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Synthetic mimics of carbohydrate-based anticancer vaccines: preparation of carbohydrate polymers bearing unimolecular trivalent carbohydrate ligands by controlled living radical polymerization†

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Synthetic methods for preparation of three different styrene-type carbohydrate monomers, containing mannose, sialic acid and *N*-acetyllactosamine were successfully developed. Diethylene glycol was used as the spacer between the styrene and carbohydrate moieties. Under the conditions of nitroxide-mediated polymerizations, controlled living radical polymerizations could be accomplished, affording well defined carbohydrate polymers with different sugar compositions. The PDIs were increased and conversions were decreased upon increasing the concentrations of carbohydrate monomers. The resulting carbohydrate polymers were characterized by NMR. Novel carbohydrate polymers bearing unimolecular trivalent carbohydrate ligands could also be achieved through the living radical process used in this study.

Introduction

Cell surface carbohydrates are involved in a variety of biological and medical functions, including cellular recognition, adhesion, cell growth regulation, cancer cell metastasis, and inflammation.[1] They also serve as recognition sites for infectious bacteria, viruses, toxins, and hormones that result in the development of a wide variety of diseases.[2] Studies of their biological and medical functions are limited because of the unavailability of sufficient amounts from natural sources or difficulties associated with preparing these complex oligosaccharides. Although the great improvements have been achieved in the chemical and enzymatic synthesis of oligosaccharides, practical and efficient synthetic methods for the preparation of these complex structures are still highly demanded.[3]

Carbohydrate polymers are synthetic polymers with a non-carbohydrate main chain, containing carbohydrate moieties as the terminals.[4] Carbohydrate polymers can be considered as alternative structures to oligosaccharides, which have been shown to mimic oligosaccharides in interactions between carbohydrates and lectins.[5] The interaction between carbohydrates and lectins is usually weak and binding ability can be enhanced *via* the use of multivalent ligands. Carbohydrate polymers with multiple carbohydrates ligands are capable of exhibiting a cluster effect, thus increasing their binding ability.[6] Due to the increasing interest in artificial materials for a variety of biomedical uses, scientific reports have been published on their use as macromolecular drugs, drug delivery systems, a stationary phase for the separation of carbohydrates binding proteins, bioassays, surface modifiers, artificial tissues.[7]

Several methods have been developed for preparing carbohydrate polymers, such as atom transfer radical polymerization (ATRP),[8] reversible addition–fragmentation chain transfer (RAFT),[9] and nitroxide-mediated polymerization (NMP).[10] NMP techniques have attracted a great deal of attention from biomedical chemists, since NMP is a method that can be used to prepare metal-free or sulfur-free carbohydrate polymers. Carbohydrate polymers prepared by NMP are much more compatible with biological and physiological conditions.[11] Several synthetic methods have been reported for preparing carbohydrate polymers containing cell surface carbohydrates, along with their biomedical applications.[12] However, the main limitation and disadvantages of current synthetic methods are that the polymerization processes or efficient ligation methods between polymers and carbohydrates moieties cannot be easily controlled. Therefore, the successful preparation of well-defined carbohydrate polymers containing cell surface carbohydrates with different compositions of carbohydrates moieties, desired molecular weights and defined PDI is still a challenge for biomedical chemists. Fully synthetic carbohydrate antigens with defined compositions of carbohydrate ligands showed

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promising results in cancer immunotherapy.[13] Synthesis of a pentavalent carbohydrate vaccine bearing five different tumor-associated carbohydrates antigens (TACAs) has been reported.[14] Installation of an appropriate linker, conjugation to a carrier protein, Keyhole Limpet Hemocyanin (KLH, an immunoenhancer), and a ratio of glycopeptides : KLH (228 : 1) were determined to be an appropriate structure for an efficient vaccine in the treatment of breast and ovarian cancer.[14a]

Herein, we report on a practical and synthetic method for the preparation of carbohydrate polymers containing different cell surface carbohydrates using nitroxide-mediated polymerization (Scheme 1). Three different cell surface carbohydrates, *i.e.*, mannose (Man), sialic acid (Sia), and *N*-acetyllactosamine (LacNAc) were attached to the polymer backbone. Carbohydrate polymers containing mannose have been used to investigate interactions between human dendritic cells associated with lectins and HIV envelop glycoproteins.[15] Carbohydrate polymers containing sialic acid have been shown to inhibit the spread of influenza virus infections.[16] Carbohydrate polymers bearing *N*-acetyllactosamine have been prepared to inhibit hemagglutination by *Erythrina corallodendron* and the specific binding ability of lectins.[17] However, only a few of these carbohydrate polymers could be prepared in a well-controlled manner in polymerization reactions or with different sugar compositions.[18] Furthermore, to be eventually used for potential administration in animals or in human testing, an ideal polymerization method that is both metal-free and sulfur-free will be needed. Our approach involved the use of nitroxide-mediated polymerization for preparing well-controlled carbohydrate polymers with defined molecular weights and PDIs. The living character of controlled radical polymerization could be further explored to prepare novel carbohydrate polymers containing multivalent carbohydrate ligands. With a suitable spacer between the carbohydrate and the polymer backbone, carbohydrate polymers bearing unimolecular trivalent carbohydrate ligands that mimic unimolecular trivalent carbohydrates-based anticancer vaccines were designed.[14]

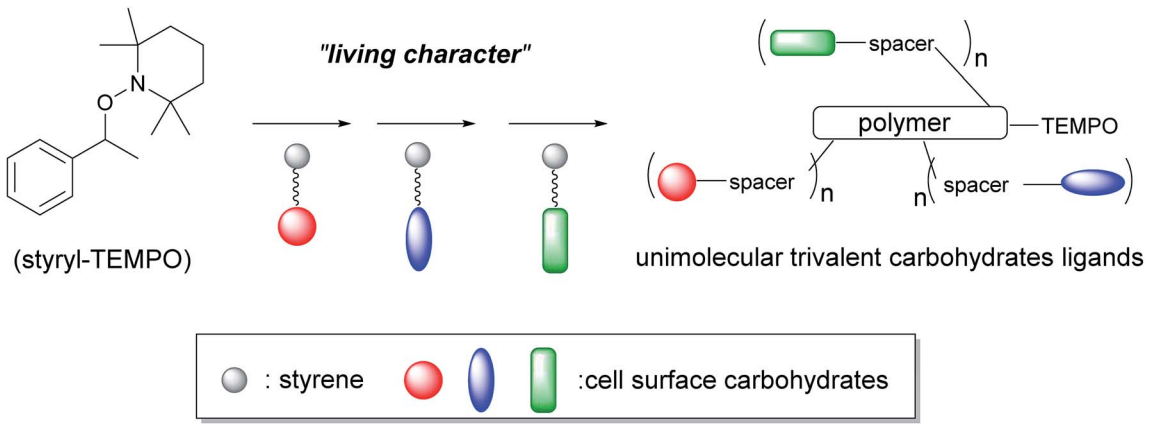
Experimental section

Materials

Styrene was distilled from CaH_2 under reduced pressure to remove the stabilizer and was stored at 4 °C under an argon atmosphere. THF were distilled by refluxing over traces of sodium metal using benzophenone as indicator under N_2. Benzene, dichloromethane, pyridine, *N*,*N*-dimethylformamide (DMF), and 1,8-diazavicyclo[5.4.0]undec-7-ene (DBU) were dried over CaH_2 and then distilled before use. Acetic anhydride, acetyl chloride, and borotrifluoride diethyletherate ($BF_3 \cdot OEt_2$) were directly distilled before use. 2,2,6,6-Tetramethyl-1-piperidinyloxy free radical (TEMPO), 4-chloromethylstyrene, diethylene glycol, mannose, sialic acid, galatose, and *N*-acetyl-glucosamine were used as received.

General

^{1}H NMR (800, 500, and 300 MHz) and ^{13}C NMR (200, 125, and 75 MHz) spectra were recorded on a Bruker AVIII-800 MHz, a Bruker AVIII-500 MHz, and a Bruker Advance-300 MHz. The NMR spectra were recorded in $CDCl_3$ or CD_3OD. Chloroform (δ = 7.26 ppm in ^{1}H NMR; δ = 77.0 ppm in ^{13}C NMR) and methanol (δ = 3.31 ppm in ^{1}H NMR; δ = 49.00 ppm in ^{13}C NMR) were used as internal standard, respectively. Splitting patterns were reported as following: s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet. Coupling constant (J) was reported in Hz. IR were recorded on a Perkin Elmer Spectrum 100 FT-IR spectrometer and reported in cm^{-1}. High resolution mass spectrometry (HRMS) were recorded on a Shimadzu LCMS-IT-TOF spectrometer (ESI-MS). Optical rotations were measured on a Horiba SEPA-300 Digital polarimeter. TLC (Merck Art. 60 F_{254}, 0.25 mm) precoated sheet was used. The reaction products were isolated by flash chromatography performed on Merck Art. Geduran Si 60 (0.040–0.063 mm) silica gel. Yields of products refer to chromatographically purified products unless otherwise stated. The benzene used for radical cyclizations was deoxygenated by passing a gentle stream of argon through for 30 min before use. All reactions were performed under a blanket of N_2 or Ar. The carbohydrate polymers were collected by centrifugal



Scheme 1 Synthetic mimics of unimolecular trivalent carbohydrate-based anticancer vaccines.

sedimentation on Eppendorf Centrifuge 5810 R as a rotation rate of 8000 rpm for 10 min and further dried in a vacuum-drying cabinet at 60 °C for 12 h. Size exclusion chromatography (SEC) was carried out with THF as eluent at a flow rate of 1.0 mL min^{-1} at room temperature on a system consisting of a PU-1580 isocratic pump (Jasco), a KF-804L column (Shodex), and a RI-71 refractometer detector (Shodex). Data were analyzed with Elite EC2000 software based upon calibration curves built upon polystyrene standards (Polymer Standards Service) with peak molecular weights ranging from 1800 to 56 000 g mol^{-1}.

Typical procedure for polymerization of carbohydrate polymers

A Schlenk-tube (thick = 1.6 mm) was charged with styryl-TEMPO (1%), carbohydrate monomer, styrene, and *N*,*N*-dimethylformamide (40 wt%). The tube was subjected to three freeze–thaw cycles and sealed off under argon. The polymerization was carried out under argon at 125 °C for an indicated period (please see Table 1). The resulting mixtures were cooled to room temperature and precipitated in methanol, diethyl ether or hexane. The carbohydrate polymers were collected by centrifugal sedimentation and dried in a vacuum-drying cabinet at 60 °C for 12 h. Conversion was evaluated gravimetrically. Molecular weight and polydispersity index (PDI) were determined by size exclusion chromatography (SEC).

2-[2-(4-Vinylbenzyloxy)ethoxy]ethanol (1). To a solution of diethylene glycol (76.0 mL, 795 mmol) was added a solution of 4-vinylbenzyl chloride (4.70 mL, 30.00 mmol), NaOH (1.20 g, 30.00 mmol), and water (0.54 mL, 30.00 mmol). The reaction mixture was stirred at 60 °C for 24 h. After cooling to room temperature, H_2O (100 mL) was added and the resulting mixture was extracted with Et_2O (3 × 250 mL). The organic layers were dried over $MgSO_4$, filtered, and concentrated in vacuum. The crude product was purified by silica gel chromatography (EtOAc : hexane = 7 : 3) to give the product as a colorless oil (6.37 g, 95%). IR (neat) 3420 (OH), 3087, 3006, 2868, 1629, 1512, 1454, 1406, 1351, 1319, 1288, 1243, 1212, 1093, 1067, 1016, 991, 907, 845, 827, 764, 729, 719, 628 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.39 (d, J = 8.1 Hz, 2H, *para*-), 7.39 (d, J = 8.1 Hz, 2H, *para*-), 6.70 (dd, J = 17.7, 10.8 Hz, 1H, ArC*H*=CH_2), 5.74 (d, J = 17.7 Hz, 1H, ArCH=CH_2), 5.23 (d, J = 10.8 Hz, 1H, ArCH=CH_2), 4.55 (s, 2H, ArCH_2O), 3.78–3.58 (m, 8H, CH_2 × 4), 2.79 (br s, 1H, OH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 137.5 (s), 137.0 (s), 136.4 (d), 127.9 (d × 2), 126.2 (d × 2), 72.9 (t), 72.4 (t), 70.3 (t), 69.3 (t), 61.7 (t); HRMS (ESI^+): m/z calcd for $C_{13}H_{18}O_3$ [M + H]$^+$: 223.1334; found: 223.1325.

2-[2-(4-Vinylbenzyloxy)ethoxy]ethyl 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranoside (3). To a solution of compound **2** (ref. 19) (6.16 g, 11.82 mmol) and freshly activated 4 Å molecular sieves (9 g) in CH_2Cl_2 (136 mL) was added a solution of compound **1** (3.15 g, 14.17 mmol) in CH_2Cl_2 (100 mL) at −10 °C. After being stirred for another 30 minutes, $BF_3{\cdot}OEt_2$ (0.30 mL, 2.36 mmol) was added dropwise at −10 °C. The reaction mixture was stirred for another 3.5 h at the same temperature, then the reaction was quenched by slow addition of MeOH (100 mL). The resulting mixture was filtrated and the residue was washed carefully with CH_2Cl_2 and MeOH. The combined organic phases were washed with concentrated $NaHCO_3$ solution (150 mL), brine (150 mL), dried over $MgSO_4$, filtered, and concentrated in vacuum. The crude product was purified by silica gel chromatography (hexane : EtOAc = 1 : 1) to give the product as a colorless oil (4.47 g, 69%). $[\alpha]_D^{26}$ +13.23 (c = 1.45, $CHCl_3$); IR (neat) 2893, 1746 (C=O), 1630, 1513, 1434, 1407, 1369, 1219, 1173, 1135, 1083, 1045, 1017, 979, 912, 847, 829, 793, 735, 689, 638 cm^{-1}; ^1H NMR (300

Table 1 Copolymerization of carbohydrate monomers and styrene[a]

Entry	Carbohydrate monomer	Ratio of carbohydrate monomer/styrene	Time (h)	Conversion (%)	M_n (g mol^{-1})	PDI
1	**3**	20/80	24	6	6237	1.11
2	**3**	20/80	30	36	7267	1.10
3	**3**	20/80	36	70	11 961	1.14
4	**3**	20/80	42	72	14 301	1.12
5	**3**	40/60	42	74	25 738	1.21
6	**3**	60/40	42	59	20 302	1.19
7	**3**	80/20	42	80	39 818	1.37
8	**3**	100/0	42	58	20 831	1.30
9	**5**	20/80	48	31	11 115	1.13
10	**5**	40/60	48	19	8363	1.55
11	**5**	60/40	48	16	10 835	1.68
12	**5**	80/20	48	22	11 997	1.74
13	**5**	100/0	48	9	4497	1.22
14	**10b**	25/75	48	65	27 348	1.15
15	**10b**	75/25	48	19	28 692	1.38
16[b]	**5**	20/80	42	33	15 082	1.24
17[c]	**10b**	20/80	42	65	24 045	1.52

[a] The ratio of styryl-TEMPO to total monomers is 1/100, ratios of different monomers in polymerization are indicated as table, and DMF was used as co-solvent (40 wt%). [b] The polymerization was initiated by the polymeric alkoxyamine **I**, obtained from entry 4 under radical living conditions. [c] The polymerization was initiated by the polymeric alkoxyamine **II**, obtained from entry 16 under radical living conditions.

MHz, $CDCl_3$) δ 7.38 (d, $J = 8.1$ Hz, 2H, *para*-), 7.29 (d, $J = 8.1$ Hz, 2H, *para*-), 6.70 (dd, $J = 17.4$, 10.8 Hz, 1H, RC*H*=CH_2), 5.73 (d, $J = 17.7$ Hz, 1H, RCH=CH_2), 5.36 (dd, $J = 9.9$, 3.3 Hz, 1H), 5.19–5.32 (m, 3H, RCH=CH_2), 4.87 (d, $J = 1.5$ Hz, 1H, H_1), 4.55 (s, 2H, ArCH_2O), 4.28 (dd, $J = 12.9$, 5.7 Hz, 1H, H_6), 4.04–4.12 (m, 2H), 3.77–3.87 (m, 1H), 3.63–3.72 (m, 2H), 2.14 (s, 3H, Ac), 2.09 (s, 3H, Ac), 1.99 (s, 3H, Ac), 1.98 (s, 3H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6 (s), 170.0 (s), 169.8 (s), 169.7 (s), 137.8 (s), 136.9 (s), 136.5 (d), 127.9 (d × 2), 126.2 (d × 2), 113.7 (t), 97.7 (d), 72.9 (t), 70.7 (t), 70.0 (t), 69.5 (d), 69.4 (t), 69.1 (d), 68.3 (d), 67.4 (t), 66.1 (d), 62.4 (t), 20.8 (q), 20.7 (q), 20.6 (q × 2); HRMS (ESI^+): *m*/*z* calcd for $C_{27}H_{36}O_{12}$ $[M + H]^+$: 575.2105; found: 575.2112.

Methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2,3,5-trideoxy-2-{2-[2-(4-vinylbenzyloxy)ethoxy]ethyl}-D-*glycero*-α-D-*galacto*-2-nonulopyranosonate (5). To a solution of compound **4** (ref. 20) (0.95 g, 1.86 mmol), compound **1** (4.15 g, 18.60 mmol) and 4 Å molecular sieve (2 g) in acetonitrile (1.9 mL) was added zinc bromide (0.84 g, 3.74 mmol) at room temperature. The reaction mixture was stirred at same temperature for 19 h. The precipitation was filtered off on celite and washed with CH_2Cl_2. The filtrate was washed with water, 50% aqueous $NaHCO_3$, dried over $MgSO_4$, and concentrated in vacuum. The crude product was purified by silica gel chromatography (hexane : EtOAc = 1 : 9) to give the product as a colorless solid (0.96 g, 75%, α : β = 91 : 9). However, only α-anomer was successfully characterized. IR (neat) 3264 (NH), 3010, 2957, 1743 (C=O), 1659 (C=O), 1546, 1445, 1407, 1369, 1303, 1220, 1199, 1177, 1130, 1095, 1074, 1039, 995, 942, 912 (CH_2=CHR), 855, 828, 787, 754, 733, 666, 648 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 (d, $J = 8.1$ Hz, 2H, *para*-), 7.28 (d, $J = 8.1$ Hz, 2H, *para*-), 6.69 (dd, $J = 17.7$, 11.0 Hz, 1H, ArC*H*=CH_2), 5.72 (d, $J = 17.7$ Hz, 1H, ArCH=CH_2), 5.42–5.27 (m, overlapped with one dd at 5.30, $J = 8.3$, 1.4 Hz, 3H, H_8, H_7, N*H*Ac), 5.21 (d, $J = 11.0$ Hz, 1H, ArCH=CH_2), 4.91–4.77 (m, 1H, H_4), 4.53 (s, 2H, ArCH_2O), 4.29 (dd, $J = 12.5$, 2.6 Hz, 1H, H_{9a}), 4.12–4.01 (m, 3H, H_5, H_6, H_{9b}), 3.94–3.83 (m, 1H, $H_{1''a}$), 3.75 (s, 3H, CO_2CH_3), 3.69–3.56 (m, 6H, CH_2 × 3), 3.51–3.40 (m, 1H, $H_{1''b}$), 2.60 (dd, $J = 12.8$, 4.7 Hz, 1H, $H_{3\text{-eq.}}$), 2.15–1.89 (m, 13H, overlapped with four s at 2.12, 2.11, 2.01, 2.00, Ac × 4 and $H_{3\text{-ax}}$), 1.86 (s, 3H, Ac); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.9 (s), 170.6 (s), 170.2 (s), 170.1 (s × 2), 168.2 (s), 137.8 (s), 136.9 (s), 136.5 (d), 127.9 (d × 2), 126.2 (d × 2), 113.7 (t), 98.8 (s), 72.9 (t), 72.4 (d), 70.5 (t), 70.1 (t), 69.3 (t), 69.1 (d), 68.6 (d), 67.3 (d), 64.4 (t), 62.3 (t), 52.7 (q), 49.3 (d), 37.9 (t), 23.1 (q), 21.0 (q), 20.8 (q × 2), 20.7 (q); HRMS (ESI^+): *m*/*z* calcd for $C_{33}H_{45}NO_{15}$ $[M + H]^+$: 696.2867; found: 696.2885, $[M + Na]^+$: 718.2687; found: 718.2681.

2-[2-(4-Vinylbenzyloxy)ethoxy]ethyl-2-acetamido-2-deoxy-β-D-glucopyranoside (7). To a solution of compound **1** (0.40 g, 1.81 mmol), compound **6** (ref. 21) (0.82 g, 2.26 mmol), and 4 Å molecular sieve (0.5 g) in dry DCM (3.3 mL) was allowed to stirred for 30 minutes at room temperature. The reaction mixture was then added mercuric cyanide (0.66 g, 4.75 mmol) and stirred at same temperature for 30 h. The precipitation was filtered off on celite and diluted with CH_2Cl_2 (150 mL). The filtrate was washed with aqueous 10% $NaHCO_3$, brine, dried over $MgSO_4$, and concentrated in vacuum. The crude product was pure enough and directly used for the next step without further purification. To a solution of 2-[2-(4-vinylbenzyloxy)ethoxy]ethyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (obtained from previous step) (0.12 g, 0.22 mmol) in MeOH (4.40 mL) was added sodium methoxide (0.006 g, 0.110 mmol). The reaction mixture was stirred at room temperature for 6 h. The solution was then neutralized with Amberlite IR 120 (H^+) ion-exchange resin, the resin filtered off and washed with MeOH. The resulting solution was concentrated under reduced pressure, and then purified by silica gel chromatography (DCM : MeOH = 7.5 : 1) to give the product as a colorless and viscous liquid (0.06 g, 85%), over two steps from compound **6**. $[\alpha]_D^{25}$ −69.78 ($c = 0.33$, CH_3OH); IR (neat) 3340, 2945, 2835, 1650 (C=O), 1566, 1450, 1408, 1378, 1318, 1258, 1211, 1078, 1022, 942, 829 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 7.37 (d, $J = 8.3$ Hz, 2H, *para*-), 7.27 (d, $J = 8.3$ Hz, 2H, *para*-), 6.68 (dd, $J = 17.7$, 11.0 Hz, 1H, ArC*H*=CH_2), 5.73 (dd, $J = 17.7$, 0.8 Hz, 1H, ArCH=CH_2), 5.17 (d, $J = 11.0$ Hz, 1H, ArCH=CH_2), 4.49 (s, 2H, ArCH_2O), 4.42 (d, $J = 8.4$ Hz, 1H, H_1, β-form), 3.92 (m, 1H, $H_{1'a}$), 3.82 (dd, $J = 11.9$, 1.7 Hz, 1H, H_{6a}), 3.70–3.52 (m, 9H), 3.40–3.32 (m, 1H), 3.30–3.19 (m, 2H), 1.89 (s, 3H, NH*Ac*); ^{13}C NMR (75 MHz, CD_3OD) δ 174.0 (s), 139.2 (s), 138.7 (s), 138.0 (d), 129.4 (d × 2), 127.4 (d × 2), 114.2 (t), 102.9 (d), 78.2 (d), 76.5 (d), 74.0 (t), 72.2 (d), 71.8 (t × 2), 70.8 (t), 70.1 (t), 62.9 (t), 57.5 (d), 23.2 (q); HRMS (ESI^+): *m*/*z* calcd for $C_{21}H_{31}NO_8$ $[M + H]^+$: 426.2128; found: 426.2138.

2-[2-(4-Vinylbenzyloxy)ethoxy]ethyl 2-acetamido-6-*O*-*tert*-butyldiphenylsilyl-2-deoxy-β-D-glucopyranoside (8). To a solution of compound **7** (1.49 g, 3.50 mmol) and imidazole (0.72 g, 10.50 mmol) in dry DMF (17.5 mL) was added TBDPSCl (1.82 mL, 7.00 mmol) dropwise. The reaction mixture was stirred at room temperature for 5 h. The excess TBDPSCl was quenched by addition of MeOH (1 mL). The resulting mixture was allowed to stir for another 30 minutes, and then concentrated under reduced pressure to give a crude product, which was then purified by silica gel chromatography (DCM : MeOH = 15 : 1) to afford the product as a pale yellow oil (1.75 g, 76%). $[\alpha]_D^{25}$ −46.51 ($c = 2.66$, $CHCl_3$); IR (neat) 3311 (OH), 3072, 3010, 2930, 2858, 1907, 1826, 1652 (C=O), 1513, 1472, 1462, 1428, 1406, 1374, 1361, 1312, 1238, 1216, 1110, 1061, 998 (RCH=CH_2), 938, 908 (RCH=CH_2), 850, 824, 750, 702, 666, 622, 604 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76–7.66 (m, 4H, Ph), 7.45–7.33 (m, 8H, Ph, *para*-), 7.29 (d, $J = 8.1$ Hz, 2H, *para*-), 6.94 (d, $J = 5.7$ Hz, 1H, NH), 6.70 (dd, $J = 17.7$, 11.1 Hz, 1H, ArC*H*=CH_2), 5.75 (dd, $J = 17.7$, 0.5 Hz, 1H, ArCH=CH_2), 5.25 (dd, $J = 11.1$, 0.5 Hz, 1H, ArCH=CH_2), 4.57 (d, $J = 12.0$ Hz, 1H, ArCH_2O), 4.53–4.48 (m, overlapping with one d at 4.50, $J = 8.4$ Hz, 2H, H_1 and ArCH_2O), 3.99 (dd, $J = 11.0$, 3.2 Hz, 1H), 3.95–3.83 (m, 2H), 3.77 (dd, $J = 8.9$, 2.3 Hz, 1H), 3.73–3.49 (m, 10H), 3.46–3.33 (m, 3H), 1.94 (s, 3H, NH*Ac*), 1.05 (s, 9H, *t*-Bu); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.1 (s), 137.4 (s), 136.8 (s), 136.2 (d), 135.6 (d × 4), 133.3 (s), 133.2 (s), 129.6 (d × 2), 128.3 (d × 2), 127.6 (d × 4), 126.3 (d × 2), 114.2 (t), 101.2 (d), 76.7 (d), 75.6 (d), 73.1 (t), 72.0 (d), 70.9 (t), 69.3 (t), 68.5 (t), 64.1 (t), 57.4 (d), 26.7 (q × 3), 22.9 (q), 19.2 (s); HRMS (ESI^+): *m*/*z* calcd for $C_{37}H_{49}NO_8Si$ $[M + H]^+$: 664.3305; found: 664.3328; $[M + Na]^+$: 686.3125; found: 686.3102.

2-[2-(4-Vinylbenzyloxy)ethoxy]ethyl-*O*-(2,3,4,6-tetra-*O*-acetyl-D-galactopyranosyl)-(1 → 4)-2-acetamido-6-*O*-*tert*-butyldiphenylsilyl-2-de-oxy-β-D-glucopyranoside (10a and 10b). To a solution of compound **9** (ref. 22) (1.17 g, 2.25 mmol), compound **8**

(0.99 g, 1.50 mmol), and 4 Å molecular sieve (2.20 g) in anhydrous DCM (25 mL) was cooled to −40 °C. The reaction mixture was allowed to stir for 20 minutes. Borontrifluoride diethyletherate (0.38 mL, 3.00 mmol) was added into the solution. The resulting mixture was allowed to stir at the same temperature for 5 h. The reaction mixture was added aqueous $NaHCO_3$ and stirred for 10 minutes. The organic layers were washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude residue was purified by silica gel chromatography (EtOAc : hexane = 9 : 1) to afford the minor isomer (α-form) **10a** as a white solid and the major isomer (β-form) **10b** as a colorless oil (1.75 g, 66%, α : β = 26% : 40%):

α-isomer **10a**: mp 158.2–161.1 °C; $[\alpha]_D^{25}$ +38.52 (c = 1.01, $CHCl_3$); IR (neat) 3519 (OH), 3273, 2860, 1743, (C═O), 1653 (C═O), 1568, 1428, 1372, 1259, 1226, 1111, 1086, 1030, 959, 909, 880, 853, 825, 810, 765, 743, 705 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.67–7.63 (two overlapping d at 7.66, J = 6.5 Hz, 7.64, J = 6.6 Hz, 4H, Ph), 7.43–7.31 (m, 8H, Ph, *para*-), 7.28 (d, J = 8.0 Hz, 2H, *para*-), 6.70–6.62 (dd at 6.68, J = 17.6, 10.9 Hz, 1H, ArC*H*═CH_2, overlapped with one d at 6.62, J = 8.15 Hz, 1H, N*H*Ac), 5.71 (d, J = 17.6 Hz, 1H, ArCH═CH_2), 5.33 (d, J = 4.5 Hz, 1H $H_{4'}$), 5.22 (d, J = 10.9 Hz, 1H, ArCH═CH_2), 4.99–4.89 (m, overlapped with one d at 4.91, J = 3.9 Hz, 3H, $H_{3'}$, $H_{1'}$, α-form), 4.86 (d, J = 8.3 Hz, 1H, H_1, β-form), 4.57 (s, 2H, ArCH_2O), 4.16 (dd, J = 8.1, 5.7 Hz, 1H, $H_{6'a}$), 4.09–3.93 (m, overlapped with one dd at 3.97, J = 10.9, 5.4 Hz, 3H, $H_{5'}$, $H_{6'b}$), 3.89 (dt, J = 11.9, 3.4 Hz, 1H), 3.81 (td, J = 11.0, 3.7 Hz, 1H), 3.77–3.71 (m, 1H), 3.71–3.58 (m, overlapped with one dd at 3.68, J = 11.6, 6.0 Hz, 8H), 3.52 (dd, J = 17.7, 8.5 Hz, 1H, H_2), 3.46–3.39 (m, 1H, $H_{2'}$), 2.06 (s, 3H, Ac), 2.01 (s, 3H, Ac), 1.98 (s, 3H, Ac), 1.97 (s, 3H, Ac), 1.87 (s, 3H, Ac), 1.02 (s, 9H, *t*-Bu); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.1 (s), 170.6 (s), 170.5 (s), 170.4 (s), 170.0 (s), 137.4 (s), 137.2 (s), 136.4 (d), 135.6 (d × 2), 135.5 (d × 2), 133.3 (s), 133.2 (s), 129.6 (d), 129.6 (d), 127.9 (d × 2), 127.6 (d × 2), 127.6 (d × 2), 126.3 (d × 2), 114.0 (t), 100.8 (d), 100.4 (d), 80.3 (d), 74.4 (d), 72.9 (t), 71.3 (d), 70.9 (t), 70.3 (t), 70.1 (d), 69.5 (t), 68.4 (t), 67.7 (d), 66.9 (d), 66.6 (d), 63.1 (t), 61.0 (t), 56.6 (d), 26.7 (q × 3), 23.4 (q), 20.8 (q), 20.7 (q), 20.6 (q), 20.5 (q), 19.2 (s); HRMS (ESI^+): m/z calcd for $C_{51}H_{67}NO_{17}Si$ $[M + H]^+$: 994.4256; found: 994.4268.

β-isomer **10b**: $[\alpha]_D^{31}$ −19.41 (c = 1.88, $CHCl_3$); IR (neat) 3395 (OH), 3007, 2933, 2859, 1755 (C═O), 1737 (C═O), 1677 (NHCO), 1540, 1429, 1367 (*t*-Bu), 1297, 1248, 1218, 1141, 1110, 1060, 1039, 954, 911, 853, 826, 794, 761, 745, 706, 665, 606 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.73–7.65 (m, 4H, Ph), 7.42–7.31 (m, 8H, Ph), 7.26 (d, J = 8.1 Hz, 2H, *para*-), 6.69 (dd, J = 17.6, 11.0 Hz, 1H, ArC*H*═CH_2), 6.20 (d, J = 8.4 Hz, 1H, N*H*Ac), 5.73 (d, J = 17.6 Hz, 1H, ArCH═CH_2), 5.33 (d, J = 3.4 Hz, 1H, $H_{4'}$), 5.22 (d, J = 11.0 Hz, 1H, ArCH═CH_2), 5.17 (dd, J = 10.5, 8.1 Hz, 1H, $H_{2'}$), 4.94 (dd, J = 10.5, 3.4 Hz, 1H, $H_{3'}$), 4.68 (d, J = 8.1 Hz, 1H, $H_{1'}$, β-form), 4.65 (d, J = 8.3 Hz, 1H, H_1, β-form), 4.54 (d, J = 12.0 Hz, 1H, ArCH_2O, AB), 4.50 (d, J = 12.0 Hz, 1H, ArCH_2O, AB), 4.15–4.07 (two overlapping dd at 4.13, J = 11.3, 6.3 Hz, and 4.10, J = 11.3, 7.0 Hz, 2H, $H_{6'a,6'b}$), 3.92–3.82 (m, 3H, $H_{1''a}$, H_{6a}, $H_{5'}$), 3.81–3.65 (m, 5H, H_{6b}, H_4, $H_{2''a}$, H_2), 3.64–3.57 (m, 6H, CH_2 × 2, $H_{2''b}$, $H_{1''b}$), 3.52 (t, J = 9.2 Hz, 1H, H_3), 3.30 (d, J = 8.1 Hz, 1H, H_5), 2.12 (s, 3H, Ac), 2.02 (s, 3H, Ac), 1.95 (s, 3H, Ac), 1.94 (s, 3H, Ac),1.69 (s, 3H, Ac), 1.04 (s, 9H, *t*-Bu); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6 (s), 170.3 (s), 170.0 (s), 170.0 (s), 169.0 (s), 137.3 (s), 137.0 (s), 136.3 (d), 135.9 (d × 2), 135.4 (d × 2), 133.5 (s), 132.5 (s), 129.7 (d × 2), 128.2 (d × 2), 127.7 (d × 2), 127.5 (d × 2), 126.3 (d × 2), 114.0 (t), 101.0 (d), 100.9 (d), 79.9 (d), 74.5 (d), 73.1 (t), 72.7 (d), 71.1 (t), 71.1 (d), 70.8 (d), 70.5 (t), 69.5 (t), 68.8 (d), 68.0 (t), 66.8 (d), 61.8 (t), 61.0 (t), 55.8 (d), 26.7 (q × 3), 23.2 (q), 20.5 (q × 2), 20.4 (q), 20.2 (q), 19.2 (s); HRMS (ESI^+): m/z calcd for $C_{51}H_{67}NO_{17}Si$ $[M + H]^+$: 994.4256; found: 994.4280, $[M + Na]^+$: 1016.4076; found: 1016.4069.

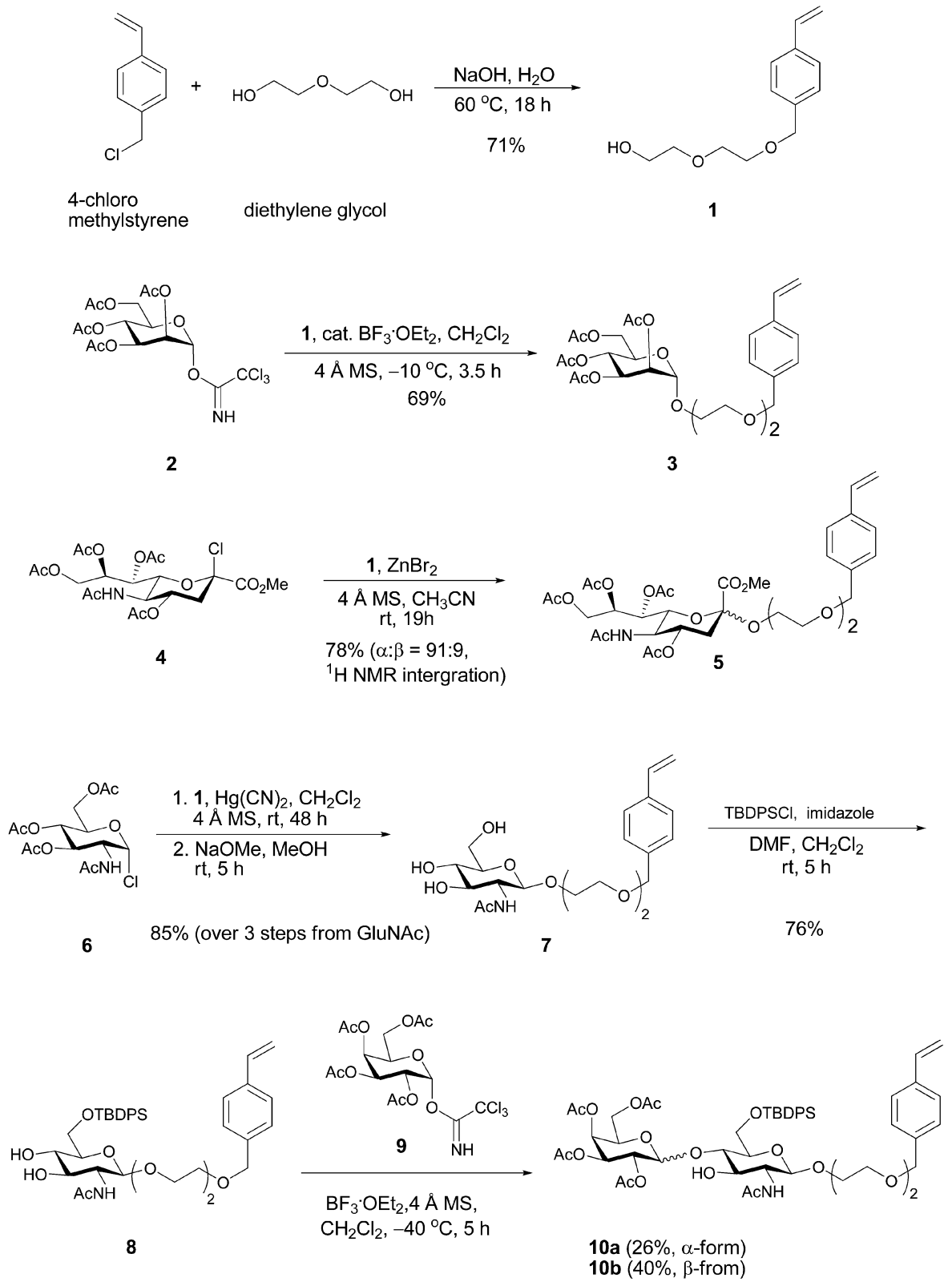
Results and discussion

Synthesis of styrene derivative 1 and carbohydrate monomer 3, 5, and 10

To fundamentally study the controlled living radical polymerization of carbohydrate polymers containing cell surface carbohydrates, we used polystyrene as the backbone. To avoid steric hindrance during the polymerization and gain more hydrophilic interactions in aqueous solution as biomaterials, diethylene glycol was used as a spacer between the styrene and carbohydrate moieties.[23] The reaction of 4-chloromethylstyrene with diethylene glycol afforded the styrene derivative **1** in 71% yield (Scheme 2).[24] To prepare the mannose-containing carbohydrate monomer, a glycosylation reaction between the mannosyl donor **2** (ref. 19) and the styrene derivative **1** was carried out using borontrifluoride-diethyletherate[25] to complete the synthesis of the carbohydrate monomer **3** containing mannose as its terminal in 69% yield (only α-form). The synthesis of a carbohydrate monomer containing sialic acid has been intensively studied.[26] Eventually, zinc bromide ($ZnBr_2$)[27] was found to be an efficient catalyst for the glycosylation between the sialyl donor **4** (ref. 20) and the styrene derivative **1**. Under optimized conditions, a partial separable mixture of carbohydrate monomers **5** in a ratio of 91 : 9 (α : β) was produced in 78% yield. More synthetic steps were needed for the synthesis of carbohydrate monomers **10** containing the disaccharide *N*-acetyllactosamine. The glycosylation reaction between the glycosyl donor **6** (ref. 21) and compound **1** was catalyzed by mercury cyanide $[Hg(CN)_2]$.[28] Deacetylation using sodium methoxide[29] afforded the carbohydrate monomer **7**, which contained an *N*-acetylglucosamine (GluNAc) unit. To complete the synthesis of the LacNAc-derived carbohydrate monomer, selective silylation at the 6-position of GluNAc moiety[30] on the compound **7** gave the glycosyl acceptor **8**. A second glycosylation reaction between the donor **9** (ref. 22) and the acceptor **8** was successfully carried out to give the disaccharide LacNAc carbohydrate monomers **10** in 66% yield (α-form: 26%; β-form: 40%).[31] It is fortunate that the major product (the β-isomer) functions as an efficient substrate for many cell surface recognition processes. Three carbohydrate monomers containing mannose, sialic acid, *N*-acetyllactosamine were successfully prepared. The methods presented herein permit the efficient preparation of carbohydrate monomers on a gram scale.

Polymerization studies

The polymerizations were conducted in sealed tubes using 1 mol% of alkoxyamine and 40 wt% of dimethylforamide (DMF)



Scheme 2 Preparation of carbohydrate monomers 3, 5, and 10.

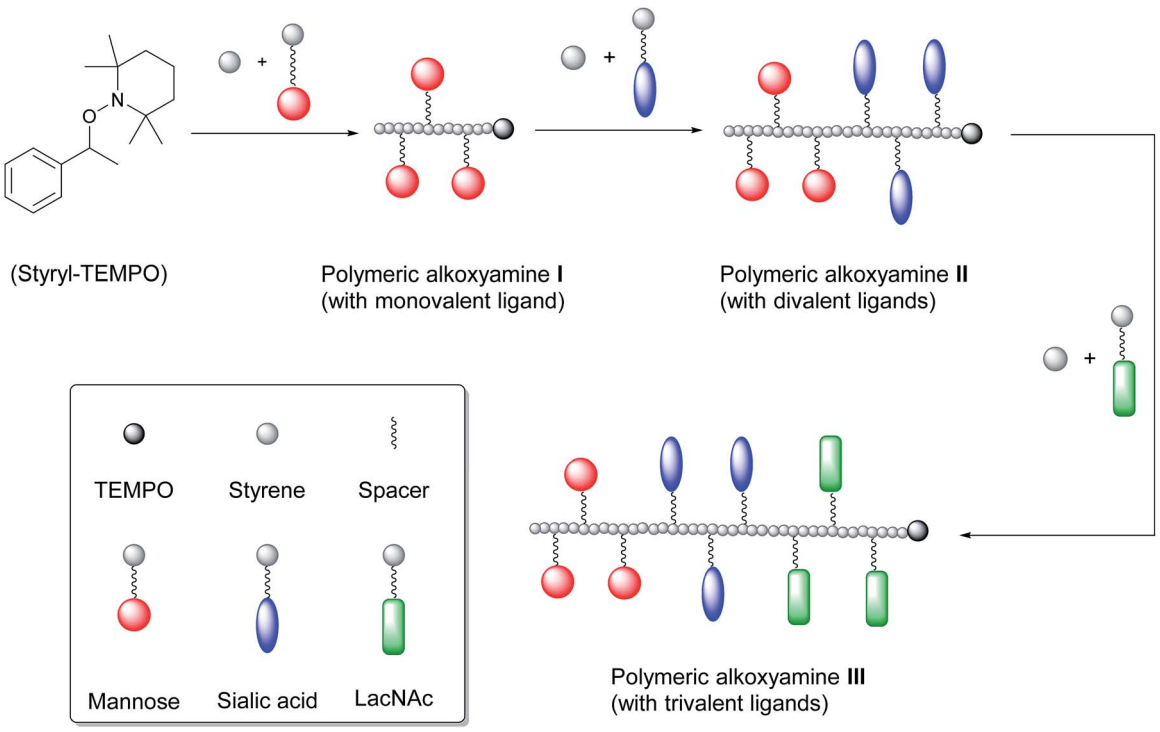
as the co-solvent with different ratios of carbohydrate monomers and styrene. The results are presented in Table 1. In some experiments (entries 1–15), styryl-TEMPO was used as the regulator. In other experiments (entries 16 and 17), polymeric alkoxyamines were used as regulators in the living radical polymerizations. The carbohydrate polymers were collected by

centrifugation and dried in a vacuum-drying cabinet at 60 °C for 12 h. Conversions were evaluated gravimetrically. Molecular weights and polydispersity indices (PDI) were determined by size exclusion chromatography (SEC). Five different ratios (20/80, 40/60, 60/40, 80/20, 100/0; ratio to styrene) of monomer compositions in polymerizations were studied in the cases of a series of carbohydrate monomers **3** and **5**, in which a mannose and a sialic acid are attached to its terminals, respectively. Because the synthesis of carbohydrate monomer **10b** bearing an *N*-acetyllactosamine was laborious, only two different ratios (25/75 and 75/25) were examined.

The copolymerization reactions of the carbohydrate monomer **3** and styrene using a ratio of 20/80 were studied first (entry 1–4) at 125 °C. As expected, the conversion was increased (from 6% to 72%) upon extending reaction time (from 24 h to 42 h) while the PDIs remained narrow (1.10–1.14). The optimized reaction time (42 h), a conversion of 72% and a narrow PDI of 1.12 were obtained (entry 4). The polymerization behavior of different concentrations in the reactions of carbohydrate monomer **3** with styrene (entry 4–8) was studied next. When the concentration of carbohydrate monomer **3** in the styrene polymerization was increased, fluctuations in conversions between 58 and 80% and in PDIs between 1.12 and 1.37 were observed. Surprisingly, a polymerization reaction using 100% of the carbohydrate monomer **3**, resulted in the formation of mannosyl-glycopolymers with a conversion of 58% and a PDI of 1.30 (entry 8). At this stage, we can confirm that, using commercially available styryl-TEMPO, controlled radical polymerizations can be achieved to produce carbohydrate polymers with different compositions, reasonable conversions and defined PDIs.

We then continued our studies on polymerization of carbohydrate monomer **5** at 125 °C for 48 h. Compared to the polymerization of carbohydrate monomer **3**, relatively low conversions were obtained. Increasing the concentration of carbohydrate monomer **5** in the styrene polymerization resulted in a decrease in conversion, from 31% to 9% and an increase in the PDI from 1.13 to 1.74 (entry 9–13). The reasons for the low conversion can be attributed to the bulky effect of the carbohydrate itself (sialic acid) in polymerizations or ineffective precipitation caused by the highly polar protecting groups (six acetyl groups and one ester group) on the sialic acid molecule. It should be noted that a low conversion (9%) and a narrow PDI (1.22) were observed in the polymerization, when 100% of the carbohydrate monomer **5** was used (entry 13).

The next series of experiments involved the use of the carbohydrate monomer **10b** (β-isomer) since it is considerably more useful than compound **10a** (α-isomer) in cell surface recognition interactions. Polymerizations at two different concentrations were studied (entry 14–15). A low concentration (25/75, ratio to styrene) of carbohydrate monomer **10b** led to a good conversion of 65% and a low PDI of 1.15 (entry 14). This good conversion can be attributed to the effect of the hydrophobic *tert*-butyldiphenylsiyl group, which enhanced the yield of precipitated product. A high concentration (75/25, ratio to styrene) of carbohydrate monomer **10b** in the polymerization resulted in decreased conversion (19%), and a larger PDI of 1.38 (entry 15). In general, the results showed that increasing the concentration of carbohydrate monomers in the reaction resulted in a decrease in conversion and an increase in PDIs.



Scheme 3 Preparation of novel carbohydrate polymers bearing unimolecular trivalent carbohydrate ligands.

The final polymerization reactions were intended to produce novel carbohydrate polymers bearing unimolecular multivalent ligands (Scheme 3). To show the living character of controlled radical polymerization, the polymerizations of the carbohydrate monomer **5** and styrene were initiated and regulated by the polymeric alkoxyamine **I** (obtained from entry 4) to delivery polymeric alkoxyamine **II** bearing a unimolecular divalent carbohydrate ligand (entry 16). The polymer weight was indeed increased and the PDI remained low (1.12 → 1.24). This carbohydrate polymeric alkoxyamine **II** could be further polymerized in the presence of the carbohydrate monomer **10b** and styrene to provide a novel polymeric alkoxyamine **III** bearing a unimolecular trivalent carbohydrate ligand with a molecular weight of 24 K and PDI of 1.52 (entry 17). This is the first report of an application of TEMPO-mediated polymerization to produce novel carbohydrate polymers bearing unimolecular trivalent cell surface carbohydrate ligands with desired molecular weights and PDIs. This synthetic approach provided an alternative method for the rapid synthesis of carbohydrate polymers with multivalent carbohydrate ligands in one single polymerization sequence. Furthermore, these novel carbohydrate polymers carrying a TEMPO as its terminal, which constitutes a living radical site for further polymerization reactions. One might imagine that using this approach, block copolymerization in the presence of three carbohydrate monomers in a one-pot polymerization reaction could be used to produce a variety of novel carbohydrate polymers with desired sugar compositions. Since multivalent carbohydrate vaccines were recently used in clinical trials for cancer therapy, these results provided an alternative and practical approach for the generation of carbohydrate polymers with desired molecular weights and controlled PDIs. Different sugar compositions of carbohydrate polymers could also be achieved to mimic tumor-associated carbohydrate antigens.

Characterization of carbohydrate polymers by NMR

^{1}H NMR measurements were also carried out to verify that the carbohydrate moieties were, in fact, attached to the polystyrene backbone, shown in Fig. 1. In spectrum 2a, the characteristic peaks of the polymer obtained from polymerization with 20% carbohydrate monomer **3** appear at around 4.8 ppm, and are assigned to the anomeric proton (H_1 of mannose), and four acetyl groups (OAc) at around 2.0–2.2 ppm. Characteristic peaks of carbohydrate polymers containing sialic acid (20% with styrene) in spectrum 2b appear at 3.7 and 2.6 ppm, and are assigned to the methyl ester (OMe) and the equatorial proton (H_3) of the sialic acid moiety, respectively. In spectrum 2c, the peaks at 7.4 and 7.7 ppm of diphenyl group, 4.7 ppm of two anomeric protons, and 1.0 ppm of *tert*-butylsilyl group clearly are present, thus verifying that carbohydrate polymers containing *N*-acetyllactosamine were successfully produced. The polymeric alkoxyamines containing divalent and trivalent ligands (**II** and **III**) are also shown in spectra 2d and e, respectively. The relatively low intensity of the characteristic peaks corresponding to sialic acid are shown in spectrum 2d, consistent with the relatively low extent of polymerization in the

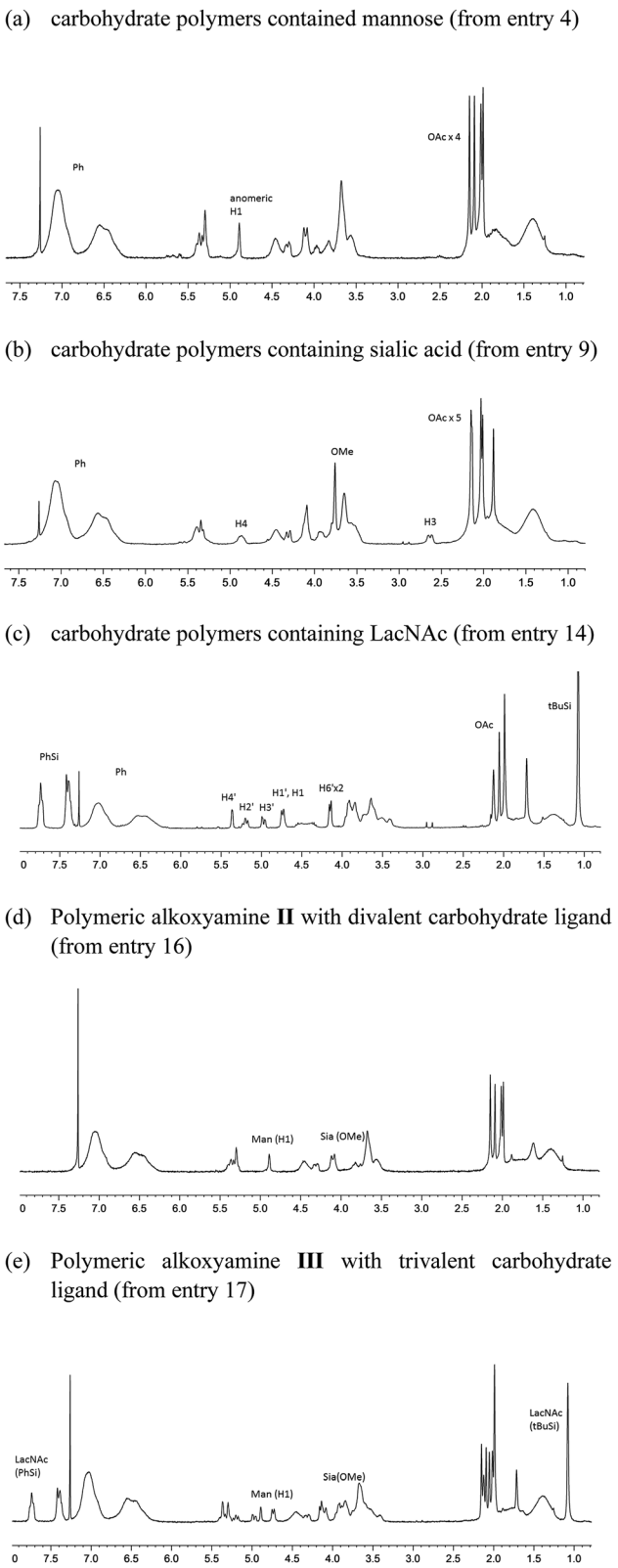


Fig. 1 ^{1}H NMR Spectra of carbohydrate polymers in $CDCl_3$ (a) polymer from entry 4. (b) Polymer from entry 9. (c) Polymer from entry 14. (d) Polymer from entry 16. (e) Polymer from entry 17.

series of carbohydrate polymer **5**. Peaks for carbohydrate polymers obtained from the polymerization in entry 17 are shown in spectrum 2e, indicating that a polymer bearing a unimolecular trivalent carbohydrate ligand was successfully produced.

Conclusions

Three different styrene-type monomers containing cell surface carbohydrates were successfully prepared for the studies of nitroxide-mediated polymerization. Diethylene glycol was used as the spacer. These new synthetic methods could be used to synthesize carbohydrate monomers on a gram scale. Under the conditions of TEMPO-mediated polymerization, controlled living radical polymerization could be achieved to produce carbohydrate polymers with defined molecular weights and PDIs. Carbohydrate polymers with different sugar compositions could also be produced through this approach. The PDIs were increased and conversions were decreased when increasing concentrations of carbohydrate monomers in polymerizations were used. Meanwhile, a radical "living" character could be exhibited to produce three different carbohydrates moieties attached to the polymers chains. To the best of our knowledge, this is the first report of the preparation of carbohydrate polymers bearing unimolecular trivalent carbohydrate ligands with defined molecular weights and PDIs. The approach presented here provides a platform to show that controlled living radical polymerization is a powerful method for preparing novel carbohydrate polymers with specific carbohydrate ligands. Polymer and sugar compositions could be adjusted through polymerization with different concentrations of initiators and carbohydrate monomers. These preliminary results provide an alternative synthetic approach for the rapid synthesis of multiple tumor-associated carbohydrates antigens in one single living polymerization sequence. Since multivalent carbohydrate vaccines are currently used in clinical trials for cancer therapy,[13,14] the preparation of carbohydrate polymers containing tumor-associated carbohydrate antigens using well-established nitroxides techniques[32] is currently underway. Our approaches should provide a potentially rewarding concept that can be applied to research related to cancer immunotherapy.

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