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Efficient and practical synthesis of Fondaparinux

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ABSTRACT

Combining advantageous sequences of Alchemia and Sanofi methods of synthesis of Fondaparinux, a more efficient and practical synthetic strategy for the synthesis of corresponding protected pentasaccharide was developed. The protected pentasaccharide was smoothly converted into Fondaparinux in overall high yield (1%).

Heparin derived from intestines of pigs or lungs of cows has been used in clinic as an anticoagulant drug for over 70 years due to its high affinity binding with anti-thrombin III.[1] Such preparations consist of a complex mixture of polysaccharides, and was always doubtful about their purity and presence of viral or prion contaminants.[2] Further, heparin as a non-specific and indirect thrombin inhibitor presents a variety of clinical challenges.[3]

In 1980, a unique pentasaccharide domain[3b,c] of heparin was found to be clinically effective, and in 2001 Fondaparinux (Fig. 1), a synthetic analogue of pentasaccharide domain of heparin was approved[4a] as the first synthetic anticoagulant heparin drug. Fondaparinux has well controlled pharmacokinetic and pharmacodynamic properties and is also free from viral or prion impurities.[4b] However, it is very expensive compared to heparin derived from animals due to its challenging and long tedious synthesis.

The successful synthesis of Fondaparinux relies heavily on the success of synthesis of its protected pentasaccharide precursor. The retrosynthetic analysis of protected pentasaccharide suggests that it can be obtained either in a linear synthesis of combining its monosaccharides in a sequential manner or from a variety of convergent combinations of its monosaccharides such as 1+2+2 or 3+2 or 4+1 etc. The first synthesis of protected pentasaccharide was reported by Petitou in 1987,[5] and subsequent pharmacokinetic and pharmacodynamic studies by Sanofi culminated in the marketing approval of synthetic pentasaccharide as Fondaparinux (Arixtra) in 2001. It was another 10 years before a generic method from Alchemia received FDA approval for production of the generic Fondaparinux in 2011.[6a,c] Further, in recent past a spate of articles describing successful total syntheses of Fondaparinux,[7–10] and few excellent review articles describing developments in the synthesis of heparin related oligosaccharides[11,12] have also been published. Given Fondaparinux synthetic complexity, not surprisingly, only few total syntheses of it were published to date. As to be expected all the synthetic strategies mainly focused on three aspects of synthesis, (i) optimizing yields of individual monosaccharides, (ii) obtaining desired stereo orientation during glycosylation steps and (iii) reducing total number of steps to obtain the protected pentasaccharide in overall high yield.

Several groups modeled their method of synthesis of protected pentasaccharide in a 3+2 convergent combination of blocks of modular saccharides. In these methods, the imidate trisaccharide (EDC) donor was usually coupled with acceptor disaccharide (BA) under mild conditions in good to moderate yields. Even though it was claimed that the syntheses were convergent the individual modular di or trisaccharides were assembled in a linear fashion which itself renders the syntheses more than 20 linear steps at a stretch. Few research groups have fashioned their syntheses of protected pentasaccharide in a true linear or sequential assembling of appropriately protected monosaccharides. A recent example for this approach was the reported synthesis of Fondaparinux by Hung[8] research group from Taiwan. They claimed 0.63% overall yield in their method, which contains a series of one pot reactions comprising overall 32 steps including a 22 linear step sequence. Thus, the analysis of syntheses of Fondaparinux by various research groups suggests that they follow either modular or linear

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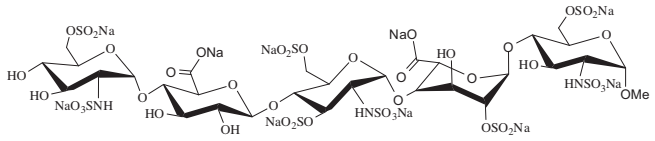


Fig. 1. Structure of Fondaparinux.

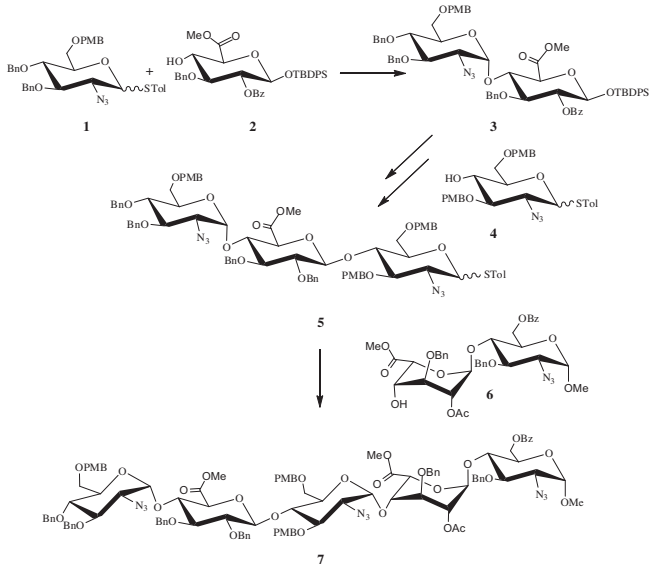
approach. In this article, we like to summarize our own findings on the synthesis of fully protected pentasaccharide and the eventual synthesis of Fondaparinux in overall high yield.

A cursory glance of hitherto reported articles suggests that all of them claim total synthesis of Fondaparinux albeit in few milligrams to gram scale. From the outset, it's our aim to synthesize Fondaparinux on large scale by focusing on the maneuvers around known strategies to achieve this target in a practical manner. This led us to revisit established procedures for its synthesis by Alchemia and Sanofi research groups.

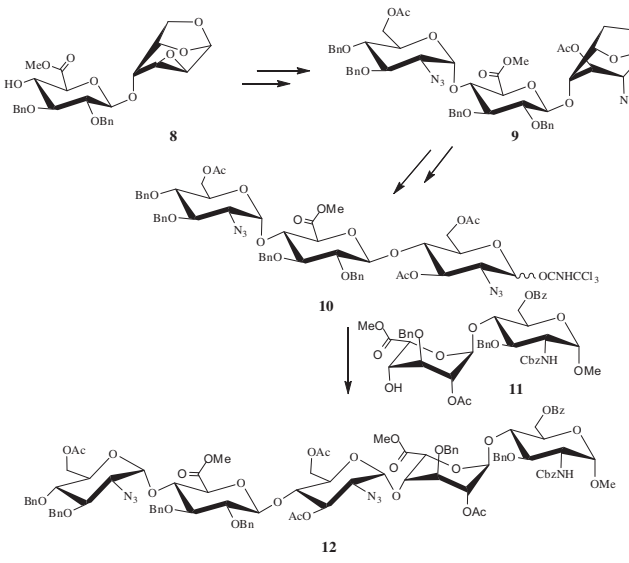
Accordingly, our initial approach for the synthesis of all protected pentasaccharide was on the lines of Alchemia's 3+2 block strategy. For assembling the desired trisaccharide, a linear strategy was followed as summarized in Scheme 1.[6a,b] This strategy was very straightforward in which the commercially available simple sugars were sequentially converted into the monosaccharides **1**, **2** and **4**, which were used in preparing the disaccharide **3** and trisaccharide donor **5**. Simplicity and practicality of all the steps involved in obtaining the above monosaccharides and trisaccharide is the hallmark of this strategy. However, very soon it became obvious for us that this patented method from Alchemia was impractical and to our dismay those attempts were met with failure which resulted in a huge loss of material and efforts. This reality struck us when the trisaccharide donor **5** was attempted to couple with the disaccharide acceptor **6**. The reaction was found to be very complicated, and only a small amount of desired pentasaccharide **7** was isolated. In order to improve the yield for the coupling reaction, multiple variables such as catalysts, solvents or reaction temperatures were studied. However, to our surprise, the yield of this coupling step did not improve significantly. Mass spectral analysis of the isolated compounds indicated that the by-products appeared to be generated from the partial cleavage of the PMB protective groups employed in the scheme. This may be due to the acidic nature of the coupling reagents or catalysts utilized in the reaction.

Having faced a setback while pursuing Alchemia method for the synthesis of protected pentasaccharide, we turned our attention to published procedure of original drug discoverer, namely Sanofi, for its adaptability in our large scale synthesis. A close examination of Sanofi method reveals that a modular strategy (3+2: tri and di saccharide combination) as summarized in Scheme 2 was followed.[5b,13] Accordingly, the key disaccharide **8** was converted into the trisaccharide **9** in three steps, and the latter one was readily transformed into the corresponding trisaccharide donor **10**, which was then coupled with the disaccharide **11** to provide the protected pentasaccharide **12** in desired manner. The salient feature of this method is the excellent stereo selectivity resulted in the products from the two key coupling reactions. However, the main disadvantage of this method is that the disaccharide **8** was obtained either from cellobiose or cerny epoxide, and both schemes employing them were found to be not amenable on large scale. The difficulty with cellobiose was its long linear route to get to the intermediate **8** with variable and inconsistent yields of important conversions, and further requiring column chromatography purifications at several stages. While cerny epoxide route was short, since it follows convergent approach for its synthesis, but riddled with unstable and volatile intermediates in the scheme. Further, the lower yield of the coupling reaction between the cerny epoxide with 2-benzylated glycosylation donor, led to failure of synthesis of intermediate **8** on large scale.[14]

At this juncture, a holistic review of both Sanofi and Alchemia approaches was considered and decided to take cognizance of simple and practical aspects from both methods. A careful comparison of these two strategies indicated that the Alchemia method attains the transformation of monosaccharides (**1**, **2** & **4**) to trisaccharide **5** in a practical manner while the Sanofi method relatively readily transforms the disaccharide **8** into the protected pentasaccharide **12**. Therefore, it was decided to obtain the trisaccharide donor based on Alchemia's strategy (using monosaccharide blocks instead of disaccharide blocks) and its conversion to the protected pentasaccharide based on Sanofi strategy. Protective groups play important role in any total synthesis and it's especially true in the case of oligosaccharide synthesis. PMB groups used in Alchemia strategy were found to be unstable under acidic glycosylation conditions and are susceptible for cleavage. This prompted us to switch to simple and acid stable acetyl groups instead of PMB groups on trisaccharide **5** as in the case of Sanofi strategy. Fur-



Scheme 1. Alchemia – Dr. Reddy's strategy for the synthesis of Fondaparinux.



Scheme 2. Sanofi strategy for the synthesis of Fondaparinux.