Synthesis, Biological Activity, and Preliminary Pharmacokinetic Evaluation of Analogues of a Phosphosulfomannan Angiogenesis Inhibitor (PI-88)

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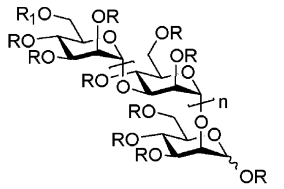
Received June 30, 2005

The phosphosulfomannan **1** (PI-88) is a mixture of highly sulfated oligosaccharides that is currently undergoing clinical evaluation in cancer patients. As well as its anticancer properties, **1** displays a number of other interesting biological activities. A series of analogues of **1** were synthesized with a single carbon (pentasaccharide) backbone to facilitate structural characterization and interpretation of biological results. In a fashion similar to **1**, all compounds were able to inhibit heparanase and to bind tightly to the proangiogenic growth factors FGF-1, FGF-2, and VEGF. The compounds also inhibited the infection of cells and cell-to-cell spread of herpes simplex virus (HSV-1). Preliminary pharmacokinetic data indicated that the compounds displayed different pharmacokinetic behavior compared with **1**. Of particular note was the *n*-octyl derivative, which was cleared 3 times less rapidly than **1** and may provide increased systemic exposure.

Introduction

The antiangiogenic phosphosulfomannan PI-88 (**1**, Figure 1)[1,2] is a promising inhibitor of tumor growth and metastasis[1,3,4] and is currently under evaluation in phase II clinical trials in cancer patients.[5] Compound **1** exerts antiangiogenic effects by antagonizing the interactions of proangiogenic growth factors (FGF-1, FGF-2, and VEGF) and their receptors with heparan sulfate (HS).[1,6] Compound **1** is also a potent inhibitor of heparanase, a glycosidase that cleaves the HS side chains of proteoglycans and plays an important role in metastasis and angiogenesis.[7−9] In addition to its anticancer effects, **1** inhibits the blood coagulation cascade,[10−12] blocks vascular smooth muscle cell proliferation and intimal thickening,[13] inhibits herpes simplex virus (HSV) infection of cells and cell-to-cell spread of HSV-1 and HSV-2,[14] and reduces proteinuria in a model of passive Heymann nephritis.[15]

Compound **1** is a mixture of highly sulfated, monophosphorylated mannose oligosaccharides ranging in size from di- to hexasaccharide.[16,17] The major components are penta- (∼60%) and tetrasaccharides (∼30%). While **1** is a promising clinical candidate,[5] the fact that it is a mixture complicates its characterization and the assessment of structure−activity relationships. The aims of this study were therefore to synthesize several simple, easier to characterize analogues of **1** and to determine if this could be accomplished while maintaining biological activity. Pentasaccharide analogues where the terminal 6-*O*-phosphate of **1** has been replaced by sulfate were chosen for synthesis because a number of studies of derivatives of the individual components of **1** indicate that the dominant pentasaccharide component is also the most biologically active and that the terminal 6-*O*-phosphate group can be replaced by a sulfo group without detriment to biological activity.[6,14,17] The compounds were further simplified by making them anomerically pure, although the final products were still mixtures of highly but incompletely sulfated forms, as has been well established for compounds of this type.[1,6,17−19] The synthetic modifications were specifically chosen with a view to altering the pharmacokinetic properties of the analogues in a favorable manner compared with the parent compound[20,21] to possibly allow for less frequent dosing while biological activity is maintained.



	n	R	R_1
1	0-4	SO_3Na or H	PO_3Na_2
2	3	H	H
3	3	Ac	Ac

Figure 1. Structures of compounds **1**−**3**.

Results and Discussion

Synthesis. The pentasaccharide α-D-Man-(1→3)-α-D-Man-(1→3)-α-D-Man-(1→3)-α-D-Man-(1→2)-D-Man (**2**) was selected as the starting point for the synthesis of analogues of **1** via modification of the reducing end. The types of modifications chosen were based on the introduction of various groups that might be expected to alter

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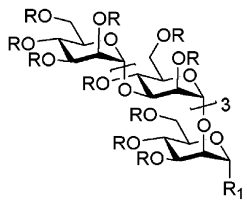
10.1021/jm050618p CCC: $30.25
Published on Web 11/17/2005



	R	R_1
4	Ac	OBn
5	Ac	$O(CH_2)_7CH_3$
6	SO_3Na or H	OBn
7	SO_3Na or H	$O(CH_2)_7CH_3$
8	Ac	$OC(=NH)CCl_3$
9	Ac	O-PEG_{5000}-OMe
10	H	O-PEG_{5000}-OMe
11	SO_3Na or H	O-PEG_{5000}-OMe
12	Ac	O-PEG_{2000}-OMe
13	H	O-PEG_{2000}-OMe
14	SO_3Na or H	O-PEG_{2000}-OMe
15	Ac	N_3
16	Ac	$NHCOCH_2OPh$
17	SO_3Na or H	$NHCOCH_2OPh$
18	Ac	NH-LC-Biotin*
19	SO_3Na or H	NH-LC-Biotin

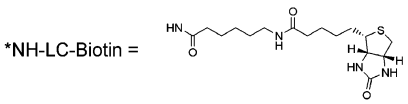


Figure 2. Structures of compounds **4**−**19**.

the pharmacokinetic properties compared to **1** itself, such as lipophilic groups (e.g., *n*-octyl) and poly(ethylene glycol) (PEG) groups.[22] It was considered that such modifications could be obtained either by glycosylation of an appropriate alcohol or by acylation of an anomeric amino group (introduced via a glycosyl azide). While **2** can be synthesized in a stepwise manner from D-mannose,[23] for the purposes of this study the starting material was sourced from the neutral oligosaccharide fraction obtained by mild acid-catalyzed hydrolysis of the extracellular phosphomannan of the yeast *Pichia (Hansenula) holstii* NRRL Y-2448.[24] The pure pentasaccharide, previously isolated by size exclusion chromatography of the above neutral oligosaccharide fraction,[24] was acetylated to give the peracetate **3**. Alternatively, **3** could be isolated by flash chromatography following acetylation of the neutral oligosaccharide fraction.

Various promoters (e.g., tin(IV) chloride, zinc chloride, TMSOTf) were investigated for direct glycosylation of peracetate **3** initially with benzyl alcohol as the glycosyl acceptor. The best conditions were found to be boron trifluoride etherate (4 equiv) in 1,2-dichloroethane at 60 °C, which cleanly gave complete conversion to product within 2 h. This protocol was used for the larger scale preparation of both the benzyl and *n*-octyl glycosides **4** and **5** (Figure 2). When the reaction with benzyl alcohol was initially scaled up, a small amount of powdered 3-Å molecular sieves was added into the reaction mixture as a precaution to trap any moisture before the addition of the promoter (BF_3 etherate). However, it was found that the molecular sieves dramatically slowed the reaction (24 h vs 2 h), while the product profiles and yields were nearly identical to the procedure excluding them. Both benzyl and *n*-octyl glycosides (**4** and **5**) were isolated in moderate to good yields (45−66%). Deprotection under Zemplén conditions followed by sulfonation and size exclusion chromatography provided the corresponding products **6** and **7** in 44% and 77% yield, respectively.

Unlike for the glycosylation of simple alcohols (benzyl and *n*-octyl), the peracetate **3** was not a powerful enough donor for condensation of the monomethyl ether of PEG_{5000} using the conditions developed above. To obtain the glycoside, a more reactive donor was required. The glycosyl imidate **8** was an obvious choice and it was prepared in a straightforward manner by anomeric deacetylation with benzylamine in THF followed by treatment with trichloroacetonitrile and DBU as a base. A TMSOTf-promoted glycosylation proceeded well and the PEGylated glycoside **9** was obtained in good yield (80%). Compound **9** was deprotected to the polyol **10** (89%) and sulfonated to give the sulfated PEG_{5000}−OMe mannopentaoside **11** in 42% yield. In a similar fashion, the sulfated PEG_{2000}−OMe mannopentaoside **14** was prepared in similar overall yield.

For the synthesis of the nitrogen-linked analogues, peracetate **3** was reacted with trimethylsilyl azide and tin(IV) chloride as catalyst to give the α-azide **15** in good yield (82%).[25] Azide **15** was subjected to the Staudinger reaction in the presence of phenoxyacetyl chloride to produce amide **16** in moderate yield. This application of the Staudinger reaction is known[26] to produce anomerization under certain conditions via ring opening of the glycosylphosphazene intermediate. Little anomerization was observed, but the contaminating triphenyl phosphine oxide could not be removed by flash chromatography. Zemplén deacetylation and sulfonation proceeded smoothly and the contaminating materials were easily separated from the desired sulfated compound **17**, obtained in 34% yield over two steps.

The biotinylated target **19** was chosen to serve two tasks: to provide a compound based on the abovementioned design requirements (i.e., enhanced lipophilicity) and to provide an analogue suitable for immobilization onto streptavidin-coated surfaces, such as a BIAcore sensor chip, for potential assay development. Literature precedent[27] exists for the in situ preparation of amides via reduction of an azide by hydrogen and palladium in the presence of an *N*-hydroxysuccinimyl (NHS) ester. In the present case, no reduction of the azide was observed under the conditions described in the literature (1 atm of H_2, 10% Pd/C). Neither increasing the H_2 pressure to 100 psi nor the use of catalytic transfer hydrogenation (NH_4·HCOO) was successful. A stepwise approach[28] was thus attempted. Hydrogenation of the azide required the use of Adam's catalyst (PtO_2) for 18 h at 100 psi of H_2. After 1 h, the azide was observed to be completely consumed, but the product appeared to be a dimerized intermediate.[29] The amine condensed with biotinamidocaproate NHS ester in py-

Table 1. Growth Factor Binding, Inhibition of Heparanase, Inhibition of HSV-1 Infectivity, and Inhibition of Cell-to-Cell Spread of HSV-1 by Compound **1** and Analogues

	K_d			IC_{50}, μM		
compd	FGF-1 (pM)	FGF-2 (nM)	VEGF (nM)	heparanase inhibn	HSV-1 infectivity	HSV-1 cell-to-cell spread
1	281−444[a]	82−140[a]	1.9−6.9[a]	0.98 ± 0.11	2[b]	1[b]
6	120 ± 25	86 ± 7	1.7 ± 0.2	1.83 ± 0.48	2	1
7	144 ± 8	68 ± 3	1.7 ± 0.1	1.64 ± 0.41	1	0.4
11	361 ± 28	150 ± 9	8.1 ± 0.6	6.03 ± 1.05	not tested	11
14	88 ± 17	114 ± 13	3.5 ± 0.8	2.12 ± 0.15	10	7
17	660 ± 40	112 ± 9	7.1 ± 0.6	2.02 ± 0.28	7	5
19	390 ± 70	84 ± 8	7.2 ± 0.6	1.85 ± 0.31	2	3

[a] Data from ref 6. [b] Data from ref 14.

ridine at 60 °C for 3 days to produce the desired amide **18** in 36% overall yield. Zemplén deacetylation and sulfonation then gave the sulfated oligosaccharide **19** in moderate yield (61%).

The sulfonation of oligosaccharides larger than disaccharides with reagents such as sulfur trioxide pyridine or trimethylamine complex is known to not go to completion.[1,6,17−19] Following literature precedent,[1,6,17] the sulfonation of the polyol precursors of the target compounds did not go to completion, and thus compounds **6**, **7**, **11**, **14**, **17**, and **19** were all obtained as reproducible mixtures of highly but incompletely sulfated forms. Compounds **11** and **14** are even more heterogeneous due to PEGylation. The purity of the compounds was therefore determined by a combination of size exclusion HPLC, capillary electrophoresis, and ^{1}H NMR spectroscopy (see Experimental Section and Supporting Information). All were of satisfactory purity (78−100%), although compound **11** contained approximately 55% salt.

Heparanase Inhibition Studies. Compounds **6**, **7**, **11**, **14**, **17**, and **19** were tested for their ability to inhibit human platelet heparanase, and the results are presented in Table 1. The assays were performed using a Microcon ultrafiltration assay,[23] which relies on the principle of physically separating HS that has been digested by heparanase from native HS to determine heparanase activity. The assay uses ultrafiltration devices (Microcon YM-10) to separate the smaller heparanase-cleaved HS fragments from native HS. Compound **1**, used as a standard in this assay, has an $IC_{50} = 0.98$ μM. All compounds tested inhibited heparanase to a similar extent as **1** with IC_{50} values of ∼1−2 μM. The exception was the PEG_{5000} derivative **11**, which was approximately six times less active than **1** ($IC_{50} =$ 6 μM).

Growth Factor Binding Studies. The binding affinities of compounds **6**, **7**, **11**, **14**, **17**, and **19** for the proangiogenic growth factors FGF-1, FGF-2, and VEGF were determined with a BIAcore (surface plasmon resonance) solution affinity assay.[6] It is important to note that ligand binding to the growth factors can only be detected when the interaction involves the HS binding site, thus eliminating the chance of evaluating nonspecific binding to other sites on the protein. A 1:1 stoichiometry was assumed for all protein:ligand interactions. The results indicate that the compounds generally retain their affinities for the growth factors compared with the parent compound and in some cases even show a modest improvement (Table 1).

Antiviral Studies. In addition to its anticancer activities, **1** is a potent inhibitor of cell infection by HSV.[14] Sulfated polysaccharides such as heparin or chondroitin sulfate E are also known inhibitors of the early step(s) of viral infection of cells, such as viral attachment and entry into cells. Their antiviral activities usually increase with increasing molecular weight, but this feature has been reported to adversely affect the ability of the polymer to penetrate into deep layers of stratified tissue,[30] thus limiting their potential antiviral application. In contrast to sulfated polysaccharides, **1** also efficiently inhibits the cell-to-cell spread of the virus, most likely because its relatively small size permits access to the narrow intercellular space.[14] The effects of compounds **1**, **6**, **7**, **11**, **14**, **17**, and **19** on infection of cells by HSV-1 and on the cell-to-cell spread of this virus are shown in Figure 3, parts A and B, respectively. The IC_{50} values for these compounds interpolated from the data are presented in Table 1. The *n*-octyl derivative **7** inhibited viral spread and viral infectivity similarly to **1**, while the benzyl derivative **6** was only slightly less active than **1**. The higher molecular weight PEGylated derivatives **11** and **14** were considerably weaker inhibitors of viral cell-to-cell spread. This observation is in line with our previous finding[14] that compounds of relatively high molecular weight were poor inhibitors of HSV-1 cell-to-cell spread, most likely because of their inability to enter the narrow intercellular space.

Pharmacokinetic Studies. Compounds **6**, **7**, **11**, **14**, and **19** were selected for preliminary pharmacokinetic analysis in male Sprague−Dawley rats. These compounds together with the parent compound **1** were radiolabeled with ^{35}S by sulfonating their respective polyol precursors with $^{35}SO_3$ pyridine complex.[31] The compounds were obtained in good chemical and radiochemical purity (as determined by HPLC; see Supporting Information). The sample of [^{35}S]**11** contained ∼45% salt (by HPLC), but this was not associated with any radioactivity, so the sample was considered suitable for use. Compounds **1**, **6**, and **14** were obtained with good specific activities (∼30 μCi/mg) while **7**, **11**, and **19** were somewhat less active (∼6 μCi/mg), but still suitable for the study.[32] Six groups of rats ($n = 4$ in each group) were dosed intravenously with the radiolabeled compounds (ca. 0.5−10 μCi/animal), plus the corresponding nonlabeled compound, to a final dose of 2.5 mg/kg. Blood samples were collected for determination of plasma radioactivity at timed intervals over a 48-h period after dosing. Urine and feces were also collected for deter-

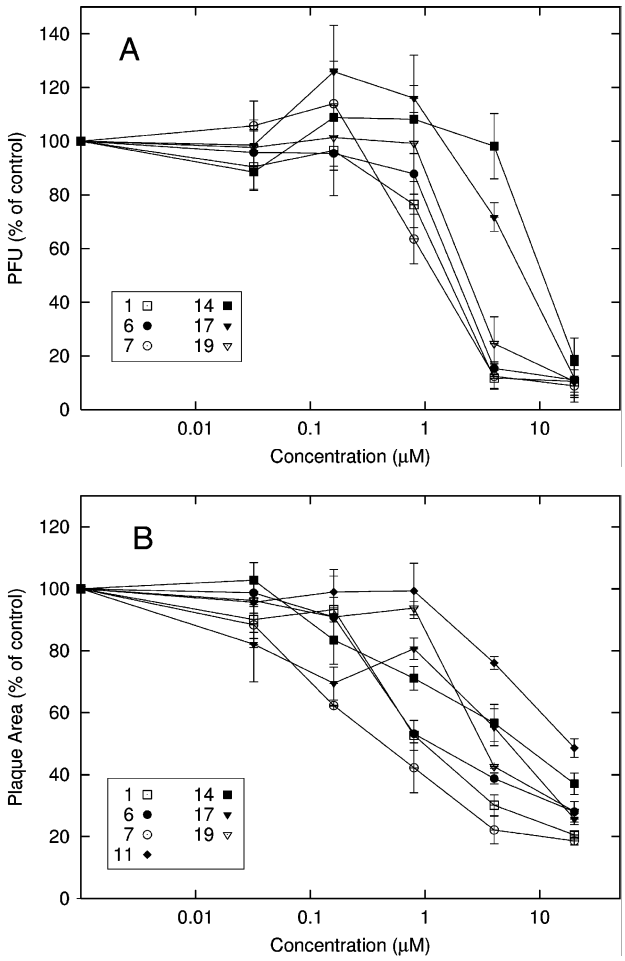


Figure 3. Effect of compounds **1**, **6**, **7**, **11**, **14**, **17**, and **19** on HSV-1 infectivity (A) and HSV-1 cell-to-cell spread (B). For explanations, see the Experimental Section. In panel A, the results are expressed as a percentage of the number of viral plaque forming units (pfu) formed in cells infected with the compound-treated virions relative to mock-treated controls. In panel B, the results are expressed as a percentage of the average area of 20 viral plaques formed in the continuous presence of compound relative to mock-treated control cells.

mination of the extent of excretion of radioactivity over this period. The data obtained are summarized in Table 2.

The log plasma concentration−time profiles were curvilinear over the period 0−12 h. A notionally linear period was observed between 0.75 and 4.0 h for all compounds, except **11**, and was used to calculate indicative pharmacokinetic parameters (see footnote *a* in Table 2) for comparison between the groups. Radiolabel was rapidly eliminated from plasma following iv dosing with all compounds studied, with half-lives of approximately 1 h or less being observed over the notionally linear 0.75−4.0 h postdose interval. Clearance of the label was most rapid for **14** (404 ± 59.5 mL/h/kg) and slowest for **7** (83.6 ± 9.1 mL/h/kg). Compound **7** also displayed the highest results for C_0 (35.6 μg equiv/mL). The overall results for **7** indicate that it is cleared three times less rapidly compared with **1** and may have an increased systemic exposure. Urinary excretion of radiolabel accounted for 40−80% of the radiolabeled doses, while excretion in the feces was low (approximately 1% of the dose).

Conclusions. Several analogues of the anticancer agent **1** were prepared and their biological activities were evaluated. The analogues were based on a single pentasaccharide backbone for ease of synthesis and to facilitate their structural characterization and the interpretation of biological results. The compounds displayed similar activities to **1** in terms of their ability to inhibit heparanase, to bind tightly to proangiogenic growth factors, and to inhibit cell infection and cell-to-cell spread of HSV-1, although the higher molecular weight PEGylated derivatives were only weak inhibitors of viral cell-to-cell spread. Of particular note was the demonstration that it is possible to alter the pharmacokinetic properties of the analogues relative to **1** by appropriate modifications at the reducing end of the carbohydrate chain. The *n*-octyl derivative **7** is particularly promising in this regard with early indications that it is cleared less rapidly than **1**. Taken together, the results indicate that it is possible to prepare simple analogues of **1** with improved pharmacokinetic properties that retain or enhance biological activity and that are easier to synthesize and to characterize. Efforts are now under way to prepare analogues with optimized properties.

Experimental Section

General. General experimental details have been given previously.[6] Heparanase was isolated from human platelets as described by Freeman and Parish.[33] PI-88 (**1**) was prepared as previously described.[17] $^{35}SO_3$·pyridine was from Amersham Biosciences, UK. The purity of polysulfated products (see Supporting Information) was determined by size exclusion HPLC and/or capillary electrophoresis (CE) as described previously.[6]

General Procedure for Deacetylation. A solution of the peracetate in anhydrous MeOH (0.1 M) 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). Acidic resin AG-50W-X8 (H^+ form) was added to adjust the pH to 6−7, the mixture was filtered and the resin was rinsed with MeOH. The combined filtrate and washings were concentrated in vacuo and dried to give the polyol product.

General Procedure for Sulfonation. A mixture of the polyol and SO_3·trimethylamine or SO_3·pyridine complex (2 equiv per alcohol) in DMF was heated (60 °C, overnight). The cooled (room temperature) reaction mixture was treated with MeOH and then made basic (to pH >10) by the addition of Na_2CO_3 (10% w/w). The mixture was filtered and the filtrate evaporated and coevaporated (H_2O). The crude polysulfated material was dissolved in H_2O and subjected to size exclusion chromatography (see below) to yield the sulfated product. After lyophilization the product was passed through an ion-exchange column (AG-50W-X8, Na^+ form, 1 × 4 cm, deionized H_2O, 15 mL) in order to transfer the product uniformly into the sodium salt form. The solution collected was evaporated and lyophilized to give the final product as a colorless glass or white power.

Size Exclusion Chromatography. Size exclusion chromatography (SEC) was performed over Bio-Gel P-2 in a 5 × 100 cm column and a flow rate of 2.8 mL/min of 0.1 M NH_4HCO_3, collecting 2.8 min (7.8 mL) fractions. Fractions were analyzed for carbohydrate content by spotting onto silica gel plates and visualization by charring, and/or analyzed for polysulfated species by the dimethyl methylene blue (DMB) test.[34] Finally, fractions were checked for purity by CE,[17] and those deemed to be free of salt were pooled and lyophilized. In cases where the presence of under sulfated byproducts or other salt contaminants was detected (normally only in small amounts), a Sephedex LH20 column chromatography step (2

Table 2. Pharmacokinetic Parameters Determined for ^{35}S-Labeled Compounds Following Iv Administration to Male Sprague−Dawley Rats (mean values ± SD, $n = 4$)

	1	**6**	**7**	**11**	**14**	**19**
C_0 (μg equiv/mL)	17.7 ± 2.23	20.5 ± 1.3	35.6 ± 3.1	30.5 ± 2.3	17.1 ± 1.8	14.0 ± 0.84
AUC_{0-12h} (μg equiv·h/mL)	9.6 ± 1.9	12.6 ± 1.2	29.7 ± 3.4	14.7 ± 1.2	6.2 ± 1.0[b]	6.5 ± 0.4[c]
$t_{1/2}$[a,d] (h)	0.83 ± 0.09	0.83 ± 0.02	1.10 ± 0.09	2.81 ± 0.04	0.59 ± 0.01	0.79 ± 0.03
k[a] (h^{-1})	0.844 ± 0.096	0.836 ± 0.024	0.633 ± 0.053	0.247 ± 0.003	1.17 ± 0.024	0.879 ± 0.028
Cl[a] (mL/h/kg)	250 ± 27.6	199 ± 13.2	83.6 ± 9.1	172 ± 11.8	404 ± 59.5	380 ± 24.3[c]
V_d[a] (mL)	43.1 ± 1.9	38.4 ± 3.8	22.9 ± 2.2	24.9 ± 2.9	44.5 ± 4.5	55.1 ± 2.6
urinary recovery (0−48 h) (% dose)	59.1 ± 13.1	39.3 ± 5.5	41.8 ± 1.5	66.5 ± 9.4	79.1 ± 3.6	80.5 ± 3.9

[a] Apparent values. [b] Calculated over 0−8 h postdose interval only. [c] Calculated over 0−4 h postdose interval only. [d] Calculated over the 0.75−4.0 h postdose interval for **1**, **6**, **7**, **14** and **19**; calculated over the 4.0−12 h postdose interval for **11**.

× 95 cm, deionized water, 1.2 mL/min, 3.5 min/fraction) was applied to remove them completely.

Peracetate 3. (a) The pentasaccharide α-D-Man-(1→3)-α-D-Man-(1→3)-α-D-Man-(1→3)-α-D-Man-(1→2)-D-Man (**2**) (1.03 g, 95% by HPLC), previously isolated by size exclusion chromatography of the neutral fraction obtained from the mild acid-catalyzed hydrolysis of the extracellular phosphomannan from *P. holstii* NRRL Y-2448 according to the literature procedure,[24] was acetylated using sodium acetate (1.2 g) in acetic anhydride (50 mL) at 140 °C for 18 h. After normal workup the crude product was purified by flash chromatography (EtOAc−hexane, 4:1) to give the peracetylated pentasaccharide **3** as a colorless gum (810 mg): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.14 (d, 0.84H, $J = 2.0$, αH1[I]), 5.71 (d, 0.16H, $J = 0.9$, βH1[I]), 5.30−5.10 (m, 8H), 5.00−4.85 (m, 7H), 4.25−3.70 (m, 19H), 2.20−1.90 (m, 51H); HRMS calcd for $C_{64}H_{87}O_{43}$ $[M + H]^+$ 1543.4623, found 1543.4599.

(b) Alternatively, the neutral fraction from the mild acid-catalyzed hydrolysis of the extracellular phosphomannan from *P. holstii* NRRL Y-2448 was directly acetylated (excess Ac_2O/pyridine/DMAP) at room temperature for 3 days. The peracetylated pentasaccharide **3** was then isolated by flash chromatography as described in method a above.

Benzyl Glycoside 4. To a solution of the peracetate **3** (225 mg, 0.146 mmol) in dry 1,2-DCE (7.3 mL, 0.02 M) was added benzyl alcohol (45 μL, 0.438 mmol). Boron trifluoride etherate (37 μL, 0.292 mmol) was added and the mixture was stirred under an atmosphere of argon at 60 °C for 2 h. The mixture was cooled and triethylamine (244 μL, 1.75 mmol) was added. The mixture was diluted with dichloromethane, washed with saturated aqueous sodium carbonate, and dried (anhydrous $MgSO_4$). The dried solution was filtered and the filter cake washed with dichloromethane. The combined filtrate and washings were concentrated, loaded onto silica gel, and purified by flash chromatography (hexanes−EtOAc, 6:1 → 1:4) to give the benzyl glycoside **4** as a colorless gum (108 mg, 46%): $R_f = 0.32$, hexanes−EtOAc, 1:3; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35−7.27 (m, 5 H, Ph), 5.30−5.12 (m, 8 H), 5.00−4.85 (m, 8 H), 4.68, 4.50 (AB q, $J = 11.8$ Hz, PhCH_2), 4.27−3.74 (m, 19 H), 2.14(4), 2.13(5), 2.13, 2.10, 2.08(4), 2.07(9), 2.07(6), 2.06(9), 2.06(6), 2.06 (×2), 2.02, 2.00, 1.99, 1.97, 1.94 (15s, 48 H, 16 × Ac); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.0, 170.5(3), 170.5(1), 170.5(0), 170.4, 170.3, 170.2, 170.0(4), 170.0-(2), 169.8(9), 169.8(8), 169.7, 169.6, 169.5(6), 169.4(6), 169.3, 136.1, 128.5, 128.2, 127.9, 99.2 (×2), 98.9, 98.8, 97.3, 76.7, 75.1, 74.9(9), 74.9(7), 71.1, 70.9, 70.8, 70.2, 69.7, 69.5(9), 69.5(6), 69.4(2), 69.3(7), 69.2, 68.6, 68.3, 67.1, 66.7(3), 66.6(7), 66.1, 65.5, 62.4, 62.1, 61.9, 61.6, 60.2, 20.9, 20.8(2), 20.8(0), 20.7(8), 20.7, 20.6, 20.5(4), 20.5(1), 20.4(9), 20.4(6).

Benzyl Glycoside Polysulfate 6. Compound **4** was deacetylated (HRMS calcd for polyol $C_{37}H_{59}O_{26}$ $[M + H]^+$ 919.3296, found 919.3279) and sulfonated according to the general procedures to give the product (**6**) as a white powder (76.1 mg, 44%): ^{1}H NMR (D_2O, 400 MHz) δ 7.35−7.26 (m, 5 H, Ph), 5.32 (s, 1 H), 5.30 (d, 1 H, $J = 1.2$ Hz), 5.26 (d, 1 H, $J = 2.0$ Hz), 5.24 (d, 1 H, $J = 1.6$ Hz), 5.05 (dd, 1 H, $J = 2.8$, 2.0 Hz), 5.00 (d, 1 H, $J = 2.0$ Hz), 4.87−4.85 (m, 2 H), 4.68−4.34 (m, 12 H), 4.32−3.86 (m, 17 H); ^{13}C NMR (D_2O, 100 MHz) δ 137.0, 129.5, 129.4, 129.1, 100.5(9), 100.5(6), 100.2, 97.9, 93.8, 76.9, 76.8, 75.6, 75.5(3), 75.4(8), 74.4, 73.8, 73.1, 73.0, 72.8, 72.7, 71.8, 71.3, 70.7, 70.6, 70.4, 69.9, 69.8, 69.7, 68.0, 67.8, 67.5, 66.6, 66.3(7), 66.3(5).

Octyl Glycoside 5. The peracetate **3** was glycosylated with octanol as described above for **4** to give the product (**5**) as a colorless gum (207 mg, 66%): $R_f = 0.41$, hexanes−EtOAc, 1:3; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.23−5.09 (m, 8 H), 4.96−4.82 (m, 8 H), 4.23−3.71 (m, 19 H), 3.59 (dt, 1 H, $J = 9.4$, 6.8 Hz, OCH_2R), 3.35 (dt, 1 H, $J = 9.4$, 6.8, OCH_2R), 2.11, 2.10(2), 2.09-(8), 2.06, 2.05, 2.04(4), 2.04(1), 2.03(8), 2.03, 2.02, 2.01, 1.99-(3), 1.98(8), 1.96, 1.94, 1.90 (16s, 48 H, 16 × Ac), 1.52 (quintet, 2 H, $J = 7.2$ Hz, CH_2), 1.27−1.18 (m, 10 H, $(CH_2)_5$), 0.80 (t, 3 H, $J = 7.2$ Hz, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.4(0) (×2), 170.3(8) (×2), 170.3, 170.2, 170.1, 169.9 (×2), 169.8(2), 169.7(5), 169.6, 169.5, 169.4(4), 169.3(5), 169.3, 99.1 (×2), 98.8, 98.7, 98.0, 77.0, 75.0, 74.8(3), 74.7(5), 71.0, 70.8, 70.7, 70.1, 69.4(9), 69.4(7), 69.3(0), 69.2(7), 69.2, 68.3, 68.2(0), 68.1(6), 67.2, 66.6(4), 66.6(0), 66.1, 65.4, 62.4, 62.3, 61.8, 61.5, 31.5, 29.1, 29.0, 28.9, 25.9, 22.4, 20.7(3), 20.7(0), 20.6(7), 20.6, 20.5, 20.4(3), 20.4(0), 20.3(9), 20.3(7), 13.8.

Octyl Glycoside Polysulfate 7. Compound **5** was deacetylated (HRMS calcd for polyol $C_{38}H_{69}O_{26}$ $[M + H]^+$ 941.40778, found 941.4060) and sulfonated according to the general procedures to give the product (**7**) as a white powder (195 mg, 72%): ^{1}H NMR (D_2O, 400 MHz) δ 5.33 (s, 1 H), 5.29 (d, 1 H, $J = 1.6$ Hz), 5.24 (d, 1 H, $J = 1.6$ Hz), 5.21 (d, 1 H, $J = 1.6$ Hz), 5.03 (dd, 1 H, $J = 2.8$, 2.0 Hz), 4.87 (d, 1 H, $J = 1.6$ Hz), 4.86−4.83 (m, 2 H), 4.70−3.92 (m, 27 H), 3.59 (dt, 1 H, $J = 9.6$, 7.0 Hz), 3.44 (dt, 1 H, $J = 9.6$, 7.0 Hz), 1.48−1.40 (m, 2 H), 1.21−1.08 (m, 10 H), 0.68 (t, 3 H, $J = 7.2$ Hz); ^{13}C NMR (D_2O, 100 MHz) δ 100.5, 100.4, 100.1, 100.0, 99.0, 98.4(1), 98.3-(8), 98.3(6), 98.3(5), 76.8(5), 76.7(9), 76.7, 76.6, 76.5(2), 76.4-(7), 76.0, 75.4(0), 75.3(5), 75.3, 75.2, 74.3, 73.0(5), 72.9(9), 72.7, 72.6, 71.7, 70.4, 70.2, 69.8(4), 69.7(5), 69.6, 69.1, 67.8(5), 67.7-(7), 66.5, 66.2, 31.5, 30.0, 28.8, 25.8, 22.5, 14.0.

Trichloroacetimidate 8. (a) A mixture of the peracetate **3** (68 mg, 51 μmol) and $BnNH_2$ (17 μL, 152 μmol) in THF (2 mL) was stirred (room temperature) for 2 d. The mixture was diluted with $CHCl_3$ (20 mL) and subjected to workup. The organic phase was evaporated and coevaporated (2 × 10 mL of MeCN) and used in the following reaction without further purification. (b) DBU (10 μL, 6.7 μmol) was added to a solution of the crude product from step a and trichloroacetonitrile (1.0 mL, 10 mmol) in 1,2-DCE (4 mL), and the combined mixture was stirred (0 °C→12 °C, overnight). The mixture was concentrated and the residue subjected to flash chromatography (50→90% EtOAc/hexanes) to yield **8** as a pale yellow oil (35 mg, 48%, two steps): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70 (s, 1 H, NH), 6.32 (d, 1 H, $J = 2.0$, H1[I]), 5.36−5.13 (m, 8 H), 5.00−4.90 (m, 6 H), 4.26−3.75 (m, 20 H), 2.15−1.94 (m, 48 H).

PEG_{5000} Polysulfate 11. (a) A mixture of the imidate **8** (33 mg, 20.2 μmol) and PEG_{5000}−monomethyl ether (151 mg, 30.3 μmol) in 1,2-DCE (3 mL) was stirred in the presence of molecular sieves (50 mg of 3-Å powder) under an atmosphere of argon (10 min). The mixture was cooled (−20 °C) with continuous stirring (10 min) prior to the addition of TMSOTf (5 μL, 2.8 μmol). After 20 min, Et_3N (10 μL) was introduced and the mixture was filtered. The solvent was evaporated and

the residue subjected to flash chromatography (0−7.5% MeOH/$CHCl_3$) to yield the glycoside **9** as a colorless glass (104 mg, 80%, based on average M_r 6483): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.28−4.87 (m, 14 H), 4.43−3.42 (m, 829 H,), 3.34 (s, 3 H, OMe), 2.15−1.94 (m, 48 H).

(b) Compound **9** (104 mg, 16 μmol) was deacetylated according to the general procedure to yield the polyol **10** as a colorless wax (82 mg, 89%, based on average M_r 5769). This residue was used in the next reaction without further purification or characterization.

(c) The polyol **10** (82 mg, 14 μmol) was sulfonated according to the general procedure to yield compound **11** as a colorless foam (45 mg, 42%, based on average M_r 7401): ^{1}H NMR (400 MHz, D_2O) δ 5.34−4.87 (m, 7 H), 4.71−3.97 (m, 20 H), 3.76−3.35 (m, 432 H), 3.23 (s, 3 H, OMe).

PEG_{2000} Polysulfate 14. (a) A mixture of the imidate **8** (60 mg, 36.5 μmol) and PEG_{2000}−OMe (110 mg, 55.0 μmol) was treated with TMSOTf as described for PEG_{5000}−OMe to yield the glycoside **12** as a colorless glass (96 mg, 74%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.28−5.13, 5.00−4.87, 4.27−3.40 (3 m, $H1^{I-V}$,2^{I-V},3^{I-V},4^{I-V},5^{I-V},$6a^{I-V}$,$6b^{I-V}$,OCH_2CH_2O), 3.34 (s, 3 H, OMe), 2.15−1.94 (16 s, 3 H each, Ac).

(b) The product from step a was deacetylated according to the general procedure to yield, presumably, the polyol **13** as a colorless wax (63 mg, 81%). This residue was used in the next reaction without further purification or characterization.

(c) The product from step b was sulfonated according to the general procedure to yield compound **14** as a colorless powder (47 mg, 68%): ^{1}H NMR (400 MHz, D_2O) δ 5.34−3.97 (m, 498 H), 3.80−3.35 (m, 81 H), 3.23 (s, 3 H, OMe).

Azide 15. A solution of peracetate **3** (270 mg, 175 μmol), $TMSN_3$ (60 mg, 525 μmol), and $SnCl_4$ (200 μL of 1 M in CH_2Cl_2) in anhydrous CH_2Cl_2 (20 mL) was stirred overnight in the dark. Additional quantities (3 equiv) of $TMSN_3$ and $SnCl_4$ were added, and stirring was continued in the dark overnight again. Ice and $NaHCO_3$ (saturated aqueous) were added, and the mixture was extracted with EtOAc, washed with brine, evaporated, and subjected to flash chromatography (EtOAc−hexane, 1:1 → 4:1) to yield azide **15** as a colorless oil (218 mg, 82%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.52 (d, 1 H, J = 2.0, $H1^I$), 5.29−5.12 (m, 8 H), 5.02−4.87 (m, 7 H), 4.29−3.76 (m, 19 H), 2.18−1.95 (m, 48 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5(9), 170.5(7), 170.5(6), 170.4, 170.3, 170.2, 170.1, 169.9(9), 169.9-(8), 169.9(5), 169.7(3), 169.6(9), 169.6(6), 169.6, 169.5, 169.3, 99.3(0), 99.2(7), 99.1, 99.0, 88.1, 75.2, 75.1, 74.8, 71.1, 70.9, 70.8, 70.6, 69.7, 69.5, 69.4, 69.2, 68.3, 67.3, 66.8, 66.7, 65.5(9), 65.5(8), 62.6, 62.2, 62.0, 61.7, 20.8(8), 20.8(6), 20.8, 20.7, 20.6-(2), 20.5(8), 20.5(7), 20.5; HRMS calcd for $C_{62}H_{84}N_3O_{41}$ $[M + H]^+$ 1526.4583, found 1526.4557.

Phenoxyacetamide 16. A solution of **15** (32 mg, 21 μmol), PPh_3 (11 mg, 42.6 μmol), and phenoxyacetyl chloride (7.3 mg, 43 μmol) in anhydrous acetonitrile (5 mL) was stirred at 0 °C for 4 h then at room temperature overnight. EtOAc and $NaHCO_3$ (saturated aqueous) were added, and the organic layer was washed with brine, dried ($MgSO_4$), and subjected to flash chromatography (EtOAc−hexane, 3:2 → 9:1) to yield 11.4 mg (33%) of amide **16** with some remaining PPh_3/PPh_3O: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36−7.32 (m, 2 H), 7.18 (br d, 1 H, J = 8.1, NH), 7.00−6.90 (m, 3 H), 5.79 (dd, 1 H, J = 3.8, 8.2, $H1^I$), 5.32−4.97 (m, 15 H), 4.60−3.76 (m, 21 H), 2.20−1.95 (m, 48 H, AcO); HRMS calcd for $C_{70}H_{92}NO_{43}$ $[M + H]^+$ 1634.5045, found 1634.5002.

Phenoxyacetamide Polysulfate 17. The peracetate **16** (11 mg, 6.7 μmol) was deacetylated and sulfonated according to the general procedures to yield 6 mg (34% for 2 steps) of **17** after lyophilization: ^{1}H NMR (400 MHz, D_2O, solvent suppressed) δ 7.30−7.21 (m, 2 H, ArH^m), 6.96−6.84 (m, 3 H, $ArH^{o,p}$), 5.56−3.59 (m, 30 H affected by suppression).

Biotinamidocaproamide 18. A mixture of **15** (70 mg, 46 μmol) and Adam's catalyst (2 mg) in EtOAc−EtOH (2:1, 3 mL) was stirred under H_2 (100 psi) overnight, filtered, evaporated, and coevaporated with anhydrous pyridine. Biotinamidocaproate *N*-hydroxysuccinimide ester (31 mg, 68 μmol) and anhydrous pyridine (1 mL) were added, and the mixture was heated to 60 °C for 3 days with stirring. The solution was evaporated and subjected to flash chromatography (Et_3N washed silica gel, EtOAc−hexane, 4:1 → MeOH-EtOAc, 3:7) to give 30.8 mg (36% over two steps) of amide **18**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (br d, 1 H, J = 9.4, NH), 6.47, 6.17 (2 × br s, 2 × 1 H, imide NHs), 5.40 (br d, 1 H, J = 9.4, $H1^I$), 5.40−4.90 (m, 16 H), 4.52 (dd, 1 H, J = 4.9, 7.5, biotin-H4), 4.36−3.72 (m, 20 H), 3.25−3.12 (m, 3 H), 2.91 (dd, 1 H, J = 5.0, 13.0, biotin-H5A), 2.75 (d, 1 H, J = 12.9, biotin-H5B), 2.27−1.96 (m, 52 H), 1.82−1.29 (m, 12 H, alkyl chains).

Biotinamidocaproamide Polysulfate 19. The peracetate **18** (30 mg, 16.3 μmol) was deacetylated and sulfonated according to the general procedures to yield 28 mg (61% for two steps) of **19** after lyophilization: ^{1}H NMR (400 MHz, D_2O, solvent suppressed, affected by amide rotamers) δ 5.60−4.75 (m, 7 H), 4.68 (dd, 1 H, J = 4.7, 7.2 Hz, biotin-H4), 4.60−3.60 (m, 26 H), 4.21 (dd, 1 H, J = 4.4, 7.2 Hz, biotin-H3), 3.33−3.16 (m, 1 H, biotin-H2), 3.07−2.97 (m, 3 H, biotin-H5A + CH_2N), 2.92 (dd, 1 H, J = 4.9, 13.5 Hz, biotin-H5B), 2.33−2.14 (m, 2 H, $COCH_2B$), 2.09 (t, 2 H, J = 7.4 Hz, $COCH_2A$), 1.63−1.15 (m, 12 H, alkyl chains).

Radiolabeling of Compounds. The polyol precursors for **1**, **6**, **7**, **11**, **14**, and **19** (2 mg of each) were desiccated under vacuum over P_2O_5 for 3 days. Into each vial was syringed 50 μL of a stock solution of $^{35}SO_3$·pyridine complex (1.77 mg, 2.0 mCi) and SO_3·Me_3N (2 mg) in anhydrous DMF (300 μL, redried over freshly activated 3-Å molecular sieves). A further 600 μL of anhydrous DMF was added to the SO_3 vial and was distributed to each sample vial. The samples were heated to 60 °C for 6 h. SO_3·Me_3N (14 mg in 300 μL of anhydrous DMF) was added to each vessel, and the resulting solutions were heated to 60 °C overnight. The vials were cooled to room temperature and each sample was quenched by addition of Na_2CO_3 (saturated aqueous adjusted to pH 8−9), evaporated to dryness, and subjected to SEC (Bio-Gel P-2, 2.6 × 90 cm, flow rate 30 mL/h, 5 min/fraction). Fractions containing the desired material were detected using a G-M counter and DMB test followed by CE. As anticipated, apart from the PEGylated compounds, all samples were detected clearly by CE in the expected fractions, and the fractions deemed to be free of contaminants were pooled and lyophilized. The PEGylated samples were pooled, based on the DMB assay and radioactivity of the fractions, and lyophilized. The specific activities and the chemical and radiochemical purities (determined by HPLC as previously described[31]) are presented in the Supporting Information.

Heparanase Inhibition Assays. The heparanase assays were performed using a Microcon ultrafiltration assay. A reaction was set up with a volume of 100 μL, 40 mM acetate buffer (pH 5.0), 0.1 mg/mL BSA, human platelet heparanase (100 ng), 5 μM [^{3}H]-labeled HS,[33] and various concentrations of inhibitors. The reactions were set up with all components except the [^{3}H]-labeled HS and allowed to equilibrate for 10 min at 22 °C. The assays were then initiated by adding the HS, and immediately 20 μL was taken and mixed with 80 μL of 10 mM phosphate (pH 7.0), and the 100 μL was transferred to a Microcon YM-10 concentrator which was then centrifuged at approximately 14 000*g* for 5 min. The solution that passed through the membrane (filtrate) was retained. This sample was considered the time = 0 sample. The assays (now 80 μL in volume) were allowed to react at 22 °C for 4 h and then the filtration step was repeated for three aliquots of 20 μL from each assay. The time = 0 filtrate and the three 4-h filtrate samples were counted for ^{3}H. The difference between the time = 0 and the averaged 4 h samples gave the amount of heparanase activity. All inhibition assays were run with a heparanase standard assay that was identical to the assay composition above, except no inhibitor was present; the amount of heparanase inhibition in the other assays was determined by comparison with this standard. The IC_{50} for **1** in this assay was 0.98 ± 0.11 μM. The results are presented in Table 1.

Growth Factor Binding Assays. Binding affinities of ligands for the growth factors FGF-1, FGF-2, and VEGF were

measured using a surface plasmon resonance solution affinity assay performed on a BIAcore 3000 (BIAcore, Uppsala, Sweden) operated using the BIAcore Control Software, as previously described.[6] Sensorgram data were analyzed using the BIAevaluation software (BIAcore). Background sensorgrams were subtracted from experimental sensorgrams to produce curves of specific binding, and baselines were subsequently adjusted to zero for all curves. Standard curves relating the relative response value to the injected protein concentration were linear, indicating that the binding response was proportional to the protein concentration, and thus suggesting that the binding experiments were conducted under mass transport conditions.[35] Therefore, the relative binding response for each injection can be converted to free protein concentration using the equation

$$[\mathrm{P}] = \frac{r}{r_\mathrm{m}}[\mathrm{P}]_\mathrm{total}$$

where r is the relative binding response and r_m is the maximal binding response.

Binding equilibria established in solution prior to injection were assumed to be of 1:1 stoichiometry. Therefore, for the equilibrium

$$\mathrm{P} + \mathrm{L} \rightleftharpoons \mathrm{P{\cdot}L}$$

where P corresponds to the growth factor protein, L is the ligand, and P·L is the protein:ligand complex, the equilibrium equation is

$$K_\mathrm{d} = \frac{[\mathrm{P}][\mathrm{L}]}{[\mathrm{P{\cdot}L}]}$$

and the binding equation[6] can be expressed as

$$[\mathrm{P}] = [\mathrm{P}]_\mathrm{total} - \frac{(K_\mathrm{d} + [\mathrm{L}]_\mathrm{total} + [\mathrm{P}]_\mathrm{total})}{2} + \sqrt{\frac{(K_\mathrm{d} + [\mathrm{L}]_\mathrm{total} + [\mathrm{P}]_\mathrm{total})^2}{4} - [\mathrm{L}]_\mathrm{total}[\mathrm{P}]_\mathrm{total}}$$

The K_d values given are the values fitted, using the binding equation, to a plot of [P] versus $[\mathrm{L}]_\mathrm{total}$. Where K_d values were measured in duplicate, the values represent the average of the duplicate measurements. The results are presented in Table 1.

Antiviral Assays. Monolayer cultures of African green monkey kidney cells[36] and herpes simplex virus (HSV-1) KOS321 strain[37] were used throughout. The antiviral assays for compounds **1**, **6**, **7**, **11**, **14**, **17**, and **19** were performed as described previously.[14] Briefly, the effects of the compounds on the infection of cells by exogenously added virus were tested by mixing serial 5-fold dilutions of compound (at 0.032−20 μM) with approximately 200 plaque forming units of the virus. Following incubation of the virus and compound for 10 min at room temperature, the mixture was added to the cells and left on the cell monolayer for 2 h at 37 °C. Subsequently, the inoculum was aspirated and replaced with an overlay medium of 1% methylcellulose solution in Eagle's minimum essential medium. The viral plaques that developed after incubation of cells for 3 days at 37 °C were stained with 1% crystal violet solution and counted. The effects of the compounds on cell-to-cell spread of HSV-1 were tested by adding serial 5-fold dilutions of compound (at 0.032−20 μM) in the serum-free overlay medium to cells after their infection with HSV-1. After incubation of the compound with the cells for 3 days at 37 °C, the images of 20 plaques were captured and subjected to area determination using IM500 software (Leica). The results for viral infection of cells and for viral cell-to-cell spread are shown in Figure 3, parts A and B, respectively, while the derived IC_{50} values are presented in Table 1. Note that compound **11** was tested only in the cell-to-cell spread assay because of lack of compound.

Pharmacokinetic Studies. Male Sprague−Dawley rats (250−350 g) were obtained from the Herston Medical Research Centre at Royal Brisbane Hospital. The animals were allowed free access to food and water before and during the experiments, during which they were maintained unrestrained in metabolism cages. Animal procedures were approved by the University of Queensland Group 5 Animal Ethics Committee. Rats were anaesthetized with isoflurane (Forthane). A catheter was inserted in the external jugular vein via an incision in the neck and was passed under the skin to a second incision in the skin of the back (midline vicinity of the scapulae). This was then exteriorized with the protection of a light metal spring. The incision was closed and the spring fixed to the skin with Michel sutures so that the rats had full range of movement. The animals were carefully monitored during recovery (1−4 h).

Stock dosing solutions were prepared by mixing appropriate amounts of unlabeled and radiolabeled drug (dissolved in phosphate-buffered saline) to give a total drug concentration of 1.25 mg/mL. All doses were administered as a bolus intravenous injection of 2.5 mg/kg in a dose volume of 2 mL/kg. The total amount of radioactivity administered to each rat was 0.5−10 μCi. The dose level used in this study is 10-fold lower than the no-effect dose previously established for acute toxicity of PI-88. Blood samples (~250 μL) were collected predose and at 5, 15, 30, and 45 min and 1, 1.5, 2, 4, 8, 12, 24, 36, and 48 h after dosing. The blood samples were immediately centrifuged, and the plasma was collected. At completion of the experiments, the animals were killed by a lethal overdose of iv pentobarbitone anaesthetic (Nembutal). Urine was collected from each animal at intervals of 0−12 h, 12−24 h, and 24−48 h after dosing. Cage washings (~15 mL of deionized water) were also collected. At the end of the experiment, bladder contents were aspirated from each animal and added to the 24−48 h voidings. Feces were collected over the same time intervals as the urine.

Aliquots of plasma (100 μL) and urine and cage washings (500 μL) were transferred directly to 6-mL polypropylene scintillation vials for determination of radioactivity. Feces collected during each time period (from one animal dosed with each compound) were weighed and homogenized in 4 volumes of deionized water using a mechanical homogenizer. Approximately 1 g (accurately weighed) of this slurry was transferred to a 20-mL glass scintillation vial, 2 mL of tissue solubilizer was added, and the vials were capped and incubated at 60 °C for at least 24 h. Radioactivity was measured following mixing of samples with Packard Ultima Gold liquid scintillation counting cocktail (2.0 mL for plasma and dose, 5.0 mL for urine and cage washings, 10 mL for feces). Counting was conducted on a Packard Tri-Carb liquid scintillation counter. Any result less than 3 times the background was considered less than the lower limit of quantification and was not used in calculations. Plasma, urine, and cage washings were counted in triplicate within 5 days of collection and were not corrected for radiochemical decay. Feces were processed as a batch at the completion of the study and the counts from these samples were corrected for radiochemical decay. Plasma pharmacokinetic parameters were calculated using PK Solutions 2.0 software (Summit Research Services) and are presented in Table 2.

Acknowledgment. We thank Drs. Brian Creese and Ian Bytheway for useful discussions and critical evaluation of the manuscript. We also thank Prof. Ron Dickinson for useful discussions and Andrew Wright and Sarika Prasad for technical assistance. This work was partially funded by an AusIndustry START grant to Progen Industries Ltd.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra for selected new compounds. Tables of HPLC and CE purity data for compounds **6**, **7**, **11**, **14**, **17**,and **19** and chemical and radiochemical purity and specific activity of radiolabeled

compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM050618P