*Bioorganic & Medicinal Chemistry Letters*, Vol.2, No.9, pp. 905-910, 1992
Printed in Great Britain

0960-894X/92 $5.00 + .00

# BIOLOGICALLY ACTIVE HEPARIN-LIKE FRAGMENTS WITH A "NON-GLYCOSAMINO"GLYCAN STRUCTURE. Part 3 : O-ALKYLATED-O-SULPHATED PENTASACCHARIDES.

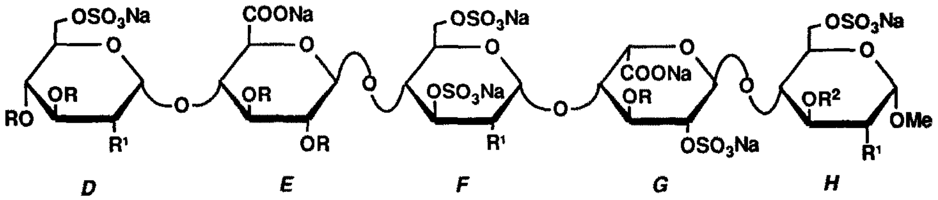
**J. Basten[a], G. Jaurand[b], B. Olde-Hanter[a], P. Duchaussoy[b], M. Petitou[b]* and C.A.A. van Boeckel[a]***

a: Organon International B.V., PO Box 20 , 5340 BH Oss, The Netherlands.
b: Sanofi Recherche; 9, rue du Président Salvador Allende; 94256 Gentilly; France.

(*Received* 7 *April* 1992)

***Abstract.*** *The synthesis of analogues of biologically active heparin pentasaccharide fragments is reported. Replacement of N-sulphate by O-sulphate esters and introduction of various O-alkyl ethers resulted in biologically active "non-glycosamino"glycans. The biological activity can be further modulated by changing the nature and the position of the alkyl chains introduced.*

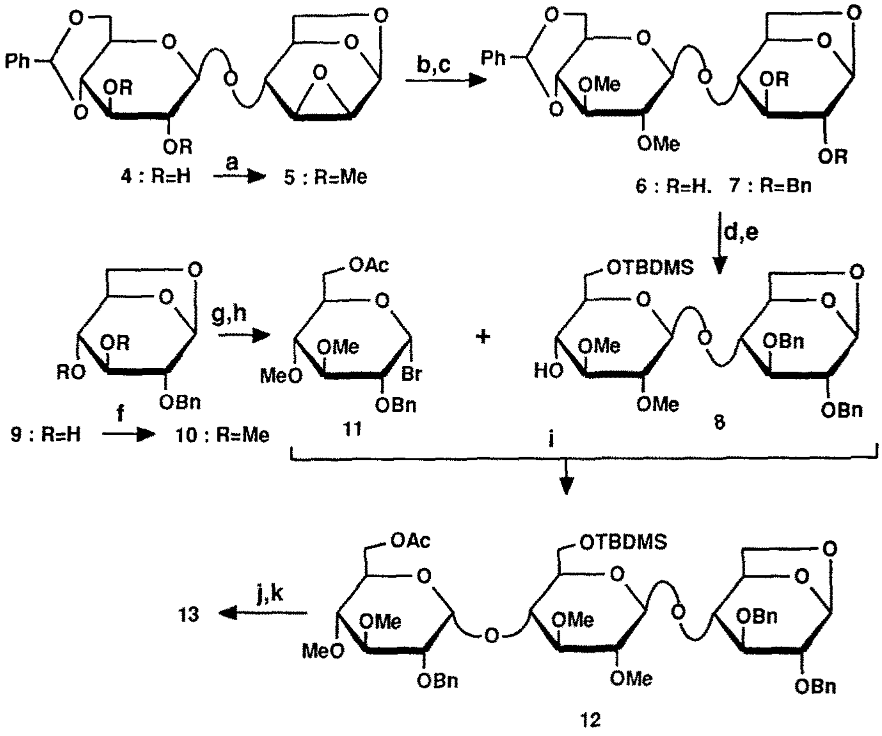
Glycosaminoglycans (heparin, heparan sulphate, dermatan sulphate, chondroitin sulphate, *etc.*) are ubiquitous compounds the biological function of which is not yet fully understood. However, there is evidence that short, precise sequences of these complex polysaccharides are involved in crucial biological processes. A clear example is the specific interaction of heparin and antithrombin III[1,2] while there are some clues for similar behaviour of other glycosaminoglycans with respect to other proteins.



1: R=R$^2$=H; R$^1$=NHSO$_3$Na
2: R=H; R$^1$=NHSO$_3$Na; R$^2$=SO$_3$Na
3: R=Me; R$^1$=OSO$_3$Na; R$^2$=SO$_3$Na

It is appealing to use these well defined, biologically active, structures as active principles for new drugs although their chemical complexity hinders an economically feasible development. To overcome this problem organic chemistry offers the possibility to substitute functional groups for other ones and we have applied this demarch to a heparin pentasaccharide sequence[3] which is a potential antithrombotic drug. Thus we prepared **1**, the methyl glycoside analogue of this sequence[4] which displayed similar biological properties. Compound **2**[5], with an additional sulphate group at 3 position of the H-unit, is of particular interest since it binds more strongly to AT III than the natural sequence, resulting in an anti-Xa activity of about 1250 U/mg. Compound **2** has become the lead compound for other analogues in our research programme. Recently, we reported the slightly shorter synthesis of a pentasaccharide in which the reducing end glucosamine unit is replaced by a 2,3,6,-tri-O-sulphated glucose residue[6] and we found that this modification did not affect the biological activity.

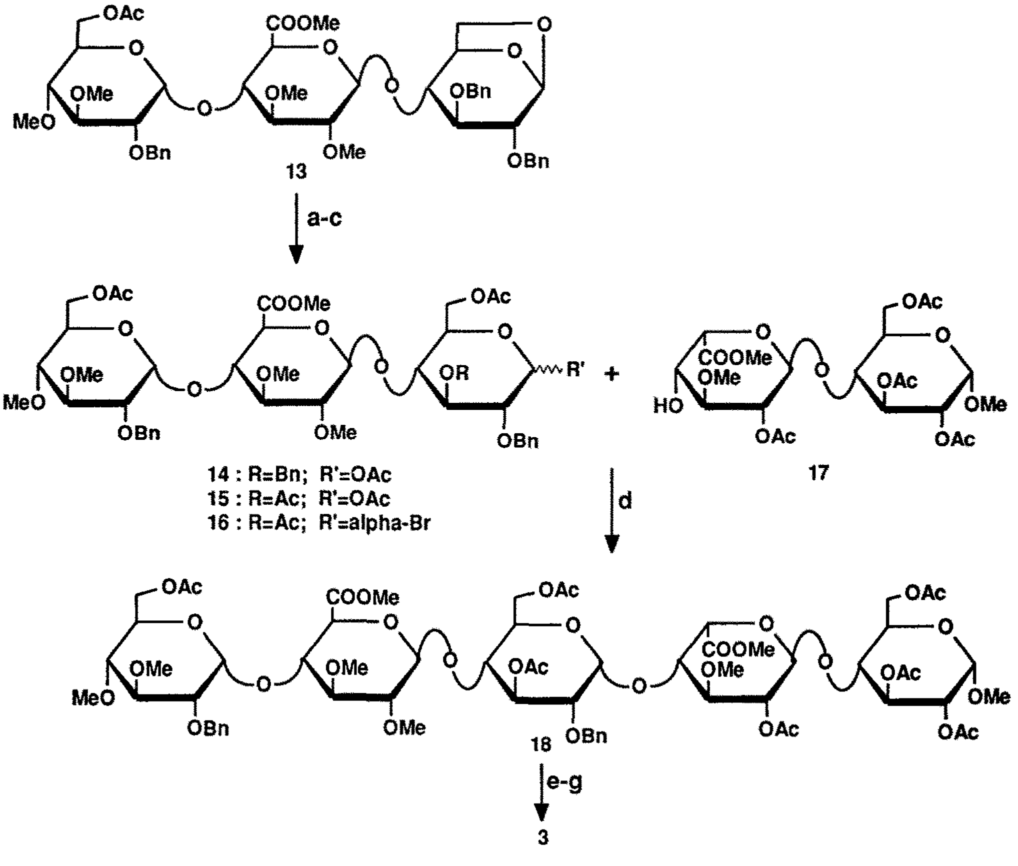
In the first two papers in this series we demonstrated that 3-O-methylation of the iduronic acid residue (G) afforded a partially alkylated pentasaccharide, still displaying high anti-factor Xa activity[1] and that methylation of the four hydroxl groups of units D and E did not have a negative effect on activation of AT III by the pentasaccharide[2]. Here we show that pentasaccharides containing solely O-sulphate esters, and O-alkyl ethers instead of hydroxyls, constitute a new class of compounds which do not belong any longer to the family of glycosaminoglycans but exhibit similar biological properties. The synthesis of such "non-glycosamino" glycans is advantageous with respect to the



**Scheme 1 : a)** $CH_3I$, NaH, THF (100%). **b)** 40% TBAOH, dioxane, 80°C, 24h **c)** BnBr, NaH, DMF (52% over 2 steps). **d)** 70% acetic acid. **e)** TBDMSCl, pyridine (100% crude over 2 steps). **f)** $CH_3I$, NaH (89%). **g)** $Ac_2O/AcOH/CF_3COOH$ (50/2/7, v/v/v)(100%). **h)** $TiBr_4$ (95%). **i)** $Hg(CN)_2$, $CH_2Cl_2$, MS 4Å, r.t. (62% $\alpha$). **j)** $CrO_3$, $H_2SO_4$, acetone. **k)** $NaHCO_3$, DMF, $CH_3I$ (57% over 2 steps).

preparation of other analogues of the natural fragment[7] since i) hydroxyl groups at defined positions are "permanently protected" by alkyl groups at an early stage of the synthesis meaning that ii) both acyl and benzyl protective groups can be used for protection of free hydroxyl groups which have to be sulphated; iii) no α-coupled glucosamines have to be introduced which require elaborate synthetic routes towards azide containing building blocks; iv) carboxylic acids can be blocked as benzyl esters (removable by catalytic hydrogenation instead of basic treatment which occasionally results in partial degradation by an elimination reaction); v) no selective N-sulphation has to be performed at the end of the synthesis.

Furthermore, we present data indicating that the choice of the substituents may influence the biological activity thereby creating a way to modulate the pharmacological parameters of the potential drugs thus obtained.



**Scheme 2:** **a)** $Ac_2O/AcOH/CF_3COOH$ (70%). **b)** $NH_2NH_2.HOAc$ (100%). **c)** Oxalyl bromide (90%). **d)** $Hg(CN)_2$, $HgBr_2$, 30h, 20°C, $CH_2Cl_2$ (62% α). **e)** LiOOH, NaOH. **f)** $H_2$, 10% Pd/C (60% over 2 steps). **g)** $(C_2H_5)_3NSO_3$, DMF, 15h 50°C (72%).

The synthesis of **3** will be described in order to illustrate the procedure used to prepare the various derivatives shown in Table 1. Compound **3** can be obtained from **18** after saponification, hydrogenolysis and O-sulphation. The preparation of **18** is achieved by condensation of the trisaccharide **16** and the disaccharide[1] **17** (scheme 2). In order to obtain rapidly the suitably protected, methylated DEF trisaccharide **12** (see scheme 1) it was most convenient for us to use the monosaccharide building block **9** and the disaccharide building block **4** which were both in stock from earlier syntheses[7]. In this respect it should be noticed, however, that much shorter synthetic routes towards the key trisaccharide **13** can be easily devised.

Methylation of the EF disaccharide **4** under standard conditions gave compound **5** quantitatively. Trans-diaxial opening of the epoxide **5** was effected with a 40% tetrabutylammonium hydroxide solution in a mixture of water and dioxane at elevated temperature (alternatively sodium benzylate was used to directly deliver the 2-O-benzylated derivative).

The diol **6** thus obtained was subsequently treated with benzyl bromide and NaH in DMF to give disaccharide **7** in 52% overall yield. After cleavage of the benzylidene protective group and selective silylation of the primary alcohol, the crude reaction product was filtered through silica gel whereupon compound **8** was isolated in almost quantitative yield.

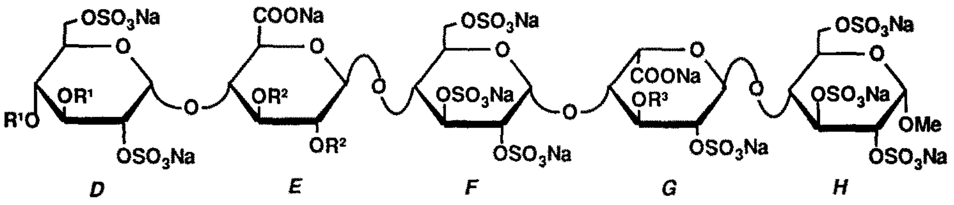
For the preparation of the D unit the 1,6-anhydro glucose unit **9** was methylated to afford **10**. After acetolysis of the 1,6-anhydro ring the mixture of anomeric α/β acetates was directly treated with titanium tetrabromide[8] to give donor **11**.

Coupling of **11** with **8** in the presence of mercury cyanide afforded trisaccharide **12** in 62% yield. Jones oxidation of **12** followed by methyl-esterification gave the DEF trisaccharide **13** in 57% yield. Treatment of this trimer with a mixture of acetic anhydride, trifluoroacetic acid and acetic acid for 16 hours at room temperature not only led to acetolysis of the 1,6-anhydro ring (compound **14**: 27% yield) but also to acetolysis of the 3-O-benzyl group at unit F (compound **15**: 45% yield).

Selective removal of the anomeric acetyl group in **15** with hydrazine acetate followed by reaction with oxalyl bromide[9] in a mixture of chloroform and DMF afforded bromide **16** in 81% overall yield. Coupling of **16** with acceptor **17** was carried out in the presence of mercury cyanide/mercury bromide to afford a 62% yield of α-coupled pentasaccharide **18**. This protected pentasaccharide was partially deblocked by saponification of the ester functions and hydrogenolysis of the benzyl ethers. O-Sulphation was then achieved using an excess of sulphating agent to give, after desalting on Sephadex G-25 fine, 72% of pentasaccharide **3**, the structure of which was confirmed by NMR[10].

Several compounds were prepared according to this procedure and their biological properties were investigated. The first data (Table 1) clearly demonstrate that the nature, number and position of alkyl substituents is a determining factor for the biological properties of the compounds. The data in Table 1 show that the antithrombin III-mediated anti-factor Xa activity varies from approximately 400 (**20**) to 1500 (**23**) units/mg according to the substitution profile. Apparently, no "key polar" hydroxyl groups[11] are involved in the interaction of heparin with AT III, suggesting that the role of the carbohydrate skeleton is to position the key sulphate and carboxylate groups[12,13] precisely in space thus allowing optimal interaction with the protein[14].

**Table 1**



| Compound | $R^1$ | $R^2$ | $R^3$ | Anti-factor Xa Activity (u/mg) |
|---|---|---|---|---|
| **3** | Me | Me | Me | 1323 |
| **19** | Me | Me | Bu | 994 |
| **20** | Me | Me | 3-Phenyl-propyl | 434 |
| **21** | Me | Bu | Me | 1100 |
| **22** | Me | Bu | Octyl | 700 |
| **23** | Bu | Me | Me | 1500 |

From a practical point of view, the possibility of modulating the pharmacological parameters of the products and their relative ease of preparation make them attractive candidates for pharmaceutical development.

## Acknowledgement

We wish to thank G. N. Wagenaars for recording NMR-spectra and Th. G. van Dinther and J.-P. Herault for determining anti-factor Xa activities. This research is sponsored by the EEC Eureka programme (project EU 237)

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10. $^1$H-nmr data for **3** (360 MHz, $D_2O$, δ ppm): Unit **D:** 5.54 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4.18 (dd, 1H, H-2), 3.68 (t, 1H, $J_{2,3}=J_{3,4}=9.6$ Hz, H-3), 3.40 (t, 1H, H-4). Unit **E**: 4.67 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 3.25 (dd, 1H, $J_{2,3}$ 9.0 Hz, H-2). Unit **F**: 5.53 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 4 55 (t, 1H, $J_{2,3}=J_{3,4}=9.8$ Hz, H-3). Unit **G**: 5.18 (d, 1H, $J_{1,2}$ 5.0 Hz, H-1), 3.80 (dd, 1H, $J_{2,3}$ 8.5 Hz, $J_{3,4}$ 3.8 Hz, H-3), 4.78 (d, 1H, $J_{4,5}$ 3.0 Hz, H-5). Unit **H**: 5.14 (d, $J_{1,2}$ 3.6 Hz, H-1). 3.46, 3.59-3.60, 3.63-3.65 (s, 5$CH_3$, $OCH_3$).
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