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Glutamyl Adenylate Analogues Are Inhibitors of Glutamyl-tRNA Synthetase

Michel Desjardins,* Sylvie Garneau,* Julie Desgagnés,* Lucille Lacoste,† Fu Yang,† Jacques Lapointe,† and Robert Chênevert*,[1]

**Département de chimie et †Département de biochimie, Faculté des sciences et de génie, Université Laval, Québec, Canada G1K 7P4*

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Glutamol adenylate **10** was a competitive inhibitor ($K_i = 3\ \mu M$) of glutamyl-tRNA synthetase from *Escherichia coli*. The N^6-benzoyl adenine derivative **9** was also an inhibitor ($K_i \sim 60\ \mu M$). Replacement of adenine by other bases (purine, cytosine, dihydrocytosine, uridine) resulted in a more than 1000-fold loss in activity, indicating the important contribution of the adenine ring to the enzyme binding.

In the first stage of protein biosynthesis, the 20 standard amino acids are esterified to their cognate transfer RNA (tRNA) by the action of a class of enzymes, the aminoacyl-tRNA synthetases (aaRS). It has been established that this reaction is a two-step event (*1*, *2*). In the first step, the amino acid reacts with ATP, with displacement of pyrophosphate (PP_i) to form an enzyme-bound mixed anhydride (aminoacyl adenylate). In this intermediate, the high-energy anhydride bond activates the carboxyl group of the amino acid. In the second step, the activated amino acid is transferred to the CCA end of the corresponding tRNA to form the aminoacyl-tRNA and AMP. This transfer is a nucleophilic attack of the 2′ or 3′ ribose hydroxyl group of the terminal AMP residue at the 3′ end of the tRNA on the activated carboxyl group of the intermediate. The two steps are the following:

$$\text{Amino acid} + \text{ATP} + \text{aaRS} \overset{Mg^{2+}}{\rightleftharpoons} \text{(aa-AMP)aaRS} + PP_i,$$

$$\text{(aa-AMP)aaRS} + \text{tRNA} \rightleftharpoons \text{aa-tRNA} + \text{AMP} + \text{aaRS}.$$

The resulting aminoacyl-tRNAs are the major activated forms of amino acids in the living cells, and they are used mostly for protein biosynthesis on the ribosomes. Selective inhibition of bacterial aaRS has proved to be a successful strategy for the production of anti-bacterial agents (*3*). Pseudomonic acid (generic name: mupirocin), isolated from *Pseudomonas fluorescens*, is a highly potent inhibitor of bacterial isoleucyl-tRNA synthetases (*4*, *5*). This antibiotic shows a very high selectivity in favor of prokaryote forms of this enzyme and plays an important clinical role.

[1] To whom correspondence should be addressed. Fax: (418) 656-7916. E-mail: robert.chenevert@chm.ulaval.ca.

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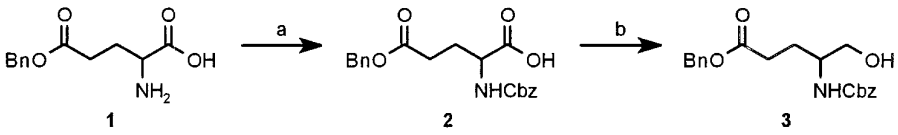


SCHEME 1. Reagents: (a) $NaHCO_3$, CbzCl, H_2O : THF (1 : 1); (b) Et_3N, ethyl chloroformate (−10°C), $NaBH_4$ (0°C), H_2O, THF.

Synthetic analogues of aminoacyl adenylates are inhibitors of the aminoacyl-tRNA synthetase (*6–12*). Prolylsulfamoyladenosine is a potent inhibitor of both human and *Escherichia coli* prolyl-tRNA synthetase (*6*). This compound is a mimic of the mixed anhydride intermediate in which the phosphate group is replaced by the isosteric but more stable sulfamoyl linkage (*7*, *8*). Replacement of the amino acid by the corresponding amino alcohol produced stable esters (aminoalkyl adenylates) which are good inhibitors of the cognate aaRS (*9*, *10*). Aminophosphonyl adenylates are also inhibitors of aaRS (*11*, *12*).

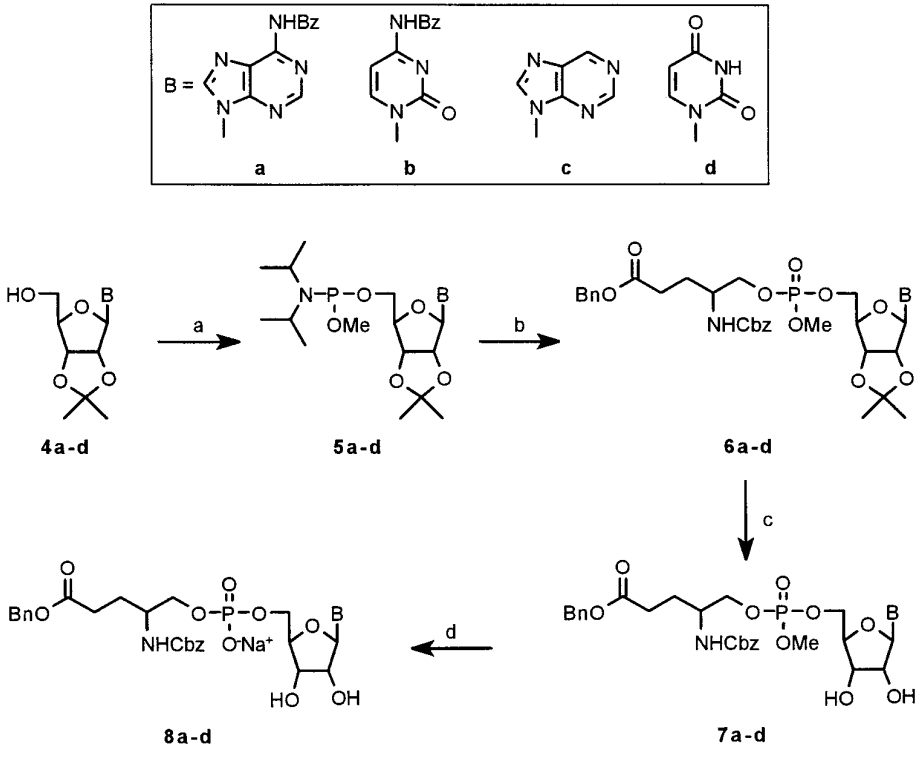
Glutamyl-tRNA synthetase (GluRS) has the characteristic, which is common also to the glutaminyl- and arginyl-tRNA synthetases from *E. coli* and all other organisms in which these enzymes have been studied, of requiring the presence of its cognate tRNA to catalyze the activation of its amino acid substrate. With the aim of obtaining inhibitors of GluRS, we have synthesized analogues of glutamyl adenylate and tested their properties in the aminoacylation reaction of $tRNA^{Glu}$ catalyzed by *E. coli* GluRS.

RESULTS AND DISCUSSION

Synthesis of Glutamyl Adenylate Analogues

The commercially available L-glutamic acid γ-benzyl ester **1** was converted into the corresponding *N*-benzyloxycarbonyl (*N*-Cbz) derivative **2** under standard conditions. Reduction of **2** via a mixed carbonic anhydride with sodium borohydride gave alcohol **3** (Scheme 1).

N^6-Benzoyl-2′,3′-*O*-isopropylideneadenosine **4a** was prepared by benzoylation of commercially available 2′,3′-isopropylideneadenosine. The phosphoramidite-phosphite triester approach was used for the condensation between alcohol **3** and adenosine derivative **4a** (Scheme 2). Compound **4a** was first phosphorylated with *N*,*N*-diisopropylmethylphosphonamidic chloride in the presence of *N*,*N*-diisopropylethylamine in dry CH_2Cl_2 to give **5a.** Phosphoramidite **5a** was coupled with **3** in dry acetonitrile using 1H-tetrazole as an activating agent and then the phosphite triester was oxidized to phosphate triester **6a** by treatment with iodine. This phosphotriester was deprotected by sequential treatment with wet trifluoroacetic acid (hydrolysis of the isopropylidene acetal group) to give **7a** and then with sodium iodide in butanone to give phosphodiester **8a.** Other compounds (**4b, c, d** to **8b, c, d**) were prepared according to the same reaction sequence (Scheme 2).



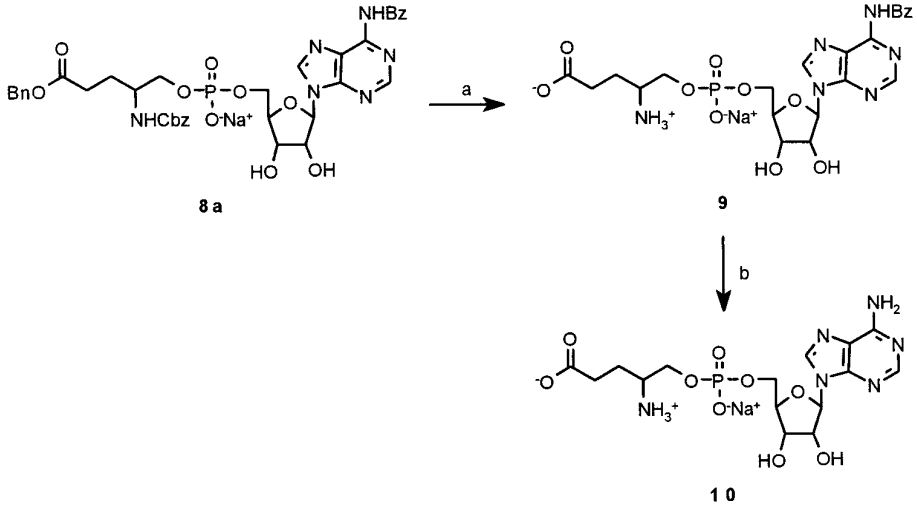
SCHEME 2. Reagents: (a) *N*,*N*-diisopropylmethylphosphonamidic chloride, *i*-Pr_2NEt, CH_2Cl_2; (b) (i) **3**, tetrazole, MeCN, (ii) I_2, H_2O : THF : pyr (1 : 1 : 1); (c) CF_3CO_2H : H_2O (9 : 1); (d) NaI, 2-butanone.

Hydrogenolysis of the benzyl ester and the benzyloxycarbonyl group of **8a** in the presence of a 10% palladium-on-charcoal catalyst provided compound **9** with the *N*-benzoyl protecting group on the purine base. This group was removed by treatment with NH_4OH to give compound **10** (Scheme 3). With compounds **8b–8d,** reduction of the bases was observed during the cleavage of protecting groups with molecular hydrogen. For instance, hydrogenation of **8b** with hydrogen followed by aminolysis of the *N*-benzoyl group gave the dihydrocytosine derivative **11** as the sole product (Scheme 4). Transfer hydrogenation with 1,4-cyclohexadiene as hydrogen donor was used in the purine, cytosine, and uracyl series (**8b–8d** to **12–14,** Schemes 4 and 5).

In structures **5, 6,** and **7,** the phosphorus is a center of chirality and these compounds were obtained as a mixture of diastereoisomers. The nonequivalence (chemical shift difference of some groups) was observed in ^1H, ^{13}C, and ^{31}P NMR spectra.

*Inhibition of GluRS by Compounds **9–14***

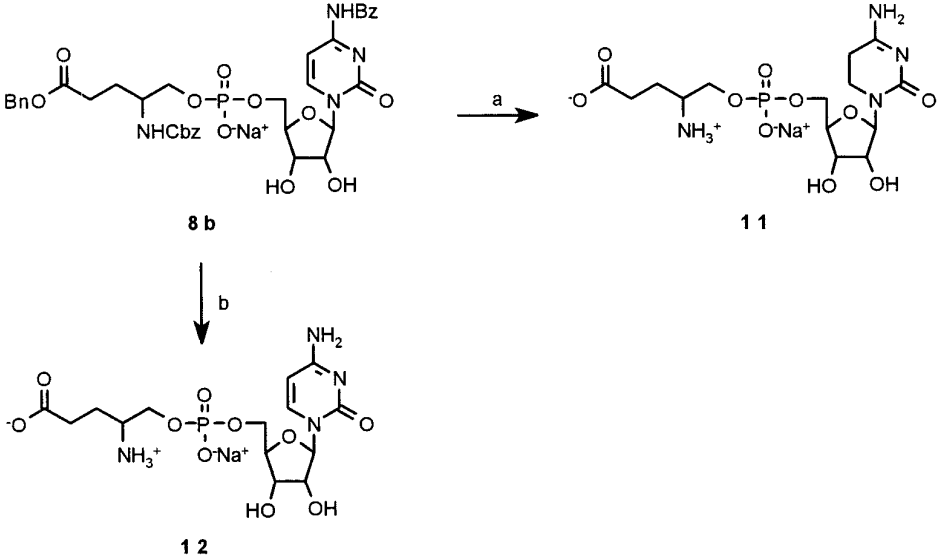
L-Glutamol-AMP **10** inhibits *E. coli* GluRS in a competitive fashion (Fig. 1), since the maximum velocity V_{max} is unchanged, whereas the apparent K_m^{app} is increased for increasing concentrations of this inhibitor [I]. The replot of K_m^{app} versus



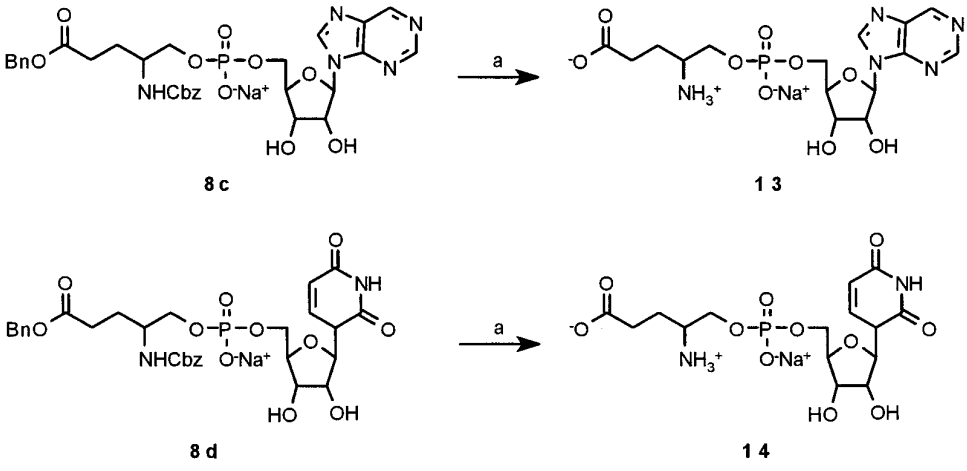
SCHEME 3. Reagents: (a) H_2, Pd/C, H_2O; (b) NH_4OH.

[I] gives the K_i value, which is 3 ± 1 μM for the two substrates studied, glutamate and ATP (Fig. 2). There is no inhibition of *E. coli* glutaminyl-tRNA synthetase by this compound.

Other compounds (**9, 11–14**) inhibit *E. coli* GluRS slightly. The K_i values are listed in Table 1. The N^6-benzoyl adenine derivative **9** was also an inhibitor ($K_i \sim$



SCHEME 4. Reagents: (a) (i) H_2, Pd/C, H_2O, (ii) NH_4OH; (b) (i) Pd/C, 1,4-cyclohexadiene, EtOH, (ii) NH_4OH.



SCHEME 5. Reagents: (a) H_2, 1,4-cyclohexadiene, EtOH.

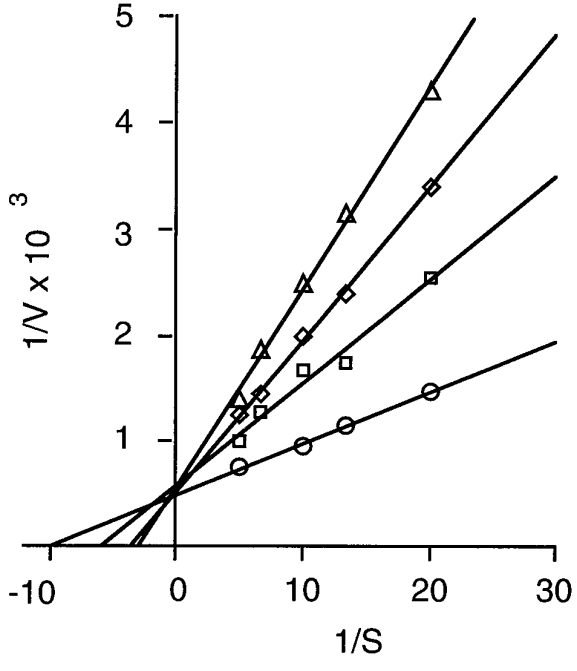


FIG. 1. Determination of the apparent K_m for glutamate of *E. coli* GluRS in the presence of different fixed concentrations of inhibitor **10**: 0 μM (○), 4 μM (□), 6 μM (◇), and 8 μM (△).

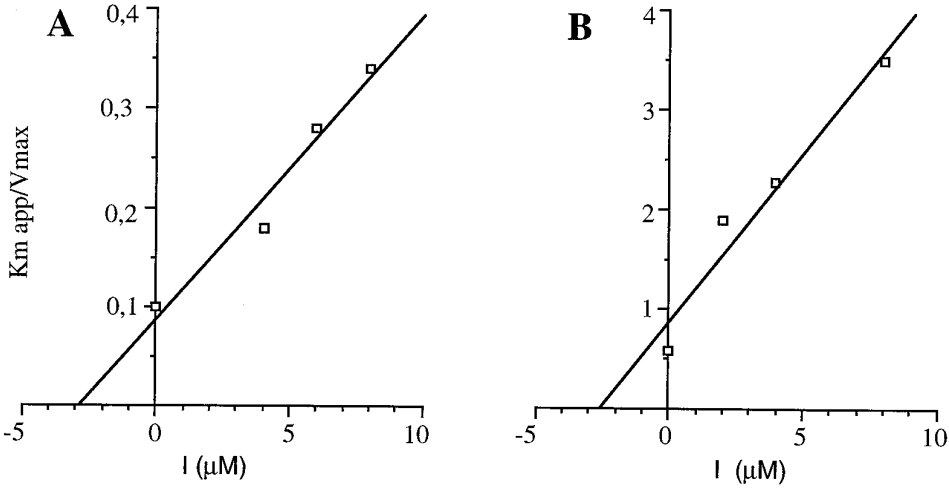


FIG. 2. Determination of the K_i values of inhibitor **10** for *E. coli* GluRS, with respect to glutamate (A) and ATP (B).

60 μM). Replacement of adenine by dihydrocytidine (**11**), cytidine (**12**), purine (**13**), and uridine (**14**) resulted in a three order of magnitude loss in activity (0.63 mM $< K_i <$ 16.7 mM), indicating the important contribution of the adenine ring to the enzyme binding.

In addition to gaining mechanistic information about *E. coli* GluRS, specific inhibitors such as **10** should facilitate the crystallization of the enzyme.

EXPERIMENTAL

Synthesis of Inhibitors

*N-Benzyloxycarbonyl-5-O-benzyl-*L*-glutamic acid* ***(2).*** To a mixture of γ-benzyl L-glutamate **(1)** (2.46 g, 10.4 mmol) and $NaHCO_3$ (1.76 g, 20.8 mmol) in 20 mL of water was added a solution of benzyl chloroformate (1.57 mL, 11 mmol) in 20 mL

TABLE 1
Inhibition of *E. coli* GluRS by Glutamyl Adenylate Analogues

Compound (base)	K_i (μM)
9 (N^6-benzoyladenine)	60
10 (adenine)	3
11 (dihydrocytidine)	16,700
12 (cytidine)	630
13 (purine)	4,100
14 (uridine)	2,750