BIOORGANIC CHEMISTRY **26,** 1–13 (1998)
ARTICLE NO. BH981082

Glutamyl Adenylate Analogues Are Inhibitors of Glutamyl-tRNA Synthetase

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Received June 29, 1997

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.

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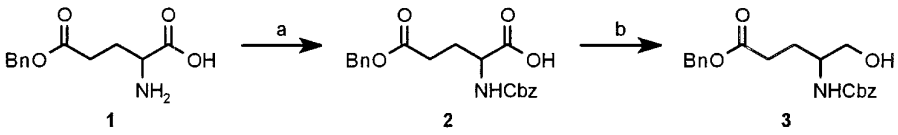
0045-2068/98 $25.00



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*).

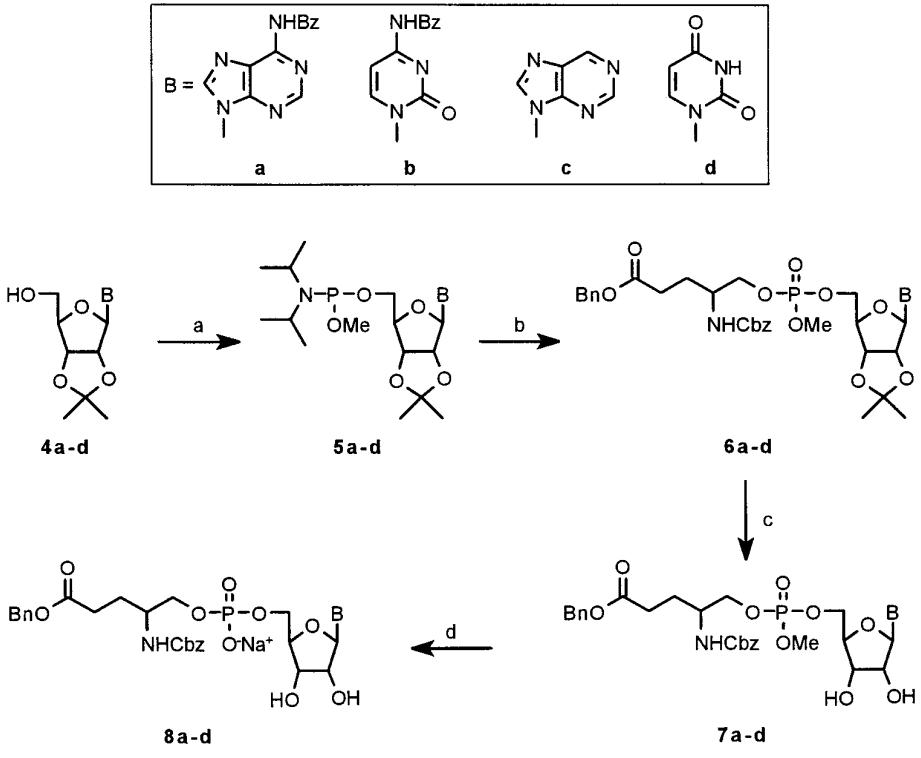
Glutamyl-tRNA synthetase (GluRS) has the characteristic, which is common also to the glutaminyl- and arginyl-tRNA synthetases from *E. coli* and all other organisms in which these enzymes have been studied, of requiring the presence of its cognate tRNA to catalyze the activation of its amino acid substrate. 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).



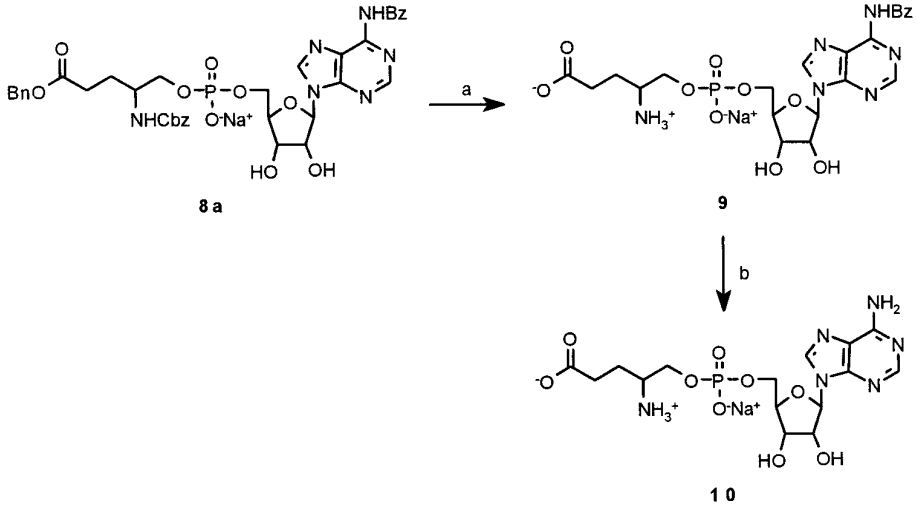
SCHEME 2. Reagents: (a) *N*,*N*-diisopropylmethylphosphonamidic chloride, *i*-Pr_2NEt, CH_2Cl_2; (b) (i) **3**, tetrazole, MeCN, (ii) I_2, H_2O : THF : pyr (1 : 1 : 1); (c) CF_3CO_2H : H_2O (9 : 1); (d) NaI, 2-butanone.

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***

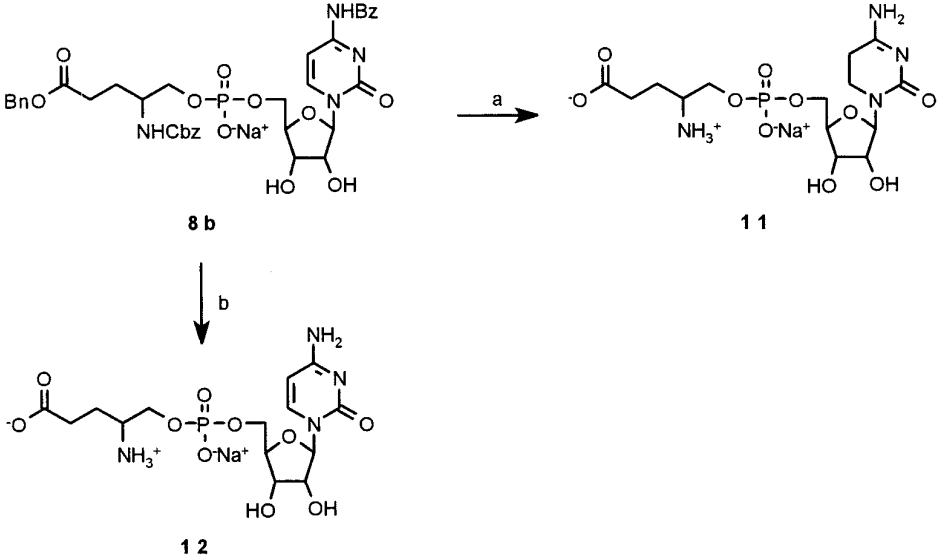
L-Glutamol-AMP **10** inhibits *E. coli* GluRS in a competitive fashion (Fig. 1), since the maximum velocity V_{max} is unchanged, whereas the apparent K_m^{app} is increased for increasing concentrations of this inhibitor [I]. The replot of K_m^{app} versus



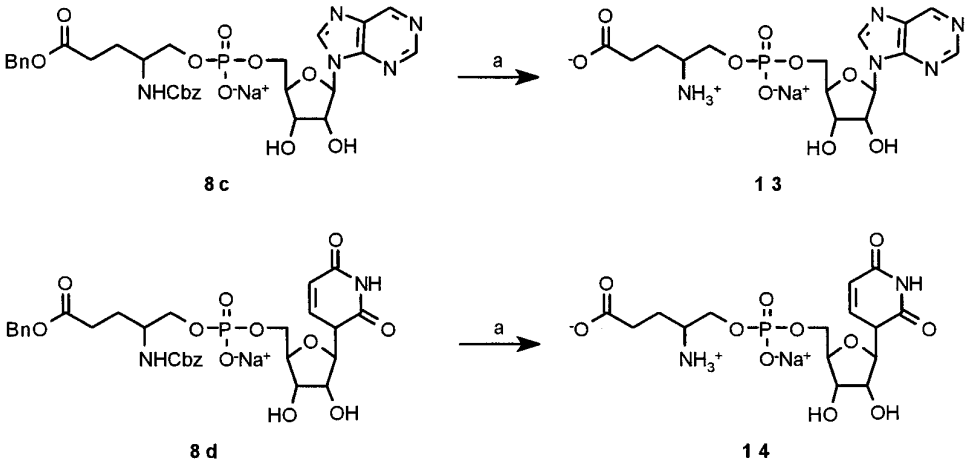
SCHEME 3. Reagents: (a) H_2, Pd/C, H_2O; (b) NH_4OH.

[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.

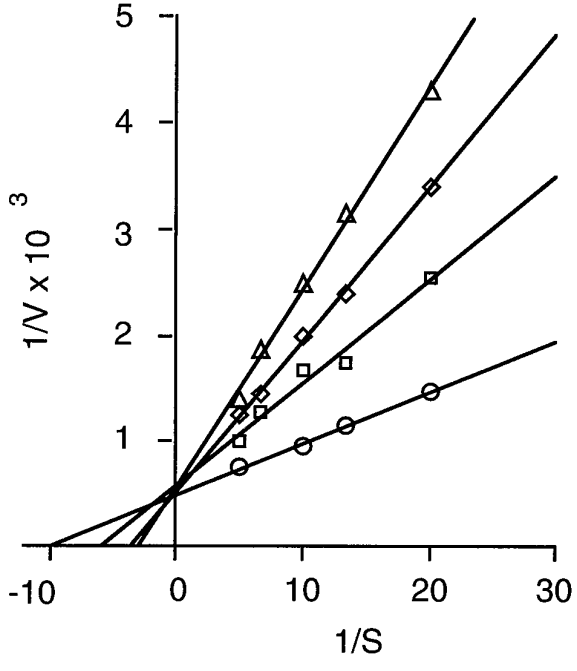


FIG. 1. Determination of the apparent K_m for glutamate of *E. coli* GluRS in the presence of different fixed concentrations of inhibitor **10**: 0 μM (○), 4 μM (□), 6 μM (◇), and 8 μM (△).

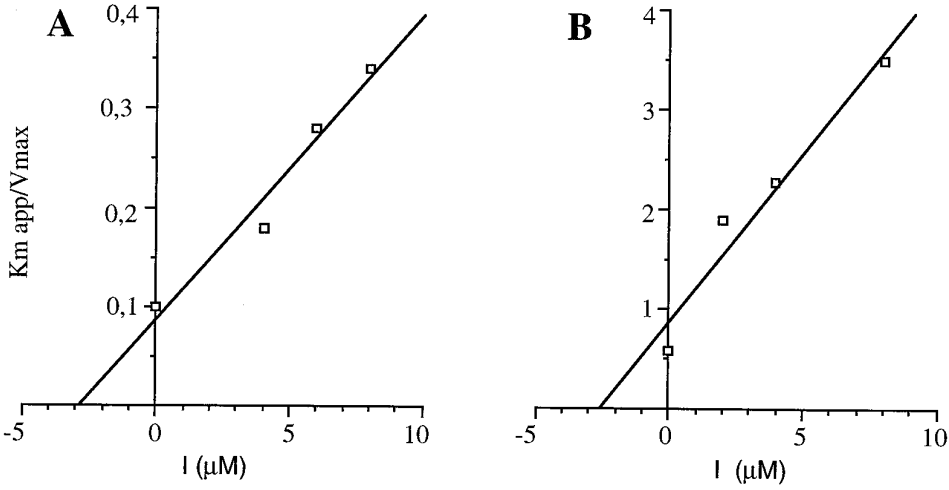


FIG. 2. Determination of the K_i values of inhibitor **10** for *E. coli* GluRS, with respect to glutamate (A) and ATP (B).

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

of THF. The mixture was stirred for 18 h at room temperature. The organic solvent (THF) was removed under reduced pressure. The aqueous layer was washed with EtOAc, acidified to pH 3 with 10% aqueous citric acid, and then extracted with EtOAc. The combined organic extracts were washed with brine, dried, and concentrated *in vacuo*. The crude product was recrystallized from CCl_4 to yield **2** as a white solid (3.40 g, 88%). IR (KBr) 3600–2800, 3300, 1730–1690, 1535, 1460, 1440, 1390, 1330, 1315, 1250, 1170, 1085, 1065, 1040, 950, 735, 690 cm^{-1}; ^1H NMR ($CDCl_3$) 2.05 (m, 1H), 2.25 (m, 1H), 2.46 (m, 2H), 4.42 (m, 1H), 5.07 (s, 4H), 5.64 (d, J = 7.9 Hz, 1H), 7.30 (ls, 10H), 10.67 (ls, 1H); ^{13}C NMR ($CDCl_3$) 27.14, 30.19, 53.12, 66.57, 67.17, 128.02, 128.16, 128.22, 128.45, 128.46, 135.52, 135.90, 156.21, 172.89, 175.89.

Benzyl 4-[(benzyloxycarbonyl)amino]-5-hydroxypentanoate ***(3).*** Ethyl chloroformate (1.26 mL, 13.26 mmol) was added at 10°C to a stirred solution of acid **2** (2.46 g, 6.63 mmol) and Et_3N (0.95 mL, 6.63 mmol) in dry THF (22 mL). The mixture was stirred at 10°C for 30 min. Then, the precipitate was filtered, and the filtrate was added during 15 min at 0°C to a solution of $NaBH_4$ (744 mg, 19.9 mmol) in 22 mL of H_2O : THF (1 : 4). The mixture was stirred for 4 h at room temperature. The mixture was acidified with 1 N HCl, and THF was evaporated. The aqueous layer was extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$, water, and brine; dried ($MgSO_4$); and concentrated *in vacuo*. The crude product was purified by flash chromatography (CH_2Cl_2 : EtOAc, 3 : 1) to yield **3** as a white solid (1.90 g, 80%). IR (KBr) 3600–3200, 3060, 3020, 2940, 1730–1680, 1525, 1450, 1240, 1160, 1065, 905, 725, 690 cm^{-1}; ^1H NMR ($CDCl_3$) 1.85 (m, 2H), 2.45 (t, J = 7.2 Hz, 2H), 2.60 (m, 1H), 3.50–3.80 (m, 3H), 5.06 (s, 2H), 5.09 (s, 2H), 5.18 (d, J = 8.3 Hz, 1H), 7.30 (m, 10H); ^{13}C NMR ($CDCl_3$) 26.13, 30.71, 52.56, 64.57, 66.39, 66.70, 127.94, 128.02, 128.13, 128.18, 128.40, 128.46, 135.64, 136.25, 156.54, 173.44.

Compound ***5a.*** N^6-Benzoyl-2′,3′-isopropylideneadenosine (**4a**) (4.84 g, 10 mmol) was dissolved in dry CH_2Cl_2 (45 mL) containing *i-Pr*$_2$EtN (8 mL, 46 mmol). *N*,*N*-Diisopropylmethylphosphonamidic chloride (3.6 mL, 18 mmol) was added rapidly with a syringe to the magnetically stirred solution at room temperature under dry atmosphere. After 12 min the solution was poured into EtOAc. The organic solution was washed with brine, dried ($MgSO_4$), and evaporated. The crude product was purified by flash chromatography on silica gel (hexane : $CHCl_3$: Et_3N, 12 : 8 : 1) to yield **5a** (4.98 g, 74%). IR (KBr) 3000–2800, 2235, 1700, 1605, 1580, 1505, 1450, 1240, 1205, 1175, 1150, 1070, 1020, 970, 905, 790, 725, 640 cm^{-1}; ^1H NMR ($CDCl_3$) 1.08–1.17 (m, 12H), 1.41 and 1.66 (2s, 6H), 3.36 and 3.41 (2d, $J_{P\text{-}H}$ = 13.2 Hz), 3.50–3.60 (m, 2H), 3.82 (m, 2H), 4.56 (m, 1H), 5.02 (m, 1H), 5.32 (m, 1H), 6.26 and 6.29 (2d, J = 2.8 Hz, 1H), 7.50–7.65 (m, 3H), 8.02 (d, J = 7.4 Hz, 2H), 8.34 and 8.40 (2s, 1H), 8.84 and 8.85 (2s, 1H), 8.98 (ls, 1H); ^{13}C NMR ($CDCl_3$) 24.50, 25.20, 27.09, 42.62, 50.40, 50.64, 63.05, 63.38, 81.60, 81.80, 84.58, 84.71, 86.19, 86.31, 91.30, 91.40, 114.05, 114.16, 123.13, 123.21, 127.77, 128.59, 132.50, 133.64, 141.79, 141.86, 149.43, 151.22, 151.33, 152.60, 164.53; ^{31}P NMR ($CDCl_3$) 150.24, 150.29.

Compound ***5b.*** Yield 78%. IR (KBr) 2950, 2225, 1650, 1550, 1370, 1300, 1250, 1060, 970, 900, 865, 780, 720, 640 cm^{-1}; ^1H NMR ($CDCl_3$) 1.04–1.13 (m, 12H), 1.30 (s, 3H), 1.53 (s, 3H), 3.31 and 3.36 (2d, $J_{P\text{-}H}$ = 13.5 Hz, 3H), 3.40–3.56 (m, 2H), 3.70–3.96 (2m, 2H), 4.44 (m, 1H), 4.75 (d, J = 3.0 Hz, 2H), 5.95 (d, J = 6.1 Hz, 1H), 7.39–7.56 (m, 4H), 7.83 (d, J = 7.3 Hz, 2H), 8.13 and 8.15 (2d, J = 3.7 Hz,

1H); ^{13}C NMR ($CDCl_3$) 24.45, 24.49, 24.53, 24.58, 24.68, 25.13, 25.17, 27.11, 42.68, 42.84, 50.40, 50.65, 62.94, 63.46, 80.37, 80.50, 85.93, 86.08, 87.08, 94.03, 94.12, 96.12, 113.74, 127.42, 128.86, 132.97, 133.08, 145.27, 147.41, 154.50, 162.19, 166.74; ^{31}P NMR ($CDCl_3$) 150.28, 150.59.

Compound ***5c.*** Yield 76%. IR (KBr) 2960, 1595, 1490, 1450, 1380, 1300, 1200, 1155, 1110, 1075, 1025, 975, 910, 865, 790, 730, 635 cm^{-1}; ^{1}H NMR ($CDCl_3$) 1.04–1.16 (m, 12H), 1.39 (s, 3H), 1.64 (s, 3H), 3.34 and 3.39 (2d, $J_{P\text{-}H}$ = 13.4 Hz, 3H), 3.48 (m, 2H), 3.74 to 3.95 (2m, 2H), 4.55 (q, J = 3.1, 1H), 5.00 (2t, J = 2.5 Hz, 1H), 5.27 (2t, J = 5.9 Hz, 1H), 6.28 and 6.31 (2d, J = 2.8 Hz, 1H), 8.33 and 8.47 (2s, 1H), 8.98 and 8.99 (2s, 1H), 9.13 and 9.14 (2s, 1H); ^{13}C NMR ($CDCl_3$) 24.61, 25.20, 27.12, 42.63, 50.52, 63.49, 81.60, 81.81, 84.78, 86.16, 86.32, 91.09, 91.21, 114.08, 114.17, 134.49, 144.04, 144.10, 148.50, 150.00, 152.62; ^{31}P NMR ($CDCl_3$) 150.31, 150.47.

Compound ***5d.*** Yield 75%. IR (KBr) 3180, 3050, 2960, 1690, 1455, 1380, 1270, 1215, 1185, 1155, 1110, 1075, 1025, 975, 910, 855, 805, 730 cm^{-1}; ^{1}H NMR ($CDCl_3$) 1.08–1.20 (m, 12H), 1.28 (s, 3H), 1.52 (s, 3H), 3.32 and 3.36 (2d, $J_{P\text{-}H}$ = 13.4 Hz, 3H), 3.39–3.61 (m, 2H), 3.73 and 3.88 (2m, 2H), 4.32 (m, 1H), 4.67 (2m, 1H), 4.76 (2m, 1H), 5.61 and 5.64 (2d, J = 1.2 Hz, 1H), 5.93 and 5.94 (2d, J = 8.1 Hz, 1H) 7.65 and 7.70 (2d, J = 8.1 Hz, 1H); ^{13}C NMR ($CDCl_3$) 24.47, 24.61, 25.15, 25.20, 27.06, 27.09, 42.61, 42.77, 50.39, 50.64, 62.92, 63.37, 80.55, 80.65, 84.72, 84.85, 85.47, 85.59, 91.70, 91.88, 102.13, 114.01, 114.03, 140.91, 140.99, 150.19, 163.43; ^{31}P NMR ($CDCl_3$) 150.48, 150.59.

Compound ***6a.*** Aminoalcohol **3** (0.426 g, 1.19 mmol), phosphoramidite **5a** (1.338 g, 2.34 mmol), and tetrazole (0.404 g, 5.77 mmol) were dissolved in dry CH_3CN. The mixture was stirred at room temperature under dry nitrogen atmosphere for 5 h. Then a solution of iodine (0.206 g) in H_2O : THF : pyridine (1 : 1 : 1, 3 mL) was added until no decoloration was observed. The mixture was diluted with CH_2Cl_2, and the organic layer was washed with brine, dried ($MgSO_4$), and evaporated. The crude product was purified by flash chromatography on silica gel (EtOAc : MeOH, 99 : 1) to give **6a** (782 mg, 78%). IR (KBr) 3600–3100, 3040, 2960, 1725, 1605, 1575, 1510, 1445, 1250, 1080–1000, 855, 790 cm^{-1}; ^{1}H NMR ($CDCl_3$) 1.38 (s, 3H), 1.60 (s, 3H), 1.84 (m, 2H), 2.41 and 2.45 (2d, J = 7.0 Hz, 2H), 3.61 and 3.63 (2d, $J_{P\text{-}H}$ = 11.0 Hz, 3H), 3.85–4.20 (m, 5H), 4.48 (m, 1H), 5.00 (d, J = 3 Hz, 1H), 5.04 (s, 4H), 5.44 (m, 1H), 6.20 (m, 1H), 7.26 (m, 10H), 7.46 (m, 2H), 7.57 (m, 1H), 8.16 and 8.18 (2s, 1H), 8.79 (s, 1H), 9.03 (m, 1H); ^{13}C NMR ($CDCl_3$) 25.16, 26.93, 25.98, 30.44, 50.49, 54.51, 66.29, 66.49, 66.57, 66.77, 69.18, 80.97, 81.42, 84.09, 84.25, 85.27, 85.36, 90.74, 90.90, 114.03, 114.58, 123.53, 123.60, 127.78, 127.87, 128.04, 128.10, 128.32, 128.39, 132.57, 135.58, 141.98, 142.51, 149.69, 150.16, 150.57, 151.14, 152.06, 152.55, 155.97, 164.81, 172.66; ^{31}P NMR ($CDCl_3$) 0.85, 0.87.

Compound ***6b.*** Yield 89%. IR (KBr) 3250, 3040, 2940, 1700, 1660, 1625, 1550, 1480, 1380, 1320, 1250, 1165, 1025, 895, 860, 785, 730, 695 cm^{-1}; ^{1}H NMR ($CDCl_3$) 1.31 (s, 3H), 1.54 (s, 3H), 1.92 (m, 2H), 2.53 (m, 2H), 3.67 and 3.71 (2d, $J_{P\text{-}H}$ = 11.1 Hz, 3H), 3.96–4.14 (2m, 3H), 4.25–4.36 (2m, 2H), 4.90 (m, 1H), 5.00–5.10 (m, 5H), 5.63 (d, J = 7.8 Hz, 1H), 5.80 and 6.01 (2m, 1H), 7.28 (m, 10H), 7.48–7.59 (m, 4H), 7.71 (d, J = 7.3 Hz, 1H), 7.92 (d, J = 3.7 Hz, 2H), 9.04 (s, 1H); ^{13}C NMR ($CDCl_3$) 26.11, 25.06, 25.13, 26.89, 26.96, 30.73, 50.70, 54.49, 66.29, 66.58, 66.90, 69.13, 69.44, 80.90, 81.08, 84.62, 86.71, 96.91, 97.54, 114.13, 127.71, 127.93, 128.10, 128.35, 128.40,

128.80, 133.07, 135.62, 147.07, 156.03, 162.06, 164.81, 169.12, 173.01; ^{31}P NMR ($CDCl_3$) 0.27, 0.76.

Compound ***6c.*** Yield 60%. IR (KBr) 2930, 2250, 1710, 1585, 1495, 1450, 1390, 1250, 1205, 1050, 905, 725 cm^{-1}; ^{1}H NMR ($CDCl_3$ 1.38 (s, 3H), 1.61 (s, 3H), 1.84, (m, 2H), 2.42 and 2.47 (2d, J = 7.4 Hz, 2H), 3.58 and 3.63 (2d, $J_{P\text{-}H}$ = 11.3 Hz, 3H), 3.80–3.86 (m, 1H), 3.88–4.10 (m, 2H), 4.13–4.26 (m, 2H), 4.49 (m, 1H), 5.06 (m, 1H), 5.08 (s, 4H), 5.40 (d, J = 6.2 Hz, 1H), 6.22 (d, J = 4.5 Hz, 1H), 7.31 (m, 10H), 8.24 (d, J = 4.8 Hz, 1H), 8.99 (d, J = 1.9 Hz, 1H), 9.09 (d, J = 8.0 Hz, 1H); ^{13}C NMR ($CDCl_3$) 25.18, 26.12, 26.97, 30.48, 50.49, 54.54, 66.54, 67.04, 69.33, 80.92, 81.05, 84.00, 84.19, 85.22, 90.57, 90.97, 114.69, 114.77, 127.96, 128.03, 128.10, 128.14, 128.39, 128.43, 134.52, 135.62, 136.18, 144.10, 148.94, 152.68, 155.99, 172.60; ^{31}P NMR ($CDCl_3$) 0.91, 0.98.

Compound ***6d.*** Yield 90%. IR (KBr) 3040, 2960, 1690, 1525, 1450, 1375, 1250, 1160, 1030, 900, 850, 725, 690 cm^{-1}; ^{1}H NMR ($CDCl_3$) 1.30 (s, 3H), 1.52 (s, 3H), 1.87 (m, 2H), 2.46 (t, J = 7.1 Hz, 2H), 3.68 and 3.71 (2d, $J_{P\text{-}H}$ = 11.2 Hz, 3H), 3.86–3.98 (m, 1H), 4.02–4.26 (2m, 5H), 4.86 (m, 1H), 5.00 (d, J = 5.2 Hz, 1H), 5.05 (s, 2H), 5.08 (s, 2H), 5.56 (m, 1H), 5.62 and 5.65 (2d, J = 2.8 Hz, 1H), 5.67 and 5.69 (2d, J = 8.1 Hz, 1H), 7.30 (m, 10H), 7.42 (d, J = 8.1 Hz, 1H), 8.79 (s, 1H), 9.76 (s, 1H); ^{13}C NMR ($CDCl_3$) 25.05, 25.10, 26.11, 26.89, 27.11, 30.56, 50.60, 54.65, 62.35, 66.60, 69.39, 80.29, 80.70, 83.75, 84.16, 86.81, 95.22, 102.34, 102.59, 114.19, 114.38, 127.92, 128.03, 128.11, 128.16, 128.39, 128.44, 135.56, 136.21, 142.60, 150.02, 156.07, 163.27, 173.02; ^{31}P NMR ($CDCl_3$) 0.36, 0.52.

Compound ***7a.*** Phosphate **6a** (0.265 g, 0.314 mmol) was dissolved in 90% (v/v) aqueous trifluoroacetic acid (10 mL) and the solution was stirred at room temperature for 10 min. The solvent was removed *in vacuo* and the residue was purified by flash chromatography on silica gel (EtOAc : MeOH, 94 : 6) to yield **7a** (236 mg, 93%). IR (KBr) 3700–2900, 1695, 1610, 1580, 1510, 1450, 1250, 1025, 630, 595 cm^{-1}; ^{1}H NMR (CD_3OD) 1.68 and 1.81 (2m, 1H), 2.39 (m, 2H), 3.64 and 3.68 (2d, $J_{P\text{-}H}$ = 11.3 Hz, 3H), 3.75–4.45 (m, 6H), 4.47 (m, 1H), 4.77 (m, 1H), 5.00 (s, 2H), 5.03 (s, 2H), 6.15 (m, 1H), 7.27 (m, 10H), 7.50 (d, J = 7.5 Hz, 2H), 7.61 (m, 1H), 8.04 (m, 2H), 8.52 and 8.53 (2s, 1H), 8.69 (s, 1H); ^{13}C NMR (CD_3OD) 27.10, 31.55, 51.97, 52.05, 55.80, 67.52, 67.70, 68.47, 70.73, 71.43, 75.52, 84.18, 90.68, 90.76, 125.02, 128.95, 129.14, 129.70, 129.91, 134.94, 137.54, 138.24, 144.32, 151.11, 153.18, 153.42, 158.53, 168.04, 174.50; ^{31}P NMR (CD_3OD) 0.81, 0.83.

Compound ***7b.*** Yield 96%. IR (KBr) 3620–3000, 2940, 2480, 1670, 1450, 1360, 1255, 1195, 1135, 1035, 835, 795, 715, 690 cm^{-1}; ^{1}H NMR (CD_3OD) 1.72 and 1.89 (2m, 2H), 2.44 (t, J = 4.3 Hz, 2H), 3.74 and 3.77 (2d, $J_{P\text{-}H}$ = 11.1 Hz, 3H), 3.88 (m, 1H), 4.05 (m, 2H), 4.16–4.41 (m, 5H), 5.02 (m, 4H), 5.89 (d, J = 2.6 Hz, 1H), 7.27 (m, 10H), 7.43–7.62 (m, 4H), 7.94 (d, J = 7.5 Hz, 2H), 8.23 (2d, J = 7.4 Hz, 1H); ^{13}C NMR 26.98, 31.34, 51.99, 55.66, 67.41, 67.60, 67.73, 70.15, 70.78, 76.02, 83.50, 93.74, 98.76, 128.80, 129.03, 129.14, 129.18, 129.31, 129.55, 129.89, 134.32, 134.44, 137.50, 138.22, 146.31, 157.64, 158.56, 162.05, 162.53, 163.01, 163.48, 164.57, 169.12, 174.49; ^{31}P NMR 0.82, 0.86.

Compound ***7c.*** Yield 97%. IR (KBr) 3250, 2920, 1675, 1600, 1450, 1200, 1135, 1040, 835, 795 cm^{-1}; ^{1}H NMR (CD_3OD) 1.69 and 1.84 (2m, 2H), 2.42 and 2.47 (2d, J = 7.4 Hz, 2H), 3.66 and 3.68 (2d, $J_{P\text{-}H}$ = 11.2 Hz, 3H), 3.70–3.82 (m, 1H), 3.84–4.00

(m, 2H), 4.26–4.40 (m, 3H), 4.47 (t, J = 4.9 Hz, 1H), 4.81 (m, 1H), 5.02 (s, 2H), 5.06 (s, 2H), 6.17 (d, J = 4.3 Hz, 1H), 7.29 (m, 10H), 8.65 (s, 1H), 8.94 (s, 1H), 9.07 (s, 1H); ^{13}C NMR (CD_3OD) 26.94, 31.33, 50.50, 54.55, 67.37, 67.54, 71.41, 75.02, 84.12, 84.22, 90.46, 90.55, 128.80, 128.99, 129.16, 129.46, 129.53, 135.57, 146.99, 149.05, 153.53, 156.00, 172.60; ^{31}P NMR (CD_3OD) 0.94, 0.98.

Compound ***7d.*** Yield 97%. IR (KBr) 3600–3150, 3040, 2930, 1685, 1525, 1450, 1380, 1250, 1190, 1130, 1030, 825, 715, 690 cm^{-1}; ^{1}H NMR (CD_3OD) 1.71 and 1.85 (2m, 2H), 2.45 (t, J = 6.5 Hz, 2H), 3.73 and 3.75 (2d, $J_{P\text{-}H}$ = 11.1 Hz, 3H), 3.86–3.95 (m, 1H), 4.00–4.30 (4m, 7H), 5.05 (s, 2H), 5.08 (s, 2H), 5.72 (d, J = 8.1 Hz, 1H), 5.86 (d, J = 3.0 Hz, 1H), 7.51 (m, 10H), 7.65 and 7.66 (2d, J = 8.1 Hz, 1H); ^{13}C NMR (CD_3OD) 27.00, 31.35, 51.92, 55.59, 62.31, 67.51, 70.76, 71.40, 75.01, 75.75, 83.39, 83.48, 86.43, 90.74, 91.61, 103.06, 128.88, 129.04, 129.21, 129.52, 129.56, 137.58, 138.29, 142.30, 142.73, 152.25, 158.56, 166.00, 174.43; ^{31}P NMR (CD_3OD) 0.93, 0.95.

Compound ***8a.*** To a stirred solution of phosphate **7a** (456 mg, 0.57 mmol) in 20 mL of dry 2-butanone was added NaI (262 mg, 1.75 mmol), and the reaction mixture was refluxed for 4 h under dry atmosphere. After evaporation of the solvent *in vacuo*, the residue was purified by flash chromatography on silica gel (acetone : MeOH, 4 : 1) to yield **8a** (363 mg, 79%). IR (KBr) 3700–2800, 1700, 1610, 1580, 1510, 1450, 1230, 1170, 1070, 850, 815, 790, 730, 690 cm^{-1}; ^{1}H NMR (CD_3OD) 1.73 (m, 1H), 1.88 (m, 1H), 2.38 (m, 2H), 3.82 (m, 2H), 4.10 (m, 2H), 4.23 (m, 1H), 4.42 (m, 1H), 4.71 (t, J = 5.0 Hz, 1H), 4.96 (s, 2H), 5.02 (s, 2H), 6.22 (d, J = 5.2 Hz, 1H), 7.24 (m, 10H), 7.52 (m, 2H), 7.62 (d, J = 7.3 Hz, 1H), 8.06 (d, J = 7.3 Hz, 2H), 8.69 (s, 1H), 8.78 (s, 1H); ^{13}C NMR (CD_3OD) 27.73, 31.70, 52.28, 52.33, 66.04, 67.37, 67.55, 72.09, 76.35, 85.74, 89.65, 124.52, 128.80, 128.99, 129.19, 129.54, 129.60, 129.85, 134.10, 134.68, 137.61, 138.85, 144.33, 150.90, 153.45, 153.61, 158.64, 168.32, 174.81; ^{31}P NMR (CD_3OD) −0.94.

Compound ***8b.*** Yield 78%. IR (KBr) 3600–3000, 2930, 1685, 1640, 1565, 1480, 1420, 1240, 1205, 1175, 1080, 780, 690 cm^{-1}; ^{1}H NMR (CD_3OD) 1.76 (m, 1H), 1.93 (m, 1H), 2.43 (t, J = 4.3 Hz, 2H), 3.87–4.23 (2m, 8H), 5.00 (s, 2H), 5.02 (s, 2H), 5.96 (d, J = 2.7 Hz, 1H), 7.28 (m, 10H), 7.49–7.57 (m, 4H), 7.93 (d, J = 7.6 Hz, 2H), 8.49 (d, J = 7.2 Hz, 1H); ^{13}C NMR (CD_3OD) 27.72, 31.62, 52.00, 67.29, 67.54, 70.21, 70.75, 76.62, 83.47, 92.69, 98.96, 128.76, 128.94, 129.11, 129.23, 129.46, 129.51, 129.82, 134.10, 134.69, 137.59, 146.62, 158.12, 159.10, 164.70, 168.95, 174.71; ^{31}P NMR (CD_3OD) 0.61.

Compound ***8c.*** Yield 76%. IR (KBr) 3640–3000, 2930, 1710, 1590, 1530, 1495, 1450, 1405, 1350, 1220, 1080, 905, 830, 785, 730, 695 cm^{-1}; ^{1}H NMR (CD_3OD) 1.67 and 1.92 (2m, 2H), 2.38 (t, J = 7.4 Hz, 2H), 3.81 (m, 3H), 4.10 (m, 1H), 4.19 (m, 2H), 4.42 (t, J = 4.4 Hz, 1H), 4.65 (t, J = 5.3 Hz, 1H), 5.00 (s, 2H), 5.03 (s, 2H), 6.21 (d, J = 4.2 Hz, 1H), 7.27 (m, 10H), 8.90 (s, 1H), 8.92 (s, 1H), 9.04 (s, 1H); ^{13}C NMR (CD_3OD) 27.56, 31.53, 50.51, 67.31, 67.66, 71.65, 76.03, 84.10, 84.23, 89.91, 128.75, 128.98, 129.12, 129.46, 129.52, 135.14, 146.92, 148.79, 153.54, 156.03, 174.64; ^{31}P NMR (CD_3OD) −1.57.

Compound ***8d.*** Yield 80%. IR (KBr) 3620–3150, 3060, 2940, 1685, 1555, 1430, 1210, 1060, 820, 730, 690 cm^{-1}; ^{1}H NMR (CD_3OD) 1.74 and 1.91 (2m, 2H), 2.43 (t, J = 7.5 Hz, 2H), 4.16–4.21 (3m, 8H), 5.04 (s, 2H), 5.08 (s, 2H), 5.78 (d, J = 8.1 Hz, 1H), 5.95 (d, J = 4.7 Hz, 1H), 7.32 (m, 10H), 7.93 (d, J = 8.1 Hz, 1H); ^{13}C NMR

(CD_3OD) 27.67, 31.61, 51.93, 65.73, 67.43, 71.37, 75.52, 84.92, 86.44, 90.06, 103.20, 128.84, 128.97, 129.14, 129.19, 129.48, 129.53, 137.64, 142.50, 152.61, 158.56, 166.17, 174.67; ^{31}P NMR (CD_3OD) 0.54.

Compound ***9.*** Compound **8a** (273 mg, 0.336 mmol), palladium (10%) on activated carbon, and water (15 mL) were shaken under H_2 in a Parr apparatus at 40 psi for 4 h. The mixture was filtered through Celite and concentrated *in vacuo* ($T < 50°C$) to yield **9** as a white solid (163 mg, quant). IR (KBr) 3600–3000, 2900, 1680, 1600, 1570, 1510, 1450, 1390, 1340, 1280, 1250–1200, 1110–1010, 940, 860, 810, 780, 690 cm^{-1}; ^1H NMR (D_2O) 1.70 (m, 2H), 2.14 (t, J = 7.5 Hz, 2H), 3.30 (m, 1H), 3.78 (m, 1H), 3.87 (m, 1H), 4.06 (m, 2H), 4.28 (m, 1H), 4.42 (t, J = 4.7 Hz, 1H), 4.69 (t, J = 5.1 Hz, 1H), 6.09 (d, J = 5.1 Hz, 1H), 7.35 (t, 7.6 Hz, 2H), 7.48 (t, J = 7.3 Hz, 1H), 7.77 (d, J = 7.6 Hz, 2H), 8.54 (s, 1H), 8.56 (s, 1H); ^{13}C NMR (D_2O) 28.05, 36.16, 54.48, 67.58, 69.36, 72.91, 76.88, 86.40, 90.51, 130.90, 131.60, 135.43, 136.15, 151.93, 154.73, 154.93, 175.05; ^{31}P NMR (D_2O) 0.49.

Compound ***10.*** Compound **9** was dissolved in a saturated solution of aqueous ammonia and the solution was stirred at room temperature for 48 h. The solvent was evaporated and the product was purified by column chromatography on cellulose (MeOH to MeOH/H_2O 4 : 1) to yield **10** (133 mg, 82%). IR (KBr) 3700–2400, 1640, 1605, 1575, 1280–1180, 1160–980 cm^{-1}; ^1H NMR (D_2O) 1.73 (m, 2H), 2.21 (t, J = 7.4 Hz, 2H), 3.37 (m, 1H), 3.69 (m, 1H), 3.86 (m, 1H), 4.08 (m, 2H), 4.30 (m, 1H), 4.45 (t, J = 4.7 Hz, 1H), 4.68 (t, J = 5.1 Hz, 1H), 5.90 (d, J = 5.1 Hz, 1H), 8.01 (s, 1H), 8.29 (s, 1H); ^{13}C NMR (D_2O) 27.55, 35.05, 54.22, 67.34, 67.60, 72.88, 76.77, 85.19, 90.02, 120.99, 142.48, 151.35, 154.72, 157.43, 182.31; ^{31}P NMR (D_2O) 0.40.

Compound ***11.*** Compound **8b** (100 mg, 0.13 mmol), palladium (10%) on activated carbon, and water (6 mL) were shaken under H_2 in a Parr apparatus at 40 psi for 4 h. The mixture was filtered through Celite and concentrated *in vacuo*. The residue was dissolved in a saturated solution of aqueous ammonia and the solution was stirred at room temperature for 48 h. The solvent was evaporated and the product was purified by column chromatography on cellulose (MeOH to MeOH/H_2O 4 : 1) to yield **11** (60 mg, 80%). IR (KBr) 3600–3000, 2940, 1665, 1575, 1400, 1220, 1070, 820 cm^{-1}; ^1H NMR (D_2O) 1.88 (q, J = 7.4 Hz, 2H), 2.36 (td, J = 7.6 and 2.9 Hz, 2H), 2.54 (t, J = 7.4, 2H), 3.50 (m, 3H), 3.86 to 4.06 (m, 5H), 4.12 to 4.22 (m, 2H), 5.54 (d, J = 7.3 Hz, 1H); ^{13}C NMR (D_2O) 27.67, 35.13, 39.24, 42.57, 51.67, 54.25, 67.35, 68.26, 72.81, 72.97, 84.32, 91.84, 163.67, 181.52; ^{31}P NMR (D_2O) 0.58.

Compound ***12.*** Compound **8b** (45 mg, 0.057 mmol), palladium (10%) on activated carbon (90 mg), and 1,4-cyclohexadiene (1.5 mL) in 4 mL of EtOH : H_2O (1 : 1) were stirred at room temperature for 48 h. The mixture was filtered through Celite and concentrated *in vacuo*. The residue was dissolved in aqueous ammonia and the solution was stirred at room temperature for 48 h. The solvent was evaporated and the product was purified by column chromatography on cellulose (MeOH to MeOH/H_2O 4 : 1) to yield **12** (22 mg, 85%). IR (KBr) 3700–3020, 2920, 1720, 1650, 1490, 1400, 1280, 1220, 1060, 780 cm^{-1}; ^1H NMR (D_2O) 1.83 (q, J = 7.4 Hz, 2H), 2.26 (t, J = 7.4 Hz, 2H), 3.54 (m, 1H), 3.84 to 4.04 (m, 5H), 4.13 to 4.25 (m, 2H), 5.89 (d, J = 3.1 Hz, 1H), 6.02 (d, J = 7.5 Hz, 1H), 7.82 (d, J = 7.5 Hz, 1H); ^{13}C NMR (D_2O) 27.80, 35.40, 54.30, 67.18, 67.37, 67.44, 71.86, 76.77, 85.18, 92.69, 98.89, 144.76, 158.28, 167.35, 182.84; ^{31}P NMR (D_2O) −0.44.

*Compound **13.*** Yield 94%. IR (KBr) 3660–2500, 1600, 1415, 1270–1180, 1160–990 cm^{-1}; ^1H NMR (D_2O) 1.72 (m, 2H), 2.16 (t, J = 7.5 Hz, 2H), 3.31 (m, 1H), 3.76 (m, 2H), 3.86 (m, 1H), 4.11 (m, 2H), 4.34 (t, 1H), 4.49 (t, J = 4.8 Hz, 1H), 6.23 (d, J = 5.1 Hz, 1H), 8.73 (s, 1H), 8.91 (s, 1H), 9.09 (s, 1H); ^{13}C NMR (D_2O) 27.95, 35.79, 53.91, 54.02, 67.22, 67.29, 67.45, 67.52, 72.53, 76.41, 86.10, 89.98, 135.96, 147.56, 150.52, 153.38, 154.62, 183.43; ^{31}P NMR (D_2O) 0.40.

*Compound **14.*** Yield 96%. IR (KBr) 3680–2500, 1690, 1560, 1410, 1205, 1080, 815, 650 cm^{-1}; ^1H NMR (D_2O) 1.83 (q, J = 7.3 Hz, 2H), 2.26 (t, J = 7.9 Hz, 2H), 3.23–3.49 (m, 1H), 3.83–4.30 (m, 7H), 5.84 (d, J = 8.1 Hz, 1H), 5.88 (d, J = 4.8 Hz, 1H), 7.82 (d, J = 8.1 Hz, 1H); ^{13}C NMR (D_2O) 27.68, 35.13, 54.30, 67.37, 67.44, 72.17, 76.40, 85.62, 91.80, 105.28, 144.35, 154.48, 168.94, 182.81; ^{31}P NMR (D_2O) 0.46.

Inhibition of E. coli Glutamyl-tRNA Synthetase by Analogues of Glutamyl Adenylate

E. coli GluRS was purified to homogeneity as previously described (*13*). The GluRS was assayed by measuring the rate of formation of [^{14}C]glutamyl-tRNA as given by the amount of [^{14}C]glutamate incorporated into the trichloroacetic acid precipitate after various incubation times at 37°C; 1 unit was defined as the amount of enzyme that aminoacylates 1 nmol of tRNA specific for glutamic acid in 1 min as previously described (*14*). Potential inhibitors of GluRS were added to the reaction mixture in various concentrations and the percentage of inhibition was calculated from the resulting loss in specific activity of the GluRS. For kinetic measurements, all substrates, except the one studied whose concentration was variable, were present at such concentrations that their specific binding sites on the GluRS were saturated as previously described (*14*) with different concentrations of the inhibitor such that a 10 to 75% decrease in GluRS activity was observed. We tested two substrates, glutamate and ATP, whose concentrations varied from 50 to 200 μM for glutamate and 0.5 to 4 mM for ATP.

The kinetic constants K_m^{Glu} and K_m^{ATP} were determined from Lineweaver–Burk plots, whereas K_i were measured from the K_m^{app} vs [I] plot, according to the equation $K_m^{app} = K_m/K_i\,[I] + K_m$, the K_m^{app} being calculated from $1/V$ vs $1/S$ reciprocal plots for various [I]. To estimate the apparent K_i values of compounds **9** and **11–14,** we measured the rate of glutamyl-tRNA formation at saturating concentrations of ATP and tRNA and at the K_m value of 100 μM for glutamate; under these conditions, K_i is the value of [I] such that $v = 0.33\ V_{max}$, since for competitive inhibition, $v/V_{max} = S/[S + K_m(1 + 1/K_i)]$.

ACKNOWLEDGMENTS

This work was supported by Grants OGP0009597 (to J.L.) and CPG0164048 (to R.C. and J.L.) from the Natural Sciences and Engineering Research Council of Canada and by Grant 98-ER-2481 from the "Fonds FCAR du Ministère de l'Éducation du Québec" (to R.C. and J.L.).

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