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HEPARIN MIMETICS WITH ANTICOAGULANT ACTIVITY

Running title: Heparin mimetics as anticoagulants

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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 manufacture, may become a very significant issue in the future. These global limitations have prompted much research, especially following the recent well-publicised 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: Heparin; heparin mimetics; anticoagulants; sulfated oligosaccharides; glycopolymers

1. INTRODUCTION

At present, globally, a large number of people are affected with different types of cardiovascular diseases (CVDs), i.e., 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, cardiovascular disease 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, i.e., fondaparinux (1, Fig. 3), see Table 1. Among these, UFH which is a sulfated polysaccharide (average 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 (or HIT, 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 2008 when over 100 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 (OSCS).¹⁰ 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 one hour), 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 e.g., 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 (TF), which activates both Factor IX (FIX) and Factor X (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), activates 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 FXIII 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 (M_n) of 12 to 16 kDa and a weight average MW (M_w) of 17 to 20 kDa, and thus a polydispersity (M_w/M_n) of about 1.3-1.4.¹⁹ The manufacturing processes for heparin have 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: Structures of the major (a) and minor (b) disaccharide sequences in heparin. 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 non-reducing 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-catalysed displacement of sulfate from Ido2S and/or enrichment of 6-O-sulfated sequences via chromatographic purification can occur at variable levels.²²

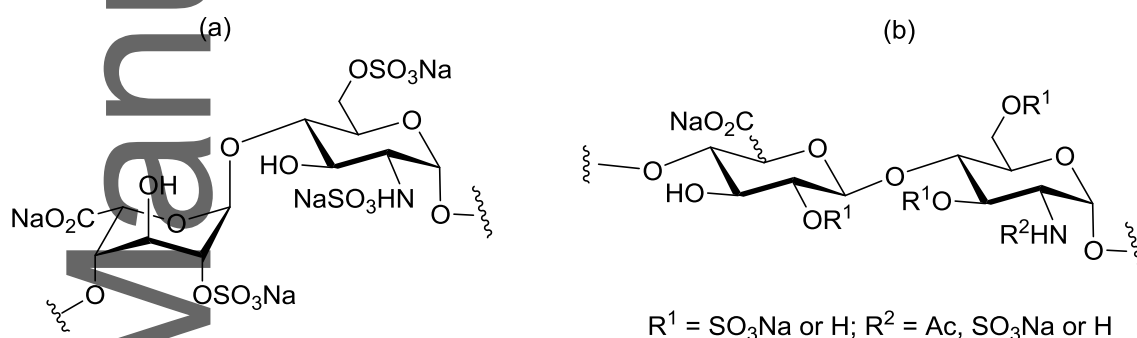


Figure 1: Structures of the major (a) and minor (b) disaccharide sequences in heparin.

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 non-reducing 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.

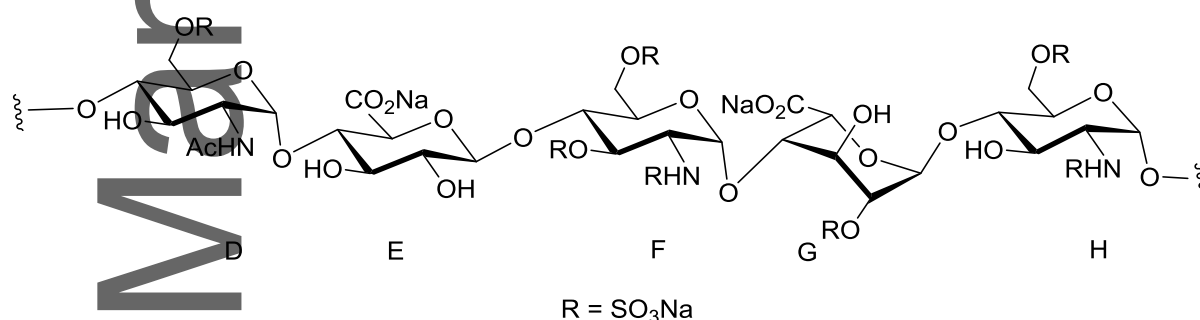


Figure 2: Structure of the unique pentasaccharide (DEFGH) sequence of heparin, also known as the antithrombin binding domain (ABD).

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 depolymerisation 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 centre of the H unit³⁰, the resultant pentasaccharide exhibited similar anticoagulant activity with additional advantages such as improved yield and a longer half-life (17 hours). This preparation was then registered as drug in the US and Europe under the trade name Arixtra®.³² Subsequently, the same investigative group developed a fully *O*-sulfated and *O*-methylated (non-glycosaminoglycan) pentasaccharide known as idraparinux (**2**), 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 h).

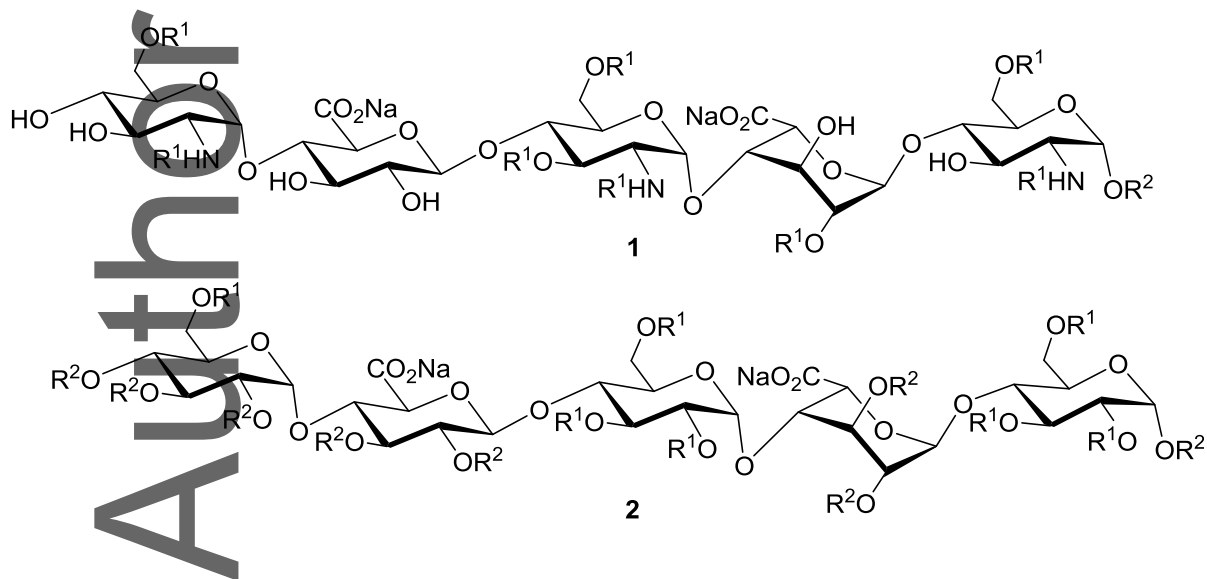


Figure 3: Structures of fondaparinux (1) and idraparinux (2). $R^1 = \text{SO}_3\text{Na}$, $R^2 = \text{Me}$.

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 thousand fold. 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. Firstly, 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 Secondly, 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} Absence of *O*-sulfates 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}

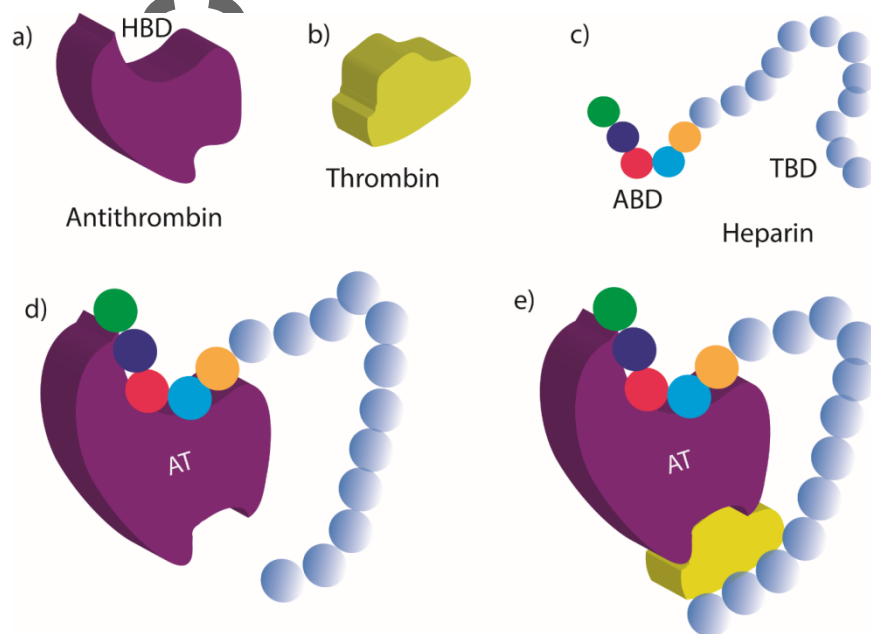


Figure 4: a) antithrombin; b) thrombin; c) long chain heparin; d) binding of heparin pentasaccharide with the antithrombin; and e) formation of ternary complex of heparin with thrombin and antithrombin. HBD: heparin binding domain; ABD: antithrombin binding domain; TBD: thrombin binding domain.

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 e.g., chitosan and hyaluronic acid; the synthesis of non-carbohydrate 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 non-sulfated anionic compounds have also been shown to have anticoagulant properties.

4.1. Synthetic heparin oligosaccharide derivatives

A series of studies to develop ultra-low molecular weight heparin/heparin oligosaccharides by chemoenzymatic 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 sized 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 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 (dnLMWH),⁶¹ using a tetrasaccharide primer which could be obtained in only two days. The *in vitro* and the *ex vivo* anticoagulation assays indicated higher potency of the dnLMWH compared with the commercially available LMWH enoxaparin.

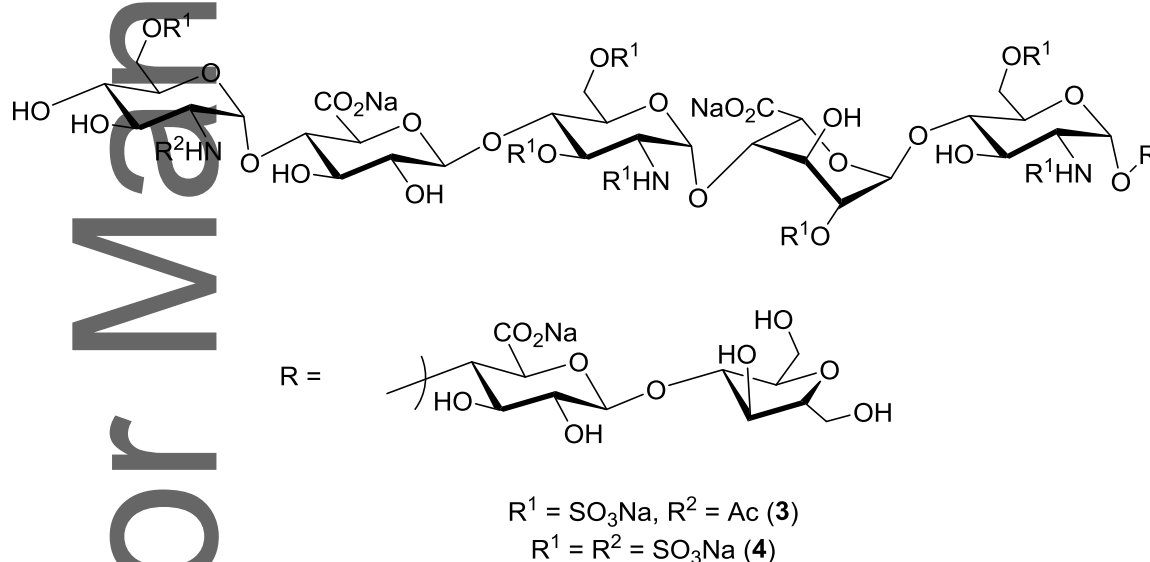


Figure 5: Structures of the synthetic sulfated oligosaccharides consisting of the ABD of porcine (3) and bovine heparin (4).

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 (Glc-pNP) 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 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 the Glc-pNP as the starting material.⁶² Three hexasaccharides were subjected to AT binding affinity testing by affinity co-electrophoresis.⁶² This study revealed that without the presence of 2-*O*-sulfated iduronic acid, the oligosaccharides are not able to bind to AT.

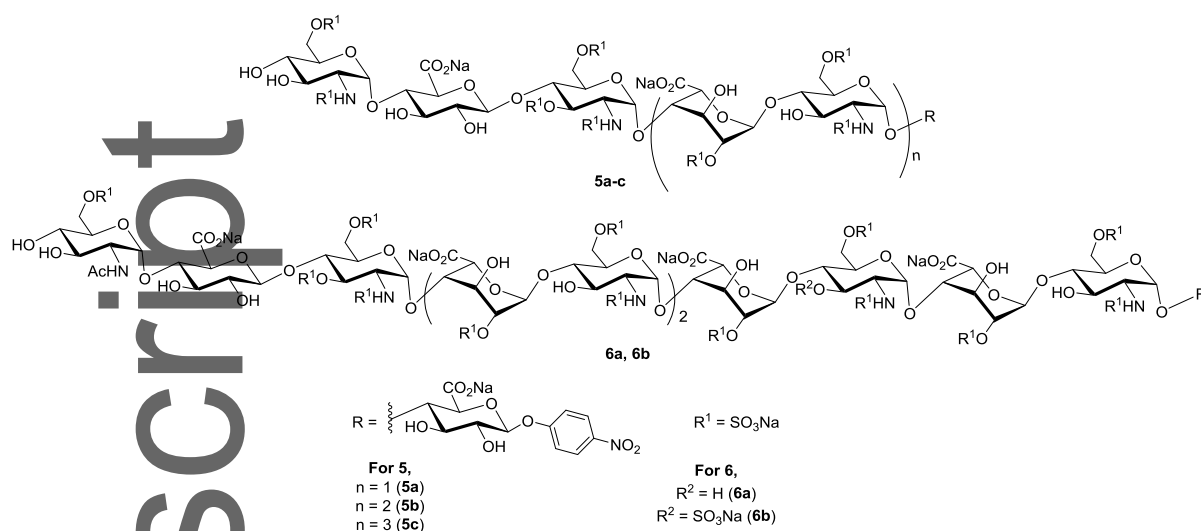


Figure 6: Structures of synthetic LMWHs.

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 non-reducing 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.

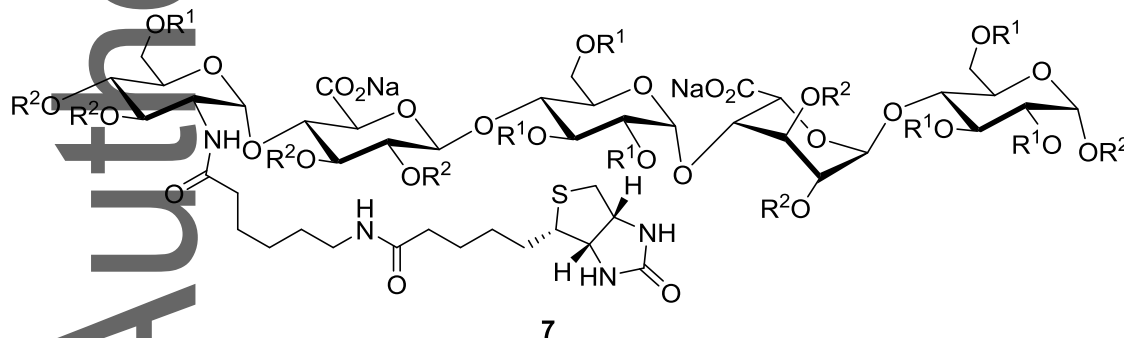


Figure 7: Chemical structure of idrabiotaparinux (**7**).⁶³ $R^1 = \text{SO}_3\text{Na}$, $R^2 = \text{Me}$.

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 glycoconjugates 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 non-glycosaminoglycan ABD pentasaccharide (i.e., idraparinux) was connected via a molecular spacer to a persulfated maltotrioxide 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.

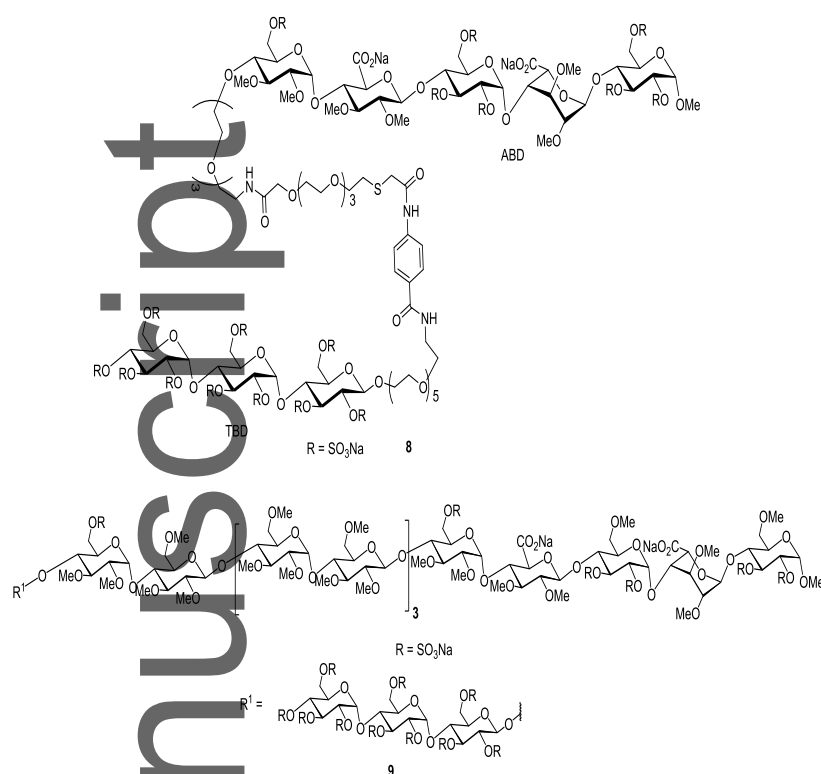
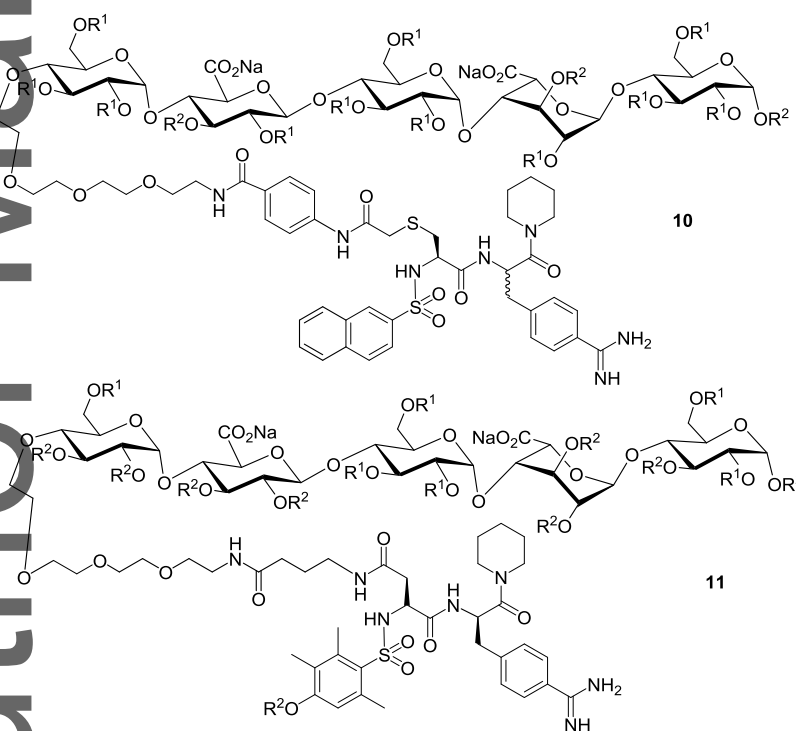


Figure 8: Synthetic tailor-made glycoconjugates consisting of ABD and TBD through flexible (**8**) and rigid (**9**) molecular spacers.^{65,66}

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 idraparinix 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 10

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 antiplatelet α IIb β 3 inhibitor tirofiban,⁷⁰ producing a conjugate with both anti-FXa and anti-platelet activity. It is noteworthy that tirofiban on its own has a very short half-life and can't be used for outpatients whereas **13** has a much longer half-life due to the presence of the idraparinux moiety.



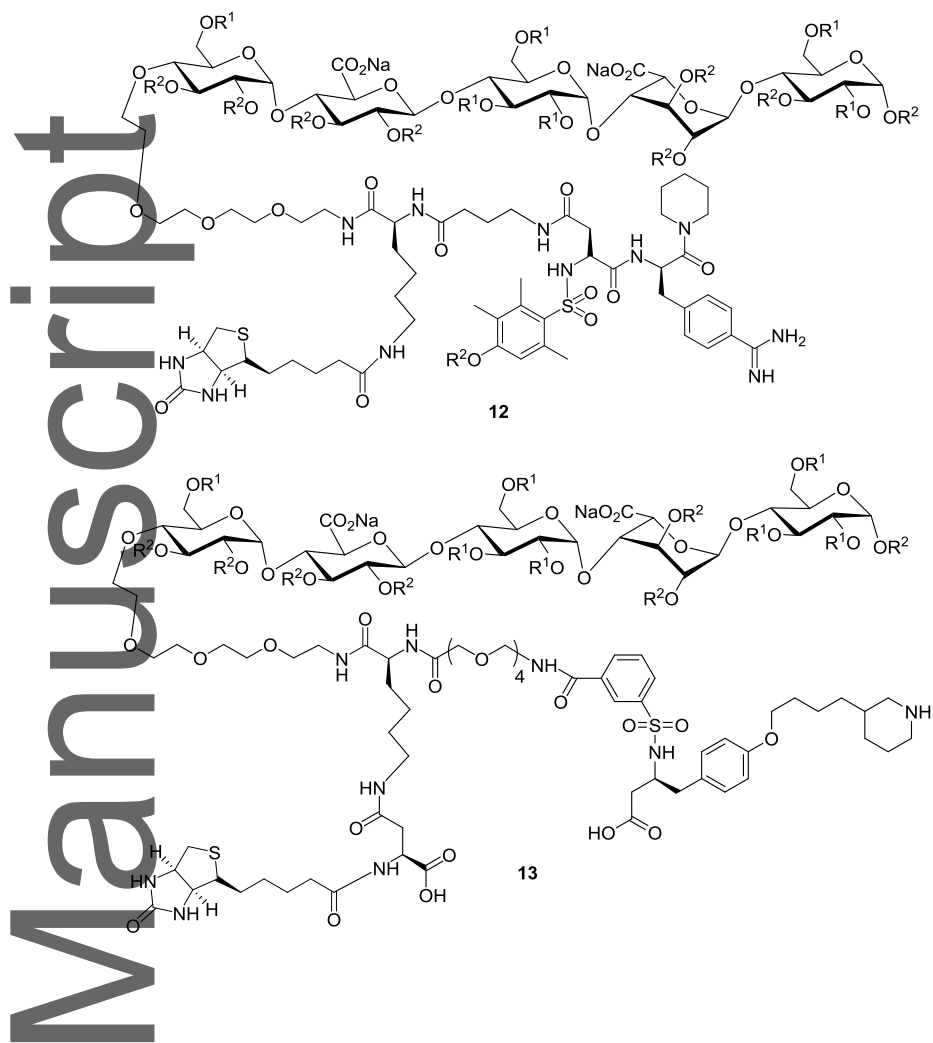


Figure 9: Structures of pentasaccharide conjugates Org39913 (**10**),⁶⁷ Org42675 (**11**),⁶⁸ EP217609 (**12**)⁶⁹ and EP224283 (**13**).⁷⁰

Recently, Oscarson and Desai generated an *in silico* library of 46 656 heparan sulfate hexasaccharides and found a rare sequence consisting of consecutive glucuronic acid 2-*O* sulfated residues (GlcA2S) 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

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 (SOS, **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

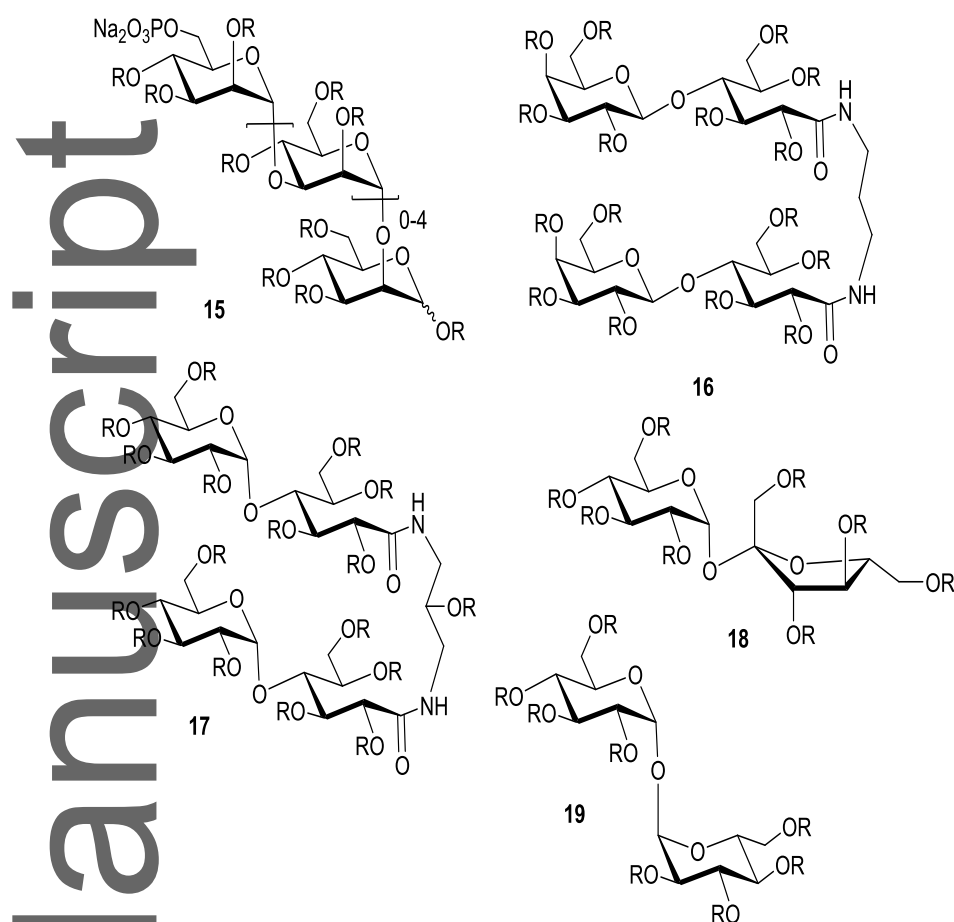


Figure 11: Structures of the polysulfated PI-88(**15**), bis-lactobionic acid amide LW10082 (**16**), maltodapoh (**17**), sucrose octasulfate (**18**) and trehalose octasulfate (**19**). R = SO₃Na.

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 chondroitin sulfate (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 β -D-glucuronic acid of CS, have been found to act as potent anticoagulants.⁹⁷⁻¹⁰⁶ 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.

Dermatan sulfate (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.^{116, 87,108,117} 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 molecular weight DS (4.2 kDa).¹²¹ Fernandez et al reported the enhancement of anticoagulant activity of 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 as the sulfating agent.

As a source of polysaccharide for sulfonation, chitosan (deacetyl chitin) has been considered due to the presence of $\beta(1\rightarrow4)$ 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 depolymerisation 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.¹³⁶ For sulfated chitosan, N-sulfation at the C-2 position is required to inhibit blood coagulation.^{143,144,145} 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 varied across different

studies, e.g., 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}

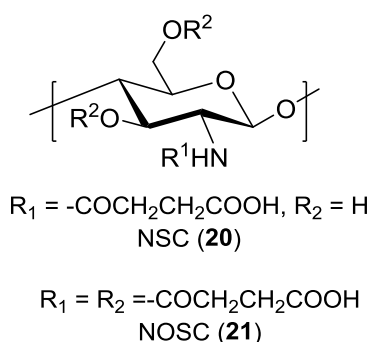


Figure 12: Structures of the N-succinyl chitosan (**20**) and N, O-succinyl chitosan (**21**).

Hyaluronic acid (HA), consisting of glucuronic acid β -(1 $\rightarrow$ 3) and N-acetylglucosamine β -(1 $\rightarrow$ 4) linkages, is a non-sulfated 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 is 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 $\rightarrow$ 6)-glycosidic linkages with α -(1 $\rightarrow$ 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 (CMDDBS),^{113,158-165} and these have recently been reviewed by Maynard and co-workers.¹⁶⁶ The CMDDBS

derivatives were found to inhibit thrombin activity via both AT and HCII.^{159,167-169} The related functionalized dextramethylcarboxylate benzylamide sulfate (DMCBSu), 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 $\beta(1\rightarrow3)$ -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 than that of UFH.¹⁷⁵

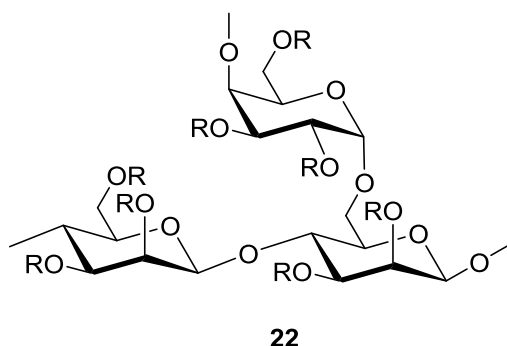


Figure 13: Structures of the sulfated galactomannan (**22**). R = SO₃Na or H.

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 μ g/ml the APTT was

226 s whereas for UFH the APTT was 125s at 10 μ g/ml. Similarly, Zhao and co-workers prepared SA of varying degrees of sulfation using sulfuric acid/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 molecular weight alginate known as PSS (**24**, Figure 14) has been used as a drug in China for over 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}

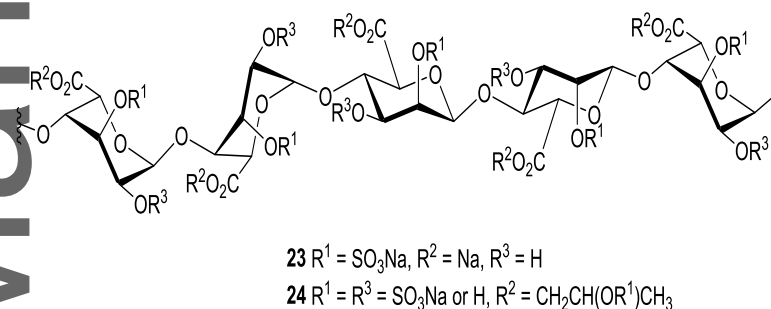


Figure 14: Structures of the synthetic sulfated alginate (**23**) and propylene glycol conjugated sulfated alginate (**24**).

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 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 $\rightarrow$ 4)- α -L-IdoA2S, shows no anticoagulant activity despite the structural similarity to heparin. However, after chemical sulfonation the polysulfated acharan sulfate, the molecule exhibited AT independent anti-IIa activity.^{184,185}

4.4. Synthetic sulfated glycopolymers

The synthesis of sulfated glycopolymers, i.e., 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 (ROP), free radical polymerization or ring-opening metathesis polymerization (ROMP), utilising as the monomers either sulfated saccharide units, or non-sulfated 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 al.¹⁸⁷ Poly(glucosyloxyethyl methacrylate) [or poly(GEMA)] 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(GEMA)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 fibrinogen¹⁸⁸ and inhibition of thrombin function via HCII.^{187,189}

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 SO₃-pyridine complex. All the sulfated glycopolymers prolonged the APTT by >300s at 500µg/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 molecular weight 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 developed lactose hepta-sulfated based homo- and co-polymers increased the coagulation time. However, the analogous trisulfated GlcNAc based homo- and co-polymers failed to show any anticoagulant activity, indicating that at least a sulfated disaccharide is required for anticoagulant activity.

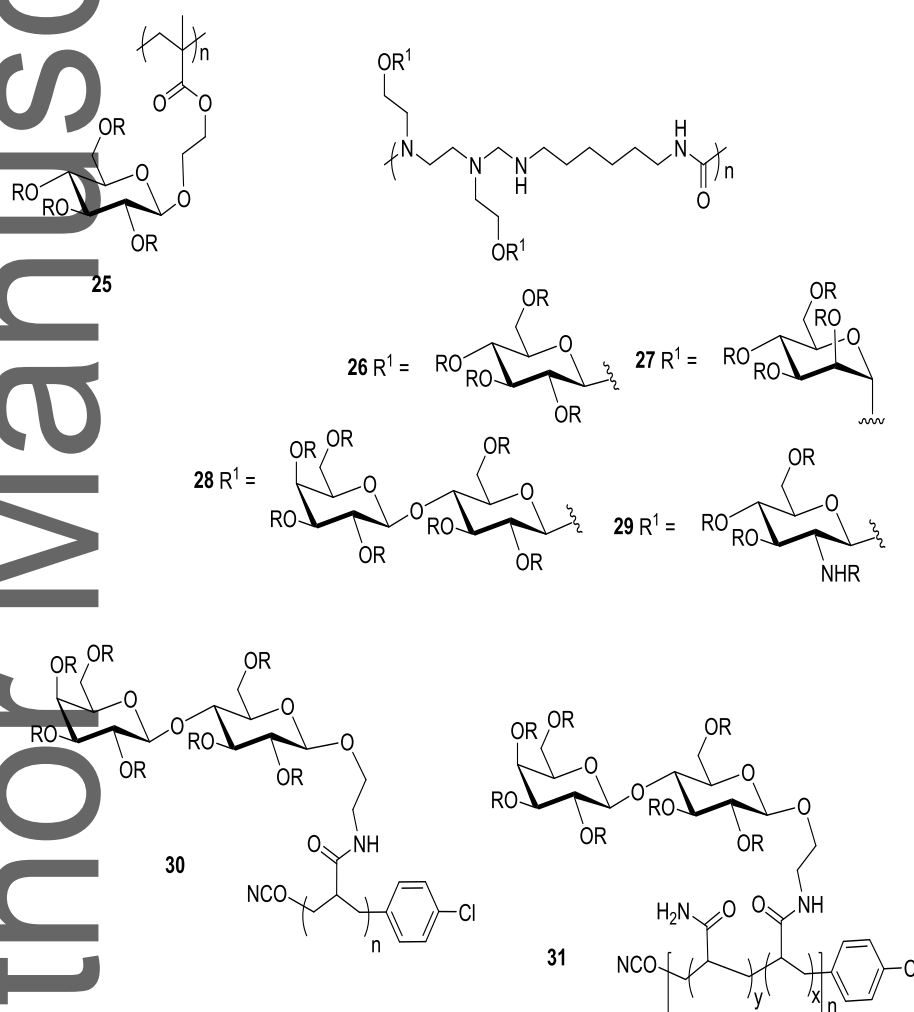


Figure 15: Structures of synthetic polysulfated glycopolymers with anticoagulant activity.

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.

4.5. Synthetic sulfated polymers

Considering the polyanionic behaviour 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 sulfonated styrene (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 s to more than 400 s 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 multiwall of carbon nanotubes were used as the macro initiator.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 amongst 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 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.

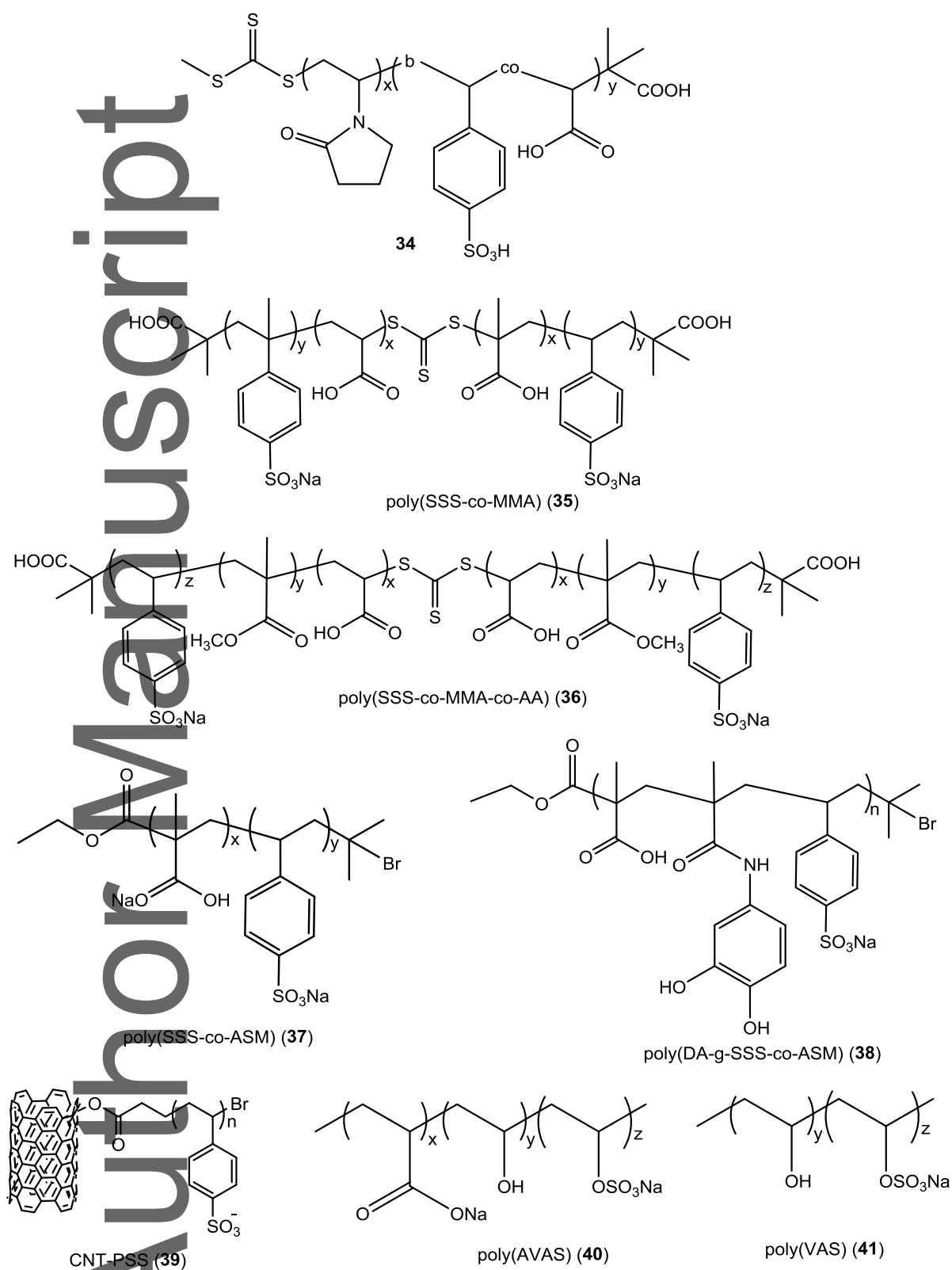


Figure 17: Chemical structures of synthetic sulfated (non-carbohydrate) polymers.

Sulfonation of polyethersulfone (PES) 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) [poly(AVAS)] (**40**) and sulfated poly(vinyl alcohol)[poly(VAS)] (**41**) having different molecular weights (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 molecular weight. 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) (PEO-SO₃) 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 (α -CD) and 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 -CDs 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 molecular weight due to the globular 3-D 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

4.6. 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, e.g., (+)-catechin sulfate (**44**) was 2 fold more potent than (-)-catechin sulfate (**45**).^{219,220}

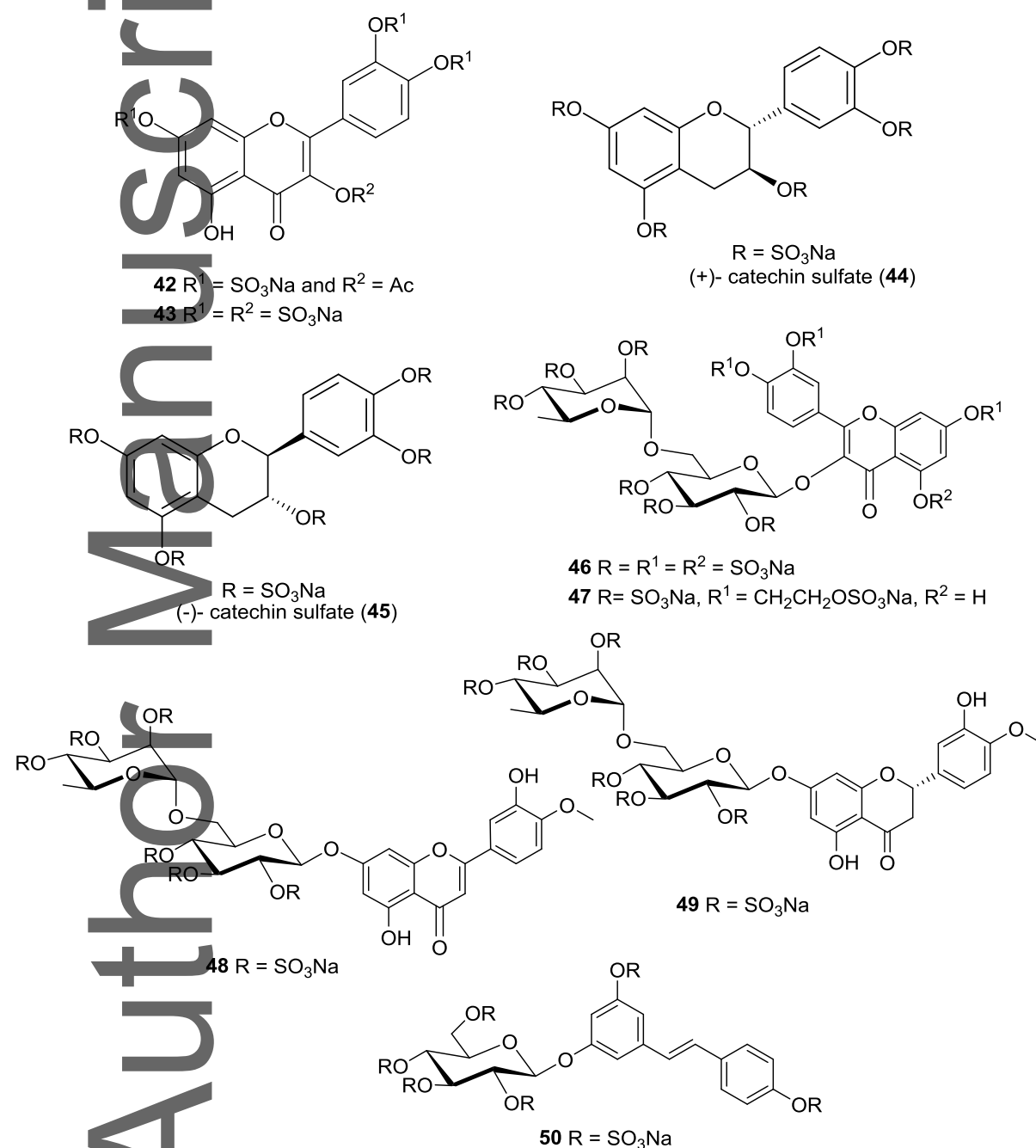


Figure 18: Structures of sulfated flavonoids and derivatives with anticoagulant activity.

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

4.7. Nonsulfated anionic compounds as anticoagulants

In addition to the above mentioned sulfated compounds, the anticoagulant activity of HS mimetic compounds without any sulfate groups has also been reported. For example, the Desai group found that poly(acrylic acid) 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.

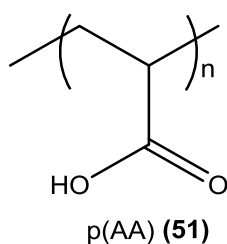


Figure 19: Chemical structure of poly(acrylic acid) p(AA) (**51**).

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-catalysed 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 towards 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 occurring 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.

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

	MW (kDa)	Anticoagulant activity	Half life (hours)	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 Hypoadoster

									onism
									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

UFH, Unfractionated heparin; LMWH, low molecular weight heparin; ULMWH, ultra-low molecular weight heparin (fondaparinux); APTT, activated partial thromboplastin time

Table 2: Summary of the properties of heparin mimetics

Structural Class	Structure	Method of preparation	Mw (kD)	Target factor	Mechanism	Half-life	<i>In vivo</i> Assay	Ref.
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			a)					
Synthetic heparin oligosacch arides	2	Synthetic	1.7	FXa	AT depende nt	60 days	✓ *	33
	3,4	Chemoenzy matic	1.7- 1.8	FXa	AT depende nt	ND	✓	59
	5,6	Chemoenzy matic	1.8- 3.6	FXa	AT depende nt	ND	✓	14
	7	Synthetic	2.0	FXa	AT depende nt	60 days	✓ *	63,64
	8,9	Synthetic		FIIa and FXa	AT depende nt	ND	x	65,66
	10	Synthetic	ND	FIIa and FXa	AT depende nt	ND	✓	67
	11,12	Synthetic	2.4- 2.6	FIIa and FXa	AT depende nt	1.5 hours	✓	68
	13	Synthetic		FXa and platele		ND	✓	70

				t				
	14	Synthetic		FIIa	HCII depende nt	ND	x	71
Polysulfate d non- heparin oligosacch arides	15	Synthetic	2.4	FIIa	HCII depende nt	1-3h (in rats and monk ey)	✓	74,75
	16	Synthetic	2.3	FIIa, FVa and FXa	HCII depende nt	ND	x	77,78
	17	Synthetic	2.0 3	FIIa and FXa	Unclear	ND	✓	79
	18	Synthetic	1.2	FIIa	Unkno wn	ND	x	80
	19	Synthetic	ND	ND	Commo n and intrinsic pathwa ys	ND	✓	81
Naturally occurring sulfated	CS	Natural	4.7 5	FIIa	AT depende	ND	x	82-84

polysaccharides					nt			
	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	ND	x	90-93
	Sulfated galactan	Extraction	~100	FIIa and FXa	AT and HCII	ND	x	88,89
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	ND	x	151,152
	CMD BS	Semisynthetic	47-50	FIIa	AT and HCII dependent	ND	x	113,158-169

					nt			
	22	Sulfation	0.9-2.4	FIIa and FXa	Differe nt than that of UFH	ND	x	174,175
	Sulfat ed alginat e	Synthetic, semi-synthetic	12-52	FIIa and FXa	AT and HCII depende nt		x	176-180
Synthetic sulfated glycopoly mers	25	Free radical polymerizat ion	ND	FIIa	HCII depende nt	ND	x	187
	26-29	Step growth polymerizat ion	27-44.0	FIIa	AT depende nt and indepen dent pathwa ys	ND	x	190
	30,31	Free radical polymerizat ion	9.0-114	Not specifi ed (intrin sic, extrins ic and comm	ND	ND	x	191

				on pathw ays)				
	32	ROMP	~43 .0	FIIa and FXa	AT depende nt	ND	x	192
Synthetic sulfated polymers	34-39	Radical polymerizat ion	>10	Intrins ic, extrins ic or comm on pathw ays)	ND	ND	x	195-199
	40, 41	Radical polymerizat ion	> 5	FIIa	AT depende nt	ND	x	203
Sulfated aromatic compound s	42-50	Natural (extraction) , synthetic	0.2- 0.7	FIIa and FXa	AT and HCII depende nt	ND	x	215-222

*These compounds have been evaluated in human clinical trials.

Note: FIIa = Thrombin; ND = not determined

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