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Synthesis of permethylated α-D-mannosylacetic acid, a new type of bioconjugate

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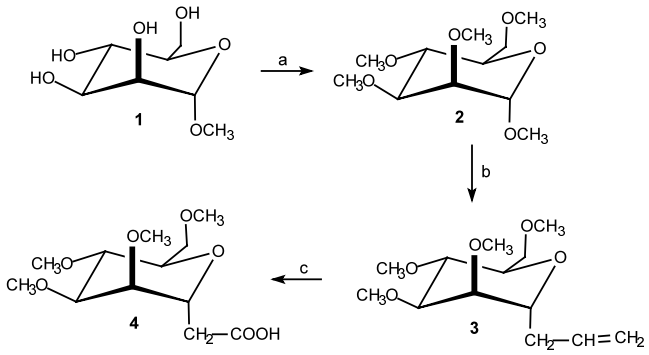
Abstract—A concise stereoselective 3-step conversion of methyl α-D-mannopyranoside to α-D-2,3,4,6-tetra-*O*-methyl-mannosylacetic acid is described. After methylation of the alcohol functions, an allylation is performed. The resulting alkene undergoes oxidative cleavage to the acid, an alkylated C-sugar, appropriate for attachment to peptides or other drug candidates for solubility enhancement.

Carbohydrates play a major role in numerous biological processes. As glycoproteins and glycolipids, they are involved in cell–cell recognition and adhesion.[1] Glycopeptides and their analogs are also useful mimics for effecting biological interactions, but their potential as biomedicinals extends beyond pharmacodynamics to the area of pharmacokinetics, especially if appropriately modified.

Carbohydrate moieties are bound to peptides or proteins by an *N*- or *O*-glycoside linkage. If the ring oxygen of a carbohydrate is replaced by a methylene unit, these stabilized derivatives are known as pseudosugars.[2] Alternatively, replacing the anomeric O by an exocyclic methylene group yields compounds known as *C*-glycosides or *C*-sugars.

C-Glycosides have proven useful as carbohydrate mimetics[3] but with improved properties; they are now stable to acid, base, and enzymatic hydrolysis and will not undergo hydrogen bonding at the former anomeric position.[4] They can replace *O*-glycosides in the investigations of various biochemical interactions. For example, the *C*-saccharide analogue of a GalNAC (*N*-acetylgalactosamine, Tn epitope) derivative has proven useful as a component of an anticancer vaccine.[5]

The chemistry of carbohydrates is dominated by the multiple hydroxyl functions. When the hydroxyl groups of sugars are alkylated, the H-bond donor character is eliminated and this change impacts overall molecular hydrophilicity. Current drug design concepts suggest that these altered properties should be beneficial with respect to oral bioavailability and for blood–brain barrier penetration.[6] Thus, alkylated *C*-sugars may be comparable to poly(ethyleneglycol) (PEG),[7] and represent a new class of solubility enhancing bioconjugates.[8] Herein we describe (as shown in Scheme 1) a simple route for the synthesis of these species in a form suitable for attachment to amino acids, peptides or other potential chemotherapeutic candidates (Scheme 1). Alkylation serves as both the synthetic end-point as well as a requisite hydroxyl protection functionality.



Scheme 1. *Reagents*: (a) NaH, CH_3I, DMF; (b) TMSOTf, allyltrimethylsilane, CH_3CN; (c) $NaIO_4$, $KMnO_4$, ^tBuOH, H_2O.

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As starting material for this transformation we selected α-D-mannose, a carbohydrate commonly present as a recognition element in biological processes. Its axial hydroxyl at C-2 provides a stereospecific outcome at C-1 during the allylation step. The methyl group was selected as the alkyl ether moiety in order to retain an overall hydrophilic character for the final bioconjugate.

The first step involved formation of the permethylated mannose from α-D-methyl mannopyranoside, slightly modified from the literature procedure of Brown et al.[9] Following dissolution of the mannopyranoside in freshly distilled DMF, 2 equiv. of sodium hydride were added. After heating at 70°C for 2–3 h, the solution was cooled to rt and 2 equiv. of methyl iodide were slowly introduced over 1 h. This two-step procedure was repeated three times.[†] After the complete cycle (a total of about 24 h), the resulting methylated mannoside was purified by column chromatography and obtained in decent yield (50–60%).[‡] The next step provided the carbon replacement for oxygen through an allylation reaction. The allylation was performed using allyltrimethylsilane in dry, freshly distilled acetonitrile;[10] trimethylsilyltriflate was used as the catalyst.[§] The reaction was almost quantitative and is highly stereoselective.[11] The product was again purified by column chromatography (yield: 97%).[¶]

The final step involved oxidative cleavage of the allyl function to produce the desired functionalized methylated *C*-sugar. According to literature reports for comparable, allylic systems,[12,13] cleavage of the allyl group was performed in a two-step fashion: OsO_4 hydroxylation–$NaIO_4$ cleavage produced the aldehyde,[12] which was oxidized to carboxylic acid by Jones reagent in a second step.[13] In our case, using the methylated derivative, we decided to avoid the osmium tetroxide due to toxicity considerations and instead utilized a one-step periodate procedure as introduced by Aristoff and coworkers.[14] Accordingly, the permethylated mannopyranoside alkene was oxidized with sodium periodate and a catalytic amount of potassium permanganate in *tert*-butanol at rt for 2 h. After standard workup, the acid was obtained in very good yield and purity (83%).[‖] In preliminary studies of **4** conjugated with the amino acid leucine, the conjugate was slightly soluble in ether and water solubility was at least doubled.[**]

In summary, we have presented an effective route to synthesize an alkylated *C*-sugar derivative of a monosaccharide. This method should be applicable to numerous other carbohydrates, although the stereochemical outcomes of the resulting products in terms of their α/β ratios will surely vary with respect to the parent sugar. When coupled to peptides or proteins, our modified sugars should offer comparable improvements in solubility as well as possible enhancements of pharmacological half-lives.

Supporting information available: ^1H, ^{13}C NMR spectra of **2**, **3**, **4**, as well as IR data and mass spectrometry results of the permethylated mannosylacetic acid **4** can be provided by request from spatola@louisville.edu.

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References

1. (a) Varki, A. *Glycobiology* **1993**, *3*, 97; (b) Lis, H.; Sharon, N. *Eur. J. Biochem.* **1993**, *218*, 1.

[†] The reaction is followed by TLC and an extra addition may be needed.

[‡] During the extraction, $CHCl_3$ was a better solvent than EtOAc; brine was used but some product was still lost in the water washes. This first extraction is tedious. Elution solvent: EtOAc/hexane (1/4). The permethylated glycoside was characterized by ^1H and ^{13}C NMR analysis (see supporting information). We clearly see 5 OCH_3 groups (^{13}C δ: 59.76, 58.35, 58.09, 56.46, 54.2 and ^1H δ: 3.35, 3.34, 3.32, 3.27, 3.25 in DMSO).

[§] The reagents were cooled to 0°C and the mixture was stirred for 2 h.

[¶] Elution solvents: EtOAc/hexane (1/2). The alkene was characterized by ^1H and ^{13}C NMR methods (see supporting information). The olefinic peaks are in the expected region (^{13}C δ: 134.43, 117.2 and ^1H δ: 5.8, 5.1 in $CDCl_3$).

[‖] **Typical procedure for the oxidation**: A solution of $NaIO_4$ (18.1 g, 8.5 mmol) in H_2O (300 mL) was treated with $KMnO_4$ (0.286 g, 1.81 mmol) for 30 min. The permethylated alkene derivative (2.48 g, 9.52 mmol), dissolved in ^{t}BuOH (80 mL), was added. The mixture was stirred at rt for 3 h. Then, ethylene glycol (3 mL, 54 mmol) was added. The greenish/brown solution was stirred for another 1.5 h, then acidified to pH 3 with 2N HCl solution. To the solution was added 80 mL of $CHCl_3$. The separated aqueous solution was further extracted with $CHCl_3$, and the combined organic phases were washed with brine twice, then dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to afford 2.2 g (83%) of a pale yellow oil. The compound was characterized by ^1H, ^{13}C, IR, and mass spectrometry (see supporting information). ^1H NMR (C_6D_6) δ 4.59 (H1, 1H, q, $J=6$ Hz), 3.36 (H2, 1H, dd, $J=2.5$, 6.5 Hz), 3.50 (H3, 1H, q, $J=3$ Hz), 3.56 (H4, 1H, t, $J=5$ Hz), 3.97 (H5, 1H, q, $J=5$ Hz), 3.67 (H6, 2H, m), 2.58 (H′1, 2H), 3.26 (OCH_3, 3H, s), 3.24 (OCH_3, 3H, s), 3.23 (OCH_3, 3H, s), 3.20 (OCH_3, 3H, s). ^{13}C NMR (C_6D_6) δ 175.81 (CO), 68.64 (C1), 78.25 (C2), 77.88 (C3), 76.95 (C4), 73.57 (C5), 71.36 (C6), 36.71 (C′1), 58.82 (OCH_3), 58.25 (OCH_3), 58.03 (OCH_3), 56.87 (OCH_3). HR-FAB m/z calcd for $[C_{12}H_{22}O_7Na]^+$ 301.1264, found 301.1275.

[**] 8 mg of leucine attached to the acid derivative could be completely dissolved in 1 mL of ether, while leucine itself is insoluble in ether. 6 mg of leucine could not be dissolved in 0.25 mL of distilled water while 12 mg of leucine attached to the acid derivative was completely soluble in the same amount of water. With the bioconjugate attached, leucine water solubility was increased to at least 48 mg/mL; leucine itself is soluble only to 23 mg/mL.[15]

2. McCaslan, G. E.; Furuta, S.; Durham, L. J. *J. Org. Chem.* **1966**, *33*, 1516.
3. Bertozzi, C. R.; Bednarski, M. D. *J. Org. Chem.* **1991**, *56*, 4326.
4. (a) Levy, D. E.; Tang, C. *The Chemistry of C-Glycosides*; Pergamon, 1995; Vol. 13; (b) Postema, M. H. D. *C-Glycoside Synthesis*; CRC Press: London, UK, 1995.
5. Peri, F.; Cipolla, L.; Rescigno, M.; La Ferla, B.; Nicotra, F. *Bioconjugate Chem.* **2001**, *12*, 325.
6. (a) Pardridge, W. M.; Mietus, L. J. *J. Clin. Invest.* **1979**, *64*, 145; (b) Burton, P. S.; Conradi, R. A.; Hilgers, A. R.; Ho, N. N. F. *J. Contr. Rel.* **1992**, *19*, 87.
7. (a) Greenwald, R. B.; Conover, C. D.; Choe, Y. H. *Crit. Rev. Ther. Drug* **2000**, *17*, 101; (b) Zalipsky, S. *Bioconjugate Chem.* **1995**, *6*, 150; (c) Matsushima, A.; Kodera, Y.; Hiroto, M.; Nishimura, H.; Inada, Y. *J. Mol. Catal. B-Enzym. 2* **1996**, *1*, 1.
8. For a review on bioconjugates, see: Hermanson, G. T. *Bioconjugate Techniques*; Academic Press: San Diego, CA, 1996.
9. Brown, C. A.; Barton, D.; Sivaram, S. *Synthesis* **1974**, *6*, 434.
10. Hosomi, A.; Sakata, Y.; Sakurai, H. *Tetrahedron Lett.* **1984**, *25*, 2383.
11. Bertozzi, C.; Bednarski, M. *Carbohydr. Res.* **1992**, *223*, 243.
12. (a) Pappo, R.; Allen, D. S.; Lemieux, R. U.; Johnson, W. S. *J. Org. Chem.* **1956**, *21*, 478; (b) Schroder, M. *Chem. Rev.* **1980**, *80*, 187.
13. Henry, J. R.; Weinreb, S. M. *J. Org. Chem.* **1993**, *58*, 4745.
14. Aristoff, P. A.; Johnson, P. D.; Harrison, A. W. *J. Am. Chem. Soc.* **1985**, *107*, 7967.
15. Greenstern, J. P.; Winitz, M. *Chemistry of the Amino Acids*; John Wiley: New York, 1961; Vol. 2, p. 547.