Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 2499–2502

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Inhibitors of Bacterial Tyrosyl tRNA Synthetase: Synthesis of Carbocyclic Analogues of the Natural Product SB-219383

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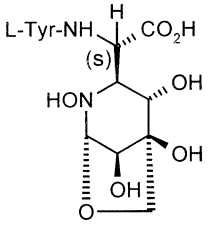
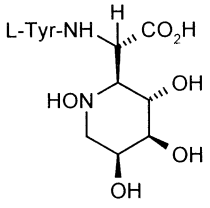
Received 16 March 2001; accepted 5 July 2001

Abstract—Carbocyclic analogues of the microbial metabolite SB-219383 have been synthesised and evaluated as inhibitors of bacterial tyrosyl tRNA synthetase. One compound showed highly potent and selective nanomolar inhibition.

Aminoacyl tRNA synthetases play a crucial role in protein synthesis in bacteria, as in all other organisms, and selective inhibition of the bacterial enzymes has potential for the treatment of bacterial infections. For example, the antibacterial agent mupirocin, marketed as Bactroban®, is an inhibitor of bacterial isoleucyl tRNA synthetase. SB-219383 **1**, identified from natural product screening, is a potent and selective bacterial tyrosyl tRNA synthetase (YRS) inhibitor (*Staphylococcus aureus* YRS, IC_{50} 0.6 nM; mammalian YRS, IC_{50} 22 μM).[1,2] Selective modification of **1** revealed that simple alkyl esters, such as the ethyl ester derivative, are equipotent to **1** and that the bicyclic scaffold is not crucial for inhibitory activity.[3] Totally synthetic analogues



SB-219383 **1**



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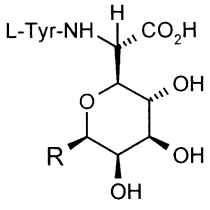
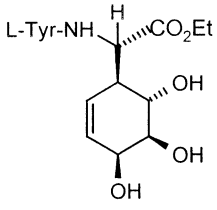
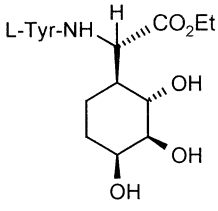


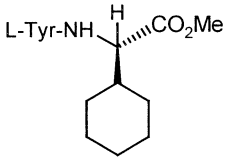
R= H : **3a**
R= Me : **4**



5a



6a



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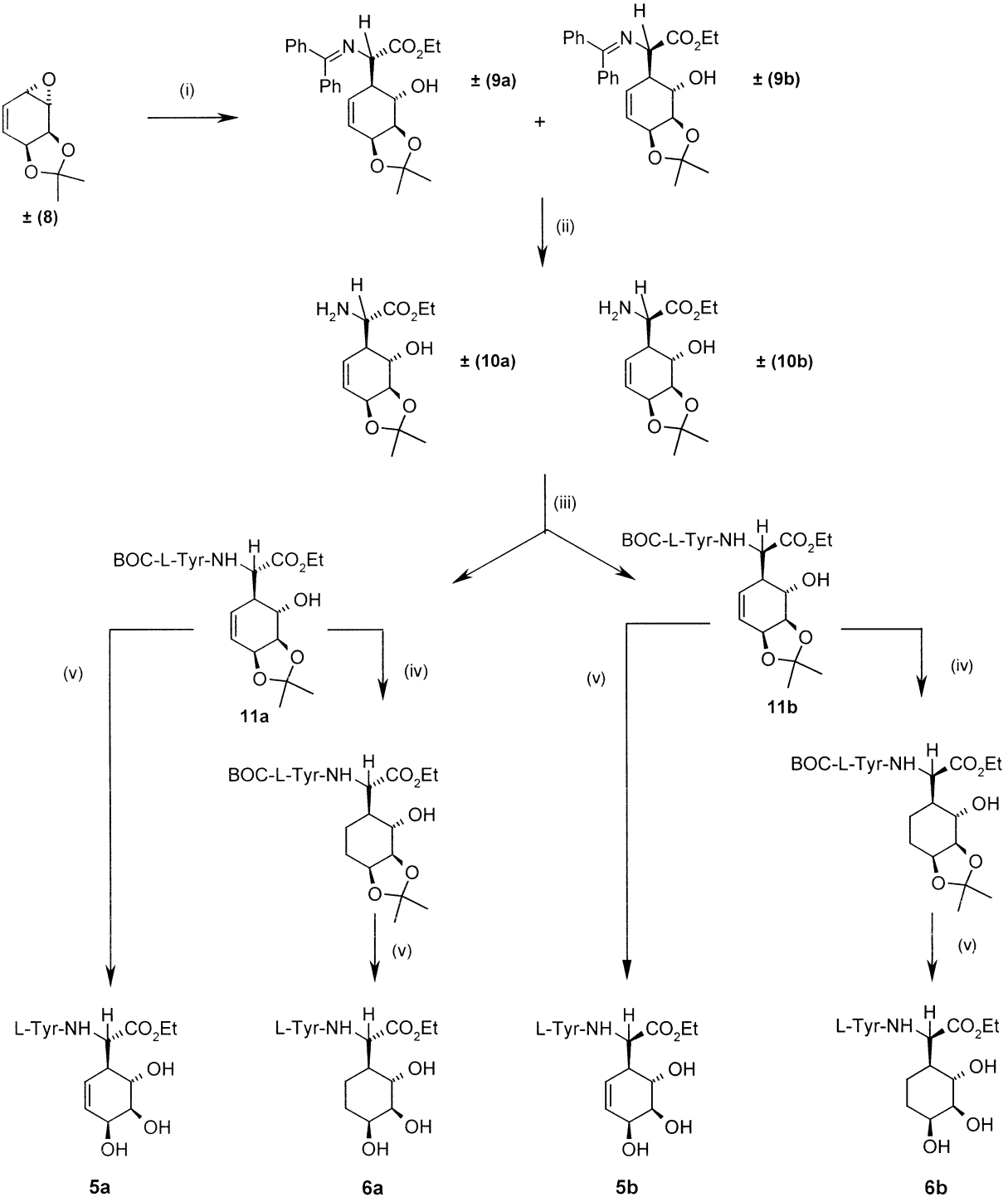
PII: S0960-894X(01)00475-9

have been prepared based on a cyclic hydroxylamine[4] or pyranose[5,6] scaffolds **2**–**4**. These compounds retain potent YRS inhibition, for example **2** and **4** have IC_{50} values of 1.2 and 4.0 nM, respectively.

These compounds, however, all show poor antibacterial activity. This could be attributed to their overall polarity reducing penetration through the bacterial cell wall. As part of the programme designed to improve the cell penetration of **1** and hence increase the antibacterial activity, the carbocyclic analogues **5a** and **6a** were identified as key targets. The unsubstituted cyclohexyl derivative **7** was also targeted as a reference compound.

Synthesis of Carbocyclic Analogues

Compound **7** was prepared by literature procedures from (*S*)-cyclohexylglycine.[7,8] For the synthesis of **5a** and **6a**, the protected racemic diol epoxide **8**[9] was



Scheme 1. (i) $Ph_2CNCHLiCOOEt$, $Et_2O{\cdot}BF_3$, Et_2O, −78 °C, 40%; (ii) pyr·HBr, EtOH, H_2O, acetone, 50%; (iii) BOC-L-TyrOH, EDAC, HOAt, DIPEA, DMF, 90%; (iv) H_2, Pd/C, MeOH, 97%; (v) TFA, 100%.

identified as a key starting material that potentially establishes the correct relative stereochemistry at all four ring positions (Scheme 1). It was anticipated that correct stereochemistry of the C–C bond would be formed by a direct *trans* ring opening of the epoxide using a glycine anion equivalent. Reaction of the epoxide **8** with the lithium enolate of *N*-(diphenylmethylene) glycine ethyl ester[10] in tetrahydrofuran at −78 °C and in the presence of a Lewis acid, $BF_3{\cdot}Et_2O$, provided the desired products **9a** and **9b** as a mixture with the 1,4-adducts formed by allylic ring opening of the epoxide. However, a regioselective introduction of the amino acid substituent was observed in diethyl ether to yield a 2:1 mixture of diastereoisomers **9a** and **9b.** Partial chromatographic separation provided **9a** as a single diastereoisomer and **9b** as a 1:1 mixture with the diastereoisomer **9a**. After deprotection of the amino function the pure diastereoisomer **10a** and the mixture **10a** plus **10b** were reacted separately with BOC-L-Tyrosine using EDAC–HOAt coupling conditions. Introduction of the additional chiral centre of L-tyrosine facilitated the complete chromatographic separation of the two pairs of diastereoisomers **11a** and **11b**, each pair containing a 1:1 mixture of the two isomers. Deprotection of the amine and diol functions using TFA gave the target compound **5a** and its diastereoisomer **5b**. Alternatively, hydrogenation of the alkene function in **11a** and **11b,** followed by TFA deprotection, produced the saturated analogue **6a** and its diastereoisomer **6b**. All four compounds, **5a**, **6a**, **5b** and **6b**, were analysed and tested as diastereoisomeric pairs.

The relative stereochemistry of these compounds was tentatively assigned by comparing the NMR data[11] of compounds **5a** and **5b** with the NMR data of compound **3a** and its diastereoisomer at position 7, **3b**.[5] The proton H-7 in compounds with the same relative stereochemistry at position 7 and all four ring positions as in SB-219383, that is compounds **3a** (4.91 ppm) and **5a** (4.87, 4.78 ppm), is found downfield compared to the corresponding proton in compounds **3b** (4.65 ppm) and **5b** (4.61, 4.68 ppm) where the stereochemistry at position 7 has been reversed. This proposed assignment of stereochemistry is in agreement with the biological activities of the corresponding compounds.

Biological Results

The carbocyclic analogues were tested as inhibitors of *S. aureus* YRS enzyme in a standard aminoacylation assay.[12] The inhibitory potencies are shown in Table 1.

The target molecule **5a**, in which one of the diastereoisomers has identical stereochemistry at each chiral centre to the natural product SB-219383, is a potent nanomolar inhibitor of *S. aureus* YRS, IC_{50} 3.4 nM.

Table 1. Tyrosyl tRNA synthetase inhibitory activities

Compound	1	5a	5b	6a	6b	7
IC_{50} (nM)	0.6	3.4	124	245	> 3000	> 3000

Compound **5a** is considerably more potent than the diastereoisomer **5b**, where at least one of the stereocentres is different from that found in SB-219383. The same stereospecificity applies to the saturated systems **6a** and **6b**. The unsaturated analogues **5a** and **5b** exhibit higher inhibitory activities compared to their saturated counterparts **6a** and **6b**, suggesting that the π orbital of the alkene could be mimicking the lone pair orbital of the heteroatom present in the heterocyclic systems **1**–**4**. Inhibition was not observed for compound **7** up to 3 μM, the highest concentration tested, confirming the importance of the three vicinal hydroxyl groups for recognition by the YRS enzyme.

Compounds **5a**, **6a**, **5b** and **6b** did not show antimicrobial activity against *S. aureus*, although compound **5a** exhibits weak activity against *Streptococcus pyogenes* (MIC 8 μg/mL).

In conclusion, this chemistry allows a rapid route to highly functionalised amino acid analogues such as **10**. Coupling to L-tyrosine yielded carbocyclic analogues of SB-219383 **1** which retained potent and selective inhibitory activity against the bacterial YRS enzyme.

Acknowledgements

We would like to thank Steven Rittenhouse for antimicrobial evaluation, Adrian Saldanha for preparation of compound **7**, and Anthony Beck and Christopher Seaman for 2D-NMR experiments.

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11. All compounds were characterised by 400 MHz ^{1}H NMR,

using 2D techniques where necessary. Comparison of ^{1}H NMR (CD_3OD) chemical shifts (ppm) and coupling constants (Hz) between compounds **5a** and **5b** is shown below:

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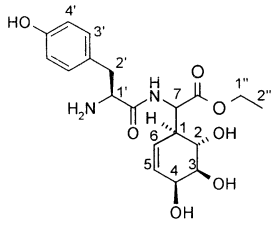

5a, 5b

H	**5a**	**5b**
1	2.6 (m)	2.54, 2.69 (2m)
2	3.4 (m)	3.69 (m)
3	3.4 (m)	3.37 (2 dd, $J=4.1$, 9.6 Hz)
4	4.0 (m)	4.05 (m)
5	5.84 (m)	5.71 (m)
6	5.42, 5.36 (2 dd, $J=2.0$, 10.0 Hz)	5.28, 5.54 (2 dd, $J=2.2$, 10.0 Hz)
7	4.87, 4.78 (2d, $J=3.3$ Hz)	4.61, 4.68 (2 d, $J=3.5$ Hz)
1′	4.1 (m)	4.05 (m)
2′	2.85 (m)	2.79, 2.82 (2 dd, $J=8.4$, 14.4 Hz)
2′	3.09 (m)	3.08 (m)
3′	7.03, 7.04 (2d, $J=8.5$ Hz)	7.00, 6.96 (2 d, $J=8.5$ Hz)
4′	6.68, 6.69 (2d, $J=8.5$ Hz)	6.66, 6.67 (2 d, $J=8.5$ Hz)
1″	4.1 (m)	4.05 (m)
2″	1.19, 1.20 (2t, $J=7.2$ Hz)	1.19 (t, $J=7.2$ Hz)