

Synthetic Oligosaccharides as Heparin-Mimetics Displaying Anticoagulant Properties

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Abstract: Heparin and low molecular weight heparins are major clinical anticoagulants and the drugs of choice for the treatment of deep venous thrombosis. The discovery of an antithrombin binding domain in heparin focused interest on understanding the mechanism of heparin's antithrombotic/ anticoagulant activity. Various heparin-mimetic oligosaccharides have been prepared in an effort to replace polydisperse heparin and low molecular weight heparins with a structurally-defined anticoagulant. The goal of attaining a heparin-mimetic with no unwanted side-effects has also provided motivation for these efforts. This article reviews structure-activity relationship (SAR) of structurally-defined heparin-mimetic oligosaccharides.

Key Words: Heparin, anticoagulant, antithrombotic, antithrombin III, heparin pentasaccharide, factor Xa, thrombin, PI-88, bis-aldonic acid amides.

INTRODUCTION

When a blood vessel is damaged, tissue factors are released from the vessel. These initiate a complex chain of cellular and molecular reactions, called the coagulation cascade, leading to the clot formation (Fig. 1). Platelets begin to stick to the damaged vessel wall and a mesh of fibrin forms, as a result of coagulation cascade, trapping more platelets in a process called thrombosis.

Blood coagulation enzymes are inhibited by serine protease inhibitors (SERPINs), mainly antithrombin III (ATIII), which serves to control the coagulation process. ATIII is a single chain, anionic glycoprotein of molecular weight 58,000. With the exception of VIIa, all of the coagulation proteases are inhibited by ATIII. While ATIII has high affinity for these serine proteases, it has a slow turnover rate. Heparin binds to thrombin and ATIII, forming a ternary complex, accelerating the rate of thrombin inhibition by 2000-fold. Heparin cofactor II (HCII) is a second SERPIN that is structurally similar to ATIII. The physiological role of HCII might be to serve as a reserve thrombin inhibitor when the plasma concentration of ATIII becomes abnormally low. Unlike ATIII, HCII can only inhibit thrombin, and it can be potentiated by glycosaminoglycans (GAGs) other than heparin, including dermatan sulfate and heparan sulfate, both which are found on the luminal surface of the endothelium [1].

Heparin is a polydisperse, highly sulfated, linear polysaccharide consisting of 1,4- linked pyranosyluronic acid and 2-amino-2-deoxyglucopyranose repeating units (Fig. 2). Heparin was discovered in 1916, and introduced clinically in 1935. The anticoagulant activity of heparin is

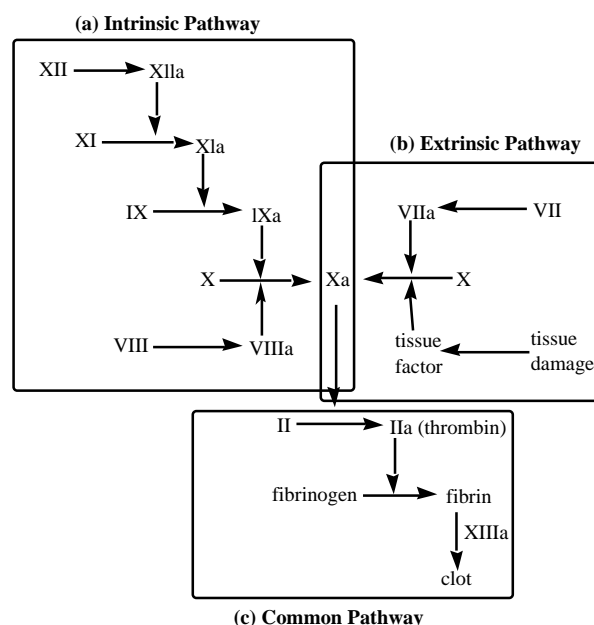
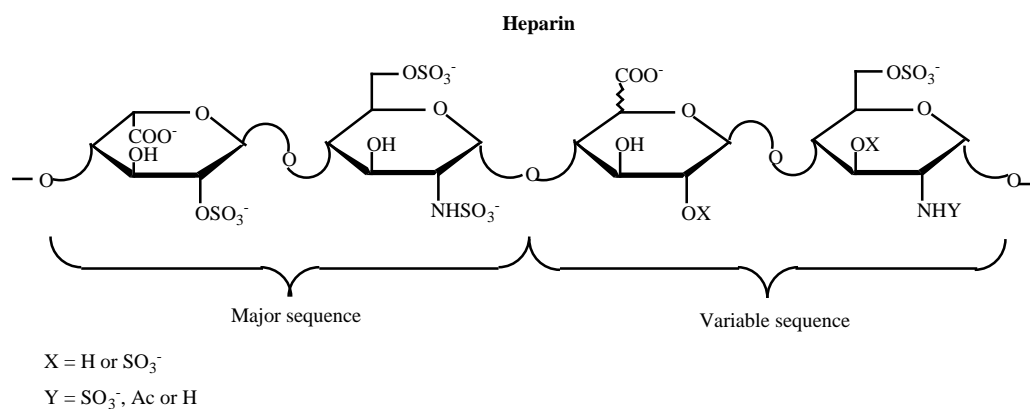


Fig. (1). The coagulation cascade is divided into the intrinsic and extrinsic pathway. (a) The intrinsic pathway is initiated by non-endothelial cells, followed by the activation of circulating coagulation factors, also leading to the formation of factor Xa. (b) The extrinsic pathway is activated by tissue damage, releasing thromboplastin, calcium and phospholipids from the cell membrane. These factors activate factor VII, transforming factor X into its proteolytically activated form, factor Xa. Both the intrinsic and extrinsic pathways merge into a final common pathway (c). The remaining steps include conversion of prothrombin (factor II) to thrombin (factor IIa) under factor Xa catalysis, then thrombin cleavage of fibrinogen to form a fibrin clot.

primarily mediated through ATIII and HCII. An understanding of its structure developed gradually and is still under intensive study [2-7].

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**Fig. (2).** General structure of heparin.

Low molecular weight heparin (LMWH) is a promising new type of heparin, which is prepared through controlled chemical or enzymatic depolymerization of heparin to lower its molecular weight. LMWHs are administered subcutaneously and do not require monitoring of the activated partial thromboplastin time (APTT), making them much easier to use. LMWHs are combined inhibitors of both thrombin and factor Xa inhibitors. Because of their reduced molecular weight, however they are 2- to 5-fold more potent inhibitors of factor Xa than thrombin.

Despite the widespread clinical use of heparin and related LMWHs as anticoagulants, heparin based drugs have some major disadvantages, such as structural diversity [8], potential pathogen contamination due to their origin from animal tissues, and the clinical problem of heparin-induced thrombocytopenia (HIT), exhibited by patients treated with these drugs [9, 10].

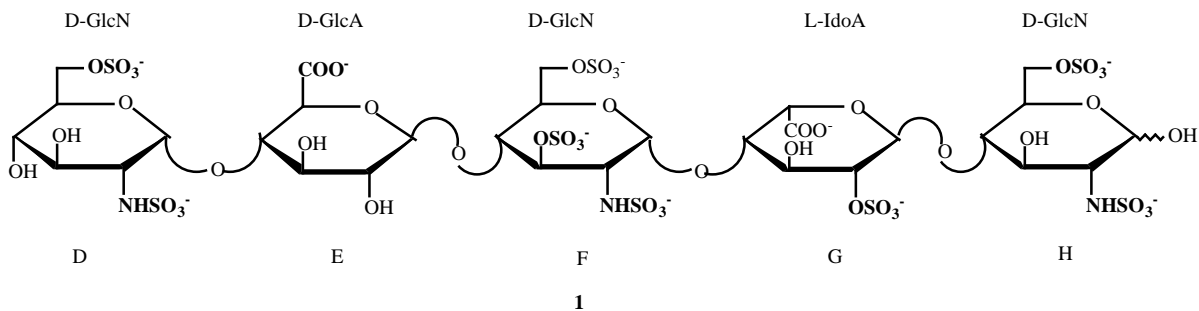
Recently, studies have focused on developing small, structurally-defined heparin-mimetics with antithrombin activity similar to natural heparin, but with reduced side effects. The potential advantages and disadvantages of such compounds are summarized in (Table 1). This review describes the anticoagulant properties of a range of selected structurally-defined, heparin-mimetic oligosaccharides. A number of other review articles covering different aspects of heparin-mimetic anticoagulant agents have recently been published [11-14].

Table 1. Advantages and Disadvantages of Small Structurally-Defined Oligosaccharides as Anticoagulants Compared to Heparin and LMWHs

Advantages	Disadvantages
Better pharmacokinetics	Potential toxicity
Better bioavailability	High cost
Synthetic or semisynthetic source	Single component may not be equivalent to multi-component regulation
Better dose control monitoring	

HEPARIN PENTASACCHARIDE

The structure of the antithrombin binding domain of heparin (Fig. 3) was discovered through the controlled enzymatic (heparinase) and chemical (nitrous acid) depolymerization of heparin followed by the separation of fractions having ATIII-binding affinity (Fig. 4). Octasaccharide 2 (heparinase digestion) and octasaccharide 3 (nitrous acid degradation), were found to have ATIII activity. The common hexasaccharide domain (4) was believed to contain the site required for ATIII binding. Altering the C residue of the hexasaccharide (4) did not result in a loss of biological activity, suggesting that DEFGH pentasaccharide is the active sequence [11, 15, 16]. The

**Fig. (3).** The role of charged groups in the ATIII binding of pentasaccharide are as follows. Removal of the *N*-sulfo group in residue D results in a 50% loss of anti-Xa activity, removal of either of the *O*-sulfo groups on residues G and H result in a 75% loss of activity, and the removal of any one of the 6-*O*-sulfo on residue D, carboxyl on residue E, 3-*O*-sulfo or *N*-sulfo on residue F, carboxyl on residue G, or *N*-sulfo group on residue H results in a 95% loss of activity (adapted from reference [van Boeckel, C.A.A. and Petitou, M., 1993]).

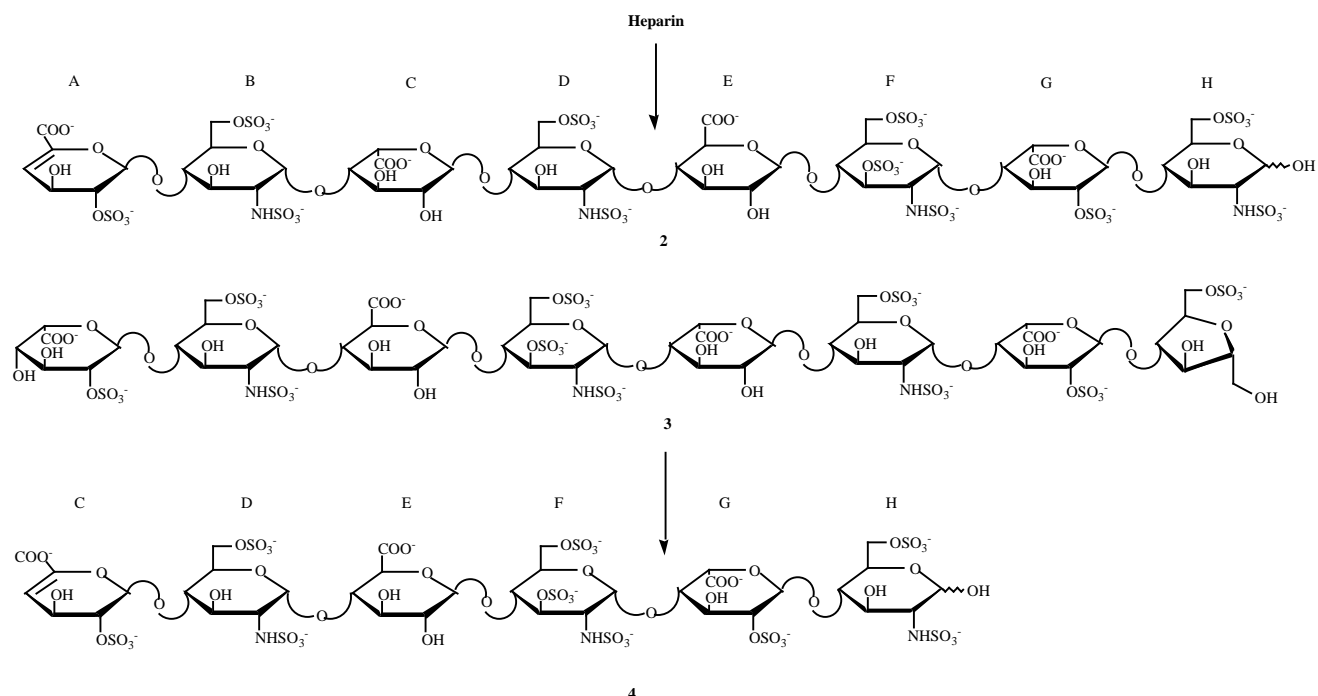


Fig. (4). Discovery of antithrombin binding domain of heparin.

sequence within heparin responsible for its anticoagulant activity was determined to be a unique pentasaccharide structure (**1**), called the antithrombin binding domain (ABD) [4, 16, 17]. This pentasaccharide sequence is composed of three glucosamine residues (D, F, and H), a glucuronic acid residue (E), and an iduronic acid residue (G) (Fig. 3). The sulfo group at C-3 of the central glucosamine residue (F) is a marker for the ATIII binding site. In the early 1980s a biologically active pentasaccharide was first synthesized from monosaccharide precursors by Sinaÿ and coworkers [18]. This synthesis was subsequently improved by Sinaÿ and others using a variety of different approaches [19-21].

The free hydroxyl group at the reducing end of the pentasaccharide caused side reactions during its preparation. Thus, a more stable analog, the methyl glycoside derivative of heparin pentasaccharide (**5**), was synthesized [22]. The antithrombotic activity (700 anti-Xa units (U)/mg) of this compound was demonstrated in animals and no bleeding was observed at therapeutic doses [23]. Pentasaccharide (**5**) is currently approved as a drug in Europe (Fondaparinux). Despite its multi-step synthesis, high cost, and selectivity for factor Xa (with no anti-IIa activity), this pentasaccharide offers the advantages of being a pure synthetic agent (Table 1).

A large number of analogs have been synthesized to better understand the structure-activity relationship (SAR) of pentasaccharide-ATIII interaction. These studies include site specific removal or addition of sulfo groups, removal of carboxyl groups, epimerization, flexible and constrained derivatives, replacement of sulfo groups with phospho groups, replacement of *N*-sulfo with *O*-sulfo groups, replacement of *N*-sulfo with *N*-acetyl groups, esterification and etherification of hydroxyl groups. An understanding of the structure-activity relationship (SAR) for the pentasaccharide

has developed about the essential structural features for pentasaccharide interaction with ATIII (Fig. 3). Several modifications have shown particularly interesting changes in biological activities, the addition of a 3-*O*-sulfo group to residue H, changes of *N*-sulfo to *O*-sulfo, changes of OH to OMe groups and studies on the uronic acid residues.

A very potent analog (**6**), having a second 3-*O*-sulfo group on the reducing end glucosamine residue H, was found to have anti-Xa activity of 1318 U/mg. Next studies were directed to simplify its synthesis. Thus, a non-glycosaminoglycan analog of (**6**), compound (**7**), was prepared in which *O*-sulfo groups replaced *N*-sulfo groups and *O*-alkyl ethers replaced hydroxyl groups, which showed anti-Xa activity of 1611U/mg (Fig. 5) [24, 25].

The importance of the uronic acid moieties in the ABD is another feature that has been intensively studied [26]. Conformational studies have shown the presence of the conformational equilibrium of 4C_1 , 1C_4 , and 2S_0 conformers at L-iduronic acid residue [27-32]. A flexible acyclic residue was used to replace either glucuronic acid (E) (**9**) or iduronic acid (G) (**10**) in the ABD (Fig. 6) [26]. The results of bioassay clearly demonstrated that residue E should be rigid while residue G should be flexible, consistent with previous studies [27-32]. The importance of conformational flexibility of L-iduronic acid was further established using conformationally locked L-iduronic acid derivatives of pentasaccharide, compounds (**11**) (2S_0 conformer), (**12**) (1C_4 conformer) and (**13**) (4C_1 conformer) (Fig. 6) [33]. Compound (**11**) showed about the same anti-factor Xa activity as heparin pentasaccharide methyl glycoside (**6**), whereas the other two conformers (**12** and **13**) showed low activity in an antithrombin-mediated anti-Xa assay. The importance of 2S_0 conformation of L-iduronic acid in antithrombin interaction was clearly demonstrated.

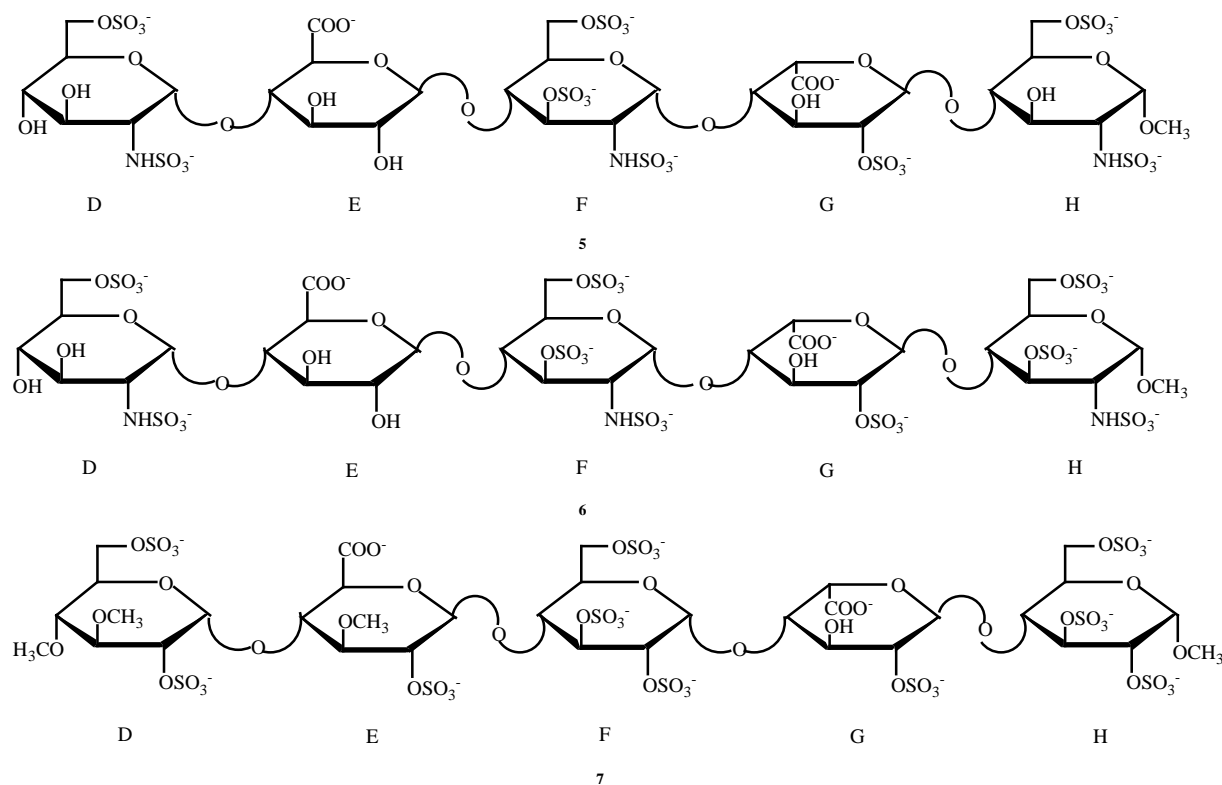


Fig. (5). Attempts to find more potent analogs of heparin pentasaccharide

The co-crystal structure of ATIII-heparin pentasaccharide complex [34, 35] revealed the interaction of heparin pentasaccharide with ATIII at the molecular level. Essential sulfo and carboxyl groups (Fig. 3) interact with the Lys and Arg residues of ATIII. The X-ray crystal structure also showed acidic amino acids close to the anomeric center of unit H. Heparin pentasaccharide analogs, containing positively charged groups at the reducing end, might be expected to interact specifically with acidic amino acids Glu113 and Asp117, in ATIII. To test this hypothesis, four heparin pentasaccharides having either one (14) and (15) or two (16) and (17) positively charged amino groups at their reducing end were synthesized (Fig. 6) [36]. The absence of anti-Xa activity in these analogs suggested that these amino acids in ATIII are not readily available for additional binding interactions. The formation of intramolecular salt bridges between the newly introduced amino functional groups and the sulfo groups of the pentasaccharide appear to be more favorable than the formation of intermolecular salt bridges between these amino groups in the pentasaccharide and Glu113 and Asp117 of ATIII.

HEPARIN OLIGOSACCHARIDES WITH THROMBIN BINDING SITE

A heparin pentasaccharide sequence corresponding to the ABD is responsible for some of heparin's ATIII mediated activity [18-20]. This pentasaccharide binds ATIII resulting in the inhibition of blood coagulation factor Xa. The inactivation of other serine proteases, including thrombin, requires a longer heparin chain. For thrombin inhibition, a heparin chain contain an ABD with adjacent repeating

trisulfated disaccharide residues corresponding to the major heparin repeating unit (Fig. 2) [37, 38].

The inhibition of thrombin requires the formation of a ternary complex involving heparin, thrombin and ATIII (Fig. 7). Thus, heparin chain is needed to be long enough to bind thrombin through thrombin binding domain (TBD) and ATIII through the ABD. The binding between heparin and thrombin is believed to occur through electrostatic interactions and to be less specific than the interaction of heparin to ATIII.

Heparin exerts its anticoagulant activity by enhancing the ATIII mediated inhibition of coagulation factor Xa and thrombin. Heparin oligosaccharides having both an ABD and a TBD should afford the full anticoagulant properties of heparin. Unfortunately the complex, multi-component structure of heparin is also believed to cause significant side-effects, such as thrombocytopenia resulting from interaction with platelet factor 4 (PF4) [39]. Homogenous synthetic oligosaccharides might promote selective binding to thrombin and ATIII, eliminating interaction with PF4, decreasing side-effects of anticoagulant therapy.

Modeling [40] and crystallography studies [35] suggest that the TBD should be located at the non-reducing end of ABD. The relative positions of the TBD and the ABD were recently experimentally confirmed [41]. The anticoagulant activities of compounds (18), (19) and (20) were, as expected, substantially higher than the activity of compound (21) (Fig. 8) (Table 2).

Heparin mimetics with the full anticoagulant properties of heparin were synthesized and their activities were studied

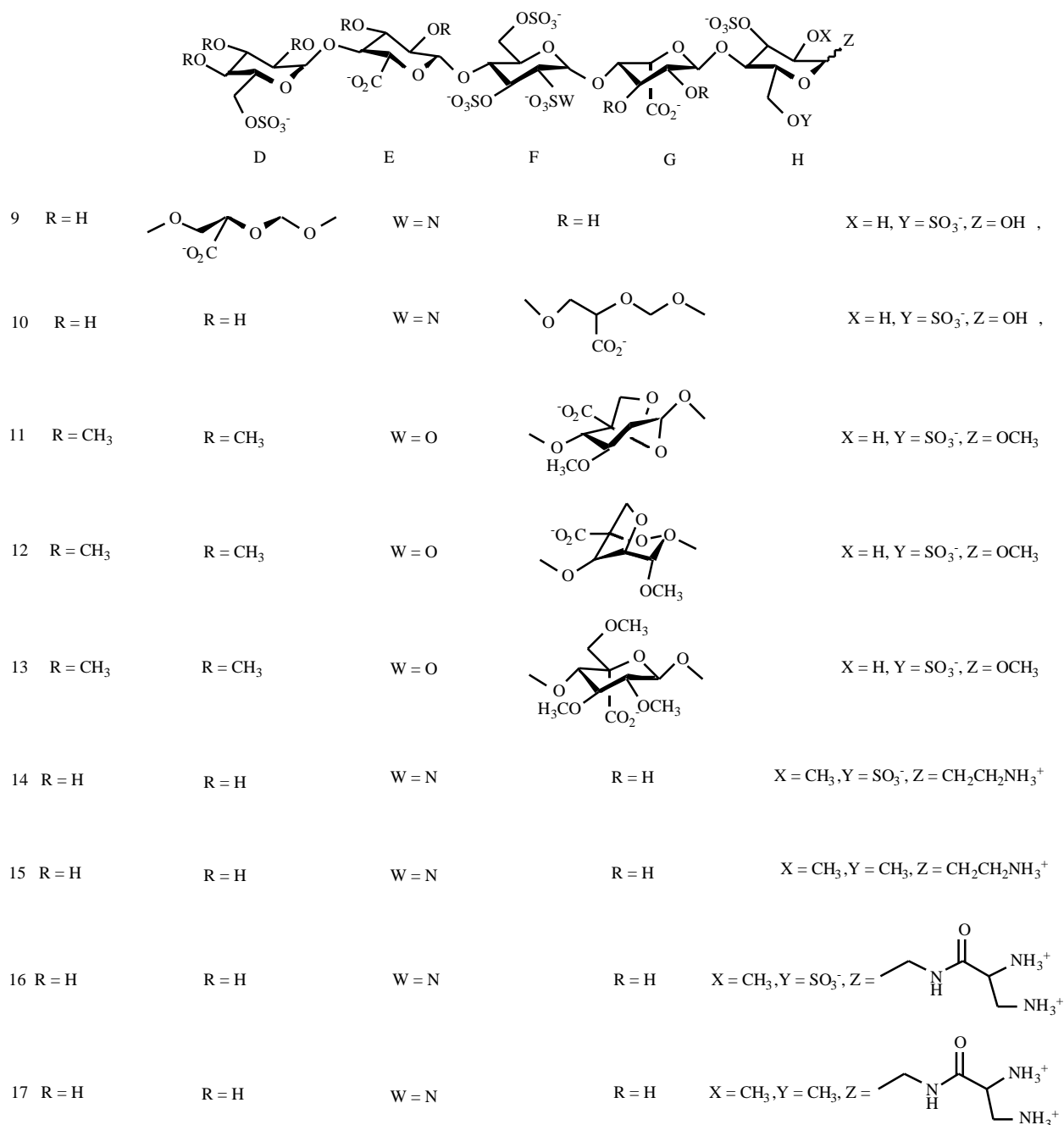


Fig. (6). Conformational and substituent studies on heparin pentasaccharide for better understanding of the ATIII interaction.

[42-47]. Compounds **22-27** consisted of multiple repeating ABDs (Fig. **9**) [45]. The ABD can also serve as a TBD since there is little specificity in this site (requiring only a high content of sulfo groups). Oligosaccharides having 16, 18 and 20 saccharide residues display a size-dependent increase in anticoagulant activity (Table **3**). This study demonstrates that while oligosaccharides containing at least 15 or 16 saccharide units display good anticoagulant activities, they also show undesirable interaction with PF4.

Compounds **18-20** were next synthesized having a single ABD at their reducing end and a TBD of various lengths,

consisting of alternating and -linked 3-*O*-methyl-2,6-di-*O*-sulfo-D-glucose residues (Fig. **8**). These compounds show *in vitro* anticoagulant activities, but their *in vivo* activity can be neutralized by PF4. Based on these experimental results, a set of criteria were established for the design of new anticoagulant agents. (1) A specific pentasaccharide sequence containing the ABD is required to activate antithrombin; (2) The TBD must be at the non reducing end of the ABD; (3) The TBD must be two to three disaccharides in length [38]; (4) A total chain length of 17 saccharide units is necessary for potent thrombin inhibition; and (5) The six

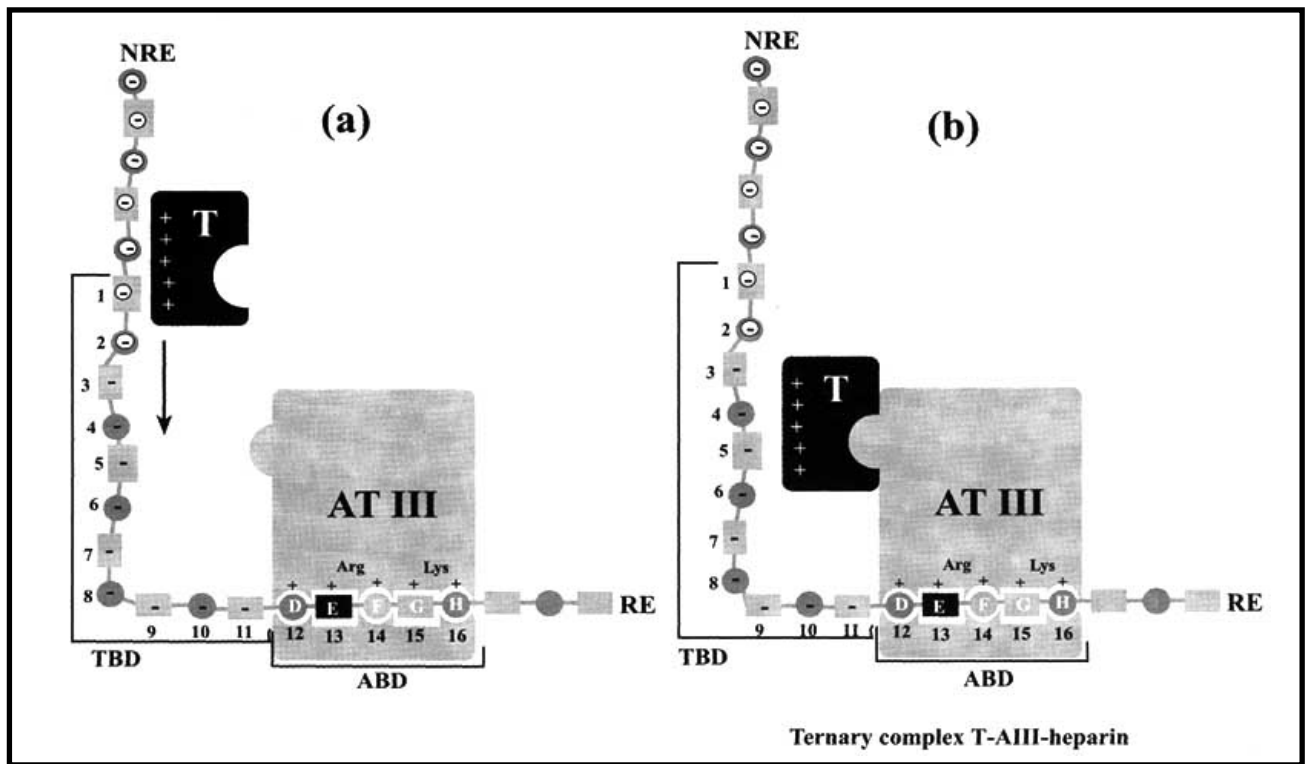


Fig. (7). Ternary complex of thrombin-heparin-ATIII responsible for anticoagulant activity. (a) Electrostatic interaction between thrombin (T) and TBD domain located to the nonreducing end side of the ABD of heparin. T slides along heparin. (b) T is bound to the TBD and to ATIII while ATIII is bound to the ABD and to T.

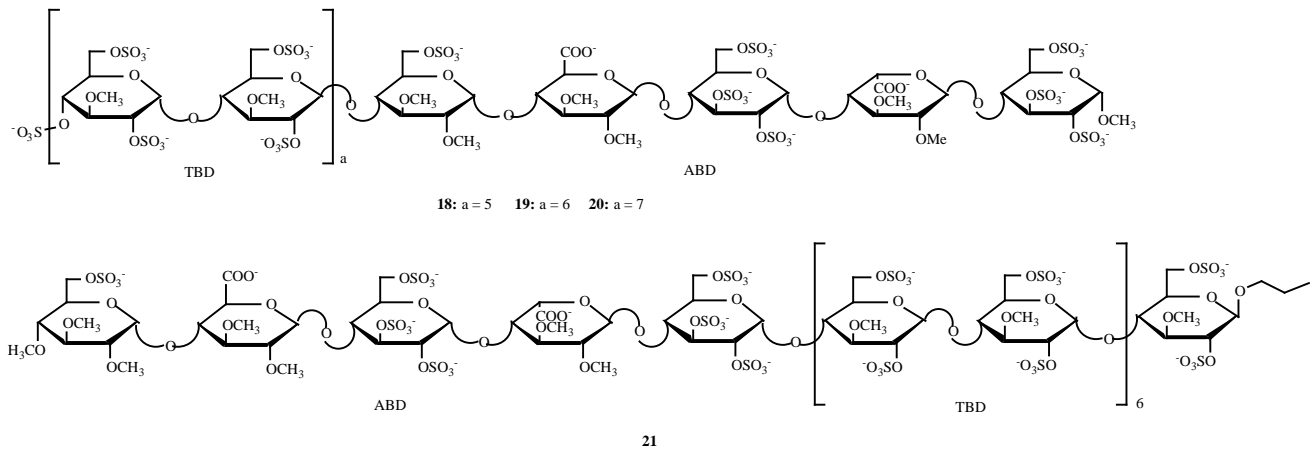


Fig. (8). The two possible ways of attaching the TBD to the ABD.

Table 2. Anti-Xa and Anti-IIa Activities of Compounds (18), (19), (20), (21) and Heparin*

Compound	18	19	20	21	heparin
Chain length (saccharide units)	15	17	19	18	~10-50
Factor Xa inhibition (units/mg)	370	270	290	230	180
Thrombin inhibition (IC ₅₀ in ng/mL)	41	5.3	1.7	164	3.3

*Petitou, M.; Duchaussoy, P.; Driguez, P.-A.; Héroult, J.-P.; Lormeau, J.-C. and Herbert, J.-M., 1999

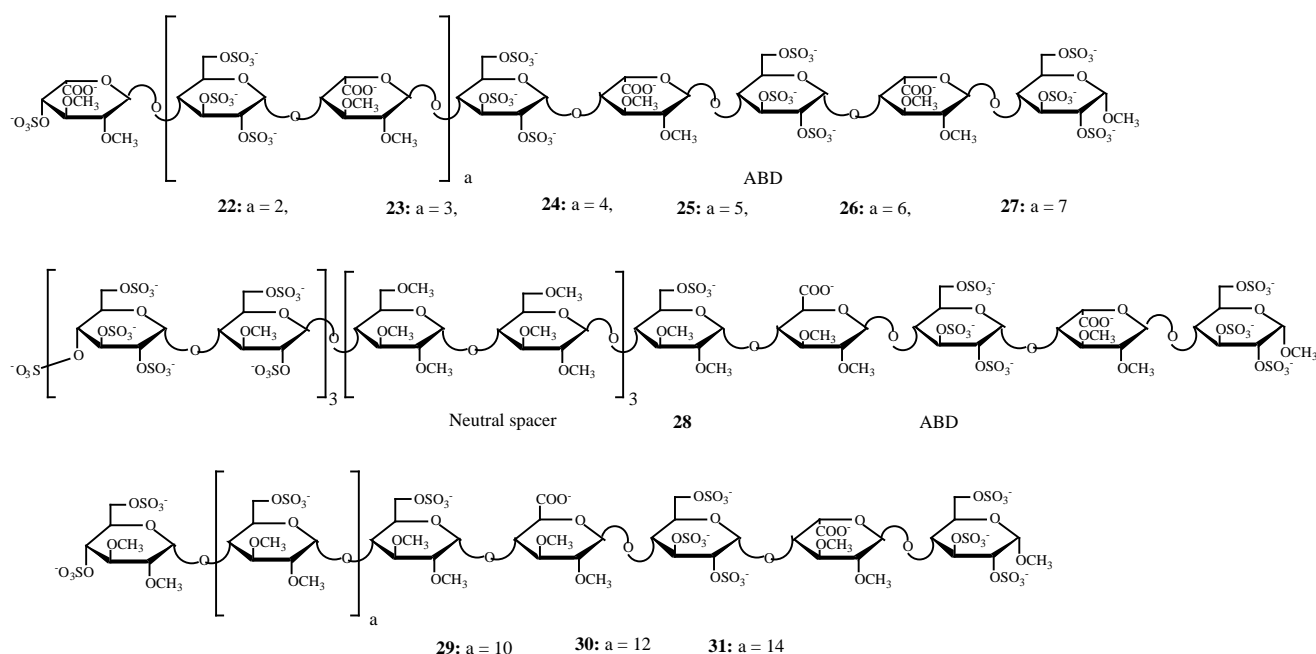


Fig. (9). Heparin mimetics with the full anticoagulant properties of heparin.

Table 3. *In Vivo* and *In Vitro* Activities of Synthetic Oligosaccharides and Heparin*

Compound	22	23	24	25	26	27	18	19	20	28	heparin
Chain length	10	12	14	16	18	20	15	17	19	17	~10-50
<i>In vitro</i> activities											
Factor Xa inhibition (units/mg)	405	360	310	359	270	236	370	270	290	270	180
Thrombin inhibition (IC ₅₀ , ng/mL)	>10,000	>10,000	>10,000	130	23	6.7	41	5.3	1.7	5.3	3.3
<i>In vivo</i> activities											
Venous thrombosis (ED ₅₀ , µg/kg)	280	360	390	150	250	110	65.5	40	38	15	80
Arterial thrombosis (ED ₅₀ , µg/kg)	>10,000	>10,000	>10,000	520	610	620	380	570	770	70	700

*Petitou, M.; Héroult, J.-P.; Bemat, A.; Driguez, P.-A.; Duchaussoy, P.; Lormeau, J.-C. and Herbert, J.-M., 1999

central saccharides are thought not to be essential in the interaction with ATIII and thrombin, so the charge can be decreased or eliminated without affecting the anticoagulant activity.

Applying the criteria above compound **28** was synthesized (Fig. 9) and showed *in vitro* and *in vivo* activity comparable to that of heparin (Table 3). One remarkable feature of compound (**28**) is that it did not interact *in vivo* with PF4 suggesting that the intervening neutral spacer prevents interaction with PF4.

An alternative approach for interrupting the interaction of heparin oligosaccharides with other proteins is to synthesize compounds with a reduced number of sulfo groups. Three oligosaccharides containing an ABD extended at the non-reducing end by a TBD (repeated 2, 3-di-*O*-methyl-6-*O*-

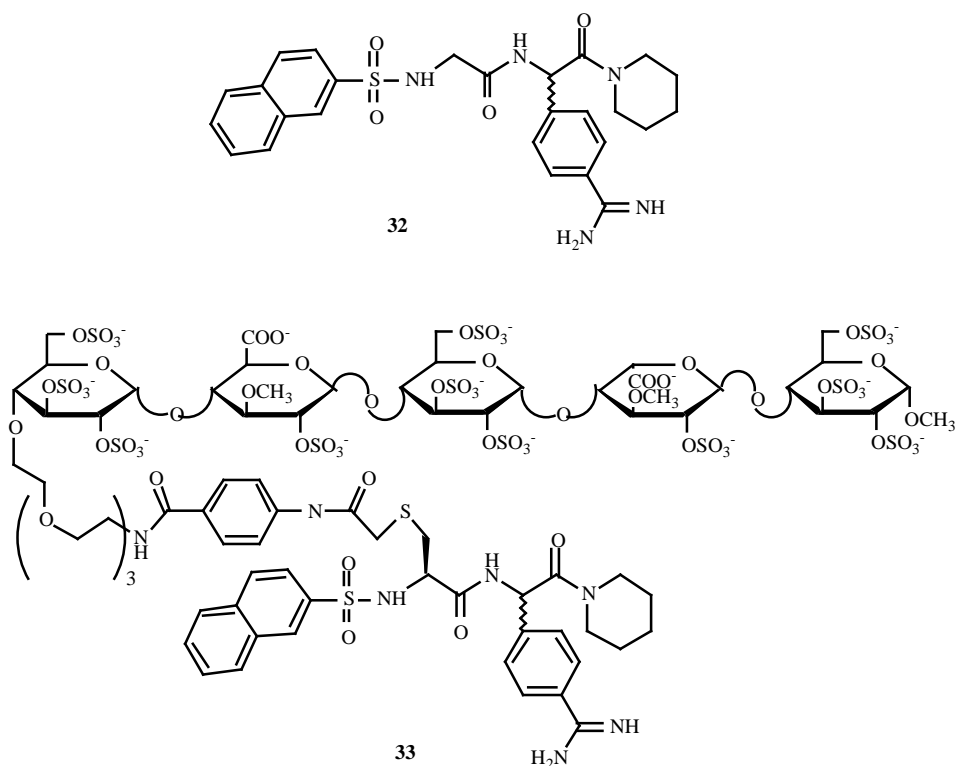
sulfo- -D-glucopyranosyl units) (**29-31**) were synthesized (Fig. 9) [46]. The 6-position was chosen for sulfonation because it could be easily differentiated, thus simplifying the synthesis. The *in vitro* anticoagulant activities of compounds **29-31** are shown in (Table 4). None of these oligosaccharides is equi-potent with heparin in inhibiting thrombin, but **31** is comparable in activity with LMWH. Most significantly, none of these compounds could be neutralized with even very high concentrations PF4.

Recently, an interesting non-sugar thrombin inhibitor, *N*-(2-naphthalenesulfonyl)-glycyl-(D)-4-aminophenyl-alanyl-piperidine (NAPAP), (**32**) was conjugated to a heparin pentasaccharide containing an ABD to obtain a dual action agent (**33**) (Fig. 10) [47]. NAPAP itself is a potent thrombin inhibitor (IC₅₀ = 0.75 µM) acting directly on thrombin's

Table 4. Anti-Xa and Anti-IIa Activities of Compounds (29), (30), (31), LMWH and Heparin*

Compound	29	30	31	LMWH	Heparin
Chain length (saccharide units)	16	18	20	~6-30	~10-50
Factor Xa inhibition (units/mg)	350	260	210	170	180
Thrombin inhibition (IC ₅₀ in ng/mL)	490	360	88	27	3.3

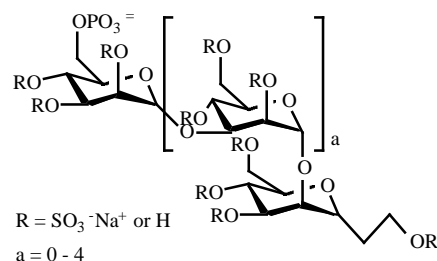
*Dríguez, P.-A.; Lederman, I.; Strassel, J.-M.; Herbert, J.-M. and Petitou, M., 1999

**Fig. (10).** NAPAP (32) and a dual action agent: NAPAP-heparin pentasaccharide complex (33).

active site, and DEFGH is a known, potent, selective anti-Xa agent. NAPAP-DEFGH conjugate (33) demonstrated both potent anti-IIa activity (IC₅₀ = 0.35 μM) and anti-Xa activity (885 U/mg). This activity is synergistic, since (33) was a stronger inhibitor than the same concentration of free pentasaccharide and NAPAP.

PI-88

PI-88 is a mixture of highly sulfated oligosaccharides prepared by sulfonation of the oligosaccharide phosphate fraction of the extra-cellular phosphomannan produced by the yeast, *Pichia holstii* [48, 49]. Analysis showed that the phosphomannan mixture is composed of Man-6-phosphate (3%), monophosphorylated disaccharide (3%), tetrasaccharide (28.5%), pentasaccharide (59%), and hexasaccharide (1%). Additionally, monophosphorylated trisaccharide and tetrasaccharylamine are present (5.5% combined) (Fig. 11) [50].

**Fig. (11).** General structure of PI-88.

PI-88 is a promising inhibitor of tumor growth and metastasis [51]. It shows its activity by inhibiting heparanase enzyme which degrades heparan sulfate proteoglycans in the extracellular matrix. These degradative activities are involved in tumor cell migration and invasion [52]. PI-88 is currently undergoing Phase II clinical evaluation in cancer patients.

The anticoagulant activity of PI-88 and its fractions PK1 (hexa- and pentasaccharides), PK3 (penta- and tetrasaccharides) PK4 (tetra) and PK5 (tri- and disaccharides) were assessed using various assays [50] (Table 5). Studies demonstrated concentration dependence [50] as well as chain length dependence [50, 53] for PI-88 anticoagulant activities. PI-88 and its fractions showed very little anti-Xa activity suggesting that PI-88 is an anti-IIa specific agent. Heparin neutralizing agents including protamine sulfate and PF4 fail to neutralize PI-88 activity [54]. Based on these studies, PI-88 was viewed as a promising anti-IIa specific anticoagulant. Because PI-88 does not contain an ABD, it is believed to inhibit thrombin through HCII. The high level of negative charge present in this highly sulfonated agent, however, raises concerns about potential side-effects associated with its interaction with other physiologically important basic proteins.

BIS-ALDONIC ACID AMIDES

Bis-aldonic acid amides are homogenous, structurally defined, synthetic carbohydrate-base compounds having interesting anticoagulant properties. They are synthesized by the reaction of aldonic acid lactones with α, ω -diamines followed by persulfonation [55].

Aprosulate sodium (**34**), a lactobionic acid amide derivative, was the first member of this class of compounds showing anticoagulant activities [56] (Fig. 12). Its biological activity is related to its high negative charge, appropriate size and structure. Aprosulat sodium, similar to PI-88, binds to HCII and inhibits the formation of factors VIII and Xa [57, 58].

Since the discovery of this class of compounds, numerous studies have been undertaken to understand their *in vivo* and *in vitro* activities [56, 57, 59-61]. The *in vivo* pharmacological properties of aprosulat sodium were investigated by administering aprosulat sodium to rats intravenously as a bolus injection [56]. Parallel experiments were performed using heparin for comparison. Experimental results suggested that although the antithrombotic ability of aprosulat sodium was modest, it had some advantages over heparin such as having a greater bleeding/ anticoagulant dose ratio, less bleeding complications, and no antiplatelet activity. The first human study with aprosulat sodium was performed on twelve healthy male volunteers [59]. Coagulation based assays were used for the identification of anticoagulant, pharmacokinetic and safety parameters. These studies suggested that twice a day aprosulat treatment was sufficient for clinical applications. Evaluation of aprosulat on platelet activation [61] showed that it inhibited thrombin-induced but not collagen-induced aggregation.

These results encouraged researchers to synthesize some other derivatives with close structural resemblance to aprosulat sodium [62] (Table 6). Both the sugar units and the chain length were varied. Their anticoagulant properties showed that all have lower activities than aprosulat sodium with the exception of compound LW 10121.

In another study, antithrombotic and anticoagulant activities of a bis-maltobionic acid amide, maltodapoh (**35**), were investigated [63]. Maltodapoh is a highly sulfated tetrasaccharide composed of two maltose sugars connected by a 1, 3-diamino-2-propanol linker (Fig. 13). Its synthesis involves electrochemical oxidation of maltose, followed by

Table 5. Anticoagulant Activity of PI-88 and Its Fractions*

Compound	APTT (sec)a, b	Heptest (sec)b, c	PT (sec)b, c	Anti-IIa (%) c
PI-88		60	17	32
PK-1		40	16	20
PK-3	300	100	14	26
PK-4	170	60	12	10
PK-5	100	40	11	6

a at 25 μ g/mL

b in the absence of substrate: APTT = 30 sec, Heptest = 10 sec, PT = 10 sec.

c at 50 μ g/mL

*Yu, G.; Gunay, N. S.; Linhardt, R. J.; Toida, T.; Fareed, J.; Hoppensteadt, D. A.; Shadid, H.; Ferro, V.; Li, C.; Fewings, K.; Palermo, M. C. and Podger, D., 2002

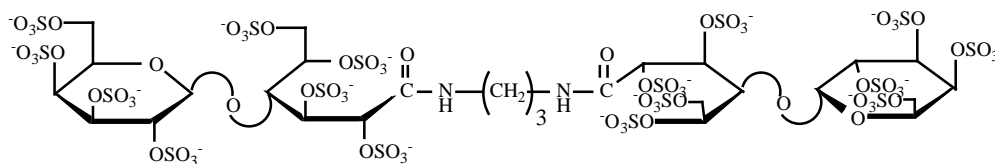


Fig. (12). Structure of aprosulat sodium.

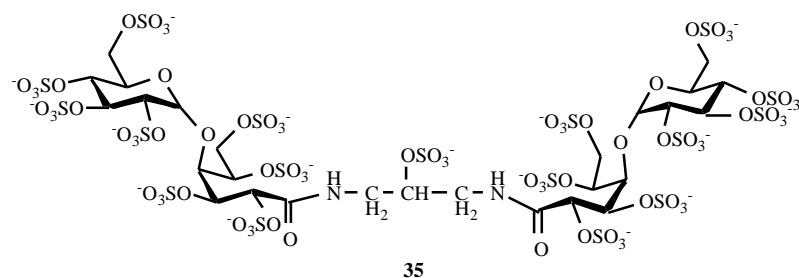
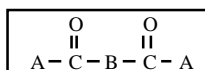


Fig. (13). Structure of maltodapoh.

Table 6. Biological Activities of Aldonic Acid Derivatives*



Compound	A (aldonic acid)	B	APTT (C ₁₅₀ (µg/mL))	anti-Xa (IC ₅₀ (µg/mL))	Thrombin Time (C ₁₅₀ (µg/mL))
Aprosulate	Lactobionic	— N — $\left(\begin{array}{c} \text{H}_2 \\ \text{C} \\ \text{H} \end{array} \right)_3$ — N —	3.4	42	195
LW 10114	Melibionic	— N — $\left(\begin{array}{c} \text{H}_2 \\ \text{C} \\ \text{H} \end{array} \right)_3$ — N —	4.5	32	168
LW 10168	Melibionic	— N — $\left(\begin{array}{c} \text{H}_2 \\ \text{C} \\ \text{H} \end{array} \right)_2$ — N —	4	19	170
LW 10125	D-Gluconic	— N — $\left(\begin{array}{c} \text{H}_2 \\ \text{C} \\ \text{H} \end{array} \right)_3$ — N —	7.5	54	720
LW 10244	L-Mannonic	— N — $\left(\begin{array}{c} \text{H}_2 \\ \text{C} \\ \text{H} \end{array} \right)_3$ — N —	13.5	>100	800
LW 10121	Lactobionic	— N — $\left(\begin{array}{c} \text{H}_2 \\ \text{C} \\ \text{H} \end{array} \right)_2$ — N —	1.5	45	215
Heparin			0.89	0.13	0.97

*Raake, W.; Klausner, R. J.; Elling, H.; Zeiller, P. and Meinetsberger, E., 1994

condensation with 1, 3 diamino-2-propanol to form the bis amide of maltobionic acid.

The *in vitro* addition of maltodapoh to plasma led to a concentration dependent increase in activated partial thromboplastin time (APTT), suggesting enhanced inhibition of thrombin by HCII. In animal studies, oral administration of the maltodapoh led to a 2- to 3-fold increase in APTT within 8 hours. Moreover, a single dose of compound (3mg/kg) resulted in anticoagulation for 24 hs.

Both aprosulate and maltodapoh are highly-charged thrombin specific agents acting through HCII. Like PI-88, there is some concern that these agents will show toxicity as a result of their highly charged nature and their ability to strongly bind to many basic proteins.

OTHER OLIGOSACCHARIDES

A recent study describes the anticoagulant activities of 17 different sulfated oligosaccharides [53]. These selected oligosaccharides differ in terms of monosaccharide residues, linkage, chain length, and degree of sulfation.

The anticoagulant activities of these agents were evaluated by APTT (Table 7). This study revealed that chain length, monosaccharide composition and linkage play important roles in anticoagulant activities of these oligosaccharides. The role of oligosaccharide chain length can be seen by a 19-fold increase in potency resulting on chain elongation from maltotetraose to maltopentaose sulfate.

Table 7. Anticoagulant activities of Oligosaccharides*

Compound	Monosaccharide constituent	Linkage	Increase in coagulation time (sec/ μ g)
Maltose sulfate	Glc	1 4	0.31
Maltotriose sulfate	Glc	1 4	0.25
Maltotetraose sulfate	Glc	1 4	0.28
Maltopentaose sulfate	Glc	1 4	5.25
Maltohexaose sulfate	Glc	1 4	5.85
Maltoheptaose sulfate	Glc	1 4	5.9
Isomaltopentaose sulfate	Glc	1 6	1.85
Isomaltohexaose sulfate	Glc	1 6	6.56
Cellotetraose sulfate	Glc	1 4	4.2
Cellopentaose sulfate	Glc	1 4	3.5
Cellohexaose sulfate	Glc	1 4	4.8
Laminaritetraose sulfate	Glc	1 3	2.9
Laminarihexaose sulfate	Glc	1 3	0.9
Laminariheptaose sulfate	Glc	1 3	0.5
Chitosan hexamer sulfate	GlcNAc	1 4	0.4
PI-88	Man	1 3, 1 2	5.67
Sucrose octasulfate	Glc, Fru	1 2	0.39
LMWH	GlcA, IdoA, GlcN	1 4, 1 4	11.20
Heparin	GlcA, IdoA, GlcN	1 4, 1 4	29.69

*Wall, D.; Douglas, S.; Ferro, V.; Cowden, W. and Parish, C., 2001

The importance of monosaccharide composition was exhibited by comparison of the low activity for the chitosan hexamer sulfate (1 4 linked glucosamine residues) with the higher activity of the cellohexaose sulfate (1 4 linked glucohexaose residues). The importance of linkage was demonstrated by the sulfated laminarin series (1 3 linked glucose units) exhibiting significantly lower activity than the 1 4, 1 6, and 1 4 linked glucose containing oligosaccharides.

None of the sulfated oligosaccharides evaluated in this study showed anticoagulant activity comparable with heparin or LMWH. Moreover, their evaluation by APTT assay fails to suggest a mechanism of action, although most of these agents are likely HCII mediated inhibitors of thrombin.

FUTURE DIRECTIONS

Many of the synthetic oligosaccharides described in this review still require *in vivo* evaluation of activity and toxicity in animal models, followed by clinical evaluation where appropriate. Among the most important new compounds for evaluation are the heparin oligosaccharides having thrombin binding sites. Future synthetic targets for *in vitro* evaluation as anticoagulants include the next generation of heparin oligosaccharide analogs having simplified backbone

structure, reduced charge character or ones having masked charge groups with potential oral bioavailability. Agents with both enhanced specificity as well as ones targeting multiple coagulation proteins are desired. Reduction of the side effects observed with LMWH and Fondaparinux might result from developing a new generation of anticoagulants. The application of combinatorial chemistry and high-throughput screening using multiple bioassays may also yield new lead compounds for chemical modification and subsequent *in vivo* evaluation.

CONCLUSION

Heparin and LMWH are the most commonly used anticoagulants despite several well-known side effects, e.g. bleeding and heparin-induced thrombocytopenia (HIT). Extensive efforts to develop better anticoagulants to replace heparin and LMWH have thus far been unsuccessful. While small synthetic heparin-mimetic oligosaccharides that combine the highly specific ABD with an adjacent TBD show activity comparable to heparin and LMWH, these molecules require a large number of synthetic steps, making them costly and possibly preventing their widespread clinical use. Other sulfated saccharides, while simpler to synthesize, require a high level of charge to show significant

anticoagulant effect. Their highly charged nature may result in significant interactions with multiple physiologically important proteins leading to many side-effects. While the studies described in this review have led to a better understanding of the SAR of heparin-mimetics, it has only led to the successful development of a single approved drug, the heparin pentasaccharide (5), Fondaparinux. More research in this area is clearly warranted to eliminate problems currently encountered with heparin and LMWHs.

ABBREVIATIONS:

SERPIN	= Serine protease inhibitor
SAR	= Structure-activity relationship
ATIII	= Antithrombin III
HCII	= Heparin cofactor II
GAG	= Glycosaminoglycan
LMWH	= Low molecular weight heparin
ABD	= Antithrombin binding domain
TBD	= Thrombin binding domain
PF4	= Platelet factor 4
NAPAP	= <i>N</i> -(2-naphthalenesulfonyl)-glycyl-(<i>D</i>)-4-aminophenyl-alanyl- piperidine
APTT	= Activated partial thromboplastin time
PT	= Prothrombin time
HIT	= Heparin-induced thrombocytopenia

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