Carbohydrate Research, 256 (1994) 59–69
Elsevier Science B.V., Amsterdam

Synthesis of a glycopeptide with phytoalexin elicitor activity I. Syntheses of a triglycosyl L-serine and a triglycosyl L-seryl-L-proline dipeptide

Tadahiro Takeda [a,*], Takuya Kanemitsu [a], Motohiro Ishiguro [a], Yukio Ogihara [a] and Machiko Matsubara [b]

[a] *Faculty of Pharmaceutical Sciences, Nagoya City University, Tanabe-dori, Mizuho-ku, Nagoya, 467 (Japan)*
[b] *Kobe Women's College of Pharmacy, Motoyama-kita-machi, Higashinada-ku, Kobe, 658 (Japan)*

(Received August 13th, 1993; accepted September 25th, 1993)

ABSTRACT

A stereocontrolled synthesis of the model compound for the phytoalexin elicitor-active glycoprotein is described. Glycosylation of the disaccharide, 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1 → 6)-2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate, with *N*-(carbobenzoxy)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1 → 3)-L-serine methyl ester or *N*-(carbobenzoxy)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl)-(1 → 3)-L-seryl-L-proline methyl ester by use of AgOTf gave the desired trisaccharide–serine or trisaccharide–seryl-proline derivatives, which were transformed into β-D-glucopyranosyl-(1 → 6)-α-D-mannopyranosyl-(1 → 6)-α-D-mannopyranosyl-(1 → 3)-L-serine and triglycosyl-(1 → 3)-L-seryl-L-proline via removal of the *N*-carbobenzoxy group, followed by deacylation.

INTRODUCTION

It is generally known that some plants synthesize antimicrobial substances as a defense mechanism against invasive microorganisms[1]. These antimicrobial substances synthesized are called phytoalexins, and their inducer is called an elicitor. When peas synthesize pisatin as a phytoalexin, it is reported that several substances secreted in the germination of fungal conidia[2,3], compounds found in culture filtrates of fungal mycelia[4,5], and cell-wall polysaccharides of the mycelia[3,4] work as elicitors. Matsubara and Kuroda found elicitor activity on a glycoprotein in a culture filtrate of germinating *Mycospharella pinodes* conidia and determined its structure to be a glycosyl chain [6]. The glycoprotein gave a single spot in high-pressure paper electrophoresis and also gave a single band at the same position on disk electrophoresis after being stained with Schiff's reagent or with 15 Amide Black

* Corresponding author.

SSDI 0008-6215(93)E0361-4

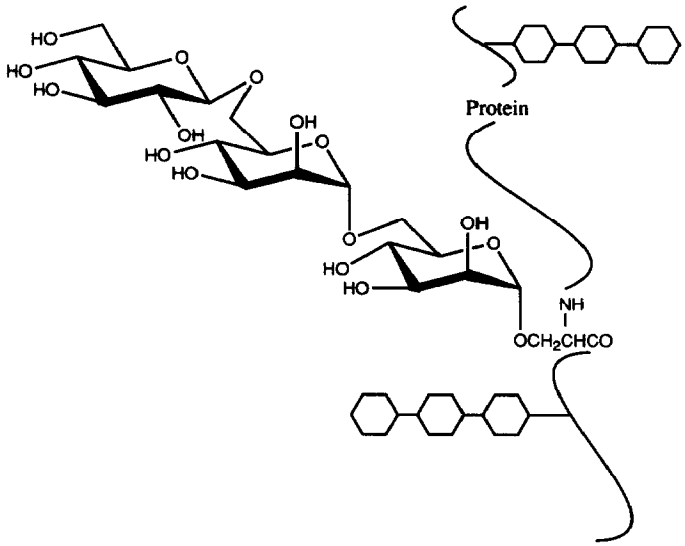


Fig. 1. Glycoprotein in which the β-D-Glc-(1 → 6)-α-D-Man-(1 → 6)-α-D-Man is glycosidically linked via an *O*-Ser residue.

10B, respectively. The molecular weight is ca. 1.3×10^6 by gel filtration. The optical rotation was $[\alpha]_D^{25}$ −122° (*c* 1, H_2O). The ratio of the sugar moiety to the protein moiety of this glycoprotein was determined to be 1:2.8. Of the composition ratios of amino acids in the glycoprotein, proline is contained in the largest quantity, followed by leucine, glycine, serine, and alanine in the order given. 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 as illustrated in Fig. 1. It has been reported that mannosyl–serine linkages can be found in yeast cell-wall mannan and yeast invertase, whereas a mannosyl–threonine linkage is present in a mycotoxin peptide[7]. Pazur et al.[8] and Lineback[9] have also reported the presence of mannose–serine or mannose–threonine linkages in a fungal glycoamylase.

In order to investigate the structural requirements for bioactive glycoprotein in detail, we have carried out synthetic studies and describe here a synthesis of triglycosyl–serine and/or triglycosyl dipeptide derivatives of a model of the glycopeptide.

RESULTS AND DISCUSSION

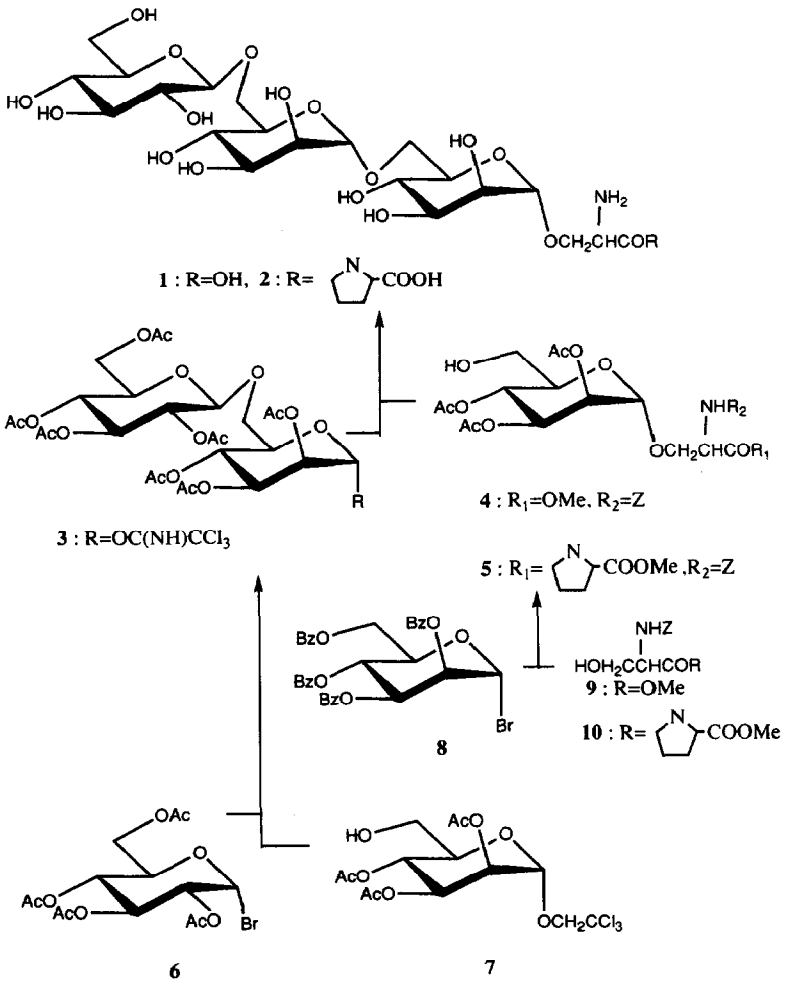
Synthesis of the target compounds **1** or **2** was carried out by employing disaccharide trichloroacetimidate **3** as glycosyl donor and mannosyl–serine or mannosyl–seryl proline derivatives **4** or **5** as glycosyl acceptors. Compound **3** was prepared from readily available tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**6**) and the 6-OH-free trichloroethyl mannoside derivative **7**. Glycosylation of compound **7**

with donor **6** in the presence of $Hg(CN)_2$ and $HgBr_2$ in benzene at room temperature gave 70.1% of the disaccharide **11**. The β-D-linked anomer showed a signal at δ 4.51 (*J* 7.6 Hz). Removal of the trichloroethyl group was achieved with zinc–copper reagent in acetate buffer, and the resultant alcohol **12** was transformed into the trichloroacetimidate **3** (ref 10). To obtain the mannosyl–serine derivative **4**, as well as the mannosyl–seryl proline derivative **5**, glycosylation between mannose and the corresponding amino acid derivatives were required. Coupling the mannosyl bromide **8** and corresponding amino acid derivatives **9** and **10** in the presence of $Hg(CN)_2$ and $HgBr_2$ in dichloromethane afforded **13** and **14** in 39.9 and 52.3% yield, respectively. The dipeptide, *N*-(carbobenzoxy)-L-seryl-L-proline methyl ester (**10**), was synthesized by coupling of *N*-(carbobenzoxy)-L-serine with L-proline methyl ester. The configuration of the α anomer **13** and **14** were confirmed by ^1H NMR spectroscopy, the signals for H-1 being observed at δ 5.08 (*J* 1.7 Hz), 5.12 (*J* 1.7 Hz), respectively. Removal of the benzoyl groups of **13** with NaOMe in MeOH gave **15** (60.7%), and the resultant compound **15** was tritylated and acetylated to give compound **17** (88.0%). Detritylation gave the 6-OH free *N*-(carbobenzoxy)-(2,3,4-tri-*O*-acetyl-α-D-mannopyranosyl-(1 → 3)-L-serine methyl ester **4** (87.0%). The reaction of disaccharide trichloroacetimidate **3** with alcohol **4**, promoted by AgOTf (silver trifluoromethanesulfonate) gave the α-(1 → 6)-linked trisaccharide derivative **19** (47.8%), from which *N*-decarbobenzoxylation followed by removal of the acetyl groups and methyl ester, afforded the desired trisaccharide serine compound **1** (Schemes 1 and 2). The anomeric configuration of compound **1** was confirmed by ^1H NMR spectroscopy, the signals for H-1, H-1′, and H-1″ being observed at δ 4.78 (*J* 1.3 Hz), 4.84 (*J* 1.2 Hz), and 4.44 (*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[11]. The HMBC experiment showed a correlation between H-1 (4.78 ppm) of the reducing end mannose and β carbon (69.0 ppm) of L-serine. A cross peak between C-6 (68.6 ppm) of the mannose and H-1′ of the inner mannose (4.84 ppm) was observed. We also observed correlations between 6-substituted mannose C-6′ (71.4 ppm) and the nonreducing end glucose H-1″ (4.44 ppm). The ^{13}C NMR data were in accordance with the proposed structure (see Table I).

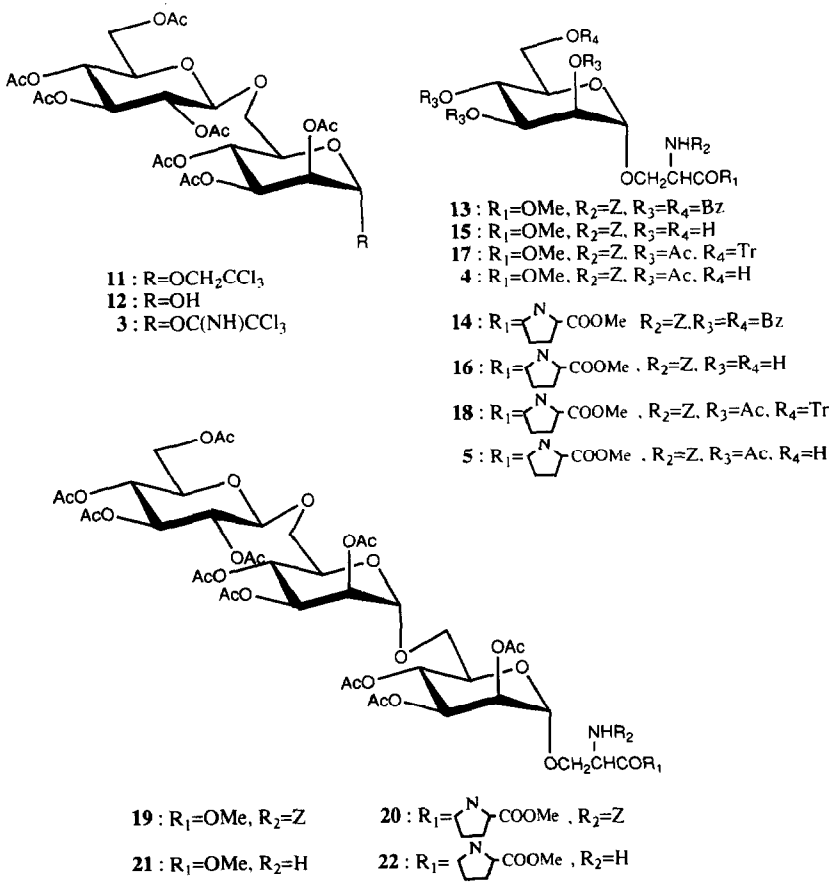
Next, we synthesized the triglycosyl dipeptide **2**. The synthesis of **2** followed a procedure analogous to that described for the preparation of **1**. Thus the reaction of the disaccharide trichloroacetimidate **3** with alcohol **5** (prepared from **14** in three steps), also promoted by AgOTf, gave the α-(1 → 6)-linked triglycosyl dipeptide derivative **20** (56.9%), which was converted to the free triglycosyl serylproline **2** in two steps, in a manner analogous to that described for deprotection of compound **19**. The ^{13}C NMR data were also in accordance with the proposed structure (see Table I). Preliminary evaluation of the triglycosyl serine and the triglycosyl dipeptides **1** and **2** shows that they, as expected, possess potential elicitor activity. These biological results will be reported in detail elsewhere.

EXPERIMENTAL

General methods.—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 Jeol GSX-400 and JNM A 500 FTNMR 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. 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 solution. Column chromatography was carried out on Silica Gel-60 (E. Merck). 2,2,2,-Trichloroethyl 2,3,4-tri-*O*-



Scheme 1.



Scheme 2.

acetyl-α-D-mannopyranoside (**7**) (ref 12), 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide (**8**) (ref 13), *N*-(carbobenzoxy)-L-serine methyl ester (**9**) (ref 14) were prepared by literature methods.

*Synthesis of the disaccharide donor. 2,2,2,-Trichloroethyl (2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1 → 6)-2,3,4,-tri-*O*-acetyl-α-D-mannopyranoside* (**11**).—To a solution of **7** (1.8 g, 3.89 mmol) in dry benzene (10 mL) were added $Hg(CN)_2$ (0.74 g), $HgBr_2$ (0.32 g), and Drierite (3.89 g). To the resulting mixture was added the tetra-*O*-acetyl-α-D-glycopyranosyl bromide (**6**) (5.19 g, 12.6 mmol). The mixture was stirred at room temperature for 10 h. The mixture was diluted $CHCl_3$ and filtered through Celite. The filtrate was washed with water and then dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography using 10:1 benzene–acetone as eluent to provide **11** (2.1 g, 70.1%); R_f 0.54 (4:1 benzene–acetone); $[\alpha]_D^{21}$ +29.5° (*c* 1.0, $CHCl_3$); ^1H NMR data ($CDCl_3$): δ 5.11 (d, 1 H, *J* 2.0 Hz, H-1), 4.51 (d, 1 H, *J* 7.6 Hz, H-1′), 4.25. 4.12 (each d, 2 H, *J* 11.6 Hz, Cl_3CCH_2), 2.19, 2.10, 2.09, 2.06, 2.03, 2.00, 1.99 (each s, 21 H, 7 × OAc); ^{13}C

TABLE I

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

Carbon atom	Compound					
	19	**21**	**1**	**20**	**22**	**2**
C-1	97.7	97.6	103.5	97.7	98.4	102.9
2	69.3 [a]	69.4	72.7	69.1 [a]	69.4	72.8 [a]
3	69.0 [b]	69.1	73.3 [a]	68.5 [b]	69.0	73.3 [b]
4	66.1	66.3	69.2	66.4 [c]	66.2 [a]	69.2 [c]
5	69.2	69.3 [a]	74.1	69.3 [a]	69.2 [b]	74.3 [d]
6	65.9	66.1	68.6	65.9	66.0	68.6
C-1′	97.3	97.3	102.6	96.6	97.4	102.6
2′	69.4 [a]	69.4 [a]	72.5	69.1 [a]	69.3 [b]	72.6 [a]
3′	69.1 [b]	69.1	73.4 [a]	68.8 [b]	69.0	73.7 [b]
4′	66.1	66.2	69.2	66.3 [c]	66.1 [a]	69.1 [c]
5′	69.6	69.4	74.6	69.5 [a]	69.2 [b]	74.6 [d]
6′	68.2	68.2	71.4	68.2	68.2	71.4
C-1″	101.1	101.1	105.6	101.1	101.1	105.6
2″	70.9	70.9	76.0	70.9	70.9	76.0
3″	72.6	72.7	78.4	72.7	72.6	78.4
4″	68.5	68.4	72.4	68.3	68.4	72.5
5″	71.9	71.9	78.8	71.9	71.9	78.8
6″	61.9	61.8	63.6	61.9	61.8	63.6
Ser-α	53.9	54.4	57.4	52.1	50.8	59.9
β	68.1	70.4	69.0	67.0	69.9	68.6
Pro-α				59.1	59.0	61.9
β				29.3	28.5	31.4
γ				24.7	22.4	24.1
δ				47.4	45.3	48.6
OMe	52.8	52.3		53.8	55.0	
Z-CH_2	67.1			66.9		

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

NMR data ($CDCl_3$): δ 97.9 (C-1), 69.0 (C-2), 68.7 (C-3), 66.1 (C-4), 70.2 (C-5), 68.0 (C-6), 101.1 (C-1′), 70.9 (C-2′), 72.6 (C-3′), 68.4 (C-4′), 71.2 (C-5′), 61.8 (C-6′). Anal. Calcd for $C_{28}H_{37}Cl_3O_{19}$: C, 43.79; H, 4.86. Found: C, 43.59; H, 4.79.

*2,3,4,6-Tetra-*O*-acetyl-β-*D*-glucopyranosyl-(1 → 6)-2,3,4-tri-*O*-acetyl-α-*D*-mannopyranose* (**12**).—A solution of compound **11** (418 mg, 0.54 mmol) in a mixture of AcOH (1 mL) and Ac_2O (0.05 mL) was added with stirring to a Zn–Cu reagent that was prepared by addition of Zn dust (1.8 g) to acetate buffer [AcONa (3.0 g)/AcOH (3.3 mL)–H_2O (4.7 mL)] containing $CuSO_4 \cdot 5H_2O$ (180 mg) solution (in 0.7 mL of H_2O). The solution was stirred at room temperature for 12 h. The mixture was diluted with acetone and filtered through Celite, and $CHCl_3$ and water were added to the filtrate. The organic layer was separated, washed with water, dried over Na_2SO_4, and then concentrated to give compound **12** (344 mg, 99.2%); R_f 0.29 (4:1 benzene–acetone); mp 85–87°C; $[\alpha]_D^{21}$ +15.6° (*c* 0.4, $CHCl_3$);

^{1}H NMR data ($CDCl_3$): δ 5.21 (br s, 1 H, H-1), 4.58 (d, 1 H, *J* 7.9 Hz, H-1′), 2.17, 2.10, 2.09, 2.06, 2.03, 2.01, 1.99 (each s, 21 H, 7 × OAc); ^{13}C NMR data ($CDCl_3$): δ 92.0 (C-1), 70.1 (C-2), 68.9 (C-3), 66.6 (C-4), 69.3 (C-5), 68.8 (C-6), 101.1 (C-1′), 71.3 (C-2′), 72.6 (C-3′), 68.3 (C-4′), 71.9 (C-5′), 61.8 (C-6′). Anal. Calcd for $C_{26}H_{36}O_{18}$: C, 49.06; H, 5.70. Found: C, 48.88; H, 5.45.

*2,3,4,6-Tetra-*O*-acetyl-β-*D*-glucopyranosyl-(1 → 6)-2,3,4-tri-*O*-acetyl-α-*D*-mannopyranosyl trichloroacetimidate* (**3**).—To a stirred solution of **12** (207 mg, 0.33 mmol) in CH_2Cl_2 (1.5 mL) were added CCl_3CN (0.3 mL) and DBU (60 μL) at 0°C. The mixture was stirred for 16 h at room temperature, then directly chromatographed on silica gel in $CHCl_3$ to give **3** (216 mg, 85.0%); R_f 0.48 (4:1 benzene–acetone); mp 79–81°C; $[\alpha]_D^{21}$ +21.0° (*c* 0.2, $CHCl_3$); ^{1}H NMR data ($CDCl_3$): δ 6.27 (d, 1 H, *J* 1.7 Hz, H-1), 4.55 (d, 1 H, *J* 7.9 Hz, H-1′), 2.21, 2.09, 2.07, 2.06, 2.02, 2.00, 1.99 (each s, 21 H, 7 × OAc); ^{13}C NMR data ($CDCl_3$): δ 94.3 (C-1), 68.8 (C-2), 67.8 (C-3), 65.6 (C-4), 72.2 (C-5), 67.6 (C-6), 100.7 (C-1′), 70.9 (C-2′), 72.1 (C-3′), 68.4 (C-4′), 71.8 (C-5′), 61.8 (C-6′), 159.8 [OC(NH)], 90.5 (CCl_3). Anal. Calcd for $C_{28}H_{36}Cl_3NO_{18}$: C, 43.06; H, 4.65; N, 1.79. Found: C, 42.64; H, 4.56; N, 1.43.

Preparation of the mannosyl–serine and mannosyl–seryl proline acceptors. *N-(Carbobenzoxy)-(2,3,4,6-tetra-*O*-benzoyl-α-*D*-mannopyranosyl)-(1 → 3)-*L*-serine methyl ester* (**13**).—Glycosylation of *N*-(carbobenzoxy)-L-serine methyl ester (1.10 g, 4.33 mmol) using 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide (17.92 g, 7.11 mmol) was carried out in the presence of $Hg(CN)_2$ (0.83 g), $HgBr_2$ (0.36 g), and 4A molecular sieves (1.22 g) in dry CH_2Cl_2 (12 mL). The mixture was stirred at room temperature for 16 h. The mixture was then diluted with $CHCl_3$ and filtered through Celite. The filtrate was washed sequentially with 0.5% HCl, satd $NaHCO_3$, and water, and then dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography using 10:1 → 4:1 benzene–acetone gradient as eluent to provide **13** (1.44 g, 39.9%); R_f 0.51 (10:1 benzene–acetone); mp 52–54°C; $[\alpha]_D^{22}$ −32.6° (*c* 0.5, $CHCl_3$); ^{1}H NMR data ($CDCl_3$): δ 8.12–7.21 (m, 25 H, Ar), 6.11 (t, 1 H, *J* 9.9 Hz, H-4), 5.85 (dd, 1 H, *J* 3.4, 9.9 Hz, H-3), 5.63 (dd, 1 H, *J* 1.7, 3.4 Hz, H-2), 5.16–5.14 (m, 2 H, Z-CH_2), 5.08 (d, 1 H, *J* 1.7 Hz, H-1), 4.66–4.62 (m, 2 H, H-6), 4.47–4.46 (m, 1 H, Ser α), 4.43–4.35 (m, 1 H, H-5), 4.12, 4.08 (m, 2 H, Ser β), 3.87 (s, 3 H, OMe); ^{13}C NMR data ($CDCl_3$): δ 98.1 (C-1), 70.2 (C-2), 69.8 (C-3), 66.6 (C-4), 69.4 (C-5), 62.6 (C-6), 54.4 (Ser α), 69.2 (Ser β), 53.1 (OMe), 67.3 (Z-CH_2). Anal. Calcd for $C_{46}H_{41}NO_{14}$: C, 66.42; H, 4.97; N, 1.68. Found: C, 66.31; H, 4.88; N, 1.48.

*N-(Carbobenzoxy)-α-*D*-mannopyranosyl-(1 → 3)-*L*-serine methyl ester* (**15**).—To a solution of **13** (2.8 g, 3.36 mmol) in MeOH (64 mL) was added NaOMe powder (0.35 g). The mixture was stirred for 6.5 h at 0°C, made neutral with Amberlite IR-120 (H^+), and evaporated in vacuo to give **15** (846 mg, 60.7%); R_f 0.28 (5:1 $CHCl_3$–MeOH); $[\alpha]_D^{22}$ +42.9°; ^{1}H NMR data ($CDCl_3$): δ 7.31–7.26 (m, 5 H, Ar), 4.76 (br s, 1 H, H-1), 3.84 (s, 3 H, OMe); ^{13}C NMR data ($CDCl_3$): δ 100.6 (C-1), 71.2 (C-2), 70.6 (C-3), 66.1 (C-4), 72.8 (C-5), 60.8 (C-6), 54.3 (Ser α), 67.7 (Ser β),

52.8 (OMe), 67.1 (Z-CH_2). Anal. Calcd for $C_{18}H_{25}NO_{10}$: C, 50.94; H, 6.18; N, 3.30. Found: C, 50.37; H, 6.06; N, 2.85.

N-*(Carbobenzoxy)-(2,3,4,-tri-*O*-acetyl-6-*O*-trityl-α-*D*-mannopyranosyl)-(1 → 3)-*L*-serine methyl ester* (**17**).—To a solution of **15** (800 mg, 1.92 mmol) in pyridine (10 mL) was added triphenylmethyl (trityl)chloride (966 mg), and the mixture was stirred for 30 h at 70°C. After the starting material disappeared, Ac_2O (12.8 mL) was added at 0°C and allowed to warm to room temperature. The mixture was extracted with $CHCl_3$, washed with water, dried with Na_2SO_4, and evaporated. The residue was chromatographed on silica gel using 10:1 benzene–acetone as eluent to provide **17** (1.33 g, 88.0%); R_f 0.48 (10:1 benzene–acetone); mp 74–76°C; $[\alpha]_D^{22}$ +44.4° (*c* 0.9, $CHCl_3$); ^{1}H NMR data ($CDCl_3$): δ 7.46–7.21 (m, 20 H, Ar), 4.85 (d, 1 H, *J* 1.7 Hz, H-1), 3.79 (s, 3 H, OMe), 2.17, 1.96, 1.71 (each s, 9 H, 3 × OAc). Anal. Calcd for $C_{43}H_{45}NO_{13}$: C, 65.89; H, 5.79; N, 1.79 Found: C, 65.61; H, 5.70; N, 1.70.

N-*(Carbobenzoxy)-(2,3,4-tri-*O*-acetyl-α-*D*-mannopyranosyl)-(1 → 3)-*L*-serine methyl ester* (**4**).—A solution of compound **17** (1.2 g, 1.53 mmol) in aq 80% 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 $CHCl_3$, washed with water and evaporated to give **4** (720 mg, 87.0%); R_f 0.25 (4:1 benzene–acetone); $[\alpha]_D^{24}$ −26.8° (*c* 0.6, $CHCl_3$); ^{1}H NMR data ($CDCl_3$): δ 7.36–7.26 (m, 5 H, Ar), 4.81 (br s, 1 H, H-1), 3.80 (s, 3 H, OMe), 2.13, 2.05, 1.99 (each s, 9 H, 3 × OAc); ^{13}C NMR data ($CDCl_3$): δ 98.1 (C-1), 71.3 (C-2), 69.4 (C-3), 66.3 (C-4), 69.2 (C-5), 61.1 (C-6), 54.3 (Ser α), 68.7 (Ser β), 52.9 (OMe), 67.2 (Z-CH_2). Anal. Calcd for $C_{24}H_{31}NO_{13} \cdot 0.5H_2O$: C, 52.36; H, 5.86; N, 2.54 Found: C, 52.18; H, 5.68; N, 2.29.

N-*(Carbobenzoxy)-L-seryl-L-proline methyl ester* (**10**).—To a solution of *N*-(carbobenzoxy)-L-serine (25 g, 0.1 mol), L-proline methyl ester hydrochloride (17.4 g, 0.1 mol) in 10:1 CH_2Cl_2–DMF (152 mL) were added Et_3N (17.1 mL) and diethylphosphorocyanidate (DEPC) (19.8 mL) at 0°C for 30 min, and the mixture was allowed to warm to room temperature for 24 h. The mixture was diluted with $CHCl_3$, and the $CHCl_3$ solution was washed sequentially with 0.5% HCl, satd $NaHCO_3$ and water, and then dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography using 20:1 $CHCl_3$–MeOH as eluent, to give **10** (13.29 g, 36.3%); R_f 0.57 (10:1 $CHCl_3$–MeOH); mp 106–108°C; $[\alpha]_D^{25}$ −49.0° (*c* 0.3, $CHCl_3$); ^{1}H NMR data ($CDCl_3$): δ 7.29 (m, 5 H, Ar), 5.06 (s, 2 H, Z-CH_2), 3.67 (s, 3 H, OMe), 2.15–1.91 (m, 4 H, Pro β, γ); ^{13}C NMR data ($CDCl_3$): δ 54.2 (Ser α), 63.1 (Ser β), 58.9 (Pro α), 28.8 (Pro β), 24.8 (Pro γ), 47.1 (Pro δ), 52.4 (OMe), 66.8 (Z-CH_2). Anal. Calcd for $C_{17}H_{22}N_2O_6 \cdot 0.5H_2O$: C, 56.81; H, 6.45; N, 7.80. Found: C, 57.15; H, 6.34; N, 7.56.

N-*(Carbobenzoxy)-(2,3,4,6-tetra-*O*-benzoyl-α-*D*-mannopyranosyl)-(1 → 3)-L-seryl-L-proline methyl ester* (**14**).—Glycosylation of **10** (1 g, 2.85 mmol) using **8** (5 g, 7.58 mmol) was carried out in the presence of $Hg(CN)_2$ (563.8 mg), $HgBr_2$ (240 mg), and 4A molecular sieves (822 mg) in dry CH_2Cl_2 (16 mL). The mixture was stirred at room temperature for 42 h. Compound **14** was prepared as described for **13**;

yield 1.38 g (52.3%); R_f 0.71 (50:1 $CHCl_3$–MeOH); mp 92–94°C; $[\alpha]_D^{23}$ −31.7° (*c* 0.4, $CHCl_3$); ^1H NMR data ($CDCl_3$): δ 7.37–7.26 (m, 25 H, Ar), 5.14–5.07 (m, 2 H, Z-CH_2), 5.12 (d, 1 H, *J* 1.7 Hz, H-1), 4.96–4.90 (m, 1 H, Ser α), 3.81–3.75 (m, 2 H, Ser β), 3.73 (s, 3 H, OMe), 2.34–2.04 (m, 4 H, Pro β, γ); ^{13}C NMR data ($CDCl_3$): δ 98.2 (C-1), 70.3 (C-2, C-3), 67.1 (C-4), 69.1 (C-5), 62.3 (C-6), 51.6 (Ser α), 69.2 (Ser β), 59.3 (Pro α), 29.3 (Pro β), 25.1 (Pro γ), 47.9 (Pro δ), 52.1 (OMe), 67.1 (Z-CH_2). Anal. Calcd for $C_{51}H_{48}N_2O_{15}$: C, 65.90; H, 5.21; N, 3.02. Found: C, 65.75; H, 5.24; N, 2.83.

N-*(Carbobenzoxy)-α-*D*-mannopyranosyl-(1 → 3)-*L*-seryl-*L*-proline methyl ester* (**16**).—This compound was prepared as described for **15**; yield 327 mg (67.4%); R_f 0.33 (5:1 $CHCl_3$–MeOH); $[\alpha]_D^{24}$ −13.4° (*c* 1.0, $CHCl_3$); ^1H NMR data ($CDCl_3$): δ 7.53–7.26 (m, 5 H, Ar), 5.06 (br s, 1 H, H-1), 3.66 (s, 3 H, OMe); ^{13}C NMR data ($CDCl_3$): δ 100.1 (C-1), 71.3 (C-2), 70.6 (C-3), 66.0 (C-4), 67.0 (C-5), 61.0 (C-6), 51.9 (Ser α), 67.0 (Ser β), 58.4 (Pro α), 29.0 (Pro β), 24.9 (Pro γ), 47.3 (Pro δ), 52.7 (OMe), 67.2 (Z-CH_2). Anal. Calcd for $C_{23}H_{32}N_2O_{11} \cdot 0.5H_2O$: C, 52.97; H, 6.38; N, 5.37. Found: C, 52.70; H, 6.50; N, 5.08.

N-*(Carbobenzoxy)-(2,3,4-tri-*O*-acetyl-6-*O*-trityl-α-*D*-mannopyranosyl)-(1 → 3)-*L*-seryl-*L*-proline methyl ester* (**18**).—This compound was prepared as described for **17**; yield 412 mg (83.9%); R_f 0.32 (10:1 benzene–acetone); $[\alpha]_D^{24}$ +23.4° (*c* 1.0, $CHCl_3$): ^1H NMR data ($CDCl_3$): δ 7.46–7.18 (m, 20 H, Ar), 5.18 (d, 1 H, *J* 1.7 Hz, H-1), 3.63 (s, 3 H, OMe), 2.19, 1.95, 1.72 (each s, 9 H, 3 × OAc); ^{13}C NMR data ($CDCl_3$): δ 98.1 (C-1), 70.3 (C-2), 69.6 (C-3), 66.3 (C-4), 69.5 (C-5), 62.2 (C-6), 51.9 (Ser α), 67.0 (Ser β), 59.2 (Pro α), 29.0 (Pro β), 24.8 (Pro γ), 47.5 (Pro δ), 52.0 (OMe), 68.5 (Z-CH_2), 86.5 (Ph_3-C). Anal. Calcd for $C_{48}H_{52}N_2O_{14}$: C, 65.44; H, 5.95; N, 3.18. Found: C, 65.59; H, 6.05; N, 3.09.

N-*(Carbobenzoxy)-(2,3,4-tri-*O*-acetyl-α-*D*-mannopyranosyl)-(1 → 3)-*L*-seryl-*L*-proline methyl ester* (**5**).—This compound was prepared as described for **4**; yield 89 mg (79.3%); R_f 0.62 (10:1 $CHCl_3$–MeOH); $[\alpha]_D^{25}$ +4.6° (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.36–7.34 (m, 5 H, Ar), 5.10 (br s, 1 H, H-1), 3.71 (s, 3 H, OMe), 2.14, 2.05, 1.98 (each s, 9 H, 3 × OAc); ^{13}C NMR data ($CDCl_3$): δ 98.6 (C-1), 70.9 (C-2), 69.5 (C-3), 66.5 (C-4), 68.9 (C-5), 61.4 (C-6), 52.0 (Ser α), 67.1 (Ser β), 59.1 (Pro α), 29.3 (Pro β), 24.9 (Pro γ), 47.5 (Pro δ), 52.1 (OMe), 69.1 (Z-CH_2). Anal. Calcd for $C_{29}H_{38}N_2O_{14}$: C, 54.54; H, 6.00; N, 4.39. Found: C, 54.40, H, 5.93; N, 4.20.

Synthesis of the triglycosyl serine. 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)-(1 → 3)-*L*-serine methyl ester* (**19**).—To a solution of **4** (200 mg, 0.37 mmol) in dry CH_2Cl_2 (20 mL) was added AgOTf (150 mg). To the resulting mixture was added the disaccharide trichloroacetimidate **3** (376 mg, 0.48 mmol), and the mixture was stirred for 2 days at room temperature. The mixture was then diluted with $CHCl_3$ and filtered through 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 100:1 $CHCl_3$–MeOH as eluent to provide **19** (200 mg, 47.8%); R_f 0.30 (4:1 benzene–acetone); R_f 0.79 (15:1

$CHCl_3$–MeOH); mp 82–84°C; $[\alpha]_D^{18}$ +25.2° (*c* 0.72, $CHCl_3$); ^1H NMR data ($CDCl_3$): δ 7.37–7.28 (m, 5 H, Ar), 4.86 (br s, 1 H, H-1′), 4.79 (br s, 1 H, H-1), 4.49 (d, 1 H, *J* 8.0 Hz, H-1″), 3.80 (s, 3 H, OMe), 2.17, 2.16, 2.09, 2.08, 2.06, 2.04, 2.02, 1.99 (3 × OAc) (each s, 30 H, 10 × OAc). Anal. Calcd for $C_{50}H_{65}NO_{30}$: C, 51.77; H, 5.65; N, 1.21. Found: C, 51.14; H, 5.66; N, 1.06.

*(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)-(1 → 3)-*L*-serine methyl ester* (**21**).—To a solution of **19** (42 mg, 0.037 mmol) in EtOH (2 mL) was added 10% Pd–C (12 mg). The mixture was stirred for 3 h under H_2 and then filtered and concentrated to dryness. The residue was chromatographed on silica gel with 10:1 $CHCl_3$–MeOH. The eluate was evaporated to dryness to give **21** (34 mg, 88.5%); R_f 0.70 (10:1 $CHCl_3$–MeOH); mp 85–87°C; $[\alpha]_D^{23}$ +44.4° (*c* 0.3, $CHCl_3$); ^1H NMR data ($CDCl_3$): δ 4.87 (d, 1 H, *J* 1.7 Hz, H-1′), 4.82 (d, 1 H, *J* 1.6 Hz, H-1), 4.49 (d, 1 H, *J* 7.9 Hz, H-1″), 3.77 (s, 3 H, OMe), 2.18, 2.16, 2.10, 2.09, 2.06, 2.05, 2.02, 2.00, 1.99, 1.96 (each s, 30 H, 10 × OAc). Anal. Calcd for $C_{42}H_{59}NO_{28}$: C, 49.17; H, 5,80; N, 1.37. Found: C, 49.35; H, 5.59; N, 1.04.

β-D-Glucopyranosyl-(1 → 6)-α-D-mannopyranosyl-(1 → 6)-α-D-mannopyranosyl-(1 → 3)-L-serine (**1**).—Compound **21** (43 mg, 0.042 mmol) was treated with Et_3N (0.1 mL) in 5:2 MeOH–H_2O (0.7 mL) at room temperature for 12 h. The solution was taken to dryness in vacuo. The residue was chromatographed on silica gel using $CHCl_3$–MeOH–H_2O (65:35:10, upper layer) to give **1** (15 mg, 59.5%); R_f 0.42 (1:2:1 $CHCl_3$–MeOH–H_2O); $[\alpha]_D^{23}$ +37.8° (*c* 0.1, H_2O); ^1H NMR data (D_2O): δ 4.84 (d, 1 H, *J* 1.2 Hz, H-1′), 4.78 (d, 1 H, *J* 1.3 Hz, H-1), 4.44 (d, 1 H, *J* 7.9 Hz, H-1″). Anal. Calcd for $C_{21}H_{37}NO_{18} \cdot 2H_2O$: C, 40.19; H, 6.58; N, 2.23, Found: C, 40.20; H, 6.67; N, 1.96.

Synthesis of the triglycosyl dipeptide. N-*(Carbobenzoxy)-(2,3,4,6-tetra-*O*-acetyl-β-*O*-glucopyranosyl)-(1 → 6)-(2,3,4-tri-*O*-acetyl-α-*D*-mannopyranosyl)-(1 → 6)-(2,3,4-tri-*O*-acetyl-α-*D*-mannopyranosyl)-(1 → 3)-*L*-serine-L-proline methyl ester* (**20**).—To a solution of **5** (85 mg, 0.13 mmol) in dry CH_2Cl_2 (1 mL) was added AgOTf (65 mg). To the resulting mixture was added **3** (160 mg, 0.20 mmol), and the mixture was stirred for 2 days at room temperature. The mixture was then diluted with $CHCl_3$ and filtered through Celite. The filtrate was washed with water, and then dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was chromatographed on silica gel using 100:1 $CHCl_3$–MeOH as eluent to provide **20** (93 mg, 56.9%); R_f 0.29 (4:1 benzene–acetone); R_f 0.80 (15:1 $CHCl_3$–MeOH); mp 97–99°C; $[\alpha]_D^{25}$ +27.8° (*c* 0.4, $CHCl_3$); ^1H NMR data ($CDCl_3$): δ 7.38–7.27 (m, 5 H, Ar), 4.85 (d, 1 H, *J* 1.3 Hz, H-1′), 4.79 (br s, 1 H, H-1), 4.49 (d, 1 H, *J* 7.9 Hz, H-1″), 3.73 (s, 3 H, OMe), 2.17, 2.16, 2.09, 2.08, 2.05, 2.04, 2.02, 2.00, 1.99, 1.98, (each s, 30 H, 10 × OAc). Anal. Calcd for $C_{55}H_{72}N_2O_{31}$: C, 52.55; H, 5.77; N, 2.23. Found: C, 52.63; H, 5.69; N, 2.12.

*(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)-(1 → 3)-*L*-seryl-L-proline methyl ester* (**22**).—This compound was prepared as described for **21**: yield

(31.7 mg, 78.9%); R_f 0.56 (8:1 $CHCl_3$–MeOH); mp 82–84°C; $[\alpha]_D^{23}$ +28.4° (*c* 0.2, $CHCl_3$); ^1H NMR data ($CDCl_3$): δ 4.91 (br s, 1 H, H-1′), 4.85 (br s, 1 H, H-1), 4.49 (d, 1 H, *J* 7.9 Hz, H-1″), 3.48 (s, 3 H, OMe), 2.18, 2.16, 2.10, 2.09, 2.08, 2.07, 2.02, 2.00, 1.99, 1.96 (each s, 30 H, 10 × OAc). Anal. Calcd for $C_{47}H_{66}N_2O_{29}$: C, 50.27; H, 5.92; N, 2.49. Found: C, 50.15; H, 5.63; N, 2.36.

β-D-Glucopyranosyl-(1 → 6)-α-D-mannopyranosyl-(1 → 6)-α-D-mannopyranosyl-(1 → 3)-L-seryl-L-proline (**2**).—This compound was prepared as described for **1**; yield (8.6 mg, 71.4%); R_f 0.81 (1:2:1 $CHCl_3$–MeOH–H_2O); $[\alpha]_D^{22}$ +25.3° (*c* 0.1, H_2O); ^1H NMR data (D_2O): δ 4.91 (d, 1 H, *J* 1.7 Hz, H-1′), 4.87 (d, 1 H, *J* 1.7 Hz, H-1), 4.53 (d, 1 H, *J* 8.1 Hz, H-1″). Anal. Calcd for $C_{26}H_{44}N_2O_{19}$: C, 45.35; H, 6.44; N, 4.07. Found: C, 45.88; H, 6.57; N, 3.85.

ACKNOWLEDGMENTS

We thank Miss S. Kato for recording the NMR spectra and Miss T. Naito for performing the microanalyses. This work was supported by a Grant-in-Aid for Scientific Research (No. 04671302) from the Ministry of Education, Science and Culture of Japan. We also gratefully acknowledge financial assistance from Hoansha.

REFERENCES

1 J.L. Ingham, *Bot. Rev.* 38 (1972) 343–424.
2 Y. Yamamoto, H. Oku, T. Shiraishi, S. Ouchi, and K. Koshizawa, *J. Phytopathol.*, 117 (1986) 136–143.
3 I.A.M. Cruickshank and M.M. Smith, *J. Phytopathol.*, 116 (1986) 48–59.
4 P.J.G.M. de Wit and P.H.M. Roseboom, *Physiol. Plant Pathol.*, 16 (1980) 391–408.
5 D.L. Daniels and L.A. Hadwiger, *Physiol. Plant Pathol.*, 8 (1976) 9–19.
6 M. Matsubara and H. Kuroda, *Chem. Pharm. Bull.*, 35 (1987) 249–255.
7 N. Sharon (translated into Japanese by T. Osawa), *Fukugo Toshitsu*, Gakkai Shupansenta, Tokyo, 1977, pp. 39–49.
8 J.H. Pazur, H.R. Knull, and D.L. Simpson, *Biochem. Biophys. Res. Commun.*, 40 (1970) 110–118.
9 D.R. Lineback, *Carbohydr. Res.*, 7 (1968) 106–108.
10 R.R. Schmidt and J. Michel, *Angew. Chem. Int. Ed. Engl.*, 19 (1980) 731–732; R.R. Schmidt, J. Michel, and M. Roos, *Liebigs Ann. Chem.*, (1984) 1343–1357.
11 A. Bax and M.F. Summers, *J. Am. Chem. Soc.*, 108 (1986) 2093–2094.
12 F.M. Winnik, J.P. Carver, and J.J. Krepinsky, *J. Org. Chem.*, 47 (1982) 2701–2707.
13 O. Kanie, T. Takeda, N. Hada, and Y. Ogihara, *J. Carbohydr. Chem.*, 10 (1991) 561–581.
14 S. Guttman, *Helv. Chim. Acta.*, 44 (1961) 721–744.