*Short Communication*

# NMR and Conformational Analysis of Two Dihydroxydecalin α-D-Glucopyranosides

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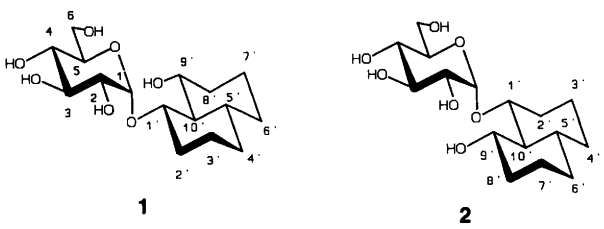
The starch-degrading enzyme amyloglucosidase (AMG, E.C. 3.2.1.3.) preferentially hydrolyses α-1,4 glycosidic linkages in amylose, although α-1,6 glycosidic linkages can also be hydrolysed.[1,2] In connection with a study of the specificity of the hydrolysis of maltose and derivatives, a rigid analogue **2** of isomaltose has been synthesized.[3] However, during the glycosylation of dihydroxydecalin, two isomeric compounds **1** and **2** are formed, structures for which were suggested.[3] The rather different chemical shifts of several protons and carbons in the two products provided an interesting opportunity for a more extensive conformational analysis, which, at the same time, would substantiate the structural assignments presented previously.[3] Therefore, the fully assigned NMR data of compound **1** and **2** together with 1D difference NOE measurements and conformational analysis based on the HSEA approach are presented in this communication.

## Results and discussion

Compounds **1** and **2** are described in the literature; the numbering of the carbon atoms is presented in Scheme 1. The $^1$H NMR data obtained at 500 MHz for solutions in $D_2O$ at 27 °C are given in Table 1. The assignments were based on phase-sensitive double-quantum filtered (DQF) COSY experiments.[4] Similarly, the $^{13}$C NMR data (125.77 MHz) are given in Table 1. The assignments were based on heteronuclear correlation spectroscopy[5] with the assigned proton signals. The results of 1D difference NOE experiments together with calculated values are presented in Table 2. Molecular modelling of the preferred conformations of compounds **1** and **2** was performed using the HSEA approach including ensemble-averaging over the whole energy surface as published previously.[6–8]



**1**



**2**

*Scheme 1.*

Inspection of the assigned chemical shifts of **1** and **2** (Table 1) reveals that the most striking differences are observed between the $^{13}$C shift of C1 (93.8 and 101.2 ppm) and C1′ (81.2 and 89.2 ppm). These differences in glycosylation shifts are in accordance with the previously proposed[9] hypothesis that glycosidic and aglyconic $^{13}$C chemical shifts are dependent on ψ torsion angles for the conformations of oligosaccharides. The HSEA calculation of the conformational preferences shown in Table 2 and the energy maps shown in Fig. 1 support the correspondence between the large glycosylation shift of **2** (8.3 ppm) and the positive value of $\psi_H$ in the minimum, and similarly, the small glycosylation shift of **1** (+ 0.9 ppm relative to α-D-$Glc_p$[10] for C1) is in good accordance with the large (negative) value of $\psi_H$ in the energy minimum for **1**.

The differences in $\psi_H$ angles are furthermore reflected in the chemical shifts of H1 and H1′ and are in good agreement with the recently proposed dependence.[11] The other chemical shift differences observed between the two compounds e.g. C2′, H5 and H2′ax cannot be accounted for by single effects, but they are most likely due to a combination of several smaller interactions.

The NOE experiments with saturation of H1 show a major difference between the two compounds, with **1** having a larger NOE to H2′eq than to the proton directly across the glycosidic linkage to H1′ (analogously to 3-deoxymaltose[12]) whereas in **2** only an NOE to H1′ is observed. This corresponds well with the calculated NOEs (Table 2) for the two compounds accommodating the proposed conformations as presented in Fig. 1. Only small

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*Table 1.* $^1H$ and $^{13}C$ NMR data of compounds **1** and **2**.

| Position | $^1H$ **1** (*J*/Hz) | $^1H$ **2** (*J*/Hz) | $^{13}C^a$ **1** | $^{13}C^a$ **2** |
|---|---|---|---|---|
| 1 | 5.27 ($J_{1,2}$ = 4.0) | 5.11 ($J_{1,2}$ = 3.8) | 93.8 (0.59) | 101.2 (0.63) |
| 2 | 3.60 ($J_{2,3}$ = 9.8) | 3.55 ($J_{2,3}$ = 9.7) | 71.5 (0.54) | 71.6 (0.58) |
| 3 | 3.51 ($J_{3,4}$ = 9.8) | 3.61 ($J_{3,4}$ = 9.0) | 74.2 (0.57) | 74.0 (0.58) |
| 4 | 3.44 ($J_{4,5}$ = 10.0) | 3.41 ($J_{4,5}$ = 9.0) | 70.2 (0.52) | 70.2 (0.59) |
| 5 | 3.65 | 3.79 | 73.5 (0.57) | 72.9 (0.56) |
| 6A | 3.77 ($J_{5,6a}$ = 5.3) | 3.76 ($J_{5,6a}$ = 4.4) | 61.3 (0.28) | 61.3 (0.39) |
| 6B | 3.88 ($J_{5,6b}$ = 2.1) ($J_{6a,6b}$ = 12.3) | 3.83 ($J_{5,6b}$ = 1.8) ($J_{6a,6b}$ = 11.5) | | |
| 1′ | 3.82 ($J_{1,2ax}$ = $J_{1,10}$ = 10.0) | 3.63 ($J_{1,2ax}$ = $J_{1,10}$ = 9.8) | 81.2 (0.59) | 89.2 (0.65) |
| 2′ax | 1.25 | 1.44 | 29.9 (0.26) | 34.7 (0.38) |
| 2′eq | 2.23 ($J_{1,2eq}$ = 4.0) | 2.27 ($J_{1,2eq}$ = 4.4) | | |
| 3′ax | 1.26 | 1.33 | 24.0 (0.35) | 23.9 (0.40) |
| 3′eq | 1.78 | 1.68 | | |
| 4′ax | 1.08 | 1.00 | 33.3 (0.28) | 33.3 (0.36) |
| 4′eq | 1.60 | 1.57 | | |
| 5′ | 1.16 | 1.08 | 40.1 (0.63) | 40.1 (0.70) |
| 6′ax | 1.02 | 1.00 | 33.0 (0.34) | 33.1 (0.34) |
| 6′eq | 1.58 | 1.57 | | |
| 7′ax | 1.27 | 1.29 | 23.6 (0.28) | 23.9 (0.36) |
| 7′eq | 1.69 | 1.69 | | |
| 8′ax | 1.28 | 1.25 | 34.7 (0.36) | 34.7 (0.36) |
| 8′eq | 1.92 ($J_{8eq,9}$ = 4.7) | 1.89 ($J_{8eq,9}$ = 4.7) | | |
| 9′ | 3.69 ($J_{8ax,9}$ = $J_{9,10}$ = 10.0) | 3.68 ($J_{8ax,9}$ = $J_{9,10}$ = 10.0) | 75.6 (0.66) | 75.6 (0.73) |
| 10′ | 1.29 | 1.22 | 53.2 (0.62) | 53.8 (0.75) |

[a] $^{13}C$ $T_1$-values (± 10 %) given in parentheses.

*Table 2.* Measured and calculated NOE[a] data of compounds **1** and **2** (in %).

| | Proton saturated / proton observed | | | | |
|---|---|---|---|---|---|
| | H1 H2 | H1 H1′ | H1 H2′eq | $\varphi_H/\psi_H$ | Energy / kcal mol$^{-1}$ |
| Observed (**1**)[b] | 10.9 | 2.7 | 5.6 | | |
| Calc. (**1**)[c] | 10.7 (10.7) | 1.0 (1.0) | 5.0 (5.3) | −65/−48 | −2.43 |
| Observed (**2**) | 10.5 | 11.0 | 0.5 | | |
| Calc. (**2**)[c] | 10.7 (10.7) | 7.1 (6.5) | 0.1 (0.5) | −44/23 | −2.34 |

[a] The NOE values were calculated using a rotational correlation time $\tau_c = 10^{-10}$ s. [b] Measured in the difference mode using an acetone-$d_6$ lock, accuracy considered +/− 5 %. [c] Calculated minimum; weighted average in parentheses.

differences are seen between the values calculated for the minima and the weighted average which accords well with the rather shallow energy surface (Fig. 1). The fact that the calculated values do not fit more closely to the experimental values is probably the result of a breakdown of one of the assumptions in the NOE calculations (isotropic motion) which is difficult to envisage in these strongly polarized molecules as is also reflected in the $^{13}C$ $T_1$-values (Table 1). Furthermore, the simple HSEA approach may not account for the actual geometries in this type of molecule. However, based on the discussion above, the structural identities of the compounds have clearly been unambiguously established.

## Experimental

*NMR spectroscopy.* Solutions of ca. 5 mg in 0.5 ml of $D_2O$ were used. Spectra were recorded in 5 mm tubes at 500.13 MHz for $^1H$ and 125.77 MHz for $^{13}C$ with a Bruker AM-500 spectrometer at 27 °C. The $^1H$ resonances were measured relative to internal acetone (2.225 ppm, DOH at 4.75 ppm at 27 °C) and coupling constants were determined on a first-order basis (+/− 0.3 Hz). The $^{13}C$ resonances were measured relative to internal dioxane (67.4 ppm). The 2D NMR spectroscopy and 1D difference NOE were performed as described earlier.[13] $^{13}C$ $T_1$-values were obtained using the inversion–recovery technique and the values

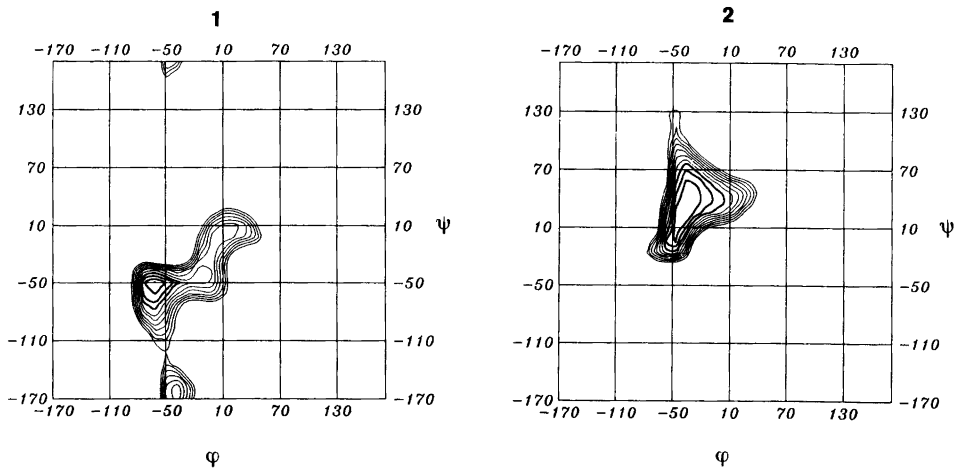


*Fig. 1.* Conformational flexibility of compounds **1** and **2**, where the 3 kcal $mol^{-1}$ limit (bold, i.e. > 98 % of the conformers) together with the 10 kcal $mol^{-1}$ limit are shown.

determined from 5 τ delays using the three-point fit routine in the Bruker DISNMR program.

*Hard sphere exo-anomeric effect (HSEA).* These were performed[14] on an IBM PS/2 system model 80 with a 387 math-coprocessor. The calculation of the ensemble-average NOEs was performed[8] on a TITAN (Ardent Computer Systems) computer as described.[6-8] The angles $\varphi_H$ and $\psi_H$ are defined as H1–C1–O1–C1′ and C1–O1–C1′–H1′. The coordinates for the α-D-$Glc_p$ unit were taken from Arnot and Scott's averaged X-ray structure coordinates[15] and the protons attached as described.[16] The coordinates for the dihydroxydecalin unit were constructed by using the molecular modelling program ALCHEMY.[17]

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