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Chemo-enzymatic synthesis of glycopolymers and sequential glycopeptides bearing lactosamine and sialyl Lewis^x unit pendant chains

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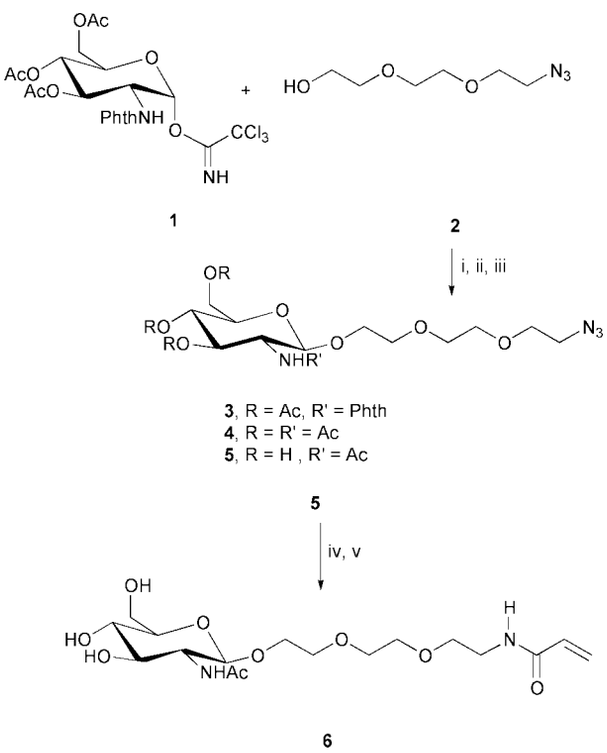
A variety of glycoconjugates bearing either *N*-acetyllactosamine or sialyl Lewis^x units have been synthesised in a chemo-enzymatic way. This includes the synthesis of glycopolymer copolymerised with acrylamide and whose glucosamine unit was substituted with different kinds of side-chain. Glycopeptides with different spacer-arm glucosamine units have also been prepared and polymerised. The sugar chain was then elongated using glycosyl transferases to afford the novel sequential glycopeptides. In these cases, the polymeric sugar cluster effect led to enzymatic glycosylation with high efficiency. Nevertheless, some differences have been noticed depending on the reaction conditions used for each substrate.

Introduction

In recent years, it has become popular to use enzymes for the construction of complex oligosaccharides[1] and glycopolymers.[2,3] Indeed, more and more enzymes such as glycosyl transferases and sugar nucleotide donors are now commercially available. Although this method offers several obvious advantages over the usual chemical one (such as stereo- and regioselectivity, specificity and shorter reaction pathways), the use of enzymes can become uncertain in some cases due to their specificity towards their natural substrate or their sensitivity to the reaction conditions. Furthermore, several studies have already shown that the sugar nucleotide donors can be modified to some extent without losing their donor properties for the enzyme,[1] but it is still of interest to get information about substrate specificity when working with an enzyme and chemically synthesised molecules as potential artificial acceptors. On the other hand, the chemistry of glycoconjugates and glycopolymers is now being developed much further.[4] Due to the polymeric glycoside cluster effect,[5] enzymes can be very useful in this area as they allow a high rate of glycosylation. As a part of our present project concerning the chemo-enzymatic synthesis of glycoconjugates as tools for glycotechnology[6] it is of the utmost importance to develop well-defined and easily handled protocols leading to a high rate of glycosylation in high yield. In this field, we wish to report, the synthesis of a variety of new glycoconjugate bearing LacNAc, sialyl α-(2,6)-LacNAc and sialyl Lewis^x pendant chains. While the glycopolymers and sequential glycopeptides[7] were prepared chemically, the subsequent elongation of the sugar chain was carried out enzymatically with good to high efficiency.



Scheme 1 *Reagents and conditions*: i, $BF_3{\cdot}Et_2O$, 4 Å molecular sieves, CH_2Cl_2, 0 °C→rt, 24 h; ii, ethylenediamine, *n*-butanol, 70 °C, 24 h then pyridine–Ac_2O, rt, 24 h; iii, MeONa, MeOH, rt, 2 h; iv, H_2, Pd–C, MeOH, rt, 4 h; v, CH_2=CHCOCl, triethylamine, MeOH, 0 °C, 3 h.

Results and discussion

The synthetic strategy involved at first the preparation of glucosamine pyranoside intermediates **4**, **7** and **8**. These were prepared using well-known glycosidation reactions either from *N*-phthalimidoglucosaminidylimidate[8] and azido(ethoxy)-ethanol **2**[9] in the presence of boron trifluoride–diethyl ether (Scheme 1) or from 2-methyl-4,5-dihydro-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyranoso)[2,1-*d*]-1,3-oxazole[10] and aminoalkyl alcohol in the presence of camphor-10-sulfonic acid as a promoter (Scheme 2). Subsequent deacetylation using Zemplen conditions and reduction of the azido group for **5** or Z-deprotection for **9** and **10** with hydrogen and palladium–carbon afforded the free amino intermediates which were both used for the synthesis of glycopolymers and sequential glycopeptides.

Synthesis of glycopolymers

Two kinds of glycopolymers were prepared: acrylamide copolymers **12** and **13** and homopolymer **14**. The synthesis of **12** and **14** involved first the reduction of the azido group of **5**

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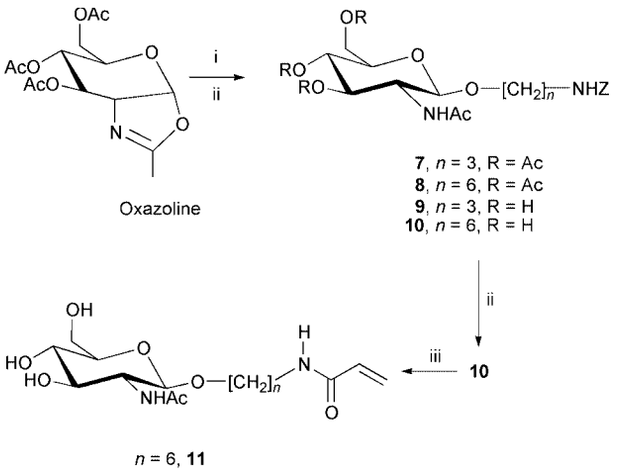
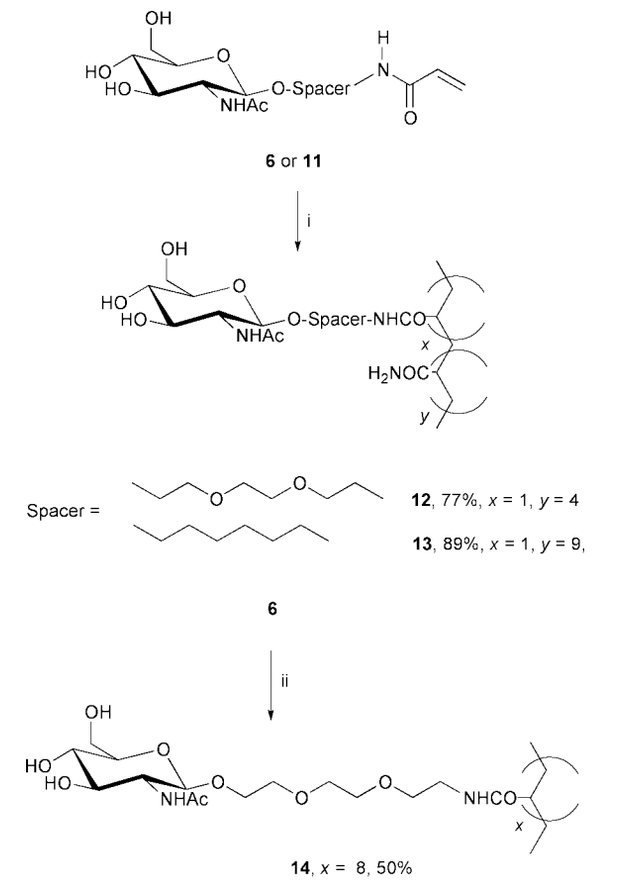


Scheme 2 *Reagents and conditions*: i, HO-$[CH_2]_n$-NHZ, CSA, $(CH_2)_2Cl_2$, 70 °C, 3 h; ii, MeONa, MeOH, 2 h, rt; iii, H_2, Pd–C, MeOH–$AcOH_{cat}$, rt, 3 h then CH_2=CHCOCl, triethylamine, MeOH, 0 °C, 3 h.

to get the free amino derivative. Then, *N*-acryloylation of this derivative was performed using acryloyl chloride and triethylamine in methanol at 0 °C[11] to afford the polymerisable monomer **6** in 68% yield (Scheme 1). Subsequent radical homopolymerisation with *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) and ammonium persulfate (APS) as promoters in deaerated water for 24 h at room temperature proceeded smoothly to give homopolymer **14** in 50% yield (Scheme 3). The average number of repeating units in **14** was evaluated to be 8 using MALDI-TOF spectroscopy.

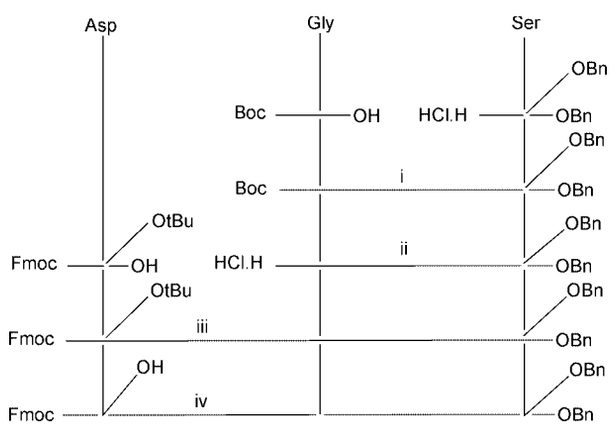


Scheme 3 *Reagents and conditions*: i, acrylamide, TEMED, APS, DMSO–H_2O (5:1), 24 h, rt; ii, TEMED, APS, H_2O, 24 h, rt.

As it was demonstrated that the efficiency of sugar elongation reactions with glycosyl transferases is strongly dependent on the sugar density and its distribution on the primer,[11] and as it was suggested that the length and flexibility of the spacer-arm were also important parameters for these reactions, two kinds of acrylamide copolymers were synthesised with different spacer-arms and different sugar densities. Thus, radical copolymerisation of monomer **6** was carried out in a mixture of deaerated DMSO–water with 4 equivalents of acrylamide, in the presence of TEMED and APS as initiators, and led to acrylamide glycopolymer **12** bearing an ethylene glycol based spacer moiety in 77% yield. The average molecular weight of **12** was estimated to be 135000 using the gel permeation chromatography (GPC) method. The ratio of glycomonomer to acrylamide in **12** was estimated to be 1:4 from the integration of ^{1}H NMR data. The synthesis of copolymer **13** started with the Z-*N*-protected *N*-acetylglucosamine derivative **10**. Removal of the Z group followed by *N*-acryloylation under the conditions described for **6** provided the polymerisable *N*-acryloylated *N*-acetylglucosamine derivative **11** in 41% yield (Scheme 2). Derivative **11** was then copolymerised using 9 equivalents of acrylamide under the conditions described above for **12** (Scheme 3). Acrylamide glycopolymer **13** was thus obtained in 89% yield. Its molecular weight was estimated to be over 380000 using the GPC method according to the previous report.[5] These values were the upper limit of our GPC method and thus it was not possible to get a calibration curve for higher molecular weight polymers. The ratio of glycomonomer to acrylamide was estimated to be 1:9 from the integration of ^{1}H NMR data. These polymers were large enough to allow a positive cluster effect for further enzymatic reactions.

Synthesis of sequential glycopeptides

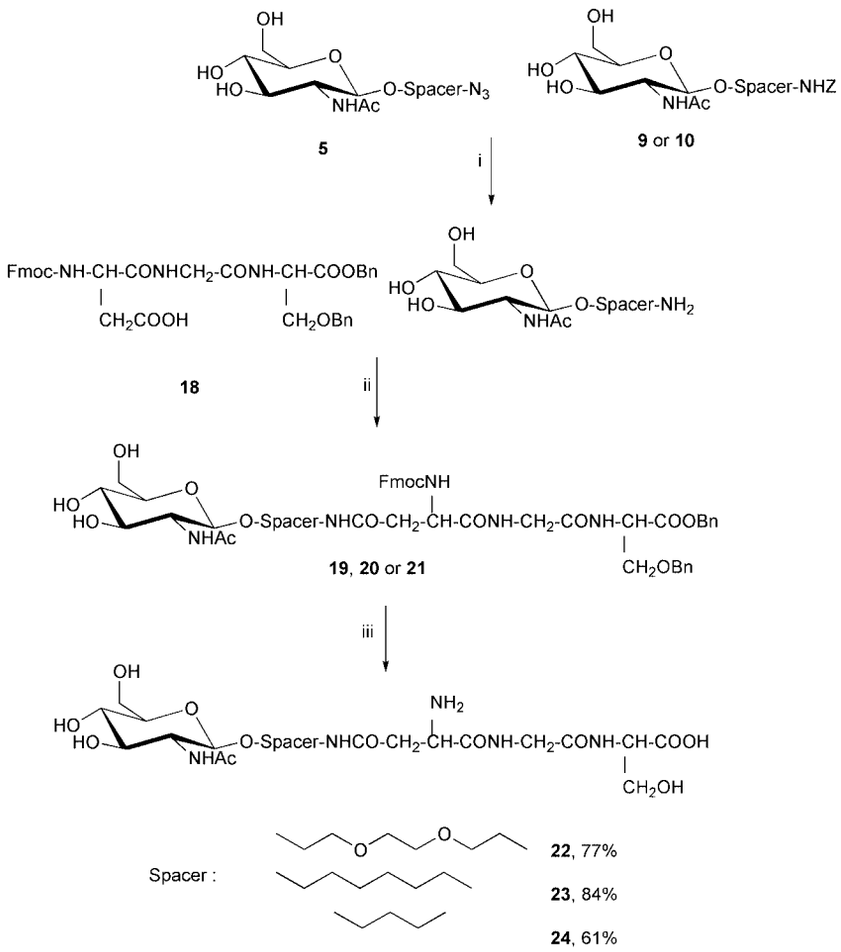
In order to evaluate the influence of the nature of the support towards the enzymatic glycosylation reactions, the protected tripeptidic sequence Fmoc-Asp-Gly-Ser$(OBn)_2$ was prepared. This sequence was chosen because it is the primary recognition motif required for the biosynthesis of *N*-linked glycoproteins process to be initiated. The synthesis of this tripeptide is shown in Scheme 4. It was obtained in 5 steps from a Boc and benzyl



Scheme 4 *Reagents and conditions*: i, DPPA, Et_3N, 0 °C→rt, 15 h (83%); ii, HCl 4 M–dioxane, 0 °C, 3 h; iii, DPPA–Et_3N, 0 °C→rt, 15 h (59%); iv, TFA, rt, 2 h (90%).

protected serine derivative **15**. The coupling reactions between the partially protected amino acid residues were achieved using diphenylphosphoryl azide (DPPA) as a convenient activator of the carboxy group. Finally, the *tert*-butyl deprotection of the β-carboxy function of the aspartic residue with TFA at room temperature led to the partially protected tripeptidic intermediate **18** required for the synthesis of glycopeptides. In this synthesis, two different kinds of protective groups were employed, first Boc and then the base-labile Fmoc because of the well-known sensitivity of the glycosidic bond to acidic conditions.

For the coupling reaction between the monosaccharidic and the peptidic part, the EEDQ (ethyl 2-ethoxy-1,2-dihydroquinoline-1-carboxylate) method was employed (Scheme 5). EEDQ is



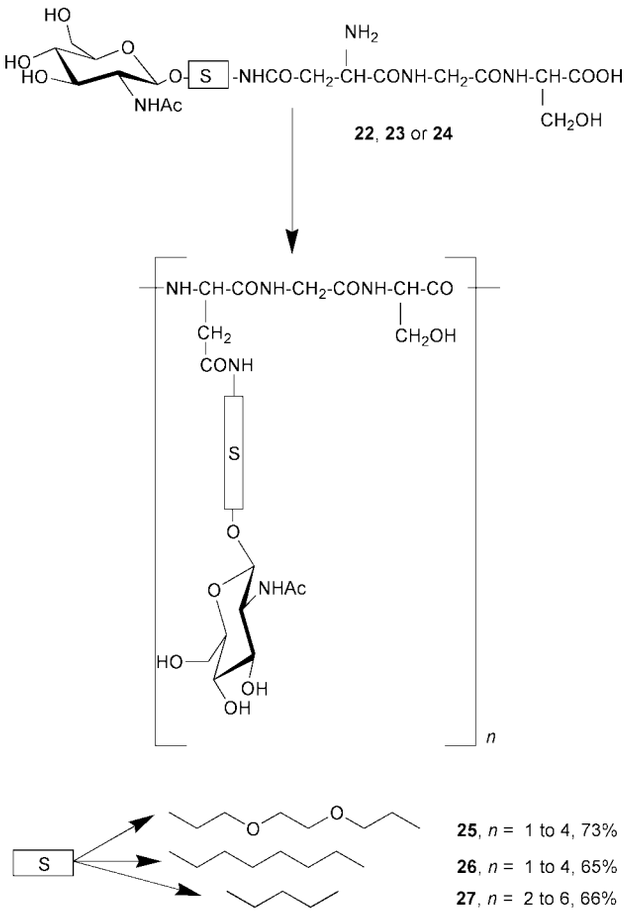
Scheme 5 *Reagents and conditions*: i, H_2, Pd–C, MeOH, rt, 4 h (for **5**) or H_2, Pd–C, MeOH–$AcOH_{cat}$, rt, 3 h (for **9** and **10**); ii, EEDQ, ethanol–benzene (1 : 1), rt, 24 h; iii, piperidine, DMF, 2 h, rt then H_2, Pd–C, AcOH–H_2O (8 : 2), rt, 24 h.

a selective activator of the carboxy groups of peptides. This reaction proceeded smoothly in a mixture of ethanol–benzene at room temperature and led to the corresponding glycopeptide after purification by simple silica gel chromatography. Thus, protected glycopeptides **19**, **20** and **21** were obtained in 77, 59 and 52% yields respectively. Finally, **19**, **20** and **21** were fully deprotected first with piperidine for removal of the Fmoc group, and then with hydrogen and palladium–carbon in a mixture of acetic acid–water (8 : 2) to remove the benzyl protecting groups. This afforded the fully deprotected glycopeptide "primer" **22** (with a propyl chain), **23** (with a hexyl chain), and **24** (with a hydrophilic chain) in 61, 84 and 77% yields respectively (Scheme 5) after simple purification by gel filtration chromatography (G-10) using deionized water as eluent. The polymerisation of these compounds was carried out in DMSO,[7] at room temperature in the presence of a slight excess of DPPA and triethylamine under a nitrogen atmosphere. The reaction usually lasted 4 days (Scheme 6). An increase in the reaction time did not lead to longer glycopeptides. The sequential glycopeptides **25**, **26** and **27** were easily purified by gel filtration chromatography (G-25) with deionized water as eluent and were thus obtained in 73, 65 and 66% isolated yield. The average number of repeating units in each compound was evaluated using MALDI-TOF spectroscopy and was found to be from 2 to 4 for **25** (*M* 1222–2444) and **26** (*M* 1158–2316) and from 2 to 5 for **27** (*M* 1076–2687).

The length of the sequential glycopeptides in our case might be due to the structure of the peptidic sequence itself. Although it was shown that long sequential glycopeptides could be obtained with a hydrophobic sequence,[7] in our case, the sequence was very hydrophilic. This fact could be a factor preventing us from getting longer sequential glycopeptides.

Enzymatic modifications of glycopolymers

The use of enzymes in organic synthesis and for sugar elongation in the construction of complex oligosaccharidic sequences is already well-documented.[1] Moreover, it has been demonstrated that enzymatic reaction with glycosyl transferases can occur with high yield using synthetic water-soluble polymer supports.[11,12] This is due to the polymeric sugar cluster effect which promotes the successful binding of the sugar residues with enzymes. Furthermore, the extent of galactosylation depends strongly on the sugar density along the polymer and on the length and flexibility of the spacer arm between the monosaccharidic unit and the polymeric backbone. Consequently we chose to perform enzymatic galactosylation reactions with homopolymer **14** having a hydrophilic spacer, with acrylamide copolymer **12** having the same side chain and with acrylamide copolymer **13** having a hexylamido side chain as substrates for the galactosyl transferase. Thus, first homopolymer **14** was used as substrate and the enzymatic galactosylation reaction was carried out as previously described[11] using only a slight excess of uridine-5′-diphosphogalactose (UDP-Gal, 1.3–1.5 equiv.) and galactosyl transferase from bovine milk in HEPES (*N*-(2-hydroxyethyl)piperazine-*N*-ethanesulfonic acid) buffer at pH 6. As no galactosylation occurred, the same reaction was attempted using as an acceptor the acrylamide copolymer **12** whose sugar density is lower. In that latter case as well, no galactosylation occured. Finally the amount of UDP-Gal was increased to 3 equivalents (Scheme 7) and thus galactosylated acrylamide copolymer **28** and homopolymer **30** were obtained in 77 and 50% yield respectively. The extent of galactosylation was estimated from the integration of ^1H NMR data to be 50% in both cases.



Scheme 6 *Reagents and conditions*: i, DPPA, Et_3N, DMSO, rt, 4 d.

To confirm the influence of the side chain on the efficiency of enzymatic galactosylation, the acrylamide copolymer **13** with a hexylamido side chain as the hydrophobic linker was then used as the substrate for enzymatic galactosylation. The reaction was conducted as described above for **28** using only a slight excess of UDP-Gal (1.3 equiv.). In that case, a fully galactosylated copolymer **29** was readily obtained as proven by ^{1}H NMR spectra. Indeed, the peak at 3.28 ppm corresponding to H-4 of the *N*-acetylglucosamine unit disappeared completely, while a peak corresponding to the anomeric proton of the galactose unit appeared at 4.35 ppm. Furthermore, the integration corresponding to H-1 and H-1′ showed two protons confirming the full substitution of the glucosamine units in the polymer. From these results, it can be assumed that, in this case, besides the sugar density, the structure and the flexibility of the linker between the *N*-acetylglucosamine unit and its polymeric anchor are the most important parameters for enzyme efficiency. Although the hydrophilic spacer-arm allowed more flexibility, the presence of heteroatoms, more so than the sugar density, seemed to be a limiting factor for these molecules to be good substrates for galactosyl transferase. Indeed, the extent of galactosylation is the same for the homo- and copolymer despite the presence of an excess of galactosyl donor. As the reaction is carried out in aqueous media, the possibility of a network of ionic bonds leading to cyclisation of the side chain and also the possibility of weak interactions between the side chain and the enzyme (preventing it from interacting properly with its substrate) have to be taken into consideration.

In the next step, galactosylated copolymer **29** was sialylated using α-(2,3)-sialyl transferase from rat liver. As the conditions described by Yamada *et al.*[11a] (sodium cacodylate buffer, pH 7.4, $MnCl_2$, Triton CF-54) for the sialylation of glycopolymers failed to provide any sialylated compound in our cases, a new set of conditions was employed. These are new, simple and efficient conditions to get polymeric sialylated compounds in good yields. The reaction proceeded smoothly in sodium cacodylate buffer in the presence of Triton X-100 at pH 6. Three equivalents of CMP-NANA were used together with α-(2,3)-sialyl transferase at 37 °C for 5 days. These very simple conditions afforded the fully sialylated polymer **31** in 75% yield. The sialylation rate was estimated to be quantitative from the integration of ^{1}H NMR data.

Finally, derivative **31** was fucosylated using α-(1,3) fucosyl transferase V and GDP-fucose in HEPES buffer[11] to give the expected glycopolymer **32** bearing the multiple sialyl Le^x antenna (Scheme 8). In this latter case, the extent of fucosylation was estimated to be 50% from NMR data and the isolated yield was only 21%.

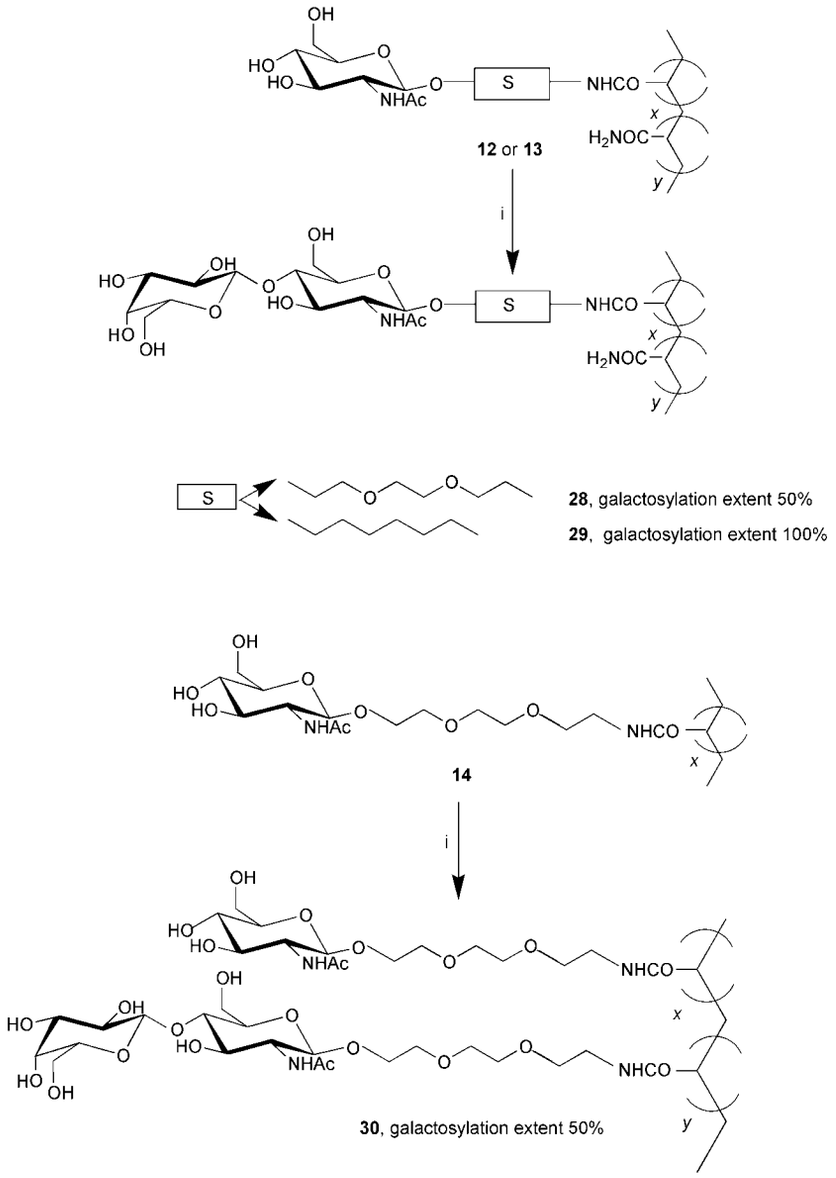
Enzymatic modifications of sequential glycopeptides

As it cannot be denied that the nature of the polymeric support is of no importance in considering the substrate specificity of an enzyme, subsequent galactosylation reactions were carried out with the sequential glycopeptides **25**, **26** and **27** (Scheme 9). A similar chemo-enzymatic approach has already been used by Baisch and Öhrlein to get sialyl Lewis^x glycopeptides.[13] Thus, galactosylation of **27** was first achieved using 3 equivalents of UDP-galactose and β-(1,4)-galactosyl transferase in HEPES buffer (50 mM, pH 7) containing manganese chloride (Method I). The solution was incubated at 37 °C for 24 hours. The galactosylated derivative **33** was then easily purified by Sephadex G-25 gel chromatography using deionized water as eluent. From the ^{1}H NMR data, the galactosylation rate was estimated to be 70%. In order to improve the galactosylation efficiency, the conditions of the reaction were improved according to a slightly modified protocol of that published by Roy *et al.* for dendritic molecules.[14] The reaction was then carried out in sodium cacodylate buffer (50 mM) and in the presence of $MgCl_2$ and $MnCl_2$ at pH 7.5 and 25 °C for 72 hours. Addition of UDP-Gal took place at 3 different stages along the reaction (Method II). These conditions furnished a fully galactosylated derivative **33′** in 84% yield. The same conditions were then employed for the enzymatic galactosylation of the other glycopeptides **25** and **26**. Thus, galactosylated derivatives **34** and **35** were obtained in 55 and 62% yield respectively. The estimated extent of galactosylation was estimated to be 80% for both compounds.

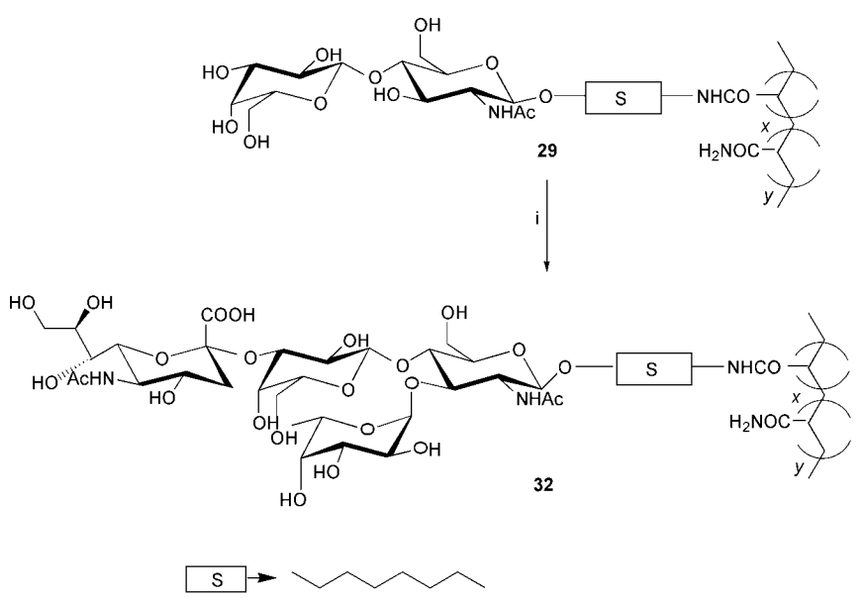
These reactions demonstrate once again that whatever the nature of the backbone, glycosyl acceptors with a hydrophilic linker are not suitable substrates for the galactosyl transferase, unlike those with an aliphatic linker.

In the next step, still with the aim of getting multivalent sialyl Lewis^x derivatives that are well-known to be highly efficient as selectin inhibitors, the sialylation reaction at position 3 was attempted with the *N*-acetyllactosamine unit compounds with either an acrylamide or peptidic support. It is already shown that α-(2,3)-sialyl transferase is suitable for modifying chemically prepared small molecules of biological interest[15] and glycopolymers.[11] Several sets of conditions including those described by Yamada *et al.*[11a] or those involving an excess of CMP-NANA and/or the use of Bovine Serum Albumin (BSA), $MnCl_2$ in solution in cacodylate buffer at different concentrations and pH values, failed to provide any sialylated derivative. Finally, α-(2,3)-sialylation of compound **33′** was achieved in sodium cacodylate buffer (50 mM, pH 6) containing 0.1% Triton X-100. It required 3 equivalents of CMP-NANA and 0.3 U of α-(2,3)-sialyl transferase. After 5 days of incubation at 37 °C and purification using G-25 gel filtration, the sialylated compound **37** was obtained in 47% yield. Its ^{1}H NMR spectra showed clearly a doublet of doublets at 2.64 ppm corresponding to H-3_{eq} of the sialyl residue. The extent of sialylation was estimated to be almost quantitative.

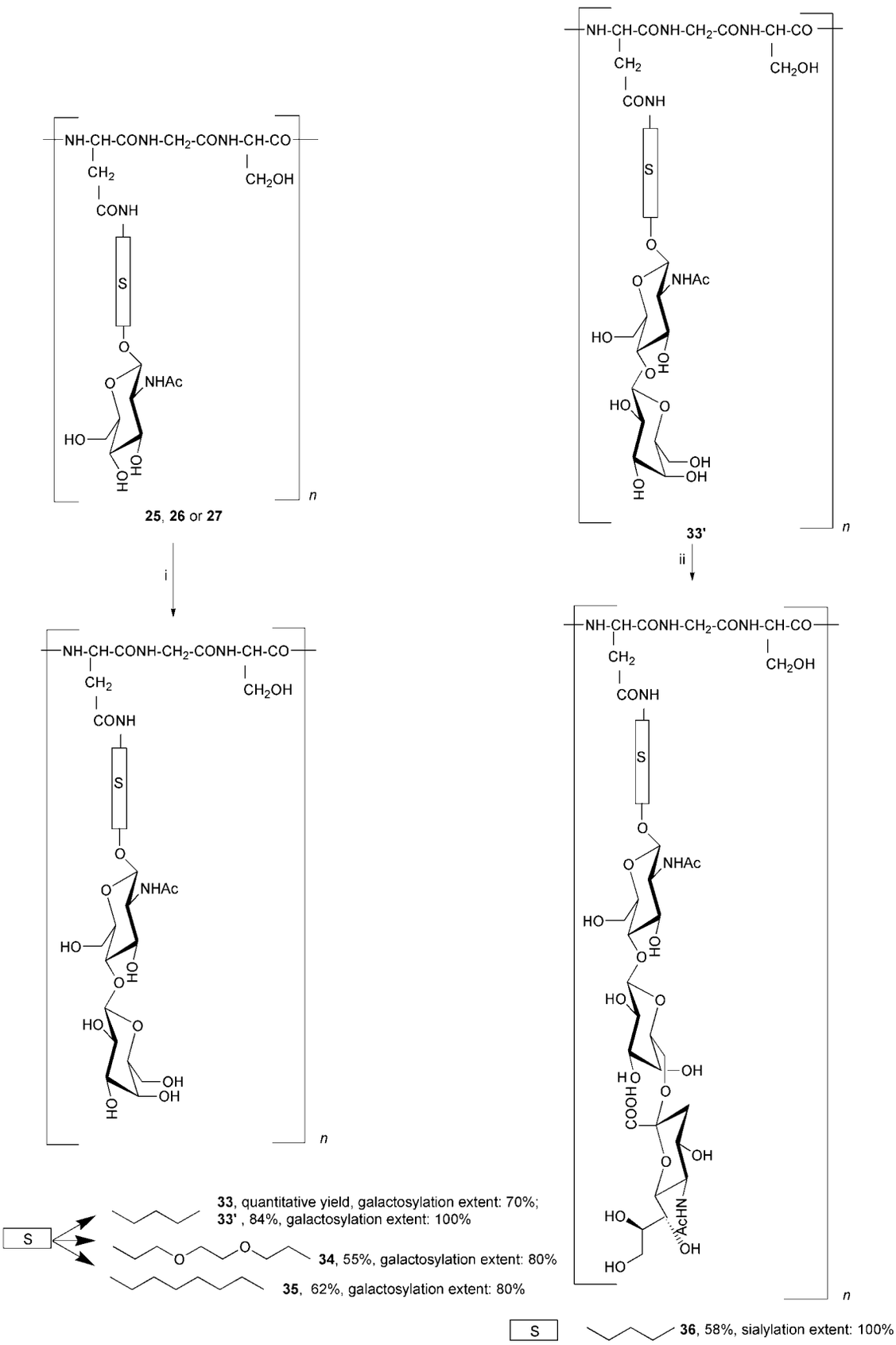
In the above case, as in the case of glycopolymers, no calf



Scheme 7 *Reagents and conditions*: i, UDP-Gal (3 equiv. for **12** and **14**, 1.3 equiv. for **13**), *α*-lactalbumin (0.2 g dm^{-3}), β-(1,4)-galactosyl transferase (1 U), HEPES buffer (pH 6.5, 50 mM, 1 cm^3), 37 °C, 72 h.

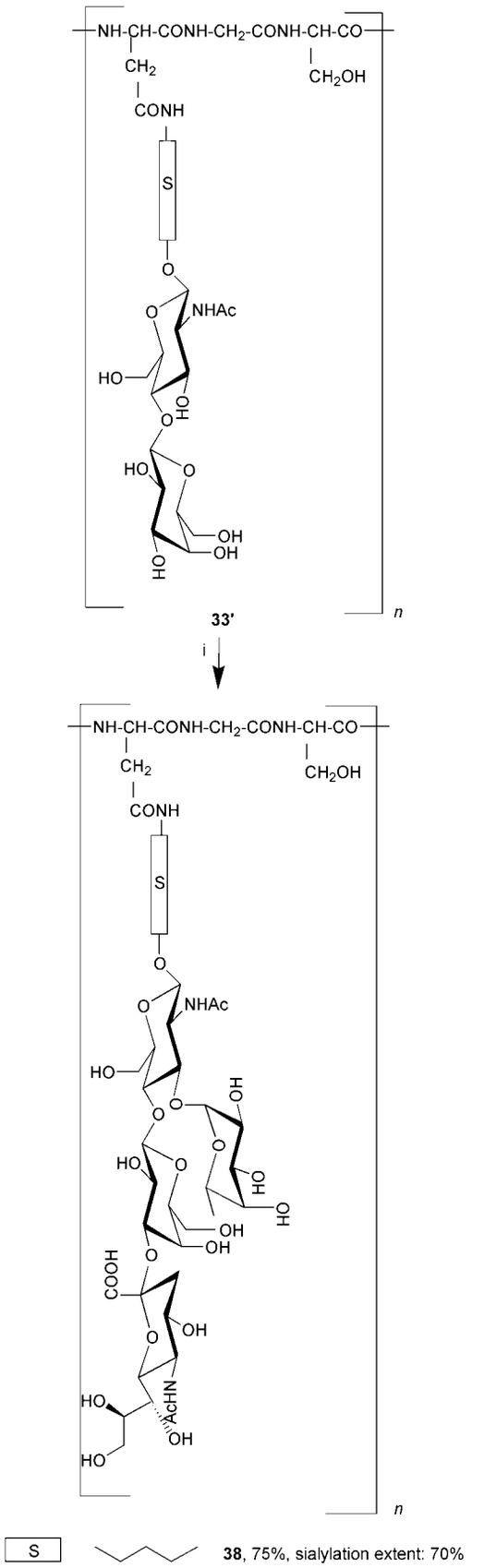


Scheme 8 *Reagents and conditions*: i, CMP-NANA (3 equiv.), α-(2,3)-sialyl transferase (0.3 U), sodium cacodylate buffer (pH 6.0, 50 mM), 0.1% Triton X-100, 37 °C, 5 d. then GDP-Fucose (2 equiv.), α-(1,3)-fucosyl transferase V (10 mU), HEPES buffer (pH 7.5, 100 mM), NaN_3, $MnCl_2 \cdot 4H_2O$, 37 °C, 3 d.



Scheme 9 *Reagents and conditions*: i, Method I: UDP-Gal (3 equiv.), α-lactalbumin (0.2 g dm^{-3}), β-(1,4)-galactosyl transferase (1 U), $MnCl_2$ (5 mM), HEPES buffer (pH 7.0, 50 mM, 1 cm^3), 37 °C, 26 h; Method II: UDP-Gal (3 equiv.), α-lactalbumin (0.2 g dm^{-3}), β-(1,4)-galactosyl transferase (1 U), $MnCl_2$ (25 mM), $MgCl_2$ (5 mM), dithiothreitol (0.2 mM), sodium cacodylate buffer (pH 7.50, 50 mM, 1 cm^3), 37 °C, 72 h; ii, BSA, NaN_3, $MnCl_2$, CMP-NANA (3 equiv.), calf intestinal alkaline phosphatase, α-(2,6)-sialyl transferase (1 U), sodium cacodylate buffer (pH 7.40, 50 mM), 37 °C, 48 h.

intestinal alkaline phosphatase was added to the reaction mixture. Although it was shown that this enzyme improved the yield of the galactosyl transferase reaction,[16] in our reaction, no significant difference was noticed. This can be explained by the polymeric nature of the support we used. Indeed, the high concentration of sugar units along the polymer chain led to a



Scheme 10 *Reagents and conditions*: i, CMP-NANA (3 equiv.), α-(2,3)-sialyl transferase (0.3 U), sodium cacodylate buffer (pH 6.0, 50 mM), 0.1% Triton X-100, 37 °C, 5 d. then GDP-fucose (2 equiv.), α-(1,3)-fucosyl transferase V (10 mU), HEPES buffer (pH 7.5, 100 mM), NaN_3, $MnCl_2 \cdot 4H_2O$, 37 °C, 3 d.

stronger association constant between sugar residues on the polymer and the enzyme.[11] This might accelerate the total reaction rate between donor and acceptor substrates.

Subsequently, compound **37** was used to obtain the expected multivalent sialyl Lewis^x derivative. The reaction was carried out as described by a previous report for acrylamide glycopolymers.[11] It took place in 100 mM HEPES buffer containing manganese chloride tetrahydrate and sodium azide at pH 7.5. Two equivalents of GDP-fucose were used and the reaction proceeded smoothly at 37 °C for 3 days in the presence of 10 mU of fucosyl transferase V and led to the *N*-acetyllactosaminyl derivative **38** bearing sialyl Lewis^x pendant chains in a quantitative yield (Scheme 10). As our aim is to produce tools for glycotechnology using easily handled and efficient protocols, the sialylation of compound **33′** in position 6 was also performed. This kind of derivative is also known to be an influenza virus inhibitor. The reaction was carried out using 2 equivalents of CMP-NANA and 0.1 U of α-(2,6)-sialyl transferase in sodium cacodylate buffer (pH 7.4, 50 mM) at 37 °C for 48 h (Scheme 9). After purification by gel filtration chromatography, ^{1}H NMR spectroscopy of compound **36** showed that the derivative had been sialylated in position 6 to an extent of 70%. A doublet of doublets corresponding to H-3_{eq} appeared at 2.53 ppm. The presence of the sialyl residue was confirmed by its ^{13}C NMR spectra.

Conclusions

Several water-soluble glycopolymers and sequential glycopeptides bearing GlcNAc units have been synthesised chemically and their sugar chain was then elongated enzymatically. All of these compounds but one turned out to be very good substrates for galactosyl transferase and high degrees of galactosylation were obtained together with good yields of *N*-acetyllactosaminated derivatives. This demonstrates that the methodology set up by Yamada *et al*. for galactosylation of glycopolymers can be easily and efficiently extended to sequential glycopeptides. Although this method could not be extended to the sialylation of sequential glycopeptides in position 3, simple and efficient new conditions were investigated that easily afforded this important intermediate in good yield in the construction of multivalent sialyl Lewis^x derivatives. This improved methodology led to the construction of polymeric derivatives **32** and **38** bearing a multivalent sialyl Lewis^x pattern after fucosylation was applied. On the other hand, sequential glycopeptide **36** was successfully sialylated in position 6 using α-(2,6)-sialyl transferase. These kinds of compounds are expected to be useful tools in the study of sugar–lectin interactions and as selectin inhibitors.

Experimental

General procedure

Unless otherwise stated all commercially available solvents and reagents were used without further purification. $CHCl_3$, dichloroethane, DMF, dimethyl sulfoxide and MeOH were stored over molecular sieves 3 Å before use. Et_3N and pyridine were stored over NaOH pellets. MS 4 Å were dried under reduced pressure at *ca*. 100 °C before use. Reactions were monitored by thin layer chromatography (TLC) on precoated plates of silica gel $60F_{254}$ (layer thickness 0.25 mm; E. Merck, Darmstadt). Column chromatography was performed on silica gel (silica gel 60N; 100–210 μm, Kanto Chemical Co., Inc.). Gel filtration chromatography was performed using Sephadex® G-10, −15 or −25 (Pharmacia Biotech.). Polymer detection polymer was performed using a UV detector (EYELA 9900) at $\lambda = 220$ nm. Average molecular weights of glycopolymers were estimated by gel permeation chromatography (GPC) with an Asahipak GS-50 column, and pullulans (5.8, 12.2, 23.7, 48.0, 100.0, 186.0, and 380K; Shodex Standard P-82) were used as standards. Enzymatic reactions were also performed using commercially available materials. Galactosyl transferase from bovine milk (Sigma) and sialyl transferase from rat liver

(Sigma) were used without further purification. Sugar composition of glycopolymers was determined from the integration data of the ^{1}H NMR spectra. Proton NMR spectra were recorded at 400 MHz using Me_4Si as internal standard and ^{13}C NMR spectra were recorded at 100 MHz on a JEOL lambda 400 spectrometer. FAB mass spectra were recorded on a JEOL JMS-SX102A spectrometer. Matrix-associated laser desorption ionization–time of flight–mass spectrometry (MALDI-TOF-MS) was performed on a Laser mat 2000 mass spectrometer. Abbreviations used for MALDI-TOF matrices are: 3-AQ, 3-aminoquinoline; cinnamic acid, α-cyano-4-hydroxycinnamic acid.

Trichloroacetimidoyl 3,4,6-tri-*O*-acetyl-2-*N*-phthalimido-2-deoxy-β-D-glucopyranoside **1**, 2-[2-(2-azidoethoxy)ethoxy]-ethanol **2** and 2-methyl-4,5-dihydro(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyranoso)[2,1-*d*]-1,3-oxazole were synthesised as previously described.[8–10]

Synthesis of the monosaccharidic part

2-[2-(2-Azidoethoxy)ethoxy]ethyl 3,4,6-tri-*O*-acetyl-2-*N*-phthalimido-2-deoxy-β-D-glucopyranoside 3. To a solution of trichloroacetimidoyl 3,4,6-tri-*O*-acetyl-2-*N*-phthalimido-2-deoxy-β-D-glucopyranoside **1** (2.85 g, 4.92 mmol) in dichloromethane (10 cm^3) containing molecular sieves 4 Å (3 g) and under nitrogen was added 2-[2-(2-azidoethoxy)ethoxy]ethanol **2** (2.6 g, 14.76 mmol). The solution was cooled to below 0 °C and $BF_3{\cdot}Et_2O$ (1.28 cm^3, 4.92 mmol) was added. The solution was stirred for 24 h under nitrogen, then triethylamine (1 cm^3) was added and the solution was stirred for another 15 min at room temperature and then diluted with chloroform. The organic layer was washed with icy saturated aq. $NaHCO_3$ (100 cm^3) and brine (100 cm^3), then dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by column chromatography (SiO_2, toluene–ethyl acetate = 80 : 20) to afford **3** (2.38 g, 79%) as a colourless oil; δ_H (400 MHz; $CDCl_3$): 1.86–2.11 (9H, all s, 3 × $COCH_3$), 3.30–3.54 (10H, m, OCH_2), 3.69 (1H, m, 5-H), 3.89 (2H, m, CH_2-N_3), 4.17 (1H, dd, *J* 2.29 and 12.21, 6-H_b), 4.32 (2H, m, 6-H_a, 2-H), 5.16 (1H, t, *J* 9.92, 4-H), 5.43 (1H, d, *J* 8.55, 1-H), 5.80 (1H, dd, *J* 9 and 10.83, 3-H), 7.79 (4H, m, Phthal.).

2-[2-(2-Azidoethoxy)ethoxy]ethyl 3,4,6-tri-*O*-acetyl-2-*N*-acetamido-2-deoxy-β-D-glucopyranoside 4. To a solution of **3** (0.911 g, 1.5 mmol) in *n*-butanol (6 cm^3) was added ethylenediamine (0.202 cm^3, 3 mmol). The mixture was heated at 70 °C under a nitrogen atmosphere for 24 h. The white precipitate that appeared was filtered off and the filtrate was evaporated under reduced pressure. After drying overnight under vacuum, the crude compound was solubilized in a mixture of pyridine and acetic anhydride (2 : 1, 15 cm^3) and was stirred at room temperature for 24 h. The solvents were evaporated under reduced pressure and the residual material was poured into ice water (50 cm^3) and extracted with chloroform (3 × 50 cm^3). The combined extracts were washed with saturated aq. $NaHCO_3$ (50 cm^3) and brine (50 cm^3), then dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by column chromatography (SiO_2, chloroform–methanol = 99 : 1) to afford **4** (0.318 g, 63%) as a white powder; δ_H (400 MHz; $CDCl_3$): 1.97–2.08 (12H, s, 4 × $COCH_3$), 3.50–3.89, (13H, m, OCH_2 and 5-H), 3.89 (1H, m, 5-H), 4.09–4.14 (2H, m, 6-H_b and 2-H), 4.25 (1H, dd, *J* 4.4 and 11.72, 6-H_a), 4.77 (1H, d, *J* 8.79, 1-H), 5.06–5.16 (2H, m, 3-H and 4-H), 6.27 (1H, d, *J* 9.53, N*H*).

2-[2-(2-Acrylamidoethoxy)ethoxy]ethyl 2-*N*-acetamido-2-deoxy-β-D-glucopyranoside 6. To a solution of **4** (0.3 g, 0.59 mmol) in methanol (5 cm^3) was added sodium methoxide (30 mg). The reaction mixture was stirred at room temperature for 3 h, then neutralized with DOWEX-50W X8 resin. The resin was filtered off and the filtrate was evaporated under reduced pressure. The residue was purified by column chromatography (SiO_2, chloroform–methanol = 90 : 10) to give **5**.

To a solution of **5** (0.25 g, 0.66 mmol) in methanol (4 cm^3) was added a suspension of Pd–C (20 mg) in methanol (1 cm^3). The reaction mixture was stirred at room temperature for 3 h under hydrogen, then the Pd–C was filtered off over Celite and the filtrate was evaporated under reduced pressure. The free amino compound thus obtained was freeze-dried and used without further purification.

To a solution of the crude amine (0.232 g, 0.66 mmol) in methanol (5 cm^3), under nitrogen and cooled to below 0 °C, were added triethylamine (0.184 cm^3, 1.32 mmol) and acryloyl chloride (0.107 cm^3, 1.32 mmol). The reaction mixture was stirred for 4 h at room temperature then the solvent was evaporated under reduced pressure. The residue was purified by column chromatography (SiO_2, chloroform–methanol = 80 : 20). The monomer-containing fractions were recombined, evaporated under reduced pressure and freeze-dried to give **6** (0.182 g, 68%) as a white powder (Found: C, 48.6; H, 7.7; N, 6.7. $C_{17}H_{30}O_9N_2{\cdot}0.8H_2O$ requires C, 48.5; H, 7.6; N, 6.65%); δ_H (400 MHz; D_2O): 1.93 (3H, s, $COCH_3$), 3.32–3.48 (5H, m, N-CH_2, 4-H, 5-H and 3-H), 3.53–3.70 (11H, m, 5 × O-CH_2 and 2-H), 3.80–3.93 (2H, m, 6-H_a and 6-H_b), 4.45 (1H, d, *J* 8.06, 1-H), 5.66 (1H, dd, *J* 1.46 and 10.36, C*H*=CH_2), 6.07–6.22 (2H, m, CH=CH_2); *m*/*z* (FAB$^+$): 407 (21%, [M + H]$^+$); (Found: *M* + H, 407.2042. $C_{17}H_{30}O_9N_2$ requires *M* + H, 407.2028).

3-[(*N*-Benzyloxycarbonyl)amino]propyl 3,4,6-tri-*O*-acetyl-2-*N*-acetamido-2-deoxy-β-D-glucopyranoside 7. To a solution of 2-methyl(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyrano)[2,1-*d*]-2-oxazole (4.23 g, 12.84 mmol) in dichloroethane (50 cm^3) was added 3-*N*-[(benzyloxy)carbonyl]aminopropanol (6.7 g, 32.0 mmol) and camphor-10-sulfonic acid until pH 2–3. The reaction mixture was heated at 70 °C for 3 h and then allowed to cool at room temperature. The solvent was evaporated under reduced pressure. The mixture was extracted with chloroform (3 × 100 cm^3) and the recombined organic layers were washed with saturated aq. $NaHCO_3$ (100 cm^3) and brine (100 cm^3) and then dried ($MgSO_4$). The solvent was evaporated under reduced pressure and the resulting material was purified by column chromatography (SiO_2, toluene–ethyl acetate = 70 : 30) to afford **7** (3.20 g, 46%) as a pale yellow powder; δ_H (400 MHz, $CDCl_3$): 1.77, 1.84 (2H, m, CH_2), 1.94, 2.03, 2.07 (12H, all s, 4 × $COCH_3$), 3.55 and 3.94 (2H, m, OCH_2), 3.56 (1H, m, 5-H), 3.96 (1H, ddd, *J* 8.9, 2-H), 4.12 (1H, dd, *J* 2.3 and 12.2, 6-H_a), 4.23 (1H, dd, *J* 4.5 and 12.2, 6-H_b), 4.31 (1H, d, *J* 8.3, 1-H), 4.95 (1H, m, N*H*COO), 5.0–5.16 (4H, m, 4-H and $PhCH_2$), 6.38 (1H, d, *J* 8.6, NH), 7.31–7.40 (5H, m, C_6H_5).

6-[(*N*-Benzyloxycarbonylamino]hexyl 3,4,6-tri-*O*-acetyl-2-*N*-acetamido-2-deoxy-β-D-glucopyranoside 8. This compound was obtained using the way described above for **7** starting from 2-methyl-4,5-dihydro-(3,4,6-tri-*O*-acetyl-1,2-dideoxy-α-D-glucopyranoso)[2,1-*d*]-1,3-oxazole and 6-[*N*-(benzyloxy)carbonylamino]hexanol (3.43 g, 46%); δ_H (400 MHz, $CDCl_3$): 1.35 and 1.50 (8H, m, CH_2), 1.93, 2.02, 2.03 and 2.08 (12H, all s, 4 × $COCH_3$), 3.19 (2H, m, N-CH_2), 3.46 and 3.80 (2H, m, OCH_2), 3.63 (1H, ddd, 2-H), 4.09 (1H, dd, *J* 2.3 and 12.2, 6-H_a), 4.25 (1H, dd, *J* 4.5 and 12.2, 6-H_b), 4.63 (1H, d, *J* 8.2, 1-H), 4.87 (1H, br s, NHCOO), 5.06 (1H, t, *J* 9.6, 4-H), 5.12 (2H, dd, *J* 16.2, $PhCH_2$), 5.29 (1H, t, *J* 9.6, 3-H), 5.93 (1H, d, *J* 8.6, NH), 7.30–7.37 (5H, m, Ph).

3-[(*N*-Benzyloxycarbonyl)amino]propyl 2-*N*-acetamido-2-deoxy-β-D-glucopyranoside 9. To a solution of **7** (3.2 g, 5.94 mmol) in methanol (10 cm^3) was added sodium methoxide (0.32 g). The solution was stirred at room temperature for 2 h then neutralized with DOWEX 50-X8 resin. The resin was filtered off and filtrate was evaporated to dryness. The resulting material was purified by column chromatography (SiO_2,

chloroform–methanol = 80:20) to afford **9** as a slightly yellow powder (1 g, 41%); δ_H (400 MHz, DMSO-d_6): 1.52 (2H, m, CH_2), 1.73 (3H, s, $COCH_3$), 2.97 (4H, m, 6-H_a, 6-H_b, N-CH_2), 3.58–3.69 (2H, m, 2-H and 3-H), 4.17 (1H, d, *J* 8.30, 1-H), 4.49 (1H, t, *J* 5.86, 4-H), 4.86 (1H, d, *J* 5.37, O-CH_2), 4.95 (3H, m, COO-CH_2-C_6H_5 and O-CH_2), 7.15 (1H, br t, NH), 7.22–7.32 (5H, m, C_6H_5), 7.60 (1H, d, *J* 9.03, NH).

6-(Acrylamido)hexyl 2-*N*-acetamido-2-deoxy-β-D-glucopyranoside 11. To a solution of **8** (3.2 g, 5.51 mmol) in methanol (10 cm^3) was added sodium methoxide (0.32 g). The solution was stirred at room temperature for 2 h then neutralized with DOWEX 50-X8 resin. The resin was filtered off and the filtrate was evaporated to dryness. The resulting material was purified by column chromatography (SiO_2, chloroform–methanol = 80:20) to afford **10** as a slightly yellow powder (1.25 g, 50%). To a solution of **10** (1.25 g, 2.75 mmol) in methanol (7 cm^3) containing a catalytic amount of acetic acid was added 10% Pd–C (0.245 g) in suspension in the same solvent (3 cm^3). The reaction mixture was stirred under hydrogen, at room temperature for 4 h then Pd–C was filtered off over Celite and the filtrate was evaporated under reduced pressure. The free amino compound thus obtained was freeze-dried and used without further purification. To a solution of crude amine (0.2 g, 0.625 mmol) in methanol (5 cm^3), under nitrogen and cooled to below 0 °C, was added triethylamine (0.147 cm^3, 1.25 mmol) and acryloyl chloride (0.102 cm^3, 1.25 mmol). The reaction mixture was stirred at room temperature for 4 h, and then the solvent was removed under reduced pressure. Monomer **11** was purified by column chromatography (SiO_2, chloroform–methanol = 80:20) and the pure compound was freeze-dried (0.097 g, 41%) (Found: C, 50.6; H, 8.3; N, 6.9. $C_{17}H_{30}O_9N_2 \cdot 1.5H_2O$ requires C, 50.9; H, 8.3; N, 7.0%); δ_H (400 MHz, D_2O): 1.15–1.41 (8H, m, 4 × CH_2), 1.77 (3H, s, $COCH_3$), 3.04–3.09 (2H, m, N-CH_2), 3.37–3.47 (2H, m, 6-H_a and 6-H_b), 3.63–3.70 (3H, m, 2-H, 3-H and 5-H), 4.23 (1H, d, *J* 8.32, 1-H), 4.52 (1H, t, *J* 5.95, 4-H), 4.89 (1H, m, O-CH_2), 4.97 (1H, m, O-CH_2), 5.54 (1H, dd, *J* 2.37 and 10.07, CH=CH_{2cis}), 6.02 (1H, dd, *J* 2.33 and 17.09, CH=CH_{2trans}), 6.18 (1H, m, C*H*=CH_2); *m*/*z* (FAB$^+$): 375 (10%, M + H$^+$); (Found: *M* + H, 375.2140. $C_{17}H_{30}O_7N_2$ requires *M* + H, 375.2131).

Synthesis of polymers

Acrylamide copolymer 12 from monomer 6. A mixture of DMSO–H_2O (5:1, 3 cm^3) was deaerated for 1 h with a water aspirator. Monomer **6** (0.130 g, 0.32 mmol) and acrylamide (0.091 g, 1.28 mmol) were added with TEMED (0.024 cm^3, 0.16 mmol) and APS (0.0365 g, 0.16 mmol). The solution was stirred for 24 h at room temperature under nitrogen and directly purified by gel filtration chromatography (Sephadex G-25, 41 × 2 cm) and eluted with deionized water. Copolymer **12** (0.17 g, 77%) was freeze-dried and obtained as a white powder; δ_H (400 MHz; D_2O): 1.5–1.62 (12H, br m, CH_2), 1.90 (3H, s, $COCH_3$), 2.05–2.20 (2H, br m, CH), 3.31 (1H, br s, 4-H), 4.42 (1H, d, *J* 8.24, 1-H); δ_C (100 MHz; D_2O): 23.02 (CO*C*H_3), 35.5 (CH_2), 39.6 (CH), 43.0 (N-CH_2), 56.2 (2-C), 61.5 (6-C), 69.4–70.4 (OCH_2), 70.6 (4-C), 74.6 (3-C), 76.7 (5-C), 101.9 (1-C), 175.2 (*C*OCH_3), 180.2 ($CONH_2$). *M* ~ 135,000 (GPC method).

Acrylamide copolymer 13 from monomer 11. A mixture of DMSO–H_2O (5:1, 3 cm^3) was deaerated for 1 h with a water aspirator. Monomer **11** (0.09 g, 0.24 mmol) and acrylamide (0.0685 g, 0.96 mmol) were added followed by TEMED (0.018 cm^3, 0.12 mmol) and APS (0.0275 g, 0.12 mmol). The solution was stirred for 48 h at room temperature under nitrogen and directly purified by gel filtration chromatography (Sephadex G-25, 42 × 2 cm) and eluted with deionized water. Copolymer **13** (0.08 g, 89%) was freeze-dried and obtained as a white powder; δ_H (400 MHz; D_2O): 1.16 (4H, br s, 4 × CH_2), 1.48–1.60 (21H, br m, CH_2), 1.87 (3H, s, CH_3), 2.0–2.14 (15H, br m, CH), 3.28 (1H, br s, 4-H), 4.33 (1H, d, *J* 8.54, 1-H); δ_C (100 MHz; D_2O): 23.0 (CO*C*H_3), 25.5–29.3 (CH_2, hex), 35.3 (CH_2), 40.2 (N-CH_2), 42.8 (CH), 56.3 (2-C), 61.4 (6-C), 70.6 (OCH_2), 71.1 (4-C), 74.6 (3-C), 76.6 (5-C), 101.8 (1-C), 175.1 (*C*OCH_3), 180.2 ($CONH_2$). *M* > 380,000 (GPC method).

Homopolymer 14 from monomer 6. H_2O (3 cm^3) was deaerated for 1 h with a water aspirator. Monomer **6** (0.168 g, 0.414 mmol) was added along with TEMED (0.0.31 cm^3, 0.207 mmol) and APS (0.047 g, 0.207 mmol). The solution was stirred for 24 h at room temperature under nitrogen and directly purified by gel filtration chromatography (Sephadex G-25, 42 × 2 cm) and eluted with water. Homopolymer **14** (0.083 g, 50%) was freeze-dried and obtained as a white powder; δ_H (400 MHz; D_2O): 1.94 (3H, s, CH_3), 3.34–3.89 (18H, m, 2-H, 3-H, 4-H, 5-H, 6-H, O-$(CH_2)_2$ and NH-CH_2), 4.45 (1H, d, *J* 8.06, 1-H); δ_C (100 MHz; D_2O): 25.5 (CO*C*H_3), 42.1 (N-CH_2), 44.5 (CH), 58.5 (2-C), 63.8 (6-C), 71.7–73.0 (OCH_2), 73.1 (4-C), 76.9 (3-C), 79.4 (5-C), 103.9 (1-C), 176.5 (*C*OCH_3); TOF-MASS (negative mode, 3-AQ): 956.0 [2M + matrix]$^+$, 1365.0 [3M + 3H + matrix]$^+$, 1787.7 [4M + matrix + H_2O]$^+$, 2174.0 [5M + matrix]$^+$, 2586.6 [6M + 6H + matrix]$^+$, 2993.2 [7M + 7H + matrix]$^+$, 3394.3 [8M + 2H + matrix]$^+$.

Synthesis of the peptidic part

Boc-Ser-(OBn)$_2$ 15. Boc-Ser-(OBn) (2 g, 6.77 mmol) was dissolved in 10% aqueous methanol (10 cm^3) and caesium carbonate (1.10 g, 3.385 mmol) was added. The clear solution was evaporated under reduced pressure several times with methanol in order to remove H_2O, then DMF (10 cm^3) was added. The reaction mixture was cooled to below 0 °C and benzyl bromide (1.2 cm^3, 10.15 mmol) was added. The mixture was stirred at room temperature overnight then DMF was evaporated under vacuum. The residue was extracted with chloroform (3 × 100 cm^3) and the organic layers were washed with water (100 cm^3) then dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by column chromatography (SiO_2, 100% hexane) to give the title compound **15** (2.6 g, 100%) as a colourless oil; δ_H (400 MHz; $CDCl_3$): 1.44 (9H, s, $C(CH_3)_3$), 3.68 (1H, dd, *J* 9.46, Serβ), 3.89 (1H, dd, *J* 9.15, Serβ), 4.41–4.51 (3H, m, Serα, O-CH_2-C_6H_5), 5.12–5.24 (2H, m, COO-CH_2-Ph), 5.43 (1H, d, *J* 8.59, NH), 7.20–7.31 (10H, m, 2 × C_6H_5).

Boc-Gly-Ser-(OBn)$_2$ 16. To a solution of **15** (2.6 g, 6.75 mmol) in dioxane (50 cm^3), cooled to below 0 °C, was added HCl (4 M) in dioxane (50 cm^3). The reaction mixture was stirred at room temperature for 3 h, and then the solvent was evaporated under reduced pressure. The free amino benzylated serine derivative crystallized from diethyl ether as a white powder (1.5 g, 69%) and was used without further purification.

A solution of this free amino derivative (1.5 g, 4.66 mmol) and Boc-Glycine (1.23 g, 6.99 mmol) in DMF (20 cm^3) was cooled to below 0 °C. DPPA (1.7 cm^3, 7.92 mmol) and triethylamine (0.975 cm^3, 6.99 mmol) were added and the solution was stirred for 2 h at 0 °C then at room temperature overnight. DMF was evaporated under vacuum and the residue was extracted with chloroform (3 × 100 cm^3). The extracts were washed successively with 5% citric acid (100 cm^3), 5% $NaHCO_3$ (100 cm^3), and brine (100 cm^3), then dried ($MgSO_4$) and evaporated under reduced pressure. The resulting material was purified by column chromatography (SiO_2, hexane–ethyl acetate = 70:30) to afford the dipeptide **16** (1.7 g, 83%) as a colourless oil; δ_H (400 MHz; $CDCl_3$): 1.43 (9H, s, $C(CH_3)_3$), 3.67 (1H, dd, *J* 3.2 and 9.61, Serβ), 3.82 (1H, br d, Glyα), 3.91 (1H, dd, *J* 3.05 and 9.61, Serβ), 4.41 (2H, dd, *J* 12.21 and 16.78, O-CH_2-C_6H_5), 4.79 (1H, m, Serα), 5.16 (2H, m, COO-CH_2-Ph), 6.95 (1H, d, *J* 7.93, NH), 7.18–7.32 (10H, m, 2 × C_6H_5).

Fmoc-Asp(OtBu)-Gly-Ser-(OBn)$_2$ 17. To a solution of **16** (1.6 g, 3.61 mmol) in dioxane (30 cm^3), cooled to below 0 °C,

was added HCl (4 M) in dioxane (30 cm^3). The reaction mixture was stirred at room temperature for 3 h, then the solvent was evaporated under reduced pressure. The free amino dipeptide crystallized from diethyl ether as a white powder (1.09 g, 80%) and was used without further purification.

A solution of this dipeptide (1.09 g, 2.89 mmol) and Fmoc-Asp(OtBu) (1.43 g, 3.47 mmol) in DMF (20 cm^3) was cooled to below 0 °C. DPPA (0.811 cm^3, 3.76 mmol) and triethylamine (0.484 cm^3, 3.47 mmol) were added and the solution was stirred for 2 h at 0 °C and then at room temperature overnight. DMF was evaporated under vacuum and the residue was extracted with chloroform. The extracts were washed with 5% citric acid (100 cm^3), and brine (100 cm^3), then dried ($MgSO_4$) and evaporated under reduced pressure. The resulting material was purified by column chromatography (SiO_2, toluene–ethyl acetate = 70 : 30) to afford the tripeptide **17** (1.26 g, 59%) as a white powder (Found: C, 68.6; H, 6.4; N, 5.5. $C_{42}H_{45}O_9N_3$ requires C, 68.6; H, 6.2; N, 5.7%); δ_H (400 MHz; $CDCl_3$): 1.42 (9H, s, $C(CH_3)_3$), 2.65 (1H, dd, *J* 5.2 and 17.8, Aspβ), 2.90 (1H, dd, *J* 6.0 and 17.2, Aspβ), 3.67 (1H, dd, *J* 3.2 and 9.60, Serβ), 3.89 (1H, dd, *J* 3.2 and 9.6, Serβ), 3.94–4.03 (2H, m, Glyα), 4.2 (1H, t, *J* 6.7, CH Fmoc), 4.44 (5H, m, Aspα, CH_2 Fmoc, O-CH_2-C_6H_5), 4.79 (1H, m, Serα), 5.15 (2H, m, COO-CH_2-Ph), 5.85 (1H, d, *J* 8.4, NH), 6.81 (1H, d, *J* 7.60, NH), 7.02 (1H, br d, NH), 7.18–7.80 (18H, m, CH_{Ar}, Fmoc and 2 × C_6H_5); *m/z* (FAB^+): 736 $[M + H]^+$, 680 $[M + H - t\text{-}Bu]^+$ (Found: *M* + H, 736.3248. $C_{42}H_{45}O_9N_3$ requires *M* + H, 736.3234).

Fmoc-Asp-Gly-Ser-(OBn)$_2$ 18. Tripeptide **17** (1.26 g, 1.70 mmol) in solution in TFA (12 cm^3) was stirred at room temperature for 2 h. TFA was evaporated under reduced pressure and the residue was purified by column chromatography (SiO_2, chloroform–methanol = 95 : 5) to afford the free β-carboxytripeptide **18** (1.04 g, 90%) as a white powder (Found: C, 65.8; H, 5.6; N, 6.0. $C_{38}H_{37}O_9N_3 \cdot 0.8H_2O$ requires C, 65.75; H, 5.6; N, 6.05%); δ_H (400 MHz; DMSO-d_6): 2.62 (1H, dd, *J* 3.17 and 16.6, Aspβ), 3.55 (1H, dd, *J* 4.15 and 9.52, Serβ), 3.67–3.83 (3H, m, Serβ and Glyα), 4.13–4.43 (6H, m, CH Fmoc, CH_2 Fmoc, Aspα, O-CH_2-C_6H_5), 4.55 (1H, br m, Serα), 5.06 (2H, dd, *J* 4.15 and 16.84, $COOCH_2C_6H_5$), 7.16–7.83 (18H, m, CH_{Ar}, Fmoc and 2 × C_6H_5), 8.11 (1H, br t, NH), 8.28 (1H, d, *J* 7.8, NH); δ_C (100 MHz; DMSO-d_6): 36.3 (Cβ, Asp), 41.9 (Cα, Gly), 46.6 (CH, Fmoc), 51.45 (Cα, Asp), 52.5 (Cα Ser), 65.85, 66.1 (CH_2 Fmoc, $COOCH_2$-C_6H_5), 69.2 (Cβ, Ser), 72.25 (O-CH_2-C_6H_5), 120.2–128.4 (2 × C_6H_5), 135.85–143.8 (CH_{Ar}, Fmoc), 156.0 (OCONH), 169.0, 169.9, 171.25, 171.8 (CO); *m/z* (FAB^+): 680 $[M + H]^+$ (Found: *M* + H, 680.2612. $C_{38}H_{37}O_9N_3$ requires *M* + H, 680.2608).

Synthesis of glycopeptides

General procedure. The free amino derivatives required were first obtained from derivative **5** by reduction of the azido group or from derivatives **9** or **10** by removal of the Z protecting group. Then, to a solution of these crude amino sugars and tripeptide **18** (1.3 equiv.) in a mixture of ethanol and benzene (1 : 1, 10 cm^3) was added EEDQ (1.4 equiv.). The reaction mixture was stirred for 24 h at room temperature under nitrogen, and then the solvents were evaporated under reduced pressure. The residues were purified by column chromatography to afford the protected glycopeptides **19**, **20** or **21**.

Fmoc-Asp(CONH-[CH_2]$_2$-O-[CH_2O]$_2$-GlcNac)-Gly-Ser-(OBn)$_2$ 19. This compound was obtained *via* the general procedure described above starting from the amino derivative afforded by **5** (0.07 g, 0.2 mmol) and acid **18** (0.177 g, 0.26 mmol). The residue was purified by column chromatography (SiO_2, chloroform–methanol = 90 : 10) to afford the protected glycopeptide **19** (0.155 mg, 77%) as a white powder (Found: C, 58.45; H, 6.1; N, 6.5. $C_{52}H_{63}O_{16}N_5 \cdot 3H_2O$ requires C, 58.5; H, 5.9; N, 6.6%); δ_C (100 MHz; $CDCl_3$): 23.4 ($COCH_3$), 38.4 (Cβ, Asp), 39.8 (CH_2-NH), 42.9 (Cα, Gly), 47.0 (CH, Fmoc), 53.0 (Cα Ser), 56.1 (2-C), 61.5 (6-C), 67.2, 68.5 (CH_2, Fmoc, O-$CH_2C_6H_5$), 70.1 (O-CH_2), 73.0 (4-C), 74.7 (3-C), 75.9 (5-C), 101.5 (1-C), 119.9–128.5 (2 × C_6H_5), 135.2–143.8 (CH_{Ar}, Fmoc), 156.4 (OCONH), 170.1(CO); *m/z* (FAB^+): 1014 $[M + H]^+$ (Found: *M* + H, 1014.4330. $C_{52}H_{63}O_{16}N_5$ requires *M* + H, 1014.4347).

Fmoc-Asp(CONH-[CH_2]$_6$-O-GlcNac)-Gly-Ser-(OBn)$_2$ 20. This compound was obtained *via* the general procedure described above starting from the amino derivative from **10** (0.181 g, 0.57 mmol) and acid **18** (0.5 g, 0.74 mmol). The residue was purified by column chromatography (SiO_2, chloroform–methanol = 90 : 10 then 85 : 15) to afford the protected glycopeptide **20** (0.344 g, 62%) as a white powder (Found: C, 62.0; H, 6.4; N, 6.7. $C_{52}H_{63}O_{14}N_5 \cdot 1.5H_2O$ requires C, 61.9; H, 6.7; N, 6.9%); δ_C (100 MHz; DMSO-d_6): 23.1 ($COCH_3$), 25.2, 26.2, 29.0, 29.1 (4 × CH_2, hexyl), 37.6 (Cβ, Asp), 38.6 (CH_2-NH), 41.9 (Cα, Gly), 46.6 (CH, Fmoc), 51.8 (Cα, Asp), 52.5 (CαSer), 55.4 (2-C), 61.0 (6-C), 65.8, 66.05, 68.2 (CH_2 Fmoc, O-CH_2-C_6H_5, COO-CH_2-C_6H_5), 69.1 (Cβ, Ser), 70.6 (OCH_2), 72.2 (4-C), 74.3 (3-C), 77.0 (5-C), 101.0 (1-C), 120.15–128.4 (2 × C_6H_5), 135.85–143.8 (CH_{Ar}, Fmoc), 155.8 (OCONH), 168.9, 169.0, 169.05, 169.9, 171.5 (CO); *m/z* (FAB^+): 982 $[M + H]^+$ (Found: *M* + H, 982.4474. $C_{52}H_{63}O_{14}N_5$ requires *M* + H, 982.4450).

Fmoc-Asp(CONH-[CH_2]$_3$-O-GlcNac)-Gly-Ser-(OBn)$_2$ 21. This compound was obtained *via* the general procedure described above starting from the amino derivative derived from **9** (0.2 g, 0.72 mmol) and acid **18** (0.636 g, 0.93 mmol). The residue was purified by column chromatography (SiO_2, chloroform–methanol = 95 : 5 then 90 : 10) to afford the protected glycopeptide **21** (0.4 g, 59%) as a white powder (Found: C, 61.2; H, 6.2; N, 7.15. $C_{49}H_{57}O_{14}N_5 \cdot 1H_2O$ requires C, 61.4; H, 6.2; N, 7.3%); δ_C (100 MHz; DMSO-d_6): 23.05 ($COCH_3$), 29.1 (CH_2, propyl), 35.9 (Cβ, Asp), 37.6 (CH_2 -NH), 41.9 (Cα, Gly), 46.6 (CH, Fmoc), 51.8 (Cα, Asp), 52.4 (Cα, Ser), 55.4 (2-C), 61.05 (6-C), 65.8, 66.1, 66.4 (CH_2, Fmoc, O-CH_2-C_6H_5, COO-CH_2-C_6H_5), 69.1 (Cβ, Ser), 70.6 (OCH_2), 72.2 (4-C), 74.3 (3-C), 76.9 (5-C), 101.2 (1-C), 120.1–143.8 (CH_{Ar}, Fmoc and 2 × C_6H_5), 155.8 (OCONH), 169.1, 169.15, 169.45, 169.9, 171.55 (CO); *m/z* (FAB^+): 940 $[M + H]^+$ (Found: *M* + H, 940.4004. $C_{49}H_{57}O_{14}N_5$ requires *M* + H, 940.3980).

General procedure for the full deprotection of glycopeptides

The Fmoc amino protecting group of the glycopeptides **19**, **20** or **21** was removed using 10% piperidine in DMF. The solution was stirred for 2 h at room temperature, then DMF was evaporated *in vacuo*. The residual material was decanted 3 times with a hexane–diethyl ether mixture (3 : 1) to remove the dibenzofulvene and dried overnight under vacuum. It was used for the next step without further purification.

Crude free amino compound was dissolved in a mixture of H_2O and acetic acid (2 : 8, 3 cm^3), and 10% Pd–C (50–70% w/w) in suspension in the same solvent (2 cm^3) was added. The reaction mixture was stirred at room temperature, under hydrogen for 24 h. Then, the Pd–C was removed by filtration over Celite and the filtrate was evaporated under reduced pressure. The residue was directly subjected to gel filtration chromatography (Sephadex G-10, 41 × 1 cm) using H_2O as eluent. The fractions containing the compound were recombined, concentrated under reduced pressure and freeze-dried to afford the deprotected glycopeptide **22**, **23** or **24** as a white powder.

Asp(CONH-[CH_2]$_2$-O-[CH_2O]$_2$-GlcNac)-Gly-Ser 22. Compound **19** (155 mg, 0.15 mmol) was used to prepare compound **22** (64 mg, 68.5%) *via* the general procedure described above (Found: C, 42.05; H, 6.6; N, 9.9. $C_{23}H_{41}O_{14}N_5 \cdot 2.5H_2O$ requires C, 42.1; H, 7.05; N, 10.7%); δ_C (100 MHz; D_2O): 22.9 ($COCH_3$), 36.2 (Cβ, Asp), 39.7 (CH_2-NH), 43.2 (Cα, Gly), 50.8 (Cα, Asp),

56.2 αSer), 57.7 (2-C), 61.4 (Cβ, Ser), 62.6 (6-C), 69.4–70.4 (OCH_2), 70.6 (4-C), 74.6 (3-C), 76.6 (5-C), 101.8 (1-C), 170.1, 170.8, 171.0, 175.2, 176.5 (CO); *m/z* (FAB$^+$): 612 [M + H]$^+$ (Found: *M* + H, 612.2719. $C_{23}H_{41}O_{14}N_5$ requires *M* + H, 612.2728).

Asp(CONH-[CH_2]$_6$-O-GlcNac)-Gly-Ser 23. Compound **20** (270 mg, 0.28 mmol) was used to prepare compound **23** (134 mg, 84%) *via* the general procedure described above (Found: C, 43.9; H, 6.7; N, 10.8. $C_{23}H_{41}O_{12}N_5 \cdot 3H_2O$ requires C, 43.6; H, 7.5; N, 11.0%); δ_C (100 MHz; D_2O): 22.9 ($COCH_3$), 25.5, 26.4, 28.9, 29.2 (4 × CH_2, hexyl), 36.3 (Cβ, Asp), 40.2 (CH_2NH), 43.2 (Cα, Gly), 50.9 (Cα, Asp), 56.3 (Cα, Ser), 57.8 (2-C), 61.4 (Cβ, Ser), 62.7 (6-C), 70.6 (OCH_2), 71.1 (4-C), 74.5 (3-C), 76.6 (5-C), 101.8 (1-C), 170.1, 170.4, 170.9, 175.1, 176.6 (CO); *m/z* (FAB$^+$): 580 [M + H]$^+$ (Found: *M* + H, 580.2845. $C_{23}H_{41}O_{12}N_5$ requires *M* + H, 580.2830).

Asp(CONH-[CH_2]$_3$-O-GlcNac)-Gly-Ser 24. Compound **21** (0.407 g, 0.43 mmol) was used to prepare compound **24** (0.142 g, 61%) *via* the general procedure described above (Found: C, 41.4; H, 6.6; N, 11.6. $C_{20}H_{35}O_{12}N_5 \cdot 2.5H_2O$ requires C, 41.2; H, 6.9; N, 12.0%); δ_C (100 MHz; D_2O): 22.8 ($COCH_3$), 28.9 (CH_2, propyl), 36.3 (Cβ, Asp), 37.1 (CH_2-NH), 43.2 (Cα, Gly), 50.8 (Cα, Asp), 56.25 (Cα, Ser), 57.7 (2-C), 61.4 (Cβ, Ser), 62.6 (6-C), 68.5 (OCH_2), 70.6 (4-C), 74.4 (3-C), 76.6 (5-C), 101.85 (1-C), 170.0, 170.5, 170.9, 175.3, 176.5 (CO); *m/z* (FAB$^+$): 538 [M + H]$^+$ (Found: *M* + H, 538.2377. $C_{20}H_{35}O_{12}N_5$ requires *M* + H, 538.2360).

General procedure for the polymerisation of glycopeptides

A solution of glycopeptide **22**, **23** or **24** (0.07 to 0.15 mmol) in DMSO (0.2 to 0.6 cm^3) was kept under nitrogen and at room temperature for a few minutes before DPPA (0.084 to 0.18 mmol) and triethylamine (0.084 to 0.18 mmol) were added. The reaction mixture was stirred vigorously at room temperature and under nitrogen for 4 days, and was then directly subjected to gel filtration chromatography (Sephadex G-25, 42 × 2 cm) using water as eluent. The fractions containing the compound were recombined, concentrated under reduced pressure and freeze-dried to afford the sequential glycopeptides **25**, **26** or **27** as white powders. The average number of repeating units was determined using MALDI-TOF mass spectrometry.

Polymerisation of 22. Compound **22** (45.2 mg, 0.074 mmol) was used to prepare compound **25** (33 mg, 73%) *via* the general procedure described above. The average number of repeating units was found to be from $n = 2$ to $n = 4$; δ_C (100 MHz; D_2O): 23.1 ($COCH_3$), 37.5 (Cβ, Asp), 39.85 (CH_2-NH), 43.6 (Cα, Gly), 51.6 (Cα, Asp), 56.3 (2-C), 61.6 (Cβ, Ser), 62.0 (6-C), 69.6–70.5 (OCH_2), 71.8 (4-C), 74.7 (3-C), 76.7 (5-C), 101.8 (1-C), 172.3, 172.4, 172.9, 173.1, 175.3 (CO); TOF-MASS (positive mode, cinnamic acid): 1223.2 [2M + H]$^+$, 1833.8 [3M + H]$^+$, 2446.4 [4M + 2H]$^+$, 3058.0 [5M + 3H]$^+$.

Polymerisation of 23. Compound **23** (66.5 mg, 0.11 mmol) was used to prepare compound **26** (43 mg, 65%) *via* the general procedure described above. The average number of repeating units was found to be from $n = 2$ to $n = 4$; δ_C (100 MHz; D_2O): 22.9 ($COCH_3$), 25.4, 26.4, 29.0, 29.2 (CH_2, hexyl), 37.3 (Cβ, Asp), 40.1 (CH_2-NH), 43.4 (Cα, Gly), 51.5 (Cα, Asp), 56.3 (Cα, Ser), 56.6 (2-C), 61.4 (Cβ, Ser), 61.6 (6-C), 70.6 (OCH_2), 71.05 (4-C), 74.5 (3-C), 76.5 (5-C), 101.8 (1-C), 171.9, 172.2, 172.3, 173.0, 175.1 (CO); TOF-MASS (positive mode, cinnamic acid): 1141.2 [2M + H − H_2O]$^+$, 1703.9 [3M + H − 2H_2O]$^+$, 2264.4 [4M + 2H − 3H_2O]$^+$.

Polymerisation of 24. Compound **24** (68 mg, 0.13 mmol) was used to prepare compound **27** (45 mg, 66%) *via* the general procedure described above. The average number of repeating units was found to be from $n = 2$ to $n = 5$; δ_C (100 MHz; D_2O): 22.85 ($COCH_3$), 29.05 (CH_2, propyl), 37.0 (Cβ, Asp), 43.4 (Cα, Gly), 51.6 (Cα, Asp), 56.3 (Cα, Ser), 57.6 (2-C), 61.45 (Cβ, Ser), 62.5 (6-C), 68.4 (OCH_2), 70.6 (4-C), 74.5 (3-C), 76.6 (5-C), 101.9 (1-C), 172.1, 172.2, 172.6, 172.9, 175.3 (CO); TOF-MASS (positive mode, cinnamic acid): 1059.4 [2M + H − H_2O]$^+$, 1579.9 [3M + 2H − 2H_2O]$^+$, 2098.9 [4M + 3H − 3H_2O]$^+$, 2619.9 [5M + 4H − 4H_2O]$^+$.

Enzymatic elongation of the primer polymer

General procedure for the enzymatic galactosylation reaction. To acrylamide copolymer **12** or **13** or homopolymer **14** in HEPES buffer (pH 6, 50 mM, 1 cm^3) were added successively UDP-galactose (3 equiv. for **12** and **14**, 1.3 equiv. for **13**), α-lactalbumin (0.2 g dm^{-3}) and β-(1,4)-galactosyl transferase (1 U). The solution was incubated at 37 °C for 72 h. The mixture was directly subjected to gel filtration chromatography (Sephadex G-25, 42 × 2 cm) and eluted with water. The fractions containing galactosylated polymer were recombined, concentrated under reduced pressure and freeze-dried to afford the copolymer derivatives **28**, **29** or the homopolymer **30** as white powders.

*Galactosylation of copolymer **12**.* Copolymer **12** (10 mg, ~ 0.014 mmol GlcNAc) was used to prepare galactosylated copolymer **28** (10.4 mg, 84%, galactosylation extent: 50%) *via* the general procedure described above; δ_H (400 MHz; D_2O): 1.86 (3H, s, CH_3), 3.28 (1H, br s, 4-H), 4.30 (1H, d, *J* 7.81, 1-H), 4.39 (1H, br t, 1′-H); δ_C (100 MHz; D_2O): 23.0 ($COCH_3$), 36.6 (CH_2), 39.7 (CH_2NH), 42.3–42.9 (CH), 55.7, 56.2 (2-C and 2-C non substituted units), 60.8, 61.5, 61.8 (6-C, 6-C non substituted units, 6′-C), 69.3 (4′-C), 70.2–70.5 (OCH_2), 71.7 (4-C non substituted), 73.2 (2′-C), 74.6 (3-C), 75.5 (5′-C), 76.1 (3′-C), 76.6 (5-C), 79.1 (4-C), 101.8 (1-C), 103.6 (1′-C), 175.2 ($COCH_3$), 177.4 (CO), 180.2 ($CONH_2$).

*Galactosylation of copolymers **13**.* Copolymer **13** (10 mg, ~ 0.01 mmol GlcNAc) was used to prepare galactosylated copolymer **29** (11.6 mg, 100%, galactosylation extent: 100%) *via* the general procedure described above; δ_H (400 MHz; D_2O): 1.55 (4H, br s, CH_2, hexyl), 1.86 (3H, s, CH_3), 4.34 (2H, m, 1-H and 1′-H); δ_C (100 MHz; D_2O): 22.8 ($COCH_3$), 25.4–29.1 (CH_2, hexyl), 35.5 (CH_2), 40.1 (CH_2NH), 42.4–42.7 (CH), 55.9 (2-C), 60.9 (6-C), 61.6 (6′-C), 69.1 (OCH_2), 72.9 (2′-C), 76.1 (3′-C), 79.2 (4-C), 101.9 (1-C), 103.6 (1′-C), 180.2 ($CONH_2$).

*Galactosylation of homopolymer **14**.* Homopolymer **14** (10 mg, ~ 0.025 mmol GlcNAc) was used to prepare galactosylated homopolymer **30** (7.1 mg, 50%, galactosylation extent: 50%) *via* the general procedure described above; δ_H (400 MHz; D_2O): 1.87 (3H, s, CH_3), 3.27 (1H, br s, 4-H), 4.29 (1H, d, 1-H), 4.38 (1H, br t, 1′-H); δ_C (100 MHz; D_2O): 23.1 ($COCH_3$), 39.6 (CH_2-NH), 55.7 (2-C), 60.8 (6-C), 61.5 (6′-C), 69.3 (4′-C), 69.7–70.7 (OCH_2), 71.7 (4-C, non substituted), 73.2 (2′-C), 74.6 (3-C), 75.5 (5′-C), 76.1 (3′-C), 76.7 (5-C), 79.1 (4-C), 101.6 (1-C), 103.6 (1′-C), 175.0 ($COCH_3$), 176.9 (CO).

*α-(2,3)-Sialylation of copolymer **29**.* To a solution of **29** (3 mg, ~ 0.006 mmol LacNAc) in sodium cacodylate buffer (50 mM, pH 6.0, 1 cm^3) containing 0.1% Triton X-100 were added successively cytidine 5′-monophospho-*N*-acetylneuraminic acid (11 mg, 0.018 mmol), and α-(2,3)-sialyl transferase (0.3 U). The reaction mixture was incubated at 37 °C for 5 days, then directly subjected to gel filtration chromatography (G-25, 42 × 2 cm, eluent H_2O) to afford the sialylated glycopolymer **31** (6.5 mg, 75%) as a white powder after freeze-drying; δ_H (400 MHz; D_2O): 1.11 (4H, br s, CH_2), 1.82 (6H, br s, 2 × CH_3), 2.54 (1H, dd, *J* 4.58 and 12.36, 3-H_{eq}), 3.27 (1H, br s, 4-H), 4.33 (2H, m, 1-H and 1′-H); δ_C (100 MHz; D_2O): 22.7, 23.0 (2 × $COCH_3$), 25.4–30.9 (CH_2, hexyl), 35.6–36.6 (CH_2), 40.1, 40.3 (3″-C, CH_2-NH), 42.05–42.8 (CH), 52.3 (5″-C), 55.8 (2-C), 60.7 (6-C), 61.7 (6′-C), 63.2 (9″-C), 68.1 (4′-C), 68.8 (7″-C), 69.1 (4″-C), 69.8–

78.9 (4′-C, 7″-C, 4″-C, 2′-C, 8″-C, 3-C, 6″-C, 5-C, 5′-C, 3′-C, 4-C), 100.5 (2″-C), 101.8 (1′-C), 103.3 (1-C), 174.6–180.2 (CO).

Sialyl Lewis^x derivative ***32.*** To a solution of HEPES buffer (100 mM, 20 cm^3) were added $MnCl_2$·$4H_2O$ (10 mM, 36.9 mg) and NaN_3 (1 mM, 1.3 mg). The resulting solution was adjusted to pH 7.5. To a solution of **31** (6.5 mg, ~0.0044 mmol SialLacNAc) in the buffer described above (1 cm^3) were added successively cytidine 5′-monophospho-*N*-acetylneuraminic acid (8.4 mg, 0.013 mmol), and α-(1,3)-fucosyl transferaseV (10 mU). The reaction mixture was incubated at 37 °C for 3 days, then directly subjected to gel filtration chromatography (G-25, 42 × 2 cm, eluent H_2O) to afford the sialyl Lewis^x glycopolymer **32** (1.5 mg, 21%) as a white powder after freeze-drying; δ_H (400 MHz; D_2O, 70 °C): 1.48 (3H, d, *J* 6.26, CH_3 Fuc), 1.63 (4H, br s, CH_2), 2.34 (6H, s, 2 × CH_3), 3.07 (1H, br dd, 3″-H_{eq}), 3.46 (2H, m, CH_2), 4.83 (1H, m, 1-H Fuc).

Enzymatic elongation of sequential glycopeptides

Galactosylation of 27 (Method I). To a solution of **27** (16 mg, ~0.03 mmol GlcNAc) in HEPES buffer (50 mM, pH 7, 1 cm^3) containing $MnCl_2$ (5 mM) were added successively UDP-galactose (54.6 mg, 0.09 mmol), α-lactalbumin (0.2 g dm^{-3}) and β-(1,4)-galactosyl transferase (1 U). The reaction mixture was then incubated at 37 °C for 26 h and then directly subjected to gel filtration chromatography (G-25, 42 × 2 cm, eluent H_2O) to afford the galactosylated sequential glycopeptide **33** (20 mg, 96%) as a white powder after freeze-drying; δ_C (100 MHz; D_2O): 22.9 (CO*C*H_3), 29.05 (CH_2, propyl), 37.05 (Cβ, Asp), 43.4 (Cα, Gly), 51.6 (Cα, Asp), 55.8 (Cα, Ser), 56.3 (Cα, Ser), 60.75 (Cβ, Ser), 61.3 (6-C), 61.7 (6′-C), 68.4 (OCH_2), 69.2 (4′-C), 71.7 (4-C, non substituted), 73.2 (2′-C), 74.5 (3-C), 75.5 (5′-C), 76.05 (3′-C), 76.55 (5-C), 79.1 (4-C), 101.8 (1-C), 103.6 (1′-C), 172.2–175.3 (CO); TOF-MASS (positive mode, cinnamic acid): 1220.8 [2M + 1Gal − H_2O]$^+$, 1381.0 [2M + 2Gal − H_2O]$^+$, 1902.7 [3M + 2Gal − 2H_2O]$^+$, 2064.0 [3M + 3Gal − 2H_2O]$^+$.

Galactosylation of 27 (Method II). To a solution of **27** (16 mg, ~0.03 mmol GlcNAc) in sodium cacodylate buffer (50 mM, pH 7.5, 1 cm^3) containing $MgCl_2$ (5 mM), $MnCl_2$ (25 mM) and dithiothreitol (0.2 mM) were added successively UDP-galactose (36.4 mg, 0.06 mmol), α-lactalbumin (0.2 g dm^{-3}) and β-(1,4)-galactosyl transferase (1 U). The reaction mixture was then incubated at 25 °C for 21 h, then UDP-Gal (9.5 mg, 0.015 mmol) was added. More UDP-Gal (9.5 mg, 0.015 mmol) was added after 25 h of incubation. The reaction mixture was incubated for 47 more hours and then directly subjected to gel filtration chromatography (G-25, 42 × 2 cm, eluent H_2O) to afford the galactosylated sequential glycopeptide **33′** (17.3 mg, 84%) as a white powder after freeze-drying; δ_C (100 MHz; D_2O): 22.9 (CO*C*H_3), 29.0 (CH_2, propyl), 37.0 (Cβ, Asp), 43.3 (Cα, Gly), 51.4 (Cα, Asp), 55.8 (Cα, Ser), 56.5 (2-C), 60.8 (Cβ, Ser), 61.4 (6-C), 61.7 (6′-C), 68.4 (OCH_2), 69.2 (4′-C), 71.7 (2′-C), 73.1 (3-C), 73.2 (5′-C), 75.5 (3′-C), 76.1 (5-C), 79.1 (4-C), 101.8 (1-C), 103.6 (1′-C), 172.1–175.2 (CO); TOF-MASS (positive mode, cinnamic acid): 1381.0 [2M + 2Gal − H_2O]$^+$, 2064.0 [3M + 3Gal − 2H_2O]$^+$, 2747.0 [4M + 4Gal − 3H_2O]$^+$.

Galactosylation of 25 (Method II). Compound **25** (10 mg, 0.016 mmol GlcNAc) was used to prepare **34** (7 mg, 55%) *via* the procedure described above for **33′**. The reaction mixture was incubated for 45 h and UDP-Gal was added after 18 h and 22 h of incubation respectively; δ_C (100 MHz; D_2O): 22.9 (CO*C*H_3), 39.7 (CH_2-NH), 43.3 (Cα, Gly), 51.4 (Cα, Asp), 55.7 (Cα, Ser), 56.2 (2-C), 60.7 (Cβ, Ser), 61.4 (6-C), 61.7 (6′-C), 68.4 (OCH_2), 69.2 (4′-C), 71.6 (2′-C), 73.2 (4-C, non substituted), 74.6 (3-C), 75.5 (5′-C), 76.1 (3′-C), 76.6 (5-C), 79.1 (4-C), 101.7 (1-C), 103.6 (1′-C), 172.1–175.2 (CO).

Galactosylation of 26 (Method II). Compound **26** (12.4 mg, 0.021 mmol GlcNAc) was used to prepare **35** (10 mg, 63%) *via* the procedure described above for **33′**. The reaction mixture was incubated for 72 h and UDP-Gal was added after 22 and 26 h of incubation respectively; δ_C (100 MHz; D_2O): 22.9 (CO*C*H_3), 25.45, 26.4, 29.0, 29.2 (CH_2, hexyl), 40.2 (N-CH_2), 43.4 (Cα, Gly), 51.5 (Cα, Asp), 55.8 (Cα, Ser), 56.3 (2-C), 60.8 (Cβ, Ser), 61.5 (6-C), 61.7 (6′-C), 69.3 (OCH_2), 71.4 (4-C, non substituted), 71.7 (4′-C), 73.2 (2′-C), 74.5 (3-C), 75.4 (5′-C), 76.1 (3′-C), 76.6 (5-C), 79.1 (4-C), 101.8 (1-C), 103.6 (1′-C), 172.25–175.0 (CO); TOF-MASS (positive mode, cinnamic acid): 1303.4 [2M + 1Gal − H_2O]$^+$, 1465.2 [2M + 2Gal − 2H_2O]$^+$, 2029.5 [3M + 2Gal − 2H_2O]$^+$, 2188.8 [3M + 3Gal − 2H_2O]$^+$.

α-2,6-Sialylation of 33′. A solution of BSA (20 mg), NaN_3 (9.34 mg) and $MnCl_2$ (312 mg) in sodium cacodylate buffer (50 mM, 10 cm^3) was adjusted to pH 7.40. To this solution (1 cm^3) was added successively **33′** (8.54 mg, ~0.012 mmol LacNAc), cytidine 5′-monophospho-*N*-acetylneuraminic acid (15 mg, 0.024 mmol), calf intestinal alkaline phosphatase (10 U) and α-(2,6)-sialyl transferase (0.1 U). The reaction mixture was incubated at 37 °C for 48 h, and then directly subjected to gel filtration chromatography (G-25, 42 × 2 cm, eluent H_2O) to afford the sequential sialylated glycopeptide **36** (7 mg, 58%) as a white powder after freeze-drying; δ_C (100 MHz; D_2O): 22.7, 23.3 (2 × CO*C* H_3), 29.0 (CH_2, propyl), 36.9 (Cβ, Asp), 40.8 (CH_2-NH), 43.3 (Cα, Gly), 48.2 (3″-C), 51.5, 51.7 (Cα, Asp, Cα, Ser), 55.6 (2-C), 61.1 (6-C), 61.8 (Cβ, Ser), 63.3 (9″-C), 64.0 (6′-C), 68.4 (OCH_2), 68.9–69.2 (4′-C, 4″-C, 2″-C), 72.4 (8″-C), 71.4 (2′-C), 73.1 (3-C), 73.2 (6″-C), 73.3 (5′-C), 75.5 (3′-C), 74.4 (5-C), 75.2 (4-C), 100.85 (2″-C), 101.6 (1-C), 104.2 (1′-C), 134.1 (COOH), 174.3, 175.6 (CO).

α-2,3-Sialylation of 33′. To a solution of **33′** (3 mg, ~0.0043 mmol LacNAc) in sodium cacodylate buffer (50 mM, pH 6.0, 0.5 cm^3) containing 0.1% Triton X-100 were added successively cytidine 5′-monophospho-*N*-acetylneuraminic acid (8 mg, 0.013 mmol), and α-(2,3)-sialyl transferase (0.3 U). The reaction mixture was incubated at 37 °C for 5 days and then directly subjected to gel filtration chromatography (G-25, 42 × 2 cm, eluent H_2O) to afford the sequential sialylated glycopeptide **37** (2 mg, 47%) as a white powder after freeze-drying; δ_H (400 MHz; D_2O): 1.65 (3H, m, 3-H_{ax}, CH_2), 1.92 (6H, br s, 2 × CH_3), 2.69 (1H, m, Aspβ, 3-H_{eq}), 3.09 (1H, br dd, Aspβ), 4.39 (2H, m, 1-H and 1′-H).

Sialyl Lewis^x derivative 38. To a solution of HEPES buffer (100 mM, 20 cm^3) were added $MnCl_2$·$4H_2O$ (10 mM, 36.9 mg) and NaN_3 (1 mM, 1.3 mg). This resulting solution was adjusted to pH 7.5. To a solution of **37** (1 mg, ~0.001 mmol SialLacNAc) in the buffer described above (0.5 cm^3) were added successively GDP-fucose (2 mg, 0.003 mmol), and α-(1,3)-fucosyl transferaseV (10 mU). The reaction mixture was incubated at 37 °C for 3 days then directly subjected to gel filtration chromatography (G-25, 42 × 2 cm, eluent H_2O) to afford the sialyl Lewis^x sequential glycopeptide **38** (1.1 mg, 100%) as a white powder after freeze-drying; δ_H (400 MHz; D_2O): 1.18 (3H, d, *J* , CH_3 Fuc), 1.80 (3H, m, 3-H_{ax}, CH_2), 2.05 (6H, br s, 2 × CH_3), 2.77 (1H, m, Aspβ, 3-H_{eq}), 3.09 (1H, br dd, Aspβ), 4.39 (2H, m, 1-H and 1′-H).

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