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NEW SYNTHETIC HEPARIN MIMETICS ABLE TO INHIBIT THROMBIN AND FACTOR Xa

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Abstract: Synthetic pentadeca-, heptadeca- and nonadecasaccharides, comprising an antithrombin III (AT III) binding pentasaccharide prolonged at the non-reducing end by a thrombin binding domain have been obtained. The pentadecasaccharide is the shortest oligosaccharide able to catalyse thrombin inhibition by AT III. The nonadecasaccharide is a more potent thrombin inhibitor than standard heparin.

Heparin, a complex anionic polysaccharide of animal origin,[1] contains a unique pentasaccharide sequence[2] that binds to, and activates the coagulation inhibitor antithrombin III (AT III). Activated AT III then irreversibly inhibits the procoagulant proteinase factor Xa.[3] It also inhibits thrombin by a slightly different mechanism that requires the formation of a ternary complex between heparin, AT III, and thrombin.[3]

We are actively looking for synthetic carbohydrate substitutes for heparin, displaying similar anticoagulant action but devoid of undesired side effects.[4] In previous publications[5] we described glycoconjugates first, then regular oligomers of an iduronic acid-containing trisulfated disaccharide, both displaying anti-Xa and anti-IIa activities. The synthesis of these compounds was relatively simple, but they did not reproduce exactly the desired pharmacological profile. For this reason we tried to identify new synthetic

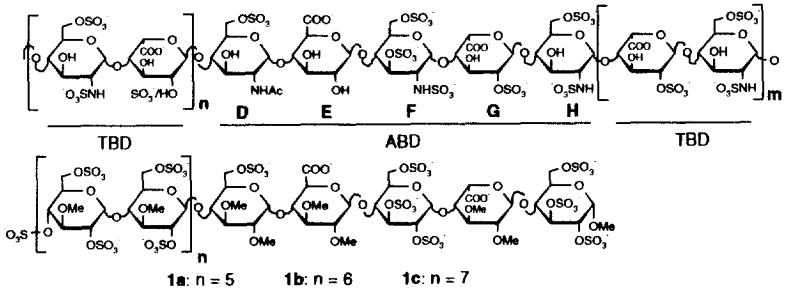

Figure 1. Structure of heparin and of **1a-c** (in heparin the iduronic acid unit next to D is not sulfated at position 2)

carbohydrate lead compounds with a structure closer to that of the original polysaccharide, i.e. possessing a specific AT III-binding domain (ABD) prolonged by a thrombin binding domain (TBD) that is not recognized by AT III, and that shows charge density and charge distribution analogous to that of heparin (Figure 1). We knew from previous work that such saccharides should be longer than a tetradecasaccharide.[5c]

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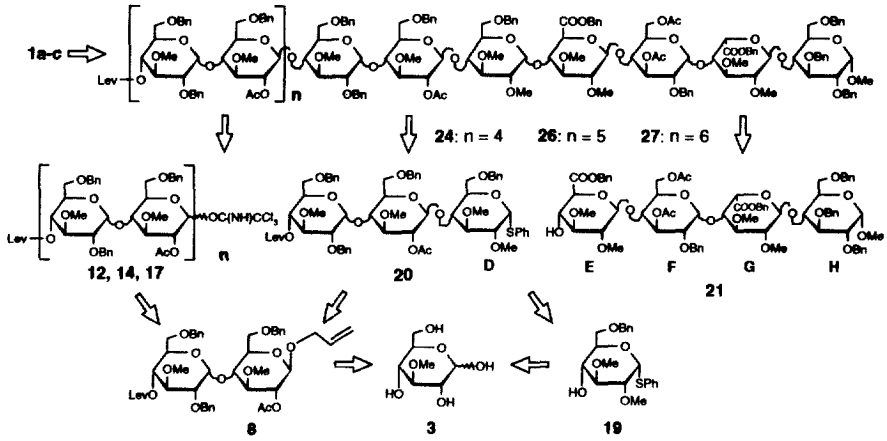
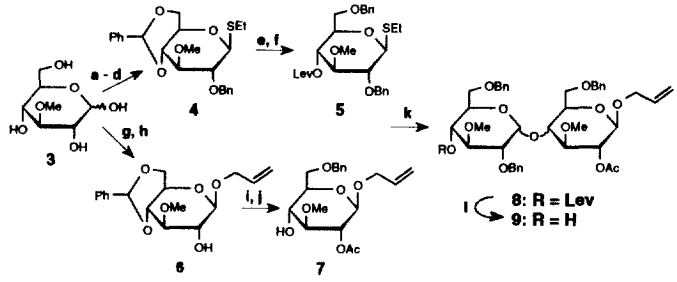
In the design of the target structures, a key issue was to attach the TBD at the correct end of the ABD to obtain efficient thrombin inhibition. Modelling studies on the ternary heparin/AT III/thrombin complex



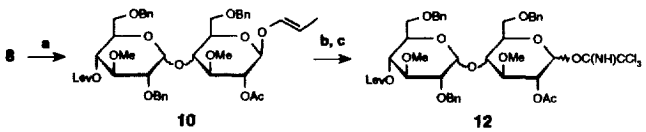
Scheme 1. Retrosynthesis of **1a-c**.

suggested that it was the non-reducing end.[5a] This view was supported by the properties of the conjugates mentioned above[5a,b] and more recently by crystallography studies.[6] The structures of **1a-c**, thus inspired by the structure of heparin itself, are depicted Figure 1. As ABD, we selected a high affinity analogue of the AT III binding sequence[7] (DEFGH). Concerning the TBD, thrombin binding being mainly a matter of electrostatic attraction of the anion binding exosite II of the protein[8] by the anionic polysaccharide, we kept the same density of charge (number of charges per saccharide unit) as that of heparin, whereas, to keep the chemistry manageable, we allowed us some laxity concerning their distribution in space. Thus, while 2,6-di-*O*-sulfo-α-D-glucose is a very close mimic of *N*-sulfo-6-*O*-sulfo-α-D-glucosamine, the space occupied by 2-*O*-sulfo-α-L-iduronic acid in a heparin chain deviates somewhat from that of 2,6-di-*O*-sulfo-β-D-glucose that we elected as a mimic; not to mention the hardly mimicable conformational flexibility of the iduronate ring.[9] Nevertheless, preliminary modelling studies on the one hand, showing that the overall shape of the molecule was similar to that of heparin, and the dramatic simplification of the chemical process expected from this choice on the other hand, led us to elect **1a-c** as our targets.

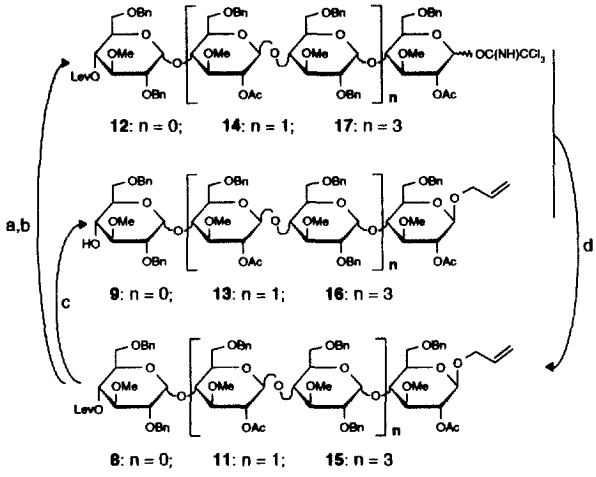


Scheme 2. (a) Ac_2O, pyridine, 16 h, quantitative; (b) EtSH, BF_3-Et_2O, toluene, 90 min, 59%; (c) MeONa, $MeOH/CH_2Cl_2$, 30 min, Dowex H^+ resin; then $PhCH(OMe)_2$, CH_3CN, CSA, 90 min, 81% overall; (d) BnBr, NaH, DMF, 2 h, 97%; (e) Et_3SiH, $ClCH_2CH_2Cl$, TFAA/TFA, 2 h, 60%; (f) LevOH, EDCI, DMAP, 3.5 h, 93%; (g) CH_2CHCH_2OH, TfOH, 120 °C, 2 h; (h) $PhCH(OMe)_2$, TsOH, DMF, 80 °C, 1 h, 57%; (i) Ac_2O, DMAP, Et_3N, CH_2Cl_2, 2 h, 95%; (j) Et_3SiH, $ClCH_2CH_2Cl$, TFAA/TFA, 4h, 82%; (k) $ClCH_2CH_2Cl$, NIS/TfOH, -25 °C, 5 min, 52%; (l) NH_2NH_2/AcOH, EtOH/toluene, 1 h, 97%

The retrosynthetic route to **1a-c**, shown in Scheme 1, takes advantage of the availability, from previous work,[7] of the expensive tetrasaccharide building block **21**, the precursor of the EFGH tetrasaccharide of the ABD part of the molecule. According to this route, the non-stereospecific coupling between **20** and **21** is first carried out, completing the ABD, and initiating the TBD. Stereospecific additions, through neighbouring group participation, of the TBD precursors (**12**, **14**, **17**) complete the elaboration of the carbohydrate backbone. The more obvious pathway that consisted in completing first the ABD part, through reaction of **21** and the monosaccharide phenyl 6-*O*-acetyl-4-*O*-levulinoyl-2,3-di-*O*-methyl-1-thio-D-glucopyranoside, and then adding the precursors of the TBD part, was ruined by the very low yield (27%) of the first reaction. Most probably the levulinoyl group was too close to the activated anomeric center in the monosaccharide, since the trisaccharide **20**, reacted well with **21** to give the expected heptasaccharide (64%) and its β-isomer (7%).



Scheme 3. (a) (1,5-cyclooctadiene)bis(methyldiphenylphosphine)iridium(I) hexafluorophosphate, THF, H_2, 10 min, 76%; (b) HgO/$HgCl_2$, acetone/H_2O, 1 h, 90%; (c) CCl_3CN, K_2CO_3, CH_2Cl_2, 16 h, 87%.

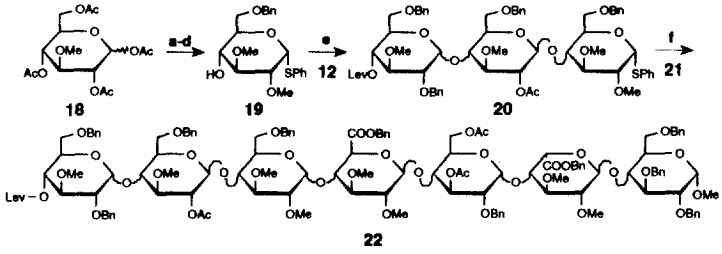


Scheme 4. (a) Ir complex, THF, H_2, 10 min; then NBS, CH_2Cl_2, 5 min; (b) CCl_3CN, K_2CO_3, CH_2Cl_2, 16 h: **14** (64% from **11**); **17** (63% from **15**); (c) NH_2NH_2/AcOH, EtOH/toluene, 1-2 h: **13** (86% from **11**); **16** (90% from **15**); (d) TBDMSOTf, CH_2Cl_2, 4Å MS, -20 °C, 10 min: **11** (80% from **9** and **12**); **15** (85% from **13** and **14**).

All the synthons required for elaboration of the TBD part of the molecule derived from the disaccharide **8**, obtained (Scheme 2) from commercially available 3-*O*-methyl-D-glucose (**3**). Thus, conversion of **3** into 1,2,4,6-tetra-*O*-acetyl-3-*O*-methyl-D-glucopyranose[10] followed by a classical series of reactions gave,[11] after reductive opening of the benzylidene of **4** and levulinoylation, the glycosyl donor thioglycoside **5**. Treatment of **3** with allyl alcohol in a Fischer glycosidation reaction using trifluoromethanesulfonic acid as catalyst[12] provided an α/β mixture (3/2) of the allyl glycosides. After benzylidenation of the crude mixture, some pure α-isomer (26%) could be isolated by selective crystallization. Column chromatography allowed separation of the remaining α- and β-isomers. We initially intended to use vinyl glycosides, obtained by isomerisation of allyl groups, as glycosyl donors[13] in an orthogonal strategy. For this reason, the more reactive β-isomer **6** was selected. Acetylation and reductive opening of the benzylidene afforded the glycosyl acceptor **7** (78%). Condensation of **5** and **7** using the NIS-trifluoromethanesulfonic acid system as activator[14] gave a mixture of the disaccharides (α/β = 7/2) easily resolved by column chromatography to give **8** (52% from **7**).

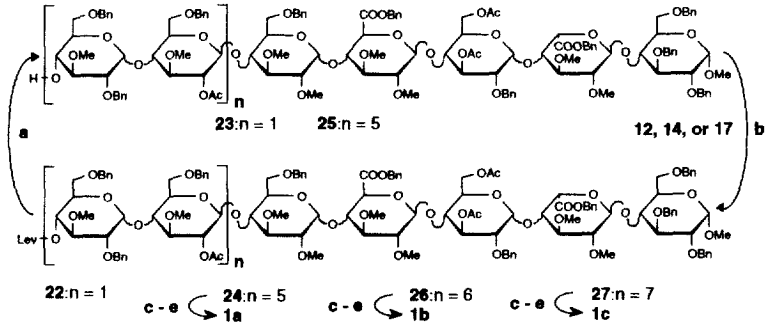
Removal of the levulinoyl group[15] gave the disaccharide acceptor **9** (97%) while isomerisation of the allyl group[16] provided the vinyl glycoside **10** (76%) together with some (5%) propyl by-product (Scheme 3). With these key building blocks in hands, we started to build up the precursors of the TBD part of the molecules.

Reaction of stoichiometric amounts of **9** and **10**, in toluene in the presence of trimethylsilyl trifluoromethanesulfonate yielded the tetrasaccharide **11** (44%). This rather low yield led us to replace **10** by the imidate **12**, obtained from **10** in two steps: hydrolysis of the prop-1'-enyl glycoside in the presence of $HgCl_2/HgO$, and treatment of the hemiacetal with trichloroacetonitrile in the presence of potassium carbonate[17] (78% overall yield). Reaction of **12** and **9** (Scheme 4) in dichloromethane in the presence of *tert*-butyldimethylsilyl trifluoromethanesulfonate gave **11** in much better yield (80%). Like **8**, the tetrasaccharide **11** was converted into the acceptor **13** (86%) and the imidate **14** (64% from **11**) which reacted together to give the octasaccharide **15** (85%) in turn converted into the acceptor **16** (90%) and the donor **17** (63%).



Scheme 5. (a) PhSH, BF_3-Et_2O, toluene, 50 °C, 1 h, (17% α-isomer, 45% β-isomer); (b) MeONa, $MeOH/CH_2Cl_2$, 1 h, Dowex H^+ resin; then $PhCH(OMe)_2$, CH_3CN, CSA, 1 h; (c) MeI, NaH, DMF, 0.5 h, 94%; (d) Et_3SiH, $ClCH_2CH_2Cl$, TFAA/TFA, 16 h, 80%; (e) TBDMSOTf, CH_2Cl_2, 4Å MS, -20 °C, 10 min, 68%; (f) NIS, TfOH, $ClCH_2CH_2Cl/Et_2O$, 4Å MS, -25 °C, 30 min, 64%.

The trisaccharide **20** was obtained (68%; Scheme 5) by reaction of **12** with the thiophenyl glycoside acceptor **19**. This latter was prepared from **18** using a similar route that led to **5**. Condensation of **20** with the tetrasaccharide **21** (obtained as described for its methyl ester counterpart[7]) in diethyl ether, in the presence of NIS and trifluoromethanesulfonic acid, gave the heptasaccharide **22** (64%). Cleavage of the levulinoyl group provided **23** (84%) which reacted with **17** to give the pentadecasaccharide **24** (76%). This latter was converted into the acceptor **25** (75%) which reacted with **12** to yield the heptadecasaccharide **26** (56%) and with **14** to



Scheme 6. (a) $NH_2NH_2/AcOH$, EtOH/toluene, 1-2 h: **23** (84% from **22**), **25** (75% from **24**); (b) TBDMSOTf, CH_2Cl_2, 4Å MS, -25 °C, 1 h: **24** (76% from **23** and **17**), **26** (56% from **25** and **12**), **27** (59% from **25** and **14**); (c) H_2, Pd/C, AcOH, 5 h; (d) NaOH; (e) $Et_3N{:}SO_3$, DMF, 55 °C, 24 h: **1a** (80% from **24**), **1b** (88% from **26**), **1c** (80% from **27**).

yield the nonadecasaccharide **27** (59%). Following a classical series of deprotection and sulfation (Scheme 6) **24**, **26**, and **27** gave (80-88% over the 3 steps) the target compounds **1a** (31 mg), **1b** (27 mg) and **1c** (44 mg).[11]

Biological tests performed on these compounds (Table 1) demonstrated their ability to bind to AT III with a high affinity and to inhibit coagulation factor Xa and thrombin. Affinity for AT III and anti-factor Xa activity were in the same range for all the compounds. Thrombin inhibition was size-dependent, as already explained for heparin by the greater ability of a longer negatively charged molecule to attract thrombin and bring it in contact with AT III. It is worthy of note that the nonadecamer **1c** was as potent as the most active fraction isolated from a standard heparin preparation,[18] thus constituting a good lead compound for structural modifications aimed at improving the biological profile of this new family of antithrombotics.

Table I. Biological properties of **1a-c**, **2**, and heparin. Affinity for AT III,[19] factor Xa inhibition,[20] and thrombin inhibition[21] were determined using published procedures.

Compound N°	**1a**	**1b**	**1c**	**heparin**
Number of saccharide units	15	17	19	≈ 10-50
Molecular weight	5618	6378	7139	≈ 15000
Affinity for AT III (Kd, nM ± SD, n = 3)	1.6 ± 0.3	3.3 ± 0.8	1.2 ± 0.2	25 ± 0.2
Factor Xa inhibition (units/mg ± SD, n = 3)	370 ± 9	270 ± 8	290 ± 29	180
Thrombin inhibition (IC50, ng/mL, 95% confidence interval)	41 (38-44)	5.3 (5-5.4)	1.7 (1.3-2.3)	3.3 (3-4)

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11. All new compounds were analysed by 300-500 MHz ^{1}H NMR, mass spectrometry and occasionally by HPLC. Combustion analyses were systematically performed on monosaccharides and disaccharides only. Selected analytical data: **4**: mp 123 °C (from diethyl ether); $[\alpha]_D$ - 42 (*c* 1.34, CH_2Cl_2). **5**: $[\alpha]_D$ - 5.1 (*c* 1.46, CH_2Cl_2). **6**: mp 131 °C (from EtOAc-cyclohexane); $[\alpha]_D$ - 43.2 (*c* 1, CH_2Cl_2). **7** : $[\alpha]_D$ - 40 (*c*, 1.06, CH_2Cl_2). **8** : $[\alpha]_D$ + 38 (*c* 1.01, CH_2Cl_2) ; ^{1}H NMR δ 5.47 (d, 1 H, $J_{1,2}$=3.5 Hz, H-1'), 4.42 (d, 1 H, $J_{1,2}$=7.9 Hz, H-1). **9**: $[\alpha]_D$ + 24.5 (*c* 1.7 CH_2Cl_2). **10**: $[\alpha]_D$ + 47 (*c* 1.16 CH_2Cl_2) ; ^{1}H NMR δ 6.19 (dd, 1 H, O(C*H*:CH)CH_3), 5.44 (d, 1 H, $J_{1,2}$=3.5 Hz, H-1'), 5.00 (m, 1 H, O(CH:C*H*)CH_3), 4.6 (d, 1 H, $J_{1,2}$=7.55 Hz, H-1), 1.55 (dd, 3 H, O(CH:CH)C*H*$_3$). **12**: ^{1}H NMR δ 5.50 (d, 1 H, $J_{1,2}$=3.5 Hz, H-1'), 6.51 (d, 1 H, $J_{1,2}$=3.7 Hz, H-1α), 5.81 (d, 1 H, $J_{1,2}$=7.1 Hz, H-1β). **19**: $[\alpha]_D$ + 243 (*c*1, CH_2Cl_2). **20**: $[\alpha]_D$+ 144 (*c* 1, CH_2Cl_2) ; ^{1}H NMR δ 5.73 (d, 1 H, $J_{1,2}$=5.2 Hz, H-1), 5.48 (d, 1 H, $J_{1,2}$=3.5 Hz, H-1''), 4.46 (d, 1 H, $J_{1,2}$=8.0 Hz, H-1').
For longer oligosaccharides, ^{1}H NMR data were collected at 500 MHz in D_2O (external TSP), δ for anomeric protons and $J_{1,2}$ are reported (detailed data are available on request). Mass Spectrometry data (ESI MS) were collected using Electron Spray Ionisation in the negative mode, monoisotopic mass/average mass/experimental mass are given. **1a**: $[\alpha]_D$ +39 (*c* 0.51, H_2O). ^{1}H NMR, monosaccharide units named MNO_4P_4DEFGH: unit M: 5.69 (3.3); unit N: 4.79 (7-8); 4 units O: 5.45 (3-4); 4 units P: 4.75 (7-8); unit D: 5.43 (3-4); unit E: 4.65 (7.3); unit F: 5.41 (3.4); unit G: 5.06 (1-2); unit H: 5.15 (3.3). ESI MS, 5613.3 / 5617.7 / 5615.5 a.m.u.. **1b**: $[\alpha]_D$ +38 (*c* 0.91, H_2O). ^{1}H NMR, monosaccharide units named MNO_5P_5DEFGH: unit M: 5.70 (3.3); unit N: 4.78 (7-8); 5 units O: 5.45 (3-4); 5 units P: 4.75 (7-8); unit D: 5.43 (3-4); unit E: 4.64 (7.3); unit F: 5.41 (3.4); unit G: 5.06 (1-2); unit H: 5.15 (3.3). ESI MS, 6373.17 / 6378.31 / 6373.5 a.m.u.. **1c**: $[\alpha]_D$ +40 (*c* 0.79, H_2O). ^{1}H NMR, monosaccharide units named MNO_6P_6DEFGH: unit M: 5.71 (3.3); unit N: 4.81 (7-8); 6 units O: 5.48 (3-4); 6 units P: 4.78 (7-8); unit D: 5.46 (3.4); unit E: 4.67 (7.3); unit F: 5.44 (3.4); unit G: 5.08 (1-2); unit H: 5.17 (3.3). ESI MS, 7133.06 / 7139.9 / 7137.26 a.m.u..
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