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

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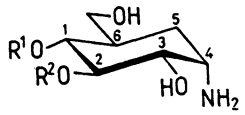
The title β-D-glucopyranoside was synthesized *via* an unequivocal route and found to be identical with β-D-glucopyranosylvalidamine derived from antibiotic validamycin A. The position at which the validamine moiety is substituted with D-glucopyranose in validamycin A is revised to C-1 on the basis of the present synthesis.

In the preceding paper,[2)] we described an unequivocal synthesis of the original structure of β-D-glucopyranosyl-validamine (**1**) and found that it was not identical with an authentic sample derived from validamycin A. These results suggested that, contrary to the previous assignment by Horii and Kameda,[3)] D-glucopyranose should be attached to the C-1 or C-3 hydroxyl group rather than to the C-2 hydroxyl group of 1L-(1,3,4/2,6)-4-amino-6-hydroxymethyl-1,2,3-cyclohexanetriol [(+)-validamine] (**2**). Therefore, in order to elucidate the position of the β-glucosidic linkage, attempts were initially made to prepare the 1-*O*-β-D-glucopyranoside (**3**) *via* an unambiguous route. In the present paper, we wish to describe the detail of the successful synthesis of **3**, identical with an authentic sample, and to discuss on the revised structure of validamycin A along with ^{13}C NMR spectral data.

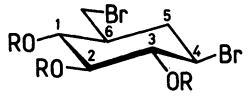


	R^1	R^2
1	H	β-D-glucopyranosyl
2	H	H
3	β-D-glucopyranosyl	H

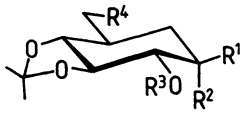
Scheme 1.

DL-7-*O*-Benzoyl-2,3-*O*-isopropylidene-(1,3,4/2,6)-4-azido-6-hydroxymethyl-1,2,3-cyclohexanetriol (**8b**) was chosen as a protected precursor of the aglycone moiety for a condensation with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide, and was prepared by starting from readily available DL-tri-*O*-acetyl-(1,3/2,4,6)-4-bromo-6-bromomethyl-1,2,3-cyclohexanetriol (**4**)[4)] in the following sequence.

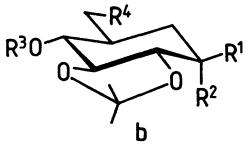
Treatment of **4** with refluxing aqueous ethanol containing 5% hydrobromic acid gave the trihydroxy compound (**5**) in 95% yield. Isopropylidenation of **5** with 2,2-dimethoxypropane in *N*,*N*-dimethylformamide (DMF) in the presence of *p*-toluenesulfonic acid at 60 °C for 2 h yielded a crystalline mixture of the 1,2-*O*- (**6a**) and 2,3-*O*-isopropylidene derivatives (**6b**) in 86% yield. Without separation, the mixture was directly treated with 2 molar equiv. of sodium benzoate in 90% aqueous DMF at 70 °C for 6 h to give through a preferential displacement of the 7-bromine atom a mixture of the benzoates (**7a** and **7b**). They were clearly separated, by chromatography on silica gel and later



	R
4	Ac
5	H

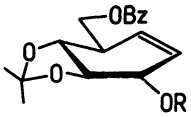


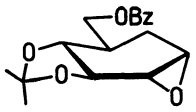
a



b

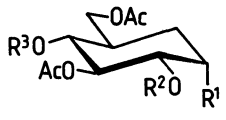
	R^1	R^2	R^3	R^4
6a,b	Br	H	H	Br
7a,b	Br	H	H	OBz
8a,b	H	N_3	H	OBz
9a,b	H	N_3	Ac	OBz
10a,b	H	N_3	Me	OBz





13

	R
11	H
12	Ac

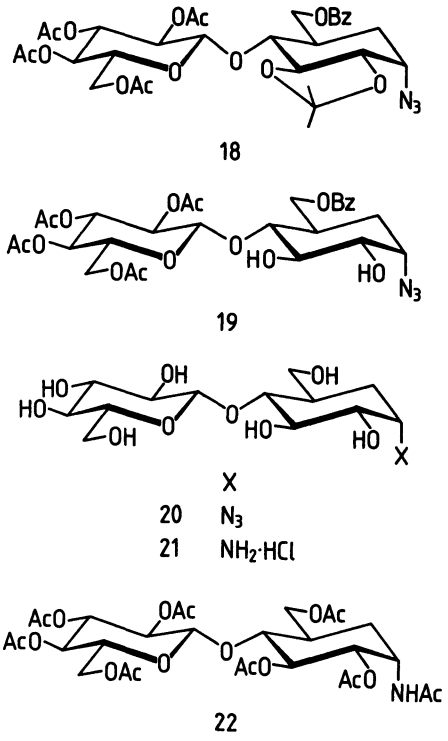


	R^1	R^2	R^3
14	NHAc	Me	Ac
15	N_3	Ac	Me
16	NHAc	Ac	Me
17	NHAc	Ac	Ac

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

by fractional crystallization, to **7a** and **7b** in 8 and 35% yields, respectively. As one of side products, the olefin (**11**) was isolated in pure form, which was further characterized as the acetate (**12**). Compound **11** was also obtained from **7a** in 12% yield by treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene in toluene, together with the epoxide (**13**, 33%). Azidolysis of **7a** with sodium azide in dimethyl sulfoxide (DMSO) at 110 °C for 20 h afforded the azide (**8a**) and **11** in 64 and 4% yields, respectively. On the other hand, a similar treatment of **7b** with an azide ion gave the azide (**8b**) selectively in 73% yield. No formation of any olefinic compound was observed in this case. The structures of **8a** and **8b** were confirmed by converting them into the corresponding *O*-acetyl (**9a** and **9b**), and *O*-methyl derivatives (**10a** and **10b**). Thus, in the ^{1}H NMR spectra, **9a** and **9b** showed a one-proton doublet of doublets (δ=5.05, J=4 and 10.5 Hz) and a one-proton triplet (δ=5.19, J=9 Hz) attributable to a proton attached to the carbon atom bearing the acetoxyl group, respectively, which indicated the location of the acetoxyl groups at C-3 in **9a** and at C-1 in **9b**. For further confirmation, both **8a** and **8b** were converted into penta-*N*,*O*-acetyl-DL-validamine (**17**) by the following sequence: *O*-deisopropylidenation, *O*-deacylation, catalytic reduction, and acetylation. Therefore, **8b** was expected to be a suitable intermediate for the synthesis of the desired β-D-glucopyranoside (**3**). In addition, in order to furnish several reference compounds for assignment of the ^{13}C NMR spectral data of the validamine derivatives, **10a** and **10b** were transformed into the peracetates of 4-*O*-methyl (**16**) and 2-*O*-methylvalidamine (**14**),[5] respectively.

Condensation of **8b** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide was conducted in dry benzene in the presence of mercury(II) cyanide and anhydrous calcium sulfate at 70 °C for 50 h. Under these conditions, only β-D-glucopyranosides were expected to be obtainable. The mixture of products was roughly separated by chromatography on silica gel with 1 : 10 2-butanone–toluene as an eluent to give a syrupy mixture of condesation products [**18**(+) and **18**(−)], showing a single spot in several solvent systems. Without further separation, it was *O*-deisopropylidenated by treatment with Amberlite IR-120B (H^+) in ethanol at ambient temperature overnight. The dihydroxy compounds thus obtained could be fractionated by a silica-gel column with 2: 5 2-butanone–toluene to afford **19**(+), $[\alpha]_D$ +27°, and **19**(−), $[\alpha]_D$ −16°, in 18 and 13% yields, respectively, based on **8b** used. Isopropylidenation of **19**(+) and **19**(−) in the usual way could regenerate crystalline **18**(+) and **18**(−) in pure forms, respectively. Judging from the optical rotations, **19**(+)



Scheme 3. Synthesis of 4-*O*-(β-D-glucopyranosyl)validamine. The formulas depict one of the respective diastereomers.

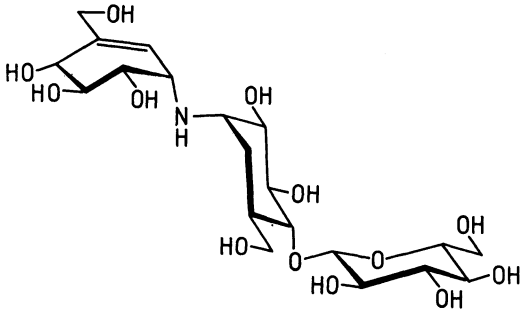
was tentatively assigned as the β-D-glucopyranoside that contained the precursor of (+)-validamine as the aglycone moiety. *O*-Deacylation of **19**(+) with methanolic sodium methoxide in methanol gave the hydroxy azide [**20**(+)], which was successively hydrogenated with 5% palladium on carbon in ethanol containing an excess of hydrochloric acid to give the amine hydrochloride [**21**(+)], $[\alpha]_D$ +22° (H_2O), as a homogeneous syrup in 94% overall yield. This compound was shown to be identical with an authentic sample of β-D-glucopyranosylvalidamine hydrochloride by comparison of chromatographic behavior (TLC on cellulose and silica gel) in several solvent systems. It was further characterized by conversion into the octa-*N*,*O*-acetyl derivative [**22**(+)], $[\alpha]_D$ +16° (lit,[3] $[\alpha]_D$ +17.6°), whose IR (in chloroform) and ^{1}H NMR spectra were superimposable on those of an authentic sample.[6]

On the other hand, the diastereomeric amine hydrochloride **21**(−) obtained similarly from **19**(−) *via* the hydroxy azide [**20**(−)] showed spectral properties similar to those of **21**(+); however, they were clearly differentiated from each other by TLC. Its octa-*N*,*O*-acetyl derivative [**22**(−)] has $[\alpha]_D$ −49°.

Table 1. ^{13}C NMR chemical shifts of peracetates of validamine derivatives in $CDCl_3$

Compound	C-1	C-2	C-3	C-4	C-5	C-6	C-7	OMe
3-*O*-Me (**14**)	71.33	72.83	79.24	45.37	27.90	34.65	63.13	57.21
1-*O*-Me (**16**)	80.24	73.15	71.60	46.32	28.42	36.13	63.64	60.16
17	←———	71.41, 71.63	———→	46.78	28.43	35.21	63.16	
22(+)	—	—	—	45.99	27.92	37.17	62.93	
2-*O*-β-D-Glc[2]	—	—	—	46.15	28.16	35.02	63.51	

A part of the ^{13}C NMR spectral data of validamine derivatives were shown in Table 1. Assignment of the resonances was carried out by comparing the spectrum of the peracetyl derivative with those of the *O*-methyl derivatives. Substitution of the acetoxyl group by methoxyl group causes a downfield shift of the signals for α-carbon atom (8—9 ppm) and β-carbon atom (1—1.5 ppm).[7] The signal for C-6 of octa-*O*-acetyl-2-*O*-β-D-glucopyranosylvalidamine[2] appears at 35.02 ppm, indicative of the location of the acetoxyl group at C-1, whereas the C-6 of **22**(+) resonates downfield at 37.02 ppm, suggesting that D-glucopyranose attaches to the C-1 hydroxyl group by way of β-glucosidic linkage. These assumptions may be compatible with the result of the present synthesis.



Scheme 4. The revised structure of validamycin A.

On the basis of the synthetic studies and the ^{13}C NMR spectral data, the structure of β-D-glucopyranosylvalidamine should be revised to 1L-1-*O*-(β-D-glucopyranosyl)-(1,3,4/2,6)-4-amino-6-hydroxymethyl-1,2,3-cyclohexanetriol. Accordingly, the structure of validamycin A was convincingly formulated as shown in Scheme 4.

Experimental

General Methods. The same method was used as described in the preceding paper.[2] ^{13}C NMR spectra were determined on a Varian FT-80 spectrometer at 20 MHz. The resonance signals are expressed in ppm downfield from the signal of tetramethylsilane.

DL-*(1,3/2,4,6)-4-Bromo-6-bromomethyl-1,2,3-cyclohexanetriol (5).* A mixture of DL-1,2,3-tri-*O*-acetyl-(1,3/2,4,6)-4-bromo-6-bromomethyl-1,2,3-cyclohexanetriol (**4**)[4] (4 g) in ethanol (160 ml) containing 48% hydrobromic acid (20 ml) was refluxed for 4 h. The reaction mixture was concentrated, and the crystallized residue was triturated with water and filtered to give a practically pure **5** (2.7 g, 95%). Recrystallization from ethanol and water gave an analytically pure sample (2 g, 71%) as prisms: mp 153.5—154 °C.

Found: C, 27.76; H, 3.92; Br, 52.48%. Calcd for $C_7H_{12}Br_2O_3$: C, 27.66; H, 3.98; Br, 52.57%.

DL-*1,2*-O- *(6a) and* DL-*2,3*-O-*Isopropylidene-(1,3/2,4,6)-4-bromo-6-bromomethyl-1,2,3-cyclohexanetriol (6b).* A mixture of **5** (2 g), *N,N*-dimethylformamide (DMF) (20 ml), and 2,2-dimethoxypropane (9 ml) was heated in the presence of *p*-toluenesulfonic acid (5 mg) at 70 °C for 2 h. After being cooled to room temperature, the reaction mixture was treated with Amberlite IRA-400 (OH^-) (1.5 ml) and then concentrated. Recrystallization of the residue gave **6a** and **6b** (2 g, 88%) as a homogeneous crystalline mixture: mp 122—125 °C; they showed similar mobilities on TLC in several solvent systems; ^1H NMR ($CDCl_3$) δ=1.42 (6H, s) and 1.48 (6H, s) (isopropylidene).

Found: C, 34.77; H, 4.60; Br, 46.69%. Calcd for $C_{10}H_{16}Br_2O_3$: C, 34.91; H, 4.69; Br, 46.45%.

DL-*1,2*-O- *(7a) and* DL-*2,3*-O-*Isopropylidene-(1,3/2,4,6)-6-benzoyloxymethyl-4-bromo-1,2,3-cyclohexanetriol (7b).* A crystalline mixture of **6a** and **6b** (2 g) was treated with sodium benzoate (1.7 g) in 90% aqueous DMF (40 ml) at 70—80 °C for 6 h. TLC showed the formation of two main components (R_f 0.38 and 0.32, developed twice in 1 : 8 2-butanone–toluene), together with several minor ones. The reaction mixture was then cooled to room temperature and diluted with ethyl acetate (50 ml), and an insoluble material was removed by filtration. The filtrate was concentrated, the residue was dissolved in chloroform, and the solution was passed through a short column of alumina. Evaporation of the solvent gave a syrupy residue, a small portion of which was fractionated on a silica-gel column with 1 : 8 2-butanone–toluene as an eluent to give pure crystals of **7a** and **7b**. The remaining syrup was dissolved in a small amount of ethanol and crystallization was induced by seeding in turn crystals of **7a** and **7b**. Compound **7b** was first obtained as needles (0.78 g, 35%): mp 130—132 °C; ^1H NMR ($CDCl_3$) δ=1.48 (6H, s, isopropylidene), 4.34 (1H, dd, J_{gem}=11 Hz, $J_{6,7}$=2 Hz, H-7), 4.68 (1H, dd, $J_{6,7'}$=4 Hz, H-7′), and 7.33—8.15 (5H, m, phenyl). The second crop of crystals was **7a** (0.29 g, 13%) obtained as prisms: mp 125—127 °C; ^1H NMR ($CDCl_3$) δ= 1.44 (6H, s, isopropylidene), 4.21 (1H, dd, J_{gem}=11 Hz, $J_{6,7}$= 6 Hz, H-7), 4.58 (1H, dd, $J_{6,7'}$=4 Hz, H-7′), and 7.35—8.15 (5H, m, phenyl).

Found for **7a**: C, 52.78; H, 5.60; Br, 20.88%, and for **7b**: C, 53.12; H, 5.44; Br, 20.59%. Calcd for $C_{17}H_{21}BrO_5$: C, 53.00; H, 5.49; Br, 20.74%.

The mother liquor from **7a** and **7b** was concentrated and the residue was chromatographed on a silica-gel column with 1 : 10 2-butanone–toluene as an eluent. DL-2,3-*O*-Isopropylidene-(1,3/2,4)-4-benzoyloxymethyl-5-cyclohexene-1,2,3-triol (**11**) was purely obtained out of four minor products and recrystallized from ethanol to give prisms (20 mg): mp 105—107 °C; ^1H NMR ($CDCl_3$, 90 MHz) δ=1.43 (6H, s, isopropylidene), 2.43 (1H, broad d, OH), 2.58—3.01 (1H, m, H-4), 3.51 (1H dd) and 3.60 (1H, dd) (J=10 and 11 Hz, H-2 and H-3), 4.27 (1H, dd, J_{gem}=11 Hz, $J_{4,7}$=6 Hz, H-7), 4.53 (1H, dd, $J_{4,7'}$=5 Hz, H-7′), 5.67 (2H, s, H-5 and H-6), and 7.28—8.08 (5H, m, phenyl).

Found: C, 66.82; H, 6.53%. Calcd for $C_{17}H_{20}O_5$: C, 67.09; H, 6.62%.

Compound **11** (41 mg) was treated with acetic anhydride (1 ml) and pyridine (1 ml) at room temperature overnight. Concentration of the reaction mixture gave a crude product, which was crystallized from ethanol to give the acetate (**12**, 20 mg, 43%) as thin needles: mp 99.5—102.5 °C; ^1H NMR ($CDCl_3$, 90 MHz) δ=1.47 (6H, s, isopropylidene), 2.13 (3H, s, OAc), 2.77—3.06 (1H, m, H-4), 3.64 (1H, t, $J_{2,3}$=$J_{3,4}$=9 Hz, H-3), 3.82 (1H, t, $J_{1,2}$=9 Hz, H-2), 4.33 (1H, dd, J_{gem}=11 Hz, $J_{4,7}$=6 Hz, H-7), 4.59 (1H, dd, $J_{4,7'}$=5 Hz, H-7′), 5.56 (1H, broad d, H-1), 5.73, (2H, broad d, H-5 and H-6), and 7.33—8.23 (5H, m, phenyl).

Found: C, 66.08; H, 6.53%. Calcd for $C_{19}H_{22}O_6$: C, 65.88; H, 6.40%.

DL-*1,2*-O-*Isopropylidene-(1,3,4/2,6)-4-azido-6-benzoyloxymethyl-1,2,3-cyclohexanetriol (8a) and 11.* A mixture of **7a** (1.5 g), sodium azide (1 g), and dry dimethyl sulfoxide (30 ml) and heated at 110 °C for 20 h. TLC indicated the formation of two components (R_f 0.40 and 0.33, 1 : 5 2-butanone–toluene). Ethyl acetate (45 ml) was added to the reaction mixture and an insoluble material was removed by filtration. The filtrate

was successively washed with aqueous sodium chloride and water, and passed through a short column of alumina. The eluate was concentrated and the residue was chromatographed on a silica-gel column (75 g) with 1 : 10 2-butanone–toluene. The first fraction was concentrated to give crystals which were recrystallized from ethanol to give **8a** (0.89 g, 64%) as prisms: mp 118.5—121 °C; IR 3460 (OH), 2150 (N_3), and 1715 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ=1.45 (6H, s, isopropylidene), 2.92 (1H, broad s, OH), 3.24 (1H, dd, $J_{1,2}$=10 Hz, $J_{1,6}$=8 Hz, H-1), 3.75 (1H, t, $J_{2,3}$=10 Hz, H-2), 4.26—4.70 (2H, m, H-7 and H-7′), and 7.33—8.16 (5H, m, phenyl).

Found: C, 58.54; H, 6.10; N, 11.85%. Calcd for $C_{17}H_{21}N_3O_5$: C, 58.78; H, 6.09; N, 12.10%.

Compound **8a** (35 mg) was acetylated as described for the preparation of **12**. The crude product was recrystallized from ethanol to give the acetate (**9a**, 25 mg, 64%) as prisms: mp 150—153 °C; ^1H NMR ($CDCl_3$) δ=1.43 (3H, s) and 1.47 (3H, s) (isopropylidene), 2.19 (3H, s, OAc), 3.34 (1H, dd, $J_{1,2}$ =8.5 Hz, $J_{1,6}$=10.5 Hz, H-1), 3.95 (1H, dd, $J_{2,3}$=10.5 Hz, H-2), 5.05 (1H, dd, $J_{3,4}$=4 Hz, H-3), and 7.35—8.16 (5H, m, phenyl).

Found: C, 58.34; H, 6.02; N, 10.67%. Calcd for $C_{19}H_{23}N_3O_6$: C, 58.60; H, 5.95; N, 10.79%.

The second fraction gave a syrup which crystallized from ethanol to give **11** (52 mg, 4.4%) as prisms, mp 103—105 °C, identical with the compound derived from **6a**.

Reaction of ***7a*** *with 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU).* A mixture of **7a** (0.2 g), DBU (0.39 ml), and toluene (6 ml) was stirred at 120 °C for 7 h. TLC showed the formation of two major components (R_f 0.58 and 0.21) together with a trace of **7a** (R_f 0.35, 1 : 5 2-butanone–toluene). The reaction mixture was concentrated and the products were fractionated on a silica-gel column (8 g) with 1 : 5 2-butanone–toluene as an eluent. The first fraction gave crystals, which were recrystallized from ethanol to give 1,2-anhydro-3,4-*O*-isopropylidene-(1,2,4/3,5)-5-benzoyloxymethyl-1,2,3,4-cyclohexanetetrol (**13**, 26 mg, 33%) as prisms: mp 87—89.5 °C; IR 1715 (C=O) and 1610 cm^{-1} (phenyl); ^1H NMR ($CDCl_3$) δ=1.45 (6H, s, isopropylidene), 4.26 (1H, dd, J_{gem}=11 Hz, $J_{5,7}$=5 Hz, H-7), 4.53 (1H, dd, $J_{5,7'}$=4 Hz, H-7′), and 7.40—8.18 (5H, m, phenyl).

Found: C, 66.81; H, 6.56%. Calcd for $C_{17}H_{20}O_5$: C, 67.09; H, 6.62%.

The second fraction gave **11** (19 mg, 12%) mp 103—105 °C, identical with the compound obtained from **6a**.

DL-*2,3-O-Isopropylidene-(1,3,4/2,6)-4-azido-6-benzoyloxymethyl-1,2,3-cyclohexanetriol (****8a****).* A mixture of **7b** (1.1 g), sodium azide (0.75 g), and dimethyl sulfoxide (10 ml) was stirred at 120 °C for 18 h. The reaction mixture was processed as described for the preparation of **8a**. The syrupy product was crystallized from ethanol to give **8b** (0.72 g, 73%) as needles: mp 105—108 °C; IR 3480 (OH), 2170 (N_3), 1730 (C=O), and 1610 cm^{-1} (phenyl); ^1H NMR ($CDCl_3$) δ=1.45 (3H, s) and 1.50 (3H, s) (isopropylidene), 3.31 (1H, m, OH), 3.52 (1H, dd, $J_{2,3}$=9 Hz, $J_{3,4}$=3 Hz, H-3), 3.67 (1H, dd, $J_{1,2}$=9 Hz, $J_{1,6}$=8 Hz, H-1), 4.03 (1H, t, H-2), 4.30 (1H, dd, J_{gem}=11 Hz, $J_{6,7}$=2 Hz, H-7), 4.77 (1H, dd, $J_{6,7'}$=4 Hz-H-7′), and 7.35—8.15 (5H, m, phenyl).

Found: C, 58.63; H, 6.11; N, 11.88%. Calcd for $C_{17}H_{21}N_3O_5$: C, 58.78; H, 6.09; N, 12.10%.

Compound **8b** (50 mg) was acetylated in the usual way and the product was recrystallized from ethanol to give the acetate (**9b**, 30 mg, 54%) as needles: mp 116—119 °C; ^1H NMR ($CDCl_3$) δ=1.49 (6H, s, isopropylidene), 2.13 (3H, s, OAc), 3.63 (1H, dd, $J_{2,3}$=9 Hz, $J_{3,4}$=3 Hz, H-3), 4.09 (1H, t, $J_{1,2}$=9 Hz, H-2), 5.19 (1H, t, $J_{1,6}$=9 Hz, H-1), and 7.35—8.20 (5H, m, phenyl).

Found: C, 58.72; H, 5.98; N, 10.84%. Calcd for $C_{19}H_{23}N_3O_6$: C, 58.60; H, 5.95; N, 10.79%.

DL-*1,2-O-Isopropylidene-3-O-methyl-(1,3,4/2,6)-4-azido-6-benzoyloxymethyl-1,2,3-cyclohexanetriol (****10a****).* A mixture of **8a** (0.3 g), methyl iodide (0.54 ml), and silver oxide (0.4 g) in dry DMF (3 ml) was stirred at room temperature for 20 h in the dark. Acetone (10 ml) was added to the reaction mixture and an insoluble material was removed by filtration. The filtrate was concentrated and the residue was coevaporated with 1-butanol several times. The product was dissolved in ethyl acetate and the solution was passed through a short column of alumina. The eluate was concentrated and the residue was crystallized from ethanol to give **10a** (0.13 g, 40%) as prisms: mp 82—85 °C; IR 2150 (N_3), 1720 (C=O), and 1610 cm^{-1} (phenyl); ^1H NMR ($CDCl_3$) δ=1.42 (6H, s, isopropylidene), 3.20 (1H, dd, $J_{1,2}$=9 Hz, $J_{1,6}$=10 Hz, H-1), 3.49 (1H, dd, $J_{2,3}$=10 Hz, $J_{3,4}$=3 Hz, H-3), 3.52 (3H, s, methoxyl), 3.84 (1H, dd, H-2), 4.18 (1H, q, $J_{4,5\,ax}=J_{4,5\,eq}$=3 Hz, H-4), 4.22 (1H, dd, J_{gem}=11.5 Hz, $J_{6,7}$=5.5 Hz, H-7), 4.50 (1H, dd, $J_{6,7'}$=4.5 Hz, H-7′), and 7.34—8.13 (5H, m, phenyl).

Found: C, 59.54; H, 6.33; N, 11.85%. Calcd for $C_{18}H_{23}N_3O_5$: C, 59.80; H, 6.42; N, 11.63%.

DL-*2,3-O-Isopropylidene-1-O-methyl-(1,3,4/2,6)-4-azido-6-benzoyloxymethyl-1,2,3-cyclohexanetriol (****10b****).* A mixture of **8b** (1 g), methyl iodide (1.8 ml), and silver oxide (1.3 g) in dry DMF (10 ml) was stirred at room temperature for 20 h in the dark. At this stage, TLC showed the presence of **9b**. Then an additional amount of methyl iodide (0.9 ml) was added to the mixture and the stirring was continued for 23 h. The reaction mixture was processed as described for the preparation of **10a**. The crude product was recrystallized from ethanol to give **10b** (0.33 g, 32%) as needles: mp 105—107 °C; IR 2140 (N_3), 1730 (C=O), and 1610 cm^{-1} (phenyl); ^1H NMR ($CDCl_3$) δ 1.48 (3H, s) and 1.51 (3H, s) (isopropylidene), 3.32 (1H, t, $J_{1,2}=J_{1,6}$=9 Hz, H-1), 3.52 (1H, dd, $J_{2,3}$=9 Hz, $J_{3,4}$ =3 Hz, H-3), 3.55 (3H, s, methoxyl), 4.03 (1H, t, H-2), 4.21 (1H,q, $J_{4,5ax}=J_{4,5eq}$=3 Hz, H-4), 4.47 (2H, d, J=3 Hz, H-7 and H-7′), and 7.35—8.13 (5H, m, phenyl).

Found: C, 59.82; H, 6.36; N, 11.39%. Calcd for $C_{18}H_{23}N_3O_5$: C, 59.80; H, 6.42; N, 11.63%.

DL-*1,2,7-Tri-O-acetyl-3-O-methyl-(1,3,4/2,6)-4-acetamido-6-hydroxymethyl-1,2,3-cyclohexanetriol (****14****).* Compound **10a** (0.1 g) was treated with Amberlite IR-120 (H^+) (1.5 ml) in ethanol (8 ml) at room temperature for 3 h. TLC indicated the formation of a single product (R_f 0.15, 1:2 2-butanone–toluene, **10a**: R_f 0.77). The resin was removed and the solution was concentrated to give a syrup which was treated with 0.3 M methanolic sodium methoxide(3 ml)(1 M=1 mol dm^{-3}) at room temperature for 0.5 h. TLC showed the presence of a single product (R_f 0.46, 5 : 1 chloroform–methanol, the starting compound: R_f 0.78). The reaction mixture was neutralized with Amberlite IR-120 (H^+) (1.5 ml) and then concentrated to give crystals (62 mg). A solution of it in methanol containing 1 M hydrochloric acid (3 ml) was hydrogenated in the presence of 5% palladium on carbon (15 mg) at room temperature for 16 h. The product showed a single spot (R_f 0.27) on TLC (4 : 1 : 1 1-propanol–acetic acid–water). The catalyst was removed by filtration and the filtrate was concentrated to give a crystalline 2-*O*-methyl-DL-validamine hydrochloride (46 mg, 78%). An aqueous solution of the hydrochloride was treated with Amberlite IRA-400 (OH^-) (1.5 ml) and then concentrated to a syrup, which was treated with acetic anhydride and pyridine in the usual way to give the total acetate. Recrystallization from ethanol gave **14** (22 mg, 22%) as prisms: mp 205.5—208 °C; IR 3350, 1645, and 1545 (amide), 1750 cm^{-1} (ester); ^1H NMR ($CDCl_3$, 90 MHz) δ=2.00 (3H, s), 2.03 (6H, s), and 2.04 (3H, s) (NAc and OAc), 3.34 (3H, s, methoxyl), 3.35 (1H, dd, $J_{2,3}$=9 Hz,

$J_{3,4}$=5 Hz, H-7), 3.86 (1H, dd, J_{gem}=11 Hz, $J_{6,7}$=4 Hz, H-7), 4.14 (1H, dd, $J_{6,7'}$=5 Hz, H-7'), 4.42 (1H, m, H-4), 4.88 (1H, t, $J_{1,2}=J_{1,6}$=9 Hz, H-1), 5.10 (1H, t, $J_{2,3}$=9 Hz, H-2), and 5.87 (1H, broad d, J=6 Hz, NH).

Found: C, 53.21; H, 6.87; N, 3.72%. Calcd for $C_{16}H_{25}NO_8$: C, 53.47; H, 7.01; N, 3.90%.

DL-*2,3,7-Tri*-O-*acetyl-1*-O-*methyl-(1,3,4/2,6)-4-azido-6-hydroxymethyl-1,2,3-cyclohexanetriol* (**15**). Compound **10b** (0.1 g) was treated with Amberlite IR-120 (H^+) (1 ml) in ethanol, and the product was successively treated with 0.3 M methanolic sodium methoxide to give a crystalline hydroxy azide (56 mg, 93%). This compounds was acetylated in the usual way and the acetate was recrystallized from ethanol to give **15** (47 mg, 50%) as needles: mp 71—73 °C; IR 2130 (N_3) and 1740 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ=2.08 (9H, s, OAc), 3.15 (1H, dd, $J_{1,2}$=9 Hz, $J_{1,6}$=10.5 Hz, H-1), 3.38 (3H, s, methoxyl), 4.05 (1H, q, $J_{3,4}=J=_{4,5ax}=J_{4,5eq}$=3 Hz, H-4), 4.13 (2H, d, J=4 Hz, H-7 and H-7'), 4.86 (1H, dd, $J_{2,3}$=10 Hz, H-3), and 5.38 (1H, dd, H-2).

Found: C, 49.08; H, 6.13; N, 12.32%. Calcd for $C_{14}H_{21}N_3O_7$: C, 48.95; H, 6.17; N, 12.24%.

DL-*2,3,7-Tri*-O-*acetyl-1*-O-*methyl-(1,3,4/2,6)-4-acetamido-6-hydroxymethyl-1,2,3-cyclohexanetriol* (**16**). Compound **15** (75 mg) was hydrogenated and then acetylated as described for the preparation of **14**. The crude product was purified on a silica-gel column (5 g) with 1 : 1 2-butanone–toluene as an eluent to give **16** (60 mg, 76%) as a homogeneous solid: IR 3290, 1655, and 1550 (amide), and 1750 cm^{-1} (ester); ^1H NMR ($CDCl_3$, 90 MHz) δ=2.00 (3H, s), 2.02 (3H, s), and 2.08 (6H, s) (NAc and OAc), 3.11 (1H, t, $J_{1,2}=J_{1,6}$=9 Hz, H-1), 3.41 (3H, s, methoxyl), 4.15 (2H, d, J=4 Hz, H-7 and H-7'), 4.48 (1H, m, H-4), 4.86 (1H, dd, $J_{2,3}$=10 Hz, $J_{3,4}$=5 Hz, H-3), 5.18 (1H, dd, $J_{1,2}$=9 Hz, H-2), and 6.03 (1H, broad d, J=8 Hz, NH).

Found: C, 53.18; H, 6.85; N, 3.76%. Calcd for $C_{16}H_{25}NO_8$; C, 53.47; H, 7.01; N, 3.90%.

1L-[19(+)] and 1D-1-O-*(2,3,4,6-Tetra*-O-*acetyl-β-D-glucopyranosyl-1,3,4/2,6-4-azido-6-benzoyloxymethyl1,-2,3-cyclohexanetriol [19(−)]*. A mixture of **8b** (1.1 g), mercury(II) cyanide (7 g), anhydrous calcium sulfate (Drierite) (7 g), and dry benzene (300 ml) was refluxed at 90 °C with stirring, and 140 ml of benzene was removed by distillation. Then freshly prepared 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (5.2 g) was added to the mixture and it was stirred vigorously at 65—70 °C for 50 h. After being cooled to room temperature, the reaction mixture was treated with triethylamine (3 ml) and an insoluble material was removed by filtration through a caoline bed and washed thoroughly with chloroform. The filtrate and washings were combined and concentrated to give a brown syrup, which was dissolved in ethyl acetate (180 ml), washed successively with aqueous sodium hydrogencarbonate (3×200 ml) and water (2×200 ml), and dried. Evaporation of the solvent gave a syrup (9.3 g) which was chromatographed on a silica-gel column (130 g) with 1 : 10 2-butanone–toluene as an eluent. Fractions having R_f 0.24 in the same solvent system (irrigated twice) were concentrated to give a pale yellow syrup (1.3 g), whose ^1H NMR spectrum showed the presence of one isopropylidene, four acetoxyl, and one benzoyloxyl groups, and a contamination of traces of unidentified side-products. A 1.3 g-portion of the syrup was dissolved in ethanol (10 ml) and treated with Amberlite IR-120B (H^+) (20 ml) at room temperature for 21 h. The resin was filtered off and the filtrate was concentrated to a syrup (1.3 g), which was chromatographed on a silica-gel column (60 g) with 2 : 5 2-butanone–toluene as an eluent. The main fraction (R_f 0.41, 1 : 1 2-butanone–toluene) gave crystals 0.363 g (18%), which were recrystallized from ethanol to give **19**(+) (0.262 g, 13%) as needles: mp 182—183.5 °C; $[\alpha]_D^{24}$ +27° (*c* 1.0, chloroform); IR 3540 (OH), 2150 (N_3), 1750 (C=O), and 1610 cm^{-1} (phenyl); ^1H NMR ($CDCl_3$) δ=2.00 (3H, s), 2.04 (3H, s), 2.07 (3H, s), and 2.16 (3H, s) (OAc), and 7.35—8.13 (5H, m, phenyl).

The second fractions (R_f 0.34) were concentrated to give an amorphous solid (0.262 g, 13%), which was recrystallized from ethanol and ether to give **19**(−) (0.162 g, 8%) as needles: mp 158—161 °C; $[\alpha]_D^{23}$ −16° (*c* 1.5, chloroform); IR 3500 (OH), 2150 (N_3), 1750 (C=O), and 1610 cm^{-1} (phenyl); ^1H NMR ($CDCl_3$) δ=1.99 (3H, s), 2.0 2(3H, s), 2.10 (3H, s), and 2.17 (3H, s) (OAc), and 7.36—8.13 (5H, m, phenyl).

Found for **19**(+): C, 52.84; H, 5.47; N, 6.32%, and for **19**(−): C, 52.50; H, 5.51; N, 6.45%. Calcd for $C_{28}H_{35}NO_{14}$: C, 52.72; H, 5.54; N, 6.59%.

Preparation of the Isopropylidene Derivative 18(+) of 19(+). A mixture of **19**(+) (50 mg), 2,2-dimethoxypropane (0.1 ml), DMF (2 ml), and *p*-toluenesulfonic acid (2 mg) was heated at 65—70 °C for 11 h. During this reaction time, 0.2 ml portions of 2,2-dimethoxypropane were added to the reaction mixture at intervals of 3 h. TLC showed one major spot (R_f 0.44) and one minor spot (R_f 0.09) due to **19**(+) in 1 : 3 2-butanone–toluene. The mixture was treated with Amberlite IRA-400 (OH^-) (1 ml) and then concentrated to a syrup ,which was chromatographed on a silica gel (2 g) with 1 : 3 2-butanone-toluene as an eluent. The main fraction gave a syrup, which crystallized from ethanol to give **18**(+) (20 mg, 38%) as plates: mp 166—170 °C $[\alpha]_D^{23}$ +21° (*c* 0.5, chloroform); ^1H NMR ($CDCl_3$) δ=1.48 (6H, broad s, isopropylidene), 1.92 (3H, s), 1.99 (3H, s), 2.02 (3H, s), and 2.06 (3H, s) (OAc), and 7.34—8.12 (5H, m, phenyl).

Found: C, 55.00; H, 5.90; N, 6.07%. Calcd for $C_{31}H_{39}N_3O_{14}$: C, 54.94; H, 5.80; N, 6.20%.

Preparation of the Isopropylidene Derivative 18(−) of 19(−). Compound **19**(−) (50 mg) was *O*-isopropylidenated as described for the preparation of **18**(+) to give **18**(−) (49 mg, 92%) as a homogeneous solid, which crystallized from ethanol to give needles (29 mg, 56%): mp 174—174.5 °C; $[\alpha]_D^{23}$ −26° (*c* 0.58, chloroform): ^1H NMR ($CDCl_3$) δ=1.48 (6H, broad s, isopropylidene), 1.99 (6H, s), 2.01 (3H, s), and 2.08 (3H, s) (OAc), and 7.32—8.12 (5H, m, phenyl).

Found: C, 55.16; H, 5.91; N, 6.10%. Calcd for $C_{31}H_{39}N_3O_{14}$: C, 54.94; H, 5.80; N, 6.20%.

Both **18**(+) and **18**(−) had similar mobilities on TLC (R_f 0.44, 1 : 3 2-butanone–toluene).

1L-1-O-*(β-D-Glucopyranosyl)-(1,3,4/2,6)-4-amino-6-hydroxymethyl-1,2,3-cyclohexanetriol Hydrochloride [21(+)]*. To a solution of **19**(+) (100 mg) in methanol (5 ml) was added 1 M methanolic sodium methoxide (8 ml) and the mixture was kept at room temperature for 24 h. The reaction mixture was treated with Amberlite IR-120B (H^+) (12 ml) and then concentrated to give the hydroxy azide **20**(+) (61 mg) as a homogeneous syrup (R_f 0.61, 4 : 1 : 1 1-propanol–acetic acid–water). This compound was directly hydrogenated in a mixture of methanol (4 ml) and water (4 ml) containing 0.5 M hydrochloric acid (0.38 ml) in the presence of 5% palladium on carbon (10 mg) at room temperature overnight. The catalyst was removed by filtration and the filtrate was concentrated to give **21**(+) [56 mg, 94% yield based on **19**(+)] as a syrup, $[\alpha]_D^{23}$ +22° (*c* 1.9, water). This compound was identical with an authentic sample derived from validamycin A, as found from comparison of the mobilities on TLC: silica gel, R_f 0.24 in 4 : 1 : 1 1-propanol–acetic acid–water and R_f 0.35 in 4 : 5 : 2 : 4 1-butanol–ethanol–acetic acid–water; cellulose, R_f 0.15 in 6 : 4 : 3 : 1 1-butanol–pyridine–water–acetic acid (ref. validamine hydrochloride, R_f 0.17).

1-D-O-(β-D-Glucopyranosyl)-(1,3,4/2,6)-4-amino-6-hydroxy-

methyl-1,2,3-cyclohexanetriol Hydrochloride [**21**(−)]. Compound **19**(−) (100 mg) was *O*-deacylated with methanolic sodium methoxide as described for the preparation of **20**(+). The hydroxy azide **20**(−) (64 mg) thus obtained was hydrogenated similarly to give **21**(−) [53 mg, 88% yield based on **19**(−)] as a syrup, which was clearly differentiated from **21**(+) on TLC, showing mobilities slightly higher than those of **21**(+) in all the solvent systems examined; silica gel, R_f 0.28 in 4 : 1 : 1 1-propanol–acetic acid–water and R_f 0.40 in 4 : 5 : 2 : 4 1-butanol–ethanol–acetic acid–water; cellulose, R_f 1.18 in 6 : 4 : 3 : 1 1-butanol–pyridine–water–acetic acid when **21**(+) was used as an reference (R_f 1.0).

1L-1,2,3,6-Tetra-O-acetyl-1-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-(1,3,4/2,6)-4-acetamido-6-hydroxymethyl-1,2,3-cyclohexanetriol [**22**(+)]. Compound **21**(+) (50 mg) was dissolved in water (5 ml) and treated with Amberlite IRA-400 (OH^-) (1.5 ml) at room temperature for 30 min. The resin was removed by filtration and the filtrate was dried by azeotropic distillation with ethanol and toluene. The residual free base was treated with acetic anhydride (2 ml) and pyridine (2 ml) at 50 °C for 20 h. The reaction mixture was concentrated and the residue was chromatographed on a silica-gel column (4 g) with 1 : 1 2-butanone–toluene as an eluent. The fraction having R_f 0.43 in 20 : 1 chloroform–methanol was concentrated to give **22**(+) (50 mg, 57%) as an amorphous solid: $[\alpha]_D^{23}$ +16° (*c* 0.75, chloroform) [lit,[3] $[\alpha]_D^{23}$ +17.6° (chloroform)]; IR ($CHCl_3$) 3440 (NH), 1770—1740 (C=O), 1680, and 1520 cm^{-1} (amide); ^1H NMR ($CDCl_3$) δ=1.98 (3H, s), 2.00 (6H, s), 2.02 (9H, s), 2.07 (3H, s), and 2.10 (3H, s) (NAc and OAc), and 6.30 (1H, d, J=7 Hz, NH). The spectrum was superimposable on that of an authentic sample.[6]

Found: C, 51.30; H, 6.19; N, 2.20%. Calcd for $C_{29}H_{41}NO_{17}$: C, 51.56; H, 6.12; N, 2.07%.

Compound **22**(+) showed a double melting point. Thus, the amorphous solid melted at 114—118 °C (lit,[3] 117—119 °C) and the melt, on continuous heating on a hot stage, crystallized at 150—160 °C to give needles, which melted sharply at 187—189 °C. The same melting and crystallization behavior was observed for an authentic sample (mp 112—119 and 183—185 °C).

1D-1,2,3,6-Tetra-O-acetyl-1-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-(1,3,4/2,6)-4-acetamido-6-hydroxymethyl-1,2,3-cyclohexanetriol [**22**(−)]. Amine hydrochloride **21**(−) (53 mg) was acetylated as described for the preparation of **22** (+) to give **22**(−) (41 mg, 44%) as a homogeneous syrup (R_f 0.51, 20 : 1 chloroform–methanol): $[\alpha]_D^{24}$ −49° (*c* 0.75, chloroform); IR ($CHCl_3$) 3440 (NH), 1740—1765 (C=O), 1680, and 1505 cm^{-1} (amide); ^1H NMR ($CDCl_3$) δ=1.99 (3H, s), 2.01 (6H, s), 2.02 (6H, s), 2.11 (3H, s), and 2.17 (6H, s) (NAc and OAc), and 6.06 (1H, broad d, J=7 Hz, NH).

Found: C, 51.48; H, 6.15; N, 1.93%. Calcd for $C_{29}H_{41}NO_{17}$: C, 51.56; H, 6.12; N, 2.07%.

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