Canadian Journal of Chemistry
Revue canadienne de chimie

Biological Chemistry of the Carbon-Sulfur Bond

Journal:	<i>Canadian Journal of Chemistry</i>
Manuscript ID	cjc-2015-0270.R1
Manuscript Type:	Award Lecture
Date Submitted by the Author:	10-Jul-2015
Complete List of Authors:	Honek, John; University of Waterloo, Chemistry
Keyword:	methionine, fluorine, bioorganic, Belleau, sulfur

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Biological Chemistry of the Carbon-Sulfur Bond

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Graphical Abstract

Abstract:

Carbon-sulfur biological chemistry encompasses a fascinating area of biochemistry and medicinal chemistry and includes the roles that methionine and *S*-adenosyl-L-methionine play in cells, as well as the chemistry of intracellular thiols such as glutathione. This article, based on the 2014 Bernard Belleau Award lecture, provides an overview of some of the key investigations that were undertaken in this area from a bioorganic perspective. The research has ameliorated our fundamental knowledge of several of the enzymes utilizing these sulfur-containing molecules, has led to the development of several novel ^{19}F biophysical probes, and has explored some of the medicinal chemistry associated with these processes.

Keywords: methionine, fluorine, unnatural amino acids, bioorganic, bioinorganic, Belleau award

The carbon-sulfur bond is an important chemical entity in Nature. It is found in numerous biological molecules, notably in the structures of the amino acid methionine, the cellular sulphonium compound *S*-adenosyl-L-methionine (AdoMet), and the various biological thiols such as ovothiol, ergothionine and the tripeptide glutathione. In order to expand our knowledge in this area, analogs of methionine were designed and synthesized to serve as potential biophysical probes and possibly inhibitors of the enzymes making use of this amino acid. Exploration of the biochemical steps involved in the incorporation of these methionine analogs into proteins was undertaken and a number of X-ray structures of the enzymes in complex with these analogs were determined. This has led to a better understanding of the substrate specificity of these enzymes and the chemical effects that the presence of fluorine atoms have on the biochemical processing of these analogs. Additionally, the bacterial resistance mechanisms that had been previously identified against the thiopeptide antibiotic thiostrepton were further investigated using analogs of thiostrepton and protein isolation and characterization techniques. Both a ribosomal RNA methyltransferase that uses AdoMet as the methylating agent, and a thiostrepton-binding protein were investigated in these studies. Lastly, Glyoxalase I, a metalloenzyme that utilizes glutathione to detoxify intracellularly-generated methylglyoxal, was studied and a new class of this enzyme was identified in bacteria which exhibits a different metal activation profile compared to previously reported Glyoxalase I enzymes. These studies have led to a better understanding of how enzyme active site structure can control metal specificity and catalytic activity in these detoxification enzymes.

Protein Biosynthesis

Methionine is an important amino acid as it is one of the “standard” twenty amino acids coded for in DNA and is introduced into a growing polypeptide chain during protein biosynthesis.¹ As it is also the “initiator” amino acid that is used as the first amino acid during protein biosynthesis, methionine is involved in additional biochemical steps compared to the other amino acids found in proteins. For example, the enzyme methionyl-tRNA synthetase (MetRS) couples L-methionine, using adenosine triphosphate (ATP), onto two different types of transfer ribonucleic acids (tRNAs) (**Scheme 1**).¹⁻² The initiator tRNA ($\text{tRNA}^{\text{initiator}}$) is the tRNA that is involved in

supplying methionine for the N-terminal position of the protein that is undergoing biosynthesis on the ribosome. On the other hand, the elongator tRNA ($\text{tRNA}^{\text{elongator}}$) supplies methionine for

the remaining positions in the protein. Additional complexity ensues as many microorganisms as well as mitochondria require formylation of the initiator methionine once it is attached to its tRNA^{initiator}. The formylation is catalyzed by the N^{10} -formyltetrahydrofolate (N^{10} -fTHF) utilizing enzyme methionyl-tRNA formyltransferase (MTF).¹

The formylated Met-tRNA^{initiator} serves as the source of the N-terminal methionine. Hydrolytic removal of the formyl group is catalyzed by the enzyme peptide deformylase (PDF).³ Frequently the N-terminal methionine is removed as well, a reaction catalyzed by methionine aminopeptidase (MAP).⁴⁻⁵ The amino acid sequence at the N-terminus is important in determining whether MAP enzymes remove the initiator methionine, with small residues adjacent to the N-terminal methionine being preferred by MAP enzymes.⁶ Methionines are susceptible to oxidation to the sulfoxide level by cellular reactive oxygen and reactive nitrogen species. It has been shown that methionine oxidation in proteins can serve as a control mechanism for the biological activity of many proteins. Various classes of enzymes termed methionine sulfoxide reductases (MSRs) exist that use the reducing equivalents from nicotinamide cofactors, through the intermediacy of thioredoxin and other redox proteins, to reduce the sulfoxide back to the sulfide oxidation level.⁷⁻⁸ Of interest is that the enzyme activity of pathogen-associated MSRs has been shown to contribute to the extent of human pathogenicity for *Streptococcus pneumoniae* and *Neisseria gonorrhoeae*, for example.⁹ Inactivation of pathogen MSRs can, in some cases, greatly reduce eukaryotic cell surface receptor binding by the pathogen and hence reduce virulence.

Methionine Analogs

The pathways discussed above for methionine's incorporation into and removal from protein are of interest as potential targets for novel drug design. With knowledge of the above rich biochemistry of the amino acid methionine, our group explored the design and synthesis of several methionine analogs and investigated their interaction with a diverse set of enzymes.¹⁰ Our first thought was to determine which methionine analogs might have interesting properties and serve as novel biophysical probes and/or enzyme inhibitors. We began our studies with the synthesis of fluorinated methionine analogs, specifically the L-monofluoromethionine (MFM), L-difluoromethionine (DFM) and L-trifluoromethionine (TFM), having one, two and three fluorines respectively present on the methyl group.¹⁰ Only TFM had been previously described, and there was only indirect evidence that this analog could be incorporated into acid-insoluble protein fractions isolated from yeast grown in its presence.¹¹ An additional report indicated that TFM could be decomposed by the enzyme γ -cystathioninase.¹² We developed facile synthetic routes to DFM and TFM (**Scheme 2**). Protected monofluoromethionine could be prepared by a

modified Pummerer rearrangement using diethylaminosulfur trifluoride and a protected methionine sulfoxide. However attempts to deprotect the monofluorinated analog resulted in hydrolysis of the fluoromethyl group, producing L-homocysteine, formaldehyde and fluoride ion.

Nevertheless the DFM and the TFM analogs were produced in good yields and were hydrolytically stable.

Our first medicinal chemistry investigation with these analogs was to explore their effects on a biologically active peptide, the formylated chemotactic peptide, f-Met-Leu-Phe.¹³ It was known that substitution of methionine in this peptide with almost any other methionine analog results in substantial loss of chemoattractant activity in neutrophil migration assays. Hence it was thought that this peptide would make an excellent indicator of how well DFM and TFM would be accepted by biological systems. The two novel formylated tripeptides, f-DFM-Leu-Phe and f-TFM-Leu-Phe were chemically synthesized and were determined to be extremely active in neutrophil migration assays. Bolstered by these early results, the question as to whether these analogs could replace normal methionine in proteins was pursued. This line of investigation would not only allow one to determine if these unnatural amino acids could be utilized in protein biosynthesis but also how might these analogs affect protein structure and function and subsequent posttranslational processing events. Bacteriophage lambda lysozyme was used as the model protein for these studies.¹⁴ Overproduction of this protein (MW 17,921 Daltons) from an auxotrophic strain of *Escherichia coli* that required exogenous methionine, in the presence of TFM resulted in the overproduction of lysozyme with TFM in place of Met to varying degrees (**Figure 1**).¹⁵⁻¹⁶ However, the yields of overproduced TFM-labeled lysozyme was found to be

lower than when normal methionine was used exclusively in the growth medium, an indication that the biosynthetic machinery, specifically the Met-tRNA synthetase, was less efficient at utilizing this analog. Interestingly, the use of DFM under the same conditions resulted in excellent protein production with all three methionine positions being completely replaced by the DFM analog.¹⁷ In all cases the isolated lysozymes containing the unnatural amino acids remained catalytically active.

As the methionine analogs contained the ^{19}F nucleus, which is nuclear magnetic resonance (NMR) active, the study of the ^{19}F NMR of the fluorinated proteins was also investigated. Since cellular biomolecules normally do not contain fluorine, the new ^{19}F probes were found to serve as excellent biophysical probes, being useful in the detection of protein-ligand binding.^{15, 17} DFM is especially interesting since the two fluorines are diastereotopic, and when incorporated into the core of a protein, the diastereotopicity of the ^{19}F NMR resonances can be clearly observed. Hence the DFM analog has proven to be a useful biophysical probe. Since those initial studies, these analogs have been further utilized. These biophysical studies have included investigations of the *Pseudomonas aeruginosa* alkaline protease (a prototypic

metzincin-class protease, whose family members are important in disease states such as cancer metathesis involving collagenases and gelatinases), the *Escherichia coli* leucine-isoleucine-valine (LIV) binding protein in collaboration with Dr. Linda Luck and co-workers, and the control of protein redox in collaboration with Dr. Yi Lu and co-workers.¹⁸⁻²⁰ In the latter case, the application of protein intein ligation to insert the methionine analogs L-norleucine, L-difluoromethionine, L-trifluoromethionine, L-selenomethionine, L-methoxinine, and L-methionine into the active site of the *Pseudomonas aeruginosa* blue copper protein azurin (Figure 2) resulted in the very selective control of the redox properties of this

protein. It should be noted that the control of redox protein properties is an important area of bioelectronics and its future applications, and unnatural amino acid substitutions can be used to extend the control of protein redox beyond what can be accomplished with just the standard twenty amino acid substitutions available by site-directed mutagenesis.²¹

The fluorinated methionines were among the first fluorinated aliphatic amino acids to be incorporated into proteins. The fluorinated aromatic amino acids such as fluorotryptophan, fluorophenylalanine and fluorotyrosine already had been successfully incorporated into proteins

and had proved useful as biophysical probes.^{15, 22} Further explorations of methionine biological chemistry focused on understanding the molecular preference for DFM over TFM by the *Escherichia coli* MetRS. Studies in collaboration with colleagues at the CNRS in France revealed that the normal aromatic side chain cascade that occurs when methionine binds into the active site of MetRS resulting in the formation of an optimal binding pocket for the thiomethyl group of methionine, also occurs in the same way for DFM, thus explaining the high incorporation levels of DFM into proteins (**Figure 3**).²³ However analyses of the X-ray structural

data on TFM bound to MetRS showed that the additional fluorine present in the methyl group of TFM blocked this aromatic residue cascade from occurring (**Figure 3**). This would result in the inability by MetRS to form the optimal thiomethyl binding pocket for the trifluoromethyl group and hence explain the poorer activation of TFM by the enzyme. We further extended our studies to nucleoside inhibitors of MetRS, such as the methionine and trifluoromethionine sulphonamoyl adenosine analogs. These compounds were determined to potently inhibit the *E. coli* MetRS.²⁴

They are thought to resemble an activated intermediate along the reaction pathway of the enzyme (Figure 4). These inhibitors were further studied by

X-ray crystallography with the *E. coli* MetRS.²³ One of the inhibitor-enzyme complexes is shown in Figure 5, and the analysis of its structure allowed for a detailed understanding of the

molecular interactions between these inhibitors and the active site. These detailed structural studies have also aided other research groups in undertaking molecular docking studies and inhibitor design to develop new antibacterial agents based on MetRS inhibition.²⁵ We further pursued mechanistic and structural studies on a number of other enzymes involved in methionine biochemistry such as the *E. coli* methionine aminopeptidase with fluorinated methionines as well as with phosphonic and phosphinic analogs, and with the enzyme bovine methionine sulfoxide reductase A.²⁶⁻²⁷

AdoMet Biological Chemistry

As one can see, the biological chemistry of methionine is quite extensive in cells, even if one focuses solely on protein biosynthesis. However methionine has additional cellular roles (Scheme 3).²⁸ Although the incorporation of methionine into proteins is obviously important, the

biosynthesis of *S*-adenosyl-L-methionine (AdoMet), through the action of the enzyme methionine adenosyltransferase (MAT), is also of critical significance to cells.²⁹ Based on the sulphonium structure of AdoMet, one might expect that Nature should be able to make use of each of the three groups that are bonded to the sulfur atom. This turns out to be true.²⁸ For example, numerous methyltransferases utilize AdoMet as a co-substrate to supply the methyl group for transfer to specific substrates such as DNA, RNA, protein, lipids and small molecules. This results in the methylated acceptor (**Scheme 3**) and the co-product, *S*-adenosyl-L-homocysteine (AdoHcy), a potent feedback inhibitor of methyltransferases. This is an important cellular reaction. Enzymes such as AdoHcy hydrolase and AdoHcy nucleosidase act to lower the concentration of AdoHcy in cells, thus preventing blockade of methyltransferase catalyzed reactions. These reactions also make available L-homocysteine, which is critical in the biosynthesis of methionine (right side of **Scheme 3**).³⁰⁻³²

The transfer of portions of the amino acid skeleton of AdoMet can also occur. For example, transfer of the entire 2-aminobutanoic acid group to a histidine side chain in eukaryotic elongation factor 2, is the first step in diphthamide biosynthesis.³³ In addition, decarboxylation of AdoMet by the enzyme AdoMet decarboxylase, followed by transfer of the propylamine moiety, is important in the biosynthetic pathway to the polyamines spermidine and spermine. This latter set of reactions is catalyzed by the enzymes spermidine and spermine synthases (left side of **Scheme 3**).^{28, 34-35} The resulting 5'-deoxy-5'-methylthioadenosine is converted back to methionine through a series of salvage steps that depend on the specific organism (**Scheme 3**).^{31, 36} The third group attached to the sulfur of AdoMet, that of the ribonucleoside itself, can also been utilized by cells. For example, the cyclopentene portion of queuosine, a modified nucleoside present in certain tRNAs, originates from the ribose portion of AdoMet.³⁷ Other

cellular roles of AdoMet have been discovered and range from the areas of riboswitches to adenosyl radical formation by AdoMet-iron/sulfur cluster cofactors.³⁸⁻³⁹ Additional studies in our laboratory have involved the design and synthesis of analogs of some of the methionine salvage pathway intermediates and the exploration of some of their antitumor/anticancer/antiviral activities.⁴⁰⁻⁴²

Methionine- γ -lyase and Antiprotozoal Agents

Parasitic diseases such as those caused by the protozoans *Trichomonas vaginalis* and *Entamoeba histolytica* can infect large numbers of humans.⁴³ *T. vaginalis* is a sexually transmitted microorganism and *E. histolytica* is the causative agent of amebic dysentery and is classified as a category B priority biodefense agent. Resistance against metronidazole and tinidazole (**Figure 6**), the current clinical drugs used to treat these infections, has been detected

R = CH₂CH₂OH metronidazole

= CH₂CH₂SO₂CH₂CH₃ tinidazole

and new antiprotozoal agents are of great interest. These important pathogens utilize a modified sulfur pathway in their cellular physiology. For example, the enzyme methionine- γ -lyase (MGL), present in these protozoa but not in humans, converts L-methionine into alpha-ketobutyrate, methylmercaptan and ammonia (**Scheme 3**).⁴⁴ Our group has explored the

contain MGL.⁴⁵⁻⁴⁶ Mechanistic details were of interest, although the release of

crosslinking agent, was suggested (**Scheme 5**).^{12, 44} Our laboratory explored the detailed processing of TFM as well as the previously unstudied DFM analog, with both the *T. vaginalis*

MGL as well as a bioorganic model system previously shown to mimic the overall chemical reaction catalyzed by MGL.⁴⁷ It was clear by ^{19}F NMR studies that both DFM and TFM were cleaved to produce a fluoride ion by MGL (and the model system), but no intermediates were detected, indicating that further breakdown is rapid. Bioorganic model studies on this cleavage reaction employed a pyridoxal analog under controlled conditions. This approach allowed for successful trapping of the highly reactive difluorothiophosgene produced from TFM cleavage, and thioformyl fluoride produced from DFM cleavage (**Scheme 5**). A suggested proposal for the toxicity of TFM was the protein crosslinking capability of the difluorothiophosgene product. However, the thioformyl fluoride produced by enzymatic processing of DFM should be a potent acylating agent but not likely a crosslinking agent. It was found that DFM is also active against intact *T. vaginalis* G3, indicating that, at least for DFM, cytotoxicity is likely due to a mechanism other than the crosslinking of proteins. It was also determined that although DFM and TFM were not cytotoxic to *E. coli* itself, the expression of the *T. vaginalis* MGL in *E. coli*, followed by challenging the organism with DFM/TFM, did result in *E. coli* cytotoxicity. Since DFM was

cytotoxic in our studies, this information should open up a new direction for the application of methionine analogs as antiprotozoal agents since it appears unnecessary for the analog to have three fluorines attached to the thiomethyl group. Structure-activity studies with analogs of DFM having the hydrogen atom on the difluoromethyl moiety replaced by other substituents may result in the discovery of compounds having greater selectivity and potency compared to TFM/DFM.

Thiostrepton: Analogs and Resistance Mechanisms

Bacterial drug resistance is a critical area of current research. The developing failure of key antibiotics in the clinic has resulted in intense research programs to develop new antibiotics as well as to understand bacterial resistance mechanisms.⁴⁸⁻⁴⁹ One aspect that we have pursued in this area is the attempt to further understand the resistance mechanisms that are involved by antibiotic-producing microorganisms. An interesting sulfur-containing antibiotic, thiostrepton, has been the focus of some of our recent research (**Figure 7**). Thiostrepton is the paradigm for

the thiopeptide class of antibiotics.⁵⁰ This molecule, produced by certain strains of *Streptomyces*, inhibits Gram +ve microorganisms and has also been shown to exhibit anticancer and antimalarial activity. Thiostrepton halts protein biosynthesis by binding to the bacterial ribosome at the GTPase center on the 50S ribosomal subunit through interactions with the 23S rRNA and ribosomal protein L11. This interaction arrests protein biosynthesis at the translocation step of the elongation cycle. However the antibiotic suffers from low aqueous solubility. Our group initially focused on computational modeling of this large (MW 1665 Daltons) antibiotic.⁵¹ Subsequent semi-synthetic strategies to maintain the antibacterial activities of thiostrepton but enhance its water solubility were undertaken.⁵² Selective chemical derivatization of the reactive dehydroalanine groups of the thiostrepton tail section with substituted thiols permitted the

synthesis of several compounds that maintained protein biosynthesis inhibition as well as activity against several Gram +ve microorganisms, yet enhanced water solubility.

In addition, biochemical studies were undertaken to explore the protein structure and function of the *Streptomyces azureus* 23S rRNA methyltransferase, which confers thiostrepton resistance to this thiostrepton-producing organism. This enzyme transfers the methyl group of AdoMet to the adenosine 1067 ribose 2'-OH. This blocks the binding of thiostrepton to its binding pocket formed between ribosomal protein L11 and a section of the 23S rRNA. In collaboration with Dr. Graeme Conn and colleagues at Emory University, the three-dimensional structure of this methyltransferase was solved (**Figure 8**).⁵³ The protein structure is interesting in

that the homodimeric structure contains two AdoMet cofactors, one in each subunit, and the interface between the two subunits is likely the binding area for the ribosomal RNA section which is the substrate for the methylation reaction. Interesting, a deep trefoil protein knot is present in the C-terminus of each subunit and this contributes to the AdoMet binding site. Follow up site-directed mutagenesis experiments have been undertaken on key active site residues to

explore the various contributions of amino acid side chains to rRNA and AdoMet binding and catalysis.

Not all *Streptomyces* synthesize thiostrepton nor have the thiostrepton-resistance methyltransferase. However these strains are still protected from the antibacterial activity of thiostrepton by a unique thiostrepton-binding protein, termed TipA.⁵⁴ This binding protein interacts with thiostrepton and forms a covalent bond between cysteine 214 in TipA and a dehydroalanine in the tail region of the antibiotic. Adduct formation leads to interaction of the complex with *Streptomyces* DNA and enhanced gene expression, an interaction which results in increased production of TipAS, the thiostrepton-binding portion of TipA. These *Streptomyces* become resistant to the effects of the antibiotic as thiostrepton becomes sequestered in the cell. Additional roles for this complex in *Streptomyces* cellular physiology may also occur. Our group has further investigated the thiostrepton-TipAS interaction with several semi-synthetic analogs of thiostrepton as well as by site-directed mutagenesis experiments focused on TipAS.⁵⁵ These studies have improved our understanding of antibiotic resistance mechanisms at the molecular level for this class of antibiotic-producing organisms.

Other Biological Systems Involving the Carbon-Sulfur Bond

Our interest in the carbon-sulfur bond also extended into the biological chemistry of several intracellular thiols⁵⁶ such as ovothiol, ergothionine,⁵⁷ mycothiol⁵⁸ (**Figure 9**) and glutathione. In the case of glutathione, the metalloenzyme Glyoxalase I,⁵⁹ part of the Glyoxalase

I/II detoxification enzyme system (**Scheme 6**) which utilizes glutathione to detoxify methylglyoxal, was investigated. Our discovery of the first Ni²⁺-activated Glyoxalase I in

Nature, detected and fully characterized from *E. coli* (**Figure 10**), led to a series of enzymological and structural studies.⁶⁰⁻⁶³ These investigations explored the structural basis for

the metal activation profile of this enzyme, and the observation that Nature can maintain a catalytic efficient active site regardless of the orientational arrangement of adjacent protein subunits. Further studies on the diverse nature of Glyoxalase I enzymes from *Pseudomonas aeruginosa*, an organism which contains three different Glyoxalase I enzymes, led to studies on shifting the metal activation profile of a Zn^{2+} -activated class Glyoxalase I to a Ni^{2+} -activated class of enzyme.⁶⁴⁻⁶⁶ A key component of this work was the identification of a peptide insert that plays a dominant role in the metal-activation profile of these enzymes. These findings have added to a better understanding of metal selectivity in metalloenzymes, and the potential of targeting the Ni^{2+} -activation class of enzymes with inhibitors. Of further interest is that the Glyoxalase I protein fold is also found in several antibiotic resistance proteins. For example, the bleomycin resistance protein from *Streptoalloteichus hindustanus*,⁶⁷ the fosfomycin resistance proteins (Fos A, B and C),⁶⁸ the mitomycin C resistance protein⁶⁹ produced by *Streptomyces lavendulae* and the thiocoraline peptide binding protein⁷⁰ produced by strains of *Micromonospora* have high structural similarity to Glyoxalase I. Hence protein structural studies on Glyoxalase I are leading to new insight into several antibiotic resistance proteins and their

common characteristics. Clearly the “simple” investigation of the biological chemistry of the carbon sulfur bond has led to numerous investigations that impact our knowledge of cellular function and health. It has been a pleasure to explore this chemical space with my students over the years. Carbon-sulfur biological chemistry will continue to be an active area of research far into the future.

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Acknowledgements

This article is based on the Bernard Belleau Award lecture presented on June 4, 2014. The research outlined herein would not have been possible without the wonderful contributions from past and present students, postdoctoral fellows, research associates and collaborators. Their enthusiasm, dedication and insights are gratefully acknowledged. The Natural Sciences and Engineering Research Council of Canada (NSERC) and the University of Waterloo are also gratefully acknowledged for financial support.

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Figure Legends:

Figure 1. Ribbon representation of the lytic lysozyme from bacteriophage lambda showing the positions of the three methionine residues (ball-and-stick) in the enzyme. (PDB 1D9U)

Figure 2. Ribbon representation of *P. aeruginosa* azurin showing the copper ion and metal ligands in the active site. Red color is the intein ligated peptide that contains the copper metal binding residues Cys112, His117 and Met121. Replacement of Met121 with unnatural methionine analogs was accomplished. (PDB 4AZU)

Figure 3. Superposition of the active site structures of the *E. coli* methionyl-tRNA synthetase complexed with either L-DFM (red) or with L-TFM (cyan) with L-DFM and L-TFM colored by element in ball-and-stick structure. Note differences in residues Tyr15, Trp253 and Phe300 and their packing differences for each structure (PDB 1PFV, 1PFW).

Figure 4. Overall reaction catalyzed by methionyl-tRNA synthetase. The chemical structures of two synthetic sulphamoyl adenosine analogs that resemble the methionine adenylylate are shown.

Figure 5. Active site structure of the *E. coli* methionyl-tRNA synthetase complexed with the methionyl sulphamoyl adenosine inhibitor (ball-and-stick) and residues Tyr15, Trp253 and Phe300 presented in stick style. (PDB 1PFY)

Figure 6. Chemical structures of metronidazole and tinidazole.

Figure 7. Chemical structure of thiostrepton.

Figure 8. Ribbon representation of the *Streptomyces azureus* thiostrepton resistance 23S rRNA methyltransferase complexed with *S*-adenosyl-L-methionine. The two subunits are colored in red and blue. The protein trefoil knot in one of the subunits, corresponding to residues 191-268, is colored in green and the two AdoMet cofactors are shown in ball-and-stick style. (PDB 3GYQ)

Figure 9. Chemical structures of ergothioneine, ovothiol and mycothiol.

Figure 10. Ribbon representation of *E. coli* Glyoxalse I. The two subunits of the enzyme are colored blue and red. Active site hexacoordinate Ni^{2+} is shown as a green sphere with His5, Glu56 contributed from one subunit and His74, Glu122 from the second subunit. Two water molecules shown as red spheres complete the active site metal coordination. (PDB 1F9Z)

Scheme Legends:

Scheme 1. Overall pathway for the incorporation of L-methionine into proteins and its post-translational modification.

Scheme 2. Chemical synthesis of fluorinated methionine analogs.

Scheme 3. Overview of L-methionine biochemistry.

Scheme 4. Chemical mechanism of the enzyme methionine- γ -lyase.

Scheme 5. Potential reaction products generated by reaction of fluorinated methionine analogs with methionine- γ -lyase.

Scheme 6. Overall reaction pathway for methylglyoxal with the Glyoxalase I and II enzyme system.