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Novel Efficient Routes to Heparin Monosaccharides and Disaccharides Achieved via Regio- and Stereoselective Glycosidation

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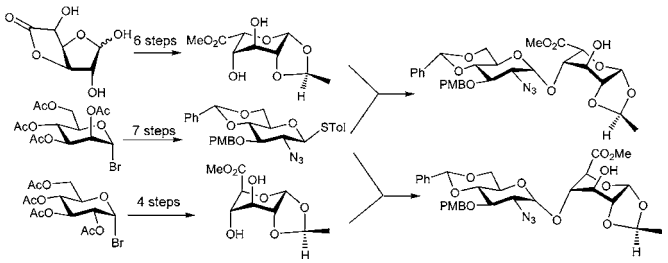
ABSTRACT



A new methodology for the synthesis of heparin building blocks has been developed. We describe novel efficient routes to both L-iduronic acid and D-glucuronic acid acceptors. Glycosylation with thioglycosides donors gave corresponding disaccharides in a regio- and stereoselective fashion. An improved approach to synthesizing azido-glucose thioglycoside donor to render azido-sugar from mannose via nucleophilic substitution is described.

Heparin belongs to the family of glycosaminoglycans (GAGs). It is a linear polymer consisting of repeating units of 1 → 4-linked pyranosyluronic acid and 2-amino-2-deoxyglucopyranose (glucosamine) residues (Figure 1).[1] The uronic acid residues consist of either L-idopyranosyluronic acid (L-iduronic acid) or D-glucopyanosyluronic acid (D-glucuronic acid). As it is the most acidic biopolymer in nature, heparin interacts with a large number of proteins, which regulate many important biological activities such as antithrombotic, antiatherosclerotic, artherogenic, antiinflammatory, angiogenic, antiangiogenic, and antiviral activities, cancer, and Alzheimer's disease.[2] With the exception of the antithrombin III−heparin interaction,[3] in which the minimal sequence of heparin required for binding is a pentasaccharide, most structure−activity relationships (SARs) between proteins and heparin are poorly understood. This is mainly due to the lack of pure defined sequences of heparin available. Since the first total synthesis of this heparin pentasaccharide was reported,[4] numerous synthetic methodologies have been disclosed for the preparation of heparin-type oligosaccha-

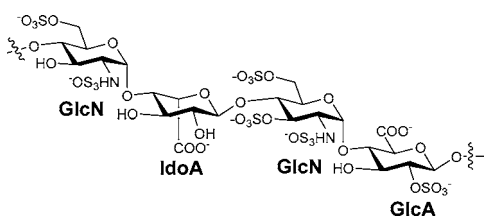


Figure 1. Schematic view of heparin.

(1) (a) Casu, B. *Carbohydr. Chem. Biochem.* **1985**, *43*, 51−134. (b) Comper, W. D. *Heparin and Related Polysaccharides*; Gordan and Breach, 1981; Vol. 7.

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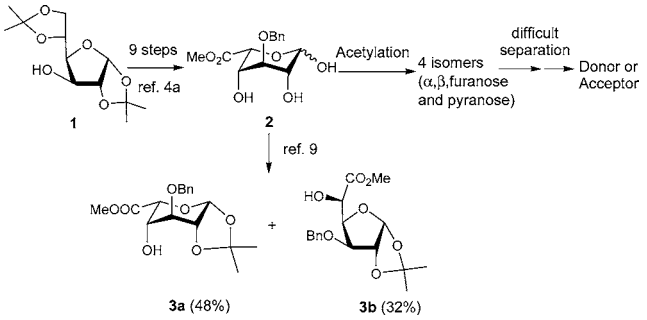


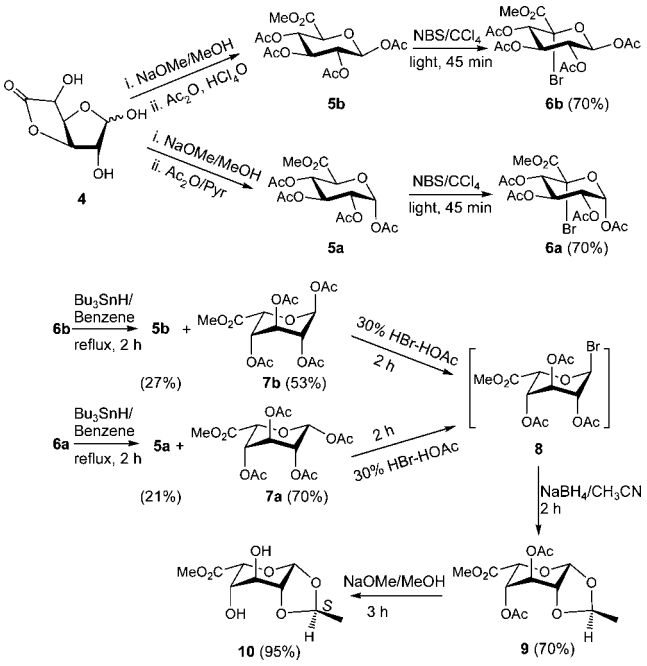
Figure 2. Common method for the synthesis of iduronic acid and an improved scheme developed by Seeberger and co-workers.

rides.[5] However, an easy and truly practical route to heparin oligosaccharides is still elusive. Good synthetic methods for the construction of heparin-based oligosaccharides, along with knowledge of the specific structural requirements for the various actions of heparin, could allow "tailor-made" sequences of the heparin template to be prepared for specific therapeutic applications.[6] In an effort to employ our effective programmable one-pot synthetic strategies[7] for the preparation of heparin and its analogues, we now describe short and straightforward routes to various synthetically relevant mono- and disaccharide building blocks.

Of the three saccharide residues, L-iduronic acid is the most challenging to synthesize.[8] Initially, we evaluated the most common protocol[4a] for its preparation, which started from diacetone glucose **1** (Figure 2). After nine synthetic steps, it yielded the hemiacetal mixture **2**. Subsequent acetylation of **2** gave four isomers consisting of an α/β mixture of pyranose and furanose forms, which required difficult chromatography to separate the desired pyranose product. A recent report[9] provided a shorter route to the acceptor **3a**, where the resulting $^1C_4$ conformation, locked by a 1,2-acetal, generally gave α-selectivity in glycosylation reactions. Unfortunately, iduronic acid **2** isomerizes to pyranose and furanose forms during installation of the 1,2-cyclic acetal systems.[10] Thus, our first goal was to develop a new effective strategy for L-iduronic acid synthesis that would yield the pyranose form exclusively.

Inexpensive D-glucuronolactone **4** was converted to methyl tetra-*O*-acetyl-α-D-glucopyranuronate **5a** according to a reported "two-step in one-pot" procedure[11] (Scheme 1). Free

Scheme 1. Preparation of Iduronic Acid **10**



radical bromination[12] at C-5 using NBS and UV light in refluxing CCl_4 gave bromide **6a** in good yield. Subsequent isomerization at C-5 by free radical reduction[13] of **6a** with tributyltin hydride gave a 1:3 ratio of D-*gluco* and L-*ido* (**7a**) isomers. Attempts to invert the C-5 carbon center with $NaBH_4$ or catalytic hydrogenation resulted in decomposition of the starting material. To investigate the isomerization outcome for the β anomer, compound **6b** was prepared. Treatment of **6b** with tributyltin hydride in refluxing benzene gave a slightly higher ratio of D-*gluco* and L-*ido* isomers (1:2, 80%). Iduronic acid derivatives (**7a** and **7b**) were then converted to glycosyl bromide **8**. Without purification, bromide **8** was subjected to treatment with sodium borohy-

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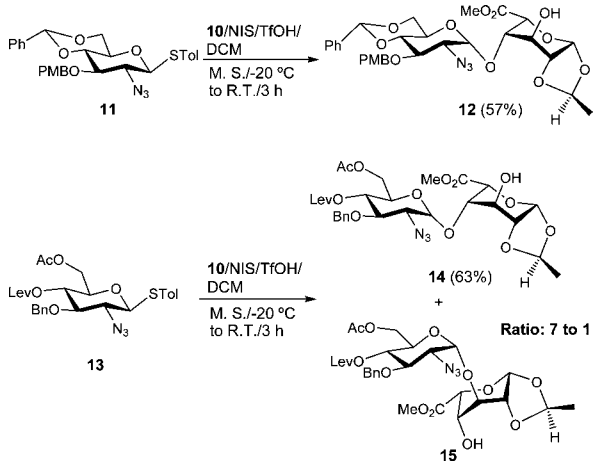
dride in acetonitrile to give the 1,2-ethylidene acetal **9** smoothly in 70% yield. Similar reaction conditions were first developed by Betaneli et al. for the preparation of 1,2-*O*-ethylidene derivatives of other common pyranoses,[14] but such conditions have not been widely adopted.[15] It is of interest to note that while (*R*)- and (*S*)-mixtures are reported in most monosaccharides, only the (*S*)-isomer was isolated here, where the (*S*)-configuration was unambiguously assigned by NOE experiments. Compound **9** was then smoothly deprotected by transesterification to afford diol **10** (95%).

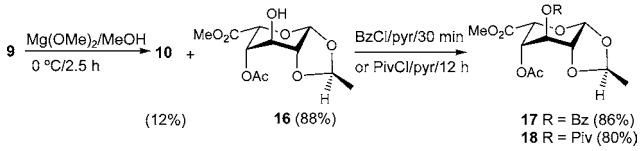
With the diol **10** in hand, a regio- and stereoselective glycosidation was attempted (Scheme 2). We were gratified

Scheme 2. Regio- and Stereoselective Glycosidation with Diol **10**



to find that the coupling reaction went swiftly to render desired 1 → 4 α-linked disaccharide **12** in 57% yield. To investigate whether a 4,6-*O*-benzylidene acetal had any effect on the glycosidation outcome, a different donor **13** was examined. Again, glycosidation was regio- and stereoselective; a 7:1 ratio of disaccharide **14** (63%) and **15** was observed. The high regioselectivity of this reaction demonstrated that 4-OH is more nucleophilic than 3-OH. These hydroxyls can also be easily differentiated by installing orthogonal protecting groups (Scheme 3). The 3-OAc of

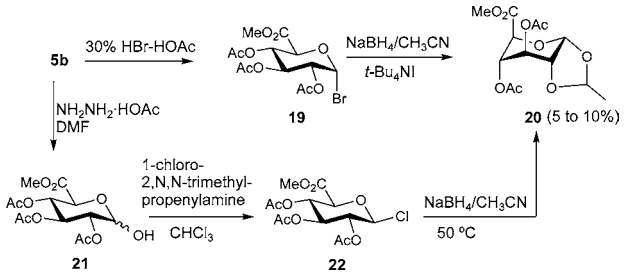
Scheme 3



compound **9** could be selectively removed by treatment with $Mg(OMe)_2$ in MeOH at 0 °C (→ **16**, 88%), followed by esterification, to give **17** (86%) or **18** (80%).

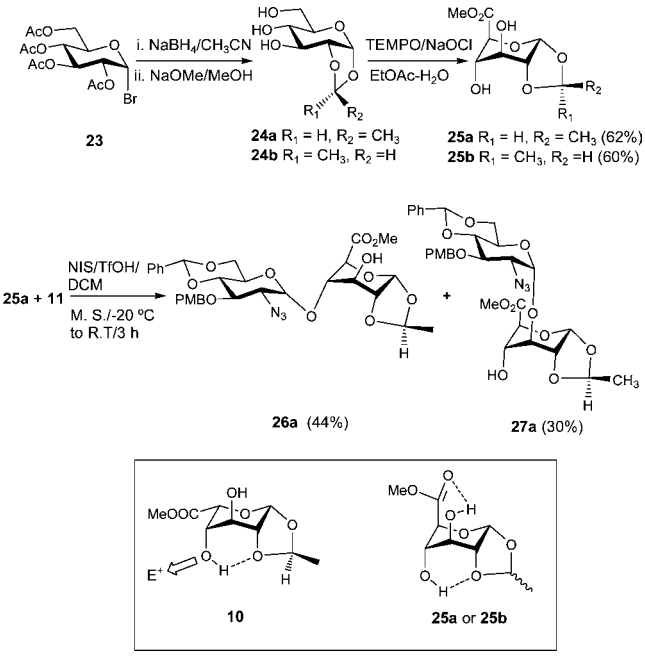
Following our initial success in the preparation of the L-iduronic acid acceptor and the various disaccharide building blocks, a similar strategy was pursued for the syntheses of the glucuronic acid-based disaccharides. To this end, bromide **19** was treated with $NaBH_4$ and *t*-Bu_4NI in acetonitrile to give acetal **20** in low yield (5−10%) (Scheme 4). Changing

Scheme 4



solvents or reaction temperatures did not improve the yield, and 1,2-*O*-ethylidene formation with β-glycosyl chloride **22**[16] was also unsuccessful. We therefore turned our attention to a selective oxidation procedure starting from readily available starting material **24a** and **24b** (Scheme 5).[14]

Scheme 5. Glycosidation with Diol **25a/25b**

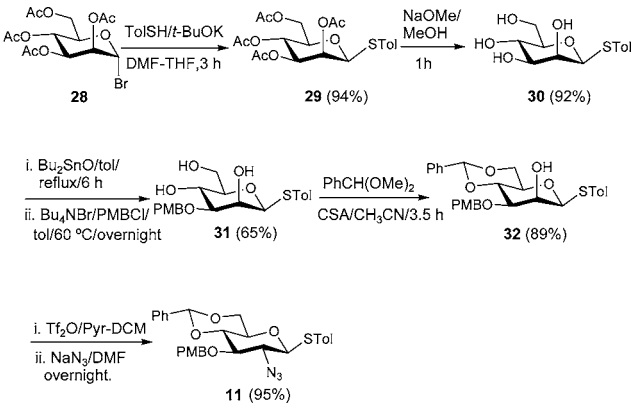


Selective oxidation of the primary hydroxyl groups was accomplished using 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) and NaOCl under phase-transfer conditions with tetrabutylammnonium chloride in $NaHCO_3$ (aq) and EtOAc.[17] Subsequent formation of the methyl ester using H^+ resin in methanol gave diol acceptors **25a** or **25b** in 62 and 60% yields, respectively. Regio- and stereoselective glycosylation

was then attempted with donor **11** and acceptor **25a**. This gave the desired 1 → 4 α-linked disaccharide **26a**, along with the regioisomer **27a**, in a 3:2 ratio. No β-anomer was found.[9] Coupling of donor **11** and acceptor **25b** was also investigated. Similar results were obtained (data not shown). Collectively these data demonstrated that the chirality of the ethylidene carbon (*R* or *S*) plays no role in determining the coupling outcome. The striking difference in regioselectivity between iduronic acid **10** and its epimer **25a/b** might arise from the different intramolecular hydrogen bonding pattern.[18] In compound **10**, the hydrogen bond 4-OH → O-2 enhances the nucleophilic reactivity of the 4-OH, which results in excellent regioselective glycosidation. On the other hand, possible intramolecular hydrogen bonding 3-OH → O═C(OMe) in **25a**/**b** counterbalances the nucleophilic reactivity in 4-OH. Thus, the glycosidation outcome is not as regioselective as the *ido* isomer.

A short route for the preparation of azidoglucosyl donor **11** has also been developed (Scheme 6). An S_N2-like reaction was carried out with mannosyl bromide **28** according to a published procedure.[19] Only β-thiomannoside **29** was obtained, which was purified by crystallization (95%). The following deacetylation product **30** also crystallized spontaneously (98%). The 3-OH was selectively protected as the *p*-methoxybenzyl ether after di-*n*-butyltin oxide activation[20] (→ **31**, 65%), which was followed by direct benzylidenation (→ **32**, 89%). Azidoglucosyl donor **11** was formed through

Scheme 6. Preparation of Donor **11**



a two-step sequence; triflation of the free hydroxyl was followed by nucleophilic substitution with NaN_3 in DMF, rendering **11** in excellent yield (95%). It is noteworthy that when the S_N2 reaction was carried out with the α-glycosides, a mixture of azidosugar and an elimination side product was obtained.

In summary, we have developed efficient routes to L-iduronic and glucuronic acid derivatives suitable for glycosylation. Both heparin disaccharide building blocks were synthesized, with **12** and **14** being achieved in a regio- and stereoselective fashion, while **26a** was formed in moderate yield. A new and short route to azidoglucosyl donor **11** is also presented.

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

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