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Synthesis of α-C(1→3)-Mannopyranoside of N-Acetylgalactosamine, a new β-Galactosidase Inhibitor.

Carla Pasquarello, Raynald Demange, and **Pierre Vogel***

Institut de chimie organique de l'Université de Lausanne, BCH, CH-1015 Lausanne-Dorigny, Switzerland

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Abstract: Radical C-glycosidation of a 3-methylidene-7-oxabicyclo[2.2.1]heptan-2-one derivative with acetobromo-mannose gave a α-C-mannopyranoside that was converted into α-D-Man*p*CH_2(1→3)-D-GalNAc, a C-disaccharide that inhibits β-galactosidase from jack bean with IC_{50} = 9.4 μM and K_i = 7.5 μM (mixed mode of inhibition).
Keywords: carbohydrate mimetics, enzyme inhibitors, C-glycosidation

The interaction between cell surface carbohydrates and their protein receptors is implicated in several important biological events.[1] Carbohydrate mimetics are potentially useful tools to study cellular interactions, the biosynthesis of glycoproteins, the catabolism of glycoconjugates,[2] and the mechanisms of digestion.[3] Inhibitors of enzymes involved in these processes such as the glycosidases and the glycosyltransferases are potential anti-cancer, antiviral, and antidiabetic agents; they can also have insect antifeedant effects.[4] Disaccharide mimetics such as the C-disaccharides[5] are potential glycosidase inhibitors and may represent non-hydrolyzable epitopes.[6] Glycosidase inhibition has been reported for dideoxyiminoalditol C-linked to monosaccharides[7] but not for other types of C-disaccharides. Except for C-trisaccharides that are mimetics of the human blood group determinant H-type II,[8] for β-D-Gal*p*CH_2(1→4)-β-D-GlcNAc-O-n-octyl[9] and for α,β-D-GalNAc*p*CH_2(1→4)-α-D-GlcOMe,[10] all the other C-disaccharides and C-trisaccharides reported thus far contain only hydroxy groups. We present here the synthesis of α-D-Man*p*CH_2(1→3)-D-GalNAc and show that it is a potent inhibitor of β-galactosidase from jack bean and a weaker inhibitor of β-galactosidase from *E. coli.*, an unexpected result considering the fact that neither D-mannose, nor N-acetyl-galactosamine (GalNAc) do inhibit these enzymes.

Giese's[11] radical C-glucosidation[12] and C-galactosidation[13] of enone (-)-**2** derived from (+)-(1*R*,4*R*)-7-oxabicyclo[2.2.1]hept-5-en-2-one ((+)-**1**: a "naked sugar" of the first generation)[14] has been shown to be highly diastereoselective, giving C-glycosides that have been converted into C-disaccharides and C-glycosides of carbapentopyranoses[15] and analogues.[16] We show now that under modified conditions the α-C-mannosylation of (-)-**2** is also possible when Ph_3SnH (instead of Bu_3SnH) and AIBN were added slowly (syringe) to a boiling benzene (instead of toluene) solution 0.16 molar in (-)-**2** and 0.2 molar in 2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl bromide. This gave α-C-mannoside (+)-**3**[17] (56%) with high diastereoselectivity. As expected, reduction of the ketone (+)-**3** with $NaBH_4$ (THF, MeOH, 0°C, 5 min) was *exo* face selective giving alcohol (-)-**4** (79%).[18] Oxidative elimination of the benzeneselenyl group with mCPBA (CH_2Cl_2, -78° to 20°C, 12 h) and

* Fax: 0041 21 692 39 75; E-mail: pierre.vogel@ico.unil.ch

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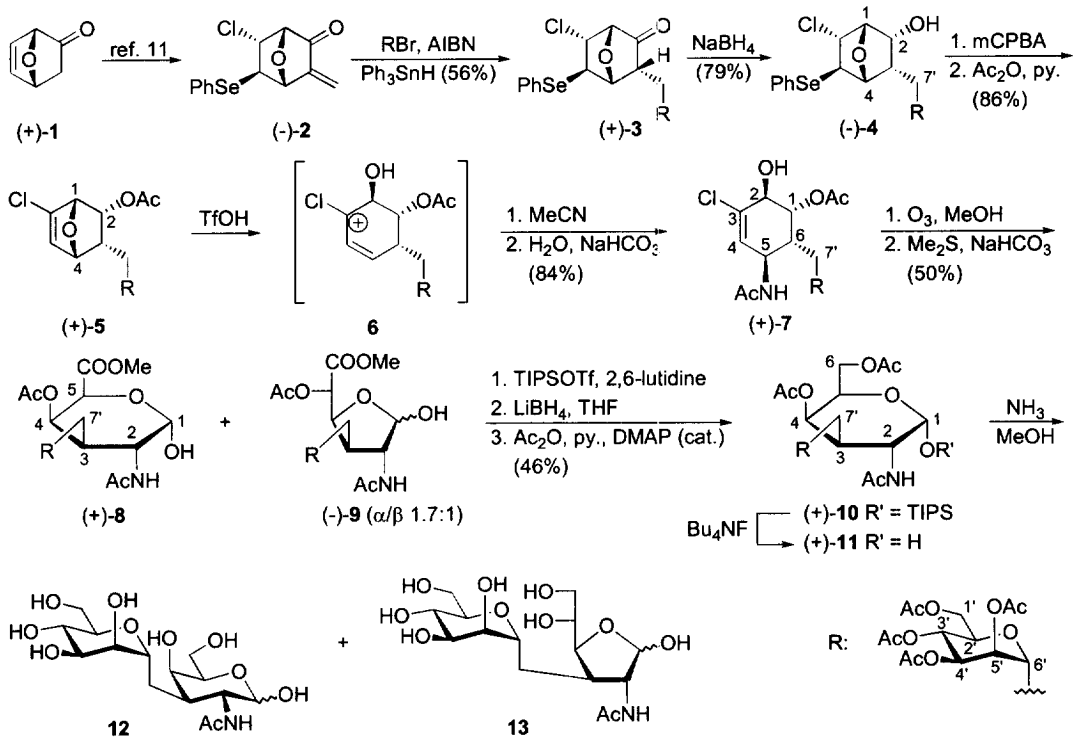
subsequent acetylation (Ac_2O, pyridine, DMAP (cat.), 20°C, 7 h) provided (+)-**5** (86%). Acid-promoted (CF_3SO_3H) oxa-ring opening of the 7-oxanorbornene (+)-**5** in anhydrous acetonitrile (0-20°C, 45 min) produced, after aqueous work-up ($NaHCO_3$), the amino-conduritol derivative (+)-**7** (84%).[19] This result can be interpreted in terms of a regioselective heterolysis of the C(4)-O(7) bond in (+)-**5** leading to the allyl cation intermediate **6** that reacts on its less sterically hindered face with MeCN (Ritter[20] reaction). Heterolysis of the C(1)-O(7) bond does not occur because the allyl cation so-obtained would not be stabilized by the chloro substituent as well as intermediate **6**. Ozonolysis of the chloroalkene (+)-**7** generated an acyl chloride-aldehyde intermediate that reacted with methanol[21] to produce a 1.4:1 mixture of the methyl uronates (+)-**8** and (-)-**9**[22] that can be separated by flash column chromatography on silica gel. Silylation of (+)-**8** with $(i\text{-Pr})_3SiOSO_2CF_3$ and 2,6-lutidine (0°C, 5 min, 20°C, 3.5 h) gave a white solid that was reacted with $LiBH_4$ (THF, 0°C, 5 min, 20°C, 23 h). The crude polyol so-obtained was then acetylated (Ac_2O, pyridine, DMAP (cat.), 20°C, 12 h) to furnish (+)-**10** (46% based on (+)-**8**). Desilylation with Bu_4NF (THF, 0°C, 5 min) delivered (+)-**11**[23] (74%). Compounds (+)-**3** – (+)-**11** were fully characterized by their spectral data and elemental analyses. Their structures were confirmed by 2D-NMR (NOESY, COSY) measurements.



Ammonolysis (NH_3, MeOH, 20°C, 10 h) of (+)-**11** and chromatographic purification (Sep-Pak® RP18, H_2O) provided a mixture of α-/β-pyranoses **12** and α-/β-furanoses **13** that was tested for its inhibitory activity toward commercially available glycosidases.[24] At 1 mM concentration the C-disaccharide **12** + **13** inhibited β-galactosidase from *E. coli.* and β-galactosidase from jack bean by 86% and 92%, respectively; further kinetic measurements gave IC_{50} = 500 μM and IC_{50} = 9.4 μM (K_i = 7.5 μM), respectively, for these two enzymes. The Lineweaver and Burk plots for the inhibition of β-galactosidase from jack bean by **12** + **13** showed a mixed

(competitive, non-competitive) type of inhibition. Neither β-galactosidases from *Aspergillus niger* and from *Aspergillus orizae*, amyloglucosidases from *Aspergillus niger* and from *Rhizopus* mold, α-mannosidases from jack bean and from almonds, β-mannosidase from *Helix* pomatia, nor α-N-acetylgalactosaminidase from chicken liver were inhibited by **12** + **13**.

Synthesis of other functional C-disaccharides and further enzymatic tests are necessary to approach an explanation of our inhibition results. This work extends the versatility of our method for the synthesis of disaccharide mimetics[12,13,15,16] and demonstrates that C-disaccharides that are not dideoxyiminoalditols can be potent glycosidase inhibitors.

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17. Data for (+)-**3**: white solid, $[\alpha]^{25}_D$ 5.8 (*c*=1.0, $CHCl_3$); IR (KBr) ν: 1745, 1370, 1225 cm^{-1}.

18. Data for (-)-**4**: white solid, $[\alpha]^{25}_D$ –7.0 (*c*=1.0, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.65-7.63 (m 2H), 7.34-7.27 (m, 3H), 5.25 (dd, ^3J=7.1, 3.3 Hz, H-4'), 5.09 (dd, ^3J=5.3, 3.3, H-5'), 5.05 (dd, ^3J=7.1, 5.9, H-3'), 4.61 (d, ^3J=5.5, H-4), 4.50-4.43 (m, H-1, H-2, H-1'), 4.33-4.30 (m, H-6), 4.10 (dd, ^2J=12.1, ^3J=3.4, H-1'), 4.00 (ddd, ^3J=9.3, 5.03, 5.0, H-6'), 3.77 (ddd, ^3J=7.2, 5.9, 3.4, H-2'), 3.65 (d, ^3J=4.9, H-5), 2.45-2.36 (m, H-3), 2.10-2.00 (m, H-7', 4 AcO), 1.47 (ddd, ^2J=14.4, ^3J=9.2, 6.7, H-7').

19. Data for (+)-**7**: white solid, $[\alpha]^{25}_D$ 16 (*c*=1.0, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.93 (d, ^3J=9.5, NH), 5.91 (d, ^3J=2.5, H-4), 5.23 (dd, ^3J=7.4, 3.1), 5.20 (dd, ^3J=3.1, 2.2, H-4', H-5'), 5.07-5.03 (m, H-1, H-3'), 4.56 (ddd, ^3J=11.0, 9.5, 2.5, H-5), 4.53 (dd, ^2J=12.0, ^3J=7.8, H-1'), 4.10-4.05 (m, H-6', H-2'), 4.02-3.98 (m, H-1', H-2), 3.50-3.40 (br. d, OH), 2.12-2.04 (m, 5 AcO, H-6), 1.82 (ddd, ^2J=14.6, ^3J=11.4, 2.9, H-7'), 1.52 (ddd, ^2J=14.6, ^3J=10.6, 3.0, H-7'); ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ: 172.1, 170.5, 170.4, 170.0, 169.7 (5s), 132.6 (s, C-3), 129.4 (d, C-4), 72.0, 70.8, 69.9, 69.8, 69.6, 68.3, 68.0 (7d), 62.8 (t, C-1'), 48.2 (d, C-5), 33.0 (d, C-6), 26.5 (t, C-7'), 23.2, 21.0, 20.9, 20.8, 20.6 (5q).

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22. Data for (+)-**8**: white solid, $[\alpha]^{25}_D$ +50 (*c*=0.5, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.81 (d, ^3J=9.8, NH), 5.57 (br. s, H-4), 5.36 (d, ^3J=3.0, H-1), 5.22 (dd, ^3J=7.2, 3.3, H-4'), 5.04 (dd, ^3J=7.2, 7.1, H-3'), 5.00 (dd, ^3J=4.9, 3.3, H-5'), 4.75 (br. s, H-5), 4.48 (dd, ^2J=12.0, ^3J=8.3, H-1'), 4.32 (ddd, ^3J=9.9, 9.8, 3.0, H-2), 4.17 (dd, ^3J=12.0, 4.9, H-6'), 4.14-4.10 (m, H-2'), 4.01-3.96 (m, H-1'), 3.74 (s, MeO), 3.70-3.60 (br. s, OH), 2.37-2.29 (dd, ^3J=9.9, 9.7, H-3), 2.14-2.04 (6 Ac), 1.80-1.60 (m, H-7'), 1.40-1.25 (m, H-7'); Data for (-)-**9**: white solid, $[\alpha]^{25}_D$ –30 (*c*=0.5, $CHCl_3$).

23. Data for (+)-**11**: white solid, $[\alpha]^{25}_D$ +37 (*c*=0.5, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 5.82 (d, ^3J=9.8, NH), 5.29 (br. s, H-4), 5.24-5.19 (m, H-1, H-4'), 5.02 (dd, ^3J=6.3, 6.2, H-3'), 4.98 (dd, ^3J=5.2, 3.3, H-5'), 4.48 (dd, ^2J=12, ^3J=8.4, H_a-1'), 4.32 (dd, ^3J=6.5, 6.4, H-5), 4.24 (ddd, ^3J=12.7, 9.8, 3.5, H-2), 4.19-4.08 (m, H_b-1', H-6, H-6'), 4.02-3.93 (m, H-2', H-6), 2.24 (dddd, ^2J=12.7, ^3J=12.0, 2.8, 2.2, H-3), 2.14-2.05 (7 Ac), 1.70 (ddd, ^2J=14.0, ^3J=12.0, 2.2, H_a-7'), 1.33 (ddd, ^2J=14.0, ^3J=10.7, 2.2, H_b-7'); ^{13}C-NMR (100.6 MHz, $CDCl_3$) δ: 171.1, 170.6, 170.4 (2C), 169.8, 169.6 (2C, 7s), 91.3, 71.1, 70.0, 68.6, 68.3, 67.9, 67.5, 66.6 (8d), 62.7, 62.4 (2t), 47.5, 33.5 (2d), 25.8 (t), 23.3, 20.9, 20.8, 20.6 (4q). CI-MS (NH_3) m/z: 634 (4, $M^{\bullet+}$), 616 (36), 71 (100).

24. Appropriate *p*-nitrophenyl glycoside substrates buffered to optimum pH of the enzymes were used; for details, see: Brandi, A.; Cicchi, S.; Cordero, F. M.; Frignoli, B.; Goti, A.; Picasso, S.; Vogel, P. *J. Org. Chem.* **1995**, *60*, 6806-6812.