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SYNTHESIS OF SULFATED GLYCOHEXAOSE OF LINKAGE REGION OF CHONDROITIN 4-SULFATE: β-D-GlcA-(1→3){(SO$_3$Na→4)}-β-D-GalNAc-(1→4)-β-D-GlcA-(1→3)-β-D-Gal-(1→3)-β-D-Gal-(1→4)-D-Xyl[1)]

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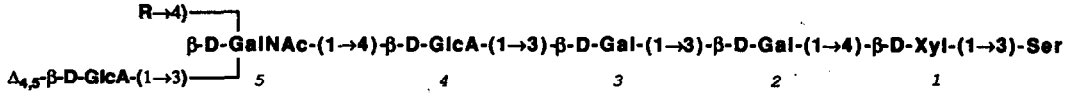
Abstract: Stereocontrolled synthesis of glycohexaoses 3 and 4 which corresponds to the linkage region of chondroitin 4-sulfate to a core protein was achieved for the first time by employing glycotriosyl donor 5 and glycotriosyl acceptor 6.

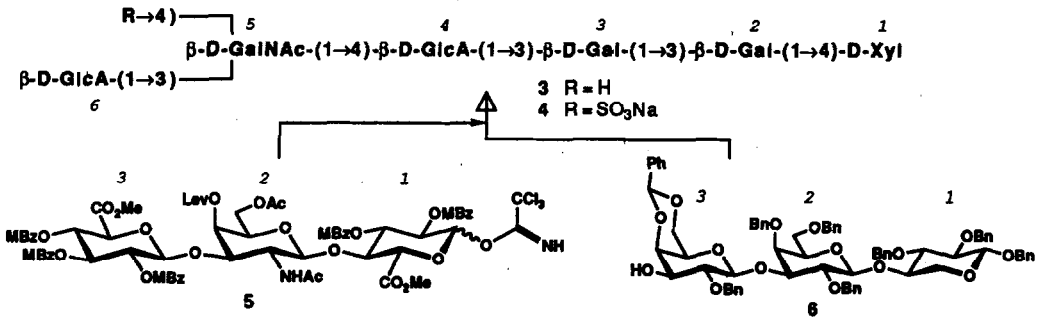
Nonsulfated and monosulfated glycosyl serine 1 and 2 were isolated[2] in 1988 as the glycosaminoglycan linkage region after exhaustive enzymic digestions of Swarm rat chondrosarcoma proteoglycans with chondroitinase ABC, papain, and Pronase. As part of our on-going project[3] on the synthesis of proteoglycan part structures, we now describe a versatile approach to the synthesis of both nonsulfated 3 and monosulfated glycohexaose 4 which, respectively, represent the glycan part of 1 and 2.



1 R = H
2 R = SO_3Na

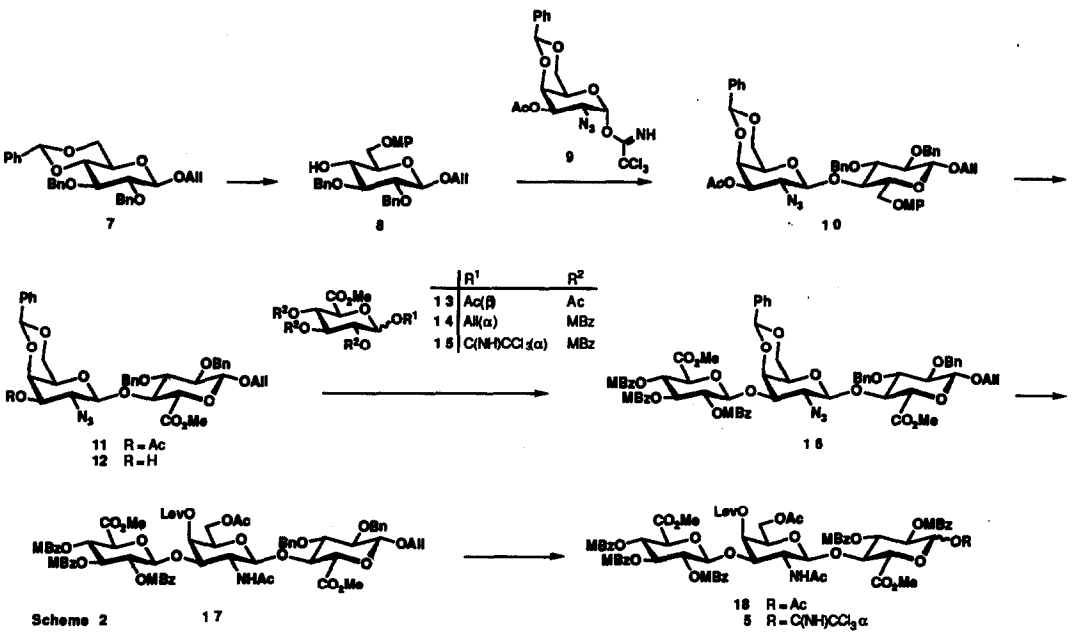
A retrosynthetic analysis of 3 and 4 led us to design a unique glycotriosyl donor 5 that carries two glucuronic acid (GlcA) residues, and a properly protected glycotriosyl acceptor 6[4]. We first describe stereocontrolled synthesis of both 5 and 6, then crucial coupling between them, and further conversion of the coupled product into target molecules 3 and 4.

Glycosylation of allyl glucopyranoside 8[5] readily obtainable from 7[6] in 2 steps (*1* CSA in 5:2 MeOH-CH_2Cl_2, *2* 4-MeOPhOH, Ph_3P, DEAD[7] in CH_2Cl_2, 84% overall) with imidate 9[8] in the presence of TMSOTf-powdered molecular sieves 4A (MS4A) in toluene of -78°~-40° afforded 62% of β-linked[9] disaccharide 10[5] along with 28% of the α-anomer[5]. Oxidative conversion of 10 into glycobiosyl acceptor 12[5] was achieved via 11[5] in 5 steps (*1* CAN in 4:1 MeCN-H_2O[10], *2* $(COCl)_2$,



Scheme 1 (MBz = 4-MeBz, Lev = $MeCOCH_2CH_2CO$)

DMSO, iPr_2EtN in CH_2Cl_2 at -78°~15°, *3* $NaClO_2$, 2-methyl-2-butene, $NaH_2PO_4 \cdot 2H_2O$ in 5:3 tBuOH-H_2O[11], *4* CH_2N_2, *5* NaOMe in 3:2 MeOH-THF; 73% overall). A glucuronic acid donor **15** was prepared from **13** via **14** in 5 steps (*1* $Bu_3SnOAll$, $SnCl_4$ in $(CH_2Cl)_2$, *2* NaOMe in 1:1 MeOH-THF, *3* MBzCl in Py, *4* $[Ir(COD)(Ph_2MeP)_2]PF_6$, H_2 in THF[12], then I_2 and H_2O, *5* Cl_3CCN, DBU in CH_2Cl_2[13], 64% overall). $BF_3 \cdot OEt_2$-molecular sieves-AW300 (MSAW300) promoted glycosylation of **12** with **15** in 1:1 toluene-CH_2Cl_2 at -25° afforded 76% of **16** which was subsequently converted into the designed glycotriosyl donor **5** via **17** and **18** in 10 steps (*1* CSA in 3:2 MeOH-CH_2Cl_2, *2* AcCl in Py at 0°, *3* Lev_2O, DMAP in 2:1 Py-$(CH_2Cl)_2$, *4* Ph_3P in 150:1 PhH-H_2O, 24 h, 50° then Ac_2O, DMAP in Py, *5* $[Ir(COD)(Ph_2MeP)_2]PF_6$, H_2 in THF, then I_2 and H_2O, *6* Ac_2O, DMAP in Py, *7* 10% Pd-C, H_2 in MeOH, *8* MBzCl, DMAP in Py, *9* piperidine•AcOH in THF[14], *10* CCl_3CN, DBU in CH_2Cl_2; 30% overall).

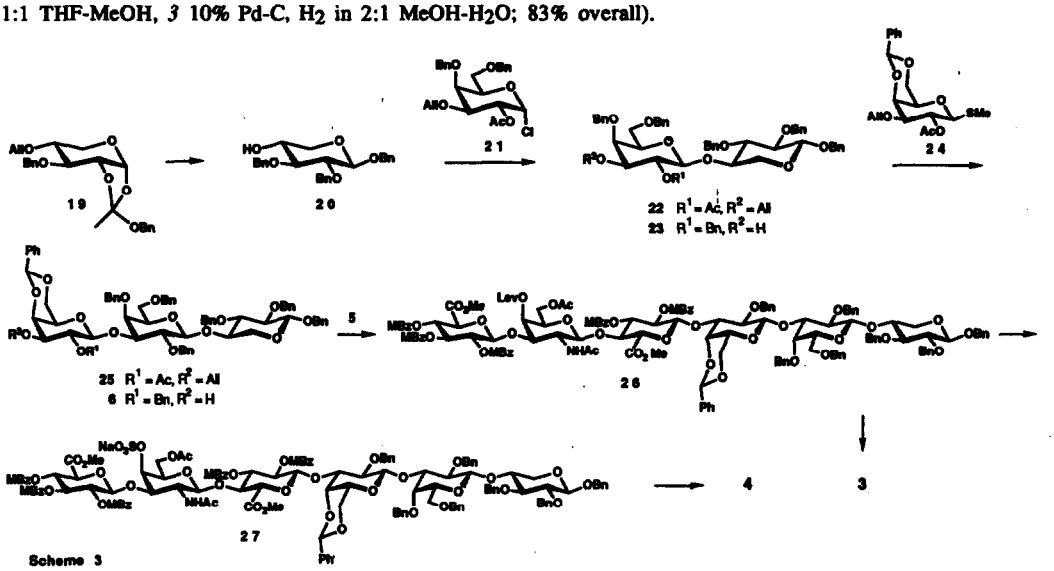


Scheme 2

Having prepared a glycotriosyl donor **5** that corresponds to the left part (*6-5-4*) of the target molecules **3** and **4**, we now describe the synthesis of a glycotriosyl acceptor **6** that corresponds to the right part (*3-2-1*) as shown in scheme 3. Xylosyl derivative **20** was readily obtained from orthoester **19**[3] in 4 steps (*1* TMSOTf-MS4A in $(CH_2Cl)_2$, *2* NaOMe in MeOH, *3* BnBr, NaH, DMF, *4* $[Ir(COD)(Ph_2MeP)_2]PF_6$, H_2 in THF, then H_2 and I_2; 73% overall). AgOTf[15]-MS4A promoted glycosylation of **20** with **21**[3] in 3:1 toluene-CH_2Cl_2 afforded 63% of β(1→4) linked **22**[5] together with 25% of the α-anomer[5]. Conversion of **22** into a glycobiosyl acceptor **23** was carried out in 3 steps (*1* NaOMe in 1:1 THF-MeOH, *2* BnBr, NaH in DMF, *3* $[Ir(COD)(Ph_2MeP)]PF_6$, H_2 in THF, then H_2O and I_2; 91% overall). $CuBr_2$-n-Bu_4NBr-AgOTf[16] promoted glycosylation of **23** with **24**[3] in $(CH_2Cl)_2$ gave 97% of **25** which was transformed into a glycotriosyl acceptor **6** in 3 steps (*1* deacetylation, *2* benzylation, *3* deallylation, 83% overall) as described for the conversion of **22** into **23**.

Crucial coupling of a glycotriosyl donor **5** (46 μmol) with a glycotriosyl acceptor **6** (42 μmol) was carried out at -20°~-30° in the presence of $BF_3 \cdot OEt_2$(14 μmol)-MSAW-300 in 1:2

toluene-CH_2Cl_2 to give 47% of **26**. The α-anomer of **26** was not detected and 20% of the hemiacetal, hydrolysis product of **5**, was recovered from the reaction mixture. **26** was smoothly deblocked in 3 steps to give the nonsulfated target molecule **3** (*1* LiOH in 20:1 THF H_2O *2* NaOMe in 1:1 THF-MeOH, *3* 10% Pd-C, H_2 in 2:1 MeOH-H_2O; 83% overall).



Scheme 3

Finally, transformation of **26** into sulfated glycohexaose **4** was achieved via **27** in 5 steps (*1* NH_2NH_2•AcOH in 5:2 EtOH-THF, *2* Me_3N•SO_3 in DMF at 60°, *3* LiOH in 15:1 THF-H_2O, *4* NaOMe in 1:1 THF-MeOH, *5* 10% Pd-C, H_2 in 2:1 MeOH-H_2O; 17% overall).

In summary, two glycohexaoses **3** and **4** that correspond to the linkage sequence of a chondroitin 4-sulfate to a core protein were synthesized for the first time by employing a glycotriosyl donor **5** and a glycotriosyl acceptor **6** as key intermediates.

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References and Notes

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4 For the synthesis of related glycotriosides, see B. Lindberg, L. Roden, and B.-G. Silvander, *Carbohydr. Res.*, **2**, 413 (1966); P. J. Garegg, B. Lindberg, and T. Norberg, *Acta Chem. Scand. B*, **33**, 449 (1979); G. Ekborg, T. Curenton, N. R. Krishna, and L. Roden, *J. Carbohydr. Chem.*, **9**, 15 (1990).

5 Physical data for new compounds are given below, values of $[\alpha]_D$ and $\delta_{H,C}$ were measured at 25°±3° for solutions in $CHCl_3$ and $CDCl_3$, respectively, unless noted otherwise. Signal assignment such as 1^3 stands for a proton at C-1 of sugar residue *3*. **4**: R_F 0.16 in 4:4:1, $CHCl_3$-MeOH-H_2O; $\delta_H(D_2O)$ 5.184 (0.3H, d, 3.7Hz, $1^1\alpha$), 4.785 (d, 1.5Hz, 4^5), 4.666 (d, 7.6Hz, 1^4), 4.660 (d, 7.6Hz, 1^3), 4.591 (0.7H, d, 7.6Hz, $1^1\beta$), 4.562 (d, 8.5Hz, 1^5), 4.522 and 4.518 (2d, 7.9Hz, $1^2\beta$ and $1^2\alpha$),

4.459 (d, 7.6Hz, 1^6), 4.180 and 4.142 (2d, 3.3Hz, 4^2 and 4^3); FABMS (M-Na)$^-$ 1152. **3**: R_F 0.13 in 4:4:1 $CHCl_3$-MeOH-H_2O; $\delta_H(D_2O)$ 5.184 (0.3H, d, 4.0Hz, $1^1\alpha$), 4.661 (d, 7.9Hz, 1^3,1^4), 4.591 (d, 7.6Hz, 1^1b), 4.522 and 4.518 (2d, 7.9Hz, 1^2), 4.510 (d, 8.2Hz, 1^6), 4.482 (d, 7.6Hz, 1^5), 4.180, 4.166, and 4.146 (3d, 3.3Hz, $4^{2,3,5}$); SIMS (M^+-Na) 1052.. **5**: R_F 0.64 in 1:2 toluene-EtOAc; $[\alpha]_D$ +21.6° (c 0.8); δ_H 8.601 (s, NH), 6.720 (d, 3.7Hz, 1^1), 5.265 (d, 3.4Hz, 4^2), 4.938 (d, 7.9Hz, 1^2), 4.790 (d, 7.6Hz, 1^3), 3.799 and 3.646 (2s, 2OMe), 2.225 (s, Lev), 2.054 and 1.576 (2s, 2Ac). **6**: R_F 0.34 in 1:1 EtOAc-hexane; $[\alpha]_D$ -20.5° (c 1.9); δ_H 5.554 (s, PhCH), 4.857 (d, 7.6Hz, 1^3), 4.472 and 4.443 (2d, 7.3Hz, 1^1 and 1^2). **8**: R_F 0.43 in 5:1 toluene-EtOAc; $[\alpha]_D$ -10.9° (c 1.8); δ_H 4.499 (d, 7.3Hz, H-1), 3.746 (s, OMe); Acetate of **8**: δ_H 5.039 (dd, 9.3 and 10.0Hz, H-4). **9**: R_F 0.43 in 7:3 hexane-EtOAc; $[\alpha]_D$ +161.3° (c 0.7); δ_H 8.755 (s, NH), 6.585 (d, 3.4Hz, H-1), 5.546 (s, PhCH), 2.178 (s, Ac). **10**: R_F 0.23 in 3:1 hexane-EtOAc; $[\alpha]_D$ +33.5° (c 1.3); δ_H 5.446 (s, PhCH), 4.511 (d, 7.6Hz, 1^1), 4.377 (d, 8.2Hz, 1^2), 3.776 (s, OMe), 2.109 (s, Ac). α-anomer of **10**: R_F 0.34; $[\alpha]_D$ +99.1° (c 1.2); δ_H 5.900 (d, 3.7Hz, 1^2), 4.534 (d, 7.6Hz, 1^1). **11**: R_F 0.65 in 1:2 toluene-EtOAc; $[\alpha]_D$ +12.4° (c 0.2); δ_H 5.482 (s, PhCH), 4.537 (d, 7.9Hz, 1^2), 4.492 (d, 7.9Hz, 1^1), 3.962 (d, 9.8Hz, 5^1), 3.840 (s, OMe), 2.132 (s, Ac). **12**: R_F 0.38 in 2:1 toluene-EtOAc; $[\alpha]_D$ -5.4° (c 0.1); mp 185-186° (hexane-Et_2O); δ_H 5.552 (s, PhCH), 4.544 (d, 7.6Hz, 1^2), 4.415 (d, 7.6Hz, 1^1), 3.973 (d, 9.5Hz, 5^1), 3.859 (s, OMe). **14**: R_F 0.42 in 3:1 hexane-EtOAc; $[\alpha]_D$ +52.8° (c 0.7); δ_H 5.456 (d, 3.7Hz, H-1), 3.676 (s, OMe), 2.359, 2.353, and 2.292 (3s, 3MBz). **15**: R_F 0.50 in 7:3 hexane-EtOAc; δ_H 8.659 (s, NH), 6.891 (d, 3.7Hz, H-1), 3.677 (s, OMe), 2.365, 2.342, and 2.300 (3s, 3MBz). **16**: R_F 0.44 in 2:1 toluene-EtOAc; $[\alpha]_D$ -23.0° (c 0.1); δ_H 5.570 (s, PhCH), 5.175 (d, 7.3Hz, 1^3), 4.497 (d, 7.6Hz, 1^2), 4.408 (d, 7.9Hz, 1^1), 3.754 and 3.644 (2s, 2OMe), 2.366, 2.345, and 2.300 (3s, 3MBz). **17**: R_F 0.34 in 1:1 toluene-EtOAc; $[\alpha]_D$ -6.1° (c 0.2); δ_H 5.409 (d, 4.8Hz, 4^2), 5.049 (d, 8.1Hz, 1^3), 4.883 (d, 7.7Hz, 1^2), 4.452 (d, 7.7Hz, 1^1), 3.758 and 3.674 (2s, 2OMe), 2.174 (s, Lev), 1.953 and 1.557 (2s, 2Ac). **18**: A 1.6:1 mixture of α and β-anomer; R_F 0.41 in 2:1 toluene-EtOAc; δ_H 6.497 (d, 3.7Hz, $1^1\alpha$), 5.919 (d, 7.3Hz, $1^1\beta$). **20**: R_F 0.46 in 3:1 toluene-EtOAc; $[\alpha]_D$ -67.8° (c 0.8); mp 120-121° (EtOAc-hexane); δ_H 4.573 (d, 6.1Hz, H-1). Acetate of **20**: δ_H 4.929 (ddd, 5.5, 8.9, and 11.9 Hz, H-4). **22**: R_F 0.40 in 7:3 hexane-EtOAc; $[\alpha]_D$ -11.5° (c 1.7); δ_H 5.292 (dd, 7.9 and 9.8Hz, 2^2), 4.472 (d, 7.9Hz, 1^2), 4.435 (d, 7.6Hz, 1^1), 2.070 (s, Ac). α-anomer of **22**: R_F 0.58; $[\alpha]_D$ +28.8° (c 2.0); δ_H 5.365 (dd, 4.0 and 10.7Hz, 2^2), 5.275 (d, 4.0Hz, 1^2), 4.412 (d, 7.6Hz, 1^1), 1.790 (s, Ac). **23**: R_F 0.38 in 2:1 toluene-EtOAc; $[\alpha]_D$ -21.0° (c 0.3); δ_H 4.474 and 4.430 (2d, 7.3 and 7.6Hz, 1^1 and 1^2). **25**: R_F 0.36 in 3:2 hexane-EtOAc; $[\alpha]_D$ -22.7° (c 0.6); δ_H 5.544 (s, PhCH), 5.442 (dd, 7.9 and 10.1Hz, 2^3), 4.902 (d, 7.9Hz, 1^3), 4.449 and 4.413 (2d, 7.3Hz, 1^1 and 1^2), 1.918 (s, Ac). **26**: R_F 0.51 and 1:2 toluene-EtOAc; $[\alpha]_D$ -4.5° (c 0.8); δ_H 5.552 Z(s, PhCH), 5.263 (d, 6.9Hz, 1^4), 5.229 (d, 3.3Hz, 4^5), 5.203 (d, 7.4Hz, 1^5), 4.788 (d, 7.7Hz, 1^6), 4.432 (d, 7.4Hz, 1^1), 4.332 (d, 8.0Hz, 1^2), 3.769 and 3.646 (2s, 2OMe), 2.208 (s, Lev), 2.010 and 1.567 (2s, 2Ac). **27**: R_F 0.33 in 10:1 $CHCl_3$-MeOH; δ_H 3.784 and 3.639 (2s, 2OMe), 2.350 x 2, 2.315, 2.291 and 2.269 (4s, 5MBz), 1.946 and 1.660 (2s, 2Ac).

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