Carbohydrate Research, 30 (1973) 109–126

SYNTHESIS OF 2,4-DIACETAMIDO-2,4,6-TRIDEOXY-L-ALTROSE, -L-IDOSE, AND -L-TALOSE FROM BENZYL 6-DEOXY-3,4-*O*-ISOPROPYLIDENE-β-L-GALACTOPYRANOSIDE

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(Received April 22nd, 1970; accepted, in revised form, March 10th, 1973)

ABSTRACT

Acetylation of benzyl 6-deoxy-3,4-*O*-isopropylidene-β-L-galactopyranoside gave benzyl 2-*O*-acetyl-6-deoxy-3,4-*O*-isopropylidene-β-L-galactopyranoside (**1**). Removal of the isopropylidene group afforded benzyl 2-*O*-acetyl-6-deoxy-β-L-galactopyranoside (**2**), which was converted into benzyl 2-*O*-acetyl-6-deoxy-3,4-di-*O*-(methylsulfonyl)-β-L-galactopyranoside (**3**). Benzyl 2,3-anhydro-6-deoxy-4-*O*-(methylsulfonyl)-β-L-gulopyranoside (**4**) was obtained from **3** by treatment with alkali. Reaction of **4** with sodium azide in *N*,*N*-dimethylformamide gave a mixture of two isomeric benzyl 2,4-diazido-2,4,6-trideoxy hexoses, the syrupy diazido derivative **5** and the crystalline benzyl 2,4-diazido-2,4,6-trideoxy-β-L-idopyranoside (**6**). Acetylation of **6** afforded a compound whose n.m.r. spectrum was completely first order and in agreement with the structure of benzyl 3-*O*-acetyl-2,4-diazido-2,4,6-trideoxy-β-L-idopyranoside (**7**). Lithium aluminium hydride reduction of **5**, followed by acetylation, afforded a crystalline product (**8**), shown by n.m.r. spectroscopy to be benzyl 2,4-diacetamido-3-*O*-acetyl-2,4,6-trideoxy-β-L-altropyranoside. Similar treatment of the diazido derivative **6** afforded benzyl 2,4-diacetamido-3-*O*-acetyl-2,4,6-trideoxy-β-L-idopyranoside (**9**). Compounds **8** and **9** could also be obtained from **4** by treatment of the crude diazido mixture with lithium aluminium hydride, with subsequent *N*-acetylation. The syrupy benzyl 2,4-diacetamido-2,4,6-trideoxy-β-L-altropyranoside (**10**) and the crystalline benzyl 2,4-diacetamido-2,4,6-trideoxy-β-L-idopyranoside (**11**) thus obtained were then *O*-acetylated to give **8** and **9** respectively. Benzyl 2,4-diacetamido-2,4,6-trideoxy-β-L-talopyranoside (**15**) was obtained from **11** by treatment with methanesulfonyl chloride and subsequent solvolysis. Compound **15** was *O*-acetylated to yield benzyl 2,4-diacetamido-3-*O*-acetyl-2,4,6-trideoxy-β-L-talopyranoside (**16**). the n.m.r. spectrum of which was in full agreement with the assigned structure. The mass spectra of compounds **8**–**11**, **15**, and **16** were also in agreement with their proposed structures. Removal of the benzyl groups from **10**, **11** and **15** afforded the corresponding 2,4-diacetamido-2,4,6-trideoxyhexoses **12**, **13**, and **17**, having the L-*altro*, L-*ido*, and L-*talo* configurations, respectively.

INTRODUCTION

Amino sugars of unusual structure are of considerable importance, as they have been found to occur as components of many antibiotics[1,2] and bacterial polysaccharides[3]. For several years our laboratory has been interested in the chemistry of some of these unusual amino sugars, in particular of 2,4-diamino-2,4,6-trideoxyhexoses. Such a diamino sugar was first isolated from a polysaccharide of *Bacillus licheniformis* (then known as *Bacillus subtilis*) by Sharon and Jeanloz[4] and has been shown recently[5] to possess the structure of 4-acetamido-2-amino-2,4,6-trideoxy-D-glucose. In an attempt to synthesize related diamino sugars, methyl 2,4-diacetamido-3-*O*-acetyl-2,4,6-trideoxy-α-L-altropyranoside and methyl 2,4-diacetamido-3-*O*-acetyl-2,4,6-trideoxy-α-L-idopyranoside were prepared from methyl 6-deoxy-3,4-*O*-isopropylidene-α-L-galactopyranoside[6]. However, acid hydrolysis of these diamino hexose derivatives led to extensive degradation. This difficulty can be avoided by using benzyl 6-deoxy-3,4-*O*-isopropylidine-β-L-galactopyranoside (benzyl 3,4-*O*-isopropylidene-β-L-fucopyranoside) as starting material instead of the analogous methyl glycoside, as the benzyl group can be easily removed under mild neutral conditions.

RESULTS AND DISCUSSION

Acetylation of benzyl 6-deoxy-3,4-*O*-isopropylidene-β-L-galactopyranoside[7] with acetic anhydride and pyridine gave the crystalline benzyl 2-*O*-acetyl-6-deoxy-3,4-*O*-isopropylidene-β-L-galactopyranoside (**1**). Examination of the 100-MHz n.m.r. spectrum of **1** (Tables I and II) showed all of the ring protons except H-4 to be axial; the C-2 acetoxyl group was equatorial (τ 7.94) and the benzylic methylene protons resonated as an AB doublet of doublets.

Treatment of **1** with Amberlite IRC-50 (H^+) resin, selectively removed the isopropylidene group, yielding benzyl 2-*O*-acetyl-6-deoxy-β-L-galactopyranoside (**2**). Compound **2** was converted by methanesulfonyl chloride in pyridine into benzyl 2-*O*-acetyl-6-deoxy-3,4-di-*O*-(methylsulfonyl)-β-L-galactopyranoside (**3**). The n.m.r. spectrum of **3** could be fully analyzed without the use of double irradiation and the coupling constants were similar to those found for compound **1**. Treatment of **3** with sodium methoxide yielded benzyl 2,3-anhydro-6-deoxy-4-*O*-(methylsulfonyl)-β-L-gulopyranoside (**4**). The n.m.r. spectrum of **4** could be interpreted readily at 100 MHz (Fig. 1). The coupling constants of the ring protons (Table II) were in agreement with the expected half-chair conformation[8–10], as well as with those found for the corresponding methyl 2,3-anhydro-6-deoxy-4-*O*-(methylsulfonyl)-α-L-gulopyranoside[6]. The H-1 signal appeared as a low-field singlet (τ 5.13), so that the one-proton doublet at τ 6.66 ($J_{2,3}$ 3.5 Hz) could be attributed to H-2. The H-5 signal appeared as an octet (τ 6.27) having $J_{5,6}$ 6.5 Hz and $J_{4,5}$ 1.7 Hz, and the triplet at τ 5.20 (J 1.7 Hz) was assigned to H-4. The remaining, unresolved multiplet at τ 6.44 is thus the H-3 signal. The foregoing interpretation was confirmed by double-resonance experiments (Fig. 1). By irradiation at the frequency of H-4, the H-5 octet collapsed into a quartet having

TABLE I

CHEMICAL SHIFTS[a] (τ UNITS) OF COMPOUNDS **1**, **3**, **4**, **7**, **8**, **9**, AND **16**

Compound	*Solvent*	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*Ph*	*CH_2*	*OAc*	*NAc*	*5-Me*	*Other*
1[b]	$CDCl_3$	5.67d	4.98q	5.91q	6.02q	6.17o	2.70s	5.28dd	7.94		8.57d	8.42, 8.65[$C(Me)_2$]
3[c]	$CDCl_3$	5.50d	4.70q	5.20q	5.00q	6.20o	2.68s	5.22dd	7.91		8.58d	6.82, 6.92(Ms)
4[b]	$CDCl_3$	5.13s	6.66d	6.44m	5.20t	6.27o	2.62s	5.16dd			8.70d	6.87(Ms)
7[c]	$CDCl_3$	5.15d	6.72q	4.70t	6.55q	5.98o	2.72s	5.30dd	7.92		8.62d	
8[b]	$CDCl_3$	5.14d	5.66–5.91m	4.75q	5.66–5.91m	6.18m	2.70s	5.30dd	7.94	8.02(2)	8.62d	3.80d (NH)
8[d]	C_5D_5N	4.83d	5.06–5.25m	4.29q	5.06–5.26m	5.94o	2.53–2.90m[e]	5.25dd	8.16	8.05(2)	8.72d	
8[d]	$(CD_3)_2CO$	5.13d	5.74–5.98m	4.96q	5.74–5.98m	6.23o	2.78s	5.37dd	8.00	{8.12}{8.20}	8.80d	
9[b]	$CDCl_3$	5.10d	5.60–5.94m	4.88q	5.60–5.94m	5.60–5.94m	2.66s	5.32dd	7.96	{8.04}{8.12}	8.72d	3.80q (NH)
9[d]	C_5D_5N	4.85d	5.20–5.42m	4.40t	5.20–5.42m	5.65m	2.44–2.82m[e]	5.26dd	8.08	8.10(2)	8.67d	
16[b]	$CDCl_3$	5.52bs	5.62–5.84m	5.16q	5.62–5.84m	6.42o	2.73s	5.34dd	7.94	7.98(2)	8.82d	3.36m (NH)

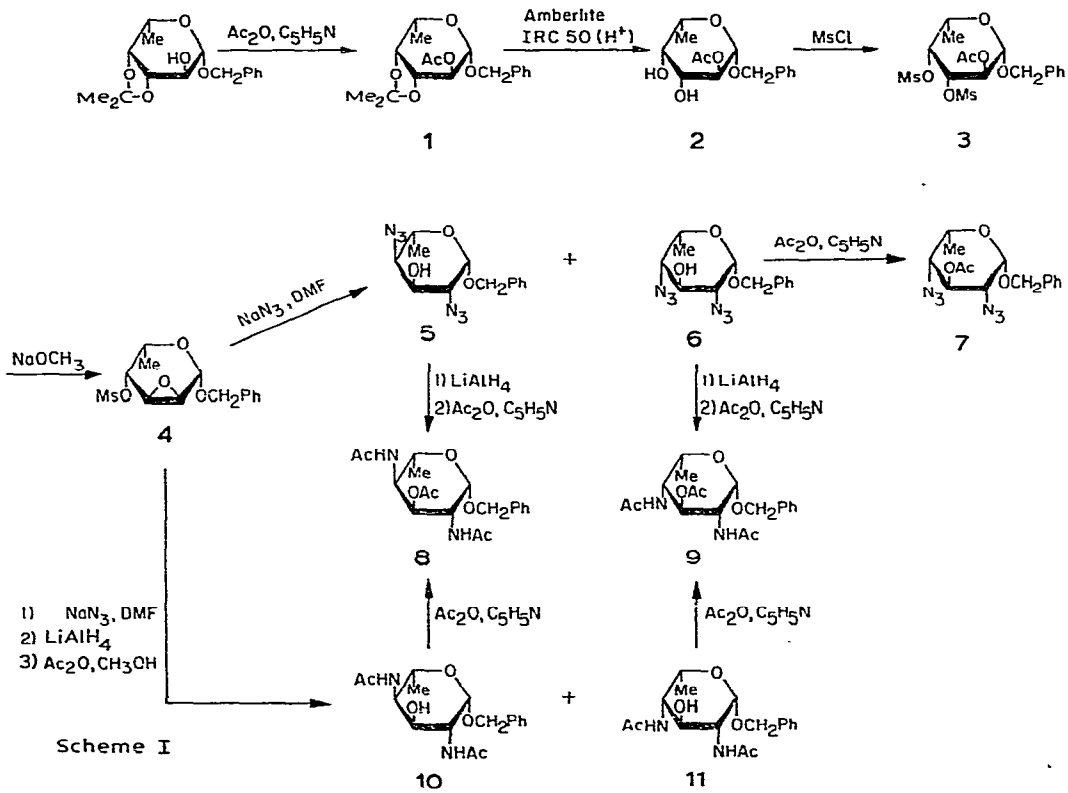
[a]Observed multiplicities: b, broadened; d, doublet; dd, doublet of doublets; m, multiplet; o, octet; q, quartet, s, singlet; t, triplet. [b]Recorded at 100 MHz. [c]Recorded at 60 MHz. [d]Recorded at 90 MHz. [e]Probably mixed with solvent signals.

TABLE II

COUPLING CONSTANTS (Hz) OF METHINE PROTONS OF COMPOUNDS **1, 3, 4, 7, 8, 9** AND **16**[a]

Compound	*Solvent*	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$
1[b]	$CDCl_3$	8.5	7.5	4.25	2.0	6.5
3[c]	$CDCl_3$	7.5	10.0	3.0	1.0	6.5
4[b]	$CDCl_3$	0	3.5	1.7	1.7	6.5
7[c]	$CDCl_3$	3.0	6.5	6.5	4.0	6.5
8[b]	$CDCl_3$	2.5	6.5	3.5	7.0	6.5
8[d]	C_5D_5N	2.5	4.5	3.5	9.25	6.5
8[d]	$(CD_3)_2CO$	2.0	4.5	3.0	9.0	6.5
9[b]	$CDCl_3$	3.5	5.5 or 8.5	5.5 or 8.5	[e]	6.5
9[d]	C_5C_5N	3.0	6.5	6.5	4.0	6.5
16[b]	$CDCl_3$	~0	4.0 or 4.5	4.0 or 4.5	2.0	6.5

[a]The benzyl methylene protons appeared as an AB pair of doublets, $J = 12$ Hz, in all of the compounds examined. [b]Recorded at 100 MHz. [c]Recorded at 60 MHz. [d]Recorded at 90 MHz. [e]Could not be obtained from the spectrum.



$J_{5,6}$ 6.5 Hz, and the H-3 multiplet changed into a clear doublet having $J_{2,3}$ 3.5 Hz. The triplet of H-4 collapsed into a doublet (J 1.7 Hz), by irradiation at either H-3 or at H-5.

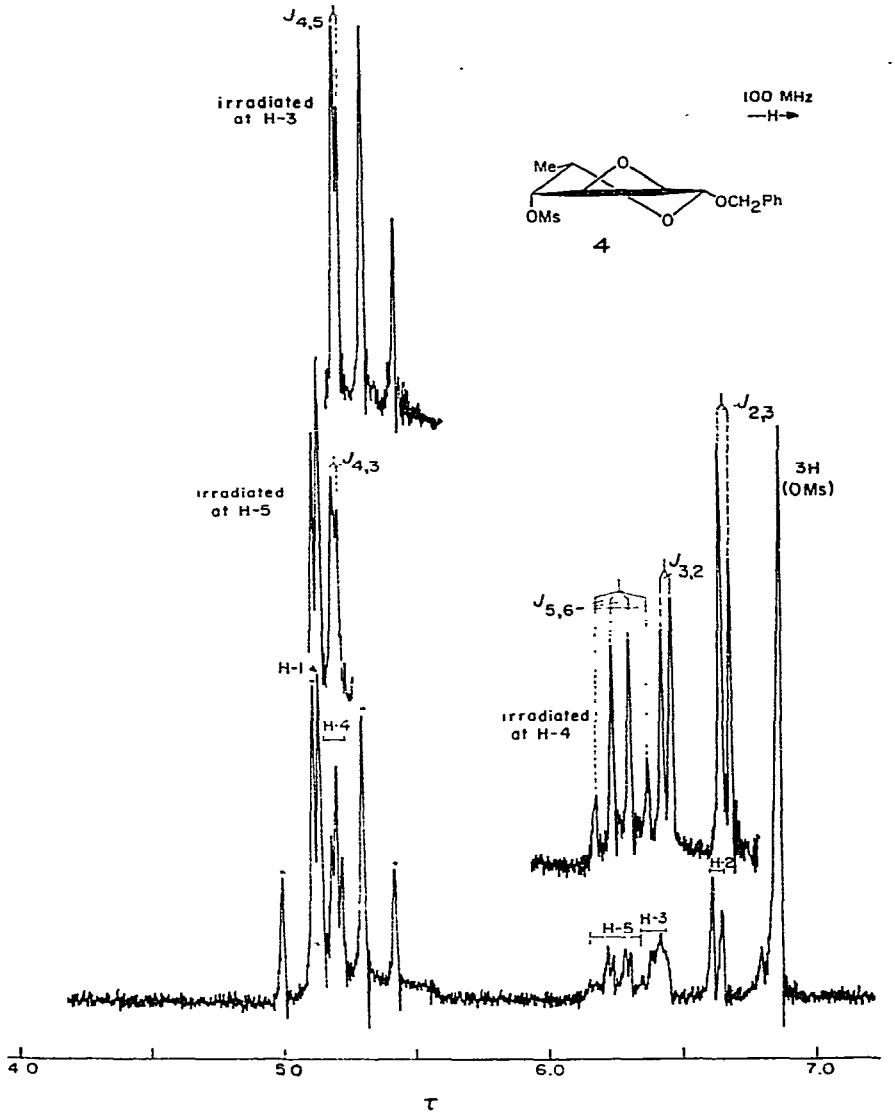


Fig. 1. Partial n.m.r. spectrum of benzyl 2,3-anhydro-6-deoxy-4-*O*-methylsulfonyl-β-L-gulopyranoside (4) at 100 MHz in $CDCl_3$. The sign * describes the AB pair of doublets of the benzylic CH_2.

Amino groups are commonly introduced into sugars by treatment of suitable derivatives with sodium azide in *N*,*N*-dimethylformamide, with subsequent reduction[11,12]. Upon treatment of compound **4** with sodium azide, two types of

reaction were expected to occur: (*a*), ring opening and (*b*), nucleophilic displacement of the methylsulfonyloxy group. With such compounds as **4**, which possess a flexible conformation, ring opening may proceed by attack either at C-2 or at C-3 (ref. 13). Such ring opening, with concomitant nucleophilic displacement (with inversion) of the methylsulfonyloxy group at C-4 (reaction *b*) should, in the present case, yield benzyl 2,4-diazido-2,4,6-trideoxy-β-L-altropyranoside and benzyl 3,4-diazido-3,4,6-trideoxy-β-L-glucopyranoside. Indeed, treatment of **4** with sodium azide in *N*,*N*-dimethylformamide yielded a mixture from which two products, syrupy **5** and crystalline **6**, could be obtained by chromatography on silica gel. However, detailed studies showed that compound **5** has the L-*altro* configuration, and the other isomer, **6**, is benzyl 2,4-diazido-2,4,6-trideoxy-β-L-idopyranoside.

Compound **5** showed a strong azide band at 2100 cm^{-1} in its i.r. spectrum, and no sulfonate band was observed. It migrated as a single spot on t.l.c. but n.m.r.

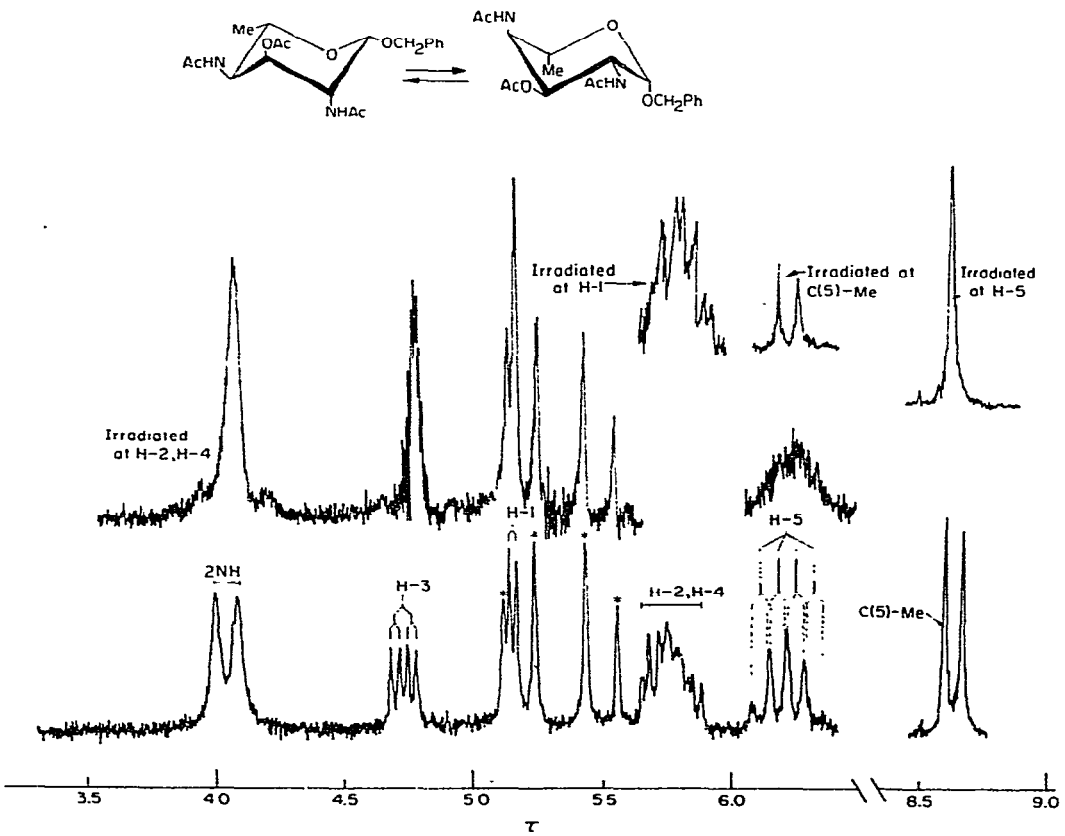


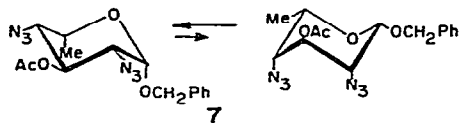
Fig. 2. Partial n.m.r. spectrum of benzyl 2,4-diacetamido-3-*O*-acetyl-2,4,6-trideoxy-β-L-altropyranoside (**8**) at 100 MHz in $CDCl_3$. The sign * describes the AB pair of doublets of the benzylic CH_2.

analysis showed it to be contaminated by a minor by-product. Attempts to purify this compound by repeated chromatography on silica gel were unsuccessful. Reduction of **5** with lithium aluminium hydride, followed by acetylation, yielded the crystalline compound **8**, which was shown to have the structure of benzyl 2,4-diacetamido-3-*O*-acetyl-2,4,6-trideoxy-β-L-altropyranoside. The 100-MHz n.m.r. spectrum of **8** in chloroform-*d* (Fig. 2, Tables I and II) was analyzed as follows: the two-proton doublet at τ 3.80 is due to the two NH protons as it disappeared after the addition of deuterium oxide. The methylene protons of the benzyl group form an AB doublet of doublets, at τ 5.30 (J 12 Hz) and the one-proton doublet at τ 5.14 is attributable, most probably, to H-1. Double-irradiation experiments showed that the multiplet at τ 6.18 is the H-5 signal, as it collapsed to a doublet upon irradiation at the C-5 methyl signal. It was also shown that the resonance at τ 4.75 is coupled neither to H-1 nor to H-5, so that it must be that of H-3. From the intermediate values of the coupling constants found in this spectrum (see Table II), it was difficult to establish unequivocally the configuration of compound **8**. However, when the n.m.r. spectrum of **8** was recorded in pyridine-d_5, remarkable changes in the values of some of the coupling constants were observed: there was a decrease in $J_{2,3}$ (4.5 Hz instead of 6.5 Hz) and an increase in $J_{4,5}$ (9.2 Hz instead of 7.0 Hz). Similar results were obtained when the spectrum of compound **8** was recorded in acetone-d_6. These data are in excellent accord with a β-L-*altro* (*1C*) structure. It appears that, in chloroform solution, both chair conformations of compound **8** (*1C* and *C1*) are present in substantial proportions in equilibrium, but that in more polar solvents there is a shift in the conformational equilibrium to favor the *1C*(L) conformation. These conclusions are in line with a report by Durette and Horton[14], who have studied the influence of solvents on the conformational equilibria of some D-aldopentopyranosides in solution. They found that for methyl tri-*O*-benzoyl-β-D-xylopyranoside there was an increase in the equilibrium proportion of that chair form having the anomeric substituent equatorial with an increase in the dielectric constant of the solvent. It has also been shown[6] that in the highly polar methyl sulfoxide-d_6, methyl 2,4-diacetamido-3-*O*-acetyl-α-L-altropyranoside exists in the *1C* conformation.

The locations of the *O*-acetyl group and of the two acetamido groups in compound **8** were also established by n.m.r. spectroscopy. *O*-Acyl groups are well known[15–17] to deshield protons attached to the same ring position; as a result these protons resonate at a lower field than the other ring protons. In the n.m.r. spectrum of compound **8** (Fig. 2), H-3 resonates at a field lower than that of all other ring protons, so that presumably the *O*-acetyl group is linked to C-3, and the two acetamido groups are attached to C-2 and C-4. Furthermore, addition of deuterium oxide (which caused the disappearance of the NH signals) changed the multiplet pattern of H-2 and H-4, but no change in the shape of the H-3 signal was observed. All of these findings support the proposed structural assignment for compound **8**.

The benzyl diazido derivative **6** was obtained crystalline from the silica gel column; it showed a strong azide band at 2100 cm^{-1} in the infrared. Acetylation of **6** gave the syrupy *O*-acetyl derivative **7**, whose n.m.r. spectrum was well resolved even

at 60 MHz (Fig. 3) and the values of the coupling constants (Table II) indicated that this compound had the L-*ido* configuration. However, as $J_{2,3}$ and $J_{3,4}$ (6.5 Hz) are somewhat low for axial–axial interactions, it appears that, although compound **7**



7

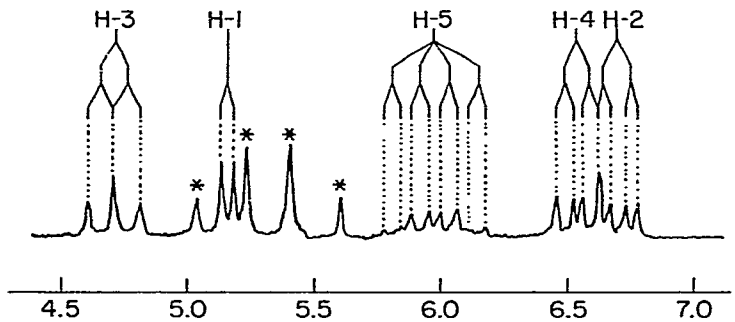
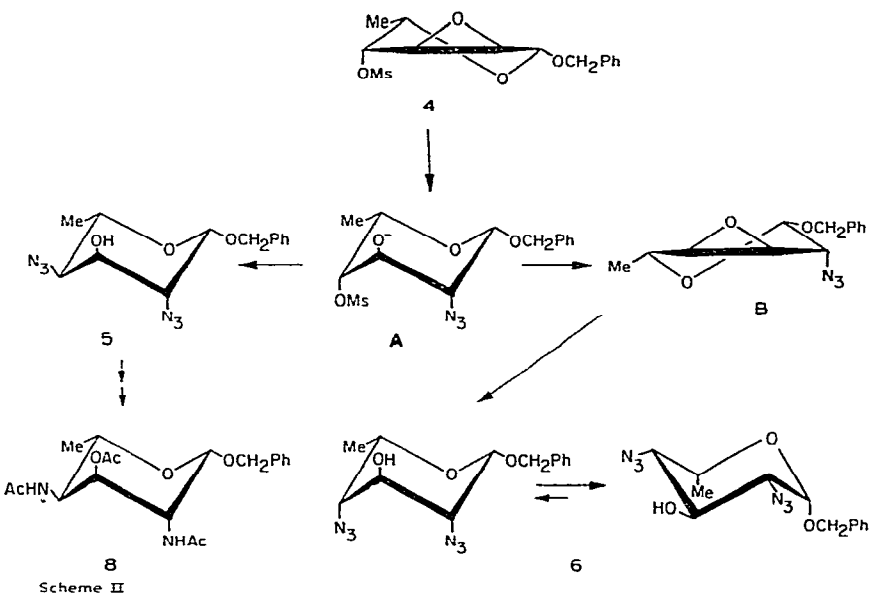


Fig. 3. Partial n.m.r. spectrum of benzyl 3-*O*-acetyl-2,4-diazido-2,4,6-trideoxy-β-L-idopyranoside (**7**) at 60 MHz in $CDCl_3$. The sign * describes the AB pair of doublets of the benzylic CH_2.

favors the *C1*(L) conformation, the other chair conformation is present in substantial proportion. This is similar to the case of methyl 2,4-diacetamido-3-*O*-acetyl-2,4,6-trideoxy-α-L-idopyranoside[6], which was also shown to exist mainly in the *C1*(L) conformation. The *O*-acetyl group in **7** is most probably attached to C-3, because H-3 resonates at a field lower than the other ring protons, as found for the H-3 resonance in the n.m.r. spectrum of compound **8**. On the basis of these data, the structure benzyl 3-*O*-acetyl-2,4-diazido-2,4,6-trideoxy-β-L-idopyranoside was assigned to compound **7**.

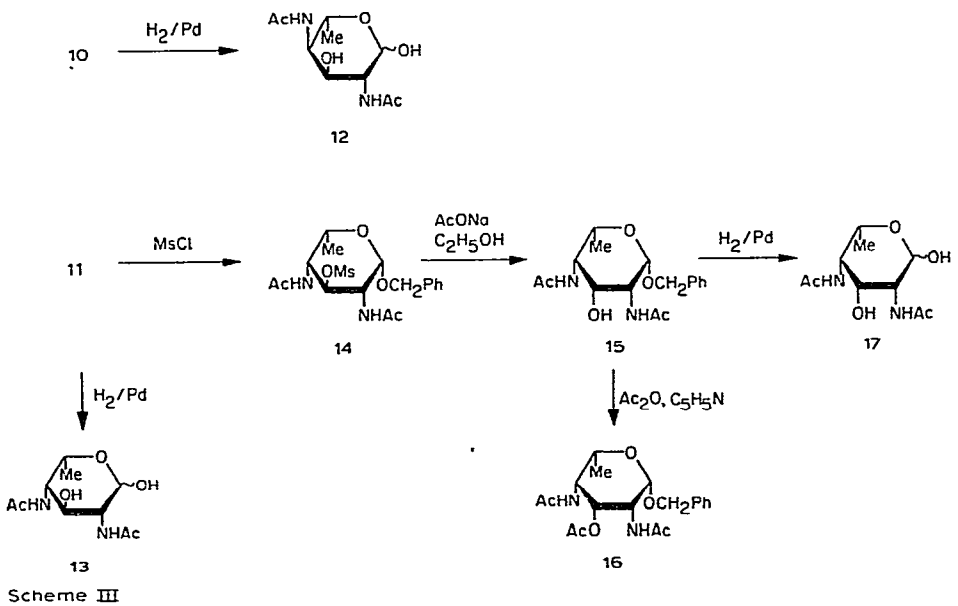
Formation of a 2,4-diazido-L-*ido* derivative from compound **4** can readily be explained by assuming a mechanism involving epoxide-ring migration[6,13,18]. According to this mechanism (scheme II), reaction of compound **4** with the azide ion results first in the *trans*-diaxial opening of the epoxy ring by attack of the nucleophile at C-2; this gives rise to an intermediate (**A**) in which the methylsulfonyloxy group is still attached to C-4. Displacement of the methylsulfonyloxy at C-4 by azide would give the benzyl 2,4-diazido-β-L-*altro* derivative; on the other hand, intramolecular displacement of the methylsulfonyloxy group by the anion at C-3 would lead to the formation of the 3,4-L-*altro* epoxide (**B**); subsequent *trans*-diaxial opening of the epoxy ring in the latter intermediate (**B**) by reaction at C-4 should give the benzyl 2,4-diazido-β-L-*ido* derivative.

Reduction of benzyl 2,4-diazido-2,4,6-trideoxy-β-L-idopyranoside (**6**) by lithium aluminium hydride and subsequent acetylation with acetic anhydride and pyridine afforded the crystalline benzyl 2,4-diacetamido-3-*O*-acetyl-2,4,6-trideoxy-β-L-idopyranoside (**9**). The n.m.r. spectrum of **9** in $CDCl_3$ showed overlapping of the signals



Scheme II

of three ring protons (complex multiplet centered at τ 5.76, probably H-2, H-4, and H-5), so that no unequivocal interpretation of the spectrum could be made. The spectrum was less complex when recorded in pyridine-d_5, and the data obtained (Tables I and II) accorded with the assigned structure. The H-3 signal resonated as a low-field triplet (τ 4.40) and the H-1 doublet was observed at τ 4.85; H-5 resonated as



Scheme III

a complex multiplet, but irradiation at the C-5 methyl group caused this multiplet to collapse to a doublet ($J_{4,5} = 4.0$ Hz).

The two benzyl diacetamido hexosides **8** and **9** could also be obtained from the epoxy derivative **4** directly when the crude diazide mixture was reduced with lithium aluminium hydride and the resultant benzyl diamino derivatives were *N*-acetylated with acetic anhydride in methanol. After chromatography, first on silica gel and then on alumina, the syrupy benzyl 2,4-diacetamido-2,4,6-trideoxy-β-L-altropyranoside (**10**) and the crystalline benzyl 2,4-diacetamido-2,4,6-trideoxy-β-L-idopyranoside (**11**) were

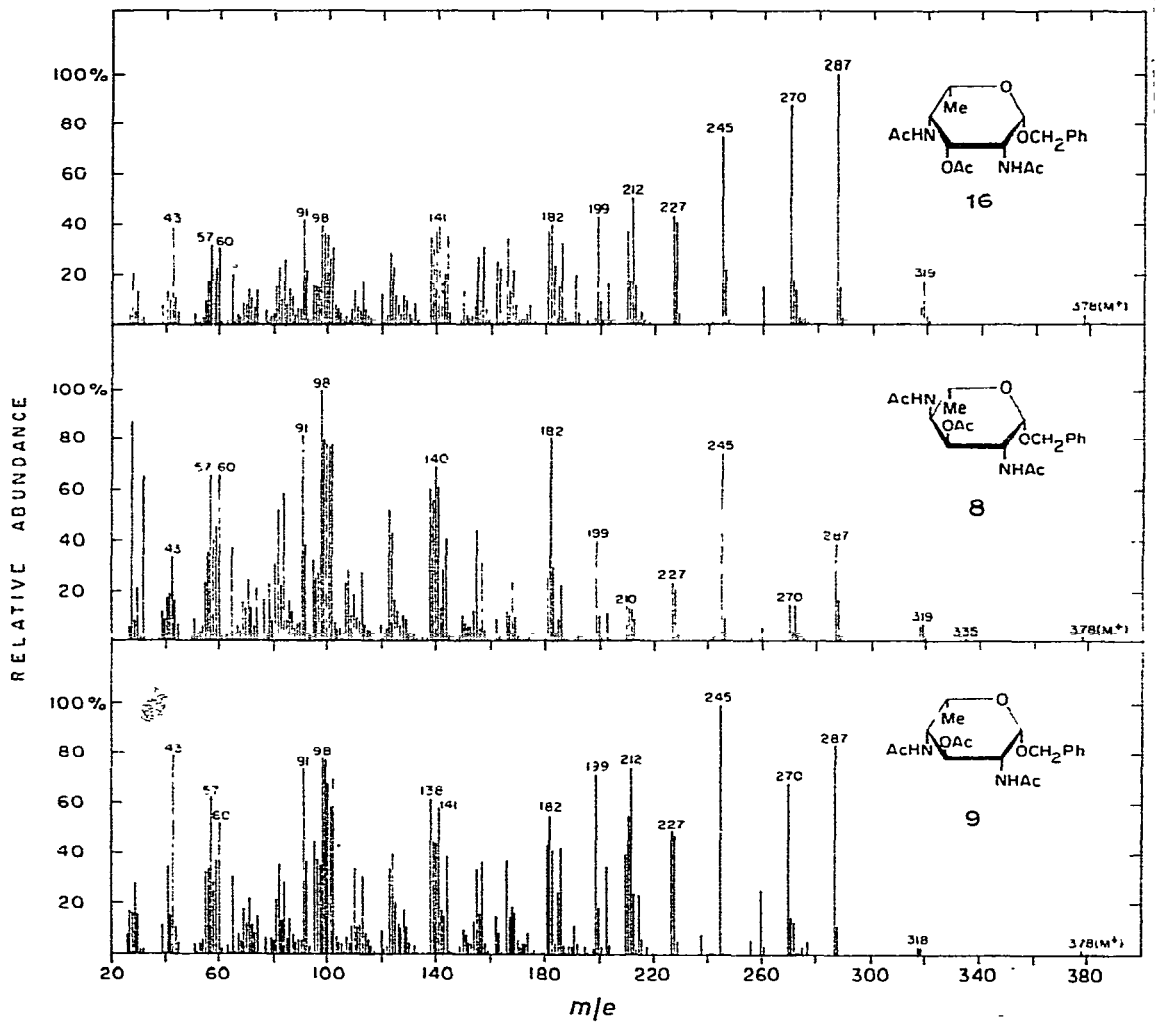
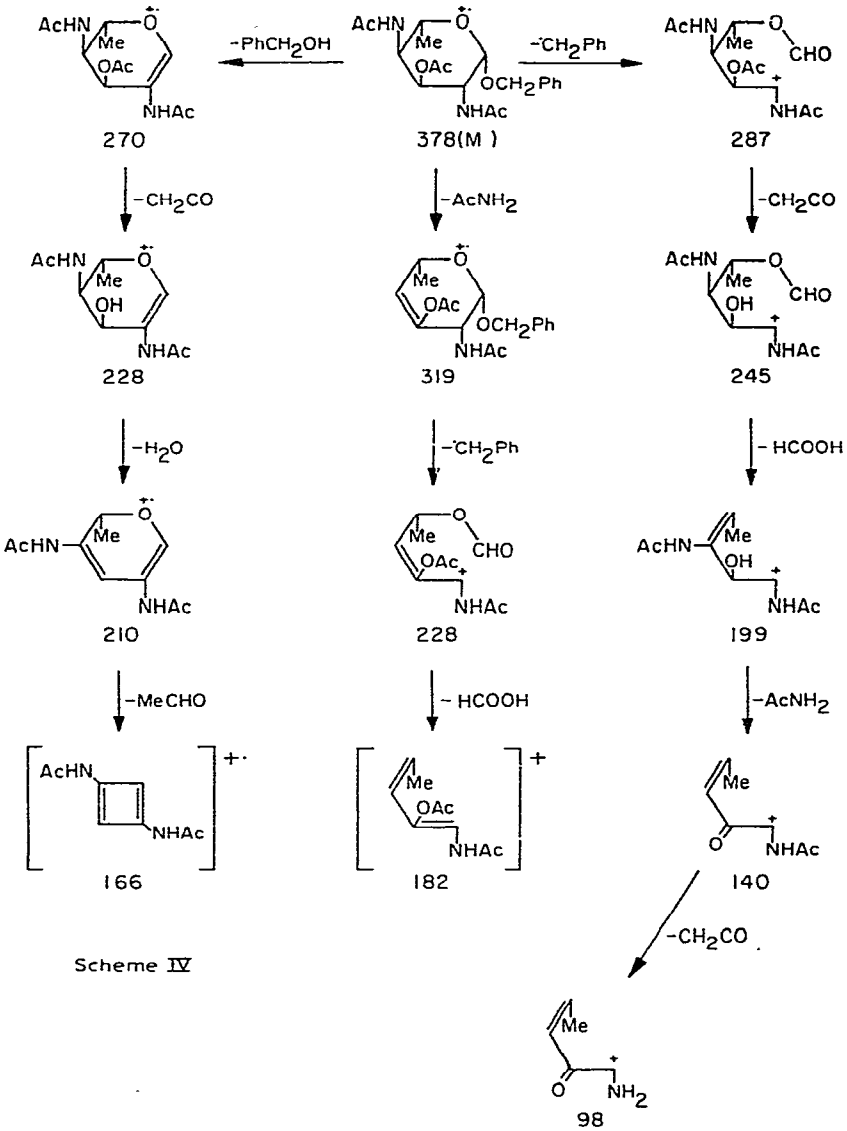


Fig. 4. The mass spectra of benzyl 2,4-diacetamido-3-*O*-acetyl-2,4,6-trideoxy-β-L-altropyranoside (**8**), benzyl 2,4-diacetamido-3-*O*-acetyl-2,4,6-trideoxy-β-L-idopyranoside (**9**), and benzyl 2,4-diacetamido-3-*O*-acetyl-2,4,6-trideoxy-β-L-talopyranoside (**16**).

isolated in an approximate 1:1 ratio. The *O*-acetyl derivatives of **10** and **11** were found to be identical with **8** and **9**, respectively.

Another benzyl 2,4-diacetamido-2,4,6-trideoxy sugar was prepared when compound **11** was treated with methanesulfonyl chloride and the resulting methylsulfonyloxy derivative **14** was solvolyzed by sodium acetate in aqueous ethanol. The solvolysis product **15** differed from **11** in its melting point, optical rotation, and rate of migration on t.l.c. Our results suggest that compounds **11** and **15** are epimers.



Scheme IV

Scheme IV. Proposed fragmentation pattern of benzyl 2,4-diacetamido-3-*O*-acetyl-2,4,6-trideoxy-*β*-L-altropyranoside (**8**).

Compound **16**, obtained by *O*-acetylation of **15**, was examined by n.m.r. spectroscopy (Tables I and II) and the spectrum was interpreted in a way similar to that described for the other two benzyl acetylated diamino sugars (**8** and **9**). The n.m.r. data of compound **16** were in good agreement with a structure of benzyl 2,4-diacetamido-3-*O*-acetyl-2,4,6-trideoxy-β-L-talopyranoside.

In accord with the structural assignments for compounds **8**, **9**, and **16**, are their mass spectra (Fig. 4). The three spectra are very similar, indicating that the compounds have a similar substitution pattern[19]. The fragmentation reactions suggested for compound **8** (Scheme IV) account for the major peaks of the mass spectra of compounds **8**, **9**, and **16**. The mass spectra of compounds **10**, **11**, and **15** were also found to be very similar.

Removal of the benzyl group from **10**, **11**, and **15** by catalytic hydrogenolysis gave the reducing sugars **12**, **13**, and **17**, respectively. The latter compounds consumed no periodate and only small molar proportions of the oxidant were taken up very slowly by their sodium borohydride reduction products, in agreement with the assigned structures. The failure of compounds **12**, **13**, and **17** to yield color in the Morgan–Elson reaction[20] is quite expected, as this reaction requires[21,22] the presence of a free hydroxyl group at C-4. The three diacetamido sugars were shown to differ in their chromatographic mobilities from the 2,4-diacetamido-2,4,6-trideoxy-D-glucose from *Bacillus licheniformis*[4,5].

EXPERIMENTAL

General. — Melting points were measured in capillary tubes on a Büchi apparatus and are corrected. Evaporations were conducted *in vacuo*, Optical rotations were determined with a Bendix polarimeter. N.m.r. spectra were recorded with Varian A-60, T-60, or HA-100 spectrometers, or with a Bruker 90-MHz spectrometer, with tetramethylsilane as internal standard and $CDCl_3$ as solvent, except where otherwise specified. Mass spectra were recorded with a MAT Atlas CH 4 mass spectrometer. The i.r. spectra were recorded with a Perkin–Elmer Infracord spectrometer, either in chloroform solution or as discs pressed in KBr. Columns were prepared from silica gel (Davison, grade 950, 60–200 mesh), unless otherwise specified. The proportion of weight of substance to weight of adsorbent added to the column was 1 to 50–100. Thin-layer chromatograms were conducted on silica gel G (E. Merck, Darmstadt), unless otherwise stated, and the spots were detected by sulfuric acid. Paper chromatograms were performed with Whatman No. 1 paper, and the following systems: (1-butanol–acetic acid–water, 25:6:25, *v/v*, upper phase, SolventA) and (1-butanol–ethanol–water 4:1:1, *v/v*, Solvent *B*), and the spots were detected by silver nitrate[4]. Periodate-oxidation studies were carried out by the spectrophotometric method[23] and the u.v. absorption was measured with a Beckman DU spectrophotometer. Analyses were performed in the Weizmann Institute Microanalytical Laboratory, under the direction of Mr. R. Heller.

Benzyl 2-O-acetyl-6-deoxy-3,4-O-isopropylidene-β-L-galactopyranoside (**1**). — To a solution of benzyl 6-deoxy-3,4-*O*-isopropylidene-β-L-galactopyranoside[7] (5.2 g)

in pyridine (20 ml), acetic anhydride (3.5 ml) was added and the solution was kept overnight at room temperature. It was then evaporated to a syrup that was crystallized from petroleum ether; yield 4.5 g (76%); m.p. 93–95°. Recrystallization from the same solvent afforded fine needles, m.p. 96–96.5°, $[\alpha]_D^{25}$ +1.3° (*c* 1.08, chloroform). The product migrated as a single spot on t.l.c (R_F 0.85 in benzene–ether 1:1). The n.m.r. data are given in Tables I and II.

Anal. Calc. for $C_{18}H_{24}O_6$: C, 64.28; H, 7.14. Found: C, 64.50; H, 6.84.

Benzyl 2-O-acetyl-6-deoxy-β-L-galactopyranoside (**2**). — A mixture of **1** (11.6 g) and Amberlite IRC-50 (H^+) resin (7.5 g) in water (1250 ml) was boiled for 4 h under reflux. The resin was filtered off and the filtrate was evaporated. Extraction of the residue with acetone and evaporation of the extract afforded a syrup that crystallized after being dried over phosphorus pentaoxide in a desiccator; yield 8 g (78%). Compound **2** migrated as a single spot on t.l.c. (R_F 0.37 in benzene–methanol 9:2). Recrystallization from benzene gave an analytical sample, m.p. 112–113°, $[\alpha]_D^{25}$ +45° (*c* 0.98, chloroform); n.m.r. data (60 MHz): τ 2.70 (5 H singlet, phenyl group), 7.92 (3 H singlet, equatorial acetoxyl group), 8.64 (3 H doublet, 5-CH_3).

Anal. Calc. for $C_{15}H_{20}O_6$: C, 60.81; H, 6.75. Found: C, 60.69; H, 6.72.

Benzyl 2-O-acetyl-6-deoxy-3,4-di-O-(methylsulfonyl)-β-L-galactopyranoside (**3**). — To an ice-cooled solution of **2** (3.6 g) in pyridine (35 ml), methanesulfonyl chloride (6 ml) was added and the mixture was stirred overnight at 3°. Water (20 ml) was added stepwise and the mixture was extracted twice with chloroform. The chloroform extract was washed with water, dried over calcium chloride and concentrated to a syrup that crystallized from benzene; yield 4.3 g (79%), m.p. 127–132°, and after recrystallization from benzene 132–133°, $[\alpha]_D^{25}$ +19.1° (*c* 0.92, chloroform). The product migrated as a single spot on t.l.c. (R_F 0.54 in benzene–ether 1:1); n.m.r. data are given in Tables I and II.

Anal. Calc. for $C_{12}H_{24}O_{10}S_2$: C, 45.13; H, 5.31; S, 14.15. Found: C, 45.39; H, 5.23; S, 14.16.

Benzyl 2,3-anhydro-6-deoxy-4-O-(methylsulfonyl)-β-L-gulopyranoside (**4**). — A solution of **3** (5.5 g) in sodium methoxide (0.5M, 40 ml) was boiled for one h under reflux. Cooling and addition of water (40 ml), caused the title compound to precipitate. Recrystallization from aqueous ethanol gave the pure compound, which migrated as a single spot on t.l.c. (R_F 0.82 in benzene–ether 1:1); yield 1.88 g (49%), m.p. 112–113°, $[\alpha]_D^{25}$ +82° (*c* 1.06, chloroform); n.m.r. data are given in Tables I and II.

Anal. Calc. for $C_{14}H_{18}O_6S$: C, 53.50; H, 5.73; S, 10.10. Found: C, 53.97; H, 5.67; S, 10.33.

Benzyl 2,4-diazido-2,4,6-trideoxy-β-L-altropyranoside (**5**) *and benzyl 2,4-diazido-2,4,6-trideoxy-β-L-idopyranoside* (**6**). — To a solution of **4** (0.9 g) in *N,N*-dimethylformamide (50 ml), was added sodium azide (1.5 g) and the resulting mixture was heated with stirring for 3 h at 130–140°. It was then cooled, the salts were filtered off, and the filtrate was evaporated yielding a syrup. The syrup, which displayed several spots on t.l.c. (benzene–methanol 9:1), was extracted with a minimal volume of benzene and applied to a column of silica gel (7734, E. Merck, 60 g). Elution with

benzene–ether (19:1, 560 ml) removed the fast-moving impurities. Continued elution with benzene–ether (19:1) afforded the syrupy **5** (140 mg, 16%) which migrated as a single spot on t.l.c. (R_F 0.86 in benzene–ether 1:1). However, examination of the n.m.r. spectrum of **5** showed it to be contaminated by a minor product (<15%). Repeated chromatography of **5** failed to yield a pure compound.

The fraction that emerged from the column after **5**, by elution with the same solvent mixture (80 ml), was found to be a mixture of **5** and the slowermoving component **6** (total 125 mg, 14%). Continued elution with benzene–ether 19:1 (160 ml) gave the crystalline **6** (200 mg, 23%), which was recrystallized from benzene–petroleum ether to give 100 mg of fine needles, m.p. 97–98°, $[\alpha]_D^{22}$ +196° (*c* 1.0, chloroform).

Anal. Calc. for $C_{13}H_{15}N_6O_3$: C, 51.31; H, 5.3; N, 27.62. Found: C, 51.10; H, 5.18; N, 27.83.

Benzyl 3-O-acetyl-2,4-diazido-2,4,6-trideoxy-β-L-idopyranoside (**7**). — A solution of **6** (55 mg) in pyridine (1 ml) and acetic anhydride (0.1 ml) was kept overnight at room temperature. It was then evaporated to a syrup that migrated as a single spot on t.l.c. (R_F 0.64 in benzene–methanol 9:1 and 0.75 in benzene–ether 1:1), $[\alpha]_D^{24}$ +192° (*c* 1.1, chloroform). It was shown to be pure by n.m.r. spectroscopy (Tables I, II and Fig. 3).

Anal. Calc. for $C_{15}H_{18}N_6O_4$: C, 52.02; H, 5.20; N, 24.56. Found: C, 51.77; H, 5.18; N, 24.24.

Benzyl 2,4-diacetamido-3-O-acetyl-2,4,6-trideoxy-β-L-altropyranoside (**8**). — A solution of the syrupy diazide **5** (140 mg) in ether (5 ml) was added dropwise to a suspension of lithium aluminium hydride (0.2 g) in ether (5 ml), and the resulting mixture was boiled for 3 h under reflux and then cooled. Excess of the hydride was destroyed by successive addition of ethanol and water, and the mixture was filtered. The filtrate was evaporated to dryness, and the residue extracted with pyridine (10 ml). To the extract was added acetic anhydride (0.2 ml); the resulting mixture was kept overnight at room temperature and then evaporated. The residue was extracted with acetone; removal of the solvent gave a glass that crystallized from ethyl acetate–petroleum ether (yield 65 mg, 38%). Recrystallization from chloroform–ether gave an analytical sample, $[\alpha]_D^{25}$ +57° (*c* 0.96, chloroform). This crystalline compound turned into a glass upon heating in a capillary tube, and no sharp m.p. could be observed; the n.m.r. spectrum recorded in several solvents (Tables I, II and Fig. 2) showed it to be homogeneous.

Anal. Calc. for $C_{19}H_{26}N_2O_6$: C, 60.31; H, 6.93; N, 7.40. Found: C, 59.85; H, 7.18; N, 6.96.

Benzyl 2,4-diacetamido-3-O-acetyl-2,4,6-trideoxy-β-L-idopyranoside (**9**). — Benzyl 2,4-diazido-2,4,6-trideoxy-β-L-idopyranoside (**6**, 77 mg) was reduced by lithium aluminium hydride and the resulting benzyl diamino derivative acetylated with pyridine and acetic anhydride as described for the preparation of compound **8**. The product obtained was recrystallized from acetone–petroleum ether; yield 35 mg

(37%), m.p. 193–194°, $[\alpha]_D^{25}$ +70° (*c* 1.1, chloroform). N.m.r. data are given in Tables I and II.

Anal. Calc. for $C_{19}H_{26}N_2O_6$: C, 60.31; H, 6.93; N, 7.40. Found: C, 59.88; H, 6.94; N, 7.05.

*Benzyl 2,4-diacetamido-2,4,6-trideoxy-β-*L*-altropyranoside* (**10**) *and benzyl 2,4-diacetamido-2,4,6-trideoxy-β-*L*-idopyranoside* (**11**). — Compound **4** (1.66 g) was treated with sodium azide in *N,N*-dimethylformamide as already described for the first stage in the preparation of the two benzyl diazido derivatives **5** and **6**. The residue obtained upon removal of the solvent was extracted with ether and the ether extract was added dropwise to a suspension of lithium aluminium hydride (1.5 g) in ether (50 ml). The suspension was boiled for 3 h under reflux and cooled. Excess of the hydride was destroyed by successive addition of ethanol and water, and the mixture was filtered. The filtrate was evaporated and the residue extracted with methanol (50 ml). *N*-Acetylation was carried out by adding silver acetate (1 g) and acetic anhydride (1.5 ml) to the foregoing methanolic extract. The resulting mixture was kept overnight at room temperature, and then boiled for a few min. and filtered through active charcoal and Celite. The filtrate was evaporated, the residue extracted with acetone and the extract was passed through a column of silica gel. Elution with acetone followed by 7:1 acetone–methanol gave fractions that were homogenous by t.l.c. on silica gel (R_F 0.63 in acetone–methanol 2:1). However, examination of these fractions on t.l.c. on alumina (aluminium oxide G, E. Merck) revealed the presence of two major components: R_F 0.60 and R_F 0.68 (in acetone–methanol 2:1). All of the fractions were therefore combined and the resulting solution was evaporated. The residue was extracted with acetone and the extract was passed through a column of alumina. Elution with 6:1 acetone–methanol gave syrup (**10**, 250 mg, 13%) that migrated as a single spot on t.l.c. (alumina) (R_F 0.60 in acetone–methanol, 2:1) but which could not be crystallized. Examination of the ratio between the integration areas of the different groups in the n.m.r. spectrum of **10** did not give satisfactory results. Compound **10** was purified by *O*-acetylation and subsequent *O*-deacetylation, and was found to be benzyl 2,4-diacetamido-2,4,6-trideoxy-β-L-altropyranoside, as described later in this article. Continued elution with the same solvent mixture gave benzyl 2,4-diacetamido-2,4,6-trideoxy-β-L-idopyranoside (**11**), which was recrystallized from acetone–petroleum ether; yield 180 mg (9%), m.p. 232–233°, $[\alpha]^{25}$ +105° (*c* 1.1, methanol), R_F 0.60 in acetone–methanol 2:1 (t.l.c., alumina).

Anal. Calc. for $C_{17}H_{24}N_2O_5$: C, 60.71; H, 7.14; N, 8.30. Found: C, 61.19; H, 7.09; N, 8.36.

*Benzyl 2,4-diacetamido-3-*O*-acetyl-2,4,6-trideoxy-β-*L*-altropyranoside* (**8**) (*from* **10**). — To a solution of syrupy **10** (36 mg) in pyridine (1 ml), acetic anhydride (0.1 ml) was added and the solution was kept overnight at room temperature. Evaporation of the solution afforded a syrup that migrated as a single spot on t.l.c. (R_F 0.72 in acetone). It was crystallized from ethyl acetate–petroleum ether; yield 20 mg (50%). Recrystallization from chloroform–petroleum ether gave a sample having physical properties the same as those described for compound **8**.

*Benzyl 2,4-diacetamido-2,4,6-trideoxy-β-*L*-altropyranoside* (**10**) (*from* **8**). — To a solution of **8** (82 mg) in methanol (1 ml), sodium methoxide solution (M, 0.1 ml) was added, and the mixture was kept overnight at room temperature. It was then neutralized with acetic acid (2M), evaporated, and the residue extracted with chloroform. Concentration of the extract afforded a pure, amorphous material, identical by t.l.c. with **10** obtained as described before: yield 58 mg (77%); R_F 0.63 in acetone–methanol 2:1, and R_F 0.25 in acetone; $[\alpha]_D^{25}$ +14° (*c* 0.84, chloroform); n.m.r. data (60 MHz): τ 2.67 (5 H singlet, phenyl group); 8.00 (6 H singlet, two acetamido groups); 8.74 (3 H doublet, *J*. 6.5 Hz, 5-Me).

Anal. Calc. for $C_{17}H_{24}N_2O_5$: N, 8.33. Found: N, 7.91.

*Benzyl 2,4-diacetamido-3-*O*-acetyl-2,4,6-trideoxy-β-*L*-idopyranoside* (**9**) *from* **11**). — To a solution of **11** (70 mg) in pyridine (2 ml), acetic anhydride (0.1 ml) was added and the mixture was kept overnight at room temperature. It was then evaporated to give a crystalline product that was recrystallized from acetone–petroleum ether as fine needles (45 mg, 57%). The physical properties (n.m.r., t.l.c., optical rotation and m.p.), as well as the elementary analysis, were identical to those of compound **9** prepared from **6**.

*Benzyl 2,4-diacetamido-2,4,6-trideoxy-3-*O*-(methylsulfonyl)-β-*L*-idopyranoside* (**14**). — To an ice-cooled solution of **11** (96 mg) in pyridine (3 ml), methanesulfonyl chloride (0.2 ml) was added. The mixture was stirred for one h in an ice-bath and then for a further 3 h at room temperature. Water (1 ml) was added and the mixture was evaporated. The residue was extracted with chloroform, washed with water, dried over calcium chloride and evaporated. The resulting syrup crystallized from ethyl acetate–petroleum ether to give the title compound (67 mg, 56%) which, when examined by t.l.c., migrated as a single spot (R_F 0.85 in acetone). An analytical sample, m.p. 248°, $[\alpha]_D^{25}$ +28° (*c* 0.7, chloroform), was obtained after recrystallization from ethanol–petroleum ether.

Anal. Calc. for $C_{18}H_{26}N_2O_6S$: C, 52.16; H, 6.32; N, 6.76. Found: C, 52.25; H, 6.15; N, 6.33.

*Benzyl 2,4-diacetamido-2,4,6-trideoxy-β-*L*-talopyranoside* (**15**). — A mixture of **14** (47 mg), sodium acetate (20 mg) and aqueous ethanol (10 ml, 80% *v/v*) was boiled for 6 h under reflux. Evaporation of the mixture followed by extraction with acetone and evaporation of the extract gave a syrup that migrated on t.l.c. as a single spot (R_F 0.23 in acetone). The syrup was crystallized from acetone–petroleum ether; yield 28 mg (73%), m.p. 226–228°. Recrystallization afforded an analytical sample, m.p. 228–229°, $[\alpha]_D^{25}$ +54° (*c* 0.95, methanol).

Anal. Calc. for $C_{17}H_{24}N_2O_5 \cdot 0.5H_2O$: C, 59.13; H, 7.24; N, 8.12. Found: C, 59.13; H, 7.39; N, 7.92.

*Benzyl 2,4-diacetamido-3-*O*-acetyl-2,4,6-trideoxy-β-*L*-talopyranoside* (**16**). — To a solution of **15** (25 mg) in pyridine (1 ml), acetic anhydride (0.1 ml) was added and the solution was kept overnight at room temperature. Evaporation of the solution afforded crystals that were recrystallized from acetone–petroleum ether; yield 15 mg

(53%), m.p. 216–217°, $[\alpha]_D^{25}$ +33° (*c* 1.07, chloroform); R_F 0.59 (t.l.c., acetone); n.m.r. data are given in Tables I and II.

Anal. Calc. for $C_{19}H_{26}N_2O_6$: C, 60.31; H, 6.93; N, 7.40. Found: C, 60.19; H, 6.76; N, 6.97.

*2,4-Diacetamido-2,4,6-trideoxy-*L*-altrose* (**12**). — Compound **10** (35 mg) was dissolved in ethanol (95%, 20 ml) and hydrogenolyzed in the presence of palladium on charcoal catalyst (10%) at 45 lb. in^{-2} for 20 h. The catalyst was removed by filtration and the solvent evaporated off. The resulting syrup (25 mg, 97%), which could not be crystallized, migrated as a single spot on t.l.c. (R_F 0.25 in acetone and R_F 0.62 in acetone–ethanol 9:1) and on paper chromatograms (R_{Glc} 2.10 in Solvent *A* and R_{Glc} 2.45 in Solvent *B*. Under the same conditions 2,4-diacetamido-2,4,6-trideoxy-D-glucose[4,5] was found to have R_{Glc} 2.26 in Solvent *A* and 2.48 in Solvent *B*). $[\alpha]_D^{25}$ −73° (*c* 1.19, water, equil.); n.m.r. data (60 MHz, D_2O, Me_4Si as external standard): τ 7.86, 7.96 (two 3 H singlets, two acetamido groups); 8.72 (3 H doublet, *J* 6.5 Hz, 5-Me).

*2,4-Diacetamido-2,4,6-trideoxy-*L*-idose* (**13**). — Compound **11** (75 mg) was dissolved in ethanol (95% *v/v*, 50 ml) and hydrogenolyzed in the presence of palladium-on-charcoal catalyst (10%) for 20 h at 45 lb.in^{-2}. The catalyst was filtered off and the solvent evaporated. The resulting syrup was crystallized from ethanol–petroleum ether; yield 26 mg (46%), m.p. 167–169°. Recrystallization from ethanol–petroleum ether afforded an analytical sample, m.p. 172–174°, $[\alpha]_D^{25}$ −45° (*c* 0.87, water, after 15 min). The product was homogeneous by t.l.c. (R_F 0.21 in acetone and R_F 0.61 in acetone–ethanol 9:1) and on paper chromatograms (R_{Glc} 2.45 in Solvent *A* and 2.70 in Solvent *B*); n.m.r. data (60 MHz, D_2O, Me_4Si as external standard): τ 7.98 (6 H singlet, two acetamido groups; 8.80 (3 H doublet, *J* 6.5 Hz, C(5)-Me).

Anal. Calc. for $C_{10}H_{18}N_2O_5$: C, 48.78; H, 7.31; N, 11.34. Found: C, 48.99; H, 7.77; N, 11.29.

*2,4-Diacetamido-2,4,6-trideoxy-*L*-talose* (**17**). — Compound **15** (50 mg) was dissolved in ethanol (95% *v/v*, 50 ml) and hydrogenolyzed in the presence of palladium-on-charcoal (10%) at 48 lb.in^{-2} for 20 h. The catalyst was filtered off and the solvent evaporated. The resulting syrup (25 mg, 86%) migrated as a single spot on t.l.c. (R_F 0.1 in acetone and R_F 0.31 in acetone–ethyl alcohol 9:1) and paper chromatograms (R_{Glc} 2.13 in Solvent *A* and R_{Glc} 2.48 in Solvent *B*); $[\alpha]_D^{25}$ −89° (*c* 1.0, water, equil.).

Periodate oxidation. — To a solution of **13** (14 mg) in water (1 ml) sodium borohydride (4 mg) was added. The solution was kept for 3 h at room temperature, and then neutralized with dilute acetic acid (2M) and passed through a column of Dowex-50 (H^+) resin (0.6 × 5 cm). The expected diacetamido hexitol was eluted with water (30 ml) and the eluent stirred for a few min. with Amberlite IR-45 (OH^-) resin. Evaporation of the solvent gave a syrup that migrated as a single spot on t.l.c. (R_F 0.10 in acetone–methanol 2:1). Periodate uptake was determined by using weighed amounts of the syrup, and assuming that it contained only diacetamido hexitol. There

was a consumption of 0.17 mole periodate/mole of diacetamido hexitol after 1 h, and 0.24 mole after 3 h.

The product obtained by reduction of **17** with sodium borohydride consumed 0.22 mole after 2 h and 0.30 after 4 h. The diacetamido hexitol derived from **12** did not consume any periodate after 24 h. No periodate consumption was observed after 24 h for compounds **12**, **13**, and **17**.

Morgan–Elson reaction. — Compounds **12**, **13**, and **17** failed to yield significant color in the Morgan–Elson reaction[20] (less than 5% of the molar color yield given by 2-acetamido-2-deoxy-D-glucose).

ACKNOWLEDGMENTS

This investigation was supported by a research grant (AI-03528) from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, U.S. Public Health Service. We thank Dr. U. Scheidegger and Dr. A. Dieffenbacher from Varian A.-G. Zürich, Switzerland, Miss Ronit Pinko of Tel Aviv University and Mr. M. Grünberg from the Weizmann Institute of Science, for the high-resolution n.m.r. measurements.

REFERENCES

1 J. D. DUTCHER, *Advan. Carbohyd. Chem.*, 18 (1963) 259.
2 K. L. RINEHART, JR., *The Neomycins and Related Antibiotics*, John Wiley and Sons, Inc., 1964.
3 N. SHARON in *The Amino Sugars*, ed. E. A. BALAZS AND R. W. JEANLOZ, Academic Press, New York, 1965, Vol. IIa, p. 1.
4 N. SHARON AND R. W. JEANLOZ, *J. Biol. Chem.*, 235 (1960) 1.
5 U. ZEHAVI AND N. SHARON, *J. Biol. Chem.*, 248 (1973) 433.
6 U. ZEHAVI AND N. SHARON, *J. Org. Chem.*, 37 (1972) 2141.
7 H. M. FLOWERS, A. LEVY, AND N. SHARON, *Carbohyd. Res.*, 4 (1967) 189.
8 D. H. BUSS, L. HOUGH, L. D. HALL, AND J. F. MANVILLE, *Tetrahedron*, 21 (1965) 69.
9 F. SWEET AND R. K. BROWN, *Can. J. Chem.*, 46 (1968) 2283.
10 J. G. BUCHANAN, R. FLETCHER, K. PARRY, AND W. A. THOMAS, *J. Chem. Soc.* (B), (1969) 377.
11 B. R. BAKER AND R. E. SCHAUB, *J. Org. Chem.*, 19 (1954) 646.
12 S. HANESSIAN AND T. H. HASKELL, *J. Org. Chem.*, 30 (1965) 1080.
13 F. H. NEWTH, *Quart. Rev.* (London), 13 (1959) 30.
14 P. L. DURETTE AND D. HORTON, *Carbohyd. Res.*, 18 (1971) 403.
15 A. C. RICHARDSON, *Carbohyd. Res.*, 4 (1967) 415.
16 T. D. INCH, J. R. PLIMMER, AND H. G. FLETCHER, JR., *J. Org. Chem.*, 31 (1966) 1825.
17 J. M. WILLIAMS AND A. C. RICHARDSON, *Tetrahedron*, 23 (1967) 1369.
18 N. R. WILLIAMS, *Advan. Carbohyd. Chem.*, 25 (1970) 109.
19 N. K. KOCHETKOV AND O. S. CHIZOV, *Advan. Carbohyd. Chem.*, 21 (1966) 39.
20 J. L. REISSIG, J. L. STROMINGER, AND L. F. LELOIR, *J. Biol. Chem.*, 217 (1955) 959.
21 R. KUHN, A. GAUHE, AND H. H. BAER, *Chem. Ber.*, 87 (1954) 1138.
22 R. W. JEANLOZ AND M. TRÉMÈGE, *Fed. Proc.*, 15 (1956) 282.
23 R. D. GUTHRIE, *Methods Carbohyd. Chem.*, 1 (1962) 435.