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INTERACTION OF TYROSYL ARYL DIPEPTIDES WITH *S. AUREUS* TYROSYL tRNA SYNTHETASE: INHIBITION AND CRYSTAL STRUCTURE OF A COMPLEX

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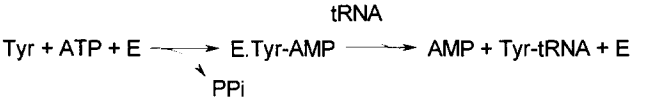
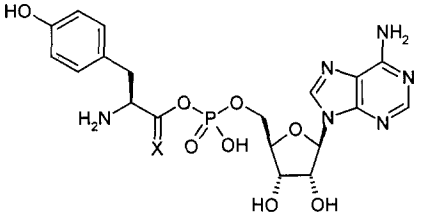
Abstract: Tyrosyl aryl dipeptide inhibitors of *S. aureus* tyrosyl tRNA synthetase have been identified with IC_{50} values down to 0.5 μM. A crystal structure of the enzyme complexed to one of the inhibitors shows occupancy of the tyrosyl binding pocket coupled with inhibitor interactions to key catalytic residues.

Aminoacyl tRNA synthetases catalyse the attachment of a proteinergic amino acid to its cognate tRNA, an essential step in protein biosynthesis. Tyrsoyl tRNA synthetase, like other members of the family, carries out the reaction in a two stage process via formation of an aminoacyl adenylate intermediate (Tyr-AMP; **1**). The aminoacyl tRNA synthetases are divided into two classes determined by different signature motifs and three dimensional structures.[1] Tyrosyl tRNA synthetase is a member of the Class I tRNA synthetases which are characterised by catalytically important HIGH and KMSKS sequence motifs.



Although aminoacyl tRNA synthetases perform an essential role in the cellular biochemistry of all organisms there are significant structural differences between bacterial and mammalian enyzmes, so that selective inhibition of tRNA synthetases offers potential for a new antibacterial therapy. *Staphylococcus aureus* is a key Gram positive bacterial pathogen and we selected the design of novel inhibitors of the *S. aureus* tyrosyl tRNA synthetase as a possible therapeutic approach . Stabilised mimics of the intermediate adenylate such as tyrosinyl adenylate are potent inhibitors of tyrosyl tRNA synthetase[2-4] but their polarity prevents their transport across the bacterial cell wall. Some tyrosyl dipeptides have been reported to inhibit *E. coli* tyrosyl tRNA synthetase with Ki values ranging from 4 – 100 : M.[3] Here we report novel sub-micromolar dipeptide inhibitors of *S. aureus* tyrosyl tRNA synthetase and the mode of inhibition as characterised by an X-ray crystal structure of an enzyme-inhibitor complex.



1 R = O
2 R = H_2

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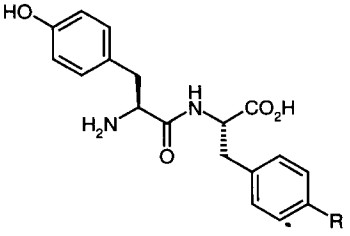
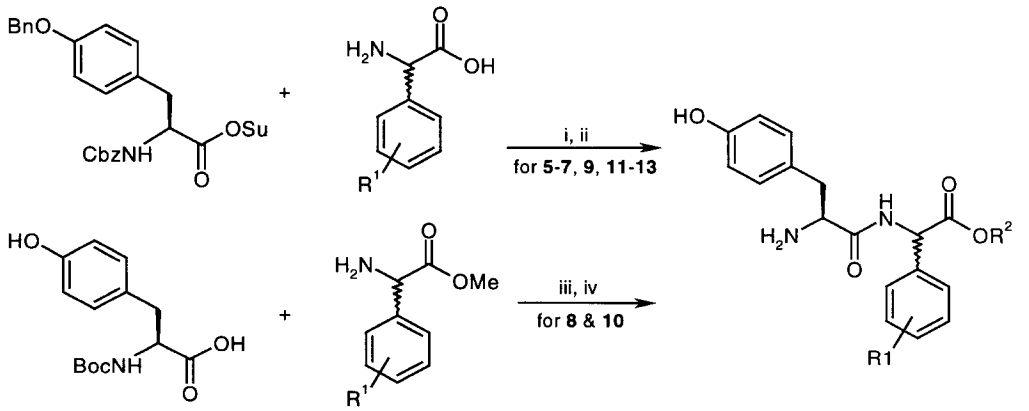
With *S. aureus* tyrosyl tRNA synthetase we found that a number of tyrosyl alkyl dipeptides did not afford any inhibition up to 3 μM, the highest concentration tested.[5] However, inhibition was observed for tyrosyl tyrosine **3**, which had an IC_{50} value of 3.8 μM. The phenolic hydroxyl appears to be important for recognition as the phenylalanine analogue **4** was inactive. Thus a series of substituted aryl dipeptide derivatives of tyrosine were targeted as potential inhibitors of the *S. aureus* tyrosyl tRNA synthetase.



3 R = OH
4 R = H

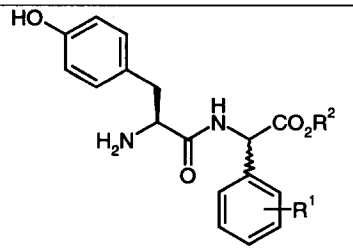
(DL)-2-Hydroxyphenylglycine[6] and (DL)-2,3,4-trihydroxyphenylglycine[7] were prepared by literature procedures, all other amino acids were sourced from commerical suppliers. The protected derivatives of dipeptides **5-7**, **9** and **11-13** were prepared from the requisite amino acid and L-*N*-Cbz-*O*-benzyltyrosine N-hydroxysuccinimide ester with sodium bicarbonate as base. Simultaneous removal of the *O*-benzyl and *N*-Cbz groups was achieved by atmospheric hydrogenation, in ethanol, over 10% palladium charcoal for 2-10 hours. The methyl esters **8** and **10** were prepared by the coupling of L-*N*-*t*-Boc-tyrosine with the amino ester hydrochloride under standard conditions using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (DEC)/HOAt/DIPEA in DMF. Treatment with TFA resulted in cleavage of the *N*-*t*-Boc group to yield the dipeptide ester.



Reagents: i) $NaHCO_3$, DMF, H_2O, 24h, RT; ii) 10% Pd-C, EtOH, H_2, 1 atm., 2-10h, RT. iii) DEC, HOAt, DIPEA, DMF, 24h, RT; iv) TFA, 2-5h, RT.

The dipeptides **5** – **13** were evaluated in assays of the aminoacylation activity of *S. aureus* tyrosyl tRNA synthetase,[5] and the results are shown in Table 1. The L-phenylglycine derivative **5** was a good inhibitor with

an IC_{50} value of 0.9 μM. The inhibition was stereoselective as the D-epimer **6** did not afford any inhibition. The 2-hydroxyphenylglycine **7** was also a good inhibitor as was its methyl ester **8**, indicating that a free carboxylate is not important for recognition. The 3 & 4-hydroxy isomers **9** and **10** were less effective and in particular the lack of inhibition by the 4-isomer contrasts with the activity of tyrosyl tyrosine.



	Stereochem.	R^1	R^2	IC_{50} (μM)
(5)	L	H	H	0.9
(6)	D	H	H	NI
(7)	DL	2-OH	H	0.8
(8)	DL	2-OH	Me	1.2
(9)	DL	3-OH	H	3.4
(10)	DL	4-OH	Me	NI
(11)	L	3,4-diOH	H	11
(12)	DL	2,3,4-triOH	H	0.51
(13)	DL	2-F	H	7.8

Table 1. Inhibition of *S. aureus* tyrosyl tRNA synthetase by tyrosyl phenylglycines. NI – no inhibition observed at 3 •M.

Trihydroxylation of the phenyl ring, compound **12**, afforded the best inhibitor of this series with an IC_{50} of 0.5 μM. The 2-phenol appears play a very specific role in inhibition, possibly as a hydrogen bond donor, as detrimental effects were observed on its removal from **12** (to give **11**) or when it is replaced by fluoro (**13** compared to **7**). The inhibitors did not show whole cell antibacterial activity against *S. aureus*.

The tyrosyl tyrosine inhibitor **3** was crystallised with *S. aureus* tyrosyl tRNA synthetase and the structure of the complex solved.[8] Electron density was very clear for both tyrosines. As anticipated, the N-terminal tyrosine occupies the tyrosyl binding pocket (Figure 1). The carbonyl group of the amide function appears to mimic the phosphoryl moiety of tyrosyl adenylate bound to *B. stearothermophilus* tyrosyl tRNA synthetase.[9] The second tyrosine makes intimate interactions with the HIGH motif in the active site: the aryl ring makes a perpendicular stacking interaction to His50 and the phenolic hydroxyl makes a hydrogen bond to His47.

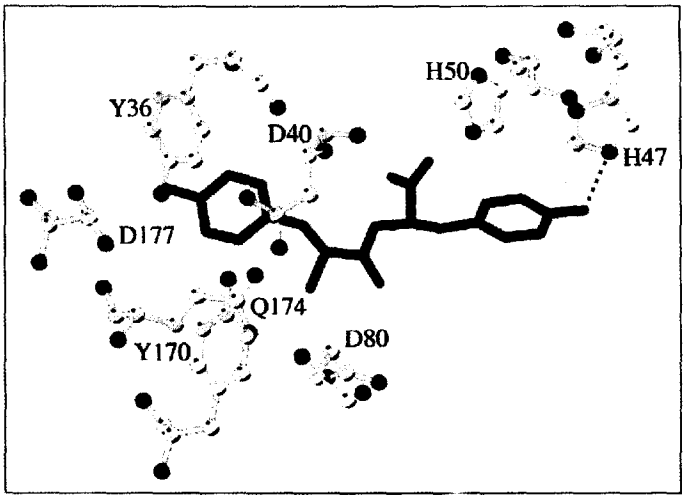


Figure 1. Binding of Tyr-Tyr in the active site of *S. aureus* tyrosyl tRNA synthetase. Protein carbons in yellow, nitrogens in blue and oxygen in red. The inhibitor is in blue and the hydrogen bond to His47 is shown in a dashed red line.

Site directed mutagenesis studies on *B. stearothermophilus* tyrosyl tRNA synthetase have shown that the two histidine residues of the HIGH site do not significantly interact with the substrates tyrosine and ATP in the ground state.[10] However, both residues contribute significantly to catalysis through interactions stabilising the transition rate of tyrosyl adenylate formation. The dipeptide inhibitor thus uses one tyrosine residue for recognition by the specific tyrosine binding pocket of tyrosyl tRNA synthetase and the other to subvert key elements of the catalytic apparatus in the enzyme active site.

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