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Synthetic Studies on the Validamycins. IV. Synthesis of DL-Valienamine and Related Branched-chain Unsaturated Aminocyclitols[1)]

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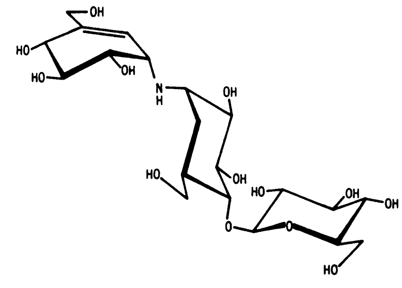
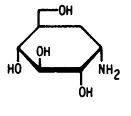
The first synthesis has been achieved of the racemates of valienamine and its related, branched-chain unsaturated aminocyclitols. Two synthetic routes for valienamine were developed, one of which is stereoselective and highly efficient. The key transformation is a stereospecific S_N2' attack (apofacial) by an azide ion on the allyl chloride, prepared by regioselective epoxidation of the conjugated diene followed by treatment with hydrochloric acid. Some information is described which is furnished from ^{13}C NMR spectra for the new branched-chain unsaturated aminocyclitols and cyclitols.

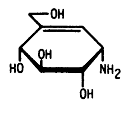
Microbial degradation of validamycin A (**1**)[2)] by several soil bracteria such as *Pseudomonas denitrificans*[3)] and *Flavobacterium saccharophilium*[4)] gave validamine (**2**) and a branched-chain unsaturated aminocyclitol, valienamine (**3**), which is a common cardinal block of the antibiotic validamycins[5)] and whose structure has been assigned as 1D-(1,3,6/2)-6-amino-4-hydroxymethyl-4-cyclohexene-1,2,3-triol by ^{1}H NMR spectroscopy.[3)] The valienamine unit serves also as an essential block of several recently-discovered oligosaccharides, highly effective α-glucosidase inhibitors.[6)] Furthermore, **3** itself has proved to exhibit an α-glucosidase inhibitory activity as well as an antibiotic activity against *Bacillus* species.[7)] The above facts have in recent years stimulated considerable interest in synthetic studies of valienamine and its analogs.[8)]



Validamycin A (1)



Validamine (2)

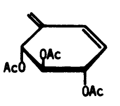


Valienamine (3)

In the present paper, as part of studies directed toward the total synthesis of validamycin A and related substances,[9)] the first synthesis of DL-valienamine and related branched-chain unsaturated aminocyclitols is described. In addition, ^{13}C NMR spectra of peracetyl derivatives of aminocyclitols synthesized were measured and part of resonances were assigned by assuming the cyclohexene ring to be in a half-chair conformation.

Results and Discussion

Synthesis of Branched-chain Unsaturated Aminocyclitols Related to Valienamine. It was previously reported[9)] that bromination of tri-*O*-acetyl-(1,3/2)-4-methylene-5-cyclohexene-1,2,3-triol (**4**)[10)] gave two 1,4-addition products **5** and **6**, from which four monobromo derivatives **7**—**10** of the branched-chain unsaturated cyclitols were prepared. Azidolysis of these bromo compounds and successive reduction readily afforded several branched-chain unsaturated aminocyclitols including DL-valienamine in good yield.



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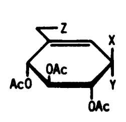
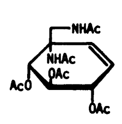


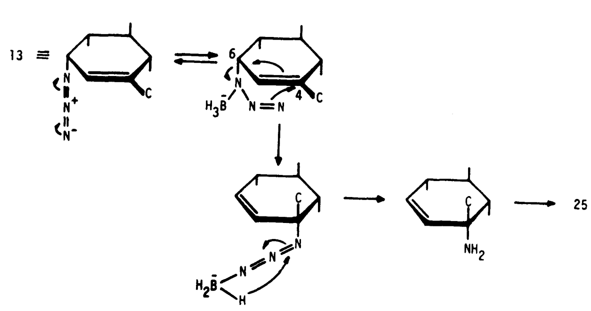


25

	X	Y	Z
5	H	Br	Br
6	Br	H	Br
7	OAc	H	Br
8	H	OAC	Br
9	H	Br	OAc
10	Br	H	OAc
11	N_3	H	N_3
12	NHAc	H	NHAc
13	H	N_3	N_3
14	H	NHAc	NHAc
15	OAc	H	N_3
16	OAc	H	NHAc
17	H	OAc	N_3
18	H	OAc	NHAc
19	N_3	H	OAc
20	NHAc	H	OAc
21	H	N_3	OAc
22	H	NHAc	OAc
23	OAc	H	OAc
24	H	OAc	OAc

When **5** was treated with sodium azide in *N*,*N*-dimethylformamide (DMF) at room temperature for 1 h,[11)] the crystalline diazide **11** was exclusively obtained in 88% yield. Similar treatment of **6** with an azide ion gave the diazide **13** as a homogeneous syrup in 96% yield. The ^{1}H NMR spectrum of **13** revealed the olefinic proton (H-5) as a doublet (J=7.5 Hz) at δ=5.94, supporting the pseudoaxial conformation for the C-6 azido group of **13**.[12)] However, in the ^{1}H NMR spectrum of **11**, the H-5 appeared as a poorly resolved multiplet overlapping with H-3. Configurational assignment of C-6 of **11** was, therefore, made by comparing the chemical shift of H-6 in **11** with that of **13**. The signal, observed at δ=4.38 in **13**, was shifted upfield in **11**, indicating that the H-6 is pseudoaxial,[13)] that is, **11** is the C-6 epimer of **13**. In both cases, azidolysis proceeded *via* a direct S_N2 mechanism with inversion of the configuration at C-6.

Selective reduction of the azido group of **11** was readily achieved by treatment with hydrogen sulfide[14)] in pyridine–water (1 : 1) at room temperature and, after treatment with acetic anhydride in pyridine, the product was isolated as the crystalline bis(acetamide) **12** in 90% yield. On the other hand, reduction of **13** with sodium boronhydride in 2-propanol at the reflux temperature followed by a similar acetylation gave, after chromatography on silica gel, two crystalline bis(acetamide)s **14** and **25** in 43 and 18% yields, respectively. The structures of **12** and **14** were confirmed by observing the expected singlet at δ=5.43[15] and doublet (J=4.5 Hz) at δ=5.65 for the olefinic protons (H-5) of **12** and **14**, respectively. The spectrum of **25** exhibited two olefinic protons as a doublet of doublets (J=1.5 and 10.5 Hz) and a broad doublet (J=10.5 Hz) at δ=5.56 and 6.21, in support of the proposed structure. The configuration at C-4 was tentatively assigned by assuming that the C-6 azido group would migrate to C-4 during the reduction through a boron-assisted allylic rearrangement (S_N2').[16]

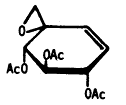


Similarly, the bromides **7** and **8** were converted into the monoazides **15** and **17** in 96 and 80% yields, respectively, which were in turn converted into the corresponding acetamides **16** and **18** in 91 and 87% yields, respectively, by reduction with hydrogen sulfide followed by acetylation.

Synthesis of DL-*Valienamine (**22**).* The synthesis of DL-valienamine starting from **6** was first worked out. The regioselective displacement reaction[9] at C-7 by an acetoxyl group was fairly improved by use of potassium acetate and 18-crown-6. Treatment of **6** with potassium acetate in DMF in the presence of 18-crown-6 at room temperature produced after 2.5 h a mixture of monobromides **9** and **10**, which, without further isolation, was subjected to azidolysis of the remaining secondary bromine atom, giving, after chromatography on silica gel, two azido compounds **19** (43%) and **21** (26%), together with a syrupy 3 : 1 mixture (9%) of **23** and **24**.[9] The structures of **19** and **21** were assigned, on the basis of a criterion similar to that used for the configurational assignment on C-6 of **11**, by use of the result that the pseudoaxial H-6 proton of **19** resonates at higher field (δ=4.12—4.30) than does the pseudoequatorial H-6 proton of **21** (δ=4.41). Further support was provided by the appearance of the doublet (J=7.5 Hz) at δ=5.96 due to the olefinic proton (H-5) of **21**, indicating that the allylic proton (H-6) is pseudoequatorial. Starting from either **5** or **6** gave almost the same distributions of products **19** and **21**.

Reduction of **19** and **21** with hydrogen sulfide followed by acetylation afforded the acetamides **20** and **22** in 69 and 70% yields, respectively. The ^{1}H NMR spectra of **22** in $CDCl_3$ and in DMSO-d_6 were superimposable on those of an authentic sample of penta-*N*,*O*-acetyl-(+)-valienamine.[17] The structure of **20** was confirmed by an observation of the coupling constant (J=8.5 Hz) between H-1 and H-6, indicative of an axial-pseudoaxial relationship between these protons. Compounds **20** and **22** were also obtained by reduction of **19** and **21** with sodium boronhydride in 2-propanol in appropriate yields without any allylic rearrangements of the azido group.

*Alternative Stereospecific Synthesis of **22**.* Although the synthesis of DL-valienamine had been accomplished, the preparation of large quantities of **21** was very tedious because of nonselectiveness of the reaction. This, coupled with the difficulty of separating **21** from **19** purely, required us to develop an alternative stereoselective route for **21**. For this purpose the spiro epoxide **26** obtainable by regioselective epoxidation of **4**[18] was chosen as the starting material. If S_N2' attack of an azide ion on allylic halides, prepared by halogenation of spiro epoxide with hydrogen halides, would proceed stereospecifically, **19** or **21** could be obtained selectively.



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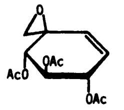

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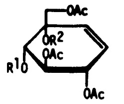
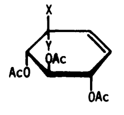
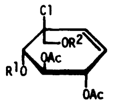


	R^1	R^2
28	H	H
29	Ac	H
30	Ac	Ac



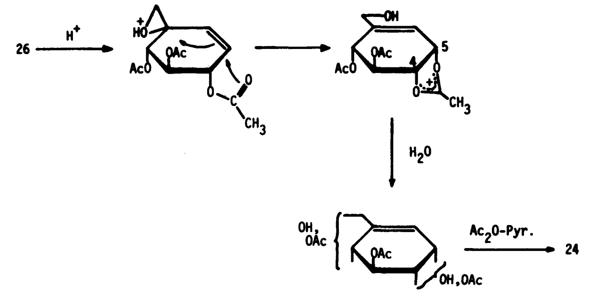
	X	Y
31	CH_2OMe	OH
32	CH_2OMe	OAc
33	CH_2N_3	OAc
34	CH_2NHAc	OH
35	CH_2NHAc	OAc
36	OAc	CH_2N_3
37	OH	CH_2NHAc
38	OAc	CH_2NHAc

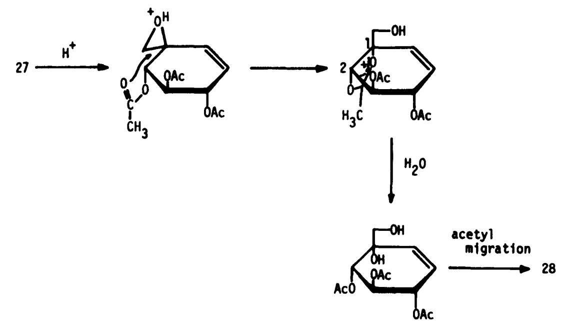


	R^1	R^2
39	Ac	H
40	H	Ac
41	Ac	Ac

Treatment of **4** with 1.2 molar equiv. of *m*-chloroperoxybenzoic acid in dichloromethane at room temperature yielded two crystalline spiro epoxides **26** (24%) and **27** (8%), together with a branched-chain cyclitol triacetate **28** (13%) and a mixture of slower-moving (on TLC) products from which pentaacetate **24**, after acetylation, was isolated (21% from **4**). TLC analysis indicated that **26** and **27** were both acid-labile and partly changed, under acidic conditions, into **24** and **28**, respectively. Therefore, the yield of **26** and especially of **27** were increased when the oxidation was carried out under buffered conditions (see experimental section).

In the ^{1}H NMR spectrum of **28**, a triplet (J=8 Hz) at δ=3.70 due to H-2 changed to a doublet (J=8 Hz) on deuteration. The structure of **28** was finally confirmed by converting it into known **29** and **30**[9] by the usual acetylation. The structures of **26** and **27** were deduced from their behavior under acidic conditions (CH_2Cl_2, TsOH monohydrate, pH *ca.* 3). The pentaacetate **24** derived from **26** was considered to be formed through an S_N1' mechanism by assistance of the C-4 acetoxyl group at C-5, leading to the 4,5-cyclic acetoxonium ion that would be opened by a trace of water. On the other hand, the pentaacetate **28** derived from **27** might be formed through an S_N1 mechanism with the participation of C-2 acetoxyl group. For further confirmation, **26** was converted into known methyl ethers **31** and **32**[9] on treatment with methanolic sodium methoxide in methanol followed by acetylation.

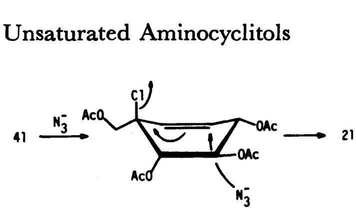




Azidolysis of **26** and **27** with sodium azide in DMF followed by acetylation gave the single azides **33** and **36** in 86 and 81% yields, respectively. They were converted, by reduction with hydrogen sulfide and successive acetylation, into the corresponding acetamides **34** and **35**, and **37** and **38**, respectively. Further acetylation of **34** and **37** yielded **35** and **38** quantitatively. Presumably the formation of **34** and **37** involved the acetyl group migration, during the reduction, from the tertiary hydroxyl to the amino group generated.

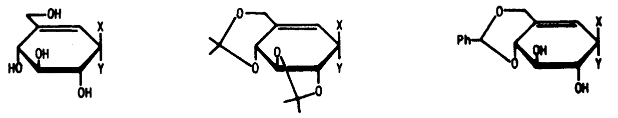
Epoxide ring opening of **26** with concd hydrochloric acid in tetrahydrofuran at 0 °C gave a sole crystalline chlorohydrin **39** quantitatively. An attempt to purify **39** with a silica-gel column resulted in the acetyl group migration giving rise to an isomer **40**. Acetylation of **39** and **40** under acidic conditions gave the same tetraacetate **41** in a quantitative yield. The ^{1}H NMR spectrum revealed the C-7 methylene protons as a singlet at δ=4.32, indicative of the presence of an acetoxymethyl group in support of the assigned structure.

Azidolysis of **41** with sodium azide in DMF at 60 °C gave only one azido product stereospecifically in 93% yield, which proved to be identical in all respects with **21**. The reaction seemed to involve an S_N2' mechanism (apofacial)[19] through an initial attack of an azide ion at C-6. When **41** was treated with sodium acetate, a complex mixture of **23**, **24**, **29**, and **30** was formed. The neighboring acetoxyl group participation was likely to predominate when a weak nucleophile was used.



Preparation of Protected Valienamine. In order to prepare the building blocks for the synthesis of validoxylamines and related biologically-important secondary amines, diisopropylidene and benzylidene derivatives of DL-valienamine were synthesized.

De-*O*-acetylation of **21** with methanolic sodium methoxide gave the hydroxy azide **43**, which was successively treated with an excess of 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid in DMF, giving the crystalline di-*O*-isopropylidene derivative **47** in 72% yield. Similarly, **44** was directly obtained, along with **47**, from the mixture of **19** and **21** prepared by azidolysis of **9**. In this case, attempted isolation of the hydroxy azides **42** and **43** failed because of their similar chromatographic mobilities.



	X	Y
42	N_3	H
43	H	N_3

	X	Y
44	N_3	H
45	NH_2	H
46	NHAc	H
47	H	N_3
48	H	NH_2
49	H	NHAc
50	H	$NHCOCF_3$

	X	Y
51	N_3	H
52	H	N_3

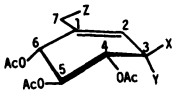
On the other hand, benzylidenation of the mixture of the hydroxy azides **42** and **43** with 1.2 molar equiv. of α,α-dimethoxytoluene gave, after a silica-gel column chromatography, **51** and **52** in 43 and 18% yields, respectively. Their ^{1}H NMR spectra revealed the vinylic protons as a broad singlet at δ=5.41 for **51** and a doublet (J=5 Hz) at δ=5.69 for **52**, confirming the assigned structures.

Compounds **44** and **47** were reduced with hydrogen sulfide in 1 : 1 pyridine–water to give **45** and **48** in quantitative yields, respectively, which were converted into the corresponding acetamides **46** and **49**. Compound **48** was further converted into the trifluoroacetamide **50** on treatment with trifluoroacetic anhydride in pyridine. Di-*O*-isopropylidene derivative **48** thus obtained is a very versatile building block for the preparation of validoxylamines and related secondary amines. Recently the synthesis of DL-validoxylamine B using **48** was accomplished.[1b]

^{13}C NMR Spectra of Valienamine and Related Compounds. On the basis of the ^{13}C NMR data for 1-methylcyclohexene,[20] the chemical shift assignment on peracetyl derivatives of valienamine (**22**) and related aminocyclitols (**12**, **14**, **16**, **18**, and **20**) and cyclitols (**23** and **24**) could be made by assuming the cyclohexene ring to be in a half-chair conformation. The results are summarized in Table 1.

The chemical shift of the methyl carbon of acetyl group is nearly indifferent to their stereochemical

TABLE 1. ^{13}C CHEMICAL SHIFT DATA IN ppm DOWNFIELD FROM INTERNAL TMS[a]



Compound[b]				Carbon atom								
No.	X	Y	Z	C-1	C-2	C-3	C-4	C-5	C-6	C-7	CH$_3$	
											OAc	NHAc
12	NHAc	H	NHAc	132.84	125.71	48.65	70.95	70.56	72.35	38.80	20.36 20.22	22.36
14	H	NHAc	NHAc	135.35	122.9	43.84	69.36	67.86	69.85	39.26	20.34	22.30 22.23
16	OAc	H	NHAc	135.40	123.02	←———	70.31,	71.41	———→	38.96	20.57 20.41 20.25	22.39
18	H	OAc	NHAc	139.60	120.12	70.19[d]	67.49	64.85	69.23[d]	39.02	20.45 20.28	22.31
20	NHAc	H	OAc	130.39	129.61	48.70	70.08[d]	69.81[d]	71.92	61.99	20.22	22.38
22	H	NHAc	OAc	132.57	126.01	43.82	69.01	67.59	69.44	62.23	20.36	22.25
23[c]	OAc	H	OAc	133.43	125.94	71.87[d]	70.67[e]	70.09[e]	72.37[d]	62.31	20.79 20.53	
24[c]	H	OAc	OAc	137.35	123.29	70.26[d]	67.99	65.19	69.78[d]	62.47	20.79 20.62	

a) Unless otherwise noted, the ^{13}C NMR spectra were measured in dimethyl-d_6 sulfoxide. b) For convenience, the carbon atoms were numbered as for derivatives of 1-methylcyclohexene. c) Measured in CDCl$_3$. d), e) Assignments may be reversed.

environment. However, it is obvious that the methyl carbon of acetamido (NHAc) group appears at lower field by 1.44—2.17 ppm than that of acetoxyl (OAc) group.

The chemical shift of C-7 is influenced not only by the substituent (OAc, NHAc) attached thereto but also by the orientation of the substituent at C-3. When an OAc group is attached (Z=OAc), the C-7 appears in the range 61.99—62.47 ppm, whereas it appears at upper field (38.80—39.26 ppm) when an NHAc group is attached (Z=NHAc). In addition, a pseudoaxial OAc or NHAc group at C-3 causes a down-field shift of 0.06—0.46 ppm for C-7.

The sp^2 carbons appear in the range 130.39—139.60 ppm for a quarternary sp^2 carbon (C-1) and in the range 120.12—129.61 ppm for a tertiary sp^2 carbon (C-2). It is noteworthy that their chemical shifts depend on substituents (OAc, NAc) at the allylic positions (C-3 or C-7) and also on the configuration at C-3. Thus, the replacement of an OAc group by an NHAc group at each position causes a downfield shift for the β sp^2 carbon by 1.97—3.67 ppm and an upfield shift for the γ sp^2 carbon by 2.56—4.78 ppm. In addition, the pseudoaxial OAc or NHAc group at C-3 (Y=OAc, NHAc) causes an upfield shift of 2.65—3.60 ppm for the β carbon (C-2) and a downfield shift of 2.18—4.20 ppm for the γ carbon (C-1). The latter γ effect on sp^2 carbon[21] is interesting because in cyclohexane rings the γ effect caused by an axial substituent is generally associated with upfield shifts.[22] This effect is presumably due to some steric interaction.[23]

Experimental

General. Melting points were determined on a Büchi 510 capillary melting point apparatus and are uncorrected. Unless otherwise noted, ^{1}H NMR spectra were taken on a Varian EM-390 (90 MHz) in chloroform-*d* (CDCl$_3$), dimethyl-d_6 sulfoxide (DMSO-d_6), or methanol-d_4 (CD$_3$OD) with reference to tetramethylsilane as an internal standard. The peak positions are given in terms of δ-values and values given for coupling constants are of first-order. ^{13}C NMR spectra were obtained on a Varian XL-100 spectrometer in CDCl$_3$ or DMSO-d_6 containing a drop of TMS as an internal standard with complete proton-decoupling. TLC was performed on a precoated silica gel 60 F-254 plaques (Merck, Darmstadt; 0.25 mm thickness), and the spots were visualized by heating above 150 °C after spraying with concd sulfuric acid. The silica gel used for a column chromatography was Wakogel C-300 (Wako Pure Chemical Industries, Ltd.). Solutions were dried over anhydrous sodium sulfate and evaporated under reduced pressure at 40—50 °C. Acetylation was conventionally carried out with acetic anhydride in anhydrous pyridine at room temperature overnight and the reaction mixture was evaporated to remove an excess of reagents. Crude syrupy products (acetates) were usually purified preliminarily by rapid passage through a short column of active alumina with chloroform.

DL-*Tri-O-acetyl-(1,3/2,6)-6-azido-4-azidomethyl-4-cyclohexene-1,2,3-triol (**11**).* A mixture of DL-tri-*O*-acetyl-(1,3,6/2)-6-bromo-4-bromomethyl-4-cyclohexene-1,2,3-triol (**5**)[9] (0.16 g), sodium azide (0.1 g, 4 molar equiv.), and *N,N*-dimethylformamide (DMF) (6 ml) was stirred at room temperature for 1 h. The mixture was diluted with ethyl acetate (20 ml), and the organic layer was thoroughly washed with cold water (3× 10 ml) and then dried. Evaporation of the solvent left a syrup which crystallized upon standing in a desiccator overnight. Recrystallization from ethanol gave **11** (0.12 g, 88%): mp 82—83 °C; ^{1}H NMR ($CDCl_3$) δ=2.03 (3H, s), 2.09 (3H, s), and 2.11 (3H, s) (OAc), 3.81 (2H, broad s, $C\underline{H}_2N_3$), 4.14—4.38 (1H, m, H-6), 5.14—5.50 (2H, m, H-1 and H-2), and 5.59—5.98 (2H, m, H-3 and H-5).

Found: C, 44.14; H, 4.60; N, 23.67%. Calcd for $C_{13}H_{16}N_6O_6$: C, 44.32; H, 4.58; N, 23.85%.

DL-*Tri-*O*-acetyl-(1,3,6/2)-6-azido-4-azidomethyl-4-cyclohexene-1,2,3-triol (**13**).* A mixture of DL-tri-*O*-acetyl-(1,3/2,6)-6-bromo-4-bromomethyl-4-cyclohexene-1,2,3-triol (**6**)[9] (0.2 g), sodium azide (0.12 g, 4 molar equiv.), and DMF (10 ml) was treated similarly as described in the preparation of **11** for 0.5 h. The crude product was purified by passing through a short column of silica gel with chloroform to give **13** (0.16 g, 96%) as a homogeneous syrup: ^{1}H NMR (60 MHz,[24] $CDCl_3$) δ= 2.03 (3H, s), 2.07 (3H, s), and 2.11 (3H, s) (OAc), 3.77 (2H, broad s, $C\underline{H}_2N_3$), 4.38 (1H, t, J=7.5 Hz, H-6), 4.95—5.80 (3H, m, H-1, H-2, and H-3), and 5.94 (1H, broad d, J=7.5 Hz, H-5).

Found: C, 44.10; H, 4.63; N, 23.55%. Calcd for $C_{13}H_{16}N_6O_6$: C, 44.32; H, 4.58; N, 23.85%.

DL-*Penta-*N,O*-acetyl-(1,3/2,6)-6-amino-4-aminomethyl-4-cyclohexene-1,2,3-triol (**12**).* Into a stirred solution of **11** (94 mg) in 1 : 1 pyridine–water (5 ml) was bubbled hydrogen sulfide at room temperature for 1.5 h, during which insoluble sulfur appeared. The yellowish mixture was evaporated and the residue was acetylated in the usual way. The crude product was transferred to the top of a column of silica gel (3 g) and first eluted with benzene, giving sulfur as colorless needles, and then with 1 : 5 ethanol–benzene, giving colorless crystals. Recrystallization from ethanol gave **12** (92 mg, 90%) as feathers: mp 229.5—230.5 °C (sintering at 228 °C): ^{1}H NMR (DMSO-d_6) δ=1.80 (6H, s), 1.94 (6H, s), and 1.98 (3H, s) (OAc and NAc), 3.45 (1H, dd, J=4 and 16.5 Hz, changing to d with J=16.5 Hz on deuteration) and 3.82 (1H, dd, J=7 and 16.5 Hz, changing to d with J=16.5 Hz on deuteration) ($C\underline{H}_2NHAc$), 4.45—4.80 (1H, m, H-6, changing on deuteration), 4.87—5.33 (2H, m, H-1 and H-2), 5.43 (1H, broad s, H-5), 5.61 (1H, broad d, J=*ca.* 6 Hz, H-3), 7.85 (1H, dd, J= 4 and 7 Hz, C-7-N$\underline{H}$Ac, disappearing on deuteration), and 8.10 (1H, d, J=9 Hz, C-6-N$\underline{H}$Ac, disappearing on deuteration).

Found: C, 53.15; H, 6.30; N, 7.20%. Calcd for $C_{17}H_{24}N_2O_8$: C, 53.12; H, 6.29; N, 7.29%.

DL-*Penta-*N,O*-acetyl-(1,3,6/2)-6-amino-4-aminomethyl-4-cyclohexene-1,2,3-triol (**14**) and* DL-*Penta-*N,O*-acetyl-(1,3,4/2)-4-amino-4-*C*-aminomethyl-5-cyclohexene-1,2,3-triol (**25**).* A suspension of **13** (144 mg), sodium boronhydride (93 mg), and 2-propanol (5 ml) was refluxed gently for 1 h. After cooling, the excess of hydride was decomposed by gradual addition of 6 M (1 M=1 mol dm^{-3}) acetic acid (*ca.* 2 ml), and then the mixture was evaporated to dryness. The resulting soild was acetylated in the usual way, and the product was chromatographed on a column of silca gel (5 g). Elution with 1 : 5 ethanol–benzene gave three fractions.

The first fraction gave a small amount of unresolved mixture, which was not further characterized.

The second fraction gave a colorless syrup, which crystallized on standing in a desiccator overnight. Recrystallization from ethanol gave **25** (29 mg, 18%): mp 178.5—181 °C (sintering at 170 °C); ^{1}H NMR ($CDCl_3$) δ=1.94 (3H, s), 1.99 (6H, s), 2.01 (3H, s), and 2.06 (3H, s) (OAc and NAc), 3.28 (1H, dd, J=6 and 13 Hz, changing to d with J=13 Hz on deuteration) and 3.81 (1H, dd, J=6.3 and 13 Hz, changing to d with J=13 Hz on deuteration) ($C\underline{H}_2NHAc$), 5.00 (1H, d, J=10.5 Hz, H-3), 5.15—5.70 (2H, m, H-1 and H-2), 5.56 (1H, dd, J=1.5 and 10.5 Hz, H-6), and 5.92—6.33 (3H, m, H-5 and 2×N$\underline{H}$Ac, changing to broad d with J=10.5 Hz at δ=6.21 on deuteration).

Found: C, 52.91; H, 6.07; N, 7.43%. Calcd for $C_{17}H_{24}N_2O_8$: C, 53.12; H, 6.29; N, 7.29%.

The third fraction gave colorless crystals, which were recrystallized from ethanol, giving **14** (67 mg, 43%) as columns: mp 253—255 °C; ^{1}H NMR (DMSO-d_6) δ=1.77 (3H, s), 1.84 (3H, s), 1.91 (3H, s), and 1.97 (6H, s) (OAc and NAc) 3.46 (1H, dd, J=5 and 15 Hz, changing to d with 15 Hz on deuteration) and (3.92 1H, dd, J=7.5 and 15 Hz, changing to d with J=15 Hz on deuteration) ($C\underline{H}_2NHAc$), 4.74 (1H, ddd, J=4.5, 5, and 9 Hz, H-6, changing to dd with J=4.5 and 5 Hz on deuteration), 4.95 (1H, dd, J=5 and 10.5 Hz, H-1), 5.34 (1H, dd, J=7.5 and 10.5 Hz, H-2), 5.55 (1H, d, J=7.5 Hz, H-3), 5.65 (1H, d, J=4.5 Hz, H-5), 7.84 (1H, dd, J=5 and 7.5 Hz, C-7-N$\underline{H}$Ac, disappearing on deuteration), and 8.11 (1H, d, J=9 Hz, C-6-N$\underline{H}$Ac, disappearing on deuteration).

Found: C, 52.84; H, 6.23; N, 6.99%. Calcd for $C_{17}H_{24}N_2O_8$: C, 53.12; H, 6.29; N, 7.29%.

DL-*Tetra-*O*-acetyl-(1,3/2,4)-5-azidomethyl-5-cyclohexene-1,2,3,-4-tetrol (**15**).* A mixture of DL-tetra-*O*-acetyl-(1,3/2,4)-5-bromomethyl-5-cyclohexene-1,2,3,4-tetrol (**7**)[9] (0.15 g), sodium azide (47 mg, 2 molar equiv.), and DMF (6 ml) was stirred at room temperature overnight. The mixture was diluted with ethyl acetate (20 ml) and successively washed with cold water (3×10 ml), and then dried. Evaporation of the solvent gave **15** (0.13 g, 96%) as an analytically pure colorless syrup: ^{1}H NMR ($CDCl_3$) δ=1.98 (6H, s) and 2.02 (6H, s) (OAc), 3.78 (2H, s, $C\underline{H}_2N_3$), 5.21—5.45 (2H, m, H-2 and H-3), 5.45—5.65 (1H, m, H-1), and 5.65—5.88 (2H, broad s, H-4 and H-6).

Found: C, 48.51; H, 5.17; N, 11.12%. Calcd for $C_{15}H_{19}N_3O_8$: C, 48.78; H, 5.19; N, 11.38%.

DL-*Tetra-*O*-acetyl-(1,2,4/3)-5-azidomethyl-5-cyclohexene-1,2,3,-4-tetrol (**17**).* A mixture of DL-tetra-*O*-acetyl-(1,2,4/3)-5-bromomethyl-5-cyclohexene-1,2,3,4-tetrol (**8**)[9] (0.15 g), sodium azide (47 mg, 2 molar equiv.), and DMF (5 ml) was treated similarly as described in the preparation of **15**. The crude product was purified by passing through a silica-gel column (5 g) with 1 : 5 2-butanone–toluene as an eluent to give **17** (108 mg, 80%) as a syrup: ^{1}H NMR ($CDCl_3$) δ=2.01 (3H, s), 2.04 (3H, s), and 2.11 (3H, s) (OAc), 3.84 (2H, s, $C\underline{H}_2N_3$), 5.15 (1H, dd, J=4 and 10.5 Hz, H-2), 5.29—5.76 (3H, m, H-1, H-3, and H-4), and 6.00 (1H, broad d, J=6 Hz, H-6).

Found: C, 48.96; H, 5.26; N, 11.25%. Calcd for $C_{15}H_{19}N_3O_8$: C, 48.78; H, 5.19; N, 11.38%.

DL-*Penta-*N,O*-acetyl-5-aminomethyl-5-cyclohexene-1,2,3,4-tetrol (**16**).* Hydrogen sulfide was bubbled into a stirred solution of **15** (0.13 g) in 2 : 1 pyridine–water (9 ml) at room temperature for 1.5 h. The mixture was evaporated and the residue was acetylated in the usual way. The product was purified by passing through a short column of silica gel with 1 : 5 ethanol–benzene as an eluent to give colorless crystals. Recrystallization from ethanol gave **16** (0.12 g, 91%): mp 131.5—132.5 °C; ^{1}H NMR ($CDCl_3$) δ=1.98 (3H, s), 2.01 (6H, s), 2.04 (3H, s), and 2.07 (3H, s) (OAc and NAc), 3.71 (1H, dd, J=5 and 15 Hz, changing to d with J=15 Hz on deuteration) and 3.98 (1H, dd, J=7 and 15 Hz, changing to d with J=15 Hz on deuteration) ($C\underline{H}_2NHAc$), 5.15—5.43 (2H, m, H-2 and H-3), and 5.43—6.02 (4H, m, H-1, H-4, H-6, and N$\underline{H}$Ac, changing to relatively narrow m at δ=5.43—5.82 on deuteration).

Found: C, 52.68; H, 5.95; N, 3.52%. Calcd for $C_{17}H_{23}NO_9$: C, 52.98; H, 6.02; N, 3.63%.

DL-*Penta-*N,O*-acetyl-(1,2,4/3)-5-aminomethyl-5-cyclohexene-1,2,-3,4-tetrol (**18**).* Reduction of **17** (69 mg) with hydrogen sulfide in 2 : 1 pyridine–water (5 ml), followed by acetylation, was carried out in the same manner as in the preparation of **16**. The product was purified by passage through a silica-gel column (3 g) with 1 : 10 ethanol–benzene to give **18** (62 mg, 87%): mp 148.5—149.5 °C; ^{1}H NMR (CDCl$_3$) δ=1.97 (3H, s), 2.00 (3H, s), 2.02 (3H, s), and 2.08 (6H, s) (OAc and NAc), 3.75 (1H, dd, J=5.5 and 15 Hz, changing to d with J=15 Hz on deuteration) and 4.00 (1H, dd, J=7 and 15 Hz, changing to d with J=15 Hz on deuteration) (C$\underline{H}_2$NHAc), 5.08 (1H, ddd, J=0.8, 3.5, and 10 Hz, H-2), 5.50 (1H, dd, J=8 and 10 Hz, H-3), 5.52—5.73 (2H, m, H-1 and H-4), and 5.73—5.97 (2H, broad d, J=*ca.* 5 Hz, H-6 and N$\underline{H}$Ac, changing to dd with J=0.8 and 6.5 Hz at δ=5.87 on deuteration).

Found: C, 53.15; H, 5.93; N, 3.69%. Calcd for $C_{17}H_{23}NO_9$: C, 52.98; H, 6.02; N, 3.63%.

DL-*Tetra*-O-*acetyl-(1,3/2,6)- (**19**) and -(1,3,6/2)-6-azido-4-cyclohexene-1,2,3-triol (**21**).* A mixture of **6** (338 mg), potassium acetate (78 mg, 1 molar equiv.), 18-crown-6 (0.1 g), and DMF (10 ml) was stirred at room temperature for 2.5 h. The sodium azide (105 mg, 2 molar equiv.) was then added to the mixture and the stirring was continued for an additional 1 h. The mixture was then diluted with ethyl acetate (30 ml) and the solution was washed with cold water several times, and dried. TLC indicated the presence of three components, together with a trace of some other products. Evaporation of the solvent and chromatography of the residue on a silica-gel column (10 g) with 1 : 10 2-butanone–toluene as an eluent gave three fractions. The first fraction gave crystals, which were recrystallized from ethanol, giving **19** (125 mg, 43%) as tiny prisms: mp 68—68.5 °C; ^{1}H NMR (CDCl$_3$) δ=2.01 (3H, s), 2.03 (3H, s), 2.05 (3H, s), and 2.08 (3H, s) (OAc), 4.12—4.30 (1H, m, H-6), 4.38 and 4.70 (AB-quartet, J=15 Hz, C$\underline{H}_2$OAc), 5.14—5.43 (2H, m, H-1 and H-2), and 5.67—5.86 (2H, m, H-3 and H-5).

Found: C, 48.68; H, 5.21; N, 11.28%. Calcd for $C_{15}H_{19}N_3O_8$: C, 48.78; H, 5.19; N, 11.38%.

The second fraction gave **21** (76 mg, 26%) as a syrup: ^{1}H NMR (CDCl$_3$) δ=2.03 (3H, s), 2.05 (6H, s), and 2.12 (3H, s) (OAc), 4.41 (1H, dd, J=4.5 and 7.5 Hz, H-6), 4.40 and 4.70 (AB-quartet, J=13 Hz, C$\underline{H}_2$OAc), 5.15 (1H, dd, J=4.5 and 10 Hz, H-1), 5.49 (1H, dd, J=7 and 10 Hz, H-2), 5.68 (1H, broad d, J=7 Hz, H-3), and 5.96 (1H, broad d, J=7.5 Hz, H-5).

Found: C, 48.64; H, 5.19; N, 11.23%. Calcd for $C_{15}H_{19}N_3O_8$: C, 48.78; H, 5.19; N, 11.38%.

The third fraction gave a homogeneous syrupy mixture of DL-penta-*O*-acetyl-(1,3/2,4)- (**23**) and -(1,2,4/3)-5-hydroxymethyl-5-cyclohexene-1,2,3,4-tetrol (**24**), weighing 26 mg (9%). By comparing its ^{1}H NMR spectrum with those of authentic samples[9] the ratio of **23** and **24** was found to be *ca.* 3 : 1.

When **5** was used instead of **6**, a similar result was obtained.

DL-*Penta*-N,O-*acetyl-(1,3,6/2)-6-amino-4-hydroxymethyl-4-cyclohexene-1,2,3-triol (penta*-N,O-*acetyl*-DL-*valienamine) (**22**).* *a)* Hydrogen sulfide was bubbled into a solution of **21** (57 mg) in 2 : 1 pyridine–water (3 ml) at room temperature for 1 h. The mixture was evaporated and the residue was acetylated in the usual way. Purification of the acetate by passage through a silica-gel column (2 g) with 1 : 10 ethanol–benzene gave crystals, which were recrystallized from ethanol to afford **22** (42 mg, 70%) as tiny prisms: mp 180—181 °C; ^{1}H NMR (CDCl$_3$) δ=2.03 (6H, s) and 2.06 (9H, s) (OAc and NAc), 4.36 and 4.66 (AB-quartet, J=14 Hz, C$\underline{H}_2$OAc), 4.89—5.23 (2H, m, H-1 and H-6), 5.69—6.00 (2H, m, H-5 and N$\underline{H}$Ac, changing to broad d with J=4.5 Hz at δ=5.90; ^{1}H NMR (100 MHz,[25] DMSO-d_6) δ=1.86 (3H, s), 1.93 (3H, s), and 2.20 (9H, s) (OAc and NAc), 4.42 and 4.67 (AB-quartet, J=14 Hz, C$\underline{H}_2$OAc), 4.81 (1H, td, J=4.5 and 9.0 Hz, H-6), 5.03 (1H, dd, J=4.5 and 9.5 Hz, H-1), 5.36 (1H, dd, J=7 and 9.5 Hz, H-2), 5.58 (1H, d, J=7 Hz, H-3), 5.87 (1H, broad d, J=4.5 Hz, H-5), and 8.15 (1H, d, J=9 Hz, N$\underline{H}$Ac, disappearing on deuteration). The spectra were superimposable on those of an authentic optically-active sample.[17]

b) A suspension of **21** (43 mg), sodium boronhydride (13 mg), and 2-propanol (3 ml) was refluxed for 3.5 h. After cooling, the excess of hydride was decomposed by gradual addition of 6 M acetic acid and the mixture was evaporated to dryness. The residue was acetylated in the usual way and the product was chromatographed on a silica-gel column (2 g) with 1 : 1 2-butanone–toluene as an eluent, giving **22** (26 mg, 65%): mp 178—179.5 °C, identical with the compound obtained above.

DL-*Penta*-N,O-*acetyl-(1,3/2,6)-6-amino-4-hydroxymethyl-4-cyclohexene-1,2,3-triol (**20**).* *a)* Compound **19** (195 mg) was similarly reduced by hydrogen sulfide to give **20** (140 mg, 69%): mp 120—121 °C; ^{1}H NMR (CDCl$_3$) δ=1.94 (3H, s), 2.00 (3H, s), and 2.03 (9H, s) (OAc and NAc), 4.35 and 4.68 (AB-quartet, J=13 Hz, C$\underline{H}_2$OAc), 4.84 (1H, dd, J=7.7 and 8.5 Hz, H-6, changing to d with J=8.5 Hz on deuteration), 5.10 (1H, dd, J=8.5 and 10.5 Hz, H-1), 5.37 (1H, dd, J=7.8 and 10.5 Hz, H-2), 5.74 (2H, broad s, H-3 and H-5), and 6.06 (1H, broad d, J=*ca.* 7.7 Hz, N$\underline{H}$Ac, disappearing on deuteration).

Found: C, 53.18; H, 6.04; N, 3.55%. Calcd for $C_{17}H_{23}NO_9$: C, 52.98; H, 6.02; N, 3.63%.

b) Compound **19** (134 mg) was treated with sodium boronhydride (41 mg) in a similar manner as in the preparation of **22** to give **20** (84 mg, 65%): mp 119—120 °C, identical with the compound obtained above.

*Epoxidation of **4**: Synthesis of* DL-*2,3,4-Tri*-O-*acetyl-1,7-anhydro-(1,2,4/3)- (**26**) and -(1,3/2,4)-1*-C-*hydroxymethyl-5-cyclohexene-1,2,3,4-tetrol (**27**).* *a)* A mixture of DL-tri-*O*-acetyl-(1,3/2)-4-methylene-5-cyclohexene-1,2,3-triol (**4**)[18] (0.3 g), 70% *m*-chloroperoxybenzoic acid (*m*CPBA) (0.33 g, 1.2 molar equiv.), and dichloromethane (5 ml) was stirred at room temperature for 12 h. The progress of reaction was monitored by TLC (1 : 5 2-butanone–toluene). Two new components (R_f 0.42 and 0.36) appeared and then were gradually converted into slower-moving components before **4** was completely consumed. The reaction mixture was cooled in an ice-bath, and the precipitates (*m*-chlorobenzoic acid) were removed by filtration. The filtrate was washed successively with 10% aqueous sodium sulfite solution, saturated aqueous sodium hydrogencarbonate solution, and water, and then dried. Evaporation of the solvent and chromatography of the residue (0.23 g) on a silica-gel column (7 g) with 1 : 5 2-butanone–toluene gave, as the first fraction, **27** (25.4 mg, 8%) as plates: mp 124.5—125.5 °C; ^{1}H NMR (CDCl$_3$) δ=2.01 (6H, s) and 2.05 (3H, s) (OAc), 2.73 and 3.18 (AB-quartet, J=5 Hz, C$\underline{H}_2$O), 5.32—5.55 (3H, m, H-3, H-4, and H-5), 5.55—5.75 (1H, m, H-2), and 5.84 (1H, dd, J=1.5 and 10 Hz, H-6).

Found: C, 54.73; H, 5.65%. Calcd for $C_{13}H_{16}O_7$: C, 54.93; H, 5.67%.

The second fraction gave crystals, which were recrystallized from ethanol to give **26** (76 mg, 24%) as plates: mp 108.5—109 °C; ^{1}H NMR (CDCl$_3$) δ=2.02 (3H, s) and 2.06 (6H, s) (OAc), 2.88 (2H, s, C$\underline{H}_2$O), 5.29—5.60 (4H, m, H-2, H-3, H-4, and H-5), and 5.96 (1H, dd, J=2 and 10.5 Hz, H-6).

Found: C, 54.73; H, 5.69%. Calcd for $C_{13}H_{16}O_7$: C, 54.93; H, 5.67%.

The third fraction gave crystals, which were recrystallized from ethanol to give DL-3,4,7-tri-*O*-acetyl-(1,2,4/3)-1-*C*-hydroxymethyl-5-cyclohexene-1,2,3,4-tetrol (**28**) (44 mg, 13%): mp 100.5—102 °C; ^{1}H NMR (CDCl$_3$) δ=2.05 (3H, s), 2.07 (3H, s), and 2.11 (3H, s) (OAc), 2.99 (1H, d, J=8 Hz, C-2-OH, disappearing on deuteration), 3.23 (1H, s, C-1-OH, disappearing on deuteration), 3.70 (1H, t, J=8 Hz, H-2, changing to d with J=8 Hz on deuteration), 4.07 and 4.25 (AB-quartet, J=12 Hz, $C\underline{H}_2OAc$), 5.28 (1H, t, J=8 Hz, H-3), 5.45 (1H, dd, J=1 and 12 Hz), and 5.69 (1H, dd, J=1 and 12 Hz) and 5.85 (1H, dd, J=1 and 12 Hz) (H-5 and H-6).

Further elution of the column gave an unresolved mixture (163 mg). After acetylation in the usual way, followed by fractionation on a silica-gel column (8 g) with 1 : 5 2-butanone–toluene, pentaacetate **24** was obtained in 91 mg (21% from **4**) as a syrup. The ^{1}H NMR spectrum was superimposable on that of an authentic sample.[9]

Acetylation of **28** (29 mg) with acetic anhydride (2 ml) and pyridine (2 ml) at room temperature for 4 d gave, after separation by a silica-gel column (3 g with) 1 : 5 2-butanone–toluene, DL-penta-*O*-acetyl- (**30**) and DL-2,3,4,7-tetra-*O*-acetyl-(1,2,4/3)-1-*C*-hydroxymethyl-5-cyclohexene-1,2,3,4-tetrol (**29**) in 19 mg (52%) and 8 mg (24%) as a syrup, respectively. These compounds were identical with the authentic samples.[9]

b) Compound **4** (0.3 g) was treated with 70% *m*CPBA (0.33 g, 1.2 molar equiv.) in dichloromethane (15 ml), buffered at pH 8 with phosphate buffer solution[26] (20 ml), with vigorous stirring at room temperature. After 20 min, an additional amount of *m*CPBA (0.28 g) was added to the reaction mixture and stirring was continued for further 40 min. The organic layer was then separated and washed similarly as described above. Evaporation of the solvent followed by fractionation of the residual products on a silica-gel column (10 g) gave **26** (124 mg,31%) and **27** (86 mg, 27%).

c) The above reaction was carried out similarly by using saturated aqueous sodium hydrogencarbonate solution as an acid scavenger, instead of the phosphate buffer solution. Compounds **26** and **27** were obtained in 37 and 29% yields, respectively.

d) A similar epoxidation reaction was carried out in the presence of solid sodium hydrogencarbonate as an acid acceptor. Compounds **26** and **27** were isolated in 39 and 28% yields, respectively.

*Reaction of Spiro Epoxides **26** and **27** under Acidic Conditions.* Spiro epoxides **26** and **27** were each dissolved in dichloromethane containing a trace of *p*-toluenesulfonic acid monohydrate (pH *ca.* 3) and each solution was left at room temperature for 30 min. Examination on TLC revealed that **26** had been converted into slower-moving products, which were convertible into pentaacetate **24** by acetylation, whereas **27** led to triacetate **28**.

*Reaction of **26** with Sodium Methoxide.* To a solution of **26** (0.11 g) in methanol (5 ml) was added 1 M methanolic sodium methoxide (1.3 ml), and the mixture was left at room temperature overnight. After neutralization with Amberlite IR-120 (H$^+$), the solution was evaporated and the residue was acetylated in the usual way for 5 d. The products were fractionated on a silica-gel column (7 g) with 1 : 5 2-butanone–toluene as an eluent to give DL-tetra-*O*-acetyl-(1,2,4/3)-1-*C*-methoxymethyl-5-cyclohexene-1,2,3,4-tetrol (**32**) (76 mg, 55%), mp 130—131 °C, and the triacetate **31** (11 mg, 9%) as a syrup. They were identical with authentic samples[9] previously obtained from **5**.

DL-*Tetra-*O*-acetyl-(1,2,4/3)-1-*C*-azidomethyl-5-cyclohexene-1,2,3,4-tetrol (33).* A mixture of **26** (125 mg), sodium azide (87 mg), ammonium chloride (24 mg), and DMF (2 ml) was stirred at room temperature for 20 h and then at 60 °C for 3 h. The mixture was evaporated to dryness and the residue was acetylated with acetic anhydride (2 ml) and pyridine (2 ml) at room temperature for 2 d and then at 40 °C overnight. The mixture was evaporated and the residue was purified by passage through a silica-gel column (10 g) with 1 : 8 2-butanone–toluene to give **33** (140 mg, 86%) as a syrup: ^{1}H NMR (CDCl$_3$) δ=2.00 (3H, s), 2.02 (3H, s), and 2.07 (6H, s) (OAc), 3.56 and 3.91 (AB-quartet, J=12.5 Hz, $C\underline{H}_2N_3$), 5.20 (1H, dd, J=1.5 and 10.5 Hz, H-2), 5.29—5.64 (2H, m, H-3 and H-4), and 5.73 (1H, dd, J=3 and 11 Hz) and 6.17 (1H, dd, J=1.5 and 11 Hz) (H-5 and H-6).

Found: C, 48.05; H, 5.21; N, 11.26%. Calcd for $C_{15}H_{19}N_3O_8$: C, 47.78; H, 5.19; N, 11.38%.

DL-*Tetra-*O*-acetyl-(1,3/2,4)-1-*C*-azidomethyl-5-cyclohexene-1,2,3,4-tetrol (36).* A mixture of **27** (126 mg), sodium azide (87 mg), ammonium chloride (24 mg), and DMF (2 ml) was treated in a similar manner as in the preparation of **33**. The crude product was subsequently acetylated with acetic anhydride (2 ml) and pyridine (2 ml) at room temperature for 2 d. The mixture was evaporated and the residue was purified by passage through a short column of alumina with ethyl acetate to give crystals, which were recrystallized from ethanol, giving **36** (132 mg, 81%) as particles: mp 99—100 °C; ^{1}H NMR (CDCl$_3$) δ=2.00 (6H, s), 2.03 (3H, s), and 2.08 (3H, s) (OAc), 3.41 and 3.72 (AB-quartet, J=13 Hz, $C\underline{H}_2N_3$), 5.27—5.79 (3H, m, H-1, H-2, and H-3), and 5.79—6.03 (2H, m, H-5 and H-6).

Found: C, 47.84; H, 5.22; N, 11.35%. Calcd for $C_{15}H_{19}N_3O_8$: C, 47.78; H, 5.19; N, 11.38%.

DL-*Penta-*N,O*-acetyl-(1,2,4/3)-1-*C*-aminomethyl-5-cyclohexene-1,2,3,4-tetrol (35).* Into a stirred solution of **32** (135 mg) in 2 : 1 pyridine–water (3 ml) was bubbled hydrogen sulfide at room temperature for 5 d. The mixture was evaporated to dryness and the residue was acetylated in the usual way for 2 d. The crude products, which were shown to contain two components (R_f 0.27 and 0.39) by TLC analysis (1 : 5 ethanol–benzene), were placed on a column of silica gel (5 g) and first eluted with toluene, giving sulfur, and then with 1 : 5 ethanol-toluene, giving desired products. The fraction having R_f 0.4 yielded **35** (40 mg, 28%) as a syrup: ^{1}H NMR (CDCl$_3$) δ= 1.93 (3H, s), 2.00 (3H, s), 2.02 (3H, s), 2.05 (3H, s), and 2.09 (3H, s) (NAc and OAc), 3.04 (1H, dd, J=6 and 14 Hz, changing to d with J=14 Hz on deuteration) and 4.44 (1H, dd, J=7.5 and 14 Hz, changing to d with J=14 Hz on deuteration) ($C\underline{H}_2NHAc$), 5.08 (1H, d, J=10.5 Hz, H-2), 5.36—5.53 (1H, m, H-4), 5.59 (1H, dd, J=7.5 and 10.5 Hz, H-3), 5.72 (1H, dd, J=3 and 10 Hz, H-5), 6.11 (1H, broad t, J=*ca.* 7 Hz, $N\underline{H}$, disappearing on deuteration), and 6.43 (1H, dd, J=1.5 and 10 Hz, H-6).

Found: C, 53.17; H, 6.03; N, 3.37%. Calcd for $C_{17}H_{23}NO_9$: C, 52.98; H, 6.02; N, 3.63%.

The fraction having R_f 0.3 yielded DL-*N*-acetyl-2,3,4-tri-*O*-acetyl-(1,2,4/3)-1-*C*-aminomethyl-5-cyclohexene-1,2,3,4-tetrol (**34**) (83 mg, 66%) as a syrup: ^{1}H NMR (CDCl$_3$) δ=1.96 (3H, s), 2.00 (3H, s), 2.02 (3H, s), and 2.10 (3H, s) (OAc and NAc), 3.00 (1H, dd, J=6 and 14 Hz, changing to d with J=14 Hz on deuteration) and 3.62 (1H, dd, J=7 and 14 Hz, changing to d with J=14 Hz on deuteration) ($C\underline{H}_2NHAc$), 3.37—3.83 (1H, m, $O\underline{H}$, disappearing on deuteration), 5.01 (1H, d, J=10 Hz, H-2), 5.29—5.62 (2H, m, H-3 and H-4), 5.63 (1H, dd, J=1.5 and 10 Hz, H-5), 5.88 (1H, dd, J=1 and 10 Hz, H-6), and 6.36 (1H, broad t, J=*ca.* 7 Hz, $N\underline{H}Ac$, disappearing on deuteration).

Found: C, 52.72; H, 6.26; N, 3.97%. Calcd for $C_{15}H_{21}NO_8$: C, 52.47; H, 6.17; N, 4.08%.

Further acetylation of **34** at 50 °C for 2 d gave **35** quantitatively.

DL-*Penta-*N,O*-acetyl-(1,3/2,4)-1-*C*-aminomethyl-5-cyclohexene-1,2,3,4-tetrol (38).* Reduction of **36** (92 mg) with hydrogen sulfide and successive acetylation, were carried out in a similar way as in the preparation of **35**. Similar fractionation of the acetylated products gave two fractions (R_f 0.25 and 0.32, 1 : 5 ethanol–benzene). The fraction having R_f 0.32 gave **38** (76 mg, 79%): mp 183—184 °C; ^{1}H NMR ($CDCl_3$) δ=1.97 (3H, s), 2.03 (9H, s), and 2.08 (3H, s) (OAc and NAc), 3.48 (1H, dd, J=5.5 and 14 Hz, changing to d with J=14 Hz on deuteration) and 3.88 (1H, dd, J=5 and 14 Hz, changing to d with J=14 Hz on deuteration) ($C\underline{H}_2$NHAc), 5.36—5.65 (2H, m, H-2 and H-3), and 5.68—6.11 (4H, m, H-4, H-5, H-6, and N$\underline{H}$Ac, changing on deuteration).

Found: C, 53.34; H, 6.14; N, 3.52%. Calcd for $C_{17}H_{23}NO_9$: C, 52.98; H, 6.02; N, 3.63%.

The fraction having R_f 0.25 gave DL-*N*-acetyl-2,3,4-tri-*O*-acetyl-(1,3/2,4)-1-*C*-aminomethyl-5-cyclohexene-1,2,3,4-tetrol (**37**) (11 mg, 13%) as a syrup: ^{1}H NMR ($CDCl_3$) δ=2.02 (9H, s) and 2.10 (3H, s) (OAc and NAc), 3.46 (1H, broad s, O$\underline{H}$, disappearing on deuteration), 3.57 (2H, d, J=6 Hz, $C\underline{H}_2$-NHAc, changing to s on deuteration), 5.13—5.53 (3H, m, H-2, H-3, and H-4), 5.62 (1H, broad d, J=*ca.* 10.5 Hz) and 5.80 (1$\underline{H}$, d, J=10.5 Hz) (H-5 and H-6), and 5.97—6.32 (1H, m, N$\underline{H}$, disappearing on deuteration).

Found: C, 52.86; H, 6.23; N, 3.88%. Calcd for $C_{15}H_{21}NO_8$: C, 52.47; H, 6.17; N, 4.08%.

Further acetylation of **37** at room temperature for 2 d yielded **38** quantitatively.

DL-*Tetra-O-acetyl-(1,3/2,4)-4-chloro-4-C-hydroxymethyl-5-cyclohexene-1,2,3-triol (**41**).* To a cooled, stirred solution of **26** (0.11 g) in tetrahydrofuran (4 ml) was added 12 M hydrochloric acid[27] (0.035 ml), and the mixture was allowed to stand at 0—5 °C for 1 h. The mixture was then neutralized by gradual addition of solid sodium hydrogencarbonate with ice-cooling. After precipitates had been removed by filtration, the filtrate was evaporated to give a syrup, which was crystallized from ethanol–water, affording **39** (124 mg, 100%) as particles: mp 125—126.6 °C; ^{1}H NMR ($CDCl_3$) δ=1.98 (3H, s), 2.02 (3H, s), and 2.10 (3H, s) (OAc), 2.78 (1H, broad s, O$\underline{H}$, disappearing on deuteration), 3.70 and 4.01 (AB-quartet, J=11.5 Hz, $C\underline{H}_2$OH), 5.25—5.67 (3H, m, H-1, H-2, and H-3), and 5.75 (2H, broad s, H-5 and H-6).

Found: C, 48.70; H, 5.40; Cl, 11.06%. Calcd for $C_{13}H_{17}ClO_7$: C, 48.68; H, 5.34; Cl, 11.05%.

Purification of **39** by chromatography on silica gel with 1 : 5 2-butanone–toluene induced an acetyl group migration, which led to partial transformation into syrupy 1,2,7-triacetate **40**: ^{1}H NMR ($CDCl_3$) δ=2.02 (3H, s), 2.08 (3H, s), and 2.10 (3H, s) (OAc), 2.99 (1H, d, J=6 Hz, O$\underline{H}$, disappearing on deuteration), 4.00—4.22 (1H, m, H-3, changing to dd with J=2 and 8 Hz at δ=4.11 on deuteration), 4.39 (2H, s, $C\underline{H}_2$OAc), 5.18—5.70 (2H, m, H-1 and H-2), and 5.57 (1H, d, J=10 Hz) and 5.75 (1H, broad d, J=10 Hz) (H-5 and H-6).

Found: C, 48.73; H, 5.46; Cl, 11.06%. Calcd for $C_{13}H_{17}ClO_7$: C, 48.68; H, 5.34; Cl, 11.05%.

Treatment of **39** and **40** with acetic anhydride containing a trace of sulfuric acid at room temperature overnight gave the tetraacetate **41** in a quantitative yield. Recrystallization from ethanol gave an analytical sample, thin plates: mp 90—91 °C; ^{1}H NMR ($CDCl_3$) δ=1.98 (3H, s), 2.01 (3H, s), 2.08 (3H, s), and 2.11 (3H, s) (OAc), 4.32 (2H, s, $C\underline{H}_2$OAc), 5.37—5.62 (3H, m, H-1, H-2, and H-3), and 5.62 (1H, d, J=10 Hz) and 5.78 (1H, d, J=10 Hz) (H-5 and H-6).

Found: C, 49.58; H, 5.13; Cl, 9.84%. Calcd for $C_{15}H_{19}ClO_8$: C, 49.66; H, 5.28; Cl, 9.77%.

*Azidolysis of **41** with Sodium Azide.* A mixture of **41** (90 mg), sodium azide (32 mg, 2 molar equiv.), and DMF (4 ml) was stirred at 60 °C for 15 h. TLC (1 : 5 2-butanone–toluene) indicated that **4** (R_f 0.47) was converted into a sole azide (R_f 0.41). The mixture was then diluted with ethyl acetate (10 ml) and the solution was washed with cold water thoroughly, and dried. Evaporation of the solvent gave **21** (85 mg, 93%) as a syrup, which was shown to be identical with the authentic sample by ^{1}H NMR analysis.

*Acetolysis of **41** with Sodium Acetate.* A mixture of **41** (53 mg), sodium acetate (48 mg, 4 molar equiv.), and DMF (2 ml) was stirred at 110 °C for 30 h. The mixture was then evaporated and the residue was acetylated in the usual way. TLC and ^{1}H NMR analyses indicated that the acetylated product contained **23**, **24**, **29**, and **30**. Further isolation was not attempted.

DL-*1,2 : 3,7-Di-O-isopropylidene-(1,3,6/2)-6-azido-4-hydroxymethyl-4-cyclohexene-1,2,3-triol (**47**).* To an ice-cooled, stirred solution of **21** (0.16 g) in methanol (3 ml) was added 1 M methanolic sodium methoxide (1 ml), and the mixture was left at 0—5 °C overnight. The reaction mixture was then treated with Amberlite IR-120 (H$^+$) and evaporated to give a syrup (97 mg). Without further purification, the syrup was dissolved in DMF (3 ml) and to the solution was added 2,2-dimethoxypropane (1 ml) and *p*-toluenesulfonic acid (8 mg). The mixture was stirred at room temperature overnight. After neutralization with solid sodium hydrogencarbonate, the mixture was diluted with ethyl acetate (30 ml), then washed with cold water (3×10 ml), and dried. Evaporation of the solvent left a syrup, which crystallized upon standing in a desiccator. Recrystallization from ethanol gave **47** (88 mg, 72% from **21**): mp 116.5—117.5 °C; ^{1}H NMR ($CDCl_3$) δ= 1.40 (3H, s), 1.47 (6H, s), and 1.52 (3H, s) (isopropylidene $C\underline{H}_3$), 3.61 (1H, dd, J=4 and 9.5 Hz, H-1), 3.97 (1H, dd, J=7.5 and 9.5 Hz, H-1), 3.97 (1H, dd, J=7.5 and 9.5 Hz, H-2), 4.15—4.57 (4H, m, H-3, H-6, and $C\underline{H}_2$O), and 5.41 (1H, broad d, J=*ca.* 6 Hz, H-5).

Found: C, 55.37; H, 6.75; N, 14.67%. Calcd for $C_{13}H_{19}N_3O_4$: C, 55.51; H, 6.81; N, 14.94%.

DL-*1,2 : 3,7-Di-O-isopropylidene-(1,3/2,6)-6-azido-4-hydroxymethyl-4-cyclohexene-1,2,3-triol (**44**).* A mixture of DL-tetra-*O*-acetyl-(1,3,6/2)-6-bromo-4-hydroxymethyl-4-cyclohexene-1,2,3-triol (**9**)[9] (0.3 g), sodium azide (72 mg, 1.5 molar equiv.), and DMF (10 ml) was stirred at room temperature for 3 h. TLC showed the formation of two products **19** (major) and **21** (minor). The mixture was diluted with ethyl acetate (40 ml) and the solution was washed with cold water, and then dried. Evaporation of the solvent gave a syrupy mixture of **19** and **21** (0.29 g, 100%).

A 0.16 g portion of the mixture was then treated with 1 M methanolic sodium methoxide (3 ml) in methanol (3 ml) at 0—5 °C overnight. The mixture was neutralized with Amberlite IR-120 (H$^+$) and evaporated, giving a colorless syrup (68 mg), which was purified by a silica-gel column (3 g) with 1 : 3 2-butanone–toluene to give a syrupy mixture of DL-(1,3/2,6)- (**42**) and (1,3,6/2)-6-azido-4-hydroxymethyl-4-cyclohexene-1,2,3-triol (**43**) (59 mg, 66%). Isolation of **42** and **43** was not achieved, because their chromatographic mobilities on silica gel were identical.

Found: C, 41.97; H, 5.50; N, 20.60%. Calcd for $C_7H_{11}N_3O_4$: C, 41.79; H, 5.51; N, 20.89%.

The syrupy mixture (85 mg) was dissolved in DMF (3 ml) and treated with 2,2-dimethoxypropane (1 ml) in the presence of *p*-toluenesulfonic acid (8 mg) at room temperature for 24 h. After neutralization with solid sodium hydrogencarbonate, the mixture was worked up similarly as described in the preparation of **47**. The crude products were fractionated on a silica-gel column (6 g) with 1 : 15 2-butanone–toluene as an eluent to give two fractions. The first fraction gave **44** (75 mg, 42% from **9**): mp 79—80 °C; ^{1}H NMR ($CDCl_3$) δ=1.40 (3H, s), 1.46 (6H, s), and 1.54 (3H, s) (isopropylidene $C\underline{H}_3$), 3.50 (1H, dd, J=8 and 10 Hz) and 3.69 (1H, dd, J=7 and 10 Hz) (H-1 and H-2), 4.00—4.67 (4H, m, H-3, H-6, and $C\underline{H}_2$O), and

5.28 (1H, s, H-5).

Found: C, 55.37; H, 6.75; N, 14.67%. Calcd for $C_{13}H_{19}N_3O_4$: C, 55.51; H, 6.81; N, 14.94%.

The second fraction gave **47** (17 mg, 9% from **9**): mp 116—117 °C, identical with the compound obtained from **21** described above.

DL-*1,2: 3,7-Di-O-isopropylidene-(1,3,6/2)-6-acetamido-4-hydroxymethyl-4-cyclohexene-1,2,3-triol (49).* Hydrogen sulfide was bubbled into a solution of **47** (80 mg) in 1 : 1 pyridine–water (3 ml) at room temperature for 1 h. The pale yellow mixture with sulfur precipitated was evaporated and the syrup residue was placed on a short column of alumina. The first fraction eluted with toluene gave sulfur as needles and the second fraction, showing a positive test of ninhydrin, eluted with ethanol gave DL-1,2 : 3,7-di-*O*-isopropylidene-(1,3,6/2)-6-amino-4-hydroxymethyl-4-cyclohexene-1,2,3-triol (**48**) (72 mg, 100%) as a syrup.

Acetylation of **48** (30 mg), without further purification, in the usual way gave, after recrystallization from ethanol, **49** (35 mg, 98%) as particles: mp 241—242 °C; ^{1}H NMR ($CDCl_3$–D_2O) $\delta=1.41$ (3H, s), 1.45 (6H, s), and 1.55 (3H, s) (isopropylidene $C\underline{H}_3$), 1.99 (3H, s, NAc), 3.50—3.86 (2H, m, H-1 and H-2), 4.13 and 4.47 (AB-quartet, $J=15$ Hz, $C\underline{H}_2O$), 4.50 (1H, broad d, $J=ca.$ 5 Hz, H-3), 4.66—4.94 (1H, m, H-6), and 5.62 (1H, broad d, $J=ca.$ 5 Hz, H-5).

Found: C, 60.85; H, 7.83; N, 4.61%. Calcd for $C_{15}H_{23}NO_5$: C, 60.59; H, 7.80; N, 4.71%.

DL-*1,2 : 3,7-Di-O-isopropylidene-(1,3,6/2)-6-trifluoroacetamido-4-hydroxymethyl-4-cyclohexene-1,2,3-triol (50).* To an ice-cooled solution of **48** (0.26 g) in pyridine (4 ml) was added dropwise trifluoroacetic anhydride (0.45 ml) and the mixture was left at room temperature overnight. Evaporation of the solvent followed by coevaporation with toluene several times gave a colorless syrup, which solidified upon standing in a desiccator to give **50** (0.34 g, 96%). It was analytically pure without further purification: ^{1}H NMR ($CDCl_3$) $\delta=1.41$ (6H, s), 1.46 (3H, s), and 1.54 (3H, s) (isopropylidene $C\underline{H}_3$), 3.56—3.82 (2H, m, H-1 and H-2), 4.11 and 4.41 (AB-quartet, $J=15$ Hz, $C\underline{H}_2O$), 4.39—4.55 (1H, m, H-6, relatively sharpened on deuteration), and 5.61 (1H, broad d, $J=ca.$ 5 Hz, H-5).

Found: C, 51.16; H, 5.77; N, 3.97%. Calcd for $C_{15}H_{20}NF_3O_5$: C, 51.28; H, 5.74; N, 3.99%.

DL-*1,2:3,7-Di-O-isopropylidene-(1,3/2,6)-6-acetamido-4-hydroxymethyl-4-cyclohexene-1,2,3-triol (46).* Compound **44** (190 mg) was reduced in a similar manner as described in the preparation of **48** to give DL-1,2 : 3,7-di-*O*-isopropylidene-(1,3/2,6)-6-amino-4-hydroxymethyl-4-cyclohexene-1,2,3-triol (**45**) (170 mg, quantitative) as a syrup, showing a positive test of ninhydrin.

Acetylation of **45** (30 mg) in the usual way gave **46** (33 mg, 92%) as a pale yellow syrup, which was crystallized from ether by scratching the flask to give **46** (22 mg, 62%): mp 178—179 °C; ^{1}H NMR ($CDCl_3$–D_2O) $\delta=1.39$ (3H, s), 1.43 (6H, s), and 1.52 (3H, s) (isopropylidene $C\underline{H}_3$), 1.96 (3H, s) (NAc), 3.53 (1H, t, $J=9$ Hz, H-2), 3.78 (1H, dd, $J=7.5$ Hz, H-1), 4.12 and 4.46 (AB-quartet, $J=12.5$ Hz, $C\underline{H}_2O$), 4.57—4.81 (2H, m, H-3 and H-6), and 5.38 (1H, broad s, H-5).

Found: C, 60.69; H, 7.64; N, 4.81%. Calcd for $C_{15}H_{23}NO_5$: C, 60.59; H, 7.80; N, 4.71%.

DL-*3,7-O-Benzylidene-(1,3/2,6)- (51) and -(1,3,6/2)-6-azido-4-hydroxymethyl-4-cyclohexene-1,2,3-triol (52).* A syrupy mixture (247 mg) of **42** and **43** was dissolved in DMF (10 ml) and treated with α,α-dimethoxytoluene (0.228 ml, 1.2 molar equiv.) in the presence of *p*-toluenesulfonic acid (12 mg) at 60—65 °C under reduced pressure (22 mmHg) (1 mmHg=133.322 Pa) for 3.5 h. TLC (1 : 2 2-butanone–toluene) indicated the formation of two products (major, R_f 0.4; minor, R_f 0.3). After neutralization with Amberlite IRA-400 (OH^-), the solvent was removed by evaporation and the residue was fractionated on a silica-gel column (5 g) with 1 : 3 2-butanone–toluene as an eluent. The first fraction gave **51** (154 mg, 43%) as needles: mp 149—150 °C; ^{1}H NMR (CD_3OD) $\delta=3.45$ (1H, dd, $J=9$ and 10.5 Hz, H-2), 3.67 (1H, dd, $J=7.5$ and 10.5 Hz, H-1), 3.98 (1H, broad d, $J=ca.$ 7.5 Hz, H-6), 4.18—4.47 (3H, m, H-3 and $C\underline{H}_2O$), 5.41 (1H, s, H-5), 5.67 (1H, s, benzylic proton), and 7.29—7.64 (5H, m, phenyl).

Found: C, 57.96; H, 5.26; N, 14.48%. Calcd for $C_{14}H_{15}N_3O_4$: C, 58.13; H, 5.23; N, 14.53%.

The second fraction gave **52** (64 mg, 18%) as feathers: decomp at 180.5 °C; ^{1}H NMR (CD_3OD) $\delta=3.62$—4.00 (2H, m, H-1 and H-2), 4.15—4.42 (2H, m, H-3 and H-6), 4.50 (2H, s, $C\underline{H}_2O$), 5.69 (1H, d, $J=5$ Hz, H-5), 5.70 (1H, s, benzylic proton), and 7.28—7.68 (5H, m, phenyl).

Found: C, 58.06; H, 5.34; N, 14.88%. Calcd for $C_{14}H_{15}N_3O_4$: C, 58.13; H, 5.23; N, 14.53%.

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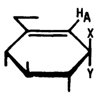

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24) Taken on a Varian EM-360A (60 MHz) spectrometer.

25) Taken on a Varian HA 100D (100 MHz) spectrometer.

26) Phosphate buffer was prepared by adding sufficient aqueous 0.1 M Na_2HPO_4 to 0.1 M NaH_3PO_4 up to pH 8.

27) When 1 M hydrochloric acid was used, the reaction required a longer time (24 h) for completion and a formation of side-products (probably by de-*O*-acetylation) was observed