Bioorganic & Medicinal Chemistry Letters, Vol.1, No.2, pp. 95-98, 1991
Printed in Great Britain

0960-894X/91 $3.00 + .00

A NEW, HIGHLY POTENT, HEPARIN-LIKE PENTASACCHARIDE FRAGMENT CONTAINING A GLUCOSE RESIDUE INSTEAD OF A GLUCOSAMINE

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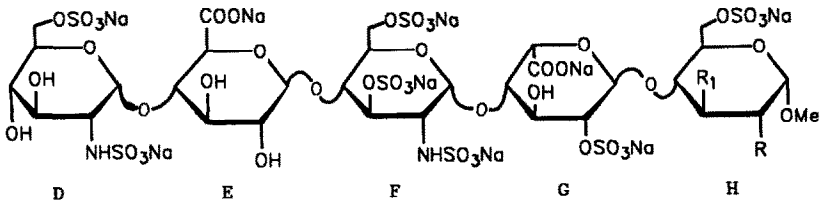
(*Received* 20 *December* 1990)

***Abstract** : The synthesis is reported of a new heparin-like pentasaccharide fragment in which the reducing end glucosamine unit is replaced by a glucose residue. This indicates that an O-sulfate can be substituted for a N-sulfate thereby allowing simpler synthesis of this kind of compounds. A new route using a trisaccharide imidate as glycosyl donor was developed for this preparation.*

Heparin is a complex anionic polysaccharide widely used as a drug for the prevention of venous thrombosis. Its action is mediated by the plasma protein antithrombin III. The recent chemical synthesis[1] of heparin oligosaccharide fragments like **1**, able to activate antithrombin III, allowed us to reveal their antithrombotic potential in animal models of thrombosis[2]. When these complex synthetic heparin fragments would display therapeutic efficacy it is attractive to have simplified analogs at one's disposal.



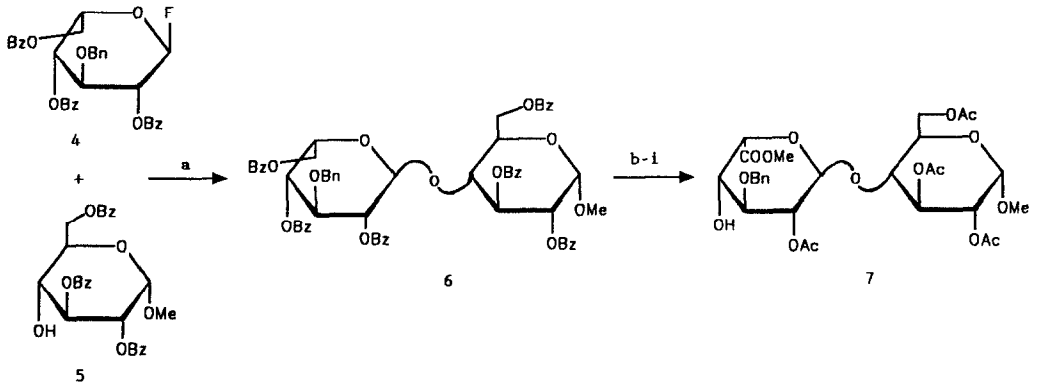
1 : R = $NHSO_3Na$; R^1 = OH
2 : R = R^1 = OSO_3Na
3 : R = $NHSO_3Na$; R^1 = OSO_3Na

The pentasaccharide **1** contains both sulfate esters (O-sulfate) and sulfate amides (N-sulfate). Different protecting groups are required to introduce all of them in the proper position, and it would obviously be simpler to deal with only O-sulfated molecules. As a step towards this simplification we report in this paper the synthesis of pentasaccharide **2** in which the reducing end N-sulfate-6-O-sulfate glucosamine unit is replaced by a 2,3,6-tri-O-sulfate glucose residue. An extra O-sulfate group was introduced at 3 position of the reducing end unit for two reasons : 1) Previous work[3] indicated that a high anionic charge at this extremity of the molecule was required for activity ; 2) The synthesis of the pentasaccharide looked simpler than the synthesis of its non 3-sulfated counterpart. Indeed a similar product (**3**) containing three N-sulfate-glucosamine units has been obtained by another group of investigators[4] while the present work was in progress, and shown to be more potent than reference compound **1** in the antithrombin III mediated inhibition of blood coagulation factor Xa (the reflect of the antithrombotic activity of this class of products).

The route towards compounds like **2** involves the key fully protected intermediate **16** which is then deprotected and functionalized. This intermediate is usually obtained from disaccharides and monosaccharides in a "2+2+1 approach" (coupling of two disaccharides EF + GH and final addition of the D non reducing end unit). In the present work we planned to prepare several analogs at the GH part of the molecule, a task that would be simplified using a different

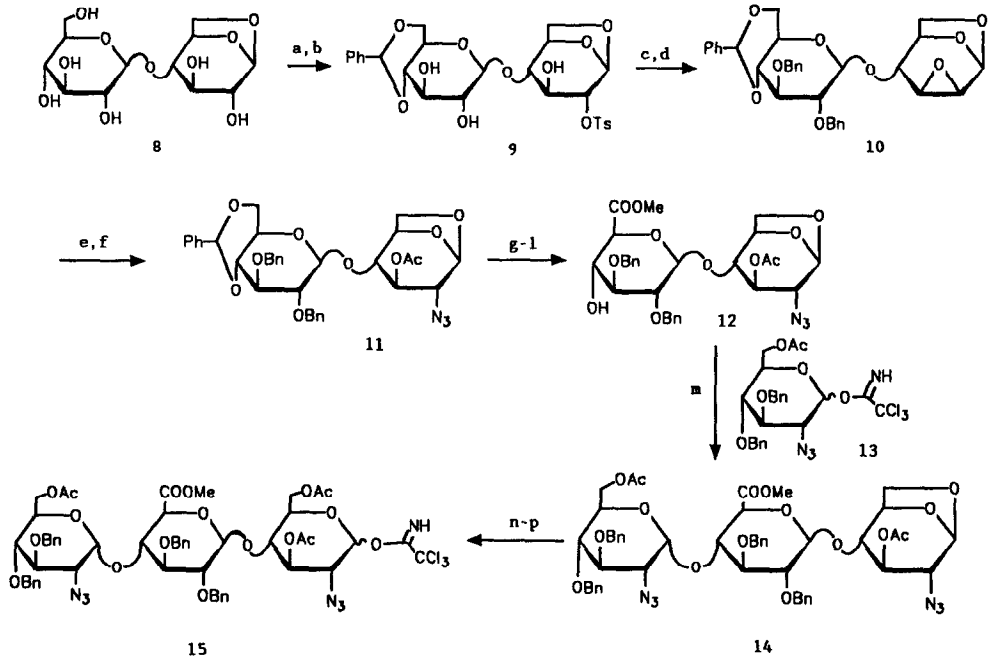
approach in which the trisaccharide precursor of DEF would be coupled to various precursors of GH. Previous attempts using this "3+2" route were unfruitful[5] presumably because bromides were employed instead of imidates for activation of the anomeric center.

GH building block 7 was prepared (scheme 1) from disaccharide **6**[6] obtained (86%) by coupling 2,4,6-tri-O-benzoyl-3-O-benzyl-α-L-idopyranosyl fluoride[7] (**4**) to methyl 2,3,6-tri-O-benzoyl-α-D-glucopyranoside[8] (**5**) in the presence of BF_3/ether. After removal of benzoyl groups the resulting polyol was selectively protected at 4',6' by a benzylidene group prepared in quantitative yield in the presence of benzaldehyde and a catalytic amount of trifluoroacetic acid. Under these experimental conditions the reaction was complete in about 30 minutes in the *ido* series while very poor results were obtained with the corresponding *gluco* compounds. Acetylation yielded 98% of cristalline compound (m.p. 155°). The benzylidene acetal was then removed by trifluoroacetic acid (78%) and the resulting diol was silylated and levulynoylated in a one pot reaction (94%). Jones oxidation, performed directly on the silyl derivative, was followed by methylation and cleavage of the levulinoyl group by hydrazine in pyridine acetate. The glycosyl acceptor 7 was thus obtained as a foam after silica gel chromatography (70% from the silylated derivative).



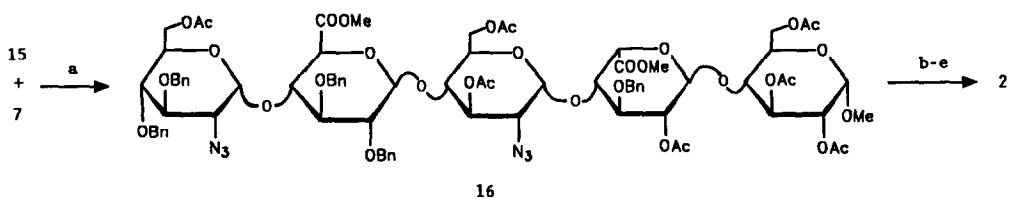
Scheme 1 : a : BF_3/Et_2O, CH_2Cl_2, -20°C (86%). b : MeONa, MeOH (95%). c : C_6H_5CHO, CF_3COOH, 30' (100%). d : Ac_2O, pyridine (98%). e : CF_3COOH/H_2O (78%). f : t-BDMSCl, Et_3N, DMAP ; then levulinic anhydride (94%). g : CrO_3/H_2SO_4, acetone/water. h : CH_3I, DMF, $KHCO_3$. i : hydrazine (70%).

For the preparation of the trisaccharide building block **15** (scheme 2) we started from 1,6-anhydro-cellobiose[9] (**8**). Selective protection at 4',6' position was achieved through benzylidenation. After several trials we found satisfactory conditions (-20°C ; 1.5 eq) for selective 2-O-tosylation that provided crude **9** which was converted into **10**[10] by treatment with sodium methoxyde and benzylation. **10** was isolated after filtration on silica gel and crystallisation (m.p. 184-5°C). Trans-diaxial opening of the epoxyde yielded the 2-azido derivative (66%) which was acetylated to yield **11** (99%). The benzylidene was cleaved (92%) and the diol was then converted into **12** by successive tritylation, levulinoylation, detritylation, oxidation, methylation and hydrazinolysis (66% over the 6 steps). Imidate **13** was prepared in the usual way from its hydroxyl precursor and coupled with **12** to give the α-linked trisaccharide **14** in 78% yield. **14** was acetolysed (91%), the anomeric acetate was cleaved by benzylamine in ether (100%) and the imidate **15** was obtained by reaction with potassium carbonate and trichloroacetonitrile at room temperature (α,ß mixture with α predominant ; 76%).



Scheme 2 : a : $C_6H_5CH(OCH_3)_2$, TsOH (80%). b : TsCl, pyridine, -20°C (60%). c : MeONa (80%). d : BnBr, DMF, NaH (76%). e : NaN_3, DMF (66%). f : Ac_2O, pyridine (99%). g : H^+ (92%). h : TrCl, pyridine ; then levulinic anhydride. i : $HClO_4$. j : CrO_3/H_2SO_4 ; acetone/water. k : MeI, $KHCO_3$. l : NH_2NH_2, H_20 (66% from the diol obtained in g). m : TMSTf, CH_2Cl_2, -20°C (78%). n : Ac_2O, CF_3COOH. o : $C_6H_5CH_2NH_2$, ether. p : CCl_3CN, K_2CO_3 (69% from 14).

Coupling of **15** with **7** was the key step of the new synthetic route (scheme 3). It was carried out at -20°C in dichloromethane and led only to the formation of an α-glycoside, as shown by NMR study of the newly created bond ($J_{1,2}$ = 3.5 Hz). This successful reaction again illustrates the value of the imidate procedure in oligosaccharide synthesis since negative results were obtained in similar cases using the trisaccharide bromide and silver triflate as catalyst[5]. The fully protected pentasaccharide **16** was then converted into the target compound **2** using classical steps : saponification, O-sulfation, cleavage of benzyl ethers with simultaneous reduction of azido into amino functions and finally N-sulfation. **2** was purified by hplc ion exchange chromatography on a mono Q column. Its structure was ascertained by NMR spectroscopy[11].



Scheme 3 : a : TMSTf, CH_2Cl_2, -20°C (74%). b: NaOH. c : Et_3N-SO_3, DMF. d : H_2 ; Pd/C. e : pyridine-SO_3, H_2O, pH9.

It is well established now that the conformation of the iduronic acid cycle is strongly influenced by the nature of neighbouring residues. Thus the equilibrium between $^1C_4$ and $^2S_0$ conformers in **1** ($^1C_4$ 40% ; $^2S_0$ 60%) is shifted toward $^2S_0$ in **3**[4]. We have obtained similar results with **2** where the simulated coupling constants for the iduronate residue ($J_{1,2}$ 5.19 Hz ; $J_{2,3}$ 9.83 Hz ; $J_{3,4}$ 4.06 Hz ; $J_{4,5}$ 3.96 Hz) indicate the strong predominance of the $^2S_0$ form : molecular mechanics calculations performed according to Ferro *et al.*[12] indicate an equilibrium between $^2S_0$ (94%) and $^1C_4$ (6%). Therefore the replacement of the N-sulfate at unit H by an O-sulfate does not appear to affect the conformation of the molecule. This is also reflected by the biological activity of **2** (1300 anti-factor Xa units/mg) which is equivalent to that of **3** and about the double of that of reference pentasaccharide **1**.

In conclusion, the replacement of the reducing end glucosamine residue H by a glucose residue does not affect the biological activity of this kind of product which opens the way towards simpler active oligosaccharides. The new "3+2" synthetic route presented here will allow a rapid synthesis of several analogs of pentasaccharide **1** having various GH residues in order to explore further structural modifications at this end of the molecule.

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6. All new compounds gave satisfactory elemental analysis and NMR data were in agreement with the expected structures.
7. This product was obtained as described for its O-acetylated counterpart, see ref. 4.
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10. Ichikawa, Y., Ichikawa, R., Kuzuhara, H. *Carbohydr. Res.*, **1985**, 141, 273. The present method gives a 34% yield of **10** from 4',6'-O-benzylidene-1,6-anhydro-cellobiose compared to 40% in ref. 10. But only two steps are required here compared to five in ref. 10.
11. ^{1}H-NMR for compound **2** (500 MHz, D_2O) : 4,68 (d, J 7.9 Hz, H-1 '") ; 5.17 (d, J 5.2 Hz, H-1') ; 5.20 (d, J 3.7 Hz, H-1) ; 5.65 (d, J 3.5 Hz, H-1") ; 5.67 (d, J 3.8 Hz, H-1"").
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