Total Synthesis of the Trehalase Inhibitor Salbostatin

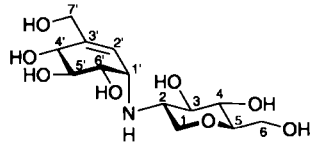
Tatsuya Yamagishi, Chikara Uchida, and Seiichiro Ogawa*

Abstract: The total synthesis of trehalase inhibitor salbostatin (**1**), recently discovered as a novel metabolite of *Streptomyces albus* species, has been achieved starting from the major product (58% yield) from the coupling of the amine di-*O*-isopropylidene-α-valienamine and the electrophile 1,5:2,3-dianhydro-D-mannitol in 2-propanol. Deprotection with aqueous acetic acid and subsequent purification on a column of acidic resin afforded **1**, which showed inhibitory activity ($IC_{50} = 8.3\ \mu M\ L^{-1}$) against silkworm trehalase.

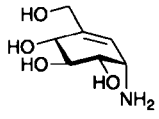
Keywords
carbasugars · cyclitols · enzyme inhibitors · salbostatin · total syntheses

Introduction

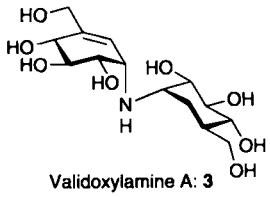
Very recently, new trehalase inhibitor salbostatin (**1**) was discovered[1] as a novel metabolite of *Streptomyces albus*, ATCC21838, and the structure has been established mainly on the basis of ^1H NMR spectroscopic data.[2] Salbostatin inhibits trehalase from porcine kidneys with an inhibition constant K_i of 1.8×10^{-7} M, and possesses a very unique pseudo-disaccharide structure composed of 2-amino-1,5-anhydro-2-deoxy-D-glucitol attached by way of an imino bridge to an unsaturated 5a-carbasugar,[**] the α-valienamine (**2**) residue. Similar pseudo-disaccharidic glycosidase inhibitors containing **2**, namely, validoxylamine A (**3**)[3] and methyl acarviosin (**4**),[4] a core component of acarbose, are known. The former is a potent trehalase inhibitor and its dihydro derivative,[3] most probably mimicking the α,α-trehalose structure, also exhibits high inhibitory activity. The latter is a strong α-glucosidase inhibitor. In both cases, the valienamine portions are thought to be mimics of the glucopyranosyl cations proposed to be formed during hydrolysis of glycosides.



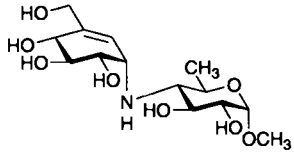
Salbostatin: **1**



α-Valienamine: **2**



Validoxylamine A: **3**

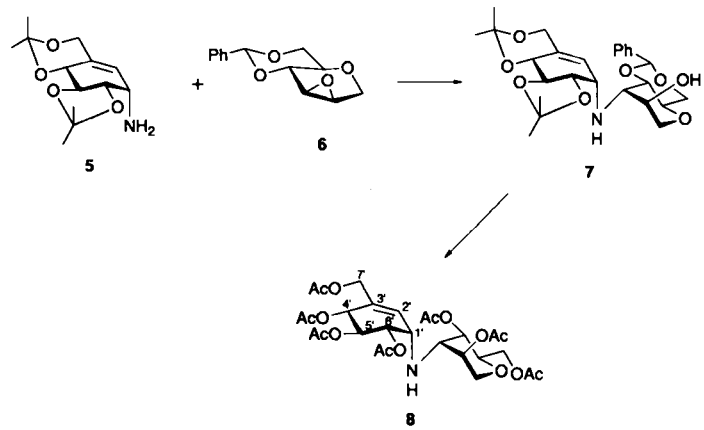


Methyl α-acarviosin: **4**

We have been studying the relationship between structure and inhibitory activity in this class of compound,[5] and our interests are now especially focused on trehalase inhibitors such as **3** and trehazolin.[3, 6] This paper describes a convenient total synthesis of salbostatin, which serves both to confirm the proposed structure and to elaborate a general method for the preparation of salbostatin analogues. The method involves coupling of an anhydro sugar with the versatile unsaturated carbasugar donor 2,3:4,6-di-*O*-isopropylidene-α-valienamine (**5**).[7]

Results and Discussion

First, coupling of the amine **5** and readily accessible 1,5:2,3-dianhydro-4,6-O-benzylidene-D-mannitol (**6**)[8] (1.3 molequiv) was carried out in 2-propanol in a sealed tube for eight days at 120 °C (Scheme 1). The reaction was very slow and, after com-



Scheme 1.

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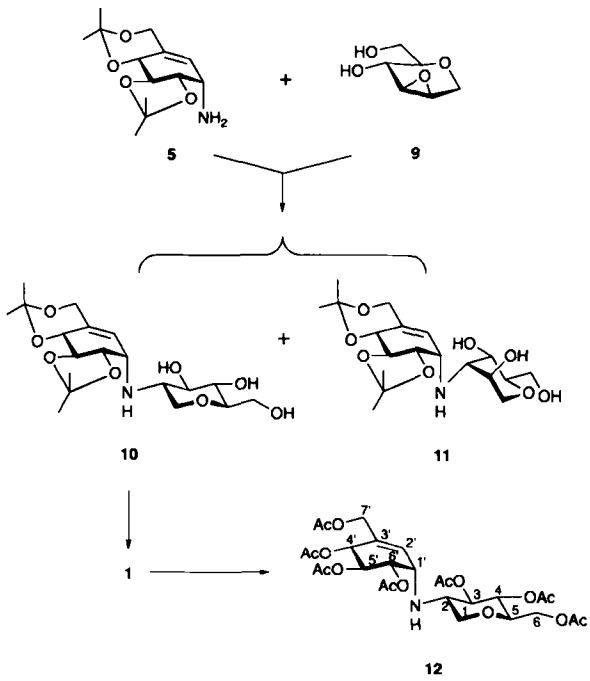
[**] In order to differentiate it from the *exo*-methylene derivative with unsaturation between C-5 and C-6, we have named the unsaturated carbasugar "α-valienamine", which has unsaturation located between C-5 and C-5a, 5a-carba-α-D-*xylo*-hex-5(5a)-enopyranosylamine.

 0947-6539/95/0109-0634 $ 10.00+.25/0

plete disappearance of **5**, the product **7**, contaminated with some deprotected derivatives, was hydrolyzed with aqueous acetic acid and acetylated. A single hepta-*N*,*O*-acetyl derivative **8** was isolated (89% yield). The opening of the epoxide proceeded with high regioselectivity. The ^{1}H NMR spectrum of **8** was, however, different from that reported for an authentic sample of peracetylated **1**,[2] and was consistent with the expected product resulting from *trans*-diaxial opening of the epoxide **6**. Thus, the ^{1}H NMR spectrum contained three coupled doublets of doublets at $\delta = 4.73$ ($J = 1.5$ and 3.7 Hz), 3.32 ($J = 3.7$ and 4.4 Hz), and 5.09 ($J =$ 4.4 and 10.3 Hz) corresponding to protons at positions 2, 3, and 4, respectively. Regioselective attack of the amine **5** at C-3 seems to be facilitated not only by steric factors, but also by hydrogen bonding between an acetal oxygen atom and the incoming amino function in the transition state.

We therefore chose to use the unprotected 1,5:2,3-dianhydro-D-mannitol (**9**) as electrophile, which was derived by hydrogenolysis (10% Pd/C) of **6** (95% yield). Removal of the benzylidene group from **6** seemed to enhance the reactivity of the 2,3-epoxide, and, it was hoped, that steric factors would now favor the desired selectivity of the ring opening. Coupling of **5** and a slight excess of **9** (1.3 molequiv) was carried out in 2-propanol at 120 °C (Scheme 2). After four days, TLC clearly



Scheme 2.

showed that two coupling products had been formed. Chromatography of the mixture on a silica gel column with butanone/toluene (3:1, v/v) as eluent afforded the diequatorially opened product **10** (58%) and the diaxially opened product **11** (25%). The structures of **10** and **11** were tentatively assigned as depicted in Scheme 2 on the basis of the ^{1}H NMR spectra. The spectrum of **10** (270 MHz, $CDCl_3$) was well resolved and was amenable to a full analysis. Thus, the coupled signals due to 1,1-H and 2-H appear as a doublet of doublets ($\delta = 4.08$, $J =$ 4.8 and 11.0 Hz), a triplet ($\delta = 3.19$, $J =$ 11.0 Hz), and a doublet of doublets of doublets ($\delta = 2.80$, $J =$ 4.8, 9.2, and 11.0 Hz), respectively; this is consistent with a 1,5-anhydroglucitol structure of the sugar moiety. The spectrum of **11**, on the other hand, consisted of complex multiplets for the ring protons of the sugar fragment. Preferential attack of the amine at C-2 of **9** might be partly rationalized, in addition to stereoelectronic effects, by assuming that **9** adopts the flipped $^1C_4$ conformation for *trans*-diaxial cleavage, favored by hydrogen bonding between the epoxide oxygen atom and the 6-hydroxyl group. *O*-Deisopropylidenation of **10** was achieved in aqueous 70% acetic acid for one hour at 60 °C to give, after purification on a column of Dowex 50W-X2 (H^+) resin with 1 M aqueous ammonia, salbostatin **1** in 97% yield. The ^{13}C and ^{1}H NMR spectra were in good agreement with those of an authentic sample.[2] To further confirm its identity, the synthetic sample of **1** was converted to the hepta-*O*-acetyl derivative **12**, the ^{1}H NMR spectrum of which was shown to be superimposable on that reported.[2] Biological assay demonstrated that **1** possesses moderate inhibitory activity ($IC_{50} = 8.3 \times 10^{-6}$ M) against silkworm trehalase, rather lower than that of validoxylamine A[3] and trehazolin.[6]

The first total synthesis of the inhibitor salbostatin **1**, presented here, confirms the structure proposed previously, and also provides a convenient method for preparation of its analogues.

Experimental Procedure

General method: Specific rotations: Jasco DIP-370 digital polarimeter in 1 dm cells. IR spectra: Jasco IR-810 spectrometer. ^{13}C and ^{1}H NMR spectry: Jeol JNM GSX-270 FT (270 MHz) instrument. TLC: silica gel 60 GF (Merck); detection by charring with conc. sulfuric acid. Column chromatrography: silica gel Wakogel C-300 (Wako, Osaka). Solvents: after drying them with anhydrous sodium sulfate, solvents were evaporated below 50 °C under reduced pressure.

2,4,6-Tri-*O*-acetyl-1,5-anhydro-3-deoxy-3-[2,3,4,6-tetra-*O*-acetyl-5a-carba-*xylo*-hex-5(5a)-enopyranosylamino]-D-altritol (8): A solution of 2,3:4,6-di-*O*-isopropylidene-α-valienamine [7] (**5**, 15.5 mg, 0.061 mmol) and 4,6-O-benzylidene-1,5:2,3-dianhydro-D-mannitol [8] (**6**, 18.5 mg, 0.079 mmol, 1.3 molequiv) in 2-propanol (0.3 mL) was heated in a sealed tube for 8 d at 120 °C and then evaporated. The residue was treated with 70% aq. acetic acid (2 mL) for 3 h at 70 °C. The mixture was evaporated and the residue was eluted from a column of silica gel (2 g) with acetone/toluene (1:2, v/v) as eluent to give a crude product, which was acetylated with acetic anhydride and pyridine. Chromatography on Dowex 50W-X2 (H^+) resin (2 mL) with 1 M aq. NH_4OH gave a single hepta-*N*,*O*-acetyl derivative **8** (33.3 mg, 89%) as a syrup; $[\alpha]_D^{22} = +78$ ($c = 1.4$ in $CHCl_3$); ^{1}H NMR (270 MHz, $CDCl_3$, TMS): $\delta = 5.98$ (1H, d, $J(1',5'a) = 5.1$ Hz, 5'a-H), 5.50 (1H, t, $J(2',3') = J(3',4') = 6.6$ Hz, 3'-H), 5.46 (1H, d, $J(3',4') = 6.6$ Hz, 4'-H), 5.09 (1H, dd, $J(3,4) = 4.4$, $J(4,5) = 10.3$ Hz, 4-H), 5.02 (1H, dd, $J(1',2') = 4.8$, $J(2',3') =$ 6.6 Hz, 2'-H), 4.73 (1H, dd, $J(1,2) = 1.5$, $J(2,3) = 3.7$ Hz, 2-H), 4.64 and 4.37 (each 1H, ABq, J(gem) = 13.2 Hz, 6',6'-H), 4.22 (1H, dd, $J(5,6a) = 5.5$, J(gem) = 12.1 Hz, 6a-H), 4.15 (1H, dd, $J(5,6b) = 4.0$, J(gem) = 12.1 Hz, 6b-H), 4.11 (1H, dd, $J(1,2) = 1.5$, J(gem) = 12.5 Hz, 1-H), 3.92 (1H, ddd, $J(5,6a) = 5.5$, $J(5,6b) =$ 4.0, $J(4,5) = 10.3$ Hz, 5-H), 3.83 (1H, d, $J(1,2) \approx 0$, J(gem) = 12.5 Hz, 1-H), 3.53 (1H, dd, $J(1',2') = 4.8$, $J(1',5'a) = 5.1$ Hz, 1'-H), 3.32 (1H, dd, $J(2,3) = 3.7$, $J(3,4) = 4.4$ Hz, 3-H), 2.13, 2.11, 2.10, 2.08, 2.07, 2.063, 2.056 (each 3H, 7s, 7 × Ac); the signal due to NH proton was not detected; $C_{27}H_{37}NO_{15}$ (615.6): calcd C 52.68, H 6.06, N 2.28; found C 52.51, H 6.17, N 2.37.

1,5:2,3-Dianhydro-D-mannitol (9): A solution of 4,6-*O*-benzylidene-1,5:2,3-dianhydro-D-mannitol (**6**, 134 mg, 0.57 mmol) in ethanol (2 mL) was hydrogenolyzed in the presence of 10% Pd/C (10 mg) under an atmosphere of hydrogen for 18 h at room temperature. The catalyst was removed by filtration, and the filtrate evaporated. Chromatography of the residue on a silica gel column (4 g) with ethanol/toluene (1:8, v/v) as eluent to give **9** (79 mg, 95%) as needles, m.p. 111–114 °C (from ethanol), $[\alpha]_D^{25} = +19$ ($c = 0.90$ in H_2O); ^{1}H NMR (270 MHz, D_2O, acetone as ref.): $\delta = 4.03$, 3.79 (2d, J(1 gem) = 13.7 Hz, each 1H, 1,1-H), 3.69 (dd, $J(5,6) = 2.4$, J(6 gem) = 12.3 Hz, 1H, 6-H), 3.51 (d, $J(4,5) = 9.5$ Hz, 1H, 4-H), 3.41 (dd, $J(5,6) = 7.1$ Hz, 1H, 6-H), 3.31–3.27 (m, 2H, H-2,3), 2.97 (ddd, 1H, 5-H); $C_6H_{10}O_4$ (146.1): calcd C 49.31, H 6.90; found C 49.05, H 7.18.

1,5-Anhydro-2-deoxy-2-[2,3:4,6-di-*O*-isopropylidene-5a-carba-*xylo*-hex-5(5a)-enopyranosylamino]-D-glucitol (10) and **1,5-Anhydro-3-deoxy-3-[2,3:4,6-di-*O*-isopropylidene-5a-carba-*xylo*-hex-5(5a)-enopyranosylamino]-D-altritol (11)**: A mixture of the epoxide **9** (29.0 mg, 0.198 mmol), valienamine **5** (39.0 mg, 0.153 mmol), and 2-propanol (0.5 mL) was heated in a sealed tube for 4 d at 120 °C, and then evaporated. TLC (butanone/toluene, 6:1, v/v) showed a formation of two products ($R_f = 0.31$ and 0.25). Chromatography of the products on a silica gel column (8 g)

with 2-butanone/toluene (3:1, v/v) as eluent gave **10** (35.7 mg, 58.2%) and **11** (15.5 mg, 25.3%) as a syrup.

Compound **10**, $[\alpha]_D^{23} = +83.4$ ($c = 0.87$ in $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$, TMS): $\delta = 5.58$ (br d, $J(1',5'a) = 4.4$ Hz, 1H, 5'a-H), 4.50 (d, $J(3',4') = 8.2$ Hz, 1H, 4'-H), 4.46, 4.19 (2d, $J(6'gem) = 14.3$ Hz, each 1H, 6',6'-H), 4.08 (dd, $J(1_{eq},2) = 4.8$, $J(1\,gem) = 11.0$ Hz, 1H, 1_{eq}-H), 3.96 (dd, $J(2',3') = 9.9$ Hz, 1H, 3'-H), 3.87 (dd, $J(5,6a) = 3.1$, $J(6\,gem) = 11.9$ Hz, 1H, 6a-H), 3.78 (dd, $J(5,6b) = 4.6$, 1H, 6b-H), 3.73 (br dd, 1H, $J(1',2') = 4.4$, $J(1',6') = 4.2$ Hz, 1H, 1'-H), 3.56 (dd, 6'-H), 3.54 (dd, $J(3,4) = 9.2$, $J(4,5) = 9.2$ Hz, 1H, 4-H), 3.38 (dd, $J(2,3) = 9.2$ Hz, 1H, 3-H), 3.35–2.40 (m, 3H, 3OH), 3.26 (ddd, 1H, 5-H), 3.19 (dd, $J(1_{ax},2) = 11.0$ Hz, 1H, 1_{ax}-H), 2.80 (ddd, 1H, 2-H), 1.56, 1.49, 1.47, 1.43 (4s, each 3H, $2CMe_2$); $C_{19}H_{31}NO_8$ (401.5): cacld C 56.84, H 7.78, N, 3.49; found C 56.42, H. 8.28, N 3.43.

Compound **11**, $[\alpha]_D^{23} = +66.5$ ($c = 0.34$ in $CHCl_3$), ^1H NMR (270 MHz, $CDCl_3$, TMS): $\delta = 5.64$ (br d, $J(1',5'a) = 3.3$ Hz, 1H, 5'a-H), 4.52 (d, 1H, $J(3',4') = 8.8$ Hz, 1H, 4'-H), 4.47, 4.21 (2d, $J(6'gem) = 14.3$ Hz, each 1H, 6',6'-H), 4.05–3.10 (m, 11H, 1_{ax},1_{eq},2,3,4,5,6,1',2',3'-H), 1.56, 1.47, and 1.43 (3s, 3, 6, and 3H, $2CMe_2$); $C_{19}H_{31}NO_8$ (401.5): found C 56.45, H 8.24, N 3.47.

1,5-Anhydro-2-deoxy-2-[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-hydroxymethylcyclohex-2-en-1-yl]amino-D-glucitol (Salbostatin) (**1**), alternatively named 1,5-anhydro-2-deoxy-2-[5a-carba-*xylo*-hex-5(5a)-enopyranosyl]amino-D-glucitol: A mixture of **10** (15.7 mg, 0.039 mmol) and aqueous 70% acetic acid (1 mL) was stirred for 1 h at 60 °C, and then evaporated. Chromatography of the product on a column of Dowex 50W-X2 (H^+) (1 mL) with aqueous 1 M ammonia as eluent afforded **1** (12.2 mg, 97%) as a white solid, $[\alpha]_D^{25} = +130$ ($c = 1.20$ in H_2O) [ref. [2]: $[\alpha]_D^{20} = +115$ ($c = 1$ in H_2O)], ^1H NMR (270 MHz, [D$_6$]DMSO/D_2O, 4:1, v/v, TMS): $\delta = 5.81$ (d, 1H, $J(1',2') = 3.3$ Hz, 1H, 2'-H), 4.05, 3.99 (2d, $J(7'gem) = 14.4$ Hz, each 1H, 7',7'-H), 3.91 (dd, $J(1_{eq},2) = 4.6$, $J(1\,gem) = 11.2$ Hz, 1H, 1_{eq}-H), 3.79 (d, $J(4',5') = 5.5$ Hz, 1H, 4'-H), 3.69 (d, $J(5,6a) \approx 0$, $J(6\,gem) = 12.1$ Hz, 1H, 6a-H), 3.48 (dd, $J(4',5') = 5.5$, $J(5',6') = 8.8$ Hz, 1H, 5'-H), 3.45 (dd, $J(5,6b) = 5.9$ Hz, 1H, 6b-H), 3.41 (dd, $J(1',6') = 4.8$, $J(5',6') = 8.8$ Hz, 1H, 6'-H), 3.23 (br dd, 1H, 1'-H), 3.16–3.11 (m, 3H, 3,4,5-H), 3.02 (dd, $J(1_{ax},2) = 10.8$ Hz, 1_{ax}-H), 2.66 (ddd, 1H, 2-H); ^{13}C NMR (67.5 MHz, [D$_6$]DMSO): $\delta = 140.38$, 120.17, 81.67, 76.60, 72.90, 70.47, 70.15, 68.97, 61.37, 61.25, 57.54, 52.76.

3,4,6-Tri-*O*-acetyl-1,5-anhydro-2-deoxy-2-[(1*S*,4*R*,5*S*,6*S*)-4,5,6-triacetoxy-3-acetoxymethylcyclohex-2-en-1-yl]amino-D-glucitol (**12**): Compound **10** (8.2 mg, 0.026 mmol) was treated with acetic anhydride (0.5 mL) in pyridine (1 mL) for 3 h at room temperature. The product was purified by chromatography on silica gel (1 g) with acetone/toluene (1:5, v/v) as eluent to yield **12** (15.4 mg, 98%) as a syrup, $[\alpha]_D^{24} = +66.6$ ($c = 0.75$ in $CHCl_3$), ^1H NMR (270 MHz, $CDCl_3$, TMS): $\delta = 5.86$ (br d, $J(1',2') = 4.8$ Hz, 1H, 2'-H), 5.53 (d, $J(4',5') = 6.6$ Hz, 1H, 4'-H), 5.48 (dd, 1H, $J(5',6') = 9.7$ Hz, 1H, 5'-H), 4.97 (dd, 1H, $J(1',6') = 4.0$ Hz, 1H, 6'-H), 4.95 (dd, $J(3,4) = 9.3$, $J(4,5) = 9.3$ Hz, 1H, 4-H), 4.87 (dd, $J(2,3) = 9.3$ Hz, 1H, 3-H), 4.64, 4.36 (2d, $J(7'gem) = 13.2$ Hz, each 1H, 7',7'-H), 4.21 (dd, $J(5,6a) = 4.8$, $J(6\,gem) = 12.5$ Hz, 1H, 6a-H), 4.09 (dd, 1H, $J(5,6b) = 2.2$ Hz, 6b-H), 4.08 (dd, $J(1_{eq},2) = 4.8$, $J(1\,gem) = 11.7$ Hz, 1H, 1_{eq}-H), 3.65 (br dd, $J(1',2') = 2.2$, $J(1',6') = 4.0$ Hz, 1H, 1'-H), 3.54 (ddd, $J(4,5) = 9.3$ Hz, 5-H), 3.14 (dd, $J(1_{ax},2) = 11.0$ Hz, 1H, 1_{ax}-H), 2.88 (ddd, 1H, 2-H), 2.10, 2.09, 2.06, 2.04, 2.03, 2.02 (6s, 3, 6, 3, 3, and 3H, 7Ac); $C_{27}H_{37}NO_{16}$ (471.6): calcd C 52.68, H 6.06, N 2.28; found C 53.08·H, 6.42, N, 2.37.

Acknowledgements: We express our sincere thanks to Dr. L. Vértesy (Hoechst AG, Frankfurt am Main, Germany) for comparing the spectra of synthetic **1** and **12** with those of the natural product and its derivative. The biological assay was carried out by Drs. O. Ando and S. Takahashi (Biomedical Research Laboratories, Sankyo, Tokyo), to whom our thanks are due.

Received: April 13, 1995 [F120]

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