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SYNTHESIS OF A DERMATAN SULFATE HEXASACCHARIDE THAT ACTIVATES HEPARIN COFACTOR II

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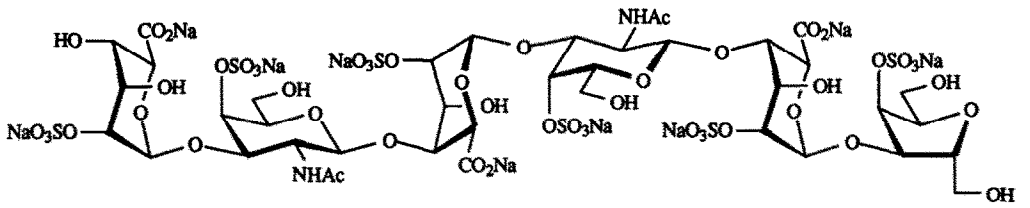
Abstract. Synthesis of α-L-IdoA(2-SO_3)-{(1→3)-β-D-GalNAc(4-SO_3)-(1→4)-α-L-IdoA(2-SO_3)-}$_2$-(1→3)-D-GalNAc(4-SO_3), was carried out for the first time in a regio- and stereocontrolled manner by use of the trichloroacetimidate technology for the carbohydrate chain extension.

Heparin cofactor II (HCII) and antithrombin III (ATIII) are present in plasma at micromolar concentrations and inhibit serine proteases involved in blood coagulation. Although ATIII inhibits factors IXa, Xa and thrombin, HCII inhibits only thrombin. Dermatan sulfate specifically increases the rate of inhibition of thrombin by HCII about 10^3 fold but not the rate of inhibition by ATIII[2].



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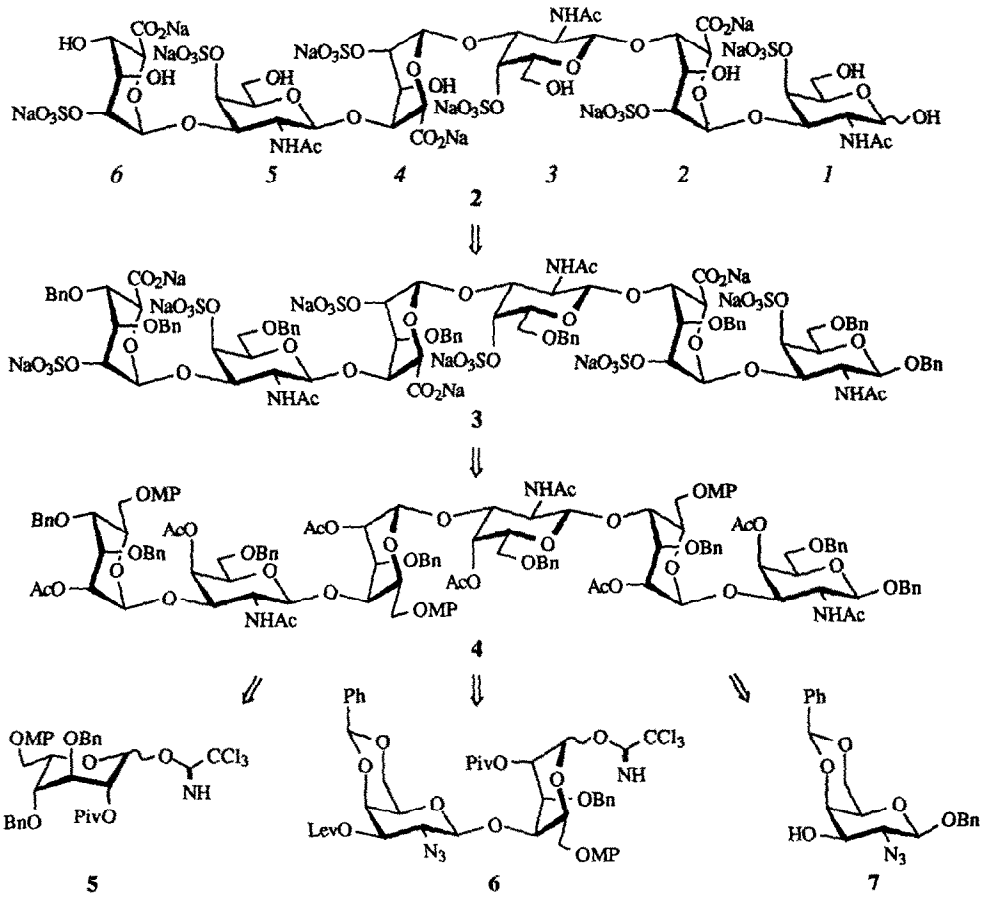
In 1990, Maimone and Tollefsen reported[3] a hexasaccharide **1** derived from pig skin dermatan sulfate as a minimum required sequence that binds HCII with high affinity and increases the rate of thrombin inhibition by about 10^2 fold. According to the reaction sequence taken by Maimone and Tollefsen[3] for the partial degradation of pig skin dermatan sulfate, the natural sequence of hexasaccharide that corresponds to **1** can be deduced as **2**. As part of our project[4] on the synthesis of functional domains of proteoglycans, we chosed **2** as a synthetic target. It is to be noted that besides our approach elegant synthetic approaches to the glycosaminoglycan chains for chondroitin and dermatan sulfate have recently been reported by Sinaÿ and co-workers[5]. Retrosynthetic analysis of **2** is depicted in scheme-1. A completely protected precursor of **2** can be designed as **3**, that in turn may be obtainable from **4** through oxidation and replacement of functional groups. The central tetrasaccharide part of **4** may be constructed by the repeated use of imidate **6**, while the non-reducing and the reducing



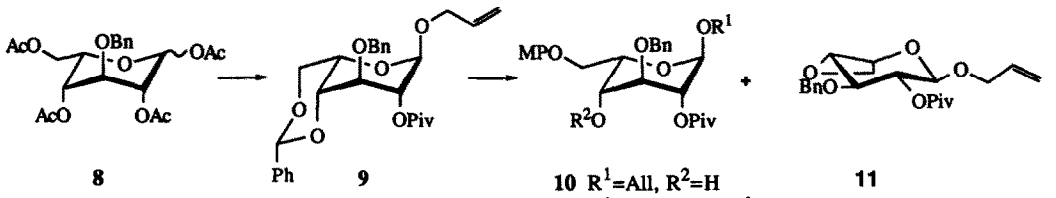
Scheme-1 (MP=4-MeOPh, Piv=tBuCO, Lev=$COCH_2CH_2COCH_3$)

end monosaccharides of **4** can be designed as **5** and **7**, respectively. Since **7** was already known[6], we first describe the preparation of two glycosyl donors **5** and **6**.

Readily obtainable L-Ido derivative **8**[7] was converted to **9**[8] in 4 steps: *1*) $Bu_3SnOCH_2CH{=}CH_2$, $SnCl_4$ in $(ClCH_2)_2$ at 0-20°; *2*) NaOMe in MeOH; *3*) $PhCH(OMe)_2$, p-TsOH•H_2O in THF; *4*) tBuCOCl, DMAP in pyridine (Py); 54% overall. Treatment of **9** with 90% aq.CF_3COOH gave 90% of diol which was then submitted to Mitsunobu reaction (p-MeOPhOH, PPh_3, DEAD in CH_2Cl_2[9]) to give **10**[8] (48%) and **11**[8] (42%). Compound **10** was converted into a 1:1 mixture of α and β-trichloroacetimidate **5** in 2 steps; *1*) $[Ir(COD)(Ph_2MeP)_2]PF_6$, H_2, THF, then I_2 in H_2O[10]; *2*) Cl_3CCN[11], DBU in CH_2Cl_2; 88% overall.

Glycobiosyl glycosyl donor **6** was prepared starting from a readily available compound **12**[12]. Conversion of **12** into **13**[8] was carried out in 3 steps: *1*) $(Lev)_2O$ in Py; *2*) $[Ir(COD)(Ph_2MeP)_2]PF_6$, H_2, THF, then I_2-H_2O-$NaHCO_3$; *3*) Cl_3CCN, DBU in CH_2Cl_2; 89% overall. TMSOTf promoted glycosylation of **10** with 1.4 equivalents of **13** in the presence of powdered molecular sieves 4A (MS4A) in PhMe at -75~-25°

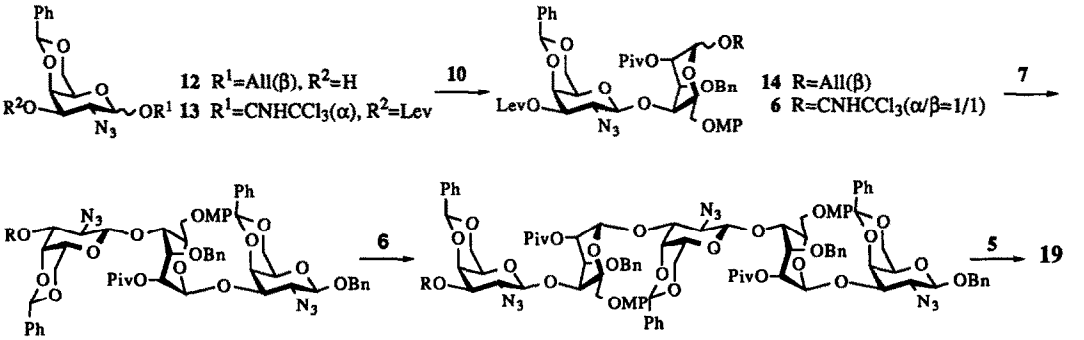
afforded **14**[8] in 83% along with 10% of the α-anomer[8]. Compound **14** was then transformed into **6**[8] in two steps as described above.



8 **9** **10** R^1=All, R^2=H **11**
5 R^1=$CNHCCl_3$, R^2=Bn
(α/β = 1/1)

Scheme-2

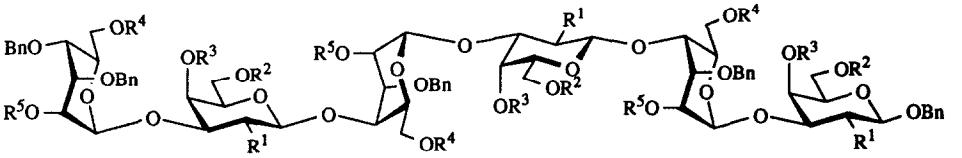
Having the designed glycosyl donors **5** and **6** as well as a glycosyl acceptor **7** in our hands, we now carried out the couplings between these synthons. The glycan chain extension was started from the reducing end. Thus TMSOTf promoted coupling of **7** with one equivalent of **6** in CH_2Cl_2 at -25° gave 86% of **15**[8]. Lev group of **15** was removed by treatment with NH_2NH_2•AcOH[13] in 1:5 PhMe-EtOH to afford 95% of **16**[8] which was then glycosylated with 1.5 equivalents of **6** in the presence of $tBuMe_2SiOTf$ in CH_2Cl_2 for 2.5 h at -23° to give 87% of pentasaccharide derivative **17**[8]. Addition of the non-reducing end L-Ido residue was achieved by use of the glycosyl donor **5**. Treatment of **17** with NH_2NH_2•AcOH in 1:5 PhMe-EtOH gave 99% of **18** that was glycosylated with 2.5 equivalents of **5** in the presence of $tBuMe_2SiOTf$ in CH_2Cl_2 to yield 99% of hexasaccharide derivative **19**. Regioselective ring opening of benzylidene group of **19** and subsequent acetylation was carried out in 3 steps to give **20**[8]: *1*) $NaBH_3CN$-MS4A in THF-HCl[14]; *2*) NaOMe in 1:1 THF-MeOH; *3*) Ac_2O, DMAP in Py; 64% overall. Transformation of **20** into **21** was achieved in two steps: *1*) AcSH and Py 24h at 25°[15]; *2*) CAN[16] in 4:1 CH_3CN-H_2O 30min at 0°; 63% overall. Oxidation of the primary hydroxyl groups of **21** into carboxylic acids was carried out in two steps and purification of the product was



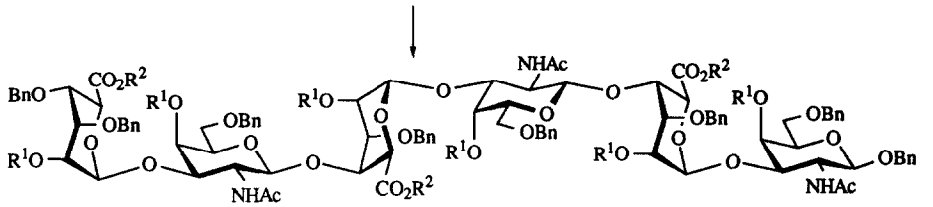
12 R^1=All(β), R^2=H
13 R^1=$CNHCCl_3$(α), R^2=Lev
14 R=All(β)
6 R=$CNHCCl_3$(α/β=1/1)
15 R=Lev
16 R=H
17 R=Lev
18 R=H
19

Scheme-3

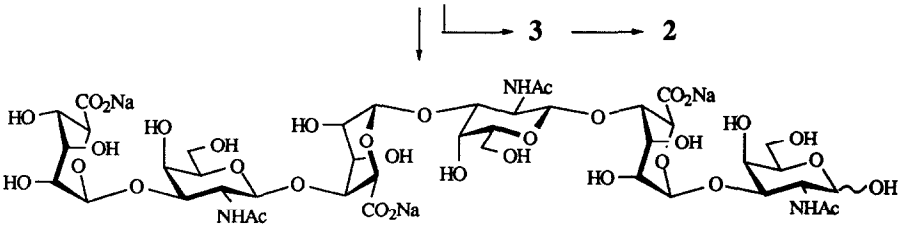
achieved after esterification to give **22**[8]: *1*) $(COCl)_2$-DMSO in CH_2Cl_2 1h at -78°, then iPr_2EtN; *2*) $NaClO_2$[17], $NaH_2PO_4 \cdot 2H_2O$ in 1:2 2-methyl-2-butene and ^tBuOH; *3*) CH_2N_2 in 1:2 MeOH-EtOAc; 39% overall. De-O-acetylation and saponification of methyl esters of **22** was carried out by treatment with NaOH in $CHCl_3$-MeOH-H_2O as described by Petitou et al[18] to give 88% of a key intermediate **23**[8] which gave 84% of free hexasaccharide **24**[8] by hydrogenolysis of benzyl groups in the presence of 10% Pd-C followed by purification through Sephadex G-10 in H_2O. Finally **23** was converted into target hexasaccharide **2**[8] via **3**[8] in two steps: *1*) $Et_3N \cdot SO_3$ in DMF at 50°, then Dowex 50W-X8 (Na^+) in 8:1 MeOH-H_2O; *2*) 10% Pd-C, H_2 in 4:1 MeOH-H_2O 20h at 25°, then in 1:3 MeOH-H_2O 19h at 25°, then Sephadex G-10 in H_2O; 50% overall.



	R^1	R^2	R^3	R^4	R^5
19	N_3	CHPh		MP	Piv
20	N_3	Bn	Ac	MP	Ac
21	NHAc	Bn	Ac	H	Ac



	R^1	R^2
22	Ac	Me
23	H	Na



24

Scheme-4

In summary, a hexasaccharide **2** that is a minimum sequence[19] of dermatan sulfate necessary for the binding to HCII was synthesized for the first time in a regio- and stereocontrolled manner by employing the glycobiosyl donor **6** as a key synthetic block for the carbohydrate chain extension.

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8. 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*. All compounds described below gave correct data for either combustion analysis or MS. **2**: R_F 0.39 in 4:4:2:1 MeOH-AcOH-H_2O-Me_2CO; δ_H (D_2O) at 22°, 5.195 (bs, two of $1^{2,4,6}$), 5.192 (d, 4.4Hz, $1^1\alpha$), 5.160 (bs, one of $1^{2,4,6}$): at 50°, 4.835 and 4.828 (2d, 2.2Hz, two of $4^{1,3,5}$), 4.803 and 4.780 (2d, 7.7 and 8.0Hz, $1^{3,5}$), 4.776 (d, 8.4Hz, $1^1\beta$), 4.729 (d, 2.9Hz, one of $4^{1,3,5}$), 4.661 (d, 1.4Hz, two of $5^{2,4,6}$), 4.638 (d, 2.2Hz, one of $5^{2,4,6}$), 4.215 (dd, 2.6 and 6.6Hz, one of $2^{2,4,6}$), 4.196 (dd, 3.3 and 7.7Hz, one of $2^{2,4,6}$); FABMS, $(M-Na)^-$ 1809, $(M-2Na+H)^-$ 1787, $(M-3Na+2H)^-$ 1765, $(M-4Na+3H)^-$ 1743. **3**: $[\alpha]_D$ -35.9° (c 0.3 in MeOH); R_F 0.54 in 4:4:1 $CHCl_3$-MeOH-H_2O; δ_H (CD_3OD) 2.009, 1.991 and 1.946 (3s, 3NHAc). **5**: R_F 0.55 in 10:1 PhMe-EtOAc; δ_H 8.614 and 8.567 (2s, N*H*, in 1:1 ratio), 6.428 (d, 0.5H, 2.6Hz, 1α), 6.259 (bs, 0.5H, 1β), 3.771 and 3.767 (2s, OMe, 1:1 ratio), 1.193 and 1.163 (2s, ^{t}Bu, 1:1 ratio). **6**: R_F 0.46 and 0.53 in 2:1 PhMe-EtOAc; δ_H (α/β=1/1), 8.633 and 8.613 (2s, C=N*H*), 6.273 (d, 2.0Hz, $1^1\alpha$), 6.239 (s, $1^1\beta$), 4.250 and 4.197 (2d, 8.3Hz, 1^2). **9**: $[\alpha]_D$ -77.6° (c 3.1); R_F 0.48 in 2:1 hexane-EtOAc; δ_H 5.522 (s, PhC*H*), 5.030 (dd, 2.0 and 2.6Hz, 2), 5.002 (bs, 1), 1.097 (s, ^{t}Bu). **10**: $[\alpha]_D$ -35.6° (c 1.0); R_F 0.29 in 3:1 hexane-EtOAc; δ_H 5.054 (dd, 1.0 and 1.3Hz, 2), 4.857 (bs, 1), 3.761 (s, *Me*OPh), 1.226 (s, ^{t}Bu). **11**: $[\alpha]_D$ -69.1° (c 1.7); R_F 0.37 in 3:1 hexane-EtOAc; FABMS $(M+1)^+$ 377; δ_H 4.855 (d, 7.6Hz, 1), 1.206 (s, ^{t}Bu). **13**: $[\alpha]_D$ +143.5° (c 0.2); R_F 0.38 in 3:1 PhMe-EtOAc; δ_H 8.756 (s, C=N*H*), 6.583 (d, 3.3Hz, 1), 5.561 (s, PhC*H*), 5.373 (dd, 3.3 and 10.9Hz, 3), 4.300 (dd, 3.3 and 10.9Hz, 2), 2.131 (s, Lev). **14**: $[\alpha]_D$ -1.6° (c 1.4); R_F 0.41 in 3:1 PhMe-EtOAc; δ_H 5.446 (s, PhC*H*), 4.959 (bs, 2^1), 4.868 (s, 1^1), 4.614 (dd, 3.3 and 10.6 Hz, 3^2), 4.179 (d, 7.9Hz, 1^2), 3.771 (s, *Me*O), 2.113 (s, Lev), 1.098

(s, ^{t}Bu); δ_C 104.2 (C-1^2), 101.0 (PhCH), 97.8 (C-1^1), 56.2 (*Me*O). α-anomer of **14**: $[\alpha]_D$ +48.1° (c 1.8); R_F 0.47 in 3:1 PhMe-EtOAc; δ_H 5.206 (d, 3.4Hz, 1^1), 4.827 (d, 3.4Hz, 1^2). **15**: $[\alpha]_D$ -2.8° (c 0.3); R_F 0.47 in 2:1 PhMe-EtOAc; δ_H 5.056 (s, 1^2), 4.308 (d, 8.3Hz, 1^1), 4.169 (d, 7.9Hz, 1^3); δ_C 104.1 (C-1^3), 101.3 (C-1^2), 100.1 (C-1^1), 100.5 and 100.2 (2PhCH). **16**: $[\alpha]_D$ -13.7° (c 1.3); R_F 0.39 in 2:1 PhMe-EtOAc; δ_H 5.632 (s, PhC*H*), 5.500 (s, PhC*H*), 5.133 (bs, 2^2), 5.063 (s, 1^2), 4.310 (d, 8.3Hz, 1^1), 4.193 (d, 6.3Hz, 1^3), 3.747 (s, *Me*O), 1.160 (s, ^{t}Bu). **17**: $[\alpha]_D$ -6.9° (c 0.6); R_F 0.47 in 2:1 PhMe-EtOAc; δ_H 5.132 and 5.081 (2bs, 2^2 and 2^4), 5.035 (bs, 1^2 and 1^4), 4.651 (dd, 3.5 and 10.8Hz, 3^5), 4.303 (d, 8.0Hz, 1^1), 4.189 (d, 8.0Hz, 1^4), 4.046 (d, 8.3Hz, 1^3), 2.119 (s, Lev); δ_C 104.0 (C-1^5), 103.9 (C-1^3), 101.4 and 101.3 (C-$1^{2,4}$), 100.1 (C-1^1), 100.4, 100.2 and 99.7 (3PhCH). **18**: $[\alpha]_D$ -19.6° (c 0.9); R_F 0.39 in 2:1 PhMe-EtOAc; δ_H 5.648, 5.577, and 5.508 (3s, 3PhC*H*), 5.152 and 5.084 (2bs, $2^{2,4}$), 5.045 (s, $1^{2,4}$), 4.304 (d, 8.3Hz, 1^1), 3.744 and 3.736 (2s, 2*Me*O), 1.170 and 1.010 (2s, 2^{t}Bu). **19**: $[\alpha]_D$ -17.9° (c 0.8); R_F 0.52 in 3:1 PhMe-EtOAc; δ_H 5.644, 5.580 and 5.532 (3s, 3PhC*H*), 5.142, 5.100, and 5.078 (3bs, $2^{2,4,6}$), 5.050, 5.034, and 5.019 (3bs, $1^{2,4,6}$), 4.300 (d, 8.2Hz, 1^1), 4.080 (d, 8.0Hz, 1^5), 4.042 (d, 8.2Hz, 1^3); δ_C 103.9 (C-$1^{3,5}$), 101.4 (C-$1^{2,4,6}$), 100.1 (C-1^1), 100.2, 99.6 and 99.5 (3PhCH). **20**: $[\alpha]_D$ -47.2° (c 4.3); R_F 0.59 in 2:1 PhMe-EtOAc; δ_H 5.540, 5.524, and 5.465 (3d, 3.1Hz, $4^{1,3,5}$), 5.102, 5.062, and 5.062 (3bs, $2^{2,4,6}$), 4.996, 4.904, and 4.892 (3bs, $1^{2,4,6}$), 4.375 (d, 8.2Hz, 1^1), 4.002 and 3.978 (2d, 7.9Hz, $1^{3,5}$), 2.065, 2.047, 2.043, 1.820, 1.741, and 1.689 (6s, 6Ac). **21**: $[\alpha]_D$ -17.0° (c 0.1); R_F 0.30 in 5:3 Me_2CO-$CHCl_3$; δ_H 2.043, 2.031, 2.031, 1.981, 1.942, 1.865, 1.765, 1.684, and 1.645 (9s, 9Ac). **22**: $[\alpha]_D$ -58.5° (c 0.1); R_F 0.63 in 5:3 Me_2CO-$CHCl_3$; δ_H 3.742, 3.739, and 3.695 (3s, 3*Me*O), 2.036, 2.024, 2.006, 1.959, 1.953, 1.886, 1.754, 1.707, and 1.653 (9s, 9Ac). **23**: $[\alpha]_D$ -43.9° (c 0.6 in MeOH); R_F 0.57 in 10:6:1 $CHCl_3$-MeOH-H_2O; δ_H (CD_3OD) 1.948, 1.867 and 1.849 (3s, 3NH*Ac*). **24**: R_F 0.56 in 4:4:2:1 MeOH-AcOH-H_2O-Me_2CO; δ_H (D_2O) at 25°, 5.194 (d, 0.5H, 3.4Hz, $1^1\alpha$), 4.929 and 4.870 (2d, 3.4Hz, two of $1^{2,4,6}$), 4.688 (d, 0.5H, 8.8Hz, $1^1\beta$), 4.584 (d, 8.8Hz, $1^{3,5}$), 2.054, 2.041, and 2.038 (3s, 3NH*Ac*); at 50° 4.819 (d, 5.1Hz, one of $1^{2,4,6}$); FABMS $(M\text{-}Na)^-$ 1198, $(M\text{-}2Na\text{+}H)^-$ 1176, $(M\text{-}3Na\text{+}2H)^-$ 1154.

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19. Private communication from Dr. D.M. Tollefsen: Synthetic **2** showed same degree of biological activity as the natural sample. The detail will be published separately.

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