Investigation of Bioisosteric Effects on the Interaction of Substrates/ Inhibitors with the Methionyl-tRNA Synthetase from *Escherichia coli*

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Abstract: Aminoacyl-tRNA synthetases catalyze the stepwise coupling of specific amino acid substrates to their cognate tRNAs. The first intermediate formed in this process is the aminoacyl-adenylate, which then subsequently reacts with the 3′-terminus of the cognate tRNA to transfer the amino acid to the tRNA. This overall reaction is critical for protein biosynthesis and is quintessential to the viability of all organisms. Therefore, the selective inhibition of bacterial amino acid-tRNA synthetases is the focus of intense current interest for the development of novel antibacterial agents. In order to elucidate some of the critical factors involved in recognition and binding of potential inhibitors to these bacterial systems, the current report has focused on the methionyl-tRNA synthetase from *Escherichia coli*. This enzyme has been studied with two sets of bioisosteric replacements in the methionine and methionyl-adenylate structures. Replacements of the carboxyl group of methionine with the phosphinic and phosphonic acid moieties were used to probe the effects of including potential transition state analogs on enzyme inhibition. The contributions of the aminoacyl-adenylate structure and the effect that fluorination has on inhibitory activity were investigated utilizing 5'-*O*-[(L-methionyl)-sulfamoyl]adenosine and 5'-*O*-[(*S*-trifluoromethyl-L-homocysteinyl)-sulfamoyl]adenosine. The K_i values for these compounds were determined to be 0.4 mM, 1.2 mM, 0.25 nM and 2.4 nM respectively. A discussion of this data in relation to structural information provided by the recent determination of the three-dimensional structures of the *E. coli* enzyme with several of these compounds is presented.

Key Words: Methionine, methionyl-tRNA synthetase, methionyl-adenylate, inhibitors, fluorine, trifluoromethionine.

INTRODUCTION

The increasingly widespread occurrence of antibiotic resistance in clinically important bacteria has necessitated the search for new enzyme targets for the design of anti-microbial agents [1-6]. Bacterial aminoacyl-tRNA synthetases (aaRSs) have emerged as promising antibiotic targets due to their importance in protein biosynthesis [7]. Notably, pseudomonic acid A (mupirocin), a natural product isolated from *Pseudomonas fluorescens*, potently and specifically inhibits bacterial isoleucyl-tRNA synthetases with minimal inhibitory effects on eukaryotic enzymes [8,9]. This compound is among the most widely used topical antibiotics worldwide, marketed under the trade name Bactroban® (GlaxoSmithKline, UK) [10]. Not surprisingly, several cases of resistance to pseudomonic acid A have been reported [11-14], and the isolation, as well as the design of inhibitors specific for other aminoacyl-tRNA synthetases has become especially pertinent [15-29].

In an attempt to expand our understanding of the key interactions involved in substrate recognition and catalysis in aminoacyl-tRNA synthetases, we have prepared a series of candidate inhibitors for *Escherichia coli* methionyl-tRNA synthetase (MetRS). As is the case for all known aminoacyl-tRNA synthetases, the MetRS-catalyzed linkage of Met to its cognate tRNA molecule proceeds through a two-step sequence (Scheme **1**). In the first step, the carboxyl group is activated by condensation with ATP, releasing inorganic pyrophosphate and generating an unstable methionyl-adenylate intermediate (**1**), which remains tightly associated with the enzyme. The $tRNA^{Met}$ then associates with the enzyme, and the adenylate moiety is displaced by the tRNA 3´-hydroxyl group to yield the aminoacyl- tRNA.

Although a number of MetRS inhibitors have been developed, these compounds often bear little similarity to the normal reaction components and provide little information regarding the key structural features necessary for binding and recognition of natural substrates or the transition state [30-35]. To address this deficiency, we have prepared a series of inhibitors which resemble intermediates formed during the course of the reaction. Specific bioisosteres were incorporated into these compounds, including 1-amino-3-(methylmercapto)propylphosphinic acid and 1-amino-3-(methylmercapto)propylphosphonic acid, the phosphinic and phosphonic acid analogs of methionine, (MetI, **2**, and MetP, **3**, respectively) to mimic the tetrahedral intermediate formed on attack of the 3'-hydroxyl group on the carboxyl group of the reactive aminoacyl adenylate intermediate. Also investigated was a stable sulfonamide linkage (**4**) in place of the labile phosphate-carboxylate anhydride of this intermediate, and a trifluoromethyl group in the methionine side chain of the aminoacyl-adenylate (**5**) in place of the normal methyl group, as a possible strategy to increase the strength of

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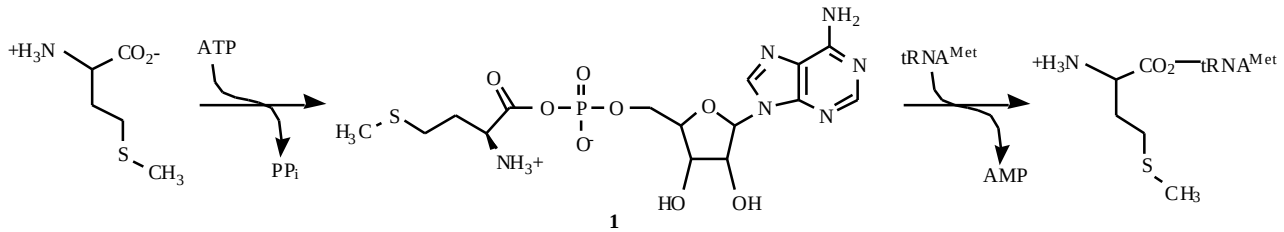


Scheme 1.

hydrophobic interactions between the enzyme and ligand. We have recently reported the crystal structure of *E. coli* MetRS in complex with several of these compounds, which has provided detailed information regarding the types of interactions responsible for substrate recognition and transition state stabilization [36]. In this report, we further characterize these interactions by examining the influence of the isosteric substitutions on the binding affinity of the compounds for the enzyme through detailed inhibition experiments. A thorough understanding of the molecular interactions which occur in this system should contribute substantial insight into the necessary structural features required for the design of effective, selective, and therapeutically viable antibiotics based on MetRS inhibition.

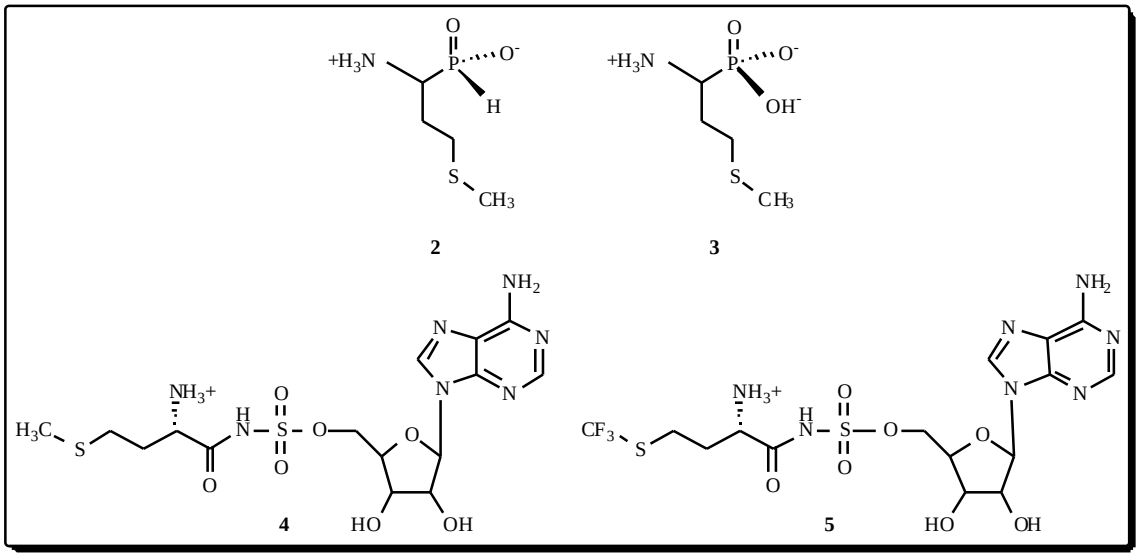
MATERIALS AND METHODS

Materials

Pwo DNA polymerase was obtained from Amersham Biosciences (Uppsala, Sweden). Boc-L-methionine, inorganic pyrophosphate, and bovine serum albumin were obtained from Sigma (St. Louis, MO). Reagent grade solvents were used throughout the course of this work. Anhydrous ethylene glycol dimethyl ether (DME) was obtained by distillation over sodium metal and benzophenone. Anhydrous *N*,*N*-dimethylformamide (DMF) was obtained by drying over 4 Å molecular sieves, followed by distillation under vacuum. The following chemicals were acquired from the Aldrich Chemical Company, Inc.: chlorosulfonyl isocyanate, 1,8-diazabicyclo[5.4.0]undec-7-ene, di-*tert*-butyl dicarbonate, 1,3-diisopropylcarbodiimide, *N*,*N*-diisopropylethylamine, *N*-hydroxysuccinimide, 2',3'-isopropylideneadenosine, sodium hydride (60% dispersion in oil). Sodium bisulfite and 1,3-dicyclohexylcarbodiimide were from Baker, Inc. (Phillipsburgh, NJ). Ammonium chloride, benzophenone, potassium hydroxide, sodium metal, and triethylamine were from BDH *via* VWR Canada (Mississauga, ON, Canada). Deuterated solvents and tetramethylsilane (TMS) were obtained from Cambridge Isotope Labs., USA. Anhydrous magnesium sulfate was purchased from Fisher Scientific (Nepean, ON, Canada). L-Trifluoromethionine (TFM) was prepared using previously reported procedures [37,38]. The methionine analogues, racemic 1-amino-3-(methylmercapto)propylphosphonic acid (MetP) and racemic 1-amino-3-(methylmercapto) propylphosphinic acid (MetI), were prepared according to the methods of Kudzin and Stec, and Baylis and co-workers, respectively [39,40]. Sulfamoyl chloride was prepared from chlorosulfonyl isocyanate and formic acid as described by Appel and Berger [41] and recrystallized from anhydrous dichloromethane. $Na_2{}^{32}P_2O_7$ ([^{32}P]-PP_i, 54.54 Ci/mM) was obtained from Perkin Elmer Life and Analytical Sciences (Boston, MA, USA). All other chemicals and reagents were of suitable purity for the applications discussed.

General Methods

Solvent evaporation was carried out under reduced pressure (Wheaton rotary evaporator). Aqueous solutions of



compounds were frozen and sublimed on a lyophilizer under reduced pressure. Merck silica gel plates were used for analytical thin layer chromatography analysis (aluminum backed, 0.2 mm layer of Kieselgel $60F_{254}$). Column chromatography was performed using 70-230 mesh silica gel, Merck 60 Aldrich cellulose, and Sephadex LH-20. HPLC separations were performed using a reversed phase μ-Bondapak C_{18} column (25 mm × 10 cm) (Waters Inc., USA). Compounds were detected by monitoring absorbance at 258 nm. Melting points were obtained on a Mel-Temp melting point apparatus and were uncorrected. Fourier transform infrared spectra were recorded on a Perkin-Elmer 1600 FT-IR in $CHCl_3$. Proton (^1H) and carbon (^{13}C) magnetic resonance spectra were obtained on Bruker AC-200, AM-250 or AM-300 spectrometers. Chemical shifts were reported downfield from tetramethylsilane (TMS) ($\delta = 0$) for ^1H NMR in $CDCl_3$ solution and (3-trimethylsilyl)-1-propanesulfonic acid sodium salt (TSP) for samples in D_2O. For ^{13}C NMR spectra, chemical shifts were reported relative to the central $CDCl_3$ resonance ($\delta = 77.0$). ^{19}F NMR spectra were recorded on a Bruker AC-200 spectrometer operating at 188.0 MHz. Chemical shifts are reported downfield relative to $CFCl_3$ ($\delta = 0.0$) using trifluoroacetic acid (TFA) as an external standard ($\delta = -76.53$). Mass spectra were recorded using electrospray mass spectrometry on a Fisons Instruments VG Quattro II. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis was performed on a Pharmacia Phast System electrophoresis apparatus, using precast gels obtained from Pharmacia (8-15% Gradient SDS-PAGE) and protein bands were visualized using Coomassie brilliant blue R-250. Fast protein liquid chromatography (FPLC) was carried out using a Pharmacia integrated FPLC system. Q-Sepharose anion exchange resin and Phenyl-Superose HR 10/10 column were obtained from Pharmacia, and CHT-II EconoPac hydroxyapatite cartridge (5 ml bed volume) was from Bio-Rad (Hercules, CA). A Beckman LS5000 TD liquid scintillation counter was used for analysis of radioactive samples, with Fisher Scintisafe Gel employed as the scintillation cocktail (Fisher Scientific, Nepean, ON). Samples were counted for 1 min each with a counting window of 1000 channels.

Synthesis

N-Boc-L-methionine Succinimide Ester (11)

This compound was prepared by modification of the method of Anderson and co-workers [42]. *N*-Boc-L-methionine (780 mg, 3.1 mmol) and *N*-hydroxysuccinimide (402 mg, 3.5 mmol) were dissolved in DME (5 ml). Dicyclohexylcarbodiimide (712 mg, 3.5 mmol) was then added at 0°C, and the mixture was stirred for 16 h at 5°C. The cloudy white suspension was then filtered and the dicyclohexylurea residue was washed with methanol (50 ml). The filtrate was concentrated and dried under vacuum. The residue was purified by silica gel chromatography (70:30 CH_2Cl_2/EtOAc) to give a white powder (540 mg, 51%).

$[\alpha]^{25}_D$ –22.4° (c 2.0, dioxane) (lit. –20.9°) [42], mp 128.5-129°C (lit. 128-129°C) [42]; R_f 0.83 (9:1 $CHCl_3$/ MeOH); ^1H NMR (300 MHz, $CDCl_3$) δ 5.21 (bs, 1H, N*H*), 4.79-4.87 (m, 1H, C*H*α), 2.88 (s, 4H), 2.62-2.68 (m, 2H, C$H_2\gamma$), 2.15-2.32 (m, 2H, C$H_2\beta$), 2.09 (s, 3H, SCH_3), 1.46 [s, 9H, C(CH_3)$_3$]; ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.5 (*C*=O), 168.1 (*C*=O), 154.7 (*C*=O), 80.6 [(*C*(CH$_3$)$_3$], 51.2 (*C*Hα), 32.1 (*C*H$_2\beta$), 29.5 (*C*H$_2\gamma$), 28.1 [C(*C*H$_3$)$_3$], 25.5 (*C*H$_2$), 15.3 (S*C*H$_3$); IR ($CHCl_3$) 1790 cm^{-1} (C=O), 1745 cm^{-1} (C=O), 1716 cm^{-1} (C=O).

2',3'-O-Isopropylidene-5'-O-sulfamoyladenosine (10)

Prepared by the method of Heacock and co-workers [43] with modification. Into anhydrous DME (100 ml) was dissolved 2', 3'-isopropylidene adenosine (9) (1.0 g, 3.3 mmol) under an argon atmosphere. Sodium hydride (196 mg of 60% dispersion in oil, 4.9 mmol) was added, and the mixture was stirred for 30 min. A solution of sulfamoyl chloride (565 mg, 4.9 mmol) in DME (30 ml) was added over 10 min, and the reaction was stirred for 20 h at room temperature. Methanol (10 ml) was added slowly and the mixture was stirred for 15 min. The solvents were removed *in vacuo.* The resulting colorless oil was purified on a silica gel column, eluting with EtOAc/MeOH 15:1. Upon removal of the solvents, a white foam was obtained that was further purified by dissolution in 2% methanol in ethyl acetate and precipitating with hexanes to give a white powder (550 mg, 47%).

R_f 0.32 (9:1 $CHCl_3$/MeOH); mp (194-196°C), lit (200-203°C) [43]; ^1H NMR (250 MHz, CD_3OD) δ 8.26 (s, 1H, H-2), 8.22 (s, 1H, H-8), 6.24 (d, 1H, *J* = 2.6 Hz, H-1'), 5.42 (dd, 1H, *J* = 6.2 Hz, 2.6 Hz, H-2'), 5.13 (dd, 1H, *J* = 6.2 Hz, 3.1 Hz, H-3'), 4.51 (m, 1H, H-4'), 4.22-4.32 (m, 2H, H_2-5'), 1.61 (s, C-CH_3), 1.39 (s, C-CH_3); ^{13}C NMR (62.8 MHz, CD_3OD) δ 157.2 (C-6), 154.0 (C-2), 150.3 (C-4), 141.5 (C-8), 120.2 (C-5), 115.6 (*C*(CH$_3$)$_2$, 91.8 (C-1'), 85.7 (C-2'), 85.4 (C-3'), 82.9 (C-4'), 69.9 (C-5'), 27.4 (*C*H$_3$), 25.4 (*C*H$_3$); ESMS (CH_3CN/ H_2O 1:1) *m/z* [387.15 (M + H); {calcd. for $C_{13}H_{18}N_6O_6S + H^+$} 387.10].

2',3'-O-Isopropylidene-5'-O-[(N-Boc-L-methionyl)-sulfamoyl]adenosine (13)

Compound **10** (377 mg, 0.98 mmol) was dissolved into anhydrous DMF (10 ml). *N*-Boc-methionine succinimide ester (**11**) (368 mg, 1.18 mmol) and 1,8-diazabicyclo [5.4.0]undec-7-ene (DBU) (0.36 ml, 2.4 mmol) were then added. The mixture was stirred at room temperature (2 h) under argon. Dimethylformamide was then removed *in vacuo* by repeated evaporation in the presence of toluene. The resulting viscous oil was purified by silica gel chromatography (elution with EtOAc/MeOH 12:1). The resulting residue was then chromatographed on a Sephadex LH-20 column with 70:30 MeOH/H_20 to yield a white powder (432 mg, 77%).

R_f 0.25 (EtOAc/ MeOH 10:1); mp 108-112°C; ^1H NMR (250 MHz, CD_3OD) δ 8.38 (s, 1H, H-2), 8.22 (s, 1H, H-8), 6.23 (d, 1H, *J* = 2.9 Hz, H-1'), 5.37 (dd, 1H, *J* = 6.1 Hz, 2.8 Hz, H-2'), 5.11 (dd, 1H, *J* = 6.1 Hz, 2.4 Hz, H-3'), 4.53 (m, 1H, H-4'), 4.28 (m, 2H, H_2-5') 4.10 (t, *J* = 8.2 Hz, C*H*α), 2.48 (t, 2H, *J* = 7.1 Hz, C$H_2\gamma$), 2.07 (m, 1H C$H_2\beta$), 2.01 (s, S-CH_3), 1.85 (m, 1H, C$H_2\beta$), 1.59 (s, 3H, C-CH_3), 1.40 [s, 9H, C-(CH_3)$_3$], 1.37 (s, 3H, C-CH_3); ^{13}C NMR (62.5 MHz,) δ 172.1 (*C*=O), 167.4 (*C*=O), 157.2 (C-6), 154.0 (C-2), 150.3 (C-4), 141.5 (C-8), 120.2 (C-5), 115.4 [*C*(CH$_3$)$_2$], 91.6 (C-

1'), 85.5 (C-2'), 83.0 (C-3'), 80.2 (C-4'), 69.9 (C-5'), 57.5 (*C*Hα), 33.8 (*C*H_2β), 31.2 (*C*H_2γ), 28.7 [C(*C*H_3)$_3$], 27.4 (*C*H_3), 25.5 (*C*H_3), 15.3 (S*C*H_3); FAB HRMS *m/z* [618. 20193 (M + H); {calcd for $C_{23}H_{36}N_7O_9S_2 + H^+$} 618.20160].

5'-O-[(L-Methionyl)-sulfamoyl]adenosine (MetSA) (4)

Compound **13** (390 mg, 0.62 mmol) was dissolved in a mixture of trifluoroacetic acid and water (8:1 TFA/ H_2O (2 ml)), and the solution was stirred for 2 h at ambient temperature. Solvents were removed *in vacuo* and the residue was co-evaporated four times with ethanol. The resulting product was dissolved in water and chromatographed on a Sephadex LH-20 column (elution with MeOH/ H_2O (7:3)) to yield a white powder (65 mg, 22%).

mp 153-154°C; ^{1}H NMR (250 MHz, D_2O) δ 8.18 (s, 1H, H-2), 8.00 (s, 1H, H-8), 5.89 (d, 1H, *J* = 5.3 Hz, H-1'), 4.54 (t, 1H, *J* = 5.3 Hz, H-2'), 4.31 (t, 1H, *J* = 4.0 Hz, H-3'), 4.21 (m, 3H, H-4' and H-5'), 3.70 (t, 1H, *J* = 6.1 Hz, C*H*α), 2.31 (t, 2H, *J* = 7.5 Hz, C*H*$_2$γ), 1.89 (m, 2H C*H*$_2$β), 1.80 (s, S-C*H*$_3$); ^{13}C NMR (62.5 MHz, DMSO) δ 172.6 (*C*=O), 158.1 (C-6), 156.5 (C-2), 149.2 (C-4), 139.3 (C-8), 119.4 (C-5), 93.2 (C-1'), 83.3 (C-2'), 83.2 (C-3'), 75.4 (C-4'), 69.9 (C-5'), 58.1 (*C*Hα), 30.7 (*C*H_2β), 25.1 (*C*H_2γ), 14.3 (S*C*H_3); ESMS (CH_3CN/ H_2O 1:1) [478.06 (M + H); {calcd. for $C_{15}H_{24}N_7O_7S_2 + H^+$} 478.117].

N-Boc-S-trifluoromethyl-L-homocysteine

L-Trifluoromethionine (609 mg, 3 mmol) was dissolved in THF/ H_2O (1:1 60 ml) at 0°C. Di-*tert*-butyl dicarbonate (720 mg, 3.3 mmol) was added followed by a dropwise addition of triethylamine (0.46 ml, 3.3 mmol) over 30 min. The solution was warmed to room temperature and stirred for 20 h. The solution was washed with hexanes (2 x 50 ml) and acidified to pH 2 with HCl (2 N) at 0°C. The aqueous solution was then extracted with EtOAc (3 x 100 ml). The organic extracts were combined and dried over $MgSO_4$. Removal of the solvents *in vacuo* gave the product as a clear yellow oil (879 mg, 97%).

$[\alpha]^{25}_D$ 16.2° (c 2.0, $CHCl_3$); R_f 0.39 (9:1 $CHCl_3$/MeOH); ^{1}H NMR (250 MHz, $CDCl_3$) δ 11.57 (bs, 1H, COO*H*), 7.22 (bd, 1H, NH, *J* = 6.2 Hz), 4.30-4.40 (m, 1H, C*H*α), 2.98 (t, 2H, *J* = 7.2 Hz, C*H*$_2$γ), 2.04-2.34 (m, 2H, C*H*$_2$β), 1.47 [s, 9H, C-(C*H*$_3$)$_3$]; ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 174.8 (*C*=O), 157.0, (*C*=O), 130.8 (q, $J_{C\text{-}F}$ = 306 Hz, *C*F_3), 82.6 [*C*(CH$_3$)$_3$], 52.3 (*C*Hα), 33.4 (CH$_2$β), 28.1 [C(*C*H$_3$)$_3$], 25.9 (CH$_2$γ); ^{19}F NMR (188 MHz, $CDCl_3$) δ -41.8; IR ($CHCl_3$) 1720 cm^{-1} (C=O), 1659 cm^{-1} (C=O).

N-Boc-S-trifluoromethyl-L-homocysteine succinimide ester (12)

N-Boc-L-trifluoromethionine (210 mg, 0.7 mmol) and *N*-hydroxysuccinimide (82 mg, 0.7 mmol) were each dissolved into DME (7 ml). 1,3-Dicyclohexylcarbodiimide (90 mg, 0.7 mmol) was then added at 0°C and the mixture stirred for 24 h at 5°C. Dicyclohexyl urea was filtered off and washed with methanol (50 ml). The filtrate was concentrated and dried under vacuum. The product was purified by silica gel chromatography (CH_2Cl_2) to give a white powder (188 mg, 67%).

$[\alpha]^{25}_D$ 8.3° (c 2.0, $CHCl_3$); R_f 0.84 (9:1 $CHCl_3$/MeOH); ^{1}H NMR (200 MHz, $CDCl_3$) δ 4.75-4.85 (m, 1H, C*H*α), 2.98-3.13 (m, 2H, C*H*$_2$γ), 2.88 (s, 4H), 2.31-2.49 (m, 1H, C*H*$_2$β), 2.15-30 (m, 1H, C*H*$_2$β), 1.47 (s, 9H, C-(C*H*$_3$)$_3$); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 175.3 (*C*=O), 172.3 (*C*=O), 157.0, (*C*=O), 130.8 (q, J_{CF} = 306 Hz, *C*F_3), 80.7 [*C*(CH$_3$)$_3$], 52.3 (*C*Hα), 33.0 (CH$_2$β), 28.1 [C(*C*H$_3$)$_3$], 25.3 (CH$_2$γ), 24.6 (2C, *C*H$_2$); ^{19}F NMR (188 MHz, $CDCl_3$) -41.5.

2',3'-O-Isopropylidene-5'-O-[(N-Boc-S-trifluoromethyl-L-homocysteinyl)-sulfamoyl]adenosine (14)

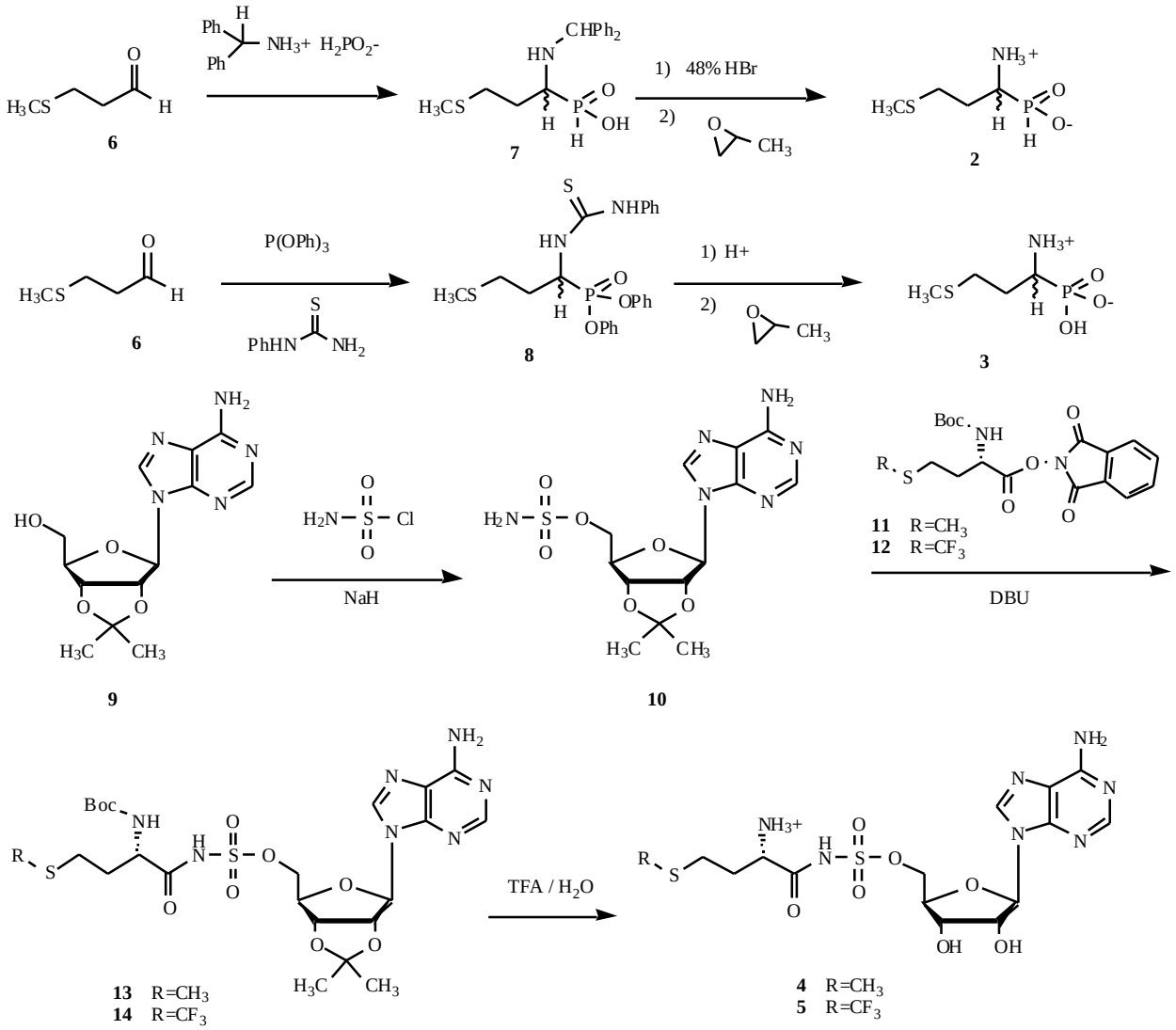
Into anhydrous DMF (5 ml) was dissolved compound **10** (65 mg, 0.17 mmol). *N*-Boc-trifluoromethionine succinimide ester **12** (68 mg, 0.17 mmol) and DBU (0.07 ml, 0.41 mmol) were then added. The mixture was stirred at room temperature for 20 h (argon). Dimethylformamide was removed *in vacuo* by repeated evaporation in the presence of toluene. The viscous oil was purified by silica gel chromatography (EtOAc/MeOH 95:5) and yielded a white powder which was recrystallized from EtOAc/ petroleum ether (52 mg, 46%).

R_f 0.47 (10:1 EtOAc/MeOH); ^{1}H NMR (200 MHz, DMSO) δ 8.37 (s, 1H, H-2), 8.29 (s, 1H, H-8), 6.64 (bs, 2H, NH_2), 6.26 (d, 1H, *J* = 2.3 Hz, H-1'), 5.94 (bd, 1H, NH, *J* = 7.6 Hz), 5.18 (dd, 1H, *J* = 6.0 Hz, 2.4 Hz, H-2'), 5.02 (dd, 1H, *J* = 5.9 Hz, 1.9 Hz, H-3'), 4.57 (d, 1H, *J* = 2.1Hz, H-4'), 4.12-4.30 (m 3H, H_2-5', C*H*α), 2.90-3.01 (m, 2H, C*H*$_2$γ), 2.06-2.23 (m, 1H, C*H*β), 1.85-2.02 (m, 1H, C*H*β), 1.61 (s, 3H, C*H*$_3$), 1.40 [s, 9H, C(C*H*$_3$)$_3$], 1.37, (s, 3H, C*H*$_3$); ^{13}C NMR (75.0 MHz, DMSO) δ 176.0 (*C*=O), 166.0 (*C*=O), 154.7 154.1, 151.7, 147.9, 129.9 (q, *J* = 306 Hz, *C*F_3), 117.8 (C-5), 112.3 [*C*(CH$_3$)$_2$], 88.8 (C-1'), 83.1 (C-2'), 83.0 (C-3'), 80.2 (C-4'), 77.2 [(*C*(CH$_3$)$_3$], 66.4 (C-5'), 54.3 (*C*Hα), 32.7 (CH$_2$β), 28.8 [C(*C*H$_3$)$_3$], 27.0 (*C*H$_2$γ); 22.2 (*C*H$_3$), 21.4 (*C*H$_3$); ^{19}F NMR δ (188 MHz, DMSO) δ -41.7; ESMS (CH_3CN/ H_2O 1:1) *m/z* [671.95 (M + 1); {calcd for $C_{23}H_{32}F_3N_7O_9S_2 + H^+$} 672.17].

5'-O-[(S-Trifluoromethyl-L-homocysteinyl)-sulfamoyl]adenosine (TFMSA) (5)

Compound **14** (50 mg, 0.07 mmol) was dissolved in 5:1 TFA/ H_2O (2 ml) at ambient temperature. Anisole (0.5 ml) was added and the mixture stirred for 2 h. The solvents were removed and the residue co-evaporated four times with ethanol. The product was then dissolved in water (4 ml) and washed with diethyl ether (2 × 5 ml). The aqueous layer was treated with decolorizing charcoal, filtered and concentrated *in vacuo*. The residue was purified by reversed phase HPLC (70:30 H_2O/ MeOH) (15 mg, 40%).

HPLC retention time: 20 min @ 4 ml/min; ^{1}H NMR (300 MHz, DMSO) δ 8.34 (s, 1H, H-2), 8.15 (s, 1H, H-8), 7.30 (bs, 2H, N*H*$_2$), 5.93 (d, 1H, *J* = 5.6 Hz, H-1'), 4.75 (t, 1H, *J* = 5.3 Hz, H-2'), 4.22 (t, 1H, *J* = 4.6 Hz, H-3'), 4.06-4.11 (m, 1H, H-4') 3.95 (m, 2H, H_2-5'), 3.60 (m, 1H, C*H*α), 2.98-3.04 (m, 2H, C*H*$_2$γ), 1.95-2.11 (m, 2H, C*H*$_2$β); ^{19}F NMR (188 MHz, DMSO) δ -41.1; ESMS (CH_3CN/ H_2O 1:1) *m/z* [531.83 (M + 1); {calcd for $C_{15}H_{20}F_3N_7O_7S_2 + H^+$} 532.08].



Scheme 2.

Cloning of the Gene Sequence for the 551-amino Acid Tryptic Fragment of *E. coli* MetRS

The gene sequence corresponding to the fully active MetRS tryptic fragment (residues 1-551) [44] was isolated from *E. coli* strain MG1655 genomic DNA using the polymerase chain reaction with the following primers:

Forward: 5' CCA GGT ACC CAT ATG ACT CAA GTC CGC AAG AAA ATT CTG G 3'

Reverse: 5' CAA GAA TTC GGA TCC TTA TTT TAC TTC TTC TTT AGA GGC 3'

Start (ATG) and stop (TTA) codons are underlined. The amplified fragment was treated with *Eco*RI and *Hin*dIII restriction endonucleases, combined with plasmid pUC18 (treated with *Eco*RI and *Hin*dIII) and ligated.

The *Nde*I restriction site which is found within the gene sequence was then removed by PCR mutagenesis to change the sequence from the normal CAT ATG to CA***C*** ATG, using the following primers (altered bases are highlighted with bold italics):

Forward: 5' ATC CAC CTC GGC CA***C*** ATG CTG GAG CAC ATC C 3'

Reverse: 5' G GAT GTG CTC CAG CAT ***G***TG GCC GAG GTG GAT 3'

The resulting plasmid (lacking the *Nde*I site in the MetRS gene) was treated with *Nde*I and *Bam*HI to generate the MetRS fragment which was purified and ligated with pET22b (Novagen) treated with *Bam*HI and *Nde*I. The resulting plasmid containing the 551-amino acid tryptic fragment of the MetRS gene was named pMTS3.

Production and Purification of MetRS Tryptic Fragment

E. coli strain BL21(λDE3) containing plasmid pMTS3 was grown aerobically at 37 ºC in Luria-Bertani (LB) medium containing 50 mg/l carbenicillin with shaking, and

allowed to reach an OD_{600} of 0.4-0.5. Isopropyl-β-D-thiogalactopyranoside (IPTG; 1 mM final concentration) was added to initiate protein production, and growth was continued for 1.5 additional hours. Cells were harvested by centrifugation at 10 000 x g and stored at -80 ºC.

For cell disruption, the frozen cells (approximately 0.8 g) were resuspended in 15-25 ml 10 mM Tris-HCl, pH 8.0, containing 10% glycerol and 10 mM β-mercaptoethanol (buffer A). Phenyl methanesulfonyl fluoride (PMSF, 2-3 mg) was added as a solid to the suspension. Cell disruption was achieved by sonication (8-10 rounds, 20 second bursts on continuous setting, intensity level 5, with 1 min cooling on ice between rounds). The cell debris was removed by centrifugation for 30 min at 48 000 x *g*. The supernatant was then filtered with a 0.22 μm syringe filter and loaded onto a Pharmacia Q-Sepharose fast flow anion exchange column (30 x 1 cm) at a flow rate of 1.5 ml/min. After the unretained protein had been eluted with buffer A, a 60 min gradient to buffer B (buffer A + 1.0 M sodium chloride) was applied at 1.5 ml/min throughout. Fractions containing MetRS eluted at approximately 0.5 M sodium chloride, and were identified using SDS-PAGE.

Pooled Q-Sepharose fractions were concentrated to < 10 ml using an Amicon stirred cell with a YM30 membrane (molecular weight cutoff 30 kDa). Ammonium sulfate was added to a final concentration of 1.0 M, and the protein solution was applied to a Pharmacia Phenyl-Superose column (10 x 1 cm) at a flow rate of 0.75 ml/min. Unretained protein was eluted from the column with buffer A (described above) containing 1.0 M ammonium sulfate. MetRS was eluted from the column by decreasing the ammonium sulfate concentration to zero over 90 min, maintaining the flow rate at 0.75 ml/min. Again, fractions containing MetRS, which eluted at approximately 0.7 M ammonium sulfate, were identified using SDS-PAGE as described above.

Phenyl-Superose MetRS fractions were dialyzed overnight against 5 mM potassium phosphate, pH 6.9, containing 10% glycerol and 10 mM β-mercaptoethanol (buffer A′) (2 × 1 l), using SpectraPor 7 dialysis tubing (Fisher Scientific). The protein was then applied to a Bio-Rad CHT-II hydroxyapatite column (5 ml bed volume) with a flow rate of 0.7 ml/min buffer A′, and eluted with a linear increase in potassium phosphate concentration to 200 mM over 90 min. Fractions containing MetRS, which eluted at approximately 80-100 mM potassium phosphate, were identified by SDS-PAGE and pooled. These fractions were subjected to buffer exchange and concentration using an Amicon stirred cell (YM30 membrane, 30 kDa MWCO) to give a final protein concentration, of approximately 1 mg/ml in 20 mM Tris-HCl, pH 7.0, containing 10 mM β-mercaptoethanol and 10% glycerol. Protein concentration was evaluated using the Bradford method [45].

Assay for MetRS Activity

The assay method used has been reported previously [46,47], and depends on the ability of the MetRS to catalyze the exchange of radiolabeled pyrophosphate into ATP in the absence of tRNA, but in the presence of the substrate methionine. The assay mixture consisted of 20 mM imidazole, pH 7.6; 2 mM ATP; 2 mM [^{32}P]-PP_i; 7 mM $MgCl_2$; 10 mM β-mercaptoethanol; 0.1 mg/ml BSA; 0.1 mM EDTA; and Met or desired analog at a range of concentrations, and was supplemented with [^{32}P]-sodium pyrophosphate to a specific activity of 4000-10 000 cpm/μl.

The total volume used for each assay was 600 μl. MetRS was added in a 15 μl aliquot to a final concentration of 1.3 nM, and the reaction was allowed to proceed at ambient temperature. At reaction times of 2, 4, 6, 8, and 10 min, 100 μl aliquots were removed and each aliquot was added to a 1 ml suspension of Norit decolourizing charcoal (1% w/v) in 300 mM sodium pyrophosphate (non-radioactive) containing 11% perchloric acid, which adsorbs radiolabeled ATP onto the charcoal. The ATP-charcoal was removed by suction filtration using Whatman GF/C glass fibre discs (2.5 cm diameter) and a Gooch funnel. The filtered material was washed first with 25 mM sodium pyrophosphate containing 1% perchloric acid (7 ml) and then with distilled water (7 ml). The filter discs were then placed in 20 ml scintillation vials, and Scintisafe Gel scintillation cocktail was added (5 ml). Additionally, for each assay mixture, a 50 μl aliquot was removed at the end of the reaction and placed directly into a scintillation vial containing 5 ml scintillation cocktail to serve as a reference standard. Identical procedures were utilized for kinetic studies on the inhibitors.

Graphical Analysis of Kinetic Data

The Grafit 3.01 software package (Erithacus Software) was used to determine the kinetic parameters K_m and V_{max}, through a non-linear regression fitting of the rate *vs*. substrate concentration curve (also referred to as the Michaelis-Menten curve). The simple, robust weighting option was employed. For each Michaelis-Menten curve, five substrate concentrations were employed and each experiment was performed in triplicate. In each case, an initial screening experiment was performed in which the substrate concentration was increased in ten-fold increments (0.01-100 mM) to determine an approximate K_m. In cases in which solubility limitations prevented the preparation of 100 mM samples, a maximum concentration of 50 mM was employed. Following this initial screening, a more refined concentration range was chosen for each substrate with concentrations above and below the expected K_m value. The K_m and k_{cat} determined for L-methionine were 10 μM and 10 s^{-1}, in very good agreement with previous reports [46-48].

In inhibition studies, two concentrations were used for each inhibitor to determine the K_i value. In all cases, the K_i values obtained from each of the two inhibitor concentrations were in good agreement with each other; as such, it was deemed that two concentrations were sufficient to obtain accurate K_i values. Dissociation constants for the binding of several of the inhibitors to MetRS as determined by fluorescence spectroscopy [36] were used as initial approximations of the inhibition constant. For each inhibitor concentration, a full Michaelis-Menten analysis by non-linear regression analysis was performed. Graphical methods were as described above.

Raw data in counts per minute (cpm) for each time point was converted to a concentration in millimolar, by dividing

each data point by the cpm value for the reference standard. Because the initial concentration of [^{32}P]-PPi in the sample was 2 mM, the cpm associated with a 100 µl aliquot can be defined as the amount corresponding to a ^{32}P concentration of 2 mM. Hence, a 50 µl aliquot corresponds to a concentration of 1 mM, and the ratio of the observed cpm for each data point to the reference 50 µl aliquot is the same as the ratio between the concentration of radiolabeled ATP in the sample and the 1 mM concentration of the standard.

Molecular Modelling

Gas phase potential energy scans of methyl 1-propyl sulfide and trifluoromethyl 1-propyl sulfide as models of methionine and trifluoromethionine, were accomplished using Jaguar 5.0 (Schrodinger , LLC, Portland, Oregon, 2002) at the B3LYP/6-31+G** level which includes the B3LYP hybrid functions and diffuse functions on all non-H atoms, and polarization functions on all atoms. DFT calculations utilized grids with medium point density. The starting structures of the two model compounds had fully extended trans geometries. Both the set of rigid (no geometry optimization), as well as relaxed (with geometry optimization) potential energy scans were calculated with the C1-S-C2-C3 dihedral angle being systematically varied from 0 (fully eclipsed) to 360 degrees in 20 degree intervals. The relative energies for each conformation (compared to the global minimum for each molecule) were then plotted for methyl 1-propyl sulfide and trifluoromethyl 1-propyl sulfide for the rigid (Fig. (**1a**)) as well as the relaxed (Fig. (**1b**)) scans.

RESULTS AND DISCUSSION

i) Inhibition of *E. coli* MetRS by Met phosphinate and Met phosphonate

During the course of the reaction catalyzed by MetRS, a transient tetrahedral intermediate is formed when the 3'-hydroxyl group of the incoming tRNA^{Met} attacks the carbonyl group of the activated methionyl adenylate intermediate. We attempted to exploit the potentially stronger binding interactions occurring between this "transition state-like" tetrahedral functionality and the enzyme by preparing Met

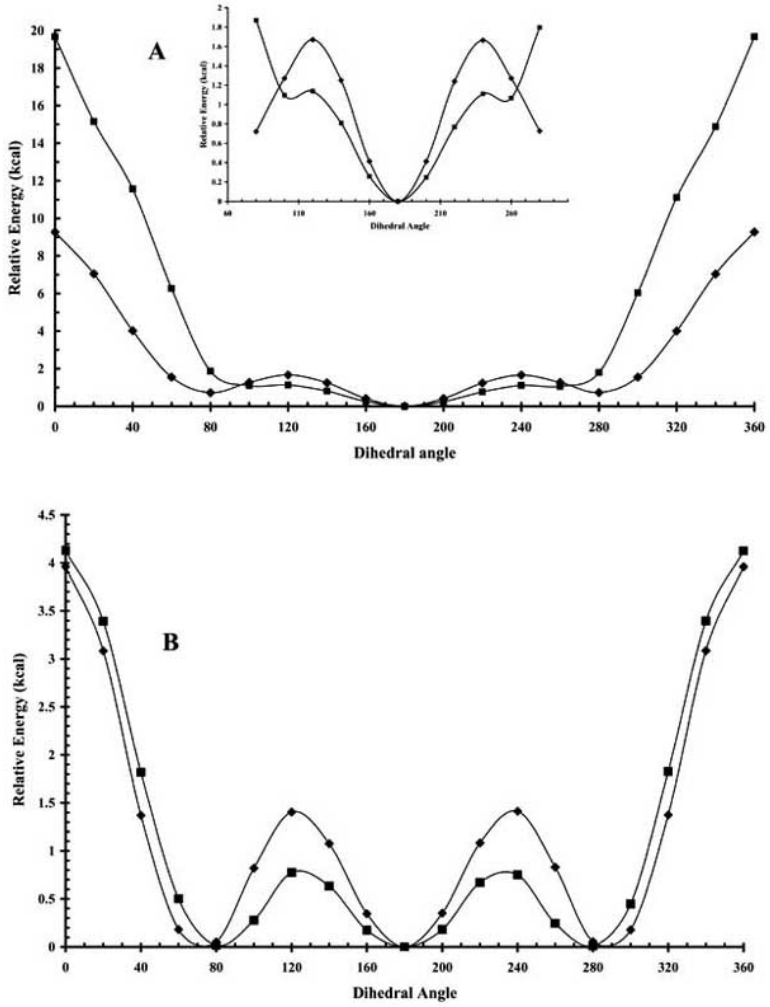


Fig. (1). Potential energy scans at the B3LYP/6-31+G** level for methyl 1-propyl sulfide (♦) and trifluoromethyl 1-propyl sulfide (■) using **A**) rigid and **B**) relaxed scans. Energies (kcal) are relative to the global minimum for each compound. Inset in **A** is expansion of region between 80-280 degrees.

analogs in which the carboxyl group is replaced with either a phosphinate or phosphonate group. Because of the tetrahedral arrangement of the oxygen atoms as well as the stable negative charge(s), compounds of this type have frequently been used to mimic similar intermediates in other enzymatic reactions [49].

Met phosphinate (**2**) and Met phosphonate (**3**) (scheme **2**) both inhibited the ATP-PP_i exchange reaction catalyzed by MetRS in a competitive fashion, with K_i values of 0.4 mM and 1.2 mM, respectively (Table **1**). The modest inhibition is consistent with results obtained for other inhibitors built upon the Met scaffold, and the value obtained for the phosphinate analog is in good agreement with the value of 0.6 mM previously obtained by Biryukov and co-workers [50]. The requirement for the adenosine portion of the reactive intermediate for tight binding is supported by previous experiments measuring the dissociation constant for **2** with MetRS by fluorescence titration, in which it was found that the dissociation constant decreased with the addition of adenosine to the mixture, and again, when both adenosine and inorganic pyrophosphate were added [36]. The binding affinity of **3** was not sufficiently strong to obtain a dissociation constant by fluorescence titration. This lowered affinity is reflected in the increased K_i value for this compound relative to **2**.

The crystallographic structures of MetRS in complex with **2** and **3** have revealed that both compounds bind to the enzyme in the Met recognition pocket and elicit the same conformational changes within this pocket that occur when Met binds, with one exception [36]. In the Met and Met phosphinate complexes, Tyr15 reorients such that the aromatic side chain forms a lid over the carboxyl or phosphinyl moiety, which presumably serves in protecting the labile phosphoric-carboxylic acid mixed anhydride from the solvent in the methionyl-adenylate intermediate. In the structure with bound **3**, however, electron density for the Tyr15 side chain is observed at positions corresponding to both the open, substrate-free conformation and the closed, bound conformation, apparently due to unfavourable steric interactions which occur between the larger phosphonate group and the Tyr15 side chain. These steric clashes are likely the basis for the weak affinities observed for the phosphinate and phosphonate, as manifested in the large K_i values.

ii) Inhibition by 5'-O-[(L-methionyl)-sulfamoyl]adenosine and 5'-O-[(S-trifluoromethyl-L-homocysteinyl)-sulfamoyl] adenosine

The methionyl adenylate intermediate formed during the course of the MetRS reaction has been shown to remain tightly associated with the enzyme, protecting the unstable anhydride linkage from solvolysis until the incoming tRNA molecule is able to associate and attack the activated carboxyl group [51]. This tight binding interaction has been used to advantage in the design of inhibitors by incorporating structural motifs resembling both the amino acid and nucleoside portions of the intermediate [31-33]. These multi-substrate/intermediate analogs are generally more potent inhibitors by several orders of magnitude, relative to compounds mimicking only one of the substrates.

An effective strategy for the design of inhibitors based on the Met~AMP intermediate scaffold has been the replacement of the labile anhydride linkage with a more stable connection, such as an ester, hydroxamate, or sulfonamide [31,52]. Based on the high similarity of the sulfonamide linkage to the anhydride, it was thought that this functionality would provide the most conservative replacement in the construction of potential inhibitors. Using this approach, we prepared two Met~AMP analogs containing sulfonamide linkages, one containing the normal Met portion (this compound was also prepared independently by Lee and co-workers [52]) and another in which the Met was replaced with L-trifluoromethione. This replacement was chosen to investigate two key aspects of the methionine recognition pocket. On one hand, the inclusion of the more hydrophobic trifluoromethyl group could lead to stronger Van der Waals' interactions with the non-polar residues in the side chain binding pocket. We have previously reported the enhanced chemotactic responses of fluorinated methionines such as L-trifluoromethionine, compared to non-fluorinated methionine incorporated into the chemotactic peptide fMet-Leu-Phe with human neutrophils [53]. Alternatively, the larger steric bulk of the trifluoromethyl group could lead to a decrease in binding affinity, due to

Table 1. Inhibition Constants for Methionine and Methionyl-adenylate Analogues in the *E. coli* MetRS ATP-PP_i Exchange Reaction. Kinetic Parameters for the Substrate, L-methionine, are Given for Reference

Compound	K_i (mM)
L-methionine	K_m: 10 μM; k_{cat}: 10 s^{-1}
2	0.4 ± 0.2
3	1.2 ± 0.4
4	0.25 ± 0.1 x 10^{-6}
5	2.4 ± 0.4 x 10^{-6}

unfavorable steric clashes between this moiety and the walls of the active site cavity. We have recently evaluated the abilities of difluoromethionine and trifluoromethionine to serve as substrates for MetRS, and found that the K_m values were highly dependent on the number of fluorines incorporated, with the K_m for difluoromethionine increased approximately 200-fold relative to Met, and the K_m for trifluoromethionine too high to be evaluated within the accessible concentration range (data not shown). However, we have successfully incorporated both of these compounds into recombinant proteins *in vivo* [38,54,55], and we sought to understand whether the decreased affinity of the fluorinated amino acid for the enzyme could be countered by the favorable interactions occurring between the enzyme and the adenosine portion of the reactive intermediate.

The inhibition constants obtained for the two Met~AMP sulfonamide-linked analogs (scheme **2**)in the ATP-PP_i exchange reaction were determined to be 0.25 nM and 2.4 nM for the methionine and trifluoromethionine derivatives respectively (Table **1**), indicating that both compounds are very potent inhibitors. Lee and co-workers also observed potent inhibition by the non-fluorinated analog, obtaining an IC_{50} value of ~ 7 nM (K_i was not determined) [52]. Interestingly, we saw only a 10-fold increase in the K_i for the fluorinated analogue relative to the non-fluorinated compound, whereas the increase in K_m for trifluoromethionine versus Met was in excess of 500-fold. These results suggest that the unfavorable contacts, which result in the dramatic increase in the K_m for trifluoromethionine, are largely paid for upon formation of the intermediate.

It is also important to evaluate the conformational preferences of the side chain of Met and TFM, as this will also play a role in the affinity of these compounds as substrates for MetRS, as well as their effectiveness as components of inhibitors such as MetSA and TFMSA. A set of high level potential energy scans (B3LYP/6-31+G**) were undertaken for the model systems methyl 1-propyl sulfide and trifluoromethyl 1-propyl sulfide, to determine if there are conformational preferences for the side chain of Met versus the side chain of TFM. Both rigid and relaxed scans are shown in Fig. (**1**). If the side chain of the amino acid is rigidified by the enzyme, there is a substantially larger energy cost for the larger TFM side chain to explore the eclipsed conformation (an energy difference of approximately 20 kcals between dihedral angles of 0 versus 180 degrees as seen for the TFM side chain versus a ~9 kcal difference for the corresponding conformations of the Met chain). As shown in the inset to Fig. (**1a**), higher energy minima occur at angles of ~105° and ~255° (at approximately 1 kcal above the global minimum) for the TFM side chain, but at ~80° and ~280° for the Met side chain (at approximately 0.7 kcal above the global minimum). The energy barriers are also different between the global minimum and the next higher energy minima for TFM and Met, being 1.14 kcals and 1.67 kcals respectively. If the potential energy scans are allowed to relax which allows for other portions of the molecule to optimize energy as the dihedral angle is frozen at a particular angle, the magnitude of the energy barriers for both side chain models are reduced (Fig. (**1b**)). The global minimum for both TFM and Met side chains are again found at 180°, however, the secondary minima, which are closer in energy to the global minima, are found at ~80°/280° for both model side chains. The energy barriers between these higher minima and the global minimum at 180° were calculated to be ~0.77 kcal and ~1.4 kcal for the TFM and the Met side chains respectively. Further evidence for the contribution of steric factors to the interaction of the fluorinated side chain with the binding pocket of MetRS can be seen in inspection of the crystal structures that have been determined with either methionine [56] or trifluoromethionine [36] bound to the enzyme. The dihedral angles (C-S-C-C) found for the side chains for Met and TFM in these two cases are 174° and 159° respectively. Hence, although the methionine side chain binds in its more stable extended structure, TFM requires a rotation of >20° from its global minimum of 180°, which produces a penalty in energy (based on the potential energy scans using a rigid approach) of ~ 0.3 kcals. The present set of calculations also indicates that the CH_3-S and the S-CH_2 bond lengths are altered upon fluorination, wherein the CH_3-S bond is predicted to be ~1.826Å versus a CF_3-S length of 1.813Å. The predicted bond lengths for the S-CH_2 bond are 1.837Å and 1.846Å for Met versus TFM respectively.

Examination of the recently reported crystal structures of MetRS in complex with Met, TFM, and MetSA, provides a possible rationale for the inhibition results obtained for the two sulfonamide compounds [36]. In the absence of Met, the side chain recognition pocket exists in a largely opened conformation. On binding of Met, however, several residues undergo reorientation to create favorable interactions between the enzyme and side chain, as well as forming new π-stacking interactions between pairs of aromatic residues which appear to stabilize this altered conformation. This transition appears to be a key step in the reaction pathway, triggering the reaction to proceed once the ATP is bound. In the case of trifluoromethionine, it appears that this closed conformation is unfavored, and the mobile residues exist exclusively in the open conformation in the MetRS-TFM structure. However, reactivity experiments with TFM and MetRS indicate that there is slow turnover with this compound, suggesting that the transition to the active conformation does in fact occur, albeit with reduced frequency (data not shown). Hence, it appears that the presence of the bulky trifluoromethyl group in the side chain creates an elevated energetic barrier, associated with assuming the activated conformation. Once this conformation is achieved and the reaction with ATP proceeds, the favorable interactions are maintained upon forming the intermediate. In the case of the TFMSA analog, these interactions between the enzyme and the adenosine portion apparently assist in lowering the thermodynamic barrier associated with the reorientation of the Met recognition residues around the larger TFM side chain. Fig. (**2**) presents a stereo view of a portion of the binding site region of the MetRS and MetSA complex, showing the binding pocket for the methionine portion of the inhibitor. As can be seen from the structure, the binding pocket for the CH_3S- moiety is very constrained and is formed by interaction with residues Leu13, Trp253, Ala256, Pro257, Tyr260 and His301. Increase in the steric size of the methyl group by replacement with the CF_3- group should result in some alteration in the arrangement of these

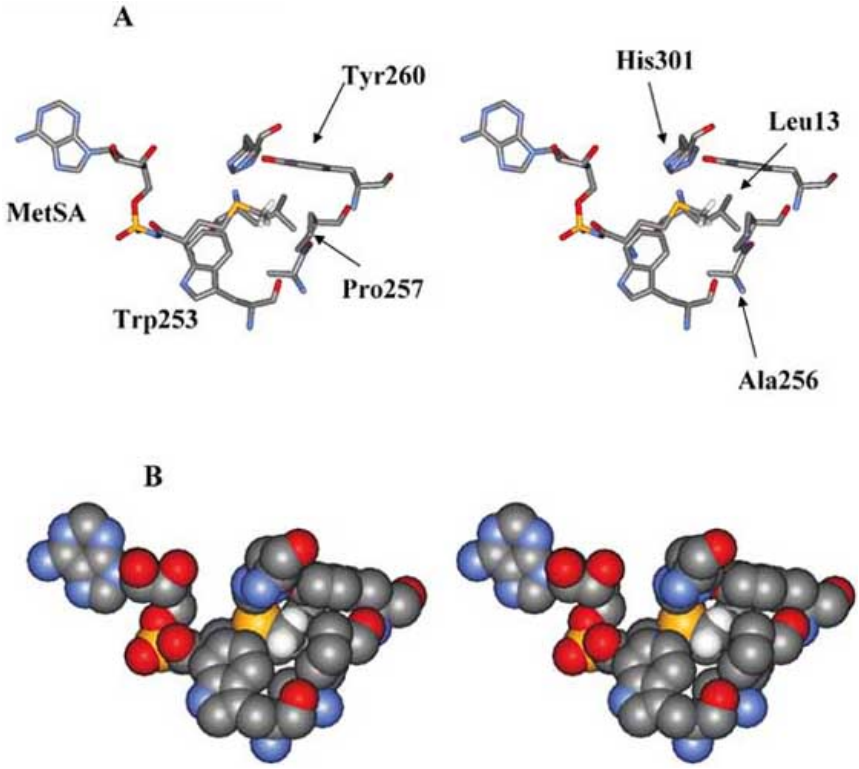


Fig. (2). Stereo view (relaxed) of a portion of the binding site region of the MetRS and MetSA complex showing the binding pocket for the methionine portion of the inhibitor. **A** and **B** are the structures in stick and spacefill respectively. Sulfur atoms are in yellow. The hydrogens (white) of the methyl group of MetSA have been added to delineate the spatial interactions in the binding pocket between the inhibitor and the protein. Based on structural coordinates from PDB file 1PFY [36].

residues surrounding the side chain, resulting in a possible lowering of the affinity of the enzyme for this derivative.

CONCLUSIONS

The results obtained with the inhibitors discussed in this report provide further insight in our developing understanding of the dynamic processes involved in catalysis by MetRS. In particular, the dramatically increased binding affinity observed for the methionyl-adenylate analogs suggests that the strong interactions between the enzyme and reactive intermediate may be used to advantage in the design of potent inhibitors of this enzyme. Although fluorination increases steric bulk and makes TFM a much less favored substrate for the enzyme than methionine, TFM linkage to adenosine in TFMSA appears to substantially improve its affinity to the enzyme.

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ABBREVIATIONS

aaRSs = Aminoacyl-tRNA synthetases

DBU = 1,8-Diazabicyclo[5.4.0]undec-7-ene

DME = Ethylene glycol dimethyl ether

DFM = *S*-Difluoromethyl-L-homocysteine; L-Difluoromethionine

DMF = N,N-Dimethylformamide

IPTG = Isopropyl-β-D-thiogalactopyranoside

LB = Luria-Bertani broth

MetI = 1-Amino-3-(methylmercapto)propylphosphinic acid

MetP = 1-Amino-3-(methylmercapto)propylphosphonic acid

MetRS = Methionyl-tRNA synthetase

MetSA = 5'-*O*-[(L-Methionyl)-sulfamoyl]adenosine

PMSF = Phenylmethylsulfonyl fluoride

TFMSA = 5'-*O*-[(*S*-Trifluoromethyl-L-homocysteinyl)-sulfamoyl]adenosine

TMS = Tetramethylsilane

TFM = *S*-Trifluoromethyl-L-homocysteine; L-Trifluoromethionine

TSP = (3-Trimethylsilyl)-1-propanesulfonic acid sodium salt

REFERENCES

[1] Levy, S. B. *N. Engl. J. Med.* **1998**, *338*, 1376.
[2] Bren, L. *FDA Consum* **2002**, *36*, 28.
[3] Harris, C. R.; Thorarensen, A. *Curr. Med. Chem.* **2004**, *11*, 2213.

[4] Jones, M. E.; Draghi, D. C.; Karlowsky, J. A.; Sahm, D. F.; Bradley, J. S. *Ann. Clin. Microbiol. Antimicrob.* **2004**, *3*, 3.
[5] Li, X. Z.; Nikaido, H. *Drugs* **2004**, *64*, 159.
[6] Spellberg, B.; Powers, J. H.; Brass, E. P.; Miller, L. G.; Edwards, J. E. Jr. *Clin. Infect. Dis.* **2004**, *38*, 1279.
[7] Schimmel, P.; Tao, J.; Hill, J. *FASEB J.* **1998**, *12*, 1599.
[8] Fuller, A. T.; Mellows, G.; Woolford, M.; Banks, G. T.; Barrow, K. D.; Chain, E. B. *Nature* **1971**, *234*, 416.
[9] Hughes, J.; Mellows, G. *Biochem. J.* **1978**, *176*, 305.
[10] Brown, M. J.; Mensah, L. M.; Doyle, M. L.; Broom, N. J.; Osbourne, N.; Forrest, A. K.; Richardson, C. M.; O'Hanlon, P. J.; Pope, A. J. *Biochemistry* **2000**, *39*, 6003.
[11] Rich, M.; Bannatyne, R. M.; Memish, Z. A. *J. Chemother.* **1999**, *11*, 414.
[12] Norazah, A.; Koh, Y. T.; Ghani Kamel, A.; Alias, R.; Lim, V. K. *Int. J. Antimicrob. Agents* **2001**, *17*, 411.
[13] Watanabe, H.; Masaki, H.; Asoh, N.; Watanabe, K.; Oishi, K.; Furumoto, A.; Kobayashi, S.; Sato, A.; Nagatake, T. *J. Hosp. Infect.* **2001**, *47*, 294.
[14] Yun, H. J.; Lee, S. W.; Yoon, G. M.; Kim, S. Y.; Choi, S.; Lee, Y. S.; Choi, E. C.; Kim, S. *J. Antimicrob. Chemother.* **2003**, *51*, 619.
[15] Berge, J. M.; Copley, R. C.; Eggleston, D. S.; Hamprecht, D. W.; Jarvest, R. L.; Mensah, L. M.; O'Hanlon, P. J.; Pope, A. J. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1811.
[16] Bernier, S.; Dubois, D. Y.; Therrien, M.; Lapointe, J.; Chenevert, R. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2441.
[17] Brown, M. J.; Carter, P. S.; Fenwick, A. S.; Fosberry, A. P.; Hamprecht, D. W.; Hibbs, M. J.; Jarvest, R. L.; Mensah, L.; Milner, P. H.; O'Hanlon, P. J.; Pope, A. J.; Richardson, C. M.; West, A.; Witty, D. R. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3171.
[18] Crasto, C. F.; Forrest, A. K.; Karoli, T.; March, D. R.; Mensah, L.; O'Hanlon, P. J.; Nairn, M. R.; Oldham, M. D.; Yue, W.; Banwell, M. G.; Easton, C. J. *Bioorg. Med. Chem.* **2003**, *11*, 2687.
[19] Decicco, C. P.; Nelson, D. J.; Luo, Y.; Shen, L.; Horiuchi, K. Y.; Amsler, K. M.; Foster, L. A.; Spitz, S. M.; Merrill, J. J.; Sizemore, C. F.; Rogers, K. C.; Copeland, R. A.; Harpel, M. R. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2561.
[20] Greenwood, R. C.; Gentry, D. R. *J. Antibiot. (Tokyo)* **2002**, *55*, 423.
[21] Houge-Frydrych, C. S.; Readshaw, S. A.; Bell, D. J. *J. Antibiot. (Tokyo)* **2000**, *53*, 351.
[22] Jarvest, R. L.; Berge, J. M.; Houge-Frydrych, C. S.; Janson, C.; Mensah, L. M.; O'Hanlon, P. J.; Pope, A.; Saldanha, A.; Qiu, X. *Bioorg. Med. Chem. Lett.* **1999**, 9,2859.
[23] Jarvest, R. L.; Berge, J. M.; Houge-Frydrych, C. S.; Mensah, L. M.; O'Hanlon, P. J.; Pope, A. J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2499.
[24 Lu, C. D.; Chen, Z. Y.; Liu, H.; Hu, W. H.; Mi, A. Q.; Doyle, M. P. *J. Org. Chem.* **2004**, *69*, 4856.
[25] Miyamoto, Y.; Machida, K.; Mizunuma, M.; Emoto, Y.; Sato, N.; Miyahara, K.; Hirata, D.; Usui, T.; Takahashi, H.; Osada, H.; Miyakawa, T. *J. Biol. Chem.* **2002**, *277*, 28810.
[26] Qiu, X.; Janson, C. A.; Smith, W. W.; Green, S. M.; McDevitt, P.; Johanson, K.; Carter, P.; Hibbs, M.; Lewis, C.; Chalker, A.; Fosberry, A.; Lalonde, J.; Berge, J.; Brown, P.; Houge-Frydrych, C. S.; Jarvest, R. L. *Protein Sci.* **2001**, *10*, 2008.
[27] Shimizu, T.; Usui, T.; Machida, K.; Furuya, K.; Osada, H.; Nakata, T. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3363.
[28] Stefanska, A. L.; Fulston, M.; Houge-Frydrych, C. S.; Jones, J. J.; Warr, S. R. *J. Antibiot. (Tokyo)* **2000**, *53*, 1346.
[29] Stefanska, A. L.; Coates, N. J.; Mensah, L. M.; Pope, A. J.; Ready, S. J.; Warr, S. R. *J. Antibiot. (Tokyo)* **2000**, *53*, 345.
[30] Lee, J.; Kang, M. K.; Chun, M. W.; Jo, Y. J.; Kwak, J. H.; Kim, S. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3511.
[31] Lee, J.; Kang, S. U.; Kang, M. K.; Chun, M. W.; Jo, Y. J.; Kwak, J. H.; Kim, S. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1365.
[32] Lee, J.; Kang, S. U.; Kim, S. Y.; Kim, S. E.; Kang, M. K.; Jo, Y. J.; Kim, S. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 961.
[33] Lee, J.; Kang, S. U.; Kim, S. Y.; Kim, S. E.; Job, Y. J.; Kim, S. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 965.
[34] Jarvest, R. L.; Armstrong, S. A.; Berge, J. M.; Brown, P.; Elder, J. S.; Brown, M. J.; Copley, R. C.; Forrest, A. K.; Hamprecht, D. W.; O'Hanlon, P. J.; Mitchell, D. J.; Rittenhouse, S.; Witty, D. R. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3937.
[35] Vaughan, M. D.; Sampson, P. B.; Honek, J. F. *Curr. Med. Chem.* **2002**, *9*, 385.
[36] Crepin, T.; Schmitt, E.; Mechulam, Y.; Sampson, P. B.; Vaughan, M. D.; Honek, J. F.; Blanquet, S. *J. Mol. Biol.* **2003**, *332*, 59.
[37] Houston, M. E.; Honek, J. F. *Jourl. Chem. Soc. Chem. Comm.* **1989**, 761.
[38] Duewel, H.; Daub, E.; Robinson, V.; Honek, J. F. *Biochemistry* **1997**, *36*, 3404.
[39] Kudzin, Z. H.; Stec, W. J. *Synthesis* **1980**, 1032.
[40] Baylis, E. K.; Campbell, C. D.; Dingwall, J. G. *Jour. Chem. Soc. Perkin Trans I* **1984**, 2845.
[41] Appel, R.; Berger, G. *Chem. Ber.* **1958**, *91*, 1339.
[42] Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M. *Jour. Amer. Chem. Soc.* **1964**, *86*, 1839.
[43] Heacock, D.; Forsyth, C. J.; Shiba, K.; Musier-Forsyth, K. *Bioorg. Chem.* **1996**, *24*, 273.
[44] Cassio, D.; Waller, J. P. *Eur. J. Biochem.* **1971**, *20*, 283.
[45] Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248.
[46] Blanquet, S.; Fayat, G.; Waller, J. P.; Iwatsubo, M. *Eur. J. Biochem.* **1972**, *24*, 461.
[47] Lawrence, F.; Blanquet, S.; Poiret, M.; Robert-Gero, M.; Waller, J. P. *Eur. J. Biochem.* **1973**, *36*, 234.
[48] Fersht, A. R.; Dingwall, C. *Biochemistry* **1979**, *18*, 1250.
[49] Oleksyszyn, J. *Aminophosphonic and Aminophosphinic Acids*; Wiley: Chichester, UK, **2000**.
[50] Biryukov, A. I.; Osipova, T. I.; Khomutov, R. M. *FEBS Lett.* **1978**, *91*, 246.
[51] Hyafil, F.; Jacques, Y.; Fayat, G.; Fromant, M.; Dessen, P.; Blanquet, S. *Biochemistry* **1976**, *15*, 3678.
[52] Lee, J.; Kim, S. E.; Lee, J. Y.; Kim, S. Y.; Kang, S. U.; Seo, S. H.; Chun, M. W.; Kang, T.; Choi, S. Y.; Kim, H. O. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1087.
[53] Houston, M. E.; Harvath, L.; Honek, J. F. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 3007.
[54] Vaughan, M. D.; Cleve, P.; Robinson, V.; Duewel, H. S.; Honek, J. F. *Jour. Amer. Chem. Soc.* **1999**, *121*, 8475.
[55] Salopek-Sondi, B.; Vaughan, M. D.; Skeels, M. C.; Honek, J. F.; Luck, L. A. *J. Biomol. Struct. Dyn.* **2003**, *21*, 235.
[56] Serre, L.; Verdon, G.; Choinowski, T.; Hervouet, N.; Risler, J. L.; Zelwer, C. *J. Mol. Biol.* **2001**, *306*, 863.

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