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TOTAL SYNTHESIS OF A SULFATED GLUCURONIC ACID CONTAINING GLYCOHEPTAOSYL CERAMIDE, A MINOR GLYCOLIPID ISOLATED FROM HUMAN CAUDA EQUINA TISSUE[1]

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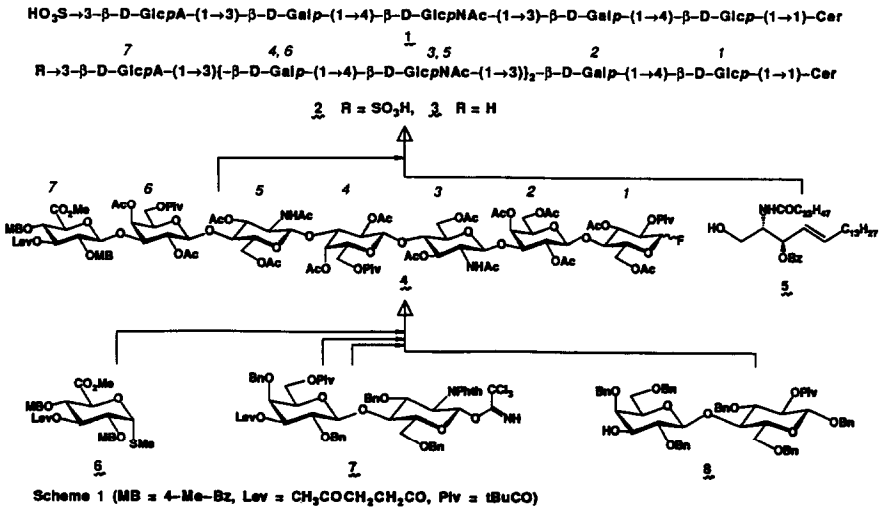
Abstract: A stereocontrolled total synthesis of sulfated glucuronic acid containing glycoheptaosyl ceramide was achieved for the first time.

In 1986, an acidic glycolipid[2], that reacted[3] with both HNK-1 (Leu 7) antibody raised against a surface antigen of natural killer cells and IgM-M protein from patients with neuropathy, was isolated from human peripheral nerves or cauda equina and chemically characterized. In 1990, the assigned structure **1** was confirmed through comparison of ^{1}H-nmr spectra between natural[4] and synthetic sample[5]. A minor acidic glycolipid which also reacted with HNK-1 and IgM-M was then isolated from human cauda equina in 1987 along with a major one **1**. The structure was proposed as **2** which has consecutive N-acetyllactosamine units[4]. Recently the presence of HNK-1 epitope was immunologically detected on poly-N-acetyllactosaminyl oligosaccharide chains linked to core proteins of chondroitin sulfate proteoglycan of rat brain[6]. As part of our continuing project[5,7] on the synthesis of carbohydrate epitopes associated with either lymphocytes or neural cells, we describe here an unambiguous synthesis of sulfated glycoheptaosyl ceramide **2** in a stereo- and regio-controlled manner.

Aiming at target molecule **2** a key glycoheptaosyl donor **4** was designed in harmony with the following working hypotheses. First, sulfate function at O-3[7] should be introduced on the glucuronic acid residue after coupling between glycoheptaosyl part **4** and ceramide **5**. Secondly, sulfate group at O-3[7] should stand the reaction conditions to deblock protective groups such as pivaloyl and toluoyl esters as well as a methyl ester at the last step of the synthetic sequence. Thirdly, in order to avoid any difficulties in later stage of the synthesis, N-protective groups should be converted into N-acetyl from N-phthaloyl groups before the coupling between a



Scheme 1 (MB = 4-Me-Bz, Lev = $CH_3COCH_2CH_2CO$, Piv = tBuCO)

glycoheptaosyl part and a ceramide derivative. Lastly, the presence of pivaloyl group at O-2[1] in the glycosyl fluoride **4** should facilitate an efficient and stereocontrolled coupling[8] with a ceramide derivative **5**.

Strategic bond disconnections of the glycosyl donor **4** led to design two glycosyl donors **6** and **7** as well as a glycosyl acceptor **8**. Since compounds **6**[5] and **8**[8a] were already reported, we first describe the synthesis of trichloroacetimidate **7**. Treatment of diol **9**[5] first with pivaloyl chloride and then with levulinic anhydride in pyridine in the presence of DMAP afforded **10**[9] in 89% overall yield. Oxidative removal[10] of methoxyphenyl group of **10** by treatment with $(NH_4)_2Ce(NO_3)_6$ gave hemiacetal which was treated with trichloroacetonitrile and DBU to give trichloroacetimidate **7**[9] in 86% yield. Glycosylation of **8** with **7** in the presence of TMSOTf and MS4A in $(ClCH_2)_2$ for 2 h at -23° afforded an 83% yield of glycotetraosyl derivative **11**[9]. Selective removal[11] of levulinoyl group by NH_2NH_2•AcOH in 4:1 EtOH-THF for 1.5 h at 20° gave a 61% yield of alcohol **12**[9] that was then glycosylated with **7** in the presence of TMSOTf in $(ClCH_2)_2$ for 1 h at -23° to afford an 81% yield of a protected glycohexaoside **13**[9]. Selective removal of two phthaloyl groups and a levulinoyl group of **13** was carried out simultaneously by treatment with NH_2NH_2•H_2O in EtOH for 50 h under reflux to give diamino alcohol that was N-acetylated with Ac_2O in MeOH for 2 h at 20° to give **14**[9] in 81% overall yield. Copper(II)bromide-nBu4NBr-AgOTf mediated glycosylation[12] of **14** with **6** in the presence of MS4A in CH_3NO_2 proceeded smoothly at 20° and gave a 62% yield of **15**[9]. Compound **15** was hydrogenolysed in the presence of 10% Pd-C in MeOH for 20 h at 50° and then acetylated with Ac_2O and DMAP in pyridine to afford a 1:1 mixture of α- and β-anomeric acetates **16**[9] in 83% overall yield. Chemoselective removal of anomeric acetate was executed in the presence of levulinoyl group at O-3[7] by piperidine-AcOH[5] in THF for 49 h at 25° to give **17**[9] which was subsequently treated with DAST[13] in $(ClCH_2)_2$ to give the designed glycoheptaosyl donor **4**[9] as a 1:4 mixture of α- and β-anomer in 81% overall yield.

Crucial coupling between **4** and **5** was carried out in the presence of $SnCl_2$-AgOTf[14] in freshly distilled $CHCl_3$ for 16 h at 20° to afford a 49% yield of **18**[9] which was completely deblocked into **3**[9] in 2 steps in 68% yield (i) 0.04*M* LiOH in 97:3 THF-H_2O at 0°, (ii) 0.3*M* MeONa in 1:1 MeOH-THF for 6 h at 15°. It is to be noted that α-fluoride **4** was not activated and quantitatively recovered from the reaction between **4** and **5**. Therefore only β-fluoride **4** was actually converted into **18** in 62% yield. Conversion of the key intermediate **18** into the target molecule was executed as follows. Selective removal of levulinoyl group at O-3[7] by NH_2NH_2•AcOH in EtOH for 16 h at 20° to give **19**[9] in 76% yield. Compound **19** was treated with Me_3NSO_3 in freshly distilled DMF for 66 h at 55°. The product was purified by Sephadex LH-20 in 1:1 $CHCl_3$-MeOH, then by Dowex 50 (Na^+) in 9:1 MeOH-H_2O and finally by preparative tlc on SiO_2 in 10:1 $CHCl_3$-MeOH to give **20**[9] in 71% yield. Complete deprotection of **20** was achieved in 2 steps, (i) 0.04 *M* LiOH in 97:3 THF-H_2O -15~0°, (ii) 0.3*M* NaOMe in 1:1 THF-MeOH for 2 h at 0~20° then neutralized with solid CO_2. The product was purified by Sephadex LH-20 in 5:5:1 $CHCl_3$-MeOH-H_2O to give **2**[9] in 37% yield.

In summary, an unambiguous synthesis of sulfated glucuronic acid containing glycoheptaosyl ceramide **2** was achieved for the first time in a stereocontrolled manner by use of a properly designed glycoheptaosyl fluoride **4** as a key glycosyl donor.

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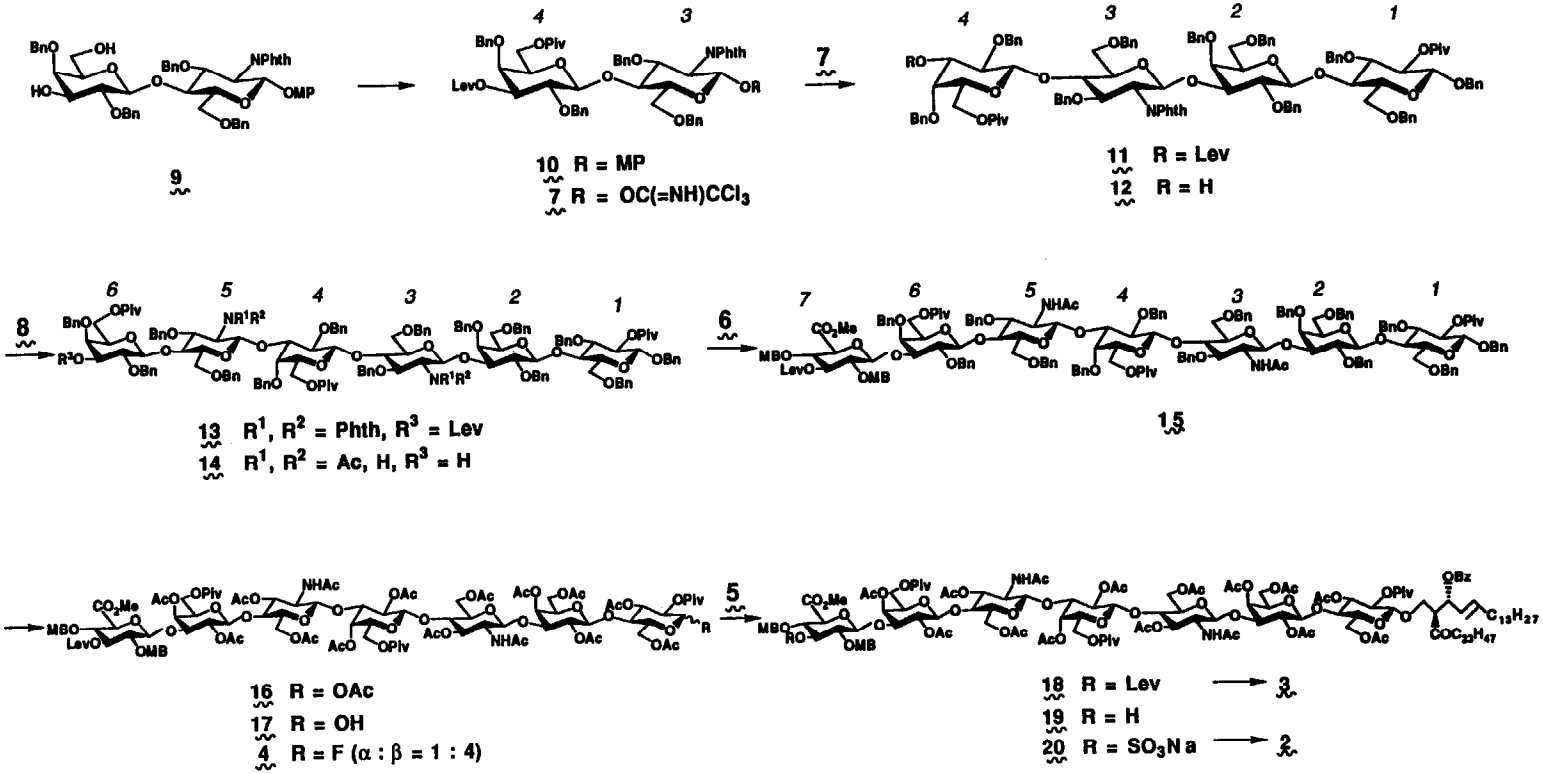
9
10 R = MP
7 R = OC(=NH)CCl3
11 R = Lev
12 R = H
13 R1, R2 = Phth, R3 = Lev
14 R1, R2 = Ac, H, R3 = H
15
16 R = OAc
17 R = OH
4 R = F (α : β = 1 : 4)
18 R = Lev
19 R = H
20 R = SO3Na
3
2

Scheme 2

Reference and Notes

1 Part 79 in the series "Synthetic Studies on Cell-Surface Glycans". For part 78, see C. Murakata and T. Ogawa, submitted for publication.

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9 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. **2**: R_F 0.42 in 30:15:4 $CHCl_3$-MeOH-H_2O; δ_H (49:1 DMSOd_6-D_2O, 60°) 5.605 (dt, 15.4 and 7.0 Hz, 5^{Cer}), 5.423 (dd, 15.4 and 7.0 Hz, 4^{Cer}), 4.730 (br. signal, 1^3 and 1^5), 4.553 (d, 7.7 Hz, 1^7), 4.359 (d, 7.3 Hz, 1^6), 4.332 (br. signal, 1^2 and 1^4), 4.221 (d, 7.7 Hz, 1^1), 4.039 (t, 8.8Hz, 3^7), 1.831 (s, NAc), 0.856 (t, 7.0 Hz, 2 x CH_2CH_3). **3**: R_F 0.48 in 30:15:4 $CHCl_3$-MeOH-H_2O; δ_H (49:1 DMSOd_6-D_2O, 60°) 5.558 (dt, 15.4 and 7.0 Hz, 5^{Cer}), 5.375 (dd, 15.4 and 7.0 Hz, 4^{Cer}), 4.688 (2d, 8.1 Hz, 1^3 and 1^5), 4.369 (d, 7.7 Hz, 1^7), 4.318 (bd, 7.7 Hz) and 4.283 (2bd, 7.7 Hz, 1^2, 1^4 and 1^6), 4.174 (d, 7.7 Hz, 1^1), 1.833 (s, NAc), 0.856 (t, 7.0 Hz, 2 x CH_2CH_3). **4**: δ_H 5.649 (dd, 0.2 H, 2.7 and 53.4 Hz, $1^1\alpha$), 5.311 (dd, 0.8 H, 5.8 and 52.8 Hz, $1^1\beta$), 3.678 (s, OMe), 2.410 and 2.402 (2s, 2 x Ph*Me*). **7**: $[\alpha]_D$ +65.1° (c 0.9); δ_H 8.521 (s, =NH), 6.386 (d, 8.2 Hz, 1^3), 4.808 (dd, 3.1 and 10.1 Hz, 3^4), 2.143 (s, COMe), 1.198 (s, COCMe3). **10**: $[\alpha]_D$ +55.3° (c 0.9); δ_H 5.602 (d, 8.2 Hz, 1^3), 4.843 (dd, 3.1 and 10.1 Hz, 3^4), 4.487 (d, 7.6 Hz, 1^4), 3.695 (s, OMe), 2.140 (s, COMe), 1.200 (s, COCMe3). **11**: $[\alpha]_D$ -0.7° (c 1.1); δ_H 5.365 (d, 8.5 Hz, 1^3), 5.011 (dd, 7.9 and 9.2 Hz, 2^1), 4.841 (dd, 3.4 and 9.2 Hz, 3^4), 2.143 (s, COMe), 1.133 and 1.082 (2s, 2 x COCMe3). **12**: $[\alpha]_D$ 0° (c 1.3); δ_H 5.379 (d, 8.5 Hz, 1^3), 5.012 (dd, 8.2 and 9.5 Hz, 2^1), 1.144 and 1.082 (2s, 2 x COCMe3). **13**: $[\alpha]_D$ -0.4° (c 0.8); δ_H 5.387 and 5.191 (2d, 8.3 Hz, 1^3 and 1^5), 4.993 (dd, 8.1 and 9.5 Hz, 2^1), 4.819 (dd, 3.2 and 10.0 Hz, 3^6), 4.267 (2d, 8.1 Hz, 1^1 and 1^2 or ^{4}), 2.146 (s, COMe), 1.139 and 1.072 (2s, 2 x COCMe3). **14**: $[\alpha]_D$ -1.8° (c 0.9); δ_H 5.125 (dd, 7.9 and 9.5 Hz, 2^1), 1.423 and 1.391 (2s, 2 x Ac), 1.143, 1.113 and 1.073 (3s, 3 x COCMe3). **15**: $[\alpha]_D$ -13.1° (c 1.3); δ_H 5.626 (t, 9.5 Hz, 3^7), 5.506 (t, 9.5 Hz, 4^7), 5.469 (dd, 7.6 and 9.5 Hz, 2^7), 5.186 (d, 7.6 Hz, 1^7), 5.124 (d, 7.9 Hz, N*H*Ac), 5.083 (dd, 7.9 and 9.8 Hz, 2^1), 5.057 (d, 8.2 Hz, N*H*Ac), 4.189 (d, 9.5 Hz, 5^7), 3.666 (s, OMe), 2.418 and 2.327 (2s, 2 x Ph*Me*), 1.885 (s, COMe), 1.393 and 1.385 (2s, 2 x NAc), 1.134, 1.113 and 1.058 (3s, 3 x COCMe3). **16**: α- and β-anomer in a ratio of 1:1; δ_H 6.288 (d, 0.5 H, 3.7 Hz, $1^1\alpha$), 5.694 (d, 0.5 H, 8.2 Hz, $1^1\beta$), 3.679 (s, OMe), 2.410 and 2.402 (2s, 2 x Ph*Me*). **17**: δ_H 3.676 (s, OMe), 2.411 and 2.403 (2s, 2 x Ph*Me*). **18**: $[\alpha]_D$ +10.5° (c 1.1); δ_H 5.870 (dt, 15.0 and 7.6 Hz, 5^{Cer}), 4.819 (d, 7.3 Hz, 1^7), 4.652 and 4.599 (2d, 7.6 and 7.3 Hz, 1^3 and 1^5), 4.412, 4.366, 4.333, and 4.284 (4d, 8.0 Hz, 1^1, 1^2, 1^4 and 1^6), 3.679 (s, OMe), 2.409 and 2.401 (2s, 2 x Ph*Me*), 1.212, 1.206 and 1.141 (3s, 3 x COCMe3), 0.879 (t, 7.0 Hz, 2 x CH_2CH_3). **19**: $[\alpha]_D$ +11.6° (c 1.0); δ_H 5.870 (dt, 15.3 and 7.6 Hz, 5^{Cer}), 5.535 (t, 7.6 Hz, 3^{Cer}), 4.884 (d, 6.4 Hz, 1^7), 4.652 and 4.610 (2d, 7.6 Hz, 1^3 and 1^5), 4.408, 4.375, 4.361 nad 4.276 (4d, 7.0~8.2 Hz, 1^1, 1^2, 1^4 and 1^6), 3.698 (s, OMe), 2.418 and 2.411 (2s, 2 x Ph*Me*), 1.214, 1.209 and 1.141 (3s, 3 x COCMe3), 0.879 (t, 7.0 Hz, 2 x CH_2CH_3). **20**: $[\alpha]_D$ +16.5° (c 0.6, MeOH); δ_H (CD_3OD) 5.883 (dt, 15.3 an 7.0 Hz, 5^{Cer}), 5.568 (t, 7.0 Hz, 3^{Cer}), 5.488 (dd, 15.3 and 7.0 Hz, 4^{Cer}), 5.339 (t, 9.5 Hz, 4^7), 5.191 (dd, 7.9 and 9.5 Hz, 2^7), 4.976 (t, 9.5 Hz, 3^7), 3.628 (s, OMe), 2.403 and 2.390 (2s, 2 x Ph*Me*), 1.235, 1.229 and 1.153 (3s, 3 x COCMe3), 0.894 (t, 7.0 Hz, 2 x CH_2CH_3).

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