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Note

A practical synthesis from siastatin B of (3*S*,4*S*,5*R*,6*R*)-4,5-dihydroxy-6-(trifluoroacetamido)piperidine-3-carboxylic acid having antimetastatic activity in mice

Takahiko Satoh, Yoshio Nishimura *, Shinichi Kondo, Tomio Takeuchi

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

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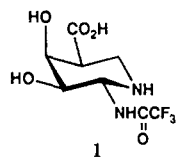
In the course of our studies [1] investigating the relationship between the structure and biological activity of glycosidase inhibitors, we previously synthesized (3*S*,4*S*,5*R*,6*R*)-4,5-dihydroxy-6-(trifluoroacetamido)piperidine-3-carboxylic acid (**1**) [2]



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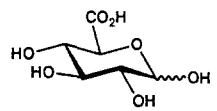
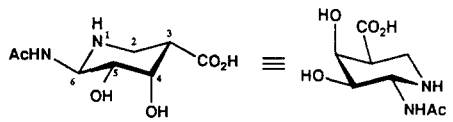


Glucuronic acid (2)



Siastatin B (3)

* Corresponding author.

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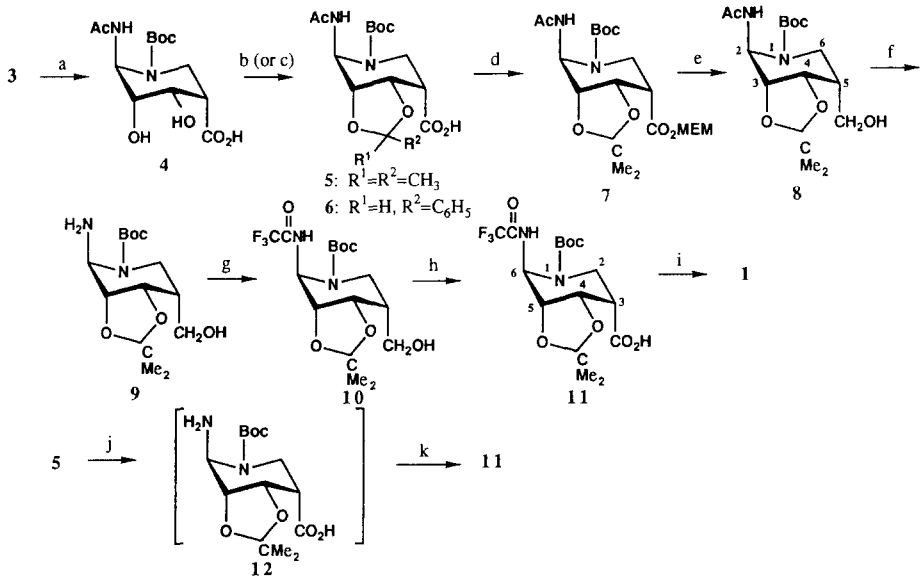

Scheme 1. (a) Ref. [1]e, 91%. (b) $(MeO)_2CMe_2$, Me_3SiCl, 98%. (c) $C_6H_5(OMe)_2$, Me_3SiCl, 92%. (d) MEMCl, i-Pr_2NEt, 83%. (e) $NaBH_4$, THF–CF_3CH_2OH, 99%. (f) $H_2NNH_2 \cdot xH_2O$, 54% (conversion yield, 80%). (g) CF_3CO_2Et, i-Pr_2NEt, 81%. (h) RuO_2, $NaIO_4$, CCl_4–CH_3CN–H_2O, 74%. (i) Ref. [2], 97%. (j) $H_2NNH_2 \cdot xH_2O$, 51%. (k) CF_3CO_2Et, i-Pr_2NEt, 4%.

from L-ribose as a 1-*N*-imino sugar inhibitor of glucosiduronase. Compound **1** has been shown to have marked inhibitory activity against β-glucosiduronase and exhibits potent inhibition of experimental pulmonary metastasis of the highly metastatic B16 line (B16 BL6) [2]. The structure and shape of **1** is highly reminiscent of glucuronic acid (**2**). Compound **1** may mimic **2** in ground-state binding to β-glucosiduronase, resulting in the strong inhibition of the enzyme.

The initial synthesis [2] requires multiple (17) steps, and the total yield is low (~ 1%), scarcely providing sufficient material for biological evaluation in in vivo assays. These facts prompted us to develop a practical synthesis of **1** utilizing siastatin B (**3**) [3]. Ketal or acetal formation between the 4-OH and 5-OH groups in **4** [1]e with 2,2-dimethoxypropane or benzaldehyde dimethyl acetal, using such acids as *p*-toluenesulfonic acid proved troublesome until we discovered that transketalization or transacetalization using chlorotrimethylsilane [4] gave the ketal **5**, or the acetal **6**, respectively in good yields. Removal of the *N*-acetyl group was best achieved by heating a solution of **5** in hydrazine hydrate [5] at 70 °C for 7 days to give the amino acid **12** (51% yield). Attempts at transforming **12** into trifluoroacetamide **11** failed due to the instability and the lack of reactivity of **12**. An alternative route was therefore devised.

The amino alcohol **9** was obtained from **5** by a sequence of esterification with (2-methoxyethoxy)methyl chloride, hydride reduction ($NaBH_4$, CF_3CH_2OH–THF) [7], and treatment with hydrazine hydrate (Scheme 1). The amino alcohol **9** smoothly underwent trifluoroacetylation with ethyl trifluoroacetate [6] (DMF, i-Pr_2NEt, 81%) to give the trifluoroacetamide **10**. Various attempts to oxidize the hydroxymethyl group in

10 led to the corresponding carboxylic acid in a low yield at best. This problem was circumvented by ruthenium tetraoxide catalyzed oxidation in the solvent system of CH_3CN–CCl_4–H_2O developed by Sharpless and co-workers [8]. The acid **11** was straightforwardly transformed into **1** by a method (HCl, 1,4-dioxane) similar to that reported previously by us [2]. Thus, **1** was efficiently obtained from **3** in a total yield of 22% (conversion yield, 32%).

Compound **1** thus obtained inhibited the invasion of B16 BL6 and Lewis lung carcinoma (3LL) cells into the reconstituted basement membranes and significantly suppressed spontaneous lung metastasis of 3LL cell by multiple i.v. administrations after the excision of primary tumors in mice [9].

This strategy should be an efficient and practical approach to various structural and biological types of imino sugar inhibitor of glycosidase enzymes. The relationship between structure and biological activity through the chemical modification of siastatin B based on this strategy is currently under further investigation.

1. Experimental

General.—Melting points were determined 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. ^{1}H NMR spectra were recorded with Jeol JNM EX 270 and 400 spectrometers. Chemical shifts are expressed in δ values (ppm) with tetramethylsilane as an internal standard. Mass spectra were taken with a Jeol JMS-SX102 in the FAB mode.

N-(tert-*Butoxycarbonyl*)-4,5-O-*isopropylidenesiastatin B* (**5**).—To a suspension of **4** (955 mg, 3 mmol) in DMF (15 mL) were added 2,2-dimethoxypropane (3.69 mL, 30 mmol) and chlorotrimethylsilane (1.9 mL, 15 mmol), and the mixture was stirred for 2 h at room temperature. After being quenched with pyridine (1.5 mL), the mixture was diluted with $CHCl_3$. The solution was washed with water and the aqueous phase was extracted three times with a 9:1 $CHCl_3$–MeOH. The organic phases were combined, dried over $MgSO_4$, and filtered. The filtrate was evaporated to give an oil, which was subjected to column chromatography on silica gel. Elution with 20:10:3 $CHCl_3$–MeOH–conc. aq NH_4OH gave **5** as a foam (1.05 g, 98%): $[\alpha]_D^{23}$ +29° (*c* 0.88, MeOH); IR (KBr) 3300 (br), 3025, 2980, 1700 (br), 1610 (s), 1560 (br), 1490, 1480, 1420 (br), 1280, 1235, 1180, 1095, 1015, 980 cm^{-1}; ^{1}H NMR (CD_3OD, 270 MHz): δ 1.32 and 1.38 (3 H, s each, isopropylidene), 1.46 (9 H, s, CO_2Bu^t), 1.94 (3 H, s, $COCH_3$), 2.96 (1 H, ddd, *J* 2.6, 5.1, and 12.5 Hz, H-3), 3.43 (1 H, t, *J* 12.5 Hz, H-2ax), 3.60 (1 H, dd, *J* 5.1 and 12.5 Hz, H-2eq), 4.47 (1 H, d, *J* 2.3 and 7.6 Hz, H-5), 4.80 (1 H, dd, *J* 2.6 and 7.6 Hz, H-4) and 5.78 (1 H, d, *J* 2.3 Hz, H-6); FABMS *m/z* 359 $(M + H)^+$. HRMS calcd for $C_{13}H_{22}N_2O_7$: (M + H), 359.1818. Found: 359.1814.

N-(tert-*Butoxycarbonyl*)-4,5-O-*benzylidenesiastatin B* (**6**).—To a suspension of **4** (19 mg, 60 μmol) in DMF (0.4 mL) were added (dimethoxymethyl)benzene (95 μL, 0.6 mmol) and chlorotrimethylsilane (15.2 μL, 0.12 mmol), and the mixture was stirred for 3 h at room temperature. After being quenched with pyridine (20 μL), the mixture was diluted with chloroform. The solution was washed with water. The aqueous phase was

extracted three times with a 9:1 $CHCl_3$–MeOH. The organic phases were combined, dried over $MgSO_4$, and filtered. The filtrate was evaporated to give a solid which was subjected to preparative TLC on silica gel developed with 20:10:3 $CHCl_3$–MeOH conc. aq NH_4OH to give **6** (22.5 mg, 92%) as an amorphous solid; $[\alpha]_D^{24}$ +39.9° (*c* 1.0, MeOH); IR (KBr) 3500 (br), 3300 (br), 3100, 3020, 2970, 1710 (br), 1610 (br), 1565 (br), 1495, 1485, 1420 (br), 1395, 1365, 1340 (sh), 1310 (sh), 1270, 1240, 1190, 1115, 1090, 1045, 990 (br), 955 (sh), 930 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz): δ 1.47 (9 H, broad s, $COOC(CH_3)_3$), 1.97 (3 H, s, $COCH_3$), 3.02 (1 H, broad t with small couplings, *J* ~ 8 Hz, H-3), 3.50–3.75 (2 H, m, H-2), 4.49 (1 H, broad d with small couplings, *J* ~ 5.9 Hz, H-5), 5.73 (1 H, s, –C*H*Ph), 5.91 (1 H, d, *J* 1.96 Hz, H-6) and 7.35–7.55 (5H, m, Ph); FABMS *m/z* 429 $(M + Na)^+$, 407 $(M + H)^+$. HRMS calcd for $C_{19}H_{26}N_2O_7$: (M–H), 405.1662. Found: 405.1664.

N-(tert-*Butoxycarbonyl*)-4,5-O-*isopropylidenesiastatin B 2-methoxyethoxymethyl ester* (**7**).—To a solution of **5** (2.51 g, 7 mmol) in DMF (50 mL) were added *N,N*-diisopropylethylamine (4.88 mL, 28 mmol) and (2-methoxyethoxy)methyl chloride (1.6 mL, 14 mmol), and the mixture was stirred for 2 h at room temperature. After being quenched with water (0.2 mL), the mixture was diluted with $CHCl_3$. The solution was washed with water and the aqueous phase was extracted three times with $CHCl_3$. The organic phases were combined, dried over $MgSO_4$, and filtered. The filtrate was evaporated to give an oil, which was subjected to column chromatography on silica gel. Elution with 20:1 CH_2Cl_2–MeOH gave **7** (2.61 g, 83%) as a colorless oil: $[\alpha]_D^{25}$ −18.7° (*c* 0.91, $CHCl_3$); IR ($CHCl_3$) 3000, 2950, 1750 (br), 1685 (s), 1530 (s), 1490 (br), 1460, 1395 (s), 1380, 1350, 1320, 1280 (br), 1260 (br), 1170, 1120, 1075, 1000, 980, 945, 915 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.32 and 1.44 (3 H, s each, isopropylidene), 1.47 (9 H, s, $COOC(CH_3)_3$), 1.98 (3 H, s, $COCH_3$), 2.99 (1 H, broad m, H-3), 3.37 (1 H, t, *J* 12.7 Hz, H-2ax), 3.39 (3 H, s, OCH_3), 3.56 (2 H, t, *J* 4.4 Hz, OCH_2–CH_2–OCH_3), 3.76–3.82 (3 H, m, O–CH_2–CH_2OCH_3 and H-2eq), 4.69 (1 H, broad d, *J* 7 Hz, H-5), 4.82 (1 H, broad dd, *J* 3 and 7 Hz, H-4), 5.40 (2 H, s, $COOCH_2O$), 5.59 (1 H, broad s, H-6) and 5.91 (1 H, broad s, –NH); FABMS *m/z* 447 $(M + H)^+$. Anal. Calcd for $C_{20}H_{34}N_2O_9$: C, 53.80; H, 7.68; N, 6.27%. Found: C, 54.21; H, 7.93; N, 6.28%.

(2S,3R,4S,5R)-2-*Acetamido*-N-(tert-*butoxycarbonyl*)-5-*hydroxymethyl*-3,4-O-*isopropylidene*-3,4-*piperidinediol* (**8**).—To a solution of **7** (2.59 g, 5.8 mmol) in a mixture of tetrahydrofuran (50 mL) and 2,2,2-trifluoroethanol (5 mL) was added $NaBH_4$ (685 mg, 17.4 mmol), and the mixture was stirred for 1 h at room temperature. After being quenched with water (1.5 mL), evaporation of the solvent gave an oil. The oil was dissolved in $CHCl_3$, and the solution was washed with water, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave an oil, which was subjected to column chromatography on silica gel. Elution with 12:1 $CHCl_3$–MeOH gave **8** (1.98 g, 99%) as a foamy solid: $[\alpha]_D^{25}$ +26.9° (*c* 0.95, $CHCl_3$); IR ($CHCl_3$) 3460, 3330 (br), 3000, 2950, 1685, 1540 (s), 1500, 1485, 1465, 1400 (s), 1390, 1375, 1360, 1330, 1260 (s), 1175, 1145, 1120, 1070, 1010, 960 (br) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.33 and 1.45 (3 H, s each, isopropylidene), 1.46 (9 H, s, $COOC(CH_3)_3$), 1.98 (3 H, s, $COCH_3$), 2.06 (1 H, m, H-5), 2.22 (1 H, t, *J* 5.6 Hz, OH), 3.16 (1 H, t, *J* 12.4 Hz, H-6ax), 3.50 (1 H, dd, *J* 3.7 and 12.4 Hz, H-6eq), 3.72–3.80 (2 H, m, $-CH_2OH$), 4.52 (1 H, d, *J* 2.4 and 7.3 Hz, H-3), 4.59 (1 H, broad d, *J* 7.3 Hz, H-4), 5.73 (1 H, broad s, H-2) and 5.84 (1

H, broad s, –NH); FABMS m/z 345 $(M + H)^+$. Anal. Calcd for $C_{16}H_{28}N_2O_6$: C, 55.79; H, 8.19; N, 8.13%. Found: C, 55.85; H, 8.39; N, 8.20%.

*(2S,3R,4S,5R)-2-Amino-*N*-(*tert*-butoxycarbonyl)-5-hydroxymethyl-3,4-O-isopropylidene-3,4-piperidinediol* (**9**).—Compound **8** (1.03 g, 3 mmol) was dissolved in hydrazine hydrate ($H_2NNH_2 \cdot xH_2O$, 20 mL), and the solution was stirred for 10 days at 70 °C. 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. The filtrate was evaporated to give an oil, which was subjected to column chromatography on silica gel. Elution with 50:1 EtOAc–MeOH gave an oil. The oil was further subjected to the preparative TLC on silica gel developed with 10:1 EtOAC–MeOH to give **9** (488 mg, 54%) as an oil and the starting **8** (332 mg, 32%). **9**: $[\alpha]_D^{26}$ −14.4° (*c* 0.99, $CHCl_3$); IR ($CHCl_3$) 3400 (br), 3010, 2905, 1690 (s), 1390, 1370, 1430, 1400 (s), 1390, 1380 (s), 1370, 1335, 1315, 1260, 1170, 1120, 1070, 1010, 960 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.35 and 1.45 (3 H, s each, isopropylidene), 1.48 (9 H, s, $COOC(CH_3)_3$), 2.40 (1 H, m, H-5), 3.19 (1 H, t, *J* 12.2 Hz, H-6ax), 3.46 (1 H, dd, *J* 3.9 and 12.2 Hz, H-6eq), 3.71–3.80 (2 H, m, $-CH_2OH$), 4.35 (1 H, dd, *J* 2.9 and 7.3 Hz, H-3), 4.57 (1 H, dd, *J* 2.0 and 7.3 Hz, H-4) and 5.08 (1 H, broad s, H-2); FABMS m/z 303 $(M + H)^+$. HRMS calcd for $C_{15}H_{26}N_2O_5$: (M + H), 303.1920. Found 303.1917.

*(2S,3R,4S,5R)-*N*-(*tert*-Butoxycarbonyl)-5-hydroxymethyl-3,4-O-isopropylidene-2-(trifluoroacetamido)-3,4-piperidinediol* (**10**).—To a solution of **9** (407 mg, 1.35 mmol) in DMF (8 mL) were added *N,N*-diisopropylamine (2.35 mL, 13.5 mmol) and ethyl trifluoroacetate (1.61 mL, 13.5 mmol), and the mixture was stirred for 36 h at 60 °C. After dilution with $CHCl_3$, the solution was washed with water. The aqueous phase was extracted with $CHCl_3$ and the organic phases were combined, dried over MgSO4, and filtered. The filtrate was evaporated to give an oil, which was subjected to column chromatography on silica gel. Elution with 5:1 toluene–acetone gave **10** (434 mg, 81%) as an oil: $[\alpha]_D^{25}$ +26° (*c* 0.94, $CHCl_3$); IR ($CHCl_3$) 3440, 3000, 2950, 1740, 1710 (s), 1550, 1520, 1480, 1460, 1400 (s), 1390, 1380, 1370 (s), 1320 (br), 1265, 1235, 1180, 1120, 1075, 1050 (s), 1000, 960 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.35 and 1.47 (3 H, s each, isopropylidene), 1.46 (9 H, s, $COOC(CH_3)_3$), 2.02 (1 H, m, H-5), 2.15 (1 H, t, *J* 5.6 Hz, –OH), 3.14 (1 H, t, *J* 12.5 Hz, H-6ax), 3.57 (1 H, dd, *J* 4.2 and 12.5 Hz, H-6eq), 3.73–3.82 (2 H, m, $-CH_2OH$), 4.56 (2 H, broad s, H-3 and H-4), 5.78 (1 H, broad s, H-2) and 6.72 (1 H, broad s, –NH); FABMS m/z 399 $(M + H)^+$. Anal. Calcd for $C_{16}H_{25}N_2O_6F_3$: C, 48.02; H, 6.34; N, 7.00%. Found: C, 48.35; H, 6.63; N, 7.24%.

*(3S,4S,5R,6S)-*N*-(*tert*-Butoxycarbonyl)-4,5-dihydroxy-4,5-O-isopropylidene-6-(trifluoroacetamido)piperidine-3-carboxylic acid* (**11**).—To a solution of **10** (398 mg, 1 mmol) in a mixture of CCl_4 (6 mL) and CH_3CN (6 mL) were added a solution of $NaIO_4$ (642 mg, 3 mmol) in water (9 mL) and RuO_2 (4 mg, 0.03 mmol), and the mixture was vigorously stirred for 2 h at room temperature. The phases were separated. The aqueous phase was extracted three times with EtOAc. To the combined organic extracts was added 2-propanol (0.5 mL), and the mixture was stirred at room temperature for 1 h. The mixture was washed with water, dried over $MgSO_4$, and filtered. Evaporation of the filtrate gave a solid, which was subjected to column chromatography on silica gel. Elution with 30:10:1 $CHCl_3$–MeOH–conc. aq ammonia gave **11** (318 mg, 77%) as an amorphous solid: mp 200 °C (dec.) (lit. [2]) 200 °C (dec.); $[\alpha]_D^{23}$ +29° (*c* 0.90, MeOH)

(lit. [2] +28° (*c* 0.24, MeOH)); IR (KBr) 3450 (br), 3250 (br), 3000, 2960, 1720 (br), 1690 (br), 1600 (s), 1570 (br), 1490, 1470, 1410, 1380 (s), 1370, 1340, 1280, 1240, 1195, 1115, 1085, 1060 (s), 1010, 965, 920 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz) δ 1.34 and 1.40 (3 H, s each, isopropylidene), 1.47 (9 H, s, $COOC(CH_3)_3$), 2.96 (1 H, ddd, *J* 2.4, 4.2 and 11.7 Hz, H-3), 3.41 (1 H, broad t, *J* 11.7 Hz, H-2ax), 3.64 (1 H, dd, *J* 4.2 and 11.7 Hz, H-2eq), 4.50 (1 H, dd, *J* 2.0 and 7.8 Hz, H-5), 4.83 (1 H, dd, *J* 2.4 and 7.8 Hz, H-4) and 5.78 (1 H, d, *J* 2.0 Hz, H-6); FABMS *m/z* 413 $(M + H)^+$. HRMS calcd for $C_{17}H_{23}N_2O_7F_3$: (M + H), 413.1536. Found 413.1523.

(3S,4S,5R,6R)-4,5-Dihydroxy-6-(trifluoroacetamido)piperidine-3-carboxylic acid (**1**).—Compound **11** (153 mg) was dissolved in HCl in 1,4-dioxane (4 M, 5 mL), and the mixture was kept at room temperature overnight. The resulting precipitate was removed by filtration and washed with 1,4-dioxane to give a colorless amorphous solid of **1** as its hydrochloride (111 mg, 97%): mp 130 °C (dec.) (lit. [2] 130 °C (dec.)); $[\alpha]_D^{23}$ +28° (*c* 0.51, H_2O) (lit. [2] +27° (*c* 0.22, H_2O)); IR and ^1H NMR spectra were identical with those of the authentic sample [2].

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