Review Article

Advances in Chemical Synthesis of Fondaparinux

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Received 13 December 2018; Revised 27 February 2019; Accepted 19 March 2019; Published 12 May 2019

Academic Editor: Gabriel Navarrete-Vazquez

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Fondaparinux (trade name Arixtra) is a safe and efficacious anticoagulant, and it is chemically related to low-molecular-weight heparins such as enoxaparin. Fondaparinux is a synthetic pentasaccharide, and its synthesis is difficult and expensive. The high cost of fondaparinux thwarts its extensive worldwide usage. Over the last two decades, several research groups and pharmaceutical companies have been interested in finding efficient and practical methods for its synthesis. The present review discusses those strategies and their pros and cons in a comparative account.

1. Introduction

According to the 2011 World Health Organization (WHO) report [1], cardiovascular disease is the world's biggest killer claiming nearly 18 million lives per year, and 80% of this staggering number of deaths arise from heart attacks and strokes alone. Myocardial infarction, cerebral infarction, atrial fibrillation, and deep vein thrombosis are all related to thrombosis and require antithrombotic therapy. Antithrombotic drugs are divided into three categories: anticoagulants, antiplatelet aggregation drugs, and thrombolytic drugs.

Since 1935, heparin has been widely used as an anticoagulant and thrombolytic drug in clinical treatment and is the longest carbohydrate drug used [2–6]. Natural heparin is extracted from the viscera of an animal and is a mixture of different active polysaccharides. During the usage, the effective dose of heparin is difficult to control and can cause dangerous side effects such as bleeding and thrombocytopenia. Heparin molecules bind nonspecifically to proteins in plasma and lead to more complex complications. Due to the animal origin of heparin, there is a potential risk of cross-species virus infection [7], and the most effective way to avoid cross-species contamination is to prepare the heparin by chemical synthesis.

In the early 1980s, a heparin pentasaccharide fragment was identified as a key binding domain for heparin anti-thrombin (ATIII) activity [8]. Based on the structure of this pentasaccharide, professor Sinaÿ's research team chemically synthesized the pentasaccharide fragment and its methyl glycoside through more than 70 chemical reactions [9, 10]. Bioactivity tests showed that the anticoagulant and thrombolytic properties of the pentasaccharide fragment were very similar to its methyl glycosides. In 2001, the methyl glycoside of the pentasaccharide fragment was marketed as a new antithrombotic drug [11], named as fondaparinux (trade name Arixtra), and its structure is shown in Figure 1.

Majority of the carbohydrate-based drugs [12, 13] are derived from natural sources, and only very few are synthetic oligosaccharides such as fondaparinux, whereas fondaparinux efficacy and safety in prevention of deep venous thrombosis and pulmonary embolism in lower extremities are better than that of the low-molecular-weight heparins (LMWH). Fondaparinux's high bioavailability, fast onset, long half-life, and less adverse reactions will make it to replace the heparin as the major anticoagulant drug in the market early or later. However, its high manufacturing cost restricts its widespread use since its chemical synthesis is long, difficult, and tedious. Arixtra,

FIGURE 1: Structure of fondaparinux.

has been off-patent since 2002 but faced slow competition due to the difficulty in fondaparinux preparation. During the last thirty years, many methods and strategies were developed for improving its synthetic process. However, most of the time, lengthy chemical processes are required because of the repeated introduction and removal of protecting groups, and the separation of side products from desired intermediates is often inefficient, both of which drastically decrease the synthetic efficiency. Therefore, it is imperative to develop highly efficient strategies for producing the homogeneous pentasaccharide for clinical applications.

Fondaparinux is the α -linked methyl glycoside of the heparin pentasaccharide fragment, which consists of three amino sugars with α -configuration (A, C, and E), one glucuronic acid unit with β -configuration (D), and one iduronic acid unit with α -configuration (B). While 1,2-*trans*-glycosylation can be accomplished by neighboring group participation of the ester group positioned at C2 of the uronic acid precursor, however 1,2-*cis* glycosylation involving the 2-deoxy 2-amino glucosyl derivative is quite difficult. The nonparticipating C2-azido group is often utilized in this case, relying mainly on the anomeric effect. Other steric effect and remote group participation were also used to help increase the preference for α -glucosaminylation. Synthesis of fondaparinux involves two stages, namely, the synthesis of protected pentasaccharide (EDCBA) and its conversion to fondaparinux. The synthesis of protected EDCBA can be achieved in multiple combinatorial ways such as linear combinations of its monosaccharides, namely, E + D + C + B + A or in convergent fashion such as E + DCBA, EDCB + A, EDC + BA, or even ED + CBA. Herein, we would like to summarize various approaches described for the synthesis of fondaparinux thus far.

2. Discussion

2.1. Synthesis of Protected Pentasaccharide EDCBA. Early approaches for the synthesis of the heparin-related protected pentasaccharide EDCBA fragment were achieved by combining disaccharide fragments DC and BA to obtain the tetrasaccharide DCBA fragment which was finally coupled with the E monosaccharide donor to give the protected pentasaccharide EDCBA in an E + DCBA strategy (vide infra).

In 1984, Sinaÿ [9] reported the first chemical synthesis of heparin pentasaccharide fragment following the E + DCBA approach in an overall yield of 0.053% (Scheme 1). The tetrasaccharide DCBA itself was assembled from DC and BA disaccharide fragments which were again constructed from the corresponding monosaccharides.

Sinaÿ's strategy involved two critical stereoselective steps, coupling reactions between disaccharides 1 and 2 and between the tetrasaccharide 3 and monosaccharide 4. Interestingly, these two coupling reactions only generated the desired α -linked products in contrast to the coupling reaction between E monosaccharide donor and DCBA tetrasaccharide acceptor described elsewhere [14].

Following the similar strategy (E + DCBA), a research group from Netherlands [15] also reported the synthesis of the heparin pentasaccharide fragment in an improved overall yield (0.22%) (Scheme 2).

Furthermore, in 1987, Petitou et al. [10] reported the synthesis of fondaparinux using the E + DCBA strategy (Scheme 3) as well albeit with a much better overall yield than that of the previously reported methods.

In 1988, Yoshitaka [14] assembled the tetrasaccharide, DCBA from the DC and BA disaccharides which were obtained from the commercially available disaccharide starting material, i.e., cellobiose. They had obtained the fully protected pentasaccharide EDCBA after coupling the DCBA with the monosaccharide E in the E + DCBA strategy.

In 2014, Chang et al. [16] also reported the synthesis of fondaparinux through coupling reaction between the tetrasaccharide EDCB and the monosaccharide A (EDCB + A strategy) in 0.63% overall yield (Scheme 4). The desired tetrasaccharide EDCB itself was assembled through sequential monosaccharide additions from the reducing to the nonreducing end. The overall strategy involved 22 linear steps from D-glucosamine hydrochloride with two critical α -linked glycosylations with complete stereoselectivity. The advantage of this strategy is to use TBDPS and Lev protecting groups to protect the hydroxyl groups which will be sulfated later, to use the acetyl protecting groups to protect the hydroxyl groups which will be oxidized to carboxylic acid, which are different from other strategies.

Synthesis of a complex molecule such as fondaparinux demands efficiency in overall approach. Usually, linear syntheses (vide supra) are less efficient in comparison to convergent syntheses of any complex molecule. Thus, assembling protected pentasaccharide EDCBA in a linear fashion from their monosaccharide building blocks is lengthy in comparison to other approaches wherein coupling of a trisaccharide with a disaccharide is executed. The next few approaches described below exemplify such convergent approaches.

In 1991, Sinaÿ and Petitou [17] reported the total synthesis of fondaparinux through a convergent (EDC + BA) strategy, as shown in Scheme 5.

Usually, when a glycosyl donor with a 2-azido group is coupled to a glycosyl acceptor, it is expected that the α -linked disaccharide should be the predominant product

SCHEME 1: Synay's E + DCBA strategy for the synthesis of heparin-related pentasaccharide fragment.

because of the lack of the neighboring group participation effect from the azido group at the 2 position in the glycosyl donor. The β -linked disaccharide may be found as the minor product, and the separation of these two isomers may be tedious and possibly challenging. However, in Synay's report [17], the coupling reaction (Scheme 5) between the trisaccharide donor **22** and the disaccharide acceptor **23** in the presence of TMSOTf described the formation of only the α -linked pentasaccharide **12** in good yield (70%).

Recently, several research groups such as Lin et al. [18], Dai et al. [19], and Li et al. [20] used the similar EDC + BA strategy to report the synthesis of protected fondaparinux-related pentasaccharide. Furthermore, 3 + 2 (EDC + BA) strategy was also utilized in the programmable one-pot method which was employed by Wong to synthesize one fondaparinux-related pentasaccharide [21]. In this method, the monosaccharide building blocks were selected from the OptiMer computer database for a given oligosaccharide. The database stores the relative reactivity values of glycosyl donors and acceptors. For a target oligosaccharide, the program selects the route of synthesis and predicts the required building blocks, monosaccharides, and disaccharides. After selecting the monosaccharides and disaccharides from the database, they are individually synthesized. These building blocks are then assembled in a one-pot method through a series of glycosylations. For the synthesis of

fondaparinux protected pentasaccharide, two crucial α -glycosylations were carried out in succession in one-pot starting from the corresponding monosaccharide and the disaccharide building blocks in 48% yield.

Alchemia Pharmaceuticals [22] of Australia had also used 3 + 2 strategy in assembling the pentasaccharide. In their procedure, they had employed EDC trisaccharide moiety donor **24** containing *p*-methoxybenzyl (PMB) protecting groups to carry out glycosylation with BA disaccharide fragment acceptor **25** (Scheme 6). In our own efforts in synthesizing the fondaparinux in an efficient and practical manner [23], we attempted to employ Alchemia intermediates **24** and **25** for assembling the pentasaccharide. To our surprise and dismay, the reaction products are very complex, and the yield of pentasaccharide **26** was very low. The purification of the reaction was quite difficult. This may be due to the instability of PMB groups under glycosylation conditions. However, it is worth noting that, when the protecting groups in EDC trisaccharide donor **24** were changed from PMB to acetyl groups, the glycosylation reaction was successful (Scheme 7).

Thus, when the EDC trisaccharide **24** was transferred into the thioglycoside **27** of the EDC trisaccharide fragment, the coupling reaction between the EDC trisaccharide donor **27** and the BA disaccharide acceptor **25** produced only a α -linked pentasaccharide **28** in 84% (Scheme 7) yield.

SCHEME 2: Van Boeckel's E + DCBA strategy for the synthesis of heparin-related pentasaccharide fragment.

SCHEME 3: Petitou's E + DCBA strategy for the synthesis of protected pentasaccharide.

SCHEME 4: Hung's EDCB + A strategy for the synthesis of protected pentasaccharide.

2.2. Synthesis of EDC Trisaccharide Fragments. In the convergent approaches, the pentasaccharide has been obtained by the coupling of trisaccharide EDC with the BA disaccharide and is most widely preferred. Between the EDC and BA fragments, the synthesis of EDC trisaccharide is quite challenging. Synthetically, the EDC trisaccharide fragment can be accessed from either of the approaches shown in Scheme 8.

In 1991, Petitou et al. [24] constructed the EDC trisaccharide fragment from the glycosylation (strategy 1) of

the monosaccharide E donor **4** with the DC disaccharide acceptor **29** (Scheme 9).

Thus, the monosaccharide bromo donor **4** with an azido group at 2 position, upon condensation with the disaccharide acceptor **29**, generated the desired α -linked EDC trisaccharide **21** in high yield. In a recent article, Dai et al. [19] demonstrated that the remote protecting groups (6-OAc/Bz) on the disaccharide acceptors also induced the exclusive α -anomeric selectivity [25] in the glycosylation

SCHEME 5: Sinaý and Petitou's EDC + BA synthetic strategy for the synthesis of the protected pentasaccharide.

SCHEME 6: Alchemia's EDC + BA strategy for the synthesis of protected pentasaccharide.

SCHEME 7: Ding's strategy for synthesis of protected pentasaccharide.

SCHEME 8: Two synthetic strategies for synthesis of trisaccharide fragment EDC.

product, i.e., trisaccharide **32** (Scheme 10). Thus, the trichloroacetimidate donor **30** was glycosylated with disaccharide donor **31** in presence of TfOH to obtain the corresponding EDC trisaccharide **32** in high yield.

In order to obtain the EDC trisaccharide adequately, the corresponding DC disaccharide should be obtained in an efficient manner. Sinay's disaccharide **29** was synthesized, as shown in Scheme 11. The benzyl protected monosaccharide

SCHEME 9: Petitou's strategy for synthesis of the α -linked trisaccharide EDC fragment. SCHEME 10: Qin's strategy for the synthesis of the α -linked trisaccharide EDC fragment.

SCHEME 11: Sinaÿ's strategy for the synthesis of the DC disaccharide fragment.

33 was condensed with the acceptor 34 to obtain the β -linked disaccharide 35 as the major product. However, this reaction was proved to be sluggish and low yielding.

In another approach for the synthesis of DC fragment 29, van Boeckel [15] employed anhydroepoxide 36 in the

condensation with the per-*O*-acetyl glucosyl bromo donor 37, as shown in Scheme 12.

The DC disaccharide 29 was also assembled from using cerny epoxide as donor by Singapore's Scinopharm [26], as shown in Scheme 13. In this method, 2-*O*-Bz in the

SCHEME 12: Synthesis of DC disaccharide by van Boeckel's strategy.

SCHEME 13: Synthesis of DC disaccharide by Scino's strategy.

thioglycoside **40** participates in coupling with monosaccharide **36** generated the β -linked disaccharide **41**. The 2-O-Bz trisaccharide fragment **41** was then converted into 2-O-Bn disaccharide **29** by debenzoylation followed by benzylation of the resulting hydroxyl group.

However, the methods described in Schemes 12 and 13 are not practical, scalable, or economical as the volatility of the key intermediate, Cerny epoxide **36**, causes serious problems (in our efforts, not reported) while handling it. Furthermore, the benzoate at the 2 position in fragment **41** should be replaced with the benzyl moiety in order to obtain DC fragment **29** (Scheme 13).

As an alternative strategy, Petitou et al. [24] developed a method to synthesize the DC fragment **46** from cellobiose in eight steps which was shown in Scheme 14.

Accordingly, the DC disaccharide fragment **46** was synthesized by taking advantage of in-built β -(1-4)glycosidic bond in commercially available cellobiose. The fixed β -glycoside bond between D and C fragments removes the

uncertainty of glycoside bond formation. However, one short coming in this strategy is that the starting material cellobiose is not cheap which in turn increases the overall cost for the synthesis of fondaparinux. Hence, it is still imperative to develop new methods to prepare the DC fragment in a practical manner.

Another approach (strategy 2 in Scheme 8) to synthesize the trisaccharide EDC is through the coupling of the ED disaccharide donor with the C monosaccharide acceptor. Alchemia Pharmaceuticals [22] constructed the EDC trisaccharide fragment **34** by using this strategy. The synthetic strategy employed is summarized in Scheme 15. Accordingly, the coupling reaction between the disaccharide donor **47** and monosaccharide **48** gave the β -linked trisaccharide **49** in high yield, after removal of the benzoyl group, methylation, and benzylation to give the trisaccharide donor **24**.

The synthesis of the corresponding disaccharide ED donor **47** is summarized in Scheme 16. It is not surprising that the coupling reaction between compound **50** and **51**

SCHEME 14: Synthesis of the DC disaccharide fragment from cellobiose.

SCHEME 15: Synthesis of the EDC trisaccharide fragment by using Alchemia's strategy.

gave a mixture of two anomeric isomers **52** and **53** and the separation of these two isomers was very difficult. The desired isomer **52** was then transferred to the corresponding disaccharide donor **47**.

Researchers at Reliable Biotech [27] screened different protecting groups for the monosaccharide donor **54** and acceptor **55** in the hope to increase stereoselectivity in the coupling reaction. It is claimed under the given reaction

SCHEME 16: Alchemia's strategy for the synthesis of the ED disaccharide fragment.

SCHEME 17: Reliable's strategy for the synthesis of the ED disaccharide.

conditions (Scheme 17), only α -linked disaccharide **56** was formed, but the reproducibility of this method is poor, and a mixture of α -linked and β -linked disaccharides were often obtained. However, by using the key disaccharide **56**, they were able to get the desired EDC trisaccharide.

As part of α -glycosylation studies of D-glucosamine derived donors, Hu et al. [28] explored α -stereoselectivity of the newly formed glycosyl bond in a glycosylation reaction. From these studies, it was concluded that excellent

α -stereoselectivity can be achieved by a combination of orthogonal protecting groups, and the stereoselectivity was found to be independent of leaving groups and activators. Furthermore, the trichloroacetimidate was found to be the optimum donor. The chosen orthogonal protecting groups were then successfully manipulated to carry out the total syntheses of several oligosaccharide analogues.

As an extension of the above studies, Chang et al. studied [16] the synthesis of EDC trisaccharide while employing the

SCHEME 18: Chang et al.'s strategy for stereoselective synthesis of ED disaccharide fragment.

SCHEME 19: Synthesis of monosaccharide B fragment from D-glucose.

ED disaccharide donor and the C monosaccharide acceptor. Accordingly, they have reported the stereoselective synthesis of ED disaccharide fragment **58** in 71% yield by using TBDPS, *p*BrBn, naphthylmethyl (2-NAP), acetyl, benzyl, and benzoate protecting groups, as shown in Scheme 18. Obviously, all the six hydroxyl groups (except anomeric hydroxyl group) in the disaccharide were differentiated with six different protecting groups. This approach may not be an efficient or practical approach as it increases overall number of steps with more protection and deprotection steps in the synthesis of fondaparinux.

The above methods summarized the syntheses of EDC trisaccharide fragment, and it will be very tempting to suggest that the best strategy would be to couple the DC disaccharide obtained from cellobiose with the E monosaccharide donor. However, this approach might not be economical as the starting cellobiose is expensive, and more

importantly, some of the crucial transformations in this approach are riddled with variable and inconsistent yields. Thus, it is believed that the best current approach for EDC trisaccharide is to assemble it from the corresponding monosaccharide building blocks [23, 25] instead of the disaccharide building blocks such as the DC fragment.

2.3. Synthesis of BA Disaccharide Fragment. Unlike the uncertainties in the synthesis of EDC trisaccharide, the synthesis of the BA disaccharide fragment is well established. When constructing the β -linked BA disaccharide fragment, the OH at 2 position of the B sugar should be selected to have a protecting group which may exert its influence in deciding the β -glycosyl bond formation. Usually, acetyl or benzoyl groups [18, 19] are employed for this cause. From the glucose, after five chemical steps, the monosaccharide B

SCHEME 20: Synthesis of monosaccharide B fragment from D-glucose.

SCHEME 21: Synthesis of the BA disaccharide fragment.

SCHEME 22: Synthesis of the BA disaccharide fragment through the trichloroacetimidate.

SCHEME 23: Transfer the protected pentasaccharide to fondaparinux.

SCHEME 24: Transfer of the protected pentasaccharide to fondaparinux through hydrogenation-sulfation-hydrogenation sequence.

fragment was obtained, which can be easily transferred to glycosylation donor **63** [18, 29] (Scheme 19).

The coupling reaction between the B fragment donor **64** with the A fragment acceptor **65** gave the β -linked BA disaccharide fragment **66** with high stereoselectivity in 92% yield (Scheme 20). After deacetylation, oxidation, and

methylation, the BA disaccharide acceptor **67** was obtained [18, 29].

It is also possible to make the methyl ester (Scheme 21) donor for fragment B monosaccharide **68** as reported by several research groups [19, 20, 23, 25, 29]. Few additional steps are needed for protections and deprotections to make

SCHEME 25: Transfer the protected pentasaccharide to fondaparinux through the hydrogenation-sulfation-hydrogenation sequence.

the methyl ester, but it is worth the effort as it avoids late-stage modifications of the pentasaccharide.

The BA disaccharide fragment was also synthesized from the trichloroacetimidate donor instead of the thioglycoside donor. Thus, when the trichloroacetimidate donor **71** was used to react with the fragment A acceptor **65**, the reaction gave a mixture of the α -linked and β -linked disaccharides **73** and **74** (Scheme 22). As can be seen, since BA disaccharide is obtained as a mixture of isomers in this method, it is not preferable.

2.4. Synthesis of Fondaparinux Sodium. Fondaparinux synthesis is achieved in two distinct stages, and both of which have challenges in their own way. In the first stage, as seen from the multiple approaches described above for the synthesis of fully protected pentasaccharide, EDCBA, that perfect approach appears to be elusive even though fondaparinux has been synthesized on a commercial scale. Even the second stage is equally challenging and requires fine tuning and optimizing a series of selective deprotections and site-specific sulfations.

The conversion of the fully protected pentasaccharide, EDCBA to fondaparinux sodium, involves the operations, namely, hydrolysis of ester groups, *O*-sulfation, hydrogenolysis, and selective sulfation of amino groups (Scheme 23). The above operations are simple from a chemical stand point, but purification [10, 17] at each stage is

really complicated due to multiple minor impurities which arise in the close range.

As an alternative strategy, Manikowski [30, 31] used alkaline hydrolysis, hydrogenation, sulfonation, and hydrogenation sequence to transfer the protected pentasaccharide to fondaparinux (Scheme 24). However, when this procedure was repeated, it was found that the reactions for removal of the Cbz group and reduction of azido groups through catalytic hydrogen transfer reactions were incomplete and not reproducible, and the sulfation on amino and hydroxyl groups was not a clean reaction.

In our own efforts to avoid above mentioned issues in the published methods, a new strategy for the last few steps (Scheme 25) was developed [23]. Accordingly, the ester groups in the protected pentasaccharide **5** were hydrolyzed and then sulfated with pyridine-sulfur trioxide complex in a mixture of pyridine and triethyl amine, to give the *O*-sulfated pentasaccharide **77** which was directly used in the next step without further purification.

The azido groups in compound **77** were reduced by trimethylphosphine in a mixture of THF and water in presence of trace amount of sodium hydroxide to afford the amino derivative, which was sulfated by reaction with the pyridine-sulfur trioxide complex in pyridine. After completion of reaction, the crude product was purified to obtain the benzyl protected *N*-sulfated pentasaccharide **76**. Finally, the compound **76** was subjected to hydrogenation over Pd/C in a mixture (1 : 1) of water and acetic acid for 2 days. After

removal of the catalyst and solvents, the crude product was purified to obtain the fondaparinux sodium in 97% purity (HPLC).

2.5. Enzymatic Synthesis of Fondaparinux. Recently, Jian Liu [32–38] had demonstrated the utility of chemoenzymatic methods in obtaining hexasaccharides and octasaccharides related to heparan sulfate by employing the heparin sulfate polymerase enzyme. However, enzymatic synthesis of fondaparinux is found to be elusive.

3. Conclusion

In conclusion, we have discussed various strategies for the synthesis of fondaparinux and also highlighted their advantages or disadvantages over the other reported methods. An attempt has also been made to suggest a practical method for the synthesis of fondaparinux from the existing approaches.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This work was supported by Key Laboratory of Preventive Veterinary Medicine, Guangdong Provincial Department of Education (2014KTSPT037).

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