EurJOC
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A Journal of
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Accepted Article

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To be cited as: *Eur. J. Org. Chem.* 10.1002/ejoc.201900540

Link to VoR: http://dx.doi.org/10.1002/ejoc.201900540

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10.1002/ejoc.201900540

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Linear Synthesis of *De novo* Oligo-Iduronic Acid

Chethan D. Shanthamurthy,[a] and Raghavendra Kikkeri*[a]

Abstract: L-Iduronic acid (IdoA) plays a pivotal role in glycosaminoglycan (GAG) protein interactions. However, the structural microheterogeneity of GAG appears to impede the systematic investigation of IdoA functions. Under such conditions, oligo-Iduronic acid (Oligo-IdoA) are ideal and straightforward heparin mimetics to unravel the relationship between IdoA structure and functions. Herein, we report for the first-time linear synthesis of rare oligo-IdoA precursor utilizing anhydrous *β*-L-idopyranosyl and IdoA thiophenol building block. After screening various synthetic strategies, we have Installed successive IdoA by 6 step reactions with 25-26% overall yield. These oligo-IdoA are expected to be excellent probes to understand conformational plasticity of IdoA and fine tune carbohydrate-protein interactions.

Introduction

L-Iduronic acid (IdoA) is key hexuronic acid component of heparin (HP), heparan sulfate (HS), and dermatan sulfate (DS). It is classified under the glycosaminoglycan (GAG) family.[1] The biosynthesis of HS and HP is initiated by the attachment of D-xylose to the prescribed serine residue of proteoglycan core proteins, followed by glycosylation of two galactose and glucuronic acid residues, leading to the formation of a tetrasaccharide linker region. In the Golgi complex, the linker region undergoes a series of enzymatic modifications, including deacetylation, sulfation, and epimerization of D-glucuronic acid into L-iduronic acid.[2] This results in highly complex and diverse HS/HP structures.[3] NMR and theoretical studies of HP and HS oligosaccharides confirmed that IdoA is predominantly presents with $^{1}C_{4}$-chair and $^{2}S_{0}$ skew-boat geometry and less abundance as a $^{4}C_{1}$-chair conformation.[4] This conformation flexibility adds additional structural diversity in HP and HS active domains and thereby fine-tunes its protein interactions. The interaction between fondaparinux (a pentasaccharide heparin mimetic) and antithrombin III is a well-studied example of the conformation plasticity of IdoA and its critical role in inhibiting blood coagulation.[5] Nieto and coworkers synthesized an HP trisaccharides library and showed that 6-*O*-sulfated and *N*-sulfated glucosamine (GlcN) residues next to IdoA significantly contribute to $^{2}S_{0}$ conformation.[6] Liu and his coworkers synthesized HP hexasaccharide and proved that 2-*O*- and 3-*O*-sulfation of GlcN and 2-*O*-sulfation of IdoA are also crucial for stabilizing $^{2}S_{0}$ geometry in oligosaccharides.[7] Even though many reports have confirmed the conformational flexibility of IdoA, the considerable microheterogeneity of GAG chains has limited our in-depth knowledge of IdoA. Because of these conditions, developing the library of oligo-IdoA is envisioned as a potent model. However, the synthesis of such oligosaccharides is highly challenging, as IdoA is not commercially available. Herein, we have succeeded developing the first synthetic protocol for oligo-IdoA.

Results and Discussion

Our synthetic strategy is based on identifying a suitable protocol for IdoA-disaccharides synthesis and extending the strategy for oligosaccahrides. The pioneer work from the lab of Hung, Seeberger, Boons, Liu, Gardiner, and others yielded gram scale synthesis of IdoA building blocks from one-pot and *de novo* methods.[8] In the present study, we employed 1,6-anhydro-*β*-L-idopyranosyl 4-alcohol (**5**) and an iduronic acid-thiophenol donor (**9**) as important precursors to synthesize oligosaccharides. The initial synthetic strategy was based on use of IdoA derivatives **9** and **1** as "donor" and "acceptor," circumvents the need for deprotection and oxidation steps. We used an amine-linker to block the reducing end IdoA residue for further conjugation. Compound **5** was synthesized with a total yield of 27% from 1,2:5,6-di-*O*-isopropylidene-*α*-D-glucofuranose by a six-step reaction.[8f] A regioselective ring opening of **5** with trimethyl(phenylthio)silane in the presence of $ZnCl_2$ at room temperature, led to thioglycoside **7** in a 75% yield. One-pot oxidation of **7** with catalytic amounts of 2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO) in the presence of bis(acetoxyiodo]benzene (BAIB) followed by esterification with benzyl bromide yielded **8** in 88% yield. It was further glycosylated with an azide-linker to yield 78% of **1**. The C4-OH of **8** was protected with levulinic acid using DCC as a coupling agent, yielding the donor **9** (Scheme.1). An attempt to glycosylate **9** and **1** in the presence of *N*-iodosuccinimide (NIS) and a catalytic amount of trimethylsilyl trifluoromethanesulfonate (TMSOTf) in DCM at -20 °C ended with 1,2-unsaturated glycosyl product **13** instead of the desired di-IdoA **2**. The use of a strong thiophilic promoter condition, such as NIS/ trifluoromethanesulfonic acid (TfOH), in a range of temperatures and time periods failed to yield di-idoA **2** (Scheme. 2). These results lead us to conclude that the neighboring 2-benzoyl substitution of donor **9** stabilizes the benzyl oxonium-ion intermediate in the presence of a thiophilic agent and facilitates 1,2-*trans* glycosylation due to the weak nucleophilicity of **1**, steroselective glycosylation did not proceed and led to lactal.

To improve the glycosylation, we have synthesized IdoA-donors with good leaving groups, such as trichloroacetimidate (**10**) and phosphorimidate (**11**). Under mild acidic conditions (catalytic amount of TMSOTf), IdoA-trichloroacetimidate (**10**) yielded **2** in 5% yield. Encouraged by this minor step forward, we examined other trichloroacetimidate promoters, such as silver(I) triflate (AgOTf), triflic acid (TfOH), and $BF_3.Et_2O$. These, unfortunately, did not improve glycosylation yield and resulted eliminated side-

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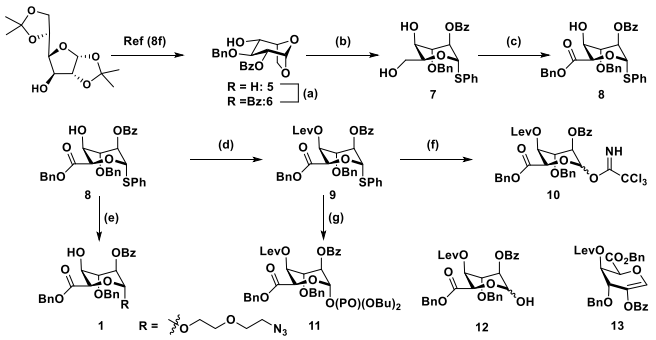
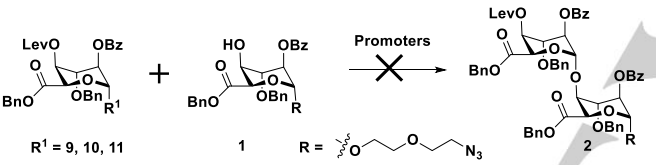
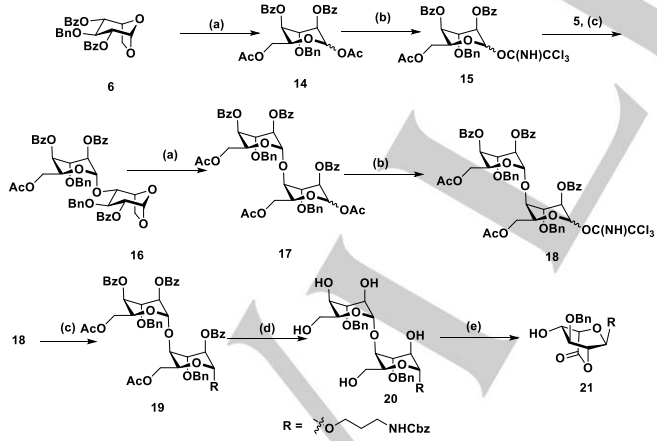
product **13**. Similarly, IdoA-phosphorimidate (**11**) failed to yield the desired **2** (Scheme. 2). Based on these unsuccessful efforts, we concluded that the electron withdrawing C5-carboxylic ester in **1** reduced the nucleophilicity of the axial C4-OH, and that **1** is a poor nucleophile for 1,2-trans glycosylation.



Scheme 1: Synthesis of acceptor molecules **1** and donor **9-11**: Reagents and conditions: (a) BzCl, DCM/Py, 85%; (b) TMSSPh, $ZnCl_2$, CH_2Cl_2, RT, 75% **(7)**; (c) TEMPO, BAIB, CH_2Cl_2/H_2O (1/1); BnBr, TBAI, $NaHCO_3$, DMF, 60 °C, (2steps), 88% **(8)**; (d) LevOH, DCC, DMAP, CH_2Cl_2, 88% **(9)**; (e) Azidoethoxyethanol, NIS, TfOH, 4 Å M.S, -5 °C-RT, 78% **(1)**; (f) NBS, Acetone/H_2O (10/1); $NCCCl_3$, DBU, 76% **(10)** (2 steps); (g) Dibutyl phosphate, CH_2Cl_2, NIS, TfOH, 4 Å M.S, -20 °C, 92% **(11)**.

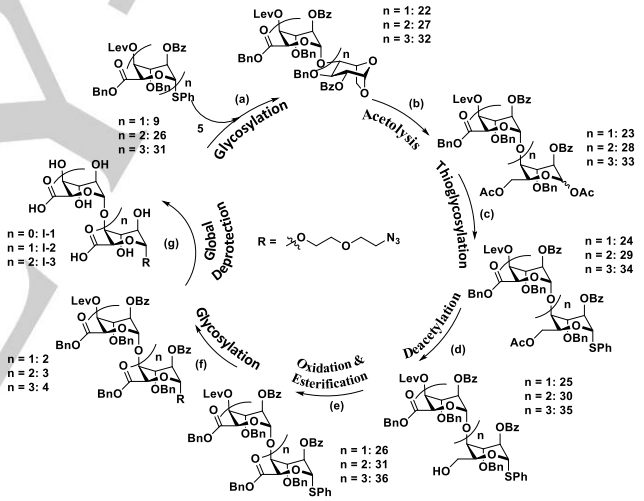


Scheme 2. Synthesis of **2** from **9-11** and **1** as donors and acceptor. Promotor: **9**: NIS/TMSOTf or NIS/TfOH; **10**: TMSOTf or AgOTf, TfOH, $BF_3.Et_2O$; **11**: TMSOTf.



Scheme 3: Synthesis of di-L-Idose precursor (**19**): Reagents and conditions: (a) $Cu(OTf)_2$, Ac_2O, 0 °C, 82% **(14)**, 78% **(17)**; (b) Bu_2SnO, MeOH, 55 °C; $NCCCl_3$, DBU, CH_2Cl_2 53% **(15)**, 43% **(18)** (2 steps); (c) TMSOTf, CH_2Cl_2, 4 Å M.S, -78 °C 59% **(16)**, 68% **(19)**; (d) NaOMe, MeOH, 88% **(20)**; (e) TEMPO, BAIB, CH_2Cl_2/H_2O (1/1), 26% **(21)**.

To improve the glycosylation of IdoA precursors, we proposed using nucleophiles **15** as donors to synthesize L-Idose disaccharide **19** and explore TEMPO oxidation of primary hydroxyl groups to yield the desired product. To achieve this, 1,2-*trans* L-Idose disaccharide **18** was synthesized from L-idose-tricholorimidate donor **15** and 1,6-anhydro-*β*-L-idopyranosyl 4-alcohol **5** using a catalytic amount of TMSOTf. This was, followed by acetolysis, and linker glycosylation to obtain di-L-Idose **19** in 23% (4 steps) overall yield. The removal of acyl groups of **18** by using sodium methoxide yielded di-L-Idose **20** (Scheme. 3). Finally, **20** was oxidized with TEMPO in the presence of BAIB, yielding unexpected lactonized IdoA monosaccharide **21** as a major product. Attempts with different TEMPO co-oxidants such as sodium hypochlorite (NaOCl) and sodium chlorite ($NaClO_2$) met similar fate. This indicates that TEMPO/BAIB/NaOCl mediated oxidation is selective for primary alcohol, but the alkaline reaction condition and radical species formed during the oxidation resulted in cleavage of the glycosidic bond, presumably by an $E1_{CB}$ elimination mechanism.[9] Overall, we concluded that oligo-L-Idose could be easily generated from **5**, but TEMPO oxidation of multiple primary hydroxy groups may be detrimental to the process and could result in cleavage of glycosidic bonds.



Scheme 4: Synthesis of oligo-IdoA (**I-1** to **I-3**): Reagents and conditions: a) NIS, TMSOTf, CH_2Cl_2, 4 Å M.S, -10 °C, 64% **(22)**, 62% **(27)**, 62% **(32)**; b) $Cu(OTf)_2$, Ac_2O, 0 °C, 94% **(23)**, 85% **(28)**, 85% **(33)**; c) TMSSPh, ZnI_2, CH_2Cl_2, RT, 89% **(24)**, 80% **(29)**, 80% **(34)**; d) *P*-TsOH, CH_2Cl_2/MeOH(1/1), 92% **(25)**, 76% **(30)**, 66% **(35)**; e) TEMPO, BAIB, CH_2Cl_2/H_2O (1/1); BnBr, TBAI, $NaHCO_3$, DMF, 60 °C, 65% **(26)**, 63% **(31)**, 58% **(36)**; f) Azidoethoxyethanol, NIS, TfOH, 4 Å M.S, -5 °C, 81% **(2)**, 76% **(3)**, 76% **(4)**. g) LiOH, THF/H_2O (4/1); $Pd(OH)_2$, MeOH, 57% **(I-1),** 53% **(I-2),** 48% **(I-3).**

To tackle the TEMPO mediated oxidation-degradation of oligosaccharides, we have decided to synthesize heterogeneous L-IdoA-L-idose disaccharide **23** composition and oxidize a single primary hydroxyl moiety at a time. We started the synthesis by glycosylating **9** and **5** with NIS and TMSOTf and obtained disaccharide **22** in 64% yield after 30 minutes (Scheme. 4). We have corroborated the *α*-configuration of glycoside **22** by ^1H and ^{13}C-NMR, showing peaks at 5.29 and 95.17 ppm, respectively. By acetolysis of the anhydro-ring of **22**, in the presence of copper (II) trifluoromethanesulfonate [$Cu(OTf)_2$] and acetic

anhydride, we have synthesized **23** in 94% yield. Successive thioglycosylation, mild deacetylation, one-pot TEMPO oxidation, and esterification of **23** yielded the target disaccharide molecule **26** in 53% (3 steps) overall yield. Glycosylation of **26** with an azide-linker yielded di-IdoA derivative **2** in 81% yields. Encouraged by the overall yield and stereoselectivity of the di-IdoA, we extended the protocol to synthesize different chain lengths of oligo-IdoA (Scheme. 4). As an example, **26** or **31** were reacted with **5** using NIS and TMSOTf. These were followed by a series of reactions, including acetolysis of the anhydro-ring, thioglycosylation, deacetylation, oxidation, esterification, and linker glycosylation. This sequence of reactions yielded compounds **3** and **4** (tri or tetra-IdoA derivatives) in 25% (6 steps) and 20% (6 steps) yields, respectively. Based on the low nucleophilicity of L-idoA and the sensitivity of L-Idose for TEMPO oxidation, we conclude that our new convergent approach is ideal for the synthesis of moderate yields of different lengths of oligo-IdoA precursors. Finally, the global deprotection yielded oligo-iduronic acids.

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Conclusions

We report for the first time a new linear approach for the synthesis of oligo-IdoA using an IdoA-thiophenol as the donor and a *β*-L-idopyranosyl derivative as the nucleophile. Sequential modifications of the L-Idose residue yielded oligo-IdoA derivatives in moderate overall yields. After screening various synthetic conditions, this is the best possible method to synthesize oligo-IdoA. This synthetic route enables us to synthesize different sulfation patterns of oligo-IdoA to study conformation plasticity of IdoA, secondary oligosaccharide structures, and thereby modulate carbohydrate-protein interactions.

Acknowledgements

Financial support from the IISER, Pune, Max-Planck partner group and DST (Grant No. SB/S1/C-46/2014) is gratefully acknowledged (to R.K).

Keywords: Carbohydrate • Oligosaccharide • Iduronic acid • Heparin • Glycosylation

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10.1002/ejoc.201900540

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Layout 1: Oligo-Iduronic acid

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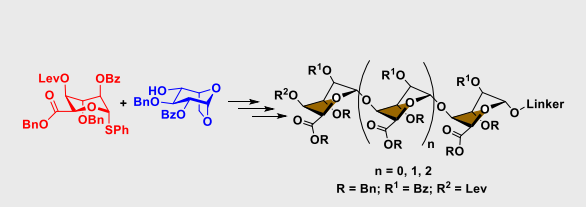

A rare iduronic acid oligosaccharides have been synthesized utilizing anhydrous *β*-L-idopyranosyl and IdoA thiophenol building block. Installation of successive IdoA residue required 6 step cycle allowed the synthesis of oligo-iduronic acid.

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Page No. – Page No.

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