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Synthesis of 4,8-anhydro-2,3-dideoxy-D-*galacto*- and -D-*gluco*-non-3-enose dimethyl acetal and their use as new probes for determining by ^{1}H-n.m.r. spectroscopy the steric course of protonation by glycoside hydrolases

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ABSTRACT

The title nonosulose derivatives **2** (D-*galacto*) and **4** (D-*gluco*) were prepared by multistep syntheses. Addition of water to the enolic double bonds of both compounds was catalyzed only by the corresponding enzymes β-D-galactosidase from *Escherichia coli*, α-D-galactosidase from green coffee beans, and β-D-glucosidase from sweet almonds, α-D-glucosidase from yeast. The enzymic hydration of **2**, performed in D_2O to analyze the steric course of the addition, gave 2,3-dideoxy-α-D-*galacto*-(3-^{2}H)-nonos-4-ulose dimethyl acetal (**5**), which when hydrolyzed gave an equilibrium mixture of the spiranes **16** and **17** as the main products (85%) and the fused-ring systems **18** and **19** as minor components (15%). Borohydride reduction of the product of enzymic hydration gave a separable mixture of the two epimers **14** and **15**, convertible in acidic methanol for 8 h at 62° into **20** and **22**, respectively. The rigid, bicyclic ring-systems allow facile assignment of the configuration at the monodeuterated C-3 as (*S*), thereby allowing determination of the steric course of the initial, enzyme-catalyzed step, the deuteration of the enolic double bond in the substrates used.

INTRODUCTION

The steric course of glycosylase action has thus far been elucidated by enzymically adding water to the probes (*Z*)-3,7-anhydro-1,2-dideoxy-D-*galacto*-oct-2-enitol[1,2] and (*Z*)-3,7-anhydro-1,2-dideoxy-D-*gluco*-oct-2-enitol[3,4], determining the initial anomeric configuration in the products [1,2-dideoxy-D-*galacto*- and -D-*gluco*-3-(2-^{2}H)octulopyranose] and the chirality at the newly formed asymmetric carbon atom 2 after oxidative degradation to (2-^{2}H)propanoate, and o.r.d. measurements thereof. Mainly because of low yields of (2-^{2}H)propanoic acid and the low sensitivity of o.r.d. measurements, relatively large amounts (> 300 mg) of the enolic substrate are needed. Enolic sugar derivatives of a new kind, such as (*Z*)-4,8-anhydro-2,3-dideoxy-D-*galacto*- (**2**) and -D-*gluco*-non-3-enose dimethyl acetal (**4**) should, after being converted into 2,3-dideoxy-α-D-*galacto*-(3-^{2}H)nonos-4-ulose dimethyl acetal (**5**) and 2,3-dideoxy-D-*gluco*-(3-^{2}H)nonos-4-ulose dimethyl acetal respectively by enzymic deuteriohydration and subsequent mild acidic hydrolysis to 2,3-dideoxy-D-*galacto*- and -D-*gluco*-(3-^{2}H)nonos-4-

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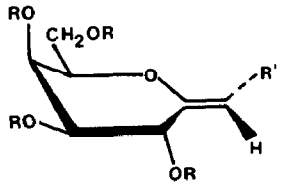
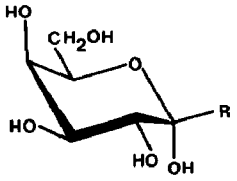
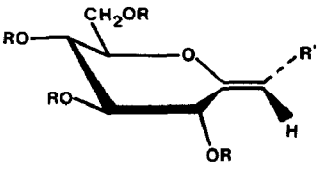


	R'	R
1	$CH_2—CH(OMe)_2$	Ac
2	$CH_2—CH(OMe)_2$	H

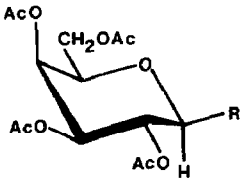


R

5 $CHD—CH_2—CH(OMe)_2$



	R'	R
3	$CH_2—CH(OMe)_2$	Ac
4	$CH_2—CH(OMe)_2$	H



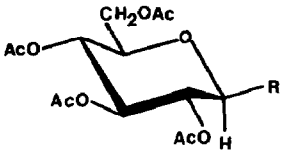
R

6 CHO

7 $CH{=}CH—CHO$

8 $\overset{Br}{\underset{|}{CH}}—CH_2—CH(OMe)_2$ *(R)

9 $\overset{Br}{\underset{|}{CH}}—CH_2—CH(OMe)_2$ *(S)



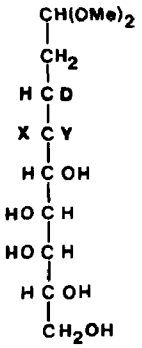
R

10 CHO

11 $CH{=}CH—CHO$

12 $\overset{Br}{\underset{|}{CH}}—CH_2—CH(OMe)_2$ *(R)

13 $\overset{Br}{\underset{|}{CH}}—CH_2—CH(OMe)_2$ *(S)



	X	Y
14	OH	H
15	H	OH

ulose, allow, after the system has spontaneously formed a bicycle, configurational assignment at C-3 by ^{1}H-n.m.r. The assumption was made that two types of structure, either A or B, or both, may be thermodynamically stable. Structure A (**18, 19**) having two six-membered fused rings (*trans*-decalin type) and the two anomeric hydroxy groups axially oriented because of anomeric effects should be conformationally favored. On the other hand, the spiro-system B (**16, 17**) might derive thermodynamical stability from a favorable stereoelectronic factor (Fig. 1). Only from the rigid structure A can the stereochemistry at the deuterated C-3 be unequivocally determined.

RESULTS AND DISCUSSION

Chemical syntheses. — 3,4,5,7-Tetra-*O*-acetyl-2,6-anhydro-D-*glycero*-L-*manno*-heptose[5] (**6**) and 3,4,5,7-tetra-*O*-acetyl-2,6-anhydro-D-*glycero*-D-*gulo*-heptose[6,13] (**10**) were treated with formylmethylenetriphenylphosphorane[7] in benzene to yield mainly 5,6,7,9-tetra-*O*-acetyl-4,8-anhydro-2,3-dideoxy-β-D-*galacto*-non-2-enose (**7**) and -β-D-*gluco*-non-2-enose (**11**). Addition of HBr to the Michael system gave in each case a pair of diastereomeric 3-bromides **8**, **9**, and **12**, **13**. Acetalation[8] of the main products **8** and **12** was carried out in MeOH containing anhydrous $CuSO_4$ and H_2SO_4. Elimination of HBr with AgF in pyridine[9] occurred smoothly. (*Z*)-5,6,7,9-Tetra-*O*-acetyl-4,8-anhydro-2,3-dideoxy-D-*galacto*-non-3-enose dimethyl acetal (**1**) and (*Z*)-5,6,7,9-tetra-*O*-acetyl-4,8-anhydro-2,3-dideoxy-D-*gluco*-non-3-enose dimethyl acetal (**3**) were isolated from the mixture by adding ether, washing the ether layer with water, evaporation and silica gel chromatography. Compounds **1** and **3** were deacetylated to yield (*Z*)-4,8-

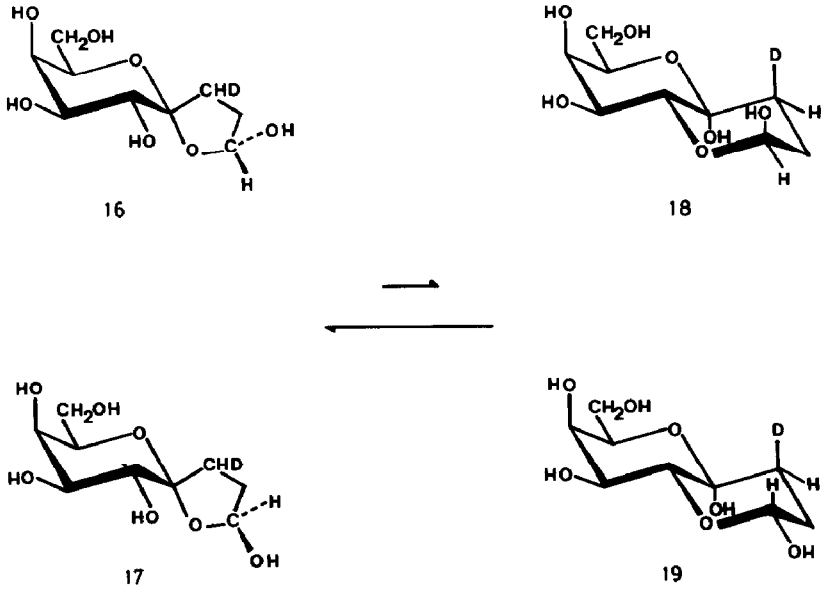


Fig. 1. Equilibrium mixture between the spirans **16** and **17** and *trans*-decalin structures **18** and **19** after hydrolysis of the acetal grouping of **5**.

anhydro-2,3-dideoxy-D-*galacto*- (**2**) and -D-*gluco*-non-3-enose dimethyl acetal (**4**). Compound **2** was purified by crystallization and **4** by flash column chromatography. The structures of **2** and **4** follow from elemental analysis and n.m.r., and the (*Z*) configuration follows from a substantial n.O.e. between the H-3 and H-5 signals.

Enzymic deuteriohydration of (Z)-4,8-anhydro-2,3-dideoxy-D-galacto-*non-3-enose dimethyl acetal* (**2**) *and determination of its steric course.* — The D-*gluco*-configured compound **4** incubated with β-D-glucosidase from sweet almonds or α-D-glucosidase from yeast and the -D-*galacto*-configurated compound **2** incubated with β-D-galactosidase from *E. coli* or α-D-galactosidase from green coffee beans gave in each case, with differing incubation times, the corresponding hydration products, as monitored by t.l.c. In no case could hydration be observed without enzyme.

In place of the other three enzymes, α-D-galactosidase from green coffee beans was selected to prove the versatility of the new system for elucidating the steric course of enzyme-catalyzed hydration and thereby shedding some light on the distribution of proton-donating groups in active sites. The steric course of hydration by α-D-galactosidase from green coffee beans has been proved[10,12] by the aforementioned method to be *cis* from the *si*-face of the enolic carbon 2 in (*Z*)-3,7-anhydro-1,2-dideoxy-D-*galacto*-oct-2-enitol.

After the unsaturated compound **2** has been completely converted into the only product, 2,3-dideoxy-α-D-*galacto*-(3-^{2}H)nonos-4-ulose dimethyl acetal* (**5**), by enzymic deuteriohydration in buffered D_2O, the latter compound was isolated. Incorporation of deuterium, as shown by ^{1}H-n.m.r., was >95%†. To the sample in D_2O was added the same volume of M deuteriotrifluoroacetic acid‡ to make up a solution of ~0.5M deuteriotrifluoroacetic acid. The acetal grouping of **5** was hydroylzed at 25° in < 10 min. This was the time needed for the first ^{1}H-n.m.r. reading. Also this time the mutarotation equilibrium was reached (Fig. 1). In contrast to our expectations that a six-membered hemiacetal would be thermodynamically more stable than a five-membered one[11] the region of anomeric protons clearly indicates predominant formation of the spiro-structures **16** and **17** along with a little of the *trans*-decalin structures **18** and **19**. Apparently the spiro-structure with a closed acetal system is favored because of stereoelectronic stabilization.

The very complex spectrum of the equilibrium mixture was useless for the determination of the stereochemistry at C-3 in both the spiro nor the *trans*-decalin components, in the latter also because of conformational instability. Acetylation of hydrolyzed and freeze-dried **5** gave exclusively the peracetylated spiro structures.

In order to avoid the formation of unwanted five-membered rings carrying the

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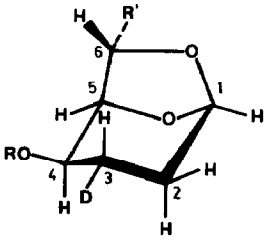
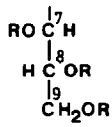
* We assume that the α anomer is initially formed by the retaining α-D-galactosidase, although the rapid mutarotation of an initially formed β anomer cannot be experimentally excluded.

† To show that no exchange of deuterium at C-3 occurred during the incubation time, the same reaction was carried out in buffered T_2O. The 2,3-dideoxy-α-D-*galacto*-[3-^{3}H]nonos-4-ulose dimethyl acetal was isolated and dissolved in water (pH 5). After 3 d, no exchange of tritium could be detected.

‡ CF_3CO_2D was prepared by letting trifluoroacetic anhydride (0.5 mmol) react portionwise with ice-cold D_2O (1 mL).

asymmetric C-3, which needs to be stereochemically assigned, the product of enzymic deuteriohydration (**5**) was reduced with sodium borohydride. The resulting epimers **14** and **15** could be separated by h.p.l.c. One of them (**15**) even crystallised from the mixture. When compound **14** was treated with CF_3CO_2H in deuteriomethanol and observed by ^{1}H-n.m.r. spectrometry, the methoxy signals of the former acetal group disappear rapidly, even at room temperature. According to the anomeric-proton region in the ^{1}H-n.m.r. spectrum, the furanosides are primarily formed. After warming up the mixture to 60°, the pyranosides are also formed (Fig. 2). All along a fifth compound gradually appeared which, after keeping the solution for 8 h at 62° remained the only product at significant concentration. This compound was isolated and shown to be 1,6-anhydro-2,3-dideoxy-3(*S*)-D-*glycero*-L-*gluco*-(3-^{2}H)nonopyranose (**20**). The epimer of **20** was formed by treating **15** under the same aforementioned conditions.



	R	R'
20	H	
21	Ac	

The structures of these compounds were deduced from extensive ^{1}H- and ^{13}C-n.m.r. investigations, also on their tetra-*O*-acetyl derivatives **21** and **23**. Relevant data are given in Tables I–III. The assignments given were verified by selective homo- and hetero-nuclear decoupling experiments. The positions of the hydroxyl groups at carbon atoms C-4, C-7, C-8, and C-9 follow from the C-H chemical-shift changes upon acetylation. The existence of a five-membered ring in **20** is also implied by the $^1J_{(^{13}C,^1H)}$ coupling constants for C-5 (156 Hz) and C-6 (151 Hz), which are appreciably larger than the coupling constants for C-4 (144 Hz), C-7 (142 Hz), and C-8 (142 Hz). The ster-

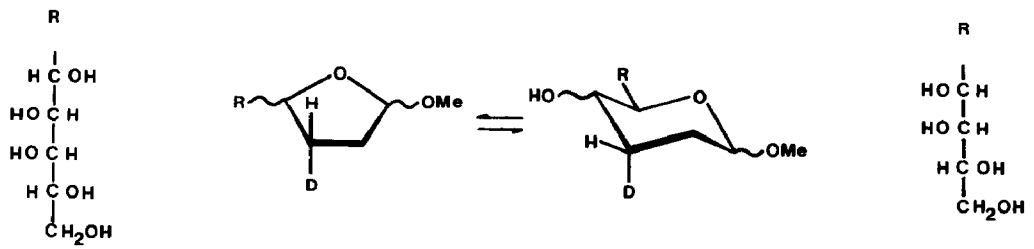


Fig. 2. Equilibrium mixture after methanolysis of the acetal grouping of **14** or **15**.

TABLE I

^{1}H-N.m.r. data (400.13 MHz) for compounds **20** and **22** in CD_3OD and **21** and **23** in $CDCl_3$[a]

Compound	*Proton*											
	H-1	*H-2*a	*H-2*e	*H-3*	*H-4*	*H-5*	*H-6*	*H-7*	*H-8*	*H-9*	*H-9'*	*OAc*
20	5.40 s	[b]	[b]	[b]	3.85 dd	4.32 d	4.25 d	3.33 dd	3.84 t	3.60 d	3.60 d	
22	5.45 s	1.89 m	1.46 m	2.00 m	3.66 dd	4.39 d	3.98 d	3.32 t	3.84 t	3.60 d	3.60 d	
21	5.54 s	[c]	[c]	[c]	4.90 dd	4.28 d	4.21 d	5.07 dd	5.42 ddd	4.00 dd	4.27 dd	2.04 s 2.06 s 2.08 s 2.14 s
23	5.57 s	1.84 t	1.58 m	1.95 m	4.67 dd	4.31 d	4.02 d	5.06 dd	5.44 ddd	4.00 dd	4.25 dd	2.04 s 2.11 s 2.11 s 2.16 s

[a] ^{1}H-N.m.r. data: δ values in p.p.m. ($\pm$0.01), internal standard CHD_2OD (δ 3.3) or $CHCl_3$ (δ 7.26), concentration ~5 mg/mL solvent, measuring temperature 23°.
[b] Multiplet from 1.58–1.7 p.p.m. (3 H). [c] Multiplet from 1.6–1.8 p.p.m. (3 H).

TABLE II

Proton–proton coupling constants for compounds **20–23**

Compound	*Coupling constants*[a]												
	$J_{1,2a}$	$J_{1,2e}$	$J_{2a,2e}$	$J_{2a,3}$	$J_{2e,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{6,7}$	$J_{7,8}$	$J_{8,9}$	$J_{8,9'}$	$J_{9,9'}$
20	1	1	[b]	[b]	*b*	10	4	0	9	1.5	6.5	6.5	[c]
22	1.5	2	13	12	5.5	[b]	2.5	0	9	1.5	6.5	6.5	[c]
21	1	1	[b]	[b]	[b]	10	4	0	7.5	3	5.5	6.5	11.5
23	1.5	2	13	13	5.5	4.5	2.5	0	7.5	2.5	5.5	7.0	11.5

[a] First order *J*-values in Hz (±0.1) at 400.13 MHz, measured from line splittings in the ^{1}H spectra, measuring conditions see [a] in Table I. [b] Coupling constants were not determined because of spectral crowding. [c] δ (H-9) = δ (H-9').

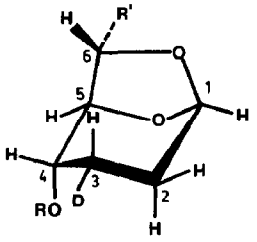
TABLE III

^{13}C-N.m.r. data (106.61 MHz) for compounds **20** and **22** in CD_3OD and for compounds **21** and **23** in $CDCl_3$[a]

Compound	*Carbon atom*										
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	*C-7*	*C-8*	*C-9*	*C–O*	*Ac*
20	102.58	31.72	[b]	66.89	79.13	75.58	71.60	71.30	64.60		
22	103.68	28.23	[b]	67.56	80.40	77.59	71.74	71.21	64.55		
21	101.95	30.02	[b]	67.68	74.69	73.79	70.53	69.06	62.00	169.74	20.58
										169.84	20.67
										170.48	20.84
											20.99
23	102.63	26.94	[b]	68.11	76.68	75.95	70.63	69.12	61.95	169.92	20.67
										170.02	20.70
										170.45	20.78
										170.66	21.21

[a] See footnote [a] of Table I. [b] Broad signal because of unresolved ^{13}C,D coupling. For the protio isotopomers of **20**: (C-3) 26.28 p.p.m., of **21**: (C-3) 21.95 p.p.m.

eochemistry at C-6 is further substantiated by a large n.O.e. between H-6 and H-3. For **20** and **21**, the equatorial disposition of the deuterium atom directly follows from the large $^3J_{(H\text{-}3,\ H\text{-}4)}$ values of 10 Hz, typical for coupling between axial protons. Further proof is given by the ^{1}H-n.m.r. spectra of the protio isotopomers of **20** and **21**, in which an additional signal at 2.00 and 1.83 p.p.m. respectively corresponds to the equatorial proton at C–3, evidenced by ^3J couplings of 6 and 4.5 Hz respectively, with the proton at H-4 and by a ^4J "W coupling" of ~1 Hz with H (C-5).



	R	R'
22	H	RO C H (7) / H C OR (8) / CH_2OR (9)
23	Ac	

For **22** and **23** the $^3J_{(H\text{-}3,\ H\text{-}4)}$ values are small (see Table II), however a large coupling exists between one of the protons at C-2 and the C-3 proton, demonstrating that this proton must be axially disposed. These results correspond with previous findings using α-D-galactosidase and (*Z*)-3,7-anhydro-1,2-dideoxy-D-*galacto*-oct-2-enitol[10,12].

CONCLUSION

Converting (*Z*)-4,8-anhydro-2,3-dideoxy-D-*galacto*- (**2**) and -D-*gluco*-non-3-enose dimethyl acetal (**4**) into their hydration products using the corresponding glycoside hydrolases allows the steric course of enzyme-catalyzed protonation to be determined with small amounts of material. The hemiacetal group, formed by enzymic deuteriohydration, is reduced and either of the epimeric nonose dimethyl acetals can be converted into the conformationally rigid bicyclic system of 1,6-anhydro-2,3-dideoxy-(3-^{2}H)nonopyranose. The configuration at the newly formed asymmetric C-3, is determined by ^{1}H-n.m.r. spectroscopy. Provided that a glycoside hydrolase hydrates the enolic substrates **2** or **4**, the nature of the product allows systematizing the enzyme as to the mode of proton transfer and nucleophile acceptance.

EXPERIMENTAL

General methods. — All reactions were monitored by t.l.c. on silica gel 60 F_{254} (Merck). Column chromatography was carried out with Silica 32-63, 60 A (ICN). Preparative h.p.l.c. separations involved Knauer components and a Hypersil column (250 × 20 mm, 5 μm, Bischoff). Melting points were recorded with a Büchi (nach Dr. Tottoli) apparatus and optical rotations with a Perkin–Elmer 241 polarimeter. ^{1}H-N.m.r. spectra were recorded with a Bruker WM 250 instrument at 250.13 MHz and a Bruker AM 400 at 400.13 MHz for solutions in $CDCl_3$ (internal Me_4Si or $CHCl_3$), CD_3OD (internal Me_4Si or CHD_2OD) or D_2O (internal sodium 4,4-dimethyl-4-silapentanesulfonate, DSS). ^{13}C-N.m.r. spectra were recorded with a Bruker AM 400 instrument at 100.614 MHz for solutions in $CDCl_3$ (internal $CDCl_3$), CD_3OD (internal CD_3OD) or D_2O (internal DSS). All enzymes were purchased from Boehringer Mannheim.

*(E)-5,6,7,9-Tetra-*O*-acetyl-4,8-anhydro-2,3-dideoxy-β-*D-galacto-*non-2-enose* (**7**). — To a solution of 2,3,4,6-tetra-*O*-acetyl-2,6-anhydro-D-*glycero*-L-*manno*-heptose[5] (**6**, 5.4 g, 14.8 mmol) in benzene (100 mL) was added formylmethylenetriphenylphosphorane (6.3 g, 20.2 mmol) and the suspension stirred at room temperature (12 h). The mixture was concentrated *in vacuo* and the residue purified by flash column chromatography (5:1 EtOAc–cyclohexane) to yield **7** (4.3 g, 75%) as colorless crystals, m.p. 115°, $[\alpha]_D^{23}$ −27.3° (*c* 0.21, $CHCl_3$); R_F 0.42 (2:1 EtOAc–cyclohexane); ^{1}H-n.m.r. (250.13 MHz, $CDCl_3$): δ 9.57 (d, 1 H, $J_{1,2}$ 7.6 Hz, H-1), 6.33 (ddd, 1 H, $J_{2,3}$ 15.7, $J_{2,4}$ 1.25 Hz, H-2), 6.66 (dd, 1 H, $J_{3,4}$ 5.7 Hz, H-3), 4.17 (ddd, 1 H, $J_{4,5}$ 7.35 Hz, H-4), 5.19 (dd, 1 H, $J_{5,6}$ 9.75 Hz, H-5), 5.11 (dd, 1 H, $J_{6,7}$ 3.15 Hz, H-6), 5.47 (dd, 1 H, $J_{7,8}$ 1.3 Hz, H-7), 4.0 (ddd, 1 H, $J_{8,9}$ 5.7, $J_{8,9'}$ 6.75 Hz, H-8), 4.1 (m, 1 H, H-9), 4.16 (m, 1 H, H-9′), 2.0, 2.06, 2.07, and 2.17 (all s, 12 H, OAc).

Anal. Calc. for $C_{17}H_{22}O_{10}$: C, 52.85; H, 5.74. Found: C, 52.66; H, 5.77.

*(E)-5,6,7,9-Tetra-*O*-acetyl-4,8-anhydro-2,3-dideoxy-β-*D-gluco-*non-2-enose* (**11**). — 2,3,4,6-Tetra-*O*-acetyl-2,6-anhydro-D-*glycero*-D-*gulo*-heptose[6,13] (**10**, 5.3 g, 14.7 mmol) was treated as described for compound **6**. The product **11** (4.75 g, 83%) was obtained as colorless crystals, m.p. 107°, $[\alpha]_D^{23}$ −38.7° (*c* 0.3, $CHCl_3$); R_F 0.41 (2:1 EtOAc–cyclohexane); ^{1}H-n.m.r. (250 MHz, $CDCl_3$): δ 9.55 (d, 1 H, $J_{1,2}$ 7.8 Hz, H-1), 6.32 (ddd, 1 H, $J_{2,3}$ 16, $J_{2,4}$ 1.2 Hz, H-2), 6.64 (dd, 1 H, $J_{3,4}$ 7.5 Hz, H-3), 4.19 (ddd, 1 H, $J_{4,5}$ 9.7 Hz, H-4), 5.11 (t, 1 H, $J_{5,6}$ 9.3 Hz, H-5), 5.28 (t, 1 H, $J_{6,7}$ 9.3 Hz, H-6), 4.98 (t, 1 H, $J_{7,8}$ 9.7 Hz, H-7), 3.77 (ddd, 1 H, $J_{8,9}$ 2.25, $J_{8,9'}$ 4.8 Hz, H-8), 4.15 (dd, 1 H, $J_{9,9'}$ 12.3 Hz, H-9), 4.26 (dd, 1 H, H-9′), 2.01, 2.03, 2.04, and 2.09 (all s, 12 H, OAc).

Anal. Calc. for $C_{17}H_{22}O_{10}$: C, 52.85; H, 5.74. Found: C, 52.66; H, 5.77.

*5,6,7,9-Tetra-*O*-acetyl-4,8-anhydro-3(*R*)-bromo-2,3-dideoxy-β-*D-galacto-*nonosedimethyl acetal* (**8**) and *5,6,7,9-Tetra-*O*-acetyl-4,8-anhydro-3(*S*)-bromo-2,3-dideoxy-β-*D-galacto-*nonose dimethyl acetal* (**9**). — To a solution of **7** (1 g, 2.6 mmol) in Ac_2O (3.5 mL) was added a solution of HBr in AcOH (33%, 11 mL) with stirring, the mixture being cooled to 0° (18 h). CH_2Cl_2 (50 mL) was added to the mixture and the solution stirred with ice-cold water to decompose Ac_2O (1.5 h). The organic layer was extracted

with satd. aqueous $NaHCO_3$ (50 mL) and then with water (3 × 30 mL). The extract was dried ($MgSO_4$) and concentrated *in vacuo* to yield a yellow syrup. To a solution of this syrup in MeOH (35 mL) was added anhydrous $CuSO_4$ (0.7 g) and 18M H_2SO_4 (0.2 mL) with stirring (14 h). The mixture was filtered off and the filtrate made neutral with pyridine. The filtrate was concentrated *in vacuo*. Flash column chromatography (1:2 EtOAc–cyclohexane) yielded **8** (540 mg, 42%) as colorless crystals; m.p. 45°; R_F 0.42 (2:1 EtOAc–cyclohexane)*; ^{1}H-n.m.r. (250.13 MHz, $CDCl_3$): δ 4.59 (dd, 1 H, $J_{1,2}$ 7.65, $J_{1,2'}$ 3.15 Hz, H-1), 2.11 (ddd, 1 H, $J_{2,2'}$ 14.7, $J_{2,3}$ 4.3 Hz, H-2), 2.34 (ddd, 1 H, $J_{2',3}$ 10.2 Hz, H-2'), 4.09 (ddd, 1 H, $J_{3,4}$ 1.5 Hz, H-3), 3.45 (dd, 1 H, $J_{4,5}$ 9.45 Hz, H-4), 5.49 (t, 1 H, $J_{5,6}$ 9.75 Hz, H-5), 5.07 (dd, 1 H, $J_{6,7}$ 3.15 Hz, H-6), 5.42 (dd, 1 H, $J_{7,8}$ 1.2 Hz, H-7), 3.94 (ddd, 1 H, $J_{8,9}$ 6.45, $J_{8,9'}$ 7.2 Hz, H-8), 4.11 (dd, 1 H, $J_{9,9'}$ 12.3 Hz, H-9), 4.17 (dd, 1 H, H-9'), 1.98, 2.042, 2.065, and 2.18 (all s, 12 H, OAc), 3.37 and 3.4 (s, 6 H, OMe).

Anal. Calc. for $C_{19}H_{29}BrO_{11}$: C, 44.46; H, 5.69; Br, 15.56. Found: C, 44.17; H, 5.63; Br, 15.26.

Compound **9** (80 mg, 6%) was not characterized. Both compounds were very unstable.

*5,6,7,9-Tetra-*O*-acetyl-4,8-anhydro-3(*R*)-bromo-2,3-dideoxy-β-*D*-gluco-nonose dimethyl acetal* (**12**) and *5,6,7,9-Tetra-*O*-acetyl-4,8-anhydro-3(*S*)-bromo-2,3-dideoxy-β-*D*-gluco-nonose dimethyl acetal* (**13**). — Compound **11** was treated as described for compound **7**. The product **12** (520 mg, 41%) was obtained as colorless crystals, m.p. 72–73°; R_F 0.42 (2:1 EtOAc–cyclohexane)**; ^{1}H-n.m.r. (250.13 MHz, $CDCl_3$): δ 4.57 (dd, 1 H, $J_{1,2}$ 7.65, $J_{1,2'}$ 3.15 Hz, H-1), 2.13 (ddd, 1 H, $J_{2,2'}$ 14, $J_{2,3}$ 4.2 Hz, H-2), 2.34 (ddd, 1 H, $J_{2',3}$ 9.8 Hz, H-2'), 4.09 (ddd, 1 H, $J_{3,4}$ 1.1 Hz, H-3), 3.48 (dd, 1 H, $J_{4,5}$ 9.1 Hz, H-4), 5.21 (t, 1 H, $J_{5,6}$ 9.3 Hz, H-5), 5.29 (t, 1 H, $J_{6,7}$ 9.3 Hz, H-6), 5.11 (t, 1 H, $J_{7,8}$ 9.75 Hz, H-7), 3.71 (m, 1 H, $J_{8,9}$ 3.8, $J_{8,9'}$ 3.8 Hz, H-8), 4.19 (d, 1 H, $J_{9,9'}$ 11 Hz, H-9), 4.19 (d, 1 H, H-9'), 2.007, 2.035, 2.056, and 2.09 (all s, 12 H, OAc), 3.38 and 3.4 (s, 6 H, OMe).

Anal. Calc. for $C_{19}H_{29}BrO_{11}$: C, 44.46; H, 5.69; Br, 15.56. Found: C, 44.73; H, 5.56; Br, 14.83.

Compound **13** (89 mg, 6.5%) was not characterized. Both compounds were very unstable.

*(Z)-5,6,7,9-Tetra-*O*-acetyl-4,8-anhydro-2,3-dideoxy-*D*-galacto-non-3-enose dimethyl acetal* (**1**). — To a solution of **8** (540 mg, 1.05 mmol) in pyridine (15 mL) was added AgF (660 mg) with stirring in the dark (1 h). Ether (50 mL) was added and the mixture stirred for another 10 min. The silver salts were filtered off and the solution washed with 1% aq. $Na_2S_2O_3$ (2 × 50 mL) then with water (2 × 50 mL). The organic layer was dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by flash column chromatography (1:2 EtOAc–cyclohexane) to yield **1** (363 mg, 80%) as colorless crystals, m.p. 93°, $[\alpha]_D^{23}$ −21° (*c* 0.50, $CHCl_3$); R_F 0.41 (2:1 EtOAc–cyclohexane); ^{1}H-n.m.r. (250.13 MHz, $CDCl_3$): δ 4.34 (t, 1 H, $J_{1,2}$ 5.7, $J_{1,2'}$ 6 Hz, H-1), 2.38 (m, 1 H, $J_{2,2'}$

* Optical rotations were therefore not measured. Because of their instability, compounds **8** and **12** were submitted directly to further reactions, except for a small amount used for n.m.r. investigations.

** See previous footnote.

14.7, $J_{2,3}$ 7, $J_{2,5}$ 1.7 Hz, H-2), 2.51 (m, 1 H, $J_{2',3}$ 9.3, $J_{2',5}$ 2.7 Hz, H-2'), 4.92 (ddd, 1 H, $J_{3,5}$ 1.9 Hz, H-3), 5.65 (ddd, 1 H, $J_{5,6}$ 10.2 Hz, H-5), 5.04 (dd, 1 H, $J_{6,7}$ 3.3 Hz, H-6), 5.50 (dd, 1 H, $J_{7,8}$ 1.5 Hz, H-7), 3.99 (ddd, 1 H, $J_{8,9}$ 5.7, $J_{8,9'}$ 7.35 Hz, H-8), 4.16 (dd, 1 H, H-9), 4.25 (dd, 1 H, H-9'), 2.011, 2.074, 2.14, and 2.17 (all s, 12 H, OAc), and 3.33 (s, 6 H, OMe).

Anal. Calc. for $C_{19}H_{28}O_{11}$: C, 52.77; H, 6.52. Found: C, 52.46; H, 6.57.

*(Z)-5,6,7,9-Tetra-*O*-acetyl-4,8-anhydro-2,3-dideoxy-*D-gluco*-non-3-enose dimethyl acetal* (**3**). — Compound **12** (520 mg, 1 mmol) was treated in the same way as compound **8**. Compound **3** (350 mg, 79%) was obtained as a colorless syrup, $[\alpha]_D^{23}$ +31.2° (*c* 0.25, $CHCl_3$); R_F 0.42 (2:1 EtOAc–cyclohexane); ^{1}H-n.m.r. (250.13 MHz, $CDCl_3$): δ 4.35 (t, 1 H, $J_{1,2}$ 5.7, $J_{1,2'}$ 5.7 Hz, H-1), 2.38 (m, 1 H, $J_{2,2'}$ 14.7, $J_{2,3}$ 7.2, $J_{2,5}$ 1.5 Hz, H-2), 2.53 (m, 1 H, $J_{2',3}$ 7.2, $J_{2',5}$ 1.2 Hz, H-2'), 4.95 (ddd, 1 H, $J_{3,5}$ 1.5 Hz, H-3), 5.43 (ddd, 1 H, $J_{5,6}$ 8.2 Hz, H-5), 5.13 (t, 1 H, $J_{6,7}$ 8.7 Hz, H-6), 5.20 (dd, 1 H, $J_{7,8}$ 9.3 Hz, H-7), 3.81 (ddd, 1 H, $J_{8,9}$ 2.5, $J_{8,9'}$ 4.8 Hz, H-8), 4.20 (dd, 1 H, $J_{9,9'}$ 11.4 Hz, H-9), 4.30 (dd, 1 H, H-9'), 2.035, 2.043, 2.11, and 2.12 (all s, 12 H, OAc), and 3.34 (s, 6 H, OMe).

Anal. Calc. for $C_{19}H_{28}O_{11}$: C, 52.77; H, 6.53. Found: C, 53.19; H, 6.67.

*(Z)-4,8-Anhydro-2,3-dideoxy-*D-galacto*-non-3-enose dimethyl acetal* (**2**). — Compound **1** (363 mg, 0.84 mmol) was deacetylated (Zemplén), and then the product purified by crystallization to yield **2** (200 mg, 90%) as colorless crystals, m.p. 121–123°, $[\alpha]_D^{21}$ +123° (*c* 0.98, H_2O); R_F 0.46 (7:2:1 EtOAc–MeOH–H_2O); ^{1}H-n.m.r. (400.13 MHz, D_2O): δ 4.52 (t, 1 H, $J_{1,2}$ 5.5, $J_{1,2'}$ 5.5 Hz, H-1), 2.4–2.59 (m, 1 H, $J_{2,2'}$ 14, $J_{2,3}$ 7, $J_{2,5}$ 1.1 Hz, H-2), 2.4–2.59 (m, 1 H, $J_{2',3}$ 7, $J_{2',5}$ 1.6 Hz, H-2'), 5.10 (dt, 1 H, $J_{3,5}$ 1 Hz, H-3), 4.19 (dd, 1 H, $J_{5,6}$ 10 Hz, H-5), 3.59 (dd, 1 H, $J_{6,7}$ 3 Hz, H-6), 4.02 (d, 1 H, H-7), 3.66 (dd, 1 H, $J_{8,9}$ 8, $J_{8,9'}$ 4.1 Hz, H-8), 3.75–3.86 (m, 1 H, $J_{9,9'}$ 12 Hz, H-9), 3.75–3.86 (m, 1 H, H-9'), and 3.37 (s, 6 H, 2 OMe); ^{13}C-n.m.r. (100.61 MHz, D_2O): δ 107.23 (C-1), 30.44 (C-2), 105.60 (C-3), 156.24 (C-4), 70.99 (C-5), 76.65 (C-6), 71.89 (C-7), 83.07 (C-8), and 64.12 (C-9).

Anal. Calc. for $C_{11}H_{20}O_7$: C, 49.98; H, 7.58. Found: C, 49.77; H, 7.58.

*(Z)-4,8-Anhydro-2,3-dideoxy-*D-gluco*-non-3-enose dimethyl acetal* (**4**). — Compound **3** (350 mg, 0.83 mmol) was deacetylated (Zemplén), and then the product purified by flash column chromatography (27:2:1 EtOAc–MeOH–H_2O) to yield **4** (191 mg, 89%) as a colorless syrup, R_F 0.46 (7:2:1 EtOAc–MeOH–H_2O); ^{1}H-n.m.r. (250.13 MHz, D_2O): δ 4.52 (t, 1 H, $J_{1,2}$ 6.3, $J_{1,2'}$ 7 Hz, H-1), 2.44 (m, 1 H, $J_{2,2'}$ 14, $J_{2,3}$ 7 Hz, H-2), 2.54 (m, 1 H, $J_{2',3}$ 8.7 Hz, H-2'), 5.11 (t, 1 H, $J_{3,5}$ 1.9 Hz, H-3), 3.92 (m, 1 H, H-5), 3.56 (d, 1 H, H-6), 3.34 (m, 1 H, H-7), 3.41 (d, 1 H, H-8), 3.78 (dd, 1 H, H-9), 3.92 (m, 1 H, H-9'), 3.37 and 3.39 (s, 6 H, OMe).

Anal. Calc. for $C_{11}H_{20}O_7$: C, 49.98; H, 7.58. Found: C, 49.32; H, 7.86. (The compound could not be freed from last traces of water).

*2,3-Dideoxy-3(*S*)-α-*D-galacto-*(3-*^{2}*H)nonos-4-ulose dimethyl acetal* (**5**). A solution of **2** (100 mg, 0.37 mmol) in Na/K-phosphate–D_2O buffer (15 mL, pH 6.8, 50 mmol) was incubated with 100 U α-D-galactosidase from green coffee beans (5 × dialyzed with Na/K-phosphate–D_2O buffer, pH 6.8, 50 mmol) at 37°. When the reaction was complete (18 h) the solution was freeze-dried. The residue was taken up in MeOH and the buffer salts were removed by passing the turbid mixture through a short silica gel column with MeOH as solvent. The solvent was evaporated under diminished pressure to yield **5** (95

mg, 94%) as a colorless syrup, $[\alpha]_D^{21}$ +19° (*c* 0.91, H_2O); R_F 0.40 (7:2:1 EtOAc–MeOH–H_2O); ^{1}H-n.m.r. (400.13 MHz, D_2O): δ 4.55 (t, 1 H, $J_{1,2}$ 5.4 Hz, H-1), 1.75–1.85 (m, 3 H, H-2, H-2', H-3), 3.60 (d, 1 H, $J_{5,6}$ 10 Hz, H-5), 3.82 (dd, 1 H, $J_{6,7}$ 3.6 Hz, H-6), 3.96 (d, 1 H, H-7), 4.00 (t, 1 H, $J_{8,9}$ 6.6 Hz, H-8), 3.70 (d, 1 H, H-9), 3.70 (d, 1 H, H-9'), and 3.38 (s, 6 H, OMe); ^{13}C-n.m.r. (100.61, D_2O): δ 101.00 (C-1), 73.14 (C-2), 73.04 (C-3), 71.96 (C-4), 73.92 (C-5), 63.86 (C-6), 34.62 (C-7), 28.34 (C-8), 107.71 (C-9), 56.07, and 56.11 (OMe).

*2,3-Dideoxy-3(*S*)-*D*-glycero-*L*-gluco-(3-^{2}H)nonose dimethyl acetal* (**14**) and *2,3-Dideoxy-3(*S*)-*D*-glycero-*L*-manno-(3-^{2}H)nonose dimethyl acetal* (**15**). — To a solution of **5** (95 mg, 0.33 mmol) in water (4 mL, pH 8) was added $NaBH_4$ with stirring (3 h). The mixture was submitted to h.p.l.c. (97:3 H_2O–MeCN) and the two epimers formed separated directly to yield **14** as a colorless syrup (41.8 mg, 44%), $[\alpha]_D^{21}$ +6° (*c* 0.91, H_2O); R_F 0.39 (7:2:1 EtOAc–MeOH–H_2O); ^{1}H-n.m.r. (400.13 MHz, CD_3OD): δ 4.55 (t, 1 H, $J_{1,2}$ 5.5 Hz, H-1), 1.43–1.85 (m, 3 H, H-2, H-2', H-3), 3.36–3.8 (m, 2 H, H-4, H-5), 3.73 (dd, 1 H, H-6), 3.95 (m, 1 H, $J_{7,8}$ 1.5 Hz, H-7), 3.97 (ddd, 1 H, $J_{8,9}$ 6.5 Hz, H-8), 3.63–3.8 (m, 2 H, H-9, H-9'), 3.38 and 3.39 (s, 6 H, OMe); ^{13}C-n.m.r. (100.61 MHz, CD_3OD): δ 107.65 (C-1), 30.95 (C-2), 29.75 (C-3), 75.16 (C-4), 74.82 (C-5), 72.79 (C-6), 72.74 (C-7), 72.23 (C-8), 65.96 (C-9), 56.12, and 55.89 (OMe).

Anal. Calc. for $C_{11}H_{22}DO_8$: C, 46.46; H/D, 8.45. Found: C, 45.33; H/D, 8.16.

Compound **15** (39 mg, 41%) was obtained as colorless crystals, m.p. 155°, $[\alpha]_D^{21}$ −5° (*c* 0.93, H_2O); R_F 0.38 (7:2:1 EtOAc–MeOH–H_2O); ^{1}H-n.m.r. (400.13 MHz, CD_3OD): δ 4.57 (t, 1 H, $J_{1,2}$ 5.5 Hz, H-1), 1.4–1.9 (m, 3 H, H-2, H-2', H-3), 3.63–3.73 (m, 2 H, H-4, H-5), 3.64 (dd, 1 H, $J_{6,7}$ 9.5 Hz, H-6), 3.93 (d, 1 H, $J_{7,8}$ 1.5 Hz, H-7), 3.97 (ddd, 1 H, $J_{8,9}$ 6.5 Hz, H-8), 3.63–3.73 (m, 2 H, H-9, H-9'), 3.38 and 3.39 (s, 6 H, OMe); ^{13}C-n.m.r. (100.61 MHz, CD_3OD): δ 107.75 (C-1), 30.92 (C-2), 30.44 (C-3), 73.09 (C-4), 74.61 (C-5), 73.00 (C-6), 72.16 (C-7), 71.07 (C-8), 66.03 (C-9), 56.40, and 55.78 (OMe).

Anal. Calc. for $C_{11}H_{22}DO_8$: C, 46.46; H/D, 8.45. Found: C, 46.01; H/D, 8.42.

*4,7,8,9-Tetra-*O*-acetyl-1,6-anhydro-2,3-dideoxy-3(*S*)-*D*-glycero-*L*-gluco-(3-^{2}H) nonopyranose* (**21**). — A solution of **14** (30 mg, 0.1 mmol) in methanolic 0.5M CF_3CO_2H was kept at 62° in a tightly stoppered flask for 8 h. The mixture was neutralized with methanolic M NaOMe, and then concentrated under diminished pressure. The residue was acetylated and then purified by flash column chromatography (1:2 EtOAc–cyclohexane) to yield **21** (16.8 mg, 41%) as a colorless syrup, $[\alpha]_D^{21}$ −1.5° (*c* 0.82, CH_2Cl_2); R_F 0.54 (2:1 EtOAc–cyclohexane).

For ^{1}H- and ^{13}C-n.m.r. see Tables I–III.

*4,7,8,9-Tetra-*O*-acetyl-1,6-anhydro-2,3-dideoxy-3(*S*)-*D*-glycero-*L*-manno-(3-^{2}H)nonopyranose* (**23**). — Compound **15** (30 mg, 0.1 mmol) was treated in the same way as compound **14** to yield **23** as colorless, crystalline solid (13.1 mg, 32%), m.p. 135–137°, $[\alpha]_D^{21}$ −28° (*c* 0.81, CH_2Cl_2); R_F 0.51 (2:1 EtOAc–cyclohexane).

Anal. Calc. for $C_{17}H_{22}DO_{10}$: C, 52.61; H, 6.19. Found: C, 51.21; H, 6.23.

For ^{1}H- and ^{13}C-n.m.r. see Tables I–III.

*1,6-Anhydro-2,3-dideoxy-3(*S*)-*D*-glycero-*L*-gluco-(3-^{2}H)nonopyranose* (**20**). — Compound **21** (10 mg, 0.025 mmol) was deacetylated (Zemplén) to give **20** (4.5 mg, 90%) as colorless crystals, m.p. 117°, R_F 0.47 (7:2:1 EtOAc–MeOH–H_2O).

For ^{1}H- and ^{13}C-n.m.r. see Tables I–III.

*1,6-Anhydro-2,3-dideoxy-3(*S*)-*D*-glycero-*L*-manno-(3-^{2}H)nonopyranose* (**22**). — Compound **23** (10 mg, 0.025 mmol) was deacetylated (Zemplén) to give **22** (4.3 mg, 89%) as colorless crystals, m.p. 172–175°; R_f 0.44 (7:2:1 EtOAc–MeOH–H_2O). For ^{1}H- and ^{13}C-n.m.r. see Tables I–III.

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