Novel Hybrid Morpholino-Glycopeptides with the Amino Acid Nitrogen Replacing C-3 of the Pyranose Ring

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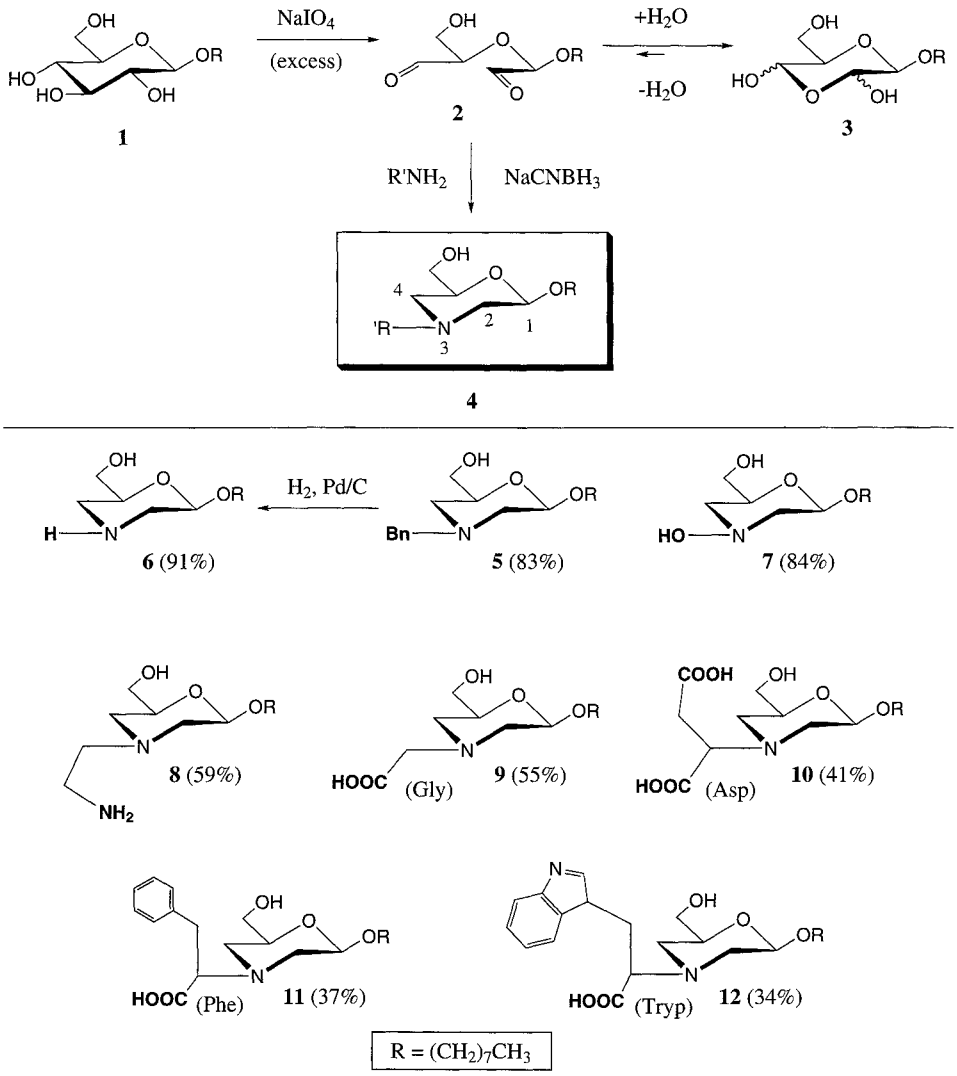
Abstract: Periodate oxidation of glycopyranosides yields dialdehydes that can be reductively aminated in the presence of amino acids to produce hybrid glycopeptides where the amino acid nitrogen is inserted into the resulting 2,4-dideoxypyranoside at position 3. Nine such 3-morpholino derivatives were prepared, including a disaccharide analog of N-acetyllactosamine (βGal(1→4)βGlcNAc) where the nitrogen of glycine was inserted into the galactose ring. The resulting hybrid disaccharide was an excellent substrate for a milk fucosyltransferase which converted it to a novel functionalized LeX mimic.

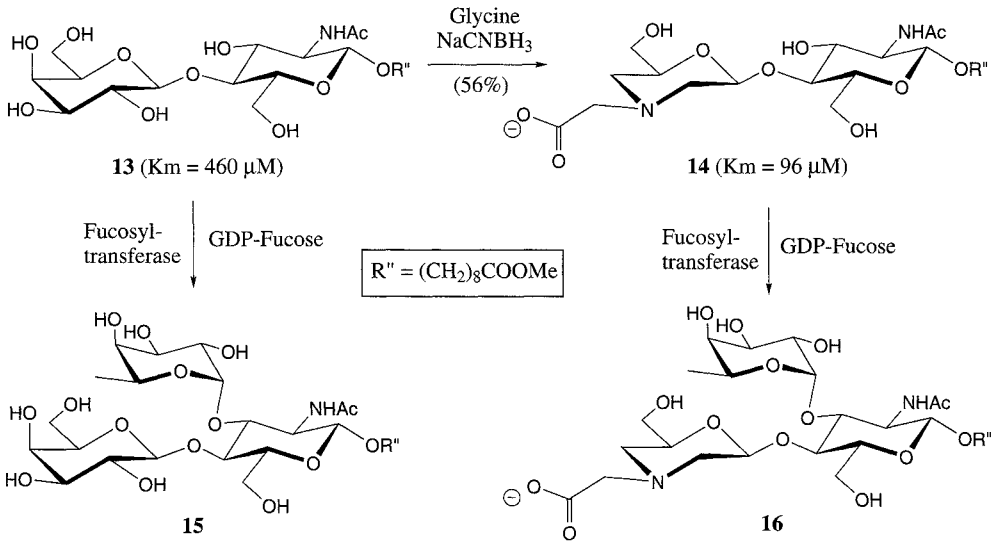
Analogs of carbohydrate-structures are of interest in the probing of protein-carbohydrate recognition[1]. In parallel with the recent surge of interest in combinatorial chemistry[2] there has been an interest in synthesizing carbohydrate analogs that are readily functionalized using high-yield established chemistry such as amide bond formation. In addition to glycopeptides themselves (ref 3), examples include the direct modification of sugars so that they bear both amine and carboxyl groups. Recent examples include uronic acid amines[3], "saccharide peptide hybrids"[4], "glycopeptoids"[5], and glycosamino acids that yield "glycotides"[6].

A novel approach to the synthesis of sugar-amino acid hybrids involves the oxidation of carbohydrates containing vicinal triols with excess sodium metaperiodate to extrude the central carbon and yield dialdehydes (Scheme). Such dialdehydes can undergo many reactions, including reductive amination to insert a nitrogen atom into the sugar ring. This procedure is commonly used in the synthesis of morpholino-DNA derivatives (using ribose oxidation)[7] and in the very elegant insertion of substituted amines into the cyclohexane ring of inositol-triphosphates[8]. We report here that simple monosaccharide glycosides, and appropriate disaccharide glycosides, are readily converted without the need for protecting groups to 3-morpholino-derivatives where the amino acid nitrogen is inserted into position 3 of the pyranose ring.

The oxidation of octyl β-D-glucopyranoside (**1**) (Scheme) produces the dialdehyde **2** which is in equilibrium with the hydrated form **3**. Reductive amination ($NaCNBH_3$) in the presence of primary amines then gave the 3-morpholino derivative **4** (Ref I, typical procedures). The reductive amination with benzylamine proceeded smoothly to give **5**. Hydrogenolysis then gave the free 3-aza-derivative **6**. Similarly, **3** was reductively aminated using hydroxylamine, ethylene diamine, glycine,



Scheme 1



Scheme 2

L-aspartic acid, DL-phenylalanine and L-tryptophan to give **6** - **12**. The yields (unoptimized) varied in the range 34 - 84%.

The reaction was extended to the N-acetyllactosamine derivative **13** wherein only the terminal Gal residue contains vicinal diols. Periodate oxidation followed by reductive amination using glycine gave the 3' aza-disaccharide analog **14** in 56% isolated yield[9].

The attractiveness of the procedure as applied here is that the octyl aglycone in **1** (or the 8-methoxycarbonyloctyl aglycone in **13**) permits the isolation of the dialdehyde from the periodate oxidation mixture by simple adsorption onto reverse-phase C-18 resins from which it can be eluted with methanol after the salts have been eluted by washing with water[10]. The final reductively aminated product is purified by conventional silica gel chromatography. No hydroxyl protecting groups are used.

The hybrid glycopetides produced are deoxygenated at C-2 and C-4 of the pyranose ring. While this may be disadvantageous in some instances, the contrary was found for the case of the recognition of the substrate LacNAc-OR" (**13**) by a human milk fucosyltransferase. This enzyme converts **13** to the LeX trisaccharide (**15**) with the kinetic constants Km = 0.46 ± 0.09 mM and Vmax = 33 ± 3 pmol/min under standard reaction conditions[10]. The 3'-aza-LacNAc derivative **14** was recognized almost 5 times more effectively than LacNAc, with Km = 0.095 ± 006 mM (Vmax of 21.3 ± 0.3 pmol/min). The product of the reaction is interesting LeX analog **16** bearing a glycine residue inserted into the galactose ring [11].

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9. **Typical experimental procedures: A) Preparation of 5:** Octyl glucopyranoside **1** (20 mg, 68 μmol) was added to a solution of sodium periodate (29.2 mg, 136 μmol) in water (0.6 mL) at 4°C. The reaction mixture was then kept at room temperature for 4 h and loaded onto two connected C-18 Sep-Pak cartridge (Waters). The dialdehyde hydrate **3** (19 mg) was obtained as a syrup after washing with water (30 mL) and elution with methanol (20 mL) followed by evaporation. The remaining syrup was dissolved in MeOH (0.6 mL) and benzyl amine (0.2 mL) was added followed by sodium cyanoborohydride (20 mg). After 4 h at room temperature, the reaction mixture was concentrated and the residue was dissolved in water and isolated on a two Sep-Paks as described above. The crude material was purified by a column of silica gel (1 : 1 EtOAc-hexane, containing 0.5% triethylamine) to provide **5** (19 mg, 83%) as a clear syrup; Rf 0.26 (5% MeOH in CH_2Cl_2); $[\alpha]_D$ -34.6° (*c* 1.6, MeOH); ^{1}H NMR (CD_3OD): δ 7.35 (m, 5 H, Ph), 4.57 (dd, 1 H, $J_{1,2eq}$ 2.5 Hz, $J_{1,2ax}$ 8.5 Hz, H-1), 3.86 (m, 1 H, OCH_2), 3.66 (m, 1 H, H-5), 3.53 (s, 2 H, $PhCH_2$), 3.51 (m, 2 H, H-6), 2.81 (dd, 1 H, $J_{2ax,2eq}$ 11.0 Hz, H-2eq), 2.76 (dd, 1 H, $J_{4ax,4eq}$ 11.0 Hz, H-4eq), 1.86 (dd, 1 H, $J_{1,2ax}$ 8.5 Hz, $J_{2ax,2eq}$ 11.0 Hz, H-2a), 1.85 (t, 1 H, $J_{4ax,4eq} = J_{4ax,5}$ = 11.0 Hz, H-4ax), 0.90 (t, 3 H, *J* 7.0 Hz, CH_3). ^{13}C NMR (CD_3COCD_3): δ 139.02 (Ph, quat.), 129.82, 128.93, 127.85 (Ph, methine), 100.19 (C-1), 75.49 (C-5), 69.13 (OCH_2), 64.01, 63.17 ($PhCH_2$, C-6), 57.86, 55.42 (C-2, C-4), 14.30 (CH_3). FAB MS; m/z 336 (17%, $[M+H^+]$).
B) Preparation of 14: Disaccharide **13** (16 mg, 29 μmol) was reacted with sodium periodate (12.4 mg, 58 μmol) in water (0.6 mL) for 2 h and worked up as described for the preparation of **3**. The dialdehyde thus obtained was dissolved in MeOH (0.6 mL) and glycine (10 mg) in sat. $NaHCO_3$-MeOH (0.3 mL) was added followed by sodium cyanoborohydride (15 mg). After 4 h at room

temperature, the reaction mixture was concentrated and the residue chromatographed on silica gel (65 : 35 : 8 $CHCl_3$-MeOH-water) to afford **14** (9 mg, 56%) as a white solid after lyophilization of the aqueous solution; Rf 0.27 (65 : 35 : 8 $CHCl_3$-MeOH-water); $[\alpha]_D$ -21° (*c* 0.7, MeOH); ^{1}H NMR (D_2O): δ 4.89 (dd, 1 H, $J_{1',2'eq}$ 1.5 Hz, $J_{1',2'ax}$ 8.5 Hz, H-1'), 4.53 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 3.71 (s, 3 H, OCH_3), 3.23 (s, 2 H, CH_2COOH), 3.22 (d, 1 H, overlapped H-2'eq), 2.96 (d, 1 H, $J_{4'ax,4'eq}$ 11.5 Hz, H-4'eq), 2.41 (t, 2 H, $COCH_2$), 2.24 (m, 2 H, H-2'ax, H-4'ax), 2.05 (s, 3 H, $COCH_3$). ^{13}C NMR (CD_3COCD_3) δ 178.74, 175.21 (CO), 101.89, 99.32 (C-1, C-1'), 79.56, 75.38, 74.65, 73.16 (C-3, C-4, C-5, C-5'), 71.37 (OCH_2), 62.59, 61.27, 61.01 (C-6, C-6', CH_2COOH), 56.14 (C-2), 55.56, 52.75 (C-2', C-4'), 52.89 (OCH_3), 34.54 ($COCH_2$), 23.05 (CH_3CO). FAB MS: m/z 619 (22.5%, $[M+Na^+]$), 597 (10.9%, $[M+H^+]$).

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11. The ^{1}H-NMR spectrum of trisaccharide analog **16** displayed chemical shifts and coupling constants suggesting that the overall conformation remains similar to that of the native LeX trisaccharide **15**. Characteristic data for **16** (D_2O): δ 5.011 (d, $J_{1'',2''}$ 4.0 Hz, H-1''), 4.887 (q, $J_{5'',6''}$ 6.7 Hz, H-5''), 4.845 (dd, $J_{1',2'eq}$ = 2.2 Hz and $J_{1',2'ax}$ = 9.0 Hz, H-1'), 4.558 (d, $J_{1,2}$ 8.5 Hz, H-1).