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Potent Synthetic Inhibitors of Tyrosyl tRNA Synthetase Derived from *C*-Pyranosyl Analogues of SB-219383

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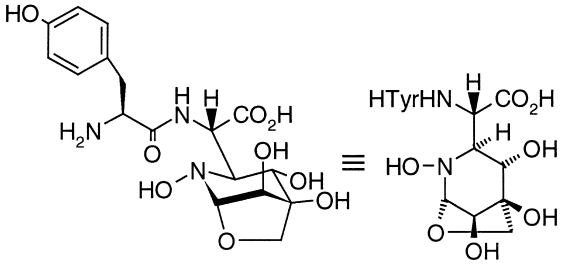
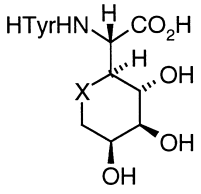
Abstract—Novel pyranosyl analogues of SB-219383 have been synthesised to elucidate the structure–activity relationships around the pyran ring. Analogues with highly potent stereoselective and bacterioselective inhibition of bacterial tyrosyl tRNA synthetase have been identified. A major reduction in the overall polarity of the molecule can be tolerated without loss of the nanomolar level of inhibition. © 2001 Elsevier Science Ltd. All rights reserved.

The increasing emergence of pathogenic bacteria resistant to conventional antibiotics has made the development of novel agents against bacterial targets ever more urgent. In our search for novel inhibitors of bacterial tRNA synthetases as potential antibacterial agents, the natural product, SB-219383 **1**,[1,2] was discovered as a potent, selective inhibitor of bacterial tyrosyl tRNA synthetase (YRS). The synthetic analogue **2** has been found to be equipotent to **1**, and has been used to infer the absolute stereochemistry of the natural product.[3] In the previous paper,[4] we showed that the pyranose analogue **3** is a selective inhibitor of bacterial YRS, although with an IC_{50} of 100 nM it was less potent than **2**. We sought to simplify the scaffold further, in order to investigate which of the features of this molecule were essential for activity and to give more scope for the variation of polarity. Here we report the synthesis and SARs of new pyranosyl ring analogues which resulted in the identification of a highly potent sub-nanomolar synthetic inhibitor of bacterial YRS.

In order to probe the requirements around the pyranosyl ring, selected sugars were taken through the Lewis acid catalysed *C*-glycosylation synthesis.[4] These included the L-fucose, D-galactose, D-glucose and L-lyxose templates. From D-fucose, the final diastereomers **4a** and **4b** were separated by preparative HPLC and isomers **a** and **b** inferred to have the (*R*) and (*S*)-stereochemistry at C1′ on the basis of the chemical shift of H1.[5] To further reduce polarity, the fucose derivative **4b**



1 SB 219383



2 X = NOH
3 X = O

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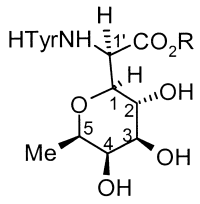
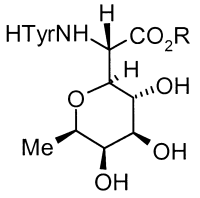
was also converted into the butyl ester **5** by treatment with butanol in the presence of HCl. Starting from the more polar D-galactose or D-glucose sugars, final epimer separation was not possible and the diastereomer ratios of **6** and **7**, respectively, were determined by NMR.[5] From L-lyxose, it was possible to reduce the diastereomer corresponding to **b** with sodium borohydride, which was then elaborated to **8** without epimerisation, as judged by NMR. In some cases, the bulky pivaloyl protecting groups resulted in reduced yields in the *C*-glycosylation step and attempts were made to improve the scope of the reaction, for example by using alternative protecting groups. However, any protecting group other than pivaloyl resulted in the formation of a bicyclic acetal such as **10** during the attempted *C*-glycosylation.

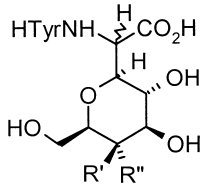
Due to the neighbouring group participation required for the *C*-glycosylation, it is not possible to synthesise analogues epimeric at the 2-position by this method. However, in order to probe further the requirements for the polar hydroxy groups in this region, the 2-deoxy



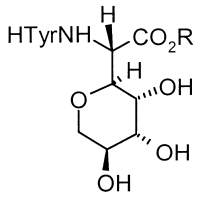
4a R = H



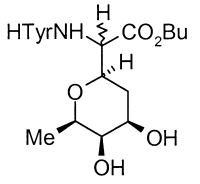
4b R = H
5 R= Bu



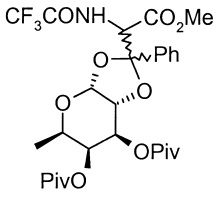
6 R' = OH , R'' = H
7 R' = H , R'' = OH



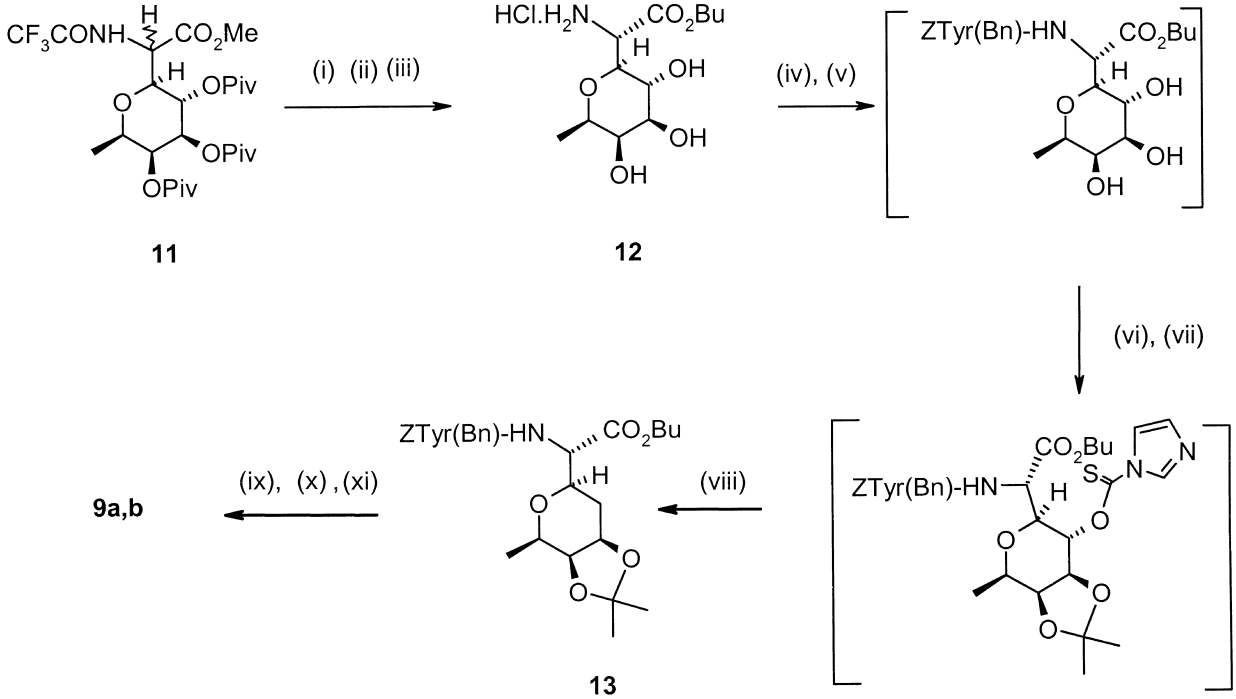
8



9



10



11 **12** **13**

9a,b

Scheme 1. (i) NaOH, MeOH, H_2O, 80 °C; (ii) HCl; (iii) *n*-BuOH, HCl, 100 °C, 16 h; (iv) $F_3CC(O)N(Me)TMS$, $N(^iPr)_2Et$, pyridine, 0 °C; (v) Z-Tyr(Bn)OH, HOBt, EDAC, DMF, $N(^iPr)_2Et$, (vi) $Me_2C(OMe)_2$ pyridinium toluene-4-sulfonate; (vii) *N*,*N*′-thiocarbonyl diimidazole, benzene, 80 °C, 38 h; (viii) Bu_3SnH, benzene, 80 °C, 18 h; (ix) DBU, 1,2-dichloroethane, 80 °C, 18 h; (x) aq HCl, dioxan; (xi) H_2, Pd/C, TFA.

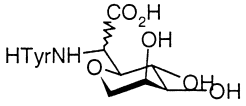
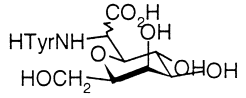
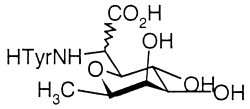
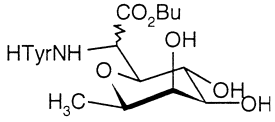
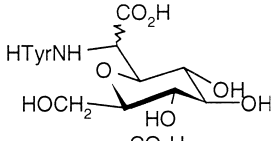
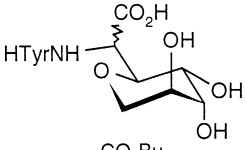
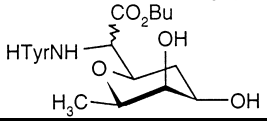
compound **9** was prepared from the fucose analogue **11** using Barton methodology[6] as shown in Scheme 1. Thus the diasteromeric mixture **11a**,**b** was completely deprotected with sodium hydroxide in aqueous dioxan, and then converted into the butyl ester **12**. This was persilylated prior to coupling to suitably protected tyrosine[3] followed by formation of the 3,4-acetonide. Conversion of the unprotected 2-hydroxy into the thiocarbonylimidazolide followed by radical de-oxygenation gave the 2-deoxy analogue **13**. This was re-epimerised at the glycine centre with DBU, followed by deprotection to afford the 2-deoxy compound **9** as a 2:1 mixture of (*R:S*) isomers as determined by NMR.

The compounds were evaluated as inhibitors of *Staphylococcus aureus* tyrosyl tRNA synthetase (YRS), in an aminoacylation assay,[7] and the results are given in Table 1. At C-5, the hydroxymethyl group of **6** results in a roughly similar level of potency to the unsubstituted compound **3**. However, introducing a methyl group at C-5, **4b**, gives rise to a 20-fold improvement, taking the inhibition to 4 nM, the level of SB-219383 itself. The potent inhibition of compound **4b** is both stereoselective (no inhibition for the epimer **4a**) and bacterioselective (no inhibition of mammalian YRS up to 3 μM). The potency of **4b** is further improved to 0.8 nM on the formation of the butyl ester **5**. The role of the hydroxyl groups around the ring in recognition by YRS was also elucidated. The axial stereochemistry at C-4 is crucial to potency (compare **7** with **6**) as is the equatorial stereochemistry at the C-3 position (compare **8** with **3**). However, removal of the 2-hydroxyl group is well tolerated as shown by the potent inhibition observed for compound **9.**

By comparison with the potent hydroxylamine, **2**, it has been inferred that the conformation at the glycine centre of **4b** is (*S*). This has been further supported by X-ray crystallography in the active site of YRS.[8,9] The importance of the hydroxyl groups has also been noted in the active site. The axial hydroxyl at C-4, which is crucial for potency, makes two significant interactions, one with the Gly193NH in the protein, and one with an ordered water molecule, while the two hydroxyl groups of the equatorial diol appear to interact with the Asp 195 side chain.

Despite the greatly reduced polarity of **9** compared to SB-219383,[10] the compound did not show any antibacterial activity against a variety of bacteria up to 32 μg mL^{-1}. Compound **5**, however, had weak antibacterial activity against the pathogens *Moraxella catarrhalis* and *Streptococcus pyogenes*, with MICs of 8 μg mL^{-1}.

Table 1. Inhibition of *S. aureus* tyrosyl tRNA synthetase

Compound	Sugar ring		Stereochemistry at C1′[5]	IC_{50} (nM)
3	L-Arabinose[3]		(*S*)	100
6	D-Galactose		3:1 (*R*):(*S*)	190
4a	L-Fucose		(*R*)	NI[a]
4b			(*S*)	4
5	L-Fucose butyl ester		(*S*)	0.8
7	D-Glucose		2:3 (*R*):(*S*)	NI
8	L-Lyxose		(*S*)	71%@ 3 μM
9	2-Deoxy-fucose		2:1 (*R*):(*S*)	3.6

[a]NI = no inhibition at 3 μM.

In conclusion, we have identified key interactions of the pyranosyl analogues of SB-219383 and synthesised compounds with highly potent and selective inhibition of bacterial tyrosyl tRNA synthetase. We have also shown that a major reduction in the overall polarity of the molecule can be tolerated without loss of the nanomolar level of inhibition.

Acknowledgements

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References and Notes

1. Stefanska, A. L.; Coates, N. J.; Mensah, L. M.; Pope, A. J.; Ready, S. J.; Warr, S. R. *J. Antibiot*. **2000**, *53*, 345.
2. Houge-Frydrych, C. S. V.; Readshaw, S. A.; Bell, D. J. *J. Antibiot*. **2000**, *53*, 351.
3. Berge, J. M.; Copley, R. C. B.; Eggleston, D. S.; Hamprecht, D. W.; Jarvest, R. L.; Mensah, L. M.; O'Hanlon, P. J.; Pope, A. J. *Bioorg. Med. Chem. Lett*. **2000**, *10*, 1811.
4. Brown, P.; Haltiwanger, R. C.; Jarvest, R. L.; Mensah, L.; O'Hanlon, P. J.; Pope, A. J. *Bioorg. Med. Chem. Lett*. **2001**, *11*, 711.
5. It was found that in any pair of diastereomers at the glycine centre, the chemical shift of the H1 proton differs by ~1 ppm, while the H1–H1′ coupling constant is small in both diastereomers. By analogy to the L(+)-arabinose derived series **3**,[4] the diastereomer with the higher-field chemical shift of H1, isomer **a,** is inferred to have the (*R*) stereochemistry at C1′.

Examples of chemical shifts (ppm) and coupling constants (Hz) in *C*-pyranosyl dipeptides (D_2O)

Compound	Isomer	H-1	H-2
3	**a**	2.68, dd, *J* 9.9, 1.5 Hz	3.00, t, *J* 9.5 Hz
	b	3.87, dd, *J* 2.0,10.0 Hz	3.62, t, *J* 9.7 Hz
4	**a**	2.67, dd, *J* 10.1, 1.9 Hz	3.83, t, *J* 9.6 Hz
	b	under m, 3.74–3.89 Hz	3.55, t, *J* 9.6 Hz
6	**a**	2.67, d, *J* 9.9 Hz	
	b	under m, 3.87–3.95 Hz	
7	**a**	2.87 d *J* 10.0 Hz	3.22, t, *J* 9.7 Hz
	b	3.88, d, *J* 9.9 Hz	3.59. t, *J* 9.1 Hz

6. Barton, D. H. R.; Motherwell, R. S. H.; Motherwell, W. B. *J. Chem. Soc., Perkin Trans. 1* **1981**, 2363.
7. Brown, P.; Mensah, L. M.; O'Hanlon, P. J.; Osborne, N. F.; Pope, A. J.; Richardson, C. M.; Walker, G. *Bioorg. Med. Chem*. **1999**, *7*, 2473.
8. Qiu, X. et. al., manuscript in preparation. Brown, P.; Berge, J. M.; Hamprecht, D. W.; Jarvest, R. L.; McNair, D. J.; Mensah, L. M.; Pope, A. J.; Qiu, X.; Richardson, C. M. Poster presentation at the 18th tRNA Workshop, Cambridge, UK, April 18–21, 2000.
9. When a 5:1 mixture of **4a:4b** was subjected to co-crystallisation with *S. aureus* YRS, the crystals obtained contained the (*S*)-isomer **4b** only, supporting the hypothesis that the active diastereomer has the (*S*)-stereochemistry at C1′.[8]
10. Calculated logP values for compounds **1** and **9** using the Pomona clogP algorithm (1998) were −4.28 and 0.22, respectively.