New Allyl Ester Linker and Solid-phase Block Synthesis of the Serglycin Core Region

Yuko Nakahara, Sumie Ando, Yukishige Ito, Hironobu Hojo, and Yoshiaki Nakahara

1The Institute of Physical and Chemical Research (RIKEN), Hirosawa 2-1, Wako-shi, Saitama 351-0198, Japan
2Department of Industrial Chemistry, Tokai University, Kitakaname 1117, Hiratsuka-shi, Kanagawa 259-1292, Japan
CREST, Japan Science and Technology Corporation (JST)

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The prototype glycopeptidyl fragments of serglycin, a proteoglycan with the characteristic peptide sequence of repeating L-seryl-L-glycine, were synthesized by a convergent method involving block condensation on a solid support. In order to facilitate detachment of the protected glycopeptides from the resin, a new allyl ester type of linker, which is cleavable by Pd(0)-catalysis, was designed and used in combination with the commercial acid-labile Sieber amide resin for the solid-phase synthesis. Glycopeptide blocks consisting of [O-(2,3,4-tri-O-acetyl-D-xylosyl)-L-seryl-L-glycine]n (n = 1 to 8) were produced in good yields. Block condensation in a solution was also successful to synthesize up to the hexadecapeptide (n = 8).

Key words: solid-phase synthesis; allyl linker; block condensation; serglycin; glycopeptide

Since the biological significance of oligosaccharides covalently linked to proteins has been recognized, numerous synthetic efforts have been made to access N- and O-glycans stereospecifically or stereoselectively by enzymatic or chemical methods. A variety of homogeneous samples thus synthesized have been extensively used for biological studies on substrate-receptor interactions with monoclonal antibodies, lectins, and glycosyltransferases with the aim of finding potential therapeutic agents. In the course of our synthetic studies on complex glycoconjugates, we have recently reported solid-phase syntheses of N- and O-glycan-bound oligopeptides, assuming that such samples would help to obtain a more in-depth understanding of the mechanism for biological interactions involving the carbohydrate surroundings. These syntheses were efficiently achieved by the stepwise addition of amino acid and glycosylated amino acid building blocks according to Fmoc methodology. However, in order to advance synthesis to a target as large as glycoprotein size, block coupling would be the method of choice, although only a few examples have so far been reported of such a convergent strategy.

In this context, we decided to focus our attention on the block synthesis of glycopeptides. The selected targets were oligomers representing the core structure of serglycin, a structurally unique proteoglycan which consists of a repeating Ser-Gly sequence. The Ser residues are heavily glycosylated with clustered chondroitin sulfate or heparin. Despite a number of studies on the synthesis of glycosaminoglycans, the huge oligosaccharide attachment of proteoglycans, only a few papers have described the synthetic assembly of glycosylated serine along the peptide backbone.

To efficiently achieve a block synthesis, it is desirable for the intermediate glycopeptide blocks to be readily split from the resin in a protected form suitable for further peptide chain-elongation reactions. Most of the conventional resins used in peptide synthesis, however, have been designed to release peptide oligomers under strongly acidic conditions which allow concomitant destruction of the side-chain functional groups. The conditions are therefore incompatible with the presence of acid-susceptible oligosaccharide linkages. On the other hand, the allyl ester type of linkers, HYCRAM and HYCRON, both reported by Kunz, can be specifically cleaved under neutral conditions by using a Pd(0) catalyst. Several glycopeptides have been synthesized on the linkers and released from the resin in the protected form.

We describe in this paper the preparation of a newly designed allyl ester anchor and the successful solid-phase synthesis of serglycin glycopeptide on it.

Results and Discussion

Our strategy for the solid-phase synthesis of sergly-
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Fig. 1. Structures of Proteoglycans and the Core Region of Serglycin.

Fig. 2. Strategy for the Solid-phase Synthesis of Glycopeptides Selectively Protected at the N- and C-terminal.

cin fragments is outlined in Fig. 2. Linear connection of this new allyl linker to the mild acid-labile amide resin allows the synthesized products to be cleaved from the resin either at site a or b by a chemoselective reaction. Cleavage at site a is possible by a Pd(O)-catalyzed reaction to release the synthesized oligomers with C-terminal carboxylic acid. On the other hand, a mild acid treatment leads to disconnection at b, and the products are obtained as the C-terminal protected oligopeptides. The resulting C-terminal allyl ester can also be cleaved by Pd-catalysis in a solution to give the same carboxylic acids in two steps. When the resin-bound glycopeptides are released by acidolysis after N-deprotection, the detached oligopeptides would be useful as the N-components in a solution-phase block condensation.

The allyl ester anchor, t-butyl 6-bromo-(E)-4-hexenoate (1), was first prepared from commercial 1,4-dibromo-2-butene and the lithium enolate of t-butyl acetate in a 60-70% yield. With bromide 1, several Fmoc amino acids could be readily esterified through their cesium salts as shown in Scheme 1. Among them, the conversion of Fmoc proline resulted in a low efficiency under these reaction conditions.
However, use of the phase-transfer procedure reported by Seitz and Kunz has improved the yield of 3.17 The solid-phase synthesis of serglycin fragments was investigated with the glycine allyl ester 2. Compound 2 was treated with TFA (trifluoroacetic acid) in CH$_2$Cl$_2$ to quantitatively cleave the t-butyl ester. Resulting carboxylic acid 9 was attached to commercial acid-labile Sieber amide resin 10 (0.6 mmol/g) via N-deprotection of the resin with 20% piperidine in NMP (N-methylpyrrolidone) and activation with HBTU (O-benzotriazol-1-yl-N, N', N'-tetramethyluronium hexafluorophosphate), HOBt (hydroxybenzotriazole), and DIEA (disopropylethylamine) in NMP to afford 12. In all the coupling and N-deprotection reactions on the resin, effective stirring of the reaction mixtures was achieved by a vortexing tube mixer. The coupling efficiency was monitored by a conventional ninhydrin test.20 The loading of 9 to the resin was estimated to proceed in a 99% yield. In contrast, an attempt to use acid-labile glycin-preloaded chlorotrityl resin 11 was unsuccessful. While ninhydrin monitoring indicated a good coupling efficiency (>99%), the gross weight of the resin decreased in the early stage of the synthetic cycle, probably due to the physical instability of the chlorotrityl resin used. Therefore, the following solid-phase synthesis of serglycin fragments was carried out by using the more stable Sieber amide resin. Resin 12 was N-deprotected with 20% piperidine and then condensed with known xylosyl serine derivative 17 under conditions using HBTU, HOBt and DIEA in NMP to give 18. Necessary glycoserine unit 17 was alternatively synthesized via glycosidation of 2,3,4-tri-O-acetyl-α-D-xylopyranosyl trichloroacetimidate 14 and Fmoc serine allyl ester 15, with subsequent

**Scheme 1.** Synthesis of the Amino Acid Derivatives Carrying an Allyl Ester Linker.

*The number in parentheses indicates the yield by procedure B (see the experimental section).

**Scheme 2.** Attachment of the Allyl Linker to the Resin and Preparation of the Xylosyl Serine Building Block.
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deleallylation\textsuperscript{23,24} of coupling product 16.

A part of glycosyl seryl glycine-loaded resin 18 was treated with Pd(Ph\textsubscript{3}P)\textsubscript{4} and dimedone in DMSO-
CH\textsubscript{2}Cl\textsubscript{2} to disconnect the allyl ester linkage. Compound 20 released from the resin was quantitatively isolated. On the other hand, treatment of resin 18 with 2\% TFA in CH\textsubscript{2}Cl\textsubscript{2} exclusively afforded 5-carbamoyl-2-pentenyl ester 21, which was readily converted to 20 by Pd(0)-catalyzed deallylation in a solution. On-resin block condensation was first examined with N-deprotected resin 19 and carboxylic acid 20 (1.5 equivalent) under the same coupling conditions. After 3 h, the reaction was almost complete and ninhydrin monitoring indicated a high efficiency (99.7\%) for the reaction. Further condensation was investigated with the resulting resin 22. Cleavage of allyl ester 22 by Pd-catalysis produced carboxylic acid 23 in a 96\% yield, while resin 22 was N-deprotected to afford 26. Carboxylic acid component 23 (1.5 equivalents) was activated and condensed with 26 under the same conditions to give tetraxylosyl octapeptide-linked resin 27. Similarly, resin 27 was cleaved at the allyl ester linkage to afford 28 in a 64\% yield. In contrast, acidic cleavage of the glycopeptide from 27 gave 29, which could be readily deallylated in a solution to produce 28 in an 85\% overall yield. The poor solubility of octapeptide 29 required the deallylation reaction to be performed in the absence of trifluoroethanol in THF. Resin 27 was N-deprotected to 31 and condensed with 28 (1.5 equivalents) to furnish tetraxylosyl hexacapeptide-linked resin 32. The coupling reaction with such large blocks proceeded smoothly, and the ninhydrin test showed 97.7\% efficiency. The product was finally released in the cleavage condition with 2\% TFA in CH\textsubscript{2}Cl\textsubscript{2} and purified by reversed-phase HPLC. Glycopeptide 33 was obtained in a 79\% yield, and its structure was elucidated by NMR and TOF mass spectra.

Attention was then turned to solution-phase block condensation between the higher oligomers. Tetrapeptide block 24 was treated with morpholine in CH\textsubscript{2}Cl\textsubscript{2} to give 25 in a 93\% yield, this then being reacted with one equivalent of 23 in the presence of the same activation reagents. The reaction was completed within 2 h, and octapeptide 29 was produced in an 84\% yield. To achieve block condensation between the octapeptide blocks, amino counterpart 30 was prepared from 31 by acidic cleavage. Solution-phase condensation of 28 and 30 (2.0 equivalents) was performed with the same reagents overnight, and coupling product 33 was isolated by HPLC in a 66\% yield.

In summary, we demonstrated the solid-phase synthesis of glycopeptides by using a newly developed allyl ester linker in combination with an acid-labile Sieber amide resin. The serglycin fragments were efficiently synthesized by means of the convergent protocol. To our knowledge, this is the first example of on-resin block synthesis with such large glycopeptide building blocks. In addition, we demonstrated that the oligoglycopeptides synthesized on the resin were also useful building blocks for solution-phase block condensation.

Experimental

Optical rotation values were determined with a Jasco DIP-370 polarimeter for solutions in CHCl\textsubscript{3} at 20±2°C, unless otherwise noted. Column chromatography was performed on Silica Gel-60 (E. Merck 70–230 mesh or 230–400 mesh), and TLC and HPTLC were performed on Silica Gel 60 F\textsubscript{254} (E. Merck). Preparative HPLC was performed with Mightsil RP-18 (10×250 mm\textsuperscript{2}; Kanto Chemical Co.). \textsuperscript{1}H- and \textsuperscript{13}C-NMR spectra were recorded with a Jeol AL400 [\textsuperscript{1}H (400 MHz), \textsuperscript{13}C (100 MHz)] spectrometer. Chemical shifts are expressed in ppm downfield from the signal for internal Me\textsubscript{4}Si for solutions in CDCl\textsubscript{3}. FAB mass spectra were obtained with a Jeol JMS-HX-110 spectrometer using 3-nitrobenzyl alcohol as a matrix. TOF mass spectra were recorded with a Shimadzu/Kratos Kompakt MALDI IV or PerSeptive Voyager-DE PRO spectrometer (α-cyano-4-hydroxycinnamic acid or 2,5-dihydroxybenzoic acid). Sieber amide resin was purchased from Novabiochem Ltd. All the solid-phase coupling and cleavage reactions were performed at room temperature in capped polypropylene test tubes while stirring with a vortex tube mixer, while N-deprotection on resin and the capping procedure were carried out with the help of an ABI model 433A peptide synthesizer with a conductivity monitoring system. The coupling reagent mixture (0.45 M HBTU/HOBt/DMF) was freshly prepared just before use according to the manual for the peptide synthesizer.

t-Butyl 6-bromo-(E)-4-hexenoate (I). To a stirred solution of N-isopropylcyclohexylamine (1.7 g, 12.0 mmol) in dry THF (10 ml) was added 1.6 N n-butyllithium/n-hexane (7.5 ml, 12.0 mmol) at −78°C under Ar, and the mixture was stirred for 1 h. A solution of t-butyl acetae (1.4 g, 12.0 mmol) in dry THF (10 ml) was then added to the mixture. The mixture was stirred for 1 h before adding a solution of 1,4-dibromo-2-butene (2.1 g, 10 mmol) in dry THF (10 ml). After stirring for 10 min at −78°C, the temperature was raised to −10°C and stirring continued for a further 1 h. The reaction was quenched with sat. NH\textsubscript{4}Cl (300 ml), and the mixture was extracted with EtOAc. The extract was successively washed with water, 10% HCl and brine, dried over Na\textsubscript{2}SO\textsubscript{4}, and concentrated in vacuo. The crude product was distilled under reduced pressure to give 1 (1.7 g, 68\%), bp 80°C at 3 mm of Hg, Rf 0.38 (hexane-EtOAc, 20:1); \textsuperscript{1}H-NMR δ: 5.82–5.67 (2H, m, H-4, H-5), 3.93 (2H, d, J = 6.3 Hz, H-6), 2.40–2.27
(4H, m, H-2, H-3), 1.45 (9H, s, t-Bu). Anal. Calcd. for C_{40}H_{42}O_{12}Br: C, 48.21; H, 6.88%. Found: C, 48.06; H, 6.90%.

Esterification of Fmoc amino acids with 1. (2-8)

Procedure A (via Cs salts). To a stirred solution of Fmoc amino acid (9.9 mmol) in MeOH (100 ml) was added a 20% Cs_{2}CO_{3} aq. solution (7.3 ml, 4.5 mmol) to adjust pH to 5-6. The mixture was concentrated in vacuo to the residual salt which was subsequently dissolved in water and lyophilized. The salt was dissolved in dry DMF (60 ml) and stirred with 1 (2.45 g, 9.9 mmol) at room temperature for 1-3 h. The mixture was concentrated in vacuo. The crude product was extracted with ether-EtOAc (1:1), successively washed with water and brine, dried over Na_{2}SO_{4}, and concentrated in vacuo. The residue was chromatographed on silica gel with toluene-EtOAc.

t-Butyl 6-[N-(9-fluorenylmethoxycarbonyl)-l-glycglycine]-(E)-4-hexenoate (2). 82% yield, Rf 0.26 (toluene-EtOAc, 1:1), mp 47-49°C; 1H-NMR δ: 7.74 (2H, d, J = 7.6 Hz, Ar), 7.59 (2H, d, J = 7.3 Hz, Ar), 7.38 (2H, t, J = 6.9 Hz, Ar), 7.29 (2H, t, J = 7.3 Hz, Ar), 5.83-5.53 (2H, m, H-4, H-5), 5.44 (1H, t, J = 5.3 Hz, NH), 4.57 (2H, d, J = 6.3 Hz, H-6), 4.39 (2H, d, J = 7.3 Hz, -CH_{2}O-), 4.21 (1H, t, J = 6.9 Hz, Ar), 3.97 (2H, d, J = 5.6 Hz, Gly-OH), 2.30 (4H, brs, H-2, H-3), 1.43 (9H, s, t-Bu). Anal. Calcd. for C_{41}H_{42}O_{12}N: C, 69.66; H, 6.71; N, 3.01%. Found: C, 69.56; H, 6.71; N, 2.92%.

t-Butyl 6-[N-(9-fluorenylmethoxycarbonyl)-l-prolyglycine]-(E)-4-hexenoate (3). 20% yield, Rf 0.18 (toluene-EtOAc, 10:1), [α]_D -41.4° (c 1.1); 1H-NMR δ: 7.76 (2H, d, J = 7.3 Hz, Ar), 7.64-7.53 (2H, m, Ar), 7.39 (2H, t, J = 7.3 Hz, Ar), 7.32-7.30 (2H, m,
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Ar), 5.75-5.50 (2H, m, H-4, H-5), 4.58 (2H, d, J = 6.3 Hz, H-6), 4.48-4.29 (4H, m, -CH(O)-, ArCH(O), Pro-oH), 3.64-3.54 (2H, m, Pro-oH), 2.30-2.23 (5H, m, H-3, H-4, Pro-bH), 2.03-1.91 (3H, m, Pro-bH, Pro-yH), 1.43 (9H, s, t-Bu). Anal. Calcd. for C39H37O6N: C, 75.55; H, 6.47; N, 3.60%. Found: C, 75.77; H, 6.50; N, 3.52%.

1-t-Butyl 6-[N², N²-bis-(9-fluorenyl methoxy carbonyl)-1-lysyl oxyl-(E)-4-hexenoate (8). 82% yield, Ref 0.21 (toulene-EtOAc, 10:1), mp 103°C, [α]D -0.5° (c 1.0); 1H-NMR δ: 7.74 (4H, d, J = 7.3 Hz, Ar), 7.57 (4H, d, J = 6.9 Hz, Ar), 7.38 (4H, t, J = 7.3 Hz, Ar), 5.81-5.53 (2H, m, H-4, H-5), 5.39 (1H, d, J = 7.3 Hz, Lys-oH), 4.90 (1H, s, Lys-eH), 4.58 (2H, d, J = 6.3 Hz, H-6), 4.38 (5H, m, -CH(O)-, Lys-aH), 4.20 (2H, t, J = 6.3 Hz, ArCH-), 3.19 (2H, m, Lys-eH), 2.30 (4H, brs, H-2, H-3), 1.56 (2H, s, Lys-CH), 1.90-1.30 (4H, m, Lys-bH, Lys-yH), 1.43 (9H, s, t-Bu). Anal. Calcd. for C44H45O11N: C, 72.80; H, 6.64; N, 3.69%. Found: C, 72.77; H, 6.65; N, 3.54%.

Procedure B (phase transfer condition). A mixture of Fmoc proline (340 mg, 1 mmol) and 1 (249 mg, 1.0 mmol) in CH2Cl2 (16 ml) was added to a stirred solution of Bu4NBr (322 mg, 1.0 mmol) in sat. NaHCO3 aq. (16 ml). The mixture was stirred at room temperature for 1 day before diluting with CHCl3. The separated organic layer was successively washed with water and brine, dried over Na2SO4, and concentrated in vacuo. The residue was chromatographed on silica gel with octane-EtOAc (10:1) to give 3 (325 mg, 65%).

6-[N²-(9-fluorenyl methoxy carbonyl)-1-glycyl oxyl-(E)-4-hexenoic acid (9). To a stirred solution of 2 (13.9 g, 29.9 mmol) in CH2Cl2 (30 ml) was added 50% aq. TFA (30 ml) at 0°C. The mixture was allowed stirred at 0°C-room temperature for 6 h before concentrating in vacuo. Toluenes was added to the residue, and the solution evaporated in vacuo to remove remaining TFA. The residue was extracted with EtOAc, washed with brine, dried over Na2SO4, and concentrated in vacuo. The product was crystallized from hexane-EtOAc to give 9 (10.1 g, 82%), Ref 0.45 (CHCl3-MeOH-AcOH, 95:5:0.1), mp 105-105.5°C; 1H-NMR δ: 7.76 (2H, d, J = 7.6 Hz, Ar), 7.59 (2H, brd, J = 7.6 Hz, Ar), 7.40 (2H, brt, J = 7.4 Hz, Ar), 7.31 (2H, brt, J = 7.2 Hz, Ar), 5.81 and 5.64 (2H, 2m, -CH=CH-), 5.33 (1H, brs, H-NH), 4.60 (2H, brd, J = 6.1 Hz, -CH2CH=), 4.41 (2H, brd, J = 7.1 Hz, -OCH2CH2), 4.24 (1H, brt, J = 6.9 Hz, -CHAr2), 4.00 (2H, d, J = 5.4 Hz, Gly-oH), 2.47-2.38 (4H, m, =CH2CH2CH2O2H). Anal. Calcd. for C45H49O12N: C, 67.47; H, 5.66; N, 3.42%. Found: C, 67.47; H, 5.63; N, 3.40%.

N-(9-Fluorenylmethoxy carbonyl)-O(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-L-serine allyl ester (16). A mixture of dried and powdered 4A molecular sieves
N-(9-Fluorenylmethoxy carbonyl)-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-L-serine (17). A mixture of 16 (111 mg, 0.18 mmol), Pd(PH3)4 (110 mg, 0.10 mmol) and N-methylaniline (1.1 ml, 10.2 mmol) in dry THF (7.5 ml) was stirred under a nitrogen atmosphere at room temperature for 2.5 h. The mixture was then acidified with dil. HCl and extracted with EtOAc. The extract was successively washed with water and brine, dried over Na2SO4, and concentrated in vacuo. The residue was chromatographed on silica gel with CHCl3-CH2Cl2 (4:1) to give 17 (100 mg, 96%). RF 0.44 (CHCl3-MeOH-CH2Cl2, 90:10:1), [α]D = -5.4° c (0.5); 1'H-NMR δ: 7.76 (2H, brd, J = 7.6 Hz, Ar), 7.60 (2H, brd, J = 6.8 Hz, Ar), 7.42–7.29 (4H, m, Ar), 7.06 (1H, d, J = 7.6 Hz, NH), 5.15 (1H, t, J = 8.1 Hz, H-3), 4.90 (2H, m, Xyl-H2, H4), 4.53 (2H, m, Ser-βH, Xyl-H1), 4.46–4.38 (2H, m, -OCH2CH2Ar5), 4.29–4.22 (2H, m, Ser-βH, Ar-C6H5), 4.09 (1H, dd, J = 4.6, 12.0 Hz, Xyl-H5), 3.81 (1H, brd, J = 8.5 Hz, Ser-βH), 3.38 (1H, dd, J = 8.1, 12.0 Hz, Xyl-H5), 2.06 (3H, s, Ac), 2.03 (3H, s, Ac), 2.02 (3H, s, Ac). Anal. Caled. for C38H34O12N2: C, 59.48; H, 5.34; N, 2.39%. Found: C, 59.30; H, 5.30; N, 2.13%.

Attachment of 9 to Sieber amide resin and coupling with 17. Commercial Sieber amide resin (1.67 g, 1.0 mmol; 0.6 mmol/g) was N-deprotected with 20% piperidine/NMP and washed with NMP by using an automated peptide synthesizer. To a solution of 9 (615 mg, 1.5 mmol) in NMP (10 ml) were added 0.45 M HBTU/HOBt/DMF (3.33 ml, 1.5 mmol) and 2 M DIEA/NMP (750 μl, 1.5 mmol). The mixture was stirred at room temperature for 10 min with a vortex tube mixer and combined with the foregoing N-deprotected resin. Mixing was continued for 3 h before filtration. The resin was washed three times each with NMP and CH2Cl2 to give 12 (1.83 g, quantitative). A resin sample (12 mg) was submitted to the Kaiser test, and the coupling efficiency was estimated to be 99.9%. The resin was placed in the reaction vessel of a peptide synthesizer, and the coupling procedure with AcO and DMAP (4-N,N-dimethylamino pyridine) was conducted. After successively washing with CH2Cl2 and NMP, resin 12 was N-deprotected through the automated program. The resin was then transferred to a polyethylene test tube. Compound 17 (878 mg, 1.5 mmol) was stirred with 0.45 M HBTU/HOBt/DMF (3.33 ml, 1.5 mmol) and 2 M DIEA/NMP (750 μl, 1.5 mmol) in NMP (10 ml) for 10 min. The mixture was added to the test tube and vortex mixing was continued for 3 h before filtration and successively washing with NMP and CH2Cl2. Drying under vacuum afforded 18 (2.26 g, quantitative). Ninhydrin monitoring of the sample (5 mg) exhibited a 99.8% yield.

N-(9-Fluorenylmethoxy carbonyl)-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-L-seryl-L-glycine (20). Resin 18 (316 mg, 0.14 mmol) was suspended in a degassed mixture of DMSO (3 ml) and CH2Cl2 (3 ml). To the suspension were added Pd(PH3)4 (34 mg, 0.03 mmol) and dimedone (200 mg, 1.40 mmol) under a nitrogen atmosphere. The mixture was stirred overnight with a vortex mixer and then filtered. The resin was washed with CH2Cl2. The combined filtrate and washings were concentrated in vacuo. The product was purified at first by gel permeation chromatography on S X 4 Bio-beads with tol uene-CH2Cl2 (1:1) and then by reversed-phase column chromatography on C-18 silica gel with 50% ac. CH3CN to give 20 (93 mg, quantitative), RF 0.23 (CHCl3-MeOH-ACOH, 90:10:0.5), [α]D = -16.3° c (0.9); 1'H-NMR δ: 7.76 (2H, d, J = 7.3 Hz, Ar), 7.58 (2H, brd, J = 7.3 Hz, Ar), 7.42–7.30 (4H, m, Ar), 6.84 (1H, brs, NH), 5.82 (1H, brd, J = 6.3 Hz, NH), 5.17 (1H, t, J = 8.6 Hz, Xyl-H1), 4.95–4.89 (2H, m, Xyl-H2, H4), 4.54–4.42 (4H, m, Ser-βH, -OCH2CH2Ar5, Xyl-H1), 4.21 (1H, brt, J = 6.7 Hz, -CHAr5), 4.13–4.07 (4H, m, Ser-βH, Xyl-H5, Gly-βH), 3.69 (1H, m, Ser-βH), 3.38 (1H, m, Xyl-H5), 2.04 (3H, s, Ac), 2.02 (6H, s, Ac). Anal. Caled. for C38H34O12N2·2/H2O: C, 56.75; H, 5.45; N, 4.27%.
Deallylation of 21 in a solution (an alternative synthesis of 20). A mixture of 21 (233 mg, 0.31 mmol), Pd(PPh₃)₄ (36 mg, 0.03 mmol) and N-methylamiline (0.33 ml, 3.04 mmol) in dry THF (10 ml) was stirred for 1 h at room temperature under nitrogen. The mixture was diluted with EtOAc, washed with brine, dried over Na₂SO₄, and concentrated in vacuo. The product was purified in the same manner as that just described to give 20 (198 mg, quantitative).

On-resin block condensation (synthesis of 22, 27 and 32). Resin 18 (1.28 g, 0.60 mmol) was N-deprotected with 20% piperidine/NMP and washed with NMP by using the automated peptide synthesizer as already described. Compound 20 (560 mg, 0.87 mmol) was activated with 0.45 M HBTU/HOBt/DMF (1.94 ml, 0.87 mmol) and 2 M DIEA/NMP (0.44 ml, 0.88 mmol) in NMP (5 ml) for 10 min. The N-deprotected resin was added to the mixture, and vortex mixing was conducted for 3 h before filtration and washing. The ninhydrin test with the sample (5.7 mg) exhibited a 99.7% conversion. After the resin had been submitted to capping with Ac₂O as already described, the collected resin was dried in vacuo to give 22 (1.64 g, quantitative).

By a similar procedure, resins 22 (646 mg, 0.25 mmol) and 27 (66 mg, 1.8 mmol) were N-deprotected and condensed with 23, respectively, and condensed with 28 (vide infra, 50 mg, 2.7 μmol), respectively. The ninhydrin test indicated the efficiency of each coupling to be 99.1 and 97.7%, respectively.

Cleavage of glycopeptide from resin by Pd(0) catalysis.

N-(9-Fluorenyl) methoxycarbonyl)-O-(2,3,4-tri-O-acetyl-β-D-xlyopyranosyl)-L-seryl-L-glucyl-0-(2,3,4-tri-O-acetyl-β-D-xlyopyranosyl)-L-seryl-L-glycine (23). Resin 22 (210 mg, 0.08 mmol) was treated with Pd(PPh₃)₄ (20 mg, 16 μmol) and dimedone (120 mg, 0.8 mmol) as described for 20. The product was chromatographed on Si 4 Bio-beads with toluene-EtOAc (1:1) to give 23 (82 mg, 9.6%), which was used for the next reaction without further purification.

Deallylation of 21 in a solution (an alternative synthesis of 20). A mixture of 21 (233 mg, 0.31 mmol), Pd(PPh₃)₄ (36 mg, 0.03 mmol) and N-methylamiline (0.33 ml, 3.04 mmol) in dry THF (10 ml) was stirred for 1 h at room temperature under nitrogen. The mixture was diluted with EtOAc, washed with brine, dried over Na₂SO₄, and concentrated in vacuo. The product was purified in the same manner as that just described to give 20 (198 mg, quantitative).

On-resin block condensation (synthesis of 22, 27 and 32). Resin 18 (1.28 g, 0.60 mmol) was N-deprotected with 20% piperidine/NMP and washed with NMP by using the automated peptide synthesizer as already described. Compound 20 (560 mg, 0.87 mmol) was activated with 0.45 M HBTU/HOBt/DMF (1.94 ml, 0.87 mmol) and 2 M DIEA/NMP (0.44 ml, 0.88 mmol) in NMP (5 ml) for 10 min. The N-deprotected resin was added to the mixture, and vortex mixing was conducted for 3 h before filtration and washing. The ninhydrin test with the sample (5.7 mg) exhibited a 99.7% conversion. After the resin had been submitted to capping with Ac₂O as already described, the collected resin was dried in vacuo to give 22 (1.64 g, quantitative).

By a similar procedure, resins 22 (646 mg, 0.25 mmol) and 27 (66 mg, 1.8 mmol) were N-deprotected and condensed with 23 (vide infra, 392 mg, 0.38 mmol) and 28 (vide infra, 50 mg, 2.7 μmol), respectively. The ninhydrin test indicated the efficiency of each coupling to be 99.1 and 97.7%, respectively.

Cleavage of glycopeptide from resin by Pd(0) catalysis.

N-(9-Fluorenyl) methoxycarbonyl)-O-(2,3,4-tri-O-acetyl-β-D-xlyopyranosyl)-L-seryl-L-glucyl-0-(2,3,4-tri-O-acetyl-β-D-xlyopyranosyl)-L-seryl-L-glycine (23). Resin 22 (210 mg, 0.08 mmol) was treated with Pd(PPh₃)₄ (20 mg, 16 μmol) and dimedone (120 mg, 0.8 mmol) as described for 20. The product was chromatographed on Si 4 Bio-beads with toluene-EtOAc (1:1) to give 23 (82 mg, 9.6%), which was used for the next reaction without further purification.

Deallylation of 21 in a solution (an alternative synthesis of 20). A mixture of 21 (233 mg, 0.31 mmol), Pd(PPh₃)₄ (36 mg, 0.03 mmol) and N-methylamiline (0.33 ml, 3.04 mmol) in dry THF (10 ml) was stirred for 1 h at room temperature under nitrogen. The mixture was diluted with EtOAc, washed with brine, dried over Na₂SO₄, and concentrated in vacuo. The product was purified in the same manner as that just described to give 20 (198 mg, quantitative).

On-resin block condensation (synthesis of 22, 27 and 32). Resin 18 (1.28 g, 0.60 mmol) was N-deprotected with 20% piperidine/NMP and washed with NMP by using the automated peptide synthesizer as already described. Compound 20 (560 mg, 0.87 mmol) was activated with 0.45 M HBTU/HOBt/DMF (1.94 ml, 0.87 mmol) and 2 M DIEA/NMP (0.44 ml, 0.88 mmol) in NMP (5 ml) for 10 min. The N-deprotected resin was added to the mixture, and vortex mixing was conducted for 3 h before filtration and washing. The ninhydrin test with the sample (5.7 mg) exhibited a 99.7% conversion. After the resin had been submitted to capping with Ac₂O as already described, the collected resin was dried in vacuo to give 22 (1.64 g, quantitative).

By a similar procedure, resins 22 (646 mg, 0.25 mmol) and 27 (66 mg, 1.8 mmol) were N-deprotected and condensed with 23 (vide infra, 392 mg, 0.38 mmol) and 28 (vide infra, 50 mg, 2.7 μmol), respectively. The ninhydrin test indicated the efficiency of each coupling to be 99.1 and 97.7%, respectively.

Cleavage of glycopeptide from resin by Pd(0) catalysis.

N-(9-Fluorenyl) methoxycarbonyl)-O-(2,3,4-tri-O-acetyl-β-D-xlyopyranosyl)-L-seryl-L-glucyl-0-(2,3,4-tri-O-acetyl-β-D-xlyopyranosyl)-L-seryl-L-glycine (23). Resin 22 (210 mg, 0.08 mmol) was treated with Pd(PPh₃)₄ (20 mg, 16 μmol) and dimedone (120 mg, 0.8 mmol) as described for 20. The product was chromatographed on Si 4 Bio-beads with toluene-EtOAc (1:1) to give 23 (82 mg, 9.6%), which was used for the next reaction without further purification.
AcOH (95:5:0.5) to give 24 (285 mg, 99%), Rf 0.35 (CHCl₃-MeOH-AcOH, 90:10:0.5), [δ]D₋21.8° (c 1.0); ¹H-NMR δ: 7.77 (2H, d, J = 7.6 Hz, Ar), 7.61 (2H, brd, J = 7.1 Hz, Ar), 7.43–7.30 (4H, m, Ar), 5.94 (1H, br, NH), 5.78 (1H, m, -CH = CH-), 5.71 (2H, brs, 2NH), 5.59 (1H, m, -CH = CH-), 5.19 and 5.18 (2H, 2t, J = 8.8 Hz, 2 Xyl-H₃), 4.99–4.88 (4H, m, 2 Xyl-H₂, 2 Xyl-H₄), 4.69 (1H, m, Ser-αH), 4.56–4.53 (3H, m, -OCH₂CH₂ = Ser-αH), 4.45 (2H, m, -OCH₂CH₂Ar), 4.23 (1H, brt, J = 6.6 Hz, -CHR₂), 4.15–3.90 (8H, m, 2 Ser-βH, 2 Xyl-H₅, 4 Gly-αH), 3.76 (2H, m, 2 Ser-βH), 3.36 (2H, m, 2 Xyl-H₅), 2.38–2.28 (4H, m, =CH₂CH₂CONH₂), 2.04, 2.03, 2.02, and 2.00 (18H, 4s, 6 Ac). Anal. Calcd for C₉₀H₄₀N₂Oₙ: C, 55.06; H, 5.67; N, 6.06%. Found: C, 55.16; H, 5.65; N, 5.89%. HR-FABMS m/z 1156.4221 ([M + H]+), 1156.4098 calcd. for C₉₃H₆₈O₇N₄.

6-[N-(9-Fluorenylmethoxycarbonyl)-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-l-seryl-l-glycyl-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-l-seryl-l-glycyl-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)]-l-seryl-l-glycyl-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-l-seryl-l-glycyl-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)]-l-seryl-l-glycyl-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-l-seryl-l-glycyl-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-l-seryl-l-glycyl-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)]-l-seryl-l-glycyl-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)]-l-seryl-l-glycyl-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)]-l-seryl-l-glycyl-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)]-l-seryl-l-glycyl-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)]-l-seryl-l-glycyl-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)-l-seryl-l-glycyl-O-(E)-4-hexanenyl (33). Resin 27 (20 mg, 3.8 μmol) was treated with 2% TFA/CH₂Cl₂ as described for 21. The crude cleavage product was purified by reversed-phase HPLC on ODS silica gel with 56% acetonitrile containing 0.1% TFA as solvent by gradient elution (25 mg, 79%), Rf 0.39 (CHCl₃-MCH₂OH, 9:1), [δ]D₋35.2° (c 0.5, CF₃CO₂H); ¹H-NMR (DMSO-d₆, 60°C) δ: 8.21 (1H, brt, J = 6.0 Hz, NH), 7.98–7.89 (6H, m, NH), 7.86 (2H, d, J = 7.3 Hz, Ar), 7.70 (3H, m, Ar, NH), 7.40 (2H, brt, J = 7.5 Hz, Ar), 7.32 (2H, brt, J = 7.5 Hz, Ar), 5.77 (1H, dt, J = 6.4, 15.4 Hz, -CH = CH-), 5.55 (1H, m, J = 6.4, 15.4 Hz, -CH = CH-), 5.11–5.06 (4H, m, 4 Xyl-H₃), 4.84–4.78 (4H, m, 4 Xyl-H₂), 4.75–4.66 (8H, m, 4 Xyl-H₁, 4 Xyl-H₄), 4.54–4.49 (6H, m, =OCH₂CH₂ =, 3 Ser-αH), 4.29–4.21 (3H, m, Ser-αH, =OCH₂CH₂Ar, =CH₂Ar), 4.00–3.93 (4H, m, 4 Xyl-H₃), 3.89–3.68 (16H, m, 8 Ser-βH, 8 Gly-αH), 3.39–3.41 (4H, m, 4 Xyl-H₅), 2.23 and 2.13 (4H, m, =CH₂CH₂CONH₂), 1.99, 1.98 (2), 1.97, 1.96, 1.95 (2), and 1.94 (2) (36H, 9s, 12 Ac); HR-FABMS m/z 1960.6472 [M + H]+, 1960.6647 calcd. for C₁₉₃H₁₀₈O₇Na₄.

6-[O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)]-l-seryl-l-glycyl-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)]-l-seryl-l-glycyl-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)]-l-seryl-l-glycyl-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)]-l-seryl-l-glycyl-O-(2,3,4-tri-O-acetyl-β-D-xylopyranosyl)]-l-seryl-l-glycyl-O-(E)-4-hexanenyl (36). To a stirred solution of 24 (251 mg, 0.22 mmol) in CH₂Cl₂ (3 ml) was added morpholine (3 ml). The mixture was stirred at room temperature for 1 h before concentrating in vacuo. The residue was concentrated to dry tolerance to remove the remaining morpholine. The crude product was chromatographed on silica gel with CHCl₃-MeOH-AcOH (95:5:0.5) and then with CHCl₃-MeOH-AcOH (70:30:1) to give 28 (188 mg, 93%), Rf 0.28 (CHCl₃-MeOH-AcOH, 90:10:1), [δ]D₋
Deallylation in a solution.

Glyco-tetrapeptide (23). Compound 24 (285 mg, 247 μmol) was stirred overnight with Pd(PPh3)4 (30 mg, 26 μmol) and N-methylaniline (265 μL, 2.45 mmol) in dry THF (10 ml) under nitrogen. The mixture was diluted with EtOAc, successively washed with 10% HCl and brine, dried over Na2SO4, and concentrated in vacuo. The residue was chromatographed on silica gel with CHCl3-MeOH-AcOH (90:10:0.5) to give 23 (241 mg, 94%).

Glyco-octapeptide (28). Compound 29 (179 mg, 91 μmol) was treated overnight with Pd(PPh3)4 (21 mg, 18 μmol) and N-methylaniline (100 μL, 0.92 mmol) in dry THF (20 ml)-CF3CH2OH (1 ml) under nitrogen. The mixture was worked up as already described. The crude product was purified by chromatography on silica gel with CHCl3-MeOH-AcOH (90:10:1) and then by reversed-phase chromatography on ODS silica gel with 50% aq. CH2CN to afford 28 (99%).

Block condensation in a solution.

Glyco-octapeptide (29). To a stirred solution of 23 (210 mg, 0.20 mmol) in dry CH2Cl2 (3 ml) were added 0.45 M HBTU/HOBt/DMF (490 μL, 0.22 mmol) and 2 M DIEA/NMP (120 μL, 0.24 mmol). The mixture was stirred at room temperature for 15 min. A solution of 25 (188 mg, 0.20 mmol) in dry CH2Cl2 (2 ml) was added to the mixture. The mixture was stirred at room temperature for 2.5 h. The mixture was diluted with EtOAc, successively washed with water and brine, dried over Na2SO4, and concentrated in vacuo. The crude product was chromatographed on silica gel with CHCl3-MeOH (90:10) to give 29 (331 mg, 84%)

Glycohexadecapeptide (33). A mixture of 28 (14 mg, 7.6 μmol), 0.45 M HBTU/HOBt/DMF (20 μL, 9 μmol) and 2 M DIEA/NMP (6 μL, 12 μmol) in dry DMF (1 ml) was stirred at room temperature for 15 min. To the mixture was added a solution of 30 (26 mg, 15 μmol) in dry DMF (1 ml). The mixture was stirred overnight before extracting with EtOAc as already described. The crude product was purified by reversed-phase HPLC to give 33 (18 mg, 66%).

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References


