JOC*Note*

Synthesis of a Novel Dicyano Abietane Analogue: A Potential Antiinflammatory Agent

Tadashi Honda,* Hidenori Yoshizawa,†
Chitra Sundararajan, and Gordon W. Gribble*

Department of Chemistry, Dartmouth College, 6128 Burke Laboratory, Hanover, New Hampshire 03755
th9@dartmouth.edu; ggribble@dartmouth.edu

Received January 10, 2006

From a structure−activity relationship perspective, the new abietane **5** having cyano groups at C-2 and C-13 and a phenolic ring C has been synthesized and evaluated biologically because the related compound **4** has high potency in inflammation models in vitro and in vivo. Compound **5** was synthesized from **8**, which was obtained in five steps from the known compound **9**, via an unexpected aromatization caused by the addition of PhSeCl and subsequent oxidation/elimination of the selenated intermediate **14** with H_2O_2.

The abietane-type diterpenoids with 20 carbon atoms are believed to result from the pyrophosphate ester of geranylgeraniol functioning as a leaving group in a complete cyclization sequence.[1] A large number of abietane-type diterpenoids having phenol [e.g., 1,2-dehydrohinokione (**1**)[2]] and quinone methide [e.g., taxodione (**2**)[3] and taxodone (**3**)[3]] structures in ring C have been reported.[1] Among them, **2** and **3**, isolated from *Taxodiaceae* species, exhibit antitumor potency.[3]

1,2-dehydrohinokione (**1**) taxodione (**2**): R = O taxodone (**3**): R = α-OH, β-H (−)-**4**

We have previously shown that tricyclic compound (±)-**4** with cyano enone functionalites in rings A and C is a novel and highly active inhibitor of nitric oxide (NO) production in mouse macrophages.[4] Compound (±)-**4** is also orally active in a preliminary in vivo inflammation model.[4] In addition, we have found that (+)-**4** having the opposite configuration to those of naturally occurring diterpenoids **1**−**3** shows 10 times higher inhibitory activity than (−)-**4** on NO production in mouse macrophages. On the contrary, (−)-**4** is active against MCF-7 mouse breast cancer cell lines, while (+)-**4** is inactive.[5]

Thus, from the perspective of structure−activity relationships, we envisioned the synthesis of a new abietane analogue **5**, which has two cyano groups at the C-2 and C-13 positions[6] and a phenolic ring C. Analogue **5** is similar in structure to that of the naturally occurring abietane, 1,2-dehydrohinokione (**1**). Analogue **5** differs from **4** in that **5** has only one Michael acceptor whereas **4** has three. Side effects and/or toxicity, which might be caused by Michael acceptors, may be reduced.

We herein describe the synthesis of compound **5** via an unexpected aromatization caused by the addition of phenylselenyl chloride (PhSeCl) and subsequent oxidation/elimination of the selenated intermediate with H_2O_2.[7] Although various syntheses of the abietane skeletons having a phenolic ring C have been reported,[1b,c] the construction of the skeleton by our synthetic sequence including this novel aromatization represents a new route to abietanes. We also briefly report the inhibitory activity of **5** against NO production in RAW cells.

Initially, we intended to synthesize **5** from acid **6** by decarboxylation at the C-8 position, but due to unsuccessful conversion of the corresponding methyl ester[8] to **6** under various conditions,[9] we were not able to obtain **6**. However, when we attempted to synthesize the new tricyclic bis-enone **7** with a methoxymethyl group at C-8 from **8** by addition with PhSeCl, followed by oxidation/elimination of the selenated intermediate with H_2O_2, the desired compound **5** was unexpectedly obtained from **8** in moderate yield. This transformation and the synthesis of **8** are shown in Scheme 1.

The direct synthesis of methyl ether **12** from keto alcohol **9**, whose synthesis[10] we explored for our projected synthesis of C-8 functionalized tricyclic bis-enone compounds, with CH_3I in the presence of NaH in DMF was unsuccessful because **12** was only obtained in low yield along with several byproducts, one of which is **12** with a C-2 methyl group. Therefore, we considered that protection of the C-3 carbonyl group of **9** is necessary for the synthesis of **12** in good yield. Ketal **10** was synthesized in 99% yield from **9** with ethylene glycol in the

* To whom correspondence should be addressed. (T.H.) Phone: 603-646-1591. Fax: 603-646-3946. (G.W.G.) Phone: 603-646-3118. Fax: 603-646-3946.
† Visiting scholar from Shionogi & Co., Ltd.

(1) (a) Devon, T. K.; Scott, A. I. *Handbook of Naturally Occurring Compounds*; Academic Press: New York, 1972; Vol. 2, pp 218−222. (b) Nakanishi, K. *Natural Products Chemistry*; Nakanishi, K., Goto, T., Ito, S., Natori, S., Nozoe, S. Eds.; Kodansha: Tokyo, 1974; Vol. 1, pp 185−312. (c) Goldsmith, D. *The Total Synthesis of Natural Products*; ApSimon, J., Ed.; John Wiley & Sons: New York, 1992; Vol. 8, pp 2−100.

(2) Chow, Y.-L.; Erdtman, H. *Acta Chem. Scand.* **1962**, *16*, 1296−1300.

(3) (a) Kupchan, S. M.; Karim, A.; Marks, C. *J. Am. Chem. Soc.* **1968**, *90*, 5923−5924. (b) Kupchan, S. M.; Karim, A.; Marks, C. *J. Org. Chem.* **1969**, *34*, 3912−3918.

(4) Favaloro, F. G., Jr.; Honda, T.; Honda, Y.; Gribble, G. W.; Suh, N.; Risingsong, R.; Sporn, M. B. *J. Med. Chem.* **2002**, *45*, 4801−4805.

(5) Honda, T.; Favaloro, F. G., Jr.; Janosik, T.; Honda, Y.; Suh, N.; Sporn, M. B.; Gribble, G. W. *Org. Biomol. Chem.* **2003**, *1*, 4384−4391.

(6) Throughout this paper except for the Experimental Section, for the ease of comparison, the same atom numbering as used with the abietane skeleton is used for our compounds.

(7) Liotta, D.; Barnum, C.; Puleo, R.; Zima, G.; Bayer, C.; Kezar, H. S., III. *J. Org. Chem.* **1981**, *46*, 2920−2923.

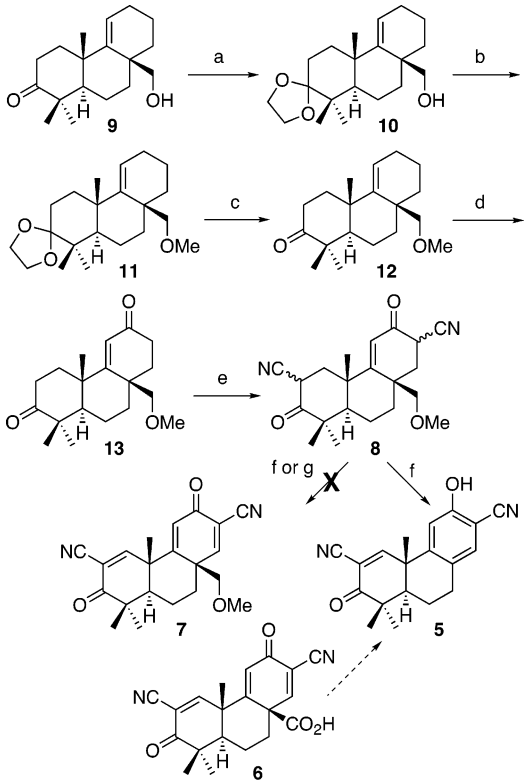
(8) Synthesis of the methyl ester of **6** will be published elsewhere.

(9) These conditions each gave a complex mixture: KOH in aqueous MeOH (reflux, overnight), LiI in DMF (reflux, 30 min), and $AlBr_3$ in Me_2S (rt, overnight). With $KOSiMe_3$ in THF (rt, overnight), the methyl ester was recovered.

(10) Honda, T.; Honda, Y.; Yoshizawa, H.; Gribble, G. W. *Org. Prep. Proced. Int.* **2005**, *37*, 546−550.

10.1021/jo060059+ CCC: $33.50
Published on Web 03/15/2006

SCHEME 1[a]



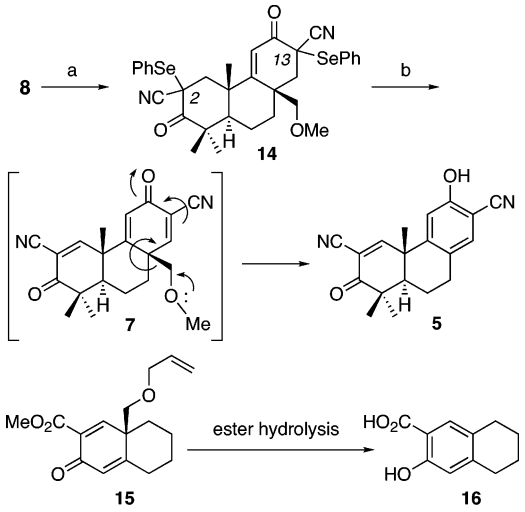
[a] Key: (a) ethylene glycol, PPTS, PhH, 99%; (b) CH_3I, NaH, DMF, 90%; (c) PPTS, aq acetone, 99%; (d) CrO_3, *t*-BuOOH, CH_2Cl_2, 77%; (e) *p*-TsCN, LDA, THF, 82%; (f) PhSeCl, pyr, CH_2Cl_2; 30% H_2O_2, 45%; (g) DDQ, 1,4-dioxane.

presence of pyridinium *p*-toluenesulfonate (PPTS) in benzene.[11] Methyl ether **11** was obtained in 90% yield from **10** using the same conditions as for converting **9** to **12**. Deketalization of **11** using PPTS in aqueous acetone[11] produced **12** in 99% yield. Thus, **12** was obtained from **9** in good overall yield (88%). A chromium-mediated allylic oxidation[12] of **12** with 70% *tert*-butyl hydroperoxide gave enone **13** in 77% yield. Double cyanation of the bis-enolate of **13** with *p*-toluenesulfonyl cyanide (*p*-TsCN) in THF[13] afforded dinitrile **8**[14] in 82% yield. The desired compound **5** was synthesized in 45% yield (overall yield from **9**, 25%) by treatment of **8** with PhSeCl in the presence of pyridine in CH_2Cl_2[7] and followed by treatment with 30% aqueous H_2O_2 solution.[7,15] The structure of compound **5** is fully characterized by ^1H and ^{13}C NMR, low- and high-resolution MS, UV, IR, and elemental analysis (see the Experimental Section).

For confirmation about which step causes aromatization of ring C, the addition step with PhSeCl, or the oxidation/elimination step of phenylselenyl group with H_2O_2, we attempted to isolate the selenated intermediate **14**. Treatment of **8** with PhSeCl gave the four possible isomers **14a**−**d** in 50% yield.[16] The major pair, **14a** and **14b** (38% yield), and the minor pair, **14c** and **14d** (12% yield), were separated by flash column chromatography. The isolated yields and ^1H NMR of each pair showed that the ratio of the four isomers (**14a**/**14b**/**14c**/**14d**) is

(11) Sterzycki, R. *Synthesis* **1979**, 724−725.
(12) Muzart J. *Tetrahedron Lett.* **1987**, *28*, 4665−4668.
(13) Kahne, D.; Collum, D. B. *Tetrahedron Lett*. **1981**, *22*, 5011−5014.

SCHEME 2[a]

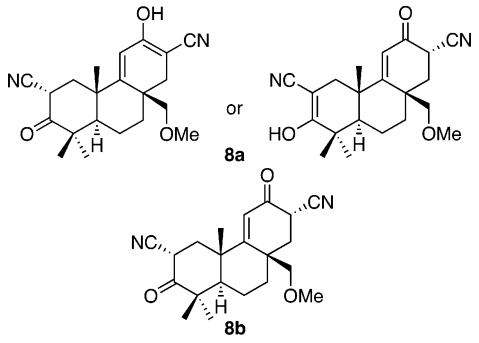


[a] Key: (a) PhSeCl, pyr, CH_2Cl_2, 50%; (b) 30% H_2O_2, 65%.

approximately 5:5:2:1. The oxidation/elimination of each pair with 30% aqueous H_2O_2 solution gave **5** in the same yield (65%). Therefore, we believe that ring C is simultaneously aromatized following oxidation/elimination of the C-2 and C-13 phenylselenyl groups, and the resulting unstable dienone **7** fragments immediately by a vinylogous retro-aldol-type pathway. Possible mechanisms are shown in Scheme 2. Our literature survey disclosed that ester **15** having a hydroxymethyl group protected with an allyl group gives phenol **16** under various ester hydrolysis conditions.[17] However, our work seems to be the first example where aromatization is accomplished from a γ-methoxymethyl-α,β-unsaturated ketone by oxidation/elimination of the selenated intermediate with H_2O_2.

We have evaluated the inhibitory activity of abietane analogue **5** and hydrocortisone, which is clinically used as an antiinflammatory drug, on NO production in RAW 264.7 cells stimulated

(14) ^1H NMR ($CDCl_3$) showed that dinitrile **8** exists as an inseparable mixture of two tautomers **8a** and **8b** at a 2:1 ratio in $CDCl_3$. Because a hydroxyl group and an α-proton of cyano group of **8a** were observed at δ 6.50 as a broad singlet and 4.21 as a double doublet (J = 14.3 and 4.76 Hz), respectively, although we concluded that **8a** has one of two possible structures as shown below, we could not determine which structure corresponds to **8a**. Because the two α-protons of the two cyano groups of **8b** were observed at δ 4.26 (J = 15.0 and 4.39 Hz) and 4.06 (J = 13.9 and 5.13 Hz) as double doublets, respectively, we concluded that **8b** has the structure as shown below with equatorial cyano groups.



(15) DDQ oxidation of **8** did not give **5** and **7** but a complex mixture product.
(16) The structure of each isomer cannot be assigned. An analytically pure sample of **14** could not be obtained.
(17) Broka, C. A. *J. Org. Chem*. **1988**, *53*, 575−583.

with interferon-γ.[18] Interestingly and importantly, we have found that **5** ($IC_{50} = 0.12\ \mu M$) is two times more potent than hydrocortisone ($IC_{50} = 0.28\ \mu M$). Thus, the abietane analogue **5** represents a new class of drug candidates for inflammatory diseases.

Experimental Section

(±)-(4aβ,8aβ,10aα)-4,4a,7,8,8a,9,10,10a-Octahydro-8a-methoxymethyl-1,1,4a-trimethylphenanthrene-2,6(1*H*,3*H*)-dione (13). To a solution of **12** (320 mg, 1.10 mmol) in CH_2Cl_2 (5.4 mL) was added *t*-BuOOH (70% aqueous solution, 1.5 mL). To the mixture was added CrO_3 (131 mg, 1.31 mmol) under cooling in an ice−water bath. The mixture was stirred at rt for 70 min. The reaction mixture was diluted with CH_2Cl_2−Et_2O (1:2, 30 mL). It was washed with 5% aqueous NaOH solution (2 × 20 mL). The basic washings were extracted with CH_2Cl_2−Et_2O (1:2, 2 × 20 mL). After the combined organic layer was washed with 5% aqueous HCl solution (twice), it was worked up by the standard method to give a residue as a brown oil. The residue was purified by flash column chromatography [hexanes−ethyl acetate (1:1)] to give **13** as a crystalline solid (259 mg, 77%): mp 123−125 °C; ^{1}H NMR ($CDCl_3$) δ 5.95 (1H,s), 3.54 (1H, d, J = 9.15 Hz), 3.41 (1H, d, J = 9.15 Hz), 3.33 (3H, s) 2.59 (3H, m), 2.30 (1H, m), 2.11 (3H, m), 1.95−1.55 (6H, m),[19] 1.24, 1.11, 1.09 (each 3H, s); ^{13}C NMR ($CDCl_3$) δ 215.7, 201.5, 173.6, 125.4, 76.0, 59.2, 52.7, 47.9, 40.4, 40.0, 37.0, 36.7, 36.2, 34.3, 34.2, 26.2, 21.9, 21.8, 19.5; HRMS (ESI+) calcd for $C_{19}H_{28}O_3$ + H 305.2117, found 305.2131. Anal. Calcd for $C_{19}H_{28}O_3$: C, 74.96; H, 9.27. Found: C, 74.68; H, 9.24.

(±)-(4aβ,8aβ,10aα)-1,2,3,4,4a,6,7,8,8a,9,10,10a-Dodecahydro-8a-methoxymethyl-1,1,4a-trimethyl-2,6-dioxophenanthrene-3,7-dicarbonitrile (8). To a solution of **13** (139 mg, 0.46 mmol) in THF (5.1 mL) was added LDA (2 M in THF/heptane, 633 μL, 1.27 mmol) at −78 °C. The mixture was stirred at rt for 20 min. Then, it was cooled at −78 °C for 10 min. To the mixture was added a cloudy solution of *p*-TsCN (95%, 348 mg, 1.82 mmol) in THF (3.9 mL) at −78 °C. The mixture was stirred at the same temperature for 30 min. To the mixture was added saturated aqueous NH_4OH solution (2.7 mL). The mixture was allowed to reach rt. The aqueous layer was acidified with 10% aqueous HCl solution. The acidic aqueous solution was extracted with ethyl acetate (3 × 30 mL). The extract was worked up by the standard method to give an amorphous solid (197 mg). The solid was purified by flash column chromatography [hexanes−ethyl acetate (1:1.5)] to give **8** (133 mg, 82%) as an amorphous solid: ^{1}H NMR ($CDCl_3$) major tautomer **8a**, δ 6.50 (1H, brs), 6.02 (1H, s), 4.21 (1H, dd, J = 14.3 and 4.76 Hz), 3.60 (1H, dd, J = 9.52 and 1.29 Hz), 3.50 (1H, d, J = 9.52 Hz), 3.32 (3H, s), 2.40 (1H, ABq, J = 14.6 Hz), 2.34 (1H, ABq, J = 14.6 Hz), 1.21, 1.17, 1.15 (each 3H, s); minor tautomer **8b**, δ 6.00 (1H, s), 4.26 (1H, dd, J = 15.0 and 4.39 Hz), 4.06 (1H, dd, J = 13.9 and 5.13 Hz), 3.69 (1H, dd, J = 9.52 and 1.83 Hz), 3.49 (1H, d, J = 9.52 Hz), 3.33 (3H, s), 1.21, 1.20, 1.16 (each 3H, s); HRMS (ESI+) calcd for $C_{21}H_{26}N_2O_3$ + H 355.2022, found 355.2012. Anal. Calcd for $C_{21}H_{26}N_2O_3$: C, 71.16; H, 7.39; N, 7.90. Found: C, 71.29; H, 7.47; N, 7.89.

(±)-(4aβ,10aα)-1,2,4a,9,10,10a-Hexahydro-6-hydroxy-1,1,4a-trimethyl-2-oxophenanthrene-3,7-dicarbonitrile (5). To a solution of PhSeCl (155 mg, 0.81 mmol) in CH_2Cl_2 (3.6 mL) was added a solution of pyridine (68 mg, 0.86 mmol) in CH_2Cl_2 (0.9 mL) in an ice bath. The mixture was stirred in the same bath for 15 min. To the mixture was added a solution of **8** (70 mg, 0.20 mmol) in CH_2Cl_2 (1.5 mL) in an ice bath. The mixture was stirred in the same bath for 1 h. After the mixture was washed with 10% aqueous HCl solution (2 × 3.6 mL), 30% H_2O_2 (155 μL) was added in an ice bath. The mixture was stirred for 10 min in the same bath. Subsequently, 30% H_2O_2 (90 μL) was added, and the mixture was stirred for additional 10 min. The reaction mixture was worked up by the standard method to give a residue (64 mg). The residue was purified by flash column chromatography [hexanes−ethyl acetate (1:1)], followed by preparative TLC [hexanes−ethyl acetate (3:2)] to give **5** (27 mg, 45%) as an amorphous solid. The solid was treated with hexanes to give crystals: mp > 220 °C dec; UV (EtOH) λ_{max} (log ϵ) 213 (4.32), 240 (4.06), 311 (3.43) nm; IR (KBr) 3304, 2976, 2930, 2228, 1672, 1615, 1507, 1417, 1388, 1286 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 8.31 (1H, s), 7.31 (1H, s), 7.14 (1H, brs), 7.10 (1H, s), 2.94 (2H, m), 2.19 (1H, dd, J = 12.1 and 2.9 Hz), 1.96 (2H, m), 1.50, 1.30, 1.25 (each 3H, s); ^{13}C NMR (CD_3OD) δ 199.4, 169.7, 160.0, 150.3, 135.8, 128.8, 117.7, 116.2, 115.9, 113.0, 100.0, 47.8, 46.3, 43.2, 29.5, 27.7, 27.3, 21.8, 19.9; MS (ESI+) *m*/*z* 307 [M + H]$^+$; HRMS (ESI+) calcd for $C_{19}H_{18}N_2O_2$ + H 307.1447, found 307.1454. Anal. Calcd for $C_{19}H_{18}N_2O_2$: C, 74.49; H, 5.92; N, 9.14. Found: C, 74.38; H, 5.90; N, 9.20.

Isolation of Phenylselenyl Adducts 14 for Confirmation of Mechanism. To a solution of PhSeCl (86 mg, 0.44 mmol) in CH_2Cl_2 (2.0 mL) was added a solution of pyridine (38 mg, 0.48 mmol) in CH_2Cl_2 (0.5 mL) in an ice bath. The mixture was stirred in the same bath for 15 min. To the mixture was added a solution of **8** (39 mg, 0.11 mmol) in CH_2Cl_2 (0.9 mL) in an ice bath. The mixture was stirred in the same bath for 1 h. After the mixture was washed with 10% aqueous HCl solution (2 × 2 mL), it was worked up by the standard method to give a yellow residue (77 mg). The residue was purified by flash column chromatography [hexanes−ethyl acetate (3:2)] to give a mixture of two isomers **14a** and **14b** (27.6 mg, 38%) and a mixture of two isomers **14c** and **14d** (8.5 mg, 12%) as a yellow amorphous solid, respectively.[16] The ratio of four isomers (**14a**/**14b**/**14c**/**14d**) was approximately 5:5:2:1. A mixture of **14a** and **14b**: ^{1}H NMR ($CDCl_3$) **14a**, δ 6.17 (1H, s), 3.46 (1H, dd, J = 9.16 and 1.46 Hz), 3.37 (1H, d, J = 9.16 Hz), 3.27 (3H, s), 1.65, 1.22, 1.18 (each 3H, s); **14b**, δ 6.90 (1H, s), 3.27 (3H, s), 3.19 (1H, d, J = 14.8 Hz), 3.11 (1H, d, J = 14.8 Hz), 1.70, 1.29, 1.19 (each 3H, s); MS (ESI+) *m*/*z* 667 [M + H]$^+$; HRMS (ESI+) calcd for $C_{33}H_{34}N_2O_3Se_2$ + H 667.0978, found 667.0974. A mixture of **14c** and **14d**: ^{1}H NMR ($CDCl_3$) **14c**, δ 6.29 (1H, s), 3.54 (1H, dd, J = 9.15 and 1.47 Hz), 3.36 (1H, d, J = 9.15 Hz), 3.27 (3H, s), 1.45, 1.26, 1.17 (each 3H, s); **14d**, δ 7.00 (1H, s), 3.35 (3H, s), 3.26 (1H, d, J = 14.3 Hz), 3.09 (1H, d, J = 14.3 Hz), 1.47, 1.20, 1.18 (each 3H, s); MS (ESI+) *m*/*z* 667 [M + H]$^+$; HRMS (ESI+) calcd for *m*/*z* 667 [M + H]$^+$; HRMS (ESI+) calcd for $C_{33}H_{34}N_2O_3Se_2$ + H 667.0978, found 667.0975.

Conversion of a Mixture of 14a and 14b to 5. To a solution of **14a** and **14b** (13.6 mg, 0.02 mmol) in CH_2Cl_2 (0.6 mL) was added 30% H_2O_2 (16 μL) in an ice bath. The mixture was stirred in the same bath for 10 min. The reaction mixture was diluted with CH_2Cl_2. The solution was worked up by the standard method to give a residue (6.4 mg). The residue was purified by preparative TLC [hexanes−ethyl acetate (3:2)] to give **5** (4.1 mg, 65%) as an amorphous solid.

Conversion of a Mixture of 14c and 14d to 5. A mixture of **14c** and **14d** gave **5** (65%) as an amorphous solid according to the same procedure as for the mixture of **14a** and **14b**.

Acknowledgment. We thank Drs. Karen T. Liby and Michael B. Sporn (Dartmouth Medical School) for the evaluation of biological potency of compound **5**. This investigation was supported by funds from NIH Grant No. 5R03-CA105294.

Supporting Information Available: General experimental procedures, experimental procedures for **10**−**12**, UV, IR, and MS spectra for **8** and **13**, and ^{1}H and ^{13}C NMR spectra for **5** and **10**−**13**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO060059+

(18) Suh, N.; Honda, T.; Finlay, H. J.; Barchowsky, A.; Williams, C.; Benoit, N. E.; Xie, Q.-W.; Nathan, C.; Gribble, G. W.; Sporn, M. B. *Cancer Res.* **1998**, *58*, 717−723.

(19) Overlapped signals which cannot be assigned.