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Total Synthesis of CRM646-A and -B, Two Fungal Glucuronides with Potent Heparinase Inhibition Activities

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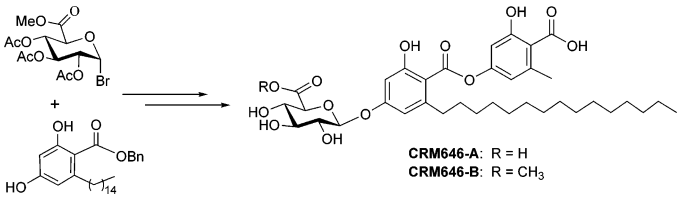

CRM646-A (**1**) and -B (**2**), two fungal glucuronides with a dimeric 2,4-dihydroxy-6-alkylbenzoic acid (orcinol *p*-depside) aglycone showing significant heparinase and telomerase inhibition activities, were synthesized for the first time. The successful approach involved construction of the phenol glucuronidic linkage, via coupling of the orsellinate derivative **27** with glucuronate bromide **7**, before assembly of the phenolic ester linkage in the depside aglycone. Attempts via direct glycosylation of the depside aglycone derivatives were not successful.

Introduction

CRM646-A (**1**) and -B (**2**) were isolated from *Acremonium* sp. MT70646 in the course of screening for heparinase and heparanase inhibitors.[1,2] Inhibitory concentrations causing 50% inhibition (IC_{50}) of the hydrolysis of porcine heparin by the heparinase (Sigma) for **1** and **2** occurred at 3 and 10 μM, respectively;[1] suramin,[3] known as a potent inhibitor of melanoma heparanase, showed an IC_{50} value of 5 μM in this assay system. The correlation between heparanase inhibition and the inhibition of tumor metastasis[4] for compounds **1** and **2** were then examined. Both compounds strongly inhibited the migration of B16-F10 melanoma cells, with IC_{50} values being 15 and 30 μM, respectively. In addition, CRM646-A (**1**) showed inhibitory activity against telomerase at a dose of 3.2 μM.[5] No cytotoxicity up to 100 μM was found for compounds **1** and **2**.[1] Therefore, these two fungal metabolites might be interesting candidates for anticancer therapeutics.

CRM646-A (**1**) and its methyl ester CRM646-B (**2**) are novel phenol glucuronides. The aglycone of the dimeric 2,4-dihydroxy-6-alkylbenzoic acid belongs to the orcinol *p*-depsides family, which are especially common and diverse in lichen genera.[6] Nevertheless, only a few of the orcinol *p*-depsides have so far been found in conjugation with sugars in the natural sources;[7] compounds **1** and **2** represent the only depsides bearing a glucuronate residue. Synthetic approaches toward orcinol *p*-depsides have been extensively studied.[6,8] However, synthesis of their glycosides has only been reported once. Thus, Dushin and Danishefsky accomplished the synthesis of the galactofuranosides KS-501 and -502 employing glycosylation of a salicylate derivative with a sugar 1,2-epoxide as a key

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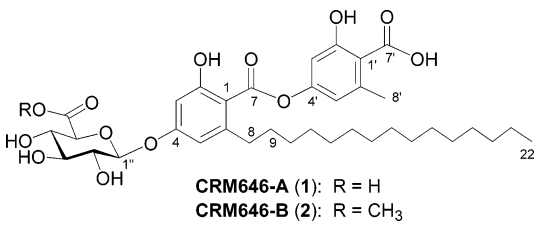
step.[9] Here we report the total synthesis of CRM646-A (**1**) and -B (**2**).



Results and Discussion

Glycosidic coupling involving either a glycosyl donor of the glucuronic acid type[10] or a phenolic acceptor[11] has long been recognized as a difficult task. Thus, a major challenge in the synthesis of CRM646-A (**1**) and -B (**2**) would be construction of the phenol glucuronidic linkage. We planned to explore this glycosidic coupling with a variety of the glycosyl donors. Methyl (2,3,4-tri-*O*-acetyl-D-glucopyranosyl trichloroacetimidate)uronate **3** (Figure 1) has been found effective in coupling with a few of the phenols under the promotion of $BF_3{\cdot}OEt_2$ (or TMSOTf).[12] Its benzyl uronate counterpart **5**[13] should behave similarly but facilitate the final release of the carboxylic acid function by hydrogenolysis under neutral conditions. Glycosyl trifluoroacetimidates are valuable alternatives to the corresponding trichloroacetimidates,[14] which have shown advantages in sialylation[15a] and glycosylation of amides.[15b] We therefore also scheduled to examine glucuronidation with trifluoroacetimidates **4** and **6**.[13] In terms of glycosylation of phenols, glycosyl bromides (under either Koenigs−Knorr or phase transfer conditions) have been proven to be the most reliable donors, although the coupling yields might be only moderate.[16] Thus, methyl 2,3,4-tri-*O*-acetyl-1-bromo-α-D-glucuronate (**7**)[17] was selected as an alternative to the imidates (i.e., **3**−**6**). In the last resort, we should be able to realize the glycosidic coupling with a glucopyranosyl donor (e.g., 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-D-glucopyranosyl trichloroacetimidate, **8**)[18] and then to elaborate the 6″-carboxylic acid function via selective oxidation of the 6″-OH group.[19]

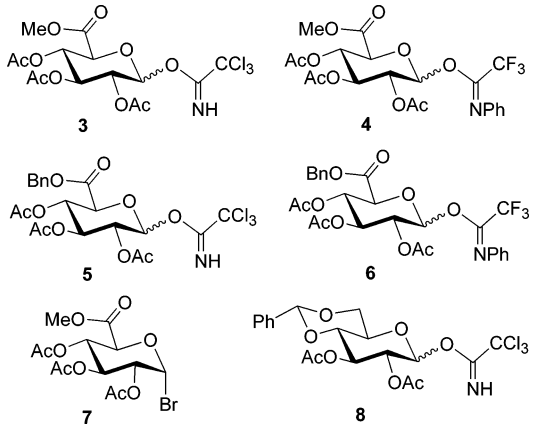


FIGURE 1. Glycosyl donors **3**−**8**.

The desired orsellinate derivatives were synthesized starting from 3,5-dihydroxytoluene, adopting modification of the literature transformations (Scheme 1). Thus, formylation of 3,5-dihydroxytoluene with DMF/$POCl_3$ gave 2,4-dihydroxy-6-methyl benzaldehyde **9** (80%).[20] Treatment of aldehyde **9** with sodium chlorite ($NaClO_2$) in a NaH_2PO_4 buffered solution of DMSO and H_2O provided benzoic acid **10** in a good 77% yield,[21] which was selectively benzylated to provide benzyl ester **11** with BnBr under the action of $KHCO_3$ in DMF (78%).[6a] Alternatively, the 2,4-dihydroxyl groups on aldehyde **9** were protected with methyl groups, providing **12** (100%), which was then oxidized into acid **13** (84%) under similar conditions used for **9** → **10**. Blocking the acid function on **13** with a methyl or an ethyl group provided **14a** or **14b**, which was subjected to LDA (1.5 equiv) followed by alkylation with 1-bromotetradecane (1.4 equiv), respectively. Unexpectedly, the desired alkylation product **15a** (from methyl ester **14a**) was isolated in a low 28% yield, whereas **15b** (from the ethyl ester counterpart **14b**) was obtained in a satisfactory 70% yield. In comparison, it was reported that treatment of **14a** with LDA and 1-bromotetradecane in the presence of HMPA provided **15a** in only 5% yield.[22] Removal of the *O*-methyl groups on **15b** was achieved with 3.0 equiv of boron tribromide at low temperature (−78 → −10 °C), providing diol **16** in 82% yield; the 4-methoxy derivative **17** was also isolated in 17% yield. The possible intermolecular Friedel−Crafts products were not detected.

The depside aglycone **21** and the required benzyl ester **22** were prepared as shown in Scheme 2. To hydrolyze the ethyl ester in **16**, we protected the 2,4-hydroxyl groups with benzyl ether first, providing **18**, to avoid the decarboxylation side reaction.[9,23] Then, treatment of **18** with KOH in a mixture solvent of DMSO and H_2O at 90 °C afforded acid **19** in nearly quantitative yield (for two steps). Coupling of acid **19** with phenol **11** under the action of trifluoroacetic anhydride provided the desired

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(14) For recent applications, see: (a) Zhang, Z.; Yu, B. *J. Org. Chem.* **2003**, *68*, 6309−6313. (b) Adinolfi, M.; Iadonisi, A.; Ravidà, A.; Schiattarella, M. *J. Org. Chem.* **2005**, *70*, 5316−5319.
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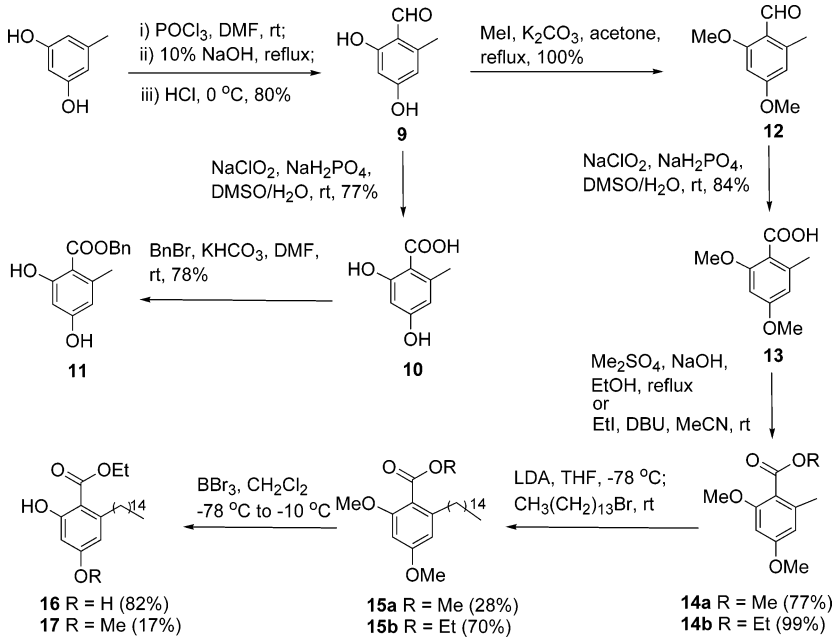
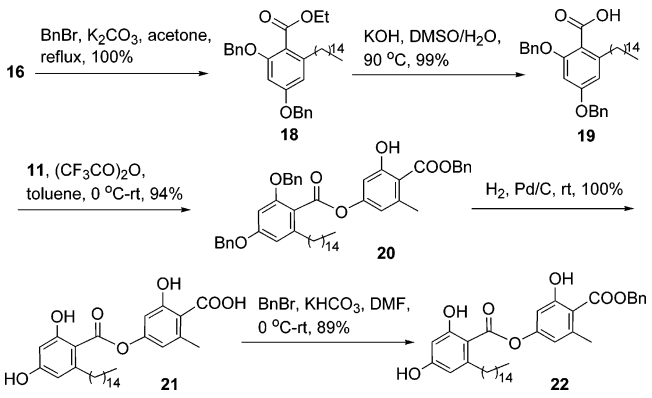
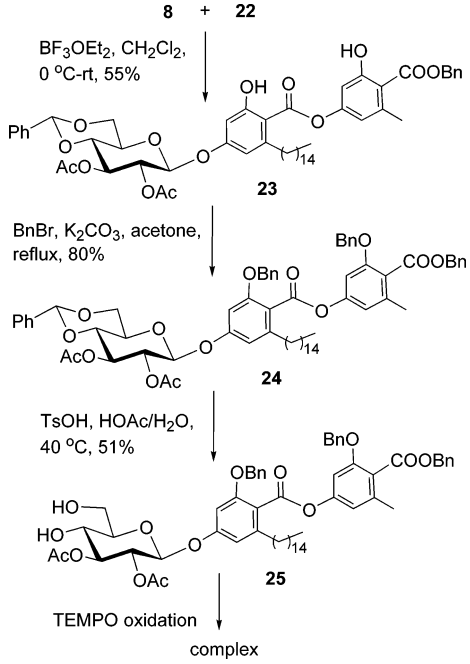
SCHEME 1. Preparation of the Orsellinate Derivatives 11 and 16



SCHEME 2. Preparation of Depside 22



SCHEME 3. Attempt To Synthesize the Glucuronide via Oxidation



phenol ester **20** in excellent yield (94%).[6a] Removal of the three benzyl groups on **20** by hydrogenolysis over Pd/C afforded the depside aglycone (**21**) of CRM646-A and -B quantitatively. Depside **21** was readily transformed into its benzyl ester **22** with BnBr in the presence of $KHCO_3$ in DMF (89%).

The 2,2′-phenolic hydroxyl groups in **22** are hydrogen-bonded with the *o*-carbonyl oxygen, as indicated by the downfielded NMR signals of the hydroxyl protons at 11.66 and 11.35 ppm, respectively. Therefore glycosylation with **22** was expected to take place selectively on the 4-OH. Unfortunately, glycosidic coupling between phenol **22** and the trichloroacetimidate/trifluoroacetimidate uronate donors **3**−**6** under a variety of conditions in the presence of $BF_3{\cdot}OEt_2$ or TMSOTf failed to provide the desired glycosides. Complex products were mostly encountered. Treatment of **22** with bromide **7** under either Koenigs−Knorr conditions (Ag_2O, CH_3CN)[16] or under phase transfer conditions (TBAB, NaOH, $CHCl_3$, H_2O)[24] led to the cleavage of the phenolic ester bond in **22**. As the last resort, glycosylation of **22** (1.2 equiv) with the glucopyranosyl trichloroacetimidate **8** under the promotion of $BF_3{\cdot}OEt_2$ (0.2 equiv) proceeded smoothly, providing the expected 4-*O*-*β*-glycoside **23** in a satisfactory 55% yield. (Scheme 3) To avoid decomposition of the phenolic ester bond during subsequent transformations, the 2,2′-hydroxyl groups on **23** were protected with benzyl groups (BnBr, K_2CO_3, acetone, reflux) to provide **24** (80%). Treatment of **24** with a catalytic amount of TsOH·H_2O (0.1 equiv) in 90% HOAc at 40 °C removed the 4″,6″-*O*-benzylidene group,[25] affording diol **25** in a moderate

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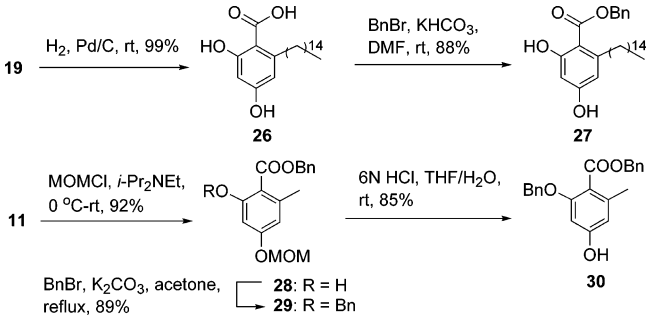
SCHEME 4. Preparation of the Orsellinate Derivatives 27 and 30

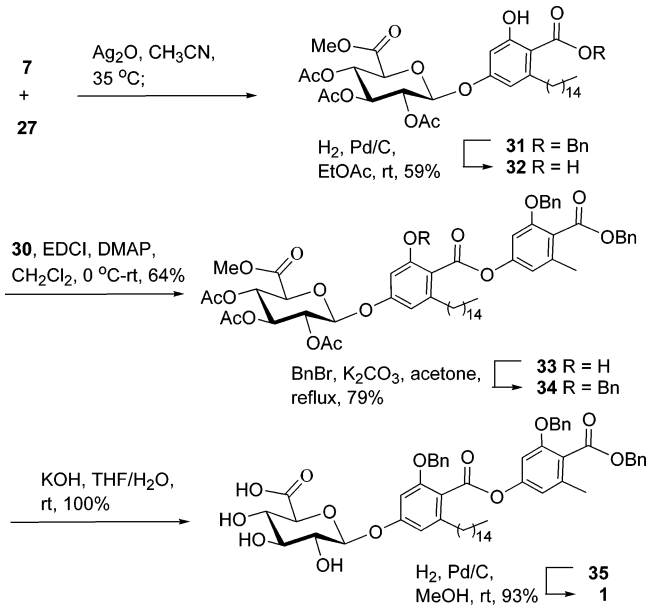


yield (51%). Unfortunately, subjection of **25** to the well-studied TEMPO oxidation protocol[19] failed to provide the desired glucuronate product. Instead, cleavage of the phenol ester was detected.

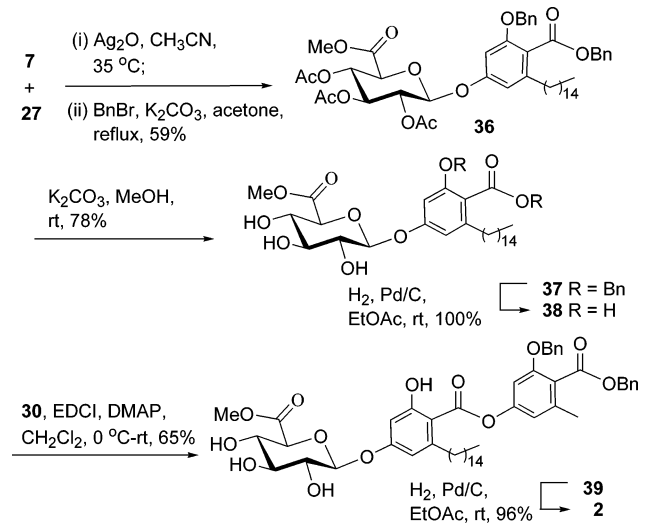
Since the above synthetic attempts toward CRM646-A and -B via direct glycosylation of the depside aglycone derivative (i.e., **22**) were found futile, we turned our attention to elaborate the phenol ester at a late stage after construction of the phenol glucuronidic linkage. Thus, benzyl 2,4-dihydroxy-6-pentadecanylbenzoate (**27**) was prepared from acid **19** by removal of the benzyl ether (H_2, Pd/C, 99%) and subsequent formation of the benzyl ester (BnBr, $KHCO_3$, DMF, rt, 88%). Benzyl 2-(benzyloxy)-4-hydroxy-6-methylbenzoate (**30**) was prepared from benzoate ester **11** by selective protection of the *p*-OH with a methoxymethyl ether (1.1 equiv MOMCl, *i*-Pr_2NEt, CH_2Cl_2, rt, 92%) followed by blocking the remaining *o*-OH with a benzyl ether (BnBr, K_2CO_3, acetone, reflux, 89%) and subsequent removal of the methoxymethyl protection (6 N HCl, THF, rt, 85%). Coupling of phenol **27** with glucuronate bromide **7** was not successful under PTC conditions (TBAB, NaOH, $CHCl_3/H_2O$). Fortunately, the glycosidic coupling of **27** with **7** (2.0 equiv) took place under the action of Ag_2O in CH_3CN at 35 °C,[16] providing the desired 4-*O*-*β*-glucuronide **31** as the major product, which however could not be separated from the starting bromide **7** (Scheme 5). Subsequent hydrogenolysis of the crude benzyl ester **31** over Pd/C afforded acid **32**, which could be readily purified (59% for two steps). Coupling of acid **32** with phenol **30** (1.5 equiv) in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and DMAP provided phenol ester **33** in a good 64% yield.[9] The 2-OH in **33** was blocked with a benzyl ether, providing **34** (BnBr, K_2CO_3, acetone, reflux, 79%), to avoid a possible cleavage of the phenolic ester bond in the subsequent hydrolytic removal of the acetyl groups on the glucuronate moiety. At this point we encountered difficulties in removing the acetyl and ester groups. Initial attempts employing K_2CO_3/MeOH or KCN/MeOH resulted in cleavage of the phenolic ester bond. DBU/MeOH or HCl/MeOH conditions led to a complex mixture of the products. Fortunately, treatment of **34** with KOH in a solvent mixture of THF and H_2O (v/v 4:1) at room temperature afforded glucuronide **35** quantitatively. Removal of the benzyl groups on **35** proceeded smoothly under 1 atm H_2 over Pd/C in methanol, furnishing the target CRM646-A (**1**) in 93% yield.

Completion of the synthesis of CRM646-B (**2**) started from the crude glucuronide **31** (Scheme 6). Thus, blocking the 2-OH with a benzyl group provided homogeneous **36**

SCHEME 5. Completion of the Synthesis of CRM646-A (1)



SCHEME 6. Completion of the Synthesis of CRM646-B (2)



(59% for two steps). Removal of the acetyl groups was achieved with K_2CO_3 in MeOH at room temperature, giving **37** in 78% yield. Hydrogenolysis of **37** afforded **38** quantitatively. Acid **38** was coupled with phenol **30** (5.0 equiv) under the action of EDCI and DMAP in CH_2Cl_2, providing the desired phenol ester **39** in a satisfactory 65% yield. Finally, removal of the two benzyl groups on **39** by hydrogenolysis over Pd/C in EtOAc furnished CRM646-B (**2**) (96%). Analytic data for the synthetic CRM646-A (**1**) and -B (**2**) were in great accordance with those reported for the natural products.[1]

Conclusion

CRM646-A (**1**) and -B (**2**), two novel glucuronides with a dimeric 2,4-dihydroxy-6-alkylbenzoic acid (orcinol *p*-depside) aglycone, are potential anticancer agents with significant heparinase and telomerase inhibition activities. Total synthesis of these two fungal metabolites were

achieved for the first time. The successful approach involved construction of the phenol glucuronidic linkage, via coupling of the orsellinate derivative **27** with glucuronate bromide **7**, before assembly of the phenolic ester linkage in the depside aglycone. It is remarkable that the phenolic ester linkage in the advanced precursor **34** remained intact in the alkaline conditions for removal of the acetyl and methyl ester groups. In contrast, the previous synthetic attempts via direct glycosylation of the depside derivatives were found futile, mostly because of the decomposition of the phenolic ester linkage. Thus, CRM646-A (**1**) and -B (**2**) were synthesized, without optimization of the transformations, in 16 linear steps and 9.1% and 9.5% overall yields, respectively, starting from 3,5-dihydroxytoluene.

Experimental Section

Benzyl 4-*O*-(Methyl 2′,3′,4′-tri-*O*-acetyl-*β*-D-glucopyranosyluronate)-2-hydroxy-6-pentadecanyl-benzoate (31). Glycosyl bromide **7** (169 mg, 0.426 mmol) and phenol **27** (100 mg, 0.220 mmol) were dissolved in dry CH_3CN (10 mL), and Ag_2O (123 mg, 1.5 eq) was added under an Ar atmosphere at 38 °C. The mixture was stirred for 6 h and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography (petroleum ether/EtOAc = 6:1) to give the crude **31** (contaminated with inseparable **7**) as a white foam. R_f 0.69 (petroleum ether/EtOAc = 2:1). ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.72 (s, 1 H), 7.44−7.38 (m, 5 H), 6.42 (d, 1 H, J = 2.1 Hz), 6.32 (d, 1 H, J = 2.7 Hz), 5.36−5.20 (m, 7 H), 4.21 (m, 1 H), 3.73 (s, 3 H), 2.77 (m, 2 H), 2.07 (s, 3 H), 2.06 (s, 3 H), 2.03 (s, 3 H), 1.40−1.04 (m, 26 H), 0.89 (t, 3 H, J = 6.8 Hz). ESIMS (*m/z*): 793.5 (M + Na^+), 809.6 (M + K^+). HR-ESIMS (*m/z*) calcd for $C_{42}H_{58}O_{13}Na$ 793.3775, found 793.3770.

4-*O*-(Methyl 2′,3′,4′-Tri-*O*-acetyl-*β*-D-glucopyranosyluronate)-2-hydroxy-6-pentadecanyl-benzoic Acid (32). The crude **31** was treated with 10% Pd/C (25 mg) in EtOAc (5.0 mL) under 1 atm H_2 for 15 h. The mixture was then filtrated and concentrated. The residue was purified by a short silica gel column (petroleum ether/EtOAc = 4:1 to 1:2) to give **32** (89 mg, 59% for two steps) as a white foam. $[\alpha]^{19}_D$ = −18.1 (*c* 1.00, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.39 (s, 1 H), 6.35 (s, 1 H), 5.36−5.21 (m, 4 H), 4.23 (d, 1 H, J = 8.7 Hz), 3.72 (s, 3 H), 2.92−2.85 (m, 2 H), 2.05 (s, 9 H), 1.55−1.45 (m, 2 H), 1.35−1.20 (m, 24 H), 0.87 (t, 3 H, J = 6.8 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.0, 170.0, 169.4, 169.3, 166.8, 165.8, 160.9, 150.0, 111.9, 101.4, 97.4, 72.5, 71.6, 70.7, 68.9, 53.1, 36.5, 31.9, 31.7, 29.8, 29.7, 29.6, 29.5, 29.3, 22.7, 20.6, 20.5, 14.1. ESIMS (*m/z*): 679.3 (M − H^-). HR-ESIMS (*m/z*) calcd for $C_{35}H_{51}O_{13}$ 679.3328, found 679.3335.

Benzyl 4′-(4-*O*-(Methyl 2″,3″,4″-Tri-*O*-acetyl-*β*-D-glucopyranosyluronate)-2-hydroxy-6-pentdecanylbenzoyloxy)-2′-benzyloxy-6′-methylbenzoate (33). A solution of acid **32** (55 mg, 0.08 mmol) and phenol **30** (42 mg, 0.122 mmol) in dry CH_2Cl_2 (2.5 mL) was treated at room temperature with DMAP (12 mg, 1.1 equiv) and EDCI (40 mg, 2.0 equiv). After stirring for 4 h, the solution was diluted with saturated aqueous NH_4Cl and extracted twice with CH_2Cl_2 (30 mL). The organic phase was washed with water and brine, respectively, and was then dried (Na_2SO_4) and concentrated. The residue was purified by silica gel column chromatography (petroleum ether/EtOAc = 6:1) to provide **33** (53 mg, 65%) as a colorless oil. R_f 0.23 (CH_2Cl_2/MeOH = 8:1); R_f 0.23 (petroleum ether/EtOAc = 4:1); $[\alpha]^{19}_D$ = −10.8 (*c* 0.96, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.32 (s, 1 H), 7.34−7.30 (m, 10 H), 6.65 (s, 2 H), 6.47 (d, 1 H, J = 2.7 Hz), 6.43 (d, 1 H, J = 2.7 Hz), 5.38−5.26 (m, 6 H), 5.07 (s, 2 H), 4.25 (d, 1 H, J = 9.0 Hz), 3.75 (s, 3 H), 3.00−2.85 (m, 2 H), 2.33 (s, 3 H), 2.08 (s, 3 H), 2.07 (s, 3 H), 2.06 (s, 3 H), 1.65−1.60 (m, 2 H), 1.38−1.20 (m, 24 H), 0.89 (t, 3 H, J = 6.8 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.0, 169.6, 169.2, 169.1, 167.3, 166.6, 165.8, 161.1, 156.8, 151.0, 148.9, 138.4, 136.0, 135.6, 128.54, 128.47, 128.39, 128.2, 128.0, 127.2, 122.4, 115.6, 112.4, 106.1, 104.3, 101.8, 97.7, 72.8, 71.7, 70.9, 70.8, 69.0, 67.1, 52.9, 37.1, 32.3, 31.9, 29.9, 29.6, 29.3, 22.6, 20.5, 20.4, 19.4, 14.0. ESIMS (*m/z*): 1033.9 (M + Na^+), 1049.8 (M + K^+). HR-ESIMS (*m/z*) calcd for $C_{57}H_{71}O_{16}H$ 1011.4744, found 1011.4737.

Benzyl 4′-(4-*O*-(Methyl 2″,3″,4″-Tri-*O*-acetyl-*β*-D-glucopyranosyluronate)-2-benzyloxy-6-pentadecanylbenzoyloxy)-2′-benzyloxy-6′-methylbenzoate (34). To a solution of **33** (64 mg, 0.063 mmol) in dry acetone (5 mL) was added K_2CO_3 (17 mg, 2.0 equiv) and BnBr (0.1 mL). The mixture was heated to reflux for 12 h. The solution was diluted with EtOAc (50 mL). The organic phase was washed with water and brine, respectively, and was then dried over Na_2SO_4 and concentrated. Chromatography over silica gel (petroleum ether/EtOAc = 4:1) gave **34** (54 mg, 78%) as a white foam. R_f 0.25 (petroleum ether/EtOAc = 4:1); $[\alpha]^{19}_D$ = −7.5 (*c* 0.65, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.45−7.27 (m, 15 H), 6.57 (d, 1 H, J = 2.1 Hz), 6.51−6.48 (m, 3 H), 5.40−5.25 (m, 5 H), 5.18 (d, 1 H, J = 6.9 Hz), 5.07 (s, 2 H), 4.82 (s, 2 H), 4.21 (d, 1 H, J = 9.3 Hz), 3.76 (s, 3 H), 2.66 (t, 2 H, J = 7.5 Hz), 2.24 (s, 3 H), 2.08 (s, 6 H), 2.07 (s, 3 H), 1.65−1.60 (m, 2 H), 1.38−1.20 (m, 24 H), 0.89 (t, 3 H, J = 6.8 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.1, 169.3, 169.2, 167.5, 166.7, 166.1, 158.5, 157.3, 156.6, 152.1, 143.7, 138.0, 136.1, 136.0, 135.6, 128.6, 128.4, 128.3, 128.1, 127.9, 127.7, 127.3, 121.7, 118.1, 115.7, 109.3, 104.2, 99.9, 98.7, 72.6, 71.7, 71.0, 70.8, 70.4, 68.9, 67.0, 53.0, 33.8, 31.9, 31.3, 29.6, 29.3, 22.6, 20.6, 20.5, 19.4, 14.1. ESIMS (*m/z*): 1023.5 (M + Na^+), 1039.5 (M + K^+). HR-ESIMS (*m/z*) calcd for $C_{64}H_{76}O_{16}Na$ 1123.5031, found 1123.5026.

Benzyl 4′-(4-*O*-(*β*-D-Glucopyranosyluronic acid)-2-benzyloxy-6-pentadecanylbenzoyloxy)-2′-benzyloxy-6′-methylbenzoate (35). To a solution of **34** (60 mg, 0.054 mmol) in THF/H_2O (10 mL, v/v = 4/1) was added KOH (30 mg) at room temperature. After stirring for 4 h, the mixture was acidified to pH = 3 with 1 N HCl and was then diluted with EtOAc (50 mL). The organic phase was washed with water and brine, respectively, and was then dried over Na_2SO_4 and concentrated. Chromatography over silica gel (CH_2Cl_2/MeOH = 4:1) provided **35** (52 mg, 100%) as a white powder. R_f 0.3 (CH_2Cl_2/MeOH = 4:1); $[\alpha]^{20}_D$ = −30.2 (*c* 0.20, CH_3OH). ^{1}H NMR (300 MHz, CD_3COCD_3−CD_3OD = 1:1): δ 7.52−7.30 (m, 15 H), 6.86 (d, 1 H, J = 1.5 Hz), 6.71 (d, 1 H, J = 1.5 Hz), 6.64 (s, 1 H), 6.54 (s, 1 H, J = 1.8 Hz), 5.33 (s, 2 H), 5.18 (s, 2 H), 5.13 (d, 1 H, J = 7.2 Hz), 4.93 (s, 2 H), 4.06 (d, 1 H, J = 9.6 Hz), 3.70 (t, 1 H, J = 9.0 Hz), 3.56 (m, 2 H), 2.69 (t, 2 H, J = 7.8 Hz), 2.20 (s, 3 H), 1.69−1.62 (m, 2 H), 1.38−1.20 (m, 24 H), 0.89 (t, 3 H, J = 6.6 Hz). ESI-MS (*m/z*): 983.5 (M + Na^+), 999.4 (M + K^+). HR-ESIMS calcd for $C_{57}H_{68}O_{13}Na$ 983.4558, found 983.4552.

CRM646-A (1). Compound **35** (50 mg, 0.052 mmol) was treated with 10% Pd/C (20 mg) in MeOH (10.0 mL) under 1 atm H_2 for 1 day. The mixture was filtered. The filtrate was concentrated and purified by a short column of silica gel (CH_2Cl_2/MeOH/H_2O = 4:1:0.1), affording CRM646-A (**1**, 33 mg, 93%) as a white solid. $[\alpha]^{20}_D$ = −41.5 (*c* 0.31, CH_3OH). ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.34 (br s, 1 H), 6.57 (s, 1 H), 6.49 (m, 3 H), 5.70−5.20 (br, 2H), 5.05 (d, 1 H, J = 7.5 Hz), 3.91 (d, 1 H, J = 9.0 Hz), 3.46−3.30 (m, 3 H), 2.65−2.60 (m, 2 H), 2.45 (s, 3 H), 1.60−1.50 (m, 2 H), 1.38−1.20 (m, 24 H), 0.86 (m, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.3, 170.2, 166.4, 160.8, 159.4, 157.5, 152.5, 143.4, 140.6, 116.5, 114.3, 113.8, 108.6, 107.4, 101.5, 100.0, 76.0, 75.8, 73.1, 71.6, 33.7, 31.5, 31.1, 29.2, 29.0, 28.9, 22.3, 21.8, 14.1.

Benzyl 4-*O*-(Methyl 2′,3′,4′-Tri-*O*-acetyl-*β*-D-glucopyranosyluronate)-2-benzyloxy-6-pentadecanyl-benzoate (36). To a solution of the crude **31** (prepared from **7** and 100 mg **27**) in dry acetone (5 mL) was added K_2CO_3 (50 mg, 2.0 equiv) and BnBr (0.1 mL). The mixture was heated to reflux for 12 h and then was diluted with EtOAc (50 mL). The organic phase was washed with water and brine, respectively, and then was

dried over Na_2SO_4 and concentrated. Chromatography over silica gel (petroleum ether/EtOAc = 4:1) gave **36** (112 mg, 59% for two steps) as a white foam. R_f 0.25 (petroleum ether/EtOAc = 4:1); $[\alpha]^{20}_D$ = 35.4 (*c* 0.65, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.37−7.25 (m, 10 H), 6.44 (d, 1 H, *J* = 1.8 Hz), 6.40 (d, 1 H, *J* = 2.1 Hz), 5.33−5.23 (m, 5 H), 5.08 (s, 1 H), 5.05 (s, 2 H), 4.12 (d, 1 H, *J* = 9.0 Hz), 3.71 (s, 3 H), 2.48 (t, 2 H, *J* = 7.5 Hz), 2.09−2.00 (s, 9 H), 1.63 (m, 2 H), 1.30−1.20 (m, 24 H), 0.89 (t, 3 H, *J* = 6.8 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.0, 169.2, 169.1, 167.7, 166.7, 158.0, 156.8, 143.3, 136.4, 135.7, 128.5, 128.4, 128.1, 127.9, 127.1, 109.5, 100.3, 100.0, 72.6, 71.8, 71.0, 70.6, 69.0, 67.0, 52.7, 33.7, 31.9, 31.1, 29.6, 29.5, 29.4, 29.3, 22.6, 20.5, 20.4, 14.0. ESIMS (*m/z*): 883.4 (M + Na^+), 899.4 (M + K^+). HR-ESIMS (*m/z*) calcd for $C_{49}H_{64}O_{13}$Na 883.4245, found 883.4239.

Benzyl 4-*O*-(Methyl *β*-D-Glucopyranosyluronate)-2-benzyloxy-6-pentadecanyl-benzoate (37). To a solution of **36** (100 mg, 0.116 mmol) in MeOH (5.0 mL) was added K_2CO_3 (10 mg) at room temperature. After stirring for 20 min, the solution was diluted with saturated aqueous NH_4Cl and extracted twice with EtOAc. The organic phase was washed with water and brine, respectively, and was then dried over Na_2SO_4 and concentrated. Chromatography over silica gel (CH_2Cl_2/MeOH = 20:1) gave **37** (67 mg, 78%) as a colorless oil. R_f 0.23 (CH_2Cl_2/MeOH = 20:1); $[\alpha]^{20}_D$ = −39.4 (*c* 1.20, $CHCl_3$). ^{1}H NMR (300 MHz, CD_3COCD_3): δ 7.45−7.37 (m, 10 H), 6.74 (d, 1 H, *J* = 1.8 Hz), 6.62 (d, 1 H, *J* = 2.1 Hz), 5.32 (s, 2 H), 5.17 (d, 1 H, *J* = 7.2 Hz), 5.14 (s, 2 H), 4.14 (d, 1 H, *J* = 9.6 Hz), 3.73 (m, 4 H), 3.59−3.54 (m, 2 H), 2.52 (t, 2 H, *J* = 7.8 Hz), 1.60−1.45 (m, 2 H), 1.33−1.20 (m, 24 H), 0.89 (t, 3 H, *J* = 6.8 Hz). ^{13}C NMR (75 MHz, CD_3COCD_3): δ 169.6, 168.2, 159.7, 157.4, 143.3, 137.7, 136.9, 129.1, 128.7, 128.5, 128.0, 119.1, 110.0, 101.2, 100.3, 76.6, 76.1, 73.8, 72.0, 70.7, 67.1, 52.4, 34.0, 32.4, 31.8, 30.5, 30.2, 30.1, 30.0, 29.5, 29.2, 29.0, 23.1, 14.2. ESIMS (*m/z*): 757.4 (M + Na^+). HR-ESIMS (*m/z*) calcd for $C_{43}H_{58}O_{10}$Na 757.3928, found 757.3922.

Benzyl 4′-(4-*O*-(Methyl *β*-D-Glucopyranosyluronate)-2-hydroxy-6-pentdecanylbenzoyloxy)-2′-benzyloxy-6′-methylbenzoate (39). Compound **37** (50 mg, 0.068 mmol) was treated with 10% Pd/C (10 mg) in EtOAc (5.0 mL) under 1 atm of H_2 atmosphere overnight. The mixture was then filtered and concentrated to dryness, affording an amorphous solid **38** (37 mg). A solution of the acid **38** (37 mg, 0.067 mmol) and phenol **30** (112 mg, 5.0 equiv) in dry CH_2Cl_2 (2.5 mL) was treated at room temperature with DMAP (9 mg, 1.1 equiv) and EDCI (25 mg, 2.0 equiv). After stirring for 4 h, the solution was diluted with saturated aqueous NH_4Cl and extracted twice with EtOAc. The organic phase was washed with water and brine, respectively, and was then dried over Na_2SO_4 and concentrated. Chromatography over silica gel (CH_2Cl_2/MeOH = 8:1) provided **39** (38 mg, 65%) as a colorless oil. R_f 0.23 (CH_2Cl_2/MeOH = 8:1). $[\alpha]^{20}_D$ = −38.7 (*c* 0.90, $CHCl_3$). ^{1}H NMR (300 MHz, CD_3COCD_3): δ 7.46−7.33 (m, 10 H), 7.03 (s, 1 H), 6.83 (s, 1 H), 6.59 (d, 1 H, *J* = 3.3 Hz), 5.38 (s, 2 H), 5.28 (d, 1 H, *J* = 7.5 Hz), 5.20 (s, 2 H), 4.22 (d, 1 H, *J* = 9.3 Hz), 3.75−3.70 (m, 4 H), 3.70−3.60 (m, 3 H), 2.93 (t, 2 H, *J* = 7.8 Hz), 2.32 (s, 3 H), 1.63 (m, 2 H), 1.38−1.25 (m, 24 H), 0.88 (t, 3 H, *J* = 6.8 Hz). ^{13}C NMR (75 MHz, CD_3COCD_3): δ 170.1, 168.1, 164.3, 162.7, 157.8, 152.8, 148.5, 138.8, 137.7, 137.3, 129.6, 129.5, 129.2, 129.1, 128.6, 116.8, 112.3, 108.8, 105.9, 102.7, 101.0, 77.0, 76.6, 74.3, 72.6, 71.6, 67.8, 52.8, 37.2, 33.1, 32.9, 31.0, 30.8, 30.7, 30.6, 29.9, 29.7, 29.4, 23.6, 19.7, 14.7.

CRM646-B (2). Compound **39** (21 mg) was treated with 10% Pd/C (10 mg) under 1 atm of H_2 in EtOAc (5.0 mL) for 24 h. The mixture was then filtered. The filtrate was concentrated and purified by a short column of silica gel (CH_2Cl_2/MeOH = 4:1) to afford CRM646-B (**2**) as a white solid (16 mg, 96%). R_f 0.23 (CH_2Cl_2/MeOH = 4:1); $[\alpha]^{20}_D$ = −29.7 (*c* 0.31, CD_3COCD_3). ^{1}H NMR (300 MHz, CD_3COCD_3): δ 6.58−6.55 (m, 3 H), 6.45 (d, 1 H, *J* = 1.8 Hz), 5.28 (d, 1 H, *J* = 7.5 Hz), 4.22 (d, 1 H, *J* = 9.6 Hz), 3.76−3.73 (m, 4 H), 3.74−3.60 (m, 3 H), 2.93 (t, 2 H, *J* = 7.8 Hz), 2.65 (s, 3 H), 1.65−1.60 (m, 2 H), 1.35−1.25 (m, 24 H), 0.88 (t, 3 H, *J* = 6.8 Hz). ^{13}C NMR (75 MHz, CD_3COCD_3): δ 170.2, 165.2, 164.4, 162.6, 153.2, 148.5, 115.5, 112.3, 109.1, 108.4, 102.8, 101.1, 77.1, 76.7, 74.3, 72.7, 52.9, 37.2, 33.1, 32.9, 31.0, 30.7, 30.6, 30.5, 30.2, 29.9, 29.7, 29.4, 24.2, 23.6, 14.7. ESIMS (*m/z*): 703.3 (M − H^-). HR-ESIMS (*m/z*) calcd for $C_{37}H_{52}O_{13}$Na 727.3306, found 727.3300.

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Supporting Information Available: Experimental procedures and analytical data for compounds **4**−**6** and **9**−**30** and reproductions of ^{1}H and ^{13}C NMR spectra for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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