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Glycodendrimer Synthesis Without Using Protecting Groups[1]

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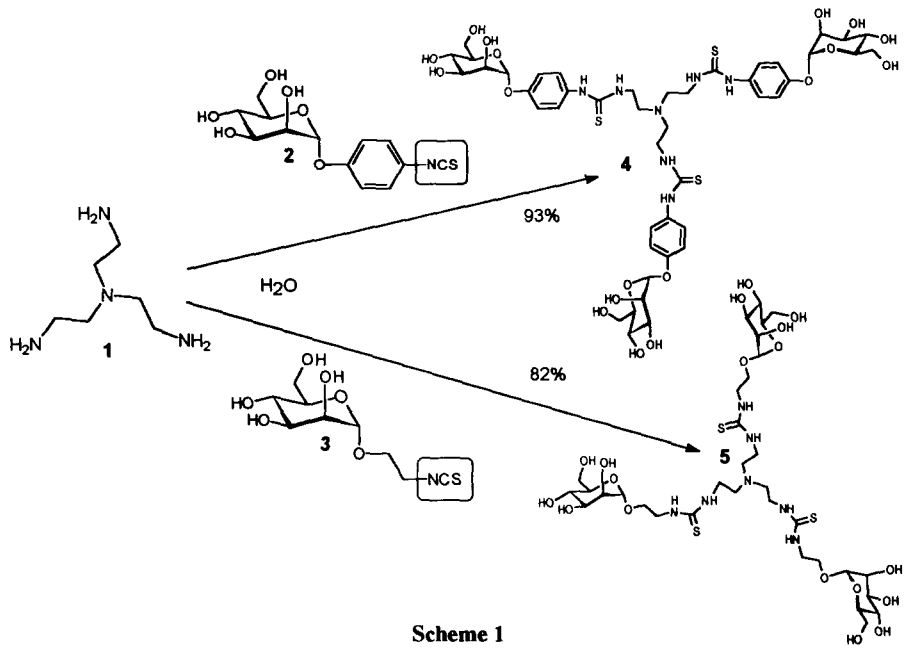
Abstract: Oligoantennary neoglycoconjugates can act as powerful inhibitors of carbohydrate-protein interactions and thus serve as antiadhesives in carbohydrate-based adhesion systems. They were obtained by a procedure that doesn't require protecting groups. Various unprotected NCS-functionalized saccharides were coupled with oligoamines in aqueous solution. This versatile method is generally applicable to the synthesis of thiourea-bridged glycodendrimers.

Multivalent glycoconjugates such as glycoclusters and glycodendrimers[2] can effectively inhibit carbohydrate-protein interactions and therefore serve as versatile tools for researchers in the field of glycobiology.[3] We have recently reported a two-step synthesis of thiourea-bridged glycodendrimers[4] which is highly flexible regarding the spacer and saccharide characteristics employed. It consists of the general reaction of acetyl-protected glycosyl isothiocyanates with oligovalent amines, followed by a base-catalyzed deprotection step. However, the final deacylation step becomes more difficult with higher dendrimer generations, often resulting in only partial deacetylation. It can be concluded that the two-step protocol for the synthesis of thiourea-bridged glycodendrimers is less feasible having more than 10 sugar moieties in the molecule.

From classical work on the synthesis of neoglycoconjugates, having carbohydrate moieties randomly attached, it is known that NCS-functionalized saccharides can be linked to proteins without using protecting groups.[5] We have applied this method to the synthesis of oligoantennary glycoconjugates with defined structures.

As we are currently investigating the mannose-specific adhesion of type 1 fimbriated *Escherichia coli*[6] we synthesized two unprotected NCS-functionalized mannose derivatives, *p*-isothiocyanato-phenyl α-D-mannopyranoside (**2**)[7] and 2-isothiocyanato-ethyl α-D-mannopyranoside (**3**)[8] for the coupling reaction with branched oligovalent amines as the core molecules. When the aryl spacer mannoside **2** and the trifunctional tris(2-aminoethyl)amine (**1**) were stirred in aqueous solution for 12 hours at rt the thiourea-bridged triantennary glycocluster **4** was obtained in 93% yield after purification by gel filtration. The analogous procedure using the alkyl spacer mannoside **3** afforded the cluster glycoside **5** in 82% yield (scheme 1).

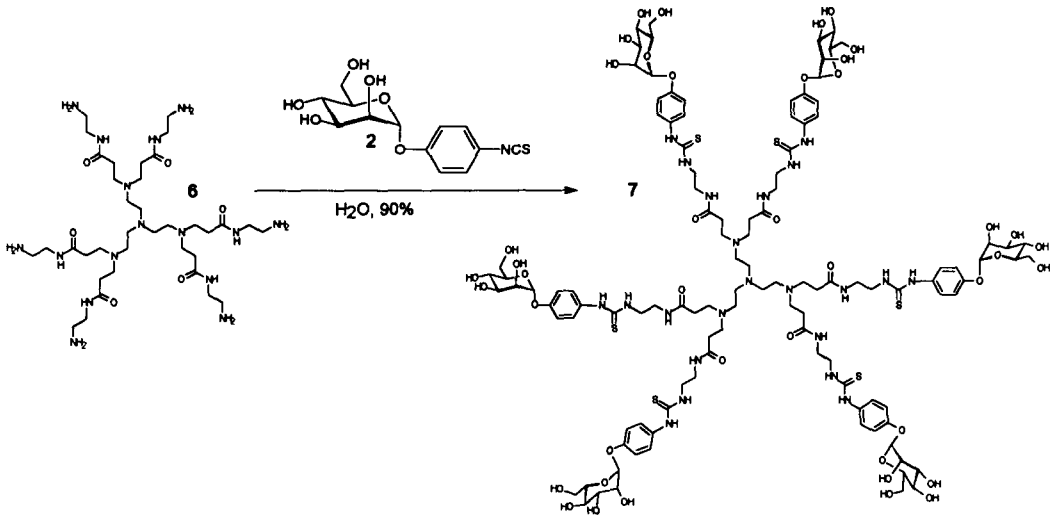


Scheme 1

The triantennary clusters **4** and **5** will be tested for their antiadhesive properties with type 1 fimbriated *E. coli* and erythrocytes.[6] Aromatic α-D-mannosides are especially potent inhibitors of mannose-specific adhesion.[9] The clustering of these compounds is therefore gaining growing attention.[10] Thus we used the phenyl mannoside **2** for the thiourea-bridging reaction in water with higher branched polyamines such as the first generation PAMAM (polyamidoamine) dendrimer **6**.[11] The coupling reaction of **2** with **6** lead to the hexavalent cluster **7** in 90% yield (scheme 2).[12] This result shows that the thiourea-bridging in water should be a versatile method also for the synthesis of larger glycodendrimers.

All glycoclusters were unequivocally characterized by means of NMR and FAB-MS spectroscopy. For the sugar moieties only one set of signals is detected in the NMR spectra due to the conformational flexibiltity of the carbohydrate antennae. The integration ratios of the core and carbohydrate peaks clearly proof the substitution pattern in each case. The conformational properties of the clusters result in line broadening of the NMR peaks but in spite of this, the carbon atoms of **4**, **5** and **7** can be fully detected in the ^{13}C NMR spectra.[13]

The described results show that the thiourea-bridging in water is an extremely easy to perform and feasible method for the preparation of glycoclusters and glycodendrimers in excellent yields. It avoids deprotection as the last yield lowering step and in addition it allows the fine tuning of the spacer characteristics of the aglycone moiety of the carbohydrate epitope.



Scheme 2

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7. **2** was synthesized from *p*-nitro-phenyl α-D-mannopyranoside by reduction of the nitro group, followed by the reaction with $CSCl_2$ in aqueous solution according to a described procedure[5] in 85 % overall yield.

8. **3** was synthesized from 2-azido-ethyl α-D-mannopyranoside (Chernyak, A. Y.; Sharma, G. V.; Kononov, L. O.; Krishna, P. R.; Levinsky, A. B.; Kochetkov, N. K.; Rao, A. V. R., *Carbohydr. Res.*, **1992**, *223*, 303-309) by catalytic reduction of the azido group followed by the reaction with $CSCl_2$ in 82% overall yield.

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12. *General coupling procedure.* The unprotected NCS-functionalized carbohydrate (1.1 equiv. per amino group of the core molecule) was added to a 3 mmolar aqueous solution of the core molecule. Then the mixture was stirred at rt for 12h. Depending on the substrates it is advisable to increase solubility by addition of water miscible organic solvents. For purification the reaction mixture is concentrated in vacuo and passed over Biogel P-2 or Sephadex G-15 as a solution in 30 mmolar NH_4HCO_3 buffer. Alternatively the products can be purified by HPLC on RP-8 or RP-18 columns with water-acetonitrile as eluent.

13. ^{13}C-NMR of **7** (100.62 MHz, D_2O): δ= 181.48 (CS), 175.44 (CO), 155.41 (ArC), 132.03 (ArC), 128.41 (ArCH), 118.85 (ArCH), 99.42 (C-1), 74.54 (C-5), 71.57 (C-3), 71.01 (C-2), 67.46 (C-4), 61.71 (C-6), 52.06 ($N(CH_2)_3$), 50.42 ($\underline{C}H_2N(CH_2)_2$), 50.33 ($CH_2N(\underline{C}H_2)_2$), 44.86 ($\underline{C}H_2NHCS$), 39.75 ($CONH\underline{C}H_2$), 33.89 ($\underline{C}H_2CO$) ppm.

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