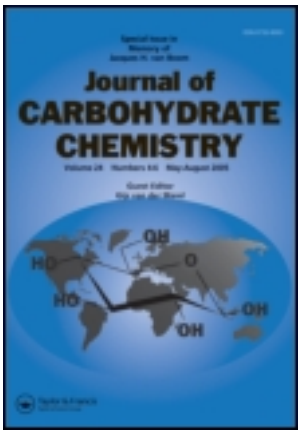

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SYNTHESIS OF ARTIFICIAL GLYCOCONJUGATE POLYMERS CARRYING 6-*O*-PHOSPHOCHOLINE α-D-GLUCOPYRANOSIDE, BIOLOGICALLY ACTIVE SEGMENT OF MAIN CELL MEMBRANE GLYCOLIPIDS OF *Mycoplasma fermentans*

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ABSTRACT

As carbohydrate probes to investigate the biological activity of novel phosphocholine-containing glycoglycerolipids of *M. fermentans*, artificial glycoconjugate polymers carrying 6-*O*-phosphocholine α-D-glucopyranoside were synthesized. The synthesis involved α-selective 1-*O*-*p*-nitrophenylation (*p*NP) of 1,6-di-*O*-acetyl-2,3,4-tri-*O*-benzyl-D-glucopyranose in the presence of a Lewis acid followed by the introduction of a phosphocholine group at position *O*-6 by an amidite method. The *p*NP group was converted into a *p*-*N*-methacrylamidophenyl group for subsequent radical polymerization.

INTRODUCTION

Artificial glycoconjugate polymers carrying biologically active carbohydrates as the pendant groups represent a new class of biomimetic compounds.[1] They have been widely used in cell cultivations,[2] tumor diagnosis,[3] and detection and trapping of viruses and toxins.[4,5] The wide-range utility can be ascribed primarily to ubiquitous lectin-like receptors on germ and mammalian cell surfaces which recognize certain types of carbohydrate chains in glycopeptides and glycolipids. Moreover, multivalence and carbohydrate cluster effects in the protein-carbohydrate binding have contributed much to extend the potential utility of the artificial glycoconjugate polymers.[6,7] As a part of our research projects to develop such glycoconjugate polymers useful to investigate germ-host cell or toxin-host cell interactions,[8-10] our interest has been directed to novel phosphocholine-containing glycoglycerolipids, GGPL-I[11,12] and GGPL-III[13-15] (Figure 1). They are main cell membrane glycolipids of *M. fermentans* which is suspected of playing certain pathogenic roles in rheumatoid arthritis[16] and the progression of HIV diseases.[17,18] Biological activities of GGPLs have revealed a close relevance to those of *M. fermentans* itself. For example, they are determined to be species-specific major immunodeterminants[19] of *M. fermentans*, and their specific antibodies are widely detectable in the sera of HIV-1 infected individuals.[20] GGPL-III is suggested also to play a key role for the adherence of *M. fermentans* to Molt-3 lymphocytes.[21] These recent biological studies have inspired us to develop a convenient synthetic pathway to artificial glycoconjugate polymers carrying GGPL-I or GGPL-III analogues. In this article, we report a facile way to prepare the polymer carrying the GGPL-I analogue starting from D-glucose.

RESULTS AND DISCUSSION

Potential biological activities of GGPLs are considered to be due to the phosphocholine group attached to the *O*-6 position of D-glucose.[19-21] This is mainly because human C-reactive protein, which increases in response to inflammation or tissue damage, has an ability to bind to phosphocholine-containing glycolipids leading to the activation of the complement system.[22,23] Moreover, it is also reported that all bacteria having a phosphocholine as the part of their surface structures show an activity to colonize a human nasopharynx.[24,25] Its existence at the position *O*-6 of α-D-glucopyranose constructs a

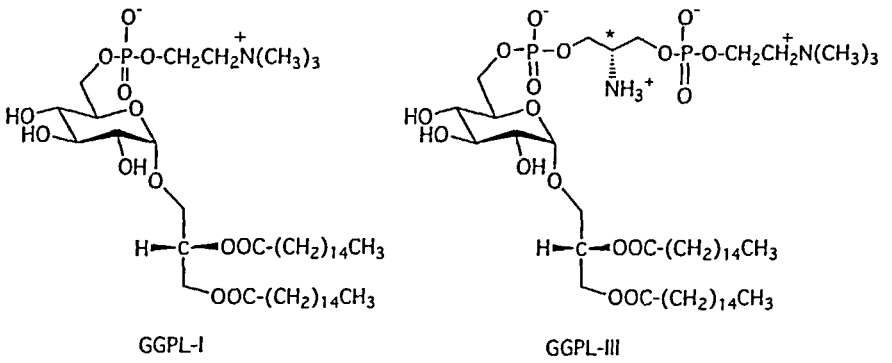
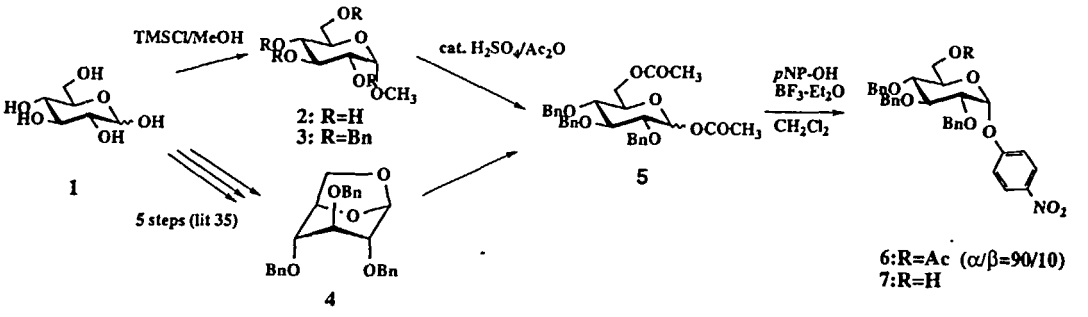


Figure 1 Absolute Structures of GGPL-I and GGPL-III.

unique structure of GGPL-I different from phosphatidylcholine and the other phosphocholine-containing glycolipids.[24,25,26] Therefore, the skeleton of 6-*O*-phosphocholine α-D-glucopyranose is considered to be the key functional group of GGPL-I for the biological activity. In this study, polymerizable GGPL-I analogues **10** (Scheme 2) carrying an *N*-methacrylamidophenyl group at the terminus were synthesized from D-glucose in a following manner.

We took a synthetic strategy starting from D-glucose *via* methyl α-D-glucopyranoside **2** leading to 1,6-di-*O*-acetyl-2,3,4-tri-*O*-benzyl-D-glucopyranose **5** (Scheme 1). Though 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose **4** provides a conventional method to afford **5**,[12] acid catalyzed acetolysis for methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside **3** using 1% H_2SO_4/acetic anhydride (0℃ to room temperature) gave **5** (α/β=85/15) directly in a high yield. The regioselective acetolysis at the primary *O*-benzyl group is considered to occur *via* a 1,6-anhydro intermediate **4**.[27] The di-*O*-acetylated derivative **5** was applied to *p*NP-glycosidation using *p*-nitrophenol (*p*NP-OH) and boron trifluoride-diethyl ether (BF_3-Et_2O) complex. After the reaction conditions were optimized by changing the solvent, reaction temperature and ratio of reagents as summarized in Table 1, *p*NP D-glucopyranoside **6** (α/β=90/10, ^{1}H NMR analysis) was obtained in 93% yields using an excess amount of BF_3-Et_2O (6 mol equiv) and *p*NP-OH (4 mol equiv) in dichloromethane. Every reaction condition studied here showed high α-selectivity probably due to the participation of the 6-*O*-acetyl group from the β-face.[12,28,29] Chromatographic separation of the desired *p*NP α-glucoside **7** was performed



Scheme 1

Table 1. 1-*O*-*p*-Nitrophenylation of 1,6-Di-*O*-acetyl-2,3,4-tri-*O*-benzyl-D-glucopyranose.

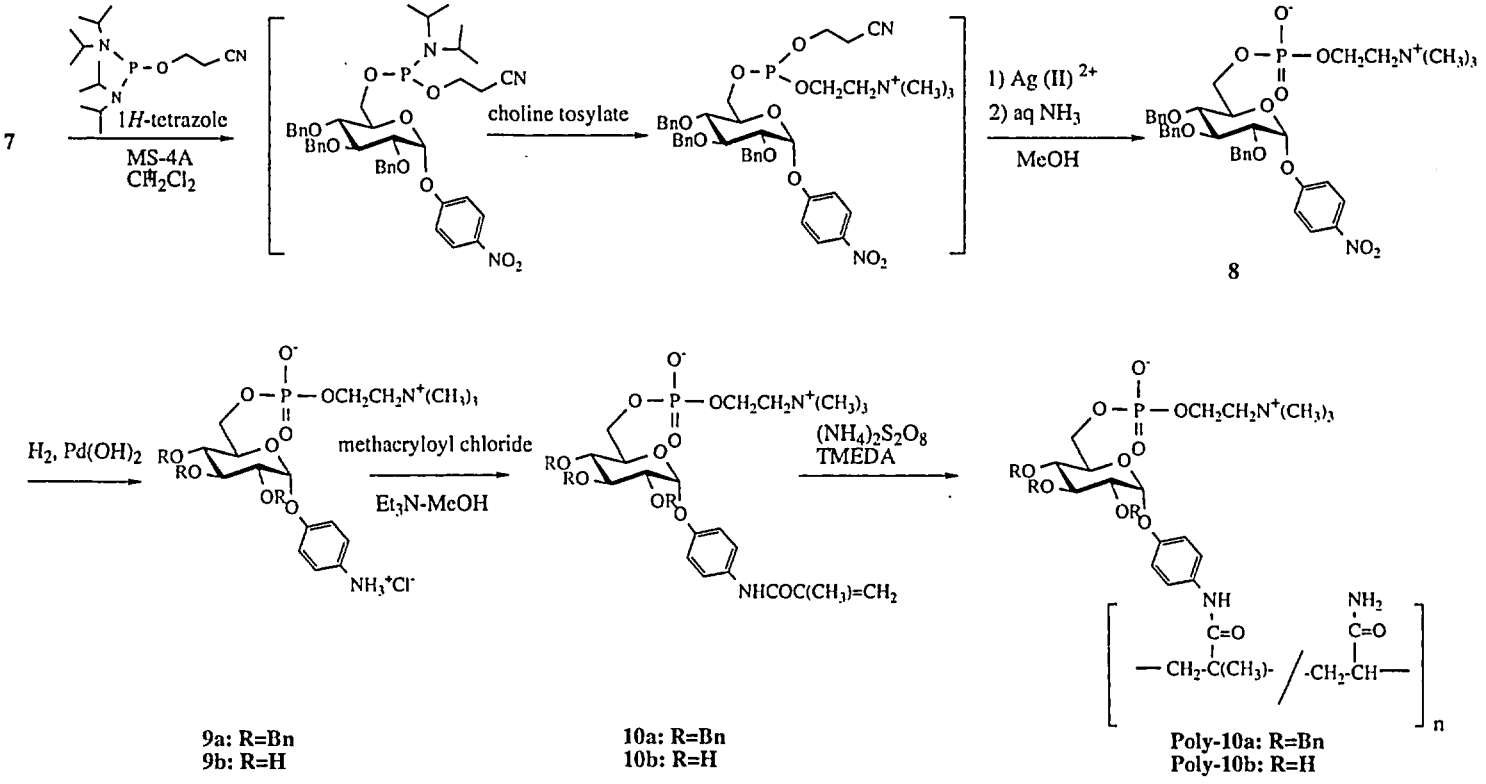
entry	5 (α/β)	$BF_3 \cdot OEt_2$ equiv	*p*-nitrophenol equiv	solvents	conditions	yield [a] %	product (α/β) [b]
1	5 (85/15)	6	4	CH_2Cl_2	0 °C, 8.5h	93	6 (90/10)
2	5 (87/13)	4	4	CH_2Cl_2	0 °C, 16h	84	6 (89/11)
3	5 (81/19)	2	2	CH_2Cl_2	rt, 2h	69	6 (75/25)
4	5 (80/20)	4	4	toluene	0 °C, 6h	70	6 (87/23)
5	5 (87/13)	4	4	CH_3CN	0 °C, 8.5h	35	6 (92/8)
6	5 (58/42)	4	4	CH_3CN	0 °C, 6h	30	6 (>99/1)

a: Isolated yield after silica gel column chromatography.
b: Determined by ^1H NMR spectroscopy.

after de-*O*-acetylation with K_2CO_3 in methanol. Here, the usual condition using sodium methoxide in methanol caused the partial decomposition of the *p*NP-glycoside linkage as well as the partial anomerization to some extents. A similar decomposition was observed upon the benzylation of a commercially available *p*NP α-D-glucopyranoside using sodium hydride and benzyl bromide in DMF. This prompted us to undertake the present synthetic approach *via* methyl α-D-glucopyranoside instead of using the *p*NP α-D-glucopyranoside.

Introduction of a phosphocholine group at the position *O*-6 of **7** was attempted using phosphorous oxychloride and 2-bromoethanol according to our previous synthesis of GGPL-I.[12] The reaction gave the desired 6-*O*-phosphocholine derivative **8**, however, in poor yield (25%) because of the partial decomposition of the *p*NP-linkage at the stage converting the 2-bromoethyl group into choline. Therefore, a phosphorodiamidite method[30] was applied as an alternative approach (Scheme 2). Successive introduction of 2-cyanoethyl *N,N*-diisopropylphosphoramidite and then choline gave a product, which was oxidized *in situ* by a silver (II) dipicolinate.[15,31-33] After a usual process to remove the cyanoethyl group in aqueous ammonia in methanol followed by chromatographic purification (silica gel column), *p*NP 6-*O*-phosphocholine α-D-glucopyranoside **8** was obtained in 54% yield. Catalytic hydrogenation using $Pd(OH)_2$ in MeOH gave selectively a *p*-aminophenyl derivative **9a**, while the reaction in aq HCl-methanol afforded a debenzylated GGPL-I mimic **9b**. The primary amino group of **9a** and **9b** was converted into *N*-methacrylamido group using methacryloyl chloride in methanol to afford polymerizable GGPL-I analogues **10a** and **10b**, respectively.

Radical copolymerization of each of **10a** and **10b** with acrylamide (molar ratio: **10a**/acrylamide = 15/60; **10b**/acrylamide = 22/43) was carried out in an aqueous solution using ammonium peroxodisulfate (APS) and *N,N,N',N'*-tetramethylethylene diamine (TMEDA).[34] The acrylamide was selected to increase not only the water solubility but also the conformational flexibility of available polymers that are favored for multivalence interactions with possible GGPL-I binding proteins. The polymerization gave desired **Poly-10a** and **Poly-10b** as colorless powders after being precipitated in methanol and dialyzed (Mw. 3500 cut-off) in water. The ^1H NMR spectra showed that they contained each of the GGPL-I analogues in ca. 30% and 50% molar ratio, respectively, suggesting the sugar monomers might be more reactive than acrylamide under the reaction conditions employed here. The molecular weight was estimated to be ca. 2×10^6 for both polymers by size exclusion chromatography.



Scheme 2

In conclusion, we have presented a convenient synthetic approach towards artificial glycoconjugate polymers carrying GGPL-I analogues starting from D-glucose. The present approach will become a general way for the synthesis of the other glycoconjugate polymers carrying 6-*O*-phosphocholine glycosides as GGPLs analogues.

EXPERIMENTAL

General Methods. Infrared (IR) spectra were recorded on a JASCO FT/IR-230 Fourier transform infrared spectrometer. ^1H and ^{13}C NMR spectra were measured on Varian INOVA-500 (500 MHz, 125 MHz) or Varian Gemini 200 (200 MHz, 50 MHz) at ambient temperature. Chemical shifts are reported in parts per million. Tetramethylsilane and residual solvent peaks were used as internal references. Coupling constants are reported in Hertz (Hz). Optical rotations were measured on a JASCO DIP-1000 digital polarimeter. Size exclusion chromatography (SEC) was conducted with a JASCO 800 high-performance liquid chromatography on Shodex B804 + 805 columns at 40 ℃ using water as the eluent and calibrated with pulluran standards. For thin-layer chromatography (TLC) analysis throughout this work, Merck precoated TLC plates (silica gel 60 F_{254}, layer thickness 0.25 mm) and Merck TLC aluminum roles (silica gel 60 F_{254}, layer thickness 0.2 mm) were used. The products were purified by preparative column chromatography on silica gel (Merck Art. No. 7734, 70-230 mesh). Reactions sensitive to moisture or air were performed under nitrogen using anhydrous solvents and reagents. The following compounds, BF_3-Et_2O complex, *p*-nitrophenol, *1H*-tetrazole, 2-cyanoethyl tetraisopropylphosphorodiamidite, and methacryloyl chloride are all commercially available. The experimental procedures for 1,6-anhydro 2,3,4-tri-*O*-benzyl-β-D-glucopyranose **4** from D-glucose[35] and silver (II) bis (pyridine-2,6-dicarboxylate) monohydrate from silver nitrate and 2,6-pyridine dicarboxylic acid[31-33] were conducted as reported. Choline tosylate was prepared from choline chloride (*p*-toluene sulfonic acid (1 equiv), MeOH, rt, 2 h, crystallized in acetone (white crystal)).

1,6-Di-O-acetyl-2,3,4-tri-O-benzyl-D-glucopyranoside (5).

a) To a solution of trifluoroacetic acid (3.25 mL, 42.2 mmol) in acetic anhydride (65.0 mL, 688 mol) was added 1,6-anhydro 2,3,4-tri-*O*-benzyl-β-D-glucopyranose **4** (6.49 g, 15.0 mmol) at room temperature. The reaction mixture was stirred for 6 h at the same temperature under dry condition ($CaCl_2$ tube). This solution was concentrated (azeotropy with ethanol and toluene). The residue was washed with water and saturated aq $NaHCO_3$ solution, and extracted with CH_2Cl_2. The organic layer was dried ($MgSO_4$) and concentrated. The residue was purified by silica gel column chromatography (AcOEt/CH_2Cl_2/*n*-hexane = 1/3/1 to 1/1/1 to 3/2/3) to give **5** (8.02 g, 15.0 mmol, 100%, α/β=85/15 (determined by 200 MHz ^1H NMR)).

b) To a solution of methyl α-D-glucopyranoside **2** (971 mg, 5.00 mmol) in 50 mL of DMF was added sodium hydride in oil, 60 % (1.20 g, 30 mmol, 6 equiv) at 0 °C. The solution was stirred for 0.5 h at the same temperature under dry conditions ($CaCl_2$ tube). To this was added benzyl bromide (3.51 mL, 30 mmol, 6 equiv) at 0 °C. The mixture was stirred for 1.5 h at 0 °C and then for 3.5 h at room temperature. The reaction was quenched by the addition of MeOH at 0 °C, filtered through a silica gel pad, and concentrated. The residual oil was washed with water and saturated aq NaCl solution, and extracted with $CHCl_3$. The organic layer was dried ($MgSO_4$) and concentrated. The residue was purified by silica gel column chromatography (AcOEt/*n*-hexane = 1/10 to 1/5 to 1/2) to give crude **3**. To the solution of crude **3** in 150 mL of Ac_2O was added 10 mol % of H_2SO_4 (26.7 μL, 0.500 mmol) at 0 °C, and stirred for 3 h at room temperature. The reaction mixture was mixed with 100 mL of water, stirred for 1 h at 0 °C, washed with saturated aq $NaHCO_3$ solution, and extracted with $CHCl_3$. The organic layer was dried ($MgSO_4$) and concentrated. The residue was purified by silica gel column chromatography (AcOEt/*n*-hexane = 1/10 to 1/7 to 1/4 to 1/2 to 2/1) to give **5** (2.46 g, 4.61 mmol, 92 % (two steps), α/β=76/24 (determined by 200 MHz ^{1}H NMR)).

***p*-Nitrophenyl 6-*O*-Acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (6).** To a solution of **5** (8.02 g, 15.0 mmol) in 250 mL of CH_2Cl_2 was added boron trifluoride ether complex (11.1 mL, 90.0 mmol, 6 equiv) and *p*-nitrophenol (8.35 g, 60.0 mmol, 4 equiv) at 0 °C under nitrogen. The reaction was stirred at 0 °C for 8.5 h, quenched by the addition of water at 0 °C, washed with saturated aq $NaHCO_3$ solution, and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by silica gel column chromatography (AcOEt/CH_2Cl_2/*n*-hexane = 1/4/15 to 1/4/10 to 1/4/7 to 1/4/5) to give **6** (8.58g, 14.0mmol, 93%, α/β=90/10 (determined by 200 MHz ^{1}H NMR)) as a pale yellow oil. Only the α-anomer crystallized (white): mp 110-111 °C; $[\alpha]_D^{23}$ +122.9° (*c* 0.10, $CHCl_3$); IR (KBr) 3072, 3030, 2894, 1727, 1590, 1517, 1494, 1454, 1346, 1234, 1111, 1066, 999, 874, 741, 700, 665, 455 cm^{-1}; ^{1}H NMR (500 MHz, rt, $CDCl_3$) δ 8.21-8.18 (m, 2H, *o*-position of nitro group), 7.42-7.25 (m, 15H, C_6H_5), 7.13-7.11 (m, 2H, *m*-position of nitro group), 5.40 (d, *J* =3.5, 1H, H-1), 5.09-4.58 (m, 6H, CH_2Ph), 4.25 (dd, *J* =12.5, 5.0, 1H, H-6), 4.20 (dd, *J* =10.0, 9.5, 1H, H-3), 4.14 (dd, *J* =13.0, 2.5, 1H, H-6), 3.83-3.80 (m, 1H, H-5), 3.73 (dd, *J*=9.5, 3.5, 1H, H-2), 3.60 (dd, *J* =9.5, 9.5, 1H, H-4), 1.97 (s, 3H, CH_3CO); ^{13}C NMR (125 MHz, rt, $CDCl_3$) δ 170.4, 161.2, 142.6, 138.3, 137.6, 137.4, 128.5, 128.5, 128.5, 128.4, 128.1, 128.1, 128.0, 127.9, 127.9, 127.8, 125.7, 116.4, 95.3, 81.6, 79.5, 76.6, 75.9, 75.2, 73.7, 70.0, 62.3, 20.7.

Anal. Calcd for $C_{35}H_{35}O_9N$: C, 68.50; H, 5.75; N, 2.28. Found: C, 68.39; H, 5.76; N, 2.20.

***p*-Nitrophenyl 2,3,4-Tri-*O*-benzyl-α-D-glucopyranoside (7).** To a solution of **6** (1.84 g, 3.00 mmol, α/β=90/10) in 100 mL of MeOH was added K_2CO_3 (580 mg, 4.20 mmol) at room temperature. This solution was stirred for 2.5 h at the same temperature. The reaction mixture was neutralized with Amberlyst^R 15E (1.50 g, rt, 1 h). This solution was filtered through a Celite pad and concentrated. The residue was purified by silica gel column chromatography (ether/*n*-hexane = 1/2 to 1/1 to 2/1 to 4/1) to give **7** (100%, α/β=>99/1 (determined by 200 MHz ^{1}H NMR), pale yellow oil). $[\alpha]_D^{23}$ +131.5° (*c* 0.10, $CHCl_3$); IR (KBr) 3448, 3030, 2927, 1593, 1516, 1495, 1342, 1244, 1103, 1072, 1020, 872, 737, 696, 638 cm^{-1}; ^{1}H NMR (500 MHz, rt, $CDCl_3$) δ 8.20-8.18 (m, 2H, *o*-position of nitro group), 7.42-7.26 (m, 15H, C_6H_5), 7.10-7.09 (m, 2H, *m*-position of nitro group), 5.40 (d, J=3.5, 1H, H-1), 5.07-4.63 (m, 6H, CH_2Ph), 4.20 (dd, J=9.5, 9.5, 1H, H-4), 3.72-3.62 (m, 5H); ^{13}C NMR (125 MHz, rt, $CDCl_3$) δ 161.3, 142.5, 138.4, 137.6, 128.48, 128.5, 128.4, 128.1, 128.0, 128.0, 127.9, 127.8, 127.7, 125.7, 116.3, 95.4, 81.4, 79.5, 76.3, 75.8, 75.1, 73.7, 72.2, 61.0.

Anal. Calcd for $C_{33}H_{33}O_8N$: C, 69.34; H, 5.82; N, 2.45. Found: C, 69.38; H, 5.78; N, 2.44.

***p*-Nitrophenyl 2,3,4-Tri-*O*-benzyl-6-*O*-phosphorylcholine-α-D-gluco-pyranoside (8).** To a solution of **7** (3.25 g, 5.69 mmol) and molecular sieves 4A (50 %w/w) in 100 mL of distilled CH_2Cl_2 was added 2-cyanoethyl-*N*,*N*,*N*',*N*'- tetraisopropyl phosphorodiamidite (2.71 mL, 8.53 mmol, 1.5 equiv) and *1H*-tetrazole (439 mg, 6.26 mmol, 1.1 equiv) at room temperature under nitrogen. The solution was stirred for 0.5 h at the same temperature. To this were added *1H*-tetrazole (1.20 g, 17.1 mmol, 3 equiv) and then (after 3 minutes) choline tosylate (6.27 g, 22.8 mmol, 4 equiv) at room temperature. This solution was stirred for 4.5 h at room temperature, filtered through silica gel, and concentrated. The residue was diluted with MeCN/H_2O/MeOH = 4/1/1 solution (120 mL). To this was added silver(II) bis(pyridine-2,6-dicarboxylate) monohydrate (7.79 g, 17.2 mmol, 3 equiv of **7**), and stirred for 1 h at room temperature. To the reaction mixture was added AcONa·3H_2O (3.10 g, 22.8 mmol, 4 equiv of **7**), and the mixture stirred for 0.5 h at room temperature. The solution was filtered through a pad of Celite and concentrated. The residual oil was diluted with 50 mL of MeOH, and to this was added 1 mL of 30 % aq NH_3 and stirred for 1 h at room temperature. This solution was concentrated and purified by silica gel column chromatography ($CHCl_3$/MeOH/H_2O = 7/1/0 to 3/1/0 to 3/2/0 to 6/4/1 to 3/3/1) to give **8** (2.20 g, 2.99 mmol, 53 % from **7**, white solid): mp 53-54 °C; $[\alpha]_D^{25}$ +106.6° (*c* 0.10, $CHCl_3$); IR (KBr) 3406, 2935, 1593, 1518, 1495, 1344, 1246, 1090, 1024, 968, 739, 698, 461 cm^{-1}; ^{1}H NMR (500 MHz, rt, $CDCl_3$) δ 8.16-8.15 (m, 2H, *o*-position of nitro group), 7.35-7.22 (m, 15H, C_6H_5), 7.15-7.14 (m, 2H, *m*-position of nitro group), 5.46 (d, J =3.0, 1H, H-1), 5.04-4.62 (m, 6H, CH_2Ph), 4.19-4.05 (m, 4H, H-6 and POCH_2CH_2), 3.90 (b, 1H, H-5), 3.76-3.70 (m, 2H, H-3 and H-4), 3.66 (dd, J =10.0,

3.0, 1H, H-2), 3.51 (b, 2H, CH_2N), 3.09 (s, 9H, $N(CH_3)_3$); ^{13}C NMR (125 MHz, rt, $CDCl_3$) δ 161.5, 142.3, 138.5, 138.3, 137.7, 128.4, 128.3, 128.3, 128.1, 127.9, 127.9, 127.6, 127.6, 127.5, 125.7, 116.6, 95.4, 81.3, 79.2, 77.2, 75.5, 74.6, 73.4, 71.5, 71.5, 66.0, 63.5, 58.9, 53.9; MS for $C_{38}H_{45}O_{11}N_2P$ 736.7596.

***p*-Aminophenyl 2,3,4-Tri-*O*-benzyl-6-*O*-phosphocholine-α-D-glucopyranoside (9a).** A mixture of **8** (970 mg, 1.32 mmol) and $Pd(OH)_2/C$ (200 mg) in 80 mL of MeOH was hydrogenated at room temperature under atmospheric pressure for 7 h. The reaction mixture was filtered through a pad of Celite and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH/H_2O = 6/4/1 to 3/3/1 to 3/5/2) to give **9a** (853 mg, 1.21 mmol, 92 %, pale brown solid): mp 153-154 °C; $[\alpha]_D^{25}$ +74.5° (*c* 0.10, $CHCl_3$); IR (KBr) 3403, 3031, 2923, 1635, 1510, 1454, 1361, 1224, 1087, 1028, 968, 921, 835, 737, 696, 532 cm^{-1}; ^1H NMR (500 MHz, rt, $CDCl_3$) δ 7.35-7.21 (m, 15H, C_6H_5), 6.84 (d, *J* =8.5, 2H, *m*-position of amino group), 6.59 (d, *J* =8.5, 2H, *o*-position of amino group), 5.17 (d, *J* =2.5, 1H, H-1), 5.02-4.56 (m, 6H, CH_2Ph), 4.32 (broad s, 2H, NH_2), 4.16-4.12 (m, 2H, H-6), 4.01-3.81 (m, 4H, H-3, H-5 and $POCH_2CH_2$), 3.60 (dd, *J* =3.0, 9.8, 1H, H-2), 3.38 (b, 3H, H-4 and CH_2N), 2.90 (s, 9H, $N^+(CH_3)_3$); ^{13}C NMR (125 MHz, rt, $CDCl_3$) δ 148.7, 143.5, 138.6, 138.1, 138.0, 128.4, 128.4, 128.3, 128.1, 128.0, 127.8, 127.8, 127.7, 127.5, 119.5, 115.6, 97.2, 81.6, 79.7, 78.2, 75.6, 74.9, 73.0, 71.3, 65.8, 65.0, 58.9, 53.8; MS for $C_{38}H_{47}O_9N_2P$ 706.7756.

***p*-Aminophenyl 6-*O*-Phosphocholine-α-D-glucopyranoside (9b).** A mixture of **8** (765 mg, 1.04 mmol), $Pd(OH)_2/C$ (150 mg) and 35 %w/w aq HCl (10.4 mmol, 10 equiv) in 50 mL of MeOH was hydrogenated at room temperature under atmospheric pressure for 7.5 h. The reaction mixture was filtered through a pad of Celite and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH/H_2O = 1/4/2 to 0/1/3 to 0/1/7) to give **9b** (441 mg, 1.01 mmol, 97 %, pale yellow solid): mp 199-200 °C; $[\alpha]_D^{25}$ +92.1° (*c* 0.10, H_2O); IR (KBr) 3349, 2937, 2489, 1637, 1511, 1479, 1222, 1083, 968, 922, 833, 768, 478 cm^{-1}; ^1H NMR (500 MHz, rt, D_2O) δ 6.85 (d, *J* =8.5, 2H, *m*-position of amino group), 6.65 (d, *J* =8.5, 2H, *o*-position of amino group), 5.26 (d, *J* =4.0, 1H, H-1), 3.97 (b, 2H, $POCH_2CH_2$), 3.92-3.86 (m, 2H, H-6), 3.82 (b, 1H, H-5), 3.70 (dd, *J* =9.5, 9.5, 1H, H-3), 3.53 (dd, *J* =9.5, 4.0, 1H, H-2), 3.37 (dd, *J* =9.5, 9.5, 1H, H-4), 3.33-3.31 (m, 2H, CH_2N), 2.91 (s, 9H, $N^+(CH_3)_3$); ^{13}C NMR (125 MHz, rt, D_2O) δ 149.3, 141.9, 119.4, 117.7, 98.7, 72.7, 71.3, 70.9, 69.0, 65.7, 64.3, 64.2, 59.2, 59.2, 53.7; MS for $C_{17}H_{29}O_9N_2P$ 436.4024.

***p*-Methacryloylamidophenyl 2,3,4-Tri-*O*-benzyl-6-*O*-phosphorylcholine-α-D-glucopyranoside (10a).** To a solution of **9a** (754 mg, 1.07 mmol) in CH_2Cl_2 (15 mL) was added methacryloyl chloride (0.210 mL, 2.13 mmol, 2 equiv) and triethylamine (1.49 mL, 10.7 mmol, 10 equiv) at room temperature. After the solution was stirred for 1 h, the mixture was extracted with $CHCl_3$, processed as usual, and purified by

silica gel column ($CHCl_3$/MeOH/H_2O = 3/2/0 to 1/1/0 to 6/4/1 to 3/3/1 to 2/5/3) to give **10a** (761 mg, 0.983 mmol, 92 %, white solid): mp 129-130 °C; $[\alpha]_D^{23}$ +55.1° (*c* 0.1, $CHCl_3$); IR (KBr) 3392, 3031, 2925, 1664, 1626, 1509, 1454, 1409, 1361, 1223, 1086, 968, 835, 737, 698, 524 cm^{-1}; ^1H NMR (500 MHz, rt, $CDCl_3$) δ 9.77 (b, 1H, CONH), 7.66 (d, J =8.5, 2H, *o*-position of amido group), 7.39-7.25 (m, 15H, C_6H_5), 6.98 (d, J =9.0, 2H, *m*-position of amido group), 5.98 (s, 1H, $COCCH_3CHH$), 5.35 (s, 1H, $COCCH_3CHH$), 5.32 (d, J =3.5, 1H, H-1), 5.05-4.57 (m, 6H, CH_2Ph), 4.18 (m, 2H, H-6), 3.93-3.77 (m, 4H, H-3, H-5 and $POCH_2CH_2$), 3.64 (dd, J =3.0, 9.5, 1H, H-2), 3.37-3.33 (m, 3H, H-4 and CH_2N), 2.93 (s, 9H, $N^+(CH_3)_3$), 1.96 (s,3H,$COCCH_3$); ^{13}C NMR (125 MHz, rt, $CDCl_3$) δ 167.4, 152.8, 140.0, 138.4, 137.8, 137.7, 133.7, 128.9, 128.4, 128.3, 128.1, 127.9, 127.8, 127.7, 127.7, 127.5, 122.6, 120.7, 117.1, 95.6, 81.5, 79.5, 78.2, 75.0, 73.2, 71.6, 65.7, 64.9, 58.7, 53.5, 18.9.

Anal. Calcd for $C_{42}H_{51}O_{10}N_2P$: C, 65.10; H, 6.63; N, 3.62. Found: C, 64.88; H, 6.76; N, 3.51.

Acrylamide Copolymer (Poly-10a). To a solution of **10a** (112 mg, 0.145 mmol) and acrylamide (32.8 mg, 0.580 mmol) in 2 mL of H_2O/DMSO = 3/1 solution was added TMEDA (32.8 µL, 0.218 mmol) and $(NH_4)_2S_2O_8$ (0.1 g/mL water solution, 49.6 µL, 21.8 µmol) at room temperature under nitrogen. This solution was stirred for 4 h at 50 °C and then for 3 h at 70 °C. To the reaction mixture was added MeOH, and the precipitate was collected by filtration to give **Poly-10a** (16.4 mg, 11 %, sugar/acrylamide = 33/67, white solid): ^1H NMR (500 MHz, 50 °C, DMSO-d_6) δ 7.80-6.62 (b, aromatic proton), 5.76 (b, H-1), 5.18-4.41 (b, CH_2Ph), 4.22-2.82 (b, H-2, H-3, H-4, H-5, H-6 and $POCH_2CH_2N$), 2.60 (b, N^+CH_3 and CH_3CCO), 2.40-1.20 (b, main chain).

***p*-Methacryloylamidophenyl 6-*O*-Phosphorylcholine-α-D-glucopyranoside (10b).** To a solution of **9b** (101 mg, 0.232 mmol) in 5 mL of MeOH was added methacryloyl chloride (45.3 µL, 0.464 mmol, 2 equiv) and triethylamine (0.323 mL, 2.32 mmol, 10 equiv) at room temperature. This solution was stirred for 1 h and concentrated to give a syrupy residue which was purified by silica gel column ($CHCl_3$/MeOH/H_2O = 3/3/1 to 2/5/3 to 0/1/3 to 0/1/7) to give **10b** (109 mg, 0.216 mmol, 93 %, yellow solid): mp 234-235 °C; $[\alpha]_D^{25}$ +76.9° (*c* 0.19, H_2O); IR (KBr) 3417, 2927, 1662, 1628, 1512, 1412, 1377, 1227, 1084, 1026, 964, 926, 872, 833, 768, 621, 521, 490 cm^{-1}; ^1H NMR (500 MHz, rt, D_2O) δ 7.25 (d, J =8.5, 2H, *m*-position of amido group), 7.03 (d, J =8.5, 2H, *o*-position of amino group), 5.63 (d,J =6.0, 1H, *cis*-$C(CH_3)CHH$), 5.45 (d, J =4.0, 1H, H-1), 5.39 (d, J =2.5, 1H, *trans*-$C(CH_3)CHH$), 3.95 (b, 2H, $POCH_2CH_2$), 3.90 (m, 2H, H-6), 3.78 (b, 1H, H-5), 3.76 (dd, J =9.5, 9.5, 1H, H-3), 3.58 (dd, J =9.5, 4.0, 1H, H-2), 3.39 (dd, J =9.5, 9.5, 1H, H-4), 3.33 (b, 2H, CH_2N), 2.93 (s, 9H, $N^+(CH_3)_3$), 1.83 (s, 3H, CH_3); ^{13}C NMR (125MHz, rt, D_2O) δ 170.7, 153.6, 139.4, 131.8, 124.5, 121.4, 118.0, 97.5, 72.7, 71.4, 70.9, 68.9, 65.7, 64.2, 59.2, 53.7, 17.6.

Acrylamide Copolymer (Poly-10b). To a solution of **10b** (109 mg, 0.216 mmol) and acrylamide (30.7 mg, 0.432 mmol) in water (2 mL) was added TMEDA (29.3 μL, 0.194 mmol) and $(NH_4)_2S_2O_8$ (1 g/L water solution, 1.48 mL, 6.48 μmol) at room temperature under nitrogen. This solution was stirred for 10 h. To the reaction mixture was added MeOH, and the precipitate was collected by filtration to afford **Poly-10b** (76 mg, 54 %, sugar/acrylamide = 49/51, white solid): Decomposition point 260 °C; $[\alpha]_D^{27}$ +62.8° (*c* 0.41, H_2O); IR (KBr) 3398, 2939, 1670, 1512, 1481, 1412, 1227, 1084, 1026, 968, 926, 872, 833, 768, 606 cm^{-1}; ^1H NMR (500 MHz, 50 °C, D_2O) δ 7.55-7.04 (b, aromatic proton), 5.72 (b, H-1), 4.32 (b, H-6), 4.20 (b, $POCH_2CH_2$), 4.05 (b, H-3 and H-5), 3.88 (b, H-2), 3.78-3.43 (b, H-4 and CH_2N), 3.24 (b, $N^+(CH_3)_3$), 3.17 (b, CH_3), 2.59-1.20 (b, main chain); ^{13}C NMR (125 MHz, 50 °C, D_2O) δ 179.5, 150.8, 125.0, 124.9, 118.3, 118.2, 98.2, 73.1, 71.8, 71.3, 69.4, 66.2, 64.6, 59.6, 54.2, 42.2, 39.9, 39.6, 35.4.

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