DOI: 10.1002/med.21489

WILEY

REVIEW ARTICLE

Heparin mimetics with anticoagulant activity

Abdullah Al Nahain[1] | Vera Ignjatovic[2,3] | Paul Monagle[2,3,4] | John Tsanaktsidis[5] | Vito Ferro[1]

[1]School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Queensland, Australia

[2]Haematology Research, Murdoch Children's Research Institute, Parkville, Victoria, Australia

[3]Department of Paediatrics, University of Melbourne, Parkville, Victoria, Australia

[4]Department of Clinical Haematology, Royal Children's Hospital, Parkville, Victoria, Australia

[5]CSIRO Materials Science and Engineering, Clayton South, Victoria, Australia

Correspondence
Vito Ferro, School of Chemistry and Molecular Biosciences, The University of Queensland, Brisbane, Queensland 4072, Australia.
Email: v.ferro@uq.edu.au

Funding information
The University of Queensland; Australian Research Council, Grant/Award Number: DP170104431; CSIRO Manufacturing

Abstract

Heparin, a sulfated polysaccharide belonging to the glycosaminoglycan family, has been widely used as an anticoagulant drug for decades and remains the most commonly used parenteral anticoagulant in adults and children. However, heparin has important clinical limitations and is derived from animal sources which pose significant safety and supply problems. The ever growing shortage of the raw material for heparin manufacturing may become a very significant issue in the future. These global limitations have prompted much research, especially following the recent well-publicized contamination scandal, into the development of alternative anticoagulants derived from non-animal and/or totally synthetic sources that mimic the structural features and properties of heparin. Such compounds, termed heparin mimetics, are also needed as anticoagulant materials for use in biomedical applications (e.g., stents, grafts, implants etc.). This review encompasses the development of heparin mimetics of various structural classes, including synthetic polymers and non-carbohydrate small molecules as well as sulfated oligo- and polysaccharides, and fondaparinux derivatives and conjugates, with a focus on developments in the past 10 years.

KEYWORDS
anticoagulants, glycopolymers, heparin, heparin mimetics, sulfated oligosaccharides

1 | INTRODUCTION

At present, globally, a large number of people are affected with different types of cardiovascular diseases (CVDs), that is, myocardial infarction (heart attack), stroke, arterial thrombosis and venous thromboembolism (deep vein thrombosis and pulmonary embolism), where the underlying etiology behind these life threatening diseases is the formation of thrombus (accumulation of aggregated platelets and cross-linked insoluble fibrin).[1–3] According to a 2017 WHO report, CVD is the leading cause of mortality throughout the world accounting for 17.7 million deaths in 2015, and equivalent to 31% of all deaths.[4] Some of the most important treatment options for these life-threatening diseases are based on unfractionated heparin (UFH), low molecular weight heparins (LMWH), and the synthetic ultra-low molecular

weight heparin (ULMWH) pentasaccharide, that is, fondaparinux (**1**, Figure 3), see Table 1. Among these, UFH which is a sulfated polysaccharide (average molecular weight [MW] of most commercial preparations ~12–30 kDa) has been in use for decades and offers advantages such as rapid onset of action after intravenous administration, reversibility, widespread availability, and low cost. However, UFH is associated with serious complications such as bleeding, heparin-induced thrombocytopenia (which can be fatal), osteoporosis (frequently in females), hypoaldosteronism, heparin-induced skin necrosis, and variable dose response in different patients, requiring special monitoring.[5–9] A serious event took place in 2007/8 when more than 200 patients died after receiving UFH and hundreds were reported to have serious adverse effects due to contamination; specifically from adulteration of the UFH preparation with oversulfated chondroitin sulfate (CS).[10,14] UFH is obtained from animal tissue (porcine, or occasionally bovine, intestinal mucosa) and so there is potential for contamination from viruses. In addition, UFH has a very short half-life (less than 1 hr), and only approximately one-third of the administered dose elicits a therapeutic response.[11] Moreover, after complex formation with antithrombin (AT), heparin cannot inhibit the function of coagulation factors Factor Xa (FXa) and thrombin (FIIa) when they are bound to platelets and fibrin, respectively.[12] Therefore, LMWHs (average MW ~3.5–6 kDa), were developed to provide some advantages over UFH, for example, increased half-life, improved bioavailability.[13] There is also the additional advantage of no additional monitoring required following LMWH administration, so that patients do not need to be hospitalized. However, LMWHs also have some limitations such as functional irreversibility and a dependence on UFH as the starting material.[14] On the other hand, synthetic fondaparinux (**1**) became commercially available in 2001 and is frequently prescribed despite the high cost due to its complex synthesis. All of these limitations associated with heparin and its derivatives have motivated the development of alternative anticoagulants with improved properties. This review focuses on different approaches for the development of heparin mimetics as alternative anticoagulants. For the purposes of this review we define heparin mimetics as compounds that mimic the structural features of heparin (principally negatively charged sulfo groups) and therefore its properties.

2 | BLOOD COAGULATION

Based on the waterfall model first proposed by Macfarlane in 1964, the zymogen Factor X (FX) is activated to FXa through two independent pathways, namely the intrinsic and extrinsic pathways.[2,15] The ultimate goal is activation of prothrombin (also known as FII and secreted from platelets) into active thrombin (FIIa), which further stimulates the generation of abundant fibrin from fibrinogen to form a stable clot. However, recent cell-based coagulation studies convey that coagulation takes place in three overlapping steps, namely initiation, amplification, and propagation.[16,17] During initiation, at the site of vascular disruption, a complex is formed between Factor VIIa (FVIIa) and subendothelial tissue factor, which activates both Factor IX (FIX) and FX; the complex also generates a small amount of thrombin to form fibrin via activation of fibrinogen. During amplification, thrombin activates platelets, Factor V (FV), Factor VIII (FVIII), and Factor XI (FXI). FIXa forms a complex (intrinsic "tenase" complex) with FVIIIa (FIXa:FVIIIa), activating a sufficient amount of FX into FXa to form a prothrombinase complex with FVa (FXa:FVa).[17] This complex generates a large amount of thrombin to convert fibrinogen into fibrin and to form a clot which becomes stable once Factor XIII forms crosslinks across fibrin strands.

3 | SOURCES, CHEMISTRY AND ANTICOAGULANT ACTIVITY OF UFH, LMWH, AND ULMWH

Heparin and specifically UFH is prepared by extraction from animal tissue, mostly porcine intestinal mucosa.[18] All heparin preparations are linear polymers with a number average MW (Mn) of 12 to 16 kDa and a weight average MW (Mw) of 17 to 20 kDa, and thus a polydispersity (Mw/Mn) of about 1.3–1.4.[19] The manufacturing processes for heparin have

TABLE 1 Summary of the properties of UFH, LMWH, and ULMWH (fondaparinux)

	MW (kDa)	Anticoagulant activity	Half-life (hr)	Production	Production concerns	Administration	Advantages	Monitoring	Side-effects/ safety issues/ disadvantage
UFH	12–30	Anti-FIIa (thrombin) Anti-FXa	<1	Extraction from porcine intestinal mucosa	Biological contamination	Intravenous Continuous infusion	Widespread availability Low cost Reversible	Frequent monitoring due to variable dose response APTT Anti-Xa	~30% of the dose is therapeutic Bleeding Heparin-induced Thrombocytopenia (HIT) Osteoporosis Hypoaldosteronism Heparin-induced skin necrosis
LMWH	3.5–6	Anti-FXa	4–5	Depolymerization of UFH	Biological contamination	Subcutaneous Once/twice daily	Bioavailability	Less frequent monitoring Anti-Xa assay APTT	Functional irreversibility Bleeding UFH as starting material
ULMWH	1.7	Anti-FXa	17–21	Chemical synthesis	None	Subcutaneous Once daily	Bioavailability	Not required except in specific populations (e.g., children)	Functional irreversibility

Notes: APTT, activated partial thromboplastin time; FIIa, Thrombin; FXa, Factor Xa; LMWH, low molecular weight heparin; MW, molecular weight, UFH, Unfractionated heparin; ULMWH, ultra-low molecular weight heparin (fondaparinux).

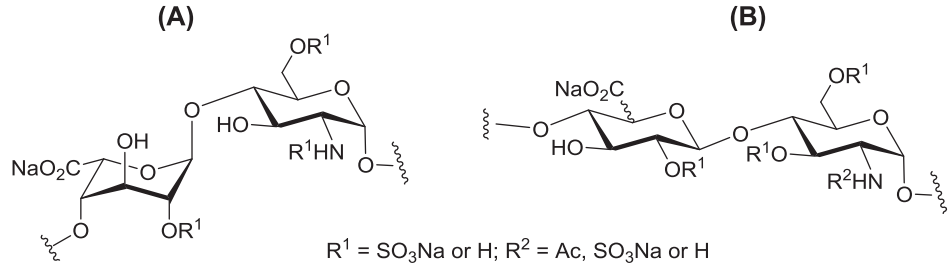


FIGURE 1 Structures of the major (A) and minor (B) disaccharide sequences in heparin

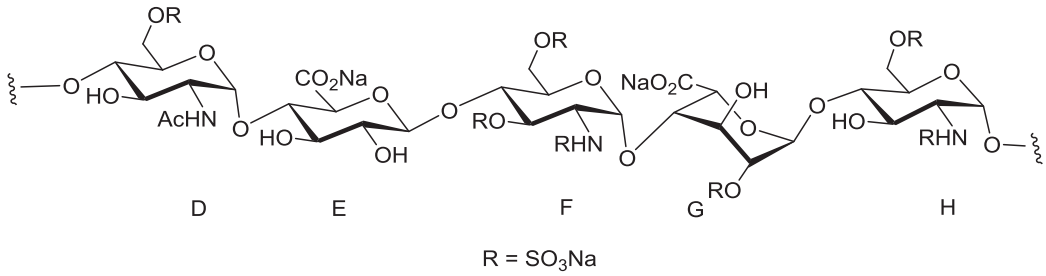


FIGURE 2 Structure of the unique pentasaccharide (DEFGH) sequence of heparin, also known as the antithrombin binding domain (ABD)

changed slightly over time as the industry has transitioned from beef lung to porcine intestine as the primary source tissue.[20]

Heparin is a highly sulfated polyanionic polysaccharide consisting of repeating disaccharide subunits of 1 → 4 linked α-D-glucosamine (GlcN) and a uronic acid, typically 90% α-L-iduronic acid (IdoA) and 10% β-D-glucuronic acid (GlcA). The most common structure occurring in heparin is the trisulfated disaccharide IdoA2S-GlcNS6S (Figure 1), however, a number of structural variations exist, leading to the microheterogeneity of heparin.[21] Different sulfation patterns are unevenly distributed along the heparin chains, with highly charged sequences mostly concentrated at the nonreducing end and less charged sequences at the reducing end, with mixed sequences between these two regions.[21] The proportions of differently charged domains and the actual composition within these domains vary depending on the animal and organ source and also on the extraction and purification procedures.[22] For example, during manufacture of UFH base-catalyzed displacement of sulfate from Ido2S and/or enrichment of 6-*O*-sulfated sequences via chromatographic purification can occur at variable levels.[22]

Heparin exerts its anticoagulant effects primarily through its interaction with the serpin (serine protease inhibitor) AT, bringing about a conformational change and thus allowing it to interact with the proteases FXa and FIIa. To induce the conformational change in AT, an AT-binding domain (ABD) comprised of a specific pentasaccharide sequence containing the 3-*O*-sulfated GlcN residue must be present (DEFGH, Figure 2). The pentasaccharide ABD stimulates exclusively the AT-mediated inactivation of FXa, whereas longer heparin fragments (at least 14–16 saccharides long) with a thrombin-binding domain (TBD) situated to the nonreducing end of the ABD, are required for inhibition of thrombin. The minimum MW of a heparin chain with anti-IIa activity is thus approximately 5 kDa.[23] The combination of ABD with extra chain length to include a TBD has been termed the "C-region".[24] Current heparin profiling methods such as NMR spectroscopy can provide an estimate of the amount of ABD content but not of the C-region.[25] The variability in the amount of ABD/C-region combined with the variation in MW leads to significant variability in bioavailability and anticoagulant effect for UFH. The anticoagulant activity of UFH strongly depends on the level of sulfation along with the amount of ABD pentasaccharide sequences.

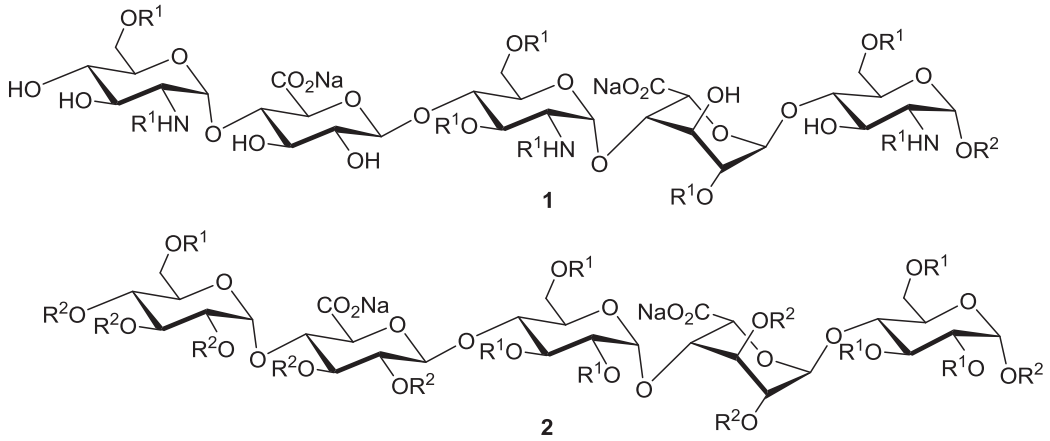


FIGURE 3 Structures of fondaparinux (**1**) and idraparinux (**2**). $R^1 = SO_3Na$, $R^2 = Me$

LMWHs are manufactured from UFH, using a variety of physical, chemical or enzymatic cleavage techniques.[20,26] The defining characteristic of all LMWH products is that 60 wt% or more must have MW below 8 kDa.[27] LMWHs produced by different depolymerization processes result in unique structural alterations to the cleaved heparin chains.[28] These structural differences give rise to differences in their in vitro and pharmacokinetic/pharmacodynamic properties.[29]

The fully synthetic methyl glycoside derivative of the ABD pentasaccharide is now marketed as the drug fondaparinux (**1**, Figure 3) following many years of development led by van Boeckel, Petitou, and co-workers.[30,31] Their earlier efforts resulted in a synthetic pentasaccharide with a low overall yield following complex synthetic procedures taking 60 steps. Following the introduction of the methyl glycoside at the anomeric center of the H unit,[30] the resultant pentasaccharide exhibited similar anticoagulant activity with additional advantages such as improved yield and a longer half-life (17 hr). This preparation was then registered as drug in the United States and Europe under the trade name Arixtra®.[32] Subsequently, the same group developed a fully *O*-sulfated and *O*-methylated (non-glycosaminoglycan) pentasaccharide known as idraparinux (**2**, Figure 3), which is an analogue of fondaparinux.[33] This pentasaccharide displayed some advantages over fondaparinux, such as ease of synthesis, improved anticoagulant activity, and a longer half-life (120 hr).

To inhibit coagulation, the serine protease inhibitor AT belonging to the serpin family, plays the central role. However, under physiological conditions, as a stand-alone inhibitor it is not sufficiently potent.[34] UFH accelerates the activity of AT several thousandfold. The anticoagulant activity of UFH was first discovered early in the 20th century, and since 1937 it has been in use in the clinic.[35] This long chain polysaccharide exhibits its effects in two ways. First, it accelerates the inhibitory activity of AT on FVIIa, FIXa, FXa, FXIa, FXIIa, and FIIa via a conformational change of AT after binding; this is known as an allosteric mechanism (Figure 4).[36–38] Second, heparin directly binds thrombin via electrostatic interactions, and reduces thrombin's activity by forming a bridge between thrombin and AT known as the ternary complex.[39–45] Sequential investigations by different groups have shown that to increase the inhibitory effect of AT on FIXa and FXa, a unique pentasaccharide sequence is needed which can bind with AT.[46,47] Moreover, to accelerate the anti-IIa activity following the ternary complex, an additional 13 monosaccharide units must be present with that pentasaccharide.[48]

Only one-third of the UFH molecules display anticoagulant activity through their interaction with AT.[40,49–52] However, the anticoagulant activity of heparin strongly depends on the presence of *N*- and *O*-sulfates.[53,54] The absence of *O*-sulfate groups on the pentasaccharide (DEFGH) dramatically reduces anticoagulant activity. In addition, esterification of the carboxyl group of the uronic acids diminishes the anticoagulant activity.[55–57] Although the bulk of the literature indicates that DEFGH is the main active sequence of UFH to exert anticoagulant activity, some studies have indicated that tetra or hexasaccharides also have anticoagulant activity.[50,58]

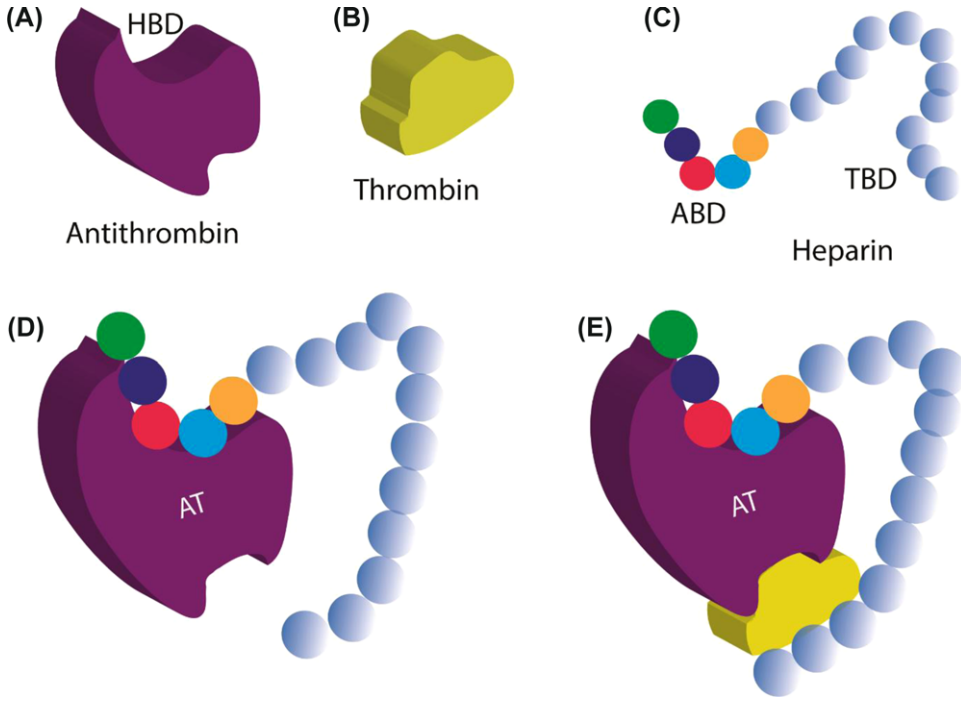


FIGURE 4 (A) antithrombin; (B) thrombin; (C) long chain heparin; (D) binding of heparin pentasaccharide with the antithrombin; and (E) formation of ternary complex of heparin with thrombin and antithrombin. HBD: heparin binding domain; ABD: antithrombin binding domain; TBD: thrombin binding domain

4 | HEPARIN MIMETICS

Different strategies have been explored to prepare heparin mimetics, such as the synthesis of heparin-related oligosaccharides and their derivatives, the sulfonation of natural polysaccharides, for example, chitosan and hyaluronic acid (HA); the synthesis of noncarbohydrate sulfated polymers; conjugation of sulfated oligosaccharides to synthetic polymers; and the isolation of sulfated polysaccharides from different natural sources (see Table 2 and below). The key structural feature behind all the above strategies is the presence of sulfo groups on a suitable scaffold. Interestingly, there have also been some reports of small sulfated molecules as potential anticoagulants; while some nonsulfated anionic compounds have also been shown to have anticoagulant properties.

4.1 | Synthetic heparin oligosaccharide derivatives

A series of studies to develop ULMWH/heparin oligosaccharides by chemoenzymetic methods has been reported by the Liu group.[59–61] Recently two synthetic sulfated oligosaccharides (**3** and **4**, Figure 5) consisting of the ABD of porcine and bovine heparin, respectively, were developed using a GlcA-anMan disaccharide (R in Figure 5) as the starting material which was selected because it could be elongated by glycosyl transferases.[59] These two oligosaccharides were found to exhibit excellent anticoagulant activities with comparable pharmacokinetic properties to **1**. Liu and co-workers also generated a library of size defined *N*-sulfo-oligosaccharides using the disaccharide GlcA-anMan as the starting material which was elongated by two bacterial glycosyltransferases.[60] After C5-epimerization and *O*-sulfonations, oligosaccharides were produced consisting of ABD and TBD connected via a linker domain (consisting of repeating disaccharides of –GlcNAc-GlcA-). This is the first report of the preparation of heparin oligosaccharides having up to 21 saccharide residues via chemoenzymatic synthesis. All the oligosaccharides displayed both anti-Xa and anti-IIa activities and showed low binding to PF4, suggesting they would be less likely to cause heparin-induced

TABLE 2 Summary of the properties of heparin mimetics

Structural class	Structure	Method of preparation	Mw (kDa)	Target factor	Mechanism	Half-life	In vivo assay	Ref.
Synthetic heparin oligosaccharides	**2**	Synthetic	1.7	FXa	AT dependent	60 days	✓*	33
	3, 4	Chemoenzymatic	1.7–1.8	FXa	AT dependent	ND	✓	59
	5, 6	Chemoenzymatic	1.8–3.6	FXa	AT dependent	ND	✓	14
	7	Synthetic	2.0	FXa	AT dependent	60 days	✓*	63,64
	8, 9	Synthetic		FIIa and FXa	AT dependent	ND	X	65,66
	10	Synthetic	ND	FIIa and FXa	AT dependent	ND	✓	67
	11, 12	Synthetic	2.4–2.6	FIIa and FXa	AT dependent	1.5 hr	✓	68
	13	Synthetic		FXa and platelet		ND	✓	70
	14	Synthetic		FIIa	HCII dependent	ND	x	71
Polysulfated non-heparin oligosaccharides	**15**	Synthetic	2.4	FIIa	HCII dependent	1–3 hr (in rats and monkey)	✓	74,75
	16	Synthetic	2.3	FIIa, FVa, and FXa	HCII dependent	ND	X	77,78
	17	Synthetic	2.03	FIIa and FXa	Unclear	ND	✓	79
	18	Synthetic	1.2	FIIa	Unknown	ND	X	80
	19	Synthetic	ND	ND	Common and intrinsic pathways	ND	✓	81
Naturally occurring sulfated polysaccharides	CS	Natural	4.75	FIIa	AT dependent	ND	X	82–84
	FCS	Isolation, depolymerization	11–100	FIIa and FXa	AT and HCII dependent	ND	✓	97–106
	DS	Extraction	12–50	FIIa	HCII dependent	ND	✓	83,86,87,108–115
	Sulfated fucans	Extraction	~100	FIIa and FXa	AT and HCII dependent	ND	X	90–93
	Sulfated galactan	Extraction	~100	FIIa and FXa	AT and HCII dependent	ND	X	88,89

(Continued)

TABLE 2 Continued

Structural class	Structure	Method of preparation	Mw (kDa)	Target factor	Mechanism	Half-life	In vivo assay	Ref.
Chemically modified natural polysaccharides	Sulfated chitosan	Sulfonation	1.9-80	FIIa and FXa	AT and HCII dependent	ND	X	126,132,134,144–150
	Sulfated HA	Sulfonation	21–3500	FIIa and FXa	AT and HCII dependent	ND	X	151,152
	CMDBS	Semisynthetic	47–50	FIIa	AT and HCII dependent	ND	X	113,158–169
	22	Sulfation	0.9–2.4	FIIa and FXa	Different than that of UFH	ND	X	174,175
	Sulfated alginate	Synthetic, semi-synthetic	12–52	FIIa and FXa	AT and HCII dependent		X	176–180
Synthetic sulfated glycopolymers	**25**	Free radical polymerization	ND	FIIa	HCII dependent	ND	X	187
	26–29	Step growth polymerization	27–44.0	FIIa	AT dependent and independent pathways	ND	X	190
	30, 31	Free radical polymerization	9.0–114	Not specified (intrinsic, extrinsic and common pathways)	ND	ND	X	191
	32	ROMP	~43.0	FIIa and FXa	AT dependent	ND	X	192
Synthetic sulfated polymers	**34–39**	Radical polymerization	>10	Intrinsic, extrinsic or common pathways)	ND	ND	X	195–199
	40, 41	Radical polymerization	>5	FIIa	AT dependent	ND	X	203
Sulfated aromatic compounds	**42–50**	Natural (extraction), synthetic	0.2–0.7	FIIa and FXa	AT and HCII dependent	ND	X	215–222

*These compounds have been evaluated in human clinical trials.

Notes: AT, antithrombin; CMDBS, carboxymethyl benzylamide sulfonate dextrans; CS, chondroitin sulfate; DS, dermatan sulfate; FCS, fucosylated chondroitin sulfate; FIIa, Thrombin; FVa, Factor Va; FXa, Factor Xa; HA, hyaluronic acid; HCII, heparin cofactor II; Mw, weight average MW; ND, not determined; UFH, unfractionated heparin.

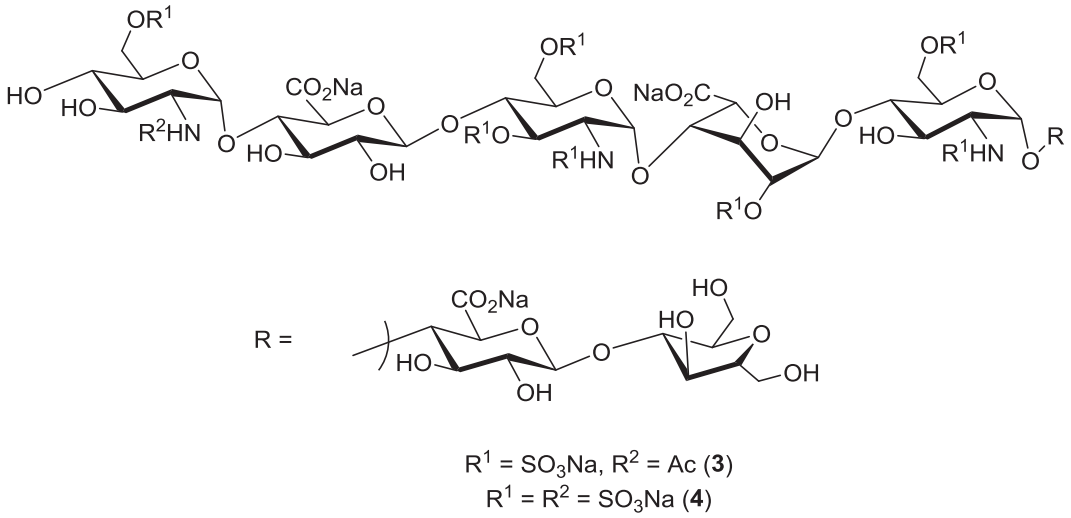


FIGURE 5 Structures of the synthetic sulfated oligosaccharides consisting of the ABD of porcine (**3**) and bovine heparin (**4**)

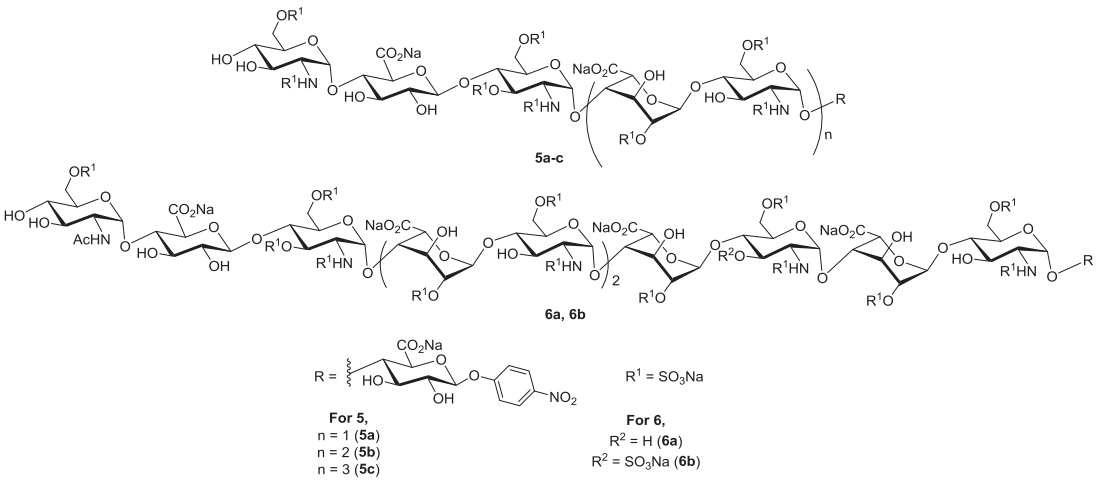
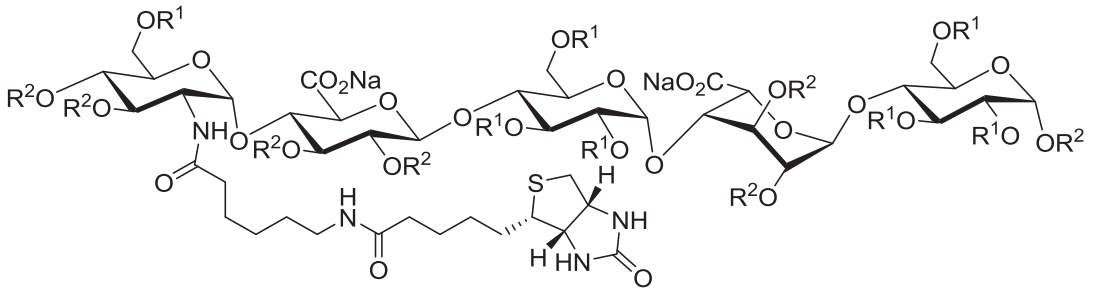


FIGURE 6 Structures of synthetic LMWHs

thrombocytopenia. Additionally, this study concluded that a minimum of 19 saccharide residues is required for the anti-IIa activity of the molecule. Although these oligosaccharides displayed strong anticoagulant activities, the synthesis took 14 days and ultimately the oligosaccharides were structurally heterogeneous. To minimize this lengthy procedure, a one-pot chemoenzymatic synthesis of LMWH with a narrow polydispersity was developed, which was named de novo LMWH,[61] using a tetrasaccharide primer which could be obtained in only 2 days. The in vitro and the ex vivo anticoagulation assays indicated higher potency of the de novo LMWH compared with the commercially available LMWH enoxaparin.

Subsequently, five LMWHs (**5a–c** and **6a,b**, Figure 6) ranging from hexasaccharide to dodecasaccharide were synthesized from commercially available monosaccharide 1-*O*-(*p*-nitrophenyl)-glucuronide as the starting material, instead of the GlcA-anMan disaccharide.[14] Each oligosaccharide consisted of the ABD from porcine (**5a–c**) or bovine (**6a, b**) heparin. Oligosaccharides **5a–c** were constructed by changing the number of IdoA2S-GlcN6S repeating units while the dodecasaccharide **6a** differs from **6b** by lack of one 3-*O*-sulfate group. The results from the



7

FIGURE 7 Chemical structure of idrabiotaparinux (**7**).[63] R1 = SO_3Na, R2 = Me

anticoagulant assays demonstrated strong binding affinity to AT and anti-FXa activity from these five oligosaccharides. The reversibility of the anticoagulation properties of these five oligosaccharides was evaluated by treatment with protamine sulfate where the dodecasaccharide **6b** displayed higher reversibility than the LMWH enoxaparin and similar to UFH. Eight related hexasaccharides containing 2-*O*-sulfated glucuronic acid (GlcA2S) were also prepared by chemoenzymatic synthesis using monosaccharide 1-*O*-(*p*-nitrophenyl)-glucuronide as the starting material.[62] Three hexasaccharides were subjected to AT binding affinity testing by affinity coelectrophoresis.[62] This study revealed that without the presence of 2-*O*-sulfated iduronic acid, the oligosaccharides are not able to bind to AT.

The development of idraparinux (**2**) was halted due to excessive bleeding complications and the very long half-life.[63] To overcome these concerns, biotin was conjugated to C-2 of the nonreducing end saccharide unit of idraparinux to give idrabiotaparinux (**7**, Figure 7) to allow for rapid neutralization with avidin.[63] This compound showed the same anticoagulant properties as idraparinux,[63,64] however, its development was also discontinued.

From the study of the formation of the ternary complex of heparin with AT and thrombin, it was found that the bridge between ABD and TBD does not interact with positively charged protein residues.[65] This finding informed the design of tailor-made glycoconjgugates such as **8** and **9** (Figure 8) with the full anticoagulant properties of heparin, consisting of synthetic ABD and TBD domains linked through a molecular spacer. Initially, a nonglycosaminoglycan ABD pentasaccharide (i.e., idraparinux) was connected via a molecular spacer to a persulfated maltotrioside as the TBD.[65,66] This study revealed that the anticoagulant activity was dependent on all three domains of the conjugate. When the spacer length was short, the conjugates failed to form any ternary complex when both the ABD and TBD were fixed. The optimum length spacer was found to be around 50 atoms long. It was subsequently found that a rigid linker such as a neutral heptasaccharide such as in conjugate **9** increased the anticoagulant activity compared with flexible ones. In addition, the charge density of the TBD was found to regulate the anti-IIa activity.

Taking advantage of the availability of direct thrombin inhibitors, the same group also developed dual active antithrombotic conjugates. A dual inhibitor Org39913 (**10**, Figure 9) which can inhibit the action of thrombin and FXa through AT was developed by conjugating the direct thrombin inhibitor α-NAPAP [α-N-(2-naphthalenesulfonyl)-glycyl-D-4-aminophenylalanyl-piperidine] and an idraparinux pentasaccharide analogue.[67] It was then optimized by decreasing the number of sulfate groups, replacing the aromatic linker by γ-aminobutyric acid and using a single enantiomer of a NAPAP analogue. The resultant conjugate Org42675 (**11**, Figure 9) exhibited similar AT-mediated anti-FXa activity to **1**, a ten times longer half-life than the direct thrombin inhibitor on its own,[68] and thrombin inhibition was enhanced 20 times compared with **10**. Conjugation of a biotin tag to Org42675 (also known as EP42675) resulted in EP217609 (**12**, Figure 9), which could retain the activities of Org42675 and could be neutralized by avidin injection.[68,69] In another study, following a similar strategy, EP224283 (**13**) was developed consisting of idraparinux conjugated to the αIIbβ3 inhibitor tirofiban,[70] in addition to biotin, producing a neutralizable conjugate with both anti-FXa and antiplatelet activity. It is noteworthy that tirofiban on its own has a very short half-life and cannot be used for outpatients whereas **13** has a much longer half-life due to the presence of the idraparinux moiety.

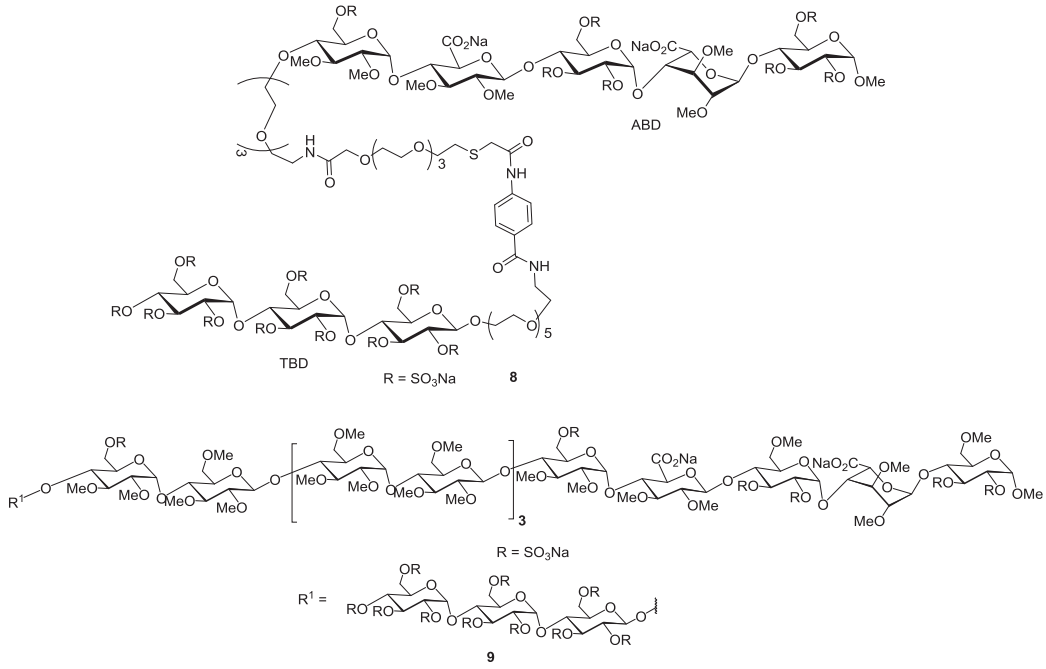


FIGURE 8 Synthetic tailor-made glycoconjugates consisting of ABD and TBD through flexible (**8**) and rigid (**9**) molecular spacers[65,66]

Recently, Oscarson and Desai generated an in silico library of 46,656 heparan sulfate hexasaccharides and found a rare sequence consisting of consecutive GlcA2S residues which could selectively target heparin cofactor II (HCII),[71] another serpin involved in the regulation of blood coagulation via inhibition of FIIa. They synthesized five unique sequences including three containing at least one GlcA2S residue (a residue rarely found in heparin). Of particular note was the hexasaccharide HX3 (**14**, Figure 10), which induced HCII activation nearly 250-fold, similar to AT activation by **1**. Compound **14**, which contains two consecutive GlcA2S residues, was a poor activator of AT (only fivefold), indicating a high selectivity for HCII.

4.2 | Polysulfated non-heparin oligosaccharides and derivatives

A series of synthetic polysulfated oligosaccharides, prepared by chemical sulfonation of various isolated oligosaccharides, was tested for anticoagulant activity by determining the activated partial thromboplastin time (APTT).[72] Anticoagulant activity was dependent on chain length, linkage, and nature of the constituent monosaccharides. One of the most potent anticoagulants was PI-88 (**15,** Figure 11), a mixture of polysulfated manno-oligosaccharides that has been in clinical development as an anticancer agent,[73,74] which was selected for further study. PI-88 was found to inhibit blood coagulation via HCII-mediated thrombin inhibition and this activity could be neutralized by protamine sulfate.[75] Raake et al. synthesized low MW polysulfated bis-lactobionic acid amides which possessed moderate to low anticoagulant activity.[76] One of the compounds, LW10082 (Aprosulate, **16**) showed similar antithrombotic activity to LMWH and was initially found to stimulate HCII[77]; but inactivation of both FV, FX,[78] and FVIII has also been reported. Abendschein and co-workers synthesized the highly sulfated tetrasaccharide derivative maltodapoh (**17**) as an anticoagulant consisting of two maltose sugars linked through 1,3-diamino-2-propanol. The mechanism of anticoagulation by maltodapoh is unclear but it was thought that this compound does not inhibit thrombin function via HCII.[79] Desai et al. found that the commercially available sucrose octasulfate (**18**) directly inhibits thrombin with high potency but low efficacy after binding with exosite II of thrombin.[80] Jairajpuri and co-workers synthesized trehalose octasulfate (**19**) as a dual anticoagulant/antiplatelet agent but the mechanism of action was not fully elucidated.[81]

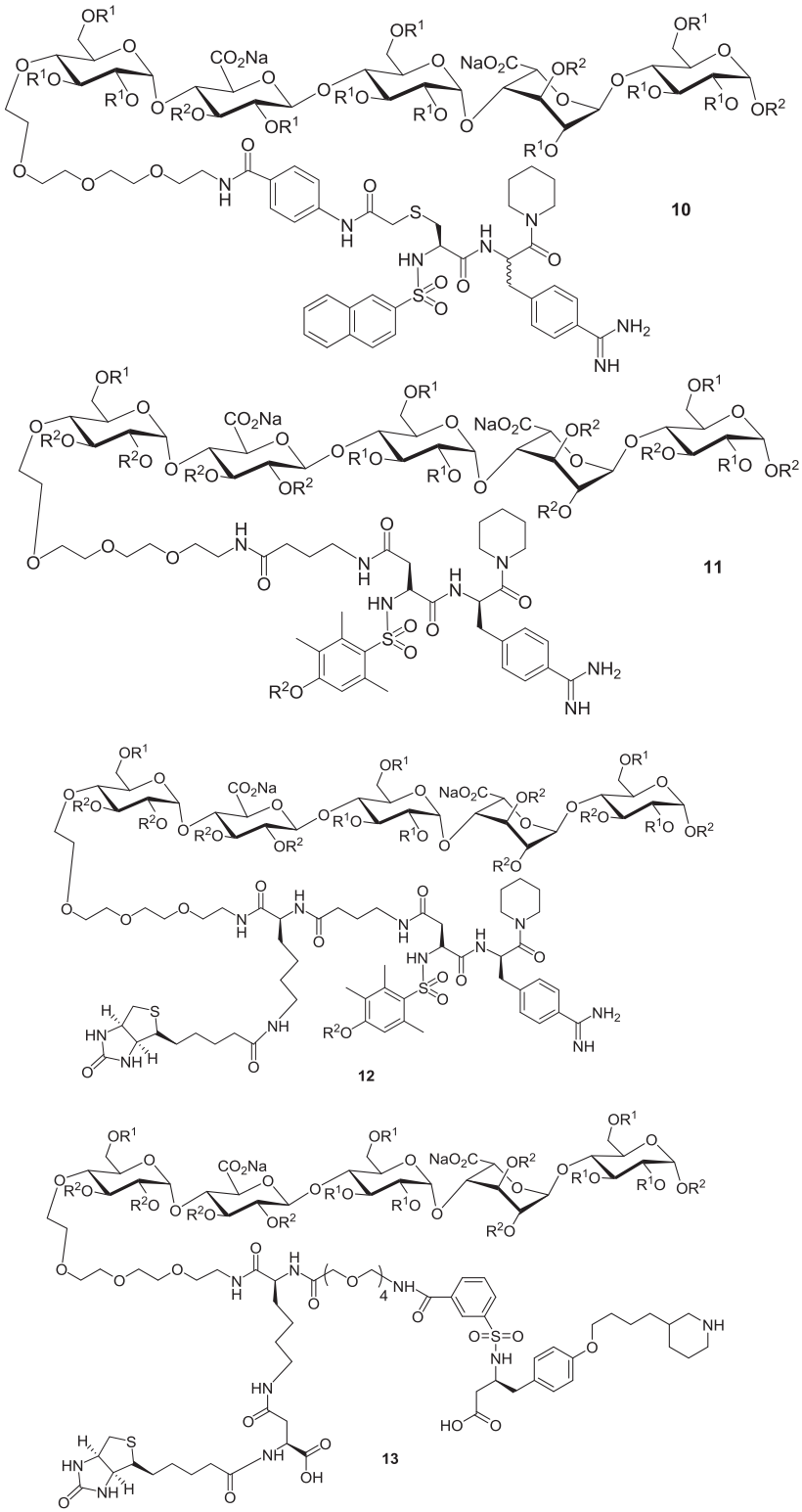
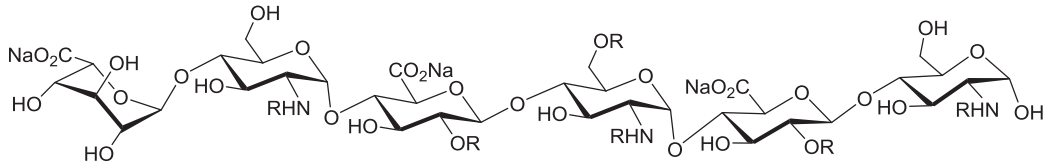


FIGURE 9 Structures of pentasaccharide conjugates Org39913 (**10**),[67] Org42675 (**11**),[68] EP217609 (**12**),[69] and EP224283 (**13**)[70]



14

FIGURE 10 Structure of synthetic hexasaccharide HX3 (**14**).[71] R = SO_3Na

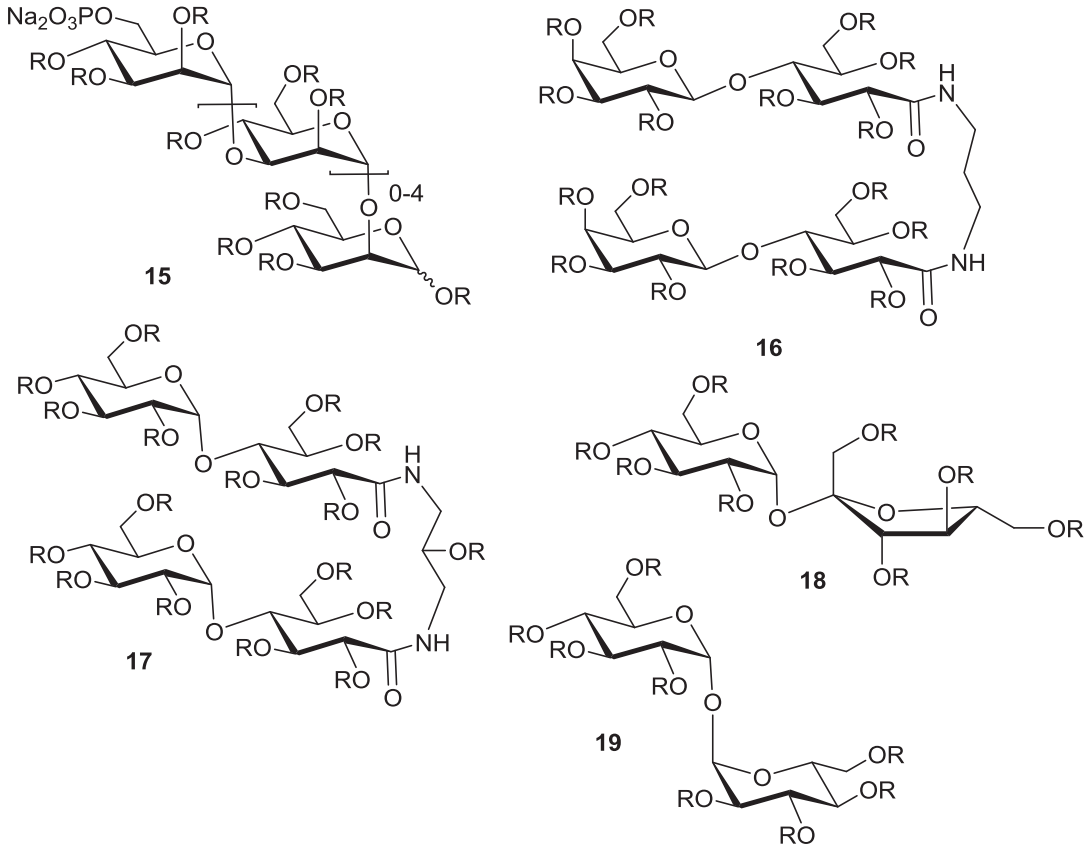


FIGURE 11 Structures of the polysulfated PI-88 (**15**), bis-lactobionic acid amide LW10082 (**16**), maltodapoh (**17**), sucrose octasulfate (**18**), and trehalose octasulfate (**19**). R = SO_3Na

4.3 | Sulfated non-heparin polysaccharides

4.3.1 | Naturally occurring sulfated polysaccharides

Besides UFH, various sulfated polysaccharides isolated from a wide range of natural sources such as CS,[82–84] dermatan sulfate (DS),[83,85–87] sulfated galactan,[88,89] and sulfated fucans[88,90–96] have been shown to have anticoagulant activity. These polysaccharides have been found to contain different degrees of sulfation at various positions in the saccharide ring which have specific effects on the coagulation time and the mechanism of action of each of the molecules.

Most early studies reported the ineffectiveness of both natural chondroitin-4-sulfate and chondroitin-6-sulfate (known CSA and CSC, respectively) as anticoagulants due to the presence of only one sulfate group per disaccharide unit,[83,84] although one study did report that CSA had significant anticoagulant activity mediated through AT.[82] Fucosylated chondroitin sulfates (FCS), which contain heavily sulfated fucose residues at the *O*-3 position of the GlcA of

CS, have been found to act as potent anticoagulants.[97–109] The naturally occurring FCSs isolated from a sea cucumber have been found to inhibit thrombin action via AT and HCII,[100,107] and could inhibit FXa by forming the intrinsic tenase complex.[106] Depending on the position of the sulfate groups, Zhao et al. have shown AT mediated anti-IIa activities by FCSs containing a higher proportion of 2,4-disulfated fucose; while 3,4-disulfated fucose functioned via a HCII dependent pathway.[100] Selective inhibition of FXa was displayed by the FCSs having at least 6–8 trisaccharide units.

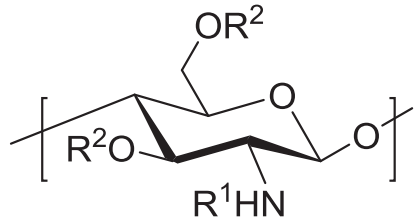
DS (previously known as chondroitin sulfate B), which contains L-IdoA instead of GlcA in the disaccharide unit, displays better anticoagulant activity although it contains a single sulfate group per disaccharide unit like CSA and CSC.[83] DS accelerates the inhibitory action on thrombin of HCII but not of AT and it takes place in the vessel wall only after vascular disruption.[83,86,87,108–115] The variation in anticoagulant activity by DS has been reasoned to be due to the structural heterogeneity caused by isolation from different sources such as porcine skin and intestinal mucosa and bovine lung.[83] For example, highly sulfated DS (25% w/w) from the skin of the ray *Raja montagui* exhibited 5–7-fold higher anticoagulant activity due to containing twofold higher sulfate and uronic acid content compared with the DS from porcine intestinal mucosa.[87,108,116,117] Previously, the authors reported more potent anticoagulant activity from the DS obtained from the skin of *Raja radula* via both HCII and to a lesser extent AT.[118,119] In another study, variations in anticoagulant activities of DS of similar structures isolated from different species of rays was observed.[120] The DS isolated from electric eel, *Electrophorus electricus* (L.), was shown to be more potent compared with porcine DS.[117] Linhardt et al. have reported anti-Xa activity by the low MW DS (4.2 kDa).[121] Fernandez et al. reported the enhancement of anticoagulant activity of activated protein C (APC) by DS.[122]

Both sulfated fucans and sulfated galactans, isolated from various species of marine organisms, have been reported to possess anticoagulant activity.[88,95] The methods of isolation of these anionic polysaccharides and their chemical compositions have been summarized in recent reviews.[95,96,123] Both AT- (30 times less potent than UFH) and HCII- (similar potency to UFH and DS) mediated thrombin inhibition have been observed from the sulfated fucans isolated from *Pelvetia canaliculata*.[90,93] Similar mechanisms were observed by Mourao et al. from sulfated fucan isolated from *Laminaria cichorioides*.[124] This compound also displayed anti-Xa activity, however, to a lesser extent. On the other hand, some sulfated fucans have been reported to inhibit FIIa function via HCII and not AT.[91,92] It has been reported that branched fucans directly inhibit FIIa, whereas both AT and HCII mediated activity have been reported for linear fucans.[125] Similarly, galactan sulfate, isolated from marine invertebrates, prolongs blood coagulation time through inhibition of thrombin (similarly to UFH) via both HCII and AT.[88,89]

4.3.2 | Chemical modification of naturally occurring polysaccharides

Polysaccharides with little or no sulfation and thus no anticoagulant activity can be converted into anticoagulants via exhaustive chemical sulfonation. Sulfonation of the polysaccharides (such as chitosan, dextran and CSs) has generally been carried out using sulfur trioxide pyridine (or triethylamine) complex, chlorosulfonic acid, or sulfuric acid/DCC (*N*,*N*′-dicyclohexylcarbodiimide) as the sulfating agent.

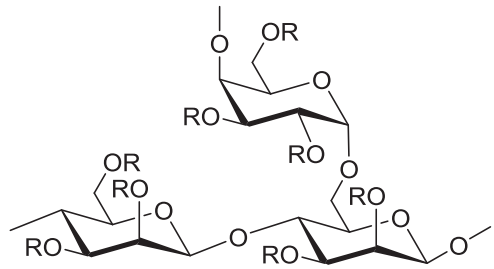
As a source of polysaccharide for sulfonation, chitosan (deacetyl chitin) has been considered due to the presence of β-(1 → 4) linkages, linearity, and the presence of amino and acetamido groups (features in common with heparin).[126] Chitosan does not have any anticoagulant effects but partial enzymatic depolymerization and sulfonation of the amino and hydroxy functional groups and/or addition of carboxyl groups endows it with anticoagulant activity.[126–142] Different methods of preparation of sulfated chitosan have been reviewed by Tamura et al. For sulfated chitosan, *N*-sulfation at the C-2 position is required to inhibit blood coagulation.[143–145] It has also been found that the 6-*O*-sulfate group is critical for anticoagulant activity and the absence of sulfation at this position totally ablates the anticoagulant activity.[132,146] On the other hand, *N*-succinyl chitosan (**20**) and *N*,*O*-succinyl chitosan (**21,** Figure 12) without any sulfate groups have been found to increase blood coagulation time.[139] Ronghua et al. reported that the anticoagulant activity of sulfated chitosan could be improved by modification of some of the amino groups with *N*-acyl groups.[134] Zou and Khor have suggested that to act as an anticoagulant, sulfated chitosan must possess at least 36 consecutive sulfate groups along the polymer backbone.[147] The reported mechanisms of action of the various sulfated chitosans have



R^1 = -$COCH_2CH_2COOH$, R^2 = H
NSC (**20**)

$R^1 = R^2$ =-$COCH_2CH_2COOH$
NOSC (**21**)

FIGURE 12 Structures of the *N*-succinyl chitosan (**20**) and *N*,*O*-succinyl chitosan (**21**)



22

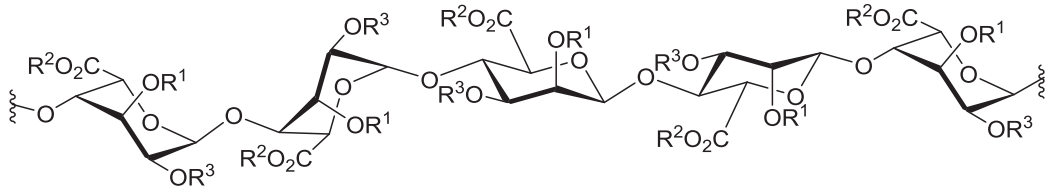
FIGURE 13 Structures of the sulfated galactomannan (**22**). R = SO_3Na or H

varied across different studies, for example, indirect inhibition of thrombin via AT[126,132,138,144,145,148,149] and HCII,[138] or direct inhibition of thrombin and AT mediated FXa inhibition.[138,149,150]

HA, consisting of glucuronic acid β-(1→3) and *N*-acetylglucosamine β-(1→4) linkages, is a nonsulfated glycosaminoglycan with no anticoagulant activity.[151] Magnani et al. developed a range of sulfated HAs which displayed anticoagulant activity dependent on the degree of sulfation.[152] These compounds inhibited FIIa function via nonspecific electrostatic interactions and FXa via AT. This study concluded at least 3.5 sulfate groups per disaccharide unit are required to enhance blood anticoagulation.[152] Subsequently, HCII and AT mediated thrombin inhibition by LMW and HMW sulfated HA, respectively, was reported.[151]

The sulfation of dextran, a branched glucan consisting of α-(1 → 6)-glycosidic linkages with α-(1 → 3)-linked branches, has long been explored for the development of heparin mimetics with anticoagulant activity.[153–157] Numerous studies have been reported to evaluate the anticoagulant activity of carboxymethyl benzylamide sulfonate dextrans (CMDBS),[113,158–165] and these have recently been reviewed by Maynard and co-workers.[166] The CMDBS derivatives were found to inhibit thrombin activity via both AT and HCII.[159,167–169] The related functionalized dextran-methylcarboxylate benzylamide sulfate, which differs from CMDBS in the preparation and the degree of sulfation,[165] displays higher anticoagulant activity than CMDBS.[170] Besides sulfated dextrans, some other semisynthetic sulfated β-glucans have been found to act as anticoagulants which accelerate thrombin inhibition via HCII.[171–173]

Chemically sulfated galactomannan (**22**, Figure 13) with various degrees of sulfation (0.7–1.4 per saccharide) displayed moderate to higher anticoagulant activity than dextran sulfate and curdlan sulfate (a sulfated β(1 → 3)-linked glucan).[174] A study has shown that sulfated galactomannan could inhibit both FIIa and FXa, via a mechanism that is thought to be different to that of UFH.[175]



23 $R^1 = SO_3Na$, $R^2 = Na$, $R^3 = H$
24 $R^1 = R^3 = SO_3Na$ or H, $R^2 = CH_2CH(OR^1)CH_3$

FIGURE 14 Structures of the synthetic sulfated alginate (**23**) and propylene glycol conjugated sulfated alginate (**24**)

Ronghua et al. expected that sulfation of alginate, consisting of β-D-mannuronic acid connected to α-L-guluronic acid via β-(1 → 4) linkage, could provide a heparin-like structure (containing both sulfates and carboxylates).[176] They prepared sulfated alginate (SA) (**23,** Figure 14) using chlorosulfonic acid in formamide, and found that at 17 μg/mL the APTT was 226 sec whereas for UFH the APTT was 125sec at 10μg/mL. Similarly, Zhao and co-workers prepared SA of varying degrees of sulfation using sulfuric acid/*N*,*N*'-Dicyclohexylcarbodiimide (DCC) as the sulfating agent. The SA also displayed excellent anticoagulant activity dependent on the degree of sulfation.[177] Fan et al. reported that SA prepared using trisulfated sodium amine as the sulfating agent inhibited the function of both FIIa and FXa.[178] A sulfated propylene glycol ester of low MW alginate known as PSS (**24,** Figure 14) has been used as a drug in China for more than 30 years for the treatment of CVDs. Lin et al. fractionated PSS and found that fractions with average MWs of ~52 or ~26 kDa inhibited FIIa mediated by AT and HCII, while the lower MW fraction (~12 kDa) weakly inhibited FXa mediated by AT.[179,180]

The naturally occurring CSs have poor anticoagulant activity. However, complete *O*-sulfonation of CSA results in enhanced anticoagulant activity (similar to that of LMWH) by inhibiting the function of FIIa via HCII,[181] while, complete sulfonation of CSC (to produce "oversulfated CS" or OCS) increases prothrombin time more than 200-fold compared with native CSC but only one fourth the activity of UFH.[182] Although OCS has anticoagulant activity, it was responsible for the deaths of more than 100 patients in 2007/8 when used to adulterate UFH, due to it causing severe anaphylactoid reactions[10,14] Therefore the application of oversulfated CSs as anticoagulant drugs is unlikely.

Fully *O*-sulfated DS containing 4.0 sulfate groups per disaccharide unit showed FIIa inhibition via HCII.[183] Acharan sulfate, a glycosaminoglycan isolated from *Achatina fulica*, with a major disaccharide repeating unit of α-D-GlcNAc(1→4)-α-L-IdoA2S, shows no anticoagulant activity despite the structural similarity to heparin . However, after chemical sulfonation the polysulfated acharan sulfate exhibited AT independent anti-IIa activity.[184,185]

4.3.3 | Synthetic sulfated glycopolymers

The synthesis of sulfated glycopolymers, that is, mono- or oligosaccharides appended to a non-carbohydrate polymer backbone, is another approach to prepare heparin mimetics possessing anticoagulant activity. The glycopolymers are generally prepared by various methods of polymerization such as ring opening polymerization, free radical polymerization, or ring-opening metathesis polymerization (ROMP), utilizing as the monomers either sulfated saccharide units, or nonsulfated saccharide units which are subsequently sulfonated after polymerization. The preparation of glycopolymers and their biological activities have recently been reviewed by Miura et al., and Maynard and co-workers.[166,186]

The anticoagulant activity of a sulfated glycopolymer was first reported by Akashi et el.[187] Poly(glucosyloxyethyl methacrylate) was prepared by free radical polymerization using ammonium peroxodisulfate as an initiator. The glycopolymer was then sulfonated using sulfur trioxide/DMF complex to give poly(glucosyloxyethyl methacrylate)sulfate (**25,** Figure 15).[187] The anticoagulant activity for **25,** evaluated as the total human blood clotting time by the method of Lee-White, was modest compared with UFH and DS, respectively. In subsequent studies, the mechanism of action of **25** was determined to be acceleration of thrombin inhibition via formation of an insoluble fibrin complex with fibrinogen[188] and inhibition of thrombin function via HCII.[187,189]

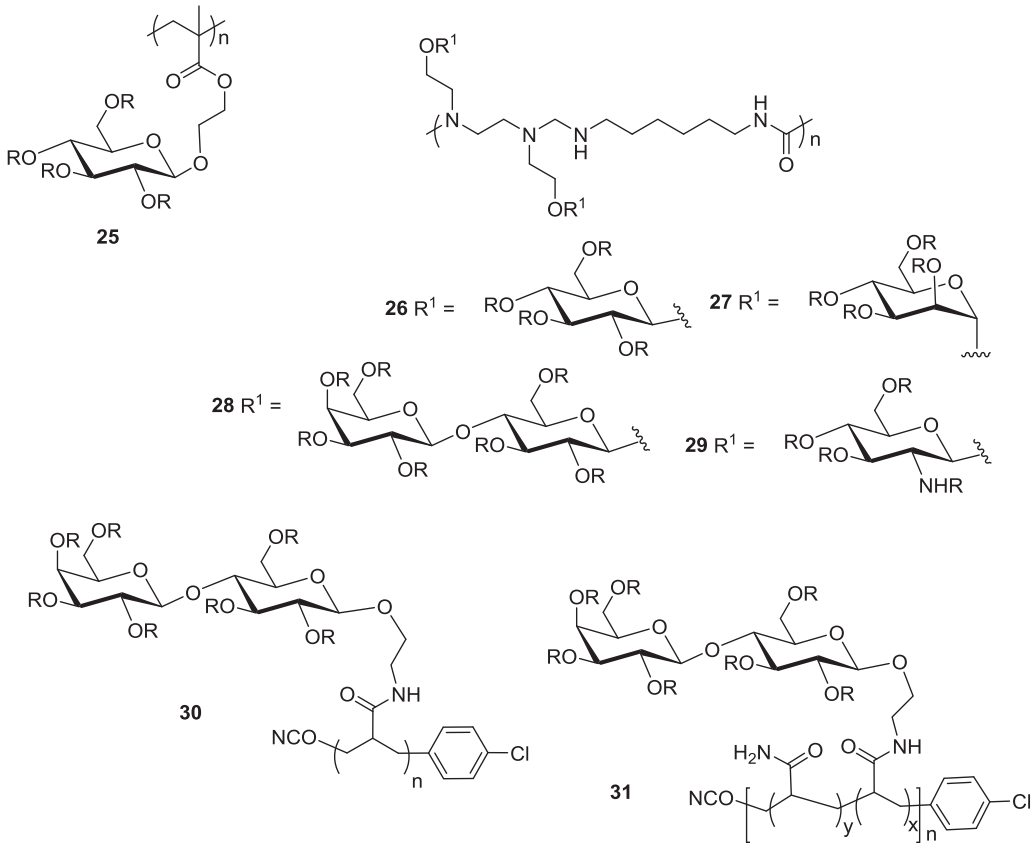


FIGURE 15 Structures of synthetic polysulfated glycopolymers with anticoagulant activity

Recently, Ayres and co-workers used a post-polymerization sulfonation strategy to prepare polyurea based glycopolymers bearing pendant sulfated glucose (**26**), mannose (**27**), lactose (**28**), or glucosamine (**29**) residues (Figure 15).[190] The polymers were synthesized by step-growth polymerization, using hexamethylene diisocyanate and the corresponding glycosylated secondary diamine dimers, followed by sulfonation with SO_3 pyridine complex. All the sulfated glycopolymers prolonged the APTT by >300 sec at 500 μg/mL, with **26** and **29** found to be the most potent. The mechanism of action for the thrombin inhibition was unclear but it was suggested that coagulation time was increased via both AT dependent and independent pathways.

The Chaikof group prepared lactose heptasulfate-based homopolymers **30** and acrylamide co-glycopolymers **31** via cyanoxyl mediated free-radical polymerization using sulfated monomers (Figure 15).[191] Before polymerization, acrylamide derivatized lactose heptasulfate was prepared as the monomer. The anticoagulant activity of **30** was found to increase with increasing MW, but the high MW **30** (114,000) was still almost 20-fold less potent than UFH. Interestingly, the low MW hetero-glycopolymer **31** (MW 9,300,) was more potent than homo-glycopolymer **30** which indicated that the acrylamide played an important role to increase coagulation time. Both of the glycopolymers acted as anticoagulants via selective sequestration of fibrinogen or potentiating the effect of other proteases associated with coagulation, such as HCII. The lactose hepta-sulfated based homo- and copolymers increased the coagulation time. However, the analogous trisulfated GlcNAc based homo- and copolymers failed to show any anticoagulant activity, indicating that at least a sulfated disaccharide is required for anticoagulant activity.

The glycopolymers **32** and **33** (Figure 16), consisting of the G (L-iduronic acid) and H (glucosamine) units of the ABD pentasaccharide, were synthesized via ROMP by Hsieh-Wilson and co-workers.[192] Although it is accepted that the full

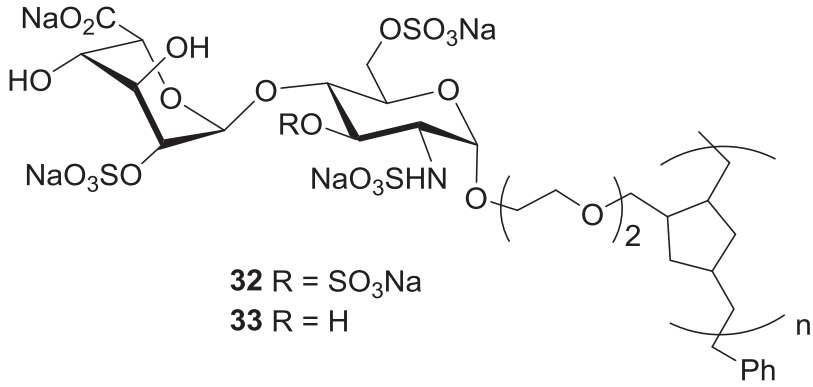


FIGURE 16 Structure of the synthetic glycopolymers **32** and **33**, synthesized via ROMP by Hsieh-Wilson and co-workers, consisting of the G (IdoA) and H (GlcN) unit of the ABD pentasaccharide

ABD pentasaccharide is required for AT-mediated anti-Xa activity, only the GH disaccharide of the ABD was utilized as the monomer in the hope that a multivalent presentation on a polymeric scaffold would enhance binding affinity to AT. Glycopolymer **32** also possesses an additional 3-*O*-sulfate on the H unit which has been shown to confer even greater specificity for AT activation. Partially benzylated sulfated monomers were polymerized in MeOH/CH_2Cl_2 and the resultant polymers were then deprotected by hydrogenolysis to give the final products. Glycopolymer **32**, consisting of 45 repeating tetrasulfated disaccharide units (MW ~ 43,000) was found to exhibit 100-fold more potent anti-Xa activity than UFH, LMWH, and Arixtra. However, the overall effect on APTT was less than for UFH (119 sec vs. > 180 sec at 150 μg/mL). The FXa activity of **32** decreased significantly with decreasing MW. The single alteration in the sulfation pattern of the H unit to give the 3-*O*-desulfated glycopolymer (**33**) totally abrogated both the anti-Xa and anti-IIa activity.

4.3.4 | Synthetic sulfated polymers

Considering the polyanionic behavior of UFH, particularly the presence of sulfate groups, a variety of anionic homopolymers and copolymers have been prepared either from polymerization of anionic monomers or sulfonation of hydroxyl groups after polymerization. The anticoagulant activity of homopolymers of water-insoluble sulfonated styrene (SS), prepared by the sulfonation of polystyrene resin, was first reported by Fougnot and Jozefonvicz.[193,194] Zhao and co-workers have developed a range of copolymers, consisting of SS (following post-polymerization sulfonation of styrene in the polymer) and other monomers, such as poly(sulfonated styrene-co-acrylic acid)-block-poly(vinyl pyrrolidone)-block-poly(sulfonated styrene-co-acrylic acid) [poly(SS-*co*-AA)-b-PVP-b-P(SS-co-AA)] (**34**),[195] poly(sulfonated styrene-co-methyl methacrylate) [poly(SS-*co*-MMA)] (**35**),[196] and poly(sulfonated styrene-co-acrylic acid-co-methyl methacrylate) [poly(SS-*co*-AA-*co*-MMA)] (**36**) by RAFT polymerization using a trithiocarbonate as the RAFT agent (Figure 17).[195,196] These polymers displayed APTT values of 300 sec to more than 400 sec at concentrations of 5.0 and 20.0 mg/mL, respectively. Subsequently, poly(sodium 4-styrene sulfonate-co-sodium methacrylate) [poly(SSS-co-SMA)] (**37**) and poly(dopamine-g- sodium 4-styrene sulfonate-co-sodium methacrylate) [poly(DA-g-SSS-co-SMA)] (**38**) (Figure 17) were found to increase blood coagulation time at much lower concentrations than that of **34**, **35** and **36**.[197] Recently, this group synthesized poly(SSS) (**39**) on carbon nanotubes by surface initiated atom transfer polymerization where bromide-functionalized multiwalled carbon nanotubes were used as the macro initiators.[198] The composite was found to inhibit the function of FXIIa, the first protease of the intrinsic pathway of the coagulation cascade. Li et al. synthesized p(AA), p(SSS), and p(SSS-co-AA), and investigated their anticoagulant activity after grafting onto poly(vinyl alcohol) p(VA).[199] The PVA-g-p(SSS) was found to be more efficient than PVA-g-p(AA), and the p(SSS-co-AA) was the most potent anticoagulant among the three.

Williams and co-workers prepared polyurethanes with varying ratios (30–80%) of propyl sulfonate groups to obtain anticoagulants.[200] The polymers displayed anticoagulant activity via thrombin inhibition, interference with fibrin

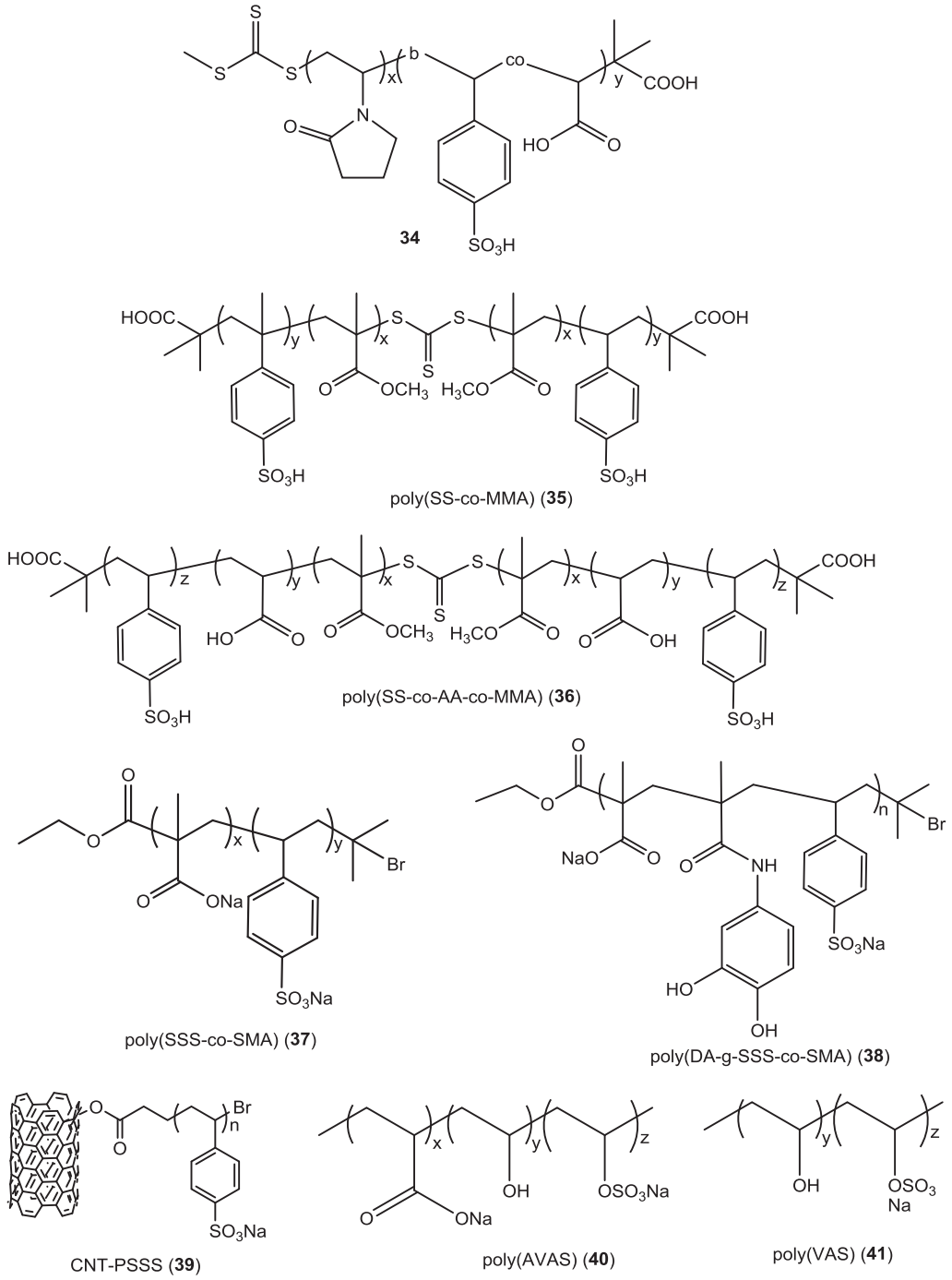


FIGURE 17 Chemical structures of synthetic sulfated (noncarbohydrate) polymers

polymerization, and by forming a complex through interaction between the polymer, thrombin, fibrin, and the plasma antiproteases. Ito et al. introduced sulfamate and carboxylate groups to their synthesized polyurethaneureas using *N*-chlorosulfonyl isocyanate as the sulfonating agent.[201] These polymers were also found to increase APTT with increasing sulfate content.

Sulfonation of polyethersulfone membranes, which was further blended with poly (acrylonitrile-*co*-acrylic acid-*co*-vinyl pyrrolidone) [poly(AN-co-AA-co-VP)] to introduce carboxyl groups, was found to exhibit significant heparin-like anticoagulant activity and to suppress platelet adhesion.[202]

Machovich et al. prepared sulfated poly(vinyl alcohol-co-acrylic acid) (**40**) and sulfated poly(viny alcohol) (**41**) having different MWs (Figure 17).[203] To exhibit effective anticoagulation, at least 20% charged groups were required. The polymers were found to accelerate thrombin inhibition via AT, and inhibit the reaction between thrombin and fibrinogen.[203,204] Polymer **40** was also found to inhibit both thrombin and plasmin activity.[205]

Tamada et al. prepared a series of sulfonated polyisoprenes (SPIPs) having various MW and different degrees of sulfonation.[206] The SPIPs were found to increase APTT values with increasing MW. Subsequently, it was found that SPIPs interact strongly with fibrinogen and fibrin monomers by forming a complex that prevents the conversion of fibrinogen to fibrin monomers and the polymerization of fibrin monomers.[207]

Min and co-workers prepared sulfonated poly(ethylene oxide) using propane sultone which displayed 14% anticoagulant activity (based on APTT test) of UFH, and inhibited thrombin function rather than FXa.[208]

Joung et al. developed supramolecular structured sulfonated polyrotaxane, (a polyrotaxane is composed of α-cyclodextrin and polyethylene glycol (PEG)), which displayed anticoagulant activity by AT mediated thrombin inhibition.[209] The most important feature of this polymer is its sliding and rotation of free α-cyclodextrins with anionic groups which played an important role to enhance the anticoagulant activity.[209,210]

Besides linear synthetic sulfonated polymers, other shaped polymers such as hyperbranched or dendritic polymers prepared from sulfonated monomers have been reported to act as anticoagulants. For example, hyper-branched sulfonated polyester nanoparticles inhibited both intrinsic and/or common pathways and thrombin activity or fibrin formation from fibrinogen.[211] Alban's group prepared tree-like structured dendritic polyglycerol sulfate whose anticoagulant activity does not depend on the MW due to the globular 3D structure.[212]

Several zwitterionic polymers have also been reported with anticoagulant activity, such as zwitterionic poly(2-oxazoline), prepared using 1,3-propane sultone and β-propiolactone,[213] and zwitterionic poly(sulfobetaine methacrylate).[214]

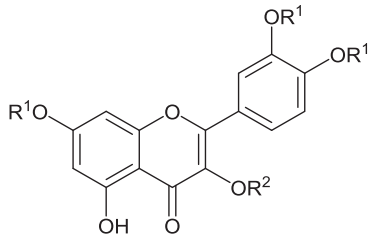
4.3.5 | Sulfated aromatic compounds/flavonoids and derivatives

Both synthetic and naturally occurring sulfated flavonoids and derivatives have been reported to possess anticoagulant activity and this area has been recently reviewed by Pinto and co-workers.[215] Of particular note are some tetrahydroisoquinolines which have been found to act as allosteric inhibitors of AT inhibition of FXa.[216,217] Sulfated benzofurans have been found to possess more potent anti-Xa activity than FIIa activity. Cabrera and co-workers reported the anticoagulant activity of trisulfated (**42,** Figure 18) and tetrasulfated quercetin (a flavonol) (**43**) which accelerated thrombin inhibition via HCII while the fully sulfated quercetin persulfate accelerated FXa inhibition via AT.[218] Sulfated flavanols were found to exhibit AT mediated anti-Xa activity where the orientation of the sulfate groups influences the potency, for example, (+)-catechin sulfate (**44**) was twofold more potent than (-)-catechin sulfate (**45**).[219,220]

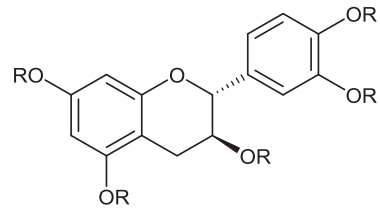
Taking advantage of the activity of sulfated flavonoids and sulfated oligosaccharides, Pinto and co-workers developed a series of persulfated flavonoid-saccharide conjugates.[221] The study found that 3-*O*-rutinosides (**46** and **47**) directly inhibited FXa and 7-*O*-rutinosides (**48** and **49**) inhibited FXa via AT. Subsequently, *trans*-resveratrol 3-β-D-glucopyranoside persulfate (**50**) was prepared as a dual anticoagulant/antiplatelet agent.[222]

4.3.6 | Nonsulfated anionic compounds as anticoagulants

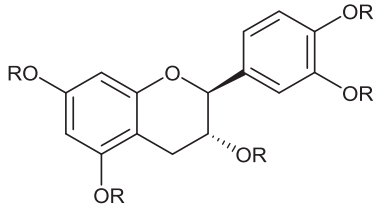
In addition to the above mentioned sulfated compounds, the anticoagulant activity of heparin mimetic compounds without any sulfate groups has also been reported. For example, the Desai group found that p(AA) (**51**, Figure 19) increased the activation of AT which subsequently accelerated the inhibitory functions of FXa and thrombin depending on pH.[223,224] At pH 6.0 poly(AA) was found to form a bridge between AT and FXa, however, this was completely abolished at pH 7.4.



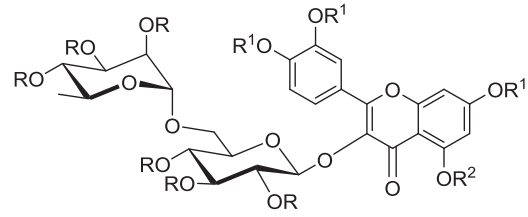
42 $R^1 = SO_3Na$ and $R^2 = Ac$
43 $R^1 = R^2 = SO_3Na$



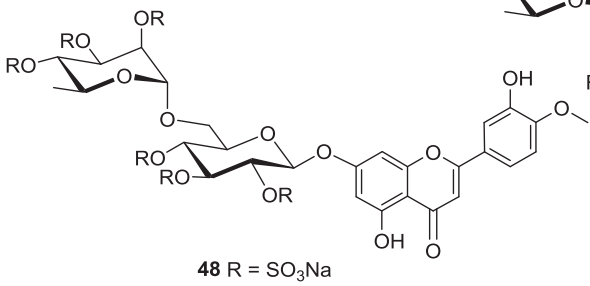
R = SO_3Na
(+)- catechin sulfate (**44**)



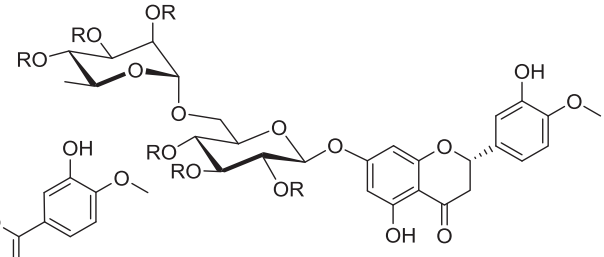
R = SO_3Na
(-)- catechin sulfate (**45**)



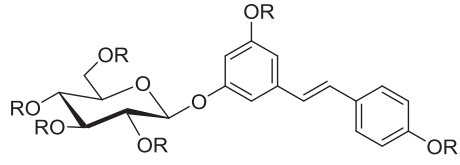
46 R = $R^1 = R^2 = SO_3Na$
47 R= SO_3Na, $R^1 = CH_2CH_2OSO_3Na$, $R^2 = H$



48 R = SO_3Na

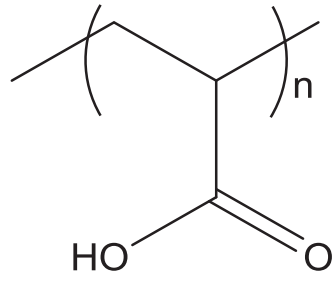


49 R = SO_3Na



50 R = SO_3Na

FIGURE 18 Structures of sulfated flavonoids and derivatives with anticoagulant activity



p(AA) **(51)**

FIGURE 19 Chemical structure of poly(acrylic acid) p(AA) (**51**)

Both DNA and RNA aptamers have also been reported to inhibit blood coagulation by increasing the inhibitory function of the coagulation factors. Bock and co-workers isolated single-stranded a 15mer-oligonucleotide consensus sequence which inhibited thrombin-catalyzed fibrin-clot formation at nanomolar concentrations and displayed its anticoagulant activity via binding with exosite I of AT.[225,226] On the other hand, RNA aptamers have also been reported to increase blood coagulation time by acceleration of the inhibitory function of FXa, FVIIa, and FIXa.[227,228]

5 | CONCLUSIONS

Since the discovery of UFH significant time and resources have been expended in the search for new anticoagulants with similar properties to UFH but without its drawbacks. These ongoing research efforts, based on a mechanistic understanding of the anticoagulant activity of UFH, have been largely directed toward mimicking the common pentasaccharide sequence ABD, TBD, and the spacing between these two domains. This has resulted in the development of commercially available LMWHs and ULMWH (fondaparinux), all of which contain the ABD of UFH, and are obtained from the chemical and/or enzymatic modification of UFH or by total synthesis. More recently, and of particular note, we have observed the development of tailor-made glycoconjugates with the full range of anticoagulant properties as UFH but with improved pharmacodynamic profiles, as well as additional useful properties such as the ability to be rapidly neutralized. Such conjugates offer an impressive array of biological properties that can be fine tuned to suit the intended cardiovascular indication and hold much promise as anticoagulant therapeutics of the future, with some having progressed to clinical trials. However, these glycoconjugates require long and complex syntheses for their manufacture. Other, more simple strategies to heparin mimetics have thus been pursued, including the modification of naturally occuring oligo- and polysaccharides and the development of heparin mimetic polymers derived from carbohydrate and non-carbohydrate monomers. The latter approaches have shown some promise with polymers identified with significant anti-Xa activity, although most of these polymers are not as potent as UFH. In the future, we expect that more structure-activity relationships will be unravelled and this may lead to the development of heparin mimetics with improved properties, suitable for progression into the clinic.

ACKNOWLEDGMENTS

We gratefully acknowledge support from the Australian Research Council (DP170104431 to VF) and the University of Queensland (IPRS/UQCent PhD Scholarship to AAN), and CSIRO Manufacturing. We thank Dr Craig Francis (CSIRO) for useful discussions.

ORCID

Vito Ferro http://orcid.org/0000-0003-3306-2550

REFERENCES

1. Gustafsson D, Bylund R, Antonsson T, et al. A new oral anticoagulant: the 50-year challenge. *Nat Rev Drug Discov*. 2004;3:649–659.
2. Furie B, Furie BC. Mechanisms of thrombus formation. *New Engl J Med*. 2008;359:938–949.
3. Raskob GE, Angchaisuksiri P, Blanco AN, et al. Thrombosis: a major contributor to global disease burden. *Thromb Res*. 2014;134:931–938.
4. Cardiovascular Diseases (CVDs). World Health Organization Fact Sheet. 2017. http://www.who.int/mediacentre/factsheets/fs317/en/.
5. Walenga JM, Bick RL. Heparin-induced thrombocytopenia, paradoxical thromboembolism, and other side effects of heparin therapy. *Med Clin North Am*. 1998;82:635–658.
6. Shen JI, Winkelmayer WC. Use and safety of unfractionated heparin for anticoagulation during maintenance hemodialysis. *Am J Kidney Dis*. 2012;60:473–486.

7. Hirsh J, Warkentin TE, Shaughnessy SG, et al. Heparin and low-molecular-weight heparin: mechanisms of action, pharmacokinetics, dosing, monitoring, efficacy, and safety. *Chest*. 2001;119:64S–94S.
8. Hirsh J, Warkentin TE, Raschke R, Granger C, Ohman EM, Dalen JE. Heparin and low-molecular-weight heparin: mechanisms of action, pharmacokinetics, dosing considerations, monitoring, efficacy, and safety. *Chest*. 1998;114:489S–510S.
9. Arepally GM, Ortel TL. Clinical practice. Heparin-induced thrombocytopenia. *New Engl J Med*. 2006;355:809–817.
10. Liu H, Zhang Z, Linhardt RJ. Lessons learned from the contamination of heparin. *Nat Prod Rep*. 2009;26:313–321.
11. Rabenstein DL. Heparin and heparan sulfate: structure and function. *Nat Prod Rep*. 2002;19:312–331.
12. Weitz JI, Crowther M. Direct thrombin inhibitors. *Thromb Res*. 2002;106:V275–V284.
13. Pineo GF, Hull RD. Low-molecular-weight heparin: prophylaxis and treatment of venous thromboembolism. *Annu Rev Med*. 1997;48:79–91.
14. Xu Y, Cai C, Chandarajoti K, et al. Homogeneous low-molecular-weight heparins with reversible anticoagulant activity. *Nat Chem Biol*. 2014;10:248–250.
15. Macfarlane RG. An enzyme cascade in the blood clotting mechanism, and its function as a biochemical amplifier. *Nature*. 1964;202:498–499.
16. Wilson MR, Campbell Tait R. Hemostasis and anticoagulants. In: Padmanabhan S, ed. *Handbook of Pharmacogenomics and Stratified Medicines*. San Diego, CA: Academic Press; 2014:479–496.
17. Adams RLC, Bird RJ. Review article: coagulation cascade and therapeutics update: relevance to nephrology. Part 1: overview of coagulation, thrombophilias and history of anticoagulants. *Nephrology*. 2009;14:462–470.
18. Walenga JM, Lyman GH. Evolution of heparin anticoagulants to ultra-low-molecular-weight heparins: a review of pharmacologic and clinical differences and applications in patients with cancer. *Crit Rev Oncol Hemat*. 2013;88:1–18.
19. Mulloy B, Gray E, Barrowcliffe TW. Characterization of unfractionated heparin: comparison of materials from the last 50 years. *Thromb Haemost*. 2000;84:1052–1056.
20. Linhardt RJ, Gunay NS. Production and chemical processing of low molecular weight heparins. *Semin Thromb Hemost*. 1999;25(Suppl 3):5–16.
21. Casu B. Structure and active domains of heparin. In: Garg HG, Linhardt RJ, Hales CA, eds. *Chemistry and Biology of Heparin and Heparan Sulfate*. Amsterdam, the Netherlands: Elsevier; 2005:1–28.
22. Bianchini P, Liverani L, Mascellani G, Parma B. Heterogeneity of unfractionated heparins studied in connection with species, source, and production processes. *Semin Thromb Hemost*. 1997;23:3–10.
23. Lane DA, Denton J, Flynn AM, Thunberg L, Lindahl U. Anticoagulant activities of heparin oligosaccharides and their neutralization by platelet factor 4. *Biochem J*. 1984;218:725–732.
24. Al Dieri R, Wagenvoord R, van Dedem GW, Beguin S, Hemker HC. The inhibition of blood coagulation by heparins of different molecular weight is caused by a common functional motif–the C-domain. *J Thromb Haemost*. 2003;1:907–914.
25. Gray E, Mulloy B, Barrowcliffe TW. Heparin and low-molecular-weight heparin. *Thromb Haemost*. 2008;99:807–818.
26. Higashi K, Hosoyama S, Ohno A, et al. Photochemical preparation of a novel low molecular weight heparin. *Carbohydr Polym*. 2012;87:1737–1743.
27. Monograph 01/2008:0828 Heparins, Low-Molecular mass. European Pharmacopoeia. 6.0 edition, 2008:2041–2042.
28. Jeske WP, Walenga JM, Hoppensteadt DA, et al. Differentiating low-molecular-weight heparins based on chemical, biological, and pharmacologic properties: implications for the development of generic versions of low-molecular-weight heparins. *Semin Thromb Hemost*. 2008;34:74–85.
29. Maddineni J, Walenga JM, Jeske WP, et al. Product individuality of commercially available low-molecular-weight heparins and their generic versions: therapeutic implications. *Clin Appl Thromb Hemost*. 2006;12:267–276.
30. Petitou M, Duchaussoy P, Lederman I, et al. Synthesis of heparin fragments: a methyl alpha-pentaoside with high affinity for antithrombin III. *Carbohydr Res*. 1987;167:67–75.
31. Petitou M, Duchaussoy P, Herbert JM, et al. The synthetic pentasaccharide fondaparinux: first in the class of antithrombotic agents that selectively inhibit coagulation factor Xa. *Semin Thromb Hemost*. 2002;28:393–402.
32. Petitou M, van Boeckel CAA. A synthetic antithrombin III binding pentasaccharide is now a drug! What comes next? *Angew Chem Int Ed*. 2004;43:3118–3133.
33. Herbert JM, Herault JP, Bernat A, et al. Biochemical and pharmacological properties of SANORG 34006, a potent and long-acting synthetic pentasaccharide. *Blood*. 1998;91:4197–4205.
34. Olson ST, Chuang Y-J. Heparin activates antithrombin anticoagulant function by generating new interaction sites (exosites) for blood clotting proteinases. *Trends Cardiovasc Med*. 2002;12:331–338.

35. Casu B, Naggi A, Torri G. Re-visiting the structure of heparin. *Carbohydr Res*. 2015;403:60–68.
36. Walker FJ, Esmon CT. The molecular mechanisms of heparin action III. The anticoagulant properties of polyanetholesulfonate. *Biochem Biophys Res Commun*. 1978;83:1339–1346.
37. Rosenberg RD, Damus PS. The purification and mechanism of action of human antithrombin heparin cofactor. *J Biol Chem*. 1973;248:6490–6505.
38. Rosenberg RD. Chemistry of the hemostatic mechanism and its relationship to the action of heparin. *Fed Proc*. 1977;36:10–18.
39. Hirsh J, Dalen JE, Deykin D, Poller L. Heparin: mechanism of action, pharmacokinetics, dosing considerations, monitoring, efficacy, and safety. *Chest*. 1992;102:337S–351S.
40. Olson ST, Shore JD. Demonstration of a two-step reaction mechanism for inhibition of alpha-thrombin by antithrombin III and identification of the step affected by heparin. *J Biol Chem*. 1982;257:14891–14895.
41. Danielsson A, Raub E, Lindahl U, Bjork I. Role of ternary complexes, in which heparin binds both antithrombin and proteinase, in the acceleration of the reactions between antithrombin and thrombin or factor Xa. *J Biol Chem*. 1986;261:15467–15473.
42. Mourier PAJ, Guichard OY, Herman F, Viskov C. Isolation of a pure octadecasaccharide with antithrombin activity from an ultra-low-molecular-weight heparin. *Anal Biochem*. 2014;453:7–15.
43. Thunberg L, Bäckström G, Lindahl U. Further characterization of the antithrombin-binding sequence in heparin. *Carbohydr Res*. 1982;100:393–410.
44. Choay J, Petitou M, Lormeau JC, Sinay P, Casu B, Gatti G. Structure-activity relationship in heparin: a synthetic pentasaccharide with high affinity for antithrombin III and eliciting high anti-factor Xa activity. *Biochem Biophys Res Commun*. 1983;116:492–499.
45. Bedsted T, Swanson R, Chuang YJ, Bock PE, Bjork I, Olson ST. Heparin and calcium ions dramatically enhance antithrombin reactivity with factor IXa by generating new interaction exosites. *Biochemistry*. 2003;42:8143–8152.
46. Whisstock JC, Pike RN, Jin L, et al. Conformational changes in serpins: II. The mechanism of activation of antithrombin by heparin. *J Mol Biol*. 2000;301:1287–1305.
47. Schreuder HA, de Boer B, Dijkema R, et al. The intact and cleaved human antithrombin III complex as a model for serpin-proteinase interactions. *Nat Struct Mol Biol*. 1994;1:48–54.
48. Petitou M, Herault J-P, Bernat A, et al. Synthesis of thrombin-inhibiting heparin mimetics without side effects. *Nature*. 1999;398:417–422.
49. Lam LH, Silbert JE, Rosenberg RD. The separation of active and inactive forms of heparin. *Biochem Biophys Res Commun*. 1976;69:570–577.
50. Oosta GM, Gardner WT, Beeler DL, Rosenberg RD. Multiple functional domains of the heparin molecule. *Proc Natl Acad Sci U S A*. 1981;78:829–833.
51. Sheehan JP, Sadler JE. Molecular mapping of the heparin-binding exosite of thrombin. *Proc Natl Acad Sci U S A*. 1994;91:5518–5522.
52. Heuck CC, Schiele U, Horn D, Fronda D, Ritz E. The role of surface charge on the accelerating action of heparin on the antithrombin III-inhibited activity of alpha-thrombin. *J Biol Chem*. 1985;260:4598–4603.
53. Hatanaka K, Yoshida T, Miyahara S, et al. Synthesis of new heparinoids with high anticoagulant activity. *J Med Chem*. 1987;30:810–814.
54. Alban S, Schauerte A, Franz G. Anticoagulant sulfated polysaccharides: part I. Synthesis and structure–activity relationships of new pullulan sulfates. *Carbohydr Polym*. 2002;47:267–276.
55. Agarwal A, Danishefsky I. Requirement of free carboxyl groups for the anticoagulant activity of heparin. *Thromb Res*. 1986;42:673–680.
56. Wessel HP, Hosang M, Tschopp TB, Weimann BJ. Heparin, carboxyl-reduced sulfated heparin, and trestatin A sulfate. Antiproliferative and anticoagulant activities. *Carbohydr Res*. 1990;204:131–139.
57. Church FC, Treanor RE, Sherrill GB, Whinna HC. Carboxylate polyanions accelerate inhibition of thrombin by heparin cofactor II. *Biochem Biophys Res Commun*. 1987;148:362–368.
58. Stone AL, Beeler D, Oosta G, Rosenberg RD. Circular dichroism spectroscopy of heparin-antithrombin interactions. *Proc Natl Acad Sci U S A*. 1982;79:7190–7194.
59. Xu Y, Masuko S, Takieddin M, et al. Chemoenzymatic synthesis of homogeneous ultralow molecular weight heparins. *Science*. 2011;334:498–501.

60. Xu Y, Pempe EH, Liu J. Chemoenzymatic synthesis of heparin oligosaccharides with both anti-factor Xa and anti-factor IIa activities. *J Biol Chem*. 2012;287:29054–29061.
61. Chandarajoti K, Xu Y, Sparkenbaugh E, Key NS, Pawlinski R, Liu J. De novo synthesis of a narrow size distribution low-molecular-weight heparin. *Glycobiology*. 2014;24:476–486.
62. Hsieh P-H, Xu Y, Keire DA, Liu J. Chemoenzymatic synthesis and structural characterization of 2-O-sulfated glucuronic acid-containing heparan sulfate hexasaccharides. *Glycobiology*. 2014;24:681–692.
63. Harenberg J. Development of idraparinux and idrabiotaparinux for anticoagulant therapy. *Thromb Haemost*. 2009;102:811–815.
64. Savi P, Herault JP, Duchaussoy P, et al. Reversible biotinylated oligosaccharides: a new approach for a better management of anticoagulant therapy. *J Thromb Haemost*. 2008;6:1697–1706.
65. de Kort M, Buijsman RC, van Boeckel CAA. Synthetic heparin derivatives as new anticoagulant drugs. *Drug Discov Today*. 2005;10:769–779.
66. Westerduin P, Basten JEM, Broekhoven MA, de Kimpe V, Kuijpers WHA, van Boeckel CAA. Synthesis of tailor-made glycoconjugates showing AT III-mediated inhibition of blood coagulation factors Xa and thrombin. *Angew Chem Int Ed Engl*. 1996;35:331–333.
67. Buijsman RC, Basten JEM, van Dinther TG, van der Marel GA, van Boeckel CAA, van Boom JH. Design and synthesis of a novel synthetic NAPAP-penta-saccharide conjugate displaying a dual antithrombotic action. *Bioorg Med Chem Lett*. 1999;9:2013–2018.
68. Vogel GMT, Meuleman DG, Van Dinther TG, Buijsman R, Princen AWM, Smit MJ. Antithrombotic properties of a direct thrombin inhibitor with a prolonged half-life and AT-mediated factor Xa inhibitory activity. *J Thromb Haemost*. 2003;1:1945–1954.
69. Olson ST, Swanson R, Petitou M. Specificity and selectivity profile of EP217609: a new neutralizable dual-action anticoagulant that targets thrombin and factor Xa. *Blood*. 2012;119:2187–2195.
70. Hechler B, Freund M, Alame G, et al. The antithrombotic activity of EP224283, a neutralizable dual factor Xa inhibitor/glycoprotein IIbIIIa antagonist, exceeds that of the coadministered parent compounds. *J Pharmacol Exp Ther*. 2011;338:412–420.
71. Sankarayanarayanan NV, Strebel TR, Boothello RS, et al. A hexasaccharide containing rare 2-O-sulfate-glucuronic acid residues selectively activates heparin cofactor II. *Angew Chem Int Ed*. 2017;56:2312–2317.
72. Wall D, Douglas S, Ferro V, Cowden W, Parish C. Characterisation of the anticoagulant properties of a range of structurally diverse sulfated oligosaccharides. *Thromb Res*. 2001;103:325–335.
73. Liao BY, Wang Z, Hu J, et al. PI-88 inhibits postoperative recurrence of hepatocellular carcinoma via disrupting the surge of heparanase after liver resection. *Tumour Biol*. 2016;37:2987–2998.
74. Parish CR, Freeman C, Brown KJ, Francis DJ, Cowden WB. Identification of sulfated oligosaccharide-based inhibitors of tumor growth and metastasis using novel in vitro assays for angiogenesis and heparanase activity. *Cancer Res*. 1999;59:3433–3441.
75. Khachigian LM, Parish CR. Phosphomannopentaose sulfate (PI-88): heparan sulfate mimetic with clinical potential in multiple vascular pathologies. *Cardiovasc Drug Rev*. 2004;22:1–6.
76. Raake W, Klauser RJ, Elling H, Meinetsberger E. Anticoagulant and antithrombotic properties of synthetic sulfated bislactobionic acid amides. *Thromb Res*. 1989;56:719–730.
77. Klauser RJ. Interaction of the sulfated lactobionic acid amide LW 10082 with thrombin and its endogenous inhibitors. *Thromb Res*. 1991;62:557–565.
78. Ofosu FA, Fareed J, Smith LM, Anvari N, Hoppensteadt D, Blajchman MA. Inhibition of factor X, factor V and prothrombin activation by the bis(lactobionic acid amide) LW10082. *Eur J Biochem*. 1992;203:121–125.
79. Martin DJ, Toce JA, Anevski PJ, Tollefsen DM, Abendschein DR. Anticoagulant and antithrombotic activity of maltodapoh, a novel sulfated tetrasaccharide. *J Pharmacol Exp Ther*. 1999;288:516–521.
80. Desai BJ, Boothello RS, Mehta AY, Scarsdale JN, Wright HT, Desai UR. Interaction of thrombin with sucrose octasulfate. *Biochemistry*. 2011;50:6973–6982.
81. Rashid Q, Abid M, Gupta N, Tyagi T, Ashraf MZ, Jairajpuri MA. Polysulfated trehalose as a novel anticoagulant agent with dual mode of action. *BioMed Res Int*. 2015;2015:1–11.
82. Bjornsson TD, Nash PV, Schaten R. The anticoagulant effect of chondroitin-4-sulfate. *Thromb Res*. 1982;27:15–21.
83. Teien AN, Abildgaard U, Höök M. The anticoagulant effect of heparan sulfate and dermatan sulfate. *Thromb Res*. 1976;8:859–867.

84. Hatton MWC, Berry LR, Ragoeczl E. Inhibition of thrombin by antithrombin III in the presence of certain glycosaminoglycans found in the mammalian aorta. *Thromb Res*. 1978;13:655–670.
85. Fernandez F, Ryn JV, Ofosu FA, Hirsh J, Buchanan MR. The haemorrhagic and antithrombotic effects of dermatan sulphate. *Br J Haematol*. 1986;64:309–317.
86. Tollefsen DM, Peacock ME, Monafo WJ. Molecular size of dermatan sulfate oligosaccharides required to bind and activate heparin cofactor II. *J Biol Chem*. 1986;261:8854–8858.
87. Maimone MM, Tollefsen DM. Structure of a dermatan sulfate hexasaccharide that binds to heparin cofactor II with high affinity. *J Biol Chem*. 1990;265:18263–18271.
88. Farias WRL, Valente A-P, Pereira MS, Mourão PAS. Structure and anticoagulant activity of sulfated galactans: isolation of a unique sulfated galactan from the red algae *Botryocladia occidentalis* and comparison of its anticoagulant action with that of sulfated galactans from invertebrates. *J Biol Chem*. 2000;275:29299–29307.
89. Pereira MS, Vilela-Silva A-CES, Valente A-P, Mourão PAS. A 2-sulfated, 3-linked α-l-galactan is an anticoagulant polysaccharide. *Carbohydr Res*. 2002;337:2231–2238.
90. Colliec S, Fischer AM, Tapon-Bretaudiere J, Boisson C, Durand P, Jozefonvicz J. Anticoagulant properties of a fucoidan fraction. *Thromb Res*. 1991;64:143–154.
91. Nishino T, Aizu Y, Nagumo T. Antithrombin activity of a fucan sulfate from the brown seaweed *Ecklonia kurome*. *Thromb Res*. 1991;62:765–773.
92. Nishino T, Aizu Y, Nagumo T. The influence of sulfate content and molecular weight of a fucan sulfate from the brown seaweed *Ecklonia kurome* on its antithrombin activity. *Thromb Res*. 1991;64:723–731.
93. Colliec S, Boisson-vidal C, Jozefonvicz J. A low molecular weight fucoidan fraction from the brown seaweed *Pelvetia canaliculata*. *Phytochemistry*. 1994;35:697–700.
94. Paulo ASM. Use of sulfated fucans as anticoagulant and antithrombotic agents: future perspectives. *Curr Pharm Des*. 2004;10:967–981.
95. Pomin VH, Mourão PAS. Structure, biology, evolution, and medical importance of sulfated fucans and galactans. *Glycobiology*. 2008;18:1016–1027.
96. Pomin V, Mourão P. Specific sulfation and glycosylation—a structural combination for the anticoagulation of marine carbohydrates. *Front Cell Infect Microbiol*. 2014;4:33.
97. Buyue Y, Sheehan JP. Fucosylated chondroitin sulfate inhibits plasma thrombin generation via targeting of the factor IXa heparin-binding exosite. *Blood*. 2009;114:3092–3100.
98. Wu M, Xu S, Zhao J, Kang H, Ding H. Physicochemical characteristics and anticoagulant activities of low molecular weight fractions by free-radical depolymerization of a fucosylated chondroitin sulphate from sea cucumber *Thelenata ananas*. *Food Chem*. 2010;122:716–723.
99. Gao N, Lu F, Xiao C, et al. β-Eliminative depolymerization of the fucosylated chondroitin sulfate and anticoagulant activities of resulting fragments. *Carbohydr Polym*. 2015;127:427–437.
100. Wu M, Wen D, Gao N, et al. Anticoagulant and antithrombotic evaluation of native fucosylated chondroitin sulfates and their derivatives as selective inhibitors of intrinsic factor Xase. *Eur J Med Chem*. 2015;92:257–269.
101. Mou J, Wang C, Li W, Yang J. Purification, structural characterization and anticoagulant properties of fucosylated chondroitin sulfate isolated from *Holothuria mexicana*. *Int J Biol Macromol*. 2017;98:208–215.
102. Mourao PAS, Pereira MS, Pavo MSG, et al. Structure and anticoagulant activity of a fucosylated chondroitin sulfate from echinoderm. Sulfated fucose branches on the polysaccharide account for its high anticoagulant action. *J Biol Chem*. 1996;271:23973–23984.
103. Fonseca RJC, Mourão PAS. Fucosylated chondroitin sulfate as a new oral antithrombotic agent. *Thromb Haemost*. 2006;96:822–829.
104. Liu X, Hao J, Shan X, et al. Antithrombotic activities of fucosylated chondroitin sulfates and their depolymerized fragments from two sea cucumbers. *Carbohydr Polym*. 2016;152:343–350.
105. Chen S, Xue C, La Y, Tang Q, Yu G, Chai W. Comparison of structures and anticoagulant activities of fucosylated chondroitin sulfates from different sea cucumbers. *Carbohydr Polym*. 2011;83:688–696.
106. Wu M, Huang R, Wen D, et al. Structure and effect of sulfated fucose branches on anticoagulant activity of the fucosylated chondroitin sulfate from sea cucumber *Thelenata ananas*. *Carbohydr Polym*. 2012;87:862–868.
107. Mourão PAS, Pereira MS, Pavão MSG, et al. Structure and anticoagulant activity of a fucosylated chondroitin sulfate from echinoderm: sulfated fucose branches on the polysaccharide account for its high anticoagulant action. *J Biol Chem*. 1996;271:23973–23984.

108. Tollefsen DM, Pestka CA, Monafo WJ. Activation of heparin cofactor II by dermatan sulfate. *J Biol Chem*. 1983;258:6713–6716.
109. Tollefsen DM. The interaction of glycosaminoglycans with heparin cofactor II: structure and activity of a high-affinity dermatan sulfate hexasaccharide. In: Lane DA, Björk I, Lindahl U, eds. *Heparin and Related Polysaccharides*. Boston, MA: Springer; 1992:167–176.
110. Mascellani G, Liverani L, Bianchini P, et al. Structure and contribution to the heparin cofactor II-mediated inhibition of thrombin of naturally oversulphated sequences of dermatan sulphate. *Biochem J*. 1993;296:639–648.
111. Mascellani G, Liverani L, Parma B, Bergonzini G, Bianchini P. Active site for heparin cofactor II in low molecular mass dermatan sulfate. Contribution to the antithrombotic activity of fractions with high affinity for heparin cofactor II. *Thromb Res*. 1996;84:21–32.
112. Maaroufi RM, Jozefowicz M, Tapon-Bretaudière J, Fischer A-M. Mechanism of thrombin inhibition by antithrombin and heparin cofactor II in the presence of heparin. *Biomaterials*. 1997;18:203–211.
113. Maaroufi RM, Jozefowicz M, Tapon-Bretaudière J, Jozefonvicz J, Fischer A-M. Mechanism of thrombin inhibition by heparin cofactor II in the presence of dermatan sulphates, native or oversulphated, and a heparin-like dextran derivative. *Biomaterials*. 1997;18:359–366.
114. Kindness G, Long WF, Williamson FB. The anticoagulant activity of dermatan sulphates: evidence against the involvement of antithrombin III. *Br J Pharmacol*. 1981;72:81–88.
115. He L, Giri TK, Vicente CP, Tollefsen DM. Vascular dermatan sulfate regulates the antithrombotic activity of heparin cofactor II. *Blood*. 2008;111:4118–4125.
116. Mansour MB, Dhahri M, Hassine M, et al. Highly sulfated dermatan sulfate from the skin of the ray *Raja montagui*: anticoagulant activity and mechanism of action. *Comp Biochem Phys B*. 2010;156:206–215.
117. Souza MLS, Dellias JMM, Melo FR, Silva L-CF. Structural composition and anticoagulant activity of dermatan sulfate from the skin of the electric eel, *Electrophorus electricus* (L.). *Comp Biochem Phys B*. 2007;147:387–394.
118. Ben Mansour M, Majdoub H, Bataille I, et al. Polysaccharides from the skin of the ray *Raja radula*. Partial characterization and anticoagulant activity. *Thromb Res*. 2009;123:671–678.
119. Ben Mansour M, Dhahri M, Bertholon I, et al. Characterization of a novel dermatan sulfate with high antithrombin activity from ray skin (*Raja radula*). *Thromb Res*. 2009;123:887–894.
120. Dellias João MM, Onofre Glaucia R, Werneck Cláudio C, et al. Structural composition and differential anticoagulant activities of dermatan sulfates from the skin of four species of rays, *Dasyatis americana*, *Dasyatis gutatta*, *Aetobatus narinari* and *Potamotrygon motoro*. *Biochimie*. 2004;86:677–683.
121. Linhardt RJ, Desai UR, Liu J, Pervin A, Hoppenstead D, Fareed J. Low molecular weight dermatan sulfate as an antithrombotic agent. Structure-activity relationship studies. *Biochem Pharmacol*. 1994;47:1241–1252.
122. Fernández JA, Petäjä J, Griffin JH. Dermatan sulfate and LMW heparin enhance the anticoagulant action of activated protein C. *Thromb Haemost*. 1999;82:1462–1468.
123. Pomin VH. Review: an overview about the structure–function relationship of marine sulfated homopolysaccharides with regular chemical structures. *Biopolymers*. 2009;91:601–609.
124. Yoon S-J, Pyun Y-R, Hwang J-K, Mourão PAS. A sulfated fucan from the brown alga *Laminaria cichorioides* has mainly heparin cofactor II-dependent anticoagulant activity. *Carbohydr Res*. 2007;342:2326–2330.
125. Pereira MS, Mulloy B, Mourão PAS. Structure and anticoagulant activity of sulfated fucans: comparison between the regular, repetitive, and linear fucans from echinoderms with the more heterogeneous and branched polymers from brown algae. *J Biol Chem*. 1999;274:7656–7667.
126. Muzzarelli RAA, Tanfani F, Emanuelli M, Pace DP, Chiurazzi E, Piani M. Sulfated *N*-(carboxymethyl)chitosans: novel blood anticoagulants. *Carbohydr. Res*. 1984;126:225–231.
127. Wolfrom ML, Shen TM, Summers CG. Sulfated nitrogenous polysaccharides and their anticoagulant activity. *J Am Chem Soc*. 1953;75:1519.
128. Wolfrom ML, Han TMS. The sulfonation of chitosan. *J Am Chem Soc*. 1959;81:1764–1766.
129. Doczi J, Fischman A, King JA. Direct evidence of the influence of sulfamic acid linkages on the activity of heparin-like anticoagulants. *J Am Chem Soc*. 1953;75:1512–1513.
130. Whistler RL, Kosik M. Anticoagulant activity of oxidized and *N*- and *O*-sulfated chitosan. *Arch Biochem Biophys*. 1971;142:106–110.
131. Horton D, Just EK. Preparation from chitin of (1→4)-2-amino-2-deoxy-β-D-glucopyranuronan and its 2-sulfoamino analog having blood-anticoagulant properties. *Carbohydr Res*. 1973;29:173–179.

132. Hirano S, Tanaka Y, Hasegawa M, Tobetto K, Nishioka A. Effect of sulfated derivatives of chitosan on some blood coagulant factors. *Carbohydr Res*. 1985;137:205–215.
133. Huang R, Du Y, Yang J, Fan L. Influence of functional groups on the in vitro anticoagulant activity of chitosan sulfate. *Carbohydr Res*. 2003;338:483–489.
134. Ronghua H, Yumin D, Jianhong Y. Preparation and anticoagulant activity of carboxybutyrylated hydroxyethyl chitosan sulfates. *Carbohydr Polym*. 2003;51:431–438.
135. Vikhoreva G, Bannikova G, Stolbushkina P, et al. Preparation and anticoagulant activity of a low-molecular-weight sulfated chitosan. *Carbohydr Polym*. 2005;62:327–332.
136. Jayakumar R, Nwe N, Tokura S, Tamura H. Sulfated chitin and chitosan as novel biomaterials. *Int J Biol Macromol*. 2007;40:175–181.
137. Alves NM, Mano JF. Chitosan derivatives obtained by chemical modifications for biomedical and environmental applications. *Int J Biol Macromol*. 2008;43:401–414.
138. Suwan J, Zhang Z, Li B, et al. Sulfonation of papain-treated chitosan and its mechanism for anticoagulant activity. *Carbohydr Res*. 2009;344:1190–1196.
139. Xiong W, Yi Y, Liu H, Wang H, Liu J, Ying G. Selective carboxypropionylation of chitosan: synthesis, characterization, blood compatibility, and degradation. *Carbohydr Res*. 2011;346:1217–1223.
140. Fan L, Wu P, Zhang J, et al. Synthesis and anticoagulant activity of the quaternary ammonium chitosan sulfates. *Int J Biol Macromol*. 2012;50:31–37.
141. Wang T, Zhou Y, Xie W, Chen L, Zheng H, Fan L. Preparation and anticoagulant activity of *N*-succinyl chitosan sulfates. *Int J Biol Macromol*. 2012;51:808–814.
142. Yang J, Luo K, Li D, et al. Preparation, characterization and in vitro anticoagulant activity of highly sulfated chitosan. *Int J Biol Macromol*. 2013;52:25–31.
143. Nishimura S-I, Nishi N, Tokura S, Okiei W, Somorin O. Inhibition of the hydrolytic activity of thrombin by chitin heparinoids. *Carbohydr Res*. 1986;156:286–292.
144. Nishimura S, Tokura S. Preparation and antithrombogenic activities of heparinoid from 6-*O*-(carboxymethyl)chitin. *Int J Biol Macromol*. 1987;9:225–232.
145. Nishimura S, Nishi N, Tokura S. Interaction of chitin heparinoids with bovine antithrombin III. *Int J Biol Macromol*. 1987;9:305–307.
146. Warner DT, Coleman LL. Selective sulfonation of amino groups in amino alcohols. *J Org Chem*. 1958;23:1133–1135.
147. Zou Y, Khor E. Preparation of sulfated-chitins under homogeneous conditions. *Carbohydr Polym*. 2009;77:516–525.
148. Subhapradha N, Ramasamy P, Srinivasan A, Madeswaran P, Shanmugam V, Shanmugam A. Sulfation of β-chitosan and evaluation of biological activity from gladius of *Sepioteuthis lessoniana*. *Int J Biol Macromol*. 2013;62:336–340.
149. Vongchan P, Sajomsang W, Subyen D, Kongtawelert P. Anticoagulant activity of a sulfated chitosan. *Carbohydr Res*. 2002;337:1239–1242.
150. Muzzarelli RA, Tanfani F, Emanuelli M, Pace DP, Chiurazzi E, Piani M. Sulfated *N*-(carboxymethyl)chitosans: novel blood anticoagulants. *Carbohydr Res*. 1984;126:225–231.
151. Magnani A, Lamponi S, Rappuoli R, Barbucci R. Sulphated hyaluronic acids: a chemical and biological characterisation. *Polym Int*. 1998;46:225–240.
152. Magnani A, Albanese A, Lamponi S, Barbucci R. Blood-interaction performance of differently sulphated hyaluronic acids. *Thromb Res*. 1996;81:383–395.
153. Ricketts CR. Dextran sulphate—a synthetic analogue of heparin. *Biochem J*. 1952;51:129–133.
154. Walton KW. The biological behaviour of a new synthetic anticoagulant (dextran sulphate) possessing heparin-like properties. *Br J Pharmacol Chemother*. 1952;7:370–391.
155. Ricketts CR. The blood anticoagulant effect of short chain-length dextran sulphates. *Br J Pharmacol Chemother*. 1954;9:224–228.
156. Forwell GD, Ingram GIC. The anticoagulant activity of dextran sulphate. *J Pharm Pharmacol*. 1956;8:530–543.
157. Ingram GIC, Forwell GD. The anticoagulant activity of dextran sulphate. *J Pharm Pharmacol*. 1956;8:589–601.
158. Chaubet F, Champion J, Maïga O, Mauray S, Jozefonvicz J. Synthesis and structure—anticoagulant property relationships of functionalized dextrans: CMDBS. *Carbohydr Polym*. 1995;28:145–152.
159. Krentsel L, Chaubet F, Rebrov A, et al. Anticoagulant activity of functionalized dextrans. Structure analyses of carboxymethylated dextran and first Monte Carlo simulations. *Carbohydr Polym*. 1997;33:63–71.

160. Maiga-Revel O, Chaubet F, Jozefonvicz J. New investigations on heparin-like derivatized dextrans: CMDBS, synergistic role of benzylamide and sulfate substituents in anticoagulant activity. *Carbohydr Polym*. 1997;32:89–93.

161. Logeart-Avramoglou D, Jozefonvicz J. Carboxymethyl benzylamide sulfonate dextrans (CMDBS), a family of biospecific polymers endowed with numerous biological properties: a review. *J Biomed Mater Res*. 1999;48:578–590.

162. Mauzac M, Aubert N, Jozefonvicz J. Antithrombic activity of some polysaccharide resins. *Biomaterials*. 1982;3:221–224.

163. Mauzac M, Jozefonvicz J. Anticoagulant activity of dextran derivatives. Part I: synthesis and characterization. *Biomaterials*. 1984;5:301–304.

164. Crepon B, Maillet F, Kazatchkine MD, Jozefonvicz J. Molecular weight dependency of the acquired anticomplementary and anticoagulant activities of specifically substituted dextrans. *Biomaterials*. 1987;8:248–253.

165. Huynh R, Chaubet F, Jozefonvicz J. Anticoagulant properties of dextranmethylcarboxylate benzylamide sulfate (DMCBSu); a new generation of bioactive functionalized dextran. *Carbohydr Res*. 2001;332:75–83.

166. Paluck SJ, Nguyen TH, Maynard HD. Heparin-mimicking polymers: synthesis and biological applications. *Biomacromolecules*. 2016;17:3417–3440.

167. Aubert N, Mauzac M, Gulino D, Jozefonvicz J. Anticoagulant hydrogels derived from crosslinked dextran. Part II: mechanism of thrombin inactivation. *Biomaterials*. 1987;8:100–104.

168. Fischer AM, Mauzac M, Tapon-Bretaudiere J, Jozefonvicz J. Anticoagulant activity of dextran derivatives. Part II: mechanism of thrombin inactivation. *Biomaterials*. 1985;6:198–202.

169. Yamagishi R, Niwa M, Sakuragawa N. Thrombin inhibitory activity of heparin cofactor II depends on the molecular weight and sulfate amount of dextran sulfate. *Thromb Res*. 1986;44:347–354.

170. Nagasawa K, Harada H, Hayashi S, Misawa T. Sulfation of dextran with piperidine-*N*-sulfonic acid. *Carbohydr Res*. 1972;21:420–426.

171. Mendes SF, Santos O Jr, Barbosa AM, et al. Sulfonation and anticoagulant activity of botryosphaeran from *Botryosphaeria rhodina* MAMB-05 grown on fructose. *Int J Biol Macromol*. 2009;45:305–309.

172. Vasconcelos AFD, Dekker RFH, Barbosa AM, et al. Sulfonation and anticoagulant activity of fungal exocellular β-(1→6)-D-glucan (lasiodiplodan). *Carbohydr Polym*. 2013;92:1908–1914.

173. Alban S, Jeske W, Welzel D, Franz G, Fareed J. Anticoagulant and antithrombotic actions of a semisynthetic β-1,3-glucan sulfate. *Thromb Res*. 1995;78:201–210.

174. Muschin T, Budragchaa D, Kanamoto T, et al. Chemically sulfated natural galactomannans with specific antiviral and anticoagulant activities. *Int J Biol Macromol*. 2016;89:415–420.

175. Mestechkina N, Shcherbukhin V, Bannikova G, et al. Anticoagulant activity of low-molecular-weight sulfated derivatives of galactomannan from *Cyamopsis tetragonoloba* (L.) seeds. *Appl Biochem Microbiol*. 2007;43:650–654.

176. Ronghua H, Yumin D, Jianhong Y. Preparation and in vitro anticoagulant activities of alginate sulfate and its quaterized derivatives. *Carbohydr Polym*. 2003;52:19–24.

177. Ma L, Cheng C, Nie C, et al. Anticoagulant sodium alginate sulfates and their mussel-inspired heparin-mimetic coatings. *J Mater Chem B*. 2016;4:3203–3215.

178. Fan L, Jiang L, Xu Y, et al. Synthesis and anticoagulant activity of sodium alginate sulfates. *Carbohydr Polym*. 2011;83:1797–1803.

179. Lin C-Z, Guan H-S, Li H-H, Yu G-L, Gu C-X, Li G-Q. The influence of molecular mass of sulfated propylene glycol ester of low-molecular-weight alginate on anticoagulant activities. *Eur Polym J*. 2007;43:3009–3015.

180. Wu J, Zhang M, Zhang Y, Zeng Y, Zhang L, Zhao X. Anticoagulant and FGF/FGFR signal activating activities of the heparinoid propylene glycol alginate sodium sulfate and its oligosaccharides. *Carbohydr Polym*. 2016;136:641–648.

181. Maruyama T, Toida T, Imanari T, Yu G, Linhardt RJ. Conformational changes and anticoagulant activity of chondroitin sulfate following its *O*-sulfonation. *Carbohydr Res*. 1998;306:35–43.

182. Amarasekara AS, Opoku G, Qiu X, Doctor V. Effect of oversulfation on the chemical and biological properties of chondroitin-6-sulfate. *Carbohydr Polym*. 2007;68:116–121.

183. Toida T, Maruyama T, Ogita Y, et al. Preparation and anticoagulant activity of fully *O*-sulphonated glycosaminoglycans. *Int J Biol Macromol*. 1999;26:233–241.

184. Li D-W, Lee IS, Sim J-S, Toida T, Linhardt RJ, Kim YS. Long duration of anticoagulant activity and protective effects of acharan sulfate in vivo. *Thromb Res*. 2004;113:67–73.

185. Wu SJ, Chun MW, Shin KH, et al. Chemical sulfonation and anticoagulant activity of acharan sulfate. *Thromb Res*. 1998;92:273–281.

186. Miura Y, Fukuda T, Seto H, Hoshino Y. Development of glycosaminoglycan mimetics using glycopolymers. *Polym J*. 2016;48:229–237.
187. Akashi M, Sakamoto N, Suzuki K, Kishida A. Synthesis and anticoagulant activity of sulfated glucoside-bearing polymer. *Bioconjugate Chem*. 1996;7:393–395.
188. Sakamoto N, Kishida A, Maruyama I, Akashi M. The mechanism of anticoagulant activity of a novel heparinoid sulfated glucoside-bearing polymer. *J Biomater Sci Polym Ed*. 1997;8:545–553.
189. Onishi M, Miyashita Y, Motomura T, Yamashita S, Sakamoto N, Akashi M. Anticoagulant and antiprotease activities of a heparinoid sulfated glucoside-bearing polymer. *J Biomater Sci Polym Ed*. 1998;9:973–984.
190. Huang Y, Shaw MA, Mullins ES, Kirley TL, Ayres N. Synthesis and anticoagulant activity of polyureas containing sulfated carbohydrates. *Biomacromolecules*. 2014;15:4455–4466.
191. Sun X-L, Grande D, Baskaran S, Hanson SR, Chaikof EL. Glycosaminoglycan mimetic biomaterials. 4. Synthesis of sulfated lactose-based glycopolymers that exhibit anticoagulant activity. *Biomacromolecules*. 2002;3:1065–1070.
192. Oh YI, Sheng GJ, Chang S-K, Hsieh-Wilson LC. Tailored glycopolymers as anticoagulant heparin mimetics. *Angew Chem Int Ed*. 2013;52:11796–11799.
193. Fougnot C, Jozefonvicz J, Samama M, Bara L. New heparin-like insoluble materials: part I. *Ann Biomed Eng*. 1979;7:429–439.
194. Fougnot C, Jozefowicz M, Samama M, Bara L. New heparin-like insoluble materials: part II. *Ann Biomed Eng*. 1979;7:441–450.
195. Ran F, Nie S, Li J, Su B, Sun S, Zhao C. Heparin-like macromolecules for the modification of anticoagulant biomaterials. *Macromol Biosci*. 2012;12:116–125.
196. Ran F, Nie S, Yin Z, et al. Synthesized negatively charged macromolecules (NCMs) for the surface modification of anticoagulant membrane biomaterials. *Int J Biol Macromol*. 2013;55:269–275.
197. Ma L, Qin H, Cheng C, et al. Mussel-inspired self-coating at macro-interface with improved biocompatibility and bioactivity via dopamine grafted heparin-like polymers and heparin. *J Mater Chem B*. 2014;2:363–375.
198. Nie C, Ma L, Xia Y, et al. Novel heparin-mimicking polymer brush grafted carbon nanotube/PES composite membranes for safe and efficient blood purification. *J Membr Sci*. 2015;475:455–468.
199. Li R, Wu G, Cai X, Ye Y. In vitro anticoagulant activity of polyanionic graft chains modified poly(vinyl alcohol) particles. *Radiat Phys Chem*. 2017;134:27–32.
200. Silver JH, Hart AP, Williams EC, et al. Anticoagulant effects of sulphonated polyurethanes. *Biomaterials*. 1992;13:339–344.
201. Ito Y, Iguchi Y, Imanishi Y. Synthesis and non-thrombogenicity of heparinoid polyurethaneureas. *Biomaterials*. 1992;13:131–135.
202. Tang M, Xue J, Yan K, Xiang T, Sun S, Zhao C. Heparin-like surface modification of polyethersulfone membrane and its biocompatibility. *J Colloid Interface Sci*. 2012;386:428–440.
203. Machovich R, Nagy M, Gyorgyi-Edelenyi J, Csomor K, Horvath I. Anticoagulant effect of sulphated poly/vinyl alcohol-acrylic acid/copolymers. *Thromb Haemost*. 1986;56:397–400.
204. Csomor K, Kárpáti E, Nagy M, Györgyi-Edelényi J, Machovich R. Blood coagulation is inhibited by sulphated copolymers of vinyl alcohol and acrylic acid under in vitro as well as in vivo conditions. *Thromb Res*. 1994;74:389–398.
205. Vörös G, Kolev K, Csomor K, Machovich R. Inhibition of plasmin activity by sulfated polyvinylalcohol–acrylate copolymers. *Thromb Res*. 2000;100:353–361.
206. Tamada Y, Murata M, Makino K, Yoshida Y, Yoshida T, Hayashi T. Anticoagulant effects of sulphonated polpisoprenes. *Biomaterials*. 1998;19:745–750.
207. Tamada Y, Murata M, Hayashi T, Goto K. Anticoagulant mechanism of sulfonated polyisoprenes. *Biomaterials*. 2002;23:1375–1382.
208. Han DK, Lee NY, Park KD, Kim YH, Ik Cho H, Min BG. Heparin-like anticoagulant activity of sulphonated poly(ethylene oxide) and sulphonated poly(ethylene oxide)-grafted polyurethane. *Biomaterials*. 1995;16:467–471.
209. Joung YK, Sengoku Y, Ooya T, Park KD, Yui N. Anticoagulant supramolecular-structured polymers: synthesis and anticoagulant activity of taurine-conjugated carboxyethylester-polyrotaxanes. *Sci Technol Adv Mater*. 2005;6:484–490.
210. Park HD, Lee WK, Ooya T, Park KD, Kim YH, Yui N. Anticoagulant activity of sulfonated polyrotaxanes as blood-compatible materials. *J Biomed Mater Res*. 2002;60:186–190.
211. Han Q, Chen X, Niu Y, et al. Preparation of water-soluble hyperbranched polyester nanoparticles with sulfonic acid functional groups and their micelles behavior, anticoagulant effect and cytotoxicity. *Langmuir*. 2013;29:8402–8409.

212. Türk H, Haag R, Alban S. Dendritic polyglycerol sulfates as new heparin analogues and potent inhibitors of the complement system. *Bioconjugate Chem*. 2003;15:162–167.
213. Tauhardt L, Pretzel D, Kempe K, Gottschaldt M, Pohlers D, Schubert US. Zwitterionic poly(2-oxazoline)s as promising candidates for blood contacting applications. *Polym Chem*. 2014;5:5751–5764.
214. Shih Y-J, Chang Y. Tunable blood compatibility of polysulfobetaine from controllable molecular-weight dependence of zwitterionic nonfouling nature in aqueous solution. *Langmuir*. 2010;26:17286–17294.
215. Correia-da-Silva M, Sousa E, Pinto MMM. Emerging sulfated flavonoids and other polyphenols as drugs: nature as an inspiration. *Med Res Rev*. 2014;34:223–279.
216. Al-Horani RA, Liang A, Desai UR. Designing nonsaccharide, allosteric activators of antithrombin for accelerated inhibition of factor Xa. *J Med Chem*. 2011;54:6125–6138.
217. Raghuraman A, Liang A, Krishnasamy C, Lauck T, Gunnarsson GT, Desai UR. On designing non-saccharide, allosteric activators of antithrombin. *Eur J Med Chem*. 2009;44:2626–2631.
218. Guglielmone HA, Agnese AM, Núñez Montoya SC, Cabrera JL. Anticoagulant effect and action mechanism of sulphated flavonoids from *Flaveria bidentis*. *Thromb Res*. 2002;105:183–188.
219. Gunnarsson GT, Desai UR. Designing small, nonsugar activators of antithrombin using hydropathic interaction analyses. *J Med Chem*. 2002;45:1233–1243.
220. Gunnarsson GT, Desai UR. Interaction of designed sulfated flavanoids with antithrombin: lessons on the design of organic activators. *J Med Chem*. 2002;45:4460–4470.
221. Correia-da-Silva M, Sousa E, Duarte B, et al. Flavonoids with an oligopolysulfated moiety: a new class of anticoagulant agents. *J Med Chem*. 2011;54:95–106.
222. Correia-da-Silva M, Sousa E, Duarte B, Marques F, Cunha-Ribeiro LM, Pinto MMM. Dual anticoagulant/antiplatelet persulfated small molecules. *Eur J Med Chem*. 2011;46:2347–2358.
223. Monien BH, Desai UR. Antithrombin activation by nonsulfated, non-polysaccharide organic polymer. *J Med Chem*. 2005;48:1269–1273.
224. Monien BH, Cheang KI, Desai UR. Mechanism of poly(acrylic acid) acceleration of antithrombin inhibition of thrombin: implications for the design of novel heparin mimics. *J Med Chem*. 2005;48:5360–5368.
225. Bock LC, Griffin LC, Latham JA, Vermaas EH, Toole JJ. Selection of single-stranded DNA molecules that bind and inhibit human thrombin. *Nature*. 1992;355:564–566.
226. Wu Q, Tsiang M, Sadler JE. Localization of the single-stranded DNA binding site in the thrombin anion-binding exosite. *J Biol Chem*. 1992;267:24408–24412.
227. Rusconi CP, Yeh A, Kim Lyerly H, Lawson JH, Sullenger BA. Blocking the initiation of coagulation by RNA aptamers to factor VIIa. *Thromb Haemost*. 2000;84:841–848.
228. Rusconi CP, Scardino E, Layzer J, et al. RNA aptamers as reversible antagonists of coagulation factor IXa. *Nature*. 2002;419:90–94.

AUTHOR'S BIOGRAPHIES

Abdullah Al Nahain is currently completing his PhD studies at the University of Queensland under the guidance of A/Prof. Vito Ferro and Dr. John Tsanaktsidis (CSIRO). His present research focuses on the development of biologically active polymers by RAFT polymerization. He completed his BPharm in 2007 from the University of Development Alternative, Bangladesh, and obtained an MSc in chemical and biological engineering from Chungju National University, South Korea, in 2012 where he conducted research into nanocarrier drug delivery systems for anticancer drugs.

Vera Ignjatovic received her PhD in 2000 from Monash University. She began her research career by establishing de novo the Haematology Research Laboratory at the Murdoch Childrens Research Institute (MCRI), a part of the Melbourne Children's Campus, in mid-2001. Since then she has developed a highly productive, internationally competitive research laboratory in the field of pediatric hemostasis. Her team is acknowledged as being at the forefront of this research field internationally, and is the only team focusing on pediatric hemostasis nationally. A/Prof Ignjatovic is in the top ranking research scientists in pediatric hemostasis research worldwide. She has published 100 papers, cited more than 1800 times, and is a principle investigator or co-investigator on projects receiving peer reviewed funding

of more than $5.2 M. A/Prof Ignjatovic has presented widely in forums ranging from Thrombosis and Haemostasis to Proteomics conferences, both nationally and internationally. Her research career has been supported by the University of Melbourne which in 2012 awarded her the title of Associate Professor and Principal Fellow, and by MCRI that promoted her to the Co-Group leader position in 2013.

Paul Monagle is a professor of Haematology at the Royal Children's Hospital Melbourne and the University of Melbourne, a recognized world leader in the field of pediatric hemostasis and antithrombotic therapy. This is evidenced by: being a past chair of the paediatric/perinatal Scientific Subcommittee of International Society of Haemostasis and Thrombosis (ISTH); leader of the pediatric chapter of the American College of Chest Physicians (ACCP) antithrombotic guidelines for more than a decade; member of the NIH working party into thrombotic disorders in pediatric cardiology patients; member of the American Society of Haematology venous thrombosis guideline steering committee, and chair of the pediatric panel; on International steering committees of multiple industry sponsored trials of anticoagulation in children; coauthor of multiple textbooks; 27 chapters in major international textbooks and 9 ISTH position statements. He has more than 200 publications, with more than 3000 citations. This includes more than 60 publications in the last 3 years and major clinical studies published in JAMA and JACC. He has given more than 100 invited presentations, locally, nationally, and internationally. He has written invited reviews/editorials for *Nature Reviews Cardiology*, *Hematology Am. Soc. Hematol. Educ. Program*, *Blood*, *Blood Reviews*, and *Thrombosis Research*.

John Tsanaktsidis is a research director at CSIRO Manufacturing. He is an experienced synthetic organic chemist focused on the development and translation of chemistry solutions to the chemical industry, by applying his extensive background and experience in scientific research, management, business development, technology commercialization, and business leadership. Dr Tsanaktsidis has authored or coauthored more than 60 peer reviewed papers, patents, and book chapters, and has an h-index of 17. He is a Distinguished Alumnus of The Flinders University of South Australia (2006), a Fellow of the RACI, the peak body for chemists in Australia, and a former Chair of the Organic Chemistry Division of the RACI.

Vito Ferro obtained his PhD from the University of Western Australia in 1992. Following postdoctoral studies at the Carlsberg Laboratory, Denmark, and the University of British Columbia, Canada, he joined Progen Pharmaceuticals Ltd where he spent 12 years in various positions, including Director of Drug Discovery. Following a brief period at Queensland University of Technology as Deputy Program Leader in the Cooperative Research Centre for Polymers, he moved to the University of Queensland in 2010 where he is Associate Professor of Biotechnology in the School of Chemistry and Molecular Biosciences and is also a member of the Australian Infectious Diseases Research Centre. His research interests are in carbohydrate and medicinal chemistry with a particular focus on glycosaminoglycans (especially heparan sulfate and heparin) and their mimetics as potential drugs for a range of diseases.

How to cite this article: Nahain AA, Ignjatovic V, Monagle P, Tsanaktsidis J, Ferro V . Heparin mimetics with anticoagulant activity. *Med Res Rev*. 2018;1–32. https://doi.org/10.1002/med.21489