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# Synthesis of Heparin Oligosaccharides

Jinq-Chyi Lee,[‡] Xin-An Lu,[†] Suvarn S. Kulkarni,[†] Yuh-Sheng Wen,[†] and Shang-Cheng Hung*,[†]

*Institute of Chemistry, Academia Sinica, Taipei 115, Taiwan, and Department of Chemistry, National Tsing Hua University, Hsinchu 300, Taiwan*

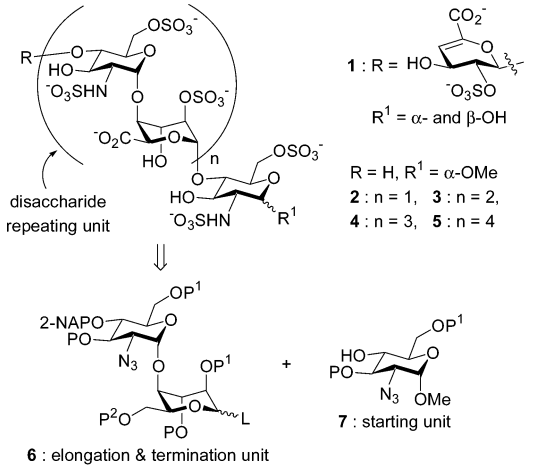
Received August 31, 2003; E-mail: schung@chem.sinica.edu.tw

Heparin and its structurally related heparan sulfate, the linear sulfated polysaccharides belonging to the family of glycosaminoglycans, play significant roles in a diverse set of biological processes, including blood coagulation, virus infection, cell growth, inflammation, wound healing, tumor metastasis, lipid metabolism, and diseases of the nervous system.[1] Heparin is widely used as an anticoagulant drug in clinic[2] and contains a trisulfated disaccharide repeating unit (Scheme 1) as the major component consisting of alternating D-glucosamine and L-iduronic acid with α1 → 4 linkages. Enzymatic degradation of heparin with heparinase gives a mixture of oligosaccharides **1** with even sugar units invariably having an unsaturated D-glucuronic acid residue at the nonreducing end.[3] Effort has been invested to determine the minimal structural requirements, mostly involving tetra- ($n = 1$) to decasaccharide ($n = 4$), for biological activities.[4] With the discovery of increasing numbers of heparin-binding proteins, there is a need to characterize the molecular properties, within the proteins and heparin, responsible for specific recognition. Toward fulfilling this goal, we have explored herein the synthesis of heparin oligosaccharides **2**−**5**.

The frequently encountered problems in the synthesis of heparin molecules[5] include the generation of rare L-idose,[6] the differentiation of all hydroxy groups on each sugar residue, the stereocontrol in the construction of all α-glycosidic bonds, the cleavage of multi-protecting groups, and the transformation of multifunctional groups. Our retrosynthetic plan of target compounds **2**−**5** entails two building blocks, an elongation and termination disaccharide unit **6** and a starting D-glucosamine unit **7**. The 2-naphthylmethyl group (2-NAP),[7] which is used to block the C4′-hydroxyl of **6**, allows chemoselective deprotection with DDQ during chain-enhancement and simultaneous removal along with the permanent benzyl groups (P) in the final termination process. The ester protecting groups ($P^1$) not only offer anchimeric assistance to generate exclusive 1,2-*trans*-glycosidic linkages, but also can be selectively removed to free those hydroxyls which would ultimately carry sulfonate groups. In addition, a temporary protection ($P^2$) is needed to mask the primary hydroxyl on L-idose that could be oxidized to carboxylic acid.

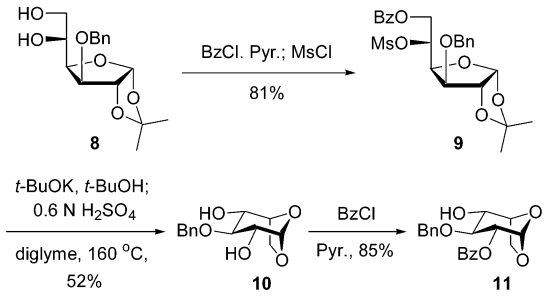
An efficient synthesis of the L-idopyranosyl sugar **11** is illustrated in Scheme 2. The 5,6-diol **8**,[8] generated from commercially available diacetone α-D-glucose in two known steps, underwent one-pot benzoylation-mesylation to yield the corresponding furanose **9** (81%) as a single isomer. Treatment of compound **9** with *t*-BuOK in *t*-BuOH followed by addition of a 1:2 mixture of 0.6 N $H_2SO_{4(aq)}$ and diglyme and subsequent heating at elevated temperature (160 °C) for 16 h led to the 2,4-diol **10** (52%) in a one-pot manner. The formation of **10** presumably takes place through an intramolecular $S_N2$ substitution to produce the C5-epimerized L-ido epoxide,[9] which concomitantly gets hydrolyzed in acidic media[10] and eliminates a water molecule via 3-*O*-benzyl-L-idopyranose intermediate. Regioselective benzoylation of **10** provided the corresponding 2-ester **11**[11] in 85% yield, exclusively.

***Scheme 1***




L = leaving group; 2-NAP = 2-naphthylmethyl group; P, $P^1$and $P^2$ are permanent, ester and temporary protecting groups, respectively.

***Scheme 2***




With the key synthon **11** in hand, we proceeded to synthesize heparin oligosaccharides **2**−**5**, as outlined in Scheme 3. The cheaply available D-glucosamine hydrochloride was first transformed into the 1,3-diol **12** (75%) employing a combination of amino-azido conversion at C2 and 4,6-*O*-naphthylidenation. Regioselective O1-benzoylation of compound **12** with 1-*N*-(benzyloxy)benzotriazole (BzOBT)[12] followed by O3-benzylation afforded the product **13**, which was subjected to sequential O6-ring opening,[13] O6-benzoylation, and anomeric debenzoylation to provide the 1-alcohol **14**. Transformation of **14** into the corresponding trichloroacetimidate and further coupling with the acceptor **11** led to the α-linked disaccharide **15α** and its *β*-isomer **15*β*** in 61% and 11% yields, respectively. The absolute configuration of **15*β*** was unambiguously determined through its X-ray single-crystal analysis (see Supporting Information). $Cu(OTf)_2$-catalyzed acetolysis of **15α** delivered the 1,6-diacetate **16** (88%), which upon selective O1-deacetylation using ammonia was similarly converted to the imidate **17** (77%). The 4-alcohol **18**, prepared by selective O6-benzoylation ($Bz_2O$, $Et_3N$,

[†] Academia Sinica.
[‡] National Tsing Hua University.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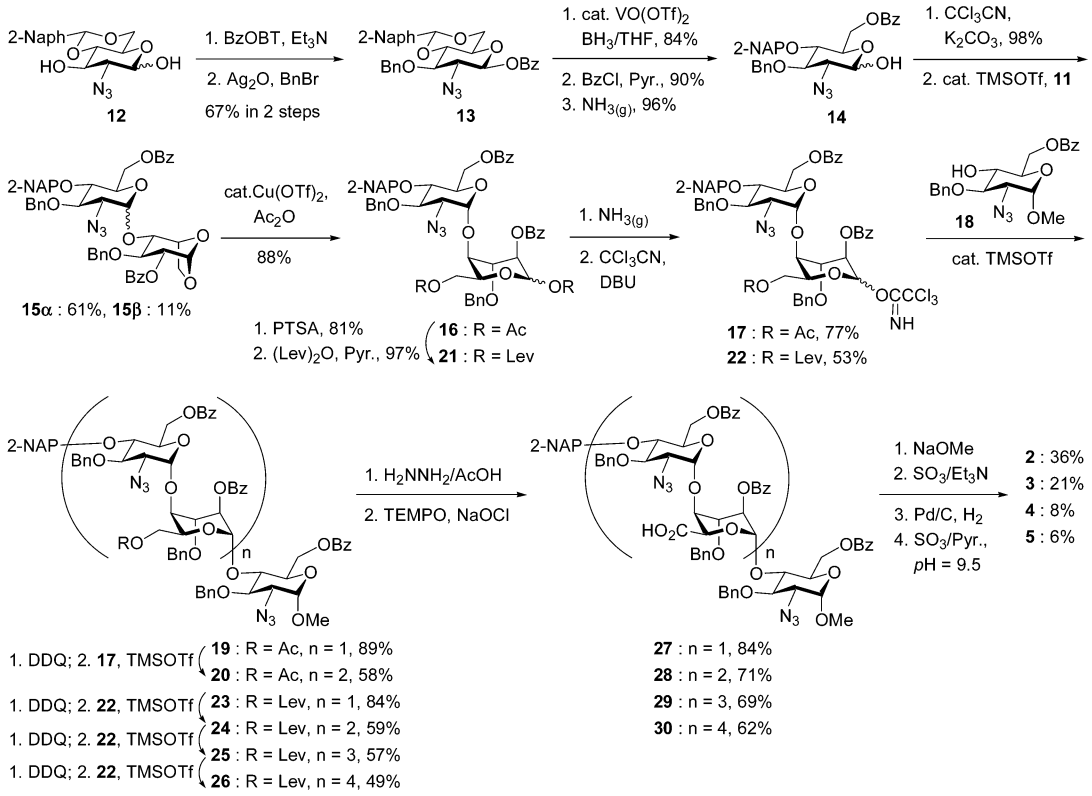
***Scheme 3***




97%) of methyl 2-azido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside,[14] was coupled with **17** to provide the α-linked trisaccharide **19** (89%) as a single isomer. Further chain-elongation sequence, involving removal of the O4-NAP using DDQ and subsequent glycosylation with the disaccharide donor **17**, posed no problems and furnished the pentasaccharide **20** (58%), exclusively. While reaction of compound **19** with $HBF_4 \cdot Et_2O$[15] gave the corresponding 6′-alcohol in excellent yield, simultaneous removal of two acetyl groups in **20** proved testing and compelled us to rethink our present strategy.

Levulinoyl (Lev) esters were thought to be better options for our purpose. Direct levulinolysis of **15α** did not work. Alternatively, deacetylation[16] of **16** afforded the 1,6-diol (81%), which was reacted with $Lev_2O$ in pyridine to get the ester **21** (97%). A similar reaction sequence of anomeric deprotection and imidate formation led to the glycosyl donor **22** (53% in two steps), which was coupled with **18** in a likewise manner to construct the α-linked trisaccharide **23** (84%). The elongation cycle was then repeated thrice to assemble the penta-, hepta-, and nonasaccharides **24**, **25**, and **26**, respectively. Cleavage of the Lev groups in **23**−**26** followed by oxidation using TEMPO,[17] individually, furnished the acids **27**−**30** in good overall yields. The corresponding *O*-sulfates, obtained by consecutive deacetylation and *O*-sulfonation of **27**−**30**, underwent hydrogenolysis to reduce the OBn, O-2-NAP, and $N_3$ groups and subsequent *N*-sulfonation to provide the desired target molecules **2**−**5**, respectively.

In summary, we successfully developed a straightforward synthesis of the L-idopyranosyl sugar **11** as a valuable building block and carried out the total synthesis of heparin oligosaccharides **2**−**5**.

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**Supporting Information Available:** Experimental procedures, $^1H$ NMR spectra, and X-ray structural information for compound **15*β*** (PDF and CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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