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Stability studies of *C*-4′,6′ acetal benzylmaltosides synthesized as inhibitors of smooth muscle cell proliferation

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Abstract—In our investigations to synthesize inhibitors of smooth muscle cell (SMC) proliferation, compound **6a** displayed submicromolar activity in in vitro antiproliferative assays and reduced intimal thickening using a rat balloon angioplasty model via iv administration. In order to identify analogs that could be administered orally, the chemical instability of the *C*-4′,6′ acetal of compound **6a** was addressed. Several novel variants with increased acid stability and comparable in vitro activity were prepared.

Restenosis, a complication of vascular wounding, is often caused by injuries from surgical procedures such as PTCA (balloon angioplasty).[1] Roughly 30–50% of the patients receiving PTCA undergo a re-narrowing of the affected vascular segment within 6 months of surgery and may require additional surgical intervention.[2] Vascular restenosis occurs when appropriate healing mechanisms become over productive and generate more than the required permanent tissue, resulting in an inadvertent re-thickening of the vascular wall. One approach[3] to controlling this problem is to selectively inhibit the growth and migration of smooth muscle cells (SMC) without affecting those of endothelial cells (EC). In the course of synthesizing a series of disaccharides that exhibit selective SMC antiproliferative activity, a tetrahydroxy carbohydrate lead (**6a**) was generated that possessed submicromolar inhibitory activity (0.020 μM, Table 1) in vitro with no apparent cytotoxicity.[4] Compound **6a** displayed 30-fold selectivity over EC proliferation through a mechanism, which may result from blocking production of lactosylceramide (LacCer). LacCer is a ceramide linked disaccharide that is thought to be linked to both proliferation and intercellular adhesion molecule expression by acting as a precursor signal to an obligatory superoxide production.[5] Thus, by either preventing the production of LacCer, or by

Table 1. SAR of *C*-4′, 6′, acetal derivatives

Compound	First step for **5a**–**h**: see below for reagents. Second step for **6a**–**h**: acylation with BzCl	Yield, % (acetal and acylation steps)	R^4 (see Scheme 1 for core structure)	SMC assay[a] [IC_{50} (μM)]	EC assay[b] [IC_{50} (μM)]
6a	Benzaldehyde dimethyl acetal, TsOH·H_2O	78/80	Ph	0.020	0.923
6b	Ph-acetaldehyde dimethyl acetal, CSA	35/63	Bn	0.035	—
6c	Acetaldehyde dimethyl acetal, TsOH·H_2O	62/54	CH_3	0.103	0.257
6d	Isobutyraldehyde diethyl acetal, CSA	45/49	Isopropyl	0.003	—
6e	Propionaldehyde diethyl acetal, TsOH·H_2O	57/47	Et	0.037	0.235
6f	4-Cl-benzaldehyde dimethyl acetal, CSA	51/50	4-Cl-Ph	0.023	0.324
6g	4-NO_2-benzaldehyde dimethyl acetal, CSA	42/35	4-NO_2-Ph	0.003	0.368
6h	4-Pyridinecarboxaldehyde, DMF, H_2SO_4, 110 °C	20/46	4-Pyridinyl	0.003	—

[a] Concentration inducing 50% inhibition of smooth muscle cell proliferation.
[b] Concentration inducing 50% inhibition of endothelial cell proliferation.

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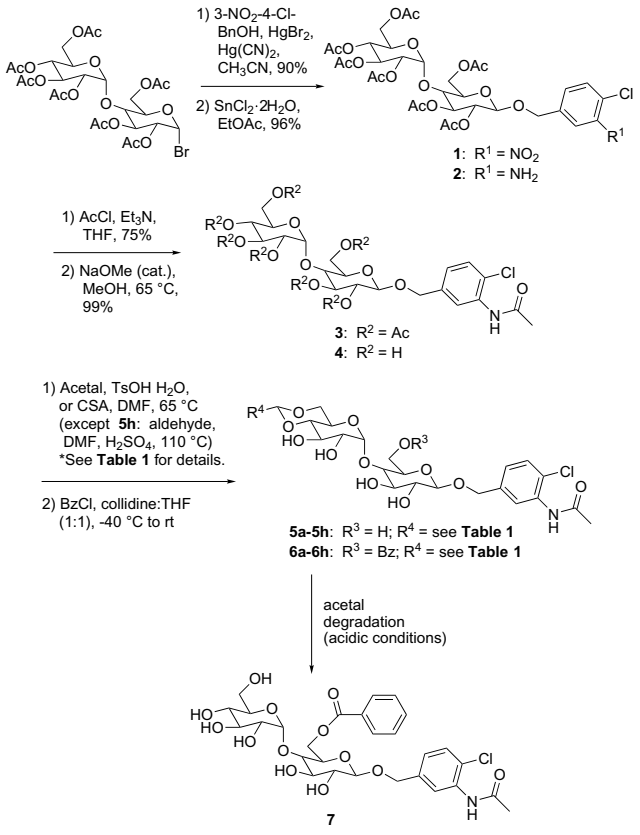
inhibiting LacCer's ability to bind to its downstream target, an inhibitory analog should have a profound effect on both proliferative and pro-inflammatory processes. To date, no other nonsulfated disaccharide has been linked to cellular events in such a manner. Therefore, the link between the proliferative pathway in SMC and the structural similarity of our disaccharides to LacCer, although speculative, could be one explanation to the inhibitory activity of these analogs and is consistent with our ongoing studies.[6] Future work in this area should help to further elucidate this mechanism.

When tested in vivo at doses of 100 mg/kg/h iv, compound **6a** also caused a statistically significant reduction of intimal thickening, ranging from 18–39%.[7] Although compound **6a** showed promising in vivo activity, the chemical instability of the *C*-4′,6′ acetal precluded its advancement as a lead. This functionality was identified to be a potential source of acid induced degradation when **6a** was dosed orally. Under conditions that mimic the stomach (pH 1), compound **6a** showed substantial degradation to **7** (Scheme 1 and Table 2). The antiproliferative activity of **7** was significantly reduced compared to parent (34.9 vs 0.020 μM). Therefore, we focused our attention on the synthesis of a library of acetals with the view of improving stability while maintaining SMC inhibitory activity.



Scheme 1.

Aromatic and aliphatic *C*-4′,6′ acetals of **6a** were synthesized according to the chemistry illustrated in Scheme 1 (**6b–h** and Table 1). Hepta-*O*-acetyl-α-maltosyl bromide was coupled with 3-nitro-4-chloro-benzyl alcohol in the presence of mercuric bromide and mercuric cyanide to yield glycoside **1**. Reduction of the nitro group of **1** with stannous chloride afforded the anilino compound **2**. Acylation of the amine with acetyl chloride in the presence of Et_3N afforded intermediate **3**, which was converted to the heptahydroxy compound **4** by reaction with catalytic sodium methoxide in methanol. In most cases, the substituted *C*-4′,6′ acetal (**5a–g**) was formed by use of a substituted aldehyde dimethyl or diethyl acetal and a catalytic acid source ($TsOH{\cdot}H_2O$ or CSA). Selective acylation of the 6-position primary alcohol using benzoyl chloride in a 1:1 mixture of tetrahydrofuran and 2,4,6-collidine afforded compounds **6a–g**.[8,9] To prepare compound **6h**, intermediate **4** was condensed with 4-pyridinecarboxaldehyde using concentrated H_2SO_4 followed by similar acylation conditions with 2,4,6-collidine.

The chemical stability of each acetal (**6b–h**) was evaluated using **6a** as a benchmark. Replacing the phenyl group of compound **6a** with a heterocycle (**6h**) or an aliphatic acetal (**6b–e**) generated more stable analogs without loss of in vitro activity (Tables 1 and 2). Analog **6f** had only a modest improvement in stability over **6a** in the pH range 1.4–2.5. Acid stability of compound **6g** could not be determined due to its precipitation under conditions of the assay. When the benzylidene at the 4′,6′-position was replaced with a methyl acetal (**6c**), the compound exhibited 5-fold less potency (0.103 vs 0.020 μM for **6a**) suggesting the need for a substituent larger than Me to retain biological activity. However, **6c** had slightly improved chemical stability compared to **6a** (32% vs 100% degradation over 18 h period at pH 1).

Table 2. Stability studies of *C*-4′,6′ acetal derivatives

Compound	R^4 (see Scheme 1 for core structure)	pH								% Remaining at 18–20 h (pH 1)	pH with ~90% at 18–20 h
		1	1.4	1.9	2.5	3	3.5	4	4.5		
		$t_{1/2}$ (h)									
6a	Ph	1.9	2.3	4.2	13	>20	>20	>20	>20	0	3.5
6b	Bn	>20	>20	>20	>20	>20	>20	>20	>20	94	1
6c	CH_3	>20	>20	>20	>20	>20	>20	>20	>20	68	1.4–1.9
6d	Isopropyl	>20	>20	>20	>20	>20	>20	>20	>20	53	2
6e	Et	>20	>20	>20	>20	>20	>20	>20	>20	52	1.9–2.5
6f	4-Cl-Ph	1.7	4	13.3	>20	>20	>20	>20	>20	0	3
6h	4-Pyridinyl	>20	>20	>20	>20	>20	>20	>20	>20	97	1

When compound **6c** was tested in vivo (iv) in the intimal thickening assay, no activity was observed. Incorporating a nicotinoyl group into the acetal functionality resulted in a compound possessing excellent acid stability without loss of in vitro activity (**6h**, 0.003 μM). Over the 18 h period at pH 1, only 3% degradation to compound **7** was detected.

In conclusion, analogs of disaccharide **6a** with improved chemical stability have been prepared. Specifically, by modifying the *C*-4′,6′ benzylidene acetal with aromatic and aliphatic variants provided more stable compounds while maintaining SMC antiproliferative activity. Incorporating a nicotinoyl acetal in this position afforded an analog (**6h**) with excellent acid stability and good in vitro activity (0.003 μM). Addressing this chemical stability issue has provided a series of stable acetal derivatives available for future in vivo studies.

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4. Procedures for SMC and EC proliferation assays:
   A. *Human SMC and EC culture conditions:* Human aortic smooth muscle (HASMC) and endothelial (HAEC) cells were obtained from Clonetics (San Diego, CA) and were routinely maintained in T-75 tissue culture flasks in either SMC or EC media supplied by Clonetics and supplemented with fetal bovine serum (10% for HASMC, 5% FBS for HAEC) at 37 °C in a humidified 5% CO_2 atmosphere. The culture medium was changed into fresh medium every 2–3 days. Both cell types were routinely sub-cultured into 24 well plates after trypsinization for use in critical growth assays. Cells used for this study were from 3rd through 6th passage.
   B. *Examination of the effects of compounds on cell proliferation using ^3H thymidine incorporation:* Cell lines were grown as described above in 24 well plates and all were assayed using an identical protocol. When cell density reached approximately 70–80% confluency, culture medium was exchanged with fresh DMEM, M199, or AIM-V (GibCo BRL) lacking any serum or growth factors, and cell growth was continued for an additional 24–48 h to induce a quiescent state. Cells were then re-stimulated with either serum or the appropriate individual growth factors for a period of 24 h in the presence of 0.5 μCi/mL ^3H thymidine before harvest.
   Although compounds were found to be more effective with longer pre-incubations, in general, experiments were initiated with the addition of compound, ^3H thymidine and serum/growth factor to serum deprived synchronized cells and results are reported in this paper accordingly.
   Compounds were added to each well at 50-fold dilution (20 μL/ well) and the plates were incubated for 24–36 h at 37 °C in 5% CO_2. Compounds were initially dissolved in 50% ethanol and serially diluted into media. Compounds were routinely assayed at concentrations from 1 to 100 μM. As a control, grade II porcine intestinal mucosal heparin (sodium salt) from Sigma (H-7005) was routinely assayed in all cell preparations at concentrations from 0.1 to 100 μg/mL.
   At the completion of the experiment, plates were placed on ice, washed three times with ice cold phosphate buffered saline (PBS), and incubated in ice cold 10% trichloroacetic acid (TCA) for 30 min to remove acid soluble proteins. Solution was transferred to scintillation vials containing 0.4 N HCl (500 μL/vial to neutralize NaOH) and each well was rinsed two times with water (500 μL) for a total volume of 2 mL/vial.
   Data was obtained, in triplicate, for both control and experimental samples. Control (100%) data was obtained from maximally stimulated cells, as the result of growth factor or serum stimulation. Experimental data was obtained from cells maximally stimulated with growth factor or serum and treated with compound. Data was expressed as a percent of control from which a percent inhibition or IC_{50} could be determined.
   C. *Cytotoxicity*: Visually, all cells were found to tolerate high levels of all compounds quite well, however to insure that no toxicity was present, cytotoxicity of compounds was examined using a commercial modification of the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. Briefly, cells were again grown in 24 well plates to 70–80% confluency and, as before, serum deprived for 24–48 h prior to initiation of the experimental protocol. To insure that the MTT assay monitored toxicity rather than proliferation, cells were incubated with 100 μg/mL of drug in fresh medium without serum for 24 h at 37 °C in a humidified CO_2 incubator. Upon completion of the compound treatment, MTT indicator dye was added for 4 h at 37 °C . Cells were then lysed and aliquots from each well were transferred to a 96 well plate for analysis. Absorbance at 570 nm wavelength with a reference wavelength of 630 nm was recorded using an ELISA plate reader. Results were determined as percent viable using no drug (100% viable) and pre-solubilization (0% viable) standards. All compounds (**6a**–**h**) exhibited no toxicity up to 100 μg/mL.
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7. Procedure for in vivo rat balloon injury model: Vascular injuries were produced by introducing and inflating a balloon catheter into the left common carotid artery of anesthetized male Sprague–Dawley rats (Charles River Breeding Laboratories). This type of injury generally damages roughly 25–30 mm of vascular wall. One hour prior to sacrificing, the animals were intravenously injected with 0.9% Evans Blue. At the time of sacrifice, the animals were further perfused with isotonic saline for 5 min, followed by 3.5% glutaraldehye. Arteries were then excised and evaluated to determine intima to media (I/M) ratio (intimal thickening).
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9. Experimental: Step 1: *4-Chloro-3-nitrobenzyl hepta-O-acetyl-β-maltoside* (**1**). To a stirred solution of 4-chloro-3-nitrobenzyl alcohol (6.70 g, 35.7 mmol) and $HgBr_2$ (14.2 g,

39.3 mmol) in freshly distilled CH_3CN (239 mL) was added in one portion $Hg(CN)_2$ (9.02 g, 35.7 mmol). After 0.5 h, hepta-*O*-acetyl-α-maltosyl bromide (25.0 g, 35.7 mmol) was added, and the mixture stirred for 18 h at rt. The reaction was then quenched with a mixture of H_2O/brine (1:1, 100 mL) and extracted with 10% CH_2Cl_2/EtOAc. The combined organic extracts were dried ($MgSO_4$) and concentrated. Purification by flash chromatography (10:90–80:20, EtOAc/petroleum ether gradient) gave 51.9 g (90%) of the title compound as a glassy oil, which was recrystallized from Et_2O/petroleum ether to afford a glassy white solid, mp 107–111 °C; ^{1}H NMR ($CDCl_3$) δ 2.00 (s, 3H), 2.02 (s, 3H), 2.03, (s, 3H), 2.04 (s, 6H), 2.11 (s, 3H), 2.15 (s, 3H), 3.70 (ddd, $J = 2.9, 4.2, 9.7$ Hz, 1H), 3.94–3.98 (m, 1H), 4.01–4.07 (m, 2H), 4.20–4.28 (m, 2H), 4.54 (dd, $J = 2.9, 12.3$ Hz, 1H), 4.63–4.68 (m, 2H), 4.84–4.94 (m, 3H), 5.06 (t, $J = 10.1$ Hz, 1H), 5.26 (t, $J = 9.2$ Hz, 1H), 5.36 (dd, $J = 9.7, 10.3$ Hz, 1H), 5.42 (d, $J = 4.2$ Hz, 1H), 7.43 (dd, $J = 2.2, 8.3$ Hz, 1H), 7.53 (d, $J = 8.3$ Hz, 1H), 7.83 (d, $J = 2.0$ Hz, 1H); IR (KBr) 3450, 2950, 1755, 1550, 1375, 1230, and 1050 cm^{-1}; mass spectrum [(+) ESI], m/z 823/825 $(M + NH_4^+)$, 828/830 $(M + Na)^+$; Anal. Calcd for $C_{33}H_{40}ClNO_{20}$: C, 49.17; H, 5.00; N, 1.74. Found: C, 49.16; H, 4.88; N, 1.71.

Step 2: *3-Amino-4-chlorobenzyl hepta-O-acetyl-β-maltoside* (**2**). A solution containing 4-chloro-3-nitrobenzyl hepta-*O*-acetyl-β-maltoside (19.3 g, 23.9 mmol) and tin(II) chloride dihydrate (37.7 g, 167 mmol) in EtOAc (479 mL) was refluxed for 2 h. The reaction was cooled to rt, carefully quenched with satd aq $NaHCO_3$ (until basic), diluted with EtOAc (250 mL), stirred for 0.5 h, and filtered. The biphasic filtrate was separated and the aqueous phase extracted with EtOAc. The combined organic extracts were dried (Na_2SO_4) and concentrated. Purification by flash chromatography (0–12% acetone/$CHCl_3$ gradient) gave 17.8 g (96%) the title compound as a glassy solid, mp 78–79 °C; ^{1}H NMR ($CDCl_3$) δ 2.00 (s, 9H), 2.026 (s, 3H), 2.032 (s, 3H), 2.11 (s, 3H), 2.16 (s, 3H), 3.00–5.00 (br s, 2H), 3.64–3.68 (m, 1H), 3.97 (ddd, $J = 2.4, 4.2, 10.1$ Hz, 1H), 4.02–4.07 (m, 2H), 4.24 (dd, $J = 2.2, 3.7$ Hz, 1H), 4.27 (dd, $J = 2.6, 4.0$ Hz, 1H), 4.50–4.57 (m, 3H), 4.74 (d, $J = 12.1$ Hz, 1H), 4.83–4.90 (m, 2H), 5.05 (t, $J = 10.1$ Hz, 1H), 5.22 (t, $J = 9.2$ Hz, 1H), 5.35 (dd, $J = 9.7, 10.5$ Hz, 1H), 5.42 (d, $J = 4.0$ Hz, 1H), 6.62 (dd, $J = 2.0, 8.1$ Hz, 1H), 6.76 (d, $J = 2.0$ Hz, 1H), 7.21 (d, $J = 8.1$, 1H); IR (KBr) 3450, 3350, 2950, 1755, 1650, 1425, 1375, 1230, and 1050 cm^{-1}; mass spectrum [(+) ESI], m/z 776/778 $(M + H)^+$, 798/800 $(M + Na)^+$; Anal. Calcd for $C_{33}H_{42}ClNO_{18}$: C, 51.07; H, 5.45; N, 1.80. Found: C, 50.94; H, 5.52; N, 1.60.

Step 3: *3-Acetamido-4-chlorobenzyl hepta-O-acetyl-β-maltoside* (**3**). To a stirred solution of 3-amino-4-chlorobenzyl hepta-*O*-acetyl-β-maltoside (20.6 g, 26.5 mmol) and triethylamine (8.13 mL, 58.3 mmol) in THF (265 mL) at 0 °C was added dropwise acetyl chloride (2.26 mL, 31.8 mmol). After 0.5 h at this temperature, it was warmed to rt, and stirred an additional 6 h. At this point, the reaction was concentrated and taken up in EtOAc (700 mL). This organic solution was washed with 1 N HCl (70 mL), satd aq $NaHCO_3$ (70 mL), and brine (70 mL) and then dried ($MgSO_4$). After concentration, the residue was purified by flash chromatography (20:80–100:0, EtOAc/petroleum ether gradient) to afford the product (16.2 g, 75%) as a glassy solid, mp 84–86 °C; ^{1}H NMR ($CDCl_3$) δ 2.00 (s, 6H), 2.020 (s, 3H), 2.027 (s, 3H), 2.03 (s, 3H), 2.11 (s, 3H), 2.16 (s, 3H), 2.24 (s, 3H), 3.66–3.69 (m, 1H), 3.94–3.98 (m, 1H), 4.00–4.06 (m, 2H), 4.22–4.28 (m, 2H), 4.50–4.61 (m, 3H), 4.80–4.91 (m, 3H), 5.05 (t, $J = 10.1$ Hz, 1H), 5.22 (t, $J = 9.2$ Hz, 1H), 5.35 (dd, $J = 9.4, 10.5$ Hz, 1H), 5.41 (d, $J = 4.0$ Hz, 1H), 6.99 (dd, $J = 2.0, 8.1$ Hz, 1H), 7.34 (d, $J = 8.1$ Hz, 1H), 7.62 (s, 1H), 8.32 (s, 1H); IR (KBr) 3400, 2950, 1750, 1690, 1600, 1540, 1425, 1375, 1230, and 1050 cm^{-1}; mass spectrum [(+) ESI], m/z 818/820 $(M + H)^+$, 840 $(M + Na)^+$; Anal. Calcd for $C_{35}H_{44}ClNO_{19}$: C, 51.38; H, 5.42; N, 1.71. Found: C, 51.03; H, 5.36; N, 1.59.

Step 4: *3-Acetamido-4-chlorobenzyl-β-maltoside* (**4**). A solution containing 3-acetamido-4-chlorobenzyl hepta-*O*-acetyl-β-maltoside (0.945 g, 1.12 mmol) and 25 wt % NaOMe in MeOH (19.2 μL, 0.336 mmol) in MeOH (27.6 mL) was refluxed for 2.5 h. The reaction was cooled to room temperature and concentrated, and the resulting residue was triturated with Et_2O to afford the product (0.583 g, 99%) as a foam; ^{1}H NMR (DMSO-d_6) δ 2.07 (s, 3H), 3.03–3.16 (m, 2H), 3.19–3.49 (m, 7H), 3.55–3.62 (m, 2H), 3.67–3.73 (m, 1H), 4.28 (d, $J = 7.7$ Hz, 1H), 4.33–5.76 (br s, 7H), 4.67 (AB_q, $J = 12.5$ Hz, $\Delta\delta = 0.22$, 2H), 5.01 (d, $J = 3.7$ Hz, 1H), 7.21 (dd, $J = 1.8, 8.1$ Hz, 1H), 7.44 (d, $J = 8.1$ Hz, 1H), 7.64 (d, $J = 1.5$ Hz, 1H), 9.33–9.69 (br s, 1H); IR (KBr) 3400, 2900, 1680, 1600, 1540, 1430, 1375, 1310, 1150, and 1035 cm^{-1}, mass spectrum [(+) ESI], m/z 524/526 $(M + H)^+$, 546 $(M + Na)^+$; Anal. Calcd for $C_{21}H_{30}ClNO_{12}$·1.0 MeOH: C, 47.53; H, 6.16; N, 2.52. Found: C, 47.94; H, 6.34; N, 2.42.

Step 5: *3-Acetamido-4-chlorobenzyl 4′,6′-O-benzylidene-β-maltoside* (**5**). To a stirred solution of 3-acetamido-4-chlorobenzyl β-maltoside (14.15 g, 27.0 mmol) in DMF (325 mL) at rt was added benzaldehyde dimethyl acetal (8.11 mL, 54.0 mmol) dropwise followed by $TsOH·H_2O$ (2.57 g, 13.5 mmol). The reaction mixture was heated to 60 °C for 6 h and then quenched with K_2CO_3 (1.87 g, 13.5 mmol) with an additional 0.5 h heating at this temperature. At this point, the solution was filtered hot, and the solvent was distilled off using the high vac. The residue was purified by flash chromatography (80:2:1–20:2:1, EtOAc/EtOH/H_2O gradient) to afford the product (10.8 g, 65%) as a white solid, mp 143–147 °C; ^{1}H NMR (DMSO-d_6) δ 2.08 (s, 3H), 3.07–3.12 (m, 1H), 3.28–3.50 (m, 5H), 3.51–3.60 (m, 2H), 3.64–3.75 (m, 3H), 4.10–4.12 (m, 1H), 4.30 (d, $J = 7.9$ Hz, 1H), 4.67 (t, $J = 5.9$ Hz, 1H), 4.68 (AB_q, $J = 12.5$ Hz, $\Delta\delta = 0.22$, 2H), 5.14 (d, $J = 4.0$ Hz, 1H), 5.25 (d, $J = 5.1$ Hz, 1H), 5.30 (d, $J = 5.3$ Hz, 1H), 5.51 (d, $J = 3.3$ Hz, 1H), 5.57 (s, 1H), 5.63 (d, $J = 6.8$ Hz, 1H), 7.22 (dd, $J = 1.5, 8.3$ Hz, 1H), 7.35–7.38 (m, 3H), 7.42–7.46 (m, 3H), 7.66 (s, 1H), 9.53 (s, 1H); IR (KBr) 3500, 3410, 2910, 2850, 1700, 1600, 1550, 1440, 1425, 1375, 1310, 1230, 1150, 1070, and 1030 cm^{-1}; mass spectrum [(+) FAB], m/z 634 $(M + Na)^+$; Anal. Calcd for $C_{28}H_{34}ClNO_{12}$·1.0 H_2O: C, 53.38; H, 5.76; N. 2.22, Found: C, 53.58; H, 5.62; N, 2.25.

Step 6: *3-Acetamido-4-chlorobenzyl 6-O-benzoyl-4′,6′-O-benzylidene-β-maltoside* (**6a**). To a stirred solution of 3-acetamido-4-chlorobenzyl 4′,6′-*O*-benzylidene-β-maltoside (5.00 g, 8.17 mmol) in THF (80 mL) at −40 °C was added collidine (80 mL, 605 mmol) dropwise followed by dropwise addition of BzCl (1.14 mL, 9.80 mmol). After 2 h at this temperature, it was warmed to rt and stirred an additional 48 h. At this point, the solvent was distilled off using the high vac, and the residue was diluted with EtOAc (700 mL). This layer was washed with 1 N HCl (70 mL), satd $NaHCO_3$ (70 mL), and brine (70 mL) and then dried ($MgSO_4$). After concentration, the oily residue was purified by flash chromatography (1–11%, MeOH/$CHCl_3$ gradient) and recrystallization (EtOAc/hexane) to afford the product (4.04 g, 69%) as a white solid, mp 185–187 °C; ^{1}H NMR (DMSO-d_6) δ 2.05 (s, 3H), 3.16–3.22 (m, 1H), 3.32–3.42 (m, 2H), 3.48–3.64 (m, 4H), 3.71 (dd, $J = 4.8, 9.7$ Hz, 1H), 3.74–3.79 (m, 1H), 4.05 (dd, $J = 4.8, 10.3$ Hz, 1H), 4.35 (dd, $J = 5.3, 12.3$ Hz, 1H), 4.39 (d, $J = 7.7$ Hz, 1H), 4.58–4.63 (m, 1H), 4.65 (AB_q, $J = 12.5$ Hz, $\Delta\delta = 0.14$, 2H), 5.14 (d, 4.0 Hz, 1H), 5.34 (t, $J = 5.1$ Hz, 2H), 5.52 (s, 1H), 5.57 (d,

$J = 3.1$ Hz, 1H), 5.79 (d, $J = 6.2$ Hz, 1H), 7.19 (dd, $J = 2.0$, 8.3 Hz, 1H), 7.32–7.38 (m, 3H), 7.38–7.45 (m, 3H), 7.51–7.55 (m, 2H), 7.63–7.68 (m, 2H), 7.98–8.01 (m, 2H), 9.49 (s, 1H); IR (KBr) 3380, 3290, 2890, 2870, 1730, 1670, 1600, 1540, 1440, 1420, 1375, 1275, 1070, 1050, 1025, 975, and 710 cm^{-1}; mass spectrum [(+) FAB], m/z 716/718 $(M + H)^+$, 738/740 $(M + Na)^+$; Anal. Calcd for $C_{35}H_{38}ClNO_{13}$: C, 58.70; H, 5.35; N, 1.96. Found: C, 58.53; H, 5.36; N, 1.94. Compounds **6b**–**h** were prepared using similar procedures to steps 5–6 of footnote 9 with the one exception that intermediate **5h** was prepared with 4-pyridinecarboxaldehyde using concentrated H_2SO_4 in DMF. All intermediates (**5b**–**h**) and final compounds (**6b**–**h**) were verified by ^1H NMR, IR, MS, and CHN.