Carbohydrate Research 361 (2012) 219–224

Contents lists available at SciVerse ScienceDirect

Carbohydrate Research

journal homepage: www.elsevier.com/locate/carres

Synthesis and conformational analysis of bicyclic mimics of α- and β-D-glucopyranosides adopting the biologically relevant $^{2,5}B$ conformation

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ARTICLE INFO

Article history:
Received 11 May 2012
Received in revised form 4 July 2012
Accepted 5 July 2012
Available online 16 July 2012

Keywords:
Boat conformation
Bicycle
Glycosidase
Glucopyranose

ABSTRACT

The synthesis of three conformationally locked D-glucopyranoside analogs displaying the hydroxyl pattern of the parent sugar is described. A two carbon bridge connecting the C-2 and C-5 atoms of the pyranose ring allows a torsion of the sugar ring toward a $^{2,5}B$ conformation as confirmed by conformational analysis. This conformation is strongly believed to be adopted by the oxacarbenium ion-like transition state of several inverting glucosidases.

1. Introduction

Glycosidases are one of the most studied classes of enzymes because of their crucial role in a large number of biological processes. More than 7000 glycosidases have been reported so far and have been classified in 130 families according to their amino acid sequence similarities.[1] While mechanistic strategies harnessed by glycosidases are fairly well understood,[2] there is still some controversy concerning the conformation of the oxacarbenium ion-like transition state[3] although distortion of the sugar ring during enzymatic hydrolysis is now well documented since the first report by Phillips 40 years ago working on complexes of HEWL.[4] Taking into account this conformational aspect could improve the design of glycosidase inhibitors as therapeutics. Historically, the dogma that all glycosidases react through a TS half-chair conformation was first ruled out by Sinnott's pioneering work on yeast α-glucosidase[5] using kinetic isotope effects, which suggests a distorted boat conformation for the substrate. Later on, Davies' group reported snapshots along several glycosidases reaction coordinates demonstrating unprecedented conformations for the corresponding transition states.[6] While several methodologies including crystallographic studies, kinetic isotope effect measurements[7] and computational chemistry[8] proved very efficient to decipher the conformation of glycoside-derived oxacarbenium ion transition state, we explored an alternative strategy based on the design and kinetic study of conformationally locked glycoside analogs toward chemical or enzymatic hydrolysis and/or glycosylation.[9] Theoretically, a glycoside analog displaying the TS conformation should be processed very fast chemically or enzymatically as the energy penalty to go from the ground state to the transition state conformation has already been paid.

Access to a defined conformation can be achieved through formation of a rigid bicycle derived from the parent sugar and several research groups have used this strategy. Compounds **1** and **2** have been designed as L-iduronic acid mimics to determine the active conformation of L-iduronic acid in heparin.[10] Bicycles **3** and **4** have been reported as possible competitive inhibitors of UDP-galactopyranose mutase through TS conformation mimicry.[11] Derivative **5** has been proposed as a glucose model in the highly reactive $^{1}C_4$ conformation to investigate the effect of conformation on glycoside reactivity.[12] More recently, boat-shaped glucopyranosyl nucleoside **6** has been incorporated in nucleic acid to investigate the importance of the lean of the base in stable duplex formation[13] (Fig. 1).

For some years, our group has been involved in the design of strained glycosides and we have previously reported the synthesis of D-glucoside **7**,[14] D-xyloside **8**,[15] D-mannoside **9**,[16] and L-iduronic acid **10**[17] (Fig. 2). While mannoside **9** was helpful to support the $^{2,5}B$ conformation adopted by the transition state in β-mannosidases,[18] xyloside **8** demonstrated a very high reactivity toward

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http://dx.doi.org/10.1016/j.carres.2012.07.005

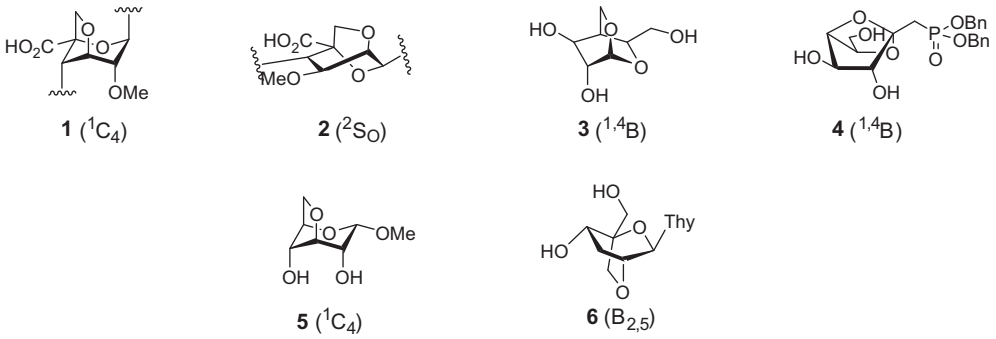


Figure 1. Structures of conformationally locked glycopyranosides **1–6**.

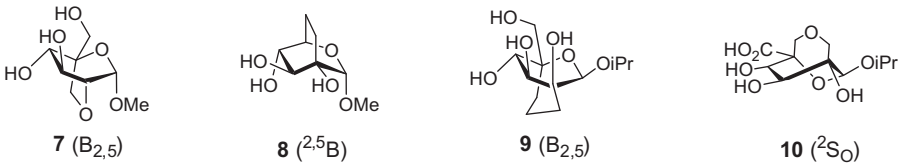


Figure 2. Structures of in house locked glycopyranosides **7–10**.

hydrolysis and glycosylation, a result in agreement with the unusual $^{2,5}B$ conformation observed in family 11 xylanases.[6b]

In this work, we focused on glucosidases and were struck by several reports mentioning a substrate distortion toward either a skewboat or a boat conformation in the Michaelis complex, suggesting a $^{2,5}B$ for the oxacarbenium ion-like transition state.[19] Such conformation was supported by ab initio metadynamics applied to β-D-glucopyranose which indicate that the $^{2,5}B$ conformer maximizes the structural/electronic requirements for efficient catalysis and is therefore preactivated for catalysis.[20] In a first step to challenge this conformation using our approach, we report herein the synthesis of several D-glucopyranoside analogs locked in a $^{2,5}B$ conformation.

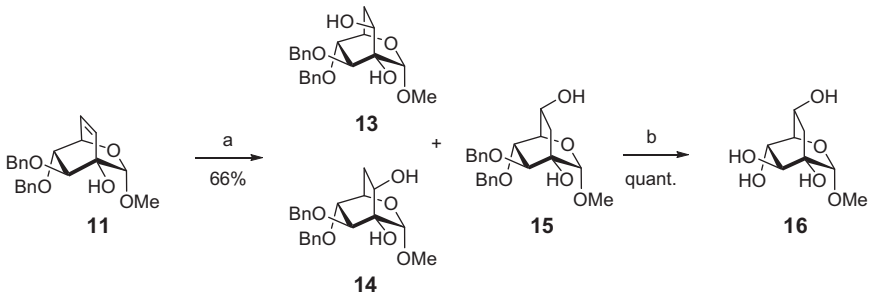
2. Synthesis

A way to force the pyranose ring toward a $^{2,5}B$ conformation is to tether the C-2 and C-5 carbon atoms with a short spacer. For our purpose, we exploited two in house strained alkenes **11** and **12**[15] that were advanced intermediates in the synthesis of bicyclic xylopyranoside mimics. Hydroboration/oxidation of **11** furnished the D-*gluco*-like diol **15** as the main product (41% yield) after purification along with the isomers **13** and **14** hydroxylated at C-7. Hydrogenolysis of **15** gave the α-D-glucopyranoside analog **16** displaying a gt like orientation (Scheme 1).

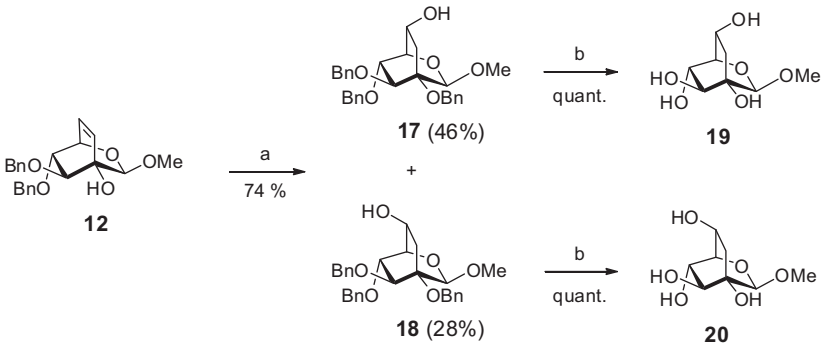
Alkene **12** was submitted to the same sequence. The hydroboration/oxidation reaction furnished the two epimers **17** and **18** that can be seen as gt and tg conformers respectively. Hydrogenation of **17** and **18** yielded the corresponding β-D-glucopyranoside analogs **19** and **20** (Scheme 2).

3. Structures confirmation

Structures of compounds **16**, **19**, and **20** were assigned by NMR combining TOCSY (60 ms), HSQC and assisted with NOESY experiments (2D and selective 1D-NOESY at different mixing times). For compound **16**, a NOE cross-peak between H6 and H4 indicates that OH at C6 is pointing toward the anomeric OMe group which confirms the (*R*)-configuration at C-6 (see Supplementary data). The coupling constant values $J_{6,7b}$ (3.7 Hz) and $J_{6,7a}$ (9.9 Hz) added to the strong NOE cross-peak between H6 and H7a also support the conclusion that compound **16** adopts the (*R*)-configuration at C-6. Similar experimental evidence was obtained in the case of compound **19** supporting a (*R*)-configuration for C-6 (see Supplementary data). Finally, comparing the key NOEs information of compound **20** with that obtained for compounds **16** and **19** it is possible to observe that for the β-derivative **20** the NOE between H6 and H4 is, in fact, absent, which could indicate that OH at C6 is pointing toward the H4 proton and the stereochemistry at C-6 should be the (*S*)-configuration. Once again, herein the coupling



Scheme 1. Synthesis of α-D-glucopyranoside analog **16**. Reagents and conditions: (a) BH_3·THF, THF, rt, 2 h then EtOH, NaOH, H_2O_2, rt, 2 h, 66% yield; (b) H_2, Pd/C, EtOAc/MeOH, overnight, quant.



Scheme 2. Synthesis of locked β-D-glucopyranosides **19** and **20**. Reagents and conditions: (a) BH_3·THF, THF, rt, 6 h then EtOH, NaOH, H_2O_2, rt, 2 h, 74% yield; (b) H_2, Pd/C, EtOAc/MeOH, overnight, quant.

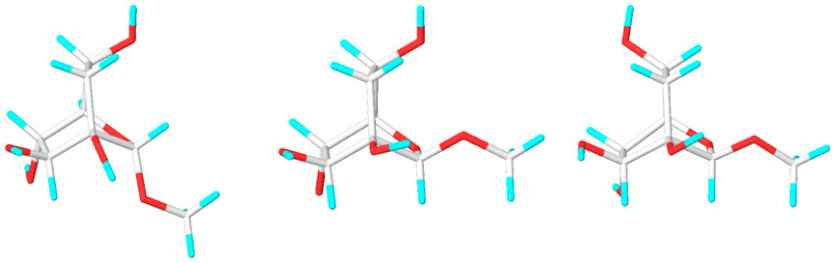


Figure 3. View of the global minimum geometry for compounds **16**, **19**, and **20** as calculated by AMBER* molecular mechanics calculations.

Table 1
Theoretical and experimental coupling constants (D_2O, 500 MHz, 298 K) of compounds **16**, **19**, and **20**

Compound **16**			Compound **19**			Compound **20**		
$^3J_{H-H}$	J_{exp}	$J_{teo\ MM}$	$^3J_{H-H}$	J_{exp}	$J_{teo\ MM}$	$^3J_{H-H}$	J_{exp}	$J_{teo\ MM}$
$J_{6,7a}$	9.9	8.2	$J_{6,7a}$	10.0	8.7	$J_{6,7a}$	9.8	9.4
$J_{6,7b}$	3.7	2.0	$J_{6,7b}$	1.8	2.0	$J_{6,7b}$	5.9	5.6
$J_{4,3}$	1.7	2.5	$J_{4,3}$	2.3	2.1	$J_{4,3}$	3.0	2.7
$J_{4,5}$	1.7	1.5	$J_{4,5}$	1.0	1.6	$J_{4,5}$	<1.0	1.4
$J_{5,6}$	1.7	2.5	$J_{5,6}$	<1.0	2.0	$J_{5,6}$	3.1	2.9
$^4J_{H-H}$	J_{exp}	$J_{teo\ MM}$	$^4J_{H-H}$	J_{exp}	$J_{teo\ MM}$	$^4J_{H-H}$	J_{exp}	$J_{teo\ MM}$
$J_{7b,3}$	1.7	W-arrangement	J_{7a-1}	1.2	W-arrangement	$J_{7b,3}$	1.7	W-arrangement
			$J_{7b,3}$	2.0	W-arrangement			

constants between H6 and H7a/H7b and the NOEs cross-peaks between H4 and H7a as well as H6 and H7b allowed to confirm that compound **20** adopts the (*S*)-configuration at C-6 (see Supplementary data).

4. Conformational analysis

The conformation of the glucopyranoside analogs **16**, **19** and **20** was investigated combining NMR data and molecular modeling protocols. Coupling constants and NOE data were analyzed and further compared with the spatial arrangement of the pyranose ring for the three methyl glucoside analogs obtained after energy minimization, using AMBER* force field integrated in Maestro package. The 3D structures of the new locked glucosides are fairly similar, and indeed demonstrated that compounds **16**, **19**, and **20** adopt a boat-like conformation (Fig. 3).

Comparison between the experimental vicinal proton–proton coupling constants and those estimated for the global minimum is given in Table 1. The analysis of the coupling constants indicates that in solution the major conformer for all the compounds adopts the desired $^{2,5}B$ conformation. The key torsion angles which define the shape of the six-membered ring were also analyzed (Table 2). They correspond to those expected for boat conformations.

Table 2
Dihedral angles from MM calculations (AMBER* force field, GB/SA solvent Model) for compounds **16**, **19**, and **20**

	α-Derivative	β-Derivative	
Dihedral angle	Compound **16**	Compound **19**	Compound **20**
C2–C1–O5–C5	14.1	10.9	5.8
C2–C3–C4–C5	12.4	13.2	9.2
C2–C7–C6–C5	11.8	15.3	7.9

Molecular dynamics calculations (MD) using the same force field were also performed to verify the structure stability of the new locked sugars. In fact, according to the AMBER* force field, the structures sampled during the MD run are basically coincident with the global minimum and display minor fluctuations around the basic structure (Fig. 4).

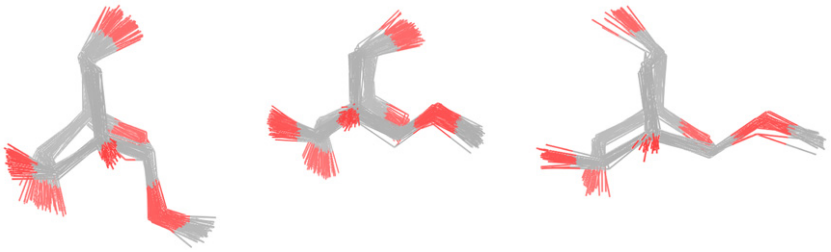


Figure 4. Superimposition of 200 structures sampled during the 10 ns MD simulation at 298 K and using the GB/SA solvent model for water. Hydrogen atoms have been removed for sake of clarity.

5. Conclusion

Three bicyclic monosaccharides **16**, **19**, and **20** locked in a $^{2,5}B$ conformation have been synthesized as D-glucopyranoside mimics from advanced intermediates. Their NMR conformational analysis confirmed that they adopt the biologically relevant $^{2,5}B$ conformation in solution. Their behavior toward enzymatic and/or chemical hydrolysis will be reported in due course.

6. Experimental section

6.1. General

Optical rotations were measured at 20 ± 2 °C with a Perkin Elmer Model 241 digital polarimeter, using a 10 cm, 1 mL cell. Mass spectra (CI (ammonia) and FAB) were obtained with a JMS-700 spectrometer. ^{1}H NMR spectra were recorded at 400 MHz with a Brüker DRX 400 or at 500 MHz (conformational studies) with a Brüker for solns in $CDCl_3$ or D_2O at room temperature. Assignments were confirmed by COSY experiments. Multiplicity is indicated as follows: s (singlet), d (doublet), t (triplet), dd (doublet of doublets), br s (broad singlet), etc. ^{13}C NMR spectra were recorded at 100.6 MHz with a Brüker DRX 400 spectrometer. Reactions were monitored by thin-layer chromatography (TLC) on a precoated plate of Silica Gel 60 F_{254} (layer thickness 0.2 mm; E. Merck, Darmstadt, Germany) and detection by charring with H_2SO_4 10% in EtOH or with 0.2% w/v cerium sulfate and 5% ammonium molybdate in 2 M H_2SO_4. Flash column chromatography was performed on silica gel 60 (230–400 mesh, E. Merck).

6.1.1. (1*R*,3*S*,4*R*,5*S*,7*R*)-5,6-bis(benzyloxy)-3-methoxy-2-oxabicyclo[2.2.2]octane-4,7-diol (15)

To a cooled solution of bicycle **11** (110 mg, 0.30 mmol) in dry THF (2.5 mL) at 0 °C under argon was added BH_3·THF (1 M solution in THF, 1,5 mL, 1.5 mmol, 5 equiv). The reaction mixture was stirred for 2 h at rt when TLC showed a complete reaction. Then EtOH (0.8 mL) was added dropwise followed by NaOH (3 M solution, 0.5 mL, 5 equiv). Finally H_2O_2 (35% v/v solution, 0.4 mL) was added dropwise at 0 °C to the solution and the reaction mixture stirred at rt for 2 h. The reaction mixture was then diluted with EtOAc (10 mL) and washed with 1 M HCl (5 mL), water (5 mL), and brine (5 mL). The organic layer was dried with $MgSO_4$ and concentrated. Purification by flash column chromatography (cyclohexane/EtOAc 2:1) afforded diol **15** (47 mg, 0.12 mmol, 41% yield). The two other isomers **13** and **14** were further eluted.

6.1.1.1. Compound 15. $[\alpha]_D$ +39.1 (*c* 1 in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): 7.40–7.30 (m, 10H, aromatic H) 4.78 (s, 1H, H-1), 4.72–4.55 (m, 4H, 4 × CHPh), 4.10 (m, 1H, H-4), 3.95–3.89 (m, 2H, H-3, H-6), 3.57 (s, 3H, OCH_3), 3.45 (t, 1H, *J* = 1.5 Hz, H-5), 2.64 (dd, 1H, *J* = 9.5 Hz, 13.8 Hz, H-7a), 2.36 (s, 1H, OH-2), 2.20 (d, 1H, *J* = 8.1 Hz, OH-6), 1.34 (ddd, 1H, *J* = 1.9 Hz, 3.0 Hz, 13.8 Hz, H-7b); ^{13}C NMR ($CDCl_3$, 100 MHz): ^{13}C NMR ($CDCl_3$, 100 MHz): 138.01, 137.68 (ipso aromatic C), 128.55–127.67 (aromatic C), 102.60 (C-1), 79.04 (C-5), 78.87 (C-3), 74.39 (C-4), 72.48 (CH_2Ph), 71.70 (C-2), 70.29 (CH_2Ph), 66.84 (OCH_3), 35.64 (C-7); *m/z* (CI, NH_3): 494 (M+NH_4^+, 100%); HRMS (CI, NH_3): Calcd for $C_{29}H_{36}O_6N$ (M+NH_4^+) 494.2537, Found 494.2533.

6.1.1.2. Spectroscopic data for (1*R*,3*S*,4*R*,5*S*,8*S*)-5,6-bis(benzyloxy)-3-methoxy-2-oxabicyclo[2.2.2]octane-4,8-diol 13. ^{1}H NMR ($CDCl_3$, 400 MHz): 7.40–7.30 (m, 10H, aromatic H), 5.01 (s, 1H, H-1), 4.72 (d, 1H, *J* = 8.1 Hz, CHPh), 4.69 (d, 1H, *J* = 12.7 Hz, CHPh), 4.60 (d, 1H, *J* = 11.6 Hz, CHPh), 4.55 (d, 1H, *J* = 12.3 Hz, CHPh), 4.35 (dd, 1H, *J* = 3.6 Hz, *J* = 9.6 Hz, H-7), 4.17 (m, 1H, H-5), 4.07 (d, 1H, *J* = 1.9 Hz, H-3), 3.55 (s, 3H, OCH_3), 3.49 (t, 1H, *J* = 1.8 Hz, H-4), 2.60 (s, 1H, OH-2), 2.30 (s, 1H, OH-7), 2.11 (ddd, 1H, *J* = 1.7 Hz, *J* = 9.7 Hz, *J* = 11.4 Hz, H-6a), 2.00 (dt, 1H, *J* = 4.0 Hz, *J* = 14.7 Hz, H-6b); ^{13}C NMR ($CDCl_3$, 100 MHz): ^{13}C NMR ($CDCl_3$, 100 MHz): 138.05, 137.85 (ipso aromatic C), 128.44–127.15 (aromatic C), 97.86 (C-1), 81.90 (C-4), 78.82 (C-3), 74.38 (C-2), 72.85 (CH_2Ph), 70.31 (CH_2Ph), 68.98 (C-5), 63.20 (C-7), 56.16 (OCH_3), 33.96 (C-6); *m/z* (CI, NH_3): 494 (M+NH_4^+, 100%).

6.1.1.3. Spectroscopic data for (1*R*,3*S*,4*R*,5*S*,8*R*)-5,6-bis(benzyloxy)-3-methoxy-2-oxabicyclo[2.2.2]octane-4,8-diol 14. ^{1}H NMR ($CDCl_3$, 400 MHz): 7.40–7.29 (m, 10H, aromatic H), 4.71 (d, 1H, *J* = 11.7 Hz, CHPh), 4.68 (d, 1H, *J* = 12.2 Hz, CHPh), 4.64 (d, 1H, *J* = 11.7 Hz, CHPh), 4.59 (s, 1H, H-1), 4.58 (d, 1H, *J* = 10.4 Hz, CHPh), 4.26 (m, 1H, H-7), 4.10 (dt, 1H, *J* = 1.2 Hz, *J* = 2.4 Hz, H-3 or H-4), 3.99 (br s, 2H, H-5 and H-3 or H-4), 3.52 (s, 3H, OCH_3), 2.39 (s, 1H, OH-2), 2.24 (dd, 1H, *J* = 3.7 Hz, *J* = 13.8 Hz, H-6a), 1.97 (dd, 1H, *J* = 9.3 Hz, *J* = 13.7 Hz, H-6b), 1.90 (d, 1H, *J* = 6.3 Hz, OH-7); ^{13}C NMR ($CDCl_3$, 100 MHz): ^{13}C NMR ($CDCl_3$, 100 MHz): 138.02, 137.94 (ipso aromatic C), 128.42–127.72 (aromatic C), 102.27 (C-1), 79.62, 77.57 (C-5 and C-3 or C-4), 72.46 (CH_2Ph), 72.22 (C-2), 71.72 (C-3 or C-4), 70.53 (CH_2Ph), 66.18 (C-7), 56.78 (OCH_3), 34.41 (C-6); *m/z* (CI, NH_3): 494 (M+NH_4^+, 100%).

6.1.2. (1*R*,3*S*,4*R*,5*S*,6*S*,7*R*)-3-methoxy-2-oxabicyclo[2.2.2]octane-4,5,6,7-tetraol (16)

Diol **15** (40 mg, 0.10 mmol) was dissolved in MeOH/EtOAc (1:1, 2 mL) and Pd black (12 mg) was added. The reaction mixture was degassed three times and stirred under H_2 atmosphere. After 7 h, TLC showed an incomplete reaction and more Pd black (25 mg) was added. The reaction mixture was stirred overnight until completion of the reaction. The reaction mixture was filtered through a 45 μM Rotilabo disk eluted with MeOH and concentrated to afford the α-D-glucopyranoside analog **16** (21 mg, 0.10 mmol, quant.) as an oil.

$[\alpha]_D$ +67.7 (*c* 1 in CH_3OH); ^1H NMR (D_2O, 400 MHz): 4.71 (s, 1H, H-1), 4.01 (ddd, 1H, *J* = 1.5 Hz, 3.7 Hz, 9.8 Hz, H-6), 3.85 (m, 1H, H-3), 3.83 (m, 1H, H-5), 3.59 (t, 1H, *J* = 1.7 Hz, H-4), 3.45 (s, 3H, OCH_3), 2.41 (dd, 1H, *J* = 9.8 Hz, 14.0 Hz, H-7a), 1.21 (ddd, 1H, *J* = 1.9 Hz, 3.7 Hz, 14.0 Hz, H-7b); ^{13}C NMR (D_2O, 100 MHz): 103.86 (C-1), 77.88 (C-5), 73.81 (C-4), 72.60 (C-3), 71.12 (C-2), 65.80 (C-6), 56.79 (OCH_3), 32.92 (C-7); *m/z* (CI, NH_3): 224 ($M+NH_4^+$, 100%); HRMS (CI, NH_3): Calcd for $C_8H_{18}O_6N$ ($M+NH_4^+$) 224.1128, Found 224.1134.

6.1.3. (1*R*,3*S*,4*R*,5*S*,7*R*)-5,6-bis(benzyloxy)-3-methoxy-2-oxabicyclo[2.2.2]octane-4,7-diol (17) and (1*R*,3*S*,4*R*,5*S*,7*S*)-5,6-bis(benzyloxy)-3-methoxy-2-oxabicyclo[2.2.2]octane-4,7-diol (18)

To a cooled solution of bicycle **12** (50 mg, 0.109 mmol) in dry THF (1.0 mL) at 0 °C under argon was added BH_3·THF (1 M solution in THF, 1.1 mL, 1.09 mmol, 10 equiv). The reaction mixture was stirred for 6 h at rt when TLC showed a complete reaction. Then EtOH (0.8 mL) was added dropwise followed by NaOH (3 M solution, 0.2 mL, 5 equiv). Finally H_2O_2 (35% v/v solution, 0.2 mL) was added dropwise at 0 °C to the solution and the reaction mixture stirred at rt for 2 h. The reaction mixture was then diluted with EtOAc (8 mL) and washed with 1 M HCl (3 mL), water (3 mL), and brine (3 mL). The organic layer was dried with $MgSO_4$ and concentrated. Purification by flash column chromatography (cyclohexane/EtOAc/DCM 6:1:1) afforded the diol **17** (25 mg, 0.052 mmol, 46% yield) and the diol **18** (15 mg, 0.031 mmol, 28% yield).

6.1.3.1. Compound 17. $[\alpha]_D$ +18.4 (*c* 1 in $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): 7.40–7.27 (m, 15H, aromatic H), 4.79 (d, *J* = 11.5 Hz, 1H, CHPh), 4.74 (app d, *J* <1 Hz, 1H, H-1), 4.68 (d, *J* = 12.0 Hz, 1H, CHPh), 4.67 (d, *J* = 11.5 Hz, 1H, CHPh), 4.68 (s, 2H, CH_2Ph), 4.53 (d, *J* = 12.0 Hz, 1H, CHPh), 4.08 (d, *J* = 2.1 Hz, 1H, H-5), 3.86 (dt, *J* = 2.1 Hz, *J* = 9.8 Hz, 1H, H-6), 3.77 (t, *J* = 2.0 Hz, 1H, H-3), 3.56 (s, 3H, OCH_3), 3.54 (app d, *J* = 2.0 Hz, 1H, H-4), 2.50 (dd, *J* = 9.8 Hz, 13.9 Hz, 1H, H-7a), 2.03 (app dt, *J* = 2.0 Hz, 13.9 Hz, 1H, H-7b); ^{13}C NMR ($CDCl_3$, 100 MHz): 138.41; 138.10; 137.38 (ipso aromatic C), 128.54–127.32 (12 aromatic C), 101.77 (C-1), 82.05 (C-4), 81.50 (C-3), 76.26 (C-2), 73.54 (C-5), 73.21 (CH_2Ph), 70.96 (CH_2Ph), 66.41 (CH_2Ph), 64.65 (C-6), 56.12 (OCH_3), 27.91 (C-7); *m/z* (CI, NH_3): 494 ($M+NH_4^+$, 100%); HRMS (CI, NH_3): Calcd for $C_{29}H_{36}O_6N$ ($M+NH_4^+$) 494.2537, Found 494.2531.

6.1.3.2. Compound 18. $[\alpha]_D$ +22.4 (*c* 1 in $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): 7.41–7.27 (m, 15H, aromatic H), 4.72 (d, *J* = 11.8 Hz, 1H, CHPh), 4.68 (s, 1H, H-1), 4.66 (d, *J* = 11.8 Hz, 1H, CHPh), 4.65 (d, *J* = 11.8 Hz, 1H, CHPh), 4.63 (s, 2H, CH_2Ph), 4.54 (d, *J* = 11.8 Hz, 1H, CHPh), 4.37 (m, 1H, H-6), 4.06 (dd, *J* ⩽ 1 Hz, 2.7 Hz, 1H, H-4), 3.96 (dd, *J* ⩽ 1 Hz, 3.9 Hz, 1H, H-5), 3.79 (app dd, *J* = 2.7 Hz, 2.0 Hz, 1H, H-3), 3.44 (s, 3H, OCH_3), 2.54 (ddd, 1H, *J* = 2.0 Hz, 9.8 Hz, 12.6 Hz, H-7b), 1.93 (dd, *J* = 5.5 Hz, 12.6 Hz, 1H, H-7a); ^{13}C NMR ($CDCl_3$, 100 MHz): 139.03; 138.11; 137.72 (ipso aromatic C), 128.45–127.31 (12 aromatic C), 100.39 (C-1), 82.01 (C-3), 80.22 (C-4), 76.90 (C-2), 72.62 (CH_2Ph), 71.53 (C-5), 70.99 (CH_2Ph), 66.17 (CH_2Ph), 64.95 (C-6), 55.54 (OCH_3), 28.27 (C-7); *m/z* (CI, NH_3): 494 ($M+NH_4^+$, 100%); HRMS (CI, NH_3): Calcd for $C_{29}H_{36}O_6N$ ($M+NH_4^+$) 494.2537, Found 494.2540.

6.1.4. (1*R*,3*S*,4*R*,5*S*,6*S*,7*R*)-3-methoxy-2-oxabicyclo[2.2.2]octane-4,5,6,7-tetraol (19)

Diol **17** (20 mg, 0.042 mmol) was dissolved in MeOH/EtOAc (1:1, 1.5 mL) and 10% Pd/C (5 mg) was added. The reaction mixture was degassed three times and stirred under H_2 atmosphere. After stirring 3 h at room temperature, TLC showed a complete reaction and the reaction mixture was filtered through a 45 μM Rotilabo disk eluted with MeOH and concentrated to afford the β-glucopyranoside analog **19** (8.6 mg, 0.042 mmol, quant.) as a colorless oil.

$[\alpha]_D$ +52.1 (*c* 1 in CH_3OH); ^1H NMR (D_2O, 400 MHz): 4.46 (app d, *J*<1 Hz, 1H, H-1), 3.87 (ddd, 1H, *J*<1 Hz, 2 Hz, 10.0 Hz, H-6), 3.66 (br s, 1H, H-5), 3.56 (app dd, *J* <1 Hz, 2 Hz,1H, H-4), 3.59 (t, 1H, *J* = 2.0 Hz, H-3), 3.39 (s, 3H, OCH_3), 2.10 (ddd, 1H, *J* ⩽ 1 Hz, 10.0 Hz, 13.8 Hz, H-7a), 1.51 (dt, 1H, *J* = 2.0 Hz, 13.8 Hz, H-7b); ^{13}C NMR (D_2O, 100 MHz): 102.20 (C-1), 76.40 (C-5 and C-3), 74.94 (C-4), 70.69 (C-2), 63.56 (C-6), 56.37 (OCH_3), 27.30 (C-7); *m/z* (CI, NH_3): 224 ($M+NH_4^+$, 100%); HRMS (CI, NH_3): Calcd for $C_8H_{18}O_6N$ ($M+NH_4^+$) 224.1128, Found 224.1125.

6.1.5. (1*R*,3*S*,4*R*,5*S*,6*S*,7*S*)-3-methoxy-2-oxabicyclo[2.2.2]octane-4,5,6,7-tetraol (20)

Diol **18** (12 mg, 0.025 mmol) was dissolved in MeOH/EtOAc (1:1, 1.0 mL) and 10% Pd/C (3 mg) was added. The reaction mixture was degassed three times and stirred under H_2 atmosphere. After stirring 3 h at room temperature, TLC showed a complete reaction and the reaction mixture was filtered through a 45 μM Rotilabo disk eluted with MeOH and concentrated to afford the β-glucopyranoside analog **20** (5.2 mg, 0.025 mmol, quant.) as colorless oil.

$[\alpha]_D$ +43.8 (*c* 1 in CH_3OH); ^1H NMR (D_2O, 400 MHz): 4.39 (s, 1H, H-1), 4.12 (app ddd, 1H, *J* = 3.4 Hz, 5.9 Hz, 10.0 Hz, H-6), 3.39 (d, *J* = 2.8 Hz 1H, H-4), 3.59 (d, *J* = 3.4 Hz, 1H, H-5), 3.44 (dd, 1H, *J* = 1.8 Hz, 2.8 Hz, H-3), 3.35 (s, 3H, OCH_3), 1.95 (ddd, 1H, *J* = 1.8 Hz, 10.0 Hz, 13.1 Hz, H-7b), 1.58 (dd, 1H, *J* = 5.9 Hz, 13.1 Hz, H-7a); ^{13}C NMR (D_2O, 100 MHz): 101.87 (C-1), 77.31 (C-3), 75.37 (C-5), 73.06 (C-4), 71.32 (C-2), 67.37 (C-6), 55.93 (OCH_3), 27.69 (C-7); *m/z* (CI, NH_3): 224 ($M+NH_4^+$, 100%); HRMS (CI, NH_3): Calcd for $C_8H_{18}O_6N$ ($M+NH_4^+$) 224.1128, Found 224.1121.

6.2. NMR conformational analysis

NMR experiments were performed on a Bruker AVANCE 500 spectrometer at 298 K and using D_2O as solvent. NMR assignments were performed using standard TOCSY and NOESY experiments. The experimental NMR parameters (*J* and NOE data) of each locked glucosides were compared to those estimated by the 3D theoretical model adopting a $^{2,5}B$ conformation. Each locked methyl glucoside model was constructed using Macromodel 9.6[21] as implemented in the Maestro suite[22] of programs (version 8.5.110) and minimized using GB/SA implicit solvent model[23] for water. Calculations were performed using AMBER*[24] force field at 298 K. The general PRCG (Polak-Ribiere Conjugate Gradient) method for energy minimization was applied for a maximum iteration number of 5000 or a convergence threshold of 0.05. For each geometry obtained for compounds **16**, **19**, and **20**, the expected vicinal coupling constants were estimated from the calculated torsion angles by using the empirical Karplus equation proposed by Haasnoot et al.[25] and compared to those obtained experimentally.

Molecular dynamics studies were also conducted using the AMBER* as force field. The starting coordinates for dynamics calculations were those obtained after energy minimization. Simulations were carried out over 10 ns at 300 K with 0.05 fs of time step and 5 ps of equilibration step. Two hundred structures were sampled for further analysis. The continuum GB/SA solvent model was employed and the general PRCG (Polak-Ribiere Conjugate Gradient) method for energy minimization was used. An extended cutoff was applied and the SHAKE procedure for bonds was not selected.

Acknowledgements

Luis Amorim and Filipa Marcelo gratefully acknowledge respectively the European Community (HPRN-CT-2002-00173) and FCT (SFRH/BD/17775/2004) for Ph.D. grants. Filipa Marcelo also thanks FCT-Portugal for a post-doctoral research grant (SFRH/BPD/65462/2009).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carres.2012.07.005.

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