

## 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. © 1999 Published by Elsevier Science Ltd. All rights reserved.

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

We are actively looking for synthetic carbohydrate substitutes for heparin, displaying similar anticoagulant action but devoid of undesired side effects.<sup>4</sup> In previous publications<sup>5</sup> 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.<sup>5c</sup>



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.<sup>5a</sup> This view was supported by the properties of the conjugates mentioned above<sup>5a,b</sup> and more recently by crystallography studies.<sup>6</sup> 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<sup>7</sup> (DEFGH). Concerning the TBD, thrombin binding being mainly a matter of electrostatic attraction of the anion binding exosite II of the protein<sup>8</sup> 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- $\alpha$ -Dglucose is a very close mimic of *N*-sulfo-6-O-sulfo- $\alpha$ -D-glucosamine, the space occupied by 2-O-sulfo- $\alpha$ -Liduronic acid in a heparin chain deviates somewhat from that of 2,6-di-O-sulfo- $\beta$ -D-glucose that we elected as a mimic; not to mention the hardly mimicable conformational flexibility of the iduronate ring.<sup>9</sup> 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<sub>3</sub>-Et<sub>2</sub>O, toluene, 90 min, 59%; (c) MeONa, MeOH/CH<sub>2</sub>Cl<sub>2</sub>, 30 min, Dowex H<sup>+</sup> resin; then PhCH(OMe)<sub>2</sub>, CH<sub>3</sub>CN, CSA, 90 min, 81% overall; (d) BnBr, NaH, DMF, 2 h, 97%; (e) Et<sub>3</sub>SiH, ClCH<sub>2</sub>CH<sub>2</sub>Cl, TFAA/TFA, 2 h, 60%; (f) LevOH, EDCI, DMAP, 3.5 h, 93%; (g) CH<sub>2</sub>CHCH<sub>2</sub>OH, TfOH, 120 °C, 2 h; (h) PhCH(OMe)<sub>2</sub>, TsOH, DMF, 80 °C, 1 h, 57%; (i)  $Ac_2O$ , DMAP, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, 2 h, 95%; (j) Et<sub>3</sub>SiH, ClCH<sub>2</sub>CH<sub>2</sub>Cl, TFAA/TFA, 4h, 82%; (k) ClCH<sub>2</sub>CH<sub>2</sub>Cl, NIS/TfOH, -25 °C, 5 min, 52%; (l) NH<sub>2</sub>NH<sub>2</sub>/AcOH, EtOH/toluene, 1 h, 97%

The retrosynthetic route to 1a-c, shown in Scheme 1, takes advantage of the availability, from previous work,<sup>7</sup> 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  $\beta$ -isomer (7%).



Scheme 3. (a) (1,5-cyclooctadiene)bis(methyldiphenylphosphine)iridium(1) hexafluorophosphate, THF, H<sub>2</sub>, 10 min, 76%; (b) HgO/HgCl<sub>2</sub>, acetone/H<sub>2</sub>O, 1 h, 90%; (c) CCl<sub>3</sub>CN, K<sub>2</sub>CO<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, 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-glucopyranose10 followed by a classical series of reactions gave,<sup>11</sup> 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<sup>12</sup> provided an  $\alpha/\beta$  mixture (3/2) of the allyl glycosides. After benzylidenation of the crude mixture, some pure  $\alpha$ -isomer (26%) could be by selective crystallization. isolated Column chromatography allowed separation of the remaining  $\alpha$ - and  $\beta$ isomers. We initially intended to use vinyl glycosides, obtained by isomerisation of allyl groups, as glycosyl donors13 in an orthogonal strategy. For this reason, the more reactive  $\beta$ -isomer 6 was selected. Acetvlation 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<sup>14</sup> gave a mixture of the disaccharides ( $\alpha/\beta = 7/2$ ) easily resolved by column chromatography to give 8(52%)from 7).

Removal of the levulinoyl group<sup>15</sup> gave the disaccharide acceptor **9** (97%) while isomerisation of the allyl group<sup>16</sup> 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<sub>2</sub>/HgO, and treatment of the hemiacetal with trichloroacetonitrile in the presence of potassium carbonate<sup>17</sup> (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<sub>3</sub>-Et<sub>2</sub>O, toluene, 50 °C, 1 h,  $(17\% \alpha$ -isomer, 45%  $\beta$ -isomer); (b) MeONa, MeOH/CH<sub>2</sub>Cl<sub>2</sub>, 1 h, Dowex H<sup>+</sup> resin; then PhCH(OMe)<sub>2</sub>, CH<sub>3</sub>CN, CSA, 1 h; (c) MeI, NaH, DMF, 0.5 h, 94%; (d) Et<sub>3</sub>SiH, ClCH<sub>2</sub>CH<sub>2</sub>Cl, TFAA/TFA, 16 h, 80%; (e) TBDMSOTf, CH<sub>2</sub>Cl<sub>2</sub>, 4Å MS, -20 °C, 10 min, 68%; (f) NIS, TfOH, ClCH<sub>2</sub>CH<sub>2</sub>Cl/Et<sub>2</sub>O, 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<sup>7</sup>) 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<sub>3</sub>N:SO<sub>3</sub>, 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).<sup>11</sup>

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,<sup>18</sup> thus constituting a good lead compound for structural modifications aimed at improving the biological profile of this new family of antithrombotics.

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

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

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- 11. All new compounds were analysed by 300-500 MHz <sup>1</sup>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<sub>2</sub>Cl<sub>2</sub>). **5**:  $[\alpha]_D 5.1$  (*c* 1.46, CH<sub>2</sub>Cl<sub>2</sub>). **6**: mp 131 °C (from EtOAc-cyclohexane);  $[\alpha]_D 43.2$  (*c* 1, CH<sub>2</sub>Cl<sub>2</sub>). **7**:  $[\alpha]_D 40$  (*c*, 1.06, CH<sub>2</sub>Cl<sub>2</sub>). **8**:  $[\alpha]_D + 38$  (*c* 1.01, CH<sub>2</sub>Cl<sub>2</sub>); <sup>1</sup>H NMR  $\delta$  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<sub>2</sub>Cl<sub>2</sub>). **10**:  $[\alpha]_D + 47$  (*c* 1.16 CH<sub>2</sub>Cl<sub>2</sub>); <sup>1</sup>H NMR  $\delta$  6.19 (dd, 1 H,  $J_{1,2}=7.55$  Hz, H-1), 1.55 (dd, 3 H, O(CH:CH)CH<sub>3</sub>). **12**: <sup>1</sup>H NMR  $\delta$  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-1 $\alpha$ ), 5.81 (d, 1 H,  $J_{1,2}=7.1$  Hz, H-1 $\beta$ ). **19**:  $[\alpha]_D + 243$  (*c*1, CH<sub>2</sub>Cl<sub>2</sub>). **20**:  $[\alpha]_D + 144$  (*c* 1, CH<sub>2</sub>Cl<sub>2</sub>); <sup>1</sup>H NMR  $\delta$  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, <sup>1</sup>H NMR data were collected at 500 MHz in D<sub>2</sub>O (external TSP),  $\delta$  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<sub>2</sub>O). <sup>1</sup>H NMR, monosaccharide units named MNO<sub>4</sub>P<sub>4</sub>DEFGH: 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<sub>2</sub>O). <sup>1</sup>H NMR, monosaccharide units named MNO<sub>5</sub>P<sub>5</sub>DEFGH: 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<sub>2</sub>O). <sup>1</sup>H NMR, monosaccharide units named MNO<sub>6</sub>P<sub>6</sub>DEFGH: 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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