

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

Ping Wang, Zhaojun Zhang, and Biao Yu*

State Key Laboratory of Bioorganic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 354 Fenglin Road, Shanghai 200032, China

byu@mail.sioc.ac.cn

Received July 5, 2005



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 (IC₅₀) of the hydrolysis of porcine heparin by the heparinase (Sigma) for 1 and 2 occurred at 3 and 10 μ M, respectively;¹ suramin,³ known as a potent inhibitor of melanoma heparanase, showed an IC₅₀ value of 5 μ M in this assay system. The correlation between heparanase inhibition and the inhibition of tumor metastasis⁴ for compounds 1 and 2 were then examined. Both compounds strongly inhibited the migration of B16-F10 melanoma cells, with IC₅₀ values being 15 and 30 μ M, respectively. In addition, CRM646-A (1) showed inhibitory activity against telomerase at a

C. A.; Nicolson, G. L. J. Bud. Chem. 1991, 200, 3001–3000.
(4) For selected reviews, see: (a) Vlodavsky, I.; Goldshmidt, O.; Zcharia, E.; Atzmon, R.; Rangini-Guatta, Z.; Elkin, M.; Peretz, T.; Friedmann, Y. Sem. Cancer Biol. 2002, 12, 121–129. (b) Parish, C. R.; Freeman, C.; Hulett, M. D. Biochim. Biophys. Acta 2001, 1471, M99–M108. (c) Bame, K. J. Glycobiology 2001, 11, 91R–98R. (d) Dempsey, L. A.; Brunn, G. J.; Platt, J. L. Trends Biochem. Sci. 2000, 25, 349–351. (e) Ferro, V.; Hammond, E.; Fairweather, J. K. Mini-Rev. Med. Chem. 2004, 4, 693–702.

dose of $3.2 \,\mu M.^5$ No cytotoxicity up to $100 \,\mu M$ 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

10.1021/jo051384k CCC: \$30.25 © 2005 American Chemical Society Published on Web 10/05/2005

 $[\]ast$ To whom correspondence should be addressed. Fax: (0086)-21-64166128.

Ko, H. R.; Kim, B. Y.; Oh, W. K.; Kang, D. O.; Lee, H. S.; Koshino,
 H.; Osada, H.; Mheen, T. I.; Ahn, J. S. J. Antibiot. 2000, 53, 211–214.
 (2) Ahn, J. S.; Kim, B. Y.; Oh, W. K.; Mheen, T. I.; Ahn, S. C.; Kang,

⁽²⁾ Ahn, J. S.; Kim, B. Y.; Oh, W. K.; Mheen, T. I.; Ahn, S. C.; Kang, D. O.; Ko, H. R.; Kim, H. M. PCT Int. Appl. WO 2001046385, 2001; *Chem. Abstr.* **2001**, 135:75833.

 ⁽³⁾ Nakajima, M.; Dechavingy, A.; Johnson, C. E.; Hamada, J.; Stein,
 C. A.; Nicolson, G. L. J. Biol. Chem. 1991, 266, 9661–9666.

⁽⁵⁾ Togashi, K.-I.; Ko, H.-R.; Ahn, J.-S.; Osada, H. *Biosci. Biotechnol. Biochem.* **2001**, *65*, 651–653.

⁽⁶⁾ For selected examples, see: (a) Elix, J. A.; Wardlaw, J. H. Aust. J. Chem. 1987, 40, 425-429. (b) Elix, J. A.; Wardlaw, J. H. Aust. J. Chem. 1997, 50, 1145-1150. (c) Elix, J. A.; Wardlaw, J. H. Aust. J. Chem. 1997, 50, 479-486. (d) Elix, J. A.; Wardlaw, J. H. Aust. J. Chem. 1996, 49, 917-924. (e) Elix, J. A.; Barclay, C. E.; Lumbsch, H. T. Aust. J. Chem. 1994, 47, 1199-1203.

 ^{(7) (}a) Ondeyka, J. G.; Zink, D. L.; Dombrowski, A. W.; Polishook,
 J. D.; Felock, P. J.; Hazuda, D. J.; Singh, S. B. J. Antibiot. 2003, 56,
 1018–1023. (b) Yasuzawa, T.; Saitoh, Y.; Sano, H. J. Antibiot. 1990,
 43, 336–343.

⁽⁸⁾ For a recent example, see: García-Fortanet, J.; Debergh, J. R.; De Brabander, J. K. Org. Lett. **2005**, 7, 685–688.

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 type¹⁰ or a phenolic acceptor¹¹ 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 BF₃·OEt₂ (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., 2,3di-O-acetyl-4,6-O-benzylidene-D-glucopyranosyl trichloroacetimidate, $8)^{18}$ and then to elaborate the 6"-carboxylic

(12) (a) Fischer, B.; Nudelman, A.; Ruse, M.; Herzig, J.; Gottlieb,
 H. E.; Keinan, E. J. Org. Chem. 1984, 49, 4988–4993. (b) Hasuoka,
 A.; Nakayama, Y.; Adachi, M.; Kamiguchi, H.; Kamiyama, K. Chem.
 Pharm. Bull. 2001, 49, 1604–1608. (c) Nakamura, S.; Kondo, M.; Goto,
 K.; Nakamura, M.; Tsuda, Y.; Shishido, K. Heterocycles 1996, 43, 2747–2756.



FIGURE 1. Glycosyl donors 3-8.

acid function via selective oxidation of the 6"-OH group.¹⁹

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/POCl₃ gave 2,4-dihydroxy-6-methyl benzaldehyde 9 (80%).²⁰ Treatment of aldehyde 9 with sodium chlorite (NaClO₂) in a NaH₂PO₄ buffered solution of DMSO and H₂O 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 KHCO₃ 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 $9 \rightarrow 10$. 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 ($-78 \rightarrow -10$ °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 H₂O 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

⁽⁹⁾ Dushin, R. G.; Danishefsky, S. J. J. Am. Chem. Soc. **1992**, 114, 655–659.

⁽¹⁰⁾ Stachulski, A. V.; Jenkins, G. N. Nat. Prod. Rep. **1998**, 173–186.

⁽¹¹⁾ For examples, see: (a) Jensen, K. J. J. Chem. Soc., Perkin Trans. 1 2002, 2219–2233. (b) Du, Y.; Wei, G.; Linhardt, R. J. J. Org. Chem. 2004, 69, 2206–2209.

⁽¹³⁾ For the preparation, see: Supporting Information.

⁽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.
(15) (a) Cai, S.; Yu, B. Org. Lett. 2003, 5, 3827-3830. (b) Tanaka,

 ^{(15) (}a) Cai, S.; Yu, B. Org. Lett. 2003, 5, 3827–3830. (b) Tanaka,
 H.; Iwata, Y.; Takahashi, D.; Adachi, M.; Takahashi, T. J. Am. Chem.
 Soc. 2005, 127, 1630–1631.

⁽¹⁶⁾ For selected examples, see: (a) Needs, P. W.; Williamson, G. Carbohydr. Res. **2001**, 330, 511–516. (b) Florent, J. C.; Dong, X.; Gaudel, G.; Mitaku, S.; Monneret, C. J. Med. Chem. **1998**, 41, 3572–3581. (c) Bellamy, F.; Horton, D.; Millet, J.; Picart, F.; Samreth, S.; Chazan, J. B. J. Med. Chem. **1993**, 36, 898–903. (d) Dawson, M. I.; Hobbs, P. D. Carbohydr. Res. **1980**, 85, 121–129.

 ⁽¹⁷⁾ Bollenback, G. N.; Long, J. W.; Benjamin, D. G.; Lindquist, J.
 A. J. Am. Chem. Soc. 1955, 77, 3310–3315.

⁽¹⁸⁾ Zimmermann, P.; Greilich, U.; Schmidt, R. R. *Tetrahedron Lett.* **1990**, *31*, 1849–1852.

⁽¹⁹⁾ Lin, F.; Peng, W.; Xu, W.; Han, X.; Yu, B. *Carbohydr. Res.* **2004**, *339*, 1219–1223 and references therein.

⁽²⁰⁾ Nielsen, S. F.; Christensen, S. B.; Cruciani, G.; Kharazmi, A.; Liljefors, T. J. Med. Chem. **1998**, 41, 4819–4832.

⁽²¹⁾ Nicolaou, K. C.; Rodríguez, R. M.; Mitchell, H. J.; van Delft, F. L. Angew. Chem., Int. Ed. **1998**, 37, 1874–1876.

⁽²²⁾ Bruce, I.; Spencer, R.; Tyman, J. J. Chem. Res., Synop. **1992**, 224–225.

⁽²³⁾ Kato, T.; Hozumi, T. Chem. Pharm. Bull. 1972, 20, 1574–1578.









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 KHCO₃ in DMF (89%).

The 2,2'-phenolic hydroxyl groups in **22** are hydrogenbonded 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 BF₃·OEt₂ or TMSOTf failed to provide the desired glycosides. Complex products were mostly encountered. Treatment of **22** with bromide **7** under either Koenigs–Knorr conditions (Ag₂O, CH₃CN)¹⁶ or under phase transfer conditions (TBAB, NaOH, CHCl₃, H₂O)²⁴ led to the cleavage of the phenolic ester bond in **22**. As the last resort, glycosylation of **22** (1.2 equiv) with

SCHEME 3. Attempt To Synthesize the Glucuronide via Oxidation



the glucopyranosyl trichloroacetimidate **8** under the promotion of BF₃·OEt₂ (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, K₂CO₃, acetone, reflux) to provide **24** (80%). Treatment of **24** with a catalytic amount of TsOH·H₂O (0.1 equiv) in 90% HOAc at 40 °C removed the 4",6"-O-benzylidene group,²⁵ affording diol **25** in a moderate

⁽²⁴⁾ Feng, I. Q.; Sun, J.; Qu, L. Q. Synth. Commun. **2002**, *32*, 3393–3398.

⁽²⁵⁾ Tsujihara, K.; Hongu, M.; Saito, K.; Kawanishi, H.; Kuriyama, K.; Matsumoto, M.; Oku, A.; Ueta, K.; Tsuda, M.; Saito, A. J. Med. Chem. **1999**, 42, 5311–5324.



yield (51%). Unfortunately, subjection of **25** to the wellstudied TEMPO oxidation protocol¹⁹ 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, KHCO₃, 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-Pr₂NEt, CH₂Cl₂, rt, 92%) followed by blocking the remaining o-OH with a benzyl ether (BnBr, K₂CO₃, 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, CHCl₃/H₂O). Fortunately, the glycosidic coupling of 27 with 7 (2.0 equiv) took place under the action of Ag₂O in CH₃CN 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, K₂CO₃, 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 K₂CO₃/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 H₂O (v/v 4:1) at room temperature afforded glucuronide 35 quantitatively. Removal of the benzyl groups on 35 proceeded smoothly under 1 atm H₂ 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**









(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 CH₂Cl₂, 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 pdepside) 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 CH₃CN (10 mL), and Ag₂O (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). ¹H NMR (300 MHz, CDCl₃): 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), $4.21\ (m,\,1\,H),\, 3.73\ (s,\,3\,H),\, 2.77\ (m,\,2\,H),\, 2.07\ (s,\,3\,H),\, 2.06\ (s,\,3\,H),\, 2.06$ 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 C₄₂H₅₈O₁₃Na 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 H₂ 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. $[\alpha]^{19}{}_{\rm D} = -18.1~(c$ 1.00, CHCl₃). ¹H NMR (300 MHz, CDCl₃): 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, 1 H)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). ¹³C NMR (75 MHz, CDCl₃): 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 C₃₅H₅₁O₁₃ 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 NH₄Cl and extracted twice with CH₂Cl₂ (30 mL). The organic phase was washed with water and brine, respectively, and was then dried (Na₂SO₄) 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. $R_f 0.23$ (CH₂Cl₂/MeOH = 8:1); $R_f 0.23$ (petroleum ether/ EtOAc = 4:1); $[\alpha]^{19}_{D} = -10.8$ (c 0.96, CHCl₃). ¹H NMR (300) MHz, CDCl₃): 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). ¹³C NMR (75 MHz, CDCl₃): 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 C₅₇H₇₁O₁₆H 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 K₂CO₃ (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 Na₂SO₄ 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, CHCl₃). ¹H NMR (300 MHz, CDCl₃): 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). ¹³C NMR (75 MHz, CDCl₃): 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 C₆₄H₇₆O₁₆Na 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/H₂O (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. $R_f 0.3$ (CH₂Cl₂/ MeOH = 4:1; $[\alpha]^{20}_{D} = -30.2$ (c 0.20, $CH_{3}OH$). ¹H 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 C₅₇H₆₈O₁₃Na 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 H₂ for 1 day. The mixture was filtered. The filtrate was concentrated and purified by a short column of silica gel (CH₂-Cl₂/MeOH/H₂O = 4:1:0.1), affording CRM646-A (1, 33 mg, 93%) as a white solid. [α]²⁰_D = -41.5 (*c* 0.31, CH₃OH). ¹H 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). ¹³C NMR (75 MHz, CDCl₃): 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 K₂CO₃ (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 Na₂SO₄ 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); $[\alpha]^{20}_{\rm D}$ = 35.4 (c 0.65, CHCl₃). ¹H NMR (300 MHz, CDCl₃): 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). ¹³C NMR (75 MHz, CDCl₃): 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 C₄₉H₆₄O₁₃-Na 883.4245, found 883.4239.

Benzyl 4-O-(Methyl β -D-Glucopyranosyluronate)-2benzyloxy-6-pentadecanyl-benzoate (37). To a solution of 36 (100 mg, 0.116 mmol) in MeOH (5.0 mL) was added K₂CO₃ (10 mg) at room temperature. After stirring for 20 min, the solution was diluted with saturated aqueous NH₄Cl and extracted twice with EtOAc. The organic phase was washed with water and brine, respectively, and was then dried over Na₂SO₄ 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 (CH₂Cl₂/MeOH = 20:1); $[\alpha]^{20}_{D} = -39.4$ (c 1.20, CHCl₃). ¹H NMR (300 MHz, CD₃COCD₃): 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). $^{13}\mathrm{C}$ NMR (75 MHz, $\mathrm{CD_3COCD_3}$): 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 C₄₃H₅₈O₁₀Na 757.3928, found 757.3922.

Benzyl 4'-(4-O-(Methyl β-D-Glucopyranosyluronate)-2hydroxy-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 H₂ 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 CH₂Cl₂ (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 NH4Cl and extracted twice with EtOAc. The organic phase was washed with water and brine, respectively, and was then dried over Na₂SO₄ and concentrated. Chromatography over silica gel (CH₂Cl₂/MeOH = 8:1) provided **39** (38 mg, 65%) as a colorless oil. $R_f 0.23$ (CH₂- $Cl_2/MeOH = 8:1$). $[\alpha]^{20}_{D} = -38.7 (c \ 0.90, CHCl_3)$. ¹H NMR (300) MHz, CD₃COCD₃): 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, 3.10)3 H), 1.63 (m, 2 H), 1.38–1.25 (m, 24 H), 0.88 (t, 3 H, J = 6.8 Hz). ¹³C NMR (75 MHz, CD₃COCD₃): 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.

 $\mathbf{CRM646\text{-}B}$ (2). Compound 39 $(21~\mathrm{mg})$ 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 $0.23 (CH_2Cl_2/MeOH = 4:1); [\alpha]^{20}_D = -29.7 (c \ 0.31, CD_3COCD_3).$ ¹H NMR (300 MHz, CD₃COCD₃): 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). ¹³C NMR (75 MHz, CD₃- $COCD_3$): 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 C₃₇H₅₂O₁₃Na 727.3306, found 727.3300.

Acknowledgment. This work is supported by the Committee of Science and Technology of Shanghai (04DZ19213, 04DZ14901), the National Natural Science Foundation of China (20321202), and the Chinese Academy of Sciences (KGCX2-SW-209).

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

JO051384K