SYNTHESES AND ACTIVITIES OF *N*-SUBSTITUTED DERIVATIVES OF SIASTATIN B†

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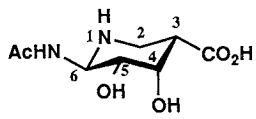
N-Substituted derivatives of siastatin B have been obtained by a chemical modification. Some derivatives showed potent inhibitory activities against *Streptococcus* sp. and *Clostridium perfringens* neuraminidases.

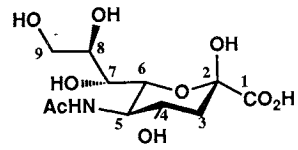
Most of the naturally occurring poly- or multifunctional piperidines are specific and potent inhibitors of glycosidases. They have many potential applications not only as molecular tools to investigate important biological processes but also as therapeutic agents such as antimetastatic, antitumor-poliferation, antiviral agents, *etc.*[1]

Such a multifunctional piperidine, siastatin B (**1**), was isolated as an inhibitor of neuraminidase by UMEZAWA *et al.*[2] from a *Streptomyces* culture. It inhibits neuraminidases isolated from microorganisms and animal tissues as well as *β*-glucuronidase and *N*-acetyl-*β*-D-glucosaminidase, and somewhat resembles structurally sialic acid (*N*-acetylneuraminic acid, **2**) (Fig. 1). After achievement of the total synthesis of **1**[3~5] and its analogues,[6~9] some *N*-(1,2-dihydroxypropyl) derivatives of **1** have been synthesized, and

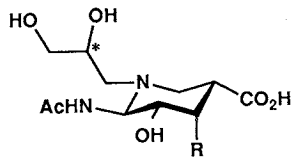
Fig. 1.



Siastatin B (**1**)



N-Acetylneuraminic acid (**2**)



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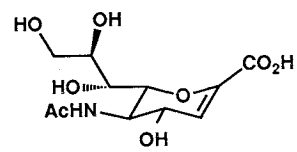
5 *=*S*, R=H
6 *=*R*, R=H



2,3-Didehydro-2-deoxy-*N*-acetylneuraminic acid (DDNA, **26**)

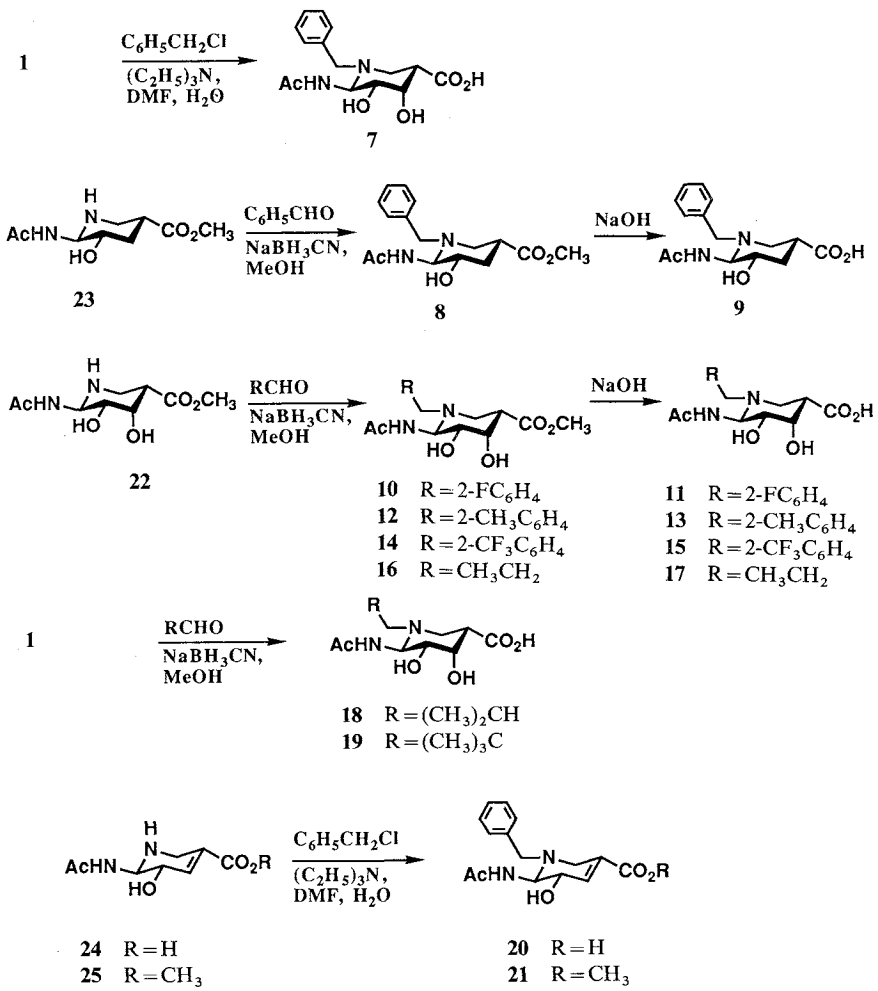
† Dedicated to the late Professor HAMAO UMEZAWA on the occasion of the 30th anniversary of the Institute of Microbial Chemistry.

N-[(2*S*)-1,2-dihydroxypropyl]siastatin B (**3**), *N*-[(2*R*)-1,2-dihydroxypropyl]siastatin B (**4**), *N*-[(2*S*)-1,2-dihydroxypropyl]-4-deoxysiastatin B (**5**) and *N*-[(2*R*)-1,2-dihydroxypropyl]-4-deoxysiastatin B (**6**), inhibitors of *Streptococcus* sp. and *Clostridium perfringens* neuraminidases, have been obtained. In this paper, the syntheses and the biological activities of *N*-substituted derivatives of siastrain B, 4-deoxysiastatin B and 3,4-didehydro-4-deoxysiastatin B (**7**～**21**) are presented.

Synthesis

In the course of our molecular graphics study[8,9] of the relationship between structure and biological activity among such inhibitors, we became interested in the substitution at the imino group of the piperidine ring which we believe interacts with the glycopyranosyl binding site to inhibit the enzymatic process. Thus, compounds **7**～**21** were prepared from **1**, its methyl ester (**22**), 4-deoxysiastatin B methyl ester[8] (**23**), 3,4-didehydro-4-deoxysiastatin B[8] (**24**) or its methyl ester[8] (**25**), by *N*-benzylation or reductive *N*-alkylation. Treatment of **1**, for example, with benzyl chloride and triethylamine in an aqueous *N*,*N*-dimethylformamide (DMF) solution gave **7** in 73% yield (Scheme 1). Compounds **20** and **21** were similarly prepared from **24**

Scheme 1.



and **25**. Reductive *N*-alkylation of **23** with benzaldehyde by sodium cyanoborohydride ($NaBH_3CN$) in methanol afforded **8** which was converted into **9** by alkaline hydrolysis in a good yield. Compounds **10**~**17** were also efficiently obtained from **22** and the corresponding aldehydes by the similar reaction sequences. On the other hand, compounds **18** and **19** were prepared by reductive *N*-alkylation from **1** with 2-methylpropanal and 2,2-dimethylpropanal, respectively, in good yields.

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

Compounds	*N*-Acetylneuraminidase	
	C. perfringens	*Streptococcus* sp.
1	18	6.29
7	3.5	3.96
9	3.5	0.58
11	1.5	4.93
13	11	1.39
15	68	16.82
17	20	0.78
18	25	5.35
19	240	15.14
20	50	14.55
26	12	1.97

Biological Activities

As shown in Table 1, compounds **7**, **9**, **11**, **13**, **15**, **17**, **18**, **19** and **20** showed inhibitory activity against *Streptococcus* sp. and *Clostridium perfringens* neuraminidases, whereas their methyl esters (**8**, **10**, **12**, **14**, **16** and **21**) did not inhibit these enzymes. Remarkably, compounds **9**, **13** and **17** strongly affected *Streptococcus* sp. neuraminidase more effectively than the well-known inhibitor, 2,3-didehydro-2-deoxy-*N*-acetylneuraminic acid[10] (DDNA, **26**). Compound **7** also inhibited yeast α-glucosidase and sweet potato β-amylase at IC_{50} values of 13.0 and 18.0 μg/ml, respectively. No other analogues showed inhibitory activity against glycosidases (α-glucosidase from yeast, β-glucosidase from almond, α-mannosidase from soybean, β-glucuronidase from bovine liver, α-amylase from porcine pancreas, β-amylase from sweet potato, α- and β-galactosidase from *Escherichia coli*, β-galactosidase from bovine liver, *N*-acetyl-α-galactosidase from chicken liver and *N*-acetyl-β-glucosaminidase from bovine liver). Further evaluation of the biological activities of these analogues are in progress.

Experimental

General Methods

Melting points were derermined with a Yanagimoto apparatus and are uncorrected. IR spectra were determined on a Hitachi Model 260-10 spectrophotometer. Optical rotations were measured with a Perkin-Elmer Model 241 polarimeter. ^1H NMR spectra were recorded with a 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 mode.

N-Benzylsiastatin B (7)

To a solution of siastatin B (**1**, 50 mg) in a mixture of DMF (3 ml) and H_2O (1 ml) were added triethylamine (0.5 ml) and benzyl chloride (0.2 ml), and 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 developed with a mixture of $CHCl_3$-MeOH-conc aq ammonia (20:10:3) to give a colorless amorphous solid of **7** (51.6 mg, 73%): $[\alpha]_D^{25}$ +3.2° (*c* 0.48, H_2O); IR (KBr) cm^{-1} 3400 (sh), 3150, 3050, 2825, 2600 (sh), 1730, 1690, 1550, 1410, 1290, 1230, 1170, 1140, 1110, 1040, 990, 960, 920, 885; ^1H NMR (400 MHz, D_2O) δ 2.05 (3H, s, $NCOCH_3$), 2.54 (1H, ddd, J=2.5, 4 and 12 Hz, 3-H), 2.75 (1H, t, J=12 Hz, 2-H_{ax}), 2.94 (1H, dd, J=4 and 12 Hz, 2-H_{eq}), 3.85 and 4.05 (2H, ABq, J=13 Hz, CH_2Ph), 3.61 (1H, d, J=9.4 Hz, 5-H), 4.37 (1H, t, J=2.5 Hz, 4-H), 4.51 (1H, d, J=9.4 Hz, 6-H), 7.35~7.45 (5H, m, Ph); MS (FAB, positive) *m/z* 309.2 $(M+H)^+$, 250.1, 207.1, 115.0, 75.0, 57.0.

N-Benzyl-4-deoxysiastatin B Methyl Ester (8)

To a solution of **23** (50 mg) in MeOH (1.3 ml) were added benzaldehyde (0.18 ml) and $NaBH_3CN$

(69 mg), and the mixture was stirred at room temperature overnight. Another portion of benzaldehyde (0.09 ml) and $NaBH_3CN$ (33 mg) were added to the mixture, and then the reaction mixture was further stirred at room temperature for 4 hours. Addition of water and evaporation of the solvent gave a solid, which was taken up in chloroform. Evaporation of the solvent gave a foam. The foam was subjected to preparative TLC developed with a mixture of $CHCl_3$ - MeOH - conc aq ammonia (20 : 10 : 3) to give a colorless amorphous solid of **8** (58 mg, 95.7%): $[\alpha]_D^{27}$ −29° (*c* 0.14, MeOH); IR (KBr) cm^{-1} 3425, 3275, 3100 (sh), 3050, 2970, 2900, 1740, 1650, 1580, 1505, 1465, 1450, 1400, 1385, 1350, 1340 (sh), 1310, 1270, 1250, 1205, 1185, 1165, 1140, 1100, 1090, 1070, 1030, 990, 970, 940; ^1H NMR (400 MHz, CD_3OD) δ 1.58 (1H, q, J=12 Hz, 4-H_{ax}), 1.98 (3H, s, $NCOCH_3$), 2.16 (1H, t, J=12 Hz, 2-H_{ax}), 2.27 (1H, dtd, J=2, 4 and 12 Hz, 4-H_{eq}), 2.57 (1H, tt, J=4 and 12 Hz, 3-H), 2.97 (1H, ddd, J=2, 4 and 12 Hz, 2-H_{eq}), 3.16 and 3.95 (2H, ABq, J=13.6 Hz, CH_2Ph), 3.45 (1H, ddd, J=4, 8 and 12 Hz, 5-H), 4.11 (1H, d, J=8 Hz, 6-H), 7.18～7.32 (5H, m, Ph); MS (FAB, positive) *m/z* 307.3 $(M+H)^+$, 248.2, 75, 57.

N-Benzyl-4-deoxysiastatin B (**9**)

To a solution of **8** (45 mg) in MeOH (2.3 ml) was added 1 M NaOH (0.59 ml), and the mixture was stirred at room temperature for 5 hours. Evaporation of the solvent gave a solid. The solid was subjected to preparative TLC on silica gel developed with a mixture of $CHCl_3$ - MeOH - conc aq ammonia (20 : 10 : 3) to give a colorless amorphous solid of **9** (43 mg, 100%): $[\alpha]_D^{25}$ −3.5° (*c* 0.79, MeOH); IR (KBr) cm^{-1} 3450, 3250, 1680, 1600, 1520, 1480, 1420, 1320, 1230, 1180, 1120, 1090 (sh), 1050 (sh), 980, 940, 910; ^1H NMR (400 MHz, CD_3OD) δ 1.64 (1H, dt, J=10.4 and 12.8 Hz, 4-H_{ax}), 1.97 (3H, s, $NCOCH_3$), 2.23 (1H, dtd, J=～2, 4 and 12.8 Hz, 4-H_{eq}), 2.27 (1H, dd, J=10 and 11.6 Hz, 2-H_{ax}), 2.42 (1H, tt, J=4 and 10 Hz, 3-H), 2.97 (1H, ddd, J=～2, 4 and 11.6 Hz, 2-H_{eq}), 3.22 and 3.89 (2H, ABq, J=13.6 Hz, CH_2Ph), 3.45 (1H, ddd, J=4, 8 and 10.4 Hz, 5-H), 4.19 (1H, d, J=8 Hz, 6-H), 7.15～7.34 (5H, m, Ph); MS (FAB, positive) *m/z* 293.2 $(M+H)^+$, 234.2, 75, 57.

N-(2-Fluorophenyl)methylsiastatin B Methyl Ester (**10**)

Compound **10** was obtained from **22** and 2-fluorobenzaldehyde by a similar procedure as was used for the preparation of **8** (yield 92.2%): $[\alpha]_D^{22}$ −22° (*c* 0.16, MeOH); IR (KBr) cm^{-1} 3340, 3070, 2975, 2930, 2840, 1950, 1660, 1595, 1550, 1500, 1470, 1440, 1420, 1400, 1390, 1360, 1345, 1325, 1300, 1280, 1260, 1230, 1220, 1180, 1150, 1120, 1110, 1105, 1090, 1080, 1030, 970, 950, 920, 900; ^1H NMR (400 MHz, CD_3OD) δ 1.99 (3H, s, $NCOCH_3$), 2.6～2.9 (3H, m, 2-H_2 and 3-H), 3.32 and 3.94 (2H, ABq, J=14 Hz, CH_2Ph), 3.39 (1H, dd, J=3 and 9 Hz, 5-H), 3.64 (3H, s, CO_2CH_3), 4.33 (1H, broad, d, J=3 Hz, 4-H), 4.47 (1H, d, J=9 Hz, 6-H), 7.0～7.5 (4H, m, Ph); MS (FAB, positive) *m/z* 341.2 $(M+H)^+$, 282.1, 180.1, 75, 57.

N-(2-Fluorophenyl)methylsiastatin B (**11**)

Compound **11** was obtained from **10** by a similar procedure to that used for the preparation of **9** (yield 91.1%): $[\alpha]_D^{22}$ −4.7° (*c* 0.97, MeOH); IR (KBr) cm^{-1} 3400, 1640, 1610, 1580, 1560, 1500, 1470, 1410, 1315, 1290, 1270, 1250, 1235, 1220, 1200, 1175, 1150, 1120, 1100, 1070, 960, 950, 930; ^1H NMR (400 MHz, CD_3OD) δ 1.99 (3H, s, $NCOCH_3$), 2.48 (1H, ddd, J=11, 4.5 and 3 Hz, 3-H), 2.72 (1H, t, J=11 Hz, 2-H_{ax}), 2.83 (1H, dd, J=11 and 4.5 Hz, 2-H_{eq}), 3.35 and 3.90 (2H, ABq, J=14 Hz, CH_2Ph), 3.38 (1H, dd, J=8 and 3 Hz, 5-H), 4.21 (1H, t, J=3 Hz, 4-H), 4.59 (1H, d, J=8 Hz, 6-H), 6.95～7.5 (4H, m, Ph); MS (FAB, positive) *m/z* 327 $(M+H)^+$, 268, 207, 109, 75, 57.

N-(2-Methylphenyl)methylsiastatin B Methyl Ester (**12**)

Compound **12** was obtained from **22** and 2-methylbenzaldehyde by a similar procedure to that used for the preparation of **8** (yield 85.6%): $[\alpha]_D^{23}$ −28° (*c* 0.31, MeOH); IR (KBr) cm^{-1} 3340, 2960, 2920, 2830, 1740, 1660, 1540, 1500, 1465, 1440, 1390, 1340, 1320, 1300, 1250, 1220, 1170, 1145, 1110, 1095, 1070, 1055, 1020, 965, 950, 940, 910; ^1H NMR (400 MHz, CD_3OD) δ 1.98 (3H, s, $NCOCH_3$), 2.32 (3H, s, CH_3), 2.56～2.78 (3H, m, 2-H_2 and 3-H), 3.13 and 3.98 (2H, ABq, J=13 Hz, CH_2Ph), 3.42 (1H, dd, J=3 and 9 Hz, 5-H), 4.32 (1H, broad t, J=3 Hz, 4-H), 4.45 (1H, d, J=9 Hz, 6-H), 7.04～7.30 (4H, m, Ph); MS (FAB, positive) *m/z* 337.2 $(M+H)^+$, 318.2, 105, 75, 57.

N-(2-Methylphenyl)methylsiastatin B (**13**)

Compound **13** was obtained from **12** by a similar procedure to that used for the preparation of **9** (yield 97.4%): $[\alpha]_D^{27}$ −15° (*c* 1.95, MeOH); IR (KBr) cm^{-1} 3400, 3250, 1690, 1635, 1610, 1590 (sh), 1570 (sh), 1550, 1470, 1410, 1380 (sh), 1340, 1310, 1290, 1210, 1170, 1150, 1110, 1090, 1050, 960, 925; ^1H NMR (400 MHz, CD_3OD) δ 1.99 (3H, s, $NCOCH_3$), 2.31 (3H, s, CH_3), 2.46 (1H, ddd, $J=3$, 4 and 11 Hz, 3-H), 2.68 (1H, t, $J=11$ Hz, 2-H_{ax}), 2.79 (1H, dd, $J=4$ and 11 Hz, 2-H_{eq}), 3.20 and 3.90 (2H, ABq, $J=13$ Hz, CH_2Ph), 3.40 (1H, dd, $J=3$ and 8 Hz, 5-H), 4.20 (1H, t, $J=3$ Hz, 4-H), 4.59 (1H, d, $J=8$ Hz, 6-H), 7.04~7.30 (4H, m, Ph); MS (FAB, positive) *m/z* 323.2 $(M+H)^+$, 264.1, 105.1, 75, 57.

N-(2-Trifluoromethylphenyl)methylsiastatin B Methyl Ester (**14**)

Compound **14** was obtained from **22** and 2-trifluorobenzaldehyde by a similar procedure to that used for the preparation of **8** (yield 89.8%): $[\alpha]_D^{25}$ −12° (*c* 0.47, MeOH); IR (KBr) cm^{-1} 3370, 2980, 2950, 2860, 1750, 1670, 1550, 1450, 1390, 1325, 1300, 1255, 1230, 1180, 1170, 1150, 1130, 1120, 1080, 1070, 1050, 1030, 980, 970, 915; ^1H NMR (400 MHz, CD_3OD) δ 1.95 (3H, s, $NCOCH_3$), 2.61~2.76 (3H, m, 2-H_2 and 3-H), 3.46 (1H, dd, $J=3$ and 8 Hz, 5-H), 3.46 and 4.06 (2H, ABq, $J=15$ Hz, CH_2Ph), 3.64 (3H, s, $COOCH_3$), 4.36 (1H, t, $J=3$ Hz, 4-H), 4.52 (1H, d, $J=8$ Hz, 6-H), 7.34~7.88 (4H, m, Ph); MS (FAB, positive) *m/z* 389.1 $(M+H)^+$, 332.1, 230.1, 159.1.

N-(2-Trifluoromethylphenyl)methylsiastatin B (**15**)

Compound **15** was obtained from **14** by a similar procedure to that used for the preparation of **9** (yield 100%): $[\alpha]_D^{23}$ −2.2° (*c* 1.96, MeOH); IR (KBr) cm^{-1} 3420 (sh), 3350, 2950, 2860, 1645, 1580, 1560, 1420, 1320, 1300, 1185, 1150 (sh), 1145 (sh), 1130, 1090, 1070, 1050, 960, 930, 910; ^1H NMR (400 MHz, CD_3OD) δ 1.95 (3H, s, $NCOCH_3$), 2.56 (1H, ddd, $J=3$, 6 and 11.5 Hz, 3-H), 2.65~2.80 (2H, m, 2-H_2), 3.46 (1H, dd, $J=3$ and 8 Hz, 5-H), 3.50 and 4.00 (2H, ABq, $J=15$ Hz, CH_2Ph), 4.27 (1H, t, $J=3$ Hz, 4-H), 4.62 (1H, d, $J=8$ Hz, 6-H), 7.3~8.0 (4H, m, Ph); MS (FAB, positive) *m/z* 377.2 $(M+H)^+$, 318.2, 159.1, 75, 57.

N-Propylsiastatin B Methyl Ester (**16**)

Compound **16** was obtained from **22** and propanal by a similar procedure to that used for the preparation of **8** (yield 64.3%): $[\alpha]_D^{29}$ −17° (*c* 0.23, MeOH); IR (KBr) cm^{-1} 3500 (sh), 3430, 3300, 2975, 2880, 2850, 1750, 1740, 1725, 1710, 1660, 1560, 1440, 1380, 1340, 1320, 1300 (sh), 1290, 1275, 1225, 1210, 1185, 1170, 1130, 1110, 1060, 1010, 980, 950, 930, 920, 910; ^1H NMR (400 MHz, D_2O) δ 0.85 (3H, t, $J=7$ Hz, CH_3), 2.09 (3H, s, $NCOCH_3$), 1.3~1.7 (2H, m, CCH_2C), 2.31 and 2.65 (each 1H, dt, $J=5$ and 12 Hz, NCH_2), 2.72 (1H, t, $J=13$ Hz, 2-H_{ax}), 2.89 (1H, ddd, $J=3$, 4 and 13 Hz, 3-H), 3.10 (1H, dd, $J=4$ and 13 Hz, 2-H_{eq}), 3.49 (1H, dd, $J=3$ and 10 Hz, 5-H), 4.35 (1H, d, $J=10$ Hz, 6-H), 4.45 (1H, t, $J=3$ Hz, 4-H); MS (FAB, positive) *m/z* 275.2 $(M+H)^+$, 216.2, 114.1, 72.1.

N-Propylsiastatin B (**17**)

Compound **17** was obtained from **16** by a similar procedure to that used for the preparation of **9** (yield 82%): $[\alpha]_D^{29}$ −2.9° (*c* 0.44, H_2O); IR (KBr) cm^{-1} 3450, 3220, 2970, 2880, 2730, 1690, 1670, 1610, 1550, 1390, 1340, 1310, 1270, 1240, 1215, 1160, 1140, 1110, 1060, 1010, 970, 920; ^1H NMR (400 MHz, D_2O with a few drops of pyridine-d_5) δ 0.74 (3H, t, CH_3), 1.24~1.56 (2H, m, CH_2), 2.01 (3H, s, $NCOCH_3$), 2.20 and 2.55 (each 1H, ddd, $J=5$, 11 and 13 Hz, NCH_2), 2.50 (1H, ddd, $J=3$, 4 and 12 Hz, 3-H), 2.63 (1H, t, $J=12$ Hz, 2-H_{ax}), 2.92 (1H, dd, $J=4$ and 12 Hz, 2-H_{eq}), 3.45 (1H, dd, $J=3$ and 9.5 Hz, 5-H), 4.33 (1H, t, $J=3$ Hz, 4-H), 4.34 (1H, d, $J=9.5$ Hz, 6-H); MS (FAB, positive) *m/z* 261.2 $(M+H)^+$, 202.1, 75, 72.1, 57.

N-2-Methylpropylsiastatin B (**18**)

Compound **18** was obtained from **1** and 2-methylpropanal by a similar procedure to that used for the preparation of **8** (yield 77.2%): $[\alpha]_D^{29}$ −11° (*c* 0.4, H_2O); IR (KBr) cm^{-1} 3400, 3350, 3320, 3260, 3225, 2975, 1700, 1620, 1550, 1470, 1410, 1390, 1350, 1310, 1270, 1250, 1220, 1170, 1140, 1115, 1065, 1010, 980, 955, 930; ^1H NMR (400 MHz, D_2O with a few drops of pyridine-d_5) δ 0.76 and 0.78 (each 3H, d, $J=7$ Hz, $CH_3\times2$), 1.67 (1H, m, $CH(CH_3)_2$), 2.00 (3H, s, $NCOCH_3$), 2.13 and 2.30 (2H, dd, $J=5$ and 13,

J=9 and 13 Hz, CH_2), 2.48~2.62 (2H, m, 2-H and 3-H), 2.88~3.00 (1H, m, 2-H), 3.47 (1H, dd, J=3 and 9 Hz, 5-H), 4.28 (1H, d, J=9 Hz, 6-H), 4.31 (1H, t, J=3 Hz, 4-H); MS (FAB, positive) m/z 275.2 $(M+H)^+$, 216.2, 143.1, 128.1, 86.1, 57.1.

N-(2,2-Dimethylpropyl)siastatin B (**19**)

Compound **19** was obtained from **1** and 2,2-dimethylpropanal by a similar procedure to that used for the preparation of **8** (yield 49.4%): $[\alpha]_D^{25}$ +13° (c 0.61, H_2O); IR (KBr) cm^{-1} 3420, 3200, 1680, 1640, 1600, 1490, 1410, 1170, 1140, 1110, 1040, 1000, 980, 940, 920; ^1H NMR (400 MHz, D_2O with a few drops of pyridine-d_5) δ 0.84 (9H, s, $CH_3 \times 3$), 2.07 (3H, s, $NCOCH_3$), 2.11 and 2.30 (2H, ABq, J=15 Hz, CH_2), 2.70 (1H, dt, J=3.5 and 11 Hz, 3-H), 2.85 (1H, t, J=13 Hz, 2-H_{ax}), 3.04 (1H, dd, J=3.5 and 13 Hz, 2-H_{eq}), 3.57 (1H, dd, J=3.5 and 8 Hz, 5-H), 4.34 (1H, br s, 4-H), 4.47 (1H, d, J=8 Hz, 6-H); MS (FAB, positive) m/z 289.2 $(M+H)^+$, 230.2, 142.2, 100.1, 71.1, 57.

N-Benzyl-3,4-didehydro-4-deoxysiastatin B (**20**) and its Methyl Ester (**21**)

Compounds **20** and **21** were obtained in yields of 65 and 78%, respectively from **24** and **25** by the similar procedures to those used for the preparation of **7** from **1**.

20: $[\alpha]_D^{22}$ +94° (c 0.24, H_2O); ^1H NMR (400 MHz, D_2O) δ 2.02 (3H, s, $NCOCH_3$), 3.37 (1H, dt, J=1.5 and 18 Hz, 2-H), 3.54 (1H, dt, J=1.5 and 18 Hz, 2-H), 3.78 and 3.90 (2H, ABq, J=13 Hz, CH_2), 4.14 (1H, td, J=1.5 and 3 Hz, 5-H), 4.80 (1H, br s, 6-H), 6.55 (1H, dt, J=1.5 and 4 Hz, 4-H), 7.3~7.7 (5H, m, Ph).

21: $[\alpha]_D^{24}$ +131° (c 0.55, H_2O); ^1H NMR (400 MHz, CD_3OD) δ 1.97 (3H, s, $NCOCH_3$), 3.17 (1H, dt, J=2 and 18 Hz, 2-H), 3.37 (1H, dt, J=1.5 and 18 Hz, 2-H), 3.65 and 3.78 (2H, ABq, J=14 Hz, CH_2), 3.72 (3H, s, CO_2CH_3), 3.97 (1H, m, 5-H), 4.91 (1H, d, J=3 Hz, 6-H), 6.88 (1H, dt, J=2 and 5 Hz, 4-H), 7.2~7.4 (5H, m, Ph).

Siastatin B Methyl Ester (**22**)

A solution of **1** (487 mg) in dry MeOH (15 ml) was stirred with Amberlist 15 (H^+) (500 mg) at room temperature for 1 day. After addition of conc aq ammonia (pH ~9), the resin was filtered off. Evaporation of the filtrate gave a solid, which was subjected to the column chromatography on silica gel. Elution with a mixture of $CHCl_3$ - MeOH - conc aq ammonia (20 : 10 : 3) gave a colorless solid of **22** (434 mg, 83.7%). The solid was crystallized from MeOH to give colorless crystals: MP 177~178°C; $[\alpha]_D^{25}$ +18° (c 0.59, H_2O); IR (KBr) cm^{-1} 3530, 3380, 3300, 3130, 3000, 2960, 2930, 1770, 1750, 1670, 1585, 1465, 1450, 1400, 1365, 1345, 1320, 1295, 1285, 1265, 1250, 1220, 1185, 1155, 1145, 1110, 1095, 1065, 1030, 1000, 980, 960, 930, 910; ^1H NMR (400 MHz, D_2O) δ 2.44 (3H, s, $NCOCH_3$), 3.85 (1H, ddd, J=2, 6 and 11 Hz, 3-H), 3.0~3.15 (2H, m, 2-H_2), 3.55 (1H, dd, J=2 and 10 Hz, 5-H), 3.75 (3H, s, $COOCH_3$), 4.49 (1H, t, J=2 Hz, 4-H) and 4.61 (1H, d, J=10 Hz, 6-H); MS (FAB, positive) m/z 233.2 $(M+H)^+$, 174.1, 75, 57.

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