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The Total Synthesis of Neoamphimedine

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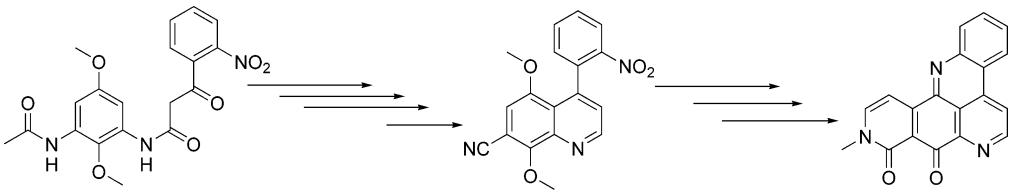


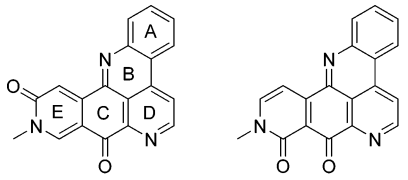
Neoamphimedine, a pyridoacridine alkaloid from *Xestospongia* sp., is a potent antitumor agent both in vitro and in vivo. Neoamphimedine can efficiently induce topoisomerase II mediated catenation of plasmid DNA in vitro and is the only member of more than one hundred pyridoacridines thus far to have this mechanism of action. Herein we report the first total synthesis of neoamphimedine.

Introduction

Pyridoacridines are a large and diverse family of marine alkaloids first described in 1983 with the report of the parent derivative amphimedine (**1**) (Chart 1).[1] Since then over one hundred derivatives have been characterized and studied displaying antitumor, antibacterial, antiviral, and antiparasitic activity.[2,3] The major biological activity of pyridoacridines described in the literature is in vitro cytotoxicity in mammalian cells. This activity is attributed to nonspecific intercalation of DNA as a result of the highly planar electron deficient aromatic ring system of the pyridoacridine pharmacophore. Although DNA intercalation is an important mechanistic feature of the pyridoacridines it is becoming evident that the range of biological activity is due to other mechanisms. These include generating reactive oxygen species (ROS), binding to transition metals, and inhibiting topoisomerases.[2−4]

Topoisomerase II (top2) is an important molecular target for chemotherapeutic drug discovery.[4−7] Besides the ability to relax supercoiled DNA, top2 also catalyzes the decatenation and catenation of DNA.[5,7] Topoisomerase II mediated decatenation of DNA is essential for G_2 to M cell cycle progression. Inhibition of top2 in mammalian cells has been shown to result in cell cycle arrest in both the S and G_2 phases suggesting a checkpoint for the completion of top2-mediated decatenation of catenated DNA.[7] Top2 poisons that stabilize the cleavable complex, such as etoposide and doxorubicin, have had the most clinical success.[2,7] Pyridoacridine derivatives have been shown to target topoisomerase function; while a few can stabilize the top2 cleavable complex, most inhibit top2 catalytic function.[2−4]

CHART 1. The Structures of Amphimedine and Neoamphimedine



Recently we reported the antineoplastic and novel top2 mediated cytotoxicity of neoamphimedine (**2**) (Chart 1).[8,9] In vitro, **2** has top2 dependent cytotoxicity in JN394T2 yeast cells as well as potent cytotoxicity in mammalian tumor cells, particularly the HCT116 cell line and the MDR1 expressing A2780AD cell line.[6,9] In vivo studies demonstrated that **2** is as efficacious as Etoposide at inhibiting the growth of epidermoid-nasopharyngeal KB tumors in nude mice. Interestingly, the

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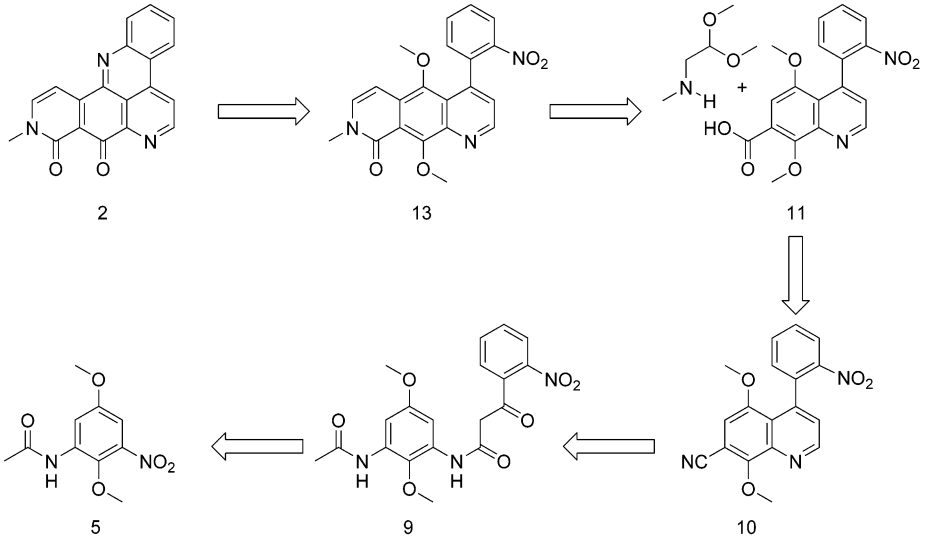
SCHEME 1. Retrosynthesis of Neoamphimedine



parent compound **1** was inactive at all doses studied, both in vitro and in vivo. Mechanism of action studies with **2** revealed that it is not a top2 cleavable complex poison; however, it efficiently induces top2-mediated catenation of plasmid DNA in vitro. Thus far, **2** is the only member of the pyridoacridine family to have this unique mechanism of action and more importantly the only small molecule known to induce top2-mediated catenation of DNA at low concentrations.[9]

The unique biological activities of the pyridoacridine family and diverse structural features have resulted in numerous synthetic studies and total syntheses. As a result, significant advances have been made with respect to synthetic methodologies in preparing pyridoacridines.[3,10−12] Despite these advances and two reported total syntheses of the parent derivative **1**,[13−15] the total synthesis of **2** has not been reported. We now report the first total synthesis of neoamphimedine.

Results and Discussion

Retrosynthetic analysis of **2** (Scheme 1) began with disconnection of the B ring. This disconnection has been described in numerous pyridoacridine syntheses giving intermediates such as **13**. Reduction of the nitro group of **13** and subsequent oxidation gives a reactive quinone intermediate that dehydrates in the presence of the amine to give **2**. In the previous synthesis reported for **1** a key hetero Diels−Alder reaction was utilized to form the 3-(2*H*)-isoquinolone E ring. There is no precedent for a hetero Diels−Alder formation of a 1-(2*H*)-isoquinolone required for the E ring of **2**, thus a different approach was taken. Disconnection of the E ring gives the acid functionality **11**, which can be derivatized with methylamino acetaldehyde dimethyl acetal to form the diazaanthracene **13**. It is important to note that this methodology facilitates derivatization of the E ring and would be useful to conduct SAR studies with various N-substituted amino acetals. The key acid intermediate **11** can be obtained from **5** through a series of reductions, amide formations, and acid-catalyzed ring closures.

The synthesis of **2** began with the known compound **5**.[16,17] Although the preparation of **5** was accomplished as described in the literature, some improvements to the synthetic procedures have been made (Supporting Information). Briefly, commercially available 4-methoxy-2-nitrophenol was nitrated with 70% nitric acid in acetic acid to afford **3** in 84−90% yield. The dinitrophenol was methylated with diazomethane to afford **4** in quantitative yield followed by selective reduction of one nitro group with 10% palladium on carbon in a boiling solution of cyclohexene and ethanol.[18] Subsequent acetylation of the resulting amine by treatment with acetic anhydride afforded **5** in 77% yield for two steps.

Catalytic reduction of **5** followed by heating the resulting amine in *m*-xylene with commercially available ethyl (2-nitrobenzoyl) acetate gave the β-keto amide **6** in 96% yield for two steps.[19] Knorr cyclization of **6** with polyphosphoric acid at 75 °C gave the quinolone **7** in 52% yield. Above 75 °C the 7-acetamido group hydrolyzes, which can influence the yield. Quinoline **9** was obtained in two steps via base-catalyzed conversion of **7** with trifluoromethanesulphonic anhydride giving the aryl triflate **8** in 70% yield.[20] The triflate ester of **8** was then removed by hydrogenolysis. In situ generation of triethyl ammonium formate in the presence of palladium catalyst and phosphine ligand gave the quinoline **9** in 87% yield (Scheme 2).[20,21]

A crucial step in this synthesis is the formation of the acid intermediate **11**, prepared via the Sandmeyer reaction (Scheme

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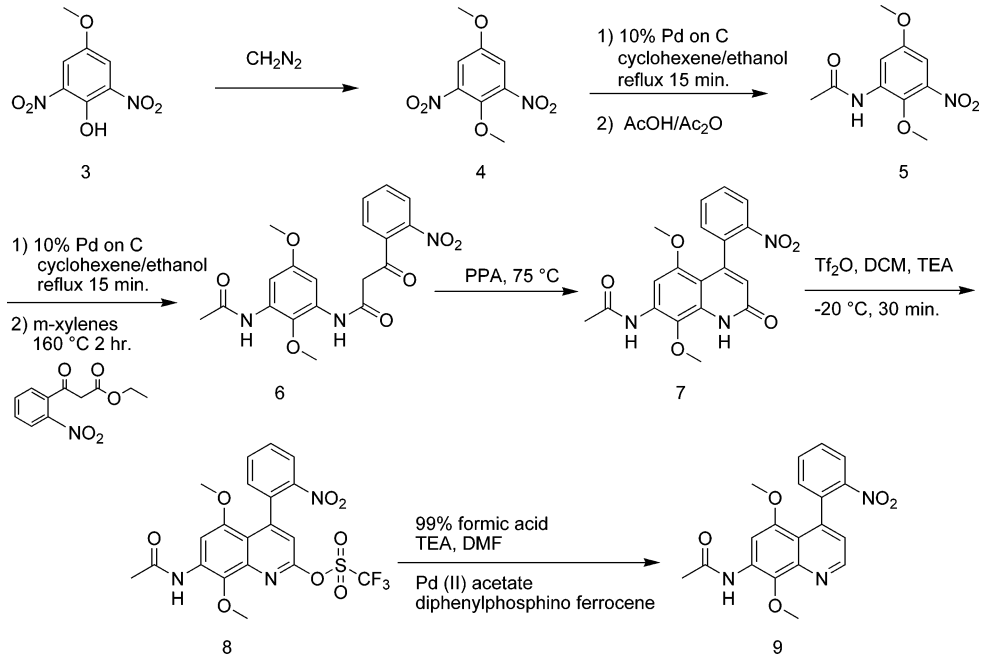
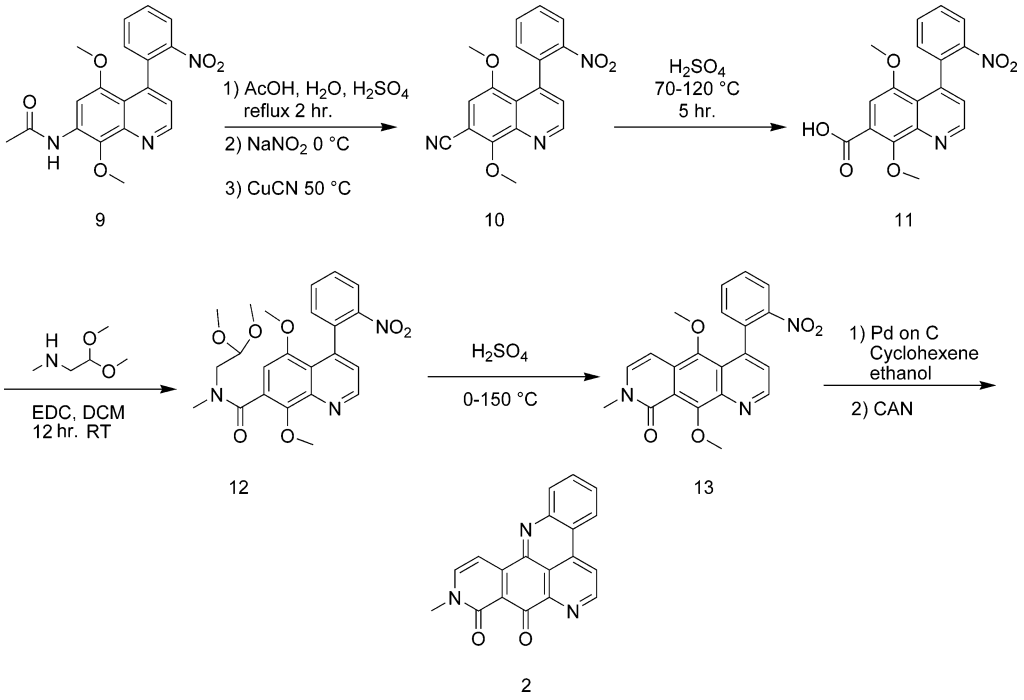
SCHEME 2. The Synthesis of Quinoline Intermediate 9



SCHEME 3. The Synthesis of Neoamphimedine



3). In one pot, the 7-acetanilide protecting group of **9** was hydrolyzed in a refluxing solution of glacial acetic acid, water, and sulfuric acid for 2 h. The corresponding aniline was then diazatized at 0 °C with sodium nitrite. The neutralized diazonium salt was added to a freshly prepared solution of potassium cupric cyanide[22] at 50 °C affording **10** in 50−58% yield. The nitrile of **10** was converted to the acid **11** by first generating the primary amide in situ at 70 °C for 3 h with concentrated sulfuric acid. The reaction was cooled and diluted in half with water followed by heating at 120 °C for 2 h to afford **11** in 80% yield.

The acid **11** was functionalized to the amide **12** with EDC and methylamino acetaldehyde dimethylacetal in 87% yield. The key isoquinolone ring of the diazaanthracene **13** was formed by converting the acetal to the aldehyde with sulfuric acid at room temperature. Once converted to the aldehyde the isoquinolone ring formation occurred smoothly at 150 °C in sulfuric acid within 10 min to form **13** in 43% yield. When ring closure of the dimethyl acetal was attempted at temperatures above 100 °C no product formed, and the compound decomposed. Neoamphimedine (**2**) was obtained by catalytic reduction of the nitro group of **13** followed by CAN oxidation in 30% yield for the

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final two steps. The synthetic product was compared with the natural product using ^{1}H NMR in chloroform and 2D NMR gHMBC and gHSQC in 2:1 chloroform/methanol. The splitting patterns, chemical shifts, and 2D proton carbon correlations were identical to the natural product.[8]

The concise synthesis of **2** was achieved in 12 steps from readily available **5**. Another important feature of this synthesis is the chemistry used to form the E ring of **2**. This ring system has proved to be the most challenging step for both the synthesis of **1** and **2**. The chemistry used in the synthesis of **2** allows for derivatization that can be useful in studying structure−activity relationships of the more biologically active **2**. Currently, we are scaling up the synthesis to conduct in vivo studies to further understand the biological mechanism of action of neoamphimedine.

Experimental

3-Acetamido-2,5-dimethoxy-(2-nitrobenzoyl)-acetanilide (6). A solution consisting of **5** (9.25 g, 38.5 mmol), 4.6 g of 10% Pd on carbon, 278 mL of cyclohexene, and 310 mL of absolute ethanol was refluxed for 2 h. The solution was filtered through celite while hot, and the catalyst was washed with ethanol. The filtrate was concentrated in vacuo giving an oil that solidified when triturated in hexanes. The white solid was filtered and air-dried (7.75 g, 96% yield). Without purification a mixture consisting of 3-acetamido-2,6-dimethoxyaniline (12 g, 57 mmol) and ethyl-(2-nitrobenzoyl)-acetate (13.5 g, 57 mmol) in 15 mL of anhydrous *m*-xylene were combined in a 100 mL round-bottom flask fitted with a short stem distillation apparatus under argon. The reaction vessel was placed on a sand bath preheated to 160−180 °C and stirred for 2 h. The temperature of the distillate reached 70 °C as ethanol was distilled off and dropped to 30 °C after distillation was complete. The reaction was cooled to room temperature and poured into 100 mL of diethyl ether. The resulting yellow oil was crystallized by trituration giving a pale yellow powder. The powder was filtered and washed 2× (25 mL) with diethyl ether and dried to afford **6** (21 g, 96% yield): mp 170−171 °C; TLC (ethyl acetate) $R_f = 0.23$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.23 (s, 1H), 8.21 (d, $J = 8.0$ Hz, 1H), 7.81 (t, $J = 7.5$ Hz, 1H), 7.75−7.67 (m, 3H), 7.59 (s, 1H), 7.49 (d, $J = 8.0$ Hz, 1H), 3.96 (s, 2H), 3.81 (s, 3H), 3.79 (s, 3H), 2.23 (s, 3H). IR (NaCl plate): 993, 1049, 1246, 1346, 1456, 1525, 1603, 1684, 2358, 3308 cm^{-1}. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{19}H_{20}N_3O_7$, 402.1301; found, 402.1299.

7-Acetamido-5,8-dimethoxy-4-(2-nitrophenyl)-2-(1*H*)quinolinone (7). Polyphosphoric acid (200 g) was prewarmed to 75 °C in a 500 mL beaker fitted with a mechanical stirrer. To the stirred viscous acid was gradually added **6** (20 g, 50 mmol). The reaction was kept at 75 °C until finished by TLC (ethyl acetate) approximately 5 h. The reaction was cooled to room temperature and poured into 500 mL of ice water. The resulting yellow solid was filtered and washed with ice water until the washings became neutral. The solid was dried under high vacuum to give 12 g of crude material. Recrystallization with ethanol and water afforded **7** as a yellow powder (10 g, 52% yield): mp >279 °C dec; TLC (10% MeOH in ethyl acetate) $R_f = 0.25$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.06 (s, 1H), 8.20 (d, $J = 8.0$ Hz, 1H), 7.87 (s, 1H), 7.71 (s, 1H), 7.67 (t, $J = 8.0$ Hz, 1H), 7.56 (t, $J = 8.0$ Hz, 1H), 7.32 (d, $J = 8.0$ Hz, 1H), 6.34 (s, 1H), 3.88 (s, 3H), 3.38 (s, 3H), 2.26 (s, 3H). IR (NaCl): 984, 1137, 1236, 1387, 1525, 1559, 1616, 1652, 3147 cm^{-1}. HRMS (*m/z*): $[M+Na]^+$ calcd for $C_{19}H_{17}N_3O_6$-Na, 406.1015; found, 406.1025.

7-Acetamido-5,8-dimethoxy-4-(2-nitrophenyl)-2-(trifluoromethane sulfonoxy)quinoline (8). Trifluoromethane sulfonic anhydride (8 mL, 48 mmol) was added dropwise over 25 min to a solution consisting of **7** (8.5 g, 22.2 mmol) in 300 mL of dry dichloromethane containing triethylamine (10 mL, 72 mmol) at −20 °C under argon. After the addition the reaction was stirred an additional 5 min, diluted with 100 mL of H_2O, and made basic with saturated aqueous $NaHCO_3$. The aqueous phase was extracted with dichloromethane 3× (50 mL). The combined dichloromethane extracts were dried with Na_2SO_4 and concentrated in vacuo. The resulting residue was chromatographed on silica gel eluting with 1:1 ethyl acetate and hexanes to afford **8** as yellow crystals (8 g, 70% yield): mp 127−128 °C; TLC (ethyl acetate) $R_f = 0.56$. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.21 (d, $J = 8.0$ Hz, 1H), 8.18 (s, 1H), 8.15 (s, 1H), 7.69 (t, $J = 8.0$ Hz, 1H), 7.60 (t, $J = 8.0$ Hz, 1H), 7.30 (d, $J = 8.0$ Hz, 1H), 6.86 (s, 1H), 4.14 (s, 3H), 3.45 (s, 3H), 2.27 (s, 3H). IR (NaCl plate): 846, 910, 1062, 1211, 1415, 1526, 1613, 1696, 2941, 3387 cm^{-1}. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{20}H_{17}N_3O_8F_3S$, 516.0688; found, 516.0671.

7-Acetamido-5,8-dimethoxy-4-(2-nitrophenyl)quinoline (9). Formic acid (99%) was added dropwise over 10 min to a solution mixture consisting of **8** (6.5 g, 12.6 mmol), triethyl amine (11.1 mL, 80 mmol), palladium(II) acetate (600 mg, 2.7 mmol) and 1,1-bis(diphosphino)ferrocene in 130 mL of dry DMF at 0 °C under argon. The reaction mixture was slowly warmed to 60 °C over 2 h, poured into 1.5 L of water, and made basic with saturated aqueous $NaHCO_3$. The aqueous phase was extracted 3× (400 mL) with dichloromethane. The dichloromethane extracts were combined, washed with 200 mL of saturated aqueous NaCl, dried with Na_2SO_4, and concentrated in vacuo resulting in a crude solid. The solid was purified by silica chromatography eluting with 1:1 DCM/ethyl acetate to afford **9** as yellow crystals (4 g, 87% yield): mp 237−238 °C; TLC (ethyl acetate) $R_f = 0.20$. ^{1}H NMR (400 MHz, $CHCl_3$): δ 8.96 (d, $J = 4.2$ Hz, 1H), 8.19 (d, $J = 8.0$ Hz, 1H), 8.16 (s, 1H), 8.13 (s, 1H), 7.67 (t, $J = 8.0$ Hz, 1H), 7.57 (t, $J = 8.0$ Hz, 1H), 7.29 (d, $J = 8.0$ Hz, 1H), 7.13 (d, $J = 4.2$ Hz, 1H), 4.14 (s, 3H), 3.46 (s, 3H), 2.29 (s, 3H). IR (NaCl plate): 858, 1257, 1347, 1391, 1419, 1456, 1522, 1616, 1684 cm^{-1}. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{19}H_{18}N_3O_5$, 368.1246; found, 368.1256.

7-Cyano-5,8-dimethoxy-4-(2-nitrophenyl)quinoline (10). A solution consisting of **9** (500 mg, 1.4 mmol), 15 mL of glacial acetic acid, 5 mL of H_2O, and 5 mL of concentrated H_2SO_4 was heated at reflux for 2 h. The resulting orange solution was cooled to room temperature and transferred (using ∼5 mL of ice water) to a 500 mL beaker containing 25 g of ice. The beaker was placed on an ice bath and cooled to 0 °C. The aniline was then diazatized by the dropwise addition of sodium nitrite (400 mg, 5.8 mmol) in 5 mL of H_2O while maintaining the temperature between 0 and 5 °C. After the addition the color of the reaction turns from dark orange to pale yellow, and the reaction is stirred at 0 °C for an additional 10 min followed by neutralization of the diazoinium salt to pH 5, at 0 °C, with solid K_2CO_3 over 30 min. Prior to neutralizing the diazonium salt the cupric cyanide solution was made fresh as follows. Copper sulfate (23 g) was dissolved in 100 mL of H_2O in a 1 L beaker and warmed to 40 °C, and KCN (25 g) in 50 mL of H_2O was added dropwise with stirring. Caution!! During this addition hydrogen cyanide is produced so this must be done in a well-ventilated hood to avoid breathing any vapors. After the addition of KCN the temperature was heated to 65 °C while the diazonium salt was being neutralized. The neutralized diazonium salt was transferred to an addition funnel and added dropwise to the cupric cyanide solution over 15 min while maintaining the temperature at 45−50 °C. The reaction mixture was then heated at 50 °C for 1 h, diluted with an equal volume of ethyl acetate, and stirred for 10 min. The aqueous phase was extracted 3× (100 mL) with ethyl acetate. The combined ethyl acetate extracts were dried with Na_2SO_4 and concentrated to give a crude oil. The oil was purified by silica flash chromatography using ethyl acetate as the eluent to afford **10** as tan crystals (265 mg, 58% yield): mp 129−130 °C; TLC (ethyl acetate/MeOH, 1:1 v/v) $R_f = 0.71$. ^{1}H NMR (500 MHz, CD_3OD): δ 8.99 (d, $J = 4.0$ Hz, 1H), 8.22 (d, $J = 8.0$ Hz, 1H), 7.73 (t, $J = 8.0$ Hz, 1H), 7.63 (t, $J = 8.0$ Hz, 1H), 7.37 (d, $J = 4.0$ Hz, 1H), 7.31 (d, $J = 8.0$ Hz, 1H), 6.82 (s, 1H), 4.26 (s, 3H), 3.44 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 155.5, 152.5,

150.8, 150.4, 150.0, 147.7, 137.9, 133.3, 130.1, 128.9, 123.9, 123.9, 122.8, 116.5, 105.6, 104.3, 63.3, 55.8. IR (NaCl plate): 990, 1072, 1139, 1243, 1346, 1389, 1465, 1526, 1716, 2359, 3342 cm^{-1}. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{18}H_{14}N_3O_4$, 336.0984; found, 336.0982.

5,8-Dimethoxy-4-(2-nitrophenyl)-7-quinolinecarboxcylic acid (11). The nitrile **10** (50 mg, 0.15 mmol) was dissolved in 750 μL of concentrated H_2SO_4 at room temperature. The acid solution was stirred at 60−70 °C for 3.5 h resulting in the primary amide. The reaction solution was cooled on ice and slowly diluted with 750 μL of ice cold water followed by heating at 120 °C under argon for 2 h. The reaction was cooled to room temperature, poured into 10 mL of ice water, and neutralized (pH = 5) with solid K_2CO_3. The neutral solution was extracted 3× (30 mL) with ethyl acetate, dried with Na_2SO_4, and concentrated in vacuo to give a pale orange powder. Recrystallization with ethyl acetate and hexanes gave pale orange needles (43 mg, 80% yield): mp >162 °C dec; TLC (ethyl acetate/MeOH, 1:1 v/v) R_f = 0.30. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.03 (s, 1H), 8.21 (d, J = 7.8 Hz, 1H), 7.68 (t, J = 7.7 Hz, 1H), 7.59 (t, J = 7.7 Hz, 1H), 7.36 (s, 1H), 7.29 (d, J = 7.8 Hz, 1H), 7.28 (s, 1H) 4.42 (s, 3H), 3.49 (s, 3H). IR (NaCl plate): 860, 996, 1134, 1236, 1346, 1393, 1456, 1524, 1575, 1699, 2514, 2938 cm^{-1}. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{18}H_{15}N_2O_6$, 355.0930; found, 355.0947.

7-[*N*-(2,2-Dimethoxyethyl)-*N*-methyl]-carboxamide-5,8-dimethoxy-4-(2-nitrophenyl)quinoline (12). To a solution consisting of **11** (30 mg, 0.085 mmol) and EDC·HCl (33 mg, 0.17 mmol) in 5 mL of dichloromethane under argon, was added (methylamino) acetaldehyde dimethyl acetal (22 μL, 20 mg, 0.17 mmol) dropwise under argon. The reaction mixture was stirred at room temperature for 15 h and quenched by the addition of 5 mL of H_2O. The aqueous phase was extracted 3× (20 mL) with dichloromethane, and the dichloromethane extracts where combined, dried with Na_2SO_4, and concentrated in vacuo. The resulting crude residue was purified by column chromatography (1.5 cm × 2.5 cm) using alumina Brockman acitivity I neutral and ethyl acetate as the eluent to afford a yellow colored oil (33.5 mg, 87% yield): TLC (ethyl acetate/MeOH 1:1 v/v) R_f = 0.56. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.97 (d, J = 4.2 Hz, 1H), 8.23−8.11 (m, 1H), 7.65 (t, J = 7.8 Hz, 1H), 7.55 (t, J = 7.8 Hz, 1H), 7.28 (m, 1H), 7.19 (d, J = 4.2 Hz, 1H), 6.59 (s, 1H), 4.90−2.79 (This region integrates to 18 protons, but they were not assigned because of cis−trans isomerization, 18H). IR (NaCl plate): 732, 1072, 1121, 1238, 1385, 1461, 1526, 1636, 2934 cm^{-1}. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{23}H_{26}N_3O_7$, 456.1771; found, 456.1790.

5,10-Dimethoxy-8-methyl-4-(2-nitrophenyl)-pyrido-[4,3-*g*]-quinoline-9(8*H*)-one (13). Dimethyl acetal **12** (30 mg, 0.065 mmol) was dissolved in 750 μL of ice cold H_2SO_4 followed by stirring at ambient temperature for 4 h to generate the reactive aldehyde intermediate. The acid solution containing the aldehyde was then placed in an oil bath preheated to 150 °C and stirred at that temperature for 10 min. The reaction was poured into 20 mL of ice water and diluted with 20 mL of ethyl acetate. The aqueous layer was made basic to pH = 9 with K_2CO_3 and extracted 3× (50 mL) with ethyl acetate. The ethyl acetate extracts were dried with Na_2SO_4 and concentrated in vacuo to give a yellow green oil. The crude oil was purified by column chromatography (1.5 cm × 2.5 cm) using alumina Brockman acitivity I neutral and ethyl acetate as the eluent, to afford a yellow to green colored oil (10 mg, 43% yield): TLC (30% MeOH in ethyl acetate) R_f = 0.41. ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.02 (d, J = 4.2 Hz, 1H), 8.27 (d, J = 8.0 Hz, 1H), 7.72 (t, J = 8.0 Hz, 1H), 7.63 (t, J = 8.0 Hz, 1H), 7.48 (d, J = 8.0 Hz, 1H), 7.26 (d, J = 4.2 Hz, 1H), 6.97, (d, J = 7.7 Hz, 1H) 6.53 (d, J = 7.7 Hz, 1H), 4.23 (s, 3H), 3.50 (s, 3H), 3.02 (s, 3H). IR (NaCl plate): 1063, 1231, 1345, 1524, 1652, 2926 cm^{-1}. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{21}H_{18}N_3O_5$, 392.1246; found, 392.1260.

Neoamphimedine (2). A reaction mixture consisting of **13** (10 mg, 0.025 mmol), 20 mg of 10% Pd on carbon, 500 μL of cyclohexene, and 1 mL of absolute ethanol was refluxed for 1 h. The reaction mixture was cooled to room temperature and filtered through a plug of Celite. The filtrate was concentrated in vacuo giving a pale yellow residue, which was dissolved into 1 mL of ACN and cooled to 0 °C on an ice bath. A solution of CAN (35 mg, 0.063 mmol) in 1 mL of H_2O was added dropwise to the ice cold ACN solution and stirred at 0 °C for 15 min. The reaction solution was allowed to warm to room temperature and stirred for an additional 45 min. The pH of the solution was adjusted with saturated aqueous $NaHCO_3$ (pH = 8) and extracted 3× (10 mL) $CHCl_3$. The $CHCl_3$ extracts were combined, dried with Na_2SO_4, and concentrated in vacuo to afford **2** as a yellow solid. The solid was recrystallized with $CHCl_3$ and hexanes to give yellow crystals (2.4 mg, 30% yield). The original publication of the structure of neoamphimedine NMR data was reported in trifluoroacedic acid (TFA). It is important to note that neoamphimedine is unstable in the presence of TFA: TLC ($CHCl_3$/MeOH 1:1 v/v) R_f = 0.43. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.32 (d, J = 5.5 Hz, 1H), 8.62 (d, J = 8.0 Hz, 1H), 8.56 (d, J = 5.5 Hz, 1H), 8.36 (d, J = 8.0 Hz, 1H), 7.97 (t, J = 8.0 Hz, 1H), 7.87 (t, J = 8.0 Hz, 1H), 7.84 (d, J = 7.0 Hz, 1H), 7.81 (d, J = 7.0 Hz, 1H), 3.74 (s, 3H). (2D gHMBC and gHSQC proton carbon correlations are in the Supporting Information.) HRMS (*m/z*): $[M+H]^+$ calcd for $C_{19}H_{12}N_3O_2$, 314.0930; found, 314.0940.

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Supporting Information Available: General experimental details, detailed procedures for the preparation of **3**−**5**, 1D and 2D NMR spectra for neoamphimedine, and 1D NMR spectra for each intermediate. This material is available free of charge via the Internet at http://pubs.acs.org.

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