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

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Abstract—Synthetic analogues of the microbial metabolite SB-219383 have been synthesised with defined stereochemistry. Densely functionalised hydroxylamine containing amino acids were prepared by the addition of a glycine anion equivalent to sugar-derived cyclic nitrones. One of four stereoisomeric dipeptides incorporating these novel amino acids was found to be a potent and selective inhibitor of bacterial tyrosyl tRNA synthetase, suggesting analogous stereochemistry of the natural product. © 2000 Elsevier Science Ltd. All rights reserved.

SB-219383 is a natural product isolated from a fermentation broth of *Micromonospora* sp. The compound was found to be a potent and selective inhibitor of bacterial tyrosyl tRNA synthetase (YRS) (*Staphylococcus aureus* YRS, IC_{50} 1.4 nM; mammalian YRS, IC_{50} 22 μM).[1] Inhibition of bacterial isoleucyl tRNA synthetase is the mode of action of the antibacterial agent mupirocin (marketed as Bactroban®) and selective inhibitors of tRNA synthetases are thus of interest as new antibacterials with a novel mode of action.[2] Extensive NMR studies and amino acid analysis showed SB-219383 to be a dipeptide with an N-terminal L-tyrosine unit coupled to a novel highly functionalised bicyclic α-amino acid. This amino acid incorporates an unprecedented *N*-hydroxylamino sugar *C*-glycosidically linked to the α-carbon of a glycine.[3] Whereas the relative stereochemical arrangement within the bicyclic moiety could be deduced from NMR experiments to be as shown in structure **1**, the relative stereochemistry between the amino acid α-stereocentre and the ring system as well as the absolute configuration of this C-terminal amino acid remained elusive. Consequently, there were four possibilities for the stereostructure of SB-219383.

We decided to use a stereoselective synthesis approach to identify the correct stereostructure and as a basis for the design of further analogues as YRS inhibitors. Previously it had been demonstrated that the monocyclic derivative **2** retains potent YRS inhibition.[4] We therefore speculated that the methyleneoxy moiety of the tetrahydrofuran ring could be omitted without loss of biological activity, thus simplifying the synthetic targets to structures such as **3** (Fig. 1). Here we report the synthesis and evaluation of four stereoisomers of **3** in which the amino acyl α-stereocenter and the absolute stereochemistry of the unnatural amino acid are defined.

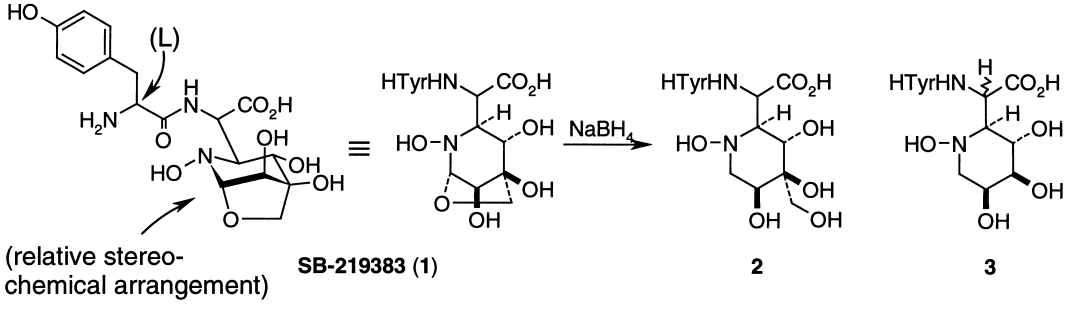

Figure 1.

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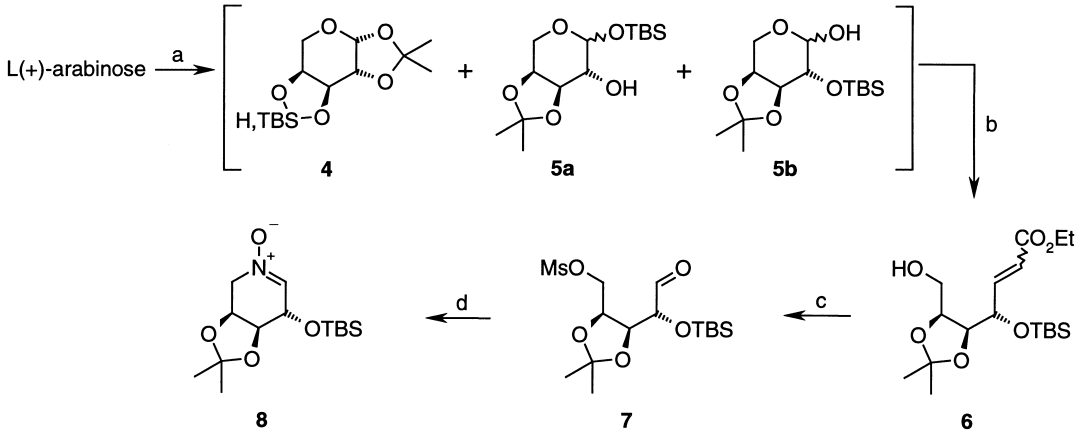
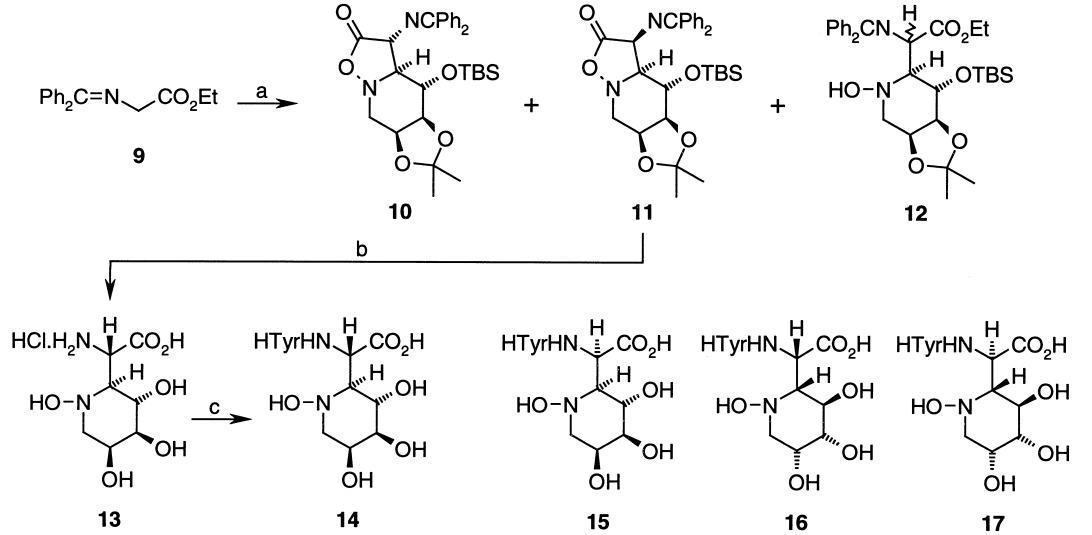


Scheme 1. (a) (i) $(MeO)_2CMe_2$, cat. TsOH, DMF; (ii) TBSCl, imidazole, CH_2Cl_2, 0 °C to 25 °C; (b) $Ph_3P{=}CHCO_2Et$, cat. $EtCO_2H$, THF, 60 °C (11% from arabinose); (c) (i) MsCl, NEt_3, cat. DMAP, CH_2Cl_2, 0 to 25 °C; (ii) O_3, EtOAc, −78 °C; Me_2S, −78 to 25 °C (79%); (d) $H_2NOH{\cdot}HCl$, NEt_3, EtOH, 25 °C (95%).

Arabinose was chosen as the starting material since it is readily available in both enantiomeric forms and three of the five stereocentres of the C-terminal amino acid are set up in the desired configuration. It was anticipated that its conversion to a suitably protected nitrone[5] would allow nucleophilic introduction[6] of the amino acid moiety. Acetonide protection of L(+)-arabinose[7] resulted in a mixture of the 1,2- and 3,4-acetonides which were difficult to separate at this stage. Reaction with *tert*-butyldimethylsilyl chloride gave the monosilylated products **4** and **5** (Scheme 1). When this mixture was submitted to acid catalysed Wittig olefination conditions, silyl migration took place to interconvert **5a** and **5b**, resulting in the efficient formation of the olefination product **6** from both isomers (73 and 79% yield, respectively, when the separated isomers were used). The enoate moiety masks the aldehyde and serves to liberate the primary hydroxyl group. Mesylation followed by ozonolysis gave rise to δ-mesyl aldehyde **7** which readily reacted with hydroxylamine to give the stable crystalline nitrone **8** in excellent yield.

For the key stereoselective C–C bond forming reaction a moderately basic glycine anion equivalent was anticipated to attack the nitrone from the face opposite to the adjacent bulky silyl ether. Gratifyingly, reaction of nitrone **8** with the lithium enolate of glycine derivative **9**[8] occurred exclusively from the less hindered side to give rise to the two diastereomeric tricyclic isoxazolidinones **10** and **11**, both possessing the desired stereochemistry at the β position (Scheme 2). Both α-epimers were obtained in similar yields, thus allowing access to both the D- and L-amino acids. The relative stereochemistry of these compounds was unambiguously established by the X-ray crystal structure determination of the opposite enantiomer to **10**.[9] Treatment of isomer **11** with aqueous HCl resulted in complete deprotection and hydrolysis of the *O*-acyl hydroxylamine to give amino acid **13**. Attempts to directly couple **13** to *N*-Boc-L-tyrosine resulted in complex product mixtures and low yields. However, silylation[10] using excess *N*-methyl-*N*-trimethylsilyl trifluoroacetamide, followed by coupling at elevated temperature, subsequent desilylation, and



Scheme 2. (a) LiHMDS, toluene, −78 °C; **8**, −78 to 0 °C (**10**: 35%, **11**: 36%, **12**: trace); (b) HCl, H_2O, dioxane, 25 °C; (c) (i) $F_3CC(O)N(Me)TMS$, $i Pr_2EtN$, pyridine, 25 °C; BocTyrOSu, 60 °C; MeOH, H_2O, 25 °C; (ii) F_3CCO_2H, 25 °C (31% from **11**).

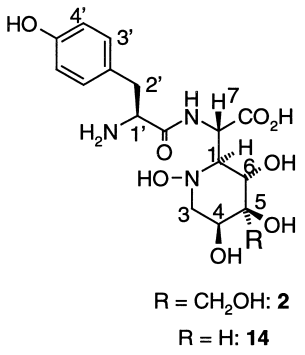


Figure 2.

Table 1. Proton NMR data (400 MHz, D_2O) of compounds **14** and **2** (Fig. 3)

H	14	2
1	3.18 (dd, J = 10.1, 1.1 Hz)	3.41 (dd, J = 10.5, 1.3 Hz)
3ax	2.83 (dd, J = 11.8, 1.2 Hz)	3.06 (dd, J = 12.4, 2.1 Hz)
3eq	3.34 (dd, J = 11.7, ca. 3 Hz)	3.39 (dd, J = 12.4, 3.2 Hz)
4	4.01 (ddd, J ca. 4.9, 1.5, 1.5 Hz)	4.06 (dd, J = 2.9, 2.2 Hz)
5	3.55 (dd, J = 9.7, 3.5 Hz)	—
CH_2OH	—	3.90 (d, J = 12.6 Hz)
$C\underline{H}_2OH$	—	3.96 (d, J = 12.6 Hz)
6	3.4 (bt, J = 9.8 Hz)	3.52 (bd, J = 9.5 Hz)
7	4.82 (d, J = 1.0 Hz)	4.65 (d, J = 1.1 Hz)
1′	4.36 (dd, J = 8.6, 5.2 Hz)	4.11 (dd, J = 7.9, 4.7 Hz)
2′	3.05 (dd, J = 14.8, 8.6 Hz)	3.04 (dd, J = 14.4, 7.9 Hz)
2′	3.32 (dd, J = 14.8, 5.2 Hz)	3.24 (dd, J = 14.4, 4.9 Hz)
3′	7.19 (d, J = 8.6 Hz)	7.25 (d, J = 8.5 Hz)
4′	6.85 (d, J = 8.6 Hz)	6.94 (d, J = 8.5 Hz)

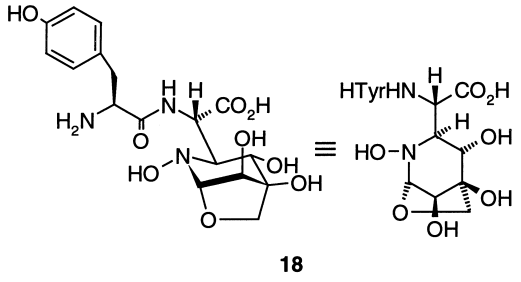


Figure 3. Likely stereostructure of SB-219383.

deprotection of the amino terminus gave the target dipeptide **14** in acceptable overall yield. Similar treatment of compound **10** gave rise to the analogous dipeptide **15** with D-configuration of the C-terminal amino acid. Additionally, compounds **16** and **17** with inverted chirality of the C-terminal amino acid were obtained when D(−)-arabinose was used as the starting material.

The four stereoisomers were tested in a standard aminoacylation assay of YRS activity.[11] Dipeptide **14** was found to be a potent inhibitor of bacterial YRS (IC_{50} 1.2 nM) with good selectivity over the mammalian enzyme (11% inhibition at 3 μM) whereas epimer **15** showed significantly weaker inhibition (IC_{50} 21.3 nM; the presence of up to 5% **14** in **15** (which would account for this level of inhibition) cannot be ruled out). Isomers **16** and **17** were inactive when tested up to 3 μM. In addition to **3** being about equipotent to SB-219383, the proton NMR spectra of **2** and **14** are almost identical (with the exception of the obvious differences resulting from the omission of the methyleneoxy unit in **14**) (Table 1, Fig. 2) Thus, the stereostructure of SB-219383 is most likely to be as shown in **18** (Fig. 3). Potent inhibition by **14** also confirms that neither the tetrahydrofuran ring of **1** nor the pendant hydroxymethyl group of **2** are important for YRS recognition. Synthesis and activity of further analogues of these compounds will be reported in due course.

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9. X-ray crystal structure determination of the opposite enantiomer to **10**. Crystals were grown via the evaporation of a $CDCl_3$/DMSO-d_6 solution at reduced pressure. Crystal data: colourless blade, 0.63×0.27×0.05 mm; $C_{29}H_{38}N_2O_5Si$, M_r = 522.70, monoclinic, C2 (no. 5), a = 30.105(5), b = 8.7694(16), c = 11.421(2) Å, β = 103.431(12)°, V = 2932.7(10) Å^3, Z = 4, D_x = 1.184 Mg m^{-3}, μ (Cu K_α, λ = 1.54178 Å) = 1.019 mm^{-1}. Data collection: Nonius MACH3 diffractometer, GX21 rotating copper anode generator, graphite monochromator, 293 K, θ_{max} = 58.91°, $\omega/2\theta$ scans, N_{ref} = 4794, N_{uniq} = 4220. Data reduction: corrections for Lorentz and polarisation effects; psi-scan absorption correction, T_{min} = 0.6774, T_{max} = 0.9206; decay correction, four standard reflections, maximum variation = 13.0%; R_{int} = 0.0325. Solution and refinement: SHELXTL V5.10 IRIX package; direct methods; full-matrix least-squares refinement on F^2; coordinates and anisotropic displacement parameters refined for the non-hydrogen atoms; hydrogen atoms in idealised positions, riding or as rigid rotating groups, with isotropic atomic displacement parameters which were an appropriate multiple of U_{eq} for the >bonded atom; N_{ref} = 4220, N_{par} = 343, $R1$ (3545 data with $I > 2\sigma(I)$) = 0.0434, w$R2$ (all data) = 0.1069, S = 1.023; $\Delta\rho_{min}$ = −0.103 e Å^{-3}, $\Delta\rho_{max}$ = 0.148 e Å^{-3}; extinction coefficient = 0.00113(9); abso-

lute structure parameter (Flack) = 0.00(4). Crystallographic data for this structure has been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44-1223-336033 or e-mail: deposit @ccdc.cam.ac.uk).

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