Synthetic Studies on the Validamycins. IX. Synthesis of Some Racemic Isomers of Validoxylamine A[1]

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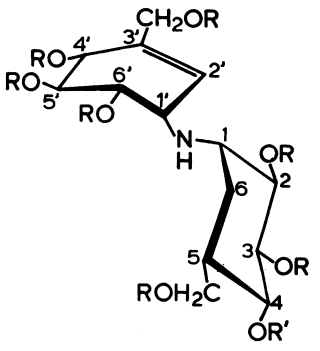
Two racemic isomers of validoxylamine A have been synthesized by use of coupling reactions of the protected DL-validamine and the allyl bromides, the precursors of the unsaturated branched-chain cyclitol moiety. The racemic diastereomers thus formed can be separated by chromatography on silica gel. It indicates that optically pure validoxylamine A analogs should be obtained if chiral validamine derivative is used in place of the racemate.

The validamycins,[2] isolated from the culture filtrate of *Streptomyces hygroscopicus* var. *limoneous*, is a complex mixture of novel aminocyclitol antibiotics[3] containing a single amino group linked to two separate branched-chain cyclitols. The antibiotics are widely used for the treatment of sheath blight disease of rice plants. Among them, validamycin A (**1**) is the major and most active component.[4]

Hydrolysis of **1** with 1 M (=1 mol dm^{-3}) sulfuric acid gave validoxylamine A (**2**)[5,6] and D-glucose. Compound **2** was assigned as *N*-[(1*S*)-(1,4,6/5)-3-hydroxymethyl-4,5,6-trihydroxycyclohex-2-enyl]-(1*S*)-(1,2,4/3,5)-2,3,4-trihydroxy-5-(hydroxymethyl)cyclohexylamine, and was also found in the validamycin producing culture.[4b] It is not only an aglycon part of **1** but also a common structural unit of other validamycins (C, D, E, and F),[7] except for validamycin B which has validoxylamine B,[8] the C-6 hydroxy analog of **2**, as an aglycon. Although **2** showed a low activity compared with validamycin A in the "dendroid-test method," it exhibits a considerable activity in the green house test.[4b]



	R	R'
Validamycin A (1)	H	β-D-glucopyranosyl
Validoxylamine A (2)	H	H
Validoxylamine A octa-O-acetate (3)	Ac	Ac

As a part of the study directed toward the total synthesis of **1** and the related substances,[9] we now wish to describe the stereoselective synthesis of two racemic isomers of **2**, that is, "reversed" validoxylamine A (**18a** and **18b**) and 1′-epivalidoxylamine A (**23a** and **23b**), in order to elucidate the structure activity relationships of this type of pseudo-disaccharides. For the synthesis of the validoxylamine A analogs, we have undertaken a coupling reaction of the protected DL-validamine (**11**) and appropriate allyl bromides (**15** and **19**),[10] the precursors of the unsaturated branched-chain cyclitol moiety.

Results and Discussion

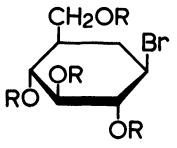
Preparation of Protected DL-*Validamine.* Di-*O*-isopropylidene derivative **11** of DL-validamine was prepared from readily available DL-1,2,3-tri-*O*-acetyl-(1,3/2,4,6)-4-bromo-6-acetoxymethyl-1,2,3-cyclohexanetriol (**4**).[11]

Hydrolysis of **4** with 2 M hydrochloric acid yielded the hydroxy compound **5**, which was then treated with 2,2-dimethoxypropane in *N*,*N*-dimethylformamide (DMF) in the presence of a small amount of *p*-toluenesulfonic acid at 60 °C to give the di-*O*-isopropylidene derivative **9** in 77% overall yield. When **9** was allowed to react with sodium azide in dimethyl sulfoxide at 110 °C, the azido compound **10** was produced in 78% yield. In agreement with the inversion of the configuration at C-4, the ^{1}H NMR spectrum of **10** showed a quartet (J= 3 Hz) at δ=4.26, assignable to the C-4 equatorial proton.

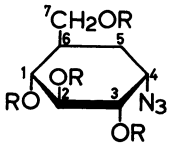
Alternatively, **10** was provided, in less satisfactory yield, from DL-1,2,3-tri-*O*-acetyl-(1,3,4/2,6)-6-acetoxymethyl-4-azido-1,2,3-cyclohexanetriol (**6**),[11] which was obtained by treatment of **4** with sodium azide. Thus, *O*-deacetylation of **6** with methanolic sodium methoxide gave the hydroxy compound **7** in 52% yield, together with elimination product, DL-(1,3/2,4)-4-hydroxymethyl-5-cyclohexene-1,2,3-triol (**8**) in 12% yield. Similar isopropylidenation of **7** afforded **10** in 83% yield.

The IR spectrum and the elemental analysis supported the assigned structure of **8**.[12] As far as we are aware, such an elimination of an azido function as hydrazoic acid under basic conditions have not hitherto been encountered in the literature except for the report by Uryu *et al.*[13]

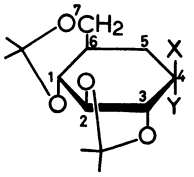
Catalytic hydrogenation of **10** with Raney nickel T-4[14] in ethanol gave the protected validamine **11** in 76% yield, which was further characterized as the *N*-acetyl derivative **12**. As it is well known that introduction of the electron-withdrawing group into an amino group increases the nucleophilicity of the nitrogen atom,[15] the *N*-(*p*-tolysulfonyl) **13** and *N*-(trifluoroacetyl) derivatives



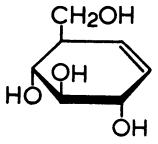
4 R=Ac
5 R=H



6 R=Ac
7 R=H

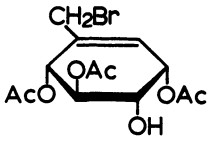


	X	Y
9	Br	H
10	H	N_3
11	H	NH_2
12	H	NHAc
13	H	NHTs
14	H	$NHCOCF_3$



8

11 +



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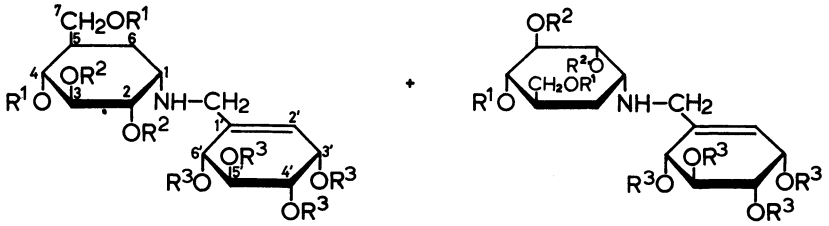


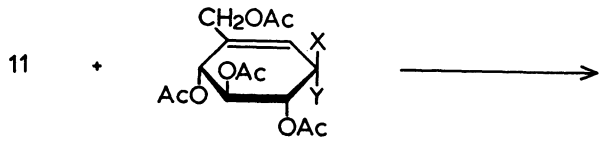
	R^1	R^2	R^3
16 a,b	$>C(CH_3)_2$	$>C(CH_3)_2$	Ac
17 a,b	Ac	Ac	Ac
18 a,b	H	H	H

14 were prepared as the nucleophiles for the allyl bromides.

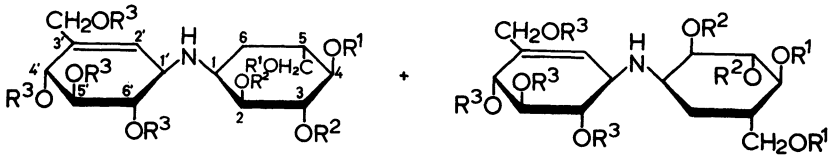
Synthesis of Racemic Reversed Validoxylamine A. The allyl bromide, DL-1,3,4-tri-*O*-acetyl-(1,2,4/3)-5-bromomethyl-5-cyclohexene-1,2,3,4-tetrol (**15**), was prepared according to the procedure described previously.[10] The coupling reaction of **11** with **15** in chloroform in the presence of *N,N*-diisopropylethylamine under reflux for 3 d, followed by the conventional acetylation, gave the expected two racemic isomers **16a** and **16b** in 80% yield. Isolation of the diastereomeric mixture by use of a silica-gel column eluting with 1 : 3 2-butanone–toluene gave **16a** (19%) and **16b** (27%). In agreement with the postulated structures, the ^{1}H NMR spectra of **16a** and **16b** showed the presence of four acetoxyl and two isopropylidene groups. The splitting pattern of the signals due to the ring protons of the unsaturated cyclitol moiety appeared to be somewhat different each other. The elemental analyses were also consistent with their structures.

Removal of the isopropylidene groups of **16a** and **16b**, followed by acetylation, gave the corresponding octa-*O*-acetyl derivatives **17a** and **17b**, respectively. The ^{1}H NMR spectra of **17a** and **17b** were very similar and the C-2′ olefinic proton of **17b** resonated at higher field (0.07 ppm) than that of **17a**. It is noteworthy that acetylation, even under the forcing conditions, gave no *N*-acetylated compound.

O-Deacetylation of **17a** and **17b** was effected by treatment with methanolic sodium methoxide in methanol to give the racemic "reversed" validoxylamine A (**18a** and **18b**), respectively. They showed the similar chromatographic behaviors (R_f 0.11) on TLC [4 : 1 : 1 1-propanol–acetic acid–water, *cf.* validoxylamine A (**2**):[16] R_f 0.33]. In the present stage, it is not possible to determine which diastereomer is which, for instance, on



	X	Y
19	H	Br
20	Br	H



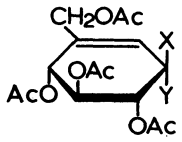
	R^1	R^2	R^3
21 a,b	$>C(CH_3)_2$	$>C(CH_3)_2$	Ac
22 a,b	Ac	Ac	Ac
23 a,b	H	H	H

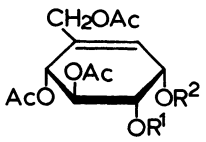
the basis of ^{1}H NMR spectroscopy.

Synthesis of Racemic 1'-Epivalidoxylamine A. Next the synthesis of the racemic 1'-epivalidoxylamine A (**23a** and **23b**) was carried out by a coupling of **11** with DL-1,2,3-tri-*O*-acetyl-(1,3,6/2)-4-acetoxymethyl-6-bromo-4-cyclohexene-1,2,3-triol (**19**).[10]

When molar equivalent of **11** and **19** were allowed to react in DMF in the presence of *N*,*N*-diisopropylethylamine at room temperature for 20 d, the pseudo-disaccharide **21a** and **21b** were obtained mainly, after chromatography on silica gel, as a homogeneous mixture in 40% yield, together with a mixture of pentaacetate **24** and **25**[10] (7%), a mixture of tetraacetate **26** and **27**[10] (23%), the *N*-acetyl derivative **12** (18%) of **11**, and recovered **11** (17%). These compounds were identified with authentic samples by comparing the ^{1}H NMR spectra. In the ^{1}H NMR spectrum of the mixture of **21a** and **21b**, the signals for the isopropylidene and acetoxymethyl groups appeared at δ=1.36—1.56 and 1.92—2.20, respectively, and that of the olefinic proton at δ=5.83.

For the synthesis of validoxylamine A, the C-6 epimer **20** of **19** would be a desirable substrate, if the nucleophilic substitution reaction with **11** proceeds *via* an S_N2 mechanism. As **20** had not been available as a pure compound, a 3.5 : 1 mixture of **19** and **20** was used in the coupling reaction with **11**. However, the reaction gave no desired products at all but the same mixture of products as was obtained from **19**. This facts indicate that the reaction pathway might be independent of the configuration of the allylic bromine atom, and the reaction mechanism may be speculated as follows: Due to a low nucleophilicity of **11**, **20** is likely to be converted into the 1,6-cyclic acetoxonium ion, by neighboring group participation of the C-1 acetoxyl group, which is cleaved by moisture or a back-side attack of **11** at C-6. On the other hand, **19** may suffer an S_N2 attack by **11** and/or by a bromide ion generated from the initial reaction of **19** with **11**, giving rise to an equilibrium mixture of **19** and **20**.





	X	Y
24	H	OAc
25	OAc	H

	R^1	R^2
26	H	Ac
27	Ac	H

The similar reaction was carried out by using **13** or **14** instead of **11**. However, desired secondary amines were not obtained at all, presumably due to the steric hindrance.

When the coupling reaction was conducted at elevated temperature (60 °C), the formation of the side-products became preferable.

Separation of the mixture of **21a** and **21b** was not possible because of their similar chromatographic behaviors. Removal of the isopropylidene groups, followed by the conventional acetylation, gave the diastereomeric mixture (**22a** and **22b**) of 1'-epivalidoxylamine A octaacetate, which were fractionated by a silica-gel column with 1 : 2 ethyl acetate–toluene to give **22a** and **22b** in 23 and 41% isolated yields, respectively. Their elemental analyses and ^{1}H NMR spectral data were consistent with the structures proposed. In both ^{1}H NMR spectra, the signals due to the acetoxymethyl protons of the saturated cyclitol moiety and those of the unsaturated cyclitol moiety could be assignable.

Compounds **22a** and **22b** showed R_f 0.40 and 0.35, respectively on TLC in 1 : 1 ethyl acetate–toluene, while validoxylamine A octaacetate (**3**)[17] R_f 0.32. The ^{1}H NMR spectrum of **3** revealed a doublet (J=5.3 Hz) at δ=5.95, ascribable to the C-2 olefinic proton, whereas those of **22a** and **22b** have a broad singlet at δ=5.70 and 5.65, respectively, indicative of the pseudoequatorial orientation of the imino group.

It is interesting to note that, in the ^1H NMR spectrum of **22b**, the chemical shifts and the splitting pattern of the signals due to the ring protons of the saturated cyclitol part as well as the acetoxylmethyl protons resemble those of **3**, suggesting that **22b** adopts more similar conformation to that of **3** in chloroform than does **22a**. Similar to the case of the reversed validoxylamine A, the *N*-acetyl derivatives of 1'-epivalidoxylamine A could not be prepared.

O-Deacetylation of **22a** and **22b** with sodium methoxide in methanol afforded the racemic 1'-epivalidoxylamine A (**23a**) and (**23b**), respectively, which showed almost similar mobilities (*Rf* 0.28) on TLC in 4 : 1 : 1 1-propanol–acetic acid–water [*cf.* validoxylamine A (**2**): R_f 0.31].

The present results indicate that resolution of the coupling products should be possible by using optically active validamine[18] instead of the racemate. Biological and biochemical studies on the isomers of validoxylamine A obtained here are on the way.

Experimental

Melting points were determined on a Büchi 510 capillary melting point apparatus and are uncorrected. ^1H NMR spectra were recorded on a Varian EM-390 (90 MHz) spectrometer in chloroform-*d*. Chemical shifts were reported as ppm relative to tetramethylsilane as an internal standard. TLC was performed on precoated silica gel 60 F-254 plates (Merck, Darmstadt; 0.25 mm thickness), and the spots were visualized by concd sulfuric acid spray (heating at 120 °C) or ninhydrin spray. Conventional chromatography was carried out with Wakogel C-300 (silica gel, Wako Pure Chemical Industries, Ltd.). Solutions were dried over anhydrous sodium sulfate and concentrated below 50 °C under reduced pressure. Catalytic hydrogenation was carried out in a Parr shaker type apparatus in the initial hydrogen pressure of 3.5 kg/cm² at room temperature.

DL-*(1,3/2,4,6)-4-Bromo-6-hydroxymethyl-1,2,3-cyclohexanetriol (5).* To a solution of DL-1,2,3-tri-*O*-acetyl-(1,3/2,4,6)-6-acetoxymethyl-4-bromo-1,2,3-cyclohexanetriol (**4**)[11] (2.84 g) in ethanol (18 ml) was added 2 M hydrochloric acid (17 ml), and the mixture was heated for 3 h at 80 °C. The mixture was evaporated and then coevaporated with ethanol several times to give a crystalline residue, which was recrystallized from ethanol giving **5** (1.5 g, 90%): mp 155.5—156 °C.

Found: C, 34.97; H, 5.26; Br, 33.28%. Calcd for $C_7H_{13}BrO_4$: C, 35.09; H, 5.43; Br, 33.15%.

DL-*1,7:2,3-Di-O-isopropylidene-(1,3/2,4,6)-4-bromo-6-hydroxymethyl-1,2,3-cyclohexanetriol (9).* A mixture of **5** (1.40 g), 2,2-dimethoxypropane (DMP) (21 ml), *p*-toluenesulfonic acid monohydrate (TsOH) (35 mg), and *N,N*-dimethylformamide (DMF) (30 ml) was heated for 3 h at 60 °C. The mixture was then neutralized with Amberlite IRA-400 (OH^-) and concentrated to dryness. Recrystallization of the crude product from ethanol gave **9** (1.56 g, 86%) as feathers: mp 148—149 °C; ^1H NMR (60 MHz)[19] δ=1.45 (6H, s) and 1.68 (6H, s) (isopropylidene), 1.71—2.31 (3H, m, H-5, H-5', and H-6), and 3.30—4.20 (6H, m, H-1, H-2, H-3, H-4, and C$\underline{H}_2$O).

Found: C, 48.59; H, 6.51; Br, 24.79%. Calcd for $C_{13}H_{21}BrO_4$: C, 48.61; H, 6.59; Br, 24.88%.

DL-*(1,3,4/2,6)-4-Azido-6-hydroxymethyl-1,2,3-cyclohexanetriol (7)* and DL-*(1,3/2,4)-4-Hydroxymethyl-5-cyclohexene-1,2,3-triol (8).* To a solution of DL-1,2,3-tri-*O*-acetyl-(1,3,4/2,6)-4-azido-6-acetoxymethyl-1,2,3-cyclohexanetriol (**6**)[11] (1.36 g) in methanol (80 ml) was added 1 M methanolic sodium methoxide (2.0 ml), and the mixture was allowed to stand at room temperature overnight. After treatment with Amberlite IR-120 (H^+) resin, the mixture was concentrated to a syrup, which was shown by TLC to contain two components (R_f 0.42 and 0.33, 3 : 1 chloroform–methanol). The products were fractionated on a silica-gel column (30 g) with 3 : 1 chloroform–methanol as an eluent. The first fraction gave **7** (378 mg, 52%) as feathers: mp 112.5—113 °C.

Found: C, 41.13; H, 6.33; N, 20.41%. Calcd for $C_7H_{13}N_3O_4$: C, 41.38; H, 6.45; N, 20.68%.

The second fraction gave **8** (70 mg, 12%): mp 136—138 °C; ^1H NMR (90 MHz)[20] δ=2.13—2.47 (1H, m, H-4), 3.39—4.15 (5H, H-1, H-2, H-3, and C$\underline{H}_2$OH), and 5.60 (2H, s, H-5 and H-6), identical with the compound obtained by the different route.[12]

DL-*1,7 : 2,3-Di-O-isopropylidene-(1,3,4/2,6)-4-azido-6-hydroxymethyl-1,2,3-cyclohexanetriol (10).* *a)*: A mixture of **7** (284 mg), DMP (5 ml), TsOH (8 mg), and DMF (9 ml) was heated for 3 h at 60 °C. After neutralization with Amberlite IRA-400 (OH^-) resin, the mixture was concentrated, and the residue was crystallized from ethanol giving **10** (329 mg, 83%) as needles: mp 109—110.5 °C; ^1H NMR (90 MHz) δ=1.45 (6H, s) and 1.50 (6H, s) (isopropylidene), 3.51 (1H, dd, J=3 and 9 Hz, H-3), 3.60—3.87 (3H, m, H-1 and C$\underline{H}_2$O), 3.98 (1H, t, J=9 Hz, H-2), and 4.26 (1H, q, J=3 Hz, H-4).

Found: C, 55.33; H, 7.46; N, 14.71%. Calcd for $C_{13}H_{21}N_3O_4$: C, 55.11; H, 7.47; N, 14.83%.

b): A mixture of **9** (1.40 g), sodium azide (1.47 g, 4 molar equivalent), and dimethyl sulfoxide (30 ml) was heated at 110 °C for 15 h. The mixture was then diluted with ethyl acetate and washed with water thoroughly. The organic layer was dried and concentrated, and the residue was recrystallized from ethanol giving **10** (960 mg, 78%), identical with the compound obtained from **9**.

DL-*1,7:2,3-Di-O-isopropylidene-(1,3,4/2,6)-4-amino-6-hydroxymethyl-1,2,3-cyclohexanetriol*[DL-*Di-O-(isopropylidene)validamine*] *(11).* A solution of **10** (946 mg) in ethanol (30 ml) was hydrogenated in the presence of Raney nickel T-4[14] (one spoonful) at room temperature overnight. The catalyst was removed by filtration and the filtrate was concentrated to give a crystalline residue, which was recrystallized from ethanol giving **11** (653 mg, 76%) as needles: mp 153—154 °C; ^1H NMR (90 MHz) δ=1.43 (9H, s) and 1.50 (3H, s) (isopropylidene), 3.42 (1H, dd, J=3 and 9 Hz, H-3), 3.53—3.81 (4H, m, H-1, H-4, and C$\underline{H}_2$O), and 3.97 (1H, t, J=9 Hz, H-2).

Found: C, 60.59; H, 8.72; N, 5.65%. Calcd for $C_{13}H_{23}NO_4$: C, 60.67; H, 9.00; N, 5.44%.

DL-*1,7: 2,3-Di-O-isopropylidene-(1,3,4/2,6)-4-acetamido-1,2,3-cyclohexanetriol (12).* Compound **11** (30 mg) was treated with acetic anhydride (1 ml) in pyridine (1 ml) at room temperature overnight. The mixture was concentrated to a syrup, which was purified by passage through a short column of alumina with chloroform. The eluate was concentrated and the residue was crystallized from ethanol giving **12** (29 mg, 84%) as prisms: mp 231—232 °C; ^1H NMR (90 MHz) δ=1.43 (3H, s), 1.47 (6H, s), and 1.51 (3H, s) (isopropylidene), 2.00 (3H, s, NAc), 3.37—3.83 (5H, m, H-1, H-2, H-3, and C$\underline{H}_2$O), 4.33—4.57 (1H, m, H-4), and 6.04 (1H, m, N$\underline{H}$).

Found: C, 60.30; H, 8.28; N, 4.68%. Calcd for $C_{15}H_{25}NO_5$: C, 60.17; H, 8.43; N, 4.68%.

DL-*1,7: 2,3-Di-O-isopropylidene-(1,3,4/2,6)-6-hydroxymethyl-4-(p-toluenesulfonamido)-1,2,3-cyclohexanetriol (13).* To a solution of **11** (100 mg) in pyridine (3 ml) was added *p*-toluenesulfonyl chloride (173 mg), and the mixture was

stirred at room temperature for 3 h. The reaction mixture was poured into ice–water (10 ml) and extracted with chloroform. The extracts were washed successively with saturated hydrogencarbonate solution and water, and concentrated. The residue was recrystallized from ethanol giving **13** (140 mg, 87%): mp 190—191 °C; ^{1}H NMR (90 MHz) δ=1.39 (6H, s), 1.41 (3H, s), and 1.46 (3H, s) (isopropylidene), 2.42 (3H, s, tosyl C$\underline{H}_3$), 3.30—3.83 (6H, m, H-1, H-2, H-3, H-4, and C$\underline{H}_2$O), and 7.27 (2H, d, J=12 Hz) and 7.54 (2H, d, J=12 Hz) (phenyl).

Found: C, 58.10; H, 6.99; N, 3.17; S, 7.62%. Calcd for $C_{20}H_{29}NO_6S$: C, 58.36; H, 7.12; N, 3.40; S, 7.79%.

DL-*1,7 : 2,3-Di-O-isopropylidene-(1,3,4/2,6)-6-hydroxymethyl-4-trifluoroacetamido-1,2,3-cyclohexanetriol (**14**).* To a stirred solution of **11** (100 mg) in pyridine (3 ml) was added dropwise trifluoroacetic anhydride (0.25 ml) under ice cooling, and the mixture was stirred at room temperature overnight. The mixture was diluted with chloroform (10 ml) and washed with water. The solution was dried and concentrated to a syrup, which was solidified upon standing in a desiccator giving **14** (129 mg, 94%): ^{1}H NMR (90 MHz) δ=1.47 (9H, s) and 1.53 (3H, s) (isopropylidene), 3.47—3.90 (6H, m, H-1, H-2, H-3, and C$\underline{H}_2$O), 4.30—4.51 (1H, m, H-4), and 6.17—6.40 (1H, m, N$\underline{H}$).

Found: C, 51.20; H, 6.39; N, 3.96%. Calcd for $C_{15}H_{22}F_3NO_5$: C, 50.98; H, 6.29; N, 3.96%.

DL-N-[*(3,4,6/5)-3,4,5,6-Tetraacetoxy-1-cyclohexenylmethyl*]-*2,3:4,7-di-O-isopropylidene-(1,2,4/3,5)-2,3,4-trihydroxy-5-(hydroxymethyl)cyclohexylamine (**16a** and **16b**).* A mixture of **11** (73 mg, 0.28 mmol), DL-1,3,4-tri-*O*-acetyl-(1,2,4/3)-5-bromomethyl-5-cyclohexene-1,2,3,4-tetrol (**15**)[10] (103 mg, 0.28 mmol)- and *N,N*-diisopropylethylamine (0.10 ml, 0.28 mmol) in chloroform (4 ml) was refluxed for 3 d. The mixture was evaporated and the residue was acetylated in the usual way. The product was shown to consist of two components (TLC: R_f 0.29 and 0.23, 1 : 3 2-butanone–toluene). It was fractionated on a silica-gel column (10 g) with 1 : 3 2-butanone–toluene as an eluent. The first fraction gave, after crystallization from ethanol, **16a** (32 mg, 19%): mp 176—177 °C; ^{1}H NMR (90 MHz) δ=1.43 (9H, s) and 1.50 (3H, s) (isopropylidene), 1.99 (3H, s), 2.02 (3H, s), 2.07 (3H, s), and 2.10 (3H, s) (OAc), 3.16—3.35 (3H, m, H-1 and C=CC$\underline{H}_2$), 3.47 (1H, dd, J=3.3 and 9 Hz, H-2), 3.55—3.83 (3H, m, H-4 and CC$\underline{H}_2$O), 3.99 (1H, t, J=9 Hz, H-3), 5.08 (1H, ddd, J=1.8, 3.8, and 9 Hz, H-4′), 5.36—5.71 (3H, m, H-3′, H-5′, and H-6′), and 5.96 (1H, br d, J=*ca.* 7 Hz, H-2′).

Found: C, 57.68; H, 7.00; N, 2.28%. Calcd for $C_{28}H_{41}NO_{12}$: C, 57.62; H, 7.08; N, 2.40%.

The second fraction gave **16b** (45 mg, 27%) as a syrup: ^{1}H NMR (90 MHz) δ=1.46 (9H, s) and 1.49 (3H, s) (isopropylidene), 2.00 (3H, s), 2.02 (3H, s), 2.07 (3H, s), and 2.10 (3H, s) (OAc), 3.12—3.32 (3H, m, H-1 and C=CC$\underline{H}_2$), 3.47 (1H, dd, J=3.3 and 9 Hz, H-2), 3.56—3.82 (3H, m, H-4 and CC$\underline{H}_2$O), 3.94 (1H, t, J=9 Hz, H-3), 5.09 (1H, dd, J=3.8 and 10 Hz, H-4′), 5.51 (1H, dd, J=6.8 and 10 Hz, H-5′), 5.47—5.65 (1H, m, H-3′), 5.74 (1H, br d, J=6.8 Hz, H-6′), and 5.85 (1H, br d, J=6 Hz, H-2′).

Found: C, 57.65; H, 6.95; N, 2.26%. Calcd for $C_{28}H_{41}NO_{12}$: C, 57.62; H, 7.08; N, 2.40%.

The fraction containing unresolved mixture of **16a** and **16b** weighed 58 mg (34%).

DL-N-[*(3,4,6/5)-3,4,5,6-Tetrahydroxy-1-cyclohexenylmethyl*]-*(1,2,4/3,5)-2,3,4-triacetoxy-5-(acetoxymethyl)cyclohexylamine (**17a** and **17b**).* Compound **16a** (21 mg) was treated with 70% aqueous acetic acid (2 ml) at room temperature overnight. The mixture was concentrated to a syrup, which was acetylated in the usual way. The product was purified by a silica-gel column with 1 : 3 2-butanone–toluene as an eluent, giving **17a** (16 mg, 65%) as a syrup: ^{1}H NMR (90 MHz) δ=1.93 (3H, s), 1.97 (6H, s), 1.98 (3H, s), 2.00 (3H, s), 2.05 (6H, s), and 2.07 (3H, s) (OAc), 2.08—3.18 (3H, m, H-1 and C=CC$\underline{H}_2$), 3.79 (1H, br dd, J=3.8 and 11 Hz) and 4.00 (1H, br dd, J=4 and 11 Hz) (CC$\underline{H}_2$OAc), 4.75—5.12 (3H, m, H-2, H-3, and H-4), 5.19—5.63 (4H, m, H-3′, H-4′, H-5′, and H-6′), and 5.81 (1H, br d, J=*ca.* 6 Hz, H-2′).

Found: C, 53.77; H, 6.03; N, 1.94%. Calcd for $C_{30}H_{41}NO_{16}$: C, 53.65; H, 6.15; N, 2.09%.

Similarly, *O*-deisopropylidenation of **16b** followed by acetylation gave **17b** (37 mg, 71%) as a syrup: ^{1}H NMR (90 MHz) δ=1.94 (3H, s), 1.97 (6H, s), 2.00 (6H, s), 2.04 (6H, s), and 2.06 (3H, s) (OAc), 2.85—3.32 (3H, m, H-1 and C=CC$\underline{H}_2$), 3.78 (1H, br dd, J=3.2 and 12 Hz) and 4.02 (1H, br dd, J=4.5 and 12 Hz) (CC$\underline{H}_2$OAc), 4.69—5.07 (3H, m, H-2, H-3, and H-4), 5.16—5.54 (4H, m, H-3′, H-4′, H-5, and H-6′), and 5.74 (1H, br d, J=5 Hz, H-2′).

Found: C, 53.64; H, 6.00; N, 1.97%. Calcd for $C_{30}H_{41}NO_{16}$: C, 53.65; H, 6.15; N, 2.09%.

DL-N-[*(3,4,6/5)-3,4,5,6-Tetrahydroxy-1-cyclohexenylmethyl*]-*(1,2,4/3,5)-2,3,4-trihydroxy-5-(hydroxymethyl)cyclohexylamine (Racemic Reversed Validoxylamine A) (**18a** and **18b**).* De-*O*-acetylation of **17a** and **17b** was carried out by treatment with 1 M methanolic sodium methoxide in methanol at room temperature overnight giving **18a** and **18b**, respectively, in quantitative yields. Both **18a** and **18b** showed the same R_f value (0.11) on TLC (4 : 1 : 1 1-propanol–acetic acid–water, *cf.* validoxylamine A,[16] R_f 0.33).

DL-N-[*(1,5/4,6)-4,5,6-Triacetoxy-3-acetoxymethyl-2-cyclohexenyl*]-*2,3:4,7-di-O-isopropylidene-(1,2,4/3,5)-2,3,4-trihydroxy-5-(hydroxymethyl)cyclohexylamine (**21a** and **21b**).* A mixture of **11** (368 mg, 1.43 mmol), DL-1,2,3-tri-*O*-acetyl-(1,3,6/2)-4-acetoxymethyl-6-bromo-4-cyclohexene-1,2,3-triol (**19**)[10] (582 mg, 1.43 mmol), and *N,N*-diisopropylethylamine (0.80 ml, 4.58 mmol) in DMF (15 ml) was stirred at room temperature for 20 d. At this time, TLC indicated the formation of many components (R_f 0.50, 0.41, 0.30, 0.27, and 0.16, 1 : 8 ethanol–toluene). The mixture was concentrated to a syrup, which was fractionated on a silica-gel column (50 g) with 1 : 5 2-butanone–toluene as an eluent. The first fraction (R_f 0.50) gave a syrupy mixture (39 mg, 7%) of DL-1,2,3,4-tetra-*O*-acetyl-(1,2,4/3)- (**24**) and -(1,3/2,4)-5-acetoxymethyl-5-cyclohexene-1,2,3,4-tetrol (**25**), identical with authentic samples.[10]

The second fraction (R_f 0.41) gave a mixture (334 mg, 40%) of **21a** and **21b** as a glass: ^{1}H NMR (90 MHz) δ=1.36—1.56 (12H, m, isopropylidene), 1.92—2.20 (12H, m, OAc), and 5.83 (1H, br s, H-2′).

Found: C, 57.39; H, 6.92; N, 2.35%. Calcd for $C_{28}H_{41}NO_{12}$: C, 57.62; H, 7.08; N, 2.40%.

The third fraction (R_f 0.30) gave a mixture (113 mg, 23%) of DL-1,3,4-tri-*O*-acetyl-(1,2,4/3)-5-acetoxymethyl-5-cyclohexene-1,2,3,4-tetrol (**26**) and DL-2,3,4-tri-*O*-acetyl-(1,2,4/3)-5-acetoxymethyl-5-cyclohexene-1,2,3,4-tetrol (**27**), identical with authentic samples.[10]

The fourth fraction (R_f 0.27) gave **12** (77 mg, 18%): mp 229—230.5 °C, identical with the compound prepared above.

The fifth fraction gave recovered **11** (63 mg, 17%).

DL-N-[*(1,5/4,6)-4,5,6-Triacetoxy-3-acetoxymethyl-2-cyclohexenyl*]-*(1,2,4/3,5)-2,3,4-triacetoxy-5-(acetoxymethyl)cyclohexylamine (**22a** and **22b**).* The syrupy mixture of **21a** and **21b** (189 mg) was treated with a small amount of *p*-toluenesulfonic acid in ethanol (5 ml) at room temperature for 1 d, and then the mixture was neutralized with Amberlite IRA-400 (OH^-). The mixture was concentrated and the residue was acetylated in the usual way. The product was shown by TLC to consist of two components (R_f 0.40 and 0.35, 1 : 1 ethyl acetate–toluene).

The mixture was fractionated on a silica-gel column (10 g) with 1 : 2 acetone–toluene as eluent. The first fraction (R_f 0.40) gave **22a** (89 mg, 41%) as a syrup: ^{1}H NMR (90 MHz) δ=1.89—2.06 (24H, m, OAc), 3.30—3.52 (2H, m, H-1 and H-1′), 3.83 (1H, dd, J=4.2 and 12 Hz) and 4.03 (1H, dd, J=4.7 and 12 Hz) (CC$\underline{H}_2$OAc), 4.24 (1H, d, J=13 Hz) and 4.60 (1H, br d, J=13 Hz) (C=C$\underline{H}_2$OAc), 4.67—5.10 (3H, m, H-2, H-3, and H-4), 5.11—5.49 (2H, m, H-5′ and H-6′), 5.53—5.69 (1H, m, H-4′), and 5.70 (1H, br s, H-2′).

Found: C, 53.74; H, 6.09; N, 2.06%. Calcd for $C_{30}H_{41}NO_{16}$: C, 53.65; H, 6.15; N, 2.09%.

The fraction (R_f 0.35) gave **22b** (50 mg, 23%) as a syrup: ^{1}H NMR (90 MHz) δ=1.90—2.07 (24H, m, OAc), 3.18—3.41 (2H, m, H-1 and H-1′), 3.79 (1H, dd, J=3.2 and 12 Hz) and 4.01 (1H, dd, J=4.5 and 12 Hz) (CC$\underline{H}_2$OAc), 4.26 (1H, d, J=14 Hz) and 4.52 (1H, br d, J=14 Hz) (C=CC$\underline{H}_2$OAc), 4.70—5.15 (3H, m, H-2, H-3, and H-4), 5.15—5.39 (2H, m, H-5′ and H-6′), 5.58 (1H, m, H-4′), and 5.65 (1H, br s, H-2′).

Found: C, 53.78; H, 6.07; N, 2.17%. Calcd for $C_{30}H_{41}NO_{16}$: C, 53.65; H, 6.15; N, 2.09%.

The fraction containing unresolved mixture of **22a** and **22b** weighed 50 mg (23%).

DL-N-[*(1,5/4,6)-3-Hydroxymethyl-4,5,6-trihydroxy-2-cyclohexenyl*]-*(1,2,4/3,5)-2,3,4-trihydroxy-5-(hydroxymethyl)cyclohexylamine (Racemic 1′-Epivalidoxylamine A) (**23a** and **23b**)*. O-Deacetylation of **22a** and **22b** in the usual way gave **23a** and **23b**, respectively, in quantitative yields. Both showed the same R_f value (0.28) on TLC (4 : 1 : 1 1-propanol–acetic acid–water, *cf.* validoxylamine A:[16] R_f 0.31).

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