Bioorganic & Medicinal Chemistry Letters, Vol.2, No.9, pp. 901-904, 1992
Printed in Great Britain

0960-894X/92 $5.00 + .00

BIOLOGICALLY ACTIVE HEPARIN-LIKE FRAGMENTS WITH A "NON-GLYCOSAMINO"GLYCAN STRUCTURE. Part 2 : A TETRA-O-METHYLATED PENTASACCHARIDE WITH HIGH AFFINITY FOR ANTITHROMBIN III.

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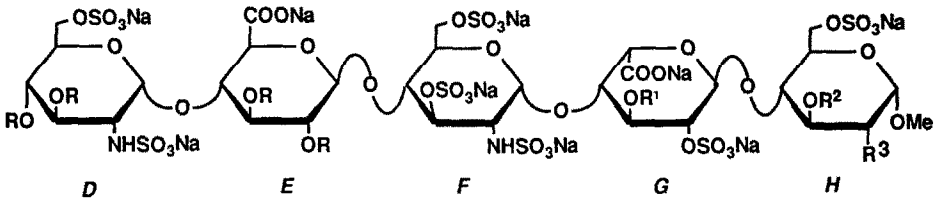
(*Received* 7 *April* 1992)

Abstract. *To simplify the preparation of biologically active oligosaccharide analogues of glycosaminoglycan fragments we describe an approach where N-sulphates are replaced by O-sulphates and free hydroxyls are substituted by methyl groups. In this communication we report on the synthesis of a pentasaccharide in which hydroxyl groups of the non-reducing end disaccharide sub-region are methylated. The biological properties of this chemically modified analogue are fully preserved.*

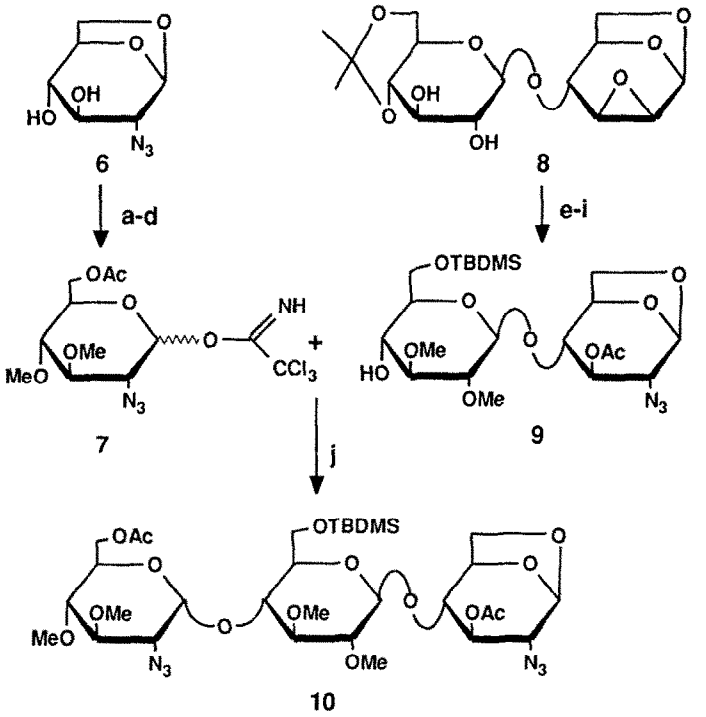
A unique pentasaccharide region of heparin is able to bind to the protease inhibitor antithrombin III (AT III) and thereby to promote selective inhibition of blood coagulation factor Xa[1]. Moreover, a synthetic counterpart shows antithrombotic activity in animal models of venous thrombosis[2,3]. After the first total chemical synthesis[4] of this pentasaccharide fragment, compound[5] **1** and various analogues[4] were prepared and used for structure-activity relationship studies. The new synthetic derivative[6] **2** which, compared to the natural pentasaccharide **1**, contains an extra sulphate ester at position 3 of the reducing end glucosamine unit H displays higher specific anti-factor Xa activity (1250 U/mg)[7] compared to the natural pentasaccharide **1** (700 U/mg). This has been ascribed to an enhanced interaction with AT-III at a second binding site[8]. Other analogues of compound **2** which can be prepared by simpler synthetic routes have been published recently. There it was shown that replacement of the reducing end N-sulphate by an O-sulphate (i.e. compound **3**) does not affect the biological activity[9].



1:	R=R¹=R²=H;	R₃=$NHSO_3Na$		
2:	R=R¹=H;	R²=SO_3Na;	R³=$NHSO_3Na$	
3:	R=R¹=H;	R²=SO_3Na;	R³=OSO_3Na	
4:	R=H;	R¹=Me;	R²=SO_3Na	R³=OSO_3Na
5:	R=Me;	R¹=H;	R²=SO_3Na;	R³=$NHSO_3Na$

In the preceding paper in this series[10] we showed that methylation of the free hydroxyl group of the crucial iduronic acid residue G (i.e. compound **4**) did not affect the biological activity. We have then focussed our attention on further alkylation of the DEF part of the pentasaccharide. It has been suggested that this "rigid" sub-region of the molecule is involved at the first stage (recognition) of the binding to AT III and an important question was whether methylation of the four hydroxyl groups of units D and E would have a negative effect on this binding. Here we report the synthesis of pentasaccharide **5** which is a tetra-O-methylated analogue of compound **2**.

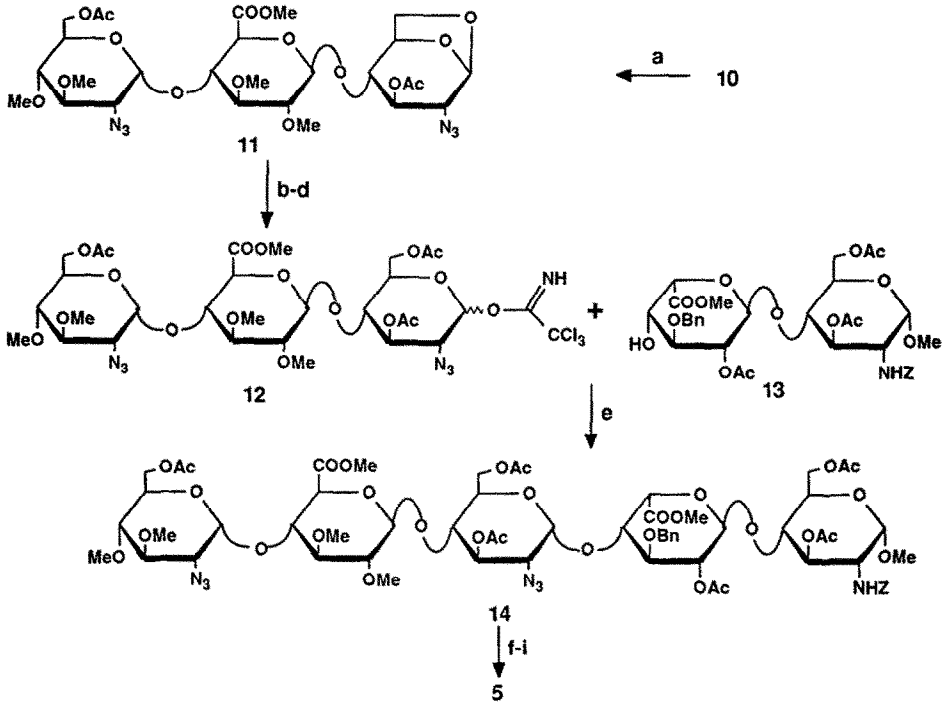
1,6-Anhydro-2-azido-2-deoxy-β-D-glucopyranose[11] **6** was used as a starting building block for the preparation of imidate **7** (scheme 1). After methylation of the 3 and 4 hydroxyl groups the crude product was subjected to acetolysis. Thereafter the anomeric acetate was cleaved by piperidine in THF followed by treatment with trichloroacetonitrile in dichloromethane in the presence of potassium carbonate which gave imidate **7**.



Scheme 1 a) CH_3I, NaH, DMF. **b)** Ac_2O : AcOH · CF_3COOH (25 . 1 : 35, v/v). **c)** Piperidine, THF (65% steps a - c). **d)** CCl_3CN, K_2CO_3, CH_2Cl_2 (99%). **e)** CH_3I, NaH, DMF (88 %). **f)** LiN_3, DMF. **g)** Ac_2O, pyridine, DMAP. **h)** 70% HOAc. **i)** TBDMSCl, pyridine (90 % steps f - i). **j)** TMSOTf, MS 4Å, CH_2Cl_2 (66%).

The synthesis of acceptor **9** started from disaccharide[12] **8**. The latter was converted into **9** by successive methylation of the hydroxyl groups, opening of the epoxide by LiN_3 treatment in DMF, acetylation of the 3-OH function, removal of the 4',6'-O-isopropylidene protective group and selective protection of the primary alcohol by a tert-butyldimethylsilyl group. TMS-triflate-promoted

glycosylation of **9** with a small excess (1.2 eq) of imidate **7** gave a mixture of α/β coupled trisaccharide. After silica gel column chromatography the pure α isomer **(10)** was isolated in 66% yield. Concomitant removal of the silyl ether and oxidation of the 6'-carbon atom was realized under Jones conditions to give the crude carboxylic acid which was then converted into **11** by esterification with CH_3I in dichloromethane (scheme 2). Following similar synthetic steps as described for glycon **7**, imidate **12** was obtained in 43% overall yield from **10**.



Scheme 2. a) CrO_3, H_2SO_4, acetone; CH_3I, $KHCO_3$, DMF (60 %). **b)** Ac_2O : AcOH : CF_3COOH 25 : 1 : 35 v/v (100 %). **c)** Piperidine, THF (98 %). **d)** CCl_3CN, K_2CO_3, CH_2Cl_2 (73%). **e)** TMSOTf, MS 4Å , CH_2Cl_2, -10°C (72%). **f)** LiOOH, THF : NaOH, CH_3OH. **g)** $Et_3N.SO_3$-complex, DMF. **h)** H_2, Pd/C 10%. **i)** pyridine SO_3-complex, H_2O, pH 9 (40% from **14**).

In the final condensation step the glycosyl donor **12** was coupled with the known acceptor[6] **13** under TMSOTf catalysis to give pentasaccharide **14** in 72% yield. Routine deblocking of the acetyl and uronic esters followed by O-sulphation, hydrogenolysis of the benzyl ethers and reduction of the azido groups and finally N-sulphation afforded pentasaccharide **5**. The crude product was desalted on Sephadex G-25 and purified by HPLC ion-exchange chromatography (mono Q column) using a linear sodium chloride gradient. The resulting pentasaccharide fractions were again desalted by gel permeation chromatography to afford pure[13] **5** in 40% yield from **14**.

Analogue **5** displayed an AT III mediated anti-factor Xa activity of 1288 U/mg thus proving that the biological activity of the alkylated heparin-like fragment is retained when the four hydroxyl groups

of the glucuronic acid E and the non-reducing glucosamine unit D are methylated. In the next paper in this series we report on the full replacement of N-sulphate groups by O-sulphate esters and alkylation of all free hydroxyl groups.

Acknowledgement

We wish to thank Mr. G.N. Wagenaars for recording the NMR-spectra and Mr. Th. G. van Dinther for determining the anti-factor Xa activities. This investigation is supported by the EEC Eureka programme (Project EU 237).

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13. ^{1}H-NMR data for **5** (360 MHz, D_2O, δ, ppm): Unit **D**, 5.44 (d, 1H, $J_{1,2}$ 3.6Hz, H-1). Unit **E**, 4.69 (d, 1H, $J_{1,2}$ 7.8Hz, H-1), 3.87 (t, 1H, H-4), 3.74 (d, 1H, $J_{4,5}$ 9.6Hz, H-4). Unit **F**, 5.63 (d, 1H, $J_{1,2}$ 3.6Hz, H-1). Unit **G**, 5.12 (d, 1H, $J_{1,2}$ 5.6Hz, H-1), 4.86 (d, 1H, $J_{4,5}$ 3.8Hz, H-5). Unit **H**, 5.01 (d, $J_{1,2}$ 3.6Hz, H-1). 3.41-3.65 (5s, 5 OCH_3).