Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 1871–1874

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Aminoalkyl Adenylate and Aminoacyl Sulfamate Intermediate Analogues Differing Greatly in Affinity for their Cognate *Staphylococcus aureus* Aminoacyl tRNA Synthetases

Andrew K. Forrest, Richard L. Jarvest,* Lucy M. Mensah, Peter J. O'Hanlon, Andrew J. Pope and Robert J. Sheppard

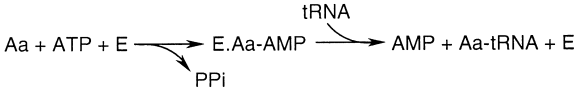
SmithKline Beecham Pharmaceuticals, New Frontiers Science Park, Third Avenue, Harlow, Essex, CM19 5AW, UK

Received 7 April 2000; revised 16 June 2000; accepted 21 June 2000

Abstract—Aminoalkyl adenylates and aminoacyl sulfamates derived from arginine, histidine and threonine, have been prepared and tested as inhibitors of their cognate *Staphylococcus aureus* aminoacyl tRNA synthetases. The arginyl derivatives were both potent nanomolar inhibitors of the Class I arginyl tRNA synthetase whereas for the Class II histidyl and threonyl tRNA synthetases, the acyl sulfamates were potent inhibitors but the adenylates had very little affinity. © 2000 Elsevier Science Ltd. All rights reserved.

Aminoacyl tRNA synthetases are essential enzymes in protein biosynthesis, catalysing the attachment of amino acids to their cognate tRNA prior to delivery to the ribosome. They are an ancient family of enzymes, lying at the interface of the RNA and protein worlds, and have been subject to considerable evolutionary divergence. Consequently, selective inhibition of the prokaryotic tRNA synthetases is attainable, as exemplified by the clinical antibiotic mupirocin, and represents a strategy for developing new classes of antibacterial agents. The enzymes fall into two distinct classes which are characterised by different structural folds and sequence motifs.[1]

The aminoacyl tRNA synthetases catalyse a two stage reaction in which the amino acid is activated as an aminoacyl adenylate intermediate (**1**), followed by attack of the 2′-OH or 3′-OH of the tRNA on the activated carboxyl group of the intermediate.



The aminoacyl adenylate intermediate (Aa-AMP; **1**) is a high energy, hydrolytically labile, species. It is thus tightly bound to the enzyme and stabilised analogues of this intermediate have the potential to be potent inhibitors. Aminoalkyl adenylates (**2**) have been described as inhibitors of several tRNA synthetases and have found many applications, from ligands for crystallography to affinity purification.[2–10] More recently, some aminoacyl sulfamoyl adenosine (aminoacyl sulfamate) derivatives (**3**) have been used in both mechanistic and crystallographic studies.[9–17] However, in many cases the inhibitory potency of the aminoacyl sulfamates has not been reported and there is very little comparative data for the two inhibitor types. Here we report the synthesis of aminoalkyl adenylates and aminoacyl sulfamates derived from some of the more highly functionalised amino acids, arginine, histidine, and threonine, and their interaction with their cognate aminoacyl tRNA synthetases.[18]

The acyl sulfamates were prepared by the general method of Castro-Pichel et al.[19] The protecting groups chosen allowed facile unblocking of the functionality using acidic treatments. Boc-Arg(Boc_2)-OH was activated as its imidazolide by reaction with carbonyl diimidazole and coupled with the sulfamate (**4**)[19] in the presence of DBU (Scheme 1). The sulfamate (**4**) was treated similarly with the *N*-hydroxysuccinimide esters of Boc-His (Boc)-OH and Boc-Thr-OH to afford the protected derivatives (**5**)–(**7**). Removal of the *N*-Boc moiety with anhydrous TFA followed by addition of water to cleave the *O*-isopropylidene group gave the required products after purification by HPLC.

A phosphoramidite strategy was used for the preparation of (**2a**–**c**).[10] The protecting groups were chosen to allow deprotection using sequential basic and acidic

*Corresponding author. Tel.: +44-1279-627629; fax: +44-1279-622260; e-mail: richard_l_jarvest@sbphrd.com

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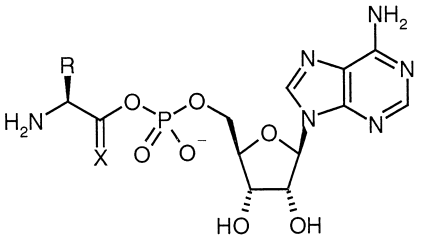
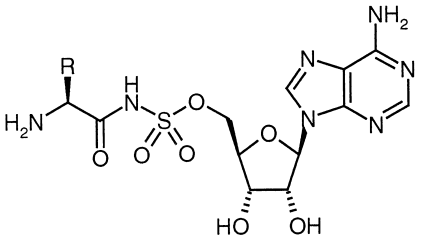
PII: S0960-894X(00)00360-7



1 X = O
2 X = H_2



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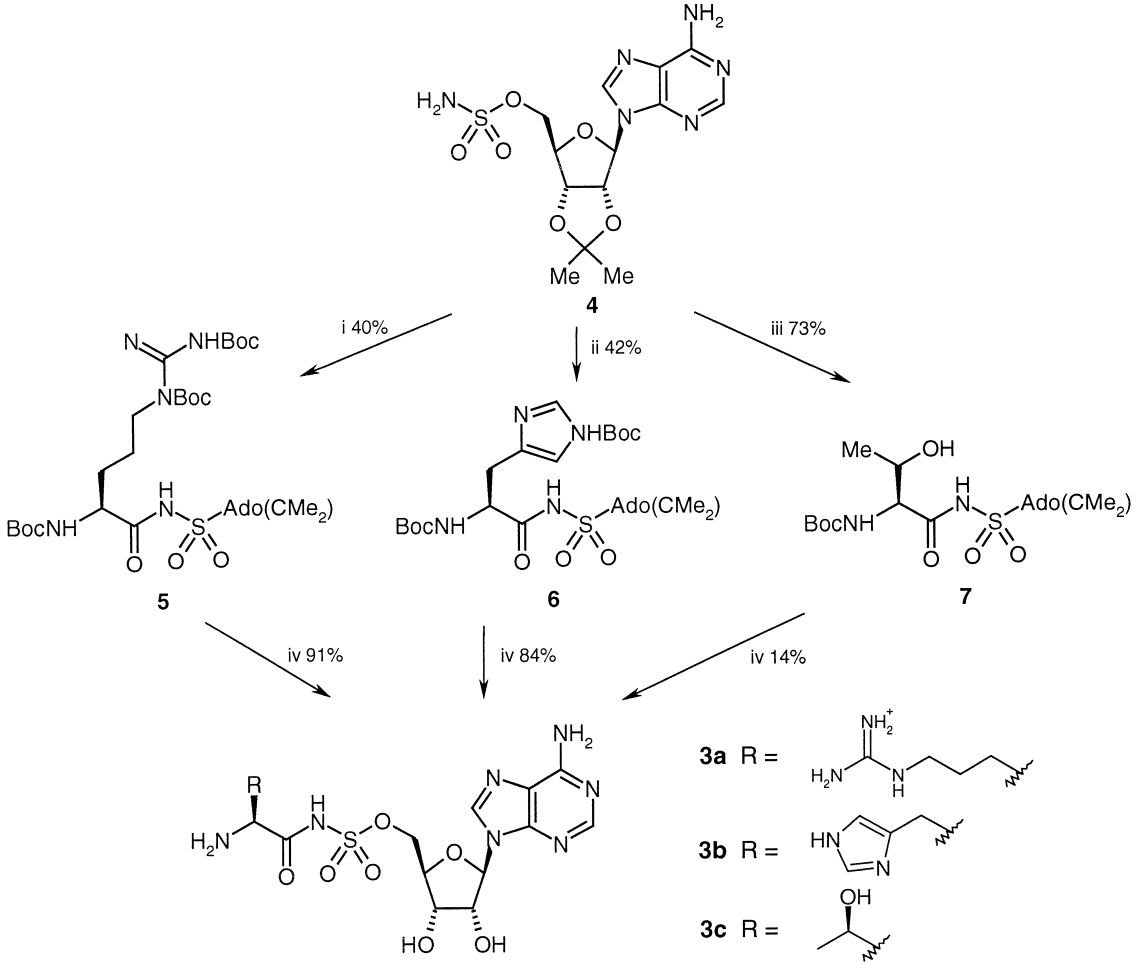
treatments. The protected histidinol (**12**)[20] was prepared by activation of Boc-His(Boc)-OH with isobutyl chloroformate followed by reduction with sodium borohydride; the other two protected amino alcohols were available commercially (Scheme 2). Coupling of the protected amino alcohols with the phosphoramidite (**8**)[10] followed by phosphorus oxidation gave the phosphotriesters (**9**)–(**11**). Appropriate deprotection treatments furnished the required aminoalkyl adenylates.

The analogues were evaluated in assays of the aminoacylation activity of their respective *Staphylococcus aureus* aminoacyl tRNA synthetase under conditions normalised for substrate K_m.[21] The results are shown in Table 1 along with reported values for the similar inhibitors of *S. aureus* isoleucyl (IRS) and tyrosyl (YRS) enzymes. Both the arginyl derivatives (**2a**) and (**3a**) were potent inhibitors of arginyl tRNA synthetase (RRS) with nanomolar potency. RRS is a Class I tRNA synthetase as are IRS and YRS, and it can be seen that all three Class I enzymes strongly interact with both aminoalkyl adenylate and aminoacyl sulfamate intermediate analogues.

For the Class II enzymes HRS and TRS, the aminoacyl sulfamate analogues are again potent inhibitors, although the histidyl sulfamate is significantly less potent against HRS than the other sulfamate–synthetase pairs. However, in contrast to the Class I enzymes, the aminoalkyl



Scheme 1. Reagents: (i) Boc-Arg(Boc_2)-imidazolide (from Boc-Arg(Boc_2)-OH, carbonyl diimidazole, THF), DBU, THF, 1.5 h, rt; (ii) Boc-His (Boc)-OSu, DBU, THF, 3 h, rt; (iii) Boc-Thr-OSu, DBU, THF, 24 h, rt; (iv) TFA, 0.5 h then TFA:H_2O (5:1), 4 h, rt.

Table 1. Inhibition of *S. aureus* cognate tRNA synthetases by intermediate analogues

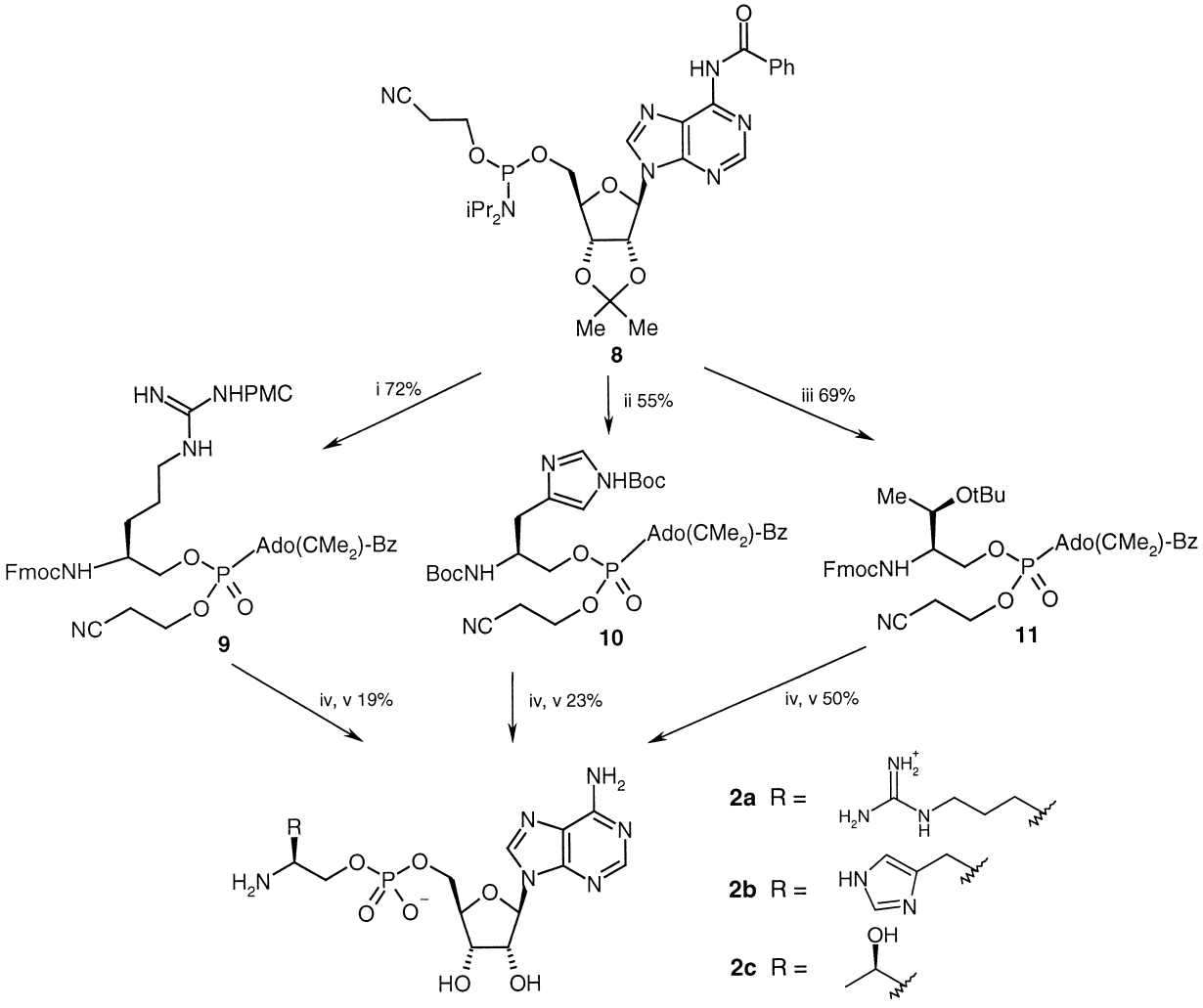
	Aminoalkyl adenylates (**2**)		Aminoacyl sulfamates (**3**)	
Enzyme	No.	IC_{50} (nM) or % inhibition	No.	IC_{50} (nM)
RRS	(**2a**)	7.5	(**3a**)	4.5
HRS	(**2b**)	20% at 300 μM	(**3b**)	130
TRS	(**2c**)	60% at 300 μM	(**3c**)	15
IRS	—	780[a]	—	4[a]
YRS	—	11[b]	—	26[b]

[a]Ref 9.
[b]Ref 10.

adenylates have very little affinity for either HRS or TRS. The difference between the two inhibitor types is very marked, with about four orders of magnitude difference for the TRS inhibitors.

Both inhibitor classes exist in solution as the anionic form of the acyl-phosphate mimic, due to the acidity of the phosphodiester and of the acyl sulfamate NH function, respectively. Although the more extended delocalisation of the negative charge in the sulfamates may better complement Class II transition state stabilisation, it seems likely that the carbonyl group which is present in the acyl sulfamate but not in the aminoalkyl adenylate, plays a crucial role in recognition by the Class II enzymes.

Class I and Class II tRNA synthetases bind the aminoacyl adenylate intermediate in different conformations. In Class I enzymes the carbonyl group is relatively exposed — in *Bacillus stearothermophilus* tyrosyl tRNA synthetase complexed with tyrosyl adenylate, the carbonyl group does not appear to undergo any direct interaction with the protein,[6] as is also the case in the structures of other Class I complexes with aminoacyl adenylate or sulfamates. The aminoalkyl adenylates are thus able to act as potent inhibitors of the Class I enzymes. By contrast, in the Class II complexes, the carbonyl of the adenylate is not only buried at the protein interface but it is also *syn* to the adjacent amino group. There is a resulting network of hydrogen bonds to the protein and to the amine. For example, in the crystal structure of *E. coli* HRS complexed to histidyl adenylate,[22] the carbonyl



Scheme 2. Reagents: (i) Fmoc-Arg(PMC)-ol, tetrazole, 6.5 h then I_2/H_2O/2,6-lutidine, 2 h, rt; (ii) Boc-His(Boc)-ol, tetrazole, 5 h then I_2/H_2O/2,6-lutidine 2 h, rt; (iii) Fmoc-Thr(*t*Bu)-ol, tetrazole, 5 h then I_2/H_2O/2,6-lutidine, 2 h, rt; (iv) NH_3, THF, H_2O, 5–17 h, rt; (v) TFA 0.5 h then TFA:H_2O (5:1), 2 h, rt.

group appears to participate in hydrogen bonds to Arg113, Gln127 and to the alpha amino group. The disruption of this network of hydrogen bonds by replacing the carbonyl with a methylene would account for the large drop in affinity of the aminoalkyl adenylate as compared to the sulfamate.

The relative affinity of the aminoalkyl adenylate and aminoacyl sulfamate inhibitors thus appears to reflect a more general difference in the interactions with the high energy intermediate of the catalytic cycle of the Class I and Class II tRNA synthetases.

Acknowledgements

We thank Mr Martin Hibbs for enzyme purification. This work was partly supported by EU project PL962188.

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