PAPER

Conformational effects due to stereochemistry and C3-substituents in xylopyranoside derivatives as studied by NMR spectroscopy

Jerk Rönnols,[a] Sophie Manner,[b] Ulf Ellervik[b] and Göran Widmalm*[a]

Glycosaminoglycans contain a β-D-xylopyranose residue at its reducing end, which links the polysaccharide to the protein in proteoglycans. 2-Naphthyl β-D-xylopyranosides have shown inhibition of tumor growth and we herein investigate conformation and dynamics of compounds structurally and stereochemically modified at the C3 position as well as the influence of solvent. The 3-deoxygenated compound, the 3-*C*-methyl-substituted β-D-xylopyranoside, β-D-ribopyranoside, the 3-*C*-methyl-substituted β-D-ribopyranoside as well as 2-naphthyl β-D-xylopyranoside were analyzed by NMR spectroscopy. Conformational equilibria were dependent on the solvent of choice, either methanol-d_4 or chloroform-*d*, with mainly $^4C_1$ and $^1C_4$ conformations present but also skew conformations to some extent. Intramolecular hydrogen bonding was concluded to be important for the 3-*C*-methyl-substituted β-D-xylopyranosides in the non-polar solvent. Dynamic NMR (DNMR) spectroscopy was carried out for the 3-deoxygenated compound, which at 25 °C in methanol-d_4 exists with equally populated states of the $^4C_1$ and the $^1C_4$ conformations, but at −100 °C only a few percent is present of the latter. Using ^{13}C NMR detection for DNMR, resonance lines were shown to broaden at −40 °C and to sharpen again below −90 °C, without the emergence of a second set of NMR resonances, a typical behavior for an unequally populated equilibrium. The enthalpy and entropy activation barriers were calculated and resulted in $\Delta H^{\ddagger}$ = 47.3 kJ mol^{-1} and $\Delta S^{\ddagger}$ = 54 J mol^{-1} K^{-1}.

Introduction

The three-dimensional structure of carbohydrates and their interactions with proteins[1] play important roles in regulation of biochemical processes. One class of highly complex carbohydrates is glycosaminoglycans (GAGs) being constituents of proteoglycans.[2] Xylopyranosides, bearing a hydrophobic aglycone, can act as an acceptor in the biosynthesis of GAGs in cells, with different results depending on the aglycone, cell-type, and the substitution pattern of the xyloside.[3] We have previously reported on the conformational properties of XylNap (**1**) (Fig. 1), with a 2-naphthyl aglycone, in methanol-d_4 (ref. 3) and a wide range of other deuterated solvents,[4] where it displayed a conformational dependence on the polarity and hydrogen bond accepting ability of the solvents. Solvents of low polarity and low hydrogen bond accepting ability induced conformational transformations from the otherwise preferred $^4C_1$ conformation to the ^2S_O and $^1C_4$ conformations, with a maximum of ~20% of the $^1C_4$ conformation in chloroform,

[a]Department of Organic Chemistry, Arrhenius Laboratory, Stockholm University, SE-106 91 Stockholm, Sweden. E-mail: gw@organ.su.se
[b]Centre for Analysis and Synthesis, Chemical Center, Lund University, PO Box 124, SE-221 00 Lund, Sweden
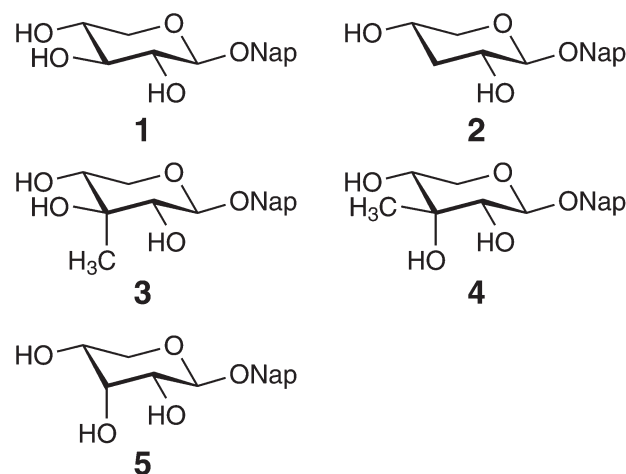
Fig. 1 Schematic of compounds **1**–**5**.

benzene, and toluene. An intramolecular hydrogen bond was detected with the HO2 hydroxyl proton as the donor and an electronegative substituent (F or OH) on C4 as an acceptor; this interaction was suggested to stabilize the $^1C_4$ conformation.

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It was observed that the 3-deoxy-compound (**2**) resides in equal amounts of the $^4C_1$ and $^1C_4$ conformations in methanol solution at 37 °C, in stark contrast to the other substances in the study.[3] In a subsequent study of the 3-*C*-methylated compounds **3** and **4** in methanol-d_4 solution, an increased conformational flexibility was observed, compared to **1**.[5] Compound **3** was the most flexible of the two, due to the larger 1,3-diaxial interactions arising from the methyl group in the $^4C_1$ conformation of **3** compared to that of the hydroxyl group in **4**. Herein, we elaborate further on the effects of the stereochemistry and substituents at C3 on the conformation of xylopyranoside derivatives (Fig. 1).

Results and discussion

^{1}H NMR spectra of compounds **1–5** in chloroform-*d* solution at 37 °C were recorded and resonances assigned; NMR assignments were also carried out for compound **5** in methanol-d_4 solution. Scalar couplings were extracted through NMR spin simulation[6] and ^{13}C NMR data were also acquired for some of the compounds (Table 1). The resulting coupling constants were fitted to coupling constants calculated from the Haasnoot–Altona equation[7] and molecular models of the canonical ring conformations $^4C_1$, $^1C_4$, $^1S_3$, $^1S_5$, $^OS_2$, $^3S_1$, $^5S_1$ and ^2S_O in accordance with the previously devised methodology.[3,4] The resulting equilibrium populations and those previously reported for compounds **1–4** in methanol-d_4 (ref. 2 and 4) and of **1** in chloroform-*d* solution[2] are listed in Table 2. In chloroform-*d* solution, compounds **2** and **3** were observed to occupy the $^1C_4$ conformation to a large extent, ~90%, supported by the long-range $^4J_{H2,H4}$ coupling in the latter, whereas the $^4C_1$ and $^1C_4$ conformations are close to equally populated for compound **4**. The intermediate $^3J_{H1,H2}$ coupling for compound **5** in methanol-d_4 solution suggests a large population of the $^1C_4$ conformation, >50%, and in the non-polar chloroform-*d* solu-

tion, $^3J_{H1,H2}$ = 2.2 Hz is consistent with a large population of the $^1C_4$ conformation, ~90%.

Compounds **2–4** were also investigated by 1D ^{1}H, [1]H-NOESY experiments.[5,8] In methanol-d_4 solution, NOE correlations were observed for compound **2** from H1 to H3$_{pro-R}$ and H5$_{pro-S}$ (Fig. 2a), which are significant in the $^4C_1$ conformation (α-face of the ring), and between H3$_{pro-S}$ and H5$_{pro-R}$ (Fig. 2b), which is significant only in the $^1C_4$ conformation (β-face of the ring), consistent with approximately equally populated $^4C_1$ and $^1C_4$ conformations. In chloroform-*d* solution, correlations between H1 and H3$_{pro-R}$ and H5$_{pro-S}$ were not observed, while a strong NOE correlation between H3$_{pro-S}$ and H5$_{pro-R}$ was present, thus verifying the strong prevalence for the $^1C_4$ conformation in the non-polar solvent. NOE experiments were pursued for compounds **3** and **4** in chloroform-*d* solution, with selective excitation of the respective methyl groups. Strong NOEs were detected for compound **3** from the methyl group to the H2 and H4 atoms (Fig. 2c), indicating a preference for the $^1C_4$ conformation. In methanol-d_4 solution, where the $^4C_1$ conformation is highly favored, strong inter-nuclear correlations were observed from the methyl group to the H1 and H5$_{pro-S}$ atoms[5] (Fig. 2d), none of which were observed in chloroform-*d* solution, further underlining the conformational dependence on solvent properties. In compound **4**, NOE correlations from the methyl group to the H2 and H4 atoms (Fig. 2e) were observed both in methanol-d_4 solution[5] and chloroform-*d* solution, consistent with a $^4C_1$ conformation. In chloroform-*d*, an NOE correlation to the H5$_{pro-R}$ resonance was also observed, which indicates a large population of the $^1C_4$ conformation (Fig. 2f).

Complementary to the NOE-based information for compound **3** in chloroform-*d* solution, chemical shift information and scalar coupling data are also illustrative. The ^{1}H NMR chemical shift for the hydroxyl proton HO3 is unusually high, δ_H 3.63, which may be compared to that of the 4-fluoro-4-deoxy-β-D-Xyl*p*-Nap derivative, having δ_H 2.92 being present to 26% in the $^1C_4$ conformation.[5] In this compound, an F4···HO2 hydrogen bond was proven by $J_{F4,HO2}$ = 2 Hz;

Table 1 δ_H, $^nJ_{HH}$ in parenthesis and δ_C in square brackets of xyloside analogs **1–5** in chloroform-*d* and **5** in methanol-d_4 at 37 °C. For methylene groups, the ^{1}H NMR chemical shift of the pro-*R* proton is given prior to that of the pro-*S* proton. n.d. = not determined

Compound	1	2	3	4	5	Me	HO2	HO3	HO4
1	5.109 (6.224) [100.91]	3.709 (7.786) [74.64]	3.675 (7.634) [74.56]	3.809 (4.501, 8.268) [69.42]	4.112, 3.468 (−11.880) [64.54]		2.704 (4.198)	2.768 (3.736)	2.266 (3.447)
2	5.513 (2.050) [98.07]	3.888 (3.092, 3.556) [66.92]	2.050, 2.267 (3.570, 3.156, −14.733) [30.82]	3.940 (1.575, 2.125) [65.77]	3.988, 3.635 (−12.427) [65.38]		3.461 (8.279)		2.452 (4.963)
3	5.590 (2.559)	3.625 (1.441)[b]		3.608 (1.937, 3.117)	4.265, 3.641 (−12.753)	1.438	3.172 (8.678)	3.631	2.282 (5.091)
4	5.555 (4.443)	3.666		3.632 (3.268, 6.124)	4.037, 3.814 (−11.956)	1.531	n.d.	n.d.	n.d.
5	5.759 (2.185)	4.067 (1.295)	4.134 (3.009)	4.012 (1.655, 2.624)	4.012, 3.905 (−12.527)		3.433 (4.544)	3.159 (3.826)	3.095 (3.815)
5[a]	5.592 (3.881) [100.65]	3.860 (3.197) [72.37]	4.074 (3.184) [68.26]	3.874 (2.662, 4.825) [70.46]	3.900, 3.815 (−11.852) [65.57]				

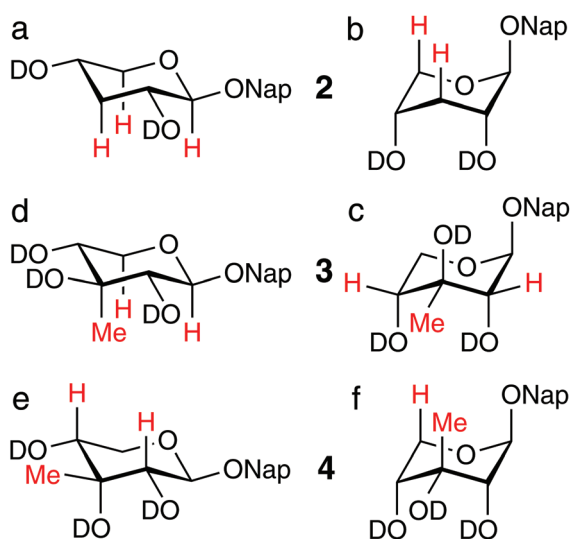[a] In methanol-d_4. [b] $^4J_{H2,H4}$.

Table 2 Populations of ring conformations of compounds **1–5** in methanol-d_4 (upper) and chloroform-d (lower), at 37 °C, in percent (%)
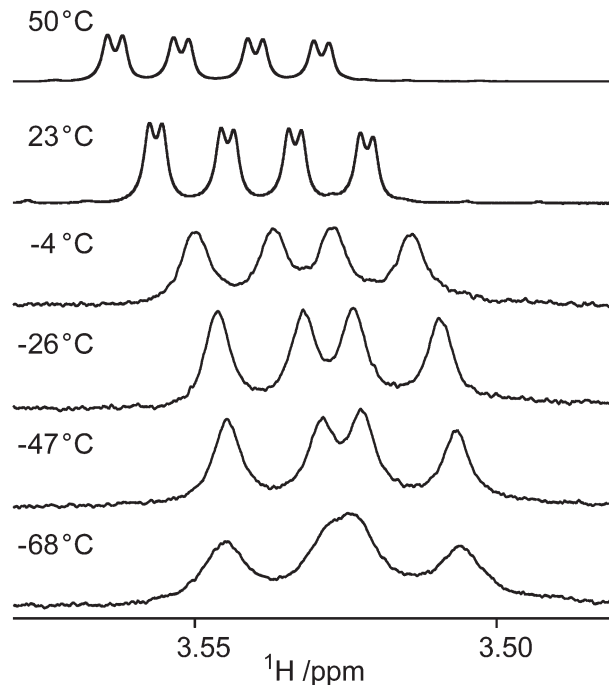
Compound	$^4C_1$	^xS_y	$^1C_4$
1	94	6[a]	0
2	48	0	52
3	70	15[b]	15
4	90	5[b]	5
5	25	20[b]	55
1	71	22[a]	7
2	0	9[a]	91
3	5	5[b]	90
4	45	5[b]	50
5	5	5[b]	90

[a] $x = 2$, $y = $ O. [b] At least two skew conformations are possible.

Fig. 2 ^{1}H,^{1}H-NOE correlations between atoms (colored red), observed from selective excitations of, *inter alia*, H1 or CH$_3$. Compound **2** in $^4C_1$ conformation (a) and in $^1C_4$ conformation (b); **3** in $^1C_4$ (c) and in $^4C_1$ (d); **4** in $^4C_1$ (e) and in $^1C_4$ (f).

Fig. 3 The ^{1}H NMR resonance at 500 MHz of H5$_{pro-S}$ of compound **2** in methanol-d_4 solution as a function of temperature.

Table 3 Experimental data and extrapolated data (italicized) of compound **2** in methanol-d_4 at different temperatures. Exchange rates (s^{-1}) were obtained as described in ref. 15

Temp/°C	$^4C_1$/%	$^1C_4$	δ_{H1}/ppm	$J_{H1,H2}$/Hz	k_{ex}/s^{-1}
50	46	54	5.279	4.416	*100 000 000*
39	48	52	5.271	4.513	*52 000 000*
23	51	49	5.255	4.717	*19 000 000*
−4	59	41	5.225	5.015	*2 500 000*
−26	65	35	5.193	5.452	*340 000*
−47	73	27	5.158	5.850	*37 000*
−66	80	20	5.13	*6.42*	2800
−68	80	20	5.127	6.580	*2500*
−76	85	15	5.10	*6.73*	840
−86	90	10	5.04	*7.03*	180
−95	92	8	5.02	*7.15*	44
−101	93	7	5.02	*7.21*	9.7

additionally, the $^3J_{H2,HO2} = 6.1$ Hz. In compound **3**, the latter coupling constant was also large being $^3J_{H2,HO2} = 8.7$ Hz, indicating hindered rotation. In order to obtain information on the conformational preference (if any) of the hydroxyl proton HO3, we rely on the heteronuclear ^{1}H,^{13}C spin–spin coupling constant to the methyl group on C3, since the compound is devoid of a hydrogen atom in this position. Employing a J-HMBC experiment[9] and detecting the interaction between the methyl ^{13}C group and the HO3 group, we obtained $^3J_{C(Me),HO3} = 6.5$ Hz, consistent with an *antiperiplanar* arrangement (*cf.* eqn (10) in ref. 10). Taken together, these results support that the $^1C_4$ conformation for compound **3** in chloroform-d solution is stabilized by the intramolecular hydrogen bonds O4···HO2 and O1···HO3.

To elaborate on the dynamics of the 3-deoxygenated compound (**2**), ^{1}H NMR spectra were recorded at a ^{1}H resonance frequency of 500 MHz in a temperature range from 50 °C to −68 °C in methanol-d_4 solution. The equilibrium populations were altered significantly with temperature, observed through changes in *e.g.* the $^3J_{H1,H2}$ and the $^3J_{H4,H5pro-S}$ coupling constants (Fig. 3). At temperatures below ~25 °C, the $^4C_1$ conformation is predominantly occupied, reflected by increased values of the $^3J_{H1,H2}$ coupling constant (Table 3). At temperatures below −68 °C, where the population of the $^4C_1$ conformation was >80%, some of the signals, *e.g.* H1 and H3$_{pro-R}$, were significantly broadened, thus disabling coupling constant analysis. The δ_{H1} and $J_{H1,H2}$ for the separate conformers could, however, be extrapolated to give for the $^4C_1$ conformation δ_{H1} 5.02 and $J_{H1,H2} = 7.64$ Hz and for the $^1C_4$ conformation δ_{H1} 5.50 and $J_{H1,H2} = 1.56$ Hz.
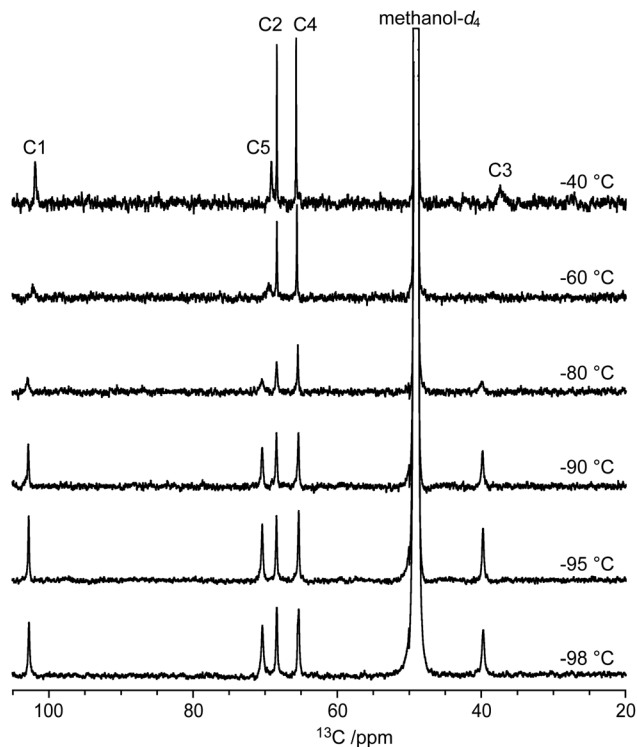
Fig. 4 The ^{13}C NMR resonances at 200 MHz of compound **2** in methanol-d_4 solution as a function of temperature.

Fig. 5 The ^{1}H NMR resonances at 800 MHz of H3$_{pro\text{-}R}$ (~1.6 ppm) and H3$_{pro\text{-}S}$ (~2.4 ppm) of compound **2** in methanol-d_4 solution as a function of temperature and the corresponding simulated spectra. Note the small peak at ~2.0 ppm in the spectrum obtained at the lowest temperature.

At this point, we turned to a higher magnetic field strength of 18.8 T, which enabled extension of the above dynamic NMR (DNMR) measurements.[11] Using ^{13}C NMR spectra at 200 MHz spectrometer frequency, we observed a broadening of the resonances corresponding to C1, C3, and C5 at −40 °C that were observed to coalesce at −60 °C (Fig. 4). Well defined sharp peaks were observed below −80 °C, corresponding to the $^4C_1$ conformer, while resonances from the less populated conformer were absent even after prolonged experimental time; a typical behavior for an unequally populated equilibrium.[12,13] The two H3 resonances of **2** were unequally broadened in ^{1}H NMR spectra below −60 °C at 800 MHz spectrometer frequency. The H3$_{pro\text{-}R}$ resonance (axially oriented in the $^4C_1$ conformation) was significantly broadened and eventually split up at −95 °C, yielding a major and a minor signal (~5%) at −101 °C (Fig. 5). The conformational exchange rates $k_{ex} = k_1 + k_{-1}$, where k_1 and k_{-1} are the forward and reverse rate constants for the $^4C_1 \rightleftharpoons {}^1C_4$ conformational equilibrium, respectively, were subsequently investigated by NMR line-shape analysis of the H3 resonances, with emphasis on exchange rates and population distribution, and yielded the data reported in Table 3. The enthalpy and entropy activation barriers were calculated from an Eyring plot[14] resulting in $\Delta H^{\ddagger} =$ 47.3 kJ mol^{-1} and $\Delta S^{\ddagger} = 54$ J mol^{-1} K^{-1}. The free energy of activation barrier $\Delta G^{\ddagger}$ can subsequently be calculated at a given temperature, under the assumption that $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ were not temperature dependent. For example, at −70 °C one obtains $\Delta G^{\ddagger} = 36.3$ kJ mol^{-1}, which can be compared to protected

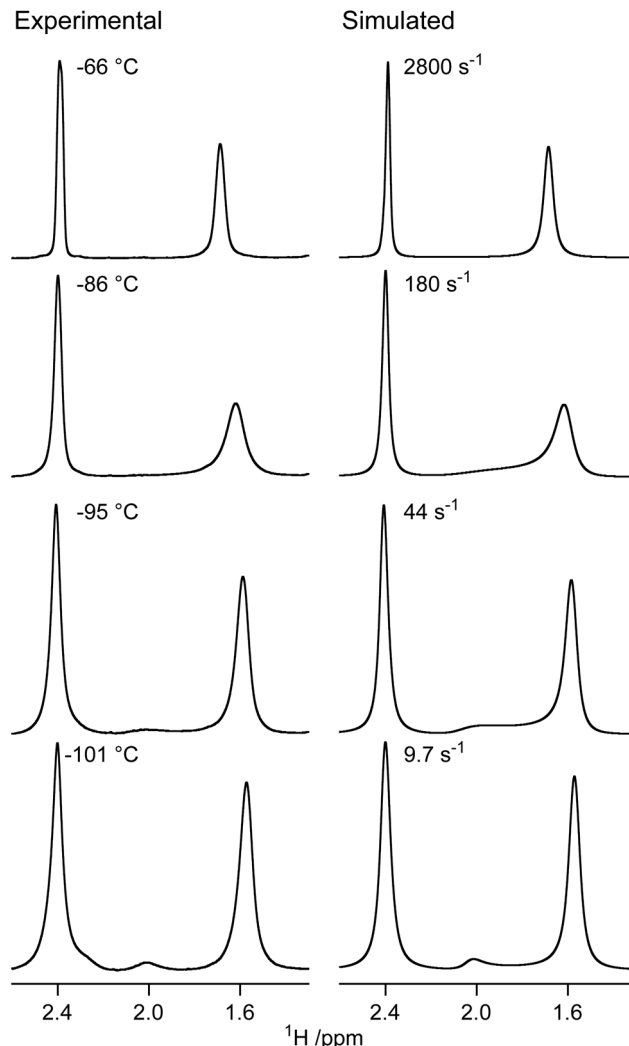*O*-methyl glycosides of α-D-mannuronic acid esters in CD$_2$Cl$_2$ at the same temperature showing $\Delta G^{\ddagger} \approx 46$ kJ mol^{-1} for the $^4C_1 \rightleftharpoons {}^1C_4$ ring interconversion.[15] Thus, the free energy of activation barrier $\Delta G^{\ddagger}$ for the 3-deoxygenated compound (**2**), is indeed low, but can be determined by DNMR spectroscopy.

Complementary spectroscopic techniques include optical rotation, which facilitates additional information about conformational preferences. The technique can be used to differentiate anomeric configuration between methyl glycosides and has been applied in conformational studies of oligosaccharides.[16,17] Additionally, it has been suggested that the extent of a particular chair conformation can be deduced in this way.[18] For compound **1** in methanol the specific rotation $[\alpha]_D^{20} =$ −30.04° corresponding to a molecular rotation $[M]_D^{20} =$ −83.0°, and is similar to that of the corresponding methyl xyloside in water, $[M]_D^{20} = -108°$, but numerically quite different to those

of other methyl pentopyranosides.[18] For β-D-Xylp-OMe in water the calculated molecular rotation for the $^1C_4$ chair conformation has a value that is more negative than that corresponding to the $^4C_1$ conformation. In methanol solution compound **3** has $[\alpha]_D^{20} = -44°$ and compound **4** has $[\alpha]_D^{20} = -18°$,[5] corresponding to $[M]_D^{20} = -128°$, and $[M]_D^{20} = -52°$, respectively. A direct comparison to compound **1**, with conformational preferences determined by NMR spectroscopy (Table 2), is difficult due to the additional 3-*C*-methyl group in **3** and **4**. However, the significantly more negative value of **3** is consistent with the presence of a larger proportion of a $^1C_4$ conformation, harmonizing with the NMR-based results (Table 2), where the extent of the $^4C_1$ conformation is 70% in **3** compared to 90% in **4**. It is anticipated that these types of changes in molecular rotation will be able to report on alterations of conformational equilibria and we foresee that NMR studies in the future may be complemented by determination of the molecular rotation.

We conclude by noting that, in comparison to the 2-naphthyl β-D-xylopyranoside, stereochemical and substituent modifications at C3 lead to large changes in flexibility and/or conformational equilibria being dependent on the solvent. Notably, the flexibility is increased if the hydroxyl group at C3 is removed, leading to lower steric interactions, or if a *C*-methyl substituent is added, leading to higher steric interactions, or if the stereochemistry is inverted at the C3 position. The conformational equilibrium of the 3-deoxygenated compound was found to be highly temperature dependent with an almost exclusive $^4C_1$ conformation present at −100 °C and only a few percent of the $^1C_4$ conformation compared to equally populated states at 25 °C. Thus, the enthalpy-favored $^4C_1$ conformer of compound **2** becomes evident at low temperature and only at high temperature is the $^1C_4$ conformer favored in methanol solution as a result of significant entropic contributions to the conformational state. The importance of sugar puckering[19] and the implications of the herein obtained results for glycosyl transferase activity and antiproliferative properties of these compounds is a challenging area for future research where progress recently has been made.[5]

Experimental section

Experimental procedures and analysis protocols were the same as previously described.[3–5,15] NMR experiments were performed on a Bruker AVANCE II 500 MHz and AVANCE III 600 MHz spectrometers and a Varian Inova 800 MHz spectrometer. ^1H and ^{13}C chemical shifts are reported in ppm using residual solvent signals as references, *viz.*, in methanol-d_4: δ_H 3.31 and δ_C 49.0; in chloroform-*d*: δ_H 7.26 and δ_C 77.16. Two J-HMBC experiments were performed as described[9,20] using a scaling factor κ of 18.1 and 21.4. For compound **1** the specific rotation was measured on five separate samples with a Perkin-Elmer 341 polarimeter at 589 nm and 20 °C in a quartz cuvette of 10 cm length; SD 0.46, *c* 1.01, MeOH.

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