Synthesis and Activity of Analogues of SB-219383:

Novel Potent Inhibitors of Bacterial Tyrosyl tRNA Synthetase

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SB-219383 is a naturally occurring antibiotic, which acts by inhibition of tyrosyl tRNA synthetase. Semi-synthetic derivatives of SB-219383 were prepared with the objective of elucidating the key features required for inhibition of tyrosyl tRNA synthetase in order to improve the antibacterial activity. Some ester and amide derivatives as well as monocyclic analogues exhibited sub-nanomolar inhibitory activity against tyrosyl tRNA synthetase.

The urgent need for novel therapies to combat bacterial infection have been well documented over the past decade with the emergence of bacteria resistant to most common classes of antibiotic, particularly *Staphylococcus* and *Enterococcus* strains[1,2]. For example many isolates of *Staphylococcus aureus* (commonly termed methicillin resistant *Staphylococcus aureus* or MRSA) are resistant to all current therapies apart from vancomycin, and recently clinical isolates with reduced susceptibility to vancomycin have been reported in Japan[3].

The discovery and structural elucidation of a novel inhibitor of tyrosyl tRNA synthetase, SB-219383, was recently reported[4,5]. In protein biosynthesis, aminoacyl tRNA synthetases are responsible for the charging of the correct amino acid onto its cognate tRNA prior to incorporation into the growing peptide chain[6]. As such they are an essential component of all living systems and inhibition of any member of the family blocks protein synthesis within the cell, which in turn inhibits cell growth. There are significant structural differences between the mammalian and bacterial enzymes and SB-219383 was demonstrated to be a highly selective inhibitor of the bacterial enzyme. Thus, SB-219383 presented a new opportunity to explore aminoacyl tRNA synthetase inhibitors as antibacterial agents. Here we report semi-synthetic derivatives of SB-219383 designed both to elucidate the key interactions with tyrosyl tRNA synthetase

and to reduce the overall polarity of the inhibitor.

Synthesis

Bicyclic Structures

Selective esterification of the carboxylate SB-219383, was achieved by direct reaction with the appropriate alcohol under acidic catalysis to afford the bicyclic esters (3a~c) (Scheme 1 and Table 1). The tyrosine amino group could be also selectively acylated to form the Cbz (1a) or acetyl (1b) derivatives. Selective protection of the aliphatic hydroxyl groups was not possible but a stable hexa-*O*-triethylsilyl derivative could be formed by reaction of (1a) with excess chlorotriethylsilane. When compound (1a) is treated with *i*-butyl chloroformate and *N*-methylmorpholine, the initially formed mixed anhydride undergoes spontaneous intramolecular cyclisation with the *N*-hydroxy group to form the tricyclic 1,2-oxazolidinone (2). This tricyclic compound is a versatile intermediate. Reaction of (2) with an appropriate primary or secondary amine followed by hydrogenation, to remove the Cbz group, gave the amides (4a~h) (Scheme 1 and Table 1). Likewise (2) was ring-opened with alkoxides as an alternative route to esters (3). The oxazolidinone ring of (2) could be cleaved by reduction modalities (see below).

Table 1. Bicyclic derivatives.
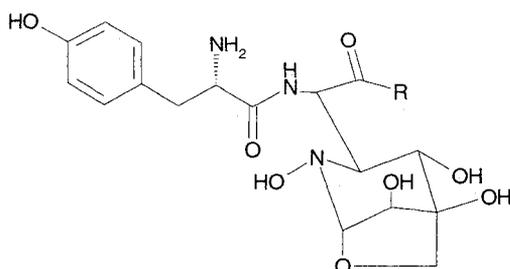
	R	YRS* IC$_{50}$(nM)	S. pneumoniae R6 MIC (µg/mL)	S .pyogenes CN10 MIC (µg/mL)
SB-219383	HO	0.6	64	32
1b	OH, NHAc	1100	-	-
3a	MeO	2.2	8	8
3b	EtO	0.63	8	4
3c	n-BuO	0.60	16	8
3d	n-hexylO	7.1	32	8
4a	n-BuNH	4.0	32	8
4b	i-BuNH	1.95	64	64
4c	PhCH$_2$NH	0.92	16	32
4d	Ph(CH$_2$)$_2$NH	8.0	>64	>64
4e	2-naphthylCH$_2$NH	0.7	>64	16
4f	2-pyridylCH$_2$NH	3.25	>64	>64
4g	morpholino	28	>64	>64
4h	azetidino	22	64	64

*S. aureus enzyme, compounds failed to show antibacterial activity against S. aureus organisms

Monocyclic Structures

The unusual bicyclic system of SB-219383 could be opened by reduction with sodium borohydride to afford the monocyclic hydroxymethyl derivative (5) (Scheme 1). The N-Cbz protected derivative (6) could be prepared by borohydride reduction of (1a) or by protection of (5). As in the bicyclic case (6) formed an oxazolidinone (7), which afforded the ester (8) on treatment with sodium n-butoxide. Under normal conditions the monocyclic analogues show good stability. However, the reductive ring-opening of SB-219383 and its N-Cbz derivative is reversible and when allowed to stand in DMSO for several days oxidative cyclisation occurs to regenerate the original bicyclic system. This possibly occurs via a nitrone intermediate[7].

Treatment of the activated tricyclic ester (2) with sodium borohydride reduced the acid to a hydroxymethyl group (9)

and subsequent removal of the Cbz group gave (10) (Scheme 2). Reduction of the activated hydroxylamine and removal of the Cbz group, with palladium gave the secondary amine (11).

Simultaneous esterification and acylation of (6) with acetyl chloride in n-butanol followed by deprotection with hydrogen over palladium yielded the 6-acetoxymethyl analogue (12). Alternatively treatment of the n-butyl ester of (6) with phenyl isocyanate and pyridine followed by deprotection gave the corresponding 4-phenylcarbamate (13). However, under identical conditions ethyl isocyanate gave only acylation at the hydroxylamine (14) (Scheme 3) suggesting that the relative reactivity of the two hydroxyl groups is finely balanced.

Acylation of the phenolic hydroxyl of the n-butyl ester of (1a) was achieved using pivaloyl chloride and Hunig's base.
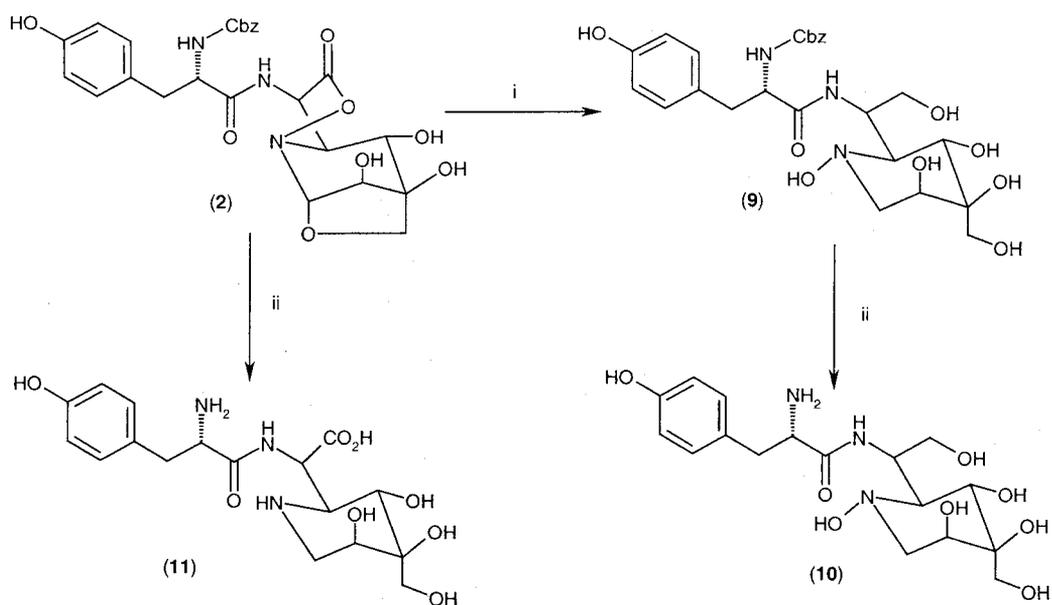
Scheme 1.
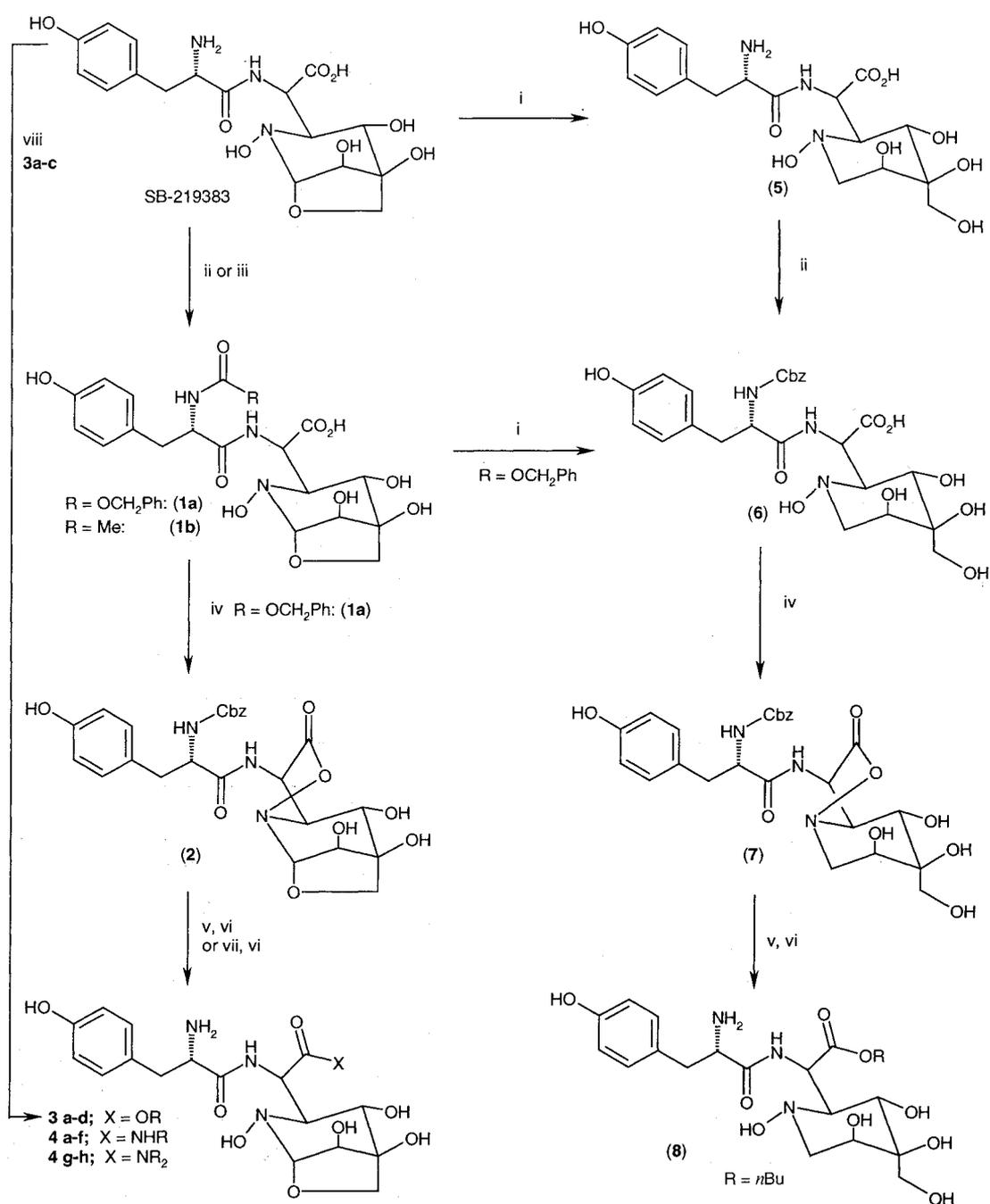
Reagents: i) $NaBH_4$, DMF/H_2O; ii) Cbz-Cl, $NaHCO_3$, H_2O; iii) Ac_2O, THF/H_2O; iv)

i-BuOCOCl, N-methylmorpholine, DMF, -20°C; v) RONa, ROH; vi) 10% Pd-C, H_2, $MeOH/H_2O$;
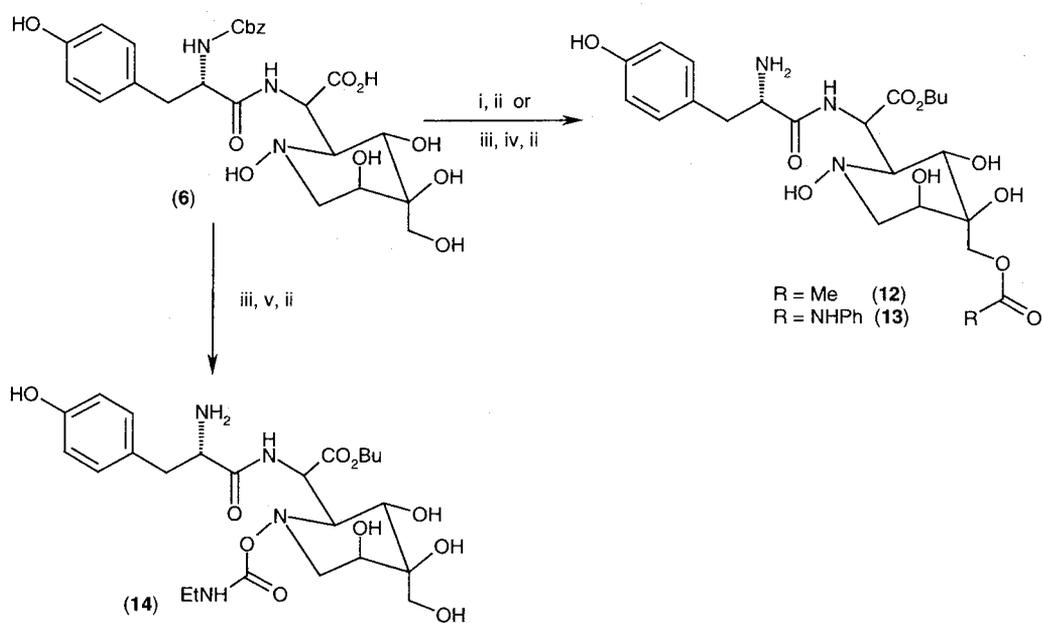
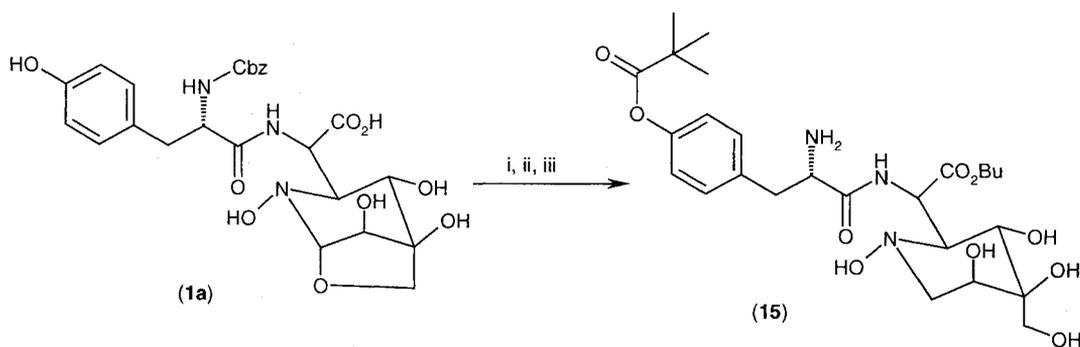vii) RNH_2 or R_2NH, H_2O; viii) ROH, 4M HCl.

Scheme 2.

Reagents: i) NaBH$_4$, DMF/H$_2$O; ii) 10% Pd-C, H$_2$, H$_2$O.

Scheme 3.

Reagents: i) AcCl, n-BuOH; ii) 10% Pd-C, H$_2$, MeOH/H$_2$O; iii) 4M HCl, n-BuOH; iv) PhNCO,

pyridine; v) EtNCO, pyridine.

Scheme 4.

Reagents: i) 4M HCl, n-BuOH; ii) Piv-Cl, i-Pr$_2$EtN, DMAP, THF, 0°C; iii) 10% Pd-C, H$_2$,

p-TSA, MeOH.

Catalytic reduction in the presence of 4-toluenesulfonic acid resulted in simultaneous removal of the Cbz group and ring-opening of the bicyclic core to give (15) (Scheme 4).

Biological Activity

Although SB-219383 is a potent inhibitor of tyrosyl tRNA synthetase, IC$_{50}$=0.6 nM, the compound shows only limited antibacterial activity. This is probably attributable to poor penetration of bacterial cells due to the highly polar nature of the natural product. The strategy adopted to improve bacterial cell penetration was to identify key functionality required for tyrosyl tRNA synthetase inhibition and to decrease the polarity of the molecule by selective derivatisation of non-essential functional groups and/or to reduce the number of hydrogen bond donor interactions. The semi-synthetic analogues were assayed against S. aureus tyrosyl tRNA synthetase[8] and tested in a standard antibacterial profile. The enzyme IC$_{50}$ values and the minimum inhibitory concentrations (MIC's) against Streptococcus pneumoniae and Streptococcus pyogenes are shown in Tables 1 and 2.

The facile reduction of SB-219383 to the monocyclic analogue (5) established the key observation that the monocyclic analogue was also a very potent inhibitor of S. aureus tyrosyl tRNA synthetase (Table 2). This suggested that neither the tetrahydrofuran ring of SB-219383 nor the hydroxymethyl group of (5) is necessary for enzyme inhibition. This was subsequently confirmed by the total synthesis of a closely related monocyclic analogue[9] and by the preparation of the acetyl derivative of the

hydroxymethyl group (12). This also enabled the role of key structural features to be determined either in the monocyclic or the bicyclic series of analogues. Not unexpectedly, in view of its increased polarity, compound (5) itself had no antibacterial activity.

In the tyrosyl moiety, derivatisation of either the phenolic hydroxyl with a pivaloyl group (15) or the amino group with an acetyl group (1b) resulted in a substantial reduction in enzyme potency, suggesting that both features are essential for good recognition. This is consistent with the tyrosyl moiety binding in the tyrosine pocket of the active site of the enzyme, as a crystal structure of tyrosyl adenylate in B. stearothermophilus tyrosyl tRNA synthetase shows strong hydrogen bond networks to both the phenol and amino functionalities.[10]

In both the bicyclic and monocyclic series conversion of the carboxylic acid group into an ester gave potent enzyme inhibitors (Tables 1 and 2). The SAR indicates the optimal alkyl ester chain length to be n-butyl (3c) and (8) and increasing the chain reduces the enzyme inhibition (3d). Despite their increased lipophilicity, the bicyclic esters showed only a slight improvement in antibacterial activity compared with SB-219383 (Table 1). One possible explanation for this could be enzymatic hydrolysis of the esters to the acid in the assay medium. Like the parent monocycle (5), the monocyclic esters possessed no significant antibacterial activity. The slight improvement in the MIC's of the bicyclic esters provided encouragement to prepare the corresponding amides of SB-219383 which should be more resistant to enzymatic hydrolysis. An identical SAR was observed for n-alkyl amides as for the

Table 2. Monocyclic derivatives.
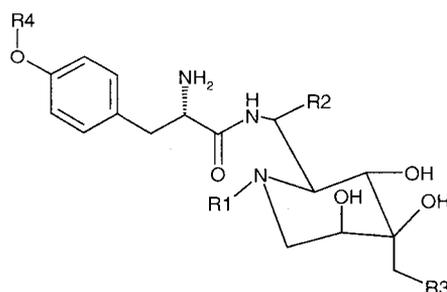
	R1	R2	R3	YRS* IC_{50} nM	S. pneumoniae R6 MIC µg/mL	S. pyogenes CN10 MIC µg/mL
5	OH	CO_2H	OH	2.90	>64	>64
8	OH	CO_2Bu^n	OH	0.18	64	>64
10	OH	CH_2OH	OH	2200	>64	>64
11	H	CO_2H	OH	591	64	64
12	OH	CO_2Bu^n	OAc	1.90	32	16
13	OH	CO_2Bu^n	OCONHPh	88% @ 3µM	>64	>64
14	EtNHC(O)O	CO_2Bu^n	OH	170	>64	>64
15	OH	CO_2Bu^n	OH, R4 = Piv	107	>64	>64

*S. aureus enzyme.

esters in that the optimal straight-chain alkyl group was n-butyl, (4a). A greater diversity of secondary amides was prepared, including branched alkyl (4b), arylalkyl (4c~e) and 2-pyridylmethyl (4f), as well as tertiary amides (4g~h). The secondary amides maintained excellent potency against the YRS enzyme (Table 1) but their antibacterial activity was reduced compared with parent acid or esters. The tertiary amides (4g~h) were significantly weaker inhibitors and showed no antibacterial activity. Although the acid functionality could be converted to esters and secondary amides whilst retaining potent inhibition, reduction of the carboxylic acid group to the corresponding hydroxymethyl functionality (10) effectively abolished activity, suggesting that the carbonyl moiety is involved in a key interaction with the enzyme.

The acetate derivative (12) maintained excellent inhibitory activity and showed some improvement in antibacterial profile over (8) (Table 2) confirming that the hydroxymethyl group is not vital for good inhibition and that antibacterial activity is improved by reducing polarity. The more bulky phenyl carbamate analogue (13), however, showed considerably reduced potency against the enzyme and no antibacterial activity, indicating that the space around the hydroxymethyl group is limited to sterically undemanding groups. A free hydroxylamine function appears to be crucial for good potency in that the amine (11) is 200-fold less potent than the corresponding hydroxylamine (5), and the ethyl carbamate analogue (14) is 1,000-fold less potent.

The analogues of SB-219383 described, define some of the essential structural moieties for potent inhibition of bacterial tryosyl tRNA synthetase. These include the phenolic and amino groups of the polyhydroxylic amino acid. Conversely, the tetrahydrofuran ring and the free acid of SB-219383 have been shown not to be essential, allowing the synthesis of simpler and less polar inhibitors. Even though potent inhibitors with significantly reduced polarity were achieved, substantial gains in antibacterial

activity were not attained.

Experimental

Enzymology

Compounds were assayed against *S. aureus* tyrosyl tRNA synthetase in a full aminoacylation assay.[8]

Antibacterial Assay

Whole-cell antibacterial activity was determined by broth microdilution. Compounds were dissolved in DMSO and diluted 1:10 in water to produce a 256 μg/ml stock solution. Using a 96 well microtitre plate, a Microlab AT Plus 2 (Hamilton Co., Reno, NV) serially diluted 50 μl of the stock solution into supplemented cation adjusted Mueller Hinton broth (Beckton Dickinson, Cockeysville, MD.). After the compounds were diluted, a 50 μl aliquot of the test isolate ($\sim 1 \times 10^6$ cfu/ml) was added to each well of the microtitre plate using the Microlab AT Plus 2. The final test concentrations ranged from 0.06 to 64 μg/ml. Inoculated plates were incubated at 35°C in ambient air for 18 to 24 hours. Following incubation, a microtitre mirror reader (Cooke Instruments, Ltd., England) was used to assist in reading the minimum inhibitory concentration (MIC). The MIC was determined as the lowest concentration of drug that inhibited visible growth of the test isolates.

Chemistry

NMR spectroscopy was performed on a Bruker AMX-250 or Avance 400 spectrometer. UV spectra were taken with a Beckman DU68 UV-visible spectrophotometer using 0.05 M pH 4.5 NH_4OAc buffer/MeOH solution unless otherwise specified. Mass spectrometry was performed on a Finnigan TSQ-700 or Micromass Platform 2 spectrometer.

2-(2,4,5,8-Tetrahydroxy-7-oxa-2-azabicyclo[3.2.1]oct-3-yl)-2-[(*N*-benzyloxy-carbonyl)tyrosylamino]acetic Acid Sodium Acetate (1a)

A solution of 2-(2,4,5,8-tetrahydroxy-7-oxa-2-azabicyclo-[3.2.1]oct-3-yl)-2-(tyrosylamino)acetic acid (SB-219383, 140 mg, 0.34 mmol) in water (15 ml) was treated with benzylchloroformate (75 μl, 0.5 mmol) and sodium bicarbonate (8.5 mg, 1 mmol). After 40 minutes the mixture was washed with diethyl ether and the aqueous phase freeze-dried to give the title compound (178 mg, 92%); λ_{max} 220, 273 nm; δ_H (D_2O) 2.84 (1H, dd, *J* 11.2 and 13.6 Hz, 3'-H), 3.32 (1H, dd, *J* 2.9 and 13.8 Hz, 3'-H), 3.49 (1H, d, *J* 8.9 Hz, 3-H), 3.68 (1H, dd, *J* 8.5 and 1.1Hz, 6-H), 3.84 (1H, bd, *J* 8.9 Hz, 4-H), 4.16 (1H, d, *J* 8.5 Hz, 6-H), 4.31 (1H, d,

J 5.4 Hz, 8-H), 4.58 (1H, dd, *J* 3.6 and 10.6 Hz, 2'-H), 4.70 (1H, s, NC*H*CO$_2$H), 4.97 (1H, d, *J* 5.2 Hz, 1-H), 5.0 (1H, d, *J* 12 Hz, COOC*H*$_2$Ph), 5.16 (1H, d, *J* 12.8 Hz, COOC*H*$_2$Ph), 6.85 (2H, d, *J* 8.4 Hz, 6'-H and 8'-H), 7.19 (2H, d, *J* 8.4 Hz, 5'-H and 9'-H), 7.22~7.55 (5H, m, COOCH$_2$*Ph*); δ_C (D_2O) 37.3 (C-3') 53.6 (N*C*HCO$_2$H), 57.5 (C-2'), 66.8 (C-3), 67.7, 68.7 (C-2", C-6), 68.2 (C-4), 72.8 (C-8), 77.2 (C-5), 93.7 (C-1), 116.3 (C-6', C-8'), 128.1, 129.0, 129.5 (COOCH$_2$*Ph*), 129.6 (C-4'), 131.4 (C-5', C-9'), 137.2 (COOCH$_2$*Ph*), 155.1 (C-7', C-1"), 174.6 (C-1'), 178.1 (*C*O$_2$H); *m/z* (ES) 548 (MH$^+$), 570 (MNa$^+$), 1095 ([2M+H]$^+$), 1117 ([2M+Na]$^+$).

2-(2,4,5,8-Tetrahydroxy-7-oxa-2-azabicyclo[3.2.1]oct-3-yl)-2-[(*N*-acetyl)tyrosylamino]acetic Acid (1b)

A solution of SB-219383 (14 mg, 0.034 mmol) in water (7 ml) was treated with a solution of acetic anhydride (0.140 ml, 1.48 mmol) in tetrahydrofuran (7 ml). After 40 minutes the reaction mixture was concentrated, the residue was dissolved in water then treated with saturated aqueous hydrogen carbonate. After 1 hour the solution was acidified to pH 2 (1 M HCl) then freeze-dried. Purification on a Sephadex® G10 column eluting with water gave the title compound (16 mg, 100%); λ_{max} 222, 274 nm; δ_H (D_2O) 1.99 (3H, s, NCOC*H*$_3$), 2.95 (1H, dd, *J* 9.8 and 14.2 Hz, 3'-H), 3.28 (1H, dd, *J* 4.8 and 14.2 Hz, 3'-H), 3.49 (1H, d, *J* 9.0 Hz, 3-H), 3.69 (1H, d, *J* 8.5 Hz, 6-H), 3.86 (1H, d, *J* 9.1 Hz, 4-H), 4.14 (1H, d, *J* 8.5 Hz, 6-H), 4.32 (1H, d, *J* 5.3 Hz, 8-H), 4.75 (1H, s, NC*H*CO$_2$H), ~4.8 (1H, hidden under HDO, 2'-H), 4.97 (1H, d, *J* 5.6 Hz, 1-H), 6.92 (2H, d, *J* 8.4 Hz, 6'-H and 8'-H), 7.25 (1H, d, *J* 8.4 Hz, 5'-H and 9'-H); δ_C (D_2O), 22.5 (NCO*C*H$_3$), 36.8 (C-3'), 52.6 (N*C*HCO$_2$H), 55.9 (C-2'), 66.5 (C-3), 67.6 (C-4), 68.7 (C-6), 72.8 (C-8), 77.1 (C-5), 93.6 (C-1), 116.3 (C-6' and C-8'), 129.5 (C-4'), 131.4 (C-5' and C-9'), 155.2 (C-7'), 174.6, 174.9 (N*C*OCH$_3$, C-1'), 176.3 (*C*O$_2$H); *m/z* (ES) 456 (MH$^+$), 473 (MNH$_4^+$).

2-(2,4,5,8-Tetrahydroxy-7-oxa-2-azabicyclo[3.2.1]oct-3-yl)-2-(tyrosylamino)acetic Acid *n*-Butyl Ester Hydrochloride Salt (3c)

A suspension of SB-219383 (70 mg, 0.17 mmol) in 4 M HCl in *n*-butanol (50 ml) was stirred for 1 hour. The reaction mixture was concentrated and chromatographed over silica gel eluting with *n*-butanol/ethanol/water mixtures to give the title compound (86 mg, 100%); λ_{max} (H_2O) 222, 271 nm; δ_H (D_2O) 0.95 (3H, t, *J* 7.4 Hz, COOCH$_2$CH$_2$CH$_2$C*H*$_3$), 1.43 (2H, qt, *J* 7.4 and 7.4 Hz, COOCH$_2$CH$_2$C*H*$_2$CH$_3$), 1.69 (2H, tt, *J* 6.8 and 6.8 Hz, COOCH$_2$C*H*$_2$CH$_2$CH$_3$), 3.03 (1H, dd, *J* 14.2 and 7.7 Hz, 3'-

H), 3.22 (1H, dd, *J* 14.2 and 5.6 Hz, 3'-H), 3.48 (1H, d, *J* 9.0 Hz, 3-H), 3.69 (1H, bd, *J* 7.4 Hz, 6-H), 3.83 (1H, d, *J* 8.5 Hz, 4-H), 4.12 (1H, d, *J* 8.5 Hz, 6-H), 4.20 (3H, m, COOC*H₂*, 2'-H), 4.33 (1H, d, *J* 5.4 Hz, 8-H), 4.91 (1H, s, NC*H*CO₂Bu), 4.95 (1H, d, *J* 5.5 Hz, 1-H), 6.95 (2H, d, *J* 8.4 Hz, 6'-H and 8'-H), 7.25 (1H, d, *J* 8.4 Hz, 5'-H and 9'-H); (Found: MH, 470.2140. C₂₁H₃₁N₃O₉ requires, MH, 470.2139).

2-(2,4,5,8-Tetrahydroxy-7-oxa-2-azabicyclo[3.2.1]oct-3-yl)-2-[(*N*-benzyloxy-carbonyl)tyrosylamino]acetic Acid *n*-Butyl Amide

A solution of (**1a**) (53 mg, 0.093 mmol) in dry DMF (4 ml) under argon at −20°C was treated with *N*-methylmorpholine (40 *μ*l, 0.33 mmol) followed by isobutylchloroformate (40 *μ*l, 0.27 mmol). After 5 minutes at −20°C, 37% aqueous *n*-butylamine (7.4 ml) was added. After 30 minutes at room temperature the reaction mixture was chromatographed over silica gel eluting with dichloromethane/methanol mixtures to give the title compound (18 mg, 32%); λ_{max} 222, 273 nm; (CD₃OD) 0.92 (3H, t, *J* 7.1 Hz, CONH(CH₂)₃*CH₃*), 1.27~1.52 (4H, m, CONHCH₂(*CH₂*)₂CH₃), 2.86 (1H, dd, *J* 9.4 and 13.7 Hz, 3'-H), 3.04~3.23 (3H, m, CONHC*H₂* and 3'-H), 3.36 (1H, d, *J* 8.8 Hz, 3-H), 3.52 (1H, d, *J* 8 Hz, 6-H), 3.79 (1H, d, *J* 8.8 Hz, 4-H), 4.05 (1H, d, *J* 8 Hz, 6-H), 4.14 (1H, d, *J* 5.3 Hz, 8-H), 4.34 (1H, dd, *J* 5.7 and 9.1 Hz, 2'-H), 4.73 (1H, s, NC*H*CONHBu), 4.78 (1H, d, *J* 5.3 Hz, 1-H), 5.01 (1H, d, *J* 12.4 Hz, COOC*H₂*Ph), 5.14 (1H, d, *J* 12.4 Hz, COOC*H₂*Ph), 6.72 (2H, d, *J* 8.5 Hz, 6'-H and 8'-H), 7.08 (2H, d, *J* 8.4 Hz, 5'-H and 9'-H), 7.75~7.90 (5H, m, COOCH₂*Ph*); (Found: MH⁺, 603.2663. C₂₉H₃₈N₄O₁₀ requires MH, 603.2666).

2-(2,4,5,8-Tetrahydroxy-7-oxa-2-azabicyclo[3.2.1]oct-3-yl)-2-(tyrosylamino)acetic Acid *n*-Butyl Amide (**4a**)

A solution of the above product (25 mg, 0.039 mmol) in methanol (3 ml) under argon was treated with 10% Pd/C (3 mg) suspended in water (3 ml) and hydrogenated at atmospheric pressure. After 7.5 hour the mixture was filtered through celite and evaporated to give the title compound (11 mg, 60%); λ_{max} 222, 273 nm; δ_H (D₂O) 0.91 (3H, t, *J* 7.3 Hz, CONH(CH₂)₃*CH₃*) 1.29 (2H, m, CONH(CH₂)₂*CH₂*CH₃), 1.37 (2H, m, CONHCH₂*CH₂*CH₂CH₃), 2.94 (1H, dd, *J* 13.7 and 7.9 Hz, 3'-H), 3.02 (2H, m, 3'-H and CONHC*H₂*(CH₂)₂CH₃), 3.20 (1H, dt, *J* 13.4 and 7.0 Hz, CONHC*H₂*(CH₂)₂CH₃), 3.36 (1H, dd, *J* 9.0 and 1.2 Hz, 3-H), 3.65 (1H, dd, *J* 8.6 and 1.7 Hz, 6-H), 3.71 (1H, dd, *J* 9.0 and 1.6 Hz, 4-H), 3.90 (1H, dd, *J* 6.4 and 7.8 Hz, 2'-H), 4.09 (1H, d, *J* 8.5 Hz, 6-H), 4.30 (1H, d, *J* 5.4 Hz, 8-H), 4.63 (1H, d, *J* 1.1 Hz,

NC*H*CONHBu), 4.91 (1H, d, *J* 5.4 Hz, 1-H), 6.92 (2H, d, *J* 8.7Hz, 6'-H and 8'-H), 7.21 (2H, d, *J* 8.7 Hz, 5'-H and 9'-H); δ_C (D₂O), 13.8 (CONH(CH₂)₃*CH₃*), 20.2 (CONH(CH₂)₂*CH₂*CH₃), 31.3 (CONHCH₂*CH₂*CH₂CH₃), 40.1, 40.2 (CONHC*H₂*(CH₂)₂CH₃ and C-3'), 52.6 (NC*H*CONHBu), 57.0 (C-2'), 65.8 (C-3), 67.8 (C-4), 68.6 (C-6), 72.7 (C-8), 77.0 (C-5), 93.3 (C-1), 116.5 (C-6', 8'), 129.2 (C-4'), 131.4 (C-5', 9'), 155.6 (C-7'), 172.9, 178.5 (C-1' and *CO₂*NHBu); (Found: MH⁺, 469.2307. C₂₁H₃₂N₄O₈ requires MH, 469.2299).

4-(Hydroxymethyl-1,3,4,5-tetrahydroxy-2-piperidinyl)-2-(tyrosylamino)acetic Acid (**5**)

A solution of SB-219383 (30 mg, 0.73 mmol) in DMF (5 ml) cooled in an ice-bath was treated with a solution of sodium borohydride (8.25 mg, 0.216 mmol) in water (1 ml). After stirring for 1 hour the reaction mixture was concentrated and the residue purified by chromatography over silica gel eluting with *n*-butanol/ethanol/water mixtures to give the title compound (19 mg, 63%); λ_{max} (H₂O) 222, 273; δ_H (D₂O) 3.04 (1H, dd, *J* 14.4 and 7.9 Hz, 3'-H), 3.06 (1H, dd, *J* 12.4 and 2.1 Hz, 6-H), 3.24 (1H, dd, *J* 14.4 and 4.9 Hz, 3'-H), 3.39 (1H, dd, *J* 12.4 and 3.2 Hz, 6-H), 3.41 (1H, dd, *J* 10.5 and 1.3 Hz, 2-H), 3.52 (1H, bd, *J* 9.5 Hz, 3-H), 3.90 (1H, d, *J* 12.6 Hz, 4-*CH₂*OH), 3.96 (1H, d, *J* 12.6 Hz, 4-*CH₂*OH), 4.06 (1H, dd, *J* 2.9 and 2.2 Hz, 5-H), 4.11 (1H, dd, *J* 4.7 and 7.9 Hz, 2'-H), 4.65 (1H, d, *J* 1.1 Hz, NC*H*CO₂H), 6.94 (2H, d, *J* 8.5 Hz, 6'-H and 8'-H), 7.25 (2H, d, *J* 8.5 Hz, 5'-H and 9'-H); δ_C (D₂O) 38.2 (C-3'), 53.2 (NC*H*CO₂H), 56.3 (C-2'), 59.9 (C-6), 61.7 (4-*CH₂*OH), 68.1 (C-5), 69.9 (C-2), 70.6 (C-3), 75.7 (C-4), 116.5 (C-6', C-8'), 128.3 (C-4'), 131.7 (C-5', C-9'), 155.5 (C-7'), 174.3, 178.4 (*CO₂*H and C-1'); (Found: MH⁺, 416.1670. C₁₇H₂₅N₃O₉ requires MH, 416.1669).

2-(4-Hydroxymethyl-1,3,4,5-tetrahydroxy-2-piperidinyl)-2-[(*N*-benzyloxycarbonyl)tyrosylamino]acetic Acid (**6**)

Using the method described for the preparation of (**1a**) (**5**) (29 mg, 0.05 mmol) was converted to the title compound (23 mg, 84%); λ_{max} 222, 273 nm; δ_H (D₂O) 2.78 (1H, dd, *J* 10.7 and 13.7 Hz, 3'-H), 2.98 (1H, dd, *J* 13.0 and 2.0 Hz, 6-H), 3.22 (1H, dd, *J* 14.1 and 3.9 Hz, 3'-H), 3.31 (1H, dd, *J* 12.5 and 2.7 Hz, 6-H), 3.35 (1H, d, *J* 11.1 Hz, 2-H), 3.52 (1H, bd, *J* 11 Hz, 3-H), 3.81 (1H, d, *J* 12.6 Hz, 4-*CH₂*OH), 3.95 (1H, d, *J* 12.5 Hz, 4-*CH₂*OH), 3.99 (1H, s, 5-H), 4.52 (1H, dd, *J* 10.0 and 4.0 Hz, 2'-H), 4.61 (1H, s, NC*H*CO₂H), 4.9 (1H, d, *J* 12.6 Hz, COOC*H₂*Ph), 5.08 (1H, d, *J* 12.8 Hz, COOC*H₂*Ph), 6.76 (2H, d, *J* 8.5Hz, 6'-H and 8'-H), 7.1 (2H, d, *J* 8.5Hz, 5'-H and 9'-H), 7.17 (2H, d, *J* 6.2 Hz, COOCH₂*Ph*), 7.36 (3H, m, COOCH₂*Ph*) (Found: MH⁺,

550.2036. $C_{25}H_{31}N_3O_{11}$, requires MH, 550.2037).

2-(4-Hydroxymethyl-1,3,4,5-tetrahydroxy-2-piperidinyl)-2-(tyrosylamino)ethanol (10)

a) 2-(4-Hydroxymethyl-1,3,4,5-tetrahydroxy-2-piperidinyl)-2-[(N-benzyloxy carbonyltyrosylamino]ethanol

A solution of (1a) (35 mg, 0.061 mmol) in dry DMF (10 ml) under argon at −20°C was treated with N-methylmorpholine (22 μl, 0.2 mmol) followed by isobutylchloroformate (22 μl, 0.17 mmol). After 5 minutes a solution of sodium borohydride (42 mg, 1.1 mmol) in water (5 ml) was added and the cooling bath removed. After stirring for 1 hour the reaction mixture was concentrated and the residue purified by chromatography over silica gel eluting with n-butanol/ethanol/water mixtures to give the title compound (10 mg, 31%); λ_{max} 222, 274 nm; δ_H (CD₃OD) 2.82 (1H, dd, J 13.9 and 9.2 Hz, 3′-H), 2.8 (1H, hidden under 3′-H, 3-H), 2.98 (1H, d, J 12.6 Hz, 6-H), 3.13 (1H, dd, J 13.9 and 5.2 Hz, 3′-H), 3.36 (1H, dd, J 12.9 and 2.8 Hz, 6-H), 3.69 (1H, dd, J 11.1 and 6.5 Hz, 1-H), 3.69 (1H, d, J 11.9 Hz, 4-CH_2OH), 3.7 (1H, hidden under NCHCH_2OH, 3-H) 3.75 (1H, dd, J 11.2 and 5.9 Hz, NCHCH_2OH), 3.93 (1H, dd, J 2.6 Hz, 5-H), 3.94 (1H, d, J 11.9 Hz, 4-CHOH), 4.35 (1H, dd, J 9.0 and 5.2 Hz, 2′-H), 4.54 (1H, m, NCHCH₂OH), 4.97 (1H, d, J 12.4 Hz, COOCH_2Ph), 5.09 (1H, d, J 12.7 Hz, COOCH_2Ph), 6.70 (2H, d, J 8.6 Hz, 6′-H and 8′-H), 7.07 (2H, d, J 8.6 Hz, 5′-H and 9′-H), 7.24 (2H, m, COOCH₂oPh), 7.28 (1H, m, COOCH₂pPh), 7.33 (2H, m, COOCH₂mPh); (Found: MH⁺ 536.2244. $C_{25}H_{33}N_3O_{10}$ requires MH, 536.2244).

b) 2-(4-Hydroxymethyl-1,3,4,5-tetrahydroxy-2-piperidinyl)-2-(tyrosylamino)ethanol (10)

The product from above (10 mg, 0.019 mmol) was hydrogenated over 10% palladium. After 3 hours the crude product was chromatographed over silica gel eluting with n-butanol/ethanol/water mixtures to give the title compound (4 mg, 53%); δ_H (D₂O) 2.87 (1H, dd, J 10.2 and 2.8 Hz, 2-H), 2.90 (1H, dd, J 14.4 and 2.0 Hz, 6-H), 2.97 (1H, dd, J 14.5 and 3.2 Hz, 6-H), 3.01 (1H, dd, J 13.9 and 7.7 Hz, 3′-H), 3.08 (1H, dd, J 13.8 and 6.6 Hz, 3′-H), 3.47 (1H, dd, J 11.5 and 6.9 Hz, NCHCH_2OH), 3.54 (1H, dd, J 11.4 and 6.4 Hz, NCHCH_2OH), 3.64 (1H, d, J 10.1 Hz, 3-H), 3.83 (1H, d, J 12.5 Hz, 4-CH_2OH), 3.89 (1H, d, J 12.5 Hz, 4-CH_2OH), 3.99 (1H, m, 2′-H), 3.99 (1H, dd, J 3.1 and 1.9 Hz, 5-H), 4.30 (1H, dt, J 6.6 and 2.9 Hz, NCHCH₂OH), 6.93 (2H, d, J 8.6 Hz, 6′-H and 8′-H), 7.21 (2H, d, J 8.6 Hz, 5′-H and 9′-H).

2-(4-Hydroxymethyl-3,4,5-trihydroxy-2-piperidinyl)-2-(tyrosylamino)acetic Acid (11)

A solution of SB-219383 (10 mg, 0.018 mmol) in DMF (2 ml) under argon at −20°C was treated with N-methylmorpholine (11 μl, 0.094 mmol) followed by isobutylchloroformate (11 μl, 0.073 mmol). After 45 minutes at room temperature, the reaction mixture was concentrated to yield crude (2). A solution of this product in methanol (2 ml) was hydrogenated with 10% Pd/C (2 mg) in water (2 ml). Chromatography over silica gel, eluting with n-butanol/ethanol/water mixtures, gave the title compound (3.6 mg, 50%); λ_{max} (H₂O pH 2) 222, 275 nm; δ_H (D₂O) inter alia 2.8~3.0 (5H, m, 6-H, 2-H and 3′-H); m/z (ES) 400 (MH⁺).

2-(4-Acetoxymethyl-1,3,4,5-tetrahydroxy-2-piperidinyl)-2-(tyrosylamino)acetic Acid n-Butyl Ester (12)

A solution of (6) (802 mg 1.46 mmol) in n-butanol (15 ml) was treated with acetyl chloride and the resultant product (44 mg, 5%) was hydrogenated over 10% palladium in methanol for 4 hours to give, after chromatography over silica gel eluting with ethyl acetate/methanol mixtures, the title compound (15 mg, 43%); δ_H (CD₃OD) 0.96 (3H, t, COOCH₂CH₂CH₂CH_3), 1.45 (2H, qt, COOCH₂CH₂CH_2CH₃), 1.65 (2H, tt, COOCH₂CH_2CH₂CH₃), 2.1 (3H, s, OCOCH₃), 2.77 (1H, dd, 3′-H), 2.97 (1H, dd, 6-H), 3.17 (1H, dd, 3′-H), 3.3 (2H hidden under MeOD, 6-H and 2-H), 3.64 (1H, d, 3-H), 3.80 (1H, dd, 2′-H), 3.92 (1H, bs, 5-H), 4.12 (2H, t, COOCH_2CH₂CH₂CH₃), 4.36 (1H, d, 4-CH_2OH), 4.42 (1H, d, 4-CH_2OH), 4.9 (1H, hidden under HDO, NCHCO₂Bu), 6.79 (2H, d, 6′-H and 8′-H), 7.14 (2H, d, 5′-H and 9′-H); m/z (CI) 514 (MH⁺).

2-(4-Phenylcarbamoyloxymethyl-1,3,4,5-tetrahydroxy-2-piperidinyl)-2-(tyrosylamino)acetic Acid n-Butyl Ester (13)

A solution of n-butyl ester of (6) (28 mg, 0.046 mmol) in pyridine (2 ml) was treated with phenyl isocyanate (22 μl, 0.173 mmol). After 40 hours the reaction mixture was concentrated. The residue was treated with ethyl acetate and aqueous 1 M HCl. The organic phase was dried and evaporated. After chromatography over silica gel eluting with dichloromethane/methanol the residue was hydrogenated over palladium for 2 hours to give the title compound (8 mg, 29%); δ_H (CD₃OD) 0.67 (3H, t, COOCH₂CH₂CH₂CH_3), 1.15 (2H, m, COOCH₂CH₂CH_2CH₃), 1.35 (2H, m, COOCH₂CH_2CH₂CH₃), 2.51 (1H, dd, 3′-H), 3.00 (1H, dd, 3′-H), 3.25 (1H, bd, 6-H), 3.45 (1H, bd, 6-H), 3.55 (1H, d, 2-H), 3.70 (1H, d, 3-H), 3.95 (3H, m, COOCH_2CH₂CH₂CH₃ and 5-H), 4.40 (1H, dd, 2′-H), 4.45 (2H, bs, 4-CH_2OH), 4.99 (1H, s, NCHCO₂Bu), 6.62

(2H, d, 6'-H and 8'-H), 6.90~7.40 (12H, m, 5'-H, 9'-H, COOCH$_2$Ph and NHPh); m/z (CI) 591 (MH$^+$).

2-[4-Hydroxymethyl-1-ethylaminocarbonyloxy-(3,4,5-trihydroxy-2-piperidinyl]-2-(tyrosylamino)acetic Acid Butyl Ester (14)

Using an identical procedure to that described above (6) was converted into (14) in 55% yield. δ_H (CD$_3$OD) inter alia 2.63 (1H, dd, J 7.9, 13.8 Hz, 3'-H), 2.97 (1H, dd, J 8.8, 13.8 Hz, 3'-H), 3.5 (2H, m, 2-H, 2'-H); (Found: MH$^+$ 543.2686. C$_{24}$H$_{39}$N$_4$O$_{10}$ requires MH 543.2666).

2-(4-Hydroxymethyl-1,3,4,5-tetrahydroxy-2-piperidinyl)-2-[(O-t-butylcarbonyl)tyrosyl-amino]acetic Acid n-Butyl Ester (15)

a) 2-(2,4,5,8-Tetrahydroxy-7-oxa-2-azabicyclo[3.2.1]oct-3-yl)-2-[(N-benzyloxycarbonyl)tyrosylamino]acetic Acid n-Butyl ester

Compound (1a) (54 mg, 0.094 mmol) was treated with a mixture of 4 M HCl in n-butanol (40 ml) after 1hour the solvent was evaporated. Chromatography over silica gel eluting with dichloromethane/methanol mixtures gave the title compound (22 mg, 39%); λ_{max} 224, 275 nm; δ_H (CD$_3$OD) 0.93 (3H, t, J 7.3 Hz, COO(CH$_2$)$_3$CH$_3$), 1.41 (2H, tq, J 7.5Hz, COO(CH$_2$)$_2$CH$_2$CH$_3$), 1.64 (2H, tt, J 6.6 and 8.3 Hz, COOCH$_2$CH$_2$CH$_2$CH$_3$), 2.78 (1H, dd, J 10.2 and 14.1 Hz, 3'-H), 3.21 (1H, dd, J 14.1 and 4.2 Hz, 3'-H), 3.4 (1H, dd, J 9.0 and 1.0 Hz, 2-H), 3.5 (1H, dd, J 8.0 and 1.2 Hz, 4-CH$_2$OH), 3.78 (1H, dd, J 9.0 and 1.0 Hz, 3-H), 4.03 (1H, d, J 8.0 Hz, 4-CH$_2$OH), 4.07~4.15 (3H, m, COOCH$_2$(CH$_2$)$_2$CH$_3$ and 5-H), 4.47 (1H, dd, J 10.2 and 4.2 Hz, 2'-H), 4.75 (1H, d, J 5.3 Hz, 6-H), 4.9 (1H, hidden under HDO, NCHCO$_2$Bu), 4.95 (1H, d, J 12.7 Hz, COOCH$_2$Ph), 5.08 (1H, d, J 12.6 Hz, COOCH$_2$Ph), 6.7 (2H, d, J 8.5 Hz, 6'-H and 8'-H), 7.09 (2H, d, J 8.4 Hz, 5'-H and 9'-H), 7.2~7.37 (5H, m, COOCH$_2$Ph); m/z (ES) 604 (MH$^+$), 621 (MNH$_4^+$)

b) 2-(2,4,5,8-Tetrahydroxy-7-oxa-2-azabicyclo[3.2.1]oct-3-yl)-2-[(O-t-butylcarbonyl)(N-benzyloxycarbonyl)tyrosyl-amino]acetic Acid n-Butyl Ester

A solution of the product from above (17 mg, 0.028 mmol) in dry THF (5 ml) at 0°C was treated with pivaloyl chloride (3.56 μl, 0.028 mmol), diisopropylethylamine (4.97 μl, 0.028 mmol) and a catalytic amount of dimethylaminopyridine. After 30 minutes the reaction mixture was concentrated. The residue was dissolved in ethyl acetate and the organic phase washed with aqueous 1 M HCl, dried and evaporated. Chromatography over silica gel

eluting with dichloromethane/methanol mixtures gave the title compound (9.5 mg, 50%); λ_{max} 223, 274 nm; δ_H (CD$_3$OD) inter alia 1.35 (9H, s, OCOC(CH$_3$)$_3$; (Found: MH$^+$, 688.3082. C$_{34}$H$_{45}$N$_3$O$_{12}$ requires MH 688.3082).

c) 2-(4-Hydroxymethyl-1,3,4,5-tetrahydroxy-2-piperidinyl)-2-[(O-t-butylcarbonyl)tyrosylamino]Acetic Acid n-Butyl Ester p-Toluenesulfonate Salt (15)

The above product (3.5 mg, 0.005 mmol) was hydrogenated over palladium in the presence of 4-toluenesulfonic acid monohydrate (0.97 mg, 0.005 mmol) for 2 hours to give the title compound (4 mg, 100%); δ_H (CD$_3$OD) 0.94 (3H, t, J 7.4 Hz, COO(CH$_2$)$_3$CH$_3$), 1.34 (9H, s, OCOC(CH$_3$)$_3$, 1.41 (2H, tq, J 7.5 Hz, COO(CH$_2$)$_2$CH$_2$CH$_3$), 1.63 (2H, tt, J 6.8 Hz, COOCH$_2$CH$_2$CH$_2$CH$_3$), 2.37 (3H, s, CH$_3$C$_6$H$_4$SO$_3$), 2.88~3.0 (2H, m, 6-H and 3'-H), 3.25~3.35 (3H, hidden under MeOD, 6-H, 2-H and 3'-H), 3.59 (1H, d, J 10.5 Hz, 3-H), 3.72 (1H, d, J 12 Hz, 4-CH$_2$OH), 3.9 (1H, d, J 12 Hz, 4-CH$_2$OH), 3.95 (2H, m, 2'-H and 5-H), 4.1 (2H, t, J 6.5 Hz, COOCH$_2$(CH$_2$)$_2$CH$_3$), 4.91 (1H, hidden under HDO, NCHCO$_2$Bu), 7.04 (2H, d, J 8.5 Hz, 6'-H and 8'-H), 7.23 (2H, d, J 8 Hz, CH$_3$C$_6$H$_4$SO$_3$), 7.37 (2H, d, J 8 Hz, 5'-H and 9'-H), 7.70 (2H, d, J 8 Hz, CH$_3$C$_6$H$_4$SO$_3$); δ_C (CD$_3$OD) 14.0 (COO(CH$_2$)$_3$CH$_3$), 20.20 (COO(CH$_2$)$_2$CH$_2$CH$_3$), 21.29 (CH$_3$C$_6$H$_4$SO$_3$), 27.44 (OCOC(CH$_3$)$_3$ 31.64 (COOCH$_2$CH$_2$CH$_2$CH$_3$), 40.06 (C-3'), ~49 (OCOC(CH$_3$)$_3$ hidden under MeOD), 52.2 (NCHCO$_2$Bu), 58.7 (C-2'), 60.9 (C-6), 62.8 (C-4-CH$_2$OH), 66.1 (COOCH$_2$(CH$_2$)$_2$CH$_3$), 69.5 (C-5), 70.6, 70.9 (C-2, C-3), 75.6 (C-4), 123.0 (C-6', C-8'), 126.9, 129.8 (CH$_3$C$_6$H$_4$SO$_3$), 131.7 (C-5', C-9'), 135.2 (CH$_3$C$_6$H$_4$SO$_3$), 141.65 (C-4'), 143.5 (CH$_3$C$_6$H$_4$SO$_3$), 151.7 (C-7'), 173.5, 174.5 (C-1', NCHCO$_2$Bu), 179.0 (OCOC(CH$_3$)$_3$); m/z (ES) 556 (MH$^+$).

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