Semisynthesis and Biological Activity of Stemofoline Alkaloids

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Received December 21, 2008

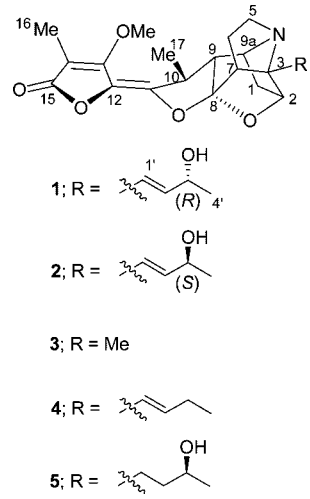
The semisynthesis of the *Stemona* alkaloids (3′*R*)-stemofolenol (**1**), (3′*S*)-stemofolenol (**2**), methylstemofoline (**3**), and (3′*S*)-hydroxystemofoline (**5**) and the unnatural analogues (11*E*)-methylstemofoline (**15**) and 3′*R*-hydroxystemofoline (**11**) has been achieved starting from (11*Z*)-1′,2′-didehydrostemofoline (**4**). This synthesis allowed, for the first time, access to diastereomerically enriched samples of **1** and **2** and the assignment of their absolute configurations at C-3′. These compounds were obtained in sufficient quantities to allow for their biological testing. In a quantitative assay as AChE inhibitors, (11*Z*)-1′,2′-didehydrostemofoline (**4**) and (3′*S*)-hydroxystemofoline (**5**) were found to be the most active.

The *Stemona* family of alkaloids includes more than 80 different natural products that have been structurally classified into eight groups.[1] The pyrrolo[1,2-*a*]azepine (5,7-bicyclic A,B-ring system) nucleus is common to all compounds in six of these groups, while a pyrido[1,2-*a*]azepine A,B-ring system (6,7-bicyclic A,B-ring system) is found in the more recently discovered Stemoncurtisine group of *Stemona* alkaloids.[1−5] A miscellaneous group comprising five *Stemona* alkaloids has also been identified.[1] The pure alkaloids derived from the extracts of the leaves and roots of *Stemona* species have insect toxicity, antifeedant, and insect repellent activities[1,3,4,6−8] and antitussive activities.[9]

In 2005, we reported the structures of six new stemofoline alkaloids, including an inseparable 1:1 mixture of (3′*R*)-stemofolenol (**1**) and (3′*S*)-stemofolenol (**2**) and the first C_{19} stemofoline alkaloid, methylstemofoline (**3**).[10] The latter alkaloid was isolated in only 1.9 mg quantity and was of limited supply for further biological screening. Three known stemofoline alkaloids were also isolated, with the major component (11*Z*)-1′,2′-didehydrostemofoline (**4**) isolated in 100 mg quantities from the root extract of an unidentified *Stemona* species (*Stemona* sp.).[10] We now have access to gram quantities of alkaloid **4** to use as a starting material to prepare sufficient quantities of the alkaloids **1**, **2**, and **3** and their analogues for biological screening. Here we report the semisynthesis of alkaloids **1**−**3** and some of their analogues and the absolute configuration at C-3′ for compounds **1** and **2**. The synthesis of the related alkaloid **5**,[11] isolated from a different *Stemona* plant species, is also described. A qualitative study of the inhibitory activities of these compounds on the nicotinic acetylcholine receptor and their antibacterial activities are also reported.

Results and Discussion

Dihydroxylation of the 3-butenyl side chain of **4** was first attempted using catalytic K_2OsO_4 and stoichiometric NMO, since diastereoselectivity was of little significance in this synthesis. Although the desired diol product was obtained, the unwanted dihydroxylated C-11−C-12 product was also observed. The alternative Sharpless asymmetric dihydroxylation reaction conditions using AD-mix-α were then attempted and resulted exclusively in the dihydroxylation of the butenyl side chain, forming the diol product **6**, which was readily recrystallized from dichloromethane. It is



suspected that the bulky DHQ_2PHAL osmium complex of AD-mix-α influenced the regioselectivity such that the more hindered C-11−C-12 double bond could no longer be approached.

The diol **6** was then oxidatively cleaved using freshly prepared $NaIO_4$ on silica gel[12] to form the aldehyde **7**, with an appropriate handle to which a new alkyl side chain can be attached. The classic Wittig reaction was carried out using 1-(triphenylphosphoranyl-diene)-2-propanone; however, the triphenylphosphine oxide byproduct proved inseparable from the desired product **8** despite washing with diethyl ether and hexane, column chromatography, and attempted recrystallization. The Horner−Wadsworth−Emmons[13] reaction was then utilized and yielded the desired α,*β*-unsaturated ketone **8** via a clean and simple method (Scheme 1).

(*R*)-(+)- and (*S*)-(−)-2-Methyl-CBS-oxazaborolidine were used to reduce the ketone **8** diastereoselectively to give the natural product alcohols **1** (dr >95:<5) and **2** (dr = 85:15), respectively.[14−16] During the isolation of the natural products from *Stemona* sp., the diastereomers **1** and **2** could not be separated. These diastereomers are so similar that their NMR data differ only in the ^{13}C NMR chemical shift of C-4′. A ^{13}C NMR experiment was run at 125 MHz with a long acquisition time so as to more accurately calculate the ratio of diastereomers (3′*R*)-**1** (23.4 ppm) and (3′*S*)-**2** (23.5 ppm) formed from the reduction of **8**. The configuration of the (*R*)-alcohol **1** was confirmed by synthesizing its (*R*)-**9** and (*S*)-**10** Mosher esters, from (*S*)- and (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPACl), respectively. Analysis of the (*R*)-Mosher ester **10** by ^{19}F NMR gave a ratio of 95:5 (3′*R*,*R*:3′*S*,*R*), a result endorsed

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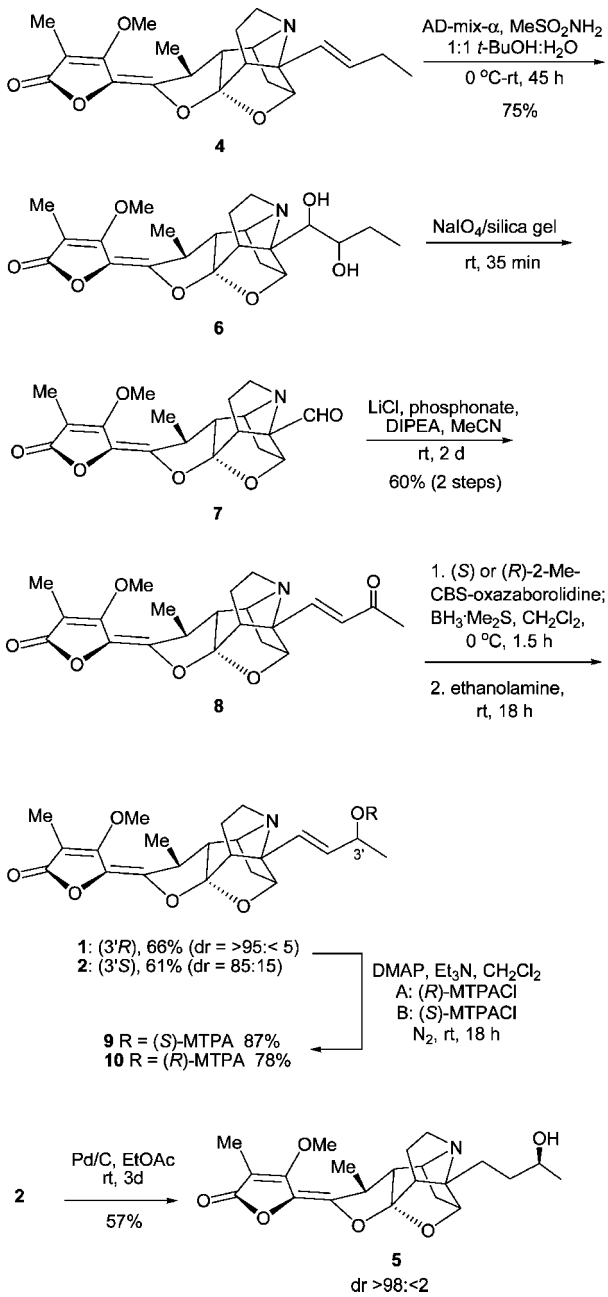
10.1021/np800806b CCC: $40.75 © 2009 American Chemical Society and American Society of Pharmacognosy
Published on Web 02/17/2009

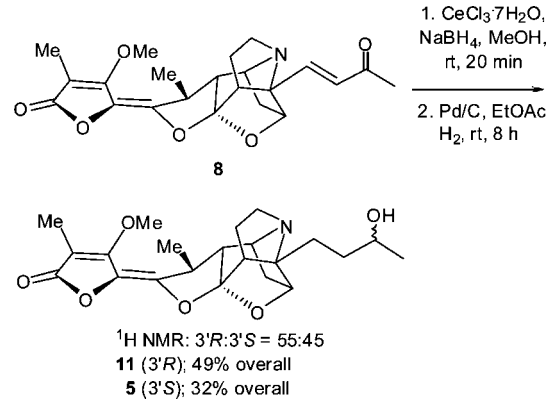
Scheme 1. Synthesis of Compounds **1**−**3**



by analysis of the (*S*)-Mosher ester **9** by ^{19}F NMR, which gave a ratio of 94:6 (3′*R*,*S*:3′*S*,*S*). For the determination of the C-3′ configuration, the method provided by Mosher[17] and modified by Ohtani[18] was followed where $\delta S - \delta R > 0$ for an alcohol of *R*-configuration and $\delta S - \delta R < 0$ for an alcohol of *S*-configuration. The C-4′ methyl group resonated at δ 1.42 for the (*S*)-Mosher ester **9** and at δ 1.38 for the (*R*)-Mosher ester **10**. Therefore, the configuration at C-3′ for **1** was assigned as *R*. According to the literature, this is the expected configuration for the reduction of **8** using the (*S*)-oxazaborolidine.[14−16]

The natural product **5** was simply prepared by hydrogenation of **2** over 10% Pd/C in the presence of H_2 gas. This compound was obtained as a single diastereomer (dr >98:<2) after purification by column chromatography (Scheme 1). Its spectroscopic data were identical to that of the natural product.[11] Although this reaction was successful and allowed for the determination of configuration, a more efficient synthesis was employed whereby the ketone **8** was first reduced using sodium borohydride to give approximately a 1:1 mixture of diastereomers (Scheme 2). This mixture was then

Scheme 2. Synthesis of (3′*S*)-Hydroxystemofoline (**5**)



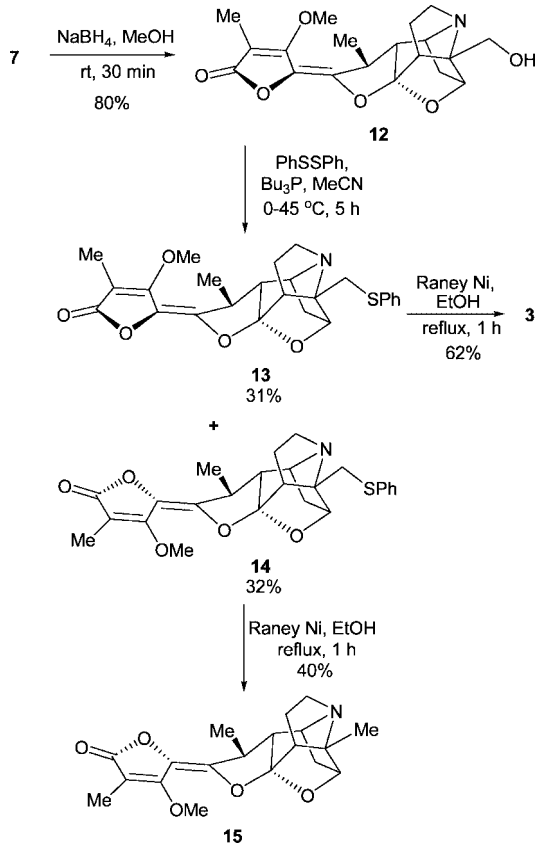
hydrogenated to give the separable isomers **11** and **5** in 49% and 32% overall yields, respectively, from **8**.

Synthesis of the natural product methylstemofoline (**3**) from the aldehyde **7** proved quite challenging. Several methods were attempted before at last achieving success. First, formation of the corresponding thioacetal (1,2-ethanedithiol, $BF_3 \cdot Et_2O$)[19] from the aldehyde **7** was achieved, but subsequent removal by Raney Ni was not successful at providing a clean sample of **3** in reasonable yield. The Wolff−Kishner reaction was then employed and the *p*-toluenesulfonyl hydrazone derivative formed easily.[20] However, reductive removal of this hydrazone group was difficult due to its high insolubility. Benzoic acid/$BH_3 \cdot THF$,[21] $NaBH(OAc)_3$,[22] and $NaBH_4$[22] all failed to displace the hydrazone. The hydrazone was then reduced to the hydrazine using $NaCNBH_3$ and *p*-toluenesulfonic acid,[23] but treatment with $NaOAc \cdot 3H_2O$,[24] then DBU, and finally NaOAc failed to produce a pure sample of **3**. As a result, the Wolff−Kishner reaction was abandoned and the aldehyde **7** was reduced to the alcohol **12**, which then underwent mesylation. The mesyl group could not be displaced by treatment with $LiEt_3BH$[25] but did react with sodium thiophenolate to give the corresponding thioether as a mixture of (*Z*)-**13** and (*E*)-**14** isomers (Scheme 3). In an attempt to reduce the number of steps involved in this synthesis, the alcohol **12** was displaced directly by diphenyldisulfide and tributylphosphine rather than going through the mesylate (Scheme 3).[26] It was observed that this reaction also resulted in isomerization of the C-11−C-12 double bond to form the separable *Z*- and *E*-isomers **13** and **14**, respectively. This isomerization was thought to have occurred by attack of the thiophenolate ion at C-11 of **13**, followed by bond rotation around the C-11−C-12 bond and then elimination of the thiophenolate ion, as illustrated by the mechanism in Scheme 4. The *Z*-isomer **13** was identified by the NOE cross-peak between the methoxy group and the C-17 methyl, a correlation that was absent in the *E*-isomer **14** (Scheme 4). Separate treatment of the thioethers (*Z*)-**13** and (*E*)-**14** with Raney Ni[19] gave methylstemofoline **3** and its unnatural *E*-isomer **15**, respectively. It must be noted that the ^{1}H NMR data reported by us[10] for the natural product **3** did not agree at first with our synthetic sample, whereas the TLC was identical. However, when base-treated $CDCl_3$ was used for NMR analysis of the authentic natural product and our synthetic sample **3**, the NMR data matched perfectly. Thus, the original ^{1}H NMR data reported for **3** require correction.[10]

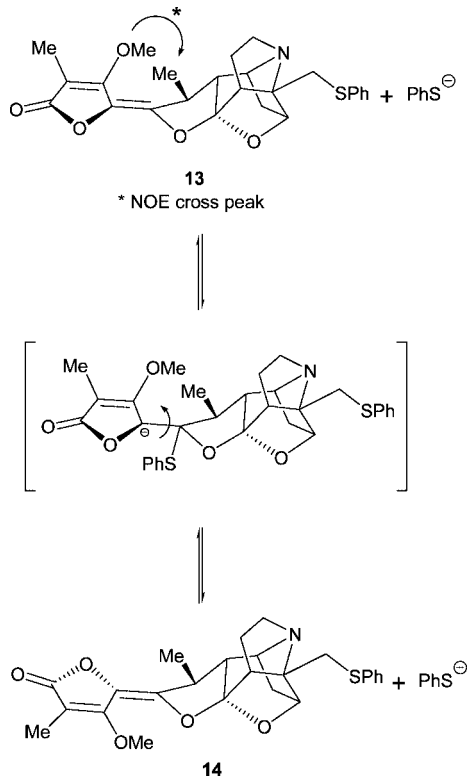
The insecticidal activity shown by the root extracts of *Stemona* plants has been associated with the acetylcholinesterase (AChE) inhibitory activities of their alkaloid components.[8,27] Compounds **1**−**5**, **11**, and **15** were therefore screened by TLC bioautography for their AChE inhibitory activities using the qualitative method of Hostettmann et al.[28] with physostigmine as a reference compound. The results are shown in Table 1.

Compound **4** showed the highest inhibitory activity of AChE with a minimum inhibitory quantity of 5 ng, followed by compound

Scheme 3. Synthesis of Methylstemofoline (**3**) and its *E*-Isomer **15**.



Scheme 4. Proposed Mechanism for Isomerization of **13**



5, having the 3′*S*-configuration, at 10 ng. The compounds **2** and **5**, also with the 3′*S*-configuration, have a higher inhibitory activity than their corresponding compounds with the 3′*R*-configuration (**1** and **11**, respectively). Methylstemofoline (**3**) has a significantly higher activity than its corresponding *E*-isomer **15** and was equally or more potent than compounds **11** and **1**, respectively, with the 3′*R*-configuration. The configuration of the 3′-hydroxy thus seems important for AChE activity. The 3′*R*-configuration seems to be deleterious for AChE activity.

Table 1. Minimum Concentration of Sample Found to Inhibit AChE as Indicated by a White Zone of Inhibition

compound	minimum inhibitory requirement	
	ng	nmol
physostigmine	0.1	3.6×10^{-4}
1 (*R*)[a]	500	1.25
2 (*S*)[b]	100	2.5×10^{-1}
3 (*Z*)	100	2.9×10^{-1}
4	5	1.3×10^{-2}
5 (*S*)[c]	10	2.5×10^{-2}
11 (*R*)[d]	100	2.5×10^{-1}
15 (*E*)	500	1.5

[a] 90:10 mixture of **1** and **2**. [b] 85:15 mixture of **2** and **1**. [c] 91:9 mixture of **5** and **11**. [d] 93:7 mixture of **11** and **4**.

Compounds **1**−**3**, **5**, **11**, and **15** were also tested for their antimicrobial activity against *E. coli, S. aerueus*, and *Ps. aeruginosa* (antibacterial) and *C. albicans* (antifungal). A broth dilution technique[29] was followed using gentamycin (MIC 1.41 μg/mL on *S. aerueus* and *Ps. aeruginosa*) and amphotericin (MIC 0.35 μg/mL on *C. albicans*) as reference compounds. Compounds **1** and **11**, having the 3′*R*-configuration, showed weak activity on *Ps. aeruginosa*, with MIC values of 62.5 μg/mL. The other compounds were not active, with MIC values equal to or greater than 125 μg/mL. Compounds **1**−**3**, **5**, **11**, and **15** were not active (MIC values equal to or greater than 125 μg/mL) on *S. aerueus* or *C. albicans.*

In summary, having access to (11*Z*)-1′,2′-didehydrostemofoline (**4**) has allowed the semisynthesis of the *Stemona* alkaloids (3′*R*)-stemofolenol (**1**), (3′*S*)-stemofolenol (**2**), methylstemofoline (**3**), and (3′*S*)-hydroxystemofoline (**5**) and their unnatural analogues (3′*R*)-hydroxystemofoline (**11**) and (11*E*)-methylstemofoline (**15**) in sufficient quantities for biological testing. This synthesis allowed, for the first time, access to diastereomerically enriched samples of **1** and **2** and the assignment of their absolute configurations at C-3′. While the alkaloids **4** and **5** appear to have the highest AChE inhibitory activities, studies are continuing on the insecticidal activity of these alkaloids on insects of importance to the agricultural industry.

Experimental Section

General Experimental Procedures. Melting points were determined by a Reichert hot-stage melting point apparatus and are uncorrected. Optical rotations were measured using a JASCO DIP-370 polarimeter. IR spectra were obtained on a Nicolet-Avatar 360 FTIR spectrophotometer. Unless otherwise noted, ^{1}H (500 MHz), ^{13}C (125 MHz), and 2D NMR spectra were recorded on a Varian Unity 500 spectrometer. ^{1}H NMR assignments were achieved with the aid of gCOSY and, in some cases, NOESY experiments. ^{13}C NMR assignments were based on DEPT, gHSQC, and gHMBC experiments. High-resolution EIMS were recorded on a Fison/VG Autospec-TOF-oa mass spectrometer (70 eV). High-resolution ESIMS were obtained with a Micromass QTOF 2 mass spectrometer using a cone voltage of 30 V and polyethyleneglycol (PEG) as an internal reference. TLC was performed on aluminum-backed Merck 60 GF_{254} silica gel, and bands were detected by UV light (λ 254 nm) and Dragendorff's reagent. Column chromatography was performed using Merck GF_{254} flash silica gel (40−63 μm). All compounds (unless specified as mixtures of diastereomers) were homogeneous by TLC analysis and judged to be of >95% purity based upon ^{1}H NMR analysis. For NMR assignments, all protons and carbons are numbered according to those of (11*Z*)-1′,2′-didehydrostemofoline (**4**). Physostigmine (esterine) and acetylcholinesterase (906 U/mg, from electric eel) were purchased from Sigma-Aldrich.

(11*Z*)-1′,2′-Dihydroxystemofoline (6). To a flask containing AD-mix-α (0.182 g) and methanesulfonamide (24.7 mg, 0.260 mmol) in 1:1 *tert*-butanol/water (1 mL) at 0 °C was added a solution of (11*Z*)-1′,2′-didehydrostemofoline (**4**) (50.0 mg, 0.130 mmol) in 1:1 ratio of *tert*-butanol/water (1 mL). The reaction mixture was allowed to warm to rt and left to stir for 3 days. Sodium sulfite (0.2 g) was then added to the reaction and left at rt for 1 h. The mixture was extracted with chloroform (3 × 20 mL), and the combined organic extracts were washed with 2 M KOH, dried over $MgSO_4$, and evaporated *in vacuo*. Product **6** was obtained as a white solid (41.0 mg, 0.0979 mmol, 75% yield) of sufficient purity (>95%) to continue to the next step. A small amount was purified by column chromatography for characterization. Diol **6** can also be recrystallized from CH_2Cl_2 to yield pure compound. R_f = 0.28 in MeOH/EtOAc (1:4); mp 188−190 °C (color changed to brown); $[\alpha]_D^{22}$ +252.9 (*c* 0.89, $CHCl_3$); IR ν_{max} 3201, 2340, 1735, 1613, 1144, 1001 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.24 (s, 1H, H-2), 4.14 (s, 3H, OCH_3), 3.74 (s, 1H, H-1′$_\beta$), 3.56 (t, *J* = *J* 6.5 Hz, 1H, H-2′$_\beta$), 3.53 (bs, 1H, H-9a), 3.24 (m, 1H, H-5$_a$), 3.09 (m, 1H, H-10), 3.02 (m, 1H, H-5$_b$), 2.98 (d, *J* = 6.0 Hz, 1H, H-7), 2.32 (m, 1H, H-6$_a$), 2.08 (s, 3H, H-16), 2.02 (d, *J* = 12.5 Hz, 1H, H-1$_a$), 1.84 (m, 2H, H-6$_b$, H-9), 1.78 (m, 1H, H-1$_b$), 1.64 (sextet, *J* = 7.0 Hz, 1H, H-3′$_a$), 1.54 (quintet, *J* = 7.0 Hz, 1H, 3′$_b$), 1.38 (d, *J* = 6.5 Hz, 3H, H-17), 0.97 (t, *J* = 7.0 Hz, 3H, H-4′); ^{13}C NMR ($CDCl_3$) δ 169.6 (C-15), 162.7 (C-13), 147.9 (C-11), 128.0 (C-12), 112.5 (C-8), 98.7 (C-14), 86.3 (C-3), 77.5 (C-2), 73.6 (C-2′), 73.0 (C-1′), 61.7 (C-9a), 58.8 (O-CH_3), 50.6 (C-7), 50.5 (C-5), 47.6 (C-9), 34.4 (C-10), 32.8 (C-1), 28.5 (C-6), 26.1 (C-3′), 18.2 (C-17), 10.0 (C-4′), 9.1 (C-16); EIMS *m/z* 419 (28%) $[M]^+$; HREIMS *m/z* 419.1949 $[M]^+$, calcd for $C_{22}H_{29}NO_7$ 419.1940.

(5*Z*)-5-[(2*S*,2a*R*,6*S*,7a*S*,7b*S*,8*R*,9*S*)-7b-(1*E*)-1-Formylhexahydro-9-methyl-4*H*-2,2,6-(epoxy[1]propanyl[3]ylidene)furo[2,3,4-*gh*]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5*H*)-furanone (7). To a vigorously stirred suspension of silica gel (1.83 g) and diethyl ether (9.2 mL) was added a warm (ca. 40 °C) solution of $NaIO_4$ (177 mg, 0.826 mmol) in water (1.5 mL). Diol **6** (266.0 mg, 0.635 mmol) was dissolved in chloroform (1 mL) and added to the silica gel/$NaIO_4$ mixture, which was left to stir at rt for 1 h. The mixture was filtered and the silica gel was washed with chloroform. Impure product **7** was obtained as a yellow oil and taken immediately through to the next step to avoid decomposition due to the instability of the aldehyde. R_f = 0.15 in MeOH/EtOAc (1:4); IR ν_{max} 2919, 2848, 1726, 1619, 1071, 1020 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.73 (s, 1H, C$\underline{H}$O), 4.61 (s, 1H, H-2), 4.15 (s, 3H, O-CH_3), 3.62 (s, 1H, H-9a), 3.26 (s, 1H, H-7), 3.15 (dd, *J* = 7.5 Hz, 6.5 Hz, 2H, 2 × H-5), 3.10 (m, 1H, H-10), 2.08 (s, 3H, H-16), 2.05 (s, 1H, H-1$_a$), 1.89 (m, 3H, 2 × H-6, H-9), 1.86 (m, 1H, H-1$_b$), 1.40 (d, *J* = 6.0 Hz, 3H, H-17); ^{13}C NMR ($CDCl_3$) δ 197.6 (CHO), 169.6 (C-15), 162.6 (C-13), 147.5 (C-11), 128.1 (C-12), 112.6 (C-8), 98.9 (C-14), 89.4 (C-3), 76.5 (C-2), 61.2 (C-9a), 58.9 (O-CH_3), 49.4 (C-7), 49.2 (C-5), 47.9 (C-9), 34.4 (C-10), 33.5 (C-1), 26.9 (C-6), 18.2 (C-17), 9.1 (C-16); EIMS *m/z* 359 (27%) $[M]^+$; HREIMS *m/z* 359.1363 $[M]^+$, calcd for $C_{19}H_{21}NO_6$ 359.1369.

(5*Z*)-5-[(2*S*,2a*R*,6*S*,7a*S*,7b*S*,8*R*,9*S*)-7b-(1*E*)-1-(3′-Oxo-1′-butenyl-)hexahydro-9-methyl-4*H*-2,2,6-(epoxy[1]propanyl[3]ylidene)furo[2,3,4-*gh*]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5*H*)-furanone (8). To a flask containing LiCl (29.6 mg, 0.699 mmol) in acetonitrile (7.1 mL) under a nitrogen atmosphere was added diethyl (2-oxopropyl)phosphonate (135.7 mg, 0.699 mmol, 0.13 mL), *N*,*N*-diisopropylethylamine (75.3 mg, 0.582 mmol, 0.10 mL), and aldehyde **7** (209.1 mg of crude from previous reaction, 0.582 mmol) dissolved in acetonitrile (1 mL). The reaction mixture was allowed to stir at rt for 2 days. The mixture was diluted with water (20 mL) and extracted with $CHCl_3$ (3 × 20 mL), and the combined organic extracts were washed with brine (20 mL), dried over $MgSO_4$, and evaporated *in vacuo*. Purification by column chromatography using EtOAc to MeOH/EtOAc (15:85) as eluent gave ketone **8** as a semisolid in 60% yield from **6** (151.9 mg, 0.381 mmol). R_f = 0.33 in MeOH/EtOAc (1:4); $[\alpha]_D^{21}$ +327.4 (*c* 0.39, $CHCl_3$); IR ν_{max} 2955, 1742, 1675, 1619, 968 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.81 (d, *J* = 15.8 Hz, 1H, H-2′), 6.38 (d, *J* = 15.8 Hz, 1H, H-1′), 4.32 (s, 1H, H-2), 4.15 (s, 3H, O-CH_3), 3.56 (bs, 1H, H-9a), 3.13−3.02 (m, 3H, 2 × H-5, H-10), 2.97 (t, *J* = 3.3 Hz, 1H, H-7), 2.29 (s, 3H, H-4′), 2.08 (s, 3H, H-16), 1.99 (d, *J* = 12.3 Hz, 1H, H-1$_a$), 1.88−1.82 (m, 3H, 2 × H-6, H-9), 1.77 (dt, *J* = 12.3, 3.3 Hz, 1H, H-1$_b$), 1.40 (d, *J* = 6.6 Hz, 3H, H-17); ^{13}C NMR (75 MHz, $CDCl_3$) δ 197.9 (CO), 169.6 (C-15), 162.7 (C-13), 147.9 (C-11), 143.8 (C-2′), 130.6 (C-1′), 128.1 (C-12), 112.7 (C-8), 98.8 (C-14), 83.2 (C-3), 80.0 (C-2), 61.0 (C-9a), 58.9 (O-CH_3), 52.4 (C-7), 48.2 (C-5), 47.6 (C-9), 34.5 (C-10), 32.7 (C-1), 27.7 (C-4′), 26.8 (C-6), 18.3 (C-17), 9.2 (C-16); EIMS *m/z* 399 (23%) $[M]^+$; HREIMS *m/z* 399.1687 $[M]^+$, calcd for $C_{22}H_{25}NO_6$ 399.1682.

(3′*R*)-Stemofolenol (1). To a solution of (*S*)-(−)-2-methyl-CBS-oxazaborolidine (4.46 mg, 0.0161 mmol) and 1 M borane-methyl sulfide complex in CH_2Cl_2 (0.01 mL) at 0 °C was added ketone **8** (32.1 mg, 0.0805 mmol) in CH_2Cl_2 (1 mL). The mixture was allowed to stir at 0 °C for 1.5 h, after which time ethanolamine (1 mL) was added and the mixture was allowed to stir at rt for 18 h. The reaction mixture was quenched with methanol (2 mL), diluted with saturated sodium bicarbonate solution (20 mL), and extracted with chloroform (3 × 20 mL). The combined organic extracts were washed with brine (40 mL), dried over $MgSO_4$, and evaporated *in vacuo*. Purification by column chromatography using EtOAc to MeOH/EtOAc (1:9) as eluent gave **1** as a white solid in 66% yield (21.4 mg, 0.0534 mmol, dr = >95:<5). R_f = 0.15 in MeOH/EtOAc (1:4); mp 180 °C (color changed to brown), 194−198 °C; $[\alpha]_D^{22}$ +227.0 (*c* 0.72, $CHCl_3$); IR ν_{max} 3649, 2950, 1737, 1618, 1007 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.86 (dd, *J* = 15.6 Hz, 5.1 Hz, 1H, H-2′), 5.75 (dd, *J* = 15.6 Hz, 0.9 Hz, 1H, H-1′), 4.36 (dquintet, *J* = 6.3 Hz, 0.9 Hz, 1H, H-3′), 4.24 (bs, 1H, C-2), 4.14 (s, 3H, O-CH_3), 3.53 (bs, 1H, H-9a), 3.09 (m, 1H, H-10), 3.02 (m, 1H, H-5$_a$), 3.00 (m, 1H, H-5$_b$), 2.88 (d, *J* = 5.1 Hz, 1H, H-7), 2.08 (s, 3H, H-16), 1.97 (d, *J* = 12.0 Hz, 1H, H-1$_a$), 1.88−1.81 (m, 3H, 2 × H-6, H-9), 1.79 (m, 1H, H-1$_b$), 1.38 (d, *J* = 6.3 Hz, 3H, H-17), 1.28 (d, *J* = 6.6 Hz, 3H, H-4′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.7 (C-15), 162.8 (C-13), 148.2 (C-11), 135.3 (C-2′), 127.9 (C-12), 127.2 (C-1′), 112.7 (C-8), 98.6 (C-14), 82.8 (C-3), 80.4 (C-2), 68.1 (C-3′), 60.9 (C-9a), 58.8 (O-CH_3), 51.4 (C-7), 48.0 (C-5), 47.6 (C-9), 34.5 (C-10), 32.8 (C-1), 26.8 (C-6), 23.4 (C-4′), 18.3 (C-17), 9.1 (C-16); NMR data agreed with that of the natural product, taken from a 1:1 mixture of **1** and **2**;[10] EIMS *m/z* 401 (28%) $[M]^+$; HREIMS *m/z* 401.1823 $[M]^+$, calcd for $C_{22}H_{27}NO_6$ 401.1838.

(3′*S*)-Stemofolenol (2). The title compound was prepared via a similiar method to the synthesis of **1**, using (*R*)-(+)-2-methyl-CBS-oxazaborolidine (13.8 mg, 0.04987 mmol) and ketone **8** (39.8 mg, 0.09975 mmol) to give **2** as a white solid in 61% yield (24.4 mg, 0.06085 mmol, dr = 85:15). R_f = 0.15 in MeOH/EtOAc (1:4); mp 180 °C (color changed to brown), 194−198 °C; $[\alpha]_D^{21}$ +212 (*c* 0.55, $CHCl_3$); IR ν_{max} 3411, 2966, 1747, 1619, 1004 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.85 (dd, *J* = 15.6 Hz, 5.7 Hz, 1H, H-2′), 5.74 (d, *J* = 15.6 Hz, 1H, H-1′), 4.36 (dquintet, *J* = 5.7 Hz, 0.9 Hz, 1H, H-3′), 4.24 (bs, 1H, C-2), 4.14 (s, 3H, O-CH_3), 3.53 (bs, 1H, H-9a), 3.10 (m, 1H, H-10), 3.03 (m, 1H, H-5$_a$), 3.00 (m, 1H, H-5$_b$), 2.88 (d, *J* = 4.8 Hz, 1H, H-7), 2.08 (s, 3H, H-16), 1.96 (d, *J* = 12.0 Hz, 1H, H-1$_a$), 1.88−1.81 (m, 3H, 2 × H-6, H-9), 1.80 (m, 1H, H-1$_b$), 1.38 (d, *J* = 6.3 Hz, 3H, H-17), 1.28 (d, *J* = 5.7 Hz, 3H, H-4′); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.7 (C-15), 162.8 (C-13), 148.2 (C-11), 135.4 (C-2′), 127.9 (C-12), 127.1 (C-1′), 112.7 (C-8), 98.6 (C-14), 82.8 (C-3), 80.4 (C-2), 68.0 (C-3′), 60.9 (C-9a), 58.8 (O-CH_3), 51.4 (C-7), 48.0 (C-5), 47.6 (C-9), 34.5 (C-10), 32.7 (C-1), 26.8 (C-6), 23.5 (C-4′), 18.3 (C-17), 9.2 (C-16); NMR data agreed with that of the natural product, taken from a 1:1 mixture of **1** and **2**;[10] EIMS *m/z* 401 (42%) $[M]^+$; HREIMS *m/z* 401.1828 $[M]^+$, calcd for $C_{22}H_{27}NO_6$ 401.1838.

Preparation of (*S*)-MTPA Ester of 1 [(*R*,*S*)-(9)]. To a solution of alcohol **1** (5.2 mg, 0.0130 mmol), 4-di(methylamino)pyridine (3.49 mg, 0.0285 mmol), and triethylamine (20.8 μL) in dry CH_2Cl_2 (0.5 mL) was added (*R*)-Mosher's acid chloride [(*R*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPACl)] (10.0 mg, 0.0396 mmol) under a N_2 atmosphere, and the mixture was allowed to stir at rt for 23 h. The solvent was removed *in vacuo* and the product was purified by column chromatography, using EtOAc to MeOH/EtOAc (1:9) as eluent to give (*R*,*S*)-**9** as an oil in 87% yield (7.0 mg, 0.0113 mmol, dr = 93:7). R_f = 0.57 in MeOH/EtOAc (1:9); ^1H NMR ($CDCl_3$) δ 7.51 (d, *J* = 7.0 Hz, 2H, ArH), 7.39 (m, 3H, ArH), 5.74 (m, 2H, H-2′, H-3′), 5.63 (t, *J* = 6.0 Hz, 1H, C-1′), 4.15 (s, 1H, H-2), 4.14 (s, 3H, O-CH_3), 3.57 (s, 3H, COC-O$\underline{C}H_3$), 3.49 (s, 1H, H-9a), 3.08 (m, 1H, H-10), 2.95 (m, 2H, 2 × H-5), 2.76 (d, *J* = 5.5 Hz, 1H, H-7), 2.08 (s, 3H, H-16), 1.94 (d, *J* = 12.5 Hz, 1H, H-1$_a$), 1.81 (m, 2H, H-1$_b$, H-9), 1.75 (m, 2H, 2 × H-6), 1.42 (d, *J* = 6.5 Hz, 3H, H-4′), 1.37 (d, *J* = 6.5 Hz, 3H, H-17); ^{13}C NMR ($CDCl_3$) δ 169.7 (C-15), 165.6 (O-$\underline{C}$O), 162.8 (C-13), 148.2 (C-11), 132.3 (C-2′), 131.2 (Ar−C), 129.7 (Ar−C), 129.4 (Ar−C), 128.4 (C-1′), 127.2 (C-12), 112.7 (C-8), 98.7 (C-14), 82.8 (C-3), 80.1 (C-2), 72.8 (C-3′), 60.8 (C-9a), 58.8 (O-CH_3), 55.4 ($CF_3CO\underline{C}H_3$), 51.8 (C-7), 48.0 (C-5), 47.6 (C-9), 34.5 (C-10), 32.6 (C-

1), 31.9, 29.7, 29.3, 26.7 (C-6), 22.7 (C-4′), 20.3, 18.3 (C-17), 14.1, 9.2 (C-16); ESIMS *m/z* 617.7 (100%), 618.8 (36%) $[MH]^+$; HRESIMS *m/z* 618.2324 $[MH]^+$, calcd for $C_{32}H_{35}NO_8F_3$ 618.2315.

Preparation of (*R*)-MTPA Ester of 1 [(*R*,*R*)-10]. To a solution of alcohol **1** (7.6 mg, 0.0190 mmol), DMAP (5.09 mg, 0.0417 mmol), and triethylamine (30.3 μL) in dry CH_2Cl_2 (0.5 mL) was added (*S*)-Mosher's acid chloride [(*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPACl)] (15.0 mg, 0.0606 mmol) under a N_2 atmosphere, and the mixture was allowed to stir at rt for 17 h. The solvent was removed *in vacuo*, and the product was purified by column chromatography, using EtOAc to MeOH/EtOAc (1:9) as eluent to give (*R*,*R*)-**10** as an oil in 78% yield (9.1 mg, 0.0147 mmol, dr = 95:5). $R_f = 0.57$ in MeOH/EtOAc (1:9); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.51 (m, 2H, ArH), 7.40 (m, 3H, ArH), 5.86 (d, *J* = 3.0 Hz, 2H, H-2′, H-3′), 5.62 (sextet, *J* ca. 3.0 Hz, 1H, C-1′), 4.19 (s, 1H, H-2), 4.14 (s, 3H, O-CH_3), 3.53 (s, 3H, COC-O$\underline{C}H_3$), 3.51 (s, 1H, H-9a), 3.09 (m, 1H, H-10), 2.99 (m, 2H, 2 × H-5), 2.83 (m, 1H, H-7), 2.08 (s, 3H, H-16), 1.95 (d, *J* = 12.3 Hz, 1H, H-1_a), 1.81 (m, 2H, H-1_b, H-9), 1.76 (m, 2H, 2 × H-6), 1.38 (d, *J* = 3.9 Hz, 3H, H-4′), 1.36 (d, *J* = 3.9 Hz, 3H, H-17); ESIMS *m/z* 618.7 (26%) $[MH]^+$, 617.7 (100%); HRESIMS *m/z* 618.2322 $[MH]^+$, calcd for $C_{32}H_{35}NO_8F_3$ 618.2315.

(3′*R*)- and (3′*S*)-Stemofolenol (1 and 2). (Scheme 2). To a solution of ketone **8** (19.6 mg, 0.049 mmol) in anhydrous methanol (3.8 mL) was added cerium(III) chloride heptahydrate (18.3 mg, 0.0491 mmol) at rt under a N_2 atmosphere. Sodium borohydride (1.86 mg, 0.0491 mmol) was slowly added, and the mixture was allowed to stir at rt for 20 min. The reaction was quenched with water (20 mL) and the product extracted with chloroform (3 × 20 mL). The combined organic extracts were dried over $MgSO_4$, and the solvent was removed *in vacuo* to give a mix of **1** and **2** in a ratio of 47:53 as an off-white solid in 98% yield (19.4 mg, 0.0484 mmol). NMR data were the same as those for **1** and **2**, respectively.

(3′*R*)-Hydroxystemofoline (11) and (3′*S*)-Hydroxystemofoline (5). (Scheme 2). To a solution of the mixture of **1** and **2** (115.7 mg, 0.289 mmol) in ethyl acetate (4.4 mL) was added Pd/C (11.6 mg, 10% w/w) and allowed to stir at rt under a H_2 atmosphere (balloon) for 7 h. The progress of the reaction was carefully monitored by MS. Once complete the mixture was filtered through Celite. ^{1}H NMR analysis of the crude reaction mixture showed the two isomer products in a ratio of 55:45 (3′*R*:3′*S*). The products were separated by column chromatography using CH_2Cl_2 to MeOH/CH_2Cl_2/NH_3 (2:98:0.5) as eluent to give **11** as a semisolid in 47% yield (57.3 mg, 0.142 mmol) and **5** as a semisolid in 32% yield (37.0 mg, 0.0918 mmol). **11**: $R_f = 0.20$ in MeOH/EtOAc (1:4); $[\alpha]_D^{22}$ +244.3 (*c* 2.1, $CHCl_3$); IR ν_{max} 2960, 1747, 1619, 989 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.30 (s, 1H, H-2), 4.14 (s, 3H, O-CH_3), 3.68 (dt, *J* = 14.0, 6.0 Hz, 1H, H-3′), 3.54 (bs, 1H, H-9a), 3.21 (ddd, *J* = 14.0, 10.5, 5.0 Hz, 1H, H-5_a), 3.11 (sextet, *J* = 6.5 Hz, 1H, H-10), 3.04 (ddd, *J* = 13.5, 8.5, 4.5 Hz, 1H, H-5_b), 2.66 (d, *J* = 6.5 Hz, 1H, H-7), 2.08 (s, 3H, H-16), 2.01−1.97 (m, 2H, H-6_a, H-1′), 1.99 (d, *J* = 12.5 Hz, 1H, H-1_a), 1.94−1.85 (m, 2H, H-1_b, H-6_b), 1.84−1.79 (m, 1H, H-9), 1.77−1.71 (m, 2H, H-1′, H-$2'_a$), 1.38 (d, *J* = 6.5 Hz, 3H, H-17), 1.33−1.26 (m, 1H, H-$2'_b$), 1.16 (d, *J* = 6.0 Hz, 3H, H-4′); ^{13}C NMR ($CDCl_3$) δ 169.6 (C-15), 162.7 (C-13), 148.0 (C-11), 128.0 (C-12), 112.3 (C-8), 98.6 (C-14), 82.2 (C-3), 78.4 (C-2), 68.7 (C-3′), 60.7 (C-9a), 58.8 (O-CH_3), 51.7 (C-7), 47.2 (C-9), 46.9 (C-5), 35.4 (C-2′), 34.3 (C-10), 32.8 (C-1), 31.2 (C-1′), 26.3 (C-6), 24.1 (C-4′), 18.3 (C-17), 9.1 (C-16); EIMS *m/z* 403 (44%) $[M]^+$; HREIMS *m/z* 403.1983 $[M]^+$, calcd for $C_{22}H_{29}NO_6$ 403.1995.

5: $R_f = 0.13$ in MeOH/EtOAc (1:4); $[\alpha]_D^{22}$ +249.9 (*c* 1.9, $CHCl_3$); lit.[11] $[\alpha]_D^{22}$ +121 (*c* 0.28, $CHCl_3$); IR ν_{max} 3150, 2955, 1737, 1619, 989 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.30 (s, 1H, H-2), 4.14 (s, 3H, O-CH_3), 3.88 (dd, *J* = 10.5, 5.5 Hz, 1H, H-3′), 3.53 (bs, 1H, H-9a), 3.19 (ddd, *J* = 14.5, 10.5, 5.5 Hz, 1H, H-5_a), 3.10 (sextet, *J* = 6.5 Hz, 1H, H-10), 3.03 (ddd, *J* = 14.0, 8.5, 4.5 Hz, 1H, H-5_b), 2.70 (d, *J* = 6.5 Hz, 1H, H-7), 2.07 (s, 3H, H-16), 2.02−1.95 (m, 1H, H-6_a), 1.98 (d, *J* = 12.5 Hz, 1H, H-1_a), 1.89−1.78 (m, 5H, H-1_b, H-6_b, H-9, 2 × H-1′), 1.69−1.63 (m, 1H, H-$2'_a$), 1.62−1.56 (m, 1H, H-$2'_b$), 1.38 (d, *J* = 6.5 Hz, 3H, H-17), 1.17 (d, *J*= 6.5 Hz, 3H, H-4′); ^{13}C NMR ($CDCl_3$) δ 169.6 (C-15), 162.7 (C-13), 148.2 (C-11), 127.9 (C-12), 112.4 (C-8), 98.5 (C-14), 82.2 (C-3), 78.3 (C-2), 65.9 (C-3′), 60.8 (C-9a), 58.8 (O-CH_3), 51.0 (C-7), 47.3 (C-9), 47.2 (C-5), 34.4 (C-10), 33.4 (C-2′), 33.1 (C-1), 26.52 and 26.45 (C-6, C-1′), 22.8 (C-4′), 18.3 (C-17), 9.1 (C-16); ^{1}H NMR data are uniformly 0.07−0.09 ppm downfield compared to the natural product; ^{13}C NMR data matched those of the natural product;[11] EIMS *m/z* 403 (67%) $[M]^+$; HREIMS *m/z* 403.1993 $[M]^+$, calcd for $C_{22}H_{29}NO_6$ 403.1995.

(5*Z*)-5-[(2*S*,2a*R*,6*S*,7a*S*,7b*S*,8*R*,9*S*)-7b-(1*E*)-1-(1′-Hydroxymethyl-)hexahydro-9-methyl-4H-2,2,6-(epoxy[1]propanyl[3]ylidene)furo[2,3,4-*gh*]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5*H*)-furanone (12). To a solution of **7** (34.5 mg, 0.0961 mmol) in dry methanol (5 mL) was added sodium borohydride (3.6 mg, 0.0961 mmol), and the mixture was allowed to stir at rt for 30 min. The reaction mixture was then diluted with water (20 mL), extracted with chloroform (3 × 20 mL), and dried over $MgSO_4$. After evaporation, the crude product was purified by flash column chromatography using MeOH/CH_2Cl_2 (2:98) to MeOH/CH_2Cl_2/NH_3 (5:95:2) as eluent to give the alcohol **12** as an off-white solid in 80% yield (25.9 mg, 0.0718 mmol). $R_f = 0.05$ in MeOH/EtOAc (1:5); mp 180 °C (color changed to brown), 186−190 °C; $[\alpha]_D^{21}$ +293.3 (*c* 0.58, $CHCl_3$); IR ν_{max} 3255, 2955,1746, 1619, 999 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.44 (s, 1H, H-2), 4.15 (s, 3H, O-CH_3), 3.67/3.64 (2 × d [ABq]), *J* = 11.3 Hz, 2H, CH_2OH), 3.53 (bs, 1H, H-9a), 3.18−3.03 (m, 3H, 2 × H-5, H-10), 2.70 (d, *J* = 5.5 Hz, 1H, H-7), 2.07 (s, 3H, H-16), 1.99 (d, *J* = 12.0 Hz, 1H, H-1_a), 1.94−1.86 (m, 3H, 2 × H-6, H-9), 1.70 (dt, *J* = 12.0, 3.5 Hz, 1H, H-1_b), 1.38 (d, *J*= 6.5 Hz, 3H, H-17); ^{13}C NMR ($CDCl_3$) δ 169.7 (C-15), 162.8 (C-13), 148.1 (C-11), 127.9 (C-12), 112.3 (C-8), 98.6 (C-14), 83.8 (C-3), 78.3 (C-2), 61.2 (C-9a), 61.0 (CH_2OH), 58.9 (O-CH_3), 48.4 (C-7), 48.1 (C-9), 47.7 (C-5), 34.4 (C-10), 33.6 (C-1), 27.1 (C-6), 18.2 (C-17), 9.1 (C-16); EIMS *m/z* 361 (42%) $[M]^+$; HREIMS *m/z* 361.1527 $[M]^+$, calcd for $C_{19}H_{23}NO_6$ 361.1525.

(5*Z*)-5-[(2*S*,2a*R*,6*S*,7a*S*,7b*S*,8*R*,9*S*)-7b-(1*E*)-1-Phenylthiomethyl-hexahydro-9-methyl-4*H*-2,2,6-(epoxy[1]propanyl[3]ylidene)furo[2,3,4-*gh*]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5*H*)-furanone (13) and (5*E*)-[(2*S*,2a*R*,6*S*,7a*S*,7b*S*,8*R*,9*S*)-7b-(1*E*)-1-Phenylthiomethyl-hexahydro-9-methyl-4*H*-2,2,6-(epoxy[1]propanyl[3]ylidene)furo[2,3,4-gh]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5*H*)-furanone (14). To a solution of alcohol **12** (80.6 mg, 0.223 mmol) and diphenyl disulfide (97.5 mg, 0.447 mmol) in acetonitrile (1.85 mL) at 0 °C was added tributylphosphine (90.3 mg, 0.447 mmol, 0.11 mL). The reaction mixture was allowed to stir at 45 °C for 5 h and then quenched with 5% aqueous NaOH solution (10 mL). The product was extracted with $CHCl_3$ (3 × 20 mL), and the combined organic extracts were washed with saturated $NaHCO_3$ (20 mL), then brine (20 mL), and then dried over $MgSO_4$. After evaporation of the solvent, the resulting mixture of **13** and **14** isomers was separated by column chromatography using EtOAc as eluent to give **13** as a semisolid in 31% yield (31.2 mg, 0.0689 mmol) and **14** as a semisolid in 32% yield (32.8 mg, 0.0724 mmol).

13: $R_f = 0.36$ in MeOH/EtOAc (1:4); $[\alpha]_D^{21}$ +232.0 (*c* 0.62, $CHCl_3$); IR ν_{max} 2918, 2849, 1743, 1621, 975 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.39 (d, *J* = 8.0 Hz, 2H, 2 × ArH-3′), 7.29 (t, *J* = 7.5 Hz, 1H, 2 × ArH-2′), 7.19 (t, *J* = 7.5 Hz, 1H, ArH-4′), 4.39 (s, 1H, H-2), 4.14 (s, 3H, O-CH_3), 3.53 (bs, 1H, H-9a), 3.28 (d, *J* = 12.5 Hz, 1H, SCH_2), 3.24−3.18 (m, 1H, H-5_a), 3.18 (d, *J* = 13.0 Hz, 1H, SCH_2), 3.12−3.04 (m, 2H, H-5_b, H-10), 2.86 (d, *J* = 6.0 Hz, 1H, H-7), 2.07 (s, 3H, H-16), 1.97 (d, *J* = 12.0 Hz, 1H, H-1_a), 1.92−1.84 (m, 3H, 2 × H-6, H-9), 1.82 (dd, *J* = 10.0, 3.5 Hz, 1H, H- H-1_b), 1.38 (d, *J*= 6.5 Hz, 3H, H-17); ^{13}C NMR ($CDCl_3$) δ 169.6 (C-15), 162.8 (C-13), 148.1 (C-11), 136.5 (ArC-1′), 129.5 (2 × ArCH-3′), 129.0 (2 × ArCH-2′), 128.0 (C-12), 126.4 (ArCH-4′), 112.5 (C-8), 98.7 (C-14), 82.7 (C-3), 78.8 (C-2), 61.2 (C-9a), 58.8 (O-$\underline{C}H_3$), 50.8 (C-7), 47.8 (C-9), 47.5 (C-5), 36.7 (CH_2S), 34.4 (C-10), 33.3 (C-1), 26.7 (C-6), 18.3 (C-17), 9.2 (C-16); EIMS *m/z* 453 (100%) $[M]^+$; HREIMS *m/z* 453.1599 $[M]^+$, calcd for $C_{25}H_{27}NO_5S$ 453.1610.

14: $R_f = 0.42$ in MeOH/EtOAc (1:4); $[\alpha]_D^{20}$ +114.2 (*c* 0.51, $CHCl_3$); IR ν_{max} 2945, 1731, 1615, 1071 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.38 (d, *J* = 7.0 Hz, 2H, 2 × ArH-3′), 7.29 (t, *J* = 7.5 Hz, 2H, 2 × ArH-2′), 7.20 (t, *J* = 7.5 Hz, 1H, ArH-4′), 4.41 (s, 1H, H-2), 4.11 (s, 3H, O-CH_3), 3.53 (bs, 1H, H-9a), 3.28 (d, *J* = 13.0 Hz, 1H, SCH_2), 3.24−3.17 (m, 2H, H-5_a, H-10), 3.17 (d, *J* = 13.0 Hz, 1H, SCH_2), 3.10−3.05 (m, 1H, H-5_b), 2.86 (d, *J* = 6.0 Hz, 1H, H-7), 2.04 (s, 3H, H-16), 1.98 (d, *J* = 12.5 Hz, 1H, H-1_a), 1.94−1.91 (m, 1H, H-1_b), 1.89−1.83 (m, 2H, 2 × H-6), 1.75 (dd, *J* = 11.0, 3.5 Hz, 1H, H-9), 1.46 (d, *J*= 6.5 Hz, 3H, H-17); ^{13}C NMR ($CDCl_3$) δ 170.5 (C-15), 163.3 (C-13), 149.7 (C-11), 136.6 (ArC-1′), 129.4 (2 × ArCH-3′), 129.0 (2 × ArCH-2′), 128.9 (C-12), 126.3 (ArCH-4′), 113.4 (C-8), 98.5 (C-14), 82.7 (C-3), 78.9 (C-2), 61.2 (C-9a), 59.4 (O-CH_3), 50.9 (C-7), 47.8 (C-5), 45.9 (C-9), 36.7 (CH_2S), 36.2 (C-10), 33.1 (C-1), 26.7 (C-6), 16.2 (C-17), 8.8 (C-

16); EIMS *m/z* 453 (100%) $[M]^+$; HREIMS *m/z* 453.1601 $[M]^+$, calcd for $C_{25}H_{27}NO_5S$ 453.1610.

Methylstemofoline (3). A solution of **13** (18.6 mg, 0.0411 mmol) and an excess of Raney Ni (ca. 1 mL of a suspension in H_2O) in ethanol (1 mL) was heated and stirred at reflux for 1.5 h. The reaction mixture was filtered, and the filtrate solvent was removed *in vacuo* and then purified by column chromatography using EtOAc to MeOH/EtOAc (1:4) as eluent to give **3** as a white solid in 62% yield (8.8 mg, 0.0255 mmol). $R_f = 0.06$ in MeOH/EtOAc (1:4); mp 170 °C (color changed to brown), 192−198 °C; $[\alpha]_D^{23}$ +122.6 (*c* 0.86, MeOH); lit.[10] $[\alpha]^{23}_D$ +125 (*c* 0.24, $CHCl_3$); IR ν_{max} (cm^{-1}): 2961, 1741, 1626, 1007, 967; ^1H NMR [$CDCl_3$ (filtered through $K_2CO_{3(s)}$)] δ 4.18 (s, 1H, H-2), 4.14 (s, 3H, O-CH_3), 3.46 (bs, 1H, H-9a), 3.19 (m, 1H, H-5_a), 3.09 (m, 1H, H-10), 3.01 (m, 1H, H-5_b), 2.65 (d, *J* 5.0 Hz, 1H, H-7), 2.07 (s, 3H, H-16), 2.01−1.92 (m, 1H, H-6_a), 1.96 (d, *J* 12.0 Hz, 1H, H-1_a), 1.86 (m, 1H, H-6_b), 1.78 (d, *J* 10.5 Hz, 1H, H-1_b), 1.78 (m, 1H, H-9), 1.37 (d, *J* 6.0 Hz, 3H, H-17), 1.34 (s, 3H, H-18); ^{13}C NMR ($CDCl_3$) δ 169.7 (C-15), 162.8 (C-13), 148.4 (C-11), 127.9 (C-12), 112.7 (C-8), 98.6 (C-14), 80.5 (C-2), 79.3 (C-3), 61.2 (C-9a), 58.8 (O-CH_3), 51.5 (C-7), 47.5 (C-5), 47.0 (C-9), 34.5 (C-10), 32.9 (C-1), 26.5 (C-6), 19.3 (C-18), 18.3 (C-17), 9.2 (C-16); ^1H NMR spectroscopic data did not agree at first, but after running a solution of the authentic sample through K_2CO_3, the NMR data of the natural product were the same as those of synthetic product; ^{13}C NMR data agreed closely with those of the natural product; EIMS *m/z* 345 (34%) $[M]^+$; HREIMS *m/z* 345.1568 $[M]^+$, calcd for $C_{19}H_{23}NO_5$ 345.1576.

(11*E*)-Methylstemofoline (15). The title compound was prepared via a similar method to the synthesis of **3**, to give the natural product **15** as a white solid in 40% yield (9.3 mg, 0.0270 mmol). $R_f = 0.10$ in MeOH/EtOAc (1:4); mp 165 °C (color changed to brown), 190−196 °C; $[\alpha]_D^{23}$ +111.7 (*c* 1.0, MeOH); IR ν_{max} 2950, 1738, 1612, 1134, 960 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.19 (s, 1H, H-2), 4.11 (s, 3H, O-CH_3), 3.47 (bs, 1H, H-9a), 3.22−3.17 (m, 2H, H-5_a, H-10), 3.03 (ddd, J = 13.5, 8.5, 4.5 Hz, 1H, H-5_b), 2.64 (d, J = 6.0 Hz, 1H, H-7), 2.05 (s, 3H, H-16), 1.973 (d, J = 12.5 Hz, 1H, H-1_a), 1.972 (m, 1H, H-6_a), 1.84 (ddd, J = 13.5, 8.5, 5.0 Hz, 1H, H-6_b), 1.78 (dt, J = 12.0, 3.5 Hz, 1H, H-1_b), 1.71 (dd, J = 10.5, 3.5 Hz, 1H, H-9), 1.45 (d, J = 6.5 Hz, 3H, H-17), 1.34 (s, 3H, H-18); ^{13}C NMR ($CDCl_3$) δ 170.6 (C-15), 163.4 (C-13), 150.0 (C-11), 128.9 (C-12), 113.7 (C-8), 98.5 (C-14), 80.8 (C-2), 79.2 (C-3), 61.1 (C-9a), 59.4 (O-CH_3), 51.7 (C-7), 47.5 (C-5), 45.4 (C-9), 36.3 (C-10), 32.8 (C-1), 26.6 (C-6), 19.2 (C-18), 16.3 (C-17), 8.8 (C-16); EIMS *m/z* 345 (28%) $[M]^+$; HREIMS *m/z* 345.1559 $[M]^+$, calcd for $C_{19}H_{23}NO_5$ 345.1576.

Bioautography Procedure. TLC bioautography was performed using the method described by Hostettmann et al.[28] TLC plates were prepared for bioautography by washing with acetone and then thoroughly dried. The samples were then applied to the plate in varying quantities and sprayed with AChE enzyme stock solution (prepared from acetylcholinesterase (906 U/mg) as described in the literature[28]). The plates were incubated at 37 °C for 20 min and then sprayed with freshly prepared indicator solution (from 1-naphthyl acetate and Fast Blue B salt prepared according to the literature[28]) to give the plate a purple coloration after 1−2 min. A white spot indicates inhibition of AChE by the sample.

Antimicrobial Activity Procedure. A series of dilutions of compounds in trypticase soy broth ranging from 500 to 1.95 μg/mL was tested against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas auruginosa* ATCC 27853, and *Candida albicans* ATCC 90028 using broth dilution techniques.[29] The solutions were incubated at 37 °C for 24 h. The minimum inhibitory concentration (MIC) was determined from the tube with the lowest concentration of culture without visible growth of organisms.

Acknowledgment. We thank the Australian Research Council and the University of Wollongong for financial support.

Supporting Information Available: Copies of the ^1H NMR and ^{13}C NMR spectra of all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Pilli, R. A.; Rosso, G. B. de Oliveira, M. C. F. *The Alkaloids*; Cordell, G. A., Ed.; Elsevier: San Diego, 2005; Vol 62, Chapter 2, pp 77−173.
(2) Mungkornasawakul, P.; Pyne, S. G.; Jatisatienr, A.; Supyen, D.; Lie, W.; Ung, A. T.; Skelton, B. W.; White, A. H. *J. Nat. Prod.* **2003**, *66*, 980–982.
(3) Kaltenegger, E.; Brem, B.; Mereiter, K.; Kalchhauser, H.; Kahlig, H.; Hofer, O.; Vajrodaya, S.; Greger, H. *Phytochemistry* **2003**, *63*, 803–816.
(4) Mungkornasawakul, P.; Pyne, S. G.; Jatisatienr, A.; Supyen, D.; Jatisatienr, C.; Lie, W.; Ung, A. T.; Skelton, B. W.; White, A. H. *J. Nat. Prod.* **2004**, *67*, 675–677.
(5) Mungkornasawakul, P.; Matthews, H.; Ung, A. T.; Pyne, S. G.; Jatisatienr, A.; Lie, W.; Skelton, B. W.; White, A. H. *ACGC Chem. Res. Commun.* **2005**, *19*, 30–33.
(6) Brem, B.; Seger, C.; Pacher, T.; Hofer, O.; Vajrodaya, S.; Greger, H. *J. Agric. Food Chem.* **2002**, *50*, 6383–6388.
(7) Jiwajinda, S.; Hirai, N.; Watanabe, K.; Santisopasri, V.; Chuengsamarnyart, N.; Koshimizu, K.; Ohigashi, H. *Phytochemistry* **2001**, *56*, 693–695.
(8) Mungkornasawakul, P.; Pyne, S. G.; Jatisatienr, A.; Lie, W.; Ung, A. T.; Issakul, K.; Sawatwanich, A.; Supyen, D.; Jatisatienr, C. *J. Nat. Prod.* **2004**, *67*, 1740–1743.
(9) Chung, H.-S.; Hon, P.-M.; Lin, G.; But, P. P.-H.; Dong, H. *Planta Med.* **2003**, *69*, 914–920.
(10) Sastraruji, T.; Jatisatienr, A.; Pyne, S. G.; Ung, A. T.; Lie, W.; Williams, M. C. *J. Nat. Prod.* **2005**, *68*, 1763–1767.
(11) Sastraruji, T.; Jatisatienr, A.; Issakul, K; Pyne, S. G.; Ung, A. T.; Lie, W.; Ung,Williams, M. C. *Nat. Prod. Commun.* **2006**, *1*, 813–818.
(12) Zhong, Y. L.; Shing, T. K. M. *J. Org. Chem.* **1997**, *62*, 2622–2624.
(13) Blanchette, M. A.; Choy, W.; Davis, J. T.; Essenfeld, A. P.; Masamune, S.; Roush, W. R.; Sakai, T. *Tetrahedron Lett.* **1984**, *25*, 2183–2186.
(14) Corey, E. J.; Bakshi, R. K.; Shibata, S.; Chen, C.-P.; Singh, V. K. *J. Am. Chem. Soc.* **1987**, *109*, 7925–7926.
(15) Cho, B. T. *Tetrahedron* **2006**, *62*, 7621–7643.
(16) Reiff, E. A.; Nair, S. K.; Reddy, B. S. N.; Inagaki, J.; Henri, J. T.; Greiner, J. F.; Georg, G. I. *Tetrahedron Lett.* **2004**, *45*, 5845–5847.
(17) Sullivan, G. R.; Dale, J. A.; Mosher, H. S. *J. Org. Chem.* **1973**, *38*, 2143–2147.
(18) Ohtani, I.; Takenori, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
(19) Fieser, L. F. *J. Am. Chem. Soc.* **1954**, *76*, 1945–1947.
(20) Kabalka, G. W.; Hutchins, R.; Natale, N. R.; Yang, D. T. C.; Broach, V. *Organic Syntheses*; Wiley: New York, 1988; Collect. Vol. 6, pp 293−296.
(21) Kabalka, G. W.; Summers, S. T. *J. Org. Chem.* **1981**, *46*, 1217–1218.
(22) Hutchins, R. O.; Natale, N. R. *J. Org. Chem.* **1978**, *43*, 2299–2301.
(23) Hutchins, R. O.; Maryanoff, B. E.; Milewski, C. A. *J. Am. Chem. Soc.* **1971**, *93*, 1793–1794.
(24) Nair, V.; Sinhababu, A. K. *J. Org. Chem.* **1978**, *43*, 5013–5017.
(25) Holder, R. W.; Matturro, M. G. *J. Org. Chem.* **1977**, *42*, 2166–2168.
(26) Kurokawa, M.; Watanabe, T.; Ishikawa, T. *Helv. Chim. Acta* **2007**, *90*, 574–587.
(27) Wang, P.; Liu, A.-L.; An, Z.; Li, Z.-H.; Du, G. -H.; Qin, H.-L. *Chem. Biodiversity.* **2007**, *4*, 523–530.
(28) Marston, S.; Kissling, J.; Hostettmann, K. *Phytochem. Anal.* **2002**, *13*, 51–54.
(29) Washington, J. A. *Laboratory Procedures in Clinical Microbiology*, 2nd ed.; Springer-Verlag: New York, 1985.

NP800806B