8.0$ Hz, 2H, 2 CH), 7.68 (d, $J = 8.2$ Hz, 2H, 2 CH), 7.71 (d, $J = 8.6$ Hz, 1H, CH), 10.20 (s (br), 1H, OH), 11.50 (s (br), 1H, OH); HRMS (ES+) calcd for [C$_{17}$H$_{19}$N$_2$O$_6$S + H] 379.0963, found 379.0964; HPLC (I) $t_R = 18.85$ min (99.13 %), (II) $t_R = 36.33$ min (100 %).

2.3, 4-(2-(N-(tert-butoxycarbonyl)-4-methylphenylsulfonamido) acetamido)-2-hydroxy benzoic acid. $\delta_{\text{H}}$ (400 MHz, d6-DMSO) 2.43 (s, 9H, CH$_3$), 4.58 (s, 2H, CH$_2$), 7.03 (d, $J = 8.4$ Hz, 1H, CH), 7.28 (s, 1H, CH), 7.47 (d, $J = 8.0$ Hz, 2H, CH), 7.73 (d, $J = 8.4$ Hz, 2H, CH), 7.94 (d, $J = 8.4$ Hz, 2H, CH), 10.48 (s, 1H, COOH); $\delta_{\text{C}}$ (100 MHz, d6- DMSO) 23.5, 27.1, 47.5, 84.8, 106.6, 108.3, 110.3, 128.3, 128.5, 128.9, 131.1, 136.4, 144.5, 150.5, 162.4, 166.7, 171.6; HRMS (ES+) calcd for [C$_{21}$H$_{24}$N$_2$O$_8$S + H] 465.1326, found 465.1307; HPLC (III) $t_R = 15.57$ min (68.82 %), (IV) $t_R = 24.49$ min (70.38 %).
2.4, 4-(N-Benzyl-2-(tosyloxy)acetamido)-2-hydroxybenzoic acid. \(\delta_H\) (400 MHz, \(d_6\)-DMSO)
2.40 (s, 3H, \(CH_3\)), 4.59 (s, 2H, \(CH_2O\)), 4.82 (s, 2H, \(CH_2\)), 6.64 (d of d, \(J = 8.2\) and 1.6 Hz, 1H, CH), 6.74 (d, \(J = 1.6\) Hz, 1H, CH), 7.13 (d, \(J = 7.0\) Hz, 2H, 2 CH), 7.20-7.30 (m, 3H, 3 CH), 7.42 (d, \(J = 8.0\) Hz, 2H, 2 CH), 7.69 (d, \(J = 8.2\) Hz, 2H ); \(\delta_C\) (100 MHz, \(d_6\)-DMSO) 21.4, 52.0, 66.4, 111.8, 116.9, 118.0, 127.6, 127.9, 128.0, 128.3, 128.6, 130.3, 131.6, 132.2, 136.8, 145.5, 162.2, 164.2, 171.3; HRMS (ES+) calcd for \([C_{23}H_{22}NO_7S + H]\) 456.1125, found 456.1111; HPLC (I) \(t_R = 21.02\) min (97.43 %), (II) \(t_R = 42.65\) min (97.93 %).

2.5, 4-(N-Benzyl-2-(4-methylphenylsulfonamido)acetamido)-2-hydroxybenzoic acid. \(\delta_H\) (400 MHz, \(d_6\)-DMSO) 2.37 (s, 3H, \(CH_3\)), 3.55 (d, \(J = 5.5\) Hz, 2H, \(CH_2NH\)), 4.78 (s, 2H, \(CH_2\)), 6.67 (d of d, \(J = 8.2\) and 2.0 Hz, 1H, CH), 6.76 (d, \(J = 2.0\) Hz, 1H ), 7.06-7.09 (m, 2H, 2 CH), 7.19-7.28 (m, 3H, 3 CH), 7.34 (d, \(J = 8.0\) Hz, 2H, 2 CH), 7.58 (d, \(J = 8.2\) Hz, 2H, 2 CH), 7.72 (d, \(J = 8.4\) Hz, 1H, CH), 7.88 (t, \(J = 5.9\) Hz, 1H, NH), 11.40 (s (br), 1H, OH); HRMS (ES+) calcd for \([C_{23}H_{23}N_2O_6S + H]\) 455.1284, found 455.1271; HPLC (I) \(t_R = 19.74\) min (97.32 %), (II) \(t_R = 39.35\) min (99.31 %).
2.6, 4-(N-Benzyl-2-(N,4-dimethylphenylsulfonamido)acetamido)-2-hydroxybenzoic acid. \( \delta_H \) (400 MHz, \( d_6 \)-DMSO) 2.37 (s, 3H, CH\(_3\)), 2.78 (s, 3H, CH\(_3\)), 3.86 (s, 2H, CH\(_2\)CO), 4.84 (s, 2H, CH\(_2\)), 6.77 (d of d, \( J = 8.4 \) and 2.0 Hz, 1H, CH), 6.86 (d, \( J = 2.0 \) Hz, 1H, CH), 7.16 (d, \( J = 8.4 \) Hz, 2H, 2 CH), 7.21-7.32 (m, 3H, 3 CH), 7.36 (d, \( J = 8.0 \) Hz, 2H, 2 CH), 7.53 (d, \( J = 8.4 \) Hz, 2H, 2 CH), 7.75 (d, \( J = 8.4 \) Hz, 1H, CH), 11.38 (s (br), 1H, OH); HRMS (ES+) calcd for \([C_{24}H_{24}N_{2}O_{6}S + H]\) 469.1431, found 469.1427; HPLC (I) \( t_R = 20.72 \) min (99.80 %), (II) \( t_R = 42.17 \) min (98.62 %).

2.7, 4-(N-Benzyl-2-(N-(tert-butoxycarbonyl)-4-methylphenyl-sulfonamido)acetamido)-2-hydroxy benzoic acid. \( \delta_H \) (400 MHz, CDCl\(_3\)) 1.31 (s, 9H, 3(CH\(_3\))), 2.41 (s, 3H, CH\(_3\)), 4.47 (s, 2H, COCH\(_2\)), 4.95 (s, 2H, CH\(_2\)), 6.69 (d, \( J = 8.4 \) Hz, 1H, CH), 6.81 (s, 1H, CH), 7.20-7.32 (m, 7H, CH), 7.87 (d, \( J = 8.4 \) Hz, 1H, CH), 8.00 (d, \( J = 8.2 \) Hz, 2H, 2 CH), 10.68 (s (br), 1H, OH); \( \delta_C \) (100 MHz, CDCl\(_3\)) 21.6, 27.7, 47.4, 53.3, 84.8, 111.6, 117.2, 119.2, 127.7, 128.4, 128.5, 128.7, 129.0, 132.3, 136.2, 136.6, 144.2, 147.7, 150.6, 162.9, 166.8, 172.6; HRMS (ES+) calcd for \([C_{28}H_{31}N_{2}O_{8}S + H]\) 557.1615, found 577.1615; HPLC (I) \( t_R = 21.67 \) min (99.02 %), (II) \( t_R = 46.05 \) min (98.14 %).
2.18a, 4-(N-(4-Bromobenzyl)-2-(N,4-dimethylphenylsulfonamido) acetamido)-2-hydroxy benzoic acid. $\delta_H$ (400 MHz, CDCl3) 2.37 (s, 3H, CH₃), 2.81 (s, 3H, CH₃), 3.75 (s, 2H, CH₂CO), 4.73 (s, 2H, CH₂), 6.49 (d, $J = 7.2$ Hz, 1H, CH), 6.61 (s, 1H, CH), 6.99 (d, $J = 8.2$ Hz, 2H, 2 CH), 7.22 (d, $J = 8.0$ Hz, 2H, 2 CH), 7.35 (d, $J = 8.2$ Hz, 2H, 2 CH), 7.58 (d, $J = 8.2$ Hz, 2H, 2 CH), 7.83 (d, $J = 7.6$ Hz, 1H, CH), 11.40 (s (br), 1H, OH); HRMS (ES+) calcd for $[C_{24}H_{24}BrN_{2}O_6S + H]$ 547.0545, found 547.0532; HPLC (I) $t_R = 20.28$ min (98.2 %), (II) $t_R = 42.13$ min (97.3 %).

2.18b, 4-(N-(3-Bromobenzyl)-2-(N,4-dimethylphenylsulfonamido) acetamido)-2-hydroxy benzoic acid. $\delta_H$ (400 MHz, CDCl3) 2.40 (s, 3H, CH₃), 2.86 (s, 3H, CH₃), 3.86 (s, 2H, CH₂CO), 4.82 (s, 2H, CH₂), 6.61 (d, $J = 8.0$ Hz, 1H, CH), 6.72 (s, 1H, CH), 7.08 (d, $J = 7.7$ Hz, 1H, CH), 7.15 (t, $J = 7.7$ Hz, 1H, CH), 7.27-7.36 (m, 3H, 3 CH), 7.39 (d, $J = 7.7$ Hz, 1H, CH), 7.65 (d, $J = 8.2$ Hz, 2H, 2 CH), 7.91 (d, $J = 8.2$ Hz, 1H, CH), 9.80 (s (br), 1H, OH), 10.68 (s (br), 1H, OH); $\delta_C$ (100 MHz, CDCl3) 21.5, 35.9, 51.6, 52.6, 111.8, 116.9, 119.0, 122.6, 127.1, 127.5, 129.5, 130.1, 131.0, 131.4, 132.5, 135.0, 138.4, 143.5, 147.4, 163.0, 167.3, 172.6; HRMS (ES+) calcd for $[C_{24}H_{24}BrN_{2}O_6S + H]$ 547.0542, found 547.0532; HPLC (I) $t_R = 20.08$ min (98.1 %), (II) $t_R = 41.53$ min (98.4 %).
2.18c, 4-(N-(4-Cyanobenzyl)-2-(N,4-dimethylphenylsulfonamido) acetamido)-2-hydroxy benzoic acid. δ\textsubscript{H} (400 MHz, CDCl\textsubscript{3}) 2.40 (s, 3H, CH\textsubscript{3}), 2.84 (s, 3H, CH\textsubscript{3}), 3.82 (s, 2H, CH\textsubscript{2}CO), 4.88 (s, 2H, CH\textsubscript{2}), 6.56 (d of d, J = 8.2 and 1.6 Hz, 1H, CH), 6.67 (d, J = 2.0 Hz, 1H, CH), 7.26 (d, J = 8.0 Hz, 2H, 2 CH), 7.29 (d, J = 8.0 Hz, 2H, 2 CH), 7.57 (d, J = 8.2 Hz, 2H, 2 CH), 7.62 (d, J = 8.2 Hz, 2H, 2 CH), 7.88 (d, J = 8.2 Hz, 1H, CH), 11.20 (s (br), 1H, OH); δ\textsubscript{C} (100 MHz, CDCl\textsubscript{3}) 21.5, 36.0, 51.5, 52.9, 111.6, 112.7, 116.5, 118.5, 118.6, 127.4, 129.1, 129.5, 132.4(2), 135.0, 141.7, 143.5, 146.6, 164.9, 165.7, 167.4; HRMS (ES+) calcd for [C\textsubscript{25}H\textsubscript{24}N\textsubscript{3}O\textsubscript{6}S +H] \textsubscript{494.1391}, found 494.1386; HPLC (I) \textit{t}_R = 22.94 min (100 %), (II) \textit{t}_R = 47.70 min (96.6 %).

2.18d, 4-(N-(3-Cyanobenzyl)-2-(N,4-dimethylphenylsulfonamido) acetamido)-2-hydroxy benzoic acid. δ\textsubscript{H} (400 MHz, CDCl\textsubscript{3}) 2.39 (s, 3H, CH\textsubscript{3}), 2.85 (s, 3H, CH\textsubscript{3}), 3.84 (s, 2H, CH\textsubscript{2}CO), 4.86 (s, 2H, CH\textsubscript{2}), 6.58 (d, J = 8.0 and 1.6 Hz, 1H, CH), 6.68 (d, J = 2.0 Hz, 1H, CH), 7.27 (d, J = 8.0 Hz, 2H, 2 CH), 7.38-7.48 (m, 3H, 3 CH), 7.56 (d, J = 7.3 Hz, 1H, CH), 7.63 (d, J = 8.2 Hz, 2H, 2 CH), 7.92 (d, J = 8.2 Hz, 1H, CH), 11.02 (s (br), 1H, OH); δ\textsubscript{C} (100 MHz, CDCl\textsubscript{3}) 21.3, 35.9, 51.5, 52.5, 112.5, 112.8, 116.6, 118.3, 118.5, 127.4, 129.4, 129.5, 131.5, 132.0, 132.5, 133.0, 135.0, 137.9, 143.5, 146.5, 163.0, 167.5, 171.6; HRMS (ES+) calcd for [C\textsubscript{25}H\textsubscript{24}N\textsubscript{3}O\textsubscript{6}S +H] 494.1384, found 494.1380; HPLC (I) \textit{t}_R = 19.98 min (98.26 %), (II) \textit{t}_R = 40.60 min (98.40 %).
2.18e, 4-(N-(cyclohexylmethyl)-2-(N,4-dimethylphenylsulfonamido) acetamido)-2-hydroxy benzoic acid. $\delta_H$ (400 MHz, $d_6$-DMSO) 1.02-1.11 (m, 3H, CH$_2$), 1.19-2.26 (m, 2H, CH$_2$), 1.53-1.64 (m, 6H, CH$_2$ and CH), 2.35 (s, 3H, CH$_3$), 2.71 (s, 3H, CH$_3$), 2.45 (d, $J$ = 7.2 Hz, 2H, CH$_2$), 3.74 (s, 2H, CH$_2$0), 5.73 (s, 2H, CH$_2$0, 6.83 (d, $J$ = 8.4 Hz 1H, CH), 6.91 (s, 1H, CH), 7.33 (d, $J$ = 8.0 Hz, 2H, CH), 7.52 (d, $J$ = 8.4 Hz, 2H, CH), 7.82 (d, $J$ = 8.4 Hz, 1H, CH); $\delta_C$ (100 MHz, $d_6$-DMSO) 20.9, 25.2, 25.9, 30.0, 35.6, 35.8, 50.9, 54.1, 112.6, 116.2, 118.7, 126.9, 129.6, 131.5, 135.1, 143.0, 147.4, 161.8, 166.3, 171.2; HRMS (ES+) calcd for [C$_{24}$H$_{30}$N$_2$O$_6$S + H] 475.1897, found 475.1905; HPLC (III) $t_R$ = 18.62 min (90.58 %), (IV) $t_R$ = 40.70 min (90.17 %).

2.18f, 4-(N-(4-tert-Butylbenzyl)-2-(N,4-dimethylphenylsulfonamido) acetamido)-2-hydroxy benzoic acid. $\delta_H$ (400 MHz, CDCl$_3$) 1.29 (s, 9H, 3(CH$_3$)), 2.39 (s, 3H, CH$_3$), 2.87 (s, 3H, CH3), 3.87 (s, 2H, COCH$_2$), 4.84 (s, 2H, CH$_2$), 6.63 (d of d, $J$ = 8.2 and 1.6 Hz, 1H, CH), 6.72 (d, $J$ = 2.0 Hz, 1H, CH), 7.08 (d, $J$ = 8.2 Hz, 2H, 2 CH), 7.25-7.31 (m, 4H, 4 CH), 7.65 (d, $J$ = 8.4 Hz, 2H, 2 CH), 7.89 (d, $J$ = 8.4 Hz, 1H, CH), 10.70 (s (br), 1H, OH); $\delta_C$ (100 MHz, CDCl$_3$) 21.5, 31.2, 34.5, 35.9, 51.6, 53.0, 111.6, 116.9, 119.1, 125.4, 127.5, 128.1, 129.5, 132.3, 133.0, 135.0,
143.5, 147.8, 150.7, 162.9, 167.2, 172.4; HRMS (ES+) calcd for \([C_{28}H_{33}N_2O_6S + H] \) 525.2033, found 525.2053; HPLC (I) \( t_R = 22.38 \text{ min} \) (98.2 %), (II) \( t_R = 47.7 \text{ min} \) (99.3 %).

2.18g, 4-(N-(Biphenyl-4-ylmethyl)-2-(N,4-dimethylphenylsulfon-amido)acetamido)-2-hydroxy benzoic acid. \( \delta_H \) (400 MHz, \( d_6\)-DMSO) 2.36 (s, 3H, CH\(_3\)), 2.80 (s, 3H, CH\(_3\)), 3.83 (s, 2H, COCH\(_2\)), 4.82 (s, 2H, CH\(_2\)), 6.52 (d, \( J = 8.0 \text{ Hz}, 1H, \text{ CH} \)), 6.59 (s, 1H, CH), 7.25 (d, \( J = 8.4 \text{ Hz}, 2H, \text{ 2 CH} \)), 7.32-7.37 (m, 3H, 3 CH), 7.44 (t, \( J = 7.3 \text{ Hz}, 2H, \text{ 2 CH} \)), 7.55 (d, \( J = 8.3 \text{ Hz}, 2H, \text{ 2 CH} \)), 7.59 (d, \( J = 8.3 \text{ Hz}, 2H, \text{ 2 CH} \)), 7.60-7.68 (m, 3H, 3 CH); \( \delta_C \) (100 MHz, \( d_6\)-DMSO) 20.4, 35.3, 50.9, 52.2, 112.6, 116.0, 118.1, 126.3, 126.5, 126.7, 126.8, 128.3, 129.0, 131.6, 134.3, 134.8, 139.9, 140.0, 143.2, 146.0, 162.0, 166.9, 170.8; HRMS (ES+) calcd for \([C_{30}H_{29}N_2O_6S + H] \) 545.1729, found 545.1740; HPLC (I) \( t_R = 21.67 \text{ min} \) (99.02 %), (II) \( t_R = 46.05 \text{ min} \) (98.14 %).

2.18h, 4-(N-(4-Cyclohexylbenzyl)-2-(N,4-dimethylphenylsulfon-amido)acetamido)-2-hydroxy benzoic acid. \( \delta_H \) (400 MHz, \( d_6\)-DMSO) 1.14-1.40 (m, 5H, CH\(_2\)), 1.64-1.81 (m, 5H,
CH$_2$), 2.36 (s, 3H, CH$_3$), 2.44 (s (br), 1H, CH), 2.77 (s, 3H, NCH$_3$), 3.86 (s, 2H, COCH$_2$), 4.79 (s, 2H, CH$_2$), 6.79 (d, $J = 8.6$ Hz, 1H, CH), 6.86 (s (br), 1H, CH), 7.06 (d, $J = 7.8$ Hz, 2H, 2 CH), 7.13 (d, $J = 7.8$ Hz, 2H, 2 CH), 7.35 (d, $J = 8.0$ Hz, 2H, 2 CH), 7.54 (d, $J = 8.0$ Hz, 2H, 2 CH), 7.77 (d, $J = 8.3$ Hz, 1H, CH), 11.30 (s (br), 1H, OH); $\delta_C$ (100 MHz, $d_6$-DMSO) 21.2, 25.1(2), 26.6, 34.2, 36.1, 42.3, 43.6, 51.2, 51.9, 112.7, 116.3, 118.9, 126.9, 127.2, 127.8, 129.9, 131.6, 134.5, 135.3, 143.4, 146.8, 147.3, 161.8, 167.0, 171.5; HRMS (ES+) calcd for [C$_{30}$H$_{35}$N$_2$O$_6$S + H] 551.2223, found 551.2210; HPLC (I) $t_R = 24.35$ min (98.11 %), (II) $t_R = 52.80$ min (98.16 %).

2.18i, 4-(2-(N,4-dimethylphenylsulfonamido)-N-(naphthalen-2-yl-methyl)acetamido)-2-hydroxy benzoic acid. $\delta_H$ (400 MHz, $d_6$-DMSO) 2.33 (s, 3H, CH$_3$), 2.79 (s, 3H, CH$_3$), 3.89 (s, 2H, CH$_2$), 5.00 (s, 2H, CH$_2$), 6.79, (d, $J = 8.4$ Hz, 1H, CH), 6.90 (s, 1H, CH), 7.32 -7.38 (m, 3H, CH), 7.45-7.52 (m, 2H, CH), 7.54 (d, $J = 8.0$ Hz, 2H, CH), 7.65 (s, 1H, CH), 7.73 (d, $J = 8.0$ Hz, 1H, CH), 7.80-7.85 (m, 1H, CH), 7.85 (d, $J = 8.4$ Hz, 2H CH); $\delta_C$ (100 MHz, $d_6$-DMSO) 21.3, 36.2, 51.3, 52.3, 112.9, 116.5, 119.0, 126.2, 126.5, 126.6, 127.3, 127.8, 127.9, 128.4, 129.9, 131.7, 132.5, 133.1, 134.8, 135.4, 143.6, 147.2, 161.9, 167.2, 171.4; HRMS (ES+) calcd for [C$_{28}$H$_{27}$N$_2$O$_6$S + H] 519.1584, found 519.1608; HPLC (I) $t_R = 21.86$ min (99.53 %), (II) $t_R = 45.92$ min (99.38 %).
2.18ja, 4-(2-(N,4-dimethylphenylsulfonamido)-N-(piperidin-4-ylmethyl)acetamido)-2-hydroxybenzoic acid. δ_H (400 MHz, d6-DMSO) 1.20-1.37 (m, 2H, CH_2), 1.61-1.80 (m, 2H, CH_2), 2.32 (s, 3H, CH_3), 2.71-2.73 (m, 4H, CH_3 and CH), 2.77 (t, J = 12.0 Hz, 2H, CH_2), 3.24 (d, J = 10.4 Hz, 2H, CH_2), 3.48 (d, J = 6.8 Hz, 2H, CH_2), 3.71 (s, 2H, CH_2), 6.55 (d of d, J = 8.4 and 2.0 Hz, 1H, CH), 6.63 (d, J = 8.0 Hz, 2H, CH), 7.32 (d, J = 8.0 Hz, 1H, CH); δ_C (100 MHz, d-CDCl3) 21.3, 26.4, 32.2, 39.2, 43.0, 48.9, 51.0, 53.1, 115.6, 115.8, 120.2, 127.2, 129.9, 131.4, 131.4, 135.6, 143.4, 143.9, 164.3, 167.0, 171.3; HRMS (ES+) calcd for [C_{23}H_{29}N_{3}O_{6}S + H] 476.1849, found 476.1850; HPLC (I) t_R = 14.74 min (98.61 %), (II) t_R = 26.80 min (100 %).

2.18jb, 4-(N-((1-(tert-butoxycarbonyl)piperidin-4-yl)methyl)-2-(N,4-dimethylphenylsulfonamido) acetamido)-2-hydroxybenzoic acid. δ_H (400 MHz, d-CDCl3) 1.44 (s, 9H, 3 CH_3), 1.58-1.78 (m, 2H, CH_2), 2.39 (s, 3H, CH_3), 2.63-2.70 (m, 2H, CH_2), 2.82 (s, 4H, CH_3 and CH), 3.59 (s (br), 2H, CH_2), 3.81 (s, 2H, CH_2), 4.06 (s br, 2H, CH_2), 6.75 (d, J = 8.2 Hz, 1H, CH), 6.81 (s (br), 1H, CH), 7.26 (d, J = 8.2 Hz, 2H, 2 CH), 7.63 (d, J = 8.2 Hz, 2H, 2 CH), 7.98 (d, J = 8.3 Hz,
1H, CH); \( \delta_C \) (100 MHz, \( d\)-CDCl3) 21.4, 28.4, 29.6, 34.6, 35.9, 51.5, 54.8, 80.0, 112.2, 116.5, 118.5, 127.4, 129.5, 132.5, 135.1, 143.4, 147.8, 155.0, 163.0, 167.4, 171.9; HRMS (ES+) calcd for \([C_{28}H_{37}N_{3}O_{8}S + Na]\) 598.2193, found 598.2177; HPLC (I) \( t_R = 19.33 \) min (98.24 %), (II) \( t_R = 39.65 \) min (97.61 %).

\[
\begin{align*}
\text{CN} & \quad \text{N} \\
\text{N} & \quad \text{O} \\
\text{O} & \quad \text{N} \\
\text{S} & \quad \text{O} \\
\text{HO} & \quad \text{O} \\
\text{HO} & 
\end{align*}
\]

2.18jc, 4-(N-(1-(4-cyanophenyl)piperidin-4-yl)methyl)-2-(N,4-di-methylphenylsulfonamido) acetamido)-2-hydroxybenzoic acid. \( \delta_H \) (400 MHz, \( d\)-CDCl3) 1.25-1.41 (m, 2H, CH\(_2\)), 1.70-1.89 (m, 2H, CH\(_2\)), 2.39 (s, 3H, CH\(_3\)), 2.80-2.90 (m, 6H, CH, CH\(_2\) and CH\(_3\)), 3.65 (d, \( J = 6.7 \) Hz, 2H, CH\(_2\)CH), 3.59-3.91 (m, 4H, CH\(_2\)), 6.77-6.90 (m, 4H, 4 CH), 7.27 (d, \( J = 8.6 \) Hz, 2H, 2 CH), 7.45 (d, \( J = 8.6 \) Hz, 2H, 2 CH), 7.63 (d, \( J = 8.6 \) Hz, 2H, 2 CH), 7.98 (d, \( J = 8.2 \) Hz, 1H, CH); \( \delta_C \) (100 MHz, \( d\)-CDCl3) 21.5, 28.9, 34.5, 36.0, 47.3, 51.7, 54.7, 99.3, 111.9, 114.3, 116.7, 118.7, 120.0, 127.4, 129.5, 132.6, 133.5, 134.9, 143.5, 148.0, 153.0, 163.1, 167.6, 172.2; HRMS (ES+) calcd for \([C_{30}H_{32}N_{3}O_{6}S + H]\) 577.2115, found 477.2093; HPLC (I) \( t_R = 20.91 \) min (98.25 %), (II) \( t_R = 43.52 \) min (98.91 %).
2.18jd, 4-(2-(N,4-dimethylphenylsulfonamido)-N-((1-(pyrimidin-2-yl)piperidin-4-yl)methyl)acetamido)-2-hydroxybenzoic acid: $\delta_H$ (400 MHz, $d_6$-DMSO) 1.60-1.73 (m, 3H, CH$_2$), 2.34 (s, 3H, CH$_3$), 2.46-2.48 (m, 2H, CH$_2$), 2.74 (s, 3H, CH$_3$), 2.80 (t, $J$ = 12.0 Hz, 1H, CH$_2$), 3.52 (d, $J$ = 7.0 Hz, 2H, CH$_2$CH), 3.79 (s, 2H, CH$_2$), 4.57 (d, $J$ = 13.0 Hz, 2H, CH$_2$), 6.57 (t, $J$ = 4.7 Hz, 1H, CH), 6.93 (d of d, $J$ = 8.4 and 2.0 Hz, 1H, CH), 7.02 (d, $J$ = 2.0 Hz, 1H, CH), 7.35 (d, $J$ = 8.4 Hz, 2H, 2 CH), 7.55 (d, $J$ = 8.2 Hz, 2H, 2 CH), 7.86 (d, $J$ = 8.4 Hz, 1H, CH), 8.32 (d, $J$ = 4.7 Hz, 2H, 2 CH); $\delta_C$ (100 MHz, $d_6$-DMSO) 21.4, 29.6, 35.9, 44.1, 51.5, 53.3, 54.9, 109.3, 113.4, 116.3, 118.4, 127.4, 129.5, 132.5, 135.2, 143.4, 147.4, 157.6, 159.9, 163.0, 167.4, 172.3; HRMS (ES+) calcd for [C$_{27}$H$_{31}$N$_5$O$_6$S + H] 554.2067, found 554.2058; HPLC (I) $t_R$ = 23.04 min (100.00 %), (II) $t_R$ = 31.37 min (98.54 %).
2.18ka, 4-(2-(N,4-dimethylphenylsulfonamido)-N-(4-(1-(2,2,2-tri-fluoroacetyl) piperidin-4-yl)benzyl)acetamido)-2-hydroxybenzoic acid. \(\delta_H\) (400 MHz, \(d_6\)-DMSO) 1.61-1.75 (m, 2H, CH\(_2\)), 1.96-2.00 (m, 2H, CH\(_2\)), 2.40 (s, 3H, CH\(_3\)), 2.82-2.87 (m, 5H, CH\(_3\) and CH\(_2\)), 3.25 (t, \(J = 12.4\) Hz, 1H, CH\(_2\)), 3.84 (s, 2H, CH\(_2\)), 4.13 (d, \(J = 12.4\) Hz, 1H, CH\(_2\)), 4.69 (d, \(J = 13.2\) Hz, 1H, CH\(_2\)), 4.84 (s, 2H, CH\(_2\)), 6.61 (d, \(J = 8.4\) Hz, 1H, CH), 6.69 (s, 1H, CH), 7.09-7.15 (m, 4H, CH), 7.27 (d, \(J = 7.2\) Hz, 2H, CH), 7.64 (d, \(J = 8.4\) Hz, 2H, CH), 7.89 (d, \(J = 8.4\) Hz, 1H, CH); \(\delta_C\) (100 MHz, \(d\)-CDCl\(_3\)) 21.4, 32.4, 33.4, 35.8, 41.8, 44.1, 46.2, 51.5, 52.8, 53.3, 115.0, 116.8, 118.9, 126.8, 127.4, 128.8, 129.4, 132.2, 134.6, 134.8, 143.4, 143.7, 147.3, 155.2, 155.6, 162.8, 167.1, 172.0; HRMS (ES+) calcd for \([C_{31}H_{32}N_3O_7S + H]\) 648.1985, found 648.1974; HPLC (I) \(t_R = 21.52\) min (95.85 %), (II) \(t_R = 45.49\) min (97.12 %).

2.18kb, 4-(2-(N,4-dimethylphenylsulfonamido)-N-(piperidin-4-yl)benzyl)acetamido)-2-hydroxybenzoic acid. \(\delta_H\) (400 MHz, \(d_6\)-DMSO) 1.63-1.78 (m, 2H, CH\(_2\)), 1.86-2.40 (m, 2H, CH\(_2\)), 2.29-2.33 (m, 1H, CH), 2.34 (s, 3H, CH\(_3\)), 2.73-2.80 (m, 5H, CH\(_3\) and CH\(_2\)), 2.90-3.02 (m, 2H, CH\(_2\)), 3.81 (s, 2H, CH\(_2\)), 4.77 (s, 2H, CH\(_2\)), 6.63 (d, \(J = 7.2\) Hz, 1H, CH), 6.69 (s, 1H, CH), 7.12 (s (br), 4H, CH), 7.33 (d, \(J = 8.0\) Hz, 2H, CH), 7.51 (d, \(J = 8.0\) Hz, 2H, CH), 7.70 (d, \(J = 8.0\) Hz, 1H, CH); HRMS (ES+) calcd for \([C_{29}H_{33}N_3O_6S + H]\) 552.2162, found 552.2149; HPLC (III) \(t_R = 17.99\) min (71.92 %), (IV) \(t_R = 35.58\) min (71.04 %).
2.18kc, 4-(N-(4-(1-tert-butoxycarbonyl)piperidin-4-yl)benzyl)-2-(N,4-dimethylphenylsulfon-amido)acetamido)-2-hydroxybenzoic acid. $\delta_H$ (400 MHz, $d_6$-DMSO)

1.38-1.39 (m, 2H, CH$_2$), 1.39 (s, 9H, CH$_3$), 1.69 (s, 2H, CH$_2$), 1.72 (s, 1H, CH$_2$), 2.36 (s (br), 2H, CH$_2$), 2.75-2.79 (m, 5H, CH$_3$ and CH$_2$), 4.05 (s, 2H, CH$_2$), 4.80 (s, 2H, CH$_2$), 6.79 (d of d, $J = 8.4$ and 2.0 Hz, 1H, CH), 6.87 (d, $J = 2.0$ Hz, 1H, CH), 7.08 (d, $J = 8.0$ Hz, 2H, CH), 7.15 (d, $J = 8.4$ Hz, 2H, CH), 7.35 (d, $J = 8.0$ Hz, 2H, CH), 7.54 (d, $J = 8.4$ Hz, 2H, CH), 7.77 (d, $J = 8.4$ Hz, 1H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$), 21.4, 28.3, 29.5, 29.8, 30.2, 32.9, 35.8, 42.1, 44.3, 51.4, 52.8, 79.9, 114.2, 116.6, 118.7, 126.8, 127.4, 128.6, 129.4, 132.1, 134.2, 135.1, 143.3, 145.0, 146.8, 155.0, 162.7, 166.9, 171.9; HRMS (ES+) calcd for $[C_{34}H_{41}N_7O_8S + H]$ 652.2687, found 652.2658; HPLC (I) $t_R = 23.65$ min (66.51 %), (II) $t_R = 50.00$ min (74.40 %).
2.18kd, 4-(N-(4-(1-(4-cyanophenyl)piperidin-4-yl)benzyl)-2-(N,4-di-methylphenylsulfonamido) acetamido)-2-hydroxybenzoic acid. $\delta_H$ (400 MHz, $d_6$-DMSO) 1.56-1.68 (m, 2H, CH$_2$), 1.80-1.83 (m, 2H, CH$_2$), 2.36 (s, 3H, CH$_3$), 2.74-2.75 (m, 1H, CH), 2.52-2.53 (m, 2H, CH$_2$), 2.77 (s, 3H, CH$_3$), 2.88-2.98 (m, 2H, CH$_2$), 3.86 (s, 2H, CH$_2$), 4.80 (s, 2H, CH$_2$), 6.79 (d of d, $J = 8.0$ and 2.0 Hz, 1H, CH), 6.87 (d, $J = 2.0$ Hz, 1H, CH), 7.03 (d, $J = 7.2$ Hz, 2H, CH), 7.09 (d, $J = 8.0$ Hz, 2H, CH), 7.17 (d, $J = 8.0$ Hz, 2H, CH), 7.35 (d, $J = 8.0$ Hz, 2H, CH), 7.53-7.56 (m, 4H, CH), 7.77 (d, $J = 8.4$ Hz, 1H, CH); $\delta_C$ (100 MHz, $d$-CDC13) 21.4, 29.5, 32.4, 35.8, 41.9, 48.2, 51.5, 52.9, 99.4, 114.3, 116.8, 118.9, 126.8, 127.4, 128.6, 129.4, 133.4, 134.3, 134.9, 143.4, 144.8, 147.4, 153.2, 162.8, 165.6, 171.8 ; LRMS (ES+) calcd for [C$_{36}$H$_{36}$N$_4$O$_6$S + H] 653.24, found 653.46 [M+H]; HPLC (I) $t_R = 23.32$ min (99.22 %), (II) $t_R = 49.33$ min (99.66 %).
2.18ke, 4-(2-(N,4-dimethylphenylsulfonamido)-N-(4-(1-(pyrimidin-2-yl)piperidin-4-yl)benzyl)-acetamido)-2-hydroxybenzoic acid. \( \delta_H \) (400 MHz, \( d \)-CDCl\(_3\)) 1.65-1.67 (m, 2H, \( CH_2 \)), 1.91-2.03 (m, 2H, \( CH_2 \)), 2.30-2.32 (m, 1H, \( CH \)), 2.39 (s, 3H, \( CH_3 \)), 2.76-2.79 (m, 2H, \( CH_2 \)), 2.85 (s, 3H, \( CH_3 \)), 2.97-3.05 (m, 2H, \( CH_2 \)), 3.83 (s, 2H, \( CH_2 \)), 4.80 (s, 2H, \( CH_2 \)), 6.48-6.59 (m, 2H, \( CH \)), 6.68 (s, 1H, \( CH \)), 7.04-7.14 (m, 4H, \( CH \)), 7.28 (d, \( J = 7.2 \) Hz, 2H, \( CH \)), 7.65 (d, \( J = 7.2 \) Hz, 2H, \( CH \)), 7.85 (s, 1H, \( CH \)), 8.39-8.50 (m, 3H, \( CH \)); \( \delta_C \) (100 MHz, \( d \)-CDCl\(_3\)) 21.5, 32.7, 35.9, 41.9, 45.8, 51.8, 53.1, 109.0, 112.4, 116.8, 119.0, 122.8, 126.9, 127.6, 129.0, 129.5, 132.1, 134.8, 135.3, 137.6, 143.5, 144.0, 152.2, 157.0, 159.2, 161.5, 167.0, 171.5; HRMS (ES+) calcd for [C\(_{33}\)H\(_{35}\)N\(_5\)O\(_6\)S + H] 630.2380, found 630.2379; HPLC (I) \( t_R = 18.68 \) min (100 %), (II) \( t_R = 37.21 \) min (95.91 %).
2.18kf, 4-(N-(4-(1-(4-cyanobenzoyl)piperidin-4-yl)benzyl)-2-(N,4-di-methylphenylsulfonamido)acetamido)-2-hydroxybenzoic acid. δ$_H$ (400 MHz, $d$-CDCl$_3$) 1.60-2.03 (m, 4H, CH$_2$), 2.39 (s, 3H, CH$_3$), 2.73-2.96 (m, 5H, CH$_2$ and CH$_3$), 3.11-3.25 (m, 1H, CH), 3.72 (d, $J = 8.0$ Hz, 2H, CH$_2$), 3.82 (s, 2H, CH$_2$), 4.82 (s, 2H, CH$_2$), 6.58 (d, $J = 8.0$ Hz, 1H, CH), 7.11 (s br, 4H, CH), 7.26 (d, $J = 8.0$ Hz, 2H, CH), 7.55 (d, $J = 7.6$ Hz, 2H, CH), 7.64 (d, $J = 8.0$ Hz, 2H, CH), 7.73 (d, $J = 8.4$ Hz, 2H, CH), 7.85 (d, $J = 7.6$ Hz, 1H, CH); δ$_C$ (100 MHz, $d$-CDCl$_3$) 21.4, 29.5, 32.5, 33.7, 35.8, 42.0, 43.0, 48.3, 51.5, 52.8, 113.5, 116.8, 117.9, 118.8, 126.7, 127.4, 127.5, 128.7, 129.4, 132.1, 132.4, 134.7, 135.0, 139.9, 143.4, 147.2, 162.7, 167.0, 168.6, 170.2; HRMS (ES+) calcd for [C$_{37}$H$_{36}$N$_4$O$_7$S + H] 681.2377, found 681.2365; HPLC (I) $t_R = 20.49$ min (99.75 %), (II) $t_R = 42.05$ min (100 %).
2.18 kg, 4-(N-(4-(1-((4-cyanophenyl)sulfonyl)piperidin-4-yl)benzyl)-2-(N,4-dimethyl-phenylsulfonamido)acetamido)-2-hydroxy-benzoic acid. \( \delta_H \) (400 MHz, \( d_6 \)-DMSO) 2.00-2.24 (m, 4H, CH\(_2\)), 2.66-2.78 (m, 6H, CH\(_3\), CH and CH\(_2\)), 3.15 (s, 3H, CH\(_3\)), 4.13 (s, 2H, CH\(_2\)), 4.24-4.27 (m, 2H, CH\(_2\)), 5.12 (s, 2H, CH\(_2\)), 6.88 (d, \( J = 8.0 \) Hz, 1H, CH), 6.97 (s, 1H, CH), 7.44 (d, \( J = 8.0 \) Hz, 2H, CH), 7.94 (d, \( J = 8.0 \) Hz, 2H, CH), 8.15-8.24 (m, 5H, CH); \( \delta_C \) (100 MHz, \( d_6 \)-DMSO) 21.4, 32.3, 35.8, 37.0, 41.2, 46.6, 51.5, 52.8, 116.3, 116.7, 116.8, 117.1, 118.7, 126.7, 127.4, 128.1, 128.7, 129.4, 132.2, 132.8, 134.7, 135.0, 140.7, 143.4, 143.8, 147.0, 162.7, 167.0, 170.6; HRMS (ES+) calcd for [C\(_{36}\)H\(_{36}\)N\(_4\)O\(_8\)S + H] 717.2047, found 717.2036; HPLC (I) \( t_R = 22.01 \) min (94.07 %), (II) \( t_R = 46.38 \) min (100 %).
2.18kh, 4-(N-(4-(1-(4-carboxyphenyl)piperidin-4-yl)benzyl)-2-(N,4-
dimethylphenylsulfonamido) acetamido)-2-hydroxybenzoic acid. δ_H (400 MHz, d6-DMSO)
1.58-1.73 (m, 2H, CH_2), 1.82 (d, J = 12.8 Hz, 2H, CH_2), 2.35 (s, 3H, CH_3), 2.69-2.79 (m, 4H, CH_3 and CH), 2.90 (t, J = 11.2 Hz, 2H, CH_2), 3.84 (s, 2H, CH_2), 4.80 (s, 2H, CH_2), 6.79 (d, J = 8.4 Hz, 1H, CH), 6.87 (s, 1H, CH), 6.99 (d, J = 8.8 Hz, 2H, CH), 7.09 (d, J = 8.0 Hz, 2H, CH), 7.18 (d, J = 8.0 Hz, 2H, CH), 7.35 (d, J = 8.0 Hz, 2H, CH), 7.54 (d, J = 8.0 Hz, 2H, CH), 7.76 (d, J = 8.8 Hz, 2H, CH), 7.78 (d, J = 8.4 Hz, 1H, CH); δ_C (100 MHz, d-CDCl3) 21.3, 32.5, 36.2, 41.4, 48.1, 48.9, 51.3, 52.0, 113.9, 116.5, 119.0, 119.3, 121.8, 127.0, 127.3, 128.0, 129.9, 131.3, 131.7, 135.0, 135.4, 143.5, 145.1, 147.5, 153.9, 161.9, 167.0, 167.6, 171.5; HRMS (ES+) calcd for [C_{36}H_{37}N_{3}O_{8}S + H] 672.2374, found 672.2372; HPLC (III) t_R = 19.17 min (82.84 %), (IV) t_R = 39.60 min (90.47 %).
2.18ki, 4-((N-(4-((1-(4-carbamoylphenyl)piperidin-4-yl)benzyl)-2-(N,4-dimethylphenylsulfonamido)acetamido)-2-hydroxybenzoic acid. $\delta_H$ (400 MHz, $d_6$-DMSO)

1.66-1.80 (m, 2H, CH$_2$), 1.82-1.91 (m, 2H, CH$_2$), 2.33 (s, 3H, CH$_3$), 2.58-2.68 (m, 1H, CH), 2.77 (s, 3H, CH$_3$), 2.86 (t, $J = 12.0$ Hz, 2H, CH$_2$), 3.72 (s, 2H, CH$_2$), 3.89 (d, $J = 12.0$ Hz, 2H, CH$_2$), 4.75 (s, 2H, CH$_2$), 6.49 (d, $J = 7.2$ Hz, 1H, CH), 6.58 (s, 1H, CH), 6.87 (d, $J = 8.8$ Hz, 2H, CH), 7.00-7.09 (m, 4H, CH), 7.20 (d, $J = 8.0$ Hz, 1H, CH), 7.54 (d, $J = 8.0$ Hz, 1H, CH), 7.67 (d, $J = 8.4$ Hz, 2H, CH), 7.80 (d, $J = 8.0$ Hz, 1H, CH); $\delta_C$ (100 MHz, $d_6$- DMSO) 21.2, 29.4, 32.5, 35.7, 41.9, 51.3, 52.7, 114.1, 116.4, 118.6, 121.6, 126.7, 127.2, 128.5, 128.9, 129.4, 132.0, 134.2, 134.9, 143.4, 144.9, 146.5, 150.2, 153.6, 162.4, 166.8, 169.2, 171.3; HRMS (ES+) calcd for [C$_{36}$H$_{38}$N$_4$O$_7$S + H] 671.2533, found 671.2545; HPLC (III) $t_R = 17.72$ min (84.23 %), (IV) $t_R = 24.81$ min (74.07 %).
2.18la, 4-(2-(N,4-dimethylphenylsulfonamido)-N-((3'-(methoxy-carbonyl)biphenyl-4-yl)methyl)acetamido)-2-hydroxybenzoic acid. δ(H) (400 MHz, d-CDCl3) 2.36 (s, 3H, CH₃), 2.78 (s, 3H, CH₃), 3.87 (s, 3H, CH₃), 3.88 (s, 2H, CH₂), 4.90 (s, 2H, CH₂), 6.82 (d of d, J = 8.4 and 2.0 Hz, 1H, CH), 6.92 (d, J = 2.0 Hz, 1H, CH), 7.29 (d, J = 8.0 Hz, 2H, CH), 7.36 (d, J = 8.0 Hz, 2H, CH), 7.56 (d, J = 8.0 Hz, 2H, CH), 7.69 (t, J = 8.0 Hz, 1H, CH), 7.64 (d, J = 8.0 Hz, 2H, CH), 7.78 (d, J = 8.4 Hz, 1H, CH), 7.93 (d of d, J = 7.6 and 1.6 Hz, 2H, CH), 8.16 (t, J = 1.6 Hz, 1H, CH); δ(C) (100 MHz, d-CDCl3) 21.4, 35.9, 51.5, 52.1, 52.8, 116.6, 117.8, 118.7, 127.2, 127.5, 128.1, 128.3, 128.8, 129.1, 129.4, 130.5, 131.4, 132.2, 135.4, 136.0, 139.4, 140.8, 143.3, 146.7, 162.9, 163.0, 167.0, 171.8; HRMS (ES+) calcd for [C₃₂H₃₁N₂O₈S + H] 603.1800, found 603.1795; HPLC (I) tᵣ = 23.65 min (100.0 %), (II) tᵣ = 48.73 min (100.0 %).
2.18lb, 4-(N-((3'-cyanobiphenyl-4-yl)methyl)-2-(N,4-dimethylphenyl sulfonamido)acetamido)-2-hydroxybenzoic acid.

δ\text{H} (400 MHz, d-CDCl₃) 2.37 (s, 3H, CH₃), 2.80 (s, 3H, CH₃), 3.90 (s, 2H, CH₂), 4.91 (s, 2H, CH₂), 6.84 (d of d, J = 8.4 and 1.6 Hz, 1H, CH), 6.93 (s, 1H, CH), 7.30 (d, J = 8.0 Hz, 2H, CH), 7.37 (d, J = 8.0 Hz, 2H, CH), 7.57 (d, J = 8.0 Hz, 2H, CH), 7.65 (t, J = 8.0 Hz, 1H, CH), 7.70 (d, J = 8.0 Hz, 2H, CH), 7.80 (t, J = 8.0 Hz, 2H, CH), 8.02 (d, J = 8.0 Hz, 1H, CH), 8.15 (s, 1H, CH); δ\text{C} (100 MHz, d-CDCl₃) 20.8, 35.8, 50.8, 51.4, 112.0, 116.0, 118.4, 118.7, 120.0, 126.8, 126.9, 128.3, 128.5, 129.5, 130.0, 130.9, 131.2, 131.3, 135.0, 136.7, 137.1, 140.6, 143.0, 146.6, 161.6, 166.7, 171.0; HRMS (ES+) calcd for [C₃₁H₂₈N₃O₆S + H] 570.1696, found 570.1693; HPLC (I) t\text{R} = 22.84 min (98.3 %), (II) t\text{R} = 47.61 min (98.43 %).

2.18lc, 4-(N-((3'-carbamoylbiphenyl-4-yl)methyl)-2-(N,4-dimethylphenylsulfonamido)acetamido)-2-hydroxybenzoic acid. δ\text{H} (400 MHz, d6-DMSO) 2.37 (s, 3H, CH₃), 2.80 (s, 3H, CH₃), 3.90 (s, 2H, CH₂), 4.91 (s, 2H, CH₂), 6.84 (d of d, J = 8.4 Hz, J = 2.0 Hz, 1H, CH), 6.93 (d, J = 2Hz, 1H, CH), 7.29 (d, J = 8.0 Hz, 2H, CH), 7.37 (d, J = 8.4 Hz, 2H, CH), 7.42 (s, 1H, CH), 7.51-7.58 (m, 3H, CH), 7.77 (d, J = 8.0 Hz, 2H, CH), 7.78-7.85 (m, 3H, CH), 8.09 (s, 1H, CH), 8.14 (s, 1H, CH); δ\text{C} (100 MHz, d-CDCl₃) 20.6, 35.6, 50.55, 51.3, 114.1, 115.5, 117.1, 125.2, 126.3, 126.32, 126.6, 128.1, 128.8, 128.9, 129.3, 130.9, 134.6, 134.8, 136.3, 138.1, 139.3, 142.8, 156.9, 162.1, 166.5, 167.4, 170.7; HRMS (ES+) calcd for [C₃₁H₃₀N₃O₇S + H] 588.1794, found 588.1794; HPLC (I) t\text{R} = 17.05 min (100 %), (II) t\text{R} = 39.80 min (98.94 %).
2.18ld, 4-(2-\((N,4\text{-dimethylphenylsulfonyl})\)-N-\((4'-(\text{methoxy-carbonyl})\text{biphenyl-4-yl})\text{methyl}\) acetamido)-2-hydroxybenzoic acid. $\delta_H (400 \text{ MHz, } d\text{-CDCl}_3) 2.40 (s, 3H, CH\text{_3}), 2.87 (s, 3H, CH\text{_3}), 3.87 (s, 2H, CH\text{_2}), 3.94 (s, 3H, CH\text{_3}), 4.91 (s, 2H, CH\text{_2}), 6.64 (d, J = 7.6, 1H, CH), 6.74 (d, J = 1.2 Hz, 1H, CH), 7.25-7.28 (m, 3H, CH), 7.53 (d, J = 8.4 Hz, 2H, CH), 7.63 (d, J = 8.4 Hz, 2H, CH); $\delta_C (100 \text{ MHz, } d\text{-CDCl}_3) 21.5, 35.9, 51.6, 52.1, 52.9, 116.9, 117.0, 119.0 126.9, 127.4, 127.5, 128.9, 129.1, 129.5, 130.1, 132.4, 135.1, 136.3, 139.4, 143.5, 144.9, 147.5, 162.9, 167.0, 167.2, 171.2; \text{HRMS (ES+) calcd for } [C_{32}H_{31}N_{2}O_{8}S + H] 603.1816, \text{found 603.1795}; \text{HPLC (I) } t_R = 23.76 \text{ min (99.37 %), (II) } t_R = 48.78 \text{ min (100.0 %).}
2.18le, 4′-((N-(4-carboxy-3-hydroxyphenyl)-2-(N,4-dimethylphenyl sulfonamido) acetamido) methyl)-[1,1′-biphenyl]-4-carboxylic acid. $\delta_H$ (400 MHz, $d_6$-DMSO) 2.37 (s, 3H, CH$_3$), 2.80 (s, 3H, CH$_3$), 3.88 (s, 2H, CH$_2$), 4.89 (s, 2H, CH$_2$), 6.72 (d of d, $J = 8.4$ Hz and 1.6 Hz, 1H, CH), 6.81 (d, $J = 1.6$ Hz, 1H, CH), 7.30 (d, $J = 8.4$ Hz, 2H, CH), 7.37 (d, $J = 8.0$ Hz, 2H, CH), 7.56 (d, $J = 8.4$ Hz, 2H, CH), 7.68 (d, $J = 8.4$ Hz, 2H, CH), 7.75 (d, $J = 8.4$ Hz, 1H, CH), 7.79 (d, $J = 8.4$ Hz, 2H, CH), 8.00 (d, $J = 8.4$ Hz, 2H, CH); $\delta_C$ (100 MHz, $d_6$-DMSO) 20.8, 35.8, 50.8, 51.5, 115.8, 117.6, 118.1, 126.5, 126.8, 126.8, 128.3, 129.4, 129.5, 129.8, 131.1, 135.0, 137.1, 137.7, 143.0, 143.7, 145.8, 162.6, 167.0; HRMS (ES+) calcd for [C$_{31}$H$_{29}$N$_2$O$_8$S + H] 589.1628, found 589.1639; HPLC (I) $t_R = 20.29$ min (98.59 %), (II) $t_R = 41.50$ min (98.69 %).

\[ \text{CN} \]
\[ \text{HO} \]
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2.18lf, 4-(N-((4′-cyanobiphenyl-4-yl)methyl)-2-(N,4-dimethylphenyl-sulfonamido) acetamido)-2-hydroxybenzoic acid. $\delta_H$ (400 MHz, $d$-CDCl$_3$) 2.36 (s, 3H, CH$_3$), 2.80 (s, 3H, CH$_3$), 3.69 (s, 2H, CH$_2$), 4.91 (s, 2H, CH$_2$), 6.78 (d of d, $J = 8.4$ and 1.6 Hz, 1H, CH), 6.89 (d, $J = 2.0$ Hz, 1H, CH), 7.30 (d, $J = 8.0$ Hz, 2H, CH), 7.37 (d, $J = 8.0$ Hz, 2H, CH), 7.57 (d, $J = 8.0$ Hz, 2H, CH), 7.70 (d, $J = 8.0$ Hz, 2H, CH), 7.78 (d, $J = 8.0$ Hz, 1H, CH), 7.86-7.92 (m, 4H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 21.2, 36.2, 51.2, 51.8, 110.2, 118.6, 118.7, 119.1, 127.2, 127.3, 127.7, 128.8, 129.9, 131.6, 131.8, 132.0, 133.1, 135.4, 137.3, 138.0, 143.4, 144.4, 146.8, 162.1, 167.1, 167.2, 171.4; HRMS (ES+) calcd for [C$_{31}$H$_{28}$N$_3$O$_6$S + H] 570.1696, found 570.1693; HPLC (I) $t_R = 23.18$ min (100.0 %), (II) $t_R = 47.81$ min (98.78 %).
2.18lg 4-((4'-carbamoylbiphenyl-4-yl)methyl)-2-((N,4-dimethylphenylsulfonamido)acetamido)-2-hydroxybenzoic acid. δ<sub>H</sub> (400 MHz, <i>d<sub>6</sub>-DMSO) 2.37 (s, 3H, CH<sub>3</sub>), 2.79 (s, 3H, CH<sub>3</sub>), 3.89 (s, 2H, CH<sub>2</sub>), 4.90 (s, 2H, CH<sub>2</sub>), 6.83 (d of d, <i>J</i> = 8.4 Hz and 2.0 Hz, 1H, CH), 6.92 (d, <i>J</i> = 2Hz, 1H, CH), 7.28 (d, <i>J</i> = 7.6 Hz, 2H, CH), 7.37, d, <i>J</i> = 8.0 Hz, CH), 7.56 (d, <i>J</i> = 8.0 Hz, 2H, CH), 7.67 (d, <i>J</i> = 8.0 Hz, 2H, CH), 7.74 (d, <i>J</i> = 8.4 Hz, 2H, CH), 7.79 (d, <i>J</i> = 8.4 Hz, 1H, CH), 7.94 (d, <i>J</i> = 8.4 Hz, 2H, CH), 8.01 (s, 1H, OH); δ<sub>C</sub> (100 MHz, <i>d<sub>6</sub>-DMSO) 21.2, 36.2, 51.1, 52.0, 116.0, 117.2, 120.4, 126.6, 127.1, 127.2, 128.4, 128.7, 129.9, 131.4, 133.3, 135.5, 137.3, 138.3, 142.5, 143.4, 163.0, 167.1, 167.8, 171.2, 172.3; HRMS (ES+) calcd for [C<sub>31</sub>H<sub>30</sub>N<sub>7</sub>O<sub>7</sub>S + H] 588.1789, found 588.1798; HPLC (I) <i>t</i><sub>R</sub> = 19.49 min (91.83 %), (II) <i>t</i><sub>R</sub> = 40.09 min (98.42 %).
2.18na, 4-(2-(N,4-dimethylphenylsulfonamido)-N-((3'-(methoxy carbonyl)terphenyl-4-yl)methyl)-acetamido)-2-hydroxybenzoic acid. $\delta_H$ (400 MHz, $d_6$-DMSO) 2.36 (s, 3H, CH$_3$), 2.81 (s, 3H, CH$_3$), 4.90 (s, 2H, CH$_2$), 6.80 (d, 1H, $J = 8.4$ Hz, CH), 6.89 (s, 1H, CH), 7.29 (d, $J = 8.4$Hz, 2H, CH), 7.36 (d, $J = 8.0$ Hz, 2H, CH), 7.61-7.67 (m, 5H, CH), 7.76-7.79 (m, 4H, CH), 7.96 (d, $J = 7.6$ Hz, 1H, CH), 8.00 (d, $J = 8.0$ Hz, 1H, CH), 8.24 (s, 1H, CH), 8.32 (s, 1H, CH); $\delta_C$ (100 MHz, $d_6$-DMSO) 20.8, 35.8, 50.8, 51.5, 52.1, 115.9, 117.4, 118.1, 126.4, 126.8, 127.1, 127.2, 127.3, 127.8, 128.0, 128.3, 129.4, 129.5, 130.3, 131.2, 135.0, 136.3, 137.8, 138.1, 139.1, 139.9, 143.0, 146.3, 161.8, 166.0, 166.7, 171.0; HRMS (ES+) calcd for [C$_{37}$H$_{32}$N$_2$O$_8$S + H] 679.2108, Found 679.2080; HPLC (I) $t_R = 23.34$ min (96.76 %), (II) $t_R = 50.50$ min (98.76 %).
2.18nb, 4'-(N-(4-carboxy-3-hydroxyphenyl)-2-(N,4-dimethylphenyl sulfinamido)acetamido)methyl)terphenyl-3-carboxylic acid. \( \delta_H \) (400 MHz, \( d_6 \)-DMSO) 2.37 (s, 3H, CH\(_3\)), 2.80 (s, 3H, CH\(_3\)), 3.87 (s, 2H, CH\(_2\)), 4.87 (s, 2H, CH\(_2\)), 6.68 (d of d, \( J = 8.4 \) and 1.2 Hz, 1H, CH), 6.76 (d, \( J = 1.2 \) Hz, 1H, CH), 7.29 (d, \( J = 8.0 \) Hz, 2H, CH), 7.37 (d, \( J = 8.0 \) Hz, 2H, CH), 7.56 (d, \( J = 8.0 \) Hz, 2H, CH), 7.62 (t, \( J = 8.0 \) Hz, 2H, CH), 7.68 (d, \( J = 8.0 \) Hz, 1H, CH), 7.73 (d, \( J = 8.0 \) Hz, 1H, CH), 7.79 (s, 4H, CH), 7.94 (d, \( J = 8.0 \) Hz, 1H, CH) 7.98 (d, \( J = 8.0 \) Hz, 1H, CH), 8.23 (s, 1H, CH); \( \delta_C \) (100 MHz, \( d_6 \)-DMSO) 20.6, 35.8, 50.8, 51.5, 113.0, 116.0, 118.3, 126.4, 126.9, 127.0, 127.1, 127.2, 128.2, 128.3, 129.2, 129.5, 130.8, 131.3, 131.4, 135.0, 136.3, 138.0, 138.2, 138.9, 139.8, 143.0, 146.6, 161.7, 166.7, 167.1, 171.0; HRMS (ES+) calcd for [C\(_{37}\)H\(_{32}\)N\(_2\)O\(_8\)S + H] 665.1952, Found 665.1957; HPLC (I) \( t_R = 21.07 \) min (96.72 %), (II) \( t_R = 44.44 \) min (97.21 %).
2.18nc, 4-((3'-Cyanoterphenyl-4-yl)methyl)-2-(N,4-dimethyl-phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. $\delta_H$ (400 MHz, $d_6$-DMSO) 2.36 (s, 3H, CH$_3$), 2.82 (s, 3H, CH$_3$), 3.92 (s, 2H, CH$_2$), 4.92 (s, 2H, CH$_2$), 6.85 (d of d, $J = 8.4$ and 1.6 Hz, 1H, CH), 6.95 (d, $J = 1.6$ Hz, 1H, CH), 7.30 (d, $J = 8.0$ Hz, 2H, CH), 7.36 (d, $J = 8.4$ Hz, 2H, CH), 7.58 (d, $J = 8.4$ Hz, 2H, CH), 7.66-7.71 (m, 3H, CH), 7.78-7.86 (m, 6H, CH), 8.05 (d, $J = 8.0$ Hz, 1H, CH), 8.20 (s, 1H, CH); $\delta_C$ (100 MHz, $d_6$-DMSO) 20.8, 35.8, 50.9, 51.5, 112.0, 112.5, 116.0, 118.5, 118.7, 126.5, 126.9, 127.0, 127.3, 128.3, 129.5, 129.9, 130.0, 130.9, 131.2, 131.3, 135.0, 135.4, 136.4, 136.8, 138.0, 139.4, 140.5, 143.0, 146.8, 161.6, 166.8, 171.1; HRMS (ES+) calcd for [C$_{37}$H$_{32}$N$_3$O$_6$S + H] 646.2006, Found 646.1986; HPLC (I) $t_R = 22.62$ min (88.65 %), (II) $t_R = 48.72$ min (90.98 %).
\[ \text{2.18nd, 4-} (N-((3'\text{-carbamoylterphenyl-4-yl})\text{methyl})\text{-2-}(N,4-}\text{dimethylphenylsulfonamido}\text{)acet-amido})\text{-2-hydroxybenzoic acid.} \]

\[ \delta_H (400 \text{ MHz, } d_6-\text{DMSO}) \]

2.36 (s, 3H, CH\text{3}), 2.81 (s, 3H, CH\text{3}), 3.91 (s, 2H, CH\text{2}), 4.91 (s, 2H, CH\text{2}), 6.91 (d of d, \( J = 7.6 \) and 1.2 Hz, 1H, CH), 6.90 (s, 1H, CH), 7.30 (d, \( J = 8.0 \) Hz, 2H, CH), 7.37 (d, \( J = 8.0 \) Hz, 2H, CH), 7.53-7.57 (m, 3H, CH), 7.68 (d, \( J = 8.4 \) Hz, 2H, CH), 7.73 (d, \( J = 7.6 \) Hz, 1H, CH), 7.75-7.88 (m, 6H, CH), 8.23 (s, 1H, CH); \[ \delta_C (100 \text{ MHz, } d_6-\text{DMSO}) \]

20.8, 35.8, 50.8, 51.5, 113.3, 115.9, 118.2, 125.4, 126.4, 126.7, 126.9, 127.0, 127.2, 128.3, 128.8, 129.1, 129.3, 129.5, 131.2, 134.9, 135.0, 136.3, 138.2, 138.4, 143.0, 146.4, 161.8, 166.7, 167.7, 171.0; HRMS (ES+) calcd for \( [C_{37}H_{33}N_3O_7S + H] \) 663.2111, Found 665.2109; HPLC (I) \( t_R = 19.89 \) min (100 %), (II) \( t_R = 41.08 \) min (100 %).
2.18ne, 4-(2-(N,4-dimethylphenylsulfonamido)-N-((4'-(methoxy-carbonyl)terphenyl-4-yl) methyl)-acetamido)-2-hydroxybenzoic acid. \( \delta_H \) (400 MHz, \textit{d}6-DMSO) 2.38 (s, 3H, CH\textsubscript{3}), 2.81 (s, 3H, CH\textsubscript{3}), 3.88 (s, 5H, CH\textsubscript{2} and CH\textsubscript{3}), 4.91 (s, 2H, CH\textsubscript{2}), 6.80 (d, \( J = 8.4 \) Hz, 1H, CH), 6.89 (s, 1H, CH), 7.30 (d, \( J = 8.4 \) Hz, 2H, CH), 7.38 (d, \( J = 8.0 \) Hz, 2H, CH), 7.57 (d, \( J = 8.4 \) Hz, 2H, CH), 7.69 (d, \( J = 8.0 \) Hz, 2H, CH), 7.75-7.84 (m, 5H, CH), 7.90 (d, \( J = 8.8 \) Hz, 2H, CH), 8.03 (d, \( J = 8.8 \) Hz, 2H, CH) \( \delta_C \) (100 MHz, \textit{d}6-DMSO) 20.8, 35.8, 50.8, 51.5, 52.0, 113.5, 115.9, 118.0, 126.4, 126.6, 126.8, 127.0, 127.2, 127.3, 128.3, 129.5, 129.7, 131.2, 135.0, 136.4, 137.5, 138.0, 139.4, 143.0, 143.9, 146.3, 161.8, 165.9, 166.7, 171.0; HRMS (ES+) calcd for [C\textsubscript{37}H\textsubscript{32}N\textsubscript{2}O\textsubscript{8}S + H] 679.2108, Found 679.2081; HPLC (I) \( t_R = 23.54 \) min (100 %), (II) \( t_R = 51.01 \) min (100 %).
2.18nf, 4’-((N-(4-carboxy-3-hydroxyphenyl)-2-(N,4-
dimethylphenylsulfonamido)acetamido)methyl)terphenyl-4-carboxylic acid. \( \delta_H \) (400 MHz, 
\( d6 \)-DMSO) 2.37 (s, 2H, CH\(_3\)), 2.80 (s, 3H, CH\(_3\)), 3.88 (s, 2H, CH\(_2\)), 4.88 (s, 2H, CH\(_2\)), 6.72 (d, \( J \) = 6.8 Hz, 1H, CH), 6.81 (s, 1H, CH), 7.29 (d, \( J \) = 6.8 Hz, 2H, CH), 7.37 (d, \( J \) = 7.6 Hz, 2H, CH), 
7.56 (d, \( J \) = 6.8 Hz, 2H, CH), 7.68 (d, \( J \) = 7.2 Hz, 2H, CH), 7.78-7.85 (m, 7H, CH), 8.03 (d, \( J \) = 7.2 Hz, 2H, CH); \( \delta_C \) (100 MHz, \( d6 \)-DMSO) 20.8, 35.7, 51.5, 55.7, 107.4, 107.7, 117.4, 126.4, 
126.5, 126.8, 127.1, 127.0, 127.3, 128.3, 129.5, 129.8, 131.1, 136.4, 137.0, 137.7, 138.0, 139.3, 
143.0, 143.5, 158.0, 162.3, 166.7, 166.9, 171.4; HRMS (ES+) calcd for [C\(_{37}\)H\(_{32}\)N\(_2\)O\(_8\)S + H] 
665.1952, Found 665.1962; HPLC (I) \( t_R \) = 17.25 min (92.99 %), (II) \( t_R \) = 37.13 min (91.78 %).
2.18 ng, 4-(N-((4′-Cyanoterphenyl-4-yl)methyl)-2-(N,4-dimethyl phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. \( \delta_H \) (400 MHz, \( d_6 \)-DMSO) 2.36 (s, 3H, CH\(_3\)), 2.81 (s, 3H, CH\(_3\)), 3.91 (s, 2H, CH\(_2\)), 4.92 (s, 2H, CH\(_2\)), 6.85 (d, \( J = 8.4 \) Hz, 1H, CH), 6.95 (s, 1H, CH), 7.30 (d, \( J = 8.0 \) Hz, 2H, CH), 7.36 (d, \( J = 8.0 \) Hz, 2H, CH), 7.58 (d, \( J = 8.0 \) Hz, 2H, CH), 7.68 (d, \( J = 8.0 \) Hz, 2H, CH), 7.78-7.82 (m, 5H, CH), 7.93-7.95 (m, 4H, CH); \( \delta_C \) (100 MHz, \( d_6 \)-DMSO) 20.8, 35.8, 50.9, 51.5, 109.9, 112.5, 116.0, 118.5, 118.7, 126.5, 126.9, 127.1, 127.2, 127.5, 128.3, 129.5, 131.3, 132.7, 135.0, 136.5, 137.0, 138.0, 139.8, 143.0, 146.8, 161.5, 166.7, 171.1. HRMS (ES+) calcd for \([C_{37}H_{32}N_3O_6S + H]\) 646.2006, Found 646.1987; HPLC (I) \( t_R \) = 22.71 min (94.15 %), (II) \( t_R \) = 49.13 min (96.29 %).
2.18nh, 4-((4''-carbamoyl-[1,1':4',1''-terphenyl]-4-yl)methyl)-2-(N,4-dimethylphenylsulfonamido)acetamido)-2-hydroxybenzoic acid. δ_H (400 MHz, d6-DMSO) 2.35 (s, 3H, CH₃), 2.79 (s, 3H, CH₃), 3.87 (s, 2H, CH₂), 4.88 (s, 2H, CH₂), 6.78 (d, J = 6.4 Hz, 1H, CH), 6.86 (s, 1H, CH), 7.29 (d, J = 8.4 Hz, 2H, CH), 7.37 (d, J = 8.0 Hz, 2H, CH), 7.55-7.69 (m, 6H, CH), 7.76-7.84 (m, 4H, CH), 7.95-8.04 (m, 3H, CH); δ_C (100 MHz, d6-DMSO) 21.3, 30.7, 51.3, 52.0, 103.0, 116.2, 118.4, 126.2, 126.5, 127.0, 127.1, 127.3, 128.2, 128.4, 128.7, 128.8, 129.6, 131.4, 131.5, 132.0, 132.1, 132.2, 133.1, 133.2, 135.1, 136.4, 138.1, 138.3, 139.1, 142.1, 143.1, 146.8, 161.7, 166.8, 167.5, 171.1, 172.0; HRMS (ES+) calcd for [C₃₇H₃₃N₃O₇S + H] 664.2111, Found 664.2141; HPLC (III) t_R = 19.22 min (76.63 %), (IV) t_R = 43.81 min (79.95 %).
2.31, 4-(N-(4-Cyclohexylbenzyl)-2-(4-methylphenylsulfonamido)acetamido)-2-hydroxybenzoic acid. \( \delta_H \) (400 MHz, d6-DMSO) 1.06-1.40 (m, 5H, CH\(_2\)), 1.66-1.76 (m, 5H, CH\(_2\)), 2.37 (s, 3H, CH\(_3\)), 2.43 (s (br), 1H, CH), 3.55 (d, \( J = 5.4 \) Hz, 2H, CH\(_2\)NH), 4.73 (s, 2H, CH\(_2\)), 6.68 (d of d, \( J = 8.4 \) and 2.0 Hz, 1H, CH), 6.77 (d, \( J = 2.0 \) Hz, 1H, CH), 6.99 (d, \( J = 8.2 \) Hz, 2H, 2 CH), 7.10 (d, \( J = 8.0 \) Hz, 2H, 2 CH), 7.34 (d, \( J = 7.8 \) Hz, 2H, 2 CH), 7.57-7.59 (m, 2H, 2 CH), 7.72 (d, \( J = 8.2 \) Hz, 1H, CH), 7.86 (t, \( J = 5.8 \) Hz, 1H, NH), 11.40 (s (br), 1H, OH); HRMS (ES+) calcd for [C\(_{29}\)H\(_{32}\)N\(_2\)O\(_6\)S + H] 537.2059, found 537.2053; HPLC (I) \( t_R = 24.12 \) min (97.43 %), (II) \( t_R = 51.54 \) min (97.70 %).

![Chemical structure](image)

2.33, 4-(2-(N-(tert-Butoxycarbonyl)-4-methylphenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)-2-hydroxybenzoic acid. \( \delta_H \) (400 MHz, CDCl\(_3\)) 1.22-1.45 (m, 14H), 1.70-1.85 (m, 5H, CH\(_2\)), 2.42 (s, 3H, CH\(_3\)), 2.46 (s (br), 1H, CH), 4.46 (s, 2H, COCH\(_2\)), 4.91 (s, 2H, CH\(_2\)), 6.70 (d, \( J = 8.0 \) Hz, 1H, CH), 6.82 (s (br), 1H, CH), 7.10-7.15 (m, 4H, 4 CH), 7.30 (d, \( J = 8.0 \) Hz, 2H, 2 CH), 7.88 (d, \( J = 8.4 \) Hz, 1H, 1 CH), 8.02 (d, \( J = 8.2 \) Hz, 2H, 2 CH), 10.66 (s (br), 1H, OH); \( \delta_C \) (100 MHz, CDCl3) 21.6, 26.0, 26.8, 27.7, 34.3, 44.2, 47.5, 53.1, 84.7, 111.6, 117.2, 119.3, 126.9, 128.3, 128.8, 129.0, 132.3, 133.5, 136.6, 144.2, 147.5, 147.9, 150.5, 162.9, 166.7, 172.6; HRMS (ES+) calcd for [C\(_{34}\)H\(_{41}\)N\(_2\)O\(_8\)S + H] 637.2547, found 637.2578; HPLC (I) \( t_R = 26.55 \) min (97.80 %), (II) \( t_R = 59.27 \) min (100 %).
2.36, 4-((N-(4-Cyclohexylbenzyl)-2-(tosyloxy)acetamido)-2-hydroxy-benzoic acid. \( \delta_H \) (400 MHz, \textit{d}6-DMSO) 1.20-1.31 (m, 5H, CH\_2), 1.70-1.75 (m, 5H, CH\_2), 2.38 (s, 3H, CH\_3), 2.41 (s (br), 1H, CH), 4.56 (s, 2H, COCH\_2), 4.75 (s, 2H, CH\_2), 6.66 (d, \( J = 8.4 \) Hz, 1H, CH), 6.75 (s (br), 1H, CH), 7.01 (d, \( J = 8.0 \) Hz, 2H, CH), 7.09 (d, \( J = 8.0 \) Hz, 2H, CH), 7.40 (d, \( J = 8.2 \) Hz, 2H, CH), 7.65-7.68 (m, 3H, 3 CH); \( \delta_C \) (100 MHz, \textit{d}6-DMSO) 21.0, 25.4, 26.2, 33.8, 43.2, 51.5, 65.9, 111.6, 115.6, 117.3, 125.4, 126.5, 127.3, 127.9, 129.9, 131.1, 131.8, 133.8, 145.0, 146.4, 162.0, 163.7, 170.9; HRMS (ES+) calcd for [C\textsubscript{29}H\textsubscript{32}NO\textsubscript{7}S + H] 538.1912, found 538.1894; HPLC (I) \( t_R = 21.56 \) min (97.25 %), (II) \( t_R = 54.14 \) min (98.36 %).
Appendix 2: Chapter 3 Experimental

1 Experimental

1.1 Chemical Methods

Anhydrous solvents methanol, DMSO, CH\(_2\)Cl\(_2\), THF and DMF were purchased from Sigma Aldrich and used directly from Sure-Seal bottles. Molecular sieves were activated by heating to 300 °C under vacuum. All reactions were performed under an atmosphere of dry nitrogen in oven-dried glassware and were monitored for completeness by thin-layer chromatography (TLC) using silica gel (visualized by UV light, or developed by treatment with KMnO\(_4\) stain or phosphomolybdic acid stain). \(^1\)H and \(^{13}\)C NMR spectra were recorded on a Bruker 400 MHz spectrometer in either CDCl\(_3\), CD\(_3\)OD or \(d_6\)-DMSO. Chemical shifts (\(\delta\)) are reported in parts per million after calibration to residual isotopic solvent. Coupling constants (\(J\)) are reported in Hz. Before biological testing, inhibitor purity was evaluated by reversed-phase HPLC (rpHPLC). Analysis by rpHPLC was performed using a Microsorb-MV 300 Å C\(_{18}\) 250 mm x 4.6 mm column run at 1 mL/min, and using gradient mixtures. The linear gradient consisted of a changing solvent composition of either (I) 100 % H\(_2\)O with 0.1 % TFA for two minutes to 100 % MeCN with 10 % H\(_2\)O and 0.1 % TFA (v/v) at 22 minutes and UV detection at 254nm or (II) 100 % H\(_2\)O with 0.1 % TFA for 2 minutes to 100 % MeCN with 10 % H\(_2\)O and 0.1 % TFA (v/v) at 62 minutes and UV detection at 214nm or (III) 100 % H\(_2\)O (0.01 M NH\(_4\)OAc) for 2 minutes to 100 % MeOH at 22 minutes and UV detection at 254nm or (IV) 100 % H\(_2\)O (0.01 M NH\(_4\)OAc) for 2 minutes to 100 % MeOH at 62 minutes and UV detection at 254nm or (V) 100 % H\(_2\)O (0.01 M NH\(_4\)OAc) for 2 minutes to 100 % MeOH at 25 minutes and UV detection at 254nm or (VI) 100 % H\(_2\)O (0.01 M NH\(_4\)OAc) for 2 mins to 100 % MeOH at 62 mins and UV detection at 254nm, each ending with 5 mins of 100 % B. For reporting HPLC data, percentage purity is given in parentheses after the retention time for each condition. All biologically evaluated compounds are > 95 % chemical purity as measured by HPLC. The HPLC traces for all tested compounds are provided in supporting information.
1.2 Stat3 Fluorescence Polarization Assay

Figure A2.1. Competitive binding of 2.18h measured by fluorescence polarization assay, with a calculated $IC_{50} = 31 \pm 9 \, \mu M$. Curve fitted using ORIGIN software.

Figure A2.2. Competitive binding of 3.7b measured by fluorescence polarization assay, with a calculated $IC_{50} = 16 \pm 5 \, \mu M$. Curve fitted using ORIGIN software.
Figure A2.3. Competitive binding of 3.7c measured by fluorescence polarization assay, with a calculated IC$_{50}$ = 12 ± 4 µM. Curve fitted using ORIGIN software.

Figure A2.4. Competitive binding of 3.7e measured by fluorescence polarization assay, with a calculated IC$_{50}$ = 53 ± 1 µM. Curve fitted using ORIGIN software.
Figure A2.5. Competitive binding of 17f measured by fluorescence polarization assay, with a calculated IC$_{50} =$ 82 ± 2 µM. Curve fitted using ORIGIN software.

Figure A2.6. Competitive binding of 3.7g measured by fluorescence polarization assay, with a calculated IC$_{50} =$ 26 ± 1 µM. Curve fitted using ORIGIN software.
Figure A2.7. Competitive binding of 3.7k measured by fluorescence polarization assay, with a calculated $IC_{50} = 22 \pm 1 \mu M$. Curve fitted using ORIGIN software.

Figure A2.8. Competitive binding of 3.7o measured by fluorescence polarization assay, with a calculated $IC_{50} = 26 \pm 1 \mu M$. Curve fitted using ORIGIN software.

1.3 General Procedures

General Procedure A (Sulfonylation of secondary amines). To a stirred solution of amine (1.0 equiv) dissolved in CH₂Cl₂ (0.1 M) was added DIPEA (1.1 equiv) and the appropriate sulfonyl chloride (1.1 equiv). After 1 hr, the reaction was diluted with CH₂Cl₂, washed with water, followed by a brine wash and dried over Na₂SO₄. The organic layer was then concentrated under reduced pressure and purified by silica gel column chromatography to yield product.
**General Procedure B** (Hydrogenolysis of benzyl ether and benzyl ester)-Global deprotection of benzylated salicylic acid. The benzyl protected salicylic acid (1 equiv) was dissolved in a stirred solution of THF/MeOH (1:1) (0.1 M). The solution was degassed thoroughly before careful addition of 10 % Pd/C (10 mg/mmol). H\(_2\) gas was bubbled through the solvent for 5 mins before the solution was put under an atmosphere of H\(_2\) gas and stirred continuously for 3 hrs. The H\(_2\) gas was evacuated and the reaction filtered (to remove the Pd catalyst) and concentrated under reduced pressure.

**General Procedure C** (Step-wise deprotection of benzyl ester and benzyl ether). The benzyl protected compound (1 equiv) was dissolved in a 3:1 mixture of THF:water (0.1 M) and was stirred overnight at rt. THF was removed under reduced pressure and the remaining solution was diluted with water and product was extracted into EtOAc. Organic fractions were combined then washed with 1 M HCl, water and brine, then dried over Na\(_2\)SO\(_4\). Solvents were removed under reduced pressure and products were used without further purification. Mono-benzyl intermediates were dissolved a 1:1 mixture of TFA:toluene (0.1 M) at rt and stirred for 5 minutes. All solvents were evaporated under reduced pressure and products were isolated using flash chromatography.

### 1.4 Characterization of Intermediates

**3.2, tert-butyl 2-(2,2,2-trifluoro-N-methylacetamido)acetate.** To a stirred solution of tert-butyl 2-(methylamino)acetate (2.00 g, 11 mmol) and DIPEA (3.65 g (4.80 ml), 27.5 mmol) in CHCl\(_3\) (0.1 M) was added triflic anhydride (2.54 g, 12.1 mmol). The solution was allowed to stir at rt for 3hr before quenching with water and extraction into CH\(_2\)Cl\(_2\). The combined organic layers were washed with water and brine, dried over Na\(_2\)SO\(_4\) and the solution concentrated under reduced pressure to give 3.2 (1.44 g, 88 %): \(\delta_H\) (400 MHz, \(d\)-CDCl\(_3\)) 1.46 (s, 9H, 3 CH\(_3\)), 3.08 (s, 1H, CH\(_3\)), 3.18 (s, 2H, CH\(_3\)), 4.04 (s, 2H, CH\(_2\)).
3.3, 2-(2,2,2-trifluoro-N-methylacetamido)acetic acid. tert-Butyl ester 3.2 (2.00 g, 11.0 mmol) was dissolved in a TFA: CH$_2$Cl$_2$ (1:1) solution (0.1 M) and allowed to stir for 5 hours at rt. The product was then concentrated under reduced pressure to yield pure compound 3.3 (2.50 g, 95 %): $\delta$H (400 MHz, d-$\text{CDCl}_3$) 3.22 (s, 3H, CH$_3$), 4.19 (s, 2H, CH$_2$).

3.4, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(2,2,2-trifluoro-N-methylacetamido)acetamido)benzoate. To a stirred solution of the secondary aniline 2.10h (0.70 g, 1.4 mmol) and carboxylic acid 3.3 (0.28 g, 1.5 mmol) in CHCl$_3$ (0.1 M) was added PPh$_3$Cl$_2$ (1.2 g, 3.4 mmol). The reaction was then heated to 60 °C and stirred overnight. The reaction was allowed to cool and the solvents removed under reduced pressure. The concentrate was absorbed directly onto silica for column chromatography purification yielding compound 3.4 (0.91 g, 97 %): $\delta$H (400 MHz, CDCl$_3$) 1.35-1.44 (m, 5H, CH$_2$), 1.71-1.90 (m, 5H, CH$_2$), 3.17 (m, 4H, CH$_3$ and CH), 3.79 (s, 2H, CH$_2$), 4.84 (s, 2H, CH$_2$), 4.97 (s, 2H, CH$_2$), 5.35 (s, 2H, CH$_2$), 6.65 (s, 1H, CH), 6.78 (dd, $J = 8.4$ and 1.6 Hz, 1H, CH), 7.10-7.19 (m, 4H, CH), 7.29-7.43 (m, 10H, CH), 7.86 (d, $J = 8.4$ Hz, 1H, CH); $\delta$C (100 MHz, CDCl$_3$) 25.8, 26.6, 34.2, 44.0, 51.2, 52.6, 66.7, 70.4, 77.2, 113.9, 115.0, 117.6, 119.8, 126.8, 126.9, 127.7, 128.0, 128.3, 128.3, 128.4, 128.6, 132.9, 133.6, 135.5, 135.6, 144.6, 147.5, 157.0, 158.4, 165.1, 165.4; LRMS (ES+) Calcd for [C$_{39}$H$_{39}$F$_3$N$_2$O$_5$ + Na] 695.27 found 695.36.
3.5, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(methylamino)-acetamido)benzoate. Compound 3.4 (2.68 mmol) was dissolved in a THF:H₂O (3:1) solution and treated with LiOH·H₂O (337 mg, 8.04 mmol). After 10 mins the reaction was completed and diluted with H₂O. The product was extracted into EtOAc and the combined extracts washed with 50 % sat. NaHCO₃, water, brine and dried over Na₂SO₄ and concentrated under reduced pressure to yield 3.5 (1.57 g, 99 %): δ_H (400 MHz, CDCl₃) 1.35 (m, 5H, CH₂), 1.66-1.84 (m, 5H, CH₂), 2.28 (s, 2H, CH₃), 2.44 (m, 1H, CH), 3.02 (s, 2H, CH₂), 4.81 (s, 2H, CH₂), 4.89 (s, 2H, CH₂), 5.30 (s, 2H, CH₂), 6.52 (s, 1H, CH), 6.54 (d, 1H, J = 8.0 Hz, CH), 7.05-7.13 (m, 4H, CH), 7.24-7.37 (m, 12H, CH), 7.80 (d, J = 8.0 Hz, 1H, CH); δ_C (100 MHz, CDCl₃) 25.6, 26.3, 34.0, 35.4, 43.7, 52.0, 52.2, 66.4, 70.0, 77.2, 113.6, 119.6, 119.9, 126.4, 126.6, 127.5, 127.6, 127.7, 128.0, 128.1, 128.3, 132.5, 133.9, 135.3, 135.43, 145.0, 147.0, 158.1, 164.8, 169.6; LRMS (ES+) Calcd for [C₃₇H₄₀N₂O₄ + H] 577.31 found 577.45.
3.6a, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(N,3-dimethylphenylsulfonamido)acetamido)benzoate. Secondary amine 3.5 was coupled to 3-methylbenzene-1-sulfonyl chloride on a 0.2 mmol scale via General Procedure A to furnish 3.6a (116 mg, 94 %): \( \delta_H \) (400 MHz, \( d-\text{CDCl}_3 \)) 1.31-1.44 (m, 5H, CH), 1.70-1.89 (m, 5H, CH), 2.36 (s, 3H, CH\(_3\)), 2.42-2.52 (m, 1H, CH), 2.83 (s, 3H, CH\(_3\)), 3.65 (s, 2H, CH\(_2\)), 4.77 (s, 2H, CH\(_2\)), 4.96 (s, 2H, CH\(_2\)), 5.35 (s, 2H, CH\(_2\)), 6.52 (s, 1H, CH), 6.69 (dd, \( J = 8.0 \) and 1.2 Hz, 1H, CH), 7.03 (d, \( J = 8.0 \) Hz, 2H, CH), 7.11 (d, \( J = 8.0 \) Hz, 2H, CH), 7.27-7.42 (m, 12 H, CH), 7.50-7.55 (m, 2H, CH), 7.84 (d, \( J = 8.0 \) Hz, 1H, CH); \( \delta_C \) (100 MHz, \( d-\text{CDCl}_3 \)) 21.2, 25.9, 26.7, 29.6, 34.2, 34.3, 35.9, 44.1, 51.3, 52.7, 66.9, 70.6, 114.2, 120.0, 120.6, 124.4, 126.9, 127.0, 127.6, 127.9, 128.1, 128.2, 128.4, 128.5, 128.8, 128.9, 133.0, 133.3, 133.8, 135.6, 135.7, 138.0, 138.9, 145.0, 147.6, 158.6, 165.3, 166.6; LRMS (ES+) Calcd for [C\(_{44}\)H\(_{46}\)N\(_2\)O\(_6\)S + Na] 753.30 found 753.18.

3.6b, benzyl 2-(benzyloxy)-4-(N-(4-Cyclohexylbenzyl)-2-(N,2,4,6-tetramethylphenylsulfonamido)acetamido)benzoate. Secondary amine 3.5 was coupled with 2,4,6-trimethylbenzenesulfonyl chloride on a 0.1 mmol scale via General Procedure A to furnish 3.6b (48 mg, 74 %): \( \delta_H \) (400 MHz, \( d-\text{CDCl}_3 \)) 1.34-1.43 (m, 5H, CH), 1.70-1.87 (m, 5H, CH), 2.29 (s, 3H, CH\(_3\)), 2.42-2.52 (m, 1H, CH), 2.56 (s, 6H, CH\(_3\)), 2.79 (s, 3H, CH\(_3\)), 3.67 (s, 2H, CH\(_2\)), 4.75 (s, 2H, CH\(_2\)), 4.90 (s, 2H, CH\(_2\)), 5.35 (s, 2H, CH\(_2\)), 6.43 (s, 1H, CH), 6.55 (dd, \( J = 8.0 \) and 1.6 Hz, 1H, CH), 6.93 (s, 2H, CH), 7.00 (d, \( J = 8.0 \) Hz, 2H, CH), 7.10 (d, \( J = 8.0 \) Hz, 2H, CH), 7.29-7.42 (m, 10 H, CH), 7.81 (d, \( J = 8.0 \) Hz, 1H, CH); \( \delta_C \) (100 MHz, \( d-\text{CDCl}_3 \)) 20.9, 22.7, 26.0, 26.7, 34.4, 34.7, 44.1, 49.6, 52.6, 69.9, 70.6, 113.9, 120.0, 120.8, 126.9, 127.0, 128.0, 128.1, 128.2,
128.5, 128.5, 128.8, 131.8, 133.1, 134.0, 135.6, 135.7, 140.5, 142.4, 147.6, 158.7, 165.3, 166.7;
LRMS (ES+) Calcd for [C_{46}H_{50}N_{2}O_{6}S + Na] 781.33 found 781.39.

3.6c, benzyl 2-(benzyloxy)-4-(N-(4-Cyclohexylbenzyl)-2-(N-methylbiphenyl-4-
ysulfonamido)acetamido)benzoate. Secondary amine 3.5 was combined with biphenyl-4-
sulfonyl chloride on a 0.1 mmol scale via General Procedure A to furnish 3.6c (50 mg, 70 %): \( \delta_{H} \) (400 MHz, \( d\text{-CDCl}_3 \)) 1.32-1.41 (m, 5H, CH\(_2\)), 1.70-1.86 (m, 5H, CH\(_2\)), 2.38-2.52 (m, 1H, CH), 2.90 (s, 3H, CH\(_3\)), 3.72 (s, 2H, CH\(_2\)), 4.77 (s, 2H, CH\(_2\)), 4.97 (s, 2H, CH\(_2\)), 4.36 (s, 2H, CH\(_2\)).
6.53 (s, 1H, CH), 6.70 (dd, \( J = 8.0 \) and 1.6 Hz, 1H, CH), 7.02 (d, \( J = 8.0 \) Hz, 2H, CH), 7.09 (d, \( J = 8.0 \) Hz, 2H, CH), 7.30-7.36 (m, 8H, CH), 7.38-7.42 (m, 2H, CH), 7.43-7.50 (m, 3H, CH), 7.60 (dt, \( J = 7.2 \) and 1.6 Hz, 2H, CH), 7.68 (d, \( J = 8.8 \) Hz, 2H, CH), 7.81 (d, \( J = 8.4 \) Hz, 2H, CH), 7.86 (d, \( J = 8.0 \) Hz, 1H, CH); \( \delta_{C} \) (100 MHz, \( d\text{-CDCl}_3 \)) 26.0, 26.7, 34.3, 36.0, 44.1, 51.3, 52.8, 66.9, 70.6, 114.1, 120.0, 120.6, 126.9, 127.0, 127.2, 127.4, 127.9, 128.1, 128.2, 128.3, 128.5, 128.8, 128.9, 132.0, 133.1, 138.8, 135.7, 135.7, 137.0, 139.3, 145.0, 145.3, 147.6, 158.7, 165.3, 166.7; LRMS (ES+) Calcd for [C_{49}H_{48}N_{2}O_{6}S + Na] 815.31 found 815.44.
3.6d, benzyl 2-(benzyloxy)-4-(2-(4-chloro-N-methylphenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)benzoate. Secondary amine 3.5 was combined with 2-naphthylsulfonyl chloride on a 0.1 mmol scale via General Procedure A to furnish 3.6d (53 mg, 79%): δ_H (400 MHz, d-CDCl_3) 1.35-1.42 (m, 5H, CH_2), 1.71-1.89 (m, 5H, CH_2), 2.42-2.52 (m, 1H, CH), 2.85 (s, 3H, CH_3), 3.70 (s, 2H, CH_2), 4.74 (s, 2H, CH_2), 4.96 (s, 2H, CH_2), 5.36 (s, 2H, CH_2), 6.50 (s, 1H, CH), 6.68 (dd, J = 8.4 and 1.6 Hz, 1H, CH), 7.01 (d, J = 8.4 Hz, 2H, CH), 7.13 (d, J = 8.0 Hz, 2H, CH), 7.29-7.36 (m, 8H, CH), 7.39-7.45 (m, 4H, CH), 7.70 (d, J = 8.4 Hz, 2H, CH), 7.84 (d, J = 8.4 Hz, 1H, CH); δ_C (100 MHz, d-CDCl_3) 25.9, 26.7, 34.3, 35.8, 44.1, 51.2, 52.7, 66.9, 70.6, 114.0, 119.9, 120.7, 126.8, 126.9, 127.9, 128.1, 128.2, 128.4, 128.5, 128.7, 128.8, 129.0, 133.1, 133.7, 135.6, 135.6, 137.1, 138.9, 144.8, 147.7, 158.6, 165.2, 166.4; LRMS (ES+) Calcd for [C_{47}H_{46}N_2O_6S + Na] 789.30 found 789.32.
3.6e, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(N-methylnaphthalene-1-sulfonamido)acetamido)benzoate. Secondary amine 3.5 was combined with 1-naphthylsulfonyl chloride on a 0.2 mmol scale via General Procedure A to furnish 3.6e (127 mg, 98 %): δ_H (400 MHz, d-CDCl_3) 1.33-1.42 (m, 5H, CH), 1.71-1.89 (m, 5H, CH), 2.42-2.52 (m, 1H, CH), 2.96 (s, 3H, CH), 3.75 (s, 2H, CH), 4.76 (s, 2H, CH), 4.92 (s, 2H, CH), 5.37 (s, 2H, CH), 6.49 (s, 1H, CH), 6.67 (d, J = 8.4 Hz, 1H, CH), 7.01 (d, J = 8.0 Hz, 2H, CH), 7.11 (d, J = 8.0 Hz, 2H, CH), 7.29-7.36 (m, 13H, CH), 7.31-7.33 (m, 3H, CH), 7.39-7.44 (m, 2H, CH), 7.55 (t, J = 8.0 Hz, 1H, CH), 7.87 (d, J = 8.0 Hz, 1H, CH), 7.96 (d, J = 8.0 Hz, 1H, CH), 8.14 (d, J = 8.0 Hz, 1H, CH), 8.45 (d, J = 7.6 Hz, 1H, CH), 8.49 (s (br), 1H, CH); δ_C (100 MHz, d-CDCl_3) 25.9, 26.7, 34.4, 36.6, 44.1, 52.7, 53.4, 66.9, 70.6, 114.2, 120.0, 120.7, 123.9, 124.9, 126.7, 127.0, 127.9, 128.1, 128.2, 128.5 (2), 128.6, 128.7, 128.8, 129.3, 133.0, 133.9, 134.0, 134.2, 134.3, 135.6, 135.7, 144.9, 147.6, 158.6, 165.3, 166.7; LRMS (ES+) Calcd for \([C_{47}H_{46}N_2O_6S + Na]\) 789.30 found 789.36.

3.6f, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(N-methylquinoline-8-sulfonamido)acetamido)benzoate. Secondary amine 3.5 was combined with quinoline-8-sulfonyl chloride on a 0.2 mmol scale via General Procedure A to furnish 3.6f (128 mg, 98 %): δ_H (400 MHz, d-CDCl_3) 1.33-1.45 (m, 5H, CH), 1.70-1.88 (m, 5H, CH), 2.42-2.52 (m, 1H, CH), 2.95 (s, 3H, CH), 4.22 (s, 2H, CH), 4.81 (s, 2H, CH), 4.96 (s, 2H, CH), 5.37 (s, 2H, CH), 6.72 (s, 1H, CH), 6.81 (d, J = 8.4 Hz, 1H, CH), 7.06 (d, J = 8.0 Hz, 2H, CH), 7.11 (d, J = 8.0 Hz, 2H, CH), 7.20-7.30 (m, 6H, CH), 7.32-7.36 (m, 3H, CH), 7.39-7.44 (m, 2H, CH), 7.55 (t, J = 8.0 Hz, 1H, CH), 7.87 (d, J = 8.0 Hz, 1H, CH), 7.96 (d, J = 8.0 Hz, 1H, CH), 8.14 (d, J = 8.0 Hz, 1H, CH), 8.45 (d, J = 7.6 Hz, 1H, CH), 8.49 (s (br), 1H, CH); δ_C (100 MHz, d-CDCl_3) 26.0,
26.7, 34.3, 36.2, 44.1, 52.6, 52.7, 66.9, 70.6, 114.4, 120.1, 120.3, 121.7, 125.2, 126.8, 127.0, 127.8, 128.1, 128.2, 128.4, 128.5, 128.8, 128.9, 132.6, 132.7, 133.2, 134.1, 135.7, 135.8, 136.3, 137.0, 143.7, 145.5, 147.4, 150.5, 158.5, 165.5, 168.0; LRMS (ES+) Calcd for \([\text{C}_{46}\text{H}_{55}\text{N}_{3}\text{O}_{6}\text{S} + \text{Na}]\) 790.29 found 790.36.

3.6g, benzyl 2-(benzylxoy)-4-(N-(4-cyclohexylbenzyl)-2-(5-(dimethylamino)-N-methyl naphthalene-1-sulfonamido)acetamido)benzoate. Secondary amine 3.5 was combined with 5-(dimethylamino)naphthalene-1-sulfonyl chloride on a 0.2 mmol scale via General Procedure A to furnish 3.6g (132 mg, 96 %): \(\delta_H (400 \text{ MHz, } d\text{-CDCl}_3)\) 1.33-1.44 (m, 5H, \(\text{CH}_2\)), 1.69-1.88 (m, 5H, \(\text{CH}_2\)), 2.42-2.52 (m, 1H, \(\text{CH}\)), 2.86 (s, 6H, \(\text{CH}_3\)), 2.95 (s, 3H, \(\text{CH}_3\)), 3.76 (s, 2H, \(\text{CH}_2\)), 4.77 (s, 2H, \(\text{CH}_2\)), 5.37 (s, 2H, \(\text{CH}_2\)), 6.50 (s, 1H, \(\text{CH}\)), 6.67 (d, \(J = 8.4 \text{ Hz, } 1\text{H, CH}\)), 7.03 (d, \(J = 8.0 \text{ Hz, } 2\text{H, CH}\)), 7.12 (d, \(J = 8.0 \text{ Hz, } 2\text{H, CH}\)), 7.23-7.38 (m, 9H, \(\text{CH}\)), 7.39-7.50 (m, 4H, \(\text{CH}\)), 7.83 (d, \(J = 8.0 \text{ Hz, } 1\text{H, CH}\)), 8.18 (d, \(J = 7.2 \text{ Hz, } 1\text{H, CH}\)), 8.23 (d, \(J = 8.4 \text{ Hz, } 1\text{H, CH}\)), 8.52 (d, \(J = 8.0 \text{ Hz, } 1\text{H, CH}\)); \(\delta_C (100 \text{ MHz, } d\text{-CDCl}_3)\) 26.0, 26.7, 34.3, 36.1, 44.1, 45.3, 50.7, 52.6, 66.9, 70.6, 114.2, 115.1, 119.6, 119.9, 120.3, 123.0, 126.9, 127.0, 127.9, 128.1, 128.2, 128.4 (br), 128.8, 129.2, 129.8, 130.0, 130.1, 133.0, 133.9, 134.4, 135.6, 144.9, 147.6, 158.6, 165.3, 166.8.
3.6h, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(N,1-dimethyl-1H-imidazole-4-sulfonamido)acetamido)benzoate. Secondary amine 43 was combined with 1-methyl-1H-imidazole-4-sulfonyl chloride on a 0.2 mmol scale via General Procedure A to furnish 3.6h (116 mg, 95%): \(\delta_H\) (400 MHz, \(d\)-CDCl\(_3\)) 1.32-1.44 (m, 5H, CH\(_2\)), 1.68-1.86 (m, 5H, CH\(_2\)), 2.41-2.51 (m, 1H, CH), 2.92 (s, 3H, CH\(_3\)), 3.63 (s, 3H, CH\(_2\)), 3.80 (s, 2H, CH\(_2\)), 4.77 (s, 2H, CH\(_2\)), 4.96 (s, 2H, CH\(_2\)), 5.32 (s, 2H, CH\(_2\)), 6.69 (d, \(J = 8.4\) Hz, 1H, CH), 6.72 (s, 1H, CH), 7.05 (d, \(J = 8.0\) Hz, 2H, CH), 7.09 (d, \(J = 8.0\) Hz, 2H, CH), 7.27-7.40 (m, 12H, CH), 7.81 (d, \(J = 8.0\) Hz, 1H, CH); \(\delta_C\) (100 MHz, \(d\)-CDCl\(_3\)) 25.9, 26.7, 33.7, 34.3, 36.2, 44.1, 51.6, 53.3, 66.8, 70.6, 114.3, 119.9, 120.2, 123.8, 126.8, 127.1, 127.8, 128.0, 128.1, 128.4, 128.5, 128.7, 132.9, 133.9, 135.7, 135.9, 138.7, 138.8, 145.3, 147.4, 158.7, 165.4, 167.1; LRMS (ES+) Calcd for [C\(_{41}\)H\(_{44}\)N\(_4\)O\(_6\)S + Na] 743.29 found 743.25

3.6i, benzyl 2-(benzyloxy)-4-(2-(4-cyano-N-methylphenylsulfonamido)-N-(4-cyclohexyl benzyl)acetamido)benzoate. Secondary amine 3.5 was combined with 4-cyanobenzene-1-
sulfonyl chloride on a 0.2 mmol scale via General Procedure A to furnish 3.5i (122 mg, 97 %):

\[ \delta_H (400 \text{ MHz}, d\text{-CDCl}_3) 1.21-1.39 (m, 5H, CH_2), 1.60-1.80 (m, 5H, CH_2), 2.38-2.58 (m, 1H, CH), 2.78 (s, 3H, CH_3), 3.65 (s, 2H, CH_2), 4.61 (s, 2H, CH_2), 4.87 (s, 2H, CH_2), 5.26 (s, 2H, CH_2), 6.39 (s, 1H, CH), 6.58 (d, J = 8.0 Hz, 1H, CH), 6.89 (d, J = 8.0 Hz, 2H, CH), 7.03 (d, J = 8.0 Hz, 2H, CH), 7.18-7.35 (m, 10H, CH), 7.65 (d, J = 8.4 Hz, 2H, CH) 7.75 (d, J = 8.0 Hz, 1H, CH), 7.79 (d, J = 8.4 Hz, 2H, CH); \delta_C (100 \text{ MHz}, d\text{-CDCl}_3) 26.1, 26.8, 34.5, 35.9, 44.2, 52.9, 53.5, 67.1, 70.7, 114.1, 116.2, 117.5, 120.0, 121.1, 127.1, 128.2 (br), 128.3, 128.4, 128.6, 128.7, 128.9, 132.6, 133.3, 133.8, 135.7, 135.8, 143.2, 144.7, 147.9, 158.8, 165.3, 166.3; LRMS (ES+) Calcd for [C_{44}H_{43}N_3O_6S + Na] 764.28 found 764.30.

3.6j, benzyl 2-(benzyloxy)-4-(2-(4-bromo-N-methylphenylsulfonamido)-N-(4-cyclohexyl benzyl)acetamido)benzoate. Secondary amine 3.5 was combined with 4-bromobenzene-1-sulfonyl chloride on a 0.2 mmol scale via General Procedure G to furnish 3.6j (123 mg, 95 %):

\[ \delta_H (400 \text{ MHz}, d\text{-CDCl}_3) 1.24-1.38 (m, 5H, CH_2), 1.61-1.80 (m, 5H, CH_2), 2.34-2.46 (m, 1H, CH), 2.75 (s, 3H, CH_3), 3.60 (s, 2H, CH_2), 4.64 (s, 2H, CH_2), 4.86 (s, 2H, CH_2), 5.27 (s, 2H, CH_2), 6.40 (s, 1H, CH), 6.58 (d, J = 8.0 Hz, 1H, CH), 6.91 (d, J = 8.0 Hz, 2H, CH), 7.03 (d, J = 8.0 Hz, 2H, CH), 7.18-7.38 (m, 10H, CH), 7.49-7.54 (m, 4H, CH) 7.75 (d, J = 8.0 Hz, 1H, CH); \delta_C (100 \text{ MHz}, d\text{-CDCl}_3) 25.9, 26.7, 34.3, 35.8, 44.1, 51.2, 52.8, 66.9, 70.6, 114.1, 119.9, 120.8, 126.9, 130.0, 127.4, 127.9, 128.1, 128.2, 128.4, 128.5, 128.8, 128.9, 132.0, 133.1, 133.7, 135.6, 135.7, 137.7, 144.8, 147.7, 158.6, 165.2, 166.4; LRMS (ES+) Calcd for [C_{43}H_{43}BrN_2O_6S + Na] 817.19 found 817.25
3.6k, benzyl 2-(benzyloxy)-4-(2-(4-chloro-N-methylphenylsulfonamido)-N-(4-Cyclohexyl benzyl)acetamido)benzoate. Secondary amine 3.5 was combined with 4-chlorobenzenesulfonyl chloride and on a 0.2 mmol scale via General Procedure A to furnish 3.6k (95 mg, 72 %): $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.35-1.42 (m, 5H, CH$_2$), 1.71-1.89 (m, 5H, CH$_2$), 2.42-2.52 (m, 1H, CH), 2.85 (s, 3H, CH$_3$), 3.70 (s, 2H, CH$_2$), 4.74 (s, 2H, CH$_2$), 4.96 (s, 2H, CH$_2$), 5.36 (s, 2H, CH$_2$), 6.50 (s, 1H, CH), 6.68 (dd, $J = 8.4$ and 1.6 Hz, 1H, CH), 7.01 (d, $J = 8.4$ Hz, 2H, CH), 7.13 (d, $J = 8.0$ Hz, 2H, CH), 7.29-7.36 (m, 8H, CH), 7.39-7.45 (m, 4H, CH), 7.70 (d, $J = 8.4$ Hz, 2H, CH), 7.84 (d, $J = 8.4$ Hz, 1H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 25.9, 26.7, 34.3, 35.8, 44.1, 51.2, 52.7, 66.9, 70.6, 114.0, 119.9, 120.7, 126.8, 126.9, 127.8, 128.1, 128.2, 128.4, 128.5, 128.7, 128.8, 129.0, 133.1, 133.7, 135.6, 135.6, 137.1 , 138.9, 144.8, 147.7, 158.6, 165.2, 166.4; LRMS (ES+) Calcd for [C$_{43}$H$_{43}$ClN$_2$O$_6$S + Na] 773.24 found 773.14.

3.6l, benzyl 2-(benzyloxy)-4-(N-(4-Cyclohexylbenzyl)-2-(4-fluoro-N-methylphenylsulfonamido)acetamido)benzoate. Secondary amine 3.5 was combined with 4-fluorobenzene-1-
sulfonyl chloride on a 0.1 mmol scale via General Procedure G to furnish 3.6l (45 mg, 71 %): $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.33-1.42 (m, 5H, CH$_2$), 1.70-1.86 (m, 5H, CH$_2$), 2.42-2.52 (m, 1H, CH), 2.84 (s, 3H, CH$_3$), 3.70 (s, 2H, CH$_2$), 4.74 (s, 2H, CH$_2$), 4.95 (s, 2H, CH$_2$), 5.35 (s, 2H, CH$_2$), 6.49 (s, 1H, CH), 6.67 (dd, $J = 8.4$ and 1.6 Hz, 1H, CH), 7.00 (d, $J = 8.0$ Hz, 2H, CH), 7.10-7.15 (m, 4H, CH), 7.29-7.38 (m, 8H, CH), 7.39-7.41 (m, 2H, CH), 7.75-7.79 (m, 2H, CH), 7.84 (d, $J = 8.0$ Hz, 1H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 26.0, 26.7, 34.4, 35.8, 44.1, 51.3, 52.8, 67.0, 70.6, 114.1, 115.5, 116.1, 120.0, 120.8, 126.9, 127.0, 128.0, 128.2, 128.5, 128.6, 128.8, 130.1, 130.2, 133.1, 133.8, 134.7, 135.7, 135.7, 144.9, 147.7, 158.7, 163.7, 165.3, 166.6; LRMS (ES+) Calcd for [C$_{43}$H$_{43}$FN$_2$O$_6$S + Na] 757.27 found 757.35.

3.6m, benzyl 2-(benzyloxy)-4-(N-(4-Cyclohexylbenzyl)-2-(4-methoxy-N-methylphenyl sulfonylamido)acetamido)benzoate. Secondary amine 3.5 was combined with 4-methoxybenzenesulfonyl chloride on a 0.1 mmol scale via General Procedure A to furnish 3.6m (56 mg, 79 %): $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.33-1.42 (m, 5H, CH$_2$), 1.80-1.86 (m, 5H, CH$_2$), 2.42-2.51 (m, 1H, CH$_2$), 2.81 (s, 3H, CH$_3$), 3.66 (s, 2H, CH$_2$), 3.84 (s, 3H, CH$_3$), 4.76 (s, 2H, CH$_2$), 4.95 (s, 2H, CH$_2$), 5.35 (s, 2H, CH$_2$), 6.52 (s, 1H, CH), 6.68 (dd, $J = 8.0$ and 1.6 Hz, 1H, CH), 6.92 (d, $J = 8.8$ Hz, 2H, CH), 7.02 (d, $J = 8.0$ Hz, 2H, CH), 7.11 (d, $J = 8.0$ Hz, 2H, CH), 7.29-7.36 (m, 8H, CH), 7.37-7.41 (m, 2H, CH), 7.68 (d, $J = 8.8$ Hz, 2H, CH), 7.84 (d, $J = 8.4$ Hz, 1H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 26.0, 26.7, 34.4, 35.4, 44.1, 51.3, 52.7, 55.4, 66.9, 70.6, 113.9, 114.1, 120.0, 120.6, 126.9, 127.0, 127.9, 128.1, 128.2, 128.5, 128.6, 128.8, 129.6, 129.9, 133.0, 133.9, 135.7, 135.7, 145.0, 147.6, 158.6, 162.7, 165.3, 166.8; LRMS (ES+) Calcd for [C$_{43}$H$_{40}$F$_3$N$_2$O$_6$S + H] 807.25 found 807.20.
3.6n, benzyl 2-(benzyloxy)-4-(N-(4-Cyclohexylbenzyl)-2-(N-methyl-4-nitrophenylsulfonamido)acetamido)benzoate. Secondary amine 3.5 was combined with 4-nitrobenzenesulfonyl chloride on a 0.2 mmol scale via General Procedure A to furnish 3.6n (88 mg, 69 %): δ_H (400 MHz, d-CDCl_3) 1.33-1.42 (m, 5H, CH_2), 1.70-1.89 (m, 5H, CH_2), 2.43-2.51 (m, 1H, CH), 2.90 (s, 3H, CH_3), 3.78 (s, 2H, CH_2), 4.70 (s, 2H, CH_2), 4.70 (s, 2H, CH_2), 5.36 (s, 2H, CH_2), 6.49 (s, 1H, CH), 6.68 (dd, J = 8.0 and 1.6 Hz, 1H, CH), 6.97 (d, J = 8.4 Hz, 2H, CH), 7.13 (d, J = 8.0 Hz, 2H, CH), 7.28-7.37 (m, 8H, CH), 7.39-7.42 (m, 2H, CH), 7.85 (d, J = 8.0 Hz, 1H, CH), 7.95 (d, J = 8.8 Hz, 2H, CH), 8.30 (d, J = 8.8 Hz, 2H, CH); δ_C (100 MHz, d-CDCl_3) 25.9, 26.7, 34.3, 35.8, 44.1, 51.4, 52.8, 67.0, 70.6, 114.0, 119.9, 121.0, 123.9, 128.0, 128.2, 128.2, 128.5, 128.5, 128.6, 128.8, 133.2, 133.6, 135.6, 135.6, 144.5, 144.7, 147.9, 149.8, 158.6, 165.2, 166.1; LRMS (ES+) Calcd for [C_{43}H_{43}N_3O_8S + Na] 784.27 found 784.25.

3.6o, benzyl 2-(benzyloxy)-4-(N-(4-Cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-methylphenylsulfonamido)acetamido)benzoate. Secondary amine 3.5 was combined with
pentafluorobenzenesulfonyl chloride on a 0.1 mmol scale \textit{via} General Procedure A to furnish \textbf{3.6o} (49 mg, 63 \%): $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.34-1.42 (m, 5H, CH$_2$), 1.70-1.86 (m, 5H, CH$_2$), 2.43-2.52 (m, 1H, CH), 3.05 (s, 3H, CH$_3$), 3.86 (s, 2H, CH$_2$), 4.67 (s, 2H, CH$_2$), 4.94 (s, 2H, CH$_2$), 5.35 (s, 2H, CH$_2$), 6.44 (s, 1H, CH), 6.66 (d, $J = 8.0$ Hz, 1H, CH), 6.96 (d, $J = 7.2$ Hz, 2H, CH), 7.12 (d, $J = 7.2$ Hz, 2H, CH), 7.30-7.41 (m, 10H, CH), 7.84 (dd, $J = 8.0$ and 1.2 Hz, 1H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 26.0, 26.7, 34.4, 35.4, 44.1, 51.9, 52.7, 67.0, 70.70, 111.8, 114.0, 115.8, 119.9, 127.0, 127.1, 128.1, 128.2, 128.5, 128.6, 128.7, 133.3, 133.4, 135.6, 135.6, 137.8, 141.6, 142.9, 144.2, 147.9, 158.7, 165.2, 165.8; LRMS (ES+) Calcd for [C$_{44}$H$_{46}$N$_2$O$_7$S + Na]$^+$ 747.31 found 747.39.

1.5 Characterization of Final Molecules

\begin{center}
\includegraphics[width=0.5\textwidth]{structure37a.png}
\end{center}

**3.7a, 4-(N-(4-cyclohexylbenzyl)-2-(N,3-dimethylphenylsulfonamido)acetamido)-2-hydroxybenzoic acid.** Benzyl protected \textbf{3.6a} was globally deprotected on a 0.13 mmol scale \textit{via} General Procedure B to furnish \textbf{3.7a} (68 mg, 91 \%): $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.31-1.41 (m, 5H, CH$_2$), 1.73-1.87 (m, 5H, CH$_2$), 2.40 (s, 3H, CH$_3$), 2.43-2.48 (m, 1H, CH), 2.89 (s, 3H, CH$_3$), 3.86 (s, 2H, CH$_2$), 4.84 (s, 2H, CH$_2$), 6.63 (d of d, $J = 8.4$ and 1.6 Hz, 1H, CH), 6.73 (d, $J = 2.0$ Hz, 1H, CH), 7.08 (d, $J = 8.0$ Hz, 2H, CH), 7.12 (d, $J = 8.0$ Hz, 2H, CH), 7.35-7.40 (m, 2H, CH), 7.57 (s, 2H, CH), 7.89 (d, $J = 8.8$ Hz, 1H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 21.4, 26.1, 26.9, 34.4, 36.0, 44.2, 51.7, 53.1, 111.2, 111.7, 119.2, 124.7, 127.1, 127.9, 128.4, 128.8, 132.2, 133.5, 133.6, 137.8, 139.1, 147.8, 148.1, 163.0, 167.0, 171.9; HRMS (ES+) calcd for [C$_{30}$H$_{34}$N$_2$O$_8$S + H] 551.2210, Found 551.2199; HPLC (III) $t_R = 22.33$ min (76.40 \%), (IV) $t_R = 50.92$ min (100.00 \%).
3.7b, 4-(N-(4-Cyclohexylbenzyl)-2-(N,2,4,6-tetramethylphenylsulfonamido)acetamido)-2-hydroxy-benzoic acid. Benzyl protected 3.6b was globally deprotected on a 0.06 mmol scale via General Procedure B to furnish 3.7b (31 mg, 89%): \(\delta_H\) (400 MHz, \(d_6\)-DMSO) 1.26-1.40 (m, 5H, CH₂), 1.64-1.81 (m, 5H, CH₂), 2.26 (s, 3H, CH₃), 2.44 (s, 7H, CH₃ and CH), 2.84 (s, 3H, CH₃), 3.84 (s, 2H, CH₂), 4.75 (s, 2H, CH₂), 6.62 (dd, \(J = 8.4\) and 2.0 Hz, 1H, CH), 6.70 (d, \(J = 2.0\) Hz, 1H, CH), 6.99 (d, \(J = 8.0\) Hz, 2H, CH), 7.03 (s, 2H, CH), 7.11 (d, \(J = 8.0\) Hz, 2H, CH), 7.75 (d, \(J = 8.4\) Hz, 1H, CH); \(\delta_C\) (100 MHz, \(d_6\)-DMSO) 20.3, 22.1, 25.4, 26.2, 33.8, 34.4, 43.2, 49.1, 51.5, 116.0, 118.4, 120.2, 126.5, 127.4, 131.2, 131.7, 132.1, 134.1, 139.4, 142.1, 146.7, 146.7, 161.5, 166.6, 171.0; HRMS (ES+) calcd for \([C_{32}H_{38}N_2O_6S + H]\) 613.2366, Found 613.2356; HPLC (V) \(t_R = 21.29\) min (96.82%), (VI) \(t_R = 37.54\) min (95.30%).

3.7c, 4-(N-(4-Cyclohexylbenzyl)-2-(N-methylbiphenyl-4-ylsulfonamido)acetamido)-2-hydroxy-benzoic acid. Benzyl protected 3.6c was globally deprotected on a 0.06 mmol scale via General Procedure B to furnish 3.7c (34 mg, 97%): \(\delta_H\) (400 MHz, \(d_6\)-DMSO) 1.23-1.38 (m, 5H,
CH₂), 1.62-1.78 (m, 5H, CH₂), 2.35-2.44 (m, 1H, CH), 2.08 (s, 3H, CH₃), 3.96 (s, 2H, CH₂), 4.78 (s, 2H, CH₂), 6.80 (dd, J = 8.4 and 2.0 Hz, 1H, CH), 6.89 (d, J = 2.0 Hz, 1H, CH), 7.04 (d, J = 8.0 Hz, 2H, CH), 7.09 (d, J = 8.4 Hz, 2H, CH), 7.44 (d, J = 8.0 Hz, 1H, CH), 7.51 (t, J = 7.6 Hz, 2H, CH), 7.68-7.81 (m, 5H, CH), 7.86 (d, J = 8.4 Hz, 2H, CH); δC (100 MHz, d⁶-DMSO) 25.4, 26.2, 33.7, 35.8, 43.2, 50.7, 51.6, 115.9, 118.4, 120.3, 126.5, 126.9, 127.2, 127.4, 127.5, 128.4, 129.0, 131.3, 134.1, 136.9, 138.3, 144.1, 146.3, 146.8, 161.6, 166.5, 171.1; HRMS (ES+) calcd for [C₃₅H₃₆N₂O₆S + H] 613.2366, found 613.2356; HPLC (V) tᵣ = 22.09 min (96.50 %), (VI) tᵣ = 38.47 min (89.47 %).

3.7d, 4-(N-(4-Cyclohexylbenzyl)-2-(N-methynaphthalene-2-sulfonamido)acetamido)-2-hydroxy-benzoic acid. Benzyl protected 3.6d was globally deprotected on a 0.07 mmol scale via General Procedure B to furnish 3.7d (35 mg, 87 %): δH (400 MHz, d⁶-DMSO) 1.28-1.39 (m, 5H, CH₂), 1.62 -1.79 (m, 5H, CH₂), 2.37-2.46 (m, 1H, CH), 2.88 (s, 3H, CH₃), 3.95 (s, 2H, CH₂), 4.76 (s, 2H, CH₂), 6.80 (dd, J = 8.4 and 2.4 Hz, 1H, CH), 6.88 (d, J = 2.0 Hz, 1H, CH), 7.02 (d, J = 8.0 Hz, 2H, CH), 7.08 (d, J = 8.0 Hz, 2H, CH), 7.62-7.73 (m, 3H, CH), 7.79 (d, J = 8.4 Hz, 1H, CH), 8.04 (d, J = 8.4 Hz, 1H, CH), 8.09 (d, J = 8.8 Hz, 1H, CH), 8.12 (d, J = 8.0 Hz, 1H, CH), 8.35 (d, J = 1.6 Hz, 1H, CH); δC (100 MHz, d⁶-DMSO) 25.4, 26.2, 33.8, 35.9, 43.2, 50.8, 51.5, 112.5, 116.0, 118.5, 119.9, 122.5, 126.5, 127.4, 127.7, 127.8, 128.7, 129.1, 131.3, 131.6, 134.1, 134.2, 135.1, 146.3 146.8, 161.5, 166.5, 171.1; HRMS (ES+) calcd for [C₃₃H₃₄N₂O₆S + H] 587.2210, Found 587.2196; HPLC (V) tᵣ = 20.69 min (98.96 %), (VI) tᵣ = 35.01 min (95.03 %).
3.7e, 4-(N-(4-cyclohexylbenzyl)-2-(N-methylnaphthalene-1-sulfonamido)acetamido)-2-hydroxy-benzoic acid. Benzyl protected 3.6e was globally deprotected on a 0.12 mmol scale via General Procedure B to furnish 3.7e (72 mg, 94 %): $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.30-1.45 (m, 5H, CH$_2$), 1.70-1.92 (m, 5H, CH$_2$), 2.42-2.52 (m, 1H, CH), 2.99 (s, 3H, CH$_3$), 3.98 (s, 2H, CH$_2$), 4.80 (s, 2H, CH$_2$), 6.58 (d of d, $J$ = 8.0 and 2.0 Hz, 1H, CH), 6.70 (d, $J$ = 2.0 Hz, 1H, CH), 7.04 (d, $J$ = 8.0 Hz, 2H, CH), 7.11 (d, $J$ = 8.0 Hz, 2H, CH), 7.51 (t, $J$ = 8.0 Hz, 1H, CH), 7.56-7.61 (m, 2H, CH), 7.87 (d, $J$ = 8.4 Hz, 1H, CH), 7.89-7.92 (m, 1H, CH), 8.04 (d, $J$ = 8.0 Hz, 1H, CH), 8.25 (d, $J$ = 7.2 Hz, 1H, CH), 8.61 (d, $J$ = 8.0 Hz, 1H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 25.9, 26.7, 34.2, 35.9, 44.1, 50.9, 53.0, 112.8, 117.2, 119.1, 123.9, 124.9, 126.7, 126.9, 128.0, 128.3, 128.6, 128.7, 129.6, 132.2, 133.2, 133.7, 134.2, 147.6, 162.8, 167.0, 172.1; HRMS (ES+) calcd for [C$_{33}$H$_{34}$N$_2$O$_6$S + H] 587.2210, Found 587.2196; HPLC (III) $t_R$ = 19.22 min (76.63 %), (IV) $t_R$ = 43.81 min (79.95 %).
3.7f, 4-(N-(4-cyclohexylbenzyl)-2-(N-methylquinoline-8-sulfonamido)acetamido)-2-hydroxybenzoic acid. Benzyl protected 3.6f was globally deprotected on a 0.29 mmol scale via General Procedure C to furnish 3.7f (123 mg, 84 %): \( \delta^H (400 \text{ MHz, } d-\text{CDCl}_3) 1.29-1.41 \) (m, 5H, CH\(_2\)), 1.69-1.86 (m, 5H, CH\(_2\)), 2.40-2.52 (m, 1H, CH), 2.77 (s, 3H, CH\(_3\)), 3.86 (s, 2H, CH\(_2\)), 4.85 (s, 2H, CH\(_2\)), 6.55 (t, \( J = 8.0 \text{ Hz}, 1\text{H, CH} \)), 6.61 (d, \( J = 8.0 \text{ Hz}, 1\text{H, CH} \)), 6.70 (s, 1H, CH), 7.04-7.14 (m, 8H, CH), 7.47 (d, \( J = 8.0 \text{ Hz}, 1\text{H, CH} \)), 7.86 (d, \( J = 8.4 \text{ Hz}, 1\text{H, CH} \)); \( \delta^C (100 \text{ MHz, } d-\text{CDCl}_3) 26.7, 34.2, 36.1, 41.5, 44.1, 50.6, 53.1, 114.9, 116.9, 119.1, 123.8, 124.8, 126.9, 128.3, 128.9 (br), 132.2, 133.1, 134.4, 147.7, 162.8, 167.8, 171.9; HRMS (ES+) calcd for \([\text{C}_{32}\text{H}_{33}\text{N}_3\text{O}_6\text{S + H}] 592.2475\), Found 592.2467; HPLC (III) \( t_R = 23.95 \text{ min (100.00 %)} \), (IV) \( t_R = 53.41 \text{ min (100.00 %)} \).

3.7g, 4-(N-(4-cyclohexylbenzyl)-2-(5-(dimethylamino)-N-methylnaphthalene-1-sulfonamido)acetamido)-2-hydroxybenzoic acid. Benzyl protected 3.6g was globally deprotected on a 0.11 mmol scale via General Procedure B to furnish 3.7g (71 mg, 92 %): \( \delta^H (400 \text{ MHz, } d-\text{CDCl}_3) 1.23-1.40 \) (m, 5H, CH\(_2\)), 1.72-1.83 (m, 5H, CH\(_2\)), 2.42-2.46 (m, 1H, CH), 3.02 (s, 3H, CH\(_3\)), 3.33 (s, 6H, CH\(_3\)), 3.92 (s, 2H, CH\(_2\)), 4.78 (s, 2H, CH\(_2\)), 6.43 (d, \( J = 8.0 \text{ Hz}, 1\text{H, CH} \)), 6.56 (s, 1H, CH), 7.04 (d, \( J = 8.0 \text{ Hz}, 2\text{H, CH} \)), 7.10 (d, \( J = 8.0 \text{ Hz}, 2\text{H, CH} \)), 7.58-7.65 (m, 3H, CH), 7.70 (t, \( J = 8.0 \text{ Hz}, 1\text{H, CH} \)), 8.31 (d, \( J = 7.2 \text{ Hz}, 1\text{H, CH} \)), 8.59 (d, \( J = 8.4 \text{ Hz}, 1\text{H, CH} \)), 8.62-8.66 (m, 1H, CH); \( \delta^C (100 \text{ MHz, } d-\text{CDCl}_3) 26.2, 27.0, 34.5, 36.0, 44.3, 46.8, 51.3, 53.2, 112.4, 117.7, 118.9, 126.2, 126.3, 127.2, 127.3, 127.5, 128.6, 130.2, 130.6, 132.2, 133.5, 135.7, 140.0, 142.1, 147.2, 147.9, 158.2, 162.7, 166.9, 171.8; HRMS (ES+) calcd for
[C₃₅H₃₉N₃O₆S + H] 630.2632, Found 630.2622; HPLC (III) \( t_R = 21.26 \text{ min (90.39 %)} \), (IV) \( t_R = 46.32 \text{ min (93.61 %)} \).

3.7h, 4-(N-(4-cyclohexylbenzyl)-2-(N,1-dimethyl-1H-imidazole-4-sulfonamido)acetamido)-2-hydroxybenzoic acid. Benzyl protected 3.6h was globally deprotected on a 0.07 mmol scale via General Procedure B to furnish 3.7h (38 mg, 98 %): \( \delta_H (400 \text{ MHz, } d-\text{CDCl}_3) 1.20-1.38 \text{ (m, 5H, CH}_2), 1.70-1.83 \text{ (m, 5H, CH}_2), 2.39-2.49 \text{ (m, 1H, CH)}, 2.91 \text{ (s, 3H, CH}_3), 3.74 \text{ (s, 3H, CH}_3), 3.96 \text{ (s, 2H, CH}_2), 4.79 \text{ (s, 2H, CH}_2), 6.55-6.64 \text{ (m, 1H, CH)}, 6.67 \text{ (s, 1H, CH)}, 7.03-7.10 \text{ (m, 4H, CH)}, 7.45 \text{ (s, 1H, CH)}, 7.74 \text{ (d, } J = 7.6 \text{ Hz, 1H, CH}); \delta_C (100 \text{ MHz, } d-\text{CDCl}_3) 26.2, 27.0, 34.5, 44.3, 52.1, 53.2, 113.1, 116.7, 119.0, 127.1, 127.2, 128.6, 132.2, 133.9, 139.2, 139.3, 146.9, 147.6, 163.0, 167.0, 172.2; HRMS (ES+) calcd for [C_{27}H_{32}N_{4}O_{6}S + H] 541.2115, Found 541.2104; HPLC (III) \( t_R = 19.10 \text{ min (100.00 %)} \), (IV) \( t_R = 42.32 \text{ min (100.00 %)} \).
3.7i, 4-(2-(4-cyano-N-methylphenylsulfonamido)\(-N\)-(4-cyclohexylbenzyl)acetamido)-2-hydroxy-benzoic acid. Benzyl protected 3.6i was globally deprotected on a 0.13 mmol scale via General Procedure B to furnish 3.7i (65 mg, 87 %): δ_H (400 MHz, d-CDCl_3) 1.25-1.41 (m, 5H, CH_2), 1.71-1.85 (m, 5H, CH_2), 2.43-2.53 (m, 1H, CH), 2.93 (s, 3H, CH_3), 3.96 (s, 2H, CH_2), 4.78 (s, 2H, CH_2), 6.61 (dd, J = 8.4 and 1.6 Hz, 1H, CH), 6.72 (d, J = 1.6 Hz, 1H, CH), 7.03 (d, J = 8.0 Hz, 2H, CH), 7.13 (d, J = 8.0 Hz, 2H, CH), 7.77 (d, J = 8.4 Hz, 2H, CH), 7.89-7.94 (m, 3H, CH); δ_C (100 MHz, d-CDCl_3) 26.2, 27.0, 34.5, 36.1, 44.4, 51.9, 53.4, 111.7, 116.4, 117.3, 117.6, 119.3, 127.3, 128.3, 128.6, 132.7, 132.8, 133.3, 143.2, 147.9, 148.2, 163.2, 166.8, 172.7; HRMS (ES+) calcd for [C_{30}H_{31}N_{3}O_{6}S + H] 562.2006, Found 562.1997; HPLC (III) t_R = 8.69 min (92.00 %), (IV) t_R = 45.44 min (91.69 %).

![Chemical Structure of 3.7i]

3.7j, 4-(2-(4-bromo-N-methylphenylsulfonamido)\(-N\)-(4-cyclohexylbenzyl)acetamido)-2-hydroxy-benzoic acid. Benzyl protected 3.6j was globally deprotected on a 0.12 mmol scale via General Procedure C to furnish 3.7j (68 mg, 87 %): δ_H (400 MHz, d-CDCl_3) 1.24-1.40 (m, 5H, CH_2), 1.68-1.84 (m, 5H, CH_2), 2.42-2.51 (m, 1H, CH), 2.90 (s, 3H, CH_3), 3.85 (s, 2H, CH_2), 4.74 (s, 2H, CH_2), 6.50 (d of d, J = 8.4 and 1.6 Hz, 1H, CH), 6.63 (d, J = 1.6 Hz, 1H, CH), 7.01 (d, J = 8.0 Hz, 2H, CH), 7.08 (d, J = 8.0 Hz, 2H, CH), 7.59 (d, J = 8.8 Hz, 2H, CH), 7.64 (d, J = 8.8 Hz, 2H, CH), 7.85 (d, J = 8.4 Hz, 1H, CH); δ_C (100 MHz, d-CDCl_3) 26.2, 27.0, 34.5, 36.0, 39.5, 44.3, 51.5, 53.1, 113.2, 116.8, 118.8, 126.1, 127.1, 127.6, 129.2, 132.2, 132.3, 133.8, 138.0, 146.9, 147.7, 163.0, 166.6, 172.0; HRMS (ES+) calcd for [C_{29}H_{31}BrN_{2}O_{6}S + H] 615.1158, Found 615.1132; HPLC (III) t_R = 23.36 min (86.52 %), (IV) t_R = 53.43 min (100.00 %).
3.7k, 4-(2-(4-chloro-N-methylphenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)-2-hydroxy-benzoic acid. Benzyl protected 3.6k was globally deprotected on a 0.12 mmol scale via General Procedure C to furnish 3.7k (49 mg, 72%): $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.34 (m, 5H, CH$_2$), 1.75 (m, 5H, CH$_2$), 2.44 (m, 1H, CH), 2.84 (s, 3H, CH$_3$), 3.96 (s, 2H, CH$_2$), 4.77 (s, 2H, CH$_2$), 6.80 (dd, $J = 8.4$ and 2.0 Hz, 1H, CH), 6.89 (d, $J = 2.0$ Hz, 1H, CH), 7.04 (d, $J = 8.0$ Hz, 2H, CH), 7.13 (d, $J = 8.0$ Hz, 2H, CH), 7.64 (d, $J = 8.8$ Hz, 2H, CH), 7.72 (d, $J = 8.8$ Hz, 2H, CH), 7.78 (d, $J = 8.4$ Hz, 1H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 25.4, 26.2, 33.8, 35.8, 43.2, 50.8, 51.6, 112.4, 115.9, 118.4, 126.5, 127.4, 128.8, 129.1, 131.2, 134.1, 137.1, 137.5, 146.4, 146.8, 161.5, 166.4, 171.0; HRMS (ES+) calcd for [C$_{29}$H$_{31}$ClN$_2$O$_6$S + H] 571.1664, Found 571.1682; HPLC (V) $t_R = 20.92$ min (96.73 %), (VI) $t_R = 35.97$ min (97.88 %).

3.7l, 4-(N-(4-cyclohexylbenzyl)-2-(4-fluoro-N-methylphenylsulfonamido)acetamido)-2-hydroxy benzoic acid. Benzyl protected 3.6l was globally deprotected on a 0.06 mmol scale via General Procedure B to furnish 3.7l (30 mg, 85%): $\delta_H$ (400 MHz, $d_6$-DMSO) 1.28-1.39 (m, 5H,
CH$_2$), 1.62-1.79 (m, 5H, CH$_2$), 2.37-2.46 (m, 1H, CH), 2.88 (s, 3H, CH$_3$), 3.95 (s, 2H, CH$_2$), 4.76 (s, 2H, CH$_2$), 6.80 (dd, $J = 8.4$ and 2.4 Hz, 1H, CH), 6.88 (d, $J = 2.0$ Hz, 1H, CH), 7.02 (d, $J = 8.0$ Hz, 2H, CH), 7.08 (d, $J = 8.0$ Hz, 2H, CH), 7.62-7.73 (m, 3H, CH), 7.79 (d, $J = 8.4$ Hz, 1H, CH), 8.04 (d, $J = 8.4$ Hz, 1H, CH), 8.09 (d, $J = 8.8$ Hz, 1H, CH), 8.12 (d, $J = 8.0$ Hz, 1H, CH), 8.35 (d, $J = 1.6$ Hz, 1H, CH); $\delta$C (100 MHz, $d$_6-DMSO) 25.4, 26.2, 33.8, 35.7, 43.2, 50.8, 51.5, 55.5, 114.2, 115.9, 118.4, 121.3, 126.5, 127.4, 129.0, 131.2, 131.4, 134.2, 146.3, 146.3, 161.5, 166.6, 177.1; HRMS (ES+) calcd for [C$_{35}$H$_{34}$N$_2$O$_7$S + H] 587.2210, Found 587.2196; HPLC (V) $t_R$ = 19.62 min (95.61 %), (VI) $t_R$ = 33.96 min (95.10 %).

3.7m, 4-(N-(4-Cyclohexylbenzyl)-2-(4-methoxy-N-methylphenylsulfonyl)acetamido)-2-hydroxybenzoic acid. Benzyl protected 3.6m was globally deprotected on a 0.08 mmol scale via General Procedure B to furnish 3.7m (40 mg, 94 %): $\delta$H (400 MHz, $d$_6-DMSO) 1.32-1.38 (m, 5H, CH$_2$), 1.63-1.78 (m, 5H, CH$_2$), 2.39-2.49 (m, 1H, CH), 2.77 (s, 3H, CH$_3$), 3.82 (s, 3H, CH$_3$), 3.86 (s, 2H, CH$_2$), 4.80 (s, 2H, CH$_2$), 6.79 (dd, $J = 8.4$ and 2.0 Hz, 1H, CH), 6.87 (d, $J = 2.0$ Hz, 1H, CH), 7.07 (d, $J = 8.8$ Hz, 4H, CH), 7.13 (d, $J = 8.0$ Hz, 2H, CH), 7.61 (d, $J = 8.8$ Hz, 2H, CH), 7.78 (d, $J = 8.4$ Hz, 1H, CH); $\delta$C (100 MHz, $d$_6-DMSO) 25.4, 26.2, 33.8, 35.7, 43.2, 50.8, 51.6, 55.5, 114.2, 115.9, 118.4, 121.3, 126.5, 127.4, 129.0, 131.2, 131.4, 134.2, 146.3, 146.9, 161.5, 162.3, 166.6, 177.1; HRMS (ES+) calcd for [C$_{39}$H$_{33}$N$_2$O$_7$S + H] 567.2159, Found 567.2170; HPLC (V) $t_R$ = 19.67 min (100 %), (VI) $t_R$ = 33.74 min (95.49 %).
3.7n, 4-(N-(4-Cyclohexylbenzyl)-2-(N-methyl-4-nitrophenylsulfonamido)acetamido)-2-hydroxy benzoic acid. Benzyl protected 3.6n was globally deprotected on a 0.12 mmol scale via General Procedure C to furnish 3.7n (52 mg, 75 %): δ_H (400 MHz, d_6-DMSO) 1.25-1.42 (m, 5H, CH₂), 1.63-1.80 (m, 5H, CH₂), 2.38-2.47 (m, 1H, CH), 2.90 (s, 3H, CH₃), 4.03 (s, 2H, CH₂), 4.74 (s, 2H, CH₂), 6.79 (dd, 1H, J = 2.0 Hz, 1H, CH), 7.02 (d, J = 8.0 Hz, 2H, CH), 7.13 (d, J = 8.0 Hz, 2H, CH), 7.78 (d, J = 8.4 Hz, 1H, CH), 7.99 (d, J = 8.8 Hz, 2H, CH), 8.38 (d, J = 8.8 Hz, 2H, CH); δ_C (100 MHz, d_6-DMSO) 25.4, 26.2, 33.8, 35.7, 43.2, 50.8, 51.7, 115.6, 115.9, 118.2, 124.2, 126.5, 127.4, 128.5, 134.0, 144.0, 146.4, 146.5, 149.5, 161.6, 166.2, 171.0; HRMS (ES+) calcd for [C_{29}H_{31}N_{3}O_{8}S + H] 582.1904, Found 598.1878; HPLC (V) t_R = 20.51 min (97.58 %), (VI) t_R = 35.00 min (95.66 %).

3.7o, 4-(N-(4-Cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-methylphenylsulfonamido)acetamido)-2-hydroxybenzoic acid. Benzyl protected 3.6o was globally deprotected on a 0.06 mmol scale via General Procedure B to furnish 3.7o (37 mg, 99 %): δ_H (400 MHz, d_6-DMSO)
1.27-7.41 (m, 5H, CH₂), 1.64-1.79 (m, 5H, CH₂), 2.40-2.49 (m, 1H, CH), 3.00 (s, 3H, CH₃), 4.13 (s, 2H, CH₂), 4.77 (s, 2H, CH₂), 6.74 (dd, J = 8.4 and 2.0 Hz, 1H, CH), 6.83 (d, J = 2.0 Hz, 1H, CH), 7.04 (d, J = 8.0 Hz, 2H, CH), 7.12 (d, J = 8.0 Hz, 2H, CH), 7.80 (d, J = 8.4 Hz, 1H, CH); δC (100 MHz, d₆-DMSO) 25.4, 26.2, 33.8, 35.5, 43.2, 51.2, 51.7, 116.1, 118.4, 118.8, 126.5, 127.5, 131.4, 133.9, 146.3, 146.5, 161.5, 165.9, 171.0; HRMS (ES+) calcd for [C₂₉H₂₇F₅N₂O₆S + H] 627.1582, Found 627.1551; HPLC (V) t_R = 22.71 min (97.47 %), (VI) t_R = 39.92 min (95.22 %).
Appendix 3: Chapter 4 Experimental

1 Experimental

1.1 Gold Docking Simulations

Inhibitors were docked using GOLD docking software to Stat3 crystal structure, pdb 1BG1. Compounds were first optimized into a low energy geometry. The compound binding site was set to an area with a 12 Å radius surrounding Ser636.

1.2 Fluorescence Polarization Assay

The fluorescence polarization assay was performed as previously reported. Briefly, fluorescently labelled peptide probe (5-FAM-GpYLPQTV-NH2, CanPeptide, Pointe-Claire (Montreal), Quebec, Canada) was incubated with Stat3 protein (SignalChem, Richmond, British Columbia, Canada), and inhibitor for 30 minutes then analyzed on a Tecan M1000 fluorimeter (Tecan, Mannedorf, Switzerland). Polarized fluorescence was plotted against concentration of inhibitor and IC_{50} values were determined by fitting to a dose response curve. Representative curves of the top compounds are shown below.

**N-Alkyl-tolyl Derivatives Fluorescence Polarization Curves**
Figure A3.1. Competitive binding of 2.18h measured by fluorescence polarization assay, with a calculated IC$_{50}$ = 30 ± 9 µM. Curve fitted using ORIGIN software.

Figure A3.2. Competitive binding of 4.2aj measured by fluorescence polarization assay, with a calculated IC$_{50}$ = 8.1 ± 0.6 µM. Curve fitted using ORIGIN software.
Figure A3.3. Competitive binding of 4.2i measured by fluorescence polarization assay, with a calculated IC$_{50} = 2.8 \pm 4.3$ μM. Curve fitted using ORIGIN software.

Figure A3.4. Competitive binding of 4.2f measured by fluorescence polarization assay, with a calculated IC$_{50} = 8.8 \pm 2.9$ μM. Curve fitted using ORIGIN software.
Figure A3.5. Competitive binding of 4.2k measured by fluorescence polarization assay, with a calculated IC$_{50}$ = 9.1 ± 8.1 µM. Curve fitted using ORIGIN software.

Figure A3.6. Competitive binding of 4.2j measured by fluorescence polarization assay, with a calculated IC$_{50}$ = 10.2 ± 0.8 µM. Curve fitted using ORIGIN software.
N-Alkyl-perfluorobenzene Derivatives Fluorescence Polarization Curves

Figure A3.7. Competitive binding of 4.7e measured by fluorescence polarization assay, with a calculated IC$_{50}$ = 11.7 ± 1.9 µM. Curve fitted using ORIGIN software

Figure A3.8. Competitive binding of 4.7h measured by fluorescence polarization assay, with a calculated IC$_{50}$ = 12.7 ± 2.8 µM. Curve fitted using ORIGIN software
Figure A3.9. Competitive binding of 4.7z measured by fluorescence polarization assay, with a calculated IC$_{50}$ = 11.0 ± 1.6 μM. Curve fitted using ORIGIN software.

Figure A3.10. Competitive binding of 4.7i measured by fluorescence polarization assay, with a calculated IC$_{50}$ = 9.4 ± 2.7 μM. Curve fitted using ORIGIN software.
### Figure A3.11. Competitive binding of 3.7o measured by fluorescence polarization assay, with a calculated IC$_{50} = 25 \pm 2 \, \mu$M. Curve fitted using ORIGIN software

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Figure A3.12. Competitive binding of 4.7j measured by fluorescence polarization assay, with a calculated IC$_{50} = 22 \pm 5 \, \mu$M. Curve fitted using ORIGIN software
1.3 MTS Assay Representative Data Plots

MDA-468 (breast), DU145 (prostate) and OCI-AML2 (Leukemia) cells were loaded in 96 well plates at 10 000 cells per well and incubated with various concentration of inhibitor for 72 hours followed by 3 hour incubation with MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium). Relative cell viability (to DMSO control) was determined colourometrically and EC\textsubscript{50} values determined by fitting to a standard dose response curve.

AML2

Figure A3.13. AML2 cell viability after 72 hour incubation with 2.18h measured by MTS assay relative to DMSO control: calculated IC\textsubscript{50} = 29 ± 1 μM, curve fitted using ORIGIN software
Figure A3.14. AML2 cell viability after 72 hour incubation with 4.2f measured by MTS assay relative to DMSO control: calculated IC$_{50}$ = 18 ± 4 µM, curve fitted using ORIGIN software.

Figure A3.15. AML2 cell viability after 72 hour incubation with 4.2i measured by MTS assay relative to DMSO control: calculated IC$_{50}$ = 12 ± 1 µM, curve fitted using ORIGIN software.
Figure A3.16. AML2 cell viability after 72 hour incubation with 4.2aj measured by MTS assay relative to DMSO control: calculated IC\(_{50}\) = 17 ± 6 μM, curve fitted using ORIGIN software.
Figure A3.17. AML2 cell viability after 72 hour incubation with 4.2k measured by MTS assay relative to DMSO control: calculated IC$_{50} = 21 \pm 1 \mu M$, curve fitted using ORIGIN software.

Figure A3.18. AML2 cell viability after 72 hour incubation with 4.2j measured by MTS assay relative to DMSO control: calculated IC$_{50} = 25 \pm 1 \mu M$, curve fitted using ORIGIN software.
Figure A3.19. AML2 cell viability after 72 hour incubation with 3.7o measured by MTS assay relative to DMSO control: calculated IC₅₀ = 10 ± 1 μM, curve fitted using ORIGIN software.

![Graph showing AML2 cell viability](image1)

Figure A3.20. AML2 cell viability after 72 hour incubation with 4.7e measured by MTS assay relative to DMSO control: calculated IC₅₀ = 3.6 ± 0.5 μM, curve fitted using ORIGIN software.

![Graph showing AML2 cell viability](image2)
Figure A3.21. AML2 cell viability after 72 hour incubation with 4.7h measured by MTS assay relative to DMSO control: calculated IC$_{50}$ = 4.6 ± 0.1 μM, curve fitted using ORIGIN software

![Graph showing viability and concentration relationship]

Figure A3.22. AML2 cell viability after 72 hour incubation with 4.7i measured by MTS assay relative to DMSO control: calculated IC$_{50}$ = 9.5 ± 1.5 μM, curve fitted using ORIGIN software

![Graph showing viability and concentration relationship]
Figure A3.23. AML2 cell viability after 72 hour incubation with 4.7j measured by MTS assay relative to DMSO control: calculated IC\textsubscript{50} = 6.4 ± 0.6 μM, curve fitted using ORIGIN software

Figure A3.24. AML2 cell viability after 72 hour incubation with 4.7z measured by MTS assay relative to DMSO control: calculated IC\textsubscript{50} = 1.9 ± 0.1 μM, curve fitted using ORIGIN software
**Figure A3.25.** MDA-468 cell viability after 72 hour incubation with 2.18h measured by MTS assay relative to DMSO control: calculated IC\textsubscript{50} = 17 ± 1 µM, curve fitted using ORIGIN software.
Figure A3.26. MDA-468 cell viability after 72 hour incubation with 4.2f measured by MTS assay relative to DMSO control: calculated IC$_{50} = 17 \pm 3$ μM, curve fitted using ORIGIN software.

Figure A3.27. MDA-468 cell viability after 72 hour incubation with 4.2i measured by MTS assay relative to DMSO control: calculated IC$_{50} = 11 \pm 3$ μM, curve fitted using ORIGIN software.
Figure A3.28. MDA-468 cell viability after 72 hour incubation with 4.2aj measured by MTS assay relative to DMSO control: calculated IC$_{50} = 17 \pm 2 \mu$M, curve fitted using ORIGIN software

![Graph showing cell viability with 4.2aj](image)

Figure A3.29. MDA-468 cell viability after 72 hour incubation with 4.2k measured by MTS assay relative to DMSO control: calculated IC$_{50} = 13 \pm 2 \mu$M, curve fitted using ORIGIN software

![Graph showing cell viability with 4.2k](image)
Figure A3.30. MDA-468 cell viability after 72 hour incubation with 4.2j measured by MTS assay relative to DMSO control: calculated IC\textsubscript{50} = 14 ± 2 μM, curve fitted using ORIGIN software

Figure A3.31. MDA-468 cell viability after 72 hour incubation with 3.7o measured by MTS assay relative to DMSO control: calculated IC\textsubscript{50} = 11 ± 1 μM, curve fitted using ORIGIN software
Figure A3.32. MDA-468 cell viability after 72 hour incubation with 4.7e measured by MTS assay relative to DMSO control: calculated IC\textsubscript{50} = 6.8 ± 1.1 μM, curve fitted using ORIGIN software.

Figure A3.33. MDA-468 cell viability after 72 hour incubation with 4.7h measured by MTS assay relative to DMSO control: calculated IC\textsubscript{50} = 8.1 ± 0.2 μM, curve fitted using ORIGIN software.
Figure A3.34. MDA-468 cell viability after 72 hour incubation with 4.7i measured by MTS assay relative to DMSO control: calculated IC\textsubscript{50} = 13.6 ± 3.5 µM, curve fitted using ORIGIN software.

Figure A3.35. MDA-468 cell viability after 72 hour incubation with 4.7j measured by MTS assay relative to DMSO control: calculated IC\textsubscript{50} = 8.5 ± 0.6 µM, curve fitted using ORIGIN software.
Figure A3.36. MDA-468 cell viability after 72 hour incubation with 4.7z measured by MTS assay relative to DMSO control: calculated IC$_{50}$ = 5.0 ± 0.5 µM, curve fitted using ORIGIN software

DU145
### Figure A3.37. DU145 cell viability after 72 hour incubation with 2.18h measured by MTS assay relative to DMSO control: calculated IC$_{50}$ = 35 ± 4 μM, curve fitted using ORIGIN software

### Figure A3.38. DU145 cell viability after 72 hour incubation with 4.2f measured by MTS assay relative to DMSO control: calculated IC$_{50}$ = 17 ± 1 μM, curve fitted using ORIGIN software
Figure A3.39. DU145 cell viability after 72 hour incubation with 4.2i measured by MTS assay relative to DMSO control: calculated IC$_{50}$ = 16 ± 1 μM, curve fitted using ORIGIN software.

Figure A3.40. DU145 cell viability after 72 hour incubation with 4.2aj measured by MTS assay relative to DMSO control: calculated IC$_{50}$ = 11 ± 2 μM, curve fitted using ORIGIN software.
Figure A3.41. DU145 cell viability after 72 hour incubation with 4.2k measured by MTS assay relative to DMSO control: calculated IC$_{50}$ = 17 ± 2 μM, curve fitted using ORIGIN software

Figure A3.42. DU145 cell viability after 72 hour incubation with 4.2j measured by MTS assay relative to DMSO control: calculated IC$_{50}$ = 19 ± 1 μM, curve fitted using ORIGIN software
Figure A3.43. DU145 cell viability after 72 hour incubation with 3.7o measured by MTS assay relative to DMSO control: calculated IC$_{50}$ = 23 ± 2 µM, curve fitted using ORIGIN software.

Figure A3.44. DU145 cell viability after 72 hour incubation with 4.7e measured by MTS assay relative to DMSO control: calculated IC$_{50}$ = 9.5 ± 0.3 µM, curve fitted using ORIGIN software.
Figure A3.45. DU145 cell viability after 72 hour incubation with 4.7h measured by MTS assay relative to DMSO control: calculated IC\textsubscript{50} = 14 ± 2 μM, curve fitted using ORIGIN software

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Figure A3.46. DU145 cell viability after 72 hour incubation with 4.7i measured by MTS assay relative to DMSO control: calculated IC\textsubscript{50} = 27 ± 6 μM, curve fitted using ORIGIN software

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Figure A3.47. DU145 cell viability after 72 hour incubation with 4.7j measured by MTS assay relative to DMSO control: calculated IC$_{50}$ = 19 ± 3 µM, curve fitted using ORIGIN software.

Figure A3.48. DU145 cell viability after 72 hour incubation with 4.7z measured by MTS assay relative to DMSO control: calculated IC$_{50}$ = 9.1 ± 4.8 µM, curve fitted using ORIGIN software.
1.4 STAT Isoform Selectivity

FP Assay

Fluorescence polarization assays with Stat1 and Stat5 were conducted with lead inhibitors \textbf{4.2i} and \textbf{4.7h} to investigate isoform selectivity (as previously reported).\textsuperscript{2} Data plots and IC\textsubscript{50} values are shown below.

![Graph showing fluorescence polarization for Stat1 and Stat5 with IC\textsubscript{50} values and model equations.](image-url)

\textbf{Model}

\textbf{Equation}

\[ y = A_1 + \frac{A_2 - A_1}{1 + 10^{(\log x_0 - x) p}} \]

\textbf{Reduced Chi-Sqr}

1.96526

\textbf{Adj. R-Square}

0.99492

\begin{tabular}
\hline
Fluorescence & Value & Standard Error \\
\hline
A_1 & 0.05413 & 1.14741 \\
A_2 & 105.22474 & 8.48984 \\
\log x_0 & 5.80386 & 0.63167 \\
p & -0.14913 & 0.03066 \\
\span & 105.17062 & \\
EC_{20} & 6.93426E9 & \\
EC_{50} & 636594.92571 & \\
EC_{80} & 58.44212 & \\
\end{tabular}

\textbf{Model}

\textbf{Equation}

\[ y = A_1 + \frac{A_2 - A_1}{1 + 10^{(\log x_0 - x) p}} \]

\textbf{Reduced Chi-Sqr}

0.20926

\textbf{Adj. R-Square}

0.982

\begin{tabular}
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Fluorescence & Value & Standard Error \\
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A_1 & -0.1633 & 0.67684 \\
A_2 & 28 & 0 \\
\log x_0 & 8.46812 & 1.11309 \\
p & -0.07677 & 0.01268 \\
\span & 28.1633 & \\
EC_{20} & 2.04296E16 & \\
EC_{50} & 2.93849E8 & \\
EC_{80} & 4.22659 & \\
\end{tabular}
Isoform Selectivity for 4.2i (FP assay)

Stat1 IC$_{50}$ = 5.8 ± 0.6 µM

Stat3 IC$_{50}$ = 2.8 ± 4.3 µM

Stat5 IC$_{50}$ = 8.5 ± 1.1 µM
**Isoform Selectivity for 4.7h (FP assay)**

Stat1 IC$_{50}$ = 10.9 ± 0.8 μM

Stat3 IC$_{50}$ = 12.8 ± 2.8 μM

Stat5 IC$_{50}$ = 13.3 ± 0.9 μM

**Phosphoflow Cytometry**

The effects of 16i and 21h on STAT1,3 and 5 phosphorylation was assessed by flow cytometry using the monocytic cell line U937. This cell line was selected as it expresses pSTAT1, 3 and 5 in response to respective cytokines. U937 cells were serum-starved overnight to reduce the basal levels of protein phosphorylation, and treated with compounds for 4 hours the following morning. During the last 15 minutes of treatment, cells were stimulated with human recombinant IFNα, IL-6 or GM-CSF to induce phosphorylation of STAT1, STAT3 and STAT5, respectively (R&D Systems). Cells were then fixed in 3 % formaldehyde at room temperature for 15 minutes and permeabilized by adding ice-cold methanol (to a final concentration of 90 %). After 30 minutes on ice, cells were washed twice in PBS, once in staining medium (PBS + 3 % FCS), and resuspended in 50 μL of staining medium containing the respective fluorophore-conjugated antibodies against pSTAT1-Alexa488 (5 μL), pSTAT3-PE (5 μL) and pSTAT5-PE (2 μL) (BD Biosciences). Cells were incubated with antibodies for 30 minutes, washed once, and resuspended in 100 μL of staining medium for flow cytometric analysis (FACSCaliber flow cytometer and FlowJo Software).
Figure A3.49. Analysis of 4.2i and 4.7h selectivity for cytokine-induced STAT phosphorylation. Serum-starved U937 cells were treated with 4.2i or 4.7h for 4 h prior to stimulation with indicated cytokine for 15 minutes. (A) Representative histograms from phospho-flow cytometric analysis of IL-6-induced Stat3 phosphorylation reveals that both 4.2i (upper) and 4.7h (lower) abrogate IL-6-stimulated pStat3. (B) Graphical comparison of 4.2i and 4.7h selectivity for inhibiting Stat1, 3, and 5 phosphorylation as assessed by phospho-flow cytometry.

1.5 Annexin V/PI Apoptosis Assay

Cell death was assessed by flow cytometric analysis of apoptosis. Target cells were washed once in PBS and resuspended at 5-10 × 10⁵ cells/ml in apoptosis binding buffer (100 mM HEPES/NaOH, pH 7.5 containing 1.4 M NaCl and 25 mM CaCl₂) from the TACS Apoptosis Detection Kit (R&D Systems, Minneapolis, MN). Approximately 100 µl of the cell suspension was transferred to a polystyrene tube and incubated with 2 µl of Annexin V-Fluorescein isothiocyanate (FITC) conjugate and 5 µl Propidium Iodide (PI) solution. Following incubation of tubes for 10 minutes at room temperature, 100 µl of binding buffer was added and samples analyzed by flow cytometry. All samples were analyzed on a BD FACSCalibur™ flow cytometer (BD Biosciences) programmed to collect a minimum of 10 000 events. Results were analyzed using Flowjo v7.6 (Tree Star, Ashland, OR) and data are presented as the sum of percent Annexin V⁺/PI⁻ and Annexin V⁺/PI⁺ cell populations. MM cell lines were seeded at a density of 5x10⁵ cells/ml and treated with increasing compound concentrations for 24 and 48 hours. Apoptosis was measured by staining with Annexin V and PI (Boehringer Mannheim, Indianapolis, IN) and samples were analyzed by FACSCalibur using CellQuest Pro software.
Figure A3.50. Compound 16i induces apoptosis in a variety of MM cell lines as evaluated in the Annexin V apoptosis assay

1.6 Evaluation of compounds against MM patient samples

Bone marrow aspirates were obtained from MM patients with consent under a protocol approved by the Research Ethic Board of University Health Network, Toronto, Canada. Samples were diluted with PBS, and mononuclear cells (MNCs) isolated by density-based cell separation (Ficoll-Paque™ PLUS, GE Healthcare, Uppsala, Sweden). Briefly, Ficoll-Paque solution was added at one third the volume and samples then centrifuged for 30 minutes at 1500 rpm to isolate the interphase MNC population. The resulting interphase layer was collected with a sterile Pasteur pipette and washed one with sterile PBS, followed by depletion of residual contaminating red blood cells using ammonium-chloride-potassium lysis buffer (0.15 M NH₄Cl, 1 mM KHCO₃, 0.1 mM Na₂EDTA, pH 7.3). After 3-5 minutes, cells were washed once with PBS and once with pre-warmed IMDM medium supplemented with 10 % fetal calf serum (FCS). Cells were counted and suspended to a final concentration of 5-10x10⁵ cells/ml in complete IMDM medium. A small aliquot of isolated MNCs (5-10 x10⁵ cells) was sampled and stained for the cell surface antigen CD138 using a mouse anti-human CD138-Phycoerythrin (PE) antibody (BD Biosciences, Mountain View, CA). The aliquoted sample was washed in PBS and re-
suspended in 50-100 μl staining buffer (PBS + 3 % FBS) prior to the addition of CD138-PE (5-10 μl). Samples were incubated with CD138-PE antibody for 30 minutes, washed with PBS and resuspended in staining buffer for analysis by flow cytometry. The remaining cells were plated in IMDM + 10 % FCS and treated with the indicated drug concentrations. After 24 hours, cells were washed in PBS and stained with CD138-PE as outline above, except that following the final wash, cells were also stained for Annexin V antigen expression. Briefly, cells were resuspended in 100 μl of apoptosis binding buffer and incubated for 10 minutes with 2 μl of Annexin V-FITC antibody. Samples were analyzed by flow cytometry as outlined previously and cytotoxicity was evaluated based on the percentage of CD138+/Annexin V- MM cells in vehicle treated samples compared to drug treated samples.

To evaluate the activity of this agent on healthy hematopoietic progenitor colony formation, MNCs from primary MM patient BM aspirates were suspended in culture medium containing 2 % FBS (2-10 × 10⁴), and combined 1:10 with methylcellulose medium (MethoCult® H4434 Classic, Stem Cell Technologies, Vancouver, BC) containing BP-4-018 (final concentration of 15 µM). Samples were then plated in sterile 35 mm culture dishes, placed inside an additional dish containing 3-4 ml of sterile water and incubated at 37 °C in 5 % CO₂. After 14 days, formed colonies were manually counted using a 60 mm gridded dish under an inverted light microscope. Note that total progenitor colonies were counted, but not discriminated by progenitor type.

1.7 Chemical Methods

Anhydrous solvents methanol, DMSO, CH₂Cl₂, THF and DMF were purchased from Sigma Aldrich and used directly from Sure-Seal bottles. All reactions were performed under an atmosphere of dry nitrogen in oven-dried glassware and were monitored for completeness by thin-layer chromatography (TLC) using silica gel (visualized by UV light, or developed by treatment with KMnO₄ stain or phosphomolybdic acid stain). ¹H and ¹³C NMR spectra were recorded on a Bruker 400 MHz spectrometer in either CDCl₃, CD₃OD or d₆-DMSO. Chemical shifts (δ) are reported in parts per million after calibration to residual isotopic peak. Coupling constants (J) are reported in Hz. Before biological testing, inhibitor purity was evaluated by reversed-phase HPLC (rpHPLC). Analysis by rpHPLC was performed using a Microsorb-MV 300 Å C18 250 mm x 4.6 mm column run at 1 mL/min, and using gradient mixtures. The linear
gradient consisted of a changing solvent composition of either (I) 100 % H2O with 0.1 % TFA for two minutes to 100 % MeCN with 10 % H2O and 0.1 % TFA (v/v) at 22 minutes and UV detection at 254nm or (I) 100 % H2O with 0.1 % TFA for two minutes to 100 % MeCN with 10 % H2O and 0.1 % TFA (v/v) at 22 minutes and UV detection at 254nm or (II) 100 % H2O with 0.1 % TFA for two minutes to 100 % MeCN with 10 % H2O and 0.1 % TFA (v/v) at 62 mins and UV detection at 254nm. For reporting HPLC data, percentage purity is given in parentheses after the retention time for each condition. All biologically evaluated compounds are > 95 % chemical purity as measured by HPLC.

1.8 General Procedures

**General Procedure A (alkylation of sulfonamide).** Activated alkyl halides (1.1 equiv.) were added to a stirred suspension of sulfonamide 2.30 or 4.5 (1 equiv.) and Cs₂CO₃ (1.5 equiv.) in DMF. After 3 hours the mixture was diluted with H2O then the product was extracted into EtOAc. Organics were combined and washed with 1 M HCl, saturated NaHCO₃, H₂O and brine, then dried over Na₂SO₄ and concentrated under reduced pressure. Crude products were purified using flash column chromatography using a mixture of hexanes and EtOAc as the eluent.

**General Procedure B (global deprotection of benzylated salicylic acid).** The dibenzyl protected salicylic acid (1 equiv.) was dissolved in a stirred solution of THF/MeOH (1:1) (0.1 M). The solution was degassed thoroughly before careful addition of 10 % Pd/C (10 mg/mmol). H₂ gas was bubbled through the solvent for 5 mins before the solution was put under an atmosphere of H₂ gas and stirred continuously for 6 hours. The H₂ gas was evacuated and the reaction filtered (to remove the Pd catalyst) and concentrated under reduced pressure. Crude product was then purified using flash column chromatography using a mixture of DCM, MeOH and AcOH as the eluent (generally 92 % DCM, 7 % MeOH and 1 % AcOH in a 1:2 ratio with DCM was utilized).

**General Procedure C (step-wise deprotection of benzylated salicylic acid).** The dibenzyl protected salicylic acid (1 equiv.) was dissolved in THF and diluted with H₂O in a 1:3 ratio. LiOH.H₂O (3 equiv.) was added and the reaction was then stirred at room temperature for 1-6 hours. THF was removed under reduced pressure then the mixture was acidified and the product was extracted into EtOAc. The combined organics were washed with 1M HCl, H₂O and brine
then dried over Na$_2$SO$_4$, concentrated and used without further purification. The mono-benzyl protected salicylic acid (benzyl ether) was then dissolved in DMF and then treated with trifluoroacetic acid in a 9:1 ratio. The reaction mixture was stirred for 1 hour at room temperature then diluted with H$_2$O and organics were extracted into EtOAc. Organics were combined and washed with 1M HCl, H2O and brine then dried over Na$_2$SO$_4$. Crude product was then purified using flash column chromatography using a mixture of DCM, MeOH and AcOH as the eluent (generally 92 % DCM, 7 % MeOH and 1 % AcOH in a 1:2 ratio with DCM was utilized).

**General Procedure D (Acylation of sulfonamide nitrogen).** Unfunctionalized sulfonamide (1 equiv.) was dissolved in DCM then treated with DMAP (0.1 equiv.) then anhydride and stirred for 30 minutes. The reaction mixture was then diluted with DCM and washed with 1M HCl, saturated NaHCO$_3$, brine, then dried over Na$_2$SO$_4$ and concentrated. Crude product was purified using flash column chromatography in a mixture of hexanes and EtOAc.

### 1.9 Characterization

![Chemical Structure](image)

**4.1a, benzyl 4-(2-(N-allyl-4-methylphenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)-2-(benzyloxy)benzoate.** Sulfonamide 2.30 was functionalized on a 0.07 mmol scale using General Procedure A to give 4.1a (53.7 mg, 100 %); $\delta_H$ (400 MHz, $d$-CHCl$_3$) 1.31-1.46 (m, 5H, CH$_2$), 1.70-1.94 (m, 5H, CH$_2$), 2.39-2.55 (m, 4H, CH and CH$_3$), 3.76 (s, 2H, CH$_2$), 3.90 (d, $J = 6.8$ Hz, 2H, CH$_2$), 3.91 (s, 2H, CH$_2$), 4.75 (s, 2H, CH$_2$), 4.92 (s, 2H, CH$_2$), 5.06-5.15 (m, 2H, CH$_2$), 5.53-5.68 (m, 1H, CH), 6.52 (s, 1H, CH), 6.66 (d of d, $J = 8.0$ and 1.4 Hz, 1H, CH), 7.01
(d, J = 8.0 Hz, 2H, CH), 7.10 (d, J = 8.0 Hz, 2H, CH), 7.24-7.42 (m, 12H, CH), 7.70 (d, J = 8.0 Hz, 2H, CH), 7.82 (d, J = 8.0 Hz, 1H, CH); δC (100 MHz, d-CHCl₃) 21.7, 26.2, 26.9, 34.6, 44.3, 47.2, 50.8, 52.9, 67.1, 70.8, 114.1, 119.5, 120.2, 127.0, 127.2, 127.6, 128.2, 128.3, 128.4, 128.7, 128.7, 129.0, 129.6, 133.0, 133.2, 134.1, 135.8, 137.1, 137.8, 143.3, 145.3, 147.8, 158.9, 165.5, 167.1; LRMS (ES+) Calcd for [C₄₀H₄₈N₂O₆S + Na] 779.31 found 779.41.

4.1b, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(N-isobutyl-4-methylphenylsulfonamido)acetamido)benzoate. Sulfonamide 2.30 was functionalized on a 0.07 mmol scale using General Procedure A to give 4.1b (22.9 mg, 43 %); δH (400 MHz, d-CHCl₃) 0.81 (d, J = 6.8 Hz, 6H, CH₃), 1.30-1.44 (m, 5H, CH₂), 1.64-1.90 (m, 6H, CH₂ and CH), 2.37-2.52 (m, 4H, CH and CH₃), 3.07 (d, J = 7.2 Hz, 2H, CH₂), 3.73 (s, 2H, CH₂), 4.71 (s, 2H, CH₂), 4.94 (s, 2H, CH₂), 5.35 (s, 2H, CH₂), 6.52 (s, 1H, CH), 6.67 (d of d, J = 8.4 and 1.6 Hz, 1H, CH), 6.98 (d, J = 8.0 Hz, 2H, CH), 7.10 (d, J = 8.0 Hz, 2H, CH), 7.23 (d, J = 8.0 Hz, 2H, CH), 7.28-7.42 (m, 10H, CH), 7.65 (d, J = 8.4 Hz, 2H, CH), 7.83 (d, J = 8.0 Hz, 1H, CH); δC (100 MHz, d-CHCl₃) 20.1, 21.7, 26.2, 26.9, 26.9, 34.6, 44.3, 48.1, 52.8, 55.8, 67.1, 70.8, 114.2, 120.2, 120.9, 127.1, 127.2, 127.7, 128.2, 128.3, 128.4, 128.7, 128.7, 129.0, 129.4, 133.3, 134.2, 135.9, 137.1, 143.8, 145.4, 147.8, 158.9, 165.6, 167.1; LRMS (ES+) Calcd for [C₄₇H₅₂N₂O₆S + Na] 795.34 found 795.56.
4.1c, benzyl 4-(2-(N-(2-amino-2-oxoethyl)-4-methylphenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)-2-(benzyloxy)benzoate. Sulfonamide 2.30 was functionalized on a 0.07 mmol scale using General Procedure A to give 4.1c (40.2 mg, 74 %); δ_H (400 MHz, _d_-CHCl_3) 1.29-1.45 (m, 5H, CH_2), 1.68-1.92 (m, 5H, CH_2), 2.36 (s, 3H, CH_3), 2.42-2.54 (m, 1H, CH), 3.56 (s, 2H, CH_2), 3.64 (s, 2H, CH_2), 4.84 (s, 2H, CH_2), 4.94 (s, 2H, CH_2), 5.36 (s, 2H, CH_2), 5.59 (s, 1H, NH), 6.58 (s, 1H, CH), 6.76 (d, _J_ = 8.0 Hz, 1H, CH), 7.08 (d, _J_ = 8.0 Hz, 2H, CH), 7.13 (d, _J_ = 8.0 Hz, 2H, CH), 7.22 (d, _J_ = 8.0 Hz, 2H, CH), 7.25-7.36 (m, 8H, CH), 7.37-7.44 (m, 2H, CH), 7.55 (d, _J_ = 8.4 Hz, 2H, CH), 7.83 (d, _J_ = 8.0 Hz, 1H, CH), 8.73 (s, 1H, NH); δ_C (100 MHz, _d_-CHCl_3) 21.7, 26.2, 26.9, 34.6, 44.3, 52.0, 53.0, 53.7, 67.3, 70.8, 114.3, 120.2, 121.4, 127.3, 127.3, 127.8, 128.3, 128.5, 128.7, 128.8, 129.0, 130.0, 133.3, 133.6, 134.4, 135.8, 135.8, 144.6, 144.7, 148.1, 158.7, 165.5, 168.3, 171.0; LRMS (ES+) Calcd for [C_{45}H_{47}N_{3}O_{7}S + Na] 796.30 found 796.43.
**4.1d, benzyl 4-(2-(N-benzyl-4-methylphenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)-2-(benzyloxy)benzoate.** Sulfonamide 2.30 was functionalized on a 0.07 mmol scale using General Procedure A to give 4.1d (49.3 mg, 87 %); $\delta_H$ (400 MHz, $d$-CHCl$_3$) 1.33-1.53 (m, 5H, CH$_2$), 1.68-1.98 (m, 5H, CH$_2$), 2.35-2.61 (m, 4H, CH and CH$_3$), 3.69 (s, 2H, CH$_2$), 4.57 (s, 2H, CH$_2$), 4.69 (s, 2H, CH$_2$), 4.78 (s, 2H, CH$_2$), 5.32 (s, 2H, CH$_2$), 6.28 (s, 1H, CH), 6.40 (s, 1H, CH), 6.89-7.49 (m, 21H, CH), 7.71 (d, $J = 6.6$ Hz, 1H, CH), 7.80 (d, $J = 5.6$ Hz, 2H, CH); $\delta_C$ (100 MHz, $d$-CHCl$_3$) 21.7, 26.2, 26.9, 34.6, 44.3, 46.8, 51.1, 52.8, 67.1, 70.8, 113.9, 120.1, 120.8, 127.0, 127.3, 127.8, 128.1, 128.2, 128.3, 128.4, 128.6, 128.7, 128.8, 128.9, 129.1, 129.6, 133.1, 134.1, 135.4, 135.8, 137.2, 143.4, 145.1, 147.8, 158.8, 165.5, 166.8; LRMS (ES+) Calcd for [C$_{50}$H$_{50}$N$_2$O$_6$S + Na] 829.33 found 829.46.

**4.1e, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(2-methylbenzyl)phenylsulfonamido)acetamido)benzoate.** Sulfonamide 2.30 was functionalized on a 0.07 mmol scale using General Procedure A to give 4.1e (45.7 mg, 80 %); $\delta_H$ (400 MHz, $d$-CHCl$_3$) 1.32-1.47 (m, 5H, CH$_2$), 1.71-1.92 (m, 5H, CH$_2$), 2.22 (s, 3H, CH$_3$), 2.42-2.54 (m, 4H, CH and CH$_3$), 3.61 (s, 2H, CH$_2$), 4.60 (s, 2H, CH$_2$), 4.64 (s, 2H, CH$_2$), 4.75 (s, 2H, CH$_2$), 5.31 (s, 2H, CH$_2$), 6.21 (s, 1H, CH), 6.34 (d, $J = 5.6$ Hz, 1H, CH), 6.94-7.20 (m, 8H, CH), 7.28-7.41 (m, 12H, CH), 7.69 (d, $J = 8.0$ Hz, 1H, CH), 7.77 (d, $J = 8.0$ Hz, 2H, CH); $\delta_C$ (100 MHz, $d$-CHCl$_3$) 19.1, 21.7, 26.2, 26.9, 34.6, 44.3, 46.9, 49.1, 52.7, 67.1, 70.8, 113.9, 120.2, 120.7, 126.0, 126.9, 127.2, 127.8, 128.2, 128.3, 128.3, 128.4, 128.6, 128.7, 129.2, 129.5, 130.1, 130.8, 133.0, 133.1,
134.0, 135.8, 137.0, 138.2, 143.3, 147.8, 158.8, 165.5, 166.7; LRMS (ES+) Calcd for [C\textsubscript{51}H\textsubscript{52}N\textsubscript{2}O\textsubscript{6}S + Na] 843.34 found 843.65.

**4.1f, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(3-methylbenzyl)phenylsulfonamido)acetamido)benzoate.** Sulfonamide 2.30 was functionalized on a 0.07 mmol scale using General Procedure A to give 4.1f (54.4 mg, 95%); δ\textsubscript{H} (400 MHz, d-CHCl\textsubscript{3}) 1.31-1.51 (m, 5H, CH\textsubscript{2}), 1.69-1.97 (m, 5H, CH\textsubscript{2}), 2.25 (s, 3H, CH\textsubscript{3}), 2.37-2.57 (m, 4H, CH and CH\textsubscript{3}), 3.70 (s, 2H, CH\textsubscript{2}), 4.54 (s, 2H, CH\textsubscript{2}), 4.70 (s, 2H, CH\textsubscript{2}), 4.78 (s, 2H, CH\textsubscript{2}), 5.32 (s, 2H, CH\textsubscript{2}), 6.30 (s, 1H, CH), 6.39 (d, J = 8.0 Hz, 1H, CH), 6.90-7.08 (m, 5H, CH), 7.08-7.22 (m, 4H, CH), 7.27-7.44 (m, 11H, CH) 7.71 (d, J = 8.0 Hz, 1H, CH), 7.80 (d, J = 8.0 Hz, 2H, CH); δ\textsubscript{C} (100 MHz, d-CHCl\textsubscript{3}) 21.4, 21.7, 26.2, 26.9, 34.6, 44.3, 47.6, 50.9, 52.7, 67.1, 70.8, 113.9, 120.1, 120.7, 126.0, 127.0, 127.3, 127.7, 128.2, 128.3, 128.4, 128.6, 128.6, 128.7, 128.8, 129.1, 129.5, 129.5, 133.1, 134.1, 135.3, 135.7, 137.4, 138.4, 143.3, 147.8, 158.8, 165.5, 166.8; LRMS (ES+) Calcd for [C\textsubscript{51}H\textsubscript{52}N\textsubscript{2}O\textsubscript{6}S + Na] 843.34 found 843.65.
4.1g, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(4-methylbenzyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 2.30 was functionalized on a 0.07 mmol scale using General Procedure A to give 4.1g (49.0 mg, 85%); δ_H (400 MHz, d-CHCl₃) 1.32-1.47 (m, 5H, CH₂), 1.71-1.91 (m, 5H, CH₂), 2.25 (s, 3H, CH₃), 2.42-2.53 (m, 4H, CH and CH₃), 3.68 (s, 2H, CH₂), 4.50 (s, 2H, CH₂), 4.68 (s, 2H, CH₂), 4.78 (s, 2H, CH₂), 5.31 (s, 2H, CH₂), 6.32 (s, 1H, CH), 6.39 (d, J = 8.0 Hz, 1H, CH), 6.97 (d, J = 8.0 Hz, 2H, CH), 7.02-7.09 (m, 4H, CH), 7.10 (d, J = 8.0 Hz, 2H, CH), 7.25-7.40 (m, 13H, CH), 7.49 (t, J = 7.6 Hz, 1H, CH), 7.60 (d, J = 7.6 Hz, 1H, CH) 7.70-7.76 (m, 2H, CH), 7.76-7.81 (m, 2H, CH); δ_C (100 MHz, d-CHCl₃) 21.2, 21.7, 26.2, 26.9, 34.6, 44.3, 46.7, 50.8, 52.8, 67.1, 70.8, 113.9, 120.2, 120.7, 127.0, 127.3, 127.8, 128.2, 128.3, 128.4, 128.6, 128.7, 128.8, 129.1, 129.4, 129.5, 132.3, 133.1, 134.1, 135.8, 137.3, 137.8, 143.3, 145.1, 145.8, 158.8, 165.5, 166.9; LRMS (ES+) Calcd for [C₅₁H₅₂N₂O₁₆S + Na] 843.34 found 843.51.

4.1h, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(2-(trifluoromethyl)benzyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 2.30 was functionalized on a 0.07 mmol scale using General Procedure A to give 4.1h (55.3 mg, 90%); δ_H (400 MHz, d-CHCl₃) 1.30-1.46 (m, 5H, CH₂), 1.70-1.91 (m, 5H, CH₂), 2.42-2.52 (m, 4H, CH and CH₃), 3.72 (s, 2H, CH₂), 4.68 (s, 2H, CH₂), 4.76 (s, 2H, CH₂), 4.80 (s, 2H, CH₂), 5.32 (s, 2H, CH₂), 6.36 (s, 1H, CH), 6.50 (d, J = 7.6 Hz, 1H, CH), 6.95 (d, J = 7.6 Hz, 2H, CH), 7.09 (d, J = 8.0 Hz, 2H, CH), 7.25-7.40 (m, 13H, CH), 7.49 (t, J = 7.6 Hz, 1H, CH), 7.60 (d, J = 7.6 Hz, 1H, CH) 7.70-7.76 (m, 2H, CH), 7.76-7.81 (m, 2H, CH); δ_C (100 MHz, d-CHCl₃) 21.8, 26.2, 26.9, 34.6, 44.3, 47.9, 52.8, 67.1, 68.1, 70.8, 114.0, 120.1, 120.8, 125.7, 127.0, 127.2, 127.7, 127.9, 128.2, 128.3, 128.4, 128.7, 128.8, 129.1, 129.6, 130.1, 132.6, 133.2, 134.0, 135.1, 135.8, 135.8,
136.9, 143.6, 145.4, 147.8, 158.8, 165.5, 166.3; LRMS (ES+) Calcd for [C_{51}H_{49}F_{3}N_{2}O_{6}S + Na] 897.32 found 897.26.

4.1i, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(3-(trifluoromethyl)benzyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 2.30 was functionalized on a 0.07 mmol scale using General Procedure A to give 4.1i (42.9 mg, 70%); δ_H (400 MHz, d-CDCl₃) 1.30-1.50 (m, 5H, CH₂), 1.69-1.95 (m, 5H, CH₂), 2.35-2.55 (m, 4H, CH and CH₃), 3.67 (s, 2H, CH₂), 4.62 (s, 2H, CH₂), 4.69 (s, 2H, CH₂), 4.81 (s, 2H, CH₂), 5.32 (s, 2H, CH₂), 6.36 (s, 1H, CH), 6.44 (d, J = 8.4 Hz, 1H, CH), 6.96 (d, J = 8.0 Hz, 2H, CH), 7.10 (d, J = 8.0 Hz, 2H, CH), 7.24-7.43 (m, 14H, CH), 7.46 (d, J = 8.0 Hz, 1H, CH), 7.51 (d, J = 8.0 Hz, 1H, CH), 7.69-7.79 (m, 3H, CH); δ_C (100 MHz, d-CDCl₃) 21.4, 25.9, 26.7, 34.3, 44.1, 46.9, 50.7, 52.6, 66.9, 70.6, 113.7, 119.7, 120.7, 122.4, 124.6, 124.7, 125.1, 125.1, 126.8, 127.0, 127.9, 128.1, 128.1, 128.4, 128.4, 128.8, 129.1, 129.4, 131.9, 132.9, 133.7, 135.5, 135.6, 136.6, 136.8, 143.4, 144.6, 147.6, 158.6, 165.2, 166.4; LRMS (ES+) calcd for [C_{31}H_{49}F_{3}N_{2}O_{6}S + Na] 897.33, found 897.41.
4.1j, benzyl 2-(benzylOxy)-4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(4-(trifluoromethyl)benzyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 2.30 was functionalized on a 0.07 mmol scale using General Procedure A to give 4.1j (46.4 mg, 76 %); $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.33-1.46 (m, 5H, CH$_2$), 1.66-1.93 (m, 5H, CH$_2$), 2.45-2.48 (m, 4H, CH and CH$_3$), 3.66 (s, 2H, CH$_2$), 4.63 (s, 2H, CH$_2$), 4.67 (s, 2H, CH$_2$), 4.86 (s, 2H, CH$_2$), 5.32 (s, 2H, CH$_2$), 6.35-6.40 (m, 2H, CH), 6.96 (d, $J = 8.0$ Hz, 2H, CH), 7.10 (d, $J = 8.0$ Hz, 2H, CH), 7.24-7.40 (m, 14H, CH), 7.52 (d, $J = 8.0$ Hz, 2H, CH), 7.72 (d, $J = 8.0$ Hz, 1H, CH), 7.74 (d, $J = 8.0$ Hz, 2H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 21.5, 25.9, 26.7, 34.3, 44.1, 47.0, 50.8, 52.6, 66.9, 70.6, 113.7, 119.8, 120.9, 125.4, 125.4, 126.8, 127.0, 127.5, 127.9, 128.1, 128.4, 128.4, 128.7, 128.7, 128.8, 129.4, 130.2, 132.9, 133.7, 135.5, 135.6, 136.7, 139.6, 143.4, 147.7, 158.6, 165.2, 166.5; LRMS (ES+) calcd for [C$_{31}$H$_{49}$F$_3$N$_2$O$_6$S + H] 875.33, found 875.24.

4.1k, benzyl 2-(benzylOxy)-4-(N-(4-cyclohexylbenzyl)-2-(N-(2-fluorobenzyl)-4-methylphenylsulfonamido)acetamido)benzoate. Sulfonamide 2.30 was functionalized on a
0.07 mmol scale using General Procedure A to give 4.1k (48.4 mg, 84%); δ_H (400 MHz, d-CHCl_3) 1.29-1.47 (m, 5H, CH_2), 1.69-1.94 (m, 5H, CH_2), 2.36-2.56 (m, 4H, CH and CH_3), 3.73 (s, 2H, CH_2), 4.61 (s, 2H, CH_2), 4.72 (s, 2H, CH_2), 4.83 (s, 2H, CH_2), 5.32 (s, 2H, CH_2), 6.40 (s, 1H, CH), 6.52 (d, J = 8.0 Hz, 1H, CH), 6.94 (d, J = 9.2 Hz, 1H, CH), 6.99 (d, J = 8.0 Hz, 2H, CH), 7.05 (t, J = 7.6 Hz, 1H, CH), 7.11 (d, J = 8.0 Hz, 2H, CH), 7.18-7.25 (m, 1H, CH), 7.26-7.39 (m, 13H, CH), 7.70-7.80 (m, 3H, CH); δ_C (100 MHz, d-CHCl_3) 21.7, 26.2, 26.9, 34.6, 44.3, 44.8, 47.8, 52.8, 67.1, 70.8, 114.0, 115.2, 115.5, 120.2, 120.8, 122.7, 122.9, 124.6, 124.6, 127.0, 127.3, 127.8, 128.2, 128.3, 128.4, 128.7, 128.7, 129.1, 129.5, 129.8, 129.9, 131.4, 131.4, 133.2, 134.1, 135.8, 137.2, 138.2, 138.3, 143.4, 147.8, 158.8, 160.0, 162.4, 165.5, 166.7; LRMS (ES+) Calcd for [C_{50}H_{49}FN_2O_6S + Na] 847.32 found 847.41.

**4.1l, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(N-(3-fluorobenzyl)-4-methylphenylsulfonamido)acetamido)benzoate.** Sulfonamide 2.30 was functionalized on a 0.07 mmol scale using General Procedure A to give 4.1l (49.8 mg, 86%); δ_H (400 MHz, d-CHCl_3) 1.30-1.54 (m, 5H, CH_2), 1.70-1.99 (m, 5H, CH_2), 2.46 (s, 4H, CH and CH_3), 3.69 (s, 2H, CH_2), 4.55 (s, 2H, CH_2), 4.69 (s, 2H, CH_2), 4.81 (s, 2H, CH_2), 5.32 (s, 2H, CH_2), 6.32 (s, 1H, CH), 6.46 (d, J = 8.0 Hz, 1H, CH), 6.84-7.05 (m, 5H, CH), 7.06-7.47 (m, 15H, CH), 7.67-7.86 (m, 3H, CH); δ_C (100 MHz, d-CHCl_3) 21.7, 26.2, 26.9, 34.6, 44.3, 46.9, 50.7, 52.8, 67.1, 70.8, 113.8, 114.9, 115.1, 115.5, 115.7, 120.1, 120.8, 124.3, 124.4, 127.1, 127.2, 127.7, 128.2, 128.3, 128.4, 128.7, 129.1, 129.6, 130.3, 130.4, 133.2, 134.0, 135.8, 135.8, 137.1, 138.2, 138.3,
4.1m, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(N-(4-fluorobenzyl)-4-methylphenylsulfonamido)acetamido)benzoate. Sulfonamide 2.30 was functionalized on a 0.07 mmol scale using General Procedure A to give 4.1m (48.3 mg, 84 %); δH (400 MHz, d-CHCl₃) 1.30-1.48 (m, 5H, CH₂), 1.68-1.95 (m, 5H, CH₂), 2.35-2.57 (m, 4H, CH and CH₃), 3.65 (s, 2H, CH₂), 4.51 (s, 2H, CH₂), 4.67 (s, 2H, CH₂), 4.81 (s, 2H, CH₂), 5.32 (s, 2H, CH₂), 6.30 (s, 1H, CH), 6.44 (d, J = 8.0 Hz, 1H, CH), 6.89-6.99 (m, 4H, CH), 7.04-7.21 (m, 4H, CH), 7.22-7.44 (m, 12H, CH), 7.69-7.80 (m, 3H, CH); δC (100 MHz, d-CHCl₃) 21.7, 26.2, 26.9, 34.6, 44.3, 46.8, 50.4, 52.8, 67.1, 70.8, 113.8, 115.5, 115.7, 120.1, 120.9, 127.0, 127.2, 127.7, 128.2, 128.3, 128.4, 128.7, 129.1, 129.6, 130.6, 130.7, 131.2, 131.2, 133.2, 134.0, 135.8, 135.8, 137.1, 143.5, 145.0, 147.9, 158.8, 161.3, 163.8, 165.5, 166.7; LRMS (ES+) Calcd for [C₅₀H₄₉FN₂O₆S + Na] 847.32 found 847.53.
4.1n, benzyl 2-(benzyloxy)-4-(2-(N-(2-chlorobenzyl)-4-methylphenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)benzoate. Sulfonamide 2.30 was functionalized on a 0.1 mmol scale using General Procedure A to give 4.1n (73.2 mg, 85 %); δH (400 MHz, d-CHCl₃) 1.31-1.48 (m, 5H, CH₂), 1.70-1.94 (m, 5H, CH₂), 2.40-2.55 (m, 4H, CH and CH₃), 3.74 (s, 2H, CH₂), 4.69 (s, 4H, 2CH₂), 4.82 (s, 2H, CH₂), 5.33 (s, 2H, CH₂), 6.41 (s, 1H, CH), 6.54 (d, J = 7.8 Hz, 1H, CH), 6.99 (d, J = 6.6 Hz, 2H, CH), 7.10 (d, J = 6.6 Hz, 2H, CH), 7.14-7.21 (m, 2H, CH), 7.27-7.46 (m, 14H, CH), 7.71 (m, 3H, CH); δC (100 MHz, d-CHCl₃) 21.6, 26.0, 26.8, 34.4, 44.2, 47.7, 48.5, 52.7, 67.0, 70.7, 113.8, 120.1, 120.7, 126.8, 127.1, 127.2, 127.7, 128.0, 128.2, 128.4, 128.5, 128.5, 129.0, 129.0, 129.1, 129.3, 129.3, 130.7, 133.0, 133.3, 133.7, 133.9, 135.6, 135.6, 137.0, 143.3, 144.9, 158.7, 165.3, 166.5; LRMS (ES+) Calcd for [C₅₀H₴₉ClN₂O₆S + Na] 863.29 found 863.54.

4.1o, benzyl 2-(benzyloxy)-4-(2-(N-(3-chlorobenzyl)-4-methylphenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)benzoate. Sulfonamide 2.30 was functionalized on a 0.1 mmol scale using General Procedure A to give 4.1o (61.6 mg, 71 %); δH (400 MHz, d-CHCl₃) 1.33-1.47 (m, 5H, CH₂), 1.70-1.96 (m, 5H, CH₂), 2.42-2.52 (m, 4H, CH and CH₃), 3.67 (s, 2H, CH₂), 4.53 (s, 2H, CH₂), 4.69 (s, 2H, CH₂), 4.81 (s, 2H, CH₂), 5.32 (s, 2H, CH₂), 6.32 (s, 1H, CH), 6.47 (d, J = 7.6 Hz, 1H, CH), 6.97 (d, J = 7.8 Hz, 2H, CH), 7.10 (d, J = 7.8 Hz, 2H, CH), 7.12-7.24 (m, 5H, CH), 7.27-7.48 (m, 12H, CH), 7.71-7.84 (m, 3H, CH); δC (100 MHz, d-CHCl₃) 21.6, 26.0, 26.8, 34.5, 44.2, 46.9, 50.5, 52.7, 67.0, 70.6, 113.8, 120.0, 120.7, 126.8, 126.9, 127.1, 127.6, 128.0, 128.1, 128.2, 128.2, 128.5, 128.5, 128.7, 128.9, 128.9, 130.0, 133.1, 133.8, 134.5, 135.7, 135.7, 137.0, 137.6, 143.4, 144.8, 147.7, 158.7, 165.3, 166.5; LRMS (ES+) Calcd for [C₅₀H₴₉ClN₂O₆S + Na] 863.29 found 863.54
4.1p, benzyl 2-(benzylloxy)-4-(2-(N-(4-chlorobenzyl)-4-methylphenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)benzoate. Sulfonamide 2.30 was functionalized on a 0.1 mmol scale using General Procedure A to give 4.1p (65.4 mg, 75.8 %); $\delta_H$ (400 MHz, $d$-CHCl$_3$) 1.32-1.47 (m, 5H, CH$_2$), 1.69-1.94 (m, 5H, CH$_2$), 2.40-2.54 (m, 4H, CH and CH$_3$), 3.65 (s, 2H, CH$_2$), 4.52 (s, 2H, CH$_2$), 4.67 (s, 2H, CH$_2$), 4.83 (s, 2H, CH$_2$), 5.33 (s, 2H, CH$_2$), 6.32 (s, 1H, CH), 6.43 (d, $J = 8.0$ Hz, 1H, CH), 6.96 (d, $J = 7.8$ Hz, 2H, CH), 7.07-7.17 (m, 4H, CH), 7.23 (d, $J = 8.0$ Hz, 2H, CH), 7.27-7.43 (m, 12H, CH), 7.70-7.83 (m, 3H, CH); $\delta_C$ (100 MHz, $d$-CHCl$_3$) 21.5, 26.0, 26.7, 34.4, 44.1, 46.7, 50.4, 52.6, 66.9, 70.6, 113.6, 119.8, 120.7, 126.8, 127.0, 127.5, 127.9, 128.1, 128.2, 128.4, 128.7, 128.9, 128.9, 129.4, 130.0, 131.9, 132.9, 133.7, 133.8, 135.6, 135.6, 136.8, 143.3, 147.7, 158.6, 165.2, 166.5; LRMS (ES+) Calcd for [C$_{50}$H$_{49}$ClN$_2$O$_6$S + Na] 863.29 found 863.48
4.1q, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(2-(trifluoromethoxy)benzyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 2.30 was functionalized on a 0.07 mmol scale using General Procedure A to give 4.1q (34.5, 56 %); \[\delta_H^{400\text{ MHz, } d-\text{CDCl}_3}\] 1.30-1.47 (m, 5H, CH), 1.67-1.92 (m, 5H, CH), 2.40-2.51 (m, 4H, CH and CH\text{ }_3\text{ }), 3.71 (s, 2H, CH\text{ }_2\text{ }, 4.64 (s, 2H, CH\text{ }_2\text{ }, 4.69 (s, 2H, CH\text{ }_2\text{ }, 4.81 (s, 2H, CH\text{ }_2\text{ }, 5.32 (s, 2H, CH\text{ }_2\text{ }, 6.38 (s, 1H, CH), 6.52 (d, J = 8.0 Hz, 1H, CH), 6.95 (d, J = 8.0 Hz, 2H, CH), 7.09 (d, J = 8.0 Hz, 2H, CH), 7.13-7.40 (m, 15H, CH), 7.49 (d, J = 8.0 Hz, 1H, CH), 7.74 (d, J = 8.0 Hz, 3H, CH); \[\delta_C^{100\text{ MHz, } d-\text{CDCl}_3}\] 21.5, 25.9, 26.7, 29.5, 34.3, 44.1, 47.6, 52.6, 66.9, 70.5, 113.8, 118.7, 119.9, 120.3, 122.6, 126.7, 127.0, 127.2, 127.5, 127.9, 128.1, 128.1, 128.4, 128.4, 128.5, 128.8, 129.1, 129.3, 130.7, 133.0, 133.8, 135.5, 135.6, 136.9, 143.3, 147.3, 147.5, 158.6, 165.2, 166.2; LRMS (ES+) calcd for [C\text{ }_{51}H_{49}F_{3}N_{2}O_{7}S + Na] 913.32, found 913.40.

4.1r, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(3-(trifluoromethoxy)benzyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 2.30 was functionalized on a 0.07 mmol scale using General Procedure A to give 4.1r (34.5 mg, 39 %); \[\delta_H^{400\text{ MHz, } d-\text{CDCl}_3}\] 1.33-1.44 (m, 5H, CH), 1.73-1.84 (m, 5H, CH), 2.42-2.52 (m, 4H, CH\text{ }_3\text{ } and CH), 3.67 (s, 2H, CH\text{ }_2\text{ }, 4.58 (s, 2H, CH\text{ }_2\text{ }, 4.69 (s, 2H, CH\text{ }_2\text{ }, 4.82 (s, 2H, CH\text{ }_2\text{ }, 5.32 (s, 2H, CH\text{ }_2\text{ }, 6.35 (s, 1H, CH), 6.44 (d, J = 8.0 Hz, 1H, CH), 6.96 (d, J = 8.0 Hz, 2H, CH), 7.01 (s, 1H, CH), 7.07-7.13 (m, 3H, CH), 7.17 (d, J = 8.0 Hz, 1H, CH), 7.26-7.38 (m, 13H, CH), 7.72 (d, J = 8.0 Hz, 1H, CH), 7.75 (d, J = 8.0, 2H, CH); \[\delta_C^{100\text{ MHz, } d-\text{CDCl}_3}\] 21.8, 26.3, 27.0, 34.7, 44.4, 47.1, 50.9, 52.9, 67.2, 70.9, 113.7, 119.8, 120.1, 120.7, 120.9, 126.8, 127.0, 127.5, 127.9, 128.1, 128.4, 128.4, 128.8, 129.4, 130.0, 132.9, 133.7, 135.5, 135.6, 136.8, 138.0, 143.4, 144.7,
147.6, 149.2, 158.6, 165.2, 166.5; LRMS (ES+) calcd for [C_{51}H_{49}F_{3}N_{2}O_{7}S + Na] 913.32, found 913.40.

4.1s, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(4-(trifluoromethoxy)benzyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 2.30 was functionalized on a 0.07 mmol scale using General Procedure A to give 4.1s (59.3 mg, 95%); \( \delta \) (400 MHz, \( d \)-CDCl\(_3\)) 1.30-1.48 (m, 5H, CH\(_2\)), 1.70-1.95 (m, 5H, CH\(_2\)), 2.35-2.55 (m, 4H, CH and CH\(_3\)), 3.65 (s, 2H, CH\(_2\)), 4.55 (s, 2H, CH\(_2\)), 4.67 (s, 2H, CH\(_2\)), 4.85 (s, 2H, CH\(_2\)), 5.32 (s, 2H, CH\(_2\)), 6.34-6.38 (m, 2H, CH), 6.96 (d, \( J = 8.0 \text{ Hz} \), 2H, CH), 7.06-7.13 (m, 4H, CH), 7.22 (d, \( J = 7.6 \text{ Hz} \), 2H, CH), 7.28-7.40 (m, 12H, CH), 7.71 (d, \( J = 8.4 \text{ Hz} \), 1H, CH), 7.75 (d, \( J = 8.4 \text{ Hz} \), 2H, CH); \( \delta \) (100 MHz, \( d \)-CDCl\(_3\)) 119.0, 119.7, 120.5, 120.7, 126.8, 127.0, 127.5, 127.9, 128.1, 128.3, 128.4, 128.6, 128.8, 128.9, 129.4, 129.9, 132.9, 133.7, 134.1, 135.5, 135.6, 136.8, 143.3, 147.7, 148.7, 148.7, 158.6, 165.2, 166.5; LRMS (ES+) calcd for [C_{51}H_{49}F_{3}N_{2}O_{7}S + H] 913.32, found 913.34.
4.1t, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(2-nitrobenzyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 2.30 was functionalized on a 0.3 mmol scale using General Procedure A to give 4.1t (194 mg, 81%); $\delta_H$ (400 MHz, $d$-CHCl$_3$) 1.31-1.51 (m, 5H, CH$_2$), 1.70-1.96 (m, 5H, CH$_2$), 2.40-2.59 (m, 4H, CH and CH$_3$), 3.83 (s, 2H, CH$_2$), 4.73 (s, 2H, CH$_2$), 4.90 (s, 2H, CH$_2$), 4.94 (s, 2H, CH$_2$), 5.35 (s, 2H, CH$_2$), 6.51 (s, 1H, CH), 6.63 (d, $J = 8.0$ Hz, 1H, CH), 6.90 (d, $J = 8.0$ Hz, 2H, CH), 7.13 (d, $J = 8.0$ Hz, 2H, CH), 7.25-7.44 (m, 14H, CH), 7.57 (t, $J = 7.6$ Hz, 1H, CH), 7.70 (d, $J = 8.0$ Hz, 1H, CH), 7.81 (d, $J = 8.0$ Hz, 2H, CH), 7.91 (d, $J = 8.10$ Hz, 1H, CH); $\delta_C$ (100 MHz, $d$-CHCl$_3$) 21.5, 26.0, 26.8, 34.4, 44.1, 49.0, 52.7, 53.5, 66.9, 70.6, 113.9, 120.1, 120.7, 124.5, 126.9, 127.0, 127.5, 128.0, 128.2, 128.4, 128.5, 128.8, 129.5, 130.6, 132.0, 133.0, 133.6, 133.8, 135.7, 136.4, 143.6, 144.8, 147.6, 148.5, 158.7, 165.3, 166.3.

4.1u, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(3-nitrobenzyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 2.30 was functionalized on a 0.3 mmol scale using General Procedure A to give 4.1u (200 mg, 84%); $\delta_H$ (400 MHz, $d$-CHCl$_3$) 1.32-1.49 (m, 5H, CH$_2$), 1.70-1.96 (m, 5H, CH$_2$), 2.41-2.57 (m, 4H, CH and CH$_3$), 3.73 (s, 2H, CH$_2$), 4.67 (s, 2H, CH$_2$), 4.72 (s, 2H, CH$_2$), 4.87 (s, 2H, CH$_2$), 5.33 (s, 2H, CH$_2$), 6.45 (s, 1H, CH), 6.56 (d, $J = 8.0$ Hz, 1H, CH), 6.98 (d, $J = 8.0$ Hz, 2H, CH), 7.12 (d, $J = 8.0$ Hz, 2H, CH), 7.25-7.41 (m, 12H, CH), 7.45 (t, $J = 8.0$ Hz, 1H, CH), 7.67 (d, $J = 7.6$ Hz, 1H, CH), 7.75 (d, $J = 8.4$ Hz, 2H, CH), 7.77 (d, $J = 8.4$ Hz, 1H, CH), 8.00 (s, 1H, CH), 8.05-8.14 (m, 1H, CH); $\delta_C$ (100 MHz, $d$-CHCl$_3$) 21.5, 26.0, 26.7, 34.4, 44.1, 47.4, 52.7, 53.5, 67.0, 70.6, 113.8, 119.9,
4.1v, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(4-nitrobenzyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 2.30 was functionalized on a 0.3 mmol scale using General Procedure A to give 4.1v (185 mg, 78 %); δ_H (400 MHz, d-CHCl_3) 1.31-1.47 (m, 5H, CH_2), 1.69-1.93 (m, 5H, CH_2), 2.40-2.56 (m, 4H, CH and CH_3), 3.69 (s, 2H, CH_2), 4.67 (s, 2H, CH_2), 4.68 (s, 2H, CH_2), 4.87 (s, 2H, CH_2), 5.33 (s, 2H, CH_2), 6.42 (s, 1H, CH), 6.50 (d, J = 8.0 Hz, 1H, CH), 6.96 (d, J = 8.0 Hz, 2H, CH), 7.10 (d, J = 8.0 Hz, 2H, CH), 7.24-7.46 (m, 14H, CH), 7.67-7.80 (m, 3H, CH), 8.11 (d, J = 8.0 Hz, 2H, CH); δ_C (100 MHz, d-CHCl_3) 21.7, 26.1, 26.8, 34.5, 44.2, 47.5, 51.1, 52.8, 67.1, 70.8, 113.9, 119.9, 121.0, 123.8, 127.0, 127.1, 127.6, 128.1, 128.3, 128.6, 128.6, 129.0, 129.1, 129.6, 133.1, 133.8, 135.7, 135.7, 136.5, 143.5, 143.8, 144.7, 147.6, 147.9, 158.8, 165.3, 165.5.
4.1w, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(pyridin-2-ylmethyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 2.30 was functionalized on a 0.07 mmol scale using General Procedure A to give 4.1w (49.5 mg, 88%); δH (400 MHz, d-CHCl3) 1.29-1.46 (m, 5H, CH2), 1.69-1.90 (m, 5H, CH2), 2.39-2.54 (m, 4H, CH and CH3), 3.87 (s, 2H, CH2), 4.61 (s, 2H, CH2), 4.70 (s, 2H, CH2), 4.87 (s, 2H, CH2), 5.32 (s, 2H, CH2), 6.49 (s, 1H, CH), 6.72 (d of d, J = 8.0 and 1.8 Hz, 1H, CH), 6.97 (d, J = 8.0 Hz, 2H, CH), 7.09 (d, J = 8.0 Hz, 2H, CH), 7.11-7.16 (m, 1H, CH), 7.27 (d, J = 8.0 Hz, 2H, CH), 7.24-7.41 (m, 10H, CH), 7.43 (d, J = 8.0 Hz, 1H, CH), 7.61 (t of d, J = 7.6 and 1.8 Hz, 1H, CH), 7.72-7.79 (m, 3H, CH), 8.41-8.45 (m, 1H, CH); δC (100 MHz, d-CHCl3) 21.7, 26.2, 26.9, 34.6, 44.3, 48.8, 52.9, 53.6, 67.1, 70.8, 114.2, 120.3, 120.7, 122.7, 123.1, 127.0, 127.3, 127.8, 128.2, 128.3, 128.4, 128.7, 128.7, 129.0, 129.6, 133.1, 134.1, 135.9, 137.0, 137.1, 143.5, 145.2, 147.7, 149.0, 156.6, 158.8, 165.6, 166.8; LRMS (ES+) Calcd for [C42H43N3O6S + H] 718.30 found 718.43.

![Chemical structure](image)

4.1x, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(pyridin-3-ylmethyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 2.30 was functionalized on a 0.07 mmol scale using General Procedure A to give 4.1x (38.2 mg, 68%); δH (400 MHz, d-CHCl3) 1.30-1.42 (m, 5H, CH2), 1.67-1.89 (m, 5H, CH2), 2.34-2.53 (m, 4H, CH and CH3), 3.87 (s, 2H, CH2), 4.61 (s, 2H, CH2), 4.70 (s, 2H, CH2), 4.87 (s, 2H, CH2), 5.33 (s, 2H, CH2), 6.49 (s, 1H, CH), 6.58 (d, J = 8.0 Hz, 1H, CH), 6.97 (d, J = 8.0 Hz, 2H, CH), 7.09 (d, J = 7.6 Hz, 2H, CH), 7.11-7.18 (m, 1H, CH), 7.23-7.40 (m, 13H, CH), 7.41-7.49 (m, 1H, CH), 7.57-7.66 (m, 1H, CH), 7.69-7.80 (m, 3H, CH); δC (100 MHz, d-CHCl3) 21.7, 26.2, 26.9, 29.8, 34.6, 44.3, 48.7,
4.1y, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(pyridin-4-ylmethyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 2.30 was functionalized on a 0.07 mmol scale using General Procedure A to give 4.1y (36.2 mg, 64 %); δ_H (400 MHz, d-CHCl_3) 1.31-1.50 (m, 5H, CH_2), 1.68-1.93 (m, 5H, CH_2), 2.37-2.57 (m, 4H, CH and CH_3), 3.68 (s, 2H, CH_2), 4.60 (s, 2H, CH_2), 4.67 (s, 2H, CH_2), 4.86 (s, 2H, CH_2), 5.33 (s, 2H, CH_2), 6.35 (s, 1H, CH), 6.42-6.54 (m, 1H, CH), 6.89-7.01 (m, 2H, CH), 7.04-7.21 (m, 4H, CH), 7.21-7.43 (m, 13H, CH) 7.68-7.80 (m, 3H, CH), 8.51 (s, 1H, CH); δ_C (100 MHz, d-CHCl_3) 21.7, 26.1, 26.9, 34.6, 44.3, 47.5, 50.6, 52.8, 67.2, 70.8, 113.8, 120.0, 121.1, 123.2, 127.0, 127.2, 127.7, 128.2, 128.3, 128.4, 128.6, 128.7, 129.0, 129.7, 133.2, 133.8, 135.7, 136.6, 143.8, 145.4, 147.9, 150.0, 158.8, 165.4, 166.5.
4.1z, benzyl 2-(benzyloxy)-4-(2-(N-(2-cyanobenzyl)-4-methylphenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)benzoate. Sulfonamide 2.30 was functionalized on a 0.3 mmol scale using General Procedure A to give 4.1z (221 mg, 93%); δH (400 MHz, d-CHCl3) 1.33-1.52 (m, 5H, CH2), 1.70-1.97 (m, 5H, CH2), 2.41-2.57 (m, 4H, CH and CH3), 3.84 (s, 2H, CH2), 4.76 (s, 2H, CH2), 4.77 (s, 2H, CH2), 4.92 (s, 2H, CH2), 5.35 (s, 2H, CH2), 6.58 (s, 1H, CH), 6.65 (d, J = 8.0 Hz, 1H, CH), 7.01 (d, J = 8.0 Hz, 2H, CH), 7.13 (d, J = 8.0 Hz, 2H, CH), 7.27-7.44 (m, 14H, CH), 7.56 (d, J = 7.6 Hz, 2H, CH), 7.73 (d, J = 8.0 Hz, 2H, CH), 7.80 (d, J = 8.0 Hz, 1H, CH); δC (100 MHz, d-CHCl3) 21.5, 26.0, 26.7, 34.4, 44.1, 48.8, 49.9, 52.7, 66.9, 70.6, 111.8, 114.0, 117.3, 120.1, 120.6, 126.8, 127.1, 127.6, 128.0, 128.1, 128.2, 128.2, 128.5, 128.5, 128.9, 129.5, 129.5, 130.0, 132.5, 133.0, 133.2, 133.2, 134.8, 135.7, 135.7, 136.5, 140.1, 143.5, 144.8, 147.5, 158.7, 165.3, 166.1; LRMS (ES+) Calcd for [C51H49N3O6S + Na] 854.32 found 854.46.

4.1aa, benzyl 2-(benzyloxy)-4-(2-(N-(3-cyanobenzyl)-4-methylphenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)benzoate. Sulfonamide 2.30 was functionalized on a 0.3 mmol
scale using General Procedure A to give 4.1aa (184 mg, 79 %); δ_H (400 MHz, d-CHCl$_3$) 1.32-1.52 (m, 5H, CH$_2$), 1.70-1.98 (m, 5H, CH$_2$), 2.38-2.59 (m, 4H, CH and CH$_3$), 3.71 (s, 2H, CH$_2$), 4.61 (s, 2H, CH$_2$), 4.72 (s, 2H, CH$_2$), 4.90 (s, 2H, CH$_2$), 5.34 (s, 2H, CH$_2$), 6.46 (s, 1H, CH), 6.55 (d, J = 8.0 Hz, 1H, CH), 6.99 (d, J = 8.0 Hz, 2H, CH), 7.14 (d, J = 7.6 Hz, 2H, CH), 7.23-7.47 (m, 14H, CH), 7.53 (t, J = 6.8 Hz, 2H, CH), 7.74 (d, J = 8.0 Hz, 2H, CH), 7.79 (d, J = 8.0 Hz, 1H, CH); δ_C (100 MHz, d-CHCl$_3$) 21.5, 26.0, 26.7, 34.4, 44.1, 47.2, 50.7, 52.7, 66.9, 70.6, 112.5, 113.8, 118.3, 119.8, 120.8, 126.9, 127.0, 127.5, 127.9, 128.1, 128.2, 128.5, 128.8, 129.5, 129.5, 130.5, 131.7, 131.7, 132.8, 133.0, 133.7, 135.6, 136.6, 137.4, 143.6, 144.7, 147.7, 158.7, 165.2, 166.4; LRMS (ES+) Calcd for [C$_{51}$H$_{49}$N$_3$O$_6$S + Na] 854.32 found 854.59.

4.1ab, benzyl 2-(benzyloxy)-4-(2-(N-(4-cyanobenzyl)-4-methylphenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)benzoate. Sulfonamide 2.30 was functionalized on a 0.3 mmol scale using General Procedure A to give 4.1ab (205 mg, 88 %); δ_H (400 MHz, d-CHCl$_3$) 1.32-1.52 (m, 5H, CH$_2$), 1.70-1.96 (m, 5H, CH$_2$), 2.41-2.57 (m, 4H, CH and CH$_3$), 3.70 (s, 2H, CH$_2$), 4.64 (s, 2H, CH$_2$), 4.70 (s, 2H, CH$_2$), 4.90 (s, 2H, CH$_2$), 5.34 (s, 2H, CH$_2$), 6.46 (s, 1H, CH), 6.50 (d, J = 8.0 Hz, 1H, CH), 6.98 (d, J = 8.0 Hz, 2H, CH), 7.13 (d, J = 8.0 Hz, 2H, CH), 7.25-7.42 (m, 14H, CH), 7.55 (d, J = 8.0 Hz, 2H, CH), 7.74 (d, J = 8.0 Hz, 2H, CH), 7.77 (d, J = 8.0 Hz, 1H, CH); δ_C (100 MHz, d-CHCl$_3$) 21.5, 26.0, 26.7, 34.4, 44.1, 47.3, 51.2, 52.7, 67.0, 70.7, 111.6, 113.8, 118.4, 119.8, 120.9, 126.9, 127.0, 127.5, 128.0, 128.2, 128.2, 128.5, 128.5, 128.9, 129.5, 132.3, 133.0, 133.7, 135.6, 136.5, 141.4, 143.6, 144.7, 147.7, 158.7, 165.2, 166.4; LRMS (ES+) Calcd for [C$_{51}$H$_{49}$N$_3$O$_6$S + Na] 854.32 found 854.59.
4.1ac, benzyl 2-(benzoxyl)-4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(2,3,4,5-tetrafluorobenzyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 2.30 was functionalized on a 0.07 mmol scale using General Procedure A to give 4.1ac (60.3 mg, 89 %); δH (400 MHz, d-CHCl3) 1.31-1.45 (m, 5H, CH2), 1.69-1.92 (m, 5H, CH2), 2.39-2.54 (m, 4H, CH and CH3), 3.81 (s, 2H, CH2), 4.57 (s, 2H, CH2), 4.73 (s, 2H, CH2), 4.90 (s, 2H, CH2), 5.34 (s, 2H, CH2), 6.50 (s, 1H, CH), 6.62-6.73 (m, 2H, CH), 6.98 (d, J = 8.0 Hz, 2H, CH), 7.11 (d, J = 8.0 Hz, 2H, CH), 7.24 (d, J = 8.0 Hz, 2H, CH), 7.28-7.41 (m, 10H, CH), 7.66 (d, J = 8.4 Hz, 2H, CH), 7.81 (d, J = 8.4 Hz, 1H, CH); δC (100 MHz, d-CHCl3) 21.7, 26.2, 26.9, 34.6, 39.8, 44.4, 49.2, 52.9, 67.2, 70.8, 101.0, 110.1, 114.1, 120.3, 121.1 127.1, 127.3, 127.6, 127.8, 128.2, 128.4, 128.5, 128.7, 128.8, 129.1, 129.5, 129.7, 133.3, 134.0, 135.8, 135.9, 136.7, 143.7, 145.0, 144.8, 147.9, 159.0, 165.5, 166.7; LRMS (ES+) Calcd for [C50H46F4N2O6S + Na] 901.29 found 901.50.
4.1ad, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(2,3,4,6-tetrafluorobenzyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 2.30 was functionalized on a 0.07 mmol scale using General Procedure A to give 4.1ad (52.9 mg, 86 %); δH (400 MHz, d-CHCl3) 1.30-1.49 (m, 5H, CH2), 1.67-1.92 (m, 5H, CH2), 2.39-2.54 (m, 4H, CH and CH3), 3.71 (s, 2H, CH2), 4.55 (s, 2H, CH2), 4.71 (s, 2H, CH2), 4.89 (s, 2H, CH2), 5.34 (s, 2H, CH2), 6.45 (s, 1H, CH), 6.61 (d, J = 8.0 Hz, 1H, CH), 6.97 (d, J = 9.0 Hz, 2H, CH), 7.04-7.16 (m, 3H, CH), 7.27-7.43 (m, 12H, CH), 7.69 (d, J = 8.0 Hz, 2H, CH), 7.80 (d, J = 8.4 Hz, 1H, CH); δC (100 MHz, d-CHCl3) 21.7, 26.2, 26.9, 34.6, 44.4, 44.6, 48.3, 52.9, 67.2, 70.8, 111.9, 112.2, 114.0, 119.9, 120.2, 121.2, 127.1, 127.2, 127.5, 127.7, 128.2, 128.4, 128.7, 128.9, 129.1, 129.7, 129.9, 133.3, 133.9, 135.8, 135.8, 136.6, 144.0, 144.8, 148.0, 158.9, 165.5, 166.4; LRMS (ES+) Calcd for [C_{50}H_{49}F_{4}N_{2}O_{6}S + Na] 901.29 found 901.56.

4.1ae, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(2,3,5,6-tetrafluorobenzyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 2.30 was functionalized on a 0.07 mmol scale using General Procedure A to give 4.1ae (61.3 mg, 100 %); δH (400 MHz, d-CHCl3) 1.30-1.46 (m, 5H, CH2), 1.69-1.90 (m, 5H, CH2), 2.39-2.53 (m, 4H, CH and CH3), 3.84 (s, 2H, CH2), 4.66 (s, 2H, CH2), 4.74 (s, 2H, CH2), 4.90 (s, 2H, CH2), 5.34 (s, 2H, CH2), 6.51 (s, 1H, CH), 6.67 (d of d, J = 8.0 and 1.6 Hz, 1H, CH), 6.92-7.04 (m, 3H, CH), 7.11 (d, J = 8.0 Hz, 2H, CH), 7.25 (d, J = 8.0 Hz, 2H, CH), 7.28-7.42 (m, 10H, CH), 7.68 (d, J = 8.4 Hz, 2H, CH), 7.82 (d, J = 8.0 Hz, 1H, CH); δC (100 MHz, d-CHCl3) 21.7, 26.2, 26.9, 34.6, 40.0, 44.4, 49.2, 52.9, 67.2, 70.8, 106.2, 106.4, 114.1, 115.9, 120.3, 121.1 127.1, 127.3, 127.6, 127.8,
128.2, 128.4, 128.7, 129.1, 129.5, 129.7, 133.3, 134.0, 135.8, 136.6, 143.8, 144.0
145.0, 147.9, 159.0, 165.5, 166.6; LRMS (ES+) Calcd for \([\text{C}_{50}\text{H}_{49}\text{F}_{4}\text{N}_{2}\text{O}_{6}\text{S} + \text{Na}]\) 901.29 found 901.50.

4.1af, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-
((perfluorophenyl)methyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 2.30 was
functionalized on a 0.07 mmol scale using General Procedure A to give 4.1af (61.8 mg, 98 %);
\(\delta_h\) (400 MHz, \(d\)-CHCl\(_3\)) 1.31-1.52 (m, 5H, CH\(_2\)), 1.65-1.97 (m, 5H, CH\(_2\)), 2.37-2.57 (m, 4H, CH and CH\(_3\)), 3.84 (s, 2H, CH\(_2\)), 4.62 (s, 2H, CH\(_2\)), 4.73 (s, 2H, CH\(_2\)), 4.91 (s, 2H, CH\(_2\)), 5.35 (s, 2H, CH\(_2\)), 6.50 (s, 1H, CH), 6.67 (d of d, \(J = 8.0\) and 1.2 Hz, 1H, CH), 6.98 (d, \(J = 8.0\) Hz, 2H, CH),
7.11 (d, \(J = 8.0\) Hz, 2H, CH), 7.21-7.45 (m, 12H, CH), 7.65 (d, \(J = 8.0\) Hz, 2H, CH), 7.83 (d, 8.0
Hz, 1H, CH); \(\delta_c\) (100 MHz, \(d\)-CHCl\(_3\)) 21.7, 26.2, 26.9, 34.6, 39.9, 44.3, 49.5, 52.9, 67.2, 70.8,
110.3, 114.0, 120.2, 127.1, 127.2, 127.7, 128.2, 128.4, 128.4, 128.7, 128.7, 129.1, 129.5, 133.3,
133.9, 135.8, 135.8, 136.4, 143.9, 147.9, 158.9, 165.5, 166.6; LRMS (ES+) Calcd for
\([\text{C}_{50}\text{H}_{43}\text{F}_{3}\text{N}_{2}\text{O}_{6}\text{S} + \text{Na}]\) 919.28 found 919.51.
4.1ag, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(naphthalen-2-ylmethyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 2.30 was functionalized on a 0.07 mmol scale using General Procedure A to give 4.1ag (60.3 mg, 100 %); δ_H (400 MHz, d-CHCl_3) 1.33-1.53 (m, 5H, CH_2), 1.69-1.98 (m, 5H, CH_2), 2.33-2.60 (m, 4H, CH and CH_3), 3.70 (s, 2H, CH_2), 4.44 (s, 2H, CH_2) 6.09 (s, 1H, CH), 6.29 (d, J = 8.0 Hz, 1H, CH), 6.97 (d, J = 7.5 Hz, 2H, CH), 7.10 (d, J = 7.4 Hz, 2H, CH), 7.12-7.46 (m, 15H, CH), 7.55 (d, J = 8.0 Hz, 1H, CH), 7.60 (s, 1H, CH), 7.68 (d, J = 8.0 Hz, 1H, CH), 7.73 (d, J = 8.4 Hz, 2H, CH), 7.85 (d, J = 8.0 Hz, 2H, CH); δ_C (100 MHz, d-CHCl_3) 21.7, 26.2, 26.9, 34.6, 44.3, 46.9, 51.2, 52.7, 67.0, 70.4, 113.5, 120.0, 120.7, 126.4, 126.5, 127.0, 127.4, 127.7, 127.8, 128.0, 128.1, 128.3, 128.3, 128.5, 128.6, 128.8, 129.1, 129.6, 132.8, 133.0, 133.1, 133.1, 134.1, 134.1, 135.7, 135.8, 137.3, 143.4, 147.8, 158.6, 165.4, 166.7; LRMS (ES+) Calcd for [C_{46}H_{48}N_{2}O_{6}S + Na] 879.34 found 879.66.
**4.1ah, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(N-(4-fluoro-2-((trifluoromethyl)benzyl)-4-methylphenylsulfonamido)acetamido)benzoate.** Sulfonamide 2.30 was functionalized on a 0.07 mmol scale using General Procedure A to give **4.1ah** (56.5 mg, 90 %); \( \delta_H \) (400 MHz, \( d-\text{CDCl}_3 \)) 1.30-1.50 (m, 5H, CH\(_2\)), 1.65-1.95 (m, 5H, CH\(_2\)), 2.35-2.50 (m, 4H, CH and CH\(_3\)), 3.68 (s, 2H, CH\(_2\)), 4.68 (s, 2H, CH\(_2\)), 4.69 (s, 2H, CH\(_2\)), 4.84 (s, 2H, CH\(_2\)), 5.33 (s, 2H, CH\(_2\)), 6.39 (s, 1H, CH), 6.53 (d, \( J = 8.0 \text{ Hz}, 1 \text{H, CH} \)), 6.95 (d, \( J = 8.0 \text{ Hz}, 2 \text{H, CH} \)), 7.10 (d, \( J = 8.0 \text{ Hz}, 2 \text{H, CH} \)), 7.16-7.48 (m, 13H, CH), 7.75 (d, \( J = 7.6 \text{ Hz}, 2 \text{H, CH} \)), 7.76 (d, \( J = 7.6 \text{ Hz}, 2 \text{H, CH} \)), 7.79 (d, \( J = 8.0 \text{ Hz}, 1 \text{H, CH} \)); \( \delta_C \) (100 MHz, \( d-\text{CDCl}_3 \)) 21.5, 25.9, 26.7, 34.3, 44.1, 47.0, 47.8, 50.8, 52.6, 66.9, 70.6, 113.2, 113.8, 119.2, 119.4, 119.9, 120.8, 121.7, 126.8, 127.0, 127.6, 127.9, 128.1, 128.2, 128.4, 128.5, 128.8, 129.4, 130.0, 132.5, 132.6, 133.0, 133.7, 135.5, 135.6, 136.5, 143.5, 144.6, 147.6, 158.6, 165.2, 166.0; LRMS (ES+) calcd for [\( \text{C}_{51}\text{H}_{48}\text{F}_4\text{N}_2\text{O}_6\text{S} + \text{Na} \)] 915.32, found 915.42.

![Chemical structure of 4.1ah](image)

**4.1ai, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(4-((trifluoromethyl)thio)benzyl)phenylsulfonamido)acetamido)benzoate.** Sulfonamide 2.30 was functionalized on a 0.07 mmol scale using General Procedure A to give **4.1ai** (37.5 mg, 60 %); \( \delta_H \) (400 MHz, \( d-\text{CDCl}_3 \)) 1.33-1.47 (m, 5H, CH\(_2\)), 1.70-1.95 (m, 5H, CH\(_2\)), 2.40-2.55 (m, 4H, CH and CH\(_3\)), 3.68 (s, 2H, CH\(_2\)), 4.60 (s, 2H, CH\(_2\)), 4.68 (s, 2H, CH\(_2\)), 4.87 (s, 2H, CH\(_2\)), 5.33 (s, 2H, CH\(_2\)), 6.38 (d, \( J = 8.0 \text{ Hz}, 1 \text{H, CH} \)), 6.42 (s, 1H, CH), 6.97 (d, \( J = 8.0 \text{ Hz}, 2 \text{H, CH} \)), 7.11 (d, \( J = 8.0 \text{ Hz}, 2 \text{H, CH} \)), 7.20-7.45 (m, 14H, CH), 7.55 (d, \( J = 8.0 \text{ Hz}, 2 \text{H, CH} \)), 7.71 (d, \( J = 8.0 \text{ Hz}, 1 \text{H, CH} \)), 7.74 (d, \( J = 8.0 \text{ Hz}, 2 \text{H, CH} \)); 21.5, 25.9, 26.7, 34.3, 44.1, 47.0, 50.8, 52.6, 66.9, 70.6,
113.7, 113.7, 119.8, 125.2, 125.4, 125.4, 125.4, 125.5, 126.8, 127.0, 127.5, 127.9, 128.1, 128.1, 128.4, 128.4, 128.7, 128.7, 128.8, 128.9, 129.4, 129.9, 130.2, 132.9, 133.7, 135.5, 135.6, 136.7, 139.6, 139.7, 143.4, 147.7, 158.6, 165.2, 166.5; LRMS (ES+) calcd for [C_{51}H_{49}F_{3}N_{2}O_{6}S + Na] 929.30, found 929.34.

**4.1aj, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(N-(4-(ethoxycarbonyl)benzyl)-4-methylphenylsulfonamido)acetamido)benzoate.** Sulfonamide **2.30** was functionalized on a 0.07 mmol scale using General Procedure A to give **4.1aj** (40.1 mg, 65%); δ_H (400 MHz, d-CDCl_3) 1.32-1.43 (m, 8H, CH_2 and CH_3), 1.70-1.89 (m, 5H, CH_2), 2.42-2.53 (m, 4H, CH_3 and CH), 3.66 (s, 2H, CH_2), 4.29 (q, J = 7.2 Hz, 2H, CH), 4.62 (s, 2H, CH_2), 4.67 (s, 2H, CH_2), 4.78 (s, 2H, CH_2), 5.31 (s, 2H, CH_2), 6.30 (s, 1H, CH), 6.44 (d, J = 8.0 Hz, 1H, CH), 6.95 (d, J = 8.0 Hz, 2H, CH), 7.10 (d, J = 8.0 Hz, 1H, CH), 7.26-7.39 (m, 15H, CH), 7.71 (d, J = 8.0 Hz, 1H, CH), 7.76 (d, J = 8.0 Hz, 2H, CH), 7.94 (d, J = 8.0 Hz, 2H, CH); δ_C (100 MHz, d-CDCl_3) 14.1 21.5, 25.9, 26.7, 34.3, 44.1, 46.9, 50.8, 51.9, 52.6, 66.9, 70.5, 113.6, 119.8, 120.7 126.3, 126.8, 127.1, 127.5, 127.9, 128.1, 128.7, 129.8, 130.7, 132.3, 132.9, 133.7, 135.5, 135.6, 136.8, 140.7, 143.3, 147.6, 158.6, 165.2, 166.4, 167.6; LRMS (ES+) calcd for [C_{53}H_{54}N_{2}O_{8}S + Na] 901.36, found 901.44.
4.1ak, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(N-(3-(methoxycarbonyl)benzyl)-4-methylphenylsulfonamido)acetamido)benzoate. Sulfonamide 2.30 was functionalized on a 0.07 mmol scale using General Procedure A to give 4.1ak (48.2 mg, 80 %); δ_H (400 MHz, d_CDCl_3) 1.30-1.47 (m, 5H, CH_2), 1.67-1.94 (m, 5H, CH_2), 2.35-2.55 (m, 4H, CH and CH_3), 3.67 (s, 2H, CH_2), 3.86 (s, 3H, CH_3), 4.60 (s, 2H, CH_2), 4.68 (s, 2H, CH_2), 4.79 (s, 2H, CH_2), 5.31 (s, 2H, CH_2), 6.34 (s, 1H, CH), 6.46 (d, J = 8.0 Hz, 1H, CH), 6.95 (d, J = 8.0 Hz, 2H, CH), 7.09 (d, J = 8.0 Hz, 2H, CH), 7.24-7.43 (m, 13H, CH), 7.47 (d, J = 7.6 Hz, 1H, CH), 7.71 (d, J = 8.0 Hz, 1H, CH), 7.76 (d, J = 8.4 Hz, 2H, CH), 7.84 (s, 1H, CH), 7.93 (d, J = 8.4 Hz, 1H, CH); δ_C (100 MHz, d_CDCl_3) 21.5, 25.9, 26.7, 34.3, 44.1, 46.8, 50.8, 52.0, 52.6, 66.9, 70.6, 113.6, 119.9, 120.6, 126.8, 127.0, 127.5, 127.9, 128.1, 128.1, 128.4, 128.4, 128.8, 129.1, 129.4, 129.5, 130.4, 132.9, 133.2, 133.7, 135.6, 135.9, 136.9, 143.3, 144.7, 147.6, 158.6, 165.2, 166.4, 166.5; LRMS (ES+) calcd for [C_{52}H_{52}N_2O_8S + Na] 887.34, found 887.40.
4.1a, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(N-(4-(methoxycarbonyl)benzyl)-4-methylphenylsulfonamido)acetamido)benzoate. Sulfonamide 2.30 was functionalized on a 0.07 mmol scale using General Procedure A to give 4.1a (51.1 mg, 84%); δ_H (400 MHz, d-CDCl_3) 1.33-1.48 (m, 5H, CH_2), 1.70-1.95 (m, 5H, CH_2), 2.38-2.60 (m, 4H, CH and CH_3), 3.67 (s, 2H, CH_2), 3.82 (s, 3H, CH_3), 4.62 (s, 2H, CH_2), 4.67 (s, 2H, CH_2), 4.76 (s, 2H, CH_2), 5.31 (s, 2H, CH_2), 6.29 (s, 1H, CH), 6.44 (d, J = 8.0 Hz, 1H, CH), 6.95 (d, J = 8.0 Hz, 2H, CH), 7.10 (d, J = 8.0 Hz, 2H, CH), 7.71 (d, J = 8.0 Hz, 1H, CH), 7.76 (d, J = 8.0 Hz, 2H, CH); δ_C (100 MHz, d-CDCl_3) 21.5, 25.9, 26.7, 34.3, 44.1, 46.9, 50.8, 51.9, 52.6, 66.9, 70.5, 113.6, 119.8, 120.7, 126.3, 126.8, 127.1, 127.5, 127.9, 128.1, 128.4, 128.7, 128.8, 129.4, 129.8, 130.7, 132.3, 132.9, 133.7, 135.5, 135.6, 136.8, 140.7, 143.3, 144.7, 129.7, 147.6, 158.6, 165.2, 166.4, 167.6; LRMS (ES+) calcd for [C_{52}H_{52}N_{2}O_{8}S + H]^+ 865.34, found 865.42.

4.2a, 4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-propylphenylsulfonamido)acetamido)-2-hydroxybenzoic acid. Compound 4.1a was globally deprotected according to general procedure B on a 0.07 mmol scale to give compound 4.2a (37.3 mg, 92%); δ_H (400 MHz, d-CDCl_3) 0.82 (t, J = 7.2 Hz, 3H, CH_3), 1.32-1.42 (m, 5H, CH_2), 1.42-1.54 (m, 2H, CH_2), 1.78-1.96 (m, 5H, CH_2), 2.38-2.52 (m, 4H, CH and CH_3), 3.25 (t, J = 7.6 Hz, 2H, CH_2), 3.93 (s, 2H, CH_2), 4.79 (s, 2H, CH_2), 6.60 (d, J = 8.0 Hz, 1H, CH), 6.70 (s, 1H, CH), 7.04 (d, J = 8.0 Hz, 2H, CH), 7.11 (d, J = 8.0 Hz, 2H, CH), 7.27 (d, J = 8.0 Hz, 2H, CH), 7.73 (d, J = 8.0 Hz, 2H, CH), 7.87 (d, J = 8.4 Hz, 1H, CH), 10.74 (s, 1H, OH); δ_C (100 MHz, d-CDCl_3) 11.2, 21.3, 21.7, 26.2, 27.0, 34.5, 44.3, 48.2, 50.1, 53.1, 111.8, 117.2, 119.3, 127.1, 127.7, 128.6, 128.7, 129.6, 132.4, 133.7, 136.9, 147.6.
143.4, 147.8, 163.1, 167.4, 172.5; HRMS (ES+) Calcd for \([C_{32}H_{38}N_2O_6S + H]\) 579.2523 found 579.2512; HPLC (I) \(t_R = 15.83\) min (97.2 %), (II) 40.90 min (96.5 %).

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\text{4.2b, 4-(N-(4-cyclohexylbenzyl)-2-(N-isobutyl-4-methylphenylsulfonamido)acetamido)-2-hydroxybenzoic acid. Compound 4.1b was globally deprotected according to general procedure B on a 0.03 mmol scale to give compound 4.2b (16.8 mg, 94 %); } \\
\text{\(\delta_H\) (400 MHz, \(d\)-CDCl$_3$) 0.8 (d, } J = 6.4 \text{ Hz, 6H, 2 CH$_3$), 1.32-1.48 (m, 5H, CH$_2$), 1.66-1.92 (m, 6H, CH and CH$_2$), 2.32-2.53 (m, 4H, CH and CH$_3$), 3.14 (d, } J = 7.2 \text{ Hz, 2H, CH$_2$), 3.92 (s, 2H, CH$_2$), 4.76 (s, 2H, CH$_2$), 6.59 (d, } J = 8.0 \text{ Hz, 1H, CH), 6.70 (s, 1H, CH), 7.01 (d, } J = 7.6 \text{ Hz, 2H, CH), 7.11 (d, } J = 7.6 \text{ Hz, 2H, CH), 7.28 (d, } J = 8.0 \text{ Hz, 2H, CH), 7.71 (d, } J = 8.0 \text{ Hz, 2H, CH), 7.87 (d, } J = 7.6 \text{ Hz, 1H, CH), 10.66 (s, 1H, OH); } \\
\text{\(\delta_C\) (100 MHz, \(d\)-CDCl$_3$) 20.1, 21.7, 26.3, 27.0, 29.8, 34.6, 44.4, 48.6, 50.1, 53.1, 111.5, 117.2, 119.4, 127.1, 127.8, 128.6, 129.5, 132.5, 133.7, 137.0, 143.3, 146.0, 147.8, 163.2, 167.2, 172.6; HRMS (ES+) Calcd for } \[C_{33}H_{40}N_2O_6S + H\] 593.2679 found 593.2653; HPLC (I) \(t_R = 23.42\) min (100 %), (II) 53.02 min (100 %).
**4.2c, 4-(2-(N-allyl-4-methylphenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)-2-hydroxybenzoic acid.** Compound 4.1a was globally deprotected according to general procedure C on a 0.06 mmol scale to give compound 4.2c (25.9 mg, 90%); $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.31-1.47 (m, 5H, CH$_2$), 1.70-1.92 (m, 5H, CH$_2$), 2.38-2.54 (m, 4H, CH and CH$_3$), 3.90 (s, 2H, CH$_2$), 3.94 (d, $J = 6.4$ Hz, 2H, CH$_2$) 4.79 (s, 2H, CH$_2$), 5.12-5.21 (m, 2H, CH$_2$), 5.59-5.71 (m, 1H, CH), 6.57 (d of d, $J = 8.4$ and 2.0 Hz, 1H, CH), 6.67 (d, $J = 2.0$ Hz, 1H, CH), 7.05 (d, $J = 8.0$ Hz, 2H, CH), 7.11 (d, $J = 8.0$ Hz, 2H, CH), 7.28 (d, $J = 8.0$ Hz, 2H, CH), 7.74 (d, $J = 8.4$ Hz, 2H, CH), 7.87 (d, $J = 8.4$ Hz, 1H, CH), 10.74 (s, 1H, OH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 21.7, 26.3, 27.0, 34.5, 44.3, 47.3, 50.9, 53.1, 111.9, 117.2, 119.3, 119.8, 127.1, 127.8, 128.6, 129.6, 132.4, 132.9, 133.7, 136.9, 143.5, 147.8, 148.0, 163.1, 167.4, 172.5; HRMS (ES+) Calcd for [C$_{32}$H$_{36}$N$_2$O$_6$S + H] 577.2366 found 577.2388; HPLC (III) $t_R$ =22.00 min (100 %), (IV) 50.92 min (100 %).

**4.2d, 4-(2-(N-(2-amino-2-oxoethyl)-4-methylphenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)-2-hydroxybenzoic acid.** Compound 4.1c was globally
deprotected according to general procedure B on a 0.05 mmol scale to give compound 4.2d (25.8 mg, 87%); \( \delta_H \) (400 MHz, \( d_6 \)-DMSO) 1.26-1.43 (m, 5H, CH\(_2\)), 1.63-1.83 (m, 5H, CH\(_2\)), 2.36 (s, 3H, CH\(_3\)), 2.40-2.49 (m, 1H, CH), 3.81 (s, 2H, CH\(_2\)), 3.96 (s, 2H, CH\(_2\)), 4.80 (s, 2H, CH\(_2\)), 6.72 (d of d, \( J = 8.4 \) and 2.0 Hz, 1H, CH), 6.79 (d, \( J = 2.0 \) Hz, 1H, CH), 7.09 (d, \( J = 8.0 \) Hz, 2H, CH), 7.14 (d, \( J = 8.0 \) Hz, 2H, CH), 7.21 (s, 1H, NH), 7.32 (d, \( J = 8.0 \) Hz, 2H, CH), 7.57 (d, \( J = 8.4 \) Hz, 2H, CH), 7.78 (d, \( J = 8.4 \) Hz, 1H, CH), 8.01 (s, 1H, NH); \( \delta_C \) (100 MHz, \( d_6 \)-DMSO) 21.0, 25.6, 26.3, 33.9, 43.4, 50.1, 51.4, 54.9, 113.4, 116.2, 118.0, 126.6, 127.1, 127.6, 129.6, 131.4, 134.2, 135.6, 143.4, 146.5, 148.1, 162.0, 167.9, 170.0, 171.0; HRMS (ES+) Calcd for \([C_{31}H_{37}N_{5}O_{7}S + H] \) 627.2500 found 627.2523; HPLC (I) \( t_R \) =20.94 min (98.5 %), (IV) 47.57 min (98.5 %).

4.2e, 4-(2-(N-benzyl-4-methylphenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)-2-hydroxybenzoic acid. Compound 4.1d was globally deprotected according to general procedure B on a 0.06 mmol scale to give compound 4.2e (34.5 mg, 92%); \( \delta_H \) (400 MHz, \( d_6 \)-CDCl\(_3\)) 1.32-1.50 (m, 5H, CH\(_2\)), 1.69-1.96 (m, 5H, CH\(_2\)), 2.37-2.55 (m, 4H, CH and CH\(_3\)), 3.78 (s, 2H, CH\(_2\)), 4.56 (s, 2H, CH\(_2\)), 4.70 (s, 2H, CH\(_2\)), 6.24 (d, \( J = 7.6 \) Hz, 1H, CH), 6.42 (s, 1H, CH), 6.99 (d, \( J = 8.0 \) Hz, 2H, CH), 7.10 (d, \( J = 8.0 \) Hz, 2H, CH), 7.16-7.29 (m, 5H, CH), 7.32 (d, \( J = 8.0 \) Hz, 2H, CH), 7.72 (d, \( J = 8.0 \) Hz, 1H, CH), 7.81 (d, \( J = 8.0 \) Hz, 2H, CH), 10.57 (s, 1H, OH); \( \delta_C \) (100 MHz, \( d_6 \)-CDCl\(_3\)) 21.8, 26.2, 27.0, 34.6, 44.4, 47.0, 51.2, 53.0, 111.6, 117.1, 119.2, 127.1, 127.8, 128.2, 128.6, 128.7, 128.8, 128.9, 129.7, 132.2, 133.6, 137.1, 143.5, 147.8, 163.0, 167.0, 172.7; HRMS (ES+) Calcd for \([C_{36}H_{38}N_{5}O_{6}S + H] \) 627.2500 found 627.2523; HPLC (I) \( t_R \) =16.48 min (97.4 %), (II) 43.18 min (98.5 %).
4.2f, 4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(2-methylbenzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. Compound 4.1e was globally deprotected according to general procedure B on a 0.06 mmol scale to give compound 4.2f (32.5 mg, 85 %); δ\textsubscript{H} (400 MHz, d\textsubscript{-}CDCl\textsubscript{3}) 1.32-1.49 (m, 5H, CH\textsubscript{2}), 1.71-1.93 (m, 5H, CH\textsubscript{2}), 2.24 (s, 3H, CH\textsubscript{3}), 2.40-2.55 (m, 4H, CH and CH\textsubscript{3}), 3.70 (s, 2H, CH\textsubscript{2}), 4.61 (s, 2H, CH\textsubscript{2}), 4.67 (s, 2H, CH\textsubscript{2}), 6.18 (d, \textit{J} = 6.8 Hz, 1H, CH), 6.37 (s, 1H, CH), 6.97 (d, \textit{J} = 8.0 Hz, 2H, CH), 7.02-7.16 (m, 5H, CH), 7.17-7.24 (m, 1H, CH), 7.32 (d, \textit{J} = 8.0 Hz, 2H, CH), 7.71 (d, \textit{J} = 8.0 Hz, 1H, CH), 7.80 (d, \textit{J} = 8.0 Hz, 2H, CH), 10.51 (s, 1H, OH); δ\textsubscript{C} (100 MHz, d\textsubscript{-}CDCl\textsubscript{3}) 19.2, 21.8, 26.3, 27.0, 34.6, 44.4, 46.9, 49.2, 52.9, 111.4, 117.2, 119.3, 126.2, 127.0, 127.9, 128.5, 128.7, 129.6, 130.1, 130.8, 132.2, 132.8, 133.6, 136.8, 138.0, 143.5, 147.8, 148.0, 163.0, 166.9, 172.7; HRMS (ES+) Calcd for [C\textsubscript{37}H\textsubscript{40}N\textsubscript{2}O\textsubscript{6}S + H] 641.2679 found 641.2650; HPLC (I) \textit{t}\textsubscript{R} =23.94 min (100 %), (II) 54.87 min (100 %).
4.2g, 4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(3-methylbenzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. Compound 4.1f was globally deprotected according to general procedure B on a 0.07 mmol scale to give compound 4.2g (38.2 mg, 85 %); $\delta_H$ (400 MHz, d-CDCl$_3$) 1.32-1.52 (m, 5H, CH$_2$), 1.70-1.97 (m, 5H, CH$_2$), 2.26 (s, 3H, CH$_3$), 2.40-2.56 (m, 4H, CH and CH$_3$), 3.79 (s, 2H, CH$_2$), 4.53 (s, 2H, CH$_2$), 4.71 (s, 2H, CH$_2$), 6.26 (d, $J$ = 7.6 Hz, 1H, CH), 6.41 (s, 1H, CH), 6.95-7.04 (m, 4H, CH), 7.06-7.21 (m, 4H, CH), 7.32 (d, $J$ = 8.0 Hz, 2H, CH), 7.72 (d, $J$ = 7.6 Hz, 1H, CH), 7.81 (d, $J$ = 8.0 Hz, 2H, CH), 10.54 (s, 1H, OH); $\delta_C$ (100 MHz, d-CDCl$_3$) 21.4, 21.7, 26.3, 27.0, 34.5, 44.4, 46.9, 51.1, 53.0, 111.5, 117.1, 119.2, 126.1, 127.1, 127.8, 128.1, 128.7, 129.0, 129.6, 132.2, 133.6, 135.1, 137.2, 138.6, 143.5, 147.8, 148.0, 163.0, 167.0, 172.8; HRMS (ES+) Calcd for [C$_{37}$H$_{40}$N$_2$O$_6$S + H] 641.2679 found 641.2648; HPLC (I) $t_R$ =23.95 min (100 %), (II) 55.00 min (100 %).

\[ \begin{array}{c}
\text{HO} \\
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4.2h, 4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(4-methylbenzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. Compound 4.1g was globally deprotected according to general procedure B on a 0.06 mmol scale to give compound 4.2h (33.2 mg, 87 %); $\delta_H$ (400 MHz, d-CDCl$_3$) 1.32-1.50 (m, 5H, CH$_2$), 1.69-1.96 (m, 5H, CH$_2$), 2.33 (s, 3H, CH$_3$), 2.38-2.60 (m, 4H, CH and CH$_3$), 3.77 (s, 2H, CH$_2$), 4.52 (s, 2H, CH$_2$), 4.71 (s, 2H, CH$_2$), 6.24 (d, $J$ = 7.8 Hz, 1H, CH), 6.36 (s, 1H, CH), 6.99 (d, $J$ = 8.0 Hz, 2H, CH), 7.04-7.09 (m, 4H, CH), 7.10 (d, $J$ = 8.0 Hz, 2H, CH), 7.31 (d, $J$ = 8.0 Hz, 2H, CH), 7.72 (d, $J$ = 8.4 Hz, 1H, CH), 7.81 (d, $J$ = 8.0 Hz, 2H, CH), 10.56 (s, 1H, OH); $\delta_C$ (100 MHz, d-CDCl$_3$) 21.3, 21.7, 26.2, 27.0, 34.6, 44.4, 46.8, 50.9, 53.0, 111.6, 117.1, 119.3, 127.1, 127.8, 128.7, 129.0, 129.5, 129.6, 132.0, 132.2, 133.6, 137.1, 138.1, 143.5, 147.8, 147.9, 163.0, 167.0, 172.7; HRMS
(ES+) Calcd for \([\text{C}_{37}\text{H}_{40}\text{N}_{2}\text{O}_{6}\text{S} + \text{H}]\) 641.2679 found 641.2654; HPLC (I) \(t_R = 16.65\) min (97.8 %), (II) 44.47 min (98.8 %).

4.2i, 4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(2-(trifluoromethyl)benzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. Compound 4.1h was globally deprotected according to general procedure B on a 0.06 mmol scale to give compound 4.2i (37.4 mg, 90 %); \(\delta_H\) (400 MHz, \(d\)-CDCl\(_3\)) 1.30-1.50 (m, 5H, CH\(_2\)), 1.67-1.91 (m, 5H, CH\(_2\)), 2.35-2.56 (m, 4H, CH and CH\(_3\)), 3.83 (s, 2H, CH\(_2\)), 4.71 (s, 2H, CH\(_2\)), 4.79 (s, 2H, CH\(_2\)), 6.88 (d, \(J = 7.2\) Hz, 1H, CH), 6.51 (s, 1H, CH), 6.97 (d, \(J = 8.0\) Hz, 2H, CH), 7.08 (d, \(J = 8.0\) Hz, 2H, CH), 7.29-7.41 (m, 3H, CH), 7.50-7.53 (m, 1H, CH), 7.62 (d, \(J = 8.0\) Hz, 1H, CH), 7.70-7.87 (m, 4H, CH), 10.63 (s, 1H, OH); \(\delta_C\) (100 MHz, \(d\)-CDCl\(_3\)) 21.8, 26.2, 27.0, 34.5, 44.3, 47.7, 47.9, 53.0, 111.8, 117.2, 119.2, 125.6, 125.8, 125.9, 127.1, 127.8, 127.9, 128.3, 128.6, 129.7, 130.1, 132.3, 132.5, 133.5, 134.9, 136.8, 143.8, 147.8, 163.0, 166.5, 172.7; HRMS (ES+) Calcd for \([\text{C}_{37}\text{H}_{37}\text{F}_{3}\text{N}_{2}\text{O}_{6}\text{S} + \text{H}]\) 695.2397 found 695.2399; HPLC (I) \(t_R = 23.90\) min (100 %), (II) 54.98 min (100 %).
4.2j, 4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(3-(trifluoromethyl)benzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. Compound 4.1i was globally deprotected according to general procedure B on a 0.04 mmol scale to give compound 4.2j (21.9 mg, 79 %); δH (400 MHz, d-CDCl3) 1.30-1.55 (m, 5H, CH₂), 1.65-1.95 (m, 5H, CH₂), 2.30-2.60 (m, 4H, CH and CH₃), 3.79 (s, 2H, CH₂), 4.64 (s, 2H, CH₂), 4.72 (s, 2H, CH₂), 6.32 (d, J = 8.0 Hz, 1H, CH), 6.47 (s, 1H, CH), 6.99 (d, J = 8.4 Hz, 2H, CH), 7.10 (d, J = 8.4 Hz, 2H, CH), 7.32 (d, J = 8.0 Hz, 2H, CH), 7.36-7.44 (m, 2H, CH), 7.46 (d, J = 7.6 Hz, 1H, CH), 7.53 (d, J = 7.6 Hz, 1H, CH), 7.65-7.95 (m, 3H, CH), 10.60 (s, 1H, OH); δC (100 MHz, d-CDCl₃) 21.7, 26.3, 27.0, 34.5, 44.4, 47.3, 51.0, 53.0, 111.7, 117.1, 119.0, 119.1, 125.1, 125.4, 127.1, 127.8, 128.6, 129.4, 129.7, 132.2, 132.3, 133.5, 136.7, 136.9, 143.8, 147.4, 147.8, 147.9, 163.1, 166.9, 172.5; HRMS (ES+) calcd for [C₃₇H₃₇N₂O₆F₃S + H] 695.2397, found 695.2406; HPLC (I) t_R = 23.87 min (100 %), (II) t_R = 54.89 min (100 %).
4.2k, 4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(4-(trifluoromethyl)benzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. Compound 4.1j was globally deprotected according to general procedure B on a 0.05 mmol scale to give compound 4.2k (28.9 mg, 83 %); δH (400 MHz, d-CDCl₃) 1.31-1.48 (m, 5H, CH₂), 1.70-1.92 (m, 5H, CH₂), 2.38-2.54 (m, 4H, CH and CH₃), 3.79 (s, 2H, CH₂), 4.64 (s, 2H, CH₂), 4.71 (s, 2H, CH₂), 6.27 (d, J = 8.0 Hz, 1H, CH), 6.46 (s, 1H, CH), 6.99 (d, J = 7.6 Hz, 2H, CH), 7.11 (d, J = 7.6 Hz, 2H, CH), 7.32 (d, J = 8.0 Hz, 2H, CH), 7.35 (d, J = 8.0 Hz, 2H, CH), 7.52 (d, J = 8.0 Hz, 2H, CH), 7.70-7.86 (m, 3H, CH), 10.60 (s, 1H, OH); δC (100 MHz, d-CDCl₃) 21.4, 26.0, 26.7, 34.3, 44.1, 47.0, 50.7, 52.8, 111.4, 116.8, 118.7, 125.2, 125.5, 126.8, 127.5, 128.4, 132.0, 132.0, 133.2, 136.6, 139.5, 143.5, 147.7, 162.8, 166.5, 166.6, 172.1; HRMS (ES+) calcd for [C₃₇H₃₇N₂O₆F₃S + H] 695.2397, found 695.2414; HPLC (I) tᵣ = 23.98 min (98.6 %), (II) tᵣ = 55.45 min (96.6 %).

4.2l, 4-(N-(4-cyclohexylbenzyl)-2-(N-(2-fluorobenzyl)-4-methylphenylsulfonamido)acetamido)-2-hydroxybenzoic acid. Compound 4.1k was globally deprotected according to general procedure B on a 0.06 mmol scale to give compound 4.2l (31.7 mg, 82 %); δH (400 MHz, d-CDCl₃) 1.32-1.47 (m, 5H, CH₂), 1.70-1.94 (m, 5H, CH₂), 2.37-2.53 (m, 4H, CH and CH₃), 3.84 (s, 2H, CH₂), 4.63 (s, 2H, CH₂), 4.75 (s, 2H, CH₂), 6.42 (d, J = 8.0 Hz, 1H, CH), 6.54 (s, 1H, CH), 6.96-7.04 (m, 3H CH), 7.06 (t, J = 7.6 Hz, 1H, CH), 7.10 (d, J = 8.0 Hz, 2H, CH), 7.21-7.27 (m, 1H, CH), 7.29 (d, J = 8.0 Hz, 2H, CH), 7.33-7.41 (m, 1H, CH), 7.47-7.84 (m, 3H, CH) 10.56 (s, 1H, OH); δC (100 MHz, d-CDCl₃) 21.7, 26.2, 27.0, 34.5, 44.3, 44.8, 47.7, 53.0, 111.6, 115.3, 115.6, 117.2, 119.3, 122.5, 122.7, 124.6, 124.6, 127.1, 127.8,
128.6, 129.6, 130.0, 131.4, 131.4, 132.3, 133.6, 137.0, 143.5, 147.8, 148.0, 160.1, 162.5, 163.0, 166.9, 172.8; HRMS (ES+) Calcd for $[\text{C}_{36}\text{H}_{37}\text{FN}_2\text{O}_6\text{S} + \text{H}]$ 645.2429 found 645.2396; HPLC (I) $t_R$ = 23.49 min (100 %), (II) 53.47 min (100 %).

4.2m, 4-((4-cyclohexylbenzyl)-2-(N-(3-fluorobenzyl)-4-methylphenylsulfonamido)acetamido)-2-hydroxybenzoic acid. Compound 4.1l was globally deprotected according to general procedure B on a 0.06 mmol scale to give compound 4.2m (36.2 mg, 94 %); $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.32-1.47 (m, 5H, CH$_2$), 1.70-1.94 (m, 5H, CH$_2$), 2.39-2.55 (m, 4H, CH and CH$_3$), 3.80 (s, 2H, CH$_2$), 4.58 (s, 2H, CH$_2$), 4.72 (s, 2H, CH$_2$), 6.33 (d, $J =$ 8.0 Hz, 1H, CH), 6.47 (s, 1H, CH), 6.91-7.05 (m, 5H CH), 7.11 (d, $J =$ 8.0 Hz, 2H, CH), 7.20-7.27 (m, 1H, CH), 7.32 (d, $J =$ 8.0 Hz, 2H, CH), 7.79 (d, $J =$ 8.0 Hz, 1H, CH), 10.56 (s, 1H, OH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 21.8, 26.2, 27.0, 34.5, 44.3, 47.1, 50.8, 53.0, 111.6, 115.1, 115.3, 115.5, 115.7, 117.0, 119.1, 124.3, 124.3, 127.1, 127.8, 128.6, 129.7, 130.3, 130.4, 132.3, 133.5, 136.9, 138.1, 138.1, 143.7, 147.8, 147.9, 161.9, 163.0, 164.3, 166.9, 172.7; HRMS (ES+) Calcd for $[\text{C}_{36}\text{H}_{37}\text{FN}_2\text{O}_6\text{S} + \text{H}]$ 645.2429 found 645.2406; HPLC (I) $t_R$ = 23.60 min (100 %), (II) 53.89 min (100 %).
**4.2n, 4-(N-(4-cyclohexylbenzyl)-2-(N-(4-fluorobenzyl)-4-methylphenylsulfonamido)acetamido)-2-hydroxybenzoic acid.** Compound **4.1m** was globally deprotected according to general procedure B on a 0.06 mmol scale to give compound **4.2n** (35.8 mg, 93 %); \( \delta_H \) (400 MHz, \( d-\text{CDCl}_3 \)) 1.32-1.47 (m, 5H, CH\(_2\)), 1.69-1.93 (m, 5H, CH\(_2\)), 2.40-2.54 (m, 4H, CH and CH\(_3\)), 3.74 (s, 2H, CH\(_2\)), 4.53 (s, 2H, CH\(_2\)), 4.69 (s, 2H, CH\(_2\)), 6.27 (d, \( J = 7.6 \) Hz, 1H, CH), 6.42 (s, 1H, CH), 6.90-7.03 (m, 4H CH), 7.10 (d, \( J = 8.0 \) Hz, 2H, CH), 7.15-7.23 (m, 2H, CH), 7.31 (d, \( J = 8.0 \) Hz, 2H, CH), 7.76 (d, \( J = 8.4 \) Hz, 1H, CH), 7.79 (d, \( J = 8.0 \) Hz, 2H, CH), 10.92 (s, 1H, OH); \( \delta_C \) (100 MHz, \( d-\text{CDCl}_3 \)) 21.8, 26.3, 27.0, 34.6, 44.4, 46.9, 50.5, 52.9, 110.8, 115.6, 115.8, 116.9, 118.9, 127.1, 127.8, 128.7, 129.7, 130.6, 130.7, 131.1 131.2, 132.2, 133.7, 137.1, 143.5, 147.8, 161.4, 163.0, 163.6, 166.8, 172.1; HRMS (ES+) Calcd for [C\(_{36}\)H\(_{37}\)FN\(_2\)O\(_6\)S + H] 645.2429 found 645.2398; HPLC (I) \( t_R = 23.63 \) min (100 %), (II) 53.98 min (100 %).
4.2o, 4-(2-(N-(2-chlorobenzyl)-4-methylphenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)-2-hydroxybenzoic acid. Compound 4.1n was globally deprotected according to general procedure B on a 0.09 mmol scale to give compound 4.2o (41.2 mg, 69%); δH (400 MHz, d-CDCl3) 1.32-1.46 (m, 5H, CH2), 1.69-1.94 (m, 5H, CH2), 2.40-2.53 (m, 4H, CH and CH3), 3.74 (s, 2H, CH2), 4.53 (s, 2H, CH2), 4.69 (s, 2H, CH2), 6.35 (d, J = 7.2 Hz, 1H, CH), 6.50 (s, 1H, CH), 7.00 (d, J = 7.8 Hz, 2H, CH), 7.09 (d, J = 7.8 Hz, 2H, CH), 7.13-7.23 (m, 2H, CH), 7.27-7.34 (m, 3H, CH), 7.40 (d, J = 6.5 Hz, 1H, CH), 7.73-7.81 (m, 3H, CH); δC (100 MHz, d-CDCl3) 21.5, 26.0, 26.7, 34.3, 44.1, 47.6, 48.5, 52.8, 112.3, 116.7, 118.8, 126.7, 127.1, 127.6, 128.5, 128.7, 129.1, 129.3, 129.4, 130.5, 133.1, 133.5, 133.8, 136.8, 143.2, 147.4, 162.7, 163.5, 166.5, 171.8; HPLC (I) tR = 28.24 min (100%). HRMS (ES+) Calcd for [C36H38N2O6SCl + H] 661.2133 found 661.2151

4.2p, 4-(2-(N-(3-chlorobenzyl)-4-methylphenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)-2-hydroxybenzoic acid. Compound 4.1o was globally deprotected according to general procedure B on a 0.07 mmol scale to give compound 4.2p (28.4 mg, 61%); δH (400 MHz, d-CDCl3) 1.30-1.46 (m, 5H, CH2), 1.70-1.94 (m, 5H, CH2), 2.39-2.52 (m, 4H, CH and CH3), 3.76 (s, 2H, CH2), 4.56 (s, 2H, CH2), 4.69 (s, 2H, CH2), 6.22 (d, J = 7.8 Hz, 1H, CH), 6.40 (s, 1H, CH), 6.99 (d, J = 8.0 Hz, 2H, CH), 7.10 (d, J = 8.0 Hz, 2H, CH), 7.16-7.23 (m, 2H, CH), 7.24-7.29 (m, 2H, CH), 7.31 (d, J = 8.0 Hz, 2H, CH), 7.71 (d, J = 7.8 Hz, 1H, CH), 7.81 (d, J = 8.0 Hz, 2H, CH); δC (100 MHz, d-CDCl3) 21.6, 26.1, 26.9, 34.4, 44.2, 46.7, 51.0, 52.8, 111.7, 116.9, 118.3, 126.9, 127.7, 128.1, 128.5, 128.5, 128.7, 128.8, 129.5, 132.0, 132.5, 133.6, 135.2, 137.1, 143.3, 147.6, 161.5, 162.8, 166.7, 170.8; HPLC (I) tR = 28.30 min
(98.40 %), (II) 62.83 min (98.25 %); HRMS (ES+) Calcd for [C\textsubscript{36}H\textsubscript{38}N\textsubscript{2}O\textsubscript{6}SCl + H] 661.2133 found 661.2137.

\begin{center}
\includegraphics{image.png}
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4.2q, 4-(2-(N-(4-chlorobenzyl)-4-methylphenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)-2-hydroxybenzoic acid. Compound 4.1p was globally deprotected according to general procedure B on a 0.08 mmol scale to give compound 4.2q (33.7 mg, 64 %); \(\delta\)\textsubscript{H} (400 MHz, \textit{d}-CDCl\textsubscript{3}) 1.31-1.47 (m, 5H, CH\textsubscript{2}), 1.70-1.92 (m, 5H, CH\textsubscript{2}), 2.41-2.54 (m, 4H, CH and CH\textsubscript{3}), 3.76 (s, 2H, CH\textsubscript{2}), 4.53 (s, 2H, CH\textsubscript{2}), 4.70 (s, 4H, 2 CH\textsubscript{2}), 6.30 (d, \(J = 7.2\) Hz, 1H, CH), 6.46 (s, 1H, CH), 6.98 (d, \(J = 7.8\) Hz, 2H, CH), 7.10 (d, \(J = 7.8\) Hz, 2H, CH), 7.15 (d, \(J = 8.2\) Hz, 2H, CH), 7.23 (d, \(J = 8.2\) Hz, 2H, CH), 7.31 (d, \(J = 8.0\) Hz, 2H, CH), 7.73-7.81 (m, 3H, CH); \(\delta\)\textsubscript{C} (100 MHz, \textit{d}-CDCl\textsubscript{3}) 21.6, 26.1, 26.8, 34.4, 44.2, 46.8, 50.5, 52.9, 110.7, 116.9, 118.9, 127.0, 127.7, 128.4, 128.8, 129.5, 130.1, 132.2, 133.4, 133.7, 134.0, 136.8, 143.5, 147.7, 162.9, 163.5, 166.8, 172.8; HPLC (I) \(t_R = 28.10\) min (100 %), (II) 62.98 min (100 %); HRMS (ES+) Calcd for [C\textsubscript{36}H\textsubscript{38}N\textsubscript{2}O\textsubscript{6}SCl + H] 661.2133 found 661.2149.
4.2r, 4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(2-(trifluoromethoxy)benzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid.

Compound 4.1q was globally deprotected according to general procedure B on a 0.05 mmol scale to give compound 4.2r (29.6 mg, 83%); $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.30-1.48 (m, 5H, CH$_2$), 1.70-1.92 (m, 5H, CH$_2$), 2.40-2.53 (m, 4H, CH and CH$_3$), 3.81 (s, 2H, CH$_2$), 4.68 (s, 2H, CH$_2$), 4.73 (s, 2H, CH$_2$), 6.40 (d, $J$ = 8.0 Hz, 1H, CH), 6.53 (s, 1H, CH), 6.99 (d, $J$ = 8.0 Hz, 2H, CH), 7.09 (d, $J$ = 8.0 Hz, 2H, CH), 7.18-7.24 (m, 2H, CH), 7.28-7.35 (m, 3H, CH), 7.51 (d, $J$ = 8.0 Hz, 1H, CH), 7.77 (d, $J$ = 8.0 Hz, 3H, CH), 10.67 (s, 1H, OH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 21.7, 26.3, 27.0, 34.5, 44.3, 45.7, 47.8, 53.0, 111.9, 117.1, 119.1, 119.3, 120.7, 121.8, 127.0, 127.4, 127.8, 128.6, 129.4, 129.6, 131.1, 132.3, 133.6, 137.0, 143.6, 147.7, 147.8, 153.4, 163.0, 166.6, 172.5; HRMS (ES+) calcd for [C$_{37}$H$_{37}$N$_2$O$_7$F$_3$S + H] 711.2346, Found 711.2348; HPLC (I) $t_R = 23.97$ min (98.3 %), (II) $t_R = 55.46$ min (96.0 %).

4.2s, 4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(3-(trifluoromethoxy)benzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid.

Compound 4.1r was globally deprotected according to general procedure B on a 0.04 mmol scale to give compound 4.2s (27.6 mg, 97%); $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.31-1.47 (m, 5H, CH$_2$), 1.69-1.94 (m, 5H, CH$_2$), 2.39-2.55 (m, 4H, CH and CH$_3$), 3.78 (s, 2H, CH$_2$), 4.61 (s, 2H, CH$_2$), 4.69 (s, 2H, CH$_2$), 6.27 (d, $J$ = 8.0 Hz, 1H, CH), 6.45 (s, 1H, CH), 6.99 (d, $J$ = 8.0 Hz, 2H, CH), 7.03 (s, 1H, CH), 7.06-7.17 (m, 3H, CH), 7.20 (d, $J$ = 7.6 Hz, 1H, CH), 7.29 (d, $J$ = 8.0 Hz, 1H, CH), 7.33 (d, $J$ = 8.4 Hz, 2H, CH), 7.73-7.85 (m, 3H, CH), 10.59 (s, 1H, OH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 21.7, 26.3, 27.0, 34.6, 44.4, 47.2, 50.9, 53.1, 111.8, 117.1, 119.0, 119.3, 120.5, 121.2, 127.1,
127.8, 128.6, 129.7, 130.3, 132.3, 133.6, 137.0, 138.1, 141.6, 143.8, 147.8, 147.9, 149.7, 163.1, 166.8, 172.3; HRMS (ES+) calcd for [C$_{37}$H$_{38}$N$_2$O$_7$F$_3$S + H] 711.2346, Found 711.2341; HPLC (I) $t_R = 23.95$ min (96.5 %), (II) $t_R = 55.18$ min (100 %).

$4.2t$, 4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(4-(trifluoromethoxy)benzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid.

Compound 4.1s was globally deprotected according to general procedure B on a 0.04 mmol scale to give compound $4.2t$ (25.1 mg, 88 %); $\delta$H (400 MHz, $d$-CDCl$_3$) 1.31-1.50 (m, 5H, CH$_2$), 1.70-1.95 (m, 5H, CH$_2$), 2.30-2.55 (m, 4H, CH and CH$_3$), 3.77 (s, 2H, CH$_2$), 4.57 (s, 2H, CH$_2$), 4.70 (s, 2H, CH$_2$), 6.26 (d, $J$ = 8.0 Hz, 1H, CH), 6.48 (s, 1H, CH), 6.99 (d, $J$ = 8.0 Hz, 2H, CH), 7.10 (d, $J$ = 8.0 Hz, 4H, CH), 7.25 (d, $J$ = 8.0 Hz, 2H, CH), 7.31 (d, $J$ = 8.0 Hz, 2H, CH), 7.50-7.56 (m, 1H, CH), 7.68-7.82 (m, 3H, CH), 10.67 (s, 1H, OH); $\delta$C (100 MHz, $d$-CDCl$_3$) 21.7, 26.3, 26.7, 27.0, 34.6, 44.4, 47.2, 53.1, 110.9, 117.1, 119.1, 121.2, 127.1, 127.8, 129.0, 130.3, 131.0, 132.3, 132.7, 133.6, 134.3, 137.0, 143.7, 147.8, 148.0, 149.1, 163.1, 166.9, 172.3; HRMS (ES+) calcd for [C$_{37}$H$_{37}$N$_2$O$_7$F$_3$S + H] 711.2346, Found 711.2333; HPLC (I) $t_R = 24.03$ min (90.9 %), (II) $t_R = 55.80$ min (96.4 %).
4.2u, 4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(2-nitrobenzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. Compound 4.1t was globally deprotected according to general procedure C on a 0.08 mmol scale to give compound 4.2u (41.6 mg, 77%); δH (400 MHz, d-CDCl3) 1.31-1.47 (m, 5H, CH2), 1.69-1.94 (m, 5H, CH2), 2.40-2.54 (m, 4H, CH and CH3), 3.92 (s, 2H, CH2), 4.74 (s, 2H, CH2) 4.95 (s, 2H, CH2), 6.49 (d, J = 8.0 Hz, 1H, CH), 6.60 (s, 1H, CH), 6.99 (d, J = 8.0 Hz, 2H, CH), 7.10 (d, J = 8.0 Hz, 2H, CH), 7.29 (d, J = 8.0 Hz, 2H, CH), 7.41 (t, J = 8.0 Hz, 1H, CH), 7.58 (t, J = 7.2 Hz, 1H, CH), 7.71 (d, J = 8.0 Hz, 2H, CH), 7.76-7.84 (m, 2H, CH), 7.93 (d, J = 8.0 Hz, 1H, CH), 10.61 (s, 1H, OH); δC (100 MHz, d-CDCl3) 21.7, 26.2, 27.0, 34.5, 44.3, 49.1, 49.2, 53.1, 111.7, 117.3, 119.3, 123.3, 124.9, 127.1, 127.8, 128.6, 128.7, 129.7, 130.7, 132.0, 132.4, 133.4, 133.7, 136.5, 143.9, 147.8, 148.8, 163.1, 166.7, 172.5; HRMS (ES+) Calcd for [C36H37N3O8S + H] 672.2374 found 672.2369; HPLC (III) tR =26.70 min (100 %), (IV) 59.40 min (100 %).
4.2v, 4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(3-nitrobenzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. Compound 4.1u was globally deprotected according to general procedure C on a 0.10 mmol scale to give compound 4.2v (60.5 mg, 90 %); δH (400 MHz, d-CDCl₃) 1.31-1.48 (m, 5H, CH₂), 2.40-2.54 (m, 4H, CH and CH₃), 3.82 (s, 2H, CH₂), 4.69 (s, 2H, CH₂) 4.73 (s, 2H, CH₂), 6.42 (d, J = 8.0 Hz, 1H, CH), 6.49 (s, 1H, CH), 6.69 (d, J = 8.0 Hz, 2H, CH), 7.10 (d, J = 8.0 Hz, 2H, CH), 7.32 (d, J = 8.0 Hz, 2H, CH), 7.47 (t, J = 8.0 Hz, 1H, CH), 7.74-7.85 (m, 3H, CH); δC (100 MHz, d-CDCl₃) 21.7, 26.2, 27.0, 34.5, 44.3, 47.6, 51.0, 53.1, 111.7, 117.0, 119.1, 123.2, 123.3, 127.2, 127.7, 128.6, 129.8, 129.9, 132.5, 133.4, 134.8, 136.6, 138.1, 144.0, 147.7, 148.0, 148.5, 163.0, 166.8, 172.5; HRMS (ES+) Calcd for [C₃₇H₃₇N₃O₈S + H] 672.2374 found 672.2375; HPLC (III) t_R = 26.74 min (100 %), (IV) 59.47 min (100 %).

4.1w, 4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(4-nitrobenzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. Compound 4.1v was globally deprotected according to general procedure C on a 0.06 mmol scale to give compound 4.2w (32.5 mg, 81 %); δH (400 MHz, d-CDCl₃) 1.31-1.48 (m, 5H, CH₂), 1.69-1.93 (m, 5H, CH₂), 2.40-2.53 (m, 4H, CH and CH₃), 3.80 (s, 2H, CH₂), 4.68 (s, 2H, CH₂) 4.70 (s, 2H, CH₂), 6.38 (d, J = 8.4 Hz, 1H, CH), 6.42 (s, 1H, CH), 6.98 (d, J = 8.0 Hz, 2H, CH), 7.10 (d, J = 8.0 Hz, 2H, CH), 7.32 (d, J = 8.0 Hz, 2H, CH), 7.42 (d, J = 8.4 Hz, 2H, CH), 7.72-7.82 (m, 3H, CH), 8.11 (d, J = 8.4 Hz, 2H, CH), 10.66 (s, 1H, OH); δC (100 MHz, d-CDCl₃) 21.7, 26.2, 26.9, 34.5, 44.3, 47.5, 51.0, 53.1, 112.0, 117.0, 118.9, 124.0, 127.1, 127.8, 128.6, 128.9, 129.3, 129.8, 132.4,
133.4, 136.5, 143.4, 144.0, 147.8, 148.0, 163.0, 166.7, 172.4; HRMS (ES+) Calcd for [C$_{36}$H$_{37}$N$_3$O$_8$S + H] 672.2374 found 672.2385; HPLC (III) $t_R$ =26.88 min (88.8 %), (IV) 59.89 min (90.8 %).

4.2x, 4-(2-(N-(2-aminobenzyl)-4-methylphenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)-2-hydroxybenzoic acid. Compound 4.1t was globally deprotected according to general procedure B on a 0.14 mmol scale to give compound 4.2x (67.2 mg, 75 %); $\delta$H (400 MHz, $d$-CDCl$_3$) 1.31-1.47 (m, 5H, CH$_2$), 1.70-1.94 (m, 5H, CH$_2$), 2.38-2.54 (m, 4H, CH and CH$_3$), 3.74 (s, 2H, CH$_2$), 4.40 (s, 2H, CH$_2$) 4.68 (s, 2H, CH$_2$), 6.21 (d, $J$ = 7.2 Hz, 1H, CH), 6.41 (s, 1H, CH), 6.70 (t, $J$ = 7.2 Hz, 1H, CH), 6.77 (d, $J$ = 8.0 Hz, 1H, CH), 6.89 (d, $J$ = 7.2 Hz, 1H, CH), 6.99 (d, $J$ = 8.0 Hz, 2H, CH), 7.10 (d, $J$ = 8.0 Hz, 2H, CH), 7.16 (t, $J$ = 7.6 Hz, 1H, CH), 7.31 (d, $J$ = 8.0 Hz, 2H, CH), 7.68 (d, $J$ = 8.4 Hz, 1H, CH), 7.79 (d, $J$ = 8.0 Hz, 2H, CH); $\delta$C (100 MHz, $d$-CDCl$_3$) 21.7, 26.2, 27.0, 34.5, 44.3, 47.1, 49.5, 53.1, 112.9, 117.0, 117.7, 119.0, 119.2, 119.7, 127.0, 127.9, 128.7, 129.7, 130.2, 131.8, 132.1, 133.5, 135.9, 143.9, 144.6, 147.0, 147.8, 162.8, 167.2, 172.4; HRMS (ES+) Calcd for [C$_{36}$H$_{39}$N$_3$O$_8$S + H] 642.2632 found 642.2647; HPLC (III) $t_R$ =22.59 min (100 %), (IV) 48.06 min (100 %).
4.2y, 4-(2-(N-(3-aminobenzyl)-4-methylphenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)-2-hydroxybenzoic acid. Compound 4.1u was globally deprotected according to general procedure B on a 0.10 mmol scale to give compound 4.2y (60.8 mg, 95\%); δ\textsubscript{H} (400 MHz, \textit{d}-CDCl\textsubscript{3}) 1.29-1.46 (m, 5H, CH\textsubscript{2}), 1.69-1.92 (m, 5H, CH\textsubscript{2}), 2.36-2.52 (m, 4H, CH and CH\textsubscript{3}), 3.78 (s, 2H, CH\textsubscript{2}), 4.42 (s, 2H, CH\textsubscript{2}) 4.66 (s, 2H, CH\textsubscript{2}), 6.19 (d, \textit{J} = 4.4 Hz, 1H, NH), 6.30 (s, 1H, NH), 6.65 (d, \textit{J} = 6.8 Hz, 1H, CH), 6.69-6.84 (m, 2H, CH), 6.93-7.12 (m, 6H, CH), 7.22-7.33 (m, 3H, CH), 7.60 (d, \textit{J} = 6.8 Hz, 1H, CH), 7.79 (d, \textit{J} = 8.0 Hz, 2H, CH); δ\textsubscript{C} (100 MHz, \textit{d}-CDCl\textsubscript{3}) 21.7, 26.2, 27.0, 34.5, 44.3, 47.1, 51.0, 53.0, 113.3, 116.7, 117.3, 118.7, 121.6, 127.0, 127.8, 128.8, 129.7, 129.8, 132.2, 133.8, 136.6, 137.0, 143.5, 146.9, 147.7, 150.6, 157.8, 162.7, 167.0, 172.4; HRMS (ES+) Calcd for [C\textsubscript{36}H\textsubscript{39}N\textsubscript{3}O\textsubscript{6}S + H] 642.2632 found 642.2641; HPLC (III) \textit{t}\textsubscript{R} = 22.38 min (90.0\%), (IV) 47.4 min (90.2\%).
4.2z, 4-(2-(N-(4-aminobenzyl)-4-methylphenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)-2-hydroxybenzoic acid. Compound 4.1v was globally deprotected according to general procedure B on a 0.04 mmol scale to give compound 4.2z (21.0 mg, 82 %); δ_H (400 MHz, d-CDCl_3) 1.32-1.45 (m, 5H, CH_2), 1.62-1.85 (m, 5H, CH_2), 2.35-2.57 (m, 4H, CH and CH_3), 3.66 (s, 2H, CH_2), 4.39 (s, 2H, CH_2) 4.65 (s, 2H, CH_2), 6.16-6.40 (m, 2H, CH), 6.81-7.15 (m, 9H, CH), 7.27 (d, J = 8.0 Hz, 2H, CH), 7.50-7.73 (m, 2H, CH), 7.79 (d, J = 8.0 Hz, 2H, CH); δ_C (100 MHz, d-CDCl_3) 21.7, 26.2, 27.0, 34.6, 44.3, 46.9, 50.4, 53.0, 110.9, 113.3, 116.7, 118.7, 127.0, 127.8, 128.7, 129.7, 130.3, 131.0, 133.8, 137.1, 143.5, 146.9, 147.7, 158.1, 162.6, 166.9, 172.4; HRMS (ES+) Calcd for [C_{36}H_{39}N_3O_6S + H] 642.2632 found 642.2644; HPLC (III) t_R =22.38 min (70.7 %), (IV) 47.38 min (74.7 %).

4.2aa, 4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(pyridin-2-ylmethyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. Compound 4.1w was globally deprotected according to general procedure C on a 0.07 mmol scale to give compound 4.2aa (28.6 mg, 65 %); δ_H (400 MHz, d_6-DMSO) 1.26-1.43 (m, 5H, CH_2), 1.63-1.83 (m, 5H, CH_2), 2.38 (s, 3H, CH_3), 2.40-2.49 (m, 1H, CH), 3.96 (s, 2H, CH_2), 4.56 (s, 2H, CH_2), 4.69 (s, 2H, CH_2), 6.47 (d, J = 7.6 Hz, 1H, CH), 6.57 (s, 1H, CH), 7.01 (d, J = 8.0 Hz, 2H, CH), 7.12 (d, J = 8.0 Hz, 2H, CH), 7.24-7.29 (m, 1H, CH), 7.52-7.68 (m, 6H, CH), 7.72 (t of d, J = 7.6 and 2.0 Hz, 1H, CH), 8.42-8.47 (m, 1H, CH); δ_C (100 MHz, d-CDCl_3) 21.7, 26.2, 27.0, 34.5, 44.3, 49.9, 53.3, 56.2, 110.6, 116.4, 118.5, 120.6, 123.7, 125.0, 127.0, 127.6, 128.6, 128.7, 129.7, 132.2, 132.3, 139.6, 143.7, 147.3, 147.6, 157.3, 162.8, 167.3, 173.0; HRMS (ES+) Calcd for [C_{35}H_{37}N_3O_6S + H] 628.2475 found 628.2467; HPLC (III) t_R =21.93 min (91.4 %), (IV) 50.10 min (91.6 %).
4.2ab, 4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(pyridin-3-ylmethyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. Compound 4.1x was globally deprotected according to general procedure C on a 0.04 mmol scale to give compound 4.2ab (19.4 mg, 77 %); $\delta_H$ (400 MHz, $d_6$-DMSO) 1.26-1.43 (m, 5H, CH$_2$), 1.63-1.83 (m, 5H, CH$_2$), 2.38 (s, 3H, CH$_3$), 2.40-2.49 (m, 1H, CH), 3.98 (s, 2H, CH$_2$), 4.56 (s, 2H, CH$_2$), 4.71 (s, 2H, CH$_2$), 6.55 (d, $J$ = 7.6 Hz, 1H, CH), 6.65 (s, 1H, CH), 7.01 (d, $J$ = 8.0 Hz, 2H, CH), 7.12 (d, $J$ = 8.0 Hz, 2H, CH), 7.24-7.29 (m, 1H, CH), 7.31-7.38 (m, 3H, CH), 7.65 (d, $J$ = 8.4 Hz, 2H, CH), 7.68 (d, $J$ = 8.4 Hz, 1H, CH), 7.73 (t of d, $J$ = 7.6 and 2.0 Hz, 1H, CH), 8.43-8.47 (m, 1H, CH); $\delta_C$ (100 MHz, $d_6$-DMSO) 21.0, 25.6, 26.3, 33.9, 43.4, 51.7, 52.9, 54.9, 111.1, 116.5, 117.5, 122.2, 122.6, 126.5, 127.2, 129.5, 131.1, 134.3, 136.7, 136.8, 143.1, 146.4, 148.9, 150.4, 151.2, 156.4, 162.3, 167.1, 171.0; HRMS (ES+) Calcd for [C$_{35}$H$_{37}$N$_3$O$_6$S + H] 628.2475 found 628.2482; HPLC (III) $t_R$ =20.58 min (100 %), (IV) 54.54 min (100 %).
4.2ac, 4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(pyridin-4-ylmethyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. Compound 4.1y was globally deprotected according to general procedure C on a 0.05 mmol scale to give compound 4.2ac (23.1 mg, 74 %); δH (400 MHz, d-CDCl3) 1.30-1.46 (m, 5H, CH2), 1.69-1.92 (m, 5H, CH2), 2.41-2.52 (m, 4H, CH and CH3), 3.79 (s, 2H, CH2), 4.67 (s, 2H, CH2), 4.69 (s, 2H, CH2), 6.27 (d, J = 8.4 Hz, 1H, CH), 6.45 (s, 1H, CH), 6.99 (d, J = 8.0 Hz, 2H, CH), 7.09 (d, J = 8.0 Hz, 2H, CH), 7.34 (d, J = 8.4 Hz, 2H, CH), 7.42 (d, J = 5.6 Hz, 2H, CH), 7.77-7.86 (m, 3H, CH), 8.60 (d, J = 5.6 Hz, 2H, CH); δC (100 MHz, d-CDCl3) 21.8, 26.3, 27.0, 34.6, 44.3, 47.7, 50.5, 53.0, 111.2, 116.5, 118.3, 124.3, 125.7, 126.0, 127.1, 127.9, 128.8, 129.8, 132.4, 133.8, 139.9, 144.0, 145.8, 146.7, 147.8, 166.2, 173.0; HRMS (ES+) Calcd for [C35H37N3O6S + H] 628.2475 found 628.2489; HPLC (I) tR =22.56 min (100 %), (II) 51.04 min (100 %).

4.2ad, 4-(2-(N-(2-cyanobenzyl)-4-methylphenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)-2-hydroxybenzoic acid. Compound 4.1z was globally deprotected according to general procedure B on a 0.16 mmol scale to give compound 4.2ad (80.5 mg, 77 %); δH (400 MHz, d-CDCl3) 1.31-1.47 (m, 5H, CH2), 1.69-1.92 (m, 5H, CH2), 2.37-2.52 (m, 4H, CH and CH3), 3.91 (s, 2H, CH2), 4.77 (s, 2H, CH2), 4.79 (s, 2H, CH2), 6.51 (d, J = 8.0 Hz, 1H, CH), 6.61 (s, 1H, CH), 7.01 (d, J = 8.0 Hz, 2H, CH), 7.10 (d, J = 8.0 Hz, 2H, CH), 7.29 (d, J = 8.0 Hz, 2H, CH), 7.36 (t, J = 7.6 Hz, 1H, CH), 7.58 (t, J =4.6 Hz, 1H, CH), 7.59 (d, J = 7.6 Hz, 1H, CH), 7.70 (d, J = 8.0 Hz, 1H, CH), 7.74 (d, J = 8.0 Hz, 2H, CH), 7.80 (d, J = 8.4 Hz, 1H, CH), 10.67 (s, 1H, OH); δC (100 MHz, d-CDCl3) 21.7, 26.2, 26.9, 34.5, 44.3, 48.9, 50.1, 53.1, 111.7, 112.0, 117.0, 117.3, 119.2, 127.0, 127.7, 128.5, 128.6, 128.6, 129.7, 130.1, 132.3,
132.8, 133.4, 136.4, 140.0, 143.8, 147.6, 147.7, 163.0, 166.6, 172.4; HRMS (ES+) Calcd for [C$_{37}$H$_{37}$N$_3$O$_6$S + H] 652.2475 found 652.2491; HPLC (III) $t_R = 26.44$ min (100 %), (IV) 58.47 min (100 %).

![Chemical structure](image)

**4.2ae, 4-(2-(N-(3-cyanobenzyl)-4-methylphenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)-2-hydroxybenzoic acid.** Compound 4.1aa was globally deprotected according to general procedure B on a 0.13 mmol scale to give compound 4.2ae (65.4 mg, 77 %); $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.31-1.46 (m, 5H, CH$_2$), 1.69-1.93 (m, 5H, CH$_2$), 2.41-2.55 (m, 4H, CH and CH$_3$), 3.79 (s, 2H, CH$_2$), 4.63 (s, 2H, CH$_2$) 4.72 (s, 2H, CH$_2$), 6.41 (d, $J = 8.0$ Hz, 1H, CH), 6.50 (s, 1H, CH), 7.00 (d, $J = 8.0$ Hz, 2H, CH), 7.12 (d, $J = 8.0$ Hz, 2H, CH), 7.32 (d, $J = 8.0$ Hz, 2H, CH), 7.39 (t, $J = 7.6$ Hz, 1H, CH), 7.47 (s, 1H, CH), 7.50-7.59 (m, 2H, CH), 7.75 (d, $J = 8.0$ Hz, 2H, CH), 7.81 (d, $J = 8.4$ Hz, 1H, CH), 10.67 (s, 1H, OH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 21.7, 26.2, 26.9, 34.5, 44.3, 47.4, 50.9, 53.1, 111.9, 112.8, 117.0, 118.4, 119.0, 127.1, 127.7, 128.6, 129.7, 129.8, 131.8, 131.9, 132.4, 133.1, 133.3, 136.6, 137.5, 144.0, 147.5, 147.9, 163.0, 166.8, 172.4; HRMS (ES+) Calcd for [C$_{37}$H$_{37}$N$_3$O$_6$S + H] 652.2475 found 652.2487; HPLC (III) $t_R = 26.44$ min (97.0 %), (IV) 58.49 min (96.5 %).
4.2af, 4-(2-(N-(4-cyanobenzyl)-4-methylphenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)-2-hydroxybenzoic acid. Compound 4.1ab was globally deprotected according to general procedure B on a 0.10 mmol scale to give compound 4.2af (58.0 mg, 89%); \( \delta_H (400 \text{ MHz, } d-\text{CDCl}_3) \) 1.32-1.48 (m, 5H, CH\(_2\)), 1.70-1.95 (m, 5H, CH\(_2\)), 2.44-2.56 (m, 4H, CH and CH\(_3\)), 3.79 (s, 2H, CH\(_2\)), 4.64 (s, 2H, CH\(_2\)) 4.70 (s, 2H, CH\(_2\)), 6.37 (d, \( J = 8.0 \text{ Hz, } 1H, \text{ CH} \)), 6.45 (s, 1H, CH), 6.98 (d, \( J = 8.0 \text{ Hz, } 2H, \text{ CH} \)), 7.11 (d, \( J = 8.0 \text{ Hz, } 2H, \text{ CH} \)), 7.20 (d, \( J = 8.0 \text{ Hz, } 2H, \text{ CH} \)), 7.37 (d, \( J = 8.0 \text{ Hz, } 2H, \text{ CH} \)), 7.51 (s, 1H, OH); \( \delta_C (100 \text{ MHz, } d-\text{CDCl}_3) \) 21.7, 26.2, 26.9, 34.5, 44.3, 47.5, 51.2, 53.1, 111.8, 112.0, 117.0, 118.6, 119.0, 127.1, 127.8, 128.6, 129.2, 129.8, 132.4, 132.6, 133.4, 136.6, 143.9, 147.6, 148.0, 163.0, 166.8, 172.5; HRMS (ES+) Calcd for [C\(_{37}\)H\(_{37}\)N\(_3\)O\(_6\)S + H] 652.2475 found 652.2481; HPLC (III) \( t_R = 26.46 \text{ min (96.5%)} \), (IV) 58.58 min (96.5%).
4.2ag, 4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(2,3,4,5-tetrafluorobenzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. Compound 4.1ac was globally deprotected according to general procedure B on a 0.07 mmol scale to give compound 4.2ag (42.2 mg, 86 %); \( \delta_H \) (400 MHz, \( d-\text{CDCl}_3 \)) 1.31-1.47 (m, 5H, CH\(_2\)), 1.70-1.94 (m, 5H, CH\(_2\)), 2.38-2.55 (m, 4H, CH and CH\(_3\)), 3.93 (s, 2H, CH\(_2\)), 4.62 (s, 2H, CH\(_2\)), 4.77 (s, 2H, CH\(_2\)), 6.56 (d, \( J = 8.0 \) Hz, 1H, CH), 6.66 (s, 1H, CH), 6.68-6.77 (m, 1H, CH), 7.02 (d, \( J = 8.0 \) Hz, 2H, CH), 7.11 (d, \( J = 8.0 \) Hz, 2H, CH), 7.28 (d, \( J = 8.0 \) Hz, 2H, CH), 7.72 (d, \( J = 8.4 \) Hz, 2H, CH), 7.86 (d, \( J = 8.4 \) Hz, 1H, CH), 10.62 (s, 1H, OH); \( \delta_C \) (100 MHz, \( d-\text{CDCl}_3 \)) 21.7, 26.2, 27.0, 34.5, 39.8, 44.3, 49.1, 53.1, 101.0, 110.2, 111.8, 117.3, 119.3, 127.1, 127.8, 128.6, 129.6, 132.5, 133.5, 136.4, 143.8, 145.1, 147.9, 163.1, 166.9, 172.8; HRMS (ES+) Calcd for [C\(_{36}\)H\(_{34}\)F\(_4\)N\(_2\)O\(_6\)S + H] 699.2146 found 699.2117; HPLC (III) \( t_R = \)23.17 min (100 %), (IV) 54.08 min (98.7 %).

4.2ah, 4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(2,3,4,6-tetrafluorobenzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. Compound 4.1ad was globally deprotected according to general procedure B on a 0.06 mmol scale to give compound 4.2ah (35.6 mg, 85 %); \( \delta_H \) (400 MHz, \( d-\text{CDCl}_3 \)) 1.31-1.47 (m, 5H, CH\(_2\)), 1.70-1.94 (m, 5H, CH\(_2\)), 2.40-2.53 (m, 4H, CH and CH\(_3\)), 3.86 (s, 2H, CH\(_2\)), 4.61 (s, 2H, CH\(_2\)), 4.76 (s, 2H, CH\(_2\)), 6.51 (d, \( J = 8.4 \) Hz, 1H, CH), 6.61 (s, 1H, CH), 7.00 (d, \( J = 8.0 \) Hz, 2H, CH), 7.07-7.17 (m, 3H, CH), 7.31 (d, \( J = 8.0 \) Hz, 2H, CH), 7.73 (d, \( J = 8.4 \) Hz, 2H, CH), 7.85 (d, \( J = 8.4 \) Hz, 1H, CH), 10.61 (s, 1H, OH); \( \delta_C \) (100 MHz, \( d-\text{CDCl}_3 \)) 21.7, 26.2, 27.0, 34.5, 44.3, 44.6, 48.2, 53.1, 111.7, 111.9, 117.2, 119.1, 119.9, 127.2, 127.8, 128.6, 129.8, 132.5, 133.3, 136.4, 143.9, 144.1, 148.0, 163.1, 166.6, 172.7; HRMS (ES+) Calcd for [C\(_{36}\)H\(_{34}\)F\(_4\)N\(_2\)O\(_6\)S + H] 699.2146 found 699.2134; HPLC (I) \( t_R = \)23.45 min (97.0 %), (II) 62.91 min (100 %).
4.2ai, 4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(2,3,5,6-tetrafluorobenzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. Compound 4.1ae was globally deprotected according to general procedure B on a 0.07 mmol scale to give compound 4.2ai (46.5 mg, 95 %); \( \delta_H \) (400 MHz, \( d_6 - \text{DMSO} \)) 1.26-1.43 (m, 5H, CH\(_2\)), 1.63-1.83 (m, 5H, CH\(_2\)), 2.37 (s, 3H, CH\(_3\)), 2.40-2.49 (m, 1H, CH), 4.00 (s, 2H, CH\(_2\)), 4.65 (s, 2H, CH\(_2\)), 4.76 (s, 2H, CH\(_2\)), 6.75 (d of d, \( J = 8.4 \) and 2.0 Hz, 1H, CH), 6.82 (d, \( J = 2.0 \) Hz, 1H, CH), 7.03 (d, \( J = 8.0 \) Hz, 2H, CH), 7.13 (d, \( J = 8.0 \) Hz, 2H, CH), 7.33 (d, \( J = 8.0 \) Hz, 2H, CH), 7.55 (d, \( J = 8.4 \) Hz, 2H, CH), 7.75-7.86 (m, 2H, CH); \( \delta_C \) (100 MHz, \( d_6 - \text{DMSO} \)) 21.0, 25.6, 26.3, 33.9, 40.5, 43.4, 49.5, 51.6, 106.6, 112.7, 115.8, 116.6, 118.6, 126.6, 126.9, 127.6, 129.4, 131.3, 131.4, 134.1, 136.4, 143.3, 146.5, 161.6, 166.6, 171.2; HRMS (ES) Calcd for \([C_{36}H_{34}F_{4}N_{2}O_{6}S + H]\) 699.2146 found 699.2176; HPLC (III) \( t_R = 22.82 \) min (100 %), (IV) 53.57 min (100 %).

4.2aj, 4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-((perfluorophenyl)methyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid.
Compound 4.1af was globally deprotected according to general procedure B on a 0.07 mmol scale to give compound 4.2aj 0.08 (47.3 mg, 82%); \( \delta_H \) (400 MHz, \( d\text{-CDCl}_3 \)) 1.32-1.46 (m, 5H, CH\(_2\)), 1.69-1.92 (m, 5H, CH\(_2\)), 2.38-2.55 (m, 4H, CH and CH\(_3\)), 3.97 (s, 2H, CH\(_2\)), 4.66 (s, 2H, CH\(_2\)), 4.77 (s, 2H, CH\(_2\)), 6.58 (d, \( J = 8.4 \text{ Hz} \), 1H, CH), 6.68 (s, 1H, CH), 7.01 (d, \( J = 8.0 \text{ Hz} \), 2H, CH), 7.12 (d, \( J = 8.0 \text{ Hz} \), 2H, CH), 7.29 (d, \( J = 8.0 \text{ Hz} \), 2H, CH), 7.71 (d, \( J = 8.0 \text{ Hz} \), 2H, CH), 7.89 (d, \( J = 8.4 \text{ Hz} \), 1H, CH), 10.62 (s, 1H, OH); \( \delta_C \) (100 MHz, \( d\text{-CDCl}_3 \)) 21.7, 26.2, 27.0, 34.5, 40.0, 44.3, 49.5, 53.1, 110.3, 111.9, 117.4, 119.2, 127.2, 127.8, 128.6, 129.6, 132.6, 133.4, 136.2, 138.9, 140.1, 144.0, 147.8, 147.9, 163.2, 166.8, 172.9; HRMS (ES+) Calcd for \([C_{36}H_{33}F_5N_2O_6S + H]\) 717.2052 found 717.2086; HPLC (I) \( t_R = \) 23.77 min (100%), (II) 54.55 min (100%).

**4.2ak, 4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(naphthalen-2-ylmethyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid.** Compound 4.1aj was globally deprotected according to general procedure B on a 0.08 mmol scale to give compound 4.2ak (40.3 mg, 74%); \( \delta_H \) (400 MHz, \( d\text{-CDCl}_3 \)) 1.32-1.48 (m, 5H, CH\(_2\)), 1.67-1.93 (m, 5H, CH\(_2\)), 2.34-2.56 (m, 4H, CH and CH\(_3\)), 3.79 (s, 2H, CH\(_2\)), 4.68 (s, 2H, CH\(_2\)), 4.73 (s, 2H, CH\(_2\)), 6.08 (s, 1H, CH), 6.37 (s, 1H, CH), 6.96 (d, \( J = 6.8 \text{ Hz} \), 2H, CH), 7.06 (d, \( J = 6.8 \text{ Hz} \), 2H, CH), 7.29-7.42 (m, 3H, CH), 7.43-7.57 (m, 3H, CH), 7.61 (s, 1H, CH), 7.66-7.77 (m, 2H, CH), 7.79-7.90 (m, 3H, CH), 10.65 (s, 1H, OH); \( \delta_C \) (100 MHz, \( d\text{-CDCl}_3 \)) 21.8, 26.3, 27.0, 34.5, 44.3, 47.0, 51.4, 52.9, 111.9, 116.9, 119.0, 126.4, 126.4, 126.5, 127.1, 127.4, 127.8, 128.1, 128.4, 128.6, 128.6, 128.8, 129.7, 132.0, 132.7, 133.2, 133.3, 133.6, 137.1, 143.5, 147.7, 162.9, 167.0, 172.3;
HRMS (ES+) Calcd for [C$_{40}$H$_{40}$N$_2$O$_6$S + H] 677.2679 found 677.2647; HPLC (I) $t_R$ =24.12 min (100 %), (II) 55.59 min (100 %).

4.2al, 4-(N-(4-cyclohexylbenzyl)-2-(N-(4-fluor-2-(trifluoromethyl)benzyl)-4-methylphenylsulfonamido)acetamido)-2-hydroxybenzoic acid. Compound 4.1ah was globally deprotected according to general procedure B on a 0.05 mmol scale to give compound 4.2al (34.5 mg, 97 %); $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.30-1.46 (m, 5H, CH$_2$), 1.70-1.93 (m, 5H, CH$_2$), 2.40-2.52 (m, 4H, CH and CH$_3$), 3.80 (s, 2H, CH$_2$), 4.71 (s, 2H, CH$_2$), 4.73 (s, 2H, CH$_2$), 6.41 (d, $J$ = 8.0 Hz, 1H, CH), 6.52 (s, 1H, CH), 6.96 (d, $J$ = 8.0 Hz, 2H, CH), 7.08 (d, $J$ = 8.0 Hz, 2H, CH), 7.19 (t of d, $J$ = 8.0 and 2.0 Hz, 1H, CH), 7.32 (d, $J$ = 8.4 Hz, 3H, CH), 7.72-7.83 (m, 4H, CH), 10.64 (s, 1H, OH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 21.8, 26.3, 27.0, 34.5, 44.4, 47.3, 48.0, 53.0, 111.8, 113.3, 113.6, 117.2, 119.2, 119.3, 119.5, 122.1, 127.1, 127.9, 128.6, 129.7, 130.8, 132.4, 132.7, 132.7, 133.5, 136.7, 143.9, 147.6, 147.8, 160.4, 162.9, 163.1, ; HRMS (ES+) calcd for [C$_{37}$H$_{36}$N$_2$O$_6$F$_4$S + H] 713.2302, found 713.2308; HPLC (I) $t_R$ = 24.22 min (100 %), (II) $t_R$ = 55.87 min (100 %).
4.2am, 4-(N-(4-cyclohexylbenzyl)-2-(4-methyl-N-(4-((trifluoromethyl)thio)benzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid.

Compound 4.1ai was globally deprotected according to general procedure B on a 0.04 mmol scale to give compound 4.2am (24.2 mg, 83 %); $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.30-1.46 (m, 5H, CH$_2$), 1.70-1.92 (m, 5H, CH$_2$), 2.41-2.53 (m, 4H, CH and CH$_3$), 3.77 (s, 2H, CH$_2$), 4.61 (s, 2H, CH$_2$), 4.71 (s, 2H, CH$_2$), 6.27 (d, $J = 8.4$ Hz, 1H, CH), 6.45 (s, 1H, CH), 6.98 (d, $J = 8.0$ Hz, 2H, CH), 7.10 (d, $J = 8.0$ Hz, 2H, CH), 7.27-7.37 (m, 4H, CH), 7.55 (d, $J = 8.0$ Hz, 2H, CH), 7.73-7.82 (m, 3H, CH), 10.59 (s, 1H, OH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 21.8, 26.3, 27.0, 34.6, 44.4, 47.3, 50.9, 53.0, 111.7, 117.2, 119.1, 127.1, 127.8, 129.7, 129.8, 132.3, 132.6, 133.4, 133.6, 136.7, 136.9, 138.9, 143.8, 144.1, 147.8, 148.0, 163.1, 167.4, 172.2; HRMS (ES+) calcd for [C$_{37}$H$_{37}$N$_2$O$_6$F$_3$S$_2$ + H] 727.2117, found 727.2096; HPLC (I) $t_R = 24.24$ min (91.4 %), (II) $t_R = 56.23$ min (90.8 %).
4.2an, 4-(N-(4-cyclohexylbenzyl)-2-(N-(4-(ethoxycarbonyl)benzyl)-4-methylphenylsulfonamido)acetamido)-2-hydroxybenzoic acid. Compound 4.1aj was globally deprotected according to general procedure B on a 0.05 mmol scale to give compound 4.2an (32.9 mg, 94 %); δH (400 MHz, d-CDCl3) 1.31-1.47 (m, 5H, CH2), 1.69-1.94 (m, 5H, CH2), 2.39-2.55 (m, 4H, CH and CH3), 3.78 (s, 2H, CH2), 4.61 (s, 2H, CH2), 4.69 (s, 2H, CH2), 6.27 (d, J = 8.0 Hz, 1H, CH), 6.45 (s, 1H, CH), 6.99 (d, J = 8.0 Hz, 2H, CH), 7.11 (d, J = 8.0 Hz, 2H, CH), 7.22-7.37 (m, 4H, CH), 7.55 (d, J = 8.0 Hz, 2H, CH), 7.69-7.86 (m, 3H, CH), 10.59 (s, 1H, OH); δC (100 MHz, d-CDCl3) 14.4, 21.8 26.3, 27.0, 34.5, 44.4, 47.2, 51.1, 53.0, 61.2, 112.0, 116.9, 119.0, 127.1, 127.8, 128.6, 129.7, 130.1, 130.3, 132.3, 133.6, 136.9, 140.7, 143.7, 146.2, 147.5, 147.9, 163.0, 165.0, 166.5, 172.3; HRMS (ES+) calcd for [C39H42N2O8S + H] 699.2734, found 699.2731; HPLC (I) tR = 23.86 min (98.4 %), (II) tR = 54.73 min (96.8 %).

4.2ao, 4-(N-(4-cyclohexylbenzyl)-2-(N-(3-(methoxycarbonyl)benzyl)-4-methylphenylsulfonamido)acetamido)-2-hydroxybenzoic acid. Compound 4.1ak was globally deprotected according to general procedure B on a 0.03 mmol scale to give compound 4.2ao (15.8 mg, 77 %); δH (400 MHz, d-CDCl3) 1.30-1.60 (m, 5H, CH2), 1.65-2.10 (m, 5H, CH2), 2.30-2.63 (m, 4H, CH and CH3), 3.78 (s, 2H, CH2), 3.90 (s, 3H, CH3), 4.61 (s, 2H, CH2), 4.71 (s, 2H, CH2), 6.34 (d, J = 8.0 Hz, 1H, CH), 6.47 (s, 1H, CH), 6.98 (d, J = 7.6 Hz, 2H, CH), 7.09 (d, J = 7.6 Hz, 2H, CH), 7.28-7.42 (m, 3H, CH), 7.49 (d, J = 7.6 Hz, 1H, CH), 7.73 (d, J = 8.4 Hz, 1H, CH), 7.80 (d, J = 8.0 Hz, 2H, CH), 7.86 (s, 1H, CH), 7.96 (d, J = 7.6 Hz, 1H, CH), 10.60 (s, 1H, OH); δC (100 MHz, d-CDCl3 21.7, 26.3, 27.0, 34.5, 44.4, 47.2, 51.1, 52.4, 53.0, 110.8, 117.0, 119.1, 127.1, 127.8, 128.6, 129.1, 129.5, 129.7, 129.8, 130.6, 132.3, 132.4, 133.5, 136.0, 137.0,
143.7, 147.7, 147.8, 163.0, 166.8, 167.0, 172.3; HRMS (ES+) calcd for [C\textsubscript{38}H\textsubscript{40}N\textsubscript{2}O\textsubscript{8}S + H]\textsubscript{685.2578}, found 685.2577; HPLC (I) \( t_R = 23.52 \text{ min (98.2 \%)} \), (II) \( t_R = 53.58 \text{ min (98.6 \%)} \).

\[ \text{4.2ap, 4-(N-(4-cyclohexylbenzyl)-2-(N-(4-(methoxycarbonyl)benzyl)-4-methylphenylsulphonamido)acetamido)-2-hydroxybenzoic acid} \] Compound \textbf{4.1al} was globally deprotected according to general procedure B on a 0.03 mmol scale to give compound \textbf{4.2ap} (19.0 mg, 70 %); \( \delta_H \) (400 MHz, \( d\text{-CDCl}_3 \)) 1.31-1.51 (m, 5H, CH\textsubscript{2}), 1.70-2.12 (m, 5H, CH\textsubscript{2}), 2.36-2.90 (m, 4H, CH and CH\textsubscript{3}), 3.79 (s, 2H, CH\textsubscript{2}), 3.92 (s, 3H, CH\textsubscript{3}), 4.64 (s, 2H, CH\textsubscript{2}), 4.70 (s, 2H, CH\textsubscript{2}), 6.32 (d, \( J = 8.0 \text{ Hz, 1H, CH} \)), 6.42 (s, 1H, CH), 6.98 (d, \( J = 8.0 \text{ Hz, 2H, CH} \)), 7.10 (d, \( J = 8.0 \text{ Hz, 2H, CH} \)), 7.21-7.44 (m, 4H, CH), 7.68-7.84 (m, 3H, CH), 7.95 (d, \( J = 8.0 \text{ Hz, 2H, CH} \)), 10.61 (s, 1H, OH); \( \delta_C \) (100 MHz, \( d\text{-CDCl}_3 \)) 21.7, 26.2, 27.0, 34.5, 44.3, 47.2, 51.1, 52.3, 53.4, 111.7, 117.1, 119.0, 127.1, 127.8, 128.7, 129.7, 130.0, 130.1, 132.2, 132.3, 133.5, 136.9, 140.9, 143.7, 147.8, 147.9, 163.0, 166.8, 167.0, 172.3; HRMS (ES+) calcd for [C\textsubscript{38}H\textsubscript{40}N\textsubscript{2}O\textsubscript{8}S + H]\textsubscript{685.2578}, Found 685.2586; HPLC (I) \( t_R = 23.55 \text{ min (100 \%)} \), (II) \( t_R = 53.68 \text{ min (100 \%)} \).
4.3, benzyl 4-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-N-(4-cyclohexylbenzyl)acetamido)-2-(benzyl)benzoate. Fmoc-glycine-OH (1.13g, 3.80 mmol) was stirred at room temperature with PPh\textsubscript{3}Cl\textsubscript{2} (2.76 g, 8.30 mmol) in CHCl\textsubscript{3} for 15 minutes to form the activated acid. Aniline 2.10h (1.75g, 3.46 mmol) was added and the solution was heated to 110 °C for 30 minutes using a Biotage Initiator microwave reactor. Upon completion, CHCl\textsubscript{3} was removed under reduced pressure and the crude product was purified using flash column chromatography using a mixture of hexanes and EtOAc as eluent, (2.08 g, 76 %).

δ\textsubscript{H} (400 MHz, d-CDCl\textsubscript{3}) 1.31-1.54 (m, 5H, CH\textsubscript{2}), 1.70-1.90 (m, 5H, CH\textsubscript{2}), 2.17 (s, 2H, CH\textsubscript{2}), 2.40-2.55 (m, 1H, CH), 3.68 (s, 2H, CH\textsubscript{2}), 4.23 (t, \textit{J} = 7.0 Hz, 1H, CH), 4.36 (d, \textit{J} = 7.0 Hz, 2H, CH\textsubscript{2}), 4.83 (s, 2H, CH\textsubscript{2}), 4.92 (s, 2H, CH\textsubscript{2}), 5.35 (s, 2H, CH\textsubscript{2}), 5.71 (s (br), 1H, NH), 6.52 (s, 1H, CH), 6.70 (d, \textit{J} = 8.0 Hz, 1H, CH), 7.07 (d, \textit{J} = 8.0 Hz, 2H, CH), 7.13 (d, \textit{J} = 8.0 Hz, 2H, CH), 7.27-7.43 (m, 14H, CH), 7.61 (d, \textit{J} = 7.4 Hz, 2H, CH), 7.77 (d, \textit{J} = 7.4 Hz, 2H, CH), 7.84 (d, \textit{J} = 8.0 Hz, 1H, CH).

4.4, benzyl 4-(2-amino-N-(4-cyclohexylbenzyl)acetamido)-2-(benzyl)benzoate.

Compound 4.3 was dissolved in a 5 % solution of piperidine in DMF and stirred for 10 minutes at room temperature. Upon completion the reaction mixture was diluted with H\textsubscript{2}O and organics were extracted into EtOAc. Combined organics were then washed with saturated NaHCO\textsubscript{3}, H\textsubscript{2}O, brine then dried over Na\textsubscript{2}SO\textsubscript{4}. Crude product was purified using flash column chromatography in a mixture of 92 % DCM, 7 % MeOH, 1 % NH\textsubscript{4}OH, and DCM in a 1:1 ratio (4.4, 62 %). δ\textsubscript{H} (400 MHz, d-CDCl\textsubscript{3}) 1.29-1.43 (m, 5H, CH\textsubscript{2}), 1.66-1.88 (m, 5H, CH\textsubscript{2}), 2.17 (s, 2H, CH\textsubscript{2}), 2.38-2.51 (m, 1H, CH), 3.06 (s, 2H, CH\textsubscript{2}), 4.80 (s, 2H, CH\textsubscript{2}), 4.90 (s, 2H, CH\textsubscript{2}), 5.31 (s, 2H, CH\textsubscript{2}), 6.50 (s, 1H, CH), 6.64 (d, \textit{J} = 8.0 Hz, 1H, CH), 7.05-7.12 (m, 4H, CH), 7.22-7.41 (m, 10H, CH), 7.81 (d,
$J = 8.0$ Hz, 1H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 25.7, 26.4, 34.0, 43.8, 43.9, 52.3, 66.5, 70.2, 113.8, 119.7, 120.0, 126.5, 126.6, 127.6, 127.8, 127.8, 128.1, 128.2, 128.4, 132.6, 134.0, 135.4, 135.4, 145.1, 147.1, 158.2, 164.9, 171.9.

\[ \text{4.5, benzyl 2-(benzylxoy)-4-(N-(4-cyclohexylbenzyl)-2-}
\text{(perfluorophenylsulfonamido)acetamido)benzoate. Compound 4.4 (0.5 g, 0.89 mmol) was}
\text{dissolved in anhydrous MeCN with K$_2$CO$_3$ (0.98 mmol) and 4 Å molecular sieves and cooled to 0}
\text{°C. 2,3,4,5,6-pentafluorobenzenesulfonyl chloride (0.89 mmol) was added and the reaction}
\text{mixture was allowed to warm to room temperature. After 6 hours the solution was filtered}
\text{through cotton to remove molecular sieves and excess solvent was removed under reduced}
\text{pressure. The concentrated mixture was diluted with DCM, then washed with 1M HCl, saturated}
\text{NaHCO$_3$, H$_2$O and brine, then dried over Na$_2$SO$_4$. Crude product was then purified using flash}
\text{column chromatography using a mixture of hexanes and EtOAc as the eluent to give compound}
\text{4.5 (0.45 g, 64%); $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.29-1.46 (m, 5H, CH$_2$), 1.69-1.90 (m, 5H, CH$_2$),}
\text{2.40-2.53 (m, 1H, CH), 3.66 (s, 2H, CH$_2$), 4.71 (s, 2H, CH$_2$), 4.91 (s, 2H, CH$_2$), 5.34 (s, 2H,}
\text{CH$_2$), 6.29 (s, 1H, NH), 6.43 (s, 1H, CH), 6.61 (d, $J = 8.0$ Hz, 1H, CH), 6.93 (d, $J = 7.8$ Hz, 2H,}
\text{CH), 7.09 (d, $J = 7.8$ Hz, 1H, CH), 7.23-7.45 (m, 11H, CH), 7.83 (d, $J = 8.0$ Hz, 1H, CH); $\delta_C$}
\text{(100 MHz, $d$-CDCl$_3$) 26.2, 26.9, 34.5, 44.3, 45.0, 53.4, 67.2, 70.8, 114.0, 120.1, 121.7, 127.2,}
\text{127.2, 128.3, 128.4, 128.5, 128.7, 128.8, 129.0, 133.3, 133.7, 135.8, 137.1, 143.8, 148.3, 159.0,}
\text{165.4, 166.3; LRMS (ES+) Calcd for [C$_{42}$H$_{37}$F$_{5}$N$_{3}$O$_{6}$S + Na] 815.22 found 815.39.}
**4.6a, benzyl 4-(2-(N-allyl-2,3,4,5,6-pentafluorophenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)-2-(benzyloxy)benzoate.** Sulfonamide 4.5 was functionalized on a 0.11 mmol scale using General Procedure A to give 4.6a (92.5 mg, 100%); \( \delta_{\text{H}} (400 \text{ MHz, } d\text{-}\text{CDCl}_3) 1.30-1.46 \text{ (m, 5H, CH}_2\text{), 1.66-1.90 \text{ (m, 5H, CH}_2\text{), 2.38-2.55 \text{ (m, 1H, CH), 3.90 (s, 2H, CH}_2\text{), 4.09 (d, } J = 4.4 \text{ Hz, 2H, CH}_2\text{), 4.65 (s, 2H, CH}_2\text{), 4.90 (s, 2H, CH}_2\text{), 5.17-5.22 \text{ (m, 2H, CH), 5.33 (s, 2H, CH}_2\text{), 5.61-5.75 \text{ (m, 1H, CH), 6.44 (s, 1H, CH), 6.63 (d, } J = 8.0 \text{ Hz, 1H, CH), 6.93 \text{ (d, } J = 7.6 \text{ Hz, 2H, CH), 7.11 (d, } J = 7.6 \text{ Hz, 2H, CH), 7.24-7.42 \text{ (m, 10H, CH), 7.82 (d, } J = 8.0 \text{ Hz, 1H, CH); } \delta_{\text{C}} (100 \text{ MHz, } d\text{-}\text{CDCl}_3) 26.1, 26.9, 34.5, 44.3, 48.0, 50.8, 52.9, 67.2, 70.8, 113.8, 120.0, 120.1, 121.3, 127.1, 127.2, 128.2, 128.3, 128.4, 128.6, 128.7, 128.8, 132.1, 133.3, 133.6, 135.6, 135.7, 144.4, 148.0, 158.9, 162.6, 165.4, 166.0; \text{ LRMS (ES+) Calcd for [C}_{45}\text{H}_{43}\text{F}_{5}\text{N}_{2}\text{O}_{6}\text{S} + \text{Na] 855.25 found 855.42}}

**4.6b, benzyl 2-(benzyloxy)-4-(2-(N-(tert-butoxycarbonyl)-2,3,4,5,6-pentafluorophenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)benzoate.** Sulfonamide
4.5 was functionalized on a 0.04 mmol scale using General Procedure D to give 4.6b (35.2 mg, 99 %); δH (400 MHz, d-CDCl3) 1.29-1.49 (m, 14H, CH₂ and 3 CH₃), 1.65-1.90 (m, 5H, CH₂), 2.36-2.54 (m, 1H, CH), 4.28 (s, 2H, CH₂), 4.80 (s, 2H, CH₂), 4.92 (s, 2H, CH₂), 5.34 (s, 2H, CH₂), 6.57 (s, 1H, CH), 6.73 (d, J = 8.0 Hz, 1H, CH), 7.00-7.15 (m, 4H, CH), 7.28-7.45 (m, 10H, CH), 7.85 (d, J = 8.0 Hz, 1H, CH); δC (100 MHz, d-CDCl3) 26.2, 26.9, 27.8, 34.6, 44.5, 53.5, 67.2, 70.9, 77.4, 114.1, 120.3, 121.3, 127.1, 127.3, 128.3, 128.4, 128.7, 128.8, 129.1, 133.3, 133.4, 135.7, 135.9, 144.8, 148.0, 155.7, 159.0, 165.4, 164.7; LRMS (ES+) Calcd for [C₄₇H₄₅F₅N₂O₈S + Na] 915.27 found 915.53.

4.6c, benzyl 4-(2-(N-benzyl-2,3,4,5,6-pentafluorophenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)-2-(benzyloxy)benzoate. Sulfonamide 4.5 was functionalized on a 0.03 mmol scale using General Procedure A to give 4.6c (25.4 mg, 94 %); δH (400 MHz, d-CDCl₃) 1.30-1.47 (m, 5H, CH₂), 1.70-1.92 (m, 5H, CH₂), 2.40-2.56 (m, 1H, CH), 3.76 (s, 2H, CH₂), 4.64 (s, 2H, CH₂), 4.70 (s, 2H, CH₂), 4.76 (s, 2H, CH₂), 5.30 (s, 2H, CH₂), 6.24 (s, 1H, CH), 6.36 (d, J = 7.6 Hz, 1H, CH), 6.94 (d, J = 7.6 Hz, 2H, CH), 7.13 (d, J = 7.6 Hz, 2H, CH), 7.20-7.43 (m, 15H, CH), 7.69 (d, J = 8.0 Hz, 1H, CH); δC (100 MHz, d-CDCl₃) 26.2, 26.9, 34.6, 44.4, 47.7, 51.4, 52.9, 67.2, 70.9, 113.7, 119.4, 121.3, 127.1, 127.3, 128.3, 128.4, 128.4, 128.6, 128.7, 128.9, 129.0, 129.1, 133.3, 133.6, 134.4, 135.6, 135.8, 144.3, 148.1, 158.9, 165.4, 165.9; LRMS (ES+) Calcd for [C₄₉H₄₃F₅N₂O₈S + Na] 905.27 found 905.51.
4.6d, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(2-methylbenzyl)phenylsulfonyl)acetamido)benzoate. Sulfonamide 4.5 was functionalized on a 0.03 mmol scale using General Procedure A to give 4.6d (18.3, 68 %); δH (400 MHz, d-CDCl3) 1.32-1.47 (m, 5H, CH2), 1.70-1.92 (m, 5H, CH2), 2.31 (s, 3H, CH3), 2.42-2.54 (m, 1H, CH), 3.66 (s, 2H, CH2), 4.62 (s, 2H, CH2), 4.72 (s, 2H, CH2), 4.74 (s, 2H, CH2), 5.30 (s, 2H, CH2), 6.15 (s, 1H, CH), 6.29 (d, J = 7.6 Hz, 1H, CH), 6.95 (d, J = 7.6 Hz, 2H, CH), 7.04-7.24 (m, 6H, CH), 7.28-7.42 (m, 10H, CH), 7.67 (d, J = 8.0 Hz, 1H, CH); δC (100 MHz, d-CDCl3) 19.2, 26.2, 26.9, 34.6, 44.4, 47.6, 49.4, 52.9, 67.2, 70.9, 113.7, 120.0, 121.2, 126.2, 127.1, 127.3, 128.3, 128.4, 128.4, 128.7, 128.8, 128.9, 129.1, 130.4, 131.2, 131.9, 133.3, 133.7, 135.8, 138.4, 144.3, 148.2, 158.9, 165.5, 165.9; LRMS (ES+) Calcd for [C50H45F5N2O6S + Na] 919.28 found 919.48.

4.6e, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(3-methylbenzyl)phenylsulfonyl)acetamido)benzoate. Sulfonamide 4.5 was functionalized
on a 0.03 mmol scale using General Procedure A to give 4.6e (21.3 mg, 79 %); δ\textsubscript{H} (400 MHz, d-CDCl\textsubscript{3}) 1.30-1.46 (m, 5H, CH\textsubscript{2}), 1.70-1.92 (m, 5H, CH\textsubscript{2}), 2.47 (s, 3H, CH\textsubscript{3}), 2.42-2.54 (m, 1H, CH), 3.76 (s, 2H, CH\textsubscript{2}), 4.64 (s, 4H, CH\textsubscript{2}), 4.75 (s, 2H, CH\textsubscript{2}), 5.30 (s, 2H, CH\textsubscript{2}), 6.25 (s, 1H, CH), 6.36 (d, J = 8.0 Hz, 1H, CH), 6.94 (d, J = 8.0 Hz, 2H, CH), 7.01 (d, J = 7.6 Hz, 1H, CH), 7.08 (d, J = 7.6 Hz, 2H, CH), 7.12 (d, J = 8.0 Hz, 2H, CH), 7.18 (t, J = 7.6 Hz, 1H, CH), 7.27-7.39 (m, 10H, CH), 7.69 (d, J = 8.0 Hz, 1H, CH); δ\textsubscript{C} (100 MHz, d-CDCl\textsubscript{3}) 21.5, 26.2, 27.0, 34.6, 44.4, 47.8, 51.3, 52.9, 67.2, 70.9, 113.7, 120.0, 121.3, 126.0, 127.2, 127.4, 128.3, 128.4, 128.4, 129.0, 129.3, 129.6, 133.3, 133.7, 134.3, 135.7, 135.8, 138.9, 144.4, 148.1, 158.9, 165.5, 166.0; LRMS (ES+) Calcd for [C\textsubscript{50}H\textsubscript{45}F\textsubscript{5}N\textsubscript{3}O\textsubscript{6}S + Na] 919.28 found 919.41.

\textbf{4.6f, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(4-methylbenzyl)phenylsulfonamido)acetamido)benzoate.} Sulfonamide 4.5 was functionalized on a 0.05 mmol scale using General Procedure A to give 4.6f (42.5 mg, 95 %); δ\textsubscript{H} (400 MHz, d-CDCl\textsubscript{3}) 1.30-1.47 (m, 5H, CH\textsubscript{2}), 1.70-1.92 (m, 5H, CH\textsubscript{2}), 2.27 (s, 3H, CH\textsubscript{3}), 2.41-2.57 (m, 1H, CH), 3.76 (s, 2H, CH\textsubscript{2}), 4.64 (s, 4H, 2 CH\textsubscript{2}), 4.77 (s, 2H, CH\textsubscript{2}), 5.30 (s, 2H, CH\textsubscript{2}), 6.28 (s, 1H, CH), 6.37 (d, J = 7.6 Hz, 1H, CH), 6.94 (d, J = 7.6 Hz, 2H, CH), 7.08-7.19 (m, 6H, CH), 7.28-7.42 (m, 10H, CH), 7.69 (d, J = 8.0 Hz, 1H, CH); δ\textsubscript{C} (100 MHz, d-CDCl\textsubscript{3}) 21.2, 26.2, 26.9, 34.6, 44.4, 47.7, 51.2, 52.9, 67.2, 70.8, 113.7, 120.0, 121.2, 127.1, 127.4, 128.3, 128.3, 128.4, 128.7, 128.9, 129.1, 129.6, 131.3, 133.3, 133.7, 135.6, 135.8, 138.4, 148.1, 159.0, 164.4, 166.0; LRMS (ES+) Calcd for [C\textsubscript{50}H\textsubscript{45}F\textsubscript{5}N\textsubscript{2}O\textsubscript{6}S + Na] 919.28 found 919.54
4.6g, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(2-(trifluoromethyl)benzyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 4.5 was functionalized on a 0.03 mmol scale using General Procedure A to give 4.6g (18.4 mg, 64%); $\delta_H$ (400 MHz, d-CDCl$_3$) 1.31-1.47 (m, 5H, CH$_2$), 1.69-1.90 (m, 5H, CH$_2$), 2.41-2.53 (m, 1H, CH), 3.77 (s, 2H, CH$_2$), 4.65 (s, 2H, CH$_2$), 4.77 (s, 2H, CH$_2$), 4.87 (s, 2H, CH$_2$), 5.30 (s, 2H, CH$_2$), 6.32 (s, 1H, CH), 6.48 (d, $J = 7.6$ Hz, 1H, CH), 6.91 (d, $J = 8.0$ Hz, 2H, CH), 7.10 (d, $J = 7.6$ Hz, 1H, CH), 7.27-7.42 (m, 11H, CH), 7.54 (t, $J = 7.6$ Hz, 1H, CH), 7.62 (d, $J = 8.0$ Hz, 1H, CH), 7.71 (d, $J = 8.0$ Hz, 1H, CH); $\delta_C$ (100 MHz, d-CDCl$_3$) 26.2, 26.9, 34.6, 44.4, 47.5, 48.5, 53.0, 67.2, 70.8, 113.8, 116.6, 120.0, 121.2, 125.8, 127.1, 127.3, 128.3, 128.4, 128.7, 128.9, 130.6, 132.9, 133.4, 133.5, 134.0, 135.6, 135.8, 144.2, 148.1, 158.9, 165.5, 166.0; LRMS (ES+) Calcd for [C$_{50}$H$_{42}$F$_8$N$_2$O$_6$S + Na] 973.25 found 973.46.

4.6h, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(3-(trifluoromethyl)benzyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 4.5 was
functionalized on a 0.03 mmol scale using General Procedure A to give 4.6h (24.2 mg, 85%); δ\textsubscript{H} (400 MHz, \textit{d}-CDCl\textsubscript{3}) 1.29-1.46 (m, 5H, CH\textsubscript{2}), 1.67-1.92 (m, 5H, CH\textsubscript{2}), 2.38-2.54 (m, 1H, CH), 3.76 (s, 2H, CH\textsubscript{2}), 4.65 (s, 2H, CH\textsubscript{2}), 4.75 (s, 2H, CH\textsubscript{2}), 4.79 (s, 2H, CH\textsubscript{2}), 5.31 (s, 2H, CH\textsubscript{2}), 6.30 (s, 1H, CH), 6.43 (d, \textit{J} = 7.6 Hz, 1H, CH), 6.92 (d, \textit{J} = 7.6 Hz, 2H, CH), 7.12 (d, \textit{J} = 7.6 Hz, 2H, CH), 7.24-7.40 (m, 10H, CH), 7.43-7.62 (m, 4H, CH), 7.71 (d, \textit{J} = 8.0 Hz, 1H, CH); δ\textsubscript{C} (100 MHz, \textit{d}-CDCl\textsubscript{3}) 26.2, 26.9, 34.5, 44.4, 47.7, 51.1, 53.0, 67.2, 70.8, 111.7, 113.6, 116.2, 119.9, 121.4, 125.5, 127.2, 127.3, 128.3, 128.4, 128.7, 128.9, 129.8, 133.2, 133.4, 135.6, 135.7, 135.8, 144.0, 148.2, 158.9, 165.4, 165.7; LRMS (ES+) Calcd for [C\textsubscript{50}H\textsubscript{42}F\textsubscript{8}N\textsubscript{2}O\textsubscript{6}S + Na\textsuperscript{+}] 973.25 found 973.39

4.6i, benzyl 2-(benzyl)oxy)-4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(4-(trifluoromethyl)benzyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 4.5 was functionalized on a 0.03 mmol scale using General Procedure A to give 4.6i (28.3 mg, 99%); δ\textsubscript{H} (400 MHz, \textit{d}-CDCl\textsubscript{3}) 1.30-1.45 (m, 5H, CH\textsubscript{2}), 1.70-1.90 (m, 5H, CH\textsubscript{2}), 2.41-2.54 (m, 1H, CH), 3.74 (s, 2H, CH\textsubscript{2}), 4.63 (s, 2H, CH\textsubscript{2}), 4.75 (s, 2H, CH\textsubscript{2}), 4.83 (s, 2H, CH\textsubscript{2}), 5.31 (s, 2H, CH\textsubscript{2}), 6.32 (s, 1H, CH), 6.36 (d, \textit{J} = 7.6 Hz, 1H, CH), 6.90 (d, \textit{J} = 8.0 Hz, 2H, CH), 7.11 (d, \textit{J} = 8.0 Hz, 2H, CH), 7.28-7.36 (m, 10H, CH), 7.38 (d, \textit{J} = 8.0 Hz, 2H, CH), 7.57 (d, \textit{J} = 8.0 Hz, 2H, CH), 7.70 (d, \textit{J} = 8.0 Hz, 1H, CH); δ\textsubscript{C} (100 MHz, \textit{d}-CDCl\textsubscript{3}) 26.2, 26.9, 34.6, 44.4, 48.0, 51.2, 53.0, 67.3, 70.9, 113.7, 119.9, 121.6, 125.9, 126.0, 126.0, 126.1, 127.2, 127.3, 128.3, 128.4, 128.4, 128.7, 128.7, 129.0, 129.0, 133.3, 133.4, 135.6, 135.7, 138.8, 148.3, 158.9, 165.4, 165.7; LRMS
4.6j, benzyl 2-(benzylidyne)4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(2-fluorobenzyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 4.5 was functionalized on a 0.03 mmol scale using General Procedure A to give 4.6j (27.0 mg, 100 %); δ_H (400 MHz, d-CDCl$_3$) 1.31-1.45 (m, 5H, CH$_2$), 1.70-1.89 (m, 5H, CH$_2$), 2.40-2.54 (m, 1H, CH), 3.83 (s, 2H, CH$_2$), 4.68 (s, 2H, CH$_2$), 4.73 (s, 2H, CH$_2$), 4.82 (s, 2H, CH$_2$), 5.31 (s, 2H, CH$_2$), 6.37 (s, 1H, CH), 6.52 (d, $J$ = 8.0 Hz, 1H, CH), 6.92-7.02 (m, 3H, CH), 7.08-7.16 (m, 3H, CH), 7.30-7.43 (m, 12H, CH), 7.74 (d, $J$ = 8.0 Hz, 1H, CH); δ_C (100 MHz, d-CDCl$_3$) 26.2, 26.9, 34.6, 44.4, 48.5, 53.0, 60.5, 67.2, 70.9, 113.9, 115.4, 115.6, 120.1, 121.8, 121.9, 125.0, 125.0, 127.2, 127.3, 128.3, 128.4, 128.7, 128.7, 128.9, 130.5, 130.6, 131.7, 131.7, 133.4, 133.6, 135.7, 135.8, 148.1, 159.0, 164.4, 165.9; LRMS (ES+) Calcd for [C$_{49}$H$_{42}$F$_8$N$_2$O$_6$S + Na] 923.26 found 923.43.

4.6k, benzyl 2-(benzylidyne)4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(3-fluorobenzyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 4.5 was functionalized on
a 0.03 mmol scale using General Procedure A to give 4.6k (26.2 mg, 97 %); δ_H (400 MHz, d-
CDCl3) 1.30-1.45 (m, 5H, CH2), 1.68-1.90 (m, 5H, CH2), 2.39-2.54 (m, 1H, CH), 3.76 (s, 2H,
CH2), 4.64 (s, 2H, CH2), 4.68 (s, 2H, CH2), 4.78 (s, 2H, CH2), 5.31 (s, 2H, CH2), 6.28 (s, 1H,
CH), 6.43 (d, J = 8.0 Hz, 1H, CH), 6.92 (d, J = 8.0 Hz, 2H, CH), 6.95-7.05 (m, 2H, CH), 7.05 (d,
J = 7.6 Hz, 1H, CH), 7.12 (d, J = 8.0 Hz, 2H, CH), 7.28-7.43 (m, 11H, CH), 7.72 (d, J = 8.0 Hz,
1H, CH); δ_C (100 MHz, d-CDCl3) 26.2, 26.9, 34.6, 44.4, 47.8, 51.0, 53.0, 67.2, 70.9, 113.7,
115.4, 115.7, 115.9, 119.9, 121.4, 124.4, 124.8, 127.2, 127.3, 128.3, 128.4, 128.4, 128.7, 128.7,
128.9, 130.7, 133.4, 133.5, 135.6, 135.8, 137.2, 137.2, 144.2, 145.1, 148.2, 158.9, 164.4,
165.8; LRMS (ES+) Calcd for [C_{49}H_{42}F_{6}N_{2}O_{6}S + Na] 923.26 found 923.51.

4.6l, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2,3,4,5,6-pentafluoro-N-(4-
fluorobenzyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 4.5 was functionalized on
a 0.03 mmol scale using General Procedure A to give 4.6l (26.8 mg, 99 %); δ_H (400 MHz, d-
CDCl3) 1.30-1.46 (m, 5H, CH2), 1.69-1.91 (m, 5H, CH2), 2.41-2.56 (m, 1H, CH), 3.73 (s, 2H,
CH2), 4.63 (s, 2H, CH2), 4.67 (s, 2H, CH2), 4.80 (s, 2H, CH2), 5.31 (s, 2H, CH2), 6.26 (s, 1H,
CH), 6.41 (d, J = 8.0 Hz, 1H, CH), 6.92 (d, J = 8.0 Hz, 2H, CH), 6.95-7.03 (m, 2H, CH), 7.11 (d,
J = 8.0 Hz, 2H, CH), 7.19-7.25 (m, 2H, CH), 7.28-7.42 (m, 10H, CH), 7.72 (d, J = 8.0 Hz, 1H,
CH); δ_C (100 MHz, d-CDCl3) 26.2, 26.9, 34.6, 44.4, 47.7, 50.7, 53.0, 67.3, 70.9, 113.7, 115.9,
116.1, 119.9, 121.2, 127.2, 127.3, 128.3, 128.4, 128.4, 128.7, 128.8, 129.0, 130.7, 130.8, 133.4,
133.5, 135.6, 135.8, 144.3, 148.2, 158.9, 165.4, 165.6; LRMS (ES+) Calcd for [C_{49}H_{42}F_{6}N_{2}O_{6}S
+ Na] 923.26 found 923.51
Sulfonamide 4.5 was functionalized on a 0.04 mmol scale using General Procedure A to give 4.6m (35.0 mg, 95%); \(\delta_H\) (400 MHz, \(d\)-CDCl\(_3\)) 1.28-1.43 (m, 5H, CH\(_2\)), 1.64-1.88 (m, 5H, CH\(_2\)), 2.36-2.52 (m, 1H, CH), 3.78 (s, 2H, CH\(_2\)), 4.64 (s, 2H, CH\(_2\)), 4.79 (s, 4H, 2 CH\(_2\)), 5.28 (s, 2H, CH\(_2\)), 6.35 (s, 1H, CH), 6.50 (d, \(J = 8.0\) Hz, 1H, CH), 6.93 (d, \(J = 7.6\) Hz, 2H, CH), 7.08 (d, \(J = 7.6\) Hz, 2H, CH), 7.15-7.38 (m, 13H, CH), 7.43 (d, \(J = 8.0\) Hz, 1H, CH), 7.71 (d, \(J = 8.0\) Hz, 1H, CH); \(\delta_C\) (100 MHz, \(d\)-CDCl\(_3\)) 26.2, 26.9, 34.6, 39.6, 44.4, 48.6, 48.7, 53.1, 67.2, 70.9, 113.9, 120.1, 121.3, 127.1, 127.3, 127.7, 128.3, 128.4, 128.4, 128.7, 128.8, 129.0, 129.6, 129.9, 131.4, 132.5, 133.4, 133.6, 134.2, 135.7, 135.8, 144.3, 148.1, 148.9, 165.4, 165.9; LRMS (ES+) Calcd for [C\(_{49}\)H\(_{42}\)ClF\(_5\)N\(_2\)O\(_6\)S + Na] 939.23 found 939.42.

Sulfonamide 4.6n, benzyl 2-(benzyloxy)-4-(2-(N-(3-chlorobenzyl)-2,3,4,5,6-pentafluorophenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)benzoate. Sulfonamide
4.5 was functionalized on a 0.04 mmol scale using General Procedure A to give 4.6n (34.5 mg, 94 %); $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.28-1.45 (m, 5H, CH$_2$), 1.66-1.90 (m, 5H, CH$_2$), 2.39-2.53 (m, 1H, CH), 3.74 (s, 2H, CH$_2$), 4.63 (s, 4H, 2 CH$_2$), 4.78 (s, 4H, 2 CH$_2$), 5.29 (s, 2H, CH$_2$), 6.26 (s, 1H, CH), 6.43 (d, $J$ = 7.8 Hz, 1H, CH), 6.91 (d, $J$ = 8.0 Hz, 2H, CH), 7.10 (d, $J$ = 8.0 Hz, 2H, CH), 7.19-7.27 (m, 4H, CH), 7.28-7.39 (m, 10H, CH), 7.72 (d, $J$ = 8.0 Hz, 1H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 26.2, 26.9, 34.6, 39.6, 44.4, 47.9, 50.9, 53.0, 67.2, 70.9, 113.6, 119.9, 121.4, 127.0, 127.2, 127.3, 128.3, 128.4, 128.7, 128.8, 128.9, 130.2, 130.5, 133.4, 133.5, 134.8, 135.6, 135.8, 136.7, 144.2, 148.2, 158.9, 165.4, 165.7; LRMS (ES+) Calcd for [C$_{49}$H$_{42}$ClF$_5$N$_2$O$_6$S + Na] 939.23 found 939.36

4.6o, benzyl 2-(benzyloxy)-4-(2-(N-(4-chlorobenzyl)-2,3,4,5,6-pentafluorophenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)benzoate. Sulfonamide 4.5 was functionalized on a 0.04 mmol scale using General Procedure A to give 4.6o (34.8 mg, 95 %); $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.28-1.43 (m, 5H, CH$_2$), 1.64-1.87 (m, 5H, CH$_2$), 2.38-2.52 (m, 1H, CH), 3.72 (s, 2H, CH$_2$), 4.54-4.71 (m, 4H, 2 CH$_2$), 4.78 (s, 4H, 2 CH$_2$), 5.28 (s, 2H, CH$_2$), 6.24 (s, 1H, CH), 6.37 (d, $J$ = 8.0 Hz, 1H, CH), 6.89 (d, $J$ = 8.0 Hz, 2H, CH), 7.09 (d, $J$ = 8.0 Hz, 2H, CH), 7.16 (d, $J$ = 8.0 Hz, 2H, CH), 7.20-7.41 (m, 11H, CH), 7.70 (d, $J$ = 8.0 Hz, 1H, CH), 7.71 (d, $J$ = 8.0 Hz, 1H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 26.2, 26.9, 34.6, 44.4, 47.6, 47.8, 50.8, 53.0, 67.3, 70.9, 113.6, 119.9, 121.4, 127.2, 127.3, 127.4, 128.3, 128.4, 128.7, 128.8, 129.0, 129.2, 130.2, 133.0, 133.4, 133.5, 134.5, 135.6, 135.7, 144.2, 148.2, 158.9, 165.4, 165.8; LRMS (ES+) Calcd for [C$_{49}$H$_{42}$ClF$_5$N$_2$O$_6$S + Na] 939.23 found 939.49
4.6p, benzyl 2-(benzyl oxy)-4-(N-(4-cyclohexylbenzyl)-2,3,4,5,6-pentafluoro-N-(2-(trifluoromethoxy)benzyl)phenylsul fonamido)acetamido)benzoate. Sulfonamide 4.5 was functionalized on a 0.04 mmol scale using General Procedure A to give 4.6p (40.2 mg, 100%);

δH (400 MHz, d-CDCl3) 1.30-1.46 (m, 5H, CH2), 1.68-1.89 (m, 5H, CH2), 2.38-2.54 (m, 1H, CH), 3.81 (s, 2H, CH2), 4.67 (s, 2H, CH2), 4.77 (s, 2H, CH2), 4.81 (s, 2H, CH2), 5.31 (s, 2H, CH2), 6.37 (s, 1H, CH), 6.52 (d, J = 7.6 Hz, 1H, CH), 6.93 (d, J = 8.0 Hz, 2H, CH), 7.11 (d, J = 7.6 Hz, 2H, CH), 7.21 (d, J = 8.0 Hz, 1H CH), 7.27-7.42 (m, 12H, CH), 7.52 (d, J = 7.2 Hz, 1H, CH) 7.75 (d, J = 8.0 Hz, 1H, CH); δC (100 MHz, d-CDCl3) 26.2, 26.9, 34.5, 44.3, 45.6, 48.4, 53.0, 67.2, 70.8, 113.8, 120.0, 120.7, 121.3, 121.9, 127.1, 127.3, 127.6, 127.8, 128.3, 128.4, 128.7, 128.9, 130.0, 131.4, 133.4, 133.5, 135.6, 135.7, 144.2, 147.7, 148.1, 158.9, 165.4, 165.7; LRMS (ES+) Calcd for [C50H42F8N2O7S + Na] 989.25 found 989.45
4.6q, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(3-(trifluoromethoxy)benzyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 4.5 was functionalized on a 0.04 mmol scale using General Procedure A to give 4.6q (38.6 mg, 100 %);
\[\delta_H (400 \text{ MHz, } d-\text{CDCl}_3) 1.30-1.46 (\text{m, 5H, CH}_2), 1.71-1.88 (\text{m, 5H, CH}_2), 2.40-2.52 (\text{m, 1H, CH}), 3.77 (\text{s, 2H, CH}_2), 4.64 (\text{s, 2H, CH}_2), 4.71 (\text{s, 2H, CH}_2), 4.80 (\text{s, 2H, CH}_2), 5.31 (\text{s, 2H, CH}_2), 6.30 (\text{s, 1H, CH}), 6.41 (\text{d, } J = 7.6 \text{ Hz, 1H, CH}), 6.92 (\text{d, } J = 8.0 \text{ Hz, 2H, CH}), 7.08-7.19 (\text{m, 4H, CH}), 7.21-7.26 (\text{m, 1H, CH}), 7.27-7.39 (\text{m, 11H, CH}), 7.71 (\text{d, } J = 8.0 \text{ Hz, 1H, CH}); \delta_C (100 \text{ MHz, } d-\text{CDCl}_3) 26.2, 26.9, 34.5, 44.4, 47.9, 51.0, 53.0, 67.2, 70.8, 113.7, 119.8, 120.9, 121.3, 121.8, 127.1, 127.2, 127.3, 128.3, 128.4, 128.4, 128.7, 128.7, 128.9, 130.6, 133.4, 133.5, 135.6, 135.7, 137.1, 144.1, 148.2, 149.6, 158.9, 165.4, 165.8; LRMS (ES+) Calcd for \([\text{C}_{50}\text{H}_{42}\text{F}_8\text{N}_2\text{O}_7\text{S} + \text{Na}] 989.25 \text{ found 989.45.}\]

4.6r, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(4-(trifluoromethoxy)benzyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 4.5 was functionalized on a 0.04 mmol scale using General Procedure A to give 4.6r (39.6 mg, 100 %);
\[\delta_H (400 \text{ MHz, } d-\text{CDCl}_3) 1.25-1.38 (\text{m, 5H, CH}_2), 1.60-1.78 (\text{m, 5H, CH}_2), 2.31-2.45 (\text{m, 1H, CH}), 4.00 (\text{s, 2H, CH}_2), 4.60 (\text{s, 2H, CH}_2), 4.72 (\text{s, 2H, CH}_2), 5.00 (\text{s, 2H, CH}_2), 5.24 (\text{s, 2H, CH}_2), 6.62 (\text{d, } J = 7.2 \text{ Hz, 1H, CH}), 6.92-7.12 (\text{m, 5H, CH}), 7.22-7.42 (\text{m, 14H, CH}), 7.60 (\text{d, } J = 8.0 \text{ Hz, 1H, CH}); \text{LRMS (ES+)} \text{ Calcd for } [\text{C}_{50}\text{H}_{42}\text{F}_8\text{N}_2\text{O}_7\text{S} + \text{Na}] 989.25 \text{ found 989.59.}]
4.6s, benzyl 2-(benzyloxy)-4-(2-(N-(2-cyanobenzyl)-2,3,4,5,6-pentafluorophenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)benzoate. Sulfonamide 4.5 was functionalized on a 0.04 mmol scale using General Procedure A to give 4.6s (36.7 mg, 100 %); δ\textsubscript{H}(400 MHz, d\textsubscript{-}CDCl\textsubscript{3}) 1.30-1.45 (m, 5H, CH\textsubscript{2}), 1.68-1.90 (m, 5H, CH\textsubscript{2}), 2.39-2.55 (m, 1H, CH), 3.90 (s, 2H, CH\textsubscript{2}), 4.71 (s, 2H, CH\textsubscript{2}), 4.86 (s, 2H, CH), 4.90 (s, 2H, CH\textsubscript{2}), 5.31 (s, 2H, CH\textsubscript{2}), 6.56 (s, 1H, CH), 6.61 (d, J = 8.0 Hz, 1H, CH), 6.93 (d, J = 8.0 Hz, 2H, CH), 7.11 (d, J = 8.0 Hz, 2H, CH), 7.27-7.45 (m, 10H, CH), 7.54-7.64 (m, 2H, CH), 7.71 (d, J = 8.0 Hz, 1H, CH), 7.75 (d, J = 8.0 Hz, 2H, CH); δ\textsubscript{C}(100 MHz, d\textsubscript{-}CDCl\textsubscript{3}) 26.2, 27.0, 34.6, 44.4, 49.1, 49.6, 53.2, 67.2, 70.9, 112.5, 114.0, 117.4, 120.2, 121.3, 127.1, 127.3, 128.2, 128.4, 128.7, 128.7, 128.9, 129.1, 130.8, 132.8, 133.3, 133.4, 133.9, 135.7, 135.8, 139.0, 144.2, 148.0, 159.0, 165.4, 165.5; LRMS (ES+) Calcd for [C\textsubscript{50}H\textsubscript{42}F\textsubscript{5}N\textsubscript{3}O\textsubscript{6}S + Na] 930.26 found 930.44.

4.6t, benzyl 2-(benzyloxy)-4-(2-(N-(3-cyanobenzyl)-2,3,4,5,6-pentafluorophenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)benzoate. Sulfonamide
4.5 was functionalized on a 0.04 mmol scale using General Procedure A to give 4.6t (33.4 mg, 92 %); δH (400 MHz, d-CDCl3) 1.30-1.46 (m, 5H, CH2), 1.68-1.91 (m, 5H, CH2), 2.38-2.55 (m, 1H, CH), 3.76 (s, 2H, CH2), 4.58-4.78 (m, 4H, 2 CH2), 4.84 (s, 2H, CH2), 5.31 (s, 2H, CH2), 6.34 (s, 1H, CH), 6.49 (d, J = 8.0 Hz, 1H, CH), 6.86-6.97 (m, 2H, CH), 7.12 (d, J = 7.8 Hz, 2H, CH), 7.23-7.40 (m, 10H, CH), 7.41-7.49 (m, 1H, CH), 7.52-7.63 (m, 3H, CH) 7.75 (d, J = 8.0 Hz, 1H, CH); δC (100 MHz, d-CDCl3) 26.2, 26.9, 34.5, 44.3, 48.0, 50.9, 53.1, 67.3, 70.9, 113.1, 113.7, 118.3, 119.8, 121.5, 127.3, 127.3, 128.3, 128.4, 128.5, 128.7, 128.9, 130.0, 131.9, 132.2, 133.0 133.3, 133.4, 135.6, 135.7, 136.6, 144.0, 148.3, 159.0, 165.3, 165.6.

4.6u, benzyl 2-(benzyloxy)-4-(2-(N-(4-cyanobenzyl)-2,3,4,5,6-pentafluorophenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)benzoate. Sulfonamide 4.5 was functionalized on a 0.04 mmol scale using General Procedure A to give 4.6u (31.5 mg, 87 %); δH (400 MHz, d-CDCl3) 1.29-1.46 (m, 5H, CH2), 1.66-1.90 (m, 5H, CH2), 2.39-2.56 (m, 1H, CH), 3.77 (s, 2H, CH2), 4.59-4.69 (m, 2H, CH2), 4.72-80 (m, 2H, CH2), 4.85 (s, 2H, CH2), 5.31 (s, 2H, CH2), 6.35 (s, 1H, CH), 6.44 (d, J = 8.2 Hz, 1H, CH), 6.93 (d, J = 7.6 Hz, 2H, CH), 6.99 (d, J = 8.0 Hz, 2H, CH), 7.11 (d, J = 8.0 Hz, 2H, CH), 7.24-7.44 (m, 12H, CH), 7.57-7.67 (m, 2H, CH), 7.73 (d, J = 8.0 Hz, 1H, CH); δC (100 MHz, d-CDCl3) 26.2, 26.9, 34.6, 44.4, 48.2, 51.4, 53.1, 67.3, 70.9, 112.5, 113.8, 118.4, 119.8, 121.6, 127.2, 127.3, 128.3, 128.5, 128.5, 128.7, 128.8, 129.0, 129.1, 132.8, 133.3, 133.4, 135.5, 135.7, 140.4, 144.0, 148.3, 159.0, 165.4, 165.6; LRMS (ES+) Calcd for [C50H42F5N3O6S + Na] 930.26 found 930.46.
4.6v, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(2,3,4,5-tetrafluorobenzyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 4.5 was functionalized on a 0.04 mmol scale using General Procedure A to give 4.6v (34.2 mg, 90%); $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.30-1.46 (m, 5H, CH$_2$), 1.67-1.91 (m, 5H, CH$_2$), 2.39-2.54 (m, 1H, CH), 3.82 (s, 2H, CH$_2$), 4.67 (s, 4H, 2 CH$_2$), 4.87 (s, 2H, CH$_2$), 5.32 (s, 2H, CH$_2$), 6.41 (s, 1H, CH), 6.59 (d, $J$ = 8.0 Hz, 1H, CH), 6.92 (d, $J$ = 8.0 Hz, 2H, CH), 7.08-7.19 (m, 3H, CH), 7.27-7.42 (m, 10H, CH), 7.80 (d, $J$ = 8.0 Hz, 1H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 26.2, 26.9, 34.6, 44.4, 44.5, 48.7, 53.1, 67.3, 70.9, 112.2, 112.3, 113.7, 113.7, 120.0, 121.6, 127.2, 127.3, 128.3, 128.4, 128.5, 128.7, 128.8, 128.9, 133.4, 133.5, 135.6, 135.7, 144.0, 148.3, 159.0, 165.4, 165.5; LRMS (ES+) Calcd for [C$_{49}$H$_{39}$F$_9$N$_2$O$_6$S + Na] 977.23 found 977.43.

4.6w, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(2,3,4,6-tetrafluorobenzyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 4.5 was functionalized on a 0.04 mmol scale using General Procedure A to give 4.6w (35.5 mg, 93%);
δ\(_H\) (400 MHz, d-CDCl\(_3\)) 1.29-1.45 (m, 5H, CH\(_2\)), 1.68-1.91 (m, 5H, CH\(_2\)), 2.36-2.52 (m, 1H, CH), 3.87 (s, 2H, CH\(_2\)), 4.68 (s, 2H, CH\(_2\)), 4.78 (s, 2H, CH\(_2\)), 4.87 (s, 2H, CH\(_2\)), 5.32 (s, 2H, CH\(_2\)), 6.42 (s, 1H, CH), 6.60 (d, J = 8.0 Hz, 1H, CH), 6.94 (d, J = 8.0 Hz, 2H, CH), 6.70-6.82 (m, 1H, CH), 7.93 (d, J = 7.6 Hz, 2H, CH), 7.12 (d, J = 8.0 Hz, 2H, CH), 7.20-7.42 (m, 10H, CH), 7.80 (d, J = 8.0 Hz, 1H, CH); δ\(_C\) (100 MHz, d-CDCl\(_3\)) 26.2, 26.9, 34.6, 39.7, 44.4, 48.9, 53.1, 67.3, 70.8, 107.0, 113.7, 114.6, 120.0, 121.5, 127.2, 127.3, 128.3, 128.4, 128.7, 128.8, 133.5, 135.6, 135.7, 144.2, 148.2, 159.0 165.4, 165.6; LRMS (ES+) Calcd for [C\(_{49}\)H\(_{39}\)F\(_9\)N\(_2\)O\(_6\)S + Na] 977.23 found 977.42.

4.6x, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(2,3,5,6-tetrafluorobenzyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 4.5 was functionalized on a 0.04 mmol scale using General Procedure A to give 4.6x (32.8 mg, 86 %); δ\(_H\) (400 MHz, d-CDCl\(_3\)) 1.30-1.45 (m, 5H, CH\(_2\)), 1.68-1.90 (m, 5H, CH\(_2\)), 2.41-2.54 (m, 1H, CH), 3.87 (s, 2H, CH\(_2\)), 4.69 (s, 2H, CH\(_2\)), 4.87 (s, 4H, 2 CH\(_2\)), 5.32 (s, 2H, CH\(_2\)), 6.43 (s, 1H, CH), 6.61 (d, J = 8.0 Hz, 1H, CH), 6.94 (d, J = 8.0 Hz, 2H, CH), 6.99-7.09 (m, 1H, CH), 7.12 (d, J = 8.0 Hz, 2H, CH), 7.28-7.42 (m, 10H, CH), 7.80 (d, J = 8.0 Hz, 1H, CH); δ\(_C\) (100 MHz, d-CDCl\(_3\)) 26.2, 26.9, 34.6, 39.7, 44.4, 48.9, 53.1, 67.3, 70.8, 107.0, 113.7, 114.6, 120.0, 121.5, 127.2, 127.3, 128.3, 128.4, 128.7, 128.8, 133.5, 133.5, 135.6, 135.8, 144.1, 148.2, 159.1 165.4, 165.5; LRMS (ES+) Calcd for [C\(_{49}\)H\(_{39}\)F\(_9\)N\(_2\)O\(_6\)S + Na] 977.23 found 977.42.
4.6y, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-((perfluorophenyl)methyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 4.5 was functionalized on a 0.04 mmol scale using General Procedure A to give 4.6y (38.4 mg, 99%); \(\delta_H\) (400 MHz, \(d\)-CDCl\(_3\)) 1.30-1.46 (m, 5H, CH\(_2\)), 1.66-1.90 (m, 5H, CH\(_2\)), 2.38-2.54 (m, 1H, CH), 3.87 (s, 2H, CH\(_2\)), 4.67 (s, 2H, CH\(_2\)), 4.72 (s, 2H, CH\(_2\)), 4.87 (s, 2H, CH\(_2\)), 5.32 (s, 2H, CH\(_2\)), 6.42 (s, 1H, CH), 6.61 (d, \(J = 8.0 \text{ Hz}\), 1H, CH), 6.92 (d, \(J = 7.6 \text{ Hz}\), 2H, CH), 7.12 (d, \(J = 7.6 \text{ Hz}\), 2H, CH), 7.27-7.42 (m, 10H, CH), 7.80 (d, \(J = 8.0 \text{ Hz}\), 1H, CH); \(\delta_C\) (100 MHz, \(d\)-CDCl\(_3\)) 26.2, 26.9, 34.6, 39.6, 44.4, 49.0, 53.1, 67.3, 70.9, 113.7, 120.0, 121.6, 127.2, 127.3, 127.4, 128.4, 128.5, 128.7, 128.8, 128.9, 133.4, 133.5, 135.6, 135.7, 144.0, 148.2, 159.1, 165.4, 165.5. LRMS (ES+) Calcd for [C\(_{49}\)H\(_{38}\)F\(_{10}\)N\(_2\)O\(_6\)S + Na] 995.22 found 995.49.

4.6z, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(naphthalen-2-ylmethyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 4.5 was functionalized on a 0.04 mmol scale using General Procedure A to give 4.6z (37.3, 100%); \(\delta_H\)
(400 MHz, d-CDCl₃) 1.33-1.50 (m, 5H, CH₂), 1.68-1.94 (m, 5H, CH₂), 2.40-2.58 (m, 1H, CH), 3.77 (s, 2H, CH₂), 4.42 (s, 2H, CH₂), 4.64 (s, 2H, CH₂), 4.87 (s, 2H, CH₂), 5.25 (s, 2H, CH₂), 6.06 (d, J = 7.2 Hz, 1H, CH), 6.26 (d, J = 7.6 Hz, 2H, CH), 7.14 (d, J = 7.6 Hz, 2H, CH), 7.17-7.24 (m, 2H, CH), 7.27-7.36 (m, 8H, CH), 7.38-7.57 (m, 4H, CH), 7.65 (s, 1H, CH), 7.72 (d, J = 8.0 Hz, 1H, CH), 7.74-7.89 (m, 2H, CH); δC (100 MHz, d-CDCl₃) 26.2, 26.9, 34.6, 44.4, 47.8, 51.5, 52.9, 67.2, 70.5, 113.3, 119.9, 121.1, 126.3, 126.7, 126.9, 127.2, 127.5, 127.7, 127.9, 128.1, 128.3, 128.4, 128.6, 129.1, 129.2, 131.7, 133.2, 133.2, 133.7, 135.5, 135.7, 158.8, 165.4, 165.9; LRMS (ES+) Calcd for [C₅₃H₄₅F₅N₂O₆S + Na] 955.28 found 955.54.

4.6aa, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(3-(methoxycarbonyl)benzyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 4.5 was functionalized on a 0.04 mmol scale using General Procedure A to give 4.6aa (36.1 mg, 96%); δH (400 MHz, d-CDCl₃) 1.29-1.46 (m, 5H, CH₂), 1.69-1.90 (m, 5H, CH₂), 2.40-2.53 (m, 1H, CH), 3.76 (s, 2H, CH₂), 3.87 (s, 3H, CH₃), 4.65 (s, 2H, CH₂), 4.73 (s, 2H, CH₂), 4.77 (s, 2H, CH₂), 5.29 (s, 2H, CH₂), 6.30 (s, 1H, CH), 6.44 (d, J = 8.0 Hz, 1H, CH), 6.91 (d, J = 8.0 Hz, 2H, CH), 7.11 (d, J = 8.0 Hz, 2H, CH), 7.28-7.37 (m, 10H, CH), 7.41 (t, J = 7.6 Hz, 1H, CH), 7.52 (d, J = 7.6 Hz, 1H, CH), 7.69 (d, J = 8.0 Hz, 1H, CH), 7.90 (s, 1H, CH), 7.97 (d, J = 7.6 Hz, 1H, CH); δC (100 MHz, d-CDCl₃) 26.2, 26.9, 32.6, 34.6, 44.4, 47.9, 51.2, 52.4, 67.2, 70.9, 113.7, 120.0, 121.4, 127.2, 127.4, 128.3, 128.4, 128.4, 128.7, 128.7, 129.3, 129.7, 129.8, 129.8, 130.2, 130.9, 133.4, 133.4, 133.6, 135.2, 135.6, 135.8, 148.1, 158.9, 165.4, 165.8, 166.6; LRMS (ES+) Calcd for [C₅₃H₄₅F₅N₂O₆S + Na] 963.27 found 963.41.
4.6ab, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(4-(methoxycarbonyl)benzyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 4.5 was functionalized on a 0.04 mmol scale using General Procedure A to give 4.6ab (35.7 mg, 95 %); δ_H (400 MHz, d-CDCl_3) 1.29-1.48 (m, 5H, CH_2), 1.66-1.90 (m, 5H, CH_2), 2.37-2.55 (m, 1H, CH), 3.63-3.88 (m, 5H, CH_2 and CH_3), 4.63 (s, 2H, CH_2), 4.75 (s, 4H, 2 CH_2), 5.29 (s, 2H, CH_2), 6.25 (s, 1H, CH), 6.41 (d, J = 8.0 Hz, 1H, CH), 6.92 (d, J = 7.4 Hz, 2H, CH), 7.11 (d, J = 8.0 Hz, 2H, CH), 7.23-7.38 (m, 11H, CH), 7.46 (d, J = 8.0 Hz, 1H, CH), 7.70 (d, J = 8.0 Hz, 1H, CH), 7.99 (d, J = 8.0 Hz, 2H, CH); δ_C (100 MHz, d-CDCl_3) 26.2, 26.9, 34.6, 44.4, 47.9, 51.2, 52.3, 53.0, 67.2, 70.8, 113.6, 119.8, 120.3, 127.2, 127.4, 128.3, 128.4, 128.7, 128.7, 128.7, 129.0, 129.1, 130.2, 130.3, 130.5, 133.3, 133.4, 133.5, 135.7, 139.7, 142.7, 148.2, 158.9, 165.4, 165.7, 166.5; LRMS (ES+) Calcd for [C_{51}H_{45}F_{5}N_{2}O_{8}S + Na] 963.27 found 963.40.

4.7a, 4-(N-(4-cyclohexylbenzyl)-2-(perfluorophenylsulfonamido)acetamido)-2-hydroxybenzoic acid. Compound 4.5 was globally deprotected according to general procedure
B on a 0.06 mmol scale to give compound 4.7a (26.8 mg, 73 %); \( \delta_H \) (400 MHz, \( d-\text{CDCl}_3 \)) 1.30-1.46 (m, 5H, CH\( _2 \)), 1.69-1.91 (m, 5H, CH\( _2 \)), 2.38-2.55 (m, 1H, CH), 3.82 (s, 2H, CH\( _2 \)), 4.76 (s, 2H, CH\( _2 \)), 5.29 (s, 1H, NH), 6.50 (s (br), 1H, CH), 6.64 (s, 1H, CH), 6.99 (d, \( J = 7.2 \) Hz, 2H, CH), 7.10 (d, \( J = 7.2 \) Hz, 2H, CH), 7.90 (s, 1H, CH), 10.91 (s, 1H, OH); \( \delta_C \) (100 Hz, \( d-\text{CDCl}_3 \)) 26.2, 27.0, 34.5, 44.3, 45.1, 53.6, 113.4, 117.1, 118.9, 127.2, 128.7, 132.8, 133.0, 143.4, 148.2, 163.2, 166.7, 172.5; HRMS (ES+) Calcd for \([\text{C}_{28}\text{H}_{25}\text{F}_5\text{N}_2\text{O}_6\text{S} + \text{H}] \) 613.1430 found 613.1426; HPLC (III) \( t_R = 25.52 \) min (88.3 %), (IV) \( t_R = 55.78 \) min (93.5 %).

\[ \begin{align*}
\text{4.7b, 4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-propylphenylsulfonamido)acetamido)-2-hydroxybenzoic acid.} & \text{ Compound 4.6a was globally deprotected according to general procedure B on a 0.03 mmol scale to give compound 4.7b (29.6 mg, 90 %); } \\
& \delta_H \text{(400 MHz, } d-\text{CDCl}_3) 0.89 \text{ (t, } J = 7.2 \text{ Hz, 3H, CH}_3) \text{, 1.30-1.46 (m, 5H, CH}_2) \text{, 1.53 (m, 2H, CH}_2) \text{, 1.69-1.92 (m, 5H, CH}_2) \text{, 2.40-2.55 (m, 1H, CH), 3.41 (t, } J = 7.2 \text{ Hz, 2H, CH}_2) \text{, 4.06 (s, 2H, CH}_2) \text{, 4.73 (s, 2H, CH}_2) \text{, 6.56 (d, } J = 6.4 \text{ Hz, 1H, CH), 6.71 (s, 1H, CH), 6.98 (d, } J = 7.6 \text{ Hz, 2H, CH), 7.11 (d, } J = 7.6 \text{ Hz, 2H, CH), 7.90 (d, } J = 6.4 \text{ Hz, 1H, CH), 10.84 (s, 1H, OH); } \\
& \delta_C \text{(100 Hz, } d-\text{CDCl}_3) 11.1, 21.3, 26.3, 27.0, 34.5, 44.4, 48.7, 49.8, 53.3, 110.6, 116.9, 119.2, 127.2, 128.5, 129.1, 133.2, 148.1, 146.3, 163.3, 166.2, 171.6; \text{ HRMS (ES+) Calcd for } \\
& [\text{C}_{31}\text{H}_{31}\text{F}_5\text{N}_2\text{O}_6\text{S} + \text{H}] 655.1881 \text{ found 655.1895; HPLC (III) } t_R = 26.85 \text{ min (100 %), (IV) } t_R = 63.45 \text{ min (98.6 %).} \end{align*} \]
4.7c, 4-(2-(N-(tert-butoxycarbonyl)-2,3,4,5,6-pentafluorophenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)-2-hydroxybenzoic acid. Compound 4.6b was globally deprotected according to general procedure B on a 0.03 mmol scale to give compound 4.7c (25.5 mg, 72 %); δH (400 MHz, d-CDCl₃) 1.32-1.52 (m, 14H, CH₂ and 3 CH₃), 1.70-1.92 (m, 5H, CH₂), 2.38-2.55 (m, 1H, CH), 4.41 (s, 2H, CH₂), 4.70 (s, 4H, 2 CH₂), 6.25 (s (br), 1H, CH), 6.49 (s, 1H, CH), 6.99 (d, J = 7.2 Hz, 2H, CH), 7.14 (d, J = 7.2 Hz, 2H, CH), 7.27-7.44 (m, 4H, CH), 7.78 (s (br), 1H, CH), 10.84 (s, 1H, OH); δC (100 Hz, d-CDCl₃) 26.3, 27.0, 27.9, 34.5, 44.4, 49.6, 53.5, 86.3, 110.6, 117.2, 119.1, 127.1, 128.7, 132.6, 133.7, 147.9, 150.3, 163.9, 165.9, 169.6, 172.4; LRMS (ESI) Calcd for [C₃₃H₃₃F₅N₂O₈S + H, BOC lost] 613.14 found 613.10; HPLC (III) tR = 27.50 min (89.0 %), (IV) tR = 61.66 min (90.2 %).

4.7d, 4-(2-(N-benzyl-2,3,4,5,6-pentafluorophenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)-2-hydroxybenzoic acid. Compound 4.6c was globally deprotected according to general procedure B on a 0.03 mmol scale to give compound 4.7d (18.0...
mg, 85 %); δH (400 MHz, d-CDCl3) 1.32-1.48 (m, 5H, CH2), 1.69-1.94 (m, 5H, CH2), 2.42-2.56 (m, 1H, CH), 3.85 (s, 2H, CH2), 4.69 (s, 4H, 2 CH2), 6.26 (s (br), 1H, CH), 6.42 (s, 1H, CH), 6.97 (d, J = 7.6 Hz, 2H, CH), 7.13 (d, J = 7.6 Hz, 2H, CH), 7.20-7.37 (m, 4H, CH), 7.75 (d, J = 8.4 Hz, 1H, CH), 10.48 (s, 1H, OH); δC (100 Hz, d-CDCl3) 26.3, 27.0, 34.5, 44.4, 47.9, 51.1, 53.1, 111.7, 119.2, 127.2, 128.6, 128.7, 129.0, 129.1, 131.9, 132.5, 133.2, 134.3, 148.1, 163.1, 166.1, 172.9; HRMS (DART) Calcd for [C35H31F5N2O6S + H] 703.1901 found 703.1903; HPLC (III) tR = 25.97 min (97.6 %), (IV) tR = 64.35 min (98.6 %).

**4.7e, 4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(2-methylbenzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid.** Compound **4.6d** was globally deprotected according to general procedure B on a 0.02 mmol scale to give compound **4.7e** (11.9 mg, 83 %); δH (400 MHz, d-CDCl3) 1.32-1.47 (m, 5H, CH2), 1.69-1.93 (m, 5H, CH2), 2.28 (s, 3H, CH3) 2.40-2.55 (m, 1H, CH), 3.72 (s, 2H, CH2), 4.65 (s, 2H, CH2), 4.72 (s, 2H, CH2), 6.11 (s (br), 1H, CH), 6.31 (s, 1H, CH), 6.97 (d, J = 7.6 Hz, 2H, CH), 7.03-7.19 (m, 5H, CH), 7.22 (t, J = 7.6 Hz, 1H, CH), 7.70 (d, J = 7.6 Hz, 1H, CH), 10.95 (s, 1H, OH); δC (100 MHz, d-CDCl3) 19.1, 25.7, 26.3, 27.0, 34.5, 44.4, 47.7, 49.4, 53.1, 110.7, 116.9, 118.7, 126.4, 127.1, 128.6, 128.8, 129.0, 130.3, 131.1, 131.7, 132.3, 133.4, 138.1, 148.0, 162.4, 166.1, 171.7; LRMS Calcd for [C36H33F3N2O6S + H] 717.21 found 717.18; HPLC (III) tR = 30.30 min (100 %), (IV) tR = 66.23 min (100 %).
**4.7f, 4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(3-methylbenzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid.** Compound 4.6e was globally deprotected according to general procedure B on a 0.02 mmol scale to give compound 4.7f (12.6 mg, 88 %); δH (400 MHz, d-CDCl₃) 1.31-1.47 (m, 5H, CH₂), 1.69-1.93 (m, 5H, CH₂), 2.28 (s, 3H, CH₃) 2.40-2.54 (m, 1H, CH), 3.50 (s, 2H, CH₂), 4.61 (s, 2H, CH₂), 4.67 (s, 2H, CH₂), 6.20 (s br, 1H, CH), 6.38 (s, 1H, CH), 6.97 (d, J = 7.6 Hz, 2H, CH), 7.03 (d, J = 7.6 Hz, 2H, CH), 7.06-7.21 (m, 3H, CH), 7.72 (s, 1H, CH), 11.08 (s, 1H, OH); δC (100 MHz, d-CDCl₃) 21.4, 25.7, 26.3, 27.0, 34.5, 44.4, 47.9, 51.3, 53.1, 113.4, 116.7, 118.6, 126.1, 127.1, 128.6, 128.9, 129.4, 129.7, 132.3, 133.5, 134.1, 138.8, 139.2, 147.9, 163.0, 166.0, 172.1; HRMS (DART) Calcd for [C₃₆H₃₃F₅N₂O₆S + H] 717.2058 found 717.2047; HPLC (III) t_R = 30.00 min (100 %), (IV) t_R = 65.94 min (100 %).

**4.7g, 4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(4-methylbenzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid.** Compound 4.6f was
globally deprotected according to general procedure B on a 0.03 mmol scale to give compound 4.7g (25.6 mg, 89 %); δH (400 MHz, d-CDCl3) 1.32-1.47 (m, 5H, CH2), 1.69-1.91 (m, 5H, CH2), 2.38-2.55 (m, 1H, CH), 3.81 (s, 2H, CH2), 4.62 (s, 2H, CH2), 4.68 (s, 2H, CH2) 6.27 (s (br), 1H, CH), 6.36 (s, 1H, CH), 6.97 (d, J = 7.6 Hz, 2H, CH), 7.06-7.18 (m, 6H, CH), 7.25 (s, 1H, CH), 10.63 (s, 1H, CH); δC (100 Hz, d-CDCl3) 21.3, 26.3, 27.0, 34.5, 44.4, 47.8, 51.1, 53.2, 110.8, 117.1, 119.1, 127.2, 128.6, 129.1, 129.8, 131.0, 132.5, 133.3, 138.6, 143.7, 148.1, 163.1, 166.1, 172.7; HRMS (DART) Calcd for [C36H33F5N2O6S + H] 717.2058 found 717.2045; HPLC (III) tR = 29.25 min (100 %), (IV) tR = 66.91 min (100 %).

4.7h, 4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(2-(trifluoromethyl)benzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. Compound 4.6g was globally deprotected according to general procedure B on a 0.02 mmol scale to give compound 4.7h (11.5 mg, 75 %); δH (400 MHz, d-CDCl3) 1.30-1.47 (m, 5H, CH2), 1.69-1.94 (m, 5H, CH2), 2.38-2.54 (m, 1H, CH), 3.85 (s, 2H, CH2), 4.68 (s, 2H, CH2), 4.87 (s, 2H, CH2) 6.33 (s (br), 1H, CH), 6.47 (s, 1H, CH), 6.95 (d, J = 7.6 Hz, 2H, CH), 7.08 (d, J = 7.6 Hz, 2H, CH), 7.39 (t, J = 7.6 Hz, 1H, CH), 7.51 (t, J = 7.6 Hz, 1H, CH), 7.64 (d, J = 8.0 Hz, 1H, CH), 7.67-7.86 (m, 2H, CH), 11.16 (s, 1H, OH); δC (100 Hz, d-CDCl3) 25.7, 26.3, 27.0, 34.5, 44.4, 47.5, 48.5, 53.2, 111.8, 116.9, 118.7, 125.6, 126.0, 127.1, 128.4, 128.6, 130.6, 132.7, 133.3, 133.9, 134.8, 139.3, 147.9, 163.0, 165.6, 172.3; LRMS Calcd for [C36H30F6N2O6S + H] 771.18 found 771.23; HPLC (III) tR = 30.27 min (100 %), (IV) tR = 66.37 min (100 %).
**4.7i, 4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(3-(trifluoromethyl)benzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid.** Compound 4.6h was globally deprotected according to general procedure B on a 0.03 mmol scale to give compound 4.7i (18.8 mg, 81 %); $\delta_H$ (400 MHz, $d_6$-DMSO) 1.27-1.42 (m, 5H, CH$_2$), 1.64-1.85 (m, 5H, CH$_2$), 2.39-2.50 (m, 1H, CH), 4.13 (s, 2H, CH$_2$), 4.69 (s, 2H, CH$_2$), 4.75 (s, 2H, CH$_2$), 6.61 (s (br), 1H, CH), 6.71 (s, 1H, CH), 7.00 (d, $J = 8.0$ Hz, 2H, CH), 7.11 (d, $J = 8.0$ Hz, 2H, CH), 7.52-7.68 (m, 4H, CH), 7.71 (d, $J = 8.4$ Hz, 1H, CH), 11.76 (s, 1H, OH); HRMS (DART) Calcd for [C$_{36}$H$_{30}$F$_{8}$N$_2$O$_6$S + H] 771.1775 found 771.1787; HPLC (III) $t_R = 29.43$ min (100 %), (IV) $t_R = 65.38$ min (97.8 %).

**4.7j, 4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(4-(trifluoromethyl)benzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid.** Compound 4.6i was globally deprotected according to general procedure B on a 0.03 mmol scale to give compound 4.7j (26.4 mg, 100 %); $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.30-1.47 (m, 5H, CH$_2$), 1.70-1.92...
(m, 5H, CH₂), 2.41-2.54 (m, 1H, CH), 3.85 (s, 2H, CH₂), 4.68 (s, 2H, CH₂), 4.73 (s, 2H, CH₂),
6.23 (s (br), 1H, CH), 6.44 (s, 1H, CH), 6.96 (d, J = 7.6 Hz, 2H, CH), 7.12 (d, J = 7.6 Hz, 2H, CH),
7.38 (d, J = 7.6 Hz, 2H, CH), 7.56 (d, J = 7.6 Hz, 2H, CH), 7.75 (s, 1H, CH); δ_C (100 Hz, d-CDCl₃) 26.3, 27.0, 34.5, 44.4, 48.1, 51.1, 53.1, 111.8, 117.0, 118.7, 125.9, 126.0, 127.2, 128.6,
129.1, 131.4, 132.5, 135.8, 138.8, 148.1, 148.2, 163.2, 165.8, 172.6; HRMS (DART) Calcd for
[C₃₆H₃₉F₈N₂O₆S + H] 771.1775 found 771.1767; HPLC (III) t_R = 29.48 min (96.6 %), (IV) t_R = 65.57 min (100 %).

4.7k, 4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(2-
fluorobenzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. Compound 4.6j was
globally deprotected according to general procedure B on a 0.04 mmol scale to give compound
4.7k (29.1 mg, 99 %); δ_H (400 MHz, d-CDCl₃) 1.30-1.47 (m, 5H, CH₂), 1.69-1.92 (m, 5H, CH₂),
2.40-2.56 (m, 1H, CH), 3.91 (s, 2H, CH₂), 4.71 (s, 4H, 2 CH₂), 6.37 (s (br), 1H, CH), 6.53 (s, 1H, CH),
6.95-7.16 (m, 6H, CH), 7.27-7.43 (m, 2H, CH), 7.79 (s, 1H, CH); HRMS (DART) Calcd for
[C₃₅H₃₀F₆N₂O₆S + H] 721.1807 found 721.1816; HPLC (III) t_R = 28.77 min (100 %), (IV) t_R = 64.18 min (100 %).
4.7l, 4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(3-fluorobenzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. Compound 4.6k was globally deprotected according to general procedure B on a 0.03 mmol scale to give compound 4.7l (21.4 mg, 99 %); δH (400 MHz, d-CDCl₃) 1.31-1.47 (m, 5H, CH₂), 1.69-1.92 (m, 5H, CH₂), 2.41-2.55 (m, 1H, CH), 3.86 (s, 2H, CH₂), 4.68 (s, 4H, 2 CH₂), 6.29 (s (br), 1H, CH), 6.45 (s, 1H, CH), 6.92-7.06 (m, 5H, CH), 7.12 (d, J = 7.6 Hz, 2H, CH), 7.25-7.31 (t, J = 7.6 Hz, 1H, CH), 7.78 (s, 1H, CH); δC (100 Hz, d-CDCl₃) 26.3, 27.0, 34.5, 44.4, 48.0, 51.0, 53.2, 111.6, 115.6, 115.6, 115.8, 116.9, 118.7, 124.4, 124.4, 127.2, 128.5, 130.6, 130.7, 132.6, 133.3, 135.0, 137.1, 137.1, 148.1, 161.9, 163.1, 164.4, 172.6; HRMS (DART) Calcd for [C₃₅H₃₀F₆N₂O₆S + H] 721.1807 found 721.1827; HPLC (III) tᵣ = 28.78 min (94.4 %), (IV) tᵣ = 64.27 min (97.1 %).

4.7m, 4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(4-fluorobenzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. Compound 4.6l was globally deprotected according to general procedure B on a 0.03 mmol scale to give compound
4.7m (20.6 mg, 95 %); $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.32-1.47 (m, 5H, CH$_2$), 1.69-1.91 (m, 5H, CH$_2$), 2.38-2.55 (m, 1H, CH), 3.81 (s, 2H, CH$_2$), 4.62 (s, 2H, CH$_2$), 4.66 (s, 2H, CH$_2$) 6.23 (s (br), 1H, CH), 6.41 (s, 1H, CH), 6.87-7.04 (m, 4H, CH), 7.11 (d, $J = 7.6$ Hz, 2H, CH), 7.21 (d, $J = 7.6$ Hz, 2H, CH), 7.41 (s, 1H, CH); $\delta_C$ (100 Hz, $d$-CDCl$_3$) 26.3, 27.0, 34.5, 44.4, 47.7, 50.8, 53.2, 111.6, 115.8, 116.6, 116.1, 118.4, 127.1, 127.6, 130.2, 132.4, 133.4, 136.6, 148.0, 163.0, 165.9, 172.2; HRMS (DART) Calcd for [C$_{35}$H$_{30}$F$_6$N$_2$O$_6$S + H] 721.1807 found 721.1798; HPLC (III) $t_R = 28.78$ min (98.5 %), (IV) $t_R = 64.27$ min (93.6 %).

![Image of compound 4.7m](image)

4.7n, 4-(2-((N-(2-chlorobenzyl)-2,3,4,5,6-pentafluorophenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)-2-hydroxybenzoic acid. Compound 4.6m was globally deprotected according to general procedure B on a 0.04 mmol scale to give compound 4.7n (21.1 mg, 72 %); $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.30-1.46 (m, 5H, CH$_2$), 1.69-1.91 (m, 5H, CH$_2$), 2.40-2.55 (m, 1H, CH), 3.90 (s, 2H, CH$_2$), 4.71 (s, 2H, CH$_2$), 4.81 (s, 2H, CH$_2$), 6.39 (s (br), 1H, CH), 6.52 (s, 1H, CH), 6.99 (d, $J = 7.6$ Hz, 2H, CH), 7.11 (d, $J = 7.6$ Hz, 2H, CH), 7.18-7.29 (m, 2H, CH), 7.34 (d, $J = 6.8$ Hz, 1H, CH), 7.44 (d, $J = 6.8$ Hz, 1H, CH), 7.79 (d, $J = 8.4$ Hz, 1H, CH), 10.70 (s, 1H, OH); $\delta_C$ (100 Hz, $d$-CDCl$_3$) 26.3, 27.0, 34.5, 39.8, 44.4, 48.7, 53.3, 112.4, 117.2, 119.1, 127.2, 127.6, 128.6, 129.8, 130.0, 131.4, 132.3, 132.5, 133.2, 134.3, 134.4, 148.0, 163.1, 166.0, 172.5; HRMS (DART) Calcd for [C$_{37}$H$_{34}$F$_6$N$_2$O$_6$S + H] 737.1503 found 737.1502; HPLC (III) $t_R = 29.83$ min (82.2 %), (IV) $t_R = 65.71$ min (80.5 %).
4.7o, 4-(2-(N-(3-chlorobenzyl)-2,3,4,5,6-pentafluorophenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)-2-hydroxybenzoic acid. Compound 4.6n was globally deprotected according to general procedure B on a 0.04 mmol scale to give compound 4.7o (21.2 mg, 72 %); δ_H (400 MHz, d-CDCl_3) 1.30-1.46 (m, 5H, CH_2), 1.70-1.92 (m, 5H, CH_2), 2.41-2.56 (m, 1H, CH), 3.86 (s, 2H, CH_2), 4.66 (s, 2H, CH_2), 4.71 (s, 2H, CH_2), 6.34 (s (br), 1H, CH), 6.49 (s, 1H, CH), 6.98 (d, J = 7.2 Hz, 2H, CH), 7.13 (d, J = 7.2 Hz, 2H, CH), 7.22-7.36 (m, 4H, CH), 7.79 (d, J = 8.0 Hz, 1H, CH), 10.69 (s, 1H, OH); δ_C (100 Hz, d-CDCl_3) 26.3, 27.0, 34.5, 44.4, 48.0, 50.9, 53.2, 112.2, 117.1, 118.9, 127.0, 127.3, 128.6, 128.9, 130.4, 132.5, 132.6, 133.1, 133.2, 135.0, 136.5, 148.2, 163.1, 166.9, 172.4; HRMS (DART) Calcd for [C_{35}H_{30}ClF_{5}N_{2}O_{6}S + H] 737.1512 found 737.1512; HPLC (III) t_R = 29.72 min (97.9 %), (IV) t_R = 65.61 min (100 %).

4.7p, 4-(2-(N-(4-chlorobenzyl)-2,3,4,5,6-pentafluorophenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)-2-hydroxybenzoic acid. Compound 4.6o was globally deprotected according to general procedure B on a 0.05 mmol scale to give compound 4.7p (27.8
mg, 75 %); $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.30-1.48 (m, 5H, CH$_2$), 1.70-1.94 (m, 5H, CH$_2$), 2.41-2.58 (m, 1H, CH), 3.86 (s, 2H, CH$_2$), 4.70 (s, 2H, CH$_2$), 4.74 (s, 2H, CH$_2$), 6.31 (s (br), 1H, CH), 6.47 (s, 1H, CH), 6.98 (d, $J$ = 7.2 Hz, 2H, CH), 7.14 (d, $J$ = 7.2 Hz, 2H, CH), 7.20 (d, $J$ = 7.2 Hz, 2H, CH), 7.30 (d, $J$ = 7.2 Hz, 1H, CH), 7.47 (s (br), 1H, OH); $\delta_C$ (100 Hz, $d$-CDCl$_3$) 26.2, 27.0, 34.5, 44.3, 47.9, 50.8, 53.2, 112.4, 117.0, 118.8, 127.2, 128.6, 129.1, 129.2, 130.2, 132.5, 132.9, 133.1, 134.6, 148.2, 163.1, 165.9, 172.3; HRMS (DART) Calcd for [C$_{35}$H$_{30}$ClF$_5$N$_2$O$_6$S + H] 737.1512 found 737.1512; HPLC (III) $t_R$ = 29.03 min (71.8 %), (IV) $t_R$ = 66.71 min (72.4 %).

4.7q. 4-((N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(2-(trifluoromethoxy)benzyl)phenylsulfonylamo)acetamido)-2-hydroxybenzoic acid.

Compound 4.6p was globally deprotected according to general procedure B on a 0.03 mmol scale to give compound 4.7q (24.1 mg, 100 %); $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.29-1.46 (m, 5H, CH$_2$), 1.68-1.90 (m, 5H, CH$_2$), 2.38-2.54 (m, 1H, CH), 3.87 (s, 2H, CH$_2$), 4.70 (s, 2H, CH$_2$), 4.75 (s, 2H, CH$_2$), 6.38 (s (br), 1H, CH), 6.51 (s, 1H, CH), 6.97 (d, $J$ = 7.6 Hz, 2H, CH), 7.09 (d, $J$ = 7.6 Hz, 2H, CH), 7.17-7.29 (m, 2H, CH), 7.32 (d, $J$ = 7.2 Hz, 1H, CH), 7.49 (d, $J$ = 7.2 Hz, 1H, CH), 7.78 (d, $J$ = 7.2 Hz, 1H, CH), 10.90 (s, 1H, OH); $\delta_C$ (100 Hz, $d$-CDCl$_3$) 26.3, 27.0, 34.5, 44.4, 45.7, 48.5, 53.2, 111.2, 117.0, 118.9, 119.3, 120.8, 121.9, 127.2, 127.5, 127.7, 128.5, 130.1, 131.5, 132.5, 133.3, 147.8, 148.0, 163.1, 165.8, 172.4; HRMS (DART) Calcd for [C$_{36}$H$_{30}$F$_8$N$_2$O$_7$S + H] 787.1724 found 787.1712; HPLC (III) $t_R$ = 29.90 min (93.6 %), (IV) $t_R$ = 66.34 min (93.1 %).
4.7r, 4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(3-(trifluoromethoxy)benzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid.

Compound 4.6q was globally deprotected according to general procedure B on a 0.03 mmol scale to give compound 4.7r (20.5 mg, 87 %); δ_H (400 MHz, d-CDCl_3) 1.30-1.48 (m, 5H, CH_2), 1.69-1.93 (m, 5H, CH_2), 2.40-2.56 (m, 1H, CH), 3.87 (s, 2H, CH_2), 4.69 (s, 2H, 2 CH_2), 6.29 (s (br), 1H, CH), 6.46 (s, 1H, CH), 6.96 (d, J = 7.6 Hz, 2H, CH), 7.07-7.25 (m, 5H, CH), 7.35 (t, J = 7.2 Hz, 1H, CH), 7.75 (s, 1H, CH), 10.88 (s, 1H, OH); δ_C (100 Hz, d-CDCl_3) 26.3, 27.0, 34.5, 44.4, 45.7, 48.0, 51.0, 53.2, 109.9, 113.5, 116.9, 118.8, 121.0, 121.3, 127.1, 127.2, 128.6, 129.0, 130.6, 132.6, 137.0, 148.2, 149.8, 156.2, 163.1, 166.0, 172.6; HRMS (DART) Calcd for \([C_{39}H_{33}F_5N_2O_6S + H]\) 787.1724 found 787.1712; HPLC (III) t_R = 29.04 min (91.5 %), (IV) t_R = 66.91 min (91.3 %).

4.7s, 4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(4-(trifluoromethoxy)benzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid.
Compound 4.6r was globally deprotected according to general procedure B on a 0.03 mmol scale to give compound 4.7s (21.2 mg, 90 %); δ\textsubscript{H} (400 MHz, d-CDCl\textsubscript{3}) 1.32-1.49 (m, 5H, CH\textsubscript{2}), 1.70-1.96 (m, 5H, CH\textsubscript{2}), 2.42-2.58 (m, 1H, CH), 3.86 (s, 2H, CH\textsubscript{2}), 4.84 (s, 2H, CH\textsubscript{2}), 6.60 (s (br), 1H, CH), 6.73 (s, 1H, CH), 7.04-7.19 (m, 4H, CH), 7.90 (d, J = 7.6 Hz, 1H, CH), 10.92 (s, 1H, OH); δ\textsubscript{C} (100 Hz, d-CDCl\textsubscript{3}) 26.2, 27.0, 34.5, 44.4, 48.0, 50.7, 53.2, 112.0, 116.9, 118.6, 121.5, 127.2, 127.3, 128.5, 128.7, 128.8, 129.0, 130.3, 133.2, 148.1, 149.4, 163.0, 165.9, 171.6; HRMS (DART) Calcd for \([C\textsubscript{36}H\textsubscript{30}F\textsubscript{8}N\textsubscript{2}O\textsubscript{7}S + H]\) 787.1724 found 787.1722; HPLC (III) \(t_R = 29.90\) min (90.4 %), (IV) \(t_R = 66.04\) min (75.0 %).

4.7t, 4-(2-(N-(2-cyanobenzyl)-2,3,4,5,6-pentafluorophenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)-2-hydroxybenzoic acid. Compound 4.6s was globally deprotected according to general procedure B on a 0.04 mmol scale to give compound 4.7t (24.9 mg, 86 %); δ\textsubscript{H} (400 MHz, d-CDCl\textsubscript{3}) 1.30-1.46 (m, 5H, CH\textsubscript{2}), 1.69-1.92 (m, 5H, CH\textsubscript{2}), 2.41-2.53 (m, 1H, CH), 3.95 (s, 2H, CH\textsubscript{2}), 4.75 (s, 2H, CH\textsubscript{2}), 4.87 (s, 2H, CH\textsubscript{2}), 6.50 (d, J = 7.2 Hz, 1H, CH), 6.62 (s, 1H, CH), 6.98 (d, J = 8.0 Hz, 2H, CH), 7.11 (d, J = 8.0 Hz, 2H, CH), 7.42 (t, J = 7.6 Hz, 1H, CH), 7.59 (t, J = 7.6 Hz, 1H, CH), 7.62-7.70 (m, 2H, CH), 7.80 (d, J = 8.4 Hz, 1H, CH), 10.73 (s, 1H, OH); δ\textsubscript{C} (100 Hz, d-CDCl\textsubscript{3}) 26.2, 27.0, 34.5, 44.3, 49.1, 49.6, 53.4, 112.1, 112.6, 116.4, 117.2, 119.1, 127.2, 128.5, 129.2, 130.6, 132.6, 132.6, 133.0, 133.1, 133.8, 138.7, 147.9, 163.1, 165.5, 172.2; HRMS (DART) Calcd for \([C\textsubscript{37}H\textsubscript{34}F\textsubscript{8}N\textsubscript{2}O\textsubscript{8}S + H]\) 728.1854 found 728.1861; HPLC (III) \(t_R = 26.47\) min (84.6 %), (IV) \(t_R = 62.94\) min (88.1 %).
4.7u, 4-(2-(N-(3-cyanobenzyl)-2,3,4,5,6-pentafluorophenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)-2-hydroxybenzoic acid. Compound 4.6t was globally deprotected according to general procedure B on a 0.04 mmol scale to give compound 4.7u (22.6 mg, 78 %); \( \delta_H \) (400 MHz, \( d \)-CDCl\(_3\)) 1.30-1.46 (m, 5H, CH\(_2\)), 1.68-1.93 (m, 5H, CH\(_2\)), 2.40-2.55 (m, 1H, CH), 3.86 (s, 2H, CH\(_2\)), 4.69 (s, 4H, 2 CH\(_2\)), 6.37 (s (br), 1H, CH), 6.47 (s, 1H, CH), 6.96 (d, \( J = 7.6 \) Hz, 2H, CH), 7.12 (d, \( J = 7.6 \) Hz, 2H, CH), 7.44 (t, \( J = 7.2 \) Hz, 1H, CH), 7.50-7.66 (m, 3H, CH), 7.81 (s (br), 1H, CH), 10.82 (s, 1H, OH); \( \delta_C \) (100 Hz, \( d \)-CDCl\(_3\)) 26.2, 27.0, 34.5, 44.3, 48.2, 50.9, 53.2, 111.5, 113.2, 116.9, 118.3, 118.7, 127.3, 128.6, 129.2, 130.0, 131.9, 132.3, 132.7, 133.0, 133.1, 136.5, 148.2, 163.1, 165.6, 172.3; HRMS (DART) Calcd for [C\(_{36}\)H\(_{30}\)F\(_5\)N\(_3\)O\(_6\)S + H] 728.1854 found 728.1861; HPLC (III) \( t_R = 27.70 \) min (78.4 %), (IV) \( t_R = 62.18 \) min (82.7 %).

4.7v, 4-(2-(N-(4-cyanobenzyl)-2,3,4,5,6-pentafluorophenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)-2-hydroxybenzoic acid. Compound 4.6u was globally
deprotected according to general procedure B on a 0.04 mmol scale to give compound 4.7v (25.8 mg, 89 %); $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.30-1.46 (m, 5H, CH$_2$), 1.68-1.91 (m, 5H, CH$_2$), 2.36-2.54 (m, 1H, CH), 3.86 (s, 2H, CH$_2$), 4.68 (s, 2H, CH$_2$), 4.73 (s, 2H, CH$_2$), 6.33 (s (br), 1H, CH), 6.43 (s, 1H, CH), 6.96 (d, $J = 7.6$ Hz, 2H, CH), 7.11 (d, $J = 7.6$ Hz, 2H, CH), 7.39 (d, $J = 7.2$ Hz, 2H, CH), 7.60 (d, $J = 7.2$ Hz, 2H, CH), 7.79 (s (br), 1H, CH), (m, 3H, CH), 7.81 (s (br), 1H, CH), 10.86 (s, 1H, OH); $\delta_C$ (100 Hz, $d$-CDCl$_3$) 26.2, 27.0, 34.5, 44.3, 48.2, 51.2, 53.2, 112.5, 112.9, 116.9, 118.4, 118.7, 127.2, 128.6, 129.2, 132.5, 132.7, 132.8, 133.1, 140.3, 148.2, 163.1, 166.1, 172.2; HRMS (DART) Calcd for [C$_{36}$H$_{30}$F$_5$N$_3$O$_6$S + H] 728.1854 found 728.1851; HPLC (III) $t_R = 27.71$ min (70.5 %), (IV) $t_R = 62.25$ min (76.1 %).

4.7w, 4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(2,3,4,5-tetrafluorobenzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. Compound 4.6v was globally deprotected according to general procedure B on a 0.03 mmol scale to give compound 4.7w (18.3 mg, 79 %); $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.30-1.46 (m, 5H, CH$_2$), 1.69-1.90 (m, 5H, CH$_2$), 2.39-2.56 (m, 1H, CH), 3.93 (s, 2H, CH$_2$), 4.68 (s, 2H, CH$_2$), 4.72 (s, 2H, CH$_2$), 6.48 (s (br), 1H, CH), 6.59 (s, 1H, CH), 6.97 (d, $J = 7.6$ Hz, 2H, CH), 7.03-7.23 (m, 3H, CH), 7.85 (s (br), 1H, CH), 10.89 (s, 1H, OH); $\delta_C$ (100 Hz, $d$-CDCl$_3$) 26.3, 27.0, 34.5, 44.4, 44.5, 48.8, 53.3, 111.9, 112.1, 117.1, 118.8, 127.2, 128.5, 132.7, 133.1, 148.2, 163.2, 165.5, 172.5; HRMS (DART) Calcd for [C$_{35}$H$_{27}$F$_9$N$_3$O$_6$S + H] 775.1524 found 775.1529; HPLC (III) $t_R = 29.48$ min (90.6 %), (IV) $t_R = 65.49$ min (91.3 %).
4.7x, 4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(2,3,4,6-tetrafluorobenzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. Compound 4.6w was globally deprotected according to general procedure B on a 0.04 mmol scale to give compound 4.7x (25.4 mg, 82 %); $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.30-1.44 (m, 5H, CH$_2$), 1.70-1.92 (m, 5H, CH$_2$), 2.40-2.58 (m, 1H, CH), 3.93 (s, 2H, CH$_2$), 4.72 (s, 2H, CH$_2$), 4.77 (s, 2H, CH$_2$), 6.47 (s (br, 1H, OH), 6.60 (s, 1H, CH), 6.92-7.15 (m, 5H, CH), 7.84 (d, $J$ = 8.0 Hz, 1H, CH), 10.90 (s, 1H, OH); $\delta_C$ (100 Hz, $d$-CDCl$_3$) 26.3, 27.0, 34.5, 39.5, 44.4, 48.8, 53.2, 112.0, 112.8, 117.1, 118.9, 127.2, 128.5, 132.7, 133.2, 148.1, 163.2, 165.6, 172.3; HRMS (DART) Calcd for [C$_{35}$H$_{27}$F$_9$N$_2$O$_6$S + H] 775.1524 found 775.1536; HPLC (III) $t_R$ = 29.02 min (98.2 %), (IV) $t_R$ = 64.7 min (100 %).

4.7y, 4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(2,3,5,6-tetrafluorobenzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. Compound 4.6x was globally deprotected according to general procedure B on a 0.04 mmol scale to give...
compound 4.7y (24.6 mg, 79 %); δ_H (400 MHz, d-CDCl_3) 1.30-1.46 (m, 5H, CH_2), 1.68-1.91 (m, 5H, CH_2), 2.38-2.55 (m, 1H, CH), 3.96 (s, 2H, CH_2), 4.72 (s, 2H, CH_2), 4.84 (s, 2H, CH_2), 6.48 (s (br), 1H, CH), 6.60 (s, 1H, CH), 6.89-7.21 (m, 5H, CH), 7.83 (s (br), 1H, CH), 10.89 (s, 1H, OH); δ_C (100 Hz, d-CDCl_3) 26.3, 27.0, 34.5, 39.8, 44.4, 49.2, 53.2, 113.0, 114.4, 117.1, 118.9, 127.2, 128.5, 132.7, 133.2, 148.1, 163.2, 165.6, 172.4; HRMS (DART) Calcd for [C_{35}H_{27}F_{9}N_{2}O_{6}S + H] 775.1524 found 775.1522; HPLC (III) t_R = 28.74 min (92.2 %), (IV) t_R = 64.29 min (96.3 %).

\[
\text{4.7z, 4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-}
\text{((perfluorophenyl)methyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid.}
\]

Compound 4.6y was globally deprotected according to general procedure B on a 0.03 mmol scale to give compound 4.7z (19.2 mg, 81 %); δ_H (400 MHz, d-CDCl_3) 1.31-1.47 (m, 5H, CH_2), 1.70-1.91 (m, 5H, CH_2), 2.41-2.55 (m, 1H, CH), 3.98 (s, 2H, CH_2), 4.74 (s, 2H, CH_2), 4.81 (s, 2H, CH_2) 6.51 (d, J = 8.0 Hz, 1H, CH), 6.63 (s, 1H, CH), 6.97 (d, J = 7.6 Hz, 2H, CH), 7.12 (d, J = 7.6 Hz, 2H, CH), 7.88 (d, J = 8.0 Hz, 1H, CH), 10.68 (s, 1H, OH); δ_C (100 Hz, d-CDCl_3) 26.3, 27.0, 34.5, 39.6, 44.4, 49.2, 53.3, 109.2, 117.3, 119.0, 127.3, 128.5, 133.0, 147.2, 130.6, 148.2, 163.4, 166.6, 172.1; HRMS (DART) Calcd for [C_{35}H_{26}F_{10}N_{2}O_{6}S + H] 793.1430 found 793.1419; HPLC (III) t_R = 28.54 min (90.6 %), (IV) t_R = 66.34 min (99.2 %).
4.7aa, 4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(naphthalen-2-ylmethyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. Compound 4.6z was globally deprotected according to general procedure B on a 0.03 mmol scale to give compound 4.7aa (18.7 mg, 83 %); δH (400 MHz, d-CDCl₃) 1.31-1.47 (m, 5H, CH₂), 1.70-1.93 (m, 5H, CH₂), 2.40-2.56 (m, 1H, CH), 3.84 (s, 2H, CH₂), 4.67 (s, 2H, CH₂), 4.83 (s, 2H, CH₂) 6.06 (s (br), 1H, CH), 6.35 (s, 1H, CH), 6.97 (d, J = 7.6 Hz, 2H, CH), 7.11 (d, J = 7.6 Hz, 2H, CH), 7.39 (d, J = 7.6 Hz, 1H, CH), 7.45-7.57 (m, 3H, CH), 7.64 (s, 1H, CH), 7.72 (d, J = 7.2 Hz, 1H, CH), 7.76-7.86 (m, 2H, CH), 10.70 (s, 1H, OH); δC (100 Hz, d-CDCl₃) 26.3, 27.0, 34.5, 44.4, 47.9, 51.7, 53.1, 112.3, 116.9, 118.8, 126.2, 126.7, 126.7, 127.2, 127.9, 127.9, 128.3, 128.7, 128.9, 129.2, 131.7, 132.3, 133.3, 146.9, 148.1, 163.0, 166.1, 172.2; HRMS (DART) Calcd for [C₃⁹H₃₃F₅N₂O₆S + H] 753.2058 found 753.2047; HPLC (III) tR = 29.97 min (98.5 %), (IV) tR = 67.73 min (98.4 %).
17ab, 4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(3-(methoxycarbonyl)benzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid.

Compound 4.6aa was globally deprotected according to general procedure B on a 0.03 mmol scale to give compound 4.7ab (18.4 mg, 80 %); $\delta_H$ (400 MHz, d-CDCl$_3$) 1.30-1.47 (m, 5H, CH$_2$), 1.70-1.94 (m, 5H, CH$_2$), 2.42-2.56 (m, 1H, CH), 3.84 (s, 2H, CH$_2$), 3.92 (s, 3H, CH$_3$), 4.70 (s, 4H, 2 CH$_2$), 6.32 (s (br), 1H, CH), 6.47 (s, 1H, CH), 6.97 (d, $J = 8.0$ Hz, 2H, CH), 7.11 (d, $J = 7.6$ Hz, 2H, CH), 7.41 (t, $J = 7.6$ Hz, 1H, CH), 7.51 (d, $J = 7.6$ Hz, 1H, CH), 7.74 (d, $J = 8.0$ Hz, 1H, CH), 7.90 (s, 1H, CH), 7.99 (d, $J = 7.6$ Hz, 1H, CH), 10.81 (s, 1H, OH); $\delta_C$ (100 Hz, d-CDCl$_3$) 26.2, 26.9, 34.5, 44.3, 48.0, 51.1, 52.5, 53.2, 112.5, 116.9, 118.8, 126.8, 127.2, 128.5, 129.3, 129.9, 130.2, 132.4, 133.4, 133.8, 135.0, 148.0, 163.0, 165.8, 166.7, 171.9; HRMS (DART) Calcd for [C$_{37}$H$_{34}$F$_5$N$_2$O$_8$S + H] 761.1956 found 761.1960; HPLC (III) $t_R = 28.47$ min (84.1 %), (IV) $t_R = 63.50$ min (89.0 %).

![Chemical Structure of Compound 4.7ab]

4.7ac, 4-(N-(4-cyclohexylbenzyl)-2-(2,3,4,5,6-pentafluoro-N-(4-(methoxycarbonyl)benzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid.

Compound 4.6ab was globally deprotected according to general procedure B on a 0.03 mmol scale to give compound 4.7ac (18.7 mg, 82 %); $\delta_H$ (400 MHz, d-CDCl$_3$) 1.31-1.47 (m, 5H, CH$_2$), 1.70-1.92 (m, 5H, CH$_2$), 2.42-2.56 (m, 1H, CH), 3.85 (s, 2H, CH$_2$), 3.92 (s, 3H, CH$_3$), 4.68 (s, 2H, CH$_2$), 4.73 (s, 2H, CH$_2$), 6.29 (s (br), 1H, CH), 6.41 (s, 1H, CH), 6.95 (d, $J = 7.6$ Hz, 2H, CH), 7.12 (d, $J = 7.6$ Hz, 2H, CH), 7.33 (d, $J = 8.0$ Hz, 2H, CH), 7.75 (d, $J = 8.0$ Hz, 1H, CH), 7.98 (d, $J = 8.0$ Hz, 2H, CH), 10.70 (s, 1H, OH); $\delta_C$ (100 Hz, d-CDCl$_3$) 26.2, 27.0, 34.5, 44.3, 48.0, 51.2, 52.5, 53.2, 112.3, 117.2, 118.9, 127.2, 128.5, 128.7, 129.2, 129.7, 130.3, 130.4, 132.5,
139.7, 148.1, 163.1, 165.8, 166.8, 172.1; HRMS (DART) Calcd for [C_{37}H_{33}F_{5}N_{2}O_{8}S + H] 
761.1956 found 761.1945; HPLC (III) \( t_R = 28.54 \text{ min} \) (88.0 %), (IV) \( t_R = 63.72 \text{ min} \) (87.7 %).
Appendix 4: Chapter 5 Experimental

1 Experimental

1.1 Chemical Methods

Anhydrous solvents: methanol, chloroform, DMSO, CH$_2$Cl$_2$, THF and DMF were purchased from Sigma Aldrich and used directly from Sure-Seal bottles. Unless otherwise indicated, reactions were performed under an atmosphere of dry nitrogen and were monitored for completeness by thin-layer silica gel chromatography (visualized by UV light, or developed by treatment with KMnO$_4$ stain). All compounds were purified by silica gel column chromatography which was performed using a Biotage Isolera One automated flash chromatography system. Intermediate compounds were characterized using $^1$H and $^{13}$C NMR. Final molecules were characterized by $^1$H and $^{13}$C NMR and high resolution mass spectrometry. Before biological testing, inhibitor purity was evaluated by analytical reversed-phase HPLC (rpHPLC). Analysis by rpHPLC was performed using a Microsorb-MV 300 Å C18 250 mm x 4.6 mm analytical column run at 1 mL/min and gradient mixtures of H$_2$O with 0.1 % (v/v) TFA, and MeCN with 10 % (v/v) H$_2$O and 0.1 % (v/v) TFA. Conditions: (I) 100 % H$_2$O with 0.1 % TFA for two minutes to 100 % MeCN with 10 % H$_2$O and 0.1 % TFA (v/v) at 22 minutes and UV detection at 254nm or (II) 100 % H$_2$O with 0.1 % TFA for two minutes to 100 % MeCN with 10 % H$_2$O and 0.1 % TFA (v/v) at 62 mins and UV detection at 254nm. For reporting HPLC data, percentage purity is given in parentheses after the retention time for each condition.

1.2 General Procedures

**General Procedure A:** Secondary Sulfonamide Coupling

Secondary amine 5.1 (1.0 equiv.) was dissolved in anhydrous DCM with DIPEA (1.3 equiv.). Sulfonyl chloride (1.2 equiv.) was added and the reaction mixture was stirred at RT for 16 hours. Upon completion, solvent was removed under reduced pressure and the crude product was then purified using flash column chromatography using a mixture of hexanes and EtOAc as the eluent.

**General Procedure B:** Global deprotection of Benzylated Salicylic Acid
The dibenzyl protected salicylic acid (1 equiv.) was dissolved in a stirred solution of THF/MeOH (1:1) (0.1 M). The solution was degassed thoroughly before careful addition of 10 % Pd/C (10 mg/mmol). H₂ gas was bubbled through the solvent for 5 mins before the solution was put under an atmosphere of H₂ gas and stirred continuously for 6 hours. The H₂ gas was evacuated and the reaction filtered (to remove the Pd catalyst) and concentrated under reduced pressure. Crude product was then purified using flash column chromatography using a mixture of DCM, MeOH and AcOH as the eluent (generally 92 % DCM, 7 % MeOH and 1 % AcOH in a 1:2 ratio with DCM was utilized).

**General Procedure C: Primary Sulfonamide Coupling**

From primary amine 6 (1.0 equiv.) which was dissolved in anhydrous MeCN with K₂CO₃ (1.1 equiv.) and 4 Å molecular sieves and cooled to 0 °C. Sulfonyl chloride (1.0 equiv.) was added and the reaction mixture was allowed to warm to room temperature. After 6 hours the solution was filtered through cotton to remove molecular sieves and excess solvent was removed under reduced pressure. The concentrated mixture was diluted with DCM, then washed with 1M HCl, saturated NaHCO₃, H₂O and brine, then dried over Na₂SO₄. Crude product was then purified using flash column chromatography using a mixture of hexanes and EtOAc as the eluent.

**General Procedure D: Sulfonamide Alkylation**

Activated alkyl halides (1.1 equiv.) were added to a stirred suspension of sulfonamide 13a-d (1 equiv.) and Cs₂CO₃ (1.5 equiv.) in DMF. After 3 hours the mixture was diluted with H₂O then the product was extracted into EtOAc. Organics were combined and washed with 1 M HCl, saturated NaHCO₃, H₂O and brine, then dried over Na₂SO₄ and concentrated under reduced pressure. Crude products were purified using flash column chromatography using a mixture of hexanes and EtOAc as the eluent.

**General Procedure E: PPh₃Cl₂ Peptide Coupling**

Carboxylic acid (1 equiv.) was stirred at room temperature with PPh₃Cl₂ in CHCl₃ for 15 minutes to form the activated acid. Aniline (1 equiv.) was added and the mixture was heated to 110 °C for 30 minutes using a Biotage Initiator microwave reactor. Upon completion, CHCl₃ was
removed under reduced pressure and the crude product was purified using flash column chromatography using a mixture of hexanes and EtOAc as eluent.

1.3 Characterization of Intermediates

5.1, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(((perfluorophenyl)methyl)amino)acetamido)benzoate. To a stirred solution of primary amine 4.4 (900 mg, 1.6 mmol) and Cs₂CO₃ (630 mg, 1.9 mmol), was added 2,3,4,5,6-pentafluorobenzyl bromide (420 mg, 1.6 mmol). The mixture was stirred at RT for 1 h, then diluted with H₂O and organics were extracted into EtOAc. Combined organic layers were washed with saturated NaHCO₃, H₂O and brine and dried over Na₂SO₄. Solvents were removed under reduced pressure and the crude product was purified using flash column chromatography with a gradient mixture of hexanes and EtOAc to give 5.1 (1.03g, 87%); δ_H (400 MHz, d-CDCl₃) 1.15-1.50 (m, 5H, CH₂), 1.67-1.96 (m, 5H, CH₂), 2.18-2.40 (m, 1H, NH), 2.41-2.59 (m, 1H, CH), 3.08 (s, 3H, C₃), 3.81 (s, 2H, CH₂), 4.79 (s, 2H, CH₂), 4.93 (s, 2H, CH₂), 5.35 (s, 2H, CH₂), 6.47 (s, 1H, CH), 6.67 (d, J = 8.0 Hz, 1H, CH), 7.03 (d, J = 7.8 Hz, 2H, CH), 7.12 (d, J = 7.9 Hz, 2H, CH), 7.28-7.48 (m, 10H, CH) 7.85 (d, J = 8.2 Hz, 1H, CH); δ_C (100 MHz, d-CDCl₃)
5.2a, benzyl 2-(benzyl)oxy)-2-(4-(N-(4-cyclohexylbenzyl)-2-(N-((perfluorophenyl)methyl)phenylsulfonamido)acetamido)benzoate. Amine 5.1 was functionalized on a 0.03 mmol scale using General Procedure A to give 5.2a (18.2 mg, 74%); δ<sub>H</sub> (400 MHz, d<sub>-CDCl<sub>3</sub></d>) 1.29-1.48 (m, 5H, CH<sub>2</sub>), 1.68-1.92 (m, 5H, CH<sub>2</sub>), 2.40-2.56 (m, 1H, CH), 3.83 (s, 2H, CH<sub>2</sub>), 4.63 (s, 2H, CH<sub>2</sub>), 4.71 (s, 2H, CH<sub>2</sub>), 4.90 (s, 2H, CH<sub>2</sub>), 5.33 (s, 2H, CH<sub>2</sub>), 6.48 (s, 1H, CH), 6.66 (d, J = 8.2 Hz, 1H, CH), 6.97 (d, J = 7.6 Hz, 2H, CH), 7.10 (d, J = 8.0 Hz, 2H, CH), 7.27-7.42 (m, 10H, CH), 7.45-7.52 (m, 2H, CH), 7.54-7.61 (m, 1H, CH), 7.77 (d, J = 7.7 Hz, 2H, CH), 7.82 (d, J = 8.2 Hz, 1H, CH); δ<sub>C</sub> (100 MHz, d<sub>-CDCl<sub>3</sub></d>) 26.2, 27.0, 34.6, 39.9, 44.4, 49.5, 53.0, 67.2, 70.9, 113.8, 120.1, 121.5, 127.1, 127.2, 127.7, 128.2, 128.4, 128.5, 128.7, 128.8, 129.0, 129.1, 129.8, 133.1, 133.4, 133.9, 135.8, 135.8, 139.5, 148.0, 159.0, 165.5, 166.5.

5.2b, benzyl 2-(benzyl)oxy)-2-(4-(N-(4-cyclohexylbenzyl)-2-(N-((perfluorophenyl)methyl)methylsulfonamido)acetamido)benzoate. Amine 5.1 was functionalized on a 0.03 mmol scale using General Procedure A to give 5.2b (22.9 mg, 80.6%);
δ\textsubscript{H} (400 MHz, \textit{d}-CDCl\textsubscript{3}) 1.30-1.46 (m, 5H, CH\textsubscript{2}), 1.74-2.15 (m, 5H, CH\textsubscript{2}), 2.32-2.63 (m, 1H, CH), 3.17 (s, 3H, C\textsubscript{3}), 3.80 (s, 2H, CH\textsubscript{2}), 4.63 (s, 2H, CH\textsubscript{2}), 4.75 (s, 2H, CH\textsubscript{2}), 4.87 (s, 2H, CH\textsubscript{2}), 5.33 (s, 2H, CH\textsubscript{2}), 6.44 (s, 1H, CH), 6.63 (d, \textit{J} = 7.7 Hz, 1H, CH), 6.98 (d, \textit{J} = 7.6 Hz, 2H, CH), 7.11 (d, \textit{J} = 7.7 Hz, 2H, CH), 7.23-7.41 (m, 10H, CH) 7.80 (d, \textit{J} = 8.1 Hz, 1H, CH); δ\textsubscript{C} (100 MHz, \textit{d}-CDCl\textsubscript{3}) 26.2, 26.9, 34.6, 39.4, 40.7, 44.4, 49.7, 53.1, 67.2, 70.8, 113.8, 120.1, 121.5, 127.2, 127.3, 128.3, 128.4, 128.5, 128.7, 128.8, 129.0, 133.4, 133.7, 135.7, 135.8, 144.5, 148.2, 159.0, 165.4, 167.0.

5.2c, benzyl 2-(benzyl)oxy)-4-(N-(4-cyclohexylbenzyl)-2-(N-((perfluorophenyl)methyl)-4-(trifluoromethyl)phenylsulfonamido)acetamido)benzoate. Amine 5.1 was functionalized on a 0.03 mmol scale using General Procedure A to give 5.2c (20.7 mg, 64 %); δ\textsubscript{H} (400 MHz, \textit{d}-CDCl\textsubscript{3}) 1.32-1.43 (m, 5H, CH\textsubscript{2}), 1.69-1.91 (m, 5H, CH\textsubscript{2}), 2.43-2.53 (m, 1H, CH), 3.86 (s, 2H, CH\textsubscript{2}), 4.62 (s, 2H, CH\textsubscript{2}), 4.70 (s, 2H, CH\textsubscript{2}), 4.91 (s, 2H, CH\textsubscript{2}), 5.34 (s, 2H, CH\textsubscript{2}), 6.47 (s, 1H, CH), 6.65 (d, \textit{J} = 8.0 Hz, 1H, CH), 6.95 (d, \textit{J} = 8.0 Hz, 2H, CH), 7.12 (d, \textit{J} = 8.0 Hz, 2H, CH), 7.27-7.41 (m, 10H, CH), 7.74 (d, \textit{J} = 8.2 Hz, 2H, CH), 7.83 (d, \textit{J} = 8.0 Hz, 1H, CH), 7.92 (d, \textit{J} = 8.2 Hz, 2H, CH); δ\textsubscript{C} (100 MHz, \textit{d}-CDCl\textsubscript{3}) 26.2, 27.0, 34.6, 40.0, 44.4, 49.6, 53.1, 67.3, 70.9, 114.0, 120.2, 121.4, 126.1, 126.1, 127.2, 127.2, 128.3, 128.4, 128.5, 128.5, 128.7, 128.8, 129.1, 129.1, 133.4, 133.8, 135.8, 135.8, 143.0, 148.2, 159.0, 165.5, 166.2.
5.2d, benzyl 2-(benzyloxy)-4-(2-(4-(tert-butyl)-N-((perfluorophenyl)methyl)phenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)benzoate. Amine 5.1 was functionalized on a 0.03 mmol scale using General Procedure A to give 5.2d (26.9 mg, 83%); $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.34-1.44 (m, 5H, CH$_2$), 1.69-1.92 (m, 5H, CH$_2$), 2.39-2.54 (m, 1H, CH), 3.86 (s, 2H, CH$_2$), 4.62 (s, 2H, CH$_2$), 4.74 (s, 2H, CH$_2$), 4.91 (s, 2H, CH$_2$), 5.34 (s, 2H, CH$_2$), 6.52 (s, 1H, CH), 6.68 (d, $J = 8.1$ Hz, 1H, CH), 7.00 (d, $J = 8.0$ Hz, 1H, CH), 7.11 (d, $J = 8.0$ Hz, 2H, CH), 7.26-7.41 (m, 10H, CH), 7.45 (d, $J = 8.5$ Hz, 2H, CH), 7.65 (d, $J = 7.9$ Hz, 2H, CH), 7.83 (d, $J = 8.2$ Hz, 1H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 26.2, 27.0, 31.2, 34.6, 35.3, 40.0, 44.4, 49.8, 53.0, 67.2, 70.9, 107.31, 114.1, 120.3, 121.1, 125.9, 127.1, 127.3, 127.5, 128.2, 128.4, 128.5, 128.7, 128.7, 129.1, 130.4, 133.3, 134.0, 135.8, 148.0, 156.9, 159.0, 165.5, 166.8.

5.2e, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(3-methyl-N-((perfluorophenyl)methyl)phenylsulfonamido)acetamido)benzoate. Amine 5.1 was
functionalized on a 0.03 mmol scale using General Procedure A to give 5.2e (21.4 mg, 68 %); δ_H (400 MHz, d-CDCl₃) 1.31-1.47 (m, 5H, CH₂), 1.70-1.90 (m, 5H, CH₂), 2.39 (s, 3H, CH₃), 2.41-2.54 (m, 1H, CH), 3.84 (s, 2H, CH₂), 4.66 (s, 2H, CH₂), 4.75 (s, 2H, CH₂), 4.92 (s, 2H, CH₂), 5.35 (s, 2H, CH₂), 6.52 (s, 1H, CH), 6.68 (d, J = 8.2 Hz, 1H, CH), 7.00 (d, J = 8.0 Hz, 2H, CH), 7.12 (d, J = 8.0 Hz, 2H, CH), 7.27-7.42 (m, 12H, CH), 7.55 (d, J = 7.6 Hz, 1H, CH), 7.57 (s, 1H, CH), 7.84 (d, J = 8.2 Hz, 1H, CH); δ_C (100 MHz, d-CDCl₃) 21.4, 26.2, 27.0, 34.6, 40.0, 44.4, 49.6, 53.0, 67.2, 70.9, 110.3, 114.1, 120.3, 124.7, 127.1, 127.3, 128.0, 128.2, 128.4, 128.5, 128.7, 128.8, 129.1, 133.3, 133.8, 133.9, 135.8, 135.8, 139.1, 139.4, 148.0, 159.0, 165.5, 166.6.

5.2f, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(2-methyl-N-((perfluorophenyl)methyl)phenylsulfonamido)acetamido)benzoate. Amine 5.1 was functionalized on a 0.03 mmol scale using General Procedure A to give 5.2f (20.4 mg, 66 %); δ_H (400 MHz, d-CDCl₃) 1.30-1.48 (m, 5H, CH₂), 1.71-1.92 (m, 5H, CH₂), 2.41-2.52 (m, 1H, CH), 2.52 (s, 3H, CH₃), 3.83 (s, 2H, CH₂), 4.71 (s, 2H, CH₂), 4.76 (s, 2H, CH₂), 4.87 (s, 2H, CH₂), 5.35 (s, 2H, CH₂), 6.45 (s, 1H, CH), 6.62 (d, J = 8.1 Hz, 1H, CH), 7.98 (d, J = 8.0 Hz, 2H, CH), 7.11 (d, J = 8.0 Hz, 2H, CH), 7.24-7.41 (m, 12H, CH), 7.45 (t, J = 6.8 Hz, 1H, CH), 7.82 (d, J = 8.1 Hz, 1H, CH), 7.96 (d, J = 7.8 Hz, 1H, CH); δ_C (100 MHz, d-CDCl₃) 20.4, 26.2, 27.0, 34.6, 39.5, 44.4, 48.9, 53.0, 67.2, 70.9, 114.0, 120.2, 121.3, 125.9, 127.1, 127.2, 128.2, 128.4, 128.5, 128.7, 128.8, 129.2, 129.3, 130.3, 132.7, 133.2, 133.4, 133.9, 135.8, 135.8, 137.5, 138.4, 148.0, 159.0, 165.5, 166.4.
5.2g, benzyl 2-(benzoyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(N-((perfluorophenyl)methyl)-3-(trifluoromethyl)phenylsulfonamido)acetamido)benzoate. Amine 5.1 was functionalized on a 0.03 mmol scale using General Procedure A to give 5.2g (19.8 mg, 52 %); δH (400 MHz, d-CDCl₃) 1.32-1.48 (m, 5H, CH₂), 1.70-1.95 (m, 5H, CH₂), 2.36-2.61 (m, 1H, CH), 3.87 (s, 2H, CH₂), 4.64 (s, 2H, CH₂), 4.71 (s, 2H, CH₂), 4.92 (s, 2H, CH₂), 5.36 (s, 2H, CH₂), 6.48 (s, 1H, CH), 6.67 (d, J = 8.1 Hz, 1H, CH), 6.98 (d, J = 7.7 Hz, 2H, CH), 7.12 (d, J = 7.7 Hz, 2H, CH), 7.30-7.48 (m, 10H, CH), 7.66 (t, 1H, CH), 7.86 (t, 2H, CH), 7.92 (d, J = 8.0 Hz, 1H, CH), 8.09 (s, 1H, CH); δC (100 MHz, d-CDCl₃) 26.2, 27.0, 34.6, 39.9, 44.4, 49.6, 53.0, 67.3, 70.9, 114.0, 120.2, 121.5, 124.9, 127.2, 127.3, 128.3, 128.4, 128.5, 128.7, 128.8, 129.1, 129.8, 131.1, 133.4, 133.7, 135.7, 135.8, 140.7, 144.5, 148.1, 159.0, 165.5, 166.1.

5.2h, benzyl 2-(benzoyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(4-isopropyl-N-((perfluorophenyl)methyl)phenylsulfonamido)acetamido)benzoate. Amine 5.1 was functionalized on a 0.03 mmol scale using General Procedure A to give 5.2h (29.8 mg, 94 %); δH
(400 MHz, $d$-CDCl$_3$) 1.27 (d, $J$ = 7.6 Hz, 6H, CH$_3$), 1.32-1.49 (m, 5H, CH$_2$), 1.70-1.92 (m, 5H, CH$_2$), 2.42-2.55 (m, 1H, CH), 2.98 (m, 1H, CH), 3.88 (s, 2H, CH$_2$), 4.65 (s, 2H, CH$_2$), 4.76 (s, 2H, CH$_2$), 4.94 (s, 2H, CH$_2$), 5.37 (s, 2H, CH$_2$), 6.54 (s, 1H, CH), 6.70 (d, $J$ = 8.0 Hz, 1H, CH), 7.03 (d, $J$ = 8.0 Hz, 2H, CH), 7.14 (d, $J$ = 8.0 Hz, 2H, CH), 7.28-7.43 (m, 12H, CH), 7.69 (d, $J$ = 8.0 Hz, 2H, CH), 7.86 (d, $J$ = 8.0 Hz, 1H, CH); $\delta$C (100 MHz, $d$-CDCl$_3$) 23.7, 26.2, 26.9, 34.3, 34.6, 40.0, 44.4, 49.7, 53.0, 67.2, 70.8, 110.3, 114.1, 120.3, 127.0, 127.1, 127.2, 127.8, 128.2, 128.4, 128.7, 128.7, 129.1, 133.3, 133.9, 135.8, 136.9, 147.9, 154.6, 159.0, 165.5, 166.8.

5.2i, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(4-ethyl-N-((perfluorophenyl)methyl)phenylsulfonamido)acetamido)benzoate. Amine 5.1 was functionalized on a 0.03 mmol scale using General Procedure A to give 5.2i (25.9 mg, 94 %); $\delta$H (400 MHz, $d$-CDCl$_3$) 127.9 (t, $J$ = 7.8 Hz, 3H, CH$_3$), 1.32-1.52 (m, 5H, CH$_2$), 1.68-1.93 (m, 5H, CH$_2$), 2.38-2.57 (m, 1H, CH), 2.73 (q, $J$ = 7.8 Hz, 2H, CH$_2$), 3.86 (s, 2H, CH$_2$), 4.64 (s, 2H, CH$_2$), 4.75 (s, 2H, CH$_2$), 4.93 (s, 2H, CH$_2$), 5.36 (s, 2H, CH$_2$), 6.53 (s, 1H, CH), 6.69 (d, $J$ = 8.1 Hz, 1H, CH), 7.01 (d, $J$ = 7.3 Hz, 2H, CH), 7.13 (d, $J$ = 7.3 Hz, 2H, CH), 7.31-7.45 (m, 10H, CH), 7.69 (d, $J$ = 7.5 Hz, 2H, CH), 7.85 (d, $J$ = 8.1 Hz, 1H, CH); $\delta$C (100 MHz, $d$-CDCl$_3$) 15.2, 26.2, 27.0, 29.0, 34.6, 40.0, 44.4, 49.6, 53.0, 67.2, 70.9, 114.1, 120.3, 121.2, 127.1, 127.3, 127.8, 128.2, 128.4, 128.7, 128.7, 129.1, 133.3, 133.9, 135.8, 136.7, 147.9, 150.0, 159.0, 165.5, 166.7.
5.2j, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(2,4,6-trimethyl-N-((perfluorophenyl)methyl)phenylsulfonamido)acetamido)benzoate. Amine 5.1 was functionalized on a 0.03 mmol scale using General Procedure A to give 5.2j (19.3 mg, 66 %); $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.31-1.48 (m, 5H, CH$_2$), 1.68-1.94 (m, 5H, CH$_2$), 2.29 (s, 3H, CH$_3$), 2.41-2.51 (m, 1H, CH), 2.53 (s, 6H, CH$_3$), 3.79 (s, 2H, CH$_2$), 4.68 (s, 2H, CH$_2$), 4.75 (s, 2H, CH$_2$), 4.87 (s, 2H, CH$_2$), 5.35 (s, 2H, CH$_2$), 6.46 (s, 1H, CH), 6.57 (d, $J = 8.0$ Hz, 1H, CH), 6.90 (s, 2H, CH), 6.98 (d, $J = 7.8$ Hz, 2H, CH), 7.11 (d, $J = 7.8$ Hz, 2H, CH), 7.27-7.44 (m, 10H, CH), 7.81 (d, $J = 8.1$ Hz, 1H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 21.0, 22.9, 26.2, 27.0, 34.6, 39.1, 44.4, 48.7, 53.0, 67.2, 70.8, 113.9, 120.2, 121.1, 127.1, 127.3, 128.2, 128.4, 128.5, 128.7, 128.7, 129.1, 131.9, 132.5, 133.2, 133.3, 134.0, 135.8, 135.8, 140.9, 143.1, 147.9, 159.0, 165.5, 166.5.

5.2k, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(N-((perfluorophenyl)methyl)-2-(trifluoromethyl)phenylsulfonamido)acetamido)benzoate. Amine 5.1 was functionalized on a 0.04 mmol scale using General Procedure A to give 5.2k (20.3 mg, 52 %); $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.31-1.48 (m, 5H, CH$_2$), 1.68-1.94 (m, 5H, CH$_2$), 2.29 (s, 3H, CH$_3$), 2.41-2.51 (m, 1H, CH), 2.53 (s, 6H, CH$_3$), 3.79 (s, 2H, CH$_2$), 4.68 (s, 2H, CH$_2$), 4.75 (s, 2H, CH$_2$), 4.87 (s, 2H, CH$_2$), 5.35 (s, 2H, CH$_2$), 6.46 (s, 1H, CH), 6.57 (d, $J = 8.0$ Hz, 1H, CH), 6.90 (s, 2H, CH), 6.98 (d, $J = 7.8$ Hz, 2H, CH), 7.11 (d, $J = 7.8$ Hz, 2H, CH), 7.27-7.44 (m, 10H, CH), 7.81 (d, $J = 8.1$ Hz, 1H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 21.0, 22.9, 26.2, 27.0, 34.6, 39.1, 44.4, 48.7, 53.0, 67.2, 70.8, 113.9, 120.2, 121.1, 127.1, 127.3, 128.2, 128.4, 128.5, 128.7, 128.7, 129.1, 131.9, 132.5, 133.2, 133.3, 134.0, 135.8, 135.8, 140.9, 143.1, 147.9, 159.0, 165.5, 166.5.
CDCl$_3$): 1.32-1.48 (m, 5H, CH$_2$), 1.70-1.93 (m, 5H, CH$_2$), 2.38-2.59 (m, 1H, CH), 3.86 (s, 2H, CH$_2$), 4.71 (s, 2H, CH$_2$), 4.74 (s, 2H, CH$_2$), 4.87 (s, 2H, CH$_2$), 5.34 (s, 2H, CH$_2$), 6.46 (s, 1H, CH), 6.64 (d, $J = 8.1$ Hz, 1H, CH), 6.97 (d, $J = 7.6$ Hz, 2H, CH), 7.11 (d, $J = 7.4$ Hz, 2H, CH), 7.25-7.44 (m, 10H, CH), 7.65-7.76 (m, 2H, CH), 7.82 (d, $J = 7.9$ Hz, 1H, CH), 7.86-7.94 (m, 1H, CH), 8.23-8.36 (m, 1H, CH); $\delta$C (100 MHz, $d$-CDCl$_3$) 26.1, 26.8, 34.5, 39.5, 44.3, 49.1, 53.0, 67.1, 70.8, 109.4, 113.9, 120.0, 121.2, 127.0, 127.1, 128.1, 128.2, 128.3, 128.6, 128.6, 129.0, 132.0, 132.1, 132.7, 133.2, 133.2, 133.7, 135.7, 135.8, 138.9, 144.5, 146.9, 147.8, 158.9, 165.4, 166.1;

![Structure](image)

**5.2l, benzyl 2-(benzyloxy)-4-(2-(4-chloro-N-((perfluorophenyl)methyl)phenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)benzoate.** Amine **5.1** was functionalized on a 0.03 mmol scale using General Procedure A to give **5.2l** (24.7 mg, 84 %); $\delta$H (400 MHz, $d$-CDCl$_3$) 1.32-1.48 (m, 5H, CH$_2$), 1.70-1.92 (m, 5H, CH$_2$), 2.41-2.57 (m, 1H, CH), 3.85 (s, 2H, CH$_2$), 4.62 (s, 2H, CH$_2$), 4.71 (s, 2H, CH$_2$), 4.92 (s, 2H, CH$_2$), 5.36 (s, 2H, CH$_2$), 6.48 (s, 1H, CH), 6.66 (d, $J = 8.1$ Hz, 1H, CH), 6.97 (d, $J = 7.8$ Hz, 1H, CH), 7.13 (d, $J = 8.0$ Hz, 2H, CH), 7.29-7.41 (m, 10H, CH), 7.45 (d, $J = 8.4$ Hz, 2H, CH), 7.74 (d, $J = 8.5$ Hz, 2H, CH), 7.84 (d, $J = 8.1$ Hz, 1H, CH); $\delta$C (100 MHz, $d$-CDCl$_3$) 26.2, 26.9, 34.6, 39.9, 44.4, 49.4, 53.1, 67.3, 70.9, 114.0, 120.2, 121.4, 127.2, 127.2, 128.2, 128.4, 128.5, 128.7, 128.8, 129.1, 129.3, 129.3, 133.4, 133.8, 135.8, 135.8, 137.9, 139.6, 144.7, 148.1, 159.0, 165.5, 166.3;
5.2m, benzyl 2-(benzyloxy)-4-(2-(4-cyano-N-((perfluorophenyl)methyl)phenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)benzoate. Amine 5.1 was functionalized on a 0.03 mmol scale using General Procedure A to give 5.2m (14.9 mg, 58 %); δ_H (400 MHz, d-CDCl_3) 1.36-1.45 (m, 5H, CH_2), 1.70-1.91 (m, 5H, CH_2), 2.40-2.56 (m, 1H, CH), 3.86 (s, 2H, CH_2), 4.61 (s, 2H, CH_2), 4.69 (s, 2H, CH_2), 4.92 (s, 2H, CH_2), 5.31 (s, 2H, CH_2), 6.47 (s, 1H, CH), 6.66 (d, J = 7.4 Hz, 1H, CH), 7.95 (d, J = 8.0 Hz, 1H, CH), 7.14 (d, J = 8.0 Hz, 2H, CH), 7.28-7.43 (m, 10H, CH), 7.76 (d, J = 8.2 Hz, 2H, CH), 7.84 (d, J = 7.4 Hz, 1H, CH), 7.92 (d, J = 8.2 Hz, 2H, CH); δ_C (100 MHz, d-CDCl_3) 25.6, 26.7, 34.5, 39.7, 44.2, 49.3, 50.8, 67.2, 70.8, 113.8, 116.6, 117.4, 120.0, 121.4, 127.0, 128.2, 128.3, 128.6, 128.7, 129.0, 132.6, 133.3, 133.5, 135.6, 135.6, 143.4, 144.3, 148.1, 158.9, 165.3, 165.8, 171.1;

5.2n, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(4-fluoro-N-((perfluorophenyl)methyl)phenylsulfonamido)acetamido)benzoate. Amine 5.1 was functionalized on a 0.03 mmol scale using General Procedure A to give 5.2n (30.2 mg, 100 %);
δ_H (400 MHz, d-CDCl_3) 1.36-1.52 (m, 5H, CH_2), 1.69-1.93 (m, 5H, CH_2), 2.40-2.60 (m, 1H, CH), 3.86 (s, 2H, CH_2), 4.62 (s, 2H, CH_2), 4.72 (s, 2H, CH_2), 4.93 (s, 2H, CH_2), 5.36 (s, 2H, CH_2), 6.49 (s, 1H, CH), 6.67 (d, _J_ = 7.8 Hz, 1H, CH), 6.97 (d, _J_ = 7.92 Hz, 1H, CH), 7.09-7.21 (m, 4H, CH), 7.30-7.48 (m, 9H, CH), 7.79-7.91 (m, 3H, CH); δ_C (100 MHz, d-CDCl_3) 26.2, 27.0, 34.6, 39.8, 44.4, 49.4, 53.1, 67.3, 70.9, 114.0, 116.1, 116.3, 120.2, 127.2, 127.2, 128.3, 128.4, 128.5, 128.7, 128.8, 129.1, 130.6, 130.7, 133.4, 133.8, 135.5, 135.8, 135.8, 148.1, 159.0, 165.5, 166.4.

5.2o, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(4-methoxy-N-((perfluorophenyl)methyl)phenylsulfonamido)acetamido)benzoate. Amine 5.1 was functionalized on a 0.03 mmol scale using General Procedure A to give 5.2o (27.8 mg, 97%); δ_H (400 MHz, d-CDCl_3) 1.35-1.49 (m, 5H, CH_2), 1.70-1.94 (m, 5H, CH_2), 2.40-2.57 (m, 1H, CH), 3.85 (s, 2H, CH_2), 3.88 (s, 3H, CH_3), 4.62 (s, 2H, CH_2), 4.74 (s, 2H, CH_2), 4.92 (s, 2H, CH_2), 5.36 (s, 2H, CH_2), 6.51 (s, 1H, CH), 6.68 (d, _J_ = 8.2 Hz, 1H, CH), 6.95 (d, _J_ = 8.8 Hz, 2H, CH), 6.99 (d, _J_ = 7.9 Hz, 2H, CH), 7.13 (d, _J_ = 7.8 Hz, 2H, CH), 7.29-7.44 (m, 10H, CH), 7.74 (d, _J_ = 8.8 Hz, 2H, CH), 7.84 (d, _J_ = 8.2 Hz, 1H, CH); δ_C (100 MHz, d-CDCl_3) 26.2, 26.9, 29.8, 34.6, 39.9, 44.4, 49.5, 53.0, 55.7, 67.2, 70.6, 114.1, 120.3, 121.2, 127.1, 127.3, 128.2, 128.4, 128.4, 128.7, 128.7, 129.1, 130.0, 130.9, 133.3, 133.9, 133.9, 135.8, 135.8, 144.9, 148.0, 159.0, 163.3, 165.5, 166.6.
**5.2p, benzyl 2-(benzylxy)-4-(N-(4-cyclohexylbenzyl)-2-(4-nitro-N-((perfluorophenyl)methyl)phenylsulfonamido)acetamido)benzoate.** Amine 5.1 was functionalized on a 0.04 mmol scale using General Procedure A to give 5.2p (24.8 mg, 75 %); $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.33-1.47 (m, 5H, CH$_2$), 1.71-1.92 (m, 5H, CH$_2$), 2.45-2.57 (m, 1H, CH), 3.88 (s, 2H, CH$_2$), 4.63 (s, 2H, CH$_3$), 4.68 (s, 2H, CH$_2$), 4.93 (s, 2H, CH$_2$), 5.36 (s, 2H, CH$_2$), 6.47 (s, 1H, CH), 6.66 (d, $J = 8.0$ Hz, 1H, CH), 6.94 (d, $J = 8.0$ Hz, 2H, CH), 7.14 (d, $J = 7.9$ Hz, 2H, CH), 7.30-7.46 (m, 10H, CH), 7.85 (d, $J = 8.2$ Hz, 1H, CH), 8.00 (d, $J = 8.7$ Hz, 2H, CH), 8.34 (d, $J = 8.8$ Hz, 2H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 26.2, 27.0, 34.6, 39.9, 44.4, 49.4, 53.2, 67.3, 70.9, 113.9, 120.1, 121.7, 124.2, 127.2, 128.3, 128.5, 128.7, 128.8, 129.1, 129.2, 133.5, 133.6, 133.9, 135.7, 135.8, 144.4, 145.1, 148.3, 150.3, 159.1, 165.4, 165.9.

**5.2q, benzyl 2-(benzylxy)-4-(N-(4-cyclohexylbenzyl)-2-(N-((perfluorophenyl)methyl)-[1,1'-biphenyl]-4-ylsulfonamido)acetamido)benzoate.** Amine 5.1 was functionalized on a 0.03 mmol scale using General Procedure A to give 5.2q (24.8 mg, 85 %); $\delta_H$ (400 MHz, $d$-CDCl$_3$)
1.32-1.48 (m, 5H, CH₂), 1.71-1.89 (m, 5H, CH₂), 2.40-2.52 (m, 1H, CH), 3.89 (s, 2H, CH₂), 4.71 (s, 2H, CH₂), 4.74 (s, 2H, CH₂), 4.94 (s, 2H, CH₂), 5.36 (s, 2H, CH₂), 6.52 (s, 1H, CH), 6.69 (d, J = 8.2 Hz, 1H, CH), 7.98 (d, J = 8.0 Hz, 2H, CH), 7.07 (d, J = 8.0 Hz, 2H, CH), 7.27-7.43 (m, 12H, CH), 7.45 (d, J = 7.2 Hz, 1H, CH), 7.51 (t, J = 7.6 Hz, 2H, CH), 7.63 (d, J = 7.2 Hz, 2H, CH), 7.69 (d, J = 8.4 Hz, 2H, CH), 7.84-7.89 (m, 3H, CH); \( \delta \)C (100 MHz, d-CDCl₃) 26.2, 27.0, 34.6, 40.0, 44.3, 49.4, 53.1, 67.2, 70.9, 110.3, 114.1, 120.3, 121.2, 127.1, 127.2, 127.5, 127.6, 128.2, 128.3, 128.4, 128.5, 128.6, 128.7, 128.8, 129.1, 129.2, 133.4, 133.9, 135.8, 135.8, 138.0, 139.5, 146.0, 148.0, 159.0, 165.5, 166.6;

5.2r, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(3,5-dimethyl-N-((perfluorophenyl)methyl)phenylsulfonamido)acetamido)benzoate. Amine 5.1 was functionalized on a 0.03 mmol scale using General Procedure A to give 5.2r (24.5 mg, 87 %); \( \delta \)H (400 MHz, d-CDCl₃) 1.44-1.57 (m, 5H, CH₂), 1.68-1.91 (m, 5H, CH₂), 2.34 (s, 6H, CH₃), 2.42-2.56 (m, 1H, CH), 3.82 (s, 2H, CH₂), 4.67 (s, 2H, CH₂), 4.78 (s, 2H, CH₂), 4.93 (s, 2H, CH₂), 5.36 (s, 2H, CH₂), 6.56 (s, 1H, CH), 6.70 (d, J = 8.0 Hz, 1H, CH), 7.02 (d, J = 7.8 Hz, 2H, CH), 7.13 (d, J = 7.8 Hz, 2H, CH), 7.17 (s, 1H, CH), 7.24-7.46 (m, 12H, CH), 7.84 (d, J = 8.0 Hz, 1H, CH); \( \delta \)C (100 MHz, d-CDCl₃) 21.1, 26.1, 26.8, 34.6, 39.9, 44.2, 49.5, 52.8, 67.1, 70.8, 114.1, 120.2, 121.0, 124.9, 127.0, 127.2, 128.1, 128.3, 128.6, 128.6, 128.9, 129.0, 133.2, 133.9, 134.1, 135.8, 135.8, 138.8, 139.1, 147.8, 158.9, 165.4, 166.7.
5.2s, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(N-((perfluorophenyl)methyl)-2,3-dihydrobenzo[b][1,4]dioxine-6-sulfonamido)acetamido)benzoate. Amine 5.1 was functionalized on a 0.04 mmol scale using General Procedure A to give 5.2s (39.0 mg, 98 %); \( \delta_H \) (400 MHz, \( d-\text{CDCl}_3 \)) 1.34-1.47 (m, 5H, CH\(_2\)), 1.69-1.92 (m, 5H, CH\(_2\)), 2.42-2.56 (m, 1H, CH), 3.82 (s, 2H, CH\(_2\)), , 4.72 (s, 2H, CH\(_2\)), 4.79 (s, 2H, CH\(_2\)), 4.87 (s, 2H, CH\(_2\)), 5.35 (s, 2H, CH\(_2\)), 6.52 (s, 1H, CH), 6.60 (d, \( J = 8.1 \) Hz, 1H, CH), 7.02 (d, \( J = 7.4 \) Hz, 2H, CH), 7.10 (d, \( J = 7.4 \) Hz, 2H, CH), 7.15 (s, 2H, CH), 7.26-7.42 (m, 10H, CH), 7.80 (d, \( J = 8.0 \) Hz, 1H, CH); \( \delta_C \) (100 MHz, \( d-\text{CDCl}_3 \)) 11.0, 14.0, 23.5, 24.8, 26.1, 26.8, 29.7, 34.5, 44.2, 47.7, 52.9, 67.1, 68.2, 70.7, 113.9, 120.2, 120.8, 123.8, 126.9, 127.2, 128.0, 128.2, 128.3, 128.6, 128.8, 129.1, 129.4, 130.8, 133.2, 133.9, 135.7, 135.7, 147.7, 151.8, 153.6, 158.9, 165.4, 166.0.

5.2t, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(N-((perfluorophenyl)methyl)naphthalene-2-sulfonamido)acetamido)benzoate. Amine 5.1 was functionalized on a 0.03 mmol scale using General Procedure A to give 5.2t (28.0 mg, 97 %); \( \delta_H \)
(400 MHz, $d$-CDCl$_3$) 1.34-1.46 (m, 5H, CH$_2$), 1.72-1.89 (m, 5H, CH$_2$), 2.41-2.54 (m, 1H, CH), 3.89 (s, 2H, CH$_2$), 4.72 (s, 4H, 2 CH$_2$), 4.92 (s, 2H, CH$_2$), 5.36 (s, 2H, CH$_2$), 6.52 (s, 1H, CH), 6.68 (d, $J = 8.0$ Hz, 1H, CH), 6.95 (d, $J = 7.8$ Hz, 2H, CH), 7.07 (d, $J = 7.8$ Hz, 2H, CH), 7.27-7.43 (m, 10H, CH), 7.57-7.76 (m, 4H, CH), 7.85 (d, $J = 8.0$ Hz, 1H, CH), 7.89-7.95 (m, 3H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 26.1, 26.8, 34.5, 39.9, 44.2, 49.4, 52.8, 67.1, 70.7, 114.0, 120.1, 121.1, 122.7, 127.0, 127.2, 127.5, 127.9, 128.1, 128.3, 128.3, 128.6, 128.6, 128.9, 129.0, 129.2, 129.2, 129.7, 130.9, 131.9, 133.2, 133.7, 134.9, 135.6, 135.7, 136.2, 144.7, 158.9, 165.4, 166.5.

5.2u, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(N-((perfluorophenyl)methyl)naphthalene-1-sulfonamido)acetamido)benzoate. Amine 5.1 was functionalized on a 0.03 mmol scale using General Procedure A to give 5.2u (15.1 mg, 48 %); $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.30-1.43 (m, 5H, CH$_2$), 1.67-1.86 (m, 5H, CH$_2$), 2.40-2.51 (m, 1H, CH), 4.00 (s, 2H, CH$_2$), 4.71 (s, 4H, 2 CH$_2$), 4.74 (s, 2H, CH$_2$), 5.35 (s, 2H, CH$_2$), 6.52 (s, 1H, CH), 6.68 (d, $J = 8.2$ Hz, 1H, CH), 6.98 (d, $J = 8.0$ Hz, 2H, CH), 7.09 (d, $J = 8.0$ Hz, 2H, CH), 7.27-7.41 (m, 10H, CH), 7.44-7.56 (m, 3H, CH), 7.81-7.88 (m, 2H, CH), 7.99 (d, $J = 8.2$ Hz, 1H, CH), 8.25-8.35 (m, 2H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 26.1, 26.8, 34.5, 39.9, 44.2, 49.6, 52.9, 67.1, 70.7, 114.0, 120.2, 121.0, 123.9, 124.4, 126.8, 127.0, 127.1, 128.1, 128.2, 128.3, 128.4, 128.6, 128.6, 128.7, 129.1, 130.6, 133.2, 133.8, 134.0, 134.2, 134.6, 135.6, 135.7, 144.8, 147.8, 158.9, 165.5, 166.6.
5.2v, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(5-(dimethylamino)-N-((perfluorophenyl)methyl)naphthalene-1-sulfonamido)acetamido)benzoate. Amine 5.1 was functionalized on a 0.03 mmol scale using General Procedure A to give 5.2v (23.4 mg, 76 %); δ_H (400 MHz, d-CDCl₃) 1.28-1.43 (m, 5H, CH₂), 1.67-1.86 (m, 5H, CH₂), 2.39-2.51 (m, 1H, CH), 2.83 (s, 6H, 2 CH₃), 4.03 (s, 2H, CH₂), 4.71 (s, 4H, 2 CH₂), 4.77 (s, 2H, CH₂), 4.89 (s, 2H, CH₂), 5.35 (s, 2H, CH₂), 6.56 (s, 1H, CH), 6.70 (d, J = 8.2 Hz, 1H, CH), 7.01 (d, J = 8.0 Hz, 2H, CH), 7.06-7.12 (m, 3H, CH), 7.22-7.47 (m, 12H, CH), 7.84 (d, J = 8.2 Hz, 1H, CH), 7.90 (d, J = 8.6 Hz, 1H, CH), 8.26 (d, J = 7.2 Hz, 1H, CH), 8.46 (d, J = 8.5 Hz, 1H, CH); δ_C (100 MHz, d-CDCl₃) 26.1, 26.8, 34.5, 39.9, 44.2, 45.3, 49.8, 53.0, 67.1, 70.7, 114.0, 115.1, 118.8, 120.2, 120.9, 122.9, 127.0, 127.2, 128.0, 128.2, 128.3, 128.4, 128.6, 128.6, 129.1, 129.7, 129.8, 130.6, 130.9, 133.2, 133.9, 134.2, 135.7, 135.7, 144.9, 147.8, 151.7, 158.8, 165.5, 166.8.

5.4, methyl 2-(N-methyl-4-(trifluoromethyl)phenylsulfonamido)acetate. Sarcosine methyl ester hydrochloride was functionalized on a 0.7 mmol scale using General Procedure A to give 5.4 (189 mg, 84 %); δ_H (400 MHz, d-CDCl₃) 2.89 (s, 3H, CH₃), 3.59 (s, 3H, CH₃), 4.01 (s, 2H, CH₂), 7.75 (d, J = 8.2 Hz, 2H CH), 7.92 (d, J = 8.2 Hz, 2H, CH); δ_C (100 MHz, d-CDCl₃) 35.4, 50.6, 52.0, 123.1 (q), 125.9 (q), 127.7, 134.1 (q), 142.0, 168.5.
5.6, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(N-methyl-4-(trifluoromethyl)phenylsulfonamido)acetamido)benzoate. Secondary aniline 2.10h and carboxylic acid 5.4 were combined according to General Procedure E to give 5.5 (211 mg, 42 %); \( \delta_H \) (400 MHz, \( d-\text{CDCl}_3 \)) 1.29-1.48 (m, 5H, CH\(_2\)), 1.71-1.94 (m, 5H, CH\(_2\)), 2.45-2.57 (m, 1H, CH), 2.90 (s, 3H, CH\(_3\)), 3.78 (s, 2H, CH\(_2\)), 4.76 (s, 2H, CH\(_2\)), 4.99 (s, 2H, CH\(_2\)), 5.38 (s, 2H, CH\(_2\)), 6.55 (s, 1H, CH), 6.67 (d of d, \( J = 8.2 \) and 1.2 Hz, 1H, CH), 7.03 (d, \( J = 8.0 \) Hz, 2H, CH), 7.14 (d, \( J = 8.0 \) Hz, 2H, CH), 7.27-7.47 (m, 10, CH), 7.75 (d, \( J = 8.4 \) Hz, 2H, CH), 7.87 (d, \( J = 8.2 \) Hz, 1H, CH), 7.92 (d, \( J = 8.2 \) Hz, 2H, CH); \( \delta_C \) (100 MHz, \( d-\text{CDCl}_3 \)) 26.0, 26.7, 34.4, 35.8, 44.1, 51.3, 52.8, 67.0, 70.6, 114.1, 120.0, 123.3 (q), 128.5 (q), 126.9, 127.0, 127.9, 128.0, 128.1, 128.2, 128.3, 128.5, 128.8, 133.1, 133.8, 135.6, 135.7, 142.4, 144.7, 147.7, 158.7, 165.2, 166.3.

5.8a, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(4-fluorophenylsulfonamido)acetamido)benzoate. Primary amine 4.4 was functionalized on a 0.3
mmol scale using General Procedure B to give 5.8a (97.4, 48 %); δ\_H (400 MHz, d-CDCl\_3) 1.26-1.46 (m, 5H, CH\_2), 1.69-1.90 (m, 5H, CH\_2), 2.41-2.54 (m, 1H, CH), 3.43 (d, J = 3.8 Hz, 2H, CH\_2), 4.71 (s, 2H, CH\_2), 4.89 (s, 2H, CH\_2), 5.36 (s, 2H, CH\_2), 5.91 (t, J = 4.6 Hz, 1H, NH), 6.40 (s, 1H, CH), 6.52 (d, J = 8.0 Hz, 1H, CH), 6.92 (d, J = 8.0 Hz, 2H, CH), 7.07-7.16 (m, 4H, CH), 7.31-7.44 (m, 10, CH), 7.76-7.85 (m, 3H, CH); δ\_C (100 MHz, d-CDCl\_3) 25.9, 26.7, 34.3, 44.1, 44.2, 53.0, 67.0, 70.7, 113.8, 116.1 (d), 119.8, 121.2, 126.9, 128.0, 128.2, 128.2, 128.5, 128.8, 129.8, 129.9, 131.2, 131.2, 135.3, 135.4, 135.5, 143.7, 147.9, 158.7, 163.7, 165.0 (d), 165.2, 166.5.

5.8b, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(4-(trifluoromethyl)phenylsulfonamido)acetamido)benzoate. Primary amine 4.4 was functionalized on a 0.28 mmol scale using General Procedure B to give 5.8b (155.4 mg, 72 %); δ\_H (400 MHz, d-CDCl\_3) 1.30-1.44 (m, 5H, CH\_2), 1.71-1.89 (m, 5H, CH\_2), 2.40-2.54 (m, 1H, CH), 3.49 (s (br), 2H, CH\_2), 4.74 (s, 2H, CH\_2), 4.90 (s, 2H, CH\_2), 5.36 (s, 2H, CH\_2), 6.18 (s (br), 1H, NH), 6.44 (s, 1H, CH), 6.56 (d, J = 8.0 Hz, 1H, CH), 6.95 (d, J = 7.4 Hz, 2H, CH), 7.10 (d, J = 8.0 Hz, 2H, CH), 7.30-7.44 (m, 10, CH), 7.73 (d, J = 8.2 Hz, 2H, CH), 7.82 (d, J = 8.2 Hz, 1H, CH), 7.93 (d, J = 8.2 Hz, 2H, CH); δ\_C (100 MHz, d-CDCl\_3) 25.9, 26.7, 34.3, 44.1, 44.2, 53.0, 67.0, 70.7, 113.8, 119.8, 121.3, 123.2 (q), 126.0 (q), 126.9, 127.6, 128.0, 128.2, 128.2, 128.5, 128.5, 128.8, 133.2, 133.2, 134.0, 134.3, 135.4, 135.5, 143.1, 143.7, 147.9, 158.7, 165.2, 166.5.
5.9a, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(4-fluoro-N-(2-(trifluoromethyl)benzyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 5.8a was functionalized on a 0.06 mmol scale using General Procedure D to give 5.9a (46.3 mg, 85 %); δH (400 MHz, d-CHCl3) 1.30-1.45 (m, 5H, CH2), 1.70-1.86 (m, 5H, CH2), 2.40-2.55 (m, 1H, CH), 3.71 (s, 2H, CH2), 4.66 (s, 2H, CH2), 4.75 (s, 2H, CH2), 4.79 (s, 2H, CH2), 5.31 (s, 2H, CH2), 6.34 (s, 1H, CH), 6.48 (d, J = 8.0 Hz, 1H, CH), 6.92 (d, J = 8.0 Hz, 2H, CH), 7.09 (d, J = 8.0 Hz, 2H, CH), 7.13-7.20 (m, 3H, CH), 7.27-7.39 (m, 12H, CH), 7.49 (t, J = 7.6 Hz, 1H, CH), 7.60 (d, J = 7.8 Hz, 1H, CH), 7.66-7.77 (m, 2H, CH), 7.85-7.92 (m, 2H, CH); δC (100 MHz, d-CHCl3) 25.9, 26.7, 34.3, 44.1, 47.4, 47.7, 52.7, 66.9, 70.5, 113.7, 115.9 (d), 119.8, 120.8, 122.6, 125.5 (d), 126.8, 126.9, 127.7, 127.9, 128.1, 128.2, 128.3, 128.4, 128.8, 129.8, 130.3, 130.4, 132.4, 133.0, 133.6, 134.5, 135.5, 135.6, 135.7, 144.6, 147.7, 158.6, 165.0 (d), 165.2, 165.9.
5.9b, benzyl 2-(benzylxy)-4-(N-(4-cyclohexylbenzyl)-2-(4-fluoro-N-(2-methylbenzyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 5.8a was functionalized on a 0.08 mmol scale using General Procedure D to give 5.9b (47.1 mg, 73 %); δH (400 MHz, d-CHCl₃) 1.30-1.48 (m, 5H, CH₂), 1.71-1.92 (m, 5H, CH₂), 2.23 (s, 3H, CH₃), 2.44-2.55 (m, 1H, CH), 3.61 (s, 2H, CH₂), 4.59 (s, 2H, CH₂), 4.64 (s, 2H, CH₂), 4.75 (s, 2H, CH₂), 5.32 (s, 2H, CH₂), 6.19 (s, 1H, CH), 6.32 (d, J = 7.8 Hz, 1H, CH), 6.96 (d, J = 8.0 Hz, 2H, CH), 7.05 (d, J = 7.2 Hz, 1H, CH), 7.09-7.21 (m, 7H CH), 7.30-7.39 (m, 10H, CH), 7.69 (d, J = 8.0 Hz, 1H, CH), 7.87-7.94 (m, 2H, CH); δC (100 MHz, d-CHCl₃) 18.9, 25.9, 26.7, 34.4, 44.1, 46.7, 49.0, 52.6, 66.9, 70.6, 113.6, 115.9 (d), 119.9, 120.7, 125.9, 126.8, 127.0, 128.0, 128.1, 128.2, 128.2, 128.9, 129.9, 129.9, 130.2, 130.3, 130.7, 132.4, 132.9, 133.7, 135.5, 135.8, 135.8, 137.9, 144.7, 147.7, 158.5, 165.0 (d), 165.3, 166.3.

5.9c, benzyl 2-(benzylxy)-4-(N-(4-cyclohexylbenzyl)-2-(4-(trifluoromethyl)-N-(2-(trifluoromethyl)benzyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 5.8b was functionalized on a 0.09 mmol scale using General Procedure D to give 5.9c (65.4 mg, 81 %); δH (400 MHz, d-CDCl₃) 1.30-1.46 (m, 5H, CH₂), 1.71-1.89 (m, 5H, CH₂), 2.42-2.53 (m, 1H, CH), 3.73 (s, 2H, CH₂), 4.65 (s, 2H, CH₂), 4.76 (s, 2H, CH₂), 4.79 (s, 2H, CH₂), 5.32 (s, 2H, CH₂), 6.34 (s, 1H, CH), 6.48 (d, J = 8.0 Hz, 1H, CH), 6.92 (d, J = 8.0 Hz, 2H, CH), 7.09 (d, J = 8.0 Hz, 2H, CH), 7.27-7.40 (m, 11H, CH), 7.50 (t, J = 7.6 Hz, 1H, CH), 7.60 (d, J = 7.8 Hz, 1H, CH), 7.69 (d, J = 7.8 Hz, 1H, CH), 7.72 (d, J = 8.0 Hz, 1H, CH), 7.78 (d, J = 8.2 Hz, 2H, CH), 8.00 (d, J = 8.2 Hz, 2H, CH); δC (100 MHz, d-CDCl₃) 25.9, 26.7, 34.3, 44.0, 47.4, 47.8, 52.7, 66.9, 70.6, 113.7, 119.8, 120.8, 125.5, 125.6, 125.8, 125.8, 126.8, 126.9, 127.8, 128.0, 128.1, 128.2, 128.4,
128.8, 129.9, 132.4, 133.0, 134.0, 134.1, 134.3, 135.5, 135.6, 137.9, 143.2, 144.5, 147.7, 158.8, 165.2, 165.7.

5.9d, benzyl 2-(benzyloxy)-4-(N-(4-cyclohexylbenzyl)-2-(N-(2-methylbenzyl)-4-(trifluoromethyl)phenylsulfonamido)acetamido)benzoate. Sulfonamide 5.8b was functionalized on a 0.10 mmol scale using General Procedure D to give 5.9d (79.7 mg, 93 %); δ<sub>H</sub> (400 MHz, d-CDCl<sub>3</sub>) 1.30-1.47 (m, 5H, CH<sub>2</sub>), 1.70-1.90 (m, 5H, CH<sub>2</sub>), 2.22 (s, 3H, CH<sub>3</sub>), 2.42-2.54 (m, 1H, CH), 3.61 (s, 2H, CH<sub>2</sub>), 4.46 (s, 2H, CH<sub>2</sub>), 4.61 (s, 2H, CH<sub>2</sub>), 4.74 (s, 2H, CH<sub>2</sub>), 5.30 (s, 2H, CH<sub>2</sub>), 6.17 (s, 1H, CH), 6.30 (d, J = 8.0 Hz, 1H, CH), 6.93 (d, J = 8.0 Hz, 2H, CH), 7.02-7.19 (m, 6H, CH), 7.29-7.37 (m, 10H, CH), 7.68 (d, J = 8.0 Hz, 1H, CH), 7.77 (d, J = 8.2 Hz, 2H, CH), 7.99 (d, J = 8.2 Hz, 2H CH); δ<sub>C</sub> (100 MHz, d-CDCl<sub>3</sub>) 18.8, 25.9, 26.7, 34.3, 44.1, 46.8, 49.0, 52.6, 66.9, 70.6, 113.6, 119.9, 120.8, 124.6, 125.7, 125.8, 125.9, 126.8, 127.0, 128.0, 128.1, 128.2, 128.3, 128.4, 128.5, 128.9, 129.9, 130.7, 132.1, 132.9, 133.6, 135.5, 135.6, 137.9, 143.2, 144.5, 147.7, 158.8, 165.2, 166.1.
1.4 Characterization of Final Molecules

\[
5.3a, 4-\text{(N-(4-cyclohexylbenzyl)-2-(N-((perfluorophenyl)methyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid.}\]
\[
\begin{align*}
\delta_H (400 MHz, d-CDCl_3) &\quad 1.34-1.50 (m, 5H, CH_2), 1.68-1.93 (m, 5H, CH_2), 2.39-2.57 (m, 1H, CH), 3.96 (s, 2H, CH_2), 4.67 (s, 2H, CH_2), 4.72 (s, 2H, CH_2), 6.50 (s (br), 1H, CH), 6.64 (s, 1H, CH), 6.99 (d, J = 7.8 Hz, 2H, CH), 7.09 (d, J = 7.8 Hz, 2H, CH), 7.47 (t, J = 7.2 Hz, 2H, CH), 7.58 (t, J = 7.1 Hz, 1H, CH), 7.82 (d, J = 6.8 Hz, 2H, CH), 7.88 (s (br), 1H, CH); \\
\delta_C (100 MHz, d-CDCl_3) &\quad 26.0, 26.7, 34.2, 39.7, 44.1, 49.2, 52.9, 110.0, 116.6, 118.3, 125.4, 126.8, 127.4, 128.4, 128.7, 132.8, 133.4, 136.0, 139.0, 147.5, 155.9, 162.8, 166.4. \text{HRMS (ESI) Calcd for [C}_{35}H_{31}F_{5}N_{2}O_{6}S + H]} 703.1895 \text{ found 703.1927; HPLC (I) } t_R = 25.06 \text{ min (91.2 %), (II) } t_R = 59.77 \text{ min (90.5 %).}
\end{align*}
\]

\[
5.3b, 4-\text{(N-(4-cyclohexylbenzyl)-2-(N-((perfluorophenyl)methyl)methylsulfonamido)acetamido)-2-hydroxybenzoic acid.}\]
\[
\begin{align*}
\delta_H (400 MHz, d-CDCl_3) &\quad 1.34-1.50 (m, 5H, CH_2), 1.68-1.93 (m, 5H, CH_2), 2.39-2.57 (m, 1H, CH), 3.96 (s, 2H, CH_2), 4.67 (s, 2H, CH_2), 4.72 (s, 2H, CH_2), 6.50 (s (br), 1H, CH), 6.64 (s, 1H, CH), 6.99 (d, J = 7.8 Hz, 2H, CH), 7.09 (d, J = 7.8 Hz, 2H, CH), 7.47 (t, J = 7.2 Hz, 2H, CH), 7.58 (t, J = 7.1 Hz, 1H, CH), 7.82 (d, J = 6.8 Hz, 2H, CH), 7.88 (s (br), 1H, CH); \\
\delta_C (100 MHz, d-CDCl_3) &\quad 26.0, 26.7, 34.2, 39.7, 44.1, 49.2, 52.9, 110.0, 116.6, 118.3, 125.4, 126.8, 127.4, 128.4, 128.7, 132.8, 133.4, 136.0, 139.0, 147.5, 155.9, 162.8, 166.4. \text{HRMS (ESI) Calcd for [C}_{35}H_{31}F_{5}N_{2}O_{6}S + H]} 703.1895 \text{ found 703.1927; HPLC (I) } t_R = 25.06 \text{ min (91.2 %), (II) } t_R = 59.77 \text{ min (90.5 %).}
\end{align*}
\]
MHZ, \( d\)-CDCl\(_3\)) 1.32-1.53 (m, 5H, CH\(_2\)), 1.68-1.97 (m, 5H, CH\(_2\)), 2.35-2.57 (m, 1H, CH), 3.15 (s, 3H, C\(_3\)), 3.89 (s, 2H, CH\(_2\)), 4.63 (s, 2H, CH\(_2\)), 4.77 (s, 2H, CH\(_2\)), 6.46(\( \text{br} \), 1H, CH), 6.60 (s, 1H, CH), 6.92-7.19 (m, 4H, CH), 7.83 (s (br), 1H, CH); \( \delta \)\( _C \) (100 MHz, \( d\)-CDCl\(_3\)) 26.2, 27.0, 34.5, 39.4, 40.6, 44.3, 49.6, 59.3, 112.3, 118.6, 119.9, 125.6, 127.1, 127.2, 128.4, 128.5, 133.4, 136.4, 138.8, 148.0, 159.2, 167.2, 169.3. HRMS (ESI) Calcd for \([C_{30}H_{29}F_5N_2O_6S + H]\) 641.1739 found 641.1748; HPLC (I) \( t_R = 24.25 \) min (98.6 %), (II) \( t_R = 56.92 \) min (98.6 %).

\[
\begin{align*}
5.3c, \text{4-(N-(4-cyclohexylbenzyl)-2-(N-((perfluorophenyl)methyl)-4-(trifluoromethyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid.} \quad & \delta_H (400 MHz, \( d\)-CDCl\(_3\)) 1.31-1.42 (m, 5H, CH\(_2\)), 1.70-1.92 (m, 5H, CH\(_2\)), 2.41-2.55 (m, 1H, CH), 4.00 (s, 2H, CH\(_2\)), 4.67 (s, 2H, CH\(_2\)), 4.73 (s, 2H, CH\(_2\)), 6.55 (s (br), 1H, CH), 6.69 (s, 1H, CH), 7.01 (d, \( J = 7.6 \) Hz, 2H, CH), 7.12 (d, \( J = 7.6 \) Hz, 2H, CH), 7.77 (d, \( J = 7.8 \) Hz, 2H, CH), 7.98 (m, 3H, CH); \( \delta \)\( _C \) (100 MHz, \( d\)-CDCl\(_3\)) 26.3, 27.0, 34.5, 40.0, 44.4, 49.5, 53.3, 113.4, 117.6, 120.4, 122.1, 122.2, 125.7, 126.2, 127.2, 128.4, 128.7, 128.7, 133.5, 142.8, 148.0, 158.6, 163.0, 165.8. HRMS (ESI) Calcd for \([C_{36}H_{30}F_8N_2O_6S + H]\) 771.1769 found 771.1791; HPLC (I) \( t_R = 26.05 \) min (97.0 %), (II) \( t_R = 62.61 \) min (97.4 %).
\end{align*}
\]
5.3d, 4-(2-(4-(tert-butyl)-N-((perfluorophenyl)methyl)phenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)-2-hydroxybenzoic acid. $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.36-1.47 (m, 5H, CH$_2$), 1.69-1.94 (m, 5H, CH$_2$), 2.39-2.55 (m, 1H, CH), 4.00 (s, 2H, CH$_2$), 4.67 (s, 2H, CH$_2$), 4.79 (s, 2H, CH$_2$), 6.58 (d, $J$ = 6.9 Hz, 1H, CH), 6.70 (s, 1H, CH), 7.05 (d, $J$ = 7.8 Hz, 2H, CH), 7.12 (d, $J$ = 7.8 Hz, 2H, CH), 7.49 (d, $J$ = 8.2 Hz, 2H, CH), 7.73 (d, $J$ = 8.1 Hz, 2H, CH), 7.91 (s, 1H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 26.3, 27.0, 31.2, 34.5, 35.3, 40.1, 44.4, 49.9, 53.2, 110.2, 117.2, 119.0, 125.9, 127.2, 127.6, 128.6, 132.5, 133.6, 133.9, 136.3, 138.8, 147.9, 157.0, 163.1, 166.9. HRMS (ESI) Calcd for [$C_{39}H_{39}F_5N_2O_6S + H$] 759.2521 found 759.2551; HPLC (I) $t_R$ = 27.38 min (97.1 %), (II) $t_R$ = 64.82 min (100 %).

5.3e, 4-(N-(4-cyclohexylbenzyl)-2-(3-methyl-N-((perfluorophenyl)methyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.31-1.43 (m, 5H, CH$_2$), 1.70-1.91 (m, 5H, CH$_2$), 2.39 (s, 3H, CH$_3$), 2.40-2.54 (m, 1H, CH), 3.96 (s, 2H, CH$_2$), 4.66 (s, 2H, CH$_2$), 4.77 (s, 2H, CH$_2$), 6.55 (s (br), 1H, CH), 6.68...
(s, 1H, CH), 7.00 (d, J = 6.8 Hz, 2H, CH), 7.10 (d, J = 6.8 Hz, 2H, CH), 7.31-7.43 (m, 2H, CH), 7.56-7.68 (m, 2H, CH), 7.90 (s, 1H, CH); δC (100 MHz, d-CDCl3) 21.1, 26.0, 26.7, 34.2, 39.7, 44.1, 49.3, 53.0, 109.8, 116.2, 118.5, 123.0, 124.6, 125.4, 126.9, 127.8, 128.3, 128.6, 132.4, 133.3, 133.7, 146.8, 147.6, 159.1, 162.8, 166.7. HRMS (ESI) Calcd for [C_{36}H_{33}F_{5}N_{2}O_{6}S + H] 717.2052 found 717.2087; HPLC (I) t_R = 25.56 min (97.3 %), (II) t_R = 61.08 min (98.2 %).

5.3f, 4-(N-(4-cyclohexylbenzyl)-2-(2-methyl-N-((perfluorophenyl)methyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. δH (400 MHz, d-CDCl3) 1.30-1.42 (m, 5H, CH2), 1.70-1.91 (m, 5H, CH2), 2.40-2.52 (m, 1H, CH), 2.54 (s, 3H, CH3), 3.93 (s, 2H, CH2), 4.73 (s, 2H, CH2), 4.77 (s, 2H, CH2), 6.48 (s, 1H, CH), 6.61 (s, 1H, CH), 7.00 (d, J = 7.2 Hz, 2H, CH), 7.09 (d, J = 7.2 Hz, 2H, CH), 7.27-7.32 (m, 2H, CH), 7.46 (t, J = 7.2 Hz, 1H, CH), 7.88 (s, 1H, CH), 7.99 (d, J = 7.4 Hz, 1H, CH); δC (100 MHz, d-CDCl3) 20.1, 26.0, 26.7, 34.2, 39.2, 44.0, 48.6, 52.9, 112.2, 118.0, 118.6, 125.7, 126.8, 128.5, 130.2, 132.5, 133.1, 133.2, 136.8, 138.3, 140.2, 144.2, 147.6, 158.9, 162.9, 166.1. HRMS (ESI) Calcd for [C_{36}H_{33}F_{5}N_{2}O_{6}S + H] 717.2052 found 717.2056; HPLC (I) t_R = 25.61 min (97.8 %), (II) t_R = 61.20 min (98.8 %).
5.3g, 4-(N-(4-cyclohexylbenzyl)-2-(N-((perfluorophenyl)methyl)-3- (trifluoromethyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. \( \delta_H \) (400 MHz, \( d \)-CDCl\(_3\)) 1.29-1.45 (m, 5H, CH\(_2\)), 1.65-1.93 (m, 5H, CH\(_2\)), 2.36-2.54 (m, 1H, CH), 3.98 (s, 2H, CH\(_2\)), 4.64 (s, 2H, CH\(_2\)), 4.72 (s, 2H, CH\(_2\)), 6.53 (s (br), 1H, CH), 6.64 (s, 1H, CH), 7.99 (d, \( J = 7.2 \) Hz, 2H, CH), 7.06 (d, \( J = 7.2 \) Hz, 2H, CH), 7.62 (t, \( J = 7.0 \) Hz, 1H, CH), 7.82 (d, \( J = 7.0 \) Hz, 1H, CH), 7.90 (s (br), 1H, CH), 8.04 (d, \( J = 7.2 \) Hz, 1H, CH), 8.07, (s, 1H, CH); HRMS (ESI) Calcd for \([C_{36}H_{30}F_8N_2O_6S + H]\) 771.1769 found 771.1772; HPLC (I) \( t_R = 25.88 \) min (97.3 %), (II) \( t_R = 62.19 \) min (97.2 %).

5.3h, 4-(N-(4-cyclohexylbenzyl)-2-(4-isopropyl-N-((perfluorophenyl)methyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. \( \delta_H \) (400 MHz, \( d \)-CDCl\(_3\)) 1.31-1.47 (m, 5H, CH\(_2\)), 1.68-1.92 (m, 5H, CH\(_2\)), 2.35-2.55 (m, 1H, CH), 2.86-3.04 (m, 1H, CH), 3.98 (s, 2H, CH\(_2\)), 4.65 (s, 2H, CH\(_2\)), 4.77 (s, 2H, CH\(_2\)), 6.56 (d, \( J = 5.5 \) Hz, 1H, CH), 6.68 (s, 1H, CH), 7.03 (d, \( J = 7.5 \) Hz, 1H, CH), 7.10 (d, \( J = 7.5 \) Hz, 2H, CH), 7.31 (d, \( J = 7.5 \) Hz, 2H, CH).
= 8.0 Hz, 2H, CH), 7.72 (d, J = 7.9 Hz, 2H, CH), 7.88 (d, J = 6.0 Hz, 1H, CH); δ_C (100 MHz, d-
CDCl_3) 14.1, 23.6, 26.1, 26.9, 34.2, 34.4, 39.0, 44.2, 49.6, 53.1, 110.1, 117.0, 118.9, 125.5,
126.9, 127.0, 127.8, 128.5, 132.4, 133.4, 136.1, 136.4, 147.8, 154.6, 163.0, 166.8, 172.7. HRMS
(ESI) Calcd for [C_{38}H_{37}F_{5}N_{2}O_{6}S + H] 745.2365 found 745.2341; HPLC (I) t_R = 26.68 min (97.4
%), (II) t_R = 63.69 min (98.1 %).

5.3i, 4-(N-(4-cyclohexylbenzyl)-2-(4-ethyl-N-
((perfluorophenyl)methyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. δ_H (400
MHz, d-CDCl_3) 1.25 (t, J = 7.2 Hz, 3H, CH_3), 1.33-1.51 (m, 5H, CH_2), 1.67-1.96 (m, 5H, CH_2),
2.40-2.57 (m, 1H, CH), 2.72 (q, J = 7.2 Hz, 2H, CH_2), 3.97 (s, 2H, CH_2), 4.65 (s, 2H, CH_2), 4.75
(s, 2H, CH_2), 6.56 (s (br), 1H, CH), 6.67 (s, 1H, CH), 7.02 (d, J = 7.5 Hz, 2H, CH), 7.10 (d, J =
7.5 Hz, 2H, CH), 7.29 (d, J = 7.8 Hz, 2H, CH), 7.71 (d, J = 7.7 Hz, 2H, CH), 7.86 (s (br), 1H,
CH); δ_C (100 MHz, d-CDCl_3) 15.2, 26.3, 27.0, 29.8, 34.5, 40.0, 44.4, 49.6, 53.2, 112.8, 117.2,
119.0, 125.7, 127.1, 127.9, 128.5, 128.6, 132.6, 133.6, 136.4, 147.9, 150.2, 163.1, 166.9. HRMS
(ESI) Calcd for [C_{37}H_{35}F_{3}N_{2}O_{6}S + H] 731.2208 found 731.2228; HPLC (I) t_R = 26.08 min (97.3
%), (II) t_R = 62.48 min (97.3 %).
5.3j, 4-(N-(4-cyclohexylbenzyl)-2-(2,4,6-trimethyl-N-((perfluorophenyl)methyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. \( \delta_H \) (400 MHz, \( d-\text{CDCl}_3 \)) 1.29–1.41 (m, 5H, CH₂), 1.67–1.91 (m, 5H, CH₂), 2.28 (s, 3H, CH₃), 2.38–2.48 (m, 1H, CH), 2.52 (s, 6H, CH₃), 3.82 (s, 2H, CH₂), 4.72 (s, 2H, CH₂), 4.74 (s, 2H, CH₂), 6.41 (s, 1H, CH), 6.51 (s, 1H, CH), 6.91 (s, 2H, CH), 6.97 (d, \( J = 6.2 \text{ Hz} \), 2H, CH), 7.07 (d, \( J = 7.4 \text{ Hz} \), 2H, CH), 7.76–7.92 (m, 1H, CH); \( \delta_C \) (100 MHz, \( d-\text{CDCl}_3 \)) 20.9, 22.7, 26.1, 26.9, 34.4, 38.9, 44.2, 48.3, 53.0, 107.2, 109.7, 117.1, 125.7, 127.1, 128.6, 128.6, 132.1, 132.1, 133.6, 141.0, 143.3, 147.3, 147.8, 163.1, 166.8. HRMS (ESI) Calcd for \([C_{38}H_{37}F_{5}N_{2}O_{6}S + H]^{+}\) 745.2365 found 745.2394; HPLC (I) \( t_R = 27.11 \text{ min} \) (97.0 %), (II) \( t_R = 64.19 \text{ min} \) (96.9 %).

5.3k, 4-(N-(4-cyclohexylbenzyl)-2-(N-((perfluorophenyl)methyl)-2-(trifluoromethyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. \( \delta_H \) (400 MHz, \( d-\text{CDCl}_3 \)) 1.32–1.52 (m, 5H, CH₂), 1.67–1.93 (m, 5H, CH₂), 2.48–2.57 (m, 1H, CH), 3.91 (s, 2H, CH₂), 4.71 (s, 2H, CH₂), 4.86 (s, 2H, CH₂), 6.48 (s, 1H, CH), 6.59 (s, 1H, CH), 6.98 (d, \( J = 7.9 \text{ Hz} \), 2H, CH).
5.3l, 4-(2-(4-chloro-N-((perfluorophenyl)methyl)phenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)-2-hydroxybenzoic acid. \( \delta_H \) (400 MHz, \( d\)-CDCl\(_3\)) 1.31-1.49 (m, 5H, CH\(_2\)), 1.67-1.92 (m, 5H, CH\(_2\)), 2.38-2.55 (m, 1H, CH), 3.95 (s, 2H, CH\(_2\)), 4.65 (s, 2H, CH\(_2\)), 4.74 (s, 2H, CH\(_2\)), 6.53 (d, \( J = 6.8 \) Hz, 1H, CH), 6.65 (s, 1H, CH), 6.99 (d, \( J = 7.4 \) Hz, 1H, CH), 7.12 (d, \( J = 7.4 \) Hz, 2H, CH), 7.46 (d, \( J = 8.0 \) Hz, 2H, CH), 7.78 (d, \( J = 8.0 \) Hz, 2H, CH), 7.89 (d, \( J = 8.0 \) Hz, 1H, CH); \( \delta_C \) (100 MHz, \( d\)-CDCl\(_3\)) 26.3, 27.0, 34.5, 40.0, 44.4, 49.4, 53.3, 112.3, 117.2, 119.0, 126.5, 127.2, 127.7, 128.7, 129.0, 129.3, 132.6, 133.1, 133.4, 137.8, 139.7, 148.0, 163.2, 166.4, 172.1; HRMS (ESI) Calcd for \([C_{35}H_{30}ClF_5N_2O_6S + H]\) 737.1506, found 737.1491; HPLC (I) \( t_R = 27.78 \) min (80.2 %), (II) \( t_R = 62.82 \) min (80.5 %).
5.3m, 4-(2-(4-cyano-N-((perfluorophenyl)methyl)phenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)-2-hydroxybenzoic acid. $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.35-1.46 (m, 5H, CH$_2$), 1.71-1.94 (m, 5H, CH$_2$), 2.40-2.56 (m, 1H, CH), 3.96 (s, 2H, CH$_2$), 4.61 (s, 2H, CH$_2$), 4.69 (s, 2H, CH$_2$), 6.48 (s (br), 1H, CH), 6.62 (s, 1H, CH), 6.98 (d, $J = 7.8$ Hz, 2H, CH), 7.11 (d, $J = 7.8$ Hz, 2H, CH), 7.71 (d, $J = 8.0$ Hz, 2H, CH), 8.11 (s, 1H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 26.1, 26.9, 34.4, 39.9, 44.2, 49.4, 53.0, 110.2, 118.9, 122.3, 122.9, 127.0, 127.7, 128.5, 129.5, 132.7, 133.4, 136.1, 143.9, 147.8, 156.4, 163.1, 166.6; HRMS (ESI) Calcd for [C$_{36}$H$_{30}$F$_3$N$_3$O$_6$S + H] 728.1848 found 728.1845. HPLC (I) $t_R = 24.72$ min (91.3 %), (II) $t_R = 59.03$ min (90.8 %).

5.3n, 4-(N-(4-cyclohexylbenzyl)-2-(4-fluoro-N-((perfluorophenyl)methyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.28-1.46 (m, 5H, CH$_2$), 1.68-1.90 (m, 5H, CH$_2$), 2.38-2.52 (m, 1H, CH), 3.96 (s, 2H, CH$_2$), 4.61 (s, 2H, CH$_2$), 4.72 (s, 2H, CH$_2$), 6.51 (s (br), 1H, CH), 6.65 (s (br), 1H, CH),
6.99 (d, J = 7.2 Hz, 2H, CH), 7.06-7.21 (m, 4H, CH), 7.78-7.95 (m, 3H, CH); HRMS (ESI)
Calcd for [C_{33}H_{30}F_{6}N_{2}O_{6}S + H] 721.1801 found 721.1820; HPLC (I) t_R = 25.26 min (92.9 %),
(II) t_R = 60.41 min (94.0 %).

5.3o, 4-(N-(4-cyclohexylbenzyl)-2-(4-methoxy-N-((perfluorophenyl)methyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. δ_H (400 MHz, d-CDCl_3) 1.35-1.49 (m, 5H, CH_2), 1.70-1.94 (m, 5H, CH_2), 2.40-2.57 (m, 1H, CH), 3.64 (s, 3H, CH_3), 3.73 (s, 2H, CH_2), 3.80 (s, 2H, CH_2), 4.40 (s, 2H, CH_2), 4.53 (s, 2H, CH_2), 6.32 (s (br), 1H, CH), 6.44 (s, 1H, CH), 6.71 (d, J = 7.6 Hz, 2H, CH), 6.79 (d, J = 8.0 Hz, 2H, CH), 6.89 (d, J = 8.0 Hz, 2H, CH), 7.54 (d, J = 7.6 Hz, 2H, CH), 7.64 (s (br), 1H, CH); δ_C (100 MHz, d-CDCl_3) 26.1, 26.9, 34.4, 39.8, 44.2, 49.4, 53.0, 55.6, 110.25, 114.1, 117.0, 118.9, 125.5, 127.0, 128.4, 128.8, 130.0, 130.5, 132.4, 133.4, 147.8, 163.2, 166.8, 172.5. HRMS (ESI) Calcd for [C_{36}H_{33}F_{6}N_{2}O_{7}S + H] 733.2001 found 733.1986; HPLC (I) t_R = 24.97 min (96.3 %), (II) t_R = 59.58 min (96.7 %).
5.3p, 4-(2-(4-amino-N-((perfluorophenyl)methyl)phenylsulfonamido)-N-(4-cyclohexylbenzyl)acetamido)-2-hydroxybenzoic acid. \( \delta_H \) (400 MHz, \( d\)-CDCl\(_3\)) 1.28-1.42 (m, 5H, CH\(_2\)), 1.65-1.90 (m, 5H, CH\(_2\)), 2.22-2.41 (m, 1H, CH), 3.91 (s, 2H, CH\(_2\)), 4.63 (s, 2H, CH\(_3\)), 4.66 (s, 2H, CH\(_2\)), 6.46 (s (br), 1H, CH), 6.59 (s, 1H, CH), 6.86-7.00 (m, 4H, CH), 7.53 (d, \( J = 7.2 \text{ Hz}, 2H, \text{ CH} \)), 7.62 (d, \( J = 7.4 \text{ Hz}, 2H, \text{ CH} \)), 7.80 (s (br), 1H, CH); \( \delta_C \) (100 MHz, \( d\)-CDCl\(_3\)) 26.7, 26.8, 34.4, 39.9, 44.2, 49.4, 53.1, 110.2, 117.0, 118.8, 126.9, 127.4, 127.5, 128.2, 128.5, 129.0, 129.7, 133.4, 137.6, 139.3, 146.0, 147.7, 162.9, 167.9. HRMS (ESI) Calcd for \([C_{35}H_{32}F_5N_3O_6S + H]\) 718.2004 found 718.2011; HPLC (I) \( t_R = 25.22 \text{ min (85.6 \%)}, \) (II) \( t_R = 55.14 \text{ min (93.0 \%)}. \)

![Structural diagram](image)

5.3q, 4-(N-(4-cyclohexylbenzyl)-2-(N-((perfluorophenyl)methyl)-[1,1'-biphenyl]-4-ylsulfonamido)acetamido)-2-hydroxybenzoic acid. \( \delta_H \) (400 MHz, \( d\)-CDCl\(_3\)) 1.37-1.45 (m, 5H, CH\(_2\)), 1.68-1.80 (m, 5H, CH\(_2\)), 2.36-2.45 (m, 1H, CH), 3.91 (s, 2H, CH\(_2\)), 4.63 (s, 2H, CH\(_2\)), 4.66 (s, 2H, CH\(_2\)), 6.46 (s (br), 1H, CH), 6.59 (s, 1H, CH), 6.85-6.99 (m, 4H, CH), 7.30-7.48 (m, 3H, CH), 7.53 (d, \( J = 7.2 \text{ Hz}, 2H, \text{ CH} \)), 7.61 (d, \( J = 8.0 \text{ Hz}, 2H, \text{ CH} \)), 7.81 (m, 3H, CH); HRMS (ESI) Calcd for \([C_{41}H_{35}F_5N_2O_6S + H]\) 779.2208 found 779.2190; HPLC (I) \( t_R = 26.77 \text{ min (93.8 \%)}, \) (II) \( t_R = 63.90 \text{ min (95.9 \%)}. \)
5.3r, 4-(N-(4-cyclohexylbenzyl)-2-(3,5-dimethyl-N-((perfluorophenyl)methyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.42-1.60 (m, 5H, CH$_2$), 1.69-1.91 (m, 5H, CH$_2$), 2.24 (s, 6H, CH$_3$), 2.39-2.52 (m, 1H, CH), 3.86 (s, 2H, CH$_2$), 4.56 (s, 2H, CH$_2$), 4.69 (s, 2H, CH$_2$), 6.46 (s, 1H, CH), 6.59 (s, 1H, CH), 6.91-7.05 (m, 4H, CH), 7.08 (s, 1H, CH), 7.29 (s, 2H, CH), 7.81 (s, 1H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 21.1, 26.1, 26.8, 34.4, 39.9, 44.2, 49.5, 53.1, 110.1, 116.9, 118.7, 125.1, 127.0, 128.4, 128.4, 133.5, 134.7, 138.6, 138.9, 146.9, 147.7, 162.9, 165.3, 167.0. HRMS (ESI) Calcd for [C$_{37}$H$_{35}$F$_5$N$_2$O$_6$S + H] 731.2208 found 731.2242; HPLC (I) $t_R = 27.74$ min (90.3 %), (II) $t_R = 62.10$ min (91.0 %).

5.3s, 4-(N-(4-cyclohexylbenzyl)-2-(N-((perfluorophenyl)methyl)-2,3-dihydrobenzo[b][1,4]dioxine-6-sulfonamido)acetamido)-2-hydroxybenzoic acid. $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.29-1.46 (m, 5H, CH$_2$), 1.70-1.92 (m, 5H, CH$_2$), 2.41-2.52 (m, 1H, CH), 3.94 (s, 2H, CH$_2$), 4.25-4.36 (m, 4H, 2 CH$_2$), 4.66 (s, 2H, CH$_2$), 4.78 (s, 2H, CH$_2$), 6.58 (d, $J = 8.2$ Hz,
1H, CH), 6.68 (s, 1H, CH), 6.92 (d, J = 8.4 Hz, 1H, CH), 7.03 (d, J = 8.0 Hz, 2H, CH), 7.11 (d, J = 8.0 Hz, 2H, CH), 7.27-7.35 (m, 2H, CH), 7.88 (d, J = 8.4 Hz, 1H, CH); δC (100 MHz, d-CDCl₃) 26.1, 26.9, 34.4, 40.0, 44.2, 49.4, 53.0, 64.2, 64.6, 110.4, 117.2, 117.3, 117.6, 119.1, 121.4, 127.1, 128.5, 131.4, 132.5, 133.3, 143.4, 147.7, 147.8, 163.0, 166.7, 172.7, 177.3; HRMS (ESI) Calcd for [C₃₇H₃₃F₅N₂O₈S + H] 761.1950 found 761.1942; HPLC (I) tₚ = 26.42 min (96.1 %), (II) tₚ = 58.72 min (96.3 %).

5.3t, 4-(N-(4-cyclohexylbenzyl)-2-(N-((perfluorophenyl)methyl)naphthalene-2-sulfonamido)acetamido)-2-hydroxybenzoic acid. δH (400 MHz, d-CDCl₃) 1.27-1.45 (m, 5H, CH₂), 1.67-1.89 (m, 5H, CH₂), 2.37-2.50 (m, 1H, CH), 4.00 (s, 2H, CH₂), 4.69 (s, 2H, CH₂), 4.72 (s, 2H, CH₂), 6.52 (d, J = 7.0 Hz, 1H, CH), 6.66 (s, 1H, CH), 6.83 (d, J = 7.4 Hz, 2H, CH), 7.02 (d, J = 7.4 Hz, 2H, CH), 7.55-7.67 (m, 2H, CH), 7.74 (d, J = 8.5 Hz, 1H, CH), 7.84-7.98 (m, 4H, CH), 8.38 (s, 1H, CH); δC (100 MHz, d-CDCl₃) 26.1, 26.9, 34.4, 40.0, 44.2, 49.4, 53.0, 110.1, 116.9, 118.8, 122.7, 127.0, 127.5, 127.9, 128.4, 129.0, 129.1, 129.2, 131.9, 132.6, 133.4, 134.9, 135.9, 144.3, 147.7, 162.9, 166.7, 173.3, 177.1; HRMS (ESI) Calcd for [C₃₉H₃₅F₅N₂O₆S + H] 753.2052 found 753.2076; HPLC (I) tₚ = 27.66 min (94.5 %), (II) tₚ = 62.02 min (94.1 %).
5.3u, 4-(N-(4-cyclohexylbenzyl)-2-(N-((perfluorophenyl)methyl)naphthalene-1-sulfonamido)acetamido)-2-hydroxybenzoic acid. \( \delta_H \) (400 MHz, \( d\)-CDCl\(_3\)) 1.28-1.46 (m, 5H, CH\(_2\)), 1.69-1.89 (m, 5H, CH\(_2\)), 2.39-2.51 (m, 1H, CH), 4.12 (s, 2H, CH\(_2\)), 4.74 (s, 4H, 2 CH\(_2\)), 6.56 (d, \( J \) = 8.0 Hz, 1H, CH), 6.67 (s, 1H, CH), 6.98 (d, \( J \) = 7.8 Hz, 2H, CH), 7.09 (d, \( J \) = 7.8 Hz, 2H, CH), 7.46-7.61 (m, 3H, CH), 7.83-7.94 (m, 2H, CH), 8.02 (d, \( J \) = 8.2 Hz, 1H, CH), 8.32 (d, \( J \) = 7.3 Hz, 1H, CH), 8.41 (d, \( J \) = 8.2 Hz, 1H, CH); \( \delta_C \) (100 MHz, \( d\)-CDCl\(_3\)) 26.1, 26.9, 34.4, 39.9, 44.2, 49.6, 53.1, 109.9, 117.1, 119.1, 124.0, 124.4, 126.8, 127.0, 128.2, 128.5, 128.8, 130.7, 132.5, 133.3, 134.0, 134.1, 134.7, 147.5, 147.7, 163.0, 166.6, 173.1, 177.3; HRMS (ESI) Calcd for [C\(_{39}\)H\(_{33}\)F\(_3\)N\(_2\)O\(_6\)S + H] 753.2052 found 753.2054; HPLC (I) \( t_R \) = 27.40 min (90.6 %), (II) \( t_R \) = 61.30 min (90.6 %).

5.3v, 4-(N-(4-cyclohexylbenzyl)-2-(5-(dimethylamino)-N-((perfluorophenyl)methyl)naphthalene-1-sulfonamido)acetamido)-2-hydroxybenzoic acid. \( \delta_H \) (400 MHz, \( d\)-CDCl\(_3\)) 1.29-1.45 (m, 5H, CH\(_2\)), 1.69-1.91 (m, 5H, CH\(_2\)), 2.40-2.53 (m, 1H, CH,
3.86 (s, 6H, 2CH₃), 4.13 (s, 2H, CH₂), 4.75 (s, 2H, CH₂), 4.77 (s, 2H, CH₂), 6.57 (d, J = 8.2 Hz, 1H, CH), 6.67 (s, 1H, CH), 7.00 (d, J = 8.0 Hz, 2H, CH), 7.10 (d, J = 8.0 Hz, 2H, CH), 7.14 (d, J = 7.6 Hz, 1H, CH), 7.42-7.52 (m, 2H, CH), 7.89 (d, J = 8.3 Hz, 1H, CH), 8.04 (d, J = 8.6 Hz, 1H, CH), 8.31 (d, J = 7.3 Hz, 1H, CH), 8.50 (d, J = 8.5 Hz, 1H, CH); δC (100 MHz, d-CDCl₃) 26.1, 26.9, 34.4, 40.0, 44.2, 49.4, 53.0, 110.1, 116.9, 118.8, 122.7, 127.0, 127.5, 127.9, 128.4, 129.0, 129.1, 129.2, 131.9, 132.1, 132.3, 133.4, 133.9, 135.9, 138.7, 144.3, 146.8, 147.7, 162.9, 166.7; HRMS (ESI) Calcd for [C₄₁H₃₈F₅N₃O₆S + H] 796.2474 found 796.2488; HPLC (I) tᵢR = 26.43 min (95.6 %), (II) tᵢR = 58.54 min (96.2 %).

5.7. 4-(N-(4-cyclohexylbenzyl)-2-(N-methyl-4-(trifluoromethyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. δH (400 MHz, d-CDCl₃) 1.29-1.46 (m, 5H, CH₂), 1.67-1.91 (m, 5H, CH₂), 2.40-2.53 (m, 1H, CH), 2.91 (s, 3H, CH₃), 3.58 (s, 2H, CH₂), 4.79 (s, 2H, CH₂), 6.58 (d, J = 8.0 Hz, 1H, CH), 6.70 (s, 1H, CH), 7.04 (d, J = 7.8 Hz, 2H, CH), 7.11 (d, J = 7.8 Hz, 2H, CH), 7.72 (d, J = 8.0 Hz, 2H, CH), 7.86-7.97 (m, 3H, CH); δC (100 MHz, d-CDCl₃) 26.1, 26.9, 34.4, 35.8, 44.0, 51.5, 53.1, 112.8, 116.7, 118.7, 121.8, 124.5, 125.9, 125.9, 126.9, 127.9, 128.3, 132.4, 133.2, 133.9, 134.2, 142.0, 146.9, 147.7, 162.7, 166.8, 172.8, 177.4; HRMS (ESI+) calcd for [C₃₀H₃₁F₃N₂O₆S + H] 605.1927, Found 605.1924; HPLC (I) tᵢR = 25.75 min (100 %), (II) tᵢR = 56.40 min (100 %).
5.10a, 4-(N-(4-cyclohexylbenzyl)-2-(4-fluoro-N-(2-(trifluoromethyl)benzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. \( \delta_H \) (400 MHz, \( d\)-CDCl\(_3\)) 1.30-1.45 (m, 5H, CH\(_2\)), 1.69-1.91 (m, 5H, CH\(_2\)), 2.39-2.52 (m, 1H, CH), 3.82 (s, 2H, CH\(_2\)), 4.70 (s, 2H, CH\(_2\)), 4.78 (s, 2H, CH\(_2\)), 6.37 (s (br), 1H, CH), 6.50 (s, 1H, CH), 6.96 (d, \( J = 7.8 \) Hz, 2H, CH), 7.09 (d, \( J = 7.8 \) Hz, 2H, CH), 7.14-7.21 (m, 2H, CH), 7.36 (t, \( J = 7.5 \) Hz, 1H, CH), 7.48 (t, \( J = 7.7 \) Hz, 1H, CH), 7.63 (d, \( J = 7.8 \) Hz, 1H, CH), 7.69 (d, \( J = 7.8 \) Hz, 1H, CH), 7.77 (d, \( J = 8.2 \) Hz, 1H, CH), 7.87-7.98 (m, 2H, CH); \( \delta_C \) (100 MHz, \( d\)-CDCl\(_3\)) 26.0, 26.7, 34.2, 44.1, 47.4, 47.7, 52.9, 111.7, 116.0 (d), 116.9, 118.8, 122.6, 125.3 (q), 125.7 (q), 126.8, 127.7, 128.4, 129.8, 130.3, 130.4, 132.1, 132.3, 133.1, 134.3, 135.6 (d), 147.6, 162.8, 163.8, 166.2, 172.3; HRMS (ESI+) Calcd for [C\(_{36}\)H\(_{34}\)F\(_4\)N\(_2\)O\(_6\)S + H] 699.2146 found 699.2160; HPLC (I) \( t_R = 27.17 \) min (99.1 %), (II) \( t_R = 60.77 \) min (98.9 %).

5.10b, 4-(N-(4-cyclohexylbenzyl)-2-(4-fluoro-N-(2-methylbenzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. \( \delta_H \) (400 MHz, \( d\)-
CDCl\textsubscript{3}) 1.31-1.45 (m, 5H, CH\textsubscript{2}), 1.70-1.94 (m, 5H, CH\textsubscript{2}), 2.19 (s, 3H, CH\textsubscript{3}), 2.40-2.56 (m, 1H, CH), 3.69 (s, 2H, CH\textsubscript{2}), 4.57 (s, 2H, CH\textsubscript{2}), 4.65 (s, 2H, CH\textsubscript{2}), 6.11 (s (br), 1H, CH), 6.35 (s, 1H, CH), 6.98 (d, J = 7.6 Hz, 2H, CH), 7.00-7.23 (m, 8H, CH), 7.71 (s (br), 1H, CH), 7.86-7.97 (m, 2H, CH); \textit{\delta}C (100 MHz, d-CDCl\textsubscript{3}) 26.0, 26.7, 34.3, 44.1, 46.8, 49.0, 52.8, 110.4, 115.9 (d), 116.7, 118.6, 126.0, 126.8, 128.4, 128.5, 129.9, 130.3, 130.4, 130.7, 132.2, 133.3, 135.5 (d), 137.6, 147.6, 162.7, 163.7, 166.2, 166.6, 172.4; HRMS (ESI+) Calcd for [C\textsubscript{36}H\textsubscript{37}FN\textsubscript{2}O\textsubscript{6}S + H] 645.2429 found 645.2411; HPLC (I) \textit{t}_{R} = 27.08 min (99.7 %), (II) \textit{t}_{R} = 60.22 min (98.4 %).

5.10c, 4-(N-(4-cyclohexylbenzyl)-2-(4-(trifluoromethyl)-N-(2-(trifluoromethyl)benzyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. \textit{\delta}H (400 MHz, d-CDCl\textsubscript{3}) 1.30-1.43 (m, 5H, CH\textsubscript{2}), 1.70-1.92 (m, 5H, CH\textsubscript{2}), 2.40-2.52 (m, 1H, CH), 3.85 (s, 2H, CH\textsubscript{2}), 4.69 (s, 2H, CH\textsubscript{2}), 4.80 (s, 2H, CH\textsubscript{2}), 6.35 (d, J = 7.4 Hz, 1H, CH), 6.50 (s, 1H, CH), 6.96 (d, J = 8.0 Hz, 2H, CH), 7.09 (d, J = 8.0 Hz, 2H, CH), 7.38 (t, J = 7.6 Hz, 1H, CH), 7.49 (t, J = 7.5 Hz, 1H, CH), 7.65 (d, J = 7.8 Hz, 1H, CH), 7.70 (d, J = 7.8 Hz, 1H, CH), 7.74-7.83 (m, 3H, CH), 8.04 (d, J = 8.2 Hz, 2H, CH); \textit{\delta}C (100 MHz, d-CDCl\textsubscript{3}) 26.0, 26.7, 34.2, 44.1, 47.5, 47.7, 52.9, 112.0, 116.8, 118.7, 121.9, 122.6, 124.6, 125.3, 125.8 (m), 126.8, 127.9, 128.1, 128.4, 129.9, 132.2, 132.3, 133.1, 133.9, 134.0, 134.3, 143.2, 147.7, 162.8, 165.9, 172.1; HRMS (ESI+) Calcd for [C\textsubscript{37}H\textsubscript{34}F\textsubscript{6}N\textsubscript{2}O\textsubscript{6}S + H] 749.2114 found 749.2120; HPLC (I) \textit{t}_{R} = 28.12 min (100 %), (II) \textit{t}_{R} = 63.17 min (100 %).
5.10d, 4-(N-(4-cyclohexylbenzyl)-2-(N-(2-methylbenzyl)-4-(trifluoromethyl)phenylsulfonamido)acetamido)-2-hydroxybenzoic acid. $\delta_H$ (400 MHz, $d$-CDCl$_3$) 1.32-1.49 (m, 5H, CH$_2$), 1.71-1.94 (m, 5H, CH$_2$), 2.23 (s,3H, CH$_3$), 2.42-2.55 (m, 1H, CH), 3.73 (s, 2H, CH$_2$), 4.63 (s, 2H, CH$_2$), 4.67 (s, 2H, CH$_2$), 6.15 (s (br), 1H, CH), 6.37 (s, 1H, CH), 6.98 (d, $J = 7.8$ Hz, 2H, CH), 7.02-7.19 (m, 5H, CH), 7.23 (t, $J = 7.8$ Hz, 1H, CH), 7.72 (d, $J = 8.2$ Hz, 1H, CH), 7.79 (d, $J = 8.2$ Hz, 2H, CH), 8.04 (d, $J = 8.0$ Hz, 2H, CH); $\delta_C$ (100 MHz, $d$-CDCl$_3$) 18.8, 26.0, 26.7, 34.3, 44.1, 46.9, 49.1, 52.9, 111.6, 116.8, 118.8, 121.9, 124.6, 125.8, 125.8, 126.1, 126.8, 128.1, 128.5, 130.0, 130.8, 131.8, 132.1, 133.1, 133.8, 134.2, 137.7, 143.1, 147.1, 147.7, 162.7, 166.4, 172.4; HRMS (ESI+) Calcd for [C$_{37}$H$_{37}$F$_3$N$_2$O$_6$S + H] 695.2397 found 695.2417; HPLC (I) $t_R = 28.07$ min (100 %), (II) $t_R = 62.81$ min (100 %).
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