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SB-219383, a Novel Tyrosyl tRNA Synthetase Inhibitor from a *Micromonospora* sp.

II. Structure Determination

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A novel tyrosyl tRNA synthetase inhibitor, SB-219383, has been isolated from a *Micromonospora* sp. The structure of SB-219383 has been elucidated by a combination of derivatisation, spectroscopic and other analytical techniques and found to be *N*-(L-tyrosyl)-2-amino[1(*S**),3(*S**),4(*S**),5(*R**),8(*R**)-2,4,5,8-tetrahydroxy-7-oxa-2-azabicyclo[3.2.1]oct-3-yl]acetic acid (**1**).

SB-219383 (**1**), isolated from fermentation broth of a novel species of *Micromonospora* sp. NCIMB 40684, is the first member of a new class of compounds having inhibitory activity against tyrosyl tRNA synthetase (IC_{50}, 2 nM). SB-219383 also exhibits moderate *in vitro* activity against some Gram-positive bacteria. The preceding paper describes the fermentation, isolation, physico-chemical and biological properties of **1**[1]. The structure elucidation of SB-219383 is reported in this paper. The structure of **1** as well as its 9-fluorenylmethoxycarbonyl (FMOC) methyl ester (**2**) are shown in Fig. 1.

Results and Discussion

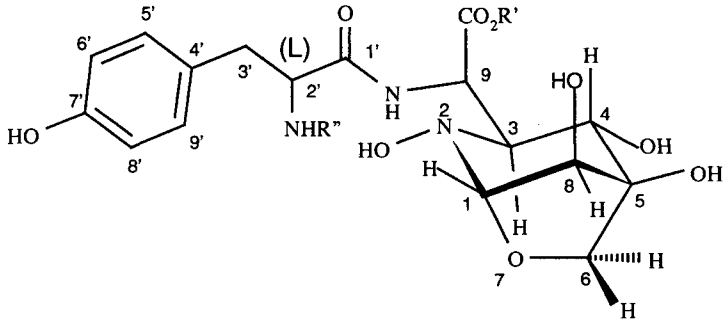
UV data [λ_{max} (H_2O) 225, 275 nm] suggested that SB-219383 contained a *para*-phenol moiety. Amino acid analysis and Edman degradation confirmed this *para*-phenol fragment to be part of a tyrosyl group. These studies also indicated that the tyrosine possessed a free amino group and was linked through its carboxylate to a "non standard" amino acid. Chiral derivatisation and ligand exchange chromatography confirmed the L-tyrosine configuration.

A molecular mass of 413 was determined for SB-219383 using electrospray and fast atom bombardment techniques both in the positive and negative ion modes. Structurally significant fragmentation ions were also generated using MS/MS with fragment ion 181 supporting the presence of a tyrosylamide. From the NMR data it was concluded that SB-219383 possessed 17 carbons and 14 non-exchangeable protons. Determination of the number of exchangeable protons could not be obtained from the NMR data but was obtained by performing electrospray mass measurements in a deuteriated solvent and then calculating the observed shift (relative to measurements in the non-deuteriated solvent). This mass deuteriation data (indicating 9 exchangeable protons) together with the accurate mass measurement (rmm 413.1434 observed) and NMR data provided a molecular formula of $C_{17}H_{23}N_3O_9$ (413.1435 calculated).

Analysis of the 1D proton and 2D COSY-45 NMR data for SB-219383 allowed a number of structural fragments to be identified including the tyrosylamide evident from other spectroscopic data (as discussed above) (Fig. 2). The tyrosylamide moiety (Fragment 1) accounts for 7 of the 14 non-exchangeable protons and 9 of the 17 carbons. The remaining 7 non-exchangeable protons were found to be associated with Fragments 2 and 3. The chemical shifts observed for the protons within Fragments 2 and 3 dictate the presence of various electronegative functionalities (denoted as X in Fig. 2). These sub-structure fragments were also fully consistent with the carbon-13 NMR data (Table 1).

Linking these structural fragments together with the

Fig. 1. Structures of SB-219383 (**1**) and SB-227871 (**2**).



SB-219383 (1) : R' = H, R" = H

SB-227871 (2) : R' = Me, R" = FMOC =

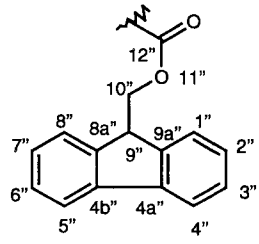
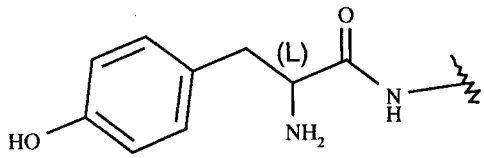
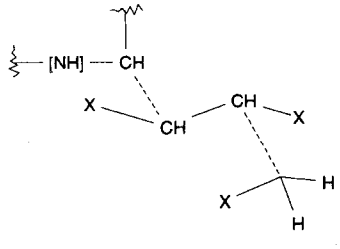


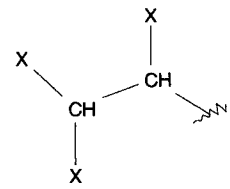
Fig. 2. NMR-derived sub-structure fragments for SB-219383. (Dotted line bonds signify "connections" through either a small 3-bond or 4-bond coupling).



Fragment 1



Fragment 2



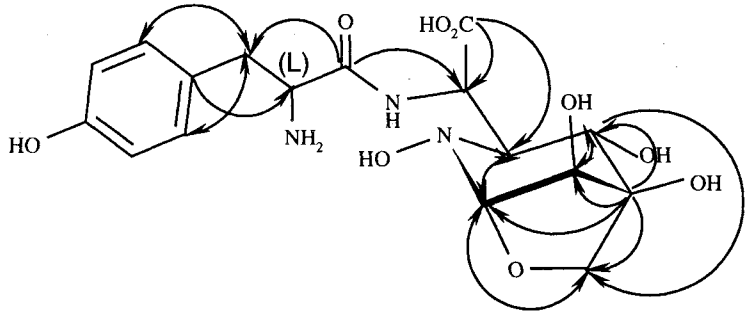
Fragment 3

incorporation of the carbonyl carbons and the sp^3 quaternary carbon was then possible using long-range, carbon-proton coupling data obtained from 2D Heteronuclear Multiple Bond Correlation (HMBC) experiments (Fig. 3). In this way the molecular structure of SB-219383 was elucidated and shown to be as depicted in Fig. 1 with a dipeptide structure containing a [3.2.1] bicyclic sugar. Interestingly the latter moiety contains two unusual features; an endocyclic nitrogen atom carrying a hydroxyl group and a quaternary carbon at one of the

Table 1. NMR chemical shift data for SB-219383 (**1**) and SB-227871 (**2**).

Position	**1** (D_2O) ^{13}C	^{1}H		**1** (DMSO-d6) ^{13}C	^{1}H		**2** (DMSO-d6) ^{13}C	^{1}H	
1	93.5	4.96	d, J=5.4Hz	92.6	4.59	d, J=5.4Hz	92.6	4.62	d, J=5.3Hz
3	66.6	3.48	dd, J=9.1,1.2Hz	65.5	3.12	dd, J=8.7,1.2Hz	65.1	3.19	dd, J=8.8,1.3Hz
4	68.0	3.81	dd, J=9.1,1.7Hz	67.0	3.62	bd, J=8.8Hz	66.2	3.63	m
5	77.0	-		76.2	-		76.1	-	
6	68.7	4.15	d, J=8.5Hz	67.8	3.83	d, J=7.7Hz	67.9	3.84	d, J=7.8Hz
		3.67	dd, J=8.5,1.7Hz		3.30	dd, J=7.8,1.6Hz		3.32	dd, J=7.9,1.5Hz
8	72.7	4.31	d, J=5.4Hz	72.8	3.91	d, J=5.3Hz	72.9	3.93	dd, J=6.3,5.4Hz
9	53.7	4.68	bs	50.5	4.58	bd, J=9Hz	50.5	4.71	dd, J=8.4,1.3Hz
10	177.8	-		173.2	-		171.7	-	
1'	170.9	-		173.9	-		172.0	-	
2'	55.5	4.37	dd, J=8.6,4.9Hz	56.4	3.39	dd, J=9.7,3.9Hz	56.1	4.27	ddd, J=10.8,8.8,3.5Hz
3'	36.9	3.40	dd, J=14.7,5.0Hz	39.8	3.03	dd, J=13.8,3.8Hz	36.3	3.09	dd, J=14.1,3.5Hz
		3.14	dd, J=14.7,8.6Hz		2.45	dd, J=13.8,9.7Hz		2.74	dd, J=14.1,10.8Hz
4'	126.8	-		128.5	-		128.1	-	
5'/9'	131.8	7.30	d, J=8.6Hz	130.1	7.04	d, J=8.5Hz	130.0	7.12	d, J=8.5Hz
6'/8'	116.7	6.95	d, J=8.6Hz	115.0	6.69	d, J=8.5Hz	114.8	6.65	d, J=8.5Hz
7'	155.9	-		155.7	-		155.6	-	
2-OH				-	nd		-	7.91	s
4-OH				-	nd		-	5.17	d, J=5.7Hz
5-OH				-	nd		-	5.09	s
8-OH				-	nd		-	4.71	d, J=6.4Hz
9-NH				-	8.03	d, J=8.9Hz	-	7.55	d, J=8.4Hz
10-OMe							51.6	3.58	s
2'-NH							-	7.50	d, J=8.8Hz
7'-OH				-	~9.20	bs	-	9.14	s
1"/8"							125.3, 125.2	7.65	m
2"/7"							127.0, 126.9	7.32	m
3"/6"							127.5	7.42	m
4"/5"							119.9	7.88	m
4a"/4b"							140.5	-	
8a"/9a"							143.8, 143.6	-	
9"							46.5	4.15	dd, J=6.5,6.5Hz
10"							65.7	4.21	dd, J=8.6,6.2Hz
								4.10	dd, J=8.5,6.7Hz
12"							155.7	-	

Fig. 3. Critical long-range carbon-proton couplings observed for SB-219383.

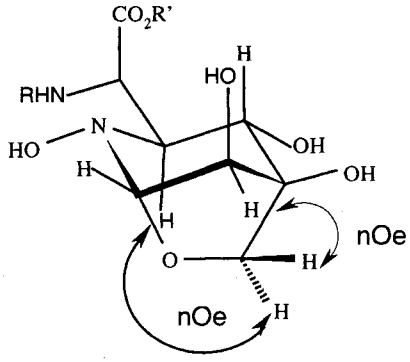


bridgehead positions.

From the NMR data available on SB-219383 and SB-227871 (Table 1) it is not possible to determine the absolute stereochemistry at all centers. However, information on the relative stereochemistry within the [3.2.1] bicyclic moiety was provided by the proton-proton coupling constant (Table 1) and nuclear Overhauser enhancement data. The large (9.1 Hz) coupling constant between H3 and H4 confirmed their relative orientation as *trans-diaxial*. The *axial* orientation of H3 was further supported by the mutual nOe observed to one of the H6 methylene protons (Fig. 4). The other H6 proton showed a 4-bond coupling of 1.7 Hz to H4 supporting their *W-type* arrangement. As the stereochemistry at C1 and C5 is fixed by the ring-junction, only the C8 stereochemistry remained undefined. Determination of the orientation of H8 followed from the nOe data (Fig. 4) with a medium-to-large nOe being observed between H8 and the H6 proton which exhibits the W-type coupling. The relative stereochemistry at this centre was also evident from a comparison of the coupling constant with literature data. The observed coupling of 5.4 Hz dictates that the H8 proton must be *equatorial* since in 1,3-anhydro-3-C-hydroxymethyl-α-glucopyranose (**3**) there is a negligibly small coupling between the equivalent *axial* proton and H1[2] (Fig. 5).

Database searching indicated that SB-219383 was a novel natural product. Sub-structure searches of the Dictionary of Natural Products and Chemical Abstracts were also carried out and no hits were obtained. However, one nucleoside antibiotic, miharamycin (**4**) was found to be of interest[3] (Fig. 5). The dipeptide moiety of miharamycin is attached *via* a α-carbon to a [3.3.0] bicyclic sugar moiety. The latter possesses a quarternary carbon at the bridgehead position and a 2-aminopurine at the anomeric

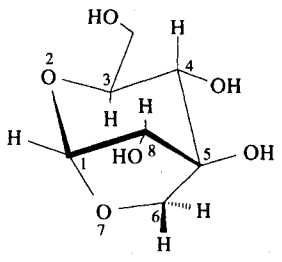
Fig. 4. Significant nOe's for SB-219383.



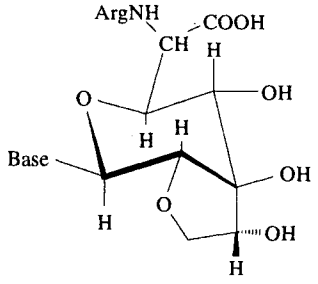
centre. NMR data of SB-219383 and miharamycin were compared. Interestingly, in both compounds, a small three bond coupling (1.2 and 2.1 Hz, respectively) was observed between the α-proton and the adjacent sugar proton. However from this data the relative stereochemistry at C-9 in SB-219383 could not be determined. The presence of a tertiary hydroxylamine function in SB-219383, α to the position of attachment of the sugar, is supported by the ^{13}C NMR chemical shifts of the linking sugar carbon.

The infra-red data for SB-219383 indicated the presence of a secondary amide (1673 and 1517 cm^{-1}), an ionised carboxylate (1617 and 1385 cm^{-1}), a phenol group and was also consistent with the presence of sugar hydroxyls. However, there was no direct IR evidence for either an ammonium or a hydroxylamine group. No additional information was forthcoming from the FT-Raman data. Furthermore, the nature of SB-219383 (*i.e.* insolubility in non-polar solvents and presence of other hydroxyl

Fig. 5. Structures of 1,3-anhydro-3-C-hydroxy-methyl-α-glucopyranose (**3**) and miharamycin (**4**).



(**3**)



Miharamycin (**4**)

functionalities) precluded the use of far infra-red spectroscopy for establishing the presence of the hydroxylamine[4]. The presence of a secondary hydroxylamine in SB-219383 was supported by a positive triphenyltetrazolium chloride test[5]. This test was also applied to reference compounds, namely a dipeptide (Tyr-Thr) and a non reducing sugar (sucrose) which gave negative results together with a secondary hydroxylamine (Et_2NOH) which was positive. Further evidence for the presence of the hydroxylamine was provided by the 2D proton-nitrogen HMBC NMR data. This data confirmed the presence of three nitrogens in the molecular structure. The dipeptide amide and amine nitrogens were readily identified from their correlations and ^{15}N chemical shifts at 116.6 and 36.4 ppm, respectively. The remaining nitrogen showed correlations to protons H9 and H8 consistent with 3-bond couplings to the hydroxylamine nitrogen N2. Furthermore the chemical shift of this nitrogen (142.9 ppm) was in good agreement with that expected for a secondary hydroxylamine (by comparison with a *N,N*-diethyl-hydroxylamine reference shift of 137.5 ppm).

One of the other major problems in confirming the elucidated structure of SB-219383 was the positioning of the exchangeable functionalities within the [3.2.1] bicyclic moiety. Attempts to observe the exchangeable protons in dimethyl sulfoxide-d_6 were thwarted by exchange rate problems which resulted in only the amide NH (*i.e.* 9-NH) and tyrosine OH (*i.e.* 7′-OH) being observed. One option available to solve the "exchangeable" issue was to obtain a derivative which would be soluble in a non-protic solvent. Using sequential FMOCCl and esterification conditions, SB-219383 was derivatised to the 2′-NHFMOC methyl ester, SB-227871. Subsequent NMR studies in dimethyl sulfoxide-d_6 solution were more informative with all 7 expected labile protons of SB-227871 being observed. The presence of the hydroxyl functionalities at C4 and C8 were readily confirmed by the proton-proton coupling of labile protons to H4 and H8 respectively. Proton-proton couplings could not however be used to confirm the presence and position of the hydroxylamine and tertiary hydroxyl. The necessary evidence for these functionalities was provided by the long-range, carbon-proton couplings observed between these labile protons and the carbons of the bicyclic sugar moiety.

Further studies on the preparation and biological activities of analogues of SB-219383 of known absolute configuration will be published elsewhere.

Experimental

Electrospray ionisation mass spectrometry (ESMS) was performed on a Finnigan TSQ-700. Fast atom bombardment (FAB) ionisation were accumulated on a JEOL SX-102 instrument. IR spectra were measured on a Bio-Rad FTS7 spectrophotometer. UV spectra were taken with a Beckman DU68 UV-visible spectrophotometer. NMR spectroscopy was performed on either a Bruker AMX 400 or DRX600 spectrometer. For dimethyl sulfoxide solutions, NMR chemical shifts were referenced to an internal standard of tetramethylsilane at 0.00 ppm. For deteurium oxide solutions, proton chemical shifts were referenced to the residual HOD signal at 4.80 ppm whilst carbon-13 chemical shifts were referenced to an external standard of dioxan at 67.4 ppm. Nitrogen-15 chemical shifts were referenced using the method of LIVE *et al.*[6].

Detection of Tyrosine

SB-219383 (**1**) (~1 mg) was hydrolysed in the vapour phase by constant boiling (5.5 N) HCl at 110°C for 24 hours under nitrogen. The free amino acid was derivatised after drying with phenylisothiocyanate (PITC) to yield PTC-AA then chromatographed on a C18 reverse phase column [Waters Novapak, 4 μm (3.9×150 mm)] at a flow rate of 1.0 ml/minute using as solvents: 0.18 M sodium acetate - 0.06% triethylamine - 6% acetonitrile adjusted to pH 6.4 with phosphoric acid and 60% acetonitrile - 40% water. Detection was carried out by UV at 254 nm.

Determination of the Absolute Configuration by Chiral HPLC

For analysis of D,L-tyrosine, the free amino acid(s) were derivatised with orthophthalaldehyde/*N*-acetyl cysteine and enantiomers separated by ligand exchange chromatography at a flow rate of 1.0 ml/minute using as solvents: 0.025 M ammonium acetate - 0.0025 M copper sulphate - 0.005 M L-proline, adjusted to pH 5.0 and 50% acetonitrile. Detection was carried out by fluorescence λ_{ex} 340 nm, λ_{em} 419 nm.

Synthesis of SB-227871 (**2**)

a) *N*-(L-(9-Fluorenylmethyloxycarbonyl)tyrosyl)-2-amino[1(*S**),3(*S**),4(*S**),5(*R**),8(*R**)-2,4,5,8-tetrahydroxy-7-oxa-2-azabicyclo[3.2.1]oct-3-yl]acetic Acid Sodium Salt

A solution of *N*-(L-tyrosyl)-2-amino[1(*S**),3(*S**),4(*S**),5(*R**),8(*R**)-2,4,5,8-tetrahydroxy-7-oxa-2-azabicyclo-[3.2.1]oct-3-yl]acetic acid (**1**) (200 mg, 0.48 mmol) in borate buffer pH 6.8 (15 ml) was treated with 9-fluorenylmethyl chloroformate (43 ml, 15 mM solution in acetone). After 2 hours the solvent was evaporated, the residue washed with pentane then purified by reverse phase silicagel chromatography to give the title compound (120 mg, 40%); UV λ_{max}^{EtOH} nm (ε) 202 (29,745), 264 (17,870), 288 (5,355), 300 (5,704); ^{1}H NMR (400 MHz, D_2O) δ 2.78 (1H, dd, J=11.2 and 13.6 Hz, 3′-H), 3.22 (1H, dd, J=2.9 and 13.8 Hz, 3′-H), 3.48 (1H, d, J=8.9 Hz, 3-H), 3.67 (1H, dd, J=8.5 and 1.1 Hz, 6-H), 3.78 (1H, bd, J=8.9 Hz, 4-H), 4.15 (1H, d, J=8.5 Hz, 6-H), 4.18 (1H, m, 9″-H), 4.28 (1H, d, J=5.4 Hz, 8-H), 4.35 (1H, dd, J=3.6 and 10.6 Hz, 2′-H), 4.45 (1H, d, J=4.6 Hz, 10″-H), 4.58 (1H, d, J=4.6 Hz, 10″-H), 4.64 (1H, s, 9-H), 4.93 (1H, d, J=5.2 Hz, 1-H), 6.82 (2H, d, J=8.4 Hz, 6′-H and 8′-H), 7.10 (2H, d, J=8.4 Hz, 5′-H and 9′-H), 7.40~8.00 (8H, m, 1″-H to 8″-H); ES-MS *m/z* 636 $(M+H)^+$, 658 $(M+Na)^+$. HRFAB-MS *m/z* calcd for $C_{32}H_{33}O_{11}N_3$ $(M+H)^+$ 636.2194, Found 636.2182.

b) *N*-(L-(9-Fluorenylmethyloxycarbonyl)tyrosyl)-2-amino[1(*S**),3(*S**),4(*S**),5(*S**),8(*S**)-2,4,5,8-tetrahydroxy-7-oxa-2-azabicyclo[3.2.1]oct-3-yl]acetic Acid Methyl Ester Hydrochloride Salt (**2**)

A solution of *N*-(L-(9-fluorenylmethyloxycarbonyl)-tyrosyl)-2-amino [1(*S**),3(*S**),4(*S**),5(*R**),8(*R**)-2,4,5,8-tetrahydroxy-7-oxa-2-azabicyclo[3.2.1]oct-3-yl]acetic acid sodium salt (110 mg, 0.17 mmol) in 4 M HCl methanol (20 ml) was stirred for 1 hour. The reaction mixture was concentrated, the residue was treated with ethyl acetate and a saturated aqueous solution of sodium bicarbonate. The organic phase was dried ($MgSO_4$) and concentrated. Flash chromatography on silica gel eluting with dichloromethane/methanol mixtures gave the title compound (56 mg, 50%); UV λ_{max}^{EtOH} nm (ε) 204 (49,957), 265 (18,295), 288 (5,526), 300 (5,825); ES-MS *m/z* 650 $(M+H)^+$. HRFAB-MS *m/z* calcd for $C_{33}H_{35}O_{11}N_3$ $(M+H)^+$ 650.2350, Found 650.2352. ^{1}H NMR data summarised in Table 1.

Acknowledgements

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