Synthesis of NK109, an Anticancer Benzo[*c*]phenanthridine Alkaloid

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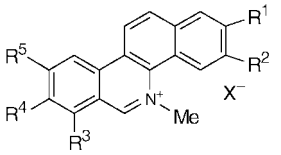
A total synthesis of NK109 (7-hydroxy-8-methoxy-5-methyl-2,3-methylenedioxybenzo[*c*]phenanthridinium hydrogensulfate dihydrate), an anticancer benzo[*c*]phenanthridine alkaloid, is reported. The primary structure of this compound was erroneously communicated in 1973 as fagaridine (from *Fagara xanthoxyloides*) which is the 8-hydroxy regioisomer. NK109 has not yet been isolated from a natural source and therefore can only be obtained by synthesis. To study a wide variety of analogues, we decided to use a synthetic route via substituted benzylamine **5**, which was obtained from the appropriate benzaldehyde and naphthylamine units. The benzo[*c*]phenanthridine ring was constructed by radical cyclization with tri-*n*-octyltin hydride and 2,2′-azobis(2-methylbutyronitrile), followed by oxidative aromatization with MnO_2. The resulting benzo[*c*]phenanthridine **6** was successfully methylated with methyl 2-nitrobenzenesulfonate. After deprotection of the benzyl group and subsequent hydration, NK109 was obtained. All reactions were performed under normal conditions. Purification was achieved only by recrystallization to give an overall yield of 40%.

Introduction

Benzo[*c*]phenanthridines are naturally occurring alkaloids with various biological activities.[1] In particular, benzo[*c*]phenanthridinium salts such as nitidine, fagaronine, sanguinarine, and chelerythrine display antitumor properties.[2] These compounds were isolated from Rutaceous and Papaveraceous plants. Nitidine and fagaronine were expected to be useful antitumor drugs because of their strong activity against several tumor cells. Preclinical studies of these compounds were performed in detail at the National Cancer Institute in the mid 1970s.[3] However, they showed that these compounds possessed a narrow antitumor spectrum as well as a certain toxicity and instability. Sanguinarine and chelerythrine exhibited cytotoxicity in vitro but had no significant antitumor activity in vivo.[4] Various analogues of these compounds have been synthesized, but none have had greater antitumor activity than the respective natural compounds.[5] Therefore, these compounds have not been the subject of a clinical evaluation. Nevertheless, there have still been many studies in this area and several authors have reported interesting chemical findings and biological effects.[6]



Nitidine	$R^1 + R^2 = OCH_2O$; $R^3 = H$; $R^4 = R^5 = OCH_3$
Fagaronine	$R^1 = OH$; $R^2 = OCH_3$; $R^3 = H$; $R^4 = R^5 = OCH_3$
Sanguinarine	$R^1 + R^2 = R^3 + R^4 = OCH_2O$; $R^5 = H$
Chelerythrine	$R^1 + R^2 = OCH_2O$; $R^3 = R^4 = OCH_3$; $R^5 = H$

Since 1987, we have sought a new benzo[*c*]phenanthridinium salt with anticancer activity. At an early stage, 18 compounds were kindly supplied by Prof. Hanaoka, Kanazawa University, Japan; these were synthesized by the transformation of protoberberine alkaloid.[7] After biological screening of the samples, we found that compound **1a** had significant antitumor activity.[8] Therefore, we developed a synthetic method more practical than the protoberberine transformation. During this process, we discovered that **1b** (7-hydroxy-8-methoxy-5-methyl-2,3-methylenedioxybenzo[*c*]phenan-

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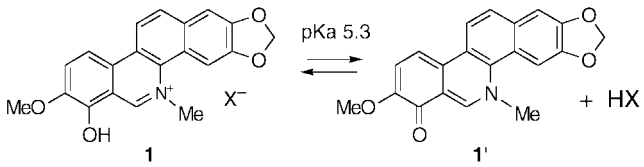
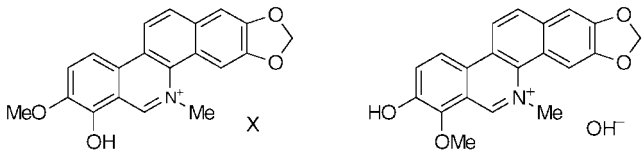
Scheme 1



thridinium hydrogensulfate dihydrate) had long-term stability for clinical use. Compound **1b**, which consists of the biologically active benzo[*c*]phenanthridinium cation, hydrogensulfate as a good counteranion, and additional dihydrate, was identified as NK109. In more detailed studies, we found that NK109 had greater antitumor activity than any known benzo[*c*]phenanthridinium in several assay systems.[9] NK109 retains full activity against cisplatin- and multidrug-resistant tumor cells.[10] Therefore, clinical studies have been carried out in Japan.

In 1973, Torto and co-workers identified a new benzo[*c*]phenanthridinium salt, fagaridine, and assigned its structure as **1c**, a different salt form of NK109.[11] Subsequently, we established that the true structure of fagaridine is not **1c**, but rather its isomer **2**. This assignment is based on the comparison of physical and chemical data.[12] Thus, NK109 has never been isolated from natural sources but is a synthetic benzo[*c*]phenanthridinium salt.



1a: X = I⁻
1b: X = HSO_4^-•$2H_2O$ (**NK109**)
1c: X = OH^-

2: Fagaridine

In this paper, we examine the effect of the acid residue on stability and present a method for synthesizing NK109.

Results and Discussion

Effect of Acid Residue on Stability. The benzo[*c*]phenanthridinium salts **1** are at equilibrium between the phenol **1** and its ketone **1′** (Scheme 1). The pK_a value at equilibrium is 5.3. Accordingly, **1** could gradually lose its acid residue in long-term storage, if it was the salt of a volatile acid such as hydrochloric acid or hydroiodic acid. Salt-free **1′** showed relatively low solubility in aqueous solution and was unstable under an oxidative atmosphere. Fixation to the quaternary salt **1** is important for maintaining solubility. In addition, the acid residue also affects stability. Experimental data are shown in Table 1. In the case of chloride, the solid changed from orange to amber, along with a decrease in purity. Although this change was rather small, it would be unsuitable for medical use. In contrast, the hydrogensulfate seemed to be completely stable under usual storage conditions. Consequently, we selected the latter. Since sulfuric acid is not volatile, **1′** was not formed unless it was neutralized. The hydrogensulfate form of the acid residue was supported by an elemental analysis.

Table 1. Stress Test of NK109[a]

time, h	purity based on the area under the HPLC curve (%) and powder color: chloride (Cl^-)	hydrogensulfate (HSO_4^-)
0	99.45 (orange)	99.47 (yellow)
10	98.78 (amber)	99.40 (yellow)

[a] Test condition: stirred at 70 °C under reduced pressure (3 mmHg).

Synthetic Investigation. Many synthetic approaches to benzo[*c*]phenanthridine alkaloid have been reported.[13] However, some of these were complex, gave a low total yield, and were limited to a particular substituent pattern. We developed a better method for large-scale synthesis and a route suitable for an analogue study, as outlined in Scheme 2. The starting materials were 6-bromo-2-hydroxy-3-methoxybenzaldehyde **3a** derived from *o*-vanillin[14] and 5-amino-2,3-methylenedioxynaphthalene **4** derived from 2,3-dihydroxynaphthalene.[5b,15]

Synthesis of Precursor 5. Hydroxy aldehyde **3a** was protected to benzyloxy aldehyde **3b**. The reaction was carried out with benzyl chloride and potassium iodide in a basic medium. Benzyl bromide was also available but was not used because of its higher cost. The aldehyde **3b** and the amine **4** were condensed to a Schiff base,[16] and its imine portion was reduced to a corresponding amine in a good yield. The resulting **5** is a precursor of the benzo[*c*]phenanthridine ring. This reduction was successfully performed with dimethylamine−borane[17] instead of conventional sodium cyanoborohydride or sodium borohydride. With sodium borohydride, a higher temperature and too much reducing agent were needed. Consequently, a large amount of hydrogen gas evolved until the termination of the reaction. Sodium cyanoborohydride caused a side reaction, that is, debromination. Dimethylamine−borane is a preferable reducing agent that does not give hydrogen gas and does not cause debromination.

Synthesis of Benzo[*c*]phenanthridine (6). Benzo[*c*]phenanthridine **6** was obtained by radical cyclization of the precursor **5** and subsequent oxidative aromatization of its cyclized intermediate. Cyclization to the benzo[*c*]phenanthridine ring system is a key step of the

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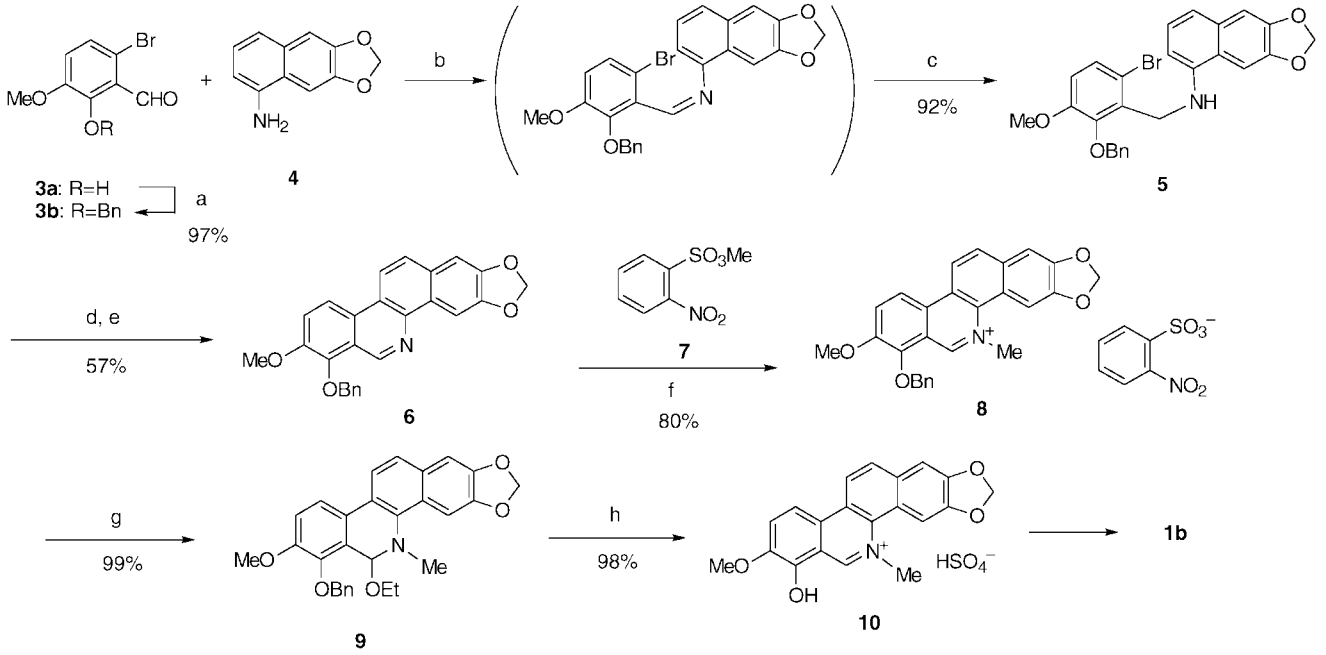
Scheme 2



(a) BnCl, KI, K_2CO_3, MeOH, reflux, 7 h; (b) toluene, reflux, 1.5h; (c) Me_2NHBH_3, AcOH, toluene, 20°C, 1 h; (d) *n*-Oct_3SnH, AMBN, toluene, 110°C, 70 min; (e) MnO_2, toluene, rt, 1 h; (f) **7**, toluene, reflux, 35 h; (g) NaOH, EtOH, rt, 2 h; (h) 6N H_2SO_4, 100°C, 1 h

Table 2. Mode of Cyclization with *n*-Bu_3SnH and AIBN[a]

entry	*n*-Bu_3SnH[b] (equiv)	AIBN[b] (equiv)	temp (°C)	time (min)	peak area of HPLC (%)[e] **6**[f]	**5**	others (dec)
1	0	2	100	60	0	20	80
2	0.6 × 5[c]	0.5 × 5[c]	100	180	15	1	84
3	3	0.1 × 7[d]	120	120	30	19	51
4	3	2	110	15	90	0	10

[a] HPLC conditions: column, LQ Pack SG-C8; mobile phase, 0.1% H_3PO_4−MeOH (30:70); flow rate, 1.0 mL/min; detection, UV (330 nm). [b] Added to a hot solution of **5** (246 mg) in toluene (25 mL). [c] Added at 30-min intervals. [d] Added at 15-min intervals. [e] This value does not indicate the actual yield (%), but rather shows the area (%) under the HPLC chromatograph obtained under the above condition. [f] They were analyzed after oxidation of cyclized intermediate. Reaction conditions: the reaction mixture (100 μL) was diluted with toluene (200 μL), MnO_2 was added (10 mg), and the solution was stirred for 5 min and then filtered through a 0.45 μm membrane filter (DISMIC-13_{HP}, ADVANTEC, Japan).

synthesis. Other cyclization methods have been described by other investigators, for example, benzyne cyclization[18] and photoradical cyclization.[19] However, the former method requires an extremely low temperature and the latter needs a special photoreaction apparatus. A method similar to our synthesis was reported by Rosa and co-workers using the radical initiator AIBN (2,2′-azobisisobutyronitrile).[20] Independently, we examined radical cyclization for large-scale synthesis. Table 2 shows the experimental results of the mode of radical cyclization by HPLC analysis. We found that compound **5** was consumed by radical species generated from the initiator. In the absence of trialkyltin hydride, **5** was decomposed and no cyclized compound was obtained (entry 1). In the presence of trialkyltin hydride, stannum radical arose and led to the formation of the cyclized intermediate, which was easily converted to compound **6** by active manganese dioxide oxidation (entries 2−4). A small amount of the initiator caused decomposition rather than cyclization (entry 3). When both reagents were divided into several portions, the decomposition rate of **5** was high (entry 2). These results indicate that **5** was consumed throughout the reaction. Accordingly, a sufficient amount of the initiator should be added all at once to a hot solution of **5** and trialkyltin hydride (entry 4). Rapid conversion into the benzo[*c*]phenanthridine ring was essential for avoiding extreme decomposition.

During our study, we confirmed that AMBN, 2,2′-azobis(2-methylbutyronitrile), and tri-*n*-octyltin hydride can be used in the same manner as AIBN and tri-*n*-butyltin hydride. These reagents offer some advantages: AMBN is highly soluble in the reaction solvent, that is, toluene, and tri-*n*-octyltin hydride is less toxic. We next sought to determine appropriate amounts of these reagents and a suitable reaction temperature (Table 3). This reaction did not proceed sufficiently at 80 °C, and a large amount of **5** remained (entry 1). Successful cyclization required a reaction temperature of over 100 °C. Although the highest yield was obtained at 100 °C (entry 2), this condition gave impurities that could not be easily removed by recrystallization. Therefore, we concluded that the best temperature for this reaction was 110 °C (entry 3). We next examined the appropriate amounts of the reagents. Entry 6 shows a high yield of **6**, but impurities formed as in entry 2. In conclusion, we decided that the best condition was 2.0 equiv of trialkyltin hydride and 1.5 equiv of the initiator at 110 °C (entry 5).

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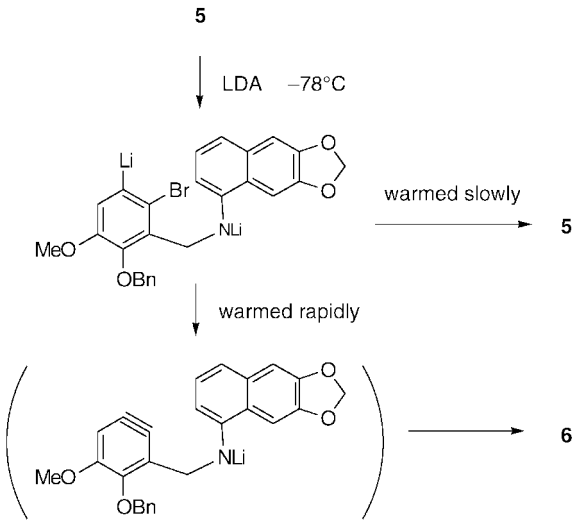
Table 3. Cyclization Conditions with *n*-Oct_3SnH and AMBN[a]

entry	*n*-Oct_3SnH (equiv)	AMBN (equiv)	temp (°C)	yield (%)[b] 6	yield (%)[b] 5	purity (%)[c]
1	2.0	2.0	80	5.6	56.3	
2	2.0	2.0	100	61.6	0	80.0
3	2.0	2.0	110	55.2	0	98.9
4	2.0	2.0	120	51.5	0	95.3
5	2.0	1.5	110	56.6	0	97.8
6	1.5	1.2	110	57.6	8.4	
7	1.2	1.2	110	47.1	12.2	

[a] Reaction conditions: **5** (492 mg) and *n*-Oct_3SnH in toluene (49 mL) were heated a each experimental temperature. An AMBN toluene solution (minimum volume needed to dissolve) was then added to the hot reaction mixture and heated for 1 h. The subsequent solution was oxidized with MnO_2 at room temperature for 1 h. [b] Calculated yield by HPLC analysis using isolated samples as a standard. HPLC conditions: column, LQ Pack ODS-M15; mobile phase, 0.1% H_3PO_4−CH_3CN (4:6); flow rate, 1.5 mL/min; detection, UV (270 nm). [c] Purity of precipitate **6** after primary purification.

Scheme 3



We also studied the cyclization of **5** with LDA[18] (Scheme 3). After lithiation of **5** at −78 °C, the reaction vessel was rapidly brought to room temperature and **5** was converted into the cyclized product **6** (34% yield). When lithiated **5** was warmed slowly, only the starting material **5** was obtained (63% recovery). This result suggests that a lithiated intermediate would be converted into benzyne at a higher temperature to form a benzo[*c*]phenanthridine ring, whereas the lithium cation would be slowly replaced by a proton at lower temperature. In a large-scale synthesis, it is difficult to control the rate of an increase in temperature. Therefore, this procedure was not suitable for our purpose.

Synthesis of Benzo[*c*]phenanthridinium (8). N-Methylation of a pyridine ring or fused aromatics is easily achieved by considering the intrinsic pK_a value but is often difficult in a steric environment.[21] In benzo[*c*]-phenanthridine **6**, we met resistance to methylation due to steric hindrance by the 4-position hydrogen. Methyl *p*-toluenesulfonate, MeOTs, required extreme conditions, that is, heating with neat MeOTs at 120 °C. Methyl trifluoromethanesulfanate, MeOTf, was much more reactive, but the undesired debenzylation of **6** proceeded simultaneously. With methyl *o*-nitrobenzenesulfonate **7**,[22] compound **6** was successfully methylated to benzo-[*c*]phenanthridinium **8**. Reagent **7** was easily prepared from *o*-nitrobenzenesulfonyl chloride and sodium methoxide in methanol. Compound **7** is not commonly used in methylation reactions but has 6-fold greater activity than conventional dimethyl sulfate.[23] Moreover, since the resulting benzo[*c*]phenanthridinium *o*-nitrobenzensulfonate **8** is barely soluble in toluene, it gradually precipitated and was removed from the reactive liquid phase. Thus, there was no further reaction.

Synthesis of NK109 (1b). Compound **8** was neutralized with sodium hydroxide in ethanol to remove the acid residue and gave the pseudobase **9**.[24] This was treated with sulfuric acid for deprotection of the benzyl group to give hydrogensulfate **10**. Subsequent hydration yielded NK109 (**1b**).

Conclusion

We have developed a versatile and convenient method for the synthesis of NK109, which exhibits superior antitumor activity and is expected to be an invaluable anticancer agent. Cyclization with *n*-Oct_3SnH−AMBN is an excellent method for constructing the benzo[*c*]-phenanthridine ring system and does not require freezing conditions. Methyl *o*-nitrobenzenesulfonate is an active, useful, and readily available agent for methylation. Furthermore, none of the steps required extreme conditions. All purification could be carried out only by recrystallization. The overall yield was approximately 40%. Thus, our procedure shows good productivity and is both safe and simple. We have already produced NK109 at a 2-kg scale for clinical studies. This method may be adaptable to the synthesis of other benzo[*c*]-phenanthridine alkaloids. In these synthetic studies we also discovered several advantages of the hydrogensulfate form of the acid residue of NK109.

Experimental Section

General. 2,2′-Azobis(2-methylbutyronitrile) and tri-*n*-octyltin hydride were purchased from Japan Hydrazine Co. Inc. and Sankyo Organic Chemicals Co. Ltd., respectively. Other materials were obtained from commercial suppliers and were used without further purification. NMR spectra were obtained on a Varian Gemini-200 spectrometer. All chemical shifts are reported in ppm relative to tetramethylsilane as an internal standard (TMS, δ 0.00). Mass spectra were recorded on a Micromass Limited Auto Spec-Q spectrometer. Elemental analyses were performed on a Yanako CHN Corder (C, H, N analysis) and a Yokogawa IC-7000D (Br, S analysis).

2-Benzyloxy-6-bromo-3-methoxybenzaldehyde (3b). To a mixture of 6-bromo-2-hydroxy-3-methoxybenzaldehyde **3a** (350 g, 1.51 mol), KI (252 g, 1.51 mol), and anhydrous K_2CO_3 (523 g, 3.78 mol) in methanol (23 L) was added 671 g of benzyl chloride (5.30 mol). After 7 h of reflux, anhydrous K_2CO_3 (209 g, 1.51 mol) was added and the solution was refluxed for another 2 h and then cooled to room temperature. After evaporation of methanol, the residue was partitioned between toluene and water. The organic extract was washed with water and dried over $MgSO_4$ in a toluene solution containing

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3b (net 467 g, 97% yield[25]). This solution was used for the next reaction.

Chromatography on silica gel with hexanes−ethyl acetate (4:1) yielded an analytical sample of **3b**, as a pale yellow semicrystalline solid: mp 49 °C; ^{1}H NMR ($CDCl_3$) δ 3.91 (s, 3H), 5.12 (s, 2H), 6.98 (d, J = 8.8 Hz, 1H), 7.33−7.48 (m, 6H), 10.25 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 56.3, 76.5, 112.5, 117.4, 128.5 × 3, 128.7 × 2, 129.1, 129.6, 136.3, 150.4, 152.9, 190.4; FAB-MS m/z 320 and 322 ($M^{+\bullet}$), 321 and 323 ($M^+ + H$). Anal. Calcd for $C_{15}H_{13}BrO_3$: C, 56.10; H, 4.08; Br, 24.88. Found: C, 55.76; H, 3.77; Br, 24.75.

***N*-(6-Bromo-2-benzyloxy-3-methoxybenzyl)-6,7-methylenedioxy-1-naphthylamine (5).** To the above-mentioned toluene solution (14 L) of **3b** (net 467 g, 1.45 mol) was added 2,3-methylenedioxy-5-naphthylamine **4** (298 g, 1.59 mol), and the mixture was refluxed for 1.5 h. Removal of toluene gave a Schiff base as an oil. The volume was brought back to 14 L with toluene, dimethylamineborane (65 g, 1.10 mol) was added, and the mixture was cooled to 20 °C. To this mixture was slowly added acetic acid (940 mL), and the resulting solution was stirred for 1 h. After reduction was complete, the reaction mixture was quenched with 1 N HCl (7 L). After being stirred for 1 h, the mixture was neutralized with 5 N NaOH and the organic layer was separated. The aqueous layer was extracted with toluene (6 L). The organic layers were combined, washed with water, dried over anhydrous $MgSO_4$, and concentrated in vacuo. The resulting residue was recrystallized with EtOH to give **5** (664 g, 92%) as a white powder: mp 126 °C; ^{1}H NMR ($CDCl_3$) δ 3.90 (s, 3H), 4.37 (br s, 1H), 4.48 (s, 2H), 5.04 (s, 2H), 5.99 (s, 2H), 6.74 (dd, J = 7.4, 1.2 Hz, 1H), 6.82 (d, J = 8.8 Hz, 1H), 7.05 (s, 1H), 7.11 (br d, J = 8.0 Hz, 1H), 7.33 (d, J = 8.8 Hz, 1H), 7.18?7.37 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 56.0, 43.8, 75.9, 97.6, 100.9, 104.7, 105.5, 113.0, 115.5, 117.6, 120.2, 125.1, 128.2 × 2, 128.4 × 2, 131.2, 132.9, 136.9, 143.0, 147.0, 147.2, 147.6, 152.5; FAB-MS m/z 491 and 493 ($M^{+\bullet}$), 492 and 494 ($M^+ + H$). Anal. Calcd for $C_{26}H_{22}BrNO_4$: C, 63.43; H, 4.50; N, 2.84; Br, 16.23. Found: C, 63.29; H, 4.37; N, 2.67; Br, 16.30.

7-Benzyloxy-8-methoxy-2,3-methylenedioxybenzo[*c*]phenanthridine (6). A solution of **5** (529 g, 1.07 mol) and *n*-Oct_3SnH (989 g, 2.15 mol) in toluene (53 L) was heated to 110 °C. A solution of AMBN (310 g, 1.61 mol) in toluene (0.53 L) was then added to the above solution. After 70 min, the mixture was cooled to room temperature, MnO_2 (636 g) was added, and the resultin solution was stirred well for 1 h. After oxidation was complete, the reaction mixture was filtered through Celite, and the filtrate was concentrated in vacuo. The residue was recrystallized with $CHCl_3$−hexane to give **6** (251 g, 57%) as a white powder: mp 172 °C; ^{1}H NMR ($CDCl_3$) δ 4.07 (s, 3H), 5.32 (s, 2H), 6.13 (s, 2H), 7.26 (s, 1H), 7.34−7.46 (m, 3H), 7.58 (dd, J = 8.0, 1.6 Hz, 2H), 7.61 (d, J = 9.0 Hz, 1H), 7.84 (d, J = 9.0 Hz, 1H), 8.34 (d, J = 9.0 Hz, 1H), 8.36 (d, J = 9.0 Hz, 1H), 8.69 (s, 1H), 9.75 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 50.0, 76.2, 101.6, 102.5, 104.7, 118.6, 118.7, 119.0, 120.3, 122.5, 127.4, 128.4, 128.7, 128.9 × 2, 129.0 × 2, 129.5, 130.1, 137.6, 140.4, 144.4, 147.2, 148.7, 148.9, 149.9; FAB-MS m/z 410 ($M^+ + H$). Anal. Calcd for $C_{26}H_{19}NO_4$: C, 76.27; H, 4.68; N, 3.42. Found: C, 76.02; H, 4.41; N, 3.25.

Methyl 2-Nitrobenzenesulfonate (7). Sodium methoxide (73 g, 1.42 mol) in methanol (0.36 L) was added in several portions to a solution of *o*-nitrobenzenesulfonyl chloride (315 g, 1.42 mol) in methanol (2.55 L) at 10 °C. After 0.5 h, the mixture was acidified with 0.1 N HCl (0.6 L), and then water (2.11 L) was added. After an additional 0.5 h of stirring, the resulting pale yellow crystal was filtered, washed with enough water to remove sodium chloride, and dried in vacuo to give **7** (285 g, 97%) as a pale yellow crystal: mp 59 °C; ^{1}H NMR ($CDCl_3$) δ 3.98 (s, 3H), 7.73−7.89 (m, 3H), 8.09−8.17 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 57.6, 124.8, 128.9, 131.3, 132.2, 134.9; FAB-MS m/z 218 ($M^+ + H$). Anal. Calcd for $C_7H_7NO_5S$: C, 38.71; H, 3.25; N, 6.45; S, 14.76. Found: C, 38.57; H, 3.04; N, 6.28; S 14.90.

7-Benzyloxy-8-methoxy-5-methyl-2,3-methylenedioxybenzo[*c*]phenanthridinium 2-Nitrobenzenesulfonate (8). A mixture of **6** (230 g, 0.56 mol) and **7** (244 g, 1.12 mol) in toluene (4.6 L) was refluxed for 35 h to yield a yellow suspension, which was then cooled, filtered, and washed with toluene. The crude product was recrystallized from DMF to give **8** (280 g, 80%) as a yellow powder: mp 227 °C; ^{1}H NMR (DMSO-d_6) δ 4.13 (s, 3H), 4.96 (s, 3H), 5.44 (s, 2H), 6.35 (s, 2H), 7.31−7.48 (m, 3H), 7.48−7.60 (m, 3H), 7.63 (dd, J = 7.8, 1.6 Hz, 2H), 7.72 (s, 1H), 7.80?7.87 (m, 1H), 8.25 (br d, J = 9.2 Hz, 2H), 8.27 (s,1H), 8.75 (d, J = 9.2 Hz, 1H), 8.77 (d, J = 9.2 Hz, 1H), 9.92 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 52.2, 56.9, 75.5, 102.7, 104.1, 105.7, 118.5, 119.5, 119.5, 119.9, 122.2, 125.0, 125.7, 127.8, 128.3 × 2, 128.4, 128.8 × 2, 128.9, 129.8, 130.5, 131.0, 131.4, 132.1, 136.3, 139.3, 143.6, 147.7, 148.6, 148.6, 150.4, 150.7; FAB-MS m/z 424 ($M^+ + H$). Anal. Calcd for $C_{33}H_{26}N_2O_9S$·DMF: C, 61.79; H, 4.75; N, 6.01; S, 4.58. Found: C, 61.78; H, 4.56; N, 5.79; S, 4.67.

7-Benzyloxy-6-ethoxy-8-methoxy-5-methyl-2,3-methylenedioxy-5,6-dihydrobenzo[*c*]phenanthridine (9). To a yellow suspension of **8** (280 g, 0.45 mol) in ethanol (4.2 L) was added 0.1 N NaOH (4.7 L), and the mixture was stirred for several hours until its color changed to white. The resulting white suspension was filtered, washed with ethanol−water (1:1), and dried in vacuo to give **9** (208 g, 99%) as a white powder: mp 141−149 °C; ^{1}H NMR ($CDCl_3$) δ 1.05 (t, J = 7.1 Hz, 3H), 2.61 (s, 3H), 3.60, 3.90 (each dq, J = 9.6, 7.1 Hz, 1H × 2), 3.94 (s, 3H), 5.09, 5.21 (AB, J = 10.9 Hz, 1H × 2), 5.64 (s,1H), 6.04 (s, 2H), 7.06 (d, J = 8.6 Hz, 1H), 7.11 (s, 1H), 7.33−7.58 (m, 5H), 7.46 (d, J = 8.6 Hz, 1H), 7.63 (s, 1H), 7.64 (d, J = 8.6 Hz, 1H), 7.78 (d, J = 8.6 Hz, 1H); minor isomer at the 6-position δ 2.52 (s), 3.96 (s), 5.21 (s), 5.90 (AB); ^{13}C NMR ($CDCl_3$) δ 15.2, 40.6, 56.0, 61.6, 75.7, 84.7, 100.6, 101.0, 104.6, 113.0, 119.1, 120.1, 122.6, 123.3, 125.0, 126.1, 126.7, 128.0, 128.3 × 2, 128.4 × 2, 131.0, 138.0, 138.7, 145.5, 147.3, 147.8, 152.2; FAB-MS m/z 424 ($M^+ + H - EtOH$). Anal. Calcd for $C_{29}H_{27}NO_5$: C, 74.18; H, 5.80; N, 2.98. Found: C, 74.36; H, 5.53; N, 2.84.

7-Hydroxy-8-methoxy-5-methyl-2,3-methylenedioxybenzo[*c*]phenanthridinium Hydrogensulfate dihydrate: NK109 (1b). A mixture of **9** (1.05 kg, 2.24 mol) and 6 N H_2SO_4 (53 L) was heated (100 °C) for 1 h. After debenzylation was complete, the mixture was cooled to 50 °C and acetone (53 L) was added. The resulting orange solid was cooled, filtered, washed with acetone, and dried in vacuo. Hydration at 75% relative humidity for several days gave NK109 (**1b**) (1.07 kg, 98%) as an orange solid: mp 256 °C (dec); ^{1}H NMR (DMSO-d_6) δ 4.08 (s, 3H), 4.92 (s, 3H), 6.34 (s, 2H), 7.73 (s, 1H), 8.13 (d, J = 9.0 Hz, 1H), 8.235 (d, J = 9.0 Hz, 1H), 8.245 (s, 1H), 8.46 (d, J = 9.0 Hz, 1H), 8.73 (d, J = 9.0 Hz, 1H), 10.05 (s, 1H), 10.5−12.3 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 51.7, 56.8, 102.6, 104.0, 105.6, 113.6, 115.1, 118.7, 120.1, 124.3, 125.0, 127.3, 130.6, 131.1, 132.0, 145.8, 145.9, 148.4 × 2, 150.9; FAB-MS m/z 334 (M^+). Anal. Calcd for $C_{20}H_{17}NO_8S$·$2H_2O$: C, 51.39; H, 4.53; N, 3.00; S, 6.86. Found: C, 51.34; H, 4.13; N, 2.66; S, 6.52.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compounds **1b**, **3b**, and **5**−**9** (14 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(25) As determined by HPLC analysis using the analytical sample as a standard.