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Supporting Information

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Molecular Transporter between Polymer Platforms: Highly Efficient Glycopeptide Synthesis by Combined Use of Solid-Phase and Water-Soluble Polymer Supports

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Materials and general procedures

Unless otherwise stated, all commercially available solvents and reagents were used without purification. Fmoc amino acid derivatives, Fmoc-Arg(Pbf)-NovaSyn®TGA resin and hydroxyethyl photolinker (4-[4-(1-hydroxyethyl)-2-methoxy-5-nitrophenoxy]-butyric acid **9**) were purchased from NOVA Biochem Co. Ltd. β-1,4-Galactocyltransferase was purchased from TOYOBO Co. Ltd. α-2,3-(*N*)-Sialyltransferase and α-1,3-fucosyltransferase V were purchased from Calbiochem Co. Ltd. Uridine-5'-diphosphogalactose, 2Na (UDP-galactose), cytidine-5'-monophospho-*N*-acetylneuraminic acid, 2Na (CMP-NANA), and guanosine-5'-diphospho-β-L-fucose, 2Na (GDP-fucose) were purchased from YAMASA CO. NMR spectra were recorded with Bruker AVANCE 400 spectrometer at 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR). Chemical reactions were monitored by thin layer chromatography (TLC) on precoated plates of silica gel 60 F_{254} (layer thickness, 0.25 mm; E. Merck, Darmstadt). Matrix-associated laser-desorption ionization time-of-fright mass spectrometry (MALDI-TOF-MS) and MALDI-TOF/TOF tandem mass spectrometry were performed by using Bruker REFLEXIII and Ultraflex. Analytical HPLC was performed on a Hitachi HPLC system equipped with an L-2130 intelligent pump and an L-2420 UV detector or with an L-7100 intelligent pump and an L-7405 UV detector, and reversed-phase (RP) C18 column, Inertsil® ODS-3 (4.6 x 250 mm) or Inertsil® ODS-3 (4.6 x 150 mm) and at a flow rate of 1.0 ml min^{-1}, and gel permeation chromatography (GPC)

column, TSK-GEL® G3000PWXL (7.8 x 300 mm) at a flow rate of 1.0 ml min^{-1}. The chromatography was monitored by using UV absorption at 220nm or 254nm. Preparative HPLC was carried out on a Hitachi HPLC system equipped with an L-7150 intelligent pump, an L-7420 UV detector and RP C18 column, Inertsil® ODS-3 (10 x 250mm) at a flow rate of 4.0 ml min^{-1}. Column chromatography was performed on silica gel (Silicagel 60N; 40-50 μm, KANTO CHEMICAL CO., INC.) using EtOAc-hexane, chloroform-EtOAc-MeOH, or chloroform-MeOH as general eluant solvents. Solvent extracts were dried with magnesium sulfate. Fast atom bombardment mass spectrometry (FAB-MS: JEOL JMS-HX110), electrospray ionization mass spectrometry (ESI-MS: JEOL JMS-700TZ), amino acid analysis (JEOL JLC-500V), and peptide sequence analysis (Procise491 cLC, Applied Biosystems) were performed by Center of Instrumental Analysis, Hokkaido University.

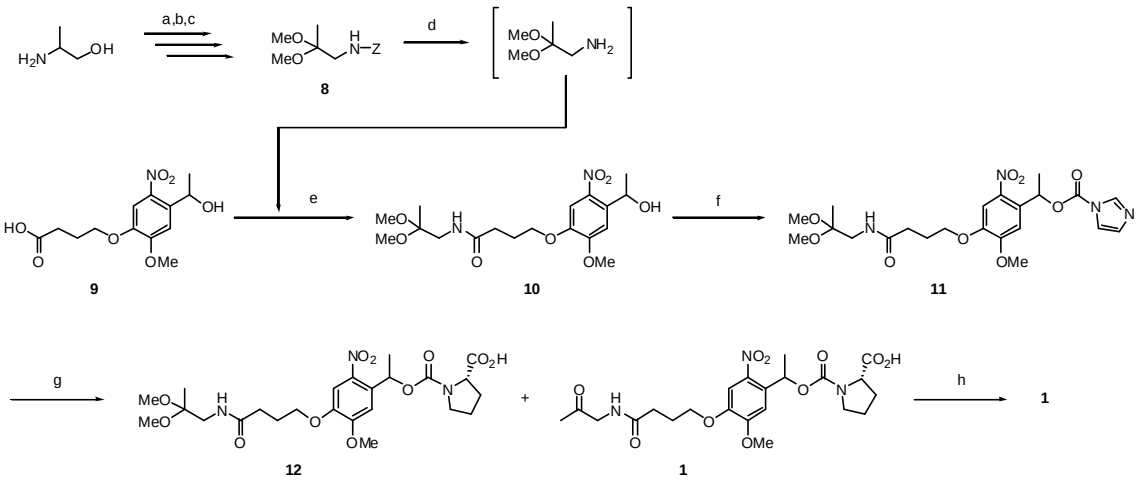
Synthesis

Pyrrolidine-1,2-dicarboxylic acid

1-(1-{5-methoxy-2-nitro-4-[3-(2-oxopropylcarbamoyl)-propoxy]-phenyl}-ethyl) ester 1

Compound **1** was synthesized according to the procedure indicated in the Scheme S-1.

Scheme S-1. Synthesis of compound **1**.



(2,2-Dimethoxypropyl)-carbamic acid benzyl ester 8

(2,2-Dimethoxypropyl)-carbamic acid benzyl ester **8** was prepared from 1-aminopropan-2-ol. To a solution of 1-aminopropan-2-ol (10.0 g, 0.13 mol) in 4.8% NaOHaq. (166 g) was added benzyloxycarbonyl chloride (Z-Cl, 27.3 g, 0.16 mol) in a portion wise maintaining at 0-10℃. The mixture was stirred for 15 min at 0-10℃, then wormed to room temperature, and stirred for 30 min. The pH of the mixture was adjusted to pH 2.5 by 4 N HClaq., and the reaction mixture was extracted

with AcOEt. The organic layer was washed with water, dried and evaporated to give a crude oil. Chromatography on silica gel gave pure (2-hydroxypropyl)-carbamic acid benzyl ester (23.7 g, 0.11 mol, 85%) as colorless oil.

To a solution of DMSO (14.0 g, 180 mmol) in dichloromethane (90 ml) was added trifluoroacetic anhydride (28.4 g, 135 mmol) in dichloromethane (60 ml) dropwisely at under -60℃. The mixture was stirred for 30 min, and to the mixture was added (2-hydroxypropyl)-carbamic acid benzyl ester (18.8 g, 90 mmol) in dichloromethane (60 ml) dropwisely, and the mixture was stirred for 40 min. Then, triethylamine (36 ml) was added dropwisely to the mixture, and the reaction mixture was wormed slowly for 1.5 hr to the room temperature. The mixture was added water (180 ml), and extracted with dichloromethane. The organic layer was dried and evaporated to give a crude oil. Chromatography on silica gel gave pure (2-oxopropyl)-carbamic acid benzyl ester (17.0 g, 82.1 mmol, 91%) as white crystal.

To a solution of (2-oxopropyl)-carbamic acid benzyl ester (10.4 g, 50 mmol) in MeOH (100 ml) was added orthoformic acid trimethyl ester (15.9 g, 150 mmol) and *p*-toluenesulfonic acid monohydrate (95 mg, 0.5 mmol), and the solution was refluxed for 2.5 hr. After cooling the mixture to the room temperature, the solvent was evaporated. The residue was added with water and extracted with AcOEt. The organic layer was washed with water, dried and evaporated to give a crude oil. Chromatography on silica gel gave pure (2,2-dimethoxy-propyl)-carbamic acid benzyl

ester **8** (12.2 g, 48 mmol, 96%) as colorless oil. ^{1}H-NMR ($CDCl_3$) : δ 7.44-7.28 (m, 5 H, Ph), 5.11 (s, 2 H, C*H*$_2$Ph), 4.86 (br t, 1 H, N*H*), 3.35 (d, 2 H, *J*=5.9 Hz, C*H*$_2$), 3.20 (s, 6 H, OC*H*$_3$), 1.29 (s, 3 H, C*H*$_3$); ^{13}CNMR ($CDCl_3$) : δ 156.4, 136.5, 128.5, 128.2, 128.1, 100.3, 66.9, 48.5, 45.1, 20.0 ; FAB-MS (pos) : 276.12 (M+Na^+), calcd 276.12.

***N*-(2,2-Dimethoxypropyl)-4-[4-(1-hydroxyethyl)-2-methoxy-5-nitro phenoxy]-butyramide 10**

To a solution of (2,2-dimethoxypropyl)-carbamic acid benzyl ester **8** (3.04 g, 12.0 mmol) in 40 ml of MeOH was added 10% (w/w) Pd-C (0.30 g). The reaction mixture was hydrogenated under H_2 gas atmosphere (balloon), and stirred vigorously for 8.5 hr. Then, Pd-C was removed by filtration and the residual solution of 2,2-dimethoxypropylamine in MeOH was subsequently added with 4-[4-(1-hydroxyethyl)-2-methoxy-5-nitrophenoxy]-butyric acid **9** (1.80 g, 6.0 mmol). To this mixture was added EEDQ (2-ethoxy-1-ethoxycarbonyl-1,2-dihydro-quinoline, 2.96 g, 12.0 mmol) and the mixture was stirred at room temperature for 18.5 hr. Solvent was evaporated to give a crude oil. Chromatography on silica gel gave pure compound **10** (1.82 g, 4.5 mmol, 75.6%) as yellow oil. ^{1}H-NMR($CDCl_3$) : δ 7.57 (s, 1 H, -C*H*$_2$(-NO_2, -OCH_3)-), 7.30 (s, 1 H, -C*H*$_2$(-NO_2, -OCH_3)-), 5.66 (br t, 1 H, N*H*), 5.56 (br dq, 1 H, C*H*), 4.11 (t, 2 H, *J*=6.2 Hz, C*H*$_2$), 3.98 (s, 3 H, OC*H*$_3$), 3.42 (d, 2 H, *J*=5.7 Hz, C*H*$_2$), 3.20 (s, 6 H, OC*H*$_3$), 2.43 (t, 2 H, *J*=7.2 Hz, C*H*$_2$), 2.37 (br d, 1 H, O*H*), 2.20 (tt, 2 H, J=6.2 Hz, 7.2 C*H*$_2$), 1.55 (d, 3 H, *J*=6.3 Hz, C*H*$_3$), 1.26 (s, 3 H, C*H*$_3$) ;

^{13}C-NMR ($CDCl_3$) : δ 172.0, 154.1, 146.9, 139.6, 137.0 109.3, 108.7, 100.2, 68.4, 65.7, 56.4, 48.6, 43.5, 32.8, 24.9, 24.3, 20.0 ; FAB-MS (pos) : 401.19 ($M+H^+$) , calcd for 401.19.

Imidazole-1-carboxylic acid

1-{4-[3-(2,2-dimethoxypropylcarbamoyl)-propoxy]-5-methoxy-2-nitrophenyl}-ethyl ester 11

To a solution of compound **10** (0.80 g, 2.0 mmol) in 20 ml of CH_2Cl_2 was added 1,1'-carbonyldiimidazole (0.36 g, 2.2 mmol). The reaction mixture was stirred at room temperature for 8 hr, and added 1,1'-carbonyldiimidazole (0.12 g, 0.73 mmol), then stirred for 15.5 hr. Water (20 ml) was added to the solution, and the mixture was extracted with CH_2Cl_2. The organic layer was washed with water, dried and evaporated to give compound **11** (1.02 g, 2.0 mmol) in quantitatively as yellow oil. ^{1}H-NMR ($CDCl_3$): 8.16 (m, 1 H, -imidazol), 7.62 (s, 1 H, -C*H*$_2$(-NO2, -OCH_3)-), 7.44 (m, 1 H, -imidazol), 7.09 (m, 1 H. -imidazol), 7.00 (s, 1 H, -C*H*$_2$(-NO2, -OCH_3)-), 6.72 (q, 1 H, *J*=6.4 Hz, C*H*), 5.67 (br t, 1 H, N*H*), 4.13 (t, 2 H, *J*=6.1 Hz, C*H*$_2$), 3.93 (s, 3 H, OC*H*$_3$), 3.42 (d, 2 H, *J*=5.7 Hz, C*H*$_2$), 3.20 (s, 6 H, OC*H*$_3$), 2.43 (t, 2 H, *J*=7.0 Hz, C*H*$_2$), 2.21 (tt, 2 H, *J*=6.1 and 7.0 Hz, C*H*$_2$), 1.95 (br , 1 H, O*H*), 1.82 (d, 3 H, *J*=6.4 Hz, C*H*$_3$), 1.25 (s, 3 H, C*H*$_3$) ; ^{13}C-NMR ($CDCl_3$) : δ 171.8, 154.1, 147.9, 147.6, 140.1, 136.9, 130.9, 130.8, 117.1, 109.2, 107.8, 100.2, 73.0, 68.5, 56.4, 48.6, 43.5, 32.6, 24.8, 21.9, 20.0 ; FAB-MS (pos) : 495.21 ($M+H^+$) , calcd for 495.21.

Pyrrolidine-1,2-dicarboxylic acid

1-(1-{4-[3-(2,2-dimethoxypropylcarbamoyl)-propoxy]-5-methoxy-2-nitro phenyl}-ethyl) ester 12

and **Pyrrolidine-1,2-dicarboxylic acid**

1-(1-{5-methoxy-2-nitro-4-[3-(2-oxo propylcarbamoyl)-propoxy]-phenyl}-ethyl) ester 1

To a solution of compound **11** (0.97 g, 1.9 mmol) in 20ml of DMF-water (4:1) was added *L*-proline (0.66 g, 5.7 mmol) and 4-methyl-morpholine (0.19 g, 1.9 mmol). The reaction mixture was stirred at room temperature for 6.5 hr, and added *L*-proline (0.33 g, 2.9 mmol) and 4-methyl-morpholine (0.19 g, 1.9 mmol). The reaction mixture was then stirred for 15 hr. Water (20 ml) was added to the solution and the pH of the solution was adjusted to pH 3 by using 4 N HClaq., then the mixture was extracted with EtOAc. The organic layer was washed with water and sat. $NaHCO_3$ aq. Water layer containing sodium salt of the products was washed with EtOAc, and organic layer was again washed with water. To a combined water layer was added EtOAc and 4 N HCl until the pH of the solution become to pH 3. Organic layer was washed with water and brine, dried and evaporated to give a mixture of compound **12** and **1** (1.02 g) as yellow solid.

A part of the mixture of dimethylacetal **12** and ketone (transporter) **1** (0.57 g) was dissolved in 10 ml of 10% TFA in dichloromethane solution, and stirred at room temperature for 10 min. After the solvent was evaporated, water was added, and the pH of the solution was adjusted to pH 2 by using saturated $NaHCO_3$, and then extracted with EtOAc. The organic layer was washed with brine,

dried and evaporated to give a quantitative yield of transporter **1** (0.56 g, 1.1 mmol) as light yellow solid as a mixture of four diastereomers. FAB-MS (pos) : 496.19 ($M+H^+$) , calcd 496.19.

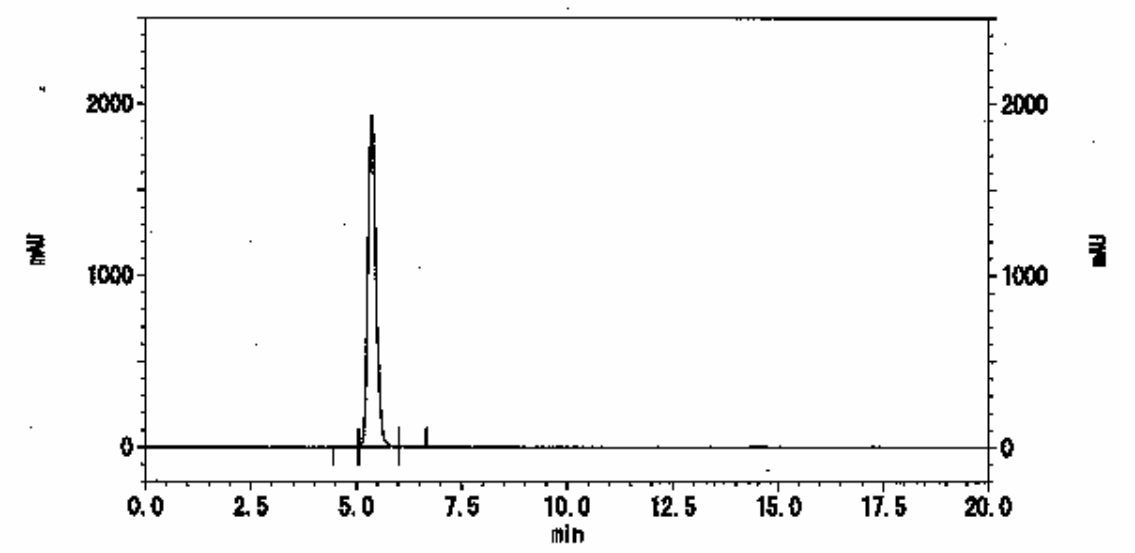


Figure S-1. RP-HPLC analysis of compound **1**. Solvent A (0.1 % TFA in H_2O) and solvent B (0.1 % TFA in acetonitrile), Solvent (A/B=50/50) was used for the chromatography with a flow rate of 1.0 ml/min.

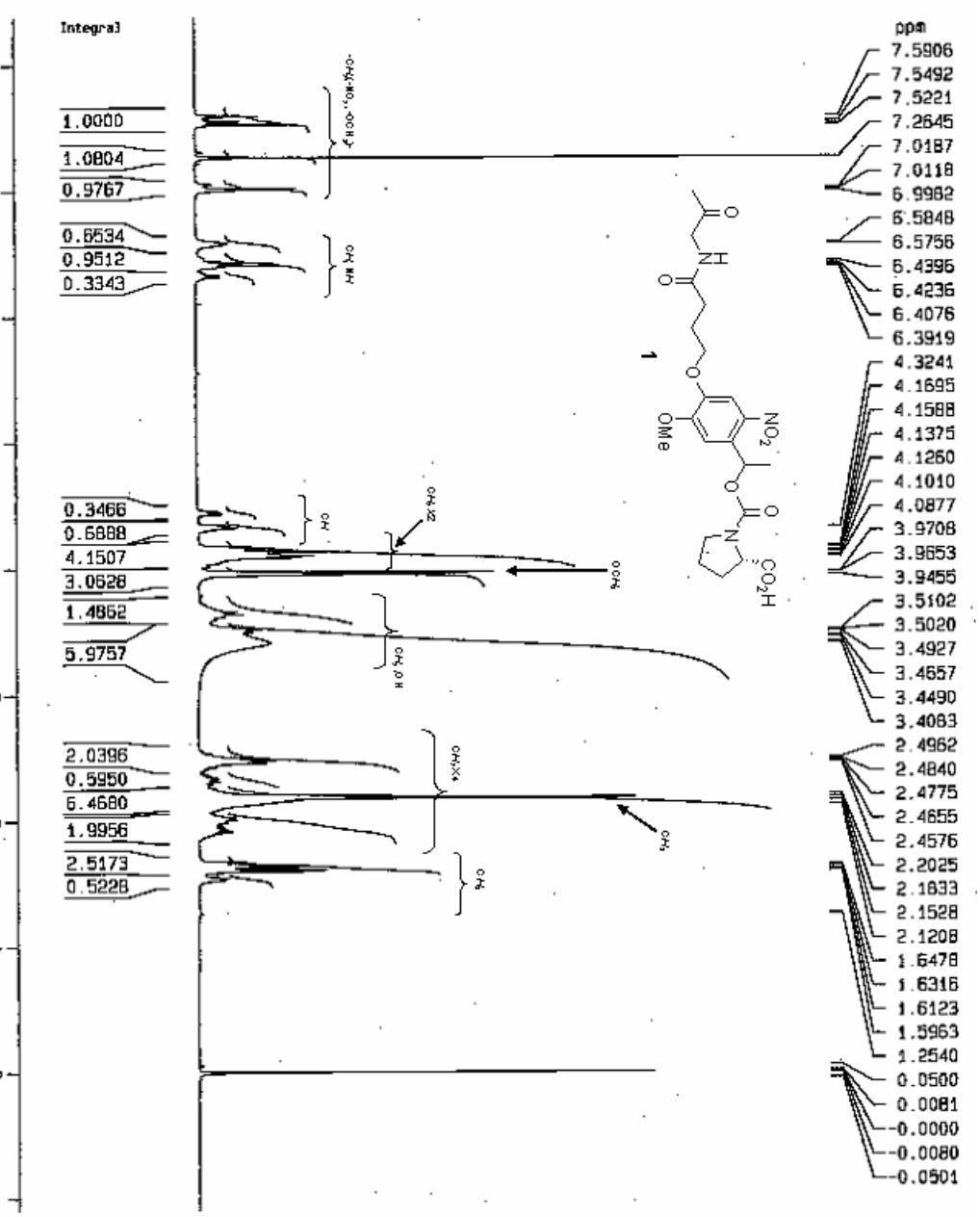
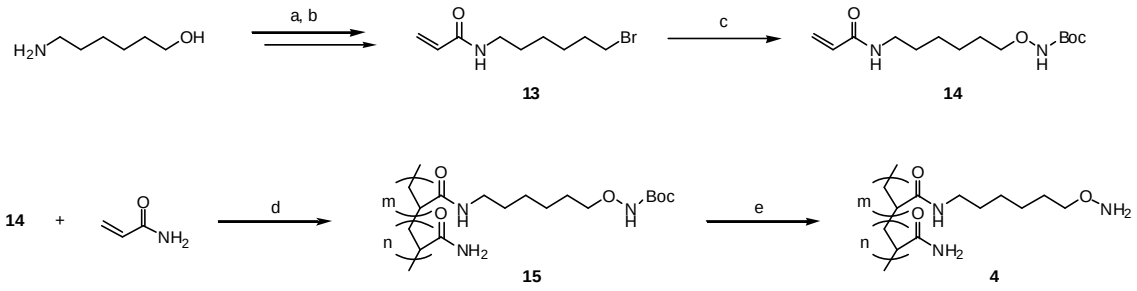


Figure S-2. ^{1}H-NMR spectrum of compound **1**.

Polyacrylamide derivative having oxylamino group 4

Scheme S-2. Synthesis of the polymer **4**.



a) Acryloyl chloride, NaOH, H_2O, b) (1)MsCl, Et_3N, CH_2Cl_2, (2) LiBr; c) NaONH-Boc, DMF; d) APS, TEMED, 50%DMSO aq.;
e) (1) 4N HCl, (2) pH control by 4N NaOH and 50mM AcOH/Na buffer (pH5.0-5.5).

***N*-(6-Bromohexyl)-acrylamide 13**

N-(6-Bromohexyl)-acrylamide **13** was synthesized from 6-aminohexan-1-ol.

To a solution of 6-aminohexan-1-ol (11.7 g, 0.1 mol) in 160 ml of water was added 50% NaOH in water (12 g, 0.15 mol), then the mixture was cooled to 0-5℃. The mixture was stirred and added with acryloyl chloride (10.9 g, 0.12 mol) in a portion wise at 0-10℃. The mixture was stirred for 20 min at 10ºC, then warmed to room temperature, and stirred for 1 hr. The mixture was poured into water, and extracted with EtOAc. The organic layer was washed with brine, dried and evaporated to give a crude oil. Chromatography on silica gel gave pure *N*-(6-hydroxyhexyl)-acrylamide (11.1 g, 65 mmol, 65%) as colorless oil. This compound was used for the next step without further purification.

To a solution of *N*-(6-hydroxyhexyl)-acrylamide (13.7 g, 80.2 mmol) in 500 ml of dichloromethane was added triethylamine (9.7 g, 96.2 mmol) and the solution was cooled to 10-15℃. The mixture was added methanesulfonyl chloride (10.1 g, 88.2 mmol) and stirred for 0.5 hr. The mixture was added LiBr (20.9 g, 0.24 mol) and 500 ml of acetone, and refluxed for 6 hr. The reaction mixture was cooled to room temperature, and stirred for 16 h. The reaction mixture was evaporated,and the residual syrup was poured into saturated $NaHCO_3$. The mixture was extracted with EtOAc. The organic layer was washed with brine, dried, and the solvent was evaporated to give a crude product. Crystallization of the crude material from EtOAc-hexane gave pure *N*-(6-bromohexyl)-acrylamide **13** (16.1 g, 68.8 mmol, 86%) as white crystal. ^{1}H-NMR ($CDCl_3$): 6.28 (dd, 1 H, *J*=1.4 and 17.0 Hz, -CH=C*H*$_2$), 6.09 (dd, 1 H, *J*=10.2 and 17.0 Hz, -C*H*=CH$_2$), 5.64 (dd, 1 H, *J*=1.4 and 10.2 Hz, -CH=C*H*$_2$; br, 1 H, N*H*), 3.41 (t, 2 H, *J*=6.7 Hz, C*H*$_2$), 3.34 (dd, 2 H, *J*=6.2 and 7.0 Hz, C*H*$_2$), 1.92-1.80 (m, 2 H, C*H*$_2$), 1.62-1.51 (m, 2 H, C*H*$_2$), 1.50-1.42 (m, 2 H, C*H*$_2$), 1.42-1.1.33 (m, 2 H, C*H*$_2$) ; ^{13}C-NMR ($CDCl_3$): δ 165.6, 130.9, 126.3, 39,4, 33.8, 32.6, 29.4, 27.8, 26.1 ; FAB-MS (pos): 234.05 ($M+H^+$) , calcd for 234.05.

***N*-[6-(*N*-*tert*-butoxycarbonylaminooxy)-hexyl]-acrylamide 14**

Butyl *N*-hydroxycarbamate (4.09 g, 30.7 mmol) was dissolved in DMF (40 ml), and the solution was treated with NaH (1.23 g, 60% dispersion in mineral oil, 30.7 mmol), and the mixture was stirred at

room temperature for 10 min under N_2. To the reaction mixture was added *N*-(6-bromohexyl)-acrylamide **13** (6.0 g, 25.6 mmol), and the mixture was stirred at 70-80℃ for 3.5 hr. The mixture was cooled to room temperature, then poured into saturated $NaHCO_3$ solution, and extracted with EtOAc. The organic layer was washed with water, dried, and evaporated to give a crude oil. Purification by silica gel chromatography using chloroform/EtOAc as eluant gave pure compound **14** (3.9 g, 13.6 mmol, 53%) as colorless oil. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.26 (br, 1 H, N*H*), 6.28 (dd, 1 H, *J*=1.5 and 17.0 Hz, -CH=CH_2), 6.11 (dd, 1 H, *J*=10.2 and 17.0 Hz, -C*H*=CH_2), 5.83 (br, 1 H, N*H*), 5.62 (dd, 1 H, *J*=1.5 and 10.2 Hz, -CH=CH_2), 3.85 (t, 2 H, *J*=6.4 Hz, CH_2), 3.34 (dd, 2H, *J*=6.1 and 6.9 Hz, CH_2), 1.68-1.32 (m, 8 H, CH_2), 1.48 (s, 9 H, CH_3) ; ^{13}C-NMR ($CDCl_3$): δ 165.6, 156.9, 131.0, 126.2, 81.6, 76.5, 39.2, 29.3, 28.2, 27.8, 26.3, 25.3 ; FAB-MS (pos): 287.20 ($M+H^+$), calcd for 287.20.

Polyacrylamide having *N*-protected oxylamino group 15

To a solution of compound **14** (229 mg, 0.8 mmol) in 50% DMSO in water (30 ml) was added acrylamide monomer (pre-purification by recrystallization from benzene, 1137 mg, 16 mmol). This clear solution was deaerated using water pump, to which was added TEMED (*N*,*N*,*N'*,*N'*-Tetramethylethylenediamine, 202μl, 1.34 mmol) and APS (ammonium persulfate, 123.2 mg, 0.54 mmol). The solution was stirred at 50-60℃ for 6 hr. The reaction mixture was directly

subjected to the purification by dialyzing with permeable membrane (Seamless Cellulose Tubing, UC20-32-100, Sanko Junyaku Co., Ltd) against water. The solution containing polymer was evaporated and lyophilized to afford a target polyacrylamide derivative **15** as an amorphous powder (1119 mg, 82%). Molecular weight of the polymer **15** was estimated as MW=22,000 by GPC-HPLC [solvent A (25 mM Na-Pi buffer pH 7.0) and solvent B (acetonitrile), A/B=90/10; flow rate, 1.0 ml/min; Chromatography was monitored at 220 nm].

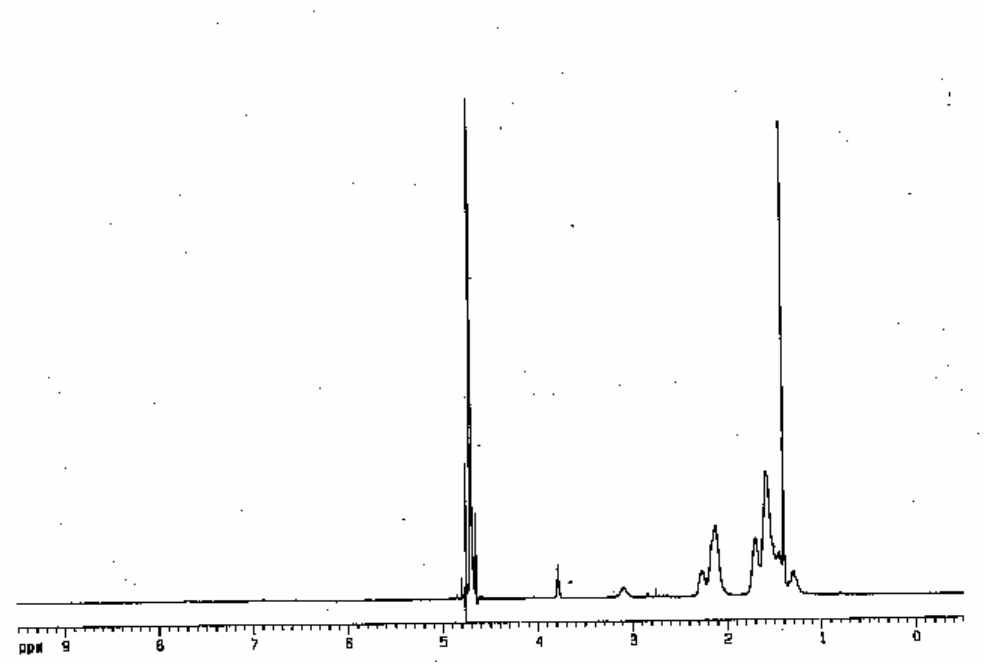


Figure S-3. ^{1}H-NMR (D_2O) spectrum of the polymer **15**.

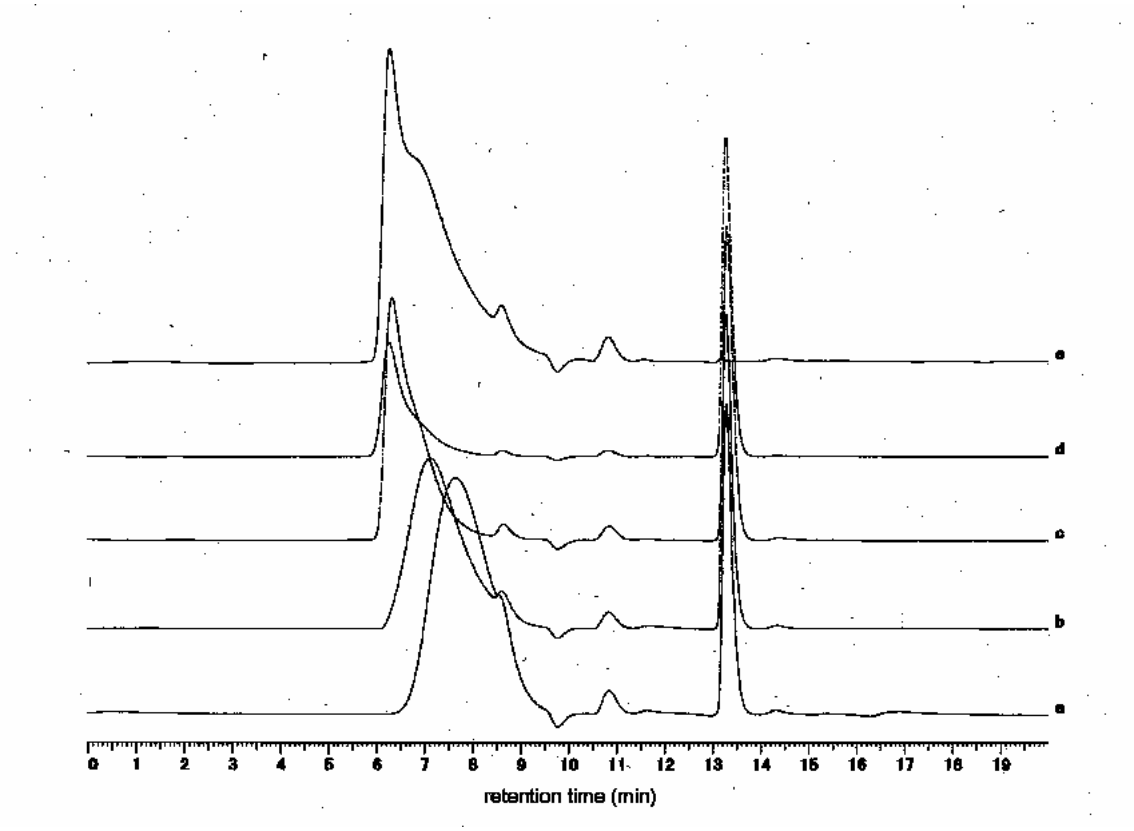


Figure S-4. GPC-HPLC analysis of polymer **15**. a) polyacrylamide (MW~12,000); b) polyacrylamide (MW~22,000); c) polyacrylamide (MW~65,000); d) polyacrylamide (MW~80,000); e) polymer **15**. Standards of polyacrylamide were obtained from Polysciences, Inc. Warrington, PA, USA.

Polyacrylamide having oxylamino group 4

Polymer **15** (136 mg, 0.08 mmol as oxylamino group) was dissolved in 4 N HCl (3.0 ml) and the solution was shaken for 6.0 hr at room temperature. To the reaction mixture was added 4 N NaOH (3.0 ml) and 50 mM AcOH/AcONa buffer (2.0 ml, pH 5.5), then the pH of the solution was controlled carefully to pH 5.0-5.5 by using 4 N NaOH and 4N HCl. This solution was used to the next blotting reaction as a stock solution of 10 mM oxylamino polymer **4** in buffer solution (pH

5.3). The content of oxylamino groups was estimated from the ratio of the monomers used for the radical copolymerization.

Preparation of water-soluble polymer containing *O*-GlcNAc-dodecapeptide 5

Solid-phase synthesis of photosensitive dodecapeptide having *O*-Ac_3GlcNAc residue 2 :

Synthesis of the photosensitive dodecapeptide carrying *O*-Ac_3GlcNAc (**2**) was performed on Fmoc-Arg (Pbf: 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl) -OH preloaded NovaSyn®TGA resin [Fmoc-Arg(Pbf)-NovaSyn®TGA resin, 0.21 mmol/g] (143mg 0.03mmol) using a automated peptides synthesizer (Advanced ChemTech, APEX396) , and off-line solid-phase peptide synthesis in the case of precious sugar amino acid and transporter molecule **1**. One cycle at automated peptide synthesizer were as followed; The resin was mixed and stirred with 20% (v/v) piperidine/DMF (*N,N*-dimethylformamide) for 5 min , and this process was repeated once more at 15min, to complete *N*-deprotection. After filtration, the resin was washed with NMP and DMF, then added to a solution of 5 equivalent of Fmoc Amino acid (0.15mmol), 5 equivalent of HBTU (: 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate), 0.15mmol), 4.3 equivalent of HOBt (*N*-hydroxybenzotriazole·H_2O, 0.13mmol), and 7 equivalent of DIEA (diisopropylethylamine, 0.21 mmol) in NMP/DMF. The mixture was stirred for 1.0 hr at ambient temperature, and this coupling process was performed two times. The reaction mixture was filtrated

and the residual resin was washed with NMP and DMF. The unreacted amino group on the resin was acetylated with 4.75% (v/v) Ac_2O–2.25% (v/v) DIEA–13 mM HOBt / NMP. After washing with NMP, the resin was subjected to deprotection of Fmoc group in the same manner as described above, and used for further peptides assembling with Fmoc-Gly-OH, Fmoc-Pro-OH, Fmoc-Ala-OH, Fmoc-Ser(*t*Bu)-OH, Fmoc-Gly-OH. At the case of Fmoc-Ser(Ac_3GlcNAcβ)-OH, coupling was performed at off-line with the solution of Fmoc-Ser(Ac_3GlcNAcβ)-OH (59.1 mg, 0.09 mmol), 0.4 M HBTU/HOBt/DMF (0.225 ml, 0.09 mmol), and DIEA (31 μl, 0.18 mmol) in NMP/DMF for 12 hr . Then, re-adopted of automated synthesizer, and used for further peptides assembling with Fmoc-Val-OH, Fmoc-Pro-OH, Fmoc-Val-OH, and Fmoc-Ser(*t*Bu)-OH. At the final step, the *N*-terminal of the peptide was capped by coupling with the solution of **1** (45 mg, 0.09 mmol), 0.4 M HBTU/HOBt/DMF (0.225 ml, 0.09 mmol), and DIEA (31 μl, 0.18 mmol) in NMP/DMF for 16 hr at off-line. The resin was washed with NMP and CH_2Cl_2, and dried *in vacuo*. The protected peptide on resin was treated with 90% TFA in water for 1.5 hr at room temperature for releasing the peptide from the resin and for deprotection of *t*Bu and Pbf groups at the same time. The mixture containing compound **2** was filtrated off and the resin was washed with TFA. The solvent was evaporated and the residual syrup was precipitated from dry diethyl ether to give crude solid of photosensitive dodecapeptide having *O*-Ac_3GlcNAc **2**.

De-*O*-acetylation of compound 2. The obtained solid was dissolved in 6 ml of methanol. After

neutralization by 1N NaOH aq., the mixture was added 60µl of 1N NaOH (final concentration of NaOH was about 10mM). The mixture was stirred for 1 h at room temperature, the solution was neutralized by addition of Dowex 50-X8 (H^+) and the resin was removed by filtration. The solvent was evaporated and the residue was dissolved in 3ml of 50mM sodium acetate buffer (pH5.5) to give the glycopeptide **3** solution (10mM theoretical concentration), and subjected to the next steps.

Chemoselective blotting of molecular transporter carrying glycopeptide primer (3) by water-soluble polymer with oxylamino functional groups (4) To a crude solution of the glycopeptide **3** (3 ml) in 50mM AcOH / AcONa buffer (pH5.5) was added 3 ml of 10 mM oxylamino polymer **4** (0.03 mmol based on oxylamino group) in water (a stock solution described above). Final concentration of each component is as follows; 5 mM (theoretical concentration) glycopeptide **3**, and 5 mM oxylamino groups on polymer **4**. After stirring at room temperature for 12 h, the reaction mixture was directly subjected to the concentration by centrifugal ultrafiltration (UF) unit (Orbital Biosciences, LLC; 10K Apollo® 20ml, high-performance centrifugal concentrators) and retentate was washed with 25 mM HEPES buffer (pH 7.0) for three times. After that, retentate polymer was collected and the volume was adjusted to 1.5ml to give the polymer containing *O*-GlcNAc-dodecapeptide **5** solution. This material was then subjected to characterized by following manner; a part of this material was changed solvent to 25mM ammonium acetate buffer (pH6.5) by using of the centrifugal UF unit (MILLIPORE;

ULTRAFREE® -MC 10,000 NMWL Filter Unit), then it was irradiated with UV at 365 nm for 3 hr. The released compound was separated from the polymer by using UF-unit, the filtrate solution was lyophilized to give the crude material containing the glycopeptide **16**, and it was measured by high resolution mass analysis and RP-HPLC (compound **16** shown in Scheme S-3) analysis. ESI-MS (pos): 1313.7 ($M+H^+$), calcd 1313.7.

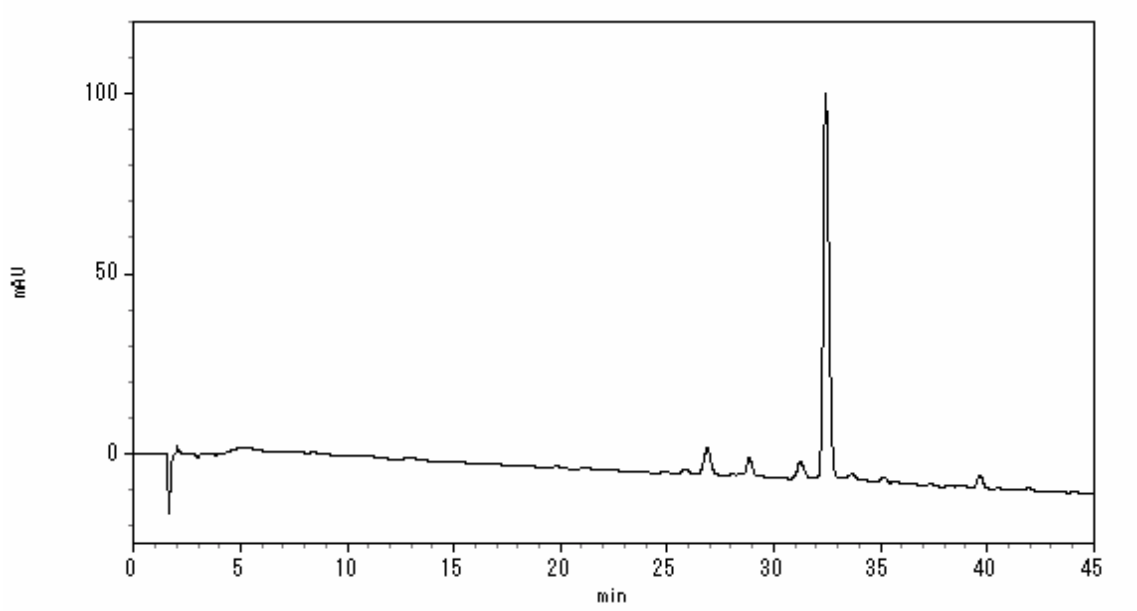
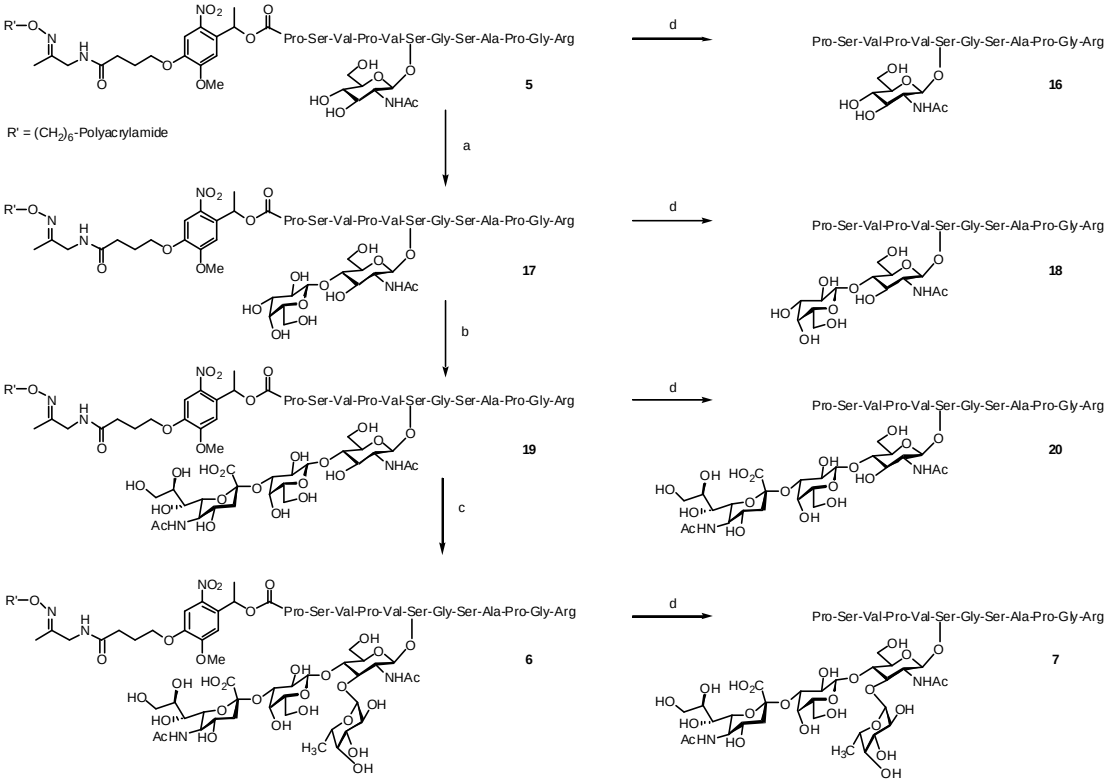


Figure S-5. RP-HPLC analysis of crude compound **16**. Solvent A (10mM ammonium acetate buffer, pH5.8) and solvent B (10% A in acetonitrile), solvent (A/B=98/2) was employed, then the ratio of B was increased from 2% to 15% over 45 min with a flow rate of 1.0 ml/min.

One-pot enzymatic sugar elongation reactions using glycosyltransferases.

Scheme S-3. Enzymatic sugar elongation reaction of the polymer **5**.



a) UDP-Gal (5mM), β1,4-Galactosyltransferases (100mU / ml), $MnCl_2$ (10mM) , BSA (0.1%) , HEPES buffer (50mM, pH7.0); b) CMP-NANA (5mM), α2,3-Sialytransferases (37mU / ml) , $MnCl_2$ (10mM) , BSA (0.1%) , HEPES buffer (50mM, pH7.0); C) GDP-Fuc (5-7mM), α1,3-Fucosyltransferases (20-30mU / ml), $MnCl_2$ (10mM) , BSA (0.1%), HEPES buffer (50mM, pH7.0); d) UV irradiation (365nm).

Galactosylation.

A solution of the polymer **5** [a stock solution described above, 0.5 ml, 9.5 μmol (theoretical molar quantity)], UDP-galactose (5 mM), and β1,4-galactosyltransferase (0.1 U/ml) in 50 mM HEPES buffer (1.5 ml, 10 mM $MnCl_2$, 0.1% BSA, pH 7.0) was shaken at 25℃. After 2 hr, MALDI-TOF / MS analysis indicated reaction finished, then 1.35 ml of the reaction mixture was subjected to the next sialylation reaction without further purification. On the other hand, residual solution of the reaction mixture was directly subjected to the concentration by centrifugal UF unit (ULTRAFREE® -MC 10,000 NMWL Filter Unit) and retentate was washed with 25 mM ammonium acetate buffer

(pH 6.5) for three times. After that, the polymer fraction was collected and the volume was adjusted to 0.1 ml to give the polymer containing *O*-(Galβ1,4GlcNAcβ1)-dodecapeptide (**17**) solution. This material was used for the characterization by the same manner as described above (characterization of compound **16**) and conversion yield of compound **18** was estimated to be 100%. ESI-MS (pos): 1475.7 ($M+H^+$), calcd 1475.7.

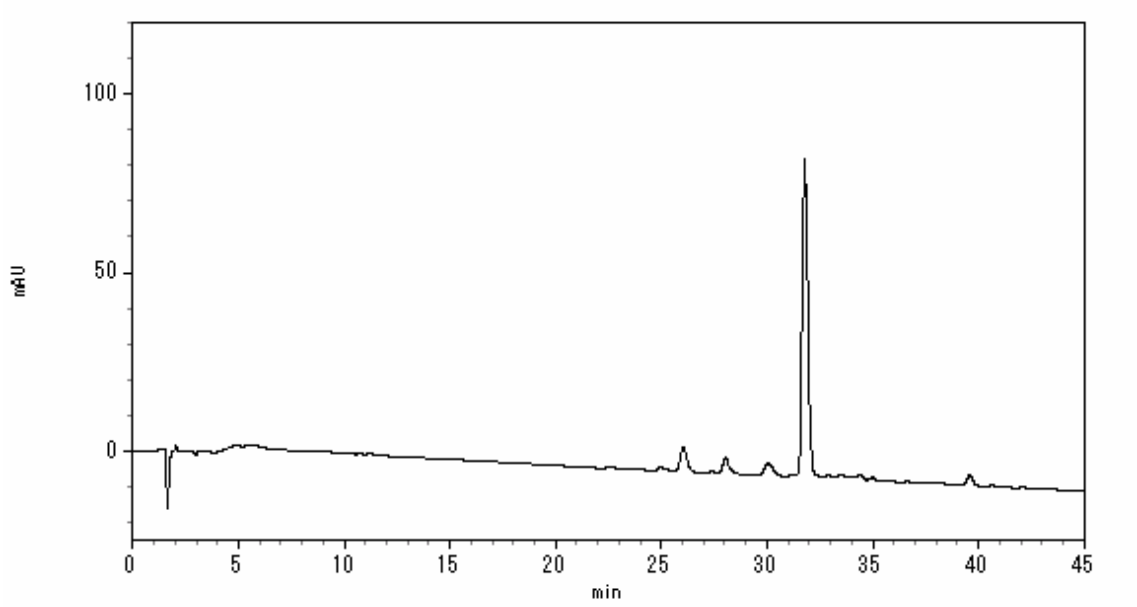


Figure S-6. RP-HPLC analysis of crude compound **18**. Solvent A (10mM ammonium acetate buffer, pH5.8) and solvent B (10% A in acetonitrile), solvent (A/B=98/2) was employed, then the ratio of B was increased from 2% to 15% over 45 min with a flow rate of 1.0 ml/min.

Sialylation.

A reaction mixture of the polymer **17** (1.35 ml) was added the solution of 50 mM CMP-NANA, and

α2,3-(N)-sialyltransferase (370 mU/ml) in 50mM HEPES buffer (0.15 ml, 10 mM $MnCl_2$, 0.1% BSA, pH 7.0) , and the solution was shaken at 25℃. After 24 hr, since MALDI-TOF/MS analysis indicated the reaction was completed, 1.35 ml of the reaction mixture was directly subjected to the next fucosylation reaction without further purification. On the other hand, residual solution of the reaction mixture was subjected to the concentration by centrifugal ultrafiltration unit (ULTRAFREE® -MC 10,000 NMWL Filter Unit) and retentate was washed with 25 mM ammonium acetate buffer (pH 6.5) for three times. The polymer fractions were collected and the volume was adjusted to 0.1 ml to give the polymer containing *O*-(Neu5Acα2,3Galβ1,4GlcNAcβ1)-dodecapeptide (**19**) solution. This material was characterized by the same manner as described above (characterization of compound **16**) and conversion yield of compound **20** was estimated to be 100%. ESI-MS (pos): 1766.8 ($M+H^+$), calcd 1766.8.

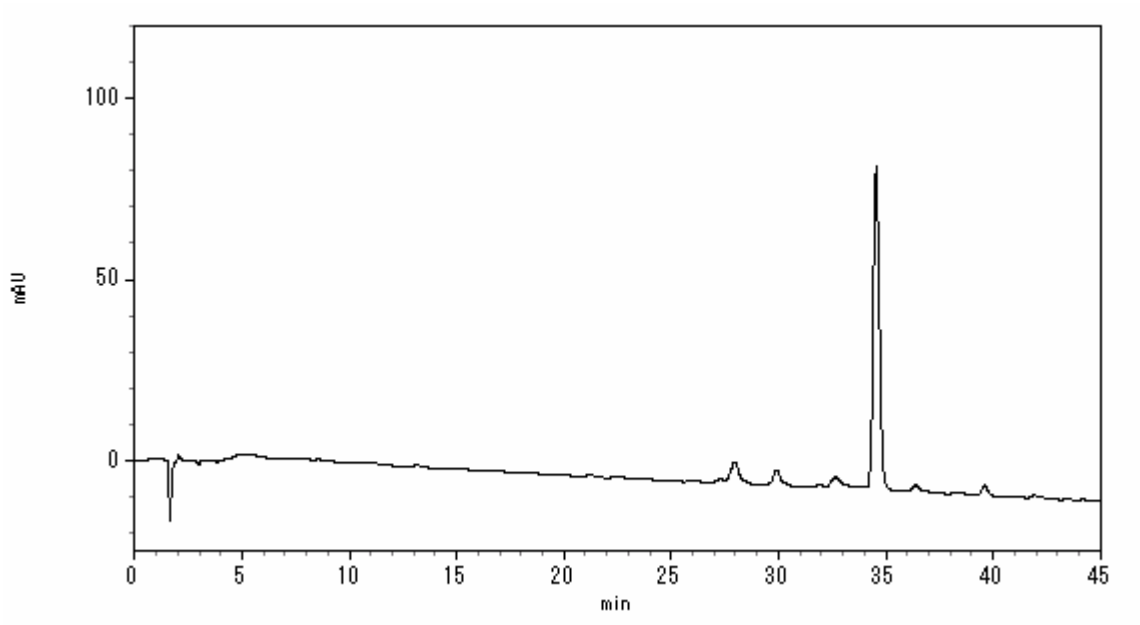


Figure S-7. RP-HPLC analysis of crude compound **20**. Solvent A (10mM ammonium acetate buffer, pH5.8) and solvent B (10% A in acetonitrile), solvent (A/B=98/2) was employed, then the ratio of B was increased from 2% to 15% over 45 min with a flow rate of 1.0 ml/min.

Fucosylation.

A reaction mixture of the polymer **19** (1.35 ml) was added the solution of 50 mM GDP-fucose and α1,3-fucosyltransferase V (200 mU/ml) in 50mM HEPES buffer (0.15 ml, 10 mM $MnCl_2$, 0.1% BSA pH 7.0) , and the solution was shaken at 25℃ for 48 hr. The progress of the glycosylation was monitored by MALDI-TOFMS of the polymer in the presence of DHB. To achieve quantitative introduction of L-fucose residues to the glycopeptide, it was suggested that one more day's incubation with 15 mU of α1,3-fucosyltransferase V and 3.0 μmol GDP-fucose was required. Finally, 0.14 ml of the reaction mixture was concentrated by centrifugal UF unit (10K Apollo® 20 ml) and retentate was washed with 25 mM ammonium acetate buffer (pH 6.5) for six times. The polymer fractions were collected and the volume was adjusted to 1.0 ml to give the polymer containing *O*-[Neu5Acα2,3Galβ1,4(Fucα1,3)GlcNAcβ]-dodecapeptide (**6**) solution. This material was also employed for further characterization by the following photolysis steps, and conversion yield of compound **7** was estimated to be 100%.

Photolysis and isolation of the products glycopeptide 7 from polymer support.

A solution of polymer **6** in water (0.95 ml) was treated by irradiation with UV laser at 365 nm (Handheld UV Lamp, 6 W, 990 μW/cm^2) for 3 hr. The released compound was separated from the polymer by using the centrifugal UF unit (10K Apollo® 20ml), the filtrate solution was lyophilized to give the crude material containing the glycopeptide **7**. This material had over 70% purity of the glycopeptide **7** which was estimated by the RP-HPLC peak area ratio (Figure S-8). More over, this material was purified by the preparative RP-HPLC to give the high purity glycopeptide **7** (Figure S-9) in 12% overall yield from the solid-phase glycopeptide synthesis calculated by amino acid analysis. ESI-MS (pos): 1912.9 ($M+H^+$), calcd 1912.9; Amino acid analysis (theoretical ratio): Ala 1.0 (1), Arg 1.0 (1), Gly 2.0 (2), Pro 3.1 (3), Ser 2.5 (3), Val 1.9 (2).

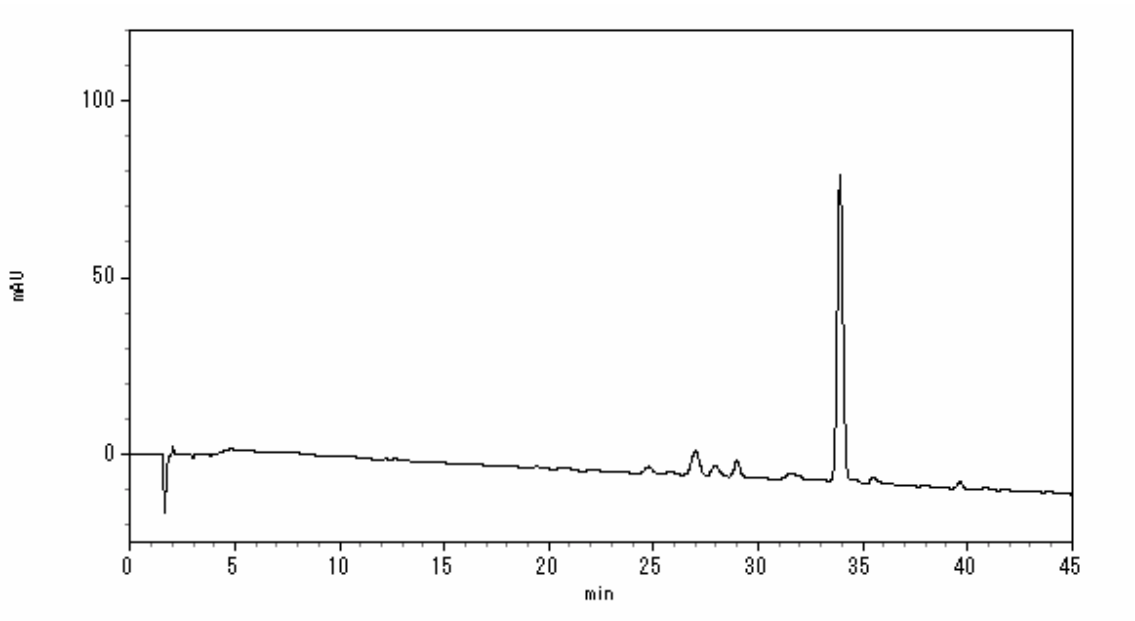


Figure S-8. RP-HPLC analysis of crude compound **7**. Solvent A (10mM ammonium acetate buffer, pH5.8) and solvent B (10% A in acetonitrile), solvent (A/B=98/2) was employed, then the ratio of B was increased from 2% to 15% over 45 min with a flow rate of 1.0 ml/min.

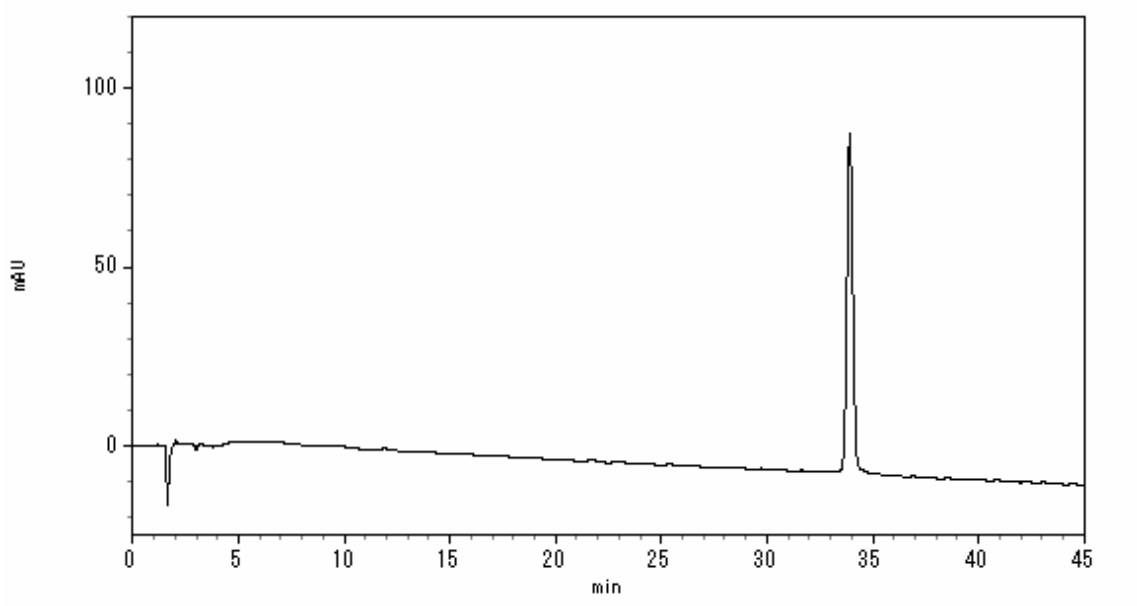


Figure S-9. RP-HPLC analysis of purified compound **7**. Solvent A (10mM ammonium acetate buffer, pH5.8) and solvent B (10% A in acetonitrile), solvent (A/B=98/2) was employed, then the ratio of B was increased from 2% to 15% over 45 min with a flow rate of 1.0 ml/min.

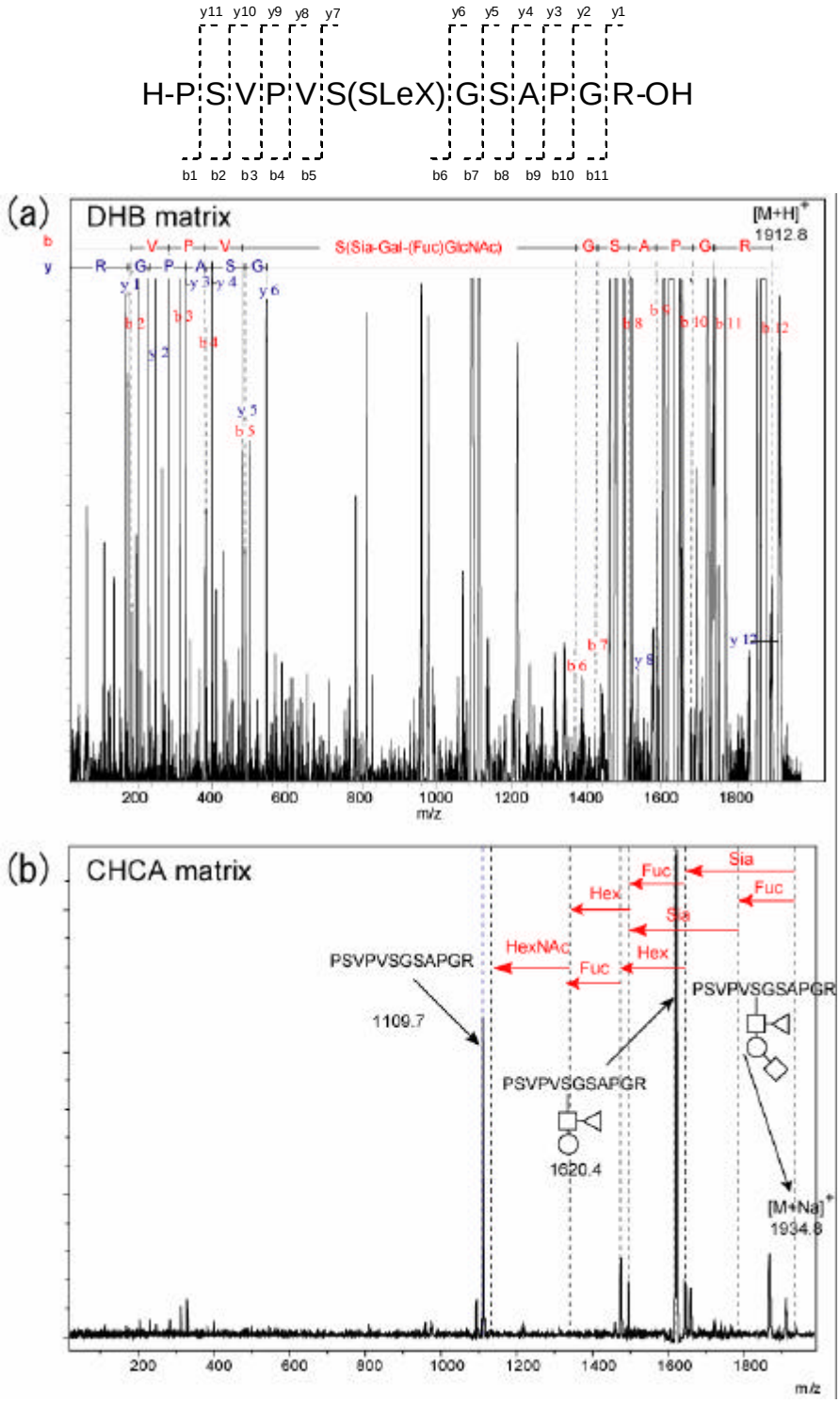


Figure S-10. Fragmentation of compound **7** by MALDI-TOF/TOF mode in the presence of DHB (a), and in the presence of CHCA (b). Structural characterization by TOF/TOF method was performed by using Bruker Ultraflex according to the procedures reported in the reference 12.