Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 9, pp. 1175-1180, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00183-2

POLYMER-SUPPORTED SOLUTION SYNTHESIS OF HEPARAN SULPHATE-LIKE OLIGOMERS[+]

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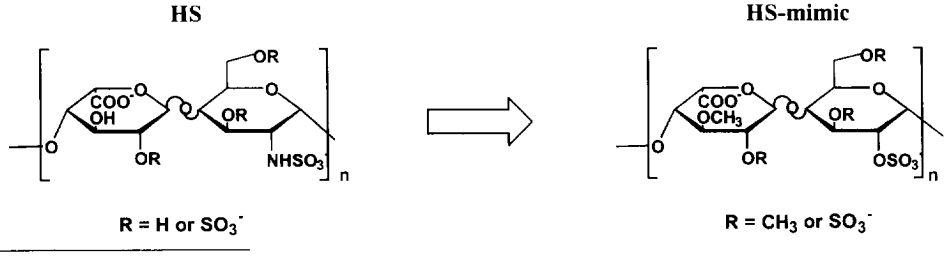
Abstract: The polyethylene glycol (PEG-polymer)-supported solution synthesis of heparan sulphate-like oligomers, which differ in length (up till 12-mers) and sulphation pattern, is described.[1]

Many types of glycosaminoglycans (e.g., heparin, dermatan sulphate, and heparan sulphate) are involved in the regulation of cell growth and blood coagulation. For instance, it has been recognized that particular heparan sulphates (HS) from mammalian tissue may inhibit the proliferation of arterial smooth muscle cells,[2] whereas other types of HS can activate the serine protease inhibitor antithrombin III.[3] The biological activity of HS is due to specific interactions of unique domains of HS with complemetary proteins. Thus it is known that HS contains a similar ATIII binding pentasaccharide domain as heparin,[3] while recent literature data[4,5] disclose that at least an octasaccharide fragment of HS with the repeating dissaccharide sequence: [Ido(2-SO_3)α1→4 GlcNSO_3(6-OSO_3)α1→4] is required for bFGF binding and interaction with the bFGF receptor. In order to study HS binding to specific proteins in more detail, the availability of well-defined heparan sulphates and analogues thereof is obligatory. To this end we intended to synthesize HS-like fragments that vary in length and sulphation pattern.

From our present knowledge on the synthesis and testing of analogues of the smallest active saccharide from heparin, it was found that the so-called 'non'glycosaminoglycans, having *O*-methyl ethers instead of hydroxyl groups and bearing solely *O*-sulphated esters, are easier to prepare while displaying enhanced biological activity.[6] It was anticipated that the introduction of similar modifications in HS-like oligosaccharides is also feasible (see Figure 1). Nevertheless, the preparation of such long oligosaccharide fragments still is laborious and time-consuming.

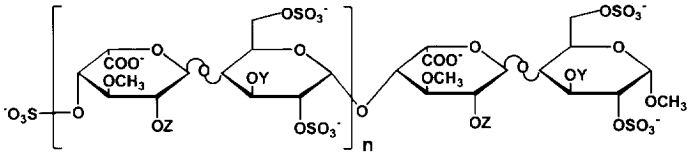
Figure 1



[*] Dedicated to Professor H. Paulsen on the occasion of his 75th anniversary.

For this reason we started to investigate the preparation of HS-like fragments via solid-phase methodologies.[7] Solid-state synthesis of oligosaccharides, although it has been described in several publications,[8] still encounters many hurdles such as decreased glycosylation rates; incomplete coupling and low stereoselectivity. Recently, Krepinsky[9] reported the successful use of polymer-supported solution synthesis for the preparation of short oligosaccharides. Via this methodology a polyethylene glycol (PEG) polymer-carbohydrate is obtained, which is soluble during glycosylation conditions but solidifies during work-up. The solubility of the reactants allows on one hand the reaction kinetics and anomeric control similar to that observed in solution-phase chemistry and on the other hand the insolubility of the polymer-carbohydrate during work-up allows the use of excess reactants and expels time-consuming chromatographic purification. Additionally, reactions can easily be studied by ^{1}H NMR spectroscopy. The added advantages of this approach over a solid-phase method prompted us to investigate the use of the PEG approach for the preparation of the *O*-methylated heparan sulphate-like oligomers **I-VII** presented in Figure 2.

Figure 2



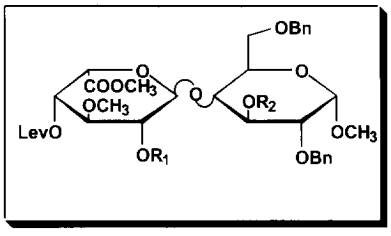
I n = 2; Y = SO_3^-; Z = CH_3
II n = 4; Y = SO_3^-; Z = CH_3
III n = 5; Y = SO_3^-; Z = CH_3

IV n = 2; Y = CH_3; Z = SO_3^-
V n = 3; Y = CH_3; Z = SO_3^-
VI n = 4; Y = CH_3; Z = SO_3^-
VII n = 5; Y = CH_3; Z = SO_3^-

From our earlier experience[6,10] it is apparent that coupling of various L-iduronic acid acceptors with D-glucopyranosyl imidate donors resulted in α-coupled products, exclusively. These findings stimulated us to use disaccharide imidates **2a,b** and the 'pegylated' iduronic acid-containing disaccharides **3a,b** as the respective donors and acceptors in the PEG-supported synthesis cycle (See Scheme). The donors **2a,b** as well as the acceptors **3a,b** were both generated from the known precursor **1a,b**.[11] Disaccharides **2a,b** were prepared according to standard procedures in high overall yields. The pegylated acceptors **3a,b** were synthesized in six steps starting from **1a,b**. Firstly, the benzyl groups were removed by hydrogenolysis and subsequent treatment with TBDMS-Cl in pyridine resulted in the 6-*O*-silylated disaccharides, exclusively. Acylation of the remaining secondary hydroxyl functions gave the fully protected disaccharide intermediates, the silyl protective group of which was hydrolyzed with acetic acid in tetrahydrofuran and water. The primary hydroxyl function was reacted with succinic anhydride in pyridine in the presence of a catalytic amount of 4-dimethylaminopyridine (4-DMAP). The carboxyl group thus introduced was activated in situ with 1-(3-dimethylaminopropyl) 3-ethylcarbodiimide hydrochloride (EDCI) and condensed with

polyethylene glycol monomethyl ether (Mw 5000) in dichloromethane to give the target acceptor disaccharides **3a** and **b** in an overall yield of 17% and 47%, respectively.

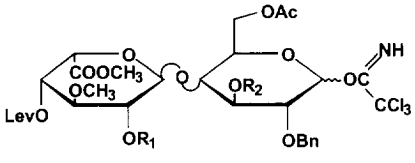
Scheme



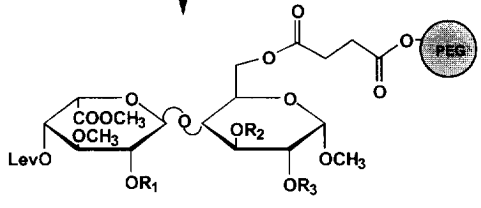
1a $R_1 = CH_3$; $R_2 = Bn$
1b $R_1 = Ac$; $R_2 = CH_3$

iv-ix

i-iii



2a $R_1 = CH_3$; $R_2 = Bn$
2b $R_1 = CH_3$; $R_2 = CH_3$

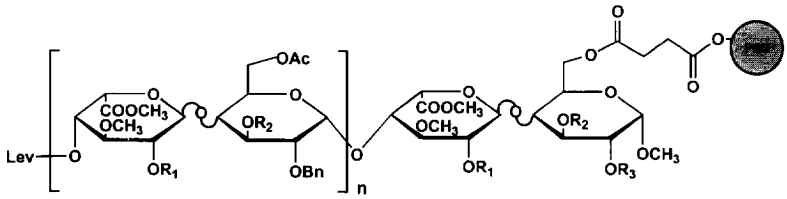


3a $R_1 = CH_3$; $R_2 = R_3 = Ac$
3b $R_1 = Ac$; $R_2 = CH_3$; $R_3 = Ac$

(i) 2% H_2SO_4, Ac_2O; (ii) 4% piperidine, THF; (iii) Cl_3CCN, CH_2Cl_2, Cs_2CO_3; (iv) H_2, PdC, EtOH; (v) TBDMS-Cl, pyridine; (vi) Ac_2O, 4-DMAP, pyridine; (vii) HOAc, H_2O, THF; (viii) succinic anhydride, 4-DMAP, pyridine; (ix) HO-PEG-OCH_3, EDCI, 4-DMAP, CH_2Cl_2

Initially the coupling of imidate **2a** with the pegylated iduronic acid **3a** was studied. In a first experiment 1.5 equiv. of **2a** was reacted with **3a** in the presence of 0.15 equiv. of TMS-OTfl and molecular sieves in dichloromethane, which gave solely the α-anomer at a reaction temperature of -20°C. However the coupling efficiency was rather low (30%), as monitored by ^{1}H NMR spectroscopy. The coupling efficiency could be doubled by raising the reaction temperature to 0°C and simultaneously using twice the amount of donor and promoter. The optimal result, more than 95% couplings efficacy, was obtained at a reaction temperature of +10°C with 2.5 equiv. of the imidate donor **2a** and 0.45 equiv. of promoter based on the acceptor **3a**. The reaction temperature turns out to be a crucial parameter in the optimization of the reaction conditions as polyethylene glycol[12] has a tendency to solidify below 0°C.

Figure 3



4 n = 2; $R_1 = CH_3$; $R_2 = R_3 = Ac$
5 n = 4; $R_1 = CH_3$; $R_2 = R_3 = Ac$
6 n = 5; $R_1 = CH_3$; $R_2 = R_3 = Ac$

7 n = 2; R_1 = Ac; $R_2 = CH_3$; $R_3 = Ac$
8 n = 3; R_1 = Ac; $R_2 = CH_3$; $R_3 = Ac$
9 n = 4; R_1 = Ac; $R_2 = CH_3$; $R_3 = Ac$
10 n = 5; R_1 = Ac; $R_2 = CH_3$; $R_3 = Ac$

The optimized coupling conditions described above were used to assemble the fully protected and pegylated oligosaccharides **4** - **10** (Figure 3). One elongation cycle comprises (see Table) the following consecutive steps; (1) removal of the levulinoyl group with hydrazine; (2) TMS-OTfl assisted coupling of the pegylated acceptors with the appropriate glycosyl donors **2a,b**; and (3) capping of unreacted 4-hydroxyl groups.[13] After each step in the elongation cycle the intermediate pegylated oligosaccharides were precipitated with diethyl ether and collected as white solids. The efficiency of each cycle was monitored by ^{1}H NMR spectroscopy. Cycles were repeated when the coupling efficiency was below 95%.

Table

Step	*manipulation*	*reagent/solvent*	*time (min)*
1	delevulinoylation	NH_2NH_2/AcOH/pyridine	5
2	coupling	**2a,b**/TMSOTfl/CH_2Cl_2/ms 4 Å/10°C	240
3	capping	Ac_2O/pyridine	30

The fully protected oligosaccharides **4** - **10** were then deprotected in two steps. Firstly all esters were saponified using lithium hydroperoxide.[14] Subsequent hydrogenolysis and O-sulphation using triethylamine-sulphur trioxide complex, afforded the crude sulphated oligosaccharides **I** - **VII**. Purification of the crude products by preparative anion exchange chromotography on a monoQ™ 5/10 column, applying a sodium chloride gradient gave after size exclusion chromatography (Sephadex G-25) the target heparan sulphate-like oligosaccharides in good yields.[15] The identity and purity of the compounds was ascertained by ^{1}H NMR spectroscopy, MALDI-mass spectroscopy,[16] HPLC analysis (anion exchange), and reversed UV capillary electrophoresis (CE).[17]

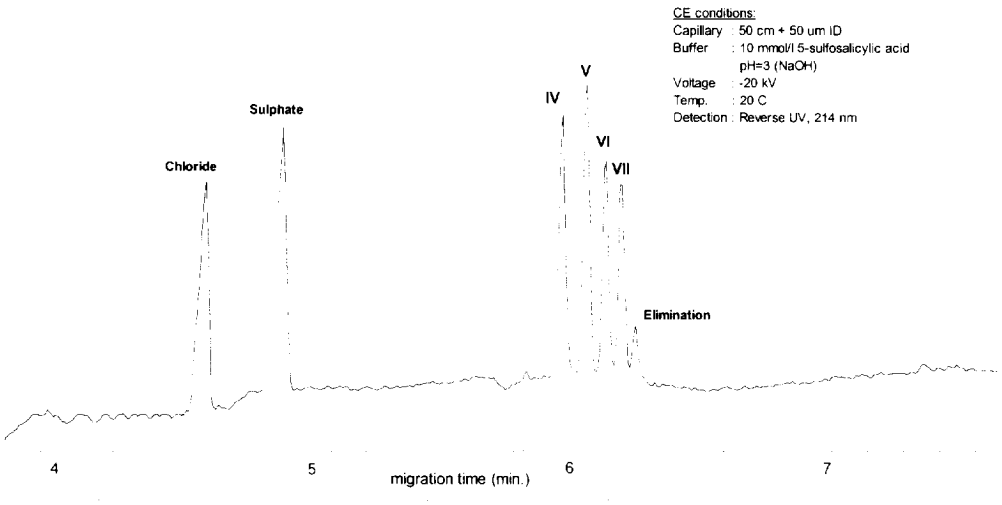


Figure 4: CE electropherogram of the sulphated oligosaccharides **IV-VII**.

The MALDI-mass experiments were performed using a new strategy[18] in which the total mass of a cluster of highly basic peptides with the sulphated oligosaccharide was determined.

In conclusion, the successful assembly of several alternating HS-like oligosaccharides via a polymer-supported solution synthesis using disaccharide synthons is demonstrated. Since, preliminary results indicated that oligosaccharides with heterogenic sequences could also be prepared,[19] it is inferred that PEG supported synthesis may provide an efficient route towards the preparation of libraries of glycosaminoglycans mimics.

Acknowledgment: The authors acknowledge Y.M. Diepeveen for recording the NMR spectra and H. Peters for performing the CE analysis.

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13. Capping was only implemented in the elongation cycle if heterogenic sequences were prepared.
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15. The sulphated oligosaccharides **IV-VII** contain small amounts of oligosaccharides of which the terminal non-reducing sugar unit is a Δ-4,5 uronic acid derivative. These impurities are formed during the sulphation *via* β-elimination and unfortunately were not separable from the final compounds.
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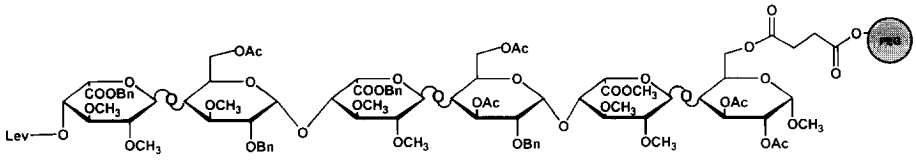
19. A fully protected pegylated hexasaccharide with a heterogenic structure prepared via PEG-supported solution synthesis using disaccharide synthons is illustrated below.



(*Received in USA* 6 *February* 1997; *accepted* 31 *March* 1997)