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Glutamylsulfamoyladenosine and pyroglutamylsulfamoyladenosine are competitive inhibitors of *E. coli* glutamyl-tRNA synthetase

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Abstract

5′-O-[N-(L-glutamyl)-sulfamoyl]adenosine is a potent competitive inhibitor of *E. coli* glutamyl-tRNA synthetase with respect to glutamic acid ($K_i = 2.8$ nM) and is the best inhibitor of this enzyme. It is a weaker inhibitor of mammalian glutamyl-tRNA synthetase ($K_i = 70$ nM). The corresponding 5′-O-[N-(L-pyroglutamyl)-sulfamoyl] adenosine is a weak inhibitor ($K_i = 15$ μM) of the *E. coli* enzyme.

Keywords: *Enzyme inhibitor, glutamylsulfamoyladenosine, glutamyl-tRNA synthetase, pyroglutamylsulfamoyl-adenosine*

Introduction

Aminoacyl-tRNA synthetases (aaRSs) are essential enzymes involved in protein biosynthesis. They catalyze the esterification of a particular tRNA with the cognate amino acid[1–2]. The overall reaction is a two-step event illustrated in Scheme 1. In the first step, the appropriate amino acid (aa) is recognized by the enzyme and reacts with ATP to form an enzyme-bound mixed anhydride (aminoacyl adenylate, aa-AMP) with displacement of pyrophosphate. In this intermediate, the high-energy anhydride bond activates the carboxyl group of the amino acid. The role of the Mg^{2+} ion is to stabilise the conformation of ATP and withdraw electrons from the β-phosphate, thus facilitating its displacement by the amino acid. In the second step, the activated amino acid is transferred to the 3′ end of the corresponding tRNA to form aminoacyl-tRNA (aa-tRNA) and adenosine monophosphate (AMP). This esterification is a nucleophilic attack by the 2′- or 3′- ribose hydroxyl group of the terminal adenosine of the tRNA on the activated carboxyl group of the mixed anhydride intermediate. These enzymes are classified into two classes of about ten members each, on the basis of common structural features[3–5]. As aaRSs have been subjected to significant evolutionary divergence, selective inhibition of bacterial enzymes is a valuable strategy for the production of antibiotics[6–10].

Potent synthetic inhibitors are usually stable analogues of the aminoacyl adenylate intermediates (aa-AMP, see Scheme 1), which are high-energy molecules sensitive to nucleophiles such as water. They are more tightly bound to the enzymes than are the substrates; therefore, stabilized analogues have the potential to be tight binding inhibitors. The stability is achieved by replacement of the labile mixed anhydride function by non-hydrolyzable bioisosteres. The replacement of the phosphate by a sulfamoyl group provides stable aminoacylsulfamoyladenosines. Several aminoacyl-sulfamoyladenosine derivatives have been synthesized and shown to be potent inhibitors of aaRSs[6,11–19].

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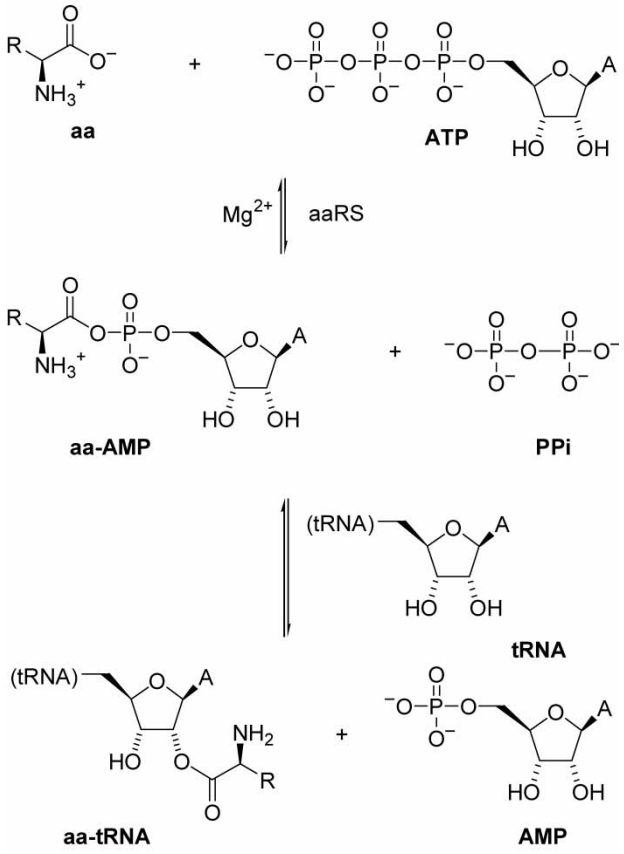


Scheme 1. Aminoacylation reaction catalyzed by aminoacyl-tRNA synthetases.

These analogues were modelled according to the natural product ascamycin[20]. SB-203207 was recently isolated from a *Streptomyces* strain and shown to inhibit bacterial and mammalian isoleucyl-tRNA synthetases ($IC_{50} = 1.7$ nM and < 2 nM respectively)[21]. In SB-203207, isoleucine is bonded to altemicidin, another natural product, via a sulfamoyl linkage. Substitution of the isoleucine residue of SB-203207 with leucine and valine produced potent inhibitors of leucyl-tRNA synthetase ($IC_{50} = 16$ nM) and valyl-tRNA synthetase ($IC_{50} = 30$ nM) respectively[22].

The molecular dimensions of the sulfamoyl group are nearly the same as those of the phosphate group. Also, the sulfamoyl can exist in solution and in the solid state in the anionic form due to the acidity of the NH function; the negative charge is delocalized over several atoms and the anion is a good mimic of the phosphate anion.

With the aim of obtaining inhibitors of GluRS, we have synthesized sulfamoyl analogues of glutamyl adenylate and tested their properties in the amino-acylation reaction of $tRNA^{Glu}$ catalyzed by *E. coli* GluRS.

Materials and methods

General

Chemical reagents were purchased from Aldrich-Sigma Chemical Company. Infrared spectra were recorded on a Bomem MB-100 spectrometer. Optical rotations were measured using a JASCO DIP-360 digital polarimeter (*c* as g of compound per 100 mL). Flash column chromatography was carried out using 40–63 μm (230–400 mesh) silica gel. NMR spectra were recorded on Bruker AC300 or Varian Inova AS400 spectrometers (300 and 400 MHz respectively).

Uniformly labelled [^{14}C]glutamate (273 mCi/mmol) and the serine protease inhibitor phenylmethylsulfonyl fluoride (PMSF) were obtained from ICN Biomedicals. Unfractionated tRNA from *E. coli* MRE 600 was purchased from Roche Diagnostics Canada. Unfractionated tRNA from bovine liver, ATP and bovine serum albumin (fraction V) were purchased from Sigma-Aldrich Canada.

Chemistry

N^6-Benzoyl-2′,3′-O-isopropylidene-5′-O-sulfamoyl-adenosine 1. Formic acid (0.365 mL, 9.67 mmol) was added to a solution of chlorosulfonyl isocyanate (0.85 mL, 9.67 mmol) in dry dichloromethane. The solution was stirred for 3 h at room temperature, for 1 h at reflux and then for 3 h at room temperature. This solution of chlorosulfonamide (8 eq.) in dichloromethane was added dropwise to a solution of N^6-benzoyl-2′,3′-*O*-isopropylidene adenosine (0.5 g, 1.21 mmol) and calcium carbonate (1.22 g, 10 eq.) in dry DMF at 0°C. After stirring at room temperature for 0.5 h, the salts were filtered and the product was diluted with ethyl acetate (75 mL). The organic phase was washed with sodium bicarbonate (2 × 20 mL), water (4 × 10 mL), dried over magnesium sulfate and evaporated. The crude product was purified by flash chromatography (75% ethyl acetate/25% CH_2Cl_2) to give **1** (0.501 g, 84%) as a white solid: m.p. 140°C (dec.); $[\alpha]_D^{20} - 26.5$ (*c* 0.54, $CHCl_3$); IR (KBr) 3265, 2992, 2939, 1698, 1606, 1377, 1100 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.26 and 1.50 (2 s, 6 H, $C(CH_3)_2$), 4.20–4.26 (m, 2 H, H5′), 4.42–4.44 (m, 1 H, H4′), 4.94 (dd, J = 2.0 and 6.2 Hz, 1 H, H3′), 5.26 (dd, J = 2.4 and 6.2 Hz, 1 H, H2′), 6.10 (d, J = 2.4 Hz, 1 H, H1′), 6.42–6.52 (br s, 2 H, SO_3NH_2), 7.31 (t, J = 7.3 Hz, 2 H, *m*-Ph), 7.42 (t, J = 7.3 Hz, 1 H, *p*-Ph), 7.83 (d, J = 7.3 Hz, 2 H, *o*-Ph), 8.23 and 8.58 (2 s, 2 H, H2 and H8); ^{13}C NMR ($CDCl_3$) δ 25.4 and 27.2 ($C(CH_3)_2$), 69.3 (C5′), 81.4 (C3′), 84.4 (C2′), 84.8 (C4′), 91.4 (C1′) 114.7 ($C(CH_3)_2$), 123.2 (C5), 128.3 (C *o*-Ph), 128.8 (C *m*-Ph), 133.0 and 133.2 (C *p*-Ph and C Ph), 142.6 (C8), 149.3, 151.5 and 152.6 (C2, C4 and C6), 165.9 (NH*C*(O)Ph).

N^6-Benzoyl-2′,3′-O-isopropylidene-5′-O-[N-(γ-tert-butyl-N-Boc-L-glutamyl)-sulfamoyl]adenosine 3. *N*-Boc-L-glutamic acid γ-t-butyl ester **2** (247 mg, 0.815 mmol) was added to a solution of sulfonamide adenosine

1 (400 mg, 0.815 mmol), DCC (168 mg, 0.815 mmol) and DMAP (99.5 mg, 0.815 mmol) in dry dichloromethane (20 mL), and the mixture was stirred at room temperature for 1.5 h. The solvent was evaporated and a solution of the residue in ethyl acetate was washed with water, dried ($MgSO_4$), and evaporated. The crude product was purified by flash chromatography (ethyl acetate) to yield **3** (506 mg, 80%) as a white solid: m.p. 164°C (dec.); $[\alpha]_D^{21}$ − 18.4 (*c* 0.425, MeOH); IR (KBr) 3416, 2982, 2934, 1703, 1610, 1148 cm^{-1}; ^1H NMR (CD_3OD) δ 1.32 − 1.36 (m, 21 H, 2 × $C(CH_3)_3$, 1 × $C(CH_3)_2$), 1.57 (s, 3 H, 1 × $C(CH_3)_2$), 1.66 − 1.75 (m, 1 H, C*H*$_2$CHNHBoc), 1.91 − 2.00 (m, 1H, C*H*$_2$CHNHBoc), 2.08 − 2.21 (m, 2 H, C*H*$_2$$CO_2$tBu), 3.88 − 3.95 (m, 1 H, C*H*NHBoc), 4.21 − 4.28 (m, 2 H, H5′), 4.53-4.58 (m, 1 H, H4′), 5.10 (dd, J = 2.2 and 6.0 Hz, 1 H, H3′), 5.39 (dd, J = 2.2 and 6.0 Hz, 1 H, H2′), 6.34 (d, J = 2.2 Hz, 1 H, H1′), 7.44–7.48 (m, 2 H, *m*-Ph), 7.56–7.60 (m, 1 H, *p*-Ph), 7.99–8.01 (m, 2 H, *o*-Ph), 8.67 and 8.71 (2 s, 2 H, H2 and H8); ^{13}C NMR (CD_3OD) δ 25.7 and 27.5 ($C(CH_3)_2$), 28.4 and 28.8 ($C(CH_3)_3$), 29.4 (*C*H$_2$ CHNHBoc), 32.9 (*C*H$_2$$CO_2$tBu), 57.5 (*C*HNHBoc), 70.1 (C5′), 80.4 and 81.6 ($C(CH_3)_3$), 83.1 (C3′), 86.0 (C2′), 86.6 (C4′), 92.2 (C1′), 115.3 ($C(CH_3)_2$), 124.6 (C5), 129.7 (C *o*-Ph), 129.8 (C *m*-Ph), 134.2 and 134.5 (C Ph and C *p*-Ph), 145.1 (C8), 150.6, 153.4 and 153.6 (C2, C4 and C6), 157.7 (NH*C*O_2tBu), 168.6 (NH*C*OPh), 174.3 (CH_2CO_2tBu), 181.2 (*C*(O)$NHSO_3$).

2′,3′-O-Isopropylidene-5′-O-[N-γ-tert-butyl-N-Boc-L-glutamyl)-sulfamoyl]adenosine 4. A solution of compound **3** (300 mg, 0.386 mmol) in methanol/ n-butylamine (3 mL/3 mL) was stirred at room temperature for 1 h. The solvents were evaporated and the residue was purified by flash chromatography (95% EtOAc / 5% MeOH to 70% EtOAc / 30% MeOH) to yield **4** (257 mg, 99%) as a white solid: m.p. 160°C (dec.); $[\alpha]_D^{21}$ − 32.1 (*c* 0.785, MeOH); IR (KBr) 3377, 2978, 1713, 1640, 1591, 1148 cm^{-1}; ^1H NMR (CD_3OD) δ 1.34–1.37 (m, 21 H, 2 × $C(CH_3)_3$, 1 × $C(CH_3)_2$), 1.57 (s, 3 H, 1 × $C(CH_3)_2$), 1.75–1.84 (m, 1 H, C*H*$_2$CHNHBoc), 1.98–2.08 (m, 1 H, C*H*$_2$CHNHBoc), 2.18–2.31 (m, 2 H, C*H*$_2$$CO_2$tBu), 3.93–3.97 (m, 1 H, C*H*NHBoc), 4.20 (dd, J = 4.2 and 10.8 Hz, 1 H, H5′), 4.26 (dd, J = 4.2 and 10.8 Hz, 1 H, H5′), 4.49–4.52 (m, 1 H, H4′), 5.07 (dd, J = 2.2 and 6.0 Hz, 1 H, H3′), 5.33 (dd, J = 3.2 and 6.0 Hz, 1 H, H2′), 6.20 (d, J = 3.2 Hz, 1 H, H1′), 8.18 and 8.38 (2 s, 2 H, H2 and H8); ^{13}C NMR (CD_3OD) δ 25.7 and 27.6 ($C(CH_3)_2$), 28.4 and 28.8 (2 × $C(CH_3)_3$), 29.5 (*C*H$_2$CHNHBoc), 32.9 (*C*H$_2$ CO_2tBu), 57.7 (*C*HNHBoc), 70.0 (C5′), 80.4 and 81.7 (2 × $C(CH_3)_3$), 83.2 (C3′), 2 × 85.7 (C2′ and C4′), 91.9 (C1′), 115.5 ($C(CH_3)_2$), 120.3 (C5), 141.6 (C8), 150.5 (C4), 154.1 (C2), 157.4 (C6), 157.9 (NH*C*O_2tBu), 174.5 (CH_2CO_2tBu), 181.3 (*C*(O)$NHSO_3$); HRMS (FAB) calcd for $C_{27}H_{42}N_7O_{11}S$ $(M + H)^+$ 672.2662; found 672.2654.

5′-O-[N-(L-Glutamyl)-sulfamoyl]adenosine 5. A solution of compound **4** (30 mg, 44.6 μmol) in trifluoroacetic acid /water (4/1, 4 mL/1 mL) was stirred at room temperature for 15 min. The solvents were evaporated to give **5** (25.8 mg, 98%) as a white solid: m.p. 99°C (dec.); $[\alpha]_D^{20} = -8.9$ (*c* 0.86, DMSO); IR (KBr) 3383, 3148, 1680, 1200, 1135 cm^{-1}; ^1H NMR (D_2O) δ 1.87–1.94 (m, 2 H, C*H*$_2$CHNH_2), 2.22 (t, J = 7.6 Hz, 2 H, C*H*$_2$$CO_2$H), 3.79 (t, J = 6.0 Hz, 1 H, CH_2C*H*NH_2), 4.16–4.19 (m, 1 H, H4′), 4.27–4.30 (m, 3 H, H3′ and H5′), 4.50 (t, J = 4.6 Hz, 1 H, H2′), 5.89 (d, J = 4.6 Hz, 1 H, H1′), 8.17 and 8.24 (2 s, 2 H, H2 and H8); ^{13}C NMR (D_2O) δ 25.4 (*C*H_2CHNH_2), 28.9 (*C*H_2CO_2H), 53.6 (CH_2*C*HNH_2), 69.7 (C3′), 70.2 (C5′), 73.9 (C2′), 81.8 (C4′), 88.5 (C1′), 118.7 (C5), 116.2 (q, J = 289.2 Hz, TFA) 142.5 (C8), 144.6 (C2), 148.3 (C4), 149.9 (C6), 162.6 (q, J = 35.8 Hz, TFA), 171.1 (*C*(O)$NHSO_3$), 175.8 (*C*O_2H); LRMS (ESI) 476 (M^+).

N^6-Benzoyl-2′,3′-O-isopropylidene-5′-O-[N-(L-pyroglutamyl)-sulfamoyl]adenosine 7. Pyroglutamic acid **6** (105 mg, 0.815 mmol) was added to a solution of sulfonamide adenosine **1** (400 mg, 0.815 mmol), DCC (168 mg, 0.815 mmol) and DMAP (99.5 mg, 0.815 mmol) in dry dichloromethane (20 mL), and the mixture was stirred at room temperature for 2 h. The solvent was evaporated and the residue was purified by flash chromatography (100% ethyl acetate to 70% ethyl acetate/30% MeOH) to give **7** (98 mg, 80%) as a white solid: m.p. 170°C (dec.); $[\alpha]_D^{20}$ − 36.5 (*c* 0.55, H_2O); IR (KBr) 3399, 2984, 2938, 1691, 1608, 1258, 1151 cm^{-1}; ^1H NMR (D_2O) δ 1.44 and 1.65 (2 s, 6 H, $C(CH_3)_2$), 1.79–1.88 (m, 1 H, H4-pyro), 2.26–2.42 (m, 3 H, H3 and H4-pyro), 4.01–4.05 (m, 1 H, H5-pyro), 4.26–4.34 (m, 2 H, H5′), 4.71–4.74 (m, 1 H, H4′), 5.18 (dd, J = 2.4 and 6.0 Hz, 1 H, H3′), 5.42 (dd, J = 2.4 and 6.0 Hz, 1 H, H2′), 6.25 (d, J = 2.4 Hz, 1 H, H1′), 7.42–7.46 (m, 2 H, *m*-Ph), 7.55–7.59 (m, 1 H, *p*-Ph), 7.84–7.86 (m, 2 H, *o*-Ph), 8.48 and 8.63 (2 s, 2 H, H2 and H8); ^{13}C NMR (D_2O) δ 24.4 (C4-pyro), 25.5 and 26.1 ($C(CH_3)_2$), 29.6 (C3-pyro), 59.2 (C5-pyro), 68.9 (C5′), 81.4 (C3′), 84.2 (C2′), 84.5 (C4′), 91.2 (C1′), 115.0 ($C(CH_3)_2$), 123.4 (C5), 128.0 (C *o*-Ph), 128.7 (C *m*-Ph), 132.5 and 133.3 (C Ph and C *p*-Ph), 143.3 (C8), 148.9, 151.3 and 152.0 (C2, C4 and C6), 168.3 (NH*C*(O)Ph), 180.6 and 181.7 (C2 and C6 pyro).

2′,3′-O-isopropylidene-5′-O-[N-(L-pyroglutamyl)-sulfamoyl]adenosine 8. A solution of compound 7 (50 mg, 83 μmol) in methanol/n-butylamine (1.5 mL/1.5 mL) was stirred at room temperature for 1 h. The solvents were evaporated and the residue was purified by reversed phase chromatography (100% H_2O to 85% H_2O/15% MeOH) to give **8** (39.3 mg, 95%) as a white hygroscopic solid: m.p. 152°C (dec.); $[\alpha]_D^{20}$ − 31.2 (*c* 0.85, H_2O); IR (KBr) 3405, 1644, 1299, 1148 cm^{-1}; ^1H NMR (D_2O) δ 1.45 and 1.66 (2 s, 6 H, $C(CH_3)_2$), 1.73–1.82 (m, 1 H, H4-pyro), 2.20–2.41 (m, 3 H, H4 and H3-pyro), 4.02–4.06 (m, 1 H, H5-pyro), 4.26–4.32 (m, 2 H, H5′), 4.70–4.72 (m, 1 H, H4′), 5.16 (dd, J = 2.2 and 6.0 Hz, 1 H, H3′), 5.39 (dd, J = 2.8 and 6.0 Hz, 1 H, H2′), 6.18 (d, J = 2.8 Hz, 1 H, H1′), 8.15 and 8.25 (2 s, 2 H, H2 and H8); ^{13}C NMR (D_2O) δ 24.4 (C4-pyro), 25.5 and 26.1 ($C(CH_3)_2$), 29.6 (C3-pyro), 59.2 (C5-pyro), 68.8 (C5′), 81.3 (C3′), 84.0 and 84.1 (C2′ and C4′), 90.7 (C1′), 115.0 ($C(CH_3)_2$), 118.7 (C5), 140.0 (C8), 148.5 (C4), 152.8 (C2), 155.4 (C6), 180.6 and 181.8 (C2 and C6-pyro); HRMS (FAB) calcd for $C_{18}H_{24}N_7O_8S$ $(M + H)^+$ 498.1407; found 498.1408.

5′-O-[N-(L-pyroglutamyl)-sulfamoyl]adenosine 9. A solution of compound **8** (35 mg, 70.3 μmol) in trifluoroacetic acid/water (4/1, 4 mL/1 mL) was stirred at room temperature for 15 min. The solvents were evaporated to give **9** (31.9 mg, 69.6 μmol) as a white solid: m.p. 95°C (dec); $[\alpha]_D^{20}$ − 10.8 (*c* 0.5, DMSO); IR (KBr) 3391, 1674, 1202, 1129 cm^{-1}; ^1H NMR (D_2O) δ 1.84–1.93 (m, 1 H, H4-pyro), 2.03-2.12 (m, 1 H, H3-pyro), 2.19–2.27 (m, 1 H, H3-pyro), 2.36–2.46 (m, 1 H, H4-pyro), 4.22 (dd, J = 5.0 and 9.4 Hz, 1 H, H5-pyro), 4.30–4.33 (m, 1 H, H4′), 4.46–4.54 (m, 3 H, H5′ and H3′), 4.70 (t, J = 4.3 Hz, 1 H, H2′), 6.05 (d, J = 4.3 Hz, 1 H, H1′), 8.33 and 8.37 (2 s, 2 H, H2 and H8); ^{13}C NMR (D_2O) δ 24.7 (C4-pyro), 28.9 (C3-pyro), 57.1 (C5-pyro), 69.4 (C3′), 71.1 (C5′), 73.6 (C2′), 81.3 (C4′), 88.3 (C1′), 116.2 (q, J = 289.2 Hz, TFA), 118.7 (C5), 142.7 (C8), 144.6 (C2), 148.4 (C4), 149.9 (C6), 162.7 (q, J = 35.8 Hz, TFA), 172.7 ($C(O)NHSO_3$), 181.7 ($CH_2C(O)NH$); LRMS (ESI) 458 (M^+).

Pharmacology

Purification of glutamyl-tRNA synthetase (GluRS) from Escherichia coli and mouse liver. GluRS from *Escherichia coli* was purified to homogeneity from the overexpressing strain *E. coli* DH5α(pLQ7612), as described previously[23]. A fraction containing GluRS and other aaRSs as part of a high molecular weight complex, was purified from mouse liver, according to the procedure described by Godar and Yang[24]. Ten mice were euthanized by CO_2 and sacrificed by cervical dislocation. Their livers were dissected out and homogenized on ice with a Polytron (3 × 25 s at 1 min intervals, with intensity 5) in 2.5 volumes of buffer T (50 mM Tris–HCl pH 7.8, 4 mM $MgCl_2$, 2 mM dithiothreitol, 10% glycerol, 0.04% NaN_3 and 0.5 mM PMSF) after increasing the PMSF concentration to 10 mM. All the following operations were conducted on ice or at 4°C. After 20 min centrifugation at 17,000*g*, solid ammonium sulfate was slowly added to the supernatant up to 30% saturation. After removing the precipitate by centrifugation (20 min, 17,000*g*), the ammonium sulfate concentration was increased to 50%, and the precipitate (the 30–50% fraction) was kept, solubilized in buffer T and further purified on an Amicon Ultra-15 (30 K) membrane to eliminate aminoacids and small metabolites. The final enzyme fraction was stored at − 20°C in buffer T containing 50% glycerol.

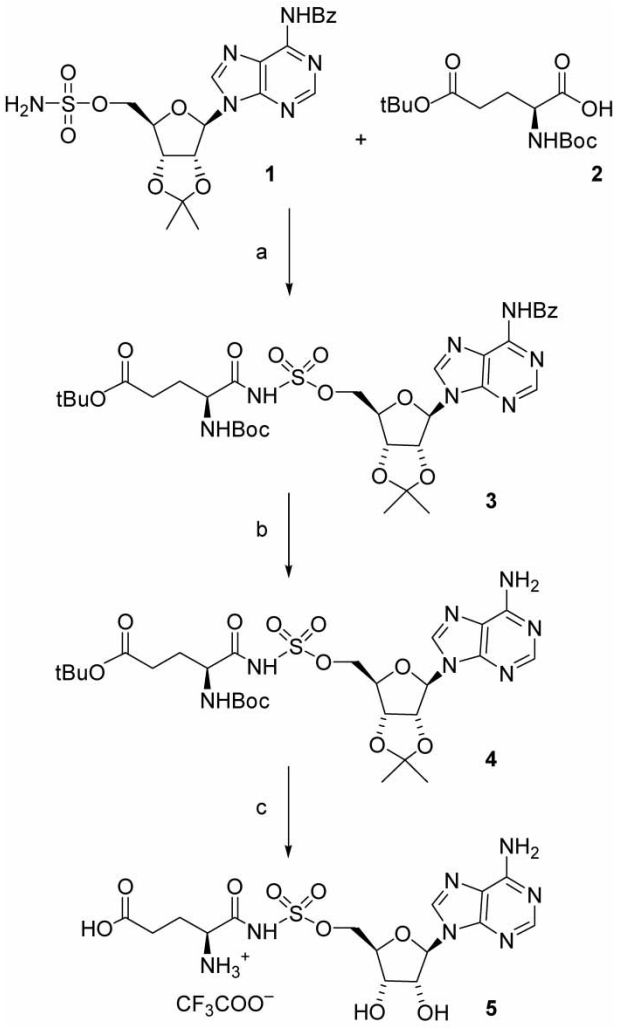
Enzyme assays, and kinetic constant measurements. *E. coli* GluRS activity was determined in the aminoacylation reaction, under the conditions described by Lapointe *et al.*[25] Mammalian GluRS aminoacylation activity was measured as previously described for bovine GluRS[26], in the presence of 125 mM Tris-acetate pH 8.0, 225 μM [^{14}C]L-glutamate, 10 mM ATP, 5 mM $MgCl_2$, 0.1 mM EDTA, 2 mM dithiothreitol, 100 to 150 μM unfractionated tRNA from bovine liver, and the partially purified mouse aaRS fraction diluted in buffer T containing 10% glycerol, 0.1 mg/mL bovine serum albumin and the "Complete EDTA-free Protease Inhibitor Cocktail" (from Roche Molecular Biochemicals). To determine the initial rate of the aminoacylation reaction, the amount of [^{14}C]glutamyl-tRNA was measured in 40 μL aliquots taken every 2 min over a 10 min incubation period at 30°C.

For the determination of the K_i value of the inhibitors with respect to glutamate for *E. coli* GluRS, we first measured the K_m^{app} values for glutamate from Lineweaver-Burk plots of initial rates of aminoacylation at various concentrations of glutamate (the variable substrate), under fixed and saturating concentrations of the two other substrates (ATP and tRNA), in the presence of various fixed concentrations of the inhibitor. From these values, K_i was measured graphically using a Dixon plot, as described by Segal[27].

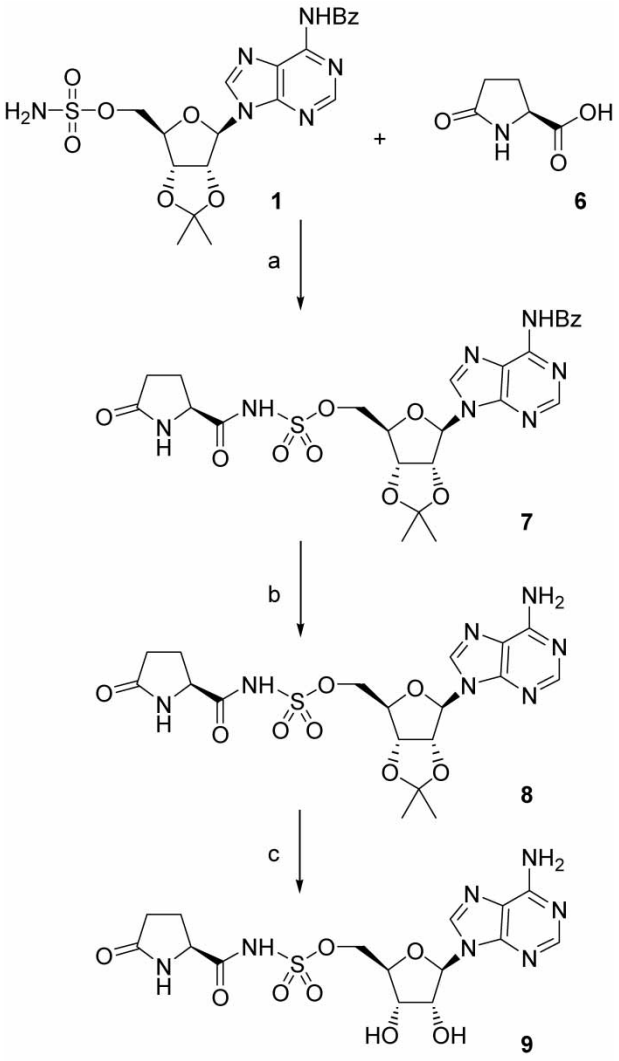
Results

Chemistry

Synthesis of 5′-O-[N-(L-glutamyl)-sulfamoyl]adenosine 5. 5′-O-Sulfamoyladenosine derivative **1** was prepared by reaction of N^6-benzoyl-2′,3′-*O*-isopropylideneadenosine [28] with sulfamoyl chloride[29,30]. Condensation of commercially



Scheme 2. (a) DCC, DMAP, CH_2Cl_2, 80%; (b) $nBuNH_2$, MeOH, 99%; (c) TFA/H_2O (4:1), 98%.



Scheme 3. (a) DCC, DMAP, CH_2Cl_2, 80%; (b) $nBuNH_2$, MeOH, 95%; (c) TFA/H_2O (4:1), 99%.

available *N*-t-Boc-L-glutamic acid γ-t-butyl ester **2** with sulfamoyladenosine derivative **1** in the presence of dicyclohexylcarbodiimide (DCC) and one full equivalent of dimethylaminopyridine (DMAP) in dichloromethane[31] gave product **3** (Scheme 2). Compound **3** was dissolved in methanol and treated with n-butylamine to remove the N-benzoyl moiety. Reaction of **4** with wet trifluoroacetic acid provoked the removal of the three remaining protecting groups (*tert*-butyl ester, N-Boc, isopropylidene) to afford compound **5**.

Synthesis of 5′-O-[N-(L-pyroglutamyl)-sulfamoyl] adenosine 9. Pyroglutamylsulfamoyladenosine **9** was prepared according to the same reaction sequence (Scheme 3). Acylation of sulfonamide **1** by pyro-L-glutamic acid **6** in the presence of DCC and DMAP yielded compound **7**. Treatment with n-butylamine to remove the N-benzoyl group, followed by acid-catalyzed hydrolysis of the 2′,3′-O-isopropylidene derivative **8** gave 5′-*O*-[*N*-(L-pyroglutamyl)-sulfamoyl] adenosine **9**. Compounds **5** and **9** slowly decompose in the solid state but are stable for several months in a buffer solution.

Pharmacology

Inhibition of Escherichia coli glutamyl-tRNA synthetase (GluRS) by glutamylsulfamoyladenosine 5 and pyroglutamylsulfamoyladenosine 9. The influence of 5′-*O*-[*N*-(L-glutamyl)sulfamoyl] adenosine **5** (Glu-AMS) and of 5′-*O*-[*N*-(L-pyroglutamyl) sulfamoyl]adenosine **9** (pGlu-AMS) on the activity of pure GluRS from *E. coli* was determined using the formation of [^{14}C]glutamyl-tRNA as an assay. Glu-AMS **5** is a very potent ($K_i = 2.8$ nM) inhibitor of this enzyme and is competitive with respect to glutamic acid (Figure 1). The related compound pGluAMS **9**, which differs from Glu-AMS only by the cyclization of the side chain of the glutamate residue is a much weaker

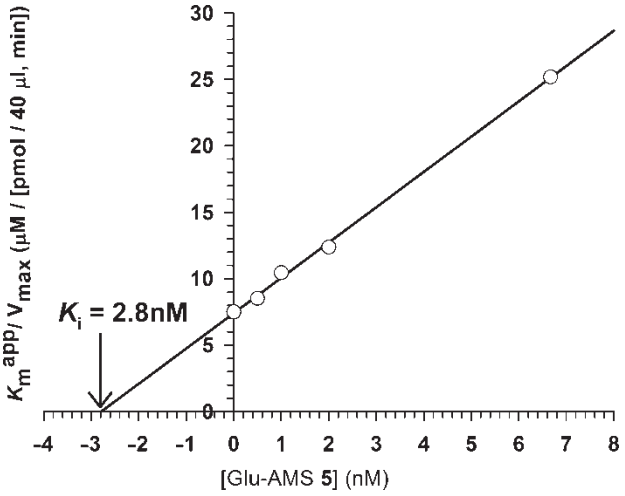


Figure 1. Competitive inhibition of *E. coli* GluRS by Glu-AMS **5**. K_m^{app} for glutamate was determined with six concentrations of glutamate (25–600 µM) in the presence of 0–6.7 nM Glu-AMS **5** and of saturating concentrations of ATP and tRNA. The aminoacylation activities were measured at 37°C. These data show that Glu-AMS **5** is a competitive inhibitor of *E. coli* GluRS with respect to glutamate, with a K_{ic} of 2.8 nM.

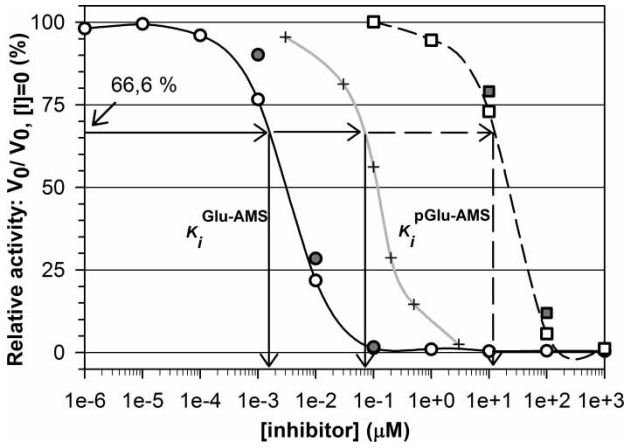


Figure 2. Estimation of the inhibition constant of pGlu-AMS **9** for *E. coli* GluRS, and of Glu-AMS **5** for murine GluRS. The relative aminoacylation activities of *E. coli* GluRS were measured at 37°C in the presence of 100 µM glutamate ($[S] = K_m^{Glu}$) and saturating concentrations of ATP and tRNA in the presence of various concentrations of pGlu-AMS **9** (squares) and, as a control, of Glu-AMS **5** (circles). Assuming that the inhibition by pGlu-AMS is competitive with respect to glutamate, the ratio of the initial rate "v" of the reaction in the absence of inhibitor, to the rate "v_i" in the presence of an inhibitor, at various concentrations of this inhibitor, was used to estimate the K_{ic} value as follows: $v = V_{max}S/(S + K_m)$, $v_i = V_{max}S/(S + K_m[1 + I/K_{ic}])$, where S and I are the substrate and inhibitor concentrations, respectively. Hence, $v_i/v = (S + K_m)/(S + K_m[1 + I/K_{ic}])$. When $S = K_m$, and $I = K_{ic}$, $v_i/v = 0.66$. The results presented here are consistent with the K_{ic} of 2.8 nM for Glu-AMS **5** shown on Figure 1, and indicate that K_{ic} is about 15 µM for p-Glu-AMS **9**. Nearly identical inhibition curves were obtained with fresh preparations of these inhibitors (empty symbols) and with solutions kept at −20°C in 50 mM potassium HEPES pH 7.5 during five months (filled symbols). The K_{ic} value of Glu-AMS **5** for murine GluRS (+) was estimated by the same approach under conditions described in the text, using a K_m^{Glu} value of 0.2 mM determined under the same conditions.

inhibitor, with a K_i value of 15 µM with respect to glutamic acid (Figure 2). The near identity of the K_i values obtained with fresh preparations of these inhibitors and with solutions kept at −20°C in 50 mM potassium HEPES pH 7.5 during five months (Figure 2) reveals the stability of Glu-AMS **5** and of pGlu-AMS **9** under these conditions.

Weaker inhibition of mammalian GluRS than of E. coli by Glu-AMS 5. The influence of Glu-AMS was also tested on murine GluRS present in high molecular weight complexes isolated from liver. The K_m^{Glu} of murine GluRS for glutamate in the aminoacylation reaction is 0.2 mM (results not shown), a value similar to that (0.14 mM) reported for the bovine enzyme[26]. Assuming that Glu-AMS **5** is a competitive inhibitor of murine GluRS, as it is for *E. coli* GluRS, the relation between Glu-AMS **5** concentration and the initial rate of glutamyl-tRNA formation at a glutamate concentration equal to K_m^{Glu}, leads to a $K_i^{Glu-AMS}$ value of about 70 nM for murine GluRS (Figure 2), which is 25-fold higher than that for *E. coli* GluRS.

Discussion

Several aminoalkyl adenylates and aminoacyl-sulfamoyladenosines (Figure 3) derived from standard amino acids have been prepared and tested for inhibition of their cognate aaRS [6]. With its K_{ic} of 2.8 nM with respect to glutamate for *E. coli* GluRS, Glu-AMS **5** is by far the best inhibitor of this class I aaRS. The corresponding aminoalkyl adenylate, glutamol-AMP is a 1000-fold weaker inhibitor of GluRS (K_i = 3000 nM)[32]. A similar though smaller difference was reported from *Staphylococcus aureus* isoleucyl-tRNA synthetase (IC_{50} = 4 nM for Ile-AMS and 780 nM for Ile-ol-AMP)[33] which is also a class I aaRS. On the other hand, the inhibition of three other class I aaRSs by the corresponding aminoalkyl adenylates and aminoacylsulfamoyladenosines are of the same order of magnitude: *S. aureus* arginyl-tRNA synthetase (IC_{50} = 7.5 and 4.5 nM, respectively)[14] and tyrosyl-tRNA synthetase (IC_{50} = 11 and 26 nM, respectively),[11] and *E. coli* glutaminyl-tRNA synthetase (GlnRS) (K_i = 280 and 1320 nM, respectively)[16,34].

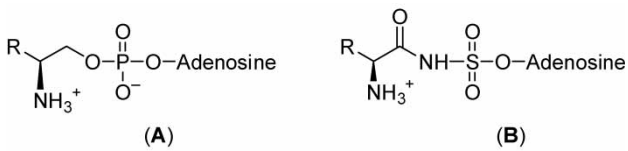


Figure 3. Structures of aminoalkyl adenylates (A) and aminoacylsulfamoyladenosines (B).

The relative weakness of glutaminylsulfamoyl-adenosine as a GlnRS inhibitor was seen as an indication that it represents accurately the GlnRS-bound amino-acyladenylate intermediate[16]. Likewise, the fact that glutamol-AMP is a weak inhibitor suggests that it represents accurately the GluRS-bound aminoacyl-adenylate intermediate. This model is supported by the comparison of the structures of GluRS/ATP/Glu and GluRS/tRNA/ATP with that of GluRS/tRNA/glutamol-AMP which shows that the latter represents the enzyme state after the glutamate activation reaction[35]. On the other hand, the 1000-fold stronger inhibitory power of Glu-AMS **5** suggests that it resembles the transition state of the glutamate activation reaction (see Scheme 1).

pGlu-AMS **9**, which differs from Glu-AMS **5** only by the cyclization of the γ-carboxylic acid side chain with the α-amino group of glutamic acid, is a weaker inhibitor ($K_i = 15\ \mu M$). This result may be explained by the fact that in pGlu-AMS **9**, a neutral amide group, replaces the negatively charged γ-carboxylate known to interact with a complementary pocket formed by four residues and the positively charged α-ammonium that hydrogen-bonds with three residues in the *Thermus thermophilus* GluRS/ATP/Glu complex[35]. Moreover, the glutamate side chain is fully extended in this complex[35]. Considering all these lost contacts, it is surprising that the pyro compound **9** binds GluRS significantly ($K_i = 15\ \mu M$) compared to ATP ($K_m = 180\ \mu M$) and AMP ($K_i = 3\ mM$).[36]

Glu-AMS inhibits the murine liver GluRS with a 25-fold lower efficiency than *E. coli* GluRS (Figure 2). This result reflects structural differences between the active sites of the bacterial and mammalian GluRSs. These differences could be exploited to design a bacterial GluRS inhibitor that would have bactericidal activity and low toxicity for the mammalian host.

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