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SYNTHESIS OF A HEPARIN PENTASACCHARIDE FRAGMENT WITH A HIGH AFFINITY FOR ANTITHROMBIN III EMPLOYING CELLOBIOSE AS A KEY STARTING MATERIAL

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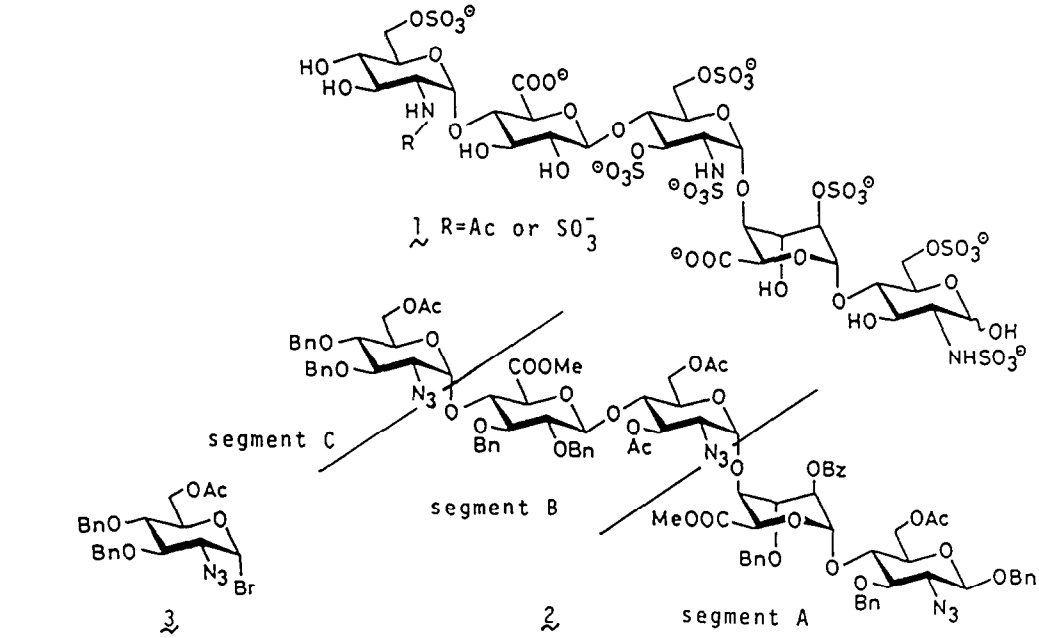
Abstract: The methodology for regio- and stereoselective modifications and transformations of cellobiose was established, and applied to the total synthesis of a heparin pentasaccharide fragment having a high affinity for antithrombin III.

Our recent findings[1,2] about the regioselective modifications of cellobiose, a disaccharide readily obtainable by enzymic or chemical degradation of cellulose, prompted us to utilize them for the preparation of complex mucopolysaccharides. Heparin is a sulfated mucopolysaccharide with potent anticoagulant activities that have been thought to be mediated by the plasma protein, antithrombin III (AT III).[3] The pentasaccharide **1** (R=Ac) was proposed by Lindahl et al.[4] in 1982 as the AT III-binding sequence of heparin. In 1984, Sinaÿ et al.[5] performed the total synthesis of **1** ($R=SO_3^-$), using five monosaccharide derivatives as building blocks and found that the association constant between **1** ($R=SO_3^-$) and AT III was the same order of magnitude as that of high-affinity heparin.[6]

This communication describes the second total synthesis of **1** ($R=SO_3^-$), employing cellobiose as a common starting material for preparation of two key synthons (**21** and **14**). The pentasaccharide **2** might be regarded as a synthetic equivalent of **1** because a wide variety of functional groups in **1** was all present in **2** as suitably protected forms ($-OSO_3^-$ → -OAc or -OBz, -OH → -OBn, $-COO^-$ → -COOMe) or a corresponding precursor ($-NHSO_3^-$ → $-N_3$). Two disaccharide segments, A and B, were derived from **21** and **14**, respectively, and segment C from **3** prepared by Paulsen et al.[7] We have succeeded in the derivation of cellobiose to the versatile intermediate **4** and its 2',3'-di-O-benzyl ether **5**.[1] Furthermore, suitably protected azido sugar **9** has been prepared from **5** in several steps of reactions.[2] For preparation of the synthon **14**, **9** underwent acetolysis (CF_3CO_2H-Ac_2O)[7] to give a 70% yield of α acetate **13**,[8] mp. 139°, $[\alpha]_D^{23}$ +38°; δ ($CDCl_3$, 400 MHz) ppm: 2.04 (s, Ac), 2.22 (s, 2×Ac), 3.54 (dd, J=3.66 and 10.74 Hz, H-2), 3.71 (s, $CO_2\underline{CH}_3$), 4.33 (d, J=7.81 Hz, H-1'), 5.45 (dd, J=8.79 and 10.74 Hz, H-3), 6.23 (d, J=3.66 Hz, H-1). The glycosyl bromide **14** was prepared from **13** by treatment with $TiBr_4$ in CH_2Cl_2-EtOAc and used for the coupling reaction without characterization.

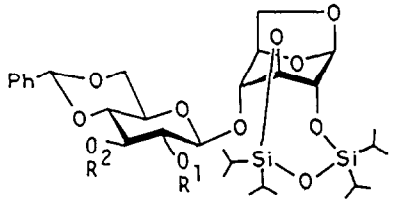
Preparation of the other synthon **21** from **4** required (i) an efficient discrimination between the 2'- and 3'-hydroxyl groups and (ii) the configurational inversion at C-5' position



1 R=Ac or SO_3^-

3

2



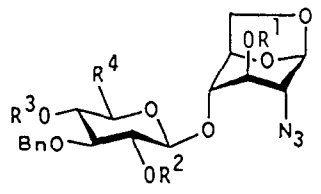
4 $R^1=R^2=H$

5 $R^1=R^2=Bn$

6 $R^1,R^2=Bu_2Sn<$

7 $R^1=H$, $R^2=Bn$

8 $R^1=Bz$, $R^2=Bn$

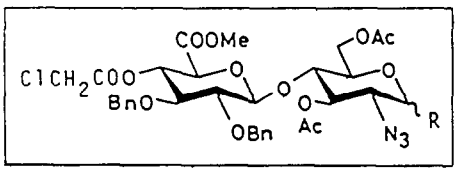


9 $R^1=Ac$, $R^2=Bn$, $R^3=ClCH_2CO$, $R^4=CO_2Me$

10 $R^1=Bn$, $R^2=Bz$, $R^3=H$, $R^4=CH_2OH$

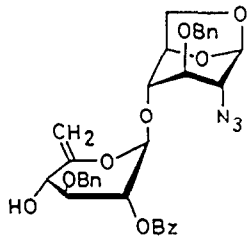
11 $R^1=Bn$, $R^2=Bz$, $R^3=H$, $R^4=CH_2OTs$

12 $R^1=Bn$, $R^2=Bz$, $R^3=H$, $R^4=CH_2I$

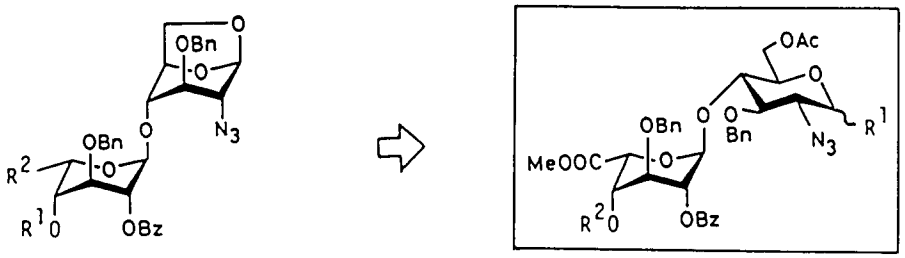


13 R=OAc

14 R=Br



15



16 = R^1=H, R^2=CH_2OH

17 R^1=$ClCH_2CO$, R^2=CH_2OTr

18 R^1=$ClCH_2CO$, R^2=CO_2Me

19 R^1=OAc, R^2=$ClCH_2CO$

20 R^1=OBn, R^2=$ClCH_2CO$

21 R^1=β-OBn, R^2=H

(D-gluco → L-ido). After several unsuccessful attempts, selective benzylation of the 3'-hydroxyl group of 4 was achieved via the dibutylstannylated intermediate 6. Thus, treatment of 4 with Bu_2SnO in toluene and subsequent alkylation with BnBr in the presence of Bu_4NI[9] at 100° gave a 97% yield of 7,[8] mp. 165°, $[\alpha]_D^{22}$ -59°; δ 3.62 (m, H-2'), which was benzoylated to afford 8,[8] mp. 90°, $[\alpha]_D^{27}$ -19°; δ 5.25-5.30 (m, H-2'). Compound 10,[8] $[\alpha]_D^{22}$ -7.3°; δ 4.68 (d, J=8.06 Hz, H-1'), 5.26 (dd, J=8.06 and 9.28 Hz, H-2'), was prepared from 8 similarly to the preparation of 9[2] (53% overall yield); i.e., a) deprotection of silyl ether with F^- in aqueous media[1] (mp. 213°, $[\alpha]_D^{22}$ -2.6°), b) tosylation of the 2-hydroxyl group (mp. 168°, $[\alpha]_D^{22}$ -0.64°), c) epoxidation with NaH (mp. 75°, $[\alpha]_D^{28}$ +6.5°), d) opening of the epoxy ring with N_3^- (mp. 175°, $[\alpha]_D^{22}$ -0.77°), e) benzylation[1] of the 3-hydroxyl group (mp. 148°, $[\alpha]_D^{22}$ -0.64°), and f) removal[2] of the benzylidene group. For the configurational inversion at C-5', hydroboration reaction[10] was to be applied. The key substrate 15,[8] $[\alpha]_D^{27}$ -4.2°; δ 5.06 (d, J=2.69 Hz, H-1'), 5.36 (dd, J=2.69 and 5.37 Hz, H-2'), was prepared from 10 as follows. Tosylation of 10 to give 11,[8] mp. 143°, $[\alpha]_D^{27}$ +5.7°, was followed by treatment with NaI, giving 12,[8] mp. 146°, $[\alpha]_D^{28}$ -22°, which was treated with DBU to afford 15 in 80% overall yield. Hydroboration of 15 with naked borane prepared from Bu_4NBH_4 and MeI[10] in CH_2Cl_2 followed by oxidative work-up with H_2O_2 and aq. $NaHCO_3$ gave a 19% yield of L-ido derivative 16,[8] $[\alpha]_D^{23}$ -20°; δ 5.22 (s, H-1'), 5,32 (br t, J=1.22 Hz, H-2'), together with a 38% yield of D-gluco derivative 10, which was recycled for the preparation of 15. Compound 16 was successively treated with TrCl and $(ClCH_2CO)_2O$ to give a 97% yield of 17,[8] $[\alpha]_D^{22}$ -2.33°; δ 5.11 (br s, H-4'), 5.28 (s, H-1'), 5.30 (br s, H-2'). Treatment of 17 with CrO_3-3.5M H_2SO_4 in acetone according to the Sinaÿ's procedure[5] for O-detritylation and the subsequent oxidation in one pot followed by esterification with CH_2N_2 afforded a 52% yield of 18,[8] $[\alpha]_D^{22}$ -20°; δ 3.73 (s, CO_2CH_3), 4.92 (d, J=1.95 Hz, H-5'), 5.26 (br s, H-4'), 5.31 (br s, H-2'), 5.38 (s, H-1'). Acetolysis[7] of 18 gave a 94% yield of 19,[8] $[\alpha]_D^{22}$ -7.9°, and bromination ($TiBr_4$) of 19 afforded a glycosyl bromide, which was condensed with BnOH in the presence of $HgBr_2$ and molecular sieves 4A in $ClCH_2CH_2Cl$ to produce a 20% yield of β anomer of 20,[8] $[\alpha]_D^{19}$ -14°; δ 3.49 (dd, J=8.06 and 9.76 Hz, H-2), together with a 20% yield of α anomer of 20,[8] $[\alpha]_D^{20}$ +38°; δ 3.42 (dd, J=3.42 and 10.01 Hz,

H-2). O-Dechloroacetylation of the β anomer of 20 with thiourea afforded a 96% yield of 21,[8] $[\alpha]_D^{25}$ -22°; δ 4.05 (br d, J=10.74 Hz, H-4'), 5.12 (br s, H-2'), 5.26 (s, H-1').

Glycosidation of 21 with 14 was conducted in the presence of AgOTf, molecular sieves 4A, and 2,4,6-collidine[5] in $ClCH_2CH_2Cl$, giving a coupled compound (segment A-B), δ 5.10 (d, J=3.42 Hz, H-1"), in 37% yield. After removal of the chloroacetyl group from the tetrasaccharide with thiourea, the resulting glycosyl acceptor, $[\alpha]_D^{23}$ +12°, was condensed with 3 in the presence of AgOTf, molecular sieves 4A, and 2,4,6-collidine[5] in $ClCH_2CH_2Cl$ for 2 days at room temperature to afford a 70% yield of fully protected pentasaccharide 2, $[\alpha]_D^{23}$ +21°; δ 2.01, 2.03, 2.04, and 2.08 (4×Ac), 3.22 (dd, J=3.66 and 10.70 Hz, H-2"), 3.26 (dd, J=3.66 and 10.50 Hz, H-2""), 3.33 (t, J=9.53 Hz, H-2), 3.69 and 3.74 ($2\times CO_2CH_3$), 4.27 (d, J=9.53 Hz, H-1), 4.34 (d, J=7.81 Hz, H-1'''), 5.10 (d, J=3.66 Hz, H-1"), 5.19 (br t, J=6.34 Hz, H-2'), 5.36 (t, J=10.50 Hz, H-3"), 5.50 (d, J=3.67 Hz, H-1""), 5.60 (d, J=5.86 Hz, H-1').

Deprotections and O- and N-sulfations of 2 were performed stepwise in the same way as the case of trisaccharide homologs reported,[2] giving a sodium salt of 1 ($R=SO_3^-$), δ(D_2O, 35 or 60°): 3.30 (br d, J=7.56 Hz, H-2""), 3.33-3.45 (m, H-2,2",2'''), 3.51 (t, J=8.79 Hz, H-4'''), 3.58 (t, J=8.78 Hz, H-3""), 4.59 (d, J=7.57 Hz, H-1'''), 4.69 (s, H-5'), 4.94 (br s, H-1β), 5.23 (d, J=3.42 Hz, H-1'), 5.47-5.53 (m, H-1α, 1"), 5.60 (br s, H-1""), which strongly binds to AT III (human). The observed association constant[11] between them was 5.2×10^6 M^{-1}, which was in good coincidence with the value reported by Sinaÿ et al.[6]

This total synthesis of 1 exemplified the usefulness and versatility of 1,6-anhydro cellobiose. The experimental details will be reported elsewhere in near future.

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8) Satisfactory spectral data (^{1}H-n.m.r.) and elemental analyses were obtained for all compounds reported herein. Values of $[\alpha]_D$ were measured for $CHCl_3$ solutions. Values of δ (^{1}H-n.m.r.), expressed in ppm downfield from TMS, were measured for $CDCl_3$ solutions unless otherwise specified, and the abbreviation "br" stands for "broad".

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