Synthetic Studies on Glycopeptides Concerned with Defense Response of Plants. I. Syntheses of Suppressins A and B

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Two glycopeptides, suppressins A and B, that suppress the production of pisatin, a phytoalexin of pea, were synthesized. In the synthesis of suppressin A, condensation of 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl trichloroacetimidate or its glycosidic β isomer with *N*-(carbobenzoxy)-L-seryl-*O*-benzyl-L-seryl-glycine methyl ester was carried out in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) to give the monoglycosyl tripeptide derivatives. For the synthesis of suppressin B, glycosylation of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide and 1,2,3,6-tetra-*O*-benzoyl-α-D-galactopyranose was promoted by silver trifluoromethanesulfonate (AgOTf) to provide a disaccharide derivative. The coupling of diglycosyl imidate, 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzoyl-2-azido-2-deoxy-D-galactopyranosyl trichloroacetimidate, and *N*-(carbobenzoxy)-L-seryl-*O*-benzyl-L-seryl-glycyl-4-benzyl-L-aspartyl-5-benzyl-L-glutamyl-*O*-benzyl-L-threonine methyl ester in the presence of TMSOTf afforded the diglycosyl hexapeptide derivatives. Reduction, followed by *N*-acetylation, and then removal of the remaining protecting groups afforded the desired suppressin B.

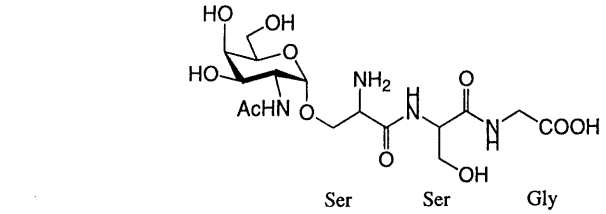
Key words defense response; glycopeptide; *Mycosphaerella pinodes*; suppressor; suppressin A, B; synthesis

A pea pathogen, *Mycosphaerella pinodes*, secretes an elicitor and a suppressor of the defense responses of pea in its pycnospore germination fluid.[1] Various plants synthesize antimicrobial substances, called phytoalexins, as a defense mechanism against invasive microorganisms.[2] The elicitor induces active defense reactions, such as the production of a major phytoalexin, pisatin. On the other hand, factors that can suppress the defense responses of plants that follow an attack by microorganisms, tentatively called suppressors, have been found in the culture filtrates and spore germination fluids of fungal pathogens. Shiraishi *et al.* characterized the chemical structures and some aspects of the biological activities of two suppressors,[3] suppressins A and B, purified from the spore germination fluid of a pea pathogen, *Mycosphaerella pinodes*. As shown in Fig. 1, both suppressors were found to be mucin-type glycopeptides composed of *N*-acetylgalactosamine and galactose as the carbohydrate moiety, and aspartic acid, glutamic acid, glycine, serine and threonine as the peptide moiety. These suppressors inhibit both the ATPase activity[4] and polyphosphoinositide metabolism[5] in pea plasma membranes, causing the temporary suppression of the signal-transduction pathway that leads to the expression of defense genes, which encode key enzymes in the biosynthetic pathway to phytoalexin.

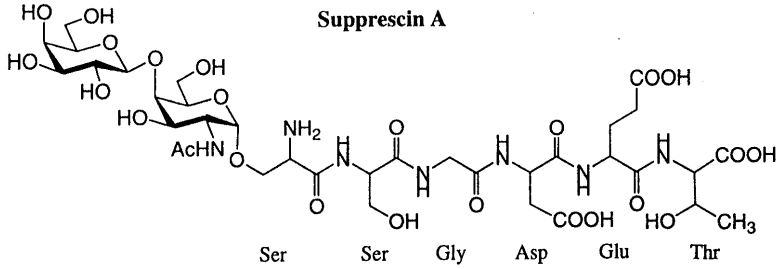
In order to investigate the structural requirements for bioactive glycopeptides in detail, we have carried out synthetic studies and we present here syntheses of suppressin A, suppressin B and their glycosidic β isomers.

Results and Discussion

The dipeptides, *N*-(*tert*-butoxycarbonyl)-*O*-benzyl-L-seryl-glycine methyl ester (**7**) and *N*-(*tert*-butoxycarbonyl)-5-benzyl-L-glutamyl-*O*-benzyl-L-threonine methyl ester (**8**) were synthesized by coupling of *N*-(*tert*-butoxycarbonyl)-*O*-benzyl-L-serine (**2**) with glycine methyl ester hydro-



Suppressin A



Suppressin B

Fig. 1

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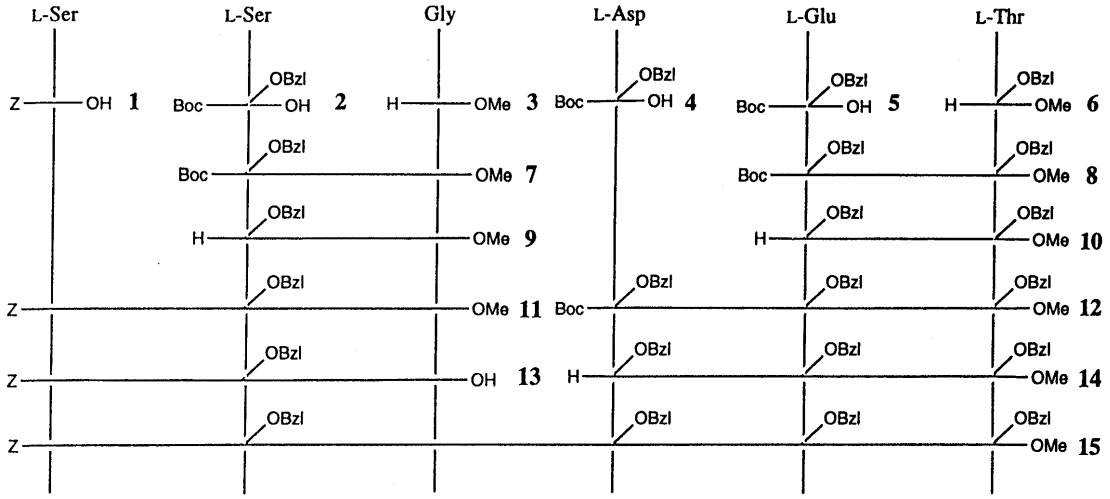

Chart 1

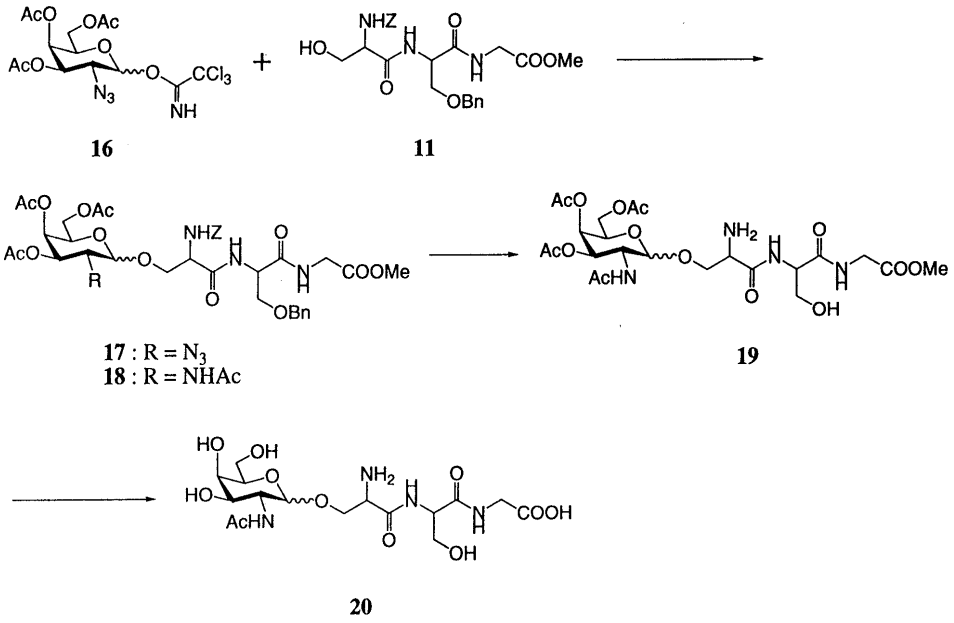

Chart 2

chloride (**3**) and *N*-(*tert*-butoxycarbonyl)-L-glutamic acid 5-benzyl ester (**5**) with *O*-benzyl-L-threonine methyl ester hydrochloride (**6**), respectively. Removal of the *tert*-butoxycarbonyl groups of **7** and **8** with trifluoroacetic acid gave compounds **9** and **10**. Combination of the dipeptide **9** with *N*-(carbobenzoxy)-L-serine (**1**), and also the dipeptide **10** with *N*-(*tert*-butoxycarbonyl)-*O*-benzyl-L-aspertic acid 4-benzyl ester (**4**) in the presence of diethylphosphorocyanidate (DEPC)[6] afforded *N*-(carbobenzoxy)-L-seryl-*O*-benzyl-L-seryl-glycine methyl ester (**11**) and *N*-(*tert*-butoxycarbonyl)-4-benzyl-L-aspartyl-5-benzyl-L-glutamyl-*O*-benzyl-L-threonine methyl ester (**12**) in 34% and 69% yields, respectively. The ^{1}H- and ^{13}C-NMR spectra of these tripeptide derivatives were in accordance with the proposed structures. Removal of the methyl ester group of **11** with NaOMe in aqueous MeOH gave the *C*-terminus-free derivative **13** (94%). A brief treatment of **12** with trifluoroacetic acid provided the *N*-terminus-free derivative **14** in 84% yield. Combination of **13** with **14** in the presence of *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ)[7] in dichloromethane gave the hexapeptide **15** in 62% yield (Chart 1). The ^{1}H-NMR spectrum of this hexapeptide showed aromatic proton signals at δ 7.31—7.23 ppm (m, 25 H), and the signal of the methyl ester appeared at δ 3.62 ppm.

In the synthesis of suppressin A, condensation of 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl trichloroacetimidate[8] (**16**α) and/or its β-isomer (**16**β) with the tripeptide **11** was carried out in the presence of TMSOTf in dichloromethane at −30 °C to give a 63% yield of a mixture of α and β glycopeptides **17**. Sodium borohydride-nickel chloride reduction of the azido group in **17**, followed by *N*-acetylation,[9] gave compound **18**. The α (**18**α) and β (**18**β) anomers of **18** could be separated by silica gel chromatography. The benzyl ester, benzyl ether and carbobenzoxy (Z) groups were cleaved from **18**α and **18**β by hydrogenolysis (Pd–C) to give **19**α and **19**β in 79% and 82% yields, respectively. Removal of the ester groups (acetyl and methyl ester) of **19**α and **19**β with NaOMe in aqueous MeOH afforded the desired 2-acet-

amido-2-deoxy-D-galactopyranosyl tripeptide **20α** (suppressin A) and **20β** in 60% and 80% yields, respectively (Chart 2). Deblocking of **19α** and **19β** with NaOMe in MeOH did not promote β-elimination of the *O*-glycosidic linkage bearing an L-seryl residue. Compounds **20α** and **20β** showed an $[M+H]^+$ ion peak at *m/z* 453 in the FAB-MS. The structures and purity of **20α** and **20β** were established by ^{1}H-, ^{13}C-NMR (Table 1) spectroscopy and FAB-MS spectrometry.

Table 1. ^{13}C-NMR Data (δ) for Compounds **18α**, **β**, **19α**, **β**, **20α**, **β**

Carbon atom	Compound					
	18α	**18β**	**19α**	**19β**	**20α**	**20β**
C-1	98.5	101.7	100.2	101.9	101.7	104.0
2	47.5	51.2	48.5	50.7	52.2	54.9
3	67.9	69.8	68.6	70.2	71.2	71.3
4	67.2	69.1	68.3	66.7	70.3	70.4
5	67.1	66.7	68.3	70.8	69.6	73.5
6	61.9	61.6	62.8	61.5	64.0	63.7
Ser-α	53.9	54.3	56.9	55.2	58.3	58.4
	52.4	53.0	54.4	54.8	55.8	56.4
β	69.3	71.2	69.8	72.1	74.2	78.0
	67.9	69.6	63.1	62.6	64.2	64.0
Gly	41.4	41.4	41.3	41.3	46.1	46.1
OMe	52.4	52.3	52.4	52.5		
Z-CH_2	67.3	67.2				
Bn-CH_2	73.6	73.5				

Synthesis of suppressin B (**32α**) and its glycosidic isomer (**32β**) was carried out by the coupling of diglycosyl imidate **28** and the hexapeptide acceptor **15**. Compound **28** was prepared by employing readily available acetobromogalactose[10] **21** as a glycosyl donor and 1,2,3,6-tetra-*O*-benzoyl-α-D-galactopyranose[11] **22** as a glycosyl acceptor. Compound **21** was coupled with **22** in the presence of silver trifluoromethanesulfonate (AgOTf) as a promoter, and 2,4,6-trimethylpyridine (collidine) as a neutralizing agent[12] of the liberated acid in toluene to afford **23** in 56% yield. The anomeric configuration of compound **23** was confirmed by ^{1}H-NMR spectroscopy, the signals for H-1 and H-1′ being observed at δ 6.76 (J=3.7 Hz) and 4.75 (J=7.9 Hz), respectively. A heteronuclear multiple-bond correlation (HMBC) experiment[13] showed a correlation between H-1′ (4.75 ppm) and the C-4-carbon (70.9 ppm). The ^{13}C-NMR data were in accordance with the proposed structure (see Table 2). Treatment of **23** with hydrogen bromide in acetic acid gave an α-bromide **24**. Elimination of the bromine and the 2-benzoyloxy group was achieved with zinc-copper reagent in acetate buffer to

Table 2. ^{13}C-NMR Data (δ) for Selected Compounds

Carbon atom	Compound								
	23	**24**	**25**	**26α**	**26β**	**27α**	**27β**	**28α**	**28β**
C-1	90.9	89.0	145.8	97.4	98.1	92.7	96.5	94.9	96.7
2	67.3	68.0	97.9	57.0	58.6	58.9	63.2	58.0	61.0
3	70.8	73.2	64.5	71.3	71.1	70.7	73.4	71.2	73.3
4	70.9	71.2	70.2	71.0	70.8	69.3	69.3	71.0	72.9
5	73.9	73.5	74.1	73.7	74.4	74.8	73.4	74.1	73.6
6	63.5	63.3	62.7	63.4	63.4	64.0	63.9	63.9	63.2
C-1′	100.2	101.2	100.3	101.4	101.3	101.4	101.2	101.4	101.2
2′	68.9	68.9	68.7	69.3	68.9	68.3	68.3	69.2	69.2
3′	70.7	70.7	70.7	70.5	70.7	70.7	70.7	70.6	70.7
4′	66.9	66.9	67.0	66.7	66.9	66.9	67.1	66.8	66.8
5′	70.9	70.9	71.0	70.9	70.8	70.7	72.4	70.9	70.9
6′	61.1	61.1	61.2	61.1	61.1	61.1	61.1	61.9	61.4
OC(NH)								160.5	160.5
CCl_3								90.7	90.7

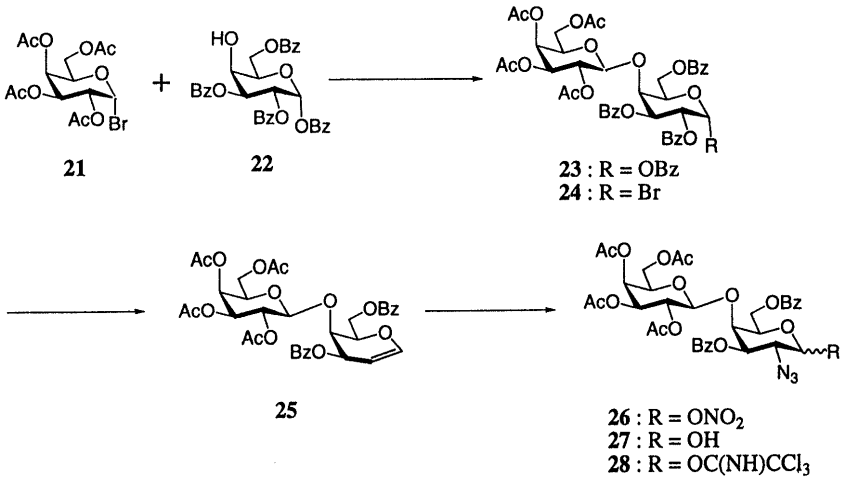

Chart 3

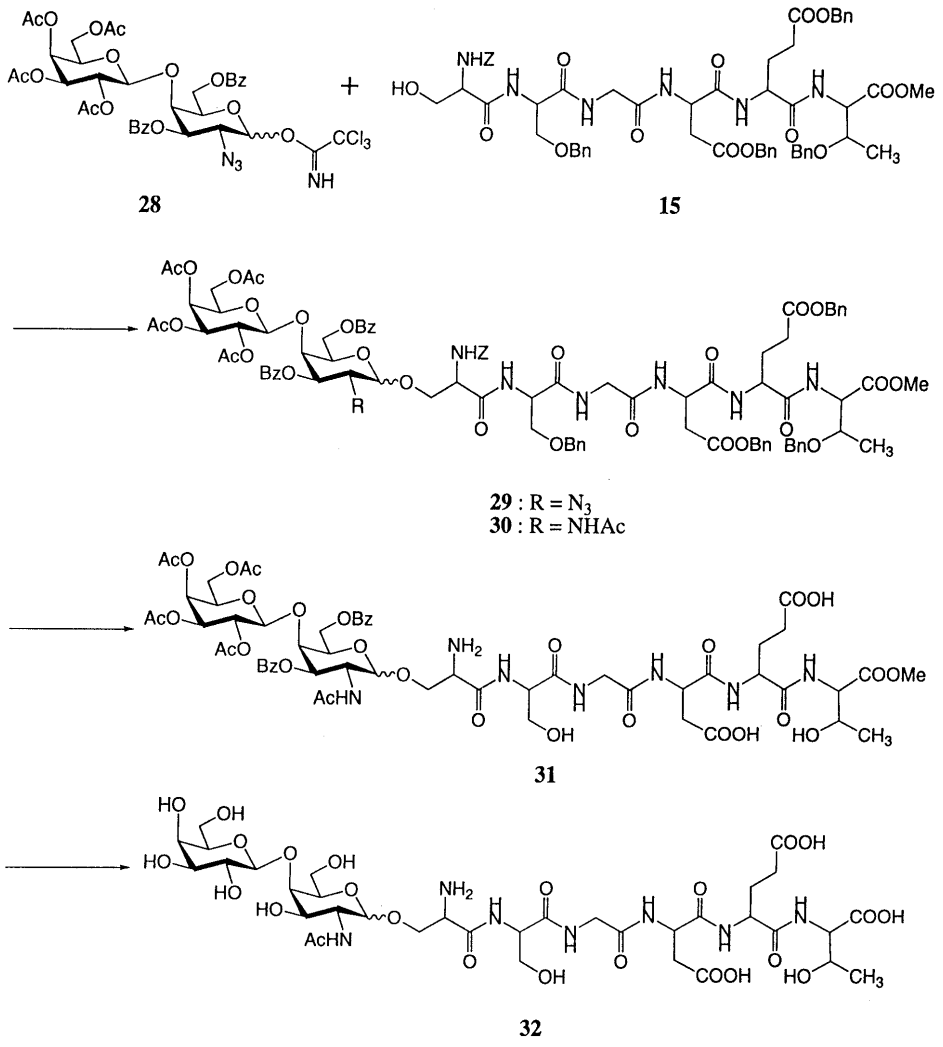

Chart 4

provide a galactal derivative **25**. The azidonitration[14] of compound **25** with NaN_3 and cerium(IV) ammonium nitrate (CAN) in CH_3CN gave a mixture of α and β nitrates **26**. Removal of the nitrate group was achieved with $NaNO_2$ in 1,4-dioxane at 80 °C, and the resultant alcohol **27** was transformed into the trichloroacetimidate **28** (Chart 3). Condensation of the diglycosyl donor **28** with the hexapeptide acceptor **15** in the presence of TMSOTf in dichloromethane at −30 °C gave the diglycosyl hexapeptide derivatives **29**α (31%) and **29**β (22%). Reduction of **29**α and **29**β, followed by *N*-acetylation afforded compounds **30**α and **30**β in 86% and 93% yields, respectively. The benzyl ester, benzyl ether and Z group were cleaved from **30**α and **30**β by hydrogenolysis (Pd–C) to give **31**α and **31**β in 66% and 68% yields, respectively. Removal of the acetyl, benzoyl and methyl ester groups of **31**α or **31**β with NaOMe in aqueous MeOH gave **32**α (suppressin B) or **32**β, respectively, each in 71% yield (Chart 4).

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. ^{1}H-NMR and ^{13}C-NMR spectra were recorded with JEOL JNM EX-270 and JNM A 500 FT NMR 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. FAB-MS 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*-(*tert*-Butoxycarbonyl)-*O*-benzyl-L-seryl-glycine Methyl Ester (7)** A solution of *N*-(*tert*-butoxycarbonyl)-*O*-benzyl-L-serine (**2**) (45 g, 150 mmol) and glycine methyl ester hydrochloride (**3**) (26 g, 210 mmol) was prepared in 6:1:1 CH_2Cl_2–DMF–THF (300 ml), then Et_3N (26 ml, 190 mmol) and diethylphosphorocyanidate (DEPC) (30 ml, 200 mmol) were added and the mixture was held at 0 °C for 1 h. It was allowed to warm to room temperature for 12 h, then diluted with $CHCl_3$, and the $CHCl_3$ solution was washed with water, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography with $CHCl_3$–MeOH (100:1) to give **7** (34 g, 44%). *Rf* 0.68 (20:1 $CHCl_3$–MeOH), 0.58 (2:1 benzene–acetone); $[\alpha]_D^{25}$ +21.0° (c=0.5, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 7.36—7.27 (m, 5 H, Ar), 7.04 (s, 1H, NH), 5.44 (s, 1H, NH), 4.56 (dd, 2H, J=11.6, 25.0 Hz, Bn-CH_2), 4.35 (br s, 1H, Ser α), 4.05 (d, 2H, J=5.5 Hz, Gly), 3.91 (d, 1H, J=6.4 Hz, Ser β-Ha), 3.74 (s, 3H, OMe), 3.61 (dd, 1H, J=6.4, 9.4 Hz, Ser β-Hb), 1.45 (s, 9H, 3 × *tert*-Bu-CH_3). ^{13}C-NMR ($CDCl_3$) δ: 53.9 (Ser α), 69.8 (Ser β), 41.3 (Gly), 52.3 (OMe), 28.3 (*tert*-Bu-CH_3), 80.3 (*tert*-Bu-C), 73.5 (Bn-CH_2). *Anal.* Calcd for $C_{18}H_{26}N_2O_6$: C, 59.00; H, 7.15; N, 7.65. Found: C, 58.63; H, 7.08; N, 7.57.

***N*-(*tert*-Butoxycarbonyl)-5-benzyl-L-glutamyl-*O*-benzyl-L-threonine Methyl Ester (8)** A solution of *N*-(*tert*-butoxycarbonyl)-*O*-benzyl-L-glutamic acid 5-benzyl ester (**5**) (2.7 g, 8.0 mmol) and *O*-benzyl-L-threonine methyl ester hydrochloride (**6**) (1.0 g, 3.9 mmol) was prepared in CH_2Cl_2 (15 ml), then Et_3N (0.6 ml, 4.2 mmol) and DEPC (1.4 ml, 9.3 mmol) were added and the mixture was held at 0 °C for 12 h. It was

diluted with $CHCl_3$, and the $CHCl_3$ solution was washed with water, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography with $CHCl_3$–MeOH (50 : 1) to give **8** (1.8 g, 88%). *Rf* 0.65 (4 : 1 benzene–acetone); $[\alpha]_D^{25}$ +2.0° (c=2.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 7.32—7.22 (m, 10H, Ar), 5.08 (s, 2H, Bn-CH_2), 4.64 (dd, 1H, J=2.4, 9.2 Hz, Thr α), 4.54, 4.35 (each d, 2H, J=11.8 Hz, Bn-CH_2), 4.33 (br s, 1H, Glu α), 4.13 (m, 1H, Thr β), 3.59 (s, 3H, OMe), 2.50 (br s, 2 H, Glu γ), 2.17, 1.98 (each m, 2H, Glu β), 1.41 (s, 9H, 3 × *tert*-Bu-CH_3), 1.18 (d, 3H, J=6.1 Hz, Thr γ). ^{13}C-NMR ($CDCl_3$) δ: 53.6 (Glu α), 27.9 (Glu β), 30.4 (Glu γ), 56.7 (Thr α), 73.9 (Thr β), 16.0 (Thr γ), 52.2 (OMe), 28.3 (*tert*-Bu-CH_3), 79.7 (*tert*-Bu-C), 70.6, 66.3 (Bn-CH_2). *Anal.* Calcd for $C_{29}H_{38}N_2O_8$: C, 64.19; H, 7.06; N, 5.16. Found: C, 64.00; H, 7.23; N, 5.46.

***O*-Benzyl-L-seryl-glycine Methyl Ester (9)** A solution of compound **7** (600 mg, 1.6 mmol) in trifluoroacetic acid (2.0 ml) was stirred for 1 h at room temperature, then diluted with $CHCl_3$, and the $CHCl_3$ solution was washed with water, dried over Na_2SO_4, filtered, and concentrated. The residue was chromatographed on silica gel with $CHCl_3$–MeOH (5 : 1) to give **9** (470 mg, quant.). *Rf* 0.32 (20 : 1 $CHCl_3$–MeOH); $[\alpha]_D^{25}$ +11.1° (c=1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 7.28 (m, 5H, Ar), 4.61 (d, 2H, J=5.5 Hz, Bn-CH_2), 4.20 (dd, 1H, J=3.7, 6.1 Hz, Ser α), 4.00 (d, 2H, J=6.7 Hz, Gly), 3.90 (dd, 1H, J=3.7, 10.4 Hz, Ser β-Ha), 3.80 (dd, 1H, J=6.1, 10.4 Hz, Ser β-Hb), 3.70 (s, 3H, OMe). ^{13}C-NMR ($CDCl_3$) δ: 53.2 (Ser α), 67.9 (Ser β), 41.2 (Gly), 52.3 (OMe), 73.6 (Bn-CH_2). *Anal.* Calcd for $C_{13}H_{18}N_2O_4$: C, 58.64; H, 6.81; N, 10.52. Found: C, 58.33; H, 6.55; N, 10.45.

5-Benzyl-L-glutamyl-*O*-benzyl-L-threonine Methyl Ester (10) A solution of compound **8** (940 mg, 1.7 mmol) in CH_2Cl_2 (4.0 ml) was treated with trifluoroacetic acid (2.0 ml) and the solution was stirred for 20 min at room temperature, then concentrated *in vacuo*. The residue was chromatographed on silica gel with $CHCl_3$–MeOH (30 : 1) to give **10** (570 mg, 74%). *Rf* 0.65 (10 : 1 $CHCl_3$–MeOH); $[\alpha]_D^{24}$ +4.8° (c=2.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 7.30—7.16 (m, 10H, Ar), 5.00 (s, 2H, Bn-CH_2), 4.64 (dd, 1H, J=2.4, 6.1 Hz, Thr α), 4.50, 4.28 (each d, 2H, J=11.9 Hz, Bn-CH_2), 4.35 (br s, 1H, Glu α), 4.11 (dd, 1H, J=1.8, 6.1 Hz, Thr β), 3.54 (s, 3 H, OMe), 2.67 (m, 2H, Glu γ), 2.22, 2.16 (each m, 2H, Glu β), 1.17 (d, 3H, J=6.1 Hz, Thr γ). ^{13}C-NMR ($CDCl_3$) δ: 52.4 (Glu α), 26.6 (Glu β), 29.2 (Glu γ), 57.2 (Thr α), 73.5 (Thr β), 15.6 (Thr γ), 52.3 (OMe), 70.5, 66.9 (Bn-CH_2). *Anal.* Calcd for $C_{24}H_{30}N_2O_6$: C, 65.14; H, 6.83; N, 6.33. Found: C, 65.30; H, 6.78; N, 6.01.

***N*-(Carbobenzoxy)-L-seryl-*O*-benzyl-L-seryl-glycine Methyl Ester (11)** A solution of *N*-(carbobenzoxy)-L-serine (**1**) (350 mg, 1.5 mmol) and compound **9** (390 mg, 1.5 mmol) was prepared in 3 : 1 CH_2Cl_2–THF (4.0 ml), then Et_3N (0.3 ml, 2.1 mmol) and DEPC (0.5 ml, 3.3 mmol) were added and the mixture was held at 0 °C for 4 h. It was diluted with $CHCl_3$, and the $CHCl_3$ solution was washed with water, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography with $CHCl_3$–MeOH (40 : 1) to give **11** (240 mg, 34%), which was recrystallized from MeOH. mp 123—125 °C. *Rf* 0.54 (10 : 1 $CHCl_3$–MeOH); $[\alpha]_D^{25}$ +7.2° (c=0.9, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 7.32—7.24 (m, 10H, Ar), 5.08 (br d, 2H, Z-CH_2), 4.71 (s, 1H, Ser α), 4.51 (br d, 2H, Bn-CH_2), 4.34 (t, 1H, J=3.7 Hz, Ser α), 3.64 (s, 3H, OMe). ^{13}C-NMR ($CDCl_3$) δ: 53.1, 55.9 (Ser α), 63.1, 69.1 (Ser β), 41.3 (Gly), 52.4 (OMe), 73.5 (Bn-CH_2), 67.2 (Z-CH_2). *Anal.* Calcd for $C_{24}H_{29}N_3O_8$: C, 59.13; H, 6.00; N, 8.62. Found: C, 59.00; H, 6.15; N, 8.39.

***N*-(*tert*-Butoxycarbonyl)-4-benzyl-L-aspartyl-5-benzyl-L-glutamyl-*O*-benzyl-L-threonine Methyl Ester (12)** A solution of *N*-(*tert*-butoxycarbonyl)-*O*-benzyl-L-aspertic acid 4-benzyl ester (**4**) (560 mg, 1.7 mmol) and compound **10** (390 mg, 0.87 mmol) was prepared in CH_2Cl_2 (5.0 ml), then Et_3N (0.13 ml, 0.91 mmol) and DEPC (0.26 ml, 1.7 mmol) were added and the mixture was held at 0 °C for 14 h. It was diluted with $CHCl_3$, and the $CHCl_3$ solution was washed with water, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography with $CHCl_3$–MeOH (50 : 1) to give **12** (450 mg, 69%). *Rf* 0.48 (4 : 1 benzene–acetone); $[\alpha]_D^{25}$ +2.8° (c=2.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 7.33—7.23 (m, 15H, Ar), 5.10 (d, 2H, J=2.4 Hz, Bn-CH_2), 5.05 (dd, 2H, J=12.2, 25.0 Hz, Bn-CH_2), 4.64—4.59 (m, 2H, Glu α, Thr α), 4.55, 4.35 (each d, 2H, J=11.9 Hz, Bn-CH_2), 4.52 (br s, 1H, Asp α), 4.13 (m, 1H, Thr β), 3.61 (s, 3H, OMe), 3.02 (dd, 1H, J=3.7, 17.1 Hz, Asp α-Ha), 2.71 (dd, 1H, J=5.5, 17.1 Hz, Asp α-Hb), 2.53 (m, 2H, Glu γ), 2.21, 2.00 (each m, 2H, Glu β), 1.43 (s, 9H, 3 × *tert*-Bu-CH_3), 1.17 (d, 3 H, J=6.7 Hz, Thr γ). ^{13}C-NMR ($CDCl_3$) δ: 50.8 (Asp α), 36.1 (Asp β), 52.4 (Glu α), 28.0 (Glu β), 30.0 (Glu γ), 56.8 (Thr α), 73.7 (Thr β), 16.2 (Thr γ), 52.3 (OMe), 28.2 (*tert*-Bu-CH_3), 80.5 (*tert*-Bu-C), 70.7, 66.8, 66.4 (Bn-CH_2). *Anal.* Calcd for $C_{40}H_{49}N_3O_{11}$: C, 64.24; H, 6.60; N, 5.62. Found: C, 65.25; H, 6.23; N, 5.32.

***N*-(Carbobenzoxy)-L-seryl-*O*-benzyl-L-seryl-glycine (13)** A solution of compound **11** (60 mg, 0.12 mmol) in 5 : 1 MeOH–H_2O (3.0 ml) was treated with NaOMe (30 mg) at room temperature for 30 min. The mixture was neutralized with Amberlite IR-120 (H^+), filtered, and concentrated. The residue was purified by column chromatography with $CHCl_3$–MeOH (10 : 1) to give **13** (55 mg, 94%), which was recrystallized from MeOH. mp 105—107 °C. *Rf* 0.37 (3 : 2 $CHCl_3$–MeOH); $[\alpha]_D^{25}$ +4.9° (c=1.0, $CHCl_3$). ^{1}H-NMR (CD_3OD) δ: 7.34—7.22 (m, 10H, Ar), 5.09 (d, J=4.9 Hz, 2H, Z-CH_2), 4.67 (br s, 1H, Ser α), 4.53 (d, J=3.7 Hz, 2H, Bn-CH_2), 4.29 (t, J=5.8 Hz, 1H, Ser α). ^{13}C-NMR (CD_3OD) δ: 58.3, 54.8 (Ser α), 70.4, 63.2 (Ser β), 41.9 (Gly), 74.2 (Bn-CH_2), 67.9 (Z-CH_2). *Anal.* Calcd for $C_{23}H_{27}N_3O_8$: C, 58.35; H, 5.75; N, 8.87. Found: C, 58.56; H, 5.77; N, 9.12.

4-Benzyl-L-aspartyl-5-benzyl-L-glutamyl-*O*-benzyl-L-threonine Methyl Ester (14) A solution of compound **12** (340 mg, 0.46 mmol) in CH_2Cl_2 (2.0 ml) was treated with trifluoroacetic acid (1.0 ml) and the solution was stirred for 20 min at room temperature, then concentrated *in vacuo*. The residue was chromatographed on silica gel with $CHCl_3$–MeOH (30 : 1) to give **14** (250 mg, 84%). *Rf* 0.69 (10 : 1 $CHCl_3$–MeOH); $[\alpha]_D^{25}$ +7.5° (c=1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 7.29—7.16 (m, 15H, Ar), 5.08—4.99 (m, 4H, 2 × Bn-CH_2), 4.62—4.57 (m, 2H, Glu α, Thr α), 4.51 (t, 1H, J=6.7 Hz, Asp α), 4.48, 4.29 (each d, 2H, J=11.9 Hz, Bn-CH_2), 4.04 (ddd, 1H, J=2.4, 6.1, 12.8 Hz, Thr β), 3.53 (s, 3H, OMe), 3.00 (d, 2H, J=6.1 Hz, Asp β), 2.48 (t, 2H, J=7.3 Hz, Glu γ), 2.14, 2.00 (each m, 2H, Glu β), 1.09 (d, 3H, J=6.1 Hz, Thr γ). ^{13}C-NMR ($CDCl_3$) δ: 49.7 (Asp α), 34.8 (Asp β), 53.3 (Glu α), 27.2 (Glu β), 30.1 (Glu γ), 56.8 (Thr α), 73.6 (Thr β), 15.9 (Thr γ), 52.2 (OMe), 70.5, 67.5, 66.5 (Bn-CH_2). *Anal.* Calcd for $C_{35}H_{41}N_3O_9$: C, 64.90; H, 6.38; N, 6.49. Found: C, 64.85; H, 6.12; N, 6.65.

***N*-(Carbobenzoxy)-L-seryl-*O*-benzyl-L-seryl-glycyl-4-benzyl-L-aspartyl-5-benzyl-L-glutamyl-*O*-benzyl-L-threonine Methyl Ester (15)** A solution of compound **13** (230 mg, 0.47 mmol) and compound **14** (230 mg, 0.36 mmol) in CH_2Cl_2 (3.0 ml) were treated with EEDQ (230 mg, 0.93 mmol) at 0 °C and the mixture was allowed to stand at room temperature for 24 h. It was diluted with $CHCl_3$, and the $CHCl_3$ solution was washed with water, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography with $CHCl_3$–MeOH (50 : 1) to give **15** (240 mg, 62%). *Rf* 0.61 (10 : 1 $CHCl_3$–MeOH); $[\alpha]_D^{24}$ +15.3° (c=1.0, $CHCl_3$). ^{1}H-NMR (CD_3OD) δ: 7.31—7.23 (m, 25H, Ar), 5.10—5.04 (m, 6H, 2 × Bn-CH_2, Z-CH_2), 4.79 (dd, 1H, J=5.5, 7.3 Hz, Asp α), 4.29 (t, J=5.5 Hz, 1H, Ser α), 4.13 (m, 1H, Thr β), 3.62 (s, 3H, OMe), 2.92 (dd, 1H, J=5.5, 16.5 Hz, Asp β-Ha), 2.81 (dd, 1H, J=7.3, 16.5 Hz, Asp β-Hb), 2.50 (m, 2H, Glu γ), 2.18, 2.04 (each m, 2H, Glu β), 1.18 (d, 3H, J=6.7 Hz, Thr γ). ^{13}C-NMR (CD_3OD) δ: 58.3, 55.8 (Ser α), 70.1, 63.4 (Ser β), 44.2 (Gly), 51.4 (Asp α), 36.9 (Asp β), 54.2 (Glu α), 28.3 (Glu β), 31.4 (Glu γ), 58.5 (Thr α), 75.6 (Thr β), 16.6 (Thr γ), 52.8 (OMe), 74.4, 72.1, 68.1, 67.5 (Bn-CH_2), 67.8 (Z-CH_2). *Anal.* Calcd for $C_{58}H_{66}N_6O_{16}$: C, 63.15; H, 6.03; N, 7.62. Found: C, 62.96; H, 5.88; N, 7.51.

***N*-(Carbobenzoxy)-*O*-(3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-L-seryl-*O*-benzyl-L-seryl-glycine Methyl Ester (17α) and *N*-(carbobenzoxy)-*O*-(3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranosyl)-L-seryl-*O*-benzyl-L-seryl-glycine Methyl Ester (17β)** A suspension of 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl trichloroacetimidate (**16α**) (220 mg, 0.45 mmol), compound **11** (220 mg, 0.45 mmol) and molecular sieves (AW 300) (250 mg) in dry CH_2Cl_2 (5.0 ml) was stirred at room temperature for 1 h, then 10% TMSOTf/CH_2Cl_2 (0.1 ml) was added at −30 °C. The mixture was stirred under an argon atmosphere for 14 h, 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 with $CHCl_3$–MeOH (100 : 1) to provide an anomeric mixture of **17α** and **17β** (230 mg, 63%).

The same procedure from 4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-galactpyranosyl trichloroacetimidate (**16β**) and compound **11** gave an anomeric mixture of **17α** and **17β** (160 mg, 52%). *Rf* 0.82 (8 : 1 $CHCl_3$–MeOH). Compound **17α**: ^{1}H-NMR ($CDCl_3$) δ: 7.36—7.26 (m, 10H, Ar), 5.11 (br s, 1H, H-1), 4.56 (s, 2H, Bn-CH_2), 3.73 (s, 3H, OMe), 2.08, 2.04, 2.01 (each s, 9H, 3 × OAc). Compound **17β**: ^{1}H-NMR ($CDCl_3$) δ: 7.36—7.26 (m, 10H, Ar), 4.45 (d, J=7.9 Hz, 1H, H-1), 4.55 (d, 2H, J=2.5 Hz, Bn-CH_2), 3.73 (s, 3H, OMe), 2.14, 2.01, 2.00 (each s, 9H,

3 × OAc).

***N*-(Carbobenzoxy)-*O*-(3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-seryl-*O*-benzyl-L-seryl-glycine Methyl Ester (18α) and *N*-(Carbobenzoxy)-*O*-(3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-galactopyranosyl)-L-seryl-*O*-benzyl-L-seryl-glycine Methyl Ester (18β)** A solution of compound **17α** and **17β** (230 mg, 0.29 mmol), $NiCl_2 \cdot 6H_2O$ (3.1 g, 12.9 mmol) and H_3BO_3 (560 mg, 9.5 mmol) in a mixture of EtOH (16 ml) and AcOEt (4.0 ml) was treated with an EtOH solution of $NaBH_4$ with stirring until the solution became black. Stirring was continued at room temperature for 1 h. Then, Ac_2O (2.3 ml) was added and the mixture was stirred for 4 h, then filtered through the 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 concentrated. The residue was chromatographed on silica gel with $CHCl_3$–MeOH (80 : 1) to provide **18α** (150 mg, 64%) and **18β** (77 mg, 33%).

Data for **18α**; *Rf* 0.64 (10 : 1 $CHCl_3$–MeOH); $[\alpha]_D^{25}$ +95.1° (*c* = 0.4, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ: 7.37—7.28 (m, 10H, Ar), 5.11 (s, 2H, Z-CH_2), 4.98 (d, 1H, *J* = 2.9 Hz, H-1), 4.58 (d, 2H, *J* = 4.8 Hz, Bn-CH_2), 3.84 (s, 3H, OMe), 2.13, 2.00, 1.96, 1.94 (each s, 12H, 4 × Ac). *Anal.* Calcd for $C_{38}H_{48}N_4O_{16}$: C, 55.88; H, 5.92; N, 6.86. Found: C, 55.54; H, 5.63; N, 6.90.

Data for **18β**; *Rf* 0.56 (10 : 1 $CHCl_3$–MeOH); $[\alpha]_D^{25}$ +0.9° (*c* = 0.5, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 7.36—7.24 (m, 10H, Ar), 5.09 (s, 2H, Z-CH_2), 4.76 (d, 1H, *J* = 8.6 Hz, H-1), 4.56 (br s, 2 H, Bn-CH_2), 3.72 (s, 3H, OMe), 2.09, 2.02, 1.98, 1.84 (each s, 12H, 4 × Ac). *Anal.* Calcd for $C_{38}H_{48}N_4O_{16}$: C, 55.88; H, 5.92; N, 6.86. Found: C, 55.58; H, 5.70; N, 6.79.

3,4,6-Tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-galactopyranosyl-L-seryl-L-seryl-glycine Methyl Ester (19α) A solution of compound **18α** (150 mg, 0.18 mmol) in 2 : 1 MeOH–AcOH (2.0 ml) was stirred with 10% Pd–C (75 mg) for 12 h under an H_2 atmosphere, then filtered through a pad of Celite and concentrated to dryness. The residue was chromatographed on silica gel with $CHCl_3$–MeOH (5 : 1) to provide **19α** (87 mg, 79%). *Rf* 0.35 (5 : 1 $CHCl_3$–MeOH); $[\alpha]_D^{25}$ +73.2° (*c* = 0.4, MeOH). ^{1}H-NMR (CD_3OD) δ: 5.00 (d, 1H, *J* = 3.6 Hz, H-1), 3.37 (s, 3H, OMe), 2.14, 2.03, 1.98, 1.94 (each s, 12H, 4 × Ac). *Anal.* Calcd for $C_{23}H_{36}N_4O_{14}$: C, 46.62; H, 6.12; N, 9.46. Found: C, 46.76; H, 5.82; N, 9.53.

3,4,6-Tri-*O*-acetyl-2-acetamido-2-deoxy-β-D-galactopyranosyl-L-seryl-L-seryl-glycine Methyl Ester (19β) A solution of compound **18β** (77 mg, 0.094 mmol) in 2 : 1 MeOH–AcOH (2.0 ml) was stirred with 10% Pd–C (38 mg) for 12 h under an H_2 atmosphere, then filtered through a pad of Celite and concentrated to dryness. The residue was chromatographed on silica gel with $CHCl_3$–MeOH (5 : 1) to provide **19β** (46 mg, 82%). *Rf* 0.25 (8 : 1 $CHCl_3$–MeOH); $[\alpha]_D^{25}$ −5.7° (*c* = 0.3, MeOH). ^{1}H-NMR ($CDCl_3$) δ: 4.66 (d, 1H, *J* = 8.0 Hz, H-1), 3.75 (s, 3H, OMe), 2.16, 2.05, 1.99, 1.97 (each s, 12H, 4 × Ac). *Anal.* Calcd for $C_{23}H_{36}N_4O_{14}$: C, 46.62; H, 6.12; N, 9.46. Found: C, 46.64; H, 5.82; N, 9.00.

2-Acetamido-2-deoxy-α-D-galactopyranosyl-L-seryl-L-seryl-glycine (20α, Supprescin A) A solution of compound **19α** (53 mg, 0.089 mmol) in 3 : 1 MeOH–H_2O (2.0 ml) was treated with NaOMe (30 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 with MeOH–H_2O (3 : 1) provide **20α** (24 mg, 60%). *Rf* 0.47 (1 : 3 : 1 $CHCl_3$–MeOH–H_2O); $[\alpha]_D^{25}$ +8.9° (*c* = 0.5, H_2O). ^{1}H-NMR (D_2O) δ: 4.92 (d, 1H, *J* = 3.7 Hz, H-1), 4.58 (t, 1H, *J* = 5.2 Hz, Ser α), 4.24 (t, 1H, *J* = 4.3 Hz, Ser α). FAB-MS *m/z*: 453 $(M+H)^+$. *Anal.* Calcd for $C_{16}H_{28}N_4O_{11}$: C, 42.48; H, 6.24; N, 12.38. Found: C, 42.66; H, 6.02; N, 12.05.

2-Acetamido-2-deoxy-β-D-galactopyranosyl-L-seryl-L-seryl-glycine (20β) A solution of compound **19β** (46 mg, 0.078 mmol) in 3 : 1 MeOH–H_2O (2.0 ml) was treated with NaOMe (26 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 with MeOH–H_2O (3 : 1) to provide **20β** (28 mg, 80%). *Rf* 0.43 (1 : 3 : 1 $CHCl_3$–MeOH–H_2O); $[\alpha]_D^{25}$ −2.5° (*c* = 0.5, H_2O). ^{1}H-NMR (D_2O) δ: 4.53 (d, 1H, *J* = 5.5 Hz, H-1), 4.51 (t, 1H, *J* = 8.5 Hz, Ser α), 4.14 (t, 1H, *J* = 5.4 Hz, Ser α). FAB-MS *m/z*: 453 $(M+H)^+$. *Anal.* Calcd for $C_{16}H_{28}N_4O_{11}$: C, 42.48; H, 6.24; N, 12.38. Found: C, 42.02; H, 6.14; N, 12.11.

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-1,2,3,6-tetra-*O*-benzoyl-α-D-galactopyranose (23) A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**21**) (1.4 g, 3.4 mmol) in dry toluene (8.0 ml) was added dropwise with stirring to a solution of 1,2,3,6-tetra-*O*-benzoyl-α-D-galactopyranose (**22**) (1.0 g, 1.7 mmol), silver trifluoromethanesulfonate (1.3 g, 5.1 mmol), 2,4,6-trimethylpyridine (500 mg) and molecular sieves (AW 300) (1.0 g) in dry toluene (8.0 ml) at −10 °C in the dark. The mixture was allowed to attain room temperature, then stirred overnight. It was 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 with benzene–acetone (40 : 1) to provide **23** (870 mg, 56%). *Rf* 0.57 (4 : 1 benzene–acetone); $[\alpha]_D^{25}$ +25.0° (*c* = 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 8.11—7.25 (m, 20H, Ar), 6.76 (d, 1H, *J* = 3.7 Hz, H-1), 4.75 (d, 1H, *J* = 7.9 Hz, H-1′), 2.27, 2.16, 2.01, 1.92 (each s, 12H, 4 × OAc). *Anal.* Calcd for $C_{48}H_{46}O_{19}$: C, 62.20; H, 5.00. Found: C, 61.95; H, 4.92.

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-galactopyranosyl bromide (24) A solution of compound **23** (680 mg, 0.73 mmol) in $CHCl_3$ (6.0 ml) was treated with 25% HBr/AcOH (5.0 ml). The mixture was stirred at 0 °C for 2 h, then diluted with $CHCl_3$, and the $CHCl_3$ solution was washed with water, dried over Na_2SO_4, filtered, and concentrated to give **24** (640 mg, 99%). *Rf* 0.59 (4 : 1 benzene–acetone); $[\alpha]_D^{25}$ +19.8° (*c* = 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 8.09—7.33 (m, 15H, Ar), 6.76 (d, 1H, *J* = 3.6 Hz, H-1), 4.68 (d, 1H, *J* = 7.9 Hz, H-1′), 2.20, 2.16, 2.00, 1.94 (each s, 12H, 4 × OAc). *Anal.* Calcd for $C_{40}H_{39}BrO_{17}$: C, 55.12; H, 4.51. Found: C, 54.92; H, 4.51.

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-1,5-anhydro-3,6-di-*O*-benzoyl-2-deoxy-D-lyxo-hex-1-enitol (25) A solution of compound **24** (910 mg, 1.0 mmol) in AcOEt (3.0 ml) was added with stirring to a Zn–Cu reagent that had been prepared by addition of Zn dust (1.9 g) to acetate buffer [AcONa · $3H_2O$ (130 mg)/60% AcOH–H_2O (13 ml)] containing $CuSO_4 \cdot 5H_2O$ (14 mg). The mixture was stirred at room temperature for 3 h, then filtered through a pad of Celite, and AcOEt and water were added to the filtrate. The organic layer was separated, washed with water, dried over Na_2SO_4, filtered, and concentrated. The residue was chromatographed on silica gel with $CHCl_3$ to provide **25** (620 mg, 88%). *Rf* 0.59 (4 : 1 benzene–acetone); $[\alpha]_D^{25}$ +15.5° (*c* = 1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 8.09—7.33 (m, 15H, Ar), 6.47 (d, 1H, *J* = 6.1 Hz, H-1), 4.77 (d, 1H, *J* = 7.9 Hz, H-1′), 2.20, 2.16, 2.00, 1.94 (each s, 12H, 4 × OAc). *Anal.* Calcd for $C_{32}H_{30}O_{15}$: C, 59.65; H, 5.30. Found: C, 59.54; H, 5.31.

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzoyl-2-azido-2-deoxy-D-galactopyranosyl Nitrate (26) A solution of compound **25** (26 mg, 0.038 mmol), NaN_3 (6.0 mg) and cerium(IV) ammonium nitrate (CAN) (60 mg) in CH_3CN (1.0 ml) was stirred at −15 °C for 2 h, and then 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 with benzene–acetone (25 : 1) to provide an anomeric mixture of **26** (19 mg, 64%). *Rf* 0.59 (4 : 1 benzene–acetone).

The α-anomer: ^{1}H-NMR ($CDCl_3$) δ: 8.17—7.33 (m, 10H, Ar), 6.43 (d, 1H, *J* = 4.0 Hz, H-1), 4.57 (d, 1H, *J* = 7.9 Hz, H-1′), 2.15, 2.13, 2.00, 1.93 (each s, 12H, 4 × OAc).

The β-anomer: ^{1}H-NMR ($CDCl_3$) δ: 8.17—7.33 (m, 10H, Ar), 5.64 (d, 1H, *J* = 8.5 Hz, H-1), 4.76 (d, 1H, *J* = 8.9 Hz, H-1′), 2.15, 2.14, 2.00, 1.94 (each s, 12H, 4 × OAc).

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzoyl-2-azido-2-deoxy-D-galactopyranose (27) A solution of compound **26** (150 mg, 0.19 mmol) in 1,4-dioxane (2.0 ml) was added with stirring to an $NaNO_2$ (250 mg) solution in 0.4 ml of H_2O. The mixture was stirred at 80 °C for 22 h, then 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 with $CHCl_3$–MeOH (100 : 1) to provide an anomeric mixture of **27** (77 mg, 54%). *Rf* 0.40 (8 : 1 $CHCl_3$–MeOH).

The α-anomer: ^{1}H-NMR ($CDCl_3$) δ: 8.19—7.40 (m, 10H, Ar), 5.51 (d, 1H, *J* = 3.1 Hz, H-1), 4.63 (d, 1H, *J* = 7.9 Hz, H-1′), 2.14, 2.13, 2.00, 1.92 (each s, 12H, 4 × OAc).

The β-anomer: ^{1}H-NMR ($CDCl_3$) δ: 8.19—7.40 (m, 10H, Ar), 4.73 (d, 1H, *J* = 7.9 Hz, H-1), 4.64 (d, 1H, *J* = 6.7 Hz, H-1′), 2.16, 2.15, 2.13, 1.91 (each s, 12H, 4 × OAc).

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-3,6-di-*O*-benzoyl-2-azido-2-deoxy-D-galactopyranosyl Trichloroacetimidate (28) A solution of compound **27** (76 mg, 0.10 mmol) and K_2CO_3 (100 mg) in CH_2Cl_2 (1.5 ml) was treated with CCl_3CN (0.1 ml). Stirring was continued at 0 °C for 4 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 with benzene–acetone (50 : 1) to provide an anomeric mixture of **28** (77 mg, 54%). *Rf* 0.64 (8 : 1 $CHCl_3$–MeOH).

The α-anomer: ^{1}H-NMR ($CDCl_3$) δ: 8.19—7.41 (m, 10H, Ar), 6.61 (d, 1H, J=3.7 Hz, H-1), 4.60 (d, 1H, J=7.9 Hz, H-1′), 2.16, 2.14, 2.00, 1.96 (each s, 12H, 4×OAc).

The β-anomer: ^{1}H-NMR ($CDCl_3$) δ: 8.19—7.41 (m, 10H, Ar), 5.77 (d, 1H, J=7.9 Hz, H-1), 4.65 (d, 1H, J=7.3 Hz, H-1′), 2.15, 2.14, 2.00, 1.92 (each s, 12H, 4×OAc).

***N*-(Carbobenzoxy)-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-*O*-benzoyl-2-azido-2-deoxy-α-D-galactopyranosyl)-L-seryl-*O*-benzyl-L-seryl-glycyl-4-benzyl-L-aspartyl-5-benzyl-L-glutamyl-*O*-benzyl-L-threonine Methyl Ester (29α) and *N*-(Carbobenzoxy)-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-*O*-benzoyl-2-azido-2-deoxy-β-D-galactopyranosyl)-L-seryl-*O*-benzyl-L-seryl-glycyl-4-benzyl-L-aspartyl-5-benzyl-L-glutamyl-*O*-benzyl-L-threonine Methyl Ester (29β)** A solution of compound **28** (230 mg, 0.26 mmol), compound **15** (240 mg, 0.22 mmol) and molecular sieves (AW 300) (400 mg) in dry CH_2Cl_2 (5 ml) was stirred at room temperature for 5 h, then 10% TMSOTf/CH_2Cl_2 (0.2 ml) was added at −30 °C. The whole was stirred under an argon atmosphere for 14 h, then diluted with $CHCl_3$ and filtered through the 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 with $CHCl_3$–MeOH (100 : 1) to provide **29α** (120 mg, 31%) and **29β** (83 mg, 22%).

Data for **29α**: *Rf* 0.65 (10 : 1 $CHCl_3$–MeOH); $[\alpha]_D^{25}$ +17.8° (c=1.0, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 8.06—7.99, 7.47—7.21 (each m, 30H, Ar), 5.24 (d, 1H, J=3.7 Hz, H-1), 5.06—5.01 (m, 6H, Z-CH_2, 2×Bn-CH_2), 4.86 (br s, 1H, Asp α), 4.52 (d, 1H, J=7.8 Hz, H-1′), 3.81 (br s, 2H, Gly), 3.60 (s, 3H, OMe), 2.50 (m, 2H, Glu γ), 2.03, 1.98, 1.96, 1.94 (each s, 12H, 4×OAc), 1.14 (dd, 3H, J=6.1, 9.2 Hz, Thr γ). *Anal.* Calcd for $C_{92}H_{101}N_9O_{31}$: C, 60.42; H, 5.57; N, 6.89. Found: C, 60.22; H, 5.65; N, 6.75.

Data for **29β**; *Rf* 0.62 (10 : 1 $CHCl_3$–MeOH); $[\alpha]_D^{25}$ +15.9° (c=0.5, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 8.06—7.99, 7.36—7.24 (each m, 30H, Ar), 5.10—5.03 (m, 6H, Z-CH_2, 2×Bn-CH_2), 4.87 (br s, 1H, Asp α), 4.60 (d, 1H, J=7.7 Hz, H-1), 4.45 (d, 1H, J=7.5 Hz, H-1′), 3.62 (s, 3H, OMe), 2.50 (m, 2H, Glu γ), 2.08, 2.03, 1.97, 1.96 (each s, 12H, 4×OAc). 1.16 (br s, 3H, Thr γ). *Anal.* Calcd for $C_{92}H_{101}N_9O_{31}$: C, 60.42; H, 5.57; N, 6.89. Found: C, 60.28; H, 5.73; N, 6.86.

***N*-(Carbobenzoxy)-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-*O*-benzoyl-2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-seryl-*O*-benzyl-L-seryl-glycyl-4-benzyl-L-aspartyl-5-benzyl-L-glutamyl-*O*-benzyl-L-threonine Methyl Ester (30α)** A stirred solution of compound **29α** (74 mg, 0.040 mmol), $NiCl_2 \cdot 6H_2O$ (360 mg, 1.5 mmol) and H_3BO_3 (180 mg, 2.9 mmol) in EtOH (15 ml) was treated dropwise with $NaBH_4$ in EtOH at 0 °C until the color changed to black. After 1 h, Ac_2O (10 ml) was added, then after 4 h, the mixture was diluted with $CHCl_3$, washed with water, dried over Na_2SO_4, filtered, and concentrated. The residue was chromatographed on silica gel with $CHCl_3$–MeOH (50 : 1) to provide **30α** (64 mg, 86%). *Rf* 0.56 (10 : 1 $CHCl_3$–MeOH); $[\alpha]_D^{25}$ −23.1° (c=0.4, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 8.04—8.01, 7.47—7.22 (each m, 30H, Ar), 5.23 (d, 1H, J=3.5 Hz, H-1), 5.10—5.03 (m, 6H, Z-CH_2, 2×Bn-CH_2), 4.87 (br s, 1H, Asp α), 4.77 (d, 1H, J=8.0 Hz, H-1′), 3.62 (s, 3H, OMe), 2.50 (m, 2H, Glu γ), 2.15, 2.08, 2.03, 1.97, 1.96 (each s, 15H, 5×Ac), 1.16 (br s, 3 H, Thr γ). *Anal.* Calcd for $C_{94}H_{105}N_7O_{32}$: C, 61.20; H, 5.74; N, 5.31. Found: C, 61.15; H, 5.55; N, 5.11.

***N*-(Carbobenzoxy)-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-(3,6-di-*O*-benzoyl-2-acetamido-2-deoxy-β-D-galactopyranosyl)-L-seryl-*O*-benzyl-L-seryl-glycyl-4-benzyl-L-aspartyl-5-benzyl-L-glutamyl-*O*-benzyl-L-threonine Methyl Ester (30β)** A solution of compound **29β** (64 mg, 0.035 mmol), $NiCl_2 \cdot 6H_2O$ (360 mg, 1.5 mmol) and H_3BO_3 (180 mg, 2.9 mmol) in EtOH (15 ml) was treated dropwise with $NaBH_4$ in EtOH at 0 °C until the color was changed to black. After 1 h, Ac_2O (10 ml) was added, then after 4 h, the mixture was diluted with $CHCl_3$, washed with water, dried over Na_2SO_4, filtered, and concentrated. The residue was chromatographed on silica gel with $CHCl_3$–MeOH (50 : 1) to provide **30β** (60 mg, 93%). *Rf* 0.53 (10 : 1 $CHCl_3$–MeOH); $[\alpha]_D^{25}$ −15.4° (c=0.5, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 8.06—8.02, 7.48—7.20 (each m, 30H, Ar), 5.11—5.03 (m, 6H, Z-CH_2, 2×Bn-CH_2), 4.87 (br s, 1H, Asp α), 4.72 (d, 1H, J=7.8 Hz, H-1′), 4.40 (d, 1H, J=7.5 Hz, H-1), 3.62 (s, 3H, OMe), 2.50 (m, 2H, Glu γ), 2.14, 2.09, 2.00, 1.98, 1.97 (each s, 15H, 5×Ac), 1.17 (br s, 3H, Thr γ). *Anal.* Calcd for $C_{94}H_{105}N_7O_{32}$: C, 61.20; H, 5.74; N, 5.31. Found: C, 61.33; H, 5.65; N, 5.22.

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-(3,6-di-*O*-benzoyl-2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-seryl-L-seryl-glycyl-L-aspartyl-L-glutamyl-L-threonine Methyl Ester (31α) A solution of compound **30α** (50 mg, 0.027 mmol) in 2 : 1 MeOH–AcOH (1.5 ml) was stirred with 10% Pd–C (25 mg) for 12 h under an H_2 atmosphere, then filtered through a pad of Celite and concentrated to dryness. The residue was chromatographed on silica gel with $CHCl_3$–MeOH (2 : 1) to provide **31α** (24 mg, 65%). *Rf* 0.74 (5 : 4 : 1 $CHCl_3$–MeOH–H_2O); $[\alpha]_D^{25}$ −10.2° (c=0.5, $CHCl_3$). ^{1}H-NMR (CD_3OD) δ: 8.04—8.00, 7.48—7.22 (each m, 10H, Ar), 5.23 (d, 1H, J=3.7 Hz, H-1), 4.85 (br s, 1H, Asp α), 4.75 (d, 1H, J=7.8 Hz, H-1′), 3.63 (s, 3H, OMe), 2.49 (m, 2H, Glu γ), 2.12, 2.05, 2.02, 1.98, 1.95 (each s, 15H, 5×Ac), 1.15 (br s, 3H, Thr γ). *Anal.* Calcd for $C_{58}H_{75}N_7O_{30}$: C, 51.59; H, 5.60; N, 7.26. Found: C, 51.67; H, 5.45; N, 7.35.

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-(3,6-di-*O*-benzoyl-2-acetamido-2-deoxy-β-D-galactopyranosyl)-L-seryl-L-seryl-glycyl-L-aspartyl-L-glutamyl-L-threonine Methyl Ester (31β) A solution of compound **30β** (40 mg, 0.022 mmol) in 2 : 1 MeOH–AcOH (1.5 ml) was stirred with 10% Pd–C (20 mg) for 12 h under an H_2 atmosphere, then filtered through a pad of Celite and concentrated to dryness. The residue was chromatographed on silica gel with $CHCl_3$–MeOH (2 : 1) to provide **31β** (20 mg, 68%). *Rf* 0.63 (5 : 4 : 1 $CHCl_3$–MeOH–H_2O); $[\alpha]_D^{25}$ −5.4° (c=0.5, $CHCl_3$). ^{1}H-NMR (CD_3OD) δ: 8.06—8.00, 7.47—7.20 (each m, 10H, Ar), 4.87 (br s, 1H, Asp α), 4.70 (d, 1H, J=8.0 Hz, H-1′), 4.42 (d, 1H, J=7.6 Hz, H-1), 3.62 (s, 3H, OMe), 2.50 (m, 2H, Glu γ), 2.15, 2.05, 2.03, 1.99, 1.98 (each s, 15H, 5×Ac), 1.16 (br s, 3H, Thr γ). *Anal.* Calcd for $C_{58}H_{75}N_7O_{30}$: C, 51.59; H, 5.60; N, 7.26. Found: C, 51.38; H, 5.80; N, 7.13.

β-D-Galactopyranosyl-(1→4)-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-seryl-L-seryl-glycyl-L-aspartyl-L-glutamyl-L-threonine (32α, Supprescin B) A solution of compound **31α** (16 mg, 0.012 mmol) in 3 : 1 MeOH–H_2O (2 ml) was treated with NaOMe (8 mg) at room temperature for 1 h. The mixture was neutralized with Amberlite IR-120 (H^+), filtered, and concentrated. The residue was chromatographed over Sephadex LH-20 with MeOH–H_2O (3 : 1) to provide **32α** (8 mg, 71%). *Rf* 0.68 (1 : 3 : 1 $CHCl_3$–MeOH–H_2O); $[\alpha]_D^{25}$ −5.4° (c=0.5, H_2O). ^{1}H-NMR (D_2O) δ: 4.87 (br s, 1H, Asp α), 4.84 (d, 1H, J=3.7 Hz, H-1), 4.41 (d, 1H, J=7.8 Hz, H-1′), 2.48 (m, 2H, Glu γ), 2.12 (s, 3H, Ac), 1.14 (br s, 3H, Thr γ). *Anal.* Calcd for $C_{35}H_{57}N_7O_{24}$: C, 43.80; H, 5.99; N, 10.21. Found: C, 43.58; H, 5.78; N, 10.46.

β-D-Galactopyranosyl-(1→4)-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-L-seryl-L-seryl-glycyl-L-aspartyl-L-glutamyl-L-threonine (32β) A solution of compound **31β** (12 mg, 0.0089 mmol) in 3 : 1 MeOH–H_2O (2 ml) was treated with NaOMe (6 mg) at room temperature for 1 h. The mixture was neutralized with Amberlite IR-120 (H^+), filtered, and concentrated. The residue was chromatographed over Sephadex LH-20 with MeOH–H_2O (3 : 1) to provide **32β** (6 mg, 71%). *Rf* 0.68 (1 : 3 : 1 $CHCl_3$–MeOH–H_2O); $[\alpha]_D^{25}$ −5.4° (c=0.5, H_2O). ^{1}H-NMR (D_2O) δ: 4.85 (br s, 1H, Asp α), 4.45 (d, 1H, J=7.9 Hz, H-1′), 4.28 (d, 1H, J=7.8 Hz, H-1), 2.50 (m, 2H, Glu γ), 2.15 (s, 3H, Ac), 1.16 (br s, 3H, Thr γ). *Anal.* Calcd for $C_{35}H_{57}N_7O_{24}$: C, 43.80; H, 5.99; N, 10.21. Found: C, 43.58; H, 6.12; N, 9.99.

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