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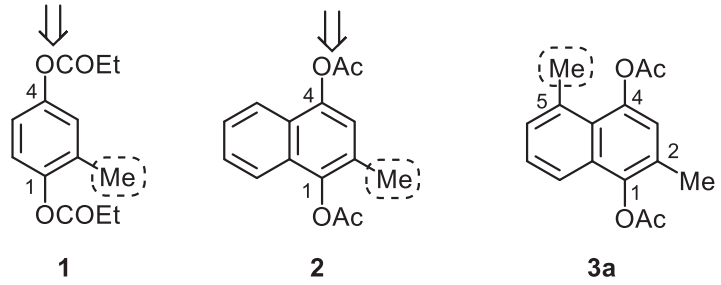
LIPASE-CATALYZED SITE-SELECTIVE DEACETYLATION OF STERICALLY HINDERED NAPHTHOHYDROQUINONE DIACETATE AND ITS APPLICATION TO THE SYNTHESIS OF A HETEROCYCLIC NATURAL PRODUCT

Riichi Hashimoto, Ayaka Sakakura, Kengo Hanaya, Shuhei Higashibayashi, and Takeshi Sugai*

Department of Pharmaceutical Sciences, Faculty of Pharmacy, Keio University, 1-5-30 Shibakoen, Minato-ku, Tokyo 105-8512, Japan. E-mail: sugai-tk@pha.keio.ac.jp

Abstract – Lipase-catalyzed site-selective deacetylation of 2,5-dimethylnaphthalene-1,4-diol diacetate was examined. With *Candida antarctica* lipase B, the suppressing effect of a methyl substituent at the *peri*-position of the α-naphthyl ester over that at the *ortho*-position was significant. This site-selectivity was in contrast to that of chemical hydrolysis reported to date. From the resulting monoacetate, mansonone F, a physiologically active heterocyclic orthoquinone, was synthesized in 38% yield in as few as three steps.

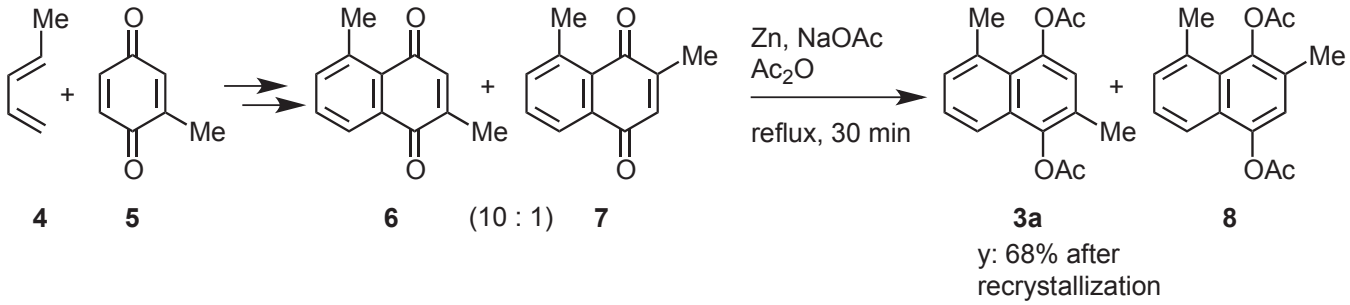
We have demonstrated lipase-catalyzed reactions in the site-selective synthesis of polyphenol natural products.[1-5] As clarified by the pioneering works by Miyazawa and coworkers,[6,7] the rate of lipase-catalyzed reactions is lowered by the introduction of neighboring substituents. For example, with hydroquinone dipropionate **1** as the substrate, *Candida antarctica* lipase B-catalyzed transesterification predominantly occurred at the less hindered C-4 position over the C-1 position, which was adjacent to the methyl group [circled at C-2 (*ortho*-) position (Figure 1)]. A similar preference was observed in naphthohydroquinone diacetate **2** upon *Pseudomonas* sp. lipase-catalyzed hydrolysis.[8] We became interested in the effect of the introduction of substituent at the *peri*-position (1,8-interaction) on lipase-catalyzed transesterification, which has not so far been examined. In order to clarify which methyl group has the stronger suppressing effect on the lipase-catalyzed transesterification at an adjacent ester, we chose naphthohydroquinone diacetate **3a** as a substrate,[9] which has two methyl groups at the *ortho*- (C-2) and *peri*- (C-5) positions (Figure 1).



1 **2** **3a**

Figure 1. Lipase-catalyzed site-selective deacetylation of **1** and **2** bearing an *ortho*-methyl group, and a newly proposed substrate **3a** with methyl groups on the *peri*- and *ortho*-positions

Toward this end, diacetate **3a** was synthesized from 1,3-pentadiene (**4**) and 2-methylbenzoquinone (**5**) based on $SbCl_5$-catalyzed regioselective Diels-Alder reaction[10] as the key step according to Bieber's report[9] with our modified recrystallization method. The desired naphthoquinone precursor **6** and its regioisomer **7** were obtained in a 10:1 ratio. This mixture was directly submitted to reductive acetylation to give a mixture of **3a** and its regioisomer **8** in a 10:1 ratio. After recrystallization under elaborated conditions (*i*-Pr_2O/AcOEt = 4:1), pure **3a** was obtained in 68% yield in the first crop. The second crop was a mixture in a **3a**:**8** = 10:1 ratio in 16% yield, which would be purified by repetitive recrystallization.



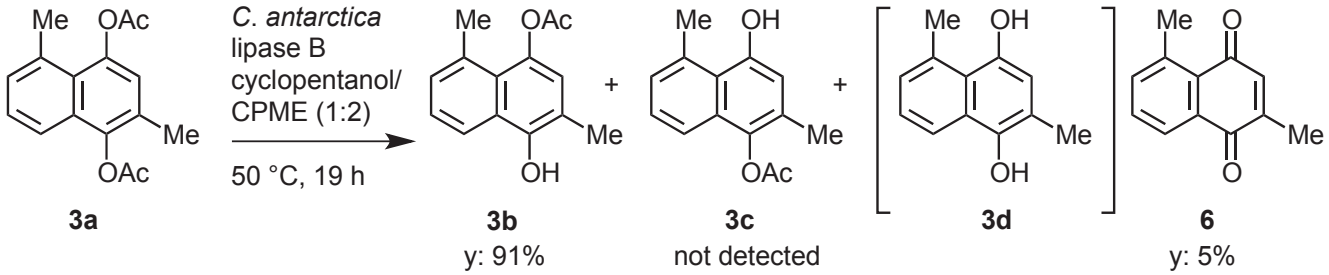
4 **5** **6** (10 : 1) **7** **3a** **8**

y: 68% after recrystallization

Scheme 1. Synthesis of the substrate **3a** based on the regioselective Diels-Alder reaction between **4** and **5**, and subsequent reductive acetylation

Deacetylation of **3a** with *C. antarctica* lipase B was first tried with 2-propanol as a nucleophile in tetrahydrofuran (THF) as a solvent[4] at room temperature. Although the reaction was sluggish and 32% of **3a** was recovered, the monoacetate **3b** was selectively obtained in 26% yield without detection of the regioisomer (C-1 acetate, **3c**).[8] The position of the remaining ester of **3b** at C-4 was confirmed by a nuclear Overhauser effect (nOe) between the proton at C-3 and the acetyl group at C-4 (Figure 2). The low efficiency of the reaction prompted us to use forced reaction conditions combining cyclopentanol as a nucleophile and cyclopentyl methyl ether (CPME) as a solvent[1] at raised temperature (50 °C). The yield

of **3b** was improved to 91% with keeping a highly site-selectivity (Scheme 2). Naphthoquinone **6** (5%) was the only observed byproduct, probably caused by the aerobic oxidation of initially formed unstable naphthohydroquinone **3d**.



Scheme 2. *C. antarctica* lipase B-catalyzed site-selective deacetylation of **3a**

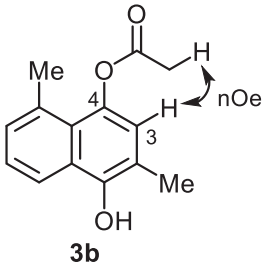


Figure 2. Elucidation of the regiochemistry of **3b** using NOESY measurement

The above-mentioned site-selectivity was in contrast to the result that had previously been reported for the hydrolysis of **3a** under alkaline conditions (K_2CO_3, $Na_2S_2O_4$ in aqueous methanol).[9] In that report, the product was a mixture of **3b** and **3c** in a 22:78 ratio. The selectivity was augmented by the aid of semi-empirical calculations.[11] Those authors claimed that the site-selectivity was ascribable to cancellation of the steric repulsion of the tetrahedral intermediate derived from the C-4 acetate ester, which was formed by the nucleophilic attack of a water molecule.

As mentioned earlier, Miyazawa and co-workers disclosed that the introduction of the substituents at the *ortho*-position lowered the rate of *C. antarctica* lipase B-mediated reactions.[6,7] In contrast, in the present case, the suppressing effect of a methyl substituent at the *peri*-position of the α-naphthyl ester exceeded that at the *ortho*-position.

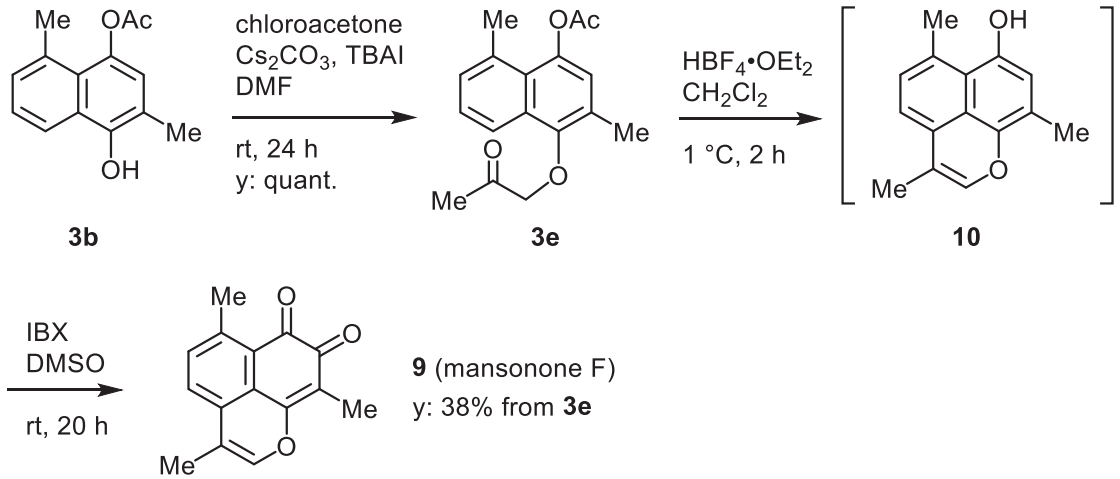
We attempted docking simulations between *C. antarctica* lipase B (PDB: 4K6G) and the substrate **3a** with CDOCKER, of the program package Discovery Studio 4.5.[3,12] Among several stable docking poses, however, there was only very small difference in the proximity between the hydroxy group of Ser105 in catalytic site and C-4 acetate ester or C-1 acetate ester. Thus, the origin of the selectivity remained unclear. Generally, the selectivity in enzyme-catalyzed reactions is responsible to the

difference of potential energy of transition states.[13,14] In our present case, the transition state which was derived between C-1 acetate ester and serine hydroxy group, may be more energetically lower than that derived from C-4 acetate ester and that hydroxy group.

We also submitted **3a** to *B. cepacia* lipase (Amano PS-IM, an equivalent enzyme of *Pseudomonas* sp. lipase for **2**). The deacetylation occurred almost exclusively on C-4 acetate ester to give **3c**. This reaction proceeded in a similar manner to diacetate **2** as the substrate,[8] under suppressing effect of substituent at the *ortho*-position.

With pure **3b** in hand, we embarked upon the transformation of **3b** to mansonone F. Mansonone F had been investigated as the irritation component in the heartwood of *Mansonia altissima* Chev. (Sterculiaceae) in West Africa,[15,16] and the structure was elucidated to be **9**, a heterocyclic orthonaphthoquinone.[17] Later, mansonone F isolated from other plants exhibited phytoalexin[18,19] and anti-MRSA[20] activities, and two total syntheses have so far been achieved.[9,21]

Our synthesis from **3b** is summarized in Scheme 3. First, the liberated hydroxy group at C-1 in **3b** was alkylated with chloroacetone and Cs_2CO_3 in *N*,*N*-dimethylformamide (DMF) in the presence of *tert*-butylammonium iodide (TBAI) to give **3e** in a quantitative manner. Intramolecular Friedel-Crafts reaction and subsequent dehydration to form the oxygen-containing heterocyclic ring was performed by HBF_4 diethyl ether complex[9] as the Lewis acid catalyst. Concomitant removal of the acetate ester at C-4 provided an unstable **10**,[9] and without isolation, the crude product was treated with 2-iodoxybenzoic acid (IBX)[22] in dimethyl sulfoxide (DMSO).[23] The desired mansonone F (**9**) was obtained in 38% yield from **3e**. Its spectral data were in good accordance with those reported previously.[9,21]



Scheme 3. Derivation of **3b** to mansonone F (**9**), an oxygen-containing heterocyclic orthonaphthoquinone

In conclusion, the suppressing effect of the methyl substituent at the *peri*-position of an α-naphthyl ester over that at the *ortho*-position in **3a** under the *C. antarctica* lipase B-catalyzed deacetylation was

disclosed. A contrasting site-selectivity between *C. antarctica* lipase B-catalyzed transesterification and chemical hydrolysis was shown. Starting from the resulting monoacetate **3b**, mansonone F was synthesized in 38% yield in three steps. Our synthesis of mansonone F through **3b** is fewer steps and higher total yield (four steps, 35% yield) than those (six steps, 8.6%) in the previously reported synthesis[9] through **3c** from the common diacetate **3a**.

EXPERIMENTAL

Lipase B from *Candida antarctica* (Novozym 435) immobilized on acrylic resin was purchased from Novozyms Inc. (Chiba, Japan). *Burkholderia cepacia* lipase (Amano PS-IM) immobilized on diatomaceous earth was purchased from Amano Enzyme Inc. (Aichi, Japan). 2-Iodoxybenzoic acid (I0791) with an estimated purity of *ca*. 40% was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Melting points were measured on a Mitamura Riken Kogyo MELTEMP and uncorrected. ^{1}H-NMR spectra were measured at 400 MHz on a VARIAN 400-MR spectrometer or at 500 MHz on a VARIAN 500-MR spectrometer, and ^{13}C-NMR spectra were measured at 125 MHz on a VARIAN 500-MR spectrometer. $CDCl_3$ and acetone-d_6 were used as a solvent and the residual solvent peaks were used as an internal standard (^{1}H-NMR: $CDCl_3$ 7.26 ppm, acetone-d_6 2.05 ppm; ^{13}C-NMR: $CDCl_3$ 77.1 ppm). IR spectra were measured as ATR on a Jasco FT/IR-4700 FT-IR spectrometer. High resolution mass spectra (HRMS) were recorded on Jeol JMS-T100LP AccuTOF. Silica gel 60N (spherical and neutral; 40-50 μm, 37563-84) from Kanto Chemical Co. was used for column chromatography. Preparative TLC was performed with Merck Silica Gel 60 F_{254} plates (0.5 mm thickness, No. 5744).

2,5-Dimethylnaphthalene-1,4-diol diacetate (3a). To a mixture of sodium acetate (697 mg, 8.49 mmol) and Zn (583 mg, 8.88 mmol) was added a mixture of **6** and **7** in *ca*. 10:1 (719 mg, 3.86 mmol), judged by the integral of signals in H-2 or H-3, in acetic anhydride (9.0 mL) under argon atmosphere at room temperature. The mixture was stirred under reflux for 30 min. Accompanied with the progress of the reaction, the yellow color ascribable to the naphthoquinones gradually disappeared. To the reaction was added acetic acid (7.7 mL) and the mixture was further stirred under reflux for 2 min. After cooling to room temperature, the mixture was poured into ice, and cooled for 20 h in a refrigerator at 4 °C. The precipitates were collected by filtration and washed with water. The precipitates were dissolved in $CHCl_3$ and filtered with a Celite pad to remove insoluble materials involving Zn. The filtrates were concentrated *in vacuo* to give a mixture of **3a** and **8** as a colorless solid in *ca*. 10:1 ratio (1.12 g), judged by the integral of signal in H-2 or H-3.

For the separation of **3a** from **8**, recrystallization was performed. From 1.12 g of the above-mentioned solid, first crop (718 mg, 68%) of pure **3** was obtained from *i*-Pr_2O/AcOEt (4:1) as colorless prisms; mp

134.5-134.9 °C. The recovered mixture in *ca*. 5:1 ratio was recrystallized from *i*-Pr_2O/AcOEt (4:1) to give the second crop (169 mg, 16%) as a mixture of **3a** and **8** in a 10:1 ratio. The recovered materials (105 mg) from mother liquor was a mixture of **3a** and **8** in a 2:1 ratio.

IR: 2925, 1749, 1370, 1195, 1152, 761 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.29 (s, 3H), 2.40 (s, 3H), 2.47 (s, 3H), 2.75 (s, 3H), 7.02 (s, 1H), 7.22 (d, *J* = 7.1 Hz, 1H), 7.36 (dd, *J* = 7.1, 8.6 Hz, 1H), 7.62 (d, *J* = 8.6 Hz, 1H); ^{13}C-NMR ($CDCl_3$) δ: 16.3, 20.6, 21.6, 23.4, 119.6, 122.3, 125.8, 126.3, 126.7, 129.2, 129.2, 132.9, 142.7, 145.0, 168.9, 170.0; HRMS (ESI) calcd for $C_{16}H_{16}NaO_4$ $[M+Na]^+$ 295.0946, Found 295.0974. Its ^{1}H and ^{13}C-NMR spectra were identified with those previously reported.[9]

From the residue remained in mother liquor from recrystallization, characteristic signals in ^{1}H-NMR ($CDCl_3$) which could be ascribable to regioisomer **8** were as follow: δ: 2.28 (3H, s), 2.41 (3H, s), 2.43 (3H, s), 2.76 (3H, s).[9]

4-Acetoxy-2,5-dimethyl-1-hydroxynaphthalene (3b). To a solution of **3a** (167 mg, 0.614 mmol) in a mixture of cyclopentanol (1.7 mL) and CPME (3.3 mL), which was pre-dried over anhydrous Na_2SO_4 at room temperature, was added *C. antarctica* lipase B (Novozym 435, 167 mg) under argon atmosphere, and the mixture was stirred for 19 h at 50 °C. After removal of insoluble materials by filtration with a Celite pad, the filtrate and washings were concentrated *in vacuo*. The residue was purified by silica gel column chromatography (5 g). Elution with hexane/CH_2Cl_2 (1:1 to 1:10) furnished monoacetate **3b** (128 mg, 91%) as a solid. Analytical sample was obtained by the recrystallization from hexane/AcOEt (4:1) as colorless prisms; mp 130.8-131.5 °C; IR: 3444, 2920, 1732, 1363, 1219, 1049, 755 cm^{-1}; ^{1}H-NMR (acetone-d_6) δ: 2.34 (s, 3H), 2.40 (s, 3H), 2.72 (s, 3H), 7.00 (s, 1H), 7.22 (d, *J* = 6.9 Hz, 1H), 7.32 (dd, *J* = 6.9, 8.6 Hz, 1H), 8.16 (d, *J* = 8.6 Hz, 1H); ^{13}C-NMR ($CDCl_3$) δ: 15.4, 21.7, 23.4, 116.5, 120.1, 122.4, 125.2, 125.6, 126.4, 129.0, 132.0, 140.1, 147.3, 171.2; HRMS (ESI) calcd for $C_{14}H_{14}NaO_3$ $[M+Na]^+$ 253.0841, found 253.0849.

Naphthoquinone **6** was also isolated (5.8 mg, 5%) from the less polar fraction of chromatographic separation.

***B. cepacia* lipase-catalyzed deacetylation of 3a.** To a solution of **3a** (16.7 mg, 61.4 μmol) in a mixture of cyclopentanol (0.17 mL) and CPME (0.33 mL), which was pre-dried over anhydrous Na_2SO_4 at room temperature, was added *B. cepacia* lipase (Amano PS-IM, 16.7 mg) under argon atmosphere, and the mixture was stirred for 19 h at 50 °C. After workup in the similar manner for C. antarctica lipase B-catalyzed deaceylation, **3c** was obtained (15.2 mg, quantitative yield) as a white solid; ^{1}H-NMR (acetone-d_6) δ: 2.17 (s, 3H), 2.42 (s, 3H), 2.90 (s, 3H), 6.76 (s, 1H), 7.14 (d, *J* = 6.8 Hz, 1H), 7.30 (dd, *J* = 6.8, 8.2 Hz, 1H), 7.56 (d, *J* = 8.2 Hz, 1H), 8.87 (s, 1H).

4-Acetoxy-1-(2-oxopropoxy)-2,5-dimethylnaphthalene (3e). To a solution of **3b** (115 mg, 0.498 mmol) in anhydrous DMF (3.0 mL) were added TBAI (47.5 mg, 0.129 mmol), chloroacetone (90 μL,

1.12 mmol) and Cs_2CO_3 (327 mg, 1.00 mmol) under argon atmosphere at room temperature, and the mixture was stirred for 24 h at room temperature. The reaction was quenched with 0.1 M phosphate buffer solution (pH 7.0), and the organic materials were extracted with Et_2O three times. The combined organic extract was washed with water and brine, and dried over anhydrous Na_2SO_4, filtered through a cotton plug, and concentrated *in vacuo* to give **3e** (149 mg, quantitative yield) as a yellow oil; IR: 2923, 1757, 1733, 1391, 1365, 1200, 1168, 1053, 766 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 2.38 (s, 3H), 2.39 (s, 3H), 2.41 (s, 3H), 2.74 (s, 3H), 4.51 (s, 2H), 6.95 (s, 1H), 7.22 (d, *J* = 7.1 Hz, 1H), 7.36 (dd, *J* = 7.1, 8.4 Hz, 1H), 7.92 (d, *J* = 8.4 Hz, 1H); ^{13}C-NMR ($CDCl_3$) δ: 15.9, 21.6, 23.3, 26.6, 77.8, 120.1, 122.8, 125.7, 126.1, 126.4, 129.2, 129.8, 132.9, 143.7, 149.8, 170.1, 204.6 ppm; HRMS (ESI) calcd for $C_{17}H_{18}NaO_4$ $[M+Na]^+$ 309.1103, found 309.1108.

Mansonone F (9). To a solution of HBF_4 diethyl ether complex (98% purity, 284 μL, 4.15 mmol) was added **3e** (32.1 mg, 0.112 mmol) in anhydrous CH_2Cl_2 (0.80 mL) at 1 °C under argon atmosphere, and the mixture was stirred for 2 h at 1 °C. The reaction was quenched with water, and the organic materials were extracted with $CHCl_3$ three times. The combined organic extract was washed with water and brine, and dried over anhydrous Na_2SO_4, filtered through a cotton plug, and concentrated *in vacuo* to give crude **10** (28.4 mg, quantitative yield). To a solution of **10** in anhydrous DMSO (2.50 mL) was added IBX (*ca*. 40% purity, 224 mg, *ca*. 0.31 mmol) under argon atmosphere at room temperature, and the mixture was stirred for 20 h at the same temperature. The reaction was quenched with saturated $NaHCO_3$ aq. solution. The organic materials were extracted with $CHCl_3$ three times. The combined organic extract was washed with saturated $NaHCO_3$ aq. solution and brine, and dried over anhydrous Na_2SO_4, filtered through a cotton plug, and concentrated *in vacuo*. The residue was purified by preparative TLC with hexane/AcOEt (3:5) to furnish **9** (10.2 mg, 38% from **3e**) as a purple solid; mp 195.1-196.4 °C [lit, 205 °C (decomposition)[21]]; IR: 2925, 1684, 1598, 1575, 1215, 758, 668 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ: 1.98 (s, 3H), 2.12 (d, *J* = 1.4 Hz, 3H), 2.72 (s, 3H), 7.09 (d, *J* = 1.4 Hz, 1H), 7.42 (d, *J* = 8.2 Hz, 1H), 7.48 (d, *J* = 8.2 Hz, 1H); ^{13}C-NMR ($CDCl_3$) δ: 7.7, 12.9, 23.1, 112.2, 113.5, 124.0, 126.3, 128.4, 129.5, 136.4, 140.4, 146.6, 161.8, 178.0, 182.0; HRMS (ESI) calcd for $C_{15}H_{12}NaO_3$ $[M+Na]^+$ 263.0684, found 263.0702. Its ^{1}H-NMR and ^{13}C-NMR spectra were in good accordance with those reported previously.[9,21]

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