An Efficient Synthesis of a Lycobetaine–Tortuosine Analogue: A Potent Topoisomerase Inhibitor

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Abstract: An efficient gram-scale synthesis that uses a Suzuki cross-coupling reaction to yield 5-methyl-2,9-dimethoxyphenanthridinium chloride, a lycobetaine–tortuosine analogue and potent topoisomerase inhibitor, is presented.

Key words: cross-coupling, Suzuki reaction, topoisomerase inhibitor, phenanthridine alkaloids, heterocycles

The pyrrolophenanthridine alkaloids lycobetaine,[1a] also called ungeremine,[1b,c] as well as tortuosine and criasbetaine[2] are minor constituents of *Amaryllidaceae*, a world-wide plant family predominantly found in warmer regions (Figure 1). Phenanthridine alkaloids and their derivatives have attracted much attention over the past years due to potent antiviral,[3a] antimicrobial[3b] and antitumor[3c] properties. Lycobetaine, particularly, has been identified as a selective topoisomerase IIβ poison, potently inhibiting the growth of human tumor cell lines.[1a]

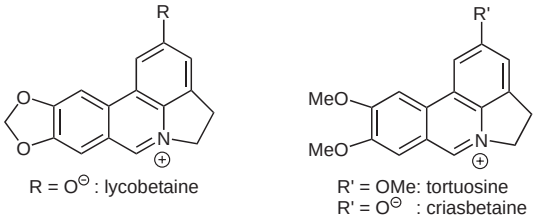

Figure 1 Naturally occurring pyrrolophenanthridine alkaloids

To further investigate these properties, comprehensive structure–activity studies were performed to identify relevant pharmacophores. Replacement of the 4,5-ethylene bridge in the pyrrolophenanthridine skeleton by a 5-methyl group and modifications of substituents in positions 2, 8 and 9 led to 2,9-dimethoxy-5-methylphenanthridinium chloride (**1**; Figure 2). This compound shows equipotent growth inhibitory properties compared to its natural analogue lycobetaine (data not shown). Chloride **1** was also found to be a potent inhibitor of human topoisomerases I and II.[4]

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Here we report an efficient gram-scale synthesis for the preparation of 2,9-dimethoxy-5-methyl-phenanthridinium chloride (**1**), a lycobetaine–tortuosine analogue and potent topoisomerase inhibitor (Figure 2).

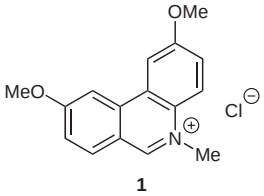

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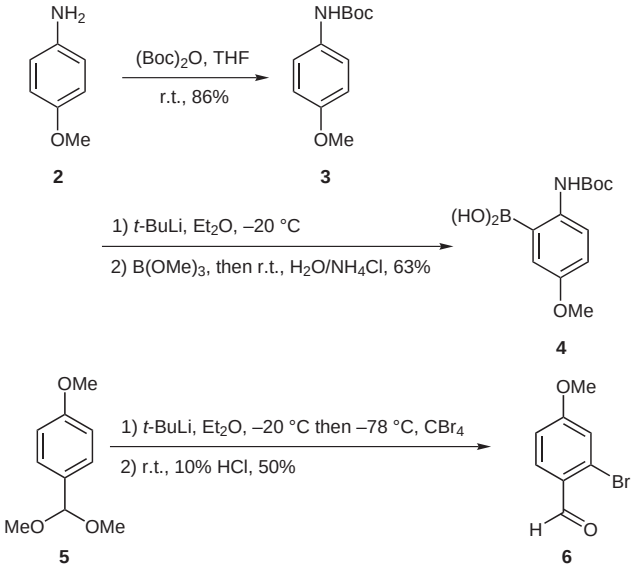
Figure 2 2,9-Dimethoxy-5-methylphenanthridinium chloride (**1**), an analogue of natural pyrrolophenanthridine alkaloids

Lycobetaine (ungeremine) was discovered in 1965[5a] and isolated in 1970.[5b] Lycorine, a more common alkaloid of *Amaryllidaceae*, provided the basis for oxidation to lycobetaine with selenium dioxide.[1c,6] In 1978, Zee-Cheng et al.[7] developed a practical photochemical reaction giving access to ungeremine and related pyrrolophenanthridines. Straightforward syntheses of ungeremine were published by Siddiqui et al.[1b] in 1990 and by Lauk et al.[8] in 1991. The former is based on a Suzuki cross-coupling reaction whereas the latter uses a radical cyclization to build the phenanthridine skeleton. Various natural pyrrolophenanthridinium alkaloids have also been prepared via a Ziegler–Ullmann reaction by Stark et al.[2]

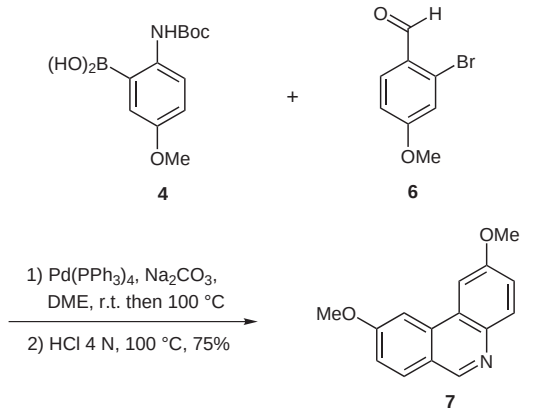
We have developed an effective large-scale synthesis of various substituted 5-methylphenanthridinium chlorides based on a Suzuki cross-coupling reaction and have applied this method to produce 5-methyl-2,9-dimethoxyphenanthridinium chloride (**1**; Figure 2).

The precursors for the Suzuki cross-coupling reaction are readily obtained. 4-Methoxyphenyl carbamic *tert*-butyl ester (**3**) was prepared by reacting *para*-anisidine (**2**) and di-*tert*-butyl dicarbonate in tetrahydrofuran[9] (Scheme 1). *Ortho*-lithiation of **3** with *tert*-butyllithium,[10] reaction with trimethyl borate, and subsequent hydrolysis yielded boronic acid **4**. 2-Bromo-4-methoxybenzaldehyde (**6**) was synthesized in one step from acetal **5** via *ortho*-lithiation[11] using *tert*-butyllithium followed by addition of tetrabromomethane[12] and subsequent acid hydrolysis (Scheme 1).



Scheme 1 Synthesis of precursors **4** and **6**

Suzuki cross-coupling reaction of **4** and **6** was performed in presence of tetrakis(triphenylphosphine)palladium and sodium carbonate in 1,2-dimethoxyethane. Acid hydrolysis of the reaction mixture afforded 2,9-dimethoxyphenanthridine (**7**) in 75% yield under optimized conditions (Scheme 2).

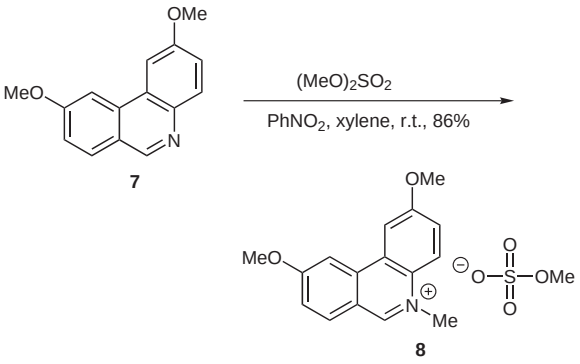


Scheme 2 Synthesis of 2,9-dimethoxyphenanthridine (**7**)

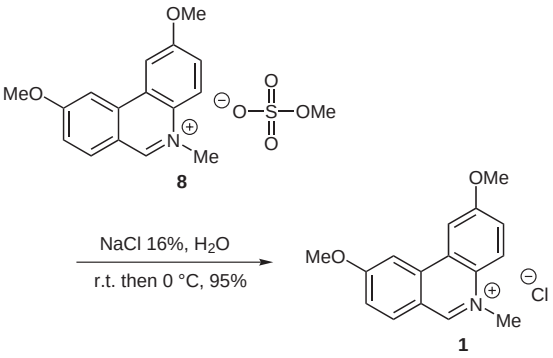
Quaternization of 2,9-dimethoxyphenanthridine (**7**) was carried out in the presence of dimethylsulfate in a mixture of nitrobenzene and xylene to yield 2,9-dimethoxy-5-methylphenanthridinium methylsulfate (**8**; Scheme 3).

Anion exchange of the methylsulfate salt **8** was achieved in an aqueous solution of sodium chloride. Target compound **1** was isolated as chloride salt by precipitation at 0 °C in nearly quantitative yield (Scheme 4).

In summary, we were able to prepare 2,9-dimethoxy-5-methylphenanthridinium chloride (**1**) on a gram scale using a six-step convergent synthesis with an acceptable overall yield of 17%. This synthetic route can be used to obtain other interesting pyrrolophenanthridine analogues,



Scheme 3 Preparation of 2,9-dimethoxy-5-methylphenanthridinium methylsulfate (**8**)



Scheme 4 Synthesis of 2,9-dimethoxy-5-methylphenanthridinium chloride (**1**)

e.g. 2,8-dimethoxy-5-methylphenanthridinium chloride, by simply adapting precursors **4** and **6**.

Synthesis of 2,9-Dimethoxyphenanthridine (7)

Tetrakis(triphenylphosphine)palladium (948 mg, 0.821 mmol, 0.05 equiv) was added to a degassed solution of 2-bromo-4-methoxybenzaldehyde (**6**; 3.53 g, 16.41 mmol, 1 equiv) in anhyd 1,2-dimethoxyethane (40 mL) and the resulting solution was stirred at r.t. After 5 min, 2-borono-4-methoxycarbanilate (**4**; 5.26 g, 19.70 mmol, 1.2 equiv) and an aq solution of Na_2CO_3 (5.22 g, 49.24 mmol, 3 equiv, 40 mL) were added and the resulting mixture was heated at 100 °C. After 5 h, a solution of concd HCl (8 mL) was slowly added to the cold solution and the resulting mixture was heated at 100 °C. After 30 min, an aq solution of 10 M NaOH was added until pH 7 was reached. The aqueous phase was extracted with Et_2O (2 × 150 mL) and $CHCl_3$ (2 × 150 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by flash column chromatography (EtOAc–cyclohexane, 7:3) to afford 2,9-dimethoxyphenanthridine (**7**; 2.94 g, 75%) as a slightly yellow solid; mp >210 °C (dec.). IR (KBr): 1030, 1501, 1516, 1614, 2831, 2935, 2974, 3009 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 4.00 (s, 3 H, OCH_3), 4.02 (s, 3 H, OCH_3), 7.28 (dd, $J_{8,7}$ = 8.9 Hz, $J_{8,10}$ = 2.4 Hz, 1 H, H-8), 7.36 (dd, $J_{3,1}$ = 2.8 Hz, $J_{3,4}$ = 9.0 Hz, 1 H, H-3), 7.75 (d, J = 2.5 Hz, 1 H), 7.76 (d, J = 2.5 Hz, 1 H), 7.92 (d, $J_{7,8}$ = 8.9 Hz, 1 H, H-7), 8.07 (d, $J_{4,3}$ = 9.0 Hz, 1 H, H-4), 9.03 (s, 1 H, H-6). ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 55.5 (OCH_3), 55.6 (OCH_3), 102.6 (C-1), 103.2 (C-10), 117.7 (C-8), 118.3 (C-3), 121.6 (C-6a), 124.8 (C-10b), 130.4 (C-7), 131.4 (C-4), 133.9 (C-10a), 139.9 (C-4a), 150.4 (C-6), 158.0 (C-2), 161.3 (C-9). MS (ES^+): *m*/*z* = 239. HRMS (ES^+): *m*/*z* calcd for

$C_{15}H_{13}NO_2$: 239.0946; found: 239.0944. Anal. Calcd for $C_{15}H_{13}NO_2$: C, 75.30; H, 5.48; N, 5.85. Found: C, 74.38; H, 5.39; N, 5.79.

Synthesis of 2,9-Dimethoxy-5-methylphenanthridinium Methylsulfate (8)

Dimethyl sulfate (8 mL) was added to a solution of compound **7** (2.6 g, 10.87 mmol, 1 equiv) in nitrobenzene (40 mL) and xylene (20 mL). The resulting mixture was stirred at r.t. After 1 h, the precipitate was filtered off, washed with cold Et_2O (2 × 50 mL) and dried under vacuum to afford 2,9-dimethoxy-5-methylphenanthridinium (**8**; 3.49, 88%) as a white solid. IR (KBr): 1005, 1221, 1253, 1614, 3008 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ = 3.63 (s, 3 H, $SOCH_3$), 3.81 (s, 3 H, OCH_3 at C-9), 3.90 (s, 3 H, OCH_3 at C-2), 4.23 (s, 3 H, NCH_3), 7.11–7.45 (m, 4 H, H-1, H-3, H-8, H-10), 7.81–7.97 (m, 2 H, H-4, H-7), 9.02 (s, 1 H, H-6). ^{13}C NMR (100.6 MHz, DMSO-d_6): δ = 46.2 (NCH_3), 55.4 ($SOCH_3$), 57.7 (2 × OCH_3), 105.0 (C-1), 106.2 (C-10), 119.1 (C-6a), 121.9 (C-3, C-8), 122.8 (C-7), 127.3 (C-10b), 129.4 (C-10a), 135.6 (C-4), 137.4 (C-4a), 150.8 (C-6), 1601.0 (C-2), 167.7 (C-9).

2,9-Dimethoxy-5-methylphenanthridinium Chloride (1)

Compound **8** (3.093 g, 8.46 mmol, 1 equiv) was added to an aq solution of 16% NaCl (80 mL) and the resulting mixture was stirred for 1 h at r.t. and for 30 min at 0 °C. The precipitate was then filtered off, washed with cold H_2O (30 mL) and dried under vacuum to afford 2,9-dimethoxy-5-methylphenanthridinium chloride (**1**; 2.34 g, 95%) as a slightly yellow solid; mp 203–204 °C. IR (KBr): 1025, 1221, 1617, 2838, 3006 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ =3.79 (s, 3 H, OCH_3 at C-9), 3.83 (s, 3 H, OCH_3 at C-2), 4.17 (s, 3 H, NCH_3), 7.24 (dd, $J_{3,1}$ = 2.2 Hz, $J_{3,4}$ = 8.9 Hz, 1 H, H-3), 7.31 (d, $J_{1,3}$ = 2.1 Hz, 1 H, H-1), 7.38 (m, 2 H, H-8, H-10), 7.87 (d, $J_{7,8}$ = 10.2 Hz, 1 H, H-7), 7.91 (d, $J_{4,3}$ = 9.0 Hz, 1 H, H-4), 9.03 (s, 1 H, H-6). ^{13}C NMR (100.6, MHz, DMSO-d_6): δ = 46.1 (NCH_3), 57.8 (2 × OCH_3), 105.1 (C-1), 106.3 (C-10), 119.0 (C-6a), 122.0 (C-3, C-8), 122.7 (C-7), 127.2 (C-10b), 129.5 (C-10a), 135.5 (C-4), 137.3 (C-4a), 150.7 (C-6), 160.9 (C-2), 167.8 (C-9).

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