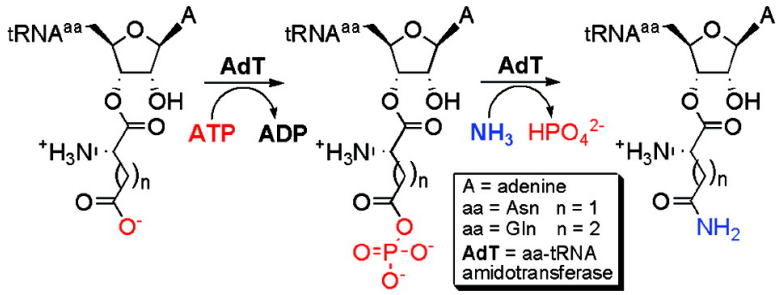
Communication

Inhibition of *Helicobacter pylori* Aminoacyl-tRNA Amidotransferase by Puromycin Analogues

Christian Balg, Jonathan L. Huot, Jacques Lapointe, and Robert Chnevert

J. Am. Chem. Soc., **2008**, 130 (11), 3264-3265 • DOI: 10.1021/ja7100714



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 02/23/2008

Inhibition of *Helicobacter pylori* Aminoacyl-tRNA Amidotransferase by Puromycin Analogues

Christian Balg,[†] Jonathan L. Huot,[‡] Jacques Lapointe,[‡] and Robert Chênevert*,[†]

Département de Chimie and Département de Biochimie et de Microbiologie, Centre de Recherche sur la Fonction, la Structure et l'Ingénierie des Protéines (CREFSIP), Faculté des Sciences et de Génie, Université Laval, Québec, Canada, G1K 7P4

Received November 6, 2007; E-mail: robert.chenevert@chm.ulaval.ca

The biosynthesis of aminoacylated tRNAs (aa-tRNA) is a critical step in the faithful translation of the genetic code into proteins. In most organisms, the 20 amino acids (aa) are esterified to their corresponding tRNA by 20 different aminoacyl-tRNA synthetases (aaRS), each of which is specific for one amino acid and a corresponding set of tRNAs (direct aminoacylation pathway).[1]

Recent genomic studies revealed the absence of glutaminyl-tRNA synthetase (GlnRS) and/or asparaginyl-tRNA synthetase (AsnRS) in archaebacteria, Gram-positive eubacteria, and many Gram-negative eubacteria. The survival of microorganisms missing one or both of these essential enzymes implies an alternative pathway for the formation of Gln-tRNA^{Gln} and Asn-tRNA^{Asn}. This indirect pathway involves the misacylation of tRNA^{Gln} with Glu (or tRNA^{Asn} with Asp) by a nondiscriminating aminoacyl-tRNA synthetase (ND-aaRS) and the subsequent transamidation of the misacylated aa-tRNA by an amidotransferase (AdT) (Figure 1).[2]

The dissemination of antibiotic resistance has become a major problem in clinical medicine, and there is a critical need to develop antibacterial agents with novel modes of action.[3] The widespread use of the indirect transamidation pathway among prominent human pathogens[4] and its absence in the mammalian cytoplasm identify AdT as an interesting target for the development of new and highly specific antimicrobial agents.[5] Here we report the synthesis and biological evaluation of puromycin analogues as mechanism-based inhibitors of aminoacyl-tRNA amidotransferases.

The proposed mechanism for the transamidation reaction catalyzed by amidotransferases is a three-step event (Figure 2). First, the hydrolysis of the amido donor, glutamine, forms glutamic acid and enzyme-bound NH_3 (glutaminase step). The second step is the activation of the side-chain carboxyl group of the amino acid fixed on the tRNA (Glu-tRNA^{Gln} or Asp-tRNA^{Asn}) resulting from the reaction of this carboxyl group with ATP to form a mixed anhydride (kinase step). In this intermediate, the high-energy anhydride bond activates the carboxyl group. Finally, the aminolysis of the activated amido acceptor by enzyme-bound NH_3 (transamidase step) forms the final product (Gln-tRNA^{Gln} or Asn-tRNA^{Asn}). The overall reaction is the simple conversion of the side chain carboxylic acid (Glu or Asp) into an amide (Gln or Asn) while the amino acid is still attached to a tRNA (pretranslational modification).

Two types of amidotransferases have been identified so far in nature. GatCAB are heterotrimeric proteins encoded by genes named gatC, gatA, and gatB. These enzymes found in both archaea and bacteria can transamidate both Glu-tRNA^{Gln} and Asp-tRNA^{Asn}. The second type, heterodimeric GatDE, occurs only in archaea and functions solely as a GluAdT. The first crystal structures of members of each type have been determined recently.[6] Up to

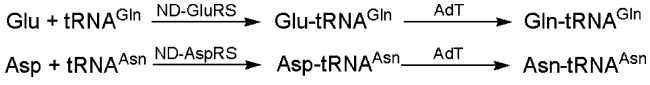


Figure 1. Indirect pathway for Gln-tRNA^{Gln} and Asn-tRNA^{Asn} biosynthesis.

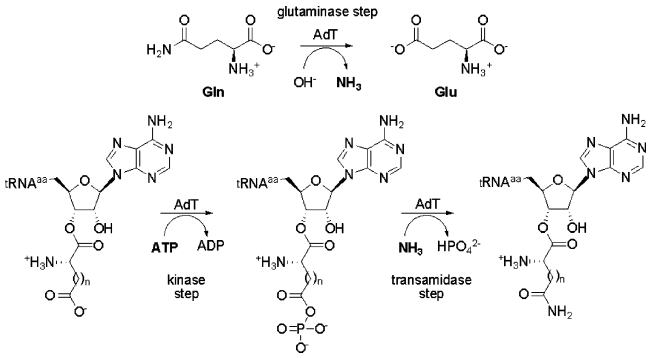


Figure 2. Reaction mechanism: Glu-tRNA^{Gln} amidotransferase (GluAdT), $n = 2$, tRNA^{aa} = tRNA^{Gln}. Asp-tRNA^{Asn} amidotransferase (AspAdT), $n = 1$, tRNA^{aa} = tRNA^{Asn}.

recently, only a few AdT inhibitors have been reported in part because of the absence of a convenient assay. Some analogues of glutamine and ATP were useful to study the reaction mechanism,[7] but these inhibitors are likely to interfere with many other enzymes acting on the same substrates.

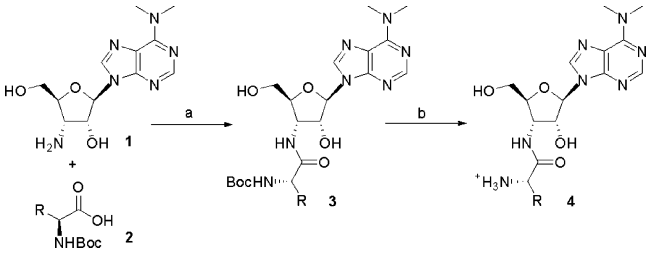
The general synthetic approach to the puromycin analogues is outlined in Scheme 1 (see Supporting Information for full details of each synthesis). The coupling of puromycin aminonucleoside **1** with *N*-*tert*-butyloxycarbonyl (*N*-Boc) protected amino acids **2** using *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide (EDC)/*N*-hydroxysuccinimide (NHS) provided amides **3** in high yields (72%−91%) despite the presence of two unprotected hydroxyl groups on the ribose. Removal of the Boc protecting group under standard conditions with CF_3COOH gave products **4**, but glycosyl bond cleavage and depurination lowered the yields. In contrast, Boc removal with 4 M HCl/dioxane proceeded without difficulty to furnish the final product **4** in high yields (89%−100%).

With a series of compounds in hand, we set out to evaluate their inhibitory activities against *Helicobacter pylori* GatCAB amidotransferase (Table 1). Enzyme production and kinetic experiments were carried out as previously described.[8] Competitive inhibition of *H. pylori* AdT by compounds **4a**−**4h**, with respect to Asp-tRNA^{Asn}, was characterized in the presence of saturating concentrations of the two other substrates (2 mM ATP and 1.28 mM L-glutamine) and of 0.50−1.25 μM Asp-tRNA^{Asn}. The decrease of the transamidation rate from its value V_o in the absence of inhibitor, to its value V_i in the presence of various inhibitor concentrations,

[†] Department of Chemistry.
[‡] Department of Biochemistry and Microbiology.

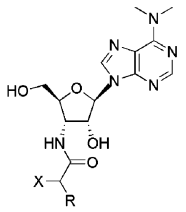
10.1021/ja7100714 CCC: $40.75

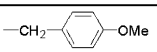
Scheme 1. General Synthetic Approach to Inhibitors[a]



[a] Conditions: (a) EDC, *N*-hydroxysuccinimide, DMF, 72−91%; (b) 4 M HCl, dioxane, 89−100%.

Table 1. Inhibition of *Helicobacter pylori* GatCAB Amidotransferase



compound	X	R	K_i (μM)
4a	(L)-NH_2	—CH_2— 	4100 ± 300
4b	(L)-NH_2	—CH_2COO^-	134 ± 4
4c	(L)-NH_2	—$(CH_2)_2$-COO^-	105 ± 2
4d	(L)-OH	—$(CH_2)_2$-COO^-	130 ± 16
4e	(L)-NH_2	—$(CH_2)_2$-$CONH_2$	45 ± 6
4f	(D,L)-NH_2	—$(CH_2)_2$- 	33 ± 3
4g	(L)-NH_2	—$(CH_2)_2$-	11 ± 0.7
4h	(L)-NH_2	—$(CH_2)_2$-	4 ± 0.2

was used to identify the competitive nature of the inhibitor and to obtain the K_i value, as described in the Supporting Information (see Figure S1).

We initially assayed the parent compound puromycin **4a**, an aminonucleoside antibiotic produced by *Streptomyces alboniger*. This natural product mimics the charged 3′-terminus of aminoacylated tRNA and has been widely used as a basic tool for the study of protein synthesis on the ribosomes. Puromycin is a very weak inhibitor of AdT (K_i = 4 mM).

The amino acid chain of puromycin is related to tyrosine and differs from the aspartic and glutamic side chains transformed by AdT (Figure 2). Replacement of the methoxyphenyl moiety of puromycin by carboxylic acid derivatives considerably enhances the ability to inhibit AdT. Compounds **4b** and **4c**, analogues of the 3′-ends of Asp-tRNA and Glu-tRNA, are competitive inhibitors with similar K_i values of 134 and 105 μM, respectively. This is consistent with the fact that the enzyme is equally efficient in transamidation of both Asp-$tRNA^{Asn}$ and Glu-$tRNA^{Gln}$.[9]

Replacement of the α-amino group by a hydroxyl (**4d**) slightly increases the K_i (K_i = 105 μM for **4c** vs 130 μM for **4d**). The amide variant **4e** has structural homology to the final product of the enzymatic reaction and shows improved activity (K_i = 45 μM) over the carboxylic acid analogues **4b** and **4c**.

Various phosphorus- and sulfur-containing derivatives have been previously proposed as analogues of tetrahedral intermediates formed transiently during enzymatic reactions involving formation or hydrolysis of amide bonds.[10] As stable analogues of the transition state in the last step of the transamidation process, we designed **4f**, **4g**, and **4h** where the carbonyl to be attacked by ammonia is replaced by a tetrahedral phosphorus or sulfur atom with a methyl group mimicking ammonia. Racemic **4f** prepared from (D,L)-phosphinothricin did inhibit transamidase activity with a K_i = 33 μM. Likewise, a K_i = 11 μM was determined for the diastereomerically mixed L-methionine (*R*,*S*)-sulfoxyde **4g**. The sulfone **4h** exhibited the highest activity with a K_i = 4 μM. Competitive inhibition with respect to Asp-$tRNA^{Asn}$ was observed for all puromycin analogues.

In conclusion, we have identified analogues of the natural product puromycin that have inhibitory activity against bacterial aminoacyl-tRNA amidotransferases. These mechanism-based inhibitors will provide useful chemical probes for further mechanistic investigations, ligands for X-ray crystallography, and a potential avenue to develop antibiotics with a novel mode of action.[11] Investigations along these lines are currently underway.

Acknowledgment. This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) and the "Fonds de recherche sur la nature et les technologies, Québec (FQRNT)".

Supporting Information Available: Experimental procedures and characterization data for all intermediates and final products; figures of ^1H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Ibba, M., Francklyn, C., Cusack, S., Eds. *The Aminoacyl-tRNA Synthetases*; Eurekah.com/Landes Bioscience: Georgetown, TX, 2005. (b) Lapointe, J., Brakier-Gingras, L., Eds. *Translation Mechanisms*; Landes Biosciences/Eurekah.com and Kluwer Academic/Plenum Publishers: 2003.
(2) Feng, L.; Tumbula-Hansen, D.; Min, B.; Namgoog, S.; Salazar, J. C.; Orellana, O.; Söll, D. Transfer RNA-dependant amidotransferases: Key enzymes for Asn-tRNA and Gln-tRNA synthesis in nature. In *The Aminoacyl-tRNA Synthetases*; Ibba, M., Francklyn, C., Cusack, S., Eds. Landes Biosciences: Georgetown, TX, 2005; pp 314−319.
(3) Walsh, C. T.; Wright, G. D. (Eds) *Chem. Rev.* **2005**, *105*, no. 2; special issue on antibiotic resistance.
(4) (a) Fritz, B.; Raczniak, G. A. *Biodrugs* **2002**, *16*, 331−337. (b) Raczniak, G.; Ibba, M.; Söll, D. *Toxicology* **2001**, *160*, 181−189.
(5) Ataide, S. F.; Ibba, M. *ACS Chem. Biol.* **2006**, *1*, 285−297.
(6) (a) Nakamura, A.; Yao, M.; Chimnaronk, S.; Sakai, N.; Tanaka, I. *Science* **2006**, *312*, 1954−1958. (b) Oshikane, H.; Sheppard, K.; Fukai, S.; Nakamura, Y.; Ishitani, R.; Numata, T.; Sherrer, R. L.; Feng, L.; Schmitt, E.; Panvert, M.; Blanquet, S.; Mechulam, Y.; Söll, D.; Nureki, O. *Science* **2006**, *312*, 1950−1954. (c) Schmitt, E.; Panvert, M.; Blanquet, S.; Mechulam, Y. *Structure* **2005**, *13*, 1421−1433.
(7) (a) Harpel, M. R.; Horiuchi, K. Y.; Luo, Y.; Shen, L.; Jiang, W.; Nelson, D. J.; Rogers, K. C.; Decicco, C. P.; Copeland, R. A. *Biochemistry* **2002**, *41*, 6398−6407. (b) Decicco, C. P.; Nelson, D. J.; Luo, Y.; Shen, L.; Horiuchi, K. Y.; Amsler, K. M.; Foster, L. A.; Spitz, S. M.; Merrill, J. J.; Sizemore, C. F.; Rogers, K. C.; Copeland, R. A.; Harpel, M. R. *Biorg. Med. Chem. Lett.* **2001**, *11*, 2561−2564. (c) Horiuchi, K. Y.; Harpel, M. R.; Shen, L.; Luo, Y.; Rogers, K. C.; Copeland, R. A. *Biochemistry* **2001**, *40*, 6450−6457.
(8) Huot, J. L.; Balg, C.; Jahn, D.; Moser, J.; Émond, A.; Blais, S. P.; Chênevert, R.; Lapointe, J. *Biochemistry* **2007**, *46*, 13190−13198.
(9) Sheppard, K.; Akochy, P. M.; Salazar, J. C.; Söll, D. *J. Biol. Chem.* **2007**, *282*, 11866−11873.
(10) Hiratake, J. *Chem. Rec.* **2005**, *5*, 209−228.
(11) In a preliminary evaluation using a standard disk diffusion assay, **4h** showed a moderate antimicrobial activity against *Staphylococcus aureus*, a Gram-positive bacteria lacking GlnRS.

JA7100714