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Total Syntheses of Lobaric Acid and Its Derivatives from the Antarctic Lichen *Stereocaulon alpinum*

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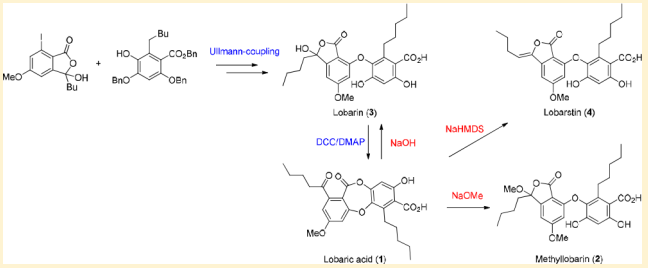
Supporting Information

ABSTRACT: The first total syntheses of the natural products lobaric acid (**1**) and its derivatives isolated from the Antarctic lichen *Stereocaulon alpinum* are reported in this study. Lobarin (**3**), with a pseudodepsidone structure, was synthesized first in 11 steps by utilizing an Ullmann aryl ether coupling reaction, and lobaric acid was synthesized in an additional three steps by a seven-membered lactonization reaction. Various derivatives were also obtained from the prepared lobaric acid, and the synthetic compounds exhibited significant PTP1B inhibitory activities.



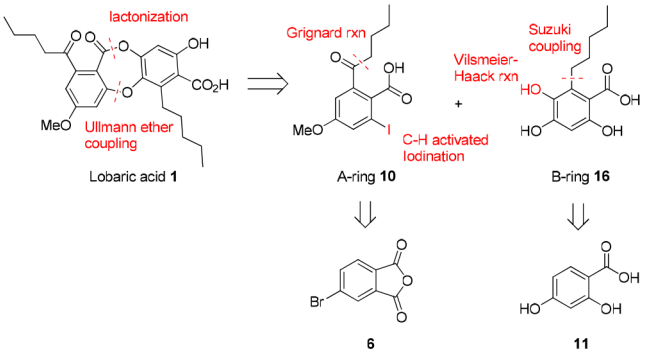
Depsidones and pseudodepsidones are commonly found as secondary metabolites in a number of lichens; several of these compounds have been isolated from various lichens in recent years, and their bioactivities have been studied. For example, the depsidone parellin, isolated from *Ochrolechia parella*, exhibits cytotoxic activity.[1] A tube-type lichen, *Hypogymnia lugubris*, produces physodic acid as a secondary metabolite that exhibits anticancer properties.[2] Furthermore, the pseudodepsidone sakisacaulon A obtained from *Stereocaulon sasakii* exhibited antimitotic activity.[3] We were particularly interested in the depsidone derivative lobaric acid (**1**), which was isolated from the Antarctic lichen *Stereocaulon alpinum*[4] and has been reported to exhibit a range of bioactivities[5] that include antimicrobial, antimitotic, cytotoxic, enzyme inhibitory, toxicological, and immunomodulatory properties.[6] Indeed, in our previous study, we evaluated the antioxidant, antimicrobial, anticancer, and protein-tyrosine phosphatase 1B (PTP1B) inhibitory activities[4] of lobaric acid and its derivatives methyllobarin (**2**), lobarin (**3**), and lobarstin (**4**).[7] In particular, lobaric acid (**1**), lobarin (**3**), and lobarstin (**4**) displayed potent PTP1B inhibitory activities.[4,8] Furthermore, these compounds did not affect other members of the protein tyrosine phosphatase family.[8] However, the extraction of these compounds from slow-growing lichens limits studies into their potential biological activities. To date, syntheses of the depsidones colensoic acid and diploicin have been reported,[9] but the developed method is not applicable to the syntheses of any derivatives owing to the large number of steps involved and the low yields obtained. As such, the syntheses of lobaric acid and its derivatives are of particular importance, as they will facilitate the production of sufficient quantities for further investigations into their biological activities.

Hence, we report herein the development of a new synthetic approach to highly substituted depsidone structures, namely, lobaric acid (**1**) and its pseudodepsidone derivatives **2**−**5**, from the same starting materials by employing similar synthetic methods.

RESULTS AND DISCUSSION

Scheme 1 shows our retrosynthetic approach to lobaric acid (**1**), in which we assume that the target is obtained through the

Scheme 1. Retrosynthetic Approach to Lobaric Acid (1)



coupling of the A- and B-rings by an Ullmann aryl ether reaction.[10] To provide the key intermediates for this reaction, the *n*-pentyl group of the B-ring is introduced by a Suzuki coupling reaction,[11] and C−H activated iodination[12] ortho to the carboxylic acid moiety in the A-ring is also required. Finally,

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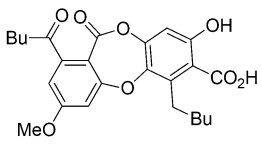
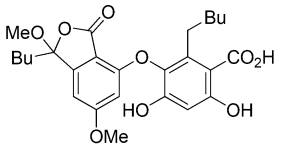
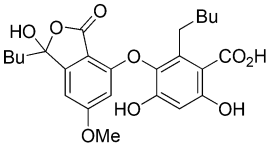


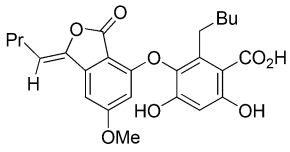
Lobaric acid **1**



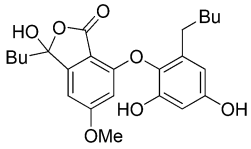
Methyllobarin **2**



Lobarin **3**



Lobarstin **4**

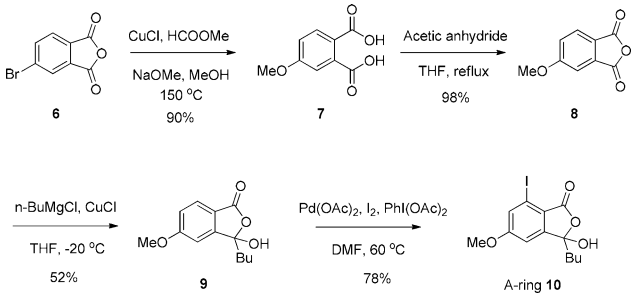


Sakisacaulon A **5**

the seven-membered-ring-containing depsidone skeleton is constructed via dicyclohexylcarbodiimide (DCC)/4-dimethylaminopyridine (DMAP) lactonization.[13]

The synthesis of lobaric acid (**1**) began with 4-bromophthalic anhydride (**6**), as outlined in Scheme 2. Accordingly, anhydride

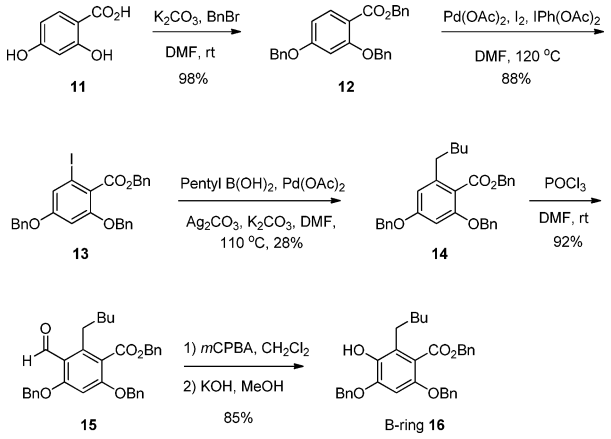
Scheme 2. Preparation of the A-Ring Fragment 10



6 was transformed into phthalic acid **7** in 90% yield through an Ullmann-type methoxylation reaction in the presence of CuCl, HCOOMe, MeONa, and MeOH and was accompanied by ring opening of the phthalic anhydride due to the presence of excess sodium methoxide.[14] The anhydride **8** was obtained in 98% yield by the treatment of **7** with acetic anhydride in tetrahydrofuran (THF) under anhydrous conditions.[15] Grignard reaction of **8** with BuMgCl and CuCl in THF afforded compound **9** in 52% yield;[16] subsequent iodination with (diacetoxyiodo)benzene in dimethylformamide (DMF) at the position ortho to the carboxylic acid moiety gave the desired A-ring fragment **10**.[12]

We then began the preparation of the B-ring starting with 2,4-dihydroxybenzoic acid (**11**), as outlined in Scheme 3. The phenolic and carboxylic acid moieties were initially protected by benzylation to give **12** in 98% yield.[17] Iodination was then carried out as described above to give iodide **13** (88%), although a higher temperature (120 °C) was required for C−H bond activation. A Suzuki alkylation reaction was then performed using pentylboronic acid to give compound **14**

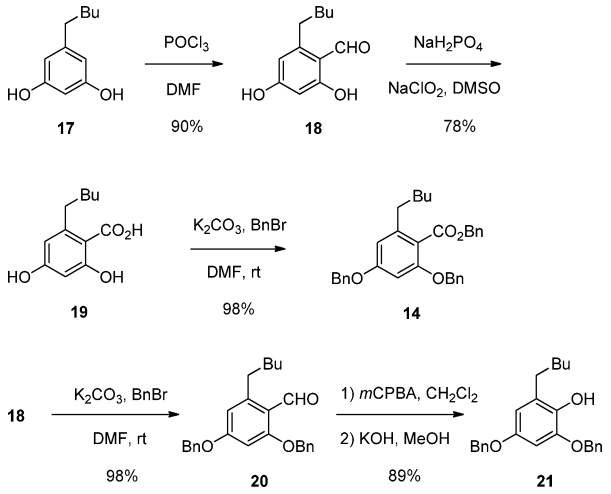
Scheme 3. Preparation of the B-Ring Fragments 16 from 11



(28%).[11] This reaction produced a lower yield than expected due to competing β-hydride elimination and the formation of undesired homocoupling products. However, the subsequent regioselective Vilsmeier−Haack formylation of **14** with phosphoryl chloride in DMF produced aldehyde **15** in 92% yield.[18] The reaction of **15** with *m*-chloroperoxybenzoic acid in CH_2Cl_2 and in the presence of KOH transformed **15** into the phenol **16** in 85% yield.[20]

However, from a commercial perspective, the synthesis of lobaric acid needs to be carried out on a large scale. Hence, as an alternative approach to the preparation of the B-ring, we employed olivetol (**17**) as the starting material (Scheme 4). In

Scheme 4. Preparation of Compounds 14 and 21 from 17



the initial step, benzaldehyde **18** was prepared from **17** through a regioselective Vilsmeier−Haack formylation, and the carboxylic acid **19** was formed in 78% yield through the oxidation of this aldehyde.[18,19] Subsequent protection of **19** with benzyl bromide in the presence of K_2CO_3 in DMF afforded benzyl ester **14** in 98% yield, which facilitated the preparation of large quantities of the B-ring fragment **16** using this new route based on olivetol (**17**) as the starting material. In addition, the synthesis of sakisacaulon A (**5**) from olivetol was also possible. In this case, the two hydroxy groups of **18** were initially protected as benzyl ethers to give compound **20** (98% yield), and the resulting protected benzaldehyde was converted

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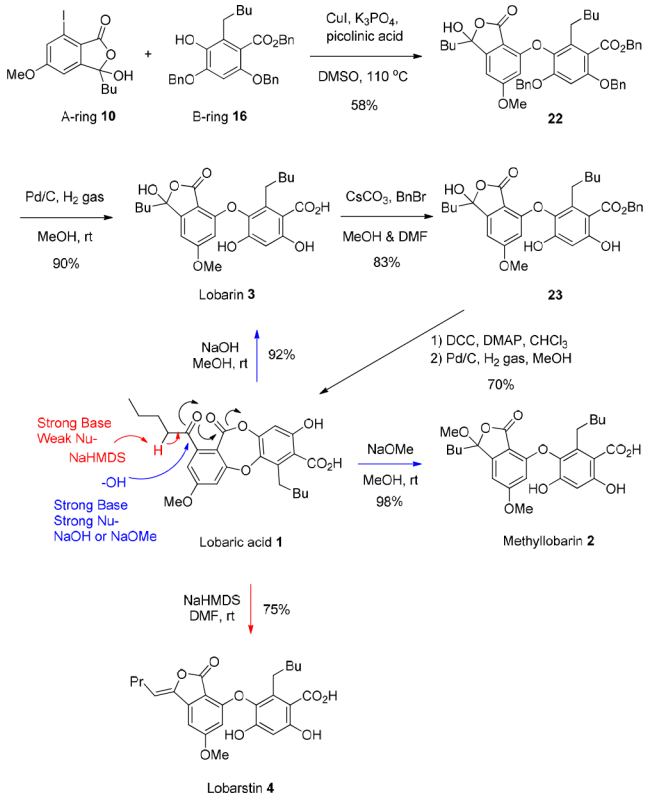
into the desired phenol derivative **21** in 89% yield, as described for **16**.

With the desired A- and B-rings in hand, the Ullmann coupling reaction was carried out under mild conditions (i.e., CuI, DMSO, K_3PO_4, and picolinic acid) to give the desired diaryl ether **22** in 58% yield. Lobarin (**3**) was then successfully obtained in 90% yield by removal of the benzyl protecting groups under standard hydrogenation conditions.[21] Protection of the carboxylic acid with cesium carbonate and benzyl bromide was required prior to the seven-membered lactonization reaction.[22] Benzyl ester **23** subsequently underwent reversible ring-opening translactonization. As such, treatment of the ring-opened form with DCC and DMAP produced a seven-membered lactone ring, and subsequent benzyl deprotection gave the desired lobaric acid (**1**) in 70% yield.[13]

As shown in Scheme 5, we subsequently used synthetic depsidone **1** to prepare a number of derivatives. More

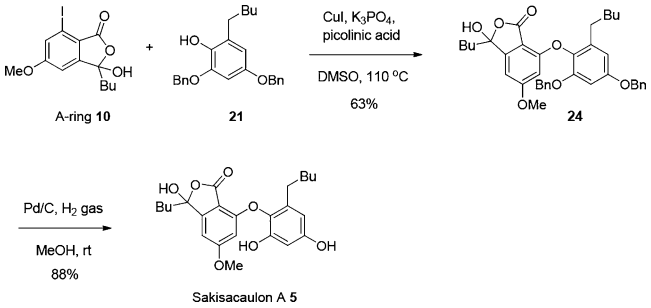
Scheme 5. Preparation of Lobaric Acid (1) and Its Derivatives



specifically, lobarin (**3**), possessing a five-membered lactone ring, was formed by reaction of the ketone carbonyl of lobaric acid (**1**) with highly nucleophilic NaOH. Similarly, methyllobarin (**2**, 98%) was synthesized from lobaric acid by treatment with NaOMe in MeOH at room temperature over 2 h. For the preparation of lobarstin **4** (only the *Z* form), a weakly nucleophilic but strong base, sodium bis(trimethylsilyl)-amide (NaHMDS), was employed to promote proton elimination, although the overall mechanism is similar to that involved in the synthesis of **3** (Scheme 5).

The synthesis of another pseudodepsidone derivative, namely, sakisacaulon A (**5**), was then carried out via the Ullmann reaction of the A-ring **10** and compound **21** to give **24** in 63% yield. The benzyl protecting groups of **24** were then removed by hydrogenation over Pd/C to give sakisacaulon A (**5**) in 88% yield (Scheme 6).

Scheme 6. Preparation of Sakisacaulon A (5)



Following the successful synthesis of lobaric acid (**1**) and its derivatives **2**–**5**, we evaluated the PTP1B inhibitory activities of these compounds (Table 1).[23,24] Among these compounds,

Table 1. PTP1B Inhibitory Activities of the Prepared Lobaric Acid and Its Derivatives

compound	PTP1B inhibitory activity (IC_{50}, μM)
lobaric acid (**1**)	0.8
methyllobarin (**2**)	1.5
lobarin (**3**)	0.9
lobarstin (**4**)	0.5
sakisacaulon A (**5**)	1.7
sumarin[a]	2.9

[a]Positive control.

lobaric acid (**1**), lobarin (**3**), and lobarstin (**4**) displayed significant PTP1B inhibitory activity with IC_{50} values in the 0.5–0.9 μM range, which are much lower than the value exhibited by sumarin (2.9 μM), used as the positive control.

In conclusion, we successfully synthesized the target compound lobaric acid (**1**) and a range of its derivatives in good yields, while also developing a method for the total synthesis of **1** and five of its derivatives using a common reaction pathway. In addition, we speculate that the similar and relatively potent PTP1B inhibitory activity of lobaric acid and its derivatives is ascribable to similarities in their natural product structures. Prior to this study, the syntheses of depsidones and their derivatives were challenging. We conclude that our developed synthetic method is suitable for the preparation of both depsidones and pseudodepsidone derivatives.

EXPERIMENTAL SECTION

General Experimental Procedures. The NMR spectra were recorded on Agilent 400 MHz and Bruker 600 and 850 MHz spectrometers as solutions in $CDCl_3$ and CD_3OD. The chemical shifts are given in ppm with respect to the residual solvent MeOH-d_4 signal (δ_H 3.31, δ_C 49.1 for ^1H and ^{13}C NMR, respectively), $CDCl_3$ (δ_H 7.27, δ_C 77.0 for ^1H and ^{13}C NMR, respectively), or tetramethylsilane. Accurate mass sepctra were obtained with an AB Sciex Triple TOF 4600 instrument with the interface in direct injection mode. Thin-layer chromatography was performed using 0.25 mm silica gel plates (Merck). Flash column chromatography was performed using silica gel 60 (230–400 mesh, Merck) with the indicated solvents. Reversed-phase column chromatography was performed on a flash chromatography system (AI-580S, Yamazen) with C18 resin (ODS-SM, 50 μm, 120A) and indicated solvents. Unless noted otherwise, commercially

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J. Nat. Prod. 2018, 81, 1460–1467

available anhydrous solvents and reagents were purchased from Aldrich or TCI and were used without further purification. All glassware was thoroughly dried in a drying oven (80 °C) or flame and cooled under a stream of dry argon just prior to use. All reactions were carried out under an inert atmosphere of argon. Solvents and liquid reagents were transferred using a syringe. Organic extracts were dried over a drying agent, $MgSO_4$, and Na_2SO_4 and concentrated under reduced pressure with the aid of a rotary evaporator.

4-Methoxyphthalic Acid (7). A flame-dried 100 mL sealed tube equipped with a magnetic stir bar was charged with MeONa (2 g, 37 mmol), CuCl (90 mg, 0.67 mmol), HCOOMe (0.4 mL, 6.7 mmol), and 4-bromophthalic anhydride (3 g, 16.8 mmol) in anhydrous MeOH (30 mL) and then heated to 150 °C, with stirring, for 16 h. After completion of the reaction, the reactor was cooled to room temperature (rt). The mixture was stirred for 30 min at rt, and then 12 N HCl was added to adjust the pH to 2. The mixture was diluted with EtOAc and H_2O, and the layers were separated. The aqueous phase was extracted with EtOAc three times (3 × 50 mL). The organic phase was washed with brine, dried over Na_2SO_4, and concentrated in a rotary evaporator. The residue was purified by EtOAc recrystallization, providing **7** as a white powder (2.3 g, 90%): TLC (EtOAc with 0.2% formic acid, UV) R_f = 0.10; ^{1}H NMR (400 MHz, CD_3OD) δ 7.84 (d, J = 8.6, 1H, H-6), 7.11 (d, J = 2.4, 1H, H-3), 7.09 (dd, J = 2.4, 8.6, 1H, H-5), 3.88 (s, 3H, 9-OMe); ^{13}C NMR (101 MHz, CD_3OD) δ 172.3 (C-8), 170.0 (C-7), 163.8 (C-4), 138.3 (C-2), 133.0 (C-1), 124.0 (C-5), 116.3 (C-6), 115.0 (C-3), 56.4 (9-OMe); HRESIMS m/z 197.0450 $[M + H]^+$ (calcd for $C_9H_9O_5$, 197.0440).

4-Methoxyphthalic Anhydride (8). A flame-dried 500 mL round-bottom flask equipped with a magnetic stir bar was charged with 4-methoxyphthalic acid (**7**) (3 g, 15.3 mmol) in anhydrous THF. Acetic anhydride (4 mL, 42.3 mmol) was added to a solution of the starting material, and the mixture was heated under reflux for 4 h. Upon cooling to rt, the solvent was removed under reduced pressure to afford 4-methoxyphthalic anhydride (**8**) as an off-white solid (2.7 g, 98%): TLC (EtOAc with 0.2% formic acid, UV) R_f = 0.50; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (d, J = 8.4, 1H, H-6), 7.40 (d, J = 2.4, 1H, H-3), 7.32 (dd, J = 2.4, 8.6, 1H, H-5), 3.98 (s, 3H, 9-OMe); ^{13}C NMR (101 MHz, CD_3OD) δ 166.1 (C-4), 162.9 (C-8), 162.4 (C-7), 134.0 (C-2), 127.2 (C-1), 123.1 (C-5), 122.8 (C-6), 108.9 (C-3), 56.4 (9-OMe); HRESIMS m/z 179.0340 $[M + H]^+$ (calcd for $C_9H_7O_4$, 179.0344).

3-Butyl-3-hydroxy-5-methoxyisobenzofuran-1(3*H*)-one (9). A flame-dried 500 mL round-bottom flask equipped with a magnetic stir bar was charged with 4-methoxyphthalic anhydride (**8**) (3 g, 16.8 mmol) and CuCl (158 mg, 1.2 mmol) in anhydrous THF (200 mL). After the mixture was cooled to −20 °C, butyl magnesium chloride (9.3 mL, 2.0 M in THF, 18.5 mmol) was added dropwise to the mixture over 1 h. The reaction mixture was stirred overnight at −20 °C and then allowed to warm to rt, and then 2 N HCl was added to adjust the pH to 2. The mixture was diluted with EtOAc and separated into two layers. The aqueous phase was extracted with EtOAc three times (3 × 50 mL). The organic phase was washed with brine, dried over Na_2SO_4, and concentrated in a rotary evaporator. Purification by flash column chromatography on a C18 resin (H_2O/CH_3CN) afforded compound **9** as a white solid (2.0 g, 52%): TLC (hexane/EtOAc, 2/1, UV) R_f = 0.32; ^{1}H NMR (850 MHz, CD_3OD) δ 7.74 (s, 1H, H-7), 7.12 (s, 1H, H-6), 7.08 (s, 1H, H-4), 3.91 (s, 3H, 5-OMe), 2.16−2.06 (m, 2H, H-9), 1.32 (m, 2H, H-10), 1.28−1.09 (m, 2H, H-11), 0.87 (t, J = 17, 3H, H-12); ^{13}C NMR (213 MHz, CD_3OD) δ 170.8 (C-1), 167.0 (C-5), 154.03 (C-8), 127.7 (C-2), 120.4 (C-3), 118.6 (C-7), 109.2 (C-4), 107.9 (C-6), 56.7 (OMe), 39.7 (C-9), 26.9 (C-10), 23.6 (C-11), 14.4 (C-12); HRESIMS m/z 237.1121 $[M + H]^+$ (calcd for $C_{13}H_{17}O_4$, 237.1127).

3-Butyl-3-hydroxy-7-iodo-5-methoxyisobenzofuran-1(3*H*)-one (10). A 500 mL round-bottom flask equipped with a magnetic stir bar was charged with 4-methoxy-2-pentanoylbenzoic acid (**9**) (6 g, 25.4 mmol), $Pd(OAc)_2$ (570 mg, 2.5 mmol), (diacetoxyiodo)benzene (9.8 g, 30.5 mmol), and iodine (7.7 g, 30.5 mmol) in DMF (100 mL). The reaction flask was sealed with a cap, and the reaction mixture was stirred at 60 °C for 12 h. The reaction mixture was cooled to rt, diluted with EtOAc, and then washed with 1 N HCl. The organic phase was washed with brine five times (5 × 50 mL), dried over Na_2SO_4, and concentrated in a rotary evaporator. Purification by flash column chromatography on a C18 resin (H_2O/CH_3CN) afforded compound **10** as a white powder (7.2 g, 78%): TLC (hexane/EtOAc, 2/1, UV) R_f = 0.51; ^{1}H NMR (600 MHz, CD_3OD) δ 7.59 (d, J = 1.0, 1H, H-6), 7.14 (d, J = 1.0, 1H, H-4), 3.93 (s, 3H, OMe), 2.19−2.03 (m, 2H, H-9), 1.33 (m, 2H, H-10; 1H, H-11), 1.09 (m, 1H, H-11), 0.89 (t, J = 6.8, 3H, H-12); ^{13}C NMR (151 MHz, CD_3OD) δ 168.7 (C-1), 166.5 (C-5), 155.3 (C-3), 129.5 (C-6), 121.5 (C-2), 108.2 (C-4), 106.5 (C-8), 93.8 (C-7), 57.1 (OMe), 39.4 (C-9), 26.6 (C-10), 23.6 (C-11), 14.3 (C-12); HRESIMS m/z 363.0084 $[M + H]^+$ (calcd for $C_{13}H_{16}IO_4$, 363.0093).

Benzyl 2,4-Bis(benzyloxy)benzoate (12). A 500 mL round-bottom flask equipped with a magnetic stir bar was charged with 2,4-dihydroxybenzoic acid (20 g, 130 mmol) in DMF (100 mL). Benzyl bromide (62 mL, 519 mmol) and potassium carbonate (72 g, 519 mmol) were added into the reaction flask at rt. After stirring for 12 h at rt, the reaction temperature was increased to 90 °C and the mixture was stirred for 1 h. The reaction mixture was cooled to rt, diluted with EtOAc, and then washed with 1 N HCl. The organic phase was washed with brine five times (5 × 50 mL), dried over Na_2SO_4, and concentrated in a rotary evaporator. Purification by recrystallization with MeOH gave compound **12** as white crystals (54.0 g, 98%): TLC (hexane/EtOAc, 10/1, UV) R_f = 0.30; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, J = 8.6, 1H, H-6), 7.40−7.26 (m, 15H, Bn), 6.61 (d, J = 2.4, 1H, H-3), 6.57 (dd, J = 2.4, 8.6, 1H, H-5), 5.32 (s, 2H, H-1 CH_2), 5.12 (s, 2H, H-4 CH_2), 5.06 (s, 2H, H-2 CH_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.6 (C-7), 163.3 (C-4), 160.3 (C-2), 136.4 (Bn), 136.3 (Bn), 134.1 (C-6), 128.7−127.0 (Bn), 113.1 (C-1), 106.0 (C-5), 101.3 (C-3), 70.6 (C-4 CH_2), 70.2 (C-2 CH_2), 66.3 (C-7 CH_2); HRESIMS m/z 425.1740 $[M + H]^+$ (calcd for $C_{28}H_{25}O_4$, 425.1753).

Benzyl 2,4-Bis(benzyloxy)-6-iodobenzoate (13). A 500 mL sealed tube equipped with a magnetic stir bar was charged with benzyl 2,4-bis(benzyloxy)benzoate (**12**) (5 g, 15 mmol), $Pd(OAc)_2$ (337 mg, 1.5 mmol), (diacetoxy)benzene (7.2 g, 22.5 mmol), and iodine (5.7 g, 22.5 mmol) in DMF (50 mL). The reaction tube was sealed with a cap, and the reaction mixture was stirred at 120 °C for 18 h. The reaction mixture was cooled to rt, diluted with EtOAc, and then washed with 1 N HCl. The organic phase was washed with brine five times (5 × 30 mL), dried over Na_2SO_4, and concentrated in a rotary evaporator. Purification by recrystallization with MeOH provided **13** as a white powder (5.7 g, 88%): TLC (hexane/EtOAc, 10/1, UV) R_f = 0.25; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (s, 1H, H-5), 7.39−7.32 (m, 15H, Bn), 6.46 (s, 1H, H-3), 5.31 (s, 2H, H-7 CH_2), 5.11 (s, 2H, H-4 CH_2), 5.08 (s, 2H, H-2 CH_2); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.3 (C-7), 161.1 (C-4), 160.6 (C-2), 142.4 (C-1), 136.1−135.6 (Bn), 128.7−126.9 (Bn; C-5), 114.9 (C-3), 99.5 (C-6), 71.1 (C-4 CH_2), 71.0 (C-2 CH_2), 66.6 (C-7 CH_2); HRESIMS m/z 551.0709 $[M + H]^+$ (calcd for $C_{28}H_{24}IO_4$, 551.0719).

Benzyl 2,4-Bis(benzyloxy)-6-pentylbenzoate (14). A flame-dried round-bottom flask equipped with a magnetic stir bar was charged with benzyl 2,4-bis(benzyloxy)-6-iodobenzoate (**13**) (1 g, 1.82 mmol), palladium acetate (41 mg, 0.18 mmol), silver carbonate (753 mg, 2.73 mmol), potassium carbonate (377 mg, 2.73 mmol), and pentylboronic acid (317 mg, 2.73 mmol) in DMF (20 mL), then purged of air with argon. The flask was then transferred to a 110 °C oil bath and stirred for 12 h. After quenching with 1 N HCl, it was extracted with Et_2O. The organic phase was washed with brine five times (5 × 20 mL), dried over Na_2SO_4, and concentrated in a rotary evaporator. Purification by flash chromatography on silica gel eluting with hexane/EtOAc, 8/1, yielded **14** as a white solid (251 mg, 28%). The procedure from **19**: A 250 mL round-bottom flask equipped with a magnetic stir bar was charged with 2,4-dihydroxy-6-pentylbenzoic acid (**19**) (5 g, 22.3 mmol), benzyl bromide (1.06 mL, 89.2 mmol), and potassium carbonate (1.23 g, 89.2 mmol) in DMF (50 mL). After stirring for 18 h at rt, the reaction mixture was diluted with EtOAc and then washed with 1 N HCl. The organic phase was washed with brine five times (5 × 30 mL), dried over Na_2SO_4, and concentrated in a rotary evaporator. Purification by flash chromatography on silica gel

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eluting with hexane/EtOAc (10/1) to yield **14** as a white solid (10.8 g, 98%): TLC (hexane/EtOAc, 10/1, UV) R_f = 0.22; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39−7.27 (m, 15H, Bn), 6.42 (d, J = 2.4, 1H, H-5), 6.41 (d, J = 2.4, 1H, H-3), 5.30 (s, 2H, H-7 CH_2), 5.02 (s, 2H, H-4 CH_2), 5.01 (s, 2H, H-2 CH_2), 2.51(t, J = 7.9, 2H, H-8), 1.53 (m, 2H, H-9), 1.22 (m, 2H H-10; 2H, H-11), 0.85 (t, J = 6.7, 3H, C-12); ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.2 (C-7), 160.4 (C-4), 157.0 (C-2), 143.2 (C-6), 136.6−135.8 (Bn), 128.60−127.1 (Bn), 116.9 (C-1), 107.2 (C-5), 98.3 (C-3), 70.4 (C-4 CH_2), 70.1 (C-2 CH_2), 66.9 (C-7 CH_2), 33.8 (C-8), 31.6 (C-9), 30.9 (C-10), 22.4 (C-11), 14.0 (C-12); HRESIMS m/z 495.2544 $[M + H]^+$ (calcd for $C_{33}H_{35}O_4$, 495.2535).

Benzyl 4,6-Bis(benzyloxy)-3-formyl-2-pentylbenzoate (15). A flame-dried round-bottom flask equipped with a magnetic stir bar was charged with DMF (10 mL) as a solvent. $POCl_3$(1.24 mL, 13.4 mmol) was added dropwise into DMF below 10 °C with rapid stirring over 30 min. Benzyl 2,4-bis(benzyloxy)-6-pentylbenzoate (**14**) (4.4 g, 8.9 mmol) in DMF (10 mL) was added slowly, keeping the temperature below 10 °C. The mixture was warmed to 70 °C and stirred for 8 h. The reaction mixture was cooled to rt, diluted with EtOAc, and then washed with 1 N HCl. The organic phase was washed with brine five times (5 × 20 mL), dried over Na_2SO_4, and concentrated in a rotary evaporator. Purification by flash chromatography on silica gel eluting with hexane/EtOAc (10/1) yielded **15** as a white solid (4.3 g, 92%): TLC (hexane/EtOAc, 10/1, UV) R_f = 0.21; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.52 (s, 1H, H-8), 7.39−7.28 (m, 15H, Bn), 6.41 (s, 1H, H-5), 5.31 (s, 2H, C-7 CH_2), 5.07 (s, 2H, H-4 CH_2), 5.06 (s, 2H, H-6 CH_2), 2.84 (t, J = 7.9, 2H, H-9), 1.47 (m, 2H, H-10), 1.25 (m, 2H, H-11; 2H, H-12), 0.86 (t, J = 7.3, 3H, H-13); ^{13}C NMR (101 MHz, $CDCl_3$) δ 189.8 (C-8), 167.4 (C-7), 164.9 (C-4), 160.1 (C-6), 146.0 (C-2), 135.5−134.4 (Bn), 128.7−127.0 (Bn), 119.1 (C-1), 116.7 (C-3), 95.5 (C-5), 70.9 (C-4 CH_2), 70.4 (C-6 CH_2), 67.3 (C-7 CH_2), 32.3 (C-9), 31.2 (C-10), 31.1 (C-11), 22.4 (C-12), 14.0 (C-13); HRESIMS m/z 523.2480 $[M + H]^+$ (calcd for $C_{34}H_{35}O_5$, 523.2484).

Benzyl 4,6-Bis(benzyloxy)-3-hydroxy-2-pentylbenzoate (16). A flame-dried round-bottom flask equipped with a magnetic stir bar was charged with benzyl 4,6-bis(benzyloxy)-3-formyl-2-pentylbenzoate (**15**) (1.0 g, 2.0 mmol) in CH_2Cl_2 (20 mL). *m*CPBA (850 mg, 4.9 mmol) in CH_2Cl_2 (10 mL) was added dropwise into the reaction mixture below 10 °C for 10 min with rapid stirring, then allowed to warmed to rt. The mixture was stirred at rt for 4 h. The mixture was extracted with H_2O and washed with concentrated $NaHCO_3$ solution three times (3 × 10 mL). The organic layer was concentrated in a rotary evaporator, and the crude oil was dissolved in MeOH (20 mL). The crude product solution was added dropwise into 10% KOH (13 mL) solution at 0 °C and stirred at 0 °C for 2 h. The MeOH was removed in a rotary evaporator; then 6 N HCl was added to adjust the pH to 2. The mixture was diluted with EtOAc, and the layers were separated. The aqueous phase was extracted with EtOAc three times (3 × 50 mL). The organic phases were washed with brine, dried over Na_2SO_4, and concentrated in a rotary evaporator. Purification by flash chromatography on silica gel eluting with hexane/EtOAc (10/1) yielded **16** as a white solid (830 mg, 85%): TLC (hexane/EtOAc, 10/1, UV) R_f = 0.25; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38−7.27 (m, 15H, Bn), 6.42 (s, 1H, H-5), 5.41 (s, 1H, OH), 5.30 (s, 2H, H-7 CH_2), 5.00 (s, 2H, H-4 CH_2), 4.94 (s, 2H, H-6 CH_2) 2.56 (t, J = 8.0, 2H, H-8), 1.51 (m, 2H, H-9), 1.22 (m, 2H, H-10; 2H, H-11), 0.84 (t, J = 6.7, 3H, H-12); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.8 (C-7), 148.9 (C-4), 146.2 (C-6), 138.2 (C-3), 137.0−135.8 (Bn), 128.7−127.2 (Bn), 117.9 (C-1), 98.9 (C-5), 71.9 (C-4 CH_2), 71.3 (C-6 CH_2), 67.0 (C-7 CH_2), 31.9 (C-8), 29.5 (C-9), 27.6 (C-10), 22.4 (C-11), 14.0 (C-12); HRESIMS m/z 511.2486 $[M + H]^+$ (calcd for $C_{33}H_{35}O_5$, 511.2484).

2,4-Dihydroxy-6-pentylbenzaldehyde (1). A flame-dried round-bottom flask equipped with a magnetic stir bar was charged with olivetol (**17**) (5 g, 27.7 mmol) and anhydrous DMF (150 mL). After cooling to 0 °C, $POCl_3$ (3.9 mL, 41.6 mmol) was added dropwise to the mixture over 5 min. The reaction mixture was stirred for 30 min at 0 °C, and the cooling bath was removed. The reaction mixture was stirred for 8 h at rt, and H_2O (300 mL) was added. The yellow mixture was extracted with CH_2Cl_2 four times (4 × 50 mL). The organic phase was washed with brine five times (5 × 20 mL), dried over $MgSO_4$, and concentrated in a rotary evaporator. Purification by flash chromatography on silica gel eluting with hexane/EtOAc (10/1) yielded **18** as a brown solid (5.2 g, 90%): TLC (EtOAc with 0.1% formic acid, UV) R_f = 0.18; ^{1}H NMR (400 MHz, CD_3OD) δ 10.02 (s, 1H, H-7), 6.21 (s, 1H, H-5), 6.10 (d, J = 2.4, 1H, H-3), 2.81 (t, J = 7.3, 2H, H-8), 1.60 (m, 2H, H-9), 1.35 (m, 2H, H-10; 2H, H-11), 0.90 (t, J = 6.7, 3H, H-12); ^{13}C NMR (101 MHz, CD_3OD) δ 194.2 (C-7), 167.8 (C-4), 167.2 (C-2), 151.5 (C-6), 113.2 (C-1), 111.2 (C-5), 101.7 (C-3), 33.7 (C-8), 32.9 (C-9), 32.8 (C-10), 23.7 (C-11), 14.5 (C-12); HRESIMS m/z 209.1175 $[M + H]^+$, (calcd for $C_{12}H_{17}O_3$, 209.1178).

2,4-Dihydroxy-6-pentylbenzoic Acid (19). A round-bottom flask equipped with a magnetic stir bar was charged with 2,4-bis(benzyloxy)-6-pentylbenzaldehyde (**18**) (5.3 g, 25.2 mmol), $NaH_2PO_4{\cdot}H_2O$ (8.7 g, 63 mmol), DMSO (120 mL), and H_2O (15 mL). $NaClO_2$ (5.7 g, 63 mmol) in H_2O (10 mL) was added dropwise to the mixture over 10 min with stirring. After stirring at rt overnight, saturated aqueous Na_2CO_3 (50 mL) was added. The resulting solution was extracted with EtOAc (15 mL). The aqueous phase was acidified to pH to 1 with concentrated HCl and then extracted with EtOAc three times (3 × 50 mL). The organic phase was washed with brine, dried over Na_2SO_4, and concentrated in a rotary evaporator. Purification by flash column chromatography on C18 resin (H_2O/CH_3CN) afforded compound **19** as a yellow solid (4.5 g, 78%): TLC (EtOAc with 0.1% formic acid, UV) R_f = 0.60; ^{1}H NMR (400 MHz, CD_3OD) δ 6.15 (s, 1H, H-5), 6.11 (s, 1H, H-3), 2.84 (t, J = 7.6, 2H, H-8), 1.53 (m, 2H, H-9), 1.31 (m, 2H, H-10; 2H, H-11), 0.87 (m, 3H, H-12); ^{13}C NMR (101 MHz, CD_3OD) δ 173.4 (C-7), 165.3 (C-4), 162.1 (C-2), 148.7 (C-6), 110.2 (C-5), 106.4 (C-1), 100.2 (C-3), 36.1 (C-8), 31.7 (C-9), 31.4 (C-10), 22.1 (C-11), 13.0 (C-12); HRESIMS m/z 225.1117 $[M + H]^+$ (calcd for $C_{12}H_{17}O_4$, 225.1127).

2,4-Bis(benzyloxy)-6-pentylbenzaldehyde (20). A 250 mL round-bottom flask equipped with a magnetic stir bar was charged with 2,4-dihydroxy-6-pentylbenzaldehyde (**18**) (10 g, 48.0 mmol) in DMF (50 mL). Benzyl bromide (7.6 mL, 64.3 mmol) and potassium carbonate (8.9 g, 64.3 mmol) were added into the reaction flask at rt. After stirring for 12 h at rt, the reaction mixture diluted with EtOAc and then washed with 1 N HCl. The organic phase was washed with brine five times (5 × 30 mL), dried over Na_2SO_4, and concentrated in a rotary evaporator. Purification by flash chromatography on silica gel eluting with hexane/EtOAc (12/1) provided **20** as a white powder (18.3 g, 98%): TLC (hexane/EtOAc, 10/1, UV) R_f = 0.43; ^{1}H NMR (850 MHz, $CDCl_3$) δ 10.56 (s, 1H, H-7), 7.35 (m, 10H, Bn), 6.47 (d, J = 2.2, 1H, H-5), 6.43 (d, J = 2.2, 1H, H-3), 5.08 (s, 2H, H-4 CH_2), 5.07 (s, 2H, H-2 CH_2), 2.96 (t, J = 8.5, 2H, H-8), 1.54 (m, 2H, H-9), 1.34 (m, 2H, H-10; 2H, H-11), 0.88 (t, J = 7.0, 3H, H-12) ; ^{13}C NMR (214 MHz, $CDCl_3$) δ 190.3 (C-7), 164.4 (C-4), 163.5 (C-2), 149.7 (C-6), 136.6 (Bn), 128.7−127.3 (Bn), 117.2 (C-1), 109.2 (C-5), 97.8 (C-3), 70.6 (C-4 CH_2), 70.1 (C-2 CH_2), 34.5 (H-8), 31.9 (H-9), 30.8 (H-10), 22.6 (H-11), 14.1 (H-12); HRESIMS m/z 389.2130 $[M + H]^+$ (calcd for $C_{26}H_{29}O_3$, 389.2116).

2,4-Bis(benzyloxy)-6-pentylphenol (21). A flame-dried round-bottom flask equipped with a magnetic stir was charged with 2,4-bis(benzyloxy)-6-pentylbenzaldehyde (**20**) (7.4 g, 19.6 mmol) in CH_2Cl_2 (100 mL). *m*CPBA (8.5 g, 49 mmol) in CH_2Cl_2 (50 mL) was added dropwise into the reaction mixture below 10 °C for 10 min with rapid stirring, then allowed to warm to rt. The mixture was stirred at rt for 4 h. The mixture was extracted with H_2O and washed with concentrated $NaHCO_3$ solution three times (3 × 30 mL). The organic layer was concentrated in a rotary evaporator, and the crude oil was dissolved in MeOH (100 mL). The crude product solution was added dropwise into 10% KOH (125 mL) solution at 0 °C and stirred at 0 °C for 2 h. The MeOH was removed in a rotary evaporator; then 6 N HCl was added to adjust the pH to 2. The mixture was diluted with EtOAc, and the layers were separated. The aqueous phase was extracted with EtOAc three times (3 × 50 mL). The organic phases were washed with brine, dried over Na_2SO_4, and concentrated in a rotary evaporator. Purification by flash chromatography on silica gel eluting with hexane/EtOAc (15/1) yielded **21** as a white solid (6.5 g,

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89%): TLC (hexane/EtOAc, 10/1, UV) R_f = 0.55; ^{1}H NMR (850 MHz, $CDCl_3$) δ 7.34 (m, 10H, Bn), 6.49 (d, J = 2.7, 1H, H-5), 6.40 (d, J = 2.7, 1H, H-3) 5.33 (s, 1H, OH), 5.03 (s, 2H, H-4 CH_2) 4.97 (s, 2H, H-2 CH_2), 2.60 (t, J = 7.7, 2H, H-7), 1.60 (m, 2H, H-8), 1.33(m, 2H, H-9; 2H, H-10), 0.89 (t, J = 6.9, 3H, H-11); ^{13}C NMR (214 MHz, $CDCl_3$) δ 151.8 (C-4), 145.8 (C-2), 137.9 (C-1), 137.3 (Bn), 136.3 (Bn), 128.9−127.6 (C-6; Bn), 107.6 (C-5), 89.9 (C-3), 71.2 (C4 CH_2), 70.8 (C-2 CH_2), 31.7 (C-7), 30.0 (C-8), 29.4 (C-9), 22.6 (C-10), 14.1 (C-11); HRESIMS m/z 377.2131 $[M + H]^+$ (calcd for $C_{25}H_{29}O_3$, 377.2116).

Benzyl 4,6-Bis (benzyloxy)-3-((1-butyl-1-hydroxy-6-methoxy- 3-oxo-1,3-dihydroisobenzofuran-4-yl)oxy)-2-pentyl Benzoate (22). A sealed tube equipped with a magnetic stir bar was charged with CuI (53 mg, 0.28 mmol), picolinic acid (53 mg, 0.55 mmol), 2-iodo-4-methoxy-6-pentanoylbenzoic acid (**10**) (500 mg, 1.38 mmol), benzyl-4,6-bis(benzyloxy)-3-hydroxy-2-pentyl benzoate (**16**) (704 mg, 1.38 mmol), and K_3PO_4 (880 mg, 4.14 mmol). The tube was then evacuated and backfilled with argon. The evacuation backfill sequence was repeated two additional times. Then the reaction mixture was dissolved in DMSO (20 mL). The tube was placed in a preheated oil bath at 110 °C, and the reaction mixture was stirred vigorously for 12 h. The reaction mixture was cooled to rt. Ethyl acetate (50 mL) and H_2O (50 mL) were added, and the mixture was stirred. The mixture was separated into two layers, and the aqueous phase was extracted with EtOAc three times (3 × 50 mL). The organic layer was dried over Na_2SO_4 and filtered through the pad of silica gel. The filtrate was concentrated in a rotary evaporator. Purification by flash column chromatography on C18 resin (H_2O/CH_3CN) afforded compound **22** as a white powder (596 mg, 58%): TLC (hexane/EtOAc, 2/1, with 0.1% formic acid, UV) R_f = 0.62; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29−7.11 (m, 10H, Bn), 6.55 (d, J = 1.8, 1H, H-5), 6.48 (s, 1H, H-3), 6.09 (d J = 1.8, 1H, H-3), 5.30 (s, 2H, H-1′ CH_2), 5.00 (s, 2H, H-4′, CH_2), 4.95 (s, 2H, H-2′ CH_2), 3.73 (s, 3H, OMe), 2.48 (m, 2H, H-8′), 2.06 (m, 2H, H-9), 1.31 (m, 2H, H-9′; 2H, H-10′; 2H, H-10), 1.13 (m, 2H, H-11; 2H, H-11′), 0.86 (t, J = 7.3, 3H, H-12), 0.75 (t, J = 6.7, 3H, H-12′); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.2 (C-7′), 166.7 (C-7), 165.1 (C-4), 158.2 (C-2), 153.9 (C-4′), 153.4 (C-2′), 151.9 (C-5′), 136.3 (Bn), 136.1 (Bn), 135.5 (Bn), 135.0 (C-6′), 128.7−127.0 (Bn; C-6), 117.7 (C-1), 106.9 (C-8), 105.0 (C-5), 102.1 (C-3), 99.7 (C-1′), 98.9 (C-3′), 71.1 (C-4′ CH_2), 71.0 (C-2′ CH_2), 67.2 (C-7′ CH_2), 56.0 (OMe), 38.5 (C-8′), 31.9 (C-9), 29.9 (C-10′), 28.2 (C-9′), 25.2 (C-10), 22.4 (C-11), 22.1 (C-11′), 13.8 (C-12′), 13.7 (C-12); HRESIMS m/z 745.3374 $[M + H]^+$ (calcd for $C_{46}H_{49}O_9$, 745.3377).

Lobarin (3). To a 100 mL round-bottom flask charged with compound **22** (100 mg, 0.211 mmol) in MeOH (20 mL) was added palladium on carbon (10%, 10 mg). The mixture was stirred under a hydrogen atmosphere (1 atm, hydrogen ballon) for 12 h. Upon completion the reaction mixture was filtered through a pad of Celite. The filtrate was concentrated in a rotary evaporator. Purification by flash column chromatography on C18 resin (H_2O/CH_3CN) afforded compound **3** as a yellow solid (57 mg, 90%): TLC (hexane/EtOAc, 2/1, with 0.1% formic acid, UV) R_f = 0.15; ^{1}H NMR (400 MHz, CD_3OD) δ 6.71 (d, J = 1.8, 1H, H-5), 6.37 (s, 1H, H-3′), 6.04 (d, J = 1.8, 1H, H-3), 3.77 (s, 3H, OMe), 2.92−2.74 (m, 2H, H-8′), 2.18−2.01 (m, 2H, H-9), 1.54 (m, 2H, H-10), 1.34 (m, 2H, H-11; 2H, H-9′), 1.22 (m, 2H, H-10; 2H, H-11′), 0.88 (t, J = 6.7, 3H, H-12), 0.80 (m, 3H, H-12′); ^{13}C NMR (101 MHz, CD_3OD) δ 174.5 (C-7′), 168.8 (C-7), 168.7 (C-4), 163.7 (C-2′), 156.7 (C-2), 156.6 (C-4′), 155.9 (C-6), 141.6 (C-6′), 134.3 (C-5′), 108.1 (C-8), 106.2 (C-1), 103.2 (C-3′), 102.6 (C-1′), 101.2 (C-3), 101.1 (C-5), 56.8 (OMe) 39.8 (C-9), 33.6 (C-9′), 31.5 (C-10′), 29.4 (C-8′), 26.9 (C-10), 23.7 (C-11), 23.3 (C-11′), 14.5 (C-12), 14.4 (C-12′); HRESIMS m/z 475.1960 $[M + H]^+$ (calcd for $C_{25}H_{31}O_9$, 475.1968).

Benzyl 3-((1-Butyl-1-hydroxy-6-methoxy-3-oxo-1,3-dihydroisobenzofuran-4-yl)oxy)-4,6-dihydroxy-2-pentylbenzoate (23). To a solution of lobarin (**3**) (50 mg, 0.11 mmol) in MeOH (5 mL) was added cesium carbonate (17 mg, 0.053 mmol), and the solution was stirred at rt for 10 min. After concentrating the solution, it was azotroped with toluene to remove remaining water. To the resulting solid were added anhydrous DMF (3 mL) and benzyl bromide (14 μL, 0.121 mmol), and then the reaction mixture was stirred at rt for 18 h. After filtering the white solid, the filtrate was concentrated in a rotary evaporator. Purification by flash column chromatography on C18 resin (H_2O/CH_3CN) afforded compound **23** (50 mg, 83%) as a white solid: TLC (hexane/EtOAc, 2/1, with 0.1% formic acid, UV) R_f = 0.44; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.44−7.33 (m, 5H, Bn), 6.61 (d, J = 1.8 1H, H-5), 6.51 (s, 1H, H-3′), 6.03 (d, J = 1.8, 1H, H-3), 5.33 (d, J = 7.7, 2H, H-7′ CH_2), 3.76 (s, 3H, OMe), 2.73−2.40 (m, 2H, H-8′), 2.15−1.98 (m, 2H, H-9), 1.29 (m, 2H, H-10; 2H, H-11), 1.15−1.03 (m, 2H, H-9′), 0.91 (m, 2H, H-10), 0.84 (t, J = 7.1, 3H, H-12), 0.78 (m, 2H, H-11′), 0.62 (t, J = 7.4, 3H, H-12′); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.9 (C-7′), 167.7 (C-7), 162.8 (C-4), 157.4(C-2′), 154.7 (C-2), 153.6 (C-4′), 139.6 (C-6′), 134.7 (Bn), 132.3 (C-5′), 129.2−128.7 (Bn), 107.4 (C-8), 106.4 (C-1), 104.8 (C-3′), 103.5 (C-1′), 101.7 (C-3), 100.6 (C-5), 67.8 (C-7′ CH_2), 56.1 (OMe), 37.8 (C-9), 31.9 (C-9′), 30.2 (C-10′), 28.6 (C-8′), 25.0 (C-10), 22.3 (C-11), 22.0 (C-11′), 13.8 (C-12), 13.7 (C-12′); HRESIMS m/z 564.2360 $[M + H]^+$ (calcd for $C_{32}H_{36}O_9$, 564.2359).

Lobaric Acid (1). To a solution of DMAP (15 mg, 0.133 mmol) and compound **23** (60 mg, 0.106 mmol) in CH_2Cl_2 (10 mL) was slowly added DCC (24 mg, 0.117 mmol) in CH_2Cl_2 (10 mL) through a syringe pump over 30 min. After the addition was completed, the mixture was stirred for another 12 h. The mixture was concentrated in a rotary evaporator. Purification by flash column chromatography on C18 resin (H_2O/CH_3CN) afforded an intermediate protected lactone (45 mg, 78%). To a round-bottom flask charged with the lactone compound (45 mg, 0.082 mmol) in MeOH (10 mL) was added palladium on carbon (10%, 4.5 mg). The mixture was stirred under a hydrogen atmosphere (1 atm, hydrogen ballon) for 12 h. Upon completion, the reaction mixture was filtered through a pad of Celite. The filtrate was concentrated in a rotary evaporator. Purification by flash column chromatography on C18 resin (H_2O/CH_3CN) afforded compound **1** (34 mg, 90%): TLC (hexane/EtOAc, 2/1, with 0.1% formic acid, UV) R_f = 0.8; ^{1}H NMR (400 MHz, CD_3OD) δ 6.92 (d, J = 2.4, 1H, H-6), 6.89 (d, J = 2.4, 1H, H-4), 6.68 (s, 1H, H-5′), 3.92 (s, 3H, OMe), 3.13 (m, 2H, H-8′), 2.82 (t, J = 7.3, 2H, H-9), 1.57 (m, 2H, H-10; 2H, H-9′), 1.48 (m, 2H, H-10′), 1.41 (m, 2H, H-11; 2H, H-11′), 0.94 (m, 3H, H-12; 3H, H-12′); ^{13}C NMR (101 MHz, CD_3OD) δ 205.9 (C-7), 172.8 (C-7′), 166.3 (C-5), 164.5 (C-3), 163.9 (C-8), 159.4 (C-6′), 150.6 (C-1), 148.6 (C-4′), 142.8 (C-3′), 139.2 (C-2′), 115.6 (C-1′), 112.8 (C-2), 112.5 (C-6), 107.9 (C-5′), 107.3 (C-4), 57.1 (OMe), 43.0 (C-9), 33.5 (C-10′), 32.3 (C-9′), 29.0 (C-8′), 27.2 (C-10), 23.7 (C-11′), 23.3 (C-11), 14.5 (C-12), 14.4 (C-12′); HRESIMS m/z 457.1846 $[M + H]^+$ (calcd for $C_{25}H_{29}O_8$, 457.1862).

Lobarstin (4). A flame-dried 250 mL round-bottom flask equipped with a magnetic stir bar was charged with lobaric acid (**1**) (100 mg, 0.22 mmol) in anhydrous DMF (5 mL), and the mixture was cooled to 0 °C. NaHMDS (48 mg, 0.26 mmol) in DMF (5 mL) was added dropwise over 5 min. The reaction mixture was stirred for 10 min at 0 °C, and the cooling bath was removed. The reaction mixture was stirred for 2 h at rt, and then 1 N HCl (20 mL) was added. The mixture was extracted with EtOAc three times (3 × 10 mL). The organic phase was washed with brine, dried over Na_2SO_4, and concentrated in a rotary evaporator. Purification by flash column chromatography on C18 resin (H_2O/CH_3CN) afforded compound **4** (90 mg, 90%) as a white powder: TLC (hexane/EtOAc (2/1) with 0.1% formic acid, UV) R_f = 0.21; ^{1}H NMR (400 MHz, CD_3OD) δ 6.94 (d, J = 1.2, 1H, H-5), 6.37 (s, 1H, H-3′), 6.01 (d, J = 1.2, 1H, H-3), 5.81 (t, J = 7.9, 1H, H-9), 3.79 (s, 3H, OMe), 2.92 (m, 2H, H-8′), 2.41 (dd, J = 7.9, 7.3, 2H, H-10), 1.57 (dq, J = 14.6, 7.3, 2H, H-9′), 1.19 (m, 2H, H-11; 2H, H-10′; 2H, H-11′), 1.01 (t, J = 7.3, 3H, H-12), 0.75 (t, J = 7.3, 3H, H-12′); ^{13}C NMR (101 MHz, CD_3OD) δ 174.6(C-7′), 168.8 (C-4), 166.7 (C-7), 163.7 (C-2), 159.8 (C-4′), 156.6 (C-2′), 147.2 (C-8), 145.4 (C-6′), 141.6 (C-6), 134.3 (C-5′), 110.8 (C-1), 106.3 (C-9), 106.2 (C-1′), 103.2(C-5), 103.0 (C-3), 97.8 (C-3′), 56.8 (OMe), 33.5 (C-9′), 34.5 (C-10′), 29.4 (C-10), 29.0 (C-8′), 23.6 (C-11), 23.3 (C-11′), 14.4 (C-12), 14.3 (C-12′); HRESIMS m/z 457.1853 $[M + H]^+$ (calcd for $C_{25}H_{29}O_8$, 457.1862).

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Methyllobarin (2). A flame-dried 250 mL round-bottom flask equipped with a magnetic stir bar was charged with lobaric acid (**1**) (100 mg, 0.22 mmol) in anhydrous MeOH (100 mL), and the mixture was cooled to 0 °C. NaOMe (18 mg, 0.33 mmol) in anhydrous MeOH (10 mL) was added dropwise over 5 min. The reaction mixture was stirred for 10 min at 0 °C, and the cooling bath was removed. The reaction mixture was stirred for 2 h at rt, and then 1 N HCl (20 mL) was added. The mixture was extracted with EtOAc three times (3 × 10 mL). The organic phase was washed with brine, dried over Na_2SO_4, and concentrated in a rotary evaporator. Purification by flash column chromatography on C18 resin (H_2O/CH_3CN) afforded compound **2** (105 mg, 98%) as a white powder: TLC (hexane/EtOAc, 2/1, with 0.1% formic acid, UV) R_f = 0.40; ^{1}H NMR (600 MHz, CD_3OD) δ 6.68 (d, J = 1.7, 1H, H-5), 6.40 (s, 1H, H-3′), 6.10 (d, J = 1.7, 1H, H-3), 3.78 (s, 3H, H-4 OMe), 3.09 (s, 3H, H-8 OMe), 3.00−2.70 (m, 2H, H-8′) 2.16−2.01 (m, 2H, H-9), 1.62−1.34 (m, 2H, H-9′), 1.37 (m, 2H, H-10; 2H, H-10), 1.19 (m, 2H, H-11, H-11′), 0.87 (t, J = 7.3, 3H, H-12), 0.77 (t, J = 7.0, 3H, H-12′); ^{13}C NMR (151 MHz, CD_3OD) δ 174.1 (C-7′), 169.0 (C-4), 168.1 (C-7), 163.9 (C-4′), 159.9 (C-2), 156.7 (C-2′), 152.7(C-6) 141.5 (C-6′), 134.1 (C-5′), 111.0 (C-8), 109.1 (C-1), 106.1 (C-1′), 103.3 (C-3′), 103.1 (C-3), 101.4 (C-5), 56.9 (C-4 OMe), 51.3 (C-8 OMe), 39.2 (C-9), 33.6 (C-10′), 31.4 (C-8′), 29.4 (C-9′), 26.5 (C-10), 23.7 (C-11), 23.2 (C-11′), 14.5 (C-12′), 14.4 (C-12); HRESIMS m/z 489.2119 $[M + H]^+$ (calcd for $C_{26}H_{33}O_9$, 489.2124).

7-(2,4-Bis(benzyloxy)-6-pentylphenoxy)-3-butyl-3-hydroxy-5-methoxyisobenzofuran-1(3*H*)-one (24). A sealed tube was charged with a magnetic stir bar, CuI (53 mg, 0.28 mmol), picolinic acid (53 mg, 0.55 mmol), 2-iodo-4-methoxy-6-pentanoylbenzoic acid (**11**) (500 mg, 1.38 mmol), 2,4-bis(benzyloxy)-6-pentylphenol (**22**) (520 mg, 1.38 mmol), and K_3PO_4 (880 mg, 4.14 mmol). The tube was then evacuated and backfilled with argon. The evacuation backfill sequence was repeated two additional times. Then the reaction mixture was dissolved in DMSO (20 mL). The tube was placed in a preheated oil bath at 110 °C, and the reaction mixture was stirred vigorously for 12 h. After cooling to rt, EtOAc (50 mL) and H_2O (50 mL) were added, and the mixture was stirred. The layers were separated, and the aqueous phsase was extracted with EtOAc three times (3 × 50 mL). The organic layer was dried over Na_2SO_4 and filtered through a pad of silica gel. The filtrate was concentrated in a rotary evaporator. Purification by flash column chromatography on C18 resin (H_2O/CH_3CN) afforded compound **24** (530 mg, 63%): TLC (hexane/EtOAc, 2/1, with 0.1% formic acid, UV) R_f = 0.70; ^{1}H NMR (850 MHz, $CDCl_3$) δ 7.34 (m, 10H, Bn), 6.55 (s, 1H, H-5), 6.52 (s, 1H, H-3) 6.50 (s, 1H, H-3′), 6.13 (s, 1H, H-5′), 5.02 (s, 2H, H-2′ CH_2), 4.96 (s, 2H, H-4′ CH_2), 3.74 (s, 3H, OMe), 2.52(m, 2H, H-7′), 2.06 (m, 2H, H-9), 1.58 (m, 2H, H-9′, 2H-8′), 1.26 (m, 2H, H-10; 2H, H-10′, 2H, H-11), 0.88 (m, 3H, H-11′), 0.82 (m, 3H, H-12); ^{13}C NMR (214 MHz, $CDCl_3$) δ 166.7 (C-7), 165,3 (C-4), 158.8 (C-2′), 156.7 (C-2), 153.3 (C-4′), 151.2 (C-6), 137.4 (C-6′), 136.7 (Bn), 136.0 (Bn), 128.6−127.6 (Bn), 127.0 (C-1′), 115.7 (C-8), 111.9 (C-1), 107.4 (C-3′), 105.0 (C-5′), 100.8 (C-3), 99.4 (C-5), 70.7 (C-2′ CH_2), 70.4 (C-4′ CH_2), 55.9 (OMe), 31.6 (C-9), 30.1 (C-8′), 29.7 (C-9′), 29.6 (C-7′), 25.2 (C-10), 22.5 (C-11), 22.4 (C-10′), 14.0 (C-12), 13.8 (C-11′); HRESIMS m/z 611.3009 $[M + H]^+$ (calcd for $C_{38}H_{43}O_7$, 611.3009).

Sakisacaulon A (5). To a 100 mL round-bottom flask charged with 7-(2,4-bis(benzyloxy)-6-pentylphenoxy)-3-butyl-3-hydroxy-5-methoxyisobenzofuran-1(3*H*)-one (**24**) (100 mg, 0.16 mmol) in MeOH (20 mL) was added palladium on carbon (10% weight %, 10 mg). The mixture was stirred under a hydrogen atmosphere (1 atm, hydrogen ballon) for 12 h. Upon completion the reaction mixture was filtered through a pad of silica. The filtrate was concentrated in a rotary evaporator. Purification by flash column chromatography on C18 resin (H_2O/CH_3CN) afforded compound **5** (62 mg, 88%): TLC (hexane/EtOAc, 2/1, with 0.1% formic acid, UV) R_f = 0.58; ^{1}H NMR (850 MHz, CD_3OD) δ 6.69 (d, J = 1.4, 1H, H-5), 6.31 (d, J = 2.6, 1H, H-5′), 6.23 (d, J = 2.6, 1H, H-3′), 6.08 (s, 1H, H-3), 3.77 (s, 3H, OMe), 2.40−2.33 (m, 2H, H-7′), 2.16−2.04 (m, 2H, H-9), 1.52 (m, 2H, H-8′), 1.34 (m, 2H, H-10; 2H, H-11), 1.21 (m, 2H, H-9′; 2H, H-10′), 0.89 (t, J = 7.1, 3H, H-12), 0.82 (t, J = 6.9, 3H, H-11′); ^{13}C NMR (214 MHz, CD_3OD) δ 168.9 (C-7), 168.8 (C-4), 106.0 (C-2), 156.9 (C-4′), 155.8 (C-6), 151.5 (C-2′), 138.3 (C-6′), 133.7 (C-1′), 108.9 (C-5′), 108.2 (C-8), 108.1 (C-1), 103.0 (C-3′), 102.7 (C-3), 101.1 (C-5), 56.7 (OMe), 39.8 (C-9), 32.9 (C-9′), 31.4 (C-7′), 31.0 (C-8′), 26.9 (C-10), 23.7 (C-11), 23.5 (C-10′), 14.5 (C-11′), 14.4 (C-12); HRESIMS m/z 431.2060 $[M + H]^+$ (calcd for $C_{24}H_{31}O_7$, 431.2070).

PTP1B Inhibition Assay. The PTP1B inhibition assay used in this study relies on the development of color resulting from the decomposition of *p*-nitrophenylphosphate (pNPP). Enzyme activity was measured using a mixture of 50 nM $PTP1B_{298}$ protein and 1.5 mM pNPP in 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer at pH 7.2, in the presence of 100 mM NaCl, 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM dithiothreitol, and 2% (v/v) DMSO. This mixture was placed in an incubator at room temperature for 30 min. The quantity of phosphate released from the pNPP was determined by measuring the increase in absorbance at 405 nm.[24]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.8b00227.

^{1}H and ^{13}C NMR spectra for compounds **1**−**24** (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Millot, M.; Tomasi, S.; Articus, K.; Rouaud, I.; Bernard, A.; Boustie, J. *J. Nat. Prod.* **2007**, *70*, 316−318.

(2) Cardile, V.; Graziano, A. C. E.; Avola, R.; Piovano, M.; Russo, A. *Chem.-Biol. Interact.* **2017**, *263*, 36−45.

(3) Morita, H.; Tsuchiya, T.; Kishibe, K.; Noya, S.; Shiro, M.; Hirasawa, Y. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3679−3681.

(4) Seo, C.; Sohn, J. H.; Ahn, J. S.; Yim, J. H.; Lee, H. K.; Oh, H. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2801−2803.

(5) (a) Kwon, I. S.; Yim, J. H.; Lee, H. K.; Pyo, S. *Biomol. Ther.* **2016**, *24*, 25. (b) Majmudar, C. Y.; Højfeldt, J. W.; Arevang, C. J.; Pomerantz, W. C.; Gagnon, J. K.; Schultz, P. J.; Tamayo-Castillo, G. *Angew. Chem., Int. Ed.* **2012**, *51*, 11258−11262. (c) Gissurarson, S. R.; Sigurdsson, S. B.; Wagner, H.; Ingolfsdottir, K. *J. Pharmacol. Exp. Ther.* **1997**, *280*, 770−773.

(6) (a) Carpentier, C.; Queiroz, E. F.; Marcourt, L.; Wolfender, J. L.; Azelmat, J.; Grenier, D.; Voyer, N. *J. Nat. Prod.* **2017**, *80*, 210−214. (b) Thadhani, V. M.; Choudhary, M. I.; Khan, S.; Karunaratne, V. *J. Natl. Sci. Found. Sri Lanka* **2012**, *40*, 43−48. (c) Brisdelli, F.; Perilli, M.; Sellitri, D.; Piovano, M.; Garbarino, J. A.; Nicoletti, M.; Celenza, G. *Phytother. Res.* **2013**, *27*, 431−437. (d) Morita, H.; Tsuchiya, T.; Kishibe, K.; Noya, S.; Shiro, M.; Hirasawa, Y. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3679−3681. (e) Ingolfsdottir, K.; Gissurarson, S. R.; Müller-Jakic, B.; Breu, W.; Wagner, H. *Phytomedicine* **1996**, *2*, 243−246.

(7) (a) Bhattarai, H. D.; Kim, T. K.; Oh, H.; Yim, J. H. *J. Antibiot.* **2013**, *66*, 559. (b) Kim, S.; Jo, S.; Lee, H.; Kim, T. U.; Kim, I. C.; Yim,

DOI: 10.1021/acs.jnatprod.8b00227
J. Nat. Prod. 2018, 81, 1460−1467

J. H.; Chung, H. *Anticancer Res.* **2013**, *33*, 5445−5451. (c) Ismed, F.; Lohézic-Le Dévéhat, F.; Delalande, O.; Sinbandhit, S.; Bakhtiar, A.; Boustie, J. *Fitoterapia* **2012**, *83*, 1693−1698.
(8) Yim, J. H.; Kim, I. C.; et al. Pharmaceutical and food compositon for preventing or treating diabetes or obesity. WO2012046945A3, Jun 14, 2012.
(9) (a) Djura, P.; Sargent, M. V.; Clark, P. D. *Aust. J. Chem.* **1977**, *30*, 1545−1551. (b) Djura, P.; Sargent, M. V. *Aust. J. Chem.* **1976**, *29*, 1069−1077. (c) Sala, T.; Sargent, M. V. *J. Chem. Soc., Perkin Trans. 1* **1979**, 2593−2598. (d) Hendrickson, J. B.; Ramsay, M. V.; Kelly, T. R. *J. Am. Chem. Soc.* **1972**, *94*, 6834−6843.
(10) (a) Maiti, D.; Buchwald, S. L. *J. Org. Chem.* **2010**, *75*, 1791−1794. (b) Miao, T.; Wang, L. *Tetrahedron Lett.* **2007**, *48*, 95−99. (c) Sperotto, E.; van Klink, G. P.; de Vries, J. G.; van Koten, G. *Tetrahedron* **2010**, *66*, 9009−9020.
(11) (a) Thuy-Boun, P. S.; Villa, G.; Dang, D.; Richardson, P.; Su, S.; Yu, J. Q. *J. Am. Chem. Soc.* **2013**, *135*, 17508−17513. (b) Suzuki, A. *J. Organomet. Chem.* **1999**, *576*, 147−168. (c) Cwik, A.; Hell, Z.; Figueras, F. *Org. Biomol. Chem.* **2005**, *3*, 4307−4309.
(12) Mei, T. S.; Giri, R.; Maugel, N.; Yu, J. Q. *Angew. Chem., Int. Ed.* **2008**, *47*, 5215−5219.
(13) Hanessian, S.; Ma, J.; Wang, W. *J. Am. Chem. Soc.* **2001**, *123*, 10200−10206.
(14) Taber, D. F.; Neubert, T. D.; Rheingold, A. L. *J. Am. Chem. Soc.* **2002**, *124*, 12416−12417.
(15) Tilvawala, R.; Pratt, R. F. *Biochemistry* **2013**, *52*, 7060−7070.
(16) Yang, G.; Shen, C.; Zhang, W. *Angew. Chem., Int. Ed.* **2012**, *51*, 9141−9145.
(17) Johnson, M. M.; Naidoo, J. M.; Fernandes, M. A.; Mmutlane, E. M.; van Otterlo, W. A.; de Koning, C. B. *J. Org. Chem.* **2010**, *75*, 8701−8704.
(18) (a) Kang, Y.; Mei, Y.; Du, Y.; Jin, Z. *Org. Lett.* **2003**, *5*, 4481−4484. (b) Ruan, B. F.; Lu, X.; Tang, J. F.; Wei, Y.; Wang, X. L.; Zhang, Y. B.; Zhu, H. L. *Bioorg. Med. Chem.* **2011**, *19*, 2688−2695. (c) Jana, N.; Nanda, S. *Eur. J. Org. Chem.* **2012**, *23*, 4313−4320. (d) Song, F.; Wang, C.; Falkowski, J. M.; Ma, L.; Lin, W. *J. Am. Chem. Soc.* **2010**, *132*, 15390−15398.
(19) Nicolaou, K. C.; Rodríguez, R. M.; Mitchell, H. J.; Suzuki, H.; Fylaktakidou, K. C.; Baudoin, O.; van Delft, F. L. *Chem. - Eur. J.* **2000**, *6*, 3095.
(20) Rathore, R.; Kochi, J. K. *J. Org. Chem.* **1995**, *60*, 7479−7490.
(21) Harris, T. D.; Oruganti, S. R.; Davis, L. M.; Keehn, P. M.; Green, B. S. *Tetrahedron* **1987**, *43*, 1519−1540.
(22) (a) Zhang, Q.; Raheem, K. S.; Botting, N. P.; Slawin, A. M.; Kay, C. D.; O'Hagan, D. *Tetrahedron* **2012**, *68*, 4194−4201. (b) Sultana, N.; Arayne, M. S.; Rizvi, S. B. S.; Haroon, U. *Bull. Korean Chem. Soc.* **2011**, *32*, 483−488.
(23) Lobaric acid (**1**) and its derivatives were partially soluble in the previous Bis-Tris buffer (ref 4) (pH 6.2, 50 mM Bis-Tris, 100 mM NaCl, 1 mM EDTA, 1 mM DTT), which gave inconsistent values. Therefore, the PTP1B assay was performed under HEPES buffer (pH 7.2, 50 mM HEPES, 100 mM NaCl, 1 mM EDTA, 1 mM DTT) conditions for the allosteric inhibitors in this study (ref 24b).
(24) (a) Cui, Y.; Yim, J. H.; Lee, D. S.; Kim, Y. C.; Oh, H. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 7393−7396. (b) Wiesmann, C.; Barr, K. J.; Kung, J.; Zhu, J.; Erlanson, D. A.; Shen, W.; McDowell, R. S. *Nat. Struct. Mol. Biol.* **2004**, *11*, 730−737.

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