Total Synthesis of Amino-Functionalized Calphostin Analogs as Potent and Selective Inhibitors of Protein Kinase C (PKC)

Hyoyeon Kim,[†] Choon-Ho Song,[†] Dae-Hyuk Kim,[‡] Nam-Gin Jung,[§] Seung-Jae Lee,[¶,*] and Beom-Tae Kim[†,‡,‖,*]

[†]*Department of Bioactive Material Sciences, Center of Bioactive Materials, Jeonju 561-756, Korea.*
**E-mail: bkim002@jbnu.ac.kr*
[‡]*Department of Molecular Biology, College of Natural Science, Jeonju 561-756, Korea*
[§]*Department of Crop Science and Biotechnology, College of Agriculture and Life Science, Jeonju 561-756, Korea*
[¶]*Department of Chemistry, College of Natural Science, Jeonju 561-756, Korea*
[‖]*Keunsaram Educational Development Institute, Chonbuk National University, Jeonju 561-756, Korea*
**E-mail: slee026@jbnu.ac.kr*
Received June 2, 2016, Accepted July 28, 2016

As potential protein kinase C (PKC) inhibitors and photodynamic agents, the novel amino-functionalized calphostin analog 1,12-bis((benzoyl-amino)methyl)-3,10-perylenequinone was successfully prepared by dimerization of the key intermediate 3-(benzoylamino)methyl-1,2-naphthoquinone (**9**), which was synthesized by an efficient and relatively short synthetic sequence (eight steps) with satisfactory overall yield. The naturally occurring form of perylenequinone **12** was prepared by consecutive methylation and demethylation reactions. In our synthetic strategy, it was beneficial that the amino functionality of 1,2-naphthoquinone **9** could be easily introduced at an early synthetic stage and subsequently dimerized to prepare various potentially bioactive perylenequinones.

Keywords: Amino-functionalized calphostin, Naphthoquinone, Perylenequinone, Protein kinase C

Introduction

Calphostins A–D are biologically significant perylenequinones that were isolated in 1989 from the fungi *Cladosporium cladosporioides* (Figure 1).[1] The calphostins were found to be potent and selective inhibitors of protein kinase C (PKC),[2] a regulatory enzyme crucial to cell division and differentiation.[3] Thus, because of their unique inhibitory activity against PKC, considerable attention has been paid to the application of calphostins as potential anticancer therapy agents for a variety of cancers.[4]

The perylenequinone core of the calphostins is a crucial chromophore to their photodynamic activity, which upon exposure to light generates reactive oxygen species with high quantum efficiencies.[5] Thus, the inhibitory potential of calphostins to PKC has been suggested as due to their light-dependent free-radical formation and the resultant local damage. However, despite the photodynamic properties of perylenequinones, including calphostins, their practical application requires overcoming several restrictions, i.e., poor aggregation tendency, relatively short absorption wavelengths, low quantum yields, low solubility, and poor stability in an aqueous environment. One of the notable endeavors dedicated to improving the properties mentioned above was the introduction of an amino functionality to the perylenequinone core to prepare various amino-substituted derivatives.[6] Representative work was performed with hypocrellin B, a similar perylenequinone natural product. Another crucial structural factor that affects photodynamic activity is substituents at the 1- and 12-positions of the perylenequinone core.[7] In fact, a previous analysis of the structural features of perylenequinones showed that the functional diversity of substituents at the 1- and 12-positions of perylenequinones is likely to play a crucial role in such activity.

Taking the factors mentioned above into consideration, we designed a calphostin derivative substituted with amino functionality at the 1- and 12-positions of the perylenequinone core as a target compound (Scheme 1, compound **12**). A literature survey of the synthesis of the perylenequinone core[7,8] showed that a synthetic strategy involving 1,2-naphthoquinone as a key intermediate, which could be converted to the dimerized 3,10-perylenequinone, would be the most promising (Scheme 1). In our synthetic approach to the target molecule, initial work focused on the preparation of 3-(benzoylamino)methyl-1,2-naphthoquinone (**9**) as the key intermediate, which was prepared starting from the readily available naphthoate[9] (**3**). Several dimerization conditions that could lead to successful preparation of 3,10-perylenequinone **10** were investigated. In an acid-catalyzed dimerization, along with the desired perylenequinone **10**, two compounds known to be key intermediates to 3,10-perylenequinone were isolated, and their structures were elucidated on the basis of spectroscopic analysis. Here, we provide the details of the synthesis of the key intermediate 3-(benzoylamino)methyl-1,2-naphthoquinone (**9**), its dimerization compound 1,12-bis((benzoyl-amino)

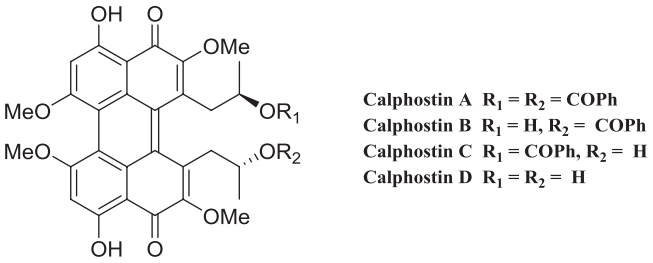


Figure 1. Structure of the calphostins A–D.

methyl)-3,10-perylenequinone (**10**), and the final naturally occurring form, 4,9-dihydroxy-2,6,7,11-tetramethoxy-3,10-perylenequinone (**12**).

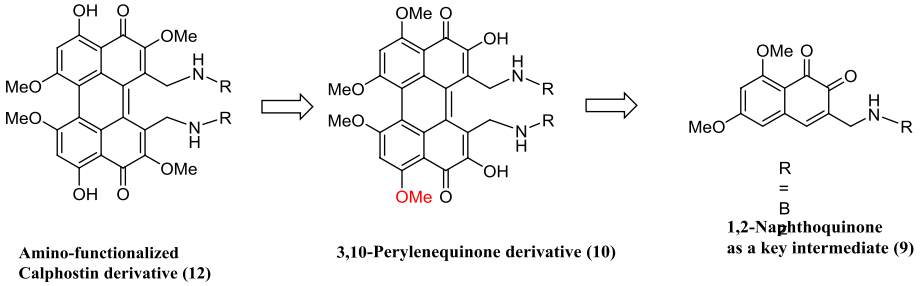
Compound **12** is a new type of nitrogen-containing perylenequninone prepared via total synthesis, which to the best of our knowledge has not been found in natural sources to date. It is notable that compound **12** could be a platform compound to develop structurally diverse and novel amino-perylenequinones.

Experimental Section

Chemicals and Instrumentation. Melting points were recorded on an MEL-TEMP melting point apparatus (Laboratory Devices Inc., USA) and were uncorrected. Mass spectra were recorded on a Synapt HDMS mass spectrometer (Waters, Milford, MA, USA). ^{1}H and ^{13}C NMR spectra were recorded on a JEOL 400 MHz or 600 MHz spectrometer (Akishima, Japan). Chemical shifts are shown in δ values (ppm), with tetramethylsilane (TMS) as the internal standard. UV spectra were recorded on an HP UV–visible Chemstation spectrophotometer (Hewlett-Packar Company, Palo Alto, CA, USA). All chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA), and all solvents for column chromatography were of reagent grade and were purchased from commercial sources.

(*E*)-4-(3,5-Dimethoxyphenyl)-3-(ethoxycarbonyl)but-3-enoic acid (2). A suspension of sodium hydride (3.01 g, 75.23 mmol, 60% dispersion in mineral oil, washed three times with toluene) in dry toluene (40 mL) was precooled in an ice bath under an Ar atmosphere and was activated with a catalytic amount of EtOH (0.5 equiv). To this suspension, a solution of 3,5-dimethoxybenzaldehyde (**1**) (5.0 g, 30.09 mmol) and diethyl succinate (14.13 mL, 84.25 mmol) in toluene (15 mL) was added dropwise. After stirring for 2.5 h, the reaction mixture was quenched by cautiously adding 1 M aqueous HCl solution (120 mL). The organic layer was separated, and the pH was adjusted to ~5 with 1 M aqueous K_2CO_3. The aqueous layer was separated and re-acidified with 10% aqueous HCl. The resulting precipitate was partitioned into diethyl ether (150 mL). The organic layer was washed with water (150 × 4 mL) and brine (150 mL), dried over Na_2CO_3, filtered, and evaporated *in vacuo* to give a yellowish solid residue. The residue was subjected to flash column chromatography (silica gel) to afford butenoic acid **2** (8.55 g, 97%) as a yellowish solid: mp 88–90°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.83 (s, 1H, H-4), 6.49 (d, 2H, J = 1.96 Hz, H-2″, 6″), 6.44 (t, 1H, J = 2.44 Hz, H-4″), 4.28 (q, 2H, J = 7.32 Hz, –CH_2–), 3.77 (s, 6H, –OCH_3), 3.69 (d, H, J = 1.48 Hz, H-2′), 3.57 (d, H, J = 2.44 Hz, H-2′), 1.32 (t, 3H, J = 6.84 Hz, –CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.80, 167.63, 160.87, 142.52, 136.52, 125.76, 106.85, 101.42, 61.52, 55.42, 33.87, 14.16.

Ethyl 4-acetoxy-5,7-dimethoxy-2-naphthoate (3). The neat mixture of butenoic acid **2** (6.98 g, 23.71 mmol), acetic anhydride (11.17 mL, 118.55 mmol), and sodium acetate (3.69 g, 45.05 mmol) was heated for 1 h at 140°C under an Ar atmosphere. After checking the completeness of the reaction by the disappearance of compound **2** in thin-layer chromatography, the reaction mixture was cooled in an ice bath and quenched by adding water (15 mL). The resulting precipitate was collected, washed thoroughly with water (50 mL × 5), and dissolved in EtOAc (100 mL). The organic layer was dried over Na_2CO_3, filtered, and evaporated *in vacuo* to give a crude product. The residue was subjected to flash column chromatography (silica gel) to afford naphthoate **3** (5.87 g, 78%) as a yellowish solid: mp 129 ~130°C; APCIMS *m/z* $[M + H]^+$ Calcd 319.1182 for $C_{15}H_{19}O_6$: Found: 318.9972; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.30 (d, 1H, J = 1.9 Hz, H-1), 7.48 (d, 1H, J = 1.9 Hz, H-3), 6.85 (d, 1H, J = 2.4 Hz, H-8), 6.58 (d, 1H, J = 2.4 Hz, H-6), 4.39 (q, 2H, J = 7.3 Hz, –CH_2-), 3.89 (d, 6H, J = 1 Hz -OCH_3), 2.35 (s, 3H, –CH_3), 1.39 (t, 3H, J = 7.3 Hz, –CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.04, 165.87, 158.74, 156.19, 146.82, 137.00, 128.74, 127.87, 117.17, 116.60, 101.55, 100.14, 61.20, 56.17, 55.43, 20.88, 14.33.



Scheme 1. Retrosynthetic analysis of amino-functionalized calphostin derivative (**12**) from 1,2-naphthoquinone (**9**).

3-Hydroxymethyl-6,8-dimethoxynaphthalen-1-ol (4). Naphthoate **3** (3.11 g, 9.76 mmol) in dry THF (25 mL) was slowly added over 30 min to a precooled suspension of lithium aluminum hydride (1.85 g, 48.81 mmol) in dry THF (20 mL). After stirring for 1.5 h at room temperature, the reaction was quenched by slowly adding sat. NH_4Cl (10 mL). The resulting sludge was removed by filtration and washed with ethyl acetate (100 mL × 3). The combined organic layers were dried over Na_2CO_3, filtered, and evaporated *in vacuo* to give naphthalenol **4** as a pale yellowish solid (2.23 g, 98%). Thin-layer chromatography (EtOAc:Hexane (3/7, v/v), R_f = 0.35) showed a single clean spot corresponding to compound **4**, which was used in the next reaction without further purification: mp 129 ~130°C; APCIMS *m/z* $[M + H]^+$ Calcd 235.0970 for $C_{13}H_{15}O_4$: Found: 235.0227; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.09 (s, 1H, −OH), 7.14 (s, 1H, H-4), 6.69 (d, 1H, J = 1.4 Hz, H-5), 6.67 (d, 1H, J = 2.4 Hz, H-2), 6.42 (d, 1H, J = 2.4 Hz, H-7), 4.70 (s, 2H, $-CH_2$-), 3.99 (s, 3H, $-OCH_3$), 3.86 (s, 3H, $-OCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.88, 157.09, 154.75, 141.26, 137.45, 115.37, 110.12, 107.17, 99.50, 97.53, 65.17, 56.09, 55.30.

3-Chloromethyl-6,8-dimethoxynaphthalen-1-ol (5). To a mixture of triphenylphosphine (4.87 g, 18.58 mmol) and carbon tetrachloride (3.59 mL, 37.15 mmol) in dry THF (5 mL) under an Ar atmosphere was added compound **4** (1.09 g, 4.64 mmol). After stirring for 1.5 h at 70°C, the reaction was quenched by adding water (150 mL), and the mixture was partitioned with EtOAc (50 mL × 4). The combined organic layers were washed with water (50 × 4 mL) and brine (100 mL), dried over Na_2CO_3, filtered, and evaporated *in vacuo* to give a yellowish solid residue. The residue was subjected to flash column chromatography (silica gel) to afford product **5** (0.91 g, 78%,) as a white solid: mp 130–131°C; APCIMS m/z $[M + H]^+$ Calcd 253.0631 for $C_{13}H_{14}ClO_3$: Found: 252.9911; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.12 (s, 1H, −OH), 7.17 (d, 1H, J = 1.4 Hz, H-4), 6.73 (d, 1H, J = 1.4 Hz, H-5), 6.68 (d, 1H, J = 2.4 Hz, H-2), 6.45 (d, 1H, J = 2.0 Hz, H-7), 4.60 (s, 2H, $-CH_2$-), 4.00 (s, 3H, $-OCH_3$), 3.87 (s, 3H, $-OCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.10, 157.07, 154.98, 137.42, 137.27, 117.53, 110.47, 108.52, 99.58, 98.09, 56.16, 55.34, 46.32.

2-((4-Hydroxy-5,7-dimethoxynaphthalen-2-yl) methyl) isoindoline-1,3-dione (6). To a solution of compound **5** (0.84 g, 3.33 mmol) in DMF (5 mL) under an Ar atmosphere, potassium phthalimide was added (1.24 g, 6.67 mmol) at room temperature. After stirring for 2 h at 50°C, the reaction mixture was cooled in an ice bath and diluted with EtOAc (100 mL). The solution was washed with water (50 × 4 mL) and brine (50 mL), dried over $MgSO_4$, filtered, and evaporated *in vacuo* to give a solid. The residue was subjected to flash column chromatography (silica gel) to afford product **6** (0.98 g, 81%) as a white solid: mp: 231–232°C; APCIMS *m/z* $[M + H]^+$ Calcd 364.1185 for $C_{21}H_{18}NO_5$: Found: 363.0417; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.07 (s, 1H, −OH), 7.83 (dd, 2H, J = 5.2; 2.8 Hz), 7.69 (dd, 2H, J = 5.6; 2.4 Hz), 7.18 (d, 1H, J = 1.0 Hz, H-1), 6.73 (d, 1H, J = 1.4 Hz, H-8), 6.66 (d, 1H, J = 2.0 Hz H-3), 6.39 (d, 1H, J = 2.0 Hz, H-6), 4.87 (s, 2H, $-CH_2$-), 3.97 (s, 3H, $-OCH_3$), 3.84 (s, 3H, $-OCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.25, 170.23, 160.88, 159.88, 157.88, 142.56, 137.54, 134.41, 134.41, 125.98, 125.94, 124.30, 123.28, 120.18, 111.06, 111.03, 103.86, 96.74, 57.04, 56.08, 45.89.

3-Aminomethyl-6,8-dimethoxynaphthalen-1-ol (7). Compound **6** (430 mg, 1.18 mmol) was dissolved in a mixed solution of ethanol/toluene (2:1, v/v), and hydrazine monohydrate (0.92 ml, 18.94 mmol) was added at room temperature under an Ar atmosphere. After stirring for 2 h at 90°C, the reaction mixture was cooled in an ice bath, and the pH was adjusted to two with 2 N aqueous HCl. The mixture was stirred for another 30 min, then neutralized to pH 7 with 1 M aqueous NaOH, and partitioned with $CHCl_3$ (20 mL × 3). The combined organic layers were washed with water (50 × 2 mL) and brine (50 mL), dried over $MgSO_4$, filtered, and evaporated *in vacuo* to give an oily residue. The residue was subjected to flash column chromatography (silica gel) to afford aminomethyl compound **7** (261 mg, yellow oil, 95%) as an oil: mp: 76–77°C; APCIMS *m/z* $[M + H]^+$ Calcd 234.1130 for $C_{13}H_{16}NO_3$: Found: 234.0664; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.05 (s, 1H, H-4), 6.63 (S, 2H, H-5,7), 6.35 (d, 1H, J = 2.0 Hz, H-2), 3.92 (s, 3H, $-OCH_3$), 3.83 (s, 2H, $-CH_2$-), 3.82 (s, 3H, $-OCH_3$) ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.87, 157.11, 154.72, 143.52, 137.56, 115.35, 109.74, 107.79, 99.34, 97.29, 56.98, 55.32, 36.39.

3-(Benzoylamino)methyl-6,8-dimethoxy-naphthalene-1-ol (8). To a solution of compound **7** (203 mg, 0.87 mmol) in CH_3CN (5 mL) was added benzoyl chloride (303 μL, 2.61 mmol) at room temperature under an Ar atmosphere. After stirring for 1 h at 50°C, K_2CO_3 (120 mg, 0.87 mmol) was added to the reaction, and the mixture was stirred for another 1 h at room temperature. The mixture was diluted with water (10 mL) and partitioned with EtOAc-$CHCl_3$ (20 mL × 3). The combined organic layers were washed with water (50 × 2 mL) and brine (50 mL), dried over $MgSO_4$, filtered, and evaporated *in vacuo* to give a solid residue. The residue was subjected to flash column chromatography (silica gel) to afford benzoylamino compound **8** (285 mg, 97%) as a colorless solid: mp 183–184°C; APCIMS *m/z* $[M + H]^+$ Calcd 338.1392 for $C_{20}H_{20}NO_4$: Found: 338.0942; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.13 (s, 1H, −OH), 7.81 (m, 2H), 7.49 (m, 1H), 7.42 (m, 2H), 7.14 (s, 1H, H-4), 6.69 (d, 1H, J = 1.5 Hz, H-5), 6.67 (d, 1H, J = 2.0 Hz, H-7), 6.50 (s, 1H, −NH), 6.43 (d, 1H, J = 2.5 Hz, H-2), 4.67 (d, 2H, J = 5.4 Hz, $-CH_2$-), 4.01 (s, 3H, $-OCH_3$), 3.87 (s, 3H, $-OCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.73, 159.63, 158.62, 156.64, 141.43, 136.45, 135.37, 133.35, 124.31, 124.02, 123.32, 121.30, 119.23, 110.18, 110.15, 103.04, 95.98, 56.59, 55.64, 45.53.

3-(Benzoylamino)methyl-6,8-dimethoxy-1,2-naphthoquinone (9). To a solution of compound **8** (285 mg, 0.84 mmol) in DMF (5 mL) was added iodoxybenzoic acid (IBX) (498 mg, 45 wt%, 0.84 mmol) at room temperature under an Ar atmosphere. After stirring for 1 h, the mixture was diluted with water (10 mL) and partitioned with EtOAc (20 mL × 3). The combined organic layers were washed with water (100 mL) and brine (100 mL), dried over Na_2SO_4, filtered, and evaporated *in vacuo* to give a dark brownish solid. The residue was subjected to flash column chromatography (silica gel) to afford 1,2-naphthoquinone **9** (260 mg, 88%) as a dark red solid: mp: 175–176°C; APCIMS *m/z* $[M + H]^+$ Calcd 352.1185 for $C_{20}H_{18}NO_5$: Found: 352.1186; IR 3337, 1674, 1651, 1594, 1327 cm^{-1}; UV λmax ($CHCl_3$) 268 (log ε = 4.45), 414 (log ε = 4.18); ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 7.78 (m, 2H), 7.45 (m, 1H), 7.37 (t, 2H, *J* = 7.0 Hz), 7.30 (s, 1H, H-4), 7.09 (t, 1H, –NH, *J* = 5.8 Hz), 6.40 (d, 1H, *J* = 2.0 Hz, H-7), 6.36 (d, 1H, *J* = 2.0 Hz, H-5), 4.33 (d, 2H, *J* = 5.8 Hz -CH_2-), 3.89 (s, 3H, –OCH_3), 3.86 (s, 3H, –OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 181.74, 175.47, 167.53, 166.56, 165.58, 143.12, 138.09, 136.06, 133.88, 131.65, 128.53, 127.05, 112.53, 110.09, 99.29, 56.27, 55.90, 38.74.

1,12-Bis((benzoylamino)methyl)-2,11-dihydroxy-4,6,7,9,-tetramethoxy-3,10-perylenequinone (10). Method A. A neat solution of 1,2-naphthoquinone **9** (80 mg, 0.23 mmol) in trifluoroacetic acid (1 mL) was stirred at 0°C for 2 h. The solution instantly turned dark green and became dark blue over 30 min. The reaction mixture was poured into ice-cold water and was extracted with chloroform (20 mL × 3). The combined organic layers were washed with water (30 mL × 3) and brine (50 mL), dried over Na_2SO_4, filtered, and evaporated *in vacuo* to afford a dark red solid. This solid was subjected to flash column chromatography (silica gel) to afford perylenequinone **10** (16 mg, 42%) as a dark red solid ($CHCl_3$:MeOH (9/1, v/v), R_f = 0.41). In addition to **10**, a mixture of two by-products was eluted, and the corresponding fractions were evaporated *in vacuo* to give a dark yellow solid (48 mg). This mixture was subjected again to careful flash column chromatography to afford compounds **13** (7 mg, R_f = 0.46, 5%) and binaphthoquinone **14** (4 mg, R_f = 0.48, 8%).

Compound **10**: mp: 171–172°C APCIMS *m/z* $[M + H]^+$ Calcd 701.2135 for $C_{40}H_{33}N_2O_{10}$: Found: 701.2138; IR 3467, 3304, 1594, 1464 cm-1; UV λmax ($CHCl_3$) 269 (log ε = 5.13), 506 (log ε = 4.90), 564 (log ε = 4.58); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.41 (s, 2H, –OH), 7.55 (d, 4H, *J* = 7.3 Hz, Hb-1,1′,5,5′), 7.37 (t, 2H, *J* = 7.3 Hz, Hb-3,3′), 7.28 (t, 4H, *J* = 7.3 Hz, Hb-2,2′,4,4′), 7.18 (t, 2H, –NH), 6.71 (s, 2H, Ha-5,5′), 5.0 (dd, 2H, *J* = 2.0 Hz, –CH_2–), 4.38 (dd, 2H, *J* = 5.9 Hz –CH_2–), 3.95 (s, 3H, –OCH_3), 3.90 (s, 3H, –OCH_3), ^{13}C NMR (100 MHz, CDCl3): δ 175.66, 166.75, 165.36, 164.87, 150.21, 134.28, 131.30, 130.74, 129.43, 128.40, 126.86, 118.88, 111.94, 106.27, 94.07, 56.62, 56.21, 39.90.

***N,N′*-(5′,6′-dihydroxy-2,2′,4,4′-tetramethoxy-5,6-dioxo-5,6-dihydro-1,1′-binaphthyl-7,7′-diyl)bis(methylene) dibenzamide (13).** mp: 158–159°C APCIMS *m/z* $[M + H]^+$ Calcd 703.2292 for $C_{40}H_{33}N_2O_{10}$: Found: 701.1942; UV λmax ($CHCl_3$) 266 (log ε = 4.57), 413 (log ε = 4.10); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.63 (d, 2H, *J* = 7.32 Hz), 7.47 (d, 2H, *J* = 7.8 Hz), 7.40 (m, 1H), 7.35 (m, 1H), 7.32 (s, 1H, H-8′), 7.28 (m, 2H), 7.21 (t, 2H, *J* = 7.8 Hz), 7.10 (m, 1H, *J* = 5.8 Hz, –NH), 4.19 (m, 4H, –CH_2–), 4.09 (s, 3H, –OCH_3), 3.89 (s, 3H, –OCH_3), 3.85 (s, 3H, –OCH_3), 3.75 (s, 3H, –OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 181.45, 180.46, 175.90, 175.15, 167.74, 166.49, 166.12, 165.83, 163.08, 147.32, 139.20, 138.11, 137.39, 136.52, 135.60, 133.90, 133.49, 131.67, 131.51, 128.50, 128.40, 126.89, 126.69, 117.34, 112.90, 112.79, 110.68, 98.16, 96.57, 56.52, 56.43, 56.36, 55.77, 38.54, 36.68.

***N,N′*-(2,2′,4,4′-tetramethoxy-5,5′,6,6′-tetraoxo-5,5′,6,6′-tetrahydro-1,1′-binaphthyl-7,7′-diyl)bis(methylene) dibenzamide (14).** mp: 180–181°C APCIMS *m/z* $[M + H]^+$ Calcd 701.2135 for $C_{40}H_{33}N_2O_{10}$: Found: 701.2132; UV λmax ($CHCl_3$) 261 (log ε = 4.56), 430 (log ε = 4.10); ^{1}H NMR (400 MHz, CDCl3): δ 7.54 (m, 6H, –NH, Hb-1,1′,5,5′), 7.39 (t, 2H, Hb-3,3′ , *J* = 7.8 Hz), 7.23 (t, 4H, Hb-2,2′4,4′, *J* = 7.8 Hz), 6.84 (s, 2H, Ha-8,8′), 6.39 (s, 2H, Ha –3,3'), 4.16 (m, 4H, –CH_2-), 3.90 (s, 6H, –OCH_3), 3.76 (s, 6H, –OCH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ179.92, 175.44, 167.97, 165.42, 164.71, 136.77, 136.32, 135.67, 133.47, 132.05, 128.63, 126.37, 116.95, 112,13, 96.09, 56.25, 56.19, 38.08. **Method B.** A solution of 1,2-naphthoquinone **8** (56 mg, 0.16 mmol) and anhydrous ferric chloride (65 mg, 0.40 mmol) in 2 mL of anhydrous acetonitrile was stirred at room temperature for 3 h. The reaction mixture was poured into 3% aqueous HCl, and then extracted with chloroform (50 mL × 3). The combined organic layers were washed with water (30 mL × 3) and brine (50 mL), dried over Na_2SO_4, filtered, and evaporated *in vacuo* to afford a dark red solid. This solid was subjected to flash column chromatography (silica gel) to afford perylenequinone **10** (8 mg, 14%).

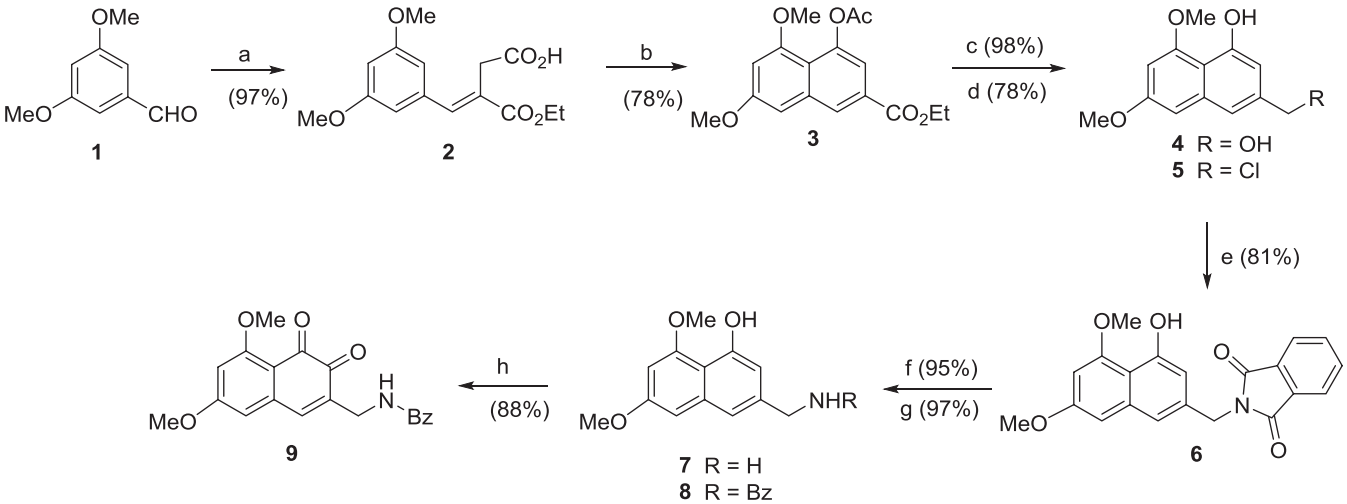
1,12-Bis((benzoylamino)methyl)-2,4,6,7,9,11-hexamethoxy-3,10-perylenequinone (11). To a solution of perylenequinone **10** (7 mg, 0.01 mmol) in THF (2 mL) was added tetrabutylammonium fluoride (TBAF; 30 μL, 0.03 mmol, 1.0 M in THF) followed by iodomethane (1 mL). After stirring at room temperature for 2 h, the mixture was diluted with $CHCl_3$ (20 mL) and washed with sat. $NaHCO_3$ (20 mL) and water, dried over Na_2SO_4, filtered, and evaporated *in vacuo* to afford a dark red solid. This solid was subjected to flash column chromatography (silica gel) to afford hexamethoxyperylenequinone **11** (6 mg, 83%) as a dark red solid: mp: 153–154°C; APCIMS *m/z* $[M + H]^+$ Calcd 729.2448 for $C_{42}H_{37}N_2O_{10}$: Found: 729.2450; IR 3440, 1606, 1461 cm-1; UV λmax (CHCl3) 266 (log ε = 3.90), 468 (log ε = 3.68); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.58 (d, 4H, *J* = 8.0 Hz, Hb-1,1',5,5'), 7.4 (t, 2H, *J* = 7.3 Hz, Hb-3,3'), 7.30 (t, 4H, *J* = 7.3 Hz, Hb-2,2',4,4'), 6.91 (t, 2H, –NH), 6.75(s, 2H,

Ha-5,5'), 4.79(d, 4H, $-CH_2-$, J = 5.8 Hz), 4.26 (s, 3H, $-OCH_3$), 4.14 (s, 3H, $-OCH_3$), 4.07 (s, 3H, $-OCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.08, 166.69, 164.26, 163.24, 154.75, 134.21, 131.27, 130.99, 130.13, 129.98, 128.38, 126.86, 111.35, 108.83, 94.95, 61.16, 56.52, 56.11, 39.84.

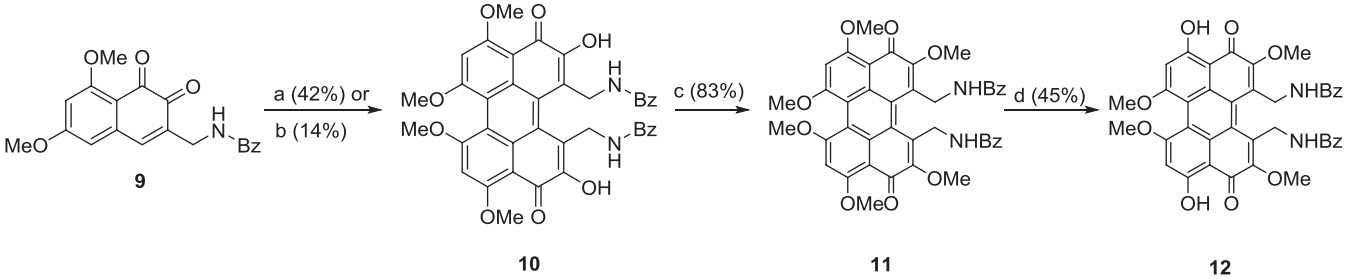
1,12-Bis((benzoylamino)methyl)-4,9-dihydroxy-2,6,7,11-tetramethoxy-3,10-perylenequinone (12). To a solution of perylenequinone **11** (21 mg, 0.03 mmol) in THF (2 mL) under an Ar atmosphere, MgI_2 in ether was added (16 mg, 0.058 mmol, 0.07 M in Et_2O). The solution instantly turned dark green. After confirming the reaction was complete by the disappearance of the starting material **13** by thin-layer chromatography, the mixture was diluted with EtOAc (25 mL) and washed with sat. NH_4Cl (20 mL) and water, dried over Na_2SO_4, filtered, and evaporated *in vacuo* to afford a dark red solid. This solid was subjected to flash column chromatography (silica gel) to afford perylenequinone **12** (9 mg, 45%) as a dark red solid: mp: 251–252°C APCIMS *m/z* $[M + H]^+$ Calcd 701.2135 for $C_{40}H_{33}N_2O_{10}$: Found: 701.2138; IR 3446, 1617, 1462 cm^{-1}; UV λmax ($CHCl_3$) 269 (log ε = 4.28), 470 (log ε = 4.00), 555 (log ε = 3.54); ^1H NMR (400 MHz, $CDCl_3$): δ 15.84 (s, 2H,-OH), 7.48 (m, 4H, Hb-1,1'5,5'), 7.33 (t, 2H, J = 7.3 Hz, Hb-3,3'), 7.24 (t, 4H, J = 7.3 Hz, Hb-2,2'4,4'), 6.90 (m, 2H, –NH), 6.36 (s, 2H, Ha-5,5'), 5.1 (dd, 2H, J = 7.3 Hz, $-CH_2-$), 4.8 (dd, 2H, J = 3.7;4.4 Hz $-CH_2-$), 4.30 (s, 3H, $-OCH_3$), 3.94 (s, 3H, $-OCH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 183.38, 167.81, 167.34, 166.74, 151.46, 134.32, 133.85, 131.51, 128.44, 126.84, 125.32, 124.06, 120.19, 107.68, 102.59, 61.79, 56.45, 40.00.

Results and Discussion

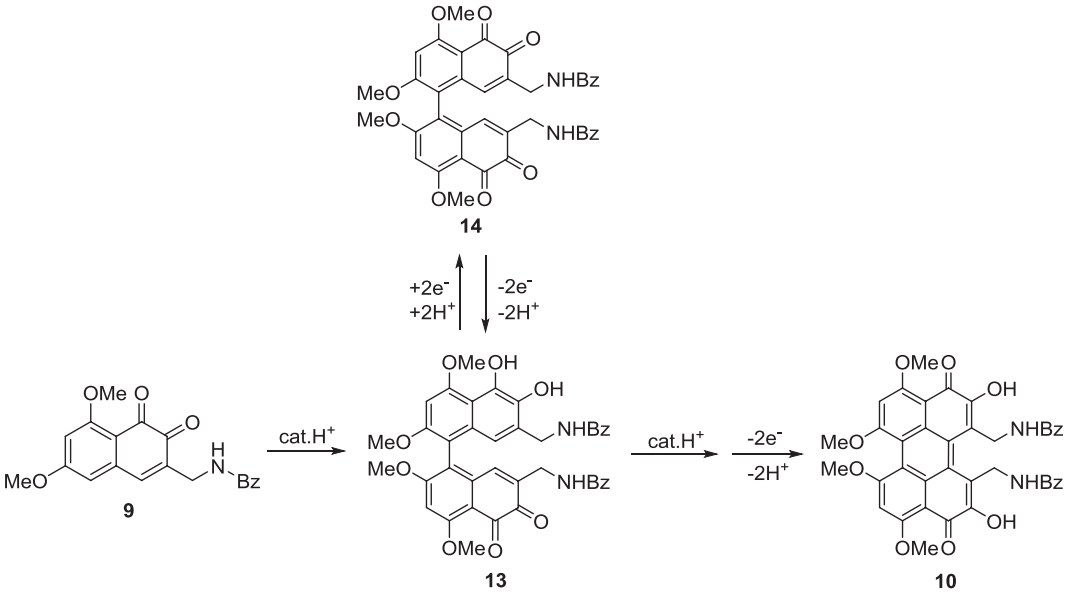
Our initial work focusing on the construction of amino-functionalized 1,2-naphthoquinone (**9**) is shown Scheme 2. The synthesis began by condensing 3,5-dimethoxybenzaldehyde (**1**) with diethyl succinate to produce butenoic acid **2**. Compound **2**, on boiling with anhydrous sodium acetate in acetic anhydride for a short time (1 h), gave the annulated product naphthoate **3** in acceptable yield (78%).[9] The ester group of naphthoate **3** was sequentially converted to alcohol **4** - (LAH/THF, 98%)[10] and subsequently to chloride **5** (CCl_4/PPH_3, 78%).[11] Conversion of chloride **5** to aminomethylene compound **7** via compound **6** was accomplished via Gabriel reaction[12] through two steps (potassium phthalimide, and then N_2H_4/H_2O) with 77% overall yield. Aminomethylene compound **7** could be a versatile platform compound to introduce diverse amino substituents to the 1- and 12-positions of the perylenequinone core (**10**) by *N*-alkylation or *N*-acylation, leading to structurally diverse amino-functionalized calphostin derivatives for investigation of structure–activity relationships. In the next step, amino-functionalized 1,2-naphthoquinone (**9**), to which an appropriate amino functionality could be introduced, was prepared in two steps. The first step involved *N*-benzoylation (BzCl, K_2CO_3) of compound **7** to afford compound **8** in excellent yield (97%). A benzoyl functionality at the amino group of compound **7** was preferentially considered because calphostins A–C have *O*-benzoyl group(s) at the sidechains of the 1- and 12-positions of the perylenequinone, as shown in the calphostin structures (Figure 1). The next step, namely oxidation of compound **8** to 1,2-naphthoquinone **9**, proceeded smoothly with a good yield (88%) through the agency of IBX in THF. IBX, a hypervalent iodine compound, is a promising reagent for regiocontrolled oxidation of polycyclic aromatic phenols to their corresponding aromatic quinones.[13] Application of IBX in this oxidation step considerably improved the yield, which was not achieved in our previous efforts to prepare 1,2-naphthoquinone derivatives using other oxidation conditions.[14]



Scheme 2. The synthetic route to 3-(benzoylamino)methyl-1,2-naphthoquinone (**9**). (a) diethyl succinate, NaH; toluene, 0°C → rt; (b) Ac_2O, AcONa, 140°C; (c) LAH, THF, rt; (d) CCl_4, PPH_3, THF, reflux; (e) Potassium phthalimide, DMF, 50°C; (f) N_2H_4/H_2O, EtOH, reflux; (g) Benzoyl chloride, K_2CO_3, CH_3CN, rt; (h) IBX, DMF, rt.



Scheme 3. Preparation of target compound, namely the naturally occurring form of amino-functionalized perylenequinone (**12**), from 1,2-naphthoquinone (**9**). (a) TFA, 0°C; (b) 10% $FeCl_3$, CH_3CN, rt; (c) TBAF, THF, MeI, rt; (d) MgI_2, THF, rt.



Scheme 4. Dimerization mechanism proposed by Merlic *et al.*[7]

With key intermediate 3-(benzoylamino)methyl-1,2-naphthoquinone (**9**) in hand, we continued the preparation of the perylenequinone skeleton, (1,12-bis((benzoylamino) methyl)-2,11-dihydroxy-4,6,7,9,-tetramethoxy-3,10-perylenequinone (**10**). In our previous work on dimerization of 1,2-naphthoquinone to construct the perylenequinone skeleton, several reaction conditions were investigated.[14a] Thus, we preferentially applied two types of reaction conditions, namely acid-catalysis and oxidative dimerization, to construct perylenequinone **10** (Scheme 3). Under the acid-catalyzed reaction conditions (trifluoroacetic acid/0°C),[8g] the desired perylenequinone **10** (mp 171–172°C) was obtained as a dark red solid in modest yield (48%). The structure of compound **10** was elucidated by analysis of ^1H and ^{13}C NMR, high-resolution mass spectrum, as well as UV and IR spectra. Three broad absorption bands at 269, 506, and 564 nm in the UV spectrum were reported to be typical for the quinone system.[15] Proton signals for the –OH (δ 8.41, broad singlet, 2H) and –NHBz (δ 7.18, triplet, 2H) in the ^1H NMR spectrum of compound **10** provide additional convincing evidence of the structure. While oxidative dimerization conditions (10% $FeCl_3$, CH_3CN)[7,8d,k,14a] generated the desired product perylenequinone **10**, these conditions were not satisfactory because of the low (<34%) and large fluctuations in yield.

The final naturally occurring form of perylenequinone **12** was prepared by consecutive methylation and demethylation reactions (Scheme 3). Thus, the hydroxyl groups at C-2 and C-11 of compound **10** were methylated by treatment with MeI and TBAF with good yield (83%). Finally, selective demethylation of the C4, C9-methyl ethers of compound **11** by treatment with MgI_2[7,8k] resulted in perylenequinone **12** with modest yield (45%).

The dimerization of 1,2-naphthoquinone **9** to 3,10-perylenequinone **10** deserves additional discussion. In this reaction, along with the dimerized product perylenequinone **10**, two other compounds were isolated as reddish solids in small amounts (Scheme 4). Their chemical structures were determined to be **13** and binaphthoquinone **14** on the basis of their spectroscopic data, which showed that these compounds were not perylenequinones but were evidently not ring-closed structures either. In several previous reports on the dimerization of 1,2-naphthoquinone under acid-catalyzed conditions,[7,8d,g] coupled but not ring-closed

intermediates were isolated, which through subsequent acid-catalyzed reaction could be closed intramolecularly to give a perylenequinone skeleton (Scheme 4). Compounds **13** and **14** identified in our experiment were shown to exactly correspond to the reported intermediates.

Conclusion

A novel amino-functionalized perylenequinone derivative, 1,12-bis((benzoyl-amino)methyl)-3,10-perylenequinone (**12**), was successfully prepared by dimerization of the key intermediate, 3-(benzoylamino)methyl-1,2-naphthoquinone (**9**), which was synthesized by an efficient and relatively short synthetic route (eight steps) with satisfactory overall yield. The naturally occurring form of perylenequinone **12** was prepared by consecutive methylation and demethylation reactions. In our synthetic strategy, it was very beneficial that the amino functionality of 1,2-naphthoquinone **9** was easily introduced in the early synthetic stages, and consequently dimerized to prepare various potentially bioactive perylenequinones as potential PKC inhibitors and photodynamic agents. However, more elaborate experimental design for the dimerization of naphthoquinone **9** should be considered to overcome the considerably low yield in this step. The inhibitory activities of three synthetic perylenequinones **10**, **11**, and **12** are currently under investigation.

Acknowledgment

This work was carried out with the support of the "Cooperative Research Program for Agricultural Science & Technology Development (Project No. PJ009998022016)," Rural Development Administration, Republic of Korea.

References

1. (a) E. Kobayashi, K. Ando, H. Nakano, T. Tamaoki, *J. Antibiot.* **1989**, *42*, 153; (b) E. Kobayashi, K. Ando, H. Nakano, T. Iida, H. Ohno, M. Morimoto, T. Tamaoki, *J. Antibiot.* **1989**, *42*, 1470; (c) T. Iida, E. Kobayashi, M. Yoshida, H. Sano, *J. Antibiot.* **1989**, *42*, 1475.
2. (a) Y. Nishizuka, *Nature* **1984**, *308*, 693; (b) Y. Nishizuka, *Science* **1986**, *233*, 305; (c) A. B. da Rocha, D. R. A. Mans, A. Regner, G. Schwartsmann, *Oncologist* **2002**, *7*, 17; (d) A. M. Gonzalez-Guerrico, J. Meshki, L. Xiao, F. Benavides, C. J. Conti, M. G. Kazanietz, *J. Biochem. Mol. Biol.* **2005**, *38*, 639; (e) E. M. Griner, M. G. Kazanietz, *Nat. Rev. Cancer* **2007**, *7*, 281; (f) H. J. Mackay, C. Twelves, *J. Nat. Rev. Cancer* **2007**, *7*, 554.
3. (a) E. Kobayashi, H. Nakano, M. Morimoto, T. Tamaoki, *Biochem. Biophys. Res. Commun.* **1989**, *159*, 548; (b) R. F. Bruns, F. D. Miller, R. L. Merriman, J. J. Howbert, W. F. Heath, E. Kobayashi, I. Takahashi, T. Tamaoki, H. Nakano, *Biochem. Biophys. Res. Commun.* **1991**, *176*, 288; (c) Z. Diwu, J. Zimmermann, T. Meyer, J. W. Lown, *Biochem. Pharmacol.* **1994**, *47*, 373; (d) V. A. Sciorra, S. M. Hammond, A. Morris, *J. Biochem.* **2001**, *40*, 2640.
4. (a) Y. Nishizuka, *Cancer* **1989**, *63*, 1892; (b) I. F. Pollack, S. Kawecki, *J. Neuro-Oncol.* **1997**, *31*, 255; (c) Z. Dubauskas, T. P. Beck, S. J. Chmura, D. A. Kovar, M. M. Kadkhodaian, M. Shrivastav, T. Chung, W. M. Stadler, C. W. Rinker-Schaefer, *Clin. Cancer Res.* **1998**, *4*, 2391; (d) D.-M. Zhu, R.-K. Narla, W.-H. Fang, N.-C. Chia, F. M. Uckun, *Clin. Cancer Res.* **1998**, *4*, 2967; (e) K. Hinterding, D. Alongso-Diaz, H. Waldmann, *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 689; (f) T. P. Beck, E. J. Kirsh, S. J. Chmura, D. A. Kovar, T. Chung, C. W. Rinker-Schaefer, W. M. Stadler, *Urology* **1999**, *54*, 573; (g) B. J. Morgan, S. Dey, S. W. Johnson, M. C. Kozlowski, *J. Am. Chem. Soc.* **2009**, *131*, 9413.
5. J. W. Lown, *Can. J. Chem.* **1997**, *75*, 99.
6. (a) Z. J. Diwu, *Photochem. Photobiol.* **1993**, *61*, 131; (b) T. Wu, S. Xu, J. Q. Shen, A. M. Song, S. Chen, M. Zhang, T. Shen, *Anticancer Drug Des.* **2000**, *15*, 287; (c) T. Wu, J. Q. Shen, A. M. Song, S. Chen, M. H. Zhang, T. Shen, *J. Photochem. Photobiol. B Biol.* **2000**, *57*, 14; (d) S. Xu, S. Chen, M. Zhang, T. Shen, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2045; (e) S. Xu, S. Chen, M. Zhang, T. Shen, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1499.
7. C. A. Merlic, C. C. Aldrich, A. W. Jennifer, S. Alan, M. Jerome, *J. Org. Chem.* **2001**, *66*, 1297.
8. (a) C. Chao, P. Zhang, *Tetrahedron Lett.* **1988**, *29*, 225; (b) R. S. Coleman, E. B. Grant, *J. Org. Chem.* **1991**, *56*, 1357; (c) C. A. Broka, *Tetrahedron Lett.* **1991**, *32*, 859; (d) Z. Diwu, J. W. Lown, *Tetrahedron* **1992**, *48*, 45; (e) R. S. Coleman, E. B. Grant, *Tetrahedron Lett.* **1993**, *34*, 2225; (f) C. Zhao, X.-S. Zhang, P. Zhang, *Liebigs Ann. Chem.* **1993**, 35–41; (g) F. M. Hauser, D. Sengupta, S. A. Corlett, *J. Org. Chem.* **1994**, *59*, 1967; (h) R. S. Coleman, E. B. Grant, *J. Am. Chem. Soc.* **1994**, *116*, 8795; (i) R. S. Coleman, E. B. Grant, *J. Am. Chem. Soc.* **1995**, *117*, 10889; (j) J. Liu, Z. Diwu, J. W. Lown, *Synthesis* **1995**, 914; (k) C. A. Merlic, C. C. Aldrich, J. Albaneze-Walker, A. Saghatelian, *J. Am. Chem. Soc.* **2000**, *122*, 3224.
9. (a) D. W. Cameron, G. I. Feutrill, L. J. H. Pannan, *Aust. J. Chem.* **1980**, *33*, 2531; (b) M. A. Rizzacasa, M. V. Sargent, *Aust. J. Chem.* **1987**, *40*, 1737.
10. R. G. F. Giles, I. R. Green, L. S. Knight, V. R. L. Son, P. R. K. Mitchell, S. C. Yorke, *J. Chem. Soc. Perkin Trans.* **1994**, *1*, 853.
11. T. Obata, T. Shimo, M. Yasutake, T. Shinmyozu, M. Kawaminami, R. Yoshida, K. Somekawa, *Tetrahedron* **2001**, *57*, 1531.
12. (a) W. G. Nigh, *J. Chem. Educ.* **1975**, *52*, 670; (b) U. Ragnarsson, L. Grehn, *Acc. Chem. Res.* **1991**, *24*, 285.
13. A. Wu, Y. Duan, D. Xu, T. M. Penning, R. G. Harvey, *Tetrahedron* **2010**, *66*, 2111.
14. (a) B.-T. Kim, H.-S. Kim, W. S. Moon, K.-J. Hwang, *Tetrahedron* **2009**, *65*, 4625; (b) K.-J. Hwang, Y.-M. Shin, D.-H. Kim, B.-T. Kim, *Bull. Korean Chem. Soc.* **2012**, *33*, 3095.
15. T. Yoshihara, T. Shimanuki, T. Araki, S. Sakamura, *Agric. Biol. Chem.* **1975**, *39*, 1683.

