Bioorganic & Medicinal Chemistry Letters, Vol.1, No.2, pp. 99-102, 1991
Printed in Great Britain

0960-894X/91 $3.00 + .00

THE FIRST TOTAL SYNTHESIS OF THE ANTITHROMBIN III BINDING SITE OF PORCINE MUCOSA HEPARIN

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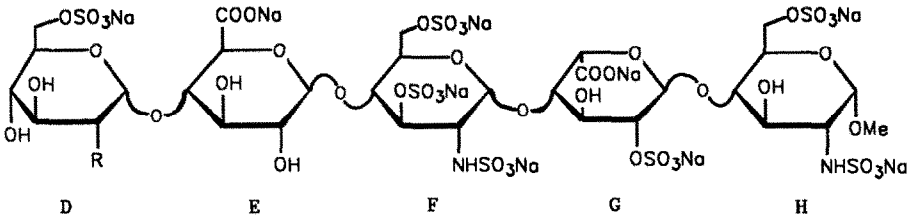
(*Received* 20 *December* 1990)

***Abstract** : The synthesis of a pentasaccharide containing a N-acetyl-glucosamine unit and corresponding to the major natural sequence required in heparin for binding to antithrombin III is reported for the first time. This compound elicits anti-factor Xa activity and high affinity for antithrombin III.*

Heparin, a highly complex anionic polysaccharide, has blood anticoagulant properties mediated by the plasma protein antithrombin III (AT III). It has been demonstrated that the interaction of the two molecules is highly specific and that a unique pentasaccharide sequence (DEFGH) in heparin is responsible for the binding to the protein[2].



1 R = NHAc

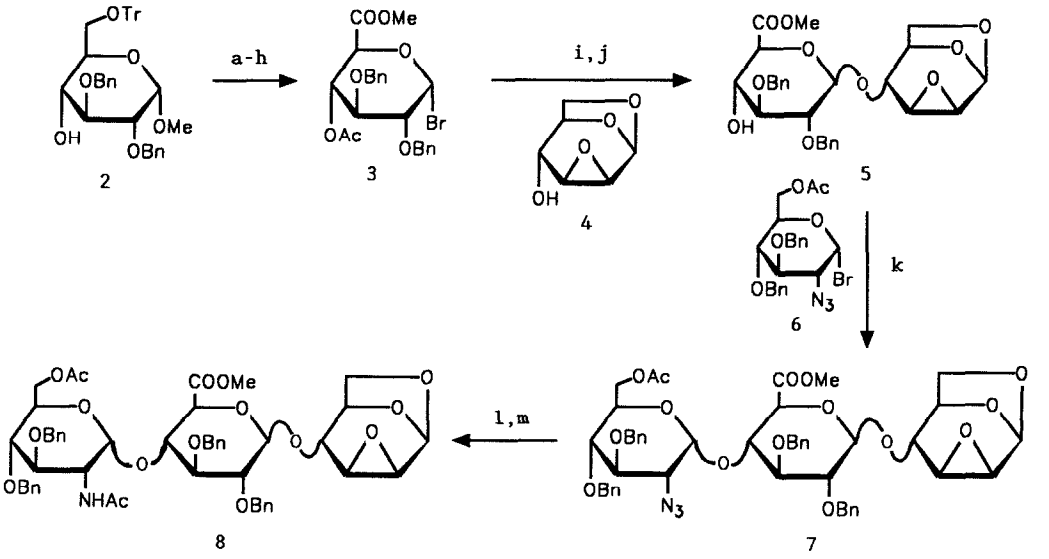
In the heparin pentasaccharide sequence, the glucosamine unit D is usually N-acetylated (R = NHAc) although it may be N-sulfated (R = $NHSO_3Na$) on some occasions, particularly when heparin is extracted from beef lung[3].

Several syntheses of the pentasaccharide fragment[4] where R = $NHSO_3Na$ and of analogs have been reported[5]. However the most frequently encountered sequence (R = NHAc) has never been synthesized[6]. We would like to report in this letter the first synthesis of the pentasaccharide **1**[7], the binding site of porcine mucosa heparin to AT III. Inasmuch as the biological properties of the DEFGH sequence arise from a precise array of sulfate groups, the availability of **1** should allow us to quantitatively estimate the contribution of the N-acetyl group to these properties.

8 was selected as a key intermediate for the following reasons : i) The single glucosamine unit is easily N-acetylated. ii) The selective trans-diaxial opening of the epoxy ring will allow the introduction of the non participating azido group as required for the construction of glucosamine unit F and the subsequent glycosylation step. iii) A "3+2" block synthesis of sequence DEFGH was found to be viable[8].

8 was synthesized according to scheme 1. The glucuronic acid derivative **3**[9] was prepared from methyl 2,3-di-O-benzyl-6-O-trityl-α-D-glucopyranoside[10] (**2**) using classical methodology. **3** was then condensed with the epoxide **4**[11] in dichloromethane, at room temperature, in the presence of solid silver carbonate. The cristalline ß-disaccharide **5** was selectively obtained in 51% yield. The α-linked disaccharide was also isolated after column chromatography (4% ; α/ß ratio : 1/12). Condensation of **5** with the known[12] bromide **6** finally gave the trisaccharide **7** in 72% yield.
^{1}H-NMR analysis of **6** confirmed the α-anomery ($J_{1,2}$ 3.5 Hz) of the newly created bond. Hydrogenation of the azido

function of 7 was performed in the presence of Pd/C in DMF using ammonium formate as the source of hydrogen. This technique[13] allows rapid reduction of the azido function without cleavage of benzyl ethers and crystalline **8** was obtained in 93% yield after N-acetylation.

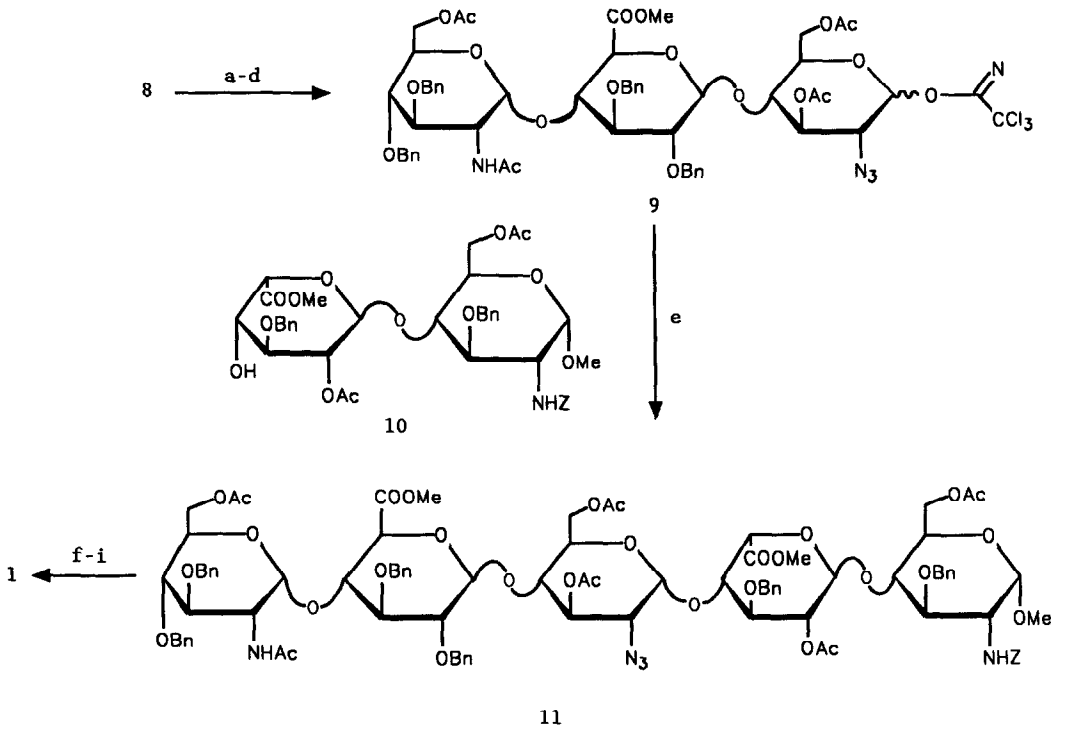


Scheme 1 : a: BzCl, pyridine. b: p-TsOH, MeOH. c: CrO_3, H_2SO_4, acetone. d: NaOH. e: CH_3COOH/CF_3COOH (15/1; v/v) 100°C. f: CH_2N_2, ether (26% from **2**). g: Ac_2O, pyridine (98%). h: $TiBr_4$, CH_2Cl_2, AcOEt (59%). i: Ag_2CO_3, drierite, 4Å mol. sieves (51%). j: NaOH then CH_2N_2, ether (72%). k: AgOTf, collidine, CH_2Cl_2 (72%). l: Pd/C, $HCOONH_4$, DMF. m: Ac_2O, MeOH (93% from **7**).

Heating compound **8** in DMF in the presence of sodium azide resulted in opening of the epoxide but also in decomposition products arising from ß-elimination of the 4-substituent of the uronic acid methyl ester. After prior saponification of the methyl ester the epoxyde could be opened in reasonable yield (65% after reesterification and O-acetylation, scheme 2). As expected the trans-diaxial product was the only one obtained. In a critical step the imidate **9** (α,ß-mixture, scheme 2) was condensed with the disaccharide **10**[4c] in dichloromethane at -20°C in presence of trimethylsilyl triflate[14] to give protected pentasaccharide **11** in 40% yield. **11** was converted in the usual way[4] into the N,O-sulfated deprotected pentasaccharide **1** which was purified by ion-exchange chromatography on a column of Q-Sepharose Fast Flow eluted by a linear gradient of sodium chloride, followed by desalting on Sephadex G25 and lyophilisation. A white fluffy material was obtained (31% from **11**).

500 MHz ^{1}H-NMR analysis[14] confirmed the structure of **1**. The coupling constants observed for the iduronic acid unit : $J_{1,2}$ 4.1 Hz; $J_{2,3}$ 8.2 Hz; $J_{4,5}$ 3.1 Hz, deserve some comments, since it is known that this sugar can exist in different conformational states in heparin[15]. From these values it was possible, using known methods[16], to compute simulated J values and the population of different conformers at equilibrium. The results indicate that $^2S_0$ and $^1C_4$ conformers are present in equilibrium in a 9/1 ratio. Compared to data obtained for the synthetic N-sulfated counterpart[16] this indicates that the N-substituent at D unit can influence the conformer population of L-iduronic acid G unit.

We determined the ability of pentasaccharide 1 to inhibit factor Xa in the presence of antithrombin III. In this assay 1 was about twice less potent than its N-sulfated counterpart (325 units/mg *vs* 600 u/mg). Finally the binding constant to antithrombin III has been measured[17]. The N-acetyl group slightly lowers the binding to ATIII compared to the N-sulfate[18] (K_D = 8.4 10^{-8} M for 1 *vs* 5.4 10^{-8} M for the N-sulfated compound). Whether these differences correspond to different functions in the natural products is unknown.



Scheme 2 : a: NaOH, MeOH; then NaN_3, DMF; then CH_2N_2, ether; then Ac_2O, pyridine (65%). b: H_2SO_4/Ac_2O, -20°C (74%). c: $C_6H_5CH_2NH_2$, ether (89%). d: CCl_3CN, K_2CO_3, CH_2Cl_2 (76%). e: TMSOTf, 4Å mol. sieves, CH_2Cl_2 (40%). f: NaOH. g: Et_3N-SO_3, DMF. h: H_2, Pd/C. i: pyridine-SO_3, water (31% from 11).

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5. For a review see M. Petitou in "Heparin", Lane, D.A. and Lindahl, U., Eds., Edward Arnold, London **1989**, 65.
6. It is chemically much simpler to obtain the N-sulfated version of this sequence in which all three glucosamine units are N-sulfated. A "2+2+1" blockwise approach has been invariably adopted (GH precursor + EF precursor + D precursor) which cannot afford a pentasaccharide in which only D is N-acetylated.
7. We chose to synthesize the α-methyl glycoside of DEFGH to avoid side reactions during the last steps of the synthesis, see reference 4c.
8. Petitou, M., Jaurand, G., Derrien, M., Duchaussoy, P., Choay, J. *Tetrahedron Lett.*, preceding paper in this issue.
9. All new compounds gave satisfactory elemental analysis. Selected analytical data of intermediates:
   3 : $[\alpha]_D$ + 98° (chloroform); ^{1}H-NMR, δ, 6.34 (d, J 3.5 Hz, H-1).
   5 : mp 169-170°C ; $[\alpha]_D$ -31° (chloroform); ^{1}H-NMR, δ, 4.62 (d, J 7.6 Hz, H-1').
   7 : $[\alpha]_D$ + 25° (chloroform); ^{1}H-NMR, δ, 5.59 (d, J 4 Hz, H-1") ; 4.69 (d, J 7.5 Hz, H-1') ; 5.77 (d, J 2.5 Hz, H-1).
   8 : mp 147-149°C ; $[\alpha]_D$ + 35° (chloroform) ; ^{1}H-NMR, δ, 4.95 (d, J 3.5 Hz, H-1") ; 4.69 (d, J 7 Hz, H-1') ; 5.77 (d, J 3 Hz, H-1) ; 5.86 (d, J 9.5 Hz, NH) ; 2.07 (s, OAc) ; 1.34 (s, NHAc).
   9 : ^{1}H-NMR, δ, 4.91 (d, J 3.5 Hz, H-1") ; 4.37 (d, J 8 Hz, H-1') ; 6.46 (d, J 3.5 Hz, H-1α) ; 5.70 (d, J 8 Hz, H-1 ß).
   11:$[\alpha]_D$ + 65° (chloroform) ; ^{1}H-NMR, δ, 4.87 (d, J 3.5 Hz, H-1"") ; 4.34 (d, J 8 Hz, H-1'") ; 4.98 (d, J 3.5 Hz, H-1") ; 5.29 (d, J 3.5 Hz, H-1') ; 4.65 (d, J 3.5 Hz, H-1) ; 5.79 (d, J 10 Hz, NHAc) ; 4.84 (d, J 10.5 Hz, NH-Z).
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14. ^{1}H-NMR data (500 MHz, D_2O) compound **1** : δ, 5.08 (d, J 3.6 Hz, H-1) ; 5.23 (d, J 4.1 Hz, H-1') ; 5.59 (d, J 3.5 Hz, H-1") ; 4.66 (d, J 8 Hz, H-1'") ; 5.49 (d, J 3.7 Hz, H-1"").
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17. We thank Dr. T. Bârzu for this determination.