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SYNTHESIS OF INHIBITORS OF GLUTAMYL-tRNA SYNTHETASE

Michel Desjardins,[a] Julie Desgagnés,[a] Lucille Lacoste,[b] Fu Yang,[b] Marie-Pascale Morin,[a] Jacques Lapointe,[b] and Robert Chênevert[a*]

Département de Chimie[a] et de Biochimie,[b] Faculté des Sciences et de Génie, Université Laval, Québec, Canada, G1K 7P4.

Abstract. Glutamic acid esters in which the alcohol moiety is ribose, prolinol or substituted piperidines are inhibitors of *E. coli* glutamyl-tRNA synthetase. © 1997 Elsevier Science Ltd.

Aminoacyl-tRNA synthetases (aaRS) catalyze the esterification of a particular tRNA with its corresponding amino acid. It has been established that this reaction is a two-step event. In the first step, the amino acid and ATP react to form an enzyme-bound mixed anhydride (aminoacyl adenylate). In the second step, the activated amino acid is transferred to the CCA-end of the cognate tRNA to form the aminoacyl tRNA and AMP.[1,2]

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

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

The resulting aminoacyl-tRNAs are the major activated forms of aminoacids in 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) is a highly potent inhibitor of bacterial isoleucyl tRNA synthetase.[4,5] This antibiotic shows a very high selectivity in favour of prokaryote forms of this enzyme and plays an important clinical role.

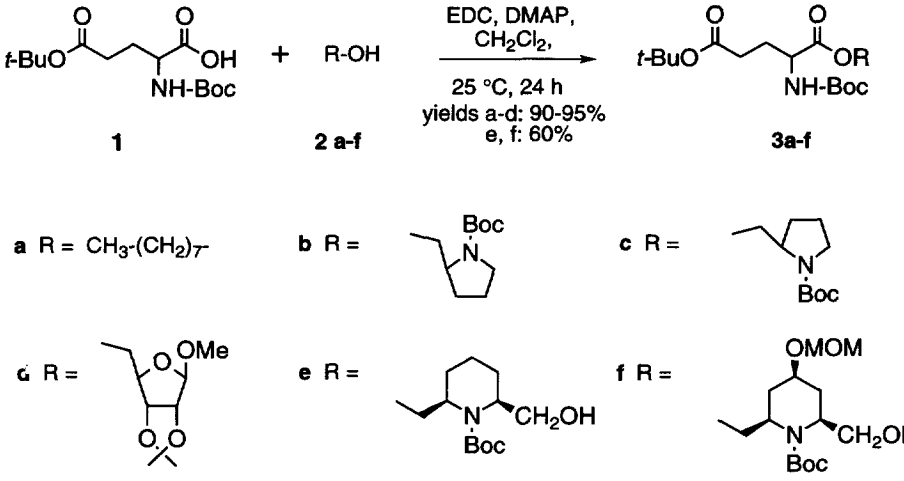
Synthetic analogs of several aminoacyl adenylates are inhibitors of the activation step catalyzed by aaRS.[6-10] We have synthesized glutamate esters in which the alcohol moiety is ribose, prolinol or substituted piperidines, and tested their inhibitory potency in the aminoacylation reaction of $tRNA^{Glu}$ catalyzed by *E. coli* glutamyl-tRNA synthetase.

Synthesis of glutamic acid esters

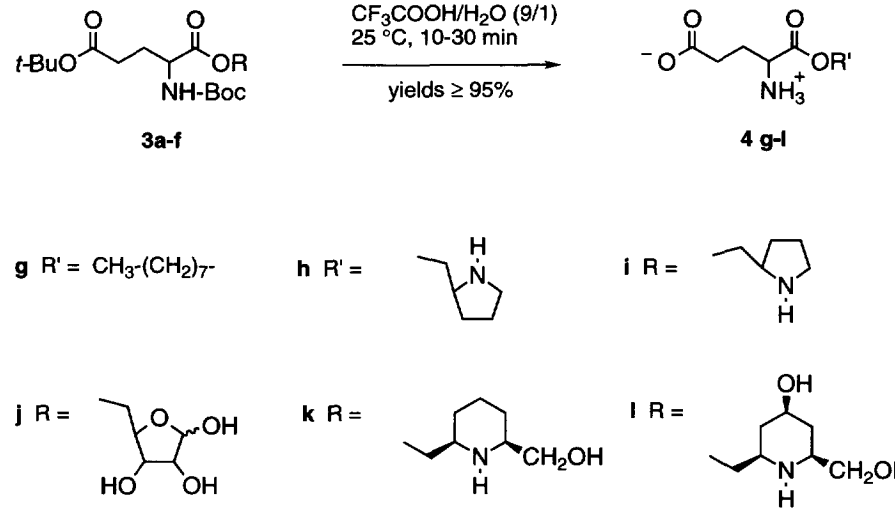
Esterification of alcohols **2a-f** with N-*tert*-butyloxy-carbonyl-L-glutamic acid γ-*tert*-butyl ester **1** in the presence of 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (EDC) and dimethylaminopyridine (DMAP) gave esters **3a-f** (Scheme 1). Ribose derivative[11] **2d** and piperidinediol[12] **2e-f** were prepared according to literature methods. Treatment of esters **3a-f** with wet trifluoroacetic acid resulted in simultaneous cleavage of protective groups to provide glutamic esters **4g-l** (Scheme 2).

Inhibition of *E. coli* glutamyl-tRNA synthetase (GluRS)

E. coli GluRS was purified to homogeneity as previously described.[13] GluRS has the characteristic, which



Scheme 1



Scheme 2

is common also to the glutaminyl- and arginyl-tRNA synthetases from *Escherichia coli* and all other organisms where these synthetases have been studied, of requiring the presence of its cognate tRNA to catalyze the activation of its amino acid substrate.[14] 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, as previously described.[15]

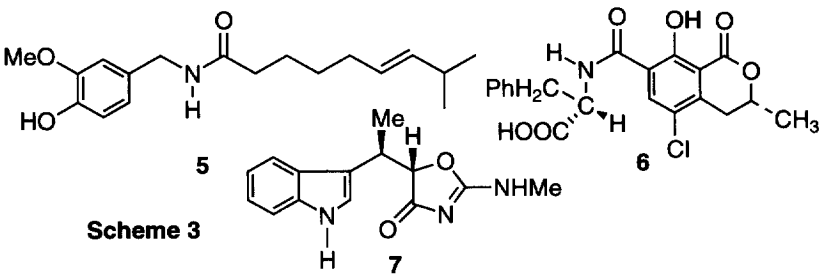
The results are reported in Table 1. *n*-Octyl L-glutamate **4g** is not an inhibitor of GluRS (entry 1). The glutamic ester of L-prolinol is 30 times more potent than the corresponding diastereomer of D-prolinol (entry 2 and 3). Ester **4j** in which the alcohol moiety is ribose has a K_i = 500 μM (entry 4). The best inhibition is obtained with piperidine derivatives **4k** (K_i = 230 μM) and **4l** (20 μM). Esters **4k,l** resulted from the esterification of meso diols and they are mixtures of diastereoisomers.

Table 1

Inhibition of *E. coli* Glutamyl-tARN Synthetase by Glutamic Esters

entry	compound	K_i (μM)
1	**4g**	no inhibition
2	**4h**	380
3	**4i**	11 400
4	**4j**	500
5	**4k**	230
6	**4l**	20

It is noteworthy that several naturally occurring analogs or derivatives of standard amino acids are specific inhibitors of the corresponding aminoacyl-tRNA synthetases. For instance, capsaicin **(5)**, a structural analog of tyrosine, inhibits (K_i = 42 μM) the aminoacylation of tRNA^{Tyr} (Scheme 3).[16] Capsaicin is the pungent ingredient found in the fruit of the genus Capsicum (paprika, cayenne). The mycotoxin ochratoxin A **(6)**, an inhibitor of phenylanalyl-tRNA synthetase, contains a phenylalanine moiety linked by its amino group to a chlorinated dihydroisocoumarin acid.[17] When phenylalanine is replaced by valine, the resulting val-ochratoxin compound is



Scheme 3

a specific inhibitor of valinyl-tRNA synthetase indicating that the specificity is due only to the amino acid and not to the dihydroisocoumarin moiety.[18] Indolmycin **(7)** has obvious structural similarities with tryptophan and this natural product is a potent and selective inhibitor of the bacterial tryptophanyl-tRNA synthetase.[19,20]

This study shows that synthetic derivatives of glutamic acid are interesting tools for the study of the mechanisms of action of GluRS. The inhibition is moderate (K_i = 20 μM for the best inhibitor) but the synthesis is simple and amenable to combinatorial synthesis, a powerful technique for the optimization of biological activity. In addition to gaining mechanistic information about *E. coli* GluRS, inhibitors such as **4l** should facilitate the crystallization of the enzyme.

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