



Synthesis of stemofoline analogues as acetylcholinesterase inhibitors

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ABSTRACT

Thirty-two new stemofoline analogues were prepared from didehydrostemofoline for studies as AChE inhibitors. C-3 Side-chain modified amino, carbamate, triazole and oxazole stemofoline derivatives were prepared. In general the amine derivatives were found to be stronger inhibitors of AChE than their alcohol analogues that we previously reported. Compounds **5** and **26**, with small C-3 side-chain substituents, were two of the most active inhibitors. Preliminary molecular docking studies suggested that these compounds may inhibit AChE by binding horizontally along the passage of the active-site gorge and block access to acetylcholine.

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1. Introduction

One of the primary roles of acetylcholinesterase (AChE) is the hydrolysis of the neurotransmitter acetylcholine (ACh) to inactive choline and acetate in cholinergic synapses. Thus AChE is essential for the rapid modulation of synaptic activity.¹ Hydrolysis of ACh occurs through a protease-like action of an active-site serine residue. This site resides at the end of a relatively long and narrow gorge. Inhibitors of AChE can bind irreversibly (e.g., some pesticides) or reversibly to the active site or at other sites in the gorge (e.g., the peripheral anionic site (PAS)) and block the access of ACh to the active site.¹ Reversible AChE inhibitors, for example, the alkaloid galanthamine (reminyl), have been used in the treatment of patients with Alzheimer's disease (AD) to alleviate the symptoms of reduced ACh concentration in the brain.² More recent AD drug development strategies involve targeting microtubule-associated τ -protein, metal ion dyshomeostasis and the various β -amyloid (A β) pathological mechanisms of this disease.^{2,3} AChE colocalizes with A β , which then promotes and accelerates A β aggregation.^{4–6} This has renewed an intense interest in AChE inhibitors, including dual binding AChE inhibitors⁷ and those that can be activated by AChE⁸ and have A β -antiaggregating action. We have recently reported that the *Stemona* alkaloids stemofoline and didehydrostemofoline and some of their C-3 side-chain derivatives are inhibitors of AChE.^{9–11} Such activity is most likely associated with

the insecticidal activity of these alkaloids and the crude extracts of *Stemona* plants. Our earlier studies focused on C-3 hydroxyalkyl derivatives, including the synthesis of rare *Stemona* alkaloids, where it was found that the AChE inhibitory activity was dependent upon the length of the C-3 alkyl chain, the position of the hydroxyl group and in some cases the configuration of the carbinol carbon.¹⁰ This study also revealed that the butyrolactone ring of stemofoline was essential for AChE inhibitory activity.¹⁰ We report here the synthesis of several novel C-3 side-chain amino, carbamate, triazole and oxazole stemofoline derivatives and their activities as AChE inhibitors.

2. Results and discussion

2.1. Synthesis of compounds

In order to prepare the amine derivatives **3–19**, the known aldehyde **2** was prepared from didehydrostemofoline **1** (Fig. 1), following our previously described procedures⁹ and used as a key

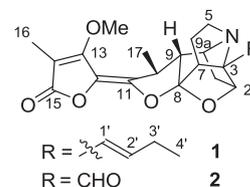
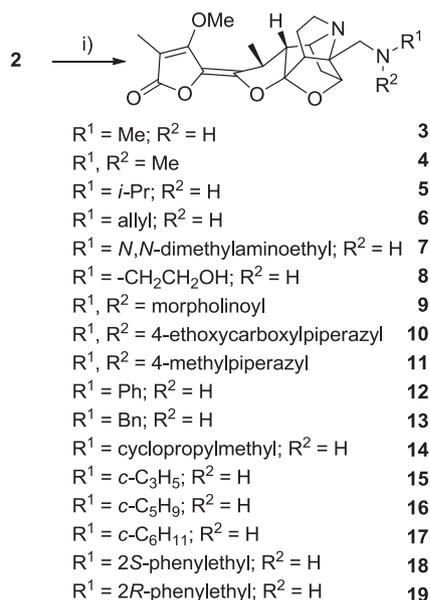


Fig. 1. Didehydrostemofoline **1** and its aldehyde derivative **2**.

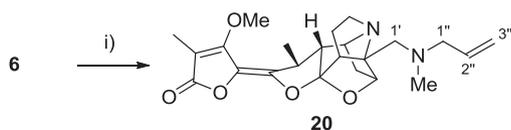
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scaffold for reductive amination reactions to prepare the 17 amine derivatives as shown in Scheme 1.

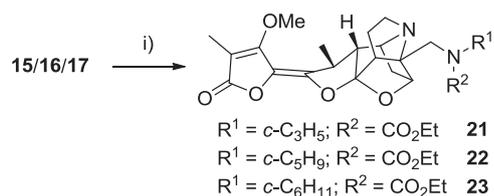


Scheme 1. Synthesis of amine derivatives **3–19**. Reagents and conditions: (i) amine, $\text{NaBH}(\text{OAc})_3$, CH_2Cl_2 , 0.1% HOAc, rt, 24 h (yields 25–93%).

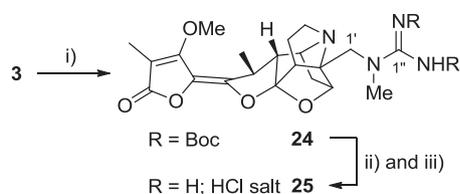
Using the reductive amination procedure described by Abdel-Magid et al.,¹² the amines **3–19** were obtained in yields ranging from 25 to 93%. A further methylation reaction of the amine **6** (Scheme 2) gave the tertiary amine **20** in 94% yield. Carbamylation reactions of the amines **15–17** (Scheme 3) gave the carbamates **21–23** in yields ranging from 56 to 80%. The HCl salt of the guanidine derivative **25** was prepared in two steps via a key guanidination reaction¹³ of the amine **3** (Scheme 4).



Scheme 2. Synthesis of amine derivative **20**. Reagents and conditions: (i) formaldehyde, $\text{NaBH}(\text{OAc})_3$, dichloroethane, 0.1% HOAc, rt, 24 h (yield 94%).

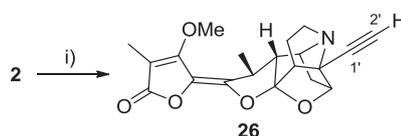


Scheme 3. Synthesis of amine derivatives **21–23**. Reagents and conditions: (i) ethyl chloroformate, THF/ NaHCO_3 (2:1), 0 °C, 3 h (yields 56–80%).



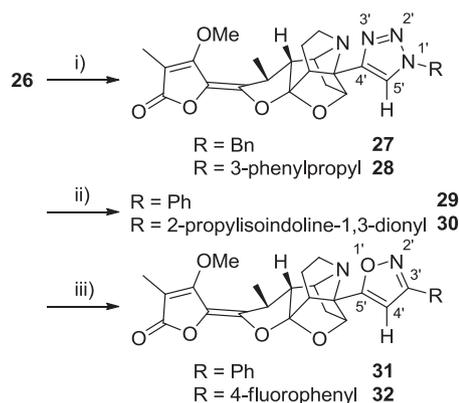
Scheme 4. Synthesis of guanidine derivative **25**. Reagents and conditions: (i) guanidine triflate, CH_2Cl_2 , rt, 24 h; (ii) TFA/ CH_2Cl_2 (1:1), rt, 3 h (yield 54%); (iii) precipitated from MeOH by HCl in ether (yield 52%).

Another key scaffold, the alkyne **26**, was prepared from a one-step alkylation reaction¹⁴ of the aldehyde **2** using the Bestmann–Ohira reagent^{15,16} (dimethyl-1-diazo-2-oxopropylphosphonate), which was prepared as described by Ghosh et al.¹⁷ (Scheme 5).



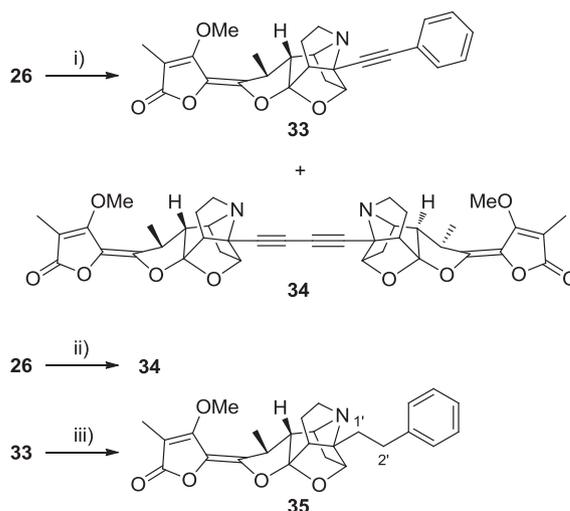
Scheme 5. Synthesis of the alkyne **26**. Reagents and conditions: (i) Bestmann–Ohira reagent ($\text{MeCOC}(\text{N}_2)\text{P}(\text{OMe})_2$), K_2CO_3 , MeOH/MeCN (1:3), rt, 24 h (yield 76%).

The 1,3-dipolar cyclization ('click') reactions of the alkyne **26** (Scheme 6) were examined under three different reaction condi-



Scheme 6. Click reactions of the alkyne **26**. Reagents and conditions: (i) RBr , NaN_3 , Cu powder, CuSO_4 , $t\text{-BuOH}/\text{H}_2\text{O}$ (1:1), MW 125 °C, 10 min (yields 18–23%); (ii) RN_3 , $\text{Cu}(\text{OAc})_2$, sodium ascorbate, MeOH/ H_2O (1:1), rt, 4 h (yields 33–46%); (iii) $\text{RC}(\text{Cl})=\text{NOH}$, $\text{Cu}(\text{OAc})_2$, sodium ascorbate, EtOH/ H_2O (1:1), NaOH, rt, 4 h (yield 59%).

tions to provide the four triazoles **27–30** and the two isoxazoles **31–32** in yields ranging from 18 to 59%. The alkyne **26** was treated under Sonogashira coupling conditions¹⁸ to yield the phenyl substituted alkyne **33** in 62% yield and the self-condensation product **34** in 4% yield (Scheme 7 (i)). The dimer **34** was more effectively obtained in 89% yield under Eglinton coupling conditions¹⁹ (Scheme 7 (ii)). The Sonogashira product **33** was



Scheme 7. Synthesis of compounds **33–35**. Reagents and conditions: (i) PhI, $\text{PdCl}_2(\text{PPh}_3)_2$, CuI, Et_3N , THF, N_2 , rt, 24 h (yields **33**: 62%, **34**: 4%); (ii) $\text{Cu}(\text{OAc})_2$, MeCN, argon gas, 40 °C, 4 h (yield 89%); (iii) Pd/C, H_2 , EtOAc, rt, 24 h (yield 74%).

hydrogenated over Pd/C under a H₂ atmosphere for 24 h to give the reduced product **35** in 74% yield (Scheme 7 (iii)).

2.2. Biological assays

We initially used the TLC bioautographic method of Hostettmann²⁰ to determine the minimum inhibitory requirements (MIRs) of these compounds against electric eel AChE (eeAChE) using galanthamine as a positive control. The results are reported in Tables 1–3. In our assay, galanthamine had a MIR of 1 ng. This assay revealed that the carbamate **22** and the tertiary amino compound **4** (Table 1, entries 1 and 2) were essentially equipotent to galanthamine and more potent than their parent compound

Table 1
AChE inhibitory activities of didehydrostemofoline **1** and the amine derivatives **3–23** and **25** against eeAChE^a

Entry	Compound	Side chain	Minimum inhibitory requirements ^b	
			ng	nmol
1	22		1	0.002
2	4		1	0.003
3	19		5	0.011
4	5		5	0.012
5	25		5	0.012
6	1		5	0.013
7	3		5	0.013
8	23		10	0.019
9	21		10	0.021
10	13		10	0.022
11	18		10	0.022
12	9		10	0.023
13	16		10	0.023
14	17		10	0.023

Table 1 (continued)

Entry	Compound	Side chain	Minimum inhibitory requirements ^b	
			ng	nmol
15	20		10	0.024
16	6		10	0.025
17	8		10	0.025
18	15		10	0.025
19	10		50	0.100
20	12		50	0.115
21	14		50	0.120
22	11		100	0.225
23	7		100	0.232

^a Galanthamine was used as a positive control having a MIR of 1 ng or 0.003 nmol.

^b Entries listed in order of decreasing activity.

didehydrostemofoline **1**. The secondary amino compounds **19**, **5** and **3** and the guanidine derivative **25** were equipotent to didehydrostemofoline **1** (Table 1, entries 3–7). The carbamates **23** and **21** and the secondary amines, **13**, **18**, **16**, **17** and **20** and the quaternary amines **9** and **20**, all had MIR values of 10 ng (Table 1, entries 8–18). The dibasic-amino compounds **11** and **17** were the least active amine derivatives (Table 1, entries 22 and 23). None of the click products **27–32** (Table 2) were as potent as didehydrostemofoline **1** or the amino derivatives in Table 1, entries 1–7. The *N*-benzyl-triazole derivative **27** was the most potent of this series of compounds while the others with longer and more flexible linkers (**28** and **30**) or no linker (**29**, **31** and **32**) between the heterocyclic triazole or isoxazole moiety and the aryl substituent were significantly less active.

The terminal alkyne **26** and the phenylethyl derivative **35** were essentially equipotent to galanthamine and didehydrostemofoline **1**, respectively (Table 3, entries 1 and 2). Substitution of the terminal alkyne CH of **26** with a phenyl group, as in compound **33**, had an adverse effect on inhibitory activity. The dimer of **26** had very poor activity (Table 3). Compared to our earlier results,¹⁰ this study showed that in general the amine derivatives were more active than our previously reported alcohol derivatives.¹⁰

We attempted to determine the IC₅₀ values of many of the more active compounds, and some of the alcohol analogues we reported earlier,¹⁰ against eeAChE and human AChE (hAChE) using Ellman's assay.²¹ We found however, that the majority of these compounds were not soluble in the assay medium (DMSO, pH 7.0 phosphate buffer). The IC₅₀ results determined on those that were soluble in the assay medium are shown in Table 4. In our assay, galanthamine

Table 2
AChE inhibitory activities of the click products **27–32** against eeAChE^a

Entry	Compound	Side chain	Minimum inhibitory requirements ^b	
			ng	nmol
1	27		10	0.020
2	30		50	0.085
3	28		50	0.097
4	32		50	0.102
5	31		50	0.105
6	29		100	0.211

^a Galanthamine was used as a positive control having a MIR of 1 ng or 0.003 nmol.^b Entries listed in order of decreasing activity.**Table 3**
AChE inhibitory activities of miscellaneous derivatives against eeAChE^a

Entry	Compound	Side chain	Minimum inhibitory requirements ^b	
			ng	nmol
1	26		1	0.003
2	35		5	0.011
3	33		50	0.116
4	34		100	0.141

^a Galanthamine was used as a positive control having a MIR of 1 ng or 0.003 nmol.^b Entries listed in order of decreasing activity.

had IC₅₀ values of 0.912 μM and 0.597 μM against eeAChE and hAChE, respectively (Table 4, entry 1). Didehydrostemofoline **1** and compound **5**, which both had MIRs of 5 ng against eeAChE, showed similar IC₅₀ values (ca. 12–25 μM) against eeAChE and hAChE, which indicated they were significantly less potent than galanthamine (Table 4, entries 2 and 3). Compounds **36**,¹⁰ **8**,⁹ and **37**,¹⁰ all with MIRs of 10 ng against eeAChE, were less active than compounds **1** and **5** (Table 4, entries 4–7). Their IC₅₀ values against eeAChE however, did not correlate as well to their corresponding

MIR values. However, their IC₅₀ values against hAChE were relatively similar (ca. 37–52 μM).

To inhibit AChE, inhibitors can bind to one or more sites of the enzyme. AChE has been reported to have at least two binding sites, the active site and the peripheral anionic site (PAS).²² The active site is buried at the bottom of a 20 Å deep narrow gorge²³ and includes four catalytic subsites, the esteratic site (which contains Ser200, His440 and Glu327), the oxyanion hole (which contains Gly118, Gly119 and Ala201), the acyl pocket (which contains Phe288 and

Table 4
Acetylcholinesterase inhibitory activity of stemofoline derivatives

Entry	Compound	Minimum inhibitory requirements		IC ₅₀ values μM (R^2)	
		ng	nmol	eeAChE	hAChE
1	galanthamine	1	0.003	0.902 \pm 0.04 (0.9953)	0.597 \pm 0.07 (0.9877)
2	1	5	0.013	19.20 \pm 0.26 (0.8749)	24.98 \pm 0.13 (0.9714)
3	5	5	0.012	12.94 \pm 0.08 (0.9883)	19.93 \pm 0.17 (0.9455)
4	36	10	0.025	302.3 \pm 0.29 (0.9245)	41.17 \pm 0.22 (0.9114)
5	8	10	0.025	52.45 \pm 0.14 (0.9668)	37.49 \pm 0.16 (0.9540)
6	9	10	0.025	77.19 \pm 0.22 (0.9274)	28.72 \pm 0.19 (0.9210)
7	37	10	0.028	108.1 \pm 0.15 (0.9659)	52.42 \pm 0.21 (0.9215)

Phe290) and the anionic subsite (which contains Trp84, Phe330 and Glu199).²⁴ In contrast, the PAS, which consists of Tyr70, Asp72, Tyr121, Tyr334 and Trp279, is located at the entrance of the active-site gorge. The binding of ligands to the PAS may block the passage of ACh or change the conformation of the active site allosterically and inhibit its function.²⁴ Preliminary molecular docking studies using GOLD suite versions 4.1 and 5.0 (CCDC, Cambridge, UK)²⁵ indicated that the more potent derivatives **5** and **26**, having small side chains, fitted 'horizontally' in the active site while those with longer side chains (e.g., **33**) fitted only 'vertically' into the active-site gorge (Fig. 2). Further studies to understand the mode of action of these compounds are in progress.

In conclusion, 32 new stemofoline analogues were prepared to study as AChE inhibitors. In general the amine derivatives were found to be stronger inhibitors of AChE than their alcohol analogues that we previously reported.^{9,10} Three compounds (tertiary

amine **4**, carbamate **22** and the terminal alkyne **26**) were found to be as active as galanthamine using the TLC bioautographic method while Ellman's assay, against eeAChE and human AChE (hAChE), identified didehydrostemofoline **1** and compound **5** as the most active of the compounds tested with IC₅₀ values in the range of 12–25 μM against eeAChE and hAChE. These activities were significantly less than that of galanthamine.

3. Experimental

3.1. General experimental procedures

All reactions, unless otherwise stated, were performed in oven dried, single-necked round bottom flasks under an atmosphere of dry nitrogen. Reagents and analytical grade solvents were purchased from commercial sources. Progress of reactions was

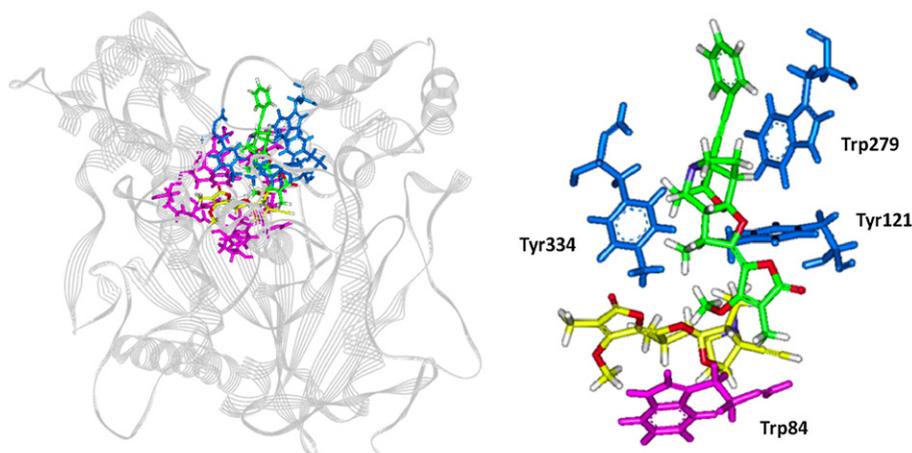


Fig. 2. Overlay of the alkyne **26** (yellow carbons, 'horizontal' binding near the active site at the base of the active-site gorge) and the phenyl alkyne derivative **33** (green carbons, 'vertical' binding, along the walls of the gorge) docked into the active-site gorge of *Torpedo californica* AChE (TcAChE). The amino acid residues at the binding sites of TcAChE are coloured pink for the active site and blue for the PAS. This view is expanded in the lower figure.

monitored by TLC using aluminium backed Merck F₂₅₄ sorbent silica gel with UV detection at 254 nm and/or Dragendorff's reagent. Compounds were purified by column chromatography using Merck flash silica gel (40–63 μm). Purity of compounds was determined by ¹H NMR spectroscopy and HPLC (see Supplementary data for details), and was always ≥95%. ¹H and ¹³C NMR spectra were recorded on a Varian Inova-500 spectrometer (500 MHz ¹H, 125 MHz ¹³C) in deuteriochloroform (CDCl₃), unless otherwise specified. NMR assignments were based on gCOSY, gHSQC, gHMBC and DEPT or APT experiments. ¹H and ¹³C NMR assignments are based on the numbering system used for stemofoline and not on the systematic name and numbering used in the naming of many stemofoline derivatives and analogues in the experimental section. Low-resolution mass spectra were obtained on a Waters LCZ single quadrupole (ESI). High-resolution mass spectra were obtained on a Waters QTOF (ESI). The microwave reactions were performed on a CEM Discovery Microwave Synthesis System (NC, USA).

3.1.1. General reaction procedure and preparation of (5Z)-5-[(2S,2aR,6S,7aS,7bR,8R,9S)-Hexahydro-7b-1-(N-methylaminomethyl)-9-methyl-4H-2,2,6-(epoxy[1]propanyl[3]ylidene)furo[2,3,4-gh]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5H)-furanone (3). To a solution of aldehyde **2** (26.4 mg, 0.074 mmol) in dry dichloroethane (2.0 mL) and acetic acid (0.2 mL) at rt was added methylamine (8.03 M in MeOH, 16.3 μL, 0.147 mmol) and then NaBH(OAc)₃ (46.7 mg, 0.220 mmol). The reaction mixture was left to stir for 24 h. The mixture was quenched with saturated aqueous NaHCO₃ solution (10 mL) and was directly extracted with CH₂Cl₂ (3×20 mL). The combined organic extracts were washed with brine and dried (MgSO₄) before being concentrated in vacuo. The concentrated residue was purified by column chromatography using gradient elution from CH₂Cl₂ to CH₂Cl₂/MeOH (9:1) to give the amine **3** (13.8 mg, 0.037 mmol, 60% yield) as a yellow gum. *R*_f=0.10 in MeOH/CH₂Cl₂ (1:9). [α]_D²⁵ +311.1 (c 0.92, CHCl₃). IR ν_{max} 3350, 2941, 2921, 2880, 2794, 1756, 1623 cm⁻¹. ¹H NMR δ 4.42 (s, 1H, H-2), 4.12 (s, 3H, O-CH₃), 3.44 (br s, 1H, H-9a), 3.12–3.07 (m, 2H, H-5a, H-10), 3.02–2.98 (m, 1H, H-5b), 2.70 (s, 2H, H-1'), 2.67 (d, J 6.5 Hz, 1H, H-7), 2.45 (s, 3H, H-3'), 2.06 (s, 3H, H-16), 1.94 (d, J 12.0 Hz, 1H, H-1b), 1.92–1.87 (m, 1H, H-6a), 1.85–1.80 (m, 2H, H-6b, H-9), 1.70 (d, J 12.0 Hz, 1H, H-1a), 1.58 (br s, 1H, NH), 1.36 (d, J 7.0 Hz, 3H, H-17). ¹³C NMR δ 169.8 (C-15), 163.0 (C-13), 148.5 (C-12), 128.0 (C-11), 112.6 (C-8), 98.7 (C-14), 82.8 (C-3), 79.0 (C-2), 61.3 (C-9a), 59.0 (O-CH₃), 52.6 (C-1'), 49.8 (C-7), 48.0 (C-9), 47.8 (C-5), 37.2 (N-CH₃), 34.7 (C-10), 33.7 (C-1), 27.2 (C-6), 18.4 (C-17), 9.3 (C-16). ESIMS *m/z* 375.0 (100%) [M+H]⁺, 376.1 (20%). HRESIMS *m/z* 375.1938 [M+H]⁺, calcd for C₂₀H₂₇N₂O₅ 375.1920.

3.1.2. (5Z)-5-[(2S,2aR,6S,7aS,7bR,8R,9S)-Hexahydro-7b-1-(N,N-dimethylaminomethyl)-9-methyl-4H-2,2,6-(epoxy[1]propanyl[3]ylidene)furo[2,3,4-gh]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5H)-furanone (4). Prepared using the general method described above, using the aldehyde **2** (16.6 mg, 0.046 mmol), dichloroethane (2.0 mL), acetic acid (0.2 mL), dimethylamine (2.0 M in THF, 47 μL, 0.092 mmol) and NaBH(OAc)₃ (29.4 mg, 0.139 mmol). The purified product was obtained as a yellow gum (7.0 mg, 0.018 mmol, 39% yield). *R*_f=0.26 in MeOH/CH₂Cl₂ (1:9). [α]_D²⁵ +281.3 (c 0.47, CHCl₃). IR ν_{max} 2936, 2768, 2356, 2337, 1743, 1620 cm⁻¹. ¹H NMR δ 4.38 (s, 1H, H-2), 4.13 (s, 3H, O-CH₃), 3.45 (br s, 1H, H-9a), 3.17–3.11 (m, 1H, H-5a), 3.11–3.06 (m, 1H, H-10), 3.03–2.97 (m, 1H, H-5b), 2.78 (d, J 6.0 Hz, 1H, H-7), 2.48 (d, J 13.5 Hz, 1H, H-1'a), 2.35 (d, J 13.5 Hz, 1H, H-1'b), 2.30 (s, 6H, N-CH₃), 2.06 (s, 3H, H-16), 1.95 (d, J 11.5 Hz, 1H, H-1a), 1.91–1.86 (m, 1H, H-6a), 1.84–1.81 (m, 1H, H-6b), 1.79–1.76 (m, 1H, H-9), 1.76–1.74 (m, 1H, H-1b), 1.36 (d, J 7.0 Hz, 3H, H-17). ¹³C NMR δ 169.8 (C-15), 163.0 (C-13), 148.6 (C-12), 128.0 (C-11), 112.7 (C-8), 98.8 (C-14), 83.2 (C-3), 79.0 (C-2), 60.8 (C-9a), 59.9 (C-1'),

59.0 (O-CH₃), 49.7 (C-7), 47.9 (C-5, C-9), 47.3 (N-CH₃), 34.7 (C-10), 33.3 (C-1), 27.1 (C-6), 18.5 (C-17), 9.3 (C-16). ESIMS *m/z* 389.0 (100%) [M+H]⁺, 390.2 (20%). HRESIMS *m/z* 389.2059 [M+H]⁺, calcd for C₂₁H₂₉N₂O₅ 389.2076.

3.1.3. (5Z)-5-[(2S,2aR,6S,7aS,7bR,8R,9S)-Hexahydro-7b-1-(N-isopropylaminomethyl)-9-methyl-4H-2,2,6-(epoxy[1]propanyl[3]ylidene)furo[2,3,4-gh]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5H)-furanone (5). Prepared using the general method described above, using the aldehyde **2** (24.3 mg, 0.068 mmol), dichloroethane (2.0 mL), acetic acid (0.2 mL), isopropylamine (11.6 μL, 0.135 mmol) and NaBH(OAc)₃ (43.0 mg, 0.203 mmol). The purified product was obtained as a colourless gum (21.1 mg, 0.052 mmol, 78% yield). *R*_f=0.23 in MeOH/CH₂Cl₂ (1:9). [α]_D²⁵ +246.9 (c 1.26, CHCl₃). IR ν_{max} 3383, 2961, 2885, 2356, 2337, 1742, 1621 cm⁻¹. ¹H NMR δ 4.43 (br s, 1H, H-2), 4.13 (s, 3H, O-CH₃), 3.45 (br s, 1H, H-9a), 3.12–3.06 (m, 2H, H-5, H-10), 3.02–2.97 (m, 1H, H-5), 2.77–2.74 (m, 1H, H-1''), 2.74 (d, J 10.0 Hz, 1H, H-1'), 2.69 (d, J 10.0 Hz, 1H, H-1'), 2.67 (d, J 5.0 Hz, 1H, H-7), 2.06 (s, 3H, H-16), 1.94 (d, J 11.5 Hz, 1H, H-1a), 1.91–1.87 (m, 1H, H-9), 1.85–1.81 (m, 2H, H-6), 1.71 (d, J 12.0 Hz, 1H, H-1b), 1.50 (br s, 1H, NH), 1.37 (d, J 7.0 Hz, 3H, H-17), 1.05 (d, J 6.5 Hz, 3H, H-4'), 1.02 (d, J 6.0 Hz, 3H, H-2''). ¹³C NMR δ 169.9 (C-15), 163.0 (C-13), 148.6 (C-12), 128.0 (C-11), 112.6 (C-8), 98.7 (C-14), 82.9 (C-3), 79.1 (C-2), 61.3 (C-9a), 59.0 (O-CH₃), 49.9 (C-7), 49.4 (C-1''), 48.0 (C-1'), 47.8 (C-5), 34.7 (C-10), 33.7 (C-1, C-9), 27.2 (C-6), 23.4 (C-2''), 22.8 (C-4'), 18.5 (C-17), 9.3 (C-16). ESIMS *m/z* 403.0 (100%) [M+H]⁺, 404.1 (20%). HRESIMS *m/z* 403.2234 [M+H]⁺, calcd for C₂₂H₃₁N₂O₅ 403.2233.

3.1.4. (5Z)-5-[(2S,2aR,6S,7aS,7bR,8R,9S)-Hexahydro-7b-1-(N-allylaminomethyl)-9-methyl-4H-2,2,6-(epoxy[1]propanyl[3]ylidene)furo[2,3,4-gh]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5H)-furanone (6). Prepared using the general method described above, using the aldehyde **2** (24.0 mg, 0.067 mmol), dichloroethane (2.0 mL), acetic acid (0.2 mL), allylamine (10.0 μL, 0.134 mmol) and NaBH(OAc)₃ (42.5 mg, 0.200 mmol). The purified product was obtained as a yellow gum (18.7 mg, 0.047 mmol, 70% yield). *R*_f=0.11 in MeOH/EtOAc (2:8). [α]_D²⁵ +437.6 (c 1.25, CHCl₃). IR ν_{max} 3402, 2952, 2925, 2880, 2847, 1742, 1619 cm⁻¹. ¹H NMR δ 5.89–5.81 (m, 1H, H-2''), 5.15 (dd, J 16.0 Hz, 1.5 Hz, 1H, H-3''(E)), 5.08 (dd, J 10.0 Hz, 1.0 Hz, 1H, H-3''(Z)), 4.44 (br s, 1H, H-2), 4.12 (s, 3H, O-CH₃), 3.44 (br s, 1H, H-9a), 3.29–3.22 (m, 2H, H-1''), 3.11–3.06 (m, 2H, H-5b, H-10), 3.02–2.96 (m, 1H, H-5a), 2.71 (ABq, J 10.0 Hz, 2H, H-1'), 2.66 (d, J 6.0 Hz, 1H, H-7), 2.05 (s, 3H, H-16), 1.94 (d, J 12.0 Hz, 1H, H-1a), 1.90–1.86 (m, 1H, H-6b), 1.84–1.79 (m, 2H, H-6a, H-9), 1.71 (dt, J 12.0 Hz, 3.0 Hz, 1H, H-1b), 1.63 (br s, 1H, NH), 1.36 (d, J 6.5 Hz, 3H, H-17). ¹³C NMR δ 169.8 (C-15), 163.0 (C-13), 148.5 (C-12), 136.9 (C-2''), 128.0 (C-11), 116.1 (C-3''), 112.6 (C-8), 98.7 (C-14), 82.9 (C-3), 79.0 (C-2), 61.3 (C-9a), 59.0 (O-CH₃), 52.8 (C-1''), 49.8 (C-7), 49.5 (C-1'), 48.0 (C-9), 47.8 (C-5), 34.6 (C-10), 33.7 (C-1), 27.2 (C-6), 18.4 (C-17), 9.3 (C-16). ESIMS *m/z* 401.0 (100%) [M+H]⁺, 402.1 (20%). HRESIMS *m/z* 401.2073 [M+H]⁺, calcd for C₂₂H₂₉N₂O₅ 401.2076.

3.1.5. (5Z)-5-[(2S,2aR,6S,7aS,7bR,8R,9S)-Hexahydro-7b-1-[N-(2-(dimethylamino)ethyl)aminomethyl]-9-methyl-4H-2,2,6-(epoxy[1]propanyl[3]ylidene)furo[2,3,4-gh]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5H)-furanone (7). Prepared using the general method described above, using the aldehyde **2** (23.9 mg, 0.067 mmol), dichloroethane (2.0 mL), acetic acid (0.2 mL), 2-(dimethylamino)ethylamine (15.3 μL, 0.133 mmol) and NaBH(OAc)₃ (42.3 mg, 0.200 mmol). The purified product was obtained as a colourless gum (8.2 mg, 0.019 mmol, 28% yield). *R*_f=0.17 in MeOH/CH₂Cl₂ (4:6). [α]_D²⁵ +178.5 (c 0.55, CHCl₃). IR ν_{max} 3384, 2962, 2932, 2360, 1740, 1618 cm⁻¹. ¹H NMR (300 MHz) δ 4.43 (br s, 1H, H-2), 4.13 (s, 3H, O-CH₃), 3.46 (br s, 1H, H-9a), 3.16–3.06 (m, 2H, H-5, H-10),

3.04–2.95 (m, 1H, H-5), 2.75 (s, 2H, H-1'), 2.74 (t, J 6.0 Hz, 2H, H-1''), 2.69 (d, J 5.4 Hz, 1H, H-7), 2.44 (t, J 6.0 Hz, 2H, H-2''), 2.25 (s, 6H, N-CH₃), 2.20 (br s, 1H, NH), 2.06 (s, 3H, H-16), 1.94 (d, J 12.0 Hz, 1H, H-1a), 1.90–1.84 (m, 2H, H-6), 1.79 (dd, J 10.2 Hz, 3.6 Hz, 1H, H-9), 1.73 (dt, J 12.3 Hz, 3.3 Hz, 1H, H-1b), 1.36 (d, J 6.6 Hz, 3H, H-17). ¹³C NMR (75 MHz) δ 169.9 (C-15), 163.0 (C-13), 148.5 (C-12), 128.0 (C-11), 112.6 (C-8), 98.7 (C-14), 82.9 (C-3), 78.9 (C-2), 61.2 (C-9a), 59.0 (O-CH₃), 58.9 (C-2''), 50.3 (C-1'), 49.8 (C-7), 48.3 (C-9), 47.9 (C-5, C-1''), 45.6 (N-CH₃), 34.6 (C-10), 33.6 (C-1), 27.1 (C-6), 18.4 (C-17), 9.3 (C-16). ESIMS *m/z* 432.0 (100%) [M+H]⁺, 433.2 (20%). HRESIMS *m/z* 432.2487 [M+H]⁺, calcd for C₂₃H₃₄N₃O₅ 432.2498.

3.1.6. (5Z)-5-[(2S,2aR,6S,7aS,7bR,8R,9S)-Hexahydro-7b-1-[N-(2-hydroxyethyl)aminomethyl]-9-methyl-4H-2,2,6-(epoxy[1]propanyl[3]ylidene)furo[2,3,4-gh]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5H)-furanone (8). Prepared using the general method described above, using the aldehyde **2** (18.6 mg, 0.052 mmol), dichloroethane (2.0 mL), acetic acid (0.2 mL), ethanolamine (8.5 μL, 0.104 mmol) and NaBH(OAc)₃ (32.9 mg, 0.155 mmol). The purified product was obtained as a colourless gum (10.0 mg, 0.025 mmol, 48% yield). *R*_f=0.20 in MeOH/CH₂Cl₂ (1:9). [α]_D²⁵+267.7 (c 0.67, CHCl₃). IR ν_{max} 3392, 2961, 2936, 1740, 1618, 1143 cm⁻¹. ESIMS *m/z* 405.0 (100%) [M+H]⁺, 406.1 (15%). HRESIMS *m/z* 405.2015 [M+H]⁺, calcd for C₂₁H₂₉N₂O₆ 405.2026. ¹H NMR δ 4.42 (br s, 1H, H-2), 4.13 (s, 3H, O-CH₃), 3.66–3.58 (m, 2H, H-2''), 3.48 (br s, 1H, H-9a), 3.16–3.12 (m, 1H, H-5a), 3.11–3.06 (m, 1H, H-10), 3.04–2.99 (m, 1H, H-5b), 2.87–2.83 (m, 1H, H-1'), 2.84 (d, J 12.0 Hz, 1H, H-1''), 2.81–2.76 (m, 1H, H-1'), 2.76 (d, J 12.0 Hz, 1H, H-1''), 2.67 (d, J 6.0 Hz, 1H, H-7), 2.11 (br s, 1H, NH), 2.06 (s, 3H, H-16), 1.96 (d, J 12.0 Hz, 1H, H-1a), 1.94–1.88 (m, 1H, H-6a), 1.88–1.85 (m, 1H, H-6b), 1.84–1.81 (m, 1H, H-9), 1.74 (d, J 12.0 Hz, 1H, H-1b), 1.37 (d, J 6.0 Hz, 3H, H-17). ¹³C NMR δ 169.9 (C-15), 163.0 (C-13), 148.4 (C-12), 128.1 (C-11), 112.5 (C-8), 98.8 (C-14), 83.2 (C-3), 78.9 (C-2), 61.2 (C-9a, C-2''), 59.0 (O-CH₃), 51.8 (C-1''), 49.7 (C-7), 49.6 (C-1'), 48.0 (C-9), 47.8 (C-5), 34.6 (C-10), 33.6 (C-1), 27.1 (C-6), 18.4 (C-17), 9.3 (C-16). ESIMS *m/z* 405.0 (100%) [M+H]⁺, 406.1 (15%). HRESIMS *m/z* 405.2015 [M+H]⁺, calcd for C₂₁H₂₉N₂O₆ 405.2026.

3.1.7. (5Z)-5-[(2S,2aR,6S,7aS,7bR,8R,9S)-Hexahydro-7b-1-morpholinomethyl-9-methyl-4H-2,2,6-(epoxy[1]propanyl[3]ylidene)furo[2,3,4-gh]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5H)-furanone (9). Prepared using the general method described above, using the aldehyde **2** (24.8 mg, 0.069 mmol), dichloroethane (2.0 mL), acetic acid (0.2 mL), morpholine (12.2 μL, 0.138 mmol) and NaBH(OAc)₃ (43.9 mg, 0.207 mmol). The purified product was obtained as a colourless gum (18.7 mg, 0.043 mmol, 63% yield). *R*_f=0.28 in MeOH/CH₂Cl₂ (1:9). [α]_D²⁵+317.6 (c 0.99, CHCl₃). IR ν_{max} 2951, 2932, 2359, 2337, 1740, 1620 cm⁻¹. ¹H NMR δ 4.35 (br s, 1H, H-2), 4.13 (s, 3H, O-CH₃), 3.69–3.63 (m, 4H, H-2''), 3.42 (br s, 1H, H-9a), 3.16–3.11 (m, 1H, H-5b), 3.09–3.05 (m, 1H, H-10), 3.01–2.96 (m, 1H, H-5a), 2.79 (br s, 2H, H-3''), 2.75 (d, J 5.5 Hz, 1H, H-7), 2.49–2.43 (m, 2H, H-1'), 2.40–2.37 (m, 2H, H-3''), 2.06 (s, 3H, H-16), 1.94 (d, J 12.5 Hz, 1H, H-1a), 1.92–1.86 (m, 1H, H-6b), 1.83–1.80 (m, 1H, H-6a), 1.79–1.75 (m, 1H, H-9), 1.71 (br s, 1H, H-1b), 1.36 (d, J 6.5 Hz, 3H, H-17). ¹³C NMR δ 169.8 (C-15), 162.9 (C-13), 148.4 (C-12), 128.0 (C-11), 112.7 (C-8), 98.7 (C-14), 83.2 (C-3), 79.0 (C-2), 67.2 (C-2''), 60.8 (C-9a), 59.0 (O-CH₃), 58.9 (C-1'), 55.2 (C-3''), 49.6 (C-7), 47.8 (C-5, C-9), 34.6 (C-10), 33.3 (C-1), 27.2 (C-6), 18.4 (C-17), 9.3 (C-16). ESIMS *m/z* 431.2 (100%) [M+H]⁺, 432.3 (5%). HRESIMS *m/z* 431.2163 [M+H]⁺, calcd for C₂₃H₃₁N₂O₆ 431.2182.

3.1.8. (5Z)-5-[(2S,2aR,6S,7aS,7bR,8R,9S)-Hexahydro-7b-1-[4-ethoxycarbonyl piperazine]methyl]-9-methyl-4H-2,2,6-(epoxy[1]propanyl[3]ylidene)furo[2,3,4-gh]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5H)-furanone (10). Prepared using the general method described above, using the aldehyde **2** (26.6 mg, 0.074 mmol),

dichloroethane (2.0 mL), acetic acid (0.2 mL), ethyl 1-piperazinecarboxylate (22.0 μL, 0.148 mmol) and NaBH(OAc)₃ (47.1 mg, 0.222 mmol). The purified product was obtained as a colourless gum (28.9 mg, 0.058 mmol, 78% yield). *R*_f=0.33 in MeOH/CH₂Cl₂ (1:9). [α]_D²⁵+236.7 (c 1.93, CHCl₃). IR ν_{max} 2964, 2932, 2356, 2337, 1743, 1694, 1620 cm⁻¹. ¹H NMR δ 4.34 (br s, 1H, H-2), 4.12 (s, 3H, O-CH₃), 4.11–4.08 (m, 2H, NCO₂CH₂CH₃), 3.42 (br s, 5H, H-9a, H-3''), 3.14–3.04 (m, 2H, H-5a, H-10), 3.00–2.95 (m, 1H, H-5b), 2.76 (br s, 2H, H-2''), 2.72 (d, J 6.0 Hz, 1H, H-7), 2.46 (s, 2H, H-1'), 2.34–2.32 (m, 2H, H-2''), 2.05 (s, 3H, H-16), 1.93 (d, J 12.0 Hz, 1H, H-1a), 1.89–1.84 (m, 1H, H-6a), 1.82–1.78 (m, 1H, H-6b), 1.75 (dd, J 10.0 Hz, 2.5 Hz, 1H, H-9), 1.69 (d, J 12.0 Hz, 1H, H-1b), 1.35 (d, J 7.0 Hz, 3H, H-17), 1.23 (t, J 7.5 Hz, 3H, NCO₂CH₂CH₃). ¹³C NMR δ 169.8 (C-15), 162.9 (C-13), 155.6 (NCO₂CH₂CH₃), 148.4 (C-12), 128.0 (C-11), 112.7 (C-8), 98.6 (C-14), 83.2 (C-3), 79.0 (C-2), 61.4 (NCO₂CH₂CH₃), 60.8 (C-9a), 59.0 (O-CH₃), 58.5 (C-1'), 54.4 (C-2''), 49.7 (C-7), 47.8 (C-5, C-9), 44.0 (C-3''), 34.6 (C-10), 33.3 (C-1), 27.2 (C-6), 18.4 (C-17), 14.8 (NCO₂CH₂CH₃), 9.3 (C-16). ESIMS *m/z* 502.0 (100%) [M+H]⁺, 503.1 (20%). HRESIMS *m/z* 502.2546 [M+H]⁺, calcd for C₂₆H₃₆N₃O₇ 502.2553.

3.1.9. (5Z)-5-[(2S,2aR,6S,7aS,7bR,8R,9S)-Hexahydro-7b-1-[4-methyl piperazine]methyl]-9-methyl-4H-2,2,6-(epoxy[1]propanyl[3]ylidene)furo[2,3,4-gh]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5H)-furanone (11). Prepared using the general method described above, using the aldehyde **2** (26.0 mg, 0.072 mmol), dichloroethane (2.0 mL), acetic acid (0.2 mL), 1-methylpiperazine (16.2 μL, 0.145 mmol) and NaBH(OAc)₃ (46.0 mg, 0.217 mmol). The purified product was obtained as a colourless gum (20.7 mg, 0.047 mmol, 64% yield). *R*_f=0.09 in MeOH/CH₂Cl₂ (1:9). [α]_D²⁵+279.4 (c 1.38, CHCl₃). IR ν_{max} 2961, 2939, 2356, 2325, 1742, 1618 cm⁻¹. ¹H NMR δ 4.42 (br s, 1H, H-2), 4.12 (s, 3H, O-CH₃), 3.41 (br s, 1H, H-9a), 3.16–3.10 (m, 1H, H-5a), 3.08–3.04 (m, 1H, H-10), 3.00–2.94 (m, 1H, H-5b), 2.80 (br s, 4H, H-2''), 2.75 (d, J 6.0 Hz, 1H, H-7), 2.46 (s, 2H, H-1'), 2.42 (br s, 4H, H-3''), 2.26 (s, 3H, N-CH₃), 2.05 (s, 3H, H-16), 1.92 (d, J 12.0 Hz, 1H, H-1a), 1.89–1.85 (m, 1H, H-6a), 1.81–1.77 (m, 1H, H-6b), 1.75 (dd, J 9.0 Hz, 2.5 Hz, 1H, H-9), 1.70 (d, J 12.0 Hz, 1H, H-1b), 1.35 (d, J 6.5 Hz, 3H, H-17). ¹³C NMR δ 169.8 (C-15), 162.9 (C-13), 148.5 (C-12), 128.0 (C-11), 112.8 (C-8), 98.6 (C-14), 83.2 (C-3), 79.0 (C-2), 60.8 (C-9a), 59.0 (O-CH₃), 58.4 (C-1'), 55.4 (C-3''), 54.5 (C-2''), 49.5 (C-7), 47.9 (C-5), 47.8 (C-9), 46.0 (N-CH₃), 34.6 (C-10), 33.2 (C-1), 27.2 (C-6), 18.4 (C-17), 9.3 (C-16). ESIMS *m/z* 444.0 (100%) [M+H]⁺, 445.2 (10%). HRESIMS *m/z* 444.2501 [M+H]⁺, calcd for C₂₄H₃₄N₃O₅ 444.2498.

3.1.10. (5Z)-5-[(2S,2aR,6S,7aS,7bR,8R,9S)-Hexahydro-7b-1-anilinomethyl-9-methyl-4H-2,2,6-(epoxy[1]propanyl[3]ylidene)furo[2,3,4-gh]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5H)-furanone (12). Prepared using the general method described above, using the aldehyde **2** (15.9 mg, 0.044 mmol), dichloroethane (2.0 mL), acetic acid (0.2 mL), aniline (13.2 μL, 0.089 mmol) and NaBH(OAc)₃ (28.2 mg, 0.133 mmol). The purified product was obtained as a colourless gum (3.0 mg, 0.007 mmol, 25% yield). *R*_f=0.74 in MeOH/CH₂Cl₂ (1:9). [α]_D²⁵+272.1 (c 0.51, CHCl₃). IR ν_{max} 3380, 2964, 2923, 2363, 2331, 1742, 1621 cm⁻¹. ¹H NMR δ 7.18 (t, J 7.5 Hz, 2H, ArH), 6.73 (t, J 7.5 Hz, 1H, ArH), 6.66 (d, J 7.5 Hz, 2H, ArH), 4.42 (s, 1H, H-2), 4.14 (s, 3H, O-CH₃), 3.62 (br s, 1H, H-9a), 3.45 (d, J 10.5 Hz, 1H, H-1'), 3.19–3.16 (m, 2H, H-5a, H-1'), 3.14–3.10 (m, 2H, H-5b, H-10), 2.84 (d, J 6.0 Hz, 1H, H-7), 2.07 (s, 3H, H-16), 2.02 (d, J 12.5 Hz, 1H, H-1a), 2.05–1.98 (m, 1H, H-6a), 1.94–1.90 (m, 1H, H-6b), 1.89 (d, J 3.5 Hz, 1H, H-9), 1.86–1.84 (m, 1H, H-1b), 1.40 (d, J 7.0 Hz, 3H, H-17). ¹³C NMR δ 169.8 (C-15), 162.8 (C-13), 148.2 (C-12, ArC-N), 129.4 (ArCH), 128.2 (C-11), 118.2 (ArCH), 113.4 (ArCH), 112.4 (C-8), 98.9 (C-14), 83.1 (C-3), 79.0 (C-2), 61.7 (C-9a), 59.0 (O-CH₃), 49.9 (C-7), 48.0 (C-9), 47.8 (C-5), 44.2 (C-1'), 34.6 (C-10), 33.7 (C-1), 26.9 (C-6), 18.5 (C-17), 9.3 (C-16). ESIMS *m/z* 437.0 (100%) [M+H]⁺,

438.1 (20%). HRESIMS m/z 437.2077 $[M+H]^+$, calcd for $C_{25}H_{29}N_2O_5$ 437.2076.

3.1.11. (5*Z*)-5-[(2*S*,2*aR*,6*S*,7*aS*,7*bR*,8*R*,9*S*)-Hexahydro-7*b*-1-(*N*-benzylaminomethyl)-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**). Prepared using the general method described above, using the aldehyde **2** (26.0 mg, 0.072 mmol), dichloroethane (2.0 mL), acetic acid (0.2 mL), *N*-benzylamine (16.0 μ L, 0.145 mmol) and $NaBH(OAc)_3$ (46.0 mg, 0.217 mmol). The purified product was obtained as a yellow gum (27.9 mg, 0.062 mmol, 86% yield). $R_f=0.46$ in MeOH/CH₂Cl₂ (1:9). $[\alpha]_D^{25} +196.9$ (c 1.86, CHCl₃). IR ν_{max} 3388, 2958, 2945, 2356, 2337, 1736, 1625 cm⁻¹. ¹H NMR (300 MHz) δ 7.34–7.29 (m, 4H, ArH), 7.25–7.23 (m, 1H, ArH), 4.43 (br s, 1H, H-2), 4.13 (s, 3H, O–CH₃), 3.82 (s, 2H, H-1''), 3.45 (br s, 1H, H-9a), 3.11–3.08 (m, 1H, H-10), 3.06–2.95 (m, 2H, H-5), 2.74 (q, J 12.0 Hz, 1H, H-1'), 2.69 (d, J 12.0 Hz, 1H, H-1'), 2.65 (d, J 6.0 Hz, 1H, H-7), 2.06 (s, 3H, H-16), 1.95 (d, J 12.0 Hz, 1H, H-1a), 1.82–1.79 (m, 3H, H-6, H-9), 1.74 (dt, J 12.0 Hz, 3.0 Hz, 1H, H-1b), 1.37 (d, J 6.0 Hz, 3H, H-17). ¹³C NMR (75 MHz, one ArCH was not observed due to peak overlap) δ 169.8 (C-15), 163.0 (C-13), 148.5 (C-12), 140.4 (ArC), 128.5 (ArCH), 128.0 (C-11), 127.0 (ArCH), 112.6 (C-8), 98.6 (C-14), 82.9 (C-3), 79.1 (C-2), 61.3 (C-9a), 59.0 (O–CH₃), 54.1 (C-1''), 49.7 (C-7), 49.2 (C-1'), 48.0 (C-9), 47.8 (C-5), 34.6 (C-10), 33.7 (C-1), 27.2 (C-6), 18.4 (C-17), 9.2 (C-16). ESIMS m/z 451.0 (100%) $[M+H]^+$, 452.3 (20%). HRESIMS m/z 451.2245 $[M+H]^+$, calcd for $C_{26}H_{31}N_2O_5$ 451.2233.

3.1.12. (5*Z*)-5-[(2*S*,2*aR*,6*S*,7*aS*,7*bR*,8*R*,9*S*)-Hexahydro-7*b*-1-(*N*-cyclopropylmethylaminomethyl)-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**). Prepared using the general method described above, using the aldehyde **2** (24.8 mg, 0.069 mmol), dichloroethane (2.0 mL), acetic acid (0.2 mL), (aminomethyl)cyclopropane (12.4 μ L, 0.138 mmol) and $NaBH(OAc)_3$ (43.9 mg, 0.207 mmol). The purified product was obtained as a colourless gum (17.3 mg, 0.042 mmol, 60% yield). $R_f=0.28$ in MeOH/CH₂Cl₂ (1:9). $[\alpha]_D^{25} +276.5$ (c 1.15, CHCl₃). IR ν_{max} 3347, 2999, 2951, 2359, 2337, 1742, 1619 cm⁻¹. ¹H NMR δ 4.45 (br s, 1H, H-2), 4.12 (s, 3H, O–CH₃), 3.46 (br s, 1H, H-9a), 3.12–3.06 (m, 2H, H-5b, H-10), 3.02–2.97 (m, 1H, H-5a), 2.77 (s, 2H, H-1'), 2.68 (d, J 6.0 Hz, 1H, H-7), 2.54 (dd, J 12.5 Hz, 6.5 Hz, 1H, H-1'a), 2.44 (dd, J 12.5 Hz, 6.5 Hz, 1H, H-1'b), 2.05 (s, 3H, H-16), 1.99 (br s, 1H, NH), 1.94 (d, J 12.5 Hz, 1H, H-1a), 1.91–1.86 (m, 1H, H-6a), 1.84–1.79 (m, 2H, H-6b, H-9), 1.73 (dt, J 12.5 Hz, 3.5 Hz, 1H, H-1b), 1.36 (d, J 6.5 Hz, 3H, H-17), 0.96–0.89 (m, 1H, H-2''), 0.46–0.45 (m, 2H, H-3''), 0.10–0.08 (m, 2H, H-3''). ¹³C NMR δ 169.8 (C-15), 163.0 (C-13), 148.5 (C-12), 128.0 (C-11), 112.6 (C-8), 98.7 (C-14), 83.9 (C-3), 79.0 (C-2), 61.3 (C-9a), 59.0 (O–CH₃), 55.5 (C-1''), 49.9 (C-1'), 49.8 (C-7), 48.0 (C-9), 47.8 (C-5), 34.6 (C-10), 33.7 (C-1), 27.2 (C-6), 18.4 (C-17), 11.1 (C-2''), 9.3 (C-16), 3.6 (C-3''), 3.4 (C-3''). ESIMS m/z 415.1 (100%) $[M+H]^+$, 416.2 (10%). HRESIMS m/z 415.2252 $[M+H]^+$, calcd for $C_{23}H_{31}N_2O_5$ 415.2233.

3.1.13. (5*Z*)-5-[(2*S*,2*aR*,6*S*,7*aS*,7*bR*,8*R*,9*S*)-Hexahydro-7*b*-1-(*N*-cyclopropylaminomethyl)-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 (**15**). Prepared using the general method described above, using the aldehyde **2** (21.6 mg, 0.060 mmol), dichloroethane (2.0 mL), acetic acid (0.2 mL), cyclopropylamine (8.2 μ L, 0.120 mmol) and $NaBH(OAc)_3$ (38.3 mg, 0.181 mmol). The purified product was obtained as a yellow gum (22.4 mg, 0.056 mmol, 93% yield). $R_f=0.36$ in MeOH/CHCl₃ (1:9). $[\alpha]_D^{25} +281.4$ (c 0.25, CHCl₃). IR ν_{max} 3364, 2961, 2936, 2356, 2334, 1742, 1620 cm⁻¹. ¹H NMR δ 4.42 (br s, 1H, H-2), 4.07 (s, 3H, O–CH₃), 3.42 (br s, 1H, H-9a), 3.10–3.01 (m, 2H, H-5a, H-10), 2.99–2.93 (m, 1H, H-5b), 2.81 (d, J 12.0 Hz, 1H, H-1'), 2.75 (d, J 12.0 Hz, 1H, H-1'), 2.63 (d, J 6.0 Hz, 1H, H-7), 2.03 (apparent sept, J 3.0 Hz, 1H, H-1''), 2.00 (s, 3H, H-16), 1.89–1.83 (m,

1H, H-6b), 1.86 (d, J 12.0 Hz, 1H, H-1a), 1.80–1.74 (m, 2H, H-6b, H-9), 1.63 (d, J 12.0 Hz, 1H, H-1b), 1.30 (d, J 6.5 Hz, 3H, H-17), 0.36 (d, J 6.5 Hz, 2H, H-2''), 0.29–0.26 (m, 1H, H-2''), 0.23–0.21 (m, 1H, H-2''). ¹³C NMR δ 169.9 (C-15), 163.0 (C-13), 148.5 (C-12), 128.0 (C-11), 112.5 (C-8), 98.6 (C-14), 82.9 (C-3), 78.9 (C-2), 61.2 (C-9a), 59.0 (O–CH₃), 49.7 (C-1'), 49.6 (C-7), 47.9 (C-9), 47.8 (C-5), 34.6 (C-10), 33.5 (C-1), 31.0 (C-1''), 27.0 (C-6), 18.4 (C-17), 9.2 (C-16), 6.4 (C-2''). ESIMS m/z 400.7 (100%) $[M+H]^+$, 401.9 (25%). HRESIMS m/z 401.2083 $[M+H]^+$, calcd for $C_{22}H_{29}N_2O_5$ 401.2076.

3.1.14. (5*Z*)-5-[(2*S*,2*aR*,6*S*,7*aS*,7*bR*,8*R*,9*S*)-Hexahydro-7*b*-1-(*N*-cyclopentylaminomethyl)-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 (**16**). Prepared using the general method described above, using the aldehyde **2** (22.6 mg, 0.063 mmol), dichloroethane (2.0 mL), acetic acid (0.2 mL), cyclopentylamine (12.9 μ L, 0.126 mmol) and $NaBH(OAc)_3$ (40.0 mg, 0.189 mmol). The purified product was obtained as a yellow gum (24.4 mg, 0.057 mmol, 90% yield). $R_f=0.21$ in MeOH/CHCl₃ (1:9). $[\alpha]_D^{25} +206.6$ (c 0.35, CHCl₃). IR ν_{max} 3351, 2951, 2885, 2359, 2337, 1743, 1620 cm⁻¹. ¹H NMR δ 4.38 (br s, 1H, H-2), 4.07 (s, 3H, O–CH₃), 3.39 (br s, 1H, H-9a), 3.06–3.00 (m, 2H, H-5, H-10), 2.98–2.94 (m, 2H, H-5, H-1''), 2.67 (s, 2H, H-1'), 2.61 (d, J 6.0 Hz, 1H, H-7), 2.18 (br s, 1H, NH), 1.99 (s, 3H, H-16), 1.87 (d, J 12.0 Hz, 1H, H-1a), 1.84–1.81 (m, 1H, H-6a), 1.76–1.74 (m, 3H, H-6b, H-2''), 1.65 (d, J 12.0 Hz, 1H, H-1b), 1.60–1.56 (m, 2H, H-3''), 1.48–1.44 (m, 2H, H-3''), 1.30 (d, J 6.5 Hz, 3H, H-17), 1.28–1.21 (m, 2H, H-2''). ¹³C NMR δ 169.8 (C-15), 162.9 (C-13), 148.5 (C-12), 127.9 (C-11), 112.5 (C-8), 98.5 (C-14), 82.8 (C-3), 79.0 (C-2), 61.2 (C-9a), 60.2 (C-1''), 58.9 (O–CH₃), 49.7 (C-7), 48.7 (C-1'), 47.9 (C-9), 47.7 (C-5), 34.6 (C-10), 33.6 (C-1), 33.1 (C-2''), 33.0 (C-2''), 27.1 (C-6), 24.1 (C-3''), 24.0 (C-3''), 18.4 (C-17), 9.2 (C-16). ESIMS m/z 428.9 (100%) $[M+H]^+$. HRESIMS m/z 429.2414 $[M+H]^+$, calcd for $C_{24}H_{33}N_2O_5$ 429.2389.

3.1.15. (5*Z*)-5-[(2*S*,2*aR*,6*S*,7*aS*,7*bR*,8*R*,9*S*)-Hexahydro-7*b*-1-(*N*-cyclohexylaminomethyl)-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 (**17**). Prepared using the general method described above, using the aldehyde **2** (23.2 mg, 0.065 mmol), dichloroethane (2.0 mL), acetic acid (0.2 mL), cyclohexylamine (15.0 μ L, 0.129 mmol) and $NaBH(OAc)_3$ (41.1 mg, 0.194 mmol). The purified product was obtained as a yellow gum (24.6 mg, 0.056 mmol, 86% yield). $R_f=0.30$ in MeOH/CHCl₃ (1:9). $[\alpha]_D^{25} +250.9$ (c 1.05, CHCl₃). IR ν_{max} 3377, 2929, 2856, 2363, 2337, 1743, 1621 cm⁻¹. ¹H NMR δ 4.43 (br s, 1H, H-2), 4.12 (s, 3H, O–CH₃), 3.45 (br s, 1H, H-9a), 3.11–3.06 (m, 2H, H-5b, H-10), 3.02–2.96 (m, 1H, H-5a), 2.79 (d, J 12.0 Hz, 1H, H-1'), 2.72 (d, J 12.0 Hz, 1H, H-1'), 2.67 (d, J 6.0 Hz, 1H, H-7), 2.42–2.38 (m, 1H, H-1''), 2.06 (s, 3H, H-16), 1.93 (d, J 11.5 Hz, 1H, H-1a), 1.90–1.87 (m, 1H, H-6b), 1.84–1.80 (m, 4H, H-6a, H-9, H-2''), 1.73–1.69 (m, 3H, H-1b, H-3''), 1.58 (d, J 12.0 Hz, 1H, H-4''), 1.36 (d, J 6.0 Hz, 3H, H-17), 1.28–1.21 (m, 2H, H-3''), 1.18–1.11 (m, 1H, H-4''), 1.09–1.04 (m, 2H, H-2''). ¹³C NMR δ 169.8 (C-15), 163.0 (C-13), 148.5 (C-12), 128.0 (C-11), 112.6 (C-8), 98.7 (C-14), 83.0 (C-3), 79.1 (C-2), 61.3 (C-9a), 59.0 (O–CH₃), 57.5 (C-1''), 49.8 (C-7), 48.1 (C-9), 47.8 (C-5), 47.3 (C-1'), 34.6 (C-10), 33.7 (C-2''), 33.6 (C-1), 33.3 (C-2''), 27.2 (C-6), 26.3 (C-4''), 25.1 (C-3''), 25.0 (C-3''), 18.4 (C-17), 9.3 (C-16). ESIMS m/z 443.0 (100%) $[M+H]^+$. HRESIMS m/z 443.2555 $[M+H]^+$, calcd for $C_{25}H_{35}N_2O_5$ 443.2546.

3.1.16. (5*Z*)-5-[(2*S*,2*aR*,6*S*,7*aS*,7*bR*,8*R*,9*S*)-Hexahydro-7*b*-1-(*N*-(2*S*-phenylethyl)aminomethyl)-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 (**18**). Prepared using the general method described above, using the aldehyde **2** (29.7 mg, 0.083 mmol), dichloroethane (2.0 mL), acetic acid (0.2 mL), (S)-1-aminoethylbenzene (21.3 μ L, 0.165 mmol) and $NaBH(OAc)_3$ (105.2 mg, 0.496 mmol). The purified

product was obtained as a yellow gum (31.1 mg, 0.067 mmol, 81% yield). $R_f=0.38$ in MeOH/CHCl₃ (1:9). $[\alpha]_D^{25} +166.0$ (c 2.07, CHCl₃). IR ν_{\max} 3015, 2958, 2359, 2334, 1742, 1621 cm⁻¹. ¹H NMR δ 7.34–7.30 (m, 2H, ArH), 7.26 (s, 2H, ArH), 7.25–7.20 (m, 1H, ArH), 4.45 (br s, 1H, H-2), 4.13 (s, 3H, O–CH₃), 3.71 (d, *J* 6.0 Hz, 1H, H-1''a), 3.44 (br s, 1H, H-9a), 3.09 (br s, 1H, H-10), 2.93 (br s, 2H, H-5), 2.64 (d, *J* 12.0 Hz, 1H, H-1'), 2.62 (d, *J* 6.0 Hz, 1H, H-7), 2.53 (d, *J* 12.0 Hz, 1H, H-1'), 2.06 (s, 3H, H-16), 1.98–1.93 (m, 1H, H-1a), 1.80–1.72 (m, 4H, H-1b, H-6a,b, H-9), 1.36 (d, *J* 6.0 Hz, 3H, H-17), 1.33 (d, *J* 6.0 Hz, 3H, 1''-CH₃). ¹³C NMR δ 169.8 (C-15), 162.9 (C-13), 148.5 (C-12), 145.9 (ArC), 128.5 (ArCH), 127.9 (C-11), 126.9 (ArCH), 126.4 (ArCH), 112.5 (C-8), 98.6 (C-14), 82.8 (C-3), 79.1 (C-2), 61.2 (C-9a), 59.0 (O–CH₃), 58.5 (C-1''), 49.6 (C-7), 47.8 (C-5, C-9, C-1'), 34.6 (C-10), 33.6 (C-1), 27.1 (C-6), 24.3 (1''-CH₃), 18.4 (C-17), 9.2 (C-16). ESIMS *m/z* 464.6 (100%) [M+H]⁺, 465.2 (25%). HRESIMS *m/z* 465.2398 [M+H]⁺, calcd for C₂₇H₃₃N₂O₅ 465.2389.

3.1.17. (5Z)-5-[(2S,2aR,6S,7aS,7bR,8R,9S)-Hexahydro-7b-1-[N-(2R-phenylethyl)aminomethyl]-9-methyl-4H-2,2,6-(epoxy[1]propanyl[3]ylidene)furo[2,3,4-gh]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5H)-furanone (19). Prepared using the general method described above, using the aldehyde **2** (30.9 mg, 0.086 mmol), dichloroethane (2.0 mL), acetic acid (0.2 mL), (R)-1-aminoethylbenzene (21.0 μ L, 0.172 mmol) and NaBH(OAc)₃ (109.5 mg, 0.517 mmol). The purified product was obtained as a yellow gum (24.7 mg, 0.053 mmol, 62% yield). $R_f=0.41$ in MeOH/CHCl₃ (1:9). $[\alpha]_D^{25} +100.9$ (c 1.65, CHCl₃). IR ν_{\max} 3030, 2961, 2936, 2356, 2340, 1742, 1620 cm⁻¹. ¹H NMR δ 7.34–7.28 (m, 2H, H-6'), 7.30 (d, *J* 2.5 Hz, 2H, ArH), 7.24–7.20 (m, 1H, ArH), 4.38 (br s, 1H, H-2), 4.12 (s, 3H, O–CH₃), 3.74 (q, *J* 6.5 Hz, 1H, H-1''b), 3.46 (br s, 1H, H-9a), 3.10–3.04 (m, 2H, H-5, H-10), 3.00–2.95 (m, 1H, H-5), 2.62 (d, *J* 12.0 Hz, 1H, H-1'), 2.55 (d, *J* 4.5 Hz, 1H, H-7), 2.46 (d, *J* 12.0 Hz, 1H, H-1'), 2.04 (s, 3H, H-16), 1.95 (d, *J* 13.0 Hz, 1H, H-1a), 1.79–1.77 (m, 4H, H-1b, H-6, H-9), 1.36 (d, *J* 6.5 Hz, 3H, H-17), 1.31 (d, *J* 6.5 Hz, 3H, 1''-CH₃). ¹³C NMR δ 170.0 (C-15), 163.1 (C-13), 148.7 (C-12), 145.9 (ArC), 128.8 (ArCH), 128.0 (C-11), 127.2 (ArCH), 126.8 (ArCH), 112.7 (C-8), 98.7 (C-14), 83.1 (C-3), 79.1 (C-2), 61.3 (C-9a), 59.1 (O–CH₃), 59.0 (C-1''), 49.8 (C-7), 48.1 (C-9, C-1'), 47.9 (C-5), 34.8 (C-10), 33.8 (C-1), 27.2 (C-6), 25.2 (1''-CH₃), 18.5 (C-17), 9.4 (C-16). ESIMS *m/z* 464.8 (100%) [M+H]⁺, 465.9 (25%). HRESIMS *m/z* 465.2390 [M+H]⁺, calcd for C₂₇H₃₃N₂O₅ 465.2389.

3.1.18. Methylation reaction: preparation of (5Z)-5-[(2S,2aR,6S,7aS,7bR,8R,9S)-hexahydro-7b-1-(N-methyl-N-allylaminomethyl)-9-methyl-4H-2,2,6-(epoxy[1]propanyl[3]ylidene)furo[2,3,4-gh]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5H)-furanone (20). Prepared using the general method described above, using formaldehyde solution (ca. 400 g/L, 3.75 μ L, 0.245 mmol) as the aldehyde component, dichloroethane (2.0 mL), acetic acid (0.2 mL), the amine **6** (9.8 mg, 0.024 mmol) as the amine component and NaBH(OAc)₃ (31.2 mg, 0.147 mmol). The reaction was left for 16 h. The purified product was obtained as a yellow gum (9.5 mg, 0.023 mmol, 94% yield). $R_f=0.37$ in MeOH/CH₂Cl₂ (1:9). $[\alpha]_D^{25} +263.6$ (c 0.63, CHCl₃). IR ν_{\max} 2955, 2920, 2847, 2359, 1743, 1621 cm⁻¹. ¹H NMR (300 MHz) δ 5.89–5.75 (m, 1H, H-2''), 5.15 (d, *J* 17.0 Hz, 1H, H-3''(E)), 5.10 (d, *J* 9.0 Hz, 1H, H-3''(Z)), 4.38 (br s, 1H, H-2), 4.13 (s, 3H, O–CH₃), 3.44 (br s, 1H, H-9a), 3.23 (dd, *J* 13.8 Hz, 6.3 Hz, 1H, H-1''b), 3.18–3.14 (m, 1H, H-5a), 3.13–3.06 (m, 1H, H-10), 3.03–2.98 (m, 1H, H-5b), 2.92 (dd, *J* 13.8 Hz, 6.6 Hz, 1H, H-1''a), 2.76 (d, *J* 5.7 Hz, 1H, H-7), 2.53 (d, *J* 13.8 Hz, 1H, H-1'), 2.42 (d, *J* 13.8 Hz, 1H, H-1'), 2.30 (s, 3H, N–CH₃), 2.06 (s, 3H, H-16), 1.94 (d, *J* 12.0 Hz, 1H, H-1a), 1.91–1.81 (m, 2H, H-6), 1.79–1.72 (m, 2H, H-1b, H-9), 1.36 (d, *J* 6.6 Hz, 3H, H-17). ¹³C NMR (75 MHz) δ 169.9 (C-15), 163.0 (C-13), 148.6 (C-12), 136.0 (C-2''), 128.0 (C-11), 117.6 (C-3''), 112.8 (C-8), 98.7 (C-14), 83.4 (C-3), 79.0 (C-2), 62.4 (C-1''), 60.8 (C-9a), 59.0 (O–CH₃), 56.9 (C-1'), 49.7

(C-7), 47.9 (C-5, C-9), 44.2 (N–CH₃), 34.7 (C-10), 33.3 (C-1), 27.1 (C-6), 18.5 (C-17), 9.3 (C-16). ESIMS *m/z* 415.2 (100%) [M+H]⁺, 416.3 (10%). HRESIMS *m/z* 415.2220 [M+H]⁺, calcd for C₂₃H₃₁N₂O₅ 415.2233.

3.1.19. Carbamylation reaction: preparation of (5Z)-5-[(2S,2aR,6S,7aS,7bR,8R,9S)-hexahydro-7b-1-(N-ethoxycarboxyl-N-cyclopropylaminomethyl)-9-methyl-4H-2,2,6-(epoxy[1]propanyl[3]ylidene)furo[2,3,4-gh]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5H)-furanone (21). To a solution of the amine **15** (11.2 mg, 0.028 mmol) in THF/saturated aqueous NaHCO₃ solution (2:1) (3.0 mL) at 0 °C was added ethyl chloroformate (4.5 μ L, 0.056 mmol) and the reaction was left to stir for 3 h. The mixture was quenched with saturated aqueous NaHCO₃ solution (10 mL) and was directly extracted with CH₂Cl₂ (3 \times 20 mL). The combined organic extracts were washed with brine and dried (MgSO₄) before being concentrated in vacuo. The concentrated residue was purified by column chromatography using gradient elution from CH₂Cl₂ to CH₂Cl₂/MeOH (9:1) to give **21** as a colourless gum (10.0 mg, 0.021 mmol, 76% yield). $R_f=0.45$ in MeOH/CHCl₃ (1:9). $[\alpha]_D^{25} +164.0$ (c 0.91, CHCl₃). IR ν_{\max} 2961, 2932, 2359, 1744, 1690, 1621 cm⁻¹. ¹H NMR δ 4.63 (br s, 1H, H-2), 4.16 (q, *J* 7.5 Hz, 2H, NCO₂CH₂CH₃), 4.13 (s, 3H, O–CH₃), 3.62 (d, *J* 13.0 Hz, 1H, H-1'), 3.44 (br s, 1H, H-9a), 3.29 (d, *J* 13.0 Hz, 1H, H-1'), 3.28–3.22 (m, 1H, H-5a), 3.10–2.99 (m, 2H, H-5b, H-10), 2.77 (d, *J* 6.0 Hz, 1H, H-7), 2.64 (br s, 1H, H-1''), 2.10–2.02 (m, 1H, H-6a), 2.05 (s, 3H, H-16), 1.94 (d, *J* 12.0 Hz, 1H, H-1a), 1.84–1.80 (m, 2H, H-6b, H-9), 1.72 (d, *J* 12.0 Hz, 1H, H-1b), 1.36 (d, *J* 6.5 Hz, 3H, H-17), 1.26 (t, *J* 7.5 Hz, 3H, NCO₂CH₂CH₃), 0.88–0.82 (m, 1H, H-2''), 0.78–0.74 (m, 1H, H-2''), 0.74–0.67 (m, 1H, H-2''), 0.65–0.56 (m, 1H, H-2''). ¹³C NMR (the carbamate C=O signal was not observed) δ 169.7 (C-15), 162.9 (C-13), 148.3 (C-12), 128.1 (C-11), 112.5 (C-8), 98.8 (C-14), 83.5 (C-3), 78.2 (C-2), 60.7 (C-9a), 61.7 (NCO₂CH₂CH₃), 59.0 (O–CH₃), 49.1 (C-7), 48.2 (C-5), 47.7 (C-9), 47.0 (C-1'), 34.6 (C-10), 33.0 (C-1), 29.3 (C-1''), 27.1 (C-6), 18.4 (C-17), 14.7 (NCO₂CH₂CH₃), 10.0 (C-2''), 9.3 (C-16), 8.8 (C-2''). ESIMS *m/z* 472.6 (100%) [M+H]⁺, 473.7 (10%). HRESIMS *m/z* 473.2300 [M+H]⁺, calcd for C₂₅H₃₃N₂O₇ 473.2288.

3.1.20. (5Z)-5-[(2S,2aR,6S,7aS,7bR,8R,9S)-Hexahydro-7b-1-(N-ethoxycarboxyl-N-cyclopentylaminomethyl)-9-methyl-4H-2,2,6-(epoxy[1]propanyl[3]ylidene)furo[2,3,4-gh]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5H)-furanone (22). Prepared using the general method described above, using the amine **16** (11.2 mg, 0.026 mmol) 2:1 THF/saturated aqueous NaHCO₃ solution (3.0 mL) and ethyl chloroformate (5.0 μ L, 0.052 mmol). The purified product was obtained as a yellow gum (7.3 mg, 0.015 mmol, 56% yield). $R_f=0.45$ in MeOH/CHCl₃ (1:9). $[\alpha]_D^{25} +205.6$ (c 0.61, CHCl₃). IR ν_{\max} 2958, 2888, 2362, 2337, 1737, 1675, 1616 cm⁻¹. ¹H NMR δ 4.50 (br s, 1H, H-2), 4.18–4.10 (m, 2H, NCO₂CH₂CH₃), 4.13 (s, 3H, O–CH₃), 3.70–3.63 (m, 2H, H-1', H-1''), 3.46 (br s, 1H, H-9a), 3.24 (d, *J* 15.5 Hz, 1H, H-1'), 3.22–3.16 (m, 1H, H-5), 3.09–3.00 (m, 2H, H-5, H-10), 2.82 (d, *J* 5.5 Hz, 1H, H-7), 2.10–2.02 (m, 1H, H-6a), 2.06 (s, 3H, H-16), 1.93 (d, *J* 11.5 Hz, 1H, H-1a), 1.84–1.72 (m, 7H, H-1b, H-6b, H-9, H-2''), 1.48 (br s, 4H, H-3''), 1.36 (d, *J* 6.0 Hz, 3H, H-16), 1.26 (t, *J* 6.0 Hz, 3H, NCO₂CH₂CH₃). ¹³C NMR (the carbamate C=O signal was not observed) δ 169.8 (C-15), 162.9 (C-13), 148.4 (C-12), 128.0 (C-11), 112.6 (C-8), 98.8 (C-14), 83.7 (C-3), 77.6 (C-2), 62.6 (C-1''), 61.4 (NCO₂CH₂CH₃), 60.9 (C-9a), 59.0 (O–CH₃), 49.4 (C-7, C-1'), 48.2 (C-5), 47.8 (C-9), 34.7 (C-10), 33.0 (C-1), 27.1 (C-6), 24.9 (C-2''), 24.8 (C-3''), 18.4 (C-17), 14.8 (NCO₂CH₂CH₃), 9.3 (C-16). ESIMS *m/z* 500.7 (100%) [M+H]⁺, 501.9 (20%). HRESIMS *m/z* 501.2622 [M+H]⁺, calcd for C₂₇H₃₇N₂O₇ 501.2601.

3.1.21. (5Z)-5-[(2S,2aR,6S,7aS,7bR,8R,9S)-Hexahydro-7b-1-(N-ethoxycarboxyl-N-cyclohexylaminomethyl)-9-methyl-4H-2,2,6-(epoxy[1]propanyl[3]ylidene)furo[2,3,4-gh]pyrrolizin-10-ylidene]-4-methoxy-

3-methyl-2(5H)-furanone (23). Prepared using the general method described above, using the amine **17** (11.1 mg, 0.025 mmol), 2:1 THF/saturated aqueous NaHCO₃ solution (3.0 mL) and ethyl chloroformate (4.0 μL, 0.050 mmol). The purified product was obtained as a yellow gum (10.2 mg, 0.020 mmol, 80% yield). *R*_f=0.45 in MeOH/CHCl₃ (1:9). [α]_D²⁵+192.6 (c 0.78, CHCl₃). IR ν_{max} 2929, 2366, 1744, 1684, 1621 cm⁻¹. ¹H NMR δ 4.50 (br s, 1H, H-2), 4.18–4.10 (m, 2H, NCO₂CH₂CH₃), 4.13 (s, 3H, O–CH₃), 3.64 (br s, 1H, H-1'), 3.46 (br s, 1H, H-9a), 3.41 (s, 1H, H-1''), 3.20 (d, *J* 15.0 Hz, 1H, H-5), 3.12–3.01 (m, 2H, H-5, H-10), 2.82 (d, *J* 6.0 Hz, 1H, H-7), 2.07 (s, 3H, H-16), 1.94 (d, *J* 12.5 Hz, 1H, H-1a), 1.87–1.72 (m, 11H, H-1b, H-6, H-9, H-3'', H-4''), 1.61 (apparent d, *J* 9.0 Hz, 1H, H-2''), 1.37 (d, *J* 6.5 Hz, 3H, H-17), 1.27 (t, *J* 7.0 Hz, 3H, NCO₂CH₂CH₃), 1.22 (apparent d, *J* 13.0 Hz, 2H, H-3''), 1.14–1.08 (m, 1H, H-2''). ¹³C NMR (the carbamate C=O signal was not observed) δ 169.8 (C-15), 162.9 (C-13), 148.4 (C-12), 128.0 (C-11), 112.7 (C-8), 98.8 (C-14), 83.7 (C-3), 77.6 (C-2), 70.8 (C-1''), 61.4 (C-9a), 61.2 (NCO₂CH₂CH₃), 59.0 (O–CH₃), 48.5 (C-1'), 48.2 (C-5, C-7), 47.8 (C-9), 34.7 (C-10), 33.0 (C-1), 27.2 (C-4''), 26.6 (C-6, C-3''), 25.6 (C-2''), 18.4 (C-17), 14.8 (NCO₂CH₂CH₃), 9.3 (C-16). ESIMS *m/z* 514.5 (100%) [M+H]⁺, 515.3 (40%). HRESIMS *m/z* 515.2726 [M+H]⁺, calcd for C₂₈H₃₉N₂O₇ 515.2757.

3.1.22. Guanidination reaction: preparation of (5Z)-5-[(2S,2aR,6S,7aS,7bR,8R,9S)-hexahydro-7b-1-[[[(1,1-dimethylethoxy)carbonyl]amino][[(1,1-dimethylethoxy)carbonyl]imino]methylamino]methyl]-9-methyl-4H-2,2,6-(epoxy[1]propanyl[3]ylidene)furo[2,3,4-gh]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5H)-furanone (24). To a solution of the amine **3** (8.8 mg, 0.024 mmol) in dry CH₂Cl₂ (2.0 mL) was added 1,3-di-Boc-2-(trifluoromethylsulfonyl)-guanidine (9.2 mg, 0.024 mmol) at rt and the reaction mixture was left to stir for 24 h. The mixture was quenched with saturated aqueous NaHCO₃ solution (10 mL) and then extracted with CH₂Cl₂ (3×20 mL). The combined organic extracts were washed with brine and dried (MgSO₄) before being concentrated in vacuo. The concentrated residue was purified by column chromatography using gradient elution from CH₂Cl₂ to CH₂Cl₂/MeOH (9:1) to give a white gum (7.9 mg, 0.013 mmol, 54% yield). *R*_f=0.45 in MeOH/CH₂Cl₂ (1:9). ¹H NMR δ 4.44 (br s, 1H, H-2), 4.15 (s, 3H, O–CH₃), 3.73 (d, *J* 13.5 Hz, 2H, H-1'), 3.54 (br s, 1H, H-9a), 3.28–3.22 (m, 1H, H-5a), 3.12–3.04 (m, 2H, H-5b, H-10), 3.08 (s, 3H, N–CH₃), 2.74 (d, *J* 5.5 Hz, 1H, H-7), 2.08 (s, 3H, H-16), 2.01 (d, *J* 12.5 Hz, 1H, H-1a), 1.99–1.89 (m, 2H, H-6), 1.83 (dd, *J* 10.0 Hz, 3.5 Hz, 1H, H-9), 1.75 (d, *J* 12.0 Hz, 1H, H-1b), 1.67 (br s, 1H, NH), 1.47 (s, 18H, CO₂C(CH₃)₃), 1.39 (d, *J* 6.5 Hz, 3H, H-17). ¹³C NMR (the signals for C-1'' and the Boc carbonyls were not observed.) δ 169.6 (C-15), 162.6 (C-13), 147.6 (C-12), 128.0 (C-11), 112.0 (C-8), 98.7 (C-14), 83.8 (C-3), 76.5 (C-2), 60.2 (C-9a), 58.9 (O–CH₃), 52.4 (C-1'), 50.4 (C-7), 47.8 (C-5), 47.5 (C-9), 40.0 (N–CH₃), 34.2 (C-10), 33.4 (C-1), 28.2 (CO₂C(CH₃)₃), 26.4 (C-6), 18.2 (C-17), 9.1 (C-16). ESIMS *m/z* 616.6 (100%) [M+H]⁺, 617.4 (80%). HRESIMS *m/z* 617.3188 [M+H]⁺, calcd for C₃₁H₄₅N₄O₉ 617.3187.

3.1.23. (5Z)-5-[(2S,2aR,6S,7aS,7bR,8R,9S)-Hexahydro-7b-1-[(aminomethyl)methylamino]methyl]-9-methyl-4H-2,2,6-(epoxy[1]propanyl[3]ylidene)furo[2,3,4-gh]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5H)-furanol hydrochloride (25). To a solution of **24** (7.9 mg, 0.013 mmol) in dry CH₂Cl₂ (1.0 mL) was added TFA (1.0 mL) at rt and the reaction mixture was left to stir for 3 h. The solvent was removed under vacuum. To the residue was added hydrogen chloride in ether (2.0 mL, 1 M) and concentrated under vacuum. The desired product was isolated as its hydrochloride salt by dissolution in MeOH and precipitation by the addition of diethyl ether as a white solid salt (4.3 mg, 0.010 mmol, 52% yield). [α]_D²⁵+191.2 (c 0.23, CH₃OH). ¹H NMR (CD₃OD, a very broad spectrum was observed, therefore only the methyl signals are reported) δ 4.23 (s, 3H, O–CH₃), 3.25 (s, 3H, N–CH₃), 2.07 (s, 3H, H-16), 1.47 (d, *J* 6.0 Hz, 3H, H-17). ¹³C NMR (CD₃OD) the signals for carbons at C-3, C-5, C-9 and C-9a were

not observed. δ 172.0 (C-15), 164.8 (C-13), 160.0 (C-1''), 149.7 (C-12), 129.4 (C-11), 112.5 (C-8), 99.6 (C-14), 76.5 (C-2), 58.9 (O–CH₃), 51.0 (C-7), 47.3 (C-1'), 38.8 (C-10), 34.3 (N–CH₃), 32.2 (C-1), 24.4 (C-6), 16.8 (C-17), 7.9 (C-16). ESIMS *m/z* 416.7 (100%) [M+H]⁺. HRESIMS *m/z* 417.2155 [M+H]⁺, calcd for C₂₁H₂₉N₄O₅ 417.2138.

3.1.24. Alkynylation reaction: preparation of (5Z)-5-[(2S,2aR,6S,7aS,7bR,8R,9S)-hexahydro-7b-1-ethynyl-9-methyl-4H-2,2,6-(epoxy[1]propanyl[3]ylidene)furo[2,3,4-gh]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5H)-furanone (26). To a mixture of the aldehyde **2** (151 mg, 0.420 mmol) and K₂CO₃ (69.7 mg, 0.504 mmol) in MeOH/MeCN (1:3) (8.0 mL) at rt was added the Bestmann–Ohira reagent (97 mg, 0.504 mmol) and the reaction mixture was left to stir for 24 h. The mixture was quenched with saturated aqueous NaHCO₃ solution (10 mL) and was extracted with diethyl ether (3×20 mL). The combined organic extracts were washed with brine and dried over MgSO₄ before being concentrated in vacuo. The concentrated residue was purified by column chromatography using gradient elution from CH₂Cl₂ to CH₂Cl₂/MeOH (98:2) to give **26** as a white gum (113.2 mg, 0.319 mmol, 76% yield). A small sample was crystallized from CH₂Cl₂. Mp 174–176 °C (decomposed). *R*_f=0.30 in MeOH/EtOAc (1:9). [α]_D²⁵+292.8 (c 0.66, CHCl₃). IR ν_{max} 3249, 2958, 2917, 2366, 1744, 1627 cm⁻¹. ¹H NMR (CDCl₃) δ 4.57 (br s, 1H, H-2), 4.13 (s, 3H, O–CH₃), 3.51 (br s, 1H, H-9a), 3.40–3.34 (m, 1H, H-5a), 3.11–3.05 (m, 2H, H-5b, H-10), 3.04 (d, *J* 6.0 Hz, 1H, H-7), 2.54 (s, 1H, H-2'), 2.17–2.10 (m, 1H, H-6a), 2.06 (s, 3H, H-16), 2.00 (d, *J* 12.5 Hz, 1H, H-1a), 1.91 (dt, *J* 12.5 Hz, 3.0 Hz, 1H, H-1b), 1.89–1.84 (m, 1H, H-6b), 1.77 (dd, *J* 10.0 Hz, 3.0 Hz, 1H, H-9), 1.36 (d, *J* 6.5 Hz, 3H, H-17). ¹³C NMR (CDCl₃) δ 169.7 (C-15), 162.8 (C-13), 147.8 (C-12), 128.2 (C-11), 112.0 (C-8), 98.9 (C-14), 81.2 (C-3), 80.9 (C-2), 75.2 (C-1'), 74.6 (C-2'), 60.7 (C-9a), 59.0 (O–CH₃), 54.4 (C-7), 48.6 (C-5), 47.3 (C-9), 34.6 (C-10), 33.5 (C-1), 27.0 (C-6), 18.3 (C-17), 9.3 (C-16). ESIMS *m/z* 356.1 (100%) [M+H]⁺. HRESIMS *m/z* 356.1459 [M+H]⁺, calcd for C₂₀H₂₂NO₅ 356.1498.

3.1.25. Click reaction method A: preparation of (5Z)-5-[(2S,2aR,6S,7aS,7bR,8R,9S)-hexahydro-7b-1-(1-benzyl-1H-1,2,3-triazol-4-yl)-9-methyl-4H-2,2,6-(epoxy[1]propanyl[3]ylidene)furo[2,3,4-gh]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5H)-furanone (27). A mixture of the alkyne **26** (12.7 mg, 0.034 mmol), benzylbromide (4.8 μL, 0.040 mmol), sodium azide (2.6 mg, 0.040 mmol) and copper powder (2.0 mg) in ^tBuOH/H₂O (1:1) (1.0 mL) was treated with 1 M aqueous CuSO₄ (0.1 mL). The mixture was stirred and heated in a microwave reactor at 125 °C for 10 min (10 W). The mixture was quenched with saturated aqueous NaHCO₃ solution (10 mL) and was directly extracted with diethyl ether (3×20 mL). The combined organic extracts were washed with brine and dried (MgSO₄) before being concentrated in vacuo. The concentrated residue was purified by column chromatography using gradient elution from CH₂Cl₂ to CH₂Cl₂/MeOH (9:1) to give a yellow gum (3.8 mg, 0.008 mmol, 23% yield). *R*_f=0.11 in MeOH/EtOAc (5:95). [α]_D²⁵+207.6 (c 0.25, CHCl₃). ¹H NMR δ 7.34 (s, 1H, C-5'), 7.32–7.28 (m, 3H, ArH), 7.22–7.21 (m, 2H, ArH), 5.44 (d, *J* 2.5 Hz, 2H, C-1''), 4.41 (br s, 1H, H-2), 4.07 (s, 3H, O–CH₃), 3.51 (br s, 1H, H-9a), 3.30 (d, *J* 5.5 Hz, 1H, H-7), 3.09–3.04 (m, 1H, H-10), 3.03–2.99 (m, 2H, H-5), 2.00 (s, 3H, H-16), 1.96 (d, *J* 12.0 Hz, 1H, H-1a), 1.92–1.86 (m, 3H, H-1b, H-6a, H-9), 1.84–1.81 (m, 1H, H-6b), 1.33 (d, *J* 6.5 Hz, 3H, H-17). ¹³C NMR δ 169.8 (C-15), 162.9 (C-13), 148.3 (C-12), 147.8 (ArC), 134.5 (C-4'), 129.2 (ArCH), 128.9 (ArCH), 128.3 (ArCH), 128.1 (C-11), 120.9 (C-5'), 112.7 (C-8), 98.7 (C-14), 81.0 (C-2), 79.9 (C-3), 61.1 (C-9a), 59.0 (O–CH₃), 54.4 (C-1''), 52.1 (C-7), 48.6 (C-5), 47.7 (C-9), 34.6 (C-10), 33.3 (C-1), 27.2 (C-6), 18.4 (C-17), 9.2 (C-16). ESIMS *m/z* 489.1 (100%) [M+H]⁺. HRESIMS *m/z* 489.2121 [M+H]⁺, calcd for C₂₇H₂₉N₄O₅ 489.2138.

3.1.26. (5Z)-5-[(2S,2aR,6S,7aS,7bR,8R,9S)-Hexahydro-7b-1-(3-phenylpropyl)-1H-1,2,3-triazol-4-yl]-9-methyl-4H-2,2,6-(epoxy[1]

propargyl[3]ylidene)furo[2,3,4-g]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5H)-furanone (28). Prepared using method A, from the alkyne **26** (15.0 mg, 0.042 mmol), bromopropylbenzene (9 μ L, 0.060 mmol), sodium azide (3.9 mg, 0.060 mmol), copper powder (2.5 mg), ^tBuOH/H₂O (1:1) (1.0 mL) and 1 M aqueous CuSO₄ (0.1 mL). The mixture was heated in a microwave reactor at 125 °C for 10 min (10 W). The product was obtained as a white gum (4.0 mg, 0.008 mmol, 18% yield). $R_f=0.22$ in MeOH/EtOAc (2:8). $[\alpha]_D^{25} +178.4$ (c 0.27, CHCl₃). ¹H NMR δ 7.40 (s, 1H, H-5'), 7.21 (d, J 8.0 Hz, 2H, ArH), 7.13 (t, J 7.5 Hz, 2H, ArH), 7.09 (d, J 7.5 Hz, 1H, ArH), 4.42 (br s, 1H, H-2), 4.27 (t, J 7.0 Hz, 2H, H-1''), 4.08 (s, 3H, O-CH₃), 3.53 (br s, 1H, H-9a), 3.30 (d, J 6.0 Hz, 1H, H-7), 3.10–3.06 (m, 1H, H-10), 3.06–3.02 (m, 2H, H-5), 2.60 (t, J 7.5 Hz, 2H, H-3''), 2.18 (quint, J 7.5 Hz, 2H, H-2''), 2.00 (s, 3H, H-16), 1.99 (d, J 14.0 Hz, 1H, H-1a), 1.90–1.87 (m, 2H, H-1b, H-6a), 1.86–1.82 (m, 2H, H-6b, H-9), 1.34 (d, J 6.5 Hz, 3H, H-17). ¹³C NMR δ 169.8 (C-15), 162.9 (C-13), 148.3 (C-12), 147.3 (C-4'), 140.2 (ArC), 128.6 (ArCH), 128.5 (ArCH), 128.0 (C-11), 126.4 (ArCH), 121.0 (C-5'), 112.6 (C-8), 98.6 (C-14), 81.0 (C-2), 79.8 (C-3), 61.1 (C-9a), 59.0 (O-CH₃), 52.1 (C-7), 49.7 (C-1''), 47.7 (C-9), 47.5 (C-5), 34.6 (C-10), 33.3 (C-1), 32.6 (C-3'), 31.6 (C-2''), 27.2 (C-6), 18.3 (C-17), 9.2 (C-16). ESIMS m/z 517.4 (100%) [M+H]⁺. HRESIMS m/z 517.2439 [M+H]⁺, calcd for C₂₉H₃₃N₄O₅ 517.2451.

3.1.27. Click reaction method B: preparation of (5Z)-5-[(2S,2aR,6S,7aS,7bR,8R,9S)-hexahydro-7b-1-(1-phenyl-1H-1,2,3-triazol-4-yl)-9-methyl-4H-2,2,6-(epoxy[1]propargyl[3]ylidene)furo[2,3,4-g]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5H)-furanone (29). To a solution of the alkyne **26** (16.2 mg, 0.046 mmol), Cu(OAc)₃ (0.8 mg, 0.005 mmol) and sodium ascorbate (13.6 mg, 0.068 mmol) in MeOH/H₂O (1:1) (1.0 mL) at rt was added 0.09 M phenylazide in MeOH (1.0 mL) and the reaction mixture was left to stir for 3 h. The mixture was quenched with saturated aqueous NaHCO₃ solution (10 mL) and was directly extracted with CH₂Cl₂ (3 \times 20 mL). The combined organic extracts were washed with brine and dried (MgSO₄) before being concentrated in vacuo. The concentrated residue was purified by column chromatography using gradient elution from CH₂Cl₂ to CH₂Cl₂/MeOH (9:1) to give a yellow gum (7.2 mg, 0.015 mmol, 33% yield). $R_f=0.37$ in MeOH/CH₂Cl₂ (1:9). $[\alpha]_D^{25} +229.9$ (c 0.48, CHCl₃). ¹H NMR δ 7.95 (s, 1H, H-5'), 7.74 (d, J 8.0 Hz, 2H, ArH), 7.54–7.51 (m, 2H, ArH), 7.44 (t, J 7.5 Hz, 1H, ArH), 4.58 (s, 1H, H-2), 4.16 (s, 3H, O-CH₃), 3.64 (br s, 1H, H-9a), 3.50 (d, J 5.5 Hz, 1H, H-7), 3.24–3.12 (m, 3H, H-5, H-10), 2.12–2.07 (m, 1H, H-1a), 2.09 (s, 3H, H-16), 2.05–2.02 (m, 2H, H-1b, H-6a), 2.00–1.98 (m, 1H, H-6b), 1.97–1.93 (m, 1H, H-9), 1.43 (d, J 7.0 Hz, 3H, H-17). ¹³C NMR (one ArCH was not observed due to peak overlap) δ 169.8 (C-15), 163.0 (C-13), 148.3 (C-12), 137.1 (ArC), 129.9 (ArCH), 128.9 (C-11), 128.2 (C-4'), 120.6 (ArCH), 119.4 (C-5'), 112.7 (C-8), 98.9 (C-14), 81.2 (C-2), 80.0 (C-3), 61.3 (C-9a), 59.0 (O-CH₃), 52.3 (C-7), 48.7 (C-5), 47.9 (C-9), 34.7 (C-10), 33.4 (C-1), 27.3 (C-6), 18.5 (C-17), 9.3 (C-16). ESIMS m/z 475.1 (100%) [M+H]⁺. HRESIMS m/z 475.1972 [M+H]⁺, calcd for C₂₆H₂₇N₄O₅ 475.1981.

3.1.28. (5Z)-5-[(2S,2aR,6S,7aS,7bR,8R,9S)-Hexahydro-7b-1-[3-(2-propylisoidindoline-1,3-dionyl)-1H-1,2,3-triazol-4-yl]-9-methyl-4H-2,2,6-(epoxy[1]propargyl[3]ylidene)furo[2,3,4-g]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5H)-furanone (30). Prepared using method B, from the alkyne **26** (12.1 mg, 0.034 mmol), *N*-propylphthalimide azide (0.50 mL, 0.187 M in THF, 0.068 mmol), sodium ascorbate (10 mg, 0.051 mmol) and Cu(OAc)₂ (0.6 mg, 0.003 mmol). The product was obtained as a colourless gum (9.1 mg, 0.016 mmol, 46% yield). $R_f=0.15$ in MeOH/EtOAc (1:9). $[\alpha]_D^{25} +131.4$ (c 0.61, CHCl₃). ¹H NMR δ 7.76–7.74 (m, 2H, ArH), 7.67–7.66 (m, 2H, ArH), 7.65 (s, 1H, H-5'), 4.41 (br s, 1H, H-2), 4.34 (t, J 7.0 Hz, 2H, H-1''), 4.08 (s, 3H, O-CH₃), 3.66 (t, J 6.5 Hz, 2H, H-3'''), 3.53 (br s, 1H, H-9a), 3.26 (d, J 6.0 Hz, 1H, H-7), 3.10–3.00 (m, 3H, H-5, H-10), 2.27 (quint, J 6.5 Hz, 2H, H-2'''), 1.98 (s, 3H, H-16),

1.93–1.89 (m, 1H, H-1b), 1.85–1.80 (m, 4H, H-1a, H-6, H-9), 1.33 (d, J 6.5 Hz, 3H, H-17). ¹³C NMR δ 169.5 (C-15), 168.0 (C-1'', C-3''), 162.7 (C-13), 148.1 (C-12), 147.0 (C-4'), 134.0 (ArCH), 131.6 (ArC), 127.6 (C-11), 123.1 (ArCH), 121.6 (C-5'), 112.4 (C-8), 98.2 (C-14), 80.7 (C-2), 79.5 (C-3), 60.8 (C-9a), 58.7 (O-CH₃), 51.8 (C-7), 48.2 (C-5), 47.6 (C-1'''), 47.3 (C-9), 34.8 (C-3'''), 34.4 (C-10), 33.0 (C-1), 29.0 (C-2'''), 26.9 (C-6), 18.0 (C-17), 8.9 (C-16). ESIMS m/z 586.4 (100%) [M+H]⁺. HRESIMS m/z 586.2318 [M+H]⁺, calcd for C₃₁H₃₂N₅O₇ 586.2302.

3.1.29. Click reaction method C: preparation of (5Z)-5-[(2S,2aR,6S,7aS,7bR,8R,9S)-hexahydro-7b-1-(3-phenylisoxazol-5-yl)-9-methyl-4H-2,2,6-(epoxy[1]propargyl[3]ylidene)furo[2,3,4-g]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5H)-furanone (31). To a solution of the alkyne **26** (15.1 mg, 0.042 mmol), Cu(OAc)₂ (3.1 mg, 0.017 mmol) and sodium ascorbate (12.6 mg, 0.064 mmol) in EtOH/H₂O (1:1) (1.0 mL) at rt was added *N*-hydroxybenzenecarboximidoyl chloride (10 mg, 0.064 mmol) and sodium hydroxide (2.6 mg, 0.064 mmol). The reaction was left to stir for 3 h. The mixture was quenched with saturated aqueous NaHCO₃ solution (10 mL) and was directly extracted with CH₂Cl₂ (3 \times 20 mL). The combined organic extracts were washed with brine and dried (MgSO₄) before being concentrated in vacuo. The concentrated residue was purified by column chromatography using gradient elution from CH₂Cl₂ to CH₂Cl₂/MeOH (9:1) to give a white gum (11.9 mg, 0.025 mmol, 59% yield). $R_f=0.37$ in MeOH/EtOAc (1:9). $[\alpha]_D^{25} +203.4$ (c 0.79, CHCl₃). ¹H NMR δ 7.82–7.79 (m, 2H, ArH), 7.45 (br s, 3H, ArH), 6.56 (s, 1H, H-4'), 4.62 (s, 1H, H-2), 4.16 (s, 3H, O-CH₃), 3.65 (br s, 1H, H-9a), 3.43 (d, J 5.5 Hz, 1H, H-7), 3.29–3.24 (m, 1H, H-5b), 3.19–3.14 (m, 2H, H-5a, H-10), 2.11–2.08 (m, 1H, H-1a), 2.09 (s, 3H, H-16), 2.03–1.96 (m, 3H, H-1a, H-6), 1.93 (dd, J 9.5 Hz, 2.5 Hz, 1H, H-9), 1.43 (d, J 6.0 Hz, 3H, H-17). ¹³C NMR δ 171.2 (C-5'), 169.7 (C-15), 162.8 (C-13), 162.6 (C-3'), 147.8 (C-12), 130.3 (ArC), 128.9 (ArC), 128.8 (ArC), 128.3 (C-11), 127.5 (ArC), 112.5 (C-8), 100.2 (C-4'), 99.0 (C-14), 80.6 (C-3), 80.1 (C-2), 61.3 (C-9a), 59.0 (O-CH₃), 52.3 (C-7), 49.0 (C-5), 47.7 (C-9), 34.6 (C-10), 33.3 (C-1), 27.2 (C-6), 18.4 (C-17), 9.3 (C-16). ESIMS m/z 475.1 (100%) [M+H]⁺. HRESIMS m/z 475.1871 [M+H]⁺, calcd for C₂₇H₂₇N₂O₆ 475.1869.

3.1.30. (5Z)-5-[(2S,2aR,6S,7aS,7bR,8R,9S)-Hexahydro-7b-1-[3-(4-fluorophenyl)isoxazol-5-yl]-9-methyl-4H-2,2,6-(epoxy[1]propargyl[3]ylidene)furo[2,3,4-g]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5H)-furanone (32). Prepared using method C, from the alkyne **26** (10.6 mg, 0.030 mmol), *N*-hydroxy-4-fluorobenzenecarboximidoyl chloride (20 mg, 0.153 mmol), sodium ascorbate (8.8 mg, 0.045 mmol), sodium hydroxide (1.8 mg, 0.045 mmol) and Cu(OAc)₂ (2.2 mg, 0.012 mmol). The product was obtained as a yellow gum (6.3 mg, 0.013 mmol, 43% yield). $R_f=0.62$ in MeOH/EtOAc (2:8). $[\alpha]_D^{25} +236.1$ (c 0.36, CHCl₃). ¹H NMR δ 7.80–7.77 (m, 2H, ArH), 7.15 (t, J 8.5 Hz, 2H, ArH), 6.52 (s, 1H, H-4'), 4.61 (br s, 1H, H-2), 4.16 (s, 3H, O-CH₃), 3.65 (br s, 1H, H-9a), 3.42 (d, J 5.5 Hz, 1H, H-7), 3.29–3.23 (m, 1H, H-5), 3.19–3.12 (m, 2H, H-5, H-10), 2.11 (s, 1H, H-1a), 2.09 (s, 3H, H-16), 2.04–1.96 (m, 3H, H-1b, H-6), 1.93 (dd, J 10.0 Hz, 3.0 Hz, 1H, H-9), 1.42 (d, J 6.5 Hz, 3H, H-17). ¹³C NMR δ 171.5 (C-5'), 169.7 (C-15), 164.0 (ArC-F, d, J_{C-F} 250 Hz), 162.8 (C-13), 161.7 (C-3'), 147.8 (C-12), 128.9 (ArCH d, J_{C-F} 9.8 Hz), 128.8 (ArCH), 128.3 (C-11), 125.1 (ArC, d, J_{C-F} 3.3 Hz), 116.3 (ArCH, d, J_{C-F} 22.0 Hz), 116.1 (ArCH), 112.4 (C-8), 100.1 (C-4'), 99.0 (C-14), 80.6 (C-3), 80.1 (C-2), 61.3 (C-9a), 59.0 (O-CH₃), 52.3 (C-7), 49.0 (C-5), 47.7 (C-9), 34.6 (C-10), 33.3 (C-1), 27.2 (C-6), 18.4 (C-17), 9.3 (C-16). ESIMS m/z 492.9 (100%) [M+H]⁺. HRESIMS m/z 493.1789 [M+H]⁺, calcd for C₂₇H₂₆N₂O₆F 493.1775.

3.1.31. Sonogashira coupling: preparation of (5Z)-5-[(2S,2aR,6S,7aS,7bR,8R,9S)-hexahydro-7b-1-(2-phenylethyn-1-yl)-9-methyl-4H-2,2,6-(epoxy[1]propargyl[3]ylidene)furo[2,3,4-g]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5H)-furanone (33). A solution

mixture of the alkyne **26** (13.4 mg, 0.038 mmol), iodobenzene (4.2 μL , 0.038 mmol), $\text{PdCl}_2(\text{PPh}_3)_2$ (0.5 mg, 0.001 mmol), CuI (0.3 mg, 0.002 mmol) and Et_3N (19 mg, 0.189 mmol) in THF (1.0 mL) in a glass tube was bubbled with argon gas and then the tube was sealed. The reaction was left to stir at rt for 24 h. The mixture was extracted with EtOAc (3×20 mL). The combined organic phase was dried (Na_2SO_4). The crude residue was purified by PTLC with $\text{MeOH}/\text{CH}_2\text{Cl}_2$ (1:99) as a mobile phase to give a phenyl alkyne product **33** as a yellow gum (10.0 mg, 0.023 mmol, 62% yield) and the dimeric by-product **34** (0.6 mg, 0.0001 mmol, 4% yield), which was directly prepared via the Eglinton coupling reaction. $R_f=0.42$ in MeOH/EtOAc (1:9). $[\alpha]_{\text{D}}^{25} +235.9$ (c 0.44, CHCl_3). $^1\text{H NMR}$ δ 7.45–7.44 (m, 2H, ArH), 7.31–7.30 (m, 3H, ArH), 4.66 (br s, 1H, H-2), 4.15 (s, 3H, O– CH_3), 3.56 (br s, 1H, H-9a), 3.48–3.42 (m, 1H, H-5a), 3.14 (d, J 6.0 Hz, 1H, H-7), 3.11–3.08 (m, 2H, H-5b, H-10), 2.23–2.17 (m, 1H, H-6a), 2.08 (s, 3H, H-16), 2.06 (d, J 12.0 Hz, 1H, H-1a), 1.98 (d, J 12.0 Hz, 1H, H-1b), 1.93–1.88 (m, 1H, H-6b), 1.83 (d, J 10.0 Hz, 1H, H-9), 1.39 (d, J 6.5 Hz, 3H, H-17). $^{13}\text{C NMR}$ δ 169.8 (C-15), 162.9 (C-13), 148.0 (C-12), 131.9 (ArCH), 128.6 (ArCH), 128.4 (ArCH), 128.2 (C-11), 122.5 (ArC), 112.2 (C-8), 98.9 (C-14), 86.3 (C-2'), 86.1 (C-1'), 81.2 (C-2), 76.0 (C-3), 60.8 (C-9a), 59.0 (O– CH_3), 54.6 (C-7), 48.7 (C-5), 47.4 (C-9), 34.6 (C-10), 33.6 (C-1), 27.1 (C-6), 18.4 (C-17), 9.3 (C-16). ESIMS m/z 432.1 (100%) $[\text{M}+\text{H}]^+$. HRESIMS m/z 432.1821 $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{26}\text{H}_{26}\text{NO}_5$ 432.1811.

3.1.32. Eglinton coupling: preparation of bis-(5Z)-5-[(2S,2aR,6S,7aS,7bR,8R,9S)-hexahydro-7b-1-ethynyl-9-methyl-4H-2,2,6-(epoxy[1]propanyl[3]ylidene)furo[2,3,4-gh]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5H)-furanone (34). A solution of the alkyne **26** (13.5 mg, 0.038 mmol) and $\text{Cu}(\text{OAc})_2$ (35 mg, 0.190 mmol) in dry MeCN (1.0 mL) in a sealed tube was bubbled with argon gas and then the tube was sealed. The reaction mixture was heated and stirred at 40 °C for 4 h. The mixture was cooled to rt and filtered through a thin pad of Celite. The filtrate was treated with an aqueous solution of NaHCO_3 and extracted with CH_2Cl_2 (3×20 mL). The combined organic phase was washed with brine and dried (MgSO_4). The evaporated residue was purified by PTLC with $\text{MeOH}/\text{CH}_2\text{Cl}_2$ (2:98) as a mobile phase to give a dimer **34** as a yellow gum (12.0 mg, 0.017 mmol, 89% yield). $R_f=0.50$ in $\text{MeOH}/\text{CH}_2\text{Cl}_2$ (1:9). $[\alpha]_{\text{D}}^{25} +286.3$ (c 0.39, CHCl_3). $^1\text{H NMR}$ δ 4.56 (br s, 2H, H-2), 4.13 (s, 6H, O– CH_3), 3.50 (br s, 2H, H-9a), 3.37–3.31 (m, 2H, H-5a), 3.10–3.02 (m, 4H, H-5b, H-10), 3.05 (d, J 6.0 Hz, 2H, H-7), 2.14–2.08 (m, 2H, H-6a), 2.06 (s, 6H, H-16), 2.00 (d, J 12.0 Hz, 2H, H-1a), 1.89–1.86 (m, 2H, H-1b), 1.85–1.83 (m, 2H, H-6b), 1.76 (dd, J 10.5 Hz, 3.5 Hz, 2H, H-9), 1.35 (d, J 6.5 Hz, 6H, H-17). $^{13}\text{C NMR}$ δ 169.7 (C-15), 162.8 (C-13), 147.6 (C-12), 128.2 (C-11), 111.9 (C-8), 99.0 (C-14), 80.9 (C-2), 77.4 (C-3), 75.8 (C-1'), 70.4 (C-2'), 60.8 (C-9a), 59.0 (O– CH_3), 54.8 (C-7), 48.9 (C-5), 47.3 (C-9), 34.6 (C-10), 33.6 (C-1), 27.0 (C-6), 18.3 (C-17), 9.3 (C-16). ESIMS m/z 709.5 (100%) $[\text{M}+\text{H}]^+$. HRESIMS m/z 709.2791 $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{40}\text{H}_{41}\text{N}_2\text{O}_{10}$ 709.2761.

3.1.33. (5Z)-5-[(2S,2aR,6S,7aS,7bR,8R,9S)-Hexahydro-7b-1-(2-phenylethyl)-9-methyl-4H-2,2,6-(epoxy[1]propanyl[3]ylidene)furo[2,3,4-gh]pyrrolizin-10-ylidene]-4-methoxy-3-methyl-2(5H)-furanone (35). To a mixture of the phenyl alkyne **33** (4.7 mg, 0.011 mmol) and Pd/C (0.5 mg, 0.002 mmol) in EtOAc (1.0 mL) at rt, a hydrogen gas was bubbled into the solution and the reaction was left to stir for 24 h. The reaction mixture was then filtered through a thin pad of Celite. The concentrated residue was purified by PTLC with MeOH/EtOAc (1:99) as a mobile phase to give a product as a white gum (3.5 mg, 0.008 mmol, 74% yield). $R_f=0.32$ in $\text{MeOH}/\text{CH}_2\text{Cl}_2$ (1:9). $[\alpha]_{\text{D}}^{25} +248.4$ (c 0.23, CHCl_3). $^1\text{H NMR}$ δ 7.20 (d, J 7.0 Hz, 2H, ArH), 7.13–7.10 (m, 3H, ArH), 4.24 (br s, 1H, H-2), 4.06 (s, 3H, O– CH_3), 3.43 (br s, 1H, H-9a), 3.12–3.06 (m, 1H, H-5), 3.05–3.00 (m, 1H, H-10), 2.99–2.93 (m, 1H, H-5), 2.72 (td, J 11.5 Hz, 5.0 Hz, 1H, H-1'), 2.66 (d, J 6.0 Hz, 1H, H-7), 2.50 (td, J 13.5 Hz, 5.5 Hz, 1H, H-1'), 2.00 (s, 3H, H-16), 1.90 (d, J 12.0 Hz, 1H, H-6a), 1.90–1.79 (m, 3H, H-1a, H-9, H-2'), 1.78–1.74 (m, 2H, H-6b, H-2'), 1.67 (dt, J 12.0 Hz, 3.5 Hz, 1H, H-1b), 1.31 (d, J 6.5 Hz, 3H, H-17).

$^{13}\text{C NMR}$ δ 169.8 (C-15), 162.9 (C-13), 148.5 (C-12), 141.7 (ArC), 128.6 (ArCH), 128.3 (ArCH), 128.0 (C-11), 126.1 (ArCH), 112.7 (C-8), 98.6 (C-14), 82.7 (C-3), 78.5 (C-2), 61.0 (C-9a), 58.9 (O– CH_3), 50.4 (C-7), 47.7 (C-5, C-9), 34.6 (C-10), 33.7 (C-2'), 33.4 (C-1), 31.4 (C-1'), 26.7 (C-6), 18.4 (C-17), 9.2 (C-16). ESIMS m/z 436.1 (100%) $[\text{M}+\text{H}]^+$. HRESIMS m/z 436.2118 $[\text{M}+\text{H}]^+$, calcd for $\text{C}_{26}\text{H}_{30}\text{NO}_5$ 436.2124.

3.1.34. AChE inhibition studies.

3.1.34.1. TLC bioautographic method. The AChE used in this assay was extracted from electric eels and purchased from Sigma–Aldrich (EC 3.1.1.7). The enzyme stock solution was prepared from a solution of AChE (1000 U) in 0.05 M tris–hydrochloric acid buffer (150 mL) at pH 7.8 to which was added bovine serum albumin (150 mg) to stabilize the enzyme. The stock solution was kept at 4 °C. TLC plates used for the bioautography were washed with acetone and then thoroughly dried. The samples were prepared as solutions in MeOH at concentrations of 1000, 100, 10, 1 and 0.1 ppm. The samples were applied to the TLC plates in varying quantities using Camag Nanomat 4 TLC spotter with 0.5 μL capillaries and sprayed with AChE enzyme stock solution and thoroughly dried again. The plates were laid flat on plastic plugs in a covered water bath (to avoid the plates from contacting the H_2O directly) and then incubated in a humid atmosphere at 37 °C for 20 min. The plates were taken out and sprayed with a freshly prepared indicator solution which was a mixture of two solutions, a solution of 1-naphthyl acetate (25 mg) in EtOH (10 mL) and a solution of Fast Blue B salt (40 mg) in H_2O (16 mL). After 1–2 min, a purple colouration on the TLC plates appeared and white spots indicated inhibition of AChE by the samples.

3.1.34.2. Spectroscopic-based method. Acetylthiocholine iodide (ATChI) was used as a substrate while 5,5'-dithiobis[2-nitrobenzoic acid] (DTNB) was used as a reagent. Two stock solutions were used in this assay, buffer solution and substrate solution. The pH 7.0 phosphate buffer solution was prepared from a mixture of 37.2 mM $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ and 62.7 mM $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, in Milli Q H_2O . The substrate solution was prepared as a 4.73 mM ATChI solution in phosphate buffer pH 7.0. The reagent solution was prepared as a 3.15 mM DTNB solution in phosphate buffer pH 7.0.

The assay was performed in 96-well plates. In each well, 120 μL of phosphate buffer pH 7.0 was mixed with 20 μL of reagent and 20 μL of substrate, then 20 μL of AChE (0.75 U/mL) in phosphate buffer pH 7.0 was added with 20 μL of the sample (which was prepared in concentrations of 5000, 1000, 200, 40, 8, 1.6 and 0.32 μM in DMSO). The final concentrations were 500, 100, 20, 4, 0.8, 0.16 and 0.032, respectively. Then the well plate was directly put into the microplate reader, which was thermostated at 25 °C. The absorbances were read using a SPECTRAMax[®] PLUS³⁸⁴ microplate thermostated spectrophotometer (California, USA) at 412 nm, every 15 s for 30 min continuously. Enzyme activity was calculated as a percentage compared to an assay using a buffer without any inhibitor. The AChE inhibitory data were analysed with the software package GraphPad Prism[®] (Graph Pad Inc., San Diego, USA). IC_{50} values are means \pm SD of three individual determinations each performed in triplicate.

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Supplementary data

Compound purity analysis by HPLC, synthesis of the Bestmann–Ohira reagent and the coupling components for the Click

reactions. Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.tet.2012.06.047>.

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