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Preparation of poly-*N*-(methyl)aminooxy serine polypeptides by NCA ring-opening polymerization for modification with reducing oligosaccharides

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N-Carboxyanhydride (NCA) ring-opening polymerization offers an attractive approach for the construction of polypeptides. Here, we report the synthesis of a serine NCA-functionalized monomer bearing a methylaminooxy group, enabling post-polymerization attachment of complex oligosaccharides *via* a neo-glycosylation reaction. It provides an attractive alternative to traditional click reactions, allowing direct conjugation of glycans with a free reducing end without requiring a reactive linker or toxic reagents. Enzymatically produced 6-sialyl lactose was efficiently conjugated to the methylaminooxy moieties of the polypeptide. The resulting neo-glycopolymer was analyzed by size-exclusion chromatography (SEC) to determine molecular weight and by diffusion-ordered spectroscopy (DOSY) nuclear magnetic resonance (NMR), which confirmed a high degree of functionalization and a substantial increase in hydrodynamic radius. Glycan attachment proceeds under mild acidic conditions highlighting the versatility of the polypeptide scaffold to yield brush-like glycosylated polypeptides.

Introduction

α-Amino acid *N*-carboxyanhydride (NCA) ring-opening polymerization is a versatile methodology for producing polypeptides with tunable properties.[1–4] The attraction of NCA polymerization is that it replicates features of natural polypeptides while maintaining the flexibility to incorporate diverse functional groups. It can be performed under mild conditions and is compatible with a variety of α-amino acids including those having unnatural functional groups.[4–7] In addition, the ring-opening polymerization allows for control over molecular weight, composition, and architecture enabling the design of sophisticated materials.[2,8–12]

NCA ring opening polymerization has been used to generate highly glycosylated polypeptides that mimic biomolecules such as mucins.[13–15] Two strategies have been pursued to prepare such polymers based on pre- or post-polymerization attachment of carbohydrates.[16] In the first approach, a fully protected glycosylated amino acid is converted into an NCA and then co-polymerized with other NCA amino acids followed by deprotection.[17] The limitation of this strategy lies in the difficulties of preparing monomers having a complex carbohydrate moiety and its use has been limited to mono- and disaccharides.[18,19] In an alternative strategy, polypeptides are prepared using amino acids having a reactive site chain such as azide, alkyne, alkene or an amine that can be derivatized with a glycan having a complementary reactive group by for example azide–alkyne cycloaddition, thiol-Michael addition or Diels–Alder reaction.[20–22] This approach made it possible to install a greater variety of oligosaccharides,[23] however, it usually involves the introduction of unnatural linkers and modification of the glycans with a suitable reactive group.

Glycosylated polypeptides prepared by NCA have provided selective ligands for various lectins, giving opportunities for targeted drug delivery and diagnostic assay development.[20,21,24,25] Such polymers have also been employed for the development of lysosome-targeting chimaeras (LYTACs) that consist of a monoclonal antibody or small molecule fused to a polypeptide functionalized with mannose-6-phosphate moieties.[26] Glycosylated polypeptides have been used as immunomodulating agents by targeting Dectin-1 and Dectin-2 to activate immune cells.[18] Other applications include glycopolypeptide-containing hydrogels for culturing hepatocytes. This application exploits the sialoglycoprotein receptor of hepatocytes to bind to galactosyl moieties of the hydrogel to increase cell viability, differentiation and proliferation.[25] Furthermore, nanogels containing glucose have been used to encapsulate

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insulin that releases at a rate depending on the concentration of glucose.[27]

Here, we describe the synthesis of a serine derived NCA (**1**) bearing a *tert*-butyloxycarbonyl (Boc) protected *N*-methyl aminooxy moiety. It can readily be polymerized and after removal of the Boc protecting group, the aminooxy containing polypeptide can be modified by reducing complex carbohydrates by oxime ligation. Aminooxy derivatives (oximes) are attractive for bioconjugation and can react with aldehydes and ketones under mild conditions.[28] *N*-Methyl aminooxy containing compounds have been attached to reducing glycans[29–32] to give conjugates preserving the cyclic pyranosyl ring structure of the conjugated saccharide[29,33] Conventional peptide chemistry has been employed to prepare glycopeptides having an *N*-methyl aminooxy moiety for attachment of a variety of different saccharides to give compounds maintaining biological activity.[32,34] These finding motivated us to develop monomer **1** for NCA ring-opening polymerization followed by conjugation of reducing saccharides to give neoglycopolymers for studying biological roles of glycosylation, probing molecular recognition events, and developing multivalent ligands for various biomedical applications (Scheme 1). It makes it possible, for the first time, to modify a polypeptide prepared by NCA with complex glycans without the need for an artificial spacer and the use of toxic reagents.

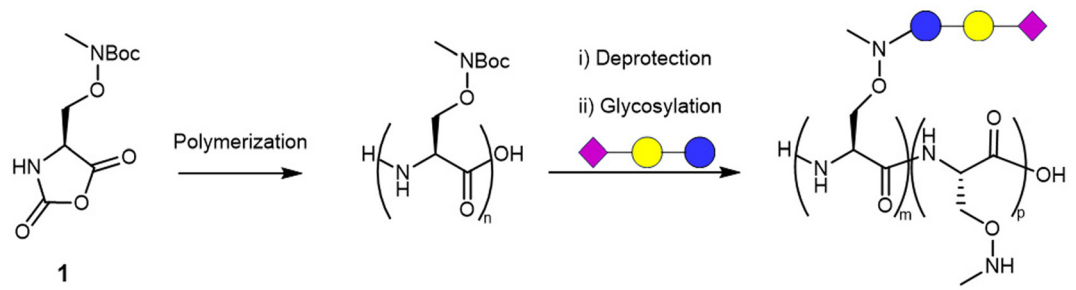
Results and discussion

Design and synthesis of the monomer

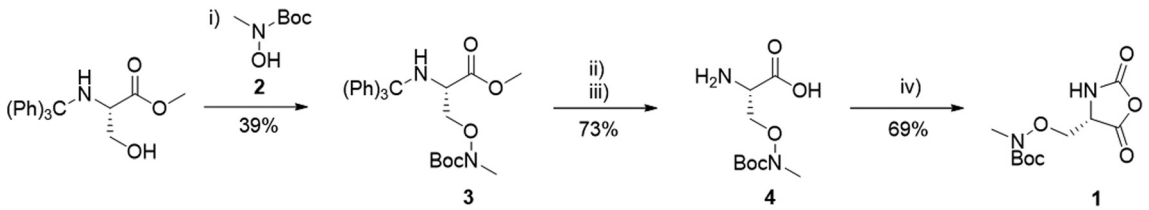
We designed a synthetic route for monomer **1** having a Boc-protected aminooxy moiety at the side chain. The *N*-methyl of the aminooxy functionality ensures preservation of the cyclic structure of the saccharide moiety after conjugation.[35] A Mitsunobu reaction of *N*-trityl-L-serine methyl ester with *tert*-butyl hydroxy(methyl)carbamate (**2**), was prepared by a literature procedure,[36] using diisopropyl azodicarboxylate (DIAD) in the presence of triphenyl phosphine gave, after purification by silica gel column chromatography, modified protected amino acid **3**. The trityl protecting group of the α-amine of **4** was selectively removed using 4% trifluoro acetic acid (TFA) in DCM at 0 °C, which was followed by hydrolysis of the methyl ester employing sodium hydroxide in THF generating compound **4** that was isolated by reverse phase silica gel chromatography. The conversion of **4** into NCA **1** was achieved using a recently reported moisture-tolerant procedure[37] entailing treatment with propylene oxide and triphosgene in THF. The purification of **1** was challenging due to its sensitivity to hydrolysis or unwanted polymerization. Ultimately, compound **1** could be obtained by purification using column chromatography on anhydrous silica gel prepared through oven desiccation (Scheme 2).[38]

Polymerization and characterization of the polypeptide

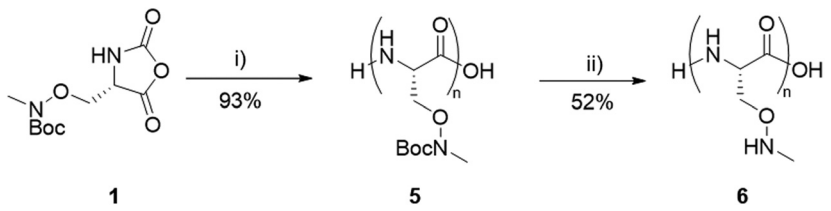
Monomer **1** was polymerized using lithium bis(trimethylsilyl) amide (LiHMDS)[12] as initiator in a ratio of 1 : 50 (mol mol^{-1} [*I*/*M*]) in anhydrous DCM for 1.5 h under an atmosphere of argon. In this reaction, the hindered base generates an N-terminal carbamate anion that serves as the reactive centre for fast chain propagation (Scheme 3). The resulting polymer (**5**) was precipitated by pouring the reaction mixture into ice cold mixture of PE/Et_2O (1 : 1 v : v). The polymer was analysed by MALDI-TOF MS and SEC. The molecular weight of the



Scheme 1 General representation of NCA-polymerization of compound **1** to generate a polypeptide-based scaffold that can be glycosylated with free reducing end oligosaccharides.



Scheme 2 *N*-Carboxy anhydride (NCA) monomer synthesis. (i) *N*-Trityl-L-serine methyl ester, PPh_3, DIAD, toluene; (ii) TFA, DCM. (iii) THF/NaOH aq. (0.3 M); (iv) propylene oxide, triphosgene, THF.

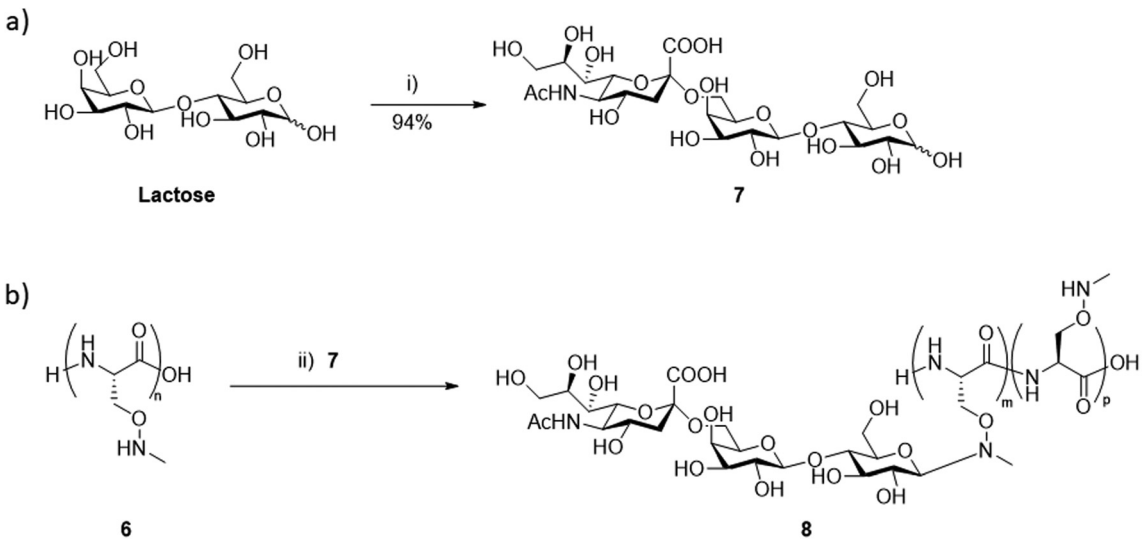


Scheme 3 i) Polymerization of the monomer (**4**) using LiHMDS in a ratio 1 : 50 (mol mol^{-1} LiHMDS/**4**) in anhydrous DCM under an argon atmosphere. (ii) Boc deprotection of polymer **5** using TFA/H_2O (1 : 1).

polymer was determined by SEC to be approximately 15 kDa ($n \approx 60$) with a polydispersity index of 1.06. MALDI-TOF MS showed signals with a difference of +217 Da, which is the molecular weight of the repeating unit of the expected polymer consistent with previously reported MALDI-TOF data of this class of polymers.[12,37] The average molecular weight determined by MALDI-TOF (2571 Da, $n = 12$) is lower than measured by SEC which most like is due to the higher ionization coefficient of smaller fragments. It is well known that MS underestimates the average molecular weight of this type of polymer.[39] The Boc protecting group of the *N*-methyl aminooxy functionality of the side chain of this polymer was removed using a mixture of TFA (50%) in water (Scheme 2). The deprotected polymer was isolated by size exclusion column chromatography using extra fine polyacrylamide beads (P2 Bio-gel) and ammonium bicarbonate buffer (20 mM) as the eluent. The resulting polymer was characterized by NMR spectroscopy to determine successful Boc deprotection and as expected the NMR signal of Boc (δ 1.45, 9H) disappeared after the acid treatment of the polymer. Moreover, an upfield shift was observed for the methyl group present on the aminooxy group (δ 3.08 to δ 2.58, N–Me, 3H) and the doublet of the beta CH_2 of the amino acid merged into a broad singlet (δ 3.97, 2H) while the alpha proton is observed at the same chemical shift without significant variation (δ4.65, 1H) indicating the integrity of the deprotected polymer after acid treatment for Boc deprotection (see SI). Unfortunately, SEC analysis was not possible for the deprotected polymer **6** due to interactions between the polymer and the stationary phase.

Ligation of enzymatically synthesized 6-sialyl-lactose

With the fully deprotected polymer **6** in hand, attention was focused on selective ligation of a 6-sialylated lactose (7) having a free reducing end that was synthesized by the reaction of lactose with Cytidine-5′-monophospho-*N*-acetylneuraminic acid (CMP-Neu5Ac) in the presence of the mutated sialyl transferase PmST1 (P34H/M144L)[40] (Scheme 4a). Polymer **6** and Neu5Ac-(2,6)-Lac (7) (1 eq. per aminooxy functionality) were dissolved in a mixture of DMF, H_2O and acetic acid (1 : 1 : 2, v/v/v) and the resulting solution was agitated for 24 h at 37 °C (Scheme 4b). The glycosylated polymer **8** was purified by P2 Biogel size exclusion chromatography using ammonium bicarbonate (50 mM, pH = 7.4) as the eluent. The resulting glycopolymer was characterized by NMR to determine the successful attachment of the glycan to the polymer and the degree of functionalization. The ^{1}H NMR and ^{1}H–^{13}C HSQC spectra of glycosylated polymer is depicted in Fig. 1, and the NMR data are given in Table 1. Compared with the spectrum of the non-



Scheme 4 a) Enzymatic synthesis of compound **7** using PmST1 (P34H/M144L), lactose, CMP-Neu5Ac in Tris buffer (100 mM pH = 8.5) containing $MgCl_2$ (20 mM). (b) Neoglycosylation of the polypeptide with oligosaccaride **7** in DMF/H_2O/AcOH (1 : 1 : 2).

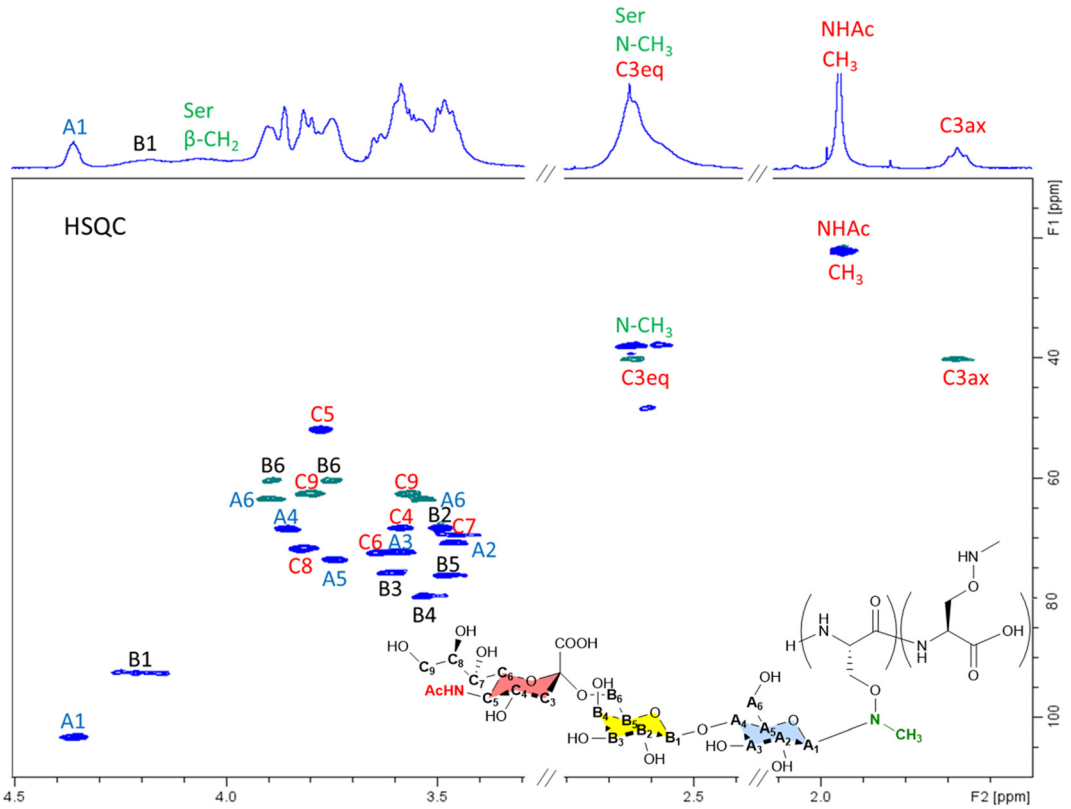


Fig. 1 ^{1}H NMR and ^{1}H–^{13}C HSQC spectra of glycosylated polymer recorded in D_2O at 298 K. Resonance assignment has been annotated.

Table 1 ^{1}H and ^{13}C chemical shifts of glycosylated polymer (**8**) recorded in D_2O at 298 K

Residue	H1/C1	H2/C2	H3/C3	H4/C4	H5/C5	H6/C6	H7/C7	H8/C8	H9/C9	NHAc
Gal	4.36	3.46	3.59	3.86	3.74	3.90, 3.53	—	—	—	—
A	103.3	70.8	72.5	68.6	73.7	63.5				
Glc	~4.18	3.49	3.61	3.53	3.48	3.89, 3.75	—	—	—	—
B	92.6	68.5	75.8	79.7	76.2	60.4				
Neu5Ac	—	—	1.68, 2.64	3.59	8.78	3.64	3.46	3.82	3.80, 3.57	1.95
C	174.0	101.3	40.1	68.4	51.9	72.5	69.6	71.9	62.6	22.1

	N–H	α-CH	β-CH_2	N–CH_3
Ser	7.95	~4.57 n.d	~4.07 n.d.	~2.64 38.0

glycosylated polymer **6** (Fig. 2), broad signals of β-CH_2 Ser observed at $\delta \sim 4.07$ have shifted downfield under influence of substitution with Glc (**B**) residues. The widely distribution of β-Glc (**B**) anomeric protons at $\delta \sim 4.18$ indicates heterogeneity in molecular weight of the glycosylated polymer (Fig. 1). Full assignment of β-Gal (**A**), β-Glc (**B**) and α-Neu5Ac (**C**) and a partial assignment of Ser was deduced from 2D TOCSY spectra (see SI, Fig. S1a). In the 2D NOESY spectrum the strong inter-residual connectivity (NOE) between H-1's of β-Glc (**B**) and *N*–CH_3 of Ser, indicate that β-Glc is attached *via* N-linkage to polypeptide backbone (see SI, Fig. S1b). The degree of functionalization was determined by the integration of signals of Ser N–CH_3 and Neu5Ac H-3eq at δ 2.62 with Neu5Ac H-3ax at δ 1.68. The peak area of the well resolved Neu5Ac H-3ax was subtracted from the peak area of Ser N–CH_3/Neu5Ac H-3eq, giving a degree of functionalization of ~50%. The use of 1.5 eq. of sialoglycan **7** did not result in a notable increase in functionalization, which most likely is due to steric hinderance caused by the already attached glycans.

Glycopolymer **8** was further analyzed and compared to the non-glycosylated polymer **6** and sialyllactose **7** by 2D ^{1}H-DOSY NMR, which provides information about the size and shape of molecules.[41,42] As expected, the glycopolymer has a lower diffusion coefficient (2.04×10^{-10} m^2 s^{-1}) compared to non-glycosylated polymer (2.44×10^{-10} m^2 s^{-1}) (Fig. 2 and Table 2). The DOSY spectrum of sialyllactose **7** displayed a single set of

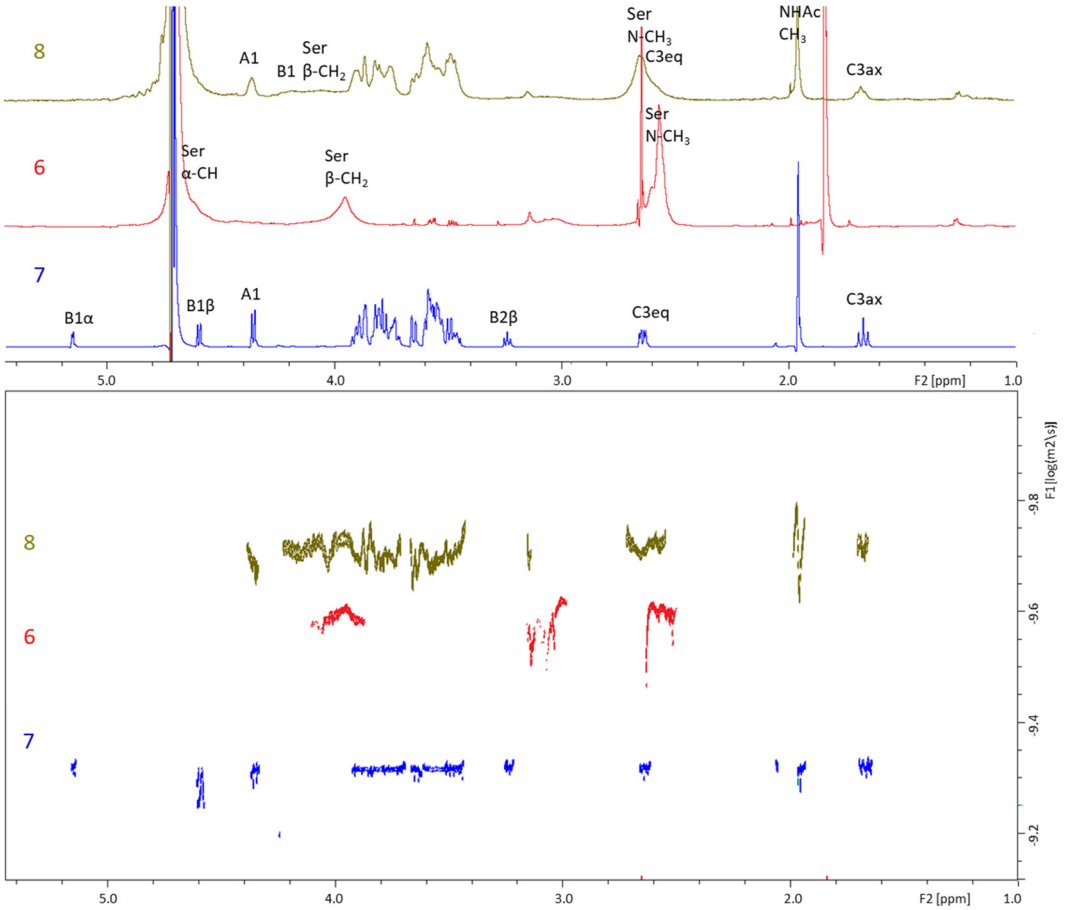


Fig. 2 2D-DOSY NMR of the glycosylated polymer **8**, the non-glycosylated polymer **6**, and sialyllactose **7**.

Table 2 Diffusion coefficients of the glycosylated polymer **8**, non-glycosylated polymer **6** and sialyllactose **7**, determined by diffusion ordered spectroscopy NMR experiments (2D ^{1}H-DOSY-NMR)

Compound	DOSY diffusion coefficient, D (m^2 s^{-1})
Glycosylated polymer (**8**)	2.04×10^{-10}
Sialyllactose (**6**)	2.96×10^{-9}
Non-glycosylated polymer (**7**)	2.44×10^{-10}

NMR signals (Fig. 2), indicating a single state, in which the molecules behave as a homogeneous unit, leading to a single diffusion coefficient. In contrast, the non-glycosylated polymer **6** and the glycosylated polymer **8**, exhibit dispersed signals, indicating they are in slow exchange and possess a wide range of molecular sizes (polydispersity), which agrees with the MALDI-TOF data. This observation made the analysis of molecular weight by DOSY difficult since polymers often do not follow expected linear trend with respect to molecular weight.[41]

The glycopolymer dissolved well in water, PBS and ammonium bicarbonate buffer (50 mM, pH = 7.4). After an incubation time of 7 days in D_2O, no notable change in the diffusion coefficient was measured by DOSY NMR indicating it had remained intact. It is well established that polypeptides have good stability under physiological conditions. Furthermore, oximes are stable at pH > 7,[43] which is also the case for sialosides, making the new materials attractive for biomedical applications.

Conclusions

In this study, we designed, synthesized and characterized an NCA having an appropriately protected aminooxy functionality. LiHMDS-mediated ring opening polymerization yielded a polypeptide that after removal of a Boc protecting group revealed an *N*-methyl aminooxy functionality suitable for neo-glycosylation. It could be functionalized with a complex oligosaccharide such as 6-sialyl lactose bearing a free reducing end under mild acidic conditions. A successful glycosylation and the degree of functionalization was confirmed by NMR and diffusion coefficients of both the glycosylated and non-glycosylated polymer. The attraction of ligation of reducing glycans with *N*-methyl aminooxy containing compounds is that the pyranosyl structure is maintained without introducing unnatural linkers. The NCA polymerization allows for functionalization of a large range of complex oligosaccharides having a reducing end, to give neoglycopolymers for various applications. Such saccha-

ides can be obtained from natural sources or through chemical- or chemoenzymatic synthesis.[44,45] The use of complex oligosaccharides is of particular interest because such compounds are expected to bind much more selectively to glycan binding proteins. The resulting highly glycosylated polymers can mimic mucins[46] that may find multiple application for example as antiviral agent.

Experimental

tert-Butyl hydroxy(methyl)carbamate (2)

tert-Butyl hydroxy(methyl)carbamate (**2**) was made according to a literary procedure.[36] *N*-Methyl hydroxylamine hydrochloride (16.72 g, 0.2 mol) was dissolved in = THF/H_2O (80 mL, 1/1, v/v). The mixture was cooled in an ice bath and K_2CO_3 (16.7 g, 0.12 mol) was added. Di-*tert*-butyl dicarbonate (48.3 g, 0.22 mol) was dissolved in THF (60 mL) and added dropwise to the mixture over a period of 45 min. The resulting reaction mixture was stirred for 1.5 h at 0 °C and then for 1.5 h at room temperature. The reaction mixture was concentrated *in vacuo* and the residue redissolved in DCM (100 mL) and then washed with $NaHCO_3$ (40 mL), twice with H_2O (40 mL) and once with brine (50 mL). The organic layer was dried ($MgSO_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified by silica gel column chromatography using a gradient of pure toluene to 10% EtOAc in toluene to yield *tert*-butyl hydroxy(methyl)carbamate **2** as a clear oil (17.6 g, 0.12 mol, 60%). ^{1}H NMR (400 MHz, Chloroform-*d*): δ 6.56 (1H, br s, OH), 3.14 (3H, s, N–CH_3), 1.47 (9H, s, CH_3, Boc). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 157.7 (C═O Boc), 82.1 (CMe_3 Boc), 37.8 (NMe), 28.4 (3 × CH_3, Boc).[47] ESI-TOF: *m*/*z* calculated for $C_6H_{14}NO_3^+$ (M + H) 148.090, found 148.092.

Methyl *O*-((*tert*-butoxycarbonyl)(methyl)amino)-*N*-trityl-L-serinate (3)

N-Trityl-L-serine methyl ester (10.02 g, 27.7 mmol), *tert*-butyl hydroxy(methyl)carbamate **2** (5.01 g, 34.0 mmol) and triphenylphosphine (8.68 g, 33.1 mmol) were dissolved in toluene (60 mL) and the resulting solution was placed under an atmosphere of N_2. The mixture was cooled in an ice bath and diisopropyl-azodicarboxylate (DIAD, 7.0 mL, 33.5 mmol) was added dropwise. The reaction mixture was heated under reflux for 2 h. Next, the reaction mixture was concentrated *in vacuo* and the residue redissolved in DCM (150 mL). The organic layer was washed twice with H_2O (150 mL) and once with brine (200 mL). The organic layer was dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo*. The residue was purified by silica gel column chromatography using a gradient from 1% EtOAc in PE to 10% EtOAc in PE to give methyl *O*-((*tert*-butoxycarbonyl)(methyl)amino)-*N*-trityl-L-serinate (Tr-Ser(N(Me)Boc)-OMe) **3** (5.32 g, 10.8 mmol, 39% as a clear oil). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.50 (d, *J* = 7.3 Hz, 6H, Tr), 7.25 (t, *J* = 7.7 Hz, 6H, Tr), 7.18 (t, *J* = 7.3 Hz, 3H, Tr), 4.18 (dd, *J* = 9.4, 4.3 Hz, 1H, β-CH_2), 3.85 (dd, *J* = 9.4, 6.7 Hz, 1H, β-CH_2), 3.54 (ddd, *J* = 10.7, 6.7, 4.3 Hz, 1H, α-CH), 3.24 (s, 3H, O–CH_3), 3.03 (s, 3H, N–CH_3), 1.48 (s, 9H, CH_3, Boc). ^{13}C NMR (151 MHz, Chloroform-*d*) δ 173.60 (C═O, Boc), 145.75(C═O, CO_2Me), 128.78 (Tr, Ar), 127.90 (Tr, Ar), 126.53 (Tr, Ar), 81.42 (C, Boc), 71.10 (C, Tr), 55.56 (β-CH_2), 51.86 (α-CH), 36.66 (CH_3, C–O), 30.95 (CH_3, C–N), 28.30 (CH_3, Boc). ESI-TOF: *m*/*z* calculated for $C_{29}H_{35}N_2O_5^+$ (M + H) 491.247, found 491.245.

O-((*tert*-Butoxycarbonyl)(methyl)amino)-L-serine (4)

Methyl *O*-((*tert*-butoxycarbonyl)(methyl)amino)-*N*-trityl-L-serinate **3** (5.32 g, 10.84 mmol) was dissolved in DCM (40 mL) and several drops of MeOH were added. The mixture was placed in an ice bath and TFA (0.8 mL) was added dropwise. After stirring the reaction mixture for 2 h, another portion of TFA (0.4 mL) was added dropwise and after another 2.5 h another portion of TFA (0.4 mL) was added dropwise. After 5 h, the reaction was quenched with saturated aqueous $NaHCO_3$ (40 mL) and the aqueous layer was washed three times with DCM (50 mL). The combined organic layers were dried ($MgSO_4$), filtered and filtrate concentrated *in vacuo.* Without further purification, the crude product (2.13 g, 8.58 mmol) was dissolved in THF (60 mL) and a solution of H_2O (21 mL) and 1 M aqueous NaOH (9 mL) was added dropwise on ice over the course of half an hour. Acetic acid was added until a neutral pH was reached, and the mixture was concentrated *in vacuo*. The residue was purified using reverse phase C_{18} chromatography using a mixture of 5% MeCN in H_2O. Fractions were checked by MS and those containing the product were combined and lyophilized to yielded *O*-((*tert*-butoxycarbonyl)(methyl)amino)-L-serine **4** (1.46, 73%) as a white amorphous solid. ^{1}H NMR (400 MHz, deuterium oxide) δ 4.34–4.28 (m, 2H, β-CH_2), 4.04–3.98 (m, 1H), α-CH_2, 3.16 (d, *J* = 1.2 Hz, 3H, N–CH_3), 1.51 (s, 9H, Boc).^{13}C NMR (101 MHz, deuterium oxide) δ 170.89 (C═O, Boc), 158.16 (C═O, CO_2H), 84.21 (C, Boc), 71.46 (β-CH_2), 53.41 (α-CH), 35.68 (N–CH_3), 27.36 (CH_3, Boc). ESI-TOF: *m*/*z* calculated for $C_9H_{19}N_2O_5^+$ (M + H) 235.122, found 235.129.

N-Carboxyanhydride *O*-((*tert*-butoxycarbonyl)(methyl)amino)-L-serine (1)

O-((*tert*-Butoxycarbonyl)(methyl)amino)-L-serine **4** (100.8 mg, 0.43 mmol) was dissolved in THF (15 mL) and propylene oxide (0.2 mL, 2.85 mmol) was added followed by triphosgene (83 mg, 0.28 mmol). The reaction mixture was stirred at room temperature for 1.5 h. The reaction mixture was cooled in an ice bath (3 mi.) and then quenched with water (15 mL). The aqueous layer was washed with EtOAc (2 × 40 mL) and the combined organic layers were washed with brine (80 mL). The organic layer was dried ($MgSO_4$), filtered and the filtrate concentrated *in vacuo*. The residue was purified using anhydrous flash silica gel column chromatography (dry solvents, oven-dried silica gel and under N_2 stream)[27] using PE/EtOAc (1/1, v/v) to yield *N*-carboxyanhydride *O*-((*tert*-butoxycarbonyl)(methyl)amino)-L-serine **1** (77.0 mg, 0.29 mmol, 69%) as a white amorphous solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.58 (dd, *J* = 10.0, 3.1 Hz, 1H, β-CH_2), 4.22 (dd, *J* = 11.8, 3.4 Hz, 1H, β-CH_2), 3.90 (dd, *J* = 11.8, 9.9 Hz, 1H, α-CH_2), 3.08 (s, 3H, N–CH_3), 1.47 (s, 9H, Boc). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 176.89, 164.16, 146.10, 90.21, 77.46, 59.41, 41.68, 33.36.

ESI-TOF: *m*/*z* calculated for $C_{10}H_{17}N_2O_6^+$ (M + H) 261.108, found 261.110.

Poly-*O*-((*tert*-butoxycarbonyl)(methyl)amino)-L-serine (5)

N-Carboxyanhydride *O*-((*tert*-butoxycarbonyl)(methyl)amino)-L-serine **1** (65 mg, 0.25 mmol) was dissolved in DCM (2.3 mL). Lithium-bis-(trimethylsilyl)-amid (0.1 mL, 1 M in THF) was diluted in anhydrous DCM (1.9 mL, 50 mM). From the resulting solution 0.1 mL (5 μmol 1 : 50 *I*/*M*) were added at once to the reaction vessel and the reaction was left stirring for 1.5 h at room temperature. The reaction mixture was then poured into cold PE/Et_2O (1 : 1, 10 mL). The mixture was centrifuged at 4500 rpm for 5 min, the supernatant was removed and the pellet was washed with PE (30 mL, 3×) to yield poly-*O*-((*tert*-butoxycarbonyl)(methyl)amino)-L-serine **5** (50 mg, 93%, 0.003 mmol) as a white powder. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.65 (bs, 1H, α-CH), 4.17 (bs, 1H, β-CH_2), 4.00 (bs, 1H, β-CH_2), 3.08 (bs, 3H, N–CH_3), 1.44 (bs, 9H, Boc). ^{1}H–^{13}C NMR (400, 101 MHz, Chloroform-*d*) δ (4.65, 52.36), (4.17, 73.21), (4.03, 73.21), (3.06, 37.15), (1.44, 28.32). The ^{13}C signals were obtained from an HSQC experiment.

Poly-*O*-(methylamino)-L-serine (6)

Poly-*O*-((*tert*-butoxycarbonyl)(methyl)amino)-L-serine **5** (5.7 mg, 0.4 μmol) was dissolved in TFA/H_2O (1.5 mL, 1/1, v/v). The reaction mixture was left stirring at room temperature for 4.5 hours. The reaction mixture was then diluted with H_2O until the TFA concentration was 10% (v/v) and then lyophilized. The residue was purified by size exclusion column chromatography using extra fine polyacrylamide beads (P2 Biogel) and ammonium bicarbonate buffer (20 mM) as the eluent to yielded poly-*O*-(methylamino)-L-serine **6** as a white powder (1.6 mg, 0.21 μmol, 52%). ^{1}H NMR (600 MHz, deuterium oxide) δ 4.69 (bs, 1H, α-CH), 4.04 (bs, 2H, β-CH_2), 2.65 (bs, 3H, N–Me). ^{1}H–^{13}C NMR (600, 151 MHz, deuterium oxide) δ (4.69, 53.20), (4.04, 71.29), (2.65, 37.67). The ^{13}C signals were obtained from an HSQC experiment.

α2,6-Sialyllactose (7)

α2,6-Sialyllactose (7) was prepared enzymatically according to a reported procedure.[40] D-Lactose monohydrate (20.2 mg, 0.056 mmol) and CMP-Neu5Ac (70.8 mg, 0.11 mmol) were dissolved in Tris-HCl (pH 8.5, 1 M, 100 μL), MilliQ (780 μL) and aqueous $MgCl_2$ (0.2 M, 100 μL). PmST1 (P34H/M144L) (20 μL, 1 mg mL^{-1} Tris HCl solution (100 mM pH 7.4)) was added, and the reaction mixture was incubated at 37 °C for 2.5 h. The reaction was incubated at 37 °C for 65 h. The reaction mixture was loaded on a P-2 Bio-gel column which was eluted with 0.1 M NH_4HCO_3 to yield α2,6-sialyl lactose **7** after combining and lyophilizing product containing fractions (33.1 mg, 94%) as a white powder. ^{1}H NMR (600 MHz, deuterium oxide) δ 5.15 (0.41H, d, *J* = 3.8 Hz, α anomer Glu), 4.59 (0.68H, d, *J* = 8.0 Hz, β anomer Glu), 4.35 (1H, d, *J* = 7.9 Hz, C_1H Gal), 3.93–3.69 (10H, m), 3.65 (1H, dd), 3.61–3.37 (8H, m,), 3.23 (0.63H, t, C_2H β Glu), 2.64 (1H, dd, H3″), 1.95 (3H, s, acetyl), 1.67 (1H, t, *J* = 12.2 Hz, C_3H_2 Sia). ^{1}H–^{13}C NMR: (600, 151 MHz, deuterium oxide) δ108.20 (C_1 Gal), 95.63 (C_1 α anomer Glu), 91.84 (C_1 β anomer Glu), 76.67, 74.60, 73.68, 72.50, 72.34, 71.78, 71.62, 71.21, 70.77, 70.65, 70.41, 69.92, 69.76, 68.36, 63.65, 62.61, 60.25, 60.09, 51.76, 40.08 (C_3 Sia), 22.03 (CH_3, acetyl). See for full assignment the Table S1 in the SI.[40] The ^{13}C signals were obtained from an HSQC experiment. ESI-TOF: *m*/*z* calculated for $C_{23}H_{40}NO_{19}^+$ (M + H) 634.218, found 634.221.

Poly-*O*-(methylamino)-L-serine modified with α2,6-sialyllactose (8)

Poly-*O*-(methylamino)-L-serine **6** (1.0 mg, 0.13 μmol) and α2,6-sialyl lactose **7** (5.5 mg, 8.68 μmol) were dissolved in a mixture of AcOH/DMF/H_2O 2/1/1 (v/v). After stirring the reaction mixture for 24 h, the solvents were evaporated, and the residue was redissolved in MilliQ (7.5 mL) and then lyophilized. The crude material was purified by size exclusion gel chromatography (Bio-Gel P6 DG) using NH_4HCO_3 (50 mM pH = 7.4) as the eluent yielding polymer **8** (3 mg, 82%). ^{1}H NMR (600 MHz, deuterium oxide) δ 4.37 (s, 1H), 3.96–3.70 (m, 9H), 3.68–3.39 (m, 12H), 2.64 (s, 5H) 2.57 (s, 2H), 1.96 (s, 3H), 1.72–1.63 (t, J_1 = 12 Hz J_2 = 24 Hz 1H). ^{1}H–^{13}C NMR (600, 151 MHz, deuterium oxide) δ (4.37, 103.35), (3.91, 63.57), (3.87, 68.60), (3.83, 71.92), (3.82, 62.73), (3.79, 51.95), (3.75, 73.77), (3.65, 72.53), (3.62, 76.37), (3.60, 72.53), (3.59, 68.60), (3.58, 62.86), (3.55, 79.76), (3.50, 68.60), (3.49, 76.37), (3.48, 70.89), (2.66, 38.06), (1.96, 22.16), (1.69, 40.25). The ^{13}C signals were obtained from an HSQC experiment.

Author contributions

FP and GJB designed the study. FP, MW, and ER performed chemical synthesis. FP performed characterization of the polymers. JMD performed the DOSY NMR experiments. TV supervised the characterization of the polymers. GJB supervised all other aspects of the study. FP, TV, and GJB wrote the paper. All authors reviewed the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

The data underlying this study are available in the article and its SI. Supplementary information: general materials and methods, copies of ^{1}H, ^{13}C and 2D NMR spectra, Table S1, Fig. S1 and HR-MS data for synthetic compounds. See DOI: **https://doi.org/10.1039/d5ob00985e**.

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