Multivalent neoglycoconjugates: solid-phase synthesis of N-linked α-sialodendrimers

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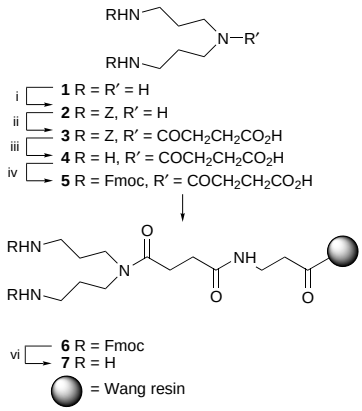
A series of N-linked α-sialodendrimers with valencies of 2, 4 and 8 has been scaffolded on an *N*,*N*-bis(3-aminopropyl)succinamic acid core using Wang resin and 9-fluorenylmethoxycarbonyl and 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate solid-phase chemistry.

Dendrimers are attractive monodispersed macromolecules with broad spectrum applications from physics to medicine.[1] The design of novel dendrimers represents an active research area and their syntheses are considered challenging owing to their size and highly branched structures. Traditionally, divergent[2] and convergent[3] strategies have been used to synthesize dendrimers. However, more recently a double exponential growth strategy[4] and a self-assembling method[5] have been developed. To date, the synthesis of most dendrimers has been carried out in solution, although Tam[6] has built hyperbranched L-lysine dendrimers on a solid-phase for multiple antigen peptide (MAP) presentation. Because of the critical role of carbohydrate-containing macromolecules in many cellular proceses, glycodendrimers have also become a growing research area.[7] There is increasing evidence that the multivalent effect displayed by these novel biopolymers may overcome the characteristic low-affinity binding interactions between carbohydrate ligands and proteins.[8,9]

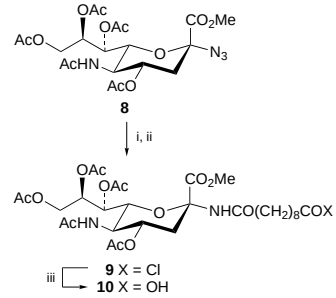
Potent L-lysine-based dendritic α-thiosialoside inhibitors of *Influenza* virus's hemagglutination to human erythrocytes have been previously described.[10] Similarly, different series of symmetrical dendrimers containing *N*-acetylneuraminic acid (NeuAc or sialic acid), representing one of the most widespread mammalian cell surface carbohydrate ligands,[11] have been successfully synthesized in solution.[12,13] Therefore, it was attractive to build a new series of sialodendrimers on a solid-phase. The present approach differs from previous S-linked sialosides[10,11] by the use of N-linked sialylated derivatives which are also expected to be sialidase resistant. Thus, the solid-phase synthesis of a new family of symmetrical N-linked sialodendrimers based on *N*,*N*-bis(3-aminopropyl)succinamic acid **4** core is described.



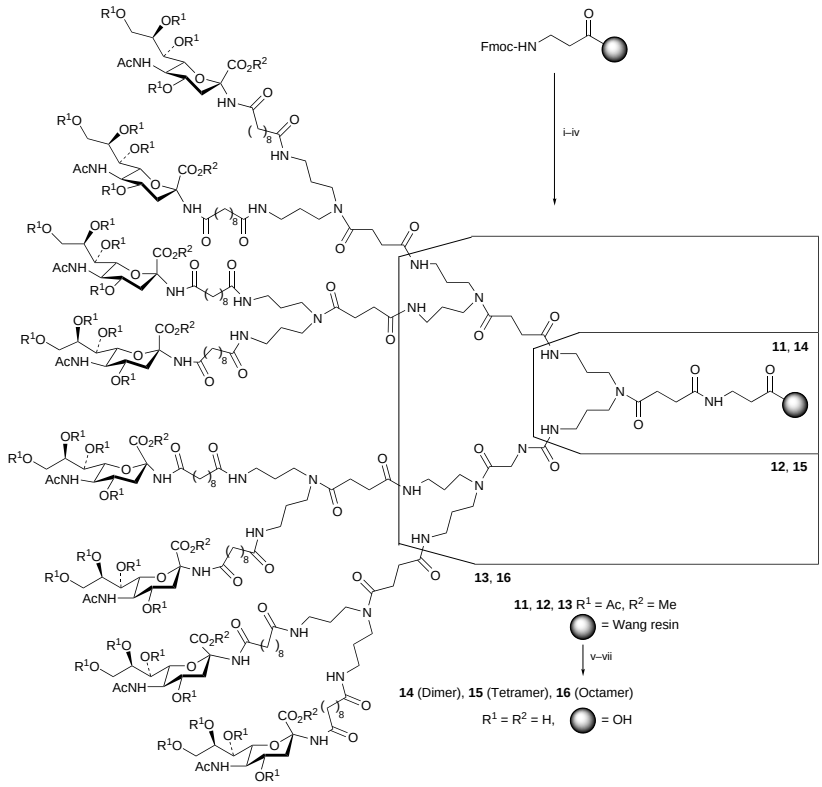
Scheme 1 *Reagents and conditions*: i, BnO_2CCN, CH_2Cl_2, 2 h, 72%; ii, succinic anhydride, Et_3N, CH_2Cl_2, 1 h, 96%; iii, H_2, 10% Pd–C, MeOH, 2 h, quant.; iv, FmocCl, 10% Na_2CO_3–dioxane, 12 h, 72%; v, H-βAla-Wang resin, TBTU, Pr^i_2EtN, DMF, 30 min; vi, 20% piperidine–DMF (1 × 5 min, 1 × 15 min)

In order to provide a strategy which could be used with other carbohydrate ligands, a divergent approach was used to build these novel poly(amidoamine) dendrimers on a solid-phase. Suitably acid-functionalized carbohydrate derivatives could then be added onto polyamino dendrimers to afford glycodendrimes with even valencies. The dendritic core was prepared as follows (Scheme 1). The primary amine groups of *N*-(3-aminopropyl)propane-1,3-diamine **1** were regioselectively protected with benzyloxycarbonyl (Z) groups using benzyl cyanoformate in CH_2Cl_2[14] to give secondary amine **2** in 72% yield. Acylation of the residual amine with succinic anhydride (triethylamine, CH_2Cl_2, 96%) provided key building block **3** having a divalent structure with a bis-Z-protected monoacid core. In order to use the Fmoc strategy, the Z-protecting groups were removed by catalytic hydrogenation (10% Pd–C, MeOH) to give **4**, which was then treated with 9-fluorenylmethyl chloroformate (FmocCl, 10% Na_2CO_3–dioxane, 0 °C) to afford **5**† in 72% yield. The dendritic polyamine scaffolds were built on a β-alanine spacer attached to Wang resin [4-(hydroxymethyl)phenoxymethyl–*co*-poly(styrene–1% divinylbenzene), 0.58 mmol g^{-1}], using the Fmoc-strategy and performing the deprotection–coupling cycles as follow: (i) Fmoc deprotection by treatment with 20% piperidine in DMF (1 × 5 min, 1 × 15 min), (ii) coupling of Fmoc–protected acid **5** (2 equiv.) using 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) (2 equiv.) as activating agent and Pr^i_2EtN (4 equiv.) in DMF during 30 min. Coupling completion was determined by a ninhydrin test for residual amino groups.[15] To characterize the dendrimers, 30 mg of resin from each sequential generation was Fmoc-deprotected and the dendrimers were released from the solid support following conventional acid treatment (95% aq. TFA, 2 h, 30–79% yield).

Glycodendrimer synthesis required sialic acid residues suitably functionalized at the anomeric position for coupling with amine-terminated dendrimers. The readily available α-sialic acid azide derivative **8**[16] was reduced by catalytic hydrogenation (10% Pd–C, MeOH) to provide α-sialic acid amine in quantitative yield (Scheme 2). To minimize anomerisation, the next acylation step was performed within 1 h. To



Scheme 2 *Reagents and conditions*: i, H_2, 10% Pd–C, MeOH, 1 h, quant.; ii, $ClCO(CH_2)_8COCl$, Pr^i_2EtN, CH_2Cl_2, 3 h; iii, H_2O, 53% overall yield



Scheme 3 *Reagents and conditions*: i, 20% piperidine–DMF (1 × 5 min, 1 × 15 min); ii, **5**, TBTU, Pr^i_2EtN, DMF, 30 min; iii, repeat cycle or 20% piperidine–DMF; iv, **10**, TBTU, Pr^i_2EtN, DMF, 0.5–8 h; v, 95% aq. TFA, 2 h; vi, 1 M NaOMe, MeOH, 2–8 h; vii, 0.05 M NaOH, 2–8 h

offer suitable carbohydrate accessibility, introduction of a spacer arm between the sialic acid residues and the dendrimer was carried out using excess $ClCO(CH_2)_8COCl$ (CH_2Cl_2, Pr^i_2EtN, 0 °C) to give unstable monoacid chloride **9** which was immediately hydrolysed to afford **10** in 53% overall yield. Attempts to isolate **9** for subsequent attachment to the resin were unsuccessful. α-Sialic acid derivative **10** was then ready to be coupled to the dendritic polyamine core on the solid-phase using the same TBTU coupling strategy described to scaffold the dendrimer generations. After coupling, peracetylated sialodendrimers with the valencies of 2, 4 and 8 (**11**, **12** and **13**) were released from the resin by treatment with 95% TFA. The sialodendrimers were then deprotected by sequential ester hydrolysis [(i) de-*O*-acetylation with 1 M NaOMe in MeOH, (ii) 0.005 M NaOH]. Purification of each independent generation by size exclusion chromatography over Biogel-P2 (H_2O as eluent) provided pure deprotected glycodendrimers **14**, **15** and **16** in moderate yields (25–56%) (Scheme 3). The purity of each compound was readily established from the relative integration of key signals in the ^1H NMR spectrum. MALDI-TOF mass spectral data (negative mode) further confirmed the integrity of the dendrimers. Biological properties of these novel N-linked sialodendrimers will be reported in due course.

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Footnotes and References

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† All compounds showed consistent NMR and mass spectral data. Because of dendrimers' repetitive structures, only selected data are reported. *Selected data* for **5**: δ_H([$^2H_6$]acetone) 1.69 and 1.91 (2 m, 4 H, β-CH_2), 2.6 [m, 2 H, succinyl C*H*$_2$C(O)OH], 4.3 (dd, 4 H, Fmoc-CH_2s); δ_C 28.7 and 30.5 (β-C), 29.8 [succinyl *C*H_2C(O)OH]; FAB-MS (positive): calc. for $C_{40}H_{41}N_3O_7$: 675.7. Found: 676.3 (M + 1). For **10** (spacer identification from anomeric position to acid is a to h): δ_H($CDCl_3$) 1.59 (m, 4 H, b-, g-CH_2s), 1.87 (s, 3 H, NAc), 2.07 (m, 1 H, H-3^{ax}), 2.74 (m, 1 H, H-3^{eq}), 3.74 (s, 3 H, OCH_3), 5.35 (dd, 1 H, $J_{6,7}$ 2.2, $J_{7,8}$ 6.1, H-7); δ_C 23.14 (NAc), 24.58 (g-C), 52.9 (OCH_3), 83.3 (C-2); FAB-MS (positive): calc. for $C_{30}H_{46}N_2O_{15}$: 674.7. Found: 675.4 (M + 1). For **14**: δ_H(D_2O) 1.62 (m, b-, g-CH_2s), 1.84 (m, 6 H, β-CH_2, H-3^{ax}), 2.08 (s, 6 H, NAc), 2.44 (m, 2 H, β-alanyl α-CH_2), 2.73 [m, 4 H, succinyl C*H*$_2$C(O)N, H-3^{eq}], 4.16 (d, 2 H, *J* 9.9, H-7); δ_C 21.5 (NAc), 24.5 (g-C), 26 and 27 (β-Cs), 36.2 (γ-Cs, β alanyl α-C and β-C), 72.4 (C-7), 84.4 (C-2); FAB-MS (positive): calc. for $C_{55}H_{94}N_8O_{24}$: 1250.64. Found: 1251.5 (M^+ + 1, 1%); MALDI-TOF (negative): Found: 1250.03 (M − 1)$^-$. For **15**: MALDI-TOF (negative): calc. for $C_{117}H_{200}N_{18}O_{48}$: 2625.37. Found: 2626 (M − 1)$^-$. For **16**: δ_H (D_2O) 1.86 (m, 28 H, β-CH_2s), 2.11 (s, 24 H, NAc), 4.18 (m, 8 H, H-7); δ_C 21.57 (NAc), 26.2 (β-Cs), 72.5 (C-7), 85.2 (C-2); MALDI-TOF (negative) calc. for $C_{241}H_{412}N_{38}O_{96}$: 5,374.85. Found: 5374 (M − 1)$^-$.

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