Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 573–581

BIOORGANIC & MEDICINAL CHEMISTRY

Preparation of Glycosylated Amino Acid Derivatives for Glycoprotein Synthesis by In Vitro Translation System

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Received 3 July 2001; accepted 23 August 2001

Abstract—General preparation of glycosylated amino acylated nucleotide for in vitro peptide synthesis was described. Both *O*-glycosylated amino acyl nucleotides and *C*-glycosylated amino acyl nucleotide were synthesized by choosing the appropriate protecting group.

Introduction

Glycosylation is one of the most prominent post-translational modifications that controls protein conformation, functions, stability, and intra/intercellular trafficking. Due to increased understanding of the biological functions of glycan chains as the recognition signals in various biological events, synthetic effort toward glycoprotein has become a major issue in organic synthesis and chemical biology.[1] Thanks to the recent advances in peptide as well as oligosaccharide synthesis technology, chemical synthesis of relatively complex glycopeptide is now a quite feasible task.[2] However, it is still considered to be a daunting challenge to produce intact glycoproteins by a purely chemical means (Schemes 1–4).

In vitro protein synthesis has proved to be a powerful method to incorporate non-natural or modified amino acids in a site-specific manner.[3] For such purpose, the use of amino acylated suppressor *t*RNA, which recognizes amber nonsense codon, has been explored. Based on this approach, Hecht et al. successfully incorporated glucosylated serine into firefly luciferase,[4] albeit in modest incorporation efficiency.

An alternative in vitro translation system which utilizes four-base codons, CGGG and AGGU, has been developed.[5,6] Since these quadruplets has been derived from the minor codons of arginine (CGG and AGG), competition between four-base and three-base should be in favor of the former. With a set of orthogonal four-base codons, incorporation of two different nonnatural amino acids was demonstrated to be possible. Considering the success of incorporating various types of modified amino acids into proteins, exploration of the possibility to produce glycoproteins with four-base codon approach seems to be highly promising (Fig. 1).

Prerequisite to the incorporation of a tailored amino acid residue (Xaa) by in vitro protein synthesis is the preparation of *t*RNA amino acylated at the terminal A residue (*t*RNA-Xaa). It has been demonstrated that *t*RNA-Xaa can be prepared by enzymatic ligation of *t*RNA lacking terminal pCpA [*t*RNA(–CA)] with amino acylated dinucleotide pCpA-Xaa[7] or pdCpA-Xaa.[8] As an active ester, cyanometyl ester has proved to be the most effective in terms of the ease of preparation and chemoselective reactivity toward ribose hydroxy groups.[9]

We report here the preparation of three types of glycosylated amino acid cyanomethyl esters **1**, **2**, and **3**, which were designed as key intermediates for the in vitro production of glycoproteins having novel carbohydrate

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0968-0896/02/$ - see front matter
PII: S0968-0896(01)00304-2

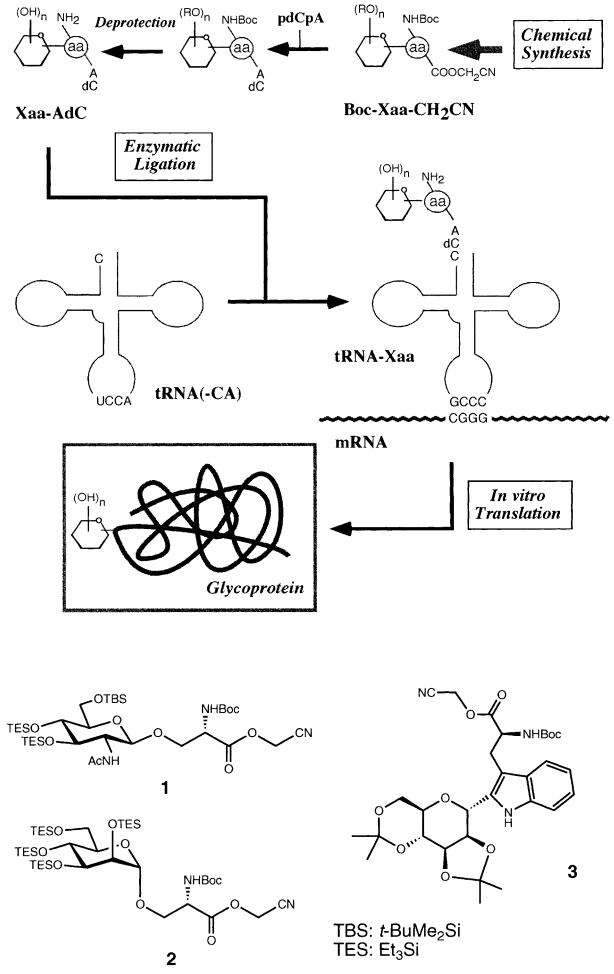


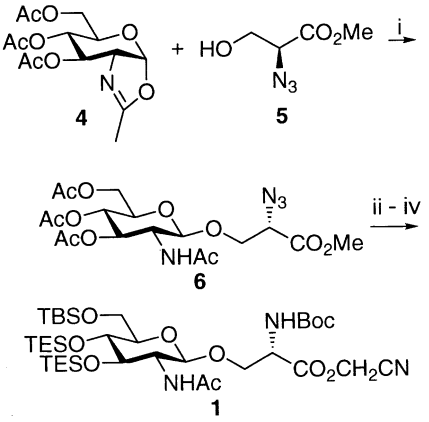
Figure 1.

structures whose biological functions are attracting recent attention.[10]

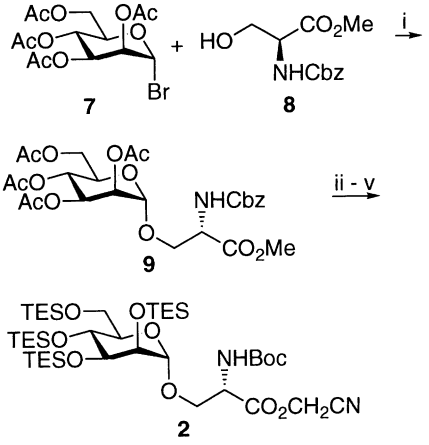
Results and Discussion

Adequate choice of protecting group for hydroxy groups of sugar part and amino group of amino acid is crucial for preparation of glycosylated aminoacyl pdCpA because of the sensitivity of the ester linkage between pdCpA and amino acid in the presence of nucleophile.[11] While Hecht et al. adopted the allyloxycarbonyl group for hydroxy protection, we focused our attention on the use of acid labile groups. In this context, the ester linkage was shown to be tolerant under *N*-Boc (*tert*-butoxycarbonyl) deprotection conditions (TFA).[12] It was then presumed that either the silyl or acetonide group can be adopted for the protection of carbohydrate components. Simultaneous removal of *N*- and *O*-protecting groups should then be possible without deteriorating the ester linkage.

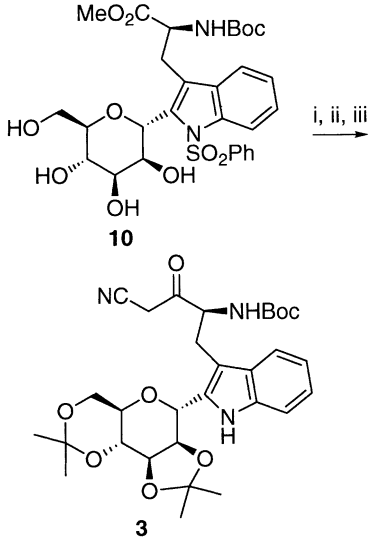
As the glycosylated amino acid residue to be incorporated by in vitro system, serine linked *N*-acetyl-D-glucosamine (*O*-GlcNAc-Ser), serine linked D-mannose (*O*-Man-Ser), and *C*-linked D-mannosyl tryptophan (*C*-Man-Trp) were selected because of their biological significance.



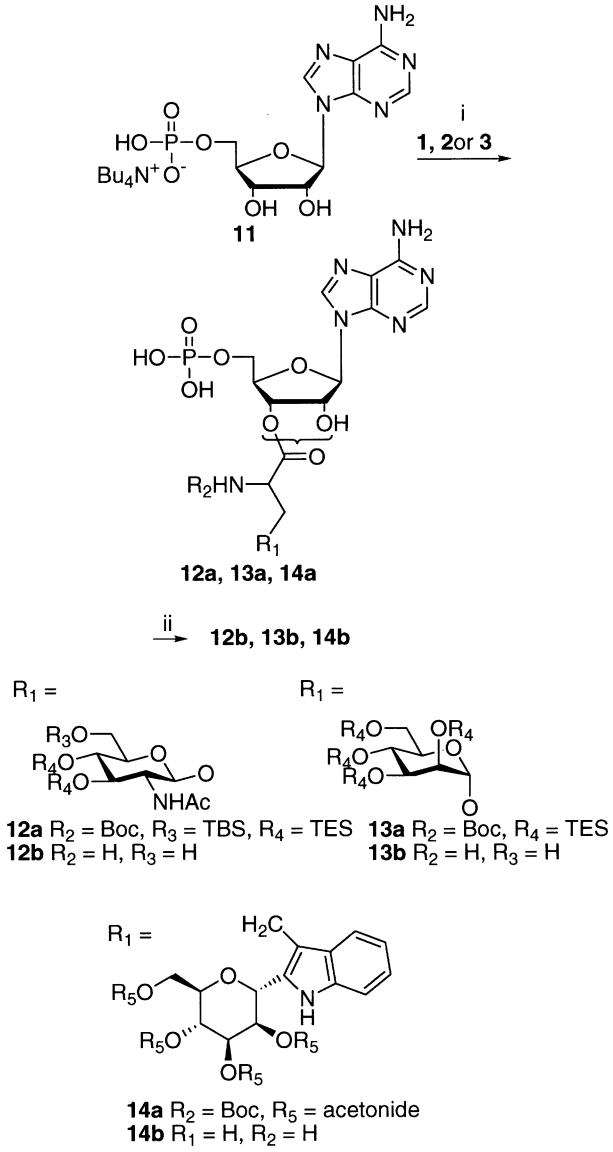
Scheme 1. Reagent and conditions. (i) TMSOTf, 1,2-dichloroethane, 50 °C, 10 h, 64%; (ii) H_2, 10% Pd/C, EtOAc, Boc_2O, rt, 3 h, 91%; (iii) NaOMe, MeOH, rt, 3 h, TBSCl, imidazole, DMF, then TESCl, imidazole, DMF, 66%; (iv) LiOH, MeOH, H_2O, then bromoacetonitrile, *i*-Pr_2NEt, CH_3CN, rt, overnight, 41%.



Scheme 2. Reagent and conditions: (i) AgOTf, CH_2Cl_2,−10 °C, overnight, 56%; (ii) 10% Pd/C, Boc_2O MeOH, rt, overnight, 92%; (iii) NaOMe, MeOH, then TESCl, pyridine, rt, overnight, 66%; (iv) LiOH, H_2O, MeOH, rt, 3 h, 84%; (v) bromoacetonitrile, *i*-Pr_2NEt, CH_3CN, rt, overnight, 84%.



Scheme 3. Reagent and conditions: (i) 2,2-dimethoxypropane, PPTS, DMF, rt, 2 days, 74%; (ii) 10% NaOH aq, EtOH, rt-reflux, overnight; (iii) bromoacetonitrile, *i*-Pr_2NEt, CH_3CN, rt, overnight, 80% (two steps).



Scheme 4. Reagent and conditions: (i) DMF, 0 °C, **15a**, 44%, **16a**, 50%, **17a**, 53%; (ii) TFA for **15b**, 42%, and **16b**, quant for **17b**, 4 M HCl dioxane, H_2O, rt, 65%.

For instance, *O*-Linked *N*-acetyl-D-glucosamine is found in a variety of nuclear and cytoplasmic proteins and functions in signal transduction.[13] *O*-Mannosyl linkage in α-dystroglycan may play a significant role in binding to laminin, which influences development and regeneration of the nervous system.[14] *C*-linked mannosyl tryptophan is a naturally occurring novel subclass of glycoprotein, which is synthesized by our group for the first time.[15] The biological role of mannosylated tryptophan is not clear at the present time, but recent study suggests that *C*-glycosylation is not a rare post-translational modification as previously thought.[16] Not only from human, *C*-Mannosylated tryptophan is also obtained from marine ascidians.[17]

Oxazoline **4** was glycosylated in the presence of TMSOTf with serine derivative **5**, which was synthesized from serine methyl ester by the action of TfN_3.[18] After transformation of azide group to Boc in one pot, the acetyl group was removed. The hydroxy groups were protected as TBS and TES group in order that the deprotection at the final stage can be performed under acidic conditions. After hydrolysis of methyl ester under alkaline conditions, the acid was converted to cyanomethyl ester **1**. Similarly, α-*O*-linked D-mannosyl serine cyanomethyl ester **2** was prepared from bromide **7** in five steps. β-Elimination during the removal of acetate could be minimized (< 3%) by controlling the NaOMe concentration (0.03%).

Mannosyl tryptophan cyanomethyl ester **3** was synthesized as below. Tetraol **10**[15] was protected as its corresponding diacetonide by 2,2-dimethoxypropane in the presence of PPTS without affecting the Boc group. After hydrolysis of methyl ester and sulfone amide at indole nitrogen, carboxylic acid was changed to cyanomethyl ester **3** in 80% yield.

Subsequently, condensation of cyanomethyl ester **1**, **2**, **3** and nucleotide was investigated. The condensation of **1** with AMP tetrabutylammonium salt **11** as a model of pdCpA was performed in 44% yield at 0 °C to reduce the amount of β-elimination of glycosylated amino ester under basic conditions. Similarly, condensation of **2** and **11** was performed at 0 °C in DMF to afford **13a** in 50% yield. When **3** was used as the glycosylated amino acid source, the reaction proceeded relatively slowly and required a high temperature (60 °C), probably because of steric hindrance of the acetonide. Deprotection of silyl group and Boc group of **12a** and **13a** were performed in TFA at the same time in a short period and **12b** and **13b** were obtained. Because TFA treatment of **14a** caused decomposition of mannosyl tryptophan moiety, acidic hydrolysis of acetonide and Boc was performed in 4 M aq HCl in dioxane to afford **14b**.

Conclusion

In conclusion, glycosylated amino acid derivatives for in vitro synthesis of glycoproteins were synthesized. Although incorporation of a glucose residue into firefly luciferase was demonstrated successfully by Hecht et al., the generality of in vitro production of glycoproteins is not clear. Since synthetic routes are now established for *O*-linked-β-D- (**1**), *O*-linked-α-D- (**2**) and *C*-linked-α-D- (**3**) glycosylated amino acids, comparative studies on incorporation efficiencies of these extremely different structures would reveal the scope of the in vitro system for the synthesis of biologically relevant glycoproteins.

Experimental

General procedures

^1H NMR spectra were taken by JEOL EX-400 as solutions in $CDCl_3$ otherwise mentioned. Chemical shifts are expressed in ppm downfield from the signal for Me_4Si. Optical rotations were measured by Jasco DIP-310 at ambient temperature. Melting points were uncorrected. Kanto silica gel (spherical, neutral, 100–210 μm) were

used for column chromatography. Merck TLC (silica gel 60F254) was used for TLC analysis. Molecular sieves were activated by heating to 180 °C just before use.

Methyl (*2S*)-2-azide-3-hydroxypropanoate (5). To a mixture of NaN_3 (66.7 g, 1.0 mol) in H_2O (160 mL) and CH_2Cl_2 (240 mL), Tf_2O (66.7 g, 0.23 mol) was added with vigorous stirring at 0 °C. The mixture was stirred for 4 h at 0 °C. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2 (70 mL × 2). The combined organic layers were washed with satd $NaHCO_3$ (120 mL) and H_2O (120 mL). The organic layer was dried over Na_2SO_4. To a solution of L-serine methyl ester·HCl salt (25.8 g, 186 mmol) and 4-(dimethylamino)pyridine (112 g, 0.92 mol) in MeCN (840 mL), TfN_3 solution (350 mL, as prepared above) was added at 0 °C, and stirred at 0 °C for 2 h. The mixture was diluted with 1,2-dichloroethane (500 mL) and concentrated at 30 °C in vacuo to ca. 300 mL, and filtered. The mother liquid was subjected to silica gel column chromatography (hexane/EtOAc = 6:4) to afford **5** (15.0 g, 62%) as a pale yellow oil: $[\alpha]_D^{25}$ −92.2 (*c* 1.0, $CHCl_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.0 (C), 63.4 (CH), 62.7 (CH_2), 52.9 (CH_3); ^1H NMR (400 MHz, $CDCl_3$) δ 4.11 (dd, 1H, *J* = 4.4, 5.4 Hz), 3.94 (d, 1H, *J* = 4.4 Hz), 3.94 (d, 1H, *J* = 5.4 Hz), 3.85 (s, 3H), 2.11 (brs, 1H); HRMS (FAB), *m*/*z* 146.0567 $(M+H)^+$, ($C_4H_8N_3O_3$ requires 146.0566).

3-*O*-(2-Acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(*2S*)-2-azide-3-hydroxypropionic acid methyl ester (6). To a solution of oxazoline **4** (15.2 g, 46.2 mmol), azide **5** (13.4 g, 92.4 mmol) and 1,1,3,3-tetramethylurea (5.5 mL, 46.2 mmol) in 1,2-dichloroethane (100 mL) was added TMSOTf (8.8 mL, 48.5 mmol) dropwise at 0 °C. After addition, the mixture was stirred at 50 °C for 10 h under N_2 atmosphere. After the reaction mixture was cooled to 0 °C, MeOH (10 mL) and Et_3N (6.8 mL, 48.5 mmol) were added. The solvent was evaporated, and the residue was purified by silica gel column chromatography (hexane/AcOEt/MeOH = 8:9:0.7) to afford **6** (14.1 g, 64%) as a colorless solid: mp 135–137 °C; $[\alpha]_D^{25}$ −22.4 (*c* 1.0, $CHCl_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3 (C), 170.3 (C), 169.0 (C), 168.1(C), 100.1 (CH), 72.0 (CH), 71.7 (CH), 68.6 (CH), 68.5 (CH_2), 62.0 (CH_2), 61.3 (CH), 54.3 (CH), 52.8 (CH_3), 23.2 (CH_3), 20.7 (CH_3), 20.6 (CH_3), 20.6 (CH_3); ^1H NMR (400 MHz, $CDCl_3$) δ 5.52 (d, 1H, *J* = 8.8 Hz), 5.32 (dd, 1H, *J* = 9.3, 10.5 Hz), 5.07 (t, 1H, *J* = 9.3 Hz), 4.83 (d, 1H, *J* = 8.3 Hz), 4.24 (m, 1H), 4.14 (dd, 1H, *J* = 2.4, 12.2 Hz), 4.04 (t, 1H, *J* = 4.0 Hz), 3.90 (dd, 1H, *J* = 3.7, 11.0 Hz), 3.82 (s, 3H), 3.80–3.75 (m, 1H), 3.71 (m, 1H), 2.09 (s, 3H), 2.03 (s, 3H), 2.03 (s, 3H), 1.98 (s, 3H). Anal. calcd for $C_{18}H_{26}N_4O_{11}$: C, 45.57; H, 5.52; N, 11.81. Found: C, 45.33; H, 5.45; N, 11.60.

***N*-(*tert*-Butoxycarbonyl)-3-*O*-(2-acetamido-2-deoxy-3,4,6-tri- *O*-acetyl-β-D-glucopyranosyl)-*S*-serine methyl ester.** To a solution of azide **6** (10.6 g, 22.3 mmol) and di-*tert*-butyl dicarbonate (5.9 g, 27 mmol) in EtOAc (200 mL) was added a suspension of 10% Pd/C (1.0 g) in EtOAc (20 mL). The mixture was stirred vigorously under H_2 atmosphere for 3 h. The catalyst was filtered off through Celite and the solvent was evaporated. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 98:2) to afford Boc derivative (11.1 g, 91%) as an amorphous: $[\alpha]_D^{25}$ −3.5 (*c* 1.1, $CHCl_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6 (C), 170.4 (C), 170.3 (C), 170.1(C), 169.1 (C), 155.2 (C), 100.9 (CH), 80.1 (C), 72.1 (CH), 71.9 (CH), 69.2 (CH_2), 68.4 (CH), 62.0 (CH_2), 54.5 (CH), 53.9 (CH), 52.7 (CH_3), 28.4 (CH_3), 23.4 (CH_3), 20.8 (CH_3), 20.8 (CH_3), 20.7 (CH_3); ^1H NMR (400 MHz, $CDCl_3$) δ 5.74 (d, 1H, *J* = 8.1 Hz), 5.43 (d, 1H, *J* = 7.6 Hz), 5.27 (dd, 1H, *J* = 9.3, 10.5 Hz), 5.05 (t, 1H, *J* = 9.6 Hz), 4.71 (d, 1H, *J* = 8.3 Hz), 4.41 (m, 1H), 4.30–4.18 (m, 2H), 4.12 (dd, 1H, *J* = 2.3, 12.3 Hz), 3.88–3.80 (m, 2H), 3.76 (s, 3H), 3.65 (m, 1H), 2.10 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 1.98 (s, 3H), 1.46 (s, 9H). Anal. calcd for $C_{23}H_{36}N_2O_{13}$: C, 50.36; H, 6.62; N, 5.11. Found: C, 50.23; H, 6.61; N, 5.13.

(*N*-*tert*-Butoxycarbonyl)-3-*O*-[2-acetamido-2-deoxy-3,4-bis-*O*-triethylsilyl-6-(*tert*-butyldimethylsilyl)-β-D-glucopyranosyl]-*S*-serine methyl ester. To a solution of acetate (8.2 g, 14.9 mmol) in dry MeOH (150 mL) was added MeONa (0.1% wt MeOH solution, 30 mL) at room temperature. The mixture was stirred for 3 h at room temperature. The mixture was neutralized with Amberlyst 15-E. The resins were filtered off and the solvent was evaporated. The material was pumped to dryness. The residue was dissolved in DMF (36 mL) and imidazole (6.1 g, 90 mmol) was added. To the solution was added *t*-butyldimethylsilyl chloride (2.0 g, 13.0 mmol) at room temperature. After the mixture was stirred for 2 h, triethylsilyl chloride (4.6 mL, 27.5 mmol) was added at 0 °C and the mixture was stirred for 12 h at room temperature. To the solution was added MeOH (4 mL) and stirred for 30 min to destroy the excess of silyl chloride. The reaction mixture was diluted with EtOAc, and H_2O was added to the solution. The aqueous layer was extracted with EtOAc. The combined organic layers were washed with aq 5% citric acid, satd. $NaHCO_3$ aq, and brine. The organic layer was dried over Na_2SO_4. The solvent was evaporated and the residue was purified by silica gel column chromatography (10–30% EtOAc in hexane) to afford silyl ether (7.5 g, 66%, 2 steps) as a colorless solid: mp 108–109 °C; $[\alpha]_D^{25}$ −9.1 (*c* 1.0, benzene); ^{13}C NMR (100 MHz, C_6D_6) δ 170.8 (C), 168.9 (C), 155.6 (C), 100.8 (CH), 79.5 (C), 78.4 (CH), 73.4 (CH), 70.8 (CH), 69.6 (CH_2), 63.6 (CH_2), 55.0 (CH), 54.7 (CH), 52.1 (CH_3), 28.7 (CH_3), 26.4 (CH_3), 23.7 (CH_3), 18.8 (C), 7.6 (CH_3), 7.5 (CH_3), 5.6 (CH_2),−4.5 (CH_3),−4.9 (CH_3); ^1H NMR (400 MHz, $CDCl_3$) δ 6.63 (d, 1H, *J* = 9.0 Hz), 5.41 (d, 1H, *J* = 8.3 Hz), 4.61 (d, 1H, *J* = 2.4 Hz), 4.40 (m, 1H), 4.20 (dd, 1H, *J* = 3.8, 9.9 Hz), 4.07–3.95 (m, 2H), 3.88 (m, 1H), 3.71 (s, 3H), 3.75–3.67 (m, 3H), 3.62 (dd, 1H, *J* = 3.9, 10.0 Hz), 1.96 (s, 3H), 1.45 (s, 9H), 1.01–0.94 (m, 18H), 0.90 (s, 9H), 0.69–0.59 (m, 12H), 0.08 (s, 3H), 0.07 (s, 3H). Anal. calcd for $C_{35}H_{72}N_2O_{10}Si_3$: C, 54.95; H, 9.48; N, 3.66. Found: C, 55.00; H, 9.59; N, 3.74.

(*N*-*tert*-Butoxycarbonyl)-3-*O*-[2-acetamido-2-deoxy-3,4-bis-*O*-triethylsilyl-6-(*tert*-butyldimethylsilyl)-β-D-glucopyranosyl]-*S*-serine cyanomethyl ester (1). The methyl ester (647 mg, 846 μmol) was dissolved in THF (8.4 mL)

and the solution was cooled to 0 °C. To the solution was added ice cooled solution of LiOH (24.3 mg, 1.02 μmol) in water (2.5 mL). The mixture was stirred at 0 °C for 90 min. The reaction was quenched with aq 10% citric acid, and aqueous layer was extracted with EtOAc three times, then the combined organic layers were washed with brine and dried over Na_2SO_4. The solvent was evaporated, and the residue was purified by silica gel column chromatography (0–4% MeOH in $CHCl_3$) to afford carboxylic acid (311 mg, 414 μmol). The obtained acid (152 mg, 202 μmol) and *i*-Pr_2NEt (37 μL, 212 μmol) were dissolved in MeCN (1.0 mL), and bromoacetonitrile (28 μL, 404 μmol) was added at room temperature. After stirring for 9 h, the solvent was evaporated. To the residue was added water and aqueous layer was extracted with EtOAc three times, then the combined organic layers were washed with brine and dried over Na_2SO_4. The solvent was evaporated, and the residue was purified by silica gel column chromatography (hexane/EtOAc = 7:3) to afford cyanomethyl ester **1** (135 mg, 41%, two steps) as a colorless solid: mp 116–118 °C; $[\alpha]_D^{25}$ −18.9 (*c* 1.0, benzene); ^{13}C NMR (100 MHz, C_6D_6) δ 169.4 (C), 169.2 (C), 155.6 (C), 114.4 (C), 100.8 (CH), 80.0 (C), 78.6 (CH), 73.9 (CH), 71.0 (CH), 68.7 (CH_2), 63.6 (CH_2), 55.4 (CH), 54.6 (CH), 48.9 (CH_2), 28.6 (CH_3), 26.4 (CH_3), 23.7 (CH_3), 18.9 (C), 7.5 (CH_3), 7.5 (CH_3), 5.7 (CH_2), 5.7 (CH_2),−4.5 (CH_3),−4.8 (CH_3); ^1H NMR (400 MHz, $CDCl_3$) δ 6.54 (d, 1H, *J* = 9.3 Hz), 5.38 (d, 1H, *J* = 8.3 Hz), 4.78 (s, 2H), 4.63 (d, 1H, *J* = 3.2 Hz), 4.48 (m, 1H), 4.19 (dd, 1H, *J* = 4.6, 9.8 Hz), 3.94 (m, 2H), 3.85–3.79 (m, 2H), 3.74–3.65 (m, 3H), 1.96 (s, 3H), 1.46 (s, 9H), 1.01–0.94 (m, 18H), 0.91 (s, 9H), 0.69–0.59 (m, 12H), 0.08 (d, 6H, *J* = 4.6 Hz). Anal. calcd for $C_{36}H_{71}N_3O_{10}Si_3$: C, 54.72; H, 9.06; N, 5.32. Found: C, 54.77; H, 9.18; N, 5.23.

***N*-(Benzyloxycarbonyl)-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-*S*-serine methyl ester (9).** To a suspension of L-Z-Ser-OMe **8** (7.76 g, 30.64 mmol), AgOTf (10.34 g, 40.24 mmol) and activated molecular sieves 4A (12.0 g) in CH_2Cl_2 (100 mL) was added a solution of bromide **7** (16.47 g, 40.05 mmol) in CH_2Cl_2 (30 mL) at −20 °C under Ar. The mixture was stirred at −10 °C overnight. The reaction mixture was quenched with triethylamine (8 mL). The mixture was filtered through Celite, and the filter cake was washed with CH_2Cl_2 (30 mL × 2). After removal of the solvent, the residue was directly subjected to silica gel column chromatography ($CHCl_3$/EtOAc 4:1–1:1) to afford **9** (9.99 g, 56%) as an amorphous: $[\alpha]_D^{25}$ 48.2 (*c* 1.1, $CHCl_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2 (C), 169.6 (C), 169.5 (C), 169.4 (C), 169.3 (C), 155.5 (C), 135.8 (C), 128.3 (CH), 128.0 (CH), 98.1 (CH), 69.2 (CH_2), 69.1 (CH), 69.0 (CH), 68.6 (CH), 67.1 (CH_2), 65.8 (CH), 62.2 (CH_2), 54.2 (CH), 52.8 (CH_3), 20.8 (CH_3), 20.7 (CH_3), 20.6 (CH_3); ^1H NMR (400 MHz, $CDCl_3$) δ 7.40–7.28 (m, 5H), 5.77 (d, 1H, *J* = 7.8 Hz), 5.26–5.22 (m, 2H), 5.18 (m, 1H), 5.14 (s, 2H), 4.79 (d, 1H, *J* = 1.5 Hz), 4.57 (m, 1H), 4.23 (dd, 1H, *J* = 5.6, 12.2 Hz), 4.09 (dd, 1H, *J* = 2.2, 12.2 Hz), 4.04–3.92 (m, 3H), 3.81 (s, 3H), 2.15 (s, 3H), 2.08 (s, 3H), 2.03 (s, 3H), 1.99 (s, 3H). Anal. calcd for $C_{26}H_{33}NO_{14}$: C, 53.51; H, 5.70; N, 2.4. Found: C, 53.65; H, 5.80; N, 2.23.

***N*-(*tert*-Butoxycarbonyl)-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-*S*-serine methyl ester.** To a solution of **9** (6.55 g, 11.22 mmol) and di-*tert*-butyl dicarbonate (3.19 g, 14.62 mmol) in MeOH (150 mL) was added 10% Pd/C (800 mg). The mixture was stirred vigorously under H_2 atmosphere for 1 h. The mixture was diluted with EtOAc (200 mL), and the catalyst was filtered off through Celite and the catalyst was washed with EtOAc. After the solvent was evaporated, the residue was purified by silica gel column chromatography (hexane/EtOAc = 6:4) to afford Boc derivative (5.65 g, 92%) as an amorphous: $[\alpha]_D^{25}$ 48.3 (*c* 1.0, $CHCl_3$); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3 (C), 170.1 (C), 169.6 (C), 169.5 (C), 169.3 (C), 155.0 (C), 98.2 (CH), 80.2 (C), 69.3 (CH), 69.2 (CH_2), 68.9 (CH), 68.8 (CH), 65.9 (CH), 62.2 (CH_2), 53.8 (CH), 52.8 (CH_3), 28.4 (CH_3), 20.9 (CH_3), 20.8 (CH_3), 20.8 (CH_3), 20.7 (CH_3); ^1H NMR (400 MHz, $CDCl_3$) δ 5.47 (d, 1H, *J* = 7.8 Hz), 5.31–5.25 (m, 2H), 5.18 (dd, 1H, *J* = 1.7, 2.9 Hz), 4.80 (d, 1H, *J* = 1.5 Hz), 4.50 (m, 1H), 4.28 (dd, 1H, *J* = 5.2, 12.3 Hz), 4.12 (dd, 1H, *J* = 2.3, 12.3 Hz), 4.00–3.92 (m, 3H), 3.80 (s, 3H), 2.15 (s, 3H), 2.12 (s, 3H), 2.05 (s, 3H), 1.99 (s, 3H), 1.47 (s, 9H). Anal. calcd for $C_{23}H_{35}NO_{14}$: C, 50.27; H, 6.42; N, 2.55. Found: C, 50.33; H, 6.47; N, 2.48.

***N*-(*tert*-Butoxycarbonyl)-3-*O*-(2,3,4,6-tetrakis-*O*-triethylsilyl-α-D-mannopyranosyl)-*S*-serine methyl ester.** To a solution of acetate (1.15 g, 2.09 mmol) in dry MeOH (30 mL) was added MeONa (0.1% wt MeOH solution, 2 mL) at room temperature in the presence of phenolphthalein as an indicator. The mixture was stirred for 2 h and then neutralized with Amberlyst 15E. The resins were filtered and the solvent was evaporated. The residue was dissolved in pyridine (30 mL) and chlorotriethylsilane (2.5 mL, 14.63 mmol) was added at 0 °C and the mixture was stirred for 8 h at room temperature. To the solution was added MeOH (3 mL) and stirred for 30 min to destroy the excess of silyl chloride, and the solvent was evaporated. The residue was dissolved into EtOAc and satd NH_4Cl. The aqueous layer was extracted with EtOAc, and the combined organic layers were washed with brine. The organic layer was dried over Na_2SO_4. The solvent was evaporated and the residue was purified by silica gel column chromatography (hexane/EtOAc = 9:1) to afford silyl ether (1.48 g, 84%, 2 steps) as a colorless oil: $[\alpha]_D^{25}$ 33.9 (*c* 1.0, benzene); ^{13}C NMR (100 MHz, C_6D_6, 70 °C) δ 170.7 (C), 155.3 (C), 101.6 (CH), 79.6 (C), 77.3 (CH), 75.5 (CH), 73.7 (CH), 70.1 (CH), 68.4 (CH_2), 63.1 (CH_2), 55.0 (CH), 51.8 (CH_3), 28.7 (CH_3), 7.6 (CH_3), 7.5 (CH_3), 7.3 (CH_3), 7.3(CH_3), 6.2 (CH_2), 6.1 (CH_2), 5.9 (CH_2), 5.5 (CH_2); ^1H NMR (400 MHz, C_6D_6, 70 °C) δ 5.44 (brs, 1H), 4.71 (d, 1H, *J* = 3.2 Hz), 4.50 (brs, 1H), 4.21 (t, 1H, *J* = 7.2 Hz), 4.03–3.91 (m, 5H), 3.81 (m, 1H), 3.61 (brs, 1H), 3.38 (s, 3H), 1.44 (s, 9H), 1.13–1.02 (m, 36H), 0.83–0.68 (m, 24H). Anal. calcd for $C_{39}H_{83}NO_{10}Si_4$: C, 55.87; H, 9.98; N, 1.67. Found: C, 55.79; H, 10.11; N, 1.76.

***N*-(*tert*-Butoxycarbonyl)-3-*O*-(2,3,4,6-tetrakis-*O*-triethylsilyl-α-D-mannopyranosyl)-*S*-serine.** To a solution of methyl ester (2.77 g, 3.31 mmol) in THF (50 mL) was added a solution of LiOH (95 mg, 3.97 mmol) in H_2O

(10 mL) at room temperature. The mixture was stirred for 3 h, and diluted with CH_2Cl_2. The reaction was quenched with aq 10% citric acid, and the aq layer was extracted with CH_2Cl_2. Then the combined organic layers were washed with brine and dried over Na_2SO_4. The solvent was evaporated, and the residue was purified by silica gel column chromatography (hexane/ EtOAc 7:1–1:1) to afford the acid (2.29 g, 84%). ^{13}C NMR (DMSO-d_6, 100 °C) δ 170.5 (C), 156.9 (C), 99.6 (CH), 77.8 (C), 75.6 (CH), 73.7 (CH), 72.0 (CH), 66.7 (CH_2), 61.5 (CH_2), 60.6 (CH), 53.8 (CH), 27.6 (CH_3), 6.1 (CH_3), 6.0 (CH_3), 6.0 (CH_3), 5.9 (CH_3), 5.9 (CH_3), 5.4 (CH_2), 4.5 (CH_2), 4.4 (CH_2), 4.3 (CH_2), 3.9 (CH_2), ^1H NMR (DMSO-d_6, 100 °C) δ 5.68 (1H, bs), 4.09 (1H, d, $J=2.8$ Hz), 3.65 (1H, m), 3.46 (1H, t, $J=7.6$ Hz), 3.3–3.15 (7H, m), 2.93 (1H, m), 0.91 (9H, s), 0.50–0.41 (36H, m), 0.18–0.10 (20H, m),−0.01 (4H, q, $J=8.0$ Hz); $[\alpha]_D^{24}$ +28.3 (*c* 0.58, $CHCl_3$); HRMS (FAB), *m*/*z* 824.5007 ($C_{38}H_{82}O_{10}NSi_4$ requires 824.5016).

***N*-*tert*-Butoxycarbonyl-3-*O*-(2,3,4,6-tetrakis-*O*-triethylsilyl-α-D-mannopyranosyl)serine cyanomethyl ester (2).** The above prepared acid (1.99 g, 2.41 mmol) and *i*-Pr_2NEt (0.84 mL, 4.82 mmol) were dissolved in 6:1 MeCN/DMF (35 mL), and bromoacetonitrile (0.58 mL, 4.82 mmol) was added at 4 °C. After stirring the mixture for 1 day at room temperature, solvent was evaporated. To the residue was added H_2O, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine and dried over Na_2SO_4. The solvent was evaporated, and the residue was purified by silica gel column chromatography (hexane/ EtOAc = 9:1–4:1) to afford cyanomethyl ester **2** (1.75 g, 84%) as a colorless oil: $[\alpha]_D^{25}$ 29.7 (*c* 1.1, benzene); ^{13}C NMR (100 MHz, C_6D_6, 70 °C) δ 169.2 (C), 155.3 (C), 113.9 (C), 101.8 (CH), 80.1 (C), 77.7 (CH), 75.6 (CH), 73.3 (CH), 70.3 (CH), 68.2 (CH_2), 63.2 (CH_2), 54.9 (CH), 48.6 (CH_2), 28.6 (CH_3), 7.6 (CH_3), 7.4 (CH_3), 7.3 (CH_3), 7.3 (CH_3), 6.2 (CH_2), 6.0 (CH_2), 5.9 (CH_2), 5.5 (CH_2); ^1H NMR (400 MHz, DMSO-d_6, 80 °C) δ 6.83 (brs, 1H), 4.95 (s, 2H), 4.77 (d, 1H, $J=3.2$ Hz), 4.59 (d, 1H, $J=3.2$ Hz), 4.32 (m, 1H), 3.95 (t, 1H, $J=7.3$ Hz), 3.86 (dd, 1H, $J=4.8$, 10.6 Hz), 3.79–3.69 (m, 5H), 3.41 (m, 1H), 1.39 (s, 9H), 0.99–0.91 (m, 36H), 0.67–0.55 (m, 24H). Anal. calcd for $C_{40}H_{82}N_2O_{10}Si_4$: C, 55.64; H, 9.57; N, 3.24. Found: C, 55.36; H, 9.69; N, 3.21.

***N*-(*tert*-Butoxycarbonyl)-[2,3;4,6-bis-*O*-(1-methylethylidene)-α-D-mannopyranosyl]-(1-phenylsulfonyl)-tryptophan cyanomethyl ester.** To a solution of tetraol **10** (121.0 mg, 0.195 mmol) and 2,2-dimethoxypropane (0.25mL) in DMF (0.7 mL), PPTS (19mg) was added at room temperature. The mixture was stirred at room temperature for 2 days. Triethylamine (0.3 mL) was added to quench PPTS, and stirred for 15 min. After the mixture was diluted with $CHCl_3$, purified by silica gel column chromatography directly ($CHCl_3$/MeOH 7:3). The material recrystallized from EtOAc–hexane to afford the diacetonide (100.1 mg, 74%): $[\alpha]_D^{24}$−11 (*c* 0.47, $CHCl_3$); mp 142–143 °C; ^{13}CNMR (67.8 MHz) δ 172.83 (C), 155.40 (C), 133.11 (CH), 128.75 (CH), 122.90 (CH), 125.71 (CH), 123.89 (CH), 120 16 (C), 119.42 (C), 115.50 (CH), 110.63 (C), 99.36 (C), 79.77 (C), 76.50 (CH), 71.96 (CH), 70.60 (CH_2), 64.82 (CH), 62.83 (CH_2), 53 22 (CH), 52.28 (CH_3), 29.05 (CH_3), 28.51 (CH_3), 27.27 (CH_2), 26.54 (CH_3), 24.35 (CH_3), 19.24 (CH_3), Anal. calcd for $C_{35}H_{44}N_2O_{11}S$ C, 59.99; H, 6.33; N, 4.00. Found C, 59.92; H, 6.33; N, 3.92.

***N*-(*tert*-Butoxycarbonyl)-[2,3;4,6-bis-*O*-(1-methylethylidene)-α-D-mannopyranosyl]-tryptophan cyanomethyl ester (3).** To the suspension of the above methyl ester (107 mg, 0.153 mmol) in EtOH (10 mL), 10% NaOH aq (1.0 mL) was added at room temperature. The mixture was stirred at room temperature for 3 h until the methyl ester was consumed on TLC. After starting material was consumed, the mixture became homogenous. Then the mixture was refluxed overnight. After cooling the mixture to room temperature, the solvent was evaporated, and the residue was diluted with $CHCl_3$. The mixture was washed with aq 10% citric acid. The aq layer was extracted with $CHCl_3$. The combined layers were washed with brine. After removal of solvent in vacuo, the residue was pumped. The residue was dissolved in CH_3CN (1 mL), then *i*-Pr_2NEt (53 μL, 0.306 mmol) and bromoacetonitrile (21 μL, 0.306 mmol) were added at room temperature. The mixture was stirred at room temperature over night. The mixture was diluted with $CHCl_3$ and brine was added. The aq layer was extracted with $CHCl_3$, then the combined layers were dried over Na_2SO_4. After evaporation, the residue was purified by silica gel column chromatography (hexane/EtOAc 3:2). The material was washed with hexane to give the cyanomethyl ester **3** (71.2 mg, 80%) : $[\alpha]_D^{24}$ +103 (*c* 1.25, PhH), ^{13}C NMR δ (C_6D_6 C_6D_6 as 128 ppm) 172.06 (C), 155.84 (C), 136.23 (C), 133.38 (C), 123.01 (CH), 120.56 (CH), 119.32 (CH), 111.58 (CH), 110.96 (C), 108.18 (C), 99.62 (C), 79.55 (C), 76.86 (CH), 76.41 (CH), 72.13 (CH), 69.18 (CH), 66.14 (CH), 63.63 (CH_2), 54.45 (CH), 48.48 (CH_2), 29.54 (CH_3), 28.55 (CH_3), 27.22 (CH_3), 27.03 (CH_2), 24.54 (CH_3), 19.25 (CH_3),^1H NMR δ 8.29 (s, 1H), 7.63 (d, 1H, $J=8.0$ Hz), 7.37 (d, 2H, $J=8.0$ Hz), 7.3–7.1 (m, 2H), 6.30 (d, 1H, $J=4.8$ Hz), 4.92–4.86 (m, 2H), 4.74–4.69 (m, 2H), 4.50–4.45 (m, 2H), 4.36–4.34 (m, 1H), 4.02 (dd, 1H, $J=10.8$ Hz, 6.2 Hz), 3.84–3.71 (m, 2H), 3.38 (d, 1H, $J=14.0$ Hz, 3.6 Hz), 3.00 (t, 1H, $J=11.6$ Hz), 1.71 (s, 3H), 1.71 (s, 3H), 1.60 (s, 3H), 1.49 (s, 3H), 1.40 (s, 3H), 1.34 (s, 9H). Anal. calcd for $C_{30}H_{39}N_3O_9$ C, 61.53; H, 6.71; N, 7.18. Found C, 61.30; H, 6.80, N, 7.00.

3-*O*-[2-Acetamido-2-deoxy-3,4-bis-*O*-triethylsilyl-6-(*tert*-butyl-dimethylsilyl)-β-D-glucopyranosyl]-*N*-*tert*-butoxycarbonyl-*S*-serine AMP ester (12a). AMP·tetra-*n*-butylammonium salt **11** (97.6 mg, 138 μmol) was dissolved in DMF (1.5 mL) and cooled to 0 °C under Ar atmosphere. To the solution was added pre-cooled (0 °C) solution of cyanomethyl ester **1** (432 mg, 547 μmol) in DMF (0.8 mL). The mixture was stirred for 2 h at 0 °C, and then 5% (v/v) acetic acid in water (221 μL) was added to neutralize the mixture. The mixture was washed with hexane (8 mL × 8) to remove the excess of cyanomethyl ester. The DMF solution was diluted with 30% MeCN in water (12 mL), and the suspension was subjected to C_{18} reverse phase column chromatography (Waters Sep-Pak Vac C_{18} 5 g/12 cc, MeCN/H_2O = 3:7→1:1→8:2). Fractions containing target molecule were

diluted with three volumes of MeCN. To the solution was added 5% (v/v) acetic acid in water (158 μL) and the solution was evaporated at 30 °C for avoiding β-elimination and saponification. The residue was pumped to give **12a** (95.5 mg, 44%). The ratio of 2′-acyl and 3′-acyl AMP was determined by NMR as 2′-acyl/3′-acyl = 1:10. The peaks corresponding to 3′-acyl AMP were picked up for NMR data: ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.8 (C), 169.2 (C), 155.8 (C), 155.1 (C), 152.3 (CH), 150.0 (C), 139.1(CH), 118.3 (C), 100.7 (CH), 85.0 (CH), 82.8 (CH), 78.5 (C), 76.3 (CH), 75.9 (CH), 75.7 (CH), 72.5 (CH), 71.4 (CH), 67.8 (CH_2), 63.6 (CH_2), 61.7 (CH_2), 57.5 (CH), 55.4 (CH), 54.1 (CH), 28.1 (CH_3), 25.7 (CH_3), 23.2 (CH_3), 23.1 (CH_2), 19.2 (CH_2), 18.1 (C), 13.5 (CH_3), 6.9 (CH_3), 6.9 (CH_3), 4.9 (CH_2), 4.7 (CH_2),−4.8 (CH_3),−5.6 (CH_3); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (s, 1H), 8.11 (s, 1H), 8.01 (d, 1H, J = 9.3 Hz), 7.22 (br s, 2H), 6.85 (d, 1H, J = 8.5 Hz), 5.98 (d, 1H, J = 7.8 Hz), 5.37 (d, 1H, J = 4.4 Hz), 5.11 (m, 1H), 4.49 (d, 1H, J = 7.8 Hz), 4.32 (m, 1H), 4.06 (br s, 1H), 3.93 (m, 1H), 3.85 (m, 1H), 3.76 (m, 2H), 3.68 (m, 1H), 3.60 (m, 1H), 3.50 (t, 1H, J = 8.5 Hz), 3.40 (m, 1H), 3.15 (m, 9H), 1.83 (s, 3H), 1.55 (m, 8H), 1.40 (s, 9H), 1.29 (m, 8H), 0.95–0.80 (m, 30H), 0.83 (s, 9H), 0.66 (q, 6H, J = 8.0 Hz), 0.57 (q, 6H, J = 7.8 Hz), 0.05 (s, 3H), 0.04 (s, 3H); HRMS (FAB), m/z 1080.4940 $(M+H)^+$, ($C_{44}H_{83}N_7O_{16}PSi_3$ requires 1080.4942).

3-*O*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-*S*-serine AMP ester (12b). To a test tube containing **12a** (34.7 mg, 22.0 μmol) was added pre-cooled (0 °C) TFA (3.0 mL), and the solution was stirred for 1 h at 0 °C. TFA was removed by blowing N_2 gas. The pellet remained was washed with ether (4 mL × 3) and dried in vacuo. The precipitate was dissolved in 3% MeCN in water (2.0 mL), and the solution was subjected to C_{18} reverse phase column chromatography (Waters Sep-Pak Vac C_{18} 10 g/35 cc, MeCN/H_2O = 3:97 → 1:9). To avoid saponification, column was performed at 4 °C. Fractions containing target molecule were diluted with 10 volumes of MeCN and evaporated at 30 °C, and the residue was pumped to give **12b** (5.9 mg, 42%). The ratio of 2′-acyl and 3′-acyl AMP was determined by NMR as 2′-acyl/3′-acyl = 1:5. The peaks corresponding to 3′-acyl AMP were picked up for NMR data: ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.8 (C), 166.7 (C), 155.7 (C), 152.5 (CH), 149.4 (C), 138.9 (CH), 118.5 (C), 100.8 (CH), 86.2 (CH), 80.9 (CH), 76.9 (CH), 75.3 (CH), 74.0 (CH), 72.2 (CH), 70.4 (CH), 66.7 (CH_2), 64.1 (CH_2), 60.9 (CH_2), 55.1 (CH), 53.1 (CH), 23.1 (CH_3); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.46 (s, 1H), 8.15 (s, 1H), 8.02 (d, 1H, J = 8.5 Hz), 7.32 (br s, 2H), 6.00 (d, 1H, J = 6.6 Hz), 5.47 (m, 1H), 4.93 (m, 1H), 4.47 (d, 1H, J = 8.3 Hz), 4.34 (br s, 1H), 4.25 (br s, 1H), 4.13 (m, 1H), 4.05 (br d, 1H, J = 9.0 Hz), 3.93 (m, 2H), 3.70 (d, 2H, J = 10.3 Hz), 3.45 (m, 2H), 3.32 (dd, 1H, J = 8.8, 9.8 Hz), 3.20 (m, 1H), 3.09 (dd, 1H, J = 8.8, 9.0 Hz), 1.85 (s, 3H); HRMS (FAB), m/z 638.1839 $(M+H)^+$ ($C_{21}H_{33}N_7O_{14}P$ requires 638.1823).

***N*-*tert*-Butoxycarbonyl-3-*O*-(2,3,4,6-tetrakis-*O*-triethylsilyl-α-D-mannopyranosyl)-*S*-serine AMP ester (13a).** AMP·tetra-*n*-butylammonium salt **11** (111 mg, 156 μmol) was dissolved in DMF (1.6 mL) and cooled to 0 °C under Ar atmosphere. To the solution was added pre-cooled (0 °C) solution of cyanomethyl ester **2** (525 mg, 608 μmol) in DMF (1.0 mL). The mixture was stirred for 2 h at 0 °C, and then 5% (v/v) acetic acid in water (250 μL) was added to neutralize the mixture. The mixture was washed with hexane (8 mL×2) to remove the excess of cyanomethyl ester. The DMF solution was diluted with 90% MeCN in water (10 mL), and the solution was subjected to C_{18} reverse phase column chromatography (Waters Sep-Pak Vac C_{18} 5 g/12 cc, MeCN/H_2O = 9:17→1:1→THF/H_2O 1:1). Fractions containing target molecule were diluted with same volume of THF and four volumes of MeCN. To the solution was added 5% (v/v) acetic acid in water (25 μL) and the solution was evaporated at 30 °C for avoiding β-elimination and saponification. The residue was pumped to give **13a** (110 mg, 50%). The ratio of 2′-acyl and 3′-acyl AMP was determined by NMR as 2′-acyl:3′-acyl = 1:10. The peaks corresponding to 3′-acyl AMP were picked up for NMR data: ^{13}C NMR (100 MHz, DMSO-d_6) δ 169.1 (C), 155.6 (C), 154.9 (C), 152.1 (CH), 150.0 (C), 139.0 (CH), 118.2 (C), 100.5 (CH), 84.9 (CH), 83.1 (CH), 78.3 (C), 76.6 (CH), 74.4 (CH), 74.1 (CH), 73.0 (CH), 72.7 (CH), 67.2 (CH), 66.9 (CH_2), 63.5 (CH_2), 61.3 (CH_2), 57.5 (CH_2), 54.3 (CH), 28.1 (CH_3), 23.1 (CH_2), 19.2 (CH_2), 13.5 (CH_3), 6.9 (CH_3), 6.9 (CH_3), 6.6 (CH_3), 4.9 (CH_2), 4.6 (CH_2), 4.6 (CH_2), 4.2 (CH_2); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.63 (s, 1H), 8.11 (s, 1H), 7.21 (br s, 2H), 7.01 (d, 1H, J = 8.1 Hz), 6.00 (d, 1H, J = 8.1 Hz), 5.32 (d, 1H, J = 4.6 Hz), 5.07 (m, 1H), 4.58 (d, 1H, J = 2.2 Hz), 4.43 (m, 1H), 4.00 (m, 2H), 3.90 (m, 1H), 3.83 (m, 1H), 3.82–3.70 (m, 6H), 3.37 (m, 1H), 3.15 (m, 8H), 1.55 (m, 8H), 1.40 (s, 9H), 1.38–1.25 (m, 8H), 0.95–0.83 (m, 48H), 0.65–0.50 (m, 24H); HRMS (FAB), m/z 1153.5546 $(M+H)^+$, ($C_{48}H_{94}N_6O_{16}PSi_4$ requires 1153.5541).

3-*O*-α-D-Mannopyranosyl-*S*-serine AMP ester (13b). To a test tube containing **13a** (28.7 mg, 20.3 μmol) was added pre-cooled (0 °C) TFA (3.0 mL), and the solution was stirred for 1 h at 0 °C. TFA was removed by blowing N_2 gas. The pellet remained was washed with ether (4 mL × 3) and dried in vacuo. The precipitate was dissolved in 1.5% MeCN in water (2.0 mL), and the solution was subjected to C_{18} reverse phase column chromatography (Waters Sep-Pak Vac C_{18} 10 g/35 cc, MeCN/H_2O = 1.5:98.5 →1:9). To avoid saponification, column was performed at 4 °C, fractions containing target molecule were diluted 10 volumes of MeCN and evaporated at 30 °C, and the residue was pumped to give **13b** (12.1 mg, quant). The ratio of 2′-acyl and 3′-acyl AMP was determined by NMR as 2′-acyl/3′-acyl = 1:5. The peaks corresponding to 3′-acyl AMP were picked up for NMR data: ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.3 (C), 155.7 (C), 152.4 (CH), 149.3 (C), 139.0 (CH), 118.6 (C), 101.0 (CH), 86.5 (CH), 80.9 (CH), 74.8 (CH), 74.0 (CH), 71.8 (CH), 70.4 (CH), 69.5 (CH), 67.0 (CH), 65.8 (CH_2), 63.7 (CH_2), 61.1 (CH_2), 52.8 (CH); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.44 (s, 1H), 8.16 (s, 1H), 7.31 (br s, 2H), 5.98 (d, 1H, J = 6.3 Hz), 5.47 (m, 1H), 4.95 (t, 1H, J = 6.1 Hz), 4.69 (s, 1H), 4.36 (br s, 1H), 4.31 (d, 1H, J = 3.2 Hz), 4.00–3.90 (m, 4H), 3.69 (m, 2H), 3.57

(m, 1H), 3.46 (m, 1H), 3.39 (m, 2H) ; HRMS (FAB), *m*/*z* 597.1551 $(M+H)^+$, ($C_{19}H_{30}N_6O_{14}P$ requires 597.1558).

***N*-(*tert*-Butoxycarbonyl)-[2,3;4,6-bis-*O*-(1-methylethylidene)-α-D-mannopyranosyl]-tryptophan AMP ester (14a).** To a solution of AMP·tetra-*n*-butylammonium salt **11** (9.3 mg, 13.1 μmol) in DMF (190 μL) was added cyanomethyl ester 3 (37.2 mg, 63.5 μmol). The mixture was stirred at 40 °C for 14 h, at 60 °C for 8 h, cooled to room temperature and poured into ether (40 mL). The precipitate was collected by centrifuge, and washed with ether (10 mL) twice. The pellet was dried in vacuo and dissolved in 10% MeCN in H_2O (2.0 mL). The solution was subjected to C_{18} reverse phase column chromatography (Waters Sep-Pak Vac C_{18} 2 g/12 cc, MeCN/H_2O = 2/8→3/7). The fractions containing target molecule were diluted with five volumes of MeCN and evaporated at 30 °C for avoiding saponification. Then **14a** was obtained as an amorphous (7.9 mg, 53%). The ratio of 2′-acyl and 3′-acyl AMP was determined by NMR as 2′-acyl/3′-acyl = 1:5. The peaks corresponding to 3′-acyl AMP were picked up for NMR data: ^{13}C NMR (100 MHz, DMSO-d_6) δ 171.5 (C), 155.6 (C), 154.9 (C), 149.9 (C), 139.1 (CH), 135.6 (C), 133.4 (C), 126.7 (C), 121.4 (CH), 118.5 (CH), 118.2 (CH), 118.2 (C), 111.3 (CH), 109.2 (C), 108.3 (C), 98.6 (C), 85.1 (CH), 82.9 (CH), 78.5 (C), 75.8 (CH), 75.5 (CH), 75.1 (CH), 72.5 (CH), 71.2 (CH), 67.6 (CH), 64.8 (CH_2), 63.6 (CH_2), 62.2 (CH_2), 57.4 (CH_2), 54.7 (CH), 28.9 (CH_3), 28.0 (CH_3), 26.9 (CH_3), 24.6 (CH_3), 23.1 (CH_2), 19.2 (CH_2), 13.5 (CH_3); ^1H NMR (400 MHz, DMSO-d_6) δ 11.11 (s, 1H), 8.62 (s, 1H), 8.12 (s, 1H), 7.58 (d, 1H, *J* = 8.1 Hz), 7.36 (d, 1H, *J* = 8.1 Hz), 7.22 (br s, 2H), 7.10 (t, 1H, *J* = 7.6 Hz), 7.02 (t, 1H, *J* = 7.6 Hz), 6.81 (d, 1H, *J* = 6.8 Hz), 5.97 (d, 1H, *J* = 7.8 Hz), 5.30 (d, 1H, *J* = 4.9 Hz), 5.10–5.00 (m, 2H), 4.61 (dd, 1H, *J* = 7.6, 8.1 Hz), 4.45 (t, 1H, *J* = 7.1 Hz), 4.45 (m, 1H), 4.30 (dd, 1H, *J* = 7.6, 10.3 Hz), 3.84–3.54 (m, 4H), 3.54 (m, 1H), 3.27 (dd, 1H, *J* = 4.6, 14.2 Hz), 3.14 (m, 8H), 3.05 (dd, 1H, *J* = 11.2, 14.6 Hz), 1.59–1.47 (m, 14H), 1.32–1.20 (m, 14H), 1.30 (s, 9H), 0.92 (t, 9H, *J* = 7.3 Hz); HRMS (FAB), *m*/*z* 876.3182 $(M+H)^+$, ($C_{38}H_{51}N_7O_{15}P$ requires 876.3181).

(α-D-Mannopyranosyl)-tryptophan AMP ester (14b). To a test tube containing **14a** (7.8 mg, 6.97 μmol) was added pre-cooled (0 °C) 4 M HCl in H_2O/dioxane = 1:1 (500 μL), and the solution was stirred for 13 h at 0 °C. The mixture was diluted with pre-cooled (0 °C) water (5.0 mL), and the solution was subjected to C_{18} reverse phase column chromatography (Waters Sep-Pak Vac C_{18} 2 g/12 cc, MeCN/H_2O = 1:1). To avoid saponification, column was performed at 4 °C, fractions containing target molecule were diluted with 10 volumes of MeCN and evaporated at 30 °C. The residues was pumped to give **14b** (4.8 mg, 65%). The ratio of 2′-acyl and 3′-acyl AMP was determined by NMR as 2′-acyl/3′-acyl = 1:5. The peaks corresponding to 3′-acyl AMP were picked up for NMR data: ^{13}C NMR (125MHz, DMSO-d_6) δ 168.5, 156.0, 152.7, 149.7, 139.0, 135.7, 135.7, 127.2, 121.3, 118.7, 117.7, 111.6, 106.0, 86.1, 80.8, 79.7, 75.2, 72.7, 71.2, 69.1, 67.9, 65.6, 64.2, 59.7, 57.5, 53.2, 25.9, 23.1, 19.2, 13.5; ^1H NMR (400 MHz, DMSO-d_6) δ 11.09 (s, 1H), 8.37 (s, 1H), 8.15 (s, 1H), 7.54 (d, 1H, *J* = 7.8 Hz), 7.38 (d, 1H, *J* = 7.8 Hz), 7.30 (br s, 1H), 7.08 (t, 1H, *J* = 7.1 Hz), 7.04 (t, 1H, *J* = 7.1 Hz), 5.89 (d, 1H, *J* = 6.6 Hz), 5.39 (d, 1H, *J* = 4.2 Hz), 4.94 (d, 1H, *J* = 8.8 Hz), 4.79 (m, 1H), 4.28 (t, 1H, *J* = 7.3 Hz), 4.16 (m, 1H), 3.95 -3.80 (m, 4H), 3.75–3.68 (m, 2H), 3.65 (dd, 1H, *J* = 4.2, 11.5 Hz), 3.42 (dd, 1H, *J* = 6.8, 14,6 Hz), 3.34 (dd, 1H, *J* = 8.05, 14.6 Hz), 3.15 (m, 8H), 1.55 (m. 8H), 1.30 (m, 8H), 0.92 (t, 12H, *J* = 7.3 Hz); HRMS (FAB), *m*/*z* 696.2024 $(M+H)^+$, ($C_{27}H_{35}N_7O_{13}P$ requires 696.2030).

Acknowledgements

We thank Dr. H. Koshino and Ms. T. Chijimatsu for NMR measurement, and Dr. T. Chihara and his staff for elemental analysis. We thank Ms. A. Takahashi for technical assistance.

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