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Synthesis of a Glycopeptide Carrying a N-Linked Core Pentasaccharide

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Abstract—A glycopeptide carrying a pentasaccharide core structure of asparagine-linked glycoproteins was synthesized. The synthesis of the carbohydrate part was performed starting from monosaccharide components in an unambiguous manner. The resultant pentaglycosyl azide was reduced into corresponding glycosyl amine and coupled with an aspartic acid derivative to furnish an Asn-linked oligosaccharide in a protected form. Subsequent coupling with a dipeptide, followed by deprotection gave the target compound.

Introduction

Glycoprotein synthesis is recognized as a challenging problem in modern synthetic organic chemistry. Naturally occurring glycoproteins typically have highly complicated oligosaccharide structures, which are linked to protein backbones via an asparagine or a serine/threonine residue.[1] In order to pursue synthetic studies toward glycoproteins, a sophisticated and interactive combination of carbohydrate chemistry and peptide chemistry is required.[2] Although recent studies have resulted in quite dramatic advancement in both of these fields, they tend to have been considered quite separately. In preparations of oligosaccharides and oligopeptides, choices of coupling conditions and protecting groups are critical factors which affect the overall efficiency. However, the most optimized strategies established for each of them may not be compatible with each other. Peptide bond formations utilizing amino acid or peptide components carrying base-labile (i.e. Fmoc) α-amino protections are performed in combination with permanent protections of side chains and C-terminal carboxyl groups, which require a variable strength of acidic conditions for their removal.[3] Since O-glycoside linkage is acid-sensitive in nature, such protecting groups should be removed under carefully controlled conditions. Although various types of C-terminal linkers have been developed that have a magnified acid sensitivity,[4] such linkers may be too labile to be compatible with oligosaccharide synthesis technology, because glycosylation reactions are often carried out under Lewis acidic conditions. On the other hand, the O-acetyl group, one of the most frequently used protecting groups in oligosaccharide synthesis, requires basic conditions for its removal, which may promote racemization and/or β-elimination of the peptide backbone. In addition, it should be pointed out that construction of a complex oligosaccharide structure itself is still a difficult task.[5] Considering such problems, the establishment of practical strategies to prepare properly protected oligopeptide fragments carrying complex oligosaccharide structures is fundamental as an initial goal which should be a prerequisite to a synthetic approach to natural glycoproteins. We report here the synthesis of a core pentasaccharide structure of asparagine (Asn)-linked glycoprotein oligosaccharide in a tripeptide linked form.

Results and Discussion

Glycopeptide **1** was chosen, as a synthetic target for the present study because of its biomedical relevance: firstly, the tripeptide sequence of **1** corresponds to amino acid 78–80 of the α-chain of human chorionic gonadotropin (hCG)[6] on which a N-linked oligosaccharide is attached to Asn(52,78). Secondly, its carbohydrate portion represents the core structure of asparagine-linked glycans which are often essential for the biological function of glycoproteins.

The synthesis of the carbohydrate part was investigated based on the retrosynthetic analysis shown in Scheme 1. Reducing-end *N*-acetylglucosamine (GlcNAc^1) was designed as a glycosyl azide **5** so that late stage coupling with aspartic acid can be performed via glycosylamine[7] to furnish the GlcNAc-Asn linkage. Coupling of **5** with the disaccharide fragment **4** (Man^3-GlcNAc^2), should be possible in a stereoselective manner, by taking advantage of the strong 1,2-*trans* directing nature of the *N*-phthaloyl

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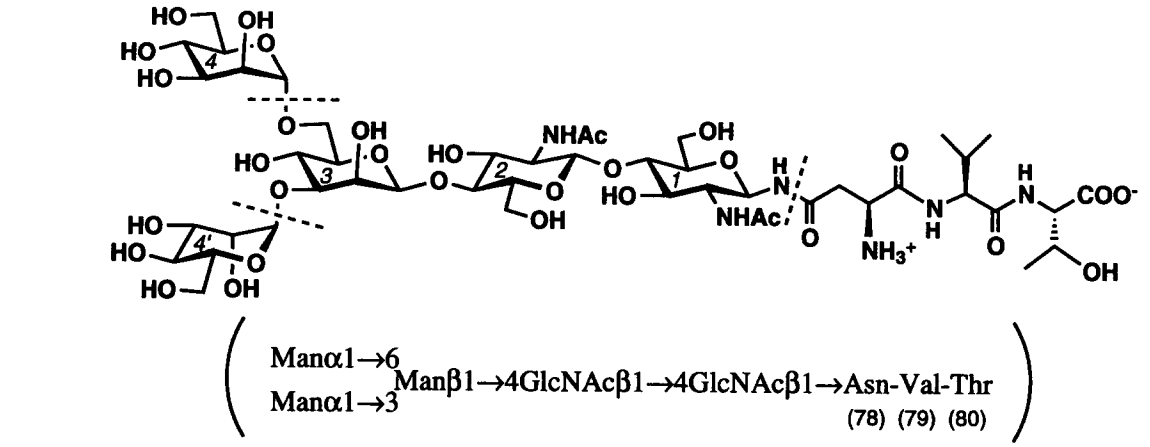
Abbreviations: Ac, acetyl; Bn, benzyl; DCC, dicyclohexylcarbodiimide; DMF, dimethylformamide; Fmoc, 9-fluorenylmethoxycarbonyl; HOBt: 1-hydroxybenzotriazole; Man, D-mannose; GlcNAc, N-acetyl-D-glucosamine; Asn, L-asparagine; Thr, L-threonine; Val, L-valine; WSCD, water-soluble carbodiimide [1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide]



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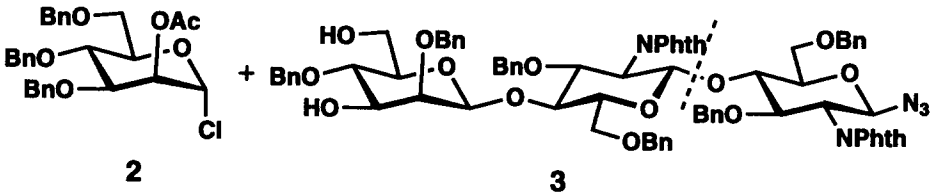

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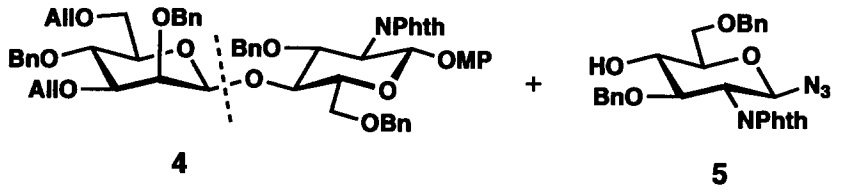

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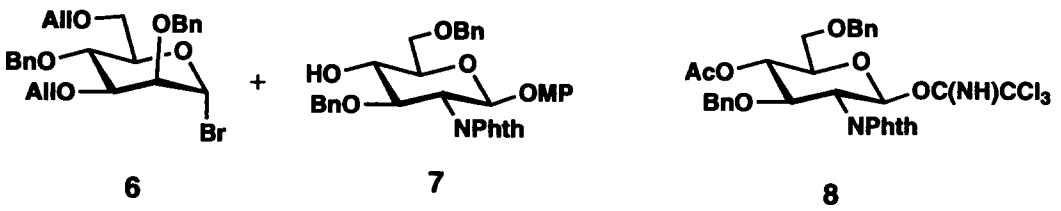


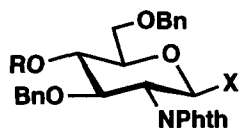
6 7 8

Scheme 1.

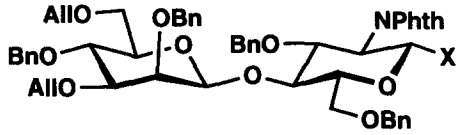
(Phth) group.[8] Compound **4**, in turn, was planned to be prepared from **6**[9] and **7**[10] by insoluble Ag salt mediated glycosylation.[11] Introduction of two additional mannose residues onto **3** (Man^4 and Man$^{4'}$) was planned to be achieved by using the chloride **2**.[12]

Preparation of azide **5** was performed in two steps starting from trichloroacetimidate **8**[13] which was first treated with Me_3SiN_3–BF_3·OEt_2 to afford **9**. Subsequent deacetylation gave **5**.

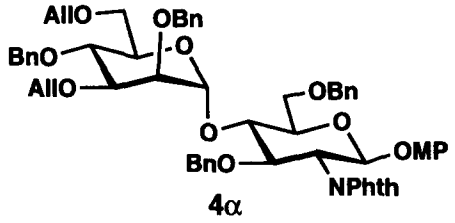
Disaccharide **4** was synthesized by the coupling of glucosamine derivative **7** and mannosyl bromide (**6**), using silver silica–alumina[11c] as a promoter. Although this particular combination of substrates gave nearly equal amounts of stereoisomers (**4**:**4**α = 8:7), the reaction was practical enough to give the desired supply of β-glycoside **4**. Subsequent deprotection of the *p*-methoxyphenyl (MP) group under Fukuyama's condition[14] afforded **10**, which was treated with dimethylaminosulfur trifluoride (DAST)[15] to give the fluoride **11**. Coupling with the aforementioned



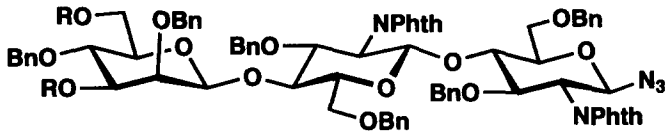
	X	R
8	$OC(NH)CCl_3$	Ac
9	N_3	Ac
5	N_3	H



	X
4	OMP
10	OH (α,β)
11	F (α,β)



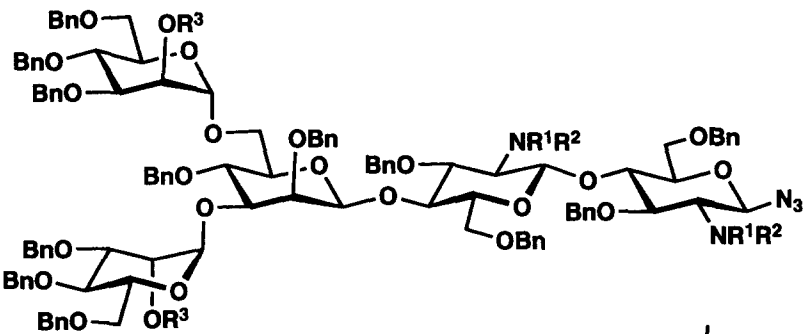
4α



	R
12	All
3	H

5 was performed under Suzuki's condition [Cp_2HfCl_2: $AgClO_4$ (1:2)/CH_2Cl_2][16] to give **12** as a single isomer in good yield. Deallylation of **12** was cleanly achieved by an iridium-catalyzed process[17] and the resultant diol (**3**) was reacted with **2**, under conditions well established for α-mannosidation ($AgOSO_2CF_3/CH_2Cl_2$) to give **13**.

Subsequent dephthaloylation of **13** was cleanly achieved by the method recently reported by Kanie *et al.* ($NH_2CH_2CH_2NH_2$/*n*-BuOH)[18] and the product was acetylated into diacetamide **14**. Attempted reduction of the azide group into the corresponding glycosyl amine turned out to be somewhat troublesome. Even under carefully controlled conditions, Lindler-catalysis mediated hydrogenation of azide was always accompanied by a substantial degree of hydrolytic cleavage of the C1-N linkage. Since it was assumed that this difficulty derives mainly from the instability of the oligosaccharide glycosylamine,[7] we expected the problem would be solved

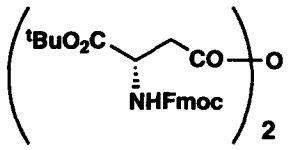


	R^1R^2	R^3
13	Phth	Ac
14	H, Ac	H

by *in situ* trapping of the amine with acid anhydride. Thus, compound **14** was treated, under an atmosphere of hydrogen, with acid anhydride **15** prepared from Fmoc/^{t}Bu-protected aspartic acid in the presence of Lindler catalyst. In accordance with our expectation, the desired coupled product **16** was obtained in quite satisfactory yield.

Further elongation of the peptide backbone was performed employing standard Fmoc based peptide chemistry.[3] Deprotection of ^{t}Bu ester gave acid **17**. Subsequent coupling with dipeptide **18**, prepared from Fmoc-Val and Thr(Bn)-OBn was achieved by the action of HOBt(1-hydroxybenzotriazole)[19]–WSC(water-soluble carbodiimide) in DMF to afford **19**. Deprotection of **19** was performed in two steps [(1) morphorine, (2) H_2, $Pd(OH)_2$], to give the target glycopeptide **1**. The 500 MHz ^{1}H NMR spectrum of synthetic **1** (Fig. 1) clearly reveals the presence of five anomeric protons with correct stereochemistry. All signals derived from the asparagine residue and carbohydrate portion are essentially identical with the data reported for the pentaglycosyl peptide obtained from natural sources.[20]

As described above, we established a reasonably practical synthetic route to the tripeptide carrying a pentasaccharide which represents the core structure of asparagine-linked glycoproteins. Concerning the low stereoselectivity observed in the preparation of the disaccharide fragment **4**, further improvement should be possible by using *p*-methoxybenzyl-assisted β-mannosidation which was recently reported from this laboratory.[21] Elongation of the peptide backbone may well be achieved based on standard Fmoc based peptide chemistry, after making a slight change to the C-terminal protecting group strategy. Since a variety of peptide ligation methodologies have been



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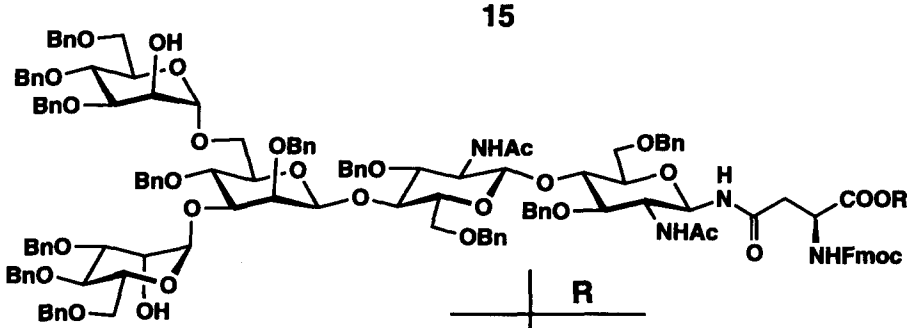
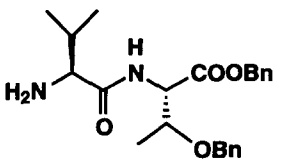


	R
16	**t-Bu**
17	**H**



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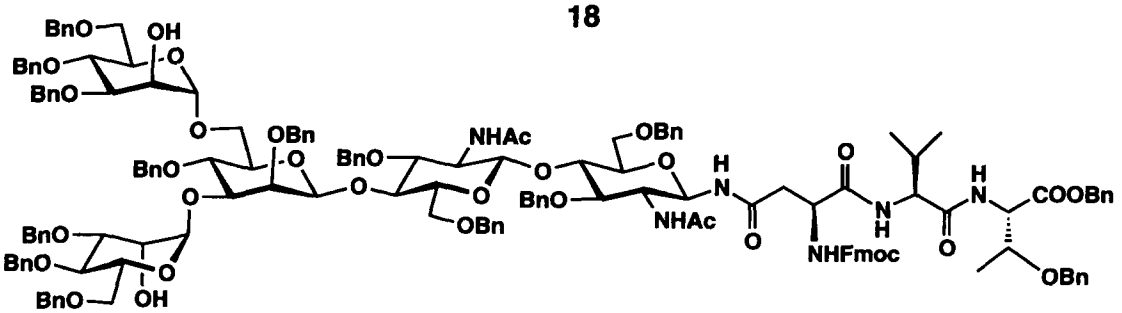

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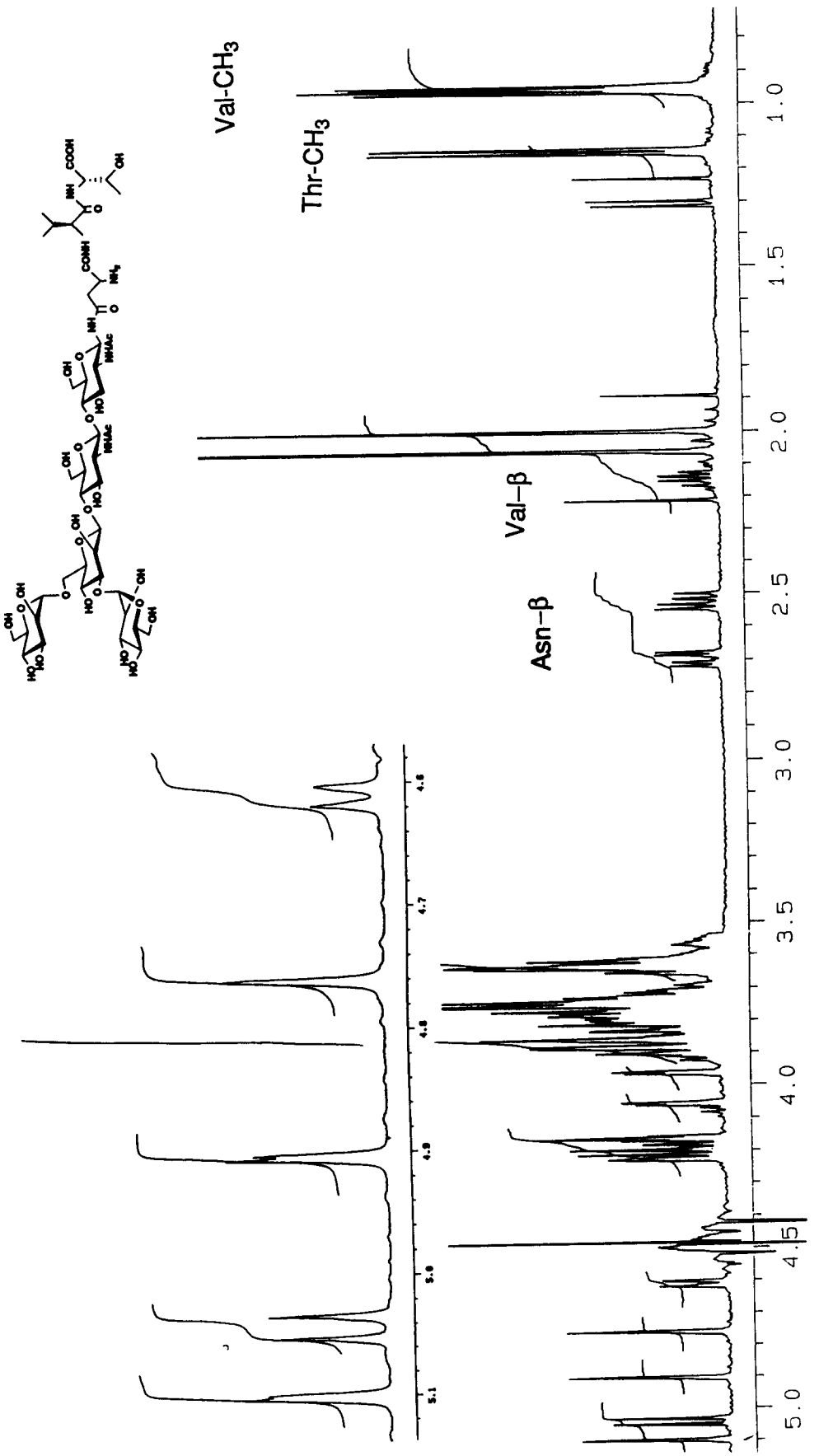

Figure 1. 500 Mz ^{1}H NMR spectrum of glycopeptide **1** (D_2O, 50 °C).

developed and successfully applied to the synthesis of large oligopeptides and proteins,[22] such glycopeptide fragment should be potentially valuable as a segment for the block condensation (chemically or enzymatically) into biologically active glycoproteins.

Experimental

General procedures

Optical rotations were measured at 20 ±3 °C with a Jasco DIP 310 polarimeter. Unless otherwise stated, ^1H and ^{13}C NMR spectra were measured either with a Jeol EX 270 or a Varian UNITY 500 spectrometer, for solutions in $CDCl_3$. Silica gel CC was performed on columns of silica gel 60 (Merck). Analytical TLC and high performance TLC were performed on glass plates coated with silica gel 60 F_{254} (Merck). Gels for size exclusion chromatography (Bio-Beads) were purchased from Bio-Rad. Molecular sieves were purchased from Nakarai Chemical and activated at 180 °C under vacuum immediately prior to use. $\{Ir(COD)[PCH_3(C_6H_5)_2]_2\}PF_6$ was prepared from $[Ir(COD)Cl]_2$ (Aldrich) according to the procedure described by Haines and Singleton.[23] Preparation of silver silica–alumina was performed as described by van Boeckel and Beetz.[11c] All reactions except hydrogenations were performed in anhydrous solvents under an atmosphere of dry N_2 or Ar.

4-O-Acetyl-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl azide (9). A mixture of trimethylsilyl azide (1.0 g, 8.7 mmol), $BF_3{\cdot}OEt_2$ (12 mg, 0.08 mmol) in CH_2Cl_2 (3 mL) containing molecular sieves AW-300 (1.5 g) was stirred at –78 °C for 0.5 h. Then, a solution of compound **8** (600 mg, 0.89 mmol) in CH_2Cl_2 (3 mL) was added dropwise. The mixture was stirred for an additional 1 h, filtered through Celite and diluted with EtOAc. The solution was washed successively with 1 N HCl, brine, aq. $NaHCO_3$, and brine, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by silica gel CC (toluene: EtOAc, 8:1) to afford 326 mg (65%) of compound **9**; mp 83–86 °C (from MeOH); $[\alpha]_D$ +50.3° (*c* 1.0); R_f 0.44 (toluene:EtOAc, 3:1); ^1H NMR (270 MHz): δ 5.38 (1H, *d*, *J* = 9.6 Hz, H-1), 5.16 (1H, *dd*, *J* = 9.9 and 8.9 Hz, H-4), 4.64 (1H, *dd*, *J* = 10.6 and 8.9 Hz, H-3), 4.17 (1H, *dd*, *J* = 10.6 and 9.6 Hz, H-2), 3.84 (1H, *m*, H-5), 3.63 (2H, *m*, H-6), 1.95 (3H, *s*, Ac); ^{13}C NMR: δ 169.49, 85.61 (C-1), 76.79 (C-3), 75.71 (C-5), 71.93 (C-4), 69.13 (C-6), 55.00 (C-2), 20.81 (Ac); IR ν 2120 cm^{-1}. Anal. Calcd for $C_{30}H_{28}N_4O_7$; C, 64.74; H, 5.27; N, 10.07. Found; C, 64.50; H, 5.06; N, 9.90.

3,6-Di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl azide (5). To a solution of compound **9** (175 mg, 0.31 mmol) in THF (5 mL) was added 0.2 M NaOMe:MeOH (1.5 mL). The mixture was stirred at room temperature for 45 min, neutralized with Amberlyst 15 (H^+) resin, and evaporated *in vacuo*. The residue was purified by silica gel CC (toluene:EtOAc, 5:1) to afford 145 mg (90%) of compound **5**; $[\alpha]_D$ +15.8° (*c* 1.0); R_f 0.31 (toluene:EtOAc 3:1); ^1H NMR (270 MHz): δ 5.37 (1H, *d*, *J* = 9.3 Hz, H-1), 4.27 (1H, *dd*, *J* = 10.5 and 8.3 Hz, H-3), 4.09 (1H, *dd*, *J* = 10.5 and 9.3 Hz, H-2), 3.83 (1H, *m*, H-5), 3.78 (2H, *m*, H-6); ^{13}C NMR: δ 85.66 (C-1), 78.33 (C-3), 75.90 (C-5), 73.66 (C-4), 69.94 (C-6), 54.79 (C-2). Anal. Calcd for $C_{28}H_{26}N_4O_6$; C, 65.36; H, 5.09; N, 10.89. Found; C, 65.10; H, 5.10; N, 10.77.

p-Methoxyphenyl O-(3,6-di-O-allyl-2,4-di-O-benzyl-β-D-mannopyranosyl)-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (4). A mixture of compound **7** (125 mg, 0.21 mmol), silver silica–alumina (1.5 g), and molecular sieves 4 Å (1.0 g) in CH_2Cl_2 (5 mL) was stirred at –20 °C. A solution of compound **6** (550 mg, 1.09 mmol) in CH_2Cl_2 (5 mL) was added and the mixture stirred at room temperature overnight and filtered through Celite. The filtrate was diluted with EtOAc, washed with brine, $NaHCO_3$ solution, and brine, successively, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was separated by silica gel CC (toluene:EtOAc, 9:1) to afford compound **4** (82 mg, 38%) together with the corresponding α-isomer **4α** (72 mg, 34%).

Compound **4**: $[\alpha]_D$ +28.1° (*c* 1.0); R_f 0.33 (toluene:EtOAc, 5:1); ^1H NMR (500 MHz): δ 5.654 (1H, *d*, *J* = 8.0 Hz, H-1^1), 4.592 (1H, *s*, H-1^2), 4.439 (1H, *dd*, *J* = 11.0 and 8.0 Hz, H-2^1), 4.361 (1H, *dd*, *J* = 11.0 and 8.6 Hz, H-3^1), 4.122 (*t*, *J* = 8.6 Hz, H-4^1), 3.855 (*t*, *J* = 9.2 Hz, H-4^2), 3.813 (1H, *d*, *J* = 3.0 Hz, H-2^2), 3.720 (3H, *s*, OMe), 3.66–3.80 (3H, *m*, H-5^1, H-6^1), 3.60–3.74 (2H, *m*, H-6^2), 3.361 (1H, *m*, H-5^2), 3.314 (1H, *dd*, *J* = 9.2 and 3.0 Hz, H-3^2); ^{13}C NMR: δ 101.42 ($J_{C\text{-}H}$ = 161 Hz, C-1^2), 97.66 ($J_{C\text{-}H}$ = 166 Hz, C-1^1), 82.45 (C-3^2), 78.35 (C-4^1), 77.41 (C-3^1), 75.98 (C-5^2), 74.99 (C-5^1), 74.65 (C-2^2), 71.83 (C-4^2), 69.36 (C-6^2), 68.56 (C-6^2), 55.60 (C-2^1). Anal. Calcd for $C_{61}H_{63}N_1O_{13}$: C, 71.96; H, 6.23: N, 1.38. Found: C, 71.62; H, 6.27; N, 1.33.

Compound **4α**: $[\alpha]_D$ +62.2° (*c* 0.9); R_f 0.46 (toluene: EtOAc, 5:1); ^1H NMR (270 MHz): δ 5.66 (1H, *d*, *J* = 8.9 Hz, H-1^1), 5.30 (1H, *s*, H-1^2), 3.68 (3H, *s*, OMe); ^{13}C NMR: δ 100.41 ($J_{C\text{-}H}$ = 171 Hz, C-1^2), 97.34 ($J_{C\text{-}H}$ = 169 Hz, C-1^1).

O-(3,6-Di-O-allyl-2,4-di-O-benzyl-β-D-mannopyranosyl)-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-D-glucopyranose (10). To a stirred solution of compound **4** (77 mg, 0.076 mmol) in toluene:MeCN (3:4; 7 mL) was added CAN (171 mg), followed by H_2O (3 mL). The mixture was stirred at room temperature for 2 h, diluted with EtOAc and washed successively with brine, aq. $NaHCO_3$, and brine. The organic layer was dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by silica gel CC (toluene:EtOAc, 3:1) to afford 48 mg (70%) of compound **10**; R_f 0.19 (toluene:EtOAc, 3:1); ^1H NMR (270 MHz): δ 4.87 (*d*, *J* = 10.8 Hz, H-1^1, β-anomer), 4.49 (1H, *s*, 1H, H-1^2), 4.34 (*dd*, *J* = 10.9 and 8.6 Hz, H-2^1, β-anomer), 3.61 (1H, *t*, *J* = 9.2 Hz, H-4^2), 3.32 (1H, *m*, H-5^2), 3.23 (1H, *dd*, *J* = 9.2 and 2.9 Hz, H-3^2). Anal. Calcd for $C_{54}H_{57}N_1O_{12}{\cdot}0.5\ H_2O$: C, 70.43; H, 6.35; N, 1.52. Found: C, 70.36; H, 6.24; N, 1.44.

O-(3,6-Di-O-allyl-2,4-di-O-benzyl-β-D-mannopyranosyl)-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-D-glucopyranosyl fluoride (11). Compound **10** (48 mg, 0.053 mmol) was dissolved in CH_2Cl_2 (2 mL) and stirred at 0 °C. To the solution was added DAST (13 μL, 0.13 mmol). The mixture was stirred for additional 10 min, and then quenched with MeOH (0.1 mL). After being diluted with EtOAc, the solution was washed successively with brine, aq. $NaHCO_3$, and brine, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was purified by silica gel CC (toluene:EtOAc, 5:1) to afford 48 mg (quantitative) of compound **11** as a mixture of anomers (α:β = 1:4.5); R_f 0.65 (toluene:EtOAc, 3:1); ^{1}H NMR (270 MHz): δ 7.65–6.65 (24H, *m*, aromatic), 5.86–5.64 (2H, *m*, $CH_2CH{=}CH_2$), 5.76 (0.8 H, *dd*, *J* = 53.4 and 5.6 Hz, H-1^1, β-anomer).

O-(3,6-Di-O-allyl-2,4-di-O-benzyl-β-D-mannopyranosyl)-(1→4)-O-(3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glycopyranosyl azide (12). A mixture of compound **5** (28 mg, 0.054 mmol), silver perchlorate (29 mg, 0.14 mmol), hafnocene dichloride (27 mg, 0.071 mmol), and molecular sieves 4 Å (0.5 g) in CH_2Cl_2 (2 mL) was stirred at –20 °C. A solution of compound **11** (48 mg, 0.053 mmol) in CH_2Cl_2 (2 mL) was added. The mixture was gradually warmed up to ambient temperature, stirred for 12 h and filtered through Celite. The filtrate was diluted with EtOAc and washed with aq. $NaHCO_3$ and brine, successively. The organic layer was dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by a column of Bio-Beads S-X2 (toluene) to afford 59 mg (79%) of compound **12**; $[\alpha]_D$ +4.5° (*c* 1.0); R_f 0.57 (toluene:EtOAc, 3:1); FTIR 2116.2 cm^{-1}; ^{1}H NMR (500 MHz): δ 5.289 (1H, *d*, *J* = 8.1 Hz, H-1^2), 5.155 (1H, *d*, *J* = 9.5 Hz, H-1^1), 4.550 (1H, *s*, H-1^3); ^{13}C NMR: δ 101.46 (C-1^3), 97.09 (C-1^2), 85.56 (C-1^1). Anal. Calcd for $C_{82}H_{81}N_5O_{17}$: C, 69.52; H, 5.77; N, 4.74. Found: C, 69.92; H, 5.79; N, 4.97.

O-(2,4-Di-O-benzyl-β-D-mannopyranosyl)-(1→4)-O-(3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glycopyranosyl azide (3). A solution of {Ir(COD)[$PCH_3(C_6H_5)_2]_2$}PF_6 (5 mg, 0.006 mmol) in THF (5 mL) was stirred until the colour disappears while the atmosphere was replaced by H_2. The solution was condensed to *ca* 3 mL and the flask was purged with N_2. Then a solution of compound **12** (51 mg, 0.036 mmol) was added and the mixture was stirred for 1 h, diluted with EtOAc and evaporated *in vacuo*. The residue was dissolved in 90% aq. Me_2CO (10 mL) and treated with $HgCl_2$ (35 mg, 0.13 mmol):HgO (0.7 mg, 0.003 mmol) for 1 h. The mixture was diluted with EtOAc, washed successively with brine, aq. $NaHCO_3$, and brine, dried over $MgSO_4$, and evaporated *in vacuo*. The residue was purified by silica gel CC (toluene:EtOAc, 3:1) to afford 48 mg (quantitative) of compound **3**; $[\alpha]_D$ +21.8° (*c* 0.4); R_f 0.10 (toluene:EtOAc, 3:1); ^{1}H NMR (500 MHz): δ 5.297 (1H, *d*, *J* = 8.2 Hz, H-1^2), 5.168 (1H, *d*, *J* = 9.5 Hz, H-1^1), 4.604 (1H, *s*, H-1^3); ^{13}C NMR: δ 101.29 (C-1^3), 97.06 (C-1^2), 85.56 (C-1^1). Anal. Calcd for $C_{76}H_{73}N_5O_{17}$: C, 68.72; H, 5.54; N, 5.27. Found: C, 68.46; H, 5.59; N, 5.09.

O-(2-O-Acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1→6)-O-[(2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1→3)]-O-(2,4-di-O-benzyl-β-D-mannopyranosyl)-(1→4)-O-(3,6-di-O-benzyl-2-deoxy-2-phthalimid-β-D-glucopyranosyl)-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl azide (13). To a stirred mixture of compound **3** (112 mg, 0.084 mmol), silver triflate (153 mg, 0.59 mmol), and molecular sieves 4 Å (1.0 g) in CH_2Cl_2 (10 mL) was added a solution of compound **2** (172 mg, 0.34 mmol) in CH_2Cl_2 (2 mL) at –40 °C. The mixture was gradually warmed up to ambient temperature and stirred for 18 h. Insoluble materials were filtered off through Celite and the filtrate was evaporated *in vacuo*. The residue was purified by a column of Bio-Beads SX-8 (toluene) and by silica gel CC (toluene: EtOAc, 7:1) to afford 154 mg (80%) of compound **13**; $[\alpha]_D$ +17.1° (*c* 0.4); R_f 0.56 (toluene:EtOAc, 2:1); ^{1}H NMR (500 MHz): δ 5.482 (1H, *s*, H-2^4), 5.312 (1H, *s*, H-2$^{4'}$), 5.223 (1H, *d*, *J* = 8.3 Hz, H-1^2), 5.138 (1H, *s*, H-1^4), 4.849 (1H, *s*, H-1^4), 4.612 (1H, *s*, H-1^3), 2.077 and 1.783 (3H × 2, *s*, Ac); ^{13}C NMR: δ 101.93 (C-1^3), 99.67 (C-1$^{4'}$), 98.24 (C-1^4), 97.04 (C-1^2), 85.53 (C-1^1), 20.08 and 20.05 (Ac). Anal. Calcd for $C_{134}H_{133}N_5O_{29}$: C, 70.67; H, 5.88; N, 3.08. Found: C, 70.98; H, 5.85; N, 2.77.

O-(3,4,6-Tri-O-benzyl-α-D-mannopyranosyl)-(1→6)-O-[(3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1→3)]-O-(2,4-di-O-benzyl-β-D-mannopyranosyl)-(1→4)-O-(2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl azide (14). A solution of compound **13** (25 mg, 0.011 mmol) in *n*-butanol containing 15 μL ethylenediamine was stirred at 90 °C for 18 h. Volatiles were removed by evaporation *in vacuo* and the residue was dissolved in MeOH (2 mL). The solution was treated at 0 °C with Ac_2O (1 mL) for 2 h and evaporated *in vacuo*. The residue was purified by preparative TLC to afford 18 mg (80%) of compound **14**; $[\alpha]_D$ –8.9° (*c* 1.0); R_f 0.48 ($CHCl_3$:MeOH, 20:1); ^{1}H NMR (500 MHz): δ 6.259 (1H, *d*, *J* = 9.0 Hz, NH), 5.16 (1H, *d*, *J* = 1.0 Hz, H-1^4), 4.96 (1H, *s*, H-1$^{4'}$), 4.65 (1H, H-1^1), 4.43 (1H, *s*, H-1^3), 4.29 (1H, H-1^2), 1.88 and 1.63 (2 × 3H, *s*, 2Ac) ; ^{13}C NMR: δ 101.40 (C-1$^{4'}$), 100.95 (C-1^3), 99.80 (C-1^4), 99.77 (C-1^2), 88.34 (C-1^1), 23.33 and 23.07 (Ac). Anal. Calcd for $C_{118}H_{129}N_5O_{25}$: C, 70.26; H, 6.44; N, 3.48. Found: C, 70.25; H, 6.51; N, 3.72.

N^2-(9-Fluorenylmethoxycarbonyl)-N^4-[O-(3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1→6)-O-[(3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1→3)]-O-(2,4-di-O-benzyl-β-D-mannopyranosyl)-(1→4)-O-(2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-O-benzyl-2-deoxy-β-D-glucopyranosyl]-L-asparagine tert-*butyl ester (16).* To a stirred solution of Fmoc-Asp-O^{t}Bu (20 mg, 0.049 mmol) was added DCC (5 mg, 0.024 mmol) at 0 °C. After being stirred for 30 min, the mixture was filtered through a membrane filter and the filtrate was evaporated *in vacuo*. Molecular sieves 3 Å (100 mg) and Lindler catalyst (13 mg) were added to the residue and a solution of compound **14** (17 mg, 0.0084 mmol) in CH_2Cl_2 (1 mL) was added. Solvent was removed under vacuum and the residue was diluted with MeOH (5

mL). The mixture was then stirred under an atmosphere of hydrogen at room temperature. After 5 h, insoluble materials were filtered off and the filtrate was evaporated *in vacuo*. The residue was purified by a column of Sephadex LH 20 in $CHCl_3$:MeOH (1:1) to afford 16 mg (79%) of compound **16**; $[\alpha]_D$ +9.0° (*c* 0.8); R_f 0.72 ($CHCl_3$:MeOH, 10:1); ^{1}H NMR (270 MHz): δ 5.96 (1H, *d*, *J* = 8.9 Hz, NH), 5.15 (1H, *s*, H-1^4), 4.94 (1H, *d*, *J* = 1 Hz, H-1$^{4'}$), 4.69 (1H, H-1^1), 4.54 (1H, H-1^3), 4.34 (1H, H-1^2), 2.82 (1H, *dd*, *J* = 14 and 4 Hz, $COCH_2$) 2.67 (1H, *dd*, *J* = 14 and 6.4 Hz, $COCH_2$), 1.74 and 1.61 (2 × 3H, *s*, 2Ac), 1.41 (Bu^t); ^{13}C NMR: δ 101.32 (C-1^4), 101.10 (C-1^3), 99.86 (C-1^2), 99.75 (C-1$^{4'}$), 79.95 (C-1^1). Anal. Calcd for $C_{141}H_{154}N_4O_{30}$: C, 71.02; H, 6.51; N, 2.35. Found: C, 71.28; H, 6.63; N, 2.15.

N^2-*(9-Fluorenylmethoxycarbonyl)*-N^4-*{*O-*(3,4,6-tri-*O-*benzyl-α-D-mannopyranosyl)-(1→6)-*O-*[(3,4,6-tri-*O-*benzyl-α-D-mannopyranosyl)-(1→3)]-*O-*(2,4-di-*O-*benzyl-β-D-mannopyranosyl)-(1→4)-*O-*(2-acetamido-3,6-di-*O-*benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O-*benzyl-2-deoxy-β-D-glucopyranosyl}-L-asparagine (17).* Compound **16** (18 mg, 0.0075 mmol) was dissolved in CH_2Cl_2:CF_3COOH (2:1; 1.5 m). The solution was stirred at room temperature for 1 h and evaporated *in vacuo*. The residue was purified by HPLC (Cosmosil 5C18-AR, Nakalai Tesque; 0.1% TFA in MeCN:0.1% TFA in H_2O, 70:30 ~ 100:0) to afford 16 mg (89%) of free acid **17**; $[\alpha]_D$ +10.4° (*c* 0.5); R_f 0.33 ($CHCl_3$:MeOH, 10:1 containing 0.1% HOAc); ^{1}H NMR (500 Mhz, $CDCl_3$:CD_3OD, 10:1): δ 4.998 (1H, *d*, *J* = 1 Hz, H-1^4), 4.754 (1H, *d*, *J* = 1 Hz, H-1$^{4'}$), 4.445 (1H, *d*, *J* = 1 Hz, H-1^3), 2.741 (1H, *dd*, *J* = 16.9 and 4.2 Hz, $COCH_2$), 2.614 (1H, *dd*, *J* = 16.9 and 7.1 Hz, $COCH_2$), 1.700 and 1.590 (2 × 3H, *s*, 2Ac); ^{13}C NMR: δ 102.45 (C-1$^{4'}$), 101.90 (C-1^3), 100.89 (C-1^4), 100.40 (C-1^2), 78.29 (C-1^1). ESI-MS m/z 2351.5 $[M+Na]^+$.

N^2-*(9-Fluorenylmethoxycarbonyl)-L-valyl-*O^3*-benzyl-L-threonine benzyl ester (Fmoc-Val-Thr(Bn)-OBn).* H_2N-Thr(Bn)-OBn (22 mg, 0.073 mmol), Fmoc-Val-OH (30 mg, 0.09 mmol), and EEDQ (26 mg, 0.10 mmol) was mixed in CH_2Cl_2 (2 mL) at –78 °C. The mixture was warmed up to room temperature, stirred for 1 h, and diluted with $CHCl_3$. After being washed with 1 N HCl, brine, aq. $NaHCO_3$, and brine, successively, the organic layer was dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by silica gel CC (hexane:EtOAc, 2:1) to afford 45 mg (quantitative) of Fmoc-Val-Thr(Bn)-OBn; $[\alpha]_D$ –15.0° (*c* 0.5); R_f 0.33 (hexane:EtOAc, 2:1); ^{1}H NMR (270 MHz): δ 7.78–7.15 (18H, *m*, aromatic), 6.42 (1H, *d*, *J* = 9.2 Hz, NH), 5.46 (1H, *d*, *J* = 8.6 Hz, NH), 4.70 (1H, *dd*, *J* = 9.2 and 2.0 Hz, α-CH_{Thr}), 2.11 (1H, *m*, β-CH_{Val}), 1.20 (3H, *d*, *J* = 6.3 Hz, MeCH(O-), 0.99–0.93 (6H, Me_2CH). Anal. Calcd for $C_{38}H_{40}N_2O_6 \cdot H_2O$:C, 71.45; H, 6.63; N, 4.38. Found: C, 71.32; H, 6.33; N, 4.38.

*L-Valyl-*O*-benzyl-L-threonine benzyl ester (18).* Fmoc-Val-Thr(Bn)-OBn (25 mg, 0.04 mmol) was dissolved in morpholine (0.5 mL). The solution was stirred at room temperature for 2 h and evaporated *in vacuo*. The residue was purified by silica gel CC (toluene:EtOAc, 10:1) to afford 16 mg (quantitative) of compound **18**; $[\alpha]_D$ –24.4° (*c* 0.6); R_f 0.44 (toluene:EtOH, 10:1); ^{1}H NMR (270 MHz): δ 7.77 (1H, *d*, *J* = 9.2 Hz, NH), 7.27–7.10 (10H, *m*, aromatic), 5.07 and 5.01 (2 × 1H, *d*, *J* = 12.2 Hz, $COCH_2Ph$), 4.64 (1H, *dd*, *J* = 9.6 and 2.3 Hz, α-CH_{Thr}), 4.12 (1H, *m*, β-CH_{Thr}), 3.24 (1H, *d*, *J* = 4.0 Hz, α-CH_{Val}), 2.31 (1H, *m*, β-CH_{Val}), 1.13 (3H, *d*, *J* = 6.3 Hz, MeCH(O-), 0.94 and 0.79 (2 × 3H, Me_2CH). Anal. Calcd for $C_{24}H_{27}N_2O_5$:C, 68.07; H, 6.43; N, 6.61. Found: C, 68.67; H, 6.38; N, 6.62.

N^2-*(9-Fluorenylmethoxycarbonyl)*-N^4-*{*O-*(3,4,6-tri-*O-*benzyl-α-D-mannopyranosyl)-(1→6)-*O-*[(3,4,6-tri-*O-*benzyl-α-D-mannopyranosyl)-(1→3)]-*O-*(2,4-di-*O-*benzyl-β-D-mannopyranosyl)-(1→4)-*O-*(2-acetamido-3,6-di-*O-*benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O-*benzyl-2-deoxy-β-D-glucopyranos-yl}-L-asparaginyl-L-valyl-*O^3*-benzyl-L-threonine benzyl ester (19).* To a solution of compounds **17** (8 mg, 0.003 mmol) and **18** (2.5 mg, 0.0062 mmol) in CH_2Cl_2 (1 mL) were added HOBt (1.8 mg, 0.01 mmol) and EDC (2.4 mg, 0.01 mmol) and the mixture was stirred at room temperature for 30 min. The resulting solution was directly subjected to a column of Sephadex LH-20 ($CHCl_3$:MeOH, 1:1) to afford 9.2 mg (90%) of compound **19**; $[\alpha]_D$ –3.8° (*c* 0.2); R_f 0.33 (toluene:EtOH, 3:1); ^{1}H NMR (270 MHz): δ 5.16 (1H, *d*, *J* = 1 Hz, H-1^4), 4.93 (1H, *d*, *J* = 1 Hz, H-1$^{4'}$), 2.70 (1H, *dd*, *J* = 16.2 and 3.2 Hz, β-CH_{Asn}), 2.49 (1H, *dd*, *J* = 16.2 and 5.9 Hz, β-CH_{Asn}), 2.11 (1H, *m*, β-CH_{Val}), 1.74 and 1.59 (2 × 3H, s, Ac), 1.16 (3H, *d*, *J* = 6.3 Hz, MeCH(O-)), 0.93–0.85 (6H, *m*, Me_2CH). ESI-MS m/z 2710.0 $[M]^+$.

N^4-*{*O-*(3,4,6-Tri-*O-*benzyl-α-D-mannopyranosyl)-(1→6)-*O-*[(3,4,6-tri-*O-*benzyl-α-D-mannopyranosyl)-(1→3)]-*O-*(2,4-di-*O-*benzyl-β-D-mannopyranosyl)-(1→4)-*O-*(2-acetamido-3,6-di-*O-*benzyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-2-acetamido-3,6-di-*O-*benzyl-2-deoxy-β-D-glucopyranosyl}-L-asparaginyl-L-valyl-L-threonine (1).* A mixture of compound **19** (9 mg, 0.0031 mmol) and morpholine (1 mL) was stirred at room temperature for 1 h. The mixture was evaporated and co-evaporated twice with toluene. The residue was diluted with MeOH:H_2O (10:1, 5 mL) and washed with hexane (2 mL). The MeOH:H_2O layer was evaporated *in vacuo* and the residue was subjected to a column of Sephadex LH-20 (MeOH). Glycopeptide containing fractions (R_f 0.63 in toluene: EtOH, 7:2) were collected and evaporated *in vacuo*. The residue was dissolved in 80% acetic acid (10 mL) and hydrogenated over 10% Pd/C under atmospheric pressure. After 19 h, the mixture was filtered through Celite and the filtrate was evaporated *in vacuo*. The residue was purified by a column of Bio-gel P-2 (H_2O) to afford 2.2 mg (58%) of compound **1**; R_f 0.26 (*n*-BuOH:MeOH:H_2O: HOAc, 5:2:2:1); ^{1}H NMR (500 MHz, D_2O): δ 5.101 (1H, *d*, *J* = 1.5 Hz, H-1^4), 5.042 (1H, *d*, *J* = 10 Hz, H-1^1), 4.904 (1H, *d*, *J* = 1.5 Hz, H-1$^{4'}$), 4.761 (1H, *s*, H-1^3), 4.610 (1H, *d*, *J* = 8 Hz, H-1^2), 2.70–2.52 (2H, *m*, β-CH_{2Asn}), 2.146 (1H, *m*, β-CH_{Val}), 2.060 and 1.999 (2 × 3H, *s*, Ac), 1.148 (3H, *d*, *J* = 6 Hz, MeCH(O-)), 0.963 and 0.950 (2 × 3H, *d*, *J* = 4 and 6.5 Hz, respectively, Me_2CH). FAB-MS (negative) m/z 1224.0 $[M-H]^-$.

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