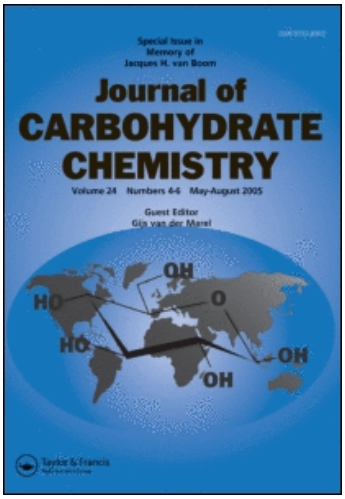

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N-Acetylneuraminic Acid: Neoglycoproteins and Pseudopolysaccharides

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Communication

N-ACETYLNEURAMINIC ACID: NEOGLYCOPROTEINS AND PSEUDOPOLYSACCHARIDES[#]

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ABSTRACT

A simplified procedure for the synthesis of acetochloroneuraminic acid is described starting from the methyl ester. N-acetylneuraminic acid α-allyl glycoside was prepared in high yield using silver salicylate as catalyst. Reductive ozonolysis of the allyl group, followed by direct reductive amination to protein carriers, gave immunogenic neoglycoproteins. Copolymerization of the α-allyl glycoside with acrylamide produced a water-soluble pseudopolysaccharide useful for the ELISA technique.

N-Acetylneuraminic acid (NeuAc, 1) is a very important carbohydrate constituent of many biologically relevant molecules.[1] It is most often found at the penultimate non-reducing end of carbohydrate residues of many glycoproteins and glycolipids, and it is also a constituent of some bacterial capsular polysaccharides.[2] Because its most important function seems to be anti-recognition, it appears fundamental, from an immunochemical point of view, to search for molecules binding and recognizing NeuAc such as serotonin, anti-NeuAc antibodies, and lectins. These molecules can serve as markers of physiological events. In order

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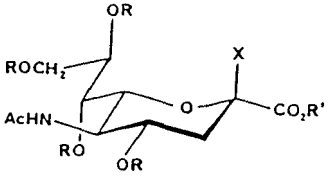
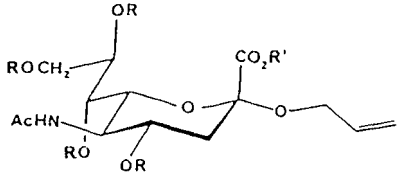
to detect such molecules, we synthesized NeuAc neoglycoproteins and a pseudopolysaccharide.

NeuAc (1) was isolated in yields of 5~6% (by weight) from edible bird's nest according to literature procedure.[3] The methyl ester of NeuAc (**2**) was obtained in quantitative yield by the method of Kuhn et al.:[4] mp 193.5-194.7 °C (MeOH-Et_2O), $[\alpha]_D$-28° (H_2O), reported[4] mp 179-180 °C, $[\alpha]_D$-28° (H_2O).[5] The transformation of the ester **2** to the acetochloroneuraminic acid **3**[4] was accomplished very efficiently through a new procedure by treating **2** with acetyl chloride in the presence of dry acetic acid in a sealed reaction vessel at room temperature for 24 h. Co-evaporations of the reaction mixture with dry toluene afforded an almost quantitative yield of **3** (>93%, ^1H NMR). The ^1H NMR data were identical with published data in both $CDCl_3$[6] and C_6D_6.[7] Treatment of the chloride **3** with dry allyl alcohol (activated molecular sieves 4A) in the presence of silver salicylate gave a 94% yield of the **α**-glycoside **4**. The exclusive formation of the α-anomer was ascertained by the ^1H NMR spectrum which showed only one doublet of doublet characteristic of the H-3eq of NeuAc derivatives in the region δ2-3 ppm (2.60 ppm).[8,9] Physical data of crystalline **4**, mp 153.6-155.7 °C, $[\alpha]_D$-14.2° ($CHCl_3$) agreed well with those of the compound previosuly prepared by Eschenfelder and Brossmer[10] using polymeric silver catalyst, mp 154-156 °C, $[\alpha]_D$-13° (MeOH). Zemplén deacetylation of **4** gave a quantitative yield of **5**, mp 143-144 °C, $[\alpha]_D$-10.1° (MeOH). Hydrolysis of the methyl ester group of **5** with 0.1 M NaOH afforded **6** almost quantitatively: mp 245-250 °C (dec), $[\alpha]_D$-9.1° (H_2O).

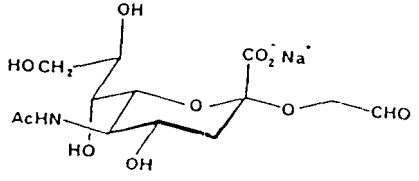
Reductive ozonolysis[11] (O_3, then Me_2S) of **6** in MeOH at -78° (5 min) gave **7** quantitatively. ^{13}C NMR of **7** in D_2O showed the aldehyde group to exist in the hydrated form (δ$\underline{C}$HO at 89.6 ppm). This reactive α-glycoside derivative of NeuAc was shown to be a very useful intermediate in the synthesis of NeuAc neoglycoproteins. For instance, **7** was directly coupled to proteins by sodium cyanoborohydride ($NaBH_3CN$)



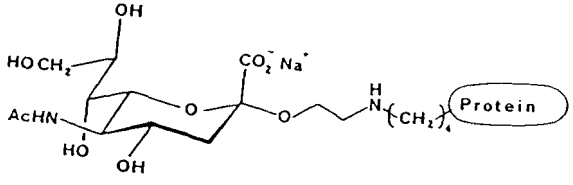
1 R=R'=H, X=OH, (NeuAc)
2 R=H, R'=Me, X=OH
3 R=Ac, R'=Me, X=Cl



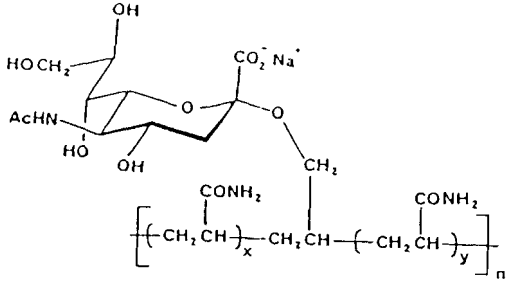
4 R=Ac, R'=Me
5 R=H, R'=Me
6 R=H, R'=Na



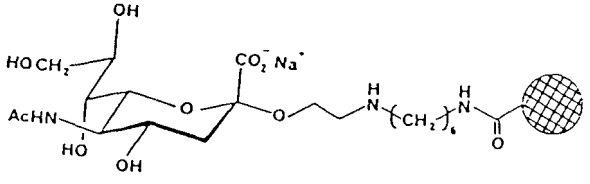
7



8 BSA
9 T. Toxoid



11



10 HAMHEX

mediated reductive amination.[12] Bovine serum albumin (BSA), tetanus toxoid (TT) and human albumin microspheres with hexanediamine spacers (HAMHEX)[13] were used as model proteins in order to prepare immunogenic NeuAc neoglycoproteins. The most efficient coupling conditions were obtained in pH 7.0 phosphate buffer when the molar ratio of reactants was the following: $CHO/NH_2/NaBH_3CN$ (5/1/15). The reactions performed at 37 °C were stopped after 3 days,[14] and the reaction mixtures were dialysed and freeze-dried. The neoglycoproteins were analysed by the Lowry method[15] for proteins and by the resorcinol method[16] for NeuAc contents. In the case of HAMHEX, the beads were exhaustively washed with 0.5 M NaCl, water, and acetone. Under these conditions, ~22 NeuAc residues were covalently coupled to one molecule of BSA (**8**) through the ε-amino groups of the lysine residues. In the case of TT (**9**) and HAMHEX beads (**10**), ~10 and ~14 NeuAc residues were respectively coupled.

A pseudopolysaccharide (**11**) of NeuAc derivative was then prepared using Kochetkov's methodology.[17] The α-allyl glycoside **6** was copolymerized with acrylamide by electron-transfer polymerization ($(NH_4)_2S_2O_8$, TEMED). The NeuAc content estimated by the resorcinol method was 20% (w/w). The molecular weight, determined by extrusion chromatography, was in the range 50-100 kDa. These polymeric NeuAc carriers were useful in some serological experiments and in the ELISA technique. The full experimental details and results of immunochemical tests will be published elsewhere.

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