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Synthesis of Heparin-Like Antithrombotics Having Perphosphorylated Thrombin Binding Domains

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Abstract—The synthesis of three heparin analogues (i.e. compounds **VI**–**VIII**) having perphosphorylated thrombin binding domains (TBDs) is reported. These compounds were tested in vitro for their antithrombin III (ATIII)-mediated anti-Xa and antithrombin activities. Conjugates **VI** and **VIII** show a remarkable increase in antithrombin activity compared to the structurally related conjugates with persulfated TBDs (i.e. compounds **IV** and **V**), whereas compound **VII** displays a diminished activity.

Introduction

For more than half a century the sulfated glycosaminoglycan heparin has been used for the prophylaxis and treatment of thrombotic disorders.[1] Heparin enhances the inhibitory potency of the plasma protein ATIII towards factor Xa and thrombin, two essential serine proteases in blood coagulation.[2]

It is well recognized that ATIII undergoes a conformational change[3–5] upon binding with a unique pentasaccharide (PS) domain of heparin[6,7] (synthetic counterpart: compound **I**, Fig. 1). This conformational transition is sufficient to promote the inhibition of factor Xa, while the activity of thrombin remains unaffected. Studies towards the structure–activity relationships (SAR)[8,9] of the PS domain not only led to more potent analogues, but also to easily accessible *O*-sulfated/*O*-methylated PS analogues (e.g. compound **II**, Fig. 1).

In order to enhance thrombin inhibition, heparin fragments should comprise in addition to the PS domain at least 10 to 12 consecutive saccharide units to allow formation of a ternary complex with ATIII and thrombin as depicted in Figure 2.[10,11] During this formation ATIII first strongly binds to the PS domain of heparin, designated as the ATIII binding domain (ABD), after which thrombin interacts with a remote thrombin binding domain (TBD) of heparin. Compared to the highly specific binding of the ABD of heparin with ATIII the interaction between thrombin and the TBD of heparin is less specific in nature.[12] The latter phenomenon was nicely illustrated[13,14] by the fact that synthetic glycoconjugates containing persulfated cellobiose and maltotriose as TBD (i.e. compounds **IV** and **V**, Fig. 1) show a significant antithrombin activity (Table 1). These heparin analogues could be obtained by attachment of a TBD to the non-reducing end of an ABD via a spacer spanning a length of about 50 atoms (Fig. 2).[11,12]

In a recent paper[15] it was reported that a T_{18}-oligodeoxynucleotide (T_{18}-ODN) could also function as TBD (PS-ODN conjugate **III**, Figs 1 and 2). The latter result was an incentive to explore in which way phosphorylated TBDs influence the pharmacological properties of the heparin analogues compared to their sulfated counterparts. In order to evaluate this effect conjugates containing perphosphorylated cellobiosyl and maltotriosyl moieties (i.e. conjugate **VI** and **VIII**, respectively, Figs 1 and 2) were prepared. In addition, it was anticipated that introduction of lipophilic groups in the TBD may facilitate interaction with thrombin as it was found that in case of the ABD *O*-alkylated analogues interact more strongly with ATIII than their natural counterparts.[8] To this end, a glycoconjugate was synthesized bearing stable isopropyl phosphodiesters on its TBD (i.e. compound **VII**).

Key words: Antithrombotics; enzyme inhibitor; heparin; oligosaccharides; thrombin.
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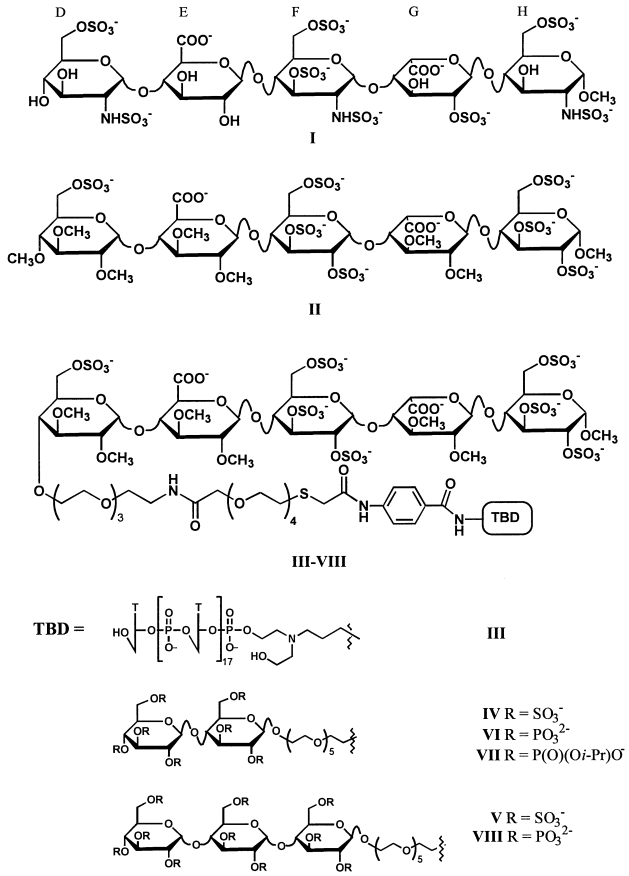


Figure 1.

Results and Discussion

The assembly of the target glycoconjugates **VI–VIII** comprises four stages: (i) synthesis of the *N*-benzyloxycarbonyl-1-amino-1-deoxyhexaethyleneglycol spacer **7** (Scheme 1); (ii) assembly of the TBDs **16a**, **16b** and **24** (Schemes 2 and 3); (iii) functionalization of the TBDs with a thiophilic iodoacetyl linker (Scheme 4); and (iv) one-pot deprotection of known ABD **28** and conjugation of its corresponding thiol with the functionalized TBDs (Scheme 4).

Synthesis of spacer 7

The requisite TBDs were equipped with the same 1-amino-1-deoxyhexaethyleneglycol spacer used earlier[14] in the construction of glycoconjugates **IV** and **V**. Since the reported[16] four-step protocol for this type of functionalized spacers requires expensive hexaethyleneglycol as starting material, an alternative route was devised using the cheap starting materials **1** and **3** (Scheme 1). Tetraethyleneglycol (**1**) was treated with *p*-tosyl chloride and a catalytic amount of 4-dimethylaminopyridine (DMAP) in pyridine to afford tetraethyleneglycol di-*p*-tosylate (**2**). Known[17] 2-(2-tetrahydropyran-2-yloxyethanoxy)ethanol (**3**) was subsequently alkylated with an excess of **2** in the presence of sodium hydride in DMF. Reaction of the intermediate tosyl derivative **4** with lithium azide gave, after removal of the tetrahydropyranyl (THP) group, the azido alcohol **5**.

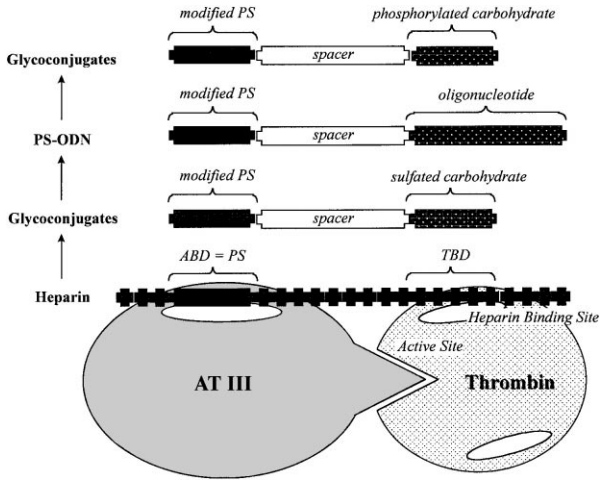


Figure 2. Schematic representation of the ternary complex of thrombin and ATII with heparin, the sulfated glycoconjugates, a PS-ODN conjugate and the proposed phosphorylated glycoconjugates.

Reduction of the azido group at this stage prevented a Staudinger reaction[18] during phosphitylation of the TBDs later on in the synthesis (Schemes 2 and 3). Thus, treatment of compound **5** with PPh_3 in pyridine and subsequently with ammonium hydroxide (25%)[19] gave amine **6**, which was protected with a benzyloxycarbonyl (Z) group using Z-OSu in H_2O/DMF under the influence of *N,N*-diisopropylethylamine (DiPEA) to yield spacer **7** (50% overall yield from **3**).

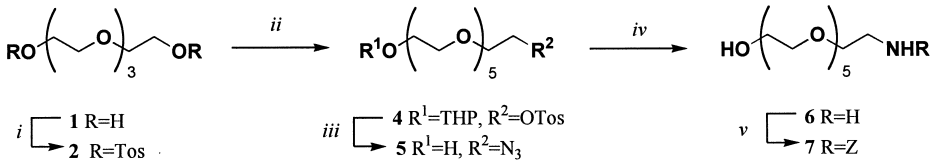
Assembly of the TBDs 16a, 16b and 24

Trichloroacetimidate donor **10** (Scheme 2) was prepared from commercially available cellobiose octaacetate (**8**) by anomeric deacetylation[20] and subsequent reaction of **9** with trichloroacetonitrile in the presence of cesium carbonate.[21] Trimethylsilyl trifluoromethanesulfonate (TMSOTf) assisted condensation of cellobiose donor **10** with spacer **7** gave the β-linked glycoside **11** exclusively. Unmasking the hydroxyl functions in **11** by Zemplén deacetylation yielded partially deprotected **12**. It was anticipated that the introduction of the seven phosphate monoesters in target TBD **16a** could be accomplished using the powerful phosphoramidite method. Indeed, 1*H*-tetrazole assisted phosphitylation of **12** with amidite **13**[22] and in situ oxidation of the intermediate phosphite-

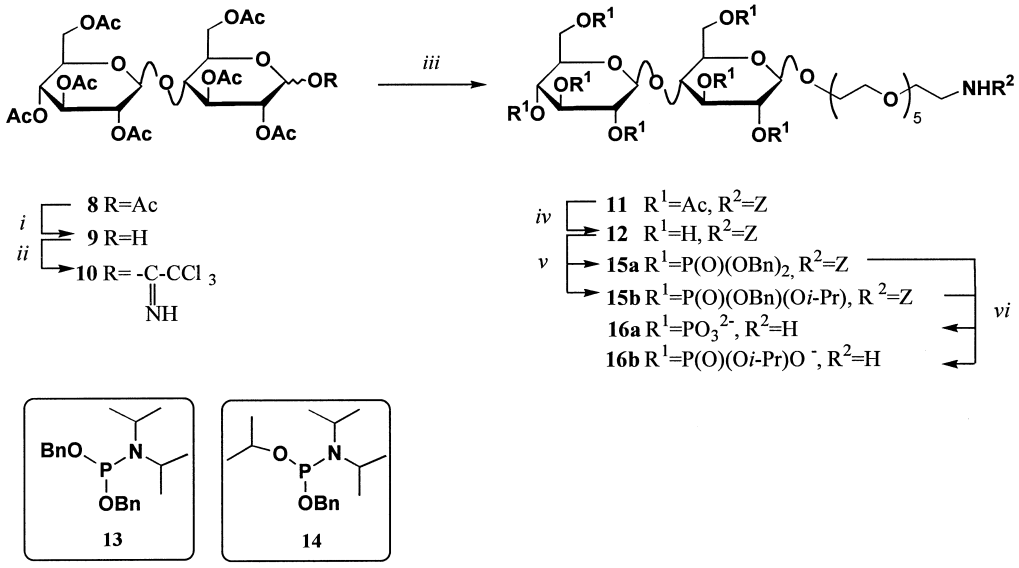
Table 1. In vitro ATIII-mediated anti-Xa and antithrombin activites of glycoconjugates **VI—VIII** and earlier reported conjugates **III–V**

Compound	TBD moiety[a]	Anti-Xa activity (U mg^{-1})	Antithrombin activity (U mg^{-1})
III	T_{18}-Oligonucleotide	173	5
IV	Cellobiose-(7-S)	740	10
V	Maltotriose-(10-S)	490	64
VI	Cellobiose-(7-P)	500	22
VII	Cellobiose-(7-IP)	690	5
VIII	Moltotriose (10-P)	1000	167

[a] The number and nature of charges in the TBD is in parentheses (S = sulfate, P = phosphate, IP = isopropyl phosphate).



Scheme 1. (i) Tos-Cl, pyridine, DMAP, 16 h, 71%; (ii) $THPO(CH_2CH_2O)_2H$ (**3**), NaH, DMF, 50°C, 2 h; then (iii) (a) LiN_3, 80°C, 16 h; (b) AcOH, THF, H_2O (4:2:1, v/v/v), 4 h, 59% (three steps); (iv) PPh_3, pyridine, 2 h; then 25% NH_4OH, 2 h; (v) Z-OSu, DiPEA, DMF/H_2O, 30 min, 85% (two steps).



Scheme 2. (i) NH_2NH_2, HOAc, DMF, 1 h; (ii) CCl_3CN, Cs_2CO_3, CH_2Cl_2, 2 h, 87% (two steps); (iii) **7**, TMSOTf, CH_2Cl_2, −20°C, 10 min, 75%; (iv) KOtBu, MeOH, 1 h, 88%; (v) (a) **13** (10 equiv), 1*H*-tetrazole, (14 equiv), CH_3CN, 1 h, then *tert*-BuOOH, 0°C, 45 min, 92%, (b) **14** (10 equiv), 1*H*-tetrazole, (14 equiv), CH_3CN, 1 h, then *tert*-BuOOH, 0°C, 45 min, 58%; (vi) H_2, Pd/C, *tert*-BuOH, H_2O, 3 h, 98% (**16a**), 97% (**16b**).

triesters with *tert*-butylhydroperoxide proceeded smoothly to give after work up and silica gel column chromatography fully protected **15a**. Removal of the benzyl and the benzyloxycarbonyl protective groups by hydrogenolysis gave TBD **16a**, which was isolated as its sodium salt (52% overall yield from **8**). The presence of the seven phosphate monoesters in **16a** was firmly established by two-dimensional (2-D) ^1H-^{31}P correlated NMR spectroscopy (Fig. 3).

Introduction of the seven isopropyl phosphodiesters in target TBD **16b** proceeded in a similar manner as described for TBD **16a**. Phosphitylation of **12** with phosphoramidite **14** (prepared according to refs 23 and 24) under the influence of 1*H*-tetrazole and subsequent *tert*-butyl hydroperoxide-mediated oxidation of the intermediate phosphite-triesters gave **15b**. Hydrogenolysis of all protecting groups in **15b** furnished homogeneous **16b** (Na^+-salt, 32% overall yield from **8**).

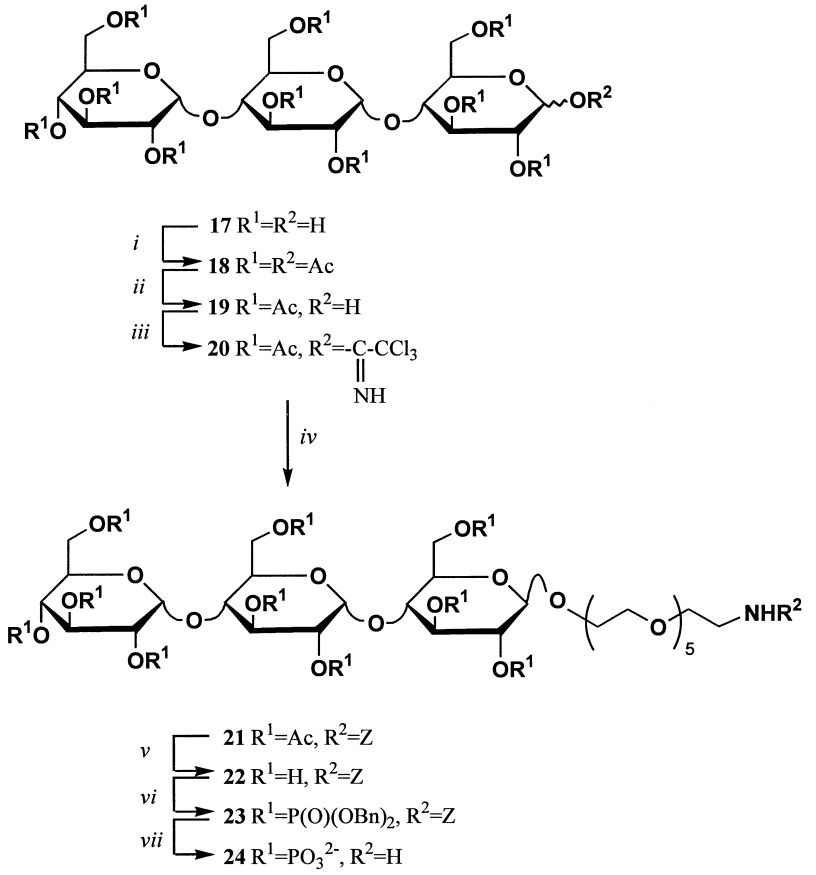
TBD **24** (Scheme 3) was prepared following the same sequence of reactions as described for the preparation of the TBD **16a**. Commercially available maltotriose (**17**) was peracetylated with acetic anhydride and a catalytic amount of DMAP in pyridine. Anomeric deacetylation of the resulting peracetate **18** and subsequent conversion into the corresponding trichloroacetimidate donor **20** was followed by glycosylation with spacer **7** to give trisaccharide **21**. Deacetylation of **21** and phosphitylation of the resulting **22** with **13** gave after oxidation the fully protected decakisphosphate **23**. Hydrogenolysis of **23** gave the requisite TBD **24** (28% overall yield from **17**).

Functionalization with thiophilic linker 25

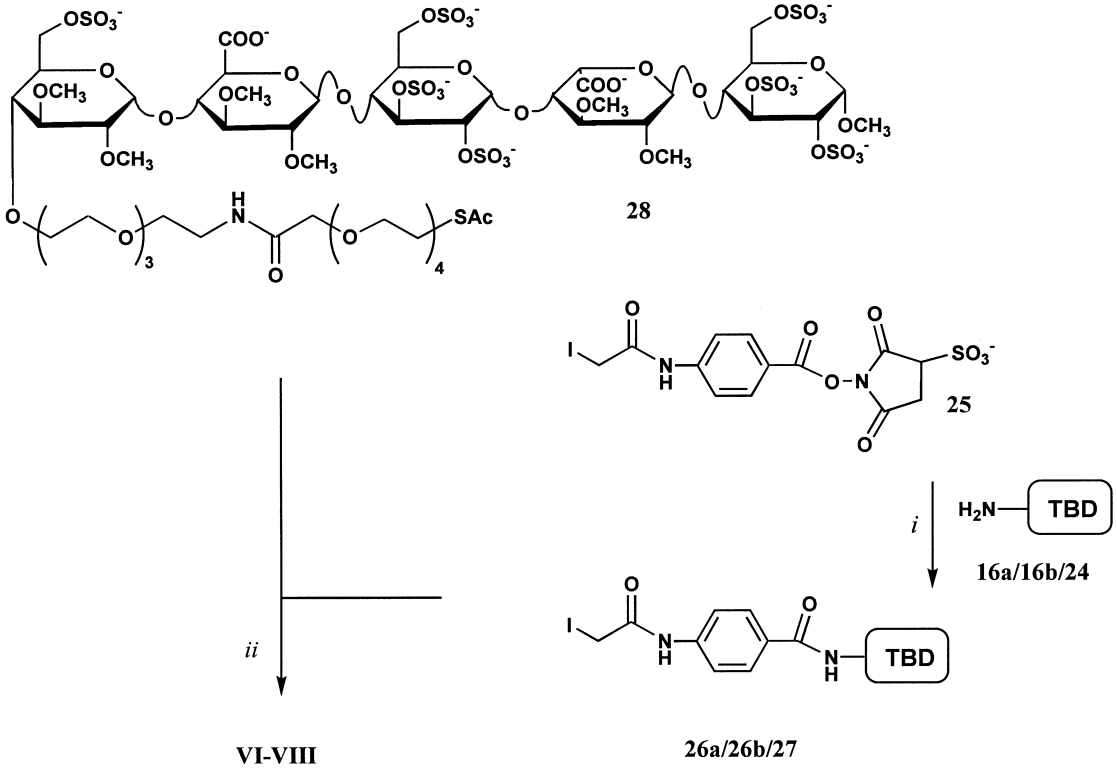
The excellent results obtained with the iodoacetyls as thiophilic groups in the synthesis of the earlier reported[14,15] conjugates urged us to exploit *sulfo*-SIAB® (**25**) in the derivatization of the TBDs **16a**, **16b** and **24** (Scheme 4). Thus, treatment of the TBDs with **25** in a buffered medium gave, after size exclusion chromatography, the iodoacetyl derivatized TBDs **26a**, **26b** and **27**.

One-pot deprotection of ABD 28 and conjugation with functionalized TBDs

The final stage in the assembly of the glycoconjugates **VI**–**VIII** entails coupling of the ABD and TBD units. In a one-pot procedure known ABD **28**[14] was deprotected using a buffered solution of 0.05 M NH_2OH and conjugated to the derivatized TBD units by forming a stable thioether bond (Scheme 4). Traces of symmetrical disulfide formed during the reaction were reduced with dithiothreitol and removed from the crude mixture by size exclusion chromatography to yield the requisite conjugates **VI**–**VIII** in excellent purity and good yield (51–80%). The analytical data of the conjugates (NMR



Scheme 3. (i) Ac_2O, pyridine, DMAP, 5 h, 91%; (ii) NH_2NH_2, HOAc, DMF, 1 h, 92%; (ii) CCl_3CN, Cs_2CO_3, CH_2Cl_2, 2 h, 71%; (iv) **7**, TMSOTf, CH_2Cl_2, −20°C, 30 min, 56%; (v) KOtBu, MeOH, 1 h, 95%; (vi) **13** (15 equiv), 1*H*-tetrazole (20 equiv), dioxane:CH_3CN (1:1, v/v) 2 h, then *tert*-BuOOH, 0°C, 45 min, 92%; (vii) H_2, Pd/C, *tert*-BuOH, H_2O, 3 h, 96%.



Scheme 4. (i) DMF, H_2O, 1 h; (ii) 0.1 M aqueous Na_2HPO_4 (pH 7.0), 0.05 M NH_2OH, 2 h (**26a**→**VI**, 68%), (**26b**→**VII**, 80%), (**27**→**VIII**, 51%).

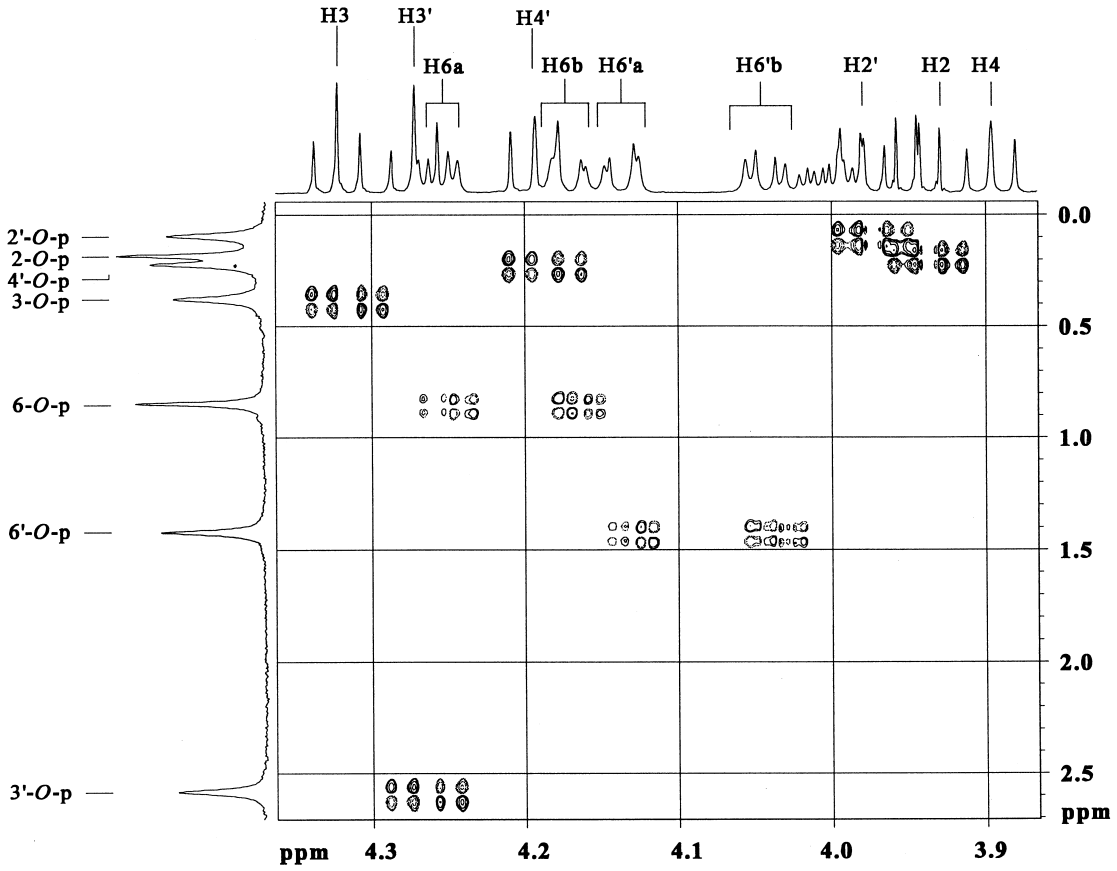


Figure 3. ^{1}H-^{31}P-Correlated NMR spectrum of compound **16a**.

spectroscopy, mass spectrometry) were in complete accordance with the proposed structures.

The glycoconjugates **VI**–**VIII** were tested[25,26] in vitro for ATIII-mediated anti-Xa and antithrombin activity and the results were compared with the conjugates **IV** and **V** (Table 1). Glycoconjugates **VI** and **VIII** having phosphate monoesters on their TBDs display strong antithrombin activities, which clearly exceed those of their counterparts with persulfated TBDs. The higher charge densities at the TBD most likely account for the increase in antithrombin activity. Conjugate **VII**, carrying phosphodiesters on its TBD, shows a surprisingly low antithrombin activity. Apparently, the bulky isopropyl groups on the TBD hamper an appropriate interaction with thrombin.

Conclusion

From the results presented in this paper it can be concluded that the TBD of heparin shows completely different structure–activity relationships than the ABD of heparin. The affinity of the TBD for thrombin is increased with the introduction of phosphate monoesters, whereas replacement of only one sulfate group for a phosphate monoester in the ABD already destroyed the affinity of the ABD for ATIII.[8] The introduction of lipophilic groups, although at different positions, showed an increase in affinity of the ABD for ATIII,[8] but resulted in an opposite effect in the affinity between TBD and thrombin. Apart from this, it should be emphasized that the thrombin inhibitory activity is mainly governed by the charge density of the TBD, a phenomenon also observed in other studies towards the SAR of the TBD.[14,15,27] Furthermore, the glycoconjugates **VI** and **VIII** are pointed out as promising antithrombotics.

Experimental

Materials and methods

^{1}H and ^{13}C NMR spectra were recorded with a Jeol JNM-FX-200 (200 and 50.1 MHz, respectively). 2-D (^{1}H-^{1}H COSY, ^{1}H-^{1}H TOCSY, ^{1}H-^{13}C COSY, ^{1}H-^{31}P COSY) NMR spectra were recorded at 300 MHz on a Bruker DPX-300 or at 600 MHz on a Bruker DMX-600. ^{1}H NMR chemical shifts in organic solvents are reported in ppm (δ relative to tetramethylsilane and ^{31}P chemical shifts are given relative to 85% H_3PO_4 as an external standard. For proton spectra in aqueous solutions (D_2O) the residual HDO peak was set at 4.80 ppm. Optical rotations were determined at 20°C by means of a Propol polarimeter. Mass spectra were recorded on a Finnigan MAT TSQ-70 equipped with an electrospray interface.

Dichloromethane, pyridine, diisopropylamine and diethyl ether were refluxed with CaH_2 for 3 h, distilled and

stored over molecular sieves (4 Å). *N,N*-Dimethylformamide was stirred with CaH_2 for 16 h and then distilled under reduced pressure and stored over molecular sieves (4 Å). Acetonitrile (Rathburn, HPLC-grade), isopropanol (Baker, p.a.), benzylalcohol (Merck, p.a.), tetrahydrofuran (THF, Biosolvent, HPLC-grade) were stored over molecular sieves (4 Å).

Trimethylsilyl trifluoromethanesulfonate, dithiothreitol (Aldrich), sodium hydride (60% dispersion in mineral oil), hydrazine monohydrate, trichloroacetonitrile, cesium carbonate, hydroxylamine hydrochloride, triphenylphosphine (Acros), *p*-toluenesulfonyl chloride, phosphor trichloride, potassium *tert*-butylate, *tert*-butylhydroperoxide (Merck), acetic anhydride (Baker, p.a.), *N*-(benzoyloxycarbonyloxy)-succinimide (Fluka) and *sulfo*-SIAB™ (Pierce) were used as received.

Column chromatography was performed on Merck Kieselgel 60 (230–400 Mesh). Gel permeation chromatography was accomplished on Sephadex LH20 (Pharmacia), Superdex 30 prep. grade (Pharmacia), Sephadex G25 and G50 (Pharmacia). TLC analysis was performed on DC Fertigfolien (Schleicher and Schüll, F1500, LS254). Compounds were visualized by UV absorption (254 nm), exposure to sublimated iodine crystals or charring with 20% H_2SO_4 in MeOH or 3% ninhydrin in ethanol.

High performance liquid chromatography (HPLC) (anion exchange) was conducted on a Waters 600E (system controller) single pump gradient system using a MonoQ prepacked HR5/5 column (Pharmacia) (anion exchange). A Waters model 484 variable wavelength UV detector was used for detection at 214 nm. An IBZ Messtechnik Chiralyser was used for optical rotation detection. Gradient elution in anion exchange was performed at 20°C by building up a gradient starting with buffer A 20% CH_3CN in H_2O and applying buffer B 2.0 M NaCl in 20% CH_3CN in H_2O at a flow rate of 1 mL/min.

Reactions were run at ambient temperature unless otherwise stated. Prior to reactions that require anhydrous conditions, traces of water were removed by coevaporation with pyridine, toluene, dioxane or 1,2-dichloroethane.

Tetraethyleneglycol-di-*p*-tosylate (2). To a stirred solution of tetraethylene glycol (**1**) (6.8 g, 35 mmol) in pyridine (175 mL) were added *p*-toluenesulfonyl chloride (20.0 g, 105 mmol) and a catalytic amount of DMAP (0.42 g, 3.5 mmol). After 16 h, TLC analysis indicated a complete conversion of **1** into **2**. The reaction mixture was concentrated and the residue was dissolved in EtOAc (400 mL) and washed three times with water (100 mL), where after the water layers were extracted twice with EtOAc (2×100 mL). The organic layers were combined, dried with $MgSO_4$ and concentrated in vacuo. The crude product was chromatographed on silica gel (50:50 to 30:70 light petroleum:EtOAc) to afford pure **2** (12 g, 71% yield). ^{1}H NMR ($CDCl_3$) δ 7.76 (d, 4H, H_{arom} Tos, J = 8.2 Hz), 7.30 (d, 4H, H_{arom} Tos, J = 8.2 Hz), 4.13 (t, 4H, CH_2OTos, J = 4.6 Hz), 3.65 (t, 4H, CH_2CH_2OTos, J = 4.6 Hz), 3.53 (bs, 8H, CH_2O TEG), 2.45 (s, 6H, CH_3 Tos); ^{13}C {^{1}H} NMR ($CDCl_3$) δ 144.5 (C_{arom} Tos), 132.5 (C_{arom} Tos), 129.5 (CH_{arom} Tos), 127.3 (CH_{arom} Tos), 69.7–68.0 (CH_2O TEG), 21.0 (CH_3 Tos).

1-Azido-1-deoxyhexaethylene glycol (5). Compound **3** (1.51 g, 8.0 mmol) and compound **2** (12 g, 23.8 mmol) were dissolved in DMF (100 mL) and heated to 50°C. Sodium hydride (0.48 g, 12 mmol, 60 wt% suspension) was added and the reaction mixture was stirred for 2 h. After TLC analysis indicated a conversion of **2** into **4**, lithium azide (3.92 g, 80 mmol) was added and the reaction temperature was elevated to 80°C. After stirring for 16 h, the excess sodium hydride was quenched with methanol (4 mL). The reaction mixture was concentrated and the residue was dissolved in diethyl ether (300 mL) and washed twice with aq $NaHCO_3$ (1 M, 2×150 mL), dried with $MgSO_4$, and concentrated in vacuo. The crude 1-azido-6-tetrahydropyranyl-1-deoxyhexaethyleneglycol thus obtained was treated with AcOH:THF:H_2O (160 mL, 4:2:1, v/v/v). After stirring for 4 h, TLC analysis revealed the reaction to be complete and the reaction mixture was neutralized with 1 N NaOH, concentrated to a smaller volume and extracted three times with diethyl ether (75 mL). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was chromatographed on silica gel (100:0 to 90:10 EtOAc:MeOH) to give pure **5** (1.34 g, 59% yield). ^{1}H NMR ($CDCl_3$) δ 3.75–3.59 (m, 22H, CH_2O HEG), 3.39 (t, 2H, CH_2N_3, J = 5.4 Hz); ^{13}C {^{1}H} NMR ($CDCl_3$) δ 72.4–69.7 (CH_2O HEG), 61.1 ($CH_2CH_2N_3$), 50.3 (CH_2N_3).

***N*-Benzyloxycarbonyl-1-amino-1-deoxyhexaethylene glycol (7).** Compound **5** (1.34 g, 4.36 mmol) and triphenylphosphine (1.83 g, 7.00 mmol) were dissolved in pyridine (5 mL). After stirring for 2 h, TLC indicated a complete conversion of the azide to the corresponding phosphinimine. Concentrated NH_4OH (25%, 10 mL) was then added and the solution was allowed to stand for 2 h, after which the reaction mixture was concentrated under reduced pressure. The crude **6** was dissolved in DMF (10 mL) and H_2O (5 mL) and treated with *N*-(benzoyloxycarbonyloxy)-succinimide (Z-OSu) (1.19 g, 4.80 mmol) and *N,N*-diisopropylethylamine (1.09 mL, 6.28 mmol). After 30 min, TLC analysis showed the complete disappearance of **6**. The reaction mixture was extracted three times with ether (25 mL), dried ($MgSO_4$), and concentrated in vacuo. The oily residue was purified by silica gel column chromatography (100:0 to 90:10 CH_2Cl_2:MeOH) to give **7** (1.53 g, 85% yield). ^{1}H NMR ($CDCl_3$) δ 7.34–7.28 (bs, 5H, H_{arom} Z), 5.29 (bs, 1H, NH), 5.10 (s, 2H, CH_2 Z), 3.72–3.53 (m, 22H, CH_2O HEG), 3.39 (t, 2H, CH_2NH); ^{13}C{^{1}H} NMR ($CDCl_3$) δ 155.9 (C=O Z), 136.1 (C_{arom} Z), 127.7–127.3 (CH_{arom} Z), 71.8–69.2 (CH_2O HEG), 65.7 (CH_2 Z), 60.7 (CH_2CH_2NH), 40.1 (CH_2NH).

Benzyl isopropyl *N,N*-diisopropylphosphoramidite (14). To a solution of phosphor trichloride (61 mL, 0.70 mol) in CH_3CN (24 mL) was added dropwise a solution of

benzylalcohol (10 mL, 0.10 mol) in 40 mL of the same solvent. After complete addition (5 min), the solution was left at ambient temperature for 10 min. After this period the reaction mixture was concentrated to remove unreacted PCl_3. ^{31}P NMR showed one signal (δ 177.7 ppm). Crude benzyl phosphorodichloridite was dissolved in Et_2O (75 mL) and cooled to −10°C. To this solution diisopropylamine (85 mL, 0.6 mol) was added dropwise and the reaction mixture was stirred overnight. ^{31}P NMR showed the presence of one resonance (δ 123.1 ppm). The reaction mixture was diluted with diethylether (100 mL) and washed with aq $NaHCO_3$ (1 M, 2×50 mL), dried with $MgSO_4$, and concentrated under reduced pressure to afford crude benzyl bis-(*N,N*-diisopropyl) phosphoramidite. To a solution of isopropanol (2.7 mL, 35 mmol) and *sym*-collidine hydrochloride (0.32 g, 2 mmol) in CH_2Cl_2 (25 mL) was added the crude bisamidite (7.5 g, 25 mmol). After 8 h, TEA (2.5 mL) was added and the mixture was concentrated. Purification of the residue by column chromatography (light petroleum:TEA 19:1, v/v) yielded pure **14** (2.1 g, 32% yield (3 steps)). ^{31}P NMR ($CDCl_3$) δ 145.75; $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 128.1–126.8 (CH_{arom} Bn), 66.8, 66.5 (O*C*H(CH_3)$_2$), 65.2, 64.9 (CH_2 Bn), 43.0, 42.7 (N*C*H(CH_3)$_2$), 24.7–24.4 (NCH(*C*H_3)$_2$, OCH(*C*H_3)$_2$).

4-*O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-2,3,6-tri-*O*-acetyl-α/β-D-glucopyranosyl trichloroacetimidate (10). Compound **8** (1.0 g, 1.5 mmol) was treated with a 0.1 M solution of hydrazine acetate in DMF (16.8 mL, 1.68 mmol) for 1 h. TLC analysis (EtOAc:light petroleum, 2:1, v/v) showed the reaction to be complete. After concentration in vacuo, the reaction mixture was diluted with EtOAc (50 mL), washed with $NaHCO_3$ (1 M, 3×25 mL), dried ($MgSO_4$), and concentrated under reduced pressure. Column chromatography (EtOAc: light petroleum, 2:1 to 1:1, v/v) of the residual oil gave pure **9**. Compound **9** was dissolved in CH_2Cl_2 (8 mL) and trichloroacetonitrile (0.76 mL) together with a catalytic amount of Cs_2CO_3 (99 mg, 0.30 mmol) were added. After 2 h, TLC analysis of the reaction mixture indicated a complete conversion of **9** into **10**. The reaction mixture was filtrated and the filtrate was concentrated under reduced pressure. Silica gel column chromatography (EtOAc:light petroleum:TEA, 50:49:1 to 75:24:1, v/v) furnished homogeneous **10** (1.04 g, 87% yield (two steps)). ^1H NMR ($CDCl_3$) δ 8.68 (s, 1H, $NH_{imidate}$), 6.48 (d, 1H, αH1, $J_{1,2}$ = 3.6 Hz), 5.57–4.89 (m, 5H, H2, H2′, H3, H3′, H4′), 4.55 (d, 1H, βH1′, $J_{1',2'}$ = 7.9 Hz), 4.57–3.66 (m, 6H, H5, H5′, H6a, H6′a, H6b, H6′b), 3.85 (dd, 1H, H4, $J_{3,4} = J_{4,5}$ = 9.3 Hz), 2.15–2.01 (7×s, 21H, CH_3 Ac); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 169.9–168.5 (7×C=O Ac), 160.2 (C=NH), 100.3 (βC1′), 92.3 (αC1), 90.2 (CCl_3), 75.6, 72.5, 71.4, 71.2, 70.5, 69.4, 68.8, 67.3 (C2, C2′, C3, C3′, C4, C4′, C5, C5′), 61.1, 61.0 (C6, C6′), 20.3–20.1 (7×CH_3 Ac).

***N*-Benzyloxycarbonyl-1-amino-1-deoxyhexaethylene glycol 4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (11).** A solution of donor **10** (1.04 g, 1.33 mmol) and acceptor **7** (0.55 g, 1.33 mmol) in CH_2Cl_2 (3 mL) was stirred for 1 h under a flow of argon in the presence of activated molecular sieves 4 Å (500 mg). The solution was cooled to −20°C and a solution of trimethylsilyl trifluoromethanesulfonate (26 μL) in CH_2Cl_2 (1 mL) was added dropwise to the reaction mixture. After 10 min, TLC analysis (5% MeOH in CH_2Cl_2) showed the presence of one product. Solid $NaHCO_3$ (0.5 g) was added to the reaction mixture, which was stirred for 10 min and then filtrated. The filtrate was diluted with CH_2Cl_2 (50 mL), subsequently washed with aq $NaHCO_3$ (1 M, 2×25 mL), dried ($MgSO_4$), and concentrated in vacuo. The residue was chromatographed on silica gel (CH_2Cl_2:MeOH, 100:0 to 95:5, v/v) yielding pure **11** (1.03 g, 75% yield). ^1H NMR ($CDCl_3$) δ 7.44–7.41 (m, 5H, H_{arom} Z), 5.15 (s, 2H, CH_2 Z), 5.36–5.11 (m, 3H, H3, H3′, H4′), 5.01–4.91 (m, 2H, H2, H2′) 4.59, 4.56 δ 2 (d, 2H, H1, H1′, $J_{1,2} = J_{1',2'}$ = 7.5 Hz), 4.61–4.16 (m, 2H, H6a, H6b, H6′a, H6′b), 3.78–3.60 (m, 25H, H4, H5, H5′, CH_2O HEG), 3.46 (t, 2H, CH_2NH, J = 5.2 Hz), 2.18–2.04 (7×s, 21H, CH_3 Ac); $^{13}C\{^1H\}$ NMR ($CDCl_3$) δ 170.4–168.9 (7×C=O Ac), 156.5 (C=O Z), 136.8 (C_{arom} Z), 128.4–127.8 (CH_{arom} Z), 100.7, 100.5 (C1, C1′), 76.5, 72.9, 72.6, 72.5, 71.8, 71.6, 67.8 (C2, C2′, C3, C3′, C4, C4′, C5, C5′), 70.5–68.9 (CH_2O HEG), 66.4 (CH_2 Z), 62.0, 61.6 (C6, C6′), 40.9 (CH_2NH), 20.8–20.2 (7×CH_3 Ac); ES–MS: $[M+H]^+$ 1034.6, $[M+Na]^+$ 1056.7, $[M+K]^+$ 1072.6.

***N*-Benzyloxycarbonyl-1-amino-1-deoxyhexaethylene glycol 4-*O*-cellobioside (12).** Compound **11** (0.40 g, 0.39 mmol) was treated with a solution of potassium *tert*-butylate (27 mg, 10 mg per mmol Ac) in MeOH (15 mL). After 1 h, TLC analysis (EtOAc:pyridine:AcOH:water, 5:7:4:1.6, v/v/v/v) indicated a complete conversion of **11** into **12**. The reaction was neutralized with Dowex 50 WX4-H^+ resin. The resin was removed by filtration and the filtrate was concentrated under reduced pressure to afford **12** (0.25 g, 88% yield), which was used without further purification. ^1H NMR (CD_3OD) δ 7.36–7.29 (m, 5H, H_{arom} Z), 4.40 (d, 1H, H1, $J_{1,2}$ = 7.9 Hz), 4.31 (d, 1H, H1′, $J_{1',2'}$ = 7.9 Hz), 3.89 (dd, 1H, H6a, $J_{5,6a}$ = 2.5 Hz, $J_{6a,6b}$ = 12.0 Hz), 3.86 (dd, 1H, H6′a, $J_{5',6'a}$ = 3.2 Hz, $J_{6'a,6'b}$ = 11.7 Hz), 3.70–3.65 (m, 2H, $H6_b$, $H6'_b$), 3.63–3.59 (m, 22H, CH_2O HEG), 3.56 (dd, 1H, H4′, $J_{3',4'} = J_{4',5'}$ = 9.0 Hz), 3.53 (t, 2H, CH_2CH$_2$NH, J = 5.8 Hz), 3.51 (dd, 1H, H3′, $J_{2',3'}$ = 8.9 Hz), 3.40 (ddd, 1H, H5′, $J_{5',6'b}$ = 6.6 Hz), 3.36 (dd, 1H, H3, $J_{2,3} = J_{3,4}$ = 8.8 Hz), 3.33 (ddd, 1H, H5, $J_{4,5}$ = 7.7 Hz, $J_{5,6b}$ = 5.7 Hz), 3.31–3.28 (m, 3H, CH_2NH, H4), 3.26 (dd, 1H, H2′), 3.22 (dd, 1H, H2); $^{13}C\{^1H\}$ NMR (CD_3OD) δ 155.9 (C=O Z), 136.1 (C_{arom} Z), 129.4–128.8 (CH_{arom} Z), 104.5, 104.1 (C1, C1′), 80.6, 78.0, 77.7, 76.4, 76.2, 74.8, 74.7 (C2, C2′, C3, C3′, C4, C4′, C5, C5′), 71.4–69.6 (CH_2O HEG), 67.3 (CH_2 Z), 62.3–61.8 (C6, C6′, *C*H_2CH$_2$NH), 41.7 (CH_2NH); ES–MS: $[M+H]^+$ 740, $[M+NH_4]^+$ 757, $[M+Na]^+$ 763.

***N*-Benzyloxycarbonyl-1-amino-1-deoxyhexaethylene glycol 4-*O*-cellobiosyl-heptakis(dibenzyl phosphate) (15a).** A solution of 1*H*-tetrazole (54 mg, 0.77 mmol) in CH_3CN (1 mL) was added to a mixture of **12** (45 mg, 56 μmol) and **13** (185 mg, 0.59 mmol) in CH_3CN (2 mL). After stirring for 1 h at 20°C, the reaction mixture was cooled with an ice bath and *tert*-butylhydroperoxide (0.5 mL)

was added. Stirring was continued for 45 min, after which TLC analysis showed the presence of one main product. Purification by silica gel column chromatography (100:0 to 95:5, CH_2Cl_2:MeOH, v/v) furnished pure **15a** (130 mg, 92% yield). ^{31}P NMR ($CDCl_3$) δ −0.09, −0.57, −0.71, −1.19, −1.45, −1.60, −2.15.

1-Amino-1-deoxyhexaethylene glycol 4-*O*-cellobiosyl-heptakisphosphate (16a). Compound **15a** (130 mg, 51 μmol) was dissolved in *tert*-butanol:H_2O (6:1, v/v, 20 mL) containing a few drops of acetic acid. The solution was stirred under a continuous stream of hydrogen in the presence of 10% Pd/C (100 mg). After 3 h the Pd/C catalyst was removed by filtration and the filtrate was concentrated in vacuo. Dowex 50 WX4-Na^+ ion-exchange then furnished **16a** (64.5 mg, 98% yield). ^{31}P NMR (D_2O, 600 MHz, ^{31}P-^{1}H COSY) δ 2.58 (3′-*O*-p), 1.42 (6′-*O*-p), 0.85 (6-*O*-p), 0.38 (3-*O*-p), 0.22 (4′-*O*-p), 0.18 (2-*O*-p), 0.09 (2′-*O*-p); ^{1}H NMR (D_2O, 600 MHz, HH-COSY) δ 4.76 (d, 1H, H1′, $J_{1',2'}$ = 8.0 Hz), 4.61 (d, 1H, H1, $J_{1,2}$ = 7.8 Hz), 4.32 (ddd, 1H, H3, $J_{2,3}$ = $J_{3,4}$ = 9.0 Hz, $^3J_{HP}$ = 18.6 Hz), 4.27 (ddd, 1H, H3′, $J_{2',3'}$ = $J_{3',4'}$ = 9.0 Hz, $^3J_{HP}$ = 18.4 Hz), 4.26 (ddd, 1H, H6a, $J_{5,6a}$ = 3.9 Hz, $J_{6a,6b}$ = 11.5 Hz, $^3J_{HP}$ = 14.2 Hz), 4.19 (ddd, 1H, H4′, $J_{3',4'}$ = $J_{4',5'}$ = 9.7 Hz, $^3J_{HP}$ = 19.1 Hz), 4.15 (ddd, 1H, H6b), 4.14 (ddd, 1H, H6′a), 4.04 (ddd, 1H, H6′b), 4.01 (m, 1H, H12a HEG), 3.97 (ddd, 1H, H2′, $^3J_{HP}$ = 10.7 Hz), 3.94 (ddd, 1H, H2, $^3J_{HP}$ = 17.4 Hz), 3.85 (m, 1H, H12b HEG), 3.79 (m, 1H, H5), 3.75–3.65 (m, 21H, H5′, CH_2O HEG), 3.18 (t, 2H, CH_2NH, J = 5.0 Hz); FAB–MS: $[M-2H+Na]^-$ 1186, $[M-3H+2Na]^-$ 1208, $[M-4H+3Na]^-$ 1230, $[M-5H+4Na]^-$ 1252, $[M-6H+5Na]^-$ 1274.

Condensation of 28 with 16a. To a solution of **16a** (12 mg, 8.3 μmol) in 0.1 M Na_2HPO_4 buffer (2.5 mL, pH 7.5) was added *sulfo*-SIAB™ (**25**) (31 mg, 62 μmol). After stirring for 3 h in the dark at 20°C, the reaction mixture was applied onto a Superdex 30 column eluted with 10% CH_3CN. The appropriate fractions were pooled and concentrated under reduced pressure at low temperature (25°C). The derivatized cellobiose-heptakisphosphate **26a** and pentasaccharide **28** (11.5 mg, 5.5 μmol) were dissolved in a 0.05 M hydroxylamine solution in 0.1 M Na_2HPO_4 buffer (1.6 mL, pH 7.0), which was degassed by passing through helium and by sonification. The reaction was stirred in an argon atmosphere. HPLC analysis (MonoQ anion exchange) indicated the reaction to be complete after 2 h. The reaction mixture was applied onto a Sephadex G-50 column eluted with 0.05 M NaCl in 10% CH_3CN in water. The appropriate fractions were pooled and concentrated to a small volume. To remove symmetrical disulfide from conjugate **VI**, the residue was treated with dithiothreitol (5 mg) dissolved in 0.1 M Na_2HPO_4 buffer (1 mL, pH 8.3). Size exclusion chromatography on a Sephadex G-50 column was followed by a gel filtration on a Superdex 30 column eluted with 10% CH_3CN in water. Concentration of the appropriate fractions and subsequent Dowex 50 WX-Na^+ ion-exchange gave after lyophilization conjugate homogeneous **VI** (13.8 mg, 68% yield). $[\alpha]_D$ +27.4 (*c* 0.5, H_2O). ^{31}P NMR (D_2O) 2.95, 2.08, 0.80, 0.77, 0.76, 0.39, 0.06; ^{1}H NMR (D_2O, 600 MHz, HH-COSY) δ 3.58, 3.57, 3.51, 3.50, 3.48, 3.47 (7×s, 21H, CH_3O), 4.40–4.00 (H6a/H6b sugar); ***ring D***: 5.41 (d, 1H, H1, $J_{1,2}$ = 3.6 Hz), 3.24 (dd, 1H, H2, $J_{2,3}$ = 9.3 Hz), 3.49 (m, 1H, H3), 3.35 (t, 1H, H4, $J_{3,4}$ = $J_{4,5}$ = 9,6 Hz), 3.84 (m, 1H, H5); ***ring E***: 4.62 (d, 1H, H1, $J_{1,2}$ = 7.5 Hz), 3.21 (dd, 1H, H2, $J_{2,3}$ = 8.5 Hz), 3.48 (m, 1H, H3), 3.86 (m, 1H, H4), 3.69 (m, 1H, H5); ***ring F***: 5.37 (d, 1H, H1, $J_{1,2}$ = 3.5 Hz), 4.27 (dd, 1H, H2, $J_{2,3}$ = 9.3 Hz), 4.49 (t, 1H, H3, $J_{3,4}$ = 9.3 Hz), 3.94 (t, 1H, H4, $J_{4,5}$ = 9.3 Hz), 4.17 (m, 1H, H5); ***ring G***: 5.04 (d, 1H, H1, $J_{1,2}$ = 3.0 Hz), 3.48 (m, 1H, H2), 3.71 (m, 1H, H3), 4.15 (m, 1H, H4), 4.73 (m, 1H, H5); ***ring H***: 5.11 (d, 1H, H1, $J_{1,2}$ = 3.5 Hz), 4.32 (dd, 1H, H2, $J_{2,3}$ = 9.3 Hz), 4.62 (t, 1H, H3, $J_{3,4}$ = 9.3 Hz), 3.94 (t, 1H, H4, $J_{4,5}$ = 9.3 Hz), 4.05 (m, 1H, H5); ***cellobiose***: (reducing end) 4.59 (d, 1H, H1, $J_{1,2}$ = 7.5 Hz), 3.97 (m, 1H, H2), 4.36 (ddd, 1H, H3, $J_{2,3}$ = $J_{3,4}$ = 10.7, $^3J_{HP}$ = 17.4 Hz), 3.92 (m, 1H, H4), 3.78 (m, 1H, H5); (non-reducing end) 3.99 (m, 1H, H2′), 4.27 (m, 1H, H3′), 4.20 (m, 1H, H4′); ***spacer***: 7.78, 7.59 (2×d, 4H, H_{arom} SIAB), 4.01 (s, 2H, $OCH_2C(O)$), 3.69–3.55 (m, 54H, CH_2O HEG/TEG), 2.84 (t, 2H, CH_2S, J = 5.0 Hz); ES–MS: $[M+H]^+$ 3265.3.

***N*-Benzyloxycarbonyl-1-amino-1-deoxyhexaethylene glycol 4-*O*-cellobiosyl-heptakis(benzyl isopropyl phosphate) (15b).** Prepared as described for **15a**, using **12** and **14** as starting materials. Purification by silica gel column chromatography (0–7% MeOH in CH_2Cl_2) gave **15b** (133 mg, 58% yield). ^{31}P NMR ($CDCl_3$) δ −1.07, −1.19, −1.40, −1.51, −1.90, −1.91, −1.92.

1-Amino-1-deoxyhexaethylene glycol 4-*O*-cellobiosyl-heptakis(isopropyl phosphate) (16b). Prepared as described for **16a**, using **15b** as starting material (94 mg, 97% yield). ^{31}P NMR (D_2O, 600 MHz, ^{31}P-^{1}H COSY) δ 0.05 (6′-*O*-p/6′-*O*-p), −0.83 (4′-*O*-p/2′-*O*-p), −1.13 (2-*O*-p), −1.30 (3-*O*-p), −1.38 (3′-*O*-p); ^{1}H NMR (D_2O, 600 MHz, HH-COSY) δ 4.94 (d, 1H, H1′, $J_{1',2'}$ = 5.7 Hz), 4.83 (d, 1H, H1, $J_{1,2}$ = 6.7 Hz), 4.54–4.04 (m, 9H, H3, H3′, C*H*$(CH_3)_2$), 4.36 (ddd, 1H, H4′, $J_{3',4'}$ = $J_{4',5'}$ = 3.5 Hz, $^3J_{HP}$ = 9.9 Hz), 4.23–4.19 (m, 3H, H2, H2′, H5), 4.15 (ddd, 1H, H6a, $J_{5,6a}$ = 7.7 Hz, $J_{6a,6b}$ = 10 Hz, $^3J_{HP}$ = 11 Hz), 4.12–4.10 (m, 2H, H6′a, H5′), 4.07 (ddd, 1H, H4), 4.06–4.02 (m, 2H, H12a HEG, H6′b), 3.87–3.77 (m, 20H, OCH_2 HEG), 3.21 (t, CH_2NH, J = 4.9 Hz), 1.32–1.27 (m, 42H, $CH(CH_3)_2$); ES–MS: $[M+H]^+$ 1461, $[M+Na]^+$ 1483, $[M+2Na-H]^+$ 1505, $[M+3Na-2H]^+$ 1527.

Condensation of 28 with 16b. Prepared as described for conjugate **VI**, using **16b** and **28** as starting materials. Purification of the crude conjugate **VII** proceeded in a similar fashion as described for conjugate **VI** (18 mg, 84% yield). $[\alpha]_D$ +22.9° (*c* 0.5, H_2O). ^{1}H NMR (D_2O, 600 MHz, HH-COSY) δ 3.60, 3.59, 3.58, 3.52, 3.51, 3.49, 3.43 (7×s, 21H, CH_3O), 4.28–4.09 (H6a/H6b sugar); ***ring D***: 5.47 (d, 1H, H1, $J_{1,2}$ = 3.5 Hz), 3.31 (dd, 1H, H2, $J_{2,3}$ = 8.7 Hz), 3.57 (m, 1H, H3), 3.40 (t, 1H, H4, $J_{3,4}$ = $J_{4,5}$ = 7.8 Hz), 3.88 (m, 1H, H5); ***ring E***: 4.68 (d, 1H, H1, $J_{1,2}$ = 7.5 Hz), 3.25 (dd, 1H, H2, $J_{2,3}$ = 8.5 Hz), 3.56 (m, 1H, H3), 3.82 (m, 1H, H4), 3.73 (m, 1H, H5); ***ring F***: 5.42 (d, 1H, H1, $J_{1,2}$ = 3.6 Hz), 4.32 (dd,

1H, H2, $J_{2,3}=8.5$ Hz), 4.56 (m, 1H, H3), 4.00 (t, 1H, H4, $J_{3,4}=J_{4,5}=8.4$ Hz), 4.21 (m, 1H, H5); ***ring G***: 5.17 (bs, 1H, H1), 3.56 (m, 1H, H2), 3.79 (m, 1H, H3), 4.19 (m, 1H, H4); ***ring H***: 5.17 (d, 1H, H1, $J_{1,2}=3.2$ Hz), 4.32 (dd, 1H, H2, $J_{2,3}=8.7$ Hz), 4.67 (m, 1H, H3), 3.98 (t, 1H, H4, $J_{3,4}=J_{4,5}=8.7$ Hz), 4.10 (m, 1H, H5); ***cellobiose***: (reducing end) 4.85 (d, 1H, H1, $J_{1,2}=6.0$ Hz), 4.23 (m, 1H, H2), 4.52 (m, 1H, H3), 4.09 (m, 1H, H4); (non-reducing end) 4.95 (d, 1H, H1′, $J_{1',2'}=7.1$ Hz), 4.22 (m, 1H, H2′), 4.53 (m, 1H, H3′), 4.36 (ddd, 1H, H4′); 4.28–4.09 (H5, cellobiose), 4.54–4.04 (m, 7H, C*H*$(CH_3)_2$), 1.38–1.31 (m, 42H, CH(C*H*$_3)_2$); ***spacer***: 7.80, 7.62 (2×d, 4H, H_{arom} SIAB), 4.09 (s, 2H, $OCH_2C(O)$), 3.75–3.62 (m, 54H, CH_2O HEG/TEG), 2.91 (t, 2H, CH_2S, $J=5.2$ Hz); ES–MS: $[M+H]^+$ 3561.2.

4-*O*-(4-*O*-(2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-2,3,6-tri-*O*-acetyl-α/β-D-glucopyranosyl trichloroacetimidate (20). To a stirred solution of maltotriose (**17**) (2.0 g, 4.0 mmol) in pyridine (100 mL) was added acetic anhydride (6.2 mL, 65 mmol) and a catalytic amount of DMAP (0.79 g, 6.5 mmol). After 5 h the reaction mixture was poured into aq $NaHCO_3$ (1 M, 250 mL) and extracted three times with EtOAc (200 mL). The combined organic layers were dried on $MgSO_4$ and concentrated in vacuo. The product was purified by column chromatography (light petroleum:EtOAc, 1:1 to 0:1, v/v) giving **18** as a white foam (3.5 g, 91% yield). Anomeric deacetylation of **18** (3.0 g, 3.1 mmol) was achieved in a similar fashion as described for the synthesis compound **9**. Purification by silica gel column chromatography (light petroleum: EtOAc, 3:2 to 1:0, v/v) gave **19** (2.7 g, 92% yield). Trichloroacetimidate **20** was subsequently prepared as described for compound **10**, using **19** as starting material. Purification of the crude **20** by column chromatography (light petroleum:EtOAc:TEA, 50:49:1 to 0:99:1, v/v/v) yielded pure **20** as white foam (1.9 g, 71% yield). ^{1}H NMR ($CDCl_3$) δ 8.67 (s, 1H, $NH_{imidaat}$), 6.48 (d, 1H, αH1, $J_{1,2}=3.7$ Hz), 5.64–5.07 (m, 6H, H2, H2′, H2″, H3, H3′, H3″, H4″), 5.02, 4.85, 4.75 (3×dd, 3H, H6a, H6′a, H6″a), 4.50 (m, 3H, H1′, H1″, H6b), 4.32–3.90 (m, 7H, H6′b, H6″b, H5, H5′, H5″, H4, H4′), 2.30–1.80 (m, 30H, CH_3 Ac); ^{13}C{^{1}H} NMR ($CDCl_3$) δ 169.9–168.7 (10×C=O Ac), 159.9 (C=NH), 95.4, 95.1 (C1′, C1″), 92.1 (C1), 90.1 (CCl_3), 73.0, 72.3, 71.1, 70.1, 69.9, 69.5, 68.7, 68.5, 67.9, 67.4 (C2, C2′, C2″, C3, C3′, C3″, C4, C4′, C4″, C5, C5′, C5″), 62.0, 61.0, 59.6 (C6, C6′, C6″), 20.2–19.7 (10×CH_3 Ac).

***N*-Benzyloxycarbonyl-1-amino-1-deoxyhexaethylene glycol 4-*O*-(4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-2,3,6-tri-*O*-acetyl-α-D-glucopyranosyl)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside (21).** Prepared as described for **11**, using **20** and **7** as starting materials. Purification by silica gel column chromatography (0–4% MeOH in EtOAc) gave **21** (0.57 g, 56% yield). ^{1}H NMR ($CDCl_3$) δ 7.35–7.29 (m, 6H, H_{arom} Z, NH), 5.66–4.96 (m, 7H, H2, H2′, H2″, H3, H3′, H3″, H4″), 5.11 (s, 2H, CH_2 Z), 4.92–4.66 (m, 3H, H6a, H6′a, H6″a), 4.62 (d, 1H, $J_{1,2}=8.5$ Hz), 4.55–4.38 (m, 3H, H1′, H1″, H6b), 4.35–3.84 (m, 7H, H4, H4′, H5, H5′, H5″, H6′b, H6″b), 3.78–3.48 (m, 22H, CH_2O HEG), 3.40 (t, 2H, CH_2NH, $J=5.2$ Hz), 2.30–1.83 (m, 30H, CH_3 Ac); ^{13}C{^{1}H} NMR ($CDCl_3$) δ 170.4–169.2 (10×C=O Ac), 154.3 (C=O Z), 136.8 (C_{arom} Z), 128.5–127.9 (CH_{arom} Z), 100.2 (C1), 95.7, 95.6 (C1′, C1″), 75.2, 73.9, 72.7, 72.1, 71.9, 71.6, 69.3, 68.9, 68.5, 68.0 (C2, C2′, C2″, C3, C3′, C3″, C4, C4′, C4″, C5, C5′, C5″), 70.4–68.9 (CH_2O HEG), 66.3 (CH_2 Z), 63.4, 63.1, 62.4, 61.4 (C6, C6′, C6″, *C*H_2CH_2NH), 40.8 (CH_2NH), 20.8–20.5 (10×CH_3 Ac); ES–MS: $[M+H]^+$ 1322.8, $[M+Na]^+$ 1344.8.

***N*-Benzyloxycarbonyl-1-amino-1-deoxyhexaethylene glycol 4-*O*-(4-*O*-(α-D-glucopyranosyl)-α-D-glucopyranosyl)-β-D-glucopyranoside (22).** Prepared as described for **12**, using **21** as starting material (177 mg, 95% yield). ^{1}H NMR (CD_3OD) δ 7.35 (bs, 5H, H_{arom} Z), 5.15 (d, 1H, H1′, $J_{1',2'}=2.9$ Hz), 5.14 (d, 1H, H1″, $J_{1'',2''}=3.8$ Hz), 5.07 (s, 2H, CH_2 Z), 4.33 (d, 1H, H1, $J_{1,2}=7.8$ Hz), 3.98 (m, 1H, H12a HEG), 3.87 (m, 1H, H6a), 3.83 (m, 1H, H6′a), 3.81 (m, 1H, H6″a), 3.77 (m, 1H, H6b), 3.76 (m, 1H, H6′b), 3.73 (m, 1H, H6″b), 3.71 (m, 1H, H12b HEG), 3.68–3.62 (m, 4H, H3, H3′, H3″, H5″), 3.62–3.58 (m, 18H, CH_2O HEG), 3.55–3.48 (m, 6H, H2′, H4, H4′, H5′, C*H*$_2CH_2NH$), 3.45 (dd, 1H, H2″, $J_{2'',3''}=9.5$ Hz), 3.38 (ddd, 1H, H5, $J_{4,5}=6.7$ Hz, $J_{5,6a}=4.7$ Hz, $J_{5,6b}=3.0$ Hz), 3.31–3.27 (m, 3H, CH_2NH, H4″), 3.25 (dd, 1H, H2, $J_{2,3}=9.3$ Hz); ^{13}C{^{1}H} NMR (CD_3OD) δ 154.4 (C=O Z), 136.8 (C_{arom} Z), 129.8–128.8 (CH_{arom} Z), 104.2 (C1), 102.7, 102.6 (C1′, C1″), 81.3, 77.6, 76.5, 75.0, 74.9, 74.8, 74.7, 74.6, 74.1, 73.7, 73.3 (C2, C2′, C2″, C3, C3′, C3″, C4, C4′, C4″, C5, C5′, C5″), 71.4–70.9 (CH_2O HEG), 67.3 (CH_2 Z), 62.7–62.1 (C6, C6′, C6″, *C*H_2CH_2NH), 41.7 (CH_2NH); ES–MS: $[M-H]^-$ 900.

***N*-Benzyloxycarbonyl-1-amino-1-deoxyhexaethylene glycol 4-*O*-maltotriosyl-decakis(dibenzyl phosphate) (23).** Prepared as described for **15a**, using **22** as starting material, which was dissolved in CH_3CN:dioxane (2:1, v/v) (216 mg, 92% yield). ^{31}P NMR: δ 0.16, −0.08, −0.76, −0.90, −0.92, −1.21, −1.25, −1.37, −1.82.

1-Amino-1-deoxyhexaethylene glycol 4-*O*-maltotriosyl-decakisphosphate (24). Prepared as described for **16a**, using **23** as starting material (131 mg, 96% yield). ^{31}P NMR (D_2O, 600 MHz, ^{31}P-^{1}H COSY) δ 1.85 (3″-*O*-p′), 1.68 (2′-*O*-p), 1.01 (2″-*O*-p), 1.60, 0.60, 0.50 (6″-*O*-p/6′-*O*-p/6-*O*-p), 0.68 (2-*O*-p), 0.21 (4″-*O*-p), −0.52 (3′-*O*-p), −0.69 (3-*O*-p); ^{1}H NMR (D_2O, 600 MHz, HH-COSY) 5.78 (d, 1H, H1″, $J_{1'',2''}=3.4$ Hz), 5.60 (d, 1H, H1′, $J_{1',2'}=3.8$ Hz), 4.68 (ddd, 1H, H3′, $J_{2',3'}=J_{3',4'}=7.3$ Hz, $^3J_{HP}=16.3$ Hz), 4.65 (d, 1H, H1, $J_{1,2}=7.2$ Hz), 4.48 (ddd, 1H, H3, $J_{2,3}=J_{3,4}=8.3$ Hz, $^3J_{HP}=16.8$ Hz), 4.43 (ddd, 1H, H3″, $J_{2'',3''}=J_{3'',4''}=7.3$ Hz, $^3J_{HP}=16.8$ Hz), 4.26 (m, 1H, H2′), 4.24 (m, 1H, H4″), 4.21–4.10 (m, 6H, H6a, H6′a, H6″a, H6b, H6′b, H6″b), 4.19 (m, 1H, H2″), 4.07 (dd, 1H, H4′, $J_{4',5'}=9.3$ Hz), 4.03 (ddd, 1H, H2, $^3J_{HP}=17.9$ Hz), 3.99 (m, 1H, H4), 3.88–3.81 (m, 3H, H5, H5′, H5″), 3.75 (t, 2H, C*H*$_2CH_2NH$, $J=5.6$ Hz), 3.70–3.66 (m, 20H, CH_2O HEG), 3.19 (t, 2H, CH_2NH); ES–MS: $[M+H]^+$ 1568, $[M+Na]^+$ 1590, $[M+2Na-H]^+$ 1612, $[M+3Na-2H]^+$ 1634.

Condensation of 28 with 24. Prepared as described for conjugate **VI**, using **24** and **28** as starting materials.

Purification of the crude conjugate **VIII** proceeded in a similar fashion as described for conjugate **VI** (10.4 mg, 51% yield). $[\alpha]_D$ +42.8° (*c* 0.5, H_2O). ^1H NMR (D_2O, 600 MHz, HH-COSY) δ 3.68, 3.67, 3.66, 3.60, 3.59, 3.57, 3.51 (7×s, 21H, CH_3O), 4.47–4.20 (H6a/H6b sugar); ***ring D***: 5.51 (d, 1H, H1, $J_{1,2}$ = 3.4 Hz), 3.34 (dd, 1H, H2, $J_{2,3}$ = 8.1 Hz), 3.67 (m, 1H, H3), 3.45 (t, 1H, H4, $J_{3,4} = J_{4,5}$ = 8.5 Hz), 3.92 (m, 1H, H5); ***ring E***: 4.70 (d, 1H, H1, $J_{1,2}$ = 7.5 Hz), 3.30 (t, 1H, H2, $J_{2,3}$ = 7.5 Hz), 3.67 (m, 1H, H3), 3.94 (m, 1H, H4), 3.78 (m, 1H, H5); ***ring F***: 5.45 (d, 1H, H1, $J_{1,2}$ = 3.5 Hz), 4.36 (dd, 1H, H2, $J_{2,3}$ = 8.4 Hz), 4.58 (t, 1H, H3, $J_{3,4}$ = 8.4 Hz), 4.02 (t, 1H, H4, $J_{4,5}$ = 8.4 Hz), 4.26 (m, 1H, H5); ***ring G***: 5.12 (bs, 1H, H1), 3.65 (m, 1H, H2), 3.83 (m, 1H, H3), 4.23 (m, 1H, H4); ***ring H***: 5.20 (d, 1H, H1, $J_{1,2}$ = 3.4 Hz), 4.40 (dd, 1H, H2, $J_{2,3}$ = 8.5 Hz), 4.71 (t, 1H, H3, $J_{3,4}$ = 8.5 Hz), 4.00 (t, 1H, H4, $J_{4,5}$ = 8.5 Hz), 4.16 (m, 1H, H5); ***maltotriose***: (reducing end) 4.05 (m, 1H, H2), 4.54 (m, 1H, H3), 3.95 (m, 1H, H4); 5.58 (bs, 1H, H1′), 4.26 (m, 1H, H2′), 4.10 (m, 1H, H4′); (non-reducing end) 5.72 (bs, 1H, H1″), 4.22 (m, 1H, H2″), 4.52 (m, 1H, H3″), 4.24 (m, 1H, H4″); 3.91–3.84 (H5, maltotriose); ***spacer***: 7.85, 7.67 (2×d, 4H, H_{arom} SIAB), 4.10 (s, 2H, $OCH_2C(O)$), 3.76–3.70 (m, 54H, CH_2O HEG/TEG), 2.93 (t, 2H, CH_2S, J = 5.0 Hz). ES–MS: $[M+H]^+$ 3668.4.

Measurements of the ATIII-mediated antithrombin and anti-Xa activities. The inhibitory potency of heparin and its analogues was measured amidolytically according to a modified procedure described by Teien and Lie.[25,26] In this procedure thrombin is incubated for a short time with a buffered solution of human ATIII and the heparin analogue (**VI**–**VIII**). After incubation the residual thrombin activity is determined by addition of the chromogenic substrate S-2238 (H-D-Phe-Pip-Arg-pNA). After 2 and 7 min, respectively, the release of *p*-nitroaniline is measured spectrophotometrically at 405 nm and the difference in optical density (ΔOD) is calculated. The antithrombin activity of the compound was read from a calibration curve of standard heparin, which has an activity of 160 U mg^{-1} according to the Fourth International Standard of Heparin Calibration.

Similar experiments were carried out to measure the anti-Xa activity of heparin and its analogues. This requires the S-2222 chromogenic substrate (Bz-Ile-Glu-Gly-Arg-pNA) to determine the residual Xa activity. In this case the anti-Xa activity was compared with the 'natural' pentasaccharide (**I**), which has an activity of 700 U mg^{-1}.

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