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An Improved Synthetic Route to the Potent Angiogenesis Inhibitor Benzyl Manα(1→3)-Manα(1→3)-Manα(1→3)-Manα(1→2)-Man Hexadecasulfate*

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An improved synthetic route to α(1→3)/α(1→2)-linked mannooligosaccharides has been developed and applied to a more efficient preparation of the potent anti-angiogenic sulfated pentasaccharide, benzyl Manα(1→3)-Manα(1→3)-Manα(1→3)-Manα(1→2)-Man hexadecasulfate, using only two monosaccharide building blocks. Of particular note are improvements in the preparation of both building blocks and a simpler, final deprotection strategy. The route also provides common intermediates for the introduction of aglycones other than benzyl, either at the building block stage or after oligosaccharide assembly. The anti-angiogenic activity of the synthesized target compound was confirmed via the rat aortic assay.

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Introduction

Recently we described the synthesis[1] of α(1→3)/α(1→2)-linked mannooligosaccharides such as **1** and **2** (Fig. 1), which were subsequently sulfonated to provide a series of heparan sulfate mimetics related to the angiogenesis inhibitor PI-88.[2–4] The synthetic strategy involved the use of only two or three building blocks in a reiterative '1 + 1' strategy that provides ready access to all homologues in the series compared with the alternative '3 + 2' block approaches to the parent pentasaccharide.[5,6] The building blocks used were the starting block **4**[7] and an orthogonally protected elongating block **6**.[8,9] The strategy allowed for a final glycosylation, if desired, with a simple capping block depending on the desired length of the target oligosaccharide. Pentasaccharide **2** is also a starting material for the preparation of various polysulfated glycosides,[10] such as **3**, which have shown potent anti-angiogenic activity in various in vitro and in vivo models of angiogenesis.[11] However, as a route to **3** the previous synthesis is somewhat inefficient as it requires complete deprotection to the polyol **2** followed by acetylation and reintroduction of the benzyl group by glycosylation. As part of our ongoing studies into anti-angiogenic heparan sulfate mimetics we sought improved, more direct routes to compounds such as **3** that are amenable to multigram scale synthesis. Herein we describe improvements to the synthesis of α(1→3)/α(1→2)-linked mannooligosaccharides and their application to the preparation of **3**.

Results and Discussion

The alcohol **5** was selected as the glycosyl acceptor for the preparation of **3**. It was considered that **5** would be a superior starting block to **4** because the protecting groups are the same as in the elongating block **6**, thus eliminating one deprotection step before sulfonation. More importantly, later in the synthesis the anomeric benzyl group can be selectively removed and activated (e.g., by hydrogenolysis followed by trichloroacetimidate formation) to provide access to various different glycosides, if desired. Alternatively, the required aglycone can be introduced into the building block itself by substituting the appropriate alcohol for benzyl alcohol (Scheme 1). For the synthesis of **5** (Scheme 1) the readily available[12] orthoester **7** was deacetylated with saturated ammonia in methanol and then treated with benzoyl chloride in pyridine at room temperature[13] to give the tribenzoate **9** in good overall yield following crystallization from ethyl acetate/hexane. The orthoester was then hydrolyzed quantitatively by treatment with 90% trifluoroacetic acid (TFA) in water at room temperature for 45 min, and the resulting hemiacetal **10** was acetylated

*Dedicated to Professor Bob Stick on the occasion of his retirement.

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to give the diacetate **11** as an anomeric mixture ($\alpha:\beta = 5:1$) in 94% yield.[14] Without further purification, diacetate **11** was treated with benzyl alcohol and BF_3 etherate in dichloromethane at room temperature for 16 h to give the glycoside **12** in 73% yield after flash chromatography. Deacetylation was smoothly carried out in quantitative yield by treatment with 0.5% acetyl chloride in methanol. The resulting building block **5**, obtained as an oil, was pure enough for use in glycosylations without further purification. This sequence represents a more efficient route to **5** than our previously published method via the corresponding benzyl orthoester[15] (overall yield from the orthoester 38% versus 23%).

An improved preparation of the elongating block **6** starting from methyl α-D-mannopyranoside (**13**) was also developed, as illustrated in Scheme 2. The key step for the synthesis of **6** is the dibutyltin oxide-mediated selective allylation of **13** to give the 3-*O*-allyl ether **14**. At the completion of the reaction the solvent was evaporated and the residue was perbenzoylated with benzoyl chloride in pyridine to give the tribenzoate **15** in 51% yield after tedious flash chromatography. The major by-product from this reaction was the tetrabenzoate **16** formed from perbenzoylation of unreacted starting material **13**. On a large scale, it was possible to remove significant quantities of **16** by crystallization from methanol/ethyl acetate before chromatography. Acetolysis of compound **15** was accomplished by stirring in acetic anhydride at room temperature for 1 h with a catalytic amount (1% v/v) of conc. sulfuric acid. The resulting product was not purified but was treated directly with 5% acetyl chloride in methanol to give the hemiacetal **18** in excellent overall yield after chromatography. This procedure avoided the use of toxic amines (e.g., benzylamine, hydrazine) typically employed in large excess for similar transformations. The trichloroacetimidate **6** was then prepared under catalysis with diazobicycloundecane (DBU, 5 mol-%).

With the building blocks in hand, **5** was glycosylated with **6** in 1,2-dichloroethane at 0°C under catalysis by trimethylsilyltriflate (TMSOTf, 0.2 equiv.) to give the disaccharide **19** in good yield (84%). The allyl group was removed by treatment with $PdCl_2$ to give the alcohol **20** in 80% yield after flash chromatography, ready for further glycosylation with **6**. Two subsequent

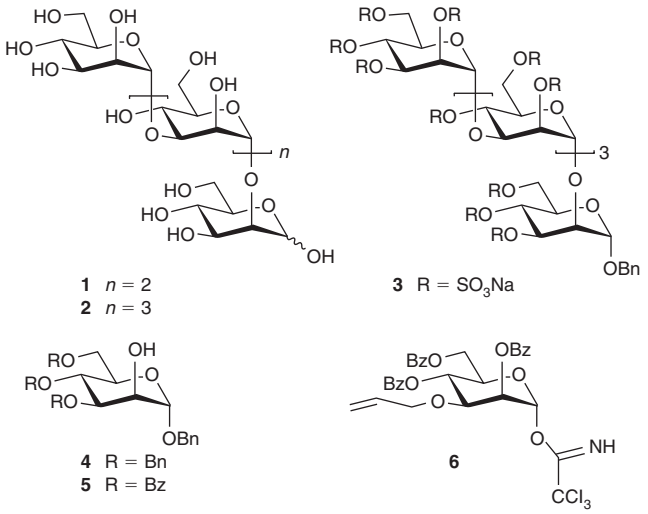
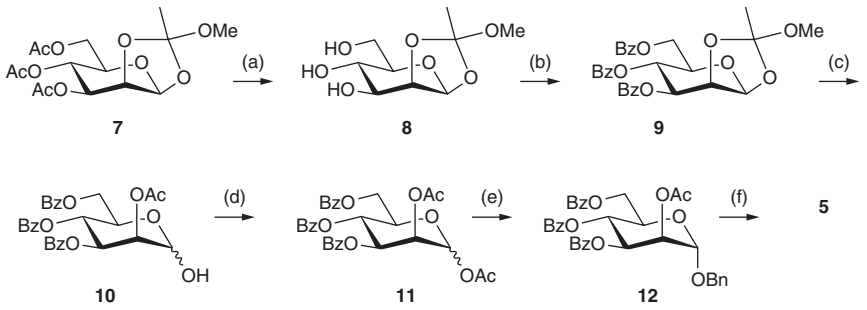
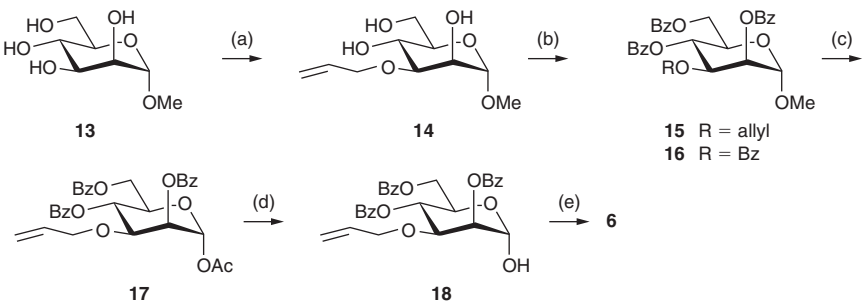


Fig. 1.



Scheme 1. *Reagents and conditions:* (a) NH_3/MeOH, 4°C, 16 h (or r.t., 4 h), 92%. (b) BzCl, DMAP, pyridine, r.t., 1–16 h, 60%. (c) ~90% TFA/water, r.t., 45 min, 100%. (d) Ac_2O, DMAP, pyridine, r.t., o/n, 94%. (e) BnOH, BF_3 etherate, CH_2Cl_2, r.t., o/n, 73%. (f) 0.5% AcCl in MeOH, r.t., o/n, 100%.



Scheme 2. *Reagents and conditions:* (a) (i) Bu_2SnO, MeOH, 3 h, 80°C, (ii) allyl-Br, Bu_4NBr, PhMe, 60°C, 2 days. (b) BzCl, pyridine, r.t., o/n, 27–51% (two steps). (c) Conc. H_2SO_4 in Ac_2O (1% v/v), r.t., 1 h. (d) 5% AcCl in MeOH, r.t., 5 h, 72% (two steps). (e) Cl_3CCN (2 equiv.), DBU (5 mol-%), CH_2Cl_2, 0°C, 5 h, 98%.

rounds of glycosylation/deallylation followed in similar yields (Scheme 3), which led to the pentasaccharide **26**. Removal of the benzoates was accomplished by treatment with NaOMe in MeOH, although the limited solubility of compound **26** resulted in incomplete deprotection, which necessitated purification of the polyol by reverse phase HPLC in this case. Sulfonation with SO_3·pyridine complex in *N*,*N*-dimethylformamide (DMF) then gave the desired sulfated pentasaccharide **3** in 72% yield following purification by dialysis, identical in all respects to a previously prepared sample.[10]

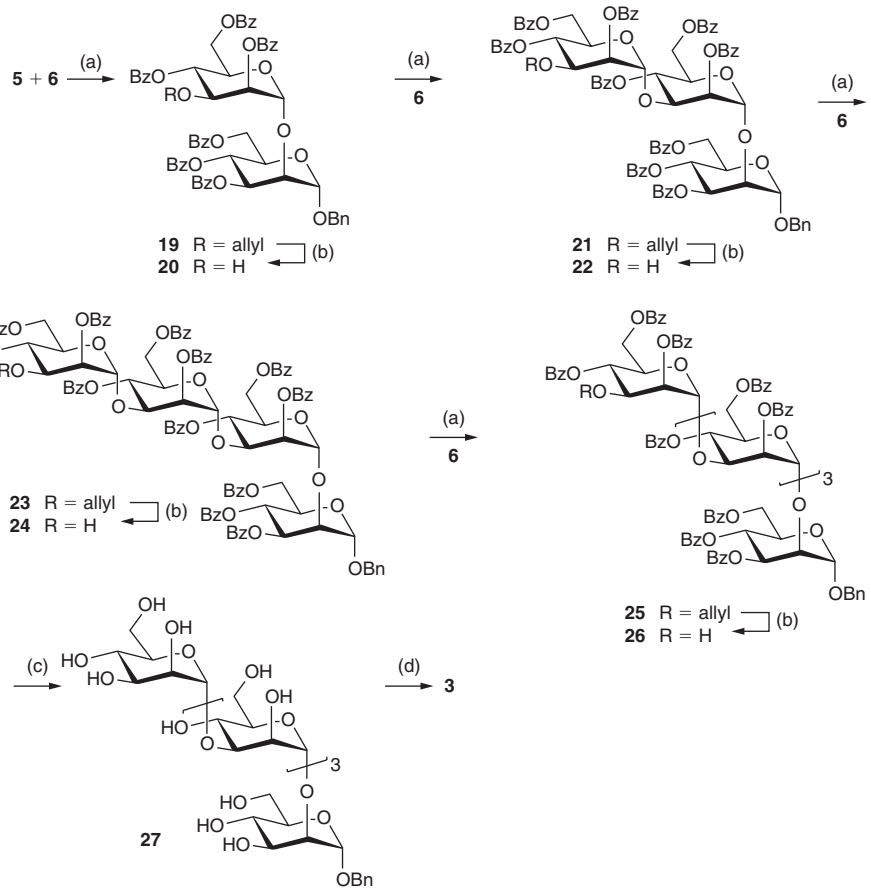
Compound **3** has displayed potent anti-angiogenic activity in several cell-based assays indicative of angiogenesis such as growth factor-induced endothelial cell proliferation assays and the endothelial cell tube formation (Matrigel) assay.[11] In addition, compound **3** has shown potent anti-angiogenic activity in vivo in two murine models.[11] In this study, compound **3** was evaluated in a modified version of the rat aortic assay[16,17] (Fig. 2), which confirmed its potent anti-angiogenic activity. This assay is considered to come closest to simulating the in vivo situation, not only because it includes the surrounding non-endothelial cells but also because the endothelial cells have not been preselected by passaging and thus are not in a proliferative state at the time of explantation.[18] The data presented supports further investigation of **3** as an anti-angiogenic agent.

In conclusion, we have developed an improved synthetic route to α(1→3)/α(1→2)-linked mannooligosaccharides and have applied this to a more efficient preparation of the anti-angiogenic sulfated pentasaccharide **3**. Of particular note are the use of a benzoyl protected starting block **5**, which results in a simpler final deprotection strategy, and improvements in both the preparation of **5** and of the elongating block **6**. The route also provides common intermediates for the introduction of any desired aglycone, either at the building block stage or after oligosaccharide assembly, without requiring complete deprotection and re-acetylation/activation/glycosylation.

Experimental

General

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 deuterium oxide (D_2O), employing residual HOD (^{1}H, δ 4.78) as an internal standard, at ambient temperatures (298 K). Where appropriate, analyses of ^{1}H NMR spectra were aided by gradient-selected correlation spectroscopy (gCOSY) experiments. Flash chromatography was performed on silica gel (40–63 μm) under a positive pressure with specified eluants. All solvents used were of analytical grade. The progress of reactions was monitored by TLC using commercially prepared silica gel 60 F_{254} aluminium-backed plates. Compounds were visualized by charring with 5% sulfuric acid in methanol and/or by visualization under ultraviolet light. HPLC was performed on a Gilson preparative HPLC system, controlled using Trilution software, running a gradient of 5 to 95% acetonitrile in water



Scheme 3. *Reagents and conditions:* (a) TMSOTf (0.2–1 equiv.), DCE (0.1 M), 3 Å MS, Ar, 0°C, 84–94%. (b) $PdCl_2$ (40 mg mmol^{-1}), MeOH/DCE (1/1, v/v, 0.05 M), 70°C, 40 min, 64–91%. (c) NaOMe, MeOH, r.t., o/n, 67%. (d) (i) SO_3·pyridine (3 equiv./OH), DMF (0.04 M), 60°C, o/n, (ii) NaOH, 72% (two steps).

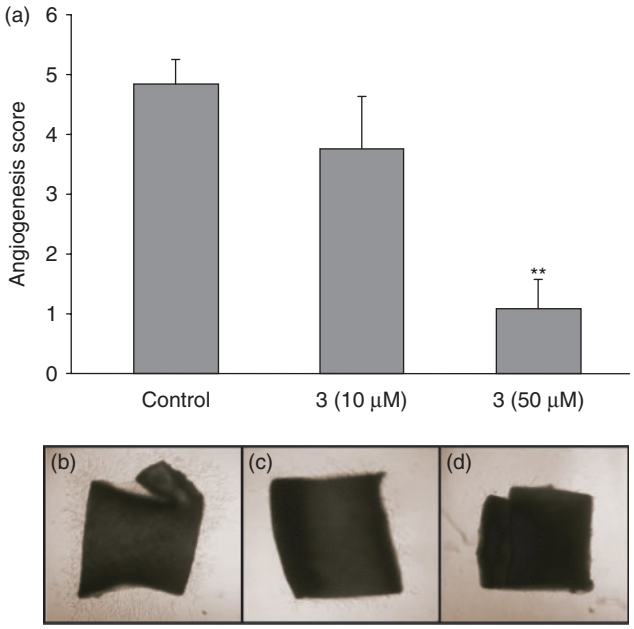


Fig. 2. Pentasaccharide **3** significantly inhibits the sprouting of new microvessels in the rat aortic assay for angiogenesis. Aortic explants were embedded in Matrigel on day 0. Treatment with **3** commenced on day 1 and the extent of angiogenesis in each group was determined on day 8 (a). The assay was scored from 0 (least positive) to 5 (most positive) and the data presented as mean ($n = 6$); bars + s.e.m.[16] $^{**}P < 0.01$ versus control. Statistical analysis was conducted using a oneway ANOVA followed by a post hoc Dunnett's test (Graphpad Instat v3.0). The photographs taken on day 4 illustrate the inhibitory effects of **3** at 10 μM (c) and 50 μM (d) in comparison to control (b).

over 30 min through an Atlantis Prep dC18 5 μm 19 × 150 mm column. HPLC detection was performed with a Gilson PrepELS detector and a Waters dual wavelength absorbance detector measuring the absorbance at 254 and 214 nm. Capillary electrophoresis (CE) was performed on an Agilent CE System with the use of 10 μm M 5-sulfosalicylic acid at pH 3 as the background electrolyte and detection by indirect UV absorbance at 214 nm, as previously described.[19] Compound homogeneity was determined by ^{1}H and/or ^{13}C NMR spectroscopy and, where appropriate, by CE.

*3,4,6-Tri-*O*-benzoyl-1,2-*O*-(methoxyethylidene)-β-D-mannopyranose* ***9***

3,4,6-Tri-*O*-acetyl-1,2-*O*-(methoxyethylidene)-β-D-mannopyranose[12] **7** (8.0 g, 0.022 mol) was dissolved in dry MeOH (70 mL). Saturated NH_3 in MeOH (25 mL) was added dropwise over 15 min at 0°C and stirring continued for 16 h at 4°C. The solvent was evaporated and the residue purified on a column of silica gel (MeOH/EtOAc 1/10 → 1/1 → EtOAc → MeOH) and co-evaporated with toluene (3 × 50 mL) to give the triol **8** as a yellow syrup (4.8 g, 92%), used directly in the next step. The triol and DMAP (cat.) were dissolved in dry pyridine (20 mL) and benzoyl chloride (6 equiv., 0.12 mol, 14 mL) was added at 0°C and stirring was continued for 16 h. The mixture was poured onto ice-water (200 mL) and stirred with MeOH/water (1/1, 200 mL). The precipitate was washed with MeOH/water (1/5, 3 × 100 mL) and water (3 × 100 mL) and then dissolved in dichloromethane (200 mL). The solution was washed sequentially with $NaHCO_3$ (sat.) (3 × 100 mL), ice-cold 0.5 M HCl (1 × 100 mL), and $NaHCO_3$ (sat.) (1 × 100 mL), dried (Na_2SO_4), filtered, and the solvent evaporated to yield an orange syrup. Recrystallization from EtOAc/hexane gave the tribenzoate **9** as needles (7.6 g, 70% from triol). ^{1}H NMR ($CDCl_3$) data were in accord with the literature.[13]

*3,4,6-Tri-*O*-benzoyl-1,2-di-*O*-acetyl-D-mannopyranose* ***11***

The tribenzoate **9** (6.5 g, 12 mmol) was treated with 90% aqueous TFA (20 mL) at 0°C and was stirred at room temperature for 45 min. The solvent was evaporated and co-evaporated with toluene (3 × 50 mL) and dried for 16 h under vacuum to give the hemiacetal **10** as a yellow foam (6.87 g, 100%), used directly in the next step. ^{1}H NMR (400 MHz) data were in accord with the literature.[14] The product and DMAP (cat.) were dissolved in dry pyridine (30 mL) and acetic anhydride (0.033 mol, 3.1 mL) in dry pyridine (6 mL) was added dropwise over 15 min at 0°C and stirring continued for 2 h at room temperature. The solution was quenched by adding dry MeOH (10 mL) at 0°C and stirred for 15 min. The solution was concentrated under vacuum, and co-evaporated with toluene (50 mL) and dichloromethane (5 mL) to give the diacetate **11** as a yellow foam (7.05 g, 94%, α:β = 5:1), used in the next step without further purification. ^{1}H NMR ($CDCl_3$) data were in accord with the literature.[14]

*Benzyl 3,4,6-Tri-*O*-benzoyl-2-*O*-acetyl-α-D-mannopyranoside* ***12***

The diacetate **11** (101 mg, 0.175 mmol) and dry benzyl alcohol (0.21 mmol, 1.2 equiv., 0.022 mL) was dissolved in dry dichloromethane (1 mL) and boron trifluoride etherate (0.875 mmol, 5 equiv., 0.124 mL) was added dropwise within 15 min at 0°C under Ar and stirring continued for 16 h at room temperature. The solution was quenched by adding triethylamine (0.175 mL) at 0°C (pH 8). The mixture was taken up in dichloromethane (50 mL), washed with $NaHCO_3$ (sat.) (3 × 20 mL), dried (Na_2SO_4), filtered, and the solvent was co-evaporated with toluene (2 × 20 mL) to yield an orange syrup. The compound was purified by flash chromatography (hexane/EtOAc 3/1 → EtOAc) to give the glycoside **12** as a colourless syrup (80 mg, 73%). ^{1}H and ^{13}C NMR ($CDCl_3$) data were in accord with the literature.[15]

*Benzyl 3,4,6-Tri-*O*-benzoyl-α-D-mannopyranoside* ***5***

A solution of the glycoside **12** (100 mg, 0.16 mmol) in dry MeOH/1,2-dichloroethane (DCE) (2/1, 3 mL) was treated with AcCl (0.3 mL, 10% v/v). The mixture was stirred at 0°C overnight and then at room temperature for 3 h. The solution was poured into a mixture of ice/water and chloroform (1/1, 100 mL). The organic phase was separated, washed with sat. $NaHCO_3$ (20 mL) and brine (20 mL), was dried (Na_2SO_4), filtered, and evaporated to give the alcohol **5** as an oil (97 mg, 100%). ^{1}H and ^{13}C NMR ($CDCl_3$) data were in accord with the literature.[15]

*Methyl 3-*O*-Allyl-2,4,6-tri-*O*-benzoyl-α-D-mannopyranoside* ***15***

A mixture of methyl α-D-mannopyranoside (2.0 g, 10.5 mmol) and Bu_2SnO (2.9 g, 11.5 mmol) in MeOH (50 mL) was heated (80°C, overnight) with azeotropic removal of H_2O throughout.

The solvent was then evaporated and co-evaporated (CH_3CN, 2×50 mL) to yield a yellow powder. Bu_4NBr (3.7 g, 11.5 mmol) and allyl bromide (4.5 mL, 50 mmol) in toluene (100 mL) were added to the above powder and the combined mixture was heated (60°C, 2 days). The solvent was evaporated and co-evaporated (CH_3CN, 2×50 mL) to yield a white powder. The powder was dissolved in pyridine (15 mL) and DCE (50 mL), and benzoyl chloride (2.4 mL, 20.9 mmol) was added. The mixture was stirred at room temperature overnight, cooled (0°C), and treated with MeOH (5 mL) with continued stirring (0°C → room temperature, 5 min) before evaporation of the solvent. The residue was subjected to workup (EtOAc) and flash column chromatography (EtOAc/hexane, 1/19 to 3/17) to give the tribenzoate **15** as a colourless oil (3.2 g, 51%, 3 steps). ^{1}H NMR ($CDCl_3$) data were in accord with the literature.[8] δ_C ($CDCl_3$) 55.59 (OMe), 63.46 (C-6), 68.68, 68.97, 69.34, 70.98, 74.63 (C-2, -3, -4, -5, OCH_2), 99.16 (C-1), 117.74 (=CH_2), 128.58–134.41 (16C, =CH, Ar), 165.59, 165.95, 166.44 (3C, C=O).

3-O-Allyl-2,4,6-tri-O-benzoyl-α-D-mannopyranose ***18***

To a solution of the methyl glycoside **15** (6.70 g, 12.26 mmol) in dichloromethane (40 mL, 0.3 M) and acetic anhydride (2.31 mL, 24.52 mmol, 2 equiv.) was added concentrated H_2SO_4 (0.4 mL, 1% v/v, ~7.52 mmol). The mixture was stirred at room temperature while the reaction was monitored by TLC (a 20 μL aliquot was treated with 20 mg of Na_2CO_3. Dichloromethane (0.2 mL) was added and the mixture was shaken well and the dichloromethane solution was used for TLC in hexane/EtOAc, 3/1). After completion (1 h), the mixture was treated with NaOAc (2.1 g, 25 mmol). After stirring at room temperature for 1 h, the mixture was filtered through a plug of celite. The solid was washed with dichloromethane (2×10 mL). The combined filtrate and washings were evaporated to dryness, co-evaporated with MeOH (2×10 mL) under vacuum to give the acetate **17** as a pale-yellow gum/foam (7.98 g), and used directly in the next step without purification. δ_H ($CDCl_3$) 8.08–8.04 (m, 6H, Bz), 7.60–7.53 (m, 3H, Bz), 7.46–7.36 (m, 6H, Bz), 6.30 (d, 1H, $J_{1,2}$ 2.4, H-1), 5.90 (dd, 1H, $J_{3,4} = J_{4,5}$ 9.8, H-4), 5.76–5.65 (m, 1H, allyl-2′), 5.60 (dd, 1H, $J_{2,3}$ 3.4, H-2), 5.17 (dm, 1H, J 17.1, allyl-3′), 5.06 (dm, 1H, J 10.3, allyl-3′), 4.66 (dd, 1H, $J_{6a,6b}$ 12.2, $J_{5,6a}$ 2.9, H-6a), 4.40 (dd, 1H, $J_{5,6b}$ 3.9, H-6b), 4.33 (ddd, 1H, H-5), 4.18–4.12 (m, 1H, allyl-1′), 4.16 (dd, 1H, H-3), 4.01 (ddm, 1H, J 12.7, 5.9, allyl-1′), 2.22 (s, 3H, Ac). The above product was suspended in MeOH (24.5 mL, 0.5 M) and acetyl chloride (1.23 mL, 5% v/v, 17.3 mmol) was added at room temperature. (Note: the solubility was improved significantly after the addition of AcCl.) The resulting slightly pink suspension was stirred at room temperature for 5 h. The mixture was cooled to 0°C and Et_3N (4.8 mL, 34.6 mmol, 2 equiv. based on AcCl) was added. The mixture was concentrated to a small volume (~14 mL). The yellow suspension was stirred at 0°C while water (42 mL) was added dropwise to result in the formation of a thick gummy precipitate. After standing at 4°C overnight, the top clear solution was decanted. The gum was dissolved in MeOH/EtOAc (2/1, 30 mL) and loaded onto silica gel. Purification by flash chromatography (hexane/EtOAc, 8/1 → 3/1) gave the hemiacetal **18** as a white foam (4.70 g, 72%, 2 steps). ^{1}H NMR ($CDCl_3$) data were in accord with the literature:[8,9] δ_H 8.12–8.01 (m, 6H, Bz), 7.60–7.52 (m, 3H, Bz), 7.47–7.31 (m, 6H, Bz), 5.87 (dd, 1H, $J_{3,4} = J_{4-5}$ 9.7, H-4), 5.76–5.64 (m, 1H, allyl-2′), 5.60 (dd, 1H, $J_{2,3}$ 3.5, $J_{1,2}$ 1.8, H-2), 5.44 (d, 1H, H-1), 5.16 (dm, 1H, J 17.5, allyl-3′), 5.04 (dm, 1H, J 10.5, allyl-3′), 4.72 (dd, 1H, $J_{6a,6b}$ 12.0, $J_{5,6a}$ 2.6, H-6a), 4.49 (ddd, 1H, $J_{5,6b}$ 3.5, H-5), 4.37 (dd, 1H, H-6b), 4.23 (dd, 1H, H-3), 4.16–4.09 (m, 1H, allyl-1′), 4.02–3.97 (m, 1H, allyl-1′), 1.65 (br s, 1H, OH).

3-O-Allyl-2,4,6-tri-O-benzoyl-α-D-mannopyranosyl Trichloroacetimidate ***6***

The hemiacetal **18** (9.4 g, 13.9 mmol) was dissolved in anhydrous dichloromethane (28 mL, 0.5 M) and trichloroacetonitrile (2.8 mL, 27.7 mmol, 2 equiv.) was added. The mixture was stirred at 0°C while DBU (104 μL, 0.69 mmol, 5 mol-%) was added. The mixture was stirred at 0°C. After 4 h, TLC (hexane/EtOAc, 4/1) indicated the presence of a small amount of starting material. Another portion of trichloroacetonitrile (1 mL) was added. After stirring at 0°C for another 1 h (TLC: no further change), the mixture was concentrated, treated with Et_3N (6 mL), and evaporated onto silica gel. Purification by flash chromatography (hexane/EtOAc/Et_3N, 450/50/1 → 360/120/1) gave the imidate **6** as a white foam (9.2 g, 98%). ^{1}H NMR ($CDCl_3$) data were in accord with the literature.[8,9]

Benzyl 2,4,6-Tri-O-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzoyl-α-D-mannopyranoside ***20***

A mixture of the imidate **6** (1.00 g, 1.36 mmol, 1.1 equiv.) and the alcohol **5** (717 mg, 1.23 mmol) in DCE (10 mL) in the presence of molecular sieves (500 mg of 3 Å powder) was treated with TMSOTf (246 μL, 1.36 mmol) at 0°C. The combined mixture was stirred at 0°C for 1.5 h at which point TLC (EtOAc/hexane, 3/7) indicated complete conversion. Et_3N (100 μL) was introduced, the mixture was filtered, and the solvent was evaporated. The residue was subjected to flash column chromatography (EtOAc/hexane, 1/9 to 1/1) to yield the disaccharide **19** as a colourless foam (1.20 g, 84%). $PdCl_2$ (50 mg) was added to a solution of the above disaccharide **19** (1.13 g, 0.97 mmol) in MeOH (10 mL) and DCE (10 mL), and the combined mixture was heated (70°C, 40 min). The mixture was cooled and filtered. The filtrate was evaporated and the residue subjected to flash column chromatography (EtOAc/hexane, 1/9 to 1/1) to yield the disaccharide alcohol **20** as a colourless glass (873 mg, 80%). δ_H ($CDCl_3$) 8.09–7.89, 7.57–7.28 (2 m, 35H, ArH), 5.98 (dd, 1H, $J_{3I,4I-4I,5I}$ 9.9, H-4^I), 5.87 (dd, 1H, $J_{2I,3I}$ 3, 7, H-3^I), 5.64 (dd, 1H, $J_{3II,4II-4II,5II}$ 9.7, H-4^{II}), 5.60 (dd, 1H, $J_{1II,2II}$ 1.8, $J_{2II,3II}$ 3.3, H-2^{II}), 5.18 (d, 1H, H-1^{II}), 5.14 (d, 1H, $J_{1I,2I}$ 1.8, H-1^I), 4.71 (d, 1H, A of ABq, $J_{A,B}$ 11.9, CH_2Ph), 4.59 (dd, 1H, $J_{5I,6I}$ 2.7, $J_{6I,6I}$ 12.2, H-6^{I}a), 4.48–4.42, 4.35–4.30 (2 m, 8H, H-2^I, -3^{II}, -5^{I,II}, -6^{I}b, -6a^{II}, -6b^{II}, CH_2Ph). δ_C ($CDCl_3$) 166.97, 166.40, 166.23, 165.90, 165.61, 165.58 (6C, C=O), 136.58 (Ar*C*CH_2), 133.78, 133.64, 133.53, 133.35, 133.30 (6C, Ar*C*CO), 130.20–128.14 (35C, ArCH), 99.79, 97.98 (C1^{I,II}), 77.43, 72.61, 70.98, 70.27, 69.97, 69.58, 69.46, 69.02, 67.49 (C-2^{I,II}, -3^{I,II}, -4^{I,II}, -5^{I,II}, CH_2Ph), 63.39, 63.13 (C-6^{I,II}). *m/z* (ESMS) 1015.2 $[M+H]^+$, 1037.2 $[M+Na]^+$.

Benzyl 2,4,6-Tri-O-benzoyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-O-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzoyl-α-D-mannopyranoside ***22***

A mixture of the imidate **6** (900 mg, 1.2 mmol, 1.1 equiv.) and the disaccharide alcohol (**20**, 1.24 g, 1.1 mmol) in DCE (8 mL) in the presence of molecular sieves (500 mg of 3 Å powder) was treated with TMSOTf (50 μL, 0.27 mmol, 0.25 equiv.) at 0°C. The combined mixture was stirred at 0°C for 4 h. TLC (EtOAc/hexane,

2/3) indicated complete conversion. Et_3N was then introduced. The mixture was filtered and the solvent was evaporated. The residue was subjected to flash column chromatography (EtOAc/hexane, 1/9 to 1/1) to yield the trisaccharide **21** as a colourless oil, which was used directly for de-*O*-allylation. $PdCl_2$ (50 mg) was added to a solution of the above trisaccharide **21** in MeOH (10 mL) and DCE (10 mL) and the combined mixture was heated (80°C, 2 h). The mixture was cooled and filtered. The filtrate was evaporated and the residue subjected to flash column chromatography (EtOAc/hexane, 1/9 to 1/1) to yield the trisaccharide alcohol **22** as a colourless oil (1.14 g, 71%, 2 steps). δ_H ($CDCl_3$) 8.07–7.78, 7.62–7.13 (2 m, 50H, ArH), 5.96 (dd, 1H, $J_{3I,4I–4I,5I}$ 9.9, H-4^I), 5.93 (dd, 1H, $J_{3III,4III–4III,5III}$ 9.7, H-4^{III}), 5.82 (dd, 1H, $J_{2I,3I}$ 3.3, H-3^I), 5.76 (dd, 1H, $J_{1III,2III}$ 2.0, $J_{2III,3III}$ 3.1, H-2^{III}), 5.65 (dd, 1H, $J_{3II,4II–4II,5II}$ 9.9, H-4^{II}), 5.32 (d, 1H, $J_{1II,2II}$ 1.5, H-1^{II}), 5.21 (d, 1H, H-1^{III}), 5.16 (d, 1H, $J_{1I,2I}$ 1.5, H-1^I), 5.08 (dd, 1H, $J_{2II,3II}$ 3.1, H-2^{II}), 4.69 (d, 1H, A of ABq, $J_{A,B}$ 11.8, CH_2Ph), 4.66 (dd, 1H, H-3^{III}), 4.60–4.30 (m, 11H, H-2^I, -$5^{I–III}$, -6a$^{I–III}$, -6b$^{I–III}$, CH_2Ph), 4.17 (dd, 1H, H-3^{II}). δ_C ($CDCl_3$) 166.64–165.22 (9C, C=O), 136.53–132.99 (10C, ArC), 130.24–128.48 (50C, ArCH), 99.78, 99.56, 97.94 (C-$1^{I–III}$), 77.64, 75.91, 72.49, 71.63, 71.17, 69.93, 69.91, 69.76, 69.57, 69.25, 68.70, 68.33, 67.70 (C-$2^{I–III}$, -$3^{I–III}$, -$4^{I–III}$, -$5^{I–III}$, CH_2Ph), 63.82, 63.07, 62.34 (C-$6^{I–III}$). *m/z* (ESMS) 1511.4 $[M + Na]^+$.

Benzyl 2,4,6-Tri-O-benzoyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-O-benzoyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-O-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzoyl-α-D-mannopyranoside ***24***

A mixture of the imidate **6** (121 mg, 0.167 mmol, 1.2 equiv.) and the trisaccharide alcohol **22** (201 mg, 0.139 mmol) in DCE (5 mL) in the presence of molecular sieves (50 mg of 3 Å powder) was treated with TMSOTf (10 µL, 0.055 mmol, 0.4 equiv.) at 0°C. The combined mixture was stirred at 0°C for 40 min. TLC (EtOAc/hexane, 40/60) indicated the complete conversion and Et_3N was introduced. The mixture was filtered and the solvent was evaporated. The residue was subjected to flash column chromatography (EtOAc/hexane, 1/9 to 1/1) to yield the tetrasaccharide **23** as a colourless oil, which was used directly for de-*O*-allylation. $PdCl_2$ (50 mg) was added to a solution of the above tetrasaccharide **23** in MeOH (10 mL) and DCE (10 mL) and the combined mixture was heated (80°C, 2 h). The mixture was cooled and filtered. The filtrate was evaporated and the residue subjected to flash column chromatography (EtOAc/hexane, 1/9 to 1/1) to yield the tetrasaccharide alcohol **24** as colourless oil (206 mg, 77%, 2 steps). δ_H ($CDCl_3$) 8.12–7.08 (m, 65H, Ph), 6.00–5.90 (m, 3H, H-4$^{I–III}$), 5.85 (dd, $J_{2I,3I}$ 3.2, $J_{3I,4I}$ 10, H-3^I), 5.80 (dd, $J_{1III,2III}$ 2.0, $J_{2III,3III}$ 3.2, H-2^{III}), 5.48 (dd, $J_{3IV,4IV}$~$J_{4IV,5IV}$ 9.6, H-4^{IV}), 5.34 (d, $J_{1II,2II}$ 1.6, H-1^{II}), 5.28 (dd, $J_{2II,3II}$ 2.8, H-2^{II}), 5.22 (d, H-1^{III}), 5.16 (d, $J_{1I,2I}$ 1.6, H-1^I), 4.92 (dd, $J_{1IV,2IV}$ 1.6, $J_{2IV,3IV}$ 3.2, H-2^{IV}), 4.88 (d, H-1^{IV}), 4.72 (d, 1H, A of ABq, J_{AB} 11.6, PhCH_2), 4.65 (dd, $J_{3III,4III}$ 9.6, H3^{III}), 4.59 (dd, $J_{5,6a}$ 3.2, $J_{6a,6b}$ 12.4, H6a), 4.55–4.20, 3.97–3.94 (2m, 13H), 4.06 (dd, H3^{IV}), 3.98 (m, H5^{IV}). δ_C ($CDCl_3$) 166.50–165.15 (12C, C=O), 136.51–132.93 (13C, ArC), 130.34–128.23 (65C, ArCH), 99.68, 99.31, 99.28, 97.89 (C-$1^{I–IV}$), 77.30, 76.48, 76.24, 72.49, 71.58, 71.43, 71.08, 69.99, 69.86, 69.72, 69.29, 69.00, 68.54, 68.17, 67.82, 67.54 (C-$2^{I–IV}$, -$3^{I–IV}$, -$4^{I–IV}$, -$5^{I–IV}$, CH_2Ph), 63.88, 63.02, 62.42, 62.31 (C-$6^{I–IV}$).

Benzyl 2,4,6-Tri-O-benzoyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-O-benzoyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-O-benzoyl-α-D-mannopyranosyl-(1→3)-2,4,6-tri-O-benzoyl-α-D-mannopyranosyl-(1→2)-3,4,6-tri-O-benzoyl-α-D-mannopyranoside ***26***

A mixture of the imidate (**6**) (161 mg, 0.223 mmol, 2 equiv.) and the tetrasaccharide alcohol **24** (229 mg, 0.112 mmol) in DCE (4 mL) in the presence of molecular sieves (100 mg of 3 Å powder) was treated with TMSOTf (2 µL, 0.012 mmol, 0.11 equiv.) at 0°C. The combined mixture was stirred at 0°C for 80 min. TLC (EtOAc/hexane, 40/60) indicated complete conversion and Et_3N was introduced. The mixture was filtered and the solvent was evaporated. The residue was subjected to flash column chromatography (EtOAc/hexane, 1/9 to 1/1) to yield the pentasaccharide **25** as a colourless oil, which was used directly for de-*O*-allylation. $PdCl_2$ (20 mg) was added to a solution of the above pentasaccharide in MeOH (5 mL) and DCE (5 mL) and the combined mixture was heated (70°C, 2 h). The mixture was cooled and filtered. The filtrate was evaporated and the residue subjected to flash column chromatography (EtOAc/hexane, 1/9 to 1/1) to yield the pentasaccharide alcohol **26** as a colourless glass (206 mg, 71%, 2 steps). δ_H ($CDCl_3$) 8.12–7.08 (m, 80H, Ph), 6.01–5.88 (m, 3H, H-4$^{I–III}$), 5.84 (dd, $J_{2I,3I}$ 3.2, $J_{3I,4I}$ 9.6, H-3^I), 5.80 (dd, $J_{1III,2III}$ 2.0, $J_{2III,3III}$ 3.6, H-2^{III}), 5.78 (dd, $J_{3IV,4IV}$~$J_{4IV,5IV}$ 9.6, H-4^{IV}), 5.44 (dd, $J_{3V,4V}$~$J_{4V,5V}$ 10, H-4^V), 5.33 (d, $J_{1II,2II}$ 1.6, H-1^{II}), 5.28 (dd, $J_{2II,3II}$ 3.2, H-2^{II}), 5.23 (d, H-1^{III}), 5.15 (d, $J_{1I,2I}$ 2.0, H-1^I), 5.10 (dd, $J_{1IV,2IV}$ 2.0, $J_{2IV,3IV}$ 2.8, H-2^{IV}), 4.88 (d, H-1^{IV}), 4.87 (dd, $J_{1V,2V}$ 1.6, $J_{2V,3V}$ 3.2, H-2^V), 4.84 (d, H-1^V), 4.72 (d, 1H, A of ABq, J_{AB} 12, PhCH_2), 4.64 (dd, $J_{3III,4III}$ 9.6, H-3^{III}), 4.59 (dd, $J_{5,6a}$ 2.8, $J_{6a,6b}$ 12, H-6a), 4.54–4.24 (m, 10H), 4.39 (dd, H-2^I), 4.24 (dd, H-3^{IV}), 4.01 (dd, H-3^V), 3.99–3.82 (m, 6H). δ_C ($CDCl_3$) 166.46–165.10 (15C, C=O), 136.51–132.92 (16C, ArC), 130.26–128.23 (80C, ArCH), 99.71, 99.29, 99.03, 97.89 (C-$1^{I–V}$), 76.98, 76.61, 76.29, 72.46, 71.66, 71.41, 71.10, 69.97, 69.85, 69.73, 69.46, 69.29, 68.91, 68.52, 68.15, 67.80, 67.51, 67.28 (C-$2^{I–V}$, -$3^{I–V}$, -$4^{I–V}$, -$5^{I–V}$, CH_2Ph), 63.86, 63.01, 62.42, 62.23 (C-$6^{I–V}$).

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→3)-2,4,6-tri-O-sulfo-α-D-mannopyranosyl-3,4,6-tri-O-sulfo-α-D-mannopyranoside, Hexadecasodium Salt ***3***

The perbenzoate **26** (172 mg, 69.3 µmol) was dissolved in dry MeOH (2.0 mL). NaOMe (11 M) in MeOH (20 µL) was added and the mixture was stirred at room temperature for 6 h, before the solution was neutralized with AG50WX8 resin (H^+ form). The solution was filtered before the solvent was evaporated and the residue taken up in water. The solution was passed through a solid phase extraction cartridge (Waters Sep-Pak Vac 6cc C_{18}) to remove methyl benzoate before purification by reverse phase HPLC. After purification the sample was lyophilized to give the polyol **27** as a white solid (43 mg, 67%, 97% pure by HPLC), identical in all respects to an authentic sample.[10] (HRMS found 919.3279. Calc. for $C_{37}H_{59}O_{26}$: 919.3296 $[M + H]^+$.)

The above polyol (43 mg, 46.8 µmol) was dissolved in DMF (1.17 mL, 0.04 M). SO_3·py (2.25 mmol, 358 mg) was added in one portion and the mixture was stirred overnight at 60°C. The mixture was cooled to 0°C before 5 M NaOH (5.4 mmol, 1.08 mL) was added in one portion. The solvent was evaporated and the residue dissolved in water and dialyzed (Slide-A-Lyzer

2K Dialysis Cassette) over 1 week with daily water changes (final water change done with HPLC-grade water). The sample was lyophilized to yield the persulfate **3** as an off-white solid (85.7 mg, 72%, 92% pure by CE). ^{1}H NMR and CE data were in accord with the literature.[10]

Rat Aortic Assay for Angiogenesis

Rat aortas were harvested from Sprague Dawley rats, 6–10 weeks of age by Tetra-Q, Brisbane, Australia and trimmed of remaining fat and connective tissue before cutting 1 mm ring sections, which were subsequently bisected and transferred to complete EBM-2 media (Lonza, Basel, Switzerland) that contained 2% fetal calf serum and all singlequot reagents except for heparin. Matrigel (BD Biosciences, San Jose, USA) was allowed to cool on ice and once in a liquid form, 180 μL was pipetted into 48-well tissue culture plates (Nunc, Rochester, USA). The plates were incubated at 37°C for 30 min to allow the Matrigel to solidify. Aortic segments were then carefully placed on top of the Matrigel in the centre of each well and 60 μL of Matrigel was pipetted to cover the aortic segment. The plate was then returned to the incubator for a further 20 min before each well was supplemented with 1.0 mL of media with or without 10 μM or 50 μM of compound **3** and replenished every 24 h with fresh media ±**3**. The stock concentration (10 mM) of **3** was prepared using sterile phosphate buffered saline, pH 7.2. As a control, medium alone as assayed was used. On day 8, the microvessels were scored from 0 (least) to 5 (most positive) by two investigators in an independent fashion. The results are presented in Fig. 2.

Accessory Publication

Copies of ^{1}H and ^{13}C NMR spectra for compounds **20**, **22**, **24**, and **26** are available from the Journal's website.

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