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Synthesis of 2,6-Aceanthrylenedione, a Cyclic Vinylog of Anthraquinone

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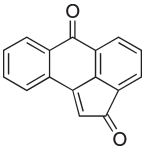
Received March 3, 2010



The first synthesis of 2,6-aceanthrylenedione (**6**), a cyclic vinylog of anthraquinone and a useful starting material for the synthesis of 1-phenylaceanthrylene-2,6-diones such as **7**, **8**, and **9**, is described. (10-Oxo-10*H*-anthracen-9-ylidene) acetyl chloride (**5**) cyclizes intramolecularly at room temperature in the presence of $AlCl_3$ to give **6**. We found that **6** is a cytotoxic compound that inhibits tubulin polymerization.

Anthracenediones constitute an important group of naturally occurring pigments that are widely distributed in nature. Substituted anthraquinones and polycyclic quinones have been reported for various uses, including cancer treatment.[1] Moreover, 9,10-anthraquinone (**1**, Figure 1) is the parent compound for a large palette of anthraquinone dyes and polycyclic quinones. Despite many attempts utilizing cyclodehydration of 1-substituted anthraquinones,[2] the 2,6-aceanthrylenedione (**6**), a cyclic vinylog of the anthraquinone, has never been prepared. Recently, we described a series of 10-(2-oxo-2-phenylethylidene)-10*H*-anthracen-9-ones as potent inhibitors of tubulin polymerization, with **2a** (Figure 1) being the most active analogue.[3] These compounds are characterized as possessing an enone moiety between the anthracenone and the terminal aromatic ring.[3] To obtain **2a** and related compounds the (10-oxo-10*H*-anthracen-9-ylidene) acetyl chloride[3] (**5**, Scheme 1) was synthesized from commercially available 10*H*-anthracen-9-one **4** via the intermediate carboxylic acid followed by reaction with suitable aromatic compounds in a Friedel—Crafts acylation reaction in the presence of 1 equiv of $AlCl_3$ at 0 °C.[3]

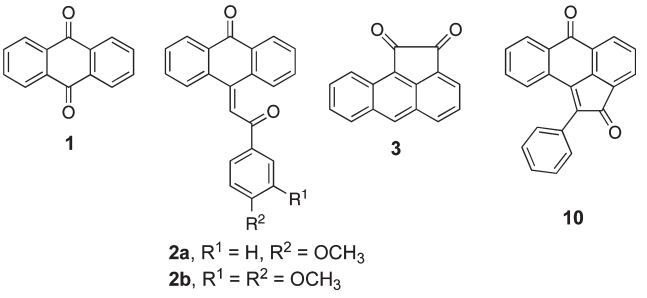


FIGURE 1. Anthraquinone (**1**), 10-(2-oxo-2-phenylethylidene)-10*H*-anthracen-9-ones **2a,b**, and 1,2-aceanthrylenedione (**3**).

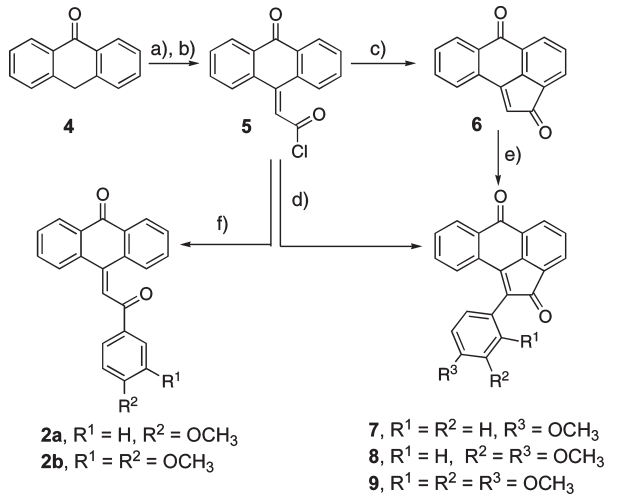
Now, we found that **5** can be readily cyclized at room temperature and in the presence of 2 equiv of $AlCl_3$ to afford 2,6-aceanthrylenedione **6** in working quantities (Scheme 1).

To our knowledge, this is the first synthesis of that particular cyclic vinylog of anthraquinone. Recently, Chunyan et al.[4] have reported the preparation of 1,2-aceanthrylenedione **3** through acylation of anthracene with oxalyl chloride catalyzed by anhydrous $AlCl_3$. It has to be pointed out that **6** was not formed during the synthesis of 10-(2-oxo-2-phenylethylidene)-10*H*-anthracen-9-ones represented by **2a** and **2b** at 0 °C. Stimulated by the synthesis of **6**, we studied the acylation reaction of reactive arenes such as anisole, veratrole, and 1,2,3-trimethoxybenzene by **5** at room temperature. Interestingly, when using **5** as a starting material in the presence of 3 equiv of $AlCl_3$, we isolated **7**, **8**, and **9** in each case as deep red products. Using veratrole as a starting material, we clearly assigned the product to the 1-(3,4-dimethoxyphenyl)aceanthrylene-2,6-dione (**8**), being deficient of one aromatic and one vinyl H atom in the proton NMR, as compared with the 10-[2-(3,4-dimethoxyphenyl)-2-oxoethylidene]-10*H*-anthracen-9-one[3] **2b**. In the same way way, we obtained **7** and **9** from the reaction of **6** with anisole and 1,2,3-trimethoxybenzene, respectively. In the light of these findings, we hypothesized that the formation of such phenylaceanthrylene-2,6-diones could possibly proceed via **6** as an intermediate step. Indeed, we found that **6** provides access to phenylaceanthrylene-2,6-diones **7**, **8**, and **9** after prolonged reaction times (24 h) in the presence of 3 equiv of $AlCl_3$, as documented by comparison with the authentic samples prepared by Friedel—Crafts acylation via **5**. Confirmation of the substitution pattern was based on ^{1}H NMR spectral analysis data. The crystal structure of 1-phenylaceanthrylene-2,6-dione (**10**, Figure 1) has recently been published,[5] with the aceanthrylene-2,6-dione unit being essentially planar. However, 1-phenylaceanthrylene-2,6-dione (**10**) cannot be obtained easily, utilizing a photoinduced reaction between anthraquinone and 1-trimethylsilyl-2-phenylacetylene.[5] Our

(1) Thomson, R. H. *Naturally Occurring Quinones*, 2nd ed.; Academic Press: New York, 1971.
(2) Klingsberg, E.; Lewis, C. E. *J. Org. Chem.* **1975**, *40*, 366.
(3) Prinz, H.; Schmidt, P.; Böhm, K. J.; Baasner, S.; Müller, K.; Unger, E.; Gerlach, M.; Günther, E. G. *J. Med. Chem.* **2009**, *52*, 1284.
(4) Chunyan, Z.; Min, C.; Yan, Z.; Xinhua, Y.; Hua, L. *Mater. Sci. Forum* **2007**, *561—565*, 683.
(5) Li, Z.; Fun, H.-K.; Chantrapromma, S.; Xu, J.-H. *Acta Crystallogr.* **2007**, *E63*, o2499.

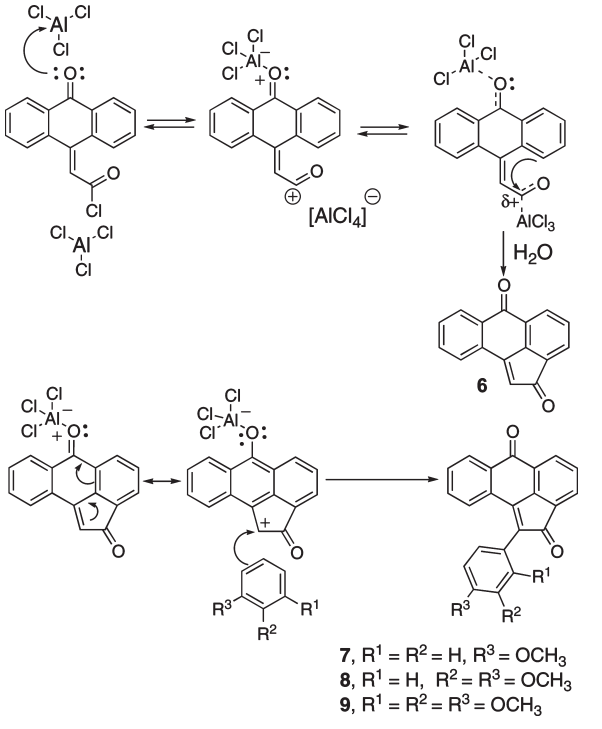
DOI: 10.1021/jo1003983 Published on Web 05/11/2010

SCHEME 1. Preparation of 2,6-Aceanthrylenedione (6) and Phenylaceanthrylene-2,6-diones 7, 8, and 9[a]



[a]Reagents and conditions: (a) OHC-COOH·H_2O, ethanol/piperidine, 90 °C, N_2; (b) $SOCl_2$, CH_2Cl_2, reflux, 2 h; (c) 1,2-DCE, 2 equiv $AlCl_3$, rt, 5 h; (d) methoxy-, 1,2-, or 1,2,3-trimethoxybenzene, 1,2-DCE, 3 equiv $AlCl_3$, rt, 4−5 h; (e) methoxy-, 1,2-dimethoxy-, or 1,2,3-trimethoxybenzene, 1,2-DCE, 3 equiv $AlCl_3$, rt, 24 h; (f) methoxy- or 1,2-dimethoxybenzene 1,2-DCE, 1 equiv $AlCl_3$, 0 °C, 4−5 h.

SCHEME 2. Proposed Reaction Mechanism of the Formation of Compounds 6, 7, 8, and 9



findings suggest that **6** is obviously able to alkylate the arene in the presence of $AlCl_3$. In a mechanistic view, the formation of **6**, **7**, **8**, and **9** could be explained as depicted in Scheme 2.

The most plausible explanation for the formation of **6** is a Friedel−Crafts intramolecular acylation reaction,[6,7] including

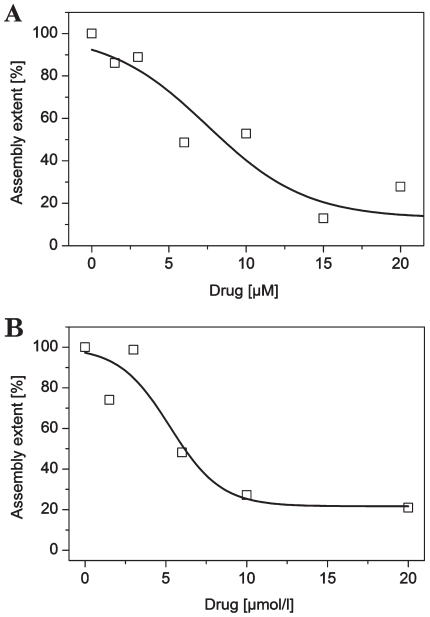


FIGURE 2. Inhibition of in vitro polymerization of tubulin (in total 1.2 mg/mL protein; ~80−90% tubulin plus ~10−20% microtubule-associated proteins) at 37 °C by various concentrations of **6**. The steady-state tubulin assembly level in the absence of inhibitor was set 100%. (A) IC_{50} value ($IC_{50} = 6.7 \pm 1.4\,\mu M$), representing the drug concentration for 50% inhibition, was determined by sigmoidal fitting of the plot of the steady-state levels of tubulin polymerization (taken at 45 min) in dependence on the drug concentration and represents the concentration for 50% inhibition of the maximum tubulin polymerization level. (B) IC_{50} value ($IC_{50} = 5.3 \pm 1.2\,\mu M$), in the presence of BSA (1.2 mg/mL).

the nucleophilic attack of the arene toward the acyl group, catalyzed by the Lewis acid $AlCl_3$. The formation of **7**, **8**, and **9** from **6** can possibly be explained through the alkylation of the arene by an intermediate carbocation in the presence of $AlCl_3$.

Because of the structural similarity of **6** to the antimitotic compounds recently described by us,[3,8] we evaluated **6** for antiproliferative activity against the human chronic myelogenous leukemia cell line K562, which is widely used for testing of potential antitumor compounds.

Cell proliferation was determined directly by counting the cells with a hemocytometer after 48 h of treatment. Compound **6** displayed strong antiproliferative activity with an IC_{50} value of 1.0 μM as compared to anthraquinone **1** (IC_{50} K562 > 100 μM). To investigate whether the antiproliferative activity of **6** was related to an interaction with tubulin, the compound was assayed for inhibition of tubulin polymerization (ITP). If an inhibitor binds to or interferes with tubulin, the assembly steady-state level is decreased, as demonstrated for **6** (Figure 2A). Our results showed that **6** is a moderate inhibitor of tubulin polymerization (ITP; $IC_{50} = 6.7 \pm 1.4\,\mu M$, Figure 2A), being 5-fold less active than the reference compound colchicine (ITP; $IC_{50} = 1.4\,\mu M$). Specific binding was confirmed by performing the tubulin polymerization assay in the presence of bovine serum albumin (BSA)[9] (Figure 2B), which gave slightly improved but comparable

(6) Holden, M. S.; Crouch, R. D.; Barker, K. A. *J. Chem. Educ.* **2005**, *82*, 934.
(7) Lan, K.; Fen, S.; Shan, Z. *Aust. J. Chem.* **2007**, *60*, 80.
(8) Prinz, H.; Ishii, Y.; Hirano, T.; Stoiber, T.; Camacho Gomez, J. A.; Schmidt, P.; Düssmann, H.; Burger, A. M.; Prehn, J. H.; Günther, E. G.; Unger, E.; Umezawa, K. *J. Med. Chem.* **2003**, *46*, 3382.

data (ITP; $IC_{50} = 5.3 \pm 1.2$ μM, Figure 1B). Therefore we conclude that inhibition of tubulin polymerization is a plausible explanation for the antiproliferative potency of **6**. Among the 1-phenylaceanthrylene-2,6-diones, only **9** also inhibited tubulin polymerization (**9**, ITP; $IC_{50} = 6.6 \pm 0.7$ μM; **7**, **8** ITP; $IC_{50} > 10$ μM) and had antiproliferative potencies comparable to **6** (**9**, IC_{50} K562 1.7 μM; **7**, **8**, IC_{50} K562 > 80 μM).

In conclusion we have presented the first protocol for the synthesis of 2,6-aceanthrylenedione, which was demonstrated to be a biologically active compound revealing a remarkably increased antiproliferative potency as compared with simple anthraquinone. This property is related to the inhibition of tubulin polymerization. In addition, we showed that the synthesis of 1-phenylaceanthrylene-2,6-diones could proceed via the 2,6-aceanthrylenedione. Given the importance of anthraquinones and related quinones for anticancer chemotherapy, we hope that the novel 2,6-aceanthrylenedione, which can be regarded as a cyclic vinylog of anthraquinone, might be of interest for the development of novel anticancer drugs. Further studies around this interesting compound are in progress and will be reported in due course.

Experimental Section

(10-Oxo-10*H*-anthracen-9-ylidene) Acetyl Chloride (5).[3] **2,6-Aceanthrylenedione (6).** Anhydrous $AlCl_3$ (2.15 g, 16 mmol) was added in one portion to a suspension of crude **5** (2.14 g, 8 mmol) in DCE (30 mL), and the solution was stirred at rt for 5 h. Then, the reaction mixture was poured into a mixture of water (150 mL)/6N HCl (50 mL), stirred for 10 min, and then extracted with CH_2Cl_2 (4 × 50 mL). The combined organic layers were washed with water, dried (Na_2SO_4), and concentrated. Thereafter, the residue was purified by silica gel chromatography (CH_2Cl_2) to afford **5** as an orange powder (0.79 g, 43%, not optimized): mp 225 °C; FTIR 1704, 1657; ^{1}H NMR ($CDCl_3$, 400 MHz, 300 K) δ 8.51−8.50 (m, 1H), 8.14 (dd, 1H, J=8.22 Hz, J=0.78 Hz), 8.06−8.04 (m, 1H), 7.81−7.73 (m, 3H), 7.61 (t, 1H, J=7.43 Hz), 6.59 (s, 1H); ^{13}CNMR ($CDCl_3$, 101 MHz) δ 195.9, 181.8, 149.6, 147.1, 133.4, 132.9, 132.7, 130.7, 130.3, 130.1, 129.1, 129.0, 127.6, 126.6, 126.1,121.2; MS m/z 232 (100). Anal. ($C_{16}H_8O_2$, 232.05): calcd C, 82.75; H, 3.47; found C, 82.42; H, 3.31.

1-(4-Methoxyphenyl)aceanthrylene-2,6-dione (7). Method A. The title compound was prepared from **5** (2.14 g, 8.00 mmol) and methoxybenzene (2.59 g, 24.00 mmol) in a similar manner as described for the preparation of **8**. Purification by silica gel chromatography (CH_2Cl_2) afforded **7** as a red-orange powder (0.38 g, 11%): mp 209 °C; FTIR 1708, 1661; ^{1}H NMR ($CDCl_3$, 400 MHz, 300 K) δ 8.46 (dd, 1H, J=7.83 Hz, J=1.56 Hz), 8.13 (dd, 1H, J = 7.83 Hz, J = 0.78 Hz), 8.09−8.08 (m, 1H), 7.76 (dd, 1H, J=7.04 Hz, J=0.78 Hz), 7.61−7.57 (m, 1H), 7.50−7.43 (m, 4H), 7.00 (d, 2H, J=9.0 Hz), 3.85 (s, 3H); ^{13}CNMR ($CDCl_3$, 101 MHz) δ 195.5, 181.8, 160.8, 147.8, 140.2, 135.9, 133.0, 132.8, 131.7, 131.66 (2 × C), 131.4, 129.7, 129.5, 129.05, 129.0, 127.8, 126.6, 126.0, 123.3, 114.6 (2 × C), 55.6; MS m/z 338 (100); high resolution ESIMS ($C_{23}H_{14}O_3$ + Na): calcd 361.08406, found 361.08368.

Method B. The title compound was prepared from **6** (0.23 g, 1 mmol), methoxybenzene (0.11 g, 1 mmol), and anhydrous $AlCl_3$ (0.40 g, 3 mmol) in DCE (15 mL) according to the preparation of **8**. Purification by silica gel chromatography (CH_2Cl_2) afforded **7** as a red-orange powder (74 mg, 22%).

(9) Morais, S.; ÒMalley, S.; Chen, W.; Mulchandani, A. *Anal. Biochem.* **2003**, *321*, 44.

1-(3,4-Dimethoxyphenyl)aceanthrylene-2,6-dione (8). Method A. Anhydrous $AlCl_3$ (3.20 g, 24.00 mmol) was added in one portion to a suspension of crude **5** (2.14 g, 8 mmol) in DCE (30 mL). Then, a solution of 1,2-dimethoxybenzene (3.22 g, 24 mmol) in 5 mL of DCE was added dropwise. After the dark red solution stirred for 4 h at rt (TLC control, CH_2Cl_2), the reaction mixture was poured into water (300 mL)/6 N HCl (50 mL), stirred for 10 min, and then extracted with CH_2Cl_2 (4 × 50 mL). The combined organic layers were washed with water, dried (Na_2SO_4), and concentrated. Purification by silica gel chromatography (CH_2Cl_2) afforded **8** as a red powder (0.45 g, 16%): mp 217 °C; FTIR 1711, 1657; ^{1}H NMR ($CDCl_3$, 400 MHz, 300 K) δ 8.54−8.52 (m, 1H), 8.21−8.19 (m, 1H), 8.18−8.16 (m, 1H), 7.84−7.82 (m, 1H), 7.69−7.65 (m, 1H), 7.58−7.52 (m, 2H), 7.19−7.16 (m, 1H), 7.07 (d, 1H, J = 1.57 Hz), 7.05 (d, 1H, J = 8.21 Hz), 3.99 (s, 3H), 3.88 (s, 3H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 195.4, 181.8, 150.4, 149.3, 147.8, 140.5, 136.0, 133.8, 131.4, 132.8, 131.8, 129.8, 129.6, 129.1, 129.0, 128.0, 126.6, 126.1, 123.6, 123.3, 112.9, 111.7, 56.3, 56.2; MS m/z 368 (100). Anal. ($C_{24}H_{16}O_4$, 368.1): calcd C, 78.25; H, 4.38; found C, 78.25; H, 4.16. Purity (HPLC): 97.51%.

Method B. Anhydrous $AlCl_3$ (0.40 g, 3 mmol) was added in one portion to a suspension of **6** (0.23 g, 1 mmol) and 1,2-dimethoxybenzene (0.14 g, 1 mmol) in DCE (15 mL). After the dark red solution stirred for 24 h at rt (TLC control, CH_2Cl_2), the reaction mixture was poured into water (100 mL)/6 N HCl (50 mL), stirred for 10 min, and then extracted with CH_2Cl_2 (4 × 50 mL). The combined organic layers were washed with water, dried (Na_2SO_4,) and concentrated. Purification by silica gel chromatography (CH_2Cl_2) afforded **8** as a red powder (97 mg, 26%).

1-(2,3,4-Trimethoxyphenyl)aceanthrylene-2,6-dione (9). Method A. The title compound was prepared from **5** (2.14 g, 8.00 mmol), 1,2,3-trimethoxybenzene (4.03 g, 24.00 mmol), and $AlCl_3$ (3.20 g, 24.00 mmol) in a similar manner as described for the preparation of **8** (method A). Purification by silica gel chromatography (CH_2Cl_2) afforded **9** as a red powder (0.44 g, 14%): mp 193 °C; FTIR 1711, 1658; ^{1}H NMR ($CDCl_3$, 400 MHz, 300 K) δ 8.54 (dd, 1H, J=8.02 Hz, J=1.17 Hz), 8.21 (dd, 1H, J=8.01 Hz, J=0.78 Hz), 7.86−7.83 (m, 2H), 7.70−7.66 (m, 1H), 7.60−7.53 (m, 2H), 7.05 (d, 1H, J=8.60 Hz, 6.84 (d, 1H, J=8.59 Hz), 3.99 (s, 3H), 3.97 (s, 3H), 3.79 (s, 3H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 195.3, 182.0, 155.3, 152.7, 147.8, 142.8, 142.1, 133.5, 133.0, 132.9, 131.7, 131.5, 129.8, 129.4, 129.3, 128.8, 128.4, 126.6, 126.1, 125.6, 118.1, 107.9, 61.7, 61.3, 56.4; MS m/z 398 (100). Anal. ($C_{25}H_{18}O_5$, 398.12): calcd C, 75.37; H, 4.55; found C, 75.30; H, 4.47. Purity (HPLC): 97.8%.

Method B. The title compound was prepared from **6** (0.23 g, 1 mmol), 1,2,3-trimethoxybenzene (0.17 g, 1 mmol), and anhydrous $AlCl_3$ (0.40 g, 3 mmol) in DCE (15 mL) in a similar manner as described for the preparation of **8**. Purification by silica gel chromatography (CH_2Cl_2) afforded **9** as a red powder (0.06 g, 15%): mp 192 °C.

Assay of Cell Growth. K562 cells were plated at 2×10^5 cells/mL in 24-well dishes (Costar, Cambridge, MA). Untreated control wells were assigned a value of 100%. Drugs were made soluble in DMSO/methanol 1:1, and control wells received equal volumes (0.5%) of vehicle alone. Drugs were dissolved in methanol/DMSO 1:1. To each well was added 5 μL of drug, and the final volume in the well was 500 μL. Cell numbers were counted with a Neubauer counting chamber (improved, double grid) after treatment with chemicals for 48 h. Each assay condition was prepared in triplicate, and the experiments were carried out three times. IC_{50} values were obtained by nonlinear regression (GraphPad Prism) and represent the concentration at which cell growth was inhibited by 50%. The adjusted cell number was calculated as a percentage of the control, which was the number of cells in wells without the addition of compound.

Isolation of MTP and in Vitro Microtubule Polymerization Assay. Microtubule protein (MTP) consisting of 80−90% tubulin and 10−20% microtubule associated proteins was isolated from porcine brain by two cycles of temperature-dependent disassembly (0 °C)/reassembly (37 °C) according to the method described by Shelanski et al.,[10] with modifications described by Vater et al.[11] Throughout the preparation, a buffer containing 20 mM PIPES (1,4-piperazine diethane sulfonic acid, pH 6.8), 80 mM NaCl, 0.5 mM $MgCl_2$, 1 mM EGTA (ethylene bis (oxyethylenenitrilo) tetraacetic acid), and 1 mM DTT was used. The protein concentration was determined by the Lowry procedure using bovine serum albumin as a standard. Antitubulin activity was assayed by recording the turbidity increase accompanying tubulin polymerization in glass cuvettes. To start tubulin polymerization, the stock MTP solutions were diluted with ice-cold preparation buffer to 1.2 mg/mL, GTP (guanosin-5′-triphosphate) was added to 0.6 mM (final concentration), and the samples were transferred into a spectrophotometer (Cary 4E, Varian Inc.) equipped with a temperature-controlled multichannel cuvette holder. Tubulin polymerization was initiated by shifting the temperature to 37 °C and turbidity was recorded over 45 min at 360 nm. The tubulin effectors were added from stock solutions in DMSO. The final DMSO concentration was 1%. Control measurements were made with DMSO only. To quantify drug activity, the turbidity signal after 45 min (plateau level, representing the assembly/disassembly steady state) was compared with that of the control samples. The IC_{50} value is defined as the drug concentration that causes a 50% inhibition in relation to the assembly level without the drug.

(10) Shelanski, M. L.; Gaskin, F.; Cantor, C. R. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 765.
(11) Vater, W.; Böhm, K. J.; Unger, E. *Acta Histochem. Suppl.* **1986**, *33*, 123.

Acknowledgment. We wish to thank Tim Schräder, Marcel Overmann, Angelika Zinner, and Marina Wollmann for excellent technical assistance.

Supporting Information Available: General experimental methods, copies of spectra, and compensation of cpd **6**-caused inhibition of tubulin polymerization by paclitaxel. This material is available free of charge via the Internet at http://pubs.acs.org.