Sequential Glycoproteins: Practical Method for the Synthesis of Antifreeze Glycoprotein Models Containing Base Labile Groups

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ABSTRACT: Practical and versatile methods for the preparation of glycopeptide polymers containing base labile moieties were established by use of 1-isobuthoxycarbonyl-2-isobuthoxy-1,2-dihydroquinoline (IIDQ) or 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) as polycondensation agents of glycopeptide units without any protection on the carbohydrate residues. These methods were applied for the synthesis of mucin-like glycoprotein mimics related to antifreeze glycoproteins (AFGPs), giving the target *O*-acetylated glycopolypeptides efficiently, while the conventional DPPA (diphenylphosphoryl azide) method gave complex mixtures of acyl migrated compounds.

Introduction

Mucins expressed on cells of epithelial tissues are known to play a wide range of biologically important roles such as a protective barrier over internal epithelial surfaces, mediation of cellular interactions and signal transduction events, and markers of tumors.[1−3] Mucins are very large molecules having a high density of *O*-linked oligosaccharide chains attached to serine or threonine, and these specific functions can be attributed to the clustered carbohydrate residues. However, detailed studies on the biological functions of mucins have not been achieved sufficiently because of the difficulties in the preparation of homogeneously pure glycoprotein that has strictly defined primary structure. During the past decade, significant progress has been made in the synthesis of mucin-type glycopeptides by incorporating properly protected *O*-glycosylated amino acid derivatives into an oligomeric peptide based on conventional solid-phase synthesis.[4] It is also known that tandem repeating peptides bearing *O*-glycans have been found in a variety of mucin-type glycoproteins, and they seem to have crucial roles in cellular adhesions. However, it is still difficult to obtain large-size glycopeptide containing a high density of carbohydrate residues. Recently, we have established an efficient methodology for the construction of mucin-like glycoprotein mimics with high molecular weights having sequential (tandem repeating) structures.[5] As a typical model for the functional mucins, we have been investigating the structure−activity relationships in antifreeze glycoproteins (AFGP, **1**, Figure 1) that consist of a simple polymer of a glycopeptide, Ala-Thr-Ala, with a disaccharide moiety (Galβ1→3GalNAcα) attached to each threonyl residue.[5,6] AFGP have been isolated from various species of Antarctic fish, and it is well-known that AFGP protect those fish from freezing by depressing the freezing point of their blood in a noncolligative manner.[7]

In the course of our study regarding AFGP, we found that the acetamide group at the C-2 position of the carbohydrate moiety plays a key role in maintaining conformational stability of AFGP and inducing the specific interaction with ice crystal.[5,6] Figure 2 is a structure of synthetic AFGP with three tripeptide repeats (*sy*AFGP$_3$: AT*AAT*AAT*A; * denotes for the glycosylated residue) determined by NMR mesurement.[6] From this structure, it was suggested that the GalNAc amide proton seems to be participate in intraresidual hydrogen bonds with the carbonyl oxygen of Thr and the oxygen atom at the glycoside bond. Furthermore, the structure seems to be stabilized by the close association of methyl groups derived from alanyl side chains and *N*-acetyl methyl groups of GalNAc. To date, several groups have also suggested that acetamide groups play an important role for the construction of the mucin architecture through specific interaction based on the noncovalent bonds.[8−11] However, the functional roles of the amide proton (hydrogen bonding) and/or *N*-acetyl methyl group (steric effect) for the conformational stability have not been concluded. These situations prompted us to synthesize AFGP analogues **2** and **3** (Figure 1) that have *O*-ethyl (**2**) or *O*-acetyl (**3**) groups instead of *N*-acetyl groups at the C-2 position, to confirm the influence of the amide proton (donor of hydrogen bonding), carbonyl oxygen (acceptor of hydrogen bonding), and steric factor caused by the *N*-acetyl group on the activity and conformation of AFGP. Since we have already confirmed that terminal galactose residues are not essential for the activity and the conformation of AFGP,[6] we designed the AFGP analogues without terminal galactose residues to simplify the synthesis. Here, we would like to report a practical methodology for the preparation of sequential glycoproteins containing base labile groups by use of 1-isobuthoxycarbonyl-2-isobuthoxy-1,2-dihydroquinoline (IIDQ)[12] or 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM)[13] for the polymerization reaction of glycopeptides. It should also be noted that this report is the first example of the use of these reagents for the polymerization of (glyco)peptides. These methodologies were also applied for the preparation of

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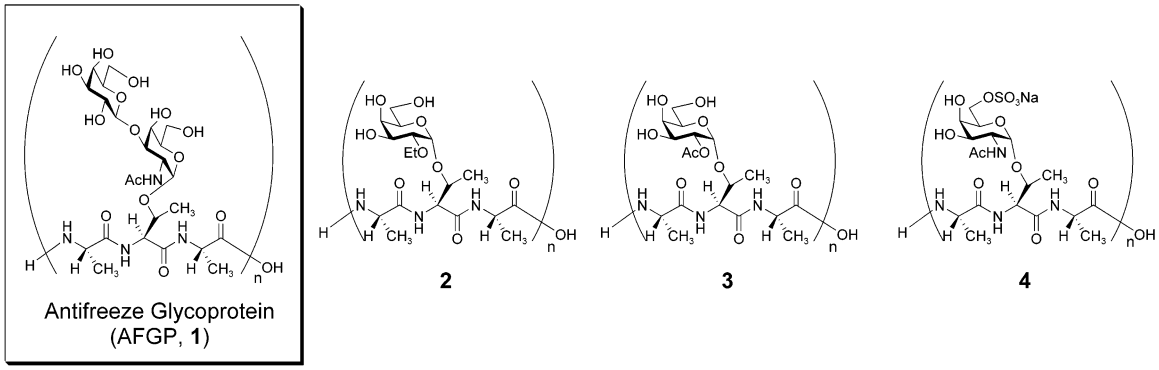


Figure 1. Glycopolypeptides synthesized in this study.

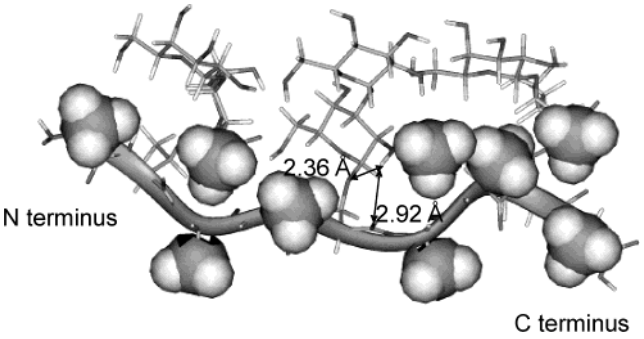


Figure 2. NMR structure of *sy*AFGP$_3$.[6] The distances between *N*-acetyl amide protons of the T5 GalNAc and T5 carbonyl or glycosidic oxygen are shown by black allows. Methyl groups of GalNAc acetamide groups and alanyl side chains are shown in van der Waals surfaces.

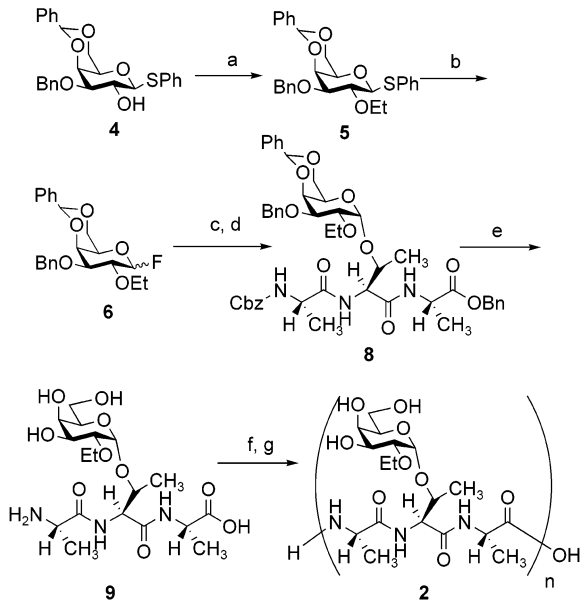
sulfated glycopolypeptide **4** (Figure 1) to elucidate the versatility of the present method.

Results and Discussion

Synthesis of Glycopeptides 2 and 3. A synthetic scheme for the synthesis of **2** is shown in Scheme 1. First, thioglycoside **5** was synthesized as previously described[14] thorough regioselective benzylation using the dibutyltin oxide method,[15] and the remaining C2 hydroxyl group was ethylated to give **6** (97%). The phenylthioglycoside was then converted into glycosyl fluoride by use of DAST-NBS,[16] since it turned out to be difficult to obtain α-glycoside with thioglycoside **7** when glycosylation reaction with the peptide unit was attempted. Compound **7**, thus obtained in 84% yield (α only), was then subjected to a glycosylation reaction with tripeptide unit **8**[5] in CH_2Cl_2 in the presence of Cp_2ZrCl_2−$AgClO_4$ (1:2)[17] to yield glycopeptide **9** (73% based on donor sugar). Then, all protective groups were removed by hydrogenolysis (90%), and the macromonomer **10** was polymerized by means of the DPPA method[5] to afford the desired glycopolypeptide **2** in 78% yield.

According to a similar synthetic strategy to compound **2**, glycopeptide **3** was successfully synthesized (Scheme 2). The *p*-methoxybenzyl (MPM) group, which can be selectively removed in the presence of the benzyl ethers and other acyl protections, was introduced to 2-OH of **5** to give **11** quantitatively. Then, glycosyl fluoride **12** was prepared from **11** (66%, $\alpha:\beta = 5:1$) and coupled with **8** in the presence of Cp_2ZrCl_2−$AgClO_4$ (1:2). Since slow reaction of the removal of the acid-sensitive *O*-MPM groups happened to accompany with this reaction, successive acetylation of the crude product was carried out to afford α-glycoside **13** in 50% yield from **12**. These

Scheme 1[a]

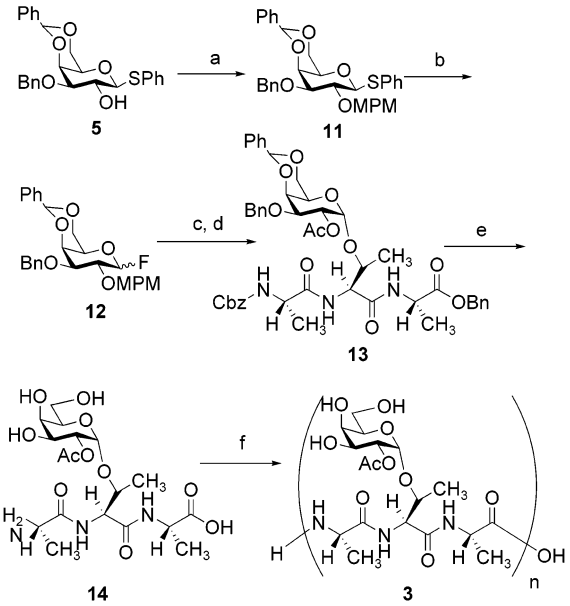


[a] Reagents: (a) NaH, EtI, DMF, −20 °C to r.t., 97%; (b) NBS, DAST, CH_2Cl_2, −15 °C to r.t., 84%; (c) Cbz-Ala-Thr-Ala-OBn (**8**), Cp_2ZrCl_2, $AgClO_4$, MS4 Å, CH_2Cl_2, −20 °C to r.t., 73%; (d) H_2, Pd/C, DMF, H_2O, AcOH, 90%; (f) DPPA, Et_3N, DMF, 0 °C to r.t., 78%.

steps were followed by hydrogenolysis, leading to macromonomer **14** in 84% yield (Figure 3A).

Glycopeptide **14** was then subjected to the polycondensation reaction using the DPPA method. However, a significant amount of acyl migration had occurred during this reaction (only ∼5% of 2-*O*-acetate were obtained as the target polymer from integral curve of ^{1}H NMR), probably due to triethylamine required for the reaction (Figure 3C). To avoid base-catalyzed side reactions during polymerization reaction, use of other coupling reagents that do not require the addition of tertiary amines for the activation was explored. As a result, we found that 1-isobuthoxycarbonyl-2-isobuthoxy-1,2-dihydroquinoline (IIDQ; mixed anhydride)[12] and 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM; active ester) can be used for this purpose.[13] Shown in Figure 4 is the reaction mechanisms of DPPA,[18] IIDQ,[19] and DMT-MM[20] mediated polymerization. It is well-known that both IIDQ and DMT-MM suppress the racemization of peptide during the coupling reaction.[12,21] At the same instance, coupling reactions promoted by these reagents can be performed in alcohol without any side reactions, indicating the applicability of these reagents for the poly-

Scheme 2[a]



[a] Reagents: (a) NaH, MPM-Cl, DMF, −20 °C to r.t., quant; (b) NBS, DAST, CH_2Cl_2, −15 °C to r.t., 75%; (c) Cbz-Ala-Thr-Ala-OBn (**8**), Cp_2ZrCl_2, $AgClO_4$, MS4 Å, CH_2Cl_2, −20 °C to r.t.; (d) Ac_2O, pyridine, 50% in two steps; (e) H_2, Pd/C, DMF, H_2O, AcOH, 84%; (f) DMT-MM, DMF, 0 °C, 55%.

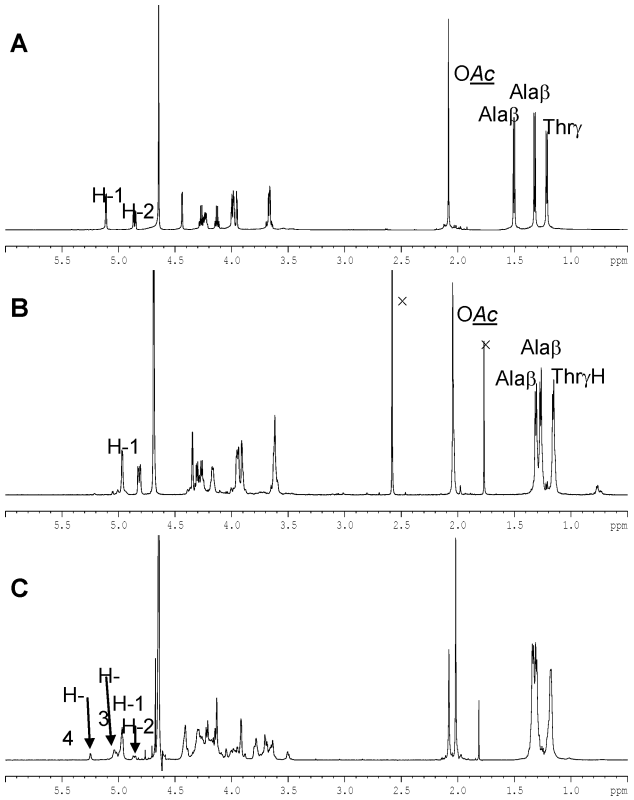


Figure 3. ^{1}H NMR spectra of (A) compound **14**, (B) compound **3** (polymerized with DMT-MM), and (C) poly-**14** prepared by means of the DPPA method.

condensation reaction of glycopeptides having unprotected hydroxyl groups. Because of these important features, they have been used for the fragment condensation reaction of peptides[22,23] or glycopeptides.[24,25]

To assess the efficiency of IIDQ and DMT-MM mediated polycondensation reaction, model reactions were carried out using H−Ala-(Galβ1→3GalNAcα)Thr-Ala-OH[5] (**1′**) as a macromonomer, and the results were compared with that obtained by means of DPPA method (Scheme 3). As anticipated, no side reactions on the hydroxyl groups of carbohydrate moieties were observed, though the average molecular weights in the IIDQ method are rather low compared with that obtained by the conventional DPPA method (Table 1). The primary reason for this is the formation of oligomers during the polymerization reaction due to the urethane formation known as a major side reaction in the mixed-anhydride method.[25] On the contrary, DMT-MM mediated polycondensation reaction proceeded smoothly, giving rise to the desired glycopolypeptide with moderate molecular weights.

Since it was suggested that the antifreeze activity of AFGP strongly depends on the conformation,[6] CD spectra of **1** prepared by means of IIDQ and DMT-MM methods were measured and compared with that obtained by the conventional DPPA method. As shown in Figure 5, all spectra showed almost the same curve, indicating the usability of these methods in the synthesis of active AFGP analogues.

Then, to verify the activity of these synthetic AFGPs, ice growth inhibition activity, which is known as a specific activity of AFGP, was also evaluated. Although the precise mechanism of action of AFGP has not been concluded yet, it is widely accepted that AFGPs interact with ice and prevent embryonic ice crystals from growing.[26] This leads to the formation of a characteristic ice crystal called "hexagonal bipyramidal ice crystal" which is stable within a certain temperature gap. As a result, solutions containing AFGP show the difference between the melting point and the freezing point, which is referred to thermal hysteresis. As shown in Figure 6, synthetic glycoproteins derived by these two alternative methods showed the same activity as previously reported,[5] showing the capability to form the hexagonal bipyramidal ice crystal.

Since the applicability of IIDQ and DMT-MM methods for the polymerization of glycopeptides were confirmed, our attention was next directed toward the polymerization of **14** bearing the *O*-acetyl group at the C-2 position. On the basis of the preliminary results described above, we chose DMT-MM as a condensing agent. As anticipated, the reaction proceeded smoothly without any evidence of acyl migration, giving the desired polymer **2** in 55% yield after a purification with Sephadex G-25 (^{1}H NMR spectra of macromonomer **14**, polymer **3**, and the polymer prepared by the DPPA method are shown in Figure 3). In a similar fashion, acyl migration was not observed in the case of IIDQ method (data not shown). The weight-average molecular weights of **3** were estimated as 7.7×10^3 by means of the GPC method.

Characterization of AFGP Analogues 2 and 3. To elucidate the importance of the *N*-acetyl group for the AFGP specific activity, ice growth inhibition activity of glycopeptides **2** and **3** was evaluated. As shown in Figure 6 (panels c and d), formation of the hexagonal bipyramidal ice crystal which is an indicator of antifreeze activity was not observed for both glycopeptides **2** and **3**, and they did not show any antifreeze activity. Then, to clarify the effect of *N*-acetyl group on the conformation of AFGP, CD measurements of **2** and **3** were performed (Figure 7). It is well-known that natural AFGPs exhibit poly(L-proline) type II (PPII) helix-like

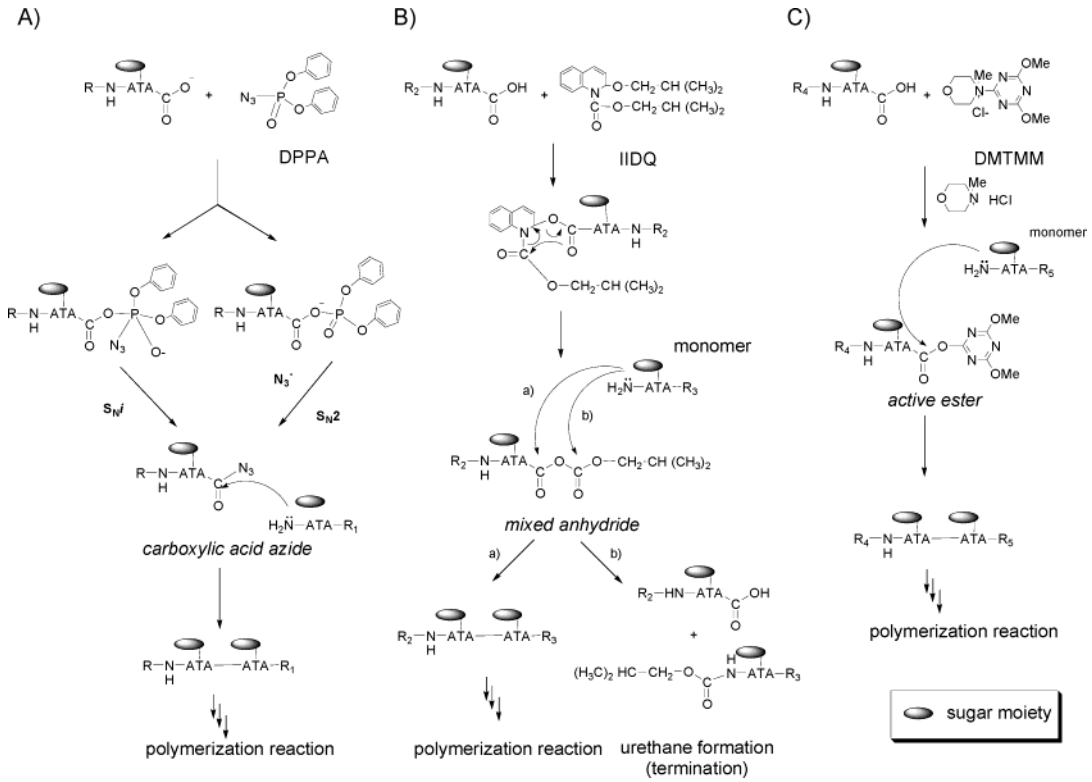
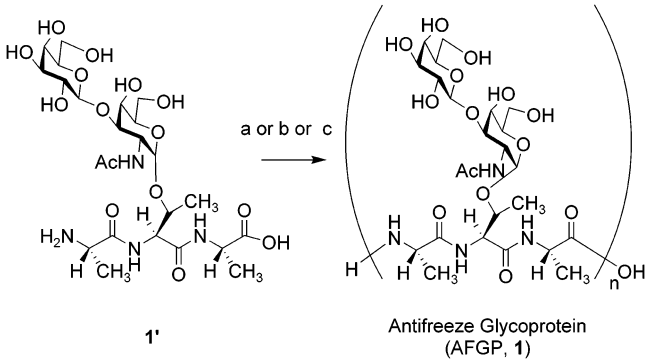


Figure 4. Mechanisms of polymerization by use of (A) DPPA,[18] (B) IIDQ,[19] and (C) DMTMM.[20]

Scheme 3[a]



[a] Reagents: (a) DPPA, Et_3N, DMF, 0 °C to r.t., 92%; (b) IIDQ, DMF, 0 °C to r.t., 97%; (c) DMT-MM, DMF, 0 °C, 82%.

spectra,[27] and synthetic AFGP **1** also showed the same pattern, with a noticeable positive band around 218 nm and a strong negative band around 190 nm.[5,6] We have already experienced to see the disruption of the conformation, namely, complete loss of the positive band around 218 nm when the GalNAc residue in AFGP was replaced by the galactose residue.[5] Incidentally, the antifreeze activity was also diminished. On the contrary, glycopeptides **2** and **3** exhibited a neither strong nor negligible positive shoulder around 218 nm, suggesting that although part of the conformation was collapsed due to the absence of the amide proton (hydrogen bond donor) at the C-2 position, some parts of these molecules still contain the nature of PPII like left-handed helical structure. The loss of antifreeze activity in **2** and **3** seemed to be owing to the small conformational changes which preclude the specific interaction with ice lattice. The existence of small positive band around 218 nm in the case of **2** and **3** should indicate the significance of the steric factor caused by *O*-acetyl groups for the stabilization of the conformation, rather than hydrogen bonding thorough carbonyl oxygen in sugar moiety. In fact, our NMR study of AFGP revealed the close association of alanyl methyl groups and *N*-acetyl groups in the carbohydrate moiety, structuring hydrophobic surface on one side of the molecule.[6] Danishefsky et al. also observed a similar type of organization in their structural studies on a mucin glycopeptide motif.[11] These results clearly indicate the importance of both amide proton and *N*-acetyl methyl group in the acetamide group at the C-2 position of the GalNAc moiety for the activity and construction of the extended conformation of mucins. It might be anticipated that amide protons in GalNAc residues decide the orientation of carbohydrate moieties through hydrogen bonding, and the resulted extended conformation of AFGPs are further stabilized by *N*-acetyl groups through close packing of methyl groups.

Synthesis of Sulfated Glycopolypeptide. In nature, a variety of mucins and proteoglycans occasionally contain base sensitive groups, more specifically, sulfates or phosphates on their carbohydrate moieties and/or peptide side chains. This fact prompted us to examine the versatility of the DMT-MM method toward the preparation of sequential glycoproteins that contain base sensitive these groups. For this purpose, we decided to synthesize a model glycopolypeptide **3** which has clustered 6-sulfated Tn antigens as carbohydrate moieties (Figure 1).

As illustrated in Scheme 4, the known glycosyl donor **15**[28] was coupled with the tripeptide unit **8** in the presence of $SnCl_2-AgClO_4$[29] to give 53% yield of α-glycoside **16**. The azide group was reduced with thiolacetic acid−pyridine[30] (65% yield) followed by quantitative removal of the benzylidene acetal under acidic condition to afford **18**. Then, the primary hydroxyl group of **18** was selectively sulfated with $SO_3 \cdot Me_3N$ in DMF to give **19** (64% yield).[31] Subsequently, hydrogenolytic removal of all protective groups was attempted without any acid

Table 1. Average Molecular Weights of Synthetic Glycoproteins Estimated from GPC Analysis[a]

entry	monomer	polymer	coupling reagents	Et_3N (equiv)	reaction time	temp	solvent	yield (%)	$M_n/10^3$ [b]	$M_w/10^3$ [c]
1	**1′**	**1**	DPPA (1.5 equiv)	2.5	3 days	0 °C → r.t.	DMF	92	5.0	7.3
2	**1′**	**1**	IIDQ (1.5 equiv)		3 days	0 °C → r.t.	DMF	97	3.5	4.4
3	**1′**	**1**	DMT-MM (1.1 equiv)		12 h	0 °C	DMF	90	4.0	7.0

[a] Experiments were carried out with TOSHOH TSKgel G3000PWXL using Pullulans (5.8, 12.2, 23.7, and 48.0 K) as standards. [b] M_n: number-average molecular weight. [c] M_w: weight-average molecular weight.

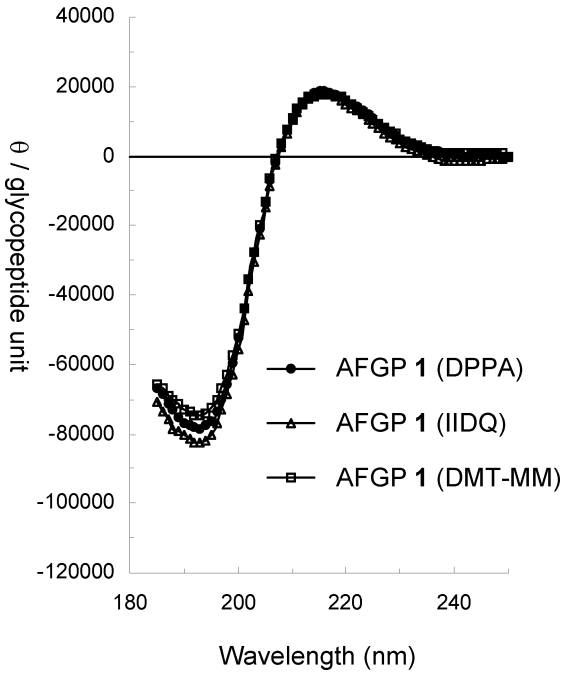


Figure 5. CD spectra of synthetic AFGP **1** prepared by the DPPA method (closed circle), IIDQ method (open triangle), and DMT-MM method (open square). All curves were measured at 4 °C in water (0.1 mg/mL).

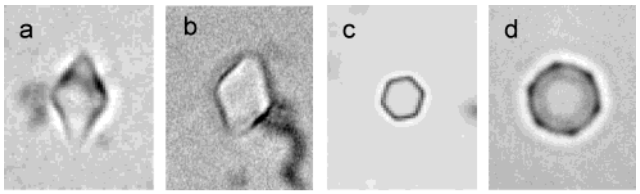


Figure 6. Ice crystal morphology in the presence of (a) synthetic AFGP **1** (IIDQ), (b) synthetic AFGP **1** (DMT-MM), (c) 2-OEt **2**, and (d) 2-OAc **3** in water (10 mg/mL). Photos were taken at −0.2 °C (a, b) or 0.0 °C (c, d). Melting points of all solutions were 0.0 °C.

to avoid removal of the sulfate group. However, the reaction was not completed even after 4 days at 50 °C. In this context, a small amount of acetic acid was added to accelerate the hydrogenolysis, and this time, the reaction proceeded smoothly, providing the macromonomer **20** after purification with gel filtration chromatography and RP-HPLC (56% yield, Figure 8A). Polymerization of **20** was furnished by means of the DMT-MM method without any problem, yielding sulfated glycopolypeptide **4** quantitatively (Figure 8B). At the same time, DPPA-mediated polymerization reaction was also attempted, and contrary to our expectations, the reaction proceeded smoothly without any side reactions, quantitatively yielding the desired glycopolypeptide **4**. These results indicate the stability of sulfate groups toward reaction conditions in which acyl migration catalyzed by bases would be promoted. To our knowledge, this is the first example of the preparation of the

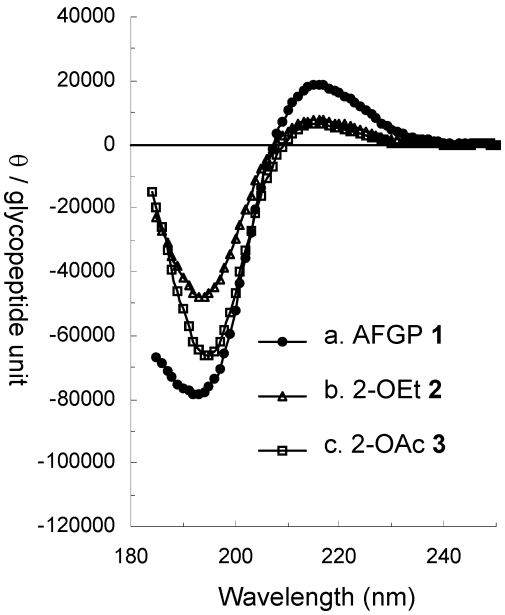


Figure 7. CD spectra of synthetic AFGP **1** (prepared by DPPA method: closed circle), 2-OEt (**2**) (open triangle), and 2-OAc (**3**) (open square). All curves were measured at 4 °C in water (0.1 mg/mL).

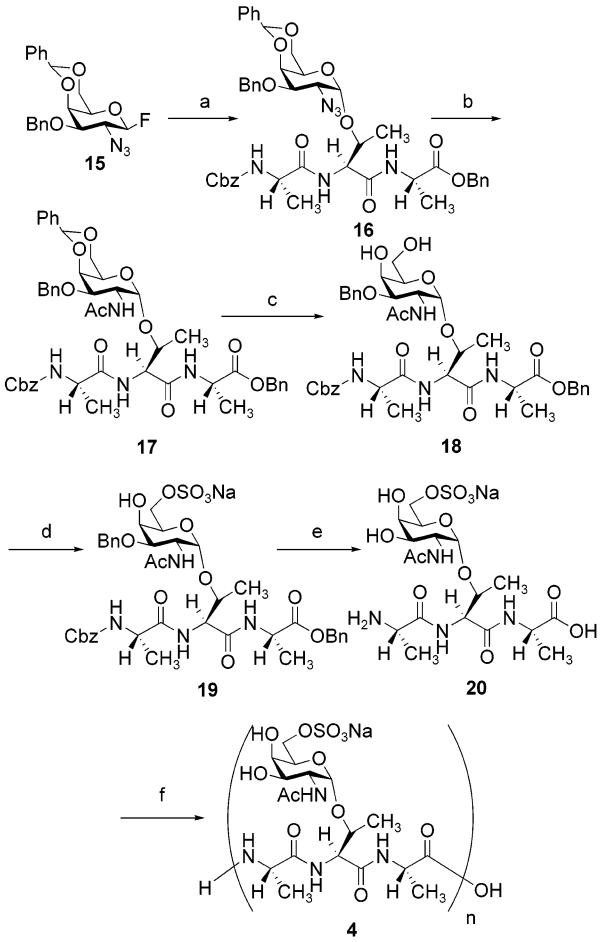
sulfated sequential glycopeptide containing clustered carbohydrate moiety.

In conclusion, it is demonstrated that DMT-MM and IIDQ can efficiently promote the polymerization reaction of glycopeptides having base labile groups without any protection of sugar hydroxyl groups. The methodologies presented here would be very useful for the construction of a variety of glycoprotein mimics containing *O*-acetate, *O*-sulfate, and/or *O*-phosphate to investigate the biological significance related to them.

Experimental Section

Thin-layer chromatography (TLC) was performed on Merck silica gel glass plates, 60F254; compounds were visualized by treatment with a solution of $(NH_4)_6Mo_7O_2$ $4H_2O$ (20 g) and $Ce(SO_4)_2$ (0.4 g) in 10% sulfuric acid (400 mL) and heating at 150 °C. Flash chromatography was performed on Kanto Chemical silica gel N60 (40−50 mm). NMR measurements were recorded at 27 °C on a BRUKER AVANCE 600 [^{1}H (600 MHz), ^{13}C (120 MHz)]. FAB-mass spectra were obtained with a JEOL JMS-HX 110 mass spectrometer, using *m*-nitrobenzyl alcohol (NBA) as matrix. Optical rotations were recorded on a Perkin-Elmer 343 polarimeter. All solvents were used as commercially received. The molecular weights of synthesized polyglycopeptides were estimated by gel permeation chromatography with a TOSOH-TSKgel G3000PW_{XL} column [pullulans (5.8, 12.2, 23.7, and 48.0 K; Shodex Standard) were used as standards]. Matrix-assisted laser desorption/ionization time-of-flight (MALDI−TOF) mass spectra were measured by using an Ultraflex TOF/TOF mass spectrometer equipped with a reflector and controlled by the Flexcontrol 1.2 software package (Bruker Daltonics GmbsH, Bremen, Germany). In MALDI−TOF MS reflector mode, ions generated by a pulsed UV laser beam (nitrogen laser, $l = 337$ nm, 5 Hz) were accelerated to a

Scheme 4[a]



[a] Reagents: (a) $SnCl_2$, $AgClO_4$, MS4 Å, CH_2Cl_2, −20 °C to r.t. 53%; (b) AcSH, pyridine, 65%; (c) CSA, MeOH, quant; (d) SO_3·Me_3N, DMF, 40 °C, 48 h, 64%; (e) H_2, $Pd(OH)_2$/C, DMF, H_2O, AcOH, 56%; (f) DPPA, Et_3N, DMF, 0 °C to r.t., quant; (g) DMT-MM, DMF, 0 °C, quant.

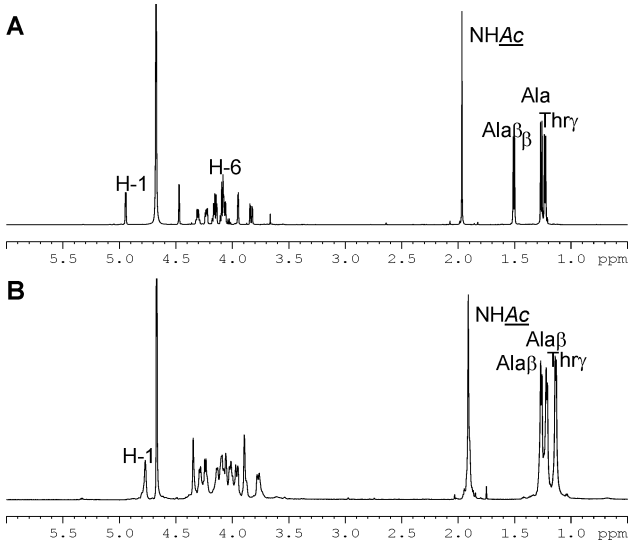


Figure 8. ^1H NMR spectra of (A) compound **20** and (B) compound **4**.

kinetic energy of 23.5 kV. 2,5-Dihydroxybenzoic acid (DHB) was used as a matrix. Circular dichroism (CD) spectra were measured in 1 mm path length quartz cells on a JASCO J-820 spectropolarimeter.

Antifreeze Activity Evaluation. The ice crystal morphology of synthetic compounds were observed using a Leica DMLB 100 photomicroscope equipped with a Linkam LK 600 temperature controller as described previously.[6] The compounds were dissolved in water (10 mg/mL), momentarily frozen (approximately −22 °C), and warmed to 0 °C on the sample stage of the optical microscope, creating several ice nuclei in the solution. This solution was then cooled at a rate of 0.07 °C/min, and the crystal morphologies were monitored. The photos were taken at −0.1 °C in the case the compound have ice crystal growth inhibition activity or at 0.0 °C for those did not have the activity.

Polymerization Reaction of 1′. Poly-[L-Alanyl-*O*-(*β*-D-galacttopyranosyl-(1→3)-2-acetamide-2-deoxy-α-D-galactopyranosyl)-L-threonyl-L-alanine] (1). (1) DPPA Method. To a stirred solution of 1′ (10.0 mg, 0.016 mmol) in DMF (200 μL) was added 10% DPPA solution in DMF (52 μL, 0.024 mmol) and 10% Et_3N solution in DMF (56 μL, 0.040 mmol) at 0 °C, and stirred at room temperature for 3 days. Then, the product in DMF was precipitated by addition of ethanol and diethyl ether and centrifuged. The crude product was separated by gel filtration chromatography (Sephadex G-25, water as eluent) and Dowex 50W-X8 [Na^+] to give 1 (9.2 mg, 92%.).

(2) IIDQ Method. To a stirred solution of **1′** (10 mg, 0.016 mmol) in DMF (240 μL) was added 10% IIDQ solution in DMF (72 μL, 0.024 mmol) at 0 °C and stirred at room temperature for 3 days. Then, the product in DMF was precipitated by addition of ethanol and diethyl ether and centrifuged. The crude product was separated by gel filtration chromatography (Sephadex G-25, water as eluent) to give **1** (9.7 mg, 97%).

(3) DMTMM Method. To a stirred solution of **1′** (5.0 mg, 8.0 μmol) in DMF (100 μL) was added DMT-MM (2.4 mg, 8.8 μmol) at 0 °C and stirred at the same temperature overnight. Then, the product in DMF was precipitated by addition of ethanol and diethyl ether and centrifuged. The crude product was separated by gel filtration chromatography (Sephadex G-25, water as eluent) to give **1** (4.5 mg, 90%).

Phenyl 3-*O*-Benzyl-4,6-*O*-benzylidene-2-*O*-ethyl-1-thio-*β*-D-galactopyranoside (6). A solution of **5**[14] (230 mg, 0.51 mmol) in DMF (25 mL) was cooled to −15 °C, 60% NaH in oil (31 mg, 0.77 mmol) was added, and the mixture was stirred at the same temperature for 30 min. Ethyl iodide (70 μL, 0.87 mmol) was added dropwisely, and the mixture was allowed to warm slowly to room temperature over 12 h. Then, MeOH was added to quench NaH, diluted with $CHCl_3$, washed with water and brine, dried ($MgSO_4$), concentrated, and purified by flash column chromatography (hexane:EtOAc = 2:1) to give **6** (236 mg,97%). **6:** $[\alpha]^{20}_D$ −7.6 (*c* 1.0, dioxane). ^1H NMR (600 MHz, $CDCl_3$) *δ*: 7.68 (d, 2 H, ArH), 7.51 (d, 2H, Ar H), 7.38−7.18 (m, 11H, ArH), 5.47 (s, 1H, Ph−C*H*−O), 4.73 (dd, 2H, Ph−CH_2−O), 4.52 (d, 1H, $J_{1,2}$ = 8.8 Hz, H-1), 4.34 (d, 1H, $J_{6a,6b}$ = 12.2 Hz, H-6a), 4.12 (d, 1H, $J_{3,4}$ = 3.4 Hz, H-4), 3.97 (d, 1H, H-6b), 3.77−3.69 (m, 3H, H-2, CH_3−CH_2−O), 3.52 (dd, 1H, $J_{2,3}$ = 9.1 Hz, H-3), 3.39 (s, 1H, H-5), 1.21 (t, 3H, *J* = 6.3 Hz, CH_3−CH_2−O). HRMS−FAB (*m*/*z*): $[M + H]^+$ calcd for $C_{28}H_{31}O_5S$, 479.1892; found, 479.1896.

3-*O*-Benzyl-4,6-*O*-benzylidene-2-*O*-ethyl-α-D-galactopyranosyl Fluoride (7). A solution of 6 (140 mg, 0.31 mmol) in CH_2Cl_2 (10 mL) was cooled to −15 °C. The stirred was then treated with DAST (62 μL, 0.47 mmol) and allowed to stir for 2 min before NBS (72 mg, 0.40 mmol) was added. After 1 h, MeOH was added to the reaction mixture to quench the excess DAST and diluted with $CHCl_3$. The organic phase was washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), and concentrated. The residual syrup was subjected to flash column chromatography (hexane:EtOAc = 6:1) to afford **7** (102 mg, 84%). α-isomer of **7** was not isolated. **7**: $[\alpha]^{20}_D$ +121.0 (*c* 1.0, dioxane). ^1H NMR (600 MHz, $CDCl_3$) *δ*: 7.53−7.24 (m, 10H, Ar H), 5.80 (dd, 1H, $J_{1,2}$ = 2.3 Hz, $J_{1,F}$ = 53.7 Hz, H-1), 5.50 (s, 1H, Ph−C*H*−O), 4.83 (d, 1H, Ph−CH_2−O), 4.74 (d, 1H, Ph−CH_2−O), 4.28 (d, 1H, $J_{6a,6b}$ = 12.6 Hz, H-6a), 4.22 (d, 1H, $J_{3,4}$ = 2.8 Hz, H-4), 4.02 (d, 1H, H-6b), 3.94 (ddd, 1H, $J_{2,3}$ = 10.1 Hz, $J_{2,F}$ = 24.3 Hz, H-2), 3.90 (dd, 1H, H-3), 3.83−3.73 (m, 3H, H-5, CH_3−CH_2−O), 1.26 (t, 3H, *J* = 7.0 Hz, CH_3−CH_2−O). HRMS−FAB (*m*/*z*): $[M + H]^+$ calcd for $C_{22}H_{26}FO_5$, 389.1764; found, 389.1750.

***N*-(Benzyloxycarbonyl)-L-alanyl-*O*-(3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-ethyl-α-D-galactopyranosyl)-L-threonyl-L-alanine Benzyl Ester (9)**. A mixture of $SnCl_2$ (68 mg, 0.36 mmol), $AgClO_4$ (75 mg, 0.36 mmol), Cbz-Ala-Thr-Ala-OBzl (8, 219 mg, 0.45 mmol), and powdered molecular sieves **4** Å (300 mg) in dry CH_2Cl_2 (10 mL) was stirred at room temperature for 2 h under a nitrogen atmosphere and then cooled to −15 °C. A solution of **7** (72 mg, 0.18 mmol) in dry CH_2Cl_2 (2 mL) was added, and the mixture was stirred at −15 °C to room temperature for 48 h, then diluted with $CHCl_3$, and filtered thorough Celite. The filtrate was washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), concentrated, and purified by flash column chromatography (toluene:EtOAc = 5:1 then 4:1) to give **9** (116 mg, 73%). **9**: $[\alpha]^{20}_D$ +57.1 (*c* 1.0, dioxane). ^{1}H NMR (600 MHz, $CDCl_3$) *δ*: 8.02 (d, 1H, *J* = 7.6 Hz, AlaN*H*), 7.53−7.22 (m, 20H, ArH), 7.02 (d, 1H, *J* = 4.8 Hz, ThrN*H*), 5.46 (s, 1H, Ph−C*H*−O), 5.34 (m, 2H, H-1, AlaN*H*), 5.17−5.08 (m, 4H, Ph−CH_2−O), 4.68−4.61 (m, 3H, Ph−CH_2−O, Alaα), 4.49 (br, 1H, Thrα), 4.25 (m, 1H, Alaα), 4.22 (d, 1H, $J_{6a,6b}$ = 12.4 Hz, H-6a), 4.15 (d 1H, H-4), 4.12 (br, 1H, Thr*β*), 3.99 (d, 1H, H-6b), 3.94−3.77 (m, 4H, H-2, H-3, CH_3−CH_2−O), 3.64 (s, 1H, H-5), 1.42 (d, 3H, *J* = 7.3 Hz, Ala*β*), 1.38 (d, 3H, *J* = 7.1 Hz, Ala*β*, 1.20 (t, 3H, *J* = 7.1 Hz,), CH_3−CH_2−O), 1.05 (d, 3H, *J* = 5.8 Hz, Thr*γ*). ^{13}C NMR (120 MHz, $CDCl_3$) *δ*: 172.8, 172.0, 168.4, 156.2 (C═O), 139.3, 138.3, 136.7, 135.9, 129.3, 129.0, 128.9, 128.8, 128.7, 128.7, 128.5, 128.0, 127.7, 126,8, 101.5 (Ph−*C*H−O), 98.8 (C-1), 76.9, 76.4, 74.7, 73.9, 72.2, 69.8, 68.5, 67.3, 63.6, 55.0, 50.9, 48.7, 30.1, 19.3, 18.2, 16.3, 15.7. HRMS−FAB (*m*/*z*): $[M + H]^+$ calcd for $C_{47}H_{56}N_3O_{12}$, 854.3864; found, 854.3851.

L-Alanyl-*O*-(2-*O*-ethyl-α-D-galactopyranosyl)-L-threonyl-L-alanine (10). To a solution of **9** (90 mg, 0.11 mmol) in DMF (5 mL), acetic acid (300 μL), and H_2O (1 mL) was added 10% Pd−C (400 mg) and stirred at room temperature for 48 h under a H_2 gas atmosphere. Then, Pd−C was removed by filtration, and the solution was evaporated. The residue was purified by gel filtration chromatography (Sephadex G-10, water as eluent) to give **10** (42.9 mg, 90%). **10**: ^{1}H NMR (600 MHz, D_2O) *δ*: 5.16 (d, 1 H, $J_{1,2}$ = 3.8 Hz, H-1), 4.44 (d, 1 H, *J* = 2.5 Hz, Thrα), 4.35 (m, 1H, Thr*β*), 4.10−4.02 (m, 2H, Alaα × 2), 3.91 (dd, 1H, $J_{5,6a}$ = 5.3 Hz, $J_{6a,6b}$ = 7.1 Hz, H-6a), 3.88 (d, 1H, $J_{3,4}$ = 3.3 Hz, H-4), 3.79 (dd, 1H, $J_{2,3}$ = 10.4 Hz, H-3), 3.67−3.60 (m, 3H, H-5, H-6b, CH_3−CH_2−O), 3.50−3.47 (m, H-2, CH_3−CH_2−O), 1.50 (d, 3H, *J* = 7.1 Hz, Ala*β*), 1.27 (d, 3H, *J* = 7.2 Hz, Ala*β*), 1.20 (d, 3H, *J* = 6.5 Hz, Thr*γ*), 1.11 (t, 3H, *J* = 7.0 Hz, CH_3−CH_2−O). ^{13}C NMR (120 MHz, D_2O) *δ*: 179.9, 172.3, 169.8, (C═O), 97.4 (C-1), 76.5, 74.5, 71.5, 69.7, 68.8, 67.2, 61.7, 57.7, 51.5, 49.6, 18.4, 18.2, 17.4, 15.0. HRMS−FAB (*m*/*z*): $[M + H]^+$ calcd for $C_{18}H_{34}N_3O_{10}$, 452.2244; found, 452.2260.

Poly[L-Alanyl-*O*-(2-*O*-ethyl-α-D-galactopyranosyl)-L-threonyl-L-alanine] (2). To a stirred solution of **10** (13.5 mg, 0.030 mmol) in DMF (500 μL) was added 10% DPPA solution in DMF (97 μL, 0.045 mmol) and 10% Et_3N solution in DMF (63 μL, 0.075 mmol) at 0 °C and stirred at room temperature overnight. Then, the product in DMF was precipitated by addition of ethanol and diethyl ether and centrifuged. The crude product was separated by gel filtration chromatography (Sephadex G-25, water as eluent) and Dowex 50W-X8 [Na^+] to give **2** (10.5 mg, 78%). The number-average molecular weight (M_n) and weight-average molecular weight (M_w) were estimated as 1.9×10^3 and 3.5×10^3, respectively, by GPC analysis as described in general procedure. In MALDI−TOF MS analysis using DHB, precursor ion peaks (*m*/*z*) at 877.72 (*n* = 2), 1311.41 (*n* = 3), 1745.42 (*n* = 4), 2179.20 (*n* = 5), 2612.74 (*n* = 6), 3045.74 (*n* = 7), and 3477.91 (*n* = 8) were observed.[32] **2**: H NMR (600 MHz, D_2O, *δ*): 5.10 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.41 (d, 1 H, *J* = 2.3 Hz, Thrα), 4.27−4.20 (m, 3H, Thr*β*, Alaα × 2), 3.91−3.85 (m, 2H, H-6, H-4), 3.77 (dd, 1H, H-3), 3.65−3.45 (m, 2H, H-6b, H-5, H-2, CH_3−CH_2−O × 2), 1.32 (d, 3H, Ala*β*), 1.30 (d, 3H, Ala*β*), 1.14 (d, 3H, Thr*γ*), 1.10 (t, 3H, *J* = 7.0 Hz, CH_3−CH_2−O).

Phenyl 3-*O*-Benzyl-4,6-*O*-benzylidene-2-methoxybenzyl-1-thio-*β*-D-galactopyranoside (11). A solution of **5** (360 mg, 0.80 mmol) in DMF (15 mL) was cooled to −15 °C, 60% NaH in oil (48 mg, 1.20 mmol) was added, and the mixture was stirred at the same temperature for 30 min. 4-Methoxybenzyl chloride (184 μL, 1.36 mmol) was added dropwisely, and the mixture was allowed to warm slowly to room temperature over 12 h. Then, diethylamine was added to quench the excess chloride, and the mixture was poured into ice−water and extracted with EtOAc. The organic pahse was washed with water and brine, dried ($MgSO_4$), concentrated, and purified by flash column chromatography (hexane:EtOAc = 6:1, then 4:1) to give **11** (440 mg, quant). **11:** $[\alpha]^{20}_D$ −19.4 (*c* 0.42, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) *δ*: 7.71 (d, 2 H, Ar H), 7.53 (d, 2H, Ar H), 7.39−7.18 (m, 13H, Ar H), 6.86 (d, 2H, Ar H), 5.48 (s, 1H, Ph−C*H*−O), 4.72 (d, 2H, Ph−CH_2−O), 4.63 (s, 2H, Ph−CH_2−O), 4.59 (d, 1H, $J_{1,2}$ = 9.5 Hz, H-1), 4.35 (d, 1H, $J_{6a,6b}$ = 12.2 Hz, H-6a), 4.14 (d, 1H, $J_{3,4}$ = 3.1 Hz, H-4), 3.97 (d, 1H, H-6b), 3.89 (dd, 1H, H-2), 3.80 (s, 3H, OCH_3), 3.61 (dd, 1H, $J_{2,3}$ = 9.2 Hz, H-3), 3.39 (s, 1H, H-5). HRMS−FAB (*m*/*z*): [M + H]$^+$ calcd for $C_{34}H_{35}O_6S$, 571.2147; found, 571.2154.

3-*O*-Benzyl-4,6-*O*-benzylidene-2-methoxybenzyl-α-D-galactopyranosyl Fluoride (12). A solution of **11** (160 mg, 0.29 mmol) in CH_2Cl_2 (10 mL) was cooled to −15 °C. The stirred was then treated with DAST (57 μL, 0.43 mmol) and allowed to stir for 2 min before NBS (67 mg, 0.37 mmol) was added. After 30 min, MeOH was added to the reaction mixture to quench the excess DAST and diluted with $CHCl_3$. The organic phase was washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), and concentrated. The residual syrup was subjected to flash column chromatography (hexane:EtOAc = 7:1) to afford **6** (101 mg, 75%). The α-isomer of **12** was not isolated. **12:** $[\alpha]^{20}_D$ +85.2 (*c* 0.64, dioxane). ^{1}H NMR (600 MHz, $CDCl_3$) *δ*: 7.52−7.24 (m, 12H, Ar H), 6.86 (d, 2H, Ar H), 5.63 (dd, 1H, $J_{1,2}$ = 2.4 Hz, $J_{1,F}$ = 53.5 Hz, H-1), 5.48 (s, 1H, Ph−C*H*-O), 4.83−4.59 (m, 4H, Ph−CH_2−O), 4.25 (d, 1H, H-4), 4.22 (br, 2H, H-6a, H-6b), 4.07 (dd, 1H, $J_{2,3}$ = 10.2 Hz, $J_{3,4}$ = 2.7 Hz,H-3), 4.03−3.94 (m, 2H, $J_{2,F}$ = 22.0, H-2, H-5), 3.79 (s, 3H, OCH_3). HRMS−FAB (*m*/*z*): [M + H]$^+$ calcd for $C_{28}H_{29}FO_6$, 481.2026; found, 481.2053.

***N*-(Benzyloxycarbonyl)-L-alanyl-*O*-(2-*O*-acetyl-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-galactopyranosyl)-L-threonyl-L-alanine Benzyl Ester (13)**. A mixture of Cp_2ZrCl_2 (126 mg, 0.23 mmol), $AgClO_4$ (178 mg, 0.86 mmol), Cbz-Ala-Thr-Ala-OBzl (**8,** 261 mg, 0.54 mmol), and powdered molecular sieves 4 Å (600 mg) in dry CH_2Cl_2 (10 mL) was stirred at room temperature for 2 h under a nitrogen atmosphere and then cooled to −40 °C. A solution of **12** (100 mg, 0.22 mmol) in dry CH_2Cl_2 (2 mL) was added, and the mixture was stirred at −40 °C to room temperature for 48 h, then diluted with $CHCl_3$, and filtered thorough Celite. The filtrate was washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), and concentrated. To the crude residue was then added acetic anhydrate (1.0 mL) and pyridine (2.0 mL) and stirred at room temperature overnight. The solution was concentrated and purified by flash column chromatography (hexane:EtOAc = 3:2) to give **13** (93 mg, 50% from **12**). **13:** $[\alpha]^{20}_D$ +96.5 (*c* 0.352, dioxane). ^{1}H NMR ($CDCl_3$) *δ*: 7.46 (d, 2H, Ar H), 7.29−7.12 (m, 19H, Ar H, AlaN*H*), 6.91 (d, 1H, *J* = 4.8 Hz, ThrN*H*), 5.39 (dd, 1H, $J_{1,2}$ = 3.7 Hz, $J_{2,3}$ = 10.5 Hz, H-2), 5.38 (s, 1H, Ph−C*H*−O), 5.32 (d, 1H, *J* = 6.1 Hz, AlaN*H*), 5.27 (d, 1H, H-1), 5.12−5.02 (m, 4H, Ph−CH_2−O), 4.64−4.56 (m, 3H, Ph−CH_2−O, Alaα), 4.33 (dd, 1H, $J_{\alpha,\beta}$ = 3.5 Hz, Thrα), 4.19 (dd, 1H, Alaα), 4.14 (d, 1H, $J_{3,4}$ = 3.2 Hz, H-4), 4.12 (dd, 1H, $J_{5,6a}$ = 0.8 Hz, $J_{6a,6b}$ = 12.5 Hz, H-6a), 4.09 (dd, 1H, Thr*β*), 4.03 (dd, 1H, H-3), 3.88 (dd, 1H, $J_{5,6b}$ = 1.3 Hz, H-6b), 3.63 (s, 1H, H-5), 1.99 (s, 3H, O*Ac*), 1.39 (d, 3H, *J* = 6.6 Hz, Ala*β*), 1.38 (d, 3H, *J* = 6.4 Hz, Ala*β*), 1.08 (d, 3H, *J* = 5.7 Hz, Thr*γ*). ^{13}C NMR (120 MHz, $CDCl_3$) *δ*: 172.5, 171.9, 169.5, 168.1, 162.6 (C═O), 138.7, 137.7, 136.2, 135.3, 129.0, 128.7, 128.6, 128.5, 128.3, 128.2, 128.1, 128.0, 127,6, 127.3, 126.4 (Ar), 101.1 (Ph−*C*H−O), 98.1 (C-1), 74.4 (Thr*β*), 74.0 (C-3, C-4), 71.5 (Ph−*C*H_2−O), 69.6 (C-2), 69.2 (C-6), 67.2, 67.0 (Ph−*C*H_2−O), 63.4 (H-5), 55.7, 50.6, 48.3, 20.0 (CO*C*H_3), 18.7, 18.5 (Ala*β*), 15.8 (Thr*γ*). HRMS−FAB (*m*/*z*): [M + H]$^+$ calcd for $C_{47}H_{54}N_3O_{13}$, 868.3657; found, 868.3678.

L-Alanyl-*O*-(2-*O*-acetyl-α-D-galactopyranosyl)-L-threonyl-L-alanine (14). To a solution of **13** (95 mg, 0.11 mmol) in DMF (4 mL), acetic acid (50 μL), and H_2O (1 mL) was added 10% Pd−C (200 mg) and stirred at room temperature for 48 h

under a H_2 gas atmosphere. Then, Pd−C was removed by filtration, and the solution was evaporated. The residue was purified by gel filtration chromatography (Sephadex G-10, water as eluent) to give **14** (41.4 mg, 82%). **14:** ^{1}H NMR (600 MHz, D_2O) δ: 5.11 (d, 1 H, $J_{1,2}$ = 3.9 Hz, H-1), 4.86 (dd, 1 H, $J_{2,3}$ = 10.6 Hz, H-2), 4.44 (d, 1 H, J = 2.9 Hz, Thrα), 4.25 (q, 1 H, J = 7.2 Hz, Alaα), 4.22 (m, 1H, Thrβ), 4.13 (q, 1 H, J = 7.1 Hz, Alaα), 4.00−3.98 (m, 2H, H-3, H-5), 3.95 (d, 1 H, $J_{3,4}$ = 2.9 Hz, H-4), 3.67 (m, 2H, H-6), 2.07 (s, 3H, O*Ac*), 1.50 (d, 3H, J = 7.1 Hz, Alaβ), 1.32 (d, 3H, J = 7.3 Hz, Alaβ), 1.18 (d, 3H, J = 6.7 Hz, Thrγ). ^{13}C NMR (120 MHz, D_2O) δ: 176.5, 173.1, 171.1, 169.7 (C═O), 96.9 (C-1), 75.3, 71.4, 70.8, 69.4, 67.1, 61.2, 57.3, 49.1, 48.8, 20.6 (COCH_3), 17.8, 17.0, 16.7. HRMS−FAB (m/z): $[M + H]^+$ calcd for $C_{47}H_{54}N_3O_{13}$, 868.2037; found, 466.2060.

Poly[L-alanyl-*O*-(2-*O*-acetyl-α-D-galactopyranosyl)-L-threonyl-L-alanine] (3). To a stirred solution of **14** (5.0 mg, 0.011 mmol) in DMF (100 μL) was added DMT-MM (3.6 mg, 0.013 mmol) at 0 °C, and the mixture was stirred at the same temperature overnight. Then, the product in DMF was precipitated by addition of ethanol and diethyl ether and centrifuged. The crude product was separated by gel filtration chromatography (Sephadex G-25, water as eluent) to give **3** (3.2 mg, 64%). The number-average molecular weight (M_n) and weight-average molecular weight (M_w) were estimated as 4.0 × 10^3 and 7.7 × 10^3, respectively, by GPC analysis as described in the general procedure. In MALDI−TOF MS analysis using DHB, precursor ion peaks (m/z) at 911.35 (n = 2), 1360.75 (n = 3), 1808.82 (n = 4), 2256.52 (n = 5), and 2707.947 (n = 6) were observed.[32] **3:** ^{1}H NMR (600 MHz, D_2O) δ: 4.97 (d, 1 H, $J_{1,2}$ = 3.2 Hz, H-1), 4.82 (dd, 1 H, $J_{2,3}$ = 10.4 Hz, H-2), 4.34 (d, 1 H, Thrα), 4.31 (q, 1 H, J = 7.3 Hz, Alaα), 4.27 (q, 1 H, J = 7.1 Hz, Alaα), 4.17 (dd, 1H, Thrβ), 3.96−3.93 (m, 2H, H-3, H-5), 3.91 (s, 1 H, H-4), 3.62 (m, 2H, H-6), 2.04 (s, 3H, O*Ac*), 1.31 (d, 3H, J = 7.1 Hz, Alaβ), 1.27 (d, 3H, J = 6.9 Hz, Alaβ), 1.18 (d, 3H, J = 7.5 Hz, Thrγ).

***N*-(Benzyloxycarbonyl)-L-alanyl-*O*-(2-azide-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranosyl)-L-threonyl-L-alanine Benzyl Ester (16)**. A mixture of $SnCl_2$ (197 mg, 1.04 mmol), $AgClO_4$ (215 mg, 1.04 mmol), **8** (630 mg, 1.30 mmol), and freshly activated powdered molecular sieves 4 Å (1.0 g) in dry CH_2Cl_2 (8 mL) was stirred at room temperature for 3 h under a nitrogen atmosphere and then cooled to −20 °C. A solution of **15**[27] (200 mg, 0.52 mmol) in dry CH_2Cl_2 (2 mL) was added, and the mixture was stirred at −20 °C to room temperature for 4 days, then diluted with $CHCl_3$, and filtered thorough Celite. The filtrate was washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), and concentrated. The crude residue was purified by flash column chromatography (Hex:EtOAc = 1:1) to give **16** (230 mg, 53%). β-Isomer of **16** was not isolated. **16:** $[\alpha]^{20}_D$ +60.1 (*c* 1.0, dioxane). ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.70 (d, 1H, J = 7.4 Hz, AlaN*H*), 7.54−7.28 (m, 20H, ArH), 6.91 (d, 1H, J = 5.4 Hz, ThrN*H*), 5.45 (s, 1H, Ph−C*H*-O), 5.31−5.30 (m, 2H, $J_{1,2}$ = 3.4 Hz, H-1, AlaN*H*), 5.19−5.05 (m, 4H, Ph−CH_2−O), 4.71 (s, 2H, Ph−CH_2−O), 4.63 (m, 1H, Alaα), 4.50 (br, 1H, Thrα), 4.27−4.20 (br, 2H, $J_{6a,6b}$ = 12.4 Hz, H-6a, Alaα), 4.16 (d, 1H, $J_{3,4}$ = 3.0 Hz, H-4), 4.12 (dd, 1H, $J_{2,3}$ = 10.5 Hz, H-2), 4.03 (dd, 1H, H-3), 3.96 (d, 1H, H-6b), 3.62 (s, 1H, H-5), 1.42 (d, 3H, J = 7.2 Hz, Alaβ), 1.39 (d, 3H, J = 7.0 Hz, Alaβ), 1.07 (d, 3H, J = 5.5 Hz, Thrγ). ^{13}C NMR (120 MHz, $CDCl_3$) δ: 172.8, 172.2, 168.1 (C═O), 138.4, 138.0, 136.5, 135.8, 129.5, 129.0, 129.0, 128.8, 128.6, 128.6, 128.5, 128.3, 128.1, 126.7 (Ar), 101.5 (Ph−*C*H−O), 97.9 (C-1), 76.3, 73.6, 73.2, 71.6, 69.7, 67.4, 63.7, 60.0, 55.3, 51.0, 48.6, 19.3, 18.4, 16.2. HRMS−FAB (m/z): $[M + H]^+$ calcd for $C_{45}H_{52}N_6O_{11}$, 851.3616; found, 851.3619.

***N*-(Benzyloxycarbonyl)-L-alanyl-*O*-(2-acetamide-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranosyl)-L-threonyl-L-alanine Benzyl Ester (17)**. A solution of **16** (120 mg, 0.14 mmol) in AcSH (1 mL) and pyridine (0.5 mL) was stirred at room temperature for 20 h, and the residue was purified by flash column chromatography (run 1; hexane: EtOAc = 5:1, then acetone, run 2; $CHCl_3$:MeOH = 99:1 then 97:3) to give **17** (85 mg, 70%). **17:** $[\alpha]^{20}_D$ +89.2 (*c* 0.37, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$) δ: 7.53−7.26 (m, 20H, ArH), 7.10 (d, 1H, J = 6.4 Hz, AlaN*H*), 6.94 (d, 1H, J = 5.6 Hz, ThrN*H*), 6.39 (d, 1H, J = 6.4 Hz, N*H*Ac), 5.46 (s, 1H, Ph−C*H*-O), 5.38 (d, 1H, J = 5.2 Hz, AlaN*H*), 5.24−5.09 (m, 5H, H-1, Ph−CH_2−O), 4.71−4.59 (m, 4H, H-2, Alaα, Ph−CH_2−O), 4.44 (dd, 1H, $J_{\alpha,\beta}$ = 2.3 Hz, Thrα), 4.28−4.22 (m, 3H, Alaα, Thrβ, H-4), 4.19 (d, $J_{6a,6b}$ = 12.3 Hz, H-6a), 3.96−3.92 (m, 2H, H-6b, H-3), 3.69 (s, 1H, H-5), 1.95 (s, 3H, NH*Ac*), 1.42 (d, 3H, J = 7.1 Hz, Alaβ), 1.39 (d, 3H, J = 7.0 Hz, Alaβ), 1.13 (d, 3H, J = 5.7 Hz, Thrγ). ^{13}C NMR (120 MHz, $CDCl_3$) δ: 173.0, 172.2, 170.5, 168.9 (C═O), 138.7, 137.7, 136.1, 135.0, 128.9, 128.7, 128.6, 128.6, 128.3, 128.3, 128.2, 128.1, 127.9, 127.5, 126.3 (Ar), 100.9 (Ph−*C*H−O), 99.6 (C-1), 74.6, 74.2, 73.4, 70.7, 69.5, 67.6, 67.0, 63.6, 56.2, 49.1, 23.3, 18.3, 18.1, 17.2. HRMS−FAB (m/z): $[M + H]^+$ calcd for $C_{47}H_{55}N_4O_{12}$, 867.3817; found, 867.3812.

***N*-(Benzyloxycarbonyl)-L-alanyl-*O*-(2-acetamide-3-*O*-benzyl-2-deoxy-α-D-galactopyranosyl)-L-threonyl-L-alanine Benzyl Ester (18)**. To a solution of **17** (50 mg, 0.058 mmol) in MeOH (2.0 mL) was added CSA (30 mg, 0.13 mmol), and the mixture was stirred for 3 h. Then, CSA was quenched with pyridine, and the solution was concentrated. The residue was purified by flash column chromatography ($CHCl_3$:MeOH = 100:2, then 100:3) to give **18** (47 mg, quant). **18:** $[\alpha]^{20}_D$ +28.6 (*c* 0.75, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$, δ): 7.36−7.26 (m, 15H, ArH), 7.11 (d, 1H, J = 6.8 Hz, AlaN*H*), 7.20 (d, 1H, J = 6.5 Hz, ThrN*H*), 6.58 (d, 1H, J = 8.2 Hz, N*H*Ac), 5.46 (d, 1H, J = 7.1 Hz, AlaN*H*), 5.20−5.05 (m, 5H, H-1, Ph−CH_2−O × 4), 4.68 (d, 1H, Ph−CH_2−O), 4.57 (m,1H, Alaα), 4.54 (d, 3H, Ph−CH_2−O), 4.48 (dd, 1H, H-2), 4.47 (dd, 1H, $J_{\alpha,\beta}$ = 2.3 Hz, $J_{\beta,\gamma}$ = 7.8 Hz, Thrβ), 4.30−4.23 (br, 2H, Alaα, Thrα), 4.08 (s, 1H, H-4), 3.92 (m, 1H, H-6a), 3.86 (br, 1H, H-5), 3.75 (m, 1H, H-6b), 3.64 (dd, 1H, $J_{2,3}$ = 10.8 Hz, $J_{3,4}$ = 2.8 Hz, H-3), 2.93 (s, 1H, 4-OH), 2.73 (s, 1H, 6-OH), 1.95 (s, 3H, NH*Ac*), 1.41 (d, 3H, J = 7.1 Hz, Alaβ), 1.37 (d, 3H, J = 7.0 Hz, Alaβ), 1.17 (d, 3H, Thrγ). ^{13}C NMR (120 MHz, $CDCl_3$) δ: 173.3, 172.9, 171.1, 169.6 (C═O), 138.3, 136.4, 135.4, 129.1, 129.1, 129.0, 128.9, 128.7, 128.6, 128.3, 127.9 (Ar), 99.1 (C-1), 76.8, 75.1, 71.8, 70.9, 68.1, 67.5, 67.5, 63.4, 56.5, 50.9, 49.0, 48.8, 23.6, 18.6, 18.3, 17.8. HRMS−FAB (m/z): $[M + H]^+$ calcd for $C_{40}H_{51}N_4O_{12}$, 779.3504; found, 779.3505.

***N*-(Benzyloxycarbonyl)-L-alanyl-*O*-(2-acetamide-3-*O*-benzyl-2-deoxy-6-*O*-sulfo-α-D-galactopyranosyl)-L-threonyl-L-alanine Benzyl Ester, Sodium Salt (19)**. To a solution of **18** (25 mg, 0.032 mmol) in DMF (2.0 mL) was added $Me_3N{\cdot}SO_3$ (9.0 mg, 0.064 mmol), and the mixture was stirred at 40 °C for 44 h. Then, the residue was purified by flash column chromatography ($CHCl_3$:MeOH = 95:5, 9:1 then 85:15) and Dowex 50W-X8 [Na^+] to give **19** as its sodium salt (64 mg, 64%). **19:** $[\alpha]^{20}_D$ +15.4 (*c* 1.0, $CHCl_3$). ^{1}H NMR (600 MHz, acetone) δ: 7.94 (d, 1H, J = 6.5 Hz, AlaN*H*), 7.69 (d, 1H, J = 8.3 Hz, ThrN*H*), 7.38−7.18 (m, 16H, Ar H, AlaN*H*), 6.90 (d, 1H, J = 6.8 Hz, N*H*Ac), 5.17−5.03 (m, 4H, Ph−CH_2−O), 4.99 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1), 4.75 (d, 1H, Ph−CH_2−O), 4.61 (dd, 1H, Thrα), 4.51−4.34 (m, 3H, Alaα × 2, Ph−CH_2−O), 4.36−4.33 (br, 2H, H-2, Thrβ), 4.26 (dd, 1H, H-4), 4.25 (dd, 1H, H-5), 4.14 (m, 2H, H-6a, 6b), 3.80 (dd, 1H, $J_{2,3}$ = 10.5 Hz, H-3), 1.95 (s, 3H, NH*Ac*), 1.38 (d, 3H, J = 7.3 Hz, Alaβ), 1.38 (d, 3H, J = 7.1 Hz, Alaβ), 1.26 (d, 3H, J = 6.1 Hz, Thrγ). ^{13}C NMR (120 MHz, acetone) δ: 172.5, 170.3 (C═O), 139.4, 137.1, 136.1, 128.4, 128.3, 128.2, 128.1, 128.0, 127.8, 127.7, 127.6, 127.5, 127.4, 127.1, 127.0 (Ar), 97.9 (C-1), 77.2, 76.1, 70.4, 69.8 (C-6), 66.7, 65.9, 65.5, 56.2, 50.4, 48.0, 21.8, 18.0, 17.1, 16.8. HRMS−FAB (m/z): $[M + H]^+$ calcd for $C_{40}H_{50}N_4NaO_{15}S$, 881.2891; found, 881.2903.

L-Alanyl-*O*-(2-acetamide-2-deoxy-6-*O*-sulfo-α-D-galactopyranosyl)-L-threonyl-L-alanine, Sodium Salt (20). To a solution of **19** (45 mg, 0.051 mmol) in DMF (3 mL), acetic acid (50 μL), and H_2O (0.5 mL) was added 10% $Pd(OH)_2$−C (100 mg), and the mixture was stirred at room temperature overnight under a H_2 gas atmosphere. Then, $Pd(OH)_2$−C was removed by filtration, and the solution was evaporated. The residue was purified by gel filtration chromatography (Sephadex G-10, water as eluent), reverse-phase HPLC (Inertsil ODS-3, GL Science Inc.; gradient 0 → 20% CH_3CN in H_2O for 60 min), and then Dowex 50W-X8 [Na^+] to give **20** as its sodium salt (16.1 mg, 56%). **20:** H NMR (600 MHz, D_2O) δ: 4.92 (d,

1H, $J_{1,2}$ = 3.7 Hz, H-1), 4.45 (d, 1H, J = 2.0 Hz, Thrα), 4.29 (m, 1H, $J_{\beta,\gamma}$ = 6.4 Hz, Thrβ), 4.21 (dd, 1H, H-5), 4.19 (q, 1H, J = 7.1 Hz, Alaα), 4.15 (dd, 1H, $J_{5,6a}$ = 4.2 Hz, $J_{6a,6b}$ = 11.3 Hz, H-6a), 4.09−4.02 (m, 3H,, Alaα H-6b, H-2), 3.93 (d, 1H, $J_{3,4}$ = 2.0 Hz, H-4), 3.81 (dd, 1H, $J_{2,3}$ = 11.0 Hz, H-3), 1.94 (s, 3H, NH*Ac*), 1.50 (d, 3H, Alaβ), 1.24 (d, 3H, Alaβ), 1.21 (d, 3H, Thrγ). ^{13}C NMR (120 MHz, D_2O) δ: 179.5, 174.9, 172.3, 170.3 (C═O), 98.4 (C-1), 75.8, 69.8, 68.8, 68.3, 68.2 (C-6), 57.8, 51.2, 50.3, 49.5, 22.7, 18.3, 18.0, 17.5. HRMS−FAB (*m*/*z*): $[M + H]^+$ calcd for $C_{18}H_{32}N_4O_{13}S$, 545.1763; found, 545.1770.

Poly[L-alanyl-*O*-(2-acetamide-2-deoxy-6-*O*-sulfo-α-D-galactopyranosyl)-L-threonyl-L-alanine, Sodium Salt] (4). *DPPA Method*: To a stirred solution of **20** (4.0 mg, 7.1 μmol) in DMF (100 μL) was added 10% DPPA solution in DMF (23 μL, 10.6 μmol) and 10% Et_3N solution in DMF (25 μL, 17.8 μmol) at 0 °C, and the mixture was stirred at room temperature overnight. Then, the product in DMF was precipitated by addition of ethanol and diethyl ether, and centrifuged. The crude product was separated by gel filtration chromatography (Sephadex G-25, water as eluent) and Dowex 50W-X8 [Na^+] to give **4** (4.0 mg, quant). The number-average molecular weight (M_n) and weight-average molecular weight (M_w) were estimated as 9.6×10^3 and 1.1×10^4, respectively, by GPC analysis as described in the general procedure.

DMTMM Method: To a stirred solution of **19** (4.0 mg, 7.1 μmol) in DMF (150 μL) was added DMT-MM (2.9 mg, 0.011 mmol) at 0 °C, and the mixture was stirred at the same temperature overnight. Then, the product in DMF was precipitated by addition of ethanol and diethyl ether and centrifuged. The crude product was separated by gel filtration chromatography (Sephadex G-25, water as eluent) to give **4** (4.0 mg, quant). The number-average molecular weight (M_n) and weight-average molecular weight (M_w) were estimated as 8.2×10^3 and 1.0×10^4, respectively, by GPC analysis as described in the general procedure. In MALDI−TOF MS analysis using DHB, precursor ion peaks (*m*/*z*) at 1115.85 (n = 2), 1667.47 (n = 3), 2215.50 (n = 4), 2765.37 (n = 5), and 3320.04 (n = 6) were observed.[32] **4:** H NMR (600 MHz, D_2O, δ): 4.77 (br, 1H, H-1), 4.35 (br, 1H, Thrα), 4.27 (m, 1H, $J_{\beta,\gamma}$ = 6.5 Hz, Thrβ), 4.14−3.95 (m, 6H, H-5, Alaα × 2, H-6a, H-6b, H-2), 3.89 (s, 1H, H-4), 3.77 (dd, 1H, $J_{2,3}$ = 11.2 Hz, H-3), 1.91 (s, 3H, NH*Ac*), 1.26 (d, 3H, J = 7.0, Alaβ), 1.21 (d, 3H, J = 6.7, Alaβ), 1.14 (d, 3H, Thrγ). ^{13}C NMR (120 MHz, D_2O): 179.3, 174.8, 172.1, 170.3 (C═O), 98.6 (C-1), 76.8, 69.6, 68.7, 68.3, 68.1 (C-6), 57.7, 50.2, 49.8, 49.7, 23.0, 18.8, 17.8, 17.3.

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