Pergamon

Bioorganic & Medicinal Chemistry, Vol. 4, No. 11, pp 1873–1880, 1996

PII: S0968-0896(96)00170-8
0968-0896/96 $15.00+0.00

Synthesis of Glycopeptides with Phytoalexin Elicitor Activity—III. Syntheses of Hexaglycosyl Hexapeptides and a Nonaglycosyl Hexapeptide[1,2]

Tadahiro Takeda,[a]* Takuya Kanemitsu[b] and Yukio Ogihara[b]

[a]*Kyoritsu College of Pharmacy, Minato-ku, Tokyo 105, Japan*
[b]*Faculty of Pharmaceutical Sciences, Nagoya City University, Tanabe-dori, Mizuho-ku, Nagoya 467, Japan*

Abstract—A block synthesis of the model compound for the phytoalexin elicitor-active glycoprotein is described. Combination of the *C*-terminus free compounds, *N*-(9-fluorenylmethoxycarbonyl)-*O*-(*tert*-butyl)-L-seryl-L-proline (**1**) or *N*-(9-fluorenylmethoxycarbonyl)-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-L-seryl-L-proline (**2**) with the *N*-terminus free compounds, 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-L-seryl-L-prolyl-L-seryl-L-proline methyl ester (**4**), *O*-(*tert*-butyl)-L-seryl-L-prolyl-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-L-seryl-L-proline methyl ester (**6**) or 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-L-seryl-L-prolyl-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-L-seryl-L-proline methyl ester (**8**), by use of *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) gave three hexaglycosyl hexapeptides and a nonaglycosyl hexapeptide derivatives (**9**, **11**, **14**, and **17**). These *N*-terminus free compounds were derived from triglycosyl tetrapeptides (**3**, and **5**) or a hexaglycosyl tetrapeptide (**7**) on selective deblock reaction by morpholine. The hexaglycosyl hexapeptides (**10**, **13**, and **16**) and the nonaglycosyl hexapeptide (**18**) have been prepared by the convergent block synthesis. Copyright © 1996 Elsevier Science Ltd

Introduction

It is generally known that some plants synthesize antimicrobial substances as a defense mechanism against invasive microorganisms.[3–11] These antimicrobial substances synthesized are called phytoalexins, and their inducer is termed an elicitor. Matsubara and Kuroda found elicitor activity on a glycoprotein in a culture filtrate of germinating *Mycosphaerella pinodes* conidia and determined its structure to be a glycoprotein.[12] This glycoprotein, whose molecular weight is about 130×10^4, with an optical rotation of $[\alpha]_D$ −122°, has a partial structure in which a reducing terminal mannosyl residue of a trisaccharide, β-D-Glc-(1→6)-α-D-Man-(1→6)-D-Man, is *O*-glycosidically attached to serine in the protein portion. The glycoprotein induces active defense reactions, such as the production of a major phytoalexin, pisatin in peaplants.[12] The glycoprotein enhanced the ATPase activities by more than 20% in cell wall fraction of all leguminous species tested.[13]

We recently reported the syntheses of several models of the glycopeptide, triglycosyl-serine, triglycosyl-seryl-proline,[1] which showed relatively high activities (accumulated pisatin μg/cm² hypocotyl; control 17.9, glycoprotein 241.1, triglycosyl-serine 115.6, triglycosyl-dipeptide 184.2),[14] triglycosyl tetrapeptides and a hexaglycosyl tetrapeptide.[2] In order to investigate the structural requirements for bioactive glycoprotein in detail, we have carried out synthetic studies and describe here a synthesis of more high molecule and also more branched molecule, hexaglycosyl hexapeptides and/or nonaglycosyl hexapeptide of a model of the glycopeptide.

This paper is dedicated to Professor Raymond U. Lemieux on the occasion of his 75th birthday.

Results and Discussion

Synthesis of *N*-terminus free units, triglycosyl tetrapeptides (**4**, and **6**) and hexaglycosyl tetrapeptide (**8**) was carried out by deblocking of compounds **3**, **5**, and **7**, which were protected by fluorenylmethoxycarbonyl (Fmoc) group.[15–17] Compounds **3**, **5**, and **7** were prepared according to the previous paper.[2] The Fmoc group could be selectively removed by treatment of morpholine.[18–21] Treating of compounds **3**, **5** and **7** with morpholine for short time provided *N*-terminus free derivatives **4**, **6** and **8** in 73, 79, and 91% yields, respectively (Fig. 1). The ^{1}H NMR spectra of these *N*-terminus free derivatives could not be detected aromatic proton of which Fmoc group was a source, and the signals for the methyl ester of the compounds **4**, **6**, and **8** appeared at 3.74, 3.70 and 3.72 ppm, respectively. Also the ^{13}C NMR data of related compounds were in accordance with the proposed structure (see Table 1). According to the combinations of the *N*-terminus free compounds **4**, **6**, and **8**, with the *C*-terminus free compounds **1**, and **2**, we could

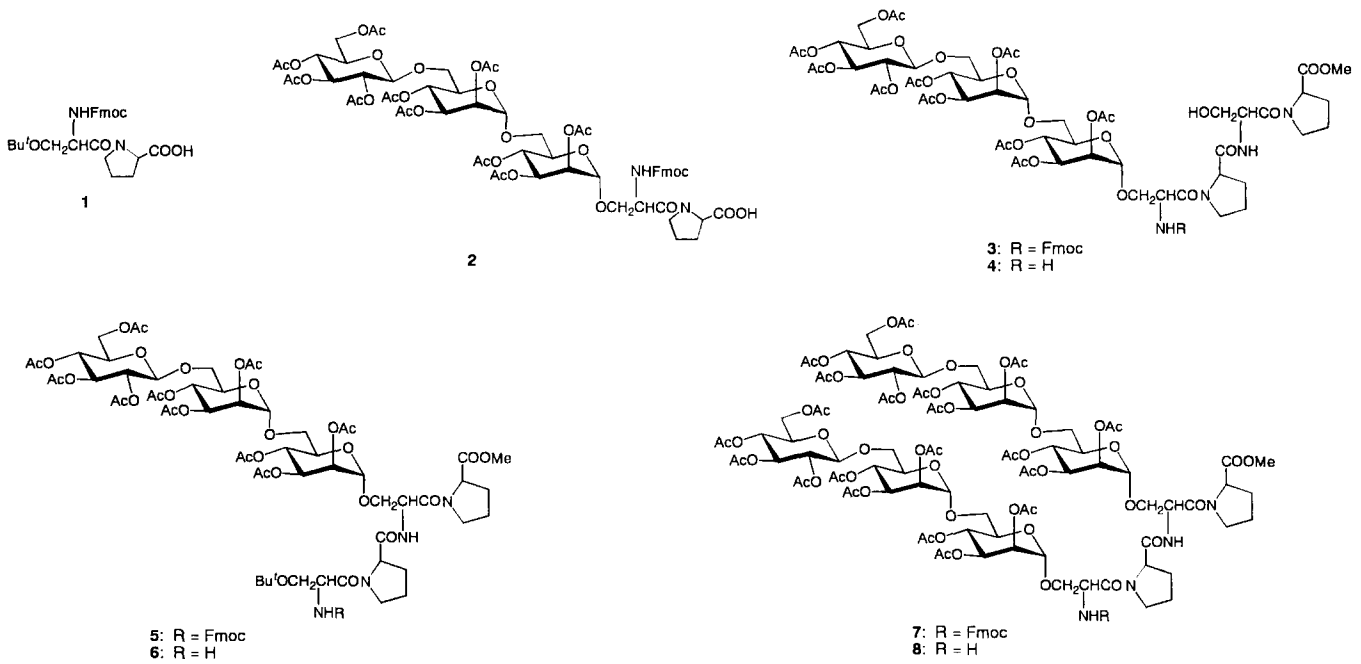


Figure 1

achieved the synthesis of the various glycopeptides which have triglycosyl branches.

Coupling the triglycosyl dipeptide **2** and triglycosyl tetrapeptide **4** in the presence of *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ)[22,23] in dichloromethane afforded **9** in 82% yield. The signal for the methyl ester of compound **9** appeared at 3.73 ppm along with the signals of aromatic proton (7.77–7.29 ppm) indicating the newly formed glycohexapeptide. EEDQ was an effective and mild condensing reagent and, during the coupling reactions, it did not affect the base-sensitive glycosyl–serine bond. The hexaglycosyl hexapeptide **9** was converted to the corresponding free hexaglycosyl hexapeptide **10** (Fig. 2). The heteronuclear multiple-bond correlation spectroscopy (HMBC) experiment showed a correlation between the H-1 (δ 4.86 × 2) of two reducing end mannoses and two β-carbons (δ 68.7 and 68.5) of serine. Cross peaks between the C-6 (δ 69.0 × 2) of two reducing end mannose residues and the H-1′ of two inner mannoses (δ 4.90 and 4.89) and between the 6-substituted mannoses C-6′ (δ 71.2 × 2) and the H-1″ (δ 4.50 × 2) of nonreducing end glucoses were observed. The assignments of these signals were achieved by analyses of detailed H—H correlation spectroscopy (COSY) and C—H COSY experiments. The ^{13}C NMR data were in accordance with the proposed structure (see Table 2). Compound 10 revealed an $[M+H]^+$ ion peak at *m/z* 1543 in FAB-MS).

Next, combination of the triglycosyl dipeptide **2** with triglycosyl tetrapeptide **6**, and also dipeptide **1** with hexaglycosyl tetrapeptide **8** in the presence of EEDQ in dichloromethane afforded **11** and **14** in 88 and 91% yield, respectively. The signal for the methyl ester of the compound **11** appeared at 3.71 ppm along with the signals of aromatic proton (7.76–7.29 ppm), indicating the newly formed glycohexapeptide, similarly the signals of the protecting group of compound **14** were also observed. Removal of the *tert*-butyl group of **11** and **14** with trifluoroacetic acid gave **12** and **15** (93,

Table 1. ^{13}C-NMR data (δ) for selected compounds

Carbon atom	Compound		
	4	**6**	**8**
C-1	98.2	97.8	98.4, 98.0
2	69.5[a]	69.2[a]	69.4[a], 69.3[a]
3	69.2[a]	69.1[a]	69.3[a], 69.3[a]
4	66.2	66.2	66.2[b], 66.2[b]
5	69.3[a]	69.3[b]	69.2[a], 69.1[a]
6	65.9	66.0	65.8, 65.7
1′	97.3	97.1	97.4, 97.3
2′	69.4[a]	69.2[a]	69.3[a], 69.3[a]
3′	69.1[a]	69.0[a]	69.2[a], 69.2[a]
4′	66.2	66.2	66.0[b], 66.0[b]
5′	69.3[a]	69.4[b]	69.2[a], 69.1[a]
6′	68.4	68.2	68.3, 68.2
1″	101.2	101.2	101.2, 101.1
2″	70.9	70.9	70.9, 70.9
3″	72.7	72.7	72.7, 72.6
4″	68.5	68.5	68.5, 68.5
5″	71.8	71.9	71.8, 71.8
6″	61.9	61.9	61.9, 61.8
Ser-α	52.8, 51.9	54.0, 53.2	51.9, 50.5
β	68.3, 63.1	65.7, 67.8	67.9, 67.4
Pro-α	60.8, 59.0	60.2, 59.2	59.2, 59.0
β	28.9, 28.8	29.1, 29.1	29.0, 28.5
γ	25.0, 24.9	24.9, 24.9	24.9, 24.9
δ	47.3, 47.1	47.3, 47.3	47.5, 47.1
Bu^t-CH_3		27.5	
C	73.6		
OMe	52.7	52.0	52.2

[a,b] These values in each column may be interchanged.

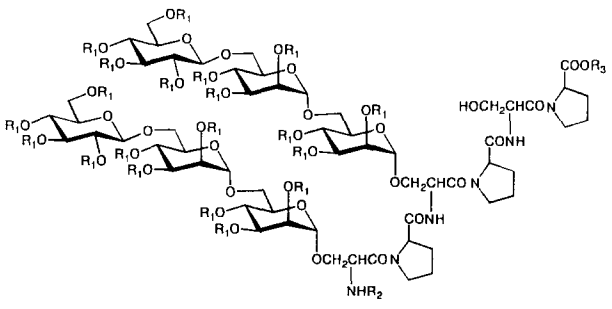
88%). And then, removal of the remaining protecting groups (Fmoc, acetyl and methyl ester groups) of the hexaglycosyl hexapeptide derivatives **12** and **15** with NaOMe in aq MeOH afforded the desired hexaglycosyl hexapeptides **13** and **16** in 85 and 77% yields (Fig. 2). The structures of compounds **13** and **16** were confirmed by ^{13}C NMR spectroscopy, in which two signals for C-1 in β-D-configuration showed at δ 105.4, four signals for C-1 in α-D-configuration showed at δ 102.3, 102.4, 102.9 and 103.6, in the case of compound **16**, the former signal at δ 105.5, the latter four signals at δ 102.4, 102.4, 102.7 and 103.5 (see Tables 2 and 3). The ^1H NMR spectra of these compounds (**13** and **16**) were in full agreement with the expected structures. The signals of anomeric proton of compound 13 were observed at δ 4.90 (br s, H-1′), 4.88 (br s, H-1′), 4.84 (br s, H-1), 4.82 (br s, H-1), 4.50 (d, $J=7.9$ Hz, H-1″) and 4.47 (d, $J=6.7$ Hz, H-1″). Compounds **13** and **16** revealed an $[M+H]^+$ ion peak at *m/z* 1543 in FAB-MS.

Finally, we synthesized the nonaglycosyl hexapeptide **18**. Coupling the *C*-terminus free triglycosyl dipeptide **2** and the *N*-terminus free hexaglycosyl tetrapeptide **8** in the presence of EEDQ in dichloromethane afforded **17** in 75% yield. Removal of the protecting groups (Fmoc, acetyl, and methyl ester groups) of **17** with NaOMe in MeOH afforded the desired nonaglycosyl hexapeptide **18** in 87% yield (Fig. 2). Deblocking of **17** with NaOMe in aq MeOH did not promote β-elimination of *O*-glycosidic linkage bearing L-seryl residue. The anomeric configuration of compound **18** was confirmed by ^1H NMR spectroscopy, the signals being observed at δ 4.92 (br s, H-1′), 4.91 (br s, H-1′), 4.89 (br s, H-1′), 4.86 (br s, H-1), 4.84 (br s, H-1), 4.83 (br s, H-1), 4.51 (d, $J=7.9$ Hz, 2×H-1″), 4.48 (d, $J=7.7$ Hz, H-1″). Compound **18** revealed an $[M+H]^+$ ion peak at *m/z* 2029 in FAB-MS. The structure and purity of **18** was demonstrated by ^1H and ^{13}C NMR (Table 3) spectroscopy and FAB-MS spectrometry. These biological results will be reported in detail elsewhere.

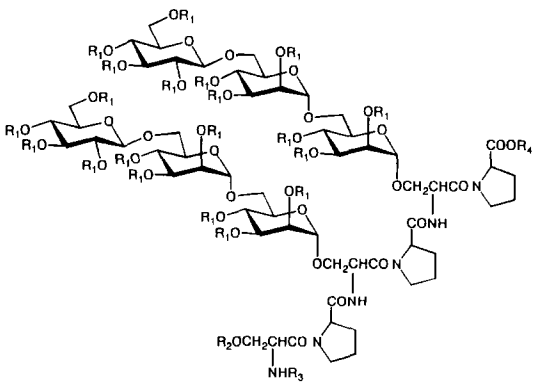
Experimental

General

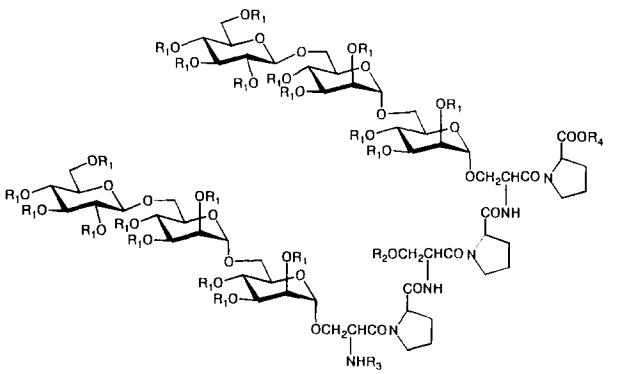
Melting points were measured with a Yanagimoto micro melting-point apparatus and are uncorrected. Optical rotations were determined with a Jasco DIP-140 digital polarimeter. ^1H NMR and ^{13}C NMR spectra were recorded with JNM A 500 FT NMR spectrometers, Me_4Si was the internal standard for solutions in $CDCl_3$, and sodium 4,4-dimethyl-4-silapentane-1-sulfonate for solutions in D_2O. FAB-MS was



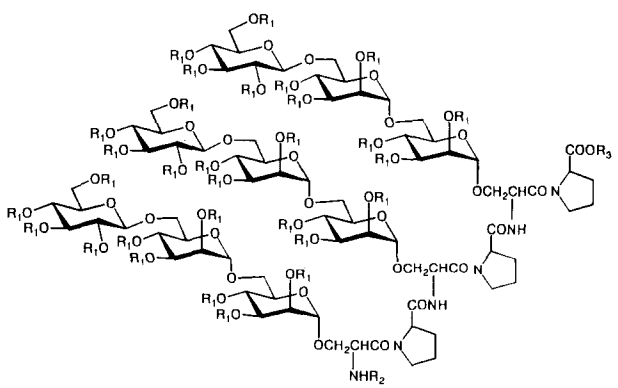
9: R_1 = Ac, R_2 = Fmoc, R_3 = Me
10: $R_1 = R_2 = R_3$ = H



14: R_1 = Ac, R_2 = Bu^t, R_3 = Fmoc, R_4 = Me
15: R_1 = Ac, R_2 = H, R_3 = Fmoc, R_4 = Me
16: $R_1 = R_2 = R_3 = R_4$ = H



11: R_1 = Ac, R_2 = Bu^t, R_3 = Fmoc, R_4 = Me
12: R_1 = Ac, R_2 = H, R_3 = Fmoc, R_4 = Me
13: $R_1 = R_2 = R_3 = R_4$ = H



17: R_1 = Ac, R_2 = Fmoc, R_3 = Me
18: $R_1 = R_2 = R_3$ = H

Figure 2

recorded on a JEOL JMS SX 102 mass spectrometer. TLC was performed on silica gel-60-F_{254} (E. Merck) with detection by quenching of UV fluorescence and by spraying with either 10% H_2SO_4 or 5% methanolic ninhydrin soln. Column chromatography was carried out on silica gel-60 (E. Merck). *N*-(9-Fluorenylmethoxycarbonyl)-*O*-(*tert*-butyl)-L-seryl-L-proline (**1**), *N*-(9-fluorenylmethoxycarbonyl)-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-manno-pyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyrano-syl)-L-seryl-L-proline (**2**), *N*-(9-fluorenylmethoxycarbonyl)-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-L-seryl-L-prolyl-L-seryl-L-proline methyl ester (**3**), *N*-(9-fluorenylmethoxycarbonyl)-*O*-(*tert*-butyl)-L-seryl-L-prolyl-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-L-seryl-L-proline methyl ester (**5**), *N*-(9-fluorenylmethoxycarbonyl)-(2, 3, 4, 6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-(2, 3, 4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-L-seryl-L-prolyl-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-(2, 3, 4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-L-seryl-L-proline methyl ester (**7**) were prepared by literature methods.[2]

2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-L-seryl-L-prolyl-L-seryl-L-proline methyl ester (4). A solution of compound **3** (116 mg, 0.076 mmol) in morpholine (2.0 mL) was stirred at room temperature for 20 min. The mixture was diluted with $CHCl_3$, and the $CHCl_3$ soln was washed with water, dried with Na_2SO_4, filtered, and evaporated. The residue was chromatographed on silica gel using 15:1 $CHCl_3$:MeOH as eluent to provide **4** (72 mg, 73%). Data for **4**: R_f ($CHCl_3$:MeOH, 15:1) = 0.23. $[\alpha]^{25}_D$ +10.6° (c = 1.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 4.91 (br s, 1 H, H-1′), 4.77 (d, 1 H, J = 1.3 Hz, H-1), 4.51 (d, 1 H, J = 7.9 Hz, H-1″), 3.74 (s, 3 H, OMe), 2.17, 2.16, 2.09, 2.061, 2.058, 2.05, 2.02, 2.00, 1.99, 1.96 (each s, 30 H, 10 × OAc). Anal.: calcd for $C_{55}H_{78}N_4O_{32}\cdot 2H_2O$ (1343.26): C, 48.98; H, 6.00; N, 4.17; found: C, 48.50; H, 5.83; N, 4.08%.

***O*-(*Tert*-butyl)-L-seryl-L-prolyl-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-(2, 3, 4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-L-seryl-L-proline methyl ester (6).** A solution of compound **5** (68 mg, 0.043 mmol) in morpholine (1.0 mL) was stirred at room temperature for 20 min. The mixture was diluted with $CHCl_3$, and the $CHCl_3$ soln was washed with water, dried with Na_2SO_4,

Table 2. ^{13}C-NMR data (δ) for selected compounds

Carbon atom	Compound				
	9	**10**	**11**	**12**	**13**
C-1	98.4, 98.0	103.3, 103.0	98.4, 98.0	98.1, 98.0	103.6, 102.9
2	69.6[a], 69.4[a]	73.9[a], 73.8[a]	69.2[a], 69.2[a]	69.3[a], 69.3[a]	73.9[a], 73.8[a]
3	69.3[a], 69.3[a]	72.7[a], 72.7[a]	69.1[a], 69.1[a]	69.1[a], 69.1[a]	72.8[a], 72.7[a]
4	66.5[b], 66.5[b]	69.4[b], 69.3[b]	66.3[b], 66.2[b]	66.4[b], 66.2[b]	69.5[b], 69.4[b]
5	69.2[a], 69.2[a]	73.3[a], 73.2[a]	69.4, 69.4	69.5, 69.5	73.3[c], 73.2[c]
6	66.0, 65.8	69.0, 69.0	66.0, 65.7	66.0, 65.8	68.9, 68.8
1′	97.4, 96.5	102.4, 102.3	97.3, 96.3	97.1, 96.7	102.4, 102.3
2′	69.5[a], 69.4[a]	74.4[a], 74.2[a]	69.2[a], 69.2[a]	69.3[a], 69.3[a]	74.5[a], 74.5[a]
3′	69.3[a], 69.3[a]	72.7[a], 72.7[a]	69.1[a], 69.1[a]	69.1[a], 69.1[a]	72.8[a], 72.6[a]
4′	66.4[b], 66.4[b]	69.4[b], 69.3[b]	66.3[b], 66.1[b]	66.0[b], 66.2[b]	69.4[b], 69.3[b]
5′	69.2[a], 69.2[a]	74.4[a], 74.2[a]	69.4, 69.4	69.5, 69.5	74.4[c], 74.2[c]
6′	68.5, 68.5	71.2, 71.2	68.4, 68.2	68.2, 68.2	71.2, 71.2
1″	101.3, 101.2	105.4, 105.4	101.3, 101.2	101.2, 101.2	105.4, 105.4
2″	70.9, 70.9	75.8, 75.8	70.9, 70.9	70.9, 70.9	75.9, 75.9
3″	72.7, 72.7	78.6, 78.6	72.7, 72.7	72.7, 72.7	78.6, 78.6
4″	68.5, 68.5	72.4, 72.4	68.5, 68.5	68.5, 68.5	72.5, 72.5
5″	71.8, 71.8	78.3, 78.3	71.9, 71.8	71.9, 71.9	78.3, 78.3
6″	61.9, 61.9	63.5, 63.3	61.9, 61.9	61.9, 61.9	63.5, 63.3
Ser-α	52.4, 52.4, 50.3	54.6, 54.4, 50.8	51.0, 50.1, 50.1	53.1, 52.5, 50.0	54.6, 54.4, 50.3
β	67.4, 67.4, 63.5	68.7, 68.5, 68.4	67.8, 67.1, 62.8	67.8, 67.3, 63.7	69.1, 68.5, 64.9
Pro-α	60.9, 60.4, 58.9	65.0, 65.0, 64.7	60.3, 60.2, 59.1	60.7, 60.5, 59.2	65.0, 64.9, 64.9
β	29.7, 29.4, 28.9	37.1, 32.0, 32.1	29.1, 28.9, 28.7	29.4, 29.1, 29.0	32.2, 32.1, 32.1
γ	25.2, 25.1, 24.9	27.4, 27.3, 27.3	25.0, 24.9, 24.7	25.1, 24.8, 24.8	27.3, 27.3, 27.2
δ	47.1, 47.1, 47.0	50.8, 50.6, 50.5	47.7, 47.2, 47.2	45.8, 47.5, 47.5	50.8, 50.6, 50.4
Bu^t-CH_3			27.3		
C			73.9		
OMe	52.4		52.1	52.1	
Fmoc-CH	47.1		47.1	47.2	
CH_2	67.2		67.4	67.5	

[a,b,c] These values in each column may be interchanged.

filtered, and evaporated. The residue was chromatographed on silica gel using 15:1 $CHCl_3$:MeOH as eluent to provide **6** (46 mg, 79%). Data for **6**: R_f ($CHCl_3$:MeOH, 15:1) = 0.37. $[\alpha]^{26}_D$ +14.8° (c = 1.0, $CHCl_3$). ^1H NMR data ($CDCl_3$): δ 4.86 (d, 1 H, J = 1.7 Hz, H-1′), 4.81 (br s, 1 H, H-1), 4.50 (d, 1 H, J = 7.9 Hz, H-1″), 3.70 (s, 3 H, OMe), 2.17, 2.16, 2.099, 2.097, 2.09, 2.07, 2.05, 2.02, 2.00, 1.97 (each s, 30 H, 10 × OAc), 1.20 (s, 9H, 3 × Bu^t-CH_3). Anal.: calcd for $C_{59}H_{86}N_4O_{32} \cdot H_2O$ (1381.35): C,51.30; H, 6.42; N, 4.06; found: C, 51.21; H, 6.40; N, 4.14%.

2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)- (1→6)-(2, 3, 4-tri-*O*-acetyl- α-D-mannopyranosyl) -L- seryl-L-prolyl-(2, 3, 4, 6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-L-seryl-L-proline methyl ester (8). A solution of compound **7** (137 mg, 0.056 mmol) in morpholine (3.0 mL) was stirred at room temperature for 20 min. The mixture was diluted with $CHCl_3$, and the $CHCl_3$ soln was washed with water, dried with Na_2SO_4, filtered, and evaporated. The residue was chromatographed on silica gel using 25:1 $CHCl_3$:MeOH as eluent to provide **8** (113 mg, 91%). Data for **8**: R_f ($CHCl_3$:MeOH, 15:1) = 0.50. $[\alpha]^{22}_D$ +29.2° (c 0.5, $CHCl_3$). ^1H NMR data ($CDCl_3$): δ 4.89 (d, 2 H, J = 1.8 Hz, 2 × H-1′), 4.86 (br s, 1 H, H-1), 4.85 (br s, 1 H, H-1), 4.50 (d, 1 H, J = 7.9 Hz, 2 × H-1″), 3.72 (s, 3 H, OMe), 2.18, 2.17, 2.162, 2.160, 2.158, 2.10, 2.094, 2.090, 2.07, 2.06, 2.05, 2.04, 2.03, 2.00, 1.99, 1.98, 1.974, 1.971, 1.96, 1.95 (each s, 60 H, 20 × OAc). Anal.: calcd for $C_{93}H_{128}N_4O_{57} \cdot 4H_2O$ (2286.09): C, 48.86; H, 6.00; N, 2.45; found: C, 48.94; H, 5.72; N, 2.64%.

***N*-(9-Fluorenylmethoxycarbonyl)-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-L-seryl-L-prolyl-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-L-seryl-L-prolyl-L-seryl-L-proline methyl ester (9).** To a solution of compound 2 (51 mg, 0.038 mmol) and compound **4** (50 mg, 0.038 mmol) in CH_2Cl_2 (4.0 mL) was added EEDQ (14 mg, 0.057 mmol) at 0°C. The mixture was stirred at room temperature for 5 h. The mixture was diluted with $CHCl_3$, and the $CHCl_3$ soln was washed with water, dried with Na_2SO_4, filtered, and evaporated. The residue was chromatographed on silica gel using 50:1 $CHCl_3$:MeOH as eluent to provide **9** (82 mg, 82%). Data for **9**: R_f ($CHCl_3$:MeOH, 10:1) = 0.65. $[\alpha]^{22}_D$ +20.8° (c = 1.0, $CHCl_3$). ^1H NMR data ($CDCl_3$): δ 7.77–7.29 (m, 8 H, arom), 4.92 (br s, 1 H, H-1′), 4.91 (br s, 1 H, H-1′), 4.89 (br s, 1 H, H-1),

Table 3. ^{13}C-NMR data (δ) for selected compounds

Carbon atom	Compound				
	14	**15**	**16**	**17**	**18**
C-1	98.4, 97.7	98.2, 97.5	103.5, 102.7	98.5, 98.4, 98.7	103.6, 103.4, 102.9
2	69.4[a], 69.4[a]	69.4[a], 69.4[a]	74.4[a], 74.4[a]	69.5[a], 69.4[a], 69.4[a]	74.5[a], 74.2a, 74.2[a]
3	69.3[a], 69.3[a]	69.3[a], 69.2[a]	72.7[a], 72.5[a]	69.4[a], 69.3[a], 69.3[a]	72.8[a], 72.7[a], 74.6[a]
4	66.3[b], 66.3[b]	66.3[b], 66.3[b]	69.3b, 69.1[b]	66.3[b], 66.3[b], 66.2[b]	69.5[b], 69.4[b], 69.4[b]
5	69.2[a], 69.1[a]	69.2[a], 69.1[a]	74.3[a], 73.2[a]	69.3[a], 69.3[a], 69.2[a]	73.3[a], 73.2[a], 73.2[a]
6	66.0, 65.8	65.9, 65.8	68.8, 68.7	66.1, 66.0, 66.0	68.9, 68.8, 68.8
1′	97.0, 96.5	96.8, 96.5	102.4, 102.4	97.2, 97.0, 96.8	102.4, 102.3, 102.2
2′	69.4[a], 69.3[a]	69.3[a], 69.3[a]	74.4[a], 74.3[a]	69.3[a], 69.2[a], 69.2[a]	74.5[a], 74.5[a], 74.2[a]
3′	69.2[a], 69.2[a]	69.2[a], 69.2[a]	72.7[a], 72.7[a]	69.2[a], 69.1[a], 69.1[a]	72.8[a], 72.6[a], 72.5[a]
4′	66.2[b], 66.2[b]	66.2[b], 66.2[b]	69.1[b], 69.1[b]	66.3[b], 66.2[b], 66.2[b]	69.4[b], 69.3[b], 69.3[b]
5′	69.1[a], 69.0[a]	69.1[a], 69.0[a]	73.2[a], 73.2[a]	69.1[a], 69.1[a], 69.0[a]	74.4[a], 74.0[a], 74.0[a]
6′	68.4, 68.4	68.3, 68.3	71.2, 71.2	68.4, 68.4, 68.4	71.2, 71.2, 71.1
1″	101.2, 101.2	101.2, 101.2	105.5, 105.5	101.2, 101.2, 101.2	105.4, 105.4, 105.3
2″	70.9, 70.9	70.9, 70.9	75.8, 75.8	70.9, 70.9, 70.9	75.9, 75.9, 75.9
3″	72.7, 72.7	72.7, 72.7	78.6, 78.6	72.7, 72.7, 72.7	78.6, 78.6, 78.6
4″	68.5, 68.5	68.5, 68.5	72.4, 72.4	68.5, 68.5, 68.5	72.5, 72.5, 72.5
5″	72.3, 72.3	72.3, 72.3	78.3, 78.3	72.3, 72.3, 72.2	78.3, 78.3, 78.3
6″	61.9, 61.9	61.9, 61.9	63.5, 63.4	61.9, 61.9, 61.9	63.5, 63.5, 63.4
Ser-α	52.7, 50.8, 49.3	52.8, 50.4, 49.8	54.7, 54.6, 50.6	52.8, 50.6, 49.6	54.6, 54.4, 51.3
β	67.9, 67.4, 65.3	67.8, 67.4, 65.2	69.1, 68.8, 66.6	67.9, 67.4, 65.3	69.1, 68.5, 65.1
Pro-α	60.8, 60.3, 59.3	60.3, 59.3, 59.1	64.9, 63.2, 62.9	60.6, 59.8, 59.2	65.0, 64.9, 64.9
β	29.5, 29.4, 29.1	30.9, 29.5, 29.1	32.2, 32.1, 32.1	30.6, 29.5, 29.1	32.2, 32.1, 32.1
γ	25.2, 25.0, 24.8	24.8, 24.8, 24.2	27.4, 27.4, 27.2	24.9, 24.9, 24.5	27.3, 27.3, 27.2
δ	47.6, 47.5, 47.4	47.6, 47.4, 46.7	50.8, 50.6, 50.4	45.6, 47.5, 47.1	50.8, 50.5, 50.4
Bu^t-CH_3	27.3				
C	74.0				
OMe	52.0	52.0		52.0	
Fmoc-CH	47.1	47.1		47.1	
CH_2	67.2	67.2		67.4	

[a,b]These values in each column may be interchanged.

4.79 (br s, 1 H, H-1), 4.51 (d, 1 H, J=8.5 Hz, H-1″), 4.48 (d, 1 H, J=7.9 Hz, H-1″), 3.73 (s, 3 H, OMe), 2.17, 2.15, 2.12, 2.11, 2.10, 2.083, 2.079, 2.075, 2.06, 2.05, 2.04, 2.023, 2.018, 2.001, 1.995, 1.99, 1.98, 1.97, 1.96, 1.94 (each s, 60 H, 20×OAc). FAB-MS: m/z 2621 $[M+H]^+$. Anal.: calcd for $C_{116}H_{150}N_6O_{62}\cdot 4H_2O$ (2692.52): C, 51.75; H, 5.91; N, 3.12; found: C, 51.26; H, 5.66; N, 3.35%.

β-D-Glucopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-L-seryl-L-prolyl-β-D-glucopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-L-seryl-L-prolyl-L-seryl-L-proline (10). To a solution of compound **9** (95 mg, 0.036 mmol) in 3:1 MeOH:H_2O (5.0 ml) was added NaOMe (90 mg) at room temperature for 12 h. The mixture was rapidly neutralized with Amberlite IR-120 (H^+), filtered, and concentrated. The residue was chromatographed over Sephadex LH-20 using 3:1 MeOH:H_2O as eluent to provide **10** (40 mg, 71%). Data for **10**: R_f ($CHCl_3$:MeOH:H_2O, 1:3:1) = 0.53. $[\alpha]^{24}_D$ +23.1° (c 0.1, H_2O). ^1H NMR data (D_2O): δ 4.90 (br s, 1 H, H-1′), 4.89 (br s, 1 H, H-1′), 4.86 (br s, 2 H, 2×H-1), 4.50 (d, 2 H, J=7.9 Hz, 2×H-1″). FAB-MS: m/z 1543 $[M+H]^+$. Anal.: calcd for $C_{60}H_{98}N_6O_{40}\cdot 8H_2O$ (1687.57): C, 42.70; H, 6.81; N, 4.98; found: C, 42.49; H, 6.66; N, 4.66%.

***N*-(9-Fluorenylmethoxycarbonyl)-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-L-seryl-L-prolyl-*O*-(*tert*-butyl)-L-seryl-L-prolyl-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-L-seryl-L-proline methyl ester (11).** To a solution of compound **2** (36 mg, 0.027 mmol) and compound **6** (37 mg, 0.027 mmol) in CH_2Cl_2 (2.0 mL) was added EEDQ (10 mg, 0.040 mmol) at 0°C. The mixture was stirred at room temperature for 4 h. The mixture was diluted with $CHCl_3$, and the $CHCl_3$ soln was washed with water, dried with Na_2SO_4, filtered, and evaporated. The residue was chromatographed on silica gel using 50:1 $CHCl_3$:MeOH as eluent to provide **11** (63 mg, 88%). Data for **11**: R_f ($CHCl_3$:MeOH, 15:1) = 0.65. $[\alpha]^{22}_D$ +15.4° (c 1.0, $CHCl_3$). ^1H NMR data ($CDCl_3$) : δ 7.76–7.29 (m, 8 H, arom), 4.86 (br s, 1 H, H-1′), 4.85 (br s, 1 H, H-1′), 4.81 (br s, 1 H, H-1), 4.80 (br s, 1 H, H-1), 4.51 (d, 1 H, J=7.9 Hz, H-1″), 4.48 (d, 1 H, J=7.9 Hz, H-1″), 3.71 (s, 3 H, OMe), 2.18, 2.17, 2.16, 2.15, 2.10, 2.093, 2.089, 2.086, 2.08, 2.062, 2.059, 2.05, 2.04, 2.023, 2.021, 2.000, 1.996, 1.99, 1.97, 1.94 (each s, 60 H, 20×OAc), 1.16 (s, 9H, 3×Bu^t-CH_3). Anal.: calcd for $C_{120}H_{158}N_6O_{62}\cdot 4H_2O$ (2748.63): C, 52.44; H, 6.09; N, 3.06: found: C, 52.35; H, 6.04; N, 3.34%.

***N*-(9-Fluorenylmethoxycarbonyl)-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-L-seryl-L-prolyl-L-seryl-L-prolyl-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-L-seryl-L-proline methyl ester (12).** A solution of compound **11** (56 mg, 0.021 mmol) in trifluoroacetic acid (1 mL) was stirred at room temperature for 3 h. The mixture was diluted with $CHCl_3$, and the $CHCl_3$ solution was washed with water, dried with Na_2SO_4, filtered, and evaporated. The residue was chromatographed on silica gel using 50:1 $CHCl_3$:MeOH as eluent to provide **12** (51 mg, 93%). Data for **12**: R_f ($CHCl_3$:MeOH, 15:1) = 0.46. $[\alpha]^{25}_D$ +16.6° (c 1.0, $CHCl_3$). ^1H NMR data ($CDCl_3$): δ 7.76–7.30 (m, 8 H, arom), 4.91 (br s, 1 H, H-1′), 4.87 (br s, 1 H, H-1′), 4.85 (br s, 1 H, H-1), 4.82 (br s, 1 H, H-1), 4.51 (d, 1 H, J=7.9 Hz, H-1″), 4.35 (d, 1 H, J=7.4 Hz, H-1″), 3.71 (s, 3 H, OMe), 2.21, 2.19, 2.16, 2.15, 2.14, 2.12, 2.094, 2.093, 2.090, 2.088, 2.06, 2.05, 2.04, 2.02, 2.00, 1.99, 1.989, 1.97, 1.96, 1.94 (each s, 60 H, 20×OAc). Anal.: calcd for $C_{116}H_{150}N_6O_{62}\cdot 5H_2O$ (2710.54): C, 51.40; H, 5.95; N, 3.10; found: C, 51.31; H, 5.99; N, 3.45%.

β-D-Glucopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-L-seryl-L-prolyl-L-seryl-L-prolyl-β-D-glucopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-L-seryl-L-proline (13). To a solution of compound **12** (52 mg, 0.020 mmol) in 3:1 MeOH:H_2O (3 mL) was added NaOMe (48 mg) at room temperature for 12 h. The mixture was rapidly neutralized with Amberlite IR-120 (H^+), filtered, and concentrated. The residue was chromatographed over Sephadex LH-20 using 3:1 MeOH:H_2O as eluent to provide **13** (26 mg, 85%). Data for **13**: R_f ($CHCl_3$:MeOH:H_2O, 1:3:1) 0.54. $[\alpha]^{23}_D$ −43.3° (c 0.4, H_2O). ^1H NMR data (D_2O): δ 4.90 (br s, 1 H, H-1′), 4.88 (br s, 1 H, H-1′), 4.84 (br s, 1 H, H-1), 4.82 (br s, 1 H, H-1), 4.50 (d, 1 H, J=7.9 Hz, H-1″), 4.47 (d, 1 H, J=6.7 Hz, H-1″). FAB-MS: m/z 1543 $[M+H]^+$. Anal.: calcd for $C_{60}H_{98}N_6O_{40}\cdot 11H_2O$ (1741.62): C, 41.38; H, 6.94; N, 4.83; found: C, 41.16; H, 6.64; N, 5.38%.

***N*-(9-Fluorenylmethoxycarbonyl)-*O*-(*tert*-butyl)-L-seryl-L-prolyl-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-L-seryl-L-prolyl-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-L-seryl-L-proline methyl ester (14).** To a solution of compound **1** (15 mg, 0.031 mmol) and compound **8** (66 mg, 0.030 mmol) in CH_2Cl_2 (3.0 mL) was added EEDQ (11 mg, 0.045 mmol) at 0°C. The mixture was stirred at room temperature for 4 h. The mixture was diluted with $CHCl_3$, and the $CHCl_3$ soln was washed with water, dried with Na_2SO_4, filtered, and evaporated. The residue was chromatographed on silica gel using 50:1 $CHCl_3$:MeOH as eluent to provide **14** (73 mg, 91%). Data for **14**: R_f ($CHCl_3$:MeOH, 15:1) = 0.57. $[\alpha]^{20}_D$ +21.9° (c 1.0, $CHCl_3$). ^1H NMR data ($CDCl_3$): δ 7.77–7.29 (m, 8 H, arom), 4.89 (br s, 1 H, H-1′), 4.85 (br s, 1 H, H-1′), 4.82 (br s, 1 H, H-1), 4.74 (br s, 1 H, H-1), 4.50 (d, 2 H, J=7.9 Hz, 2×H-1″), 3.72 (s, 3 H, OMe), 2.18, 2.170, 2.165, 2.16, 2.15, 2.109, 2.106, 2.10, 2.093, 2.090, 2.070, 2.065, 2.060, 2.057, 2.05, 2.024, 2.020, 2.00, 1.99, 1.96 (each s, 60 H, 20×OAc), 1.21 (s,

9H, 3 × Bu^t-CH3). Anal.: calcd for $C_{120}H_{158}N_6O_{62}\cdot 4H_2O$ (2748.63): C, 52.44; H, 6.09; N, 3.06; found: C, 52.28; H, 5.85; N, 3.23%.

***N*-(9-Fluorenylmethoxycarbonyl)-L-seryl-L-prolyl-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-L-seryl-L-prolyl-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-L-seryl-L-proline methyl ester (15).** A solution of compound **14** (27 mg, 0.010 mmol) in trifluoroacetic acid (1 mL) was stirred at room temperature for 4 h. The mixture was diluted with $CHCl_3$, and the $CHCl_3$ soln was washed with water, dried with Na_2SO_4, filtered, and evaporated. The residue was chromatographed on silica gel using 50:1 $CHCl_3$:MeOH as eluent to provide **15** (23 mg, 88%). Data for **15**: R_f ($CHCl_3$:MeOH, 15:1) = 0.50. $[\alpha]^{27}_D$ +21.6° (*c* 0.5, $CHCl_3$). ^{1}H NMR data ($CDCl_3$): δ 7.76–7.27 (m, 8 H, arom), 4.89 (br s, 1 H, H-1′), 4.85 (br s, 1 H, H-1′), 4.80 (br s, 1 H, H-1), 4.76 (br s, 1 H, H-1), 4.50 (d, 2 H, J = 7.9 Hz, 2 × H-1″), 3.72 (s, 3 H, OMe), 2.182, 2.178, 2.17, 2.16, 2.15, 2.104, 2.101, 2.095, 2.09, 2.08, 2.071, 2.066, 2.06, 2.054, 2.048, 2.02, 2.01, 2.00, 1.98, 1.90 (each s, 60 H, 20 × OAc). Anal.: calcd for $C_{116}H_{150}N_6O_{62}\cdot 4H_2O$ (2692.52): C, 51.75; H, 5.91; N, 3.12; found: C, 51.62; H, 5.62; N, 3.47%.

L-Seryl-L-prolyl-β-D-glucopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-L-seryl-L-prolyl-β-D-glucopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-L-seryl-L-proline (16). To a solution of compound **15** (84 mg, 0.032 mmol) in 3:1 MeOH:H_2O (4 mL) was added NaOMe (80 mg) at room temperature for 12 h. The mixture was rapidly neutralized with Amberlite IR-120 (H^+), filtered, and concentrated. The residue was chromatographed over Sephadex LH-20 using 3:1 MeOH:H_2O as eluent to provide **16** (38 mg, 77%). Data for **16**: R_f ($CHCl_3$:MeOH:H_2O, 1:3:1) = 0.33. $[\alpha]^{26}_D$ −19.0° (*c* 0.4, H_2O). ^{1}H NMR data (D_2O): δ 4.92 (br s, 1 H, H-1′), 4.89 (br s, 1 H, H-1′), 4.86 (br s, 1 H, H-1), 4.83 (br s, 1 H, H-1), 4.51 (d, 1 H, J = 7.9 Hz, H-1″), 4.47 (d, 1 H, J = 6.8 Hz, H-1″). FAB-MS: *m/z* 1543 $[M^+H]^+$. Anal.: calcd for $C_{60}H_{98}N_6O_{40}\cdot 11H_2O$ (1741.62): C, 41.38; H, 6.94; N, 4.83; found: C, 40.94; H, 6.63; N, 5.13%.

***N*-(9-Fluorenylmethoxycarbonyl)-(2, 3, 4, 6-tetra-*O*-acetyl-β-D-glucopyranosyl) - (1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-L-seryl-L-prolyl- (2, 3, 4, 6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)- (1→6) - (2, 3, 4 -tri-*O*-acetyl-α-D-mannopyranosyl)-L-seryl-L-prolyl-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→6)- (2, 3, 4-tri-*O*-acetyl-α -D- mannopyranosyl)-(1→6)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-L-seryl-L-proline methyl ester (17).** To a solution of compound **2** (18 mg, 0.014 mmol) and compound **8** (30 mg, 0.014 mmol) in CH_2Cl_2 (2.0 mL) was added EEDQ (5 mg, 0.020 mmol) at 0°C. The mixture was stirred at room temperature for 5 h. The mixture was diluted with $CHCl_3$, and the $CHCl_3$ soln was washed with water, dried with Na_2SO_4, filtered, and evaporated. The residue was chromatographed on silica gel using 50:1 $CHCl_3$:MeOH as eluent to provide **17** (36 mg, 75%). Data for **17**: R_f ($CHCl_3$:MeOH, 15:1) = 0.50. $[\alpha]^{25}_D$ +34.4° (*c* 1.0, $CHCl_3$). ^{1}H NMR data ($CDCl_3$): δ 7.76–7.29 (m, 8 H, arom), 4.90 (br s, 1 H, H-1′), 4.88 (br s, 2 H, 2 × H-1′), 4.86 (br s, 2 H, 2 × H-1), 4.81 (br s, 1 H, H-1), 4.50 (d, 2 H, J = 7.9 Hz, 2 × H-1″), 4.47 (d, 1 H, J = 7.9 Hz, H-1″), 3.72 (s, 3 H, OMe), 2.16, 2.15, 2.12, 2.104, 2.096, 2.09, 2.084, 2.078, 2.068, 2.066, 2.062, 2.059, 2.05, 2.04, 2.033, 2.029, 2.021, 2.017, 1.998, 1.995, 1.99, 1.982, 1.976, 1.970, 1.965, 1.96, 1.951, 1.945, 1.94, 1.93 (each s, 90 H, 30 × OAc). Anal.: calcd for $C_{154}H_{200}N_6O_{87}\cdot 4H_2O$ (3599.32): C, 51.39; H, 5.82; N, 2.33; found: C, 51.08; H, 5.50; N, 2.61%.

β-D-Glucopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-L-seryl-L-prolyl-β-D-glucopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-L-seryl-L-prolyl-β-D-glucopyranosyl-(1→6)-α-D-mannopyranosyl- (1→6) -α-D-mannopyranosyl-L- seryl-L-proline (18). To a solution of compound **17** (129 mg, 0.037 mmol) in 3:1 MeOH:H_2O (5 mL) was added NaOMe (111 mg) at room temperature for 12 h. The mixture was rapidly neutralized with Amberlite IR-120 (H^+), filtered, and concentrated. The residue was chromatographed over Sephadex LH-20 using 3:1 MeOH:H_2O as eluent to provide **18** (64 mg, 87%). Data for **18**: R_f ($CHCl_3$:MeOH:H_2O, 1:3:1) = 0.31. $[\alpha]^{25}_D$ −8.7° (*c* 0.4, H_2O). ^{1}H NMR data (D_2O): δ 4.92 (br s, 1 H, H-1′), 4.91 (br s, 1 H, H-1′), 4.89 (br s, 1 H, H-1′), 4.86 (br s, 1 H, H-1), 4.84 (br s, 1 H, H-1), 4.83 (br s, 1 H, H-1), 4.51 (d, 2 H, J = 7.9 Hz, 2 × H-1″), 4.48 (d, 1 H, J = 7.7 Hz, H-1″). FAB-MS: *m/z* 2029 $[M+H]^+$. Anal.: calcd for $C_{78}H_{128}N_6O_{55}\cdot 13H_2O$ (2264.07): C, 41.38; H, 6.86; N, 3.71; found: C, 41.09; H, 6.70; N,3.91%.

Acknowledgments

We thank Miss S. Kato for recording the NMR spectra, Miss T. Naito for performing the microanalyses, and Miss K. Takahashi for measurements of FAB-MS at Nagoya City University, Nagoya, Japan. This work was supported by a Grant-in-Aid for scientific Research on Priority Areas No.06240105 and also for scientific Research (No.07672286) from the Ministry of Education, Science and Culture of Japan.

References

1. Takeda, T.; Kanemitsu, T.; Ishiguro, M.; Ogihara, Y.; Matsubara, M. *Carbohydr. Res.* **1994**, *256*, 59.

2. Part II. Synthesis of glycopeptides with phytoalexin elicitor activity II. Takeda, T.; Kanemitsu, T.; Shimizu, N.; Ogihara, Y.; Matsubara, M. *Carbohydr. Res.* **1996**, *283*, 81.

3. Ingham, J. L. *Bot. Rev.* **1972**, *38*, 343.

4. Darvill, A. G.; Albersheim, P. *Ann. Rev. Plant Physiol.* **1984**, *35*, 243.

5. Sharp, J. K.; Valent, B.; Albersheim, P. *J. Biol. Chem.* **1984**, *259*, 11312.

6. Sharp, J. K.; McNeil, M.; Albersheim, P. *J. Biol. Chem.* **1984**, *259*, 11321.

7. Ossowski, P.; Pilotti, A.; Garegg, P.; Lindberg, B. *J. Biol. Chem.* **1984**, *259*, 11337.

8. Sharp, J. K.; Albersheim, P.; Ossowski, P.; Pilotti, A.; Garegg, P.; Lindberg, B. *J. Biol. Chem.* **1984**, *259*, 11341.

9. Shiraishi, T.; Oku, H.; Yamashita, M.; Ouchi, S. *Ann. Phytopath. Soc. Jpn.* **1978**, *44*, 659.

10. Yamamoto, Y.; Oku, H.; Shiraishi, T.; Ouchi, S.; Koshizawa, K. *J. Phytopathol.* **1986**, *117*, 136.

11. Cruickshank, I. A. M.; Smith, M. M.; *J. Phytopathol.* **1986**, *116*, 48.

12. Matsubara, M.; Kuroda, H. *Chem. Pharm. Bull.* **1987**, *35*, 249.

13. Kiba, A.; Toyoda, K.; Ichinose, Y.; Yamada, T.; Shiraishi, T. *Plant Cell Physiol.* **1995**, *36*, 809.

14. Takeda, T. The Abstracts Paper of the Chemistry of the Natural Supramolecule, **1995**, 180.

15. Carpino, L. A.; Han, G. Y. *J. Org. Chem.* **1972**, *37*, 3404.

16. Carpino, L. A. *Acc. Chem. Res.* **1987**, *20*, 401.

17. Paquet, A. *Can. J. Chem.* **1982**, *60*, 976.

18. Schultheiss-Reimann, P.; Kunz, H. *Angew. Chem.* **1983**, *95*, 64.

19. Schultheiss-Reimann, P.; Kunz, H. *Angew. Chem. Int. Ed. Engl.* **1983**, *22*, 62.

20. Paulsen, H.; Schults, M. *Carbohydr. Res.* **1987**, *159*, 37.

21. Kunz, H.; Birnbach, S.; Wenig, P. *Carbohydr. Res.* **1990**, *202*, 207.

22. Belleau, B.; Malek, G. *J. Am. Chem. Soc.* **1968**, *90*, 1651.

23. Yajima, H.; Kawatani, H. *Chem. Pharm. Bull.* **1971**, *19*, 1905.

(*Received in U.S.A.* 26 *January* 1996; *accepted* 17 *May* 1996)