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Deoxyribosyl analogues of methionyl and isoleucyl sulfamate adenylates as inhibitors of methionyl-tRNA and isoleucyl-tRNA synthetases

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Abstract—2′-Deoxy, 3′-deoxy, and 2′,3′-dideoxyribosyl surrogates of isoleucyl and methionyl sulfamate adenylates have been investigated to identify the pharmacophoric importance of the ribose group for the inhibition of *Escherichia coli* methionyl-tRNA (MRS) and isoleucyl-tRNA (IRS) synthetases. Molecular modeling of 2′,3′-dideoxyribosyl Met-$NHSO_2$-AMP (**9**) with the crystal structure of *E. coli* MRS revealed that the lack of the two hydroxyl groups on ribose was compensated by the formation of an extra hydrogen bond between the ring oxygen and His24, resulting in a small activity reduction.

The aminoacyl-tRNA synthetases (aaRSs) are a family of enzymes that precisely transfer amino acids to their corresponding tRNA molecules to form aminoacyl-tRNAs, which are substrates for translation in protein synthesis and are pivotal in determining how the genetic code is interpreted as amino acids.[1] Although the activities of aminoacyl-tRNA synthetases are essential in all living organisms, the selective inhibition of pathogen synthetases over their human cellular counterparts provides an attractive antibacterial mode of action for discovering novel classes of antibiotics, particularly for the treatment of antibiotic-resistant bacterial strains, such as methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant enterococci (VRE).[2–4]

The aaRS reaction comprises two steps including the formation of an aminoacyl adenylate (aa-AMP, **1**) from an amino acid and ATP and the transfer of the aminoacyl moiety to the 3′-terminal adenosine of tRNA. The aminoacyl adenylate, an active intermediate, has been exploited as a starting prototype in the search for novel aaRS inhibitors because of its tight binding affinity, which is generally 2 or 3 orders of magnitude greater than those of the substrates, amino acid and ATP.

Modifications of aminoacyl adenylates have been extensively investigated for the purpose of improving chemical stability, tight binding, and pathogen selectivity. Many of the modifications have focused on the linker region, because the mixed anhydride acylphosphate bond of the intermediate is readily susceptible to hydrolysis. Substitutions with non-hydrolyzable isosteres have been tested to identify stable surrogates of aminoacyl adenylates as potent aaRS inhibitors. To date, alkylphosphate,[5–7] ester (aa-CO_2-AMP),[8–10] amide (aa-CONH-AMP),[8] hydroxamate (aa-CON(OH)-AMP),[8–10] sulfamate (aa-$NHSO_3$-AMP),[5,6,11–14] sulfamide (aa-$NHSO_2NH$-AMP),[6] *N*-alkoxysulfamide (aa-$NHSO_2NHO$-AMP)[15], and *N*-hydroxysulfamide (aa-$NHSO_2N(OH)$-AMP)[15] surrogates have been studied as candidates for the replacement of the labile acylphosphate of the aminoacyl adenylate. Among them, the sulfamate analogues consistently exhibited

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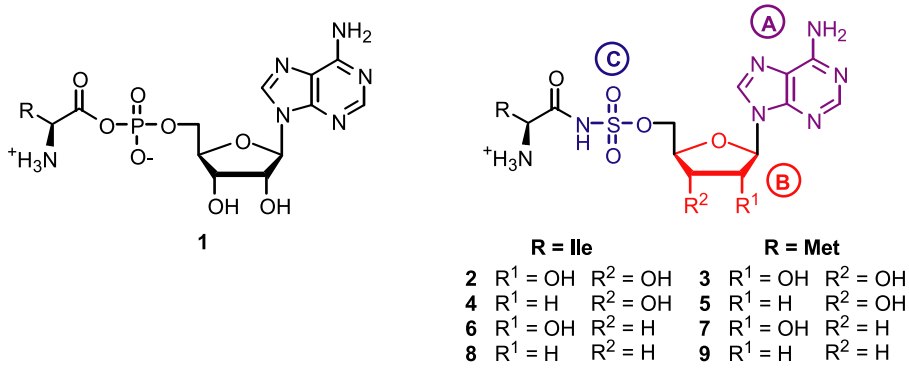


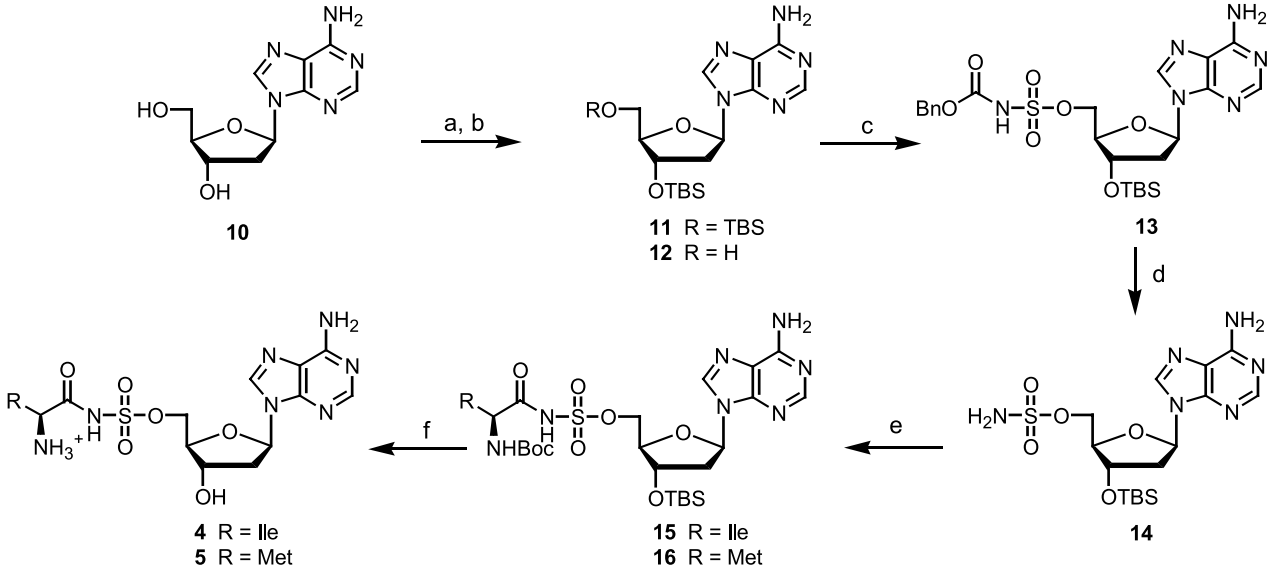
Figure 1.

the most potent inhibition of enzymes, including AlaRS,[11] ArgRS,[5] HisRS,[5] IleRS,[12,15] MetRS,[15] ProRS,[13] SerRS,[14] ThrRS,[5] and TyrRS.[6]

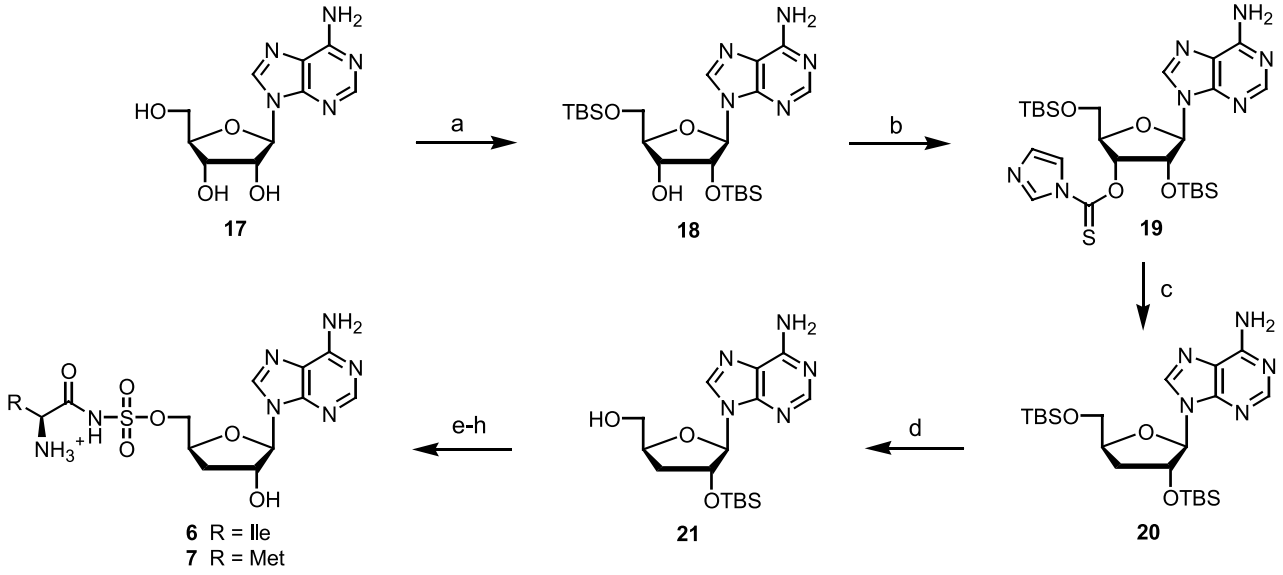
Over the past few years, for the purpose of discovering aaRS inhibitors as novel antibiotic candidates, we have systematically modified isoleucyl and methionyl adenylates based on three pharmacophoric regions, including the adenine, ribose, and acylphosphate moieties (designated as the A, B, and C regions in Fig. 1, respectively), and have reported that their sulfamate surrogates were potent IRS and MRS inhibitors.[8–10,15] In particular, 2-iodo and 2-acetylenic Ile-NHSO$_2$-AMP were found to be highly potent *E. coli* IRS inhibitors, with IC_{50} values of 14 and 39 nM, which were 7- and 2.5-fold more potent than the parent sulfamate analogue, respectively.[15]

As a continuation of these studies, we have now examined the effect of the 2′- and 3′-hydroxyl groups of ribose on the inhibition of IRS and MRS, through the syntheses of the 2′-deoxyribosyl, 3′-deoxyribosyl, and 2′,3′-dideoxyribosyl analogues of isoleucyl and methionyl sulfamate adenylates. Interestingly, we found that the 2′,3′-dideoxyribosyl analogues were much more potent than the 2′-deoxy or 3′-deoxy surrogates for both aaRSs.

The syntheses of the 2′-deoxyribosyl analogues (**4**, **5**) of isoleucyl and methionyl sulfamate adenylates are outlined in Scheme 1. Commercially available 2′-deoxyadenosine (**10**) was converted to 3′-protected 2′-deoxyadenosine (**12**) by two steps. Treatment of **12** with *N*-(carbobenzyloxy)sulfamoyl chloride, prepared from chlorosulfonyl isocyanate and benzyl alcohol at −78 °C,[16] and subsequent catalytic hydrogenation provided the 5′-sulfamoyl intermediate **14**. The condensation of **14** with *N*-Boc-isoleucine and *N*-Boc-methionine by 1,3-dicyclohexylcarbodiimide afforded the penultimate intermediates (**15**, **16**), whose two protecting groups were hydrolyzed under acidic conditions to produce the final 2′-deoxyribosyl sulfamate adenylates (**4**, **5**), respectively. The syntheses of the 3′-deoxyribosyl analogues (**6**, **7**) are represented in Scheme 2, and were accomplished from 2′-protected 3′-deoxyadenosine (**21**), which was prepared from adenosine in four con-



Scheme 1. Reagents and conditions: (a) TBSCl, imidazole, DMF, rt, 95%; (b) AcOH/H_2O/THF (3:1:1), 0 °C to rt, 80%; (c) (i) BnOH, CSI, CH_2Cl_2, −78 °C; (ii) NEt_3, rt, 75%; (d) H_2, Pd/C, MeOH, rt, 99%; (e) BocNHCHRCO$_2$H, DCC, DMAP, DMF, rt, 70% for Ile, 72% for Met; (f) 0.1 M HCl in MeOH, 0 °C to rt, 39% for Ile, 45% for Met.

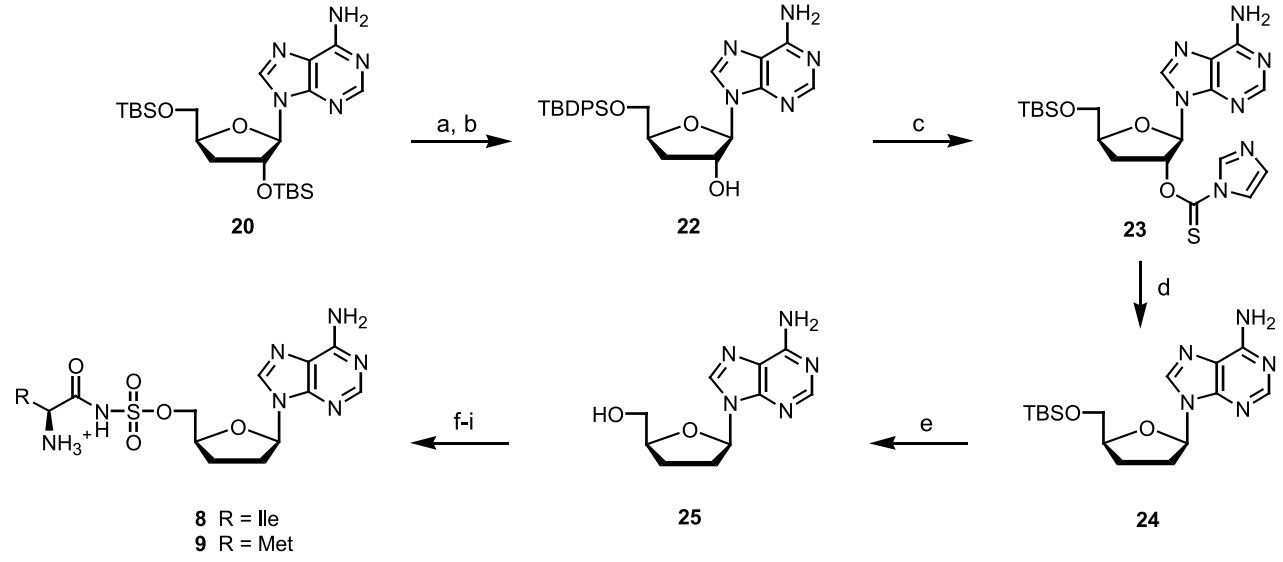


Scheme 2. Reagents and conditions: (a) TBSCl, pyridine, DMF, rt, 40%; (b) thiocarbonylimidazole, DMF, rt, 80%; (c) (n-Bu)$_3$SnH, AIBN, toluene, reflux, 87%; (d) AcOH/H_2O/THF (3:1:1), 0 °C to rt, 80%; (e) (i) BnOH, CSI, CH_2Cl_2, −78 °C; (ii) NEt_3, rt, 71%; (f) H_2, Pd/C, MeOH, rt, 99%; (g) BocNHCHRCO_2H, DCC, DMAP, DMF, rt, 70% for Ile, 72% for Met; (h) 0.1 M HCl in MeOH, 0 °C to rt, 40% for Ile, 45% for Met.

ventional steps, by following the same route described in Scheme 1. The syntheses of the 2′,3′-dideoxyribosyl analogues (**8**, **9**), shown in Scheme 3, were completed by employing 2′,3′-dideoxyadenosine (**25**), which was prepared from the 3′-deoxyadenosine intermediate (**20**) in five steps, using the above protocol.

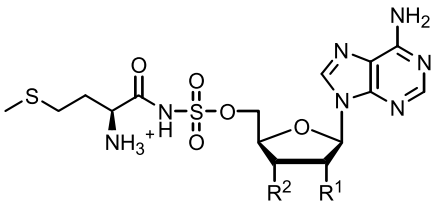
The synthesized methionyl and isoleucyl adenylate analogues (**4**–**9**) were evaluated as inhibitors of *E. coli* MetRS and IleRS, respectively. The inhibitory activities were determined by measuring the decrease in the generation of the aminoacylation product, [^{3}H]isoleucyl *E. coli*-tRNA^{Ile} or [^{35}S]methionyl *E. coli*-tRNA^{Met}, in the presence of different chemical concentrations, as described.[17] The previously reported sulfamate analogues of isoleucyl adenylate (Ile-NHSO$_3$-AMP) (**2**) and methionyl adenylate (Met-NHSO$_3$-AMP) (**3**) were also evaluated as references in our assay protocol, and showed IC_{50} values of 0.265 and 0.083 μM, respectively (see Table 1).

In the deoxy series of isoleucyl sulfamate adenylate, we found that the 2′-deoxyribosyl isoleucyl sulfamate adenylate displayed a three order of magnitude reduction in enzyme inhibition (**4**, IC_{50} = 280 μM) and the 3′-deoxyribose analogue showed an over 100-fold reduction in

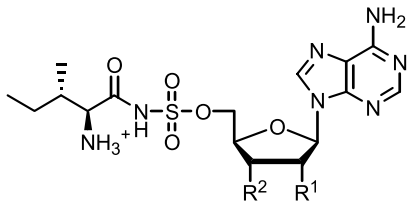


Scheme 3. Reagents and conditions: (a) Bu_4NF, THF, rt, 98%; (b) TBDPSCl, imidazole, DMF, rt, 90%; (c) thiocarbonylimidazole, DMF, rt, 92%; (d) (n-Bu)$_3$SnH, AIBN, toluene, reflux, 63%; (e) CF_3CO_2H, THF, rt, 94%; (f) (i) BnOH, CSI, CH_2Cl_2, −78 °C; (ii) NEt_3, rt, 77%; (g) H_2, Pd/C, MeOH, rt, 97%; (h) BocNHCHRCO_2H, DCC, DMAP, DMF, rt, 78% for Ile, 81% for Met; (i) CF_3CO_2H, CH_2Cl_2, 0 °C to rt, 71% for Ile, 69% for Met.

Table 1. Inhibitory activities of the synthesized compounds toward *Escherichia coli* MetRS and IleRS



Met-AMP



Ile-AMP

Compound	R^1	R^2	IC_{50} (μM)
Ile-AMP			
2	OH	OH	0.265
4	H	OH	280
6	OH	H	35
8	H	H	6.4
Met-AMP			
3	OH	OH	0.083
5	H	OH	110
7	OH	H	220
9	H	H	0.383

activity (**6**, IC_{50} = 35 μM). However, the 2′,3′-dideoxyribosyl congener exhibited a moderate 24-fold decrease in activity (**8**, IC_{50} = 6.4 μM), which is relatively less loss as compared to the 2′-deoxyribose and 3′-deoxyribose surrogates.

The deoxy series of methionyl sulfamate adenylate showed a similar SAR activity pattern. Whereas both the 2′-deoxyribose (**5**, IC_{50} = 110 μM) and 3′-deoxyribose analogues (**7**, IC_{50} = 220 μM) displayed dramatic decreases in enzyme inhibition, of 1300-fold and 2600-fold, respectively, the 2′,3′-dideoxyribose surrogate showed a small loss in activity, with ca. 4-fold reduction (**9**, IC_{50} = 0.383 μM).

These results indicate that the deoxyribose series of sulfamate analogues displayed reduced potencies as compared to the parent compounds, and interestingly, the 2′,3′-dideoxyribosyl analogues were found to be much more potent than the 2′-deoxy or 3′-deoxy analogues in both IRS and MRS.

Recently, the 3D structure of methionyl-tRNA synthetase from *E. coli* complexed with compound **3** (Met-$NHSO_2$-AMP) was determined by X-ray crystallography.[18] The methionine-binding pocket was reportedly composed of residues Ala12, Leu13, Tyr15, Trp253, Ala256, Pro257, Tyr260, Ile297, His301, and Trp305. The adenine base formed two hydrogen bonds with the main-chain NH group of Val326 and the δ-nitrogen atom of the His323 imidazole ring, respectively. The 2′-OH group of the ribose was hydrogen bonded to the side-chain of Asp296, and the 3′-OH group made bifurcated hydrogen bonds with Glu27 and Gly294. Several water molecules formed a hydrogen-bond network with the adenosine moiety. The sulfamoyl group acted as hydrogen bond acceptors for the δ-nitrogen atom of the imidazole ring in His24 and water molecules. The sulfur atom of the methionine interacted with the phenolic hydroxyl of Tyr260 and the protonated *N*-epsilon atom of His301 by hydrogen bonding. The ammonium group coordinated with both the carbonyl of Leu13 and the carboxylate of Asp52 by dipole–dipole ionic interactions.

In order to investigate the binding mode of 2′,3′-dideoxyribosyl Met-$NHSO_2$-AMP (**9**), it was docked in the methionyl adenylate-binding site of *E. coli* MetRS.[19] The docked model of compound **9** is shown in Figure 2. In the proposed model, the adenine moiety forms hydrogen bonds with water molecules. The distance and the angle between the adenine N1 atom and the main-chain NH group of Val326, which was forming a hydrogen bond in compound **3**, were measured as 2.47 Å and 141.13°, respectively. This result indicates that the side-chains of the residues within the binding site undergo a large conformational change, as compared with the conformation within the X-ray structure of compound **3** bound into MetRS (weighted rms value = 1.47 Å). The sulfonyl oxygen atom acts as a hydrogen bond acceptor for a water molecule, and the sulfonamide NH group interacts with the carbonyl group of Leu13 by hydrogen bonding (1.8 Å). The ammonium group makes bifurcated hydrogen bonds with both carbonyl groups of Leu13 and Pro14. We also found a particular hydrogen bond in the ribose ring, whose oxygen atom interacts with the NH of His24 (1.8 Å). The inhibitory activity of 2′,3′-dideoxyribosyl Met-$NHSO_2$-AMP (**9**, IC_{50} = 0.383 μM) represents only a 5-fold decrease as compared to Met-$NHSO_2$-AMP (**3**, IC_{50} = 0.083 μM), while it lacks two hydroxyl groups. The high potency

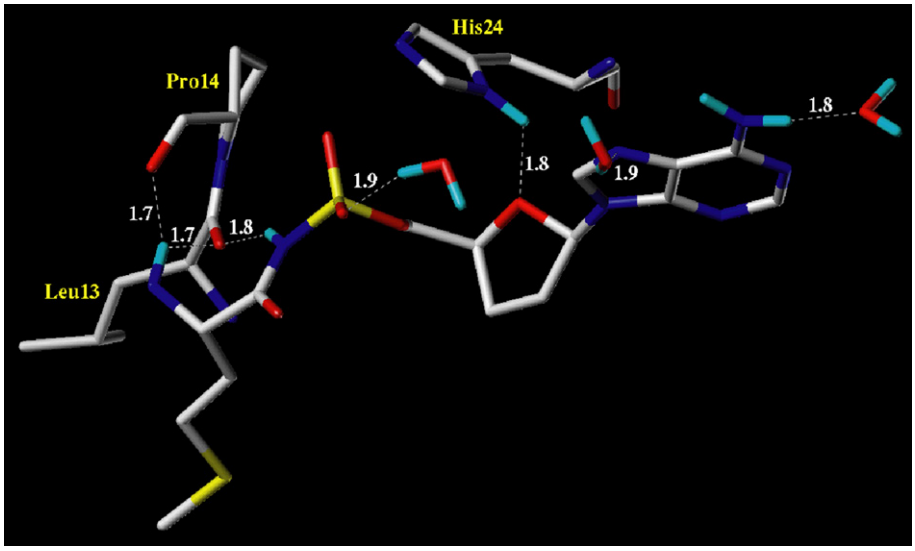


Figure 2. Proposed model of compound **9** bound to the *Escherichia coli* MetRS binding site.

of compound **9** may be acquired by the formation of an extra hydrogen bond between the oxygen atom of the ribose ring and His24.

In summary, we have synthesized 2′-deoxy, 3′-deoxy, and 2′,3′-dideoxyribosyl surrogates of isoleucyl and methionyl sulfamate adenylates, which were previously described as potent IRS and MRS inhibitors, from adenosine and 2′-deoxyadenosine to identify the pharmacophoric importance of the two hydroxyl groups on the ribose for the inhibition of the *E. coli* methionyl-tRNA and isoleucyl-tRNA synthetases. All of the deoxy analogues displayed reduced potencies as compared to the parent compounds. Interestingly, the 2′,3′-dideoxyribosyl analogues were found to be much more potent than the 2′-deoxy or 3′-deoxy analogues in both IRS and MRS. The molecular modeling study of 2′,3′-dideoxyribosyl Met-$NHSO_2$-AMP (**9**) with the crystal structure of *E. coli* MRS revealed that an extra hydrogen bond between the ring oxygen of **9** and His24 compensated for the dramatic loss of activity, due to the lack of the two hydroxyl groups on ribose, resulting in only a small reduction in the activity.

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19. Compound **9** was modeled in the binding pocket of *E. coli* MetRS, using the X-ray structure of the MetRS–compound **3** complex. All of the hydrogen atoms were added to MetRS, and then the obtained complex was fully optimized by energy minimization using the Tripos force field and Gasteiger–Hückel partial atomic charges. All computational work was done on a Silicon Graphics O_2 R10000 workstation.