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Synthesis of Hyper-branched Dendritic Lactosides

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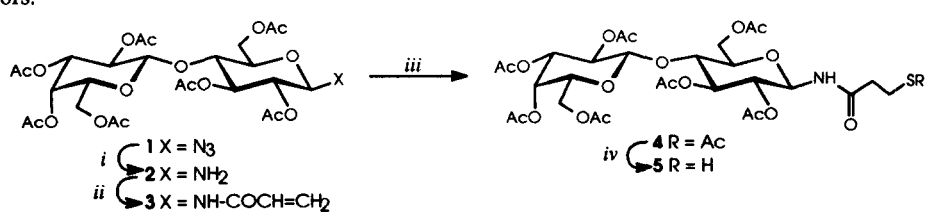
Abstract: Hyper-branched dendritic lactosides with a valency of three (**16**) and nine (**21**) residues were synthesized using gallic acid (**6**) as trivalent core structure and tetraethyleneglycol as hydrophilic spacer.

Cell surface glycolipids and glycoproteins are playing key roles in cellular recognition, adhesions and cell growth regulations.[1] However, intrinsic binding interactions between most carbohydrate ligands and their receptors are comparatively weaker than common protein-protein interactions.[2] One can therefore conclusively hypothesize that, if such carbohydrate-based functions still occur, the multivalent nature of the cell surface carbohydrates may act cooperatively to increase the overall binding avidity. Carbohydrate clusters have been demonstrated to produce noticeable increases in their inhibitory properties relative to those of their monovalent units.[3,4] More recently, we[5] and others[6-7] have suggested that glycopolymers, which are naturally multivalent, represent powerful synthetic carbohydrate clusters having dramatically increased inhibitory properties as shown by the inhibition of hemagglutination of human erythrocytes by influenza viruses. We have also described the first synthesis of sialic acid-containing dendrimers which had similar inhibitory properties to those of the glycopolymers mentioned above.[8,9] Therefore, the potential of chemically well-defined "glycodendrimers" as multivalent inhibitors in cell adhesion processes is extremely high.

The strategy described herein relies on the synthesis of a thiolated lactoside derivative (**5**) to be added to a pre-formed dendrimer (Scheme 1). This strategy offers the advantage that any carbohydrate ligands can be used at a late stage of the synthesis. Furthermore, the precursor of **5** is an N-acryloylated derivative which can also be used for both glycopolymer[10] and neoglycoprotein syntheses.[11] Lactosylated dendrimers were first chosen as model, since such clusters are well known to bind efficiently to hepatic asialoglycoprotein receptors.[4]

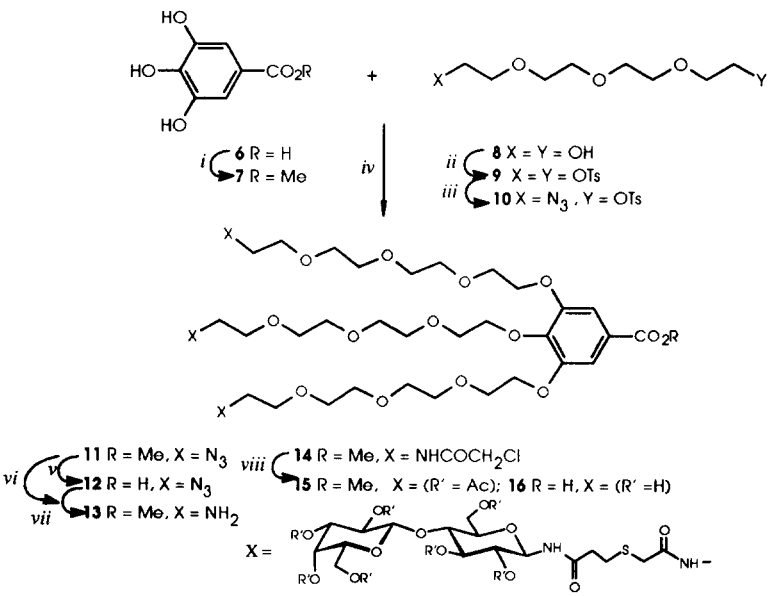


Scheme 1. i) H_2, 10% Pd-C, EtOH, 3 h, 91%; ii) CH_2=CHCOCl, EtOAc, Et_3N, 0°C, 1 h, 95%; iii) HSAc, EtOAc, Et_3N, 25°C, 5 h, 95%; iv) H_2NNH_2-HOAc, DMF, 25°C, 30 min, N_2, quantitative.

β-D-Lactosyl azide (**1**), readily obtained under stereospecific phase transfer catalysis,[12] was reduced (H_2, 10% Pd-C, 91%) to the unstable lactosylamine **2** which was immediately N-acryloylated (CH_2=CHCOCl, EtOAc, Et_3N) to provide key precursor **3** in 95% yield. Thiolation of **3** was achieved by Michael addition with thioacetic acid (EtOAc, Et_3N) to provide **4** (95%). Chemoselective de-S-acetylation of **4** with hydrazinium acetate in DMF under nitrogen gave **5** (quantitative).[13]

The construction of the core unit on which the dendrimers were scaffolded was initiated with gallic acid **6** and its subsequent transformation into methyl ester **7** (MeOH, PTSA, 95%).[14] This choice allows the scaffolding of the dendrimer valency to be 3^n, where n represents the n'th generation and complements well our previously synthesized dendrimers[8,9] having L-lysine core units and valencies of 2^n. Tetraethylene glycol **8** was then chosen as an hydrophilic spacer of sufficient lenght to allow carbohydrate ligands to be readily accessible by their receptors. Diol **8** was tosylated (TsCl, Et_3N, Et_2O) to give **9** in 81% yield which was transformed into mono-tosylated azide **10** (NaN_3 , EtOH, reflux, 39%). Gallic acid methyl ester **7** was then alkylated with a slight excess of azido tosylate **10** (3.6 equivalents, K_2CO_3 , DMF, 80 °C, 24 h) to afford the key dendrimer precursor **11** in 68% yield after silica gel column chromatography. Reduction of triazide **11** as described above for **1** provided triamino ester **13** (62%) suitable for both attachment to thiolated lactoside **5** after N-chloroacetylation to **14** with chloroacetic anhydride ($(ClCH_2CO)_2O$, Et_3N, 72%) and for further dendrimer scaffoldings.

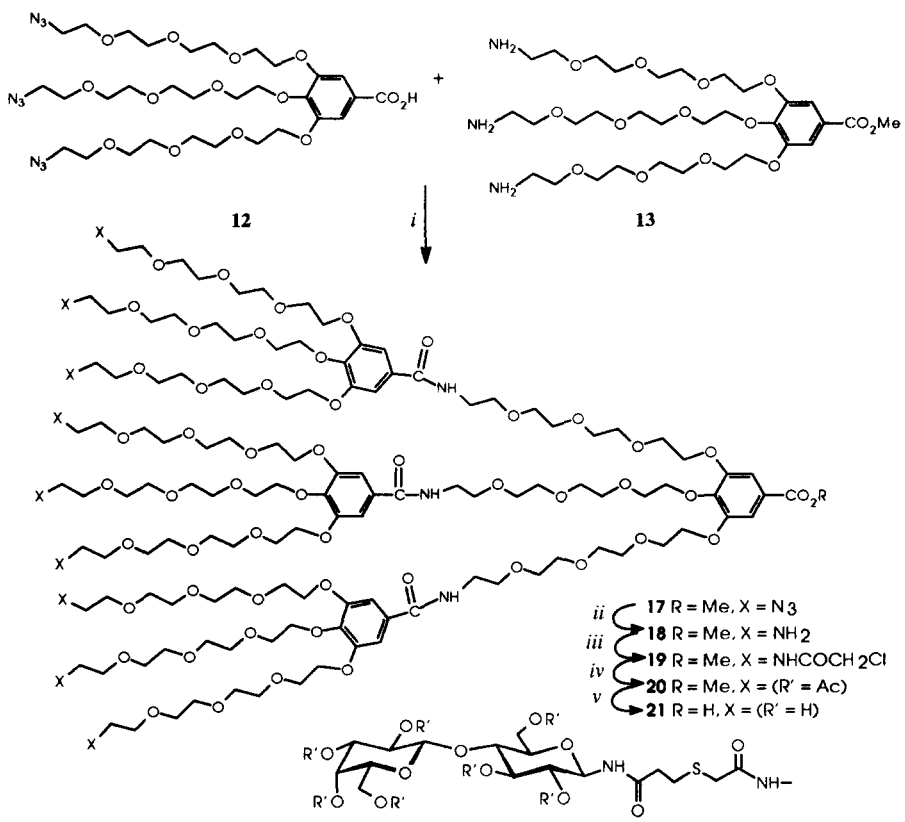
Attachment of thiol **5** to N-chloroacetylated ester **14** (CH_3CN, Et_3N, 25°C, o. n.) gave **15** as the first generation of trivalent lactosides in 98% yield. The level of lactoside incorporation was readily established by ^{1}H-NMR spectroscopy which revealed the absence of the well separated N-chloroacetyl signal at 4.01 ppm together with a new thiomethylene signal at 3.20 ppm which was integrated relative to those of the anomeric glucosyl signals at 5.23 ppm (H-1, dd) and the aromatic protons at 7.37 ppm. Treatment of peracetylated **15** with 1M NaOH in ethanol (1:10, v/v) furnished **16** (quant.). Alternatively, the methyl ester of triazide **11** was hydrolyzed (KOH, EtOH, reflux, 2 h, 100%) to give azido acid **12**.



Scheme 2. i) MeOH, PTSA, reflux, 2 h, 95%; ii) TsCl, Et_3N, Et_2O, 0°C, 1h, 25°C, 3 h, 81%; iii) NaN_3, 95% EtOH, reflux, 4 h, 39%; iv) K_2CO_3, DMF, 80°C, 24 h, 68%; v) KOH, EtOH, reflux, 2 h, quantitative; vi) H_2, 10% Pd-C, EtOH, 2 h, 62%; vii) $(ClCH_2CO)_2O$, Et_3N, EtOH, 25°C, 2 h 72%; viii) **5**, Et_3N, N_2, CH_3CN 25°C, o. n., 98%.

Similarly, construction of the second generation was accomplished by coupling a slight excess of azido acid **12** to amino ester **13** using carbodiimide chemistry and hydroxybenzotriazole (HOBt) activator (EDC, HOBt, DIPEA, 25°C, 3h) to provide nona-azido ester **17** in 83% yield (Scheme 3). Reduction of the nine azide groups of **17** by catalytic hydrogenation (H_2, 10% Pd-C) as above provided nona-amine **18**. The integrity of **18** was verified by the complete absence of azide band in its infrared spectrum (2106 cm^{-1}) and the corresponding amine was directly N-chloroacetylated as above to give **19** in 78% yield after silica gel column chromatography.

Coupling of thiolactoside **5** (11 equiv., Et_3N, CH_3CN, DMSO, 1:5 (v/v), 25°C, o.n.) with **19** under nitrogen afforded fully protected nona-lactoside **20** in 88% yield. The reaction was monitored by TLC and the level of lactoside incorporation was monitored by ^{1}H-NMR spectroscopy which showed the well separated aromatic protons of the inner and outer residues at 7.22 and 7.16 ppm respectively in a ratio of 3 to 1. All of the ester protecting groups of compound **20** were removed by treatment with 1M NaOH in ethanol (1:10, v/v) to give lactosylated "glycodendrimer" **21** in quantitative yield.



Scheme 3. *Reagents and conditions:* i, EDC, HOBt, EtOH/CH_3CN (1:1), DIPEA, 25°C, 3 h, 83%; ii, H_2, 10% Pd-C, EtOH, 3 h; iii, $(ClCH_2CO)_2O$, EtOH, Et_3N, 25°C, 3 h, 70%; iv, **5**, Et_3N, CH_3CN/DMSO (1:5), 25°C, overnight, 88%; v, NaOH, EtOH, 25°C, overnight, quantitative.

In conclusion, non-peptidyl "glycodendrimers" were synthesized in good yields using a convergent approach which will allow the incorporation of more complex oligosaccharides such as those involved in various adhesion and inflammation processes.

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14. All new compounds showed satisfactory spectral and/or elemental/mass analysis. Selected spectroscopic an analytical data are as follow. Compound **4**: m.p. 77-79°C, ^{1}H-NMR ($CDCl_3$, δ ppm): 6.24 (d, 1H, NH), 5.17 (dd, 1H, $J_{1,2}$ = 9.4 Hz, $J_{NH,1}$ = 9.4 Hz, H-1), 4.42 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1'), 3.03-3.08 (m, 2H, CH_2-SAc), 2.35-2.51 (m, 2H, $\underline{CH_2}CH_2SAc$), 2.28 (s, 3H, SAc); ^{13}C-NMR: 100.9 (C-1'), 78.0 (C-1), 36.2 (CH_2 -SAc), 30.6 (SAc), 24.2 ($\underline{CH_2}CH_2$ -SAc); E.A. calcd. for C, 48.66, H, 5.66, N, 1.83, found C, 48.40, H, 5.51, N, 1.77. Compound **10**: ^{1}H-NMR ($CDCl_3$, δ ppm): 7.35-7.80 (4H, Ar), 4.09-4.16 (2H), 3.54-3.68 (12H), 3.30-3.40 (2H), 2.41 (s, 3H, $ArCH_3$); ^{13}C-NMR: 145.4, 133.5, 130.4, 128.5, 71.3, 71.2, 71.1, 70.6, 69.9, 69.2, 51.2, 22.2. Compound **11**: ^{1}H-NMR ($CDCl_3$, δ ppm): 7.27 (s, 2H, Ar), 4.13-4.25 (6H), 3.60-3.92 (39H), 3.31-3.42 (t, 6H, CH_2N_3); ^{13}C-NMR: 167.1, 152.8, 143.0, 125.5, 109.4, 72.9, 71.3, 71.2, 71.1, 71.0, 70.5, 70.1, 69.4, 52.7, 51.2; FAB-MS calcd. for $C_{32}H_{53}N_9O_{14}$, 787.4, found, 788.4 (M+1). Compound **14**: ^{1}H-NMR ($CDCl_3$, δ ppm): 7.28 (s, 2H, Ar), 7.10 (b, 3H, NH), 4.12-4.24 (m, 4H), 4.01 (s, 6H, CH_2Cl); FAB-MS for $C_{38}H_{62}Cl_3N_3O_{17}$ 939.3, found 940.3 (M+1). Compound **15**: ^{1}H-NMR ($CDCl_3$, δ ppm): 7.27 (s, 2H, Ar), 5.21-5.26 (m, 2H, H-1, H-3), 4.45 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1'), 4.17 (m, 6H, ArO-CH_2), 3.86 (s, 3H, OMe), 3.20 (m, 6H, SCH_2); 2.60-2.82 (m, 2H, $\underline{CH_2}CH_2$-SAc), 2.43-2.56 (m, 2H, CH_2SAc); ^{13}C-NMR: 152.1, 124.1 (Ar), 100.8 (C-1'), 77.7 (C-1), 52.2 (OMe), 42.5 (CH_2NHCO), 36.2 ($CH_2\underline{CH_2}S$), 36.1 (SCH_2CO), 27.5 ($\underline{CH_2}CH_2S$). Compound **20**: ^{1}H-NMR (DMSO-d_6, δ ppm): 7.22 (s, 2H, inner Ar), 7.16 (s, 6H, outer Ar), 5.27 (dd, 1H, $J_{1,2}$ = 9.4 Hz, H-1), 4.71 (d, $J_{1',2'}$ = 9.4 Hz, H-1'), 3.15 (s, 18H, SCH_2), 2.66-2.72 (m, 18H, $CH_2\underline{CH_2}S$), 2.35-2.45 (m, 18H, $\underline{CH_2}CH_2S$). Compound **21**: ^{1}H-NMR (DMSO-d_6, δ ppm): 7.22 (s, 2H, inner Ar), 7.16 (s, 6H, outer Ar), 4.74 (dd, 1H, $J_{1,2}$ = 9.0 Hz, H-1), 4.20 (d, 1H, $J_{1',2'}$ = 7.0 Hz, H-1'), 3.10 (s, 18H, SCH_2CO), 2.71-2.76 (m, 18H, $\underline{CH_2}CH_2S$), 2.37-2.42 (m, 18H, $CH_2\underline{CH_2}S$).

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