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N-(2,2-Dimethoxyethyl)-, N-(2,2-Diethoxyethyl)-, and N-(4,4-Diethoxybutyl)-6-hydroxy Hexanamides as New Linking Agents for Carbohydrates and Proteins[1]

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Abstract: The title compounds have been conveniently synthesized by the addition reaction of ε-caprolactone and the corresponding, readily available ω-aminoalkyl dialkyl acetals. The free aldehydes, formed from sugar glycosides bearing the described hexanamides as aglycons, can be readily linked to proteins by reductive amination to give neoglycoconjugates.

Neoglycoconjugates are important tools in glycobiology.[2] They result from covalent attachment of carbohydrates to proteins. Such conjugates of bacterial polysaccharides and proteins have been successfully used as a new generation of conjugate vaccines.[3,4] In neoglycoconjugates, carbohydrates and proteins are normally connected via a several atoms long spacer (linker). Among many methods available for conjugation of carbohydrates to proteins, reductive amination[5,6] is very popular because of its experimental simplicity and high yields. The method involves the reaction of amino groups present in proteins with various forms of aldehydes present or generated in carbohydrates, in the presence of a suitable reducing agent. The initially formed Shiff's base is thereby converted into a stable product. In the case of antigenic polysaccharides, the aldehydo groups required by the process can be generated, for example, by treatment of vicinal diols in the polymer with a limited amount of periodate. Subsequent reaction, in the presence of a suitable reducing agent, of the (poly)aldehyde thus formed with amino functions in the protein then yields a neoglycoconjugate. Reducing mono- and oligosaccharides are amenable to direct conjugation by reductive amination but the process is very slow, because the concentration of the acyclic aldehydo form of the sugar is very low. Activation of low molecular weight carbohydrates, such as mono- or oligosaccharides, by periodate oxidation or many other

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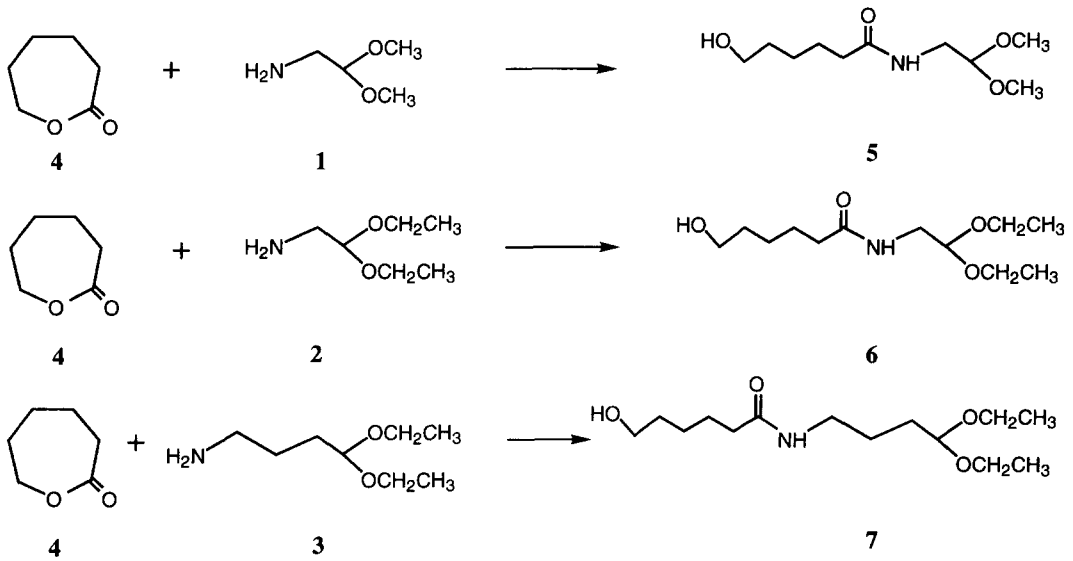
means[7] is impractical, as it might compromise their structural integrity, which could considerably decrease their antigenicity. The use of functionalized linkers in these situations is far more preferable. The requisite sugar is then, almost as a rule, prepared in the form of a glycoside whose aglycon contains functional groups suitable for linking to proteins. Many compounds that can serve as linkers in the preparation of neoglycoconjugates by reductive amination have been described (for examples, see ref. 8-12). Their preparation often involves multi step syntheses, and isolation of intermediates and/or products by chromatography is almost always required, making these procedures unattractive for large scale work. Preparations of the title compounds (**5-7**) do not suffer from such drawbacks. They are obtained by a simple addition reaction from inexpensive, commercially available starting materials, and can be isolated in the analytically pure state by distillation.

Guided by our extensive work on high yielding *N*-acylation of the aminosugar perosamine with lactones,[13-18] we focussed our attention on the acylation of commercially available amines containing a masked aldehydo group. We have treated three such amines (**1** - **3**) with the readily available 6-hexanolactone (ε-caprolactone, **4**).

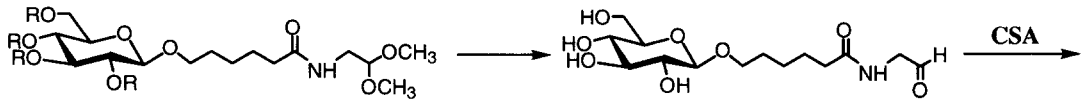


Products **5-7** can be most conveniently obtained by treatment, at room temperature, of the requisite amine with excess of **4** until satisfactory conversion is achieved (5-7 days). The rate of the conversion can be increased by the presence of a base, *e.g.* pyridine, but variable amounts of byproducts are then formed. Fractionation of the crude reaction mixture by vacuum distillation gives the desired, functionalized, hitherto unknown[19] dialkyl acetals **5-7** in excellent yields. The preparations (see below) are suitable for large scale work, and isolation/purification by chromatography is not required.

In a typical experiment, a mixture of **4** (5-10 molar excess) and the amine **1** (5 g) was kept at room temperature until TLC showed satisfactory conversions (5-7 days), and the product was fractionally distilled. Excess of **4** and the unchanged amine, if present, were collected in the forerun. Obtained next, was the target product **5** (8.5 g, 90%).[20]

Compounds **6**[21] and **7**[22] were similarly obtained in 93 and 90% yields, respectively.

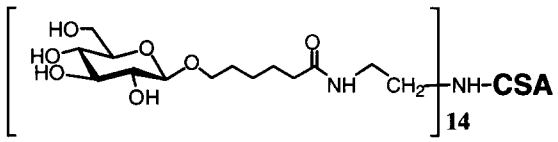
To demonstrate the utility of the title hexanamides, a neoglycoconjugate was prepared from chicken serum albumin (CSA). Thus, silver trifluoromethanesulfonate-promoted reaction, under base-deficient conditions,[23] of 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide and **5** gave **8**[24] (64%) which was debenzoylated, to give **9** {87%, CIMS: *m/z* 382 ([M + 1]$^+$), 399 ([M + 18]$^+$}. CSA was coupled with **10** (1 eqv/NH_2), obtained by deacetalization of **9** with 0.02 M trifluoroacetic acid[9], by borane-pyridine complex-mediated reductive amination.[10] The product was purified by dialysis, to remove the unchanged **10** and excess of reagents, to give the neoglycoconjugate **11**. The material produced a single band, when analyzed by polyacrylamide gel electrophoresis in sodium dodecyl sulfate buffer. The MALDI-TOF mass spectral analysis of **11** showed it to contain, on average, 14 D-glucose residues/mol CSA.



R **10**

8 **Bz**

9 **H**



11

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19. All new compounds gave correct microanalyses and/or exhibited ^{1}H, ^{13}C-NMR and mass spectral characteristics in accord with their structures.
20. Bp 153-157 °C/8 Pa; ^{1}H NMR ($CDCl_3$): δ 5.99 (m, 1 H, NH), 4.38 (t, 1 H, *J* 5.2 Hz, H-7), 3.62 (t, 2 H, *J* 6.5 Hz, H-1a,b), 3.39 (m, 8 H, incl 2 s, overlapped, 2 OCH_3, and t, H-6a,b), 2.52 (s, 1 H, OH), 2.21 (t, 2 H, *J* 7.15, H-5a,b), 1.72–1.61 (m, partially overlapped, H-4a,b), 1.63–1.54 (m, partially overlapped, H-2a,b), 1.45–1.34 (m, 2 H, H-3a,b); ^{13}C NMR ($CDCl_3$): δ 173.41 (CO), 102.58 (C-7), 62.22 (C-1), 54.25 (2 C, 2 OCH_3), 40.77 (C-6), 36.32 (C-5), 32.16 (C-2), 25.22 (C-3), 25.15 (C-4); CIMS: *m/z* 220 ($[M + 1]^+$), 237 ($[M + 18]^+$). Anal. Calcd for $C_{10}H_{21}NO_4$: C, 54.79; H, 9.59; N, 6.39. Found: C, 54.54; H, 9.66; N, 6.30
21. Bp 155–160 °C/8Pa, Anal. Calcd for $C_{12}H_{25}NO_4$: C, 58.30; H, 10.12; N, 5.67. Found: C, 58.31; H, 10.11; N, 5.60.
22. Bp 185–190 °C/6 Pa, Anal. Calcd for $C_{14}H_{29}NO_4$: C, 61.09; H, 10.55; N, 5.09. Found: C, 61.18; H, 10.58; N, 5.00.
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24. $[\alpha]_D$ +15° (c 1, $CHCl_3$); Calc for $C_{44}H_{47}NO_{13}$: C, 66.25; H, 5.90; N, 1.76. Found; C, 66.00; H, 5.92; N, 1.69.