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α-Mannosyl Clusters Scaffolded on Azamacrocycles:Synthesis and Inhibitory Properties in the Adhesion of Type 1 Fimbriated *Escherichia coli* to Guinea Pig Erythrocytes

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Abstract

The reaction of azamacrocycles, such as **1a** and **1b**, with acetyl-protected α-D-mannosyl isothiocyanate yields thiourea-bridged tetravalent glycoclusters **3** as novel neoglycoconjugates in one step. The deprotected compounds **4** showed significant inhibition of the adhesion of type 1 fimbriated *Escherichia coli* to Guinea pig erythrocytes.

Keywords: Macrocycles; Glycosides; Isothiocyanates; Supramolecular chemistry

Specific interactions of carbohydrate ligands and protein receptors are a key step in many biological recognition processes.[1] Their importance in cell-cell interactions such as immune response, the regulation of inflammatory processes[2] and microbial adhesion[3] suggests the synthesis of suitable carbohydrate mimetics with possible medicinal applicability.[4] An important characteristic of carbohydrate-protein interactions is their multivalency.[5] Consequently it has been shown, that structurally well-defined multivalent glycomimetics such as small glycoclusters[6] as well as sugar-functionalized dendrimers (glycodendrimers)[7] possess highly increased binding potencies for their specific receptors compared to the monovalent analogues (cluster effect).

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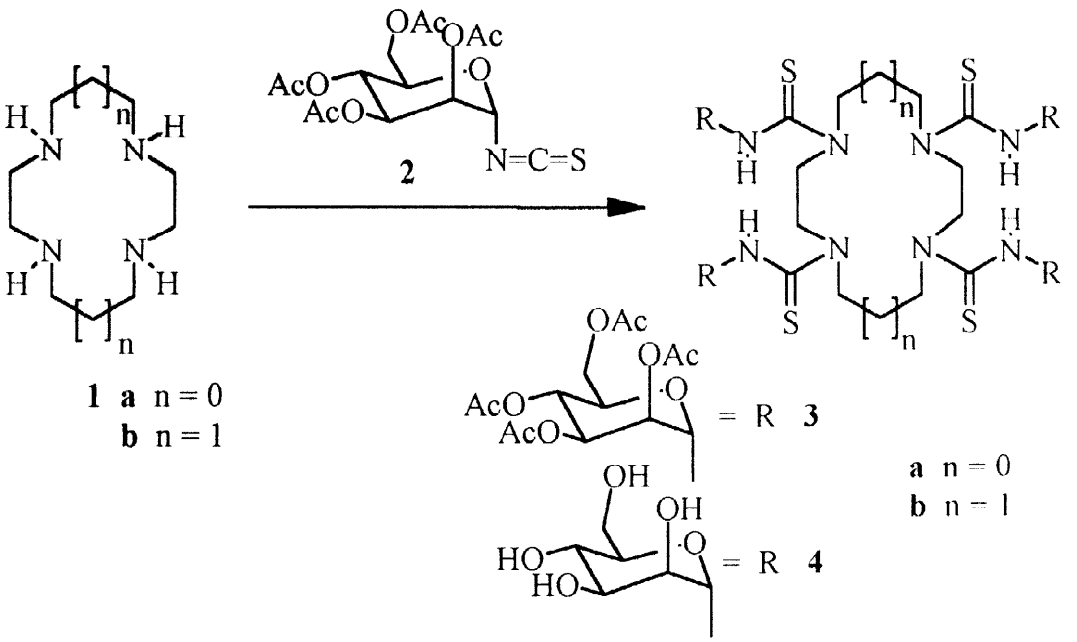
[8] This effect might eventually lead to the interception of biological response by competitive binding to lectin or selectin receptors on cell surfaces.

A defined arrangement of multiple sugar moieties does not necessarily require a large dendrimer core. It is well known from supramolecular chemistry that macrocycles with much simpler structure also provide some preorganization.[9] Previously calixarenes have been used to arrange sugar moieties, however, the water solubility of such systems is limited.[10] We report here the synthesis of α-mannosyl-functionalized azamacrocycles and the evaluation of their ability to inhibit the type 1 fimbriae-mediated adhesion of *Escherichia coli* to Guinea pig erythrocytes.

Following the thiourea-bridging strategy[11] the target compounds **3a** and **3b** were synthesized from the commercially available azamacrocycles 1,4,7,10-tetraaza-cyclododecane (cyclen) **1a**, 1,4,8,11-tetraaza-cyclotetradecane **1b** (cyclam) and acetyl-protected α-mannosyl isothiocyanate **2**.[12] The reaction proceeds cleanly in dichloromethane at room temp. without additional base and gave thiourea-bridged tetra mannosyl clusters **3a** and **3b** in 80 and 91 % yield, respectively.[13]



The ^{1}H NMR spectra of both compounds **3a** and **3b** in [D_6]-DMSO showed broad and unresolved signals, indicating a restricted mobility on the NMR time scale. When measured at +90°C the ^{1}H NMR spectra become clearer and the typical signals of the mannosyl moieties and the aza crown ether part can be identified. ^{13}C NMR spectra show, even at this elevated temperature, more signals than required for ideal fourfold symmetry. This may indicate that the interconversion of some conformers is still slow on the NMR time scale under these conditions. The constitution of the two molecules was confirmed by the observation of their intense molecular ions in MALDI-TOF and ESI mass spectra and correct elemental analyses.

The acetyl protecting groups were removed with sodium methanolate in methanol. During the deprotection water must be added to dissolve partially deprotected intermediates. Gel filtration on Sephadex G-15 and lyophylization gave the unprotected tetravalent mannosyl clusters **4a** (52 %) and **4b** (60 %).[14]

The binding potency of the compounds for the carbohydrate recognition domain on type 1 fimbriae was tested. Fimbriae are long filamentous protein appendages on the surface of bacteria, which carry carbohydrate-specific recognition domains (CRDs).[15] They enable the microbe to adhere to potential host cells through

multivalent carbohydrate-protein interactions. Type 1 fimbriae are mannose-specific and widely distributed among enterobacteria. Among those *Escherichia coli* are one of the most important ones. Type 1 fimbriae on *E. coli* are believed to significantly enhance bacterial virulence in uropathogenic infections.[16] The binding potencies of the compounds were determined by inhibiting the hemagglutination of Guinea pig erythrocytes by *E. coli* on microtiter plates by serially twofold diluted inhibitor solutions.[17] This inhibition agglutination test is a standard method in immunology[18] and yields half quantitative inhibitory potencies, determined as inhibition titres (IT: the lowest sugar concentration that inhibits hemagglutination). The obtained values are given in Table 1:

Table 1. Inhibition Titres (ITs) and Relative ITs of **4a** and **4b** Compared to Methyl α-D-mannoside and *p*-Nitrophenyl α-D-mannoside

	Methyl α-D-mannoside	*p*-Nitrophenyl α-D-mannoside	**4a**	**4b**
IT [mmol/L]	3.9 +/-0.1	0.041 +/-0.005	0.020 +/-0.010	0.005 +/-0.003
Relative IT	1	94 +/-10	195 +/-60	780 +/-300

p-Nitrophenyl α-D-mannoside (pNPMan) is one of the most potent inhibitors of the type 1 fimbrial lectin which is believed to be due to its hydrophobic, aromatic moiety.[19] It binds about 100 times better than methyl α-D-mannoside, which is usually used as reference glycoside. Our azamacrocyle clusters show significantly better binding to the type 1 fimbrial lectin in the inhibition agglutination test than pNPMan. The unsymmetrical macrocycle **4b** performs even better than the symmetrical analogue **4a**. This might be explained by a better conformational availability of the mannosyl residues provided by greater flexibility of the macrocyclic scaffold. This may allow a better fit of the mannosyl moieties to the carbohydrate recognition site. The binding potency of the synthesized compounds **4** for the lectin on type 1 fimbriae is remarkable high compared to the so far known inhibitors.[19] Even the inhibitory potencies of other multivalent mannosyl clusters which have been reported are significantly lower.[17]

In summary, we have shown that aza crown carbohydrate conjugates can be synthesized efficiently by reaction of mannosyl isothiocyanates with azamacrocycles. The so obtained tetravalent glycoclusters are potent inhibitors of the hemagglutination of erythrocytes by type 1 fimbriated *E. coli*. This indicates that the macrocyclic scaffold provides significant preorganization of the mannosyl moieties to support their binding to the fimbrial receptor.

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[13] Experimental procedure and selected analytical data. *1,4,7,10-Tetraaza-cyclododecane-1,4,7,10-tetra-N-[2,3,4,6-tetra-O-acetyl-α-D-mannosyl-thioformamide*]: A mixture of **1a** (30 mg, 0.18 mmol) and **2** (290 mg, 0.74 mmol) in 15 ml of CH_2Cl_2 was stirred for 16 h at room temp. and the solvent was removed *in vacuo*. Column chromatography (SiO_2, ethyl acetate) yielded 250 mg (80%) of **3a** (R_f = 0.76), as a white solid, m.p. 152°C; IR (KBr): 3430 cm^{-1}, 1750, 1230; UV/Vis (CH_3CN): λ_{max} (lg ε) 192 nm (4.497), 210 (4.589), 256 (4.614); ^1H NMR (400 MHz, DMSO, +90 °C): δ 7.70 (m, 2 H), 7.20 (m, 2 H), 6.21 (m, 4 H), 5.50 (m, 4 H), 5.29 (m, 4 H), 5.10 (m, 4 H), 4.11 (m, 28 H), 2.03 (s, 12 H), 2.02 (s, 12 H), 2.01 (s, 12 H), 1.93 (s, 12 H); ^{13}C NMR (100 MHz, DMSO, +90 °C) δ 181.0 (C_{quat}), 169.6 (C_{quat}), 169.4 (C_{quat}), 168.9 (C_{quat}), 168.7 (C_{quat}), 82.8 (+), 73.2 (+), 70.5 (+), 68.3 (+), 65.5 (+), 61.9 (-), 49.1 (-), 48.9 (-), 20.3 (+), 19.9 (+), 19.8 (+), 19.6 (+); MS (MALDI-TOF); m/z (%): 1768.5 (100) $[M + K]^+$, 1752 (45) $[M + Na]^+$. - $C_{68}H_{96}N_8O_{36}S_4$ (1729.78): calcd. C 47.21, H 5.60, N 6.48; found C 46.97, H 5.56, N 6.32. Molar mass 1730 (MS); *1,4,8,11-Tetraaza-cyclotetradodecane-1,4,8,11-tetra-N-[2,3,4,6-tetra-O-acetyl-α-D-mannosyl-thioformamide*] (**3b**): R_f = 0.69 (SiO_2, ethyl acetate), white solid, m.p. 154°C; IR (KBr): 3380 cm^{-1}, 1750, 1230; UV/Vis (CH_3CN): λ_{max} (lg ε) 194 nm (4.518), 210 (4.679), 256 (4.713); ^1H NMR (400 MHz, DMSO, +90 °C): δ 7.85 (m, 2 H), 7. 60 (m, 2 H), 6.25 (m, 4 H), 5.51 (m, 4 H), 5.31 (m, 4 H), 5.08 (m, 4 H), 4.00 (m, 28 H), 2.18 (s, 4 H), 2.04 (s, 12 H), 2.03 (s, 12 H), 2.03 (s, 12 H), 2.02 (s, 12 H); ^{13}C NMR (100 MHz, DMSO, +90 °C): δ 182.5 (C_{quat}), 169.4 (C_{quat}), 169.3 (C_{quat}), 168.9 (C_{quat}), 168.7 (C_{quat}), 83.0 (+), 73.2 (+), 70.5 (+), 68.3 (+), 65.5 (+), 62.0 (-), 48.7 (-), 47.7 (-), 25.9 (-), 19.9 (+), 19.9 (+), 19.8 (+), 19.6 (+); MS (ESI+); m/z (%): 1779.5 (100) $[M + Na]^+$, 1757.6 (80) $[M + H]^+$, 901.4 (40) $[M + 2 Na]^{2+}$, 890.3 (20) $[M + Na + H]^{2+}$; $C_{70}H_{100}N_8O_{36}S_4$ (1757.84): calcd. C 47.83, H 5.73, N 6.37; found C 47.51, H 5.72, N 6.32. Molar mass 1757.8 (MS).

[14] *General procedure for deprotection:* The acetyl-protected glycoclusters **3** were dissolved in dry methanol (ca. 5 ml for 50 mg compound), 1M of sodium methanolate solution (100 µl) was added and the mixture was stirred at r.t. When a precipitate was formed, the minimum amount of distilled water was added to obtain a clear solution. After complete deprotection (TLC ethyl acetate/methanol/water, 7:2:1), it was neutralized with 1N HCl, methanol was removed *in vacuo* and the residue was submitted to gel filtration chromatography on Sephadex G-15 and eluted with 15 mM ammonium hydrogen carbonate solution. Product fractions were collected and repeatedly freeze dried with deionized distilled water. *1,4,7,10-Tetraaza-cyclododecane-1,4,7,10-tetra-N-α-D-mannosyl-thioformamide* (**4a**): Yield 52 %. - MS (ESI+), m/z (%): 1079.4 (100) $[M + Na]^+$, 1056.4 (10) $[M]^+$. - $C_{36}H_{64}N_8O_{20}S_4$ (1056.32). *1,4,8,11-Tetraaza-cyclotetradodecane-1,4,8,11-tetra-N-α-D-mannosyl-thioformamide* (**4b**): Yield 60 %. - MS (ESI+), m/z (%): 1107.4 (100) $[M + Na]^+$, 1084.4 (5) $[M]^+$. - $C_{38}H_{68}N_8O_{20}S_4$ (1084.34).

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