Pergamon

Tetrahedron Letters, Vol. 38, No. 41, pp. 7211-7214, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)01718-8

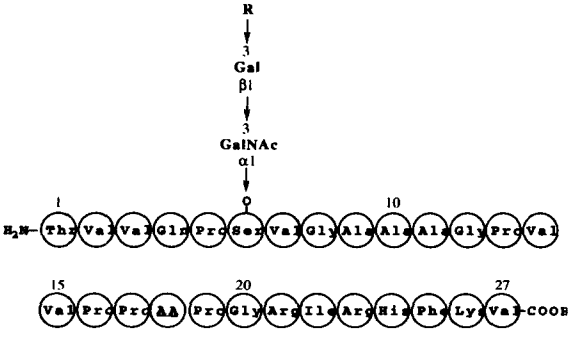
Total Synthesis of B-Chain of Human α2HS Glycoprotein

Yoshiaki Nakahara [a,b,*], Yuko Nakahara [a], Yukishige Ito [a]
and Tomoya Ogawa [a,c,*]

[a] *The Institute of Physical and Chemical Research (RIKEN), Hirosawa 2-1, Wako-shi, Saitama, 351-01, Japan*
[b] *Department of Industrial Chemistry, Tokai University, Kitakaname 1117, Hiratsuka-shi, Kanagawa, 259-12, Japan*
[c] *Graduate School for Agricultural and Life Sciences, The University of Tokyo, Yayoi, Bunkyo-ku, Tokyo, 113, Japan*

Abstract: B-chain of human α2HS glycoprotein **1**, a cystein-containing heptacosapeptide carrying a trisaccharide (sialyl T) side chain, was synthesized for the first time by the solid-phase method utilizing a glycosyl serine building block **11** with benzyl protecting groups. © 1997 Elsevier Science Ltd.

Present-day synthesis of glycopeptides relies heavily on solid-phase methods, which provide not only rapid assembly of peptide backbones but also high coupling efficiency with sophisticated and fully automated instruments.[1] However, synthesis of a longer oligopeptide (>25 amino acid-peptide) possessing both multiple functional side chains and oligosaccharide attachment has rarely been achieved.[2] Recently we have disclosed a solid-phase synthesis of heptacosapeptide **2**[3] carrying an O-linked disaccharide (Galβ1→3GalNAcα1→, Thomsen-Friedenreich antigen), an asialo demercapto [Ala[18]] analogue of B-chain[4] (**1**) of human α2HS glycoprotein, which is a human plasma globulin behaving as one of the negative acute-phase reactants.[5]



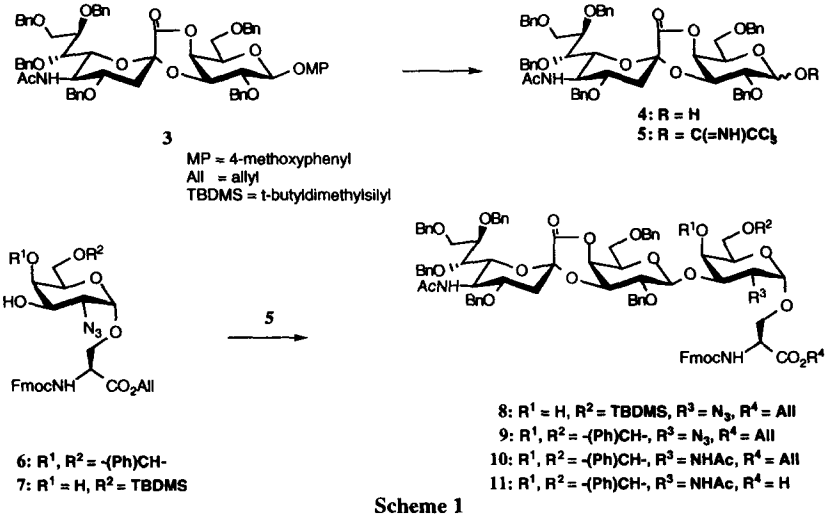
1 : R = Neu5Acα2, AA = Cys
2 : R = H, AA = Ala

The synthesis was performed on the basis of Fmoc method using an automated peptide synthesizer. Only at the coupling step with the benzylated disaccharide-linked serine unit, the reaction was carried out utilizing a mechanical shaker in order to make easy the recovery of the unreacted glycoserine, though efficiency

of the condensation was moderate (55%). The ultimate deprotection was accomplished by hydrogenolysis to give the target compound **2**. The fact that a glycoserine-deleted peptide was formed in 45% and the unreacted glycoserine building block was recovered in a reasonable amount even after running the reaction for 64 h led us to conclude that more effective mixing would be necessary to complete the solid-phase reaction.

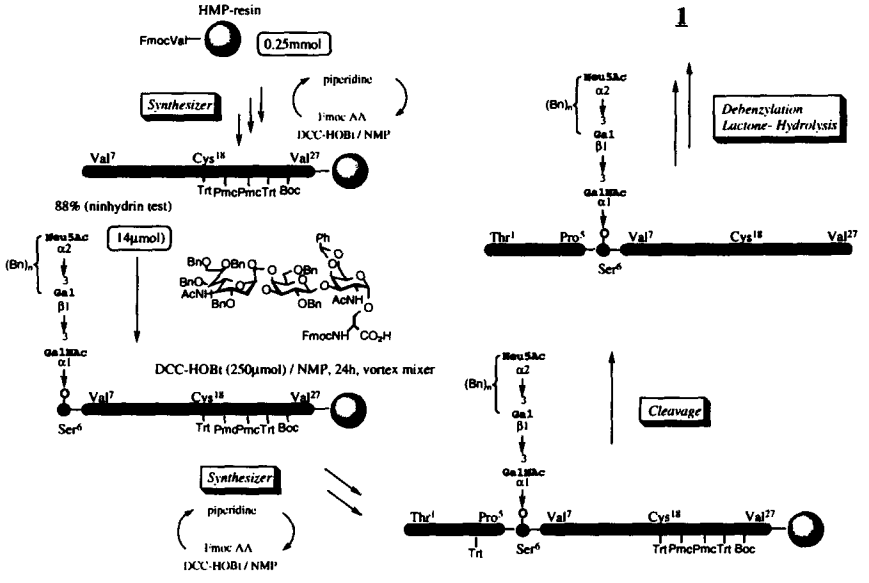
In this communication, we report a first total synthesis of the native B-chain of α2HS glycoprotein involving an improved procedure by the use of a vortexing mixer with a suitably protected glycoserine building block **11**.

The trisaccharide-linked serine **11** was synthesized as follows. The disaccharide **3**[6] was treated with ceric ammonium nitrate in a mixture of toluene:CH_3CN:H_2O (3 : 4 : 3) to afford hemiacetal **4** (77%), which was converted into trichloroacetimidate **5** (CCl_3CN, DBU, α/β 3/1, 97%). The glycosyl acceptor **7** was prepared in 2 steps from the known glycosylated serine derivative **6**[7] (1. 80% CF_3CO_2H-CH_2Cl_2, 97%, 2. TBDMSCl, imidazole, DMF, 91%). Glycosylation of **7** with the α-trichloroacetimidate **5** was promoted by BF_3•OEt_2 (0.3 equiv.) in toluene-CH_2Cl_2 at -15 – -5° C to give a β 1→3 linked product **8**[8] (54%) and its α-isomer (7%). Desilylation of **8** (aq. CF_3CO_2H-CH_2Cl_2, 83%) followed by benzylidenation [$PhCH(OMe)_2$, p-TsOH, CH_3CN, 95%] afforded **9**, which upon treatment with AcSH in pyridine[9] (→**10**) and then with $Pd(PPh_3)_4$-MeNHPh[10] in THF was converted into **11**[8] (70%).



Scheme 1

The henicosapeptide (7 - 27)-linked HMP resin (4-hydroxymethylphenoxymethyl-copolystylene-1% divinylbenzene) was synthesized in a 0.25 mmol scale on an automated peptide synthesizer according to the Fmoc protocol, the side-chain functional groups being blocked with Trt groups for cysteine, glutamine, and histidine, Boc group for lysine, and Pmc (2,2,5,7,8-pentamethylchroman-6-sulfonyl) group for arginine. The coupling reactions were performed with DCC-HOBt in NMP (N-methylpyrrolidone), while the N-terminal Fmoc group was removed with piperidine in NMP. Coupling yield in each step was determined by ninhydrin method and the estimated overall yield after 20 couplings was 88%. The glycoserine building block **11** (2.6 equiv) was thus activated with DCC-HOBt and condensed to the deFmoc henicosapeptide-resin (14 μmol). The mixture was shaken on a vortex test tube-mixer for 24 h at room temperature.



Scheme 2

Then the resin was put back into a small scale reaction vessel for the synthesizer and the N-terminal amino acid residues (1 - 5) were coupled under the automatic program. After completing the peptide chain elongation, treatment of the deFmoc glycopeptide-resin with a TFA-based cleavage reagent (95% aq. TFA–phenol–thioanisole–1,2-ethanedithiol, 42:3:2:1) followed by gel-permeation chromatography afforded the resin-free glycopeptide. In contrast to the prior experiment[3], HPLC analysis on a reversed-phase column demonstrated that no detectable amount of glycoserine-deleted byproduct was formed in the reaction and that the product consisted mainly of hexa- (52%, MALDI-TOF•MS : 3920 $[M+1]^+$) , penta- (31%, 3829 $[M+1]^+$), and tetrabenzylated trisaccharide-bound peptides (4%, 3740 $[M+1]^+$). The latter two were formed by partial debenzylation during the TFA-cleavage procedure. The glycopeptides were collected by preparative HPLC and treated with TMSOTf/TFA[11] in the presence of thioanisole to give the debenzylated product, which upon gel-permeation chromatography afforded two fractions corresponding to the dimeric (58%, 6760 $[M+1]^+$) and the monomeric (42%, 3363 $[M+1]^+$) glycopeptides, respectively. Both fractions were further purified by reversed-phase HPLC. The dimeric compound retained about half of the lactone linkage which was evidenced by the characteristic proton signal at δ 5.30 ppm for Gal H-4, while the monomer had no lactone and was the target compound **1**. The dimer was treated with 0.2M $NaHCO_3/D_2O$ (pH 7.5) for 4 days to cleave the lactone[6] and then with 1,4-dithiothreitol overnight to give **1** exclusively (85% total yield for deprotection). The ^{1}H-NMR signals for the carbohydrate part of synthesized **1**[8] were in good agreement with those reported for the trisaccharide derived from natural **1**.[6] ESI•MS showed m/z 1133.1 as $[(M+3)/3]^{3+}$. Finally, it was observed that the isolated **1** was prone to dimerize in the absence of antioxidant (e.g. dithiothreitol).

In conclusion, the B-chain of α2HS glycoprotein, a heptacosapeptide carrying an O-linked sialotrisaccharide, cystein, and several basic amino acid residues, was synthesized for the first time, in good

yield by solid-phase method. The benzyl groups on the carbohydrate residues were efficiently removed under acidic condition without any significant side-reaction on the carbohydrate linkages.

Acknowledgment

This work was financially supported by the Grant-in-Aid for Scientific Research on Priority Areas No. 06240105 from the Ministry of Education, Science and Culture, Japan and partly by CREST program of Japan Science and Technology Corporation. We are grateful to Drs. J. Uzawa, H. Koshino and Ms T. Chijimatsu for NMR, Drs. K. Takio and N. Dohmae for MALDI-TOF MS, Dr. S. Kurono for ESI MS measurements. We thank Ms M. Yoshida and her staff for elemental analyses, and Ms A. Takahashi for technical assistance.

References and Notes

1. For reviews see: Meldal, M.; Bock, K. *Glycoconjugate J.* **1994**, *11*, 59-63; Meldal, M. In *Neoglycoconjugates*, Lee, Y. C.; Lee, R. T., Ed.; Academic Press Inc: San Diego, **1994**; pp 145–198; Norberg, T.; Lünig, B.; Tejbrant, J. *Methods Enzymol.*, **1994**, 247, 87-106.
2. Hietter, H.; Schultz, M.; Kunz, H. *SYNLETT* **1995**, 1219-1220; Jansson, A. M.; Jensen, K. J.; Meldal, M.; Lomako, J.; Lomako, W. M.; Olsen, C. E.; Bock, K. *J. Chem. Soc., Perkin Trans. 1* **1996**, 1001-1006; Liebe, B.; Kunz, H. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 618-621.
3. Nakahara, Y.; Nakahara, Y.; Ogawa, T. *Carbohydr. Res.* **1996**, *292*, 71-81.
4. Gejyo, F.; Chang, J.-L.; Bürgi, W.; Schmid, K.; Offner, G. D.; Troxler, R. F.; van Halbeek, H.; Dorland, L.; Gerwig, G. J.; Vliegenthart, J. F. G. *J. Biol. Chem.* **1983**, *258*, 4966-4971.
5. Gejyo, F.; Schmid, K. *Biochim. Biophys. Acta* **1981**, *671*, 78-84 and references cited therein.
6. Nakahara, Y.; Iijima, H.; Ogawa, T. *Tetrahedron Lett.* **1994**, *35*, 3321-3324.
7. Macindoe, W. M.; Iijima, H.; Nakahara, Y.; Ogawa, T. *Tetrahedron Lett.* **1994**, *35*, 1735-1738; *Carbohydr. Res.* **1995**, *269*, 227-257.
8. Selected ^{1}H-NMR data are given below. Compound **8** (270 MHz, $CDCl_3$): δ 7.75 (d, 2 H, *J* = 7.3 Hz, Ar), 7.61 (d, 2 H, *J* = 7.3 Hz, Ar), 7.4-7.1 (m, 34 H, Ar), 5.93 (m, 1 H, -C*H*=CH_2), 5.83 (d, 1 H, *J* = 8.6 Hz, NH), 5.34 (brd, 1 H, *J* = 17.2 Hz, =CH_2), 5.26 (brd, 1 H, *J* = 10.2 Hz, =CH_2), 5.21 (d, 1 H, *J* = 4.0 Hz, H-4b), 4.95 (d, 1 H, *J* = 3.3 Hz, H-1a), 2.18 (dd, 1 H, *J* = 4.6, 11.9 Hz, H-3c eq), 1.71 (s, 3 H, Ac), 0.85 (s, 9 H, t-Bu), 0.03 and 0.02 (2 s, 6 H, 2 Me); ^{13}C NMR (68 MHz): δ 103.0 (C-1b), 99.4 (C-1a), 95.3 (C-2c). Compound **11** (270 MHz, $CDCl_3$): δ 7.72 (d, 2 H, *J* = 7.6 Hz, Ar), 7.54 (m, 4 H, Ar), 7.4-7.1 (m, 37 H, Ar), 6.12 (brd, 1 H, *J* = 6.9 Hz, NH), 5.93 (d, 1 H, *J* = 7.3 Hz, NH), 5.39 [brs, 1 H, PhC*H*$(O)_2$], 5.12 (d, 1 H, J = 3.6 Hz, H-4b), 5.04 (brs, 1 H, H-1a), 2.13 (brd, 1 H, *J* = 13.0 Hz, H-3c eq), 1.82 and 1.72 (2 s, 6 H, 2 Ac). Compound **1** [600 MHz; D_2O, 25 °C (or 60 °C), *t*-BuOH (δ 1.23)]: δ 7.62 (brs, 1 H, His), 7.31 (brt, 2 H, Phe), 7.27 (brt, 1 H, Phe), 7.19 (d, 2 H, *J* 7.3 Hz, Phe), 6.85 (brs, 1 H, His), 4.89 (d, 1 H, *J* 2.4 Hz, H-1:GalNAc, 60 °C), 4.45 (d, 1 H, *J* 7.3 Hz, H-1:Gal), 4.19 (brd, 1 H, *J* 2.4 Hz, H-4:GalNAc), 3.51 (brt, 1 H, H-2:Gal), 2.74 (dd, 1 H, *J* 4.3, 12.2 Hz, H-3eq:NeuAc), 2.02 and 1.99 (2s, 6 H, 2Ac:GalNAc and NeuAc), 1.77 (t, 1 H, *J* 12.2 Hz, H-3ax: NeuAc).
9. Nakahara, Y.; Iijima, H.; Ogawa, T. *Carbohydr. Lett.* **1994**, *1*, 99-106.
10. Ciommer, M.; Kunz, H. *SYNLETT* **1991**, 593-595.
11. Fujii, N.; Otaka, A.; Ikemura, O.; Akaji, K.; Funakoshi, S.; Hayashi, Y.; Kuroda, Y.; Yajima, H. *J. Chem. Soc., Chem. Commun.* **1987**, 274-275.

(*Received in Japan* 14 *July* 1997; *revised* 14 *August* 1997; *accepted* 18 *August* 1997)