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A SYNTHETIC APPROACH TO KERATAN SULFATE I: SYNTHESIS OF TRISULFATED GLYCOTETRAOSE[1]

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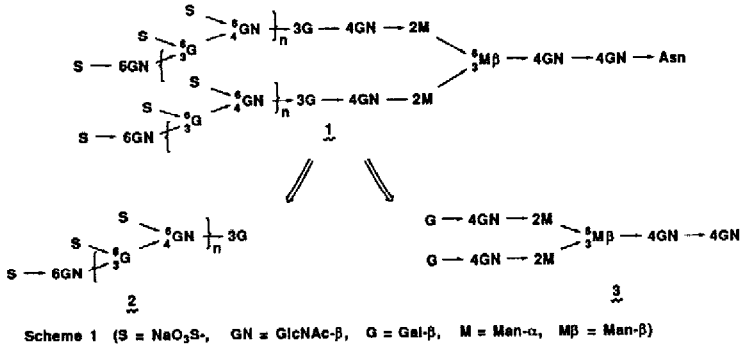
Abstract: A stereocontrolled synthesis of O-(6-O-sulfo-β-D-GlcNAc)-(1→3)-O-(6-O-sulfo-β-D-Gal)-(1→4)-O-(6-O-sulfo-β-D-GlcNAc)-(1→3)-Gal trisodium salt, a part structure of the acidic glycan of bovine corneal keratan sulfate, was achieved for the first time.

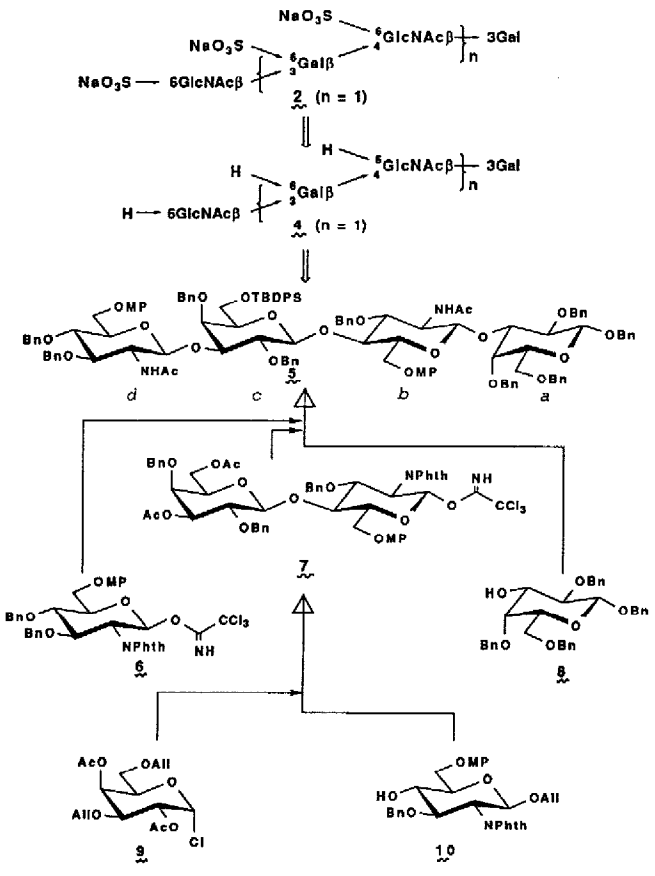
Keratan sulfate I occurs as a major component of the extracellular matrix of the cornea[2] and carries sulfated poly N-acetyllactosamine chains. Fucosylated structures of poly N-acetyllactosamine chains of glycoconjugates were identified as stage-specific antigens[3] in the developing mouse embryo and a characteristic of certain embryonic and tumor cells of man[4]. Sulfation of poly N-acetyllactosamine chains is also regulated developmentally. Upon retinoic acid treatment, mouse tetratocarcinoma cells were induced to differentiate and synthesized an increased amount of a proteoglycan[5] that are structually very similar to keratan sulfate I. The polysulfated glycan chains of keratan sulfate I are elongated from complex type oligosaccharide core structures that are linked to L-asparagine through N-glycosidic linkage[6]. The structures of sulfated oligosaccharides enzymatically released from bovine corneal keratan sulfate were recently chemically characterized[7].



Scheme 1 (S = NaO_3S-, GN = GlcNAc-β, G = Gal-β, M = Man-α, Mβ = Man-β)

A plausible synthetic approach to a putative sturcture **1** for keratan sulfate I is shown in Scheme 1. Since the complex type glycononaose core structure **3** has already been synthesized[8], synthetic experiments directed to the sulfo-glycan **2** should be exploited. We now describe a stereo-controlled synthesis of tri-O-sulfo-glycotetraose **2**(n=1) according to the synthetic plan shown in Scheme 2. The key intermediate glycotetraoside **5** was designed so as to function after partial deprotection as a suitable substrate **4** for sulfation, and may be synthesized by sequential glycosylations of a glycosyl acceptor **8**[9] with glycosyl donors **7** and **6**. The lactosaminyl donor **7** should be prepared from known monosaccharide synthons **9**[10] and **10**[11].

Conversion of allyl glycoside **10** into the glycosyl donor **6**[12] was achieved in 3 steps in 62% overall yield via compounds **11**[12] and **12**[12]; 1 Ag_2O, KI, and benzyl bromide in DMF, 2 $(Ph_3P)_3RhCl$ and DABCO in 7:3:1 EtOH-PhH-H_2O, then HgO and $HgCl_2$ in aq. Me_2CO[13], 3 CCl_3CN and DBU[14].

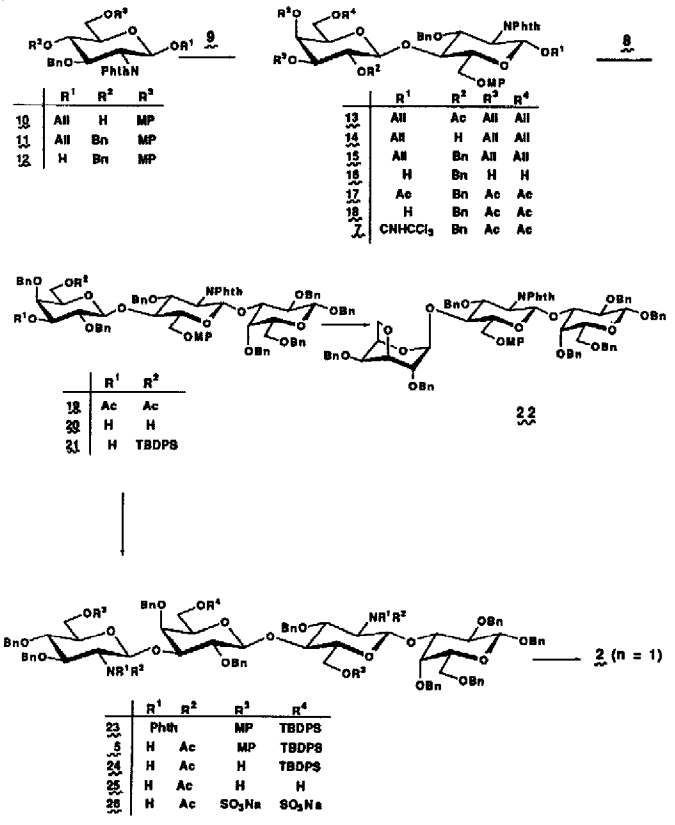


Scheme 2 (MP = p-MeOPh, TBDPS = Bu^tPh_2Si)

Conversion of allyl glycoside **10** into the glycobiosyl donor **7** was performed in 34% overall yield in 7 steps as follows. Silver triflate promoted glycosylation of **10** with the chloride **9** gave a 92% yield of β-(1→4) linked glycobioside **13**[12]. The β-D-configuration at a newly generated stereocenter C-1b in **13** was anticipated by the presence of an acetyl auxiliary at O-2 in **9** and was confirmed by ^1H n.m.r. data. Deacetylation of diacetate **13** with $LiOH\text{-}H_2O_2$[15] gave a 91% yield of the diol **14**[12] which was benzylated to afford tri-O-benzyl derivative **15**[12] in 90% yield. Deallylation of **15** with Wilkinson's catalyst as described before and acetylation of the product **16** gave a 71% overall yield of triacetate **17**[12] as a mixture of β- and α-anomer in a ratio of 11:1. Chemoselective cleavage of anomeric acetate was achieved by $H_2NNH_2\cdot AcOH$ in DMF[16] to give a 73% yield of hemiacetal **18** which was then converted to the imidate **7**[12] in 87% yield.

Having prepared the glycosyl donors **6** and **7**, glycosylation of benzyl galactoside **8** was now examined. Trifluoroborane etherate promoted glycosylation of **8** with the glycosyl donor **7** gave an 83% yield of desired glycotriaoside **19**[12]. The configuration of C-1b was expected as β-D according to the presence of C-2 N-phthaloyl group[17] in **7** and confirmed by ^1H n.m.r. data for **19**. Deacetylation of **19** by NaOMe-MeOH gave a 92% yield of the diol **20**[12]. Attempted introduction of a p-methoxyphenyl group at O-6c by Mitsunobu reaction failed and gave quantitatively 3,6-anhydro derivative **22**[12]. Alternatively the diol **20** was treated with Bu^tPh_2SiCl[18] and imidazole to afford a glycosyl acceptor **21**[12] in 78% yield.

Trifluoroborane etherate promoted glycosylation of **21** with **6** afforded glycotetraoside **23**[12] in 72% yield based on consumed glycosyl acceptor **21**. Dephthaloylation with $NH_2NH_2\cdot H_2O$[17] and acetylation afforded a 63% yield of the designed key intermediate **5**, that was further transformed into target molecule **2** as follows. Ammonium cerium(IV) nitrate treatment[19] of **5** in aq.CH_3CN gave a 75% yield of the diol **24**[12], which was desilylated with Bu_4NF[20] in THF to yield a 78% of the triol **25**[12] suitable as a substrate for sulfation. Treatment with $SO_3\cdot NMe_3$ complex in DMF at 50° afforded tri-sulfo compound **26**[12] in 93% yield, which was hydrogenolyzed in the presence of 10% Pd-C in 9:1 MeOH-H_2O to give the target **2**(n=1) in



Scheme 3

92% yield. ^{1}H-N.m.r. data for synthetic **2** was in good agreement with those reported[7] for the sample isolated from bovine corneal keratan sulfate I.

In conclusion, by combining a versatile synthetic route to a sulfo-glycan **2** described here with the already available synthetic route to a core glycononaose **3**, a reasonable possibility for the assemblage of a whole structure of keratan sulfate I may be emerged as shown in Scheme 1.

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Reference and Notes

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12) Physical data for synthetic compounds are given below. Values of $[\alpha]_D$ and $\delta_{H,C}$ were measured for the solution in $CHCl_3$ and $CDCl_3$, respectively, at 25°, unless noted otherwise.
2(n=1): $[\alpha]_D$ +12° (c 0.1, H_2O); δ_H (D_2O, $\delta_{acetone}$ 2.225) 2.032 and 2.043 (2s, Ac x 2), 4.531 (d, 8.1 Hz) and 4.534 (d, 7.7 Hz, H-1c), 4.561 (d, 8.1 Hz) and 5.226 (d, 3.3 Hz, H-1a). **5**: $[\alpha]_D$ -6.4° (c 0.3); δ_H 0.884 (s, Bu^t), 1.382 and 1.438 (2s, Ac x 2), 3.715 and 3.737 (2 s, OMe x 2); δ_C 101.7 (161 Hz) and 101.8 (161 Hz, C-1bd), 102.6 (158 Hz) and 103.2 (162 Hz, C-1ac). **6**: $[\alpha]_D$ +73.7° (c 0.8); δ_H 3.762 (s, OMe), 6.453 (d, 8.7 Hz, H-1), 8.554 (s, C=NH). **7**: $[\alpha]_D$ +57.9° (c 0.5); δ_H 1.939 and 2.002 (2 s, Ac x 2), 3.764 (s, OMe), 6.443 (d, 8.4 Hz, H-1a), 8.535 (s, C=NH). **11**: m.p. 54-55°; $[\alpha]_D$ +50.0° (c 0.4); δ_H 3.771 (s, OMe), 5.210 (d, 8.3 Hz, H-1). **12**: m.p. 176-178°; $[\alpha]_D$ +87.1° (c 0.6); δ_H 3.740 (s, 2.4 H, OMe), 3.755 (s, 0.6 H, OMe), 5.374 (t, 0.2 H, 3.7 Hz, H-1α), 5.444 (t, 0.8 H, 8.1 Hz, H-1β). **13**: m.p. 55-56°; $[\alpha]_D$ +40.1° (c 0.9); δ_H 2.042 and 2.060 (2 s, Ac x 2), 3.785 (s, OMe), 4.537 (d, 7.9 Hz, H-1b), 5.184 (d, 8.5 Hz, H-1a), 5.380 (d, 2.4 Hz, H-4b). **14**: $[\alpha]_D$ +42.5° (c 1.3); δ_H 3.777 (s, OMe), 4.438 (d, 7.9 Hz, H-1b), 5.201 (d, 8.2 Hz, H-1a). **15**: $[\alpha]_D$ +32.7° (c 0.5); δ_H 3.748 (s, OMe), 4.346 (d, 7.9 Hz, H-1b), 5.197 (d, 8.2 Hz, H-1a). **17** β-anomer: m.p. 153-154°; $[\alpha]_D$ +41.7° (c 0.4); δ_H 1.928, 1.940 and 1.993 (3 s, Ac x 3), 3.766 (s, OMe), 4.413 (d, 7.9 Hz, H-1b), 6.320 (d, 8.8 Hz, H-1a), **17** α-anomer: m.p. 55-57°; $[\alpha]_D$ +56.1° (c 1.5); δ_H 1.931, 1.951, and 2.097 (3 s, Ac x 3), 3.773 (s, OMe), 6.270 (d, 3.7 Hz, H-1a). **18**: $[\alpha]_D$+49.3° (c 0.5); δ_H 1.931 and 1.979 (2 s, 4 H, Ac x 2), 1.943 and 1.966 (2 s, 2 H, Ac x 2), 3.754 (s, 2 H, OMe), 3.766 (s, 1 H, OMe), 5.297 (d, 0.33 H, 3.5 Hz, H-1a), 5.378 (d, 0.67 H, 8.8 Hz, H-1a). **19**: m.p. 56-58°; $[\alpha]_D$ +9.1° (c 0.7); δ_H 1.943 and 1.954 (2 s, Ac x 2), 3.748 (s, OMe), 5.478 (d, 8.2 Hz, H-1b). **20**: $[\alpha]_D$ -3.7° (c 0.6); δ_H 3.742 (s, OMe), 4.298 (d, 7.6 Hz) and 4.348 (d, 7.6 Hz, H-1ac), 5.499 (d, 8.2 Hz, H-1b). **21**: $[\alpha]_D$ -0.2° (c 1.1); δ_H 1.018 (s, Bu^t), 3.757 (s, OMe), 5.453 (d, 8.5 Hz, H-1b). **22**: m.p. 40-41°; $[\alpha]_D$ -17.4° (c 1.0); δ_H 3.731 (s, OMe), 5.015 (s, H-1c), 5.478 (d, 8.2 Hz, H-1b); δ_C 99.7 (166 Hz, C-1b), 101.3 (166 Hz, C-1c), 102.6 (158 Hz, C-1a). **23**: $[\alpha]_D$ -4.4° (c 0.9); δ_H 0.838 (s, Bu^t), 3.704 and 3.789 (2 s, OMe x 2), 5.266 (d, 8.2 Hz) and 5.435 (d, 8.2 Hz, H-1bd); δ_C 99.2 (162 Hz, C-1d), 99.8 (166 Hz, C-1b), 102.5 (156 Hz, C-1a), 102.9 (159 Hz, C-1c). **24**: $[\alpha]_D$ -19.2° (c 0.7); δ_H 1.016 (s, Bu^t), 1.431 and 1.466 (2s, Ac x 2); δ_C 101.6 and 101.7 (C-1bd), 102.6 and 103.1 (C-1ac). **25**: $[\alpha]_D$ -17.3° (c 0.4); δ_H 1.474 and 1.506 (2 s, Ac x 2); δ_C 101.8 (C-1), 102.1 (C-1 x 2), 102.2 (C-1). **26**: $[\alpha]_D$ -10.5° (c 1.2); δ_H 100.9, 101.8, 102.0 and 102.6 (C-1 x 4).

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