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Synthesis of site-specific deuterium substituted methyl 6-*O*-[(*R*)- and (*S*)-1-carboxyethyl]-α-D-galactopyranoside and conformational analysis thereof based on *J* couplings

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Abstract

Methyl 6-(*S*)-^{2}H-6-*O*-[(*R*)- and (*S*)-1-carboxyethyl]-α-D-galactopyranoside have been synthesized from 6-(*S*)-^{2}H-1,6-anhydro-β-D-galactopyranoside. Conformational analysis of the exocyclic dihedral angles has been performed based on $^3J_{H,H}$ and $^3J_{C,H}$ coupling constants of the title compounds together with their non-deuterated counterparts. The ω dihedral angle (O-5–C-5–C-6–O-6) can be described by a conformational equilibrium where the order of conformers is *gauche-trans* > *trans-gauche* > *gauche-gauche*, i.e., the same as for galactose but with a different population distribution. The C-5–C-6–O-6–C-2′ torsion populates only the *gauche* states, as deduced from *J* couplings. This is at variance with previous Langevin dynamics simulations in which the proportion of the *trans* state is significant. For the C-6–O-6–C-2′–H2′ torsion an equal population of the three staggered conformers is compatible with experimental data, although previous simulations have indicated *gauche* conformers to be preferred. Since about half the number of the possible conformational states for the major degrees of freedom are significantly populated, as corroborated by experimental data, the flexibility of the substituting 1-carboxyethyl group is large.
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Keywords: 1-Carboxyethyl; *J* Coupling; Deuterium; NMR

1. Introduction

The three-dimensional structure of saccharides is determined by the constituent sugars, their absolute and anomeric configuration as well as by the sites of substitution. In addition, modification of the sugars can occur, e.g., by substitution with *O*-acetyl, phosphate or sulfate groups, and other groups such as pyruvate acetals and 1-carboxyethyl ethers. The latter, mostly found in bacterial polysaccharides, occur both with the (*R*)- and

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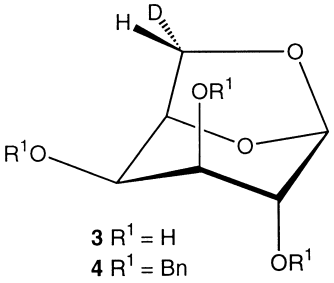
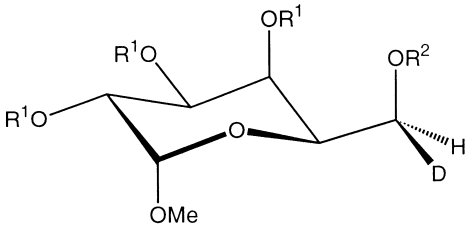
(*S*)-configuration. Different substituents will alter the shape and the electrostatics as well as the hydrophobicity of the saccharides. These groups may also change the conformation of the saccharide and may themselves exhibit internal flexibility.

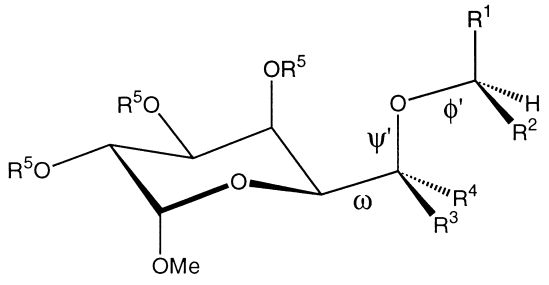
We have previously synthesized and studied 1-carboxyethyl substituted sugars by NMR and CD spectroscopy [1]. Two of these, methyl 6-*O*-[(*R*)- and (*S*)-1-carboxyethyl]-α-D-galactopyranoside $\mathbf{1_R}$ and $\mathbf{1_S}$, were further studied by molecular mechanics and Langevin dynamics (LD) simulations to investigate the conformational flexibility and the dynamics of the major degrees of freedom in the molecules [2]. LD is a technique which differs from molecular dynamics simulations in that the solvent is modelled by random and frictional forces. This facilitates longer simulations to be carried out than with explicit solvent. In addition to the simulation study, the (*R*)-isomer could be crystallized and showed an extended conformation [3]. In the present study we have synthesized methyl 6-(*S*)-^{2}H-6-*O*-[(*R*)- and (*S*)-1-carboxyethyl]-α-D-galactopyranoside $\mathbf{2_R}$ and $\mathbf{2_S}$ and together with the protiated compounds $\mathbf{1_R}$, $\mathbf{1_S}$ studied them with NMR spectroscopy. The $^3J_{H,H}$ and $^3J_{C,H}$ coupling constants have been measured and used, via Karplus relationships, to describe the conformational equilibria.



3 R^1 = H
4 R^1 = Bn



5 R^1 = Bn, R^2 = H



1_R R^1 = COOH, R^2 = CH_3, R^3 = R^4 = R^5 = H
1_S R^1 = CH_3, R^2 = COOH, R^3 = R^4 = R^5 = H
2_R R^1 = COOH, R^2 = CH_3, R^3 = D, R^4 = R^5 = H
2_S R^1 = CH_3, R^2 = COOH, R^3 = D, R^4 = R^5 = H
6_R R^1 = COOH, R^2 = CH_3, R^3 = D, R^4 = H, R^5 = Bn
6_S R^1 = CH_3, R^2 = COOH, R^3 = D, R^4 = H, R^5 = Bn

2. Results and discussion

Methyl 6-(*S*)-^{2}H-6-*O*-[(*R*)- and (*S*)-1-carboxyethyl]-α-D-galactopyranoside ($\mathbf{2_R}$ and $\mathbf{2_S}$) were synthesized from 6-(*S*)-^{2}H-1,6-anhydro-β-D-galactopyranoside (**3**), which was prepared via photobromination and reduction with tri-*n*-butyltindeuteride as described previously [4]. The incorporation of deuterium at C-6 was >95% as judged by ^{1}H NMR. Benzylation of **3** using sodium hydride and benzyl bromide gave **4**. Acetolysis gave an anomeric mixture which upon methanolysis afforded **5**, which was separated from its β-anomer by silica gel chromatography. Treatment of **5** with sodium hydride and (*S*)- or (*R*)-2-chloropropanoic acid gave methyl 2,3,4,-tri-*O*-benzyl-6-(*S*)-^{2}H-6-*O*-[(*R*)- and (*S*)-1-carboxyethyl]-α-D-galactopyranoside, respectively ($\mathbf{6_R}$ and $\mathbf{6_S}$). Hydrogenolysis and purification by gel permeation chromatography gave $\mathbf{2_R}$ and $\mathbf{2_S}$, which were used in the sodium salt form for the NMR studies.

Stereospecific NMR assignments could be performed for the signals from the H-6 protons since in the deuterated analogues H-6 *pro*-S has been substituted with a deuterium atom. In $\mathbf{1_R}$ the proton signals at δ_H 3.76 and 3.59 are assigned to *pro*-R (H-6_R) and *pro*-S (H-6_S), respectively, whereas in $\mathbf{1_S}$ the signals appear at δ_H 3.56 and 3.70, respectively. Thus, the order of the chemical shifts are reversed for the signals of the H-6 protons in the two isomers. Such an effect is also observed when substitution in disaccharides takes place at C-6 by α- or β-linked sugar residues [5]. The $^3J_{H,H}$ couplings between H-5 and H-6_R/H-6_S (Table 1) were measured to be used in the subsequent conformational analysis of the ω dihedral angle defined by O-5–C-5–C-6–O-6. In $\mathbf{1_R}$ and $\mathbf{1_S}$ two more dihedral angles are important, namely ψ′ defined by C-5–C-6–O-6–C-2′ and φ′ defined by C-6–O-6–C-2′–H-2′

Table 1
Coupling constants in Hz for $\mathbf{1_R}$ and $\mathbf{1_S}$ measured at 30 °C

Compound	H-6$_R$, H-6$_S$	H-5, H-6$_R$	H-5, H-6$_S$	H-6$_R$, C-2′	H-6$_S$, C2′	C-6, H-2′
$\mathbf{1_R}$	11.0	7.1	4.6	4.9	3.7	3.7
$\mathbf{1_S}$	10.4	7.6	4.6	4.8	4.2	3.3 [a]

[a] From ref. [2].

in order to describe the conformational flexibility of the 1-carboxyethyl group. The conformational preferences of ψ' and φ' can be investigated by analysis of the heteronuclear $^3J_{C,H}$ coupling constants. Carbon atoms C-6 and C-2′ were site-selectively excited and the long-range $^3J_{C,H}$ couplings [6,7] to H-2′ and H-6$_R$/H-6$_S$, respectively, could be measured (Table 1). For the ω and ψ' dihedral angles two three-bond coupling constants were obtained whereas for φ' only one was acquired. These J values were then used in the subsequent conformational analysis.

The preferred dihedral angle can be studied using Karplus equations. For the ω dihedral angle the following equation [8] can be used for defining the population distribution of staggered conformers from $^3J_{H5,H6R}$ and $^3J_{H5,H6S}$ values.

$$^3J_{HH} = P_1\cos^2\theta + P_2\cos\theta + P_3 + \Sigma\Delta\chi_i \left\{P_4 + P_5\cos^2(\zeta\theta + P_6|\Delta\chi_i|)\right\} \quad (1)$$

In all analyses a restriction is also imposed, viz., that the sum of the fractional populations is equal to unity. The two other major degrees of freedom in the molecule, ψ' and φ', were analyzed by long-range heteronuclear $^3J_{C,H}$ coupling constants. The following Karplus type relationship was used [9].

$$^3J_{HCOC} = 5.7\cos^2\theta - 0.6\cos\theta + 0.5 \quad (2)$$

For the φ' dihedral angle the magnitude of the $^3J_{C,H}$ coupling constants in $\mathbf{1_R}$ and $\mathbf{1_S}$ are similar and are consistent with equal populations in all three rotameric states. In the LD simulations [2] lasting 30 ns for each isomer, *gauche* states were observed for 98 and 100% of the time for $\mathbf{1_R}$ and $\mathbf{1_S}$, respectively. The value of the $^3J_{C,H}$ coupling constants between H-2′ and C-6 were calculated to be 3.1 and 2.6 Hz, to be compared to those measured from NMR which were 3.7 and 3.3 Hz for $\mathbf{1_R}$ and $\mathbf{1_S}$, respectively. The $^3J_{C,H}$ value for $\mathbf{1_R}$ was larger than for $\mathbf{1_S}$ both in the simulation and as determined by experiment. Although the simulations led to *gauche* states, the experimental values are compatible with an equal population of the three staggered φ' conformers.

The conformational preference of the ψ' dihedral angle was calculated from the $^3J_{C,H}$ values in Table 1 and eq (2). The results are given in Table 2. For both isomers the $^3J_{C,H}$ coupling constants between C-2′ and H-6$_R$ are of the same magnitude (within experimental error) and quite large. The LD simulation showed the *trans* conformer to be most abundant, 68 and 60%, followed by the g^- state with 18 and 38% for $\mathbf{1_R}$ and $\mathbf{1_S}$, respectively. The difference in the $J_{C\text{-}2,H\text{-}6_S}$ values between $\mathbf{1_R}$ and $\mathbf{1_S}$ is larger than the experimental error showing that conformational differences between the molecules do exist. Analysis of the NMR data leads to a somewhat higher population of the g^+ state compared to the g^- state, for both isomers. In contrast to the simulations no significant population of a *trans* state can be corroborated by experimental data.

In galactopyranose the hydroxymethyl group populates all three staggered conformers [10] as opposed to glucopyranose in which only the *gt* and *gg* conformers are significantly populated. The distribution of the ω dihedral angle in $\mathbf{1_R}$ and $\mathbf{1_S}$ (Table 2) was calculated from the $^3J_{H,H}$ coupling constants in Table 1 and eq (1). The preferred orientation of ω is similar for the two isomers of **1**. The *gt* conformer is most abundant, followed by the *tg* and then the *gg* conformers, as in galactose, but the relative proportions are changed. The agreement between the LD simulation and experiment was good for $\mathbf{1_R}$ with relative proportions between *gt*:*tg*:*gg* of 68:25:7 in the simulation. For $\mathbf{1_S}$ the *gt* conformer was populated 95% of the time in the simulation. It is the major conformer as determined by experiment, albeit not that pronounced.

Table 2
Preferred orientation [a] in $\mathbf{1_R}$ and $\mathbf{1_S}$ of ω and ψ' dihedral angles calculated from $^3J_{H,H}$ and $^3J_{C,H}$ values, respectively

Compound	Dihedral angle	P_{g^+}	P_t	P_{g^-}
$\mathbf{1_R}$	ω	57	26	18
$\mathbf{1_R}$	ψ'	63	−3	40
$\mathbf{1_S}$	ω	62	26	12
$\mathbf{1_S}$	ψ'	61	−11	50

[a] ω refers to the dihedral angle O-5–C-5–C-6–O-6 and ψ' to the dihedral angle C-5–C-6–O-6–C-2′.

If instead the experimental data are compared to the low energy conformers identified previously by energy minimizations [2], *gauche* states of ψ' are observed for the lowest energy conformers of both isomers, although these states were not the most populated in the LD simulation. The outcome of the simulation is sensitive to small changes in the potential energy surface as the energy difference between conformational states is ~1 kcal/mol or less, with low barriers for interconversion. Hence, the poor agreement between simulation and experiment.

In summary, the 1-carboxyethyl substituted galactose residue in this study is characterized by large conformational flexibility of its exocyclic dihedral angles. For the ω angle all staggered conformers are significantly populated. The ψ' angle has both *gauche* conformers equally populated and the φ' angle has at least two staggered conformers populated to a large extent.

3. Experimental

General.—Sodium hydride (55–60%, suspension in oil) was washed with hexane prior to use. Thin layer chromatography (TLC) was performed on pre-coated plates (Merck Silica Gel 60 F_{254}) which were developed by charring with aqueous 8% H_2SO_4. Column chromatography of synthetic intermediates was performed on Matrex silica gel 60 (35–70 μm, Amicon). Gel permeation chromatography of deprotected products was carried out on polyacrylamide gel (Bio-Gel P-2, 2.6×90 cm) in 0.1 M pyridinium acetate buffer. Solutions were concentrated under reduced pressure at temperatures not exceeding 50 °C. Atoms in the 1-carboxyethyl group are numbered from the carboxyl group (1′). A g^+ state (*gt* for ω) denotes a dihedral angle of +60°, a g^- state (*gg*) has its dihedral around −60° and a *t* state (*tg*) has its dihedral around −180°.

NMR spectroscopy.—NMR spectra were recorded at 30 °C on JEOL GSX-270 MHz, Varian Unity 500 MHz or Varian Inova 600 MHz NMR spectrometers. For solutions in $CDCl_3$ or D_2O tetramethylsilane (δ_H/δ_C 0.00 ppm) or sodium 3-trimethylsilylpropanoate-$^2H_4$ (δ_H 0.00 ppm) were used as internal references, respectively. Measurement of long-range $^{13}C,^1H$ coupling constants were performed for D_2O solutions of the sodium salt of $\mathbf{1_R}$ and $\mathbf{1_S}$ (pD 8, 40 and 60 mM, respectively) using a gradient enhanced version for ^{13}C site selective excitation as devised by Nishida et al. [7] For C-6 the selective excitation used a Gaussian shaped pulse of 200 ms duration and a spectral width of 4000 Hz was sampled with 16,384 data points (accuracy ±0.3 Hz) using 23,000 transients. Band-selective decoupling of the methyl group (δ_H 1.33) was applied during the acquisition period. For C-2′ the selective excitation used a half-Gaussian shaped pulse of 50 ms duration and a spectral width of 1200 Hz was sampled with 8192 data points using 19,000 and 33,000 transients for $\mathbf{1_R}$ and $\mathbf{1_S}$. The accuracy is estimated to be ±0.2 Hz, except for C-2′ to H-6 pro-R in $\mathbf{1_S}$ where it is ±0.5 Hz. The FIDs were processed using Varian VNMR software. An exponential weighting function with a line broadening of 0.6 Hz was applied prior to Fourier transformation. The $^3J_{C,H}$ value on excitation of C-6 was measured directly from the separation of the anti-phase components in the H2′ signal. Otherwise the *J*-doubling procedure was used with zero-filling eight times of the FID and eight delta functions in the frequency domain [11,12].

Synthesis.—6-(S)-^{2}H-1,6-anhydro-2,3,4-tri-O-benzyl-β-D-galactopyranoside (**4**). A solution of 6-(*S*)-^{2}H-1,6-anhydro-β-D-galactopyranoside [4] (**3**, 780 mg, 4.8 mmol, >95% ^{2}H according to ^{1}H NMR) in DMF (10 mL) was added dropwise to a suspension of NaH (1.0 g) in DMF (10 mL), followed by benzyl bromide (1.9 mL, 16.0 mmol) in DMF (10 mL). After 3 h water was added dropwise and the reaction mixture was extracted with $CHCl_3$. The organic phases were dried with anhydrous $MgSO_4$ and evaporated to yield (2.01 g, 97%) crude **4** which was used without further purification. ^{13}C NMR data were in good agreement with published values for the non-deuterated derivative [13].

Methyl 6-(S)-^{2}H-2,3,4-tri-O-benzyl-α-D-galactopyranoside (**5**). 6-(*S*)-^{2}H-1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-galactopyranoside (**4**, 340 mg, 0.79 mmol) was dissolved in warm acetic anhydride (1 mL). The solution was cooled to 0 °C and 0.05 mL of a freshly prepared mixture of concentrated H_2SO_4 (0.1 mL) in acetic anhydride (5 mL) was added. After completion of the reaction as indicated by TLC (toluene–EtOAc, 4:1), the mixture was poured into ice water (6 mL). The water was decanted after 1 h from the oily product and replaced. After 20 h the water was decanted again and the product dissolved in $CHCl_3$. The organic layer was

washed with aqueous 10% sodium bicarbonate, water, dried ($MgSO_4$) and evaporated to dryness. Column chromatography (toluene–EtOAc, 9:1) yielded an anomeric mixture of 6-(*S*)-^{2}H-1,6-di-*O*-acetyl-2,3,4-tri-*O*-benzyl-D-galactopyranoside (308 mg, 73%, $\alpha/\beta \approx 3{:}1$). ^{13}C NMR ($CDCl_3$) showed, inter alia, signals at 94.2 (C-1-β), 90.8 (C-1-α) and 63.0 (triplet, C6).

The anomeric mixture of 6-(*S*)-^{2}H-1,6-di-*O*-acetyl-2,3,4-tri-*O*-benzyl-D-galactopyranoside (2.9 g, 5.4 mmol) was dissolved in dry methanol (80 mL) and treated with 2M HCl (80 mL) in MeOH. After 10 h the reaction mixture was concentrated to yield an anomeric mixture of methyl glycosides (94% 2.4 g, $\alpha/\beta \approx 1{:}1$). Column chromatography ($CHCl_3$–EtOAc, 5:1) afforded the pure α-anomer **5** (0.99 g, 41%). ^{13}C NMR ($CDCl_3$): 139.0–127.4 (Bn), 98.6 (C-1), 78.8, 76.2, 74.8, 73.4, 69.8 (C-2–C-5 and $3\times CH_2$, overlapping signals), 55.1 (OMe). The β-anomer was obtained as a by-product (0.74 g, 31%).

Methyl 6-(S)-*^{2}H-6-*O-*[*(R)*-1-carboxyethyl]*-α-D-*galactopyranoside* (**2_R**). A solution of methyl 6-(*S*)-^{2}H-2,3,4-tri-*O*-benzyl-α-D-galactopyranoside (**5**, 106 mg, 0.23 mmol) and (*S*)-chloropropanoic acid (50 mg, 0.46 mmol) in 1,4-dioxane (20 mL) was stirred with NaH (0.6 g) at 50 °C for 16 h, whereafter the mixture was cooled and water (20 mL) was added. Extraction with petroleum ether (bp 60–70 °C, 2×25 mL) was followed by acidification of the water phase (pH ≈ 3, AcOH). The water phase was extracted with $CHCl_3$ (3×25 mL) and the combined $CHCl_3$ phases were dried ($MgSO_4$), filtered, and concentrated to give methyl 6-(*S*)-^{2}H-6-*O*-[(*R*)-1-carboxyethyl-2,3,4-tri-*O*-benzyl-α-D-galactopyranoside **6_R** (90 mg, 74%) as a syrup. ^{13}C NMR data were in agreement with those of the non-deuterated compound [1].

Compound **6_R** (88 mg, 0.17 mmol) in MeOH was subjected to hydrogenolysis for 25 h at atmospheric pressure using Pd–C as catalyst. Filtration and concentration gave **2_R** (40 mg, 98%). ^{13}C NMR data were in agreement with those of the non-deuterated compound [1]. Prior to NMR studies compound **2_R** was treated with Chelex-100 cation exchange resin, filtered, purified by gel permeation chromatography and converted to its sodium salt by ion-exchange (Dowex-50, Na^+).

Methyl 6-(S)-*^{2}H-6-*O-*[*(S)*-1-carboxyethyl]*-α-D-*galactopyranoside* (**2_S**). Starting from methyl 6-(*S*)-^{2}H-2,3,4-tri-*O*-benzyl-α-D-galactopyranoside (**5**, 116 mg, 0.24 mmol), (*R*)-chloropropanoic acid (300 mg, 2.76 mmol) and NaH (1.15 g), using the same experimental conditions as in the synthesis of **6_R**, methyl 6-(*S*)-2H-6-*O*-[(*R*)-1-carboxyethyl]-2,3,4-tri-*O*-benzyl-α-D-galactopyranoside **6_S** was obtained (108 mg, 82%). ^{13}C NMR data were in agreement with those of the non-deuterated compound [1].

Compound **6_S** (92 mg, 0.18 mmol) in MeOH was subjected to hydrogenolysis for 25 h at atmospheric pressure using Pd–C as catalyst. Filtration and concentration gave **2_S** (42 mg, 98%). ^{13}C NMR data were in agreement with those of the non-deuterated compound [1]. Prior to NMR studies compound **2_S** was treated with Chelex-100 resin, filtered, purified by gel permeation chromatography and converted to its sodium salt by ion-exchange (Dowex-50, Na^+).

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References

[1] M. Andersson, L. Kenne, R. Stenutz, and G. Widmalm, *Carbohydr. Res.*, 254 (1994) 35–41.
[2] R. Stenutz and G. Widmalm, *Glycoconjugate J.*, 14 (1997) 973–981.
[3] L. Eriksson, Å. Pilotti, R. Stenutz, and G. Widmalm, *Acta. Cryst.*, C52 (1996) 2285–2287.
[4] H. Ohrui, Y. Nishida, and H. Meguro, *Agric. Biol, Chem.*, 48 (1984) 1049–1053.
[5] P.-E. Jansson, L. Kenne, and I. Kolare, *Carbohydr. Res.*, 257 (1994) 163–174.
[6] T. Nishida, G. Widmalm, and P. Sándor, *Magn. Reson. Chem.*, 33 (1995) 596–599.
[7] T. Nishida, G. Widmalm, and P. Sándor, *Magn. Reson. Chem.*, 34 (1996) 377–382.
[8] C.A.G. Haasnoot, F.A.A.M. de Leeuw, and C. Altona, *Tetrahedron*, 36 (1980) 2783–2792.
[9] I. Tvaroska, M. Hricovíni, and E. Petráková, *Carbohydr. Res.*, 189 (1989) 359–362.
[10] Y. Nishida, H. Ohrui, and H. Meguro, *Tetrahedr. Lett.*, 25 (1984) 1575–1578.
[11] L. McIntyre and R. Freeman, *J. Magn. Reson.*, 96 (1992) 425–431.
[12] F. del Rio-Portilla, V. Blechta, and R. Freeman, *J. Magn. Reson.*, A111 (1994) 132–135.
[13] P.-M. Åberg and B. Ernst, *Acta. Chem. Scand.*, 48 (1994) 228–233.