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RCM as a tool to freeze conformation of monosaccharides: synthesis of a β-mannopyranoside mimic adopting a conformation close to the biologically relevant $B_{2,5}$ boat

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Abstract—The synthesis of a β-D-mannopyranoside analog, fully identical to the naturally occurring D-mannopyranose in terms of hydroxyl pattern, and displaying a skew-boat conformation close to a $B_{2,5}$ boat strongly believed to be adopted by the oxycarbenium transition state during glycosidic bond cleavage of β-mannane by family 26 β-mannanase, is described. The conformationally locked analog has been obtained by tethering the C-2 and C-5 carbon atoms of the sugar ring with a three carbon bridge using RCM methodology. Conformation of the mannose analog has been confirmed by NMR and molecular modelling.

Glycosidases are amongst the most studied classes of enzymes because of their involvement in an amazing number of biological processes including glycoprotein biosynthesis and cell–cell or cell-host recognition.[1] A detailed understanding of their mechanisms along with the design of potent inhibitors is of great interest and should lead to chemotherapeutic applications.[2] More than 6000 glycosidases have been reported so far and have been classified in many families according to their aminoacid sequence similarities.[3] While the mechanistic strategies used by glycosidases are fairly well understood,[4] there is still some debate concerning the conformation of the oxycarbenium-like transition state. A half-chair conformation was generally accepted for the oxycarbenium-like transition state until the pioneering work of Sinnott[5] based on kinetic isotope effects and suggesting a boat conformation for yeast α-glucosidase. More recently, the group of Davies has developed a methodology enabling to take snapshots along the glycosidase reaction coordinate.[6] For several glycosidases, a substrate distorsion towards unusual skew-boat conformations was unambiguously observed in the Michaelis complex and leading to oxycarbenium transition state adopting boat conformations. In the specific case of the family 26 β-mannanase, a retaining endo-β-(1,4)-mannosidase,[7] an unprecedented $B_{2,5}$ boat conformation was proposed for the mannopyranosyl cation TS conformation (Fig. 1).[8]

This $B_{2,5}$ conformation was also invoked for the strong inhibition observed on β-mannosidases with the transition state analogs D-manno-1,5-lactone **1**,[9] D-manno-1,5-lactam **2**[10] and D-mannoamidine **3**[11] (Fig. 2). Vasella has explored the close ^{1,4}B conformation[12] and obtained very significant results with conformationally locked azasugars but no pyranosidic analog displaying the hydroxyl pattern of D-mannose and freezed in the $B_{2,5}$ boat conformation has been reported so far to the best of our knowledge. Such structure is of interest since it could be used to probe mannosidases that perform such a substrate distorsion and help in the design of more selective inhibitors of these enzymes. A way to force the sugar ring towards a $B_{2,5}$ boat conformation consists in tethering the C-2 and C-5 carbon atoms with a short spacer (Fig. 2), a strategy previously used by our group with L-iduronic acid in the field of heparin.[13]

Keywords: Conformation; Glycosidase; Mannopyranose; Ring closing metathesis.
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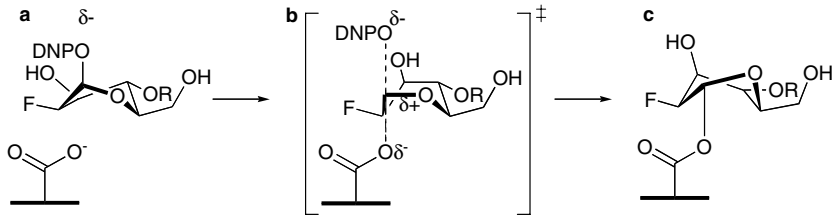


Figure 1. Mannopyranose conformation along the glycosidic bond cleavage of Man26A E212A. (a) Michaelis complex ($^1S_5$), (b) transition state ($B_{2,5}$), (c) covalent intermediate ($^OS_2$).

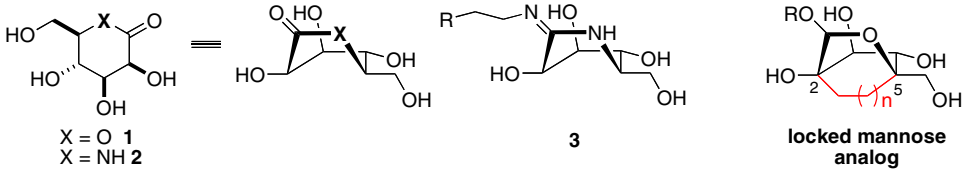


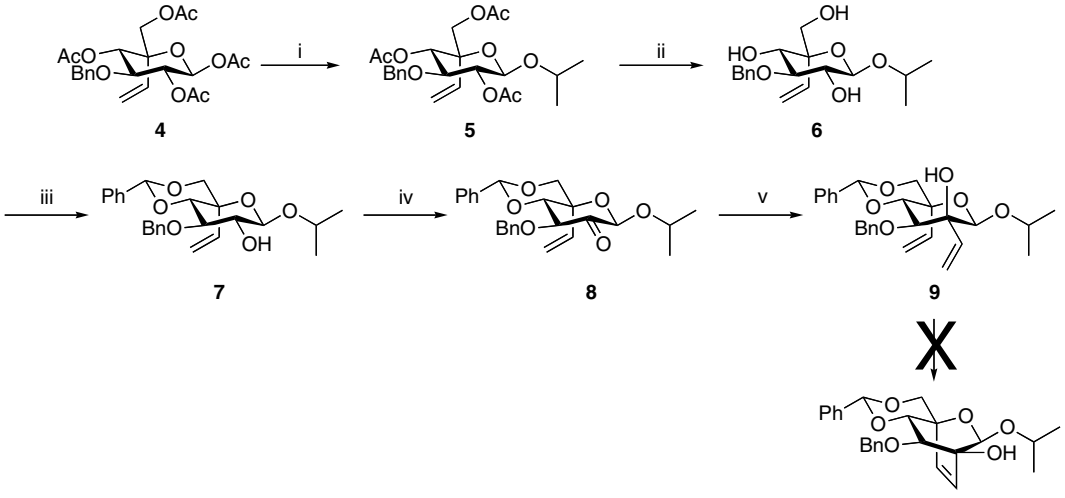
Figure 2. Structures and conformation of lactone **1**, lactam **2**, amidine **3** and target locked mannopyranose analog.

This strategy requires the installation of two moieties at these positions which will be later connected and we used the powerful RCM which is now becoming the method of choice to achieve cyclization in organic chemistry via construction of a C=C bond from terminal alkenes.[14] The synthesis, taking advantage of our previously reported D-glucose derivative **4**[15] which displays a vinyl group at C-5, consists in the stereoselective installation of the alkenyl moiety at C-2 followed by the RCM step. Tetracetate **4** was glycosylated with isopropanol and trimethylsilyl triflate to yield exclusively the β-glycoside **5** in 72% yield. Deacetylation under Zemplen conditions yielded triol **6** which was protected as its 4,6-O-benzylidene derivative **7** in 77% yield over two steps. Swern oxidation of free alcohol at C-2 furnished the rather unstable ketone **8** which was directly engaged in the next step without purification. Grignard reaction with vinyl magnesium bromide in THF afforded the single diene **9** with the vinyl group at C-2 pointing down and being *trans* to the anomeric isopropyl group as expected.[16] Ring closing metathesis of diene **9** using either the 1st or 2nd generation Grubbs' catalysts did not afford the expected cyclized derivative and only starting material was recovered (Scheme 1).

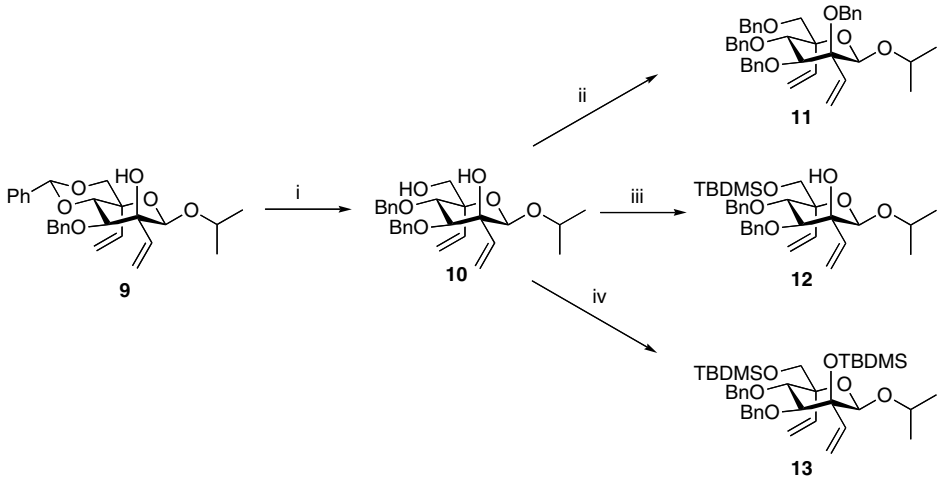
To check wether this result could be attributed to the conformational strain imposed by the benzylidene group or to the chelation of ruthenium by the free allylic alcohol,[17] three analogs of diene **9**, compounds **11**–**13**, displaying various protecting groups, were uneventfully synthesized from **9** (Scheme 2) and submitted to RCM.

Unfortunately no cyclized product was detected whatever the protectings groups pattern used even in the presence of the more powerful 2nd generation Grubbs or Hoveyda–Grubbs' catalysts (Table 1).

A conformational study of compound **12** by NMR and molecular modelling shows that the vinyl moiety at C-5



Scheme 1. Reagents and conditions: (i) isopropanol, TMSOTf, 4 Å MS, anhydrous CH_2Cl_2, 72%; (ii) NaOMe, MeOH; (iii) $PhCH(OMe)_2$, CSA, toluene, 77% over two steps; (iv) oxalyl chloride, DMSO, Et_3N, anhydrous CH_2Cl_2; (v) CH_2=CHMgBr, anhydrous THF, 84% over two steps; (vi) 1st or 2nd generation Grubbs' catalyst (5–10 mol %), anhydrous CH_2Cl_2, reflux.



Scheme 2. Reagents and conditions: (i) $LiAlH_4$, $AlCl_3$, EtOAc, Et_2O, 72%; (ii) NaH, BnBr, anhydrous DMF, 91%; (iii) TBDMSCl, pyridine, 2 h, 96%; (iv) TBDMSCl, pyridine, overnight, 20% (88% based on the recovered starting material).

Table 1. RCM attempts with dienes **9**,**11**,**12** and **13**

Diene	Entry	Conditions	Catalyst	Result
9	1	CH_2Cl_2, reflux, 20 h	Grubbs'II	No reaction
	2	$PhCH_3$, reflux, 20 h	Grubbs'II	No reaction
11	3	CH_2Cl_2, reflux, 20 h	Grubbs'II	No reaction
	4	$PhCH_3$, reflux, 20 h	Grubbs'I	No reaction
	5	$PhCH_3$, reflux, 20 h	Grubbs'II	No reaction
	6	$PhCH_3$, reflux, 96 h	Hoveyda–Grubbs	No reaction
12	7	CH_2Cl_2, reflux, 20 h	Grubbs'II	No reaction
	8	$PhCH_3$, reflux, 20 h	Grubbs'II	No reaction
	9	$PhCH_3$, reflux, 20 h	Hoveyda–Grubbs	No reaction
13	10	CH_2Cl_2, reflux, 20 h	Grubbs'II	No reaction
	11	$PhCH_3$, reflux, 20 h	Grubbs'II	No reaction

is axial while the vinyl moiety at C-2 is equatorial, pointing away from the ring (Fig. 3).

To circumvent this problem, the C-2 alkenyl arm was homologated as follows: ketone **8** was alkylated with allyl magnesium bromide to afford a separable mixture of tertiary alcohols **14** and **15** (5:1 ratio) in favor of the D-*manno* configured isomer **14**. Compound **14** was submitted to RCM using 1st generation Grubbs' catalyst and the expected tricyclic alkene **16** was obtained in a satisfactory 87% yield. Final hydrogenolysis yielded the desired bicyclic β-D-mannopyranose analog **17**[18] (Scheme 3).

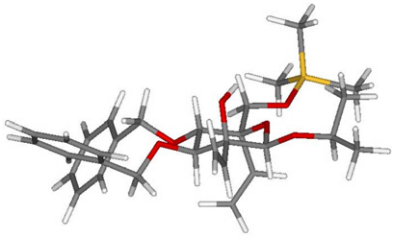
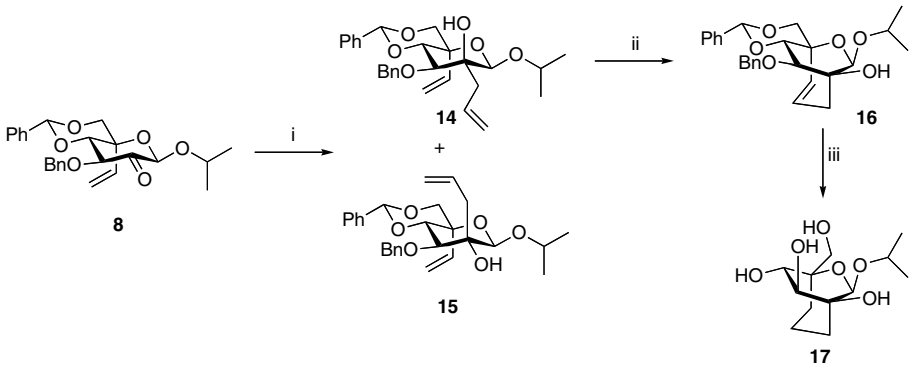


Figure 3. Conformation of compound **12** obtained by NMR and molecular modelling.

Conformation of compound **17** was investigated by NMR (NOE and $J_{3,4}$ 5.4 Hz data) and molecular modelling (MM3* calculations). The comparison between the experimental NMR observations with those derived from the molecular mechanics allowed to unequivocally deduce that this D-mannose analogue exists as an equilibrium of two conformers, **A** and **B**, both adopting a skew-boat conformation close to the desired $B_{2,5}$ geometry (Fig. 4). Conformers **A** and **B** just differ on the orientation of the hydroxymethyl C6 moiety. The observed shift from boat to skew-boat is probably due to the length of the spacer, which allows **17** to adopt a skew-boat geometry, more relaxed with respect to the boat. According to molecular modelling, the use of the shorter two carbon tether would indeed force the six-membered ring to adopt the boat conformation.

Analog **17** was assayed towards the relevant family 26 mannanase and a range of available glycosidases including α-L-fucosidase from bovine kidney, α-galactosidase from coffee beans, β-galactosidases from bovine liver, *Escherichia coli* and *Aspergillus oryzae*, α-glucosidases from yeast and rice, amyloglucosidase from *Aspergillus niger*, almonds β-glucosidase, jack bean α-mannosidase, β-mannosidase from *Helix pomatia*, β-xylosidase from *Aspergillus niger* and β-*N*-acetylglucosaminidase from



Scheme 3. Reagents and conditions: (i) CH_2=$CHCH_2MgBr$, anhydrous THF, 72% from **7**; (ii) 1st generation Grubbs' catalyst (5–10 mol %), anhydrous CH_2Cl_2, reflux, 87%; (iii) H_2, Pd black, methanol/ethyl acetate, 97%.

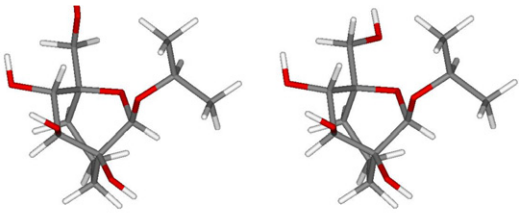


Figure 4. Structure of conformers **A** and **B** for **17**. They only differ on the orientation around the exocyclic C5–C6 bond. Torsion angles for the pyranoid ring are O1–C1–C2–C3, 34°; C1–C2–C3–C4, 22°; C2–C3–C4–C5, 62°; C3–C4–C5–O, 40°; C4–C5–O–C1, 19°; C5–O–C1–C2, 58°.

jack bean and bovine kidney but did not show significant activity on these enzymes, a result that can be tentatively explained by a steric clash caused by the three carbon bridge precluding entry of the analog in the glycosidases active site.

In conclusion, the conformational aspect in the glycosidase-mediated glycosidic bond cleavage is crucial not only for the rational design of potent glycosidase inhibitors but also to have insights into the stereoelectronic effects involved in the glycosidase mechanisms. A D-mannopyranose analog adopting a skew-boat conformation close to the family 26 β-mannanase relevant $B_{2,5}$ boat conformation has been obtained using RCM as the key step. Unfortunately this compound did not exhibit any effect towards glycosidases which might be due to the steric clash caused by the three carbon bridge.

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18. Spectroscopic data for compound **17**: $[\alpha]_D$ −30 (*c* 1 in CH_3OH); ^1H NMR (D_2O, 400 MHz): 4.73 (d, 1H, $J = 0.9$ Hz, H-1), 4.18 (d, 1H, $J = 5.4$ Hz, H-4), 4.02 (m, 1H, $J = 6.2$ Hz, CH isopropyl), 3.80 (dd, 1H, $J = 0.9$ Hz, $J = 5.4$ Hz, H-3), 3.56 (d, 1H, $J = 12.3$ Hz, H-6), 3.44 (d, 1H, $J = 12.3$ Hz, H-6′), 1.96 (m, 1H, H-7), 1.82–1.63 (m, 4H, H-7′, H-8, H-8′, H-9), 1.36 (m, 1H, H-9′), 1.22 (d, 3H, $J = 6.2$ Hz, CH_3 isopropyl), 1.13 (d, 3H, $J = 6.2$ Hz, CH_3 isopropyl); ^{13}C NMR (D_2O, 100 MHz): 100.71 (C-1), 79.68 (C-2), 76.44 (C-3), 73.55 (C-4), 71.04 (C-5), 70.21 (CH isopropyl), 65.81 (C-6), 35.48 (C-7), 28.89 (C-9), 23.08 (CH_3 isopropyl), 20.68 (CH_3 isopropyl), 17.48 (C-8); *m*/*z* (CI, NH_3): 280 ($M+NH_4^+$, 100%); HRMS (CI, NH_3): calcd for $C_{12}H_{26}O_6N$ ($M+NH_4^+$) 280.1760, found 280.1757.