Pergamon

Tetrahedron Letters, Vol. 36, No. 41, pp. 7383-7386, 1995
Elsevier Science Ltd
Printed in Great Britain
0040-4039/95 $9.50+0.00

0040-4039(95)01547-7

Synthesis of Novel Dendritic Glycosides

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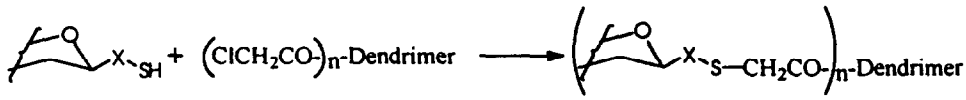
Abstract: Dendritic thiolated glycosides, with and without a spacer moiety, exhibiting valencies of two, four and eight were synthesized using L-lysine as a divalent core structure via solid-phase chemistry.

Carbohydrates have paramount roles in biological systems including cellular recognitions, adhesions, and cell growth regulations.[1] These areas are under active investigation and include syntheses of well-known complex sialylated oligosaccharides such as G_{M3}, sialyl Lewis^X and others. Complete chemical syntheses of such oligosaccharides are extremely labor-intensive and, thus, purely chemical strategies for their syntheses represent a difficult task. Chemo-enzymatic strategies have provided an attractive alternative.[2]

In addition, binding interactions between single glycosides and their receptors are very weak.[3] Multivalent glycopolymers have been used to show that specific carbohydrate-protein interactions may be amplified based on the cluster effect of glycosides.[4-6] Glycopolymers, however, are too variable in nature, their lengths not being consistent, and so they cannot represent chemically well-defined entities with pre-designed carbohydrate densities. Our group has already been the first to design[7] and synthesize chemically well-defined glycotelomers[8] and glycodendrimers.[9,10] Some of these glycodendrimers contained pendant sialic acid residues and clearly exhibited improved inhibitory properties when used as inhibitors of carbohydrate/receptor interactions. Naturally, our attention shifted to glycodendrimers containing carbohydrate moieties other than sialic acid.

As an extension of our previous approach, we propose herein a strategy (Scheme 1) leading to chemo-enzymatic syntheses of dendritic complex oligosaccharides. Described is the syntheses of various thiolated glycosides, with and without a spacer moiety (**3a**, **4a**, **7a**, and **8a**) to be coupled to a pre-formed N-chloroacetylated L-lysine dendrimer on solid-phase. After cleavage from the solid support and appropriate protecting group removal, these dendritic glycoforms (**14** to **17**) represent novel precursors into chemo-enzymatic syntheses of glycodendrimers containing G_{M3}, and sialyl Lewis^X.[2]



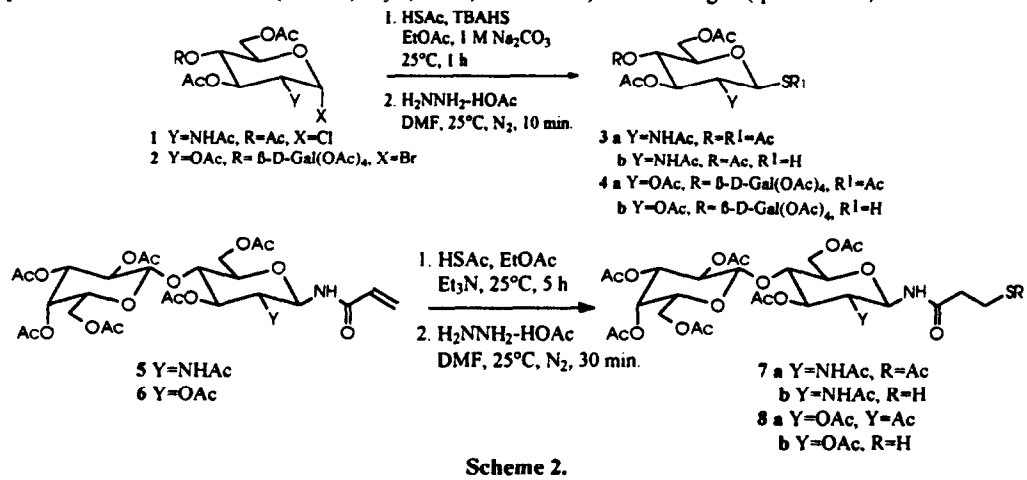
X = no spacer
X = NH-CO-CH_2CH_2-
3b, **4b**, **7b**, **8b**

15, **16**, **17**

Scheme 1.

Glycosides with thioacetate functionality were either directly obtained under stereospecific phase transfer catalysis (HSAc, TBAHS, EtOAc, 1 M Na_2CO_3, 25°C, 1 h)[11,12] to thiolated **3a** (58% yield) and **4a**

(79%) or obtained by Michael addition of N-acryloylated precursors **5** and **6** with thioacetic acid (EtOAc, Et_3N, 25°C, 5 h) to give **7a** (97%) and **8a** (95%). All glycosides were chemoselectively de-S-acetylated with hydrazinium acetate in DMF (EtOAc, Et_3N, 25°C, 10-30 min.) under nitrogen (quantitative).[12]



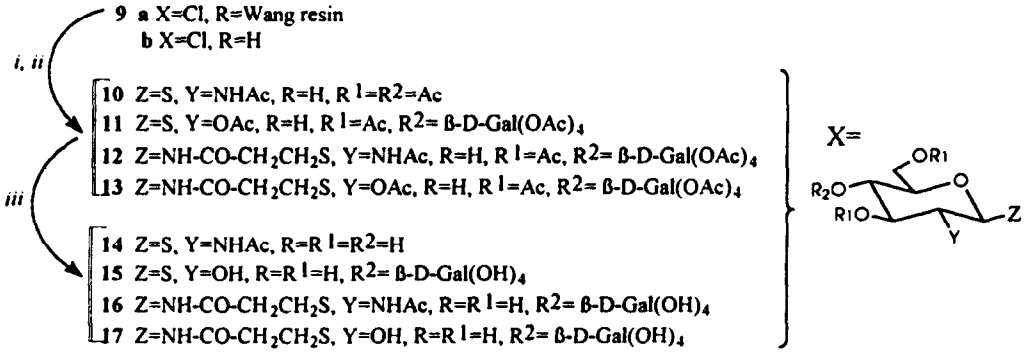
Scheme 2.

N-Chloroacetylated hyper-branched dendritic structures, to which the thioglycosides were to be coupled, were based on the rational scaffolding of L-lysine core structures using well-established Fmoc chemistry and benzotriazolyl esters as coupling procedures on solid phase.[9] Dendritic L-lysine cores were elaborated on *p*-benzyloxybenzyl alcohol resin (Wang resin) to which was anchored a ß-alanyl spacer via Fmoc/HOBt chemistry. Coupling of L-lysine to ß-alanine was carried out with freshly prepared N^{α}-N^{ε}-di-Fmoc-L-lysine-OBt ester. After each cycle, Fmoc removal was achieved by the usual ß-elimination process (20% piperidine in DMF). Products from each sequential generation were treated directly with N-chloroacetylglycylglycine benzotriazolyl ester, prepared from commercially available

$(XCH_2CO\text{-}Gly\text{-}Gly\text{-})_2\text{-}Lys\text{-}ß\text{-}Ala\text{-}O\,R$

$(XCH_2CO\text{-}Gly\text{-}Gly\text{-})_4\text{-}Lys_2\text{-}Lys\text{-}ß\text{-}Ala\text{-}O\,R$

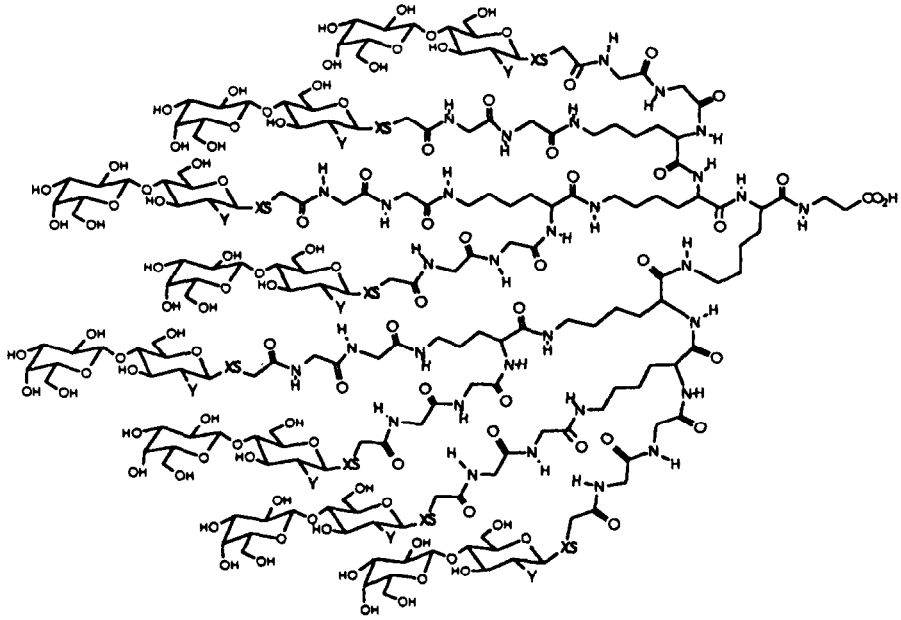
$(XCH_2CO\text{-}Gly\text{-}Gly\text{-})_8\text{-}Lys_4\text{-}Lys_2\text{-}Lys\text{-}ß\text{-}Ala\text{-}O\,R$



Scheme 3. i) **3**, **4**, **7** or **8**, 1% Et_3N/DMF, 25°C, 16 h; ii) 95% TFA, 25°C, 1.5 h; iii) NaOMe/MeOH, 25°C, 1.5 h, then H^+ resin.

N-chloroacetylglycylglycine. Ninhydrin tests determined completion of each coupling cycle. Using this solid phase approach, di-, tetra- and octa-valent N-chloroacetylated dendrimers (**9a**) were obtained in the first, second and third generations respectively. For quality and structural determination purposes, the corresponding N-chloroacetylated dendrimers (**9b**) were released from the polymer support with aqueous trifluoroacetic acid (95% TFA, 1.5 h, >90% yields).

While still attached to the resin, each dendrimer generation was treated with an excess of thioglycosyl derivatives **3b**, **4b**, **7b** or **8b**, (1.2 equiv. per N-chloroacetyl group, 1% Et_3N/DMF, 25°C, 16 h). Before the bulk of the dendrimers were released from the polymeric support, aliquots were withdrawn and hydrolyzed as above. The completeness of the couplings were estimated from the ^{1}H-NMR spectra of the glycosylated dendrimers which showed characteristic signals for any residual N-chloroacetyl methylene groups at 4.12 ppm (DMSO-d^6). Where required couplings were repeated.



Scheme 4. Third Generation Glycodendrimers **15** (Y=OH, X=no spacer), **16** (Y=NHAc, X=NH-CO-CH_2CH_2) and **17** (Y=OH, X=NH-CO-CH_2CH_2)

Polymer bound glycodendrimers (**10** to **13**) were released from the polymer support as above for **9b** and obtained in 65-99% yields after dissolution in minimum amount of neat TFA and precipitation in ether. The ^{1}H- and ^{13}C-NMR spectra (DMSO-d^6, 500 MHz) of the dendrimers revealed the integrity of the α-glycoside linkages as well as the ratio of the ß-alanyl residues (2.36 ppm) relative to both the L-lysyl (2.99 ppm) and glycosyl signals.[13]

Each of the polymer free peracetylated glycodendrimers (**10** to **13**) were de-O-acetylated with NaOMe/MeOH (25°C, 1.5 h) followed by H^+ resin treatment to give dendrimers **14** to **17** (**15**, **16**, and **17** depicted in scheme 4). When the N-acryloylated spacer (**7** and **8**) was used some ß-elimination of the sugar to afford N-acryloylated mono-glycoside (**5** and **6**) was observed and care must be taken to minimize exposure to high concentrations of NaOMe/MeOH.

Preliminary biological testing of dendrimers **14** to **17** included double immunodiffusion assays using wheat germ agglutinin lectin for **14** and *Arachis hypogaea* lectin (peanut lectin) for dendrimers **15** to **17**. All dendrimers, except those in which ß-elimination of the glycoside residue was extensive, exhibited

precipitation bands. Precipitation bands for divalent dendrimers were transient and as valency increased, precipitation lines noticeably became stronger and less diffuse.

In conclusion, novel glycodendrimers were synthesized in moderate to good yields, with high purity, using a solid phase, convergent approach. This approach has demonstrated that a variety of glycosides may be incorporated and allows an entry into the synthesis of even more complex dendritic oligosaccharide compounds by well established enzymatic transformations.[2]

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13. All compounds showed satisfactory NMR spectral (Bruker 500 MHz AMX) and, where possible, mass spectral data. Due to the repetitive nature of glycodendrimers and space constraints, selected spectroscopic and analytical data are as follows. **3a**: FAB-MS (pos.) calcd for $C_{16}H_{21}NO_9S$: 405.1; found: 406.1 (M + 1, 31.4%); ^{1}H-NMR ($CDCl_3$) δ: 2.34 (s, 3H, SAc), 5.08-5.15 (m, 3H, H-1, H-3, H-4); ^{13}C-NMR: 81.6 (C-1). **4a**: FAB-MS (pos.) calcd for $C_{28}H_{38}O_{16}S$: 694.2; found: 695.3 (M + 1, 0.9%); ^{1}H-NMR ($CDCl_3$) δ: 2.20 (s, 3H, SAc), 5.05-5.11 (m, 2H, H-1, H-3); ^{13}C-NMR: 79.8 (C-1). **7a**: FAB-MS (pos.) calcd for $C_{31}H_{44}N_2O_{18}S$: 764.3; found: 765.2 (M + 1, 34.6%); ^{1}H-NMR ($CDCl_3$) δ: 1.93 (s, 3H, NAc), 2.27 (s, 3H, SAc), 4.98-5.02 (m, 2H, H-1, H-3); ^{13}C-NMR: 80.8 (C-1). **8a**: FAB-MS (pos.) calcd for $C_{31}H_{43}NO_{19}S$: 765.2; found: 766.2 (21.4%);^{1}H-NMR ($CDCl_3$) δ: 2.28 (s, 3H, SAc), 5.17 (dd, 1H, $J_{1,2}$=9.4 Hz, H-1); ^{13}C-NMR: 78.0 (C-1). Divalent **9**: FAB-MS (pos.) calcd for $C_{21}H_{33}Cl_2N_7O_9$: 598.4; found 598.3 (7.9% base peak); ^{1}H-NMR (DMSO-d^6) δ: 2.36 (t, 2H, J=7 Hz, ß-alanyl α-CH_2), 2.99 (m, 2H, lysyl ε-CH_2), 4.12 (d, 4H, J=2.6 Hz, $ClCH_2$). Tetravalent **9**: FAB-MS (pos.) calcd for $C_{45}H_{71}Cl_4N_{15}O_{17}$: 1236.0; found: 1236.9 (M + 1, 0.7%). Divalent **10**: FAB-MS (pos.) calcd for $C_{49}H_{73}N_9O_{25}S_2$: 1252.3; found: 1253.2 (M + 1, 19.8%); ^{1}H-NMR (DMSO-d^6) δ: 1.75 (s, 6H, NAc), 2.36 (t, 2H, J=7 Hz, ß-alanyl α-CH_2), 3.00 (m, 2H, lysyl ε-CH_2), 4.77 (d, 2H, $J_{1,2}$=10.3 Hz, H-1); ^{13}C-NMR: 82.8 (C-1). Tetravalent **10**: FAB-MS (pos.) calcd for $C_{101}H_{151}N_{19}O_{49}S_4$: 2543.6, found: 2544.3 (M + 1, 0.5%). Divalent **11**: FAB-MS (pos.) calcd for $C_{73}H_{103}N_7O_{43}S_2$: 1830.8; found 1853.7 (M + Na, 7.4%); ^{1}H-NMR (DMSO-d^6) δ: 2.36 (t, 2H, J=7 Hz, ß-alanyl α-CH_2), 2.99 (m, 2H, lysyl ε-CH_2), 4.89 (d, 2H, $J_{1,2}$=10.1 Hz, H-1); ^{13}C-NMR: 81.2 (C-1). Divalent **12**: ^{1}H-NMR (DMSO-d^6) δ: 1.71 (s, 6H, NAc) 2.36 (t, 2H, J=7 Hz, ß-alanyl α-CH_2), 2.99 (m, 2H, lysyl ε-CH_2), 5.05-5.10 (m, 4H, H-1, H-3); ^{13}C-NMR (HMQC): 77.8 (C-1). Divalent **13**: ^{1}H-NMR (DMSO-d^6): 2.36 (t, 2H, J=7 Hz, ß-alanyl α-CH_2), 2.99 (m, 2H, lysyl ε-CH_2), 5.27 (dd, 2H, $J_{1,2}$=9.3 Hz, H-1); ^{13}C-NMR (HMQC): 76.6 (C-1). Divalent **14**: FAB-MS (pos.) calcd for $C_{37}H_{61}N_9O_{19}S_2$: 999.4; found: 1000.6 (M + 1, <1%); ^{1}H-NMR (D_2O) δ: 2.09 (s, 6H, NAc), 2.66 (t, 2H, J=6.6 Hz, ß-alanyl α-CH_2), 3.26 (t, 2H, J=6.9 Hz, lysyl ε-CH_2), 4.74 (2d, 2H, 2 x H-1, unequiv.); ^{13}C-NMR: 2 x 83.5 (2 x C-1, unequiv.). Divalent **15**: ^{1}H-NMR (D_2O) δ: 2.64 (t, 2H, J=6.6 Hz, ß-alanyl α-CH_2), 3.28 (t, 2H, J=6.8 Hz, lysyl ε-CH_2), 4.68 (d, 2H, $J_{1,2}$=9.9 Hz, H-1); ^{13}C-NMR: 84.4 (C-1). Divalent **16**: ^{1}H-NMR (D_2O) δ: 2.70 (m, 2H, ß-alanyl α-CH_2), 3.34 (m, 2H, lysyl ε-CH_2), 5.07 (d, 2H, $J_{1,2}$=9.3 Hz, H-1); ^{13}C-NMR (HMQC): 77.8 (C-1). Divalent **17**: ^{1}H-NMR (D_2O) δ: 2.05 (s, 6H, NAc), 2.70 (m, 2H, ß-alanyl α-CH_2), 3.34 (m, 2H, lysyl ε-CH_2), 5.07 (d, 2H, $J_{1,2}$=9.7 Hz, H-1); ^{13}C-NMR (HMQC): 80.8 (C-1).

(*Received in USA* 29 *June* 1995; *accepted* 10 *August* 1995)