DOI: 10.1002/cmdc.201400019

Total Synthesis of Anticoagulant Pentasaccharide Fondaparinux

Tiehai Li, Hui Ye, Xuefeng Cao, Jiajia Wang, Yonghui Liu, Lifei Zhou, Qiang Liu, Wenjun Wang, Jie Shen,* Wei Zhao,* and Peng Wang*[a]

The anticoagulant pentasaccharide fondaparinux was synthesized using an improved and optimized synthetic strategy including a convergent [3+2] coupling approach, orthogonal protecting groups and various glycosyl donors. The new methods of glycosylation were also used for controlling the stereochemical configuration and improving the yield of the glycosylation. In addition, HPLC and NMR methods to monitor the process of total synthesis of fondaparinux were employed. This work provides a comprehensive elaboration for the synthesis and analysis of fondaparinux based on related literature, as well as abundant information for the synthesis of heparin-like oligosaccharides.

Introduction

Heparin and heparan sulfate, members of the glycosaminoglycans (GAGs) family, are structurally related linear polyanionic polysaccharides, consisting of α-1,4-linked glucosamine and uronic acid (either D-glucuronic or L-iduronic) disaccharide repeating units, which are heavily O- and N-sulfated.[1] They are present on the surface of most animal cells as well as in the basement membranes and extracellular matrices.[2] It is well established that heparin and heparan sulfate play significant roles in various biological processes such as blood coagulation, bacterial and viral infection, inflammation, growth factor regulation, cell adhesion, cell growth, tumor metastasis, lipid metabolism and diseases of the nervous system.[3]

Heparin and heparan sulfate are widely used as anticoagulant drugs for major cardiovascular and orthopedic surgeries such as hip fractures, knee surgery or hip replacement, preventing the occurrence of venous thrombosis.[4] Current heparin-based treatment in the clinic mainly relies on the heterogeneous unfractionated (UF, molecular weight average ~14000) or low-molecular-weight (LMW, molecular weight average ~6000) heparins that are either extracted from natural animal sources (porcine intestine or bovine lung) or degraded forms of such extractions.[5] The problems associated with UF and LMW heparin therapies are the challenges in product quality control in the process of its preparation owing to the non-uniformity of the enormous bulk of sources. The problem manifested with the case of worldwide distribution of contaminated animal-sourced heparin in 2007, which caused hundreds of patient deaths in the United States, and raised the concerns over the reliability and safety of animal-sourced heparins and LMW heparins.[6]

A unique pentasaccharide domain in some heparin chains was discovered that blocks factor Xa in the coagulation cascade. In light of the discovery, a homogeneous chemically synthesized this pentasaccharide was patented by Choay[7a] and Sanofi,[7b] and later led to the debut of antithrombotic drug fondaparinux (Arixtra®, Figure 1) on the market by GlaxoSmithKline in the USA in 2002.[7] Fondaparinux has been demonstrated to display superior antithrombotic activity and brings about antithrombin-mediated activity against factor Xa exclusively, not against thrombin.[8] As a pure synthetic pentasaccharide, it is much easier to control product quality in terms of reproduction reliability and purity. Furthermore, the synthetic homogenous pentasaccharide has multiple advantages over the aforementioned two forms in terms of pharmacokinetics (such as longer circulation half-life and low IC_{50} value) and product quality control.[9] Therefore, it is very significant and urgent for clinical applications to synthesize the homogeneous pentasaccharide with a highly efficient approach. In 2011, Dr. Reddy's Laboratories Ltd marketed fondaparinux in the USA, a bioequivalent generic version of Arixtra® using a different patented

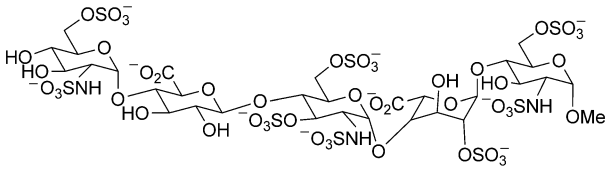


Figure 1. Structure of fondaparinux (Arixtra®).

[a] *Dr. T. Li,[+] H. Ye,[+] X. Cao, J. Wang, Y. Liu, L. Zhou, Q. Liu, W. Wang, Dr. J. Shen, Dr. W. Zhao, Prof. P. Wang*
State Key Laboratory of Medicinal Chemical Biology and College of Pharmacy, Nankai University
Synergetic Innovation Center of Chemical Science and Engineering (Tianjin)
94 Weijin Road, Tianjin 300071 (P.R. China)
E-mail: jieshen@nankai.edu.cn
wzhao@nankai.edu.cn
pwang@nankai.edu.cn

[+] *These authors contributed equally to this work.*

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cmdc.201400019.

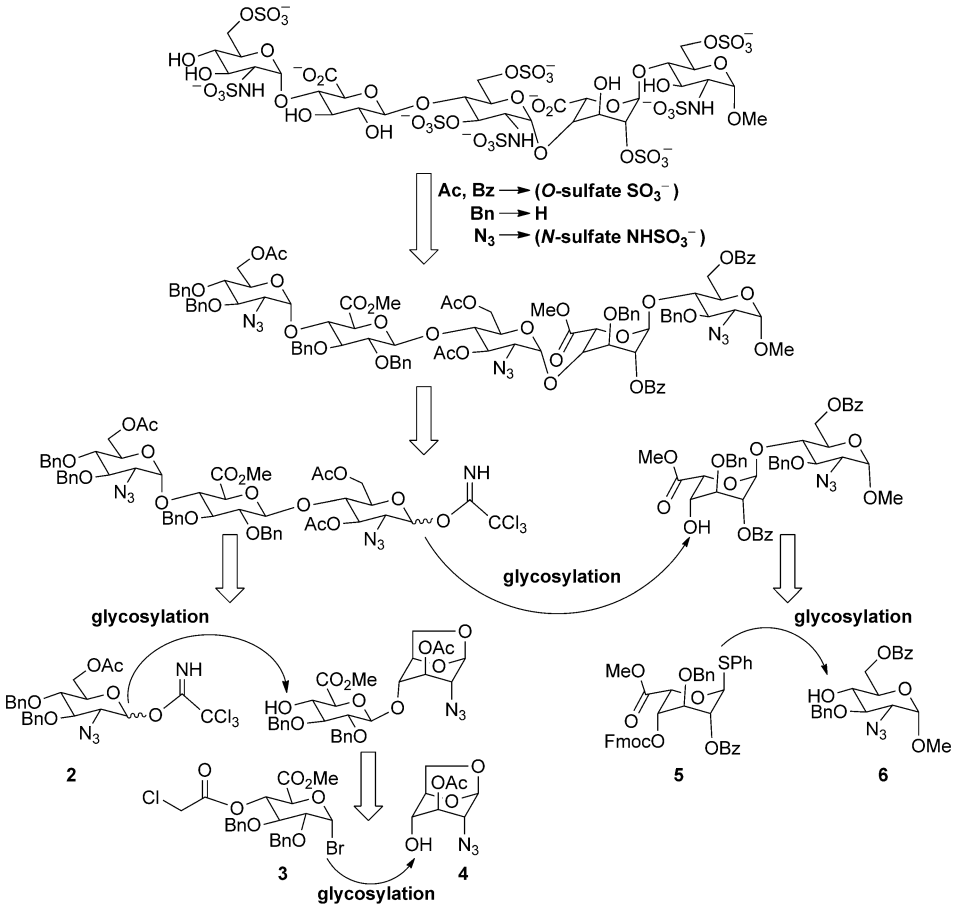
synthetic process developed by Alchemia Ltd.[7c] In addition, a variety of synthetic methods for the preparation of heparin-like oligosaccharides have been developed over the past two decades.[7f–h, 10] Various analogues of the antithrombin III binding pentasaccharide domain of heparin were synthesized by the van Boeckel and Petitou labs, and structure–activity relationship (SAR) studies revealed that essential sulfate and carboxylate substituents were located at opposite sides of the pentasaccharide molecule.[10b] Using a straightforward synthesis of the L-idopyranosyl building block and α-glycosylation by glucosamine-derived donors for the total synthesis of heparin oligosaccharides was accomplished by Hung and co-workers.[10j,k] A modular strategy toward the synthesis of heparin-like oligosaccharides in a sequential glycosylation protocol using sulfonium triflate activator systems was developed in the van der Marel lab.[10a] Boons and co-workers developed a modular approach for the parallel combinatorial synthesis of well-defined heparin oligosaccharide library for SAR studies by utilizing a relatively small number of selectively protected and flexible disaccharide building blocks.[10i] A highly efficient one-pot methodology for the synthesis of heparin-like oligosaccharides utilizing thioglycosides with well-defined reactivity as building blocks was developed by Wong's lab.[10h] Moreover, Huang's lab also developed the preactivation-based one-pot approach combinatorial synthesis of heparin-like oligosaccharides for the analysis of heparin–protein interactions.[10f] Based on these synthetic strategies of heparin-like oligosaccharides, we established an improved and optimized synthetic strategy that exploited a set of closely related building blocks and new methods of glycosylation to assemble fondaparinux pentasaccharide. We also applied HPLC and NMR methods to monitor the process of total synthesis of fondaparinux. Furthermore, the glycosylation of uronic acid building blocks with low reactivity imposed by the C-5 ester was investigated.

Results and Discussion

Synthetic strategy for fondaparinux

Fondaparinux consists of α-1,4-linked glucosamine with uronic acids (D-glucuronic acid and L-iduronic acid), as well as β-1,4-linked D-glucuronic and α-1,4-linked L-iduronic acid with D-glucosamine. The positions of O-sulfations are C-6 of glucosamine, C-2 of iduronic acid, and rarely C-3 of glucosamine. C-2 amino groups of glucosamine are all N-sulfated (Figure 1).

As illustrated in Scheme 1, we envisaged the preparation of fully protected pentasaccharide to be prepared by a convergent and stereocontrolled [3+2] approach. The protecting group strategy was established for O-sulfation and selective N-sulfation, as well as stereoselectivity of glycosylation. Acetyl and benzoyl protecting hydroxy groups intended to be O-sulfated, benzyl ethers were used as permanent protection groups for other free hydroxy groups. Azido groups were used as amino functionalities for subsequent N-sulfation, as well as performing non-neighboring group participation for the introduction of 1,2-*cis*-glycosidic linkages. The benzoyl protecting C-2 hydroxy of iduronic acid (building block **5**) allowed the stereoselective introduction of 1,2-*trans*-glycosidic linkages by



Scheme 1. Synthetic strategy of fondaparinux.

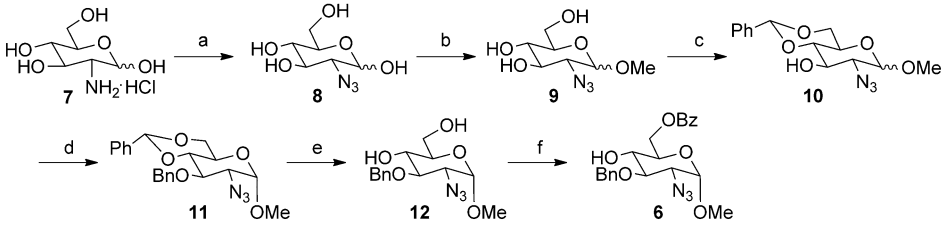
neighboring group participation. The 1-thio iduronic acid building block **5** displayed a highly disarmed class of glycosyl donor, it was activated by 1-benzene sulfinyl piperidine/triflic anhydride (BSP/Tf_2O) to couple with building block **6**.[10a,11] It is worth mentioning that levulinoyl (Lev) protecting the C-2 hydroxy group of glucuronic acid, which can be selectively removed and transferred to other protecting hydroxy groups, was envisaged to construct β-1,4-linked D-glucuronic with glucosamine.[10j] However, due to the inherent low reactivity imposed by the C-5 ester and the disarmed Lev protecting group, coupling this lowly active glucuronic acid donor with glucosamine acceptor led to unsuccessful glycosylation. Therefore, using the armed benzyl ether (Bn) protecting C-2 hydroxy group of glucuronic acid (building block **3**) to enhance the activity as the glycosyl donor, a Ag_2CO_3-mediated coupling of glycosyl bromide (building block **3**) with building block **4** under mild condition slowly led to the desired disaccharide.[10e] In addition, the 9-fluorenylmethyl carbonate (Fmoc) protecting the C-4 hydroxy group of building block **5** and the chloracetyl group protecting building block **3** can be easily removed with triethylamine and thiourea, respectively, to give the corresponding glycosyl acceptors for glycosylation without affecting other protecting groups.[10e,i]

Preparation of monomeric building blocks

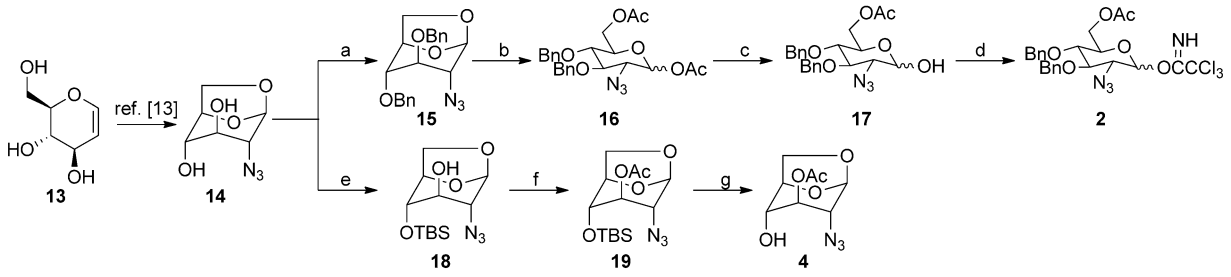
Based on the synthetic strategy for fondaparinux, five strategically chosen monosaccharide building blocks **2**, **3**, **4**, **5** and **6** were initially synthesized. The glucosazide building block **6** was synthesized starting from commercially available D-glucosamine hydrochloride **7** in six steps (Scheme 2). Treatment of D-glucosamine hydrochloride with imidazole-1-sulfonyl azide as a diazotransfer reagent gave 2-azido-2-deoxy-D-glucopyranose **8** in 85% yield using our previously reported methods.[12] Bringing a solution of **8** in methanol with 10% HCl to reflux afforded methyl glycoside **9** as a mixture of anomers, which were treated with $PhCH(OMe)_2$ and camphorsulfonic acid (CSA) in acetonitrile to give 4,6-*O*-benzylidence **10**.[10d] Benzylation of the remaining 3-hydroxy group with BnBr and NaH in THF gave the corresponding α/β anomers. The desired α anomer **11** was separated by crystallization from petroleum ether/ethyl acetate mixtures in 49% yield. Hydrolysis of the 4,6-*O*-benzylidene acetal gave diol **12** using 80% AcOH. Selective protection of the 6-hydroxy group with benzoate ester gave the desired building block **6** in 69% yield over two steps.

Glucosazide building blocks **2** and **4** were synthesized using the common intermediate 1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranose **14** (Scheme 3), which can be easily prepared from readily available D-glucal **13** based on previously reported methods.[13] Benzylation of intermediate **14** using benzyl bromide and NaH in *N*,*N*-dimethylformamide (DMF) gave the benzyl ether **15** in 92% yield. 1,6-anhydride **15** was subjected to acetolysis in the presence of catalytic amount of *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) to afford 1,6-diacetate **16** in 92% yield. Selective removal of the anomeric acetyl group using NH_3 in the presence of THF/MeOH (7:3) gave lactol **17** in 85% yield; the resulting lactol was converted into desired trichloroacetimidate **2** using trichloroacetonitrile and 1,8-diazazdicycloundec-7-ene (DBU) in 93% yield.[10d] In addition, selective silylation of the C-4 hydroxy of intermediate **14** gave silyl ether **18** using *tert*-butyldimethylsilyl chloride (TBDSCl) and imidazole in 90% yield.[13b] The remaining C-3 hydroxy group of **18** was protected with acetyl ester giving **19**, which was then subjected to desilylation with TBAF and AcOH to provide desired building block **4** in 94% yield.[13b,14]

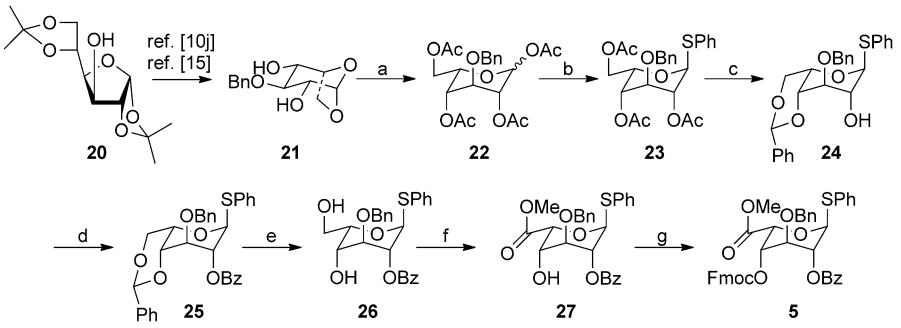
The L-iduronic acid building block **5** was efficiently synthesized as illustrated in Scheme 4. The commercially available diacetone α-D-glucose **20** was converted into 1,6-anhydro-β-D-ido-



Scheme 2. *Reagents and conditions*: a) Imidazole-1-sulfonyl azide, K_2CO_3, $CuSO_4$, MeOH, RT, overnight, 85%; b) 10% HCl/MeOH, reflux, 90 °C, 24 h; c) CSA, $PhCH(OMe)_2$, CH_3CN, 50 °C, 5 h, 65% (two steps); d) NaH, BnBr, THF, 0 °C to RT, overnight, 49%; e) 80% HOAc, 60 °C, 6 h; f) BzCl, pyridine,CH_2Cl_2, −20 °C, 3 h, 69% (two steps).



Scheme 3. *Reagents and conditions*: a) NaH, BnBr, DMF, 0 °C to RT, overnight, 92%; b) TBSOTf, Ac_2O, 0 °C, 20 min, 92%; c) NH_3, THF/MeOH (7:3), 0 °C, 85%; d) CCl_3CN, DBU, CH_2Cl_2, RT, 3 h, 93%; e) TBDSCl, imidazole, DMF, 0 °C to RT, overnight, 90%; f) Ac_2O, pyridine, CH_2Cl_2, 0 °C to RT, overnight, 89%; g) TBAF, AcOH, THF, RT, overnight, 94%.



Scheme 4. *Reagents and conditions*: a) TMSOTf, Ac_2O, 0 °C to RT, 2 h, 81%; b) $BF_3{\cdot}Et_2O$, PhSH, CH_2Cl_2, 0 °C to RT, 4 h, 82%; c) 1. $NaOCH_3$, CH_3OH, RT, 12 h; 2. $PhCH(OCH_3)_2$, CSA, DMF, RT, 6 h; d) BzCl, pyridine, CH_2Cl_2, 0 °C to RT, overnight, 77% (three steps); e) 80% AcOH, 80 °C, 2 h, 72%; f) 1. TEMPO, BAIB, CH_2Cl_2/H_2O, RT, 4 h; 2. CH_3I, $KHCO_3$, DMF, 0 °C to RT, 4 h, 50% (two steps); g) FmocCl, pyridine, DMAP, CH_2Cl_2, 0 °C to RT, 3 h, 78%.

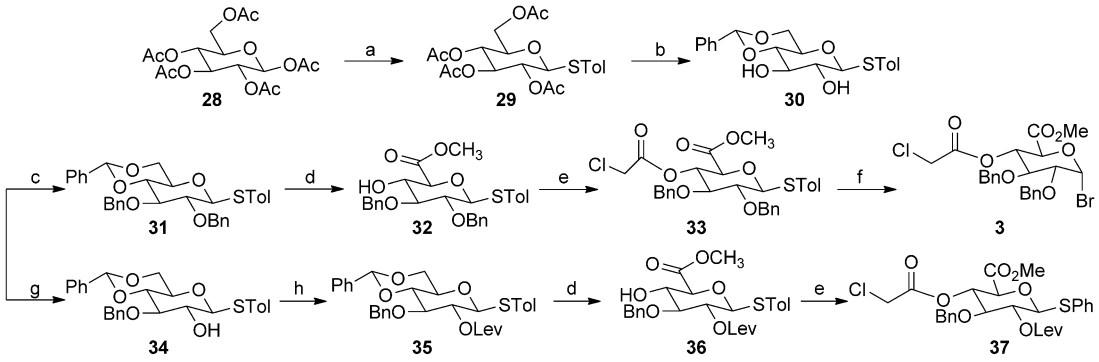
pyranose derivative **21** by the methods of Hung and Fügedi.[10j, 15] Treatment of **21** with Ac_2O and catalytic amounts of trimethylsilyl trifluoromethanesulfonate (TMSOTf) gave tetraacetate **22** in 81% yield, which was readily converted into thioglycoside **23** in the presence of thiophenol and $BF_3{\cdot}Et_2O$ in 82% yield.[10d] Using standard methods, the acetate esters of **23** were removed with catalytic amounts of $NaOCH_3$. Then, 4,6-*O*-benzylidene acetal **24** was formed using $PhCH(OCH_3)_2$ and CSA in DMF. The remaining hydroxy group of **24** was benzoylated to give **25** in 77% yield over three steps. Removal of the 4,6-*O*-benzylidene group of **25** using 80% AcOH gave diol **26** in 72% yield, which was subjected to selective oxidation of the resulting primary hydroxy group with 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) in the presence of iodobenzene diacetate (BAIB) as co-oxidant.[16] The resulting carboxyl groups were then esterified in the presence of $KHCO_3$ and MeI to give desired **27** in 50% yield over two steps.[10h] Blocking the remaining 4-hydroxy group with Fmoc in the presence of FmocCl, 4-dimethylaminopyridine (DMAP) and pyridine gave L-iduronic acid building block **5** in 78% yield.[17]

It was initially envisaged to synthesize glucouronic acid building block **37** with levulinoyl (Lev) protecting the C-2 hydroxy group for the construct β-1,4-linked D-glucuronic acid with **4** due to neighboring group participation (Table 1). Diphenylsulfoxide/triflic anhydride (Ph_2SO/Tf_2O) as a powerful promoter system can activate disarmed 1-thio uronic acid building blocks **37**, facilitating its condensation with glucosazide building block **4**.[10a, 18] Unfortunately, the strategy of glycosylation could not give the desired product, but substantial decomposition of the glycosyl donor and acceptor were observed by TLC. It was considered that this glucuronic acid donor was too inactive due to the inherent low reactivity imposed by the C-5 ester and the disarmed Lev protecting group at the C-2 position to lead to unsuccessful glycosylation.[19] In order to improve the reactivity of the glucouronic acid building block, disarmed levulinoyl (Lev) protecting C-2 hydroxy group was changed to armed benzyl ether giving **33**. Coupling **33** with **4** gave the corresponding disaccharide **40** as a mixture of anomers ($\alpha/\beta = 4.6{:}1$) using the same glycosylation conditions; however, the ratio of desired β anomer was too low. It was observed that Ag_2CO_3-mediated coupling of glycosyl bromide **3** with **4** under mild condition can slowly form disaccharide **40** as a mixture of anomers ($\alpha/\beta = 1{:}7$).[7c, 10e] The glucouronic acid building blocks **3, 33** and **37** were synthesized as described in Scheme 5. The treatment of commer-

Table 1. Glycosylation of glucouronic acid derivates with acceptor **4**.

Donors	Activators	Yield [%] (α/β)[c]
37	$Ph_2SO/Tf_2O/TTBP$[a]	ND[d]
33	$Ph_2SO/Tf_2O/TTBP$[a]	58 (4.6:1)
3	Ag_2CO_3[b]	72 (1:7)

[a] *Conditions*: 4 Å MS, CH_2Cl_2, −60 °C, then −40 °C to RT. [b] *Conditions*: 4 Å MS, CH_2Cl_2, RT. [c] Anomeric ratio determined from anomeric mixture by ^1H NMR. [d] ND: no desired product.

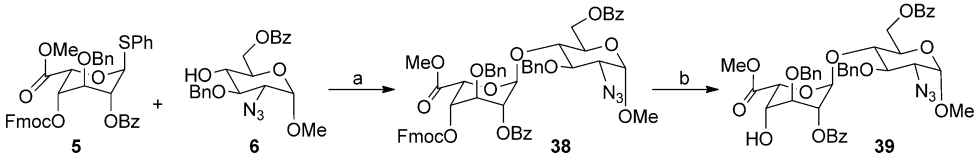


Scheme 5. *Reagents and conditions*: a) $BF_3{\cdot}Et_2O$, TolSH, CH_2Cl_2, 0 °C to RT, 4 h, 86%; b) 1. $NaOCH_3$, CH_3OH, RT, 2 h; 2. $PhCH(OCH_3)_2$, CSA, DMF, RT, 6 h, 83% (two steps); c) NaH, BnBr, DMF, 0 °C to RT, overnight, 85%; d) 1. $CH_2Cl_2/TFA/H_2O$, 10:1:0.1, RT, 20 min; 2. TEMPO, BAIB, CH_2Cl_2/H_2O, RT, 5 h; 3. CH_3I, $KHCO_3$, DMF, 0 °C to RT, 4 h, 42% for **32**, 40% for **36** (three steps); e) $(ClCH_2CO)_2O$, pyridine, CH_2Cl_2, 0 °C to RT, 6 h, 86% for **33**, 82% for **37**; f) IBr, CH_2Cl_2, RT, 4 h, 72%; g) 1. $(Bu_2Sn)O$, toluene, reflux, 6 h; 2. BnBr, CsF, DMF, RT, overnight, 63% (two steps); h) LevOH, DCC, DMAP, CH_2Cl_2, 0 °C to RT, overnight, 83%.

cially available β-peracetylated glucose **28** with *para*-toluenethiol (TolSH) in the presence of $BF_3{\cdot}Et_2O$ afforded **29** in 86% yield. Using standard methods, the acetate esters of **29** were removed with catalytic amounts of $NaOCH_3$, and then 4,6-*O*-benzylidene acetal **30** was generated using $PhCH(OCH_3)_2$ and CSA in DMF. Benzylation of **30** using benzyl bromide and NaH in DMF gave the benzyl ether **31** in 85% yield. The benzylidene acetal of **31** was removed by TFA in CH_2Cl_2 and water, followed by selective oxidation of the resulting primary hydroxy group with TEMPO in the presence of BAIB as co-oxidant.[10j] The resulting carboxyl groups were then esterified in the presence of $KHCO_3$ and MeI to afford desired **32** in 42% yield over three steps.[10h] The free C-4 hydroxy group of this glucouronic acid building block was protected with a chloracetyl group in the presence of chloroacetic acid anhydride and pyridine. The chloracetyl group can be readily removed with thiourea as an acceptor for glycosylation.[7c] Thioglycoside **33** was converted into the corresponding glycosyl bromide **3** with IBr in 72% yield.[20] In addition, regioselective benzylation of the C-3 hydroxy of **30** by treatment with dibutyltin oxide, followed by reaction with benzyl bromide in the presence of CsF in DMF afforded **34** in 63% yield. The C-2 hydroxy of **34** was protected with Lev to give **35** by reaction with levulinic acid (LevOH) in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC) and DMAP.[10d] Subsequently, glucouronic acid building block **37** can be readily obtained using the same methods of synthesis of **33** from **31**.

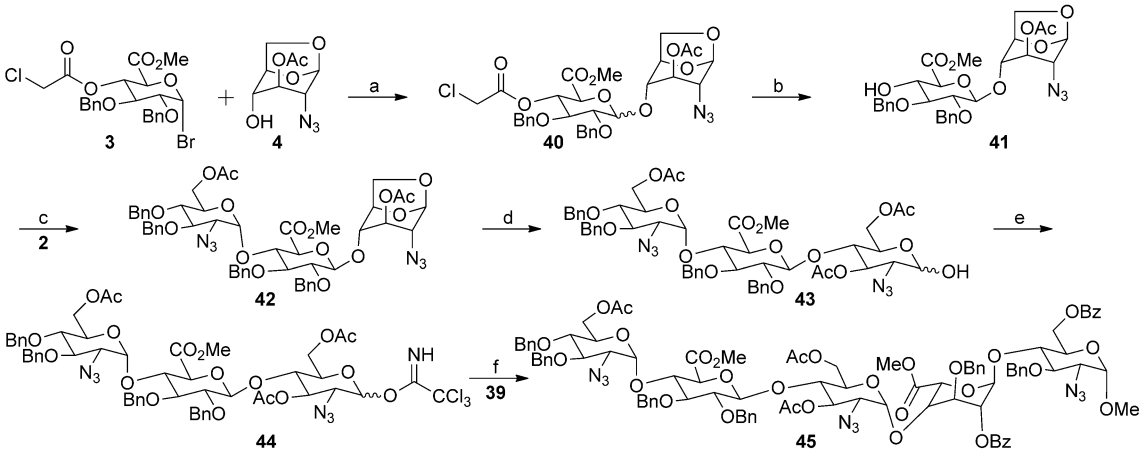
Glycosylation and assembly of fully protected pentasaccharide

Thioglycosides of the idouronate were reported by van der Marel and co-workers to be effective glycosyl donors under the activation of BSP,[10a] a powerful promoter system developed by the Crich laboratory.[11] Activation of the anomeric thiophenyl group of idouronate **5** with the BSP/Tf_2O reagent system proceeded efficiently for condensation of the glucosazide building block **6** to afford the desired disaccharide **38** in 56% yield. Subsequently, the Fmoc group can be readily removed with triethylamine to give **39** as an acceptor for the next glycosylation[10j] (Scheme 6).



Scheme 6. *Reagents and conditions*: a) BSP, Tf_2O, 4 Å MS, CH_2Cl_2, −60 °C, then −40 °C to RT, 4 h, 56%; b) Et_3N, CH_2Cl_2, RT, 2 h, 88%.

Condensation of glucouronic acid building block **3** with glucosazide building block **4** slowly formed disaccharide **40** as a mixture of anomers (α/β = 1:7) in the presence of silver carbonate[7c,10e] (Scheme 7). The resulting mixture of anomers was subjected to removal of the chloracetyl group using thiourea to give the corresponding disaccharide anomers, which can be separated by silica column chromatography to give desired β-1,4-linked disaccharide **41** as an acceptor for next coupling in 55% yield over two steps. A TBSOTf-mediated coupling of the trichloroacetimidate donor **2** with acceptor **41** afforded desired trisaccharide **42** in 78% yield. Opening of the anhydro bridge of **42** and subsequent deacetylation of the anomeric center gave lactol **43** in 64% yield over two steps.[10k] The resulting lactol was converted into desired trichloroacetimidate **44** using trichloroacetonitrile and K_2CO_3 in 81% yield. A convergent [3+2] coupling of this trichloroacetimidate donor **44** and acceptor **39** in the presence of TMSOTf afforded the fully protected pentasaccharide **45** in 68% yield. The convergent [3+2] coupling approach improved the utilization of difficultly

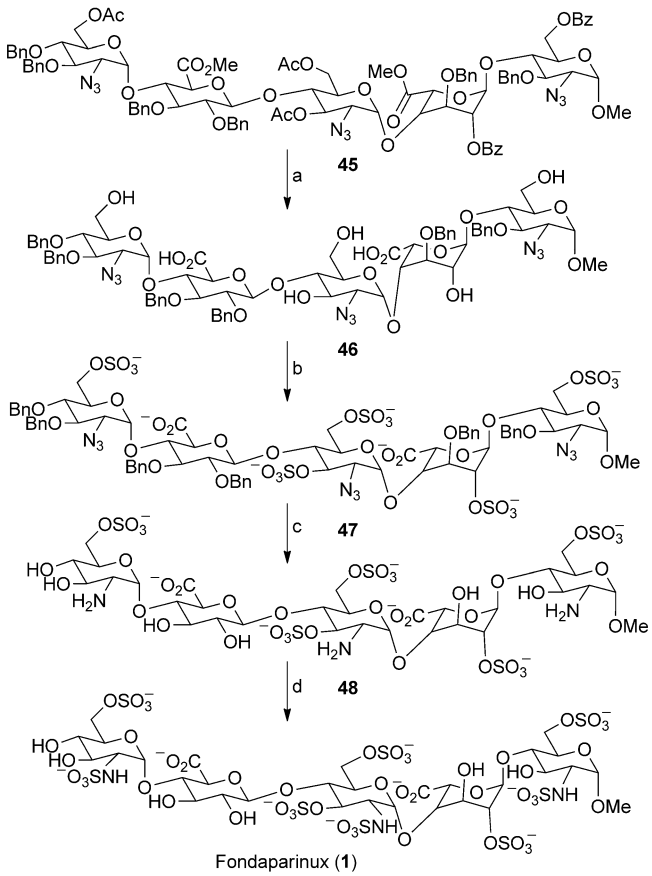


Scheme 7. *Reagents and conditions*: a) Ag_2CO_3, 4 Å MS, CH_2Cl_2, RT, 4 days; b) thiourea, $CHCl_3$, 60 °C, 24 h, 55% (two steps); c) TBSOTf, 4 Å MS, toluene, −20 °C to RT, 2 h, 78%; d) 1. TBSOTf, Ac_2O, 0 °C, 20 min; 2. NH_3, THF/MeOH (7:3), 0 °C, 5–10 min, 64% (two steps); e) CCl_3CN, K_2CO_3, CH_2Cl_2, 4 h, 81%; f) TMSOTf, 4 Å MS, CH_2Cl_2, −20 °C to RT, 2 h, 68%.

prepared L-iduronic acid building block due to the use of disaccharide **39** as an acceptor and efficient synthesis of **39** by the BSP/Tf_2O-mediated promoter system.

Preparation of fondaparinux

With the fully protected pentasaccharide **45** in hand, the acetyl, benzoyl and methyl esters of **45** were saponified by a two-step procedure using LiOH and H_2O_2 in THF, and then NaOH in methanol to afford partially deprotected **46** (Scheme 8).[10i] O-sulfation of the resulting hydroxy groups of **46** was achieved with sulfur trioxide trimethylamine complex in DMF. The crude of O-sulfated product was purified to give **47** by Dowex 50WX4 Na^+-form and Sephadex LH-20 columns, respectively. Removal of the benzyl ethers and reducing azido of **47** gave amine **48** by catalytical hydrogenation in the presence of Pd/C, followed by selective N-sulfation of **48** employing pyridinium sulfur trioxide by maintaining pH 9.5 with 2 M NaOH. Finally, the crude N-sulfated product was purified to give fondaparinux (**1**) by Dowex 50WX4 Na^+-form and Sephadex G-25 columns, respectively.[10e,h]



Scheme 8. *Reagents and conditions*: a) 1. LiOH, H_2O_2, THF, −5 °C to RT, 16 h; 2. 4 M NaOH, MeOH, RT, 24 h, then 35 °C, 12 h; b) SO_3·NMe_3, DMF, 50 °C, 24 h, Dowex 50WX4 Na^+-form and Sephadex LH-20, 66% (two steps); b) H_2, Pd/C, *t*BuOH/H_2O, 4 days; (d) SO_3·Pry, 2 M NaOH, 4 h, Dowex 50WX4 Na^+-form and Sephadex G-25, 62% (two steps).

Conclusions

In summary, the total synthesis of anticoagulant pentasaccharide fondaparinux has been improved and optimized. We established an effective protecting group strategy for O-sulfation and selective N-sulfation, as well as, stereoselective glycosylation. The BSP/Tf_2O-mediated promoter system contributed to coupling disarmed thioglycosides of idouronate with appropriate glucosazide building blocks. The convergent [3+2] coupling approach also improved the utilization of difficultly prepared L-iduronic acid building block. In addition, the HPLC and NMR methods were used to monitor the process of total synthesis of fondaparinux. Overall, we report a comprehensive elaboration for the synthesis and analysis of fondaparinux. We believe that the continued improvement of the multistep production of fondaparinux will be a critical task for medicinal and process chemistry worldwide.

Experimental Section

General procedures: All reagents were purchased from commercially sources and used without further purification. All solvents were available commercially dried or freshly dried and distilled prior to use. Reactions were monitored by thin-layer chromatography (TLC) using silica gel GF_{254} plates with detection using short-wave UV light ($\lambda = 254$ nm) and staining with 10% phosphomolybdic acid in EtOH or a *p*-anisaldehyde solution (EtOH/*p*-anisaldehyde/AcOH/H_2SO_4, 135:5:4:1.5), followed by heating on a hot plate. Column chromatography was conducted using silica gel (200–300 mesh) with EtOAc and petroleum ether or EtOAc (or CH_2Cl_2) and MeOH as eluent. ^1H NMR and ^{13}C NMR were recorded with a Bruker AV 400 spectrometer at 400 MHz and 100 MHz, respectively, using $CDCl_3$, CD_3OD and D_2O as solvents. Chemical shifts (δ) are reported in ppm from $CDCl_3$ ($\delta = 7.26$ ppm for ^1H NMR, $\delta = 77.00$ ppm for ^{13}C NMR), CD_3OD (3.31 ppm for ^1H NMR, $\delta = 49.00$ ppm for ^{13}C NMR). Coupling constants are reported in Hertz. High-resolution mass spectra (HRMS) were obtained on a Varian QFT-ESI mass spectrometer. Compound purity was determined by HPLC analysis on a Waters e2695 system with a Waters Atlantis T3 column (4.6 mm × 150 mm, 5 μm) and Dinoex Carbopac PA column (14 mm × 250 mm, 5 μm) at 1.0 mL min^{-1} using a Waters 2998 photodiode array detector at 220 nm.

Methyl *O*-(methyl 2-*O*-benzoyl-3-*O*-benzyl-4-*O*-(4-(((9*H*-fluoren-9-yl)methoxy)carbonyloxy)-α-L-idopyranosyluronate)-(1→4)-2-azido-6-*O*-benzoyl-3-benzyl-2-deoxy-α-D-glucopyranoside (38): A solution of **5** (430 mg, 0.6 mmol), 1-benzene sulfinyl piperidine (BSP; 176 mg, 0.9 mmol) and 4 Å molecular sieves (500 mg) in anhyd CH_2Cl_2 (10 mL) was stirred at RT under Ar atmosphere for 30 min. The reaction mixture was cooled −60 °C, and Tf_2O (139 μL, 0.84 mmol) was added. The temperature was slowly raised to −40 °C and stirred at this temperature for 15 min. Then a solution of **6** (372 mg, 0.9 mmol) in CH_2Cl_2 (2.0 mL) was added, and the reaction mixture was allowed to slowly warm to RT. TLC analysis showed complete conversion of starting material to a major product (petroleum ether/EtOAc 8:1, $R_f = 0.22$). The reaction was quenched by the addition of Et_3N (234 μL, 1.68 mmol) and filtered. The filtrate was concentrated in vacuo and purified by silica gel column chromatography (petroleum ether/EtOAc, 10:1) to afford compound **38** as a white solid (343 mg, 56%): ^1H NMR (400 MHz, $CDCl_3$): $\delta = 3.47$ (s, 6H), 3.51 (dd, $J = 3.6$ Hz, $J = 10.0$ Hz, 1H), 3.92 (t, $J = 9.6$ Hz, 1H), 4.02–4.12 (m, 3H), 4.18 (t, $J = 7.6$ Hz, 1H), 4.31 (dd,

J=8.4, 10.0 Hz, 1H), 4.38 (dd, J=8.0, 10.0 Hz, 1H), 4.52 (dd, J=4.0, 12.0 Hz, 1H), 4.74–4.87 (m, 6H), 5.08 (d, J=2.4 Hz, 1H), 5.11 (d, J=3.6 Hz, 1H), 5.30 (s, 1H), 5.50 (s, 1H), 7.24–7.36 (m, 14H), 7.41–7.47 (m, 6H), 7.55–7.61 (m, 2H), 7.79 (d, J=7.2 Hz, 2H), 8.08 ppm (d, J=7.6 Hz, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=46.58, 52.19, 55.43, 62.61, 63.78, 67.18, 67.78, 69.34, 70.32, 71.50, 73.09, 73.15, 74.97, 75.37, 78.70, 97.75, 98.43, 120.08, 125.07, 125.20, 127.22, 127.52, 127.71, 127.96, 128.11, 128.17, 128.26, 128.38, 128.50, 129.04, 129.76, 129.84, 130.03, 133.06, 133.48, 137.06, 137.63, 141.20, 141.24, 142.92, 143.22, 154.30, 165.32, 166.06, 168.42 ppm; HRMS (ESI): m/z $[M+NH_4]^+$ calcd for $C_{57}H_{57}N_4O_{15}$: 1307.3815, found: 1307.3823; Anal. RP-HPLC: t_R=21.13 min ($MeOH/H_2O$, 78:22; purity: 95.52%).

Methyl *O*-(methyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-2-azido-6-*O*-benzoyl-3-benzyl-2-deoxy-α-D-glucopyranoside (39): Et_3N (2.1 mL, 15 mmol) was added to a solution of compound **38** in CH_2Cl_2 (8 mL). The mixture was stirred at RT for 2 h. TLC analysis showed complete conversion of starting material to a major product (petroleum ether/EtOAc, 4:1, R_f=0.21). The mixture was concentrated in vacuo, and the crude product was purified by silica gel column chromatography (petroleum ether/EtOAc, 4:1) to yield **39** as a white solid (211 mg, 88%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=3.45–3.47 (m, 7H), 3.86–3.91 (m, 2H), 4.01–4.04 (m, 3H), 4.48 (dd, J=3.2, 12.0 Hz, 1H), 4.68–4.4.72 (m, 2H), 4.77–4.84 (m, 4H), 4.97 (s, 1H), 5.21 (s, 1H), 5.40 (s, 1H), 7.26–7.43 (m, 14H), 7.51–7.57 (m, 2H), 7.91 (d, J=7.6 Hz, 2H), 8.03 ppm (d, J=7.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=52.08, 55.46, 62.74, 63.86, 67.97, 68.14, 68.84, 69.30, 72.70, 74.88, 75.03, 75.41, 78.77, 98.10, 98.49, 127.46, 127.66, 128.14, 128.21, 128.44, 128.56, 128.58, 129.81, 133.13, 133.71, 137.24, 137.75, 165.06, 166.07, 169.52 ppm; HRMS (ESI): m/z $[M+NH_4]^+$ calcd for $C_{42}H_{47}N_4O_{13}$: 815.3134, found: 815.3137; Anal. RP-HPLC: t_R=34.52 min ($MeOH/H_2O$, 85:15; purity: 92.06%).

***O*-(Methyl 2,3-di-*O*-benzyl-4-*O*-chloroacetyl-β-D-glucopyranosyluronate)-(1→4)-2-*O*-acetyl-1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranose (40)**: A solution of **39** (620 mg, 1.18 mmol), compound **4** (809 g, 3.54 mmol) and 4 Å molecular sieves (800 mg) in anhyd CH_2Cl_2 (15 mL) was stirred at RT under Ar atmosphere for 30 min, then Ag_2CO_3 (650 mg, 2.36 mmol) was added. The mixture was stirred at RT under Ar atmosphere for 4 days. TLC analysis showed complete conversion of starting material to a major product (CH_2Cl_2/CH_3OH, 50:1, R_f=0.61). The reaction mixture was filtered through Celite. The filtrate was concentrated in vacuo and purified by silica gel column chromatography (petroleum ether/EtOAc, 4:1 and then CH_2Cl_2/CH_3OH 100:1→50:1) to yield a mixed product with α/β anomers as a white solid (523 mg, 66%): β anomer: ^{1}H NMR (400 MHz, $CDCl_3$): δ=2.13 (s, 3H), 3.26 (s, 1H), 3.67–3.71 (m, 4H), 3.75 (s, 3H), 3.80–3.87 (m, 2H), 3.98 (d, J=14.0 Hz, 1H), 4.03 (d, J=7.6 Hz, 1H), 4.60 (d, J=5.2 Hz), 4.65 (d, J=11.6 Hz), 4.70 (d, J=6.8 Hz, 1H), 4.81 (d, J=10.8 Hz), 4.87 (d, J=11.6 Hz, 1H), 5.03 (d, J=11.2 Hz), 5.21 (dd, J=10.0, 9.2 Hz, 1H), 5.30 (s, 1H), 5.53 (s, 1H), 7.26–7.41 ppm (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=20.01, 40.40, 52.87, 58.95, 64.96, 70.70, 72.06, 72.40, 73.81, 75.25, 75.30, 76.12, 80.81, 81.21, 100.29, 102.10, 128.00, 128.24, 128.47, 138.04, 138.08, 166.00, 167.28, 169.25 ppm; HRMS (ESI): m/z $[M+NH_4]^+$ calcd for $C_{31}H_{38}ClN_4O_{12}$: 693.2169, found: 693.2169; Anal. RP-HPLC: t_R=23.82 min ($MeOH/H_2O$, 70:30; purity: 97.84%).

***O*-(Methyl 2,3-di-*O*-benzyl-β-D-glucopyranosyluronate)-(1→4)-2-*O*-acetyl-1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranose (41)**: Thiourea (189 mg, 2.48 mmol) was added to the solution of compound **40** (419 mg, 0.62 mmol) in $MeOH/CHCl_3$ (1:1, 10 mL). The mixture was stirred at 60 °C for 24 h. TLC analysis showed complete conversion of starting material to a major product (CH_2Cl_2/CH_3OH, 50:1, R_f=0.49). The reaction mixture was concentrated in vacuo, and after the addition of CH_2Cl_2 (20 mL), washed with saturated $NaHCO_3$ solution (12 mL). The organic phase was dried over Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by silica gel column chromatography (petroleum ether/EtOAc/CH_2Cl_2, 3:1:1) to yield **41** as a white solid (310 mg, 83%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=2.03 (s, 3H), 3.13 (s, 1H), 3.43–3.52 (m, 2H), 3.58 (s, 1H), 3.69 (dd, J=1.6, 7.6 Hz, 1H), 3.72 (s, 3H), 3.76 (d, J=9.6 Hz, 1H), 3.84 (dd, J=8.4, 9.2 Hz, 1H), 3.95 (d, J=7.6 Hz, 1H), 4.50 (d, J=5.6 Hz, 1H), 4.57 (d, J=7.2 Hz, 1H), 4.70 (d, J=10.8 Hz, 1H), 4.75–4.80 (m, 2H), 4.91 (d, J=10.8 Hz, 1H), 5.22 (s, 1H), 5.41 (s, 1H), 7.18–7.30 ppm (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=20.97, 52.69, 58.83, 64.87, 70.69, 71.54, 74.01, 74.09, 75.12, 75.34, 76.21, 80.86, 82.98, 100.20, 103.66, 127.72, 127.75, 127.88, 128.12, 128.37, 128.43, 138.31, 138.41, 169.10, 169.51 ppm; HRMS (ESI): m/z $[M+NH_4]^+$ calcd for $C_{29}H_{37}N_4O_{11}$: 617.2453, found: 617.2447; Anal. RP-HPLC: t_R=9.27 min (CH_3CN/H_2O, 45:55; purity: 99.09%).

***O*-(6-*O*-Acetyl-2-azide-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(Methyl 2,3-di-*O*-benzyl-β-D-glucopyranosyluronate)-(1→4)-2-*O*-acetyl-1,6-anhydro-2-azido-2-deoxy-β-D-glucopyranose (42)**: A solution of **2** (881 mg, 1.54 mmol), compound **41** (0.72 g, 1.28 mmol) and 4 Å molecular sieves (500 mg) in anhyd toluene (15 mL) was stirred at RT under Ar atmosphere for 30 min. The reaction mixture was cooled to −20 °C, TBSOTf (0.136 mL, 0.593 mmol) was added, and the reaction mixture was allowed to slowly warm to RT within 2 h. TLC analysis showed complete conversion of starting material to a major product (CH_2Cl_2/acetone, 50:1, R_f=0.44). The reaction was quenched by the addition of Et_3N and filtered. The filtrate was concentrated in vacuo and purified by silica gel column chromatography (petroleum ether/EtOAc/CH_2Cl_2 5:1:1→CH_2Cl_2/acetone=300:1) to yield **42** as a white solid (1.01 g, 78%): ^{1}H NMR (400 MHz, $CDCl_3$): δ=2.03 (s, 3H), 2.09 (s, 3H), 3.21 (s, 1H), 3.26 (dd, J=4.0, 10.0 Hz, 1H), 3.51 (t, J=9.6 Hz, 1H), 3.59–3.66 (m, 3H), 3.75–3.79 (m, 5H), 3.85–3.91 (m, 1H), 3.96 (d, J=9.6 Hz, 1H), 4.01 (d, J=7.2 Hz, 1H), 4.15 (t, J=9.6 Hz, 1H), 4.23–4.24 (m, 2H), 4.53–4.56 (m, 2H, H-5), 4.67 (d, J=7.6 Hz, 1H), 4.73 (d, J=10.4 Hz, 1H), 4.79–4.86 (m, 4H), 5.01–5.04 (m, 2H), 5.21 (s, 1H), 5.47 (s, 1H), 5.53 (d, J=4.0 Hz, 1H), 7.24–7.35 ppm (m, 20H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=20.82, 20.98, 52.75, 58.80, 62.22, 63.24, 64.90, 69.59, 70.49, 73.71, 74.46, 74.88, 74.93, 75.07, 75.09, 75.45, 75.85, 77.43, 79.99, 81.40, 83.72, 97.72, 100.20, 103.13, 127.38, 127.55, 127.77, 127.90, 127.97, 128.03, 128.13, 128.35, 128.43, 128.49, 137.51, 137.59, 138.05, 138.14, 168.15, 169.11, 170.64 ppm; HRMS (ESI): m/z $[M+NH_4]^+$ calcd for $C_{51}H_{60}N_7O_{16}$: 1026.4091, found: 1026.4090; Anal. RP-HPLC: t_R=27.55 min ($MeOH/H_2O$, 82:18; purity: 84.57%).

***O*-(6-*O*-Acetyl-2-azide-3,4-di-*O*-benzyl-2-deoxy-2-α-D-glucopyranosyl)-(1→4)-*O*-(Methyl 2,3-di-*O*-benzyl-β-D-glucopyranosyluronate)-(1→4)-3,6-di-*O*-acetyl-2-azido-2-deoxy-α/β-D-glucopyranose (43)**: TBSOTf (21.4 μL, 0.1 mmol) was added to a solution of compound **42** (1.01 g, 1.0 mmol) in Ac_2O (10 mL) under Ar atmosphere in an ice bath for 20 min. TLC analysis showed complete conversion of starting material to a major product (petroleum ether/EtOAc/CH_2Cl_2, 3:1:1, R_f=0.56). The reaction was quenched by the addition of Et_3N (0.1 mL). The mixture was concentrated in vacuo with an oil pump. The crude product was purified by silica gel column chromatography (petroleum ether/EtOAc/CH_2Cl_2, 6:1:1) to yield a white solid (966 mg, 87%): HRMS (ESI): m/z $[M+NH_4]^+$ calcd for $C_{55}H_{66}N_7O_{19}$: 1128.4408, found: 1128.4409.

A solution of the above white solid (888 mg, 0.8 mmol) in THF/MeOH (*v/v* 7:3, 30 mL) was stirred under NH_3 in an ice bath for about 5–10 min. TLC analysis showed complete conversion of starting material to a major product (petroleum ether/EtOAc/CH_2Cl_2, 3:1:1, $R_f = 0.18$). The mixture was concentrated in vacuo, and the crude product was purified by silica gel column chromatography (petroleum ether/EtOAc/CH_2Cl_2, 6:1:1→3:1:1) to yield **43** as a white solid (735 mg, 86%): HRMS (ESI): *m/z* $[M+NH_4]^+$ calcd for $C_{53}H_{64}N_7O_{18}$: 1086.4302, found: 1086.4312; Anal. RP-HPLC: $t_R =$ 35.02 and 37.09 min (CH_3CN/H_2O, 70:30; purity: 97.15%).

***O*-(6-*O*-Acetyl-2-azide-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(Methyl 2,3-di-*O*-benzyl-β-D-glucopyranosyluronate)-(1→4)-3,6-di-*O*-acetyl-2-azido-2-deoxy-α/β-D-glucopyranosyl trichloroacetimidate (44)**: Cl_3CCN (1.0 mL) was added to a solution of compound **43** (532 mg, 0.5 mmol) and K_2CO_3 (138 mg, 1.0 mmol) in anhyd CH_2Cl_2 (10 mL) under Ar atmosphere at RT. The mixture was stirred at RT for 2 h. TLC analysis showed complete conversion of starting material to a major product (petroleum ether/EtOAc/CH_2Cl_2, 3:1:1, $R_f = 0.69$). The reaction mixture was concentrated in vacuo, and the crude product was purified by silica gel column chromatography (petroleum ether/EtOAc/CH_2Cl_2, 6:1:1) to yield **44** with α/β anomers as a white solid (490 mg, 81%): β anomer: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.03$ (s, 3H), 2.05 (s, 3H), 3.27 (dd, $J = 3.6$ Hz, $J = 10.0$ Hz, 1H), 3.42 (t, $J = 8.8$ Hz, 1H), 3.50–3.51 (m, 2H), 3.58–3.60 (m, 1H), 3.69–3.75 (m, 2H), 3.77 (s, 3H), 3.80–3.87 (m, 3H), 4.05 (t, $J = 9.2$ Hz, 1H), 4.11 (dd, $J = 7.2$, 14.4 Hz, 1H), 4.18–4.21 (m, 2H), 4.26 (d, $J = 11.6$ Hz, 1H), 4.33 (d, $J = 8.0$ Hz, 1H), 4.41 (d, $J = 12.0$ Hz, 1H), 4.55 (d, $J = 11.6$ Hz, 1H), 4.68 (d, $J =$ 11.6 Hz, 1H), 4.76 (d, $J = 11.2$ Hz, 1H), 4.80–4.86 (m, 3H), 4.96 (d, $J =$ 10.8 Hz, 1H), 5.09 (t, $J = 9.6$ Hz, 1H), 5.50 (d, $J = 3.2$ Hz, 1H), 5.66 (d, $J = 8.4$ Hz, 1H),7.22–7.35 (m, 20H), 8.77 ppm (br s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 20.68$, 20.85, 52.72, 60.41, 61.40, 62.22, 63.24, 63.30, 69.71, 71.94, 73.73, 74.39, 74.98, 75.03, 75.27, 75.53, 80.13, 81.92, 83.82, 90.26, 96.35, 97.58, 103.13, 127.30, 127.72, 127.82, 127.90, 128.05, 128.08, 128.39, 128.48, 128.54, 137.56, 138.03, 160.62, 168.31, 169.97, 170.13, 170.67 ppm; HRMS (ESI): *m/z* $[M+NH_4]^+$ calcd for $C_{55}H_{64}Cl_3N_8O_{18}$: 1229.3399, found: 1229.3393.

Methyl *O*-(6-*O*-Acetyl-2-azide-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(Methyl 2,3-di-*O*-benzyl-β-D-glucopyranosyluronate)-(1→4)-*O*-(3,6-di-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(Methyl 2-*O*-benzoyl-3-*O*-benzyl-α-L-idopyranosyluronate)-(1→4)-2-azido-6-*O*-benzoyl-3-benzyl-2-deo-xy-α-D-glucopyranoside (45): A solution of donor **44** (310 mg, 0.25 mmol), disaccharide acceptor **39** (168 mg, 0.21 mmol) and 4 Å molecular sieves (0.5 g) was stirred in anhyd CH_2Cl_2 (8 mL) under Ar atmosphere for 30 min. Then reaction mixture was cooled to −20 °C, and after the addition of TMSOTf (3.8 μL, 0.021 mmol), was allowed to slowly warm to RT within 2 h. TLC analysis showed complete conversion of starting material to a major product (petroleum ether/EtOAc/CH_2Cl_2, 3:1:1, $R_f = 0.55$). The reaction was quenched by the addition of Et_3N (0.1 mL) and filtered. The filtrate was concentrated in vacuo and purified by silica gel column chromatography (petroleum ether/EtOAc/CH_2Cl_2 7:1:1→CH_2Cl_2/acetone 80:1) to yield **45** as a white solid (262 mg, 68%): ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 2.02$–2.23 (3 s, 9H), 3.21 (dd, $J = 3.2$, 10.8 Hz, 1H), 3.26 (dd, $J = 3.6$, 10.0 Hz, 1H,), 3.35 (s, 3H), 3.38–3.43 (m, 2H), 3.53 (s, 3H), 3.66–3.72 (m, 2H), 3.74 (s, 3H), 3.82–3.92 (m, 4H), 3.97–4.06 (m, 3H), 4.10–4.21 (m, 4H), 4.26 (d, $J = 12.0$ Hz, 1H), 4.34 (d, $J =$ 8.0 Hz, 1H), 4.42–4.47 (m, 2H), 4.53–4.65 (m, 4H), 4.72–4.86 (m, 9H), 4.95–4.97 (m, 2H), 5.10 (d, $J = 3.2$ Hz, 1H), 5.25–5.34 (m, 2H), 5.49 (d, $J = 3.6$ Hz), 5.72 (d, $J = 6.0$ Hz, 1H), 7.20–7.44 (m, 34H), 7.49–7.53 (m, 2H), 8.00 (d, $J = 7.6$ Hz, 2H), 8.10 ppm (d, $J = 7.2$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 20.65$, 20.83, 52.25, 52.66, 55.41, 60.71, 61.35, 62.22, 62.37, 63.28, 63.37, 69.15, 69.30, 69.72, 71.52, 72.17, 73.05, 74.42, 74.47, 74.99, 75.08, 75.21, 75.44, 75.52, 75.73, 76.57, 76.87, 77.26, 78.50, 80.13, 81.72, 83.73, 97.57, 97.63, 98.27, 98.43, 103.23, 127.23, 127.56, 127.72, 127.88, 127.96, 128.07, 128.27, 128.36, 128.54, 128.57, 128.67, 129.07, 129.85, 129.92, 133.00, 133.46, 137.32, 137.36, 137.56, 137.97, 138.05, 165.35, 166.07, 168.35, 168.35, 169.72, 170.00, 170.10, 170.63 ppm; HRMS (ESI): *m/z* $[M+NH_4]^+$ calcd for $C_{95}H_{105}N_{10}O_{30}$: 1866.7032, found: 1866.7004; Anal. RP-HPLC: $t_R = 24.17$ min (CH_3CN/H_2O, 90:10; purity: 98.95%).

Methyl *O*-(2-azide-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-β-D-glucopyranosyluronic acid)-(1→4)-*O*-(2-azido-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(3-*O*-benzyl-α-L-idopyranosyluronic acid)-(1→4)-2-azido-3-benzyl-2-deoxy-α-D-glucopyranoside (46): A solution of H_2O_2 (30%, 5.1 mL) was added to a cooled (−5 °C) solution of compound **45** (185 mg, 0.1 mmol) in THF (10 mL), and $LiOH_{(aq)}$ (1.25 M, 2.4 mL) was added dropwise. After 16 h at RT, MeOH (6 mL) and NaOH (4 M, 3 mL) were added, and the reaction mixture stirred for 24 h at RT. In the case that the reaction had not gone to completion, stirring was continued at 35 °C for an additional 12 h. The reaction mixture was acidified (6 M HCl) and diluted with H_2O. The compound was extracted with CH_2Cl_2, washed with 10% $Na_2SO_{3(aq)}$ and H_2O. The aqueous layer was extracted with CH_2Cl_2 (2×). The organic layers were combined, dried (Na_2SO_4) and filtered. The filtrate was concentrated in vacuo to give **46** as white powder (135 mg, 91%): ^{1}H NMR (400 MHz, CD_3OD): $\delta = 3.28$ (dd, $J = 3.2$, 10.4 Hz, 1H), 3.36 (dd, $J = 3.6$, 10.0 Hz, 1H), 3.41 (s, 3H), 3.50 (t, $J = 8.0$ Hz, 1H), 3.64–3.72 (m, 6H), 3.74–3.82 (m, 6H), 3.88–3.97 (m, 7H), 3.99–4.08 (m, 4H), 4.55–4.61 (m, 2H), 4.67–4.70 (m, 2H), 4.72 (d, $J = 8.0$ Hz, 1H), 4.77–4.79 (m, 3H), 4.84–4.68 (m, 2H), 4.86–4.91 (m, 3H), 5.00 (d, $J = 10.8$ Hz, 1H), 5.13 (d, $J = 3.2$ Hz, 1H), 5.30 (d, $J = 4.4$ Hz, 1H), 5.54 (d, $J = 3.6$ Hz, 1H), 7.19–7.36 (m, 28H), 7.44 ppm (d, $J = 7.2$ Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 55.57$, 60.61, 61.12, 61.61, 64.53, 64.61, 64.73, 71.31, 71.94, 72.43, 72.76, 73.18, 73.52, 74.88, 75.40, 75.55, 75.73, 75.93, 76.18, 76.25, 76.29, 77.41, 78.49, 79.12, 79.61, 79.72, 80.94, 83.17, 85.29, 98.95, 99.18, 100.05, 102.32, 103.99, 128.48, 128.52, 128.62, 128.83, 128.93, 129.03, 129.18, 129.36, 129.40, 129.48, 139.38, 139.53, 139.63, 139.69, 139.73, 172.57 ppm; HRMS (ESI): *m/z* $[M+NH_4]^+$ calcd for $C_{73}H_{87}N_{10}O_{25}$: 1503.5838, found: 1503.5833; Anal. RP-HPLC: $t_R = 31.40$ min (Eluent A: CH_3CN, Eluent B: 10 mM NH_4HCO_3 buffer; gradient: 0–10 min 10% A, 10–35 min 10–80% A, 35–38 min 80% A; purity: 91.99%).

Methyl *O*-(2-azide-3,4-di-*O*-benzyl-2-deoxy-6-*O*-sulfo-α-D-glucopyranosyl)-(1→4)-*O*-(2,3-di-*O*-benzyl-β-D-glucopyranosyluronic acid)-(1→4)-*O*-(2-azido-2-de-oxy-3,6-di-*O*-sulfo-α-D-glucopyranosyl)-(1→4)-*O*-(3-*O*-benzyl-2-*O*-sulfo-α-L-ido-pyranosyluronic acid)-(1→4)-2-azido-3-*O*-benzyl-2-deoxy-6-*O*-sulfo-α-D-glucopyranoside heptasodium salt (47): A solution of **46** (45 mg, 0.03 mmol) and trimethylamine sulphur trioxide complex (209 mg, 0.15 mmol) in *N*,*N*-dimethylformamide (DMF; 3 mL) was stirred for 24 h at 50 °C. The reaction mixture was cooled to RT and then concentrated in vacuo. The resulting residue was passed through a column of Dowex® 50WX4 Na^+-form using CH_3OH as eluent. The fractions containing the product were concentrated in vacuo, and the residue was passed through a column of Sephadex LH-20 using MeOH/$CHCl_3$ (1:1) as eluent to afford **47** as a white powder (24 mg, 72%): ^{1}H NMR (400 MHz, CD_3OD): $\delta = 3.34$–3.86 (m, 2H), 3.45 (s, 3H), 3.50 (dd, $J = 3.6$, 10.4 Hz, 1H), 3.58–3.64 (m, 2H), 3.72 (t, $J = 9.6$ Hz, 1H), 3.83–3.95 (m, 6H), 3.98–4.06 (m, 3H), 4.11–4.19

(m, 3H), 4.26–4.33 (m, 4H), 4.39 (d, J=11.2 Hz, 1H), 4.50 (d, J=10.4 Hz), 4.59–4.61 (m, 2H), 4.63 (d, J=4.0 Hz, 1H), 4.66–4.78 (m, 4H), 4.81–4.84 (m, 4H), 4.93–5.00 (m, 5H), 5.20 (d, J=3.6 Hz, 1H), 5.42 (s, 1H), 5.52 (d, J=4.0 Hz, 1H), 7.13–7.37 (m, 28H), 7.47 ppm (d, J=7.2 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD): δ=55.76, 64.40, 64.76, 65.03, 66.15, 67.00, 67.48, 67.87, 71.01, 71.15, 71.36, 71.41, 71.56, 71.85, 73.57, 74.08, 74.75, 75.24, 75.92, 76.06, 76.17, 76.21, 76.25, 77.72, 79.32, 79.91, 81.21, 83.21, 85.29, 95.92, 98.91, 99.21, 99.99, 103.40, 128.19, 128.27, 128.44, 128.46, 128.65, 128.74, 128.94, 129.17, 129.23, 129.30, 129.33, 129.34, 129.43, 129.55, 129.95, 138.95, 139.05, 139.54, 140.01, 140.12, 172.58 ppm; HRMS (ESI): m/z calcd for $C_{73}H_{82}N_9O_{40}S_5$ $[M-H]^-$: 1884.3268, found: 1884.3083, calcd for $C_{73}H_{85}N_{10}O_{40}S_5$ $[M-2H+NH_4]^-$: 1901.3534, found: 1901.3312, calcd for $C_{73}H_{81}N_9O_{40}S_5$ $[(M-2H)/2]^-$: 941.6592, found: 941.6575; Anal. RP-HPLC: t_R=20.33 min (Eluent A: CH_3CN, Eluent B: 10 mM Na_2HPO_4 buffer; gradient: 0–30 min 10–70% A, 35–40 min 70% A; purity: 88.50%).

Methyl *O*-(2-deoxy-2-sulfamido-6-*O*-sulfo-α-D-glucopyranosyl)-(1→4)-*O*-(β-D-glucopyranosyluronic acid)-(1→4)-*O*-(2-deoxy-2-sulfamido-3,6-di-*O*-sulfo-α-D-glucopyranosyl)-(1→4)-*O*-(2-*O*-sulfo-α-L-idopyranosyluronic acid)-(1→4)-2-de-oxy-2-sulfamido-6-*O*-sulfo-α-D-glucopyranoside decasodium salt (1): A solution of compound **47** in MeOH/*t*BuOH/H_2O (2:1:1, 4 mL) was hydrogenated in the presence of 10% Pd/C (40 mg) and 8 atm H_2 at RT for 4 days. A ^{1}H NMR spectrum showed no signals of aryl groups. The mixture was filtered through Celite, and the filtrate was concentrated in vacuo to give **48** as a gray-green solid (16 mg, 100%): HRMS (ESI): m/z calcd for $C_{31}H_{51}N_3O_{40}S_5$ $[(M-2H)/2]^-$: 632.5326, found: 632.5329, calcd for $C_{31}H_{50}N_3NaO_{40}S_5$ $[(M-3H+Na)/2]^-$: 643.5236, found: 643.5239, calcd for $C_{31}H_{49}N_3Na_2O_{40}S_5$ $[(M-4H+2Na)/2]^-$: 654.5151, found: 654.5152, calcd for $C_{31}H_{48}N_3Na_3O_{40}S_5$ $[(M-5H+3Na)/2]^-$: 665.5061, found: 654.5058.

The above amino alcohol **48** was dissolved in H_2O (2 mL), and the solution was adjusted to pH 9.5 through addition of 2 M $NaOH_{(aq)}$. Sulfur trioxide pyridine complex (32 mg, 0.2 mmol) was added in five equal portions at half-hour intervals at RT, and the solution was maintained at pH 9.5 via calibration with 2 M $NaOH_{(aq)}$. After stirring for 4 h, the solution was adjusted to pH 7–8 using 0.1 M HCl, and passed through a column of Dowex 50WX4 Na^+-form using H_2O as eluent. The fractions containing the product were lyophilized, and the residue was passed through a column of Sephadex G-25 using 0.2 M $NaCl_{(aq)}$ as an eluent. The fractions containing product were lyophilized, and the crude product was subjected to desalting through a Sephadex G-25 column eluted with H_2O to give compound **1** as a white solid (12 mg, 62%, over two steps): ^{1}H NMR (400 MHz, D_2O): δ=3.22–3.28 (m, 2H), 3.40–3.45 (m, 5H), 3.53–3.66 (m, 3H), 3.73–3.86 (m, 5H), 3.93–3.97 (m, 2H), 4.10–4.17 (m, 4H), 4.23–4.40 (m, 6H), 4.46 (d, J=10.8 Hz, 1H), 4.61 (d, J=7.6 Hz, 1H), 4.79 (m, 1H), 5.00 (d, J=3.2 Hz, 1H), 5.17 (d, J=2.8 Hz, 1H), 5.48 (d, J=2.8 Hz, 1H), 5.60 ppm (d, J=3.6 Hz, 1H); ^{13}C NMR (100 MHz, D_2O): δ=58.15, 59.36, 60.42, 60.64, 68.69, 69.04, 69.42, 71.24, 71.72, 72.31, 72.53, 72.63, 72.78, 73.83, 75.45, 75.80, 78.73, 78.77, 78.89, 79.08, 79.41, 79.65, 99.02, 100.39, 100.99, 102.24, 103.88 ppm; HRMS (ESI): m/z calcd for $C_{31}H_{50}N_3NaO_{49}S_8$ $[(M+Na-3H)/2]^-$ 763.4594, found: 763.4598; calcd for $C_{31}H_{49}N_3Na_2O_{49}S_8$ $[(M+2Na-4H)/2]^-$ 774.4504, found: 774.4500; calcd for $C_{31}H_{48}N_3Na_3O_{49}S_8$ $[(M+3Na-5H)/2]^-$ 785.4413, found: 785.4414; calcd for $C_{31}H_{47}N_3Na_4O_{49}S_8$ $[(M+4Na-6H)/2]^-$ 796.4323, found: 796.4318; calcd for $C_{31}H_{46}N_3Na_5O_{49}S_8$ $[(M+5Na-7H)/2]^-$ 807.4233, found: 807.4249; calcd for $C_{31}H_{45}N_3Na_6O_{49}S_8$ $[(M+6Na-8H)/2]^-$ 818.4142, found: 818.4142; calcd for $C_{31}H_{44}N_3Na_7O_{49}S_8$ $[(M+7Na-9H)/2]^-$ 829.4070, found: 829.4053; calcd for $C_{31}H_{43}N_3Na_8O_{49}S_8$ $[(M+8Na-10H)/2]^-$ 840.3962, found: 840.4034; Anal. RP-HPLC: t_R=16.18 min (Eluent A: 0.001% DMSO in H_2O, Eluent B: 11.7% NaCl in H_2O; gradient: 0–5 min 50% B, 5–25 min 50–90% B, 25–30 min 90% B; purity: 95.50%).

Acknowledgements

This work was supported by the National Basic Research Program of China (973 Program, No. 2010CB529106, 2012CB910300) and the Natural Science Foundation of China (No. 21332006, 21372130). The authors would like to thank Jeffrey Meisner (Emory University, Atlanta, GA, USA) for proof reading.

Keywords: fondaparinux · glycosylation · heparin · pentasaccharides

[1] a) N. S. Gandhi, R. L. Mancera, *Chem. Biol. Drug Des.* **2008**, *72*, 455–482; b) U. R. Desai, H. M. Wang, R. J. Linhardt, *Biochemistry* **1993**, *32*, 8140–8145; c) I. Capila, R. J. Linhardt, *Angew. Chem.* **2002**, *114*, 426–450; *Angew. Chem. Int. Ed.* **2002**, *41*, 390–412.

[2] J. A. Loeb, G. D. Fischbach, *J. Cell Biol.* **1995**, *130*, 127–135.

[3] a) M. Bernfield, M. Gotte, P. W. Park, O. Reizes, M. L. Fitzgerald, J. Lincecum, M. Zako, *Annu. Rev. Biochem.* **1999**, *68*, 729–777; b) S. Tumova, A. Woods, J. R. Couchman, *Int. J. Biochem. Cell Biol.* **2000**, *32*, 269–288; c) J. D. Esko, S. B. Selleck, *Annu. Rev. Biochem.* **2002**, *71*, 435–471; d) U. Lindahl, M. Kusche-Gullberg, L. Kjellen, *J. Biol. Chem.* **1998**, *273*, 24979–24982.

[4] a) N. Mackman, *Nature* **2008**, *451*, 914–918; b) D. S. Weitz, J. I. Weitz, *J. Thromb. Thrombolysis* **2010**, *29*, 199–207.

[5] Y. M. Xu, S. Masuko, M. Takieddin, H. M. Xu, R. P. Liu, J. Jing, S. A. Mousa, R. J. Linhardt, J. Liu, *Science* **2011**, *334*, 498–501.

[6] a) M. Guerrini, D. Beccati, Z. Shriver, A. Naggi, K. Viswanathan, A. Bisio, I. Capila, J. C. Lansing, S. Guglieri, B. Fraser, A. Al-Hakim, N. S. Gunay, Z. Q. Zhang, L. Robinson, L. Buhse, M. Nasr, J. Woodcock, R. Langer, G. Venkataraman, R. J. Linhardt, B. Casu, G. Torri, R. Sasisekharan, *Nat. Biotechnol.* **2008**, *26*, 669–675; b) D. B. Blossom, A. J. Kallen, P. R. Patel, A. Elward, L. Robinson, G. P. 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.

[7] a) *Process for the organic synthesis of oligosaccharides and derivatives thereof*, J. Choay, J.-C. Jacquinet, J.-C. Lormeau, M. Nassr, M. Petitou, P. Sinay, (Choay, S.A., Paris, France) US Pat. US4818816A, April, **1989**.; b) *New pentasaccharides*, C. A. A. van Boeckel, T. Beetz, M. Petitou, (N. V. Akzo, The Netherlands, Sanofi, S. A., France) Eur. Pat. Appl. EP0300099A1, July, **1987**.; c) *Synthetic heparin pentasaccharides*, J. Seifert, L. Singh, T. E. Ramsdale, M. L. West, N. B. Drinnan, (Alchemia Ltd, Australia), Int. Pat. Appl. WO2003022860A1, March, **2003**.; d) J. M. Herbert, J. P. Herault, A. Bernat, R. G. M. van Amsterdam, J. C. Lormeau, M. Petitou, C. van Boeckel, P. Hoffmann, D. G. Meuleman, *Blood* **1998**, *91*, 4197–4205; e) M. M. Samama, G. T. Gerotziafas, *Thromb. Res.* **2003**, *109*, 1–11; f) M. Petitou, P. Duchaussoy, I. Lederman, J. Choay, J.-C. Jacquinet, P. Sinaÿ, G. Torri, *Carbohydr. Res.* **1987**, *167*, 67–75; g) C. A. A. van Boeckel, M. Petitou, *Angew. Chem.* **1993**, *105*, 1741–1761; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1671–1690; h) M. Petitou, C. A. A. van Boeckel, *Angew. Chem.* **2004**, *116*, 3180–3196; *Angew. Chem. Int. Ed.* **2004**, *43*, 3118–3133.

[8] a) J. M. Walenga, W. P. Jeske, M. M. Samama, F. X. Frapaise, R. L. Bick, J. Fareed, *Expert Opin. Invest. Drugs* **2002**, *11*, 397–407; b) A. G. G. Turpie, *Expert Opin. Pharmacother.* **2004**, *5*, 1373–1384.

[9] a) M. Petitou, P. Duchaussoy, J. M. Herbert, G. Duc, M. El Hajji, J. F. Branellec, F. Donat, J. Necciari, R. Cariou, J. Bouthier, E. Garrigou, *Semin. Thromb. Hemostasis* **2002**, *28*, 393–402; b) J. I. Weitz, L. A. Linkins, *Expert Opin. Invest. Drugs* **2007**, *16*, 271–282.

[10] a) J. D. C. Codée, B. Stubba, M. Schiattarella, H. S. Overkleeft, C. A. A. van Boeckel, J. H. van Boom, G. A. van der Marel, *J. Am. Chem. Soc.* **2005**, *127*, 3767 – 3773; b) P. D. J. Grootenhuis, C. A. A. Van Boeckel, *J. Am. Chem. Soc.* **1991**, *113*, 2743 – 2747; c) J. Tatai, P. Fugedi, *Tetrahedron* **2008**, *64*, 9865 – 9873; d) Y. Zhou, F. Lin, J. Chen, B. Yu, *Carbohydr. Res.* **2006**, *341*, 1619 – 1629; e) P. Sinaÿ, J.-C. Jacquinet, M. Petitou, P. Duchaussoy, I. Lederman, J. Choay, G. Torri, *Carbohydr. Res.* **1984**, *132*, C5 – C9; f) Z. Wang, Y. Xu, B. Yang, G. Tiruchinapally, B. Sun, R. Liu, S. Dulaney, J. Liu, X. Huang, *Chemistry* **2010**, *16*, 8365 – 8375; g) M. Petitou, P. Duchaussoy, P. A. Driguez, G. Jaurand, J. P. Herault, J. C. Lormeau, C. A. A. van Boeckel, J. M. Herbert, *Angew. Chem.* **1998**, *110*, 3186 – 3191; *Angew. Chem. Int. Ed.* **1998**, *37*, 3009 – 3014; h) T. Polat, C. H. Wong, *J. Am. Chem. Soc.* **2007**, *129*, 12795 – 12800; i) 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; j) J.-C. Lee, X.-A. Lu, S. S. Kulkarni, Y.-S. Wen, S.-C. Hung, *J. Am. Chem. Soc.* **2004**, *126*, 476 – 477; k) J.-C. Lee, C.-A. Tai, S.-C. Hung, *Tetrahedron Lett.* **2002**, *43*, 851 – 855.
[11] D. Crich, M. Smith, *J. Am. Chem. Soc.* **2001**, *123*, 9015 – 9020.
[12] H. Ye, R. H. Liu, D. M. Li, Y. H. Liu, H. X. Yuan, W. K. Guo, L. F. Zhou, X. F. Cao, H. Q. Tian, J. Shen, P. G. Wang, *Org. Lett.* **2013**, *15*, 18 – 21.
[13] a) J. Xue, Z. W. Guo, *Tetrahedron Lett.* **2001**, *42*, 6487 – 6489; b) A. R. S. Ganguli, J. K. Coward, *Tetrahedron: Asymmetry* **2005**, *16*, 411 – 424.
[14] M. Izumi, O. Tsuruta, S. Harayama, H. Hashimoto, *J. Org. Chem.* **1997**, *62*, 992 – 998.
[15] J. Tatai, G. Osztrovszky, M. Kajtár-Peredy, P. Fügedi, *Carbohydr. Res.* **2008**, *343*, 596 – 606.
[16] a) A. De Mico, R. Margarita, L. Parlanti, A. Vescovi, G. Piancatelli, *J. Org. Chem.* **1997**, *62*, 6974 – 6977; b) L. J. van den Bos, J. D. Codee, J. C. van der Toorn, T. J. Boltje, J. H. van Boom, H. S. Overkleeft, G. A. van der Marel, *Org. Lett.* **2004**, *6*, 2165 – 2168.
[17] T. Zhu, G.-J. Boons, *Tetrahedron: Asymmetry* **2000**, *11*, 199 – 205.
[18] J. D. Codée, R. E. Litjens, R. den Heeten, H. S. Overkleeft, J. H. van Boom, G. A. van der Marel, *Org Lett.* **2003**, *5*, 1519 – 1522.
[19] H. A. Orgueira, A. Bartolozzi, P. Schell, R. E. Litjens, E. R. Palmacci, P. H. Seeberger, *Chemistry* **2003**, *9*, 140 – 169.
[20] H. Osman, D. S. Larsen, J. Simpson, *Tetrahedron* **2009**, *65*, 4092 – 4098.

Received: January 7, 2014
Published online on April 11, 2014