Solid-phase Synthesis of Dendritic Sialoside Inhibitors of Influenza A Virus Haemagglutinin

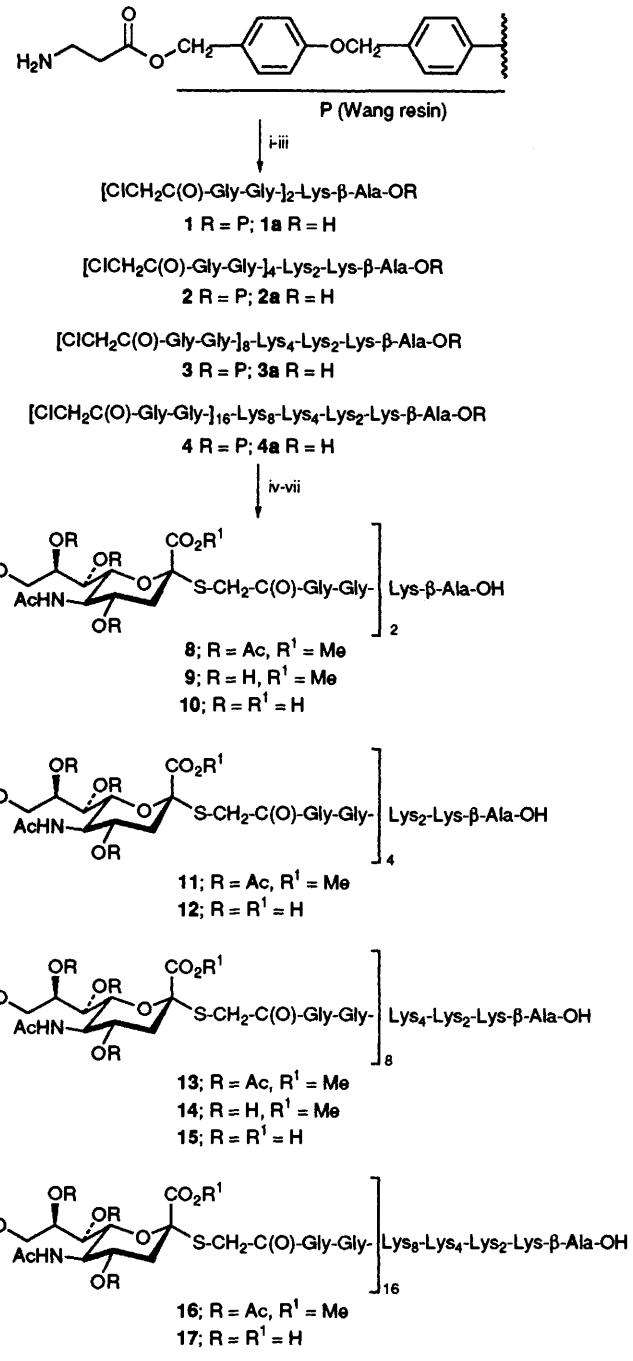
René Roy,* Diana Zanini, Serge J. Meunier and Anna Romanowska

Department of Chemistry, University of Ottawa, Ottawa, Ontario, Canada K1N 6N5

Efficient solid-phase synthesis of dendritic α-thiosialoside inhibitors of influenza A virus haemagglutinin are described using hyperbranched L-lysine core structures.

The prime event in host cell infections by influenza viruses is triggered by the recognition and binding of viral haemagglutinins (HAs) to α-sialosides present on the cell surface glycolipids and glycoproteins.[1] The binding affinity of monomeric HA to single α-sialoside is however rather weak (mmol dm^{-3} range).[2] In order to circumvent the poor affinities of α-sialosides in the design of potential inhibitors of viral adhesions, a number of different approaches have been established.[3-7] The most striking improvement was achieved using the multivalency concept ('cluster effect').[8] A number of years ago, we synthesized sialylated glycopolymers to fulfill this requirement.[3] The improved cooperativity of binding set by the multivalency of the glycopolymers allowed for a thousand-fold increase in inhibitory activities (μmol dm^{-3} range).[4] Other investigators have followed a similar approach in the preparation of other sialylated polymers[5] and liposomes.[7]

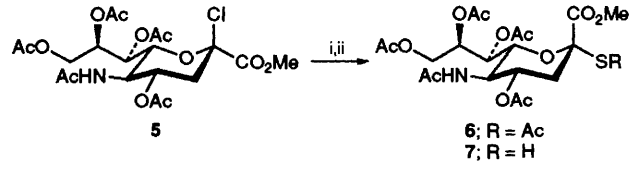
In line with the previous multivalent α-sialosides,[3-5] we present herein a conceptually new approach designed to improve the therapeutic potential of viral HA inhibitiors. The new strategy is based on the efficient solid-phase synthesis of water-soluble dendritic α-thiosialosides. Dendritic macromolecules with hyperbranched fractal structures are likely to mimic multi-antennary glycoproteins. The solid-phase dendrimer synthesis described herein eliminates some of the technical drawbacks associated with the starburst[9] or con-



Scheme 1 *Reagents and conditions*: i, Nα-Nε-di-fmoc-L-Lys-OBt, DMF; ii, 20% piperidine (3 × 10 min); iii, repeat cycle or $ClCH_2C(O)$-Gly-Gly-OBt, DMF; iv, **7**, 1% Et_3N, DMF, 16 h, 25 °C; v, 95% aq. TFA, 1.5 h, 66–99%; vi, NaOMe, MeOH, 1 h; vii, 0.05 mol dm^{-3} NaOH, 2 h, 25 °C, then H^+ resin treatment, quantitative

vergent approaches.[10] Because influenza A viruses also possess receptor destroying enzymes with sialidase activity, the dendritic sialosides were synthesized in the form of α-thioketosides in order to provide combined haemagglutinin and sialidase inhibition properties.

In the actual synthetic scheme, dendritic L-lysine cores[11] were constructed on β-alanyl spacer anchored to poly[styrene-co-4-(hydroxymethyl)phenoxymethyl] resin (Wang resin, 0.58 mmol g^{-1}) using fluoren-9-ylmethoxycarbonyl (fmoc) chemistry (Scheme 1). The hyperbranched poly-L-lysine scaffolding was done using preformed Nα-Nε-di-fmoc-L-lysine benzotriazolyl ester (2 equiv.) [HOBt, diisopropylcarbodiimide (DIPC), *N*,*N*-dimethylformamide (DMF), 0 °C, then 25 °C, 1 h]. Removal of the fmoc protecting group was effected by



Scheme 2 *Reagents and conditions*: i, AcSNa, TBAHS, EtOAc, 1 mol dm^{-3} Na_2CO_3, 25 °C, 30 min, 66%; ii, NaOMe, MeOH, −40 °C, 5 min, then H^+ resin

treatment with 20% piperidine in DMF (3 × 10 min). The products resulting from each sequential generation were then treated with preformed benzotriazolyl ester of chloroacetylglycylglycine as described above. In this manner, di-**1**, tetra-**2**, octa-**3** and hexadeca-valent **4** chloroacetylated dendrimers were obtained in the first, second, third and fourth generations, respectively. For structural determination purposes, compounds **1a**–**4a** were released from the polymer support by treatment with 95% aq. trifluoroacetic acid (TFA, 1.5 h).†

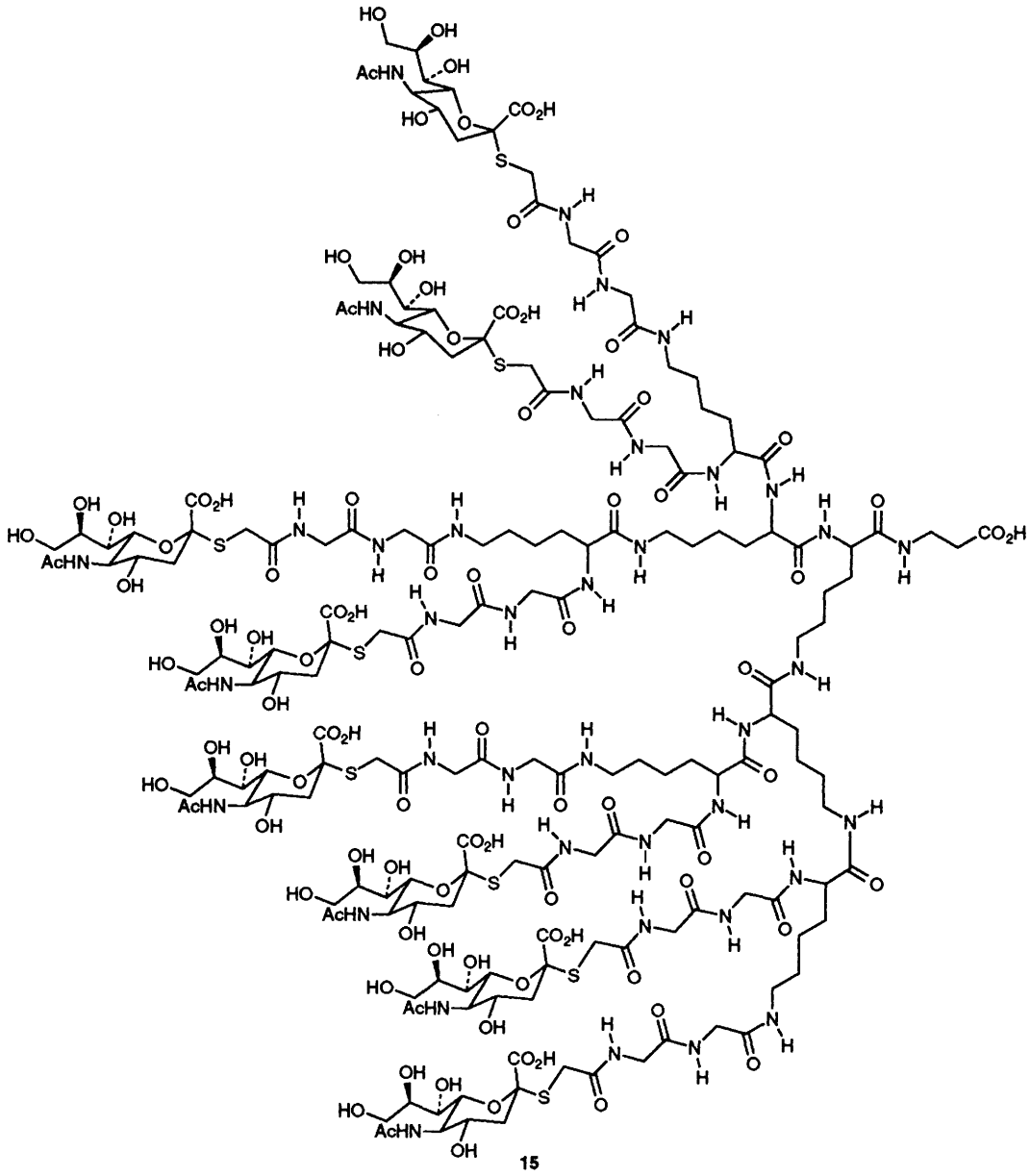
† All new compounds gave consistent NMR and mass spectral data. ^1H and ^{13}C NMR data were obtained on a Brüker 500 MHz AMX NMR spectrometer using [$^2H_6$]DMSO (ref. at δ 2.49 and 39.5 respectively) or D_2O (ref. at δ 4.75) as solvents. Mass spectra were recorded with a Kratos 11 H instrument (glycerol or thioglycerol matrices for FAB-MS). Owing to the repetitive structures of the dendrimers, selected data only are reported. Compound **1a**: ^1H NMR ([$^2H_6$]DMSO) δ 1.16 (m, 2H, lysyl γ-CH_2), 1.33 (m, 2H, lysyl δ-CH_2), 1.46 and 1.60 (2m, 2H, lysyl β-CH_2, unequiv.), 2.36 (t, 2H, *J* 7 Hz, β-alanyl α-CH_2), 2.99 (m, 2H, lysyl ε-CH_2), 3.20 (m, 2H, β-alanyl β-CH_2), 3.65 and 3.74 (2d, 4H, *J* 5.8 Hz, glycyl CH_2s), 3.77 (d, 4H, *J* 5.8 Hz, glycyl CH_2s), 4.12 (d, 4H, *J* 2.6 Hz, $ClCH_2$), 4.13 (m, 1H, lysyl α-CH), 7.72 (t, 1H, *J* 5.6 Hz, lysyl ε-NH), 7.92 (m, 2H, β-alanyl NH and lysyl α-NH), 8.18 (m, 2H, glycyl NH), 8.45 (m, 2H, glycyl NH), 10.80 (bs, 1H, CO_2H); ^{13}C NMR (by HMQC) δ 22.7 (lysyl γ-C), 28.7 (lysyl δ-C), 31.7 (lysyl β-C), 33.8 (β-alanyl α-C), 34.3 (β-alanyl β-C), 38.7 (lysyl ε-C), 41.7 (4 × glycyl CH_2), 42.4 ($ClCH_2$), 52.5 (lysyl α-C); FAB-MS (pos.) calc. for $C_{21}H_{33}Cl_2N_7O_9$ 598.4 found 598.3 (% base peak 7.9).

Compound **8**: peptide backbone, i.d. except for $-SCH_2$ at 3.37 and 3.47 (2d, 2 × 2H, *J* 7.1 Hz), NeuAc residues: 1.78 (dd, 2H, 2 × *J* 12.1 Hz, H-3ax), 1.65 (s, 6H, NAc), 1.91, 1.96, 1.99, 2.06 (4s, 24H, OAc), 2.64 (dd, 2H, *J* 4.6 and 12.5 Hz, H-3eq), 3.72 (s, 6H, CO_2Me), 3.77 (m, 2H, H-6), 3.86 (m, 2H, H-5), 4.02 (dd, 2H, *J* 5.8 and 12.2 Hz, H-9), 4.16 (dd, 2H, *J* 3.1 and 12.2 Hz, H-9′), 4.70 (ddd, 2H, *J* 10.8 and 4.6 Hz, H-4), 5.12 (dd, 2H, *J* 2.0 and 8.3 Hz, H-7), 5.25 (m, 2H, H-8), 7.62–8.16 (m, 9H, NHs); ^{13}C NMR δ: 20.6 (OAcs), 22.6 (NAc), 22.8 (lysyl γ-C), 28.8 (lysyl δ-C), 31.7 (lysyl β-C), 32.1 (2 × SCH_2), 33.8 (β-alanyl α-C), 34.9 (β-alanyl β-C), 37.4 (C-3), 38.5 (lysyl ε-C), 42.0, 42.5, and 42.6 (glycyl CH_2s), 47.8 (C-5), 52.5 (lysyl α-C), 53.1 (MeO), 61.9 (C-9), 67.1 (C-7), 67.9 (C-4), 69.5 (C-8), 73.7 (C-6), 82.4 (C-2), 167.7 to 172.8 (C=Os).

Compound **10**: ^1H NMR (D_2O) δ peptide backbone 1.42 (m, 2H, lysyl γ-CH_2), 1.64 (m, 2H, lysyl δ-CH_2), 1.78 and 1.84 (2m, 2H, lysyl β-CH_2, unequiv.), 2.69 (m, 2H, β-alanyl α-CH_2), 3.34 (m, 2H, lysyl ε-CH_2), NeuAc residues: 1.90 and 1.96 (2dd, 2H, 2 × *J* 12.1 Hz, H-3ax), 2.12 and 2.13 (2s, 6H, NAc), 2.91 and 2.96 (2dd, 2H, *J* 12.6 and *J* 4.7 Hz, H-3 eq.), 3.50–4.33 (m, 29H, peptide backbone, NeuAc residues excluding above and NHs and CO_2Hs); ^{13}C NMR (D_2O, by HMQC) δ: 18.8 (NAc), 19.2 (lysyl γ-C), 24.6 (lysyl δ-C), 27.4 (lysyl β-C), 29.5 (2 × SCH_2), 30.8 (β-alanyl α-C), 32.4 (β-alanyl β-C), 35.8 (lysyl ε-C), 36.7 and 37.1 (C-3 and C-3′), 39.4 (4 × glycyl CH_2s), 48.2 (C-5), 50.7 (lysyl α-C), 59.9 (C-9), 65.0 (C-7), 69.1 (C-4), 72.1 (C-8), 72.9 (C-6); FAB-MS (pos.) calc. for $C_{43}H_{69}N_9O_{25}S_2$ 1176.2 found 1176.4 (% base peak 0.8).

Compound **3a**: Consistent spectral data with compound **1a** including corresponding integration signals.

Compound **13**: Consistent spectral data with compound **8** except in the ^{13}C NMR ([$^2H_6$]DMSO, 600 MHz), β-alanyl residues become part of the background, ^{13}C NMR δ: 20.7, 20.7, 20.9, 21.0 (OAc), 22.7 (NAc), 23.0 (lysyl γ-C), 28.9 (lysyl δ-C), 31.9 (lysyl β-C), 32.2 (S-CH_2), 37.5 (C-3), 38.6 (lysyl ε-C), 42.1 and 42.6 (4 × glycyl CH_2s), 47.9 (C-5), 52.7 (lysyl α-C), 53.2 (MeO), 62.0 (C-9), 67.1 (C-7), 68.0 (C-4), 69.6 (C-8), 73.8 (C-6), 82.5 (C-2), 167.8 to 170.3 (C=Os).



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While still attached to the resin, each dendrimer generation was treated with an excess of 2-thiosialic acid derivative **7** (1% triethylamine–DMF, 16 h, 25 °C). Before the bulk of the dendrimers were released from the polymeric support, aliquots were withdrawn and hydrolysed as above. The completeness of the couplings was estimated from the ^{1}H NMR spectrum of the sialylated dendrimers which showed characteristic signals for any residual chloroacetylmethylene groups δ 4.2, dimethylsulfoxide-d_6 ([$^2H_6$]DMSO)]. Where required, the couplings were repeated.

The 2-α-thioacetylsialic acid precursor **6** was prepared from acetochloroneuraminic acid **5** under stereospecific phase-transfer catalysed conditions [tetrabutylammonium hydrogen sulfate (TBAHS), ethyl acetate, 1 mol dm^{-3} sodium carbonate, sodium thioacetate, 25 °C, 30 min, 66% yield, crystalline, m.p. 89–92 °C].[12] Chemoselective hydrolysis of the thioacetate group in the presence of other ester functionalities was achieved by mild transesterification (NaOMe 0.95 equiv., MeOH, −40 °C, <5 min, then H$^+$ resin treatment)[13] to afford **7** (quantitative) which was used directly for the sialylation reaction (Scheme 2).

The peracetylated sialyl dendrimers **8**, **11**, **13** and **16** were released from the polymer support as above for **1a–4a** and obtained in 66–99% yields after removal of the solvent under reduced pressure. The ^{1}H and ^{13}C NMR spectra ([$^2H_6$]DMSO, 500 MHz) of the dendrimers revealed the integrity of the α-sialoside linkages as well as the ratio of the β-alanyl residues relative to those of both L-lysyl and sialyl signals. Each of the protected dendrimers (**8**, **11**, **13** and **16**) were de-esterified with NaOMe–MeOH (25 °C, 1 h) (**9** and **14**) followed by 0.05 mol dm^{-3} NaOH (25° C, 2 h, then H$^+$ resin treatment) to afford dendrimers **10**, **12**, **15** and **17** in essentially quantitative yields. The larger octa- **15** and hexadeca-meric **17** dendrimers can be dialysed using benzoylated dialysis tubing (M.W. cutoff 2000).

The binding properties of the sialylated dendrimers **10**, **12**, **15**, **17** were first established using the plant lectin wheat germ agglutinin (WGA) in a direct enzyme linked lectin assay (ELLA) in microtiter plates using horseradish peroxidase (HRPO) labelled WGA. While the di- and tetra-valent dendrimers **10** and **12** showed low coating capacities on the microtiter plates, the octa- and hexadeca-valent dendrimers (**15** and **17**) showed binding properties only slightly less than that of an homologous sialylated polymer poly(acrylamide-co-*p*-*N*-acrylamidophenyl α-thiosialoside).[4] Each dendrimer (**10**, **12**, **15** and **17**) was then evaluated for its inhibition properties using the sialylated glycopolymer as coating antigen and HRPO-WGA. The result of these inhibition experiments is illustrated in Fig. 1. All the dendrimers showed excellent inhibitory capacities (*ca.* 10^6 times better than a monosialo-

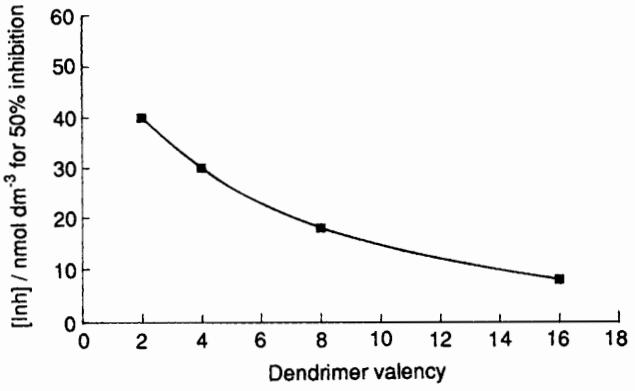

Fig. 1 Inhibition curve of horseradish peroxidase (HRPO) labelled wheat germ agglutinin (WGA) with poly(acrylamide-co-*p*-*N*-acrylamidophenyl α-thiosialoside) (10 μg/well) by the sialylated dendrimers **10**, **12**, **15** and **17** in a direct microtiter plate enzyme linked lectin assay (ELLA). The HRPO substrate was 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and the absorbances were read at 410 nm.

side) thus demonstrating the efficiency of dendritic clustered structures.

Preliminary experiments with influenza A virus (strain X-31) showed that the dendrimers **10**, **12**, **15** and **17** exhibited powerful inhibition of haemagglutination of erythrocytes at concentrations of 625, 312.5, 156 and 19 μmol dm^{-3}, respectively. The results with the divalent dendrimer **10** (625 μmol dm^{-3}) demonstrated that even at such a low level of clustering, the divalent species is five times more potent than a monosialoside (*ca.* 3 mmol dm^{-3}). Dendrimer **17** is thus as potent as previous sialylated glycopolymers.[3–5]

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