Synthetic Studies on the Validamycins. I. Synthesis of β-D-Glucopyranosylvalidamine: 1L-2-O-(β-D-Glucopyranosyl)-(1,3,4/2,6)-4-amino-6-hydroxymethyl-1,2,3-cyclohexanetriol[1]

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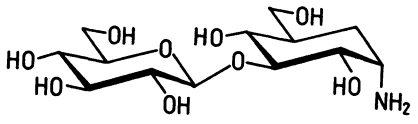
β-D-Glucopyranosylvalidamine, the structure of which had first been assigned to a degradation product of validamycin A, was synthesized by condensation of a protected validamine with 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide, followed by removal of protecting groups. The synthesized β-D-glucopyranoside was not identical with an authentic sample derived from the antibiotic.

Validamycin A is a main component of the validamycin complex which is used to control sheath blight of rice plants and produced by *Streptomyces hygroscopicus* var. *limoneus*. It was isolated by Iwasa *et al.*[2] in 1970 and the structure was first assigned by Horii and Kameda[3] in 1972 on the basis of degradative studies. The unique structural feature of the validamycins prompted us to study the synthesis and structure-activity relationship of this class of antibiotics.



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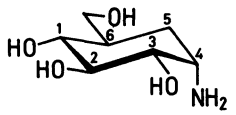

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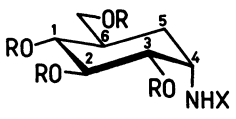
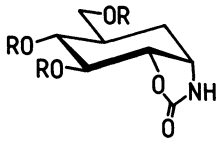
Scheme 1.

The hydrogenolitic cleavage of validamycin A yields β-D-glucopyranosylvalidamine (**1**), validatol, and deoxyvalidatol.[4] Compound **1** was hydrolyzed to afford validamine (**2**) and D-glucose, and its structure was formulated as shown in Scheme 1 based on results of the periodate oxidation of its *N*-acetyl derivative.[4] The present paper, as part of studies directed toward total synthesis of validamycin A and related substances, describes the synthesis of the original structure of **1**, 1L-2-O-(β-D-glucopyranosyl)-(1,3,4/2,6)-4-amino-6-hydroxymethyl-1,2,3-cyclohexanetriol, by condensation of a properly protected DL-validamine with 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide. In addition, two diastereomeric β-D-glucopyranosides thus obtained were hydrolyzed to generate enantiomeric validamines, which constituted an optical resolution of racemic validamine.

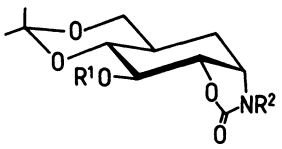
Acid hydrolysis of DL-penta-*N*,*O*-acetyl-(1,3,4/2,6)-4-amino-6-hydroxymethyl-1,2,3-cyclohexanetriol (peracetyl-DL-validamine) (**3**)[5] with 4 M hydrochloric acid (1 M=1 mol dm^{-3}) at reflux temperature gave the amine hydrochloride (**4**) as an amorphous solid in quantitative yield. Treatment of **4** with benzyloxycarbonyl chloride in an alkaline solution gave a crystalline *N*-benzyloxycarbonyl derivative (**5**), which was further characterized as the tetra-O-acetyl derivative (**6**). Compound **5** was then converted into the *N*,*O*-carbonyl derivative (**7**) in 75% yield by treatment with 10% aqueous sodium hydroxide, whose IR spectrum revealed an absorption at 1730 cm^{-1}, indicative of the cyclic carbamate function. The structure of **7** was confirmed by the ^{1}H NMR spectrum of the corresponding tri-O-acetyl derivative (**8**). Thus, the signals for H-1, H-2, and H-3 protons appeared as a triplet (J=9 Hz), a doublet of doublets (J=6.5 and 9 Hz), and a doublet of doublets (J=6.2 and 6.5 Hz) at δ 4.99, 5.26, and 4.60, respectively, being consistent with the chair conformation slightly distorted by the introduction of the cyclic carbamate portion. Isopropylidenation of **7** with 2,2-dimethoxypropane in *N*,*N*-dimethylformamide (DMF) in the presence of *p*-toluenesulfonic acid gave the 1,7-O-isopropylidene derivative (**9**) in 97% yield. The assigned structure was supported by the ^{1}H NMR spectra of the corresponding O-acetyl (**10**), di-*N*,*O*-



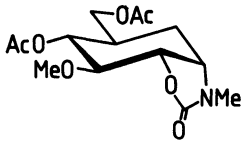
	R	X
3	Ac	Ac
4	H	H·HCl
5	H	Cbz
6	Ac	Cbz



	R
7	H
8	Ac



	R^1	R^2
9	H	H
10	Ac	H
11	Ac	Ac
12	Me	Me



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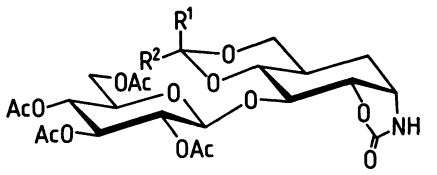
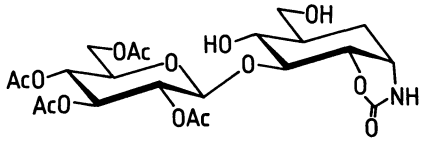
Scheme 2. Synthesis of protected DL-validamine. All compounds are racemic. The formulas depict one of the respective enantiomers.



	R^1	R^2
14	Me	Me
15	H	Ph



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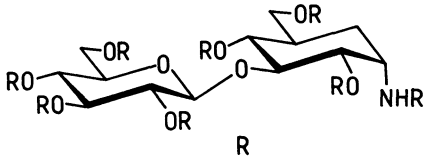

	R
17	H
18	Ac

Scheme 3. Synthesis of β-D-glucopyranosylvalidamine. The formulas depict one of the respective diastereomers.

acetyl (**11**), and di-*N*,*O*-methyl derivatives (**12**). For further confirmation, **9** was transformed into DL-1,7-di-*O*-acetyl-3,4-*N*,*O*-carbonyl-2,4-di-*N*,*O*-methyl-(1,3,4/2,6)-4-amino-6-hydroxymethyl-1,2,3-cyclohexanetriol (**13**), whose ^{1}H NMR spectrum was fully consistent with the assigned structure. Thus, there appeared two coupled doublets of doublets (J=6 and 8 Hz) attributable to H-2 and H-3 at δ 3.36 and 4.50, respectively. Therefore, **9** was shown to possess a free hydroxyl group at C-2, being a suitable intermediate for the synthesis of **1**.

Condensation of **9** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide was carried out in a mixture of benzene and dioxane (2 : 1, v/v) in the presence of mercury(II) cyanide and anhydrous calcium sulfate at 65 °C for 6 d. Formation of two components was observed and they were clearly separated by chromatography on silica gel with 2-butanone–toluene (1 : 1, v/v) as an eluent, giving the protected β-D-glucopyranosides [**14**(+)], $[\alpha]_D$+77.8°, and [**14**(−)], $[\alpha]_D$−30.4°, as a syrup in 47 and 50% yields, respectively. They were shown to have four acetoxyl, one carbonyl, and one isopropylidene groups by the IR and ^{1}H NMR spectra, and their analytical data supported the assigned structures. The β-configurations were proposed both by the optical rotations and by the reaction conditions employed for the condensation reaction. Treatment of **14**(+) and **14**(−) with 80% aqueous acetic acid at ambient temperature afforded the corresponding dihydroxy compounds [**16**(+)] and [**16**(−)] in 44 and 76% yields, respectively. The presence of two hydroxyl groups at C-1 and C-7 was evidenced by the exclusive transformation into the 1,7-*O*-benzylidene derivatives [**15**(+) and **15**(−)] in 74 and 66% yields, respectively, by treatment with 1,1-dimethoxy-1-phenylmethane in DMF in the presence of acid catalyst. The ^{1}H NMR spectra of **15**(+) and **15**(−) exhibited one-proton sharp singlets at δ 5.56 and 5.55, respectively, due to the benzylic protons. Removal of the protecting groups of **16**(+) and **16**(−) was effected by treatment with refluxing 10% aqueous barium hydroxide. The free bases [**17**(+) and **17**(−)] thus obtained as a homogeneous syrup were further characterized as the corresponding octa-*N*,*O*-acetyl derivatives [**18**(+) and **18**(−)]. Acid hydrolysis of **17**(+) with 6 M hydrochloric acid gave D-glucose and validamine hydrochloride, which were detected by TLC on cellulose. They were acetylated in the usual way to give, after chromatography, penta-*O*-acetyl-α-D-glucopyranose and penta-*N*,*O*-acetyl-(+)-validamine [**3**(+)], $[\alpha]_D$ +60.2°, mp 146—148 °C. The latter compound was identified with an authentic sample, $[\alpha]_D$ +61.6°, mp 147—149 °C, prepared from (+)-validamine hydrochloride,[6,7] by the mixed melting point method and by comparison of their IR (in chloroform) and ^{1}H NMR spectra, and behavior on TLC. The enantiomer **3**(−), $[\alpha]_D$ −59.8°, mp 147—149 °C, similarly obtained from **17**(−), was shown to be identical with an authentic sample except for the optical rotations being opposite in sign. Therefore, the optical resolution of racemic validamine was accomplished by the above experiments.

Attempts were then made to compare **18**(+) with an authentic sample[6] derived from validamycin A, and they were found to be completely different from each other, on the basis of ^{1}H and ^{13}C NMR spectra, and chromatographic behavior. Consequently, we concluded that the original structure of β-D-glucopyranosylvalidamine must be reinvestigated and revised. We will report an unequivocal synthesis of β-D-glucopyranoside identical with an authentic sample in all respects in the succeeding paper.

Experimental

Unless otherwise noted, melting points were determined on a Mitamura Riken micro hot stage and uncorrected. The ^{1}H NMR spectra were taken on a Varian EM-360 (60 MHz) in chloroform-*d* ($CDCl_3$) or dimethyl-d_6 sulfoxide (DMSO-d_6) with reference to tetramethylsilane as an internal standard and the peak positions are given in terms of δ-values. Values given for coupling constants are of first-order. The IR spectra were measured on a Hitachi 225 spectrometer in potassium bromide pellets or in chloroform. Optical rotations were measured on a Japan Spectroscopic DIP-4 polarimeter in a 10 mm cell. TLC was performed on a precoated silica gel 60 F-254 plate or cellulose F-254 plate (Merck, Darmstadt). The silica gel used for column chromatography was Wakogel C-300.

DL-*(1,3,4/2,6)-4-Amino-6-hydroxymethyl-1,2,3-cyclohexanetriol Hydrochloride (*DL-*Validamine Hydrochloride) (**4**).* A solution of DL-tetra-*O*-acetyl-(1,3,4/2,6)-4-acetamido-6-hydroxymethyl-1,2,3-cyclohexanetriol (**3**)[5] (3.8 g) in 4 M hydrochloric acid (240 ml) was refluxed for 2 h. The reaction mixture was concentrated to give a pale yellow syrup, which was dissolved in water and decolorized with active carbon. Evaporation of the solvent gave **4** (2.1 g, 100%) as a chromatographically homogeneous glass, which showed a single spot at R_f 0.41 on TLC [cellulose, 1-butanol–ethanol–water–17% aqueous ammonia (4 : 5 : 2 : 4)].

Found: C, 39.07; H, 7.74; N, 6.39; Cl, 16.33%, Calcd for $C_7H_{15}NO_4 \cdot HCl$: C, 39.35; H, 7.55; N, 6.56; Cl, 16.59%.

DL-*(1,3,4/2,6)-4-Benzyloxycarbonylamino-6-hydroxymethyl-1,2,3-cyclohexanetriol (5).* To a stirred solution of **4** (2.1 g) in a mixture of acetone–water (2 : 1) (220 ml) containing 1 M aqueous sodium hydroxide (30 ml) was added dropwise 30% benzyloxycarbonyl chloride in toluene (11 ml) at ambient temperature. The reaction mixture was agitated for 17 h at ambient temperature, and then neutralized with 1 M hydrochloric acid and concentrated. The residue was extracted with acetone and the extracts were concentrated to give a white solid, which was crystall ized from ethyl acetate to give **5** (1.51 g, 52.3%) as prisms: mp 148—150 °C; IR (KBr disk) 1710 cm^{-1} (C=O).

Found: C, 57.69; H, 6.72; N, 4.35%. Calcd for $C_{15}H_{21}NO_6$: C, 57.86; H, 6.80; N, 4.50%.

DL-*Tetra-O-acetyl-(1,3,4/2,6)-4-benzyloxycarbonylamino-6-hydroxymethyl-1,2,3-cyclohexanetriol (6).* Compound **5** (0.1 g) was treated with acetic anhydride (1 ml) in pyridine (2 ml) at ambient temperature overnight. The reaction mixture was poured into ice–water and the resulting gum was collected by decantation. The product was dried over phosphorus pentaoxide under vacuum to give **6** (0.1 g, 69%) as a homogeneous syrup: ^1H NMR ($CDCl_3$) δ=1.91 (3H, s), 1.99 (3H, s), 2.00 (3H, s), and 2.05 (3H, s) (OAc), 4.32 (1H, m, H-4), 5.06 (2H, s, $C\underline{H}_2Ph$), and 7.29 (5H, broad s, phenyl).

Found: C, 57.58; H, 6.12; N, 2.82%. Calcd for $C_{23}H_{29}NO_{10}$: C, 57.61; H, 6.10; N, 2.92%.

DL-*3,4-N,O-Carbonyl-(1,3,4/2,6)-4-amino-6-hydroxymethyl-1,2,3-cyclohexanetriol (7).* To a solution of **5** (0.8 g) in acetone–water (3 : 1) (30 ml) was added 1 M aqueous sodium hydroxide, the pH of the solution being adjusted to 11. After having been stood at ambient temperature for 4 h, the reaction mixture was neutralized with 1 M hydrochloric acid, concentrated, and dried by coevaporation with ethanol. The residue was extracted with hot 1,4-dioxane (5 ml × 3) and, after being cooled, the extracts gave **7** (0.39 g, 75%) as prisms: mp 164—165 °C; IR (KBr disk) 3500—3200 (OH) and 1730 cm^{-1} (cyclic carbamate).

Found: C, 47.29; H, 6.52; N, 6.71%. Calcd for $C_8H_{13}NO_5$: C, 47.29; H, 6.45; N, 6.89%.

Compound **7** (1.2 g) was directly obtained in 77% yield from **3** (2.9 g) without isolation of the intermediates.

DL-*Tri-O-acetyl-3,4-N,O-carbonyl-(1,3,4/2,6)-4-amino-6-hydroxymethyl-1,2,3-cyclohexanetriol (8).* Compound **7** (0.1 g) was acetylated in the conventional manner and the product was crystallized from ethanol to give **8** (0.14 g, 85%) as needles: mp 133—134 °C; ^1H NMR ($CDCl_3$) δ=2.04 (6H, s) and 2.06 (3H, s) (OAc), 3.90 (2H, q) and 4.22 (2H, q) ($J_{6,7}$=3 Hz, $J_{6,7'}$=5 Hz, J_{7gem}=11.5 Hz, H-7 and H-7'), 4.60 (1H, dd, $J_{2,3}$=6.2 Hz, $J_{3,4}$=6.5 Hz, H-3), 4.99 (1H, t, $J_{1,2}$=$J_{1,6}$=9 Hz, H-1), 5.26 (1H, dd, H-2), and 6.40 (1H, s, NH).

Found: C, 50.80; H, 5.67; N, 4.11%. Calcd for $C_{14}H_{19}NO_8$: C, 51.06; H, 5.82; N, 4.25%.

DL-*3,4-N,O-Carbonyl-1,7-O-isopropylidene-(1,3,4/2,6)-4-amino-6-hydroxymethyl-1,2,3-cyclohexanetriol (9).* To a solution of **7** (1.2 g) in dry *N,N*-dimethylformamide (8 ml) was added 2,2-dimethoxypropane (3.5 ml) and *p*-toluenesulfonic acid (20 mg), and the mixture was heated at 80 °C for 2 h. The mixture was treated with Amberlite IRA 400 (OH^-) (2.5 ml) and then concentrated to give a crystalline product. Recrystallization from ethanol gave **9** (1.1 g, 79%) as crystals: mp 243—244 °C (decomp); IR (KBr disk) 3400—3300 (OH and NH) and 1740 cm^{-1} (cyclic carbamate); ^1H NMR (DMSO-d_6) δ=1.27 (3H, s) and 1.37 (3H, s) (isopropylidene), 4.16 (1H, t, $J_{2,3}$=$J_{3,4}$=6.5 Hz, H-3), 5.28 (1H, d, $J_{2,OH}$=5 Hz, OH), and 7.29 (1H, broad s, NH).

Found: C, 54.05; H, 6.89; N, 5.89%. Calcd for $C_{11}H_{17}NO_5$: C, 54.31; H, 7.04; N, 5.76%.

DL-*2-O-Acetyl-3,4-N,O-carbonyl-1,7-O-isopropylidene-(1,3,4/2,6)-4-acetamido-6-hydroxymethyl-1,2,3-cyclohexanetriol (11).* Compound **9** (0.1 g) was treated with acetic anhydride (2 ml) in pyridine (3 ml) at 50 °C for 3 d. The reaction mixture was poured into saturated aqueous sodium hydrogencarbonate and the precipitates were collected by decantation. The product was dissolved in ethyl acetate, and the solution was washed with water and dried. Evaporation of the solvent gave a syrup (0.12 g), which was crystallized from ethanol to give **11** (30 mg, 22%) as needles: mp 142—144 °C; ^1H NMR ($CDCl_3$) δ=1.34 (3H, s) and 1.41 (3H, s) (isopropylidene), 2.10 (3H, s, OAc), 2.51 (3H, s, NAc), 3.58 (1H, t, $J_{1,2}$=$J_{1,6}$=10 Hz, H-1), 4.52 (2H, d, H-3 and H-4), and 5.03 (1H, m, H-2).

Found: C, 54.77; H, 6.40; N, 4.10%. Calcd for $C_{15}H_{21}NO_7$: C, 55.04; H, 6.47; N, 4.28%.

DL-*2-O-Acetyl-3,4-N,O-carbonyl-1,7-O-isopropylidene-(1,3,4/2,6)-4-amino-6-hydroxymethyl-1,2,3-cyclohexanetriol (10).* Compound **9** (61 mg) was treated with acetic anhydride (1 ml) in pyridine (2 ml) at ambient temperature for 25 h. TLC indicated the formation of one major component (R_f 0.28) and one minor component (R_f 0.77) [silica gel, 2-butanone–toluene (1 : 1)]. The reaction mixture was concentrated and the crude product, without further purification, was subjected to measurement of ^1H NMR spectrum. The product was shown to be contaminated with **11**: ^1H NMR ($CDCl_3$) δ=1.34 (3H, s) and 1.41 (3H, s) (isopropylidene), 2.06 (3H, OAc), 3.50 (1H, t, $J_{1,2}$=$J_{1,6}$=10 Hz, H-1), 4.48 (1H, t, $J_{2,3}$=$J_{3,4}$=7 Hz, H-3), 5.14 (1H, dd, H-2), and 6.37 (1H, s, NH).

DL-*1,7-Di-O-acetyl-3,4-N,O-carbonyl-2,4-di-N,O-methyl-(1,3,4/2,6)-4-amino-6-hydroxymethyl-1,2,3-cyclohexanetriol (13).* To a solution of **9** (0.22 g) in dry *N,N*-dimethylformamide (10 ml) was added silver oxide (1 g) and methyl iodide (1 ml), and the mixture was vigorously stirred at ambient temperature in the dark for 14 h. An insoluble material was removed by filtration and washed thoroughly with ethyl acetate. The filtrate and washings were combined and concentrated. The residue was dissolved in ethyl acetate and filtered through a short column of alumina. The filtrate was concentrated to give DL-3,4-*N,O*-carbonyl-1,7-*O*-isopropylidene-2,4-di-*N,O*-methyl-(1,3,4/2,6)-4-amino-6-hydroxymethyl-1,2,3-cyclohexanetriol (**12**) as a homogeneous syrup: ^1H NMR δ=1.40 (3H, s) and 1.47 (3H, s) (isopropylidene), 2.75 (3H, s, NCH_3), 3.55 (3H, s, OCH_3), and 4.37 (1H, t, $J_{2,3}$=$J_{3,4}$=7 Hz, H-3).

Crude **12** was treated with 80% aqueous acetic acid (10 ml) at 65 °C for 3 h. The reaction mixture was concentrated and dried by coevaporation with toluene. The product was acetylated in the conventional manner to give a syrup, which was crystallized from ethanol to give **13** (90 mg, 31% yield based on **7** used) as needles: mp 108—110 °C; ^1H NMR ($CDCl_3$) δ=2.02 (3H, s) and 2.05 (3H, s) (OAc), 2.78 (3H, s, NCH_3), 3.36 (1H, dd, $J_{1,2}$=8 Hz, $J_{2,3}$=6 Hz, H-2), 3.48 (3H, s, OCH_3), 4.50 (1H, dd, $J_{3,4}$=8 Hz, H-3), and 4.85 (1H, t, $J_{1,6}$=8 Hz, H-1).

Found: C, 53.00; H, 6.65; N, 4.34%. Calcd for $C_{14}H_{21}NO_7$: C, 53.33; H, 6.71; N, 4.44%.

Koenigs-Knorr Reaction of 9 and 2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl Bromide. A mixture of **9** (1.0 g), 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (6 g, 3.6 molar equiv.), and benzene–1,4-dioxane (2 : 1) (120 ml) was heated at 65 °C in the presence of mercury(II) cyanide (7 g) and anhydrous calcium sulfate (Drierite) (7.2 g) for 140 h. During the course of reaction, additional bromide (3 g) was added at an interval of 50 h. TLC indicated the disappearance of **9** and the formation of two new components in an approximately equal proportion, together with several side

products. An insoluble material was removed by filtration through a bed of caoline and washed thoroughly with chloroform (50 ml×4). The filtrate and washings were combined and concentrated. The residue was dissolved in ethyl acetate (200 ml) and the solution was washed successively with saturated aqueous sodium hydrogencarbonate and water, dried over anhydrous sodium sulfate, and then concentrated to give a pale yellow syrup (11 g). The product was chromatographed on a silica-gel column (100 g, 35 mm×300 mm) packed with 2-butanone–toluene (3 : 8). The first fractions obtained by elution with the same solvent (1 l) were discarded. Succeeding elution with methanol (500 ml) gave a homogeneous mixture of two β-D-glucopyranosides, which was again chromatographed on a silica-gel column (150 g, 30 mm×500 mm) with 2-butanone–toluene (1 : 1) as an eluent. The first fraction [R_f 0.38, silica gel, 2-butanone–toluene (2 : 1)] gave 1L-3,4-*N,O*-carbonyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-1,7-*O*-isopropylidene-(1,3,4/2,6)-4-amino-6-hydroxymethyl-1,2,3-cyclohexanetriol[**14**(+), 1.09 g, 47%] as a homogeneous glass: $[\alpha]_D^{20}$ +77.8° (*c* 1.02, chloroform); ^{1}H NMR ($CDCl_3$) δ=1.33 (3H, s) and 1.44 (3H, s) (isopropylidene), 1.99 (6H, s), 2.00 (3H, s), and 2.09 (3H, s) (OAc), 6.22 (1H, s, NH).

Found: C, 52.56; H, 6.20; N, 2.46%. Calcd for $C_{25}H_{35}NO_{14}$: C, 52.35; H, 6.15; N, 2.44%.

The second fraction (R_f 0.31) gave the diastereomer [**14**(−), 1.16 g, 50%] as a homogeneous glass: $[\alpha]_D^{20}$ −30.4° (*c* 1.02, chloroform); ^{1}H NMR ($CDCl_3$) δ=1.42 (6H, broad s, isopropylidene), 2.01 (9H, s) and 2.08 (3H, s) (OAc), and 6.18 (1H, broad s, NH).

Found: C, 52.48; H, 6.32; N, 2.49%. Calcd for $C_{25}H_{35}NO_{14}$: C, 52.35; H, 6.15; N, 2.44%.

1L-*3,4*-N,O-*Carbonyl-2*-O-*(2,3,4,6-tetra*-O-*acetyl-β-D-glucopyranosyl)-(1,3,4/2,6)-4-amino-6-hydroxymethyl-1,2,3-cyclohexanetriol* [***16***(+)]. A solution of **14**(+) (0.45 g) in 80% aqueous acetic acid (10 ml) was allowed to stand at ambient temperature for 18 h. The reaction mixture was concentrated to give a crystalline residue (0.36 g), which was recrystallized from ethanol to give **16**(+) (0.18 g, 44%): mp 180—182 °C; $[\alpha]_D^{20}$ +73.4° (*c* 1.01, chloroform); ^{1}H NMR ($CDCl_3$) δ=1.99 (3H, s), 2.02 (3H, s), 2.07 (3H, s), and 2.12 (3H, s) (OAc), and 6.31 (1H, s, NH).

Found: C, 49.29; H, 5.80; N, 2.49%. Calcd for $C_{22}H_{31}NO_{14}$: C, 49.53; H, 5.86; N, 2.63%.

1D-*3,4*-N,O-*Carbonyl-2*-O-*(2,3,4,6-tetra*-O-*acetyl-β-D-glucopyranosyl-(1,3,4/2,6)-4-amino-6-hydroxymethyl-1,2,3-cyclohexanetriol* [***16***(−)]. Compound **14**(−) (0.39 g) was treated with 80% aqueous acetic acid (10 ml) as in the preparation of **14**(+). The product (0.35 g) was recrystallized from ethanol to give **16**(−) (0.27 g, 76%) as needles: mp 216—219 °C; $[\alpha]_D^{20}$ −62.8° (*c* 0.85, methanol); ^{1}H NMR (DMSO-d_6) δ=1.98 (3H, s), 2.00 (3H, s), 2.03 (3H, s), and 2.12 (3H, s) (OAc), and 7.47 (1H, s, NH).

Found: C, 49.75; H, 5.83; N, 2.45%. Calcd for $C_{22}H_{31}NO_{14}$: C, 49.53; H, 5.86; N, 2.63%.

Compounds **16**(+) and **16**(−) showed single spots at R_f 0.43 and 0.38, respectively, on TLC [silica gel, chloroform–methanol (5 : 1)], and were clearly differentiated from each other.

1L-*1,7*-O-*Benzylidene-3,4*-N,O-*carbonyl-2*-O-*(2,3,4,6-tetra*-O-*acetyl-β-D-glucopyranosyl)-(1,3,4/2,6)-4-amino-6-hydroxymethyl-1,2,3-cyclohexanetriol* [***15***(+)]. To a solution of **16**(+) (63 mg) in dry *N,N*-dimethylformamide (3 ml) were added 1,1-dimethoxy-1-phenylmethane (0.06 ml) and a catalytic amount of *p*-toluenesulfonic acid, and the mixture was heated at 60 °C for 1.5 h. TLC indicated the formation of a single product [R_f 0.40, silica gel, 2-butanone–toluene (2 : 1)]. The reaction mixture was neutralized with Amberlite IRA-400 (OH^-) (1.2 ml), and then concentrated to give a colorless glass, which was crystallized from ethanol to give **15**(+) (54 mg, 74%) as needles: mp 240—243 °C; $[\alpha]_D^{20}$ +56° (*c* 0.80, chloroform); ^{1}H NMR ($CDCl_3$) δ=1.97 (9H, s) and 2.14 (3H, s) (OAc), 5.56 (1H, s, benzylic), 5.91 (1H, s, NH), and 7.39 (5H, broad s, phenyl).

Found: C, 56.08; H, 5.67; N, 2.25%. Calcd for $C_{29}H_{35}NO_{14}$: C, 56.04; H, 5.68; N, 2.25%.

1D-*1,7*-O-*Benzylidene-3,4*-N,O-*carbonyl-2*-O-*(2,3,4,6-tetra*-O-*acetyl-β-D-glucopyranosyl)-(1,3,4/2,6)-4-amino-6-hydroxymethyl-1,2,3-cyclohexanetriol* [***15***(−)]. Compound **16**(−) (70 mg was treated with 1,1-dimethoxy-1-phenylmethane (0.1 ml) and *p*-toluenesulfonic acid in DMF (3 ml) as in the preparation of **15**(+). The crude product was recrystallized from ethanol to give **15**(−) (54 mg, 66%) as prisms: mp 192—194 °C; $[\alpha]_D^{20}$ −74° (*c* 0.60, chloroform); ^{1}H NMR ($CDCl_3$) δ=1.52 (3H, s), 1.98 (3H, s), 2.02 (3H, s), and 2.09 (3H, s) (OAc), 5.55 (1H, s, benzylic), and 6.16 (1H, s, NH).

Found: C, 56.01; H, 5.59; N, 2.26%. Calcd for $C_{29}H_{35}NO_{14}$: C, 56.04; H, 5.68; N, 2.25%.

Compounds **15**(+) and **15**(−) showed single spots at R_f 0.40 and 0.36, respectively, on TLC [silica gel, 2-butanone–toluene (2 : 1)] and were clearly differentiated from each other.

1L-*2*-O-*β-D-Glucopyranosyl-(1,3,4/2,6)-4-amino-6-hydroxymethyl-1,2,3-cyclohexanetriol* [***17***(+)] *and Its Peracetate* [***18***(+)]. A mixture of **16**(+) (0.142 g), barium hydroxide (0.5 g), and 1,4-dioxane–water (1 : 1) (50 ml) was heated under reflux for 24 h. The reaction mixture was neutralized by addition of solid carbon dioxide and the precipitates were removed by filtration The filtrate was concentrated to give **17** (+) as a syrup, which was shown to be homogeneous on TLC [R_f 0.18, cellulose, 1-butanol–pyridine–water–acetic acid (6 : 4 : 3 : 1)]. The syrup was treated with acetic anhydride (4 ml) in pyridine (4 ml) at ambient temperature for 18 h. The reaction mixture was concentrated and the residue was dissolved in chloroform and the solution was passed through a short column of alumina. The eluate was concentrated to give **18**(+) (0.11 g, 59%) as a homogeneous syrup: $[\alpha]_D^{20}$ −9.5° (*c* 0.89, chloroform): ^{1}H NMR ($CDCl_3$) δ=1.92, 1.96, 2.00, 2.03, and 2.05 (24H, NAc and OAc), and 5.77 (1H, d, $J_{4,NH}$=7.5 Hz, NH); IR (KBr disk) 3400 (NH), 1755 (ester), 1660, and 1535 cm^{-1} (amide).

Found: C, 51.79; H, 6.33; N, 1.93%. Calcd for $C_{29}H_{41}NO_{17}$: C, 51.56; H, 6.12; N, 2.07%.

1D-*2*-O-*β-D-Glucopyranosyl-(1,3,4/2,6)-4-amino-6-hydroxymethyl-1,2,3-cyclohexanetriol* [***17***(−)] *and Its Peracetate* [***18***(−)]. Compound **16**(−) (0.264 g) was hydrolyzed with aqueous barium hydroxide as in the preparation of **17**(+). The free base **17**(−) showed a single spot on TLC [R_f 0.18, cellulose, 1-butanol–pyridine–water–acetic acid (6 : 4 : 3 : 1)]. It was acetylated in the conventional way to give **18**(−) (0.31 g, 92%) as a homogenous syrup: $[\alpha]_D^{20}$ −41.7° (*c* 1.07, chloroform); ^{1}H NMR ($CDCl_3$) δ=1.93, 1.99, 2.04, and 2.09 (total 24H, s, NAc and OAc), and 6.02 (1H, d, $J_{4,NH}$=7 Hz, NH); IR (KBr disk) 3400 (NH), 1755 (ester), 1660, and 1535 cm^{-1} (amide).

Found: C, 51.60; N, 6.21; N, 2.10%. Calcd for $C_{29}H_{41}NO_{17}$: C, 51.56; H, 6.12; N, 2.07%.

Attempts were made to identify **17**(+) and **18**(+) with authentic samples derived from validamycin A. The IR and ^{1}H NMR spectra of **17**(+) and **18**(+) were both shown to be different from those of authentic samples. Compound **17**(+) revealed a single spot at R_f 0.18, while the corresponding authentic sample showed a single spot at R_f 0.12 on TLC [cellulose, 1-butanol–pyridine–water–acetic acid (6 : 4 : 3 : 1)]. Compound **18**(+) revealed a single spot at R_f 0.32 and the authentic sample at R_f 0.51 [silica gel, chloroform–methanol

(10: 1)].

Acid Hydrolysis of 17(+) and 17(−). Preparation of Enantiomeric Validamines. A mixture of **17**(+) (0.13 g) and 4 M hydrochloric acid (6 ml) was heated under reflux for 1 h. The reaction mixture was concentrated and the residue was dried by coevaporation successively with water, ethanol, and toluene. A partly crystalline product was acetylated in the conventional way and the product was chromatographed on a silica-gel column (20 g) with 2-butanone–toluene (1 : 4). The fractions first eluted gave penta-*O*-acetyl-D-glucopyranose (68 mg) as a pale yellow syrup, homogeneous on TLC [R_f 0.52, 2-butanone–toluene (1 : 2)], identical with an authentic sample. The second fractions gave tetra-*O*-acetyl-1L-(1,3,4/2,6)-4-acetamido-6-hydroxymethyl-1,2,3-cyclohexanetriol [(+)-validamine] (73 mg, 40%) as a syrup: $[\alpha]_D^{20}$ +42.6° (*c* 1.08, chloroform). The syrupy product gradually crystallized from ethanol to give prisms or plates: mp 197—202 °C. On the other hand, crystallization from ether gave needles: mp 146—148 °C; $[\alpha]_D^{20}$ +60.2° (*c* 0.60, chloroform). These two crystals were shown to be dimorphic crystals by spectroscopic methods. The compound (needles) was identified with an authentic sample by comparison of IR (in chloroform) and ^{1}H NMR (in $CDCl_3$) spectra and TLC behavior.

Found: C, 52.92; H, 6.41; N, 3.70%. Calcd for $C_{17}H_{25}NO_9$: C, 52.71; H, 6.51; N, 3.62%.

A reference compound was prepared by the conventional acetylation of validamine hydrochloride kindly provided by Dr. Satoshi Horii. The peracetate slowly crystallized from a small amount of chloroform to give needles, which were again recrystallized from ether: mp 147—149 °C; $[\alpha]_D^{20}$ +61.6° (*c* 0.71, chloroform). Found: C, 52.46; H, 6.39; N, 3.57%.

A crude **17**(−) (0.15 g) was treated with 4 M hydrochloric acid as described above. TLC indicated the formation of D-glucose (R_f 0.45) and (−)-validamine hydrochloride (R_f 0.25) [cellulose, 1-butanol–pyridine–water–acetic acid (6: 4: 3: 1)]. The mixture was acetylated in the conventional way and the products were fractionated on a silica-gel column to give penta-*O*-acetyl-D-glucopyranose (57 mg) as a syrup and tetra-*O*-acetyl-1D-(1,3,4/2,6)-4-acetamido-6-hydroxymethyl-1,2,3-cyclohexanetriol [(−)-validamine] (0.13 g, 76%) as a syrup: $[\alpha]_D^{20}$ −54.1° (*c* 0.98, chloroform). This compound crystallized from ethanol to give plates, mp 197—199.5 °C, and from ether to give needles: mp 147—149 °C; $[\alpha]_D^{20}$ −59.8° (*c* 1.3, chloroform). Its IR (in chloroform) and ^{1}H NMR (in $CDCl_3$) spectra were superimposable on those of an authentic sample.

Found: C, 52.95; H, 6.41; N, 3.66%. Calcd for $C_{17}H_{25}NO_9$: C, 52.71; H, 6.51; N, 3.62%.

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7) The absolute configuration of (+)-validamine was established as depicted in Scheme 1 by X-ray spectroscopic analysis of its hydrobromide [K. Kamiya, Y. Wada, S. Horii, and M. Nishikawa, *J. Antibiot.*, **24**, 317 (1971)].