

Activity of Lycorine Analogues against the Fish Bacterial Pathogen *Flavobacterium columnare*

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ABSTRACT: In a continuing effort to discover natural products and natural product-based compounds for the control of columnaris disease in channel catfish (*Ictalurus punctatus*), 17 lycorine analogues were synthesized, including new benzoyl analogues 6–16, and evaluated for antibacterial activity against two isolates (ALM-00-173 and BioMed) of *Flavobacterium columnare* using a rapid bioassay. Two of the lycorine analogues had greater antibacterial activity than 1-*O*-acetyllycorine, an analogue of lycorine evaluated previously that is highly active against both isolates. Carbamate analogue 18 (1*S*,2*S*,3*a*¹*S*,12*b**S*)-2,3*a*¹,4,5,7,12*b*-hexahydro-1*H*-[1,3]dioxolo[4,5-*j*]pyrrolo[3,2,1-*de*]phenanthridin-1,2-diylbis(*o*-tolylcarbamate) had the strongest antibacterial activity toward both *F. columnare* isolates ALM-00-173 and BioMed, with 24-h IC₅₀ values of 3.0 ± 1.3 and 3.9 ± 2.2 mg/L, respectively, and a MIC of 5.5 ± 0 mg/L for both isolates. Compound 18 appears to be the most promising lycorine analogue for future efficacy studies to determine its potential for use as an alternative to the currently used compounds to control columnaris disease in channel catfish.

KEYWORDS: antibacterial activity, channel catfish, columnaris disease, *Flavobacterium columnare*, lycorine

INTRODUCTION

Columnaris disease is a worldwide bacterial fish disease that results in heavy economic losses to the aquaculture industry.¹ The bacterial pathogen *Flavobacterium columnare* is the cause of columnaris disease, and this Gram-negative rod-shaped bacterium is in the family Flavobacteriaceae.² Columnaris disease may cause severe gill-rotting and possible skin ulceration in catfish. Epidemics of columnaris disease can occur in populations of pond-raised channel catfish (*Ictalurus punctatus*) in the southeastern United States, and, therefore, this common disease is a major concern for the channel catfish aquaculture industry.

Catfish producers currently have several available management approaches including the application of medicated feeds, attenuated vaccines,³ and nonantibiotic therapeutic agents such as 35% Perox-Aid for external columnaris. However, Perox-Aid is not recommended for use in earthen ponds without water exchange. Although additional agents such as copper sulfate pentahydrate (CuSO₄·5H₂O) and potassium permanganate (KMnO₄) have been mentioned as potential treatment options for columnaris,⁴ the efficacy of these therapeutants can be adversely affected by certain water quality variables. In addition, these compounds must be applied carefully due to their broad-spectrum toxicity toward nontarget organisms (e.g., channel catfish).⁵

Previously, a rapid 96-well microplate bioassay developed by Schrader and Harries⁶ has been utilized to evaluate numerous natural products and natural product-based compounds for antibacterial activity toward *F. columnare*. Subsequently, promising compounds have been identified; for example, tannic acid is highly toxic toward an isolate of *F. columnare* obtained from an infected catfish in Mississippi.⁷ A more recent study by Schrader et al.⁸ discovered that lycorine (1) (Figure 1) and two lycorine derivatives, 1,2-*O*,*O'*-diacetyllycorine (2) and 1-*O*-acetyllycorine (4), possess strong antibacterial activity toward two genomovars

of *F. columnare*, BioMed (genomovar I) and ALM-00-173 (genomovar II).

Lycorine is a pyrrolo[*de*]phenanthridine ring-type alkaloid extracted from various genera of plants in the Amaryllidaceae, and its structure was first elucidated by Nagakawa et al.⁹ The various biological properties of lycorine include inhibition of the following: (1) ascorbic acid (AA) biosynthesis;^{10,11} (2) growth and cell division in higher plants, algae, and yeasts;¹² (3) cyanide-insensitive respiration;¹³ (4) *Trichomonas vaginalis* nucleoside triphosphate diphosphohydrolase and ecto-5'-nucleotidase activities;¹⁴ and (5) apoptosis-resistant cancer cells.¹⁵ In addition, lycorine is antiplasmodial¹⁶ and has significant cytostatic activity toward cancer cells.¹⁷ These interesting properties make lycorine a valuable tool for studying a number of important physiological processes.

Because of our interest in improving the activity of lycorine and discovering promising compounds with the potential to control columnaris disease, we pursued the synthesis of various lycorine analogues. Two genomovars of *F. columnare* were included in the bioassay to identify potential selective toxicity of the lycorine analogues. Here we report the synthesis and antibacterial activity of 17 analogues, many of which are novel and, therefore, have not been previously evaluated against the two *F. columnare* genomovars.

MATERIALS AND METHODS

General Methods. General laboratory chemicals and solvents were purchased from commercial sources and used without purification.

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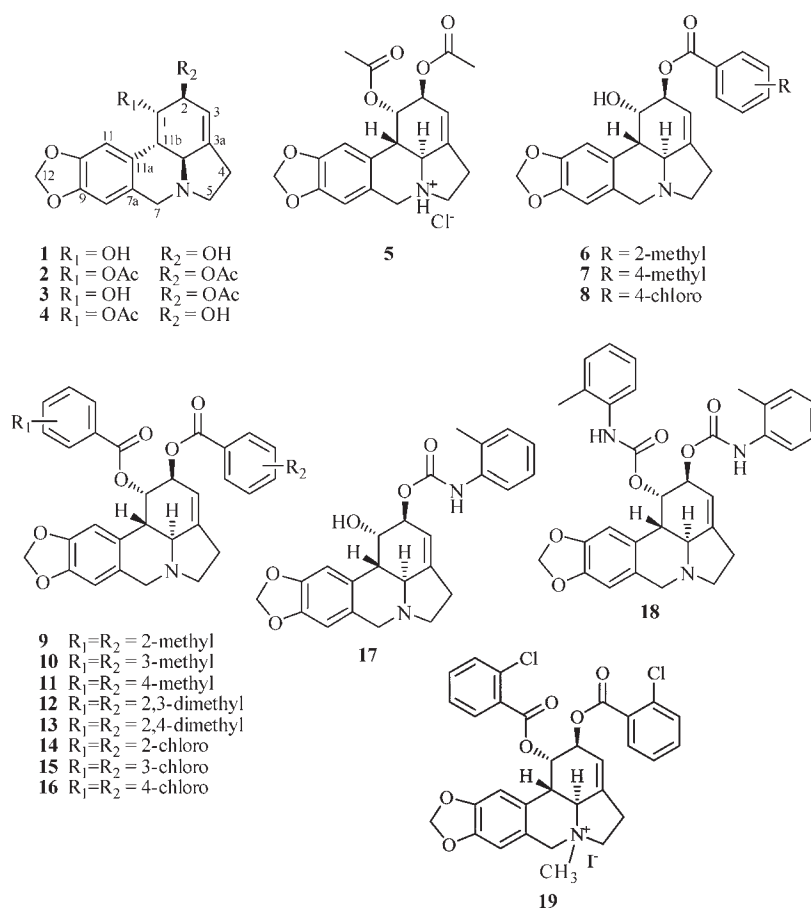


Figure 1. Chemical structures of lycorine analogues under study.

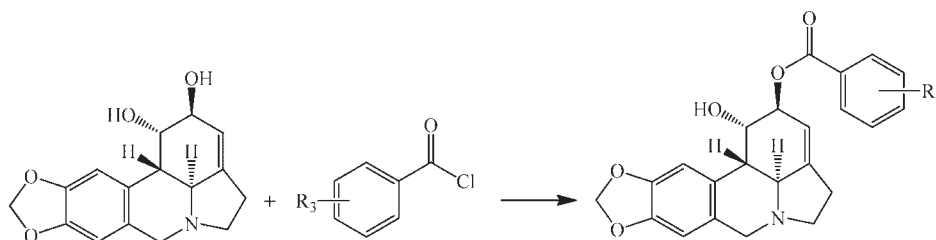
Reactions were performed in a round-bottom flask, under N₂ atmosphere, and were monitored by thin-layer chromatography (TLC). ¹H and ¹³C NMR spectra were recorded at 400 MHz on an AVANCE III Bruker spectrometer (Bruker Daltonics Inc., Billerica, MA). ESI MS spectra were recorded on a LC-MS system, that is, an Agilent 1100 HPLC (Agilent Technologies Inc., Santa Clara, CA) coupled to a JEOL AccuTOF (JMS-T100LC) mass spectrometer (JEOL USA, Inc., Peabody, MA). Analytical and preparative TLCs were performed on silica gel GF plates (Analtech uniplat, scored 10 × 20 cm, 250 μm, and 20 × 20 cm, 250 μm, respectively); the spots were visualized under UV light.

Antibacterial Bioassay. Two isolates of *F. columnare* [BioMed (genomovar I) and ALM-00-173 (genomovar II)] were obtained from Dr. Covadonga Arias (Department of Fisheries and Allied Aquacultures, Auburn University, Alabama). To ensure purity, cultures of both strains were maintained separately on modified Shieh agar plates (pH 7.2–7.4).¹⁸ Prior to the bioassay, single colonies of the test cultures were used to prepare the assay culture materials by separately culturing each genomovar isolate in 75 mL of modified Shieh broth (18 h for BioMed and 24 h for ALM-00-173) at 29 ± 1 °C at 150 rpm on a rotary shaker (model C24KC; New Brunswick Scientific, Edison, NJ).

Compounds were evaluated for antibacterial activity using a rapid 96-well microplate bioassay following the procedures of Schrader and Harries.⁶ Florfenicol and oxytetracycline HCl were included as positive controls for each assay. Also, control wells (no test compound added) and wells with 1-*O*-acetyllycorine were included in each assay. The analogue 1-*O*-acetyllycorine was included in the bioassay for comparison because it was more active than lycorine against both genomovars of *F. columnare*,⁸ and it was more readily available in sufficient quantities for

testing. To determine the 24-h 50% inhibition concentration (IC₅₀) and minimum inhibition concentration (MIC), sterile 96-well polystyrene microplates (type Costar; Corning Corp., Acton, MA) with flat-bottom wells were used to conduct the bioassay. Technical grade methanol was used to dissolve the test compounds. Final concentrations of test compounds, 1-*O*-acetyllycorine, and drug controls in the microplate wells were 0.01, 0.1, 1.0, 10.0, and 100.0 μM. Dissolved test compounds and drug controls were added separately to appropriate microplate wells (10 μL/well), and only methanol was added to control wells (10 μL/well). Solvent was allowed to completely evaporate before 0.5 McFarland bacterial culture (see ref 6) was added to the microplate wells (200 μL/well). The 0.5 McFarland standard provided a culture suspension of actively growing cells of *F. columnare* at 10⁸ colony forming units (CFU)/mL. Three replications were used for each dilution of each test compound and controls; each bioassay was repeated. Microplates were incubated at 29 °C (VWR model 2005 incubator; Sheldon Manufacturing, Inc., Cornelius, OR). A Packard model SpectraCount microplate photometer (Packard Instrument Co., Meriden, CT) was used to measure the absorbance (630 nm) of the microplate wells at times 0 and 24 h. Final results were converted to units of milligrams per liter to allow comparison with previous studies.

The means and standard deviations of absorbance measurements were calculated, graphed, and compared to controls to help determine the 24-h IC₅₀ and MIC for each test compound.⁶ The 24-h IC₅₀ and MIC results for each compound tested were divided by the respective 24-h IC₅₀ and MIC results obtained for the positive controls florfenicol (RDCF) and relative to drug control oxytetracycline (RDCCO) values.

Scheme 1^a

^a Conditions: DMAP, pyridine; lycorine/substituted benzoylchloride (1:1.05).

Preparation and Synthesis of Lycorine Analogues 2–5.

Compounds **2** and **3** were prepared from **1** (Sigma-Aldrich Co., St. Louis, MO), whereas **4** and **5** were prepared from **2** by following published procedures.^{17,19–21}

(1*S*,2*S*,3*a*¹*S*,12*bS*)-2,3*a*¹,4,5,7,12*b*-Hexahydro-1*H*-[1,3]dioxolo[4,5-*j*]pyrrolo[3,2,1-*de*]phenanthridine-1,2-diyl-diacetate (**2**) and (1*S*,2*S*,3*a*¹*S*,12*bS*)-1-Hydroxy-2,3*a*¹,4,5,7,12*b*-hexahydro-1*H*-[1,3]dioxolo[4,5-*j*]pyrrolo[3,2,1-*de*]phenanthridin-2-yl-acetate (**3**). Synthesis of **2** and **3** was according to published procedures.²² A mixture of **1** (30 mg, 0.10 mmol), pyridine (3 mL), and acetic anhydride (5 mL) in a sealed flask was stirred at room temperature for 30 h, and then the reaction mixture was quenched with cold water and extracted with chloroform (3 × 10 mL). The combined organic layers were washed with brine and dried over anhydrous magnesium sulfate. After removal of the volatiles, the crude product was purified by SiO₂ open column chromatography (EtOAc/CHCl₃/MeOH, 40:40:20) to give **2** (14.5 mg, 38% yield) and **3** (7.0 mg, 21% yield), both as white needles.

(1*S*,2*S*,3*a*¹*S*,12*bS*)-2-Hydroxy-2,3*a*¹,4,5,7,12*b*-hexahydro-1*H*-[1,3]dioxolo[4,5-*j*]pyrrolo[3,2,1-*de*]phenanthridin-1-yl acetate (**4**). A solution of **2** (18 mg, 0.05 mmol) in methanol (3 mL) and 35% hydrochloric acid (0.5 mL) was heated on an oil bath for 1 h.²³ After cooling, the mixture was cautiously basified (pH 8) with aqueous ammonia solution, diluted with water, and then extracted with chloroform (3 × 10 mL). The combined organic layers were washed with brine and dried over anhydrous magnesium sulfate. After evaporation, the residue was chromatographed over silica gel (EtOAc/CHCl₃/MeOH, 40:40:20) to give **4** (9 mg, 55% yield) as a colorless solid.

(1*S*,2*S*,3*a*¹*S*,12*bS*)-1,2-Diacetoxy-1,2,3*a*¹,4,5,6,7,12*b*-octahydro-[1,3]dioxolo[4,5-*j*]pyrrolo[3,2,1-*de*]phenanthridin-6-ium chloride (**5**). To a solution of **2** (18 mg, 0.05 mmol) in ethanol (3 mL) was added hydrogen chloride (2 mL, 10%), and the reaction mixture was stirred at room temperature for 10 h. Then, the mixture was cautiously basified (pH 7.5) with aqueous NaHCO₃ solution, diluted with water, and extracted with chloroform (3 × 10 mL). After removal of the volatiles, the crude product was purified by SiO₂ open column chromatography (CHCl₃/MeOH, 60:40) to give **5** (17 mg, 82% yield) as a white solid.

General Procedure for the Synthesis of Compounds 6–8.

To a solution of **1** (25 mg, 0.085 mmol) in dry pyridine (2 mL) was added substituted benzoyl chloride and 4-dimethylaminopyridine (DMAP) (1.05 mg, 0.01 mmol) (Scheme 1). The reaction mixture was stirred at room temperature for 12 h and then carefully poured into ice water (30 mL), causing precipitation. The precipitate was filtered, washed with brine, dried, and purified by flash column chromatography (SiO₂, EtOAc/hexane = 60:40).

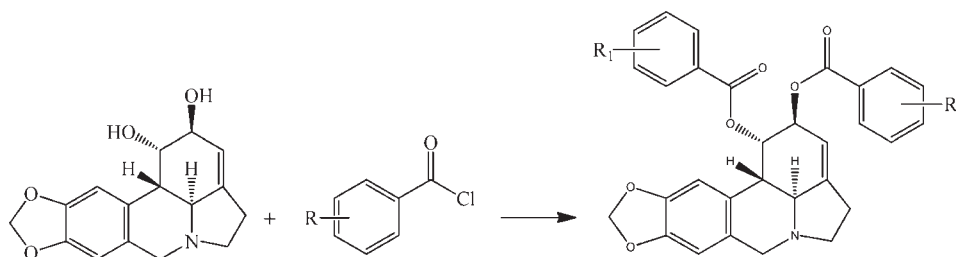
(1*S*,2*S*,3*a*¹*S*,12*bS*)-1-Hydroxy-2,3*a*¹,4,5,7,12*b*-hexahydro-1*H*-[1,3]dioxolo[4,5-*j*]pyrrolo[3,2,1-*de*]phenanthridin-2-yl 2-methylbenzoate (**6**). The reaction of **1** (25 mg, 0.085 mmol) and 2-methylbenzoyl chloride (14 mg, 0.09 mmol) afforded **6** (15 mg, 43% yield) as a white solid: ¹H NMR (400 MHz, CDCl₃) δ 7.90 (1H, d, *J* = 7.7 Hz, Ph-H-2), 7.40 (1H, t, *J* = 7.6, 7.4 Hz, Ph-H-4'), 7.23 (2H, m, Ph-H-3, 5), 6.81 (1H, s, H-11), 6.60 (1H, s, H-8), 5.91 (1H, br s, -OCH₂O-), 5.90

(1H, br s, -OCH₂O-), 5.60 (1H, br s, H-3), 5.58 (1H, br s, H-2), 4.67 (1H, br s, H-1), 4.16 (1H, d, *J* = 14.2 Hz, H-7b), 3.58 (1H, d, *J* = 14.1 Hz, H-7a), 3.40 (1H, m, H-5b), 2.92 (1H, d, *J* = 10.4 Hz, H-11b), 2.82 (1H, d, *J* = 10.4 Hz, H-11c), 2.70 (2H, m, H-2-4), 2.61 (3H, s, Ph-CH₃), 2.45 (1H, ddd, *J* = 8.1, 8.1, 8.1 Hz, H-5a); ¹³C NMR (100 MHz, CDCl₃) δ 166.9 (C-1''), 146.7 (C-3a), 146.4 and 145.9 (C-10, C-9), 140.4 (Ph-C-2), 132.2 (Ph-C-4), 131.8 (Ph-C-3), 130.9 (Ph-C-1), 129.8 (Ph-C-6), 127.2 (Ph-C-5), 129.2 (C-7a), 125.8 (C-11a), 113.9 (C-3), 107.7 (C-8), 104.7 (C-11), 101.0 (-OCH₂O-), 73.9 (C-1), 69.2 (C-2), 64.4 (C-11c), 56.9 (C-7), 53.7 (C-5), 41.8 (C-11b), 28.8 (C-4), 22.0 (Ph-CH₃). HRMS: calcd for C₂₄H₂₄NO₅ [M + H], 406.1655; found, 406.1634.

(1*S*,2*S*,3*a*¹*S*,12*bS*)-1-Hydroxy-2,3*a*¹,4,5,7,12*b*-hexahydro-1*H*-[1,3]dioxolo[4,5-*j*]pyrrolo[3,2,1-*de*]phenanthridin-2-yl-4-methylbenzoate (**7**). The reaction of **1** (25 mg, 0.085 mmol) and 4-methylbenzoyl chloride (14 mg, 0.09 mmol) afforded **7** (17 mg, 49% yield) as a white solid: ¹H NMR (400 MHz, CDCl₃) δ 7.93 (2H, d, *J* = 8.2 Hz, Ph₂-H-2,6), 7.23 (2H, d, *J* = 8.2 Hz, Ph₂-H-3,5), 6.82 (1H, s, H-11), 6.61 (1H, s, H-8), 5.93 (1H, d, *J* = 1.3 Hz, -OCH₂O-), 5.92 (1H, d, *J* = 1.3 Hz, -OCH₂O-), 5.60 (2H, br s, H-2, 3), 4.67 (1H, br s, H-1), 4.19 (1H, d, *J* = 14.1 Hz, H-7b), 3.58 (1H, d, *J* = 14.1 Hz, H-7a), 3.42 (1H, m, H-5b), 3.14 (1H, d, *J* = 10.4 Hz, H-11b), 2.88 (1H, d, *J* = 10.4 Hz, H-11c), 2.70 (2H, m, H-2-4), 2.45 (1H, ddd, *J* = 8.2, 8.2, 8.2 Hz, H-5a), 2.41 (3H, s, Ph-CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 164.3 (C-1''), 146.5 (C-3a), 146.4 and 146.2 (C-10, C-9), 139.3 (Ph₂-C-4), 131.1 (Ph₂-C-2,6), 129.2 (C-7a), 128.5 (Ph₂-C-3,5), 128.3 (Ph₂-C-1), 126.0 (C-11a), 113.3 (C-3), 107.2 (C-8), 105.3 (C-11), 100.0 (-OCH₂O-), 71.2 (C-1), 70.0 (C-2), 61.2 (C-11c), 56.5 (C-7), 53.4 (C-5), 41.4 (C-11b), 28.4 (C-4), 22.1 (Ph-CH₃). HRMS: calcd for C₂₄H₂₄NO₅ [M + H], 406.1655; found, 406.1614.

(1*S*,2*S*,3*a*¹*S*,12*bS*)-1-Hydroxy-2,3*a*¹,4,5,7,12*b*-hexahydro-1*H*-[1,3]dioxolo[4,5-*j*]pyrrolo[3,2,1-*de*]phenanthridin-2-yl-4-chlorobenzoate (**8**). The reaction of **1** (25 mg, 0.085 mmol) and 4-chlorobenzoyl chloride (16 mg, 0.09 mmol) afforded **8** (24 mg, 66% yield) as a white solid: ¹H NMR (400 MHz, CDCl₃) δ 8.00 (2H, d, *J* = 8.6 Hz, Ph-H-2,6), 7.42 (2H, d, *J* = 8.6 Hz, Ph-H-3,5), 6.82 (1H, s, H-11), 6.62 (1H, s, H-8), 5.94 (1H, d, *J* = 1.3 Hz, -OCH₂O-), 5.93 (1H, d, *J* = 1.3 Hz, -OCH₂O-), 5.92 (1H, br s, H-3), 5.60 (1H, br s, H-2), 4.67 (1H, br s, H-1), 4.18 (1H, d, *J* = 14.1 Hz, H-7b), 3.65 (1H, d, *J* = 14.1 Hz, H-7a), 3.41 (1H, m, H-5b), 3.14 (1H, d, *J* = 10.4 Hz, H-11b), 3.00 (1H, d, *J* = 10.4 Hz, H-11c), 2.73 (2H, m, H-4), 2.52 (1H, ddd, *J* = 8.3, 8.3, 8.3 Hz, H-5a); ¹³C NMR (100 MHz, CDCl₃) δ 164.5 (C-1''), 146.3 (C-3a), 146.6 and 146.4 (C-10, C-9), 139.5 (Ph-C-4), 131.2 (Ph-C-2,6), 128.6 (Ph-C-3,5), 128.2 (Ph-C-1), 129.1 (C-7a), 126.0 (C-11a), 113.5 (C-3), 107.3 (C-8), 105.1 (C-11), 100.1 (-OCH₂O-), 71.3 (C-1), 70.1 (C-2), 61.3 (C-11c), 56.6 (C-7), 53.6 (C-5), 41.2 (C-11b), 28.6 (C-4). HRMS: calcd for C₂₃H₂₂ClNO₅ [M + H], 426.1108; found, 426.1117.

General Procedure for the Synthesis of Compounds 9–16. Substituted benzoyl chloride (0.18 mmol) and DMAP (1.05 mg, 0.01 mmol) were added to a solution of **1** (25 mg, 0.085 mmol) in dry pyridine (2 mL) (Scheme 2). The reaction mixture was stirred at

Scheme 2^a

^a Conditions: DMAP, pyridine; lycorine/substituted benzoyl chloride (1:2.1), 24 h, 30–80% yield.

room temperature for 24 h and then carefully poured into ice water (30 mL). The precipitate was filtered and washed with brine. Purification was by flash column chromatography (SiO₂, EtOAc/hexane = 52: 48).

(1*S*,2*S*,3*a*¹*S*,12*bS*)-2,3*a*¹,4,5,7,12*b*-Hexahydro-1*H*-[1,3]dioxolo[4,5-*j*]pyrrolo[3,2,1-*de*]phenanthridine-1,2-diyl-bis(2-methylbenzoate) (**9**). The reaction of **1** (25 mg, 0.085 mmol) and 2-methylbenzoyl chloride (28 mg, 0.18 mmol) afforded **9** (24 mg, 54% yield) as a white solid: ¹H NMR (400 MHz, CDCl₃) δ 7.95 (1*H*, d, *J* = 7.6 Hz, Ph₂-H-2), 7.67 (1*H*, d, *J* = 7.6 Hz, Ph₁-H-2), 7.35–7.43 (2*H*, m, Ph₂-H-4, Ph₁-H-4), 7.16–7.25 (4*H*, m, Ph₂-H-3, 5, Ph₁-H-3, 5), 6.88 (1*H*, s, H-11), 6.60 (1*H*, s, H-8), 6.15 (1*H*, br s, H-1), 5.90 (1*H*, d, *J* = 1.2 Hz, -OCH₂O-), 5.88 (1*H*, d, *J* = 1.2 Hz, -OCH₂O-), 5.74 (1*H*, br s, H-3), 5.69 (1*H*, br s, H-2), 4.23 (1*H*, d, *J* = 14.2 Hz, H-7b), 3.64 (1*H*, d, *J* = 14.1 Hz, H-7a), 3.48 (1*H*, m, H-5b), 3.19 (1*H*, d, *J* = 9.3 Hz, H-11b), 3.07 (1*H*, d, *J* = 9.3 Hz, H-11c), 2.75 (2*H*, m, H₂-4), 2.65 (3*H*, s, Ph₂-CH₃), 2.58 (1*H*, ddd, *J* = 8.1, 8.1, 8.1 Hz, H-5a), 2.47 (3*H*, s, Ph₁-CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 166.5 (C-1'), 166.1 (C-1''), 146.5 (C-3a), 146.4 and 145.9 (C-10, C-9), 140.4 (Ph₂-C-2), 139.4 (Ph₁-C-2), 132.2 (Ph₂-C-4), 132.1 (Ph₁-C-4), 131.7 (Ph₂-C-3), 131.6 (Ph₁-C-3), 131.0 (Ph₂-C-1), 130.9 (Ph₁-C-1), 129.4 (C-7a), 129.3 (Ph₂-C-6), 129.1 (Ph₁-C-6), 127.2 (Ph₂-C-5), 127.0 (Ph₁-C-5), 125.7 (C-11a), 113.2 (C-3), 107.4 (C-8), 105.0 (C-11), 100.0 (-OCH₂O-), 70.9 (C-1), 68.9 (C-2), 61.6 (C-11c), 56.6 (C-7), 53.6 (C-5), 40.5 (C-11b), 28.1 (C-4), 22.0 (Ph₂-CH₃), 21.6 (Ph₁-CH₃). HRMS: calcd for C₃₂H₃₀NO₆ [M + H], 524.2073; found, 524.2090.

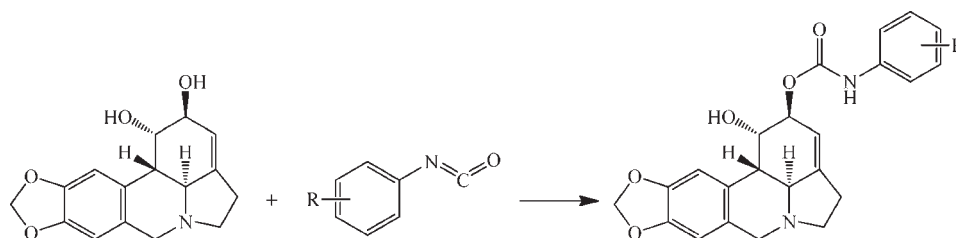
(1*S*,2*S*,3*a*¹*S*,12*bS*)-2,3*a*¹,4,5,7,12*b*-Hexahydro-1*H*-[1,3]dioxolo[4,5-*j*]pyrrolo[3,2,1-*de*]phenanthridine-1,2-diyl-bis(3-methylbenzoate) (**10**). The reaction of **1** (25 mg, 0.085 mmol) and 3-methylbenzoyl chloride (28 mg, 0.18 mmol) afforded **10** (26 mg, 59% yield) as a white solid: ¹H NMR (400 MHz, CDCl₃) δ 7.88 (2*H*, br s, Ph₂-H-2,6), 7.74 (1*H*, s, Ph₁-H-2), 7.69 (1*H*, d, *J* = 7.6 Hz, Ph₁-H-6), 7.39–7.24 (4*H*, m, Ph₂-H-4,5, Ph₁-H-4,5), 6.89 (1*H*, s, H-11), 6.58 (1*H*, s, H-8), 6.16 (1*H*, br s, H-1), 5.89 (1*H*, d, *J* = 1.0 Hz, -OCH₂O-), 5.85 (1*H*, d, *J* = 1.0 Hz, -OCH₂O-), 5.70 (2*H*, br s, H-2, 3), 4.22 (1*H*, d, *J* = 14.1 Hz, H-7b), 3.62 (1*H*, d, *J* = 14.1 Hz, H-7a), 3.45 (1*H*, m, H-5b), 3.18 (1*H*, d, *J* = 10.4 Hz, H-11b), 3.04 (1*H*, d, *J* = 10.4 Hz, H-11c), 2.75 (2*H*, m, H₂-4), 2.52 (1*H*, ddd, *J* = 8.2, 8.2, 8.2 Hz, H-5a), 2.39 (3*H*, s, Ph₂-CH₃), 2.36 (3*H*, s, Ph₁-CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 165.5 and 165.4 (C-1', C-1''), 146.5 and 146.4 (C-10, C-9), 146.2 (C-3a), 138.2 and 138.1 (Ph₁-C-3, Ph₂-C-3), 133.9 and 133.8 (Ph₁-C-4, Ph₂-C-4), 130.4 and 130.3 (Ph₁-C-1, Ph₂-C-1), 129.9 and 129.6 (Ph₁-C-2, Ph₂-C-2), 129.3 (C-7a), 128.3 and 128.2 (Ph₁-C-5, Ph₂-C-5), 127.0 and 126.9 (Ph₁-C-6, Ph₂-C-6), 126.5 (C-11a), 114.1 (C-3), 107.3 (C-8), 105.2 (C-11), 100.9 (-OCH₂O-), 71.0 (C-1), 69.5 (C-2), 61.6 (C-11c), 56.9 (C-7), 53.9 (C-5), 41.1 (C-11b), 28.8 (C-4), 21.3 and 21.2 (Ph₁-CH₃, Ph₂-CH₃). HRMS: calcd for C₃₂H₃₀NO₆ [M + H], 524.2073; found, 524.2097.

(1*S*,2*S*,3*a*¹*S*,12*bS*)-2,3*a*¹,4,5,7,12*b*-Hexahydro-1*H*-[1,3]dioxolo[4,5-*j*]pyrrolo[3,2,1-*de*]phenanthridine-1,2-diyl-bis(4-methylbenzoate) (**11**).

The reaction of **1** (25 mg, 0.085 mmol) and 4-methylbenzoyl chloride (28 mg, 0.18 mmol) afforded **11** (23 mg, 52% yield) as a white solid: ¹H NMR (400 MHz, CDCl₃) δ 7.94 (2*H*, d, *J* = 8.2 Hz, Ph₂-H-2,6), 7.80 (2*H*, d, *J* = 8.1 Hz, Ph₁-H-2,6), 7.23 (2*H*, d, *J* = 8.1 Hz, Ph₂-H-3,5), 7.18 (2*H*, d, *J* = 8.1 Hz, Ph₁-H-3,5), 6.87 (1*H*, s, H-11), 6.57 (1*H*, s, H-8), 6.12 (1*H*, br s, H-1), 5.89 (1*H*, d, *J* = 1.3 Hz, -OCH₂O-), 5.85 (1*H*, d, *J* = 1.3 Hz, -OCH₂O-), 5.68 (1*H*, br s, H-3), 5.67 (1*H*, br s, H-2), 4.22 (1*H*, d, *J* = 14.1 Hz, H-7b), 3.62 (1*H*, d, *J* = 14.1 Hz, H-7a), 3.44 (1*H*, m, H-5b), 3.14 (1*H*, d, *J* = 10.4 Hz, H-11b), 3.03 (1*H*, d, *J* = 10.4 Hz, H-11c), 2.73 (2*H*, m, H₂-4), 2.50 (1*H*, ddd, *J* = 8.3, 8.3, 8.3 Hz, H-5a), 2.42 (3*H*, s, Ph₂-CH₃), 2.38 (3*H*, s, Ph₁-CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 164.4 and 164.2 (C-1', C-1''), 146.4 (C-3a), 146.5 and 146.3 (C-10, C-9), 139.6 and 139.5 (Ph₁-C-4, Ph₂-C-4), 131.2 and 131.1 (Ph₁-C-2,6, Ph₂-C-2,6), 129.8 (C-7a), 128.6 and 128.5 (Ph₁-C-3,5, Ph₂-C-3,5), 128.3 and 128.1 (Ph₁-C-1, Ph₂-C-1), 126.0 (C-11a), 113.5 (C-3), 107.3 (C-8), 105.1 (C-11), 100.0 (-OCH₂O-), 71.3 (C-1), 70.1 (C-2), 61.5 (C-11c), 56.6 (C-7), 53.5 (C-5), 41.0 (C-11b), 28.2 (C-4), 21.3 and 21.2 (Ph₁-CH₃, Ph₂-CH₃). HRMS: calcd for C₃₂H₃₀NO₆ [M + H], 524.2073; found, 524.2095.

(1*S*,2*S*,3*a*¹*S*,12*bS*)-2,3*a*¹,4,5,7,12*b*-Hexahydro-1*H*-[1,3]dioxolo[4,5-*j*]pyrrolo[3,2,1-*de*]phenanthridine-1,2-diyl-bis(2,3-dimethylbenzoate) (**12**). The reaction of **1** (25 mg, 0.085 mmol) and 2,3-dimethylbenzoyl chloride (30 mg, 0.18 mmol) afforded **12** (15 mg, 32% yield) as a white solid: ¹H NMR (400 MHz, CDCl₃) δ 7.66 (1*H*, d, *J* = 7.3 Hz, Ph₂-H-6), 7.35 (1*H*, d, *J* = 8.2 Hz, Ph₂-H-4), 7.30 (1*H*, d, *J* = 7.5 Hz, Ph₁-H-6), 7.24 (1*H*, d, *J* = 7.4 Hz, Ph₁-H-4), 7.13 (1*H*, t, *J* = 7.8, 7.6 Hz, Ph₂-H-5), 7.06 (1*H*, t, *J* = 7.8, 7.6 Hz, Ph₁-H-5), 6.88 (1*H*, s, H-11), 6.58 (1*H*, s, H-8), 6.13 (1*H*, br s, H-1), 5.91 (1*H*, s, -OCH₂O-), 5.89 (1*H*, s, -OCH₂O-), 5.73 (1*H*, br s, H-3), 5.66 (1*H*, br s, H-2), 4.21 (1*H*, d, *J* = 13.3 Hz, H-7b), 3.56 (1*H*, d, *J* = 13.3 Hz, H-7a), 3.43 (1*H*, m, H-5b), 3.12 (1*H*, d, *J* = 10.4 Hz, H-11b), 2.97 (1*H*, br s, H-11c), 2.72 (2*H*, m, H₂-4), 2.52 (3*H*, s, Ph₂-3-CH₃), 2.46 (1*H*, ddd, *J* = 8.1, 8.1, 8.1 Hz, H-5a), 2.34 (3*H*, s, Ph₁-3-CH₃), 2.30 (3*H*, s, Ph₂-2-CH₃), 2.27 (3*H*, s, Ph₁-2-CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 165.9 and 165.7 (C-1', C-1''), 149.7 and 149.3 (C-10, C-9), 146.2 (C-3a), 141.7 and 141.6 (Ph₁-C-2, Ph₂-C-2), 140.5 and 140.3 (Ph₁-C-3, Ph₂-C-3), 137.4 and 137.0 (Ph₁-C-6, Ph₂-C-6), 132.0 and 131.8 (Ph₁-C-4, Ph₂-C-4), 131.3 and 130.9 (Ph₁-C-1,5, Ph₂-C-1,5), 129.3 (C-7a), 126.6 (C-11a), 113.4 (C-3), 107.0 (C-8), 104.9 (C-11), 101.0 (-OCH₂O-), 70.6 (C-1), 69.3 (C-2), 61.5 (C-11c), 56.4 (C-7), 53.3 (C-5), 41.2 (C-11b), 28.0 (C-4), 21.6, 21.3, 21.0, and 20.9 (Ph₁-2,3-CH₃, Ph₂-2,3-CH₃). HRMS: calcd for C₃₄H₃₄NO₆ [M + H], 552.2308; found, 552.2317.

(1*S*,2*S*,3*a*¹*S*,12*bS*)-2,3*a*¹,4,5,7,12*b*-Hexahydro-1*H*-[1,3]dioxolo[4,5-*j*]pyrrolo[3,2,1-*de*]phenanthridine-1,2-diyl-bis(2,4-dimethylbenzoate) (**13**). The reaction of **1** (25 mg, 0.085 mmol) and 2,4-dimethylbenzoyl chloride (30 mg, 0.18 mmol) afforded **13** (14 mg, 30% yield) as a white solid: ¹H NMR (400 MHz, CDCl₃) δ 7.85 (1*H*, d, *J* = 7.9 Hz, Ph₂-H-6), 7.59 (1*H*, d, *J* = 7.9 Hz, Ph₁-H-6), 6.97–7.06 (4*H*, m, Ph₂-H-3,5, Ph₁-H-3,5),

Scheme 3^a

^a Conditions: Pyridine, lycorine/isocyanate (1:1.1), room temperature, 20 h, 31% yield.

6.87 (1H, s, H-11), 6.58 (1H, s, H-8), 6.12 (1H, br s, H-1), 5.90 (1H, d, $J = 1.3$ Hz, $-\text{OCH}_2\text{O}-$), 5.87 (1H, d, $J = 1.3$ Hz, $-\text{OCH}_2\text{O}-$), 5.71 (1H, br s, H-3), 5.65 (1H, br s, H-2), 4.21 (1H, d, $J = 13.3$ Hz, H-7b), 3.58 (1H, d, $J = 13.3$ Hz, H-7a), 3.43 (1H, m, H-5b), 3.14 (1H, d, $J = 10.4$ Hz, H-11b), 3.00 (1H, br s, H-11c), 2.72 (2H, m, H2-4), 2.61 (3H, s, Ph₂-1-CH₃), 2.55 (1H, ddd, $J = 8.0, 8.0, 8.0$ Hz, H-5a), 2.44 (3H, s, Ph₁-1-CH₃), 2.36 (3H, s, Ph₂-4-CH₃), 2.31 (3H, s, Ph₁-4-CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 166.4 and 166.1 (C-1', C-1''), 150.4 and 149.5 (C-10, C-9), 146.4 (C-3a), 142.7 and 142.6 (Ph₁-C-4, Ph₂-C-4), 140.5 and 140.3 (Ph₁-C-2, Ph₂-C-2), 132.5 and 132.3 (Ph₁-C-3,6, Ph₂-C-3,6), 131.1 and 130.8 (Ph₁-C-1,5, Ph₂-C-1,5), 129.4 (C-7a), 126.4 (C-11a), 113.8 (C-3), 107.3 (C-8), 105.2 (C-11), 100.9 ($-\text{OCH}_2\text{O}-$), 70.9 (C-1), 69.6 (C-2), 61.8 (C-11c), 56.9 (C-7), 53.7 (C-5), 41.0 (C-11b), 28.2 (C-4), 21.9, 21.6, 21.3, and 21.2 (Ph₁-2,4-CH₃, Ph₂-2,4-CH₃). HRMS: calcd for C₃₄H₃₄NO₆ [M + H], 552.2308; found, 552.2320.

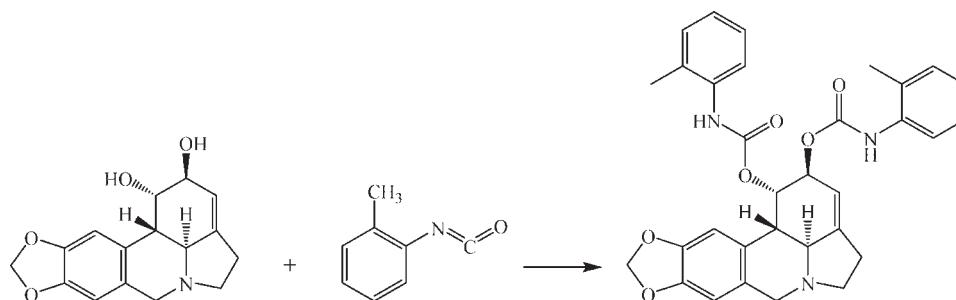
(1*S*,2*S*,3*a*'*S*,12*b**S*)-2,3*a*'*1*,4,5,7,12*b*-Hexahydro-1*H*-[1,3]dioxolo[4,5-*j*]pyrrolo[3,2,1-*de*]phenanthridine-1,2-diyl-bis(2-chlorobenzoate) (**14**). The reaction of **1** (25 mg, 0.085 mmol) and 2-chlorobenzoyl chloride (32 mg, 0.18 mmol) afforded **14** (35 mg, 74% yield) as a white solid: ¹H NMR (400 MHz, CDCl₃) δ 7.88 (1H, d, $J = 7.6$ Hz, Ph₂-H-6), 7.62 (1H, d, $J = 7.6$ Hz, Ph₁-H-6), 7.47-7.41 (2H, m, Ph₂-H-3, Ph₁-H-3), 7.35-7.39 (2H, m, Ph₂-H-4, Ph₁-H-4), 7.30-7.34 (1H, m, Ph₂-H-5), 7.22-7.27 (1H, m, Ph₁-H-5), 6.88 (1H, s, H-11), 6.58 (1H, s, H-8), 6.19 (1H, br s, H-1), 5.90 (1H, d, $J = 1.2$ Hz, $-\text{OCH}_2\text{O}-$), 5.88 (1H, d, $J = 1.2$ Hz, $-\text{OCH}_2\text{O}-$), 5.72 (2H, br s, H-2, 3), 4.19 (1H, d, $J = 14.2$ Hz, H-7b), 3.57 (1H, d, $J = 14.1$ Hz, H-7a), 3.41 (1H, m, H-5b), 3.15 (1H, d, $J = 9.3$ Hz, H-11b), 3.02 (1H, d, $J = 9.0$ Hz, H-11c), 2.71 (2H, m, H2-4), 2.45 (1H, d, $J = 8.3$ Hz, H-5a); ¹³C NMR (100 MHz, CDCl₃) δ 164.9 (C-1'), 164.4 (C-1''), 146.7 (C-3a), 146.6 and 146.4 (C-10, C-9), 134.0 (Ph₂-C-4), 133.7 (Ph₁-C-4), 132.8 (Ph₂-C-2), 132.7 (Ph₁-C-2), 131.8 (Ph₂-C-1), 131.6 (Ph₁-C-1), 131.2 (Ph₂-C-6), 131.0 (Ph₁-C-6), 130.9 (Ph₂-C-1), 129.8 (Ph₂-C-3), 129.7 (Ph₁-C-3), 129.3 (C-7a), 126.6 (Ph₂-C-5), 126.5 (Ph₁-C-5), 126.4 (C-11a), 113.5 (C-3), 107.3 (C-8), 105.1 (C-11), 101.0 ($-\text{OCH}_2\text{O}-$), 71.9 (C-1), 70.5 (C-2), 61.6 (C-11c), 56.9 (C-7), 53.7 (C-5), 40.8 (C-11b), 28.8 (C-4). HRMS: calcd for C₃₀H₂₄Cl₂NO₆ [M + H], 564.0981; found, 564.0992.

(1*S*,2*S*,3*a*'*S*,12*b**S*)-2,3*a*'*1*,4,5,7,12*b*-Hexahydro-1*H*-[1,3]dioxolo[4,5-*j*]pyrrolo[3,2,1-*de*]phenanthridine-1,2-diyl-bis(3-chlorobenzoate) (**15**). The reaction of **1** (25 mg, 0.085 mmol) and 3-chlorobenzoyl chloride (32 mg, 0.18 mmol) afforded **15** (33 mg, 70% yield) as a white solid: ¹H NMR (400 MHz, CDCl₃) δ 8.03 (1H, s, Ph₂-H-2), 7.95 (1H, d, $J = 7.8$ Hz, Ph₂-H-6), 7.85 (1H, s, Ph₁-H-2), 7.79 (1H, d, $J = 7.8$ Hz, Ph₁-H-6), 7.54 (1H, d, $J = 8.0$ Hz, Ph₂-H-4), 7.49 (1H, d, $J = 8.0$ Hz, Ph₁-H-4), 7.39 (1H, t, $J = 7.9$ Hz, Ph₂-H-5), 7.33 (1H, t, $J = 7.9$ Hz, Ph₁-H-5), 6.84 (1H, s, H-11), 6.59 (1H, s, H-8), 6.13 (1H, br s, H-1), 5.89 (1H, d, $J = 1.3$ Hz, $-\text{OCH}_2\text{O}-$), 5.86 (1H, d, $J = 1.3$ Hz, $-\text{OCH}_2\text{O}-$), 5.67 (2H, br s, H-2, 3), 4.22 (1H, d, $J = 14.2$ Hz, H-7b), 3.62 (1H, d, $J = 14$ Hz, H-7a), 3.44 (1H, m, H-5b), 3.16 (1H, d, $J = 10.4$ Hz, H-11b), 3.01 (1H, d, $J = 10.4$ Hz, H-11c), 2.74 (2H, m, H2-4), 2.54 (1H, ddd, $J = 8.2, 8.2, 8.2$ Hz, H-5a); ¹³C NMR

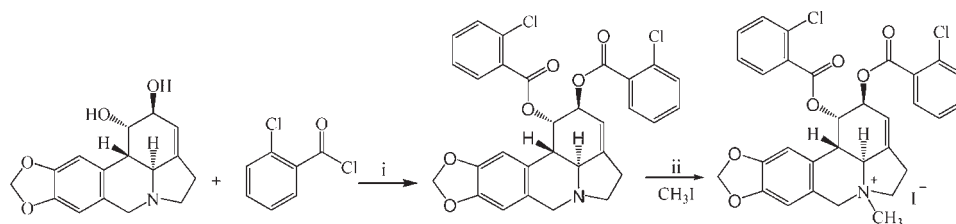
(100 MHz, CDCl₃) δ 164.3 and 164.0 (C-1', C-1''), 146.5 (C-3a), 146.8 and 146.5 (C-10, C-9), 134.6 and 134.5 (Ph₁-C-3, Ph₂-C-3), 133.3 and 133.2 (Ph₁-C-4, Ph₂-C-4), 131.6 and 131.3 (Ph₁-C-1, Ph₂-C-1), 129.9 and 129.8 (Ph₁-C-2, Ph₂-C-2), 129.7 and 129.6 (Ph₁-C-5, Ph₂-C-5), 129.3 (C-7a), 128.1 and 128.0 (Ph₁-C-6, Ph₂-C-6), 126.1 (C-11a), 113.6 (C-3), 107.4 (C-8), 105.0 (C-11), 101.0 ($-\text{OCH}_2\text{O}-$), 71.4 (C-1), 70.0 (C-2), 61.5 (C-11c), 56.8 (C-7), 53.6 (C-5), 41.0 (C-11b), 28.8 (C-4). HRMS: calcd for C₃₀H₂₄Cl₂NO₆ [M + H], 564.0981; found, 564.0989.

(1*S*,2*S*,3*a*'*S*,12*b**S*)-2,3*a*'*1*,4,5,7,12*b*-Hexahydro-1*H*-[1,3]dioxolo[4,5-*j*]pyrrolo[3,2,1-*de*]phenanthridine-1,2-diyl-bis(4-chlorobenzoate) (**16**). The reaction of **1** (25 mg, 0.085 mmol) and 4-chlorobenzoyl chloride (32 mg, 0.18 mmol) afforded **16** (37 mg, 77% yield) as a white solid: ¹H NMR (400 MHz, CDCl₃) δ 8.00 (2H, d, $J = 8.6$ Hz, Ph₂-H-2,6), 7.82 (2H, d, $J = 8.6$ Hz, Ph₁-H-2,6), 7.42 (2H, d, $J = 8.6$ Hz, Ph₂-H-3,5), 7.35 (2H, d, $J = 8.6$ Hz, Ph₁-H-3,5), 6.83 (1H, s, H-11), 6.58 (1H, s, H-8), 6.11 (1H, br s, H-1), 5.89 (1H, d, $J = 1.3$ Hz, $-\text{OCH}_2\text{O}-$), 5.86 (1H, d, $J = 1.3$ Hz, $-\text{OCH}_2\text{O}-$), 5.67 (2H, br s, H-2,3), 4.22 (1H, d, $J = 14.1$ Hz, H-7b), 3.62 (1H, d, $J = 14.1$ Hz, H-7a), 3.44 (1H, m, H-5b), 3.14 (1H, d, $J = 10.4$ Hz, H-11b), 3.01 (1H, d, $J = 10.4$ Hz, H-11c), 2.75 (2H, m, H2-4), 2.53 (1H, ddd, $J = 8.4, 8.4, 8.4$ Hz, H-5a); ¹³C NMR (100 MHz, CDCl₃) δ 164.6 and 164.4 (C-1', C-1''), 146.5 (C-3a), 146.6 and 146.4 (C-10, C-9), 139.7 and 139.6 (Ph₁-C-4, Ph₂-C-4), 131.3 and 131.2 (Ph₁-C-2,6, Ph₂-C-2,6), 128.7 and 128.6 (Ph₁-C-3,5, Ph₂-C-3,5), 128.3 and 128.0 (Ph₁-C-1, Ph₂-C-1), 129.2 (C-7a), 126.1 (C-11a), 113.6 (C-3), 107.4 (C-8), 105.0 (C-11), 100.0 ($-\text{OCH}_2\text{O}-$), 71.2 (C-1), 70.0 (C-2), 61.4 (C-11c), 56.7 (C-7), 53.7 (C-5), 41.1 (C-11b), 28.7 (C-4). HRMS: calcd for C₃₀H₂₄Cl₂NO₆ [M + H], 564.0981; found 564.0973.

(1*S*,2*S*,3*a*'*S*,12*b**S*)-1-Hydroxy-2,3*a*'*1*,4,5,7,12*b*-hexahydro-1*H*-[1,3]dioxolo[4,5-*j*]pyrrolo[3,2,1-*de*]phenanthridin-2-yl-*o*-tolylcarbamate (**17**). 2-Methylphenyl isocyanate (14 mg, 0.11 mmol) was added to a solution of **1** (30 mg, 0.10 mmol) in dry pyridine (2 mL) (Scheme 3). The reaction mixture was stirred at room temperature for 20 h and then carefully poured into ice water (30 mL), causing precipitation. The precipitate was filtered, washed with brine, and purified by flash column chromatography (SiO₂, EtOAc/hexane = 70:30) to afford **17** (13 mg, 31% yield) as a white solid: ¹H NMR (400 MHz, CDCl₃) δ 7.82 (1H, br s, NH), 7.23-7.15 (2H, m, Ph-H-5,6), 7.04 (1H, t, $J = 7.4, 7.4$ Hz, Ph-H-3), 6.81 (1H, s, H-11), 6.62 (1H, s, H-8), 6.59 (1H, m, Ph-H-4), 5.94 (1H, d, $J = 1.4$ Hz, $-\text{OCH}_2\text{O}-$), 5.92 (1H, d, $J = 1.4$ Hz, $-\text{OCH}_2\text{O}-$), 5.58 (1H, br s, H-3), 5.33 (1H, br s, H-2), 4.60 (1H, br s, H-1), 4.16 (1H, d, $J = 14.2$ Hz, H-7b), 3.56 (1H, d, $J = 14.1$ Hz, H-7a), 3.38 (1H, m, H-5b), 2.90 (1H, d, $J = 10.4$ Hz, H-11b), 2.74 (1H, d, $J = 10.4$ Hz, H-11c), 2.67 (2H, m, H2-4), 2.45 (1H, ddd, $J = 8.1, 8.1, 8.1$ Hz, H-5a), 2.25 (3H, s, Ph-CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 153.3 (C-1''), 146.7 (C-3a), 146.4 and 145.9 (C-10, C-9), 135.7 (Ph-C-1), 130.4 (Ph-C-2), 130.0 (C-7a), 129.8 (Ph-C-3), 127.4 (Ph-C-5), 126.9 (Ph-C-4), 125.3 (C-11a), 124.3 (Ph-C-6), 114.0 (C-3), 107.6 (C-8), 104.8 (C-11), 101.0 ($-\text{OCH}_2\text{O}-$), 74.6 (C-1), 69.3 (C-2), 60.7 (C-11c), 56.8 (C-7), 53.6 (C-5), 41.6 (C-11b),

Scheme 4^a

^a Conditions: Pyridine, lycorine/isocyanate (1:2.2) room temperature, 40 h, 29% yield.

Scheme 5^a

^a Conditions: (i) DMAP, pyridine, lycorine/substituted benzoyl chloride (1:2); (ii) CH₃I, CH₃CN, 24 h, 40 °C.

28.7 (C-4), 17.6 (Ph-CH₃). HRMS: calcd for C₂₄H₂₃N₂O₅ [M - H], 419.16070; found, 419.16072.

(1*S*,2*S*,3*a*'*S*,12*bS*)-2,3*a*'*A*,4,5,7,12*b*-Hexahydro-1*H*-[1,3]dioxolo[4,5-*j*]pyrrolo[3,2-*l*]phenanthridin-1,2-diyl-bis(*o*-tolylcarbamate) (**18**). 2-Methylphenyl isocyanate (28 mg, 0.22 mmol) was added to a solution of **1** (30 mg, 0.10 mmol) in dry pyridine (2 mL) (Scheme 4). The reaction mixture was stirred at room temperature for 40 h and then carefully poured into ice water (30 mL). The precipitate that formed was filtered and washed with brine. Purification by flash column chromatography (SiO₂, EtOAc/hexane = 60:35) afforded **18** (16 mg, 29% yield) as a white solid: ¹H NMR (400 MHz, CDCl₃) δ 7.84 (1H, br s, 2'-NH), 7.67 (1H, br s, 1'-NH), 7.23–7.11 (4H, m, Ph₁-H-5,6, Ph₂-H-5,6), 7.05–6.94 (2H, m, Ph₁-H-3, Ph₂-H-3), 6.81 (1H, s, H-11), 6.60 (1H, s, H-8), 6.47 (2H, m, Ph₁-H-4, Ph₂-H-4), 6.28 (1H, br s, H-1), 5.93 (1H, d, *J* = 1.4 Hz, -OCH₂O-), 5.91 (1H, d, *J* = 1.4 Hz, -OCH₂O-), 5.71 (1H, br s, H-3), 5.43 (1H, br s, H-2), 4.19 (1H, d, *J* = 14.2 Hz, H-7b), 3.57 (1H, d, *J* = 14.1 Hz, H-7a), 3.40 (1H, m, H-5b), 2.95 (1H, d, *J* = 10.4 Hz, H-11b), 2.85 (1H, d, *J* = 10.4 Hz, H-11c), 2.69 (2H, m, H₂-4), 2.45 (1H, ddd, *J* = 8.1, 8.1, 8.1 Hz, H-5a), 2.27 (3H, s, Ph₂-CH₃), 2.16 (3H, s, Ph₁-CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 152.8 (C-1''), 152.5 (C-1'), 146.6 (C-3a), 146.4 and 145.9 (C-10, C-9), 135.7 (Ph₂-C-1), 135.4 (Ph₁-C-1), 130.4 (Ph₂-C-2, Ph₁-C-2), 130.3 (Ph₂-C-3, Ph₁-C-3), 129.3 (C-7a), 126.9 (Ph₂-C-5, Ph₁-C-5), 126.8 (C-11a), 126.7 (Ph₂-C-4, Ph₁-C-4), 124.5 (Ph₂-C-6), 124.2 (Ph₁-C-6), 114.4 (C-3), 107.3 (C-8), 105.3 (C-11), 101.0 (-OCH₂O-), 71.8 (C-1), 68.8 (C-2), 61.2 (C-11c), 56.8 (C-7), 53.7 (C-5), 40.5 (C-11b), 28.7 (C-4), 17.7 (Ph₂-CH₃), 17.6 (Ph₁-CH₃). HRMS: calcd for C₃₂H₃₀N₃O₆ [M - H], 552.21020; found, 552.21073.

(1*S*,2*S*,3*a*'*S*,12*bS*)-1,2-bis((2-chlorobenzoyl)oxy)-6-methyl-1,2,3*a*'*A*,5,6,7,12*b*-octahydro-[1,3]dioxolo[4,5-*j*]pyrrolo[3,2-*l*]phenanthridin-6-ium iodide (**19**). Iodomethane (0.5 mL, excess) via cannula was added to a solution of **14** (20 mg, 0.035 mmol) in acetonitrile (2 mL, anhydrous) (Scheme 5). The reaction mixture was stirred under N₂ and heating at 40 °C for 24 h. After completion of the reaction, the

reaction mixture was concentrated, and the residue was purified by SiO₂ open column chromatography (CHCl₃/MeOH, 80:20), affording **19** as a white solid (13 mg, 52% yield): ¹H NMR (400 MHz, CDCl₃) δ 8.02 (1H, s, Ph₂-H-2), 7.93 (1H, d, *J* = 7.6 Hz, Ph₂-H-6), 7.90 (3H, s, N-CH₃), 7.83 (1H, s, Ph₁-H-2), 7.80 (1H, d, *J* = 7.6 Hz, Ph₁-H-6), 7.52 (1H, d, *J* = 8.1 Hz, Ph₂-H-4), 7.47 (1H, d, *J* = 8.1 Hz, Ph₁-H-4), 7.40 (1H, t, *J* = 7.7 Hz, Ph₂-H-5), 7.32 (1H, t, *J* = 7.7 Hz, Ph₁-H-5), 6.82 (1H, s, H-11), 6.60 (1H, s, H-8), 6.12 (1H, br s, H-1), 5.90 (1H, d, *J* = 1.2 Hz, -OCH₂O-), 5.88 (1H, d, *J* = 1.2 Hz, -OCH₂O-), 5.68 (2H, br s, H-2, 3), 4.43 (1H, d, *J* = 14.0 Hz, H-7b), 3.84 (1H, d, *J* = 14.0 Hz, H-7a), 3.54 (1H, m, H-5b), 3.25 (1H, d, *J* = 10.2 Hz, H-11b), 3.14 (1H, d, *J* = 10.2 Hz, H-11c), 2.88 (2H, m, H₂-4), 2.64 (1H, ddd, *J* = 8.0, 8.0, 8.0 Hz, H-5a); ¹³C NMR (100 MHz, CDCl₃) δ 164.4 and 164.1 (C-1', C-1''), 146.5 and 146.3 (C-10, C-9), 135.5 (C-3a), 134.7 and 134.4 (Ph₁-C-3, Ph₂-C-3), 133.6 and 133.3 (Ph₁-C-4, Ph₂-C-4), 131.7 and 131.4 (Ph₁-C-1, Ph₂-C-1), 129.7 and 129.5 (Ph₁-C-2, Ph₂-C-2), 129.3 and 129.0 (Ph₁-C-5, Ph₂-C-5), 128.9 (C-7a), 128.3 and 128.1 (Ph₁-C-6, Ph₂-C-6), 127.1 (C-11a), 121.6 (C-3), 110.3 (C-8), 108.1 (C-11), 100.9 (-OCH₂O-), 82.5 (C-11c), 72.4 (C-1), 71.0 (C-2), 64.8 (C-7), 61.7 (C-5), 47.6 (N-CH₃), 32.0 (C-11b), 23.8 (C-4). HRMS: calcd for C₃₁H₂₆Cl₂NO₆ [M⁺ + H], 578.1137; found, 578.1127.

RESULTS AND DISCUSSION

It was previously shown that 1-*O*-acetyllycorine (**4**) is more active than lycorine against both isolates of *F. columnare*, ALM-00-173 and BioMed.⁸ Therefore, in this study, **4** was selected as a standard to gauge the activities of the lycorine analogues synthesized for evaluation against both genomovars of *F. columnare*. The IC₅₀, MIC, RDCE, and RDCO values obtained for **4** in this study were very similar to those of previous studies;⁸ therefore, results from this study can be compared with those from the previous study with confidence.

Analogues of lycorine were prepared by introducing diversity at the C1-*O*- and C2-*O*-positions while the [1,3]dioxolo-pyrrolo-*[de]*phenanthridine ring was kept intact. Acetylation at the

Table 1. Results of the Bioassay Evaluation of Lycorine Analogues for Toxicity toward *Flavobacterium columnare* ALM-00-173^a

compd	24-h IC ₅₀ ^b	MIC ^c	24-h IC ₅₀ ^b		MIC ^c	
			RDCF ^d	RDCO ^e	RDCF ^d	RDCO ^e
2 ^f	2	4	3	3	10	11
3	17.7 (8.8)	16.6 (16.3)	27.8 (13.8)	26.4 (13.1)	50.5 (49.5)	54.3 (53.2)
4	7.3 (1.1)	3.3 (0)	10.4 (1.8)	9.9 (1.7)	10.0 (0)	10.8 (0)
5	12.2 (0)	40.7 (0)	15.5 (0)	14.8 (0)	100.0 (0)	107.5 (0)
6	>52.3	46.4 (5.9)	>51.8	>49.3	100.0 (0)	107.5 (0)
7	14.6 (1.6)	4.7 (0.6)	16.4 (0.3)	15.6 (0.3)	10.0 (0)	10.8 (0)
8	13.0 (0.2)	42.6 (0)	15.8 (0.3)	15.1 (0.3)	100.0 (0)	107.5 (0)
9	16.6 (7.7)	4.1 (0)	21.3 (9.9)	20.2 (9.4)	10.0 (0)	10.8 (0)
10	>52.3	5.2 (0)	>51.8	>49.3	10.0 (0)	10.8 (0)
11	13.8 (2.9)	46.4 (5.9)	15.3 (1.3)	14.6 (1.3)	100.0 (0)	107.5 (0)
12	13.8 (0)	30.4 (24.9)	13.0 (0)	12.3 (0)	55.0 (45.0)	59.2 (48.4)
13	15.8 (0.9)	5.5 (0)	14.8 (0.8)	14.1 (0.8)	10.0 (0)	10.8 (0)
14	9.4 (5.8)	31.0 (25.4)	8.7 (5.4)	8.3 (5.1)	55.0 (45.0)	59.2 (48.4)
15	13.6 (4.0)	5.6 (0)	12.5 (3.7)	11.9 (3.5)	10.0 (0)	10.8 (0)
16	21.8 (11.6)	31.0 (25.4)	20.0 (10.7)	19.0 (10.1)	55.0 (45.0)	59.2 (48.4)
17	11.2 (1.5)	23.2 (19.0)	13.7 (1.8)	13.1 (1.7)	55.0 (45.0)	59.2 (48.4)
18	3.0 (1.3)	5.5 (0)	2.8 (1.2)	2.6 (1.1)	10.0 (0)	10.8 (0)
19	>70.6	>70.6	>51.8	>49.3	>100.0	>107.5

^a Numbers in parentheses are the standard error of the mean. ^b 24-h IC₅₀ = 50% inhibition concentration in mg/L. ^c Minimum inhibition concentration in mg/L. ^d Relative to drug control florfenicol; numbers closer to "1" indicate greater activity because a value of "1" indicates the same activity compared to drug control. ^e Relative to drug control oxytetracycline; numbers closer to "1" indicate greater activity because a value of "1" indicates the same activity compared to drug control. ^f Values are from ref 10.

Table 2. Results of the Bioassay Evaluation of Lycorine Analogues for Toxicity toward *Flavobacterium columnare* BioMed^a

compd	24-h IC ₅₀ ^b	MIC ^c	24-h IC ₅₀ ^b		MIC ^c	
			RDCF ^d	RDCO ^e	RDCF ^d	RDCO ^e
2 ^f	10	37	11	15	100	108
3	6.1 (0.8)	3.3 (0)	7.8 (1.1)	10.9 (1.5)	10.0 (0)	10.8 (0)
4	9.4 (1.2)	3.3 (0)	12.1 (1.5)	16.9 (2.1)	10.0 (0)	10.8 (0)
5	12.8 (0.2)	40.7 (0)	13.4 (0.2)	18.5 (0.3)	100.0 (0)	107.5 (0)
6	>52.3	22.9 (17.7)	>42.4	>58.8	55.0 (45.0)	59.2 (48.4)
7	14.8 (1.0)	22.9 (17.7)	13.6 (0.9)	18.9 (1.2)	55.0 (45.0)	59.2 (48.4)
8	14.5 (1.3)	42.6 (0)	14.4 (1.3)	20.0 (1.8)	100.0 (0)	107.5 (0)
9	9.3 (0.8)	4.1 (0)	9.8 (0.9)	13.6 (1.2)	10.0 (0)	10.8 (0)
10	>52.3	28.8 (23.6)	>42.4	>58.8	55.0 (45.0)	59.2 (48.4)
11	5.8 (4.3)	22.9 (17.7)	11.7 (1.1)	16.2 (1.5)	100.0 (0)	107.5 (0)
12	14.6 (0.3)	55.2 (0)	11.2 (0.2)	15.6 (0.3)	100.0 (0)	107.5 (0)
13	14.9 (1.7)	30.4 (24.9)	11.4 (1.3)	15.9 (1.8)	55.0 (45.0)	59.2 (48.4)
14	8.5 (6.2)	5.6 (0)	6.4 (4.7)	8.9 (6.5)	10.0 (0)	10.8 (0)
15	13.6 (2.3)	5.6 (0)	10.2 (1.7)	14.2 (2.4)	10.0 (0)	10.8 (0)
16	15.0 (4.3)	5.6 (0)	11.3 (3.2)	15.6 (4.4)	10.0 (0)	10.8 (0)
17	15.0 (1.1)	42.1 (0)	15.0 (1.1)	20.9 (1.5)	100.0 (0)	107.5 (0)
18	3.9 (2.2)	5.5 (0)	3.0 (1.7)	4.1 (2.4)	10.0 (0)	10.8 (0)
19	>70.6	>70.6	>42.4	>58.8	>100.0	>107.5

^a Numbers in parentheses are the standard error of the mean. ^b 24-h IC₅₀ = 50% inhibition concentration in mg/L. ^c Minimum inhibition concentration in mg/L. ^d Relative to drug control florfenicol; numbers closer to "1" indicate greater activity because a value of "1" indicates the same activity compared to drug control. ^e Relative to drug control oxytetracycline; numbers closer to "1" indicate greater activity because a value of "1" indicates the same activity compared to drug control. ^f Values are from ref 10.

C2-O-position was performed initially, yielding 3, to compare the antibacterial effect of the two monoacetylated analogues 3 and 4.

Acetylation at the C2-O-position resulted in weaker activity against *F. columnare* ALM-00-173 (Table 1); the activity against

F. columnare BioMed was similar for both compounds (Table 2). The lycorine diacetate **2** is more active against genomovar ALM-00-173 than monoacetate **3** (Table 1). It must be noted that *F. columnare* ALM-00-173 is considered to be potentially more problematic for catfish producers because genomovar II isolates are more pathogenic for channel catfish.²⁴ To improve the solubility of 1,2-*O,O'*-diacetyllycorine, its hydrochloride salt (**5**) was synthesized. However, this approach did not result in increased antibacterial activity (see Tables 1 and 2).

On the basis of the preceding discussions, esterification at C1 and C2 was performed. Esterification reaction between benzoyl chloride and lycorine yielded novel compounds **6–16**. Of these analogues, **9** and **14** showed activities similar to those of **3** and **4** against *F. columnare* BioMed (see IC₅₀, MIC, RDFC, RDCO values in Table 2). Noticeably, **9** and **14** are distinct from the other benzoyl chloride ester analogues in having a single substituent at the C2-position of the benzoyl ring. This structural feature, however, did not seem to be of significance for activity against *F. columnare* ALM-00-173 as the IC₅₀, MIC, RDFC, and RDCO values were not consistently lower than those of **4** (see Table 1). Compound **9**, which is substituted at both the C1-*O*- and C2-*O*-positions, is more active than the monosubstituted analogue **6**. This is similar to results observed with the acetylated analogues. To improve water solubility, an iodo-salt of **14**, the most active compound among the benzoyl chloride analogues, was prepared, yielding **19**. However, improved solubility did not correspond to improved antibacterial activity.

Carbamates **17** and **18** were synthesized from lycorine and the appropriate isocyanate, via a Curtius reaction mechanism, converting the isocyanate to an amine by nucleophilic addition on the isocyanate. Compound **18** demonstrated high activity against both *F. columnare* isolates, and **18** appears to have stronger antibacterial activity than **4** (Tables 1 and 2). Consistent with the observation with the acetylated analogues, the monosubstituted analogue **17** was less active (Tables 1 and 2). Interestingly, **18** differs from **9** only in having nitrogen between the carbonyl and the toluene group. It appears that the addition of a “spacer unit” between these two moieties might be responsible for improved activity. Notably, **18** inhibited the growth of *F. columnare* ALM-00-173 and *F. columnare* BioMed.

In conclusion, the lycorine analogues synthesized having substitution at both the C1-*O*- and C2-*O*-positions have better antibacterial activity compared to having only one substitution at either carbon. Salt preparations of the disubstituted analogues have better solubility but no enhanced antibacterial activity. The carbamate is more effective than the benzoyl chloride analogues. 1,2-*O,O'*-Diacetyllycorine is highly active and selective toward the genomovar II isolate ALM-00-173; however, we have succeeded in preparing a lycorine analogue, **18**, which had high antibacterial activity against both isolates and considerably better than that of **4**.

Efficacy studies still need to be conducted to determine the potential of **18** for use as an alternative to the antibiotic florfenicol, currently under U.S. Food and Drug Administration conditional approval, or other management approaches (e.g., use of 35% Perox-Aid) for controlling columnaris disease in channel catfish. In addition, palatability studies would need to be conducted to determine the potential for incorporation of **18** into catfish feed. However, results from our in vitro study certainly indicate **18** to be the most promising of the lycorine analogues evaluated in this study.

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