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Facile Synthesis of 2'-Substituted Lactoses

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Isopropylidenation of lactose with 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid gave two products, which were identified by ^{1}H- and ^{13}C-NMR as 2,3:5,6:3',4'-tri-*O*-isopropylidenelactose dimethyl acetal (1) and its 6'-*O*-(2-methoxy)-isopropyl derivative (2). These products were used for the synthesis of 2'-*O*-methyllactose (7), 2',6'-di-*O*-methyllactose (9) and 2'-*O*-benzyllactose (13).

Sialic acid is most frequently linked α-glycosidically to D-galactose in position 3 or 6 as the terminal sugar of oligosaccharides or glycoconjugates [1]. Removal of this residue in the course of pathological processes or *in vitro* exposes the penultimate D-galactose, which is then recognized by specific lectins leading, for example, to the removal of asialoglycoproteins and erythrocytes from the circulation [2, 3]. This interaction can be inhibited by D-galactose and related compounds. Generally, D-galactose-terminated oligosaccharides show a more pronounced effect than the free monosaccharide or the methyl glycosides [3, 4]. As an approach to study the binding mechanism in detail on a chemical basis, different partially methylated lactoses were needed for inhibition experiments. We looked for synthetic routes leading to these compounds. Here we describe a simple synthesis of 2'-*O*-methyllactose (**7**), 2',6'-di-*O*-methyllactose (**9**) and 2'-*O*-benzyllactose (**13**).

Results and Discussion

Hough and coworkers [5] reported the isopropylidenation of lactose with an excess of 2,2-dimethoxypropane in the presence of *p*-toluenesulfonic acid, giving the tri-*O*-isopropylidene dimethylacetal (**1**) as the major product. However, further products were not isolated and characterized. In our laboratory, isopropylidenation of lactose was

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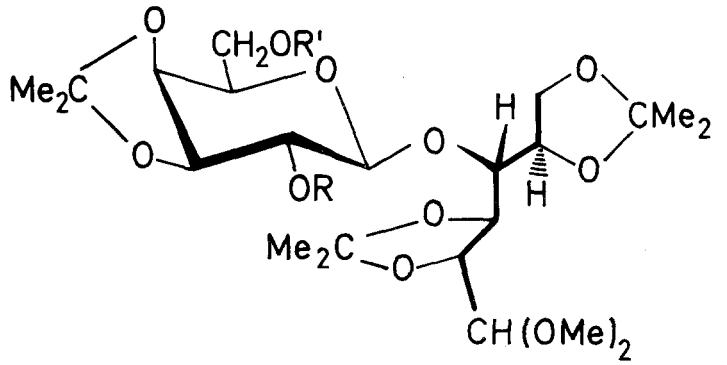
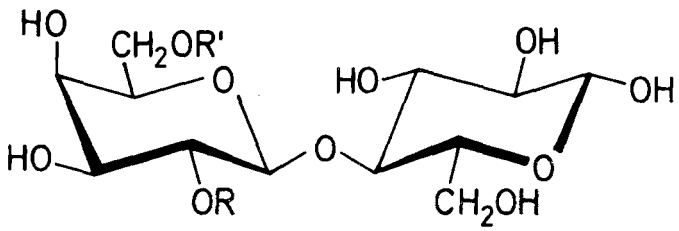


1 R = R' = H
2 R = H, R' = C(OMe)Me$_2$
3 R = R' = Ac
4 R = Ac, R' = C(OMe)Me$_2$
5 R = H, R' = Tr
6 R = Me, R' = Tr
8 R = R' = Me
10 R = Me, R' = C(OMe)Me$_2$
11 R = Bn, R' = C(OMe)Me$_2$
12 R = Bn, R' = H



7 R = Me, R' = H
9 R = R' = Me
13 R = Bn, R' = H

Figure 1. Reaction scheme for the synthesis of 2'-mono- and 2',6'-disubstituted lactoses.

done according to Hough's procedure and two products were isolated by column chromatography on silica gel. The acetal **1** was the major product (54%) and 2,3:5,6:3',4'-tri-*O*-isopropylidene-6'-*O*-(2-methoxy)-isopropyllactose dimethylacetal (**2**) (29%) was the minor compound which was characterized by the following data: the acetal **2** readily changed to **1** by standing overnight at room temperature or by boiling for 1 h in methanol. Treatment of **1** with 2,2-dimethoxypropane partially generated the acetal **2** as detected by TLC. These data suggest that **2** is a ketal derivative of **1** which is also supported by the similarity of the i.r. spectra.

The structure of **2** was finally determined by ^{1}H- and ^{13}C-NMR spectroscopy of the acetate **4** (see Tables 1 and 2). The ^{1}H-NMR spectrum shows 18 protons from the three isopropylidene groups. In addition, the signals of one *O*-methyl group (δ 3.21) and of two methyl residues (δ 1.35) are found, indicating the presence of a newly introduced 2-methoxyisopropyl group. The location of the 2-methoxyisopropyl group was determined as the O-6' position rather than O-2', since signals due to H-2' (δ 5.01, $J_{1,2}$ = 8.2, $J_{2,3}$ = 7.5 Hz) of **4** had the same chemical shift as the acetate of the acetal **3**, whereas the methylene protons on C-6' of **4** (δ 3.59, 3.73) were shifted upfield when compared to **3** (δ 4.24, 4.40). The structure of **4** was more definitely proven by comparison of its proton-decoupled ^{13}C-NMR spectrum with that of **3**. New signals due to a 2-methoxyisopropyl group at O-6' appeared at 48.7 (O**Me**), 100.2 (**C**Me$_2$) and about 27 ppm (**Me**$_2$C), and signals due to an acetyl group at O-6' of **3** (20.6 and 169.5 ppm) were not observed. Furthermore, signals of C-6' shifted from 63.4 in **3** to 59.8 ppm in **4**.

Table 1. ^{13}C-NMR Chemical shifts (ppm) of the ring carbon atoms of **1** and the acetal acetates **3** and **4** (75 MHz).

	Chemical shifts											
Compound	C-1	C-2	C-3	C-4	C-5	C-6	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′
1	107.4	74.7	77.6	78.2	79.5	64.6	103.5	74.2	75.5	73.6	75.9	62.4
3	105.1	73.7	77.3	78.0	78.2	64.7	100.2	70.9	75.3	72.7	73.9	63.4
4	105.4	73.8	76.7	78.2	78.2	64.9	100.9	72.3	75.6	73.1	73.9	59.8

The data of the other carbon atoms are given in the "Experimental" section.

The tri-*O*-isopropylidene acetal **1** has been used by Abbas *et al.* [6] for the preparation of 2′-*O*-methyllactose through the 6′-*O*-benzoate of **1**. Although the benzoylation of **1** gives the 6′-*O*-benzoate as the major product, 2′,6′-di-*O*- and 2′-*O*-benzoate are also formed. To avoid the occurrence of these byproducts, tritylation of **1** was used in the present study, followed by methylation with methyl iodide-KOH in dry dioxane, and hydrolysis with 60% acetic acid, giving 2′-*O*-methyllactose (**7**) in high yield. 2′,6′-Di-*O*-methyllactose (**9**) was also synthesized by the same procedure omitting the tritylation step.

The isopropylidene acetal **2** was considered to be a precursor superior to **1** for the synthesis of 2′-substituted lactoses. Methylation of **2** with methyl iodide-KOH in dioxane gave 86% of the corresponding 2′-*O*-methyl acetal **10**, which migrated faster than **2** on TLC. This compound (**10**) was then readily converted with 60% acetic acid to 2′-*O*-methyllactose (**7**), which was identical with the product obtained by the former procedure.

2′-*O*-benzyllactose (**13**) was also synthesized from **2** in high yield by benzylation with benzyl bromide-KOH in dioxane, followed by treatment with methanol and acid hydrolysis with 60% acetic acid.

Table 2. ^{1}H-NMR data of acetal acetates **3** and **4** (300 MHz).[a]

	Chemical shifts (δ)—									Coupling constant (Hz)			
Compound	H-1 (d)	H-2 (dd)	H-3 (dd)	H-4 (dd)	H-5 (dd)	OAc (s)	CMe_2 (s)		OMe (s)	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$
3	4.37	4.47	3.97	4.09	4.35	2.13 2.09	1.33, 1.48,	1.37, 1.56	3.42	6.1	7.2	1.5	2.6
4	4.36	4.45	3.98	4.09	4.31	2.15	1.33, 1.48,	1.35, 1.56	1.37, 3.43 3.21	6.0	7.2	1.6	2.7

	Chemical shifts (δ)							Coupling constant (Hz)					
	H-1′ (d)	H-2′ (dd)	H-3′ (dd)	H-4′ (dd)	H-5′ (ddd)	H-6′a (dd)	H-6′b (dd)	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'}$	$J_{5',6a'}$	$J_{5',6b'}$	$J_{6',6'}$
3	4.78	5.01	4.14	4.15	3.95	4.24	4.40	8.2	7.2	5.4	2.6	4.8	7.1	11.6
4	4.77	5.01	4.10	4.20	3.85	3.59	3.73	8.2	7.5	5.3	2.0	6.2	8.0	10.0

[a] d=doublet, dd=doublet of doublets, ddd=doublet of dd, s=singlet; H-6a and b could not be assigned.

The structures of **7**, **9** and **13** were further analyzed by mass spectrometry of the corresponding trimethylsilyl derivatives. The glucose moiety, which is the same for all three compounds, gives similar fragmentation patterns. The type and number of substituents in the galactose moiety, however, can be easily discriminated by sets of fragments showing the expected shifts for the m/z values of the corresponding fragments (see Fig. 2).

These results demonstrate that the isopropylidene acetal **2** is a remarkably useful precursor for the synthesis of 2'-substituted lactoses. The use of these compounds for studying the interaction of erythrocytes and macrophages has been reported [4].

Experimental

General Methods

Melting points are uncorrected. TLC was carried out on plastic sheets coated with 0.2 mm silica gel 60 (Merck); the components were located by spraying with 0.2% orcinol in 1.5 M sulfuric acid and heating. The solvent systems used for chromatography were toluene/ethyl acetate, 1/1 by vol (solvent A); and chloroform/methanol/water, 60/35/8 by vol (solvent B). Column chromatography was done under slightly increased pressure using an air-compressor. Infra-red spectra were recorded in KBr discs with a Perkin-Elmer 457 spectrometer and the data given (cm^{-1}) represent the peak maxima. Both ^{1}H- and ^{13}C-NMR spectra were recorded with a Bruker AM-300 instrument at 300 MHz and 75 MHz, respectively, for solutions in [^{2}H]chloroform with tetramethylsilane as the internal standard. Assignments were made according to [6] and by decoupling experiments.

*2,3:5,6:3',4'-Tri-O-isopropylidenelactose Dimethylacetal (**1**) and its 6'-O-(2-Methoxy)-isopropyl Derivative (**2**)*

A suspension of anhydrous lactose (0.5 g, 15 mmol) in 2,2-dimethoxypropane (4 ml) containing *p*-toluenesulfonic acid (20 mg) was heated at 80°C with magnetic stirring. The solution became clear during the course of the reaction. Progress of the reaction was monitored by TLC (solvent A). After 1.5 h lactose disappeared completely and two products (R_F 0.23 and 0.08) became visible on TLC. The mixture was cooled, neutralized with triethylamine, and then concentrated under reduced pressure. The syrup obtained was coevaporated with toluene (10 ml), applied to a column (2.5 × 20 cm) of silica gel, and eluted with a gradient solvent system of toluene/ethyl acetate (4/1 to 1/1). On evaporation, compound **1** was obtained in 59% yield (0.45 g), which was then crystallized from ether/*n*-hexane, 1/2 by vol; m.p. 129-130°C; compare with m.p. 129-131°C [6]. I.r. 3450 cm^{-1} (OH), 1385 and 1375 cm^{-1} (isopropylidene). ^{1}H-NMR: δ 4.42 (d, 1H, *J* 7.8 Hz, H-1'), 4.37 (d, 1H, *J* 6.1 Hz, H-1); 3.49 (s, 6H, $(OMe)_2$); 1.50, 1.40 and 1.33 (s, each 3H × 2, $\mathbf{C}Me_2$). ^{13}C-NMR: see Table 1 and δ 110.4 and 109.9 ($\mathbf{C}Me_2$); 55.9 and 53.7 (OMe) and 28.17, 27.12, 26.30, 25.74 and 24.04 ($Me_2\mathbf{C}$).

The acetal **2** which moved faster on TLC was eluted first and obtained as a syrup (0.18 g, 29%); i.r. 3400 cm^{-1} (OH), 1385 and 1375 cm^{-1} (isopropylidene). NMR data of **2** were not obtained, since **2** was unstable in [^{2}H]chloroform and gave a mixture of **1** and **2**.

Compound **2** (20 mg) was treated with methanol at room temperature overnight or in boiling methanol (1 h), giving **1** quantitatively. A solution of **1** (100 mg) in 2,2-dimethoxypropane (4 ml) containing *p*-toluenesulfonic acid (25 mg) was heated (60°C) and the reaction monitored by TLC (solvent A). After 3 h, about 40% of **1** (R_F 0.08) was converted to **2** (R_F 0.23), along with some decomposition products which migrated slower than **1**.

Conventional acetylation (pyridine/acetic anhydride) of **1** gave **3**. ^{13}C-NMR: see Table 1 and δ 170.7 and 169.5 (CO); 110.8, 110.7 and 108.0 (**C**Me_2); 55.6 and 53.2 (OMe); 27.6, 27.5, 26.4, 26.3, 26.1 and 24.7 (**Me**$_2$C); 20.9 and 20.8 (**C**H_3CO). Conventional acetylation of **2** gave **4**. ^{13}C-NMR: see Table 1 and 169.9 (CO); 110.8 and 110.3 (**C**Me_2); 55.9 and 53.7 (OMe); 28.0, 27.7, 26.5, 26.4, 26.3, 25.0, 24.6 and 24.4 (**Me**$_2$C); 21.1 (**C**H_3CO).

*2,3:5,6:3′4′Tri-O-isopropylidene-6′O-tritylactose Dimethylacetal (**5**)*

To a solution of **1** (102 mg, 2 mmol) in dry pyridine (5 ml) trityl chloride (65 mg, 2.4 mmol) was added, and the solution heated (70°C) for 3 h with magnetic stirring. TLC (solvent A) showed the presence of one major product (R_F 0.53); there were also small amounts of a faster-moving product and unchanged **1**. The mixture was extracted with chloroform (100 ml) and washed successively with 0.5 M HCl and water, dried over $MgSO_4$, and concentrated. The residue was subjected to column chromatography on silica gel (*n*-hexane/ethyl acetate, 3/2 by vol), which gave **5** (60 mg, 40%); homogeneous on TLC (solvent A), R_F 0.56. I.r. 3450 cm^{-1} (OH), 1385 and 1375 cm^{-1} (isopropylidene), 710 cm^{-1} (Ph).

*2,3:5,6:3′4′Tri-O-isopropylidene-2′O-methyl-6′O-tritylactose Dimethylacetal (**6**)*

Ground KOH (0.5 g) was added with stirring to a solution of **5** (50 mg; 67 μmol) in dry dioxane (5 ml) and kept for 30 min at 70°C. Then methyl iodide (2 ml) was added. The mixture was stirred for 3 h, extracted with chloroform (70 ml), neutralized with 0.5 M HCl and washed twice with water. After drying over $MgSO_4$, the organic layer was concentrated under reduced pressure. The resulting syrup was subjected to column chromatography on silica gel (toluene/ethyl acetate, 3/2 by vol) to give **6** (50 mg. 98%) as a syrup; homogeneous on TLC (solvent A), R_F 0.75. I.r. [no absorption near 3500 cm^{-1} (OH)].

*2′O-Methyllactose (**7**)*

A solution of **6** (50 mg, 65 μmol) in 60% acetic acid (5 ml) was heated with stirring for 1 h at 80°C. The mixture was diluted with water and lyophilized to give a white solid. The solid was suspended in water (3 ml) and subjected to filtration using a small pipette with cotton wool to remove trityl alcohol, and then lyophilized again, giving **7** (25 mg, quantitative yield) as a white solid. M.p. 155-157°C, compare with m.p. 156-158°C [6]; homogeneous on TLC (solvent B), R_F 0.28, $R_{lactose}$ 1.78. For MS see Fig. 2.

*2,3:5,6:3′4′Tri-O-isopropylidene-2′6′di-O-methyllactose Dimethylacetal (**8**)*

The tri-*O*-isopropylidene acetal **1** (100 mg) was methylated with methyl iodide-KOH in dry dioxane as described for **6**. The resulting syrup was purified by column chromatography on silica gel to give **8** (107 mg, 96%) as a syrup; homogeneous on TLC (solvent A), R_F 0.48. I.r. [no absorption near 3500 cm^{-1} (OH)], 1385 and 1375 cm^{-1} (isopropylidene).

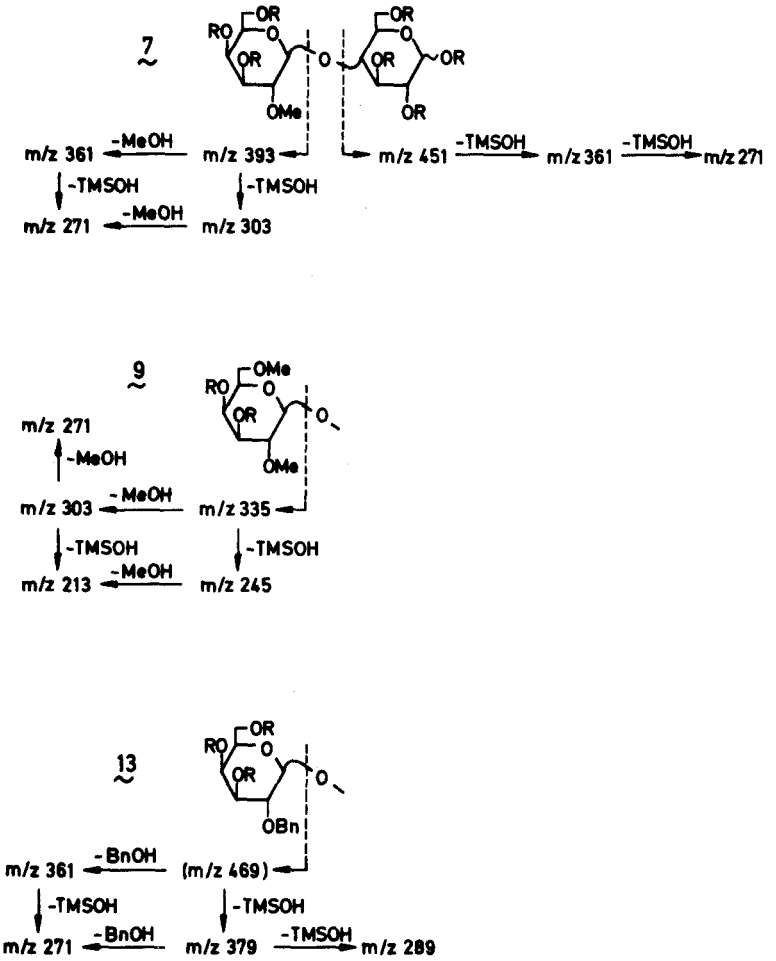


Figure 2. Mass chromatographic fragmentation pattern of compounds **7**, **9** and **13** as their trimethylsilyl derivatives (70 eV, electron impact); R = trimethylsilyl (TMS).

*2'6'Di-O-methyllactose (**9**)*

Hydrolysis of **8** (70 mg, 130 μmol) was carried out essentially as described for **7**. After lyophilization, the resulting amorphous solid was subjected to column chromatography on silica gel and gave **9** (46 mg, 95%); homogeneous on TLC (solvent B), R_F 0.29, $R_{lactose}$ 3.28. For MS see Fig. 2.

*2,3:5,6:3'4Tri-O-isopropylidene-2'O-methyl-6'O-(2-methoxy)-isopropyl-lactose Dimethylacetal (**10**)*

The isopropylidene acetal **2** (90 mg, 148 μmol) was methylated with methyl iodide-KOH in dry dioxane as described for **6** (reaction time, 1.5 h). The resulting syrup was purified

by column chromatography on silica gel to give **10** (79 mg, 90%) as a syrup; homogeneous on TLC (solvent A), R_F 0.53. I.r. [no absorption near 3500 cm^{-1} (OH)], 1385 and 1375 cm^{-1} (isopropylidene).

The 2'-*O*-methylisopropylidene acetal **10** (70 mg, 118 μmol) was hydrolyzed with 60% acetic acid (1 h, 80°C), and lyophilized to give **7** (35 mg, 83%) as a white solid, which was identical with the product described above.

*2'-O-Benzyl-2,3:5,6:3',4'-tri-O-isopropylidene-6'-(2-methoxy)-isopropyl-lactose Dimethylacetal (**11**)*

Ground KOH (1.0 g) was added to the solution of **2** (150 mg, 248 μmol) in dry dioxane (8 ml) and heated for 30 min at 60°C with stirring. Benzyl bromide (1 ml) was added and the solution stirred for 1.5 h at 90°C. TLC (solvent A) showed a single product (R_F 0.49). The mixture was extracted with chloroform (100 ml), neutralized with 0.5 M HCl, washed twice with water, and then dried. The chloroform layer was concentrated under reduced pressure to remove unreacted benzyl bromide and benzyl alcohol, giving a syrup. The syrup was subjected to column chromatography on silica gel (toluene/ethyl acetate, 4/1 by vol), to give **11** (145 mg, 87%). I.r. [no absorption near 3500 cm^{-1} (OH)], 750 and 705 cm^{-1} (Ph).

*2'-O-Benzyl-2,3:5,6:3',4'-tri-O-isopropylidenelactose Dimethylacetal (**12**)*

A solution of **11** (80 mg) in 95% methanol was boiled for 2 h. The mixture was evaporated under reduced pressure to give a syrup, which was purified by column chromatography on silica gel (toluene/ethyl acetate, 7/3 by vol); yielding **12** (49 mg, 67%); homogeneous on TLC (solvent A), R_F 0.19. I.r. 3500 cm^{-1} (OH), 1388 and 1375 cm^{-1} (isopropylidene), 750 and 705 cm^{-1} (Ph).

*2'-O-Benzyllactose (**13**)*

The compound **12** (15 mg) was hydrolyzed with 60% acetic acid essentially as described for **7**. The hydrolysate was lyophilized to give **13** (10 mg, 97%) as a white solid; homogeneous on TLC (solvent B), R_F 0.33. For MS see Fig. 2.

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