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UMI
Synthesis of α,α-Difluoromethylenesulfonic Acids on a Soluble Polymer Support and Their Evaluation as Inhibitors of Protein Tyrosine Phosphatases and Aryl Sulfatases

by

Carmen Leung

A thesis submitted in conformity with the requirements for the degree of Masters of Science
Graduate Department of Chemistry
University of Toronto

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Abstract

Synthesis of α,α-Difluoromethylenesulfonic Acids on a Soluble Polymer Support and Their Evaluation as Inhibitors of Protein Tyrosine Phosphatases and Aryl Sulfatases

Degree of Master of Science, 2000

by Carmen Leung

Department of Chemistry, University of Toronto

A small molecule library of biaryl derivatives bearing the α,α-difluoromethylenesulfonic acid (DFMS) group was prepared on non-crosslinked polystyrene (NCPS), a soluble polymer support. The liquid phase organic synthesis approach was employed which enabled the reactions to be carried out under homogeneous conditions and monitored by conventional 19F NMR. To prepare the library, (3-bromophenyl)(difluoro)methanesulfonates were attached to 5% 4-hydroxylated NCPS via an ether linkage. Suzuki cross-coupling was then used to form the polymer-bound biaryl compounds. The products were cleaved off the polymer support by mild basic hydrolysis. Product yields ranged from 34-97%, with most of them being suitable for biological screening (95-100% pure). The biaryl DFMS compounds were examined as inhibitors of two protein tyrosine phosphatases, PTP1B and CD45, as well as with crude aryl sulfatase from H. pomatia. The most potent inhibitor for these three enzymes had an IC50 of 14 µM, 31 µM, and 334 µM respectively.
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Dedicated to my parents

and

my best friend Tom
1 Introduction

1.1 Overview and Global Objectives

Many biological processes that take place in eukaryotic cells are controlled by the phosphorylation and dephosphorylation of serine, threonine, and tyrosine residues in certain proteins.\(^1\), \(^2\), \(^3\), \(^4\), \(^5\), \(^6\) The two classes of enzymes that control the phosphorylation state of these residues are the kinases, which introduce the phosphate group, and the phosphatases, which hydrolyze the phosphate groups off. Together, they act as "on and off switches" for regulating cellular signal transduction pathways. Over the past decade, considerable attention has been given to the study of protein tyrosine phosphatases (PTPases). They have been shown to have fundamental roles in regulating numerous cell functions, including the cell cycle, cytoskeletal organization, immune response, and insulin signaling.\(^1\), \(^2\), \(^3\), \(^4\), \(^5\), \(^6\) Overexpression, deletion, or malfunction of certain phosphatases may lead to disease states such as diabetes, cancers, and immune dysfunctions.\(^7\) As a result, there is much interest in developing inhibitors for these enzymes since they could be used as novel therapeutics, as well as tools for studying specific signal transduction pathways. Our global objective is to develop inhibitors for a PTPase known as PTP1B. There is now considerable evidence implicating PTP1B as a negative regulator for insulin receptor signaling. Consequently, the development of inhibitors for this enzyme could be used as therapeutics for certain types of diabetes. With this in mind, we present here our initial studies on developing methodologies for rapidly constructing potential inhibitors of PTPases. More specifically, we describe herein the construction of a small molecule library of non-peptidyl compounds containing the difluoromethanesulfonic acid (DFMS) moiety, a non-hydrolyzable phosphotyrosine
mimetic, using polymer supported organic synthesis techniques. The methodology developed here should significantly decrease the time and effort required for obtaining inhibitors for PTP1B and possibly other therapeutically important phosphatases.

1.2 Protein Tyrosine Phosphatases (PTPases)

1.2.1 Classes of PTPases

In eukaryotic cells, the majority of protein phosphorylation occurs on serine and threonine residues. Tyrosine phosphorylation only accounts for 0.01 to 0.05% of the total protein phosphorylation. PTPases are responsible for hydrolyzing phosphate groups off of phosphotyrosine residues in peptides and/or proteins (Scheme 1). There are now more than 100 PTPases identified. They are nonmetalloenzymes comprised of a single polypeptide chain. Although there is little sequence similarity among PTPases, there is one unique feature that defines them and that is the relative placements of the conserved cysteine and arginine residues found in the active site. These two residues, separated by five amino acids, constitute the PTP signature motif (H/V)C(X)₂R(S/T), where X is any amino acid.⁴

![Scheme 1. Action of PTPases](image-url)
The tyrosine phosphatase superfamily can be categorized into four major groups: (1) the tyrosine-specific phosphatases (PTPases), (2) the VH-1-like dual specificity phosphatases, (3) the cdc25 (cell division control), and (4) the low-molecular-weight phosphatases. The tyrosine-specific phosphatase can be further sub-categorized into receptor-like and intracellular PTPases. The receptor-like PTPases, such as the leukocyte phosphatase, CD45, generally have an extracellular domain, a single transmembrane region, and one or two cytoplasmic PTPase domains. Some receptor-like PTPases have two tandem homologous PTPase domains, and whether only one or both of the domains are catalytically active is still being investigated. The intracellular PTPases, such as PTP1B and the Yersinia PTPase, contain a single catalytic domain and various amino and carboxyl terminal extenstions. The VH-1-like dual specificity phosphatases can hydrolyze phosphotyrosine residues, as well as phosphoserine and phosphothreonine residues. The cdc25 PTPases are important for cell control. Lastly, low-molecular-weight phosphatases have unknown function and seem to be composed of the catalytic domain alone.

1.2.2 Structural Features of PTPases

Crystal structures of several different PTPases have been determined. From these studies, two important structural features that are common among the superfamily of PTPases were found, and that is the phosphate binding loop (PTP loop) and the movable loop (WpD loop).

The PTP loop is found in the active site of phosphatases. It is located within a crevice, that is about 9 Å deep, and is made up of a β strand-loop-α helix element. Centered within the loop (PTP loop) is the PTPase signature motif (H/V)C(X)₅R(S/T).
which contains the essential cysteine and arginine residues required for catalytic activity. Mutation or deletion studies that have been carried out on both these residues either abolished or severely reduced the PTPase activity. Found within the signature motif, and extending two amino acids from it, is the submotif GXGXXG. The first glycine residue has shown to be essential since mutation or deletion of it also abolishes PTPase activity. Phosphotyrosine residues, and other substrates that are structurally similar, bind within the PTP loop. The substrates situate themselves such that the phosphorus atom is ideally positioned for nucleophilic attack from the invariant cysteine residue. At physiological pH, the cysteine residue exists as a thiolate anion, which attacks the phosphotyrosine residue and forms a covalent cysteinyl phosphoenzyme intermediate. The cysteine residue is located at the very center of the PTP loop and within close proximity to every amide nitrogen of the PTP loop peptide backbone. The orientation of the polarized microdipoles arising from the amide nitrogens has been shown to be essential for phosphate binding and thiolate stabilization. The arginine residue plays a role in substrate binding and transition state stabilization. The guanidinium group of the invariant arginine provides stabilization by forming two hydrogen bonds with the oxygen atoms on the substrate. The oxygens are also hydrogen bonded to the amide groups of the PTP loop peptide backbone.

Adjacent to the PTP loop along the surface of PTPases is the movable loop, also referred to as the WpD loop. The sequence of this loop is quite different among the PTPases except for the WpD sequence, which includes an essential catalytic aspartic acid residue and a tryptophan residue located near the hinge position of the loop. When a substrate binds to the PTP loop, the WpD loop moves like a “flap” to cover the
active site. In the absence of a substrate, Asp356 found in *Yersinia* PTPase is located approximately 10 Å away from the phosphate-binding site. However, once substrate binding occurs, the conformation of the enzyme changes such that the Asp356 moves about 6 Å closer to the phosphate binding site.\(^{10, 18}\) Similarly, substrate binding in PTP1B induces closing of the WpD loop. As a result, Asp181 is brought closer to the catalytic site and forms a network of hydrogen bonds with the phenolic oxygen of phosphotyrosine and a buried water molecule.\(^{12}\) Closing of the active site subsequently positions the aspartic acid residue opposite the invariant cysteine residue in the PTP loop and close to the scissile oxygen of the substrate. The change in conformation allows for the aspartic acid residue to act as a general acid during catalysis.\(^{10, 12, 14, 18}\) Overall, there are two states the WpD loop can exist in, and that is the “open” state in which there are negligible interactions between the WpD loop and binding site, and the “closed” state in which the aspartatic acid residue in the WpD loop can hydrogen bond to the substrate. In the absence of substrates, there are equal distributions of PTPases in the “open” and “closed” states, while in the presence of substrates, the PTPases are predominantly found in the “closed” state.\(^{14}\)

### 1.2.3 Catalytic Mechanism

The PTPase catalyzed reaction proceeds through a double displacement mechanism and is carried out by invariant residues found in the PTP loop and the WpD loop. When a substrate binds to PTPase, the phosphoryl group is first transferred to the active site cysteine residue forming a covalent thiophosphate enzyme intermediate. Following this step is hydrolysis of the thiophosphate enzyme intermediate by a water molecule yielding the free enzyme and inorganic phosphate (Scheme 2).\(^{4}\)
Scheme 2. Proposed catalytic mechanism of PTPases
The cysteine residue found in the signature motif, located at the base of the active site, is absolutely essential for PTPase activity. Guan and Dixon demonstrated that when Cys215 was mutated to Ser215 (one atom substitution), there was total loss of enzyme activity, although the substrate still bound. The cysteine residue acts as a nucleophile by attacking the phosphorus of the phosphotyrosine residue of a substrate, which in turn, results in the formation of a thiophosphate enzyme intermediate. Evidence for the covalent thiol phosphate linkage came from the work of Guan and Dixon. They showed that $^{32}\text{P}$-labeled phosphoprotein has a bell-shaped pH-rate profile, with maximal hydrolysis in the pH range of 2.5-3.5. As well, the thiol phosphate linkage hydrolyzed in the presence of iodine or bromine. These two observations suggest the formation of a covalent thiol phosphate linkage. The bond energy of the P-S bond (45-50 kcal/mol) is less than the P-O bond energy (95-100 kcal/mol). Therefore, P-S bond cleavage is much more facile and this is probably one of the reasons nature chose cysteine instead of serine as the crucial nucleophilic residue.

The pKₐ of the cysteine residue in the active site of PTPase is 4.67, thus, at physiological pH it exists as a thiolate anion. Normally, a free cysteine has a pKₐ of 8.5. A reduction in the pKₐ as seen for the invariant cysteine, would require stabilization of the thiolate anion. Most of the stabilizing forces come from the invariant histidine residue that precedes the cysteine in the PTPase signature motif. Substitutions of the histidine to asparagine and alanine increases the pKₐ of the active site thiol from 4.67 to 5.99 and 7.35 respectively. The role of histidine is structural by defining the conformation of the cysteine and PTP-loop. The histidine residue does not have a catalytic role since it interacts with only the carbonyl oxygen of the cysteine and not
directly with the cysteine side chain.\textsuperscript{9, 10} Besides the histidine residue, there are other electrostatic interactions that contribute to the stabilization of the thiolate anion since replacement of the histidine with alanine still results in a $pK_a$ that is one unit lower than free cysteine.\textsuperscript{21, 22} In comparing the $pK_a$ of serine to cysteine, serine has a much higher $pK_a$ of 14. This may explain why there is no PTPase activity in the cysteine to serine mutants since the hydroxyl group would probably not be ionized at physiological pH. Essentially, a hydroxyl group would be a worse nucleophile than a thiolate.\textsuperscript{22}

The conserved arginine residue of the PTPase signature motif plays a role in substrate binding and an even more important role in transition state stabilization.\textsuperscript{23} Mutagenesis studies carried out on the arginine residue resulted in either loss of enzyme activity, or severely reduced activity.\textsuperscript{4, 15, 16, 23, 24} The guanidinium group present in the side chain of the arginine residue provides transition state stabilization by forming ion pairs with the two oxyanion oxygens found on the phosphoryl group.\textsuperscript{4, 23} Studies have shown that a guanidinium group is ideally suited for interaction with phosphate by virtue of its planar structure and its ability to form multiple hydrogen bonds with the phosphate moiety.\textsuperscript{17} Lysine, which also has a cationic side chain, has shown to partially replace arginine in terms of substrate and inhibitor binding. However, lysine cannot replace the catalytic activity of PTPase.\textsuperscript{23} Therefore, the trigonal bipyramidal transition state is likely stabilized by the unique structural properties of the guanidinium group of arginine. As well, the arginine residue is most likely positioned such that it interacts more favorably with the transition state than the ground state.\textsuperscript{4, 17}

Most PTPases, with the exception of cdc25 which is less reactive, has a serine or threonine immediately proceeding the conserved arginine residue. The serine or
threonine residues are positioned such that they can form S-HO hydrogen bonds with the invariant cysteine residue. Mutagenesis studies have shown that the loss of the hydroxyl moiety results in no change in substrate or inhibitor binding, but dramatically affects the rate of intermediate hydrolysis. Therefore, the role of serine or threonine in PTPase activity is most likely to facilitate the breakdown of the thiol phosphate enzyme intermediate by stabilizing the enzyme thiolate leaving group.

Found within the WpD loop is the conserved aspartic acid residue. Upon substrate binding, the WpD loop acts like a "flap" by folding over the active site and swinging the aspartic acid over such that it sits directly above the scissile oxygen of the substrate. Studies have shown that the aspartic acid acts as a general acid by donating a proton to the oxygen of the phenolate leaving group during the formation of the thiol phosphate enzyme intermediate. The aspartic acid may also act as a general base during the dephosphorylation step by activating a water molecule for hydrolysis of the thiol phosphate enzyme intermediate.

As mentioned above, the PTPase reaction proceeds through an in-line double displacement mechanism. In the first transition state, the thiol phosphate enzyme intermediate is formed and it occurs by a dissociative mechanism (Scheme 3). In other words, bond formation to the incoming cysteine nucleophile is minimal and bond breakage between the phosphorus and the leaving group is substantial. To promote and stabilize the dissociative transition state, the invariant aspartic acid residue donates a proton to the phenolate leaving group to reduce the buildup of negative charge.

In the second transition state, the phosphate group is hydrolyzed off the enzyme by a water molecule that is activated by the conserved aspartic acid. Once again, the
Scheme 3. Possible transition state structures for the phosphorylation and dephosphorylation of the invariant cysteine in PTPases
transition state is dissociative where the P-S bond breakage is substantial (Scheme 3). In
this case, the transition state is promoted and stabilized by the serine or threonine residue
found in the PTPase signature motif. The hydroxyl group is able to form an S-OH
hydrogen bond to the leaving thiolate, thus reducing the buildup of negative charge.4, 26, 27, 34

In summary, the common catalytic mechanism carried out by most PTPases
involves the cysteine residue acting as a nucleophile, forming the thiol phosphate enzyme
intermediate. The driving forces behind the formation of the phosphoenzyme
intermediate is the aspartic acid acting as a general acid, and the arginine residue
stabilizing the trigonal bipyramidal transition state. Once the phosphoenzyme
intermediate is formed, a water molecule approaches the side of the intermediate in which
the leaving group has just vacated. When the water molecule becomes activated and/or
positioned correctly by the aspartic acid, dephosphorylation of the phosphoenzyme takes
place and inorganic phosphate is subsequently released out of the active site.

1.3 PTP1B

PTP1B was the first PTPase to be obtained in pure form.35, 36 The purified
enzyme was shown to have 321 amino acids, however, the cDNA indicated that it is
really comprised of 435 residues. The conserved PTP domain is located within residues
30 to 278. The thirty-five carboxyl terminal residues target the enzyme to the
cytoplasmic face of the endoplasmic reticulum membranes.

The crystal structure of human PTP1B has been determined.9 It has a single
catalytic domain which is made up of eight α helices and twelve β strands. The PTPase
signature motif (His214 to Ser222) is situated in loop 15 (PTP loop) connecting the
carboxyl terminus of β-12 with the amino terminus of α-4. PTP1B has the structural and mechanistic characteristics as described above for PTPases.

Research has shown that free phosphotyrosine is a poor substrate for PTP1B. However, when there are residues flanking both sides of the phosphotyrosine residue, binding affinity increases. More specifically, acidic residues positioned on the amino terminal side of the phosphotyrosine enhances binding affinity, while replacement of the acidic residues with arginine have the opposite affect. Zhang et al have shown that a minimum of six amino acids, including a phosphotyrosine residue, are required for efficient substrate binding and catalysis. Upon assigning the phosphotyrosine residue at position 0, the hexapeptide should be comprised of four amino terminal residues positioned from -4 to -1, and one carboxyl terminal residue at position +1.

Jia et al determined the crystal structures of the catalytically inactive Cys215 to Ser215 mutant complexed with the high affinity peptides DADePYL-NH₂ and DDeY-LNH₂ to study the structural basis of substrate recognition by PTP1B. They found that there are numerous ways in which PTP1B recognizes phosphotyrosine peptides. Firstly, the depth of the binding pocket is the same length as a phosphotyrosine residue (9 Å). Phosphoserine and phosphothreonine residues are too short to reach the catalytic cysteine residue at the base of the pocket. Secondly, there are residues with nonpolar side chains lining the binding pocket such that they can form hydrophobic interactions with the phenyl group of the phosphotyrosine residue. Lastly, there are hydrogen bonds and electrostatic interactions formed between the peptide and the enzyme. The crystal structures show that hydrogen bonds are formed between the main chain carbonyl of
peptide residue -2 with the main chain nitrogen of Arg47. As well, the guanidinium side chain of Arg47 forms salt bridges with the side chains at positions -2 and -1, and forms a long hydrogen bond with the main chain carbonyl at -4. These interactions are consistent with the preference of PTP1B for peptides with acidic amino acids amino terminal to the phosphotyrosine residue. Van der Waals contacts are observed between the side chain of Leu at position +1 of the peptide with the side chains of Val49, Ile219, and Gln262. There was also a water-mediated hydrogen bond between the amide group at +1 and the side chain of Gln262.

Further studies were carried by Sarmiento et al to determine the roles of Tyr46, Arg47, Asp48, Phe182, and Gln262 and their influence on substrate recognition.\textsuperscript{42} PTP1B mutants were created by changing Ty46 to Phe and Ala, Arg47 to Glu and Asp, Asp48 to Ala, Phe182 to Ala, and Gln262 to Ala. The effects of the substitutions were evaluated by using p-nitrophenyl phosphate and several phosphotyrosine-containing peptides as substrates. From their results they concluded the following. The aromaticity of Tyr46 is important for substrate recognition and optimal substrate orientation, since the side chain of Tyr46 forms hydrophobic stacking interactions with the phenyl ring of phosphotyrosine as seen in crystal structures. Arg47 interacts with acidic residues in the substrate and is considered an important substrate specificity determinant. Substitution of Arg47 with glutamic acid resulted in a severe decrease in PTP1B activity, likely due to the charge repulsion between the glutamic acid and acidic residues in the substrate. As well, substitutions of the peptide residues at position -2 and -1 with alanine resulted in a decrease in activity, thus further demonstrating the preference of Arg47 for acidic residues. The role of Asp48 is to position the substrates in an optimal conformation for
substrate binding and/or nucleophilic attack by the active site cysteine residue. Phe182 is required for substrate binding and Enzyme-Product formation since its hydrophobic stacking interactions with the phenyl ring of the phosphotyrosine residue brings the general acid Asp181 into proper position for proton donation. Lastly, Gln262 may be important for aligning the phenyl ring of the phosphotyrosine residue in the Enzyme-Product formation step, as well as for optimum positioning of the nucleophilic water molecule for efficient Enzyme-Product hydrolysis.

Recently, Sarmiento et al have learned that Arg47 can adopt two conformations for substrate interaction depending on the nature of the substrate. They observed that when there is an acidic residue at position -1 of the peptide substrate, there exists a preference for a second acidic residue at position -2. The guanidium group of Arg47 allows for favorable electrostatic interactions with the side chains of residues at -1 and -2. From the crystallographic studies carried out by Sarmiento et al. they found that Arg47 can change conformations such that it can also accommodate aromatic groups at position -1. When a phenylalanine or p-benzoylphenylalanine residue was at -1, hydrophobic interactions were observed between the aliphatic part of the Arg47 side chain and peptide positions -1 and -3. The ability of PTP1B to change conformations without disrupting the catalytic activity demonstrates the plasticity in PTP1B substrate recognition. This unique feature should assist in the design of PTP1B specific inhibitors.

1.3.1 PTP1B and Diabetes

Type 2 or non-insulin-dependent diabetes mellitus (NIDDM) is a very serious disease state that affects 80% of people with diabetes. It is characterized by insulin resistance, where the cells no longer respond to normal levels of circulating insulin, and
hyperglycemia (high blood glucose levels). Insulin is a hormone that binds to the insulin receptor, which in turn, activates the receptor to control blood glucose levels. The insulin receptor is a transmembrane glycoprotein comprised of two α-subunits that are each linked to a β-subunit and to each other by disulfide bonds. The α-subunits are extracellular and contain the insulin binding site(s). The β-subunits, which are linked to the α-subunits by a single transmembrane segment, are intracellular and contain intrinsic protein tyrosine kinase activity. Thus, the insulin receptor is also referred to as the insulin receptor kinase (IRK). When insulin binds to its receptor, the IRK activity is activated so that autophosphorylation on residues Tyr1146, Tyr1150, and Tyr1151 of the receptor can take place. Following full activation, IRK phosphorylates or binds to other proteins, such as the insulin receptor substrate 1 (IRS-1), which results in a signaling cascade of secondary phosphorylation and dephosphorylation reactions that eventually leads to glycogen synthesis and lowering of blood glucose levels (Scheme 1).44, 45, 46, 47

To terminate insulin action, even after insulin has left the receptor, dephosphorylation of both the insulin receptor and IRS-1 is required. Several PTPases have been implicated as the negative regulators of the insulin receptor signaling, one of them being PTP1B.4 Kenner et al examined the effects of insulin and insulin-like growth factor (IGF-I) on PTPase activity.48 They found that when there was chronic stimulation of PTPase activity, there was enhanced expression of PTP1B mRNA and protein. They proposed that PTP1B opposed IRK and in turn, desensitized cells to long term action by insulin and IGF-I. Other studies carried out by Kenner et al provided more evidence that PTP1B acts
As a negative regulator of insulin signaling. In their work, they used cell lines that overexpressed insulin and IGF-I with either PTP1B, or the inactive Cys215 to Ser215 mutant of PTP1B. They found that in the cells containing PTP1B, IRK autophosphorylation and IRS-1 phosphorylation were inhibited, whereas the cells with the inactive PTP1B mutant had increased activity. Thus demonstrating the role of PTP1B in the negative regulation of insulin signaling. Ahmad et al demonstrated that IRK autophosphorylation and IRS-1 phosphorylation could be enhanced if PTP1B activity was repressed. In their studies, they used antibodies that immunoprecipitated and inactivated PTP1B by sterically blocking catalytic interactions between PTP1B and its substrates. Their results led them to conclude that PTP1B has a role in the negative regulation of insulin signaling, and also suggested that inhibition of PTP1B could be used as a therapeutic tactic for treatment of type 2 diabetes.

Recently, scientists at McGill University and Merck Frosst Canada collaborated and provided compelling evidence that PTP1B plays a major role in modulating both insulin signaling and fuel metabolism. Elchebly et al first created PTP1B knockout mice, where the mice lacked the gene that encoded for PTP1B. They mated the PTP1B knockout mice with wild type mice to obtain PTP1B+/− (wild type), PTP1B−/−
(heterozygotes for PTP1B gene and expressed half the amount of PTP1B as the wild type mice), and PTP1B\textsuperscript{−−} (homozygotes for the lack of PTP1B) progeny mice. The three types of mice were subjected to glucose and insulin tolerance tests. In comparison to the wild type mice, PTP1B\textsuperscript{−−} mice were found to be highly insulin sensitive since the blood glucose levels were slightly lower and maintained with half the level of circulating insulin. These results seen in the PTP1B\textsuperscript{−−} mice was most likely due to prolonged phosphorylation of the receptor. It suggests that the role of PTP1B is to turn off the insulin signal by dephosphorylating the activated insulin receptor, thus acting as the insulin receptor PTPase. To further probe the role of PTP1B, Elchebly et al. measured the levels of tyrosine phosphorylation of the insulin receptor and IRS-1 in the liver and muscle tissue of the mice. After injection of insulin, they found the PTP1B\textsuperscript{−−} mice had increased levels of phosphorylation in comparison to the wild type mice, which is consistent with the suggestion of PTP1B acting as the insulin receptor PTPase. The mice were also subjected to a high fat diet to determine the effect of obesity on insulin sensitivity, since obesity results in insulin resistance. While on the high fat diet, the wild type mice became fat and insulin resistant, while the PTP1B\textsuperscript{−−} mice were obesity resistant and maintained insulin sensitivity.\textsuperscript{51} The results observed in the PTP1B\textsuperscript{−−} mice were surprising since insulin is a highly potent agent that promotes the storage of carbohydrates and fat.\textsuperscript{46} From the work of Elchebly et al., PTP1B has been implicated as a highly potential therapeutic target for the treatment of type 2 diabetes and obesity since the absence of PTP1B activity causes enhanced insulin sensitivity and resistance to weight gain in mice.\textsuperscript{51}
1.4 PTPase Inhibitors

1.4.1 Peptide-Based Inhibitors

The first approach towards designing inhibitors for enzymes is usually taking the natural peptide substrate and replacing the reactive residue with a nonreactive one. In the case of PTPases, the scissile oxygen of phosphotyrosine (pTyr. 3) would have to be replaced with a non-hydrolyzable functionality. One of the first potent PTP1B peptide-based inhibitor was developed by Burke et al.\textsuperscript{52} They synthesized two hexameric peptides Ac-D-A-D-E-X-L-amide. where X was a non-hydrolyzable phosphoryltyrosyl mimetic. The specific sequence of the hexamer has been shown to be a very good substrate for PTP1B. The first peptide contained a phosphonomethyl phenylalanine (Pmp. 4) residue where the scissile oxygen was replaced with a methylene moiety. The peptide had an IC\textsubscript{50} of 200 \( \mu \)M. However, when a difluoromethylene group was put in place of the methylene group, making it a phosphonodifluoromethyl phenylalanine (F\textsubscript{2}Pmp. 5) residue, the IC\textsubscript{50} was decreased to a remarkable 100 nM.\textsuperscript{52} The 2000-fold enhancement was originally thought to be due to the fluorines lowering the pK\textsubscript{a2} of the phosphonate group, as well as the introduction of specific interactions between the fluorines and the active site. However, inhibition studies have shown that the lower pK\textsubscript{a2} is not a contributing factor to inhibitor potency because both the monoanionic and dianionic forms of F\textsubscript{2}Pmp bind equally well to PTP1B. Therefore, the F\textsubscript{2}Pmp-containing peptide is most likely a better inhibitor than the Pmp-containing peptide because of the fluorines being able to form specific interactions with the active site residues, or restore the hydrogen bonding interactions that exists between the scissile oxygen of phosphotyrosine and the side chains of the residues in the active site.\textsuperscript{53}
Although F₂Pmp-containing peptides can be very potent inhibitors, there is a disadvantage to using them in cellular studies. At physiological pH, the difluoromethyleneephosphonic acid (DFMP) moiety is di-ionized, which decreases the ability of the peptides to penetrate cellular membranes. Therefore, Kole et al designed L-O-malonyltyrosine (L-OMT, 6), which has two carboxylic acids in place of the phosphate group, as an alternative phosphotyrosine mimic. The advantage of using a malonyl structure is that the di-acid can be protected as the di-ester for delivery across the cell membranes. Once the substrate is inside the cell, the di-acid can be deprotected by esterases. L-OMT-containing peptides were prepared and tested against PTP1B. It demonstrated significantly higher potency (IC₅₀ = 10 μM) than the analogous Pmp-containing peptides. Based on the observation that F₂Pmp is more effective than Pmp, Burke et al introduced a fluorine to L-OMT to give 4'-O-[2-(2-fluoromalonyl)-L-tyrosine, (FOMT, 7). They found a 10-fold enhancement (IC₅₀ = 1 μM) in inhibitory potency, which once again was probably due to new bonding interactions and not due to the lowering of pKₐ values.
In an attempt to further enhance effects of OMT-containing peptides, researchers have designed cyclized peptides to restrain conformational flexibility.\textsuperscript{56, 57} When the hexamer D-A-D-E-OMT-L was cyclized by means of a sulfide bridge between the side chain of a C-terminally added cysteine residue and the β-carbon of a N-terminal acetyl residue (9a), it exhibited a $K_i$ of 0.73 μM. The cyclic peptides were screened against PTP1 which is the rat equivalent of PTP1B found in humans. In comparison, the linear counterpart of the cyclized OMT-containing peptide (8) had a $K_i$ of 13 μM which was 18-fold worse. It was suggested that the conformation restriction allow for a better fit of the
OMT residue within the binding pocket.\textsuperscript{56} A cyclized FOMT-containing peptide (9b) was also synthesized and tested against PTP1. It had a $K_i$ of 0.17 $\mu$M, which was a 4-fold enhancement in comparison to the OMT variant.\textsuperscript{57}

The FOMT residue contains only one fluorine atom. Thus, the introduction of a second fluorine atom could possibly enhance the inhibitor potency of peptides. Fretz has developed the synthesis for $O$-(carboxydifluoromethyl)-L-tyrosine (10), in which a carboxyl group in FOMT is replaced with a fluorine.\textsuperscript{58} In his studies, he found that the $pK_a$ and van der Waals' volume of the difluoroacetic acid group of 10 and the phosphate group of phosphotyrosine were similar to each other. This implies that 10 could be a very good phosphotyrosine mimetic. Unfortunately, there has yet to be any reports on the screening of such peptides against PTP1B.

The inhibitor effects of OMT- or FOMT-containing peptides relies on the action of cellular esterase to deprotect the peptides when the OMT or FOMT are protected as the diester. However, studies have shown that esterase treatment results in the removal of only one ester.\textsuperscript{59} The limitation to mono de-esterfication may be due to the close proximity of the malonyl $\alpha,\alpha$-carboxyl groups. Thus, Burke \textit{et al} designed malonyl analogues where the two carboxyl groups are spatially separated to make di de-esterfication more amenable, but within close proximity to maintain the phosphate-mimicking character. Their best inhibitor against PTP1 was the hexamer Ac-D-A-D-E-X-L-amide, where X was 3-carboxy-4-(O-carboxymethyl)-L-tyrosine (11). A $K_i$ of 3.6 $\mu$M was determined for 11, which was not as potent as the FOMT-containing peptide.\textsuperscript{59}
Researchers at Ontagen Corp. found that para-substituted cinnamates have moderate inhibition towards PTP1B. As such, they prepared a library of tripeptides acylated at the amino terminus with ρ-carboxycinnamic acid. Their results showed that inhibitors containing glutamate residues were the most potent inhibitors, while the lysine containing inhibitors were the poorest. This is consistent with the enzyme's preference for acidic residues. The best inhibitor in their library had a $K_i$ of 79 nM (12). Although they were able to develop a highly potent inhibitor, it appears that these compounds may be irreversible inhibitors with the cinnamoyl group acting as a Michael acceptor for the invariant cysteine residue.
Another non-phosphorus containing phosphotyrosine mimetic is the sulfotyrosine residue (sY. 13). Studies have shown that a tris-sulfotyrosyl-containing peptide can enhance insulin signaling by inhibiting PTP1B. Desmarais et al investigated other sulfotyrosyl-containing peptides as potent inhibitors of PTP1B and CD45, a receptor type PTPase. The IC₅₀'s of their substrates were found in the low to mid-micromolar range. In comparison to the F₂Pmp-containing peptides, the sulfotyrosyl derivatives were less potent. Interestingly, there has been no report of a sulfonate analogue of F₂Pmp where the introduction of fluorine atoms could significantly enhance inhibitor potency.

![Chemical Structure of sY](image)

1.4.2 Non-Peptidyl Metal-Containing Inhibitors

A number of metal-containing small molecules have shown to competitively inhibit PTPases, including gallium nitrate, phenylarsine oxide, vanadate, and pervanadate (complexes of vanadate with hydrogen peroxide). These molecules are thought to inhibit PTPases by either binding as a transition state analogue, that is, adopting a trigonal bipyramidal structure to mimic the transition state of the phosphoryl transfer reactions, or by oxidizing the invariant cysteine residue to prevent nucleophilic
attack. Unfortunately, these metal-containing small molecules are nonselective and are potentially toxic.\textsuperscript{7,52,65,66}

1.4.3 Non-Peptidyl Irreversible Inhibitors

Attempts have been made to design irreversible PTPase inhibitors. By taking advantage of the nucleophilic nature of the invariant cysteine residue, introduction of a reactive electrophilic center in the inhibitors could lead to covalent bond formation between the nucleophile and inhibitor, resulting in irreversible inhibition.

4-Difluoromethylphenyl bis(cyclohexylammonium) phosphate (14) has been shown to be a suicide inhibitor of SHP, a cytoplasmic PTPase. The mechanism involves the enzymatic release of difluoromethyl phenol which rapidly eliminates fluoride, generating a reactive quinone methide. This species can then be attacked by nucleophilic residues at the enzyme active site, resulting in a covalent attachment (15) and subsequently the inactivation of the enzyme (Scheme 5).\textsuperscript{67}

![Scheme 5. Mechanism of inhibition of PTPases by 4-difluoromethylphenyl bis(cyclohexylammonium) phosphate (14)](image-url)
Quiescent affinity labels are reagents that contain weakly electrophilic groups that normally participate poorly in $S_N2$ reactions. As well, these types of reagents are only chemically reactive when bound to the enzyme active site. Widlanski and coworkers developed three potential quiescent affinity reagents (16a-c) and tested them against a truncated recombinant form of Yop51 PTPase.$^{68}$ Compound 16a caused little or no inactivation. 16b gave modest inactivation, and 16c was by far the best inactivator. Two mechanisms have been proposed for the inactivation process, both involving the highly nucleophilic cysteine residue and subsequent formation of a covalent bond. The first mechanism involves a $S_N2$ reaction in which the active site nucleophile displaces the halogen (Scheme 6A). The second mechanism involves addition of the cysteine residue to the phosphonate to give a phosphorane, followed by closure to a three-membered ring which may further react (Scheme 6B).$^{68}$
Menadione (17) and naphthoquinone analogues have been shown to inhibit PTPase cdc25a. On the other hand, the sulfone analogue of naphthoquinone (18) is unable to inhibit cdc25a, as well as cdc25b, cdc25c, LAR, and *Yersinia* PTP. However, it is potent towards PTP1B with a \( K_i \) of 3.5 \( \mu M \). The proposed mechanism of inactivation by these compounds involves a Michael addition of the nucleophilic cysteine thiol to the enone, thus covalently modifying the enzyme active site.
Although the above compounds have shown to inactivate PTPases irreversibly. they suffer from low potency, non-selective inactivation of enzymes, and/or possible non-specific interaction(s) with other cellular nucleophiles.7

1.4.4 Non-Peptidyl Reversible Organic Inhibitors

Peptide based inhibitors are less attractive as lead compounds for designing drugs because they are usually susceptible to proteolytic degradation and often exhibit poor cellular uptake. As a result, there is more attention in developing small, organic, reversible, competitive inhibitors as drug candidates.

The DFMP moiety proved to be very potent towards PTPases when incorporated into peptides. As such, Kole et al examined several phenyl- (19a-d) and naphthylphosphonates (20. 21a) against PTP1B and PP2A, a serine/threonine phosphatase.71 The phenylphosphonates were very poor inhibitors. However, there was an increase in inhibitory activity as the methylene unit (19a) was replaced with -CHOH-(19b). -CHF-(19c) and -CF2- (19d), the most potent inhibitor being the phenyl derivative bearing the -CF2- unit. The naphthyl derivatives, on the other hand, were much more potent. Upon introducing the second aryl ring, both 20 and 21a inhibited PTP1B and PP2A effectively and equally well. The Ks for 20 and 21a against PTP1B was determined to be 255 and 179 μM respectively, suggesting that changing the position of the DFMP group in the naphthylphosphonates does not have any significant effect on the potency of inhibition.71,72 X-ray studies of mutant PTP1B (Cys215 to Ser) complexed with 21a revealed that the enhanced inhibitor activity was due to extensive hydrophobic interactions formed with the naphthalene ring. The same interactions were not possible with the phenyl analogue.72 The studies also revealed two water molecules that
interacted with Tyr46, Lys120, and Asp181 of the enzyme and were in close proximity to the naphthalene ring. Thus, Burke et al introduced a hydroxyl group to the 4-position of the naphthalene ring (21b). The result was a $K_i$ that was 2-fold lower ($K_i = 93 \, \mu M$) in comparison to the parent molecule 21a. Molecular modeling studies confirmed that the enhanced inhibitor activity was due to new hydrogen bonds formed between the hydroxyl group and enzyme residues Tyr46 and Lys120.\(^7\)

![Chemical structures](image)

19a $X = \text{CH}_2$
19b $X = \text{CHOH}$
19c $X = \text{CHF}$
19d $X = \text{CF}_2$

20

21a $X = \text{H}$
21b $X = \text{OH}$

Taylor et al have shown that the meta-biphenyl DFMP 22 is a good inhibitor of PTP1B ($K_i$ of 17 $\mu M$).\(^7\) The introduction of a second aryl ring resulted in a 17-fold enhancement in potency in comparison to the phenyl DFMP (19d). Based on these observations, Hum et al prepared a series of biaryl derivatives bearing the DFMP group either at the meta or para position (23a, b).\(^7\) The most potent inhibitor against PTP1B was a triphenyl derivative (24) from the meta series of biphenyl DFMP compounds. Compound 24 was a competitive inhibitor and had a $K_i$ of 1.7 $\mu M$.\(^7\) In general, Hum et al found the meta series of inhibitors more potent than the para series\(^7\), which is consistent with the results obtained from Taylor et al.\(^7\)
The introduction of a second DFMP group to naphthyl derivatives already bearing one DFMP group has been shown to enhance inhibitor activity and PTPase selectivity. \cite{76} DFMP substitutions at the 2,7-positions (25a) and 2,6-positions (25b) had IC$_{50}$ values of 26 and 29 μM respectively when screened against PTP1B. An IC$_{50}$ of 27 μM was observed against CD45 when there were DFMP groups at the 1,7-positions (25c). All three compounds showed a small degree of selectivity between PTP1B and CD45, and a large degree of selectivity against PP2A (IC$_{50}$ > 300 μM for 25a, b, c). To further investigate the effects of introducing a second DFMP group, Taylor \textit{et al} examined bis-DFMP diphenylmethyl derivatives (26) with varying lengths of the hydrocarbon chain linking the two aryl groups (n = 1, 2, 3, or 4). \cite{73} When screened against PTP1B, the 1,4-diphenylbutane (26, n = 4) derivative was the best inhibitor, exhibiting an IC$_{50}$ of 4.4 μM and a K$_{i}$ of 1.5 μM.
Phenyl DFMP is a poor inhibitor. However, when a second aryl ring with a DFMP group is added on, the inhibitor potency increases dramatically. To understand the structural basis of increased binding affinity with biphenyl derivatives, X-ray studies were carried out on mutant PTP1B (Cys215 to Ser) complexed with either phosphotyrosine or bis-(para-phosphophenyl) methane (BPPM, 27). Analysis of the crystal structures revealed a second aryl phosphate-binding site. It is a low-affinity, non-catalytic binding site adjacent to the active site, which suggests that high affinity binding of bis-DFMP diphenylmethyl derivatives may be a result of one of the phosphate groups occupying the catalytic site and the other occupying the low affinity non-catalytic site. However, recent X-ray analysis of the PTP1B-26 complex revealed that one DFMP
group binds at the catalytic site, while the other DFMP group forms non-specific interactions with an arginine residue and does not interact with the second "phosphate"-binding site. There appears to be two mutually exclusive binding modes. One of the DFMP groups bind in either the catalytic site or the non-catalytic site, while the second DFMP group is bound by a combination of electrostatic, hydrophobic, aromatic-aromatic, and water-mediated hydrogen bonds in an area slightly removed from both the catalytic and non-catalytic sites.

Small, organic, reversible, competitive inhibitors do not necessarily need the phosphate moiety to inhibit PTPases. Some examples are presented below.

Nitroarylhydroxymethylphosphonic acids (28a, b and 29), where the nitro and α-hydroxy groups are essential for activity, have shown to inhibit CD45. Frechette et al examined a series of α-hydroxyphosphonates and found that 28a was the best inhibitor of CD45 with an IC₅₀ of 1.2 μM. Similar studies were carried out by Beers et al. The best inhibitors they found were 28b and 29, both displaying an IC₅₀ of 2 μM.

\[
\begin{align*}
\text{28a} & \quad X = \text{C}_6\text{H}_{11}-\text{S}- \\
\text{28b} & \quad X = 4-\text{Br-C}_6\text{H}_4-\text{O}- \\
\end{align*}
\]

Some studies have modeled on natural products in designing PTPase inhibitors. Based on natural product inhibitors of phosphothreonine phosphatases, Rice et al
found that out of a series of structurally similar compounds, 30 was the most potent.\textsuperscript{81} It had a $K_i$ of 0.85 $\mu$M against PTP1B and 8 $\mu$M, 7 $\mu$M, and 11 $\mu$M against cdc25A, cdc25B, and cdc25C respectively. Although 30 showed moderate selectivity for PTP1B relative to the cdc PTPases, there was significant selectivity in comparison to PP1 and PP2A since it was unable to inhibit both PP-type enzymes at 100 $\mu$M.\textsuperscript{81} Sulfurcin (31a), a marine natural product isolated from a deep-water sponge, has shown to inhibit cdc25A, PTP1B, and VHR with IC\textsubscript{50} values of 7.8 $\mu$M, 29.8 $\mu$M, and 4.7 $\mu$M respectively.\textsuperscript{82} However, when the methyl group was replaced with a hydrogen (31b), the IC\textsubscript{50} values decreased to 2.8 $\mu$M, 4.4 $\mu$M, and 4.6 $\mu$M for cdc25A, PTP1B, and VHR respectively. Removal of the methyl group resulted in enhanced potency towards PTP1B. Unfortunately, enzyme selectivity decreased as a result of it.\textsuperscript{82}
Suramin (32) is a reversible and competitive inhibitor of PTPases. It has a $K_i$ of 4 $\mu$M and 1.3 $\mu$M against PTP1B and Yersinia PTP respectively, and a $K_i$ that is 10-fold higher for VHR.$^{83}$

Kotoris et al examined naphthyl and biphenyl derivatives containing $\alpha,\alpha$-difluorosulfonate (33a, 34a), $\alpha,\alpha$-difluorocarboxylate (33b, 34b), or $\alpha,\alpha$-difluorotetrazole (33c, 34c) groups as potential phosphate biosteres for PTP1B inhibition.$^{84}$ These three groups would be more amenable for cellular studies since they are monoanionic. The most potent inhibitor was 34a, displaying a modest $K_i$ of 49 $\mu$M and demonstrating that the CF$_3$-sulfonate group was the most effective phosphate biostere.
1.5 Combinatorial Chemistry

Finding a highly active compound for a specific biological target is a very challenging process, as is the case for PTPases. Thousands of compounds are typically synthesized and screened before finding one, a lead compound, that exhibits some degree of activity. From there, more compounds are made that are structurally similar to the lead compound(s) in hopes of finding a highly active one that is specific, potent, and non-toxic. Synthesizing thousands and thousands of compounds individually in solution, that is, working on one reaction, on one substrate, in one reaction vessel at a time to give one product, can be a very slow and time-consuming process. Combinatorial chemistry is a synthetic strategy that has the potential to make large number of compounds (chemical libraries) in a faster, cheaper, and more efficient manner.\textsuperscript{85} As such, we wish to take this approach for the search of potent and highly selective PTP1B inhibitors.

Combinatorial chemistry involves a systematic and repetitive, covalent connection of a set of different “building blocks” of varying structures to each other to
yield a large array of diverse molecular compounds.\textsuperscript{86, 87} The chemical libraries can be synthesized individually in parallel such that the structures of all compounds are known. Or, the library can be prepared as a mixture of “tagged” compounds and the identity of any active compound is determined by an iterative process of resynthesis and screening.\textsuperscript{85}

In the drug discovery process, combinatorial chemistry can be applied to either lead discovery or lead optimization. In the lead discovery area, random screening takes place where the objective is to identify a lead compound in the absence of any structural information about active molecules. However, when a lead compound is known, then lead optimization takes place by preparing libraries of molecules that are structurally related, otherwise known as establishing SAR (related structural analogues). The objective in lead optimization is to optimize biological potency of the active compound(s).\textsuperscript{85, 86} In our studies, we wish to prepare a SAR library via parallel synthesis.

1.6 Polymer-Supported Organic Synthesis

Typically, combinatorial chemistry takes place on polymer supports since they would allow for rapid purification of the compounds after each step of the synthesis. Polymers that are used for polymer-supported organic synthesis (PSOS) must possess certain properties. They must be commercially available or rapidly and conveniently prepared, exhibit good stabilities in a wide range of reaction conditions, and provide appropriate functional groups for easy attachment of organic compounds. Although compounds can be directly attached to the polymer, a linker chain is often used to ensure anchor stability, improve accessibility to reagents, and improve the ease of cleavage of the final product off the polymer.\textsuperscript{88}
There are two types of polymers used for PSOS. The most commonly used supports are "insoluble" polymers. These types of polymers are insoluble in almost all solvents. The other type of support that has recently been introduced to the area of PSOS is "soluble" polymers where the polymers are soluble in the reaction medium. In the presence of solvents other than that used for the reaction, the "soluble" polymer precipitates out. For both types of polymers, rapid purification takes place simply by filtering out the polymer and washing away byproducts and excess reagents.

1.6.1 Insoluble vs. Soluble Polymer Supports

The use of insoluble polymer supports for organic synthesis is referred to as Solid Phase Organic Synthesis (SPOS). The first type of insoluble polymer used for SPOS was the Merrifield resin (35), first introduced by Merrifield in the 1960's for peptide synthesis. Since then, it has become one of the most widely used crosslinked polystyrene resin. The polymer can be readily synthesized by copolymerizing styrene (36) with 3% of chloromethylated styrene (37) and 1 to 2% of divinylbenzene (38). (Scheme 7).

![Scheme 7. Synthesis of Merrifield resin (35)](image-url)
The \(-\text{CH}_2\text{Cl}\) moiety allows for easy attachment of organic molecules. Divinylbenzene acts as a crosslinking agent to provide mechanical stability and insolubility properties (in most organic solvents) of the resin. As a result of the crosslinking, reactions are carried out under heterogeneous conditions and the polymer or polymer-bound product can be isolated by filtration.\(^{89}\)

Although insoluble polymers have proven to be invaluable in PSOS, there are disadvantages to using them. Under heterogeneous reaction conditions, there is nonlinear kinetic behavior, unequal distribution and/or access to the chemical reaction, and the inability to follow the reaction by conventional analytical techniques, to name but a few.\(^{88}\) As a consequence, the idea of using soluble polymers to allow for homogeneous reaction conditions have attracted attention.

The use of soluble polymer supports for organic synthesis is referred to as Liquid Phase Organic Synthesis (LPOS). Soluble supports allow for homogeneous conditions, and consequently linear kinetic behavior, greater access of reagents to all reactive sites on the polymer, and most importantly, reactions can be monitored to determine the completeness of reactions and purity of polymer bound products. Reaction monitoring can be accomplished with analytical methods such as NMR spectroscopy, IR, and even TLC. Moreover, these conventional methods allow for samples to be taken from reactions for characterization and returned to the reaction in a nondestructive manner.\(^{88}\)

Several different types of soluble polymers have been utilized in LPOS of peptides, oligonucleotides, oligosaccharides, as well as small molecules.\(^{88}\) However, the most commonly used polymer is poly(ethylene glycol) (PEG. 39a, b). PEG is formed by polymerization of ethylene oxide, which yields polyether structures with either hydroxyl
groups at both ends (39a), or a methoxy group at one end and a hydroxyl group at the other (39b). Because of the polyether chains, PEG possesses a polar, hydrophilic nature which consequently limits the use of PEG for certain types of reaction conditions. Thus, Janda and other researchers have explored the use of linear, non-crosslinked polystyrene (NCPS, 40) which can be thought of as a soluble version of the Merrifield resin. NCPS has been used in a variety of studies either as a support for synthesizing organic molecules or for immobilizing reagents/catalysts. NCPS can be readily prepared by copolymerizing styrene with functionalized styrene, providing freedom for the type of functional groups desired for attachment of organic molecules. The absence of a crosslinking agent results in the solubility of the polymer in certain organic solvents. For both PEG and NCPS, dilution of the homogeneous polymer solution with solvents that the polymers are insoluble in induces precipitation of the support, which can be subsequently filtered. NCPS is nonpolar and hydrophobic, thus complementing the nature of PEG. Theoretically, these two polymers together can cover a wide array of chemistry for LPOS.
1.7 Specific Objectives

The DFMP group has been shown to be an effective phosphate mimetic, as discussed in sections 1.4.1 and 1.4.4. However, the dianionic nature of the phosphonate group makes the DFMP moiety less amenable for cellular studies. Kotoris et al have found that the \( \alpha,\alpha \)-difluoromethylenesulfonic acid (DFMS) group is an effective phosphate mimetic.\(^8\) As such, we wish to prepare a library of structurally related DFMS-bearing compounds in hopes of finding a potent and selective PTP1B inhibitor.

Previous work has been carried out by Hum et al in the Taylor lab where a small library of biphenyl DFMP-bearing compounds (42) were prepared using a PSOS approach (Scheme 8).\(^7\) Instead of the more traditional solid phase methodology to PSOS, the LPOS tactic was used in preparing this library since it allowed for homogeneous reactions conditions and monitoring of the reactions by conventional \(^{19}\)F NMR. \(^{19}\)F NMR spectroscopy is a very useful tool for monitoring reactions since it is a very sensitive nucleus, from an NMR perspective, and the chemical shifts are spread over a wide frequency range. Thus, chemical transformations even fairly remote from the fluorine(s) would result in a change in the \(^{19}\)F-NMR chemical shift.\(^6\) The specific approach involved attaching an appropriately functionalized aryl difluorophosphonic acid of type 41 to NCPS via a phosphate ester linkage. The second aryl ring was then introduced by Suzuki coupling, which is a reaction that has been widely used in the area of polymer-supported synthesis.\(^7\) The ethyl protecting group and cleavage of the phosphonic acid products from the support was carried out in one step with either TMSBr or TMSI.
Scheme 8. Approach for LPOS of biphenyl DFMP’s

The methodology developed for preparing the biphenyl DFMP library had proven to be a very powerful synthetic strategy for rapid construction of the compounds. However, the types of biphenyl DFMP compounds synthesized in the library were limited to ones that were stable under the deprotection and cleavage conditions (TMSBr or TMSI). As such, our specific objective is to develop a methodology for preparing a library of biaryl compounds bearing the DFMS moiety (44) using LPOS. Although, the DFMS group is not quite as effective a phosphate mimic as the DMFP group, it is still the most effective monoanionic phosphate mimic for obtaining PTP1B inhibitors. We anticipated that the DFMS compounds could be removed from the support by basic hydrolysis, as opposed to using highly reactive TMSBr or TMSI. This would mean that the diversity of compounds prepared on the support could be expanded. In addition, the DFMS group is monoanionic, which may prove to be important from a therapeutic perspective.
The specific objective of this thesis was to examine whether the LPOS approach to compound construction could be applied to the synthesis of DFMS derivatives. DFMS compounds resulting from this approach would then be screened as inhibitors of PTP1B. To test this approach, we chose to prepare a small library of biaryl compounds in a manner analogous to that developed in our lab by Hum et al (Scheme 9). An appropriately functionalized aryl difluoromethanesulfonylic acid of type 43 would be attached to an appropriately modified soluble polymer support via a sulfate ester linkage. A Suzuki coupling would be used to form the polymer-bound biaryl compounds. The products would then be cleaved off the polymer support by hydrolysis. Here we report that this is indeed a feasible approach to the synthesis of biaryl DFMS compounds and that some of the compounds prepared in this manner are good inhibitors of PTP1B.

Scheme 9. General approach for LPOS of biaryl DFMS's
2 Experimental

General: All starting materials were obtained from commercial suppliers (Aldrich Chemical Company, Oakville, Ontario, Canada or Lancaster Synthesis Incorporated, Windham, New Hampshire, USA). Solvents were purchased from Caledon Laboratories (Georgetown, Ontario, Canada). Lancaster Synthesis Incorporated, or BDH Canada (Toronto, Canada). Tetrahydrofuran (THF) was distilled from sodium metal in the presence of benzophenone under argon. Dichloromethane (CH₂Cl₂) was distilled from calcium hydride under argon. Dimethylformamide (DMF) was distilled under reduced pressure from calcium hydride and stored over 4 Å sieves under argon. Reactions involving moisture-sensitive reagents were executed under an inert atmosphere of dry argon or nitrogen. All glassware was pre-dried prior to use and all liquid transfers were performed using dry syringes and needles. Silica gel chromatography was performed using silica gel 60A (Silicycle, 230–400 mesh). ¹H, ¹⁹F, ³¹P, and ¹³C NMR spectra were recorded on a Varian 200-Gemini, Bruker AC-200, or Bruker AC-300 NMR spectrometer. The abbreviations s, d, t, q, m, dd, dt, and br are used for singlet, doublet, triplet, quartet, multiplet, doublet of doublets, doublet of triplets, and broad respectively. Coupling constants are reported in Hertz (Hz). Chemical shifts (δ) for ¹H NMR spectra run in CDCl₃ are reported in ppm relative to the internal standard tetramethylsilane (TMS). Chemical shifts (δ) for ¹H NMR spectra run in CD₃OD are reported in ppm relative to residual solvent protons (δ 3.30). Chemical shifts (δ) for ¹H NMR spectra run in D₂O are reported in ppm relative to residual solvent protons (δ 4.79). For ¹³C NMR spectra run in CDCl₃, chemical shifts are reported in ppm relative to the CDCl₃ residual carbons (δ 77.0 for central peak). For ¹³C NMR spectra run in CD₃OD, chemical shifts
are reported in ppm relative to the CD$_3$OD residual carbons (δ 49.0 for central peak). For $^{31}$P NMR spectra, chemical shifts are reported in ppm relative to 85% phosphoric acid (external). $^{19}$F NMR spectra, chemical shifts are reported in ppm relative to trifluoroacetic acid (external). Low resolution (MS) and high resolution (HRMS) electron impact mass spectra were obtained on a Micromass 70-S-250 mass spectrometer. Low resolution electrospray mass spectra (LRESMS) were obtained on a Micromass Quatro II mass spectrometer. High resolution electrospray mass spectra (HRESMS) were obtained on a Bruker Daltonics Apex II fourier transform ion cyclotron resonance spectrometer equipped with a 7.0 tesla superconducting magnet. All melting points were taken on a Mel-temp melting point apparatus and are uncorrected. Analytical HPLC was performed on a Waters LC 4000 System using a Vydac 218TP54 analytical C-18 reverse phase column and a Waters 86 tunable absorbance detector set at 254 nm. All HPLC analysis was performed using the isocratic gradient (solvent A: acetonitrile; solvent B: water with 0.1% TFA): 0 min: 77% A, 23% B; 30 min: 77% A, 23% B; 35 min: 100% A; 45 min: 100% A; 50 min: 77% A, 23% B; 65 min: 77% A, 23% B. Buffer chemicals were obtained from Sigma Chemical Company. Enzyme assay solutions were prepared with deionized/distilled water. Fluorescein diphosphate (FDP), human PTP1B and CD45 were gifts from Merck-Frosst Canada Inc (Montreal, Quebec, Canada). H. pomatia aryl sulfatase type H5 was obtained from Sigma Chemical Company.

2-(Bromomethyl)naphthalene (47). A solution of 2-methylnaphthalene (46, 10.0 g, 70.3 mmol, 1 eq) and N-bromosuccinimide (13.8 g, 77.3 mmol, 1.1 eq) in benzene (80 mL) was irradiated to reflux for 1 hr using an IR lamp. The reaction was cooled to rt
and washed with water (3 x 80 mL). The organic layer was dried (MgSO₄), filtered, and concentrated by rotary evaporation. Column chromatography (100% hexane, R_f = 0.3) of the crude residue yielded a white solid which was a mixture of mono (47a) and dibrominated (47b) products. No further purifications were carried out since the dibrominated product (47b) is unreactive in the subsequent reaction. ¹H NMR (CDCl₃) δ 4.68 (2H, s, CH₂ of monobrominated product, 47a); 6.84 (1H, s, CH of dibrominated product, 47b).

**Sodium 2-naphthylmethanesulfonate (48).** To a solution of 47a and b (47a, 6.75 g, 30.54 mmol 1 eq) in acetone (57 mL) was added an aqueous solution of Na₂SO₃ (34 mL, 0.9 M, 1 eq). The reaction was refluxed for 1 hr and the acetone was then removed by rotary evaporation. A precipitate formed during the evaporation process. The precipitate was collected by filtration, rinsed with water (50 mL) and CH₂Cl₂ (50 mL), and then dried under high vacuum. The product 48, which was a white solid, was obtained in 81% yield: ¹H NMR (DMSO-d₆) δ 7.80-7.85 (4H, m, Ar-H), 7.48-7.56 (3H, m, Ar-H), 3.90 (2H, s, CH₂).

**2-Naphthylmethanesulfonyl chloride (49).** To a solution of 48 (4.0 g, 16.38 mmol, 1 eq) in 1:1 acetonitrile/tetramethylene sulfone (14 mL) was added phosphorus oxychloride (6.9 mL, 11.35 g, 73.69 mmol, 4.5 eq). The reaction was heated at 55°C for 6 hrs, cooled to rt, and then poured into cold water (500 mL). The resulting precipitate was collected by filtration, dissolved in CH₂Cl₂ (50 mL), and washed with water (3 x 50 mL). The organic layer was dried (MgSO₄), filtered, and concentrated by rotary evaporation to give pure 49 as an off white solid in 62% yield: ¹H NMR (CDCl₃) δ 7.90-7.97 (4H, m, Ar-H), 7.53-7.60 (3H, m, Ar-H), 5.03 (2H, s, CH₂).
Neopentyl 2-naphthylmethanesulfonate (50). To a solution of 49 (3.10 g., 12.87 mmol, 1 eq) and neopentyl alcohol (1.70 g. 19.30 mmol, 1.5 eq) in anhydrous THF (25 mL) at 0°C was added a solution of triethylamine (2.33 mL, 1.69 g. 16.73 mmol, 1.3 eq) in anhydrous THF (15 mL). The reaction was stirred overnight at rt. concentrated by rotary evaporation, diluted with water (40 mL), and extracted with CH₂Cl₂ (3 x 40 mL). The combined organic layers were dried (MgSO₄), filtered, and concentrated by rotary evaporation. Column chromatography (60:40 CH₂Cl₂/hexane. Rf = 0.3) of the crude residue yielded pure 50 as a white solid in 77% yield: ¹H NMR (CDCl₃) δ 7.81-7.90 (4H, m, Ar-H). 7.50-7.55 (3H, m, Ar-H). 4.53 (2H, s, CH₂). 3.75 (2H, s, CH₂O). 0.91 (9H, s, CH₃).

Neopentyl difluoro(2-naphthyl)methanesulfonate (45). To a solution of 50 (1.0 g. 3.42 mmol, 1 eq) in anhydrous THF (20 mL) at -78°C was added t-BuLi (2.21 mL, 1.7 M, 3.76 mmol, 1.1 eq) dropwise over a period of 5 min. The reaction was stirred at -78°C for 2 hrs. A solution of NFSi (1.19 g, 3.76 mmol, 1.1 eq) in anhydrous THF (20 mL) was then added dropwise over a period of 2 min. and the reaction was stirred at -78°C for 1 hr. This process was repeated (1.2 eq of t-BuLi, 1.2 eq of NFSi). The reaction was stirred at rt overnight then quenched with water (80 mL) and extracted with CH₂Cl₂ (3 x 80 mL). The combined organic layers were dried (MgSO₄), filtered, and concentrated by rotary evaporation. Column chromatography (30:70 CH₂Cl₂/hexane, Rf = 0.2) of the crude residue yielded pure 45 as a white solid in 69% yield: ¹H NMR (CDCl₃) δ 8.23 (1H, s, Ar-H). 7.90-7.98 (3H, m, Ar-H). 7.58-7.74 (3H, m, Ar-H). 4.14 (2H, s, CH₂O). 1.02 (9H, s, CH₃): ¹⁹F NMR (CDCl₃) δ -23.63.
Lithium Difluoro(2-naphthyl)methanesulfonate (53). A solution 45 (0.2 g, 0.65 mmol, 1 eq) and LiBr (0.068 g, 0.79 mmol, 1.2 eq) in redistilled butanone (10 mL) was refluxed for 48 hrs. The reaction mixture was concentrated by rotary evaporation. The crude product was dissolved in water (20 mL) and washed with CH2Cl2 (3 x 20 mL). Lyophilization of the aqueous layer followed by HPLC purification yielded pure 53 as a white solid in 88% yield: 1H NMR (D2O) δ 8.26 (1H, s, Ar-H), 8.00 (3H, t, J = 8.8 Hz, Ar-H), 7.62-7.75 (3H, m, Ar-H).

2,2-Dimethyl-3-((tetrahydro-2H-and 2-pyran-4-yl)oxy)-1-propanol (62). The following procedure was adapted from the work of Kato et al.104 To a solution of neopentyl glycol (61, 5.00 g, 48.01 mmol, 1 eq) and p-toluenesulfonic acid monohydrate (0.027 g, 0.14 mmol, 0.003 eq) in anhydrous THF (135 mL) at −25 °C was added 3,4-dihydro-2H-pyran (4.38 mL, 4.04 g, 48.01 mmol, 1 eq). The reaction was stirred for 3 hrs at −25 °C, then warmed to rt and stirred for 14 hrs. The reaction was quenched with triethylamine (0.5 mL), diluted with ether (200 mL), and washed with brine (3 x 200 mL). The organic layer was dried (MgSO4), filtered, and concentrated by rotary evaporation to give a yellow oil. Vacuum distillation of the crude residue yielded pure 62 as a colorless oil in 22% yield: 1H NMR (CDCl3) δ 4.54 (1H, br s, CH), 3.80-3.90 (1H, m, CH2O), 3.63 (1H, d, J = 8.8 Hz, CH2O), 3.36-3.57 (3H, m, CH2O), 3.22 (1H, d, J = 8.8 Hz, CH2O), 2.77 (1H, t, J = 6.6 Hz, OH), 1.55-1.77 (6H, br m, CH2), 0.92 (6H, s, CH3); 13C NMR (CDCl3) δ 99.44, 75.48, 70.56, 62.50, 36.14, 30.61, 25.37, 21.76, 19.69; MS m/z (relative intensity) 187 (54), 103 (100), 101 (43); HRMS calcd for C10H19O3 (M – H+) 187.1334, found 187.1325.
3-(tert-Butoxy)-2,2-dimethyl-1-propanol (63). A solution of neopentyl glycol (61. 10.0 g, 96 mmol, 1 eq) and t-butanol (10.5 mL, 8.14 g, 110 mmol, 1.1 eq) in CHCl₃ (20 mL) was added to 50% H₂SO₄ (20 mL) at 0°C. The reaction was stirred at rt for 48 hrs. the aqueous layer was separated from the organic layer, and then extracted with CHCl₃ (2 x 20 mL). The combined organic layers were dried (MgSO₄), filtered, and concentrated by rotary evaporation. Column chromatography (10:90 EtOAc/hexane, Rf = 0.3) of the crude residue yielded pure 63 as a pale yellow oil in 10% yield: ¹H NMR (CDCl₃) δ 3.44 (2H, d, J = 5.8 Hz, CH₂OH), 3.33 (1H, br s, OH), 3.23 (7H, s, CH₂), 1.18 (9H, s, CH₃), 0.91 (6H, s, CH₃).

3-{{[1-(tert-Butyl)-1,1-dimethylsilyloxy]-2,2-dimethyl-1-propanol (64). To a solution of neopentyl glycol (61. 1.00 g, 9.60 mmol, 1 eq) and imidazole (1.31 g, 10.56 mmol, 1.1 eq) in DMF (5 mL) at 0 °C was added a solution of tert-butyldimethylsilyl chloride (1.31 g, 19.24 mmol, 2 eq) in DMF (5mL). The reaction was stirred at rt overnight, concentrated by rotary evaporation, diluted with water (20 mL), and extracted with ether (3 x 20 mL). The organic layers were combined, dried (MgSO₄), filtered, and concentrated by rotary evaporation to give a yellow oil. Column chromatography (10:90 EtOAc/hexane, Rf = 0.2) of the crude residue yielded pure 64 as a colorless oil in 30% yield: ¹H NMR (CDCl₃) δ 3.47 (4H, s, CH₂), 0.90 (15H, s, CH₃), 0.07 (6H, s, CH₃Si); ¹³C NMR (CDCl₃) δ 71.82, 71.27, 36.61, 25.80, 21.34, 18.12; MS m/z (relative intensity) 161 (25), 105 (33), 75 (100); HRMS calcd for C₇H₁₇O₂Si (M-C₄H₉) 161.0998, found 161.1002.

3-{{[1-(tert-Butyl)-1,1-dimethylsilyloxy]-2,2-dimethylpropyl 2-naphthylmethanesulfonate (65). The following procedure was adapted from the work of Kotoris
To a solution of 49 (1.00 g, 4.15 mmol, 1 eq) and 64 (1.35 g, 6.23 mmol, 1.5 eq) in anhydrous THF (6 mL) at 0 °C was added a solution of triethylamine (0.75 mL, 0.54 g, 5.40 mmol, 1.3 eq) in anhydrous THF (5 mL). The reaction was stirred at rt overnight, concentrated by rotary evaporation, quenched with water (40 mL) and extracted with CH₂Cl₂ (3 x 40 mL). The combined organic layers were dried (MgSO₄), filtered, and concentrated by rotary evaporation. Column chromatography (gradient from 40:60 to 70:30 CH₂Cl₂/hexane, Rf = 0.5 in 70:30 CH₂Cl₂/hexane) of the crude residue yielded 65 as a white solid in 77% yield: mp 44-45.5 °C; ¹H NMR (CDCl₃) δ 7.80-7.89 (W, m, Ar-H), 7.49-7.55 (3H, m, Ar-H), 4.51 (2H, s, CH₂), 3.90 (2H, s, CH₂O), 3.28 (2H, s, CH₂O), 0.85 (15H, s, CH₃), -0.02 (6H, CH₃Si); ¹³C NMR (CDCl₃) δ 133.36, 133.30, 130.41, 128.66, 128.03, 127.79, 127.66, 126.79, 126.59, 125.57, 75.57, 68.04, 56.77, 36.77, 25.91, 21.14, 18.27, -5.55; MS m/z (relative intensity) 423 (48), 365 (57), 141 (100); HRMS calcd for C₇₂H₁₅₂O₄Si₁₁ (M+H⁺) 423.2023, found 423.2034.

3-[[1-(tert-Butyl)-1,1-dimethylsilyloxy]-2,2-dimethylpropyl] 1,2-di(2-naphthyl)-1-ethene-1-sulfonate (66). The following procedure was adapted from the work of Kotoris et al. To a solution of 65 (0.5 g, 1.19 mmol, 1 eq) in anhydrous THF (13 mL) at -78°C was added t-BuLi (0.94 mL, 1.39 M, 1.30 mmol, 1.1 eq) dropwise over a period of 5 min. The reaction was stirred for 2 hrs. A solution of NFSi (0.411 g, 1.30 mmol, 1.1 eq) in anhydrous THF (13 mL) was added over a period of 2 min. The reaction was stirred for 1 hr at -78°C, and the process was repeated (1.2 eq of t-BuLi, 1.2 eq of NFSi). After warming to rt and stirring for an additional 2 hrs, the reaction was quenched with water (50 mL), extracted with ether (50 mL), and washed with brine (2 x 50 mL). The organic layer was dried (MgSO₄), filtered, and concentrated by rotary
evaporation. Column chromatography (1:99 EtOAc/hexane, $R_f = 0.05$. but in 10:90 EtOAc/hexane. $R_f = 0.4$) of the crude residue yielded pure 66 as a hydroscopic white solid in 39% yield: $^1$H NMR (CDCl$_3$) $\delta$ 8.06 (2H, d, $J = 6.6$ Hz, Ar-H). 7.90 (2H, d, $J = 8.3$ Hz, Ar-H). 7.85 (1H, d, $J = 7.6$ Hz, Ar-H), 7.79 (1H, s, Ar-H), 7.68 (2H, t, $J = 8.6$ Hz, Ar-H). 7.41-7.60 (6H, m, Ar-H). 7.00 (1H, d, $J = 8.7$ Hz, CH), 3.94 (2H, s, CH$_2$O), 3.26 (2H, s, CH$_2$O). 0.83 (9H, s, CH$_3$). 0.82 (6H, s, CH$_3$), -0.03 (6H, s, CH$_3$Si): $^{13}$C NMR (CDCl$_3$) $\delta$ 139.88, 136.37, 133.70, 133.46, 133.32, 132.83, 132.34, 130.28, 130.10, 128.85, 128.54, 128.47, 128.02, 127.79, 127.53, 127.09, 126.56, 126.50, 126.01, 75.63, 67.90, 36.68, 25.77, 21.08, 18.13. -5.68: MS $m/z$ relative intensity 503 (8), 417 (13), 279 (100), 140 (9): HRMS calcd for C$_{33}$H$_{41}$O$_4$Si$_1$S$_1$ (M + H$^+$) 561.2495. found 561.2489.

2,2-Dimethyl-1,5-pentanediol (71).$^{107}$ To a solution of LiAlH$_4$ (3.5 g, 87.5 mmol, 2 eq) in anhydrous THF (140 mL) at 0°C was added a solution of 2,2-dimethyl-glutaric acid (70, 7.0 g, 43.75 mmol, 1 eq) in anhydrous THF (55 mL) dropwise over a period of 20 min. The reaction was stirred at rt for 2 hrs. cooled to 0°C, and slowly quenched with water (3.5 mL), 15% NaOH (3.5 mL), and then water (10.5 mL). A small amount of K$_2$CO$_3$ was added to the reaction mixture and then filtered to remove the precipitate and LiAlH$_4$ salts. The precipitate was washed with THF (200 mL), and the filtrate was concentrated by rotary evaporation. Column chromatography (solvent gradient from 2:98 to 10:90 MeOH/CHCl$_3$. $R_f = 0.5$ in 10:90 MeOH/CHCl$_3$) of the crude residue yielded pure 71 as a colorless oil in 67% yield: $^1$H NMR (CDCl$_3$) $\delta$ 3.64 (2H, t, $J = 6.6$ Hz, CH$_2$OH). 3.33 (2H, s, CH$_2$OH). 1.73 (2H, br s, OH). 1.27-1.58 (4H, m, CH$_2$). 0.89 (6H, s, CH$_3$).
A solution of 71 (4.1 g. 31.01 mmol. 1 eq) and trityl chloride (9.5 g. 34.11 mmol. 1.1 eq) in anhydrous pyridine (80 mL) was stirred at rt overnight. The reaction mixture was concentrated by high vacuum rotary evaporation. The oily residue was diluted with ether (50 mL), washed with saturated aqueous solution of NH₄Cl (3 x 50 mL), and brine (2 x 50 mL). The organic layer was dried (MgSO₄), filtered, and concentrated by rotary evaporation. Column chromatography (5:95 EtOAc/hexane, Rf = 0.1) of the crude residue yielded pure 73 as a white solid in 49% yield: ¹H NMR (CDCl₃) δ 7.30-7.47 (15H, m, Ar-H), 3.31 (2H, br s, CH₂OH), 3.06 (2H, t, J = 6.6 Hz, CH₂O), 1.50-1.63 (2H, m, CH₂), 1.29 (1H, s, OH), 1.21-1.30 (7H, m, CH₂), 0.87 (6H, s, CH₃).

The following procedure was adapted from the work of Kotoris et al. To a solution of 49 (2.44 g. 10.15 mmol. 1 eq) and 73 (5.70 g. 15.22 mmol. 1.5 eq) in anhydrous THF (50 mL) at 0 °C was added a solution of triethylamine (1.78 mL, 1.29 g, 13.19 mmol, 1.3 eq) in anhydrous THF (50 mL). The reaction was stirred at rt overnight, concentrated by rotary evaporation, quenched with water (50 mL), and extracted with CH₂Cl₂ (3 x 50 mL). The combined organic layers were dried (MgSO₄), filtered, and concentrated by rotary evaporation. Column chromatography (5:95 EtOAc/hexane, Rf = 0.1, followed by 50:50 CH₂Cl₂/hexanes, Rf = 0.3) of the crude residue yielded pure 74 as a colorless hydroscopic solid in 91% yield: ¹H NMR (CDCl₃) δ 7.75-7.85 (4H, m, Ar-H), 7.22-7.55 (18H, m, Ar-H), 4.51 (2H, s, CH₂), 3.77 (2H, s, CH₂O), 2.99 (2H, t, J = 6.6 Hz, CH₂O), 1.41-1.58 (2H, m, CH₂), 1.18-1.26 (2H, m, CH₂), 0.86 (6H, s, CH₃); ¹³C NMR (CDCl₃) δ 143.95, 132.76, 132.69, 130.01, 128.24, 127.53, 127.35, 127.21, 126.49, 126.37, 126.18.
124.89. 85.98. 77.62. 63.59. 56.28. 34.43. 33.65. 23.92. 23.31: MS m/z (relative intensity) 578 (86). 501 (86). 336 (17). 243 (54). 141 (100). 77 (12); HRMS calcd for C_{37}H_{38}O_{4}Si 478.2491: found 578.2466.

5-[[1-(tert-Butyl)-1,1-dimethylsilyl]oxy]-2,2-dimethyl-1-pentanol (76). To a solution of 71 (1.00 g. 7.56 mmol. 1 eq) and imidazole (1.03 g. 15.13 mmol. 1.1 eq) in DMF (5 mL) at 0 °C was added a solution of tert-butyldimethylsilyl chloride in DMF (5 mL). The reaction was stirred at rt overnight then concentrated by rotary evaporation. To the residue was added water (40 mL) and the mixture was extracted with ether (3 x 40 mL). The combined organic layers were dried (MgSO_{4}), filtered, and concentrated by rotary evaporation to give a yellow oil. Column chromatography (gradient from 5:95 to 20:80 EtOAc/hexanes. Rf = 0.5 in 20:80 EtOAc/hexanes) of the crude residue yielded pure 76 as a colorless oil in 66% yield: \(^1\)H NMR (CDCl_{3}) \(\delta\) 3.63 (2H. t. J = 6.7 Hz. CH_{2}OSi). 3.32 (2H. d. J = 5.9 Hz. CH_{2}O). 1.40–1.60 (2H. m. CH_{2}). 1.20–1.30 (2H. m. CH_{2}). 0.89 (6H. s. CH_{3}). 0.87 (9H. s. CH_{3}). 0.06 (6H. s. CH_{3}Si); \(^{13}\)C NMR (CDCl_{3}) \(\delta\) 71.69, 63.98, 34.79, 34.63, 27.35. 25.95. 23.93. 18.31. -5.31: MS m/z (relative intensity) 159 (9). 97 (79). 83 (31). 75 (47). 69 (29). 55 (100); HRMS calcd for C_{13}H_{31}O_{2}Si (M + H\(^{+}\)) 247.2093. found 247.2084.

5-[[1-(tert-Butyl)-1,1-dimethylsilyl]oxy]-2,2-dimethylpentyl 2-naphthylmethanesulfonate (77). The following procedure was adapted from the work of Kotoris et al.\(^8\) To a solution of 49 (0.65 g. 2.70 mmol. 1 eq) and 76 (1.00 g. 4.06 mmol. 1.5 eq) in anhydrous THF (5 mL) at 0 °C was added a solution of triethylamine (0.50 mL. 0.36 g. 3.51 mL. 1.3 eq) in anhydrous THF (5 mL). The reaction was stirred at rt overnight. concentrated by rotary evaporation, quenched with water (20 mL), and extracted with
CH₂Cl₂ (3 x 20 mL). The combined organic extracts were dried (MgSO₄), filtered, and concentrated by rotary evaporation. Column chromatography (gradient from 30:70 to 50:50 CH₂Cl₂/hexane. Rf = 0.3 in 60:40 CH₂Cl₂/hexane) of the crude residue yielded pure 77 as a white solid in 69% yield: mp 35-38 °C; ¹H NMR (CDCl₃) δ 7.82-7.88 (W. m. Ar-H). 7.49-7.53 (3H. m. Ar-H). 4.51 (2H. S. CH₂). 3.72 (2H. S. CH₂O). 3.18 (3H. t. J = 5.9 Hz. CH₂O). 1.32-1.45 (2H. m. CH₂). 1.16-1.26 (2H. m. CH₂). 0.87 (6H. s. CH₃). 0.86 (9H. s. CH₃). 0.02 (6H. s. CH₃Si); ¹³C NMR (CDCl₃) δ 133.12, 133.05, 130.33, 128.55, 127.87, 127.66, 127.54, 126.72, 126.51, 125.24, 78.04, 63.36, 56.59, 34.39, 33.89, 26.97, 25.87, 23.65, 18.23. -5.38: MS m/z (relative intensity) 451 (2). 393 (7). 336 (18). 311 (37). 141 (100). 115 (11). 97 (46). 55 (22): HRMS calcd for C₂₃H₃₉O₃Si₂S (M + H⁺) 451.2338. found 451.2323.

5-[[1-(tert-Butyl)-1,1-dimethylsilyloxy]-2,2-dimethylpentyl fluoro(2-naphthyl)methanesulfonate (78). The following procedure was adapted from the work of Kotori et al.⁹⁸ To a solution of 77 (0.211 g. 0.47 mmol. 1 eq) in anhydrous THF (15 mL) at -78°C was added t-BuLi (1.7 M. 0.30 mL. 0.51 mmol. 1.1 eq) over a period of 2 min. The reaction was stirred at -78°C for 30 min. A solution of NFSi (0.148 g. 0.47 mmol. 1 eq) in anhydrous THF (5 mL) was added dropwise to the reaction mixture over a period of 2 min. and the mixture was stirred for 2 hrs at -78°C. The process was repeated (1.1 eq of t-BuLi. 1 eq of NFSi). The reaction mixture was warmed to room temperature, stirred for 1 hr. quenched with water (30 mL), and extracted with CHCl₃ (3 x 30 mL). The combined organic layers were dried (MgSO₄), filtered, and concentrated by rotary evaporation. Column chromatography (5:95 EtOAc/hexane. Rf = 0.2. followed by 40:60 CH₂Cl₂. Rf = 0.2) of the crude residue yielded pure 78 as a pale yellow oil in 50% yield:
$^1$H NMR (CDCl$_3$) δ 8.07 (1H. s. Ar-H), 7.87-7.96 (3H. m. Ar-H), 7.55-7.67 (3H. m. Ar-H), 6.36 (1H. d. J = 46.9 Hz. CHF), 4.07 (2H. s. CH$_2$), 3.58 (2H. t. J = 6.6 Hz. CH$_2$). 1.41-1.54 (2H. m. CH$_2$), 1.26-1.35 (6H. m. CH$_2$), 0.96 (6H. s. CH$_3$), 0.90 (9H. s. CH$_3$), 0.05 (6H. s. CH$_3$); $^{19}$F NMR (CDCl$_3$) δ -97.49 (d. J$_{CF}$ = 45.8 Hz); $^{13}$C NMR (CDCl$_3$) δ 134.29, 132.51, 128.71, 128.47, 128.19 (d. J$_{CF}$ = 7.3 Hz), 127.81, 127.62, 126.87, 126.03 (d. J$_{CF}$ = 19.8 Hz), 123.57 (d. J$_{CF}$ = 5.9 Hz), 100.58 (d. J$_{CF}$ = 216.7 Hz), 81.34, 63.44, 34.40, 34.18, 27.05, 25.91, 23.64, 18.29. -5.34: MS m/z (relative intensity) 411 (32), 327 (45), 309 (18), 235 (29), 221 (48), 159 (100); HRMS calcd for C$_{24}$H$_{30}$F$_4$Si$_3$S (M + H$^+$) 469.2244, found 469.2246.

5-[(1-(tert-Butyl)-1,1-dimethyisilyl]oxy]-2,2-dimethylpentyl difluoro(2-naphthyl)methanesulfonate (79). The following procedure was adapted from the work of McAtee et al.$^{100}$ To a solution of 77 (0.216 g. 0.48 mmol. 1 eq) and NFSi (0.453 g. 1.44 mmol. 3 eq) in anhydrous THF (12 mL) at -78°C was added NaHMDS (1.0 M. 1.20 mL. 1.20 mmol. 2.5 eq) dropwise over a period of 1 hr. The reaction mixture was stirred for 2 hrs at -78°C, warmed to rt. stirred for 1 hr. quenched with water (30 mL), and extracted with CHCl$_3$ (3 x 30 mL). The combined organic layers were dried (MgSO$_4$), filtered, and concentrated by rotary evaporation. Column chromatography (2.5:97.5 EtOAc/hexane. $R_f$ = 0.7 in 15:85 EtOAc/hexane) of the crude residue yielded pure 79 as a white solid in 91% yield: mp 45-47 °C; $^1$H NMR (CDCl$_3$) δ 8.23 (1H. s. Ar-H). 7.95 (3H. t. J = 9.5 Hz. Ar-H), 7.58-7.75 (3H. m. Ar-H), 4.16 (2H. s. CH$_2$O), 3.58 (2H. t. J = 5.9 Hz. CH$_2$O), 1.32-1.54 (4H. m. CH$_2$), 0.99 (6H. s. CH$_3$), 0.90 (9H. s. CH$_3$), 0.05 (6H. s. CH$_3$); $^{19}$F NMR (CDCl$_3$) δ -23.33; $^{13}$C NMR (CDCl$_3$) δ 134.98, 132.32, 128.99, 128.77, 128.43, 128.30, 128.16, 127.86, 127.19, 122.68 (t. J$_{CF}$ = 5.5 Hz), 121.39, 82.92.
63.47, 34.72, 34.39, 27.22, 25.97, 23.69, 18.32, -5.30; MS \( m/z \) (relative intensity) 177 (100), 171 (23), 97 (95), 55 (14); HRMS calcd for \( C_{30}H_{27}O_4F_2Si_2S_i \) (\( M - C_4H_9 \)) 429.13674, found 429.13580.

**5-Hydroxy-2,2-dimethylpentyl difluoro(2-naphthyl)methanesulfonate** (82). The following procedure was adapted from the work of Corey et al.\(^{111}\) To a solution of 79 (1.87 g, 3.80 mmol, 1 eq) in THF (19 mL) was added 3:1 acetic acid/water (76 mL). The reaction was stirred at rt for 3 hrs. The reaction mixture was diluted with ether (200 mL), washed with water (3 x 200 mL), 5% NaHCO\(_3\) (3 x 200 mL), and brine (3 x 200 mL). The organic layer was dried (MgSO\(_4\)), filtered, and concentrated by rotary evaporation. Column chromatography (gradient from 20:80 EtOAc/hexane to 30:70 EtOAc/hexane, \( R_f = 0.10 \) in 20:80 EtOAc/hexane) of the crude residue yielded pure 82 as a white solid: mp 48-50 °C; \(^1H\) NMR (CDCl\(_3\)) \( \delta \) 8.23 (1H, s, Ar-H), 7.95 (3H, t, \( J = 9.5 \) Hz, Ar-H), 7.60-7.73 (3H, m, Ar-H), 4.18 (2H, s, CH\(_2\))O), 3.63 (2H, t, \( J = 5.9 \) Hz, CH\(_2\)O), 2.26 (1H, s OH), 1.30-1.65 (4H, m, CH\(_2\)), 0.99 (6H, s, CH\(_3\)): \(^19\)F NMR (CDCl\(_3\)) \( \delta \) -23.31; \(^13\)C NMR (CDCl\(_3\)) \( \delta \) 134.96, 132.30, 128.97, 128.81, 128.48, 128.30 (t, \( J_{CF} = 6.9 \) Hz). 127.88, 127.25, 122.62 (t, \( J_{CF} = 5.5 \) Hz), 121.44, 115.80, 82.70, 63.27, 34.50, 34.37, 26.82, 23.62; MS \( m/z \) (relative intensity) 372 (8.4), 177 (100), 127 (8), 84 (5.6), 55 (13). HRMS calcd for \( C_{18}H_{22}O_4F_2S_i \) 372.12069, found 372.12155.

**3-{[1-(tert-Butyl)-1,1-dimethylsilyl oxy]-2,2-dimethylpropyl} difluoro(2-naphthyl)methanesulfonate** (83). The following procedure was adapted from the work of McAtee et al.\(^{109}\) To a solution of 65 (0.303 g, 0.72 mmol, 1 eq) and NFSi (0.678 g, 2.15 mmol, 3 eq) in anhydrous THF (15 mL) at -78°C was added NaHMDS (1.79 mL, 1.0 M, 1.79 mmol, 2.5 eq) dropwise over a period of 30 min. The reaction was stirred for
2 hrs at -78°C, warmed to rt. and then stirred for 1 hr. The reaction was quenched with water (40 mL), extracted with ether (40 mL), and the combined organics were washed with water (2 x 40 mL). The organic layer was dried (MgSO₄), filtered, and concentrated by rotary evaporation. Column chromatography (20:80 CH₂Cl₂/hexane. Rf = 0.1) of the crude residue yielded pure 83 as a white solid in 84% yield: mp = 35.36°C; ¹H NMR (CDCl₃) δ 8.23 (1H. s. Ar-H). 7.90-8.00 (3H. m. Ar-H). 7.55-7.75 (3H. br m. Ar-H). 4.28 (2H. s. CH₂O). 3.36 (2H. s. CH₂O). 0.95 (6H. s. CH₃). 0.89 (9H. s. CH₃). 0.04 (6H. s. CH₃); ¹⁹F NMR (CDCl₃) δ -23.42; ¹³C NMR (CDCl₃) δ 134.81. 132.14. 128.94. 128.76. 128.41. 128.26 (t. JCF = 6.9 Hz). 127.83. 127.16. 125.13 (t. JCF = 22.1 Hz). 122.60 (t. JCF = 5.1 Hz). 121.26 (t. JCF = 283.6 Hz). 80.14. 67.53. 36.99. 25.80. 20.94. 18.20. -5.68; MS m/z (relative intensity) 177 (100). 127 (6). 75 (13); HRMS calcd for C₁₈H₂₃O₄F₂Si₂S₁ (M - C₄H₄) 401.1054. found 401.1026.

**3-Hydroxy-2,2-dimethylpropyl difluoro(2-naphthyl)methanesulfonate (84).**

The following procedure was adapted from the work of Corey et al. To a solution of 83 (0.167 g. 0.36 mmol. 1 eq) in THF (1 mL) was added 3:1 acetic acid/water (4 mL). The reaction was stirred for 4 days. diluted with ether (25 mL), washed with water (3 x 25 mL). 5% NaHCO₃ (3 x 25 mL). and brine (2 x 25 mL). The organic layer was dried (MgSO₄), filtered, and concentrated by rotary evaporation. Column chromatography (15:85 EtOAc/hexane. Rf = 0.1) of the crude residue yielded pure 84 as a white solid in 53% yield: mp = 80.5-81.5°C; ¹H NMR (CDCl₃) δ 8.23 (1H. s. Ar-H). 7.87-7.96 (3H. m. Ar-H). 7.71 (1H. dd. J = 1.8. 8.8). 7.54-7.63 (2H. m. Ar-H). 4.31 (2H. s. CH₂O). 3.42 (2H. s. CH₂O). 1.88 (1H. br s. OH). 0.97 (6H. s. CH₃); ¹⁹F NMR (CDCl₃) δ -23.19; ¹³C NMR (CDCl₃) δ 134.82. 132.10. 128.91. 128.80. 128.47. 128.29 (t. JCF = 6.8 Hz). 127.82.
127.21, 124.82 (t. \( J_{CF} = 22.1 \) Hz), 122.49 (t. \( J_{CF} = 5.2 \) Hz), 121.29 (t. \( J_{CF} = 283.8 \) Hz), 79.96, 67.26, 36.89, 20.79; MS \( m/z \) (relative intensity) 344 (8), 177 (100), 127 (11); HRMS calcd for \( C_{16}H_{16}O_2F_2S \) 344.0894, found 344.0894.

2,2-Dimethyl-5-phenoxypentyl difluoro(2-naphthyl)methanesulfonate (87). The following procedure was adapted from the work of Rano et al.\textsuperscript{112} To a solution of phenol (0.013 g, 0.13 mmol, 1 eq) in 1:1 anhydrous \( CH_2Cl_2/THF \) (10 mL) was added TMAD (86, 0.046 g, 0.27 mmol, 2 eq). Once all the TMAD dissolved, 82 (0.100 g, 0.27 mmol, 2 eq) was added, followed by tributylphosphine (0.070 mL, 0.057 g, 0.27 mmol, 2 eq) dropwise over a period of 2 min. The reaction was stirred at rt overnight and concentrated by rotary evaporation. Column chromatography (50:50 \( CH_2Cl_2/hexane \). \( R_f = 0.5 \)) of the crude residue yielded pure 87 as a white solid in quantitative yield: mp 75.5-76.5 °C; \( ^1H \) NMR (CDCl\textsubscript{3}) \( \delta \) 8.23 (1H, s, Ar-H), 7.86-7.95 (3H, m, Ar-H), 7.72 (1H, d, \( J = 8.4 \) Hz, Ar-H), 7.53-7.62 (2H, m, Ar-H), 7.27 (2H, t, \( J = 7.7 \) Hz, Ar-H), 6.86-6.96 (3H, m, Ar-H), 4.19 (2H, s, CH\textsubscript{2}O), 3.90 (2H, t, \( J = 6.6 \) Hz, CH\textsubscript{2}O), 1.70-1.80 (2H, m, CH\textsubscript{2}), 1.44-1.50 (2H, m, CH\textsubscript{2}), 1.01 (6H, s, CH\textsubscript{3}); \( ^{19}F \) NMR (CDCl\textsubscript{3}) \( \delta \) -23.21; \( ^{13}C \) NMR (CDCl\textsubscript{3}) \( \delta \) 158.88, 134.81, 132.11, 129.41, 128.93, 128.81, 128.46, 128.26 (t, \( J_{CF} = 6.8 \) Hz), 127.83, 127.20, 124.97 (t, \( J_{CF} = 22 \) Hz), 122.54 (t, \( J_{CF} = 5.7 \) Hz), 121.30 (t, \( J_{CF} = 284 \) Hz), 120.61, 114.42, 82.79, 67.93, 34.55, 34.43, 23.72, 23.58; MS \( m/z \) (relative intensity) 448 (17), 177 (100), 94 (14), 83 (9), 55 (24); HRMS calcd for \( C_{24}H_{26}O_4F_2S \) 448.1520, found 448.1523.

\( N,N,N',N'-\)Tetramethyl-1,2-hydradinedicarboxamide (91).\textsuperscript{117} To a solution of hydrazine (89, 2.9 mL, 3.0 g, 93.6 mmol, 1 eq) and triethylamine (27.4 mL, 19.9 g, 196.6 mmol, 2.1 eq) in anhydrous \( CH_2Cl_2 \) (150 mL) was slowly added dimethyl carbamoyl
chloide (90. 18.1 mL, 21.1 g, 196.6 mmol, 2.1 eq). The reaction was refluxed for 5 hrs and cooled to rt. The triethylamine hydrochloride salt (Et₃N·HCl) and the desired product (91) precipitated out and were collected by filtration. The crude product was used in the subsequent steps without further purification. A white solid was obtained in 75% yield based on ¹H NMR: ¹H NMR (D₂O) δ 3.18 (6H, q, J = 7.3 Hz, CH₂ of Et₃N·HCl), 2.91 (12 H, s, CH₃), 1.26 (9H, t, J = 7.3 Hz, CH₃ of Et₃N·HCl).

**N,N,N',N'-Tetramethylazodicarboxamide (TMAD, 86)** To a solution of crude 91 (5.90 g, 33.67 mmol, 1 eq) in anhydrous CH₂Cl₂ (100 mL) at 20°C was added a solution of lead (IV) acetate (15.7 g, 33.67 mmol, 1 eq) in anhydrous CH₂Cl₂ (180 mL) over a period of 1 hr. The reaction was stirred at rt for 1 hr, diluted with water (225 mL), washed with 10% NaHCO₃ (85 mL), and brine (85 mL). The organic layer was dried (MgSO₄), filtered, and concentrated by rotary evaporation. Column chromatography (70:30 EtOAc/hexane, Rᵣ = 0.2) of the crude residue yielded pure 86 as a yellow solid in 61% yield: ¹H NMR (CDCl₃) δ 3.06 and 3.15 (2 s).

**Sodium (3-bromophenyl)methanesulfonate (93).** The following procedure was adapted from the work of Kotoris et al.⁹⁸ To a solution of 3-bromobenzyl bromide (92, 13.0 g, 52 mmol, 1 eq) in acetone (51 mL) was added an aqueous solution of Na₂SO₃ (51 mL, 1 M, 1 eq). The reaction was refluxed for 48 hrs. The mixture was concentrated by rotary evaporation during which the product formed as a precipitate. The mixture was filtered and the filter cake washed with CH₂Cl₂ to give pure 93 as a white solid in 81% yield: ¹H NMR (D₂O) δ 7.56-7.63 (1H, m, Ar-H), 7.25-7.38 (2H, m, Ar-H), 4.16 (2H, s, CH₂); ¹³C NMR spectra could not be obtained due to solubility problems; LRESMS m/z (relative intensity) 251 (100).
(3-Bromophenyl)methanesulfonyl chloride (94). The following procedure was adapted from the work of Kotoris et al. To a solution of 93 (12.05 g, 44 mmol, 1 eq) in 1:1 acetonitrile/tetramethylsulfolane (44 mL) was added phosphorus oxychloride (17.3 mL, 28.46 g, 185.3 mmol, 4.2 eq). The reaction was stirred for 3 hrs at 70°C. cooled to rt. and then poured into cold water (300 mL) which resulted in the precipitation of the crude product. The precipitate was filtered off and then dissolved in CH₂Cl₂ (50 mL) and washed with water (3 x 100 mL). The organic layer was dried (MgSO₄), filtered, and concentrated by rotary evaporation to give pure 94 as a white solid in 85% yield: mp 90-91°C; ¹H NMR (CDCl₃) δ 7.62 (1 H, d, J = 6.6 Hz, Ar-H), 7.30-7.46 (34, m, Ar-H), 4.82 (2H, s, CH₂); ¹³C NMR (CDCl₃) δ 134.21, 133.45, 130.68, 129.98, 128.45, 123.08, 70.09; MS m/z (relative intensity) 268 (9), 169 (100), 90 (45), 63 (26); HRMS calcd for C₁₉H₁₆O₂S⁺ ClBr 267.8960, found 267.8951.

5-[[1-(tert-Butyl)-1,1-dimethylsilyl]oxy]-2,2-dimethylpentyl (3-bromophenyl)-methanesulfonate (95). The following procedure was adapted from the work of Kotoris et al. To a solution of 94 (9.00 g, 33.5 mmol, 1 eq) and 76 (12.40 g, 50.3 mmol, 1.5 eq) in anhydrous THF (60 mL) at 0°C was added a solution of triethylamine (6.1 mL, 4.43 g, 43.5 mmol, 1.3 eq) in anhydrous THF (60 mL). The reaction was stirred at rt overnight, concentrated by rotary evaporation, quenched with water (50 mL), and extracted with CH₂Cl₂ (3 x 50 mL). The combined organic layers were dried (MgSO₄), filtered, and concentrated by rotary evaporation. Column chromatography (75:15 CH₂Cl₂/hexane, Rf = 0.3) of the crude residue yielded pure 95 as a white solid in 86% yield: mp 41-43°C; ¹H NMR (CDCl₃) δ 7.25-7.60 (4H, br m, Ar-H), 4.31 (2H, s, CH₂O), 3.80 (2H, s, CH₂), 3.56 (2H, t, J = 6.2 Hz, CH₂O), 1.15-1.55 (4H, m, CH₂), 0.89 (15H, s, CH₃), 0.05 (6H, s,
CH$_3$; $^{13}$C NMR (CDCl$_3$) δ 133.65, 132.10, 130.47, 130.27, 129.27, 122.71, 78.11, 63.50, 56.04, 34.83, 34.06, 27.24, 25.98, 23.80, 18.31. -5.26; MS m/z (relative intensity) 309 (3), 169 (28), 97 (100), 75 (21), 55 (71); HRMS calcd for C$_{20}$H$_{36}$O$_4$Si$_2$S$_1$Br$_1$ (M + H$^+$) 479.1287, found 479.1293.

5-[(1-(tert-Butyl)-1,1-dimethylsilyloxy)-2,2-dimethylpentyl (3-bromophenyl)-(difluoro)methanesulfonate (96). The following procedure was adapted from the work of McAtee et al. To a solution of 95 (0.50 g, 1.05 mmol, 1 eq) and NFSi (1.00 g, 3.15 mmol, 3 eq) in anhydrous THF (28 mL) at -78°C was added NaHMDS (2.63 mL, 1.0 M, 2.63 mmol, 2.5 eq) dropwise over a period of one hr. The reaction was stirred at -78°C for 2 hrs. warmed to rt. and stirred overnight. The reaction was then quenched with water (30 mL) and extracted with ether (3 x 30 mL). The combined organic layers were dried (MgSO$_4$), filtered, and concentrated by rotary evaporation. Column chromatography (1:99 EtOAc/hexane. $R_f = 0.81$ in 15:85 EtOAc/hexane) of the crude residue yielded pure 96 as a pale yellow oil in 88% yield: $^1$H NMR (CDCl$_3$) δ 7.83 (1H. s. Ar-H). 7.71 (1H. d. J = 7.9 Hz. Ar-H). 7.64 (1H. d. J = 7.9 Hz. Ar-H). 7.38 (1H, t. J = 8.2 Hz. Ar-H). 4.16 (2H. s. CH$_2$O), 3.60 (9H. s. CH$_3$). 0.06 (6H. s. CH$_3$); $^{19}$F NMR (CDCl$_3$) δ -24.28; $^{13}$C NMR (CDCl$_3$) δ 135.64, 130.25, 130.06 (t. J$_{CF} = 6.3$ Hz), 129.95 (t. J$_{CF} = 23$ Hz), 125.79 (t. J$_{CF} = 5.8$ Hz), 122.69, 119.98 (t. J$_{CF} = 285$ Hz), 83.36, 63.34, 34.37, 34.33, 27.05, 25.91, 23.55, 18.28, -5.34; MS m/z (relative intensity) 383 (3), 207 (100), 171 (11), 147 (23), 126 (41), 115 (14), 97 (49), 83 (63), 69 (27), 55 (70); HRMS calcd for C$_{20}$H$_{34}$O$_4$F$_2$Si$_2$S$_1$Br$_1$ 515.1099, found 515.1120.

5-Hydroxy-2,2-dimethylpentyl (3-bromophenyl)(difluoro)methanesulfonate (97). The following procedure was adapted from the work of Corey et al. To a
solution of 96 (1.15 g, 2.24 mmol, 1 eq) in THF (6 mL) was added a mixture of 3:1 acetic acid/water (24 mL). The reaction was stirred at rt for 3 hrs, diluted in ether (25 mL), washed with water (3 x 25 mL), 5% NaHCO$_3$ (3 x 25 mL), and brine (3 x 25 mL). The organic layer was dried (MgSO$_4$), filtered, and concentrated by rotary evaporation. Column chromatography (25:75 EtOAc/hexane, R$_f$ = 0.3) of the crude residue yielded pure 97 as a pale yellow oil in 51-89% yield: $^1$H NMR (CDCl$_3$) $\delta$ 7.82 (1H, s, Ar-H). 7.72 (1H, d, $J$ = 8.3 Hz, Ar-H). 7.63 (1H, d, $J$ = 7.9 Hz, Ar-H). 7.39 (1H, t, $J$ = 7.8 Hz). 4.16 (2H, s, CH$_2$O). 3.62 (2H, t, $J$ = 6.4 Hz, CH$_2$O). 2.22 (1H, s, OH). 1.49-1.59 (2H, m, CH$_2$). 1.33-1.39 (2H, m, CH$_2$). 0.98 (6H, s, CH$_3$): $^{19}$F NMR (CDCl$_3$) $\delta$ -24.28. $^{13}$C NMR (CDCl$_3$) $\delta$ 135.58, 130.27, 130.01 (t, $J_{CF}$ = 6.3 Hz). 129.79 (t, $J_{CF}$ = 24 Hz). 125.77 (t, $J_{CF}$ = 6.3 Hz). 122.67, 119.97 (t, $J_{CF}$ = 284 Hz). 83.22, 63.01, 34.32, 34.20, 26.77, 23.50; MS m/z (relative intensity) 205 (100). 126 (39). 115 (8). 101 (30). 83 (64). 69 (21). 55 (56); HRMS calcd for C$_{14}$H$_{19}$O$_3$F$_2$SiBr$_1$ 400.0156, found 400.0131.

2,2-Dimethyl-5-phenoxypentyl (3-bromophenyl)(difluoro)methanesulfonate (98). The following procedure was adapted from the work of Rano *et al.*$^{112}$ To a solution of phenol (0.055 g, 0.58 mmol, 1 eq) in 1:1 anhydrous THF/CH$_2$Cl$_2$ (26 mL) was added TMAD (86. 0.20 g. 1.16 mmol. 2 eq). Once the TMAD was completely dissolved, a solution of 97 (0.47 g, 1.16 mmol, 2 eq) in 1:1 anhydrous THF/CH$_2$Cl$_2$ (14 mL) was added. Tributylphosphine (0.30 mL, 0.24 g, 1.16 mmol, 2 eq) was added dropwise over a period of 5 min. The reaction was stirred at rt for 1.5 hrs and then concentrated by rotary evaporation. Column chromatography (50:50 CH$_2$Cl$_2$/hexane, R$_f$ = 0.6) of the crude residue yielded pure 98 as a colorless oil in quantitative yield: $^1$H NMR (CDCl$_3$) $\delta$ 7.83 (1H, s, Ar-H). 7.71 (1H, d, $J$ = 8.3 Hz, Ar-H). 7.64 (1H, d, $J$ = 7.9 Hz, Ar-H). 7.37 (1H, t.
J = 8.1 Hz, Ar-H). 7.24-7.30 (2H, m, Ar-H), 6.95 (1H, d, J = 7.6 Hz, Ar-H). 7.39 (1H, J = 7.4 Hz, Ar-H), 7.24-7.30 (3H, m, Ar-H), 6.95 (1H, d, J = 7.6 Hz, CH₂). 1.45-1.51 (2H, m, CH₂), 1.02 (6H, s, CH₃); ¹⁹F NMR (CDCl₃) δ -24.19; ¹³C NMR (CDCl₃) δ 159.20, 135.94, 130.64, 130.40 (t, JCF = 6.8 Hz), 130.20 (t, JCF = 23 Hz), 129.75, 126.14 (t, JCF = 6.4 Hz), 123.06, 120.95, 120.36 (t, JCF = 285 Hz), 114.74, 83.44, 68.23, 34.86, 34.78, 24.04, 23.86: MS m/z (relative intensity) 476 (20), 205 (85), 126 (24), 97 (37), 94 (100). 83 (24), 55 (57); HRMS calcd for C₂₀H₂₃O₄F₂S·BrI 476.0469, found 476.0475.

4-Acetoxy non-crosslinked polystyrene polymer (NCPS, 103). The following procedure was adapted from the work of Hum et al. A solution of styrene (46 mL, 41.81 g, 401 mmol, 1 eq), 4-acetoxystyrene (5.9 mL, 6.25 g, 37.02 mmol, 0.09 eq), and VAZO (0.49 g, 2.01 mmol, 0.005 eq) in deoxygenated and anhydrous toluene (120 mL) was heated at 95 °C for 48 hrs. The toluene was deoxygenated by bubbling nitrogen through it overnight. Once the reaction cooled to rt. it was diluted with CH₂Cl₂ (75 mL) and then added dropwise, using an addition funnel, to a solution of MeOH (1.2 L) and brine (10 mL). The polymer (103) was collected by filtration, washed with MeOH (300 mL), and dried under high vacuum to give a white solid (44 g): ¹H NMR (CDCl₃) δ 6.30-7.60 (br d, Ar-H), 2.36 (br s, CH₃), 1.30-2.25 (br d, -CH₂-CH-).

4-Hydroxylated NCPS (104). To a solution of polymer 103 (5 g) in THF (40 mL) was added an aqueous solution of NaOH (5 mL, 5 M). The reaction was refluxed for 24 hrs, cooled to rt, diluted with CH₂Cl₂ (10 mL), and then added dropwise, using an addition funnel, to a solution of MeOH (300 mL) and brine (3 mL). The polymer (104) was collected by filtration, washed with MeOH (100 mL), and dried under high vacuum.
to give a white solid (4.5 g, 90% polymer recovered): $^1$H NMR (CDCl$_3$) $\delta$ 6.20-7.50 (br d, Ar-H), 4.48 (br s, OH), 1.10-2.30 (br d, -CH$_2$-CH-).

5-(4-ethylphenoxy)-2,2-dimethylpentyl (3-bromophenyl)(difluoro)methanesulfonate (106). The following procedure was adapted from the work of Rano et al.$^{112}$

To a solution of 4-ethylphenol (105, 0.101 g, 0.83 mmol, 1 eq) in 1:1 anhydrous THF/CH$_2$Cl$_2$ (45 mL) was added TMAD (86, 0.287 g, 1.66 mmol, 2 eq). Once the TMAD was completely dissolved, a solution of 97 (0.665 g, 1.66 mmol, 2 eq) in 1:1 anhydrous THF/CH$_2$Cl$_2$ (25 mL) was added. Tributylphosphine (0.427 mL, 0.35 g, 1.66 mmol, 2 eq) was then added dropwise to the reaction mixture over a period of 5 min. The reaction was stirred at rt for 3 hrs and then concentrated by rotary evaporation. Column chromatography (50:50 CH$_2$Cl$_2$/hexane. R$_f$ = 0.6) of the crude residue yielded pure 106 as a white solid in 96% yield: mp 36-37°C; $^1$H NMR (CDCl$_3$) $\delta$ 7.84 (1 H, s, Ar-H), 7.72 (1H, d, J = 8.0 Hz, Ar-H), 7.64 (1H, d, J = 7.3 Hz, Ar-H), 7.39 (1H, t, J = 8.1 Hz, Ar-H), 7.11 (2H, d, J = 8.0 Hz, Ar-H), 6.82 (2H, d, J = 8.8 Hz, Ar-H), 4.19 (2H, s, CH$_2$O), 3.93 (2H, t, J = 6.2 Hz, CH$_2$), 2.59 (2H, q, J = 7.6 Hz, CH$_2$), 1.73-1.85 (2H, m, CH$_2$), 1.44-1.54 (2H, m, CH$_2$), 1.21 (3H, t, J = 7.7 Hz, CH$_3$), 1.03 (6H, s, CH$_3$); $^{19}$F NMR (CDCl$_3$) $\delta$ -24.19; $^{13}$C NMR (CDCl$_3$) $\delta$ 157.18, 136.53, 135.62 (t, $J_{CF}$ = 1.8 Hz), 130.28, 130.21 (t, $J_{CF}$ = 6.4 Hz), 128.70, 125.86 (t, $J_{CF}$ = 6.0 Hz), 122.81, 120.15, 114.65, 82.99, 63.38, 34.85, 34.52, 28.00, 23.95, 23.64, 15.72; MS m/z (relative intensity) 504 (28), 205 (59), 122 (100), 107 (65), 97 (29), 83 (25), 55 (52); HRMS calcd for C$_{22}$H$_{27}$O$_4$F$_2$S$_1$Br$_1$ 504.0782, found 504.0774.

Coupling of 97 to 5% 4-hydroxylated NCPS (110). To a solution of 4-hydroxylated NCPS (104, 12.3 g, 6.2 mmol, 1 eq) in an anhydrous mixture of 1:1
CH$_2$Cl$_2$/THF (50 mL) was added TMAD (86.4 g, 24.8 mmol, 4 eq). The reaction was stirred until TMAD was completely dissolved. A solution of 97 (10.0 g, 24.8 mmol, 4 eq) in an anhydrous mixture of 1:1 CH$_2$Cl$_2$/THF (25 mL) was then added, followed by tributylphosphine (6.2 mL, 5.0 g, 24.8 mmol, 4 eq) added dropwise over a period of 5 min. The reaction was stirred at rt overnight, diluted with CH$_2$Cl$_2$ (35 mL), and then added dropwise, using an addition funnel, to a solution of MeOH (650 mL) and brine (7 mL). The resulting polymer (110) was stirred for 3 hrs, collected by filtration, washed with MeOH (250 mL), and dried under high vacuum to give a white solid (11.8 g, 96% polymer recovery): $^1$H NMR (CDCl$_3$) δ 7.84 (br s, Br-Ar-H), 7.66 (br t, Br-Ar-H), 7.34 (br t, Br-Ar-H), 6.20-7.22 (br d, Ar-H), 4.21 (br s, CH$_2$O), 3.84 (br s, CH$_2$O), 1.10-2.30 (br d, -CH$_2$-CH- and CHI), 1.02 (br s, CH$_3$); $^{19}$F NMR (CDCl$_3$) δ -24.18. The aryl bromide content was determined by $^1$H NMR to be 0.54 mmol per 1 g of the polymer.

**Coupling of 108 to 4-Hydroxylated NCPS (111).** To a solution of 4-hydroxylated NCPS (104, 0.5 g, 0.27 mmol, 1 eq) in anhydrous CH$_2$Cl$_2$ (3 mL) was added pyridine (0.109 mL, 0.107 g, 1.35 mmol, 5 eq) followed by 4-nitrocinnamoyl chloride (108, 0.285 g, 1.35 mmol, 5 eq). The reaction was stirred at rt overnight, diluted with CH$_2$Cl$_2$ (1 mL), and then added dropwise, using an addition funnel, to a solution of MeOH (25 mL) and a few drops of brine. The polymer (111) was collected by filtration, washed with MeOH (50 mL), and dried under high vacuum to give a white solid in quantitative yield: $^1$H NMR (CDCl$_3$) δ 8.26 (br d, NO$_2$-Ar-H), 7.85 (br d, CH), 7.70 (br d, NO$_2$-Ar-H), 6.20-7.30 (br d, Ar-H), 1.10-2.30 (br d, -CH$_2$-CH-). The nitrocinnamoyl content was determined by $^1$H NMR to be 0.64 mmol per 1 g of the polymer.
4-Ethylphenyl (E)-3-(4-nitrophenyl)-2-propenoate (112). To a solution of 4-ethylphenol (105. 0.5 g, 4.09 mmol. 1 eq) and 4-nitrocinnamoyl chloride (108. 1.30 g. 6.14 mmol. 1.5 eq) in anhydrous THF (8 mL) was added a solution of triethylamine (0.74 mL. 0.54 g. 5.32 mmol. 1.3 eq) in anhydrous THF (2 mL). The reaction was stirred at rt overnight. concentrated by rotary evaporation, quenched with water (15 mL), and extracted with CH$_2$Cl$_2$ (3 x 15 mL). The combined organic layers were dried (MgSO$_4$), filtered, and concentrated by rotary evaporation. Column chromatography (75:25 CH$_2$Cl$_2$/hexane, R$_r$ = 0.4) of the crude residue yielded pure 112 as a yellow solid in 89% yield: mp = 148.5-149.5 °C; $^1$H NMR (CDCl$_3$) $\delta$ 8.29 (2H. d. J = 8.9 Hz, NO$_2$-Ar-H). 7.89 (1H. d. J = 16.2 Hz, CH). 7.74 (2H. d. J = 8.8 Hz, NO$_2$-Ar-H). 7.25 (2H. d. J = 8.0 Hz, Et-Ar-H). 7.08 (2H. d. J = 8.2 Hz, Et-Ar-H). 6.76 (1H. d. J = 16.2 Hz, CH). 2.67 (2H. q. J = 7.5 Hz, CH$_2$). 1.25 (3H. t. J = 7.7 Hz, CH$_3$); $^{13}$C NMR (CDCl$_3$) $\delta$ 164.57. 148.55, 148.34, 143.21, 141.94, 140.11, 128.75, 124.10, 121.63, 121.05, 28.19, 15.45; MS m/z (relative intensity) 297 (15), 176 (100), 130 (27), 102 (25); HRMS calec for C$_{17}$H$_{15}$N$_1$O$_4$ 297.1001. found 297.1006.

General method for Suzuki cross coupling on polymer 110. The following procedure was adapted from the work of Hum et al.$^{74}$ Polymer 110 (0.500 g. 0.27 mmol. 1 eq). arylboronic acid (0.81 mmol. 3 eq). K$_2$CO$_3$ (0.112 g. 0.81 mmol. 3 eq). and (PhCN)$_2$PdCl$_2$ (0.021 g. 0.054 mmol. 0.2 eq) were placed in a round bottom flask, flushed with nitrogen. Deoxygenated DMF (3 mL) was added followed by the addition of water (0.049 mL. 2.7 mmol. 10 eq). The reaction mixture was stirred at rt and monitored by $^{19}$F NMR. Upon completion, the reaction mixture was diluted with CH$_2$Cl$_2$ (10 mL) and centrifuged twice to remove the palladium catalyst. The supernatants were combined
and concentrated by rotary evaporation. The resulting polymer was redissolved in CH₂Cl₂ (3 mL), precipitated in a mixture of MeOH (25 mL) and a few drops of brine, collected by filtration, and washed with MeOH. Percent recovery of polymer ranged from 85%-95%. Only a single polymer-bound species was evident by ¹⁹F NMR. However, there was 2-7% hydrolysis of the polymer-bound sulfonates from the support.

**General method for cleaving the product from the polymer (general structure 113).** The polymer-bound biaryl derivatives (general structure 113, 1 eq) and K₂CO₃ (3 eq) were placed in a 50 mL test tube, equipped with a stir bar and stopper, and dissolved in DMF (3 mL). Water (10 eq) was then added to the reaction mixture and heated at 80 °C for 17 hrs. The reaction was diluted with CH₂Cl₂ (3 mL) and the polymer was precipitated out in a mixture of MeOH (25 mL) and a few drops of brine. The polymer was separated from the product by filtration, and the filtrate was concentrated by rotary evaporation. To remove trace amounts of polymer and other organic impurities, the following wash procedure was performed. The crude reaction product was dissolved in an aqueous solution of NaOH (10 mL, 1.0 N) and washed with CH₂Cl₂ (3 x 10 mL). The aqueous layer was acidified to pH~0.5 with HCl (10 N). NaCl was added until a saturated solution was obtained. The sulfonic acids were then extracted with EtOAc (3 x 15 mL). The combined organic layers were dried (MgSO₄), filtered, diluted with toluene (50 mL), and concentrated on a high vacuum rotary evaporator. The sulfonic acids were then dissolved in water (3 mL) and treated with NH₄HCO₃ (2.5 eq). After repeated lyophilizations, the biaryl sulfonates were obtained as off-white solids (purity as obtained by HPLC).
Ammonium salt of [3-(3'-chlorophenyl)phenyl](difluoro)methylsulfonic acid (120). 120 was obtained in 62% yield (90% pure): \(^1\)H NMR (D\(_2\)O) δ 7.89 (1H. d. J = 7.7 Hz. Ar-H). 7.68 (2H. d. J = 8.0 Hz. Ar-H). 7.55-7.62 (2H. m. Ar-H). 7.40-7.43 (2H. m. Ar-H); \(^19\)F NMR (D\(_2\)O) δ -25.23: LRESMS m/z (relative intensity) 317 (100); HRESMS calcd for C\(_{13}\)H\(_8\)F\(_2\)Cl\(_3\)S\(_3\)I\(_3\) 316.9851. found 316.9856. HPLC retention time = 16.7 min.

Ammonium salt of [3-(3'-fluorophenyl)phenyl](difluoro)methylsulfonic acid (121). 121 was obtained in 72% yield (94% pure): \(^1\)H NMR (D\(_2\)O) δ 7.82 (1H. s. Ar-H). 7.83 (1H. d. J = 7.0 Hz. Ar-H). 7.40-7.71 (5H. br m. Ar-H). 7.10-7.19 (1H. m. Ar-H); \(^19\)F NMR (D\(_2\)O) δ -25.25. -35.23: LRESMS m/z (relative intensity) 301 (100); HRESMS calcd for C\(_{13}\)H\(_8\)F\(_3\)S\(_3\)I 301.0146. found 301.0152. HPLC retention time = 13.7 min.

Ammonium salt of [3-(4'-chlorophenyl)phenyl](difluoro)methylsulfonic acid (122). 122 was obtained in 84% yield (100% pure): \(^1\)H NMR (D\(_2\)O) δ 7.83 (1H. s. Ar-H). 7.64 (2H. d. J = 7.0 Hz. Ar-H). 7.47-7.54 (3H. m. Ar-H). 7.36 (2H. d. J = 8.3 Hz. Ar-H); \(^19\)F NMR (D\(_2\)O) δ -25.03: LRESMS m/z (relative intensity) 317 (100); HRESMS calcd for C\(_{13}\)H\(_8\)F\(_2\)Cl\(_3\)S\(_3\)I\(_3\) 316.9851. found 316.9856. HPLC retention time = 16.8 min.

Ammonium salt of [3-(4'-phenyl)phenyl](difluoro)methylsulfonic acid (123). 123 was obtained in 67% yield (95% pure): \(^1\)H NMR (D\(_2\)O) δ 7.88 (1H. s. Ar-H). 7.78 (1H. d. J = 7.4 Hz. Ar-H). 7.53-7.69 (4H. br m. Ar-H). 7.20 (2H. t. J = 8.92 Hz. Ar-H); \(^19\)F NMR (D\(_2\)O) δ -25.18. -37.15: LRESMS m/z (relative intensity) 301 (100); HRESMS calcd for C\(_{13}\)H\(_8\)F\(_3\)S\(_3\)I 301.0146. found 301.0152. HPLC retention time = 13.8 min.

Ammonium salt of [3-(3'-chloro-4'-fluorophenyl)phenyl](difluoro)methylsulfonic acid (124). 124 was obtained in 88% yield (100% pure): \(^1\)H NMR (D\(_2\)O) δ 7.79 (1H. s. Ar-H). 7.43-7.66 (5H. br m. Ar-H). 7.22 (1H. t. J = 8.9 Hz. Ar-H); \(^19\)F NMR
(D₂O) δ -25.15, -40.14: LRESMS m/z (relative intensity) 335 (100); HRESMS calcd for 
C₁₃H₁₇F₃Cl₁₃S₀₃ 334.9757, found 334.9762. HPLC retention time = 16.7 min.

**Ammonium salt of [3-(3',4'-dichlorophenyl)phenyl](difluoro)methylsulfonic acid (125).** 125 was obtained in 81% yield (99% pure): ¹H NMR (D₂O) δ 7.85 (1H. s. Ar-H). 7.67-7.75 (3H. m. Ar-H). 7.45-7.59 (3H. m. Ar-H); ¹⁹F NMR (D₂O) δ -25.10: LRESMS m/z (relative intensity) 351 (100); HRESMS calcd for C₁₃H₁₇F₂Cl₂S₀₃ 350.9461, found 350.9466. HPLC retention time = 21.0 min.

**Ammonium salt of [3-(3',5'-dichlorophenyl)phenyl](difluoro)methylsulfonic acid (126).** 126 was obtained in 97% yield (98% pure): ¹H NMR (D₂O) δ 7.83 (1H. s. Ar-H). 7.64-7.72 (2H. m. Ar-H). 7.51-7.59 (3H. m. Ar-H). 7.37 (1H. t. J = 1.91. Ar-H); ¹⁹F NMR (D₂O) δ -25.13: LRESMS m/z (relative intensity) 463(4). 351 (100); HRESMS calcd for C₁₃H₁₇F₂Cl₂S₀₃ 350.9461, found 350.9466. HPLC retention time = 22.1 min.

**Ammonium salt of [3-(3'-trifluoromethylphenyl)phenyl](difluoro)methylsulfonic acid (127).** 127 was obtained in 73% yield (93% pure): ¹H NMR (D₂O) δ 7.93 (2H. d. J = 6.4 Hz. Ar-H). 7.82 (2H. t. J = 7.5 Hz. Ar-H). 7.55-7.70 (4H. br m. Ar-H); ¹⁹F NMR (D₂O) δ 16.10. -25.20: LRESMS m/z (relative intensity) 351 (100); HRESMS calcd for C₁₄H₈F₃S₀₃ 351.0114, found 351.0120. HPLC retention time = 15.5 min.

**Ammonium salt of [3-(4'-trifluoromethylphenyl)phenyl](difluoro)methylsulfonic acid (128).** 128 was obtained in 79% yield (100% pure): ¹H NMR (D₂O) δ 7.92 (1H. s. Ar-H). 7.69-7.80 (6H. m. Ar-H). 7.58 (1H. t. J = 7.8 Hz. Ar-H); ¹⁹F NMR (D₂O) δ 16.21. -25.08: LRESMS m/z (relative intensity) 351 (100); HRESMS calcd for C₁₄H₈F₃S₀₃ 351.0114, found 351.0120. HPLC retention time = 16.0 min.
Ammonium salt of [3-(4'-acetylphenyl)phenyl](difluoro)methylsulfonic acid (129). 129 was obtained in 88% yield (100% pure): $^1$H NMR (D$_2$O) $\delta$ 7.75-7.79 (3H. m. Ar-H), 7.64 (1H. d. J = 7.5 Hz. Ar-H), 7.57 (1H. d. J = 7.5. Ar-H), 7.44-7.49 (3H. m. Ar-H), 2.50 (3H. s. CH$_3$); $^{19}$F NMR (D$_2$O) $\delta$ -24.94; LRESMS $m/z$ (relative intensity) 325 (100); HRESMS calcd for C$_{15}$H$_{11}$F$_2$S$_1$O$_4$ 325.0346. found 325.0352. HPLC retention time = 11.9 min.

Ammonium salt of (3-phenylphenyl)(difluoro)methylsulfonic acid (130). 130 was obtained in 97% yield (99% pure): $^1$H NMR (D$_2$O) $\delta$ 7.95 (1H. s. Ar-H), 7.85 (1H. d. J = 7.6 Hz. Ar-H), 7.41-7.73 (7H. br m. Ar-H); $^{19}$F NMR (D$_2$O) $\delta$ -25.03: LRESMS $m/z$ (relative intensity) 283 (100); HRESMS calcd for C$_{13}$H$_9$F$_2$S$_1$O$_3$ 283.0241. found 283.0246. HPLC retention time = 13.7 min.

Ammonium salt of [3-(4'-biphenyl)phenyl](difluoro)methylsulfonic acid (131). 131 was obtained in 34% yield (99% pure): $^1$H NMR (CD$_2$OD) $\delta$ 7.98 (1H. s. Ar-H), 7.65-7.82 (8H. br m. Ar-H), 7.33-7.57 (4H. br m. Ar-H); $^{19}$F NMR (D$_2$O) $\delta$ -24.41: LRESMS $m/z$ (relative intensity) 359 (100); HRESMS calcd for C$_{16}$H$_{13}$F$_2$S$_1$O$_3$ 359.0554. found 359.0559. HPLC retention time = 22.1 min.

Ammonium salt of [3-(2'-naphthyl)phenyl](difluoro)methylsulfonic acid (132). 132 was obtained in 91% yield (100% pure): $^1$H NMR (D$_2$O) $\delta$ 7.62-7.75 (5H. m. Ar-H), 7.22-7.49 (6H. br m. Ar-H); $^{19}$F NMR (D$_2$O) $\delta$ -25.04: LRESMS $m/z$ (relative intensity) 333 (100); HRESMS calcd for C$_{17}$H$_{11}$F$_2$S$_1$O$_3$ 333.0397. found 333.0402. HPLC retention time = 18.4 min.

Ammonium salt of [3-(1'-naphthyl)phenyl](difluoro)methylsulfonic acid (133). 133 was obtained in 92% yield (99% pure): $^1$H NMR (D$_2$O) $\delta$ 7.88 (1H. s. Ar-H),
7.62 (2H, d, \( J = 10.3 \text{ Hz, Ar-H} \)), 7.18-7.54 (8H, br m, Ar-H); \(^{19}\text{F NMR (D}_2\text{O}) \delta -24.73; \)

LRESMS \( m/z \) (relative intensity) 333 (100); HRESMS calcd for C\(_{17}\)H\(_{11}\)F\(_2\)S\(_3\)O\(_3\) 333.0397, found 333.0402. HPLC retention time = 18.9 min.

**Ammonium salt of [3-(2'-methylphenyl)phenyl](difluoro)methylsulfonic acid** (134). 134 was obtained in 89% yield (99% pure): \(^1\text{H NMR (D}_2\text{O}) \delta 7.53-7.71 (4H, br m, Ar-H), 7.29-7.39 (4H, br m, Ar-H), 2.23 (3H, s, \text{CH}_3); \(^{19}\text{F NMR (D}_2\text{O}) \delta -25.08; \)

LRESMS \( m/z \) (relative intensity) 297 (100); HRESMS calcd for C\(_{14}\)H\(_{11}\)F\(_2\)S\(_3\)O\(_3\) 297.0397, found 297.0402. HPLC retention time = 15.2 min.

**Ammonium salt of [3-(4'-ethylphenyl)phenyl](difluoro)methylsulfonic acid** (135). 135 was obtained in 71% yield (98% pure): \(^1\text{H NMR (D}_2\text{O}) \delta 7.85 (1H, s, Ar-H), 7.61 (2H, d, \( J = 8.0 \text{ Hz, Ar-H} \)), 7.41-7.49 (3H, m, Ar-H), 7.16 (2H, d, \( J = 8.2 \text{ Hz, Ar-H} \)), 2.49 (2H, q, \( J = 7.67 \text{ Hz, CH}_2 \)), 1.06 (3H, t, \( J = 6.7 \text{ Hz, CH}_3 \)); \(^{19}\text{F NMR (D}_2\text{O}) \delta -24.90; \)

LRESMS \( m/z \) (relative intensity) 311 (100); HRESMS calcd for C\(_{15}\)H\(_{13}\)F\(_2\)S\(_3\)O\(_3\) 311.0554, found 311.0559. HPLC retention time = 18.2 min.

**Ammonium salt of [3-(4'-methylphenyl)phenyl](difluoro)methylsulfonic acid** (136). 136 was obtained in 63% yield (100% pure): \(^1\text{H NMR (D}_2\text{O}) \delta 7.91 (1H, s, Ar-H), 7.80 (1H, d, \( J = 7.6 \text{ Hz, Ar-H} \)), 7.56-7.68 (4H, br m, Ar-H), 7.33 (2H, d, \( J = 7.9 \text{ Hz, Ar-H} \)), 2.36 (3H, s, \text{CH}_3); \(^{19}\text{F NMR (D}_2\text{O}) \delta -25.02; \)

LRESMS \( m/z \) (relative intensity) 297 (100); HRESMS calcd for C\(_{14}\)H\(_{11}\)F\(_2\)S\(_3\)O\(_3\) 297.0397, found 297.0402. HPLC retention time = 16.0 min.

**Ammonium salt of [3-(4'-methoxyphenyl)phenyl](difluoro)methylsulfonic acid** (137). 137 was obtained in 92% yield (95% pure): \(^1\text{H NMR (D}_2\text{O}) \delta 7.80 (1H, s, Ar-H), 7.60 (2H, t, \( J = 7.0 \text{ Hz, Ar-H} \)), 7.43-7.50 (3H, m, Ar-H), 6.93 (2H, d, \( J = 8.7 \text{ Hz, Ar-H} \)).
Ar-H). 3.75 (3H, s, CH3); 19F NMR (D2O) δ -24.98: LRESMS m/z (relative intensity) 313 (100); HRESMS calcd for C14H11F2S1O4 313.0346, found 313.0352. HPLC retention time = 13.8 min.

**Ammonium salt of [3-(4'-tert-butylphenyl)phenyl](difluoro)methylsulfonic acid (138).** 138 was obtained in 85% yield (100% pure): 1H NMR (D2O) δ 7.79 (1H, s, Ar-H), 7.59 (1H, d, J = 5.6 Hz, Ar-H), 7.08-7.34 (6H, br m, Ar-H), 0.95 (9H, s, CH3); 19F NMR (D2O) δ -24.51: LRESMS m/z (relative intensity) 339 (100); HRESMS calcd for C17H17F2SiO3 339.0867, found 339.0872. HPLC retention time = 22.3 min.

**Ammonium salt of [3-(5'-acetyl-2'-thiophene)phenyl](difluoro)methylsulfonic acid (139).** 139 was obtained in 54% yield (77% pure): 7.77 (1H, s, Ar-H), 7.55-7.68 (3H, m, Ar-H), 7.41 (1H, t, J = 7.4 Hz, Ar-H), 7.25-7.27 (1H, m, Ar-H), 2.44 (3H, s, CH3); 19F NMR (D2O) δ -25.34: LRESMS m/z (relative intensity) 331 (100), 287 (25); HRESMS calcd for C13H8F2S2O4 330.9910, found 330.9916. HPLC retention time = 11.8 min.

**Ammonium salt of [3-(2'-benzo[b]furan)phenyl](difluoro)methylsulfonic acid (140).** 140 was obtained in 82% yield (100% pure): 1H NMR (D2O) δ 7.87 (1H, s, Ar-H), 7.43-7.53 (2H, m, Ar-H), 7.09-7.27 (3H, br m, Ar-H), 6.88-7.00 (2H, br m, Ar-H), 6.55 (1H, s, Ar-H); 19F NMR (D2O) δ -25.06: LRESMS m/z (relative intensity) 323 (100); HRESMS calcd for C15H9F2S1O4 323.0190, found 323.0195. HPLC retention time = 17.0 min.

**Ammonium salt of [3-(2'-benzo[b]thiophene)phenyl](difluoro)methylsulfonic acid (141).** 141 was obtained in 56% yield (98% pure): 1H NMR (D2O) δ 7.78 (1H, s, Ar-H), 7.50 (1H, d, J = 7.3 Hz, Ar-H), 7.37 (1H, d, J = 6.9 Hz, Ar-H), 7.29 (2H, m, Ar-
H). 7.17 (1H, t, J = 7.7 Hz, Ar-H), 7.08 (1H, s, Ar-H), 6.94-7.03 (2H, m, Ar-H); $^{19}$F NMR (D$_2$O) $\delta$ -24.91; LRESMS m/z (relative intensity) 339 (100); HRESMS calcd for C$_{15}$H$_6$F$_2$S$_2$O$_3$ 338.9961, found 338.9967. HPLC retention time = 21.3 min.

Ammonium salt of [3-(2'-thioophene)phenyl](difluoro)methylsulfonic acid (142). 142 was obtained in 64% yield (100% pure): $^1$H NMR (D$_2$O) $\delta$ 7.94 (1H, s, Ar-H), 7.84 (1H, d, J = 7.0 Hz, Ar-H), 7.45-7.63 (4H, br m, Ar-H), 7.15 (1H, t, J = 4.4 Hz, Ar-H); $^{19}$F NMR (D$_2$O) $\delta$ -25.30; LRESMS m/z (relative intensity) 289 (100); HRESMS calcd for C$_{11}$H$_7$F$_2$S$_2$O$_3$ 288.9805, found 288.9810. HPLC retention time = 13.4 min.

Ammonium salt of [3-(2'-furanphenyl)phenyl](difluoro)methylsulfonic acid (143). 143 was obtained in 39% yield (100% pure): $^1$H NMR (D$_2$O) $\delta$ 8.02 (1H, s, Ar-H), 7.93 (1H, d, J = 6.6 Hz, Ar-H), 7.56-7.64 (3H, m, Ar-H), 6.93 (1H, d, J = 3.6 Hz, Ar-H), 6.62 (1H, s, Ar-H); $^{19}$F NMR (D$_2$O) $\delta$ -25.38; LRESMS m/z (relative intensity) 273 (100); HRESMS calcd for C$_{11}$H$_7$F$_2$S$_2$O$_4$ 273.0033, found 273.0039. HPLC retention time = 12.1 min.

Ammonium salt of [3-(3'-thiophene)phenyl](difluoro)methylsulfonic acid (144). 144 was obtained in 72% yield (100% pure): $^1$H NMR (D$_2$O) $\delta$ 7.95 (1H, s, Ar-H), 7.84 (1H, d, J = 7.84 Hz, Ar-H), 7.71-7.73 (1H, m, Ar-H), 7.50-7.63 (4H, br m, Ar-H); $^{19}$F NMR (D$_2$O) $\delta$ -25.11; LRESMS m/z (relative intensity) 289 (100); HRESMS calcd for C$_{11}$H$_7$F$_2$S$_2$O$_3$ 288.9805, found 288.9810. HPLC retention time = 12.8 min.

3,5-Dichloro-3'-methyl-1,1'-biphenyl (151). The following procedure was adapted from the work of O'Shea et al. A solution of 3-bromotoluene (150. 0.171 g, 1.00 mmol, 1 eq) and Pd(PPh$_3$)$_4$ (0.035 g, 0.03 mmol, 0.03 eq) in DME (4 mL) was stirred for 20 min at rt. An aqueous solution of Na$_2$CO$_3$ (3 mL, 0.35 M, 1.05 eq) was
added to the reaction mixture, followed by a solution of 3,5 dichlorobenzene boronic acid (0.200 g, 1.05 mmol, 1.05 eq) in DME (1 mL). The reaction was refluxed for 3 hrs. cooled to rt. and diluted with ether (15 mL). The resulting reaction mixture was washed with 5% NaHCO₃ (3 x 15 mL) and brine (2 x 15 mL). The organic layer was dried (MgSO₄), filtered, and concentrated by rotary evaporation. Column chromatography (100% hexane. Rᵣ = 0.5), by atmospheric pressure, of the crude residue yielded pure 151 as a white solid in 87% yield: mp 43-45°C; ¹H NMR (CDCl₃) δ 7.19-7.45 (7H, m, Ar-H). 2.41 (3H, s, CH₃); ¹³C NMR (CDCl₃) δ 144.30, 138.67, 138.46, 135.14, 129.13, 128.88, 127.74, 126.97, 125.59, 124.12, 21.44: MS m/z relative intensity 236 (100). 201 (23), 165 (63); HRMS calcd for C₁₃H₁₀Cl₂ 236.0160. found 236.0159.

3′-(Bromomethyl)-3,5-dichloro-1,1′-biphenyl (152). The following procedure was adapted from the work of Kotoris et al.⁹⁸ A solution of 151 (0.125 g, 0.53 mmol, 1 eq) and N-bromosuccinimide (0.103 g, 0.58 mmol, 1.1 eq) in benzene (5 mL) was irradiated to reflux for 1 hr using an IR lamp. Once the reaction cooled to rt. the mixture was washed with water (3 x 5 mL). The organic layer was dried (MgSO₄), filtered, and concentrated by rotary evaporation. Column chromatography (100% hexane. Rᵣ = 0.2) of the crude residue yielded a mixture of mono- 152a and dibrominated 152b products as a white solid (75% yield of 152a based on ¹H NMR). This material was used in the next step without any further purification: ¹H NMR (CDCl₃) δ 4.54 (2H, s, CH₂, monobrominated product, 152a). 6.69 (1H, s, CH₂, dibrominated product, 152b).

Diethyl (3′,5′-dichloro-1,1′-biphenyl-3-yl)methylphosphonate (153). The following procedure was adapted from the work of Hum et al.⁷⁴ To a solution of 152a and b (152a, 0.53 g, 1.67 mmol, 1 eq) in benzene (1.5 mL) was added triethyl phosphite.
(1.5 mL, 1.46 g, 8.37 mmol, 5 eq). The reaction was refluxed overnight. Benzene was removed by distillation, and unreacted triethyl phosphite was removed by vacuum distillation. The resulting crude residue was then subjected to high vacuum for 1 hr. Column chromatography (50:50 EtOAc/hexane, Rf = 0.1) of the crude residue yielded pure 153 as a yellow oil in 92% yield: \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 7.31-7.47 (7H, m, Ar-H). 3.97-4.2 (4H, m, CH\(_2\)). 3.21 (2H, d, \(J_{HP} = 21.7\) Hz, CH\(_2\)). 1.26 (6H, t, \(J = 7.1\) Hz, CH\(_3\)). \(^{31}\)P NMR (CDCl\(_3\)) \(\delta\) 27.24: \(^{13}\)C NMR (CDCl\(_3\)) \(\delta\) 143.67. 138.61 (d). 135.12, 132.53 (d). 129.73 (d). 129.10. 128.35 (d). 127.09. 125.50. 62.07 (d). 33.65 (d, \(J_{CP} = 138.2\) Hz). 16.28 (d); MS \(m/z\) relative intensity 372 (100). 344 (43). 316 (32). 249 (23). 235 (79). 199 (35). 165 (93). 109 (55). 81 (40). 65 (13); HRMS calcd for C\(_{17}\)H\(_{19}\)O\(_3\)P\(_1\)Cl\(_2\) 372.0449. found 372.0433.

Diethyl (3',5'-dichloro-1,1'-biphenyl-3-yl)(difluoro)methylphosphonate (148). The following procedure was adapted from the work of McAtee et al.\(^{109}\) To a solution of 153 (0.400 g, 1.07 mmol, 1 eq) and NFSi (1.014 g, 3.23 mmol, 3 eq) in anhydrous THF (10 mL) at -78°C was added NaHMDS (2.68 mL, 1.0 M, 2.68 mmol, 2.5 eq) over a period of 20 min. The reaction was stirred at -78°C for 2 hrs, warmed to rt, and then stirred for an addition hr. The reaction was then quenched with water (15 mL), extracted with ether (15 mL), and washed with brine (2 x 15 mL). The organic layer was dried (MgSO\(_4\)), filtered, and concentrated by rotary evaporation. Column chromatography (20:80 EtOAc/hexane, Rf = 0.1) of the crude residue yielded pure 148 as a yellow oil in 95% yield: \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 7.78 (1H, s, Ar-H). 7.46-7.68 (5H, m, Ar-H). 7.36-7.38 (1H, m, Ar-H). 4.14-4.31 (4H, m, CH\(_2\)). 1.34 (6H, t, \(J = 7.2\) Hz, CH\(_3\)); \(^{19}\)F NMR (CDCl\(_3\)) \(\delta\) -32.62 (d, \(J_{FP} = 117.1\) Hz); \(^{31}\)P NMR (CDCl\(_3\)) \(\delta\) 7.33 (t, \(J_{PP} = 114.9\) Hz); \(^{13}\)C NMR
Diammonium (3\textsuperscript{'},5\textsuperscript{'}-dichloro-1,1\textsuperscript{'}-biphenyl-3-yl)(difluoro)methylphosphonate (154). The following procedure was adapted from the work of Hum \textit{et al.}\textsuperscript{74} To a solution of 148 (0.300 g, 0.73 mmol, 1 eq) in anhydrous CH\textsubscript{2}Cl\textsubscript{2} (12 mL) was added TMSBr (1.0 mL, 1.16 g, 7.33 mmol, 10 eq). The reaction was refluxed for 48 hrs. Once the reaction cooled to rt, it was concentrated by rotary evaporation, and then subjected to high vacuum overnight. The crude residue was dissolved in 1 N NaOH (15 mL) and washed with CH\textsubscript{2}Cl\textsubscript{2} (3 x 15 mL). The aqueous layer was acidified with 10 N HCl until pH~0.5, and then extracted with ether (3 x 20 mL). The combined organic layers were dried (MgSO\textsubscript{4}), filtered, and concentrated by rotary evaporation. The resulting phosphonic acid was then dissolved in water (5 mL) and treated with NH\textsubscript{4}HCO\textsubscript{3} (0.145 g, 1.83 mmol, 2.5 eq). After repeated lyophilizations, pure 154 was obtained as a white solid in 73\% yield: \textsuperscript{1}H NMR (D\textsubscript{2}O) \( \delta \) 7.82 (1H, s, Ar-H), 7.51-7.61 (5H, m, Ar-H), 7.37 (1H, s, Ar-H); \textsuperscript{19}F NMR (D\textsubscript{2}O) \( \delta \) -30.88 (d, \( J_{FP} \) = 93.5 Hz); \textsuperscript{31}P NMR (D\textsubscript{2}O) \( \delta \) 6.14 (t, \( J_{PF} \) = 93.3 Hz); \textsuperscript{13}C NMR spectra could not be obtained due to solubility problems; LRESMS \( m/z \) (relative intensity) 351 (100).

Sodium (3\textsuperscript{'},5\textsuperscript{'}-dichloro-1,1\textsuperscript{'}-biphenyl-3-yl)methanesulfonate (158). The following procedure was adapted from the work of Kotoris \textit{et al.}\textsuperscript{98} To a solution of 152a and b (152a, 0.144 g, 0.41 mmol, 1 eq) in acetone (2 mL) was added an aqueous solution of Na\textsubscript{2}SO\textsubscript{3} (2 mL, 0.2 M, 1 eq). The reaction was refluxed for 24 hrs, concentrated by
rotary evaporation, and then diluted with acetone until precipitate formed. The precipitate was filtered and the filter cake washed with CH$_2$Cl$_2$. Recrystallization of the crude residue in ethanol yielded pure 158 as a white solid in 65% yield: $^1$H NMR (D$_2$O) δ7.40-7.70 (7H, m, Ar-H), 4.15 (2H, s, CH$_2$); $^{13}$C NMR spectra could not be obtained due to solubility problems; LRESMS m/z (relative intensity) 315 (100).

**Kinetic Studies with PTP1B and CD45.** Rates of PTP1B- and CD45-catalyzed dephosphorylation in the presence or absence of inhibitors were determined using fluorescein diphosphate (FDP) as substrate in assay buffer containing 50 mM Bis-Tris (pH 6.3), 2 mM EDTA, 5 mM N.N'-dimethyl-N.N'-bis(mercaptoacetyl)hydrazine (DMH) or dithiothreitol (DTT), and 0.001% triton X-100. Assays were carried out at 25 °C in 96-well plates with total volume of 200 µL per well. Reactions were initiated by the addition of PTP1B (final concentration 0.5 µg/mL) or CD45 (final concentration 0.075 µg/mL). The phosphatase activity was followed by monitoring the production of the fluorescent product fluorescein monophosphate (FMP) continuously for 10 min using the Cytofluor II plate reader (PerSeptive Biosystems), with excitation at 440 nm (slit width 20 nm) and emission 530 nm (slit width 25 nm), or the SPECTRAmax GEMINI XS (Molecular Devices) dual-scanning microplate spectrofluorometer. with excitation at 485 nm and emission at 538 nm. IC$_{50}$ determinations were determined at ten or thirteen different inhibitor concentrations (done in duplicate for compound 154) with FDP at $K_M$ concentration (20 µM) and 5% DMSO.

**Kinetic Studies with H. pomatia Aryl Sulfatase A and B.** Rates of $H. pomatia$ aryl sulfatase-catalyzed desulfurylation in the presence or absence of inhibitors were determined using $p$-nitrophenyl sulfate ($p$-NPS) as substrate in assay buffer containing 20
mM MOPS (pH 7.0) and 0.1 M KCl. Assays were carried out at 25 °C in 1 mL cuvettes with total volumes of 1.03 mL. Reactions were initiated by the addition of *H. pomatia* aryl sulfatase type H5 (final concentration 0.03 mg/mL). The sulfatase activity was followed by monitoring the production of p-nitrophenol continuously for 20 min at 400 nm using a Varian Cary 1 spectrophotometer. The percent inhibition in the presence of inhibitors were performed in duplicate using p-NPS at K_M concentration (0.83 mM) with 500 μM inhibitor. IC_{50} determinations were determined in duplicate at eight or eleven different inhibitor concentrations with p-NPS at K_M concentration (0.70 mM).

3 Results and Discussion

3.1 Stability of Sulfonate Ester Linkage

Before PSOS was initiated, the stability of the sulfonate ester linkage was examined in solution to ensure that the polymer-bound sulfonates would be stable to the reaction conditions we wished to employ for the polymer-supported chemistry. Model compound 45 was prepared (Scheme 10) and tested in acidic and basic conditions.

The model sulfonate 45 was prepared in five steps, based on a synthesis developed by Kotoris *et al.* The commercially available 2-methylnaphthalene (46) was brominated with N-bromosuccinimide (NBS) in benzene. A mixture of the mono- (major product) 47a and dibrominated (minor product) 47b products was obtained and used directly in the following step without any further purification. Refluxing crude 47a and 47b with sodium sulfite (Na_2SO_3) in a 1:1 mixture of acetone and water for 1 hr. gave the sulfonate sodium salt 48 in 81% yield. No products resulting from the reaction of sulfite ion with 47b were detected indicating that sulfite ion does not react with the dibrominated species 47b. Subsequent heating of 48 in phosphorus oxychloride (POCl_3)
in a 1:1 mixture of acetonitrile (CH$_3$CN) and tetramethylene sulfone at 55 °C for 6 hrs resulted in a 62% yield of the sulfonyl chloride 49. The sulfonyl chloride was then converted to the neopentyl protected sulfonate 50 by reacting it with neopentyl alcohol in the presence of triethylamine (NEt$_3$). Compound 50 was obtained in 77% yield. The model α, α-difluoromethylenesulfonate 45 was prepared by electrophilic fluorination of 50 using N-fluorobenzenesulfonyl fluoride (NFSi, 51), a relatively cheap and commercially available electrophilic fluorinating reagent.

Scheme 10. Synthesis of model sulfonate 45
The fluorination of 50 took place in a stepwise manner. At \(-78^\circ\text{C}\), the sulfonate ester 50 was deprotonated at the methylene position with 1.1 eq of \(t\)-BuLi. A fluorine atom was then introduced by adding 1.1 eq of NFSi at \(-78^\circ\text{C}\). By repeating these two steps (1.2 eq \(t\)-BuLi, 1.2 eq NFSi) the difluorinated sulfonate 45 in 69% yield. The exact mechanism for electrophilic fluorination is unknown, however two mechanisms have been proposed. One mechanism involves a single electron transfer, where the radical anion species \(R_2NF^-\) is formed (SET, Scheme 11A). The second mechanism involves an \(S_N2\) nucleophilic displacement at the fluorine, resulting in the displacement of \(R_2N^-\) (\(S_N2\), Scheme 11B):

**A** Single electron transfer (SET)

\[
\begin{align*}
R_2NF + Nu^- & \rightarrow [R_2N-F^- + Nu^-] \rightarrow R_2N^- + NuF
\end{align*}
\]

**B** Nucleophilic displacement at the fluorine (\(S_N2\))

\[
\begin{align*}
R_2N-F + Nu^- & \rightarrow [R_2N-F-\cdots Nu] \rightarrow R_2N^- + NuF
\end{align*}
\]

**Scheme 11.** SET and \(S_N2\) proposed mechanisms for electrophilic fluorination

The model sulfonate 45 was subjected to different acidic and basic conditions in order to test the stability of the sulfonate ester linkage. We found that 45 was stable in
acidic conditions (2N HCl, 25%TFA/THF) and mild basic conditions (Na$_2$CO$_3$, H$_2$O, DMF). However, heating 45 in the presence of Na$_2$CO$_3$, water, and DMF at 80 °C for 24 hrs resulted in almost complete hydrolysis of the sulfonate ester to the sulfonate sodium salt 52 (Scheme 12). This was confirmed by comparing the $^{19}$F NMR spectra of the crude product 52 with the sulfonate lithium salt 53. The sulfonate lithium salt can be prepared by heating the model sulfonate 45 with lithium bromide at 90 °C (Scheme 12).

Thus, the sulfonate linkage is very stable to acid and base at room temperature but will undergo hydrolysis in the presence of base with heating. These studies indicated that our polymer-bound sulfonates would be stable to the Suzuki coupling conditions (boronic acid, palladium catalyst, base, room temperature) we wished to employ for library construction (see Scheme 9, section 1.7). As well, the desired sulfonate products could be released from the support under relatively mild conditions (mild base, heating).

**Scheme 12.** Synthesis of the sulfonate sodium salt 52 and the sulfonate lithium salt 53
3.2 **Strategy for Polymer Loading**

As discussed previously in section 1.6, a linker chain is often used to ensure anchor stability, improve accessibility to reagents, and improve the ease of cleavage of the final product off the polymer.\(^8\) Thus, in our methodology for preparing the small molecule library, we wished to use a linker chain between the aryl DFMS group and the polymer support (see Scheme 9, section 1.7). We envisioned two possible routes by which the aryl DFMS group could be linked to the support.

3.2.1 **Strategy #1 for Polymer Loading**

The first strategy for linking the aryl DFMS group to the support involved coupling a linker arm to the support and then coupling the linker arm to the DFMS moiety (Scheme 13). The most obvious method in coupling the linker chain to the DFMS

![Scheme 13. Strategy #1 for polymer loading](image)

\(^8\) Reference or citation needed.
group was to convert the sulfonic acid 55 to the sulfonyl chloride 56 and then react the sulfonyl chloride with an appropriate functionalized polymer-bound linker arm. However, previous attempts to convert the sulfonic acids of type 55 to the sulfonyl chlorides 56 failed. This may be due to the presence of the fluorines alpha to the sulfonate. In the absence of the fluorines, sulfonyl chlorides can be prepared from reacting sulfonic acids with thionyl chloride, phosphorus pentachloride, or phosphoryl chloride (see Scheme 10). These approaches require an initial nucleophilic attack of the sulfonic acid hydroxyl group on the chlorinating agent. However, with the α-fluorinated sulfonates, the attack would less likely take place due to decreased nucleophilicity of the sulfonate hydroxyl group. Thus, this strategy was not attempted.

3.2.2 Strategy #2 for Polymer Loading

\[ \text{Scheme 14. Strategy #2 for polymer loading} \]
The second strategy for polymer loading involved coupling the aryl DFMS moiety to a linker chain and then coupling the linker arm to a polymer support (Scheme 14). In this strategy, a monoprotected linker chain would be coupled to a sulfonyl chloride 57, forming a sulfonate ester (58) which would be fluorinated. The fluorinated sulfonate ester 59 would then be coupled to a polymer support.

3.3 Synthesis of DFMS-Bearing Naphthalene Coupled to Linker Chain

To work out the conditions for preparing the sulfonates of type 58, we used naphthyl derivative 49 as a model system since this compound had already been prepared in multigram quantities (see Scheme 10).

![Chemical structures](image)

Previous studies by Kotoris et al on methyl- (60a), ethyl- (60b), isopropyl- (60c), and neopentyl-protected sulfonate esters (60d) of phenylmethanesulfonate demonstrated that only the neopentyl-protected sulfonate (60d) would undergo electrophilic fluorination. There are several possible reasons for this. For the methyl esters, the nucleophilic base may be attacking at the carbon adjacent to the oxygen atom of the ester bond and displacing the benzylsulfonate moiety (Scheme 15A). For the ethyl and isopropyl esters, the base may be deprotonating a β proton of the alkoxy group which would be followed by elimination of the benzylsulfonate moiety (Scheme 15B).
case of the neopentyl ester. steric hindrance and lack of β protons would prevent these two reactions from occurring.

![Scheme 15. Mechanisms for the nucleophilic displacement (A) and elimination (B) of the benzylsulfonate moiety](image)

3.3.1 Synthesis of Monoprotected Linker Chain

Based on the observations made by Kotoris et al. an appropriate linker chain would be one that lacked β protons. Thus, we first attempted to use commercially available neopentyl glycol (61). Before coupling the linker chain to the sulfonyl chloride 49, the linker chain had to first be monoprotected. Three different protecting groups were examined. A tetrahydropyranyl (THP) protected linker (62) was prepared using a procedure that was adapted from Kato et al. By reacting neopentyl glycol with dihydropyran and a catalytic amount of p-toluenesulfonic acid, the desired linker was obtained in a low yield of 22% (Scheme 16A). In an attempt to find an appropriate protecting group that resulted in a higher yield, a t-butyl protected linker (63) was prepared using t-butanol (t-BuOH) and 50% H₂SO₄ (Scheme 16B). a procedure developed by Richardson et al. However, a disappointing yield (10%) was obtained.
Since the yield was again very low, we prepared the tert-butyldimethylsilyl (TBS) protected derivative (64) by reacting a solution of neopentyl glycol, tert-butyldimethylsilyl chloride (TBDMS-Cl), and imidazole (imid), in DMF. Once again, the yield of the monoprotected linker was low (30%, Scheme 16c). The low yields obtained for all three monoprotected linker chains were likely due to steric factors from the two methyl groups at position 2 of the linker. Nevertheless, since the highest yield was obtained using the TBS group, we decided to proceed using this protecting group.

Scheme 16. Synthesis of THP (A), tert-butyl (B), and TBS monoprotected (C) linker chain
3.3.2 Electrophilic Fluorination of Naphthylmethanesulfonate Ester 65

Once the TBS monoprotected linker chain 64 was prepared, it was coupled to the sulfonyl chloride 49 in the presence of triethylamine, resulting in a 77% yield of the naphthylmethanesulfonate ester 65 (Scheme 17).

Scheme 17. Synthesis of naphthylmethanesulfonate ester 65

We then attempted a stepwise electrophilic fluorination 65 using NFSi. The procedure, which was adapted from the work and Kotoris et al.98. involved dissolving 65 in THF at −78 °C and then introducing 1.1 eq of base. The reaction would be stirred for 2 hrs at −78 °C, followed by addition of 1.1 eq of NFSi dissolved in THF. The reaction would be stirred an additional hour at −78 °C, and the above steps would be repeated except that 1.2 eq of base and NFSi would be added. Three different bases (t-BuLi, LDA, and NaHMDS) were used and all three were unsuccessful. From the TLC and $^{19}$F NMR spectra of the crude products, the reactions were very messy and little to none of the desired difluorinated product was obtained.

Although the electrophilic fluorinations were unsuccessful, a more careful examination of the crude products led to the discovery that there was one common byproduct that was formed each time the reactions were carried out. Spectral data has suggested that the binaphthylethenesulfonate 66 was the byproduct. Compound 66
may be the result of a Ramberg-Backlund type reaction, which is the rearrangement of an \( \alpha \)-halo sulfone with a base to give an olefin.\(^{106}\) The proposed mechanism is outlined in Scheme 18. After the addition of the first equivalent of \( \tau \)-BuLi, the reaction mixture had gone from pale yellow to bright yellow/green. This was an indication that highly conjugated anionic molecules were present, such as the intermediate (67) formed from the nucleophilic displacement of the linker chain from 65 by an anionic variant of 65. When the first equivalent of NFSi was added, the reaction mixture turned back to yellow. This may be a result of the fluorines quenching the negative charges, resulting in the monofluorinated intermediate 68. When the second equivalent of \( \tau \)-BuLi was added, each drop resulted in the reaction mixture turning from yellow to bright red and then back to yellow. The bright red was probably the result of the methylene proton(s) between the naphthyl group and sulfur atom being deprotonated. Since the reaction returned to its yellow color, the anionic methylene carbon was most likely quenched by displacing the fluorine and subsequently forming a three-membered cyclic intermediate (69). Extrusion of \( \text{SO}_2\text{g} \) would result in the final product 66. Addition of the second equivalent of NFSi resulted in no visible change in the reaction. Thus the synthesis of 66 likely occurred via a Ramberg-Backlund type reaction since the rearrangement of \( \alpha \)-halosulfone to a three-
membered cyclic intermediate, followed by cheletropic extrusion of $\text{SO}_2$, is characteristic of such reactions. The major product of the reaction was 66 with a yield of 39%.

\[ \text{Scheme 18. Proposed mechanism for the synthesis of 66} \]
3.3.3 Synthesis of Alternative Monoprotected Linker Chain

Due to the problems associated with the neopentyl glycol linker arm, an alternative linker arm was examined. This linker chain (71) was similar to the neopentyl linker arm except that it was two carbons longer. Using a literature procedure, lithium aluminum hydride (LiAlH₄) reduction of commercially available 2,2-dimethylglutaric acid (70) gave 71 in 67% yield (Scheme 19).

![Scheme 19. Synthesis of the alternative linker chain 71](image)

Once again, the linker chain had to be monoprotected before coupling it to the sulfonyl chloride 49. The linker was first protected with a t-butyl group (72) using t-BuOH and 50% H₂SO₄ (Scheme 20A) as described by Richardson et al. However, the

![Scheme 20. Synthesis of the t-butyl (A) and trityl monoprotected (B) linker chain 71](image)
desired product could not be isolated and purified. A trityl protected linker was then examined (73) since the trityl group can be removed under mild acidic conditions. Based on a procedure developed by Nagatsugi et al. 73 was synthesized by reacting the linker 71 with trityl chloride (trityl-Cl) and pyridine (pyr) (Scheme 20B). A moderate yield of 49% was obtained.

3.3.4 Electrophilic Fluorination of Naphthylmethanesulfonate Ester 74

The trity protected linker 73 was coupled to the sulfonyl chloride 49 with triethylamine (Scheme 21). The resulting naphthylmethanesulfonate ester 74 was obtained in a high yield of 91%.

![Scheme 21. Synthesis of the naphthylmethanesulfonate ester 74](image)

Stepwise electrophilic fluorination of 74 with t-BuLi and NFSi was unsuccessful. Based on the crude $^{19}$F NMR spectrum, the desired difluorinated product was not obtained. As well, a Ramberg-Backland type product was not detected. Therefore, we attempted to fluorinate 74 using an alternative electrophilic fluorination procedure which was adapted from the work of McAtee et al. This involved dissolving the naphthylmethanesulfonate ester in THF at -78 °C along with 3 eq of NFSi. A base was then introduced over a period of 30 min to 1 hr. The reaction was stirred for 2 hrs at -
78 °C. warmed to rt, and stirred for an additional hour. In this procedure (one-step fluorination), since NFSi is introduced at the start of the reaction, fluorination would likely occur more readily than nucleophilic attack to another naphthylmethanesulfonate ester molecule once the anion is formed. One-step electrophilic fluorinations were carried out with t-BuLi, LDA, LiHMDS, KHMDS, and NaHMDS (Scheme 22). Based

\[
\text{O} \quad \text{OTriyl} \quad \text{NFSi (3 eq)} \quad \text{Base (2.5 eq), THF} \quad -78^\circ C. \quad 2 \text{hrs} \rightarrow \text{rt, 1hr}
\]

Base = LiHMDS, KHMDS, NaHMDS

**Scheme 22. Synthesis of the DFMS-bearing naphthalene 75**

on the $^{19}$F NMR spectrum of the crude product from each reaction, the HMDS bases were successful in obtaining the difluorinated product 75 as the major product. However, we were unable to obtain accurate yields since deprotection of the linker chain was occurring during the work-up. Nevertheless, it appeared that the fluorination yields were rather low. Therefore, we examined the TBS moiety as a protecting group. Linker chain 71

\[
\text{OH} \quad \text{OH} \quad \text{TBDMS-Cl (1.1 eq)} \quad \text{imid (2 eq)} \quad \text{DMF, rt. O/N} \quad \text{70%}
\]

**Scheme 23. Synthesis of the TBS protected linker chain 76**
was monoprotected with a TBS group using TBDMS-Cl, imidazole, and DMF (Scheme 23). The desired product 76 was obtained in a respectable 70% yield, the highest yield observed among all the monoprotected linker chains prepared.

3.3.5 **Electrophilic Fluorination of Naphthylmethanesulfonate Ester 77**

Once the linker chain 76 was prepared, it was coupled to the sulfonyl chloride 49 in the presence of triethylamine (Scheme 24). The resulting naphthylmethanesulfonate ester 77 was obtained in 69% yield.

![Scheme 24. Synthesis of naphthylmethanesulfonate ester 77](image)

One-step electrophilic fluorinations were carried out on the naphthylmethanesulfonate ester 77. When t-BuLi was used as the base, the monofluorinated variant 78 was the major product with a yield of 50% (Scheme 25A). In attempt to introduce the second fluorine, 78 was subjected to another one-step electrophilic fluorination. However, the reaction only yielded unreacted starting material. The desired difluorinated sulfonate ester 79 was successfully obtained when LiHMDS, KHMDs, and NaHMDS were used as the base (Scheme 25B). The reactions resulted in high yields of 74%, 86%, and 91% respectively, with NaHMDS being the best base.
Scheme 25. Synthesis of the monofluorosulfonate ester 78 (A) and the difluorosulfonate ester 79 (B)

3.3.6 Deprotection of the DFMS-Bearing Naphthalene 79

Before the DFMS-bearing naphthalene 79 could be loaded onto the polymer, the linker chain had to be deprotected. Typically, tetra-n-butylammonium fluoride (TBAF) is used for silyl ether bond cleavage. However, when 79 was treated with TBAF the linker arm was cleaved off rather than the TBS group. Three mechanisms have been proposed for this occurrence. One involves deprotection of the linker arm, followed by an intramolecular cyclization of the linker arm to form a six-membered ring (80) and release of the sulfonate 81 (two-step displacement. Scheme 26A). The intramolecular cyclization was likely promoted by the “gem-dimethyl effect” which is brought about by the two methyl groups at carbon position 2 of the linker chain. It appears that alkyl substitutions accelerate cyclization of acyclic systems because it would be more
Scheme 26. Proposed mechanisms for the two-step (A) and one-step (B) displacement of sulfonate 81

enertically favorable. The second mechanism has the nucleophilic fluoride attacking at the methylene carbon adjacent to the sulfonate ester bond, thus displacing the sulfonate 81 (one-step displacement. Scheme 26B). The third mechanism involves both the one- and two-step displacements, which was likely occurring for 79 since treatment of TBAF with the neopentyl protected naphthalene 45 also resulted in displacement of the
sulfonate 81, however at a slightly slower rate (Scheme 26B). After 24 hrs, 94% of 79 had reacted whereas for 45 only 62% had reacted.

Removal of the TBS group was accomplished by using a procedure developed by Corey et al. Treatment of 79 with acetic acid-H2O-THF (3:1:1) for 3 hrs at rt (Scheme 27) gave the deprotected naphthalene 82 in 75% yield.

3.3.7 Electrophilic Fluorination and Deprotection of Naphthylmethanesulfonate Ester 65

Having worked out suitable conditions for fluorinating compound 77, we decided to examine whether these conditions could be used to fluorinate ester 65 which had the shorter neopentyl glycol linker arm. To determine which linker chain would be more suitable for coupling the aryl DFMS to the polymer support, the one-step electrophilic fluorination and deprotection reactions were carried out on the ester 65 (Scheme 28). Electrophilic fluorination of 65 using the one-step fluorination procedure and NaHMDS as the base gave an 84% yield of the difluorinated product 83, which was comparable to the yield obtained for the DFMS-bearing naphthalene 79. However, deprotection of 83 (to give 84) under the identical acidic conditions used for 79 resulted in a lower yield of 53% and the reaction required 4 days as opposed to 3 hrs for complete reaction. The
Scheme 28. Electrophilic fluorination and deprotection of the naphthylmethanesulfonate ester 65

difference in reactivity between the DFMS-bearing naphthalenes 79 and 83 was likely due to steric factors. Hydrolysis of the t-butyldimethylsilyloxy group would less readily take place with the shorter linker chain since there is a quaternary carbon adjacent to the site of hydrolysis. The longer linker chain was chosen over the shorter one since the time required for preparing the deprotected DFMS-bearing naphthalene 82 was shorter, and the yields were better.

3.4 Mitsunobu Coupling of the DFMS-Bearing Naphthalene 82 to Phenol

We reasoned that the best way of attaching the linker arm of the sulfonate to the support was by an ether linkage since ether functionalities are highly stable to a variety of reaction conditions. Thus, NCPS would be prepared such that a small percentage of the aryl groups were functionalized at the para position with a hydroxyl group. The ether linkage would be formed by a Mitsunobu reaction between the hydroxyl group on the
linker arm and the phenolic groups on the polymer. We chose a Mitsunobu reaction since Rano et al\textsuperscript{112} as well as Krchnak et al\textsuperscript{113} have used this reaction for forming aryl ethers on polymer supports in high yield. Indeed, the Mitsunobu reaction is ideal for polymer-supported chemistry because products are typically obtained in high yields under mild conditions.

In the preliminary studies, phenol (85) was used as a model for the polymer support. The first attempts in coupling the DFMS-bearing naphthalene 82 to phenol using typical Mitsunobu conditions. that is, 2 eq of DIAD or DEAD with 2 eq of triphenylphosphine (PPh\textsubscript{3}). were unsuccessful. The reactions did not go cleanly or to completion. For polymer-supported chemistry, all loading sites on the polymer should be used since higher yields would be obtained and any undesired reactions with the unloaded sites would be prevented.

Rano et al have demonstrated that polymer-bound phenols react rapidly and cleanly with alcohols to form aryl ethers in high yields when N,N',N',N'-tetramethylazodicarboxamide (TMAD, 86) and tributylphosphine (PBu\textsubscript{3}) are used.\textsuperscript{112}

\begin{center}
\begin{tikzpicture}
  \node (n1) at (0,0) {N\hspace{0.5cm}N=\hspace{0.5cm}N\hspace{0.5cm}N};
  \node (n2) at (0,-1) {O}
  \node (n3) at (0,1) {O}
  \draw (n1) -- (n2);
  \draw (n1) -- (n3);
\end{tikzpicture}
\end{center}

Thus, we attempted to couple 82 to phenol with 2 eq of both TMAD and PBu\textsubscript{3} (Scheme 29). The reaction proceeded very cleanly and the product 87 was obtained in quantitative yields.
Scheme 29. Mitsunobu coupling of the deprotected DFMS-bearing naphthalene 82 to phenol

The proposed mechanism for the Mitsunobu reaction is shown in Scheme 30.\textsuperscript{114} PBu\textsubscript{3} first forms an adduct with TMAD where nucleophilic attack of the phosphine to TMAD is followed by deprotonation of phenol by TMAD. Phenol would have a lower pH\textsubscript{a} (pK\textsubscript{a} of 10)\textsuperscript{115} than the DFMS-bearing naphthalene 82 (comparable to ethanol, pH\textsubscript{a} of 16)\textsuperscript{115}. Thus it would be expected to deprotonate first. The phenolate would then deprotonate 82 to initiate nucleophilic attack of 82 to the PBu\textsubscript{3}/TMAD adduct. In this step, an oxyphosphonium ion intermediate (88) is formed to activate 82 for the final step. Once phenol is deprotonated again by TMAD, tributylphosphine oxide is displaced from the intermediate 88 by phenolate in an S\textsubscript{N}2 manner resulting in the coupled product 87.

Of the three Mitsunobu reagents we tried, TMAD was probably the best reagent because the basicity of it was strong enough to efficiently activate phenol. Similar observations were made by Tsunoda \textit{et al.}\textsuperscript{116} They found TMAD to be a better reagent than DEAD for Mitsunobu reactions that involved nucleophiles with higher pH\textsubscript{a}’s, such as \textit{N}-benzyltrifluoroacetamide that has a pH\textsubscript{a} of 13.6.
Scheme 30. Proposed mechanism for the Mitsunobu reaction.
3.4.1 Synthesis of TMAD

Although TMAD is commercially available, it is rather an expensive reagent. Thus, we prepared TMAD in two simple steps (Scheme 31). The reduced form of TMAD (91) was synthesized using a procedure adapted from Crawford et al.\textsuperscript{117} Hydrazine (89) was reacted with carbamoyl chloride (90) in the presence of triethylamine. The product isolated, which was a mixture of 91 and triethylamine hydrochloride salt, was used directly in the subsequent oxidation step to form TMAD (86). Using lead (IV) acetate (Pb(OAc)\textsubscript{4}) as the oxidant\textsuperscript{118}, TMAD was obtained in 61% yield.

\begin{center}
\begin{tikzpicture}
\node[align=center] (A) at (0,0) {H\textsubscript{2}N—NH\textsubscript{2}};
\node[align=center] (B) at (2,0) {90 (2.1 eq)};
\node[align=center] (C) at (4,0) {\text{NET\textsubscript{3} (2.1 eq)}};
\node[align=center] (D) at (6,0) {\text{CH\textsubscript{2}Cl\textsubscript{2}, r. 5 hrs}};
\node[align=center] (E) at (0,-1) {89};
\node[align=center] (F) at (2,-1) {91};
\node[align=center] (G) at (4,-1) {Pb(OAc)\textsubscript{4} (1 eq)};
\node[align=center] (H) at (6,-1) {CH\textsubscript{2}Cl\textsubscript{2}, -20\textdegree C r. 2 hrs};
\node[align=center] (I) at (8,-1) {61\%};
\node[align=center] (J) at (8,-2) {86};
\end{tikzpicture}
\end{center}

\textbf{Scheme 31.} Synthesis of TMAD (86)

3.5 Synthesis of DFMS-Bearing Aryl Bromide Coupled to Linker Chain 76

Once the chemistry was developed using our model system, we then focussed our attention on preparing the desired substrate (e.g. an aryl halide as outlined in Scheme 9, section 1.7) and loading it onto the polymer support.

The Suzuki reaction is a palladium-catalyzed cross-coupling reaction between aryl halides or aryl triflates and boronic acids.\textsuperscript{119, 120} For aryl halides, iodides and bromides are often used due to their high reactivity.\textsuperscript{119, 120} We chose to prepare aryl bromides as opposed to the iodides because Hum et al have observed that during
electrophilic fluorination with NFSi and NaHMDS, a significant loss of iodine from aryl iodides occurs. As a result, the product would be contaminated with the noniodinated material.

\[ \text{Scheme 32. Synthesis of DFMS-bearing aryl bromide 97} \]
The synthesis of aryl bromides with the linker chain (76) attached is outlined in Scheme 32. The commercially available 3-bromobenzyl bromide (92) was refluxed for 48 hrs with Na$_2$SO$_3$ in 1:1 mixture of acetone and H$_2$O. An 81% yield was obtained for the product 93. 3-Bromobenzyl bromide, as opposed to the 4-bromo derivative, was used because research has shown that biphenyl derivatives substituted with the DFMP moiety at the meta-position are more potent inhibitors than the para variant towards PTP1B. Heating the sodium sulfonate salt 93 with phosphorus oxychloride in a 1:1 mixture of acetonitrile and tetramethylene sulfone at 70 °C for 3 hrs resulted in the sulfonyl chloride (94) in 85% yield. The TBS monoprotected linker chain 76 was then coupled to the sulfonyl chloride 94 in the presence of triethylamine. The resulting sulfonate ester 95 was obtained in 86% yield. Electrophilic fluorination with NFSi and NaHMDS gave the difluorinated product 96 in 88% yield. Lastly, the desired DFMS-bearing aryl halide (97) was prepared for Mitsunobu coupling by deprotection of 96 in a 3:1:1 mixture of acetic acid/H$_2$O/THF. Yields for 97 ranged from 51% to 89%.

3.6 Suzuki Reactions on a Model System

Before the DFMS-bearing aryl bromide 97 was loaded onto the polymer, we first prepared compound 98 and used this as a model system for working out the Suzuki reaction conditions. Using the optimized Mitsunobu conditions described above (TMAD, PBU$_3$), compound 98 was obtained in quantitative yields (Scheme 33).

The Suzuki cross-coupling reaction is a carbon-carbon bond forming reaction between an aryl halide or triflate and a boronic acid or boronate ester in the presence of a palladium (Pd) catalyst and base. The availability of the reagents and mild reaction conditions makes this reaction one of the most widely used methods in both laboratories.
Scheme 33. Mitsunobu coupling of DFMS-bearing aryl bromide 97 to phenol

and industrial processes.\textsuperscript{119,120} It has also been shown to be amenable for SPOS.\textsuperscript{97} The Suzuki reaction can tolerate a broad range of functional groups. Steric hindrance of the aryl halides and boronic acids do not have any significant effect on the reaction. The inorganic byproducts from the reaction are non-toxic and can be easily removed from the reaction mixture. The reaction is unaffected by the presence of water. However water has been shown to enhance the rate of the reaction\textsuperscript{74} or even be used as a solvent for reactions\textsuperscript{121}.

The general catalytic cycle of the Suzuki cross-coupling reaction is shown in Scheme 34. It involves three steps: oxidative addition, transmetalation, and reductive elimination.\textsuperscript{119} In the first step, oxidative addition of aryl halides to a palladium (0) species occurs. Insertion of the palladium into the aryl halide results in the formation of a stable \textit{trans}-\sigma-organopalladium (II) halide intermediate (I). The rate-determining step is often the oxidative addition step where relative reactivity decreases in the order of I\textgreater OTf\textgreater Br\textgreater Cl. On the other hand, the presence of electron withdrawing groups on the aryl halide can enhance the rate. The next step involves a transmetalation between the organopalladium (II) halide intermediate I and a boronic acid to give a new intermediate II. This step is enhanced when a base is added. There are two proposals to explain the role of the base. The negatively charged bases may quaternize boron to form boronate...
Scheme 34. General catalytic cycle of the Suzuki cross-coupling reaction

complexes and subsequently increase the nucleophilicity of the organic group (Scheme 35A). Transfer of the organic group would likely occur more readily in this situation.

The alternative explanation is displacement of the halide ligand in intermediate I by an alkoxy, hydroxy, or acetoxy anion to give a reactive Pd-OR complex III (Scheme 35B).

Scheme 35. Proposed roles of the base in the Suzuki reaction
In the final step, reductive elimination yields the biaryl coupled product and regenerates the palladium (0) species. The reaction takes place once the trans-diarylpalladium (II) species isomerizes to the cis species, where favorable \( \pi \)-orbital interactions can occur between the aryl groups (Scheme 36).\textsuperscript{119}

\[ \begin{align*}
\text{trans} & \iff \text{cis} \\
\text{cis} & \rightarrow \text{Ar-} \text{Ar}^+ + \text{PdL}_2
\end{align*} \]

**Scheme 36.** Cis-trans isomerization of diarylpalladium intermediates in the Suzuki reaction

The key feature in our methodology is the Suzuki cross-coupling reaction since diversity in our small molecule library would be achieved by using various boronic acids. To complete the final stages of our preliminary studies, we needed to work out the Suzuki reaction conditions. The DFMS-bearing aryl halide 98 was used as our model compound.

### 3.6.1 Room Temperature Suzuki Reactions

The conditions we first examined for room temperature Suzuki reactions were developed in the Taylor group for the preparation of a library of biphenyl DFMP's.\textsuperscript{74} The protocol requires 20 mol\% of the palladium catalyst (PhCN)\textsubscript{2}PdCl\textsubscript{2}, 3 eq of boronic acid, 3 eq of K\textsubscript{2}CO\textsubscript{3}, 10 eq of H\textsubscript{2}O, and degassed DMF. Solvents are typically degassed since the presence of air can result in the formation of deboronated compounds and
symmetrical biaryl compounds. By monitoring the reaction by $^{19}$F NMR, Suzuki coupling of the model compound 98 with phenylboronic acid successfully went to completion within 18 hrs at room temperature. We also attempted the same reaction except in the absence of water. The reaction had only gone to 77% completion after 28 hrs. Thus, water can have a rate enhancing effect, which is consistent with the results of Hum et al where the addition of water increased their Suzuki coupling rate 1.7-fold.

During the progress of our research project, Buchwald et al developed impressive room temperature Suzuki coupling conditions where aryl chloride substrates, that weren't even activated, could be used. Their conditions required only 0.5-1.5 mol% of Pd(OAc)$_2$ along with an electron-rich, biaryl phosphine ligand (2 ligands/Pd), such as 99 and 100. They have proposed the following roles for the phosphine ligands when it coordinates to palladium. The electron-richness is thought to enhance oxidative addition and increase catalyst lifetime by binding tightly to the palladium and keeping it in solution. The steric bulk is to promote reductive elimination. In their studies, they found that KF and CsF were the most effective bases and the reactions were most efficient when carried out in THF or dioxane. This may be due to the fluoride ions being a
stronger base in THF$^{111}$ and subsequent formation of reactive organo(trifluoro)borate ions for the transmetalation step (Scheme 37)$^{119}$.

\[
\text{ArB(OH)$_2$} \xrightarrow{\text{CsF}} \begin{array}{c|c|c}
\text{F} & \text{B} & \text{F} \\
\hline
\text{F} & \text{F} & \text{X} \\
\end{array} \xrightarrow{\text{Ar'-Pd-X}} \text{Ar-Pd(II)-Ar'}
\]

**Scheme 37.** Proposed role of organo(trifluoro)borate ions in the Suzuki reaction

To determine whether the conditions developed by Buchwald *et al* could be applied to our substrates, eight different reactions were set up using Pd(OAc)$_2$, ligand 99 or 100, phenylboronic acid (PhB(OH)$_2$), KF or CsF, and dioxane or THF. The reactions were monitored by $^{19}$F NMR and the results are listed in Table 1. All the reactions were unsuccessful in going to completion within 24 hrs. even when 5 mol% of the palladium catalyst was used. The percent conversion of the DFMS-bearing aryl bromide 98 to the biaryl product ranged from 30%-93%. $^{19}$F NMR analysis of the reactions showed the rates to exponentially decrease over the 24 hr time period. Thereafter, the palladium catalyst was dead. Adding more catalyst at that point did not significantly push the reaction closer to completion. The most efficient base/solvent system was CsF and dioxane. We also noticed that the reactions were sensitive to the solvent and whether it was degassed. Reactions went further to completion when THF was degassed, however degassing wasn’t necessary for dioxane.

Further attempts to push the Suzuki reaction to completion with the conditions developed by Buchwald *et al*$^{123,124}$ were not carried out as it would have required more of the palladium catalyst and phosphine ligands. Using more of the phosphine ligands
Table 1. Room Temperature Suzuki Coupling of DFMS-Bearing Aryl Bromide 98 with PhB(OH)₂²

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Phosphine Ligand</th>
<th>Base</th>
<th>Solvent</th>
<th>% Conversion²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>89⁶</td>
<td>CsF</td>
<td>Dioxane</td>
<td>72%</td>
</tr>
<tr>
<td>2⁴</td>
<td>90</td>
<td>CsF</td>
<td>Dioxane</td>
<td>76%</td>
</tr>
<tr>
<td>3</td>
<td>90</td>
<td>CsF</td>
<td>Dioxane</td>
<td>93%</td>
</tr>
<tr>
<td>4</td>
<td>90</td>
<td>CsF</td>
<td>Dioxane (degassed)</td>
<td>78%</td>
</tr>
<tr>
<td>5⁵</td>
<td>90</td>
<td>CsF</td>
<td>Dioxane</td>
<td>79%</td>
</tr>
<tr>
<td>6</td>
<td>90</td>
<td>KF</td>
<td>THF (degassed)</td>
<td>30%</td>
</tr>
<tr>
<td>7</td>
<td>90</td>
<td>KF</td>
<td>THF (degassed)</td>
<td>68%</td>
</tr>
<tr>
<td>8⁵</td>
<td>90</td>
<td>KF</td>
<td>THF (degassed)</td>
<td>54%</td>
</tr>
</tbody>
</table>

²Reaction conditions: 5 mol% of Pd(OAc)₂, 1 eq of DFMS aryl bromide 98, 1.5 eq of PhB(OH)₂, 0.1 eq of phosphine ligand, 3 eq of base, rt., 24 hrs. ³Percent conversion of 98 into biaryl product after 24 hrs as determined by ¹H NMR. ⁴0.075 eq of phosphine ligand 99 was used. ⁵2 mol% of Pd(OAc)₂ was used. ⁶3 eq of PhB(OH)₂ was used.

would not have been ideal since they are either difficult to prepare or expensive reagents. In comparison, the conditions developed in our laboratory by Hum et al.⁷⁴ requires a rather inexpensive palladium catalyst ((PhCN)₂PdCl₂) and no phosphine ligand. Thus, we settled on their room temperature Suzuki conditions, which requires 1 eq of 98, 20 mol% of (PhCN)₂PdCl₂, 3 eq of PhB(OH)₂, 3 eq of K₂CO₃, 10 eq of H₂O, and degassed DMF.⁷⁴

3.7 Polymer Synthesis and Loading

With the Suzuki conditions worked out, we were ready to prepare the library using the LPOS approach. The two most common polymer supports used for LPOS are polyethylene glycol (PEG) and non-crosslinked polystyrene (NCPS).⁸⁸,⁹⁰ However, there are advantages to using NCPS as opposed to PEG. NCPS is soluble in polar, aprotic solvents such as THF, CH₂Cl₂, CHCl₃, EtOAc, and benzene, yet insoluble in polar, protic solvents such as MeOH, EtOH, and water.⁹² Therefore, homogeneous reactions can be
carried out in a broad range of organic solvents and reactions can be rapidly purified by precipitation of the polymer in MeOH, EtOH, or water and subsequent filtration. PEG on the other hand, is slightly soluble in water which poses a problem for solvent extraction purification of intermediates and products.\(^9\) The functional group(s) on NCPS and polymer loading can be easily controlled, whereas the nature of PEG allows for only a maximum of two molecules to be attached to the hydroxyl groups at the termini of each polymer molecule.\(^9\) Lastly, PEG is insoluble in THF at low temperatures and has been found to complex with metal cations.\(^9\) Besides these advantages to using NCPS, we chose to use NCPS as our polymer support because it has not been widely used in the area LPOS of small molecule libraries and we wished to explore the scope of this polymer as a support in LPOS.

The synthesis of the desired 4-hydroxylated NCPS (104) is outlined in Scheme 38. To prepare our polymer, we first copolymerized styrene (36) with 9 mol% of 4-acetoxy styrene (102) in the presence of the free radical initiator VAZO at 110°C for 48

\[
\text{36} + \text{9 mol\% of 4-acetoxy styrene (102)} \longrightarrow \text{VaZO (0.005 eq)} \quad \text{toluene} \quad 110^\circ C, 48 \text{ hrs} \quad \text{103} \quad \text{NaOH (aq) / THF} \quad \text{1.5 hrs, 24 hrs} \quad \text{104}
\]

**Scheme 38.** Synthesis of 4-hydroxylated NCPS (104)
hrs to obtain the 4-acetoxy-functionalized NCPS (103). The polymer was then saponified by refluxing it in an aqueous solution of NaOH in THF for 24 hours to give the 4-hydroxylated NCPS (104). At each step, the polymer was purified by diluting the reaction mixture with CH$_2$Cl$_2$ and then precipitating out the polymer in MeOH, filtering it off, and washing it with MeOH. The recovery of polymer 104 was approximately 90%.

3.7.1 Determining Loading of the Polymer

The loading of the polymer is defined as the number of anchoring sites per gram of polymer and is expressed in units of millimoles per gram (mmol/g). Before any reaction is carried out on a polymer, the loading must first be determined. This was done so in the following manner. The aryl bromide 106 was prepared by coupling the deprotected sulfonate ester 97 to 4-ethylphenol (105) with TMAD and PBu$_3$ (Scheme 39).

Ten standards were then prepared with varying ratios of the monomers styrene and aryl bromide 106. The total mass for each of the standards was approximately 25 mg and the ratios ranged from 0.1 to 1.0 mmol of the aryl bromide 106 per gram of styrene and 106. Deuterated-chloroform (0.5 mL) was added to each of the standards and $^1$H NMR spectra

![Scheme 39. Synthesis of DFMS-bearing aryl bromide 106]
were acquired. Integration ratios of the averaged (-CH$_2$O-) peak area to the aromatic proton peak area were calculated and then plotted against the mmol of 106/g of styrene and 106. A linear relationship was found, where the equation of the line ($y = mx + b$) was subsequently used for determining the loading of the polymer loaded with the initial starting functionality (aryl bromide 97). From the equation found, $y$ would be the integration ratio CH$_2$O (average)/Ar-H of the polymer, $m$ would be the slope of the line, $b$ would be the y-intercept of the graph, and $x$ would be the ratio of mmol of 106/g of styrene and 106. Therefore, the loading of the polymer-bound aryl bromide would be determined by first calculating the integration ratio CH$_2$O (average)/Ar-H of the polymer ($y$ value), and then using the equation of the line from the above studies to find the polymer loading ($x$ value).

### 3.7.2 Incomplete Loading of the Polymer

Once 4-hydroxylated NCPS 104 was prepared, the aryl bromide 97 was coupled to the polymer using Mitsunobu conditions from the preliminary studies (assuming 0.3 mmol/g loading, 1 eq of 104, 2 eq of 97, 2 eq of TMAD, and 2 eq of PBu$_3$). Surprisingly, the polymer was not fully loaded which was unexpected since quantitative yields were obtained in the preliminary studies. Incomplete loading was discovered when the loaded polymer (107) was subjected to a reaction with pyridine and 4-nitrocinnamoyl chloride (108) (Scheme 40).$^{126}$ $^1$H NMR analysis of the resulting product (109) revealed a signal corresponding to the aryl protons ortho to the nitro group of the 4-nitrocinnamoyl functionality.$^{127}$ Further studies needed to be carried out until maximum loading was obtained.
3.7.3 Loading of Aryl Bromide 97 onto 4-Hydroxylated NCPS

In order to obtain maximum loading of the aryl bromide 97 onto polymer 104, different Mitsunobu coupling conditions were carried out. The results are listed in Table 2. The difference in loading was significant when the number of equivalents for all of 97.

\[ \text{Scheme 40. Coupling 4-nitrocinnamoyl chloride 108 to loaded polymer 107} \]

**Table 2. Effect of Mitsunobu Coupling Reagents on Polymer Loading\(^{a}\)**

<table>
<thead>
<tr>
<th>Polymer 104 (eq)</th>
<th>Aryl Bromide 97 (eq)</th>
<th>TMAD (eq)</th>
<th>PBu(_3) (eq)</th>
<th>Reaction Time (hrs)</th>
<th>Polymer Loading(^{b}) (mmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>O/N(^{c})</td>
<td>0.18</td>
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<td>2</td>
<td>24</td>
<td>0.28</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>O/N(^{c})</td>
<td>0.30</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
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<td>3</td>
<td>24</td>
<td>0.36</td>
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<tr>
<td>1</td>
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<td>4</td>
<td>4</td>
<td>O/N(^{c})</td>
<td>0.42</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>24</td>
<td>0.43, 0.54(^{d})</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>48</td>
<td>0.47</td>
</tr>
</tbody>
</table>

\(^{a}\)All Mitsunobu reactions were carried out in (1:1) CH\(_2\)Cl\(_2\)/THF at rt. \(^{b}\)Polymer loading was determined by the \(^{1}\)H NMR integration ratio of the (-CH\(_2\)-) to the combined aryl proton signals. \(^{c}\)The reaction was stirred overnight. \(^{d}\)Results from two determinations.
TMAD and PBu₃ was changed from two through to four. However, there was little change observed when 5 equivalents of each reagent was used. Thus for maximum loading, the polymer 104 was stirred with 4 equivalents of each reagent at room temperature for 24 hrs (Scheme 41). Any hydroxyl groups left unreacted on the resulting polymer (110) was not a concern to us thereafter since they were not expected to participate in any subsequent reactions. Incomplete loading may have been due to non-random polymerization. If a series of hydroxyl moieties were grouped together in a contiguous manner, then steric crowding would likely prevent neighboring sites from coupling with the aryl bromide.

Scheme 41. Loading of aryl bromide 97 onto 4-hydroxylated NCPS

Upscaling the Mitsunobu coupling of the aryl bromide 97 to 4-hydroxylated NCPS resulted in a polymer loading of 0.54 mmol/g. To determine the percentage of unreacted loading sites, 4-nitrocinnamoyl chloride (108) was reacted with 4-hydroxylated NCPS in the presence of pyridine (Scheme 42). A polymer loading of 0.64 mmol/g was obtained for 111. Assuming that 0.64 mmol/g is a fully loaded polymer, the Mitsunobu conditions we use results in a loaded polymer that has at least 16% of the anchoring sites left unreacted.
Suzuki cross-coupling reactions were carried out on the polymer-bound aryl bromides (110) using the room temperature Suzuki conditions described above (Scheme 43). The reactions were performed in parallel, using boronic acids that were commercially available. A total of 26 different aryl boronic acids were used, of which 7 were heterocyclic. The reactions were monitored by $^{19}$F NMR and within 9 hrs or less, all reactions had successfully gone to completion, with the exception of the Suzuki reaction outlined in Scheme 44. This reaction, which involved 2-furanboronic acid (114), required 24 hrs. and then another 24 hrs with fresh reagents. Although all reactions went to completion, $^{19}$F NMR analysis of the reactions indicated that 2-7% of the polymer-bound sulfonates were lost from the support (15.5% for reaction in Scheme 44), most
likely a result of slow hydrolysis. Boronic acids that were activated with electron-withdrawing groups (such as trifluoromethyl, acetyl) went to 100% completion within 3-4 hrs. while those without substituents or with electron-donating groups (such as methyl, ethyl, t-butyl) took longer. Once the palladium catalyst was removed by centrifugation, all polymer-bound biaryl products were purified by diluting the reaction mixture with CH$_2$Cl$_2$, precipitating out the polymer in MeOH (and a few drops of brine), filtering off the polymer, and rinsing the collected polymer with MeOH. The polymer recovery ranged from 90%-95%. During the workup, no hydrolysis of the sulfonate ester linkage took place as determined by $^{19}$F NMR of the filtrate from the precipitated polymer.

Scheme 44. Suzuki cross-coupling of polymer-bound aryl bromide with 2-furanboronic acid

Suzuki reactions were attempted with four other heterocyclic boronic acids (116-119), however all were unsuccessful. The boronic acids 116, 118, and 119 were surprisingly unreactive, especially since 116 and 118 had electron-withdrawing groups present. The other thiopheneboronic acid 117 could not be pushed to 100% completion even when 1 eq of the palladium catalyst was used. As well, adding fresh reagents did not have any significant affect on the reaction.
3.9 Cleavage of the Product off the Polymer Support

Based on the stability studies of the sulfonate ester linkage, the procedure in which the biaryl products were cleaved off the polymer support and purified is outlined in Scheme 45. The reactions were set up in parallel, in test tubes equipped with a stir bar.

**Scheme 45.** Cleavage of biaryl DFMS compounds off the polymer support
and septum. The products were hydrolyzed off the polymer support with 3 eq of K$_2$CO$_3$. 10 eq of H$_2$O. in DMF. at 80 °C for 17 hrs. The crude reaction mixture was then diluted with CH$_2$Cl$_2$ and added to a solution of MeOH and a few drops of brine. The precipitated polymer was filtered off, leaving the filtrate which contained the crude potassium salt of the biaryl DFMS compounds. The filtrate was concentrated down, redissolved in 1N NaOH, washed with CH$_2$Cl$_2$ to remove any residual polymer and organic impurities, and acidified to pH of approximately 0.5. The sulfonic acids were then extracted out with EtOAc, concentrated down, treated with an aqueous solution of NH$_4$HCO$_3$, lyophilized down repeatedly to obtain the ammonium salts of the biaryl DFMS compounds in yields ranging from 34-97%. Although 26 polymer-bound Suzuki reactions took place, only 25 of the biaryl DFMS's were isolated, starting with 120 in Table 3. Suzuki coupling of the aryl bromide with 3-formylfuran-2-boronic acid (145) resulted in a biaryl DFMS that was very unstable to air and the acidic conditions used in the workup following the cleavage step. Nevertheless, the mild cleavage conditions proved to be compatible for coupling heterocyclic boronic acids to the polymer-bound aryl bromides. In contrast, the heterocycles would have been unstable under the conditions used by Hum et al (TMSBr or TMSI) for preparing their library of DFMP compounds.$^{74}$
<table>
<thead>
<tr>
<th>Product</th>
<th>% Yield</th>
<th>% Purity</th>
<th>% Purity</th>
<th>Product</th>
<th>% Yield</th>
<th>% Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( ^{19}F ) NMR</td>
<td>HPLC</td>
<td></td>
<td></td>
<td>( ^{19}F ) NMR</td>
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</table>

Table 3. Percentage Yields and Purities of Products 120-144 Cleaved off the Polymer Support
and HPLC analysis of the ammonium salts indicated that 19 of
the 25 compounds were at least 96% pure, while 5 of the other 6 were 90-95% pure. All
compounds in the biaryl DFMS library, with the exception of 139, could be used directly
for biological screening without any further purification. Compound 139 was only 77% pure as a result of its instability under acidic conditions, but it was used in the biological screenings anyhow. If the sulfonic acid was a potent inhibitor, then it would have been resynthesized or further purified.

3.10 Enzyme Inhibition Studies with PTP1B

IC₅₀ values for PTP1B were determined for the sulfonic acids 120-144. Fluorescein diphosphate (FDP) was used as the substrate at Kₘ concentration (20 μM) in assay buffer containing 50 mM Bis-Tris (pH 6.3), 2 mM EDTA, 5 mM DTT, 2% glycerol, and 0.001% triton, at 25 °C. All inhibitors were dissolved in DMSO, with a final concentration of 5% for the assays. The reactions were followed by monitoring the appearance of the fluorescent product fluorescein monophosphate (FMP) on a plate reader with excitation at 440 nm or 485 nm and emission at 530 nm or 538 nm. The results are listed in Table 4. The unsubstituted biaryl DFMS 130 had the lowest IC₅₀ value of 483 μM. All other sulfonic acids were better inhibitors. Of the biphenyl DFMS compounds that contained a fluorine or chlorine, the chlorine-containing sulfonic acids were more potent than their fluorine counterparts, and the addition of a second chlorine atom enhanced their potency significantly. In fact, the most potent inhibitor in our library was 126 (IC₅₀ of 14 μM, Figure 1), which has chlorines substituted at positions 3 and 5 on the second aryl ring. The presence of the chlorines at the meta-positions was
important for binding affinity, since the 3,4-substituted counterpart (125) resulted in a 2-fold decrease in potency.

![Biaryl DFMS 126, (µM)]

**Figure 1.** Inhibition of PTP1B by compound 126. The activity of PTP1B (0.5 µg/mL) in the presence of 126 at various concentrations (0-500 µM) were measured at 25 °C as described in the Experimental (Chapter 2).

Among the collection of biphenyl sulfonic acids, the hydrophobic DFMS compounds 131, 133, and 138 were fairly decent inhibitors, with IC$_{50}$ values of 80 µM, 98 µM, and 78 µM respectively. Their enhanced potency, in comparison to the biphenyl DFMS 130, was probably due to π-stacking abilities and/or hydrophobic interactions from the extra aryl or t-butyl moiety.

The heterocyclic DFMS compounds (139-144) were generally better inhibitors than the biphenyl compounds, likely due to the ability of the heterocycles forming both electrostatic and hydrophobic interactions. Interestingly, the thiophenes were more potent than the furans, with the exception of the benzo-derivatives 140 and 141. Both sulfonic acids inhibited PTP1B equally well, with IC$_{50}$ values of 49 µM and 56 µM respectively. Although the oxygen atom of furans may not be able to form efficient
Table 4. IC\textsubscript{50} Values of Biaryl DFMS Compounds 120-144 for PTP1B and CD45

<table>
<thead>
<tr>
<th>Product</th>
<th>IC\textsubscript{50}</th>
<th>Product</th>
<th>IC\textsubscript{50}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PTP1B (μM)</td>
<td>CD45 (μM)</td>
<td>PTP1B (μM)</td>
</tr>
<tr>
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<td>148</td>
<td>69</td>
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<td>130</td>
<td>483</td>
<td>&gt;500</td>
<td>80</td>
</tr>
<tr>
<td>132</td>
<td>154</td>
<td>83</td>
<td>98</td>
</tr>
<tr>
<td>134</td>
<td>286</td>
<td>&gt;500</td>
<td>182</td>
</tr>
<tr>
<td>136</td>
<td>202</td>
<td>&gt;500</td>
<td>175</td>
</tr>
<tr>
<td>138</td>
<td>78</td>
<td>99</td>
<td>32</td>
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<tr>
<td>140</td>
<td>49</td>
<td>46</td>
<td>56</td>
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<tr>
<td>142</td>
<td>58</td>
<td>49</td>
<td>291</td>
</tr>
<tr>
<td>144</td>
<td>116</td>
<td>81</td>
<td></td>
</tr>
</tbody>
</table>
electrostatic interactions with the enzyme, as opposed to thiophenes, the loss of potency may be compensated by hydrophobic interactions introduced from an extra aryl moiety.

3.11 Enzyme Inhibition Studies with CD45

To examine enzyme selectivity, the sulfonic acids 120-144 were also screened against another PTP, CD45. CD45 is a transmembrane PTP expressed on the surface of hematopoietic cells. It is believed to be responsible for the dephosphorylation of Src PKKs which results in the up-regulation of their catalytic activity that eventually leads to cell activation.\textsuperscript{76} The results are listed in Table 4. In comparison to the PTP1B studies, similar observations were made for CD45. The unsubstituted biphenyl DFMS 130 was a poor inhibitor towards CD45. Compound 130, along with 3 other sulfonic acids (124, 134, and 136), had an IC\textsubscript{50} greater than 500 \(\mu\)M. All other sulfonic acids were relatively better. Once again, the 3,5-dichloro-biphenyl DFMS 126 was the most potent inhibitor, with an IC\textsubscript{50} of 31 \(\mu\)M (Figure 2). Interestingly, the 3,4-substituted counterpart 125

![Figure 2](image-url)

\textbf{Figure 2.} Inhibition of CD45 by compound 126. The activity of CD45 (0.075 \(\mu\)g/mL) in the presence of 126 at various concentrations (0-500 \(\mu\)M) were measured at 25 \(^{\circ}\)C as described in the Experimental (Chapter 2).
resulted in a 5-fold decrease in potency, as opposed to a 2-fold difference for PTP1B. Thus for CD45, having halogens (Cl of F) at the meta-position on the second aryl ring is important for enhanced binding affinity. Similar to the PTP1B studies, the hydrophobic DFMS compounds 131, 132, 133, and 138 were decent inhibitors with IC\textsubscript{50} values of 67 \(\mu\text{M}\), 83 \(\mu\text{M}\), 81 \(\mu\text{M}\), and 99 \(\mu\text{M}\) respectively. As well, similar trends were seen with the heterocyclic sulfonic acids (139-144).

Overall, the sulfonic acids with IC\textsubscript{50} values in the low micromolar range were generally more potent towards CD45. However, little selectivity was seen between the two enzymes in terms of inhibition. This is consistent with the studies of Wang et al. where similar observations were made with their bis-DFMP naphthalene substrates.\textsuperscript{76}

3.12 DFMP Analogue of Biphenyl DFMS 126

The DFMS-bearing compounds are typically not as effective PTP1B inhibitors than their DFMP analogues. This has been demonstrated by Kotoris et al.\textsuperscript{84} In their PTP1B inhibition studies, the naphthyl and biphenyl DFMS's were 5-fold and 7.7-fold poorer inhibitors of PTP1B than their DFMP counterparts. Similarly, the triphenyl DFMP 24 from the library of biphenyl DFMP compounds had an IC\textsubscript{50} of 8.6 \(\mu\text{M}\) for PTP1B\textsuperscript{75}, while the DFMS analogue 131 that we prepared was 9-fold worse (IC\textsubscript{50} of 80 \(\mu\text{M}\)). As such, we wished to prepare a DFMP analogue of the biphenyl DFMS 126 in hopes of finding a submicromolar PTP1B inhibitor.

\[\text{DFMS} \text{ analogue of Biphenyl DFMS 126}\]

\[\text{DFMP} \text{ analogue 131}\]
We first attempted to prepare the DFMP analogue by carrying out a room temperature Suzuki coupling of diethyl protected 3-bromophenyl DFMP 146 with 3,5-dichlorobenzeneboronic acid (147) (Scheme 46). However, the reaction was unsuccessful because the desired biphenyl DMFP product 148 could not be separated from a byproduct, which was later determined by GCMS analysis to be the triphenylphosphonic acid 149. The byproduct was likely a result of two Suzuki couplings that took place, the second coupling arising between the biaryl chloride (148) and the boronic acid 147.

![Scheme 46. Room temperature Suzuki coupling of aryl bromide 146 with boronic acid 147](image)

The synthesis outlined in Scheme 47 was successful for preparing the desired DFMP analogue (154). The commercially available 3-bromotoluene (150) was coupled to 3,5-dichlorobenzeneboronic acid (147) by refluxing the reaction in DME in the presence of Pd(PPh₃)₄, Na₂CO₃, and water. The product 151 from the Suzuki coupling was obtained in 87% yield. Compound 151 was then converted to the benzyl bromide.
152 with NBS. A mixture of the mono- (major product) 152a and dibrominated (minor product) 152b products was obtained and not further purified since the dibrominated 152b byproduct was unreactive in the subsequent reaction. The benzyl bromide 152 was treated with triethylphosphite (P(OEt)₃) to give the phosphonate 153 in 92% yield. A one-step electrophilic fluorination with NFSi and NaHMDS resulted in the difluorinated phosphonate 148 in 95% yield. Deprotection of 148 with TMSBr, followed by conversion of the phosphonic acid to the ammonium salt, gave the desired biphenyl DFMP analogue 154 in 73% yield.

Scheme 47. Synthesis of the biphenyl DFMP 154
3.12.1 Enzyme Inhibition Studies of 154 with PTP1B

The biphenyl DFMP analogue 154 was screened against PTP1B using the same assay conditions as previously described in Section 3.10. Surprisingly, an IC\textsubscript{50} of 47 \textmu M was obtained (Figure 3). The DFMP analogue was expected to be more potent than the DFMS counterpart, however it was 3-fold worse. This suggests that the DFMS group of the sulfonic acid may be more suitable for placing the \textit{meta}-chlorines on the second aryl ring, in an optimum position for enhanced hydrogen bonding and/or electrostatic interactions with the enzyme.

![Figure 3. Inhibition of PTP1B by compound 154. The activity of PTP1B (0.5 \textmu g/mL) in the presence of 154 at various concentrations (0-250 \textmu M) was measured at 25 °C as described in the Experimental (Chapter 2).](image-url)
3.13 A Word about Aryl Sulfatases

It has not escaped our attention that our library of sulfonic acids may have had potent inhibitors of aryl sulfatases. Aryl sulfatases are enzymes that hydrolyze aryl sulfates to their non-sulfated counterparts. Among this family is steroid sulfatase, which has recently attracted much interest. The enzyme is responsible for hydrolyzing estrogen sulfate (155) to estrogen (156). (Scheme 48). Estrogens are essential for supporting the growth of approximately one-third of all breast tumors. thus, there is considerable interest in developing inhibitors for this enzyme. One of the most potent, irreversible steroidal inhibitor to date for steroid sulfatase is estrone-3-O-sulfamate with an IC$_{50}$ of 80 nM (EMATE. 157). However, EMATE has been found to be
estrogenic. Scientists are now looking at developing non-steroidal type inhibitors that should have the following properties for high-affinity binding to steroid sulfatase: (1) an oxygen or an electronically similar link between the steroid ring and sulfonate moiety that can form hydrogen bonds. (2) an oxygen anion or an uncharged but highly electronegative substituent at the sulfur atom that can take part in ionic interactions and (3) a large carbon skeleton that can provide hydrophobic interactions. Our library of sulfonic acids met all three criteria. Thus, we were interested in screening our compounds against steroid sulfatase.

3.13.1 Enzyme Inhibition Studies with Aryl Sulfatase

Human steroid sulfatase is not commercially available. The enzyme has never been overexpressed and is very difficult to purify. However, the degree of primary sequence homology is high among the family of aryl sulfatases, which includes steroid sulfatase and aryl sulfatase A, B, and C (some studies have suggested that steroid sulfatase and aryl sulfatase C are the same enzyme). Thus, the enzyme studies were carried out with commercially available *H. pomatia* aryl sulfatase, which is a mixture of aryl sulfatase A and B. We assumed that any compounds that are inhibitors of the *H. pomatia* aryl sulfatase A and B may be potent towards human steroid sulfatase.

Rapid screens of the sulfonic acids against *H. pomatia* aryl sulfatase were carried out at 500 μM of inhibitor. *p*-Nitrophenyl sulfate (*p*-NPS) was used as the substrate at the *K_m* concentration (0.83 mM) in assay buffer containing 20 mM MOPS (pH 7.0) and 0.1 KCl at 25 °C. The enzyme activity was monitored spectrophotometrically at 400 nM. The results are listed in Table 5. Percent inhibition
Table 5. Percent Inhibition of *H. pomatia* Aryl Sulfatase with 500 μM of the Sulfonic Acids

<table>
<thead>
<tr>
<th>Product</th>
<th>% Inhibition at 500 μM</th>
<th>Product</th>
<th>% Inhibition at 500 μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>73</td>
<td>121</td>
<td>63</td>
</tr>
<tr>
<td>122</td>
<td>50</td>
<td>123</td>
<td>56</td>
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<tr>
<td>124</td>
<td>66</td>
<td>125</td>
<td>71</td>
</tr>
<tr>
<td>126</td>
<td>95</td>
<td>127</td>
<td>57</td>
</tr>
<tr>
<td>128</td>
<td>52</td>
<td>129</td>
<td>37</td>
</tr>
<tr>
<td>130</td>
<td>61</td>
<td>131</td>
<td>ND*</td>
</tr>
<tr>
<td>132</td>
<td>69</td>
<td>133</td>
<td>76</td>
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<tr>
<td>134</td>
<td>57</td>
<td>135</td>
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<tr>
<td>136</td>
<td>49</td>
<td>137</td>
<td>56</td>
</tr>
<tr>
<td>138</td>
<td>ND*</td>
<td>139</td>
<td>77</td>
</tr>
<tr>
<td>140</td>
<td>63</td>
<td>141</td>
<td>ND*</td>
</tr>
<tr>
<td>142</td>
<td>71</td>
<td>143</td>
<td>54</td>
</tr>
<tr>
<td>144</td>
<td>144</td>
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</tbody>
</table>

*Percent inhibition could not be determined due to solubility problems.
values with sulfonic acids 131, 138, and 141 were not obtained due to solubility problems. All of the compounds were poor inhibitors. The most potent inhibitor was once again the 3.5-dichloro-biaryl DFMS 126 with 95% inhibition of *H. pomatia* aryl sulfatase. An IC\textsubscript{50} value was obtained for the sulfonic acid using the same assay conditions for the rapid screens except with a different K\textsubscript{M} concentration of \( p \)-NPS. We had later realized that the \( p \)-NPS used in the rapid screens was partially hydrolyzed, resulting in a higher K\textsubscript{M} concentration than expected. Thus, before determining the IC\textsubscript{50} of compound 126, the K\textsubscript{M} concentration was redetermined with a new bottle of \( p \)-NPS. The new K\textsubscript{M} concentration was 0.70 mM (Figure 4). Compound 126 was found to have an IC\textsubscript{50} of 334 \( \mu \)M (Figure 5), making it a modest, reversible, non-steroidal, aryl sulfatase inhibitor.

![Figure 4. K\textsubscript{M} concentration for *H. pomatia* aryl sulfatase with the substrate \( p \)-NPS. The assays were carried out as described in the Experimental (Chapter 2).](image-url)
Figure 5. Inhibition of *H. pomatia* aryl sulfatase by compound 126. The activity of *H. pomatia* aryl sulfatase (0.03 mg/mL) in the presence of 126 at various concentrations (0-922 µM) was measured at 25 °C as described in the Experimental (Chapter 2).

3.13.2 Synthesis of the Non-Fluorinated Sulfonate 158 and Enzyme Inhibition Studies with Aryl Sulfatase

To determine the significance of the fluorines of 126 on binding affinity, a nonfluorinated analogue 158 was prepared and screened. The synthesis is outlined in scheme 49. The precursor for 158, which was 152, was prepared in the same manner as described above for the synthesis of the phosphonic acid. The benzyl bromide was converted to the sodium sulfonate salt by refluxing it in 1:1 mixture of acetone and water for 24 hrs in the presence of Na₂SO₃. The nonfluorinated sulfonate 158 was obtained in 65% yield.

The IC₅₀ of 158 for *H. pomatia* aryl sulfatase was determined under the same conditions used for the biaryl DFMS 126. Compound 158 had an IC₅₀ of 742 µM. In
comparison to the fluorinated counterpart 126, which had an IC₅₀ of 334 μM. the fluorines only resulted in a 2.2-fold increase in inhibitory potency. Thus, the very large effect of fluorines observed with DFMP or DFMS inhibitors and PTPs is not as pronounced with the DFMS compounds and aryl sulfatases A and B from *H. pomatia*.

![Chemical diagram]

Scheme 49. Synthesis of the non-fluorinated sulfonate 158

4 Conclusion

In conclusion, LPOS has proven to be an effective method for rapid construction of our small molecule library of α,α-difluoromethylenesulfonic acids. All reactions were
carried out under homogeneous conditions, thus allowing us to monitor the reactions by conventional $^{19}$F NMR. The products were rapidly purified by precipitation and subsequent filtration, and most of the compounds could be directly screened for biological activity without further purification. Lastly, the mild cleavage conditions enabled us to use heterocyclic boronic acids, which resulted in greater structural diversity of our library.

From the phosphatase biological studies, the DFMS group has proven to be an effective monoanionic phosphate mimetic. We even found that the DFMS moiety can be more potent than the DFMP moiety as demonstrated by the sulfonic acid 126 and the phosphonic acid 154. As well, sulfonic acids bearing a heterocyclic ring enhances binding affinity since they were generally more potent inhibitors of both PTP1B and CD45.

5 Future Direction

Since the start of our research project, many reports have appeared in the literature describing inhibitors of PTPases. Desmarais et al prepared several tripeptides containing the non-hydrolyzable phosphotyrosine analogue, F$_2$Pmp (5), and screened them against PTP1B, CD45, PTPβ, LAR and SHP-1.$^{140}$ They found that the tripeptide Glu-F$_2$Pmp- F$_2$Pmp was most potent towards PTP1B, with an IC$_{50}$ of 40 nM. and was at least 100-fold more selective for PTP1B than the other PTPs. Speculations were made that one of the F$_2$Pmp residues may have been binding in the second non-catalytic arylphosphate binding site found on PTP1B.

To take advantage of the second arylphosphate binding site which is found in close proximity to the active site of PTP1B, Taing et al prepared a series of
bis(aryldifluorophosphonates) designed to simultaneously occupy both sites. Their most potent inhibitor, 159, had a $K_i$ of 0.93 mM and displayed greater than 100-fold preference for PTP1B relative to VHR, LAR and PTPα. The likelihood that 159 was simultaneously binding to both arylphosphate binding sites is unlikely since the bis-DFMP diphenylmethyl derivatives (26, $n = 4$) developed by Taylor and coworkers had similar potency and was found not to bind in both arylphosphate binding sites.

The bis(aryloxymethylphosphonate) 160 has been shown to be more potent towards PTP1B ($K_i$ of 0.047 mM) than VHR due to possible interactions of the second aryloxymethylphosphonate group with the second phosphate binding site. From these studies, the aryloxymethylphosphonate was found to be a very good nonhydrolyzable phosphotyrosine mimetic.
Researchers at Wyeth-Ayerst Research Inc. prepared a series of large molecular weight organic molecules and screened them against several PTPs.\textsuperscript{143, 144, 145} Among their collection of compounds they found that polyaromatic molecules such as 161 (IC\textsubscript{50} of 61 nM)\textsuperscript{143}, 162a-d (IC\textsubscript{50} values ranging from 0.12 to 0.3 \textmu M)\textsuperscript{144}, and 163 (0.32 \textmu M)\textsuperscript{145} were selective and potent inhibitors of PTP1B. Their enhanced binding affinity and specificity were attributed to increased hydrophobic interactions with residues just outside the catalytic pocket\textsuperscript{144}. Phe\textsubscript{182} and/or a hydrophobic region in the immediate vicinity of the catalytic site\textsuperscript{143, 145}. This hydrophobic region is defined on one side by residues 215-221 of the PTP loop and on the other by Phe\textsubscript{182} and Asp\textsubscript{181}\textsuperscript{145}. They found

\[
\begin{align*}
161 & \quad \text{Br} \quad \text{O} \quad \text{Br} \\
162a & \quad X = \text{n-octyl} \quad \text{Y} = \text{H} \\
162b & \quad X = \text{n-octyl} \quad \text{Y} = \text{H} \\
162c & \quad X = \text{n-octyl} \quad \text{Y} = \text{CH}_2\text{COOH} \\
162d & \quad X = \text{n-octyl} \quad \text{Y} = \text{CH}_2\text{COOH} \\
\end{align*}
\]
interactions between the benzyl group, at the α-carbon of the oxo-acetic acid moiety (161 and 163). with the hydrophobic region had an important effect on PTP1B binding and inhibition. For 161 and 163, the absence of the benzyl group resulted in a 6-fold and 4-fold decrease in potency respectively. Implications were made that the hydrophobic region may be a selective determinant for PTP1B binding.

Very recently, 2-(oxalylamino)-benzoic acid (OBA, 164) was found to be a very good phosphotyrosine mimetic. A crystal structure of OBA complexed with PTP1B revealed that OBA forms similar interactions with PTP1B in comparison to the natural phosphotyrosine substrate. Some of these include a salt bridge between the carboxy group of oxamic acid and the guanidinium group of Arg221, and a hydrogen bond between the carbonyl group of the oxamic acid and the main chain amide of Gly220. Additional interactions were also found, which were important for binding affinity to PTP1B. Upon OBA binding, the WpD loop was found in the closed conformation where favorable aromatic-aromatic interactions between Phe182 and the aryl ring of OBA could take place. As well, the α-carboxy group formed hydrogen bonds with Asp181 and Tyr46, and a salt bridge with Lys120. OBA was found to have a $K_i$ of 23 μM, and was more selective towards PTP1B than PTPα, PTPε, PTPβ, CD45, LAR, and SHP1.

\[ 164 \text{ OBA} \quad 165 \]
Further studies were carried out to enhance the binding affinity and specificity of OBA for PTP1B. Iversen and coworkers examined residues at positions 47, 48, 258, and 259 of PTP1B and several other PTPs. These residues were found in the active site or within close proximity. They found residue 48 to be of interest because Asp exists at this position for PTP1B and is an Asn in many other PTPs. Thus, they developed the OBA variant, compound 165, which was designed to form a salt bridge between the basic nitrogen and Asp48, and cause repulsion in other PTPs containing an Asn in the equivalent position. Indeed, the salt bridge was formed with PTP1B as well as interactions between the saturated ring and several nonpolar residues, including van der Waals interactions with Ala217, Ile219, and Val49, and aromatic-aromatic interactions with Tyr46. The combined features resulted in a $K_i$ of 0.29 μM for 165 which was 79-fold more potent than 164. As well, 165 was at least 1900-fold more selective for PTP1B than for several other PTPs that has the basic residue Asn at position 48. CD45 on the other hand, has an Asp at position 48 and so not surprisingly the selectivity for PTP1B (200-fold) was significantly lower. Nevertheless, Asp48 may be another selective determinant for PTP1B.

Iversen and coworkers also found residue 259 to be a key selectivity determinant between PTP1B and PTPα. In PTP1B, residue 259 is a glycine, whereas PTPα has glutamine in the equivalent position. By screening different analogs of the hexapeptide DADE(pY)L, they found the size of residue 259 to be important for substrate recognition. Introducing glycine at position 259 of PTPα resulted in an enzyme with the same broad substrate recognition and catalytic activity as PTP1B. Conversely, when Gly259 in PTP1B was replaced with glutamine, the enzyme turned into a PTPα-like enzyme.
Iversen and coworkers proposed that the bulky glutamine in position 259 causes steric hindrance and would therefore limit the substrate recognition capability of PTPα. whereas a glycine would allow for broader substrate recognition, as seen by PTP1B. Thus, designing bulkier substrates may enhance selectivity of the inhibitor for PTP1B than for PTPα.

Sarmiento et al have found that compounds 166, 167, and 168 are potent and selective inhibitors for PTP1B, each binding in their own distinct manners. The sulfate moiety of 166 did not interact with the PTP binding loop as well as the phosphate group of phosphotyrosine. However, the decreased binding affinity was compensated by extensive hydrophobic interactions between the polyaromatic ring and residues surrounding the active site pocket of PTP1B. Due to the size of the polyaromatic ring, Sarmiento et al suggested that the PTP1B active site must possess significant plasticity to accommodate such a large molecule. Compound 166 had a \( K_i \) of 39 \( \mu \)M. The salicylic moiety of 167 was found to be a good phosphotyrosine mimetic, since it maintained the electrostatic interactions seen between the phosphate moiety of phosphotyrosine and the PTP binding loop. Interestingly, the positions of the hydroxyl and carboxyl groups were important since reversal of the two groups resulted in a 12-fold reduction in potency. The 2-thioxo-1-benzimidazoyl fused ring portion of 167 was also found to be important, since hydrophobic interactions between the ring system and surface residues of PTP1B resulted in a 320-fold binding enhancement. A \( K_i \) of 61 \( \mu \)M was obtained for 167. Lastly, the nitrophenyl moiety of 168 may also act as a phosphotyrosine mimetic. The nitro group of the 3-nitrophenyl acryloyl moiety was able to form hydrogen bonds with the PTP binding loop, where as the phenyl ring formed hydrophobic interactions. As well, the nitro group
in the quinolon moiety may have formed hydrogen bonds with the basic side chain of Arg47, resulting in the selectivity (greater than 2-fold) of 168 for PTP1B than for LAR or PTPα. The latter two phosphatases lack the basic residue at the equivalent position. Compound 168 had a $K_i$ of 54 μM. From these studies, Sarmiento et al demonstrated that selective and potent non-phosphorus inhibitors of PTP1B can be obtained if properly functionalized phosphate surrogates are attached to appropriate aromatic frameworks which effectively occupies the pTyr pocket and forms interactions with residues immediately outside the active site.\textsuperscript{149}

Based on these studies that have recently come out in the literature, there is potential for finding more potent and selective DFMS-bearing PTP1B inhibitors. Future work in the area of low-molecular weight, non-peptidyl, organic DFMS inhibitors may include designing aryl DFMS derivatives with hydroxyl and or carboxyl groups ortho to the DFMS moiety (general structure 169) in order to increase electrostatic interactions between the inhibitor and active PTP binding loop. Just as equally important is forming interactions with residues within or immediately outside the active site in order to
enhance enzyme selectivity. Since the heterocyclic sulfonic acids from the DFMS library were generally more potent inhibitors of PTP1B and CD45, the introduction of different functionalities on the heterocyclic ring may enhance their potency and selectivity by forming new electrostatic interactions with active site and/or surface residues. Enzyme selectivity may be enhanced just by appropriately positioning the functional groups such that they can interact with the key residues Arg47 and Asp48 of PTP1B. Introducing more heterocycles or aryl rings to sulfonic acids in our library may also result in increased hydrophobic interactions, subsequently leading to increased binding affinity.

The LPOS approach in preparing the biaryl DFMS compounds has proven to be an effective method for rapid construction of the library. However, because the sulfonate ester linkage was only stable at room temperature, the type of chemistry that could be carried out on the polymer-bound substrates was limited. Based on our studies though, one possible route to forming polyaromatic DFMS compounds is by carrying out multiple room temperature Suzuki couplings. Suzuki reactions take place more readily if aryl bromides and iodides are used. Aryl chlorides are generally less reactive. Thus, by loading a dihalogenated aryl sulfonate onto the polymer, such as 3-bromo-5-chlorophenyl
DFMS (170), different Suzuki couplings can be carried out by using appropriate palladium catalysts. In comparison to the highly reactive palladium catalyst used in our studies, (PhCN)$_2$PdCl$_2$, a less reactive catalyst could be used to selectively carry out the Suzuki coupling with the bromide. Once the first Suzuki coupling takes place, than a more reactive catalyst/ligand system (such as those described by Buchwald and coworkers$^{123, 124}$) could be used for the second coupling with the chloride. Since three "building blocks" would be used in this methodology, the size of the library would be considerably greater, resulting in greater odds for finding a substrate that is potent and selective towards PTP1B or other phosphatases.

\[
\begin{align*}
\text{O} & \quad \text{linker} \quad \text{O} \\
\text{F} & \quad \text{F} \\
\text{Cl} & \quad \text{Br}
\end{align*}
\]

170

The sulfonic acids in our library were only moderate inhibitors of *H. pomatia* aryl sulfatase. However, they may be more potent towards human steroid sulfatase. Isolation of this enzyme from human placenta is currently taking place in the Taylor group, thus screening of the DFMS library with steroid sulfatase will take place in due time.

Future studies to improve the potency of the sulfonic acids with aryl sulfatase may include preparing the sulfamate derivatives (general structure 171a). Many of the non-steroidal steroid sulfatase inhibitors that have recently appeared in the literature have
incorporated the sulfamate moiety due to EMATE being a highly potent inhibitor of steroid sulfatase.\textsuperscript{131} The sulfamate moiety of EMATE was responsible for inactivation of the enzyme.\textsuperscript{132} Introducing alkyl chains or aryl rings to the sulfonic acids may also further enhance binding affinity by the increasing hydrophobic interactions between the inhibitor and the residues immediately outside the active site (171a and b).

Recently, post-translational modification of many secreted and membrane-bound proteins by sulfation of tyrosine residues has attracted much attention.\textsuperscript{150} Little is known about the biological roles of these proteins, as well as the enzymes responsible for transferring the sulfate group to tyrosine (tyrosyl protein sulfotransferases, TPSTs). However, tyrosine sulfate has been implicated as a determinant of protein-protein interactions involved in the inflammatory and immune response. The compounds described in this study and future compounds of this type may be useful as tools for studying these and other biological processes involving tyrosine sulfation.
References


78. Personal communication from A. Nicole Dinaut.


100. Performed by M-J. Chen from the Taylor group.


125. Personal communication from G Hum.
126. 4-Nitrocinnamoyl chloride was prepared from a procedure developed by White et al. White, W. N.; Fife, W. K. J. Am. Chem. Soc. 1961. 83, 3846-3853.

127. Other reagents were considered before using 4-nitrocinnamoyl chloride as the reagent for detecting the extent of polymer loading. Acetic anhydride would readily undergo nucleophilic attack by the hydroxyl functionality of the polymer. However, the methyl group of the acetoxy moiety would have a $^1$H NMR signal overlapping the broad doublet arising from the (-CH$_2$CH-) signal from the polymer. In attempt to shift the methyl $^1$H NMR signal downfield from the broad doublet, we tried chloroacetic anhydride. However, the methylene $^1$H NMR signal from the chloroacetoxy moiety overlapped with one of the (-CH$_2$O-) signal of the polymer.

128. Polymer loading was determined in the same manner as that used for determining loading of polymer 110. However, the monomer 112 was used instead of monomer 106.

129. Prepared by G. Hum from the Taylor group. For the literature procedure, refer to reference 74.


