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Carbohydrate Research 283 (1996) 81–93

CARBOHYDRATE RESEARCH

Syntheses of triglycosyl tetrapeptides and a hexaglycosyl tetrapeptide [1]

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Received 27 September 1995; accepted 19 November 1995

Abstract

A stereo-controlled synthesis of the model compound for the phytoalexin elicitor-active glycoprotein is described. Glycosylation of the trisaccharide, 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 trichloroacetimidate (**12**), with *N*-(9-fluorenylmethoxycarbonyl)-L-seryl-L-proline benzyl ester (**3**) or *N*-(carbobenzoxy)-L-seryl-L-proline methyl ester (**4**) by use of $BF_3 \cdot OEt_2$ gave the triglycosyl-seryl-proline derivatives. The *N*- as well as *C*-terminus of these triglycosyl dipeptides could be deblocked selectively to give compounds **14** and **16**, which are versatile intermediates for the completion of model compound synthesis of glycopeptide. Triglycosyl tetrapeptides (**18**, **21**) and hexaglycosyl tetrapeptide (**23**) have been prepared by the convergent block synthesis.

Keywords: Phytoalexin; Elicitor; Glycopeptide

1. Introduction

It is generally known that some plants synthesize antimicrobial substances as a defense mechanism against invasive microorganisms [2]. These antimicrobial substances synthesized are called phytoalexins, and their inducer is called 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 glycosyl chain [3].

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[1] Synthesis of glycopeptides with phytoalexin elicitor activity. Part II. For part I, see ref. [1].

PII S0008-6215(96)00004-3

The glycoprotein 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.

We recently reported [1] the synthesis of triglycosyl-serine, namely β-D-glucopyranosyl-(1 → 6)-α-D-mannopyranosyl-(1 → 6)-α-D-mannopyranosyl-(1 → 3)-L-serine, and triglycosyl L-seryl-L-proline dipeptide. In order to investigate the structural requirements for bioactive glycoprotein in detail, we have carried out synthetic studies and describe herein a synthesis of triglycosyl tetrapeptides and/or hexaglycosyl tetrapeptide of a model of the glycopeptide.

2. Results and discussion

Synthesis of the target triglycosyl tetrapeptides **18** and **21** was carried out by employing triglycosyl dipeptides **14** or **16** and seryl-proline dipeptide derivatives **5** or **2**. Compounds **14** or **16** were prepared from trisaccharide trichloroacetimidate **12** as a glycosyl donor and each seryl-proline dipeptide derivatives (**3**, **4**) as glycosyl acceptors. Coupling the mannosyl bromide and 2,2,2-trichloroethyl 2,3,4-tri-*O*-acetyl-α-D-mannopyranoside in the presence of $Hg(CN)_2$ and $HgBr_2$ in dichloromethane afforded **6** in 92.2% yield. Removal of the acetyl groups of **6** with NaOMe in MeOH gave **7** (88.7%), and the resultant compound **7** was tritylated and acetylated to give compound **8**. Detritylation gave the 6-OH-free trichloroethyl mannopyranosyl-(1 → 6)-α-D-mannopyranoside derivative **9**. Glycosylation of compound **9** with readily available tetra-*O*-acetyl-α-D-glucopyranosyl bromide in the presence of $Hg(CN)_2$ and $HgBr_2$ in dichloromethane at room temperature gave 45.3% of the trisaccharide **10**. The β-D-linked anomer showed a signal at δ 4.49 (*J* 7.9 Hz). Removal of the trichloroethyl group was achieved with zinc–copper reagent in acetate buffer, and the resultant alcohol **11** was transformed into the trichloroacetimidate **12** [4]. In a parallel route, trisaccharide **10** was obtained by condensation with the disaccharide trichloroacetimidate, 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1 → 6)-2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate, which was reported in a previous paper [1], and 2,2,2-trichloroethyl 2,3,4-tri-*O*-acetyl-α-D-mannopyranoside, in the presence of silver triflate (AgOTf) in 73.3% yield. The ^1H and ^{13}C NMR spectra of these trisaccharide derivatives were superimposable on each other, and the ^{13}C NMR data of related compounds were in accordance with the proposed structure (see Table 1). The dipeptide, with the *N*-terminal fluorenylmethoxycarbonyl (Fmoc) group being used in conjunction with the *C*-terminal benzyl ester as *N*-(9-fluorenylmethoxycarbonyl)-*O*-(*tert*-butyl)-L-seryl-L-proline benzyl ester (**1**), was synthesized by coupling of *N*-(9-fluorenylmethoxycarbonyl)-*O*-(*tert*-butyl)-L-serine with L-proline benzyl ester. Another dipeptide, with the *N*-terminal carbobenzoxy (Z) group being used in conjunction with the *C*-terminal methyl ester as *N*-(carbobenzoxy)-L-seryl-L-proline methyl ester (**4**), was prepared according to the previous report [1]. The reaction of trisaccharide trichloroacetimidate **12** with dipeptide **3**, promoted by $BF_3 \cdot OEt_2$ gave the trisaccharide derivative **13** (75.3%), from which debenzylation, afforded the triglycosyl dipeptide **14**. The anomeric configuration of compound **13** was confirmed by ^1H NMR spectroscopy, the signals for H-1, H-1′, and

Table 1
^{13}C NMR data (δ) for compounds **6–12**

Carbon atom	Compound						
	6	**7**	**8**	**9**	**10**	**11**	**12**
C-1	98.0	102.2	98.1	98.0	98.0	92.1	94.4
C-2	69.0	72.6	69.6	69.4	69.0	70.0	69.0
C-3	69.3	74.1	70.3	70.3	68.8	68.8	67.9
C-4	66.4	68.5	66.6	66.4	66.1	67.4	65.5
C-5	70.4	74.3	70.4	71.0	70.3	68.8	71.9
C-6	66.1	67.1	65.8	66.3	66.1	67.8	65.7
C-1′	97.5	101.3	97.0	97.9	97.5	97.6	97.4
C-2′	68.8	72.0	69.0	68.7	69.3	69.3	69.3
C-3′	68.9	72.5	69.2	69.0	69.0	69.0	69.0
C-4′	66.2	68.1	66.5	66.5	66.1	66.3	66.2
C-5′	68.7	71.5	68.8	68.7	69.3	69.3	69.2
C-6′	62.5	62.7	62.6	61.4	68.3	68.4	68.2
C-1″					101.1	100.9	101.1
C-2″					70.9	71.1	71.0
C-3″					72.6	72.6	72.6
C-4″					68.4	68.5	68.4
C-5″					71.9	71.9	71.8
C-6″					61.8	61.9	61.8
OCH_2	79.4	80.0	79.2	79.4	79.4		
OC(NH)							159.8
CCl_3	95.8	97.8	95.9	95.8	95.8		90.6
Ph_3C			86.6				

H-1″ being observed at δ 4.83 (br s), 4.93 (br s), and 4.47 (J 7.9 Hz), respectively. The carbon signals were identified by carbon-13–proton correlation spectroscopy (^{13}C–^1H COSY) and by analysis of a detailed heteronuclear multiple-bond correlation (HMBC) experiment [5]. The HMBC experiment showed a correlation between H-1 (4.83 ppm) of the reducing end mannose and β-carbon (67.2 ppm) of L-serine. A cross peak between C-6 (65.5 ppm) of the reducing end mannose residue and H-1′ of the inner mannose (4.93 ppm) was observed. We also observed correlations between 6-substituted mannose C-6′ (68.3 ppm) and the nonreducing end glucose H-1″ (4.47 ppm). The ^{13}C NMR data were in accordance with the proposed structure (see Table 2). Next, we synthesized the other triglycosyl dipeptide (**15**) [2]. The synthesis of **15** followed a procedure analogous to that described for the preparation of **13**. Thus the reaction of the trisaccharide trichloroacetimidate **12** with dipeptide **4** gave the triglycosyl dipeptide derivative **15**

[2] Compound **15** was already obtained by condensation with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1 → 6)-2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate and *N*-(carbobenzoxy)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1 → 3)-L-seryl-L-proline methyl ester in our previous paper [1]. Removal of the Z group on **15** with Pd/C gave compound **16**. Chemical and physical data of these compounds were in accordance with those reported in the literature [1].

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

Carbon atom	Compound								
	13	**14**	**17**	**18**	**19**	**20**	**21**	**22**	**23**
C-1	97.7	98.7	98.0	103.5	98.0	97.8	102.6	98.4	103.7
($J_{C\text{-}1,\,H\text{-}1}$ 171.7 Hz)								97.7	103.3
C-2	69.3 [a]	69.3 [a]	69.5 [a]	73.3	69.3	69.3 [a]	72.0	69.4 [a]	69.5
								69.4 [a]	69.4
C-3	68.9 [b]	69.2 [a]	69.3 [a]	72.8 [a]	69.0 [a]	69.1 [b]	72.5 [a]	69.3 [a]	72.8 [a]
								69.3 [a]	72.8 [a]
C-4	66.5	66.3	66.4	69.3 [b]	66.2	66.2 [c]	68.7 [b]	66.3 [b]	69.4 [b]
								66.3 [b]	69.3 [b]
C-5	69.4 [c]	69.2	69.4	73.3	69.3 [b]	69.2 [d]	73.4 [c]	69.2	73.3
								69.2	73.3
C-6	65.5	65.5	66.2	68.7	66.0	65.9	67.3	65.9	68.7
								65.7	68.6
C-1′	96.3	97.2	97.1	102.6	97.3	97.0	101.2	97.1	102.6
($J_{C\text{-}1',\,H\text{-}1'}$ 175.8 Hz)								96.6	102.5
C-2′	69.2 [a]	69.4 [a]	69.5 [a]	74.6	69.3	69.4 [a]	72.0	69.4 [a]	74.6
								69.4 [a]	74.6
C-3′	68.5 [b]	69.1 [a]	69.3 [a]	72.5 [a]	69.3 [a]	69.2 [b]	72.6 [a]	69.3 [a]	72.8 [a]
								69.3 [a]	72.7 [a]
C-4′	66.3	66.0	66.4	69.2 [b]	66.2	66.3 [c]	68.8 [b]	66.2 [b]	69.3 [b]
								66.1 [b]	69.1 [b]
C-5′	69.6 [c]	69.5	69.4	74.3	69.4 [b]	69.3 [d]	73.3 [c]	69.2 [a]	74.1
								69.2 [a]	74.0
C-6′	68.3	68.7	68.3	71.4	68.3	68.2	69.9	68.3	71.4
								68.2	71.4
C-1″	101.3	101.1	101.3	105.6	101.2	101.2	104.9	101.3	105.6
($J_{C\text{-}1'',\,H\text{-}1''}$ 163.4 Hz)								101.2	105.6
C-2″	70.9	70.9	71.0	76.0	70.9	70.9	75.2	70.9	76.0
								70.9	76.0
C-3″	72.7	72.6	72.8	78.5	72.7	72.7	77.9	72.8	78.5
								72.7	78.5
C-4″	68.5	68.4	68.6	72.6	68.5	68.5	71.7	68.5	72.6
								68.5	72.6
C-5″	71.8	71.9	71.9	78.8	71.8	71.9	78.1	71.8	78.8
								71.8	78.8
C-6″	62.0	61.9	62.0	63.6	61.9	61.9	62.8	61.9	63.6
								61.9	63.6
Ser α	51.1	51.0	51.6	54.9	53.1	53.2	55.4	49.9	54.9
			53.5	56.7	50.0	49.8	52.8	49.2	54.8
Ser β	67.2	67.3	67.5	67.3	63.1	64.1	66.1	67.7	68.8
			63.6	63.5	67.8	67.7	67.9	67.3	68.7
Pro α	59.2	59.0	58.9	65.2	60.3	60.5	66.7	59.1	65.2
			60.7	63.5	59.2	59.2	60.7	59.0	65.1
Pro β	29.1	28.9	28.9	32.2	28.8	29.0	30.1	29.1	32.3
			28.7	32.3	29.1	29.4	30.6	29.0	32.2
Pro γ	24.8	22.4	25.1	27.5	24.9	24.6	25.8	24.8	27.6
			24.9	27.7	25.0	24.8	25.9	24.7	27.4
Pro δ	47.5	45.3	47.1	50.7	47.8	47.6	50.8	47.6	50.8
			48.0	50.9	47.3	47.5	50.7	47.3	50.6

Table 2 (continued)

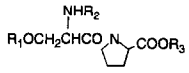
Carbon atom	Compound								
	13	**14**	**17**	**18**	**19**	**20**	**21**	**22**	**23**
Bu^t-CH_3					27.3				
Bu^t-C					74.0				
OMe			52.5		52.1	52.2		52.0	
Fmoc-CH	47.1	47.1	47.2		47.2	47.1		47.1	
Fmoc-CH_2	66.5	66.0	67.2		67.1	67.2		67.1	
Bn-CH_2	68.5								

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

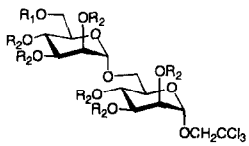
(71.4%). To obtain the two kinds of triglycosyl tetrapeptides, which extend to *C*-terminus peptide **17**, as well as to extend to *N*-terminus peptide derivative **19**, condensation between corresponding triglycosyl dipeptides (**14** and **16**) and the corresponding dipeptide derivatives (**5** and **2**) were each required. The benzyl ester and Z group were cleaved from **1** and **4** by hydrogenolysis (Pd/C) to give **2** and **5**. Coupling the triglycosyl dipeptide **14** and dipeptide **5**, and also compound **16** and compound **2** in the presence of *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) in dichloromethane, afforded **17** and **19** in 61.9% and 69.6% yield, respectively. The signal for the methyl ester of the compound **17** appeared at δ 3.74 along with the signals of aromatic proton (δ 7.76–7.27), indicating the newly formed tetrapeptide; similarly the signals of protecting group of compound **19** were also observed. EEDQ was an effective and mild condensing reagent, and during the coupling reactions it did not affect the base-sensitive *O*-glycosidic bonds. Triglycosyl tetrapeptides (**17** and **19**) were converted to the corresponding free triglycosyl tetrapeptides **18** and **21**, respectively. The ^{13}C NMR data were in accordance with the proposed structure (see Table 2). Compound **18** and **21** revealed an $[M + H]^+$ ion peak at m/z 873 in the fast-atom-bombardment mass spectrum (FABMS). Finally, we synthesized hexaglycosyl tetrapeptide **23**. Coupling the *N*-terminus free triglycosyl dipeptide **16** and *C*-terminus free triglycosyl dipeptide **14** in the presence of EEDQ in dichloromethane afforded **22** in 73.7% yield. Removal of the protecting groups (Fmoc, acetyl and methyl ester groups) of **22** with NaOMe in MeOH afforded the desired hexaglycosyl tetrapeptide **23** in 86.2% yield. Deblocking of **22** with NaOMe in MeOH did not promote β-elimination of *O*-glycosidic linkage bearing L-seryl residue. Compound **23** revealed an $[M + H]^+$ ion peak at m/z 1359 in FABMS.The structure and purity of **23** was demonstrated by ^1H and ^{13}C NMR spectroscopy and by FABMS spectrometry. These biological results will be reported in detail elsewhere.

3. Experimental

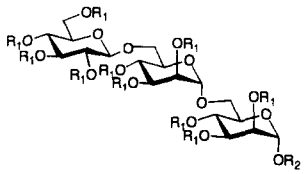
General methods.—Melting points were measured with a Yanagimoto micromelting-point apparatus and are uncorrected. Optical rotations were determined with a Jasco DIP-140 digital polarimeter. ^1H and ^{13}C NMR spectra were recorded



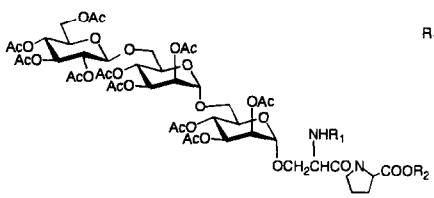
1: R_1 = Bu^t, R_2 = Fmoc, R_3 = Bn
2: R_1 = Bu^t, R_2 =Fmoc, R_3 = H
3: R_1 = H, R_2 = Fmoc, R_3 = Bn
4: R_1 = H, R_2 = Z, R_3 = Me
5: R_1 = R_2 = H, R_3 = Me



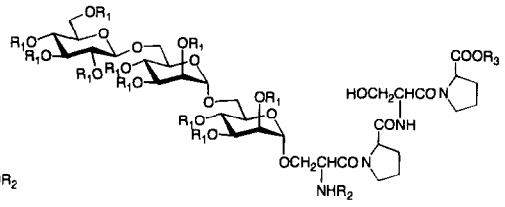
6: R_1 = R_2 = Ac
7: R_1 = R_2 = H
8: R_1 = Tr, R_2 = Ac
9: R_1 = H, R_2 = Ac



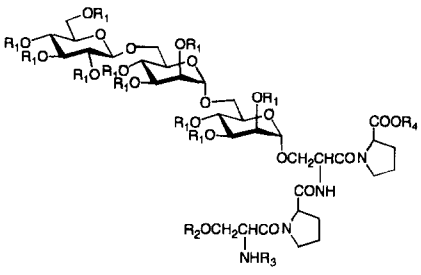
10: R_1 = Ac, R_2 = CH_2CCl_3
11: R_1 = Ac, R_2 = H
12: R_1 = Ac, R_2 = $C(NH)CCl_3$



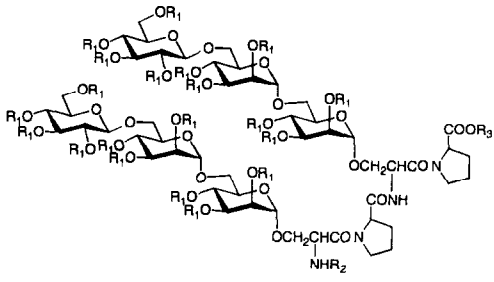
13: R_1 = Fmoc, R_2 = Bn
14: R_1 = Fmoc, R_2 = H
15: R_1 = Z, R_2 = Me
16: R_1 = H, R_2 = Me



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



19: R_1 = Ac, R_2 = Bu^t, R_3 = Fmoc, R_4 = Me
20: R_1 = Ac, R_2 = H, R_3 = Fmoc, R_4 = Me
21: R_1 = R_2 = R_3 = R_4 = H



22: R_1 = Ac, R_2 = Fmoc, R_3 = Me
23: R_1 = R_2 = R_3 = H

with Jeol JNM EX-270, GSX-400 and JNM A 500 FTNMR spectrometers. Me_4Si was the internal standard for solutions in $CDCl_3$ and/or CD_3OD, and sodium 4,4-dimethyl-4-silapentane-1-sulfonate for solutions in D_2O. FABMS was recorded on a Jeol JMS SX 102 mass spectrometer. TLC was performed on Silica Gel-60F_{254} (E. Merck) with detection by quenching of UV fluorescence and by spraying with either 10% H_2SO_4 or 5% methanolic ninhydrin solution. Column chromatography was carried out on Silica Gel-60 (E. Merck). *N*-(Carbobenzoxy)-L-seryl-L-proline methyl ester (**4**), 2,2,2-trichloroethyl 2,3,4-tri-*O*-acetyl-α-D-mannopyranoside, 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1 → 6)-2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate were prepared by literature methods [1].

N-*(9-Fluorenylmethoxycarbonyl)*-O-(tert-*butyl)*-L-*seryl*-L-*proline benzyl ester* (**1**).—To a solution of *N*-(9-fluorenylmethoxycarbonyl)-*O*-(*tert*-butyl)-L-serine (1 g, 2.6 mmol), L-proline benzyl ester hydrochloride (650 mg, 2.7 mmol) in CH_2Cl_2 (5 mL) were added Et_3N (0.5 mL, 3.5 mmol) and diethyl phosphorocyanidate (DEPC) (0.6 mL, 4.0 mmol) at 0 °C for 2 h, and the mixture was allowed to warm to room temperature for 16 h. The mixture was diluted with $CHCl_3$, and the $CHCl_3$ solution was washed with water and dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography using $CHCl_3$ as eluent, to give **1** (1.34 g, 90.0%); R_f 0.36 (10:1 benzene–acetone); mp 46–48 °C; $[\alpha]_D^{20}$ −32.3° (*c* 0.4, $CHCl_3$); ^1H NMR data ($CDCl_3$): δ 7.74–7.23 (m, 13 H, Ar), 5.72 (d, 1 H, *J* 7.9 Hz, Ser-NH), 5.16 (s, 2 H, Bn-CH_2), 4.69–4.64 (m, 2 H, Ser α, Pro α), 4.34 (d, 2 H, *J* 7.9 Hz, Ser β), 4.27–4.14 (m, 1 H, Fmoc-CH), 2.25–1.78 (m, 4 H, Pro β, γ), 1.17 (s, 9 H, 3 × Bu^t-CH_3); ^{13}C NMR data ($CDCl_3$): δ 52.9 (Ser α), 67.1 (Ser β), 59.1 (Pro α), 29.1 (Pro β), 24.8 (Pro γ), 47.2 (Pro δ), 27.2 (Bu^t-CH_3), 73.5 (Bu^t-C), 47.1 (Fmoc-CH), 62.8 (Fmoc-CH_2), 66.6 (Bn-CH_2). Anal. Calcd for $C_{34}H_{38}N_2O_6$: C, 70.56; H, 6.71; N, 4.91. Found: C, 70.22; H, 6.59; N, 4.78.

N-*(9-Fluorenylmethoxycarbonyl)*-O-(tert-*butyl)*-L-*seryl*-L-*proline* (**2**).—To a solution of compound **1** (1.35 g, 2.37 mmol) in 50% AcOH–MeOH (6 mL) was added 10% Pd–C (1 g). The mixture was stirred for 25 min under H_2 and then filtered through a pad of Celite and concentrated to dryness. The residue was chromatographed on silica gel using 20:1 $CHCl_3$–MeOH as eluent to give **2** (1.13 g, 99.7%); R_f 0.28 (10:1 $CHCl_3$–MeOH); mp 78–79 °C; $[\alpha]_D^{26}$ −67.6° (*c* 0.5, $CHCl_3$); ^1H NMR data ($CDCl_3$): δ 7.77–7.26 (m, 8 H, Ar), 5.72 (d, 1 H, *J* 8.2 Hz, Ser-NH), 4.71 (dd, 1 H, *J* 8.2, 14.0 Hz, Ser α), 4.63–4.61 (m, 1 H, Pro α), 1.15 (s, 9 H, 3 × Bu^t-CH_3); ^{13}C NMR data ($CDCl_3$): δ 52.5 (Ser α), 67.2 (Ser β), 59.8 (Pro α), 27.9 (Pro β), 24.7 (Pro γ), 47.9 (Pro δ), 27.2 (Bu^t-CH_3), 73.9 (Bu^t-C), 47.1 (Fmoc-CH), 62.9 (Fmoc-CH_2).

N-*(9-Fluorenylmethoxycarbonyl)*-L-*seryl*-L-*proline benzyl ester* (**3**).—A solution of compound **2** (1.30 g, 2.28 mmol) in trifluoroacetic acid (2 mL) was stirred for 12 h at room temperature. The mixture was concentrated in vacuo, and recrystallized from MeOH gave **3** (1.02 g, 87.2%); R_f 0.48 (2:1 benzene–acetone); mp 161–163 °C; $[\alpha]_D^{20}$ −21.8° (*c* 0.4, $CHCl_3$); ^1H NMR data ($CDCl_3$): δ 7.76–7.25 (m, 13 H, Ar), 5.86 (d, 1 H, *J* 8.5 Hz, Ser-NH), 5.23, 5.12 (each d, 2 H, *J* 12.2 Hz, Bn-CH_2), 4.69–4.64 (m, 2 H, Ser α, Pro α), 4.36 (d, 2 H, *J* 7.3 Hz, Ser β), 4.21–4.19 (m, 1 H, Fmoc-CH), 3.90–3.72 (m, 4 H, Pro δ, Fmoc-CH_2), 3.18 (s, 1 H, OH), 2.24 (d, 1 H, *J* 2.2 Hz, Pro β-Ha), 1.99–1.97 (m, 3 H, Pro β-Hb, Pro γ); ^{13}C NMR data ($CDCl_3$): δ 53.8 (Ser α),

67.2 (Ser β), 59.1 (Pro α), 28.9 (Pro β), 24.9 (Pro γ), 47.3 (Pro δ), 47.1 (Fmoc-CH), 64.0 (Fmoc-CH_2), 67.4 (Bn-CH_2). Anal. Calcd for $C_{30}H_{30}N_2O_6$: C, 70.02; H, 5.88; N, 5.47. Found: C, 69.72; H, 5.87; N, 5.21.

L-*Seryl*-L-*proline methyl ester* (**5**).—To a solution of *N*-(carbobenzoxy)-L-seryl-L-proline methyl ester (**4**) (500 mg, 1.43 mmol) in 5:3:2 MeOH–H_2O–AcOH (6 mL) was added 5% Pd–C (50 mg). The mixture was stirred for 40 min under H_2, then filtered through a pad of Celite and concentrated to dryness. The residue was chromatographed on Sephadex LH-20 with MeOH. The eluate was evaporated to dryness to give **5** as a syrup (260 mg, 84.3%); R_f 0.29 (4:1 $CHCl_3$–MeOH); $[\alpha]_D^{26}$ −91.2° (*c* 0.3, $CHCl_3$); ^{1}H NMR data ($CDCl_3$): δ 4.16–4.13 (m, 1 H, Ser α), 4.01–3.99 (m, 1 H, Pro α), 3.48 (s, 3 H, OMe); ^{13}C NMR data ($CDCl_3$): δ 56.4 (Ser α), 61.2 (Ser β), 59.1 (Pro α), 28.2 (Pro β), 22.6 (Pro γ), 45.4 (Pro δ), 50.7 (OMe).

2,2,2-Trichloroethyl (2,3,4,6-tetra-O-*acetyl*-α-D-*mannopyranosyl)-(1 → 6)-2,3,4-tri*-O-*acetyl*-α-D-*mannopyranoside* (**6**).—To a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide (21.78 g, 0.053 mol), 2,2,2-trichloroethyl 2,3,4-tri-*O*-acetyl-α-D-mannopyranoside (12.57 g, 0.029 mmol) in dry CH_2Cl_2 (120 mL) were added $Hg(CN)_2$ (7.5 g), $HgBr_2$ (3.75 g) and molecular sieves (12.57 g). The mixture was stirred under N_2 at room temperature for 5 h. The mixture was diluted with $CHCl_3$ and filtered. The filtrate was washed with water, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography using 100:1 $CHCl_3$–MeOH as eluent to give **6** (20.33 g, 92.2%); R_f 0.52 (4:1 benzene–acetone); mp 66–68 °C; $[\alpha]_D^{26}$ +52.3° (*c* 4.0, $CHCl_3$); ^{1}H NMR data ($CDCl_3$): δ 5.11 (br s, 1 H, H-1), 4.87 (d, 1 H, *J* 1.2 Hz, H-1′), 4.29, 4.19 (each d, 2 H, *J* 11.3 Hz, CCl_3CH_2), 2.18, 2.16, 2.11, 2.07, 2.06, 2.01, 1.98 (each s, 21 H, 7 × OAc). Anal. Calcd for $C_{45}H_{49}Cl_3O_{17}$: C, 55.82; H, 5.10. Found: C, 55.71; H, 5.15.

*2,2,2-Trichloroethyl (*α-D-*mannopyranosyl)-(1 → 6)-*α-D-*mannopyranoside* (**7**).—To a solution of compound **6** (19.58 g, 0.026 mol) in MeOH (70 mL) was added NaOMe (577 mg). The mixture was stirred at room temperature for 12 h, then neutralized with Amberlite IR-120 (H^+), filtered, and concentrated. The residue was purified by chromatography over Sephadex LH-20 with MeOH as eluent to give **7** (10.72 g, 88.7%); R_f 0.12 (3:1 $CHCl_3$–MeOH); $[\alpha]_D^{26}$ +73.1° (*c* 3.2, MeOH); ^{1}H NMR data (CD_3OD): δ 5.04 (br s, 1 H, H-1), 4.54 (br s, 1 H, H-1′), 4.30, 4.21 (each d, 2 H, *J* 11.0 Hz, CCl_3CH_2).

2,2,2-Trichloroethyl (2,3,4-tri-O-*acetyl-6*-O-*trityl*-α-D-*mannopyranosyl)-(1 → 6)-2,3,4-tri*-O-*acetyl*-α-D-*mannopyranoside* (**8**).—To a solution of compound **7** (2.28 g, 5.1 mmol) in pyridine (17 mL) was added chlorotriphenylmethane (3.37 g, 12.1 mmol), and the mixture was stirred for 30 h at 70 °C. After the starting material disappeared, Ac_2O (10 mL) was added at 0 °C, and the mixture was allowed to warm to room temperature. The mixture was extracted with $CHCl_3$, washed with water, dried with Na_2SO_4, filtered, and evaporated. The residue was chromatographed on silica gel with 10:1 benzene–acetone as eluent to provide **8** (3.53 g, quant); R_f 0.75 (4:1 benzene–acetone); mp 105–107 °C; $[\alpha]_D^{26}$ +57.3° (*c* 1.3, $CHCl_3$); ^{1}H NMR data ($CDCl_3$): δ 7.46–7.21 (m, 15 H, Ar), 5.15 (d, 1 H, *J* 1.8 Hz, H-1), 4.92 (d, 1 H, *J* 1.8 Hz, H-1′), 4.31, 4.19 (each d, 2 H, *J* 11.6 Hz, CCl_3CH_2), 2.15, 2.01, 2.00, 1.95, 1.87, 1.75 (each s, 18 H, 6 × OAc). Anal. Calcd for $C_{45}H_{49}Cl_3O_{17}$: C, 43.79; H, 4.86. Found: C, 43.32; H, 4.86.

*2,2,2-Trichloroethyl (2,3,4-tri-*O*-acetyl-α-*D*-mannopyranosyl)-(1 → 6)-2,3,4-tri-*O*-acetyl-α-*D*-mannopyranoside* (**9**).—A solution of **8** (7.4 g, 7.6 mmol) in 80% aq AcOH was stirred for 6 h at 55 °C. The resultant solution was poured into ice–water. The precipitate was filtered, and the filtrate was extracted with $CHCl_3$. The $CHCl_3$ solution washed with water, dried with Na_2SO_4, filtered, and evaporated to give **9** (6.58 g, quant); R_f 0.26 (4:1 benzene–acetone); ^{1}H NMR data ($CDCl_3$): δ 5.11 (d, 1 H, *J* 1.5 Hz, H-1), 4.87 (d, 1 H, *J* 1.8 Hz, H-1′), 4.29, 4.17 (each d, 2 H, *J* 11.5 Hz, CCl_3CH_2), 2.19, 2.14, 2.09, 2.07, 2.02, 1.99 (each s, 18 H, 6 × OAc).

*2,2,2-Trichloroethyl (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*-mannopyranoside* (**10**). —*Method 1.* To a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (acetobromoglucose) (21.61 g, 53 mmol) and compound **9** (6.58 g, 9.1 mmol) in dry CH_2Cl_2 (60 mL) was added $Hg(CN)_2$ (22.03 g), $HgBr_2$ (12.55 g), and molecular sieves (6 g). The mixture was stirred under N_2 at room temperature for 5 h. The mixture was diluted $CHCl_3$ and filtered. The filtrate was washed with water, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography using 200:1 $CHCl_3$–MeOH as eluent to provide **10** (4.35 g, 45.3%).

Method 2. To a solution of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1 → 6)-2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate (580 mg, 0.74 mmol) and 2,2,2-trichloroethyl 2,3,4-tri-*O*-acetyl-α-D-mannopyranoside (244 mg, 0.56 mmol) in dry CH_2Cl_2 (10 mL) was added AgOTf (223 mg, 0.87 mmol) in the dark. The mixture was stirred under argon for 2 days at room temperature, then diluted with $CHCl_3$ and filtered through a pad of Celite. The filtrate was washed with water, and then dried over Na_2SO_4, filtered, and concentrated. The residue was chromatographed on silica gel using 200:1 $CHCl_3$–MeOH as eluent to provide **10** (432 mg, 73.3%).; R_f 0.42 (4:1 benzene–acetone); mp 65–67 °C; $[\alpha]_D^{21}$ +18.6° (*c* 0.2, $CHCl_3$); ^{1}H NMR data ($CDCl_3$): δ 5.10 (d, 1 H, *J* 1.7 Hz, H-1), 4.84 (d, 1 H, *J* 1.7 Hz, H-1′), 4.49 (d, 1 H, *J* 7.9 Hz, H-1″), 4.28, 4.18 (each d, 2 H, *J* 11.5 Hz, CCl_3CH_2), 2.20, 2.16, 2.09, 2.08, 2.07, 2.06, 2.02, 2.01, 2.00, 1.97 (each s, 30 H, 10 × OAc).

*2,3,4,6-Tetra-*O*-acetyl-β-*D*-glucopyranosyl-(1 → 6)-2,3,4-tri-*O*-acetyl-α-*D*-mannopyranosyl-2,3,4-tri-*O*-acetyl-α-*D*-mannopyranose* (**11**).—A solution of compound **10** (79 mg, 0.075 mmol) in a mixture of AcOH (0.2 mL) and Ac_2O (0.1 mL) was added with stirring to a Zn–Cu reagent that was prepared by addition of Zn dust (540 mg) to acetate buffer [AcONa (900 mg)/AcOH (1.0 mL)–H_2O (1.4 mL)] containing $CuSO_4 \cdot 5H_2O$ (54 mg) solution (in 0.2 mL of H_2O). The solution was stirred at room temperature for 20 h. The mixture was diluted with acetone and filtered through a pad of Celite, and $CHCl_3$ and water were added to the filtrate. The organic layer was separated, washed with water, dried over Na_2SO_4, filtered, and then concentrated. The residue was chromatographed on silica gel using 200:1 $CHCl_3$–MeOH as eluent to provide **11** (63 mg, 92.53%); R_f 0.48 (2:1 benzene–acetone); mp 105–108 °C; $[\alpha]_D^{23}$ +12.3° (*c* 0.3, $CHCl_3$); ^{1}H NMR data ($CDCl_3$): δ 5.21 (br s, 1 H, H-1), 4.82 (d, 1 H, *J* 1.9 Hz, H-1′), 4.53 (d, 1 H, *J* 8.0 Hz, H-1″), 2.17, 2.15, 2.10, 2.09, 2.08, 2.07, 2.03, 2.01, 2.00, 1.98 (each s, 30 H, 10 × OAc). Anal. Calcd for $C_{38}H_{52}O_{26}$: C, 49.35; H, 5.67. Found: C, 48.90; H, 5.47.

*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-*mannopyranoyl trichloroacetimidate* (**12**).—To a solution of compound **11** (34 mg, 0.037 mmol) in dry CH_2Cl_2 (0.3 mL) was added CCl_3CN (30 μL, 0.30 mmol) and DBU (7 μL, 0.035 mmol) at 0 °C for 2 h, and the mixture was allowed to warm to room temperature for 12 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 100:1 $CHCl_3$–MeOH as eluent to provide **12** (31 mg, 78.9%); R_f 0.40 (4:1, benzene–acetone); mp 89–92 °C; $[\alpha]_D^{21}$ +10.5° (*c* 0.2, $CHCl_3$); ^{1}H NMR data ($CDCl_3$): δ 8.82 (s, 1 H, NH), 6.24 (d, 1 H, *J* 1.8 Hz, H-1), 4.83 (d, 1 H, *J* 1.9 Hz, H-1′), 4.48 (d, 1 H, *J* 8.0 Hz, H-1″), 2.23, 2.16, 2.09, 2.08, 2.07, 2.06, 2.02, 2.01, 2.00, 1.97 (each s, 30 H, 10 × OAc). Anal. Calcd for $C_{40}H_{52}ClNO_{26}$: C, 44.93; H, 4.90; N, 1.31. Found: C, 44.72; H, 4.73; N, 1.38.

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 benzyl ester* (**13**).—To solution of compound **12** (2 g, 1.87 mmol), compound **3** (480 mg, 0.93 mmol) and molecular sieves (AW 300) (2 g) in dry CH_2Cl_2 (4.0 mL) was stirred at room temperature for 3 h. To the stirred suspension was added $BF_3 \cdot EOEt_2$ (1.0 mL, 8.1 mmol) at −20 °C. The mixture was stirred under argon for 2 h, then diluted with $CHCl_3$ and filtered through a the pad of Celite. The filtrate was washed with water, then dried over Na_2SO_4, filtered, and concentrated. The residue was chromatographed on silica gel using 50:1 $CHCl_3$–MeOH as eluent to provide **13** (998 mg, 75.3%); R_f 0.39 (4:1 benzene–acetone); mp 115–117 °C; $[\alpha]_D^{21}$ +25.7° (*c* 0.1, $CHCl_3$); ^{1}H NMR data ($CDCl_3$): δ 7.76–7.27 (m, 13 H, Ar), 4.93 (br s, 1 H, H-1′), 4.83 (br s, 1 H, H-1), 4.47 (d, 1 H, *J* 7.9 Hz, H-1″), 2.17, 2.08, 2.07, 2.02, 2.01, 2.00, 1.99, 1.98, 1.97, 1.94 (each s, 30 H, 10 × OAc). Anal. Calcd for $C_{68}H_{80}N_2O_{31} \cdot 2H_2O$: C, 56.04; H, 5.80; N, 1.92. Found: C, 56.25; H, 5.51; N, 1.74.

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* (**14**).—To a solution of compound **13** (39 mg, 0.027 mmol) in 50% AcOH–MeOH (0.5 mL) was added 10% Pd–C (50 mg). The mixture was stirred for 20 min under H_2, and then filtered through a pad of Celite and concentrated to dryness. The residue was chromatographed on silica gel using 50:1 $CHCl_3$–MeOH as eluent to provide **14** (35 mg, 96.6%); R_f 0.29 (10:1 $CHCl_3$–MeOH); mp 121–124 °C; $[\alpha]_D^{21}$ +45.1° (*c* 0.2, $CHCl_3$); ^{1}H NMR data ($CDCl_3$): δ 7.76–7.27 (m, 8 H, Ar), 4.88 (br s, 1 H, H-1′), 4.82 (br s, 1 H, H-1), 4.51 (d, 1 H, *J* 8.5 Hz, H-1″), 2.18, 2.17, 2.11, 2.09, 2.07, 2.06, 2.03, 2.01, 1.99, 1.96 (each s, 30 H, 10 × OAc). Anal. Calcd for $C_{61}H_{74}N_2O_{31} \cdot 2H_2O$: C, 53.59; H, 5.75; N, 2.05. Found: C, 53.36; H, 5.54; N, 2.27.

N-*(Carbobenzoxy)-(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 **12** (1 g, 0.94 mmol), compound **4** (160 mg, 0.46 mmol) and molecular sieves (AW 300) (1 g) in dry CH_2Cl_2 (4.0 mL) was stirred at room temperature for 4 h. To the stirred suspension was added $BF_3 \cdot OEt_2$ (0.5 mL) at −20 °C. The mixture was stirred under argon for 2 h, then diluted with $CHCl_3$ and filtered through a pad of Celite. The filtrate was washed with water, then dried over

Na_2SO_4, filtered, and concentrated. The residue was chromatographed on silica gel using 5:1 benzene–acetone as eluent to provide **15** (410 mg, 71.4%).

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 (**17**).—To a solution of compound **14** (225 mg, 0.17 mmol), compound **5** (207 mg, 0.96 mmol) in CH_2Cl_2 (4.0 mL) was added *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) (125 mg, 0.50 mmol) at 0 °C. The mixture was stirred at room temperature for 2 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 **17** (160 mg, 61.9%); R_f 0.68 (15:1 $CHCl_3$–MeOH), 0.17 (2:1 benzene–acetone); mp 119–121 °C; $[\alpha]_D^{25}$ +9.8° (*c* 0.6, $CHCl_3$); ^{1}H NMR data ($CDCl_3$): δ 7.76–7.27 (m, 8 H, Ar), 4.92 (br s, 1 H, H-1′), 4.84 (br s, 1 H, H-1), 4.51 (d, 1 H, *J* 7.9 Hz, H-1″), 3.74 (s, 3 H, OMe), 2.18, 2.10, 2.09, 2.02, 2.01, 2.00 (2 × OAc), 2.00, 1.99, 1.97, 1.95 (each s, 30 H, 10 × OAc). Anal. Calcd for $C_{70}H_{88}N_4O_{34} \cdot 4H_2O$: C, 52.50; H, 6.04; N, 3.50. Found: C, 52.14; H, 5.63; N, 3.50.

β-D-glucopyranosyl-(1 → 6)-α-D-mannopyranosyl-(1 → 6)-α-D-mannopyranosyl-L-seryl-L-prolyl-L-seryl-L-proline (**18**).—To a solution of compound **17** (52 mg, 0.034 mmol) in 3:1 MeOH–H_2O (2.0 ml) was added NaOMe (50 mg) at room temperature for 12 h. The mixture was neutralized with Amberlite IR-120 (H^+), filtered, and concentrated. The residue was chromatographed on Sephadex LH-20 using 3:1 MeOH–H_2O as eluent to provide **18** (23 mg, 76.8%); R_f 0.52 (1:3:1 $CHCl_3$–MeOH–H_2O); mp 185–187 °C; $[\alpha]_D^{21}$ −28.7° (*c* 0.5, H_2O); ^{1}H NMR data (D_2O): δ 4.96 (br s, 1 H, H-1′), 4.95 (br s, 1 H, H-1), 4.56 (d, 1 H, *J* 7.9 Hz, H-1″). FABMS: *m/z* 873 $[M + H]^+$. Anal. Calcd for $C_{34}H_{56}N_4O_{22} \cdot 4H_2O$: C, 43.22; H, 6.83; N, 5.93. Found: C, 43.45; H, 6.32; N, 5.88.

*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* (**19**).—To a solution of compound **16** (60 mg, 0.053 mmol) and compound **2** (28 mg, 0.058 mmol) in CH_2Cl_2 (2.0 mL) was added EEDQ (20 mg, 0.081 mmol) at 0 °C. The mixture was stirred at room temperature for 21 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 **19** (59 mg, 69.6%); R_f 0.39 (2:1 benzene–acetone); mp 123–125 °C; $[\alpha]_D^{26}$ +9.4° (*c* 0.5, $CHCl_3$); ^{1}H NMR data ($CDCl_3$): δ 7.76–7.28 (m, 8 H, Ar), 4.86 (br s, 1 H, H-1′), 4.83 (br s, 1 H, H-1), 4.49 (d, 1 H, *J* 7.9 Hz, H-1″), 3.71 (s, 3 H, OMe), 2.17, 2.16, 2.09, 2.08, 2.06, 2.05, 2.02, 2.00, 1.97, 1.95 (each s, 30 H, 10 × OAc), 1.20 (s, 9H, 3 × Bu^t-CH_3). Anal. Calcd for $C_{74}H_{96}N_4O_{34} \cdot 2H_2O$: C, 54.81; H, 6.22; N, 3.46. Found: C, 54.74; H, 6.08; N, 3.01.

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-proline methyl ester (**20**).—A solution of compound **19** (59 mg, 0.037 mmol) in trifluoroacetic acid (1 mL) was stirred for 45 min at room

temperature. 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 **20** (51 mg, 90.0%); R_f 0.22 (2:1 benzene–acetone); mp 128–131 °C; $[\alpha]_D^{26}$ +10.2° (*c* 0.4, $CHCl_3$); ^1H NMR data ($CDCl_3$): δ 7.76–7.29 (m, 8 H, Ar), 4.85 (d, *J* 1.2 Hz, 1 H, H-1′), 4.83 (br s, 1 H, H-1), 4.49 (d, 1 H, *J* 7.9 Hz, H-1″), 3.72 (s, 3 H, OMe), 2.17, 2.09, 2.06, 2.05, 2.02, 2.00, 1.99, 1.98, 1.97, 1.96 (each s, 30 H, 10 × OAc). Anal. Calcd for $C_{70}H_{88}N_4O_{34} \cdot 2H_2O$: C, 53.71; H, 5.92; N, 3.58. Found: C, 53.38; H, 5.69; N, 3.72.

L-*Seryl*-L-*prolyl*-(β-D-*glucopyranosyl*)-(1 → 6)-(α-D-*mannopyranosyl*)-(1 → 6)-(α-D-*mannopyranosyl*)-L-*seryl*-L-*proline* (**21**).—To a solution of compound **20** (10 mg, 6.5 μmol) in 3:1 MeOH–H_2O (0.5 mL) was added NaOMe (10 mg) at room temperature for 12 h. The mixture was 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 **21** (5 mg, 92.9%); R_f 0.47 (1:3:1 $CHCl_3$–MeOH–H_2O); mp 189–191 °C; $[\alpha]_D^{23}$ −24.8° (*c* 0.5, H_2O); ^1H NMR data (D_2O): δ 4.87 (br s, 1 H, H-1′), 4.81 (br s, 1 H, H-1), 4.38 (d, 1 H, *J* 7.3 Hz, H-1″). FABMS: *m*/*z* 873 $[M + H]^+$. Anal. Calcd for $C_{34}H_{56}N_4O_{22} \cdot 4H_2O$: C, 43.22; H, 6.83; N, 5.93. Found: C, 42.98; H, 6.75; N, 5.74.

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* (**22**).—To a solution of compound **16** (107 mg, 0.095 mmol), compound **14** (130 mg, 0.098 mmol) in CH_2Cl_2 (5.0 mL) was added EEDQ (37 mg, 0.15 mmol) at 0 °C. The mixture was stirred at room temperature for 8 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 **22** (171 mg, 73.7%); R_f 0.34 (2:1 benzene–acetone); mp 130–132 °C; $[\alpha]_D^{27}$ +21.0° (*c* 1.0, $CHCl_3$); ^1H NMR data ($CDCl_3$): δ 7.76–7.28 (m, 8 H, Ar), 4.91 (br s, 1 H, H-1′), 4.90 (br s, 1 H, H-1′), 4.88 (br s, 1 H, H-1), 4.74 (br s, 1 H, H-1), 4.52 (d, 1 H, *J* 7.9 Hz, H-1″), 4.51 (d, 1 H, *J* 7.9 Hz, H-1″), 3.72 (s, 3 H, OMe), 2.164, 2.159, 2.151, 2.090, 2.075, 2.059, 2.053, 2.042, 2.039, 2.032, 2.017, 2.012, 1.996, 1.993, 1.987, 1.972, 1.965, 1.957, 1.949, 1.943 (each s, 60 H, 20 × OAc). FABMS: *m*/*z* 2435 $[M + H]^+$. Anal. Calcd for $C_{108}H_{138}N_4O_{59} \cdot 3H_2O$: C, 52.09; H, 5.83; N, 2.25. Found: C, 51.83; H, 5.68; N, 2.33.

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* (**23**).—To a solution of compound **22** (62 mg, 0.025 mmol) in 3:1 MeOH–H_2O (4.0 mL) was added NaOMe (60 mg) at room temperature for 12 h. The mixture was 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 **23** (30 mg, 86.2%); R_f 0.46 (1:3:1 $CHCl_3$–MeOH–H_2O); mp 205–207 °C; $[\alpha]_D^{24}$ +10.9° (*c* 0.5, H_2O); ^1H NMR data (D_2O): δ 4.97 (br s, 1 H, H-1′), 4.94 (br s, 1 H, H-1′), 4.95 (br s, 1 H, H-1), 4.90 (br s, 1 H, H-1), 4.56 (d, 2 H, *J* 7.9

Hz, 2 × H-1″). FABMS: m/z 1359 $[M + H]^+$. Anal. Calcd for $C_{52}H_{86}N_4O_{37} \cdot 8H_2O$: C, 41.54; H, 6.84; N, 3.73. Found: C, 41.18; H, 6.97; N, 4.04.

Acknowledgements

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

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