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Preliminary communication

Total synthesis of a heparin pentasaccharide fragment having high affinity for antithrombin III

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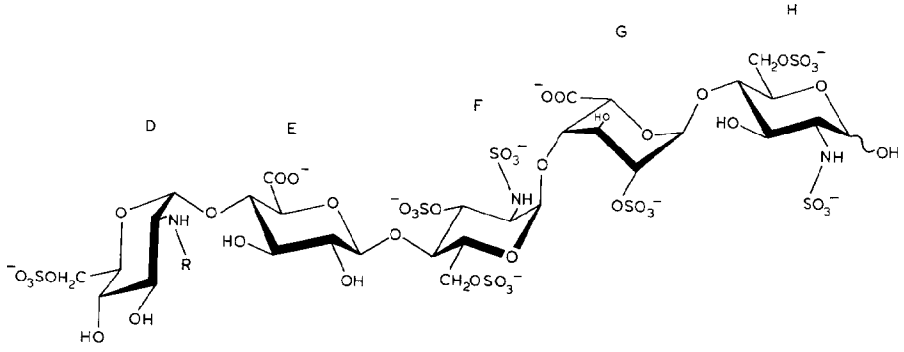
Heparin is a sulfated glucosaminoglycan with a well-known anticoagulant activity[1], and the active molecules have a high affinity for antithrombin III (AT-III), thereby enhancing the effects of this inhibitor on procoagulant proteases. The structures of high-affinity oligosaccharides, prepared from heparin by extraction, partial deaminative cleavage, or partial depolymerisation with bacterial heparinase, have been studied[2]. This work led to the hypothesis[3] that the minimum sequence that binds to AT-III was contained in the pentasaccharide **1**.



1 R = SO_3^- or Ac

In order to confirm this conclusion unambiguously, we have synthesised **1** (R = SO_3^-). The monosulfated α-L-idopyranosyluronate residue of **1** was derived from the orthoacetate **6**, m.p. 67–68°, $[\alpha]_D^{23}$ +19° (*c* 1, chloroform), which was prepared from 3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-glucofuranose[4] (**2**), as illustrated in Scheme 1.

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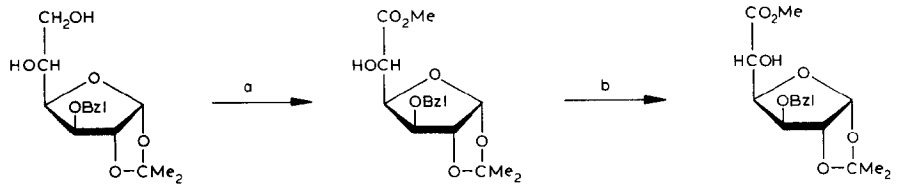

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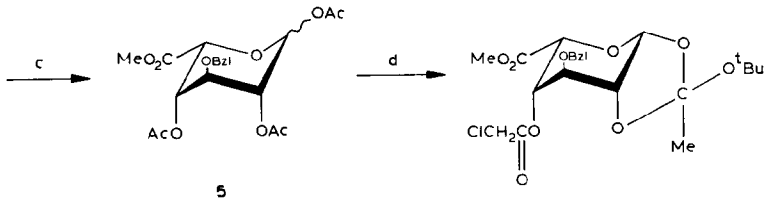

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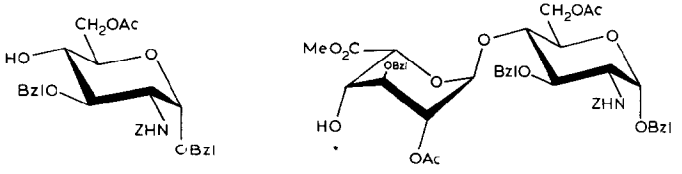
Scheme 1. Reagents: (*a*) TrCl–pyridine; Ac_2O–pyridine; CrO_3–H_2SO_4; CH_2N_2; yield 51%; (*b*) $(CF_3SO_2)_2O$–CH_2Cl_2–pyridine, –10°, 1 h; CF_3CO_2Na–$HCONMe_2$, 80°, 12 h; yield 56%; (*c*) CF_3CO_2H–H_2O (9:1), room temperature, 15 min; Ac_2O–pyridine, room temperature, 5 h; yield 66%; (*d*) $TiBr_4$–CH_2Cl_2–ethyl acetate, room temperature, 24 h; ^tBuOH–2,4,6-trimethylpyridine–CH_2Cl_2, room temperature, 15 h; K_2CO_3–methanol, –20°, 5 h; $CH_2ClCOCl$–CH_2Cl_2–pyridine, –20°, 20 min; yield 47%.

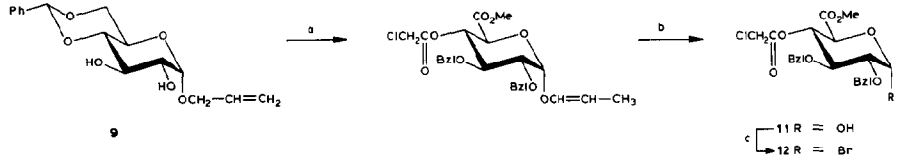
Benzyl 3-*O*-benzyl-2-(benzyloxycarbonyl)amino-2-deoxy-α-D-glucopyranoside[5] was selectively acetylated (*N*-acetylimidazole, CH_2Cl_2, reflux, 30 h) to give the alcohol **7** (71%), m.p. 114–115°, $[\alpha]_D^{23}$ +88° (*c* 1, chloroform). Condensation of **6** (1 equiv.) with **7** (4 equiv.) in refluxing chlorobenzene for 15 min in the presence of 2,6-dimethylpyridinium perchlorate[6] gave, after selective *O*-dechloroacetylation (thiourea, pyridine–methanol, 100°, 30 min), the disaccharide derivative **8** (40% from **6**), m.p. 146–147°, $[\alpha]_D^{23}$ +44° (*c* 1, chloroform).



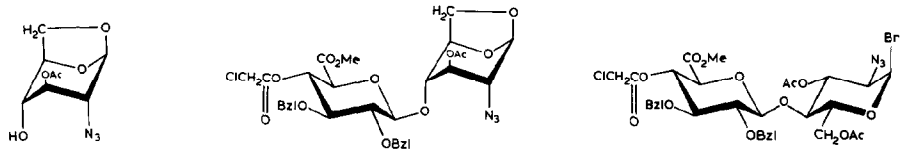
7 8

The synthesis of the bromide **15** was accomplished as follows. The bromide **12**, $[\alpha]_D^{23}$ +82.5° (*c* 1.5, chloroform), was prepared from allyl 4,6-*O*-benzylidene-α-D-glucopyranoside[7] (**9**) according to Scheme 2. The alcohol **13**, $[\alpha]_D^{23}$ –6° (*c* 1, chloroform), was prepared (50%) in three steps from 1,6:2,3-dianhydro-4-*O*-(tetrahydropyran-2-yl-β-D-mannopyranose[8] [(*a*) NaN_3–$HCONMe_2$, 110°, 12 h; (*b*) Ac_2O–pyridine; (*c*) toluene-*p*-sulfonic acid–MeOH, room temperature, 3 h]. Reaction of **12** with **13** (Ag_2CO_3, CH_2Cl_2,

molecular sieve 4 Å, room temperature, 6 days) gave the disaccharide derivative **14** (50%), $[\alpha]_D^{23}$ −17° (*c* 1, chloroform). Acetolysis (Ac_2O, trifluoroacetic acid, 18°, 18 h, yield 86%) of **14** and treatment of the product with titanium tetrabromide (CH_2Cl_2 – ethyl acetate, 17°, 20 h, 50%) then gave **15**.

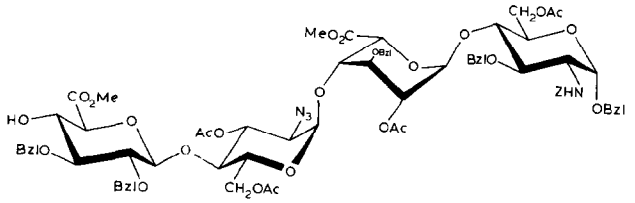


Scheme 2. Reagents: (*a*) $PhCH_2Br$–NaH–$HCONMe_2$, room temperature, 3 h; *p*-TsOH–MeOH–H_2O, 80°, 2 h; TrCl–pyridine; Ac_2O–pyridine; AcOH–H_2O, room temperature, 12 h; CrO_3–H_2SO_4; NaOH–MeOH; ether–CH_2N_2; EtOH–H_2O–PhH, $(PPh_3)_3Rh(I)Cl$, 1,4-diazabicyclo[2.2.2] octane, reflux, 4 h; $CH_2ClCOCl$–pyridine, room temperature, 30 min; (*b*) acetone-H_2O–$HgCl_2$, room temperature, 5 min, yield 80%; (*c*) CH_2Cl_2–$[Me_2N^+CHBr]Br^-$, 0°, 5 h, yield 90%.



The alcohol **8** was condensed with the bromide **15** (silver triflate, 2,4,6-trimethylpyridine, 1,2-dichloroethane, −20° → room temperature, 18 h) to give, after selective *O*-dechloroacetylation, the tetrasaccharide derivative **16** (30%), $[\alpha]_D^{23}$ +62° (*c* 1, chloroform). The known[9] bromide **17** was then condensed with **16** (silver triflate, 2,4,6-trimethylpyridine, 1,2-dichloroethane, −20° → room temperature, 12 h) to give the pentasaccharide derivative **18** (89%), $[\alpha]_D^{23}$ +65° (*c* 1, chloroform). Deprotection and selective *O*- and *N*-sulfations [(*a*) NaOH–$HCONMe_2$–MeOH, 0°, 6 h; (*b*) HCl; (*c*) CH_2N_2; (*d*) SO_3–Me_3N–$HCONMe_2$, 50°, 20 h; (*e*) MeOH–H_2O, H_2, Pd/C, room temperature, 36 h; (*f*) SO_3–Me_3N–NaOH, pH 9, room temperature, 48 h; (*g*) 0.5M NaOH, 2 h] gave the sodium salt of the pentasaccharide **1**, $[\alpha]_D^{23}$ +42° (*c* 1, water). The ^{1}H-n.m.r. spectrum (300 MHz, D_2O, 35°, internal TSP) was in full agreement with the structure: fragment D, δ 5.63 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1), 3.25 (dd, 1 H, $J_{1,2}$ 3.5, $J_{2,3}$ 10.5 Hz, H-2); fragment E, 4.63 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 3.42 (dd, 1 H, $J_{1,2}$ 7.9, $J_{2,3}$ 9.0 Hz, H-2); fragment F, 5.52 (d, 1 H, $J_{1,2}$ 3.4 Hz, H-1), 4.37 (dd, 1 H, $J_{2,3}$ 10.6, $J_{3,4}$ 9 Hz, H-3), 3.97 (t, 1 H, $J_{3,4} = J_{4,5}$ = 9 Hz, H-4), 3.44 (dd, 1 H, $J_{1,2}$ 3.4, $J_{2,3}$ 10.6 Hz, H-2); fragment G, 5.21 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 4.31 (dd, 1 H, $J_{1,2}$ 3.6, $J_{2,3}$ 7 Hz, H-2), 4.17 (dd, 1 H, $J_{2,3}$ 8.2, $J_{3,4}$ 3.6 Hz, H-3), 4.15 (dd, 1 H, $J_{3,4}$ 3.6, $J_{4,5}$ 2.7 Hz, H-4), 4.78 (d, 1 H, $J_{4,5}$ 2.7 Hz, H-5); fragment H, 5.44 (d, $J_{1,2}$ 3.5 Hz, H-1α), 4.71 (dd, $J_{1,2}$ 8 Hz, H-1β), 3.26 (dd, $J_{1,2}$ 3.5, $J_{2,3}$ 10.5 Hz, H-2α), 3.06 (dd, $J_{1,2}$ 8.2, $J_{2,3}$ 10.5 Hz, H-2β).

The synthetic tri-[10] and tetra-saccharides[11] F–G–H and E–F–G–H (this letter code was proposed by Choay *et al.* [2i]) do not bind to AT-III, whereas the synthetic pentasaccharide **1** ($R = SO_3^-$) binds strongly. The complex between **1** and AT-III has an



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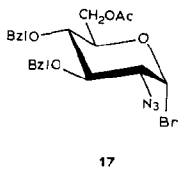

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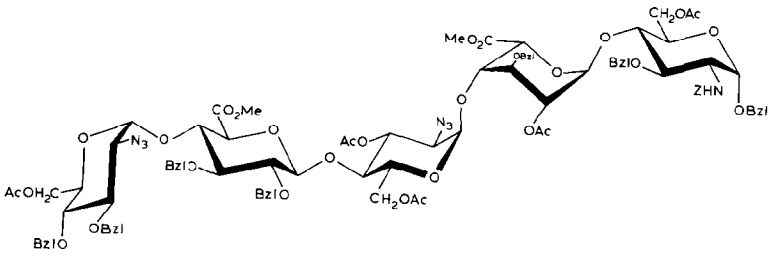

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association constant of 7.10^6 M^{-1}, which is the same order of magnitude as that of high-affinity heparin. Furthermore, in the presence of AT-III, **1** displays a high inhibitory activity with respect to factor Xa but, as expected, is unable to activate AT-III in the thrombin inhibition process[12].

On the assumption[2h,3a] that residue H is important for high biological activity, our work demonstrates that **1** probably corresponds to the minimum sequence required in heparin species for strong binding to AT-III and to specifically induce high anti-Xa activity.

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