# Programmable One-pot Synthesis of Heparin Pentasaccharide Fondaparinux

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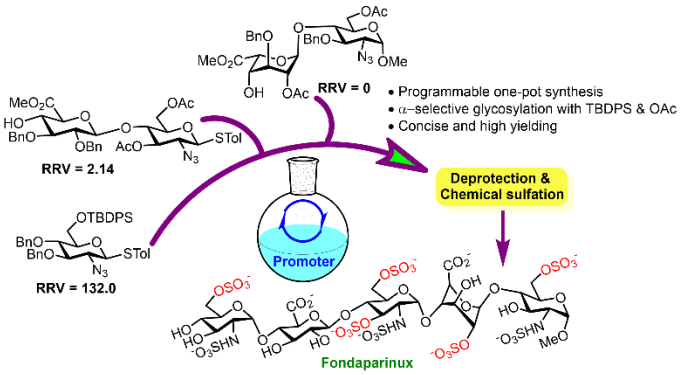



**ABSTRACT:** The clinically approved Fondaparinux (Arixtra) has been used for the treatment of deep vein thrombosis (DVT) and acute pulmonary embolism (PE) since 2002, and considered to be better than the low molecular-weight heparin (LMWH) in terms of anticoagulation response, duration of action and biosafety. However, the synthetic methods developed for its manufacture in the past years are relatively complicated thus restricting its extensive use. We reported here a potentially scalable and programmable one-pot synthesis of Fondaparinux using the [1,2,2] strategy and designed thioglycosides with well-defined reactivity as building blocks.

Fondaparinux **1**, a synthetic pentasaccharide with the brand name Arixtra, is a heparin-based anti-coagulant which has been used for the treatment of deep vein thrombosis (DVT) and acute pulmonary embolism (PE) since 2002. Two types of heparins, namely, high molecular weight heparin (HMWH) and low molecular weight heparin (LMWH) have been used as injectable anticoagulants which bind to antithrombin III (AT) and exhibit selective inhibition of factor Xa and thrombin in blood clotting cascade.[1] However, active monitoring is required for the patients administrated with heparins as serious complications like heparin-induced thrombocytopenia bleeding may occur. The sulfate-containing synthetic pentasaccharide **1** with the sequence D-GlcNS6S-α-(1,4)-D-GlcA-β-(1,4)-D-GlcNS3,6S-α-(1,4)-L-IdoA2S-α-(1,4)-D-GlcNS6S-OMe was identified as AT-binding sequence[2] and later was introduced to the market in 2002 with a trade name "Fondaparinux (Arixtra)"(**Figure 1**).[3] Fondaparinux was shown to have faster anticoagulation response, higher and more predictable anti-Xa activity, longer half-life and duration of action, lower risk of heparin-induced thrombocytopenia (HIT), and better biosafety than LMWH, making it a more acceptable anti-coagulant.[4] In addition, the contamination in naturally occurring heparins that caused several deaths[5] in 2008 led to the increasing clinical use of Fondaparinux as an alternative and perhaps better anti-coagulant.

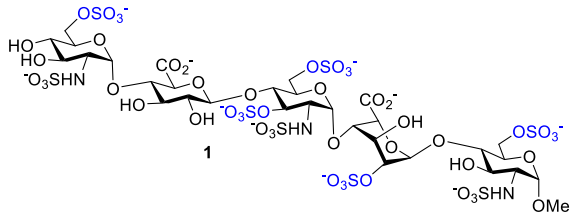



Figure 1: Structure of Fondaparinux.

For the treatment of DVT and acute PE, the recommended dose of **1** ranges from 5mg-10mg/daily based on the body weight. However, the high-cost treatment (ranging from $600-1400 in the USA), mainly due to its complicated and high-cost manufacturing process, has limited the availability of Fondaparinux.

Thus, development of an efficient and cost- effective synthesis of **1** is highly desirable to meet the clinical demand. The synthesis of Fondaparinux is very challenging due to the difficulty in the regio- and stereoselective glycosylation between the glucosamine, glucuronic acid and iduronic acid building blocks and the strategic installation of $OSO_3^-$ and $NHSO_3^-$ groups. In particular, the 1,2-*cis* or α-glycosylation between a glucosamine and an uronic acid building block without the formation of the unwanted β-isomer as well as improvement of the overall yield in a shortest possible synthetic route represents a major challenge. In the past years, many groups, including that of Petitou,[6] Lin,[7] Hung,[8] Wang,[9] Qin,[10] Manikowski,[11]and Ding[12] have reported the synthesis of Fondaparinux, but the procedures still encounter problems of long stepwise process, non-stereoselective glycosylation and low yield and efficiency. Zhao and co-workers recently reported a pre-activation based iterative one-pot synthesis of Fondaparinux with less than 40% yield.[13] We thought the method of programmable one-pot synthesis of oligosaccharides using designed thioglycoside building blocks with defined relative reactivity values (RRVs) developed by us[14, 15] could be useful for the practical synthesis of **1**.

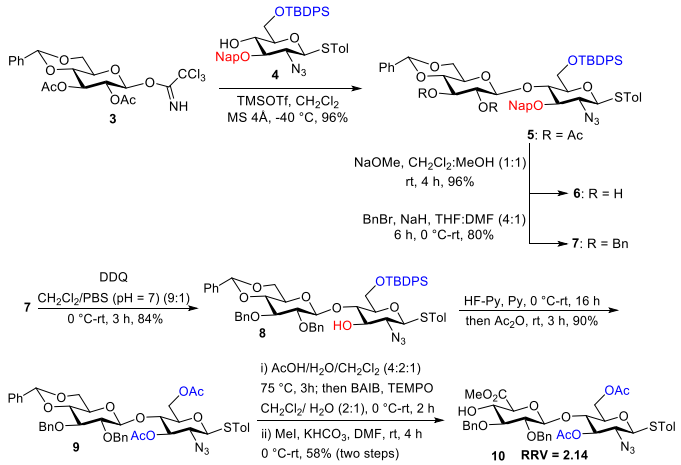
The concept of RRV is based on the quantitative determination of the reactivity of a thioglycoside donor with methanol as compared to the reactivity of the thioglycoside donor of *per*-acetyl mannose. RRV is measured using HPLC to determine the amount of leaving group released and the starting donor left in the reaction time course. With the RRV of various thioglycoside building blocks (BBLs) available, one can design a computer software to guide the selection of appropriate BBLs with well-differentiated RRVs for the one-pot assembly of oligosaccharides. We developed the first computer program, "Optimer" in 1999[14] as a database search tool for the rapid one-pot assembly of large numbers of linear and branched complex oligosaccharides including N-glycans[15] and glycosaminoglycans.[16] In 2018, we reported an upgraded version of this software, namely, Auto CHO with a library of 150 building blocks (BBLs) with experimentally measured RRVs and 50,000 BBLs with predicted RRVs by machine learning (including those with RRV predicted by chemical shifts by NMR)[14] to diversify the applicability of the software for the synthesis of oligosaccharides. To use either "Optimer" or "Auto CHO" software, the user needs to input the desired oligosaccharide structure then the software will generate one or more synthetic routes based on the RRVs of the BBLs needed for the synthesis of the oligosaccharide as output. Once the user chooses a specific synthetic route from the output, BBLs are required to be synthesized in the laboratory and then one-pot synthesis can be performed by sequential addition of BBLs starting from the most reactive from the non-reducing end unit toward the less reactive, least reactive and so on in the reducing end. The one-pot strategy was successfully applied to the synthesis of heparin-like oligosaccharides[16a,b] and the heparin-based anticoagulant Idraparinux.[16c]

The building blocks used in this one-pot strategy allow differential removal of the protecting groups for the regioselective introduction of sulfate groups to evaluate their role in biological functions. Following this strategy, we report here an efficient and scalable programmable one-pot synthesis of Fondaparinux **1** using the [1,2,2] strategy and designed thioglycosides (**2**, **10** and **18**) as building blocks.

All the building blocks are readily attainable from commercially available monosaccharides. The synthetic design involves the use of our established programmable one-pot method to conduct highly α-selective glycosylation using TBDPS and Ac groups at O6 and late stage introduction of the acidic functionalities (glucuronic and Iduronic). For the selective installation of the 3-$SO_3^-$ group, we masked the C3-hydroxyl group (C3-OH) with an orthogonal protecting group, namely, 2-napthyl ether (Nap). The synthesis of 2-azido thioglycoside donor **2** was achieved from D-glucosamine hydrochloride using our previous reported procedure (Supporting Information, Scheme S1).[16b] The RRVs of the newly synthesized building blocks were measured by HPLC analysis in a competition assay with a reference thioglycoside donor of known RRV (Supporting Information).[14,15]

The synthesis of disaccharide **10** involved the glycosylation between glycosyl trichloroacetimidate **3**[17] and thioglycoside acceptor **4**[18] in the presence of TMSOTf to generate **5** in 96% yield. Zémplen de-acetylation gave **6** and *O*-benzylation of 2',3'-OH led to the formation of **7** in 80% yield. Removal of the 2-Nap protecting group using DDQ[19] furnished disaccharide **8** with free hydroxyl group at C3 in 84% yield. Removal of the silyl protecting group under $F^-$ source (HF-Py) followed by protection of 3,6-OH as acetyl ester using $Ac_2O$/py led to **9** in 90% yield. Hydrolysis of the 4',6'-O-benzylidene acetal using 80 % AcOH-$H_2O$ produced the crude dihydroxy derivative for the selective oxidation of the primary hydroxyl group to carboxylic acid using TEMPO/BAIB and subsequent esterification with MeI/$KHCO_3$ to give disaccharide acceptor **10** in 58 % yield (Scheme 1).

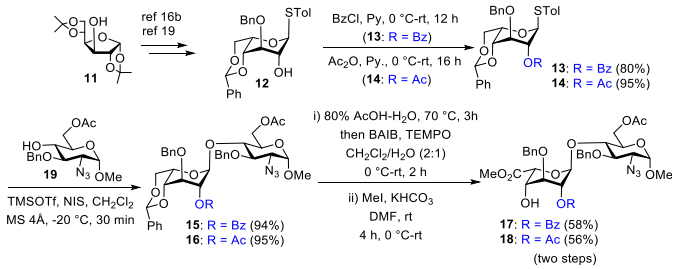
Scheme 1. Synthesis of D-Glc-β-(1→4)-D-Glc$N_3$ Disaccharide Building Block



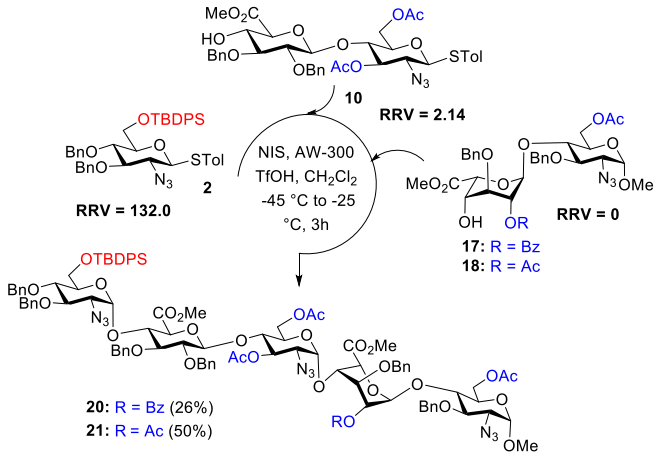

For the synthesis of Ido-Glc$N_3$ disaccharide derivatives (**17** & **18**), we used commercially available diacetone glucose **11** which was converted to α-L-idopyranoside **12** using known procedures.[20] The 2-OH group of **12** was protected both as benzoyl (Bz) and acetyl ester (Ac) to generate **13**[16b] and **14**, in 80% and 95% yield, respectively. NIS/TMSOTf-Mediated glycosylation of **13** with α-methyl acceptor **19**[16b]

generated disaccharide **15** in 94% yield. The 4′,6′-*O*-benzylidene acetal was hydrolyzed using 80% AcOH and the crude dihydroxy derivative was treated with TEMPO/BAIB to oxidize the primary hydroxyl group to acid and subsequent esterification of the acid with MeI/$KHCO_3$ generated L-iduronic acid-containing disaccharide acceptor **17** in 58% yield (Scheme 2). It is noted that we have also reported the synthesis of **17** using a different synthetic route.[16b]

Scheme 2. Synthesis of L-Ido-α-(1→4)-D-Glc$N_3$ Disaccharide Building Block

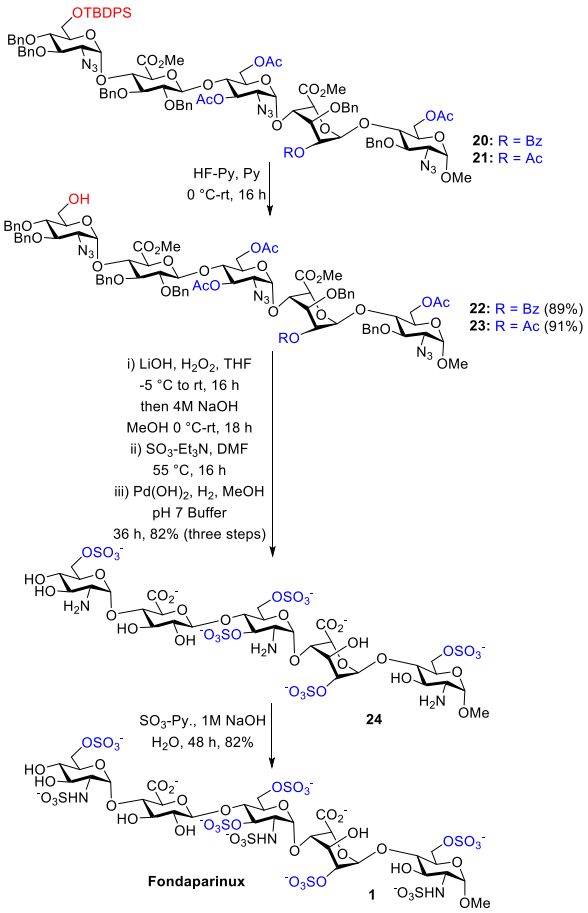



Scheme 3. One-pot Synthesis of Protected Fondaparinux




We measured the RRVs of glycosyl donors (**2** and **10**),[14,15] and found that the RRV of **2** was 132.0 whereas for **10** it was 2.14. After synthesizing all the required building blocks (**2**, **10** and **17**), we attempted the programmable one-pot synthesis of the protected pentasaccharide **20**. However, the yield was only 26%. To improve the yield of the one-pot synthesis, we changed the disaccharide acceptor **17** to **18**, in which the 2-OH is protected as acetyl ester. The 2-*O*-acetyl protected donor **14** was coupled with α-methyl acceptor **19** in the presence of NIS/TMSOTf to generate disaccharide **16** in 95% yield. Hydrolysis of the 4′,6′-*O*-benzylidene acetal, TEMPO/BAIB oxidation of primary alcohol to acid and subsequent esterification of the acid using MeI/$KHCO_3$ generated **18** (56%) which was then used in the one-pot synthesis of protected Fondaparinux **21** in 50 % yield (Scheme 3). The concept of RRV is based on the measurement of the reactivity of thioglycoside donor. Both **17** & **18** are glycosyl acceptors without leaving group, so the RRV were not measured and assigned to "zero". Thus, it was difficult to foresee the lower yield (26%) of the pentasaccharide **20** using 2-*O*-Bz protected disaccharide **17** (Scheme 3) and higher yield of **21** (50%) using 2-OAc containing disaccharide **18**.

Scheme 4. Synthesis of Fondaparinux




We used the protected pentasaccharides **20** and **21** for differential deprotection and chemical sulfation. The silyl group (TBDPS) in **20** and **21** was removed using HF-Py to generate compounds **22** and **23**, in 89% and 91% yield, respectively. Protection group free *O*-sulfated pentasaccharide **24** was obtained in three steps sequences. Saponification of **22** and **23** using LiOH/$H_2O_2$[21] in the presence of NaOH/MeOH hydrolyzed all ester functional groups. For the installation of the $OSO_3^-$ groups, it was further treated with excess $SO_3$-$Et_3N$ followed by unmasking all *O*-benzyl groups and reduction of $N_3$ to amine under catalytic hydrogenation with Pd $(OH)_2$/C to give **24** in 82% yield. The selective N-sulfation was performed at pH 9.5 with $SO_3$-Py, and the *pH* of the reaction was controlled by slow addition of 1M NaOH(aq) from time to time. The crude product was passed through size-exclusion (Sephadex G-25) and ion-exchange (Dowex 50WX8$Na^+$) columns to furnish Fondaparinux **1** (Scheme 4). The reported NMR and mass spectrometry data are well matched with the reported.[8] All newly synthesized derivatives were characterized by $^1H$, $^{13}C$ NMR spectra and high-resolution mass spectra (HRMS). $^1J_{C\text{-}H}$ Coupling constants were measured from the 2D-NMR to determine the α- and β-linkages between the building blocks (Supporting Information).

In conclusion, we have developed a programmable one-pot synthesis of the clinically important anticoagulant Fondaparinux using designed thioglycoside building blocks with well-defined RRVs for α-selective glycosylation guided by silyl ether and acetyl ester functionality at O6 in the one-pot sequence. The introduction of 3-$OSO_3^-$ was performed with the aid of 2-napthyl ether (Nap). The carefully selected orthogonal protecting groups which can be differentially deprotected and readily accessible thioglycoside building blocks in the one-pot synthesis effectively reduce the number of synthetic steps and eliminate the multiple purification steps. In addition, the advantage of programmable approach is to allow a pre-evaluation of the building blocks to be used in a one-pot manner. The total synthesis was accomplished in 22 longest linear route with 4.2% overall yield from diacetone glucose which is a very significant improvement compare to previously reported synthetic methodologies. The protected pentasaccharide was synthesized for more than 200 mg and can be performed in gram scales. The synthetic route reported here is scalable and should be useful for the synthesis of Fondaparinux and closely related structures decorated with regiodefined *O*-and *N*-sulfation.[16b]

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Supporting Information [Experimental and purification procedures, characterization data ($^1$H and $^{13}$C (ppm) and high-resolution mass spectra). Copies of $^1$H and $^{13}$C spectra are also provided for all newly synthesized compounds.

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### Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. †These authors contributed equally. (Supriya Dey, Hong-Jay Lo)

## ABBREVIATIONS

DDQ: 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone; NIS: N-Iodosuccinimide; TEMPO: 2,2,6,6-Tetramethylpiperidin-1-yl)oxyl or (2,2,6,6-tetramethylpiperidin-1-yl)oxidanyl; BAIB:Diacetoxyiodo benzene; RRV = Relative Reactivity Values; BBLs: Building blocks

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