Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 13, pp. 1351-1354, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00222-7

A REGIO- AND STEREOSELECTIVE SYNTHESIS OF 4-*O*-SULFATED CHONDROITIN DI- AND TETRASACCHARIDES BASED ON THE STRATEGY DESIGNED FOR THE ELONGATION OF THE REPEATING UNIT[1]

Jun-ichi Tamura[a], Klaus W. Neumann[a] and Tomoya Ogawa*[,a,b]

[a]) *The Institute of Physical and Chemical Research (RIKEN), Wako-shi, Saitama, 351-01 Japan.*
[b]) *Department of Cellular Biochemistry, University of Tokyo, Yayoi, Bunkyo-ku, Tokyo, 113 Japan.*

Abstract. 4-*O*-Sulfated chondroitin di- and tetrasaccharide [β-D-GalNAc(4-SO_3)(1→4)β-D-GlcA and β-D-GalNAc(4-SO_3)(1→4)β-D-GlcA(1→3)β-D-GalNAc(4-SO_3)(1→4)β-D-GlcA] were regio- and stereoselectively synthesized based on the synthetic route suitably designed for the elongation of the repeating unit.

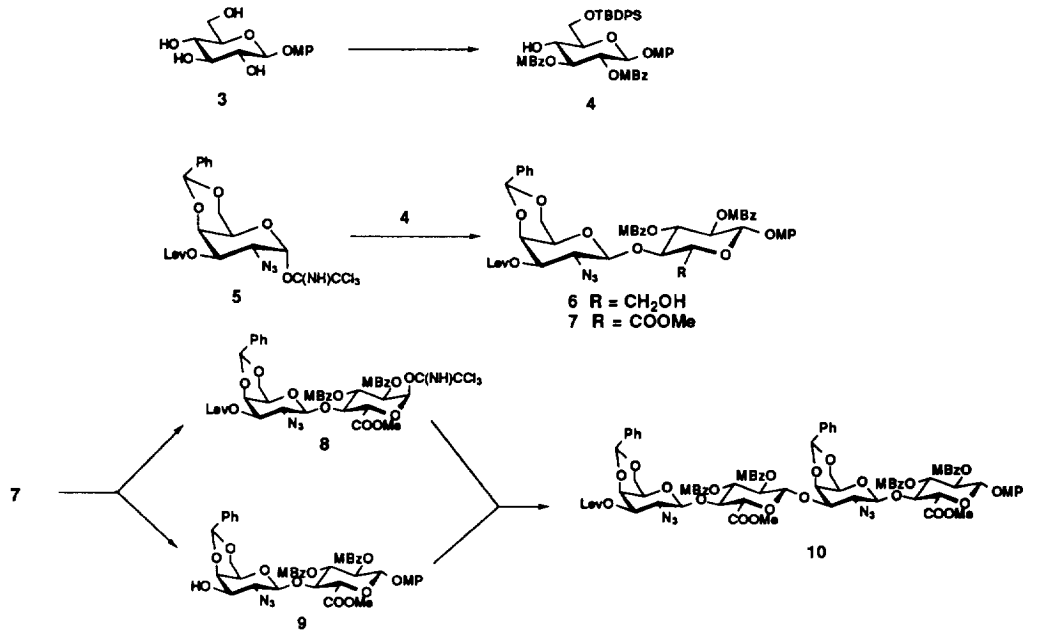
Recently various studies indicated that sulfated glycosaminoglycan chains of proteoglycans should be essential for some of their biological functions. For example, oligosaccharide domains of heparin,[2] dermatan sulfate,[3] and heparan sulfate[4] have been reported to have specific interaction with antithrombin III, heparin cofactor II, and basic fibroblast growth factor, respectively. Despite of the biological functions those sulfated chains may have, their biosynthesis mechanism, particularly, control of selective sulfation has not been clarified.[5] This prompted us to investigate the synthesis of chondroitin and its sulfated oligosaccharides as substrates for enzymatic sulfation relevant to their biosynthesis.

It is noted that the synthesis of chondroitin-4-*O*-sulfated disaccharide [β-GlcA(1→3)β-GalNAc(4-SO_3)] was reported in 1989 by Sinaÿ et al..[6] In the following year Jacquinet[7] reported the synthesis of the reverse disaccharide sequence [β-GalNAc(4-SO_3)(1→4)β-GlcA]. However, chondroitin repeating oligosaccharides longer than tetrasaccharide have not yet been synthesized to our knowledge.

A key synthetic intermediate for the chondroitin oligosaccharide chain elongation was designed as follows. Having a β-GalN3(1→4)GlcA type disaccharide as repeating intermediate, we have chosen p-methoxyphenyl (MP) and levulinyl (Lev) moieties as chemoselectively removable protecting groups at the reducing end (GlcA 1-OH) and at the non-reducing end (GalN3 3-OH), respectively, as designed in scheme-1. According to this strategy, we have synthesized 4-*O*-sulfated chondroitin di- and tetrasaccharide (**1** and **2**) regio- and stereoselectively as follows.

The glycosyl acceptor **4** was synthesized from readily available p-methoxyphenyl β-D-glucopyranoside **3**.[8] Conversion of **3** into **4** was carried out in 4 steps; *1*) $Me_2C(OMe)_2$ / Me_2CO, p-TsOH, *2*) p-MeC_6H_4COCl / pyridine, *3*) camphorsulfonic acid / CH_2Cl_2-MeOH, *4*) TBDPSCl, imidazole / DMF; 81% overall. The known glycosyl imidate **5**[9] (1.8 equiv.), prepared from D-galactose in 11 steps, and an acceptor **4** were subjected to condensation in PhMe at -50 ~ -40 °C in the presence of BF_3•OEt_2 (0.1 equiv.) and MS4A. After a rough separation by column chromatography on silica gel the crude disaccharide was treated with Bu_4NF and AcOH in THF[10] to give **6** in 70% yield in 2 steps. No formation of the isomeric α-linked disaccharide was observed

when the above described glycosylation conditions were used. Oxidation at *C*-6 of **6** was carried out by means of Swern oxidation [$(COCl)_2$-DMSO-NEt^iPr_2] followed by treatment with $NaClO_2$ in *t*-BuOH-H_2O in the presence of 2-methyl-2-butene and NaH_2PO_4.[11] The crude carboxylic acid was esterified with diazomethane to give methyl ester **7**[12] which was isolated in 96% yield in 3 steps. Having in hand a key intermediate **7**, a common precursor for glycosyl donor **8** and acceptor **9**, MP group was removed by treatment with cerium ammonium nitrate.[13] The intermediate hemiacetal was converted into α-imidate **8** using CCl_3CN and DBU in CH_2Cl_2 in 94% yield. Applying the same key intermediate **7** Lev group was easily removed by the action of H_2NNH_2•AcOH[14] in PhMe-EtOH resulting in a quantitative yield of **9**. Coupling of **8** and **9** was performed in the presence of BF_3•OEt_2 (1 equiv.) and MS4A in PhMe at -20 °C to furnish the desired tetrasaccharide **10**[12] in 50 % yield.



Scheme - 1 **(Lev = $CH_3COCH_2CH_2CO$, MP = p-MeOPh, MBz = p-MePhCO, TBDPS = *t*-$BuMe_2Si$)**

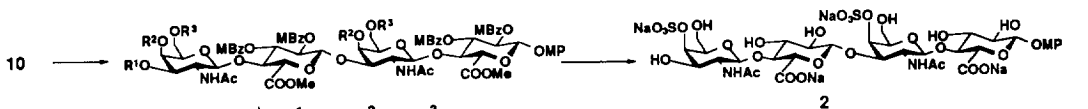
Next, 4-*O*-sulfation was first examined for the intermediate disaccharide **9** as depicted in scheme-2. The azide group of disaccharide **9** was reduced and simultaneously acetylated by exposure to AcSH[15] in pyridine to obtain 3'-*O*-acetylated acetamide **11** in 75% yield.[16] Reductive opening of the benzylidene group of **11** proceeded regioselectively when using $NaBH_3CN$ in THF and ethereal HCl in the presence of MS3A[17] to give **12** in 87%. The 4-OH of **12** was sulfated with SO_3•NMe_3 complex in DMF at 50 ~ 60 °C to afford **13** quantitatively. Hydrolysis of the ester group of **13** with LiOH in aqueous THF followed by treatment with NaOMe in MeOH was carried out in 91% yield. Finally, the product was hydrogenolized in the presence of $Pd(OH)_2$ in H_2O to yield **1**[12] (74%) which could be purified by HPLC gel permeation using a Shodex 310H column (H_2O).

In a similar manner, tetrasaccharide **10** was subjected to the transformation from azide to acetamide to yield **14** in 42% as shown in scheme-3. Lev group at 3-OH of GalNAc was removed as described for the synthesis of **9**, and the alcohol was masked as acetyl group by conventional method to obtain **15** (64% yield, 2 steps). Reductive opening of bis-benzylidene acetals of **15** afforded **16** in 51% yield together with a readily separable by-product **17** (31%) derived from acetyl migration. Sulfation was carried out for **16** as described for disaccharide **12** to give **18** in 92% yield. Deprotection of the di-sulfated tetrasaccharide was executed as described in the case of disaccharide to give the target compound **2**[12] in 70% yield (3steps). Final purification of **2** was carried out by gel permeation using a sephadex G-50 column (H_2O). Both target compounds, **1** and **2** gave satisfactory ^{1}H-NMR spectra, where the characteristic deshielded *H*-4 signal(s) for galactosamine residue(s) were observed as doublets at 4.68 ppm (for **1**) and 4.67 and 4.74 ppm (for **2**), respectively.

In summary, 4-*O*-sulfated chondroitin di - and tetrasaccharides have been synthesized based on a new synthetic route which allows oligosaccharide chain elongation using a disaccharide repeating unit as building block and multiple sulfation of suitably deprotected intermediates.

	R^1	R^2
11	PhCH<	
12	H	Bn
13	SO_3Na	Bn

Scheme - 2



	R^1	R^2	R^3
14	Lev	PhCH<	
15	Ac	PhCH<	
16	Ac	H	Bn
17	H	Ac	Bn
18	Ac	SO_3Na	Bn

Scheme - 3

Acknowledgement: A part of this work was financially supported by a Grant-in Aid for Scientific Research on Priority Areas No. 06240105 from the Ministry of Education, Science and Culture, and also by the Special Coordination Funds of the Science and Technology Agency of Japanese Government. Authors thank Dr. J. Uzawa, Dr. H. Koshino and Ms. T. Chijimatsu for the NMR measurement, Mr. H. Shimizu for the measurement of FABMS spectra and Ms. A. Takahashi for technical assistance. J. T. and K. W. N. thank Science and Technology Agency of Japanese Government for Basic Science Program as a special researcher and STA fellowship award, respectively.

References and Notes

1. Part 101 in the series of "Synthetic Studies on Cell-surface Glycans". For part 100 see; Wang, Z. -G.; Ito, Y.; Nakahara, Y.; Ogawa,T. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2805.

2. Lindahl, U.; Bäckström, G.; Thumberg, L.; Leder I. G. *Proc. Natl. Acad. Sci. USA* **1980**, *77*, 6551.
3. Tollefsen, D. M.; Peacock, M. E.; Monafo, W. J. *J. Biol. Chem.* **1986**, *261*, 8854.
4. Maccarana, M.; Casu, B.; Lindahl, U. *ibid.* **1993**, *268*, 23898.
5. Silbert, J. E. *Trend Glycosci. Glycotech.* **1991**, *3*, 343.
6. Marra, A.; Dong, X.; Petitou, M.; Sinaÿ, P. *Carbohydr. Res.* **1989**, *195*, 39.
7. Jacquinet, J.-C. *ibid.* **1990**, *199*, 153.
8. Yamaguchi, M.; Horiguchi, A.; Fukuda, A.; Minami, T. *J. Chem. Soc., Perkin Trans. 1*, **1990**, 1079.
9. Goto, F.; Ogawa, T. *Bioorg. Med. Chem. Lett.* **1994**, *4* , 619.
10. Ogilvie, K. K.; Beaucage, S. L.; Entwistle, D. W. *Tetrahedron Lett.* **1976**, *16*, 1255.
11. Nakahara, Y.; Ogawa, T. *Carbohydr. Res.* **1990**, *205*, 147.
12. Physical data for key compounds are given below, values of δ_H were measured at 25°C. Chemical shifts are expressed in p.p.m. downfield from the signal for internal Me4Si for solutions in CDCl3, and for the solutions in D2O, in p.p.m. downfield from the signal for Me4Si, by reference to internal *t*-BuOH (1.23). Signal assignment such as 1^3 stands for a proton at C-1 of sugar residue *3*. **7**: δ_H (CDCl3) 2.07 (s, *CH3*CO), 2.35, 2.27 (2s, 2Ph*CH3*), 2.57-2.72 (m, 2*CH3*), 3.12 (s, 5^2), 3.59 (s, $6a^2$), 3.74 (s, Ph*CH3*), 3.73-3.82 (m, 2^2, $6b^2$), 3.82 (s, COO*CH3*), 4.10 (d, $J_{3,4}$ = 3.30 Hz, 4^2), 4.34 (d, $J_{4,5}$ = 9.24 Hz, 5^1), 4.45 (d, $J_{1,2}$ = 7.92 Hz, 1^2), 4.55 (t, 4^1), 4.60 (dd, $J_{2,3}$ = 10.89 Hz, 3^2), 5.23 (d, $J_{1,2}$ = 7.26 Hz, 1^1), 5.26 (s, Ph*CH*), 5.59 (dd, $J_{2,3}$ = 8.91 Hz, 2^1), 5.80 (dd, 3^1), 6.77, 6.93 (2d, MeO*C6H4*), 7.07, 7.16 (2d, Me*C6H4*), 7.27-7.31 (m, Ph), 7.83-7.88 (m, Me*C6H4*). **10**: δ_H (CDCl3) 2.08(s, *CH3*CO), 2.28, 2.33, 2.34, 2.36 (4s, 4Ph*CH3*), 2.58-2.62, 2.70-2.73 (m, 2*CH3*), 2.66 (s, 5^4), 3.08(s, 5^2), 3.41 (dd, $J_{2,3}$ = 10.73, $J_{3,4}$ = 3.42, Hz, 3^2), 3.44 (s, 6a,b^4), 3.52(d, $J_{6a,6b}$ = 11.22 Hz, 6^2), 3.63 (dd, $J_{1,2}$ = 7.81 Hz, 2^2), 3.64 (d, $6b^2$), 3.69 (dd, $J_{1,2}$ = 8.3, $J_{2,3}$ = 10.74 Hz, 2^4), 3.74, 3.75 (2s, Ph*CH3*,COO*CH3*), 3.81 (s, COO*CH3*), 3.99 (d, $J_{3,4}$ = 3.42 Hz, 4^4), 4.09 (dd, 3^4), 4.28 (d, 4^2), 4.50 (d, $J_{3,4}$ = 8.78 Hz, 4^1), 4.57 (d, $J_{3,4}$ = 8.30 Hz, 4^3), 5.13 (d, $J_{1,2}$ = 5.85 Hz, 1^3), 5.20 (d, $J_{1,2}$ = 7.32 Hz, 1^1), 5.23 (s, Ph*CH*), 5.34 (dd, $J_{2,3}$ = 6.34 Hz, 2^3), 5.42 (s, Ph*CH*), 5.57 (dd, $J_{2,3}$ = 9.23 Hz, 2^1), 5.60 (dd, 3^3), 5.75 (dd, 3^1), 6.75-6.92 (m, MeO*C6H4*), 7.04-7.18 (m, Me*C6H4*), 7.30-7.38 (m, Ph), 7.77-7.86 (m, Me*C6H4*). **1**: δ_H(D2O) 2.05 (s,NH*Ac*), 3.80 (s, PhO*Me*), 4.56 (d, $J_{1,2}$ = 7.59 Hz, 1^2), 4.68 (d, $J_{3,4}$ = 2.31 Hz, 4^2), 5.00 (d, $J_{1,2}$ = 7.59 Hz, 1^1), 6.95-6.98 (m, MeO*C6H4*), 7.07-7.11 (m, MeO*C6H4*); FABMS, 650.1 $(M+Na)^+$, 628.2 $(M+H)^+$. **2**: δ_H (D2O) 2.03, 2.04 (2s, 2NH*Ac*), 3.80 (s, PhO*Me*), 4.45 (d, $J_{1,2}$ = 7.59 Hz, 1^3), 4.53, 4.58 (2d, $J_{1,2}$ = 6.92, 7.58 Hz, $1^{2,4}$), 4.67, 4.74 (2d, $J_{3,4}$ = 2.31 Hz, $4^{2,4}$), 4.99 (d, $J_{1,2}$ = 7.59 Hz, 1^1), 6.95-6.98 (m, MeO*C6H4*), 7.07-7.11 (m, MeO*C6H4*); FABMS, 1153.0 $(M+Na)^+$, 1131.1 $(M+H)^+$.
13. Fukuyama, T.; Laird, A. A.; Hotchkiss, L. M. *Tetrahedron Lett.* **1985**, *26*, 6291.
14. Nakano, T.; Ito, Y.; Ogawa, T. *Tetrahedron Lett.* **1991**, *32*, 1569.
15. Rosen, T.; Lico, I. M.; Chu, D. T. W. *J. Org. Chem.* **1988**, *53*, 1580.
16. 3'-*O*-Acetylated acetamide **11** was also obtained from **7** in 88% yield albeit it took 3 steps; *1*) AcSH / pyridine, *2*) H2NNH2•AcOH / PhMe-EtOH, *3*) Ac2O / pyridine.
17. Garegg, P. J.; Hultberg, H.; Wallin, S. *Carbohydr. Res.* **1982**, *108*, 97.

(*Received in Japan* 11 *May* 1995; *accepted* 22 *May* 1995)