Pergamon

Tetrahedron 55 (1999) 11253–11266

TETRAHEDRON

A Novel Silyl Linker: Motif for Side Chain Tethered Approach to Solid-Phase Glycopeptide Synthesis

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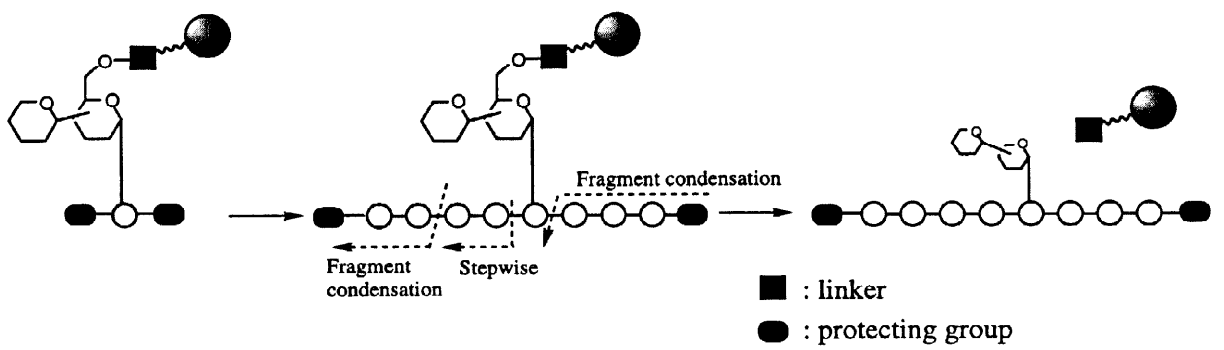
Received 5 July 1999; accepted 26 July 1999

Abstract: In order to facilitate the solid-phase syntheses of protected glycopeptide blocks, we designed a novel silyl linker, which allows the alcoholic side chain (carbohydrate, serine, or threonine) of (glyco-)peptides to link to the solid support. Utilizing this linker, peptide coupling reactions at both the N- and the C-termini were successful. Synthesis of the glycophorin AM fragment corresponding to the N-terminal glycoheptapeptide is demonstrated.

Key words: Glycopeptides, solid-phase synthesis, silicon halides

Introduction

In the conventional solid-phase synthesis of peptides, the first (C-terminal) amino acid is linked to the resin via a benzyl ester-type linker and then the N and side-chain protected amino acids are sequentially assembled by either a manual or machine-aided procedure. The crucial step for cleavage of the synthesized peptides is usually combined with deprotection of most of the side chain functional groups. This strategy has also been employed in glycopeptide synthesis.[1] On the other hand, current interest in the solid-phase synthesis stems from the combinatorial approach to small-molecule libraries for pharmacological use, and various specific linkers have been designed to adapt to the diverse target molecules.[2] Among the reported linkers, those consisting of arylsilane or silyl-ether[3] are particularly intriguing, because their chemoselectively cleavable properties would also provide a new procedure for the glycopeptide synthesis.



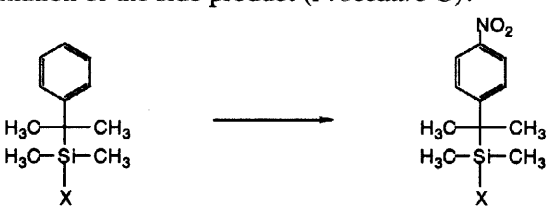
Scheme 1

PII: S0040-4020(99)00649-3

In the course of our studies aiming at synthesis of larger glycopeptides,[4] an efficient method to prepare the fully protected glycopeptide segments was required. Here we propose a novel silyl-ether type linker which enables the side-chain hydroxyl groups of the peptide or glycopeptide to bind to the solid support. A key feature of this approach is the feasibility of peptide chain-elongation at both the N- and C-termini by block- or step-wise condensation. The synthesized oligomer in a protected form should be released from the resin by fluoridolysis. Scheme 1 illustrates the outline of this procedure. Our preliminary investigations on this work have already been reported.[5] It is noteworthy that based on a similar concept Danishefsky and co-workers have recently reported the synthesis of oligosaccharides and their transformation to glycopeptides on a solid support by using silylated polystyrene resin.[3i, 3n]

Results and Discussion

Our studies started with the preparation of an appropriately functionalized silicon derivative. Chloro(α,α-dimethylbenzyl)dimethylsilane **1**[6] was hydrolyzed with aq. KOH to silanol **2**, nitration of this with ammonium nitrate-trifluoroacetic anhydride in CH_3CN afforded *p*-nitro compound **3** in 61% yield. Chlorination of **3** was readily achieved by treatment with oxalyl chloride and a catalytic amount of DMF in CH_2Cl_2 to give the key compound **4** as colourless plates. Silyl-etherification of β-hydoxy amino acids **5**, **6** and O-linked glycosyl amino acids **7**, **9** with **4** was examined under several conditions. The conventional silylation procedure using silyl chloride and imidazole in DMF was not very successful (*Procedure B*). The desired silyl ethers were obtained in moderate to low yields by this method. In most cases, the major side reaction was the formation of silyloxysilyl ethers as represented by compound **14** and the concomitant elimination of *p*-nitrocumene. The mechanism of this undesired side-reaction is unclear. Bis[(α,α-dimethyl-*p*-nitrobenzyl)dimethyl]disiloxane **15**, prepared from **3** and **4**, did not react with the serine hydroxyl group in the presence of imidazole in DMF. The once-formed silyl ether **10** was no longer convertible into **14** under the silylation conditions. In contrast, acceptable yields were obtained when the alcohols were treated with **4**, NaI, and N-methylmorpholine in DMF (*Procedure A*). Silylation of **9** using imidazole in THF also gave a good yield without formation of the side product (*Procedure C*).

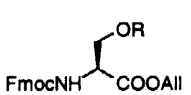


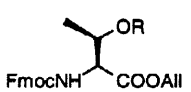
1: X = Cl

2: X = OH

3: X = OH

4: X = Cl



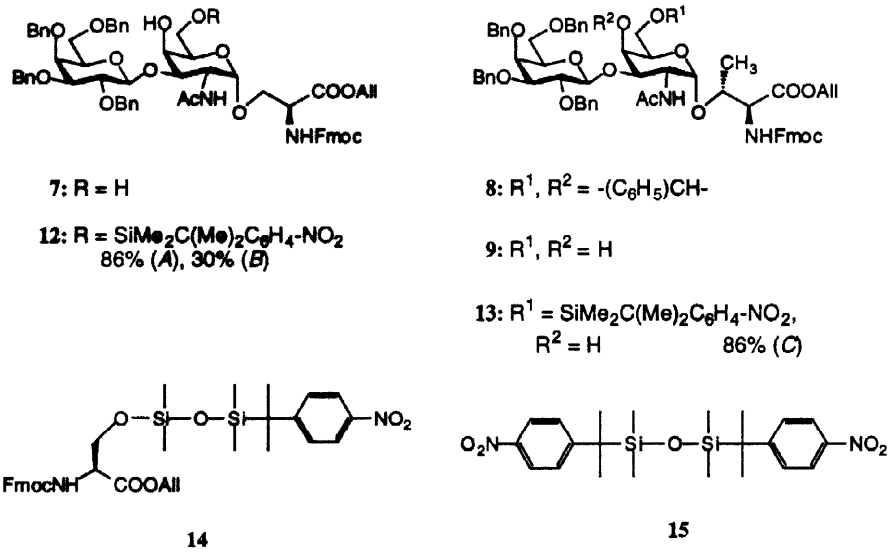


5: R = H

6: R = H

10: R = $SiMe_2C(Me)_2C_6H_4$-NO_2
97-100% (*A*), 37% (*B*)

11: R = $SiMe_2C(Me)_2C_6H_4$-NO_2
73% (*A*), 9% (*B*)



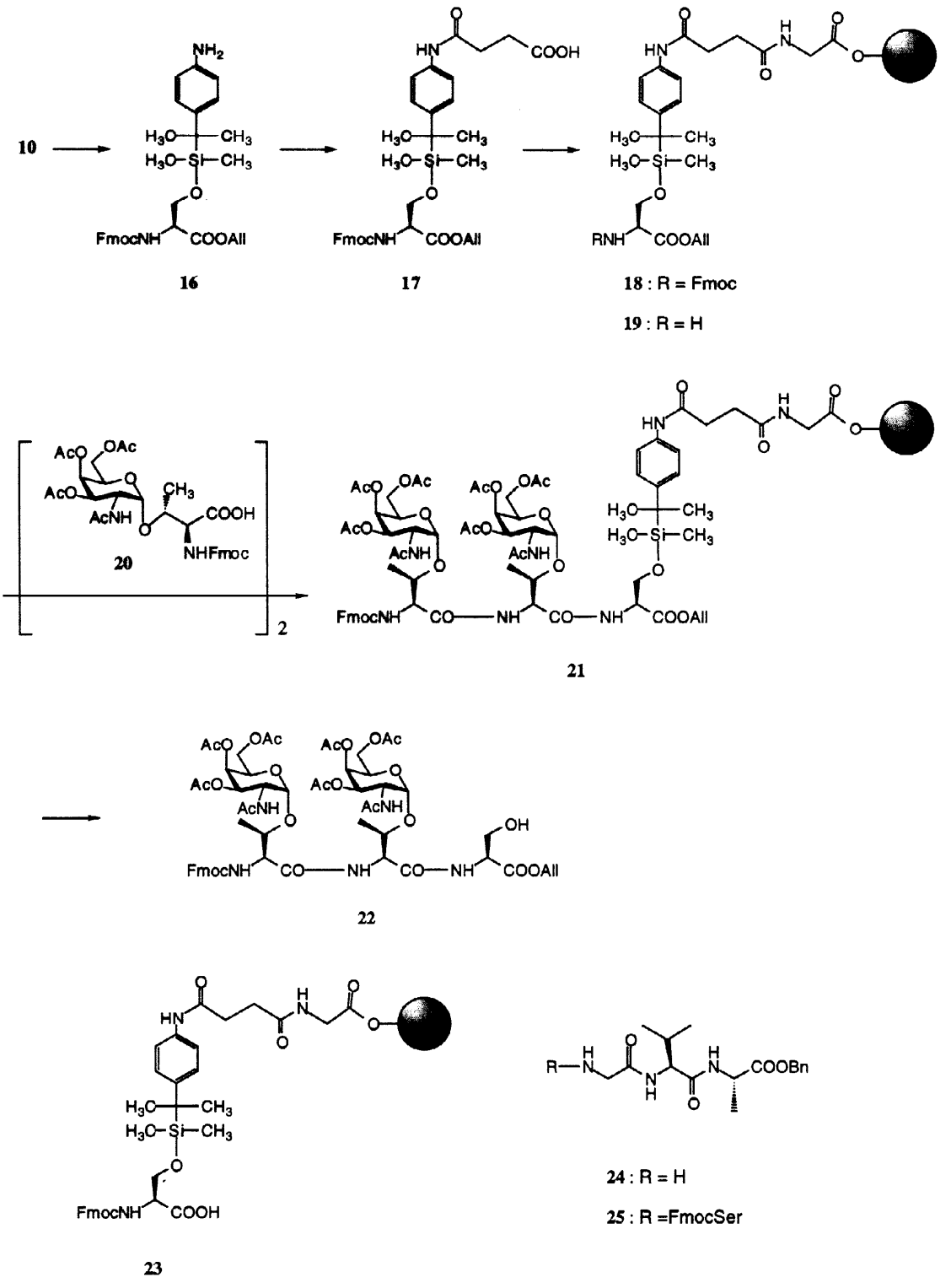
Scheme 2

A first example of the solid-phase synthesis using this linker was displayed by the growing chain in the N-terminal direction. The silyl ether **10** was reduced with Zn-AcOH to the aniline derivative **16**, which was treated with succinic anhydride to afford succinanilic acid **17** in 87% yield (2 steps). The carboxylic acid **17** was activated with HBTU (O-benzotriazol-1-yl-N, N, N', N'-tetramethyluronium hexafluorophosphate) and HOBt (1-hydroxybenzotriazole), and attached to the amino resin prepared by N-deprotection of the commercial FmocGly-preloaded Wang resin. The reaction was performed in NMP (N-methylpyrrolidone) as the solvent using a vortex tube-mixer. The efficiency of this acylation was estimated to be 99.9% by ninhydrin test.

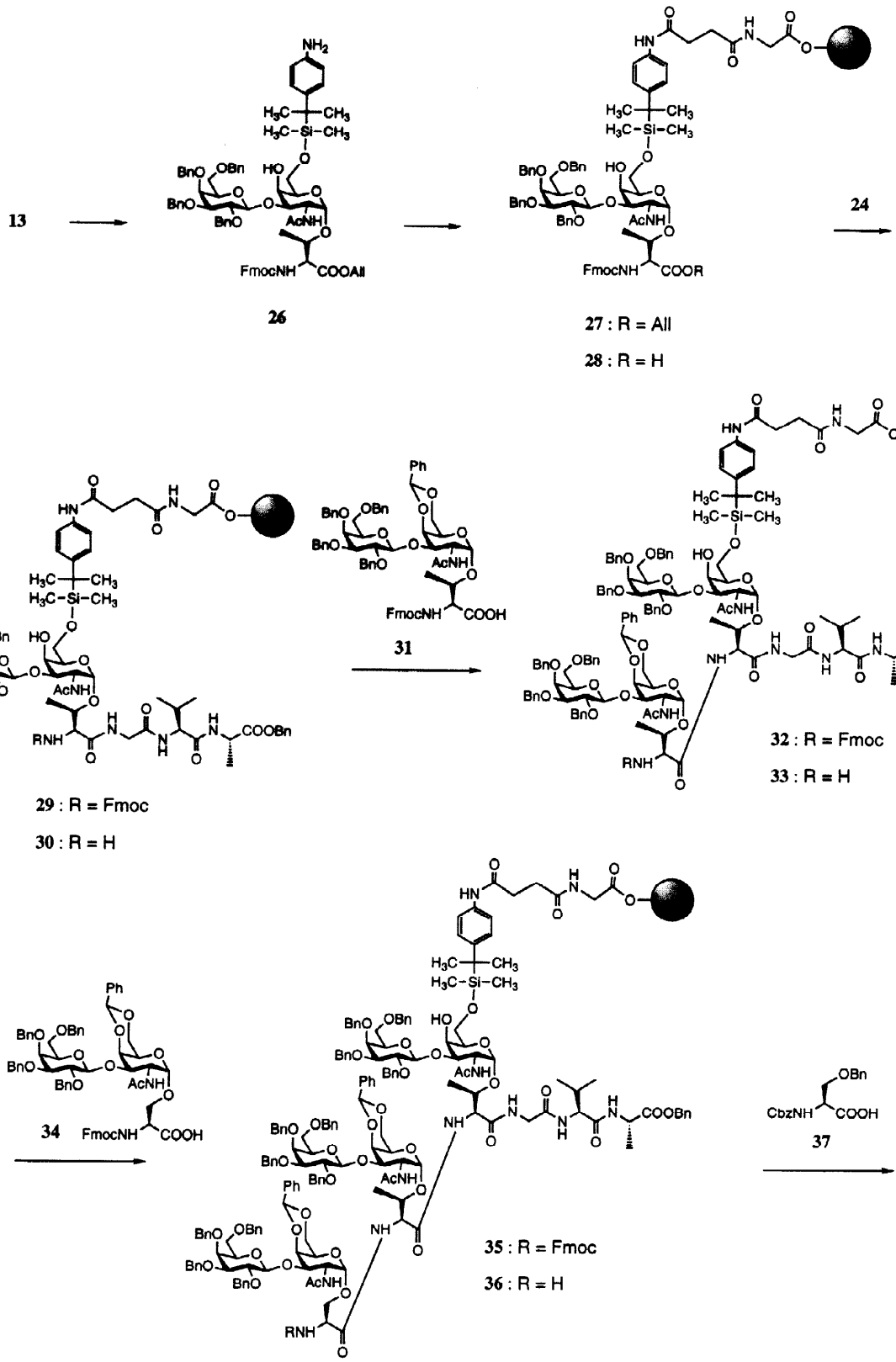
The serine-bound resin was N-deprotected with 50% piperidine in NMP and coupled with the N-acetylgalactosaminyl threonine derivative **20**[7] in the HBTU-HOBt activation conditions. The reaction was completed by vortex-mixing overnight and ninhydrin monitoring displayed the coupling efficiency to be 99.9%. N-Deprotection and the coupling procedures were repeated to produce disaccharyl tripeptide **21**. Cleavage of the synthesized glycopeptide from the resin was achieved by fluoridolysis using CsF and AcOH in DMF. The product **22** was readily isolated by simple chomatography and the overall yield from **19** through two coupling steps was 73%. The use of TBAF (tetrabutylammonium fluoride) in the place of CsF partly resulted in cleavage of the Fmoc group.

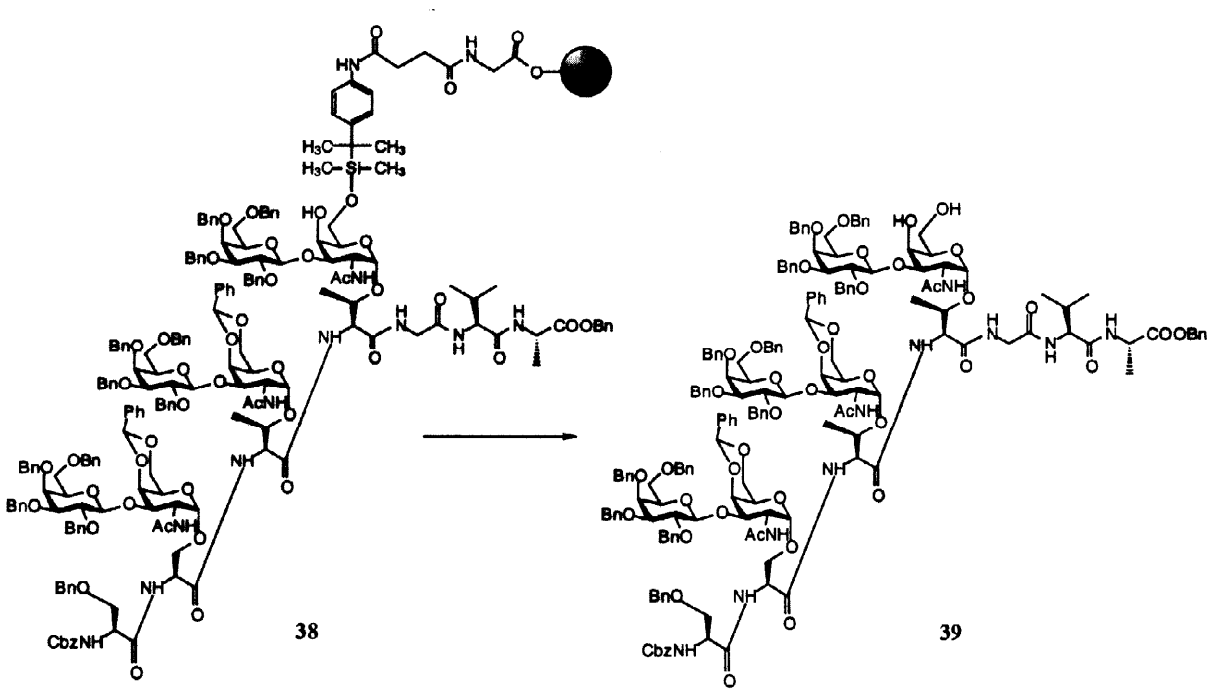
C-Terminal peptide chain elongation was next examined. The serine-linked resin **18** underwent ready deallylation on treatment with $Pd(PPh_3)_4$ and dimedone in THF. Activation of the liberated resin-bound carboxylic acid **23** followed by coupling with tripeptide **24**[8] was performed using HBTU-HOBt and *i*-Pr_2NEt in NMP. The resulting resin was treated under fluoride condition to afford tetrapeptide **25** in 76% yield.

Convinced of the potential usefulness of this side chain-tethering strategy for glycopeptide synthesis, we embarked on the solid-phase synthesis of an N-terminal heptapeptide fragment of glycophorin AM in the asialo form. With respect to the related fragment bearing the disialylated tetrasaccharides, we have previously accomplished the total synthesis by stepwise solution techniques.[8]



Scheme 3


13
26
27 : R = All
28 : R = H
24
29 : R = Fmoc
30 : R = H
31
32 : R = Fmoc
33 : R = H
34
35 : R = Fmoc
36 : R = H
37



Scheme 4

In a similar manner as above, the silyl ether **13** was attached to the resin through reduction, succinoylation, and activation with HBTU-HOBt. Pd(0)-catalyzed cleavage of the allyl ester **27** was followed by condensation with **24** to afford **29**. The resin was then submitted to the standard Fmoc chemistry for an elongation along the N-terminal. The synthetic cycles involving N-deprotection and peptide coupling were carried out with **31**, **34**, and **37** as acyl donors. In each peptide-coupling reaction, two equivalents of the soluble substrate were used and ninhydrin test exhibited high efficiency. The glycopeptide thus synthesized was detached from the solid support using TBAF and AcOH in THF. Purification by gel filtration and preparative TLC afforded the target glycoheptapeptide **39** in 55% overall yield. The compounds **39** was characterized by ^{1}H-NMR and high resolution FAB mass spectra. Any glycoamino acid unit-deleted analog was not detected from this synthesis.

In conclusion, we developed a novel approach to the glycopeptide synthesis utilizing a newly designed silyl linker, which allowed elongation at both the C- and N-terminal of the peptide chain on the solid support. High efficiency was obtained in each peptide coupling on the basis of Fmoc methodology. Isolation of the synthesized glycopeptide oligomers was readily accomplished by fluoride ion-mediated hydrolysis and simple chromatographic purification. Because of easy installation of the silyl linker and enough stability in the conditions of Fmoc chemistry, this side chain-tethered strategy should be of great utility in the synthesis of various glycopeptides.

Experimental

Optical rotations were determined with a Jasco DIP-370 polarimeter for solutions in $CHCl_3$, unless noted otherwise. Column chromatography was performed on Silica Gel-60 (E. Merck 70-230 mesh or 230-400

mesh). TLC and HPTLC were performed on Silica Gel 60 F_{254} (E. Merck). ^1H and ^{13}C NMR spectra were recorded with a JEOL AL400 [^1H (400 MHz), ^{13}C (100 MHz)] spectrometer. Chemical shifts are expressed in ppm downfield from the signal for internal Me_4Si for solutions in $CDCl_3$. Fab mass spectra were obtained with a JEOL spectrometer (3-nitrobenzyl alcohol was used as a matrix). Fmoc Gly-preloaded HMP resin and the reagents for the peptide synthesis were purchased from Perkin-Elmer Applied Biosystems, Div. All the solid-phase reactions were performed at room temperature in the capped polypropylene test tubes with stirring on a vortex tube-mixer.

(α,α-Dimethylbenzyl)dimethylsilanol 2

To an ice-cooled solution of chloro(α,α-dimethylbenzyl)dimethylsilane (26.6 g, 0.13 mol) in ether (75 ml), was added with stirring a solution of KOH (8.4 g, 0.15 mol) in 80% aq. MeOH (100 ml) by portions. The mixture was then stirred at room temperature for 20 h. Ether layer was separated and aqueous layer was extracted with ether. The combined etherial extracts were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude product was distilled to give **2** as a colorless oil (22.9 g, 91%), bp. 58-59 °C / 0.1 mmHg. ^1H NMR: δ 7.28-7.21 (m, 4H, Ar), 7.09 (m, 1H, Ar), 1.57 (brs, 1H, O*H*), 1.38 (s, 6H, CMe_2), 0.03 (s, 6H, $SiMe_2$); ^{13}C NMR: -3.4 (Si-Me), 23.5 (C-Me), 28.8 (C-Me), 124.3 (Ar), 126.0 (Ar), 127.7 (Ar), 147.8 (Ar). Anal. Calcd. for $C_{11}H_{18}OSi$: C, 67.98; H, 9.34%. Found: C, 67.51; H, 9.40%.

(α,α-Dimethyl-4-nitrobenzyl)dimethylsilanol 3

To a stirred mixture of **2** (5.0 g, 27.2 mmol) and NH_4NO_3 (2.6 g, 32.5 mmol) in CH_3CN (35 ml), was added $(CF_3CO)_2O$ (5.8 ml, 41.0 mmol) at –15 °C. After stirring for 1 h, an additional amount of $(CF_3CO)_2O$ (4.0 ml, 28.2 mmol) was added to the mixture and stirring was continued overnight at –15 °C – room temperature. The mixture was diluted with water and extracted with EtOAc. The extract was washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was chromatographed on silica gel with hexane-EtOAc (4 : 1) to give **3** (4.0 g, 61%) as colorless crystals, mp. 117-119 °C. ^1H NMR: δ 8.15 (brd, 2H, *J* 9.2 Hz, Ar), 7.41 (brd, 2H, *J* 9.2 Hz, Ar), 1.45 (s, 6H, -CMe_2), 0.05 (s, 6H, -$SiMe_2$); ^{13}C NMR: -3.5 (Si-Me), 23.3 (C-Me), 30.8 (C-Me), 123.1 (Ar), 126.8 (Ar), 145.0 (Ar), 156.8 (Ar). Anal. Calcd. for $C_{11}H_{17}NO_3Si$: C, 55.28; H,7.17; N, 5.86%. Found: C, 55.15; H, 7.15; N, 5.86%.

Chloro(α,α-dimethyl-4-nitrobenzyl)dimethylsilane 4

To an ice-cooled mixture of **3** (3.6 g, 15.0 mmol) and oxalyl chloride (1.5 ml, 17.9 mmol) in dry CH_2Cl_2 (50 ml), was added a drop of DMF. Then the mixture was stirred at 0 °C-room temperature for 1 d and concentrated *in vacuo*. The resulting solid was recrystallized from hexane to give **4** (3.6 g, quant.) as colorless plates, mp 114.5-117 °C. ^1H NMR: δ 8.13 (brd, 2H, *J* 8.8 Hz, Ar), 7.41 (brd, 2H, *J* 8.8 Hz, Ar), 1.49 (s, 6H, -CMe_2), 0.29 (s, 6H, -$SiMe_2$); ^{13}C NMR: -1.2 (Si-Me), 23.4 (C-Me), 30.8 (C-Me), 123.0 (Ar), 127.2 (Ar), 145.4 (Ar), 154.3 (Ar). Anal. Calcd. for $C_{11}H_{16}NO_2SiCl$: C, 51.31; H, 6.26; N, 5.44; Cl, 13.77%. Found: C, 51.41; H, 6.29; N, 5.43; Cl, 13.66%.

N-(9-Fluorenylmethoxycarbonyl)-O-[2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-galactopyranosyl]-L-serine allyl ester 7

A mixture of known N-(9-Fluorenylmethoxycarbonyl)-O-[2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl-(1→3)-2-acetamido-4,6-O-benzylidene-2-deoxy-α-D-galactopyranosyl]-L-serine allyl ester[1] (150 mg, 0.13 mmol) and 80% aq. trifluoroacetic acid was stirred for 2 h. The mixture was concentrated with water and toluene *in vacuo*. The residue was chromatographed on silica gel with $CHCl_3$-EtOH (95 : 5) to afford **7** (115 mg, 83%), Rf 0.40 ($CHCl_3$-MeOH, 9 : 1), $[\alpha]_D$ +55.9° (c 0.5). ^1H NMR: δ 7.76 (d, 2H, *J* 7.3 Hz, Ar), 7.60 (brd, 2H, *J* 6.2 Hz, Ar), 7.34-7.24 (m, 28H, Ar), 5.88 (brd, 1H, *J* 7.5 Hz, N*H*), 5.86 (m, 1H, C*H*=CH_2), 5.48 (d, 1H, *J* 8.8 Hz, NH), 5.31 (brd, 1 H, *J* 16.8 Hz, =C*H*2), 5.25 (brd, 1 H, *J* 10.5 Hz, =CH_2), 4.86 (brs, 1H, H-1a), 1.57 (s, 3H, Ac). Anal. Calcd. for $C_{63}H_{68}N_2O_{15}$•H_2O: C, 68.09; H, 6.34; N, 2.52%. Found: C, 68.14; H, 6.33; N, 2.41%.

N-(9-Fluorenylmethoxycarbonyl)-O-[2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl-(1→3)-2-acetamido-4,6-O-benzylidene-2-deoxy-α-D-galactopyranosyl]-L-threonine allyl ester 8

A mixture of Cp_2ZrCl_2 (1.20 g, 4.7 mmol), $AgClO_4$ (0.97 g, 4.7 mmol), and powdered molecular sieves 4A (10 g) in dry CH_2Cl_2 (90 mL) was stirred at room temperature for 30 min under Ar, then cooled on dry ice-CH_3CN bath (-40 °C). A solution of 2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl-(1→3)-2-azido-4,6-O-benzylidene-2-deoxy-α-D-galactopyranosyl fluoride[1] (2.50 g, 3.00 mmol) and FmocThr-OAll (1.20 g, 3.27 mmol) in dry CH_2Cl_2 (100 mL) was added, and the mixture was stirred between -40 °C and room temperature overnight, then diluted with EtOAc, and filtered through Celite. The filtrate was washed with aq $NaHCO_3$, water, and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by chomatography on silica gel with toluene-EtOAc (4 : 1) to give α-glycoside (2.90 g, 81%) and β-isomer (0.22 g, 6%).

α-glycoside: Rf 0.63 (toluene-EtOAc, 7:3), $[\alpha]_D$ + 83.9° (c 5.8). ^1H NMR: δ 7.75 (d, 2 H, *J* 6.5 Hz,Ar), 7.62 (d, 2 H, *J* 7.3 Hz, Ar), 7.52 (brd, 2 H, Ar), 7.4-7.2 (m, 27H, Ar), 5.90 (m, 1 H, C*H*=CH_2), 5.81 (d, 1 H, *J* 9.5 Hz, N*H*), 5.49 [s, 1 H, PhC*H*$(O)_2$], 5.34 (brd, 1 H, *J* 17.1 Hz, =C*H*2), 5.24 (brd, 1 H, *J* 10.5 Hz, =CH_2), 5.10 (d, 1 H, *J* 3.4 Hz, H-1a), 1.32 (d, 3 H, *J* 6.4 Hz, Thr-γH); ^{13}C NMR: δ 19.1 (Thr-βC), 99.9 (C-1a), 100.4 [Ph*C*H(O)2], 104.8 (C-1b), 156.6 (NH*C*O), 169.7 (*C*O2All). Anal. Calcd for $C_{69}H_{70}N_4O_{14}$: C, 70.27; H, 5,98; N, 4.75%. Found: C, 70.28; H, 5,94; N, 4.54%.

β-isomer: Rf 0.47 (toluene-EtOAc, 7:3), $[\alpha]_D$ + 21.26° (c 1.3). ^1H NMR: δ 7.74 (d, 2 H, *J* 7.6 Hz,Ar), 7.61 (d, 2 H, *J* 7.1 Hz, Ar), 7.52 (brd, 2 H, Ar), 7.4-7.2 (m, 27H, Ar), 5.88 (m, 1 H, C*H*=CH_2), 5.78 (d, 1 H, *J* 9.6 Hz, N*H*), 5.48 [s, 1 H, PhC*H*$(O)_2$], 5.27 (brd, 1 H, *J* 17.3 Hz, =C*H*2), 5.12 (brd, 1 H, *J* 10.3 Hz, =CH_2), 5.10 (d, 1 H, *J* 3.4 Hz, H-1a), 1.34 (d, 3 H, *J* 6.4 Hz, Thr-γH); ^{13}C NMR: δ 16.8 (Thr-βC), 100.3 (C-1a), 100.6 [Ph*C*H(O)2], 104.7 (C-1b), 156.8 (NH*C*O), 170.0 (*C*O2All). Anal. Found: C, 70.20; H, 5,99; N, 4.58%.

The α-glycoside (167 mg, 0.14 mmol) was stirred with Zn powder (1.0 g, 15.3 mmol), AcOH (1.0 ml), and Ac_2O (0.3 ml) in THF at room temperature for 1 h. The mixture was diluted with EtOAc and filtered through Celite. The filtrate was washed with water, aq $NaHCO_3$, and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by chomatography on silica gel with $CHCl_3$-MeOH (9 : 1) to give **8** (174 mg, quant.), Rf 0.49 (toluene-EtOAc, 1 : 1), $[\alpha]_D$ +76.2° (c 0.5). ^1H NMR: δ 7.76 (d, 2 H, *J* 7.6 Hz, Ar), 7.63 (d, 2 H, *J* 7.1 Hz, Ar), 7.53 (brd, 2 H, Ar), 7.5-7.2 (m, 27H, Ar), 5.86 (m, 1 H, C*H*=CH_2), 5.69 (d, 1 H, *J* 8.3 Hz, N*H*), 5.55 (d, 1 H, *J* 9.2 Hz, N*H*), 5.46 [s, 1 H, PhC*H*$(O)_2$], 5.32 (brd, 1 H, *J* 16.9 Hz, =C*H*2), 5.26 (dd, 1 H, *J* 1.1, 10.5 Hz, =CH_2), 5.06 (brd, 1 H, *J* 2.2 Hz, H-1a), 1.71 (s, 3H, Ac), 1.25

(d, 3 H, *J* 6.1 Hz, Thr-γH). Anal. Calcd. for $C_{71}H_{74}N_2O_{15}$•1/2H_2O: C, 70.80; H, 6.27; N, 2.32%. Found: C, 70.77; H, 6.26; N, 2.46%.

N-(9-Fluorenylmethoxycarbonyl)-O-[2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-galactopyranosyl]-L-threonine allyl ester 9

The compound **8** was debenzylidenated as described for **7** to give **9**, Rf 0.21 (toluene-EtOAc, 3 : 7), [α]D +56.0 (c 1.0). ^{1}H NMR: δ 7.77 (d, 2 H, *J* 7.6 Hz, Ar), 7.62 (d, 2 H, *J* 6.9 Hz, Ar), 7.5-7.2 (m, 24H, Ar), 5.83 (m, 1 H, C*H*=CH_2), 5.61 (d, 1 H, *J* 8.3 Hz, N*H*), 5.42 (d, 1 H, *J* 9.2 Hz, N*H*), 5.31 (brd, 1 H, *J* 16.9 Hz, =CH_2), 5.27 (dd, 1 H, *J* 1.2, 10.2 Hz, =CH_2), 4.87 (brd, 1 H, *J* 3.9 Hz, H-1a), 1.68 (s, 3H, Ac), 1.28 (d, 3 H, *J* 6.3 Hz, Thr-γH). Anal. Calcd. for $C_{64}H_{70}N_2O_{15}$: C, 69.42; H, 6.37; N, 2.53%. Found: C, 69.03; H, 6.42; N, 2.62%.

N-(9-Fluorenylmethoxycarbonyl)-O-[(α,α-dimethyl-4-nitrobenzyl)dimethylsilyl]-L-serine allyl ester 10

Procedure A (with NaI-NMM in DMF)

A mixture of **4** (420 mg, 1.63 mmol), **5** (500 mg, 1.36 mmol), and NaI (610 mg, 4.06 mmol) in dry DMF (20 ml) was stirred at room temperature for 15 min. Then N-methylmorpholine (NMM, 179 μl, 1.62 mmol) was added to the mixture. After stirring for 20 min, the mixture was diluted with ether, washed with sat. $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was chromatographed on silica gel with hexane-EtOAc (2 : 1) to afford **10** (775 mg, 97%), Rf 0.85 (hexane-EtOAc, 1 : 1), [α]D +13.4° (c 3.0). ^{1}H NMR: δ 8.13 (bd, 2H, *J* 9.0 Hz, Ar), 7.77 (brd, 2H, *J* 7.6 Hz, Ar), 7.58 (brd, 2H, *J* 6.5 Hz, Ar), 7.40 (brt, 2H, *J* 6.8 Hz, Ar), 7.36 (brd, 2H, *J* 9.0 Hz, Ar), 7.31 (brt, 2H, *J* 7.5 Hz, Ar), 5.89 (m, 1 H, C*H*=CH_2), 5.49 (d, 1 H, *J* 8.0 Hz, N*H*), 5.33 (dd, 1 H, *J* 1.5, 17.1 Hz, =CH_2), 5.26 (dd, 1 H, *J* 1.0, 10.5 Hz, =CH_2), 4.66 (d, 2H, *J* 5.9 Hz, -CH_2CH=CH_2), 4.45-4.35 (m, 3H, Ser-αH, OCH_2CHAr_2), 4.25 (brt, 1H, *J* 7.2 Hz, -C*H*Ar_2), 4.00 (dd, 1H, *J* 2.7, 10.3 Hz, Ser-βH), 3.78 (dd, 1H, *J* 3.3, 10.3 Hz, Ser-βH), 1.40 (s, 6H, CMe_2), 0.00 (2s, 6H, $SiMe_2$). Anal. Calcd. for $C_{32}H_{36}N_2O_7Si$•3/2H_2O: C, 62.42; H, 6.38; N, 4.55%. Found: C, 62.12; H, 6.37; N, 4.91%.

Procedure B (with imidazole in DMF)

A mixture of **4** (95 mg, 0.37 mmol), **5** (100 mg, 0.27 mmol), and imidazole (91 mg, 1.40 mmol) in dry DMF (1 ml) was stirred at room temperature for 3 h. The mixture was diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. Chromatography of the residue on silica gel with hexane-EtOAc (4 : 1) gave **14** (29 mg, 16%) and **10** (59 mg, 37%). compound **14**: Rf 0.88 (hexane-EtOAc, 1 : 1), [α]D +12.4° (c 0.4). ^{1}H NMR: δ 8.12 (brd, 2H, *J* 9.3 Hz, Ar), 7.76 (brd, 2H, *J* 7.6 Hz, Ar), 7.60 (brt, 2H, *J* 6.6 Hz, Ar), 7.40 (brt, 2H, *J* 7.6 Hz, Ar), 7.36 (brd, 2H, *J* 9.0 Hz, Ar), 7.30 (brt, 2H, *J* 7.5 Hz, Ar), 5.89 (m, 1 H, C*H*=CH_2), 5.61 (d, 1 H, *J* 8.3 Hz, N*H*), 5.33 (dd, 1 H, *J* 1.4, 17.1 Hz, =CH_2), 5.25 (dd, 1 H, *J* 1.4, 10.5 Hz, =CH_2), 4.67 (m, 2H, -CH_2CH=CH_2), 4.49-4.43 (m, 2H, Ser-αH, OCH_2CHAr_2), 4.34 (dd, 1H, *J* 7.3, 10.4 Hz, OCH_2CHAr_2), 4.24 (brt, 1H, *J* 7.2 Hz, -C*H*Ar_2), 4.09 (dd, 1H, *J* 2.7, 10.4 Hz, Ser-βH), 3.87 (dd, 1H, *J* 3.5, 10.4 Hz, Ser-βH), 1.40 (s, 6H, CMe_2), 0.00 (s, 6H, $SiMe_2$). Anal. Calcd. for $C_{34}H_{40}N_2O_8Si_2$: C, 61.61; H, 6.39; N, 4.23%. Found: C, 61.89; H, 6.36; N, 4.24%.

N-(9-Fluorenylmethoxycarbonyl)-O-[(α,α-dimethyl-4-nitrobenzyl)dimethylsilyl]-L-threonine allyl ester 11

Compound **11** was prepared from **6** in 73% yield by the same procedure (*A*) as described for **10**. compound **14**: mp 78-80 °C (recrystallized from hexane-EtOAc), Rf 0.62 (hexane-EtOAc, 1 : 1), $[\alpha]_D$ –4.7 °(c 1.1), ^{1}H NMR: δ 8.11 (brd, 2H, *J* 8.8 Hz, Ar), 7.77 (brd, 2H, *J* 7.6 Hz, Ar), 7.61 (brdd, 2H, *J* 4.6, 7.3 Hz, Ar), 7.41-7.29 (m, 6H, Ar), 5.86 (m, 1H, C*H*=CH_2), 5.29 (dd, 1 H, *J* 1.2, 17.1 Hz, =CH_2), 5.23 (dd, 1 H, *J* 1.2, 11.7 Hz, =CH_2), 4.52-4.25 (m, 6H, -CH_2CH=CH_2, Thr-αH, OCH_2CHAr$_2$, -C*H*Ar$_2$), 1.38 (brs, 6H, CMe_2), 0.01 & 0.03 (2s, 6H, $SiMe_2$). Anal. Calcd. for $C_{33}H_{38}N_2O_7Si$: C, 65.76; H, 6.35; N, 4.65%. Found: C, 65.58; H, 6.33; N, 4.47%.

N-(9-Fluorenylmethoxycarbonyl)-O-[2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-6-O-(α,α-dimethyl-4-nitrobenzyl)dimethylsilyl-α-D-galactopyranosyl]-L-serine allyl ester 12

Compound **12** was prepared from **7** in 86% yield by the same procedure (*A*) as described for **10**. compound **12**: Rf 0.42 (toluene-EtOAc, 1 : 1), $[\alpha]_D$ +49.8° (c 0.4), ^{1}H NMR: δ 8.03 (d, 2H, *J* 8.8 Hz, Ar), 7.71 (d, 2H, *J* 7.6 Hz, Ar), 7.55 (brd, 2H, *J* 6.8 Hz, Ar), 7.36-7.16 (m, 26H, Ar), 5.81 (m, 1H, C*H*=CH_2), 5.66 (d, 1H, *J* 7.8 Hz, N*H*), 5.50 (d, 1H, *J* 8.8 Hz, N*H*), 5.26 (brd, 1 H, *J* 17.1 Hz, =CH_2), 5.21 (brd, 1 H, *J* 10.5 Hz, =CH_2), 1.51 (s, 3H, Ac), 1.32 & 1.30 (2s, 6H, CMe_2), -0.04 & -0.09 (2s, 6H, $SiMe_2$). Anal. Calcd. for $C_{74}H_{83}N_3O_{17}Si•H_2O$: C, 66.69; H, 6.42; N, 3.15%. Found: C, 66.96; H, 6.43; N, 3.11%.

N-(9-Fluorenylmethoxycarbonyl)-O-[2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-6-O-(α,α-dimethyl-4-nitrobenzyl)dimethylsilyl-α-D-galactopyranosyl]-L-threonine allyl ester 13

Procedure c (with imidazole in THF)

A mixture of **4** (97 mg, 0.38 mmol), **9** (271 mg, 0.25 mmol), and imidazole (93 mg, 1.37 mmol) in dry THF (5 ml) was stirred at 0 °C for 30 min. The mixture was diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, and concentrated *in vacuo*. Chromatography of the residue on silica gel with toluene-EtOAc (1 : 1) afforded **13** (273 mg, 86%), Rf 0.47 (toluene-EtOAc, 1 : 1), $[\alpha]_D$ +55.7° (c 0.5), ^{1}H NMR: δ 8.07 (d, 2H, *J* 8.7 Hz, Ar), 7.75 (d, 2H, *J* 7.6 Hz, Ar), 7.61 (d, 2H, *J* 7.3 Hz, Ar), 7.38-7.20 (m, 26H, Ar), 5.82 (m, 1H, C*H*=CH_2), 5.60 (d, 1H, *J* 9.1 Hz, N*H*), 5.43 (d, 1H, *J* 9.3 Hz, N*H*), 5.30 (brd, 1 H, *J* 17.1 Hz, =CH_2), 5.25 (brd, 1 H, *J* 10.3 Hz, =CH_2), 1.69 (s, 3H, Ac), 1.37 & 1.36 (2s, 6H, CMe_2), 1.27 (d, 3H, J 6.1 Hz, Thr-γH), 0.00 & -0.04 (2s, 6H, $SiMe_2$). Anal. Calcd. for $C_{75}H_{85}N_3O_{17}Si$: C, 67.80; H, 6.45; N, 3.16%. Found: C, 67.59; H, 6.45; N, 3.16%.

N-(9-Fluorenylmethoxycarbonyl)-O-[(4-amino-α,α-dimethylbenzyl)dimethylsilyl]-L-serine allyl ester 16

A mixture of **10** (780 mg, 1.32 mmol), Zn powder (5.0 g) and AcOH (1 ml) in dry THF was stirred at room temperature for 45 min. The mixture was diluted with EtOAc, filtered through Celite, washed with sat. $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was chromatographed on

silica gel with hexane-EtOAc (1 : 1) to afford **16** (650 mg, 88%), Rf 0.56 (toluene-EtOAc, 1 : 1), $[\alpha]_D$ +7.1° (c 3.0). ^{1}H NMR: δ 7.77 (bd, 2H, *J* 7.5 Hz, Ar), 7.61 (brt, 2H, *J* 7.2 Hz, Ar), 7.41 (brd, 2H, *J* 7.4 Hz, Ar), 7.32 (brt, 2H, *J* 7.4 Hz, Ar), 7.02 (brd, 2H, *J* 8.5 Hz, Ar), 6.62 (brt, 2H, *J* 8.8 Hz, Ar), 5.91 (m, 1 H, C*H*=CH$_2$), 5.55 (d, 1 H, *J* 8.8 Hz, N*H*), 5.35 (dd, 1 H, *J* 1.2, 17.1 Hz, =C*H*$_2$), 5.26 (dd, 1 H, *J* 1.2, 10.5 Hz, =C*H*$_2$), 4.67 (brd, 2H, *J* 5.9 Hz, -C*H*$_2$CH=CH$_2$), 4.45-4.40 (m, 2H, Ser-αH, OC*H*$_2$CHAr$_2$), 4.27 (dd, 1H, *J* 7.3, 10.2 Hz, OC*H*$_2$CHAr$_2$), 4.27 (brt, 1H, *J* 7.3 Hz, -C*H*Ar$_2$), 4.00 (dd, 1H, *J* 2.7, 10.3 Hz, Ser-βH), 3.72 (dd, 1H, *J* 3.2, 10.3 Hz, Ser-βH), 1.30 & 1.29 (2s, 6H, CMe$_2$), -0.02 & -0.04 (2s, 6H, SiMe$_2$).
Anal. Calcd. for $C_{32}H_{38}N_2O_5Si$: C, 68.79; H, 6.86; N, 5.01%. Found: C, 68.77; H, 6.89; N, 4.82%.

N-(9-Fluorenylmethoxycarbonyl)-O-[(α,α-dimethyl-4-succin-mono-amidobenzyl)dimethylsilyl]-L-serine allyl ester 17

A mixture of **16** (650 mg, 1.16 mmol), succinic anhydride (120 mg, 1.20 mmol), and NMM (111μl, 0.89 mmol) in CH_2Cl_2 (20 ml) was stirred at room temperature for 4 h and then concentrated *in vacuo*. The residue was chromatographed on Biobeads S-X3 with toluene-EtOAc (1 : 1) to give **17** (760 mg, 99%), Rf 0.37 ($CHCl_3$-MeOH, 9 : 1), $[\alpha]_D$ +2.6° (c 1.0). ^{1}H NMR: δ 7.74-7.13 (m, 12H, Ar), 5.87 (m, 1H, C*H*=CH$_2$), 5.56 (d, 1H, *J* 8.8 Hz, N*H*), 5.31 (brd, 1H, *J* 16.1 Hz, =C*H*$_2$), 5.23 (brd, 1H, *J* 10.5 Hz, =C*H*$_2$), 4.64 (d, 2H, *J* 5.6 Hz, -C*H*$_2$CH=CH$_2$), 4.40 (m, 1H, Ser-αH), 4.39, 4.31 & 4.24 (3brt, 3H, Ar$_2$C*H*C*H*$_2$-), 3.95 (dd, 1H, *J* 2.2, 10.0 Hz, Ser-βH), 3.69 (dd, 1H, *J* 3.2, 10.3 Hz, Ser-βH), 2.63 & 2.50 (2m, 4H, -COC*H*$_2$C*H*$_2$CO$_2$H), 1.29 & 1.30 (2s, 6H, CMe$_2$), -0.50 & -0.59 (2s, 6H, SiMe$_2$).
Anal. Calcd. for $C_{36}H_{42}N_2O_8Si \cdot H_2O$: C, 63.69; H, 6.55; N, 4.13%. Found: C, 63.94; H, 6.31; N, 4.18%.

Attachment of 17 to resin (synthesis of 18)

Commercial FmocGly-HMP-resin (1g, 0.78 mmol/g) was stirred with 50% piperidine/NMP solution (7 ml) for 2 h. Then the mixture was filtered on a sintered glass disk, washed successively with NMP, 2-propanol, CH_2Cl_2, and ether. The resulting resin was dried *in vacuo* to give N-deprotected H-Gly-HMP-resin (806 mg), which was used for further solid-phase synthesis. A mixture of **17** (250 mg, 0.38 mmol), HBTU (288 mg, 0.76 mmol), 0.5 M HOBt/DMF (0.76 ml, 0.38 mmol), and 2M N,N-diisopropylethylamine/DMF (0.19 ml, 0.38 mmol) in NMP (3.0 ml) was stirred at room temperature for 80 min. Then the above H-Gly-HMP-resin (0.95 mmol/g, 271 mg, 0.25 mmol) was added and the mixture was stirred overnight. The resin was collected by filtration, washed successively with NMP, 2-propanol, CH_2Cl_2, and ether, and dried *in vacuo* to give **18** (439 mg, quantitative)

N-(9-Fluorenylmethoxycarbonyl)-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-threonyl-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-threonyl-L-serine allyl ester 22

The resin **18** (0.59 mmol/g, 151 mg, 0.09 mmol) was N-deprotected with 50% piperidine/NMP to produce **19** (134 mg) in the similar manner as described above. A mixture of **20** (33 mg, 0.05 mmol), HBTU (37 mg, 0.1 mmol), HOBt (66 mg, 0.49 mmol), and 2M *i*-Pr$_2$NEt /DMF (0.49 μl, 0.1 mmol) in NMP (1.0 ml) was stirred at room temperature for 35 min. Then the resin **19** (0.47 mmol/g, 66 mg, 0.04 mmol) was added and the mixture was stirred overnight. The resin was collected by filtration, washed successively

with NMP, 2-propanol, CH_2Cl_2, and ether, and dried *in vacuo* to afford glycopeptide-linked resin (89 mg). The procedures for N-deprotection and coupling with **20** were repeated once more to produce **21** (112 mg). A mixture of 0.2 M-CsF/1 M-AcOH/DMF and **21** (77 mg) was stirred overnight and filtered. The resin was washed with NMP, 2-propanol, CH_2Cl_2, and ether. The combined filtrate and washings were concentrated *in vacuo*. The residue was diluted with EtOAc, washed with water, brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude product was chromatographed on a preparative TLC plate (1 mm thick, 20 x 20 cm) with $CHCl_3$-MeOH (9 : 1) to give **22** (27 mg, 73%), Rf 0.64 ($CHCl_3$-EtOH, 9 : 1), $[\alpha]_D$+83.0° (c 1.0). ^1H NMR: δ: 7.78 (d, 2H, *J* 7.6 Hz, Ar), 7.64 (d, 2H, *J* 7.3 Hz, Ar), 7.43-7.32 (m, 4H, Ar), 7.02 (d, 1H, *J* 7.3 Hz, N*H*), 6.75 (d, 1H, *J* 8.8 Hz, N*H*), 5.92 (m, 1H, C*H*=CH_2), 5.84 (d, 1H, *J* 8.3 Hz, N*H*), 5.40-5.36 (m, 1H, =CH_2), 5.36 (brd, 2H, *J* 2.9 Hz, GalNAc H-4). 5.29 (dd, 1H, *J* 1.0, 10.5 Hz, =CH_2), 5.21 (dd, 1H, *J* 3.2, 11.7 Hz, GalNAc H-3), 5.17 (d, 1H, *J* 3.7 Hz, GalNAc H-1), 5.10 (dd, 1H, J 2.9, 11.7 Hz, GalNAc H-3), 4.99 (d, 1H, *J* 3.4 Hz, GalNAc H-1), 4.69 (brd, 2H, J 5.6 Hz, -CH_2CH=CH_2), 2.16 (s, 3H, Ac), 2.15 (s, 3H, Ac), 2.04 (s, 3H, Ac), 2.00 (s, 9H, 3Ac), 1.99 (s, 6H, 2Ac), 1.28 (d, 3H, Ac), 1.24 (d, 3H, Ac).

N-(9-Fluorenylmethoxycarbonyl)-L-seryl-L-glycyl-L-valyl-L-alanine benzyl ester 25

The resin **18** (50 mg, 30 μmol) was stirred with $Pd(PPh_3)_4$ (7 mg, 6 μmol) and dimedone (85 mg, 600 μmol) in dry THF (2.0 ml) for 3.5 h, then washed with NMP, 2-propanol, CH_2Cl_2, and ether, and dried *in vacuo* to give **23** (53 mg). A mixture of **23** (53mg, 0.03 μmol), HBTU (22 mg, 0.06 μmol), HOBt (40 mg, 0.30 mmol), *i*-Pr_2NEt (15 μl, 0.03 mmol) in NMP (1 ml) was stirred for 1 h. Then **24** (15 mg, 0.05 mmol) was added to the mixture and stirring was continued overnight. The reaction was worked up as descrived above to give tetrapeptide-linked resin (57 mg), which was submitted to the cleavage conditions using CsF/AcOH/DMF. The crude product was purified by preparative TLC with $CHCl_3$-MeOH (9 : 1) to give **25** (14 mg, 76%), Rf 0.49 ($CHCl_3$-MeOH, 4 : 1), $[\alpha]_D$ -21.2° (c 0.5, $CHCl_3$-MeOH, 4 : 1). ^1H NMR (DMSO-d_6): δ: 8.41 (d, 1H, *J* 6.6 Hz, N*H*), 8.13 (t, 1H, *J* 6.6 Hz, N*H*), 7.82 (d, 2H, *J* 7.3 Hz, Ar), 7.68-7.65 (m, 3H, Ar, N*H*), 7.36-7.23 (m, 9H, Ar), 5.02 (s, 2H, -CH_2Ph), 1.84 (m, 1H, Thr-βH), 1.22 (d, 3H, *J* 7.3 Hz, Ala-βH), 0.75 & 0.69 (2d, 6H, *J* 6.9 Hz, Thr-γH). Fab•MS: m/z 667.2 (M+Na).

N-(9-Fluorenylmethoxycarbonyl)-O-[2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-6-O-(α,α-dimethyl-4-aminobenzyl)dimethylsilyl-α-D-galactopyranosyl]-L-threonine allyl ester 26

Compound **13** (147 mg, 0.11 mmol) was reduced in the same manner as described for **16**. Chromatographic purification on silica gel with toluene-EtOAc (1 : 1) afforded **26** (116 mg, 81%), Rf 0.25 (toluene-EtOAc 1 : 1), $[\alpha]_D$+49.0° (c 1.0). ^1H NMR: δ: 7.74 (d, 2H, *J* 7.4 Hz, Ar), 7.61 (d, 2H, *J* 7.3 Hz, Ar), 7.40-7.20 (m, 24H, Ar), 6.98 (brd, 2H, *J* 8.5 Hz, Ar), 6.53 (brd, 2H, *J* 8.5 Hz, Ar), 5.81 (m, 1 H, C*H*=CH_2), 5.61 (d, 1 H, *J* 9.0 Hz, N*H*), 5.58 (d, 1 H, *J* 9.5 Hz, N*H*), 5.28 (brd, 1 H, *J* 17.3 Hz, =CH_2), 5.23 (dd, 1 H, *J* 1.2, 10.5 Hz, =CH_2), 4.74 (d, 1H, *J* 3.7 Hz, H-1a), 1.27 (s, 6H, CMe_2), 1.24 (d, 3H, *J* 6.1 Hz, Thr-γH), -0.03 & -0.05 (2s, 6H, SiMe_2). Anal. Calcd. for $C_{75}H_{87}N_3O_{15}Si$: C, 69.37; H, 6.75; N, 3.24%. Found: C, 69.01; H, 6.79; N, 3.24%.

N-(9-Fluorenylmethoxycarbonyl)-O-[2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl-(1→3)-2-acetamido-4,6-O-benzylidene-2-deoxy-α-D-galactopyranosyl]-L-threonine 31

A mixture of **8** (138 mg, 0.12 mmol), $Pd(PPh_3)_4$ (2 mg, 1.7 μmol), and N-methylaniline (125 μl, 1.15 mmol) in dry THF (3 ml) was stirred under Ar at room temperature for 30 min. The mixture was diluted with EtOAc, washed with 1N HCl and brine, dried over Na_2SO_4, and concentrated *in vacuo*. The crude product was chromatographed on Bio-beads S X3 to give **31** (127 mg, 95%), Rf 0.30 ($CHCl_3$-MeOH, 9 : 1), $[\alpha]_D$ +100.4 °(c 0.5). ^1H NMR: δ: 7.75 (d, 2H, *J* 6.8 Hz, Ar), 7.61 (d, 2H, *J* 6.1 Hz, Ar), 7.51 (m, 3H, Ar), 7.40-7.18 (m, 25H, Ar), 6.13 (br, 1H, N*H*), 5.52 (br, 1H, N*H*), 5.40 [s, 1H, PhC*H*$(O)_2$], 1.81 (s, 3H, Ac), 1.19 (d, 3H, *J* 6.1 Hz, Thr-γH). Anal. Calcd. for $C_{68}H_{70}N_2O_{15}$•1/2H_2O: C, 70.14; H, 6.14; N, 2.40%. Found: C, 70.04; H, 6.13; N, 2.37%.

N-(Benzyloxycarbonyl)-O-benzyl-L-seryl-O-[2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl-(1→3)-2-acetamido-4,6-O-benzylidene-2-deoxy-α-D-galactopyranosyl]-L-seryl-O-[2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl-(1→3)-2-acetamido-4,6-O-benzylidene-2-deoxy-α-D-galactopyranosyl]-L-threonyl-O-[2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-α-D-galactopyranosyl]-L-threonyl-L-glycyl-L-valyl-L-alanine benzyl ester 39

A mixture of **26** (192 mg, 0.15 mmol) and succinic anhydride (16 mg, 0.16 mmol) in dry CH_2Cl_2 (3 ml) was stirred at room temperature for 2 h, and then concentrated *in vacuo*. The residue was submitted to gel filtration on Bio-beads S X3 with toluene-EtOAc (1 : 1) and the obtained succin-mono-amide derivative (185 mg, 90%) was used for the next reaction without further purification. A mixture of the above succin-mono-amide (84 mg, 0.06 mmol), H-Gly-HMP-resin (32 mg, 0.03 mmol), HBTU (45 mg, 0.12 mmol), HOBt (81 mg, 0.60 mmol), and *i*-Pr_2NEt (89 μl, 0.18 mmol) in NMP (1 ml) was stirred overnight. After filtration, washing with NMP, 2-propanol, and CH_2Cl_2, and drying *in vacuo*, the resin **27** (66 mg) was obtained. The resin **27** (63 mg, 26 μmol) was stirred with $Pd(PPh_3)_4$ (6 mg, 5 μmol), and dimedone (72 mg, 512 μmol) in dry THF (1 ml) for 5 h. Filtration of the mixture and washing with THF, 2-propanol, CH_2Cl_2, and ether afforded **28** (65 mg), which was stirred with **24** (17 mg, 51 μmol), HBTU (20 mg, 52 μmol), HOBt (35 mg, 259 μmol), and *i*-Pr_2NEt (39 μl, 79 μmol) in NMP (1.5 ml) overnight. The resin **29** (68 mg), obtained via filtration and washing, was treated with 50% piperidine/NMP (1.5 ml) for 2 h, and filtered off to give **30** (61 mg). The resin **30** was reacted with **31** (59 mg, 51 μmol) using HBTU (39 mg, 103 μmol), HOBt (69 mg, 510 μmol), and *i*-Pr_2NEt (38 μl, 77 μmol) in NMP (2 ml) overnight. Filtration and washing afforded **32** (84 mg). The resin **32** (80 mg, 22 μmol) was N-deprotected to **33** (75 mg) as described above, and then coupled with **34** (50 mg, 44 μmol) by stirring with HBTU (34 mg, 90μmol), HOBt (60 mg, 444 μmol), and *i*-Pr_2NEt (39 μl, 79 μmol) in NMP (1.5 ml) overnight to furnish **35** (96 mg). In a similar manner, **35** (93 mg, 21 μmol) was converted to **36** (88mg) by treatment with 50% piperidine/NMP for 2 h and then condensed with **37** (14 mg, 43 μmol) in the presence of HBTU (31mg, 82 μmol), HOBt (56 mg, 414 μmol), and *i*-Pr_2NEt (31 μl, 63 μmol) in NMP (2 ml) to give **38** (93 mg). To a mixture of **38** (47 mg, 10 μmol) and AcOH (60 μl, 1 mmol) in THF (1 ml) was added 1M TBAF/THF (1 ml, 1 mmol). The mixture was stirred overnight and filtered. The resin was washed successively with THF, 2-propanol, CH_2Cl_2, and ether. The combined filtrate and washings were concentrated *in vacuo*. The product was extracted with EtOAc, washed with brine,

dried over Na_2SO_4, and concentrated *in vacuo*. The residue was chromatographed on Bio-beads S X1 with toluene-EtOAc (1 : 1). The glycopeptide fraction was further purified by preparative TLC with $CHCl_3$-MeOH (9 : 1) to give **39** (18 mg, 55% overall), Rf 0.23 ($CHCl_3$-MeOH, 9 : 1), $[\alpha]_D$ +79.5° (c 0.7). ^1H NMR (50 °C): δ 5.42 [s, 1H, PhC*H*$(O)_2$], 5.41 [s, 1H, PhC*H*$(O)_2$], 6.69, 6.68 & 6.65 (3s, 9H, 3Ac), 5.13 & 5.06 (2d, 4H, *J* 12.5 Hz, CO_2CH_2Ph x 2), 2.00 (m, 1H, Val-βH), 1.25 (brd, 3H, *J* 7.8 Hz, Ala-βH), 1.11 (brd, 3H, *J* 5.6 Hz, Thr-γH), 1.06 (brd, 3H, *J* 6.1 Hz, Thr-γH), 0.86 (brd, 3H, *J* 6.8 Hz, Val-γH), 0.83 (brd, 3H, *J* 6.6 Hz, Val-γH), HRMS Calcd for $C_{186}H_{211}O_{44}N_{10}$ (M+H), m/z 3288.4581 (relative intensity 41.7%). Found 3288.4521 (46.5%).

Acknowledgments

We thank Shin-Etsu Chemical Co., Ltd. for the generous gift of silyl derivatives. We thank Dr. Ohnishi for Fab MS measurement, Dr. T. Chihara and his staff for elemental analyses, Mr. H. Nakano and Ms. A. Takahashi for technical assistance.

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