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Carbohydrate Research 303 (1997) 373–377

CARBOHYDRATE RESEARCH

Note

Solid-phase synthesis of a glycosylated peptide fragment of the IL-8 receptor containing two vicinal oligosaccharide chains

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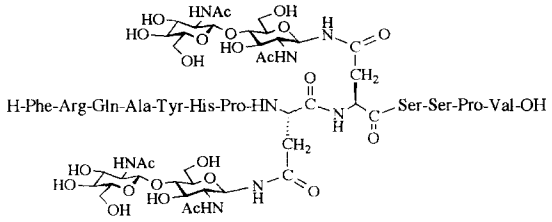
Received 23 April 1997; accepted 12 June 1997

Abstract

A vicinally glycosylated peptide fragment which corresponds to one of the exoloops of the IL-8 receptor was synthesized by use of a solid-phase approach. © 1997 Elsevier Science Ltd.

Keywords: Solid-phase synthesis; N-Linked glycopeptide; IL-8; Reduction of tyrosine side-chain

Interleukin-8 (IL-8) is a pro-inflammatory polypeptide chemokine that is related to several disease states [1], and inhibition of its action can lead to beneficial effects [2]. Recently, Wilson and coworkers [3] have discovered that some peptide fragments of the extracellular regions of IL-8 receptors are also potent inhibitors of IL-8. What is of particular interest to us is a peptide fragment $AA^{174-185}$ of the 3rd extracellular domain (ECD) that possesses two possible N-glycosylation sites vicinal to each other, although the inhibitory activity of this fragment was relatively low ($K_i = 10\ \mu M$). In order to study the influences of glycosylation on the biological activities of this peptide and also to find more potent inhibitors of IL-8, we decided to synthesize the glycoform (**1**) of the 3rd ECD fragment $AA^{174-186}$ that contains two disaccharide chains. In addition, the synthesis of a glycopeptide containing two vicinal oligosaccharide chains itself is also an interesting subject.



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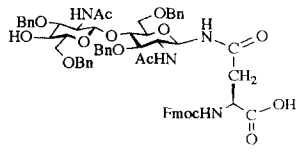

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The oligosaccharide chains of glycoproteins and glycopeptides play a significant role in cell recogni-

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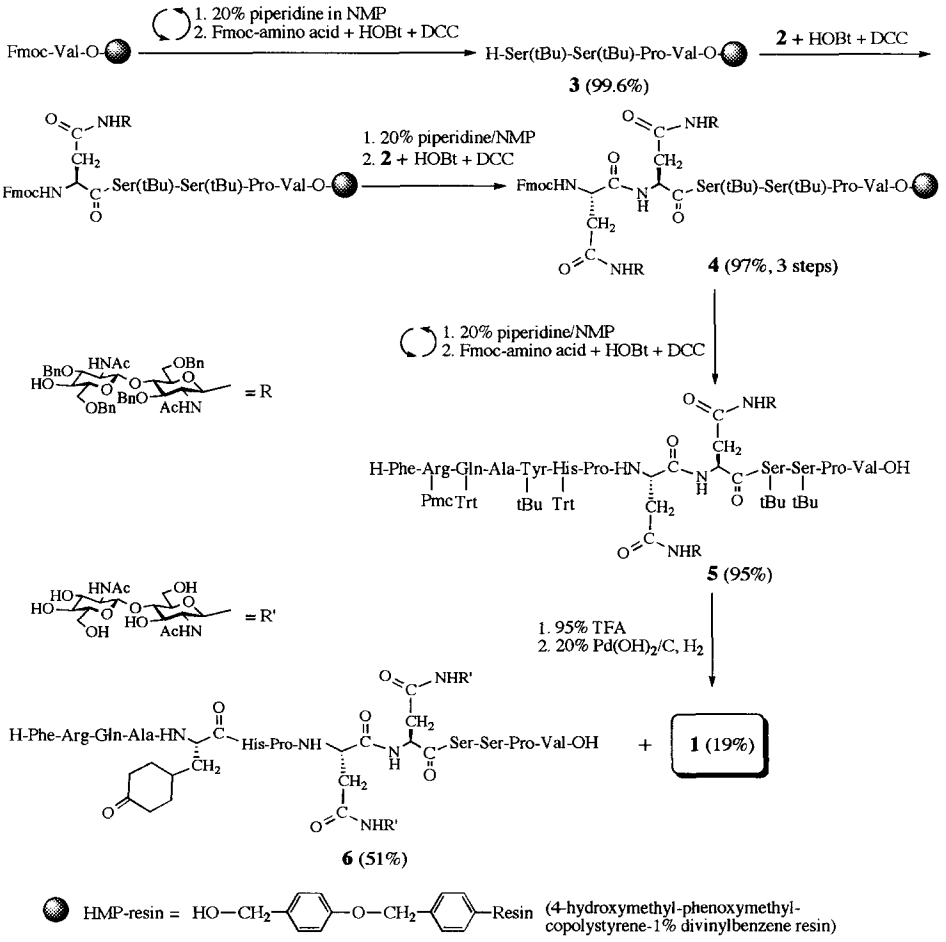
PII S0008-6215(97)00180-8

tion and signal transduction [4]; therefore, the synthetic and biological studies on glycopeptides and glycoproteins have been a very active area in current organic and bioorganic chemistry (see ref. [5] for recent reviews). Nevertheless, the synthesis of glycopeptides still remains a challenging problem to organic chemists. What is of special interest is the synthesis of glycopeptides containing two vicinal oligosaccharide chains, a process that is not well documented. Therefore, whether sugar chains will affect the peptide coupling reactions and how these sugar chains interfere with each other are still open questions.

For the synthesis of target compound **1**, we employed a strategy (shown in Scheme 1) that was based on solid-phase synthesis involving Fmoc-amino acids and a benzyl-protected oligosaccharide that has been successfully applied to the synthesis of several complex glycopeptides (for solid-phase synthesis of glycopeptides, see ref. [7] and references cited in ref. [6]). Here, we employed the benzylated chitobiose–asparagine conjugate **2** as the key building block. Thus, peptide fragment $Ser^{10} \sim Val^{13}$ linked to the HMP-resin (**3**) was first constructed on valine-preloaded HMP-resin (0.7 mmol/g) with an automatic synthesizer using the ready-made program and standard Fmoc method on a 0.25-mmol scale to give **3** with an overall efficiency of 99.6% (monitored by the ninhydrin reaction). The coupling reactions were pursued by using DCC–HOBt as the activating agent in *N*-methylpyrrolidone (NMP), and deprotection of N-terminals was accomplished by using 20% piperidine in NMP. *tert*-Butyl was employed to protect the side-chain of serine. Coupling between resin **3** and glycopeptide **2** was carried out manually, i.e. the mixture of **2** (1.5 equiv) activated by DCC–HOBt (3.0 equiv) and **3** (1.0 equiv) suspended in NMP was shaken on a vortex mixer overnight. Then, the glyco-



Scheme 1. Solid-phase synthesis of a glycosylated peptide fragment (**1**) of the IL-8 receptor.

sylated peptide fragment linked to the resin was obtained in quantitative yield (by weight). Its N-terminus was deprotected by treatment with 20% piperidine in NMP at room temperature for 1 h (98%) and then coupled again with glycopeptide **2** manually as mentioned above to give the doubly glycosylated peptide-resin **4** (99%). Compound **4** was applied to the automatic synthesizer again, and the peptide chain elongation was carried out on a 0.1-mmol scale, with *tert*-butyl, trityl, and 2,2,5,7,8-pentamethylchroman-6-sulfonyl as protective groups for the side-chains of tyrosine, histidine/glutamine, and arginine, respectively, according to the ready-made program, to give **5** (95% by weight). Releasing the glycopeptide from resin was realized by treating the loaded resin **5** with a mixture of TFA, 1,2-dimercaptoethane, water, thioanisole, and phenol (40:1:2:2:3) at room temperature for 1 h, under which conditions the protective groups on the amino acid residues were removed concomitantly. After a simple workup (filtration, washing with ether, and concentration in vacuo), the resulting product was directly subjected to reductive debenzylation using 20% $Pd(OH)_2$-on-charcoal in 50% aq ethanol (6 days). The final product was purified by reversed-phase HPLC (C_{18} column) to afford the expected glycopeptide **1** (19% from **5**) as a white solid, together with a substantial amount of side product **6** (51%) with the tyrosine side-chain reduced to cyclohexanone. No effort was made to optimize the yield of expected product **1**, but it might be improved by shortening the reduction time. In a similar reaction, a glycopeptide containing one chitobiose chain at Asn^9 was debenzylated under the same conditions in 5 days to give the expected product in 50% yield.

In conclusion, a glycosylated peptide fragment of the IL-8 receptor containing two vicinal disaccharide chains (**1**) was prepared by means of solid-phase synthesis. It is worthy to mention that two vicinal glycosylated asparagine residues could be consequently coupled with resin-supported peptides in excellent yields, showing no obstacle for the elongation of the peptide chain, but that the tyrosine residue was susceptible to the hydrogenolysis conditions used to remove benzyl groups. To avoid or reduce the complication of the tyrosine reduction, one must monitor the progress of catalytic debenzylation more carefully or to choose other debenzylation conditions. Studies concerning the influences of sugar chains in **1** on the conformation of peptide backbone and the studies about the influences of sugar chains in **1** and corresponding mono-glycosylated peptides at either Asn^8 or Asn^9 as well as the tyrosine side-chain on the biological activities of this peptide fragment will be presented separately.

1. Experimental

General methods.—^{1}H NMR spectra (600 MHz) were recorded with a Varian Unity 600 spectrometer in solns of deuterium oxide. Automatic peptide synthesis was carried out with an Applied Biosystems 431A Peptide Synthesizer. All reagents and solvents, except distilled water, HPLC grade 2-propanol, and deuterium oxide used for NMR measurements, were peptide synthesis grade purchased from Applied Biosystems.

L-*Phenylalanyl*-L-*arginyl*-L-*glutamyl*-L-*alanyl*-L-*tyrosyl*-L-*histidyl*-L-*prolyl*-[N^4-*2-acetamido-2-deoxy-β*-D-*glucopyranosyl*-(*1 → 4*)-*2-acetamido-2-deoxy-β*-D-*glucopyranosyl*]-L-*asparaginyl*-[N^4-*2-acetamido-2-deoxy-β*-D-*glucopyranosyl*-(*1 → 4*)-*2-acetamido-2-deoxy-β*-D-*glucopyranosyl*]-L-*asparaginyl*-L-*seryl*-L-*seryl*-L-*prolinyl*-L-*valine* (**1**).—An Fmoc-protected tetrapeptide linked to HMP-resin (**3**, 390 mg) was synthesized, starting from FmocVal-preloaded HMP-resin (357 mg, 0.25 mmol), after three cycles of the standard synthesizer program of condensation with the DCC–HOBt-activated FmocPro, FmocSer(*t*-Bu), and FmocSer(*t*-Bu) (1 mmol each), subsequently. Efficiency of the condensation at each step was monitored by utilizing the ninhydrin test, and the overall yield of **3** was 99.6%.

A mixture of compound **2** (25 mg, 22.3 μmol) in NMP (0.5) mL, M DCC–NMP (40 μL, 40 μmol), and M HOBt–NMP (40 μL, 40 μmol) was stirred at room temperature for 1 h and was then added to peptide-resin **3** (30 mg, 18.6 μmol). The suspension was shaken on a vortex mixer (Iwaki TM-252) for 24 h at room temperature, followed by filtration and washing with NMP and CH_2Cl_2. The resulting resin was dried under vacuum overnight (50 mg, quant), and a part of it (25 mg) was treated with 20% piperidine in NMP (1 mL) at room temperature for 1.5 h. The resin obtained after washing subsequently with NMP, 50% MeOH–CH_2Cl_2, and CH_2Cl_2 and drying under vacuum overnight (23 mg, 98%) was added to a soln of activated **2** (15 mg, 13.4 μmol) by HOBt–DCC as above. The mixture was shaken on a vortex mixer for 38 h at room temperature, and glycosylated peptide-resin **4** (33 mg) was obtained in 98% yield after filtration, washing with NMP, and CH_2Cl_2 and drying (95% from **3**). Compound **4** was

applied to the automatic synthesizer again, and Pro, His(Trt), Tyr(*t*-Bu), Ala, Gln(Trt), Arg(Pmc), and Phe were subsequently introduced by a standard, 0.1 mmol small-scale program. After deprotection of Fmoc with piperidine, washing, and drying, glycopeptide-linked resin **5** (41 mg, 95%) was obtained.

Compound **5** (40 mg) was treated with a mixture of phenol (150 mg) in TFA (2.0 mL), $HSCH_2CH_2SH$ (0.05 mL), water (0.1 mL), and thiolanisole (0.1 mL) at room temperature for 1.5 h. The mixture was filtered off, washed by TFA (2 × 2 mL), CH_2Cl_2 (4 × 2 mL), and the washings were combined and concd to dryness. The residue was suspended in water (5 mL) and extracted with cold ether (5 × 10 mL). The water layer was concd, and the resulting residue was coevaporated with water four times and then dissolved in 50% aldehyde-free EtOH aq soln (10.0 mL) and stirred with 20% $Pd(OH)_2$-on-charcoal (40 mg) under hydrogen at ~ 1 atm at 22–24 °C for 6 days. After filtration and concn in vacuo, the residue obtained was separated by reversed-phase HPLC (column: LichroCART®, 10 × 250 mm) to produce the expected glycopeptide **1** (3.5 mg, 19% from **5**) and the glycopeptide with the reduced tyrosine side-chain **6** (9.2 mg, 51% from **5**). **1**: HPLC retention time = 28.29 min (eluent: gradient program A, 5 mL/min); ^{1}H NMR (600 MHz, in D_2O, HDO δ 4.43 as standard, 60 °C): δ 8.44 (s, 1 H, H-Ar^{His}), 7.21–7.05 (m, 6 H, H-Ar^{His,Phe}), 6.91 (d, *J* 8.1 Hz, 2 H, H-Ar^{Tyr}), 6.62 (d, 2 H, H-Ar^{Tyr}), 4.89 (d, 1 H, $J_{1,2}$ 8.9 Hz, H-1^1), 4.87 (d, 1 H, $J_{1,2}$ 8.8 Hz, H-1$^{1'}$), 4.67–4.50 (m, 4 H, H-$\alpha^{His,Ser,2\times Asn}$), 4.43 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1^2), 4.40 (bd, 1 H, $J_{1,2}$ 8.2 Hz, H-1$^{2'}$), 4.34–4.29 (m, 3 H, H-$\alpha^{Pro,Tyr,Ser}$), 4.15–4.08 (m, 5 H, H-$\alpha^{Pro,Arg,Phe,Glu,Ala}$), 3.93 (d, 1 H, *J* 5.6 Hz, H-α^{Val}), 3.75–3.30 (m, 35 H, H-β, $\beta'^{2\times Ser}$, H-$\delta^{2\times Pro}$, H-2, 3, 4, 5, 6, and 6′ of 4 sugar rings, δ'^{Pro}), 3.15 (m, 2 H, H-δ'^{Pro}, H-β^{His}), 3.04–2.91 (m, 5 H, H-β, β'^{Phe}, H-δ,δ'^{Arg}, H-β'^{His}), 2.80 (m, 1 H, H-β^{Tyr}), 2.70–2.63 (m, 5 H, H-β^{Tyr}, H-β, $\beta'^{2\times Asn}$), 2.23–2.20 (m, 2 H, H-γ, γ'^{Gln}), 2.12–2.09 (m, 2 H, H-$\gamma^{2\times Pro}$), 1.89 (s, 6 H, 2 × Ac), 1.84 (s, 3 H, Ac), 1.83 (s, 3 H, Ac), 1.95–1.75 (m, 9 H, H-β^{Val}, H-β, β'^{Gln}, H-β, $\beta'^{2\times Pro}$, *H*-$\gamma'^{2\times Pro}$), 1.60 (m, 1 H, H-β^{Arg}), 1.53 (m, 1 H, H-β'^{Arg}), 1.41 (m, 2 H, H-γ, γ'^{Arg}), 1.15 (d, 3 H, *J* 5.7 Hz, H-β^{Ala}), 0.75, 0.72 (2 d, 2 × 3 H, *J* 6.4, 5.9 Hz, H–H-γ, γ'^{Val}); MALDI-TOF-MS ($C_{99}H_{149}N_{25}O_{40}$, average MW 2329.4): *m/z* (positive-ion mode) 2330.8 $[M + H]^+$, 2352.4 $[M + Na]^+$. **6**: HPLC t_R = 17.96 min (eluent: gradient program A, 5 mL/min); ^{1}H NMR (600 MHz, in D_2O, HDO δ 4.43 as standard, 60 °C): δ 8.46 (s, 1 H, H-Ar^{His}), 7.23–7.09 (m, 6 H, H-Ar^{His,Phe}), 4.87 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1^1), 4.85 (d, 1 H, $J_{1,2}$ 8.3 Hz, H-1$^{1'}$), 4.82 (dd, 1 H, *J* 8.3, 5.4 Hz, H-α^{His}), 4.61 (dd, 1 H, *J* 6.1 Hz, H-α^{Ser}), 4.55 (m, 2 H, H-$\alpha^{2\times Asn}$), 4.42 (d, 2 H, $J_{1,2}$ 8.3 Hz, H-1$^{2,2'}$), 4.31 (dd, 1 H, *J* 8.0, 5.0 Hz, H-α^{Pro}), 4.29 (dd, 1 H, *J* 5.2 Hz, H-α^{Ser}), 4.23 (dd, 1 H, *J* 8.3, 5.4 Hz, H-α^{His}), 4.18 (dd, 1 H, *J* 10.1, 5.2 Hz, H-α^{Arg}), 4.14–4.07 (m, 4 H, H-$\alpha^{\underline{Tyr},Phe,Glu,Ala}$), 3.98 (d, 1 H, *J* 5.9 Hz, H-α^{Val}), 3.75–3.27 (m, 36 H, H-β, $\beta'^{2\times Ser}$, H-$\delta^{2\times Pro}$, H-2, 3, 4, 5, 6, and 6′ of 4 sugar rings, $\delta'^{2\times Pro}$), 3.07–2.96 (m, 6 H, H-β, β'^{His}, H-β, β'^{Phe}, H-δ, δ'^{Arg}), 2.71–2.60 (m, 4 H, H-β, $\beta'^{2\times Asn}$), 2.20–2.07 (m, 6 H, H-γ, γ'^{Gln}, H-$\gamma^{2\times Pro}$, H-2 × B$^{\underline{Tyr}}$), 1.88 (s, 6 H, 2 × Ac), 1.82 (s, 3 H, Ac), 1.81 (s, 3 H, Ac), 1.96–1.77 (m, 12 H, H-β^{Val}, H-β, β'^{Gln}, H-β, $\beta'^{2\times Pro}$, *H*-$\gamma'^{2\times Pro}$, H-2 × B′$^{\underline{Tyr}}$, H-D$^{\underline{Tyr}}$), 1.61–1.12 (m, 8 H, H-β, β'^{Arg}, H-γ, γ'^{Arg}, H-4 × C$^{\underline{Tyr}}$), 1.18 (d, 3 H, *J* 6.8 Hz, H-β^{Ala}), 0.75, 0.73 (2 d, 2 × 3 H, *J* 6.8, 6.8 Hz, H–H-γ, γ'^{Val}); MALDI-TOF-MS ($C_{99}H_{153}N_{25}O_{40}$, average MW 2333.4): *m/z* (positive-ion mode) 2334.5 $[M + H]^+$, 2356.1 $[M + Na]^+$. [Gradient program A: 0 ~ 20 min, 7% 2-PrOH aq soln; 20 ~ 30 min, 7 ~ 9% 2-PrOH aq soln; 31 ~ 40 min, 14% 2-PrOH aq soln. $\underline{Tyr}$ represents the reduced tyrosine residue in the assignment of NMR signals.]

Acknowledgements

This work was financially supported by the Special Co-ordination Funds of the Science and Technology Agency of the Japanese Government and partly by the CREST programme of the Japan Science and Technology Corporation. Z.-W.G. is grateful for a RIKEN Fellowship and thanks Dr. Y. Ito for many helpful discussions. We thank Dr. N. Dohmae for MALTI-TOF-MS measurement, Dr. J. Uzawa and his staff for NMR measurements and Ms. A. Takahashi for technical assistance.

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