Carbohydrates

DOI: 10.1002/anie.201404154

Synthesis of the Heparin-Based Anticoagulant Drug Fondaparinux**

*Cheng-Hsiu Chang, Larry S. Lico, Teng-Yi Huang, Shu-Yi Lin, Chi-Liang Chang, Susan D. Arco, and Shang-Cheng Hung**

Abstract: *Fondaparinux, a synthetic pentasaccharide based on the heparin antithrombin-binding domain, is an approved clinical anticoagulant. Although it is a better and safer alternative to pharmaceutical heparins in many cases, its high cost, which results from the difficult and tedious synthesis, is a deterrent for its widespread use. The chemical synthesis of fondaparinux was achieved in an efficient and concise manner from commercially available* D*-glucosamine, diacetone* α*-*D*-glucose, and penta-O-acetyl-*D*-glucose. The method involves suitably functionalized building blocks that are readily accessible and employs shared intermediates and a series of one-pot reactions that considerably reduce the synthetic effort and improve the yield.*

Heparin is a polysulfated polysaccharide with alternating D-glucosamine (GlcN) and either D-glucuronic acid (GlcA) or L-iduronic acid (IdoA) units. Within its microheterogeneous chain is a particular pentasaccharide motif that binds and activates antithrombin, an inhibitor of the blood coagulation cascade.[1] This property forms the basis for the clinical potency of heparin in the treatment of thromboembolic disorders.[2] Nevertheless, active monitoring is needed during heparin therapy because serious complications such as heparin-induced thrombocytopenia, uncontrolled bleeding, and osteoporosis may occur.[3] Moreover, the safety and quality of the heparin supply chain was called into question when a recent contamination with oversulfated chondroitin sulfate turned fatal.[4] The quest for precisely targeted anticoagulant activity that avoids the problems associated with nature-sourced heparins led to the development of fondaparinux (**1**), a synthetic pentasaccharide derived from the aforementioned antithrombin-binding sequence.[5] Fondaparinux is safer and displays comparable to superior efficacy and pharmacological properties to unfractionated heparin and its low-molecular-weight variants.[6] However, nature-sourced heparins are preferred over fondaparinux because of their lower price.

Fondaparinux synthesis is made very demanding by the regio- and stereochemical aspects of the assembly and the strategic placement of multiple sulfonate groups. The established route involves approximately 55 steps, which drastically decrease the overall yield.[5,7] α-Glucosaminylation, in particular, mainly relies on the anomeric effect, which is insufficient in curbing production of the unwanted β isomer. The 1,2-*trans* glycosylation involving the uronic acid precursors, although feasible through neighboring group participation, is confounded by the different C2 functionalizations in the final product. Hence, as often is the case in general heparin syntheses,[8] protecting-group selection is critical. Recent efforts to improve fondaparinux synthesis have only showed limited success.[9]

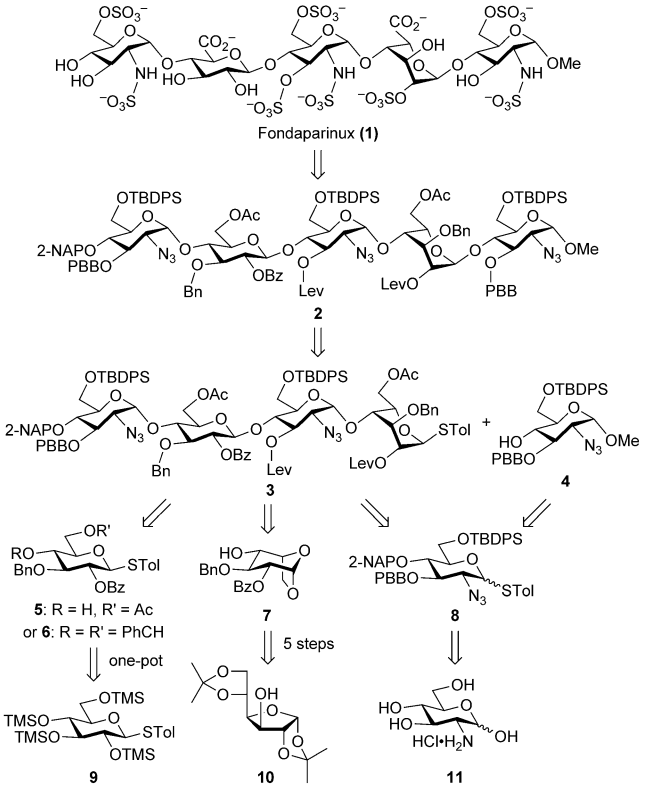
To provide an efficient and concise access to fondaparinux, we conceived a route through the building blocks **5** or **6**, **7**, and **8** (Scheme 1), all readily attainable from commercially available starting materials. The approach followed the formation of the tetrasaccharide donor **3** and its subsequent coupling with the reducing end acceptor **4** to generate the fully protected precursor (**2**) of the target compound **1**. Shared intermediates and one-pot reactions are used throughout the process to reduce the steps and minimize wasteful purification stages. The synthetic design exploits our established methods, especially regioselective one-pot multiprotection,[10] highly stereoselective α-glucosaminylation influenced by the orthogonal protecting groups in **8** (N_3, PBB, 2-NAP, and TBDPS),[11] and late-stage oxidation[11a,c,12] to avoid the low reactivity and base sensitivity associated with uronate esters. The glucose derivatives **5** and **6** can be prepared in one pot from the tetrasilylated compound **10**, which can be obtained from penta-*O*-acetyl-D-glucose in two steps.[10] The anhydro-L-idose **7** can be acquired from diacetone D-glucose (**10**) in five steps with 37% overall yield.[12a,13] Lev and Bz groups were selected to facilitate neighboring-group participation and allow the introduction of the crucial sulfate groups at O2 and O3 of the respective IdoA and GlcN units, while ensuring that the O2 position of GlcA would be free. Ac groups were used to protect the primary alcohols destined for oxidation.

The GlcN building blocks **4** and **8** were prepared from D-glucosamine·HCl (**11**; Scheme 2). Conversion of the amino group into an azide, followed by peracetylation yielded compound **12**. Next, a BF_3-assisted one-pot thioglycosylation and deacetylation supplied the triol **13**. A new extended one-pot procedure starting from **13** that includes per-O-silylation with HMDS,[14] 4,6-O-napthylmethylidene formation, and regioselective ring opening installed the 2-NAP group at

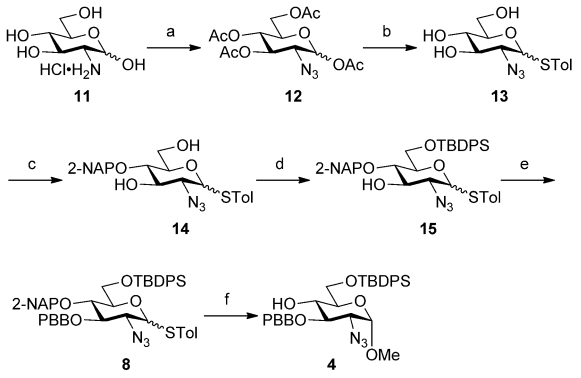
[*] Dr. C.-H. Chang, L. S. Lico, Dr. T.-Y. Huang, Dr. S.-Y. Lin, Dr. C.-L. Chang, Prof. Dr. S.-C. Hung
Genomics Research Center, Academia Sinica
No. 128 Academia Road, Section 2, Taipei 115 (Taiwan)
E-mail: schung@gate.sinica.edu.tw
Dr. C.-H. Chang
Department of Chemistry, National Tsing Hua University
No. 101, Section 2, Kuang-Fu Road, Hsinchu 300 (Taiwan)
L. S. Lico, Prof. Dr. S. D. Arco
Institute of Chemistry, University of the Philippines
Diliman, Quezon City 1101 (Philippines)

[**] This work was supported by the Ministry of Science and Technology (NSC 100-2113-M-001-019-MY3 and NSC 101-2628-M-001-006-MY3), National Health Research Institutes (NHRI-EX101-10146NI), and Academia Sinica.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201403154.



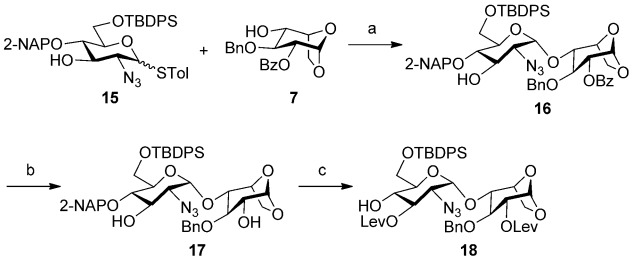
Scheme 1. A retrosynthetic analysis of fondaparinux. 2-NAP = 2-naphthylmethyl, Ac = acetyl, Bn = benzyl, Bz = benzoyl, Lev = levulinyl, PBB = *p*-bromobenzyl, TBDPS = *tert*-butyldiphenylsilyl, TMS = trimethylsilyl, Tol = 4-tolyl.



Scheme 2. Preparation of the glucosamine building blocks. Reagents and conditions: a) 1) TfN_3, $CuSO_4$, Et_3N, MeCN, MeOH, 0 °C to RT, 2 d; 2) Ac_2O, DMAP, 0 °C to RT, 16 h, 84%; b) *p*-thiocresol, $BF_3{\cdot}Et_2O$, RT, 8 h; MeOH, reflux, 16 h, 78% ($\alpha/\beta = 3.5/1$); c) HMDS, TMSOTf, CH_2Cl_2, 0 °C, 1 h; 2-NAPCHO, TMSOTf, 0 °C, 2 h; $BH_3{\cdot}THF$, TMSOTf, 0 °C, 8 h; TBAF, RT, 16 h, 88%; d) TBDPSCl, imidazole, DMAP, CH_2Cl_2, 91%; e) NaH, PBBBr, DMF, 95%; f) MeOH, NIS, TfOH, Et_2O, −60 to −40 °C, 3 h; DDQ, MeCN, RT, 2 d, 57%. DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone, DMAP = 4-*N*,*N*-dimethylaminopyridine, DMF = dimethylformamide, HMDS = hexamethyldisilazane, NIS = *N*-iodosuccinimide, TBAF = tetrabutylammonium fluoride, Tf = trifluoromethanesulfonyl.

O4.[10] The sequential protection of the diol **14** with TBDPS and PBB groups gave the donor **8**. Finally, the α-methyl glycoside **4** (57%, $J_{1,2} = 3.2$ Hz) was prepared by glycosylation with methanol and 2-NAP cleavage with DDQ in one pot. For this reaction, the β product was also produced in 15% yield.

Evidently, tetrasaccharide **3** could be produced in a [2 + 2]-manner in a reaction requiring a dilevulinylated disaccharide acceptor (**18**) and a benzoylated disaccharide donor (**21**). The acceptor can be prepared from monosaccharides **7** and **8** through a known diol intermediate (**17**), which we have previously accessed[11a] by using a palladium- and tin-catalyzed reaction. This time, we decided to avoid these metal catalysts by following a simpler approach (Scheme 3). When

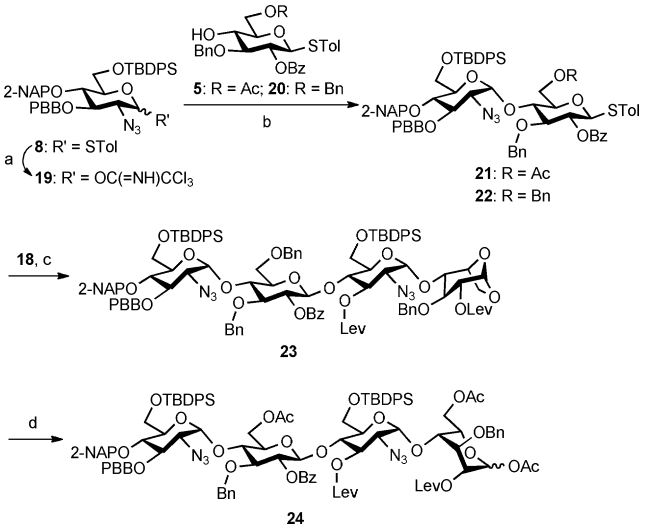


Scheme 3. Synthesis of the disaccharide acceptor **18**. Reagents and conditions: a) NIS, TfOH, CH_2Cl_2, −60 to −40 °C, 3 h, 70%; b) NaOMe, MeOH, 92%; c) LevOH, EDC, DMAP, CH_2Cl_2, 0 °C, 16 h; DDQ, H_2O, RT, 4 h, 70%. EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide.

using the thioglycoside **15** instead of **8**, the glycosylation of idose **7** (1.5 equiv) led to the α disaccharide **16** ($J_{1',2'} = 3.5$ Hz) in 70% yield, together with its easily separable β isomer (10%). Self-condensation of the donor was not observed. Zemplén conditions lead to the removal of the Bz group to afford the diol **17**, which underwent one-pot levulinylation and denaphthylmethylation to furnish compound **18**.

The construction of compound **3** was initially planned to include the glucosyl acceptor **5**, but this proved troublesome upon the condensation of **18** with donor **21**[11c] (Scheme 4). Glycosylation efficiency decreased, presumably as a result of the electron-withdrawing esters in both compounds. To circumvent this problem, we temporally used Bn in place of Ac in the glucosyl building block (**20**[10a]). Fortunately, the coupling of **22** and **18** effectively produced the tetrasaccharide **23**. Further exposure to Ac_2O and TMSOTf led to the concurrent primary Bn-to-Ac exchange[15] and anhydro-ring acetolysis. Although the triacetate **24** was ultimately obtained, the [2+2]-coupling required 3 equiv of the donor **22** to make up for the low reactivity of the acceptor **18**. The selective conversion of the 6-O-Bn group also required a large excess of TMSOTf (10 equiv) in a reaction that must be maintained at −40 °C to avoid side products. These issues prompted us to look for an alternative approach to construct **24** and eventually reach **3**.

This time the assembly of tetrasaccharide **3** was carried out through sequential monosaccharide addition from the reducing to the nonreducing end (Scheme 5). With the glucosyl donor **6** (1.3 equiv), the stereoselective extension of **18** worked successfully. The addition of aqueous TFA to the same reaction vessel resulted in the hydrolysis of the

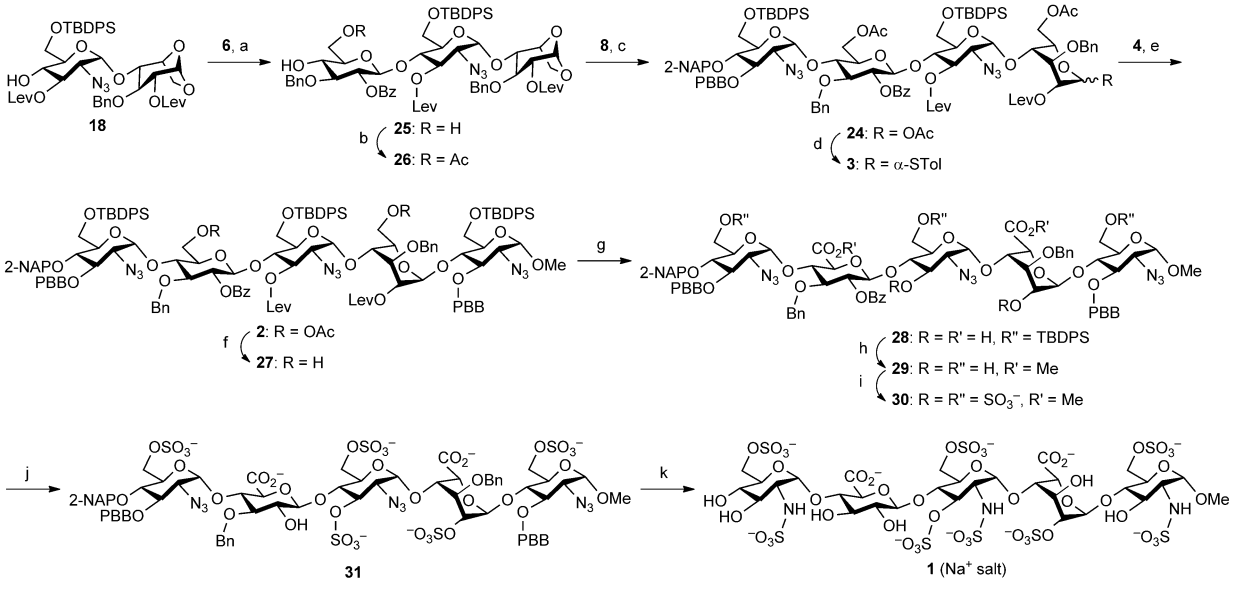


Scheme 4. Synthesis of the tetrasaccharide **24**. Reagents and conditions: a) 1) NIS, acetone, H_2O, 0 °C, 2 h, 81 %; 2) K_2CO_3, CCl_3CN, CH_2Cl_2, RT, 16 h, 95 %; b) AgOTf, CH_2Cl_2, −40 to −20 °C, 4 h, **21**: 70 %, **22**: 72 %; c) NIS, TfOH, CH_2Cl_2, −40 to −20 °C, 4 h, 71 % (when using **22**); d) TMSOTf, Ac_2O, −40 °C, 4 h, 61 %.

benzylidene ring to produce the diol **25** ($J_{1'',2''} = 8.5$ Hz). Regioselective acetylation at the primary alcohol delivered the trisaccharide acceptor **26**. The stereoselective α-glucosaminylation of **26** with thioglycoside **8** (2 equiv) and the acetolysis of the anhydro ring were accomplished in one pot to furnish compound **24**. This new route towards **24** is more straightforward and gave better overall yield compared to our initial procedure (13.9 % versus 10.8 % from the component building blocks). Treatment with TMSSTol and ZnI_2[11a] led directly to the desired thioglycoside **3**. NMR data showing $J_{1''',2'''} = 3.8$ Hz and the W-coupling between 1-H and 3-H[11c] (see the Supporting Information) confirmed the stereochemistry of the newly formed glycosidic bonds. The coupling of this donor with the acceptor **4** (1.2 equiv) furnished the pentasaccharide **2**, the structure of which was supported by NMR analysis as above.

Functional-group transformations were then carried out. The Ac groups in **2** were selectively removed by using $Mg(OMe)_2$ to get the diol **27**. TEMPO oxidation of the freed alcohol functionalities, followed by hydrazine-mediated cleavage of the Lev groups furnished the diol **28**. Further methyl ester formation and desilylation delivered the pentaol **29**. The exposure of **29** to $SO_3{\cdot}Et_3N$ led to the corresponding pentasulfate **30**, which was saponified to afford compound **31**. Hydrogenolysis cleaved all of the remaining protecting groups and simultaneously reduced the azide to the amine. Final N-sulfonation supplied the crude material, which was passed through size-exclusion and ion-exchange columns to isolate fondaparinux (**1**) as a sodium salt. NMR and mass spectrometry analyses and HPLC comparison with the commercial product confirmed the structure of **1** (see the Supporting Information).

In summary, we synthesized fondaparinux in 32 collective steps from commercially available starting materials and in 0.63 % overall yield over 22 linear steps from D-glucosamine·HCl. The approach benefitted from carefully selected orthogonal protecting groups and readily accessible monosaccharide building blocks. The use of common intermediates and one-pot reactions effectively reduced the synthetic and purification steps. This method represents an improved route



Scheme 5. Synthesis of fondaparinux. Reagents and conditions: a) NIS, TfOH, CH_2Cl_2, −60 to −40 °C, 3 h; TFA, H_2O, RT, 1 h, 58 %; b) Ac_2O, Et_3N, CH_2Cl_2, 0 °C to RT, 16 h, 82 %; c) NIS, TfOH, CH_2Cl_2, −78 °C to −20 °C, 4 h; TMSOTf, Ac_2O, −20 °C, 8 h, 65 %; d) TMSSTol, ZnI_2, CH_2Cl_2, RT, 1 h, 85 %; e) NIS, TfOH, CH_2Cl_2, −20 °C to 0 °C, 4 h, 61 %; f) $Mg(OMe)_2$, MeOH, CH_2Cl_2, RT, 16 h, 83 %; g) 1) TEMPO, BAIB, CH_2Cl_2, H_2O, RT, 24 h; 2) NH_2NH_2, Pyr, AcOH, 0 °C, 2 h; h) 1) CH_2N_2, Et_2O, CH_2Cl_2, RT, 16 h; 2) HF·Pyr, Pyr, RT, 3 d, 60 % from **27**; i) $SO_3{\cdot}Et_3N$, DMF, 60 °C, 73 %; j) LiOH, H_2O_2, THF, 37 °C, 3 d, 71 %; k) 1) $Pd(OH)_2$, H_2, phosphate buffer (pH 7), MeOH, RT, 2 d, 80 %; 2) SO_3·Pyr, NaOH, H_2O, RT, 2 d, 81 %. BAIB = bis(acetoxy)iodobenzene, TEMPO = 2,2,6,6-tetramethyl-1-piperidinyloxyl free radical, Pyr = pyridine.

that may prove a useful basis for producing fondaparinux for pharmaceutical use.

Received: April 10, 2014
Published online: ■■ ■■, ■■■■

Keywords: anticoagulant · carbohydrates · heparin · one-pot reactions · total synthesis

[1] a) I. Capila, R. J. Linhardt, *Angew. Chem.* **2002**, *114*, 426 – 450; *Angew. Chem. Int. Ed.* **2002**, *41*, 390 – 412; b) R. J. Linhardt, *J. Med. Chem.* **2003**, *46*, 2551 – 2564.

[2] a) C. A. A. van Boeckel, M. Petitou, *Angew. Chem.* **1993**, *105*, 1741 – 1761; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1671 – 1690; b) M. Petitou, B. Casu, U. Lindahl, *Biochimie* **2003**, *85*, 83 – 89.

[3] J. I. Weitz, L.-A. Linkins, *Expert Opin. Invest. Drugs* **2007**, *16*, 271 – 282.

[4] D. B. Blossom, A. J. Kallen, P. R. Patel, A. Elward, L. Robinson, G. Gao, R. Langer, K. M. Perkins, J. L. Jaeger, K. M. Kurkjian, M. Jones, S. F. Schillie, N. Shehab, D. Ketterer, G. Venkataraman, T. K. Kishimoto, Z. Shriver, A. W. McMahon, K. F. Austen, S. Kozlowski, A. Srinivasan, G. Turabelidze, C. V. Gould, M. J. Arduino, R. Sasisekharan, *N. Engl. J. Med.* **2008**, *359*, 2674 – 2684.

[5] M. Petitou, C. A. A. van Boeckel, *Angew. Chem.* **2004**, *116*, 3180 – 3196; *Angew. Chem. Int. Ed.* **2004**, *43*, 3118 – 3133.

[6] a) J. M. Walenga, W. P. Jeske, F. X. Frapaise, R. L. Bick, J. Fareed, M. M. Samama, *Expert Opin. Invest. Drugs* **2002**, *11*, 397 – 407; b) S. Middeldorp, *Thromb. Res.* **2008**, *122*, 753 – 762; c) M. Nagler, M. Haslauer, W. A. Wuillemin, *Thromb. Res.* **2012**, *129*, 407 – 417.

[7] a) C. A. A. van Boeckel, T. Beetz, J. N. Vos, A. J. M. de Jong, S. F. van Aelst, R. H. van den Bosch, J. M. R. Mertens, F. A. van der Vlugt, *J. Carbohydr. Chem.* **1985**, *4*, 293 – 321; b) M. Petitou, J.-C. Jacquinet, P. Sinay, J. Choay, J.-C. Lorraeau, M. Nassr, US Patent 4,818,816, **1989**.

[8] a) C. Noti, J. L. de Paz, L. Polito, P. H. Seeberger, *Chem. Eur. J.* **2006**, *12*, 8664 – 8686; b) F. Baleux, L. Loureiro-Morais, Y. Hersant, P. Clayette, F. Arenzana-Seisdedos, D. Bonnaffé, H. Lortat-Jacob, *Nat. Chem. Biol.* **2009**, *5*, 743 – 748; c) S. Arungundram, K. Al-Mafraji, J. Asong, F. E. Leach III, I. J. Amster, A. Venot, J. E. Turnbull, G.-J. Boons, *J. Am. Chem. Soc.* **2009**, *131*, 17394 – 17405; d) Z. Wang, Y. Xu, B. Yang, G. Tiruchinapally, B. Sun, R. Liu, S. Dulaney, J. Liu, X. Huang, *Chem. Eur. J.* **2010**, *16*, 8365 – 8375; e) S. U. Hansen, G. J. Miller, C. Cole, G. Rushton, E. Avizienyte, G. C. Jayson, J. M. Gardiner, *Nat. Commun.* **2013**, *4*, 2016; f) M. M. L. Zulueta, S.-Y. Lin, S.-C. Hung, *Trends Glycosci. Glycotechnol.* **2013**, *25*, 141 – 158; g) M. M. L. Zulueta, S.-Y. Lin, Y.-P. Hu, S.-C. Hung, *Curr. Opin. Chem. Biol.* **2013**, *17*, 1023 – 1029.

[9] a) Y. Xu, S. Masuko, M. Takieddin, H. Xu, R. Liu, J. Jing, S. A. Mousa, R. J. Linhardt, J. Liu, *Science* **2011**, *334*, 498 – 501; b) F. Lin, G. Lian, Y. Zhou, *Carbohydr. Res.* **2013**, *371*, 32 – 39; c) R. Kovi, A. Naik, B. Patel, M. Madala (Apicore), WO 2013/003001 A1, **2013**; d) P. P. Patel, C. Ma, K. K. Ohrr, S. Nadji (Reliable Pharmaceutical), WO 2013/115817 A1, **2013**; e) T. Li, H. Ye, X. Cao, J. Wang, Y. Liu, L. Zhou, Q. Liu, W. Wang, J. Shen, W. Zhao, P. Wang, *ChemMedChem* **2014**, *9*, 1071 – 1080.

[10] a) C.-C. Wang, J.-C. Lee, S.-Y. Luo, S. S. Kulkarni, Y.-W. Huang, C.-C. Lee, K.-L. Chang, S.-C. Hung, *Nature* **2007**, *446*, 896 – 899; b) C.-C. Wang, S. S. Kulkarni, J.-C. Lee, S.-Y. Luo, S.-C. Hung, *Nat. Protoc.* **2008**, *3*, 97 – 113; c) T.-Y. Huang, M. M. L. Zulueta, S.-C. Hung, *Org. Lett.* **2011**, *13*, 1506 – 1509; d) T.-Y. Huang, M. M. L. Zulueta, S.-C. Hung, *Org. Biomol. Chem.* **2014**, *12*, 376 – 382.

[11] a) Y.-P. Hu, S.-Y. Lin, C.-Y. Huang, M. M. L. Zulueta, J.-Y. Liu, W. Chang, S.-C. Hung, *Nat. Chem.* **2011**, *3*, 557 – 563; b) C.-C. Chung, M. M. L. Zulueta, L. T. Padiyar, S.-C. Hung, *Org. Lett.* **2011**, *13*, 5496 – 5499; c) M. M. L. Zulueta, S.-Y. Lin, Y.-T. Lin, C.-J. Huang, C.-C. Wang, C.-C. Ku, Z. Shi, C.-L. Chyan, D. Irene, L.-H. Lim, T.-I. Tsai, Y.-P. Hu, S. D. Arco, C.-H. Wong, S.-C. Hung, *J. Am. Chem. Soc.* **2012**, *134*, 8988 – 8995.

[12] a) S.-C. Hung, X.-A. Lu, J.-C. Lee, M. D.-T. Chang, S.-l. Fang, T.-c. Fan, M. M. L. Zulueta, Y.-Q. Zhong, *Org. Biomol. Chem.* **2012**, *10*, 760 – 772; b) Y.-P. Hu, Y.-Q. Zhong, Z.-G. Chen, C.-Y. Chen, Z. Shi, M. M. L. Zulueta, C.-C. Ku, P.-Y. Lee, C.-C. Wang, S.-C. Hung, *J. Am. Chem. Soc.* **2012**, *134*, 20722 – 20727.

[13] L.-D. Lu, C.-R. Shie, S. S. Kulkarni, G.-R. Pan, X.-A. Lu, S.-C. Hung, *Org. Lett.* **2006**, *8*, 5995 – 5998.

[14] A. A. Joseph, V. P. Verma, X.-Y. Liu, C.-H. Wu, V. M. Dhurandhare, C.-C. Wang, *Eur. J. Org. Chem.* **2012**, 744 – 753.

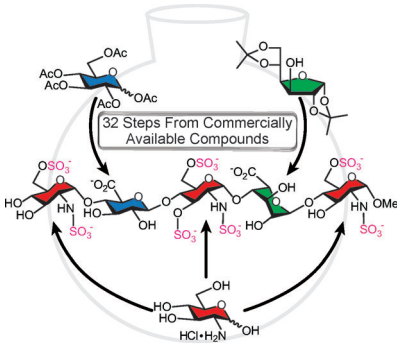
[15] P. Angibeaud, J.-P. Utille, *Synthesis* **1991**, 737 – 738.

Communications

Carbohydrates

C.-H. Chang, L. S. Lico, T.-Y. Huang, S.-Y. Lin, C.-L. Chang, S. D. Arco, S.-C. Hung* _____ ■■■■–■■■■

Synthesis of the Heparin-Based Anticoagulant Drug Fondaparinux



Working against the clot: The synthetic anticoagulant fondaparinux, a pentasaccharide based on the antithrombin-binding domain of heparin, was prepared in a concise and efficient manner in the shortest route reported to date. The application of one-pot strategies, the use of common intermediates, and the efficient preparation of monosaccharide building blocks from commercial sources are key features of this approach.