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Synthesis of heparin-like oligosaccharides on a soluble polymer support†

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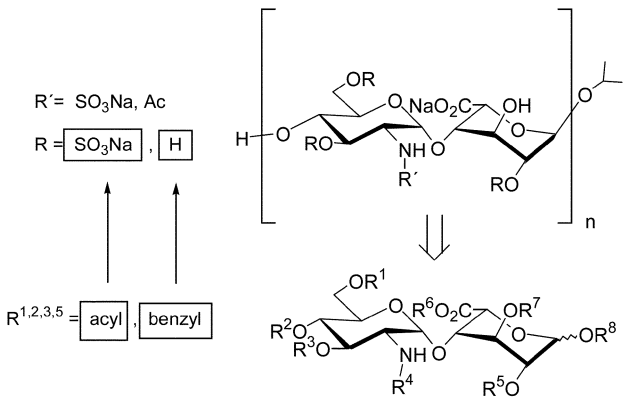
Based on previously developed solution phase chemistry, an effective general approach to the synthesis of heparin-like oligosaccharides on a soluble polymer support is reported.

The biological functions of heparin-binding proteins are regulated by heparan sulfate glycosaminoglycans (HS-GAGs).[1] The recognition that a structurally unique heparin pentasaccharide binds antithrombin with high selectivity[2] suggested that the interactions of GAGs with heparin-binding proteins[3] are saccharide specific. However, the minimal structural requirements for GAGs to bind other heparin-binding proteins have not been as precisely determined. Thus, for fibroblast growth factors 1 and 2 (FGF-1 and FGF-2)[4] minimal binding motifs have been reported[5] but the information available from crystallographic analysis of FGF complexes with heparin fragments reveals highly diverse patterns of stoichiometry, contact sites and orientation.[6] The heterogeneity of the GAG fragments used in these studies is thought to be partially responsible for this apparently conflicting evidence. Indeed, HS is recognised to be a family of closely related polysaccharides composed of unsulfated and variously sulfated sequences of alternating 1 → 4 linked D-glucosamine and L-iduronic or D-glucuronic acid units.[7] It is generally agreed that the availability of specifically designed heparin-like oligosaccharides with precisely defined molecular structures will constitute a crucial step in studying and understanding the molecular basis of the diverse heparin (HS) regulated biological activities.[3,4]

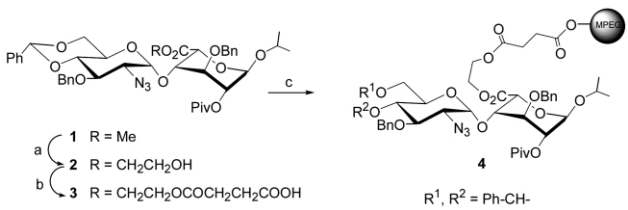
In the frame of a programme on the activation of FGFs we have synthesised several heparin-like oligosaccharides using a convergent *n* + 2 approach (Scheme 1) that permits us to control the size, the sequence and the pattern of sulfation in the final product.[8] Effective as this approach might be, the synthesis of the diversity of oligosaccharides required for investigating the molecular recognition events involved in the interaction of heparin (HS) with heparin-binding proteins demands further simplification and automation. Here, as in most areas in Glycobiology, the development of efficient solid phase synthesis conditions to construct oligosaccharides on automated synthesisers is much needed. However, solid phase methodologies for the synthesis of such complex oligosaccharides have to be further developed.[9] In spite of the considerable effort devoted to the synthesis of GAG oligosaccharides,[10] only a preliminary communication on solid phase synthesis of *O*-methylated HS-like oligomers has appeared in the literature.[11]



Scheme 1

As a consequence of our interest in the molecular mechanism of FGF activation we are involved in work directed towards developing a general solid phase synthesis approach based on our previous solution phase syntheses.[8] As a result we report in this communication an effective preparation of heparin-like oligosaccharides on a soluble polymer support as a key step in simplifying the synthetic process. The effectiveness of this approach is illustrated by the synthesis of hexasaccharide **14** which contains the structural motif of the regular region of heparin.

The extension of our solution phase chemistry[8] to the polymer support has involved a careful study of the compatibility of all parameters involved such as type of support, linker and attachment as well as capping, glycosylation and cleavage strategies. Problems associated with the solid phase reactivity of the key disaccharide building blocks, and particularly of the increasing oligosaccharide constructs, in the formation of the α(1 → 4) glycosidic linkage decided us to use a soluble polymer support such as the readily available polyethylene glycol ω-monomethyl ether (MPEG).[12] On the other hand, our solution phase chemistry conditions were compatible with an ester linker, the crucial choice being the attachment position. Regarding the sulfation patterns of natural GAGs it seemed advisable to avoid positions R^1, R^3, R^4, and R^5. Problems associated with the well established preparation of the key L-iduronate monosaccharide building bocks[13] precluded R^7 and previous experience also advised us to avoid R^8. Therefore, we envisaged attaching the starting disaccharide building block (**1**) to the support through the carboxylate group (Scheme 2). For the sake of practicality and in order to make as much use as possible of well established solution phase chemistry the methyl ester group was transformed into the 2-hydroxyethyl uronate (**2**) using recently reported mild transesterification conditions.[14]. Compound **2** was then attached to MPEG through hemisuccinate **3**. The MPEG-bound disaccharide **4** was then transformed into a glycosyl acceptor using the previously established[8] sequence **4** → **5** → **6** (Scheme 3). The outcome of this and the ensuing reaction sequences was directly followed using NMR spectroscopy. Glycosylation with glycosyl donor **7**[8] gave the MPEG bound tetrasaccharide **8**.[15] This glycosylation step was



Scheme 2 a) Ethylene glycol, Bu_2SnO, toluene, 135 °C, 81%; b) succinic anhydride, DMAP, Py, 98%; c) MPEG, DIC, DMAP, CH_2Cl_2, 100%.

† Electronic supplementary information (ESI) available: NMR spectra. See http://www.rsc.org/suppdata/cc/b3/b307259b/

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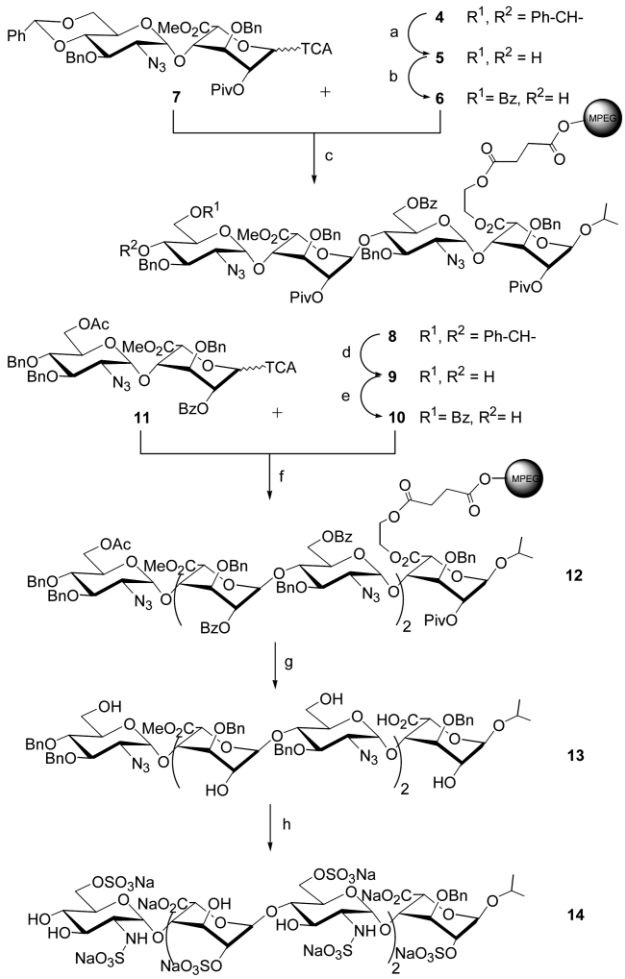

Scheme 3 a) EtSH, *p*TsOH, CH_2Cl_2; b) BzCN, Et_3N (cat.), CH_3CN, −20 °C; c) i. TMSOTf, CH_2Cl_2 (four cycles); ii. PS-Suc-COOH, DMAP, DIC, CH_2Cl_2; d) EtSH, *p*TsOH, CH_2Cl_2; e) BzCN, Et_3N (cat.), CH_3CN, −15 °C; f) i. TMSOTf, CH_2Cl_2 (four cycles); ii. PS-Suc-COOH, DMAP, DIC, CH_2Cl_2; g) i. LiOH, H_2O_2, THF; ii. MeOH, KOH 3M, 37% from **3**, 8 steps; h) see reference [8*a*].

followed by capping using a Merrifield type resin functionalised with an acid-ended tether to esterify unreacted hydroxyl groups.[15] The MPEG-bound tetrasaccharide glycosyl acceptor was then generated using the sequence **8** → **9** → **10**. Glycosylation of **10** with donor **11**[8] afforded bound hexasaccharide **12**.[15] Although cleavage from the support could be performed by dibutyltin oxide mediated transesterification with methanol,[14] cleaner results were obtained by directly submitting **12** to basic conditions (Scheme 3). The obtained hexasaccharide **13** showed an NMR spectrum that was identical in all respects to that of a sample previously prepared using solution phase chemistry.[8] From **13** the target hexasaccharide **14** can be directly prepared.[8*a*]

In conclusion, the solution phase synthetic approach previously developed by us has allowed for the construction of the basic heparin-like hexasaccharide backbone on a polymer support. Using this approach, the diversity of disaccharide building blocks already prepared will permit the straightforward preparation of a diversity of GAG oligosaccharides for biological investigation.

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15 The glycosylation reactions were carried out using the following general protocol: To a mixture of the MPEG-bound glycosyl acceptor (0.1 mmol) and glycosyl donor (0.2 mmol) in dry CH_2Cl_2 (3 mL), TMSOTf (0.03 mmol) was added. After stirring for 1–2 h, Et_3N was added and the volume reduced to 2 mL. Et_2O (30 mL) was added and the precipitate removed. Capping was then performed by swelling a mixture of the formed MPEG-bound oligosaccharide, PS-Suc-COOH (0.49 mmol) and DMAP in dry CH_2Cl_2 (7 mL), then adding DIC (0.8 mmol) and shaking overnight. The mixture was filtered and the filtrate concentrated to 3–4 mL. Et_2O (40–50 mL) was added until precipitation of the pure MPEG-bound oligosaccharide occurred.