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Note

Glycosidation of 2,5-anhydro-3,4-di-*O*-benzyl-D-mannitol with different glucopyranosyl donors. A comparative study

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Abstract—2,5-Anhydro-3,4-di-*O*-benzyl-D-mannitol was glycosylated using different donors such as tetra-*O*-acetyl-α-D-glucopyranosyl bromide in the presence of $Hg(CN)_2$, the corresponding β-thiophenylglycoside in the presence of NIS and TfOH as well as the α- and β-trichloroimidate with TMSOTf as promoter. The resulting mixtures were analyzed by HPLC and the following main components were isolated and characterized: 2,5-anhydro-3,4-di-*O*-benzyl-1-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-D-mannitol; 6-*O*-acetyl-2,5-anhydro-3,4-di-*O*-benzyl-1-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-D-mannitol; 2,5-anhydro-3,4-di-*O*-benzyl-1,6-bis-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-D-mannitol; 2,5-anhydro-3,4-di-*O*-benzyl-1-*O*-[-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl]-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-D-mannitol and 2,5-anhydro-3,4-di-*O*-benzyl-1,6-bis-*O*-(3,4,6-tri-*O*-acetyl-1,2-*O*-ethylidene-2′-yl-α-D-glucopyranosyl)-D-mannitol. The latter compound representing a bis-orthoester might be a common intermediate in all the investigated reactions, as its rearrangement and/or decomposition can yield all of the isolated compounds.

Keywords: 2,5-Anhydro-D-mannitol derivatives; Mono-, di- and triglycosylated derivatives; Orthoesters and their decomposition products

Recently, we reported[1] the synthesis of several 1,6-di-O-glycosylated 2,5-anhydro-D-mannitol derivatives using 2,5-anhydro-3,4-di-*O*-benzoyl-D-mannitol **3** as acceptor and the following donors: acetobromo derivatives in the presence of $Hg(CN)_2$ or thioglycosides in the presence of NIS and TfOH, respectively. In every case complex mixtures were formed from which the 1,5-di-O-glycosylated trisaccharides could be obtained after column chromatography in disappointingly low yields only. This is more inconceivable as the primary OH groups of **3** should react more readily than the secondary OH groups of 2,5-anhydro-1,6-di-*O*-benzoyl-D-mannitol, which afforded the corresponding 3-O-glycosides in high yields.[2] For getting a better insight into the reaction mentioned above, a systematic investigation was started. To avoid a possible interference of the *O*-benzoyl groups of **3**, the corresponding 3,4-di-*O*-benzyl analogue **2** was used as acceptor and acetobromo α-D-glucopyranose **5** in the presence of $Hg(CN)_2$, the corresponding β-thiophenylglycoside **6** in the presence of NIS and TfOH as donors as well as the α- and β-trichloroimidate **7** and **8** with TMSOTf as promoter. The molar ratio of the acceptor and donor was 1:2.2 and the reactions were quenched after the donor had been consumed (TLC). The crude mixtures obtained after the usual processing were analyzed by HPLC and submitted thereafter to preparative column chromatography. In most cases the multi-component mixtures could only be separated partially in one run and the main components of the so obtained fractions were isolated usually by repeated column chromatography. The structures of the purified components were established by NMR spectroscopy and the corresponding data, including the conditions of the reactions are listed in Tables 1–5.

As can be seen from Table 1, the 1,6-di-O-glycosylated compound **11** was formed in highest yield (44%), when bromide **5** was used as donor, but a substantial amount (9%) of the O-monoglycosylated compound **9**

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Table 1. Glycosidation of **4** with different donors applied in a molar ratio of 1:2.2

Run	Donor	Temp	Time	Promoter	% Of products according to HPLC				
					9	**10**	**11**	**12**	**13**
1	**5**	rt	20 h	$Hg(CN)_2$	9	15	44	—	—
2	**6**	−40 °C	2 h	NIS; TfOH	—	26	21	15	—
3	**7**	−40 °C	25 min	TMSOTf[a]	—	2	18	—	21
4	**7**	−40 °C	10 min	TMSOTf[b]	—	9	24	24	1
5	**8**	−40 °C	10 min	TMSOTf[b]	—	10	25	23	1

[a] 10 mol %.
[b] 20 mol %.

Table 2. Data of the isolated compounds

	9	**10**	**11**	**12**	**13**
$[\alpha]_D$ ($CHCl_3$)	0	+2	+5	+15	+40
R_f (TLC)[a]	0.4	0.7	0.5	0.4	0.6
t_R (HPLC) min	3.6	6.6	8.0	8.8	9.6
Anal. Calcd for	$C_{34}H_{42}O_{14}$	$C_{36}H_{44}O_{15}$	$C_{48}H_{60}O_{23}$	$C_{60}H_{76}O_{31}$	$C_{48}H_{60}O_{23}$
C	60.53	60.33	57.37	55.73	57.37
H	6.27	6.19	6.02	5.92	6.02
Found: C	60.37	60.21	57.13	55.40	57.24
Found: H	6.42	6.30	6.14	6.09	6.26

[a] Solvent: EtOAc–hexane 2:1.

Table 3. Characteristic ^1H NMR chemical shifts

	2,5-Anhydro-D-mannitol unit									Glucopyranosyl unit(s)						
	H-1a	H-1b	H-2	H-3	H-4	H-5	H-6a	H-6b		H-1	H-2	H-3	H-4	H-5	H-6a	H-6b
9	3.63	3.92	4.18	4.01	4.03	4.08	3.64	3.70		4.54	5.00	5.19	5.08	3.66	4.10	4.24
10	3.63	3.94	4.19	4.03	3.96	4.21	4.15–4.18			4.54	5.02	5.20	5.08	3.67	4.11	4.25
11	3.61	3.91	4.12	3.99	3.99	4.12	3.61	3.91		4.52	5.00	5.20	5.07	3.66	4.10	4.24
12	3.65	3.93	4.12	4.11[a]	4.04[a]	4.12	3.56	3.92	′	4.79	3.71	5.16	4.96	3.63	4.07	6.03
									″	4.44	4.96	5.13	5.02	3.70	4.11	6.03
									‴	4.47	5.00	5.18	5.08	3.67	4.11	6.04
13	3.55	3.58	4.12	3.96	3.96	4.12	3.55	3.58		5.57	4.34	5.17	4.89	3.94	4.19	

[a] Interchangeable assignation.

Table 4. Characteristic $J_{H,H}$ couplings

	2,5-Anhydro-D-mannitol unit									Glucopyranosyl unit(s)						
	$^2J_{1a,1b}$	$^3J_{1a,2}$	$^3J_{1b,2}$	$^3J_{2,3}$	$^3J_{3,4}$	$^3J_{4,5}$	$^3J_{5,6a}$	$^3J_{5,6b}$	$^2J_{6a,6b}$	$^3J_{1,2}$	$^3J_{2,3}$	$^3J_{3,4}$	$^3J_{4,5}$	$^3J_{5,6a}$	$^3J_{5,6b}$	$^2J_{6a,6b}$
9	10.6	6.3	5.5	3.7	3.0	4.2	~6.0	3.7	11.9	8.0	9.5	9.7	9.7	2.8	4.7	12.3
10	10.5	5.8	6.1	3.6	3.6	3.2				8.0	9.6	9.6	9.7	2.4	4.6	12.3
11	10.6	5.9	5.7	7.0		7.0	5.9	5.7	10.6	8.0	9.6	9.6	9.7	2.3	4.7	12.3
12	10.6						5.5		10.4	′ 7.5	9.3	9.6	9.7			
										″ 8.0	9.6	9.6	9.7			
										‴ 7.9	9.6	9.6	9.7			
13	10.1	6.1	6.2	4.1		4.1	6.1	6.2	10.1	5.2	2.0	2.0	9.5			

and its 6-*O*-acetylated derivative **10** (15%) could also be detected in the reaction mixture. Formation of the latter can be explained by the formation of an orthoacetate **14**, the decomposition of which can lead according to the mechanism suggested by Garegg et al.[3] either to the O-acetylated derivative **10**, to the O-glycoside **11** or to an intermediate, in which a free OH group is present at C-2 of the glycosyl moiety (**15**).[4–7] Although this latter could not be detected in the aforementioned reaction, when the thioglycoside **6** was used as donor and NIS as promoter (Table 1, Run 2) the 1,6-bis-glycosylated anhydro derivative **12** could be isolated from the resulting mixture carrying a 2-*O*-glycosido-glycosyl unit at one of the terminal positions of the anhydro-mannitol

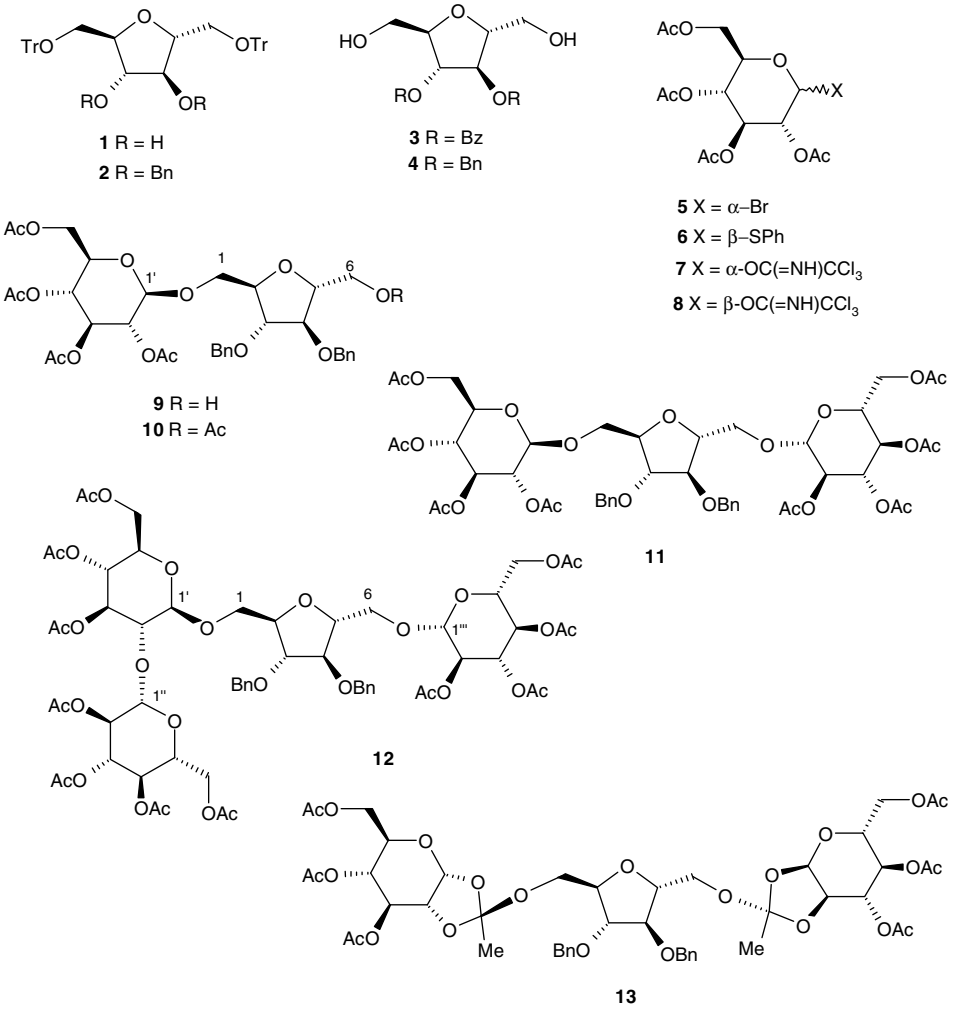
Table 5. Characteristic ^{13}C NMR chemical shifts

	2,5-Anhydro-D-mannitol unit							Glycopyranosyl unit(s)					
	C-1	C-2	C-3	C-4	C-5	C-6		C-1	C-2	C-3	C-4	C-5	C-6
9	69.4	81.6	84.3	84.0	83.3	62.6		100.9	71.2	72.7	68.3	71.8	61.9
10	69.4	81.8	84.4	84.4	80.7	64.1		101.0	71.2	72.8	68.4	71.9	61.9
11	69.4	81.7	84.5	84.5	81.7	69.4		100.9	71.2	72.8	68.4	71.8	61.9
12	69.6	81.5[a]	84.5[b]	84.0[b]	81.4[a]	69.3	′	101.5	77.6	74.1	68.6	71.5	61.8
							″	100.2	71.7	73.0	68.3	72.0	62.0
							‴	100.9	71.1	72.8	68.4	71.8	62.0
13	63.5	81.6	84.6	84.6	81.6	63.5		97.0	73.2	70.1	68.2	67.1	63.1

[a,b]Arbitrary assignments.

residue. That means that, in this case, the orthoester **14** must have been formed as an intermediate, which collapsed to the 2-OH glycoside **15** and this reacted further with the donor affording **12**. It should be mentioned that because of the C_2 symmetry of the acceptor molecule **4**, the two terminal positions (C-1 and C-6) are equal and can be distinguished from each other by NMR spectroscopy only after an asymmetric substitution. The connection of the additional glucose unit to O-2 in case of compound **12** was proven by the anomalous chemical shifts of H-2′, C-2′ and the H-1″ signals (see Tables 3–5) as well as the heteronuclear long-range couplings H-1″/C-2′, H-2′/C-1″ detected by HMBC (Scheme 1).

When the α-imidate **7** was used as donor and a catalytic amount (10 mol %) of TMSOTf as promoter in the presence of a relatively large amount of a molecule sieve (Table 1, Run 3), besides the 1,6-di-*O*-glycopyranoside **11**, the symmetric bis-orthoester **13** was formed



Scheme 1.

in almost equal amount. The orthoester structure of **13** is evident from its NMR spectra as proved by the anomalous chemical shifts and coupling pattern of the glucose units, the characteristic chemical shifts of the quaternary carbon signal at 121.3 ppm, and anomalous methyl signal at 1.69 ppm (see Tables 3–5). The couplings H-1′/$C_{orthoacetate}$ and H_2-1,6/$C_{orthoacetate}$ detected by the HMBC also prove this structure. The formation of **13** is a direct proof of the hypothesis[3] according to which orthoesters are formed in the Koenigs–Knorr type glycosidation reactions as intermediates, which are relatively stable but collapse in the presence of strong acids. The large amount of the used molecular sieve absorbed obviously a substantial part of the promoter, therefore the rearrangement of the orthoester into the normal glycoside was a slow process. This could be proved indirectly, as when the amount of the promoter was raised to 20 mol %, and simultaneously the amount of the molecular sieve lowered (see Table 1, Run 4), the orthoester could be detected in traces only, but instead of **11** the triglucosyl derivative **12** was formed unexpectedly in amounts almost equal to **11**. On the other hand, a substantial amount of the monoglycosylated derivative **9** with a free terminal OH group could also be detected, which is probably formed via collapse of the mono-orthoester **14**. Change of the anomeric configuration of the donor molecule, using the β-anomer **8** instead of **7** did not change substantially the product distribution (Scheme 2).

As a conclusion, It is likely that in all the investigated reactions the bis-orthoester **13** was formed as a common intermediate, as the rearrangement and/or decomposition of it can result in all of the isolated derivatives (**9**–**12**). Furthermore, other anomers and related compounds are probably also formed via the same pathways[3] as by-products, but they could not be isolated in pure state from the multi-component mixture. The different donors and promoters can shift the composition of the resulting mixtures by influencing the reaction mechanism but the relatively low yield of the expected product **11** is a result of the complexicity of this reaction.

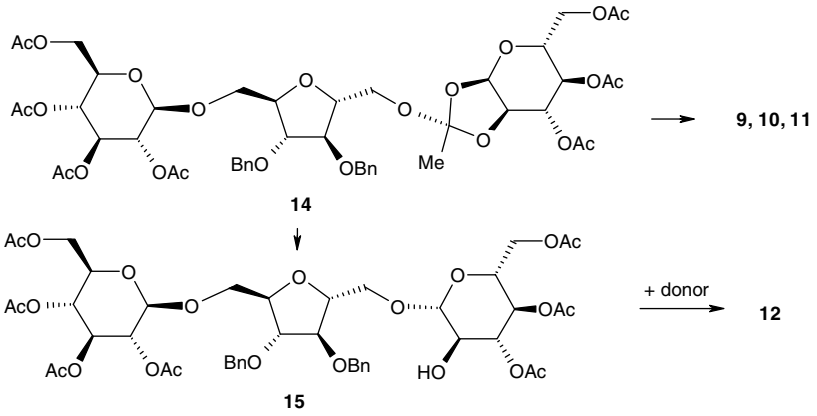
1. Experimental

1.1. General methods

Organic solns were dried over $MgSO_4$ and concentrated under diminished pressure at or below 40 °C. TLC used E. Merck precoated Silica Gel 60 F_{254} plates, with 1:19 EtOAc–hexane and detection by spraying the plates with a 0.02 M soln of I_2 and a 0.3 M soln of KI in 10% aq H_2SO_4 followed by heating at ca. 200 °C. For column chromatography, Kieselgel 60 was used. The mp are uncorrected. Optical rotations were determined on 1.0% solns in $CHCl_3$ at 20 °C. The NMR spectra were recorded on a Bruker Avance 500 spectrometer at 500 (^{1}H) and 125 (^{13}C) MHz, respectively, in $CDCl_3$ soln at ambient temperature. The chemical shifts were referenced to δ_{TMS} 0 ppm. For structure determination, ^{1}H,^{1}H-COSY, TOCSY, HMQC, HMBC as well as selective 1D TOCSY and NOESY spectra were recorded. For HPLC, Eurospher-100 C18 column (5 μm, 125 × 4 mm I.D., Knauer, Berlin) was used with a Waters 600 E pump and system controller and a Waters 996 PDA detector (at 256 nm). For elution, 3:2 acetonitrile–0.1% ammonium acetate (pH 4.5) was used at a speed of 1 mL/min^{-1}. The retention time for the isolated compounds is given in Table 2.

1.2. 2,5-Anhydro-3,4-di-*O*-benzyl-1,6-di-*O*-trityl-D-mannitol (2)

(a) To a stirred soln of **1**[1] (12.7 g, 20 mmol) in Me_2SO (50 mL), a soln of NaOH (5 g) in water (50 mL) and benzyl chloride (5 mL, 60 mmol) were added simultaneously. Stirring was continued at 50 °C for 3 h. Thereafter the mixture was cooled, poured into water and extracted with $CHCl_3$. The residue obtained after



Scheme 2.

concentration gave, after column chromatography (1:4 EtOAc–hexane) **2** (12 g, 72%); $[\alpha]_D$ +10 (*c* 1, $CHCl_3$). Anal. Calcd for $C_{58}H_{52}O_5$: C, 84.03; H, 6.32. Found: C, 83.97; H, 6.50.

(b) 10 g NaH (0.4 mol) was dissolved in DMF (150 mL) and a soln of **1** (64 g, 0.1 mol) was added gradually with stirring. Thereafter benzyl chloride (26 mL, 0.3 mol) was added and the mixture was stirred at 90 °C for 2 h. Thereafter MeOH (20 mL) was added and the mixture was kept at rt overnight. The residue of the concentrated mixture was dissolved in $CHCl_3$, washed with water, dried and concentrated to give crude **2** (83 g, ~100%) as a brown oil. This was used without purification in the next step.

1.3. 2,5-Anhydro-3,4-di-*O*-benzyl-D-mannitol (4)

A soln of crude **2** (56.5 g, 68 mmol) in AcOH (300 mL) was cooled to +10 °C and treated with 33% HBr in AcOH (50 mL). The resulting precipitate was filtered off after 1 min and the filtrate was diluted with ice-water (1 L). The precipitated oil was extracted with $CHCl_3$, washed with cold water, 5% $NaHCO_3$ and water, dried and concentrated. The residue was separated by column chromatography (2:1 EtOAc–hexane). The fractions having R_f 0.2 afforded on concentration crystalline **4** (1.4 g, 30%); mp 76–78 °C (ether–hexane); $[\alpha]_D$ +40 (*c* 1, $CHCl_3$). Anal. Calcd for $C_{20}H_{24}O_5$: C, 69.75; H, 7.02. Found: C, 69.70; H, 7.12.

No improvement was obtained when 80% aq AcOH was used for the hydrolysis of **2** at 80 °C for 1 h.

1.4. Glycosidation of 4 using bromide 5 as donor

A soln of **4** (6.88 g, 20 mmol) in dry MeCN (100 mL) was stirred in the presence of molecular sieves (4 Å) (7 g) for 30 min at rt. Thereafter **5** (18.1 g, 44 mmol) and $Hg(CN)_2$ (12.6 g, 50 mmol) were added and stirring was continued at rt for 20 h. The filtered mixture was diluted with 3-fold $CHCl_3$, washed with 5% $NaHCO_3$ and a 10% aq soln of KBr, dried and concentrated. The resulting oily residue was investigated by HPLC. For results, see Table 1, Run 1.

1.5. Glycosidation of 4 using thioglycoside 6 as donor

A soln of **4** (4.64 g, 13.5 mmol) in dry MeCN (100 mL) was stirred in the presence of molecular sieves (4 Å) (5 g) for 30 min at rt. Thereafter **6** (13.5 g, 29.6 mmol), and after cooling to −40 °C, NIS (9.51 g, 42.3 mmol) and TfOH (0.25 mL, 2.8 mmol) were added. After 2 h the temperature was raised to rt, the mixture was filtered, washed with 5% aq $Na_2S_2O_3$, 5% $NaHCO_3$ and water, dried and concentrated. The residue was investigated by HPLC. For results see Table 1, Run 2.

1.6. Glycosidation of 4 using imidate 7 as donor

(a) A soln of **4** (7.94 g, 23.1 mmol) and **7** (25 g, 50.7 mmol) in dry CH_2Cl_2 (400 mL) was stirred in the presence of molecular sieves (4 Å) (20 g) for 30 min at rt. Thereafter the mixture was cooled to −40 °C and TMSOTf (0.4 mL, 2.2 mmol) was added. Stirring was continued at −40 °C for 25 min. Thereafter Et_3N (2 mL) was added and the temperature was raised to rt. The filtered mixture was washed with water, dried and concentrated. The residue was investigated by HPLC. For results, see Table 1, Run 3.

(b) A soln of **4** (1.72 g, 5 mmol) and **7** (5.42 g, 11 mmol) in dry CH_2Cl_2 (60 mL) was stirred in the presence of molecular sieves (4 Å) (5 g) for 30 min at rt. Thereafter the mixture was cooled to −40 °C and TMSOTf (0.2 mL, 1.1 mmol) was added. The mixture was stirred at −40 °C for 10 min and processed as described for a. The residue was investigated by HPLC. For results see, Table 1, Run 4.

1.7. Glycosidation of 4 using imidate 8 as donor

The process described above in method b was repeated using **8** as donor. The obtained residue was investigated by HPLC. For results see Table 1, Run 5.

The following compounds were isolated from the different glycosidation reactions in pure state:

1.8 2,5-anhydro-3,4-di-*O*-benzyl-1-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-D-mannitol (**9**);

1.9 6-*O*-acetyl-2,5-anhydro-3,4-di-*O*-benzyl-1-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-D-mannitol (**10**);

1.10 2,5-anhydro-3,4-di-*O*-benzyl-1,6-bis-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-D-mannitol (**11**);

1.11 2,5-anhydro-3,4-di-*O*-benzyl-1-*O*-[-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl]-6-*O*-(2,3,4,6-tetra-*O*-acetyl-β-glucopyranosyl)-D-mannitol (**12**);

1.12 2,5-anhydro-3,4-di-*O*-benzyl-1,6-bis-*O*-(3,4,6-tri-*O*-acetyl-1,2-*O*-ethylidene-2-yl-α-D-glucopyranosyl)-D-mannitol (**13**).

Compounds **9** and **12** had the same R_f values, but they were never present simultaneously in the different reaction mixtures. For further structural identification, an aliquot part of the reaction mixtures was acetylated: when **9** was converted into its 6-*O*-acetate **10**, **12** remained unchanged.

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