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A Modular Strategy Toward the Synthesis of Heparin-like Oligosaccharides Using Monomeric Building Blocks in a Sequential Glycosylation Strategy

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Abstract: A novel flexible assembly strategy is described for the modular synthesis of heparin and heparan sulfates. The reported strategy uses monomeric building bocks to construct the oligosaccharide chain to attain a maximum degree of flexibility. In the assembly, 1-hydroxyl glucosazido- and 1-thio uronic acid donors are combined in a sequential glycosylation protocol using sulfonium triflate activator systems. The key 1-thio uronic acids were obtained in an efficient manner from diacetone glucose employing a chemo- and regioselective oxidation of partially protected glucose and idose thioglycosides.

Introduction

Heparin and heparan sulfate (H and HS) are present in many living organisms and are the most complex members of the glycosaminoglycan (GAG) superfamily.[1] They are composed of a linear backbone built up from 20 to 200 α-1,4-linked disaccharide moieties, which in turn are constructed from an uronic acid (either D-glucuronic or L-iduronic) and a glucosamine residue (Figure 1). H and HS are characterized by the high degree of sulfation at both the hydroxyl and the amino groups of the polymer. Sulfate groups have been found on the 3- and/or 6-position of the glucosamine residue and on the 2-position of the uronic acids. In addition, the amino groups of the polysaccharide can be sulfated, acetylated, or unsubstituted. The different substitution patterns of the disaccharide units, together with the variant alternation of the GlcA−GlcN and IdoA−GlcN moieties, give rise to innumerable H and HS structures. This microheterogeneity is at the basis of the plethora of regulatory functions H and HS fulfill, and it is now well-established that H and HS play a pivotal role in processes such as blood coagulation, inflammation, homeostasis, cell adhesion, and cell growth.[1,2] However, the microheterogeneity is also the origin of the lack of detailed knowledge of the mode of H and HS action at the molecular level. To elucidate the selective mode of binding of specific H and HS fragments with their protein targets, well-defined H and HS sequences are required. One of the most powerful tools to obtain these complex oligosaccharides is evidently organic synthesis.[3] This is nowhere better illustrated than by the anticoagulant heparin pentasaccharide. Since the first heroic total syntheses[4] two decades ago, an analogue to this highly functionalized pentamer has been developed into a novel antithrombotic drug (Arixtra),[5] which very selectively targets the anticoagulation factor Xa through activation of the protease inhibitor antithrombin III (AT III). Although these total syntheses represent a milestone in heparin synthesis, they were highly target-orientated.[6] To grant access to a wide range of differentially substituted H and HS fragments, a general modular synthetic strategy is required.[7−9] Such a strategy uses a set of

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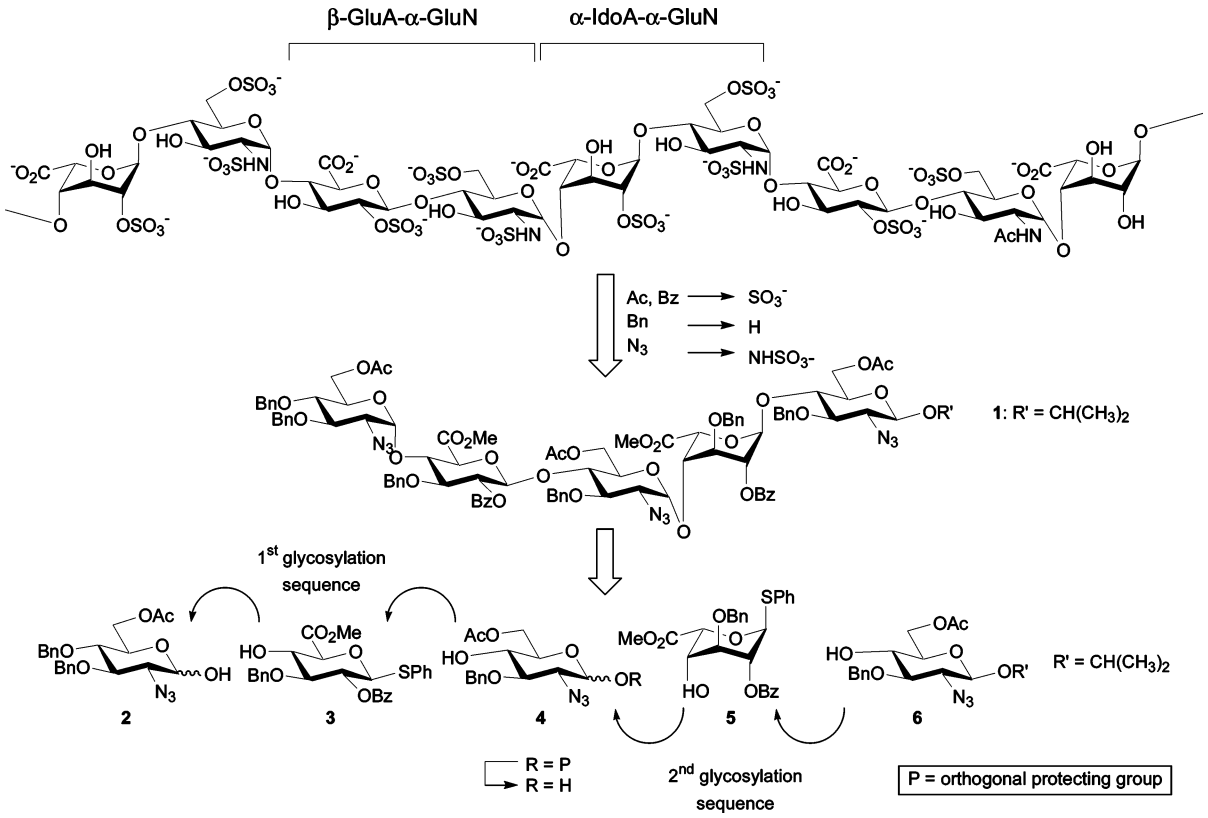

Figure 1. Synthetic strategy.

closely related building blocks, which should allow for standardized coupling conditions and provide flexibility and variety in use. To attain a maximum degree of flexibility in the assembly of H and HS fragments, we present here the first modular strategy that employs monomeric building blocks. The use of monomeric modules will open up possibilities for the incorporation of a diverse set of easily available building blocks both "natural" and "unnatural" (for example, conformationally restricted, flexible, epimeric, and "non-GAG") synthons, and it avoids the use of far-advanced and therefore precious dimer or oligomer motives. Efficiency in the monomeric assembly strategy of the H and HS fragments is warranted by the application of an iterative sequential glycosylation strategy, which precludes excessive synthetic steps at the oligosaccharide level.

Synthetic Strategy

Any H and HS synthesis has to overcome a range of synthetic hurdles imposed by the complex structure of the H and HS saccharides. Besides the elaborate protecting group strategy that has to enable the installation of sulfate groups on selected hydroxyl and amino functions, the stereoselective construction of the glucosamine−uronic acid backbone forms a major obstacle. The inherent low reactivity of the uronic acid building blocks (as compared to their non-oxidized glucose and idose counterparts)[11] requires a glycosylation methodology that at the same time is robust enough to effectuate an efficient glycosylation and watches over the stereocontrol in the formation of the glycosidic linkages. In this respect, the α-glucosamine−glucuronic acid linkage is of special interest, since the stereoselective construction of this linkage, in contrast to its α-glucosamine−iduronic acid counterpart, has received very little attention of the scientific community.[12] Finally, an efficient route of synthesis should be devised for the necessary building blocks. The latter holds especially true for the iduronic acid synthons, since idose or iduronic acid are not readily available from natural or commercial sources.[13,14]

The synthetic approach presented here is based on a sequential glycosylation strategy using monomeric building blocks and

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capitalizes on the use of 1-thio uronic acid synthons and the recently introduced sulfonium activator systems, as is depicted in Figure 1.[15] The 1-thio uronic acid building blocks represent a highly disarmed class of donor glycosides and require a very potent activator system to promote their condensation.[13a] It was reasoned that the thiophilic sulfonium activator systems diphenylsulfoxide/triflic anhydride (Ph_2SO/Tf_2O)[16,17] and 1-benzenesulfinyl piperidine/triflic anhydride (BSP/Tf_2O)[18] should be potent enough to activate these highly disarmed 1-thio uronic acids,[13a] facilitating their condensation with the appropriate glucosazide building blocks and therefore allowing their effective use in the assembly of H and HS fragments. To introduce the α-glucosamine linkages, we selected 1-hydroxyl glucosazide donors, which can be activated by the same sulfonium activator systems.[19] It is well-known that the stereochemical outcome of any condensation greatly depends on the reactivity of the coupling partners involved and that glycosyl donors and acceptors of low reactivity generally benefit the formation of the more thermodynamically favored product. Indeed, a very high degree of α-selectivity was recurrently observed in the condensation of 1-hydroxyl donors bearing a nonparticipating C2-hydroxyl protecting group with a variety of rather unreactive acceptors.[15,20] Accordingly, the glycosylations of the unreactive C4-hydroxyl of glucuronic acid acceptors with their designated 1-hydroxyl glucosazide donors should also predominantly provide the α-linked products. Furthermore, the condensation of L-iduronic acid acceptors and their D-glucosazide coupling partners has been shown to proceed in a highly stereoselective fashion to provide the desired α-glucosamine linkage guided by double stereodifferentiation (matched pair) in the transition state leading to the interglycosidic bond.[21]

To probe the above-described assembly strategy, fully protected pentasaccharide **1** (Figure 1) was selected as a model saccharide. Thus, the construction of target compound **1** will start with the condensation of 1-hydroxyl glucosazide donor **2** and suitably protected 1-thio uronic acceptor **3**, having a free 4-OH function. The resulting disaccharide can then immediately be used in the next glycosylation event, in which the thio uronic acid is condensed with glucosamine building block **4**. Unmasking of the anomeric hydroxyl function then paves the way for a second glycosylation sequence in which the trisaccharide is elongated with the second uronic acid building block **5** and the third glucosamine **6** to furnish the pentasaccharide **1**. Throughout this synthetic approach we adopted a well-established protecting group strategy:[4−9] hydroxyls meant to be sulfated are protected with acyl (acetyl and benzoyl) groups, benzyl groups are installed on the remaining hydroxyls, and the amino functionalities are masked as azides.

Synthesis of the Monomeric Building Blocks. The 1-thio uronic acid synthons take up crucial positions in the strategy outlined above. It is therefore of paramount importance to have an efficient route of synthesis for these building blocks. Although great improvements in the construction of 1-hydroxyl glucuronic and iduronic acid building blocks have recently been disclosed,[13] no productive syntheses for their 1-thio counterparts have appeared.[22] Evidently, the presence of both the anomeric thio function and the C5 carboxylic acid function within the same glycoside significantly complicates their construction. To this end, a new synthetic route to access these crucial building blocks was devised. As is depicted in Scheme 1, we took full advantage of the 2,2,6,6-tetramethyl piperidinyloxy free radical (TEMPO)-[bis(acetoxy)iodo] benzene (BAIB) reagent combination,[23] which we recently introduced as an efficient means for the chemo- and regioselective oxidation of thioglycosides into their corresponding 1-thio uronic acids.[24,25] Partially protected 1-thio glucuronic acid precursor **10** was obtained in a straightforward manner from 1,2:5,6-di-*O*-isopropylidene-3-*O*-benzyl-glucofuranose **7**. Acidic hydrolysis of both acetonide functions in **7** and ensuing acetylation provided the β-tetraacetate **8**,[26] which was transformed into thioglycoside **9**. Saponification of the acetate esters in **9** and formation of the 4,6-*O*-benzylidene acetal was followed by protection of the C2-hydroxyl with a benzoyl function to provide anchimeric assistance in the forthcoming glycosylation reactions. Subsequent acidolysis of the cyclic acetal furnished the phenyl thioglucoside **10**.[27] The crucial oxidation step, in which the primary C6-alcohol was selectively oxidized in the presence of both the anomeric thiophenyl moiety and the secondary C4-alcohol function, was accomplished by treatment of **10** with a catalytic amount of TEMPO and a slight excess of BAIB as a co-oxidant in a biphasic dichloromethane/water solvent system to provide the glucuronic acid **11** in a yield of 83%. Transformation of the carboxylic acid into the corresponding methyl ester completed

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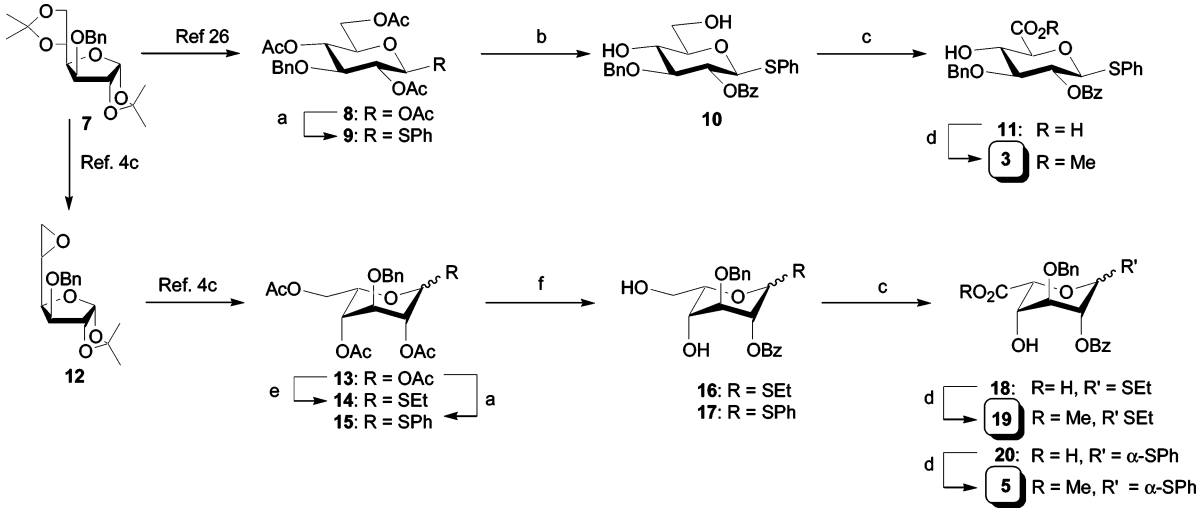
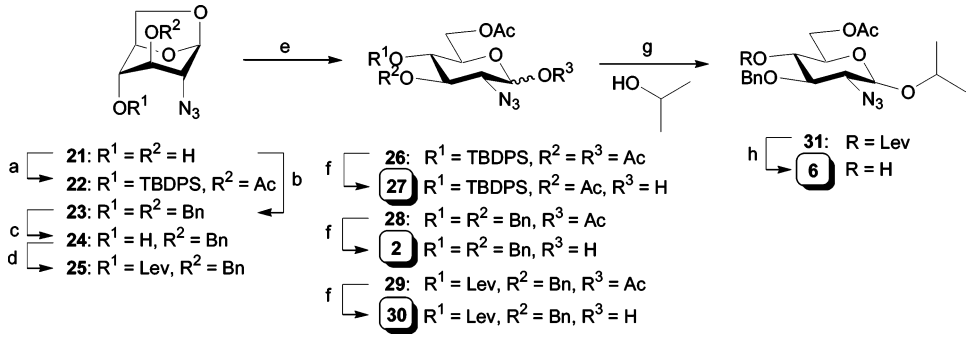
Scheme 1. Synthesis of the 1-Thio Uronic Acid Building Blocks[a]



[a] Reagents and conditions: (a) PhSH, BF_3·OEt_2, DCM, 0 °C to room temperature, **9**: 85%, **15**: 91% (4:1 α/β); (b) i. KO*t*Bu, MeOH/DCM; ii. $PhCH(OMe)_2$, *p*-TsOH, ACN/DMF, 50 °C; iii. BzCl, pyridine, 0 °C to room temperature; iv. 60% TFA in H_2O, DCM, **10**: 80%; (c) TEMPO, BAIB, DCM, H_2O, **11**: 83%, **18**: 83% **20**: 81%; (d) CH_2N_2, DMF, **3**: 91%, **19**: 96%, **5**: 83%; (e) EtSH, BF_3.OEt_2, DCM, 0 °C to room temperature, 79% (1.5:1 α/β); (f) i. KO*t*Bu, MeOH/DCM; ii. DMP, *p*-TsOH, DMF; iii. BzCl, pyridine, 0 °C to room temperature; iv. 80% HOAc in H_2O, 60 °C, **16**: 79%; **17**: 81% (anomers partially separated).

Scheme 2. Synthesis of the Glucosazide Building Blocks[a]



[a] Reagents and conditions: (a) i. TBDPSCl, pyridine; ii. Ac_2O, pyridine, 96%; (b) BnBr, NaH, DMF, 89%; (c) $TiCl_4$, DCM, 85%; (d) Lev_2O, dioxane, pyridine, 100%; (e) 10% TFA in Ac_2O, 0 °C to room temperature; (f) 6% piperidine in THF, **27**: 87% (two steps), **2**: 94% (two steps), **30**: 91% (two steps); (g) **30**, Ph_2SO, Tf_2O, TTBP,[38] DCM, −40 °C, then 2-propanol, 82%, (2:3 α/β); (h) H_2NNH_2·H_2O, HOAc, pyridine, 86%.

the synthesis of the glucuronic acid building block **3**. This highly efficient strategy was also applied to the construction of the 1-thio iduronic acids **5** and **19**. Glucose **7** was converted into its C5-epimer, as reported in the literature,[4c] and transformed into the idopyranose **13**. The anomeric thio function was installed to provide idopyranoses **14** and **15**, which were subjected to a similar sequence of reactions as their glucose congener, to give the 1-thio idose derivatives **16** and **17**. BAIB-mediated TEMPO oxidation of C6 in **16** and **17** proceeded uneventfully to afford the desired iduronic acids **18** and **20**, which were treated with diazomethane to afford the uronic acid ester building blocks **19** and **5**, respectively.

The construction of the glucosazide building blocks used in this study is depicted in Scheme 2 and followed straightforward synthetic methodologies. Thus, regioselective introduction of the *tert*-butyldiphenylsilyl group[28] in **21** and acetylation of the remaining hydroxyl furnished the first fully protected glucosazide **22**. Benzylation of the two hydroxyls in **21** gave compound **23**, of which the C4-hydroxyl was selectively liberated[29] to provide **24**, which will function as a masked equivalent of compound **4** in the assembly of the target pentasaccharide **1**.[30] The orthogonally protected **25** was obtained after protection of the 4-OH in **24** with a levulinoyl group. Acidolysis of the 1,6-anhydro bridge in **22**, **23**, and **25** and selective unmasking of the anomeric hydroxyl functions gave the corresponding glucosazide building blocks **27**, **2**, and **30**. Capping of the reducing end of glucosazide **30** with an 2-propanol moiety provided, after delevulinoylation, the terminal building block **6** for the oligosaccharide assembly.

Glycosylations and Assembly of the Oligosaccharides

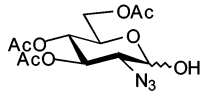
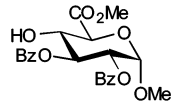
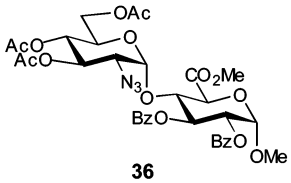
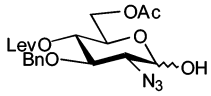
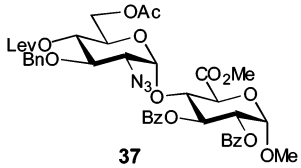
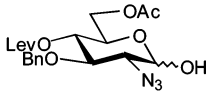
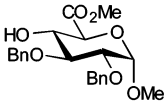
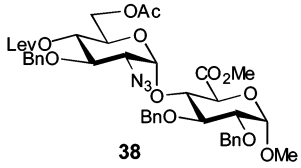
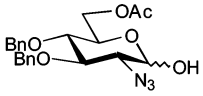
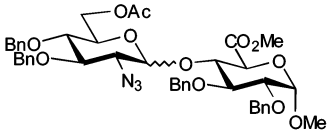
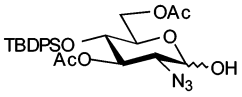
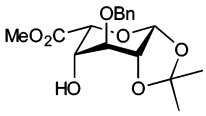
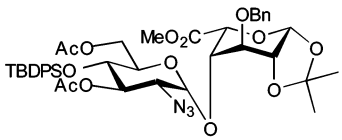
With all the required building blocks in hand, the formation of the crucial α-glucosamine linkages was examined. It is now well-established that the condensation of a D-glucopyranoside donor, bearing a C2-nonparticipating group and a L-idose or L-iduronic acid acceptor, proceeds highly stereoselectively to

(28) When the *tert*-butyldimethylsilyl group was employed for the protection of compound **21** both the OH-3 and OH-4 silylated products were obtained: Izumi, M.; Tsuruta, O.; Harayama, S.; Hashimoto, H. *J. Org. Chem.* **1997**, *62*, 992−998.

(29) Hori, H.; Nishida, Y.; Ohrui, H.; Meguro, H. *J. Org. Chem.* **1989**, *54*, 1346−1353.

(30) Glucosazide **24** presents a practical glycosyl acceptor, combining orthogonal protection with efficient reactivity. Furthermore, the locked anomeric configuration of **24** simplifies the characterization of reaction products as compared to the analogous glucosazide **4** (R = Ac), being a mixture of anomers.

Table 1. Dehydrative Glycosylations Using 1-Hydroxyl Donors and Uronic Acid Acceptors[a]

Entry	Donor	Acceptor	Disaccharide	Yield (α/β)[b]
1	 **32**	 **33**	 **36**	59% (1:0)
2	 **30**	**33**	 **37**	54% (1:0)
3	 **30**	 **34**	 **38**	63% (1:0)
4a	 **2**	**34**	 **39**	a: 76% (7:1)
4b[c]				b: 54% (8:1)
5	 **27**	 **35**	 **40**	50% (1:0)

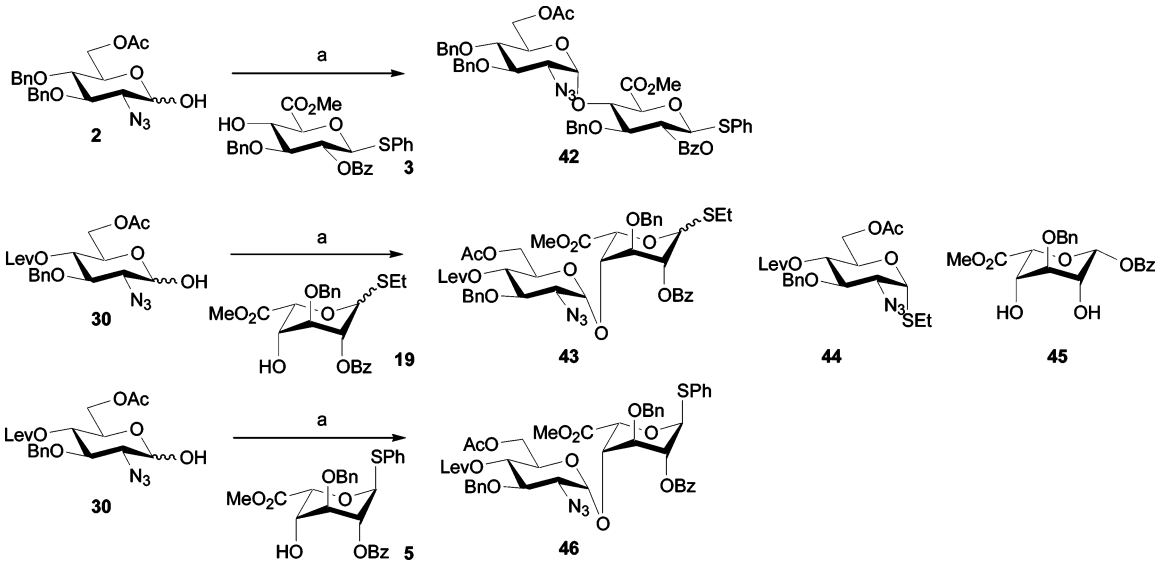
[a] Ph_2SO, Tf_2O, TTBP, DCM, −40 °C to room temperature. [b] anomeric ratio determined from anomeric mixture by NMR. [c] BSP, Tf_2O, TTBP, DCM, −40 °C to room temperature.

form the α-linked products,[4,6,7b,10,12] which is further guided by favorable steric effects in the transition state leading to the glycosidic bond. Analogous condensations employing D-glucose or D-glucuronic acid acceptors generally proceed less stereoselectively to provide α/β-mixtures. From a stereochemical viewpoint, the formation of the α-D-glucosazide-(1 → 4)-L-iduronic acid linkage thus poses no problems, whereas the formation of the corresponding α-D-glucosazide-D-glucuronic acid glycosidic bond presents a challenge, particularly in "armed" glycosylation systems. Consequently, we were eager to find out how glucuronic acid acceptors behave in the dehydrative glycosylations with 1-hydroxyl glucosazide donors, and therefore a series of model glycosylations were conducted, the outcome of which are summarized in Table 1. As can be seen from entries 1−4, the model glucuronic acids **33** and **34** performed well in the Ph_2SO/Tf_2O-mediated condensations, which all proceeded in good yield. In addition, the stereochemical outcome of the condensations proved to be very satisfactory: in all cases the condensation proceeded with a high degree of α-selectivity. In line with the expectations (vide supra), the increased reactivity of the coupling partners in entry 4 led to the formation of some β-linked disaccharide. When the reactivity of the dibenzylated donor **2** was tempered by employing the less reactive BSP/Tf_2O activator system,[31] (entry 4b) a scarcely better α/β-ratio was obtained at the expense of a slightly lower yield. Iduronic acid acceptor **35**[7b] and donor **27** also performed well under the dehydrative conditions to furnish the α-linked disaccharide **40** with complete stereoselectivity (entry 5).

Encouraged by these results, we returned to the 1-thio uronic acid acceptors **3**, **5**, and **19** (Scheme 3). Glucosazide **2** was activated using Ph_2SO in combination with Tf_2O and subsequently condensed with glucuronic acid **3** to provide the α-linked target disaccharide **42** as the sole product in a very rewarding 91% yield. Unfortunately, the condensation of

(31) The glycosylating species that arises from reaction with the BSP/Tf_2O system (i.e., glucosazide **52**) is more stable and therefore less reactive than the corresponding glucosazide that is generated using the Ph_2SO/Tf_2O activator (i.e., **53**).

Scheme 3. Glycosylations with 1-Thio Uronic Acid Acceptors **3**, **19**, and **5**[a]



[a] Reagents and conditions: (a) Ph_2SO, Tf_2O, TTBP, DCM, −40 °C to room temperature, **42**: 91%; **43**: 31% (2.5:1 α/β), **44**: 19%, **45**: 24%; **46**: 43%.

glucosazide **30** and iduronic acid acceptor **19** was less successful and led to a mixture of products. Three major products were isolated: in addition to the desired disaccharide **43** (31% yield), the α-thioglycoside **44** and the 1-β-*O*-benzoyl-L-iduronic acid **45** were formed in 24 and 19% yield, respectively. Apparently the OH-4 in **19** is not nucleophilic enough compared to the ethylthio function at the relatively reactive anomeric center in **19**, and therefore a substantial amount of aglycon transfer takes place.[32] Unwanted intermolecular aglycon transfer reactions have previously been circumvented by changing the protecting groups in the acceptor glycosides.[32b] Such a solution could not be applied here since the protecting group strategy prescribes a benzyl group[33] and a participating acyl function at the C3- and C2-hydroxyls of the iduronic acid synthon, respectively. Therefore, it was decided to tune the reactivity of the anomeric thio function in the acceptor iduronic acid building block, and the α/β-thioethyl function in **19** was changed to an α-thiophenyl group in **5**.[34,35] This subtle modification indeed proved effective in avoiding the undesired aglycon transfer and led to a more productive glycosylation, as shown in Scheme 3.

Now the stage was set to probe the glycosylating properties of the thio uronic disaccharides (Scheme 4). At first, the highly reactive Ph_2SO/Tf_2O thiophilic promotor system[17] was applied for the activation of the disarmed thio disaccharides. It was found that this reagent system very effectively activated the anomeric center of glucuronic acid **42** at low temperature (−60 °C), as judged by TLC analysis. Surprisingly, no ensuing condensation took place with a choice of acceptor glucosazides (i.e., **24** and **50**). Gratifyingly, the BSP/Tf_2O system, developed in the Crich laboratory,[16] also proved to be potent enough for the activation of 1-thio glucuronic acid **42**, and this time addition of glucosazide **24** to the activated thioglycoside led to the desired condensation reaction to stereoselectively afford trisaccharide **47** in 76% yield.[36] The BSP/Tf_2O activator could also be employed to smoothly activate 1-thio iduronic acid **43** (Scheme 4). We were pleased to establish that glucosamine acceptor **50**,[37] which is sterically rather encumbered and bears a carbamate function, was compatible with the developed condensation conditions and could be coupled with the activated iduronic acid **43** to provide trisaccharide **51**, having two orthogonally protected amino functionalities, in 55% yield.

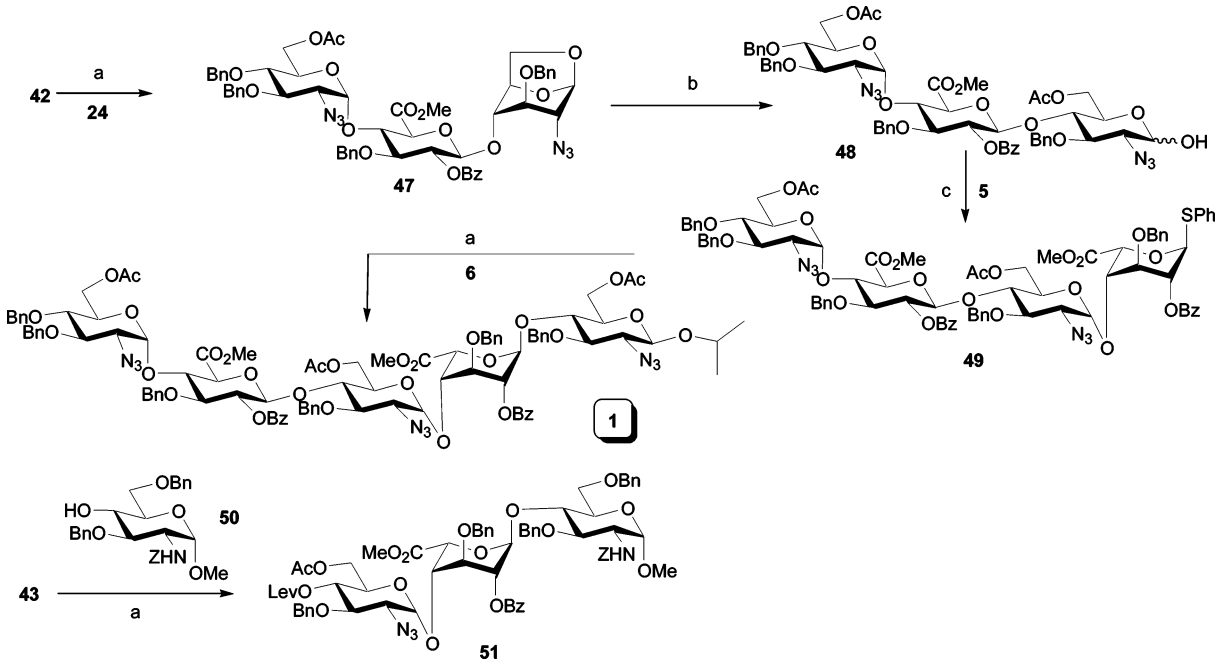
Having explored both the acceptor and donor properties of the 1-thio iduronic acid building blocks, the assembly of the target pentasaccharide **1** was continued. To this end, trimer **47** was transformed into a donor amenable for the second glycosylation sequence. Thus, the anomeric hydroxyl function of trisaccharide **47** was unmasked by opening of the anhydro bridge and subsequent deacetylation of the anomeric center. The chain elongation of compound **48** commenced by extending the trisaccharide with the phenyl 1-thio iduronic acid building block **5**. The dehydrative condensation proceeded with excellent stereoselectivity to yield tetrasaccharide **49** in 51% yield. No aglycon transfer was observed during the glycosylation. The tetrasaccharide **49** could directly be used in the second condensation event of this glycosylation sequence. Activation of the anomeric thiophenyl group in **49** with the BSP/Tf_2O reagent system again proceeded efficiently, and ensuing addition of the terminal glucosamine building block **6** completed the assembly of target pentasaccharide **1**.

Conclusion

In conclusion, we have developed the first modular assembly strategy for the synthesis of heparin and heparan sulfates that

(32) (a) Belot, F.; Jacquinet, J.-C. *Carbohydr. Res.* **1996**, *290*, 79−86. (b) Zhu, T.; Boons, G.-J. *Carbohydr. Res.* **2000**, *329*, 709−715.
(33) Naturally ocurring H and HS contains only uronic acid residues which bear unsulfated hydroxyl functions at the C-3 position. Therefore, a benzyl group is required during the assembly of the oligosaccharides. For the synthesis of "non-natural" H and HS sequences bearing sulfates at the OH-3 of the uronic acids, a benzoyl group can be introduced in the building blocks.
(34) Thiophenyl glycosides are slightly less reactive than their thioethyl counterparts: (a) Fügedi, P.; Garegg, P. J. *Carbohydr. Res.* **1986**, *149*, C9−C12. (b) Zuurmond, H.; Van der Laan, S. C.; Van der Marel, G. A.; Van Boom, J. H. *Carbohydr. Res.* **1991**, *215*, C1−C3. (c) Lahmann, M.; Oscarson, S. *Can. J. Chem.* **2002**, *80*, 889−893.
(35) The anomeric configuration of the glycosides has an effect on their reactivity, with the β-glycoside being the more reactive donor of the two anomers: Lemieux, R. U.; Hendricks, K. B.; Stick, R. V.; James, K. *J. Am. Chem. Soc.* **1975**, *97*, 4056−4062.
(36) Premixing of the donor and acceptor glycoside led to the formation of the acceptor BSP−OTf adduct **54**. Similar results were obtained previously, as reported in ref 16b.
(37) Beetz, T.; Van Boeckel, C. A. A. *Tetrahedron Lett.* **1986**, *27*, 5889−5892.
(38) Crich, D.; Smith, M.; Yao, Q.; Picione, J. *Synthesis* **2001**, 323−326.

Scheme 4. Assembly of the Oligosaccharides[a]



[a] Reagents and conditions: (a) BSP, Tf_2O, DCM, −60 °C to room temperature, **47**: 76%, **1**: 53%, **51**: 55%; (b) i. 10% TFA in Ac_2O, 0 °C to RT; ii. 6% piperidine in THF, 92%; (c) Ph_2SO, Tf_2O, TTBP, DCM, −40 °C to room temperature, 51%.

employs monomeric building blocks. The development of this strategy has been made possible by the identification of the 1-thio glucuronic and iduronic acids as powerful synthons for H and HS assembly. First, a very efficient route of synthesis was disclosed for these building blocks. Next they were combined with 1-hydroxyl glucosazides in a sequential glycosylation protocol, in which both donor types were transformed into efficient glycosylating agents by the application of the powerful electrophilic sulfonium activator systems Ph_2SO/Tf_2O and BSP/Tf_2O, which are introduced for the first time in H and HS assembly. In addition, application of these sulfonium activator systems for the condensation of the 1-hydroxyl donors and their uronic acid acceptors led to highly stereoselective glycosylations, required for the construction of the oligosaccharide backbone. The reported strategy thus presents all requirements for the modular assembly of H and HS fragments as well as other members of the glycosaminoglycan superfamily, such as the chrondoitin and dermatan sulfates: efficient synthesis of building blocks, rapid and stereoselective assembly of the oligosaccharide chain, and a high degree of flexibility, offered by the monomeric synthons. It should be noted that the intermediate oligomeric building blocks (e.g., **42**, **48**, and **49**) can also find potential in a more convergent block strategy, which uses, for example, tri- and disaccharides. However, a linear strategy that iteratively uses closely related (monomeric) building blocks benefits from standardized condensation procedures and the ability to interchange the building blocks in a very straightforward manner and hence streamlines the oligomer assembly process.

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Supporting Information Available: Full experimental and characterization details for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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