SYNTHESES OF THE POTENT INHIBITORS OF NEURAMINIDASE, *N*-(1,2-DIHYDROXYPROPYL) DERIVATIVES OF SIASTATIN B AND ITS 4-DEOXY ANALOGS

TOSHIAKI KUDO, YOSHIO NISHIMURA*, SHINICHI KONDO and TOMIO TAKEUCHI

Institute of Microbial Chemistry,
3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141, Japan

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Potent inhibitors of neuraminidase, 3,4-didehydro-4-deoxysiastatin B, 4-deoxysiastatin B and *N*-[(*S* and *R*)-1,2-dihydroxypropyl] derivatives of siastatin B, 3,4-didehydro-4-deoxysiastatin B and 4-deoxysiastatin B have been synthesized by the chemical modifications of siastatin B.

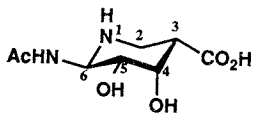
Poly- and multifunctional piperidines isolated from microorganisms and plants are powerful and specific glycosidase inhibitors, and they have the potential to produce a number of kinds of beneficial therapeutic effects such as antihyperglycemic, antiviral and anticancer activity, *etc.*[1]

A multifunctional piperidine, siastatin B (**1**) which was isolated as an inhibitor of neuraminidase by H. UMEZAWA *et al.*[2] from a *Streptomyces* culture, resembles structurally sialic acid (*N*-acetylneuraminic acid, **2**). After achievement of the total synthesis[3~5] and several totally-synthetic analogs,[6,7] we have designed potential neuraminidase inhibitors,[8,9] transition-state analogs and substrate analogs of neuraminic acid aided by molecular graphics tools. Here we wish to report the syntheses of the inhibitors, 3,4-didehydro-4-deoxysiastatin B (**4**), 4-deoxysiastatin B (**5**) and *N*-[(*S* and *R*)-1,2-dihydroxypropyl] derivatives of siastatin B (**13** and **14**), 4-deoxysiastatin B (**17** and **18**) and 3,4-didehydro-4-deoxysiastatin B (**21** and **22**), and their methyl esters (**6**, **7**, **10**, **11**, **12**, **15**, **16**, **19** and **20**) and amides (**8** and **9**) by a chemical modification of **1** with the full experimental details (Schemes 1 and 2).[8,9]

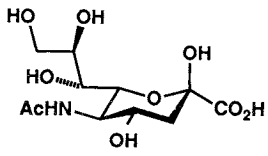
Synthesis

In the course of our studies[6~10] on the relationships between structure and biological activity of siastatin B, we became interested in the syntheses of rationally designed analogs (**4**, **5**, **13**, **14**, **17** and **18**) of siastatin B able to mimic oxocarbonium ion (**3**) of neuraminic acid, an intermediate in the reaction catalyzed by neuraminidase (Fig. 1), obtained from molecular modeling[8,9] using molecular orbital calculations by the AM1 method.[11]

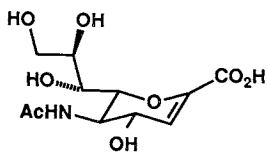
Treatment of **1** with di-*tert*-butyl dicarbonate (Boc-dimer) at 70°C gave the 3,4-didehydro-4-deoxy



Siastatin B (**1**)

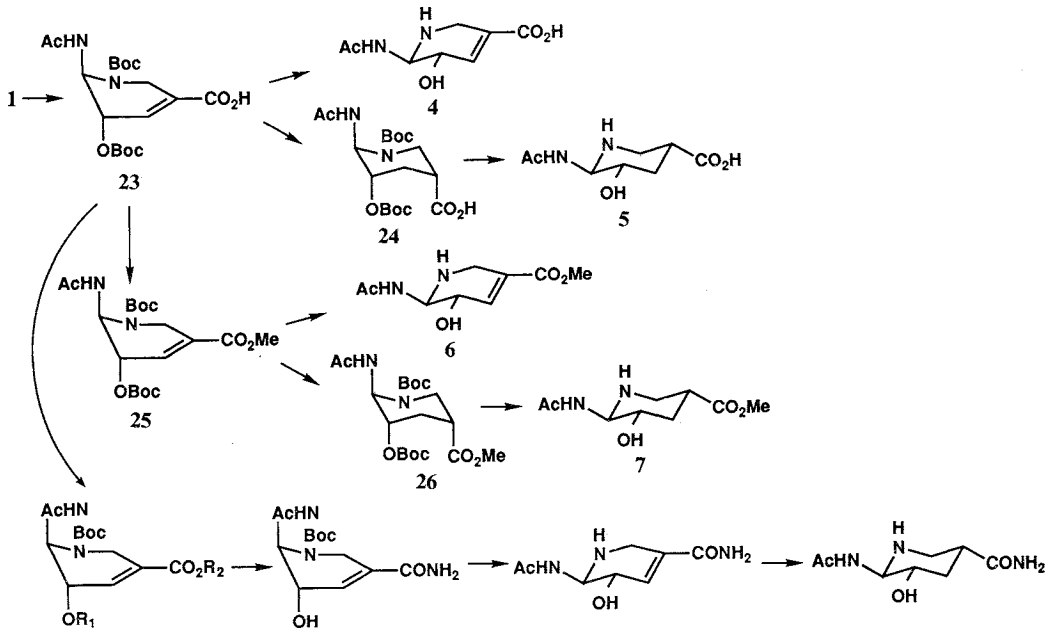


N-Acetylneuraminic acid (**2**)



2,3-Didehydro-2-deoxy-*N*-acetylneuraminic acid (DDNA, **30**)

Scheme 1.



Scheme 2.

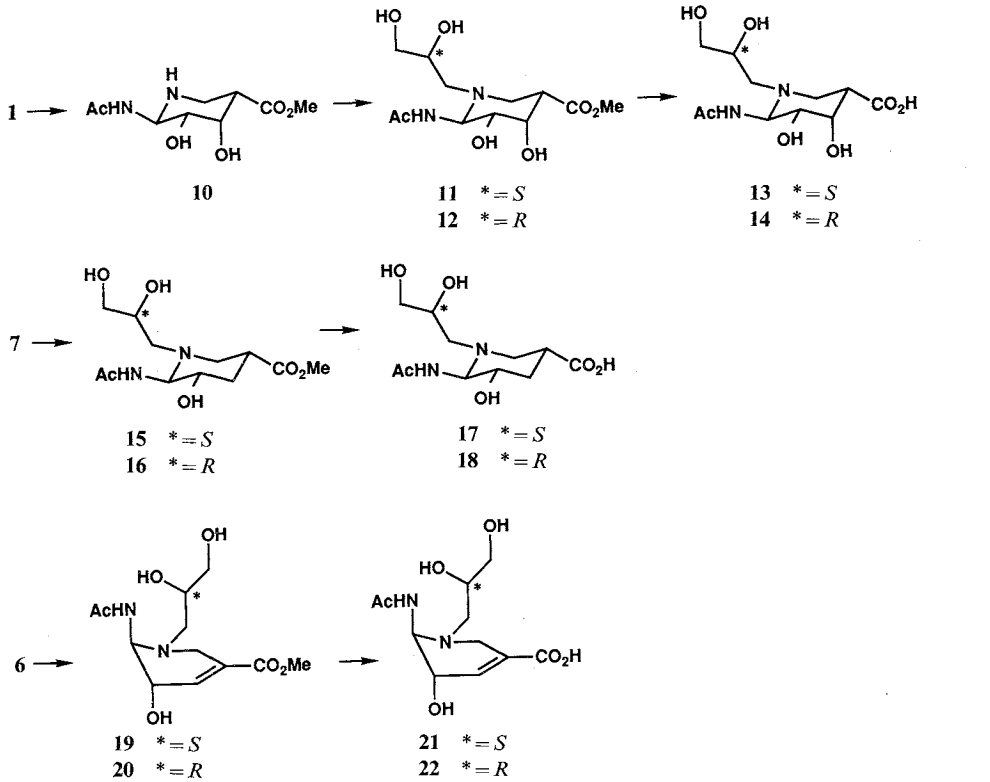


Fig. 1. Mechanism of action of neuraminidase.

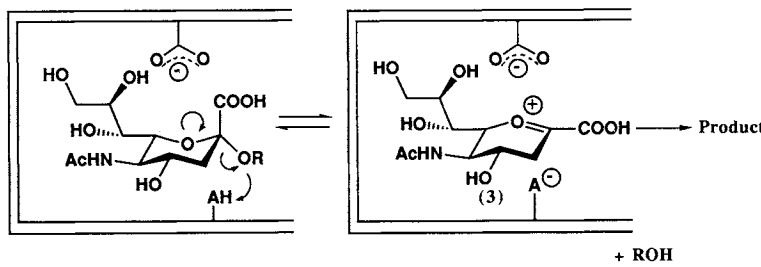


Table 1. IC_{50} (μg/ml) of siastatin B (**1**) and its analogs against *N*-acetylneuraminidases.

Compound	*N*-Acetylneuraminidase	
	C. perfringens[2]	*Streptococcus*[a]
1	50	6.3
4	32	1.1
5	20	1.8
13	7.8	3.0
14	130	>100
17	14	1.3
18	22	12.4
21	80	9.0
30	12	2.0

[a] Assay was carried out using *Streptococcus* sp. sialidase (Seikagaku Kogyo Co. Ltd., Tokyo) and bovine colostrum *N*-acetylneuraminlactose (Sigma Chemical Co., St. Louis), and the liberated *N*-acetylneuraminic acid was determined by the method of AMINOFF.[13]

Table 2. IC_{50} (μg/ml) of siastatin B (**1**) and its analogs against glycosidases.

Compound	α-Glucosidase[14] (yeast)	β-Glucuronidase[15] (bovine liver)	β-Amylase[16] (sweet potato)
1	>100	15.5	>100
4	16.0	22.5	>100
5	5.3	12.0	>100
6	>100	>100	46
17	12.0	>100	64
18	8.5	>100	>100

compound **23** with β-elimination of the 4-OH group. Removal of protecting groups (Boc-groups) of **23** with 4 M hydrogen chloride in dioxane afforded 3,4-didehydro-4-deoxysiastatin B (**4**). Hydrogenation of **23** with palladium on carbon yielded in the 4-deoxy compound **24** which was converted into 4-deoxysiastatin B (**5**) by de-protection. Treatment of **23** with methyl iodide afforded **25** which was converted into 3,4-didehydro-4-deoxysiastatin B methyl ester (**6**) upon removal of Boc groups. Reduction of **25** to **26** followed by acid treatment gave 4-deoxysiastatin B methyl ester (**7**). Selective removal of *O*-Boc group of **23** with potassium carbonate followed by esterification with methyl iodide gave **28**. Ammonolysis of **28** with ammoniacal methanol to the corresponding amide **29**, and subsequent removal of Boc-group furnished 3,4-didehydro-4-deoxysiastatin B amide (**8**). Reduction of **8** gave 4-deoxysiastatin B amide (**9**).

Treatment of **1** with Amberlist 15 (H^+) in methanol afforded siastatin B methyl ester (**10**). Reductive *N*-alkylation of **10** with D-glyceraldehyde by sodium cyanoborohydride in methanol gave **11**. Hydrolysis of **11** with aqueous sodium hydroxide solution afforded *N*-[(*S*)-1,2-dihydroxypropyl]siastatin B (**13**). The similar reductive *N*-alkylation of **10** with L-glyceraldehyde furnished **12** which was converted into *N*-[(*R*)-1,2-dihydroxypropyl]siastatin B (**14**) upon hydrolysis. *N*-[(*S* and *R*)-1,2-Dihydroxypropyl]-4-deoxysiastatin B (**17** and **18**) were also obtained through their methyl esters **15** and **16** from **7**, respectively, by the similar reductive *N*-alkylation followed by hydrolysis. *N*-[(*S* and *R*)-1,2-Dihydroxypropyl]-3,4-didehydro-4-deoxysiastatin B (**21** and **22**) were also synthesized *via* their methyl esters **19** and **20** from **6**, respectively, by the similar reaction sequences.

Biological Activities

As shown in Table 1, compounds **1**, **4**, **5**, **13**, **14**, **17**, **18** and **21** showed inhibitory activity against *Streptococcus* sp. and *Clostridium perfringens* neuraminidases, whereas their methyl ester (**6**, **7**, **10**, **11**, **12**, **15**, **16**, **19** and **20**) and the amides (**8** and **9**) did not inhibit these enzymes. Remarkably, compounds **4**, **5**, **13** and **17** affected these neuraminidases as strongly as the well-known inhibitor, 2,3-didehydro-2-deoxy-*N*-acetylneuraminic acid[12] (DDNA, **30**). Compounds **4**, **5**, **17** and **18** inhibited yeast α-glucosidase, and compounds **1**, **4** and **5** also affected bovine liver β-glucuronidase. Compounds **6** and **17** showed weak inhibitory activity against sweet potato β-amylase. Further evaluation of the biological activities of these analogs is in progress.

Experimental

General Methods

Melting points were determined with a Yanagimoto apparatus and were uncorrected. IR spectra were determined on a Hitachi Model 260-10 spectrophotometer. Optical rotations were measured with a Perkin-Elmer Model 241 polarimeter. ^{1}H NMR spectra were recorded with JEOL JNM-GX400 spectrometer. Chemical shifts are expressed in δ values (ppm) with tetramethylsilane as an internal standard. Mass spectra were taken by a JEOL JMS-SX102 in the FAB and SI modes.

3,4-Didehydro-4-deoxysiastatin B (**4**)

Compound **23** (300 mg) was dissolved in 4 M hydrogen chloride in dioxane (9 ml), and the mixture was allowed to stand at room temperature for 1 hour. The crystals were taken by centrifugation and washed throughly with dioxane to give colorless crystals of **4** as the hydrochloride (170 mg, 96%): MP 165~166°C (dec); $[\alpha]_D^{21}$ +116° (*c* 0.18, H_2O); IR (KBr) cm^{-1} 3450, 3320, 3200, 2930, 2925, 2830, 2750, 1735, 1680, 1530, 1400, 1265, 1210, 1195, 1160, 1145, 1105, 1055, 1010, 990, 935, 910, 885; NMR (400 MHz, D_2O) δ 2.11 (3H, s, $NCOCH_3$), 3.98 (1H, dt, J=17 and ~2 Hz, 2-H), 4.09 (1H, dt, J=17 and 2 Hz, 2-H), 4.66 (1H, ddt, J=6, 4 and 2 Hz, 5-H), 5.17 (1H, d, J=6 Hz, 6-H), 7.01 (1H, m, 4-H); MS (FAB, positive) *m/z* 201 $(M+H)^+$, 142, 75, 57.

4-Deoxysiastatin B (**5**)

Compound **24** (30 mg) was dissolved in 4 M hydrogen chloride in dioxane (0.9 ml), and the mixture was allowed to stand at room temperature for 3 hours. Evaporation of the solvent gave an amorphous solid, which was throughly washed with ether. The solid was subjected to preparative TLC on silica gel, developing with a mixture of $CHCl_3$-MeOH-conc NH_4OH (20:10:3), to give a colorless solid (15 mg, 99.5%): MP 158~160°C (dec); $[\alpha]_D^{24}$ +48° (*c* 0.19, H_2O); IR (KBr) cm^{-1} 3425, 2930, 2840, 2770, 2700, 2550, 2420, 1725, 1695, 1660, 1645, 1555, 1440, 1420, 1380, 1330, 1295, 1280, 1235, 1210, 1160, 1090, 1070, 1030, 990, 985, 935, 890; NMR (400 MHz, D_2O) δ 1.89 (1H, ddd, J=14, 11.5 and 10 Hz, 4-H_{ax}), 2.11 (3H, s, $NCOCH_3$), 2.56 (1H, ddt, J=14, 5 and 2 Hz, 4-H_{eq}), 3.00 (1H, tt, J=11.5 and 5 Hz, 3-H), 3.31 (1H, dd, J=13 and 11.5 Hz, 2-H_{ax}), 3.59 (1H, ddd, J=13, 5 and 2 Hz, 2-H_{eq}), 4.02 (1H, ddd, J=10, 9 and 5 Hz, 5-H), 4.87 (1H, d, J=9 Hz, 6-H); MS (FAB, positive) *m/z* 203 $(M+H)^+$, 144, 115, 75, 57.

3,4-Didehydro-4-deoxysiastatin B Methyl Ester (**6**)

Compound **6** was obtained as crystals of hydrochloride from **25** by a similar procedure to that used for the preparation of **4** (80%): MP 155~156°C (dec); $[\alpha]_D^{22}$ +133° (*c* 0.94, H_2O); IR (KBr) cm^{-1} 3400 (sh), 3290, 3230 (sh), 3060, 2920, 2825, 2730, 2700, 2630, 2570, 2525, 2420, 2350, 1745, 1720, 1695, 1580, 1550, 1430, 1390, 1380, 1340, 1320, 1305, 1285, 1250, 1220, 1160, 1150, 1115, 1090, 1060, 1020, 1015, 995, 955, 935, 915, 890, 885, 830; NMR (400 MHz, D_2O) δ 2.11 (3H, s, $NCOCH_3$), 3.85 (3H, s, CO_2CH_3), 3.99 (1H, dt, J=17 and ~2 Hz, 2-H), 4.10 (1H, dt, J=17 and 2 Hz, 2-H), 4.66 (1H, ddt, J=6, 4 and ~2 Hz, 5-H), 5.14 (1H, d, J=6 Hz, 6-H), 7.06 (1H, dt, J=4 and ~2 Hz, 4-H); MS (FAB, positve), *m/z* 215 $(M+H)^+$, 156, 75, 57.

4-Deoxysiastatin B Methyl Ester (7)

Compound **7** was obtained as colorless crystals of hydrochloride from **26** by a similar procedure to that used for the preparation of **4** (93%): MP 176~177°C; $[\alpha]_D^{24}$ +58° (*c* 0.13, H_2O); IR (KBr) cm^{-1} 3300, 2970, 2920, 2830, 2750, 2550, 2425, 1745, 1695, 1605, 1590, 1540, 1470, 1440, 1410, 1375, 1310, 1290, 1275, 1255, 1220, 1180, 1165, 1110, 1090, 1070, 1040, 1000, 980, 920, 895, 880; NMR (400 MHz, D_2O) δ 1.94 (1H, q, J=11 Hz, 4-H_{ax}), 2.13 (3H, s, $NCOCH_3$), 2.57 (1H, dt, J=11 and 4 Hz, 4-H_{eq}), 3.08 (1H, tt, J=11 and 4 Hz, 3-H), 3.37 (1H, t, J=11 Hz, 2-H_{ax}), 3.62 (1H, dd, J=11 and 4 Hz, 2-H_{eq}), 3.79 (3H, s, CO_2CH_3), 4.04 (1H, dt, J=~10 and 4 Hz, 5-H), 4.91 (1H, d, J=9 Hz, 6-H); MS (FAB, positive) *m/z* 217.2 $(M+H)^+$, 158.1, 141.1, 75.0, 57.0.

3,4-Didehydro-4-deoxysiastatin B Amide (8)

Compound **8** was obtained as a amorphous solid from **29** by a similar procedure to that used for the preparation of **5** (100%): MP 158~159°C (dec); $[\alpha]_D^{21}$ +109° (*c* 0.11, H_2O); IR (KBr) cm^{-1} 3370, 3310, 3200, 1660, 1620, 1540, 1420, 1380, 1300, 1280, 1255, 1225, 1150, 1130, 1090, 1040, 1020, 1000, 940, 920, 875; NMR (400 MHz, D_2O) δ 2.06 (3H, s, $NCOCH_3$), 3.54 (1H, dt, J=18 and ~2 Hz, 2-H), 3.64 (1H, dt, J=18 and 2.4 Hz, 2-H), 4.25 (1H, dq, J=7.2 and ~2 Hz, 5-H), 4.53 (1H, d, J=7.2 Hz, 6-H), 6.54 (1H, dt, J=2.4 and ~2 Hz, 4-H); MS (FAB, positive) *m/z* 200 $(M+H)^+$, 141, 75, 57, 45.

4-Deoxysiastatin B Amide (9)

The solution of **8** (178 mg) in H_2O (0.3 ml) was stirred with 10% Pd/C (3.1 mg) under atmospheric pressure of hydrogen at room temperature for 3 hours. After filtration, evaporation of the filtrate gave a solid. The solid was subjected to preparative TLC on silica gel, developing with a mixture of $CHCl_3$-MeOH-conc NH_4OH (10:10:1), to give **9** as an amorphous solid (131 mg, 74%): MP 208~209°C; $[\alpha]_D^{21}$ +27° (*c* 0.11, H_2O); IR (KBr) cm^{-1} 3420, 3380, 3320, 3275, 3210, 2950, 1670, 1550, 1475, 1455, 1420, 1375, 1340, 1300, 1285, 1270, 1240, 1200, 1160, 1130, 1110, 1080, 1055, 1035, 1010, 995, 965, 950, 920, 890, 850; NMR (400 MHz, D_2O) δ 1.87 (1H, dt, J=12.4 and 11.6 Hz, 4-H_{ax}), 2.11 (3H, s, $NCOCH_3$), 2.48 (1H, ddt, J=12.4, 4 and 2 Hz, 4-H_{eq}), 2.96 (1H, tt, J=11.6 and 4 Hz, 3-H), 3.27 (1H, t, J=11.6 Hz, 2-H_{ax}), 3.53 (1H, ddd, J=11.6, 4 and 2 Hz, 2-H_{eq}), 4.02 (1H, ddd, J=12.4, 10 and 4 Hz, 5-H), 4.87 (1H, d, J=10 Hz, 6-H); MS (FAB, positive) *m/z* 202 $(M+H)^+$, 143, 115, 75, 57, 45.

Siastatin B Methyl Ester (10)

The solution of **1** (487 mg) in dry MeOH (15 ml) was stirred with Amberlist 15 (H^+) (500 mg) at room temperature overnight. After addition of conc NH_4OH (pH ~8), the resin was filtered off. Evaporation of the filtrate gave a solid, which was subjected to column chromatography on silica gel. Elution with a mixture of $CHCl_3$-MeOH-conc NH_4OH (20:10:3) gave a solid. The solid was crystallized from MeOH to give colorless crystals (434 mg, 84%): MP 172~174°C (dec); $[\alpha]_D^{26}$ +18° (*c* 0.59, H_2O); IR (KBr) cm^{-1} 3500, 3340, 3270, 3080, 3010, 2960, 2920, 1740, 1640, 1555, 1445, 1420, 1380, 1320, 1305, 1280, 1240, 1230, 1220, 1205, 1170, 1140, 1125, 1105, 1080, 1040, 1020, 995, 980, 960, 920, 875; NMR (400 MHz, D_2O) δ 2.04 (3H, s, $NCOCH_3$), 2.85 (1H, ddd, J=11, 6 and 3 Hz, 3-H), 3.0~3.15 (2H, m, 2-H_2), 3.55 (1H, dd, J=10 and 3 Hz, 5-H), 3.75 (3H, s, CO_2CH_3), 4.48 (1H, t, J=3 Hz, 4-H), 4.61 (1H, d, J=10 Hz, 6-H); MS (FAB, positive) *m/z* 233.2 $(M+H)^+$, 174.1, 75.0, 57.0.

N-[(*S*)-1,2-Dihydroxypropyl]siastatin B Methyl Ester (11)

To MeOH (1 ml) containing 80% aqueous solution of D-glyceraldehyde (169 mg) were added **10** (100 mg) and $NaBH_3CN$ (34 mg), and the mixture was stirred at room temperature for 2 hours. Evaporation of the solvent gave a solid. The solid was subjected to preparative TLC on silica gel, developing with a mixture of $CHCl_3$-MeOH-conc NH_4OH (15:10:2), to give a hygroscopic solid (80 mg, 61%): $[\alpha]_D^{24}$ −25° (*c* 0.18, MeOH); IR (KBr) cm^{-1} 3520, 3380, 3310, 3070, 2970, 2940, 2870, 2840, 1740, 1655, 1550, 1450, 1440, 1380, 1330, 1310, 1270, 1245, 1200, 1170, 1150, 1130, 1110, 1105, 1070, 1050, 1010, 965, 945, 910, 895, 880, 865; NMR (400 MHz, CD_3OD), δ 2.00 (3H, s, $NCOCH_3$), 2.30 (1H, dd, J=13 and 3 Hz, 7-H), 2.71 (1H, dd, J=13 and 9 Hz, 7-H), 2.75~3.08 (3H, m, 2-H_2 and 3-H), 3.34 (1H, dd, J=8.5 and 2.5 Hz, 5-H), 3.45 (1H, dd, J=11 and 5 Hz, 9-H), 3.51 (1H, dd, J=11 and 4.5 Hz, 9-H), 3.69 (3H, s, CO_2CH_3), 3.73 (1H, m, 8-H), 4.32 (1H, broad t, J=2.5 Hz, 4-H), 4.39 (1H, d, J=8.5 Hz, 6-H); MS (FAB,

positive) m/z 307 $(M+H)^+$, 248, 207, 115, 75, 57.

N-[(*R*)-1,2-Dihydroxypropyl]siastatin B Methyl Ester (**12**)

Compound **12** was obtained as a hygroscopic solid from **10** with L-glyceraldehyde by a similar procedure to that used for the preparation of **11** (23%): $[\alpha]_D^{21}$ $-10°$ (c 0.62, MeOH); IR (KBr) cm^{-1} 3400, 2960, 2900, 1730, 1650, 1550, 1450, 1380, 1320, 1290, 1250, 1215, 1175, 1140, 1110, 1085, 1045, 1020, 975, 940, 890; NMR (400 MHz, CD_3OD) δ 1.99 (3H, s, $NCOCH_3$), 2.25 (1H, dd, $J=13.6$ and 6.4 Hz, 7-H), 2.73 (1H, ddd, $J=11.6$, 4 and 2.8 Hz, 3-H), 2.77 (1H, dd, $J=13.6$ and 7.2 Hz, 7-H), 2.84 (1H, t, $J=11.6$ Hz, 2-H_{ax}), 3.02 (1H, dd, $J=11.6$ and 4 Hz, 2-H_{eq}), 3.32 (1H, dd, $J=8.8$ and 3.2 Hz, 5-H), 3.42 (1H, dd, $J=11.6$ and 6 Hz, 9-H), 3.57 (1H, dd, $J=11.6$ and 4 Hz, 9-H), 3.65~3.75 (1H, m, 8-H), 3.69 (3H, s, CO_2CH_3), 4.30 (1H, t, $J=3.2$ Hz, 4-H), 4.39 (1H, d, $J=8.8$ Hz, 6-H); MS (FAB, positive) m/z 307.2 $(M+H)^+$, 248.2, 207.2, 115.1, 75.0, 57.0.

N-[(*S*)-1,2-Dihydroxypropyl]siastatin B (**13**)

To a solution of **11** (41 mg) in MeOH (1.6 ml) was added 1 M NaOH (0.3 ml), and the mixture was stirred at room temperature for 1 hour. Evaporation of the solvent gave a solid. The solid was subjected to preparative TLC on silica gel, developing with a mixture of $CHCl_3$ - MeOH - conc NH_4OH (15 : 10 : 3), to give a hygroscopic solid (30 mg, 71%): $[\alpha]_D^{23}$ $-2.3°$ (c 1.02, H_2O); IR (KBr) cm^{-1} 3400, 3250, 1690, 1660, 1600, 1550, 1400, 1310, 1280, 1205, 1180, 1150, 1100, 1070, 1050, 1005, 975, 920, 890, 860; NMR (400 MHz, D_2O with a few drops of py-d_5, 40°C) δ 2.10 (3H, s, $NCOCH_3$), 2.37 (1H, dd, $J=14$ and 3.2 Hz, 7-H), 2.63 (1H, ddd, $J=12.4$ and 3 Hz, 3-H), 2.70 (1H, dd, $J=14$ and 8.8 Hz, 7-H), 2.74 (1H, t, $J=12$ Hz, 2-H), 3.06 (1H, dd, $J=12$ and 4 Hz, 2-H), 3.50 (1H, dd, $J=12$ and 6 Hz, 9-H), 3.54 (1H, dd, $J=8.8$ and 3 Hz, 5-H), 3.61 (1H, dd, $J=12$ and 4 Hz, 9-H), 3.91 (1H, m, 8-H), 4.38 (1H, t, $J=3$ Hz, 4-H), 4.42 (1H, d, $J=8.8$ Hz, 6-H); MS (FAB, positive) m/z 293 $(M+H)^+$, 234, 207, 115, 75, 57, 45.

N-[(*R*)-1,2-Dihydroxypropyl]siastatin B (**14**)

Compound **14** was obtained as a hygroscopic solid from **12** by a similar procedure to that used for the preparation of **13** (86%): $[\alpha]_D^{24}$ $+16°$ (c 0.44, H_2O); IR (KBr) cm^{-1} 3400 (broad), 3250 (broad), 1660, 1600, 1410, 1320 (sh), 1170 (sh), 1140 (sh), 1110, 1060, 1010, 920, 890; NMR (400 MHz, D_2O with a few drops of py-d_5, 40°C) δ 2.05 (3H, s, $NCOCH_3$), 2.36 (1H, dd, $J=14$ and 7 Hz, 7-H), 2.59 (1H, m, 3-H), 2.71 (1H, dd, $J=14$ and 6 Hz, 7-H), 2.79 (1H, t, $J=12$ Hz, 2-H_{ax}), 3.01 (1H, dd, $J=12$ and 4 Hz, 2-H_{eq}), 3.45 (1H, dd, $J=12$ and 5 Hz, 9-H), 3.48 (1H, dd, $J=8.5$ and 3 Hz, 5-H), 3.59 (1H, dd, $J=12$ and 4 Hz, 9-H), 3.80 (1H, m, 8-H), 4.31 (1H, t, $J=3$ Hz, 4-H), 4.42 (1H, d, $J=8.5$ Hz, 6-H); MS (FAB, positive) m/z 293 $(M+H)^+$, 234, 207, 115, 75, 57, 45.

N-[(*S*)-1,2-Dihydroxypropyl]-4-deoxysiastatin B Methyl Ester (**15**)

Compound **15** was obtained as a hygroscopic solid from **7** by a similar procedure to that used for the preparation of **11** (91%): $[\alpha]_D^{24}$ $-8°$ (c 0.43, MeOH); IR (KBr) cm^{-1} 3400, 2960, 1725, 1660, 1550, 1445, 1405, 1290 (broad), 1220, 1160, 1110, 1070, 1050, 1020, 990, 940, 930, 880; NMR (400 MHz, CD_3OD) δ 1.65 (1H, dt, $J=13$ and 10.5 Hz, 4-H_{ax}), 2.01 (3H, s, $NCOCH_3$), 2.24 (1H, ddt, $J=13$, 4.5 and 1.8 Hz, 4-H_{eq}), 2.36 (1H, dd, $J=13$ and 4 Hz, 7-H), 2.47 (1H, t, $J=11$ Hz, 2-H_{ax}), 2.6~2.7 (2H, m, 3-H and 7-H), 2.22 (1H, ddd, $J=11$, 4 and 1.8 Hz, 2-H_{eq}), 2.42 (1H, ddd, $J=10.5$, 7.6 and 4.5 Hz, 5-H), 2.46 (1H, dd, $J=11$ and 6 Hz, 9-H), 2.51 (1H, dd, $J=11$ and 5 Hz, 9-H), 2.68 (3H, s, CO_2CH_3), 2.7~2.8 (1H, m, 8-H), 4.14 (1H, d, $J=7.6$ Hz, 6-H); MS (FAB, positive) m/z 291 $(M+H)^+$, 232, 207, 115, 75, 57, 45.

N-[(*R*)-1,2-Dihydroxypropyl]-4-deoxysiastatin B Methyl Ester (**16**)

Compound **16** was obtained as a hygroscopic solid from **7** by a similar procedure to that used for the preparation of **12** (69%): $[\alpha]_D^{23}$ $+11°$ (c 0.34, MeOH); IR (KBr) cm^{-1} 3400, 2970, 1730, 1660, 1540, 1445, 1405, 1270, 1215, 1160, 1110, 1070, 1045, 1020, 1000, 990, 980, 950, 930, 870; NMR (400 MHz, CD_3OD) δ 1.69 (1H, dt, $J=13$ and 9 Hz, 4-H_{ax}), 2.00 (3H, s, $NCOCH_3$), 2.21 (1H, ddt, $J=13$, 4 and 1.5 Hz, 4-H_{eq}), 2.29 (1H, dd, $J=13$ and 7 Hz, 7-H), 2.54 (1H, dt, $J=11.5$ and 9 Hz, 2-H_{ax}), 2.63 (1H, tt, $J=10$ and 4 Hz, 3-H), 2.71 (1H, dd, $J=13$ and 6 Hz, 7-H), 3.13 (1H, ddd, $J=11.5$, 4 and 1.5 Hz, 2-H_{eq}), 3.41 (1H, ddd, $J=9$, 7 and 4 Hz, 5-H), 3.44 (1H, dd, $J=11$ and 5.5 Hz, 9-H), 3.56 (1H, dd, $J=11$ and

4.5 Hz, 9-H), 3.65~3.75 (1H, m, 8-H), 3.67 (3H, s, CO_2CH_3), 4.18 (1H, d, J=7 Hz, 6-H); MS (FAB, positive) m/z 291 $(M+H)^+$, 232, 207, 115, 75, 57, 45.

N-[(*S*)-1,2-Dihydroxypropyl]-4-deoxysiastatin B (**17**)

Compound **17** was obtained as a hygroscopic solid from **15** by a similar procedure to that used for the preparation of **13** (96%): $[\alpha]_D^{24}$ −5.4° (*c* 1.28, H_2O); IR (KBr) cm^{-1} 3400, 3230, 1680, 1660, 1635, 1590, 1415, 1315, 1160, 1110, 1080, 1060, 1020, 970, 935, 910, 870; NMR (400 MHz, D_2O) δ 1.60 (1H, q, J=12 Hz, 4-H_{ax}), 2.10 (3H, s, $NCOCH_3$), 2.30 (1H, broad d with small couplings, J=12 Hz, 4-H_{eq}), 2.35~2.55 (2H, m, 2-H_{ax} and 7-H), 2.53 (1H, tt, J=12 and 3 Hz, 3-H), 2.73 (1H, dd, J=14 and 10 Hz, 7-H), 3.28 (1H, broad d with small couplings, J=11 Hz, 2-H_{eq}), 3.48 (1H, dd, J=12 and 6 Hz, 9-H), 3.53~3.63 (1H, m, 5-H), 3.59 (1H, dd, J=12 and 4 Hz, 9-H), 3.87~3.97 (1H, m, 8-H), 4.13 (1H, d, J=8 Hz, 6-H); MS (FAB, positive) m/z 299 $(M+Na)^+$, 277 $(M+H)^+$, 218, 207, 185, 110, 75, 57, 45.

N-[(*R*)-1,2-Dihydroxypropyl]-4-deoxysiastatin B (**18**)

Compound **18** was obtained as a hygroscopic solid from **16** by a similar procedure to that used for the preparation of **14** (64%): $[\alpha]_D^{24}$ +13° (*c* 0.93, H_2O); IR (KBr) cm^{-1} 3400, 3200, 1690, 1640, 1600, 1415, 1320, 1210, 1150, 1110, 1080, 1050, 1020, 980, 960, 935, 900; NMR (400 MHz, D_2O) δ 1.58 (1H, q, J=10.5 Hz, 4-H_{ax}), 2.09 (3H, s, $NCOCH_3$), 2.28 (1H, dt, J=10.5 and 4.5 Hz, 4-H_{eq}), 2.38 (1H, dd, J=14 and 7 Hz, 7-H), 2.45~2.57 (2H, m, 2-H_{ax} and 3-H), 2.76 (1H, dd, J=14 and 5.8 Hz, 7-H), 3.24 (1H, broad d with small couplings, J=9 Hz, 2-H_{eq}), 3.47 (1H, dd, J=12 and 6 Hz, 9-H), 3.52 (1H, ddd, J=10.5, 8.5 and 4.5 Hz, 5-H), 3.61 (1H, dd, J=12 and 4 Hz, 9-H), 3.8~3.9 (1H, m, 8-H), 4.11 (1H, d, J=8.5 Hz, 6-H); MS (FAB, negative) m/z 275.2 $(M-H)^-$, 206.1, 184.2, 130.1, 112.1.

N-[(*S*)-1,2-Dihydroxypropyl]-3,4-didehydro-4-deoxysiastatin B Methyl Ester (**19**)

Compound **19** was obtained as a hygroscopic solid from **6** by a similar procedure to that used for the preparation of **11** (87%): $[\alpha]_D^{23}$ +136° (*c* 0.12, MeOH); IR (KBr) cm^{-1} 3400, 1710, 1660, 1640, 1540, 1445, 1405, 1380, 1280, 1200, 1155, 1115, 1050, 1010, 970, 950, 910, 860; NMR (400 MHz, CD_3OD) δ 1.96 (3H, s, $NCOCH_3$), 2.45 (1H, dd, J=13 and 9 Hz, 7-H), 2.74 (1H, dd, J=13 and 4 Hz, 7-H), 3.30 (1H, dt, J=18 and 2 Hz, 2-H), 3.47 (1H, dd, J=11.5 and 6 Hz, 9-H), 3.81 (1H, dt, J=18 and 1.2 Hz, 2-H), 3.87 (1H, dd, J=11.5 and 4.5 Hz, 9-H), 3.77 (3H, s, CO_2CH_3), 3.85~3.93 (1H, m, 8-H), 3.94 (1H, dt, J=5 and 2 Hz, 5-H), 4.97 (1H, d, J=2 Hz, 6-H), 6.88 (1H, dt, J=5 and 2 Hz, 4-H); MS (FAB, positive) m/z 289 $(M+H)^+$, 230, 207, 110, 75, 57, 45.

N-[(*R*)-1,2-Dihydroxypropyl]-3,4-didehydro-4-deoxysiastatin B Methyl Ester (**20**)

Compound **20** was obtained as a hygroscopic solid from **6** by a similar procedure to that used for the preparation of **12** (83%): $[\alpha]_D^{23}$ −151° (*c* 0.32, MeOH); IR (KBr) cm^{-1} 3400, 3300, 2960, 1720, 1660, 1540, 1445, 1410, 1380, 1280, 1200, 1155, 1110, 1070, 1050, 1010, 950, 910, 865; NMR (400 MHz, CD_3OD) δ 1.95 (3H, s, $NCOCH_3$), 2.60 (1H, dd, J=13 and 4 Hz, 7-H), 2.72 (1H, dd, J=13 and 9 Hz, 7-H), 3.15 (1H, dt, J=18 and 2 Hz, 2-H), 3.51 (1H, dd, J=11 and 5.5 Hz, 9-H), 3.55 (1H, dd, J=11 and 5 Hz, 9-H), 3.61 (1H, dd, J=18 and 1 Hz, 2-H), 3.77 (3H, s, CO_2CH_3), 3.8~3.9 (1H, m, 8-H), 3.93 (1H, dt, J=5 and 2 Hz, 5-H), 4.92 (1H, d, J=2 Hz, 6-H), 6.89 (1H, dt, J=5 and 2 Hz, 4-H); MS (FAB, positive) m/z 289 $(M+H)^+$, 230, 207, 110, 75, 57, 45.

N-[(*S*)-1,2-Dihydroxypropyl]-3,4-didehydro-4-deoxysiastatin B (**21**)

Compound **21** was obtained as a hygroscopic solid from **19** by a similar procedure to that used for the preparation of **13** (66%): $[\alpha]_D^{22}$ +108° (*c* 0.12, H_2O); IR (KBr) cm^{-1} 3400, 3250, 1660, 1560, 1400, 1360, 1290, 1155, 1110, 1075, 1045, 970, 900, 870; NMR (400 MHz, D_2O) δ 2.03 (3H, s, $NCOCH_3$), 2.56 (1H, dd, J=13.5 and 9 Hz, 7-H), 2.73 (1H, dd, J=13.5 and 4 Hz, 7-H), 3.32 (1H, dt, J=18 and 2 Hz, 2-H), 3.51 (1H, d, J=18 Hz, 2-H), 3.53 (1H, dd, J=12 and 6 Hz, 9-H), 3.66 (1H, dd, J=12 and 4 Hz, 9-H), 3.97~4.05 (1H, m, 8-H), 4.12 (1H, m, 5-H), 4.91 (1H, d, J=3 Hz, 6-H), 6.54 (1H, dt, J=5 and 2 Hz, 4-H); MS (FAB, positive) m/z 275 $(M+H)^+$, 216, 207, 110, 75, 57, 45.

N-[(*R*)-1,2-Dihydroxypropyl]-3,4-didehydro-4-deoxysiastatin B (**22**)

Compound **22** was obtained as a hygroscopic solid from **20** by a similar procedure to that used for the preparation of **14** (51%): $[\alpha]_D^{23}$ +143° (*c* 0.13, H_2O); IR (KBr) cm^{-1} 3400, 3240 (sh), 1660, 1565, 1400, 1360 (sh), 1280, 1160, 1115, 1080, 1075, 975, 910, 880; NMR (400 MHz, D_2O) δ 2.03 (3H, s, $NCOCH_3$), 2.56 (1H, dd, J=13.5 and 9 Hz, 7-H), 2.73 (1H, dd, J=13.5 and 4 Hz, 7-H), 4.32 (1H, dt, J=18 and 1.5 Hz, 2-H), 4.51 (1H, broad d, J=18 Hz, 2-H), 4.53 (1H, dd, J=12 and 6 Hz, 9-H), 4.66 (1H, dd, J=12 and 4 Hz, 9-H), 3.97~4.5 (1H, m, 8-H), 4.12 (1H, m, 5-H), 4.91 (1H, d, J=3 Hz, 6-H), 6.54 (1H, dt, J=5 and 2 Hz, 4-H); MS (FAB, negative) m/z 273.2 $(M-H)^-$, 214.1, 175.2, 130.1, 112.1, 43.0.

N,5-*O*-Bis(*tert*-butoxycarbonyl)-3,4-didehydro-4-deoxysiastatin B (**23**)

To a solution of **1** (1 g) in *N*,*N*-dimethylformamide (DMF, 38 ml) were added diisopropylethylamine (7.2 ml) and Boc-dimer (4.5 ml), and the mixture was stirred at 70°C for 5 hours. Evaporation of the solvent gave an oil, which was dissolved in $CHCl_3$. The solution was washed with water, dried over $MgSO_4$, and filtered. Evaporation of the filtrate afforded an oil, which was subjected to the column chromatography on silica gel. Elution with a mixture of $CHCl_3$-CH_3OH-conc NH_4OH (20:10:3) gave an amorphous solid of **23** (984 mg, 54%): MP 192~193°C (dec); $[\alpha]_D^{21}$ +145° (*c* 0.19, MeOH); IR (KBr) cm^{-1} 3390, 2980, 2940, 1720, 1670, 1630, 1535, 1480, 1460, 1440, 1400, 1375, 1325, 1285, 1250, 1170, 1130, 1095, 1080, 1040, 1000, 990, 920, 880, 860; NMR (400 MHz, CD_3OD) δ 1.47 and 1.48 (each 9H, s, $OC(CH_3)_3 \times 2$), 1.93 (3H, s, $NCOCH_3$), 4.7~4.85 (1H, broad m, 2-H), 4.49 (1H, d, J=18 Hz, 2-H), 4.97 (1H, m, 5-H), 6.21 (1H, broad s, 6-H), 6.81 (1H, m, 4-H); MS (FAB, positive) m/z 401 $(M+H)^+$, 345, 289, 124, 57.

N,5-*O*-Bis(*tert*-butoxycarbonyl)-4-deoxysiastatin B (**24**)

The solution of **23** (90 mg) in MeOH (9 ml) was stirred with 10% Pd/C (30 mg) under atmospheric pressure of hydrogen at room temperature overnight. After filtration, evaporation of the filtrate gave a solid. The solid was subjected to preparative TLC on silica gel, developing with a mixture of $CHCl_3$-MeOH (2:1), to give **24** as an amorphous solid (83 mg, 92%): $[\alpha]_D^{24}$ +29° (*c* 0.49, $CHCl_3$); IR (KBr) cm^{-1} 3430, 2980, 2930, 1740, 1680, 1545, 1480, 1460, 1420, 1395, 1370, 1280, 1255, 1150, 1100, 1050, 1040, 1010, 955, 920, 880, 860, 845; NMR (400 MHz, CD_3OD) δ 1.46 (18H, s, $OC(CH_3)_3 \times 2$), 1.96 (3H, s, $NCOCH_3$), 2.13 (1H, ddd, J=15, 7 and 3 Hz, 4-H), 2.35 (1H, broad dt, J=15 and 3 Hz, 4-H), 2.59 (1H, m, 3-H), 3.31 (1H, broad m, 2-H), 4.14 (1H, broad d, J=13 Hz, 2-H), 4.67 (1H, q, J=3 Hz, 5-H), 5.86 (1H, d, J=3 Hz, 6-H); MS (FAB, positive) m/z 425 $(M+Na)^+$, 403 $(M+H)^+$, 347, 291, 126, 57.

N,5-*O*-Bis(*tert*-butoxycarbonyl)-3,4-didehydro-4-deoxysiastatin B Methyl Ester (**25**)

To a solution of **23** (100 mg) in DMF (1 ml) were added diisopropylethylamine (0.59 ml) and MeI (0.086 ml), and the mixture was stirred at room temperature for 24 hours. Evaporation of the solvent gave an oil, which was dissolved in CH_2Cl_2. The solution was washed with H_2O, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil. The oil was subjected to preparative TLC on silica gel, developing with a mixture of $CHCl_3$-MeOH (10:1), to give a foamy glass (87.5 mg, 84.5%): $[\alpha]_D^{24}$ +137° (*c* 0.19, MeOH); IR (KBr) cm^{-1} 2980, 1730, 1700 (sh), 1505, 1480, 1465, 1445, 1410, 1400, 1375, 1320, 1270 (sh), 1260, 1165, 1095, 1040, 1000, 975, 950, 910 (broad), 860; NMR (400 MHz, CD_3OD) δ 1.48 and 1.49 (each 9H, s, $OC(CH_3)_3 \times 2$), 1.93 (3H, s, $NCOCH_3$), 3.79 (1H, broad d with small couplings, J=19 Hz, 2-H), 3.82 (3H, s, CO_2CH_3), 4.51 (1H, dd, J=19 and 2 Hz, 2-H), 5.01 (1H, broad d, J=6 Hz, 5-H), 6.24 (1H, m, 6-H), 6.96 (1H, dt with small couplings, J=6 and 3 Hz, 4-H); MS (FAB, positive) m/z 415 $(M+H)^+$, 303, 241, 200, 154, 138, 124, 75, 57.

N,5-*O*-Bis(*tert*-butoxycarbonyl)-4-deoxysiastatin B Methyl Ester (**26**)

Compound **26** was obtained as a foamy glass from **25** by a similar procedure to that used for the preparation of **24** (80%): $[\alpha]_D^{24}$ +28° (*c* 0.4, MeOH); IR ($CHCl_3$) cm^{-1} 3000, 2970, 2950, 1735, 1690, 1515, 1485, 1465, 1450, 1420, 1400, 1375, 1360, 1325, 1280, 1275, 1150, 1100, 1075, 1055, 1000, 950, 920, 895, 860, 835, 820; NMR (400 MHz, CD_3OD) δ 1.47 (18H, s, $OC(CH_3)_3 \times 2$), 1.97 (3H, s, $NCOCH_3$), 2.11 (1H, ddd, J=16, 6 and 2.8 Hz, 4-H_{ax}), 2.53 (1H, broad d with small couplings, J=16 Hz, 4-H_{eq}), 2.64 (1H, m, 3-H), 3.11 (1H, dd, J=14 and 4 Hz, 2-H_{ax}), 3.69 (3H, s, CO_2CH_3), 4.49 (1H, d with small couplings, J=14 Hz, 2-H_{eq}), 4.60 (1H, dt, J=6 and 2.8 Hz, 5-H), 6.00 (1H, d, J=2.8 Hz, 6-H); MS

(FAB, positive) *m/z* 417 $(M+H)^+$, 361, 305, 243, 202, 172, 158, 140, 80, 57.

N-(*tert*-Butoxycarbonyl)-3,4-didehydro-4-deoxysiastatin B (**27**)

Compound **23** (300 mg) was dissolved in saturated K_2CO_3 solution in MeOH (3 ml), and the mixture was allowed to stand at room temperature. Evaporation of the solvent gave a solid, which was dissolved in $CHCl_3$. The solution was washed with H_2O, dried over $MgSO_4$, and filtered. Evaporation of the solvent gave a solid. The solid was crystallized from a mixture of MeOH - ethyl acetate (20 : 1) to give colorless crystals (220 mg, 98%): MP >175°C (dec); $[\alpha]_D^{21}$ +124° (*c* 0.2, MeOH); IR (KBr) cm^{-1} 3330, 2980, 1700, 1660, 1590, 1560, 1480, 1410, 1380, 1370, 1340, 1320, 1305, 1250, 1210, 1175, 1140, 1120, 1090, 1040, 1000, 965, 905, 890, 860, 820; NMR (400 MHz, CD_3OD) δ 1.48 (9H, s, $OC(CH_3)_3$), 1.91 (3H, s, $NCOCH_3$), 3.78 (1H, broad d, J=20 Hz, 2-H), 4.06 (1H, m, 5-H), 4.46 (1H, dd, J=20 and 2 Hz, 2-H), 6.04 (1H, broad s, 6-H), 6.59 (1H, dt, J=6 and 2 Hz, 4-H); MS (SI) *m/z* 301 $(M+H)^+$, 245, 142, 75, 57.

N-(*tert*-Butoxycarbonyl)-3,4-didehydro-4-deoxysiastatin B Methyl Ester (**28**)

Compound **28** was obtained as a foamy glass from **27** by a similar procedure to that used for the preparation of **25** (99%): MP 90~91°C; $[\alpha]_D^{25}$ +103° (*c* 0.71, $CHCl_3$); IR (KBr) cm^{-1} 3440, 3070, 3000, 2950, 1720, 1665, 1550, 1485, 1445, 1420, 1400, 1380, 1335, 1280 (sh), 1250, 1170, 1140, 1085, 1055, 1000, 965, 950, 890 (sh), 875, 815; NMR (400 MHz, CD_3OD) δ 1.48 (9H, s, $OC(CH_3)_3$), 1.91 (3H, s, $NCOCH_3$), 3.74 (1H, broad d, J=19 Hz, 2-H), 3.78 (3H, s, $COOCH_3$), 4.13 (1H, broad d, J=4 Hz, 5-H), 4.42 (1H, dd, J=19 and 2 Hz, 2-H), 6.07 (1H, broad s, 6-H), 6.96 (1H, m, 4-H); MS (FAB, positive) *m/z* 315 $(M+H)^+$, 259, 138, 75, 57.

N-(*tert*-Butoxycarbonyl)-3,4-didehydro-4-deoxysiastatin B Amide (**29**)

Anhydrous ammonia was bubbled into a solution of **28** (151 mg) in MeOH (30 ml) under stirring for 1.5 hours, and then the mixture was stirred at room temperature overnight. Evaporation of the solvent gave an oil. The oil was subjected to preparative TLC on silica gel, developing with a mixture of $CHCl_3$ - MeOH - conc NH_4OH (20 : 10 : 3), to give a foamy glass (99 mg, 91%): MP 137~138°C; $[\alpha]_D^{21}$ +123° (*c* 0.76, MeOH); IR (KBr) cm^{-1} 3400, 2980, 2930, 1685, 1655, 1610, 1550, 1420, 1375, 1255, 1170, 1140, 1080, 1050, 995, 960, 860; NMR (400 MHz, CD_3OD) δ 1.49 (9H, s, $OC(CH_3)_3$), 1.92 (3H, s, $NCOCH_3$), 3.77 (1H, broad d with small couplings, J=19.6 Hz, 2-H), 4.12 (1H, broad, d, J=5.2 Hz, 5-H), 4.44 (1H, dd, J=19.6 and 2 Hz, 2-H), 6.05 (1H, broad s, 6-H), 6.65 (1H, dt, J=5.2 and 2 Hz, 4-H); MS (FAB, positive) *m/z* 300 $(M+H)^+$, 244, 207, 168, 141, 115, 75, 57, 45.

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