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Synthesis and NMR studies of methyl 3-*O*-[(*R*)- and (*S*)-1-carboxyethyl]-α-D-gluco-, galacto- and manno-pyranosides

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Abstract

The title compounds were prepared by condensation of a suitably protected monosaccharide and (*R*)- or (*S*)-2-chloropropionic acid followed by removal of the protecting groups. Characterisation by ^{1}H and ^{13}C NMR spectroscopy and NOE difference experiments revealed both chemical shift and enhancement differences between the diastereomers which could be used in determination of the absolute configuration of the 1-carboxyethyl group.

Keywords: Diastereomers; Methyl glucosides; Monosaccharide derivatives

1. Introduction

Sugar residues etherified with lactic acid are important components in several bacterial polysaccharides [1–3]. The biosynthetic pathway to the lactic acid derivatives involves the conversion of a phosphoenol pyruvate into an enol of pyruvic acid which is then reduced to the (*R*)- or (*S*)-1-carboxyethyl group [1]. Both the (*R*)- and (*S*)-forms of the 1-carboxyethyl groups substituting the 3-, 4- or 6-positions of different monosaccharide units have been found [1–4]. The substitution position of the 1-carboxyethyl group has mainly been determined, after reduction of the carboxylic acid group, by mass spectrometry of the derived alditol acetates or the partly methylated alditol acetates formed. The substitution position can also be determined by NMR spectroscopy as the ^{13}C chemical shift of the signal for the carbon substituted with the 1-carboxyethyl group will appear 8–9 ppm downfield, relative to the corresponding signal from a non-substituted sugar. The (*R*)- or (*S*)-configuration of the 1-carboxyethyl group was usually determined, after reduction of the carboxylic acid group, by gas chromatographic comparison of the derived alditol acetates with those of synthetic samples. To avoid synthesis of reference substances, the need for more direct methods for determination of the configuration of the 1-carboxyethyl group is obvious. So far two different alternative methods have been described. These are based on (i) NOE measurements on the conformationally rigid lactones formed between the carboxyl group and an adjacent hydroxyl group [5] and (ii) circular dichroism (CD) spectroscopy on methyl glycosides of 1-carboxyethyl substituted sugars [6]. These methods are not entirely general so there is still the need for reference substances

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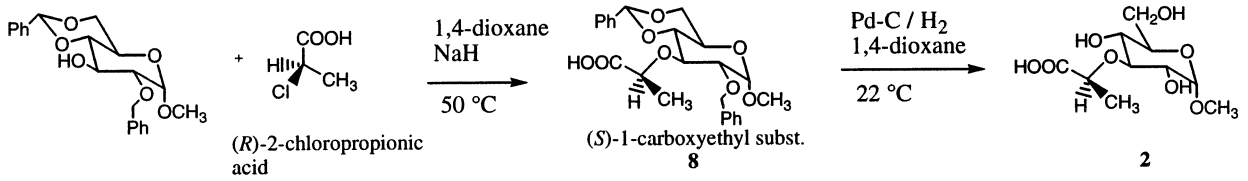

Scheme 1.

and for further improvements of alternative methods.

At our department the capsular polysaccharides from several strains of *Butyrivibrio fibrisolvens* are under investigation and 1-carboxyethyl substituted sugars are common components of these polysaccharides [3]. Thus, it is an advantage for us to have different 1-carboxyethyl substituted sugars available in order to facilitate the structural studies, and for further development of more convenient methods for determination of the configuration of the 1-carboxyethyl group. We report herein the synthesis of the (*R*)- and (*S*)- forms of the methyl 3-*O*-(1-carboxyethyl)-α-D-gluco- (**1** and **2**), galacto- (**3** and **4**) and manno- (**5** and **6**) pyranosides.

2. Results and discussion

The 1-carboxyethyl substituted methyl glycosides **1**, **2**, **3**, **4**, **5** and **6** were synthesised by the condensation of (*S*)- or (*R*)-2-chloropropionic acid [7,8] with protected monosaccharide derivatives in which all hydroxyl groups but that of the position for substitution were blocked. This method was used previously for the synthesis of other 1-carboxyethyl substituted sugars [6,9] (Scheme 1).

Inversion of the configuration at C-2′ takes place when the hydroxy group of the sugar reacts with the 2-chloropropionic acid forming an ether. The monosaccharide derivatives chosen were the readily available methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-gluco- and manno-pyranosides [10,11]. The galactose derivative chosen was methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranoside which was prepared from methyl 4,6-*O*-benzylidene-α-D-galactopyranoside [12] by partial benzylation of the 2-position as described by Garegg et al. [10]. In addition to the expected product in the condensation minor amounts, 1–6% as determined by ^{1}H NMR spectroscopy, of the other diastereomer was formed during the reaction conditions used. These amounts varied with respect to the absolute configuration of the 2-chloropropionic acid and the stereochemistry of the adjacent hydroxyl groups of the sugar. The protected 3-substituted methyl glycopyranosides of glucose and galactose were purified from the contaminating diastereomers by crystallisation before removal of the benzyl ether and the 4,6-*O*-benzylidene acetal protecting groups. The latter groups were removed by hydrogenolysis to give the 3-substituted methyl glycopyranosides **1**, **2**, **3** and **4**. We were not able to purify the protected 3-substituted mannose derivatives from the contaminating diastereomers before deprotection. Therefore, isomeric mixtures (98/2 and 94/6, respectively) were obtained from the deprotected mannosides **5** and **6**.

The 1-carboxyethyl substituted methyl glycosides were investigated by NMR spectroscopy and the ^{1}H and ^{13}C NMR chemical shifts are given in Table 1. When the ^{13}C chemical shifts are compared with those of the unsubstituted methyl glycosides significant shifts are only observed for the C-2, C-3 and C-4 signals with a large downfield shift for the signal of the substituted carbon and upfield shifts for the signals of the two adjacent carbons. The magnitude of these shifts is dependent on the configuration of the 1-carboxyethyl group and differences between the (*R*)- and (*S*)-forms are thus observed. For the methyl glucosides **1** and **2** an upfield shift ($-0.6/-0.9$ ppm) for the C-2 signal of **1** and the C-4 signal of **2** was observed and almost no shift for the C-4 signal of **1** and the C-2 signal of **2**. In addition to the shift differences for the (*R*)- and (*S*)-forms the galactosides and mannosides show a shift pattern for the stereochemically similar compounds **3** and **6**,

Table 1
^{1}H and ^{13}C chemical shifts of 3-*O*-[(*R*)- and (*S*)-1-carboxyethyl]-substituted methyl α-D-glycosides **1**, **2**, **3**, **4**, **5** and **6** for solutions in D_2O

Compound	H-1 C-1	H-2 C-2	H-3 C-3	H-4 C-4	H-5 C-5	H-6a/H-6b C-6	OCH_3 OCH_3	C-1′	H-2′ C-2′	H-3′ C-3′
(*R*)-Glc*p* (**1**)[a]	4.83	3.63	3.52	3.48	3.62	3.86/3.75	3.41		4.26	1.40
	99.62	71.46	82.79	70.56	72.54	61.24	55.64	182.70	79.62	19.58
(*S*)-Glc*p* (**2**)	4.79	3.64	3.52	3.46	3.64	3.85/3.73	3.41		4.23	1.39
	100.29	72.31	82.66	69.54	72.65	61.42	55.85	183.00	79.48	19.55
Me Glc*p*[b]	4.80	3.55	3.66	3.40	3.64	3.87/3.75	3.42			
	100.10	72.05	73.92	70.40	72.41	61.40	55.84			
(*R*)-Gal*p* (**3**)	4.87	3.92	3.60	4.12	3.85	3.75/3.74	3.40		4.11	1.37
	99.79	67.96	78.06	67.30	71.47	62.09	55.66	182.41	76.42	19.70
(*S*)-Gal*p* (**4**)	4.84	3.91	3.63	4.04	3.89	3.74/3.74	3.41		4.06	1.37
	100.18	68.12	78.95	67.62	71.09	62.14	55.79	182.79	77.45	19.40
Me Gal*p*	4.84	3.81	3.81	3.97	3.89	3.74/3.74	3.41			
	100.25	69.03	70.32	70.06	71.56	62.06	55.86			
(*R*)-Man*p* (**5**)	4.79	4.01	3.58	3.71	3.63	3.90/3.76	3.40		4.08	1.38
	101.21	68.50	79.97	66.70	73.26	61.83	55.51	182.52	77.61	19.35
(*S*)-Man*p* (**6**)	4.78	4.05	3.54	3.73	3.61	3.88/3.74	3.39		4.10	1.38
	101.57	67.97	79.40	66.34	73.61	61.80	55.49	182.22	76.39	19.64
Me Man*p*	4.76	3.93	3.76	3.64	3.62	3.90/3.76	3.41			
	101.69	70.74	71.38	67.60	73.38	61.78	55.53			

[a] (*R*)-Glc*p*, methyl 3-*O*-[(*R*)-1-carboxyethyl]-α-D-glucopyranoside, etc.
[b] Me Glc*p*, methyl α-D-glucopyranoside, etc.

and **4** and **5** in which the adjacent carbons have the hydroxyl groups in axial and equatorial positions. If only the stereochemistry nearest to the 1-carboxyethyl substituent is considered, the pairs C-2, C-3 and C-4 are mirror images (Fig. 1). This means that the (*R*)-form of the galactoside **3** has the same stereochemistry around C-3 as the (*S*)-form of the mannoside **6** and this is reflected in similar shifts for the signals of the axially substituted carbons and the equatorially substituted carbons compared with those of the unsubstituted methyl glycosides. The shifts for the signals of **3** C-2/**6** C-4, **3** C-3/**6** C-3 and **3** C-4/**6** C-2 are shifted by approximately the same amount (~ -1.2, ~ 7.9 and -2.8 ppm) and this is also observed for the signals of **4** C-2/**5** C-4, **4** C-3/**5** C-3 and **4** C-4/**5** C-2 (-0.9, 8.6 and ~ -2.3 ppm).

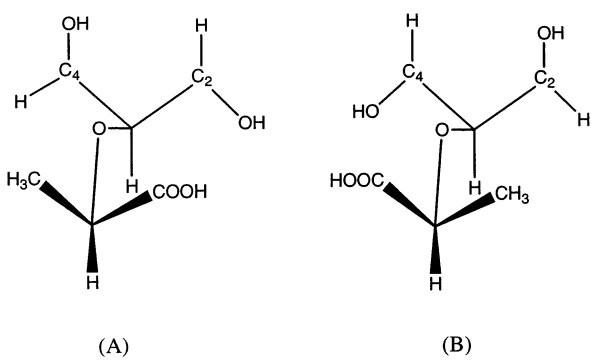

Fig. 1. The stereochemistry around C-3 in the 1-carboxyethyl substituted methyl D-galactosides and D-mannosides. The stereochemistry for **3** (A) and **6** (B) is shown.

In comparison with the ^{1}H chemical shifts from the pertinent methyl glycosides only the signals from H-2, H-3 and H-4 are affected. A minor downfield shift (0.06–0.15 ppm) for the H-2 and H-4 signals and an upfield shift (between -0.14 and -0.22 ppm) for the H-3 signal are observed. No significant differences were observed for the (*R*)- and (*S*)-forms of the methyl glycosides but a similar shift pattern to that obtained for the ^{13}C signals could be observed for the stereochemically similar compounds of the methyl galactosides and mannosides, **3** and **6**, and **4** and **5**, respectively.

Thus the chemical shifts of both the ^{1}H and ^{13}C signals are dependent on the stereochemistry around the substitution position as well as on the configuration of the 1-carboxyethyl group. The configurational dependence of the ^{1}H signals is minor but the ^{13}C signals are

Table 2
Observed NOE[a] in % of [(*R*)- and (*S*)-1-carboxyethyl]-substituted methyl α-D-glycosides **1**, **2**, **3**, **4**, **5** and **6** when H-3′ of the 1-carboxyethyl group was presaturated (86–98%)

Compound	H-2	H-3	H-4	H-2′
(*R*)-Glc*p* (**1**)[b]	0.033	0.087	0.067	1.30
(*S*)-Glc*p* (**2**)	0.073	0.073	0.060	1.30
(*R*)-Gal*p* (**3**)	0.063	0.090	0.113[c]	1.17
(*S*)-Gal*p* (**4**)	0.077	0.210	0.046[c]	1.14
(*R*)-Man*p* (**5**)	0.077	0.223	0.067	1.03
(*S*)-Man*p* (**6**)	0.120	0.077	0.053	1.00

[a] Data were obtained from NOE difference experiments at 400 or 600 MHz, setting the intensity of the H-3′ signal to −300%.

[b] (*R*)-Glc*p*, methyl 3-*O*-[(*R*)-1-carboxyethyl]-α-D-glucopyranoside, etc.

[c] Overlap of signals resolved by addition of pyridine-d_5.

significantly changed and thus they could be used for the determination of the relative configuration of the 1-carboxyethyl group.

The methyl glycosides **1**, **2**, **3**, **4**, **5** and **6** were subjected to NOE difference experiments at both 400 and 600 MHz with presaturation of H-3′ of the 1-carboxyethyl group. Only minor differences in magnitude of the enhancements were observed for the experiments at the two magnetic fields and the relative enhancements between the signals were the same. The observed enhancements for the signals of the ring protons H-2, H-3 and H-4 and the H-2′ signal are given in Table 2. No significant difference was observed for the (*R*)- and (*S*)-forms of the methyl glucosides **1** and **2** with the adjacent hydroxyl groups in equatorial positions. However, for the different forms of the methyl galactosides, **3** and **4**, and mannosides, **5** and **6**, which have the adjacent hydroxy groups in axial and equatorial positions, there are similar enhancements of the signals of the ring protons for the compounds with similar stereochemistry (Fig. 1), i.e., compounds **3** and **6**, and **4** and **5**, respectively.

When adding the information obtained from all the ^{1}H and ^{13}C NMR chemical shifts and the NOE experiments then there are significant differences between the (*R*)- and (*S*)-forms of the methyl 3-*O*-(1-carboxyethyl)-α-glycosides and it should be possible to identify the configuration of the 1-carboxyethyl group if the absolute configuration of the sugar is known. However, there could be many other contributions influencing the NMR data when the sugar substituted with a 1-carboxyethyl group is a component in a polysaccharide, making the identification based on these data difficult.

An additional difference between the (*R*)- and (*S*)-1-carboxyethyl substituted methyl α-D-glycosides is the values for the optical rotation, $[\alpha]_D$. All pairs show a 60° difference with the (*R*)-form having the most positive value. Similar differences have been observed for other 1-carboxyethyl substituted methyl glycosides [6].

3. Experimental

General methods.—NaH (55–60% suspension in oil) was washed with petroleum ether prior to use. TLC was performed on precoated plates (E. Merck Silica Gel 60 F_{254}), detection being afforded with 5% H_2SO_4 and heating. Column chromatography of synthetic intermediates was performed on Matrex silica gel 60 Å (35–70 μm, Amicon). Gel permeation chromatography of deprotected products was carried out on a column (2.6 × 80 cm) of Bio-Gel P-2, using H_2O as eluent. Solvents were used as purchased except for 1,4-dioxane which was dried with 3 Å molecular sieves prior to use. The petroleum ether fraction used had a bp of 60–70 °C. Solutions were concentrated under reduced pressure at temperatures not exceeding 50 °C. Optical rotations were recorded at 20–25 °C using Perkin–Elmer 141 or Perkin–Elmer 341 polarimeters. The $[\alpha]_D$-values for the mannose derivatives **5**, **13**, **6** and **14** have been corrected since the analytical samples contained small but controlled amounts of the contaminating diastereomer. High-resolution mass spectrometry (HRMS) was applied in the FAB positive mode on a Jeol JMS-SX/SX-102A four-sector tandem instrument with either glycerol or polyethyleneglycol as the matrix. NMR spectra were recorded at 400 MHz (^{1}H) and 100 MHz (^{13}C) with a Bruker DRX 400 instrument except for some of the NOE-difference spectra, which were recorded on a Bruker

DRX 600 spectrometer. Chemical shifts are given relative to Me_4Si using internal acetone (δ_H 2.225, δ_C 31.07) for D_2O solutions at 30 °C, whereas NMR spectra recorded for samples in $CDCl_3$ are referenced to internal Me_4Si (δ 0.00). NMR spectra on all fully protected 1-carboxyethyl substituted sugars were recorded in $CDCl_3$ containing 1.5% pyridine-d_5 due to poor stability in $CDCl_3$. NMR spectra were recorded at 30 °C where nothing else is stated. Assignments of signals were done from standard H,H-COSY and HMQC experiments. ^{1}H NMR chemical shifts and coupling constants of overlapping signals were obtained from the cross-peaks in the 2D spectra. Melting points were determined on a METTLER FP62 instrument and are uncorrected.

*Methyl 3-*O*-[(*R*)-1-carboxyethyl]-α-*D*-glucopyranoside* (**1**).—A solution of methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside [10] (1.00 g, 2.69 mmol) and (*S*)-2-chloropropionic acid [7,8] (1.26 g, 11.6 mmol) in 1,4-dioxane (50 mL) was stirred with NaH (1.8 g, 75 mmol) for 18 h at 50°C, whereupon the mixture was cooled and water (15 mL) added to destroy excess NaH. A two-phase mixture was obtained. The upper phase, containing the product, was a 1,4-dioxane–water mixture and the lower viscous phase contained mostly salt. The upper phase was collected, washed with petroleum ether (3 × 15 mL), and acidified (pH 2–3, HCl), and the product was extracted with CH_2Cl_2 (3 × 30 mL). The CH_2Cl_2 solution was washed with H_2O (15 mL), dried (Na_2SO_4), filtered, and concentrated to give crude methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-[(*R*)-1-carboxyethyl]-α-D-glucopyranoside (**7**), which was contaminated by 6% of methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-[(*S*)-1-carboxyethyl]-α-D-glucopyranoside (**8**). Recrystallisation from toluene–petroleum ether gave pure **7** (814 mg, 68%) as white needles; mp 171–172 °C, $[\alpha]_D$ +26° (*c* 0.5, $CHCl_3$); HRMS: Calcd for $C_{24}H_{28}O_8$ + Na: 467.1682, found 467.1663 $[M + Na]^+$; ^{1}H NMR: δ 10.25 (s, 1 H, COOH), 7.48–7.28 (m, 10 H, Ar–H), 5.54 (s, 1 H, PhCH), 4.81 (d, 1 H, J_{gem} 12 Hz, $PhCH_2$), 4.70 (d, 1 H, J_{gem} 12 Hz, $PhCH_2$), 4.64 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.40 (q, 1 H, J 7 Hz, carboxyethyl-CH), 4.26 (dd, 1 H, $J_{6a,6b}$ 9.5, $J_{5,6a}$ 4.5 Hz, H-6a), 3.92 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3), 3.78 (ddd, 1 H, $J_{4,5}$ 10 Hz, H-5), 3.72 (dd, 1 H, H-6b), 3.59 (m, 2 H, H-2, 4), 3.33 (s, 3 H, OCH_3), 1.47 (d, 3 H, *J* 7 Hz, carboxyethyl–CH_3); ^{13}C NMR: δ 174.7 (COOH), 137.0–125.8 (aromatic), 101.3 (PhCH), 98.1 (C-1), 81.6 (C-4), 78.4 (C-2), 78.1 (C-3), 77.3 (carboxyethyl–CH), 73.1 ($PhCH_2$), 68.9 (C-6), 62.2 (C-5), 55.3 (OCH_3), 18.9 (carboxyethyl–CH_3).

Hydrogenolysis of compound **7** (112 mg, 252 μmol) dissolved in 10 mL 1,4-dioxane was catalysed by Pd–C (50 mg) and carried out for 4 h at 20–25 °C. The catalyst was removed by filtration and one drop of aq conc NH_3 was added to the filtrate to avoid accidental hydrolysis and possible lactone formation under the following concentration to dryness. This gave the crude ammonium salt of **1** which was purified on a column of Bio-Gel P-2 followed by passage through a column (1 × 5 cm) of cation exchanger (Dowex 50, Na^+). After lyophilisation, the Na salt of **1** (66 mg, 91%) was afforded as a white amorphous powder; $[\alpha]_D$ +144° (*c* 1.0, H_2O); HRMS: Calcd for $C_{10}H_{18}O_8$ + H: 267.1079, found 267.1102 $[M + H]^+$.

*Methyl 3-*O*-[(S)-1-carboxyethyl]-α-*D*-glucopyranoside* (**2**).—Starting from methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (1.03 g, 2.76 mmol), (*R*)-2-chloropropionic acid (1.00 g, 9.21 mmol) and NaH (1.65 g, 69 mmol), using the same experimental and work-up conditions as in the synthesis of **7**, methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-[(*S*)-1-carboxyethyl]-α-D-glucopyranoside (**8**) contaminated by less than 1% of **7** was obtained. Recrystallisation from toluene–petroleum ether yielded pure **8** (890 mg, 73%) as colourless crystals; mp 167.5–168.5 °C, $[\alpha]_D$ +18° (*c* 1.0, $CHCl_3$); HRMS: Calcd for $C_{24}H_{28}O_8$ + H: 445.1862, found 445.1902 $[M + H]^+$. ^{1}H NMR: δ 8.4 (s, 1 H, COOH), 7.48–7.27 (m, 10 H, Ar–H), 5.54 (s, 1 H, PhCH), 4.81 (d, 1 H, J_{gem} 12 Hz, $PhCH_2$), 4.67 (d, 1 H, J_{gem} 12 Hz, $PhCH_2$), 4.59 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.43 (q, 1 H, *J* 7 Hz, carboxyethyl–CH), 4.26 (dd, 1 H, $J_{6a,6b}$ 10, $J_{5,6a}$ 4.5 Hz, H-6a), 3.95 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$

Hz, H-3), 3.82 (ddd, 1 H, H-5), 3.71 (dd, 1 H, $J_{5,6b}$ 10 Hz, H-6b), 3.64 (dd, 1 H, $J_{4,5}$ 9 Hz, H-4), 3.59 (dd, 1 H, H-2), 3.38 (s, 3 H, OCH_3), 1.48 (d, 3 H, J 7 Hz, carboxyethyl–CH_3); ^{13}C NMR: δ 174.8 (COOH), 137.8–126.1 (aromatic), 101.8 (PhCH), 98.8 (C-1), 80.8 (C-4), 79.5 (C-2), 78.9 (C-3), 77.4 (carboxyethyl–CH), 73.7 ($PhCH_2$), 69.0 (C-6), 62.1 (C-5), 55.5 (OCH_3), 19.1 (carboxyethyl–CH_3).

Hydrogenolysis of **8** (121 mg, 272 μmol), followed by purification and exchange of cations, was done in the same way as for compound **7**. This gave the Na salt of **2** (75 mg, 95%) as a white amorphous powder; $[\alpha]_D$ +81° (*c* 1.0, H_2O); HRMS: Calcd for $C_{10}H_{18}O_8$ + Na: 289.0899, found 289.0895 $[M + Na]^+$.

*Methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-*D*-galactopyranoside* (**9**).—To a solution of methyl 4,6-*O*-benzylidene-α-D-galactopyranoside (4.00 g, 14.2 mmol) prepared according to [12], tetrabutylammoniumhydrogen sulfate (1.00 g, 2.95 mmol), and benzyl bromide (4.17 g, 24.4 mmol) in CH_2Cl_2 (100 mL) were added in aq 5% NaOH (10 mL) as described by Garegg et al. [10]. The mixture was boiled under reflux and vigorous stirring for 4 days, allowed to cool, and the aqueous phase was removed. The organic phase was dried (Na_2SO_4), filtered, and concentrated. The crude product was a mixture of mainly the 3-*O*- and the 2-*O*-benzyl derivatives, but minor amounts of the 2,3-di-*O*-benzyl derivative and starting material were also present. To be able to separate the 3-*O*-benzyl- from the 2-*O*-benzyl derivative on a silica gel column, the mixture was acetylated (Ac_2O in pyridine; 25 °C, overnight). After concentration, the products were separated using 3:2 petroleum ether–EtOAc as the eluent. The pooled fractions containing the 3-*O*-acetyl derivative were concentrated to dryness to give methyl 3-*O*-acetyl-2-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranoside (**10**) contaminated with 12–15% of the 2,3-di-*O*-benzyl derivative according to ^1H NMR. The mixture was deacetylated with NaOMe in MeOH, neutralised (Dowex 50 H^+), and after evaporation the residue was purified on a silica gel column eluted with 1:2 petroleum ether–EtOAc to give pure **9** (2.41 g, 46% over all yield). Crystallisation of **9** from toluene–petroleum ether gave white crystals: mp 100.6–101.5 °C; $[\alpha]_D$ +59° (*c* 1.1, $CHCl_3$); HRMS: Calcd for $C_{21}H_{24}O_6$ + H: 373.1651, found 373.1642 $[M + H]^+$; ^1H NMR ($CDCl_3$, 25 °C): δ 7.55–7.25 (m, 10 H, Ar–H), 5.54 (s, 1 H, PhCH), 4.80 (d, 1 H, J_{gem} 12 Hz, $PhCH_2$), 4.78 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.65 (d, 1 H, J_{gem} 12 Hz, $PhCH_2$), 4.27 (dd, 1 H, $J_{3,4}$ 3.5, $J_{4,5}$ 1.0 Hz, H-4), 4.24 (dd, 1 H, $J_{6a,6b}$ 12.5, $J_{5,6a}$ 1.5 Hz, H-6a), 4.13 (dd, 1 H, $J_{2,3}$ 10 Hz, H-3), 4.05 (dd, 1 H, $J_{5,6b}$ 1.5 Hz, H-6b), 3.82 (dd, 1 H, H-2), 3.66 (m, 1 H, H-5), 3.36 (s, 3 H, OCH_3), 2.46 (s, 1 H, HO-3); ^{13}C NMR ($CDCl_3$, 25 °C): δ 137.6–126.3 (aromatic), 101.2 (PhCH), 98.9 (C-1), 76.8 (C-2), 76.1 (C-4), 73.3 ($PhCH_2$), 69.3 (C-6), 68.5 (C-3), 62.3 (C-5), 55.6 (OCH_3).

Acetylation of a sample of pure **9** gave, after purification, an analytical sample of **10** as a syrup; $[\alpha]_D$ +128° (*c* 1.0, $CHCl_3$); HRMS: Calcd for $C_{23}H_{26}O_7$ + H: 415.1756, found 415.1850 $[M + H]^+$; ^1H NMR ($CDCl_3$): δ 7.55–7.24 (m, 10 H, Ar–H), 5.49 (s, 1 H, PhCH), 5.28 (dd, 1 H, $J_{2,3}$10.5, $J_{3,4}$ 3.5 Hz, H-3), 4.80 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.74 (d, 1 H, J_{gem} 12 Hz, $PhCH_2$), 4.62 (d, 1 H, J_{gem} 12 Hz, $PhCH_2$), 4.45 (dd, 1 H, $J_{4,5}$ ~1 Hz, H-4), 4.21 (dd, 1 H, $J_{6a,6b}$ 12.5, $J_{5,6a}$ 1.5 Hz, H-6a), 4.10 (dd, 1 H, H-2), 4.05 (dd, 1 H, $J_{5,6b}$ 1.5 Hz, H-6b), 3.71 (m, 1 H, H-5), 3.40 (s, 3 H, OCH_3), 2.08 (s, 3 H, OAc); ^{13}C NMR ($CDCl_3$): δ 170.7 (OAc), 138.3–126.2 (aromatic), 100.8 (PhCH), 99.2 (C-1), 74.3 (C-4), 73.6 (C-2), 73.5 ($PhCH_2$), 70.9 (C-3), 69.3 (C-6), 62.1 (C-5), 55.6 (OCH_3), 21.1 (OAc).

*Methyl 3-*O*-[(*R*)-1-carboxyethyl]-α-*D*-galactopyranoside* (**3**).—Starting from **9** (0.80 g, 2.15 mmol), (*S*)-2-chloropropionic acid (0.49 g, 4.5 mmol) and NaH (1.54 g, 64 mmol) using the same experimental and work-up conditions as in the synthesis of **7**, methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-[(*R*)-1-carboxyethyl]-α-D-galactopyranoside (**11**), contaminated with ca. 5% of methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-[(*S*)-1-carboxyethyl]-α-D-galactopyranoside (**12**), was obtained. Recrystallisation from toluene–petroleum ether yielded **11** (0.63 g, 66%) as white crystals: mp 167.5–168.5 °C; $[\alpha]_D$ +104° (*c* 1.0, $CHCl_3$);

HRMS: Calcd for $C_{24}H_{28}O_8 + H$: 445.1862, found 445.1857 $[M + H]^+$; ^1H NMR: δ 9.79 (s, 1 H, COOH), 7.54-7.25 (m, 10 H, Ar-H), 5.57 (s, 1 H, PhCH), 4.84 (d, 1 H, J_{gem} 12 Hz, $PhCH_2$), 4.77 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.65 (d, 1 H, J_{gem} 12 Hz, $PhCH_2$), 4.34 (dd, 1 H, $J_{4,5}$ 1 Hz, H-4), 4.26 (q, 1 H, J 7 Hz, carboxyethyl–CH), 4.26 (dd, 1 H, $J_{6a,6b}$ 12.5, $J_{5,6a}$ 1.5 Hz, H-6a), 4.07 (dd, 1 H, H-2), 4.07 (dd, 1 H, $J_{5,6b}$ 2 Hz, H-6b), 3.98 (dd, 1 H, $J_{2,3}$ 10, $J_{3,4}$ 3.5 Hz, H-3), 3.62 (m, 1 H, H-5), 3.32 (s, 3 H, OCH_3), 1.51 (d, 3 H, J 7 Hz, carboxyethyl–CH_3); ^{13}C NMR: δ 174.3 (COOH), 137.6-126.1 (aromatic), 100.8 (PhCH), 98.4 (C-1), 75.9 (C-3), 75.0 (C-2), 74.2 (carboxyethyl–CH), 73.8 (C-4), 73.2 ($PhCH_2$), 69.4 (C-6), 62.3 (C-5), 55.5 (OCH_3), 19.2 (carboxyethyl–CH_3).

Deprotection of **11** (105 mg, 236 μmol) followed by purification and cation exchange, in the same way as for compound **7**, gave the Na salt of **3** (64 mg, 94%) as a white amorphous powder; $[\alpha]_D$ +183° (*c* 1.1, H_2O); HRMS: Calcd for $C_{10}H_{18}O_8 + Na$: 289.0899, found 289.0910 $[M + Na]^+$.

Methyl 3-O-[(S)-1-carboxyethyl]-α-D-galactopyranoside (**4**).—Starting from **9** (1.16 g, 3.11 mmol), (*R*)-2-chloropropionic acid (0.71 g, 6.5 mmol) and NaH (2.28 g, 95 mmol), using the same experimental and work-up conditions as in the synthesis of **7**, methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-[(*S*)-1-carboxyethyl]-α-D-galactopyranoside (**12**), contaminated with ~2% of **11**, was obtained. Recrystallisation from toluene–petroleum ether yielded **12** (1.15 g, 83%) as white crystals; mp 138.5–139.5 °C; $[\alpha]_D$ +71° (*c* 1.0, $CHCl_3$); HRMS: Calcd for $C_{24}H_{28}O_8 + H$: 445.1862, found 445.1886 $[M + H]^+$; ^1H NMR: δ 10.6 (s, 1 H, COOH), 7.55–7.25 (m, 10 H, Ar-H), 5.57 (s, 1 H, PhCH), 4.81 (d, 1 H, J_{gem} 12 Hz, $PhCH_2$), 4.78 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 4.63 (d, 1 H, J_{gem} 12 Hz, $PhCH_2$), 4.51 (bs, 1 H, H-4), 4.39 (q, 1 H, J 7 Hz, carboxyethyl–CH), 4.23 (dd, 1 H, $J_{6a,6b}$ 12.5, $J_{5,6a}$ 1.5 Hz, H-6a), 4.05 (dd, 1 H, $J_{5,6b}$ 2 Hz, H-6b), 4.00 (m, 2 H, H-2, 3), 3.65 (bs, 1 H, H-5), 3.39 (s, 3 H, OCH_3), 1.52 (d, 3 H, J 7 Hz, carboxyethyl–CH_3); ^{13}C NMR: δ 175.6 (COOH), 138.3–126.5 (aromatic), 101.2 (PhCH), 99.2 (C-1), 77.3 (C-2), 76.3 (carboxyethyl–CH), 75.6 (C-3), 75.3 (C-4), 73.7 ($PhCH_2$), 69.3 (C-6), 62.5 (C-5), 55.5 (OCH_3), 18.8 (carboxyethyl–CH_3).

Hydrogenolysis of **12** (103 mg, 232 μmol) followed by purification and cation exchange, in the same way as for compound **7**, gave the Na salt of **4** (65 mg, 98%) as a white amorphous powder; $[\alpha]_D$ +118° (*c* 1.2, H_2O); HRMS: Calcd for $C_{10}H_{18}O_8 + Na$: 289.0899, found 289.0844 $[M + Na]^+$.

Methyl 3-O-[(R)-1-carboxyethyl]-α-D-mannopyranoside (**5**).—Starting from methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside [11] (1.04 g, 2.78 mmol), (*S*)-2-chloropropionic acid (0.75 g, 6.9 mmol) and NaH (1.93 g, 80 mmol), using the same experimental and work-up conditions as in the synthesis of **7**, crude methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-[(*R*)-1-carboxyethyl]-α-D-mannopyranoside (**13**) contaminated with 2% of methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-[(*S*)-1-carboxyethyl]-α-D-mannopyranoside (**14**) and some aliphatic contaminants, was obtained as a syrup in ~95% yield as estimated from ^1H NMR. It was not possible to separate compounds **13** and **14** on silica gel, but the aliphatic contaminants were removed by passage through a silica column (99:1, $CHCl_3$–MeOH). Purification of **13** from the isomer **14** by crystallisation with different counter ions was tried and column chromatography on Sephadex LH-20 (3 × 25 cm), using EtOAc as eluent was also tried, but without success. The analytical sample of **13** was thus contaminated by 2% of **14** as determined by ^1H NMR; $[\alpha]_D$ +48° (*c* 1.1, $CDCl_3$); HRMS: Calcd for $C_{24}H_{28}O_8 + H$: 445.1862, found 445.1899 $[M + H]^+$; ^1H NMR: δ 9.5 (s, 1 H, COOH), 7.5–7.2 (m, 10 H, Ar–H), 5.62 (s, 1 H, PhCH), 4.98 (d, 1 H, J_{gem} 12 Hz, $PhCH_2$), 4.82 (d, 1 H, J_{gem} 12 Hz, $PhCH_2$), 4.65 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.43 (q, 1 H, J 7 Hz, carboxyethyl–CH), 4.24 (dd, 1 H, $J_{6a,6b}$ 10, $J_{5,6a}$ 4.5 Hz, H-6a), 4.17 (t, 1 H, $J_{3,4} = J_{4,5} = 10$ Hz H-4), 4.11 (dd, H-2), 3.98 (dd, 1 H, $J_{2,3}$ 3.5 Hz, H-3), 3.86 (t, 1 H, $J_{5,6b}$ 10 Hz, H-6b), 3.76 (ddd, 1 H, H-5), 3.30 (s, 3 H, OCH_3), 1.52 (d, 3 H, J 7 Hz, carboxyethyl–CH_3); ^{13}C NMR: δ 176.3 (COOH), 138.7–126.0 (aromatic), 101.5 (PhCH), 101.1 (C-1), 79.4 (C-4), 77.5 (C-2), 76.8 (C-3), 76.3 (carboxyethyl–CH), 74.0 ($PhCH_2$), 68.9 (C-6), 64.0 (C-5), 54.8 (OCH_3), 19.2 (carboxyethyl–CH_3).

Hydrogenolysis and purification of **13** (128 mg, 288 μmol) was done on the isomeric mixture (98:2) followed by purification and cation exchange in the same way as for compound **7.** This gave the Na salt of **5** (75 mg, 90%) as a white amorphous powder; $[\alpha]_D$ +77° (*c* 1.0, H_2O); HRMS: Calcd for $C_{10}H_{18}O_8 + Na$: 289.0899, found 289.0898 $[M + Na]^+$.

Methyl 3-O-[(S)-1-carboxyethyl]-α-D-mannopyranoside (**6**).—Starting from methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (1.03 g, 2.76 mmol), (*R*)-2-chloropropionic acid (0.75 g, 6.9 mmol) and NaH (1.65 g, 69 mmol), using the same experimental and work-up conditions as in the synthesis of **7**, crude **14**, contaminated with 6% of **13** and some unidentified aliphatic contaminants, was obtained in ~80% yield, according to ^1H NMR. The aliphatic contaminants were removed by chromatography on silica gel (99:1, $CHCl_3$–MeOH). It was not possible to separate **14** from **13**, so the analytical sample of **14** contained 6% of **13** according to ^1H NMR; $[\alpha]_D$ +1° (*c* 1.5, $CHCl_3$); HRMS: Calcd for $C_{24}H_{28}O_8 + H$: 445.1862, found 445.1883 $[M + H]^+$; ^1H NMR: δ 8.5 (s, 1 H, COOH), 7.5–7.3 (m, 10 H, Ar–H), 5.62 (s, 1 H, PhCH), 4.81 (d, 1 H, J_{gem} 12 Hz, $PhCH_2$), 4.74 (d, 1 H, J_{gem} 12 Hz, $PhCH_2$), 4.73 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.25 (dd, 1 H, $J_{6a,6b}$ 10, $J_{5,6a}$ 4,5 Hz, H-6a), 4.22 (t, 1 H, $J_{4,5}$ 10 Hz, H-4), 4.21 (q, 1 H, *J* 7 Hz, carboxyethyl–CH), 3.93 (dd, 1 H, $J_{2,3}$ 3.5, $J_{3,4}$ 10 Hz, H-3), 3.86 (dd, 1 H, $J_{5,6b}$ 10 Hz, H-6b), 3.84 (d, 1 H, H-2), 3.79 (ddd, 1 H, H-5), 3.35 (s, 3 H, OCH_3), 1.43 (d, 3 H, *J* 7 Hz, carboxyethyl–CH_3); ^{13}C NMR: δ 174.1 (COOH), 137.8–126.1 (aromatic), 102.1 (PhCH), 100.3 (C-1), 77.9 (C-4), 77.2 (C-3), 76.3 (C-2), 75.5 (carboxyethyl–CH), 73.8 ($PhCH_2$), 68.8 (C-6), 64.1 (C-5), 55.0 (OCH_3), 19.1 (carboxyethyl–CH_3).

Hydrogenolysis of **14** (116 mg, 261 μmol) was done on the isomeric mixture (94:6) followed by purification and cation exchange in the same way as for compound **7** to give the Na salt of **6** (63 mg, 84%) as a white amorphous powder; $[\alpha]_D$ +12° (*c* 1.0, H_2O); HRMS: Calcd for $C_{10}H_{18}O_8 + H$: 267.1079, found 267.1083 $[M + H]^+$.

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