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Synthesis of poly(aspartimide)-based bio-glycoconjugates

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ABSTRACT

The purpose of this programme was to synthesize and analyze new bioconjugates of interest for the potential inhibition of the influenza virus, using poly(aspartimide) as a polymer support. The macromolecular targets were obtained by attaching various sialic acid-linker-amine compounds to poly(aspartimide). ^{1}H and ^{13}C NMR studies were then performed to analyze the degree of incorporation of the sialic acid-linker-amine compounds within the poly(aspartimide). These studies illustrated that the incorporation was dependent on the nature of the spacer between the sugar and the amine functionality. Thus aliphatic spacers favoured the inclusion of sialic acid onto the polymer support whereas compounds having only an aromatic moiety between the sialic acid and the amine could not be easily incorporated.

1. Introduction

One of the most promising methods to prevent infection of hosts by the influenza virus is to inhibit the interaction of the virus with sialic acid residues on host cells.[1] In this regard, the development of polyvalent inhibitors for the influenza virus has attracted recent attention.[2–6] Polyvalent binding is characterized by the simultaneous interactions between multiple ligands on one entity and multiple receptors on the target biomolecule. In order to have an enhanced inhibition activity, the synthetic inhibitors must have a greater affinity towards the virus than the natural receptors. The connection of sialic acid molecules to a polyvalent backbone potentially increases the avidity of these materials for the virus, by taking advantage of the multivalent effect. Two types of carriers have been studied for the preparation of glycoconjugates bearing multiple sialic acid residues: natural backbones, such as proteins[7–9] or polysaccharides[10–12] and synthetic backbones, such as spherical dendrimers,[13–15] liposomes[16–20] and linear polymers.[21–29] Compared to other scaffolds, polymeric supports offer several advantages such as their ease of synthesis and capacity to display multiple sialic acid residues.[30]

The purpose of this paper is to report the design and synthesis of new polyvalent sialosides, obtained by functionalization of poly(aspartimide) (PAI) with compounds containing sialic acid. Our aim is to make new bioconjugate drugs that can potentially act as inhibitors of hemagglutinin. The poly(aspartimide) was selected as the polyvalent support because it affords linear biodegradable compounds, it presents immunological activity and, more importantly, it affords water soluble compounds.

2. Results and discussion

In order to obtain polymeric glycoconjugates with antiviral activity, we took into consideration the fact that infection with influenza virus occurs through attachment to the cell surface of hemagglutinin, the major component of the influenza virus.

Literature studies have shown that hemagglutinin (HA) recognizes the carboxylic acid function and the alcoholic chain of the sialic acid (Fig. 1) while neuraminidase (NA) recognizes, in particular, the hydroxyl group on carbon 4 (C-4) of sialic acid. We therefore chose to synthesize a series of compounds which contained sialic acid linked to a spacer that contained a terminal amine functionality. Within the derivatives, the nature of the aglyconic chain was varied to both probe the ease with which the sialic acid-linker-amine units could be incorporated within the polymer, and the importance of these structural changes on the biological activities of the materials. Also, to minimize the potential for hydrolysis of the derivatives, via the action of neuraminidase, a sulfur atom was introduced into the structure.

Four new polyvalent compounds have been obtained by coupling of sialic acid attached to four different linkers to poly(aspartimide). The spacers connected to sialic acid were of varying length and functionality (Schemes 1–3) but each spacer group

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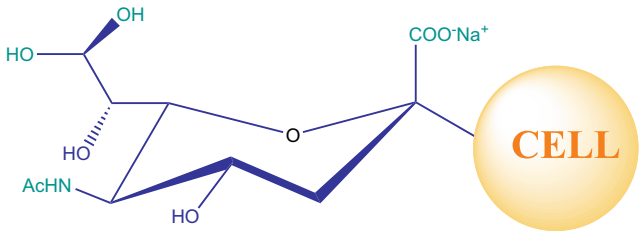


Figure 1. Representation of the sialic acid linked to body cell.

contained an amine functional group at its terminus to allow attachment to the polymer. The syntheses of the amino spacer functionalized sialic acid derivatives are described in detail below.

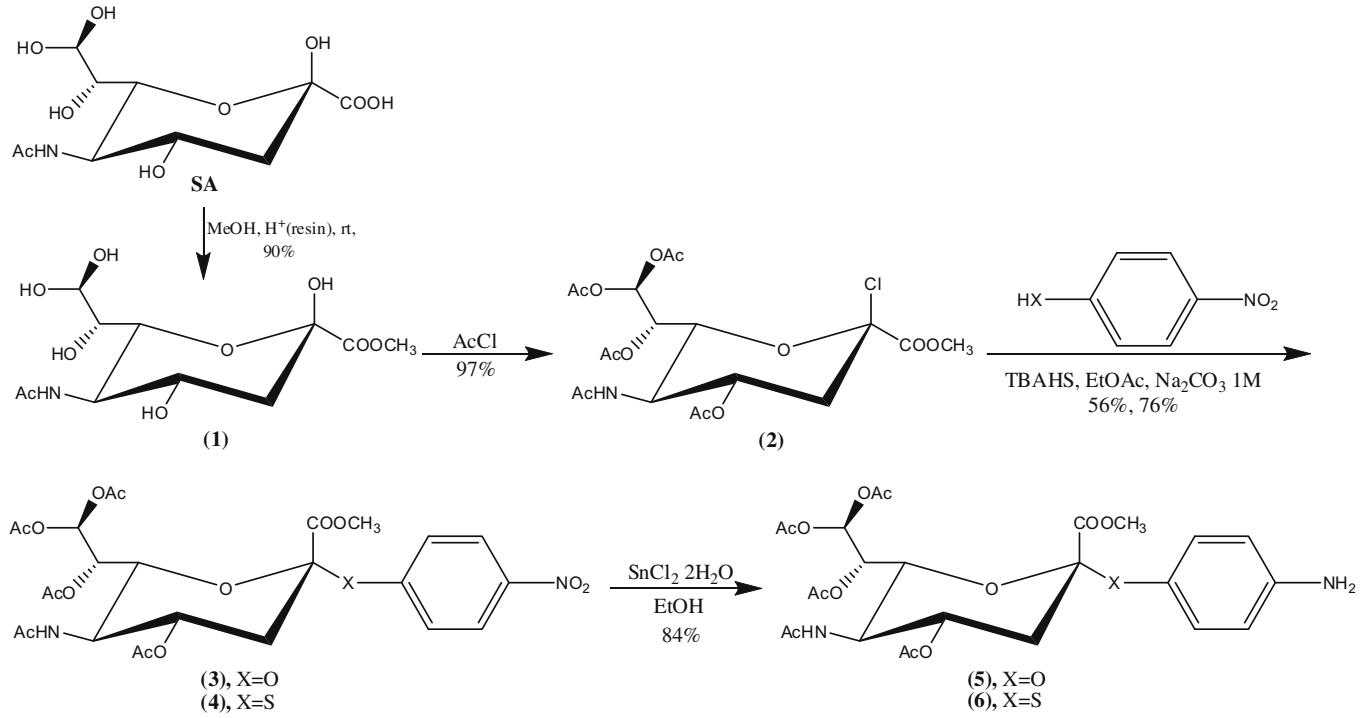
2.1. Glycoconjugate synthesis

In order to attach the linker unit to the sialic acid, it was initially necessary to protect and activate the sialic acid to allow for its subsequent glycosylation. This was achieved by reaction of sialic acid with methanol, under acidic conditions, to form the methyl ester **1**. The reaction took place with good yield (90%) and the obtained compound was sufficiently pure to be used in the subsequent reaction without further purification. This was then treated with acetyl chloride to form the fully acetylated chloroneuraminic acid methyl ester **2** which proved a key intermediate for the synthesis of the amino spacer functionalized sialic acid derivatives **5**, **6**, **8** and **11**. For entry to **3** and **4**, chloride **2** was reacted with p-nitrophenol or *p*-nitrobenzenethiol under phase transfer conditions[22,31–33] to afford **3** and **4**, respectively. The nitro functionality was then reduced by treatment with tin(II) chloride to afford the amines **5** and **6**, ready for coupling to poly(aspartimide). Amine **6** was also used in the preparation a further amine-spacer-sialic acid derivative **8**, as it was anticipated that the sulfur-linked derivatives may offer better profiles in vivo, due to their enhanced stability to glycosidase enzymes compared with the O-linked glycosides. Thus **6** was coupled to protected valerianic acid **12** (Scheme 2) to afford amide **7**. This was then treated with TFA to effect removal of the *N*-Boc group and to afford amine **8**.

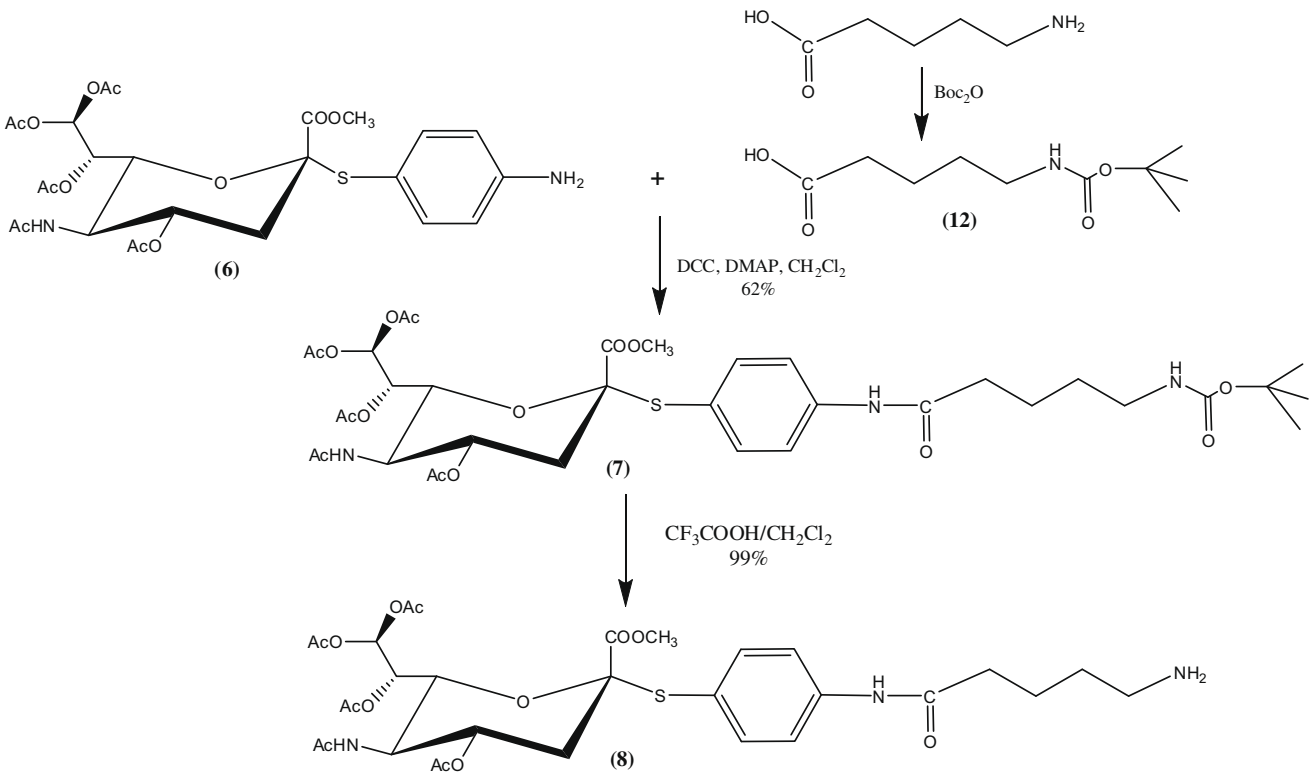
For the synthesis of the final sialic acid-spacer-amine derivative **11**, the methyl ester of acetochloroneuraminic acid **2** was glycosylated with 4-hydroxy benzaldehyde under phase transfer conditions[34] (Scheme 3). The aldehyde functionality within the obtained compound was coupled to the free amine functionality within **13** under reductive amination conditions to afford amine **10**. The terminal Boc-protecting group was then removed by treatment with TFA, to afford the final sialic acid-spacer-amine derivative **11**.

The obtained amino sialic acid intermediates **5**, **6**, **8** and **11** were then coupled in turn to poly (aspartimide) (Scheme 4) according to the procedure described by Thoma et al.[35] The reactions were carried out in DMF at 50 °C in the presence of Et_3N for 5 days and this was followed by treatment with an excess of 3-amino-propanol at rt for 1 day. The acetate and methyl esters within the compounds were then removed by treatment of the glycopolymers with 1 M NaOH and the reaction products were then purified by dialysis against water. After lyophilization, the structures of the glycopolymers **18**–**21** were confirmed by spectroscopic methods.

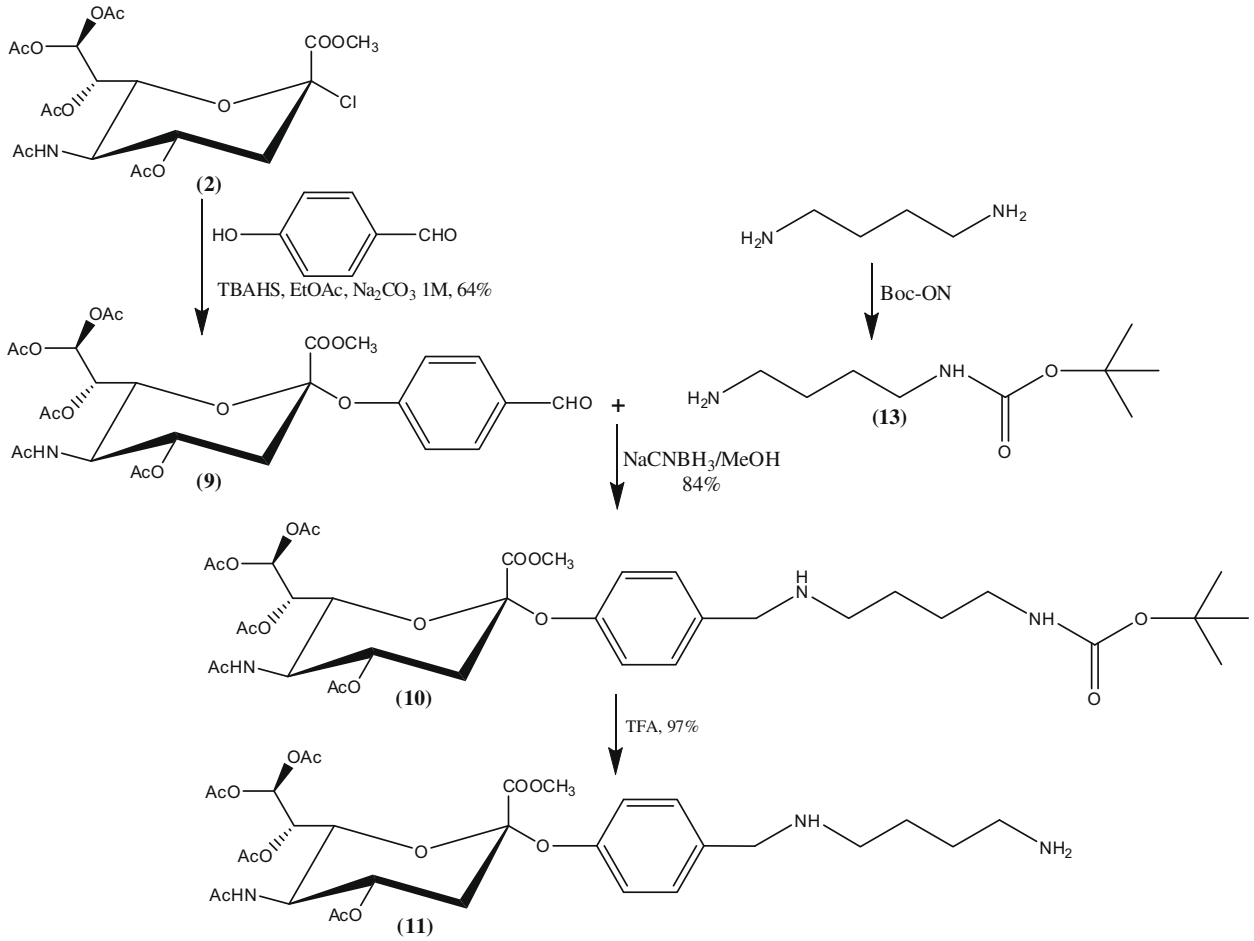
The integration of signals from the ^1H NMR spectra corresponding to the aromatic protons within the sialic acid-linker-amine intermediates (7.42–7.28 ppm) and protons from polymer chain (2.66 ppm) allowed the quantitative analysis of the product composition. The results for this analysis are indicated in Table 1 and these illustrate that incorporation of sialic acid into the poly(aspartimide) backbone was of varying success for each sialic acid-spacer-amine derivative. A satisfactory incorporation was obtained for compounds **8** and **11** that contained an aliphatic spacer within the structure. An insignificant degree of incorporation of the sialic



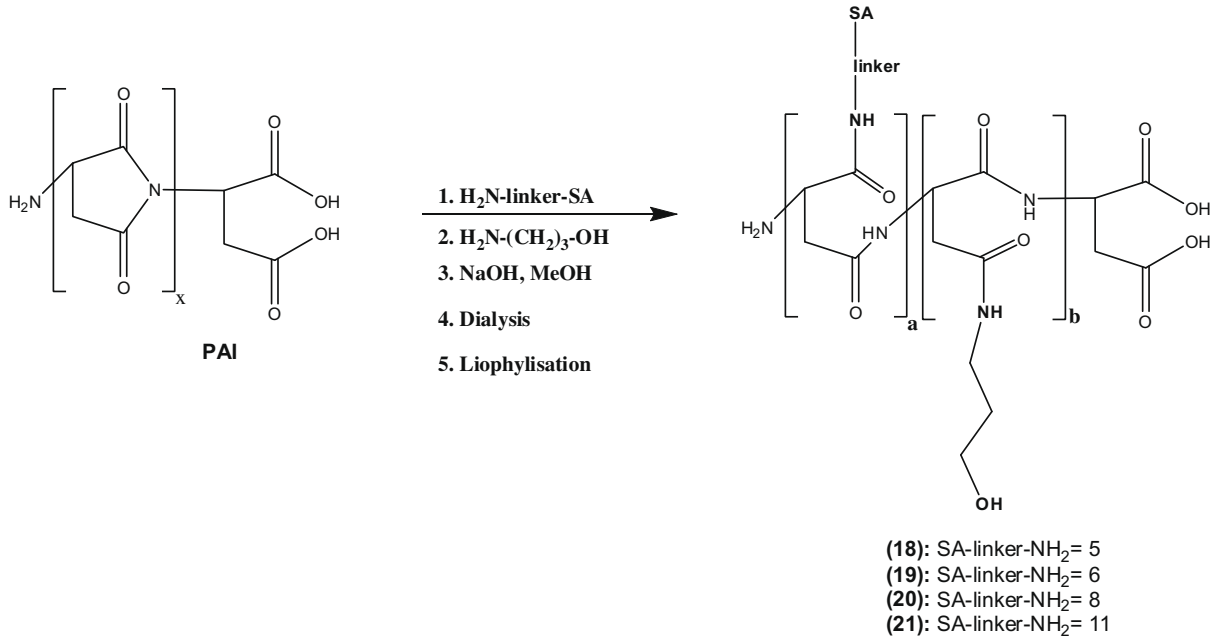
Scheme 1. Synthesis of sialic acid-linker-amine intermediates **5** and **6**.



Scheme 2. Synthesis of sialic acid-linker-amine intermediate **8**.



Scheme 3. Synthesis of sialic acid-linker-amine intermediate **11**.



Scheme 4. Glycopolymer synthesis.

acid derivative into the polymer structure was obtained for amine derivatives **5** and **6**, which correlates to glycopolymers **18** and **19**. The introduction of an aliphatic chain within the linker improves the incorporation, as illustrated for **20** and **21** (Table 1). This may be due to less steric hinderance for these compounds. By increasing the chain flexibility, which is the case for compounds with terminal aliphatic chains, the inclusion of sialic acid increased to 2%.

It is important to mention that all the glycopolymers are water soluble, which makes these compounds suitable for in vivo studies. The presence of poly(aspartimide) also adds other characteristics to polyvalent glycoconjugates such as easy biodegradability and immunological properties.[36,37]

The weight-average molar mass, radius of gyration and the polydispersities of glycopolymers are presented in Table 2. The polydispersity index values (M_W/M_n) indicate relatively narrow molecular weight distributions.

The degrees of polymerization obtained from size exclusion chromatography are consistent with the data obtained for the original polymer from NMR experiments in dimethyl sulfoxide. From these spectra it is possible to determine the number-average degree of polymerization comparing the integral of signals assigned to NH_2 protons (at chain ends) at 8.5 ppm and the single proton of cycle belonging to the polymer chain at 5.3 ppm (see Scheme 4). It is equal to 220. As a conclusion because the degree of polymerization of polymers **20** and **21** determined by SEC is 203 and 210, respectively (Table 2), there is no polymer degradation during the chemical modification.

Table 1
Results of the synthesis of poly(aspartic acid)–sialic acid conjugates[a]

Glycopolymer	Sialic acid-linker-amine intermediates	PAI:SA ratio	Incorporation of SA (mol %)	Yield (%)
18	5	10:1	ND	44
19	6	10:1	ND	35
20	8	10:1	2	55
21	11	10:1	1	55

[a] Conditions: DMF/NEt_3/50 °C/5 days and then excess of 3-aminopropanol for 1 day. Incorporation of ligand units was estimated on the basis of the results of ^{1}H NMR spectra. ND: not determined due to small amount.

3. Conclusions

In this work we have described the synthesis and characterization of two new glycopolymers with potential antiviral properties. In general, glycosylation of the *N*-acetylneuraminic acid has been achieved in good yields, using phase transfer catalyzed reactions. ^{1}H NMR spectroscopic studies have shown that the efficiency of incorporating the sialyl derivatives into the poly(aspartimide) increases with the introduction of an aliphatic chain into the structure. This has resulted in the preparation of water soluble compounds of interest for the inhibition of the influenza virus. The assessment of the properties of these compounds to inhibit influenza virus is currently under investigation in our laboratories.

4. Experimental

4.1. General methods

All reagents, solvents and starting materials were purchased from Aldrich and Fluka and were used without further purification unless otherwise noted. Poly(aspartimide) was prepared according to the procedure described by Neri et al.[38]

Table 2
The calculations of molar mass and radius of gyration were carried out according to the Zimm fit method

Glycopolymer	M_W (g/mol)	M_n (g/mol)	M_W/M_n	R_g (nm)	dn/dc	I_p	DP
20	36,910	20.853	1.77	8.2	0.1420	1.77	203
21	38,020	18.367	2.07	8.2	0.145	2.07	210

Thin-layer chromatography was performed on Silica Gel 60-F254 (Merck) and detection was realized by either of the following methods: charring with a solution of $KMnO_4$ (aq), ninhydrin reagent spray, 5% (v/v) H_2SO_4 in EtOH and subsequent heating or UV detection. Column chromatography was performed on Silica Gel (Merck, Kieselgel 60). Dialysis was performed using dialysis membranes (M_W cutoff of 12–14,000 Da). ^{1}H NMR spectra were recorded at 250 MHz on a Bruker spectrometer at room temperature. Chemical shifts are reported in ppm relative to the solvent: $CHCl_3$ in $CDCl_3$ at 7.24 ppm, CD_2HOD in CD_3OD at 3.30 ppm and HOD in D_2O at 4.8 ppm (internal Me_4Si, $\delta = 0$), or D_2O. For carbon spectra the references are 77.0 ppm for $CDCl_3$ and 49.9 ppm for CD_3OD. Infrared spectra were recorded on a Perkin–Elmer 1720-X series Fourier Transform spectrometer as thin films, Nujol mulls or KBr discs, all absorptions are quoted in cm^{-1}.

Size Exclusion Chromatography (SEC) analysis was conducted using multiple detectors. Waters Alliance 2690 (USA) chromatograph was equipped with two Shodex OHpak columns (SB-804HQ and SB-802.5HQ) and two online detectors: a differential refractometer (Waters 2410) and a Dawn Helios light scattering detector (MALS) from Wyatt (USA) equipped with a K5 cell and 120 mW solid-state laser operating at 658 nm. A 0.1 M solution of $NaNO_3$ was used as the eluent at a flow rate of 0.5 mL/min. The weight-average molar mass, radius of gyration and polymolecularity were obtained from data collected and analyzed using ASTRA SEC-software (version 5.3.4, Wyatt Technology Corp., USA). The calculations of molar mass (Fig. 2) and radius of gyration were carried out according to the Zimm fit method (angles 5–16).

4.2. Methyl 5-acetamido-3,5-dideoxy-D-glycero-β-D-galacto-2-nonulopyranosylonate (1)

To a stirred solution of Neu5Ac (3 g, 9.7 mmol) in dry methanol (100 mL) at room temperature under N_2 was added Amberlite IR-120 (H^+) (1.5 g). After stirring for 48 h, the resin was filtered off and evaporated to a volume of ~5 ml. Ether was added to turbidity and **1** crystallized as a white solid on standing. It was recuperated by filtration (2.82 g, 90%) and used in the subsequent reaction without further purification; mp 191–193 °C, lit.[39] 179–180 °C. ^{1}H NMR and ^{13}C NMR spectral data matched those reported.

4.3. Methyl (5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-D-glycero-β-D-galacto-2-nonulopyranosyl)onate chloride (2)

A suspension of **1** (1.5 g, 4.64 mmol) in freshly distilled acetyl chloride (50 mL) was stirred in a securely stoppered flask at room temperature for 72 h and then concentrated under reduced pressure (azeotroped three times with toluene). Drying under high vacuum afforded **2** (2.29 g, 97%) as a foam and this was used directly in the subsequent reaction without further purification. ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.92 (3H, s, NAc), 2.06, 2.07, 2.09, 2.13 (12H, 4 × s, 4 × OAc), 2.29 (1H, dd, $J_{3a,3e}$ = 13.9 Hz, H-3a), 2.80 (1H, dd, $J_{3e,3a}$ = 13.9 Hz, H-3e), 3.89 (3H, s, CO_2Me), 4.07 (1H, dd, J_9 = 12.5 Hz, H-9), 4.24 (1H, t, J_5 = 10.2 Hz, H-5), 4.36 (1H, dd, J_6 = 10.2 Hz, H-6), 4.43 (1H, dd, $J_{9'}$ = 12.5 Hz, H-9′) 5.18 (1H, ddd, J_8 = 7.2 Hz, H-8), 5.33 (1H, d, J_{NH} = 10.2 Hz, NH), 5.41 (1H, ddd, J_4 = 11.2 Hz, H-4), 5.49 (1H, dd, J_7 = 7.2 Hz, H-7).

4.4. General method of glycosylation of acetochloroneuraminic acid

To a solution of freshly prepared SA_2 in ethyl acetate was added a solution of the phenol compound and TBAHS in Na_2CO_3 (1 M solution). The mixture was stirred vigorously for 3 h at room temperature and next diluted with equal amounts of ethyl acetate and $NaHCO_3$ (saturated solution). The organic phase was separated and washed twice with $NaHCO_3$ (saturated solution), water and brine. The organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using the mixture ethyl acetate/dichloromethane/triethylamine = 1:1:1%.

4.4.1. Methyl (4-nitrophenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-oxy-α-D-glycero-D-galacto-2-nonulopyranosid)onate (3)

By using the above-mentioned procedure, **2** (1 g, 1.96 mmol), EtOAc (30 ml), *p*-nitrophenol (0.545, 3.92 mmol), Na_2CO_3 (1 M, 30 mL), TBAHS (0.798 g, 2.354 mmol) were combined to afford **3** (0.68 g, 56%) as a yellow foam mp 88–90 °C, lit.[22] 87–88 °C. ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.94 (3H, s, NAc), 2.05, 2.06, 2.07, 2.12 (12H, 4s, 4 × OAc), 2.31 (1H, dd, $J_{3a,3e}$ = 12.5 Hz, H-3a), 2.79 (1H,

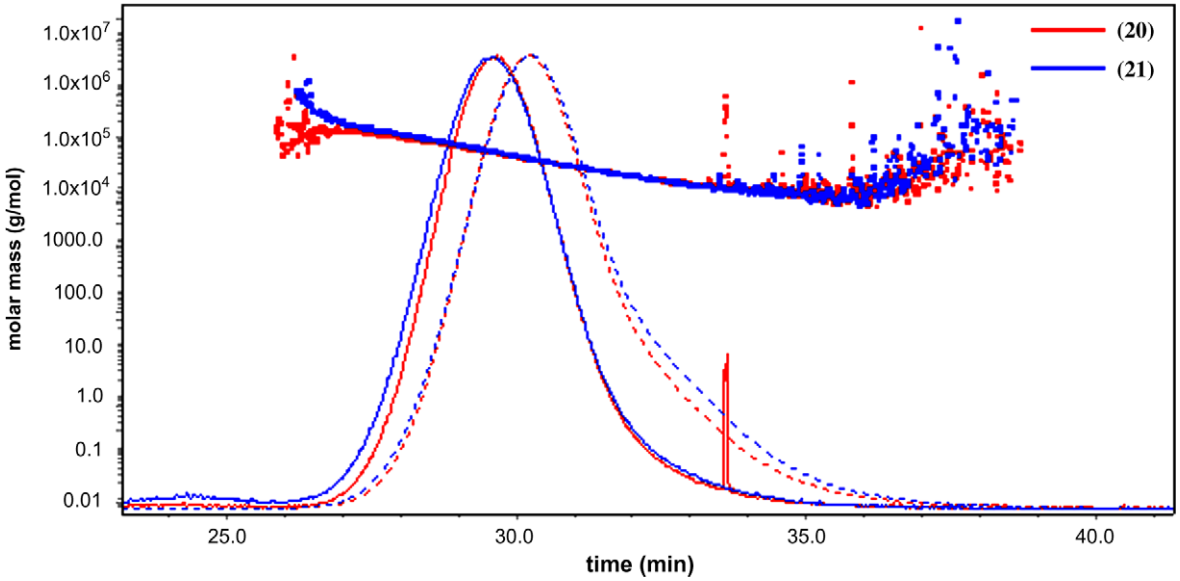


Figure 2. Fractograms of polymers **20** and **21**, concentrations (line) and molar masses (dots).

dd, $J_{3e,3a}$ = 4.7 Hz, H-3e), 3.67 (3H, s, CO_2Me), 4.07–5.37 (8H, m), 7.14 (2H, d, J ($H_{o,m}$) = 9.3 Hz, ArH-*ortho*), 8.21 (2H, d, ArH-*meta*). ^{13}C NMR ($CDCl_3$): 170.94–168.12 (6 × C=O), 159.03, 143.41, 125.70, 118.57, 99.52, 68.35, 68.20, 67.01, 62.14, 60.44 (OMe), 49.32, 38.76, 23.26–20.78 (5 × Ac).

4.4.2. Methyl (4-nitrophenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-D-glycero-α-D-galacto-2-nonulopyranosid)onate (4)

By using the above-mentioned procedure, **2** (0.5 g, 0.98 mmol), EtOAc (15 mL), *p*-nitrobenzenethiol (0.304, 1.96 mmol), 15 mL Na_2CO_3 1 M, TBAHS (0.332 g, 0.98 mmol) (0.41 g, 67%) mp 168–170 °C, lit.[22] 170–171 °C. ^1H NMR (250 MHz, $CDCl_3$) δ 1.89 (3H, s, NAc), 2.06–2.04 (12H, 4s, 4 × OAc), 2.17 (1H, dd, coupling constants as for **3**, H-3a), 2.84 (1H, dd, H-3e), 3.61 (3H, s, CO_2Me), 3.98–5.51 (8H, m), 7.64 (2H, d, ArH), 8.21 (2H, d, ArH). ^{13}C NMR ($CDCl_3$): 171.23–167.78 (6 × C=O), 148.59, 138.22, 135.47, 124.23, 87.35, 74.92, 69.23, 68.89, 67.36, 62.15, 60.43, 53.10 (OMe), 49.17, 38.34, 23.16–20.82 (5 × Ac).

4.4.3. Methyl (4-formylphenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyt-3,5-dideoxy-D-glycero-α-D-galacto-2 nonulopyranosid)onate (9)

By using the above-mentioned procedure, **2** (0.5 g, 0.98 mmol), EtOAc (15 mL), *p*-hydroxybenzaldehyde (0.24, 1.96 mmol), 15 ml Na_2CO_3 1 M, TBAHS (0.399 g, 1.17 mmol) (0.37 g, 64%) mp 88–90 °C, lit.[34] 90.4–91.6 °C. ^1H NMR (250 MHz, $CDCl_3$) δ 1.93 (3H, s, NAc), 2.05–2.20 (12H, 4s, 4 × OAc), 2. 31 (1H, dd, $J_{3a,e}$ = 13.1 Hz, H-3a), 2.70 (1H, dd, $J_{3e,a}$ = 13.1 Hz, H-3e), 3.65 (3H, s, CO_2Me), 4.08 (1H, dd, H-9′), 4.11 (1H, ddd, H-5), 4.17 (1H, ddd, J_9 = 10.3 Hz, H-9), 4.58 (1H, dd, J_6 = 10.8 Hz, H-6), 4.92 (1H, ddd, J_4 = 12.2 Hz, H-4), 5.27 (1H, d, J_{NH} = 10.0 Hz, NH), 5.38 (1H, m, J_7 = 1.4 Hz, H-7), 7.15 (2H, d, J ($H_{o,m}$) = 8.64 Hz, ArH-*ortho*), 7.82 (2H, d, ArH-*meta*), 9.93 (1H, –CHO). ^{13}C NMR ($CDCl_3$): 191.48 (CHO), 171.36–168.69 (6 × C=O), 159.41, 132.34, 132.13, 119.22, 99.89, 74.03, 69.05, 68.77, 67.49, 62.43, 53.64 (OMe), 49.75, 39.07, 23.65–20.17 (5 × Ac).

4.5. General method of reduction of nitro compounds: 4-nitrophenyl derivatives

Compounds **3** and **4** were suspended in absolute ethanol to which was added tin(II) chloride dihydrate. The reaction mixture was stirred at 70 °C for 2 h and then cooled and poured onto ice-water and the final pH was adjusted to 8 with $NaHCO_3$. The resulting mixture was filtered and the clear filtrate extracted with EtOAc. The organic layers were combined, washed successively with saturated $NaHCO_3$, H_2O and saturated NaCl, dried over $MgSO_4$ and concentrated under reduced pressure. The compounds **5** and **6** were obtained as off yellow foams and were used directly in subsequent reactions without further purification.

4.5.1. Methyl (4-aminophenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-oxy-α-D-glycero-D-galacto-2-nonulopyranosid)-onate (5)

Compound **3** (0.66 g, 1.077 mmol), $SnCl_2 \times 2H_2O$ (1.526 g, 6.767 mmol), ethanol (40 ml), (0.52 g, 83%). ^1H NMR (250 MHz, $CDCl_3$) δ 1.93 (3H, s, NAc), 2.05–2.12 (12H, 4s, 4 × OAc), 2.31 (1H, dd, $J_{3e,a}$ = 12.9 Hz, H-3a), 2.7 (1H, dd, $J_{3e,a}$ = 5.1 Hz, H-3e), 3.66 (3H, s, CO_2Me), 4.07–5.36 (8H, m), 6.62 (2H, d, J ($H_{o,m}$) = 8.48 Hz, ArH), 6.89 (2H, d, ArH).

4.5.2. Methyl (4-aminophenyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-α-D-glycero-D-galacto-2-nonulopyranosid)onate (6)

Compound **4** (0.4 g, 0.637 mmol), $SnCl_2 \times 2H_2O$ (0.9 g, 4 mmol), ethanol (30 ml), (0.32 g, 84%). ^1H NMR (250 MHz, $CDCl_3$) δ 1.86 (3H, s, NAc), 2.02–2.10 (12H, 4s, 4 × OAc), 2.14 (1H, m, H-3a), 2.72 (1H, dd, H-3e), 3.62 (3H, s, CO_2Me), 3.81–5.32 (8H, m), 6.59 (2H, d, coupling constant as for **5**, ArH), 7.25 (2H, d, ArH).

4.6. Boc-5-aminopentanoic acid (12)

To 5-aminopentanoic acid (0.5 g, 4.268 mmol) dissolved in water (12 mL) was added $NaHCO_3$ (0.717 g, 8.54 mmol). The mixture was stirred at 5 °C and added to Boc_2O (1.397 g, 6.402 mmol) in previously cooled dioxane (5 ml). The mixture was stirred at 0 °C for 1 h and then allowed to heat to room temperature overnight. Water (10 ml) was added. The aqueous layers were extracted twice with ethyl acetate. The organic layers were extracted twice with $NaHCO_3$ (saturated solution). The combined aqueous layers were acidified to a pH 1 with 10% HCl. The aqueous layers were extracted again three times with ethyl acetate, dried over $MgSO_4$ and concentrated under reduced pressure. The compound (0.85 g, 91.7%) was obtained as a white powder and used directly in subsequent reactions without further purification, mp 45–48 °C. ^1H NMR (250 MHz, $CDCl_3$) δ 1.45 (9H, s), 1.54 (4H, m), 2.32 (2H, t), 3.06 (2H, t), 3.32 (1H, s). ^{13}C NMR ($CDCl_3$) 158.56, 117.36, 79.85, 40.93, 34.53, 30.42, 28.79, 23.26.

4.7. *N*-Boc-1,4-diaminobutane (13)

1,4-Diaminobutane (0.25 g, 2.836 mmol) dissolved in 12 N HCl (0.3 ml) was added to a solution of dioxane and water in equal proportion (5 ml) and cooled down to 0 °C. To this mixture Boc-ON (0.23 g, 0.945 mmol) was added and allowed to warm at room temperature. The solution was suspended in H_2O (2 ml), acidified with 6 N HCl to pH 4 and washed with EtOAc (3 × 4 ml). The remaining aqueous phase was adjusted to pH 11 with 10 N NaOH. The organic phase was extracted with EtOAc, dried over $MgSO_4$ and concentrated under reduced pressure to give a pale yellow oil (0.13 g, 23%). ^1H NMR (250 MHz, $CDCl_3$) δ 1.44 (9H, s), 1.52 (4H, m), 1.97 (2H, s), 2.72 (2H, m), 3.11 (2H, m), 4.29 (1H, s). ^{13}C NMR ($CDCl_3$) 156.43, 77.40, 41.65, 40.27, 28.34, 27.38.

4.8. Methyl(*tert*-butyl-5-(4-aminophenyl-5-oxopentylcarbamate)-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-α-D-glycero-D-galacto-2-nonulopyranosid)onate (7)

Compound **6** (0.31 g, 0.518 mmol), **12** (0.113 g, 0.518 mmol), DCC (0.118 g, 0.57 mmol) and catalytic amount of DMAP in anhydrous CH_2Cl_2 were stirred at room temperature overnight. The dicyclohexylurea was filtered off and the solution was concentrated. The foam residue was purified by silica gel column chromatography using the mixture ethyl acetate: triethylamine in a ratio 1:1%. The compound **7** was obtained as a pale yellow foam (0.26 g, 62%). ^1H NMR (250 MHz, $CDCl_3$) δ 1.44 (9H, s), 1.85 (3H, s, NAc), 2.07–2.00 (12H, 4s, 4 × OAc), 2.41 (2H, t), 2.56 (1H, s), 2.80 (1H, dd, J_{3e} = 17.27 Hz, H-3e), 3.17 (2H, m), 3.58 (3H, s, CO_2Me), 3.93–5.31 (8H, m), 5.83 (1H, m), 7.44 (2H, d, J ($H_{o,m}$) = 8.47 Hz, ArH-*ortho*), 7.56 (2H, d, ArH-*meta*), 8.44 (1H, s). ^{13}C NMR ($CDCl_3$): 172.13–168.34 (8 × C=O), 156.76, 149.05, 140.62, 138.69, 137.69, 123.09, 119.56, 114.93, 88.12, 75.08, 70.46, 68.13, 62.40, 60.83, 53.15 (OMe), 49.39, 38.36, 28.80, 23.47–21.22 (5 × Ac).

4.9. Methyl (5-amino-*N*-(4-phenyl)pentanamide-5-acetamido-4,7,8,9-tetra-*O*-acetyl-3,5-dideoxy-2-thio-α-D-*glycero*-D-galacto-2-nonulopyranosid)onate (8)

Compound **7** (0.33 g, 0.413 mmol) and TFA (6.20 g, 54.38 mmol) were stirred in anhydrous CH_2Cl_2 (8 ml) at room temperature for 1.5 h then concentrated under reduced pressure. Drying under high vacuum afforded **8** (0.285 g, 99%) as a foam mass and was

used in the subsequent reaction without further purification. ^{1}H NMR (250 MHz, MeOD) δ 1.76 (4H, m), 1.84 (3H, s, NAc), 2.12–1.98 (12H, 4s, 4 × OAc), 2.13 (1H, m), 2.48 (2H, m), 2.86 (1H, dd, H-3e), 2.99 (2H, m), 3.60 (3H, s, CO_2Me), 3.83–4.80 (8H, m), 7.47 (2H, d, J ($H_{o,m}$) = 8.49 Hz, ArH-*ortho*), 7.61 (2H, d, ArH-*meta*). ^{13}C NMR ($CDCl_3$) 173.95–169.38 (7 × C═O), 141.85, 136.47, 124.39, 121.09, 88.83, 75.37, 71.25, 70.76, 68.97, 63.11, 53.36 (OMe), 40.44, 39.18, 37.01, 28.03, 23.28–20.87 (5 × Ac).

4.10. Methyl (*tert*-butyl-4-benzylamino)butylcarbamate-5-acetamido-4,7,8,9-tetra-*O*-acetyt-3,5-dideoxy-2-oxy-α-D-glycero-D-galacto-2 nonulopyranosid)onate (10)

To **9** (0.37 g, 0.62 mmol) dissolved in methanol (20 ml), **13** (0.28 g, 1.49 mmol) was added. After 1 h of stirring at room temperature $NaCNBH_3$ (0.187 g, 2.976 mmol) and AcOH (0.178 g, 2.976 mmol) were added. After 24 h of stirring at room temperature, the reaction mixture was diluted with EtOAc (30 ml) and Na_2CO_3 saturated solution (30 ml). After the filtration of salts, the organic phase was separated, washed with Na_2CO_3 saturated solution (30 ml), dried over $MgSO_4$ and concentrated under reduced pressure. The compound **10** (0.4 g, 84%) was used directly in subsequent reaction without further purification. ^{1}H NMR (250 MHz, MeOD) δ 1.38 (s, 9H), 1.45 (4H, m), 1.82 (3H, s, NAc), 1.95–2.08 (12H, 4s, 4 × OAc), 2.56 (2H, m), 2.64 (1H, s), 2.71 (1H, dd, J_{3e} = 13.34 Hz, H-3e), 2.99 (2H, t), 3.59 (2H, s), 3.67 (3H, s, CO_2Me), 3.93–4.83 (8H, m), 5.32 (2H, m), 7.23 (2H, d, J ($H_{o,m}$) = 8.63 Hz, ArH-*ortho*), 6.98 (2H, d, ArH-*meta*). ^{13}C NMR (MeOD): 176.81–172.38 (7 × C═O), 161.43, 157.76, 138.54, 133.74, 126.28, 123.96, 104.39, 82.81, 77.30, 72.90, 66.20, 56.44, 52.76 (OMe), 50.09, 44.10, 31.87, 24.19–23.66 (5 × Ac).

4.11. Methyl (*N*-(4-benzyl)butane-1,4-diamine-5-acetamido-4,7,8,9-tetra-*O*-acetyt-3,5-dideoxy-2-oxy-α-D-glycero-D-galacto-2 nonulopyranosid)onate (11)

Compound **10** (0.4 g, 0.52 mmol) and TFA (6.14 g, 53.85 mmol) were stirred in anhydrous CH_2Cl_2 (8 ml) at room temperature for 1.5 h then concentrated under reduced pressure. Drying under high vacuum afforded **11** (0.34 g, 97%) which was used in the subsequent reaction without further purification. ^{1}H NMR (250 MHz, MeOD) δ 1.78–1.81 (4H, m), 1.90 (3H, s, NAc), 1.99–2.12 (12H, 4s, 4 × OAc), 2.82 (1H, dd, H-3e), 3.00 (2H, m), 3.36 (2H, m), 3.64 (3H, s, CO_2Me), 3.77–4.55 (7H, m), 4.92 (1H, ddd), 5.37 (2H, m), 7.12 (2H, d, J ($H_{o,m}$) = 8.64 Hz, ArH-*ortho*), 7.44 (2H, d, ArH-*meta*). ^{13}C NMR (MeOD) 174.01–169.35 (6 × C═O), 162.23, 161.63, 156.27, 132.46, 121.66, 101.34, 74.01, 70.28, 69.59, 68.55, 63.19, 53.58 (OMe), 40.19, 39.43, 30.66, 29.01, 24.16–20.82 (5 × Ac).

4.12. General method for the binding of ligands to poly(aspartimide)

The SA-linker-amine intermediates (5 ÷ 11), poly(aspartimide) and distilled NEt_3 (1:10:1.5 ratio) were stirred in absolute DMF at 50 °C under argon. After stirring at 50 °C for 5 days, the solution was cooled down to ambient temperature and an excess of 3-amino-propanol was added. After 24 h the clear, colourless solution was added dropwise to 200 ml of ether/ethanol (1:1). The precipitate was filtered off and washed with ethanol and ether. The crude products were dissolved in methanol to which 1 N NaOH solution was added. After stirring for 2 h at room temperature the solution was further purified by ultrafiltration (Dialysis Tubing—'Visking') (four times with distilled water on each occasion). Following lyophilization, polymers (18 ÷ 21) were isolated as white powders.

4.12.1. Polymer (18)

Compound **5** (0.22 g, 0.37 mmol), (PAI) (0.36 g, 3.71 mmol), NEt_3 (0.06 g, 0.56 mmol), DMF (4 ml), 3-amino-propanol (0.836 g, 11.14 mmol), methanol (10 ml), NaOH (2 ml), (0.21 g, 44%).

4.12.2. Polymer (19)

Compound **6** (0.36 g, 0.60 mmol), (PAI) (0.58 g, 5.98 mmol), NEt_3 (0.09 g, 0.9 mmol), DMF (10 ml), 3-amino-propanol (1.35 g, 17.98 mmol), methanol (15 ml), NaOH (3 ml), (0.26 g, 35%).

4.12.3. Polymer (20)

Compound **8** (0.37 g, 0.53 mmol), (PAI) (0.51 g, 5.29 mmol), NEt_3 (0.08 g, 0.79 mmol), DMF (7 ml), 3-amino-propanol (1.19 g, 15.95 mmol), methanol (16 ml), NaOH (3 ml), (0.43 g, 55%).

4.12.4. Polymer (21)

Compound **11** (0.54 g, 0.81 mmol), (PAI) (0.79 g, 8.09 mmol), NEt_3 (0.123 g, 1.216 mmol), DMF (16 ml), 3-amino-propanol (1.83 g, 24.36 mmol), methanol (21 ml), NaOH (4 ml), (0.56 g, 55%).

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