Carbohydrate Research, 237 (1992) 115–129
Elsevier Science Publishers B.V., Amsterdam

D-Galacturonic acid derivatives as acceptors and donors in glycosylation reactions *

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(Received September 10th, 1991; accepted in revised form April 14th, 1992)

ABSTRACT

Jones oxidation of suitably protected allyl β-D-galactopyranosides and subsequent esterification were reinvestigated. Partial deprotection of the resulting D-galacturonic acid derivatives afforded compounds suitable for transformation into glycosyl acceptors. The synthesis of 2-, 3-, and 4-trityl ethers, relying on efficient differential protecting-group strategies, is described. Trityl-cyanoethylidene condensation of these trityl ethers, leading to the protected disaccharide units β-D-Gal*p*A-(1 → 2)-D-Gal*p*A and β-D-Gal*p*A-(1 → 3)-Gal*p*A with high stereoselectivity, is demonstrated. A β-D-Gal*p*A-(1 → 4)-D-Gal*p*A disaccharide was also prepared.

INTRODUCTION

D-Galacturonic acid-containing carbohydrate residues have been frequently isolated from such natural sources as bacteria[2] or plants[3]. Pectic polysaccharides containing oligogalactosiduronic acids have been found to possess, as so called dietary fibres, human-physiological significance[4]. Additionally, homogalacturonan fragments of plant cell-wall are known to induce, via enzymic reactions, the production of defence substances against invading pathogens[5]. However, the molecular mechanisms of these biological processes are far from being clarified. The synthesis of authentic materials as well as of structural analogues is therefore required. We have investigated glycosylation reactions using D-galacturonic acid derivatives by application of the trityl-cyanoethylidene condensation (TCC) method[6]. This reaction has been employed for the preparation of oligosaccharides as well as of polysaccharides built up from repeating units or blocks.

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* Presented at the VIth European Carbohydrate Symposium, Edinburgh, Scotland, September 1991; abstract B.141, Part IV of the series Galacturonic Acid Derivatives. For Part III see ref. 1.

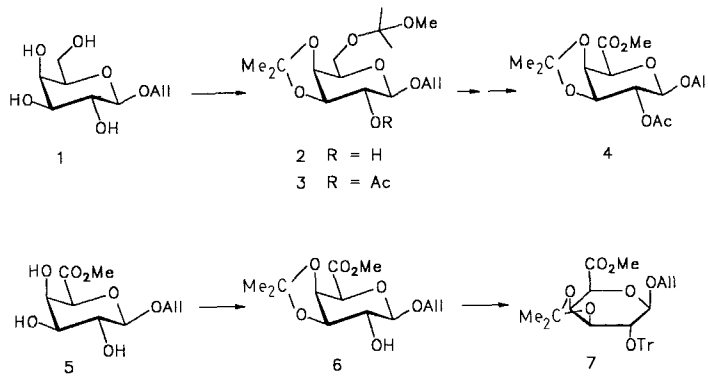
RESULTS AND DISCUSSION

Recently, the reinvestigation and some new syntheses of D-galactose-derived galacturonic acid derivatives suitable as glycosyl donors and acceptors have been reported[7–10]. The glycosylation procedure using TCC requires a tritylated hydroxyl group in the acceptor molecule. This requires an additional synthetic step when compared with other glycosylation reactions. However, alternative glycosylation procedures, e.g., the Mukaiyama method, were hitherto not successful[10].

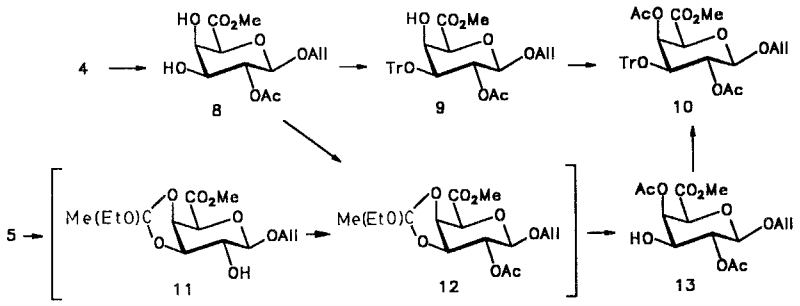
The previously reported methyl (allyl β-D-galactopyranosid)uronate derivatives **4** and **5** (ref. 9) have been used as starting materials in the synthesis of suitable glycosyl acceptors in TCC reactions. We investigated here the synthesis of **4** via the acyclic acetals **2** (ref. 11) and **3**. It seems that this approach is the most convenient one despite the considerably smaller yield compared to our findings with the corresponding benzyl β-D-galactopyranoside[9]. Thus, allyl β-D-galactopyranoside **1** on reaction with 2,2-dimethoxypropane with rigorous exclusion of moisture led to allyl 3,4-*O*-isopropylidene-6-*O*-(1-methoxy-1-methylethyl)-β-D-galactopyranoside[11] (**2**). Sequential acetylation of non-isolated **2**, Jones oxidation[12], and esterification afforded the desired key intermediate **4** in 14% overall yield (Scheme 1).

Glycosyl acceptors at positions 2 (**7**) and 3 (**10**) were prepared in the following manner: treatment of triol **5** with 2,2-dimethoxypropane in acetone gave compound **6** (92%). Tritylation of **6** with tritylium perchlorate–2,4,6-collidine led to the trityl ether **7** (90%, Scheme 1). The 3-*O*-trityl derivative **10** was synthesized starting from diol **8** obtained by *O*-deisopropylidenation of key compound **4** with trifluoroacetic acid in 90% aq acetic acid at room temperature. Direct tritylation of diol **8** led regioselectively to derivative **9** (86%). Acetylation (Ac_2O–pyridine) catalyzed by *N*,*N*-dimethyl-4-aminopyridine gave then the protected trityl ether **10** (95%, Scheme 2).

Alternative routes to glycosyl acceptor **10** started from compound **5** or **8** via the cyclic ortho ester **12**, which was opened by treatment with aq acetic acid to form,



Scheme 1.

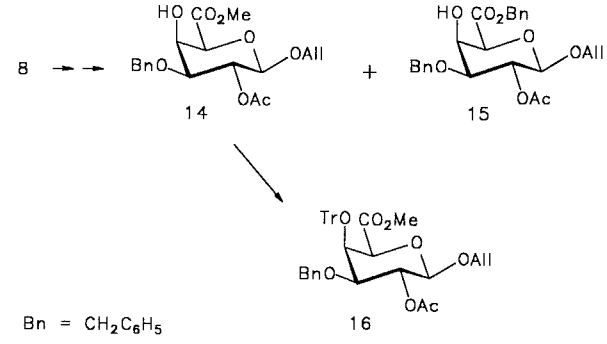


Scheme 2.

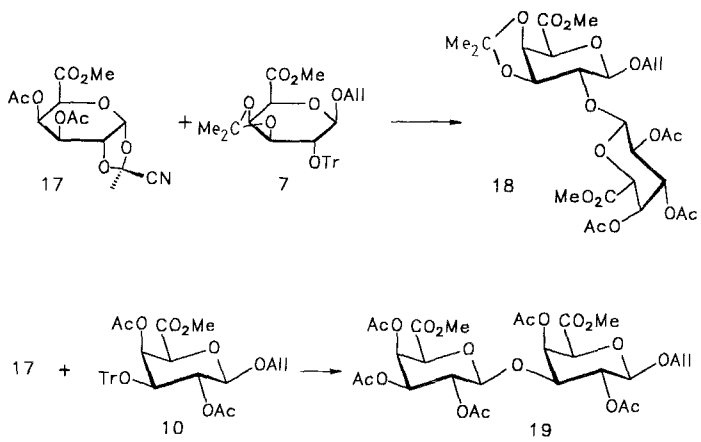
as expected[13], the diacetate **13** (75%). Tritylation of the remaining hydroxyl group gave acceptor **10** (96%, Scheme 2).

Regioselective alkylation of the free 3,4-*cis*-diol of the D-galacturonic acid derivative **8** via a 4,6-di-*O*-butylstannyl ether[14] was used in order to prepare an acceptor molecule (**16**) for glycosylation at C-4. Therefore, compound **8** was treated with dibutyltin oxide in refluxing toluene, and subsequently with benzyl chloride in the presence of tetrabutylammonium iodide, yielding a mixture of the 3-*O*-benzyl derivatives **14** (55%) and **15** (30%). Although, the benzylation reaction could be then carried out with total regioselectivity, a side reaction caused partial formation of a benzyl ester. Eventually, compound **14** was tritylated. In this case, complete tritylation was only achieved in the presence of *N*,*N*-dimethyl-4-aminopyridine yielding 80% of the trityl ether **16** (Scheme 3).

The synthesis of cyanoethylidene derivatives (CED) of uronic acid is known[15] and was recently improved[7]. Thus, methyl 3,4-di-*O*-acetyl-1,2-*O*-[1-(*exo*-cyano)ethylidene]-α-D-galactopyranuronate (**17**) was prepared from the 6-*O*-trityl-CED of the corresponding neutral sugar by oxidation and then esterification[7]. We used uronic acid-CED **17** as the glycosyl donor in all glycosylation reactions, whereas trityl ethers **7**, **10**, and **16** were used as glycosyl acceptors. The structures of all monosaccharide derivatives were confirmed by their ^{1}H and ^{13}C NMR spectra.



Scheme 3.



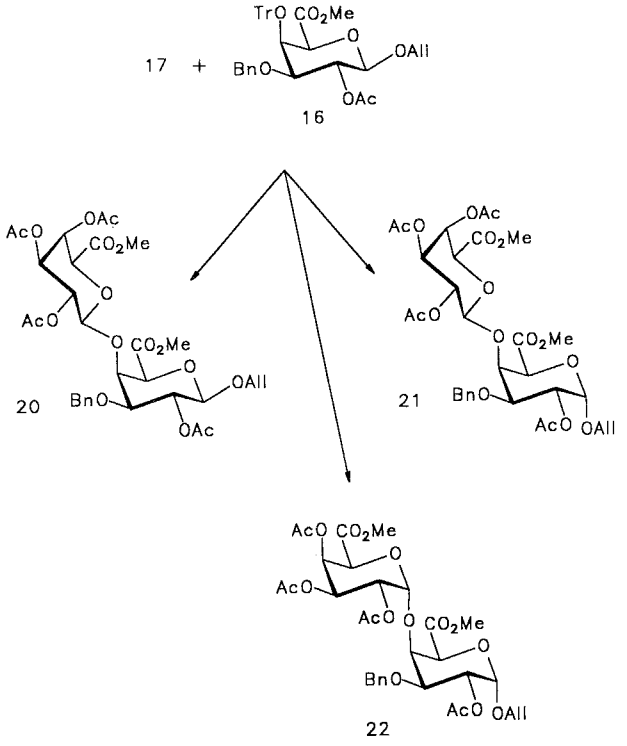
Scheme 4.

The synthesis of disaccharides was carried out in the presence of tritylium perchlorate in dichloromethane using the vacuum technique under the standard conditions[6] of the TCC. The mixture of resulting disaccharides was isolated by column chromatography, and the ratio of 1,2-*trans*- to 1,2-*cis*-linked disaccharides was estimated by comparing the integrated intensities of the signals for H-1 or H-5 in the ^{1}H NMR spectra.

The coupling of **17** with **7** and **10** gave the expected β-(1 → 2)- and β-(1 → 3)-linked disaccharides **18** (58%) and **19** (57%) containing no more than 1% of the α anomer (Scheme 4). The value of $J_{1',2'}$ in the ^{1}H NMR spectra (8.0 and 7.9 Hz) and the signal for C-1′ in the ^{13}C NMR spectra (102.1 and 100.5 ppm) proved the 1,2-*trans*-glycosidic linkage. The remaining NMR data also agreed with the depicted structures of disaccharides **18** and **19**.

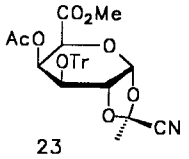
An experiment with **10** and a mixture of the *exo*/*endo*-isomers of the CED instead of pure *exo*-isomer **17** carried out under the same conditions showed a similar outcome.

In the case of glycosylation at C-4 (TCC of **16** and **17**, Scheme 5) two phenomena were observed. First, 1′,2′-*trans* (**20**, **21**)- and 1′,2′-*cis* (**22**)-disaccharides were formed, demonstrating a low stereoselectivity as previously reported for some neutral sugar derivatives[6]. Secondly, the partial anomerisation at the glycosidic centre of the 1,2-*trans*-allyl glycoside **16** led to the formation of 1,2-*cis*-allyl biosides **21** and **22**. An analogous anomerisation of alkyl-β-D-glucopyranosides under the action of a system containing 1,2-*O*-cyanoalkylidene sugar derivative–$TrClO_4$ was observed earlier[16]. The structures of the disaccharides **20** (10%), **21** (28%), and **22** (7%) are in accordance with the spectroscopic data. Thus, in the ^{1}H NMR spectra, the small coupling constants $J_{1,2}$ 3.3 Hz (**21**), $J_{1,2}$ 3.5 Hz and $J_{1',2'}$ 3.6 Hz (**22**) give evidence of the *cis*-glycosidic bonds. The values for **20** ($J_{1,2}$ 7.7 Hz, $J_{1',2'}$ 7.5 Hz) and **21** ($J_{1',2'}$ 7.7 Hz) indicate *trans*-glycosidic bonds. Key ^{1}H-reso-



Scheme 5.

nances were assigned by NOE experiments. Signals of anomeric carbon atoms of *cis*-glycosidic bonds were observed in ^{13}C NMR spectra at 95.9 (C-1 of **21**), 95.7 and 96.9 (C-1 and C-1′ of **22**) ppm. The downfield shift of the C-4 signals at 79.3 (**20**), 76.1 (**21**), and 75.0 (**22**) ppm compared to that of the non-glycosylated compound **14** (67.4 ppm) and a slight upfield shift of the signals for C-3 (74.0, 74.5, and 75.6 ppm) and for C-5 (70.4, 70.5, and 70.0 ppm) of **20**, **21**, and **22**, respectively, compared with C-3 (77.9 ppm) and C-5 (73.8 ppm) of **14** confirmed the glycosylation at C-4.



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The glycosylation reactions at C-3 and C-4 should permit prediction of the result of a polycondensation based on the same principle (e.g., polycondensation[17] of monomer **23**). Additionally, the disaccharides now prepared will be used as model compounds, especially for the interpretation of the NMR spectra of the

TABLE I

^{1}H NMR data [a] (250 MHz) for **2**, **7–10**, and **13–16**

Compound	Chemical shift (δ)											
	H-1	H-2	H-3	H-4	H-5	H-6	H-6′	Me_2C [b]	Ac [b]	OMe [b]	CH_2Ph	Ar
2	4.21d	3.55dd	4.04dd	4.14dd	3.83m	3.67s	3.69s	1.30		3.20		
								1.35 [c]				
								1.50				
7	4.57d	4.58dd	3.95dd	3.98dd	4.53d			1.18		3.84		7.31m
								1.30				7.47m
8	4.45d	5.03dd	3.74m	4.31m	4.15d				2.13	3.83		
9	4.27d	5.44dd	3.71dd	3.15m	2.51d				1.94	3.71		7.31m
												7.51m
10	4.16d	5.54dd	3.56dd	4.57d	3.59d				1.88,	3.62		7.28m
									2.23			7.43m
13	4.50d	5.06dd	3.89m	5.57dd	4.21d				2.13,	3.76		
									2.15			
14	4.45d	5.24dd	3.57dd	4.41dd	4.09m				2.00	3.80	4.61dd	7.32m
15	4.42d	5.27dd	3.55dd	4.42dd	4.11d				2.03		4.65dd	7.35m
											5.21m	
16	4.44d	5.81dd	3.21dd	4.50m	3.88d				2.00	3.27	3.89dd	7.20m

[a] In $CDCl_3$ with Me_4Si as reference. [b] Singlets. [c] s (6 H).

polycondensation products. The relevant data for all compounds is given in Tables I–V.

EXPERIMENTAL

General methods.—See ref. 9. All washing solutions were cooled to ~5°C. The $NaHCO_3$ solution was saturated. NMR data of the allyl group: ^1H NMR ($CDCl_3$): δ 4.0–4.1 (m, 1 H), 4.3–4.4 (m, 1 H), 5.1 (m, 1 H), 5.3 (m, 1 H), 5.8 (m, 1 H); ^{13}C NMR ($CDCl_3$): δ 69.9 ($CH_2CH=CH_2$), 117.5 ($CH_2CH=CH_2$), 133.7 ($CH_2CH=CH_2$). The following solvent systems (v/v) were used for chromatography: (A_1) 2:1, (A_2) 4:1, (A_3) 7:2, (A_4) 10:1, (A_5) 1:1, (A_6) 1:2, and (A_7) 30:1 PhMe–EtOAc; (*B*) 4:2:2:1 PhMe–EtOH–EtOAc–AcOH; (*C*) 1:2:0.1 PhMe–EtOAc–EtOH; (*D*) 5:1 PhMe–EtOAc, 2% pyridine; (E_1) 1:1, and (E_2) 10:1 PhMe–Me_2CO; and (*F*) 5:1:1 PhMe–heptane–EtOH.

Methyl (allyl 2-O-acetyl-3,4-O-isopropylidene-β-D-galactopyranosid)uronate (**4**).—To an anhydrous solution of TsOH (157 mg, 0.82 mmol) in 2,2-dimethoxypropane (50 mL) allyl β-D-galactopyranoside[9] **1** (4.25 g, 19.3 mmol) was added, and the mixture was stirred under Ar for 20 h at room temperature with exclusion of moisture. Pyridine (0.67 mL, 8.2 mmol) was then added, the mixture was evaporated and the resulting residue dissolved in an ice-cold solution of dry pyridine (13 mL) and Ac_2O (7.5 mL). After 3 h at room temperature (TLC, solvent A_1, 1% pyridine), EtOH (3.7 mL) was added dropwise at 0°C, and, after 30 min, the mixture was diluted with $CHCl_3$ (40 mL), poured into ice–water and extracted with $CHCl_3$ (3 × 20 mL). The combined organic layers were diluted with hexane (200 mL), washed with aq $NaHCO_3$ (2 × 50 mL), water (2 × 50 mL), dried, and evaporated with repeated addition of PhMe. To a solution of the residue (crude **3**) in Me_2CO (33 mL) and CH_2Cl_2 (48 mL), a solution of CrO_3 (4.5 g, 45 mmol) in H_2SO_4 (3.5 M, 19.3 mL) was added dropwise during 30 min, at ~0°C. The mixture was stirred for 30 min at 0°C, then for 1.5 h at room temperature (TLC, solvent *B*). EtOH (38 mL) was then added at 0°C, and, after 30 min, the separated solid was filtered off, washed thoroughly with Me_2CO, and the combined organic phases were neutralized by addition of aq $NaHCO_3$ (100 mL). The volume was reduced under diminished pressure to 100 mL (first at room temperature as long as gas evolvment occurred, then at ~40°C).

For esterification of the carboxylic group, the residue was vigorously stirred in CH_2Cl_2 (100 mL), Bu_4NBr (5 g, 15 mmol) and MeI (8 mL, 126 mmol) for 20 h at ambient temperature (TLC, solvent A_1). The phases were separated, and the aqueous phase was extracted with $CHCl_3$ (3 × 30 mL). The combined organic layers were washed with water (30 mL), dried, and evaporated. The syrupy residue was dissolved in a minimum of CH_2Cl_2 and Et_2O was added, whereupon the ammonium salts precipitated. The filtrate was evaporated and processed by column chromatography (solvent A_2) to yield 935 mg (14%) of **4**; R_F 0.4; mp

TABLE II

^{1}H NMR data [a] (400 MHz) for **18–22**

Compound	Chemical shift (δ)									
	H-1 H-1′	H-2 H-2′	H-3 H-3′	H-4 H-4′	H-5 H-5′	Me_2C [b]	Ac [b]	OMe [b]	CH_2Ph	Ar
18	4.48d 4.68d	3.60dd 5.27dd	4.19t 5.08dd	4.44dd 5.67dd	4.32d 4.26d	1.32 1.48	2.00, 2.09, 2.11	3.73 3.83		
19	4.45d 4.64d	5.33dd 5.13dd	4.04dd 5.00dd	5.71d 5.67dd	4.22s 4.27d		1.99, 2.05, 2.10, 2.12 [c]	3.75 [c]		
20	4.41d 4.78d	3.70dd 5.16dd	3.57dd 5.02dd	4.51t 5.59dd	4.08d 4.08d		1.97, 2.03, 2.07, 2.10	3.70 3.85	4.62d 4.74d	7.30m
21	5.15d 4.84d	5.20dd 5.13dd	4.02dd 4.95dd	4.62dd 5.58dd	4.49d 4.01d		1.98, 2.02, 2.07, 2.09	3.73 3.90	4.66d 4.72d	7.33m
22	5.32d 5.26d	5.18dd 5.13dd	3.97dd 5.37dd	4.64d 5.74dd	4.38s 5.25d		1.98, 2.04, 2.11, 2.17	3.30 3.74	4.57d 4.76d	7.27m

[a] In $CDCl_3$ with Me_4Si as reference. [b] Singlets. [c] s (6 H).

TABLE III

Coupling constants [$J_{H,H}$ values (Hz)] for **2**, **7**–**10**, and **13**–**22**

Compound	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'}$	J_{CH_2Ph}
2	8.2	7.3	5.3	2.2					
7	2.2	7.6	3.6	2.1					
8	7.7	9.8		1.3					
9	7.5	9.5	3.4	1.4					
10	7.9	9.9	2.9	0.8					
13	8.0	10.1	3.6	1.3					
14	7.9	9.7	3.2	1.3					12.3
15	7.7	9.9	3.3	1.6					12.2
16	7.8	10.0	3.0	1.4					12.3
18	7.7	6.7	5.9	2.3	8.0	10.5	3.6	1.6	
19	8.0	10.4	3.4		7.9	10.4	3.4	1.0	
20	7.7	9.5	2.9	0.5	7.5	10.4	3.2	1.0	11.8
21	3.3	9.9	2.5	1.2	7.7	10.1	3.4	0.8	11.5
22	3.5	10.4	2.9		3.6	11.1	3.0	1.5	12.2

98–100°C (from EtOAc–hexane), $[\alpha]_D^{20}$ −21.3° (*c* 1.0, $CHCl_3$); lit.[9], mp 98–101°C (from EtOAc–hexane); $[\alpha]_D^{20}$ −21.3° (*c* 1.0, Me_2CO).

Allyl 3,4-O-isopropylidene-6-O-(1-methoxy-1-methylethyl)-β-D-galactopyranoside (**6**).—To an anhydrous solution of TsOH (163 mg, 0.86 mmol) in 2,2-dimethoxypropane (50 mL) allyl β-D-galactopyranoside[9] **1** (4.41 g, 20 mmol) was added, and the mixture was stirred under Ar for 20 h at room temperature with exclusion of moisture. The reaction was monitored by TLC (solvent E_1, 1% pyridine). After adding pyridine (0.7 mL, 8.6 mmol), the mixture was evaporated and the resulting residue purified by column chromatography (solvent A_1) to give syrupy **2** (3.9 g, 58%); R_F 0.7, $[\alpha]_D^{30}$ −2.0° (*c* 2.0, $CHCl_3$).

Methyl (allyl 3,4-O-isopropylidene-β-D-galactopyranosid)uronate (**6**).—To a suspension of **5** (ref. 9) (1.92 g, 7.8 mmol) in dry Me_2CO (62.4 mL) and 2,2-dimethoxypropane (15.6 mL), TsOH (312 mg, 1.6 mmol) was added, and the mixture was stirred for 24 h at ambient temperature (TLC, 6:1 $CHCl_3$–MeOH). The mixture was then passed through a layer of alkaline alumina (2 × 3 cm), the eluate evaporated, and the residue was crystallized from EtOAc–hexane to give **6** (2.07 g, 92%); R_F 0.8; mp 97°C; $[\alpha]_D^{29}$ −33.5° (*c* 1.0, $CHCl_3$). *Anal.* Calcd for $C_{13}H_{20}O_7$: C, 54.16; H, 6.99. Found: C, 53.65; H, 6.82.

Methyl (allyl 3,4-O-isopropylidene-2-O-trityl-β-D-galactopyranosid)uronate (**7**).—To a solution of **6** (500 mg, 1.73 mmol) in CH_2Cl_2 (52 mL) were added $TrClO_4$ (739 mg, 2.15 mmol) and 2,4,6-collidine (0.37 mL, 2.8 mmol). The mixture was kept for 15 min at room temperature (TLC, solvent *D*), diluted with a mixture of hexane (180 mL) and $CHCl_3$ (40 mL), and washed with water (3 × 50 mL). The organic layer was dried and evaporated. The crude material was purified by column chromatography (first with hexane, then with PhMe) to give syrupy **7** (823 mg, 90%); R_F 0.6; $[\alpha]_D^{30}$ −34.9° (*c* 2.7, $CHCl_3$). *Anal.* Calcd for $C_{32}H_{34}O_7$: C, 72.43; H, 6.46. Found: C, 72.64; H, 6.72.

TABLE IV

^{13}C NMR data [a] for **2**, **7–10**, and **13–16**

Compound	Chemical shifts (ppm)												
	C-1	C-2	C-3	C-4	C-5	C-6	CH_3CO	CH_3CO	$(CH_3)_2C$	$(CH_3)_2C$	OCH_3	$PhCH_2$	C-Ar
2	101.2	73.7	78.8	73.7	72.5	60.3			24.3 24.4 26.2 28.1	100.1, 110.1	48.5		
7	102.6	71.7	74.7	71.2	73.1	168.7			25.4 25.9	110.9	52.1		88.7 127.3–143.9
8	99.7	72.4	72.2	70.0	74.1	168.6	21.0	171.2			52.8		
9	99.9	70.3	73.7	68.9	73.8	168.1	21.1	169.5			52.4		88.6 127.6–144.5
10	100.4	70.1	72.4	70.5	72.6	167.1	21.1 [b]	169.6 169.9			52.5		88.2, 127.6–144.2
13	99.5	72.3	71.2	70.9	72.5	167.2	20.8 21.0	170.7 171.2			52.7		
14	99.8	70.1	77.9	67.4	73.8	168.3	20.9	169.8			52.7	71.5	127.9–128.7
15	99.7	70.2	78.2	67.4	73.8	167.5	20.9	169.8				67.3	127.8–128.6
16	100.4	71.0	79.8	69.8	74.6	168.2	20.9	169.4			52.0	72.1	89.3 126.5–144.5

[a] In $CDCl_3$ with Me_4Si as reference. [b] Signal includes C-2.

TABLE V

^{13}C NMR data [a] (62.9 MHz) for **18–22**

Compound	Chemical shifts (ppm)												
	C-1 C-1′	C-2 C-2′	C-3 C-3′	C-4 C-4′	C-5 C-5′	C-6 C-6′	CH_3CO	CH_3CO	$(CH_3)_2C$	$(CH_3)_2C$	OCH_3	CH_2Ph	C-Ar
18	100.2	82.9	67.6	73.6	71.8	166.4	20.6 [b],	169.6	26.2	110.6	52.5 [b]		
	102.1	68.9	70.4	68.5	72.6	167.4	20.8	169.8	27.7				
								170.0					
19	99.5	70.4	75.4	69.8	72.6	166.0	20.5	168.8			52.7 [b]		
	100.5	68.3	70.3	67.9	72.4	166.8	20.6 [b]	169.1					
							20.9	169.8					
							21.4	170.0					
								170.1					
20	101.0	70.2	74.0	79.3	70.4	166.8	20.5	169.4			52.4	72.4	126.8–129.2
	99.6	68.7	72.1	68.4	72.6	167.7	20.6	169.6			52.7		
							20.8	170.2					
							21.1	170.6					
21	95.9	69.7	74.5	76.1	70.5	166.2	20.5 [b]	169.4			52.4	72.1	127.1–138.0
	100.7	68.7	70.7	68.4	72.2	167.9	20.8	169.9 [b]			52.8		
							21.5	170.1					
22	95.7	69.4	75.6	75.0	70.0	167.7	20.5	169.5			52.2	72.8	126.8–129.5
	96.9	67.1	66.9	69.1	68.2	167.6	20.6	169.6			52.3		
							20.9	170.1					
							21.0	170.9					

[a] In $CDCl_3$ with Me_4Si as reference. [b] Signals includes C-2.

Methyl (allyl 2-O-acetyl-β-D-galactopyranosid)uronate (**8**).—A solution of **4** (928 mg, 2.81 mmol) in aq AcOH (90% 56.2 mL) and aq CF_3CO_2H (90% 14.0 mL) was kept for 40 min at ambient temperature (TLC, solvent *C*), diluted with PhMe (100 mL), evaporated in vacuo at 50°C, and evaporated with repeated addition of solvent *F*, whereupon the product crystallized (730 mg, 90%), R_F 0.34, mp 102–104°C (from EtOAc–hexane); $[\alpha]_D^{20}$ −26.4° (*c* 0.92, $CHCl_3$). *Anal.* Calcd for $C_{12}H_{18}O_8$: C, 49.65; H, 6.25. Found: C, 49.60; H, 6.59.

Methyl (allyl 2-O-acetyl-3-O-trityl-β-D-galactopyranosid)uronate (**9**).—To a solution of **8** (416 mg, 1.4 mmol) in CH_2Cl_2 (10 mL), 2,4,6-collidine (0.45 mL, 3.4 mmol) and $TrClO_4$ (0.9 g, 2.6 mmol) were added in portions, and the mixture was stirred for 15 min (TLC, solvent A_3) at room temperature. Then, a mixture of hexane (60 mL) and $CHCl_3$ (20 mL) was added, and the solution was washed with water (3 × 30 mL), dried, and evaporated. The residue was processed by column chromatography (EtOAc gradient 15–35% in hexane) to afford **9** (644 mg, 86%), R_F 0.26, mp 171–172°C (from EtOAc–hexane); $[\alpha]_D^{20}$ +12.3° (*c* 1.0, $CHCl_3$). *Anal.* Calcd for $C_{31}H_{32}O_8$: C, 69.91; H, 6.06. Found: C, 69.74; H, 6.40.

Methyl (allyl 2,4-di-O-acetyl-3-O-trityl-β-D-galactopyranosid)uronate (**10**).—(*Via* **9**): Compound **9** (533 mg, 1 mmol) was dissolved in a mixture of Ac_2O (6 mL), pyridine (18 mL) and *N*,*N*-dimethyl-4-aminopyridine (120 mg) and kept for 24 h at room temperature (TLC, 3:1 PhMe–Et_2O). EtOH (4 mL) was added at 0°C, and, after 20 min at room temperature, the mixture was diluted with $CHCl_3$ (100 mL) and poured into ice–water. The phases were separated, and the aq phase was extracted with $CHCl_3$ (50 mL). The combined organic solutions were washed with aq $NaHCO_3$ (3 × 50 mL), water (2 × 50 mL), dried, and evaporated. Traces of pyridine were removed by evaporation with repeated addition of PhMe. The residue was purified by column chromatography (solvent E_2) to yield 564 mg (98%) of **10**, R_F 0.5, mp 174–175°C (from EtOAc–heptane), $[\alpha]_D^{20}$ +58.7° (*c* 0.88, $CHCl_3$).

(*Via* **13**): To a solution of **13** (665 mg, 2 mmol) in CH_2Cl_2 (15 mL) and 2,4,6-collidine (0.4 mL, 3 mmol) was added $TrClO_4$ (900 mg, 2.6 mmol) with stirring at ambient temperature. After 20 min, further amounts of 2,4,6-collidine (0.2 mL, 1.5 mmol) and $TrClO_4$ (400 mg, 1.2 mmol) were added. When the reaction was complete (TLC, solvent A_3), the mixture was diluted with $CHCl_3$ (100 mL). The organic solution was washed with water (3 × 30 mL), dried, and evaporated. Column chromatography (solvent A_4) was performed to give 1.1 g (96%) of **10**, R_F 0.4, mp 174–175°C (from EtOAc–heptane); $[\alpha]_D^{20}$ +58.3° (*c* 0.8, $CHCl_3$). *Anal.* Calcd for $C_{33}H_{34}O_9$: C, 68.98; H, 5.96. Found: C, 68.72; H, 6.20.

Methyl (allyl 2,4-di-O-acetyl-β-D-galactopyranosid)uronate (**13**).—(*Via* **8**): Compound **8** (1.7 g, 6 mmol) and TsOH (9 mg, 0.05 mmol) were dried by a threefold distillation with PhMe. Ethyl orthoacetate (8 mL) was added, and the suspension was stirred for 8 h at room temperature (TLC, solvent *C*, R_F 0.6). After adding Et_3N (2.5 mL), the mixture was diluted with Et_2O (100 mL), washed with water (4 × 30 mL), dried, and evaporated. The syrupy residue was dissolved in aq AcOH

(80%, 25 mL), kept for 10 min at room temperature (TLC, solvent *C*), diluted with PhMe (40 mL), and evaporated. Distillation of solvent *F* (4 × 60 mL) from the residue caused crystallization of **13** in a yield of 1.5 g (75%), R_F 0.5, mp 130–132°C (from EtOAc–heptane); $[\alpha]_D^{20}$ −7.3° (*c* 1.0, $CHCl_3$).

(*Via* **5**): To a solution of **5** (1.27 g, 5.2 mmol) in CH_2Cl_2 (31 mL) were added in portions $(EtO)_3CMe$ (5 × 0.96 mL) and TsOH (2 × 32 mg, total 0.34 mmol) with stirring at room temperature. After overall 100 min (TLC, solvent *C*, R_F 0.6), Et_3N (16.1 mL) was added to the reaction mixture which was diluted with $CHCl_3$ (150 mL), washed with water (2 × 50 mL), dried, and evaporated. Conventional acetylation (10:1 pyridine–Ac_2O, 11 mL) led to a residue which was codistilled with solvent *F* (3 × 20 mL) and dried in high vacuum (TLC, 2:1:0.02 PhMe–EtOAc–EtOH, R_F 0.6). Column chromatography was performed with solvent *D* to yield 1.4 g (75% overall yield) of **12**, which was immediately dissolved in aq AcOH (80%, 14.5 mL) and kept for 2 h at room temperature. Repeated evaporation of solvent *F* from the product caused crystallization of 1.25 g (72% overall yield) of **13**, R_F 0.4; mp 128–131°C (from EtOAc–hexane); $[\alpha]_D^{20}$ −7.0° (*c* 1.0, $CHCl_3$). *Anal.* Calcd for $C_{14}H_{20}O_9$: C, 50.60; H, 6.07. Found: C, 50.91; H, 6.20.

*Methyl (***14***) and benzyl (allyl 2-*O*-acetyl-3-*O*-benzyl-β-*D*-galactopyranosid)uronate* (**15**).—Compound **8** (244 mg, 0.84 mmol), Bu_2SnO (246 mg, 1 mmol), and PhMe (10 mL) were heated under reflux for 2 h, whereas the water formed during the reaction was removed by 4A molecular sieves (especially for small quantities, it is advantageous to place the molecular sieve in a Mini-Soxhlet extractor[18]). Then, the temperature was reduced to 50°C, BnCl (0.69 mL, 6 mmol) and Bu_4NI (370 mg, 1 mmol) were added, and the mixture was kept for 3 h at 50–60°C and for an additional 1 h at 90°C (TLC, solvent *C*). After cooling to room temperature, MeOH (2 mL) was added, the mixture was evaporated and the residue was processed by column chromatography (Et_2O) to give pure **15** (115 mg, 30%; R_F 0.8) and **14** (175 mg, 55%; R_F 0.6).

Compound **15** had mp 100°C (from EtOAc–hexane); $[\alpha]_D^{30}$ +11.6° (*c* 3.4, $CHCl_3$). *Anal.* Calcd for $C_{25}H_{28}O_8$: C, 65.78; H, 6.18. Found: C, 65.29; H, 6.28.

Compound **14** had mp 87–89°C (from Et_2O–hexane); $[\alpha]_D^{26}$ +10.7° (*c* 1.4, $CHCl_3$). *Anal.* Calcd for $C_{19}H_{24}O_8$: C, 59.99; H, 6.36. Found: C, 59.92; H, 6.15.

*Methyl (allyl 2-*O*-acetyl-3-*O*-benzyl-4-*O*-trityl-β-*D*-galactopyranosid)uronate* (**16**). —A solution of **14** (367 mg, 0.96 mmol), $TrClO_4$ (617 mg, 1.8 mmol) and *N*,*N*-dimethyl-4-aminopyridine (70.9 mg, 0.58 mmol) in CH_2Cl_2 (20 mL) and 2,4,6-collidine (0.29 mL, 2.2 mmol) was kept for 5 h at ambient temperature (TLC, solvent *D*). The reaction was terminated by addition of aq pyridine (1% water, 0.08 mL). The mixture was diluted with CH_2Cl_2, washed with 5% aq $KHSO_4$ (20 mL) and water (3 × 20 mL). The organic layer was dried and evaporated. The residue was dissolved in a minimum of PhMe. Precipitated crystals contained no carbohydrates and were separated by filtration. The filtrate was evaporated and processed by column chromatography (solvent A_7) to yield 479 mg (80%) of **16** as a syrup; R_F 0.6; $[\alpha]_D^{26}$ −27.3° (*c* 1.0, $CHCl_3$). *Anal.* Calcd for $C_{38}H_{38}O_8$: C, 73.29; H, 6.15. Found: C, 73.55; H, 6.02.

Synthesis of disaccharides.—General procedure. Solutions of the reagents (CED and 10 mol% excess of trityl ether) in CH_2Cl_2 (1 mL/mmol) and $TrClO_4$ (10 mol% of the amount of CED) in $MeNO_2$ (1 mL/0.1 mmol) were placed in separate limbs of a tuning-fork-shaped tube. The reaction components were dried by a twofold lyophilization (0.4 Pa) with dry PhH and kept for 2 h at 50°C in high vacuum. Then the components were dissolved in CH_2Cl_2 ($\sim$ 2 mL) under reduced pressure, mixed, and kept overnight at ambient temperature in the dark. When the reaction was complete (TLC, solvent A_5), aq pyridine (2% water, 0.05 mL/mmol) was added. The mixture was filtered, diluted with $CHCl_3$ (50 mL), and washed with water (3 × 20 mL). The organic phase was dried and evaporated. The residue was purified by column chromatography (PhMe–solvent A_6).

Methyl {allyl 3,4-O-isopropylidene-2-O-[methyl (2,3,4-tri-O-acetyl-β-D-galactopyranosyl)uronate]-β-D-galactopyranosid}uronate (**18**).—Reagents: **7** (424.5 mg, 0.8 mmol), CED **17** (247 mg, 0.72 mmol); initiator: $TrClO_4$ (24.7 mg, 0.072 mmol); product: **18** (254 mg, 58% with respect to **17**) containing not more than 1% of the α anomer; R_F 0.4; mp 152°C (from Et_2O), $[\alpha]_D^{21}$ +5.2° (*c* 1.0, $CHCl_3$). *Anal.* Calcd for $C_{26}H_{36}O_{16}$: C, 51.65; H, 6.00. Found: C, 51.52; H, 5.93.

Methyl {allyl 2,4-di-O-acetyl-3-O-[methyl (2,3,4-tri-O-acetyl-β-D-galactopyranosyl)uronate]-β-D-galactopyranosid}uronate (**19**).—Reagents: **10** (575 mg, 1 mmol), CED (309 mg, 0.9 mmol), (a): **17**, (b): mixture of *exo*(**17**)/*endo*-isomer; initiator: $TrClO_4$ (30.8 mg, 0.09 mmol); product: (a): **19** (293 mg, 49% with regard to CED); (b): **19** (339 mg, 57% with respect to CED) containing not more than 1% of the α anomer, R_F 0.3, syrup, $[\alpha]_D^{24}$ +35.3° (*c* 1.0, $CHCl_3$). *Anal.* Calcd for $C_{27}H_{36}O_{18}$: C, 50.00; H, 5.59. Found: C, 50.25; H, 5.82.

Methyl {allyl 2-O-acetyl-3-O-benzyl-4-O-[methyl (2,3,4-tri-O-acetyl-β-D-galactopyranosyl)uronate]-β-D-galactopyranosid}uronate (**20**).—Reagents: **16** (315 mg, 0.5 mmol), CED **17** (155 mg, 0.45 mmol); initiator: $TrClO_4$ (15.5 mg, 0.045 mmol); product: syrupy **20** (33 mg, 10% with respect to **17**); R_F 0.4; $[\alpha]_D^{22}$ +50.6° (*c* 2.4, $CHCl_3$). *Anal.* Calcd for $C_{32}H_{40}O_{17}$: C, 55.17; H, 5.79. Found: C, 55.45; H, 6.02.

Methyl {allyl 2-O-acetyl-3-O-benzyl-4-O-[methyl (2,3,4-tri-O-acetyl-β-D-galactopyranosyl)uronate]-α-D-galactopyranosid}uronate (**21**).—Yield of syrupy **21** (88 mg, 28% with respect to **17**); R_F 0.45; $[\alpha]_D^{21}$ +77.3° (*c* 2,6, $CHCl_3$). *Anal.* Calcd for $C_{32}H_{40}O_{17}$: C, 55.17; H, 5.79. Found: C, 54.85; H, 5.60.

Methyl {allyl 2-O-acetyl-3-O-benzyl-4-O-[methyl (2,3,4-tri-O-acetyl-α-D-galactopyranosyl)uronate]-α-D-galactopyranosid}uronate (**22**).—Yield of syrupy **22** (22 mg, 7% with respect to **17**); R_F 0.50; $[\alpha]_D^{21}$ +108.5° (*c* 1.9, $CHCl_3$). *Anal.* Calcd for $C_{32}H_{40}O_{17}$: C, 55.17; H, 5.79. Found: C, 55.00; H, 5.51.

ACKNOWLEDGMENT

The authors are indebted to the Fonds der Chemischen Industrie for financial support. We are grateful to Dr. A.S. Shashkov (N.D. Zelinsky Institute of Organic

Chemistry, Russian Academy of Sciences, Moscow) and Dr. V. Sinnwell (University of Hamburg) for recording the NMR spectra.

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