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SYNTHESIS OF (*Z*)-3,7-ANHYDRO-1,2-DIDEOXY-2-DEUTERIO-D-*gluco*-OCT-2-ENITOL, A PROCHIRAL SUBSTRATE FOR PROBING THE CATALYTIC FUNCTIONING OF GLUCOSYLASES

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ABSTRACT

Synthesis of the title compound provides a prochiral, glycosyl-donor substrate well suited for use as a probe of the catalytic functioning of D-glucosyl-mobilizing enzymes, because the full stereochemistry of enzymic reactions at its double bond may be unambiguously determined by examining the reaction products. The starting material for the synthesis was 2,6-anhydro-D-*glycero*-D-*gulo*-heptonic acid, from which 3,7-anhydro-4,5,6,8-tetra-*O*-benzyl-1-deoxy-D-*glycero*-D-*gulo*-2-octulose was prepared in eight steps. Reduction with lithium aluminum deuteride, and conversion of the resulting diastereomeric alcohols into (*Z*)-3,7-anhydro-4,5,6,8-tetra-*O*-benzyl-1,2-dideoxy-2-deuterio-D-*gluco*-oct-2-enitol (**11**) and 3,7-anhydro-4,5,6,8-tetra-*O*-benzyl-1,2-dideoxy-2-deuterio-D-*glycero*-D-*gulo*-oct-1-enitol (**16**), was carried out. By-products were 3,7-anhydro-2-*O*-benzoyl-4,5,6,8-tetra-*O*-benzyl-1,2-dideoxy-2-deuterio-D-*erythro*-L-*galacto*-octitol and 3,7-anhydro-2-*O*-benzoyl-4,5,6,8-tetra-*O*-benzyl-1,2-dideoxy-2-deuterio-D-*erythro*-L-*talo*-octitol, which could, like compound **16**, be recycled. On debenzylation the oct-2-enitol **11** yielded (*Z*)-3,7-anhydro-1,2-dideoxy-2-deuterio-D-*gluco*-oct-2-enitol.

INTRODUCTION

Stereochemical studies of glycosylation reactions catalyzed with nonglycosidic substrates have in recent years provided a substantial advance with regard to appreciating the catalytic capabilities of glycosylases. Such studies, employing glycosyl fluorides and available enolic glycosyl donors as substrates, have revealed the ability of various well known glycosylases to catalyze different stereochemical reactions with different substrates. In reactions with D-glycals, for example, an α-

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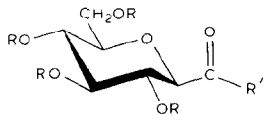
and a β-D-glucosidase[1], a β-D-galactosidase[2], and several D-glucanases[3,4] were found to protonate a D-glycal substrate from a direction opposite, to that generally assumed for protonation of the glycosidic substrates of the enzyme. An especially clear example of such catalytic flexibility was uncovered by the use of (*Z*)-3,7-anhydro-1,2-dideoxy-2-deuterio-D-*galacto*-oct-2-enitol[5], the first sugar donor (apart from the glycals) to yield products allowing determination of the full steric course of an enzymically catalyzed reaction. Lehmann and Schlesselmann[6] found that the 2-deuterio-D-*galacto*-octenitol is protonated by *E. coli* β-D-galactosidase from a direction opposite that found for the protonation of 2-deuterio-D-galactal by the same enzyme[2].

The desirability of investigating an analogous compound to probe the catalytic functioning of D-glucosyl-mobilizing enzymes led to the synthesis of (*Z*)-3,7-anhydro-1,2-dideoxy-D-*gluco*-oct-2-enitol and to the observation that its hydration is catalyzed by several D-glucosylases, including rice α-D-glucosidase and *Trichoderma reesei* trehalase[7]. Analysis of the 2-deuterated octulose formed (in D_2O) in digests of the octenitol with the latter two enzymes (unpublished observations) suggested that deuteration of the substrate had, in each case, occurred from above its *re*-face. However, the finding that some H_2O had entered the reactions complicated this interpretation and promoted the present synthesis of 2-deuterio-D-*gluco*-oct-2-enitol so that the stereochemistry of D-glucosylase-catalyzed reactions might be unambiguously determined.

We now describe a novel and more effective synthesis pathway for the preparation of 2-deuterio-D-*gluco*-oct-2-enitol as a probe of the mechanism of enzyme action. The synthesized compound was recently found to contribute toward understanding the process of catalysis by glycosylases[8].

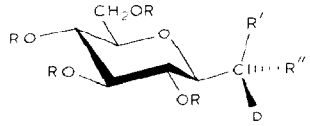
RESULTS

Synthesis of (Z)-*3,7-anhydro-1,2-dideoxy-2-deuterio*-D-gluco-*oct-2-enitol* (**10**). — 2,6-Anhydro-D-*glycero*-D-*gulo*-heptonic acid[9] (**1**) was treated with acetic anhydride in the presence of zinc chloride, to give the per-*O*-acetylated heptonic acid[10] (**2**), which was then converted with phosphorus pentachloride into 3,4,5,7-tetra-*O*-acetyl-2,6-anhydro-D-*glycero*-D-*gulo*-heptonic acid chloride (**3**). Compounds **2** and **3** were both characterized by conversion into the crystalline methyl ester **4**. Acylation of isopropylidenemalonate (Meldrum's acid) with **3** in dichloromethane and pyridine[11] at low temperature gave a condensation product **13** which, without isolation, was hydrolyzed by aqueous acetic acid to give, after reacetylation and purification, crystalline 4,5,6,8-tetra-*O*-acetyl-3,7-anhydro-1-deoxy-D-*glycero*-D-*gulo*-2-octulose (**5**). Beyond these initial steps which parallel those employed in preparing the 2-deuterio-D-*galacto*-octenitol analog[5], the synthesis follows an entirely new path that provides for a process of by-product recycling that greatly enhances the efficiency of preparing the desired product.



1 R = H, R′ = OH
2 R = Ac, R′ = OH
3 R = Ac, R′ = Cl
4 R = Ac, R′ = OCH_3
5 R = Ac, R′ = CH_3
6 R = Bzl, R′ = CH_3
Bzl = $PhCH_2$

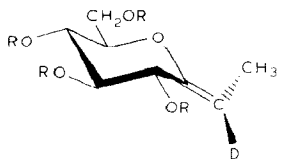
A solution of the octulose **5** in dry methanol was treated with anhydrous copper sulfate and sulfuric acid for two days. Following the addition of pyridine (with ice cooling), and solvent removal, in order to replace partly lost acetyl groups, the residue was reacetylated with 1:1 pyridine–acetic anhydride, to yield a syrup comprising ~60% of 4,5,6,8-tetra-*O*-acetyl-3,7-anhydro-1-deoxy-D-*glycero*-D-*gulo*-2-octulose 2,2-dimethyl acetal (**14**); separation on a column of silica gel, yielded the pure crystalline compound. This was deacetylated, and the dried product subjected to benzylation[12] to introduce a differential blocking group. 3,7-Anhydro-4,5,6,8-tetra-*O*-benzyl-1-deoxy-D-*glycero*-D-*gulo*-2-octulose 2,2-dimethyl acetal (**15**) was isolated in the ordinary way without further purification and treated with 1:1 acetic acid–water for 2 h at 65–70°. Complete solvent removal gave 3,7-anhydro-4,5,6,8-tetra-*O*-benzyl-1-deoxy-D-*glycero*-D-*gulo*-2-octulose (**6**) as an oil. For analytical purposes, a sample of **6** was chromatographed on a column of silica gel; the crystals separated had properties conforming to the assigned structure of **6**. Reduction of the benzylated octulose with lithium aluminum deuteride yielded a syrupy mixture of 3,7-anhydro-4,5,6,8-tetra-*O*-benzyl-1-deoxy-2-deuterio-D-*erythro*-L-*galacto*-octitol (**7a**) and -L-*talo*-octitol (**7b**). Structural assignments were made for the nondeuterated analogs[7] of **7a** and **7b**. The mixed isomers were tosylated. The product was a syrupy mixture of 3,7-anhydro-4,5,6,8-tetra-*O*-benzyl-1-deoxy-2-deuterio-2-*O*-*p*-tolylsulfonyl-D-*erythro*-L-*galacto*-octitol (**8a**) and -L-*talo*-octitol (**8b**). To introduce a double bond between[5] C-2 and C-3 by solvolysis, a solution of **8a** and **8b** in *N*,*N*-dimethylformamide was boiled under reflux with sodium benzoate. Working up gave a mixture of the two elimination products (*Z*)-3,7-anhydro-4,5,6,8-tetra-*O*-benzyl-2-deuterio-D-*gluco*-oct-2-enitol (**11**) and 3,7-anhydro-4,5,6,8-tetra-*O*-benzyl-1,2-dideoxy-2-deuterio-D-*glycero*-D-*gulo*-oct-1-enitol (**16**), plus the two substitution products, 3,7-anhydro-2-*O*-benzoyl-4,5,6,8-tetra-*O*-benzyl-1,2-dideoxy-2-deuterio-D-*erythro*-L-*galacto*-octitol (**9a**) and 3,7-anhydro-2-*O*-benzoyl-4,5,6,8-tetra-*O*-benzyl-1,2-dideoxy-2-deuterio-D-*erythro*-L-*talo*-octitol (**9b**). In order to separate the two alkenes from the products of substitution, the mixture was deacylated. Chromato-



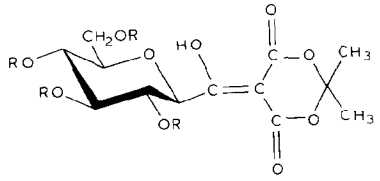
7a R = Bzl, R′ = OH, R″ = CH_3
7b R = Bzl, R′ = CH_3, R″ = OH
8a R = Bzl, R′ = OTs, R″ = CH_3
8b R = Bzl, R′ = CH_3, R″ = OTs
9a R = Bzl, R′ = OBz, R″ = CH_3
9b R = Bzl, R′ = CH_3, R″ = OBz

Bz = PhCO
Ts = $O_2SC_6H_4$-Me*p*

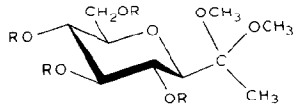
graphy on a column of silica gel, with 1:5 ethyl acetate–petroleum ether, separated the two elimination products **11** and **16** from the two deacylated substitution products **7a** and **7b**. The enitols were then separated from each other by chromatography on silica gel. Compound **16** crystallized spontaneously. It could also the characterized as the peracetate **18**. The desired compound **11** was obtained as a syrup (29%). For conversion into the title compound **10**, debenzylation was carried out under controlled conditions. The unsubstituted free enitol **10**, like all related compounds having an exocyclic, enol ether grouping, is stable; it was acetylated to afford the pure stable, crystalline (*Z*)-4,5,6,8-tetra-*O*-acetyl-3,7-anhydro-1,2-dideoxy-2-deuterio-D-*gluco*-oct-2-enitol (**12**). ^{1}H-N.m.r. spectra recorded in $CDCl_3$ at 250 MHz (see Table I), and elemental analyses, agreed with the structure assigned to the final product **12** and to the synthetic intermediates. The *Z*-configuration for compound **12** was deduced from a thorough spectral investigation on (*Z*)-4,5,6,8-tetra-*O*-acetyl-3,7-anhydro-1,2-dideoxy-D-*galacto*-oct-2-enitol[5], as well as (*Z*)-4,5,6,8-tetra-*O*-acetyl-3,7-anhydro-1,2-dideoxy-D-*gluco*-oct-2-enitol[7]. In particular, the absence of vicinal coupling of the H-1 resonance of **12** confirms the presence of a deuteron at C-2.



10 R = H
11 R = Bzl
12 R = Ac

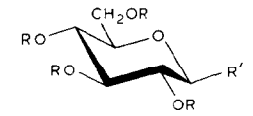


13 R = Ac



14 R = Ac
15 R = Bzl

Conversion of compounds **16**, **9a**, *and* **9b** *into the octitols* **7a** *and* **7b**. — Whereas, in the original approach[7] for the preparation of (*Z*)-3,7-anhydro-1,2-dideoxy-D-*gluco*-oct-2-enitol, the substitution products in the solvolysis reaction were lost because deacylation removed not only the *O*-benzoyl groups introduced by the displacement reaction but also the acetyl protecting groups on O-4, -5, -6, and -8, thereby abolishing the differential protection, with benzyl ether protection, the by-products **9a** and **9b** can be reconverted into the original starting compounds **7a** and **7b**. This allows recycling and improvement of overall yields. Also, the oct-1-enitol by-product **16** was converted, by treatment with *m*-chloroperoxybenzoic acid in dichloromethane, into a mixture of the two isomeric ethylene oxides 1,2;3,7-dianhydro-4,5,6,8-tetra-*O*-benzyl-2-deuterio-D-*erythro*-L-*galacto*-octitol (**17a**) and -L-*talo*-octitol (**17b**), which were then reduced with lithium aluminium hydride to yield a mixture of the diastereoisometric octitols **7a** and **7b**.



16 R = Bzl, R′ = DC=CH$_2$

17a R = Bzl, R′ = 

17b R = Bzl, R′ = 

18 R = Ac, R′ = DC=CH$_2$

The present synthesis provides an effective route for preparing (*Z*)-2-deuterio-D-*gluco*-octenitol **10**, a potential substrate for D-glucosylases of different types. Use of the compound as a probe of the catalytic functioning of such enzymes was recently demonstrated[8] in a study of its hydration, catalyzed by *Aspergillus niger* and rice α-D-glucosidases and by *Trichoderma reesei* trehalase.

TABLE I

^{1}H-N.M.R. DATA ($CDCl_3$, 250 MHz) FOR COMPOUNDS **2, 4, 5, 6, 7a, 7b, 8a, 8b, 11, 12, 14, 15, 16, 17a, 17b,** AND **18** (RELATIVE TO Me_4Si). COMPARE DATA FOR ANALOGOUS COMPOUNDS[7]

Proton	*Compound*															
	2	**4**	**5**	**6**	**7a**	**7b**	**8a**	**8b**	**11**	**12**	**14**	**15**	**16**	**17a**	**17b**	**18**
H-1			2.25	2.19	1.30	1.15	1.35	1.04	1.66	1.63	1.38	1.40	5.3	2.79	2.66	5.29
H-1′													5.46	2.87	2.81	5.35
H-2	4.08	4.02														
H-3	5.11	5.11	3.77	3.57	3.11	3.32	3.20	3.42			3.62	3.53	3.13	3.15	3.35	3.87
H-4	5.30	5.27	5.10		3.57	3.45	3.57	3.26	3.92	5.40	5.03			3.48	3.48	4.94
H-5	5.24	5.20	5.26	to	to	to	to	3.62	3.64	5.11	5.25	to	to	to	to	5.23
H-6	3.78	3.73	5.11	4.08	3.77	3.78	3.76	3.56	to	5.19	5.16	3.78	3.80	3.76	3.76	5.09
H-7	4.17	4.15	3.74	3.51	3.45	3.46	3.41	3.32	3.83	3.80	3.67	3.43	3.49	3.42	3.40	3.71
H-7′	4.28	4.27														
H-8			4.19	3.58-	3.57-	3.70	3.57-	3.69	3.64-	4.22	4.13	3.69	3.62-	3.48-	3.48-	4.12
H-8′			4.27	4.08	3.77	3.70	3.76	3.69	3.83	4.32	4.23	3.77	3.80	3.76	3.76	4.26
$COCH_3$	2.03	2.02	2.03							2.03	2.01					2.00
	2.04	2.03	2.03							2.04	2.01					2.01
	2.05	2.03	2.05							2.11	2.04					2.03
	2.10	2.10	2.11							2.12	2.09					2.09
$-CH_2-$				4.52-	4.51-	4.5-	4.52-	4.49-	4.49			4.55-	4.52-	4.48-	4.48-	
				4.90	4.90	4.97	4.99	4.93	4.87			4.90	4.95	4.92	4.92	
C_6H_5				7.15-	7.16-	7.17-	7.17-	7.17-	7.13-			7.17-	7.13-	7.12-	7.12	
				7.34	7.35	7.37	7.39	7.32	7.37			7.37	7.37	7.36	7.36	
C_6H_4							7.17-	7.17-								
							7.39	7.32								
							7.80	7.76								
$-CH_3$							2.41	2.4								
$-OCH_3$		3.76									3.17	3.27				
											3.21	3.27				
$-CO_2OH$	7.38															

$J_{H,H}$																
1,1′														5.4	5.7	
1,4									1.5	2						
2,3	9.6	9.7														
3,4	9.3	9.7	9.7		7.2	9.7	8.3	9.7			9.7	9.4	9.4	9.7	10	9.9
4,5	9.3	9.0	9.7					8.2	6.7	8.7	9.7					9.9
5,6	9.5	9.3	9.1					8.2		9.1	9.3					9.9
6,7	4.6	4.8	9.3	9.1	9.3	9.3	9.3	9.3		9.4	9.3	9.6	9.4	9.4		9.5
6,7′	2.2	2.2														
7,7′	12.6	12.4														
7,8			4.8	3.8	4.7	3	6.2	2.4		4.8	5.2	4.5	3.5	3.7		4.8
7,8′			2.7	2.3	2.7	3		2.4		2.7	2.4	2.2	2.7	2.4		2.4
8,8′			12.3							12.1	12.2	11.2				12.3

EXPERIMENTAL

Methods. — Solutions were evaporated *in vacuo*. Optical rotations were measured with a Perkin–Elmer 141 polarimeter. Melting points are uncorrected. All reactions were monitored by t.l.c. on silica gel 60 F_{254} (Merck) using as solvents: for benzylated compounds, 1:2 EtOAc–light petroleum; and for acetylated compounds 1:1 EtOAc–light petroleum. Preparative column chromatography was performed on silica gel 60 (0.063–0.2 mm, Merck) by applying the "flash" technique[13] and using the solvents indicated. ^{1}H-N.m.r. spectra were recorded with a Bruker WM 250 spectrometer at 250 MHz for solutions in $CDCl_3$ (internal Me_4Si). Unless otherwise indicated, light petroleum refers to the fraction having b.p. 60–70°.

*3,4,5,7-Tetra-*O*-acetyl-2,6-anhydro-*D-glycero-D-gulo-*heptonic acid*[10] (**2**). — 2,6-Anhydro-D-*glycero*-D-*gulo*-heptonic acid[9] (**1**; 6.16 g, 29.58 mmol) was suspended in Ac_2O (30 mL); the mixture was stirred with $ZnCl_2$ (2.3 g) for 24 h at room temperature, poured into ice–water (200 mL), stirred for 4 h, and extracted with $CHCl_3$ (9 x 70 mL). The extracts were combined, washed twice with water (300 mL), dried ($MgSO_4$), and evaporated. The resulting slightly yellow syrup crystallized spontaneously (7.7 g, 69%). Recrystallization from toluene gave **2** as a monohydrate.

Anal. Calc. for $C_{15}H_{20}O_{11}\cdot 1\ H_2O$ (394.33): C, 45.69; H, 5.62. Found: C, 45.94; H, 5.49.

Drying under diminished pressure for 7 h at 50° yielded crystals of anhydrous compound **2**, m.p. 137° (lit.[10] 138–140°), $[\alpha]_{589}^{23}$ −4° (*c* 1.0, $CHCl_3$), lit.[10] $[\alpha]_{589}^{25}$ +1.7° (*c* 1.04, $CHCl_3$); R_F 0.008 for ^{1}H-n.m.r. data see Table I.

Anal. Calc. for $C_{15}H_{20}O_{11}$ (376.32): C, 47.88; H, 5.36. Found: C, 47.78; H, 5.26.

*3,4,5,7-Tetra-*O*-acetyl-2,6-anhydro-*D-glycero-D-gulo-*heptonic acid chloride* (**3**). — Compound **2** (4 g, 10.6 mmol) suspended in absolute Et_2O (55 mL) was treated with PCl_5 (3g, 14.4 mmol), and boiled under reflux until a clear solution was obtained; this was cooled, and light petroleum (b.p. 30–60°; 100 mL) was added. On storage at 0°, colorless crystals of compound **3** (3.1 g, 74%), R_F 0.14, were obtained. Because of its sensitivity to hydrolysis, the compound was not further purified and submitted to microanalysis, but was converted into the methyl ester.

*Methyl 3,4,5,7-tetra-*O*-acetyl-2,6-anhydro-*D-glycero-D-gulo-*heptonate* (**4**). — *(a)* Compound **3** (3.4 g, 8.6 mmol) was dissolved in C_5H_5N (25 mL), and CH_3OH (50 mL) was added. The mixture was heated overnight at 50°, cooled, poured into ice–water (150 mL), and extracted with CH_2Cl_2 (3 x 60 mL). The extract was successively washed with 5% $NaHCO_3$ solution (100 mL) and water (2 x 100 mL), dried ($MgSO_4$), and evaporated. The last traces of C_5H_5N were removed by codistillation with toluene. Flash chromatography[13] in a column (15 x 3 cm) of silica gel with 1:1 EtOAc–light petroleum as the solvent gave syrupy **4** which crystallized from CH_3OH (2.4 g, 71.5%); m.p. 149°, R_F 0.28.

(b) Compound **2** (300 mg, 0.76 mmol) in absolute CH_3OH (15 mL) was boiled under reflux for 24 h. After solvent removal *in vacuo*, the resulting, pale-yellow syrup was submitted to flash chromatography in a column (14 x 1 cm) of silica gel

with 1:1 EtOAc–light petroleum, to give syrupy **4** that crystallized from Et_2O (186 mg, 63%), m.p. 149°, $[\alpha]_{589}^{23}$ −23° (*c* 0.2, $CHCl_3$); R_F 0.28; for ^{1}H-n.m.r. data, see Table I.

Anal. Calc. for $C_{16}H_{22}O_{11}$ (390.34): C, 49.23; H, 5.68. Found: C, 49.04; H, 5.61.

*4,5,6,8-Tetra-*O*-acetyl-3,7-anhydro-1-deoxy-*D-glycero-D-gulo-*2-octulose* (**5**). — Compound **3** (25.5 g, 64.5 mmol) in CH_2Cl_2 (200 mL) was added dropwise during 1.5 h at 0° to a stirred solution of isopropylidenemalonate (9.3 g, 65.5 mmol) in C_5H_5N (10.5 mL) and CH_2Cl_2 (150 mL). After 2 h at 0°, the mixture was washed with water (200 mL), dried ($MgSO_4$), and evaporated. The last traces of C_5H_5N were removed by codistillation with toluene. The red syrup (19 g) of the condensation product **13** was dissolved in 1:2 glacial AcOH–water (200 mL) and boiled under reflux for 4 h; the solution turned light-yellow. After evaporation, the resulting syrup was dissolved in $CHCl_3$ (300 mL). The solution was successively washed with an aqueous 5% solution of $NaHCO_3$ (200 mL) and water (2 x 150 mL), dried ($MgSO_4$), and evaporated. The syrup obtained was reacetylated with 1:1 Ac_2O–pyridine (100 mL). Working up procedures yielded a yellow syrup. Purification was carried out by flash chromatography on a column (15 x 5 cm), of silica gel with 1:2 EtOAc–light petroleum as solvent. Crystallization from Et_2O gave pure **5** (14.2 g, 59%); m.p. 110°, $[\alpha]_{589}^{23}$ +31° (*c* 0.5, $CHCl_3$); R_F 0.28; for ^{1}H-n.m.r. data, see Table I.

Anal. Calc. for $C_{16}H_{22}O_{10}$ (374.37); C, 51.33; H, 5.92. Found: C, 51.51; H, 5.90.

*4,5,6,8-Tetra-*O*-acetyl-3,7-anhydro-1-deoxy-*D-glycero-D-gulo-*2-octulose 2,2-dimethyl acetal* (**14**). — Compound **5** (6.3 g, 16.8 mmol) was dissolved in absolute CH_3OH (150 mL). Anhydrous $CuSO_4$ (10.7 g) and concentrated H_2SO_4 (0.5 mL) were added, and the mixture was stirred for 2 d at room temperature. After addition of C_5H_5N (30 mL) (ice cooling), the resulting deep-blue solution was evaporated. The obtained residue was acetylated in 1:1 Ac_2O–C_5H_5N (60 mL) in order to replace any acetyl groups lost during the foregoing procedure. Working up in the normal way yielded a syrup which consisted mainly of compound **14** (~60%) and was purified by flash chromatography on a column (15 x 5 cm) of silica gel with 1:3 EtOAc–light petroleum as the solvent. Compound **14** crystallized spontaneously. Recrystallization from Et_2O gave analytically pure **14** (4.2 g, ~60%); m.p. 98°, $[\alpha]_{589}^{23}$ +22° (*c* 0.5, $CHCl_3$); R_F 0.21; for ^{1}H-n.m.r data, see Table I

Anal. Calc. for $C_{18}H_{28}O_{11}$ (420.42): C, 51.42; H, 6.71. Found: C, 51.31; H, 6.47.

*3,7-Anhydro-4,5,6,8-tetra-*O*-benzyl-1-deoxy-*D-glycero-D-gulo-*2-octulose 2,2-dimethyl acetal* (**15**). — Compound **14** (6.7 g, 15.9 mmol) was *O*-deacetylated with 0.02M $NaOCH_3$ (100 mL). The solution was evaporated to dryness *in vacuo*. A solution of the residue (4 g) in absolute *N,N*-dimethylformamide (200 mL) was treated with NaH (5 g) under stirring. After 2 h, $PhCH_2Br$ (27 mL) was added dropwise during1.5 h, and the mixture was stirred for another 3 h.[12] CH_3OH (20 mL)

was then added cautiously, and the mixture was evaporated at 13.3 Pa. The yellow syrup was dissolved in water, the solution extracted with $CHCl_3$ (4 x 100 mL), and the extract washed with water (3 x 200 mL), dried ($MgSO_4$), and evaporated. Crude, oily **15** (9.3 g) was used in the following step without further purification. A small amount (0.5 g) was purified by column chromatography on a column (16 x 2 cm) of silica gel using 1:5 EtOAc–light petroleum as the solvent, to afford pure **15** as a colorless syrup (~450 mg), $[\alpha]_{589}^{23}$ +26° (*c* 0.5, $CHCl_3$); R_F 0.36; for ^{1}H-n.m.r. data, see Table I.

Anal. Calc. for $C_{38}H_{44}O_7$ (612.77): C, 74.48; H, 7.24. Found: C, 74.51; H, 7.02.

3,7-Anhydro-4,5,6,8-tetra-O-*benzyl-1-deoxy*-D-glycero-D-gulo-*2-octulose* (**6**). — Crude **15** (9.3 g) was hydrolyzed with 1:1 AcOH–water (150 mL) under stirring for 2 h at 65–70°. AcOH was removed by repeated addition and evaporation of water (3 x 100 mL). After the final evaporation, a small portion (0.5 g) of the residual, yellow oil (8.9 g) was submitted to flash chromatography on a column (18 x 2 cm) of silica gel, using 1:5 EtOAc–light petroleum. After evaporating the corresponding fractions to dryness compound **6** crystallized on addition of Et_2O (440 mg, 88%); m.p. 78°, $[\alpha]_{589}^{23}$ +10° (*c* 0.25, $CHCl_3$); R_F 0.42; for ^{1}H-n.m.r. data, see Table I.

Anal. Calc for $C_{36}H_{38}O_6$ (566.7): C, 76.30; H, 6.75. Found: C, 76.17; H, 6.78.

3,7-Anhydro-4,5,6,8-tetra-O-*benzyl-1-deoxy-2-deuterio*-D-erythro-L-galacto-*octitol* (**7a**) *and 3,7-anhydro-4,5,6,8-tetra*-O-*benzyl-1-deoxy-2-deuterio*-D-erythro-L-talo-*octitol* (**7b**). — Compound **6** (5 g, 8.82 mmol) in absolute Et_2O (100 mL) was reduced with $LiAlD_4$ (350 mg). After stirring for 1.5 h at 25°, the excess of reductant was decomposed by adding EtOAc (2 mL) and CH_3OH (10 mL). The mixture was washed with water (2 x 100 mL), dried ($MgSO_4$), and evaporated *in vacuo* to a pale-yellow syrup compromising compounds **7a** and **7b** (4.55 g). The mixture of the two alcohols (**7a** and **7b**) (600 mg) was separated by chromatography on a column (15 x 2 cm) of silica gel, using 1:3 EtOAc–light petroleum as the solvent. The (faster-eluted) **7a** crystallized on addition of Et_2O (190 mg, 31%);0.29; m.p. 58–59°, $[\alpha]_{589}^{23}$ + 6° (*c* 0.5, $CHCl_3$); R_F 0.29; for ^{1}H-n.m.r. data, see Table I.

Anal. Calc for $C_{36}H_{39}DO_6$ (569.72): C, 75.89; H + D, 7.25. Found: C, 75.73; H + D, 7.06. Further elution gave **7b** as a colorless syrup (290 mg, 48%); $[\alpha]_{589}^{23}$ −2.1° (*c* 0.57, $CHCl_3$); R_F 0.19; for ^{1}H-n.m.r. data, see Table I.

Anal. Calc for $C_{36}H_{39}DO_6$ (569.72): C, 75.89; H + D, 7.25. Found C, 76.05; H + D, 7.10.

3,7-Anhydro-4,5,6,8-tetra-O-*benzyl-1-deoxy-2-deuterio-2*-O-p-*tolylsulfonyl*-D-erythro-L-galacto-*octitol* (**8a**) and *3,7-anhydro-4,5,6,8-tetra*-O-*benzyl-1-deoxy-2-deuterio-2*-O-*tolylsulfonyl*-D-erythro-L-talo-*octitol* (**8b**). — A mixture of compounds **7a** and **7b** (4.55 g, 7.89 mmol) was treated with *p*-toluenesulfonyl chloride (5 g) in C_5H_5N (100 mL). After 48 h, the mixture was processed in the usual way. Flash chromatography on a column (18 x 5 cm) of silica gel with 1:1 EtOAc–light petroleum as solvent gave the product mixture **8a** and **8b** as a colorless syrup (5.14 g,

89%); R_F 0.41 for both components.

Compounds **8a** and **8b** could also be separately synthesized from the corresponding alcohol as described here for **8b**. Compound **7b** (180 mg, 0.31 mmol) was treated with an excess of *p*-toluenesulfonyl chloride (148 mg) in pyridine (5 mL) for 2 d. The usual work-up followed by flash chromatography on a column (16 x 2 cm) of silica gel with 1:3 EtOAc–cyclohexene gave **8b** as an amorphous solid (192 mg, 85.5%); $[\alpha]_{589}^{23}$ −21.8° (*c* 0.33, $CHCl_3$); for ^{1}H-n.m.r. data, see Table I.

Anal. Calc. for $C_{43}H_{45}DO_8S$ (723.91): C, 71.34; H + D, 6.54; S, 4.43. Found: C, 71.44; H + D,6.40; S, 4.18.

*(Z)-3,7-Anhydro-4,5,6,8-tetra-*O*-benzyl-1,2-dideoxy-2-deuterio-*D-gluco-*oct-2-enitol* (**11**), *and 3,7-anhydro-4,5,6,8-tetra-*O*-benzyl-1,2-dideoxy-2-deuterio-*D-glycero-D-gulo-*oct-1-enitol* (**16**). — To a solution of the **8a** and **8b** mixture (3.5 g) in absolute *N,N*-dimethylformamide (130 mL) was added sodium benzoate (4.5 g). The suspension was boiled under reflux, with stirring, for 1.5 h, cooled, and evaporated at 13.3 Pa. A solution of the residue in water (200 mL) was extracted with $CHCl_3$ (3 x 100 mL). The extracts were combined, successively washed with saturated aqueous $NaHCO_3$ solution (200 mL) and water (3 x 200 mL), dried ($MgSO_4$), and evaporated, to yield a mixture of the two elimination products (**11** and **16**) and the two substitution products (**9a** and **9b**) (R_F 0.58 for both compounds). The mixture was deacylated with 0.02M $NaOCH_3$. After 24 h, the solution was de-ionized by passing it through a column (10 x 2 cm) of silica gel, and evaporated. The yellow syrup obtained was submitted to flash chromatography on a column (18 x 3 cm) of silica gel with 1:5 EtOAc-light petroleum as the solvent. The elimination products, **11** and **16**, were separated completely from the two deacylated substitution products **7a** and **7b**; the latter were then recycled. Compounds **11** and **16** were separated by flash chromatography on a column (18 x 3 cm) of silica gel with 1:9 EtOAc–light petroleum as the eluant. Compound **13** was obtained as a syrup (772 mg, 29%); R_F 0.63; for ^{1}H-n.m.r. data, see Table I. Compound **16** crystallized spontaneously and was recrystallized from hexane (932 mg, 35%); m.p. 65°, $[\alpha]_{589}^{23}$ −10° (*c* 0.2, $CHCl_3$); R_F 0.61; for ^{1}H-n.m.r. data, see Table I.

Anal. Calc for $C_{36}H_{37}DO_5$ (551.71): C, 78.37; H + D, 7.13. Found C, 78.65; H + D, 7.13.

*(Z)-4,5,6,8-Tetra-*O*-acetyl-3,7-anhydro-1,2-dideoxy-2-deuterio-*D-gluco-*oct-2-enitol* (**12**). — Dry NH_3 was passed into a stirred solution of compound **11** (1.2 g, 2.175 mmol) in absolute oxolane (20 mL) for 2.5 h at −78°. Sodium (0.5 g), cut into small pieces, was added under a stream of nitrogen. The solution turned deep blue. After 1.5 h, the mixture was treated with absolute CH_3OH (10 mL) until the blue color disappeared, and the solution was stirred overnight at room temperature. The mixture was then evaporated; absolute CH_3OH (10 mL) was added, and the solution de-ionized by passing it through a column (10 x 1 cm) of silica gel. The residue obtained on evaporating the eluate was acetylated in 1:1 Ac_2O–C_5H_5N (10 mL). On working up as usual, the product crystallized from Et_2O, to yield **12** (0.61 g, 78%); m.p. 120°, $[\alpha]_{589}^{23}$ +52.6° (*c* 0.5, $CHCl_3$); R_F 0.40; for ^{1}H-n.m.r. data, see Table I.

Anal. Calc. for $C_{16}H_{21}DO_9$ (359.35): C, 53.47; H + D, 6.45. Found: C, 53.49; H + D, 6.16.

*4,5,6,8-Tetra-O-acetyl-3,7-anhydro-1,2-dideoxy-2-deuterio-*D-glycero-D-gulo-*oct-1-enitol* (**18**). — Compound **16** (450 mg, 0.81 mmol) was dissolved in saturated HBr–AcOH (5 mL) under ice-cooling. After 18 h at +10°, the reaction was complete. The mixture was poured into ice-water (~100 mL), made neutral with $NaHCO_3$, and extracted with CH_2Cl_2 (4 x 50 mL). The extract was washed with water (100 mL), dried ($MgSO_4$), and evaporated. The syrupy residue was submitted to flash chromatography on a column (15 x 2 cm) of silica gel, using 1:3 EtOAc–light petroleum as solvent. Evaporation gave a syrup that crystallized spontaneously. Recrystallization from Et_2O–light petroleum yielded colorless crystals of **18** (214 mg, 71%); m.p. 98–99°, lit.[14] m.p. 102.5–103°, $[\alpha]_{589}^{23}$ −11.5° (*c* 0.2 $CHCl_3$); R_F 0.4; for ^{1}H-n.m.r. data, see Table I.

Anal. Calc. for $C_{16}H_{21}DO_9$ (359.35) : C, 53.47; H + D, 6.45. Found: C, 53.42; H + D, 6.11.

Conversion of compound **16** *into the diastereoisomeric alcohols* **7a** *and* **7b**. — A solution of compound **16** (546 mg, 0.99 mmol) in CH_2Cl_2 (20 mL) was treated with *m*-chloroperoxybenzoic acid (1.12 g, 6.5 mmol), and stirred for 2 d at room temperature. The mixture was diluted with CH_2Cl_2 (50 mL), successively washed with aqueous 10% Na_2SO_3 solution (40 mL), saturated $NaHCO_3$ solution (40 mL), and water (2 x 50 mL), and dried ($MgSO_4$). Evaporation followed by flash chromatography on a column (18 x 2 cm) of silica gel with 1:5 EtOAc–cyclohexene yielded a colorless syrup consisting of the two epoxides **17a** and **17b** (470 mg, 83%); R_F 0.52 for both compounds; for ^{1}H-n.m.r. data, see Table I.

Anal. Calc. for $C_{36}H_{37}DO_6$ (567.71): C, 76.16; H + D, 6.92. Found: C, 75.93; H + D, 6.96. The syrupy **17a** plus **17b** (18 mg) was dissolved in Et_2O (3 mL), and treated with a few milligrams of $LiAlH_4$. After 15 min, the usual work-up procedure yielded a mixture of the diastereomeric alcohols **7a** and **7b** (16.5 mg) in the ratio of 2:1.

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REFERENCES

1 E. J. HEHRE, D. S. GENGHOF, H. STERNLICHT, AND C. F. BREWER, *Biochemistry*, 16 (1977) 1780–1786.
2 J. LEHMANN AND B. ZIEGER, *Carbohydr. Res.*, 58 (1977) 73–78.
3 E. J. HEHRE, S. KITAHATA, AND C. F. BREWER, *J. Biol. Chem.*, 261 (1986) 2147–2153.
4 T. KANDA, C. F. BREWER, G. OKADA, AND E. J. HEHRE, *Biochemistry*, 25 (1986) 1159–1165.
5 H. FRITZ, J. LEHMANN, AND P. SCHLESSELMANN, *Carbohydr. Res.*, 113 (1983) 71–92.

6 J. LEHMANN AND P. SCHLESSELMANN, *Carbohydr. Res.*, 113 (1983) 93–99.
7 C. F. BREWER, E. J. HEHRE, J. LEHMANN, AND W. WEISER, *Justus Liebigs Ann. Chem.*, (1984) 1078–1087, 1626.
8 W. WEISER, J. LEHMANN, S. CHIBA, H. MATSUI, C. F. BREWER, AND E. J. HEHRE, *Biochemistry*, 27 (1988) 2294–2300.
9 E.-F. FUCHS AND J. LEHMANN, *Chem. Ber.*, 108 (1975) 2254–2260.
10 R. W. MYERS AND Y. C. LEE, *Carbohydr. Res.*, 152 (1986) 143–158.
11 T. A. HASE AND K. SALONEN, *Synth. Commun.*, 10 (1980) 221–224.
12 J. S. BRIMACOMBE, *Methods Carbohydr. Chem.*, 6 (1972) 366–378.
13 W. C. STILL, M. KAHN, AND A. MITRA, *J. Org. Chem.*, 43 (1978) 2923–2925.
14 YU. A. ZHDANOV, G.A. KOROLCHENKO, L. A. KUBASSKAYA, AND R. M. KRIVORUCHKO, *Dokl. Akad. Nauk SSSR*, 129 (1959) 1049; M. L. SHULMAN, S.D. SHIYAN, AND A.YA. KHORLIN, *Carbohydr. Res.*, 33 (1974) 229–235.