HHS Public Access

Author manuscript

Org Biomol Chem. Author manuscript; available in PMC 2018 July 21.

Published in final edited form as:

Org Biomol Chem. 2017 July 21; 15(27): 5656–5668. doi:10.1039/c7ob01058c.

Targeting Heparin– and Heparan Sulfate–Protein Interactions

Ryan J. Weiss[a], **Jeffrey D. Esko**[a], and **Yitzhak Tor**[b]

[a]Department of Cellular and Molecular Medicine, Glycobiology Research and Training Center, University of California, San Diego, La Jolla, CA, 92093-0358, USA

[b]Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA, 92093-0358, USA

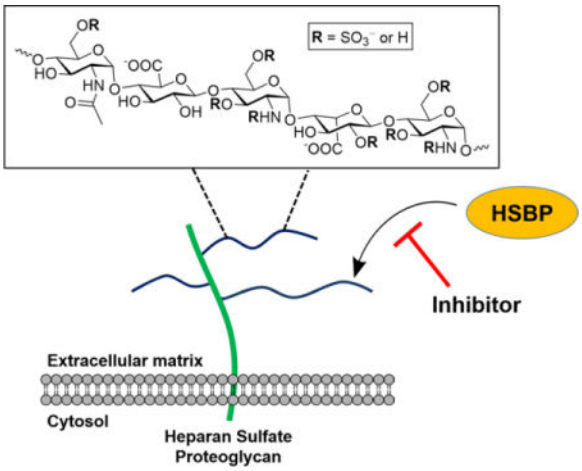
Abstract

Heparin and heparan sulfate glycosaminoglycans are long, linear polysaccharides that are made up of alternating dissacharide sequences of highly sulfated uronic acid and amino sugars. Unlike heparin, which is only found in mast cells, heparan sulfate is ubiquitously expressed on the cell surface and in the extracellular matrix of all animal cells. These negatively-charged carbohydrate chains play essential roles in important cellular functions such as cell growth, adhesion, angiogenesis, and blood coagulation. However, these biomolecules are also involved in pathophysiological conditions such as pathogen infection and human disease. This review discusses past and current methods for targeting these complex biomolecules as a novel therapeutic strategy to treating disorders such as cancer, neurodegenerative diseases, and infection.

Graphical Abstract

Heparan sulfate is ubiquitously expressed on the cell surface and in the extracellular matrix of all animal cells. These negatively-charged carbohydrate chains play essential roles in important cellular functions such as cell growth, adhesion, angiogenesis, and blood coagulation by interacting with various heparan sulfate binding proteins (HSBP). This review discusses methods for targeting these complex biomolecules, as a strategy for treating disorders such as cancer, neurodegenerative diseases, and infection.

Correspondence to: Yitzhak Tor.



Introduction

Heparan sulfate proteoglycans (HSPGs) are glycoconjugates found in the glycocalyx that surround virtually all mammalian cells.[1] Each HSPG consists of a core protein linked to one or more linear heparan sulfate (HS) chains. The chains are composed of alternating D-glucosamine and uronic acids (D-glucuronic and L-iduronic acids) that can be variably *N*- and *O*-sulfated (Figure 1). The presence of sulfate groups at specific positions in HS imparts an overall high negative charge and their arrangement in short segments of the chain create binding sites for protein ligands.[2] HSPGs are assembled in the endoplasmic reticulum and Golgi apparatus by glucosyltransferases, sulfotransferases and an epimerase and can be processed further by plasma membrane bound endosulfatases that remove sulfate groups from specific sites.[3] The composition of HS varies spatially and temporally in different cell types and during development, but the factors involved in regulating its biosynthesis remain poorly defined.[2] Nevertheless, the HSPG's expressed on the cell surface and in the surrounding extracellular matrix tend to be heritable and impart the capacity for selective cell signaling and cell-to-cell crosstalk.[4]

There are at least 300 known HS-binding proteins (HSBPs) with varying affinity, specificity, and function.[5] The family of HS-binding proteins includes growth factors, cytokines, chemokines, enzymes, enzyme inhibitors, and extracellular matrix proteins.[6] Binding to HS can have profound effects on deposition and presentation of HSBPs at their sites of production, protection of HSBPs against proteolysis, functioning of complexes with signaling receptors, protein oligomerization, and allosteric effects on enzyme activity.[4b]

There has been a considerable amount of research investigating the nature of HS–protein interactions.[5, 7] Certain proteins preferentially bind spatial arrangements of the sulfated sugar subunits of HS rather than specific sequences. In other cases, a lock and key fit occurs, such as found for antithrombin-heparin.[4b] Over two decades ago, HS was shown to act as a co-receptor for the binding of growth factors to receptor tyrosine kinases, such as fibroblast growth factor 2 (FGF2) and vascular endothelial growth factor (VEGF) binding to FGF and VEGF receptors, respectively.[8] For example, a complex is formed between HS, FGF2 and the FGF2 receptor (Figure 2), resulting in FGF/FGFR dimerization and activation of the

tyrosine kinase domain and signal transduction.[9] Whereas FGF2 binding to HS depends on *N*-sulfation of glucosamine residues and 2-*O*-sulfation of iduronic acids, additional 6-*O*-sulfated sites on glucosamine HS are essential for its interaction with the FGF receptor.[4b, 9–10] Thus, the analysis of HS–protein interactions are often complex and require different contiguous sequences of sulfated domains.

HSPGs have also been implicated in pathophysiological conditions.[11] For example, changes in HS composition occur in tumors, and tumor growth and angiogenesis depend on HS–growth factor interactions.[11b, 12] Changes in the expression level and composition of HS have been shown to correlate with tumor cell transformation and invasiveness.[12a] The HSPG syndecan-1 is expressed abundantly on the cell surface of epithelial tumors[13] and multiple myeloma cells[14] and is up regulated in pancreatic cancer.[15] Additionally, HS has been implicated in neurodegenerative disorders including prion disease (e.g., mad cow disease),[16] Parkinson's,[17] and Alzheimer's disease.[18] Many pathogens, including viruses and bacteria, are able to display HS-binding proteins that help facilitate their attachment to host cells, mediate cell entry, and increase infectivity.[4b, 30] Some examples are illustrated in Table 1. In most cases, HSPGs on the plasma membrane act as initial, low affinity co-receptors that concentrate the pathogen on the cell surface and promote binding to secondary receptors, which stimulates infection. Due to the apparent role of these complex polysaccharides in infection and disease, HS is a viable drug target.

Heparin, a fractionated form of HS from porcine entrails or horse lungs, is a powerful anticoagulant used in surgery and for treatment of clotting disorders.[31] The purification of heparin involves a series of steps that enrich for highly sulfated oligosaccharide fragments with high anticoagulant activity. Its anticoagulant activity is due to its high affinity to the serine protease inhibitor antithrombin III (AT).[31b–d] Heparin has the highest negative charge density of any known biomolecule (~3.3 negative charges per disaccharide) and is related in structure to HS (2.3 sulfate groups per disaccharide in heparin vs. ~0.8 sulfate groups per disaccharide in typical HS). While HS is ubiquitously expressed in all animal cells, heparin is produced and stored selectively in the secretory granules of connectivetissue mast cells.[32] Low molecular weight (e.g. enoxaparin) and ultralow molecular weight heparins (fondaparinux) are also in clinical use.[33] Progress has also been made in making non-anticoagulant forms of heparin that might be useful as competitive ligands for treating other types of disorders.[34]

While excellent reviews have been published on HS structure, biosynthesis, and function,[1, 4a, 7b, 35] and heparin/HS binding proteins,[5, 7a, 36] the purpose of this contribution is to provide an overview of various approaches and therapeutic agents used to target heparin and heparan sulfate.

Targeting heparan sulfate-protein interactions

Several different strategies have been explored to target HS– protein interactions including enzymatic methods to remove or modify HS, the use of glycosaminoglycan (GAG) mimetics to competitively block HS–protein interactions, and the utilization of large cationic proteins or small molecules to antagonize HS-protein interactions. The following sections will

provide an overview of each strategy and will give details of diverse agents that have been designed over recent years.

Enzymatic targeting of HS

Enzymes such as bacterial heparinases and mammalian endosulfatases have been explored as potential agents for the treatment of disorders involving HS–protein interactions. Heparinases I (Hep I) and III (Hep III) from *Flavobacterium heparinum* specifically cleave the highly sulfated (Hep I) and poorly sulfated (Hep III) regions of the HS polysaccharide backbone (Hep II cleaves both regions),[37] while endosulfatases remove specific sulfate residues located in HS chains (Figure 3).[3, 38] These enzymes serve as useful tools for biologists probing the role of HS in homeostasis and disease. Some groups have looked at their effect on preventing infection and other processes dependent on the interaction with cell-surface HS. Treatment of cells with heparinases inhibits the attachment or entry of several HS-binding pathogens including viruses,[39] bacteria,[40] and parasites.[41] Heparinase treatment has also been explored in tumor growth/metastasis[42] and amyloid-related diseases in mice.[25f, 43] Early clinical trials demonstrated that a single intravenous injection of recombinant heparinase-I (Neutralase) could dose-dependently neutralize anticoagulant heparin in heart surgery patients.[44] However, later trials were terminated due to ineffectiveness and safety concerns. Endosulfatases are important enzymes that edit the sulfated domains of HS by removing the 6-*O*-sulfate groups.[45] Modulation of endosulfatases in cells has been shown to limit *Chlamydia muridarum* bacterial infection.[46] Sulfatase 1 (*Sulf1*) might prove useful as a tool to modulate the capacity of HSPGs to bind to growth factors up regulated in tumors such as FGF2[47] and hepatocyte growth factor.[48] However, targeting these agents to selectively modulate HS on tumor cells without affecting normal tissues remains a challenge.

GAG mimetics

Another approach being explored for inhibiting HS–protein interactions is the use of exogenous heparin/HS or synthetic GAG mimetics as competitive inhibitors. Exogenously added heparin and HS chains inhibit infection of host cells by HS-binding pathogens including HSV,[49] HPV,[39b] hepatitis B,[39c, 50] and various bacteria.[51] Additionally, exogenous HS and heparin have been used to block cancer cell growth and metastasis.[52] However, there are major drawbacks associated with using heparin due to its anticoagulant activity and the risk of heparin-induced thrombocytopenia.[53] The use of soluble HS and heparin also can result in undesirable effects on normal cells and physiology. To circumvent some of these issues, GAG derivatives have been generated (Figure 4). Some of these polyanionic molecules can be synthesized by digestion of heparin polysaccharides. In one example, a nonsulfated K5 polysaccharide from *E. coli* was engineered to inhibit viral infection.[54] Another study examined a synthetic 3-*O*-sulfated octasaccharide to block infection by HSV, which depends on a 3-*O*-sulfated domain in HS.[55] Chemically modified heparin derivatives were shown to inhibit influenza H5N1 virus attachment.[56] Selective desulfation of heparin minimized its anticoagulant effect while maintaining inhibitory activity. Partially depolymerized GAGs have been made for the treatment of malaria.[57]

Low molecular weight heparin and HS mimetics, such as rhamnan sulfate,[58] PG545,[59] and PI-88 (**1**),[60] have displayed potent antiviral and anticancer activity. HS-imitative sulfated polysaccharides isolated from seaweed known as carrageenans (**2**) have also shown the ability to inhibit dengue virus[61] and HSV infection.[62]

Sulfated small molecules have been constructed from commercially available starting materials to avoid the heterogeneity implicit in using naturally occurring GAGs and GAG fragments (Figure 4). GAG mimetics such as 3-amino-1-propanesulfonic acid (**3**)[63] and eprodisate disodium (**4**)[64] have been tested as anti-amyloid agents for the treatment of Alzheimer's disease and amyloidosis. Polysulfonated compounds such as suramin (**5**),[65] poly(sodium-4-styrene sulfonate) (**6**),[66] and sulfonate polymers have been tested as well.[67]

GAG mimetic compounds have shown promise as therapeutics when tested *in vitro*, but translation of these findings *in vivo* have not yet met with success. Several of these compounds, such as PI-88 and PG545, are currently in clinical trials for blocking tumor growth.[68] PG545 exhibited tolerability and a long plasma half-life when administered by intravenous infusion for treatment of advanced solid tumors (ClinicalTrials.gov NCT02042781). However, later clinical trials were terminated due to negative reactions upon injection.[69] Daily injections of PI-88 have shown preliminary efficacy as an adjuvant therapy for hepatocellular carcinoma and melanoma in Phase I and II clinical trials.[70] Further studies are still ongoing to determine its safety and efficacy.[71] Additionally, carrageenan has been formulated as a prophylactic microbicidal gel to block HIV and HPV infection.[72] Unfortunately, it failed in a Phase III trial and has been discontinued.

Cationic proteins and polymers as HS antagonists

Other types of agents used as antagonists of HS–protein interactions include cationic proteins, foldamers, and small molecules. These molecules rely on electrostatic interactions between their positively charged functional groups and the highly anionic sulfate and carboxylate moieties of heparin and HS. Lactoferrin, a heparin- and iron-binding protein found in the secretory granules of neutrophils, has been shown to neutralize heparin and antagonize certain HS–protein interactions.[73] Lactoferrin has proven to be an effective antimicrobial agent[74] and inhibitor of HSV,[75] hepatitis C (HCV),[76] HIV, and human cytomegalovirus (HCMV) infection.[77] However, clinical trials observing the oral treatment of HCV with a combination of lactoferrin and interferon[78] or interferon alpha-2b and ribavirin[79] showed no added benefits compared to treatments without lactoferrin. Other proteins have been tested as potent inhibitors of heparin and its derivatives, including inactive recombinant antithrombin (AT) variants designed to bind heparin.[80] These modified proteins have shown promise *in vitro* and *in vivo* in mice, but they may prove expensive to produce in large quantities for clinical use.

Other cationic macromolecules have proven to be potent antagonists of GAG–protein interactions. Positively-charged arginine-rich proteins isolated from the sperm of salmon and other fish, known as protamine, have long been used clinically to reverse the anticoagulant activity of heparin, despite undesired side effects and allergic reactions observed in some patients.[81] Protamine binds to heparin and blocks its interaction with antithrombin in the coagulation cascade. Protamine was also shown to inhibit hepatitis B viral infection through

blocking viral interaction with cell-surface HSPGs.[50] Protamine inhibits host cell infection of Pseudomonas aeruginosa by preventing bacterial-enhanced HSPG shedding.[82] Low molecular weight protamine (LMWP) has also been explored as a safer alternative to protamine *in vitro*[83] and *in vivo* in dogs.[84] An arginine-rich protamine variant (VSRRRRRRGGRRRR) is currently being investigated as a nontoxic and non-immunogenic protamine substitute for neutralization of heparin and LMWH.[85]

Protamine, despite potential undesirable side effects, is currently the only heparin reversal agent used clinically.[86] Many other polycationic agents have therefore been investigated as alternatives to protamine for heparin neutralization. Hexadimethrine bromide (i.e. polybrene) (**7**) was one of the first protamine alternatives investigated *in vivo*.[87] This fully synthetic cationic polymer showed promise, but was eventually abandoned due to toxicity and lower efficacy compared to protamine. Histones, polycationic proteins that normally function in DNA packaging, and synthetic poly-DL-lysine (**8**), have also been explored as heparin neutralization agents, but proved to be inferior to protamine or exhibited high toxicity (Figure 5).[88] A more recent study utilized poly-L-lysine dendrimers with glycine cores [Gly–$Lys_{63}(NH_2)_{64}$] as neutralization agents. These dendrimers neutralized heparin activity in rats.[89] Another study utilized arginine-substituted poly(allylamine hydrochloride) derivatives (**9**) for potent reversal of heparin *in vitro* and *in vivo*.[90] The polycationic macrocyclic calix[8]arenes (**10**), also have been shown to rapidly neutralize heparin in blood through tight electrostatic interactions.[91] A subsequent study showed these molecules, immobilized onto polymer matrix filters, were able to remove heparin from blood, therefore minimizing side effects associated with administration of heparin reversal agents.[92]

Polyvalent peptide-derivatized dendrimers that display multiple cationic motifs have also been investigated for interaction with heparin and HS. Arginine-rich polycationic virus-like particles neutralize the heparin-antithrombin interaction in human plasma,[93] branched dendrimer-based peptides inhibited HSV-1 and 2 infection,[94] and a large peptide isolated from human hemofiltrate blocked HCMV infection *in vitro* through a HS-dependent pathway.[95] Synthetic ligands (e.g., **11**) that form micelles can bind heparin through electrostatic interactions of their protonated amines (Figure 6).[96] A universal synthetic heparin reversal agent (UHRA) was developed based on a branched dendritic polyglycerol core containing multiple hexamethylated tris(2-aminoethylamine) binding groups (**12**).[97] These UHRA molecules show great promise as safer alternatives to protamine for neutralization of heparin and its analogs; however, their application and safety in humans need to be explored.

Small molecule antagonists of heparin and HS

Small, low molecular weight compounds make up 90% of approved drugs and have the advantage of versatility, simplified manufacturing, formulation, delivery, and stability when compared to biologics.[98] As such, small molecules (MW < 900 Da) have also been exploited as antagonists of HS and heparin. A dispirotripiperazine derivative, (DSTP 27) (**13**), was discovered to bind to HS on the cell surface and inhibit attachment, absorption, and replication of a wide range of viruses (Figure 7).[99] This compound proved to be a potent antiviral in a structure-activity relationship study with a novel class of antiviral compounds

containing the *N,N'*-bis-5-pyrimidyl moiety.[100] A subsequent study demonstrated the ability of DSTP 27 to block HS-dependent viral attachment of an HPV virus with long-term efficacy.[101]

A water soluble small molecule heparin antagonist, ciraparantag (PER977) (**14**), was recently found to potently bind and neutralize the activity of multiple anticoagulants including unfractionated heparin and the LMWH enoxaparin.[102] In an early phase I/II trial, a single dose of ciraparantag restored baseline homeostasis within 10–30 minutes and the reversal effects were sustained for up to 24 hours in patients.[103] This promising compound is currently being studied in phase III clinical trials as an intravenously administered universal antagonist of heparinoids and other novel oral anticoagulants.

A new family of aromatic cationic small molecule antagonists of HS were synthesized and probed as anti-inflammatory agents (e.g. **15**, **16**).[104] They were first investigated as inhibitors of protein binding to HS in a 96-well ELISA-based assay. The most potent compounds were also evaluated for their ability to block acute inflammation in mice with positive results. A catechin extract from green tea, epigallocatechin gallate (EGCG) (**17**), was shown to inhibit virion attachment of many unrelated viruses to HS and sialic acid, another type of charged glycan found on the cell surface.[105] This broad-spectrum antiviral is one of the first small molecules effective against enveloped and nonenveloped viruses such as SV-1, HCV, and influenza A virus (IAV). EGCG blocked the binding of HSV-1 and HCV to HS, while also inhibiting attachment of IAV to sialic acid.

Synthetic small molecule peptide mimics known as foldamers (e.g., **18**), based on amine and guanidinium-substituted arylamides, have also been developed for *in vitro* neutralization of heparin (Figure 8). These molecules self-assemble to form β-like sheets with enhanced association to fondaparinux, a synthetic pentasaccharide analog of heparin.[106] A novel salicylamide derivative, known as delparantag (**19**), was also developed as an alternative to protamine administration.[107] This compound, displaying multiple cationic lysines and aromatic amino acid units, neutralized heparin and enoxaparin, a low molecular weight heparin (LMWH), in rats and later showed promise in a small trial in humans.[108] However, phase II clinical trials revealed unsafe toxicity profiles and hypotension in patients upon intravenous infusion of delparantag (plasma half-life of 3–5 minutes), thus halting further development of this compound.[109]

A high-throughput screen of the National Cancer Institute's (NCI) small molecule diversity set identified *bis*-2-methyl-4-amino-quinolyl-6-carbamide (**20**), a dimeric aminoquinoline called surfen (NSC 12155), as a potent small molecule antagonist of HS (Figure 9).[110] Surfen binds to multiple types of GAGs in solution, but most potently to heparin and HS. Surfen neutralized the ability of heparin to activate antithrombin, blocked the sulfation and degradation of GAG chains by bacterial lyases *in vitro*, and inhibited angiogenesis initiated by the binding of HS-dependent growth factors. In addition, surfen blocked HS-mediated HSV-1 infection and the enhancement of HIV-1 infection by amyloid fibrils found in semen (known as SEVI).[111] Interestingly, surfen not only inhibited HS-mediated viral attachment but also directly bound to SEVI fibrils and disrupted their interaction with the virus.

Other studies have utilized surfen's ability to antagonize HS as a tool to study HS interactions in biological systems. Surfen has been shown to regulate murine T-cell activation and proliferation,[112] stimulate chondrogenesis *in vitro*,[113] inhibit highly anionic polyphosphates involved in the inflammatory process,[114] and reduce lesion formation in demyelination of a multiple sclerosis mouse model.[115] Surfen also acts as an inhibitor of vascular endothelial growth factor receptor (VEGFR) phosphorylation and vascular hyperpermeability in mice.[116]

The biocompatibility of surfen, based on early studies of surfen as an excipient for depot insulin,[117] encourages further research of its neutralizing activity. However, due to this compound's relatively low efficacy (micromolar range), the design and synthesis of more selective and potent analogs was undertaken (Figure 9). A structure-activity relationship was established by measuring the inhibition of HS-dependent FGF binding to CHO cells.[118] In addition, their activity as inhibitors of HS binding to receptor for advanced glycation endproducts (RAGE) *in vitro* and neutralization of the anticoagulant activity of unfractionated heparin and low molecular weight heparins were investigated. The dimeric molecular structure of surfen and its aminoquinoline ring systems were found to be essential for its interaction with HS. Dimeric analogs displayed higher inhibitory potency than surfen and blocked downstream FGF signaling in mouse embryonic fibroblast cells.[118] Importantly, oxalyl surfen (**26**) was shown to neutralize both *in vitro* and in mice the synthetic heparin analog fondaparinux, for which no antidote exists.[118] These findings illustrate the therapeutic potential of small molecules as antagonists of HS and raise the possibility of using surfen-type compounds as biochemical tools and as potential effectors in disorders that involve GAG-protein interactions.

Summary and Prospects

GAGs are vital components of the glycocalyx and are involved in many important biological processes that are essential for maintaining homeostasis. HSPGs, in particular, perform vital roles in cell signaling and cell-to-cell interactions. The heterogeneity and anionic nature of HS polysaccharides allow them to interact with a large number of proteins, including viral and bacterial proteins. Their apparent pathophysiological roles in disease and infection have stimulated research into the development of agents that can antagonize HS–protein and HS–pathogen interactions. As mentioned above, different methods for blocking these interactions have been explored including enzymatic alteration of HS, GAG mimetic compounds as competitive protein binders, and various types of cationic molecules that bind competitively to HS and inhibit its binding to proteins.

There is still much to uncover about HS–protein interactions, as illustrated by the discovery of new HS-binding proteins.[5] As in all areas of medicinal chemistry, there are numerous challenges associated with the development of novel therapeutics that target these types of biomolecules and their biomolecular interactions. Most of the agents developed so far have failed to yield approved treatments, in part because it has been difficult to identify agents that target only undesirable interactions while leaving intact other vital HS-protein interactions.

Currently, the only clinically approved drug known to block GAG–protein interactions is the heparin-neutralizing agent protamine.[119] The success of protamine stems from its potent activity and acute application in patients to reverse heparin overdose. Nevertheless, protamine can exhibit adverse side effects in some patients due to anaphylactic reaction to foreign protein.[120] Protamine also lacks the ability to neutralize newly developed ultra-low molecular weight heparin derivatives, such as the synthetic pentasaccharide fondaparinux.[121] Thus, finding safer alternatives to protamine that can neutralize heparin and LMWHs is an important area of research. It remains to be seen whether the answer to this challenge will be found in small molecules or larger heparin-neutralizing oligomeric materials.

Developing effective agents to target GAG–protein interactions in disease by focusing on GAG-selective agents is a formidable challenge due to the ubiquitous expression of GAGs in different tissues and the likelihood of adverse effects. Most GAG inhibitors rely on blocking electrostatic interactions that dominate GAG–protein interactions, without any sequence specificity. The dearth of structural information available about the nature of these binding sites makes it difficult to model and identify selective inhibitors. Advances should be forthcoming as new schemes for synthesizing chemically defined HS oligosaccharides emerge,[122] as glycan array technology becomes refined,[123] and with the development of new top down methods for sequencing HS oligosaccharides.[124] Up to now, the discovery of novel inhibitors of heparin– and HS–protein interactions has been an exciting area of research. We hope that continued development of drugs targeting these complex carbohydrates and the proteins they interact with will lead to new therapeutic approaches for treating human disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors acknowledge grants CA199292 and HL107150 (to J.D.E.) and GM077471 (to Y.T.) from the National Institutes of Health.

Notes and references

1. Bishop JR, Schuksz M, Esko JD. Nature. 2007; 446:1030–1037. [PubMed: 17460664]
2. Esko JD, Lindahl U. J Clin Invest. 2001; 108:169–173. [PubMed: 11457867]
3. Dhoot GK, Gustafsson MK, Ai X, Sun W, Standiford DM, Emerson CP Jr. Science. 2001; 293:1663–1666. [PubMed: 11533491]
4. (a) Sarrazin S, Lamanna WC, Esko JD. Csh Perspect Biol. 2011; 3(b) Varki, A. Essentials of glycobiology. 2nd. Cold Spring Harbor Laboratory Press; Cold Spring Harbor, N.Y.: 2009.
5. Xu D, Esko JD. Annual Review of Biochemistry. 2014; 83:129–157.
6. Sarrazin S, Lamanna WC, Esko JD. Cold Spring Harbor Perspectives in Biology. 2011; 3:a004952. [PubMed: 21690215]
7. (a) Kreuger J, Spillmann D, Li J-p, Lindahl U. The Journal of Cell Biology. 2006; 174:323–327. [PubMed: 16880267] (b) Bernfield M, Götte M, Park PW, Reizes O, Fitzgerald ML, Lincecum J, Zako M. Annual Review of Biochemistry. 1999; 68:729–777.(c) Kjellen L, Lindahl U. Annual Review of Biochemistry. 1991; 60:443–475.(d) Lindahl, U., Li, Jp. International Review of Cell and

Molecular Biology. Kwang, WJ., editor. Vol. 276. Academic Press; 2009. p. 105-159.(e) Proudfoot AEI. Biochemical Society Transactions. 2006; 34:422–426. [PubMed: 16709177]

8. (a) Grünewald FS, Prota AE, Giese A, Ballmer-Hofer K. Biochimica et Biophysica Acta (BBA) – Proteins and Proteomics. 2010; 1804:567–580. [PubMed: 19761875] (b) Yayon A, Klagsbrun M, Esko JD, Leder P, Ornitz DM. Cell. 1991; 64:841–848. [PubMed: 1847668]
9. Mohammadi M, Olsen SK, Ibrahimi OA. Cytokine & Growth Factor Reviews. 2005; 16:107–137. [PubMed: 15863029]
10. Yayon A, Klagsbrun M. Cancer and Metastasis Reviews. 1990; 9:191–202. [PubMed: 2292136]
11. (a) Lindahl U, Kjellen L. Journal of Internal Medicine. 2013; 273:555–571. [PubMed: 23432337] (b) Knelson EH, Nee JC, Blobe GC. Trends in biochemical sciences. 2014; 39:277–288. [PubMed: 24755488]
12. (a) Blackhall FH, Merry CLR, Davies EJ, Jayson GC. British Journal of Cancer. 2001; 85:1094–1098. [PubMed: 11710818] (b) Okolicsanyi RK, van Wijnen AJ, Cool SM, Stein GS, Griffiths LR, Haupt LM. Journal of cellular biochemistry. 2014; 115:967–976. [PubMed: 24357546] (c) Sasisekharan R, Shriver Z, Venkataraman G, Narayanasami U. Nat Rev Cancer. 2002; 2:521–528. [PubMed: 12094238]
13. Szatmári T, Dobra K. Frontiers in Oncology. 2013; 3:310. [PubMed: 24392351]
14. Ridley RC, Xiao H, Hata H, Woodliff J, Epstein J, Sanderson RD. Blood. 1993; 81:767–774. [PubMed: 8427968]
15. Conejo JR, Kleeff J, Koliopanos A, Matsuda K, Zhu ZW, Goecke H, Bicheng N, Zimmermann A, Korc M, Friess H, Büchler MW. International Journal of Cancer. 2000; 88:12–20. [PubMed: 10962434]
16. (a) Ben-Zaken O, Tzaban S, Tal Y, Horonchik L, Esko JD, Vlodavsky I, Taraboulos A. Journal of Biological Chemistry. 2003; 278:40041–40049. [PubMed: 12871949] (b) Warner RG, Hundt C, Weiss S, Turnbull JE. Journal of Biological Chemistry. 2002; 277:18421–18430. [PubMed: 11882649] (c) Horonchik L, Tzaban S, Ben-Zaken O, Yedidia Y, Rouvinski A, Papy-Garcia D, Barritault D, Vlodavsky I, Taraboulos A. Journal of Biological Chemistry. 2005; 280:17062–17067. [PubMed: 15668247]
17. (a) Cohlberg JA, Li J, Uversky VN, Fink AL. Biochemistry. 2002; 41:1502–1511. [PubMed: 11814343] (b) Holmes BB, DeVos SL, Kfoury N, Li M, Jacks R, Yanamandra K, Ouidja MO, Brodsky FM, Marasa J, Bagchi DP, Kotzbauer PT, Miller TM, Papy-Garcia D, Diamond MI. Proceedings of the National Academy of Sciences. 2013; 110:E3138–E3147.
18. van Horssen J, Wesseling P, van den Heuvel LPWJ, de Waal RMW, Verbeek MM. The Lancet Neurology. 2003; 2:482–492. [PubMed: 12878436]
19. (a) Ellis LM. American Association for Cancer Research. 2006; 5:1099–1107.(b) Turner N, Grose R. Nature Reviews Cancer. 2010; 10:116–129. [PubMed: 20094046] (c) Rapraeger A, Krufka A, Olwin B. Science. 1991; 252:1705–1708. [PubMed: 1646484] (d) Thacker BE, Seamen E, Lawrence R, Parker MW, Xu Y, Liu J, Vander Kooi CW, Esko JD. ACS Chemical Biology. 2016; 11:971–980. [PubMed: 26731579] (e) Krilleke D, Ng YS, Shima DT. Biochemical Society transactions. 2009; 37:1201–1206. [PubMed: 19909247]
20. (a) Sertie AL, Sossi V, Camargo AA, Zatz M, Brahe C, Passos-Bueno MR. Human molecular genetics. 2000; 9:2051–2058. [PubMed: 10942434] (b) Sherman-Baust CA, Weeraratna AT, Rangel LB, Pizer ES, Cho KR, Schwartz DR, Shock T, Morin PJ. Cancer cell. 2003; 3:377–386. [PubMed: 12726863] (c) Harisi R, Jeney A. OncoTargets and therapy. 2015; 8:1387–1398. [PubMed: 26089687]
21. (a) Bendas G, Borsig L. International Journal of Cell Biology. 2012; 2012:10.(b) Ley K. Trends in molecular medicine. 2003; 9:263–268. [PubMed: 12829015]
22. (a) Otsuki S, Hanson SR, Miyaki S, Grogan SP, Kinoshita M, Asahara H, Wong CH, Lotz MK. Proc Natl Acad Sci U S A. 2010; 107:10202–10207. [PubMed: 20479257] (b) Huegel J, Sgariglia F, Enomoto-Iwamoto M, Koyama E, Dormans JP, Pacifici M. Developmental dynamics : an official publication of the American Association of Anatomists. 2013; 242:1021–1032. [PubMed: 23821404] (c) Zak BM, Schuksz M, Koyama E, Mundy C, Wells DE, Yamaguchi Y, Pacifici M, Esko JD. Bone. 2011; 48:979–987. [PubMed: 21310272]

23. (a) Baggiolini M. J Intern Med. 2001; 250:91–104. [PubMed: 11489059] (b) Monneau Y, Arenzana-Seisdedos F, Lortat-Jacob H. Journal of leukocyte biology. 2016; 99:935–953. [PubMed: 26701132]
24. (a) Kelton JG, Warkentin TE. Blood. 2008; 112:2607–2616. [PubMed: 18809774] (b) Jin L, Abrahams JP, Skinner R, Petitou M, Pike RN, Carrell RW. Proceedings of the National Academy of Sciences. 1997; 94:14683–14688.
25. (a) Gonzales JC, Gordts PLSM, Foley EM, Esko JD. The Journal of Clinical Investigation. 2013; 123:2742–2751. [PubMed: 23676495] (b) Gordts PLSM, Foley E, Lawrence R, Sinha R, Lameda-Diaz C, Deng L, Nock R, Glass CK, Erbilgin A, Lusis AJ, Witztum JL, Esko JD. Cell metabolism. 2014; 20:813–826. [PubMed: 25440058] (c) Stanford KI, Bishop JR, Foley EM, Gonzales JC, Niesman IR, Witztum JL, Esko JD. The Journal of Clinical Investigation. 2009; 119:3236–3245. [PubMed: 19805913] (d) MacArthur JM, Bishop JR, Stanford KI, Wang L, Bensadoun A, Witztum JL, Esko JD. Journal of Clinical Investigation. 2007; 117:153–164. [PubMed: 17200715] (e) Noborn F, Ancsin JB, Ubhayasekera W, Kisilevsky R, Li JP. J Biol Chem. 2012; 287:25669–25677. [PubMed: 22654109] (f) Li JP, Galvis MLE, Gong F, Zhang X, Zcharia E, Metzger S, Vlodavsky I, Kisilevsky R, Lindahl U. P Natl Acad Sci USA. 2005; 102:6473–6477.(g) Eisenberg S, Sehayek E, Olivecrona T, Vlodavsky I. J Clin Invest. 1992; 90:2013–2021. [PubMed: 1430223]
26. (a) Freeman CG, Parish CR, Knox KJ, Blackmore JL, Lobov SA, King DW, Senden TJ, Stephens RW. Biomaterials. 2013; 34:5670–5676. [PubMed: 23623426] (b) Stewart MD, Sanderson RD. Matrix Biology. 2014; 35:56–59. [PubMed: 24309018] (c) Brockstedt U, Dobra K, Nurminen M, Hjerpe A. Experimental cell research. 2002; 274:235–245. [PubMed: 11900484]
27. (a) Small DH, Nurcombe V, Reed G, Clarris H, Moir R, Beyreuther K, Masters CL. The Journal of neuroscience : the official journal of the Society for Neuroscience. 1994; 14:2117–2127. [PubMed: 8158260] (b) Small DH, Williamson T, Reed G, Clarris H, Beyreuther K, Masters CL, Nurcombe V. Annals of the New York Academy of Sciences. 1996; 777:316–321. [PubMed: 8624106] (c) Zhang X, Li JP. Progress in molecular biology and translational science. 2010; 93:309–334. [PubMed: 20807650] (d) Holmes BB, DeVos SL, Kfoury N, Li M, Jacks R, Yanamandra K, Ouidja MO, Brodsky FM, Marasa J, Bagchi DP, Kotzbauer PT, Miller TM, Papy-Garcia D, Diamond MI. Proc Natl Acad Sci U S A. 2013; 110:E3138–3147. [PubMed: 23898162]
28. (a) Spear PG, Shieh MT, Herold BC, WuDunn D, Koshy TI. Adv Exp Med Biol. 1992; 313:341–353. [PubMed: 1332443] (b) Shukla D, Liu J, Blaiklock P, Shworak NW, Bai X, Esko JD, Cohen GH, Eisenberg RJ, Rosenberg RD, Spear PG. Cell. 1999; 99:13–22. [PubMed: 10520990] (c) Chen Y, Maguire T, Hileman RE, Fromm JR, Esko JD, Linhardt RJ, Marks RM. Nature medicine. 1997; 3:866–871.(d) Patel M, Yanagishita M, Roderiquez G, Bou-Habib DC, Oravecz T, Hascall VC, Norcross MA. AIDS Research and Human Retroviruses. 1993; 9:167–174. [PubMed: 8096145] (e) Vivès RR, Imberty A, Sattentau QJ, Lortat-Jacob H. J Biol Chem. 2005; 280:21353–21357. [PubMed: 15797855] (f) Connell BJ, Lortat-Jacob H. Frontiers in Immunology. 2013; 4(g) Joyce JG, Tung JS, Przysiecki CT, Cook JC, Lehman ED, Sands JA, Jansen KU, Keller PM. J Biol Chem. 1999; 274:5810–5822. [PubMed: 10026203] (h) Boyle KA, Compton T. J Virol. 1998; 72:1826–1833. [PubMed: 9499033]
29. (a) Freissler E, Meyer auf der Heyde A, David G, Meyer TF, Dehio C. Cellular Microbiology. 2000; 2:69–82. [PubMed: 11207564] (b) Wuppermann FN, Hegemann JH, Jantos CA. Journal of Infectious Diseases. 2001; 184:181–187. [PubMed: 11424015] (c) Frick IM, Schmidtchen A, Sjobring U. European journal of biochemistry/FEBS. 2003; 270:2303–2311.(d) Vogt AM, Barragan A, Chen Q, Kironde F, Spillmann D, Wahlgren M. Blood. 2003; 101:2405–2411. [PubMed: 12433689] (e) Pinzon-Ortiz C, Friedman J, Esko J, Sinnis P. J Biol Chem. 2001; 276:26784–26791. [PubMed: 11352923] (f) Bishop JR, Crawford BE, Esko JD. Infection and Immunity. 2005; 73:5395–5401. [PubMed: 16113255] (g) Oliveira FO Jr, Alves CR, Calvet CM, Toma L, Boucas RI, Nader HB, Castro Cortes LM, Krieger MA, Meirelles Mde N, Souza Pereira MC. Microbial pathogenesis. 2008; 44:329–338. [PubMed: 18037261]
30. Bartlett AH, Park PW. Expert Reviews in Molecular Medicine. 2010; 12 1-null.
31. (a) Hirsh J, Anand SS, Halperin JL, Fuster V. Circulation. 2001; 103:2994–3018. [PubMed: 11413093] (b) Wardrop D, Keeling D. British Journal of Haematology. 2008; 141:757–763. [PubMed: 18355382] (c) McLean J. Circulation. 1959; 19:75–78. [PubMed: 13619023] (d) Björk I, Lindahl U. Mol Cell Biochem. 1982; 48:161–182. [PubMed: 6757715]
32. Guyton, AC., Hall, JE. Textbook of Medical Physiology. Elsevier Saunders; 2006.

33. Kakkar AK. Best Practice & Research Clinical Haematology. 2004; 17:77–87. [PubMed: 15171959]
34. (a) Alekseeva A, Casu B, Cassinelli G, Guerrini M, Torri G, Naggi A. Analytical and bioanalytical chemistry. 2014; 406:249–265. [PubMed: 24253408] (b) Naggi A, Casu B, Perez M, Torri G, Cassinelli G, Penco S, Pisano C, Giannini G, Ishai-Michaeli R, Vlodavsky I. J Biol Chem. 2005; 280:12103–12113. [PubMed: 15647251] (c) Casu B, Naggi A, Torri G. Matrix biology : journal of the International Society for Matrix Biology. 2010; 29:442–452. [PubMed: 20416374] (d) Babazada H, Yamashita F, Yanamoto S, Hashida M. Journal of controlled release : official journal of the Controlled Release Society. 2014; 194:332–340. [PubMed: 25234820] (e) Liu J, Linhardt RJ. Nat Prod Rep. 2014; 31:1676–1685. [PubMed: 25197032] (f) Mousa SA, Linhardt R, Francis JL, Amirkhosravi A. Thromb Haemost. 2006; 96:816–821. [PubMed: 17139378] (g) Chen J, Avci FY, Munoz EM, McDowell LM, Chen M, Pedersen LC, Zhang L, Linhardt RJ, Liu J. J Biol Chem. 2005; 280:42817–42825. [PubMed: 16260789] (h) Xu Y, Masuko S, Takieddin M, Xu H, Liu R, Jing J, Mousa SA, Linhardt RJ, Liu J. Science. 2011; 334:498–501. [PubMed: 22034431] (i) Hu YP, Lin SY, Huang CY, Zulueta MM, Liu JY, Chang W, Hung SC. Nature chemistry. 2011; 3:557–563.
35. (a) Casu B, Lindahl U. Advances in carbohydrate chemistry and biochemistry. 2001; 57:159–206. [PubMed: 11836942] (b) Hacker U, Nybakken K, Perrimon N. Nat Rev Mol Cell Biol. 2005; 6:530–541. [PubMed: 16072037] (c) Esko JD, Selleck SB. Annual Review of Biochemistry. 2002; 71:435–471.(d) Sasisekharan R, Venkataraman G. Current Opinion in Chemical Biology. 2000; 4:626–631. [PubMed: 11102866]
36. Capila I, Linhardt RJ. Angewandte Chemie (International ed in English). 2002; 41:391–412. [PubMed: 12491369]
37. (a) Ernst S, Langer R, Cooney CL, Sasisekharan R. Critical reviews in biochemistry and molecular biology. 1995; 30:387–444. [PubMed: 8575190] (b) Pojasek K, Shriver Z, Hu Y, Sasisekharan R. Biochemistry. 2000; 39:4012–4019. [PubMed: 10747789] (c) Ernst S, Venkataraman G, Winkler S, Godavarti R, Langer R, Cooney CL, Sasisekharan R. Biochemical Journal. 1996; 315:589–597. [PubMed: 8615834]
38. (a) Morimoto-Tomita M, Uchimura K, Werb Z, Hemmerich S, Rosen SD. J Biol Chem. 2002; 277:49175–49185. [PubMed: 12368295] (b) Freeman SD, Keino-Masu K, Masu M, Ladher RK. Developmental dynamics : an official publication of the American Association of Anatomists. 2015; 244:168–180. [PubMed: 25370455]
39. (a) de Witte L, Bobardt M, Chatterji U, Degeest G, David G, Geijtenbeek TBH, Gallay P. Proceedings of the National Academy of Sciences. 2007; 104:19464–19469.(b) Giroglou T, Florin L, Schäfer F, Streeck RE, Sapp M. Journal of Virology. 2001; 75:1565–1570. [PubMed: 11152531] (c) Leistner CM, Gruen-Bernhard S, Glebe D. Cell Microbiol. 2008; 10:122–133. [PubMed: 18086046] (d) Tiwari V, Clement C, Xu D, Valyi-Nagy T, Yue BY, Liu J, Shukla D. J Virol. 2006; 80:8970–8980. [PubMed: 16940509] (e) Broutian TR, Brendle SA, Christensen ND. The Journal of General Virology. 2010; 91:531–540. [PubMed: 19846678] (f) Nasimuzzaman M, Persons DA. Mol Ther. 2012; 20:1158–1166. [PubMed: 22434139]
40. (a) Alvarez-Dominguez C, Vazquez-Boland JA, Carrasco-Marin E, Lopez-Mato P, Leyva-Cobian F. Infect Immun. 1997; 65:78–88. [PubMed: 8975895] (b) de Vries FP, Cole R, Dankert J, Frosch M, van Putten JP. Mol Microbiol. 1998; 27:1203–1212. [PubMed: 9570405] (c) Moelleken K, Hegemann JH. Mol Microbiol. 2008; 67:403–419. [PubMed: 18086188]
41. Vogt AM, Barragan A, Chen Q, Kironde F, Spillmann D, Wahlgren M. Heparan sulfate on endothelial cells mediates the binding ofPlasmodium falciparum–infected erythrocytes via the DBL1α domain of PfEMP1. 2003
42. (a) Liu D, Shriver Z, Venkataraman G, El Shabrawi Y, Sasisekharan R. Proceedings of the National Academy of Sciences. 2002; 99:568–573.(b) Sasisekharan R, Moses MA, Nugent MA, Cooney CL, Langer R. P Natl Acad Sci USA. 1994; 91:1524–1528.(c) Liu D, Pojasek K, Shriver Z, Holley K, El-Shabrawi Y, Venkataraman G, Sasisekharan R. Journal. 2011
43. (a) Bateman D, McLaurin J, Chakrabartty A. BMC Neuroscience. 2007; 8:29. [PubMed: 17475015] (b) Kanekiyo T, Zhang J, Liu Q, Liu CC, Zhang L, Bu G. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2011; 31:1644–1651. [PubMed: 21289173]

44. Heres EK, Horrow JC, Gravlee GP, Tardiff BE, Luber J Jr, Schneider J, Barragry T, Broughton R. Anesthesia and analgesia. 2001; 93:1446–1452. table of contents. [PubMed: 11726421]
45. Ai X, Do AT, Kusche-Gullberg M, Lindahl U, Lu K, Emerson CP Jr. J Biol Chem. 2006; 281:4969–4976. [PubMed: 16377625]
46. Kim JH, Chan C, Elwell C, Singer MS, Dierks T, Lemjabbar-Alaoui H, Rosen SD, Engel JN. Cell Microbiol. 2013; 15:1560–1571. [PubMed: 23480519]
47. Wang S, Ai X, Freeman SD, Pownall ME, Lu Q, Kessler DS, Emerson CP. P Natl Acad Sci USA. 2004; 101:4833–4838.
48. Lai J, Chien J, Staub J, Avula R, Greene EL, Matthews TA, Smith DI, Kaufmann SH, Roberts LR, Shridhar V. J Biol Chem. 2003; 278:23107–23117. [PubMed: 12686563]
49. (a) Feyzi E, Trybala E, Bergström T, Lindahl U, Spillmann D. J Biol Chem. 1997; 272:24850–24857. [PubMed: 9312084] (b) Laquerre S, Argnani R, Anderson DB, Zucchini S, Manservigi R, Glorioso JC. J Virol. 1998; 72:6119–6130. [PubMed: 9621076]
50. Schulze A, Gripon P, Urban S. Hepatology (Baltimore, Md). 2007; 46:1759–1768.
51. (a) Henry-Stanley M, Hess DJ, Erickson E, Garni RM, Wells C. Medical microbiology and immunology. 2003; 192:107–115. [PubMed: 12684756] (b) Henry-Stanley MJ, Hess DJ, Erlandsen SL, Wells CL. Shock (Augusta, Ga). 2005; 24:571–576.(c) Yan Y, Silvennoinen-Kassinen S, Leinonen M, Saikku P. Microbes and infection/Institut Pasteur. 2006; 8:866–872.
52. (a) Zacharski LR, Lee AY. Expert opinion on investigational drugs. 2008; 17:1029–1037. [PubMed: 18549339] (b) Lim HC, Multhaupt HA, Couchman JR. Molecular Cancer. 2015; 14:15. [PubMed: 25623282] (c) Mousa SA, Petersen LJ. Thromb Haemost. 2009; 102:258–267. [PubMed: 19652876] (d) Nagy Z, Turcsik V, Blasko G. Pathology oncology research : POR. 2009; 15:689–692. [PubMed: 19757196]
53. Ahmed I, Majeed A, Powell R. Postgraduate Medical Journal. 2007; 83:575–582. [PubMed: 17823223]
54. Rusnati M, Vicenzi E, Donalisio M, Oreste P, Landolfo S, Lembo D. Pharmacology & Therapeutics. 2009; 123:310–322. [PubMed: 19447134]
55. Copeland R, Balasubramaniam A, Tiwari V, Zhang F, Bridges A, Linhardt RJ, Shukla D, Liu J. Biochemistry. 2008; 47:5774–5783. [PubMed: 18457417]
56. Skidmore MA, Kajaste-Rudnitski A, Wells NM, Guimond SE, Rudd TR, Yates EA, Vicenzi E. MedChemComm. 2015; doi: 10.1039/C4MD00516C
57. Vogt AM, Pettersson F, Moll K, Jonsson C, Normark J, Ribacke U, Egwang TG, Ekre H-P, Spillmann D, Chen Q, Wahlgren M. PLoS Pathog. 2006; 2:e100. [PubMed: 17009869]
58. Lee JB, Hayashi K, Hayashi T, Sankawa U, Maeda M. Planta Med. 1999; 65:439–441. [PubMed: 10418331]
59. (a) Ferro V, Liu L, Johnstone KD, Wimmer N, Karoli T, Handley P, Rowley J, Dredge K, Li CP, Hammond E, Davis K, Sarimaa L, Harenberg J, Bytheway I. Journal of Medicinal Chemistry. 2012; 55:3804–3813. [PubMed: 22458531] (b) Dredge K, Hammond E, Davis K, Li CP, Liu L, Johnstone K, Handley P, Wimmer N, Gonda TJ, Gautam A, Ferro V, Bytheway I. Investigational New Drugs. 2010; 28:276–283. [PubMed: 19357810] (c) Said JS, Trybala E, Görander S, Ekblad M, Liljeqvist J-Å, Jennische E, Lange S, Bergström T. Antimicrobial agents and chemotherapy. 2016; 60:1049–1057. [PubMed: 26643323]
60. (a) Nyberg K, Ekblad M, Bergström T, Freeman C, Parish CR, Ferro V, Trybala E. Antiviral Research. 2004; 63:15–24. [PubMed: 15196816] (b) Lee E, Pavy M, Young N, Freeman C, Lobigs M. Antiviral Research. 2006; 69:31–38. [PubMed: 16309754]
61. Talarico LB, Damonte EB. Virology. 2007; 363:473–485. [PubMed: 17337028]
62. Carlucci MJ, Scolaro LA, Damonte EB. Chemotherapy. 1999; 45:429–436. [PubMed: 10567773]
63. Tremblay P, Aisen P, Garceau D. Alzheimer's & Dementia. 2005; 1:S2.
64. Rumjon A, Coats T, Javaid MM. International Journal of Nephrology and Renovascular Disease. 2012; 5:37–43. [PubMed: 22427728]
65. Garson JA, Lubach D, Passas J, Whitby K, Grant PR. Journal of Medical Virology. 1999; 57:238–242. [PubMed: 10022794]

66. (a) Herold BC, Bourne N, Marcellino D, Kirkpatrick R, Strauss DM, Zaneveld LJ, Waller DP, Anderson RA, Chany CJ, Barham BJ, Stanberry LR, Cooper MD. The Journal of infectious diseases. 2000; 181:770–773. [PubMed: 10669374] (b) Kisilevsky R, Lemieux LJ, Fraser PE, Kong X, Hultin PG, Szarek WA. Nature medicine. 1995; 1:143–148.

67. (a) Cheshenko N, Keller MJ, MasCasullo V, Jarvis GA, Cheng H, John M, Li JH, Hogarty K, Anderson RA, Waller DP, Zaneveld LJ, Profy AT, Klotman ME, Herold BC. Antimicrobial agents and chemotherapy. 2004; 48:2025–2036. [PubMed: 15155195] (b) Keller MJ, Zerhouni-Layachi B, Cheshenko N, John M, Hogarty K, Kasowitz A, Goldberg CL, Wallenstein S, Profy AT, Klotman ME, Herold BC. The Journal of infectious diseases. 2006; 193:27–35. [PubMed: 16323128] (c) Taylor DL, Brennan TM, Bridges CG, Mullins MJ, Tyms AS, Jackson R, Cardin AD. Antiviral Res. 1995; 28:159–173. [PubMed: 8585769]

68. (a) Liu CJ, Lee PH, Lin DY, Wu CC, Jeng LB, Lin PW, Mok KT, Lee WC, Yeh HZ, Ho MC, Yang SS, Lee CC, Yu MC, Hu RH, Peng CY, Lai KL, Chang SSC, Chen PJ. Journal of Hepatology. 2009; 50:958–968. [PubMed: 19303160] (b) Kudchadkar R, Gonzalez R, Lewis KD. Expert opinion on investigational drugs. 2008; 17:1769–1776. [PubMed: 18922112] (c) Basche M, Gustafson DL, Holden SN, O'Bryant CL, Gore L, Witta S, Schultz MK, Morrow M, Levin A, Creese BR, Kangas M, Roberts K, Nguyen T, Davis K, Addison RS, Moore JC, Eckhardt SG. Clinical cancer research : an official journal of the American Association for Cancer Research. 2006; 12:5471–5480. [PubMed: 17000682]

69. Winterhoff B, Freyer L, Hammond E, Giri S, Mondal S, Roy D, Teoman A, Mullany SA, Hoffmann R, von Bismarck A, Chien J, Block MS, Millward M, Bampton D, Dredge K, Shridhar V. European journal of cancer (Oxford, England : 1990). 2015; 51:879–892.

70. Liu CJ, Chang J, Lee PH, Lin DY, Wu CC, Jeng LB, Lin YJ, Mok KT, Lee WC, Yeh HZ, Ho MC, Yang SS, Yang MD, Yu MC, Hu RH, Peng CY, Lai KL, Chang SSC, Chen PJ. World Journal of Gastroenterology : WJG. 2014; 20:11384–11393. [PubMed: 25170226]

71. Gnoni A, Santini D, Scartozzi M, Russo A, Licchetta A, Palmieri V, Lupo L, Faloppi L, Palasciano G, Memeo V, Angarano G, Brunetti O, Guarini A, Pisconti S, Lorusso V, Silvestris N. Expert opinion on therapeutic targets. 2015; 19:1623–1635. [PubMed: 26212068]

72. (a) Singh O, Garg T, Rath G, Goyal AK. Journal of Pharmaceutics. 2014; 2014:18.(b) Marais D, Gawarecki D, Allan B, Ahmed K, Altini L, Cassim N, Gopolang F, Hoffman M, Ramjee G, Williamson AL. Antiviral therapy. 2011; 16:1219–1226. [PubMed: 22155903]

73. (a) Metz-Boutigue MH, Jolles J, Mazurier J, Schoentgen F, Legrand D, Spik G, Montreuil J, Jolles P. European journal of biochemistry/FEBS. 1984; 145:659–676.(b) Wu H, Lundblad R, Church F. Neutralization of heparin activity by neutrophil lactoferrin. 1995(c) Sánchez L, Calvo M, Brock JH. Archives of Disease in Childhood. 1992; 67:657–661. [PubMed: 1599309]

74. Farnaud S, Evans RW. Molecular Immunology. 2003; 40:395–405. [PubMed: 14568385]

75. (a) Fujihara T, Hayashi K. Archives of Virology. 1995; 140:1469–1472. [PubMed: 7661698] (b) Andersen JH, Jenssen H, Sandvik K, Gutteberg TJ. J Med Virol. 2004; 74:262–271. [PubMed: 15332275]

76. EL-Fakharany E, Sanchez L, Al-Mehdar H, Redwan E. Virology Journal. 2013; 10:199. [PubMed: 23782993]

77. Harmsen MC, Swart PJ, Béthune M-Pd, Pauwels R, Clercq ED, The TB, Meijer DKF. Journal of Infectious Diseases. 1995; 172:380–388. [PubMed: 7622881]

78. Hirashima N, Orito E, Ohba K, Kondo H, Sakamoto T, Matsunaga S, Kato A, Nukaya H, Sakakibara K, Ohno T, Kato H, Sugauchi F, Kato T, Tanaka Y, Ueda R, Mizokami M. Hepatology Research. 2004; 29:9–12. [PubMed: 15135340]

79. Ishibashi Y, Takeda K, Tsukidate N, Miyazaki H, Ohira K, Dosaka-Akita H, Nishimura M. Hepatology Research. 2005; 32:218–223. [PubMed: 15936247]

80. (a) Bianchini EP, Fazavana J, Picard V, Borgel D. Development of a recombinant antithrombin variant as a potent antidote to fondaparinux and other heparin derivatives. 2011(b) Fazavana J, Bianchini EP, Saller F, Smadja C, Picard V, Taverna M, Borgel D. Journal of Thrombosis and Haemostasis. 2013; 11:1128–1136. [PubMed: 23581397]

81. Carr JA, Silverman N. The Journal of cardiovascular surgery. 1999; 40:659–666. [PubMed: 10596998]

82. Park PW, Pier GB, Hinkes MT, Bernfield M. Nature. 2001; 411:98–102. [PubMed: 11333985]
83. Chang LC, Liang JF, Lee HF, Lee LM, Yang VC. AAPS pharmSci. 2001; 3:E18. [PubMed: 11741269]
84. (a) Chang LC, Wrobleski S, Wakefield TW, Lee LM, Yang VC. AAPS pharmSci. 2001; 3:24–31. (b) Byun Y, Singh VK, Yang VC. Thrombosis Research. 1999; 94:53–61. [PubMed: 10213181]
85. He H, Ye J, Liu E, Liang Q, Liu Q, Yang VC. Journal of Controlled Release. 2014; 193:63–73. [PubMed: 24943246]
86. Sokolowska E, Kalaska B, Miklosz J, Mogielnicki A. Expert Opinion on Drug Metabolism & Toxicology. 2016; 12:897–909. [PubMed: 27223896]
87. (a) Weiss WA, Gilman JS, Catenacci AJ, Osterberg AE. Journal of the American Medical Association. 1958; 166:603–607. [PubMed: 13502035] (b) Lillehei CW, Sterns LP, Long DM, Lepley D. Annals of Surgery. 1960; 151:11–16. [PubMed: 14416954]
88. Fabian I, Aronson M. Thrombosis Research. 17:239–247.
89. Al-Jamal KT, Al-Jamal WT, Kostarelos K, Turton JA, Florence AT. Results in Pharma Sciences. 2012; 2:9–15. [PubMed: 25755989]
90. Kaminski K, Kalaska B, Koczurkiewicz P, Michalik M, Szczubialka K, Mogielnicki A, Buczko W, Nowakowska M. MedChemComm. 2014; 5:489–495.
91. Mecca T, Consoli GML, Geraci C, La Spina R, Cunsolo F. Organic & Biomolecular Chemistry. 2006; 4:3763–3768. [PubMed: 17024282]
92. Mecca T, Cunsolo F. Polymers for Advanced Technologies. 2010; 21:752–757.
93. Udit AK, Everett C, Gale AJ, Kyle JR, Ozkan M, Finn MG. Chembiochem. 2009; 10:503–510. [PubMed: 19156786]
94. Luganini A, Nicoletto SF, Pizzuto L, Pirri G, Giuliani A, Landolfo S, Gribaudo G. Antimicrobial agents and chemotherapy. 2011; 55:3231–3239. [PubMed: 21576438]
95. Borst EM, Ständker L, Wagner K, Schulz TF, Forssmann W-G, Messerle M. Antimicrobial agents and chemotherapy. 2013; 57:4751–4760. [PubMed: 23856778]
96. (a) Bromfield SM, Wilde E, Smith DK. Chemical Society Reviews. 2013; 42:9184–9195. [PubMed: 24019031] (b) Rodrigo AC, Barnard A, Cooper J, Smith DK. Angewandte Chemie International Edition. 2011; 50:4675–4679. [PubMed: 21506216]
97. (a) Shenoi RA, Kalathottukaren MT, Travers RJ, Lai BFL, Creagh AL, Lange D, Yu K, Weinhart M, Chew BH, Du C, Brooks DE, Carter CJ, Morrissey JH, Haynes CA, Kizhakkedathu JN. Science Translational Medicine. 2014; 6:260ra150.(b) Kalathottukaren MT, Abraham L, Kapopara PR, Lai BF, Shenoi RA, Rosell FI, Conway EM, Pryzdial EL, Morrissey JH, Haynes CA, Kizhakkedathu JN. Blood. 2017; 129:1368–1379. [PubMed: 28034889]
98. Overington JP, Al-Lazikani B, Hopkins AL. Nat Rev Drug Discov. 2006; 5:993–996. [PubMed: 17139284]
99. Schmidtke M, Karger A, Meerbach A, Egerer R, Stelzner A, Makarov V. Virology. 2003; 311:134–143. [PubMed: 12832211]
100. Schmidtke M, Riabova O, Dahse HM, Stelzner A, Makarov V. Antiviral Res. 2002; 55:117–127. [PubMed: 12076756]
101. Selinka HC, Florin L, Patel HD, Freitag K, Schmidtke M, Makarov VA, Sapp M. Journal of Virology. 2007; 81:10970–10980. [PubMed: 17686860]
102. Smythe MA, Trujillo T, Fanikos J. American Journal of Health-System Pharmacy. 2016; 73:S27–S48. [PubMed: 27147456]
103. Ansell JE, Laulicht BE, Bakhru SH, Hoffman M, Steiner SS, Costin JC. Thrombosis Research. 146:113–118.
104. Harris N, Kogan F, Il'kova G, Juhas S, Lahmy O, Gregor YI, Koppel J, Zhuk R, Gregor P. Biochimica Et Biophysica Acta-General Subjects. 2014; 1840:245–254.
105. Colpitts CC, Schang LM. Journal of Virology. 2014; 88:7806–7817. [PubMed: 24789779]
106. (a) Choi S, Clements DJ, Pophristic V, Ivanov I, Vemparala S, Bennett JS, Klein ML, Winkler JD, DeGrado WE. Angewandte Chemie-International Edition. 2005; 44:6685–6689. [PubMed: 16094685] (b) Montalvo GL, Zhang Y, Young TM, Costanzo MJ, Freeman KB, Wang J,

Clements DJ, Magavern E, Kavash RW, Scott RW, Liu D, DeGrado WF. Acs Chemical Biology. 2014; 9:967–975. [PubMed: 24491145]

107. Kuziej J, Litinas E, Hoppensteadt DA, Liu Dahui, Walenga JM, Fareed J, Jeske W. Clinical and Applied Thrombosis/Hemostasis. 2010; 16:377–386. [PubMed: 20460347]
108. McAllister RE. Thrombosis Research. 125:S162–S163.
109. Mahan CE. Journal of thrombosis and thrombolysis. 2014; 37:271–278. [PubMed: 23653171]
110. Schuksz M, Fuster MM, Brown JR, Crawford BE, Ditto DP, Lawrence R, Glass CA, Wang L, Tor Y, Esko JD. Proceedings of the National Academy of Sciences. 2008; 105:13075–13080.
111. (a) Roan NR, Sowinski S, Muench J, Kirchhoff F, Greene WC. Journal of Biological Chemistry. 2010; 285:1861–1869. [PubMed: 19897482] (b) Castellano LM, Shorter J. Biology. 2012; 1:58–80. [PubMed: 24832047]
112. Warford J, Doucette CD, Hoskin DW, Easton AS. Biochemical and Biophysical Research Communications. 2014; 443:524–530. [PubMed: 24315874]
113. (a) Huegel J, Mundy C, Sgariglia F, Nygren P, Billings PC, Yamaguchi Y, Koyama E, Pacifici M. Developmental biology. 2013; 377:100–112. [PubMed: 23458899] (b) Huegel J, Sgariglia F, Enomoto-Iwamoto M, Koyama E, Dormans JP, Pacifici M. Developmental dynamics : an official publication of the American Association of Anatomists. 2013; 242:1021–1032. [PubMed: 23821404]
114. Smith SA, Choi SH, Collins JNR, Travers RJ, Cooley BC, Morrissey JH. Inhibition of polyphosphate as a novel strategy for preventing thrombosis and inflammation. 2012
115. Warford J, Madera L, Hoskin D, Easton A. Journal of Neuroimmunology. 2014; 275:73.
116. Xu D, Fuster MM, Lawrence R, Esko JD. Journal of Biological Chemistry. 2011; 286:737–745. [PubMed: 20974861]
117. Umber F, Störring FK, Föllmer W. Klinische Wochenschrift. 1938; 17:443–446.
118. Weiss RJ, Gordts PLSM, Le D, Xu D, Esko JD, Tor Y. Chemical Science. 2015; doi: 10.1039/C5SC01208B
119. Pai, M., Crowther, M. Heparin – A Century of Progress. Lever, R.Mulloy, B., Page, CP., editors. Vol. 207. Springer; Berlin Heidelberg: 2012. p. 265-277.ch. 11
120. (a) Portmann AF, Holden WD. Journal of Clinical Investigation. 1949; 28:1451–1458. [PubMed: 15395947] (b) Weiler JM, Freiman P, Sharath MD, Metzger WJ, Smith JM, Richerson HB, Ballas ZK, Halverson PC, Shulan DJ, Matsuo S, et al. J Allergy Clin Immunol. 1985; 75:297–303. [PubMed: 2857186]
121. (a) Nagler M, Haslauer M, Wuillemin WA. Thrombosis Research. 2012; 129:407–417. [PubMed: 22133273] (b) van Veen JJ, Maclean RM, Hampton KK, Laidlaw S, Kitchen S, Toth P, Makris M. Blood Coagul Fibrinolysis. 2011; 22:565–570. [PubMed: 21959588] (c) Schroeder M, Hogwood J, Gray E, Mulloy B, Hackett AM, Johansen KB. Anal Bioanal Chem. 2011; 399:763–771. [PubMed: 20922518]
122. (a) Arungundram S, Al-Mafraji K, Asong J, Leach FE, Amster IJ, Venot A, Turnbull JE, Boons GJ. Journal of the American Chemical Society. 2009; 131:17394–17405. [PubMed: 19904943] (b) Liu R, Xu Y, Chen M, Weïwer M, Zhou X, Bridges AS, DeAngelis PL, Zhang Q, Linhardt RJ, Liu J. J Biol Chem. 2010; 285:34240–34249. [PubMed: 20729556] (c) Chen Y, Li Y, Yu H, Sugiarto G, Thon V, Hwang J, Ding L, Hie L, Chen X. Angewandte Chemie International Edition. 2013; 52:11852–11856. [PubMed: 24038939]
123. (a) Dulaney SB, Xu Y, Wang P, Tiruchinapally G, Wang Z, Kathawa J, El-Dakdouki MH, Yang B, Liu J, Huang X. The Journal of Organic Chemistry. 2015; 80:12265–12279. [PubMed: 26574650] (b) Yang J, Hsieh PH, Liu X, Zhou W, Zhang X, Zhao J, Xu Y, Zhang F, Linhardt RJ, Liu J. Chemical Communications. 2017; 53:1743–1746. [PubMed: 28106177] (c) Puvirajesinghe, Tania M., Ahmed, Yassir A., Powell, Andrew K., Fernig, David G., Guimond, Scott E., Turnbull, Jeremy E. Chemistry & Biology. 2012; 19:553–558. [PubMed: 22633407] (d) de Paz JL, Noti C, Seeberger PH. Journal of the American Chemical Society. 2006; 128:2766–2767. [PubMed: 16506732] (e) Noti C, Seeberger PH. Chemistry & Biology. 2005; 12:731–756. [PubMed: 16039522]
124. (a) Guimond SE, Puvirajesinghe TM, Skidmore MA, Kalus I, Dierks T, Yates EA, Turnbull JE. J Biol Chem. 2009; 284:25714–25722. [PubMed: 19596853] (b) Thanawiroon C, Rice KG, Toida

T, Linhardt RJ. J Biol Chem. 2004; 279:2608–2615. [PubMed: 14610083] (c) Hu H, Huang Y, Mao Y, Yu X, Xu Y, Liu J, Zong C, Boons GJ, Lin C, Xia Y, Zaia J. Molecular & Cellular Proteomics. 2014; 13:2490–2502. [PubMed: 24925905] (d) Huang R, Zong C, Venot A, Chiu Y, Zhou D, Boons GJ, Sharp JS. Analytical Chemistry. 2016; 88:5299–5307. [PubMed: 27087275] (e) Li L, Zhang F, Zaia J, Linhardt RJ. Analytical Chemistry. 2012; 84:8822–8829. [PubMed: 22985071]

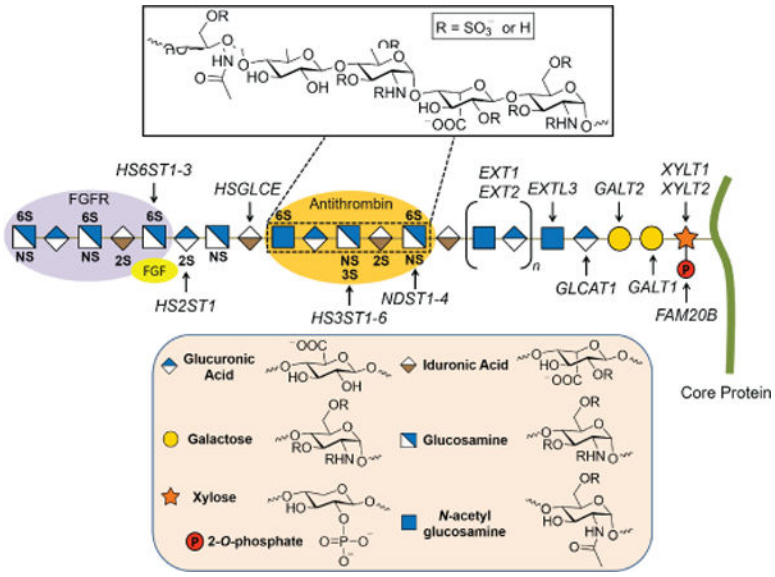


Figure 1.

Heparan sulfate structure and biosynthesis. Respective *O*- and *N*-sulfotransferases (HS2ST, HS3ST, HS6ST, and NDST) sulfate specific sites along the HS chain (shown in bold). The purple and orange oval shapes depict protein binding sites along the HS chain. *Inset*: Pentasaccharide antithrombin (AT) binding sequence.

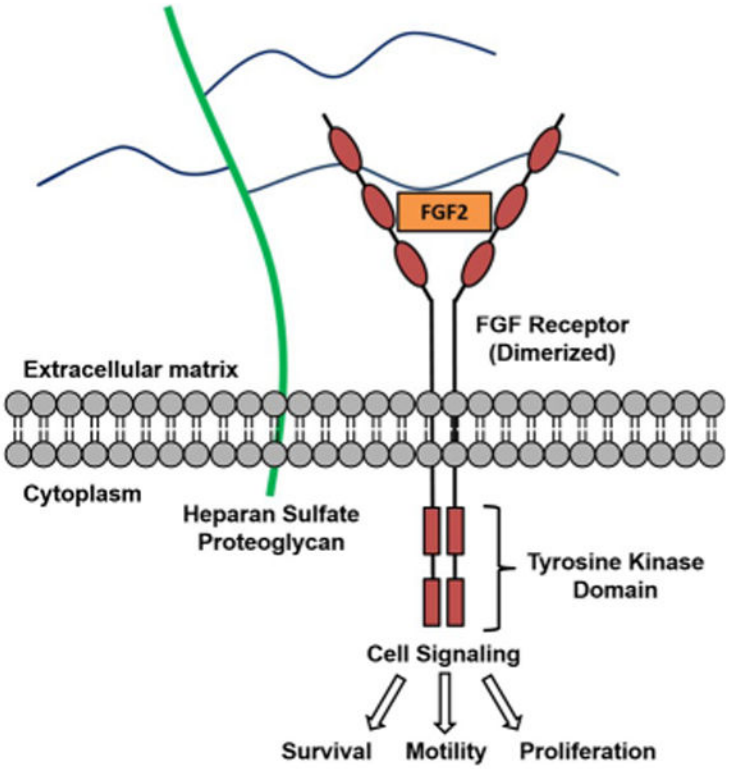


Figure 2.
FGF2 binds to HS (blue lines) and forms a ternary complex with its receptor.

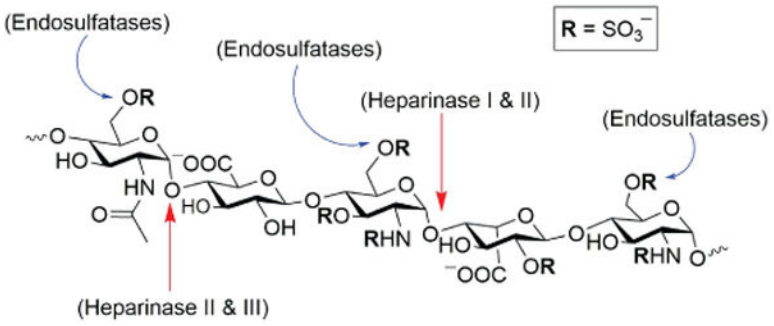


Figure 3.
Heparinase (I-III) and endosulfatase (*Sulf1, Sulf2*) activity on a pentasaccharide region of heparin/HS.

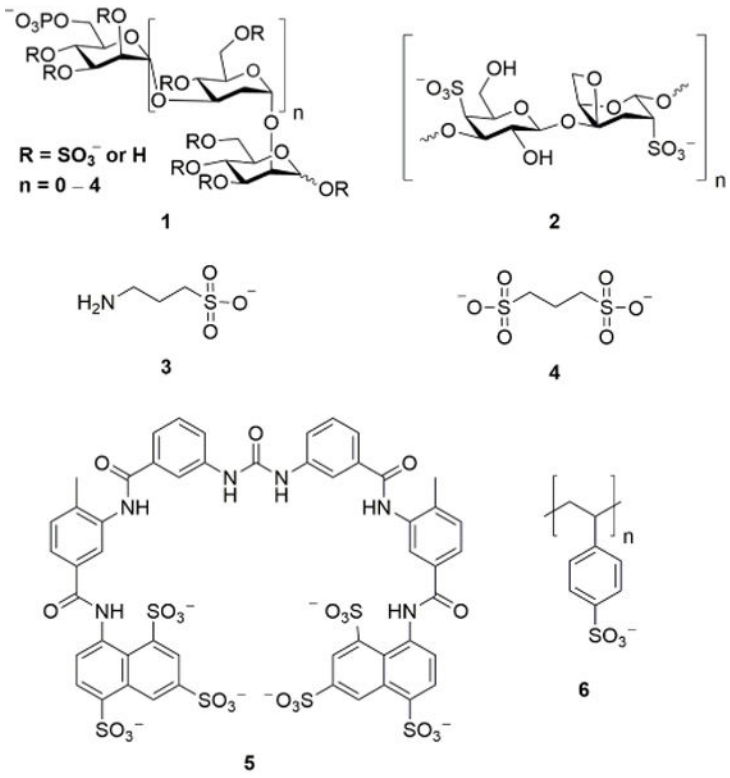


Figure 4.
Examples of GAG mimetic compounds.

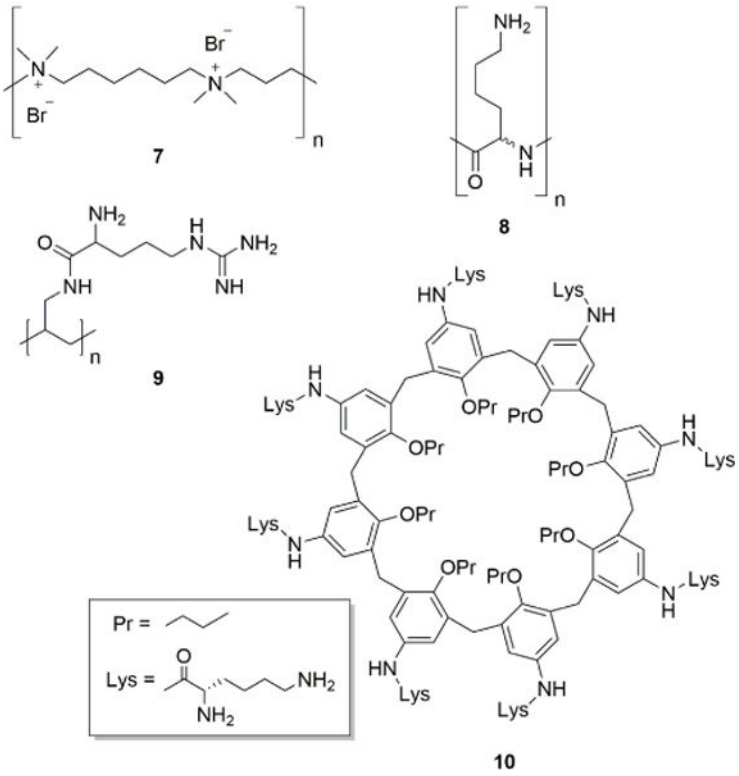


Figure 5.
Examples of cationic antagonists of heparin and HS.

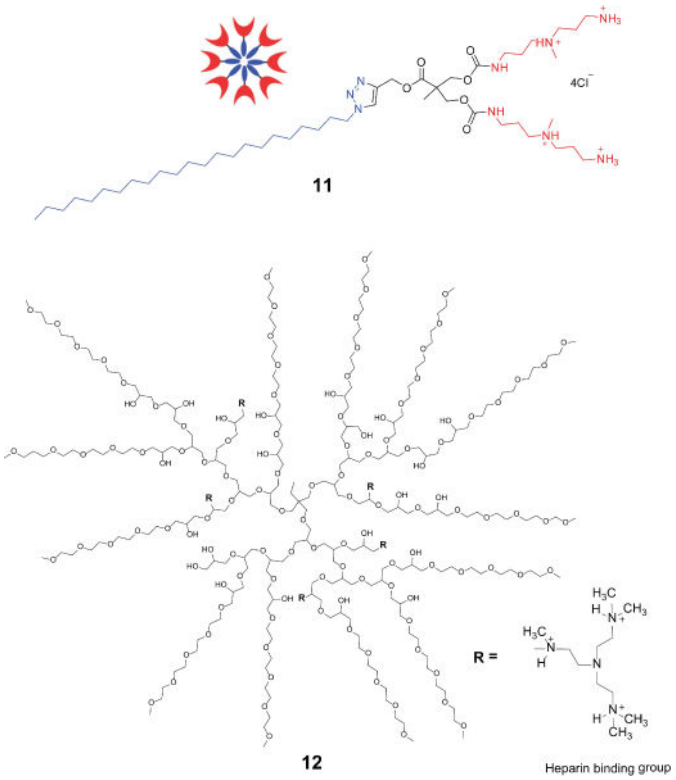


Figure 6.

Synthetic self-assembling ligands (**11**) and UHRA (**12**) for multivalent binding to heparin. Adapted from Ref. 96 with permission from the Royal Society of Chemistry.

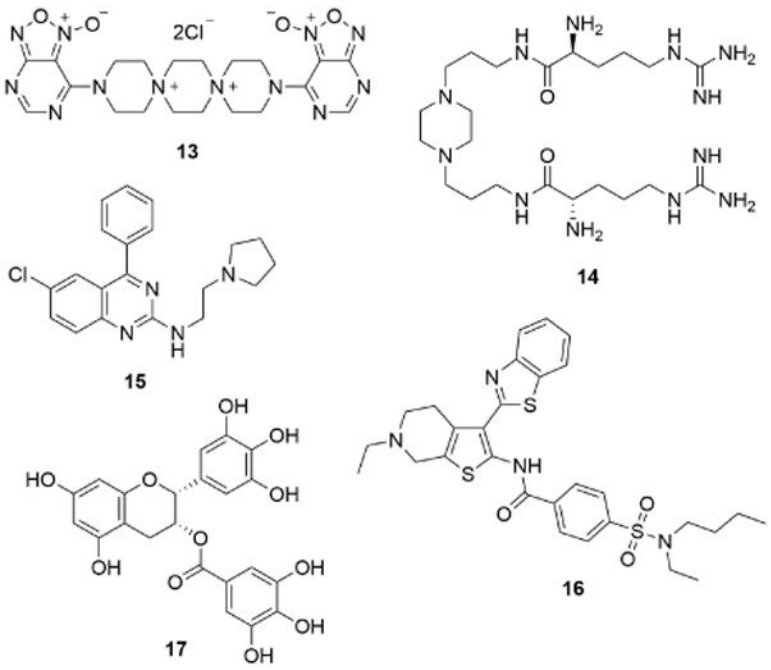


Figure 7.
Small molecule antagonists of heparin and HS-protein interactions.

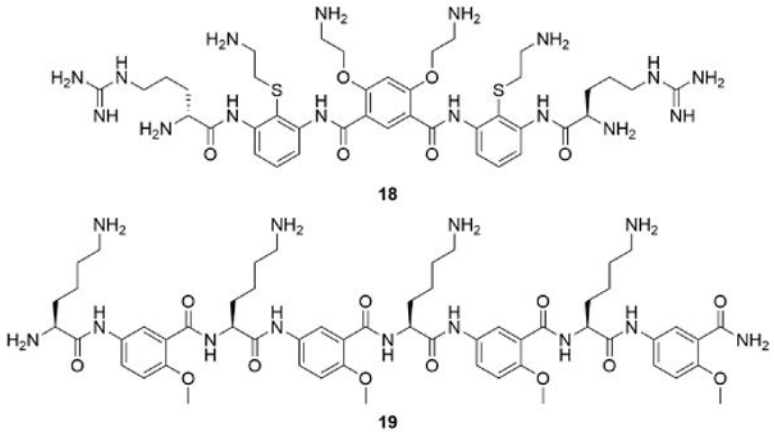


Figure 8.
Foldamers: small molecule peptide mimetics for neutralizing heparin.

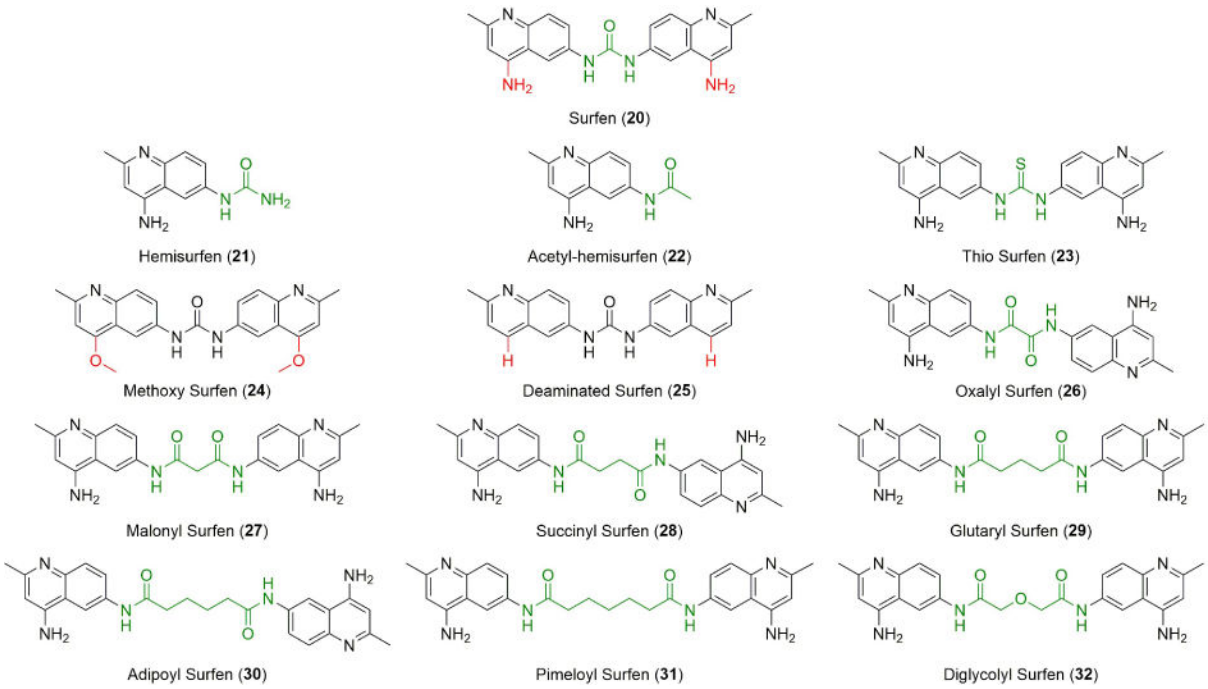


Figure 9.

Structures of surfen (**20**) and its analogs. Modifications are highlighted in red and green. Reproduced from Ref. 118 with permission from the Royal Society of Chemistry.

Table 1

Examples of heparin/HS-binding proteins and their biological activity.

Class	Examples	Physiological Function	Pathophysiology	Refs
Growth factors	FGF2, $VEGF_{165}$, neuropilin-1	mitogenesis, development, wound healing, angiogenesis, axon guidance	cancer	8b, 19
ECM proteins	collagen, fibrinogen, laminin	cell adhesion, migration, differentiation, blood coagulation	cancer, Knobloch syndrome	20
Cell adhesion proteins	P-selectin, L-selectin, integrins	cell adhesion, inflammation	cancer	21
Morphogens	activin, BMP-2, sonic hedgehog	development, regeneration, bone formation	multiple hereditary exostoses, osteoarthritis	22
Chemokines	platelet factor 4, IL-8, TNF-α, CXCL12	chemotaxis, cell migration, immune response, angiogenesis	inflammation, arteriosclerosis, cancer	23
Blood coagulation factors	antithrombin III, factor Xa, leuserpin-2	regulation of clotting cascade	heparin-induced thrombocytopenia	24
Lipoproteins	ApoE, ApoB, lipoprotein lipase	lipid metabolism, cell membrane functions	atherosclerosis, Alzheimer's disease, AA amyloidosis	25
Nuclear proteins	histones, transcription factors	unknown	cancer	26
Amyloid proteins	APP, Aβ, tau protein, α-synuclein, PrP^{Sc}	synapse organization, brain development, memory, circadian rhythm	Alzheimer's/Parkinson's disease, prion disease	27
Viral proteins	gB, gC, gD, gp120, Tat, E protein, L1 capsid protein	-----	HIV-1, HPV, HSV-1, HCMV, Dengue virus	28
Microbial proteins	M protein, PfEMP1, Opa, circumsporozoite protein	-----	bacterial/parasite infection, inflammation	29