Tetrahedron Vol. 46, No. 24, pp. 8207-8228, 1990
Printed in Great Britain

0040-4020/90 $3.00+.00

SYNTHESES OF HEPARIN - LIKE PENTAMERS CONTAINING "OPENED" URONIC ACID MOIETIES.

H. Lucas, J. E. M. Basten, Th. G. van Dinther, D. G. Meuleman, S. F. van Aelst and C. A. A. van Boeckel
AKZO Pharma Division, Organon International B. V.,
Scientific Development Group,
P. O. Box 20, 5340 BH Oss, The Netherlands.

(*Received in UK* 10 *August* 1990)

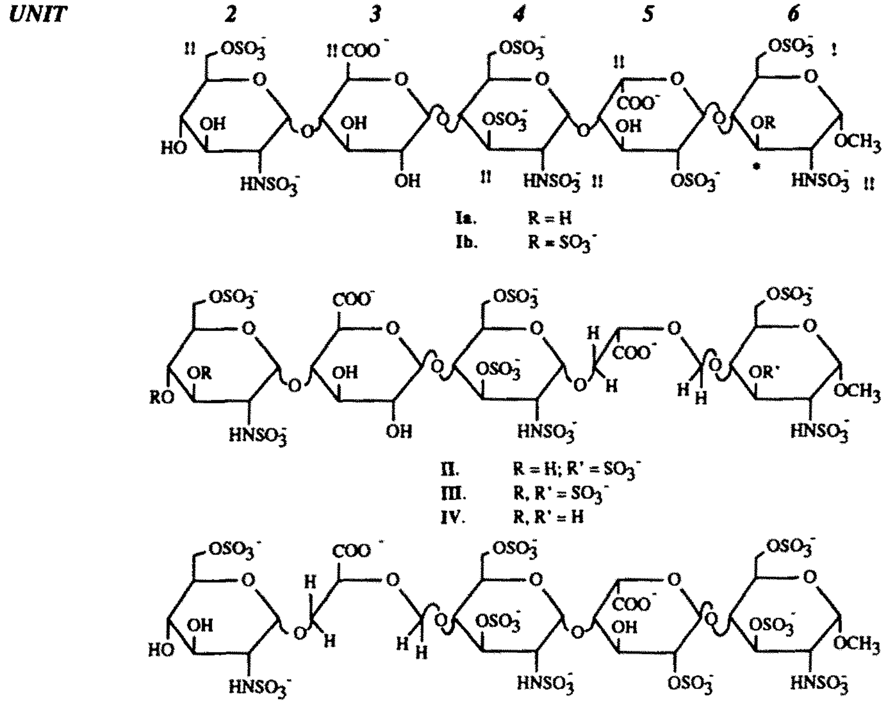
Abstract. The syntheses of four analogues of pentasaccharide **Ia**, which corresponds to the minimal AT III binding region of heparin, are presented and the biological activities of these analogues will be discussed. Three of these analogues (i.e. compounds **II**, **III** and **IV**) contain an *R*-glyceric acid oxymethylene residue (*i.e.* **B** in *fig.3*) instead of α-*L*-iduronic acid and in the other analogue (*i.e.* compound **V**) the β-*D*-glucuronic acid unit has been replaced by an *S*-glyceric acid oxymethylene residue (*i.e.* **A** in *fig.3*). The *R* and *S*-glyceric acid oxymethylene residues represent an "opened" iduronic acid unit and an "opened" glucuronic acid unit, respectively, containing the essential carboxylate function in the appropriate configuration. The crucial step in the syntheses of these "opened" uronic acid pentamer analogues, was the preparation of the required glyceric acid oxymethylene residues **8a**, **8b** and **8c**.

Analogues **II** and **III**, containing an "opened" iduronic acid moiety, display a significant AT III mediated αXa activity. Compound **III** contains two extra sulphate groups at *unit 2*. Removal of the contributing O-sulphate groups at position 3 and 6 of *unit 6* of compound **II** (*i.e.* compound **IV**) results in a seven-fold drop in αXa activity. Replacement of the β-*D*-glucuronic acid unit by an *S*-glyceric acid oxymethylene residue (*i.e.* compound **V**) leads to almost a complete loss of αXa activity, notwithstanding the fact that all the essential and contributing charged groups are present in the molecule.

Introduction.

The last few years much knowledge has been gained concerning the structure - activity relationships of the anti-thrombotic drug heparin. The activity of heparin is mainly based on the binding of part of the sulphated polysaccharide with the protease inhibitor antithrombin III (AT III)[1-4], thereby accelerating inactivation of serine proteases in the coagulation cascade. It is well known that the minimal AT III binding region of heparin consists of a unique pentasaccharide fragment[2,5]. This pentasaccharide and its synthetic counterpart[6-11] (*i.e.* compound **Ia**) catalyse the AT III - mediated inactivation of factor Xa (αXa activity) but not of thrombin.

By determining the αXa activity of a series of synthetic analogues[12-20] it has been elucidated that most of the charged groups play an important role in the activation of AT III. Some of these groups are strictly required for the activation of AT III (!! in compound **Ia**) in that the removal of one of these functions leads to at least 90% or complete loss of the αXa activity. Other groups (! in compound **Ia**) contribute significantly during the AT III activation process, since removal of one of these groups is accompanied by a serious decrease (70 - 80%) of αXa activity.



Ia. R = H
Ib. R = SO_3^-

II. R = H; R' = SO_3^-
III. R, R' = SO_3^-
IV. R, R' = H

V.

Fig. 1.

Taking into account these structure activity relationships and by contemplating molecular modelling data we postulated a heparin - AT III interaction model (see *fig.2*)[20]. On the basis of this model we introduced an extra sulphate group at position 3 of *unit 6* (* in compound **Ia**) of the naturally occurring fragment to give analogue **Ib**[19]. This extra-sulphated analogue displays higher affinity towards AT III, an enhanced AT III mediated αXa activity and a prolonged biological half life[21].

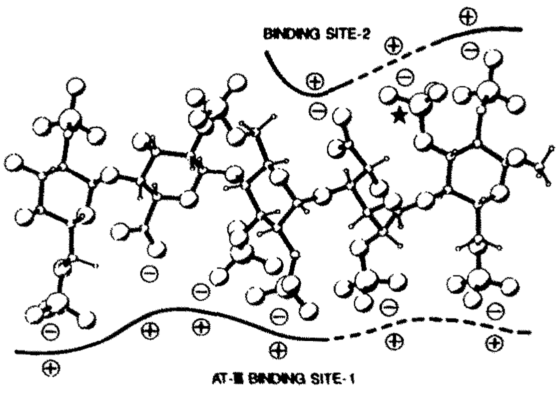


Fig. 2 Proposed binding model[20] of the pentasaccharide with AT III, in which the star indicates the position of the extra 3-O-sulphate group that enhances the AT III mediated αXa activity. The solid lines represent the essential binding areas, whereas the broken lines represent the contributing areas.

The interaction model depicted in *fig.2* suggests that, with respect to uronic acid units *3* and *5*, only the carboxylate functions are strictly required for binding. The point in question is if the ring structures of the pyranuronate residues are also essential for the activation of AT III. To answer this question we synthesized three heparin analogues (**II**, **III** and **IV**) in which the iduronic acid unit is replaced by an *R*-glyceric acid oxymethylene residue and one analogue in which an *S*-glyceric acid oxymethylene residue is present instead of glucuronic acid (*i.e.* compound **V**) (see *fig.1*). These "ring opened" analogues still contain the essential carboxylate function in the appropriate configuration, although in the meantime the flexibility is increased. However, in analogues **II**, **III** and **IV** the contributing sulphate group at position 2 of the iduronic acid *unit 5* is intrinsically absent. For the first analogue to be synthesized (i.e. compound **II**), we reasoned that the absence of the sulphate group at *unit 5* may be compensated by the introduction of an extra sulphate group at position 3 of *unit 6*.

When compound **II**[22] was found to be biologically active, we synthesized two analogues of this compound : one containing two extra sulphate groups at *unit 2* (*i.e.* compound **III**) and another one lacking two sulphate groups at *unit 6* (*i.e.* compound **IV**).

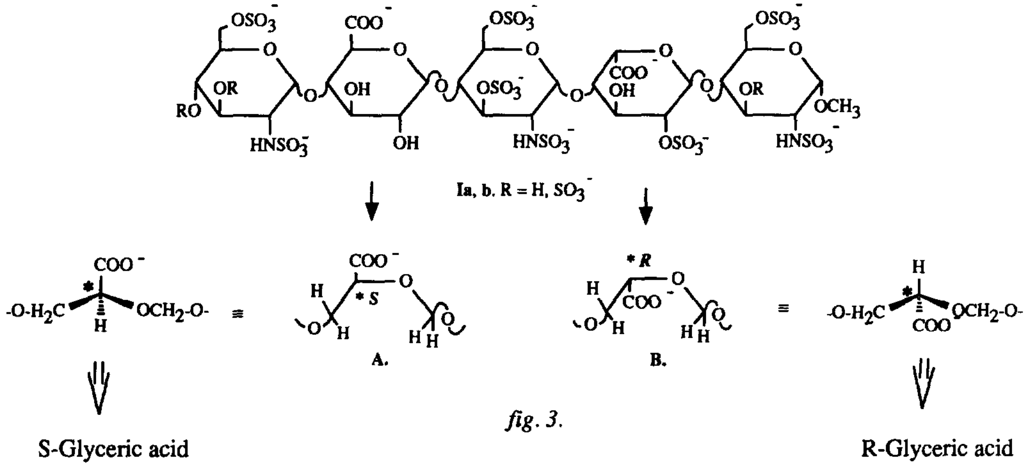


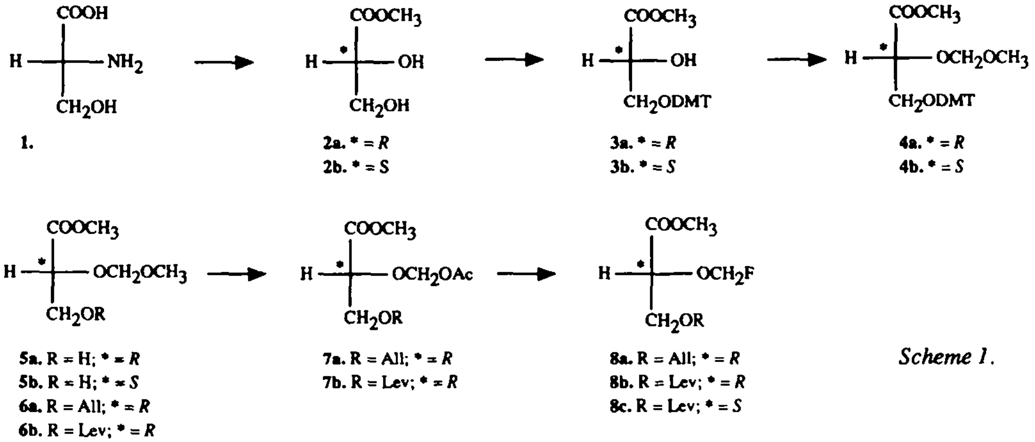
fig. 3.

Results and Discussion.

The crucial steps in the synthesis of heparin analogues containing "opened" uronic acid moieties such as the pentamers **II**, **III**, **IV** and **V**, involves the syntheses and the incorporation of the glyceric acid oxymethylene moieties **A** and **B** (see *fig.3*). The moieties **A** and **B**, replacing the uronic acid units, expose the essential carboxylate groups in the appropriate configuration. Coupling of these "ring opened" carbohydrate fragments (*i.e.* "pseudo" uronic acids) with suitably protected building blocks, which have been used previously in the syntheses of heparin fragments[8,13-15], provide fully protected pentamers (*i.e.* compounds **16**, **17**, **21** and **34**). The latter derivatives are then converted into the desired heparin analogues **II**, **III**, **IV** and **V** using established reaction sequences.

Preparation of the "opened iduronic acid"- and "opened glucuronic acid" building blocks (8a,b and 8c respectively). (Scheme 1.)

The preparation of the "ring opened" building blocks **8a,b,c** is depicted in scheme 1. As is outlined in *fig.3* the building blocks (*i.e.* compounds **8a,b**) that have to replace the *L*-iduronic acid moiety can be synthesized from *R*-glyceric acid methylester (*i.e.* compound **2a**). Compound **2a** was obtained in a one pot reaction from D-serine (**1**) following the procedure described by Lok *et al*[23]. (see scheme 1). Alternatively compound **2a** could be afforded more easily by treating commercially available 2,3-*O*-isopropylidene-*R*-glyceric acid with 80% aqueous acetic acid. In this way compound **2a** was obtained in 84% yield.



Scheme 1.

The primary hydroxyl function of compound **2a** was then selectively protected with a 4,4'-dimethoxytrityl (DMT) ether by treating **2a** with 4,4'-dimethoxytrityl chloride in a mixture of tetrahydrofuran (THF) and pyridine at -15 °C to afford compound **3a** in 47% yield after chromatographic purification. Next the free secondary hydroxyl group was functionalised with a methoxymethyl (MOM) ether to introduce the required oxymethylene function in compound **4a**. For this purpose compound **3a** was treated with chloromethyl methyl ether to obtain compound **4a** in 87% yield after purification. In this reaction the presence of a large excess of diisopropylethylamine was found to be necessary in order to avoid premature cleavage of the DMT protecting group.

In the next step the DMT ether function of compound **4a** was selectively cleaved under mild acidic conditions (80% aqueous acetic acid) to give compound **5a** in a yield of 89%. The free hydroxyl function of compound **5a** should then be blocked with a temporary protecting group that is not affected during the subsequent steps. To this end we could either select an allyl ether or a levulinoyl ester[24]. In view of the low reactivity of the used glycosyl acceptors (*i.e.* compounds **9** and **18**, schemes 2 and 3 respectively) we initially selected the allyl protective group to increase the reactivity of the glycosyl donor[25] (*i.e.* compound **8a**). At a later stage, however, we found that the more easily available levulinoylated glycosyl donor **8b** can also be used.

The introduction of the allyl ether should be accomplished under mild conditions, because the basic conditions which are routinely used for allylation procedures, may lead to epimerization of the chiral centre of the glyceric acid methyl ester. Thus the *R*-isomer **5a** was first treated with allylchloroformate in pyridine followed by the decarboxylation of the resulting allyloxycarbonyl ester in the presence of a catalytic amount of tetrakis(triphenylphosphine)palladium(0)[26] in dioxane at elevated temperature, to give compound **6a** in an overall yield of 65%.

For the synthesis of the "under sulphated" pentamer analogue (*i.e.* compound **IV**) we resorted to the use of the levulinoyl ester as temporary protecting group for the "opened iduronic acid" building block. Treatment of the *R*-isomer **5a** with levulinic anhydride in pyridine in the presence of a catalytic amount of N,N-dimethylaminopyridine gave compound **6b** in quantitative yield.

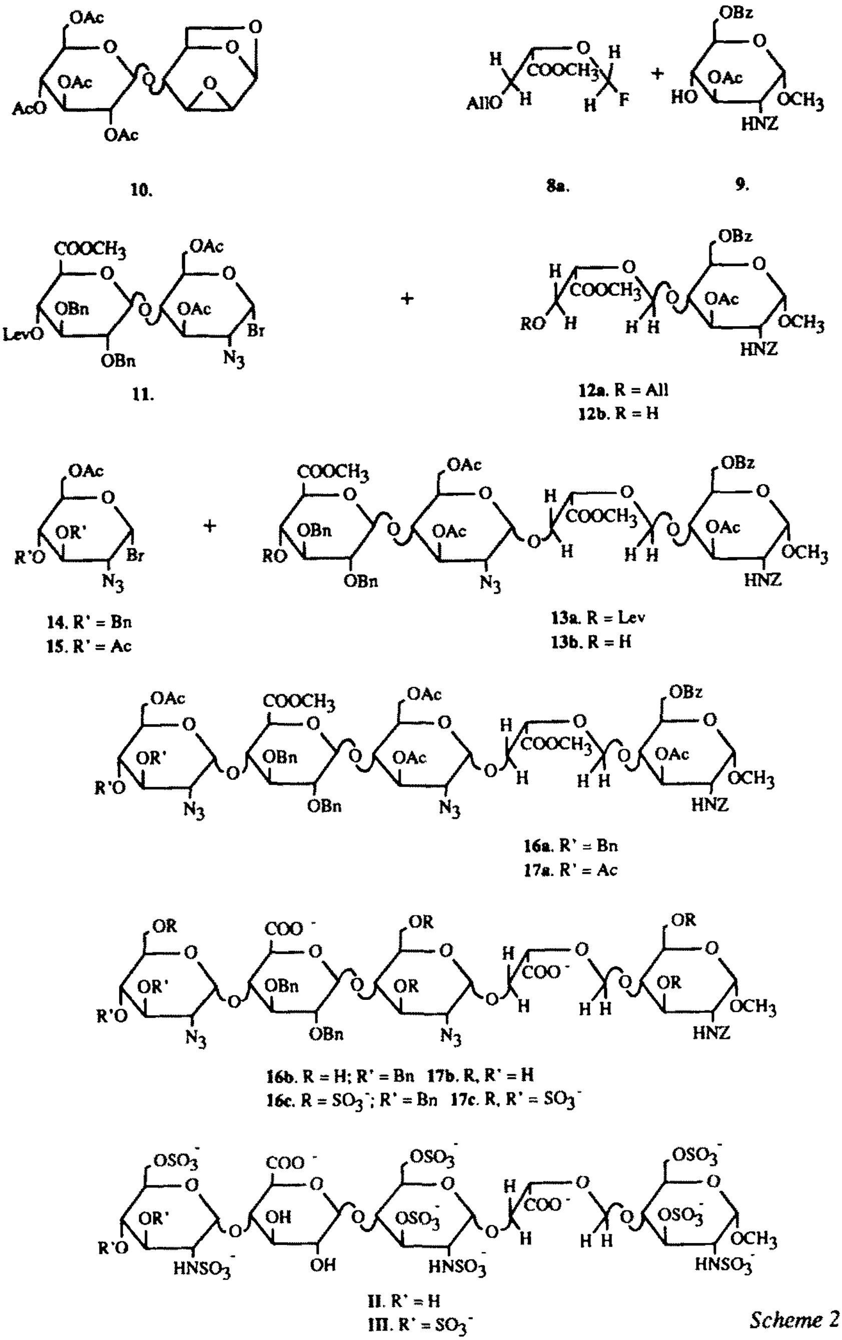
To finalize the synthesis of the "opened uronic acid" building blocks the following steps had to be performed: *i)* acetolysis of the MOM ether, to give the oxymethyl acetate moiety; *ii)* fluorination of the oxymethyl acetate moiety, to give the desired "glycosyl" donors **8a** and **8b**. The first step, the acetolysis of the MOM ether, is based on a fortuitous observation in our laboratory. It appeared that the MOM ether was selectively converted into the oxymethyl acetate ($-OCH_2OAc$) function under acetolysis conditions which are routinely used for the opening of 1,6 anhydro sugars[27]. Thus treatment of compounds **6a** and **6b** with acetic acid, acetic anhydride and trifluoroacetic acid (12/80/8, v/v/v) gave compounds **7a** and **7b** in yields of 99% and 58%, respectively. The oxymethyl acetate function shows a strong resemblance to an anomeric acetate of a carbohydrate. Accordingly, using a known fluorination methodology in carbohydrate chemistry (HF/pyridine in dichloromethane)[28], we could easily convert the oxymethyl acetate functions of compounds **7a** and **7b** into the activated oxymethyl fluoride derivatives compounds **8a** and **8b** in about 70% yield.

For the synthesis of the "opened glucuronic acid" pentamer we used building block **8c** which was prepared from the *S*-isomer **5b** following the same route as described for compound **8b**.

Syntheses of pentamers **II** *and* **III**. *(Scheme 2)*

An important part of the syntheses of pentamers **II** and **III** (see scheme 2) involves the preparation of dimer **12b** which next should be coupled to disaccharide **11** via an α-glycosidic bond to afford the required tetramer **13a**. Disaccharide **11** and its precursor compound **10**[8] are building blocks which have been used previously in the syntheses of various heparin-like derivatives. Glycosylation of tetramer **13b** with the known glycosyl donors **14** or **15** furnished the fully protected pentamers **16a** and **17a**, respectively.

The key intermediate **12b** was obtained after the condensation of glycosyl donor **8a** with glycosyl acceptor **9** followed by deprotection of the allyl ether. Thus, the coupling reaction was carried out in dry dichloromethane at -20 °C in the presence of boron trifluoride etherate as promoter[29] to afford dimer **12a** in 84% yield after chromatographic purification. Unfortunately, isomerization of the allyl protecting group of compound **12a** into the acid labile propenyl by the action of 1,5-cyclooctadiene-bis-[methyldiphenylphosphine]-iridium hexafluorophosphate[30] as a catalyst failed. On the other hand treatment of **12a** at room temperature with palladium(II)chloride[31] in a solution of sodium acetate in acetic acid for 16 hours furnished dimer **12b** in 40% yield.


10.
8a.
9.
11.
12a. R = All
12b. R = H
14. R' = Bn
15. R' = Ac
13a. R = Lev
13b. R = H
16a. R' = Bn
17a. R' = Ac
16b. R = H; R' = Bn 17b. R, R' = H
16c. R = SO3-; R' = Bn 17c. R, R' = SO3-
II. R' = H
III. R' = SO3-

Scheme 2.

Next the reactive primary hydroxyl function of **12b** was stereoselectively coupled with the known glycosyl donor **11** in dichloromethane at room temperature using a mixture of $HgBr_2/Hg(CN)_2$ as promoter according to the *in situ* anomerisation procedure as described by Paulsen[25]. Pure α-coupled tetramer **13a** was afforded in a yield of 65% after column chromatography. Cleavage of the levulinoyl group of compound **13a** with hydrazine acetate afforded the glycosyl acceptor **13b**. Coupling of tetramer **13b** with the known glycosyl bromides **14**[32] or **15** in dichloromethane at -35 °C, using silver triflate as promoter and 2,6-di-t-butylpyridine[8] as acid scavenger, gave after purification the fully protected pentamers **16a** and **17a** in yields of 50% and 45%, respectively. Finally the fully protected pentamers **16a** and **17a** were converted in four steps into the desired analogues **II** and **III** respectively.

The first step was the treatment of the protected pentamers with base to saponify the acetyl and methyl esters simultaneously. For this purpose both pentamers (*i.e.* compounds **16a** and **17a**) were dissolved in chloroform and added dropwise to a mixture of sodium hydroxide (4n) and methanol. After 16 hours at room temperature TLC analysis revealed complete saponification. The reaction mixtures were acidified to pH≈2 followed by extraction with dichloromethane to give the saponified pentamers **16b** and **17b** which could be purified by short column chromatography. Due to the high polarity of compound **17b**, which provoked difficulties in the extraction and purification procedures, the yield of the saponification step was only 37%, whereas compound **16b** could be isolated in 76%. In the next step the free hydroxyl groups of the saponified pentamers (*i.e.* **16b** and **17b**) were sulphated by the action of trimethylamine-sulphur trioxide complex in dry N,N-dimethylformamide at 50 °C for 24 hours. To remove the excess of sulphating reagent the crude reaction mixture was directly applied on a gel permeation column (LH-20) and eluted with N,N-dimethylformamide containing 0.5% (v/v) triethylamine. The fractions which contained sulphated pentamer (*i.e.* **16c** and **17c**) were pooled and evaporated to dryness, followed by elution in water from a DOWEX 50WX4-Na^+ ion-exchange column. Next, the benzyl ethers and the azido groups were simultaneously hydrogenolyzed in a mixture of water and t-butanol in the presence of palladium on activated charcoal. Subsequently, N-sulphation was carried out in water, under buffered conditions (pH≈9.5), at room temperature for 20 hours using pyridine-sulphur trioxide complex. The crude reaction mixtures were exchanged over a DOWEX 50WX4-Na^+ column and the eluate was concentrated to a small volume and desalted on a Sephadex G-25 column.

After gel-permeation chromatography (G-25), analogue **II** was isolated in an overall yield of 22% (over the last four steps) and showed a purity of 97% by ^1H-NMR spectroscopy. The ^1H-NMR spectrum of compound **III**, however, clearly revealed the presence of approximately 15% of side-products, probably under O-sulphated material. Apparently, the O-sulphation of the three hydroxyl groups of the non-reducing glucosamine unit (*i.e. unit 2*) of analogue **III** proceeds extremely laboriously. Purification of analogue **III**, using standard Sephadex-DEAE followed by Sephadex Q-Sepharose (fast flow) ion exchange chromatography raised the purity to only 90% as indicated by ^1H-NMR spectroscopy (see *fig. 4a*). Fortunately, further purification of analogue **III** could be realized on an HPLC system equipped with a mono-Q ion exchange column and a Chiramonitor detector[33]. With this method we could achieve a purity of about 95% according to ^1H-NMR spectroscopy (see *fig. 4b*). Because of the extensive purification procedure the overall yield over the four last steps was only 12% after desalting by gel-permeation chromatography (G-25).

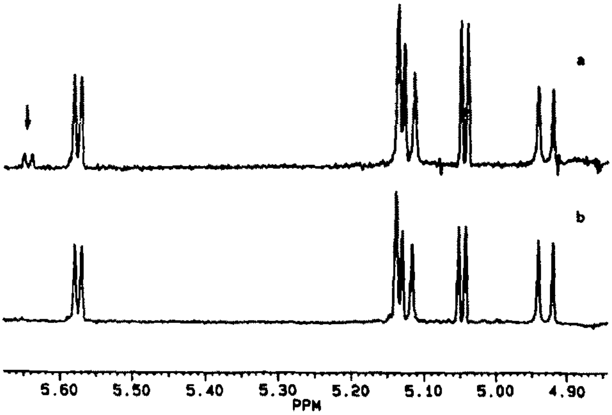


Fig. 4 Anomeric region of 360 MHz ^{1}H-NMR spectra of compound III before (a.) and after (b.) purification on an HPLC system equipped with a Mono-Q ion exchange colum and chira-monitor detection[33].

Synthesis of pentamer IV. (Scheme 3)

The synthesis of the "under sulphated" pentamer IV started with the preparation of a suitably protected dimer **19b** which was coupled to trisaccharide **20** to give the fully protected pentamer **21** as outlined in scheme 3.

Thus condensation of the "ring opened" carbohydrate fragment **8b** with monosaccharide **18** in dry dichloromethane at -10 °C using boron trifluoride etherate as promoter afforded dimer **19a** in a yield of 33% after purification on silicagel. Delevulinoylation of **19a** by treatment with hydrazine acetate gave the required glycosyl acceptor **19b**.

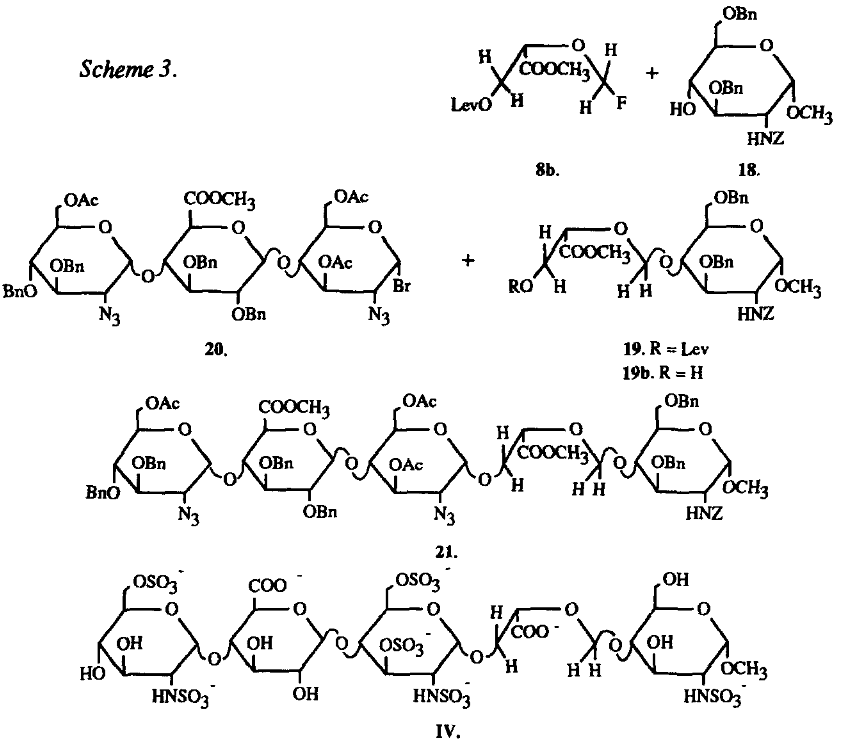
Glycosylation of **19b** with glycosyl bromide **20**[34], which was available from the syntheses of other heparin analogues, gave the fully protected pentamer **21**. The glycosylation reaction was carried out in dry dichloromethane using an mixture of $HgBr_2/Hg(CN)_2$ as promoter after which the fully protected pentamer **21** was isolated in 41% yield by silicagel chromatography.

Next saponification, O-sulphation, reduction/debenzylation and N-sulphation of pentamer **21**, according to the same procedures as described for the syntheses of pentamers II and III, afforded the desired analogue IV in an overall yield of 47% after gel-permeation chromatography. Further purification of the end product by ion-exchange chromatography was not required, because ^{1}H-NMR analysis revealed a purity of 95%.

Synthesis of pentamer V. (Schemes 4, 5 and 6)

For the preparation of pentamer V we had to consider a different synthetic strategy with regard to that one followed for analogues II, III and IV. The use of intermediate 1,6-anhydro sugars is not possible, since *e.g.* the acetolysis of compound **22** to give compound **23** (see scheme 4) would be accompanied by simultaneous cleavage of the oxymethylene acetal moiety.

Scheme 3.



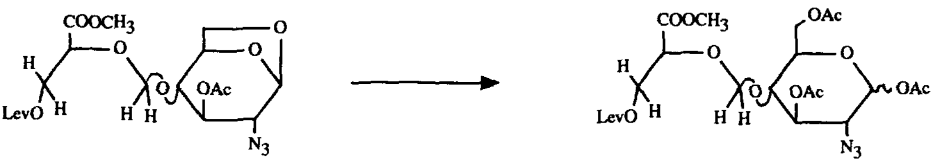
8b. 18.

20. 19. R = Lev
19b. R = H

21.

IV.

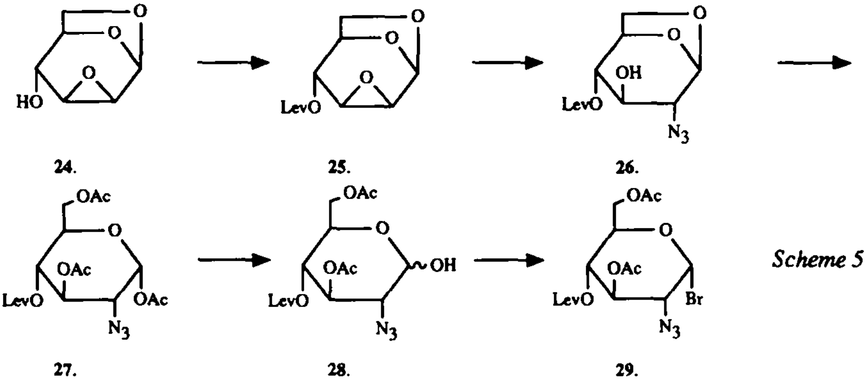
For this reason the synthesis of analogue **V** starts from the reducing end disaccharide (*i.e.* compound **30** in scheme 6) followed by the stepwise chain elongation with suitably protected monomers (*i.e.* compounds **29**, **8c** and **33**, respectively) to afford the fully protected pentamer **34**.



22. *Scheme 4.* 23.

To this end we had to prepare the suitably protected monosaccharide **29** which could be easily obtained from the Cerny epoxide **24**[35] (see scheme 5).

The free hydroxyl function of compound **24** was temporally protected with a levulinoyl ester in quantitative yield. Opening of the epoxide of compound **25** was accomplished by the action of sodium azide in N,N-dimethylformamide in the presence of para-toluenesulfonic acid and 2,6-lutidine[8]. After 3 days at 100 °C compound **26** was furnished in a yield of 64% after silicagel chromatography. Acetolysis of **26** at 40 °C for 24 hours gave compound **27** in a yield of 87%. Anomeric saponification of compound **27** was conducted in THF using piperidine as base[36] to afford compound **28** in 71% yield after column chromatography.



Scheme 5.

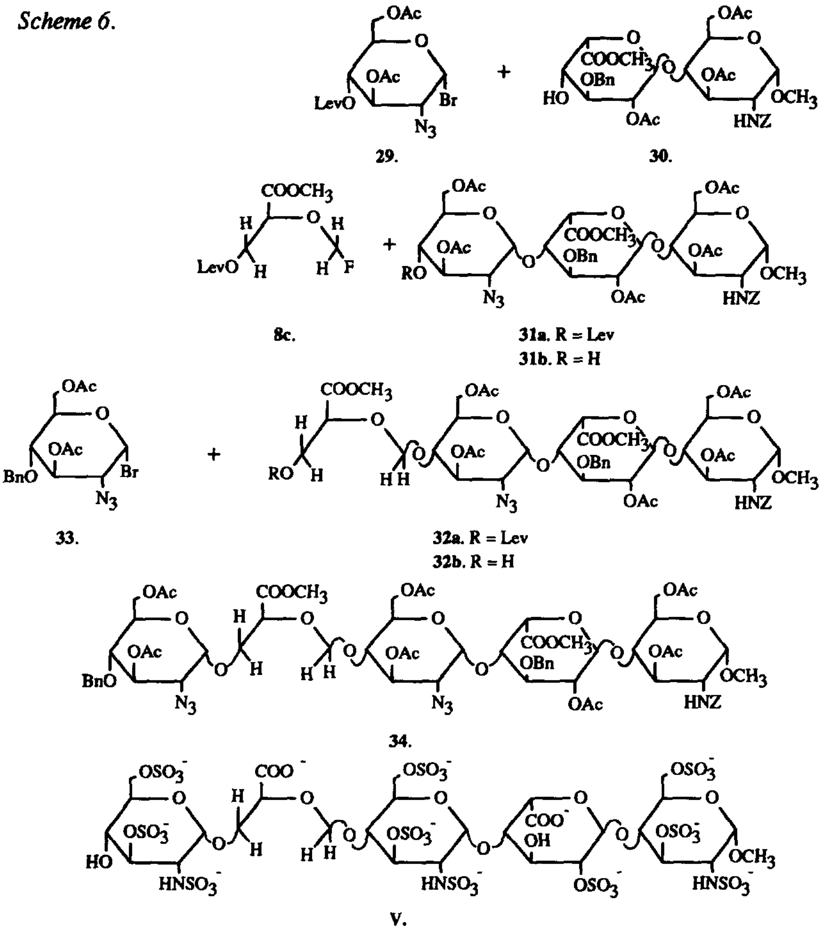
Finally the free anomeric centre of **28** was brominated by the action of oxalyl bromide in dry dichloromethane in the presence of N,N-dimethylformamide[37] to give after purification the desired glycosyl bromide **29** in 89% yield.

Condensation of the glycosyl donor **29** with the known glycosyl acceptor **30** in dichloromethane, promoted by silver triflate in the presence of molecular sieves 10 Å, furnished trisaccharide **31a** in a yield of 44%. After cleavage of the levulinoyl group, trisaccharide **31b** was coupled with the *S*-glyceric acid building block **8c** in dichloromethane using boron trifluoride etherate as promoter to afford tetramer **32a** in 77% yield after purification on silicagel. Selective removal of the levulinoyl ester of **32a** provided the required glycosyl acceptor **32b**, which in the next step could be glycosylated with the monosaccharide **33**. The α-coupling of the reactive primary hydroxyl function was performed under the mild conditions ($HgBr_2/Hg(CN)_2$), described above, to give the fully protected pentamer **34** in a yield of 75%. ^{1}H-NMR analysis of pentamer **34** revealed that the product contained 15-20% of another compound, probably the β-isomer. Unfortunately, we were not able to purify pentamer **34** at this stage of the synthesis.

In the next step, compound **34** had to be treated with base to saponify simultaneously the methyl- and acetyl esters. Since the iduronic acid methyl ester of the fully protected pentamer is prone to β-elimination[38], upon treatment with sodium hydroxide, a modified saponification procedure had to be found. We reasoned that a rapid saponification of the methyl ester would solve this problem because the presence of the carboxylate group suppresses the β-elimination. Thus, pentamer **34** was treated with a mixture of $LiOH/H_2O_2$ in THF[39] at low temperature to cleave the methyl ester rapidly, after which sodium hydroxide and methanol were added to complete the saponification. After 16 hours at room temperature the mixture was worked-up to afford the saponified product in nearly quantitative yield.

After O-sulphation, reduction/debenzylation and N-sulphation, according to the procedures previously described, analogue **V** could be isolated, but showed a purity of only 85% by ^{1}H-NMR spectroscopy. Further purification of analogue **V** was performed on a Sephadex DEAE ion-exchange column, followed by gel-permeation chromatography (G-25), to afford analogue **V** in an overall yield of 35% (starting from compound **34**) displaying a purity of about 95% by ^{1}H-NMR spectroscopy.

Scheme 6.



29.

30.

8c.

31a. R = Lev
31b. R = H

33.

32a. R = Lev
32b. R = H

34.

V.

Biological activities and structure - activity relationships of heparin analogues II - V.

The anti-coagulant properties of the synthetic heparin analogues presented here (*i.e.* compounds **Ia** - **V**, see Table I) are measured as the AT III mediated αXa[40] activity and the Heparin Cofactor II (HC II) mediated αIIa[41] activity. Both activities are determined in an amidolytic assay[42]; the αXa activity is determined with the chromogenic substrate S2222 and the αIIa activity with the chromogenic substrate S2238.

In Table I the AT III mediated αXa activities and the HC II mediated αIIa activities of the "opened" uronic acid analogues (*i.e.* compounds **II**, **III**, **IV** and **V**) are listed together with the biological activities found for pentamer **Ia** and its extra 3-O-sulphated analogue **Ib**.

Table I. Measured anti-coagulant activities of the heparin-like fragments **Ia - V**.

entry	compound	AT III mediated anti-Xa activity in U/mg	HC II mediated anti-IIa activity in U/mg (N)[a]
1	**Ia**	700	0.5 (10)
2	**Ib**	1250	1.0 (11)
3	**II**	150	0.4 (10)
4	**III**	162	1.5 (12)
5	**IV**	21	0.1 (9)
6	**V**	19	1.6 (12)

[a] *number of negative charges.*

From Table I, we may conclude that of the new analogues only compounds **II** and **III** (entry 3 and 4, Table I) elicit a significant αXa activity. This proofs that the idopyranuronate ring of the AT III binding heparin fragment is not essential for the activation of AT III. The glucopyranuronate ring, on the contrary, seems to be irreplaceable as is reflected by the low biological activity of pentamer **V** (entry 6, Table I). In this analogue the glucuronic acid unit has been replaced by an *S*-glyceric acid oxymethylene residue whilst all the essential and contributing groups, as are present in compound **Ib**, have been maintained.

Thus, the introduction of flexible "opened" uronic acid functions (*i.e. R* or *S* glyceric acid oxymethylene residues) in heparin like analogues is only allowed at the level of iduronic acid. These results can be understand when we contemplate the conformational properties of the idopyranuronate - and glucopyranuronate ring, respectively. It is well established that iduronic acid (*unit 5*) is the only flexible sugar in the AT III binding heparin fragment[43-46]. Conformational analysis, under low ionic conditions, reveals that the iduronic acid moiety occurs in an equilibrium between a ^2S_O skewboat and a $^1C_4$ chair conformation. In fact the conformational diversity of the heparin fragment is mainly controlled by the iduronic acid unit.

On the other hand, the glucuronic acid unit is fixed in the rigid $^4C_1$ chair conformation, just like the glucosamine units *2*, *4* and *6*. Most likely, the replacement of the rigid glucuronic acid unit by the flexible *S*-glyceric acid oxymethylene residue brings about a drastic change in the conformational behaviour[47,48] of the heparin fragment which causes the drop in biological activity.

Concerning the role of the individual sulphate groups in the class of "opened" iduronic acid pentamer analogues, we observed the same tendency as was found for the synthetic analogues of the natural product. For instance, the absence of the two contributing O-sulphate groups at position 3 and 6 of the reducing glucosamine unit in pentamer **IV** results in a 7-fold drop in the αXa activity. A similar drop in αXa activity was reported by us previously for an analogue of the natural product lacking also these O-sulphate groups at the reducing glucosamine unit[14].

Although the inactivation of factor II (thrombin) by HC II is only weakly catalysed by the synthetic pentamers (see table I), the αIIa activity is slightly raised when the amount of sulphate groups in the pentamer is increased. This is in agreement with other reports in which it was stated that the HC II mediated αIIa activity is proportional to the polyanionic character (number of negative charges) of the catalyst involved[49]. Others claim that the length of the polyanion is also of importance[50]. Furthermore, the introduction of two extra sulphate groups at *unit 2* of pentamer **II** (i.e. compound **III**) does not significantly affect the αXa activity.

In conclusion, the α-*L*-iduronic acid unit of the AT III binding heparin fragment (*i.e.* compound **Ia**) can be replaced by an *R*-glyceric acid oxymethylene residue with retention of a significant AT III mediated αXa activity. This modification simplifies the synthesis of the corresponding heparin like fragment, because the *R*-glyceric acid oxymethylene building block is easier to synthesize than α-*L*-iduronic acid.

A similar modification of the β-*D*-glucuronic acid unit by an *S*-glyceric acid oxymethylene residue provides a virtually inactive analogue.

Acknowledgement We wish to thank Mr. G. N. Wagenaars (Organon analytical R&D laboratories) for recording the NMR-spectra, Miss C. Smits (General Pharmacoloy R&D laboratories) for measuring the biological activities of the mentioned pentamers and Drs. R. Gebhardt for the assistance with the preparation of pentamers II and III.

Experimental part

General procedures.

Dioxane and pyridine were dried by heating with CaH_2 under reflux and then distilled; N,N-dimethylformamide (DMF) was stirred with CaH_2 at room temperature and distilled under reduced pressure. Tetrahydrofuran was distilled from $LiAlH_4$. Dichloromethane, chloroform, ether and toluene were distilled from P_2O_5. Pyridine was stored over molecular sieves 4Å, toluene and ether over sodium wire and dichloromethane over basic alumina. Optical rotations were recorded at ambient temperature with a Perkin Elmer 241 polarimeter. TLC analysis was performed on Merck - Fertigplatten (Kieselgel 60 F254, 5 x 10 cm.). Compounds were visiualized by spraying with sulphuric acid/ethanol (1/9;v/v) or by Usui (110 g of molybdate phosphoric acid dissolved in 2200 ml ethanol and 110 ml sulphuric acid). Column chromatography was performed on Kieselgel 60, 70-230 mesh (Merck). Gel filtration was performed on Sephadex LH-20 (Pharmacia). The purifications of the end products were performed on a Sephadex DEAE-A25 or Q-sepharose ion exchange column (Pharmacia) and in some cases on a Waters HPLC system equipped with a Mono-Q ion exchange column (Pharmacia). Desalting of the end products was performed on Sephadex G-25 (Pharmacia). ^{1}H-NMR spectra were recorded on a Bruker WM 360 spectrometer equipped with an ASPECT 3000 computer or a Bruker WM 200 spectrometer; chemical shifts are given in ppm (δ) relative to TMS as internal reference, or relative to D_2O.

General deprotection/sulphation procedures.

Saponification.

To a mixture of chloroform (5.5 ml), methanol (27 ml) and sodium hydroxide (4n, 5.5 ml) was added at room temperature a solution of the fully protected pentamer (0.1 mmole) in chloroform (5.5 ml). After 16 hours the reaction mixture was cooled to 0 °C and acidified to pH≈2 with hydrochloric acid (6n). The reaction mixture was extracted with dichloromethane (5 X 5 ml) and the organic layers were washed with ice water (5 ml). The extract was dried ($MgSO_4$) and evaporated to dryness. The residue was purified over silicagel (4 g, dichloromethane/methanol, 85/15 → 8/2) to give the saponified pentamer.

O-sulphation.

The saponified pentamer (0.1 mmole) was dissolved in N,N-dimethylformamide (6.5 ml). Under nitrogen atmosphere, trimethylamine sulphur trioxide complex (5 eq. for each hydroxyl group) was added and the mixture was stirred for 16 hours at 50 °C. Next, the reaction mixture was directly applied on a Sephadex LH-20 column and eluted with N,N-dimethylformamide containing 0.5% (v/v) triethylamine. The fractions which contained sulphated pentamer were pooled and evaporated to dryness. The residue was eluted from a DOWEX 50WX4-Na^+ ion-exchange column in water and the eluate was condensed to dryness to give the O-sulphated pentamer.

Hydrogenolysis.

A solution of the O-sulphated pentamer (0.1 mmole) in a mixture of water (25 ml) and t-butanol (9 ml) was stirred under hydrogen atmosphere in the presence of 10% palladium on activated charcoal (75% w/w with regard to the pentamer) for 20 hours. After filtration of the suspension, the filtrate was concentrated. Then, the residue was directly used for the N-sulphation.

N-sulphation.

After hydrogenolysis the pentamer was dissolved in water (10 ml) and stirred in the presence of pyridine sulphur trioxide complex (50 mg) and sodium carbonate (50 mg). A second, third and fourth portion of pyridine sulphur trioxide complex and sodium carbonate were added after 2,4 and 6 hours stirring, respectively. After stirring for 20 hours the reaction mixture was applied on a DOWEX 50WX4-Na^+ ion-exchange column and eluted with water. The eluate was concentrated and desalted on a column of Sepahadex G-25. The appropriate fractions were pooled and concentrated to a small volume which was lyophilized to give the desired pentamer. Purification, if necessary, was conducted on Sephadex DEAE A-25 or Q-Sepharose (ion exchange chromatography) with a salt gradient (0.5 M → 2.0 M NaCl).

*(R)-glyceric acid methyl ester (***2a***).* -- Compound **2a** can be obtained from D-serine (i.e. compound **1**) as described by Lok et al[22]. or by treatment of 2,3-*O*-isopropylidene-(*R*)-glyceric acid methyl ester (4.05 g, 25.3 mmole) in 70 % acetic acid (70 ml) at room temperature overnight. The reaction mixture was evaporated to dryness and coevaporated with toluene. The residue was eluted from a silicagel column (200 g, CH_2Cl_2/acetone, 98/2 → 8/2) to afford compound **2a** in 84% yield (2.55 g, 21.3 mmole). R_f 0.45 (CH_2Cl_2/acetone 9/1,); $[\alpha]^{20}$ +7.7° (c 0.94; CH_2Cl_2).

*(S)-glyceric acid methyl ester (***2b***).* -- The (*S*)-glyceric acid methyl ester **2b** was obtained from 2,3-O-isopropylidene-(*S*)-glyceric acid methyl ester according to the same procedure as described for its R-isomer compound **2a**. Yield 98%; R_f 0.23 (CH_2Cl_2/CH_3OH, 95/5,); $[\alpha]^{20}$ -7.1° (c 0.94; CH_2Cl_2).

*3-O-dimethoxytrityl-(R)-glyceric acid methyl ester (***3a***) and 3-O-dimethoxytrityl-(S)-glyceric acid methyl ester (***3b***).* -- A solution of 4,4'-dimethoxytrityl chloride (3.88 g, 11.46 mmole) in dry tetrahydrofuran (51 ml) was added dropwise to a mixture of compound **2a** (1.25 g, 10.42 mmole) in dry pyridine (51 ml) and stirred at a temperature of -15 °C. After 16 hours the mixture was diluted with dichloromethane, washed with water, saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over a Whatmann phase separator filter. The filtrate was condensed to dryness and coevaporated with dry toluene. The crude product was purified by silicagel chromatography (200 g) with toluene/acetone 95/5 → 9/1 to give compound **3a** in 47% yield (2.07 g, 4.93 mmole). R_f 0.67 (toluene/acetone, 9/1,). ^{1}H-NMR (200 MHz) ($CDCl_3$): δ 3.14 (d, 1H, $O\underline{H}$, $J_{H,OH}$ 8.0 Hz); 3.40 (dq, 2H, $-C\underline{H}_2$-ODMT, $J_{gem.}$ 10.0 Hz, $J_{vic.}$ 3.0 Hz and 4.0 Hz); 3.76 (s, 3H, $-COOC\underline{H}_3$); 3.78 (s, 6H, 2 X $OC\underline{H}_3$ DMT); 4.27 (dt, 1H, $C\underline{H}$); 6.79 - 7.45 (m, 13H, DMT).

Compound **3b** was obtained following the same route as described above starting from compound **2b**. Yield 45%; R_f 0.60 (toluene/acetone, 9/1,). ^{1}H-NMR (220 MHz) ($CDCl_3$): δ 3.13 (d, 1H, $O\underline{H}$, $J_{H,OH}$ 8.0 Hz); 3.41 (dq, 2H, $-C\underline{H}_2$-ODMT, $J_{gem.}$ 9.4 Hz, $J_{vic.}$ 3.0 Hz and 3.4 Hz); 3.72 (s, 3H, $-COOC\underline{H}_3$); 3.78 (s, 6H, 2 X $OC\underline{H}_3$ DMT); 4.26 (dt, 1H, $C\underline{H}$); 6.79 - 7.45 (c, 13H, DMT).

*3-O-dimethoxytrityl-2-O-methoxymethyl-(R)-glyceric acid methyl ester (***4a***)* and *3-O-dimethoxytrityl-2-O-methoxymethyl-(S)-glyceric acid methyl ester (***4b***).* -- Compound **3a** (2.07 g, 4.93 mmole) was dissolved in dichloromethane (20 ml) and diisopropylethylamine (8.5 ml, 4.9 mmole) and stirred for 5 minutes at ambient temperature. Next chloromethyl methyl ether (1.58 ml, 19.6 mmole) was added dropwise to the mixture where after the mixture was refluxed at 40 °C during 4 hours. The reaction mixture was cooled to room temperature and diluted with dichloromethane. The organic layers were washed with saturated aqueous $NaHCO_3$, water and brine and dried over a Whatmann phase separator filter. The filtrate was evaporated to dryness and the residue was purified on a silicagel column (60 g, toluene/acetone, 95/5) to give compound **4a** in 87% yield (1.98 g, 4.25 mmole); R_f 0.44 (toluene/acetone, 95/5); ^{1}H-NMR (200 MHz) ($CDCl_3$): δ 3.39 (s, 3H, $CH_2OC\underline{H}_3$); 3.41 (d, 2H, $-C\underline{H}_2$-ODMT, J 5.0 Hz); 3.72 (s, 3H, $-COOC\underline{H}_3$); 3.79 (s, 6H, 2 X $OC\underline{H}_3$ DMT); 4.32 (t, 1H, $-C\underline{H}$); 4.76 (AB, 2H, $-OC\underline{H}_2O-$, J 5.5 Hz); 6.78 - 7.45 (c, 13H, DMT).

Starting from **3b**, compound **4b** could be prepared according to the same procedure as described for compound **4a**. Compound **4b** was however not isolated but directly used in the next step.

*2-O-methoxymethyl-(R)-glyceric acid methyl ester (***5a***)* and *2-O-methoxymethyl-(S)-glyceric acid methyl ester (***5b***)*. -- Compound **4a** (3.5 g, 7.5 mmole) was dissolved in 80% acetic acid (50 ml) and stirred at room temperature. After 2 hours the mixture was diluted with water and pyridine and evaporated to a small volume. Next, the mixture was diluted once more with pyridine and evaporated to dryness. Elution of the crude product from a silicagel column (50 g, CH_2Cl_2/acetone, 99/1 → 9/1) afforded compound **5a** (1.1 g, 89%). R_f 0.12 (toluene/acetone, 9/1). ^{1}H-NMR (200 MHz) ($CDCl_3$): δ 3.43 (s, 3H, $-CH_2OC\underline{H}_3$); 3.78 (s, 3H, $-COOC\underline{H}_3$); 3.88 (dq, 2H, $-C\underline{H}_2OH$, $J_{gem.}$ 12 Hz, $J_{vic.}$ 4 Hz and 5.8 Hz); 4.27 (dd, 1H, $-C\underline{H}$); 4.79 (AB, 2H, $-OC\underline{H}_2O-$).

Compound **5b** was prepared according to the same procedure with this difference that intermediate **4b** was not purified. After two steps compound **5b** could be isolated in 62.5%. R_f 0.15 (toluene/acetone, 9/1). The ^{1}H-NMR data of compound **5b** were identical as those found for compound **5a**.

*3-O-allyl-2-O-methoxymethyl-(R)-glyceric acid methyl ester (***6a***)*. -- A solution of allyloxycarbonyl chloride (320 µl, 3.0 mmole) in acetonitrile (5 ml) was added dropwise to a mixture of compound **5a** (415 mg, 2.5 mmole) in dry pyridine (20 ml) in approximately 30 minutes at a temperature of -35 °C. After 2 hours the mixture was diluted with dichloromethane, washed with saturated aqueous $NaHCO_3$, water and brine. The organic layer was dried over $MgSO_4$ and evaporated to dryness. The residue was purified over silicagel (10 g, dichloromethane/acetone, 99/1 → 98/2) and the isolated allyloxycarbonyl intermediate (450 mg, 73%) was dissolved in dry dioxane (8 ml) after which , under argon, a catalytic amount of tetrakis(triphenylphosphine)paladium(0) was added. Then the mixture was refluxed during 20 minutes at a temperature of 110 °C. Next, the mixture was cooled to room temperature and evaporated to dryness. Purification over silicagel (7.5 g, dichloromethane/acetone 99/1 → 98/2) gave compound **6a** (330 mg, 65% overall yield). R_f 0.32 (CH_2Cl_2/acetone, 98/2). ^{1}H-NMR (200 MHz) ($CDCl_3$): δ 3.41 (s, 3H, $-OC\underline{H}_3$); 3.76 (d, 2H, $-C\underline{H}_2OCH_2CHCH_2$, $J_{vic.}$ 4.2 Hz); 3.77 (s, 3H, $-COOC\underline{H}_3$); 4.04 (m, 2H, $O-C\underline{H}_2CHCH_2$); 4.35 (t, 1H, $-C\underline{H}$); 4.75 (t, 2H, $-O-C\underline{H}_2-O$, J 7.2 Hz); 5.23 (c, 2H, $O-CH_2CHC\underline{H}_2$); 5.88 (m, 1H, $O-CH_2C\underline{H}CH_2$).

*3-O-levulinoyl-2-O-methoxymethyl-(R)-glyceric acid methyl ester (***6b***)*. -- Compound **5a** (1.07 g, 6.5 mmole) was dissolved in dry pyridine (15 ml). At 0 °C, levulinic anhydride in ether (10 ml, 1 M) was added, together with a catalytic amount of N,N-dimethylaminopyridine, whereupon the mixture was stirred for 2 hours at room temperature. Next, water (10 ml) was added to the reaction mixture followed by dilution with dichloromethane. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine and dried over a whatmann phase separator filter. The filtrate was evaporated to dryness and purified by silicagel chromatography (70 g, toluene/ethyl acetate, 3/2 → 2/3) to give compound **6b** in quantitative yield (1.7 g, 6.5 mmole). R_f 0.44 (toluene/ethyl acetate, 1/1). ^{1}H-NMR (200 MHz) ($CDCl_3$): δ 2.19 (s, 3H, $C\underline{H}_3COCH_2CH_2CO-$); 2.56 - 2.80 (m, 4H, $CH_3COC\underline{H}_2C\underline{H}_2CO-$); 3.42 (s, 3H, $-CH_2OC\underline{H}_3$); 3.78 (s, 3H, $-COOC\underline{H}_3$); 4.30 - 4.46 (c, 3H, $\underline{H}C-C\underline{H}_2-$); 4.72, 4.77 (d, d, 2H, $-OC\underline{H}_2O-$, $J_{gem.}$ 6.6 Hz).

*2-O-acetoxymethyl-3-O-allyl-(R)-glyceric acid methyl ester (***7a***)*. -- Compound **6a** (120 mg, 0.60 mmole) was dissolved in a mixture of acetic anhydride, acetic acid and trifluoroacetic acid (24 ml, 80/12/8, v/v/v) and stirred for 1 hour at room temperature. Thereafter the mixture was concentrated and twice coevaporated with toluene to give compound **7a** in quantitative yield (140 mg, 0.60 mmole). R_f 0.44 (toluene/acetone, 9/1). ^{1}H-NMR (200 MHz) ($CDCl_3$): δ 2.09 (s, 3H, $-COC\underline{H}_3$); 3.76 (c, 2H, $HC-C\underline{H}_2-$); 4.04 (m, 2H, $-C\underline{H}_2CHCH_2$); 4.40 (t, $\underline{H}C-CH_2-$, J 4.4 Hz); 5.15 - 5.34 (c, 2H, $-CH_2CHC\underline{H}_2$); 5.37 (s, 2H, $-OC\underline{H}_2O-$); 5.87 (m, 1H, $-CH_2C\underline{H}CH_2$).

*2-O-acetoxymethyl-3-O-levulinoyl-(R)-glyceric acid methyl ester (***7b***)*. -- Compound **6b** (1.7 g, 6.5 mmole) was dissolved in a mixture of acetic anhydride (21 ml), acetic acid (3 ml) and trifluoroacetic acid (2.1 ml) and stirred at room temperature for 2 hours. After that the reaction mixture was evaporated to dryness and the residue was eluted from a silicagel column (70 g, toluene/acetone, 9/1 → 8/2) to give compound **7b** (1.1 g, 58%). R_f 0.35 (toluene/acetone, 8/2). ^{1}H-NMR (200 MHz) ($CDCl_3$): δ 2.10 (s, 3H, $-COC\underline{H}_3$); 2.55 - 2.80 (c, 4H, $CH_3COC\underline{H}_2C\underline{H}_2CO-$); 3.79 (s, 3H, $-COOC\underline{H}_3$); 4.28 - 4.55 (c, 3H, $\underline{H}C-C\underline{H}_2-$); 5.16 (s, 2H, $-OC\underline{H}_2O-$).

3-O-allyl-2-O-fluoromethyl-(R)-glyceric acid methyl ester (**8a**). -- Compound **7a** (760 mg, 3.3 mmole) was dissolved in dichloromethane (15 ml) and cooled to 0 °C. At this temperature, 70 % HF/pyridine (3.5 ml) was added dropwise to the reaction mixture. After 1 hour the reaction mixture was poured out in aqueous sodium acetate and dichloromethane and stirred for 5 minutes. The organic layer was washed with saturated aqueous $NaHCO_3$ and brine and dried over $MgSO_4$. The crude product was purified over silicagel (30 g, toluene/acetone, 99/1 → 95/5) to afford compound **8a** in 68% yield (430 mg, 2.24 mmole). R_f 0.76 (toluene/ethyl acetate, 1/1); $[\alpha]^{20}$ +49.0° (c 0.77; CH_2Cl_2). ^{1}H-NMR (200 MHz) ($CDCl_3$): δ 3.80 (s, 3H, $-COOC\underline{H}_3$); 3.81 (dd, 2H, $HC-C\underline{H}_2-$, $J_{gem.}$ 5.0 Hz, $J_{vic.}$ 1.0 Hz); 4.05 (m, 2H, $-C\underline{H}_2CHCH_2$); 4.46 (dq, 1H, $\underline{H}C-CH_2-$); 5.15 - 5.33 (c, 2H, $-CH_2CHC\underline{H}_2$); 5.20 - 5.58 (ABX, 2H, $-OC\underline{H}_2F$, $J_{H,H}$ 3.0 Hz, $J_{H,F}$ 15.6 Hz); 5.88 (m, 1H, $-CH_2C\underline{H}CH_2$).

2-O-fluoromethyl-3-O-levulinoyl-(R)-glyceric acid methyl ester (**8b**) and *2-O-fluoromethyl-3-O-levulinoyl-(S)-glyceric acid methyl ester* (**8c**). -- Compound **7b** (1.07 g, 3.7 mmole) was treated, according to the same procedure as described above, with 70% HF/pyridine (4.5 ml) to give after work-up and purification on silicagel (70 g, toluene/ethyl acetate, 3/2 → 2/3) compound **8b** (651 mg, 70%). R_f 0.56 (toluene/ethyl actate, 1/1); $[\alpha]^{20}$ +30.3° (c 1.4; CH_2Cl_2). ^{1}H-NMR (200 MHz) ($CDCl_3$): δ 2.18 (s, 3H, $C\underline{H}_3COCH_2CH_2CO-$); 2.56 - 2.80 (c, 4H, $CH_3COC\underline{H}_2C\underline{H}_2CO-$); 3.82 (s, 3H, $-COOC\underline{H}_3$); 4.35 - 4.55 (c, 3H, $\underline{H}C-C\underline{H}_2-$); 5.18 - 5.56 (ABX, 2H, $-OC\underline{H}_2F$, $J_{H,H}$ 3.0 Hz, $J_{H,F}$ 15.4 Hz and 13.8 Hz).

Compound **8c** was synthesized according to the same procedure as described for compound **8b** starting from compound **5b**. R_f 0.50 (toluene/ethyl acetate, 1/1); $[\alpha]^{20}$ -29.1° (c 1.4; CH_2Cl_2). The ^{1}H-NMR data are identical as the data found for compound **8b**.

Dimer **12a**. -- A mixture of compound **8a** (340 mg, 1.75 mmole) and **9** (820 mg, 1.73 mmole) in dichloromethane (28 ml) was stirred for 1 hour at room temperature in the presence of activated molecular sieves 4Å. At a temperature of -20 °C a solution of 1 M $BF_3.Et_2O$ in dichloromethane (1.75 ml) was added to the reaction mixture in approximately 5 minutes. After 1 hour the reaction mixture was diluted with dichloromethane, filtered and washed with aqueous $NaHCO_3$ and brine. The organic layer was dried ($MgSO_4$) and evaporated to dryness. The residue was purified by silicagel chromatography (35 g, toluene/acetone, 99/1 → 9/1) to afford the desired dimer **12a** in 84% yield (910 mg). R_f 0.73 (toluene/acetone, 8/2), $[\alpha]^{20}$ +107.9° (c 1.06; CH_2Cl_2).

Dimer **12b**. -- Compound **12a** (400 mg, 0.62 mmole) was dissolved in acetic acid (1.4 ml) and water (0.07 ml). Under nitrogen, sodium acetate (132 mg, 1.61 mmole) and palladium chloride (133 mg, 0.74 mmole) were added and the mixture was stirred overnight. Then the mixture was diluted with dichloromethane , filtered, washed with water, aqueous $NaHCO_3$ and brine and subsequently dried ($MgSO_4$). The crude product was purified over SiO_2 (15 g, dichloromethane/acetone, 98/2 → 9/1) to give compound **12b** (112 mg, 40%). R_f 0.29 (dichloromethane/acetone, 9/1). ^{1}H-NMR (200 MHz) ($CDCl_3$): δ 1.92 (s, 3H, $-OC\underline{H}_3$); 2.82 (t, 1H, $O\underline{H}$); 3.42 (s, 3H, $-COOC\underline{H}_3$); 4.28 (dd, 1H, $-C\underline{H}$, J 3.8 Hz and 6.0 Hz); 4.62 (c, 2H,, H-6a, H-6b); 4.75 (d, 1H, H-1, J 4.0 Hz); 4.78, 4.92 (d, d, 2H, $-OC\underline{H}_2O-$, $J_{gem.}$ 7.0 Hz); 5.09 (AB, 2H, $-OC\underline{H}_2Ph$, J 12.0 Hz); 5.05 (d, 1H, $-N\underline{H}$, J 10.0 Hz); 5.23 (dd, 1H, H-3, J 9.0 Hz and 11.0 Hz).

Tetramer **13a**. -- To a solution of compound **12b** (400 mg, 0.66 mmole) in dichloromethane (16 ml) was added, under nitrogen, molecular sieves 4Å (1.2 g). After stirring the mixture for 2 hours at room temperature $HgBr_2$ (190 mg, 0.53 mmole) and $Hg(CN)_2$ (134 mg, 0.53 mmole) were added. The reaction mixture was cooled to -20 °C where after a solution of glycosyl bromide **11** (650 mg, 0.80 mmole) in dichloromethane (10 ml) was added dropwise over 30 minutes. After 48 hours at room temperature, the mixture was filtered over hyflo. The organic solution was washed with aqueous KBr (2.0 M), $NaHCO_3$ solution and brine. The organic layer was dried ($MgSO_4$) and evaporated to dryness. The crude product was purified over silicagel (50 g, toluene/acetone, 95/5 → 8/2) to give compound **13a** (600 mg, 65%). R_f 0.60 (toluene/acetone, 8/2).

Tetramer **13b**. -- To a solution of compound **13a** (460 mg, 0.34 mmole) in dry pyridine (2.0 ml) was added 4 ml of a mixture of pyridine, acetic acid and hydrazine hydrate (6/4/0.5, v/v/v). The mixture was stirred for 6 minutes at room temperature. Next, dichloromethane (50 ml) was added and the mixture was washed with water, $NaHCO_3$ solution and brine. The organic layer was evaporated to dryness and the residue was purified by chromatography on silicagel (20 g, dichloromethane/acetone, 97/3 → 9/1) to afford compound **13b** (370 mg, 87%). R_f 0.42 (dichloromethane/actone, 9/1); $[\alpha]^{20}$ +187.4° (c 1.0; CH_2Cl_2). ^{1}H-NMR (360 MHz) ($CDCl_3$):
UNIT 3: δ 4.32 (d, 1H, H-1, $J_{1,2}$ 7.8 Hz); 3.36 (dd, 1H, H-2, $J_{2,3}$ 9.4 Hz); 3.79 (s, 3H, $-COOC\underline{H}_3$).
UNIT 4: δ 4.98 (d, 1H, H-1, $J_{1,2}$ 3.8 Hz); 3.17 (dd, 1H, H-2, $J_{2,3}$ 10.8 Hz); 5.39 (dd, 1H, H-3, $J_{3,4}$ 9.0 Hz); 3.71 (dd, 1H, H-4, $J_{4,5}$ 10.0 Hz); 4.21 (dd, 1H, H-6a, $J_{5,6a}$ 4.2 Hz, $J_{6a,6b}$ 12.0 Hz).
UNIT 5: δ 3.80 (s, 3H, $-COOC\underline{H}_3$); 5.02 , 5.14 (d, d, 2H, $-OC\underline{H}_2O-$, $J_{gem.}$ 12.0 Hz).

UNIT 6: δ 3.39 (s, 3H, $-OC\underline{H}_3$); 4.72 (d, 1H, H-1, $J_{1,2}$ 2.0 Hz); 5.22 (dd, 1H, H-3, $J_{2,3}$ 10.9 Hz, $J_{3,4}$ 9.0 Hz).

Pentamer **16a** *and* **17a**. -- A mixture of glycosyl acceptor **13b** (180 mg, 0.14 mmole), silver triflate (93 mg, 0.36 mmole), activated molecular sieves (4Å, 600 mg) and 2,6-di-tert-butylpyridine (55 µl, 0.30 mmole) in dichloromethane (5 ml) was stirred at -35 °C under nitrogen. Glycosyl bromide **14** (140 mg, 0.29 mmole), dissolved in dichloromethane (3 ml), was added dropwise over 15 minutes. The reaction mixture was stirred for 1 hour at -35 °C, then diluted with dichloromethane and filtered over hyflo. The organic solution was washed with an aqueous solution of $NaHCO_3$ and brine, dried over $MgSO_4$ and evaporated to dryness. The crude reaction product was eluted from a silicagel column (9.0 g, dichloromethane/acetone, 98/2 → 9/1) to give pure compound **16a** (130 mg, 50%). R_f 0.70 (toluene/ethyl acetate, 1/1); $[\alpha]^{20}$ +105.4° (c 1.0; CH_2Cl_2). ^{1}H-NMR (360 MHz) ($CDCl_3$):
UNIT 2: δ 5.49 (d, 1H, H-1, $J_{1,2}$ 4.0 Hz); 3.26 (dd, 1H, H-2, $J_{2,3}$ 10.0 Hz).
UNIT 3: δ 4.32 (d, 1H, H-1, $J_{1,2}$ 8.0 Hz); 3.42 (dd, 1H, H-2, $J_{2,3}$ 9.4 Hz); 4.04 (dd, 1H, H-4, $J_{3,4}$ 9.0 Hz, $J_{4,5}$ 10.0 Hz); 3.75 (s, 3H, $-COOC\underline{H}_3$).
UNIT 4: δ 4.95 (d, 1H, H-1, $J_{1,2}$ 3.8 Hz); 3.15 (dd, 1H, H-2, $J_{2,3}$ 10.4 Hz); 5.37 (dd, 1H, H-3, $J_{3,4}$ 9.6 Hz); 3.68 (dd, 1H, H-4, $J_{4,5}$ 8.0 Hz).
UNIT 5: δ 5.13, 5.02 (d, d, 2H, $-OC\underline{H}_2O-$, $J_{gem.}$ 12.2 Hz); 3.71 (s, 3H, $-COOC\underline{H}_3$).
UNIT 6: δ 4.72 (d, 1H, H-1, $J_{1,2}$ 4.0 Hz); 3.39 (s, 3H, $-OC\underline{H}_3$).

Pentamer **17a** was prepared according to the same procedure as described for pentamer **16a** by coupling of glycosyl acceptor **13b** with glycosyl bromide **15**.
After silicagel chromatography pentamer **17a** could be isolated in 45% yield (0.058 mmole, 90 mg). R_f 0.44 (dichloromethane/ acetone, 93/7); $[\alpha]^{20}$ +117.13° (c 1.12; CH_2Cl_2). ^{1}H-NMR (360 MHz) ($CDCl_3$):
UNIT 2: δ 5.63 (d, 1H, H-1, $J_{1,2}$ 3.9 Hz); 3.25 (dd, 1H, H-2, $J_{2,3}$ 10.6 Hz); 5.35 (dd, 1H, H-3, $J_{3,4}$ 9.0 Hz); 4.17 (dd, 1H, H-6a, $J_{gem.}$ 12.2 Hz, $J_{vic.}$ 4.2 Hz); 4.38 (dd, 1H, H-6b, $J_{vic.}$ 2.2 Hz).
UNIT 3: δ 4.34 (d, 1H, H-1, $J_{1,2}$ 8.0 Hz); 3.43 (dd, 1H, H-2, $J_{2,3}$ 9.2 Hz); 3.80 (s, 3H, $-COOC\underline{H}_3$).
UNIT 4: δ 4.97 (d, 1H, H-1, $J_{1,2}$ 3.8 Hz); 3.17 (dd, 1H, H-2, $J_{2,3}$ 10.6 Hz); 5.37 (dd, 1H, H-3, $J_{3,4}$ 9.0 Hz).
UNIT 5: δ 5.13, 5.02 (d, d, 2H, $-OC\underline{H}_2O-$, $J_{gem.}$ 12.0 Hz); 3.71 (s, 3H, $-COOC\underline{H}_3$).
UNIT 6: δ 4.72 (d, 1H, H-1, $J_{1,2}$ 3.8 Hz); 3.39 (s, 3H, $-OC\underline{H}_3$).

Pentamers **16b** *and* **17b**. -- Pentamer **16a** (80 mg, 0.050 mmole) was saponified according to the method as described in the general procedures. After working-up and purification over silicagel (3 g, dichloromethane/methanol/acetic acid, 80/20/1) pentamer **16b** was isolated in a yield of 76% (52 mg). R_f 0.70 (dichloromethane/methanol/acetic acid, 80/20/1).
Analogous, pentamer **17a** (80 mg, 0.051 mmole) could be converted into pentamer **17b**. The purification of the end product over silicagel (2 g) required, however, the more polar eluent system dichloromethane/methanol/acetic acid, 70/20/1. Pentamer **17b** was isolated in a yield of 37% (22 mg). R_f 0.58 (dichloromethane/methanol/acetic acid, 70/20/1).

Pentamers **16c** *and* **17c**. -- Pentamer **16b** (52 mg, 0.038 mmole) was O-sulphated as described in the general procedures to give after work-up and purification pentamer **16c** (42 mg, 58%). R_f 0.60 (ethyl acetate/pyridine/acetic acid/water, 11/7/1.6/4).
The same procedure was used to convert pentamer **17b** (22 mg, 0.019 mmole) into pentamer **17c** (25 mg, 68%). R_f 0.11 (ethyl acetate/pyridine/acetic acid/water, 5/7/1.6/4).

Pentamer **II**. -- Compound **16c** (42 mg, 0.022 mmole) was hydrogenolyzed and subsequently N-sulphated according to the methods as described in the general procedures. Pentamer **II** was isolated in 50% yield (18.2 mg). $[\alpha]^{20}$ +92.7° (c 0.91; H_2O). ^{1}H-NMR (360 MHz) (D_2O):
UNIT 2: δ 5.12 (d, 1H, H-1, $J_{1,2}$ 3.6 Hz); 3.42 (dd, 1H, H-2, $J_{2,3}$ 9.4 Hz); 4.37 (dd, 1H, H-3, $J_{3,4}$ 9.9 Hz).
UNIT 3: δ 4.61 (d, 1H, H-1, $J_{1,2}$ 7.9 Hz).
UNIT 4: δ 5.03, (d, 1H, H-1, $J_{1,2}$ 3.6 Hz); 3.48 (dd, 1H, H-2, $J_{2,3}$ 9.4 Hz); 4.45 (dd, 1H, H-3, $J_{3,4}$ 9.9 Hz).
UNIT 5: δ 4.91, 5.11 (d, d, 2H, $-OC\underline{H}_2O-$, $J_{gem.}$ 7.5 Hz); 3.85, 4.12 (m, 2H, $-OC\underline{H}_2C-$); 4.41 (t, 1H, $-C\underline{H}COO^-$, J 2.4 Hz).
UNIT 6: δ 5.61 (d, 1H, H-1, $J_{1,2}$ 3.6 Hz); 3.25 (dd, 1H, H-2, $J_{2,3}$ 9.4 Hz); 3.62 (t, 1H, H-3, $J_{3,4}$ 9.9 Hz); 3.56 (t, 1H, H-4, $J_{3,4}$ 9.9 Hz); 3.42 (s, 3H, $-OC\underline{H}_3$).

Pentamer **III**. -- After hydrogenolysis and N-sulphation (see general procedures) compound **17c** (25 mg, 0.013 mmole) could be converted into pentamer **III** according to the methods described in the general procedures. After purification on an HPLC system (Waters) equipped with a mono-Q ion exchange column (Pharmacia) - NaCl-gradient of 1.0 M to 1.6 M - and a Chiramonitor (ACS) detector, pentamer **III** could be isolated in an overall yield of 12% (13 mg). $[\alpha]^{20}$ +71.1° (c 0.27; H_2O); ^{1}H-NMR (360 MHz) (D_2O):
UNIT 2: δ 5.57 (d, 1H, H-1, $J_{1,2}$ 3.4 Hz); 3.48 (c, 1H, H-2); 4.52 (dd, 1H, H-3, $J_{2,3}$ 9.0 Hz, $J_{3,4}$ 10.4 Hz); 4.10 (dt, 1H, H-5, $J_{4,5}$ 10.0 Hz, $J_{5,6a}$ and $J_{5,6b}$ 2.4 Hz); 4.28 (dd, 1H, H-6a, $J_{gem.}$ 11.0 Hz); 4.38 (dd, 1H,

H-6b).
UNIT 3: δ 4.63 (d, 1H, H-1, $J_{1,2}$ 8.0 Hz); 3.45 (c, 1H, H-2).
UNIT 4: δ 5.13 (d, 1H, H-1, $J_{1,2}$ 3.0 Hz); 3.46 (c, 1H, H-2); 4.39 (t, 1H, H-3, J 8.4 Hz); 4.27 (c, 1H, H-6a); 4.50 (c, 1H, H-6b).
UNIT 5: δ 4.93, 5.12 (d, d, 2H, -OC$\underline{H}_2$O-, $J_{gem.}$ 7.4 Hz).
UNIT 6: δ 5.04 (d, 1H, H-1, $J_{1,2}$ 3.6 Hz); 3.49 (c, 1H, H-2); 4.46 (dd, 1H, H-3, $J_{2,3}$ 10.8 Hz, $J_{3,4}$ 8.4 Hz); 3.78 (dd, 1H, H-4, $J_{4,5}$ 10.0 Hz); 3.45 (s, 3H, -OC$\underline{H}_3$).

Dimer **19a**. -- A mixture of compound **8b** (50 mg, 0.20 mmole) and compound **18** (100 mg, 0.20 mmole) in dichloromethane (3 ml) was stirred for 1 hour at room temperature in the presence of molecular sieves (4Å). The mixture was cooled to -10 °C where after $BF_3.Et_2O$ in dichloromethane (0.4 ml of a 0.5 M solution) was added under nitrogen. After 1 hour at -10 °C the reaction mixture was diluted with dichloromethane and filtered over hyflo. The organic layer was washed with aqueous $NaHCO_3$ and brine and dried over a Whatmann phase separator filter. The filtrate was evaporated to dryness and the crude product was purified on silicagel (4 g, toluene/ethyl acetate, 2/1 → 1/1) to afford pure dimer **19a** (50 mg, 33%). R_f 0.56 (toluene/ethyl acetate, 1/1).

Dimer **19b**. -- Compound **19a** (280 mg, 0.38 mmole) was dissolved in a 1 M solution of hydrazine acetate (6.3 ml) and stirred for 6 minutes at room temperature. The reaction mixture was next diluted with dichloromethane and washed with water, aqueous $NaHCO_3$ and brine. The organic layer was dried over a Whatmann phase separator filter and evaporated to dryness. The crude product was purified on a short silicagel column (5 g, toluene/ethyl acetate, 2/1 → 1/1) to give compound **19b** (100 mg, 41%). R_f 0.43 (toluene/ ethyl acetate, 1/1). $[\alpha]^{20}$ +143.2° (c 0.57; CH_2Cl_2), ^{1}H-NMR (360 MHz) ($CDCl_3$): δ 3.34 (s, 3H, -OC$\underline{H}_3$); 3.70 (s, 3H, -COOC$\underline{H}_3$); 3.97 (ddd, 1H, H-2, $J_{1,2}$ 3.6 Hz, $J_{2,3}$ 10.4 Hz, J_{HNH} 11.0 Hz); 4.16 (dd, 1H, -C$\underline{H}$, J 3.6 Hz, J 7.2 Hz); 4.68 (d, 1H, H-1, $J_{1,2}$ 3.6 Hz); 5.08, 5.12 (d, d, 2H, -OC$\underline{H}_2$O-, $J_{gem.}$ 5.8 Hz).

Pentamer **21**. -- A solution of compound **19b** (84 mg, 0.13 mmole) in dichloromethane (2.0 ml) was stirred for 1 hour at room temperature in the presence of 4Å molecular sieves (100 mg). Under nitrogen, $HgBr_2$ (46.7 mg, 0.13 mmole) and $Hg(CN)_2$ (32.8 mg, 0.13 mmole) were added where after the mixture was cooled to 0 °C. At this temperature, a solution of compound **20** (222 mg, 0.20 mmole) in dichloromethane (1.0 ml) was added to the reaction mixture. After 16 hours at room temperature the reaction mixture was diluted with dichloromethane, filtered over hyflo, washed with a 2M solution of KBr (2 X 10 ml) and finally with $NaHCO_3$ and brine. The organic layer was dried (Whatmann phase separator filter) and evaporated to dryness. The crude product was purified on silicagel (5 g, toluene/ethyl acetate, 4/1 → 1/1) to give pentamer **21** (90 mg, 41%). R_f 0.60 (toluene/ ethyl acetate, 1/1). ^{1}H-NMR (360 MHz) ($CDCl_3$):
UNIT 2: δ 5.50 (d, 1H, H-1, $J_{1,2}$ 4.0 Hz); 3.27 (dd, 1H, H-2, $J_{2,3}$ 10.2 Hz); 3.85 (dd, 1H, H-3, $J_{3,4}$ 8.4 Hz); 4.19 (dd, 1H, H-6a, $J_{gem.}$ 12.4 Hz, $J_{vic.}$ 3.8 Hz); 4.26 (dd, 1H, H-6b, $J_{vic.}$ 1.4 Hz).
UNIT 3: δ 4.32 (d, 1H, H-1, $J_{1,2}$ 7.8 Hz); 3.42 (dd, 1H, H-2, $J_{2,3}$ 9.4 Hz); 4.05 (dd, 1H, H-4, $J_{3,4}$ 9.0 Hz, $J_{4,5}$ 10.0 Hz); 3.75 (s, 3H, -COOC$\underline{H}_3$).
UNIT 4: δ 4.94 (d, 1H, H-1, $J_{1,2}$ 3.8 Hz); 3.14 (dd, 1H, H-2, $J_{2,3}$ 10.6 Hz); 5.36 (dd, 1H, H-3, $J_{3,4}$ 8.8 Hz); 4.15 (dd, 1H, H-6a, $J_{gem.}$ 12.4 Hz, $J_{vic.}$ 4.0 Hz); 4.36 (dd, 1H, H-6b, $J_{vic.}$ 2.0 Hz).
UNIT 5: δ 3.67 (s, 3H, -COOC$\underline{H}_3$); 4.30 (t, 1H, -C$\underline{H}$COOCH$_3$, J 4.0 Hz); 3.78, 3.65 (c, c, 2H, -OC$\underline{H}_2$CH-).
UNIT 6: δ 4.68 (d, 1H, H-1, $J_{1,2}$ 4.0 Hz); 3.97 (c, 1H, H-2); 3.35 (s, 3H, -OC$\underline{H}_3$).

Pentamer **IV**. -- Fully protected pentamer **21** (90 mg, 0.053 mmole) was deprotected and sulphated as described in the general procedures to obtain, after desalting on Sephadex G-25 in water, the desired analogue **IV** in 47% overall yield (37 mg). $[\alpha]^{20}$ +72.7° (c 0.77; H_2O); ^{1}H-NMR (360 MHz) (D_2O):
UNIT 2: δ 5.63 (d, 1H, H-1, $J_{1,2}$ 3.9 Hz); 3.26 (dd, 1H, H-2, $J_{2,3}$ 9.0 Hz); 4.17 (dd, 1H, H-6a, $J_{gem.}$ 11.0 Hz, $J_{vic.}$ 2.2 Hz); 4.38 (dd, 1H, H-6b, $J_{vic.}$ 2.2 Hz).
UNIT 3: δ 4.63 (d, 1H, H-1, $J_{1,2}$ 7.8 Hz); 3.41 (c, 1H, H-2).
UNIT 4: δ 5.16 (d, 1H, H-1, $J_{1,2}$ 3.8 Hz); 3.46 (dd, 1H, H-2, $J_{2,3}$ 10.6 Hz); 4.29 (dd, 1H, H-6a, $J_{gem.}$ 11.0 Hz, $J_{vic.}$ < 1Hz); 4.34 (dd, 1H, H-6b, $J_{vic.}$ < 1Hz).
UNIT 5: δ 4.96, 5.09 (d, d, 2H, -OC$\underline{H}_2$O-, $J_{gem.}$ 7.2 Hz); 4.34 (dd, 1H, -C$\underline{H}$COO$^-$, J 2.4 Hz, J 5.6 Hz).
UNIT 6: δ 5.04 (d, 1H, H-1, $J_{1,2}$ 3.8 Hz); 3.28 (dd, 1H, H-2, $J_{2,3}$ 9.2 Hz); 3.41 (s, 3H, -OC$\underline{H}_3$).

1,6;2,3-di-anhydro-4-O-levulinoyl-β-D-mannopyranose **25**. -- Compound **24** (5 g, 34.7 mmole) and a catalytic amount of N,N-dimethylaminopyridine were dissolved in pyridine (50 ml). At a temperature of 0 °C a solution of levulinic anhydride in ether (1M, 60 ml) was added to the reaction mixture. After 3 hours at room temperature water (10 ml) was added and the mixture was stirred for a further 10 minutes. Then, the mixture was diluted with dichloromethane and washed with aqueous $NaHCO_3$ and brine. The organic layer was dried (Whatmann phase separator) and evaporated to dryness followed by coevaporation with toluene to afford compound **25** in quantitative yield (8.5 g). R_f 0.58 (dichloromethane/acetone, 9/1).

1,6-anhydro-2-azido-2-deoxy-4-O-levulinoyl-β-D-glucopyranose **26**. -- A solution of compound **25** (2 g, 8.3 mmole), sodium azide (5.41 g, 83 mmole), para-toluenesulphonic acid (1.58 g, 8.3 mmole) and 2,6-lutidine (1 ml, 8.3 mmole) in N,N-dimethylformamide (36) was stirred for 3 days at a temperature of 100 °C. The mixture was concentrated to a small volume and diluted with water (200 ml) followed by the extraction with dichloromethane. The organic layer was washed with $NaHCO_3$ and brine and dried ($MgSO_4$). Filtration and subsequent evaporation gave the crude product which was purified on silicagel (200 g, toluene/ethyl acetate, 3/2 → 2/3) to afford compound **26** (1.4 g, 64%). R_f 0.38 (toluene/ethyl acetate, 1/1). ^{1}H-NMR (200 MHz) ($CDCl_3$): δ 5.51 (s, 1H, H-1); 3.26 (s, 1H, H-2); 3.98 (s, 1H, H-3); 4.68 (s, 1H, H-4); 4,59 (d, 1H, H-5, $J_{5,6a}$ 5.4 Hz); 3.77 (dd, 1H, H-6a, $J_{5,6a}$ 5.4 Hz, $J_{gem.}$ 8.0 Hz); 4.19 (d, 1H, H-6b); 2.20 (s, 3H, $C\underline{H}_3$ Lev.); 2.60 - 2.87 (c, 4H, $-C\underline{H}_2C\underline{H}_2-$ Lev.).

1,3,6-tri-O-acetyl-2-azido-2-deoxy-4-O-levulinoyl-α-D-glucopyranose **27**. -- Compound **26** (1.5 g, 5.3 mmole) was dissolved in a mixture of acetic anhydride (28 ml), acetic acid (1.2 ml) and trifluoroacetic acid (3.8 ml) and stirred for 20 hours at 40 °C. The mixture was evaporated to dryness and coevaporated twice with toluene to give compound **27** (1.96 g, 87%). R_f 0.35 (toluene/ethyl acetate, 1/1).

3,6-di-O-acetyl-2-azido-2-deoxy-4-O-levulinoyl-α/β-D-glucopyranose **28**. -- At 0 °C, piperidine (1.9 ml) was added to a solution of compound **27** (1.5 g, 3.5 mmole) in THF (48 ml). The mixture was stirred overnight at room temperature and the next day poured out in a diluted HCl solution (0.24n, 100 ml). The mixture was extracted with ethyl acetate and the organic layers were dried ($MgSO_4$), filtered and evaporated. The residue was purified on silicagel (70 g, toluene/ethyl acetate, 3/2 → 2/3) to give compound **28** (1.0 g, 71%). R_f 0.27 (toluene/ethyl acetate, 1/1).

3,6-di-O-acetyl-2-azido-2-deoxy-4-O-levulinoyl-α-D-glucopyranosyl bromide **29**. -- To a solution of compound **28** (1.2 g, 3.1 mmole) in chloroform (27.6 ml) and N,N-dimethylformamide (4.8 ml), a solution of oxalyl bromide (1M, 9.6 ml) was added at ambient temperature. After 1.5 hour the mixture was diluted with ether and washed with aqueous $NaHCO_3$ and brine. The organic layer was dried ($MgSO_4$) and evaporated to dryness. The residue was purified on a short silicagel column (35 g, toluene/ethyl acetate, 3/2 → 2/3) to afford glycosyl bromide **29** (1.2 g, 89%). R_f 0.57 (toluene/ethyl acetate, 1/1).^{1}H-NMR (200 MHz) ($CDCl_3$): δ 6.41 (d, 1H, H-1, $J_{1,2}$ 3.9 Hz); 3.77 (dd, 1H, H-2, $J_{2,3}$ 10.0 Hz); 5.16 (t, 1H, H-3, $J_{3,4}$ 10.0 Hz); 5.54 (t, 1H, H-4, $J_{4,5}$ 10.0 Hz); 2.08, 2.16 (s, s, 6H, 2 X $C\underline{H}_3$-OAc); 2.18 (s, 3H, $C\underline{H}_3$-OLev).

Trimer **31a**. -- To a solution of compound **30** (250 mg, 0.34 mmole) in dichloromethane (2.0 ml) was added activated molecular sieves 10 Å (600 mg) after which the mixture was stirred at room temperature for 1 hour. Then, silver triflate (219 mg, 0.85 mmole) was added and the mixture was cooled to -30 °C. At this temperature a solution of glycosyl bromide **29** (300 mg, 0.70 mmole) in dichloromethane (1.0 ml) was added dropwise under nitrogen atmosphere, to the reaction mixture in about 30 minutes. After 1 hour the reaction mixture was diluted with dichloromethane and filtered over hyflo. The filtrate was washed with aqueous $NaHCO_3$ and brine and then dried ($MgSO_4$). Evaporation afforded the crude product which was purified over silicagel (35 g, toluene/ethyl acetate, 3/2 → 2/3) to give pure compound **31a** (163 mg, 44%). R_f 0.45 (toluene/ethyl acetate, 1/1).

Trimer **31b**. -- Compound **31a** (190 mg, 0.18 mmole) was dissolved in a solution of hydrazine acetate in pyridine (1.0 M, 3.0 ml) and stirred for 6 minutes at room temperature. Then, the reaction mixture was diluted with dichloromethane and washed with water, aqueous $NaHCO_3$ and brine and dried over a whatmann phase separator filter. The filtrate was evaporated to dryness and the residue was chromatographed on silicagel (8 g, toluene/ethyl acetate, 1/1 → 2/3) to give pure compound **31b** (161 mg, 89%). $[\alpha]^{20}$ + 38.0° (c 1.0; CH_2Cl_2), R_f 0.41, (toluene/ethyl acetate, 1/2). ^{1}H-NMR (360 MHz) ($CDCl_3$):
UNIT 4: δ 4.93 (d, 1H, H-1, $J_{1,2}$ 3.8 Hz); 3.20 (dd, 1H, H-2, $J_{2,3}$ 10.4 Hz); 5.21 (dd, 1H ,H-3, $J_{3,4}$ 9.0 Hz); 3.44 (m, 1H, H-4); 3.89 (c, 1H, H-5); 4.28 (dd, 1H, H-6a, $J_{vic.}$ 2.5 Hz); 4.49 (dd, 1H, H-6b, $J_{gem.}$ 12.4 Hz, $J_{vic.}$ 4.0 Hz); 3.09 (d, 1H, $-O\underline{H}$, J 5.8 Hz).
UNIT 5: δ 5.04 (d, 1H, H-1, $J_{1,2}$ 3.0 Hz); 4.79 (t, 1H, H-2, J 3.0 Hz); 3.90 (c, 1H, H-3); 4.05 (t, 1H, H-4, J 3.8 Hz); 4.75 (d, 1H, H-5, $J_{4,5}$ 3.2 Hz); 3.83 (s, 3H, $-COOC\underline{H}_3$).
UNIT 6: δ 4.69 (d, 1H, H-1, $J_{1,2}$ 3.8 Hz); 3.93 (dd, 1H, H-2, $J_{2,3}$ 10.8 Hz); 5.20 (dd, 1H, H-3, $J_{3,4}$ 8.4 Hz); 4.29 (c, 2H, H-6a, H-6b); 3.36 (s, 3H, $-OC\underline{H}_3$).

Tetramer **32a**. -- A mixture of compound **8c** (40 mg, 0.16 mmole) and compound **31b** (160 mg, 0.16 mmole) in dichloromethane was stirred for 1 hour at room temperature in the presence of molecular sieves 4Å (200 mg). The reaction mixture was cooled to -10 °C where after a solution of $BF_3.Et_2O$ in dichloromethane (0.5 M, 0.32 ml) was added under nitrogen atmosphere. After 1 hour the reaction mixture was diluted with dichloromethane and filtered over hyflo. The filtrate was washed with aqueous $NaHCO_3$ and brine and dried ($MgSO_4$). After evaporation the crude reaction product was purificated on silicagel (5 g, dichloromethane/acetone, 93/7 → 8/2) to give tetramer **32a** (150 mg, 77%). R_f 0.27 (dichloromethane/acetone, 9/1).

Tetramer **32b**. -- Tetramer **32a** (80 mg, 0.07 mmole) was dissolved in a solution of hydrazine acetate in pyridine (1 M, 1.5 ml) and stirred at room temperature for 6 minutes. The reaction mixture was diluted with dichloromethane and washed with water, aqueous $NaHCO_3$ and brine and dried ($MgSO_4$). The crude reaction mixture was purified over silicagel (4 g, toluene/ethyl acetate, 1/1 → 1/3) to give tetramer **32b** (50 mg, 68%). $[\alpha]^{20}$ + 41.2° (c 1.0; CH_2Cl_2), R_f 0.36 toluene/ethyl acetate, 1/2). ^{1}H-NMR (360 MHz) ($CDCl_3$):
UNIT 3: δ 4.83, 4.87 (d, d, 2H, -OC$\underline{H}_2$O-, $J_{gem.}$ 7.2 Hz); 3.76 (s, 3H, -COOC$\underline{H}_3$).
UNIT 4: δ 5.02 (d, 1H, H-1, $J_{1,2}$ 3.8 Hz); 3.04 (dd, 1H, H-2, $J_{2,3}$ 10.4 Hz); 5.35 (dd, 1H, H-3, $J_{3,4}$ 9.0 Hz); 3.59 (dd, 1H, H-4, $J_{4,5}$ 10.0 Hz); 3.97 (c, 1H, H-5); 4.28 (dd, 1H, H-6a, $J_{gem.}$ 12.6 Hz, $J_{vic.}$ 4.0 Hz); 4.37 (dd, 1H, H-6b, $J_{vic.}$ 2.6 Hz).
UNIT 5: δ 5.08 (d, 1H, H-1, $J_{1,2}$ 3.0 Hz); 4.80 (c, 1H, H-2); 4.08 (t, 1H, H-4, J 4.0 Hz); 4.74 (d, 1H, H-5, J 4.0 Hz); 3.76 (s, 3H, -COOC$\underline{H}_3$).
UNIT 6: δ 4.69 (d, 1H, H-1, $J_{1,2}$ 3.8 Hz); 3.92 (c, 1H, H-2); 5.18 (dd, 1H, H-3, $J_{2,3}$ 11.0 Hz, $J_{3,4}$ 8.6 Hz); 4.22 (dd, 1H, H-6a, $J_{gem.}$ 6.2 Hz, $J_{vic.}$ 3.0 Hz); 4.29 (c, 1H, H-6b); 3.36 (s, 3H, -OC$\underline{H}_3$).

Pentamer **34**. -- A mixture of tetramer **32b** (45 mg, 0.040 mmole) and molecular sieves 4Å (100 mg) in dichloromethane (2 ml) was stirred at room temperature for 1 hour. Under nitrogen atmosphere, $HgBr_2$ (11.5 mg, 0.032 mmole) and $Hg(CN)_2$ (8 mg, 0.032 mmole) were added whereupon the mixture was cooled to 0 °C. At this temperature a solution of glycosyl bromide **33** (44.2 mg, 0.1 mmole) in dichloromethane (0.5 ml) was added dropwise to the reaction mixture. The mixture was allowed to come to room temperature. After 16 hours the mixture was diluted with dichloromethane and filtered over hyflo. The filtrate was washed with 2 M KBr (2 X 10 ml), aqueous $NaHCO_3$ and brine and dried ($MgSO_4$). Evaporation afforded the crude reaction product. The residue was purified by silicagel chromatography (4 g, toluene/ethyl acetate, 3/2 → 2/3) to give pentamer **34** (44 mg, 75%). R_f 0.47 (toluene/ethyl acetate, 1/1), $[\alpha]^{20}$ +90.5° (c 1, CH_2Cl_2). ^{1}H-NMR (360 MHz) ($CDCl_3$):
UNIT 2: δ 5.01 (d, 1H, H-1, $J_{1,2}$ 3.9 Hz); 3.06 (dd, 1H, H-2, $J_{2,3}$ 11.0 Hz); 5.30 (dd, 1H, H-3, $J_{3,4}$ 9.0 Hz); 3.65 (dd, 1H, H-4, $J_{4,5}$ 10.0 Hz); 4.21 (dd, 1H, H-6a, $J_{vic.}$ 4.6 Hz, $J_{gem.}$ 12.2 Hz); 4.39 (dd, 1H, H-6b, $J_{vic.}$ 2.0 Hz).
UNIT 3: δ 4.86, 4.81 (d, d, 2H, -OC$\underline{H}_2$O-, $J_{gem.}$ 6.6 Hz), 3.82 (s, 3H, -COOC$\underline{H}_3$).
UNIT 4: δ 4.95 (d, 1H, H-1, $J_{1,2}$ 3.6 Hz); 3.14 (dd, 1H, H-2, $J_{2,3}$ 10.6 Hz); 5.49 (dd, 1H, H-3, $J_{3,4}$ 9.0 Hz); 3.59 (dd, 1H, H-4, $J_{4,5}$ 10.1 Hz); 4.24 (dd, 1H, H-6a, $J_{vic.}$ 4.0 Hz, $J_{gem.}$ 12.2 Hz); 4.32 (dd, 1H, H-6b, $J_{vic.}$ 2.4 Hz).
UNIT 5: δ 5.09 (d, 1H, H-1, $J_{1,2}$ 3.6 Hz); 4.80 (t, 1H, H-2, J 3.6 Hz); 4.08 (t, 1H, H-4, J 3.8 Hz); 4.74 (c, 1H, H-5); 3.78 (s, 3H, -COOC$\underline{H}_3$).
UNIT 6: δ 4.69 (d, 1H, H-1, $J_{1,2}$ 3.9 Hz); 5.18 (dd, 1H, H-3, $J_{2,3}$ 11.0 Hz, $J_{3,4}$ 8.6 Hz); 4.29 (c, 2H, H-6a, H-6b); 3.35 (s, 3H, -OC$\underline{H}_3$).

Pentamer V. -- Compound **34** (45 mg, 0.031 mmole) was dissolved in THF (3.16 ml) and cooled to -5 °C. At this temperature, a solution of 30% H_2O_2 (1.55 ml) and an aqueous solution of LiOH (0.71 ml, 1.25 M) were added. The mixture was heated to 0 °C and stirred for 3 hours where after methanol (1.69 ml) and a solution of sodium hydroxide (0.79 ml, 4n) were added. Then, the mixture was stirred for 16 hours at room temperature. The reaction mixture was acidified to pH≈2 with diluted HCl (6n) at 0 °C and poured out in ice-water, followed by the extraction with dichloromethane. The organic layers were washed with ice-water and with a 5% aqueous solution of Na_2SO_3 (acidified to pH=3), dried over $MgSO_4$ and concentrated to dryness. The residue was then used in the O-sulphation, reduction/debenzylation and N-sulphation procedures as described in the general procedures. After purification pentamer V could be isolated in 35% overall yield (20 mg). $[\alpha]^{20}$ + 35.8° (c 0.33; H_2O); ^{1}H-NMR (360 MHz) (D_2O):
UNIT 2: δ 5.62 (d, 1H, H-1, $J_{1,2}$ 3.6 Hz); 3.45 (dd, 1H, H-2, $J_{2,3}$ 11.0 Hz); 4.38 (dd, 1H, H-3, $J_{3,4}$ 9.0 Hz); 3.79 (t, 1H, H-4, J 9.0 Hz); 4.18 (c, 1H, H-5); 4.29 (c, 2H, H-6a, H-6b).
UNIT 3: δ 4.86, 5.09 (d, d, 2H, -OC$\underline{H}_2$O-, $J_{gem.}$ 7.0 Hz); 3.94 (dd, 1H, -OC$\underline{H}_a$CH-, $J_{vic.}$ 3.8 Hz, $J_{gem.}$ 11.0 Hz); 4.03 (c, 1H, -OC$\underline{H}_b$CH-); 4.51 (c, 1H, -C$\underline{H}$COO$^-$).
UNIT 4: δ 5.11 (d, 1H, H-1, $J_{1,2}$ 3.8 Hz); 3.49 (dd, 1H, H-2, $J_{2,3}$ 10.6 Hz); 4.43 (dd, 1H, H-3, $J_{3,4}$ 9.0 Hz): 4.07 (dd, 1H, H-4, $J_{4,5}$ 11.0 Hz); 4.33 (dd, 1H, H-6a, $J_{vic.}$5.0 Hz, $J_{gem.}$ 10.0 Hz); 4.53 (dd, 1H, H-6b, $J_{vic.}$ 3.0 Hz).
UNIT 5: δ 5.12 (d, 1H, H-1, $J_{1,2}$ 5.0 Hz); 4.32 (dd, 1H, H-2, $J_{2,3}$ 9.5 Hz); 4.09 (dd, 1H, H-3, $J_{3,4}$ 4.5 Hz); 4.24 (t, 1H, H-4, J 4.5 Hz); 4.86 (d, 1H, H-5, J 2.4 Hz).
UNIT 6: δ 5.01 (d, 1H, H-1, $J_{1,2}$ 3.6 Hz); 3.36 (dd, 1H, H-2, $J_{2,3}$ 10.4 Hz); 4.45 (dd, 1H, H-3, $J_{3,4}$ 8.4 Hz); 3.76 (dd, 1H, H-4, $J_{4,5}$ 10.2 Hz); 4.03 (c, 1H, H-5); 4.25 (c, 1H, H-6a); 4.43 (c, 1H, H-6b); 3.43 (s, 3H, -OC$\underline{H}_3$).

References

1. Casu, B.; Oreste, P.; Torri, G.; Zopetti, G.; Choay, J.; Lormeau, J.-C.; Petitou, M. and Sinay, P. *Biochem. J.* **1981**, *197*, 599.
2. Choay, J.; Lormeau, J.-C.; Petitou, M.; Sinay, P. and Fareed, *J. Ann. N. Y. Acad. Sci.* **1981**, *370*, 664.
3. Lindahl, U.; Backstrom, G.; Thunberg, L. and Leder, I.G. *Proc. Nat. Acad. Sci. USA* **1980**, *77*, 6551.
4. Rosenberg, R. D. and Lam, L. *Proc. Nat. Acad. Sci. USA* **1979**, *76*, 1218.
5. Thunberg, L.; Backstrom, G. and Lindahl, U. *Carbohydr. Res.* **1982**, *100*, 393.
6. Petitou, M.; Duchaussoy, P.; Lederman, I.; Choay, J.; Sinay, P.; Jacquinet, J.-C. and Torri, G. *Carbohydr. Res.* **1986**, *147*, 221.
7. Jacquinet, J.-C.; Petitou, M.; Duchaussoy, P.; Lederman, I.; Choay, J.; Torri, G. and Sinay, P. *Carbohydr. Res.* **1984**, *130*, 221.
8. Van Boeckel, C. A. A.; Beetz, T.; Vos, J. N.; de Jong, A. J. M.; van Aelst, S. F.; van den Bosch, R. H.; Mertens, J. M. R. and van der Vlugt, F. A. *J. Carbohydr. Chem.* **1985**, *4*, 293.
9. Ichikawa, Y.; Monden, R. and Kuzuhara, H. *Tetrahedron Lett.* **1986**, *27*, 611.
10. Sinay, P.; Jacquinet, J.-C.; Petitou, M.; Duchaussoy, P.; Lederman, I.; Choay, J. and Torri, G. *Carbohydr. Res.* **1984**, *132*, C5.
11. Petitou, M.; Duchaussoy, P.; Lederman, I.; Choay, J.; Jacquinet, J.-C.; Sinay, P. and Torri, G. *Carbohydr. Res.* **1987**, *167*, 67.
12. Petitou, M.; Duchaussoy, P.; Lederman, I.; Choay, J. and Sinay, P. *Carbohydr. Res.* **1988**, *179*, 163.
13. Van Boeckel, C. A. A.; Lucas, H.; van Aelst, S. F.; van den Nieuwenhof, M. W. P.; Wagenaars, G. N. and Mellema, J.-R. *Recl. Trav. Chim. Pays-Bas* **1987**, *106*, 501.
14. Beetz, T. and van Boeckel, C. A. A. *Tetrahedron Lett.* **1986**, *27*, 5889.
15. Van Aelst, S. F. and van Boeckel, C. A. A. *Recl. Trav. Chim. Pays-Bas* **1987**, *106*, 593.
16. Petitou, M. *Nouv. Rev. Fr. Hematologie* **1984**, *26*, 221.
17. Choay, J.; Petitou, M.; Lormeau, J.-C.; Sinay, P.; Casu, B. and Gatti, G. *Biochem. Biophys. Res. Commun.* **1983**, *116*, 492.
18. Petitou, M.; Lormeau, J.-C. and Choay, J. *Eur. J. Biochem.* **1988**, *176*, 637.
19. Van Boeckel, C. A. A.; Beetz, T. and van Aelst, S. F. *Tetrahedron Lett.* **1988**, *29*, 803.
20. Van Boeckel, C. A. A.; van Aelst, S. F.; Beetz, T.; Meuleman, D. G.; van Dinther, Th. G. and Moelker, H. C. T. *Ann. N. Y. Acad. Sci.* **1988**, *556*, 489.
21. Meuleman, D. G.; Hobbelen, P. M. J.; van Dinther, Th. G.; Vogel, G. M. T.; van Boeckel, C. A. A. and Moelker, H. C. T. *Seminars in Thrombosis and Haemostasis* **1990**, *In press.*
22. Van Boeckel, C. A. A.; Basten, J. E. M.; Lucas, H. and van Aelst, S. F. *Angew. Chem.* **1988**, *100*, 1217.
23. Lok, C. M.; Ward, J. P. and van Dorp, D. A. *Chem. Phys. Lipids* **1976**, *16*, 115.
24. Hassner, A.; Rubinstein, M. and Patchornik, A. *J. Am. Chem. Soc.* **1975**, *97*, 1614.
25. Paulsen, H. *Angew. Chem. (Engl. ed.)* **1982**, *21*, 155.
26. Guibe, F. and Saint M'leux, Y. *Tetrahedron Lett.* **1981**, *22*, 3591. Oltvoort, J. J.; Kloosterman, M. and van Boom, J. H. *Recl. Trav. Chim. Pays-Bas* **1983**, *102*, 501.
27. Vos, J. N.- non published results.
28. Hayashi, M.; Hashimoto, S. and Noyori, R. *Chem. Lett.* **1984**, 1747.
29. Nicolaou, K. C.; Chuchowlowski, A.; Dolle, R. E. and Randall, J. L. *J. Chem. Soc. Chem. Commun.* **1984**, 1155.
30. Oltvoort, J. J.; van Boeckel, C. A. A.; de Koning, J. H. and van Boom, J. H. *Synthesis* **1982**, *308.*
31. Ogawa, T. and Nakabayashi, S. *Carbohydr. Res.* **1981**, *93*, C1.
32. Paulsen, H. and Stenzel, W. *Chem. Ber.* **1978**, *111*, 2334.
33. Vos, J. N.; Kat-van den Nieuwenhof, M. W. P.; Basten, J. E. M. and van Boeckel, C. A. A. *J. Carbohydr. Chem. In press.*
34. Petitou, M.; Jaurand, G.; Derrien, M.; Duchaussoy, P. and Choay, J. *Prec. V EUROCARB Symposium Prague* **1989**, A-68.
35. Stanek, J. and Cerny, M. *Synthesis* **1972**, 698.
36. Bovin, N. V.; Zurabyan, S. E. and Khorlin, A. Ya. *Izv. Akad. Nauk. SSSR Ser. Khim.* **1981**, 2806.
37. Sabesan, S. and Lemieux, R. U. *Can . J. Chem.* **1984**, *62*, 644.
38. Kiss, J. *Adv. Carbohydr. Chem.* **1974**, *29*, 229.
39. Evans, D. *Tetrahedron Lett.* **1987**, *28*, 6141.

40. Teien, A. N. and Lie, M. *Thromb. Res.* **1977**, *10*, 399.
41. Van Dinther, Th. G.; Hol, F. and Meuleman, D. G. *Thromb. Heamost.* **1987**, *58*, 423.
42. Hobbelen, P. M. J.; van Dinther, Th. G.; Vogel, G. M. T.; van Boeckel, C. A. A.; Moelker, H. C. T. and Meuleman, D. G. *Thrombosis and Haemostasis* **1990**, *63*, 265.
43. Ferro, D. R.; Provasoli, A.; Ragazzi, M.; Torri, G.; Casu, B.; Gatti, G.; Jacquinet, J.-C.; Sinay, P.; Petitou, M. and Choay, J. *J. Am. Chem. Soc.* **1986**, *108*, 6773.
44. Torri, G.; Casu, B.; Gatti, G.; Petitou, M.; Choay, J.; Jacquinet, J.-C. and Sinay, P. *Biochem. Biophys. Res. Commun.* **1985**, *128*, 134.
45. Van Boeckel, C. A. A.; van Aelst, S. F.; Wagenaars, G. N.; Mellema, J.-R.; Paulsen, H.; Peters, T.; Pollex, A. and Sinnwell, V. *Recl. Trav. Chim. Pays-Bas* **1987**, *106*, 19.
46. Sanderson, P. N.; Huckerby, T. N. and Nieduszynski, I. A. *Glycoconjugate J.* **1985**, *2*, 109.
47. Lucas, H.; van Boeckel, C. A. A.; Wagenaars, G. N. and Mellema, J.-R. *Prec. V EUROCARB Symposium Prague* **1989**, B-12.
48. Van Boeckel, C. A. A.; Wagenaars, G. N. and Mellema, J.-R. *Recl. Trav. Chim. Pays-Bas* **1988**, *107*, 649.
49. Church, F. C.; Treanor, R. E.; Bradly Sherill, G. and Whinna, H. C. *Biochem. Biophys. Res. Commun.* **1987**, *148*, 362.
50. Bray, B.; Lane, D. A.; Freyssinet, J.-M.; Pejler, G. and Lindahl, U. *Biochem. J.* **1989**, *262*, 225.