Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 7, pp. 893-898, 1994
Elsevier Science Ltd
Printed in Great Britain
0960-894X/94 $6.00+0.00

0960-894X(94)E0071-L

SYNTHESIS OF A DERMATAN SULPHATE-LIKE HEXASACCHARIDE WITH A "NON-GLYCOSAMINO"GLYCAN STRUCTURE

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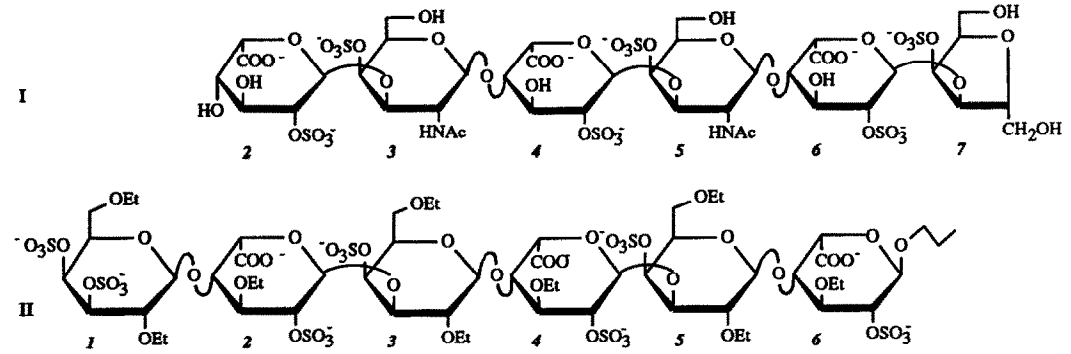
Abstract. *The synthesis of a "non-glycosamino"glycan counterpart (i.e. compound II) of a naturally occurring dermatan sulphate hexasaccharide that binds with high affinity to heparin cofactor II is described.*

Dermatan sulphate (DS) exerts part of its anticoagulant activity by stimulating the heparin cofactor II (HC-II) mediated inactivation of thrombin. Although the chemical structure of dermatan sulphate is heterogeneous it mainly consists of repeating disaccharide sequences of O-β-D-GalNAc-(4-SO_4)-(1→4)α-L-IdoA(1→3), which are not sulphated at iduronic acid.



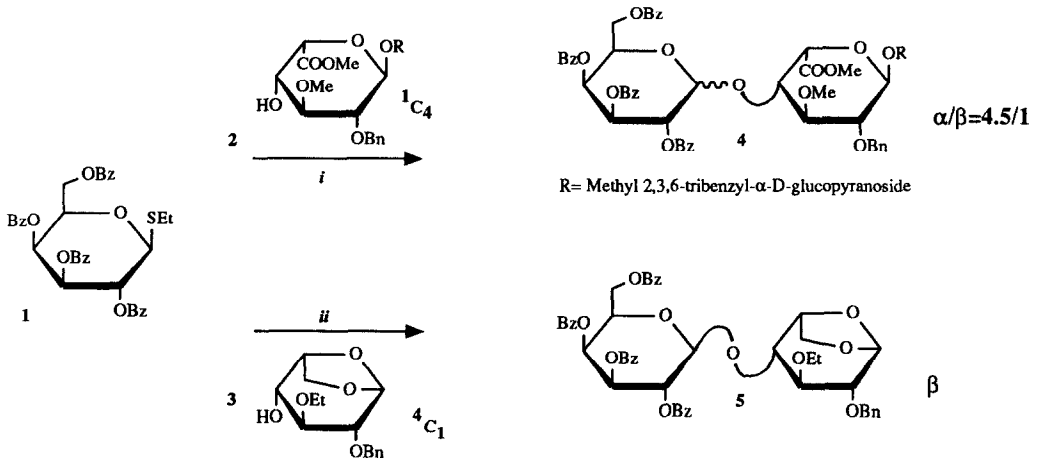
Compound I : Chemical structure of the high affinity dermatan sulphate hexasaccharide. The reducing terminal $ATal_r(4\text{-}SO_4)$ is a product of the deaminative cleavage reaction and corresponds to GalNAc(4-SO_4).

Compound II: "Non-glycosamino"glycan counterpart of I.

Recently[1] the chemical structure of a unique hexasaccharide fragment (*i.e.* compound I) in DS was reported representing the minimal sequence for high affinity binding to heparin-cofactor II. An important structural feature of this sequence is the presence of extra O-sulphate groups at the 2 position of the iduronic acid moieties. During our studies towards simplified heparin-like fragments we demonstrated[2] that substitution

of various functional groups (*i.e.* replacement of N-sulphate groups by O-sulphate esters and alkylation of free hydroxyl groups) in the naturally occurring antithrombin binding pentasaccharide fragment did not result in a decrease of the antithrombotic activity. These results prompted us to investigate the effect of similar modifications in DS fragments closely resembling the high affinity dermatan sulphate hexasaccharide **I**. As part of our research program on the design and preparation of such "non-glycosamino"glycan derivatives we now report[3] on the synthesis of hexasaccharide **II**, in which acetamido[4] and hydroxyl groups are replaced by ethoxy groups.

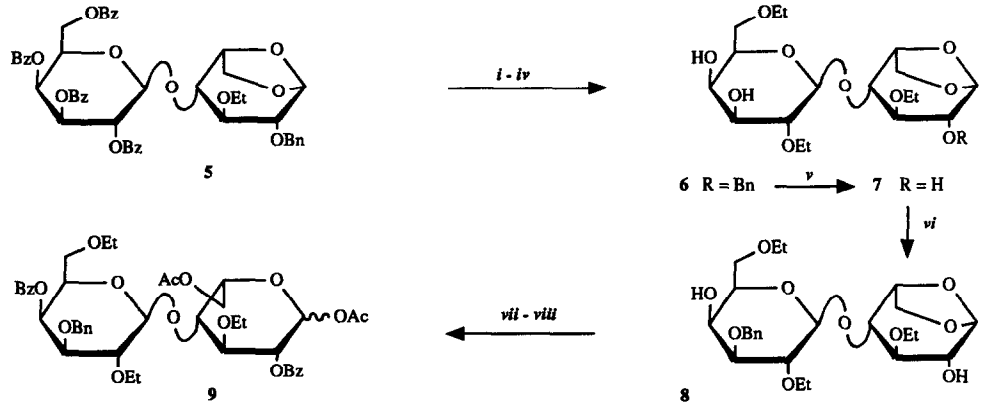
Since the required hexasaccharide **II** consists of three repeating disaccharide sequences we devised a strategy that is based on the multiple application of one Galβ(1→4)IdoA building block. In order to introduce the β-interglycosidic bond of this disaccharide, various galactosyl donors (*e.g.* **1**) were coupled with iduronic acid acceptor **2**[5] (displaying the $^1C_4$ conformation) under various conditions (Scheme 1). Unexpectedly, in all these attempts mainly the undesired α-coupled product was formed[6]. The formation of the α-coupled product has been explained by unfavourable steric interaction of donor **1**[7] with acceptor **2** in the transition state leading to the β-coupled product.



Scheme 1. *i)* NIS, TfOH, CH_2Cl_2, MS 4Å, 0°C (41%, α/β=4.5/1). *ii)* NIS, TfOH, CH_2Cl_2, MS 4Å, 0°C (90%,β).

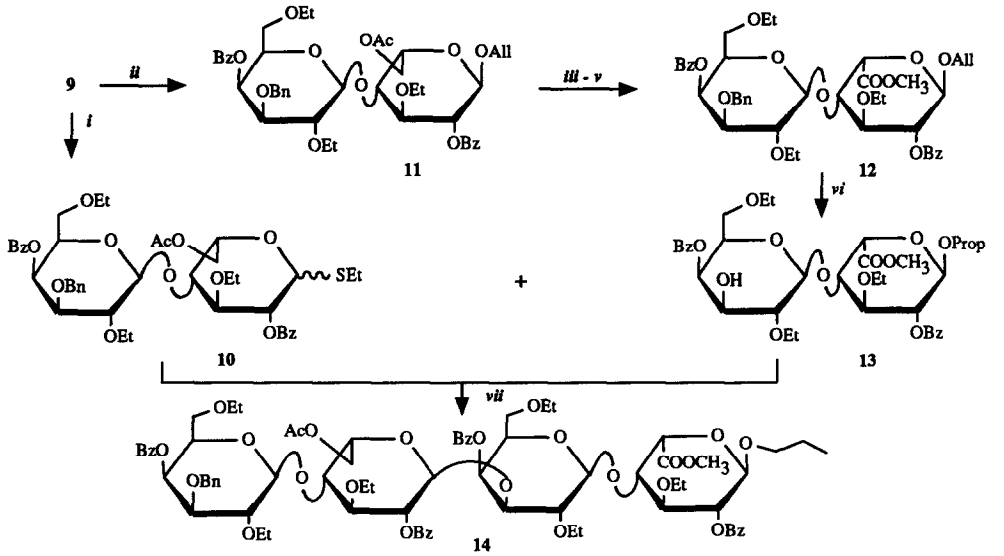
We previously confirmed this assumption by studying double stereodifferentiation[8]. Moreover we found that the unfavourable steric interaction between donor and acceptor could be diminished by changing the conformation of the acceptor[8,9]. This was also found to be the case in the synthesis of the Galβ(1→4)IdoA disaccharide. Thus, coupling of **1** with 1,6-anhydro-idose **3**[6] (displaying the $^4C_1$ conformation) in the presence of N-iodocuccinimide (NIS) and a catalytic amount of trifluoromethanesulphonic acid (TfOH)[7] afforded exclusively the β-coupled disaccharide **5** in 90% yield (Scheme 1). Disaccharide **5** was converted into **6** in an excellent yield by successively removal of the benzoate esters, introduction of the 3',4'-O-isopropylidene protective group[10], alkylation of the remaining 2',6' hydroxyl functions and subsequent removal of the isopropylidene group (Scheme 2). For the introduction of the 1,2-trans

interglycosidic bonds between the individual Galβ(1→4)Ido disaccharides a participating benzoate ester at position 2 of the idose moiety is desired. Therefore we first removed the 2-O-benzyl group in compound **6** by



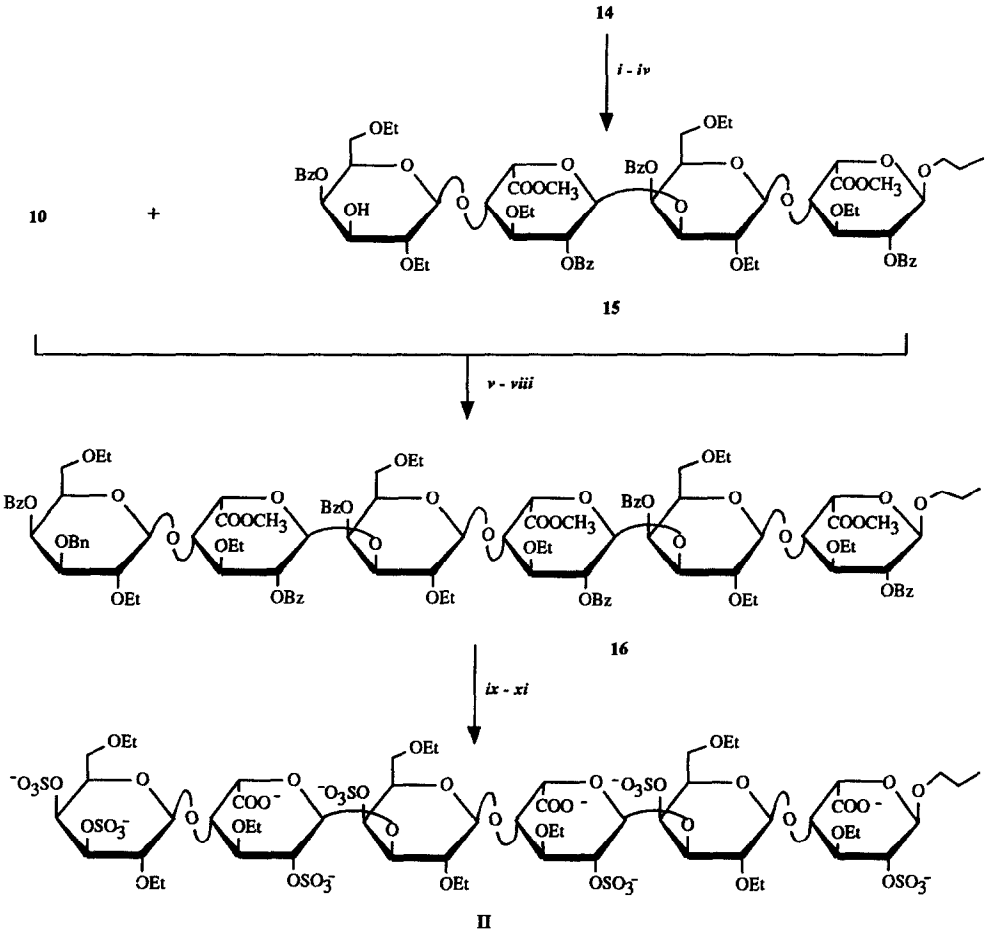
Scheme 2. *i)* KOtBu, MeOH, dioxane, 2h, r.t. (100%); *ii)* Dimethoxypropane, pTosOH (93%); *iii)* C_2H_5I, NaH, DMF, 3h, r.t. (100%); *iv)* 70% HOAc, 50°C, 4h (98%); *v)* Pd on charcoal, H_2, MeOH, 1h (100%); *vi)* Bu_2SnO, MeOH, Δ, then BnBr, TBAB, DMF (70%); *vii)* Benzoyl chloride, pyridine, 20h, r.t. (99%); *viii)* HOAc/Ac_2O/TFA 1/25/3.5, 20h at 20°C (95%).

hydrogenolysis to give **7**. Moreover this replacement now allows the use of a temporary 3'-O-benzyl protective group. Regioselective benzylation of **7** was effected by reaction of the stannylidene complex[11] of **7** with benzyl bromide in the presence of tetrabutylammonium bromide (TBAB) to give **8** in a yield of 70%.



Scheme 3. *i)* Ethanethiol, toluene, $BF_3.Et_2O$, 2.5h, r.t. (70%); *ii)* Allyl alcohol, $BF_3.Et_2O$, CH_2Cl_2, 0°C, 4h (62%); *iii)* HCl in MeOH, CH_2Cl_2, 5h, r.t. (95%); *iv)* CrO_3, H_2O, H_2SO_4, acetone, 3h, r.t.; *v)* $KHCO_3$, CH_3I, DMF, 3h, r.t.; *vi)* Pd on charcoal, H_2, MeOH, 3h (66%, step *iv* - *vi*); *vii)* NIS, TfOH, toluene, CH_2Cl_2, MS 4Å, -15°C, 1h (90%).

Treatment of compound **8** with benzoyl chloride in pyridine followed by ring opening of the 1,6-anhydro functionality under acetolysis conditions gave key-intermediate **9** in an overall yield of 70% (based on **6**).This intermediate could be used for the preparation of glycosyl donor **10** as well as for glycosyl acceptor **13** (Scheme 3).Treatment of **9** with ethanethiol in the presence of $BF_3.Et_2O$ gave donor **10** in 70% yield (α/β = 7/3). On the other hand the anomeric centre of **9** was blocked by condensation with allyl alcohol in the presence of $BF_3.Et_2O$ to give the reducing end building block **11**. Besides the formation of the desired α-O-allyl derivative **11** (62% yield) a small quantity (3%) of the β-coupled product was isolated. In order to convert the idose moiety into the iduronic acid derivative the 6-O-acetyl protective group in **11** was saponified selectively in quantitative yield by the action of hydrogen chloride in methanol[12]. Jones oxidation



Scheme 4. *i)* HCl in MeOH, CH_2Cl_2, 24h, r.t. (92%); *ii)* CrO_3, H_2O, H_2SO_4, acetone, 3h; *iii)* $KHCO_3$, CH_3I, DMF, 3h, r.t.; *iv)* Pd on charcoal, H_2, MeOH, 30 min. (80%, step *ii* - *iv*); *v)* NIS, TfOH, toluene, CH_2Cl_2, MS 4Å, -15°C, 1h (89%); *vi)* HCl in MeOH, CH_2Cl_2, 48h, r.t. (100%); *vii)* CrO_3, H_2O, H_2SO_4, acetone, 3h; *viii)* $KHCO_3$, CH_3I, DMF, 3h, r.t. (70%, step *vii* - *viii*); *ix)* Pd on charcoal, H_2, MeOH, (93%); *x)* LiOOH, THF, 16h, 0°C, NaOH, MeOH, 16h, r.t. (78%); *xi)* $Et_3N.SO_3$-complex, DMF, 16h, 55°C (80%).

followed by esterification of the obtained carboxylic acid with methyl iodide and $KHCO_3$ gave compound **12** in 68% yield[13]. Removal of the benzyl group and concomitant reduction of the allyl group provided compound **13** in 95% yield. Glycosylation of acceptor **13** with donor **10** in the presence of NIS and a catalytic amount of trifluoromethanesulphonic acid at -15°C afforded exclusively the α-coupled tetrasaccharide **14** in high yield (90%). This tetrasaccharide was now subjected to the earlier mentioned deacetylation, oxidation, methylation and hydrogenolysis steps to give glycosyl acceptor **15** in an overall yield of 74% (see Scheme 4). Hexasaccharide **16** was prepared in the same way as described for the synthesis of tetrasaccharide **15**. Thus glycosylation of compound **15** with thioglycoside **10** followed by deacetylation, oxidation and methylation afforded the fully protected hexasaccharide **16** in 62% yield (based on **15**). This protected hexasaccharide was successively hydrogenolyzed, saponified and sulphated to give the required target molecule **II**. Desalting of the crude product was then performed on a Sephadex G-25 column to give 60% of hexasaccharide **II**, the structure of which was corroborated by NMR spectroscopy[14] and FAB Mass spectrometry. An excellent purity of >97% was confirmed by capillary electrophoresis using indirect U.V. detection[15] (see Fig. 1).

Preliminary pharmacology showed that compound **II** binds and activates HC II indeed.

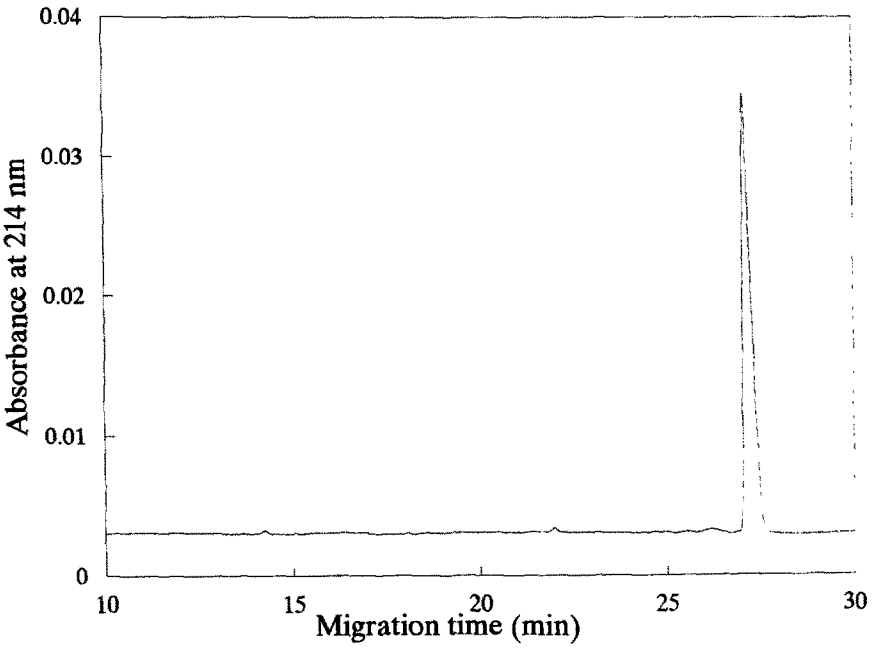


Fig. 1: HPCE electropherogram of hexasaccharide II
5 mM SSA pH=3, Rev.–UV, 5 kV, Rev. Polarity, 2 sec inj.

Acknowledgement

We wish to thank G. N. Wagenaars, J. Damm, G. Overklift (Organon, Department of Analytical Chemistry) and P. Sizun (Sanofi) for recording the NMR and CE spectra and J. Spit for preparing the manuscript.

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3. A paper on a alkylated analogue of hexasaccharide **I** containing O-α-L-IdoA(2-SO_4)-(1→3)-β-D-Gal(2,4-SO_4)(1→4) repeating units, instead of O-β-D-Gal(4-SO_4)-(1→4)α-L-IdoA-(2-SO_4)(1→3) sequences reported by us, will be published by G. Jaurand et al.
4. Replacement of an N-Acetyl by an O-alkyl group excludes the formation of minor amounts of N-sulphated compounds during the functionalization procedure, as has been observed on model compounds.
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13. The presence of the allyl protective group allows also selective deprotection of the anomeric centre and application of building block **12** in other block-synthesis.
14. ^{1}H-NMR data (360 MHz, D_2O, δ, ppm): the ^{1}H-NMR spectrum of compound **II** was completely assigned using 2D-COSY techniques. The following resonances, denoted from the non-reducing end ***1*** to the reducing end ***6***, are important; *unit 1*: 4.69 (d, *J*=8.0Hz, 1H, H-1), 3.49 (dd, *J*=8.0, 10Hz, 1H, H-2), 4.39 (dd, *J*=3.6, 10Hz, 1H, H-3), 4.94 (d, *J*=3.6Hz, 1H, H-4); *unit 2*: 5.22 (br.s, H-1), 4.38 (m, H-2), 4.11 (m, H-3), 4.29 (br.q, H-4), 4.89 (d, *J*=3.5Hz, H-5); *unit 3*: 4.62 (d, *J*=7.9Hz, 1H, H-1), 3.52 (dt, *J*=7.9Hz, *J*=2.0Hz, H-2), 4.68(d, *J*=3.6Hz, H-4); *unit 4*: 5.22 (br.s, H-1), 4.38 (m, H-2), 4.11(m, H-3) 4.29 (br.q, H-4), 4.89 (d, *J*=3.5Hz, H-5); *unit 5*: 4.64 (d, *J*=7.9Hz, 1H, H-1), 3.52 (dt, *J*=7.9Hz, *J*=2.0Hz, H-2), 4.68 (d, *J*=3.6Hz, H-4); *unit 6*: 5.09 (br.s, 1H, H-1), 4.23 (t, 1H, H-2), 4.11 (m, H-3), 4.25 (t, 1H, H-4), 4.44 (d, *J*=2.0Hz, 1H, H-5). Estimated purity = >97%. $[\alpha]_D^{20}$= -18.0 (c=1, H_2O).
FAB(+): 2129 $(M+Na)^+$; 2107 $(M+H)^+$; FAB(-): 2083 $(M-Na)^-$; 2061 $(M-2Na+H)^-$
15. Damm, J.B.L.; Overklift, G.T., submitted for publication.

(*Received in USA* 20 *January* 1994; *accepted* 1 *March* 1994)