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Article

Synthesis and Biological Evaluation of Polysulfated Oligosaccharide Glycosides as Inhibitors of Angiogenesis and Tumor Growth

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A series of polysulfated penta- and tetrasaccharide glycosides containing $\alpha(1\rightarrow3)/\alpha(1\rightarrow2)$-linked mannose residues were synthesized as heparan sulfate (HS) mimetics and evaluated for their ability to inhibit angiogenesis. The compounds bound tightly to angiogenic growth factors (FGF-1, FGF-2, and VEGF) and strongly inhibited heparanase activity. In addition, the compounds exhibited potent activity in cell-based and ex vivo assays indicative of angiogenesis, with tetrasaccharides exhibiting activity comparable to that of pentasaccharides. Selected compounds also showed good antitumor activity in vivo in a mouse melanoma (solid tumor) model resistant to the phase III HS mimetic **1** (muparfostat, formerly known as PI-88). The lipophilic modifications also resulted in reduced anticoagulant activity, a common side effect of HS mimetics, and conferred a reasonable pharmacokinetic profile in the rat, as exemplified by the sulfated octyl tetrasaccharide **5**. The data support the further investigation of this class of compounds as potential antiangiogenic, anticancer therapeutics.
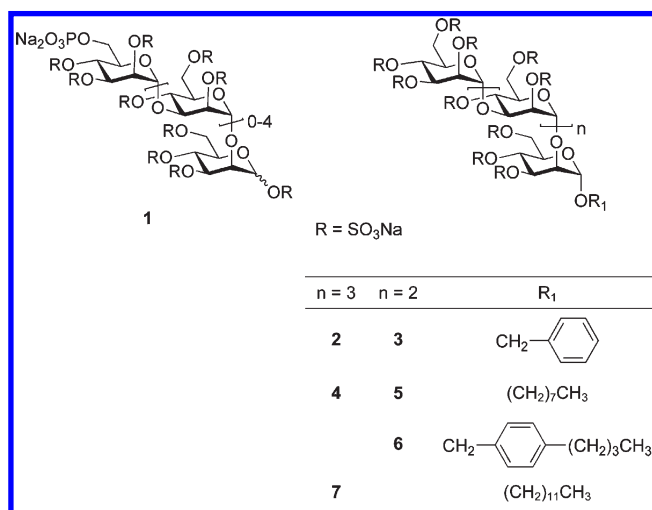
Introduction

To grow and metastasize, tumors are critically dependent on angiogenesis,[1,2] that is, the growth of new blood vessels from preexisting ones surrounding a tumor, and inhibition of angiogenesis is now well established as an important therapeutic strategy for cancer patients.[3] Cell surface and extracellular matrix (ECM)[a] heparan sulfate (HS) glycosaminoglycans are complex polysaccharides that are ubiquitous in nature and play important roles in the regulation of several aspects of cancer biology, including angiogenesis, tumor progression, and metastasis.[4,5] The use of HS mimetics to modulate these processes is a promising approach for new cancer therapeutics.[4-7] This is well-illustrated by **1** [muparfostat (PI-88) (Chart 1)],[8,9] a complex mixture of highly sulfated oligosaccharides that recently progressed to phase III clinical trials in postresection hepatocellular carcinoma.[10]

HS mimetics such as **1** are promising as anticancer therapeutics because they simultaneously inhibit both angiogenesis

Chart 1

and metastasis.[8] **1** inhibits angiogenesis directly by antagonizing the interactions of angiogenic growth factors such as FGF-2 and VEGF with HS, thus blocking the formation of the ternary HS−growth factor receptor complexes[11] that initiate the angiogenic cell signaling cascade. Compound **1** blocks metastasis by potently inhibiting heparanase,[12,13] an endo-β-glucuronidase that cleaves HS side chains of proteoglycans

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[a] Abbreviations: HS, heparan sulfate; FGF, fibroblast growth factor; VEGF, vascular endothelial growth factor; ECM, extracellular matrix; CMC, chemistry, manufacturing, and controls; APTT, activated partial thromboplastin time; HUVEC, human umbilical vein endothelial cell; TGI, tumor growth inhibition.

that are a major component of the ECM and basement membranes, thus allowing metastatic tumor cells to escape into the bloodstream. Heparanase also plays a key role in angiogenesis;[14] thus, its inhibition by **1** contributes indirectly to the antiangiogenic activity of **1**.
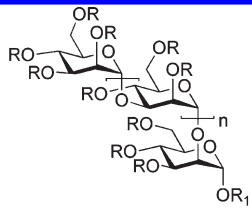
Compound **1** is still being assessed in a phase II trial for melanoma. Its administration is associated with side effects such as anticoagulant activity[15] and immune-mediated thrombocytopenia.[16] The pharmacokinetic profile[17] supports daily dosing, but because it is a complex mixture, its development has been challenging compared with that of typical small molecules that are single chemical entities. To improve upon this technology, HS mimetics such as **2** and **4** (Chart 1) were prepared and evaluated for their ability to inhibit heparanase and to bind to angiogenic growth factors.[18] The compounds in this small initial series were mostly glycosides of the major pentasaccharide found in **1**. Compounds such as **2** and **4** exhibited biological activity similar to that of **1**, but compound **4** in particular, with its more lipophilic octyl group, showed improved pharmacokinetics in a rat model (cleared from the plasma approximately 3 times more slowly than **1**). Both **2** and **4** were subsequently shown to be potent inhibitors of in vivo angiogenesis in two separate murine models when administered as subcutaneous injections at a dose of 30 mg/kg/day (b.i.d.).[19] To build on these promising early results, herein we describe the synthesis of analogues of compounds **2** and **4** and their biological evaluation as potential antiangiogenic agents.

Results and Discussion

Analogues of **2** and **4** in which the length and nature of the aglycone were altered for the purpose of examining the effects of such changes on the activity of the compounds were prepared [**3** and **5**−**17** (Charts 1 and 2)]. In some cases, the aglycones contained various chromophores in the anticipation that they would render the compounds easier to detect in biological fluids (Chart 2). In the earlier, preliminary series,[18] the length of the oligosaccharide portion was limited to pentasaccharide because other studies had shown that the lower homologues were less potent. For example, highly sulfated di- and trisaccharides are only partial competitive inhibitors of heparanase and do not completely inhibit the enzyme, even at very high concentrations.[20] However, in this study, tetrasaccharide glycosides were also prepared because sulfated tetrasaccharides have exhibited antiangiogenic[8] and anti-heparanase[18,20] activity comparable to that of pentasaccharides but lower anticoagulant activity.[15,21] It was also desirable to test whether the greater lipophilicity of the tetrasaccharide glycosides would have any significant effect on the pharmacokinetic profile of these compounds. In addition, from a drug development perspective, such compounds would be prepared by stepwise total synthesis[20,22] from monosaccharide building blocks, and thus, tetrasaccharides would be cheaper to manufacture, requiring one fewer round of glycosylation.

The compounds were prepared from the penta- and tetrasaccharides **28** and **29**, respectively (Scheme 1), which were available as byproducts[23] from the manufacture of **1**, or by total synthesis using readily prepared monosaccharide building blocks.[20,22] The starting materials were converted into their peracetates **30** and **31**, respectively, by treatment with excess acetic anhydride in pyridine. Glycosylations with the more reactive alcohols benzyl alcohol, octanol, and 8-azido-1-octanol
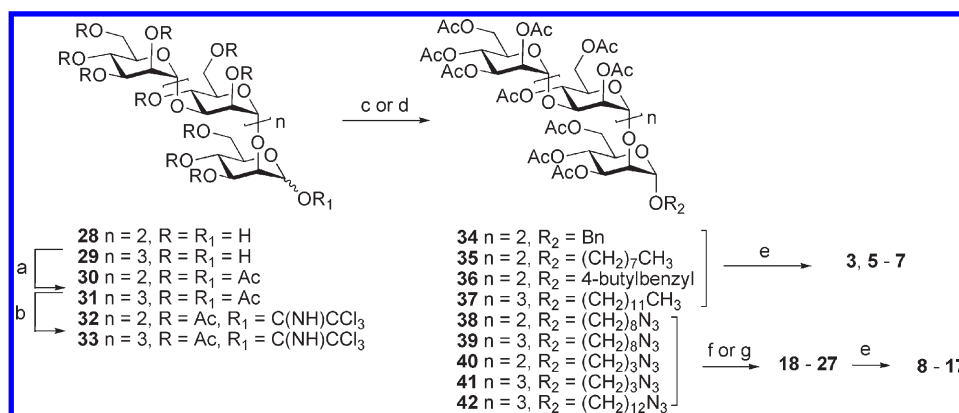
Chart 2

R = SO3Na		R = Ac		
n = 3	n = 2	n = 3	n = 2	R1
8	9	18	19	$(CH_2)_7$—triazole—phenyl
10	11	20	21	$(CH_2)_7$—triazole—naphthyl
-	12	-	22	$(CH_2)_7$—NH—SO2—naphthyl—NMe2
13	14	23	24	$(CH_2)_2$—triazole—phenyl
15	-	25	-	$(CH_2)_2$—NH—SO2—naphthyl—NMe2
16	-	26	-	$(CH_2)_{11}$—triazole—phenyl
17	-	27	-	$(CH_2)_{11}$—triazole—naphthyl

were conducted via treatment of a mixture of the peracetate and the alcohol with BF_3 etherate at 60 °C for 2−6 h, which resulted in the desired glycoside in 48−75% yield after chromatography. The expected exclusive formation of the α-glycoside, due to blocking of the β-face by the tri- or tetrasaccharide substituent at the 2-position, was confirmed by NMR spectroscopy (e.g., a measured $^1J_{C-1,H-1}$ value of 170 Hz for compound **39** is characteristic[24] for the α-linkage). Glycosylation of 4-butylbenzyl alcohol, dodecanol, and 12-azido-1-dodecanol required the more reactive trichloroacetimidates **32** and **33** as the glycosyl donors.[18] These donors were readily prepared from their corresponding peracetates **30** and **31**, respectively, by anomeric deacetylation with benzylamine followed by reaction with trichloroacetonitrile and base; however, the products contained traces of the byproduct benzylacetamide which could not be completely removed by chromatography. The benzylacetamide was carried through with the glycoside but was easily removed at the polyol stage by washing with EtOAc. The glycosylation reactions were conducted in dichloromethane at −20 °C with catalysis by TMSOTf and once again gave exclusively the α-anomer as the sole product in moderate yield. The dansyl derivatives **22** and **25** were prepared by Staudinger reaction of the corresponding azides **38** and **39** with triphenylphosphine in aqueous THF followed by acylation with dansyl chloride in the presence of triethylamine.

Compounds **18**−**21**, **23**, **24**, **26**, and **27** contain a phenyl- or naphthyltriazole group at the end of the alkyl chain introduced via a Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition reaction[25,26] between the azidoalkyl glycoside and phenyl- or naphthylacetylene. In the first instance, the cycloaddition

Scheme 1. Synthesis of Polysulfated Glycosides **3** and **5−17**[a]

28 n = 2, R = R₁ = H
29 n = 3, R = R₁ = H
30 n = 2, R = R₁ = Ac
31 n = 3, R = R₁ = Ac
32 n = 2, R = Ac, R₁ = C(NH)CCl₃
33 n = 3, R = Ac, R₁ = C(NH)CCl₃

34 n = 2, R₂ = Bn
35 n = 2, R₂ = (CH₂)₇CH₃
36 n = 2, R₂ = 4-butylbenzyl
37 n = 3, R₂ = (CH₂)₁₁CH₃
38 n = 2, R₂ = (CH₂)₈N₃
39 n = 3, R₂ = (CH₂)₈N₃
40 n = 2, R₂ = (CH₂)₃N₃
41 n = 3, R₂ = (CH₂)₃N₃
42 n = 3, R₂ = (CH₂)₁₂N₃

[a] (a) Ac₂O, Py; (b) (i) BnNH₂, (ii) Cl₃CCN, Cs₂CO₃ (10 mol %), K₂CO₃ (2 equiv); (c) alcohol (3 equiv), BF₃·Et₂O, 1,2-DCE, 60 °C, 48−75%; (d) alcohol (2 equiv), TMSOTf, DCM, −20 °C; (e) (i) NaOMe/MeOH, (ii) SO₃·Me₃N (or ·pyridine) complex (3 equiv/OH), DMF, 60 °C, (iii) NaOH (aqueous); (f) (i) Ph₃P, THF/H₂O, (ii) dansyl chloride, Et₃N; (g) phenyl- or naphthylacetylene (2 equiv), t-BuOH, CuSO₄ (10 mol %), sodium ascorbate (30 mol %).

Table 1. Inhibition of Heparanase,[a] Growth Factor Binding,[b] and Anticoagulant Activity (at 0.1 mg/mL)[c] of Test Compounds[d]

compd	heparanase K_i (nM)	K_d for FGF-1 (pM)	K_d for FGF-2 (nM)	K_d for VEGF (nM)	APTT (s)	heptest (s)
1	240 ± 30	240 ± 40	61 ± 9	2.2 ± 2.3	> 500	> 500
2	370 ± 120	160 ± 60	95 ± 22	1.8 ± 0.5	> 500	> 500
3	280 ± 60	240 ± 50	129 ± 12	21 ± 4	417.9	281.1
4	350 ± 90	161 ± 22	82 ± 21	1.5 ± 0.4	250.8	124.4
5	330 ± 100	118 ± 9	160 ± 40	18 ± 5	96.7	79.5
6	310 ± 70	210 ± 160	160 ± 40	12.0 ± 1.1	73.1	49.8
7	280 ± 90	6000 ± 3000	480 ± 70	12 ± 3	125.9	64.2
8	420 ± 110	160 ± 80	43 ± 10	0.62 ± 0.24	96.9	38.4
9	310 ± 60	280 ± 60	140 ± 21	10.4 ± 2.2	53.0	24.4
10	360 ± 80	120 ± 50	35 ± 9	0.70 ± 30	89.0	20.0
11	270 ± 50	190 ± 120	110 ± 40	7.1 ± 2.0	59.5	28.8
12	330 ± 70	200 ± 70	130 ± 60	8.9 ± 2.0	nd[e]	nd[e]
13	350 ± 110	100 ± 40	83 ± 14	1.4 ± 0.8	nd[e]	nd[e]
14	610 ± 120	360 ± 130	550 ± 80	25 ± 17	nd[e]	nd[e]
15	570 ± 140	790 ± 100	86 ± 11	12.2 ± 0.4	nd[e]	nd[e]
16	270 ± 90	2300 ± 1300	253 ± 25	5.1 ± 1.8	nd[e]	nd[e]
17	270 ± 70	190 ± 100	73 ± 23	0.79 ± 0.24	nd[e]	nd[e]

[a] K_i values ± the standard error for inhibition of recombinant human heparanase as determined from inhibition response curves as previously described.[20] [b] Binding affinities for growth factors FGF-1, FGF-2, and VEGF were determined using a surface plasmon resonance-based solution affinity assay, as previously described.[28,29] The K_d values represent the average of at least duplicate measurements ± the standard deviation. [c] The normal ranges for coagulation values in pooled human plasma are 26−36 s for APTT and 13−20 s for heptest. APTT and heptest values are the means of two determinations. [d] See the Experimental Section for assay details. [e] Not determined.

reaction was performed on the aglycone in a convenient one-pot procedure[27] whereby the azido alcohol, initially formed by displacement of the corresponding bromide by azide ion, reacted in situ with the alkyne in the presence of a copper catalyst. This simple procedure provided ready access to the triazole-functionalized alcohols for glycosylation with the trichloroacetimidate donor **32** or **33**. Unfortunately, these alcohols proved to be completely unreactive under our glycosylation conditions, even at elevated temperatures. The compounds were therefore prepared via the corresponding azidoalkyl glycosides **38−42** which underwent a Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition with phenyl- or naphthylacetylene in aqueous *tert*-butanol. The cycloadditions generally proceeded satisfactorily and gave a moderate yield (43−75%) of product after overnight reaction; however, it is noteworthy that some reactions with naphthylacetylene were rather sluggish and required prolonged reaction times and an excess of reagents to produce a reasonable yield of product, e.g., 3 days for **21** (50%) and 11 days for **27** (26%).

The peracetylated glycosides were then converted into the polysulfated final products **3** and **5−17** by deprotection with sodium methoxide in methanol followed by sulfonation with excess sulfur trioxide pyridine (or dimethylamine) complex in DMF at 60 °C for 16 h. The polysulfates were obtained in good yield following purification by size exclusion chromatography or dialysis to remove salts and low-molecular weight impurities. Product purity was determined by a combination of CE[21] and size exclusion HPLC and was generally > 95%. Mass spectrometric analysis (ESMS) was conducted for some compounds and, in accord with previous observations,[28] gave spectra that consisted of clusters of peaks corresponding to the loss of sulfo groups. However, it was evident from the ¹H and ¹³C NMR spectra that the compounds generally were fully sulfated (single sets of peaks with resonances shifted downfield). This is in contrast to polysulfated oligosaccharides that are sulfated at the reducing end such as **1**, which contain a small subpopulation of slightly undersulfated species. Such compounds are difficult to obtain fully sulfated because they are susceptible to anomeric hydrolysis and are poorly soluble

in DMF, typically forming insoluble gums at the completion of the sulfonation reaction,[21] resulting in incomplete sulfonation. The improved level of sulfation may thus be due to the greater solubility conferred on the compounds by the lipophilic aglycones as well as the increased stability to hydrolysis.

The compounds were initially evaluated for their ability to inhibit the hydrolysis of ^{3}H-labeled HS by recombinant human heparanase using an ultrafiltration assay[20] (Table 1). The results indicate that the compounds are potent inhibitors of heparanase (K_i = 270−610 nM), with tetrasaccharides displaying activity comparable to that of pentasaccharides. With the exception of **14** and **15**, which were approximately 2.5-fold weaker inhibitors than **1**, the compounds had K_i values similar to those of **1**, indicating that the presence of a lipophilic aglycone does not greatly impair the compounds' ability to inhibit heparanase.

The affinity of the compounds for angiogenic growth factors FGF-1, FGF-2, and VEGF was then determined via a surface plasmon resonance-based solution affinity assay.[28,29] All compounds bound tightly to the growth factors (K_d values in the nanomolar to picomolar range), in some cases with slightly better affinities than **1**, with tetrasaccharides displaying an affinity comparable to that of pentasaccharides (Table 1). With the exception of **8** and **10**, which showed improved affinity for all three growth factors compared with **1**, the aglycone modifications had variable effects on affinity depending on the growth factor, with FGF-1 seemingly more sensitive (10 of 16 compounds exhibited improved affinity).

The compounds were subsequently subjected to a suite of cell-based assays indicative of angiogenesis in which the improved antiangiogenic activity of many compounds compared with that of **1** became apparent. The first of these focuses on the ability of the compounds to inhibit the proliferation of endothelial cells in response to angiogenic growth factors. In human umbilical vein endothelial cell (HUVEC) proliferation assays, many compounds inhibited FGF-1-, FGF-2-, and VEGF-induced endothelial cell proliferation with IC_{50} values similar to or lower than that of **1** (Figure 1a). These data and the K_d data from Table 1 were also expressed as a ratio to provide a measure of the efficiency with which growth factor affinity is translated into antiproliferative activity against HUVECs whose division is being promoted by the same growth factor (Figure 1b). The ratios are normalized with respect to **1**, allowing for easier comparison with this compound, and the data indicate that compounds **3**, **5**, **7**, and **16** show significant improvement in the translation of growth factor affinity to antiproliferative activity. This suggests that the aglycone groups used in these compounds facilitate significantly higher bioavailability in these cell experiments which, if translated to increased bioavailability in organisms, could significantly improve the efficacy of this potent class of HS mimetics.

Endothelial cell tube formation on an ECM-derived protein matrix using HUVECs was also strongly inhibited by several compounds at concentrations of 10, 50, and 100 μM (Figure 2a), with many compounds achieving 100% inhibition at concentrations of 50 μM (Figure 2b and data not shown). In contrast, **1** has only modest activity in this assay and does not reach 100% inhibition, even at very high concentrations (e.g., only 58% inhibition at 100 μM).[19] It is important to note that the compounds do not induce cytotoxicity at the concentrations tested in the models described above with the LC_{50} values (lethal concentration required to kill 50% of the endothelial cells) for all the compounds
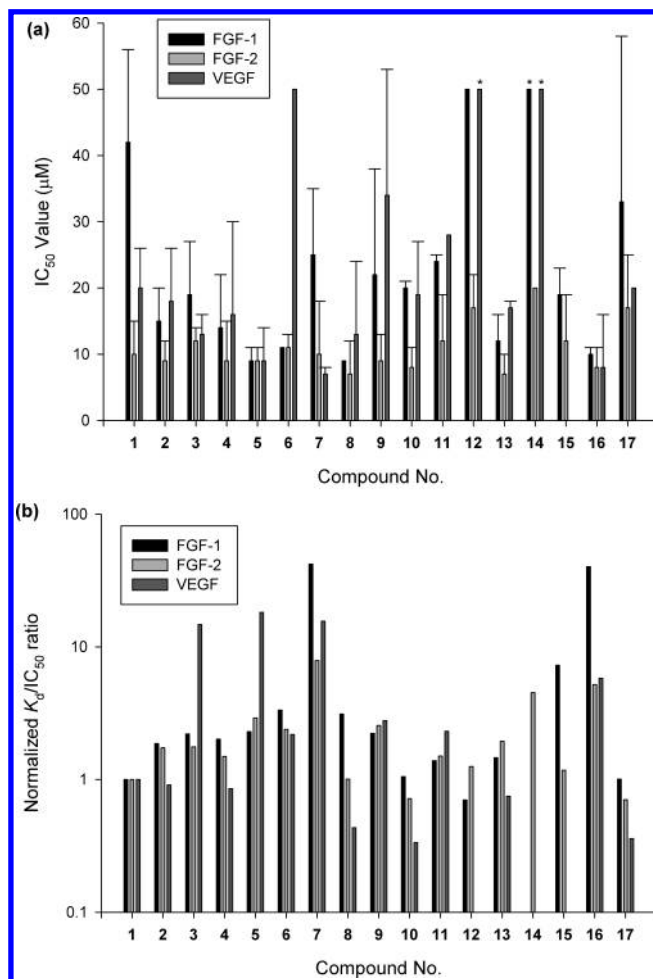

Figure 1. Inhibition of growth factor-induced HUVEC proliferation by test compounds. (a) The IC_{50} data represent the mean and standard deviation to indicate interexperimental error where selected compounds were tested in two to eight independent experiments. Data without error bars are from single experiments. An asterisk indicates IC_{50} > 50 μM (50 μM was typically the highest concentration tested). (b) These data and those in Table 1 have been used to calculate the ratio of growth factor affinity (K_d) to cell proliferation efficacy (IC_{50}). The ratio, which has been normalized with respect to compound **1** to ease comparison, indicates the efficiency with which affinity for a growth factor carries over to the cell proliferation experiment specific for that same growth factor. A ratio of >1 indicates that that compound has a higher efficiency than **1**.

being > 100 μM (data not shown). The LC_{50} for compound **5**, for example, is more than 10-fold higher than its IC_{50} concentration in the endothelial cell proliferation assays.

Selected compounds were also examined in the ex vivo rat aortic angiogenesis assay. In this model, rat aortic endothelium exposed to a three-dimensional matrix of ECM-derived proteins switches to a microvascular phenotype, generating branching networks of microvessels. Angiogenesis is triggered by the injury caused by the dissection procedure and does not require stimulation by exogenous growth factors. With the exception of **2** and **3**, the tested compounds significantly inhibited angiogenesis at a concentration of 10 μM, with compound **4** showing the most potent inhibition at 50 μM of all compounds tested (see Figure 3). To examine the viability of the aortic tissue following treatment with the test compounds, withdrawal of these compounds on day 6 or 7 (depending on the individual experiment) was followed by
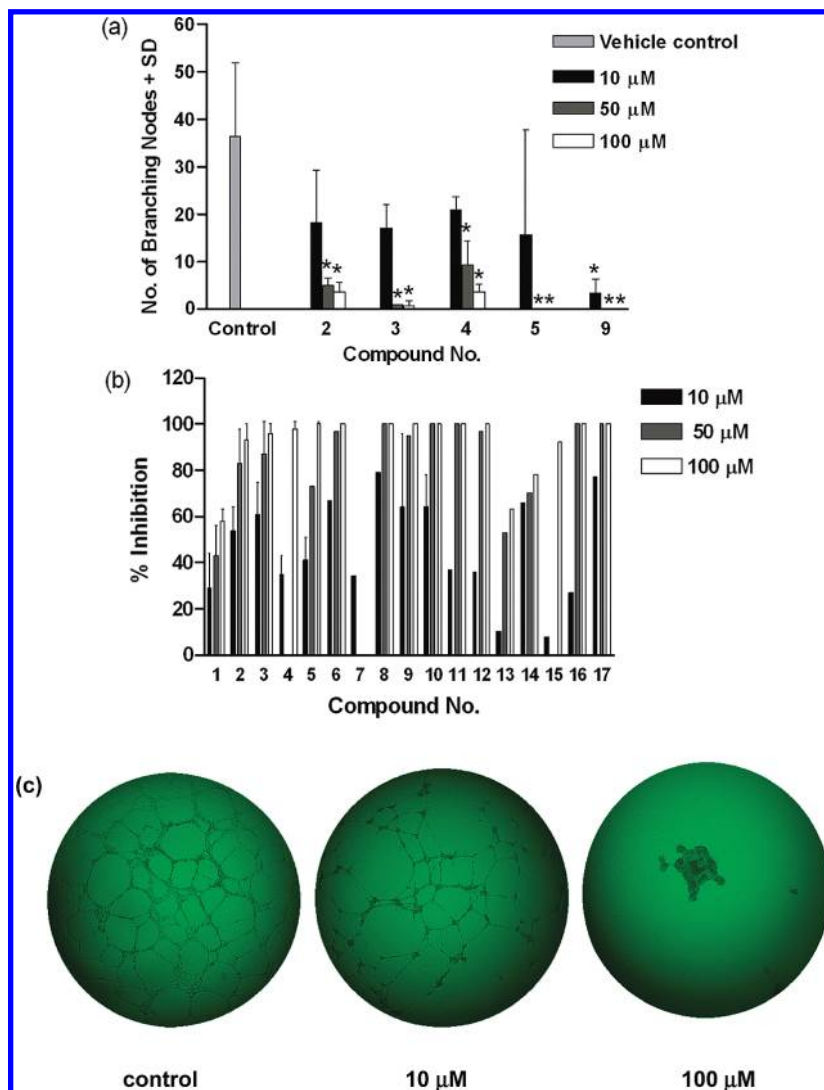
Figure 2. (a) Inhibition of HUVEC tube formation on a Matrigel by selected test compounds at 10, 50, and 100 μM taken from a representative experiment (control $n = 9$, treatment groups $n = 3$). An asterisk indicates $P < 0.05$ vs vehicle control. Statistics were determined using a one-way ANOVA followed by a Dunnett's post hoc test (Graphpad Instat, version 3.05). (b) Percentage inhibition of selected compounds in the tube formation assay. For compounds tested on multiple occasions, standard deviations are shown to indicate interexperimental error. (c) Some representative images of control and compound **5** at 10 and 100 μM.
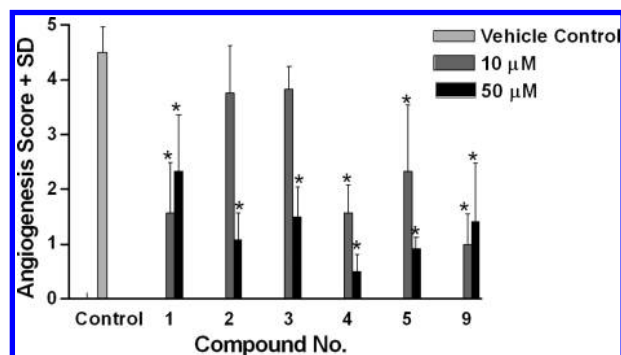

Figure 3. Inhibition of angiogenesis by selected compounds at concentrations of 10 and 50 μM in the rat aortic assay. An asterisk indicates $P < 0.05$ vs vehicle control. Statistics were determined using a one-way ANOVA followed by a Dunnett's post hoc test (Graphpad Instat, version 3.05). The data are representative of an individual angiogenesis experiment with each concentration assessed in triplicate.

treatment with VEGF for up to an additional 7 days. The appearance of microvessel sprouts demonstrated that the compounds exert their inhibitory effects via an antiangiogenic mechanism as opposed to the induction of a toxic effect on the tissue (data not shown).

The anticoagulant activity of selected test compounds was determined by measuring the effect of a solution of a compound at a concentration of 0.1 mg/mL in PBS on the elevation of the activated partial thromboplastin time (APTT) of pooled normal human plasma. The compounds were also evaluated in the heptest, a clot-based assay which measures both anti-Xa and anti-IIa activity but reflects predominantly anti-Xa activity at low concentrations. The data are presented in Table 1 and clearly show that the presence of the aglycone attenuates the anticoagulant activity. The compounds with the smallest aglycones, such as **2** and **3** which have only a benzyl group, still have some significant anticoagulant activity, but in general, the remaining compounds could be described as possessing only modest anticoagulant activity. The APTT data were independently confirmed by testing in another laboratory.

To test the effectiveness of these compounds in a tumor model, the B16 melanoma model was chosen. B16 melanoma
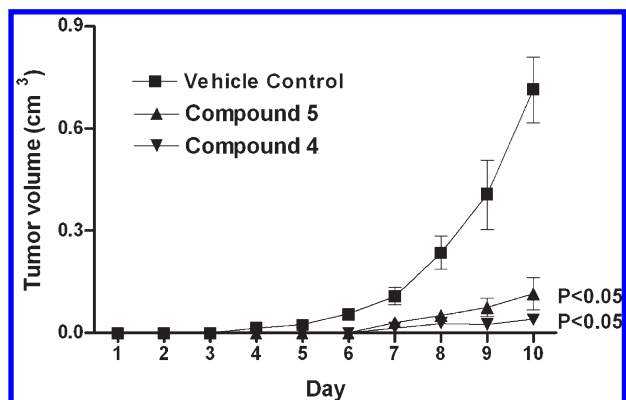
Figure 4. Antitumor activity in the B16 mouse melanoma model. Compounds **4** and **5** were administered for 7 days at 30 mg/kg/day (b.i.d.) to C57 mice ($n = 10$), 3 days after tumor challenge at day 0. Both **4** and **5** significantly reduced the tumor size up to day 10. $P < 0.05$ is considered significant.

is a commonly used cell line for the induction of tumors in syngeneic C57/BL6 mice. B16 melanoma is a nonmetastatic, fast-growing tumor unresponsive to most anticancer agents, including **1**. The tetrasaccharide **5** and its pentasaccharide homologue, the previously prepared **4**, were tested in this model because they exhibited good activity across all the assays and are among the simplest to synthesize in this series. The activities of **4** and **5** in cell-based assays were similar, so the tumor study would be useful for the examination of whether any differences between a tetra- and a pentasaccharide became apparent in vivo. The compounds were administered as a subcutaneous injection in PBS for 7 days at 30 mg/kg/day (b.i.d.) to C57 mice ($n = 10$), 3 days after tumor challenge at day 0. Treatment with both **4** and **5** significantly reduced the tumor size [percentage of tumor growth inhibition (TGI) values of 94 and 86%, respectively] up to day 10 (Figure 4). Both compounds significantly inhibited tumor progression in this model, indicating that HS mimetics have the potential to inhibit tumor growth. The experiments were repeated at a daily dose of 15 mg/kg, i.e., half the previous dose, and both compounds once again significantly inhibited tumor growth, producing TGI values of 42% for compound **4** and 64% for compound **5**. No appreciable differences were noted between the two compounds in this model, indicating that tetra- and pentasaccharides have similar activity in vivo.

The pharmacokinetics of **5** were investigated in male Sprague-Dawley rats following intravenous (iv) and subcutaneous (sc) administration at a nominal dose of 10 mg/kg (Figure 5). Measurable concentrations of **5** were detected in plasma up to 6−8 h postdose, and the apparent half-life was approximately 1.7 h for both routes of administration. The apparent volume of distribution was very low (26 mL/kg) and comparable to the total plasma volume in the rat ($\sim$31 mL/kg),[30] suggesting that **5** was not extensively distributed out of the vasculature into peripheral tissue compartments. The total plasma clearance was low (12 mL h^{-1} kg^{-1}), and renal elimination of intact **5** was not a significant clearance pathway, as **5** was not detected in urine after either sc or iv administration. These data suggest that **5** may have improved pharmacokinetics versus those of **4**, which was previously found to exhibit a slightly shorter half-life (1.1 h) and a higher plasma clearance ($\sim$84 mL h^{-1} kg^{-1}).[18] In addition, **5** was well absorbed following subcutaneous dosing, with a bioavailability of approximately 67%.
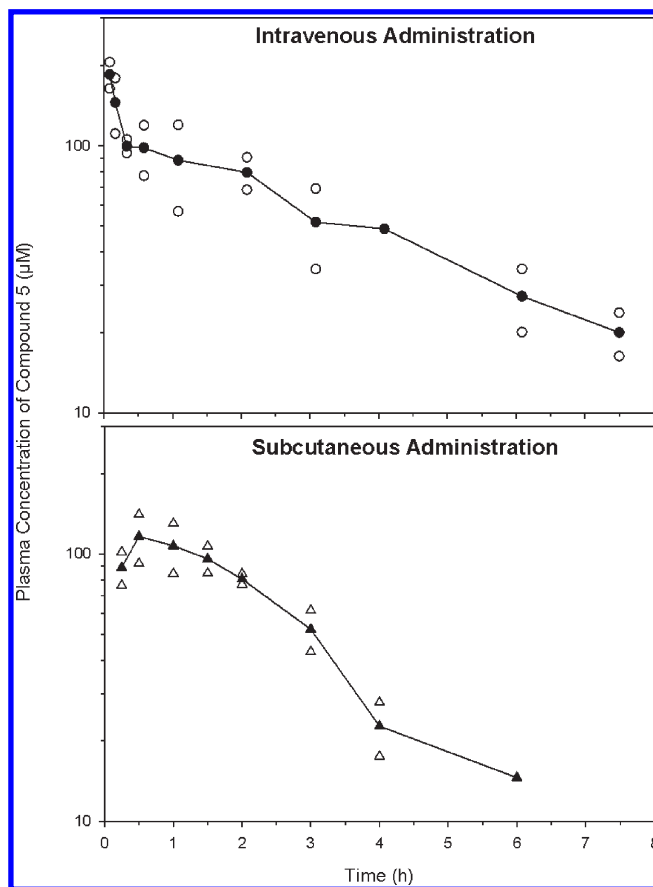

Figure 5. Plasma concentrations of **5** following iv and sc administration to male Sprague-Dawley rats at a nominal dose of 10 mg/kg ($n = 2$ rats per dosing route). Concentrations observed in individual rats are presented (empty symbols) together with the mean for each dosing route (filled symbols).

Conclusions

A series of polysulfated glycosides of $\alpha(1\rightarrow3)/\alpha(1\rightarrow2)$-linked mannopenta- and -tetrasaccharides were readily synthesized in four or five steps from the parent oligosaccharides. These HS mimetics were evaluated for their antiangiogenic activity in a series of in vitro and ex vivo assays. In general, the compounds exhibited superior activity to the phase III HS mimetic **1** in cell-based assays indicative of angiogenesis, and importantly, tetrasaccharides, which are likely to be cheaper to manufacture than pentasaccharides, were shown to have activity similar to that of pentasaccharides. Moreover, compounds **4** and **5** showed potent antitumor activity in vivo in a mouse melanoma model which is resistant to **1**. The lipophilic modifications also resulted in significant attenuation of anticoagulant activity (a common side effect of HS mimetics) and improved in vivo pharmacokinetics, with compound **5** exhibiting a good pharmacokinetic profile in rats. Taken together, the data support the continued development of HS mimetics of this type as antiangiogenic, anticancer agents.

Experimental Section

General Information. Unless otherwise stated, nuclear magnetic resonance (NMR) spectra were recorded at 400 MHz for ^{1}H and 100 MHz for ^{13}C, either in deuteriochloroform (CDCl$_3$) with residual CHCl$_3$ (^{1}H, δ 7.26) or in deuterium oxide (D$_2$O), employing residual HOD (^{1}H, δ 4.78) as the internal standard, at

ambient temperatures (298 K). Where appropriate, analyses of ^{1}H NMR spectra were aided by gCOSY experiments. Flash chromatography was performed on silica gel (40−63 μm) under a positive pressure with specified eluants. All solvents used were analytical grade. The progress of reactions was monitored by thin layer chromatography (TLC) using commercially prepared silica gel 60 F_{254} aluminum-backed plates. Compounds were visualized by charring with 5% sulfuric acid in methanol and/or under ultraviolet light. Analytical HPLC was performed on a Waters Alliance 2690 Separations Module equipped with a Waters 2410 refractive index detector, using a Phenomenex PolySep-GFC-P2000 (300 mm × 7.8 mm) column and 0.1 M $NaNO_3$ as the mobile phase. The flow rate was 0.8 mL/min, and the column temperature was set to 35 °C. Capillary electrophoresis (CE) was performed in reverse polarity mode on an Agilent CE System using 10 mM 5-sulfosalicylic acid (pH 3) as the background electrolyte and detection by indirect UV absorbance at 214 nm, as previously described.[21] Compound homogeneity was determined by ^{1}H and/or ^{13}C NMR spectroscopy and for the final products by CE and/or HPLC. Unless indicated otherwise, the purity of the final products was >95%.

General Procedure for Deacetylation. A solution of the peracetate in anhydrous MeOH (0.1 M) (or MeOH-THF) was treated with a solution of NaOMe in MeOH (1.35 M, 0.2−0.6 equiv). The mixture was stirred at room temperature for 1−3 h (monitored by TLC) and then neutralized with ion-exchange resin AG-50W-X8 (H^{+} form). The mixture was filtered, and the resin was rinsed with MeOH. The combined filtrate and washings were concentrated in vacuo and thoroughly dried to give the polyol product.

General Procedure for Sulfonation. A mixture of the polyol and SO_3·pyridine (or trimethylamine) complex (2 equiv per hydroxyl group) in DMF (0.04 M) was heated (60 °C, overnight). The ice-cooled reaction mixture was treated with MeOH (3 volumes) and then made basic (to pH > 10) by the addition of aqueous Na_2CO_3 (10%, w/w). The mixture was filtered and the filtrate evaporated and coevaporated (H_2O). The crude polysulfated product was dissolved in H_2O and purified by size exclusion chromatography (see below). When required, after lyophilization the product was passed through an ion-exchange resin column (AG-50W-X8, Na^{+} form, 1 cm × 4 cm, deionized H_2O, 15 mL) to transfer the product uniformly into the sodium salt form. The solution collected was evaporated and lyophilized to give the final product.

Size Exclusion Chromatography. Size exclusion chromatography (SEC) was performed on Bio-Gel P-2 in a 5 cm × 100 cm column at a flow rate of 2.8 mL/min with 0.1 M ammonium bicarbonate as the eluant, collecting 2.8 min (7.8 mL) fractions. Fractions were analyzed for carbohydrate content by being spotted onto silica gel plates and visualization by charring, and/or analyzed for polyanionic species by the dimethyl methylene blue (DMB) test.[31] Finally, fractions were checked for purity by CE, and those deemed to be free of salt were pooled and lyophilized.

2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-1,3,4,6-tetra-*O*-acetyl-D-mannopyranose (30). The polyol **28**[23] (2.3 g, 3.45 mmol) was acetylated [excess Ac_2O (9.4 mL, 99.4 mmol, 2.06 equiv per hydroxyl group), pyridine (15 mL), and DMAP (10 mg) at room temperature for 4 days] and purified by flash chromatography (3:2 → 19:1 EtOAc:hexane) to give the peracetate **30** as an oil (4.11 g, 95%): ESMS *m/z* 1277.19 [M + Na][+]; ^{1}H NMR (CDCl₃) δ 6.20 (d, 1 H, $J_{1,2}$ = 1.8 Hz, H-1[1]), 5.35−5.15 (m, 7 H), 5.05−4.92 (m, 5 H), 4.30−3.85 (m, 15 H), 2.18, 2.14, 2.12, 2.10, 2.08, 2.06, 2.03, 2.02, 1.97 (9 s, 42 H, 14 × Ac).

Benzyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranoside (34). A solution of peracetate **30** (150 mg, 120 μmol), benzyl alcohol (38 mg, 360 μmol), and BF₃·Et₂O (34 mg, 240 μmol) in dry DCE

(3.5 mL) was stirred at 60 °C under argon for 5 h. The solution was cooled to room temperature, and Et₃N (250 μL) was added before the mixture was poured into brine and extracted with DCM. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated and the residue purified by flash chromatography (1:1 → 7:3 EtOAc:hexane) to give the glycoside **34** (102 mg, 64%) as a colorless oil: ^{1}H NMR (CDCl₃) δ 7.36−7.28 (m, 5 H, Ph), 5.33−5.14 (m, 7 H), 5.02 (dd, 1 H, J = 3.1, 1.9 Hz), 4.99−4.96 (m, 2 H), 4.95 (d, 1 H, J = 1.5 Hz), 4.94 (d, 1 H, J = 1.6 Hz), 4.89 (d, 1 H, J = 1.5 Hz), 4.70, 4.52 (ABq, J_{AB} = 11.9 Hz, PhC*H₂*), 4.30−3.83 (m, 12 H), 2.17, 2.15, 2.12, 2.11, 2.10, 2.09, 2.08, 2.04, 2.03, 2.02, 1.99, 1.96 (12 s, 39 H, 13 × Ac); ^{13}C NMR (CDCl₃) δ 170.7, 170.6, 170.5, 170.4, 170.2, 170.1, 169.9, 169.7(1), 169.6(9), 169.6, 169.5, 169.3, 136.2, 128.6, 128.3, 127.9, 99.4, 99.0, 98.9, 97.4, 76.7, 75.3, 75.2, 71.0, 70.9, 70.2, 69.7, 69.6(5), 69.5(8), 69.4, 69.2, 68.7, 68.3, 67.1, 66.7, 66.2, 65.6, 62.5, 62.2, 62.0, 61.8, 20.9, 20.8(2), 20.7(9), 20.6(3), 20.6(0), 20.5(7), 20.5.

Benzyl 2,3,4,6-Tetra-*O*-sulfo-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-sulfo-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-sulfo-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-sulfo-α-D-mannopyranoside, Tridecasodium Salt (3). Glycoside **34** (102 mg, 78 μmol) was sequentially deacetylated and sulfonated according to the general procedures and then purified by SEC to give the polysulfate **3** (104 mg, 64%) as a white amorphous solid: ^{1}H NMR (D_2O, solvent suppressed on HOD) δ 7.37−7.24 (m, 5 H, Ph), 5.36 (br s, 1 H), 5.28 (d, 1 H, J = 1.4 Hz), 5.26 (d, 1 H, J = 1.1 Hz), 5.06 (t, 1 H, J = 2.0 Hz), 5.01 (d, 1 H, J = 1.8 Hz), 4.87 (br s, 1 H), 4.69−4.65 (m, 2 H affected by suppression), 4.59−3.68 (m, 22 H affected by suppression); ^{13}C NMR (125 MHz, D_2O) δ 136.6, 129.0, 128.9, 128.6, 99.6, 97.4, 76.7, 76.5, 76.1, 75.0, 74.9, 74.0, 72.8, 72.5, 72.2, 71.4, 70.2, 69.9, 69.4, 69.2, 67.5, 67.4, 66.1, 66.0.

Octyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranoside (35). 1-Octanol (47 mg, 360 μmol, dried over 3 Å sieves) was glycosylated with peracetate **30** (150 mg, 120 μmol) as described above for compound **34** to give, following flash chromatography (2:3 → 3:2 EtOAc:hexane), glycoside **35** (100 mg, 63%) as a colorless oil: ^{1}H NMR (CDCl₃) δ 5.32−5.13 (m, 7 H), 5.01 (dd, 1 H, J = 2.9, 1.9 Hz), 4.99−4.96 (m, 2 H), 4.93 (d, 1 H, J = 1.4 Hz), 4.89 (d, 1 H, J = 1.2 Hz), 4.86 (d, 1 H, J = 1.1 Hz), 4.28−3.95 (m, 12 H), 3.92−3.82 (m, 3 H), 3.63 (dt, 1 H, J = 9.5, 6.8 Hz, OC*H₂*CH₂ A), 3.39 (dt, 1 H, J = 9.4, 6.6 Hz, OC*H₂*CH₂ B), 2.15(3), 2.14(6), 2.11, 2.09, 2.08, 2.06, 2.04, 2.03, 2.00, 1.98, 1.95 (11 s, 39 H, 13 × Ac), 1.60−1.52 (m, 2 H, OCH₂C*H₂*), 1.31−1.21 (m, 10 H), 0.85 (t, 3 H, J = 6.8 Hz, CH₃); ^{13}C NMR (CDCl₃) δ 170.6, 170.5(4), 170.4(5), 170.3, 170.2, 170.1, 170.0, 169.9, 169.7(4), 169.6, 169.5, 169.4, 99.4, 99.0, 98.8, 98.1, 77.0, 75.2, 71.0, 70.8, 70.2, 69.6, 69.5, 69.4, 69.3, 68.5, 68.3, 67.3, 66.7, 66.3, 65.6, 62.5(4), 62.4(7), 62.0, 61.8, 31.7, 29.2(4), 29.2(0), 29.1, 26.0, 22.5, 20.9, 20.7(9), 20.7(6), 20.6(1), 20.5(8), 20.5(5), 20.5, 14.0.

Octyl 2,3,4,6-Tetra-*O*-sulfo-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-sulfo-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-sulfo-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-sulfo-α-D-mannopyranoside, Tridecasodium Salt (5). Glycoside **35** (97 mg, 73 μmol) was sequentially deacetylated and sulfonated according to the general procedures and then purified by SEC to give polysulfate **5** (100 mg, 65%) as a white amorphous solid: ^{1}H NMR (D_2O, solvent suppressed on HOD) δ 5.38 (br s, 1 H), 5.28 (m, 2 H), 5.07 (br t, 1 H, J = 2.3 Hz), 4.91 (d, 1 H, J = 1.7 Hz), 4.88 (br s, 1 H), 4.70 (br s, 1 H), 4.66 (dd, ^{1}H affected by suppression, J = 8.0, 2.7 Hz), 4.58−4.19 (m, ~16 H affected by suppression), 4.04−3.95 (m, 4 H), 3.62 (dt, 1 H, J = 9.4, 6.9 Hz, OCH₂ A), 3.48 (dt, 1 H, J = 9.5, 6.1 Hz, OCH₂ B), 1.53−1.42 (m, 2 H), 1.25−1.10 (m, 10 H), 0.71 (t, J = 6.7 Hz, CH₃); ^{13}C NMR (125 MHz, D_2O) δ 99.6, 98.0, 76.8, 76.5, 76.1, 75.0, 74.9, 73.9, 72.8, 72.6, 72.2, 71.4, 70.0, 69.9, 69.4, 69.2, 68.7, 67.6, 66.1, 65.9, 31.4, 25.6, 28.4(3), 28.4(1), 25.4, 22.0, 13.5.

2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranosyl Trichloroacetimidate (32). Peracetate **30** (500 mg, 398 μmol) in

diethyl ether (3.0 mL) and THF (750 μL) was treated with benzylamine (0.137 g, 1.3 mmol, 139 μL) at 0 °C. The mixture was allowed to warm slowly to room temperature and react overnight. The solvent was evaporated and the residue dissolved in DCM and washed with cold 0.5 M HCl (three times), followed by brine, and the organic solution was dried (Na_2SO_4), filtered, and evaporated. The residue was dissolved in dry DCM (10 mL), and molecular sieves (3 Å, 30 mg), anhydrous cesium carbonate (12.9 mg, 39.8 μmol), and potassium carbonate (110 mg, 796 μmol) were added. The mixture was stirred at 0 °C before trichloroacetonitrile (115 mg, 80 μL, 796 μmol) was added. The mixture was stirred for 5 h at room temperature until complete conversion by TLC. The mixture was filtered, and the solvent was evaporated to give the crude product which was subjected to flash chromatography (1:6 → 3:1 EtOAc:hexane) to give the trichloroacetimidate **32** (307.5 mg, 57%) as a clear oil which solidified on standing in the refrigerator. Note that the product contains benzylacetamide which is best removed with an EtOAc wash after polyol formation: ^{1}H NMR (CDCl$_3$) δ 6.40 (d, 0.7 H, $J_{1,2}$ = 1.5 Hz, H-1[1]α), 6.22 (d, 0.3 H, $J_{1,2}$ = 1.5 Hz, H-1[1]β), 5.40−5.14 (m, 7 H), 5.05−4.89 (m, 5 H), 4.31−3.84 (m, 15 H), 2.19−1.98 (m, 39 H, Ac).

4-Butylbenzyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranoside (36). To a solution of trichloroacetimidate **32** (200 mg, 147 μmol) in DCM (5 mL) was added 4-butylbenzylalcohol (48.3 mg, 50.2 μL, 294 μmol). The mixture was stirred at −20 °C for 20 min. TMSOTf (10 μL, 56 μmol) was added. The mixture was stirred for a further 20 min at −20 °C. Triethylamine (17 μL, 122 μmol) was added, before the mixture was warmed to room temperature and filtered. The solution was evaporated and the crude product purified by flash chromatography (1:4 → 5:1 EtOAc:hexane) to give glycoside **36** (106 mg, 53%) as a clear oil containing traces of benzyl acetamide: ^{1}H NMR (CDCl$_3$) δ 7.20 (2 H, d, Ar), 7.14 (2 H, d, Ar), 5.32−5.14 (7 H, m), 5.02−4.87 (6 H, m), 4.65, 4.46 (2 H, AB q, PhCH_2), 4.28−3.83 (15 H, m), 2.58 (2 H, t, CH$_2$), 2.16, 2.15, 2.12, 2.10, 2.09, 2.08 (×2), 2.03, 2.01 (×2), 2.00(6), 1.98, 1.95 (39 H, 11s, OAc), 1.56 (2 H, m, CH$_2$), 1.32 (2 H, m, CH$_2$), 0.89 (3 H, t, CH$_3$).

4-Butylbenzyl 2,3,4,6-Tetra-*O*-sulfo-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-sulfo-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-sulfo-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-sulfo-α-D-mannopyranoside, Tridecasodium Salt (6). Glycoside **36** (106 mg, 78.3 μmol) was sequentially deacetylated and sulfonated according to the general procedures and then purified by SEC to give polysulfate **6** (64%) as an amorphous solid: ^{1}H NMR (D$_2$O) δ 7.30 (2 H, d, Ar), 7.21 (2 H, d, Ar), 5.39 (1 H, m), 5.33 (2 H, m), 5.13 (1 H, m), 5.06 (1 H, m), 4.91 (1 H, m), 4.73−3.93 (22 H, m), 2.53 (2 H, t, CH$_2$), 1.48 (2 H, m, CH$_2$), 1.20 (2 H, m, CH$_2$), 0.78 (3 H, t, CH$_3$).

Dodecyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranoside (37). 1-Dodecanol (0.849 mmol, 3 equiv) was glycosylated with trichloroacetimidate **33**[18] (0.469 g, 0.285 mmol) as described for compound **36** to give, following flash chromatography (99:1 → 98:2 CHCl$_3$:MeOH), glycoside **37** as a colorless gum. Two fractions were obtained each containing the byproduct BnNHAc (76 mg, 5:3 **37**:BnNHAc ratio; 179 mg, 5:11 **37**:BnNHAc ratio): ^{1}H NMR (CDCl$_3$) δ 5.30−5.16 (m, 8 H), 4.99−4.87 (m, 8 H), 4.31−3.77 (m, 19 H), 3.68−3.61 (m, 1 H, OCH$_2$), 3.44−3.36 (m, 1 H, OCH$_2$), 2.18, 2.17, 2.15, 2.11, 2.10, 2.09, 2.07, 2.06, 2.05, 2.02, 2.01, 1.97, 1.95 (13 s, 48 H, 16 × Ac), 1.58 (quintet, 2 H, J = 6.7 Hz, CH$_2$), 1.33−1.22 (m, 18 H, 9 × CH$_2$), 0.86 (t, 3 H, J = 6.7 Hz, CH$_3$).

Dodecyl 2,3,4,6-Tetra-*O*-sulfo-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-sulfo-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-sulfo-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-sulfo-α-D-mannopyranoside, Hexadecasodium Salt (7). Glycoside **37** (72 mg, 0.043 mmol) was sequentially deacetylated

and sulfonated according to the general procedures and then purified by SEC to give the polysulfate **7** (64%) as a white powder (77 mg): ^{1}H NMR (D$_2$O, internal DOH at 4.60 ppm) δ 5.19 (s, 1 H), 5.15 (d, 1 H, J = 1.9 Hz), 5.10 (d, 1 H, J = 1.9 Hz), 5.07 (d, 1 H, J = 1.9 Hz), 4.89 (m, 1 H), 3.77−3.64 (m, 30 H), 3.48−3.41 (m, 1 H, OCH$_2$), 3.33−3.27 (m, 1 H, OCH$_2$), 1.30 (m, 2 H, CH$_2$), 1.10−0.90 (m, 18 H, 9 × CH$_2$), 0.54 (t, 3 H, J = 6.7 Hz, CH$_3$).

8-Azidooctyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranoside (38). 8-Azidooctanol[32] (171 mg, 1.2 mmol, 3 equiv) was glycosylated with peracetate **30** (500 mg, 0.398 mmol) as described for compound **34** to give, following flash chromatography (1:4 → 3:1 EtOAc:hexane), glycoside **38** (331 mg, 61%) as a glass: ^{1}H NMR (CDCl$_3$) δ 5.34−5.16 (7 H, m), 5.03−4.87 (6 H, m), 4.30−3.84 (15 H, m), 3.64 (1 H, m, CH$_2$), 3.39 (1 H, m, CH$_2$), 3.24 (2 H, t, CH$_2$N$_3$) 2.17, 2.16, 2.13, 2.11 (×2), 2.10 (×2), 2.07, 2.06, 2.05, 2.02, 2.00, 1.96 (39 H, 11s, OAc), 1.60−1.56 (4 H, m, CH$_2$), 1.39−1.29 (8 H, m, CH$_2$).

8-(4-Phenyl[1,2,3]triazol-1-yl)octyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranoside (19). Glycoside **38** (74.5 mg, 54.5 μmol), phenylacetylene (16.7 mg, 164 μmol), *tert*-butanol (600 μL), CuSO$_4$ (20 μL of a 0.3 M solution), and sodium ascorbate (25 μL of a 1 M solution) were shaken at room temperature overnight. The solvent was evaporated and the crude mixture subjected to flash chromatography (1:4 → 3:1 EtOAc:hexane) to give the phenyltriazole **19** as a glass (34.4 mg, 43%): R_f = 0.36 (3:1 EtOAc:hexane); ^{1}H NMR (CDCl$_3$) δ 7.82 (2 H, d, Ph-H), 7.76 (1 H, s, triazole-H), 7.42 (2 H, t, Ph-H), 7.32 (1 H, t, Ph-H), 5.35−5.14 (7 H, m), 5.04−4.88 (6 H, m), 4.39 (2 H, t, CH$_2$N), 4.30−3.86 (15 H, m), 3.65 (1 H, m, CH$_2$), 3.39 (1 H, m, CH$_2$), 2.18, 2.17, 2.14, 2.11(9), 2.11(5), 2.10(6), 2.10(3), 2.07, 2.06 (×2), 2.03, 2.00, 1.97 (39 H, 12s, OAc), 1.95 (2 H, m, CH$_2$), 1.57 (2 H, m, CH$_2$), 1.38−1.30 (8 H, m, CH$_2$).

8-(4-Phenyl[1,2,3]triazol-1-yl)octyl 2,3,4,6-Tetra-*O*-sulfo-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-sulfo-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-sulfo-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-sulfo-α-D-mannopyranoside, Tridecasodium Salt (9). Phenyltriazole **19** (34.4 mg, 23.4 μmol) was sequentially deacetylated and sulfonated according to the general procedures and then purified by SEC to give the polysulfate **9** (43.7 mg, 85%) as a white powder: ^{1}H NMR (D$_2$O) δ 8.30 (1 H, s, triazole-H), 7.70 (2 H, m, phenyl-H), 7.45−7.34 (3 H, m, phenyl-H), 5.39 (1 H, m), 5.32 (2 H, m), 5.12 (1 H, m), 4.89 (1 H, m), 4.74−3.95 (22 H, m), 4.38 (2 H, m, CH$_2$N), 3.59 (1 H, m, CH$_2$O), 3.44 (1 H, m, CH$_2$O), 1.84 (2 H, m, CH$_2$), 1.45 (2 H, t, CH$_2$), 1.19 (8 H, m); ^{13}C NMR (125 MHz, D$_2$O) δ 147.4, 129.7, 129.3, 128.8, 125.7, 122.4, 99.6, 98.8, 97.9, 76.7, 76.5, 76.1, 75.0, 74.9, 74.0, 72.8, 72.6, 72.2, 71.4, 70.0, 69.4, 69.2, 68.6, 67.6, 66.1, 66.0, 50.8, 29.1, 28.3, 27.9, 27.7, 25.3, 25.1.

8-Azidooctyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranoside (39). 8-Azido-1-octanol (51 mg, 0.356 mmol) was glycosylated with peracetate **31**[18] (200 mg, 0.13 mmol) as described for compound **34** to give, after flash chromatography (1:2 → 3:1 EtOAc:hexane), glycoside **39** (103 mg, 48%) as a glass: ^{1}H NMR (CDCl$_3$) δ 5.28−5.14 (8 H, m), 5.02−4.86 (8 H, m), 4.27−3.75 (19 H, m), 3.63 (1 H, m, CH$_2$), 3.38 (1 H, m, CH$_2$), 3.23 (2 H, t, CH$_2$N$_3$), 2.15, 2.14(5), 2.14(3), 2.11, 2.10, 2.09, 2.08(8), 2.08(3), 2.07(9), 2.07(0), 2.06, 2.04, 2.03(5), 2.00, 1.99, 1.95 (48 H, 16s, OAc), 1.60−1.54 (4 H, m, CH$_2$), 1.35−1.28 (8 H, m, CH$_2$); ^{13}C NMR (150 MHz, CDCl$_3$) δ 170.6(4), 170.6(2), 170.6(1), 170.5, 170.4, 170.3, 170.2, 170.1, 170.0(2), 169.9(8), 169.8, 169.7, 169.6, 169.5, 169.4, 99.3, 99.0, 98.9, 98.2, 77.2, 75.2, 75.1, 75.0, 71.1, 71.0, 70.9, 70.3, 69.7, 69.6, 69.5, 69.4, 69.3, 68.4(5), 68.3(8), 68.3(5), 67.3, 66.8, 66.7, 66.3, 65.6, 62.6, 62.5, 62.0, 61.7, 51.4, 29.6, 29.2, 29.1, 29.0, 28.7,

26.6, 26.0, 20.9(2), 20.8(9), 20.8(7), 20.8, 20.7, 20.6(2), 20.5(8), 20.5.

8-(4-Phenyl[1,2,3]triazol-1-yl)octyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranoside (18). Azide **39** (25 mg, 0.015 mmol) was reacted with phenylacetylene (5 mg, 0.045 mmol) as described for compound **19** to give, after flash chromatography (2:1 EtOAc:hexane, 1:2 → 4:1), phenyltriazole **18** (20 mg, 75%) as a glass: R_f = 0.30 (3:1 EtOAc:hexane); ^{1}H NMR (CDCl$_3$) δ 7.82 (2 H, d, Ph-H), 7.74 (1 H, s, triazole-H), 7.41 (2 H, t, Ph-H), 7.31 (1 H, t, Ph-H), 5.30−5.15 (8 H, m), 5.02−4.87 (8 H, m), 4.39 (2 H, t, CH$_2$N), 4.29−3.76 (19 H, m), 3.63 (1 H, m, CH$_2$), 3.39 (1 H, m, CH$_2$), 2.17, 2.16, 2.15(6), 2.13, 2.11, 2.10(6), 2.10(3), 2.09(5) (×2), 2.08, 2.06, 2.05, 2.03, 2.02, 2.00, 1.96 (48 H, 15s, OAc), 1.94 (2 H, m, CH$_2$), 1.57 (2 H, m, CH$_2$), 1.35−1.30 (8 H, m, CH$_2$).

8-(4-Phenyl[1,2,3]triazol-1-yl)octyl 2,3,4,6-Tetra-*O*-sulfo-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-sulfo-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-sulfo-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-sulfo-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-sulfo-α-D-mannopyranoside, Hexadecasodium Salt (8). Phenyltriazole **18** (57.5 mg, 0.033 mmol) was sequentially deacetylated and sulfonated according to the general procedures and then purified by SEC to give polysulfate **8** (58.6 mg, 66%) as a white powder: 93% pure by CE; ^{1}H NMR (D$_2$O) δ 8.43 (s, =CH), 7.90−7.88 (m, 2 H, Ph), 7.62−7.57, 7.32−7.49 (2m, 3 H, Ph), 5.55 (s, 2 H), 5.50 (s, 2 H), 5.29 (s, 1 H), 5.08 (s, 2 H), 5.06 (s, 1 H), 4.91−4.35 (m, 25 H), 4.25−4.10 (m, 4 H), 3.76, 3.61 (2m, OC*H*$_2$), 2.01 (m, 2 H, CH$_2$), 1.61 (m, 2 H, CH$_2$), 1.36 (m, 8 H, CH$_2$).

8-(4-Naphthalen-1-yl[1,2,3]triazol-1-yl)octyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranoside (20). Azide **39** (27 mg, 0.016 mmol), 1-ethynylnaphthalene (8 mg, 0.05 mmol), *tert*-butanol (200 μL), CuSO$_4$ (5 μL of a 0.3 M solution), and sodium ascorbate (6.25 μL of a 1 M solution) was shaken at room temperature overnight. The solvent was evaporated and the crude mixture subjected to flash chromatography (1:1 → 4:1 EtOAc:hexane) to give naphthyltriazole **20** (18 mg, 64%) as a glass: R_f = 0.20 (3:1 EtOAc:hexane); ^{1}H NMR (CDCl$_3$) δ 8.35 (1 H, m, naphth-H), 7.87−7.90 (2 H, m, naphth-H), 7.82 (1 H, s, triazole-H), 7.73 (1 H, d, naphth-H), 7.54−7.50 (3 H, m, naphth-H), 5.30−5.15 (8 H, m), 5.02−4.87 (8 H, m), 4.48 (2 H, t, CH$_2$N), 4.29−3.76 (19 H, m), 3.65 (1 H, m, CH$_2$), 3.39 (1 H, m, CH$_2$), 2.17, 2.16, 2.15, 2.13, 2.11, 2.10(6), 2.10(3), 2.09(5) (×2), 2.08, 2.06, 2.05, 2.04, 2.02, 1.99, 1.96 (48 H, 15s, OAc), 2.03 (2 H, m, CH$_2$), 1.61 (2 H, m, CH$_2$), 1.39 (2 H, m, CH$_2$), 1.37−1.29 (6 H, m, CH$_2$).

8-(4-Naphthalen-1-yl[1,2,3]triazol-1-yl)octyl 2,3,4,6-Tetra-*O*-sulfo-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-sulfo-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-sulfo-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-sulfo-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-sulfo-α-D-mannopyranoside (10). Naphthyltriazole **20** (22.6 mg, 0.0125 mmol) was sequentially deacetylated and sulfonated according to the general procedures and then purified by SEC to give polysulfate **10** (86.9 mg, 80%) as an off-white powder: ^{1}H NMR (D$_2$O) δ 8.24 (1 H, s, triazole-H), 8.02 (1 H, m, naphth-H), 7.93 (2 H, m, naphth-H), 7.73 (1 H, d, naphth-H), 7.55−7.48 (3 H, m, naphth-H), 5.37 (2 H, m), 5.32 (2 H, m), 5.12 (1 H, m), 4.91 (2 H, m), 4.82 (1 H, m), 4.72−3.89 (27 H, m), 4.42 (2 H, m, CH$_2$N), 3.53 (1 H, m, CH$_2$O), 3.38 (1 H, m, CH$_2$O), 1.88 (2 H, m, CH$_2$), 1.42 (2 H, t, CH$_2$), 1.19 (8 H, m).

8-(4-Naphthalen-1-yl[1,2,3]triazol-1-yl)octyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranoside (21). Azide **38** (76.4 mg, 55.9 μmol) was reacted with 1-ethynylnaphthalene (25.5 mg, 168 μmol) as described for compound **20** for 3 days (extra 25 μL portions of sodium ascorbate were added each day) to give, after

flash chromatography (1:1 → 3:1 EtOAc:hexane), naphthyltriazole **21** (42.5 mg, 50%) as a glass: R_f = 0.37 (3:1 EtOAc:hexane); ^{1}H NMR (CDCl$_3$) δ 8.35 (1 H, m, naphth-H), 7.90−7.87 (2 H, m, naphth-H), 7.82 (1 H, s, triazole-H), 7.73 (1 H, d, naphth-H), 7.54−7.50 (3 H, m, naphth-H), 5.30−5.15 (7 H, m), 5.04−4.88 (6 H, m), 4.48 (2 H, t, CH$_2$N), 4.31−3.85 (15 H, m), 3.65 (1 H, m, CH$_2$), 3.40 (1 H, m, CH$_2$), 2.18, 2.17, 2.13, 2.12, 2.11, 2.10(4), 2.10(2), 2.07, 2.06, 2.05, 2.03, 2.00, 1.97 (39 H, 13s, OAc), 2.03 (2 H, m, CH$_2$), 1.59 (2 H, m, CH$_2$), 1.39 (2 H, m, CH$_2$), 1.36−1.30 (6 H, m, CH$_2$).

8-(4-Naphthalen-1-yl[1,2,3]triazol-1-yl)octyl 2,3,4,6-Tetra-*O*-sulfo-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-sulfo-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-sulfo-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-sulfo-α-D-mannopyranoside, Tridecasodium Salt (11). Naphthyltriazole **30** (42.5 mg, 28 μmol) was sequentially deacetylated and sulfonated according to the general procedures and then purified by SEC to give polysulfate **11** (48.8 mg, 83%) as a white powder: ^{1}H NMR (D$_2$O) δ 8.20 (1 H, s, triazole-H), 7.93 (3 H, m, naphth-H), 7.57−7.48 (4 H, m, naphth-H), 5.39 (1 H, m), 5.34 (2 H, m), 5.13 (1 H, m), 4.91 (1 H, m), 4.85 (1 H, m), 4.74−3.93 (22 H, m), 4.42 (2 H, m, CH$_2$N), 3.52 (1 H, m, CH$_2$O), 3.40 (1 H, m, CH$_2$O), 1.85 (2 H, m, CH$_2$), 1.41 (2 H, t, CH$_2$), 1.18 (8 H, m).

8-(5-Dimethylaminonaphthalene-1-sulfonamido)octyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranoside (22). (a) Azide **38** (161 mg, 118 μmol) and triphenylphosphine (92.7 mg, 353 μmol, 3 equiv) were dissolved in THF (3 mL). Water (63.7 mg, 64 μL, 3.54 mmol, 30 equiv) was added and the mixture stirred at 50 °C for 2 h. The solvent was evaporated to give the crude amine which was used without further purification or characterization. (b) The product from part a (70 mg, 52.2 μmol) was dissolved in DMF (1.3 mL). Dansyl chloride (42.2 mg, 156.6 μmol) was added, followed by triethylamine (11 μL). The solution was stirred for 3 h at room temperature. The solvent was evaporated and the crude product purified by flash chromatography (1:2 → 3:1 EtOAc:hexane) to give sulfonamide **22** (24.3 mg, 25%) as a pale yellow oil: ^{1}H NMR (CDCl$_3$) [first set of rotamer aromatic peaks (integration of 0.4 per proton)] δ 8.61 (1 H, m-broad, Ar), 8.32 (1 H, m-broad, Ar), 7.58−7.51 (2 H, m-broad, Ar), 7.45 (1 H, m-broad, Ar), 7.24 (1 H, m-broad, Ar); ^{1}H NMR (CDCl$_3$) [second set of rotamer aromatic peaks (integration of 0.6 per proton)] δ 8.23 (1 H, d, Ar), 7.66 (1 H, m, Ar), 7.64 (1 H, m, Ar), 7.58−7.51 (2 H, m, Ar), 7.44 (1 H, m, Ar); ^{1}H NMR (CDCl$_3$) (nonaromatic peaks) δ 5.34−5.15 (7 H, m), 5.03−4.86 (6 H, m), 4.79 (1 H, t, NH), 4.30−3.85 (15 H, m), 3.64 (1 H, m, CH$_2$), 3.37 (1 H, m, CH$_2$), 2.93 [6 H, s, (CH$_3$)$_2$N], 2.86 (2 H, q, C*H*$_2$NH) 2.17, 2.16, 2.13, 2.11 (×2), 2.10 (×2), 2.07, 2.05, 2.04(7), 2.02, 2.00, 1.96 (39 H, 11s, OAc), 1.52 (2 H, m, CH$_2$), 1.37 (2 H, m, CH$_2$), 1.24 (2 H, m, CH$_2$), 1.20−1.08 (6 H, m, CH$_2$).

8-(5-Dimethylaminonaphthalene-1-sulfonamido)octyl 2,3,4,6-Tetra-*O*-sulfo-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-sulfo-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-sulfo-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-sulfo-α-D-mannopyranoside, Tridecasodium Salt (12). Sulfonamide **22** (24.3 mg, 15.4 μmol) was sequentially deacetylated and sulfonated according to the general procedures and then purified by SEC to give polysulfate **12** (29.4 mg, 82%) as an off-white powder: ^{1}H NMR (D$_2$O) δ 8.71 (1 H, d, naphth-H), 8.33 (2 H, m, naphth-H), 8.05 (1 H, d, naphth-H), 7.85 (2 H, m, naphth-H), 5.36−5.29 (3 H, m), 5.12 (1 H, m), 4.88 (2 H, m), 4.74−3.90 (22 H, m), 3.50 (1 H, m, CH$_2$O), 3.45 [6 H, s, (CH$_3$)$_2$N], 3.34 (1 H, m, CH$_2$O), 2.87 (2 H, m, CH$_2$N), 1.22 (2 H, m, CH$_2$), 0.97 (2 H, t, CH$_2$), 0.81 (2 H, m), 0.66−0.40 (6 H, m).

3-Azidopropyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranoside (41). 3-Azidopropanol[33,34] (98 mg, 972 μmol) was glycosylated with peracetate **31** (500 mg, 324 μmol) as described for compound **34** to give, following flash chromatography (3:2 → 3:1 EtOAc:

hexane), glycoside **41** (387 mg, 75%) as an oil: ESMS *m/z* 1601.81 [M + NH$_4$]$^+$; ^{1}H NMR (CDCl$_3$) δ 5.30−5.13 (m, 8 H), 5.02−4.88 (m, 8 H), 4.28−3.75 (m, 20 H), 3.49 (dt, 1 H, *J* = 9.8, 6.1 Hz, OC*H*$_2$CH$_2$B), 3.42−3.35 (m, 2 H, CH$_2$N$_3$), 2.16, 2.14(9), 2.14(7), 2.11, 2.10, 2.09(2), 2.08(8), 2.08(7), 2.08, 2.07, 2.06, 2.05, 2.04, 2.00, 1.99, 1.95 (16s, 16 × 3 H, 16 × AcO), 1.89−1.84 (m, 2 H, CC*H*$_2$C); ^{13}C NMR (CDCl$_3$) δ 170.4, 170.3, 170.2, 170.1, 169.9, 169.8(2), 169.7(7), 169.6, 169.5, 169.4, 169.3, 169.2, 99.1(2), 99.1(0), 98.8(4), 98.7(7), 98.1, 76.7, 75.0, 74.9, 74.7, 71.0, 70.8, 70.7, 70.0, 69.5, 69.3, 69.2, 68.5, 68.2, 67.2, 66.7, 66.6, 66.0, 65.4, 64.6, 62.4, 62.3, 61.9, 61.5, 47.9, 28.5, 20.7(4), 20.7(2), 20.6(9), 20.6, 20.4(9), 20.4(6), 20.4.

3-(4-Phenyl[1,2,3]triazol-1-yl)propyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranoside (23). Azide **41** (59 mg, 37 μmol) was reacted with phenylacetylene (13.5 mg, 132 μmol) as described for compound **19** to give, after flash chromatography (4:1 → 1:0 EtOAc:hexane), phenyltriazole **23** (47 mg, 75%) as an oil: ESMS *m/z* 1686.94 [M + H]$^+$; ^{1}H NMR (CDCl$_3$) δ 7.83−7.78 (m, 3 H, triazole-H + ArH), 7.43−7.30 (m, 3 H, ArH), 5.31−5.14 (m, 8 H), 5.03−4.90 (m, 8 H), 4.52 (t, 2 H, *J* = 6.6 Hz, triazole-C*H*$_2$), 4.30−3.73 (m, 19 H), 3.46 (dt, 1 H, *J* = 10.1, 5.8 Hz, OC*H*$_2$B), 2.30−2.24 (m, 2H, CH$_2$C*H*$_2$CH$_2$), 2.18, 2.17, 2.16, 2.13, 2.12, 2.11(1), 2.10(8), 2.10(2), 2.10(0), 2.09, 2.06, 2.03, 2.02(3), 2.01(8), 2.00, 1.97 (16s, 16 × 3 H, 16 × Ac).

3-(4-Phenyl[1,2,3]triazol-1-yl)propyl 2,3,4,6-Tetra-*O*-sulfo-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-sulfo-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-sulfo-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-sulfo-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranoside, Hexadecasodium Salt (13). Phenyltriazole **23** (45 mg, 27 μmol) was sequentially deacetylated (ESMS of polyol *m/z* 1014.59 [M + H]$^+$) and sulfonated according to the general procedures and then purified by SEC to give polysulfate **13** (31 mg, 43%) as a white powder: ^{1}H NMR (D$_2$O) δ 8.25−8.19 (m, 1 H), 7.72−7.67 (m, 2 H), 7.41−7.34 (m, 2 H), 7.32−7.27 (m, 1 H), 5.43−4.64 (m, 7 H), 4.55−3.59 (m, 23 H), 3.55−3.49 (m, 1 H), 2.17−2.10 (br s, 2 H).

3-Azidopropyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranoside (40). 3-Azidopropanol (67 mg, 660 μmol) was glycosylated with peracetate **30** (276 mg, 220 μmol) as described for compound **34** to give, following flash chromatography (3:2 → 4:1 EtOAc:hexane), glycoside **40** (176 mg, 61%) as an oil: ESMS *m/z* 1319.69 ([M + Na]$^+$); ^{1}H NMR (CDCl$_3$) δ 5.31−5.12 (m, 7 H), 5.00−4.87 (m, 6 H), 4.26−3.94 (m, 11 H), 3.90−3.81 (m, 2 H), 3.77 (dt, 1 H, *J* = 9.9, 6.0 Hz), 3.47 (dt, 1 H, *J* = 9.9, 6.0 Hz), 3.42−3.32 (m, 2 H), 2.14(1), 2.13(5), 2.10, 2.08 (×2), 2.06 (×2), 2.05, 2.03(2), 2.02(5), 1.99, 1.98, 1.93 (13s, 13 × 3 H, 13 × OAc), 1.84 (pentet, 1 H, *J* = 6.2 Hz); ^{13}C NMR (CDCl$_3$) δ 170.6, 170.5, 170.4, 170.3, 170.1(4), 170.0(9), 169.9(2), 169.8(8), 169.7, 169.6, 169.5(4), 169.4(5), 169.3, 99.4, 98.9, 98.8, 98.1, 76.8, 75.1(5), 75.1(1), 70.9, 70.8, 70.1, 69.6, 69.5, 69.4, 69.2, 68.5, 68.3, 67.3, 66.6, 66.1, 65.5, 64.7, 62.5, 61.9, 61.7, 48.0, 28.5, 20.8(4), 20.7(6), 20.7, 20.6, 20.5(4), 20.5(2), 20.4(9), 20.4(7).

3-(4-Phenyl[1,2,3]triazol-1-yl)propyl 2,3,4,6-Tetra-*O*-sulfo-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-sulfo-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-sulfo-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-sulfo-α-D-mannopyranoside, Tridecasodium Salt (14). Azide **40** (174 mg, 134 μmol) was reacted with phenylacetylene as described for compound **19** to give, following flash chromatography (4:1 → 17:3 EtOAc:hexane), the phenyltriazole (109 mg, 58%) as an oil, used directly in the next step without further characterization. This material (109 mg, 78 μmol) was sequentially deacetylated and sulfonated according to the general procedures and then purified by dialysis (Pierce Slide-A-Lyzer 2000 MW cutoff cartridge, 10 × 2 L HPLC grade water, pH adjusted to 8 with 3 M NaHCO$_3$) to give polysulfate **14** (10.5 mg,

6%) as an off-white solid: 95% pure by CE; ^{1}H NMR (D$_2$O) δ 8.22−8.26 (1 H, m), 7.69−7.71 (2 H, d), 7.29−7.40 (3 H, m), 3.52−5.33 (30 H, m), 2.14−2.17 (2 H, m).

3-(5-Dimethylaminonaphthalene-1-sulfonamido)propyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranoside (25). (a) A mixture of polymer-bound triphenylphosphine (124 mg, 194 μmol, Fluka catalog no. 93094) and azide **41** (105 mg, 66 μmol) in anhydrous THF (2 mL) was stirred for 2 h; then water (36 μL, 2.0 mmol) was added, and the mixture was stirred at 50 °C for 4 h. The mixture was cooled to room temperature, filtered, and evaporated in vacuo to give the amine, used without further purification: APCI-MS *m/z* 1558.25 [M + H]$^+$. (b) A solution of the amine (47 mg, 30.2 μmol), dansyl chloride (16.3 mg, 60.3 μmol), Et$_3$N (15.3 mg, 21 μL, 150 μmol), and DMAP (12 μL of a 135 mM solution in CHCl$_3$, 1.5 μmol) in CHCl$_3$ (900 μL) was stirred for 4 h. Phenomenex StrataNH$_2$ medium (30 mg) was added, and the mixture was stirred for 30 min then applied directly to a prepared flash chromatography column (4:1 → 9:1 EtOAc:hexane) to give sulfonamide **25** (18 mg, 30%) as an oil: ^{1}H NMR (CDCl$_3$) δ 8.68 (br s, 1 H), 8.36 (br s, 1 H), 8.23 (dd, 1 H, *J* = 7.3, 1.1 Hz), 7.61−7.53 (m, 2 H), 7.29 (br s, 1 H), 5.31−5.15 (m, 8 H), 5.03 (dd, 1 H, *J* = 3.2, 1.9 Hz), 5.00−4.93 (m, 5 H), 4.91 (d, 1 H, *J* = 1.6 Hz), 4.88 (d, 1 H, *J* = 1.7 Hz), 4.30−3.87 (m, 19 H), 3.80 (ddd, 1 H, *J* = 2.3, 4.7, 10.1 Hz), 3.74 (dt, 1 H, *J* = 10.0, 5.5 Hz), 3.48 (dt, 1 H, *J* = 10.0, 5.8 Hz), 3.00−2.97 (br s, 8 H), 2.18, 2.17(1), 2.16(6), 2.14, 2.12(1), 2.11(7), 2.11(2), 2.11(1), 2.10(5), 2.10, 2.09, 2.06, 2.05, 2.04, 2.03, 2.01, 1.97 (17s, 17 × 3 H), 1.77−1.71 (m, 2 H); APCI-MS *m/z* 1791.35 [M + H]$^+$.

3-(5-Dimethylaminonaphthalene-1-sulfonamido)propyl 2,3,4,6-Tetra-*O*-sulfo-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-sulfo-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-sulfo-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-sulfo-α-D-mannopyranoside (15). Sulfonamide **25** (18 mg, 10.1 μmol) was sequentially deacetylated and sulfonated according to the general procedures and then purified by dialysis against purified water (Pierce Slide-A-Lyzer, 2000 MWCO, 4 × 5 L) to yield **15** (3 mg, 11%) as a white amorphous solid: 95% pure by CE; ^{1}H NMR (D$_2$O) δ 8.75−8.69 (m, 1 H), 8.40−8.32 (m, 2 H), 8.03−7.82 (m, 3 H), 5.45−4.67 (m, 7 H affected by irradiation), 4.60−3.52 (m, 15 H affected by irradiation), 3.46 (s, 6 H), 3.07−2.87 (m, 4 H), 1.45−1.30 (m, 2 H).

12-Azidododecanol. To a mixture of 12-bromo-1-dodecanol (246 mg, 0.927 mmol) in DMF (1.8 mL, 0.5 M) were added sodium azide (121 mg, 1.855 mmol, 2 equiv) and tetrabutylammonium iodide (17 mg, 0.0464 mmol, 0.05 equiv), and the mixture was stirred at 80 °C for 3 h. The mixture was filtered through a plug of Celite and the cake rinsed with EtOAc (20 mL). The combined filtrate and washings were evaporated onto silica gel and purified by flash chromatography (6:1 → 2:1 hexane:EtOAc) to give 12-azido-1-dodecanol as a colorless oil (193 mg, 92%). The ^{1}H NMR (CDCl$_3$) spectrum was in accord with the literature.[35]

12-Azidododecyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranoside (42). 12-Azidododecanol (1.5 equiv) was glycosylated with trichloroacetimidate **33**[18] (0.325 g, 0.197 mmol) as described for compound **36** to give, following flash chromatography (CHCl$_3$ → CHCl$_3$:MeOH, 99:1 → 98:2), glycoside **42** as a colorless gum containing the byproduct BnNHAc (71.1 mg, 1:1 **42**:BnNHAc ratio): ^{1}H NMR (CDCl$_3$) δ 5.29−5.14 (m, 8 H), 5.01−4.86 (m, 8 H), 4.28−3.75 (m, 19 H), 3.64 (dt, 1 H, *J* = 9.5, 7.0 Hz, OCH$_2$), 3.39 (dt, 1 H, *J* = 9.5, 7.0 Hz, OCH$_2$), 3.22 (t, 2 H, *J* = 7.0 Hz, NCH$_2$), 2.16, 2.15, 2.11, 2.10, 2.09, 2.09, 2.09, 2.09, 2.07, 2.06, 2.04, 2.04, 2.01, 1.95 (14s, 48 H, 16 × Ac), 1.57 (m, 4 H, 2 × CH$_2$), 1.36−1.22 (m, 16 H, 8 × CH$_2$).

12-(4-Phenyl[1,2,3]triazol-1-yl)dodecyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranoside (26). Azide **42** (71 mg, 41.5 μmol) was reacted with phenylacetylene (83 μmol, 2 equiv) as described for compound **19** to give, following flash chromatography (1:6 → 3:1 EtOAc: hexane), phenyltriazole **26** as a colorless gum (46.3 mg, 62%): ^{1}H NMR (CDCl$_3$) δ 7.82 (d, 2 H, *J* = 7.2 Hz, Ph), 7.75 (s, 1 H, triazole-CH), 7.41 (t, 2 H, *J* = 7.2 Hz, Ph), 7.31 (t, 1 H, *J* = 7.2 Hz, Ph), 5.30−5.15 (m, 8 H), 5.03−4.87 (m, 8 H), 4.38 (t, 2 H, *J* = 7.2 Hz, NCH$_2$), 4.29−3.77 (m, 19 H), 3.64 (dt, 1 H, *J* = 9.6, 6.8 Hz, OCH$_2$), 3.40 (dt, 1 H, *J* = 9.6, 6.8 Hz, OCH$_2$), 2.17, 2.16, 2.16, 2.13, 2.11, 2.11, 2.11, 2.10, 2.09, 2.07, 2.05, 2.05, 2.02, 2.00, 1.96 (15s, 48 H, 16 × Ac), 1.93 (m, 2 H, CH$_2$), 1.57 (m, 2 H, CH$_2$), 1.34−1.24 (m, 16 H, 8 × CH$_2$).

12-(4-Phenyl[1,2,3]triazol-1-yl)dodecyl 2,3,4,6-Tetra-*O*-sulfo-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-sulfo-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-sulfo-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-sulfo-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-sulfo-α-D-mannopyranoside, Hexadecasodium Salt (16). Phenyltriazole **26** (46 mg, 25.4 μmol) was sequentially deacetylated and sulfonated according to the general procedures and then purified by SEC to give polysulfate **16** as a white powder (45 mg, 64%): 94% pure by CE; ^{1}H NMR (D$_2$O, internal DOH at 4.60 ppm) δ 7.88 (s, 1 H, triazole-CH), 7.47−7.44 (m, 2 H), 7.21−7.10 (m, 3 H), 5.23 (br s, 1 H), 5.19 (d, 1 H, *J* = 1.5 Hz), 5.12 (d, 1 H, *J* = 1.8 Hz), 5.10 (d, 1 H, *J* = 1.5 Hz), 4.91 (m, 1 H), 4.76−3.72 (m, 32 H), 3.37−3.30 (m, 1 H, OCH$_2$), 3.23−3.17 (m, 1 H, OCH$_2$), 1.51 (m, 2 H, CH$_2$), 1.12 (m, 2 H, CH$_2$), 0.90−0.63 (m, 16 H, 8 × CH$_2$).

12-(4-Naphthalen-1-yl[1,2,3]triazol-1-yl)dodecyl 2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-acetyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-acetyl-α-D-mannopyranoside (27). Azide **42** (86 mg, 50.3 μmol) was reacted with 1-ethynylnaphthalene (83 μmol, 2 equiv) as described for compound **20** (11 days). The mixture was then evaporated onto silica gel and purified by flash chromatography (1:6 → 3:1 EtOAc: hexane) to give naphthyltriazole **27** as a colorless gum (24.2 mg, 26%): ^{1}H NMR (CDCl$_3$) δ 8.38−8.33 (m, 1 H), 7.92−7.86 (m, 2 H), 7.80 (s, 1 H, triazole-CH), 7.72 (dd, 1 H, *J* = 7.3, 1.5 Hz), 7.54−7.48 (m, 3 H), 5.31−5.15 (m, 8 H), 5.03−4.87 (m, 8 H), 4.47 (t, 2 H, *J* = 7.3 Hz, N-CH$_2$), 4.30−3.77 (m, 19 H), 3.64 (dt, 1 H, *J* = 9.7, 6.8 Hz, OCH$_2$), 3.40 (dt, 1 H, *J* = 9.7, 6.8 Hz, OCH$_2$), 2.17, 2.16, 2.16, 2.13, 2.12, 2.11, 2.11, 2.10, 2.09, 2.07, 2.06, 2.05, 2.02, 2.00, 1.97, 1.57 (16s, 48 H, 16 × Ac), 2.07−1.95 (m, overlapped with Ac singlets, 2 H, CH$_2$), 1.57 (m, 1 H, CH$_2$), 1.42−1.23 (m, 16 H, 8 × CH$_2$).

12-(4-Naphthalen-1-yl[1,2,3]triazol-1-yl)dodecyl 2,3,4,6-Tetra-*O*-sulfo-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-sulfo-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-sulfo-α-D-mannopyranosyl-(1→3)-2,4,6-tri-*O*-sulfo-α-D-mannopyranosyl-(1→2)-3,4,6-tri-*O*-sulfo-α-D-mannopyranoside, Hexadecasodium Salt (17). Naphthyltriazole **27** (39.6 mg, 0.0213 mmol) was sequentially deacetylated and sulfonated according to the general procedures and then purified by SEC to give polysulfate **17** as a white powder (40 mg, 68%): ^{1}H NMR (D$_2$O, internal DOH at 4.60 ppm) δ 8.05 (s, 1 H, triazole-CH), 7.97 (d, 1 H, *J* = 8.3 Hz), 7.90 (d, 2 H, *J* = 7.8 Hz), 7.55−7.40 (m, 4 H), 5.44−5.22 (m, 4 H), 5.09−3.82 (m, 33 H), 3.41−3.33 (m, 1 H, OCH$_2$), 3.25−3.16 (m, 1 H, OCH$_2$), 1.78 (quintet, 2 H, CH$_2$), 1.14−0.79 (m, 18 H, 9 × CH$_2$).

Heparanase Inhibition Assay. The inhibitory potencies of the compounds toward recombinant human heparanase[36] were determined using a Microcon ultrafiltration assay, which utilizes ^{3}H-labeled HS as the substrate (K_m = 1.64 μM), as previously described.[20] The results are presented in Table 1.

Growth Factor Binding Assays. The binding affinities of the compounds for growth factors FGF-1, FGF-2, and VEGF were determined on a BIAcore 3000 instrument (BIAcore, Uppsala, Sweden) using a surface plasmon resonance-based solution affinity assay, as previously described.[28,29] Heparin-coated CM4 sensor chips were prepared via reductive amination of the reducing end aldehyde with 1,4-diaminobutane, as previously described.[29] Ligands binding to FGF-1 and VEGF were assessed in HBS-EP buffer [10 mM HEPES (pH 7.4), 150 mM NaCl, 3.0 mM EDTA, and 0.005% (v/v) polysorbate 20], while binding to FGF-2 was assessed in HBS-EP buffer containing 300 mM NaCl. Sensorgram data were analyzed using BIAevaluation (BIAcore) and K_d values determined as previously described.[29] The K_d values represent the average of at least duplicate measurements ± the standard deviation. The results are presented in Table 1.

Growth Factor-Induced Endothelial Cell Proliferation Assay. (a) Endothelial Cell Culture. HUVECs were maintained and subcultured according to standard cell culture protocols essentially as described by Lonza (Basel, Switzerland). Briefly, cells were maintained in Lonza endothelial growth medium (EGM) with recommended supplements and growth factors [VEGF, FGF-2, EFG, IGF, hydrocortisone, fetal bovine serum (FBS), ascorbic acid, heparin, and gentamicin]. Cells were subcultured when they reached 70−80% confluence by trypsinization and reseeding in fresh growth medium in new culture vessels at densities of 2500−5000 cells/cm^2 of vessel surface area. Cell counts were performed using a hemocytometer, and viable cells were visualized with trypan blue. Medium for the proliferation studies was prepared using EGM with 2% FBS and gentamicin only. Test compounds were weighed out from powder stocks and diluted in PBS to 10 mM stock solutions and stored at −80 °C. For experiments, compounds were subsequently diluted in EBM-2 medium (supplemented with 2% FBS and gentamicin) to various working concentrations as required. **(b) Proliferation Assay.** Proliferation was induced in HUVECs using various concentrations of growth factor VEGF, FGF-1, or FGF-2 over a period of 72 h. In the first of a series of experiments, the assay was further optimized by examination of the cell density and growth factor concentration required to induce maximal proliferation by growth factors. Briefly, 100 μL of cells was added to each well at concentrations between 1 and 3 × 10^3 per well. Growth factors and test compounds were then added in 50 μL volumes at specified concentrations (typically seven ranging from 1 to 50 μM in triplicate) to yield a final volume of 200 μL. Following incubation for 70 h, 20 μL of the CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega) was added for 2 h prior to the absorbance at 490 nm being read, yielding OD values. Once these values were subtracted from the blanks (medium only), the data were imported into BIAevaluation to determine IC$_{50}$ values for each curve. The data are presented in Figure 1.

Matrigel Microtubule Formation Assay. The tube formation assay was performed essentially as described by Malinda et al.,[37] with modifications. HUVECs in the fourth or fifth passage at 70−80% confluence were harvested and resuspended in Lonza endothelial growth medium (EGM2) containing all supplements as directed by the manufacturer, except heparin, at a cell density of 4 × 10^5 cells/mL. For each set of triplicate wells, 200 μL of cells (4 × 10^5 cells/mL) was treated with an equal volume of compound to produce final concentrations of 10, 50, and 100 μM (thus ensuring 1 × 300 μL is available for each condition). A 100 μL aliquot of cells was then plated onto 96-well plates precoated with growth factor-reduced Matrigel (50 μL for 30 min followed by a further 30 μL for 1 h) and incubated for 18 h. Tube formation was examined by phase-contrast microscopy, and images were collected using an Olympus C5050 digital camera. Tube formation inhibition was quantified manually from images by recording the total number of branching nodes connecting three or more tubules. Results are expressed as the number of branching nodes for individual experiments (Figure 2a) or percentage inhibition compared to control to facilitate interexperiment comparison (Figure 2b). Untreated HUVECs were used as a control for normal cell

growth and tube formation in Matrigel. Representative images are shown in Figure 2c.

Ex Vivo Rat Aortic Angiogenesis Assay. Explants from rat aortas were prepared by a modification of protocols previously described.[38−41] Briefly, thoracic aortas were excised from 2−4-month-old Sprague-Dawley rats and trimmed of remaining fat and connective tissue. Great care was taken at every stage to reduce physical damage of the aorta. Tissue was transferred to complete EBM-2 medium (Cambrex) containing 2% FCS and all singlequots (Cambrex) reagents except for heparin. Meanwhile, Matrigel (BD Biosciences) was allowed to cool on ice, and once in a liquid form, 180 μL was pipetted into 48-well tissue culture plates (Nunc). The plates were incubated at 37 °C for 30 min to allow Matrigel to solidify.

We prepared aortas by cutting 1 mm ring sections and then bisecting them. Aortic segments were then carefully placed on top on the Matrigel in the center of each well, and once they were orientated as required, 60 μL of extra Matrigel was placed on top and the plate returned to the incubator for a further 20 min. Each well was then supplemented with 1.0 mL of medium in the absence (control) or presence of test compounds usually at two concentrations within the range of 1−50 μM, depending on the particular compound or experiment. Cultures were replenished as appropriate every 48 h, and scoring of microvessels was conducted at various time points up to 8 days by two assessors. The extent of microvessel sprouting was determined as previously described[41] and expressed as a semiquantitative score. Sprouting vessels were photographed using the 4× objective with an Olympus C-7070 camera and an adaptor for the eyepiece.

In some instances, to determine the potential toxicity of compounds in this assay, we assessed the viability of the tissue by withdrawing the compound or medium from the culture on day 6 or 7 and adding complete medium with VEGF (typically 10 ng/mL) for up to an additional 7 days. In the absence of toxicity, the viable tissue should sprout microvessels in response to the exogenous growth factor.

Anticoagulant Activity. The anticoagulant activity of the test compounds was determined by measuring the effect of various concentrations of the compound (0−100 μg/mL in PBS) on the elevation of the APTT of pooled normal human plasma. APTT measurements were performed on a STAGO STA-Compact Coagulation Analyzer using standard protocols according to the manufacturer's instructions. Unfractionated heparin (UFH) was used as the control. The normal range of APTT for pooled normal human plasma is 26−36 s. The results are presented in Table 1. The coefficient of variation for APTT coagulation values of 36 s is 2% and for coagulation values of ≥90 s is 5−7%.

The compounds were also evaluated in the heptest[42] as follows. Venous blood was collected by clean venipuncture using 3.8% sodium citrate as an anticoagulant. One volume of anticoagulant was gently mixed with 9 volumes of blood in plastic tubes. Plasma was prepared by centrifugation at 1800g for 10 min. Plasma samples were analyzed within 30 min; 50 μL of plasma was incubated with 10 μL of each anticoagulant thereafter for 2 min at 37 °C, and 50 μL of factor Xa (Haemachem, St. Louis, MO) was added. After incubation for an additional 2 min at 37 °C, coagulation was induced by addition of 50 μL of Recalmix (Haemachem). Clotting assays were performed with a ball coagulameter KC10 (Amelung, Lemgo, Germany). Heptest values in normal plasma samples ranged from 13 to 20 s (mean of 17.1 ± 2.1 s). The coefficient of variation for heptest coagulation values is 1.8% for values of 20−90 s and 3−4% for values of >90 s.

Pharmacokinetic Studies. Studies were conducted in accordance with the Australian and New Zealand Council for the Care of Animals in Research and Training Guidelines and were approved by the Monash University animal experimentation ethics committee. Adult male Sprague-Dawley rats (weighing 279−303 g) were supplied by Monash University Animal Services (Clayton, Victoria, Australia). Jugular vein and carotid artery cannulations were performed on the day prior to dosing to allow iv dosing and blood sampling, respectively. Compound **5**, formulated as a 2.8 mg/mL solution in PBS, was infused intravenously over a period of 5 min (1.0 mL/rat; n = 2 rats) or injected subcutaneously between the scapulae (1.0 mL/rat; n = 2 rats) to achieve a nominal dose of 10 mg/kg. Samples of blood [collected from the arterial cannula using a Culex autosampler (BASi)] and total urine were collected periodically for up to 8 and 24 h postdose, respectively. To minimize the potential for ex vivo degradation of **5** in blood/plasma samples, blood samples were collected directly into heparinized borosilicate vials (at 4 °C) containing Complete (a protease inhibitor cocktail, 80 mg/mL, Roche, Mannheim, Germany), potassium fluoride (4 mg/mL), and EDTA (0.1 M). Once collected, blood samples were centrifuged, and the plasma supernatant was removed and stored at −20 °C for subsequent analysis by LC−MS.

The terminal elimination half-life, total plasma clearance, and apparent volume of distribution were estimated by standard noncompartmental analysis using WinNonlin (version 4.0, Pharsight Corp., Mountain View, CA).

Analysis of Compound 5 in Rat Plasma. LC−MS analysis of **5** was performed on a Waters Micromass Quattro Ultima Pt triple quadrupole instrument coupled with a Waters 2795 HPLC system. Mass spectrometry was performed with negative mode electrospray ionization with a capillary voltage of 2.7 kV, a detector multiplier gain of 650 V, and source block and desolvation temperatures of 90 and 350 °C, respectively. Elution of the analyte was monitored using selected ion recording (SIR) using the combined signals of the seven strongest doubly charged fragment ions (m/z 708.5, 668.5, 628.5, 588.5, 548.5, 508.5, and 468.5) corresponding to the loss of 5−11 sulfate units from the molecular ion of **5**. The molecular ion was not observed due to extensive in-source fragmentation. Chromatographic separation was performed on a Shodex Ohpak 6 μm SB-802.5 HQ 80 Å 250 mm × 2 mm column equipped with a 0.22 μm particle filter. The mobile phase consisted of 20% acetonitrile, 50% ammonium formate (0.1 M), and 30% water delivered at a rate of 0.6 mL/min. Leucine enkaphalin was used as the internal standard.

Plasma samples were prepared for analysis by protein precipitation using acetonitrile under alkaline conditions. Solution standards were diluted from a stock solution (prepared in a 10% methanol/water mixture) with a 10% methanol/water mixture. Plasma standards were prepared by spiking blank plasma (100 μL) with solution standards (20 μL in a 10% methanol/water mixture) and the internal standard (IS) leucine enkephalin (20 μL in a 50% acetonitrile/water mixture) to give a final IS concentration of 0.2 μg/mL. Protein precipitation for plasma samples was conducted via the addition of sodium hydroxide (40 μL, 0.1 M) and acetonitrile (180 μL) followed by vortexing (60 s) and centrifugation (10000 rpm) in a microcentrifuge for 3 min. The supernatant was transferred to a clean Eppendorf tube and freeze-dried. Subsequently, the residue was reconstituted in 10% aqueous methanol (100 μL) and 50 μL injected onto the column for LC−MS analysis. Calibration standards were prepared in the range of 20−400 μg/mL. Plasma samples from the dosing studies were prepared similarly except the solution standard was replaced with a blank solvent. Extraction recovery from rat plasma was confirmed by comparison of the peak area of the spiked plasma samples to the peak area of the control sample where the spike was added to a blank plasma extract.

In Vivo Mouse Melanoma Model. B16F1 cells were cultured in complete DMEM medium containing 10% FCS, penicillin/streptomycin, L-glutamine, sodium pyruvate, and 2-mercaptoethanol. Cells were harvested for tumor inoculation, B16F1 cells by disruption with trypsin/EDTA, washed with HBSS, and centrifuged for 5 min at 1500 rpm. Cells were then resuspended in PBS to ensure 5 × 10^5 cells were injected in a volume of 50 μL. The tumor was implanted between the shoulder blades. Three days following tumor inoculation, each treatment group was

injected subcutaneously at different sites each day and at a dose of 30 mg/kg twice daily for 7 days in a volume of injection of 100 μL. Injections continued until day 15, providing a 12-day treatment period. Mice were monitored daily from the start of injections, and palpable tumors were measured daily. Tumor size was determined from the measurement in two dimensions, $l \times w$, where l is the longest dimension and w the shortest dimension. To estimate tumor volume, the formula $0.5l(w^2)$ was employed. Data are presented as the median tumor growth (Figure 4) and were analyzed by a one-way analysis of variance (ANOVA) followed by a post hoc Newman-Keuls multiple-comparison test (Graphpad Prism, version 3). Data are presented as means $\pm$ the standard error of the mean. Differences with a p value of less than 0.05 were considered statistically significant. The percentage of tumor growth inhibition (%TGI) was also calculated to correct for interexperimental differences. TGI values were calculated using the formula TGI $= (1 - \Delta T/\Delta C) \times 100$, where ΔT and ΔC represent the change in mean tumor mass between the last day of therapy and the first day of therapy in the sample compound-treated (T) and vehicle control (C) groups, respectively.

Supporting Information Available: Copies of ^{1}H and ^{13}C NMR spectra for selected new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Carmeliet, P.; Jain, R. K. Angiogenesis in cancer and other diseases. *Nature* **2000**, *407*, 249–257.
(2) Bergers, G.; Benjamin, L. E. Tumorigenesis and the angiogenic switch. *Nat. Rev. Cancer* **2003**, *3*, 401–410.
(3) Herbst, R. S. Therapeutic options to target angiogenesis in human malignancies. *Expert Opin. Emerging Drugs* **2006**, *11*, 635–650.
(4) Sasisekharan, R.; Shriver, Z.; Venkataraman, G.; Narayanasami, U. Roles of heparan-sulphate glycosaminoglycans in cancer. *Nat. Rev. Cancer* **2002**, *2*, 521–528.
(5) Wegrowski, Y.; Maquart, F. X. Involvement of stromal proteoglycans in tumour progression. *Crit. Rev. Oncol. Hematol.* **2004**, *49*, 259–268.
(6) Lever, R.; Page, C. P. Novel drug development opportunities for heparin. *Nat. Rev. Drug Discovery* **2002**, *1*, 140–148.
(7) Presta, M.; Leali, D.; Stabile, H.; Ronca, R.; Camozzi, M.; Coco, L.; Moroni, E.; Liekens, S.; Rusnati, M. Heparin derivatives as angiogenesis inhibitors. *Curr. Pharm. Des.* **2003**, *9*, 553–566.
(8) Parish, C. R.; Freeman, C.; Brown, K. J.; Francis, D. J.; Cowden, W. B. Identification of sulfated oligosaccharide-based inhibitors of tumor growth and metastasis using novel in vitro assays for angiogenesis and heparanase activity. *Cancer Res.* **1999**, *59*, 3433–3441.
(9) Kudchadkar, R.; Gonzalez, R.; Lewis, K. D. PI-88: A novel inhibitor of angiogenesis. *Expert Opin. Invest. Drugs* **2008**, *17*, 1769–1776.
(10) Liu, C. J.; Lee, P. H.; Lin, D. Y.; Wu, C. C.; Jeng, L. B.; Lin, P. W.; Mok, K. T.; Lee, W. C.; Yeh, H. Z.; Ho, M. C.; Yang, S. S.; Lee, C. C.; Yu, M. C.; Hu, R. H.; Peng, C. Y.; Lai, K. L.; Chang, S. S.; Chen, P. J. Heparanase inhibitor PI-88 as adjuvant therapy for hepatocellular carcinoma after curative resection: A randomized phase II trial for safety and optimal dosage. *J. Hepatol.* **2009**, *50*, 958–968.
(11) Eswarakumar, V. P.; Lax, I.; Schlessinger, J. Cellular signaling by fibroblast growth factor receptors. *Cytokine Growth Factor Rev.* **2005**, *16*, 139–149.
(12) Parish, C. R.; Freeman, C.; Hulett, M. D. Heparanase: A key enzyme involved in cell invasion. *Biochim. Biophys. Acta* **2001**, *1471*, M99–M108.
(13) Hammond, E.; Bytheway, I.; Ferro, V. Heparanase as a target for anticancer therapeutics: New developments and future prospects.

In *New Developments in Therapeutic Glycomics*; Delehedde, M., Lortat-Jacob, H., Eds.; Research Signpost: Trivandrum, India, 2006; pp 251–282.
(14) Ilan, N.; Elkin, M.; Vlodavsky, I. Regulation, function and clinical significance of heparanase in cancer metastasis and angiogenesis. *Int. J. Biochem. Cell Biol.* **2006**, *38*, 2018–2039.
(15) Wall, D.; Douglas, S.; Ferro, V.; Cowden, W.; Parish, C. Characterisation of the anticoagulant properties of a range of structurally diverse sulfated oligosaccharides. *Thromb. Res.* **2001**, *103*, 325–335.
(16) Rosenthal, M. A.; Rischin, D.; McArthur, G.; Ribbons, K.; Chong, B.; Fareed, J.; Toner, G.; Green, M. D.; Basser, R. L. Treatment with the novel anti-angiogenic agent PI-88 is associated with immune-mediated thrombocytopenia. *Ann. Oncol.* **2002**, *13*, 770–776.
(17) Basche, M.; Gustafson, D. L.; Holden, S. N.; O'Bryant, C. L.; Gore, L.; Witta, S.; Schultz, M. K.; Morrow, M.; Levin, A.; Creese, B. R.; Kangas, M.; Roberts, K.; Nguyen, T.; Davis, K.; Addison, R. S.; Moore, J. C.; Eckhardt, S. G. A phase I biological and pharmacologic study of the heparanase inhibitor PI-88 in patients with advanced solid tumors. *Clin. Cancer Res.* **2006**, *12*, 5471–5480.
(18) Karoli, T.; Liu, L.; Fairweather, J. K.; Hammond, E.; Li, C. P.; Cochran, S.; Bergefall, K.; Trybala, E.; Addison, R. S.; Ferro, V. Synthesis, biological activity, and preliminary pharmacokinetic evaluation of analogues of a phosphosulfomannan angiogenesis inhibitor (PI-88). *J. Med. Chem.* **2005**, *48*, 8229–8236.
(19) Ferro, V.; Dredge, K.; Liu, L.; Hammond, E.; Bytheway, I.; Li, C.; Johnstone, K.; Karoli, T.; Davis, K.; Copeman, E.; Gautam, A. PI-88 and novel heparan sulfate mimetics inhibit angiogenesis. *Semin. Thromb. Hemostasis* **2007**, *33*, 557–568.
(20) Fairweather, J. K.; Hammond, E.; Johnstone, K. D.; Ferro, V. Synthesis and heparanase inhibitory activity of sulfated manno-oligosaccharides related to the antiangiogenic agent PI-88. *Bioorg. Med. Chem.* **2008**, *16*, 699–709.
(21) Yu, G.; Gunay, N. S.; Linhardt, R. J.; Toida, T.; Fareed, J.; Hoppensteadt, D. A.; Shadid, H.; Ferro, V.; Li, C.; Fewings, K.; Palermo, M. C.; Podger, D. Preparation and anticoagulant activity of the phosphosulfomannan PI-88. *Eur. J. Med. Chem.* **2002**, *37*, 783–791.
(22) Liu, L.; Johnstone, K.; Fairweather, J. K.; Dredge, K.; Ferro, V. An improved synthetic route to the potent angiogenesis inhibitor benzyl manα(1→3)-manα(1→3)-manα(1→3)-manα(1→2)-man hexadecasulfate. *Aust. J. Chem.* **2009**, *62*, 546–552.
(23) Ferro, V.; Fewings, K.; Palermo, M. C.; Li, C. Large-scale preparation of the oligosaccharide phosphate fraction of *Pichia holstii* NRRL Y-2448 phosphomannan for use in the manufacture of PI-88. *Carbohydr. Res.* **2001**, *332*, 183–189.
(24) Bock, K.; Pedersen, C. A study of ^{13}CH coupling constants in hexopyranoses. *J. Chem. Soc., Perkin Trans. 2* **1974**, 293–297.
(25) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. A stepwise Huisgen cycloaddition process: Copper(I)-catalyzed regioselective "ligation" of azides and terminal alkynes. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596–2599.
(26) Tornøe, C. W.; Christensen, C.; Meldal, M. Peptidotriazoles on solid phase: [1,2,3]Triazoles by regiospecific copper(I)-catalyzed 1,3-dipolar cycloadditions of terminal alkynes to azides. *J. Org. Chem.* **2002**, *67*, 3057–3064.
(27) Chittaboina, S.; Xie, F.; Wang, Q. One-pot synthesis of triazole-linked glycoconjugates. *Tetrahedron Lett.* **2005**, *46*, 2331–2336.
(28) Cochran, S.; Li, C.; Fairweather, J. K.; Kett, W. C.; Coombe, D. R.; Ferro, V. Probing the interactions of phosphosulfomannans with angiogenic growth factors by surface plasmon resonance. *J. Med. Chem.* **2003**, *46*, 4601–4608.
(29) Cochran, S.; Li, C. P.; Ferro, V. A surface plasmon resonance-based solution affinity assay for heparan sulfate-binding proteins. *Glycoconjugate J.* **2009**, *26*, 577–587.
(30) Davies, B.; Morris, T. Physiological parameters in laboratory animals and humans. *Pharm. Res.* **1993**, *10*, 1093–1095.
(31) Farndale, R. W.; Buttle, D. J.; Barrett, A. J. Improved quantitation and discrimination of sulphated glycosaminoglycans by use of dimethylmethylene blue. *Biochim. Biophys. Acta* **1986**, *883*, 173–177.
(32) Boigegrain, R.; Castro, B.; Selve, C. Sels d'alkyloxyphosphonium XIII. Monoactivation selective des diols-1,n biprimaires symetriques dans les reactions de substitutions nucleophiles. *Tetrahedron Lett.* **1975**, 2529–2530.
(33) Gluchowski, C.; Wetzel, J. M.; Chiu, G.; Marzabadi, M. R.; Wong, W. C.; Nagarathnam, D. Dihydropyridines and new uses thereof. U.S. Patent US6608086, **2003**.

(34) Matyjaszewski, K.; Sumerlin, B. S.; Tsarevsky, N.; Spanswick, J. Preparation of functional polymers. PCT Int. Appl. WO2005/087818, **2005**.

(35) Murozuka, Y.; Kasuya, M. C. Z.; Kobayashi, M.; Watanabe, Y.; Sato, T.; Hatanaka, K. Efficient sialylation on azidododecyl lactosides by using B16 melanoma cells. *Chem. Biodiversity* **2005**, *2*, 1063–1078.

(36) McKenzie, E.; Young, K.; Hircock, M.; Bennett, J.; Bhaman, M.; Felix, R.; Turner, P.; Stamps, A.; McMillan, D.; Saville, G.; Ng, S.; Mason, S.; Snell, D.; Schofield, D.; Gong, H.; Townsend, R.; Gallagher, J.; Page, M.; Parekh, R.; Stubberfield, C. Biochemical characterization of the active heterodimer form of human heparanase (Hpa1) protein expressed in insect cells. *Biochem. J.* **2003**, *373*, 423–435.

(37) Malinda, K. M.; Nomizu, M.; Chung, M.; Delgado, M.; Kuratomi, Y.; Yamada, Y.; Kleinman, H. K.; Ponce, M. L. Identification of laminin α1 and β1 chain peptides active for endothelial cell adhesion, tube formation, and aortic sprouting. *FASEB J.* **1999**, *13*, 53–62.

(38) Nicosia, R. F.; Ottinetti, A. Growth of microvessels in serum-free matrix culture of rat aorta. A quantitative assay of angiogenesis in vitro. *Lab. Invest.* **1990**, *63*, 115–122.

(39) Dredge, K.; Marriott, J. B.; Macdonald, C. D.; Man, H. W.; Chen, R.; Muller, G. W.; Stirling, D.; Dalgleish, A. G. Novel thalidomide analogues display anti-angiogenic activity independently of immunomodulatory effects. *Br. J. Cancer* **2002**, *87*, 1166–1172.

(40) Ng, S. S.; MacPherson, G. R.; Gutschow, M.; Eger, K.; Figg, W. D. Antitumor effects of thalidomide analogs in human prostate cancer xenografts implanted in immunodeficient mice. *Clin. Cancer Res.* **2004**, *10*, 4192–4197.

(41) Min, J. K.; Han, K. Y.; Kim, E. C.; Kim, Y. M.; Lee, S. W.; Kim, O. H.; Kim, K. W.; Gho, Y. S.; Kwon, Y. G. Capsaicin inhibits in vitro and in vivo angiogenesis. *Cancer Res.* **2004**, *64*, 644–651.

(42) Harenberg, J.; Giese, C.; Dempfle, C. E.; Stehle, G.; Heene, D. L. Monitoring of heparin and low molecular weight heparin with capillary and venous whole blood. *Thromb. Haemostasis* **1988**, *60*, 377–381.