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J. Am. Chem. Soc., **Just Accepted Manuscript** • DOI: 10.1021/jacs.9b03266 • Publication Date (Web): 03 Jun 2019

An Efficient Modular One-pot Synthesis of Heparin-Based Anticoagulant Idraparinux

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KEYWORDS: Heparin, Idraparinux, Anticoagulant, Modular one-pot Synthesis.

ABSTRACT: Idraparinux is a fully *O*-sulfated α-methyl glycoside of heparin pentasaccharide motif known to interact with the antithrombin III domain and act as anti-coagulant. The current most effective synthesis of Idraparinux is complicated and non-stereoselective, requiring numerous step-wise procedures with low yields. We report here an efficient modular one-pot synthesis of Idraparinux, involving the use of a glycosyl phosphate with 6-*O*-*tert*-butyl diphenyl silyl group and a D-glucuronic acid-containing disaccharide thioglycoside with 6-*O*-acetyl group as donor building blocks for the α-directing one-pot glycosylations with an L-iduronic acid-containing disaccharide acceptor building block. The uronic acid was incorporated in a disaccharide module used in the one-pot synthesis to avoid the complicated late-stage installation of these acidic sugars. The one-pot synthesis of Idraparinux demonstrated here is an effective strategy and should be applicable to the modular assembly of other heparan sulfates with regiodefined sulfation pattern for functional study.

INTRODUCTION

Heparin (H) and heparan sulfate (HS) are linear polyanionic molecules of glycosaminoglycans (GAGs), known to interact with several biologically important proteins and play a major role in various biological processes.[1] Heparin-based drugs have been used in the clinics for the treatment of thromboembolic diseases[2a] since 1930 even though the structure and activity relationship (SAR) was not known at that time.[2b] Within its heterogeneous chain, a pentasaccharide motif binds to the serine protease inhibitor antithrombin III (AT III) and blocks thrombin and factor Xa involved in the process of the blood coagulation cascade.[3,4] Since the ground breaking work of van Boeckel and Petitou[3] on the SAR study of heparin in late 1990s, the development of heparin-based drugs has been increasingly important. Remarkably, Fondaparinux (**1C**) (**Fig. 1**), the unique AT-binding pentasaccharide motif was introduced to the market in 2001 due to its higher anti-Xa activity and longer half-life compared to the low molecular weight heparin.[5] Further research in the field has led to the development of the next generation anticoagulant drug Idraparinux (**1A**), a synthetic pentasaccharide analogue of Fondaparinux, which contains *O*-sulfation and *O*-methyl functionalities instead of *N*-sulfation and free hydroxyl groups (**Fig 1**). Compared to **1C**, **1A** was known to have higher anti-Xa activity (K_d = 1.4 ± 0.3 nM) and longer half-life and was in phase III clinical trial for the treatment of patients with atrial fibrillation and venous thromboembolic events.[6] The conformation and sulfation pattern of L-iduronic acid is important for the anticoagulation properties of heparin sulfates, including the clinically important drugs Fondaparinux and Idraparinux.[7] Recent study further showed that replacement of the L-iduronic acid unit by 6-deoxy-L-talopyranose completely diminished the anticoagulant activity of **1A**.[8]

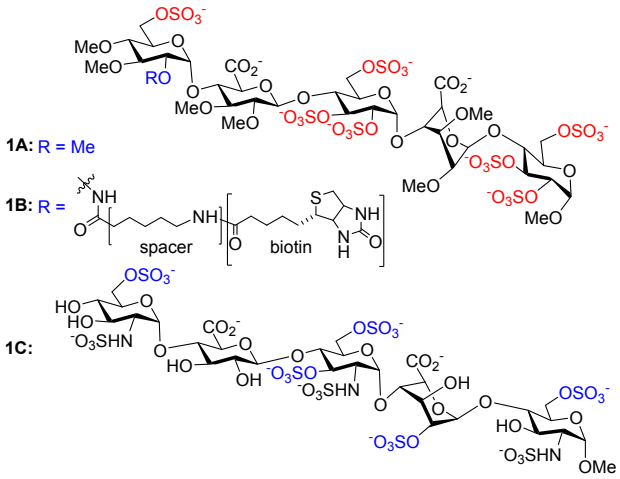


Figure 1: Synthetic pentasaccharide with anticoagulant activity: Idraparinux (1A), Idrabiotaparinux (1B) and Fondaparinux (1C)

However, like Fondaparinux, the long term (more than 6 months) use of **1A** also led to intracranial bleeding in elderly patients and those with renal impairment.[9] The incidence of major bleeding complication was later controlled by introducing a biotin moiety at the C2 position of the non-reducing end glucose of **1A**, to generate the second generation anticoagulant Idrabiotaparinux **1B**, which can be removed from the patient in case of overdose or bleeding by injection of avidin.[10]

In addition, the safety and quality of heparins from natural sources must be monitored to avoid any impurity contamination; e.g. the oversulfated chondroitin sulfate led to more than 200 deaths in the USA in 2008.[11] Thus, the anticoagulants made by chemical synthesis have attracted more attention recently[12]. However, the synthesis of **1A** and related substances is always challenging due to the possible contamination of heterogeneous glycosidic linkages and negatively charged sulfates.[13] To date, there are only four different procedures reported for the synthesis of Idraparinux. The first synthesis was reported by Westerduin *et al.* in 1994.[14] Fifteen years later, Yu and Chen developed an improved synthesis of **1A** in 51 steps with 4 % overall yield.[15b] More recently, Borbás and co-workers achieved the synthesis of **1A** in 39 steps from D-glucose.[16] In 2017, Mlynarski and co-workers reported the synthesis of a protected Idraparinux in 32 steps, including a 23-step linear route from D-cellobiose and D-glucose in 1.7 % overall yield.[17] The deprotection and chemical sulfation of the protected pentasaccharide to **1A** was not demonstrated in this report. However, most of the reported synthetic methods still involve long step-wise procedure and non-stereoselective glycosylation, with low efficiency and low yield, making the SAR study and development of new heparin-based pharmaceuticals difficult to pursue. In order to overcome this challenge, we have developed a programmable one-pot oligosaccharide synthesis method, using thioglycoside donors with known relative reactivity values (RRV) to simplify the synthesis of complex oligosaccharides,[18] and have extended the method to the synthesis of a heparin-like oligosaccharide[19a] and related pentasaccharides[19b] with regioselective *O*-sulfation pattern. Continuing our effort to develop a general and effective method for the synthesis of heparan sulfates with regiodefined sulfation pattern, we direct our effort to explore better building blocks with appropriate differential protecting groups and leaving groups (including the thioglycoside and phosphate[20]) to be used for the efficient modular assembly of this class of molecules. We herein report an efficient modular one-pot method for the synthesis of the clinically important Idraparinux as a representative example using designed glycosyl phosphate and thioglycoside building blocks as donors for α-selective glycosylation.

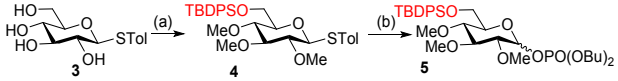
RESULTS AND DISCUSSION

Idraparinux **1A** contains fully *O*-methylated uronic acids and other sugar units connected to each other with 1,4-α-β-α-α glycosidic linkages. We envisioned that the protected Idraparinux **23** could be obtained from one-pot synthesis using the [1,2,2] approach and the building blocks (**5**, **13** and **22**) shown in the schemes. To generate stereoselective glycosidic linkages in the one-pot sequence, we designed the synthetic route with the following considerations: (i) highly stereoselective α-glycosylation influenced by the TBDPS and OAc protecting groups at *O*-6 position (ii) use of the built-in β-1,4-glycosidic linkage of D-cellobiose to design a new D-glucuronic acid containing disaccharide thioglycoside building block (**22**) as donor for α-selective glycosylation, (iii) construction of an L-iduronic acid-containing disaccharide as acceptor,

(iv) masking the hydroxyl groups with TBDPS group, benzyl ether or acetyl ester for *O*-sulfation at the later stage.

Synthesis of monosaccharide and disaccharide building blocks. The synthesis of monosaccharide phosphate donor **5** was achieved in two steps from the glucose thioglycoside **3**[19b] (**Scheme 1**). The primary hydroxyl group of **3** was initially protected with TBDPS[21] followed by protection of the rest hydroxyl groups as methyl ether using MeI/NaH to generate **4**. Later, the anomeric phosphate group[20a] was installed to yield **5**.

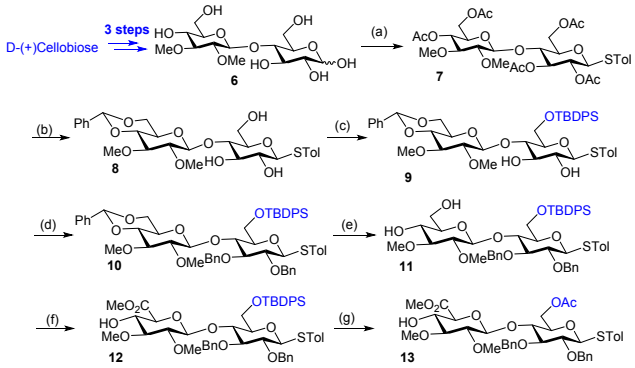
Scheme 1. Synthesis of monosaccharide donor



Reaction conditions: (a) (i) TBDPSCl, Imidazole, CH_2Cl_2, rt, 3h; then MeI, NaH, DMF, 2h, 0 °C-rt, 85 %; (b) $HOPO(OBu)_2$, NIS,TfOH, CH_2Cl_2, 4Å MS, -40 °C, 1 h, 89%.

The synthesis of the two disaccharide building blocks Glc-Glc (**13**) and Ido-Glc (**22**) were performed next. The synthesis of **13** was initiated from commercially available D-cellobiose. Compound **6** was obtained from D-cellobiose in 3 steps using the literature procedure developed by Mlynarski and co-workers.[17] The hydroxyl groups in **6** were protected with acetyl (Ac) and readily converted into **7** using *p*-toluenethiol (*P*-CrSH)/BF_3-OEt2. Later, Zémplen de-acetylation was performed, followed by installation of the 4′,6′-*O*-benzylidene protection group leading to **8** in excellent yield. In order to protect the 2,3-OH groups as benzyl ether, the primary hydroxyl group of the reducing end was protected as TBDPS ether to generate **9**. Next, the NaH/BnBr mediated *O*-benzylation led to compound **10** in excellent yield. Hydrolysis of the 4′,6′-*O*-benzylidene acetal using 80 % AcOH led to the dihydroxy compound **11**; upon selective oxidation using TEMPO/BAIB and subsequent esterification with iodomethane in the presence of $KHCO_3$ furnished the D-glucuronic acid-containing disaccharide building block **12** **(Scheme 2)**. Finally, the TBDPS ether was readily deprotected using HF-Py with concurrent selective *O*-acetylation to generate disaccharide **13** in excellent yield.

Scheme 2. Synthesis of D-Glc-β-(1→4)-D-Glc disaccharide building block

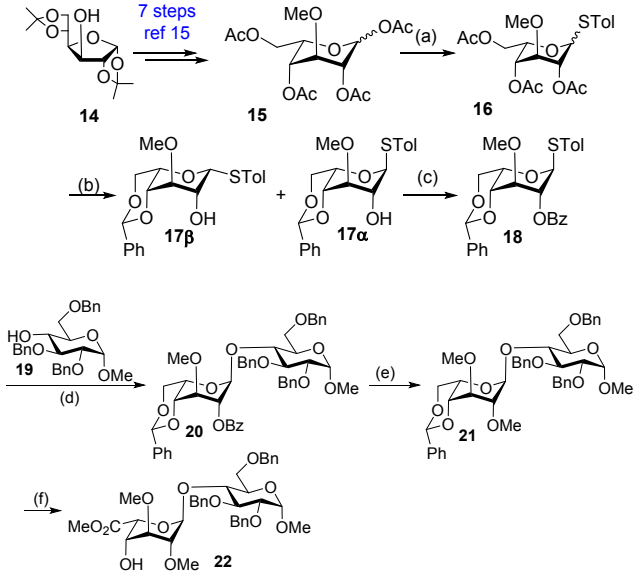


Reaction conditions: (a) (i) Ac_2O, Py (1:2), 0 °C-rt, 12 h; (ii) *p*-CrSH, BF_3-Et_2O, CH_2Cl_2, 0 °C-rt, 12 h, 68% (two steps); (b) (i) NaOMe, MeOH, rt, 12h; (ii) $PhCH(OMe)_2$, CSA, CH_3CN, rt, 12 h, 82 % (two steps); (c) TBDPSCl, Imidazole, CH_2Cl_2, 3h, 84 %; (d) BnBr, NaH, DMF, 3h, 0 °C-rt, 89 %; (e) 80 % AcOH-H_2O, 70 °C, 3h, 89 %; (f) (i) BAIB, TEMPO, CH_2Cl_2: H_2O (2:1), 0 °C-rt, 2 h; (ii) MeI, $KHCO_3$, DMF, rt, 4 h, 0 °C-rt, 77% (two steps); (g) (i) HF-Py, Py, 0 °C-rt, 12 h; (ii) Ac_2O, Et_3N, CH_2Cl_2, 0 °C-rt, 2 h, 86% (two steps).

For the synthesis of Ido-Glc disaccharide derivative **22**, we used NIS/TMSOTf-mediated coupling of suitably functionalized idose donor **18** and protected α-methyl glucoside acceptor **19**.[22] The synthesis of L-Idose donor **18** was commenced from diacetone glucofuranose **14** which was converted to the L-idose building block **15** using the known procedure.[15] It was further treated with *p*-toluenethiol in the presence of $BF_3{\cdot}OEt_2$ to furnish **16** as a non-separable anomeric mixture (α:β = 8:1).[15b] The formation of the anomeric mixture was not surprising as a recent study showed that neighboring-group participation has a minor role for idose donors.[23] Removal of the *O*-acetyl groups under Zémplen reaction condition followed by 4,6-*O*-benzylidine protection led to the chromatographically separable mixture of **17** in good yield. The α-anomer **17α** was treated with BzCl/Py to furnish the fully protected glycosyl donor **18** (**Scheme 3**). Glycosylation of donor **18** with α-methyl acceptor **19** led to disaccharide **20**.[15b] The *O*-2-benzoyl group in disaccharide **20** was removed and further *O*-methylated to furnish **21**. Hydrolysis of the 4′,6′-*O*-benzylidene acetal using 80 % AcOH led to the crude dihydroxy derivative which was

converted to the L-iduronic acid-containing disaccharide building block acceptor **22** using TEMPO/BAIB mediated selective oxidation of the primary hydroxyl group to acid and subsequent esterification using MeI/$KHCO_3$ (Scheme 3).[15b]

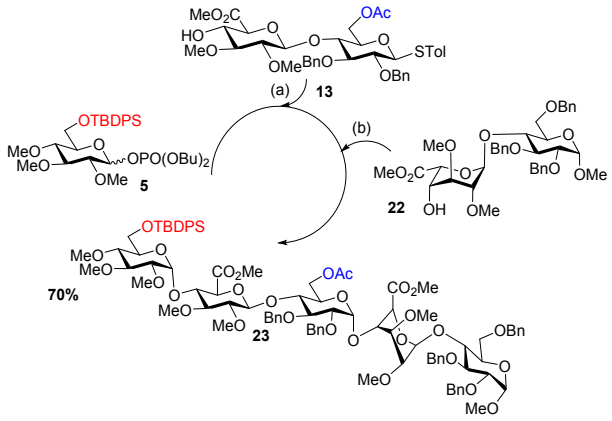
Scheme 3. Synthesis of L-Ido-α-(1→4)-D-Glc disaccharide building block



Reaction conditions: (a) *p*-CrSH, BF_3-Et_2O, CH_2Cl_2, 0 °C-rt, 12 h, 88%; (b) (i) NaOMe, MeOH:CH_2Cl_2(3:1), rt, 12h; (ii) $PhCH(OMe)_2$, CSA, CH_3CN, rt, 12 h, **17α** (82 %); **17β** (10 %); (c) BzCl, Py, 0 °C-rt, 12 h, 84%; (d) NIS, TMSOTf, CH_2Cl_2, 4ÅMS , -20 °C to 0 °C, 25 min, 93%; (e) (i) NaOMe, MeOH:CH_2Cl_2 (3:1), rt, 1h, (ii) MeI, NaH, DMF, 0 °C-rt, 3h, 90 % (2 steps); (f) (i) 80 % AcOH-H_2O, 70 °C; (ii) BAIB, TEMPO, CH_2Cl_2: H_2O (2:1), 0 °C-rt, 2 h; (iii) MeI, $KHCO_3$, DMF, rt, 4 h, 0 °C-rt, 68% (3 steps).

With all the building blocks in hand, we attempted the one-pot synthesis of protected Idraparinux **23**. The fully protected thioglycoside donor **4** was first coupled with acceptor **13** in the presence of NIS/TfOH. After the complete consumption of the donor, disaccharide **22** was added along with an additional amount of NIS and TfOH. However, the formation of desired pentasaccharide **23** was not observed even though the trisaccharide was formed initially in ~20%. The exact reason for the failure was unknown, probably due to the protecting groups and promoter. Therefore, we replaced the anomeric *p*-toluenethiol group of **4** by phosphate to generate the corresponding glycosyl phosphate donor **5** (**Scheme 1)**. Next, one-pot synthesis was performed using phosphate donor **5** along with the designed disaccharides (**13** and **22)** to yield pentasaccharide **23** in 70% yield (**Scheme 4**). The TBDPS ether group of **23** was removed selectively using HF-Py for the subsequent sulfation. This method eliminates the problem associated with *O*-sulfation[19b] to generate **2** (**Scheme 5**).

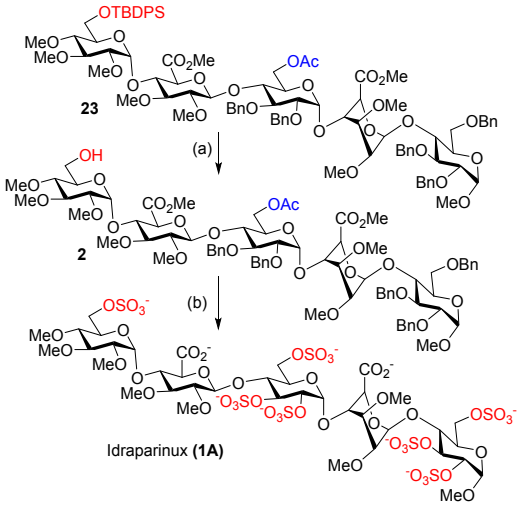
Scheme 4. One-pot synthesis of protected Idraparinux 23.



Reaction conditions: (a) TMSOTf (1.0 equiv.), CH2Cl2, 4Å MS,-45 °C; (b) NIS, -45 °C to -25 °C, 80 min.

The *O*-sulfated pentasaccharide **1A** was obtained from pentasaccharide **2** in a two-step sequences: (i) saponification of the ester functionalities using $LiOH/H_2O_2$ in THF followed by NaOH in methanol and unmasking of all OBn groups to OH under catalytic hydrogenation in the presence $Pd(OH)_2/C$ and (ii) global *O*-sulfation was performed using excess SO_3-Et_3N. Finally, the crude pentasaccharide was passed through an ion exchange Dowex 50WX8Na^+ and then purified by Sephadex-G25 gel chromatography using water as eluent. The pure fractions were concentrated and lyophilized to furnish compound **1A** in 59 % yield over 2 steps (Scheme 5).

Scheme 5. Synthesis of Idraparinux



Reaction conditions: (a) HF-Py, Py, 0 °C-rt, 12 h, **77 %**; (b) (i) 1M LiOH, H_2O_2, THF, -5 °C-rt, 8 h then 4M NaOH, MeOH, rt, 18 h; then H_2, 20 % $Pd(OH)_2$-C, MeOH : HCOOH (1:0.1), 24 h, rt; (ii) SO_3-Et_3N, DMF, 55 °C, 12 h, 59 % (2 steps).

CONCLUSION

In conclusion, we have developed an efficient modular one-pot synthesis of the heparin-based anticoagulant Idraparinux, using a set of well-designed glycosyl phosphate and thioglycoside building blocks in a [1,2,2] approach. The use of D-cellobiose, containing a 1,4-β-glycosidic linkage between the two glucose monomers, reduced the number of steps and enabled easy access to the disaccharide building block **13**. The constructions of D-glucuronic acid- and L-iduronic acid-containing disaccharide building blocks were performed prior to the modular assembly of the pentasaccharide backbone, as this is more effective than the late-stage introduction of these two acidic sugars.[19b] The α-selective glycosylation in the one-pot synthesis is driven by silyl and acetyl groups at *O*-6 position in spite of the presence of non-directing OMe and OBn protecting groups at C2 of the building blocks. The main advantages of this synthetic strategy as compared to the reported methods are: 1) the stereoselective construction of glycosidic linkages using glycosyl phosphate and thioglycoside building blocks (**5** and **13**) with TBDPS and Ac protecting groups at *O*-6 to direct the α-selective glycosylation; 2) the design of disaccharide building block **13** from cellobiose and the disaccharide acceptor **22** from glucofuranose for the one-pot synthesis. While Mlynarski and co-workers reported the use of cellobiose as starting material for conversion to an 1,6-anhydride as acceptor for glycosylation, the glycosylation reaction was not α-selective[17].

The synthetic route described here demonstrates an effective strategy for the modular one-pot synthesis of the clinically important anti-coagulant Idraparinux, and we believe this strategy is also applicable to other related substances. In 2013, the non-heparin based oral anticoagulant drugs dabigatran, apixaban, edoxaban, and rivaroxaban were approved for clinical use.[24] However, the side effects[25] of these drugs clearly indicated that the use of classical heparin-based anticoagulants is still important for the antithrombotic therapy.[26]

In summary, the modular one-pot synthesis of Idraparinux described in this work involves the use of building blocks with differential protecting groups, and this strategy should be complementary to the chemical [27] and chemoenzymatic methods [28] developed by many other groups and applicable to the synthesis of other heparin-based structures with regiodefined sulfation pattern for the SAR study and for the development of new heparin-based medicines.

Supporting Information: The Supporting Information is available free of charge on the ACS publications website http://pubs.acs.org. (Experimental procedures, copies of NMR spectra for all new compound).

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

This work was supported by the National Institutes of Health (AI072155), the National Science Foundation (CHE-1664283), Academia Sinica and the Kwang Hua Foundation.

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