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Assembly of glycoaminoacid building blocks: A new strategy for the straightforward synthesis of heparan sulfate mimics†

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A new strategy that enables a modular straightforward synthesis of heparan sulfate oligosaccharide mimics by the assembly of simple glycoaminoacid building blocks is described. The coupling between units is readily carried out by an amidation reaction. Several glycoamino acid oligomers were prepared and their interaction with the FGF2 protein was analyzed.

Heparan sulfate (HS) is a linear polysaccharide composed of repeating disaccharide units of uronic acid (either D-glucuronic, GlcA, or L-iduronic acid, IdoA) linked to 2-amino-2-deoxy D-glucopyranose (GlcN) (Figure 1a). These units may be sulfated at *C*-3 and *C*-6 of GlcN and at *C*-2 of the uronic acid unit. The nitrogen of the GlcN unit can be sulfonated, acetylated or unsubstituted. HS does not usually occur as a free molecule but rather it forms conjugates with proteins called HS proteoglycans, which are present in the extracellular matrix (ECM) or on the cell surface. HS has structural functions and regulates a wide range of biological processes.[1-3] HS binds to proteins mainly through electrostatic interactions between the sulfate groups and positively charged residues in the protein. The specificity and the affinity of a protein for HS are highly dependent on its sulfation pattern and chain length. [2,4] The wide range of HS interactions, which are relevant to many disease processes, have made these biomolecules attractive targets for drug development.[5,6]

An important challenge for structure–activity relationship studies of HS and to the development of new HS-based drugs is the limited availability of homogeneous HS with well-defined sulfation patterns. Although there have been major advances in the chemical synthesis of HS oligosaccharides that have led to the access of complex structures,[7-9] the preparation of these molecules is still challenging. An alternative approach is to synthesize compounds of similar structure that are easier to prepare and can mimic the biological activity of natural HS.[10] For example, the semisynthesis of HS mimics by regioselective sulfation of more simple and readily available polysaccharides.[11,12] Although in this approach it is possible to obtain complex structures with control over the total sulfation degree, the sulfation sequence along the sugar chain is uncertain and it is not achieved with the precision of the typical synthesis of oligosaccharides. The synthesis of HS-mimicking polymers offers better control over sulfation profile. Several examples of sulfated glycopolymers obtained by polymerization of glycomonomers have been described,[13] including libraries of glycopolymers obtained from the disaccharide units with sulfate groups at specific positions.[14-16] In these polymers the saccharide residues are not linearly linked, as occurs in natural HS, but they are pending from the polymeric backbone providing a multivalent presentation of the sugar units. Similarly, a library of glycomimetic clusters capped with a variety of HS saccharide fragments have been described with the ability to mimic the activity of natural HS.[17]

In the present work, we have designed and synthesized a new class of linear HS oligosaccharide mimics that can simplify and reduce the number of steps of the conventional HS oligosaccharide synthesis and can generate molecular diversity. The synthetic strategy is based on the assembly of glycoamino acid building blocks through amide linkages.[18] In the structure of the new mimics (Figure 1b), the uronic acid of HS is replaced by an amino acid having a hydroxyl group, namely serine (L-Ser), which is α-glycosylated with GlcNAc. This substitution will significantly shorten the number of synthetic steps since the preparation of a single uronic acid building block is usually cumbersome.[19] The assembly of the building blocks is carried out by amide bond formation between the carboxylic acid

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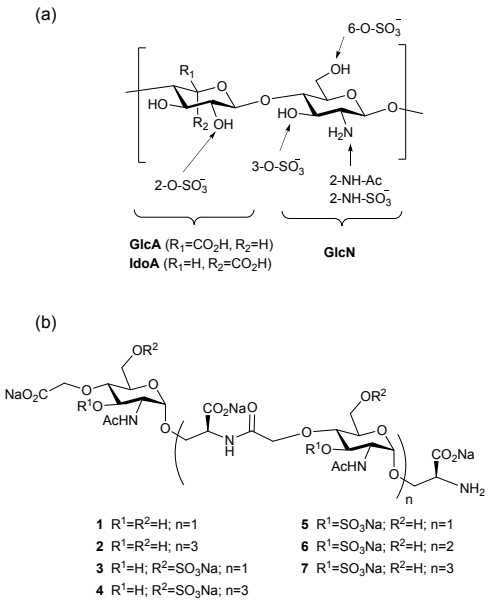

Figure 1. Chemical structure of HS (a) and of the mimics (b) synthesized in this work.

attached to *O*-4 of the GlcN moiety with the amine of the amino acid. This reaction circumvents the limitations of stereocontrolled glycosylations and facilitates the automation of the process. Here we present the synthesis of glycoamino acid oligomers with variable chain length, without sulfate groups (**1** and **2**, in Figure 1b) or with sulfate groups at *C*-6 (**3** and **4**) or *C*-3 (**5**-**7**) of the GlcN moiety. The binding affinity of the compounds with the fibroblast growth factor-basic (FGF2) protein was evaluated using surface plasmon resonance (SPR).

The synthesis starts from derivatives **8**-**10** bearing a thiophenyl group at the anomeric position (Scheme 1), which can be activated for glycosylation under a variety of conditions and is chemically stable before activation. Alkylation of **8**-**10** with *tert*-butyl bromoacetate allowed us to introduce the protected carboxylic group at the *O*-4 position of the sugar. Glycosylation of L-serine derivative **14** with donors **11**-**13** was carried out in the presence of NIS/TMSOTf as a promoter in a mixture of Et_2O/CH_2Cl_2 at low temperature, to give the α-glycosides **15**-**17** stereoselectively. Treatment of **15**-**17** with trifluoroacetic acid afforded the free carboxylic acids **18**-**20**, whereas the amines **21**-**23** were obtained by reaction of **15**-**17** with piperidine. Amide coupling of carboxylic acids with amines was first attempted using EDC in the presence of a catalytic amount of DMAP; but poor yields (30-40%) were obtained. Conversion of the carboxylic acid to the *N*-hydroxysuccinimide-activated ester and subsequent treatment with the amine did not lead to a significant improvement in yield. We found the best conditions to be when the coupling was carried out in the presence of hydroxybenzotriazole, affording the products **24**-**26** in higher yields (86-96%) and shorter reaction times (2-4 h). For the synthesis of longer oligomers (**2**, **4** and **6**), the process of side-chain deprotection and subsequent coupling was repeated on **24**-**26** to give **33**, **34**, and **35** in 82, 77, and 80% yields, respectively. Reduction of azides **24** and **33** with AcSH, which proceeded with concomitant acetylation,[20] gave the corresponding acetamides **36** and **37** in 94 and 86 % yields, respectively. Subsequent deprotection steps afforded the nonsulfated oligomers **1** and **2**.

Sulfated oligomers were prepared from intermediates **25**-**26** and **34**-**35** (Scheme 1). Initial attempts to sulfate *C*-3 in compound **26**, after conversion of the azide group into acetamide and then removal of NAP group, gave complex mixtures. We reasoned that the presence of the acetamide could affect the sulfation step, and thus we changed the reaction sequence so that the azide group was kept during the sulfation reaction. To remove the *C*-3 naphthyl ether, compound **26** was treated with DDQ in a biphasic CH_2Cl_2/H_2O solvent system to give the diol **40** in 65% yield. When the oxidative cleavage was carried out in a phosphate buffer[21] at pH 7.5 the yield was increased to 75%. A similar increase in yield was obtained using the CH_2Cl_2/H_2O solvent system in the presence of β-pinene as an acid scavenger.[22] Sulfation of **40** was carried out with SO_3·Py complex at 50 °C to give disulfate derivative **44** in 90% yield, which was then treated with AcSH to give diacetamide **48**. Removal of the Fmoc group, followed by hydrolysis of *tert*-butyl ester and hydrogenolysis of the benzyl groups furnished short oligoamide 3-*O*-sulfonated **5**. A similar protocol was applied to intermediates **25**, **34** and **35** to afford sulfated oligomers **3**, **4** and **6**, respectively.

When assessed the interaction of the oligomers with FGF2 by SPR, the results indicated a tendency to an increased binding with the size of the chain and with the sulfation at *C*-3 (see below). In light of these results, we decided to synthesize an oligomer of longer chain length with six glucosamine units bearing sulfate groups at the *C*-3 position (compound **7**, Scheme 2). The synthesis used the intermediates which have sulfate and acetamide groups installed (**48** and **49**), and were used for the preparation of oligomers with two and four glucose units **5** and **6**, respectively. The reaction between the carboxylic acid **50**, generated from **48**, and the amine **51**, obtained from **49**, gave compound **52** (60% yield). Subsequent deprotection steps led to oligomer **7**. Interestingly, the synthetic approach used for **7** shows the compatibility of the coupling reaction with substrates having sulfate groups, thus allowing the chain assembly using building blocks with preinstalled sulfate groups. In a typical chemical synthesis of heparin oligosaccharides, the sulfate groups are introduced after the assembly of the oligosaccharide chain. This can be problematic with larger oligosaccharides where multiple sulfations are required since the late-stage sulfation can be difficult to reach completion. Moreover, the use of building blocks already having the required functional groups should facilitate synthesis automation.

To evaluate the ability of the glycoamino acid oligomers **1**-**7** to interact with growth factor proteins, we performed a binding study with FGF2 using SPR technique. FGF2 is an important protein involved in the regulation of cell proliferation and differentiation.[2] Compounds **1**-**7** were flowed on the immobilized FGF2 at a 200 μM concentration. As can be appreciated in Figure 2, the binding of the oligomers to FGF2 depends on the presence and the position of sulfate groups, and on the length of the chain. Non-sulfated compounds **1** and **2**, and the short 6-*O*-sulfonated oligoamide **3**

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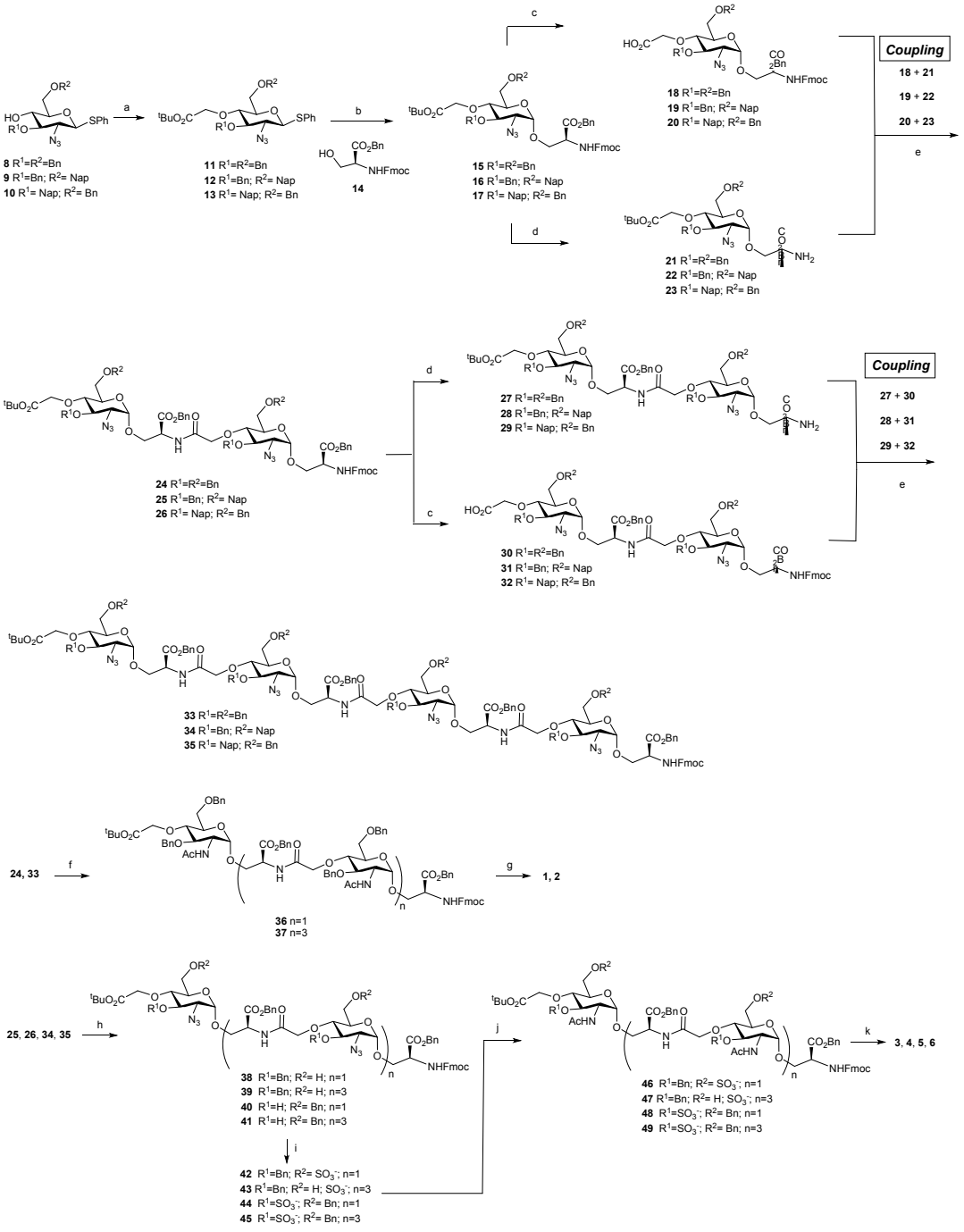

Scheme 1 Reagents and conditions: a) *t*BuO_2CCH_2Br, NaH, TBAI (**11**, 85%; **12**, 90%; **13**, 93%); b) **14**, TMSOTf, Et_2O: CH_2Cl_2, -30 °C (**15**, 65%; **16**, 53%; **17**, 65%); c) TFA, CH_2Cl_2; d) piperidine, DMF; e) EDC, DMAP, HOBt, THF (**24**, 96%; **25**, 93%; **26**, 86%; **33**, 87%;**34**, 76%; **35**, 75%); f) AcSH, py (**36**, 94%; **37**, 94%); g) 1) piperidine, DMF; 2) TFA, CH_2Cl_2; 3) H_2, Pd/C, MeOH (**1**, 56% from **36**; **2**, 54% from **37**); h) DDQ, CH_2Cl_2, buffer pH 7.5 (**38**, 71%; **39**, 65%; **40**, 75%; **41**, 69%); i) SO_3·py, 50 °C (**42**, 75%; **43**, 60%; **44**, 90%; **45**, 67%); j) AcSH, py (**46**, 75%; **47**, 82%; **48**, 91%; **49**, 66%); k) 1) piperidine, DMF; 2) TFA, CH_2Cl_2; 3) H_2, Pd/C, MeOH (**3**, 73%; **4**, 57%; **5**, 63%; **6**, 75%).

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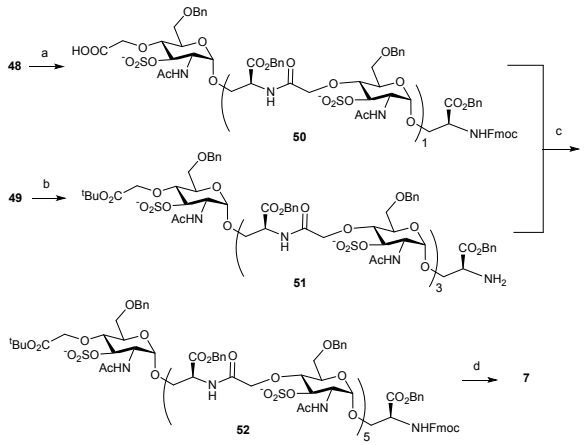

Scheme 2 a) TFA, CH2Cl2; b) piperidine, DMF; c) EDC, DMAP, HOBt, THF (60%); d) i) piperidine, DMF; ii) TFA, CH2Cl2; iii) H2, Pd/C, MeOH (64% from **52**).

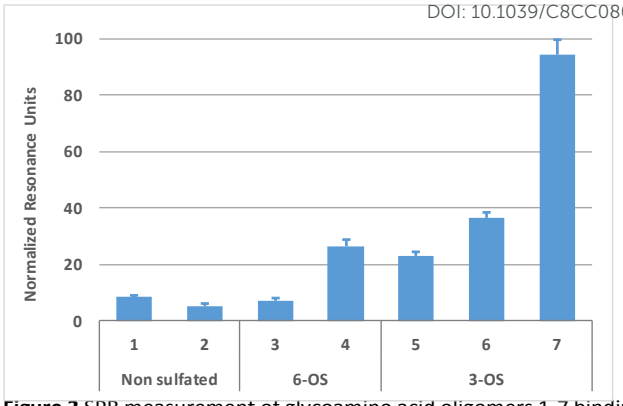

Figure 2 SPR measurement of glycoamino acid oligomers 1-7 binding to immobilized FGF2.

showed negligible binding response with FGF2. On the other hand, a clear effect was observed as a function of the sulfate group position. Thus, compounds with a sulfate group at *C*-3 (**5** and **6**) showed stronger binding than the *C*-6 sulfated derivatives of equal chain length (**3** and **4**, respectively). More significant was the effect due to the chain length. The four-block oligomers **4** and **6** had a greater interaction with FGF2 than their corresponding two-block oligomers **3** and **5**. The longest compound **7**, with six glycoamino acid blocks, showed a substantial increase in the interaction, supporting the positive correlation between chain length and interaction intensity. Using steady-state analysis of SPR measurements for the interaction between **7** and FGF2, the dissociation constant (K_D) was found to be 448 µM.

In summary, we have described the synthesis of novel structural mimics of HS that can potentially provide a high degree of molecular diversity. These compounds are readily obtained through a synthetic route that has been optimized by choosing appropriate protecting groups and reaction conditions. The coupling of building blocks is performed by a simple and efficient amidation that allows the use of preinstalled sulfate groups. Although the binding affinity with FGF-2 is relatively low compared to other described HS oligosaccharides or HS mimics (which generally have more sulfate groups), we observed a structure-dependent interaction of the compounds with FGF2, which suggests that the strategy is appropriate to obtain model compounds for interaction studies with HS-binding proteins.

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Conflicts of interest

There are no conflicts to declare.

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