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DIASTEREOTOPIC SUBSTRATES OF β-D-GALACTOSIDASE FROM *Escherichia coli* AS PROBES FOR A CATALYTICALLY ACTIVE, PROTONATING GROUP

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ABSTRACT

With 3,4,5,7-tetra-*O*-acetyl-2,6-anhydro-D-*glycero*-L-*manno*-heptononitrile as the starting material, the diastereotopic, enolic sugar derivatives (*Z*)-3,7-anhydro-2-deoxy-D-*galacto*-oct-2-enononitrile (**2**) and (*Z*)- (**5**) and (*E*)-3,7-anhydro-1,2-dideoxy-D-*galacto*-oct-2-enitol (**8**) were prepared by multistep syntheses, and structurally investigated by ^{1}H- and ^{13}C-n.m.r. spectroscopy. Compounds **2** and **5** are susceptible to β-D-galactosidase-catalyzed hydration of the enolic double bond, whereas the isomer of **5**, compound **8**, cannot be enzymically hydrated. Because the α-protons in the hydration product of nitrile **2** do exchange in protic media, only **5** can be used as a diastereotopic probe for elucidating the stereochemistry of enzymic protonation.

INTRODUCTION

Indication for proton-donating groups at active sites of glycosidases, normally meant to protonate glycosidic oxygen atoms, was obtained by using substrate models as proton probes. Such compounds as[1] 2,6-anhydro-1-deoxy-D-*galacto*-hept-1-enitol** (**1**) or -D-*gluco*-hept-1-enitol[2] carry, instead of a glycosidic oxygen atom, an electron-rich carbon atom attached to the pyranoid ring. Protonation of these nucleophilic sites triggers the addition of water or an alcohol to the double bond. The generally accepted view[3] that an α-glycosidase protonates an α-glycosidic oxygen atom, or a corresponding carbon atom, from "below" the pyranoid plane, and a β-glycosidase, a β-glycosidic oxygen atom, or a corresponding carbon atom, from "above" the pyranoid plane (see Fig. 1) originates from the mechanism proposed for lysozyme action[4]. Although plausible mechanisms for all glycosylases can be formulated on the foregoing assumption, nevertheless stringent experimental evidence is lacking. We now describe syntheses of new, potential substrates for β-D-galactosidase which, as

*To whom reprint requests should be addressed.
**Referred to herein as heptenitol.

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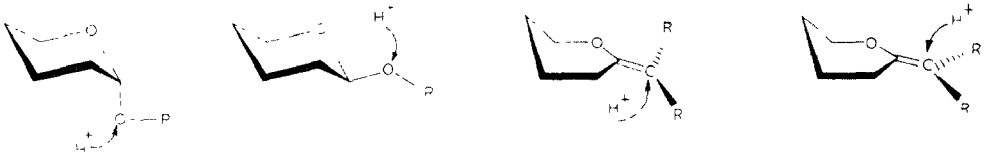

Fig. 1 Proposed protonation.

diastereotopic probes, should each be convertible by enzymic protonation into one, or two, diastereomeric product(s). Product analysis should then give evidence as to the direction of protonation.

RESULTS AND DISCUSSION

Incubation of heptenitol **1** with β-D-galactosidase from *E. coli*[5] in sodium phosphate buffer (pH 6.8) containing magnesium chloride leads to 1-deoxy-D-*galacto*-heptulose* (**10**). The reaction is initiated by enzymic protonation of the enolic sugar bond, whereby a stable 1-methyl group is formed. The stereochemistry of the protonation step may be investigated with analogs of **1** carrying two different substituents at the methylene carbon atom. Neither substituent ought to be such as to prevent enzymic protonation, either sterically or chemically. The newly formed, asymmetric carbon atom has to be optically stable, and the configurational assignment thereof, unequivocal and relatively easy.

The synthesis of (Z)-3,7-anhydro-2-deoxy-D-galacto-*oct-2-enononitrile* (**2**). — A compound that only partially meets the given requirements is **2**, which may be enzymically hydrated. It is to be expected that the cyano group would strongly deactivate the double bond, as well as facilitate proton or deuteron exchange in the reaction product, and thereby cause optical instability. The comparatively straightforward concept of synthesis, starting with a mixture of 4,5,6,8-tetra-*O*-acetyl-3,7-anhydro-D-*threo*-L-*talo*-octononitrile[6] (**12**) and 4,5,6,8-tetra-*O*-acetyl-3,7-anhydro-D-*threo*-L-*galacto*-octononitrile[6] (**13**), precursors already available in our laboratory, decided us try to prepare **2** for possible use as a diastereotopic probe for β-D-galactosidase.

A double bond could be introduced between C-2 and C-3 of compounds **12** and **13** *via* the 2-bromo derivatives **14** and **15**, which underwent smooth dehydrohalogenation when treated with silver fluoride in pyridine. Bromination could be achieved either with triphenylphosphine dibromide in benzene[7] or triphenylphosphine and carbon tetrabromide in *N,N*-dimethylformamide (DMF)[8]. The mixture of unsaturated nitriles was separated by chromatography on silica gel. One isomer (**3**) crystallized, and its structure was proved by n.m.r. spectroscopy to be (*E*)-4,5,6,8-tetra-*O*-acetyl-3,7-anhydro-2-deoxy-D-*galacto*-oct-2-enononitrile. The other (the ma-

*Referred to herein as heptulose

jor) component was (*Z*)-4,5,6,8-tetra-*O*-acetyl-3,7-anhydro-2-deoxy-D-*galacto*-oct-2-enononitrile (**4**), deacetylated with ammonia in methanol to give **2**. The (*E*) isomer **3** could not be deacetylated without major decomposition. The 2-deuterio compound of **2**, namely, **2**-*d**, was prepared by equilibrating the mixture of α-bromonitriles (**6** and **15**) for 24 h in pyridine–D_2O. After removal of the solvent, this procedure was repeated three times, and the deuterium content at C-2 was then 95%. Some of the deuterium, ~15%, was lost during formation of the unsaturated compound **2**-*d*. The remaining 80% of deuterium was, however, stable in protic solvents.

The nitrile **2** could not be hydrated in acidic solution. Acetic acid (80%) did not affect the compound, even after boiling for 2 h under reflux. Also, hydration with 0.1M hydrochloric acid could not be observed before general decomposition took place. The double bond could only be hydrated with high concentrations of β-D-galactosidase from *E. coli* in sodium phosphate buffer, pH 6.8, containing magnesium chloride, during a long incubation-time (14 d), to give **11** as the sole reaction-product, identified by t.l.c. and n.m.r. spectroscopy. The product still contained the cyano group intact, but no double bond. The newly formed, asymmetric C-2 adjacent to the cyano group in **10**-*d* is, however, not optically stable enough to survive either the long incubation-times or the further treatment with D_2O necessary for the n.m.r. investigation. This was proved by the following experiment: when **2** and **2**-*d* were incubated under the same conditions, the products isolated by dialysis, and then repeatedly lyophilized with D_2O, C-2 was completely deuterated.

Attempts to convert the cyano group into a less activating one, such as a carbonamido group or a hydroxymethyl group, *via* a carboxylic ester group, failed. The cyanide could not be saponified without occurrence of side reactions.

The synthesis of (Z)-*3,7-anhydro-1,2-dideoxy*-D-galacto-*oct-2-enitol*** (**5**). — Except for an analog of **1** wherein the substituents at C-1 were deuterium and tritium, the octenitol **11**, or its geometrical (*E*) isomer, would be most closely related structurally to **1**, and would promise similar properties when applied as a substrate for the β-D-galactosidase from *E. coli*. Two different pathways lead to the formation of **5**.

Pathway I. 1,3-Diphenyl-2-(3,4,5,7-tetra-*O*-acetyl-2,6-anhydro-D-*glycero*-L-*manno*-heptosyl)imidazolidine[9] (**28**) served as the starting material. It could be converted, by conventional *O*-deacetylation and subsequent *O*-benzylation, into the *O*-benzyl derivative **29**. Cleavage of the aldehyde protecting group with *p*-toluenesulfonic acid monohydrate, and Grignard reaction with methylmagnesium iodide, resulted in the formation of an almost equimolar mixture of the diastereomeric alcohols **16** and **17**, which could be separated by "flash chromatography" (ref. 10). Pure **16** was tosylated to give **18**, subsequently debenzylated by catalytic hydrogenolysis, and the product acetylated to yield **19**. Treatment of **19** with sodium benzoate in hot DMF gave two products in equal amounts: the desired (*Z*)-octenitol acetate **16** as the product of E_2-elimination, and **19**, which resulted from bimolecular, nucleophilic displacement.

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*All deuterium-labelled compounds discussed herein will be denoted by the suffix *d*.

**Referred to herein as (*Z*)-octenitol.

No trace of the 1-octenitol acetate, the constitutional isomer of **6**, could be found. Compounds **6** and **20** could not be separated on a large scale. After deacylation, however, compound **5** was separated from the by-product by column chromatography, and crystallized. The tosylate **21**, obtained from the secondary alcohol **17**, was treated as described for **18**, to give **22**. Its favored reaction with sodium benzoate in hot DMF is substitution. Only a small amount of the (*E*)-octenitol acetate **7** could be isolated. This selectivity can well be explained by conformational strain between the methyl group and the 4-*O*-acetyl group, 1,3-*syn*-disposed in the transition state of isomer **22** leading to **7**.

Pathway II. The difficulties encountered in the separation of the benzylated alcohols **16** and **17** were the especial reason for the following alternative synthesis. The acetate **31** of 2,6-anhydro-D-*glycero*-L-*manno*-heptonic acid[11] (**30**) was converted into its acid chloride **32** by treatment with phosphorus pentachloride in ether. Acylation of isopropylidenemalonate (Meldrum's acid)[12] in dichloromethane and pyridine at low temperature with **32** yielded the condensation product **33**, which, on hydrolysis with aqueous acetic acid, was decarboxylated. The resulting ketone (**34**) could be reduced, either with sodium cyanoborohydride, or with hydrogen over platinum. The former reduction yielded the alcohols **23** and **24** in the ratio of 4.89 : 1; the latter, in the ratio of 1 : 3. Separation of the acetylated alcohols was much easier than that of its benzylated counterparts. *O*-Tosylation gave compounds **19** and **22**, already described. A higher overall yield makes pathway II preferable to pathway I for the preparation of **5**. As expected, **5** is converted by the β-D-galactosidase from *E. coli*, in 0.05M sodium phosphate buffer containing 1mM magnesium chloride, with a velocity similar to that for **1***.

The synthesis of (Z)-*3,7-anhydro-2-deuterio-1,2-dideoxy*-D-galacto-*oct-2-enitol* (**5-*d***). — Although glycosidases equilibrated in D_2O catalyze hydration of enolic sugar derivatives[1], we preferred to conduct the enzymic hydration in H_2O with a 2-deuterated probe. 3,7-Anhydro-4,5,6,8-tetra-*O*-benzyl-1-deoxy-D-*threo*-L-*galacto*-octitol (**16**), or its 2-epimer (**17**), was oxidized with pyridinium dichromate in *N,N*-dimethylformamide[14]. The resulting ketone **35** could be reduced with lithium aluminum deuteride in ether, to yield the deuterated epimers **16-*d*** and **17-*d*** in the ratio of 1 : 2.4. Separation was conducted as described for their isotopomers **16** and **17**. Therefrom, the unsaturated end-product **5-*d*** was prepared in the same way as its nondeuterated counterpart.

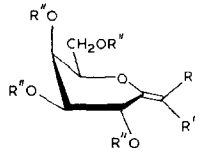
The synthesis of (E)-*3,7-anhydro-1,2-dideoxy*-D-galacto-*oct-2-enitol*** (**8**). — For the isomer of **5**, namely, (*E*)-octenitol (**8**), compound **23** was used as the starting material. Bromination with triphenylphosphine dibromide in benzene[8] yielded 4,5,6,8-tetra-*O*-acetyl-2-bromo-1,2-dideoxy-D-*threo*-L-*talo*-octitol (**25**), with inversion of configuration. Dehydrobromination with silver fluoride in pyridine, or 1,5-diaza-

*For details, see ref. 13.
**Referred to herein as (*E*)-octenitol.

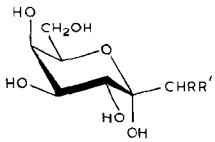
[5.4.0]bicycloundec-5-ene in *N,N*-dimethylformamide, yielded, after deacetylation, crystalline **8**. Under conditions the same as applied for **5**, the isomer **8** was not converted by β-D-galactosidase.

Structural investigations by n.m.r. spectroscopy

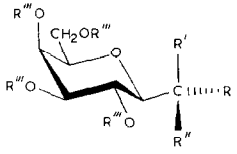
(*a*) *Saturated, eight-carbon derivatives.* — The 3,7-anhydro-octononitriles(**1–4** and **11**), as well as the 3,7-anhydro-1-deoxy-octitols (**12–27**) carry a newly formed, asymmetric C-2 atom. The following investigations are concerned with the assignment of either the D-*threo*-L-*galacto* or the D-*threo*-L-*talo* configuration to the aforementioned compounds.



1 R = R′ = R″ = H
2 R = CN, R′ = H, R″ = H
2d R = CN, R′ = D, R″ = H
3 R = H, R′ = CN, R″ = Ac
4 R = CN, R′ = H, R″ = Ac
5 R = CH_3, R′ = H, R″ = H
5d R = CH_3, R′ = D, R″ = H
6 R = CH_3, R′ = H, R″ = Ac
7 R = H, R′ = CH_3, R″ = Ac
8 R = H, R′ = CH_3, R″ = Ac
9 R = R′ = H, R″ = Ac



10 R = H, R′ = H
11 R = CN, R′ = H
11d R = CN, R′ = D



12 R = CN, R′ = OH, R″ = H, R‴ = Ac
13 R = OH, R′ = CN, R″ = H, R‴ = Ac
14 R = Br, R′ = CN, R″ = H, R‴ = Ac
15 R = CN, R′ = Br, R″ = H, R‴ = Ac
16 R = CH_3, R′ = OH, R″ = H, R‴ = Bzl
16d R = CH_3, R′ = OH, R″ = D, R‴ = Bzl
17 R = OH, R′ = CH_3, R″ = H, R‴ = Bzl
17d R = OH, R′ = CH_3, R″ = D, R‴ = Bzl
18 R = CH_3, R′ = OTs, R″ = H, R‴ = Bzl
18d R = CH_3, R′ = OTs, R″ = D, R‴ = Bzl
19 R = CH_3, R′ = OTs, R″ = H, R‴ = Ac
19d R = CH_3, R′ = OTs, R″ = D, R‴ = Ac
20 R = OBz, R′ = OTs, R″ = H, R‴ = Ac
21 R = OTs, R′ = CH_3, R″ = H, R‴ = Bzl
22 R = OTs, R′ = CH_3, R″ = H, R‴ = Ac
23 R = CH_3, R′ = OH, R″ = H, R‴ = Ac
24 R = OH, R′ = CH_3, R″ = H, R‴ = Ac
25 R = Br, R′ = CH_3, R″ = H, R‴ = Ac
26 R = CH_3, R′ = OAc, R″ = H, R‴ = Ac
27 R = OAc, R′ = CH_3, R″ = H, R‴ = Ac

Bz = PhCO
Bzl = $PhCH_2$
Ts = $SO_2C_6H_4Me$-*p*

The n.m.r. spectra all showed small coupling-constants for the vicinal 2,3-protons (see Table I). These values indicate the gauche orientation of H-2, H-3. Of the different H-2,H-3 gauche orientations possible, A and B (see Fig. 2), obtained by rotation around the C-2–C-3 bond, A has the least 1,3-interaction of R or R′, respectively, with the acetoxyl group on C-4. Such favored orientations as A have

TABLE I

VALUES OF $^3J_{2,3}$ FOR 3,7-ANHYDROOCTONONITRILES AND 3,7-ANHYDRO-1-DEOXY-D-*threo*-L-*talo*- AND -L-*galacto*-OCTITOLS (IN Hz)

Compound	$^3J_{2,3}$	*Compound*	$^3J_{2,3}$
14	3.5	**21**	1.5
15	1.9	**22**	2.0
16	4.8	**23**	1.5
17	1.5	**25**	2.0
18	1.5	**26**	2.4
19	3.0	**27**	1.9

Fig. 2. Orientations having H-2,H-3 gauche.

TABLE II

^{13}C–^1H COUPLING CONSTANTS FOR C-1 IN COMPOUNDS **14**, **15**, **26**, AND **27**

Compound	$^3J_{C\text{-}1,H\text{-}3}$ (*Hz*)	$^2J_{C\text{-}1,H\text{-}2}$ (*Hz*)	$J_{C\text{-}1,H\text{-}1}$ (*Hz*)
14	6	8	
15	2.5	8	
26	1	3	128
27	3	3	128

been described[15]. It is, therefore, justified to suggest structure A for compounds **12**–**27**.

^{13}C–^1H coupling-constants, like ^1H–^1H couplings, depend on the interplanary angle[16]. The signals of the nitrile carbon atom in the proton-undecoupled, ^{13}C-n.m.r. spectra of compounds **14** and **15** are doublets of doublets (through coupling with H-2 and H-3) (see Table II). By selective decoupling, $^3J_{C\text{-}1,H\text{-}3}$ can be determined to be 6 and 2.5 Hz, respectively. The larger value corresponds to the antiperiplanar, and the smaller to the synclinal orientation. Analogously, the configuration of C-2 in the isomers **26** and **27** can be determined. In this case, values of 1 and 3 Hz, respectively, are found. The larger coupling-constant again corresponds to the antiperiplanar, and the smaller to the synclinal orientation of C-1 and H-3. Therefore, **26** has the

D-*threo*-L-*galacto* and **27**, the D-*threo*-L-*talo*, configuration. All other compounds listed in Table I can be stereochemically correlated with **26** and **27**.

Independent of these investigations, the configuration of C-2 in compound **27** can be determined through the dibenzylidene derivative **36**. The vicinal coupling-constant $^3J_{2,3}$ of 3,7-anhydro-2,4:6,8-di-*O*-benzylidene-D-*threo*-L-*talo*-octitol (**36**), a rigid ring-system, unequivocally proves the configuration of C-2. Thus, compound **36** has the D-*threo*-L-*talo* configuration, proved separately by the aforementioned method.

(*b*) *Unsaturated, eight-carbon derivatives.* — The configuration of C-2 of the unsaturated nitriles **2**, **3**, and **4** can be deduced from increment calculations for the chemical shifts of H-2, as well as from data for comparable pyran derivatives[17] (see Table III). These indicate that H-2 in the (*Z*) isomer must absorb at a higher field than H-2 in the (*E*) isomer (see Table III). According to investigations of Reist *et al.*[18] and other authors[19], the elimination of *p*-toluenesulfonic acid from **19** under the conditions used must occur through an E_2 mechanism. From **19**, therefore, only the (*Z*) isomer **6** should be formed. By the same argument, formation of the (*E*) isomer from **25** by dehydrobromination can be predicted. The structural assignments for **5** and **8** were achieved by ^{1}H- and ^{13}C-n.m.r.-spectral investigations.

As in the nitriles **2**, **3**, and **4**, H-2 in the octenitols absorbs at a higher field when the configuration is (*Z*), and *vice versa*. This can again be deduced from increment calculations[20], as well as by comparison with data taken from the literature[21] (see Table IV). A further indication of the correctness of this argument can be de-

TABLE III

CHEMICAL SHIFTS FOR H-2 IN COMPOUNDS **3** AND **4**

	Increment for	
	3	**4**
Calc.	5.07	5.16
Found	4.85	5.20

TABLE IV

CHEMICAL SHIFTS OF H-2 (δ, RELATIVE TO Me_4Si)

	Increment for	
	(E)	*(Z)*
Calc.[17]	4.96	4.18
Found	**(7)** 5.44	**(6)** 4.95
	(8) 5.39	**(5)** 5.22

TABLE V

^{1}H-N.M.R. DATA (D_2O, 250 MHz) FOR COMPOUNDS **1**, **5**, **5-d**, **8** AND ($CDCl_3$, 360 MHz) FOR COMPOUNDS **6**, **7**, AND **9**

Proton	**1**	**9**	*Proton*	**5**	**5-d**	**6**	**7**	**8**
H-1	4.84	4.82	H-1	1.64	1.63	1.63	1.68	1.72
H-1′	4.75	4.51	H-2	5.22	—	4.95	5.44	5.39
H-3	4.24	5.68	H-4	4.12	4.19	5.61	5.72	4.50
H-4	3.64	5.06	H-5	3.57	3.56	5.02	5.22	3.85
H-5	4.05	5.52	H-6	4.04	4.04	5.50	5.54	4.16
H-6	~3.76[a]	4.03	H-7	3.69	3.68	3.99	4.24	3.92
H-7	~3.78[a]	4.16	H-8	3.78	3.77	4.17	4.17	3.71
H-7′	~3.85[a]	4.18	H-8′	3.90	3.89	4.27	4.24	3.85
J (H,H) (in Hz)								
H-1,H-1′	2	2	H-1,H-2	7	—	7	6.5	7.5
H-1,H-3	2	2	H-1,H-4	2	2	2	0	0
H-1′,H-3	2	2	H-2,H-4	2	—	1.8	0	~1
H-3,H-4	10	10.5	H-4,H-5	10	10	10	5.5	7
H-4,H-5	3.5	3	H-5,H-6	3	3	3.5	3.5	3
H-5,H-6	1	1.5	H-6,H-7	1	1	1.8	4.5	3
H-6,H-7	[a]	~6	H-7,H-8	4	4	6	~3.5	3
H-6,H-7′	[a]	~7	H-7,H-8′	8	8	7	~8	8.5
H-7,H-7′	[a]	11	H-8,H-8′	12	12	11.5	11.5	11

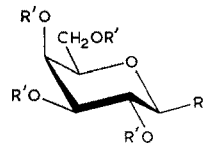
[a]Spectrum of higher order.

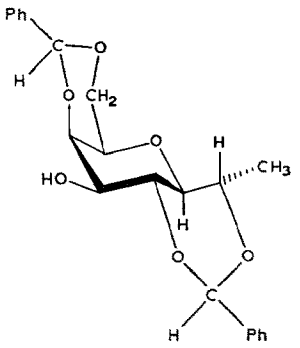
TABLE VI

^{1}H-N.M.R. DATA (CD_2Cl_2, 360 MHz) FOR COMPOUND **7** AT 223, 273, AND 310 K (δ RELATIVE TO Me_4Si), *J* (H,H) IN Hz

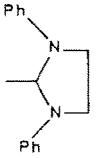
Proton	*Temperature (K)*			*J (H,H)*	*Temperature (K)*		
	223	*273*	*310*		*223*	*273*	*310*
H-1	1.70	1.675	1.66	H-1,H-2	7.5	7.5	7.5
H-2	5.47	5.43	5.40	H-4,H-5	4.7	5.7	6.0
H-4	5.65	5.68	5.69	H-5,H-6	3.5	3.5	3.5
H-5	5.23	5.20	5.19	H-6,H-7	[a]	4.5	4.5
H-6	5.49	5.49	5.48	H-7,H-8	[a]	2.5	3.1
H-7	4.37	4.28	4.25	H-7,H-8′	[a]	8.5	8.0
H-8	4.22	4.17	4.16	H-8,H-8′	[a]	11.5	11.5
CH_3CO	2.16	2.11	2.08				
	2.145	2.075	2.06				
	2.14	2.07	2.04				
	2.00	2.07	2.04				

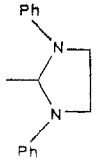
[a]Spectrum of higher order.





36

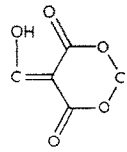
28 R =  , R′ = Ac

29 R =  , R′ = Bzl

30 R = CO_2H , R′ = H

31 R = CO_2H , R′ = Ac

32 R = COCl , R′ = Ac

33 R =  , R′ = Ac

34 R = $COCH_3$, R′ = Ac

35 R = $COCH_3$, R′ = Bzl

duced from the ^{1}H-n.m.r. spectrum of the heptenitol acetate **9**. Here, the signals for the enolic H-1 and H-1′ can be clearly assigned. Atom H-1 (*cis* to C-3) absorbs at δ 4.51, and H-1′ (*trans* to C-3), at a lower field (δ 4.81). According to Pascual *et al.*[22], an alkyl group attached to a double bond generally causes a shift of δ ~0.45 of the geminal-proton signal to lower field. The H-2 atom in the (*Z*) isomer **6**, having a shift of δ 4.95, therefore corresponds with H-1 (δ 4.51). The same proton in the (*E*) isomer **7** (δ 5.44) corresponds with H-1′ in the heptenitol acetate **9**.

Judging by the coupling constants of the pyranoid-ring protons, the octenitols **5** and **6** have the same conformation ($^6C_3$) as the heptenitols **1** and **9** ($^5C_2$) (see Tables IV and V). For the isomeric octenitols **7** and **8**, however, the unusually small $^3J_{4,5}$ values of 7 and 5.5 Hz are striking. Equally unusual are the relatively large H-6–H-7 couplings, compared with that for the heptenitols **1** and **9**. A plausible explanation for this phenomenon would be a change of conformation induced by a strong interaction between the 1-methyl group and the substituents at C-4. ^{1}H-N.m.r.

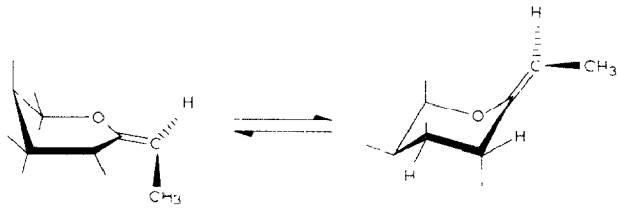


Fig. 3. Equilibration of conformers.

measurements on compound **7** at different temperatures (see Table VI), which, for the H-4,H-5 and H-7,H-8 couplings show a temperature-dependent change, indicate an equilibrium between the $^{0}C_{3}$ and the energetically favored $^{3}C_{6}$ conformation[23]. In the $^{3}C_{6}$ conformation, small coupling constants for the diequatorial H-4,H-5 are to be expected. The energy barrier for the conformational change is so low that no temperature-dependent broadening of the signals can be observed. The barrier is estimated to be $\sim$25.1 kJ/mol ($\sim$6 kcal/mol). The ^{13}C-n.m.r. chemical shifts of C-4 in **6** and C-3 in **9** show only a slight difference. The same applies to the corresponding carbon atom in the deacetylated products **5** and **1** (see Table VII). Obviously, the introduction of a *trans* methyl group can be of no great influence. For the same conformation, a *cis* methyl group, however, ought to cause a significant shift ($\sim$5 p.p.m.) of the C-4 signal to higher field ("syn-γ-shift")[24]. The observed value of only 1.2 p.p.m. (see Table VII) indicates a difference in conformation between the (*E*)-octenitol **7** and the heptenitol **9**.

In the heptenitol **9**, the long-range ^{13}C–^{1}H coupling constants for C-3 can be

TABLE VII

^{13}C-N.M.R. DATA ($CDCl_3$, 90.52 MHz) FOR COMPOUNDS **1**, **5**, **5-d**, **6**, **7**, AND **9** (δ RELATIVE TO Me_4Si)

Atom	**1**	**9**	*Atom*	**5**	**5-d**	**6**	**7**
			C-1	9.73	9.61	9.53	10.95
C-1	94.55	95.84	C-2	105.54	105.27	107.06	114.79
C-2	160.36	154.08	C-3	151.54	151.92	145.95	142.75
C-3	69.07	66.93	C-4	69.28	69.27	67.47	65.72
C-4	74.45	71.28	C-5	74.99	74.99	71.48	69.35
C-5	70.07	67.66	C-6	70.11	70.11	67.71	66.05
C-6	81.23	75.60	C-7	80.93	80.93	75.25	74.64
C-7	62.19	61.56	C-8	62.28	62.27	61.76	60.98
$COCH_3$	—	170.30	$COCH_3$	—		170.41	170.86
	—	170.03		—	—	170.10	169.76
	—	169.87		—	—	169.91	169.54
	—	169.39		—	—	169.47	169.39
$COCH_3$	—	20.70	$COCH_3$	—	—	20.76	20.92
	—	20.64		—	—	20.62	20.87
	—	20.58		—	—	20.60	20.80
							20.71

TABLE VIII

LONG-RANGE, ^{13}C–^{1}H, COUPLING CONSTANTS FOR C-3 IN COMPOUNDS **1** AND **9**, AND FOR C-4 IN COMPOUNDS **5**, **5-d**, **6**, AND **7** (IN Hz)

Atoms	**1**	**9**		
C-3,H-1 (*trans*)	5	5[a]		
C-3,H-1′ (*cis*)	2	2[a]		
C-3,H-5	5	5		
C-3,H-6	5	5		
	5	**5-d**	**6**	**7**
C-4,H-2 (*trans*)	—	—	—	~5[b]
C-4,H-2 (*cis*)	2	0	2	—
C-4,H-5	5	5	5	~5[b]
C-4,H-6	5	5	5	~2[b]

[a]Determined by selective decoupling of H-1 and H-1′. [b]Exact measurement was not possible (spectrum of higher order).

TABLE IX

^{1}H-N.M.R. DATA FOR COMPOUND **2** (D_2O, 250 MHz), COMPOUNDS **14** AND **15** ($CDCl_3$, 250 MHz), AND ($CDCl_3$, 90 MHz) COMPOUNDS **3** AND **4** (δ RELATIVE Me_4Si)

Proton	**2**	**14**	**15**	**3**	**4**	*J (H,H)*	**2**	**14**	**15**	**3**	**4**
H-2	5.28	4.43	4.51	5.20	4.85	H-2,H-3	—	4	2.5	—	
H-3	—	3.78	3.81	—	—	H-2,H-4	2	—	—	2	1.8
H-4	4.38	5.35	5.35	5.96	5.76	H-3,H-4	—	10	10	—	—
H-5	3.79	5.09	5.09	5.28	5.13	H-4,H-5	10	10	10	10	10
H-6	4.17	5.45	5.45	5.60	5.56	H-5,H-6	3	3.0	3.0	3	3.3
H-7	4.10	4.06	4.04	4.13	4.15	H-6,H-7	1	1	1	1.2	1
H-8	3.82	4.14	4.12	to	4.16	H-7,H-8	4	6	—	—	—
H-8′	3.96	4.23	4.23	4.46	to	H-7,H-8′	8	7	—	—	—
$COCH_3$	—	2.00	2.01	2.00	2.00	H-8,H-8′	12	11			
	—	2.06	2.06	2.06	2.08						
	—	2.10	2.12	2.16	2.15						
	—	2.18	2.19	2.20	2.18						

assigned by selective decoupling (see Table VIII). The assignment is confirmed by the rule[25] that vicinal, ^{13}C–^{1}H, *trans* coupling-constants are larger than the corresponding *cis* couplings. By comparison of the C-4,H-2 couplings in the octenitols **6**, **5**, and **5**-*d* with the corresponding C-3,H-1 and C-3,H-1′ coupling-constants of **9**, the structure of the (*Z*)-octenitols was further verified. The configuration around the double bond of the (*E*) isomer **7** cannot be determined by long-range, ^{13}C–^{1}H coupling; H-4 and H-2 do not differ sufficiently in their chemical shifts to allow exact determination of coupling constants (see Tables V and VIII).

TABLE X

^{1}H-N.M.R. DATA ($CDCl_3$, 90 MHz) FOR COMPOUND **34** AND ($CDCl_3$, 250 MHz) FOR COMPOUNDS **18**, **19**, **20**, **21**, **22**, **23**, **35**, **25**, **26**, AND **27** (δ RELATIVE TO Me_4Si)

Proton	*Compound*										
	18	**19**	**20**	**21**	**22**	**34**	**23**	**35**	**25**	**26**	**27**
H-1	1.36	1.31	1.46	1.10	1.30	~2	1.29	2.13	1.70	1.32	1.29
H-2	5.28	4.84	5.21	4.79	4.52		3.76	-	4.01	5.06	4.92
H-3	3.19	3.64	3.63	3.41	3.66	3.75	3.25	3.72	3.77	3.44	3.63
H-4	4.04	5.32	5.40	3.65	5	5.30	5.32	4.06	5.15	5.32	5.15
H-5	3.63	5.04	5.09	3.55	5	5.07	5.11	3.63	5.03	5.04	5.05
H-6	3.99	5.39	5.46	3.96	5.38	5.48	5.44	3.96	5.41	5.44	5.42
H-7	3.48	3.87	3.97	3.42	3.82	3.9	3.86	3.50	3.94	3.89	3.92
H-8	to	4.02	4.16	to	4.02	to	4.10	to	4.09	4.13	3.97
H-8′	3.60	4.07	4.24	3.50	4.08	4.3	4.21	3.62	4.24	4.22	4.19
$COCH_3$	-	1.99	1.96		1.97	2.00[a]	2.00		1.96	1.99	2.00
	—	2.04	1.99	---	2.04	2.06[a]	2.05	-	1.99	2.02	2.06
	—	2.05	2.06	--	2.05	2.10[a]	2.10	--	2.06	2.05	2.08
	—	2.11	2.19	--	2.12	2.20[a]	2.16	—	2.16	2.10	2.09
	—	--		—	--	2.30[a]	- -	-		2.18	2.17
$-CH_2-$	4.38–	-	-	4.40–	--		-	4.38	-	-	
	5.00	---	-	4.96	—	- -	--	4.99	-	-	--
C_6H_5	7.21–	--	7.44	7.18	-	- --	-	7.19	-	--	-
	7.46	-	7.56	7.45	-		-	7.41		-	
	—	- -	8.07	-	- -		-		--	- -	-
C_6H_4	7.16	—	--	7.16	—	- -	—	-	- -	—	-
	7.77	—	—	7.72	—	-	—	- -	- -	--	- -
CH_3	2.43	2.45	2.45	2.47							
J (H,H)[b]											
H-1,H-2	6.5	6.5	6.5	6.8	6.5	---	6	--	6.5	6.5	6.5
H-2,H-3	1.5	3	2	1.5	2	---	2	-	2	2.1	2.1
H-3,H-4	9.5	10	10	9.5	10	9.7	9.8	9.5	10	10	10
H-4,H-5	9.5	10	10	9.5	10	10.5	10	9.5	10	10	10
H-5,H-6	2.8	3.5	3.5	--	3.5	3	3.5	3.8	3.5	3.5	3.5
H-6,H-7	1	1	1	- -	1	2	2	1	1	1	1
H-7,H-8	—	6.5	4.5	—	7	—	7		6.5	6.5	6.5
H-7,H-8′	—	7	7	--	6.5	- -	6.5	- -	11	7	7
H-8,H-8′	—	11	11	-	11	-	11		-	11	11
OH-2–H-2	- -	-		-		-	7	-		- -	- -

[a]Acetyl protons and H-1 of **34**. [b]In Hz

EXPERIMENTAL

General methods. – Melting points are uncorrected. Optical rotations were measured with a Perkin–Elmer 141 polarimeter. ^{1}H-N.m.r. spectra* were recorded with Varian EM 390 (90 MHz), Bruker WM 250 (250 MHz), and Bruker HX (360

*With several exceptions, all n.m.r. data are listed in Tables I–XII.

TABLE XI

^{13}C-N.M.R. DATA ($CDCl_3$, 20.15 MHz) FOR COMPOUNDS **14**, **26**, AND **27** (δ RELATIVE TO Me_4Si)

Atom	*Compound*		
	14	**26**	**27**
C-1	114.96	15.59	13.39
C-2	67.34[a]	69.22[a]	66.47[a]
C-3	76.43[a]	75.53[a]	75.56[a]
C-4	67.54[a]	66.53[a]	65.57[a]
C-5	71.69[a]	72.36[a]	72.50[a]
C-6	67.34[a]	67.45[a]	67.70[a]
C-7	74.77[a]	73.93[a]	75.02[a]
C-8	61.17	61.31	61.59
$COCH_3$	170.33	170.52	170.33
	170.05	170.32	170.16
	169.93	170.24	170.02
	169.35	170.13	169.82
	—	170.13	—
	—	169.43	—
$COCH_3$	20.73	21.03	21.17
	20.56	20.61	20.73
	20.47	20.56	20.61

[a]Tentative assignment.

TABLE XII

^{1}H-N.M.R. DATA ($CDCl_3$, 250 MHz) FOR COMPOUNDS **31** AND **32** (δ RELATIVE TO Me_4Si)

Proton	*Compound*	
	31	**32**
H-2	4.07	3.9
H-3	5.41	5.42
H-4	5.14	5.15
H-5	5.46	5.40
H-6	4.01	3.9
H-7	4.20	to
H-7′		4.3
$COCH_3$	2.02	2.00
	2.07	2.06
	2.19	2.20
J (H,H) in Hz		
H-2,H-3	10	10.5
H-3,H-4	10.5	10.5
H-4,H-5	3	1
H-5,H-6	1	1
H-6,H-7	6.5	—

MHz) spectrometers for solutions in $CDCl_3$ (internal standard, Me_4Si) or D_2O (internal standard, sodium 2,2,3,3-tetradeuterio-4,4-dimethyl-4-silapentanoate). For ^{13}C-n.m.r. spectra, Bruker WP 80 (20.15 MHz) and Bruker HX 360 (90.53 MHz) spectrometers were used. All reactions were monitored by t.l.c. on silica gel 60 F_{254} (Merck), using 1:1 (v/v) ethyl acetate–petroleum ether (b.p. 60–70°) for acetylated compounds, 1:3 (v/v) ethyl acetate–petroleum ether (b.p. 60–70°) for benzylated compounds, and 1:3 (v/v) ethyl acetate–methanol for nonacetylated sugars. Column chromatography was performed in the "flash chromatography" mode[10], using silica gel (230–400 mesh, Merck).

4,5,6,8-Tetra-O-acetyl-3,7-anhydro-2-bromo-2-deoxy-D-threo-L-galacto- *and* -L-talo-*octononitrile* (**14** *and* **15**). – *Method a.* A mixture of 4,5,6,8-tetra-*O*-acetyl-3,7-anhydro-D-*threo*-L-*talo*-octononitrile[6] (**12**) and 4,5,6,8-tetra-*O*-acetyl-3,7-anhydro-D-*threo*-L-*talo*-octononitrile[6] (**13**) (11.162 g, 3 mmol) in *N,N*-dimethylformamide (DMF; 50 mL) was treated with carbon tetrabromide (2.01 g) and triphenylphosphine (1.885 g) for 1.5 h at 60°, cooled, and evaporated to dryness under diminished pressure. The dark-brown residue was taken up in a little ether, and submitted to chromatography in a column (30 × 3 cm) of silica gel with ether as the solvent. Fractions containing **14** and **15** were collected, and evaporated *in vacuo*. From ether, crystals were obtained at −20° that, according to ^1H-n.m.r. spectroscopy, consisted of both **14** and **15**. Recrystallization (7 ×) from ethyl acetate–petroleum ether (b.p. 60–70°) yielded pure **14** (120 mg, 9%). The mother liquor contained **14** and **15**.

Method b. To a solution of triphenylphosphine (9.967 g) in benzene (150 mL) was added benzene (30 mL) containing bromine (5.91 g), dropwise, under nitrogen during 15 min. A mixture of **12** and **13** (12.8 g, 33 mmol) dissolved in benzene (100 mL) was added, and the solution was boiled under reflux for 30 min. After cooling, the solid was filtered off, and the filtrate evaporated under diminished pressure. The residue was purified by chromatography in a column (10 × 5 cm) of silica gel with 1:2 (v/v) ethyl acetate–petroleum ether (b.p. 60–70°) as the solvent. Fractions containing **14** and **15** were collected, and evaporated *in vacuo*, to yield a colorless syrup (11.7 g, 78%) of **14** and **15** (1:1). Crystallization from ethyl acetate–petroleum (b.p. 60–70°) yielded pure **14** (1.33 g, 9%); m.p. 137°, $[\alpha]_{578}^{22}$ −4.91° (*c* 1.1, $CHCl_3$).

Anal. Calc. for $C_{16}H_{23}BrNO_9$: C, 42.68; H, 4.48; Br, 17.57; N, 3.26. Found: C, 42.89; H, 4.62; Br, 17.57; N, 3.26.

(E)-*4,5,6,8-Tetra*-O-*acetyl-3,7-anhydro-2-deoxy*-D-galacto-*oct-2-enononitrile* (**3**) *and* (Z)-*3,7-anhydro-2-deoxy*-D-galacto-*oct-2-enononitrile* (**2**). – A solution of compound **14** (1 g, 2.22 mmol) in pyridine (3 mL) was shaken vigorously with silver fluoride (technical grade, 1 g) for 1 min, poured into ether (300 mL), and the insoluble matter filtered off. The filtrate was successively washed with aqueous sodium thiosulfate (2%, 100 mL) and water (2 × 100 mL), dried (magnesium sulfate), and evaporated *in vacuo*, to yield a brown syrup (600 mg). The mixture of products was separated by chromatography in a column (25 × 2.5 cm) of silica gel with 4:1 (v/v) ether–petroleum ether (b.p. 60–70°). The first fraction contained **3**, and, after crystalli-

zation from ether, yielded colorless crystals (100 mg, 12.2%); m.p. 60°, $[\alpha]_{578}^{22}$ +47.2° (*c* 1.0, $CHCl_3$).

Anal. Calc. for $C_{16}H_{19}NO_9$: C, 52.03; H, 5.19; N, 3.79. Found: C, 52.05; H, 5.25; N, 3.71.

From the second fraction was obtained a syrup consisting of the (*Z*) isomer **4**. Attempts to crystallize **4** failed. To a solution of compound **4** in methanol (15 mL) was added a solution of ammonia in methanol (5 mL, 25%) at 0°. After 4.5 h, the solvent was evaporated under diminished pressure, and **2** was separated from acetamide by chromatography in a column (15 × 0.8 cm) of silica gel with 25:14:7 (v/v/v) ethyl acetate–isopropyl alcohol–water. Compound **2** crystallized from ethyl acetate at 0° after the addition of a little methanol; yield, 85 mg (40.3%); m.p. 144°, $[\alpha]_{578}^{22}$ +272.5° (*c* 1.0, H_2O).

Anal. Calc. for $C_8H_{11}NO_5$: C, 47.76; H, 5.51; N, 6.96. Found: C, 47.43; H, 5.51; N, 7.04.

(Z)-3,7-Anhydro-2-deoxy-2-deuterio-D-galacto-*oct-2-enononitrile* (**2-*d***). — A solution of compound **14** (1.0 g, 2.22 mmol) in 5:1 (v/v) pyridine–D_2O (6 mL) was kept for 24 h at room temperature, and then evaporated under diminished pressure. This procedure was repeated three times, whereafter the ^{1}H-n.m.r. spectrum showed a mixture of **14-*d*** and **15-*d*** having a deuterium content of 95% at C-2. A solution of this mixture in pyridine (3 mL) was treated with silver fluoride (1 g) as for **14** and **15**. The product was deacetylated with methanolic ammonia. After separation from acetamide, **2-*d*** was obtained by crystallization from ethyl acetate–methanol; yield, 78 mg (17.5%) having a deuterium content of ~80% at C-2.

2-(2,6-Anhydro-3,4,5,7-tetra-O-*benzyl*-D-glycero-L-manno-*heptosyl)-1,3-diphenylimidazolidine* (**29**). — 1,3-Diphenyl-2-(tetra-*O*-acetyl-2,6-anhydro-D-*glycero*-L-*manno*-heptosyl)imidazolidine[9] (**28**) (15 g, 27.05 mmol) was deacetylated with 0.02M sodium methoxide. The mixture was evaporated to dryness *in vacuo*, and the residue dissolved in absolute *N,N*-dimethylformamide (250 mL) and treated with sodium hydride (10 g) under stirring. After 1 h, benzyl bromide (50 mL) was added dropwise during 2 h, and the mixture was stirred overnight, treated cautiously with methanol (10 mL), and evaporated *in vacuo* (0.1 Torr). A solution of the residue in chloroform (300 mL) was washed with water (2 × 200 mL), dried (magnesium sulfate), evaporated to dryness *in vacuo*, and the benzyl alcohol removed by steam distillation. The light-brown oil obtained (16.97 g, 84%) was used in the next step without purification.

3,7-Anhydro-4,5,6,8-tetra-O-*benzyl-1-deoxy*-D-threo-L-galacto-*octitol* (**16**) *and 3,7-anhydro-4,5,6,8-tetra*-O-*benzyl-1-deoxy*-D-threo-L-talo-*octitol* (**17**). — A solution of compound **29** (18 g, 24 mmol) in dichloromethane was treated at 0° with a solution of *p*-toluenesulfonic acid monohydrate (18 g) in acetone (200 mL). The precipitate was filtered off after 1 h, and washed with dichloromethane (200 mL). The filtrate and washings were combined, washed successively with sodium hydrogencarbonate solution and water (200 mL each), dried (magnesium sulfate), and evaporated under diminished pressure to a colorless syrup (13.5 g). At 0°, a solution of the syrupy aldehyde in ether (150 mL) was added dropwise to a solution of methylmagnesium

iodide from magnesium (3 g) and methyl iodide (6 mL) in ether (150 mL), and the mixture was boiled for 1 h under reflux, and cooled. Ice-cold aqueous ammonium chloride solution (300 mL, 20%) was added cautiously, and the organic compound was extracted with ether (300 mL); the extract was washed with water (200 mL), dried (magnesium sulfate), and evaporated *in vacuo* to a faintly brown oil containing, as its main components, **16** and **17** [t.l.c. with 1:2 (v/v) ethyl acetate–petroleum ether (b.p. 60–70°), R_F 0.15 and 0.28]. Separation was achieved by flash chromatography[10] in a column (15 × 7 cm) of silica gel with 1:3 (v/v) ethyl acetate–petroleum ether (b.p. 60–70°) as the solvent, to give syrupy **16** (5.41 g, 39%); $[\alpha]_{578}^{22}$ +28.9° (*c* 1.23, $CHCl_3$); ^{1}H-n.m.r. data ($CDCl_3$, 90 MHz): δ 1.23 (d, 3 H, H-1), 2.2 (d, 1 H, OH-2), 3.06 (dd, 1 H, H-3), 3.4–3.73 (m, 4 H, H-2,7,8,8'), 3.74–4.26 (m, 3 H, H-5,6), 4.3–5.1 (m, 8 H, -CH_2-), and 7.1–7.4 (m, 20 H, 4 C_6H_5-), $J_{1,2}$ 6.7, $J_{2,3}$ 1.5, $J_{3,4}$ 9, $J_{4,5}$ 9, $J_{5,6}$ 1, and $J_{6,7}$ 1 Hz. After further elution with 1:1 (v/v) ethyl acetate–petroleum ether (b.p. 60–70°) and evaporation, **17** was obtained as a colorless syrup (4.29 g, 31%); $[\alpha]_{578}^{22}$ +8.5° (*c* 1.31, $CHCl_3$); ^{1}H-n.m.r. data ($CDCl_3$, 90 MHz): δ 1.14 (d, 3 H, H-1), 2.83 (bs, 1 H, OH-2), 3.27 (dd, 1 H, H-3), 3.5–3.66 (m, 3 H, H-7,8,8'), 3.67 (dd, 1 H, H-5), 3.97 (dd, 1 H, H-4), 3.87–4.16 (m, 2 H, H-2,6), 4.43–5.16 (m, 8 H, 4 -CH_2-), and 7.23–7.53 (m, 20 H, 4 C_6H_5), $J_{1,2}$ 6.1, $J_{2,3}$ 4.8, $J_{3,4}$ 9, $J_{5,6}$ 2.6, and $J_{6,7}$ 1 Hz.

3,7-Anhydro-4,5,6,8-tetra-O-*benzyl-1-deoxy-2*-O-p-*tolylsulfonyl*-D-threo-L-galacto-*octitol* (**18**). — A solution of compound **16** (4.8 g, 8.44 mmol) in pyridine (80 mL) was treated with *p*-toluenesulfonyl chloride (1.93 g), and kept for 15 h at room temperature. After the usual processing, **18** was isolated as a colorless oil (5.47 g, 93%); $[\alpha]_{578}^{22}$ −3.5° (*c* 1.71, $CHCl_3$).

3,7-Anhydro-4,5,6,8-tetra-O-*benzyl-1-deoxy-2*-O-p-*tolylsulfonyl*-D-threo-L-talo-*octitol* (**21**). — A solution of compound **17** (4.8 g, 8.44 mmol) in pyridine (80 mL) was treated with *p*-toluenesulfonyl chloride (1.93 g), kept for 15 h at room temperature, and processed as usual, yielding **21** as a colorless syrup (5.3 g, 87%); $[\alpha]_{578}^{22}$ +9.74° (*c* 1.15, $CHCl_3$).

4,5,6,8-Tetra-O-*acetyl-3,7-anhydro-1-deoxy-2*-O-p-*tolylsulfonyl*-D-threo-L-talo-*octitol* (**22**). — Compound **21** (3.57 g, 5 mmol) in 10:1 (v/v) methanol–ethyl acetate (150 mL) was hydrogenolyzed in the presence of palladium–charcoal as the catalyst. When no further hydrogen was taken up, the catalyst was filtered off, and the filtrate was evaporated under diminished pressure, to give crystalline 3,7-anhydro-1-deoxy-2-*O*-*p*-tolylsulfonyl-D-*threo*-L-*talo*-octitol (1.71 g, 94.6%), which was acetylated with 1:1 (v/v) acetic anhydride–pyridine (50 mL) for 15 h, and processed in the usual way. From ether were obtained colorless crystals (1.82 g, 71%); m.p. 128°, $[\alpha]_{578}^{22}$ +4.2° (*c* 1.0, $CHCl_3$).

Anal. Calc. for $C_{23}H_{30}O_{12}S$: C, 52.07; H, 5.70. Found: C, 52.07; H, 5.87.

4,5,6,8-Tetra-O-*acetyl-3,7-anhydro-1-deoxy-2*-O-p-*tolylsulfonyl*-D-threo-L-galacto-*octitol* (**19**). — *Method a.* 4,5,6,8-Tetra-*O*-acetyl-3,7-anhydro-1-deoxy-D-*threo*-L-*galacto*-octitol (**23**) (4.09 g, 10.74 mmol) in pyridine (100 mL) was treated with

p-toluenesulfonyl chloride (3.1 g) for 24 h at room temperature, and the mixture was processed in the usual way, to give **19** as a colorless syrup (5.1 g, 89%).

Method b. Compound **16** (4.98 g, 6.98 mmol) in 10:1 (v/v) methanol–ethyl acetate (200 mL) was hydrogenolyzed with palladium–charcoal as the catalyst. When the reaction was complete, the catalyst was filtered off, and the filtrate was evaporated under diminished pressure, to yield a colorless syrup (2.54 g) to which was added 1:1 (v/v) acetic anhydride–pyridine (100 mL). After 15 h, the acetylation was complete, and the mixture was processed in the usual way, to give **19** (3.34 g, 92.3%) as a colorless syrup, $[\alpha]_{578}^{22}$ +15.7° (*c* 0.97, $CHCl_3$).

(Z)-*3,7-Anhydro-1,2-dideoxy*-D-galacto-*oct-2-enitol* (**5**) *and 2,4,5,6,8-penta*-O-*acetyl-3,7-anhydro-1-deoxy*-D-threo-L-talo-*octitol* (**27**). — To a solution of compound **19** (4.01 g, 7.5 mmol) in absolute DMF (150 mL) was added sodium benzoate (5 g), and the mixture was boiled under reflux with stirring, cooled, and evaporated *in vacuo* (0.1 Torr); a solution of the residue in water (200 mL) was extracted with chloroform (3 × 200 mL), and the extracts were combined, successively washed with saturated, aqueous sodium hydrogencarbonate solution (200 mL) and water (2 × 200 mL), dried (magnesium sulfate), and evaporated under diminished pressure, to yield a dark-brown oil which was deacetylated with 0.02M sodium methoxide (120 mL). After 6 h, the solution was de-ionized by passage through a column (10 × 1 cm) of silica gel, and the eluate was evaporated to dryness under diminished pressure. The residue was chromatographed in a column (10 × 5 cm) of silica gel with 10:1 (v/v) ethyl acetate–methanol as the solvent, yielding **5**, crystallized from 20:1 (v/v) ethyl acetate–methanol (580 mg, 40.2%); m.p. 162°, $[\alpha]_{578}^{22}$ +184.6° (*c* 1.0, H_2O).

Anal. Calc. for $C_8H_{14}O_5$: C, 50.52; H, 7.42. Found: C, 50.35; H, 7.63.

Elution with 2:1 (v/v) ethyl acetate–methanol yielded syrupy 3,7-anhydro-1-deoxy-D-*threo*-L-*talo*-octitol, which was acetylated with 1:1 (v/v) acetic anhydride–pyridine (20 mL). After 18 h the reaction was processed as usual, and the product crystallized from ether, to give **27** (880 mg, 27.7%); m.p. 96°, $[\alpha]_{578}^{22}$ −4.4° (*c* 1.0, $CHCl_3$).

Anal. Calc. for $C_{18}H_{26}O_{11}$: C, 51.67; H, 6.26. Found: C, 51.38; H, 6.24.

(Z)-*4,5,6,8-Tetra*-O-*acetyl-3,7-anhydro-1,2-dideoxy*-D-galacto-*oct-2-enitol* (**6**). — A solution of compound **5** (190.2 mg, 1 mmol) in 1:1 (v/v) acetic anhydride–pyridine (8 mL) was kept for 18 h at room temperature, when the reaction was complete, and the mixture was processed as usual, to give a colorless syrup (328 mg, 92%).

3,4,5,7-Tetra-O-*acetyl-2,6-anhydro*-D-glycero-L-manno-*heptonic acid* (**31**). — 2,6-Anhydro-D-*glycero*-L-*manno*-heptonic acid[11] (**30**); 44 g, 211 mmol) was suspended in acetic anhydride (200 mL), and the mixture was stirred with zinc chloride (16 g) for 24 h at room temperature, poured into ice–water (1 L), stirred for 3 h, and extracted with chloroform (10 × 100 mL). The extracts were combined, washed with water, dried (magnesium sulfate), and evaporated to dryness *in vacuo*. The resulting,

colorless syrup crystallized slowly on addition of toluene, to yield **31** (50.7 g, 65.5%); m.p. 136°, $[\alpha]_{578}^{22}$ +17.9° (*c* 1.0, $CHCl_3$).

Anal. Calc. for $C_{15}H_{20}O_{11}$: C, 47.87; H, 5.36. Found: C, 47.70, H, 5.32.

*3,4,5,7-Tetra-O-acetyl-2,6-anhydro-*D*-glycero-*L*-manno-heptonic acid chloride* (**32**). — Compound **31** (18.81 g, 50 mmol) was suspended in absolute ether (250 mL), treated with phosphorus pentachloride (11 g), and boiled under reflux until a clear solution was obtained (~3 h). After cooling, petroleum ether (b.p. 30–60°; 400 mL) was added. At 0°, colorless crystals of **32** (16 g, 80%) were obtained: m.p. 100°, $[\alpha]_{578}^{22}$ +24.8° (*c* 1.0, $CHCl_3$).

Anal. Calc. for $C_{15}H_{19}ClO_{10}$: C, 45.64; H, 4.85. Found: C, 45.76; H, 5.01.

*4,5,6,8-Tetra-O-acetyl-3,7-anhydro-1-deoxy-*D*-glycero-*L*-manno-2-octulose* (**34**). — A solution of compound **32** (16.5 g, 41.8 mmol) in dichloromethane (200 mL) was added dropwise during 0.5 h at 0° to a mixture of isopropylidenemalonate[12] (6.03 g, 41.8 mmol) and pyridine (6.76 mL, 83.6 mmol) in dichloromethane (100 mL). After 2 h at 0°, the mixture was washed with water (100 mL), dried (magnesium sulfate), and evaporated to dryness *in vacuo*. Codistillation with toluene removed last traces of pyridine. A solution of the residue in 1:2 (v/v) glacial acetic acid–water (150 mL) was boiled under reflux for 2.5 h, cooled, evaporated under diminished pressure, and the resulting light-brown oil dissolved in chloroform (300 mL). The solution was successively washed with an aqueous solution of sodium hydrogencarbonate (150 mL) and water (150 mL), dried (magnesium sulfate), and evaporated *in vacuo* under diminished pressure. The syrup obtained was purified by flash chromatography in a column (10 × 5 cm) of silica gel with 1:2 (v/v) ethyl acetate–petroleum ether (60–70°) as solvent. Fractions containing **34** were collected, and evaporated *in vacuo*. The solid residue was recrystallized from 1:3 (v/v) ethyl acetate–petroleum ether (b.p. 60–70°) to give **34** (9.84 g, 62%); m.p. 123°, $[\alpha]_{578}^{22}$ +61.7° (*c* 1.0, $CHCl_3$).

Anal. Calc. for $C_{16}H_{22}O_{10}$: C, 51.34; H, 5.92. Found: C, 51.31; H, 5.90.

*4,5,6,8-Tetra-O-acetyl-3,7-anhydro-1-deoxy-*D*-threo-*L*-galacto-octitol* (**23**). — A solution of compound **34** (4.8 g, 12.8 mmol) in absolute methanol (200 mL) was treated with glacial acetic acid (15 mL) and sodium cyanoborohydride (1.6 g). After 18 h at room temperature, chloroform (200 mL) and water (100 mL) were added, and the mixture was shaken vigorously. The organic layer was successively washed with saturated, aqueous sodium hydrogencarbonate solution (100 mL) and water (100 mL), dried (magnesium sulfate), and evaporated *in vacuo*, to yield a colorless oil. According to n.m.r.-spectral analysis, its components were, 83% of **23** and 17% of **24**. On addition of ether, **23** crystallized slowly at −20° (3.46 g, 71%); m.p. 87°, $[\alpha]_{578}^{22}$ +11.1° (*c* 1.0, $CHCl_3$).

Anal. Calc. for $C_{16}H_{24}O_{10}$: C, 51.06, H, 6.43. Found: C, 50.90; H, 6.63.

*4,5,6,8-Tetra-O-acetyl-3,7-anhydro-2-bromo-1,2-dideoxy-*D*-threo-*L*-talo-octitol* (**25**). — Triphenylphosphine (1.311 g, 5 mmol) was dissolved under stirring in absolute benzene (15 mL), and a solution of bromine (800 mg, 5 mmol) in benzene (10 mL) was added dropwise under a stream of nitrogen during 15 min at 15°, then, com-

pound **23** (1.2 g, 3.19 mmol) was added, and the mixture was boiled under reflux for 1.5 h, cooled, and evaporated under diminished pressure. The dark syrup resulting was dissolved in ethyl acetate (5 mL), and purified by flash chromatography on a column (10 × 3 cm) of silica gel, with ethyl acetate–petroleum ether (b.p. 60–70°) as the solvent. Fractions containing **25** were evaporated *in vacuo*, to give a colorless syrup that crystallized on adding a little 1:4 (v/v) ethyl acetate–petroleum ether, and storing at 0° (870 mg, 62.3%); m.p. 127°, $[\alpha]_{578}^{22}$ +8.7° (*c* 1.0, $CHCl_3$).

Anal. Calc. for $C_{16}H_{23}BrO_9$: C, 43.75; H, 5.28. Found: C, 43.89; H, 5.38.

(E)-*4,5,6,8-Tetra*-O-*acetyl-3,7-anhydro-1,2-dideoxy*-D-galacto-*oct-2-enitol* (**7**). — A solution of compound **25** (200 mg, 0.46 mmol) in pyridine (10 mL) was stirred vigorously with silver fluoride (400 mg, technical grade) for 4 h. The suspension was poured into ether (150 mL), stirred for 0.5 h, and filtered. The filtrate was successively washed with aqueous sodium thiosulfate solution (100 mL, 2%) and water (100 mL), dried (magnesium sulfate), and evaporated under diminished pressure to a light-brown syrup that was purified by flash chromatography in a column (10 × 0.8 cm) of silica gel with 1:2 (v/v) ethyl acetate–petroleum ether (b.p. 60–70°) as solvent. A colorless syrup of **7** was obtained (104 mg, 63%). For characterization, **7** was deacetylated.

(E)-*3,7-Anhydro-1,2-dideoxy*-D-galacto-*oct-2-enitol* (**8**). — A solution of compound **25** (700 mg, 1.59 mmol) in absolute *N,N*-dimethylformamide (10 mL) was treated with 1,8-diazobicyclo[5.4.0]undec-7-ene (DBU) (1 mL), and kept for 3 d at 65°. After cooling, ether (300 mL) was added, and the mixture was washed with water (100 mL), dried (magnesium sulfate), and evaporated to dryness *in vacuo*. The syrup obtained was dissolved in 0.02M sodium methoxide, and after deacetylation was complete, the solution was passed through a column (7 × 0.7 cm) of silica gel for neutralization of the base. The eluate was evaporated under diminished pressure, and the resulting dark-brown syrup was submitted to flash chromatography in a column (7 × 3 cm) of silica gel with 10:1 (v/v) ethyl acetate–methanol as the solvent. Fractions containing **8** were collected, and evaporated to dryness *in vacuo*. The resulting residue was dissolved in ethyl acetate; and on adding petroleum ether (b.p. 60–70°) to incipient turbidity, **8** crystallized slowly (130 mg, 43%); m.p. 107°, $[\alpha]_{578}^{22}$ +44.3° (*c* 1.0, H_2O).

Anal. Calc. for $C_8H_{14}O_5$: C, 50.52; H, 7.42. Found: C, 50.27; H, 7.55.

3,7-Anhydro-4,5,6,8-tetra-O-*benzyl-1-deoxy*-D-glycero-L-manno-*octulose* (**35**). — A solution of compound **16** (7.2 g, 12.66 mmol) or compound **17** (7.2 g, 12.66 mmol) in absolute *N,N*-dimethylformamide (80 mL) was treated at room temperature with pyridinium dichromate[14] (20 g). After 18 h, the suspension was poured into ether (300 mL), washed with water (150 mL), dried (magnesium sulfate), and evaporated to dryness *in vacuo*. The light-yellow syrup obtained was purified by flash chromatography in a column (5 × 3 cm) of silica gel, with 1:3 (v/v) ethyl acetate–petroleum ether (b.p. 60–70°) as the solvent, to give **35** as a colorless syrup (6.39 g, 89%); $[\alpha]_{578}^{22}$ +30.6° (*c* 1.4, $CHCl_3$).

3,7-Anhydro-4,5,6,8-tetra-O-*benzyl-1-deoxy-2-deuterio*-D-threo-L-galacto-*octitol*

(**16**-*d*) *and 3,7-anhydro-4,5,6,8-tetra*-O-*benzyl-1-deoxy-2-deuterio*-D-threo-L-talo-*octitol* (**17**-*d*). — Compound **35** (7.5 g, 13.23 mmol) in absolute ether (150 mL) was reduced with lithium aluminum deuteride (450 mg). After boiling overnight under reflux, the excess of reductant was decomposed by cautiously adding ethyl acetate (3 mL) and then methanol (10 mL). The mixture was washed with water (2 × 150 mL), dried (magnesium sulfate), and evaporated to dryness under diminished pressure. The colorless syrup obtained was submitted to flash chromatography in a column (14 × 5 cm) of silica gel. As the eluant, 1:2 (v/v) ethyl acetate–petroleum ether (b.p. 60–70°) was used to elute **16**-*d*, which after evaporation of the eluate was obtained as a syrup (1.6 g, 21.3%); ^{1}H-n.m.r. data ($CDCl_3$, 90 MHz): δ 1.23 (s, 3 H, H-1), 2.26 (bs, 1 H, OH), 3.08 (d, 1 H, H-3), 3.47–3.73 (m, 4 H, H-5,7,8,8'), 3.97 (dd, 1 H, H-6), 4.10 (dd, 1 H, H-4), 4.37–5.06 (m, 8 H, 4 CH_2), and 7.1–7.5 (m, 20 H, 4 C_6H_5); $J_{3,4}$ 9.8, $J_{4,5}$ 9, $J_{5,6}$ 3, and $J_{6,7}$ 1 Hz.

Compound **17**-*d*, subsequently eluted with 3:1 (v/v) ethyl acetate–petroleum ether (b.p. 60–70°), was obtained as a syrup (3.8 g, 50.5%); ^{1}H-n.m.r. data ($CDCl_3$, 90 MHz): δ 1.15 (s, 3 H, H-1), 2.63 (bs, 1 H, OH), 3.18 (d, 1 H, H-3), 3.36–3.56 (m, 3 H, H-7,8,8'), 3.58 (dd, 1 H, H-5), 3.85 (dd, 1 H, H-4), 3.93 (dd, 1 H, H-6), 4.1–4.5 (m, 8 H, 4 CH_2), and 7.1–7.5 (m, 20 H, 4 C_6H_5); $J_{3,4}$ 9, $J_{5,6}$ 3, and $J_{6,7}$ 1 Hz.

3,7-Anhydro-4,5,6,8-tetra-O-*benzyl-1-deoxy-2-deuterio-2*-O-p-*tolylsulfonyl*-D-threo-L-galacto-*octitol* (**18**-*d*). — Compound **16**-*d* (4.8 g, 8.44 mmol) was treated with *p*-toluenesulfonyl chloride (1.93 g) in pyridine (80 mL), and after 15 h was processed in the usual way: **18**-*d* was obtained as a colorless syrup (5.65 g, 93%); ^{1}H-n.m.r. data ($CDCl_3$, 90 MHz): δ 1.33 (s, 3 H, H-1), 2.32 (s, 3 H, CH_3), 3.47–3.70 (m, 4 H, H-5,7,8,8'), 3.88–4.13 (m, 2 H, H-4,6), 4.37–5.03 (m, 8 H, 4 CH_2), and 7.0–7.8 (m, 24 H, C_6H_4, 4 C_6H_5); $J_{3,4}$ 9.9, $J_{4,5}$ 9, $J_{5,6}$ 3, and $J_{6,7}$ 1 Hz.

4,5,6,8-Tetra-O-*acetyl-3,7-anhydro-1-deoxy-2-deuterio-2*-O-p-*tolylsulfonyl*-D-threo-L-galacto-*octitol* (**19**-*d*). — From **18**-*d* (7.9 g, 11.12 mmol), compound **19**-*d* (5.63 g, 94%) was obtained as described for the preparation of **19**; ^{1}H-n.m.r. data ($CDCl_3$, 90 MHz): δ 1.28 (s, 3 H, H-1), 1.95 (s, 3 H, $COCH_3$), 2.01 (s, 6 H, 2 $COCH_3$), 2.06 (s, 3 H, $COCH_3$), 2.42 (s, 3 H, CH_3), 3.55 (d, 1 H, H-3), 3.7–4.1 (m, 3 H, H-7,8,8'), 4.93 (dd, 1 H, H-5), 5.23 (dd, 1 H, H-4), 5.28 (dd, 1 H, H-6), and 7.22 and 7.68 (2 d, 4 H, C_6H_4); $J_{3,4}$ 9.5, $J_{4,5}$ 10, $J_{5,6}$ 3, and $J_{6,7}$ 1 Hz.

(Z)-*3,7-Anhydro-1,2-dideoxy-2-deuterio*-D-galacto-*oct-2-enitol* (**5**-*d*). — Compound **5**-*d* was synthesized from **19**-*d* (4.01 g, 7.5 mmol) as described for **5**, giving crystalline **5**-*d* (570 mg, 40%).

2,4,5,6,8-Penta-O-*acetyl-3,7-anhydro-1-deoxy*-D-threo-L-galacto-*octitol* (**26**). Compound **23** (500 mg, 1.33 mmol) was treated with 1:1 (v/v) acetic anhydride–pyridine (10 mL), and kept for 15 h at room temperature. Conventional isolation of the acetate yielded crystalline **26** (417 mg, 75%) from ethyl acetate–petroleum ether (b.p. 60–70°): m.p. 90°, $[\alpha]_{578}^{22}$ +36.7° (*c* 1.0 $CHCl_3$).

Anal. Calc. for $C_{18}H_{26}O_{11}$: C, 51.67; H, 6.26. Found: C, 51.37; H, 6.15.

2,4,5,6,8-Penta-O-*acetyl-3,7-anhydro-1-deoxy*-D-threo-L-talo-*octitol* (**27**). — Compound **17** (1 g, 1.76 mmol), dissolved in methanol (100 mL), was hydrogenolyzed

in the presence of palladium–charcoal as the catalyst. When the reaction was complete, the insoluble material was filtered off, and the filtrate evaporated under diminished pressure. The residue was acetylated with 1:1 (v/v) acetic anhydride–pyridine (10 mL). Conventional isolation yielded colorless crystals of **27** (544 mg, 74%) from ether; m.p. 96°, $[\alpha]_{578}^{22}$ −4.4° (*c* 1.0, $CHCl_3$).

Anal. Calc. for $C_{18}H_{26}O_{11}$: C, 51.67; H, 6.26. Found: C, 51.38; H, 6.24.

*3,7-Anhydro-1-deoxy-2,4:6,8-di-*O*-benzylidene-*D-threo-L-talo-*octitol* (**36**). — Compound **26** (1.47 g, 3.5 mmol) was treated with 0.02M sodium methoxide solution (10 mL). After 4 h the solution was de-ionized by passing it through a column (7 × 0.5 cm) of silica gel, and evaporated to dryness *in vacuo*. The residue was dissolved in water (10 mL), and the solution lyophilized. The colorless powder resulting was quickly added to a mixture of benzaldehyde (5 mL) and anhydrous zinc chloride (2 g), and the mixture shaken vigorously in the dark for 6 h. When the reaction was complete, ice (20 mL) was added, and the mixture was immediately extracted successively with petroleum ether (b.p. 30–50°; 2 × 40 mL) and chloroform (2 × 50 mL). The extracts were combined, successively washed with aqueous sodium hydrogencarbonate solution (25 mL) and water (25 mL), dried (magnesium sulfate), and evaporated to dryness under diminished pressure. The residue was purified by chromatography in a column (9 × 2.5 cm) of silica gel with 1:1 (v/v) ethyl acetate–petroleum ether (b.p. 60–70°) as the solvent. Compound **36** was isolated as a colorless syrup (990 mg, 70.1%); $[\alpha]_{578}^{26}$ +35.0° (*c* 1.08, $CHCl_3$); ^{1}H-n.m.r. data ($CDCl_3$, 90 MHz): δ 1.36 (d, 3 H, H-1), 2.66 (bs, 1 H, OH-5), 2.94 (dd, 1 H, H-3), 3.3 (m, 1 H, H-7), 3.6–4.4 (m, 8 H, H-2,4,5,6,8,8′), 5.43 (s, 1 H, -CH-), 5.56 (s, 1 H, -CH-), and 7.1–7.5 (m, 10 H, 2 C_6H_5).

*3,4,5,7-Tetra-*O*-acetyl-2,6-anhydro-1-deoxy-*D-galacto-*hept-1-enitol* (**9**). — This compound was prepared according to Brockhaus and Lehmann[1], but unlike those authors, we were able to obtain compound **9** crystalline from ether by carefully adding petroleum ether (b.p. 60–70°). Colorless crystals formed at −20° (81.9%); m.p. 65°, $[\alpha]_{578}^{22}$ +69° (*c* 1.0, $CHCl_3$); lit.[1] $[\alpha]_{578}^{25}$ +70° (*c* 1.0).

Anal. Calc. for $C_{15}H_{20}O_9$: 52.32; H, 5.86. Found: C, 52.29; H, 5.86.

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