Expedient synthesis and structure–activity relationships of phenanthroindolizidine and phenanthroquinolizidine alkaloids

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The total synthesis of alkaloids phenanthroindolizidine **1a**, tylophorine **1b**, and phenanthroquinolizidine **1c**, has been achieved in 46%, 49%, and 42% overall yield, respectively, starting from the corresponding phenanthrene-9-carboxaldehyde. Compound **1c** exhibited potent inhibition activity in three human cancer cell lines, with IC_{50} values ranging from 104 to 130 nM. The structure–activity relations of these alkaloids and some of their synthetic intermediates against the three cell lines were also described.

Introduction

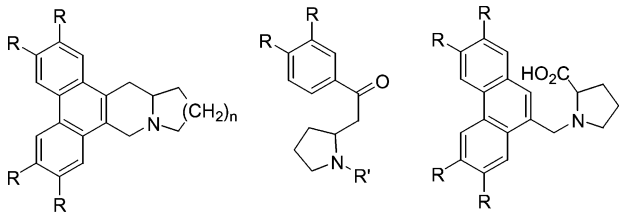
Since the first isolation of tylophorine **1b** in 1935,[1] the phenanthroindolizidine alkaloids have been a focus of study in the fields of medicinal and synthetic chemistry. These alkaloids are well known for their cytotoxic activity, due to the inhibition of protein[2] and nucleic acid[3] synthesis.[4] In addition, some of these alkaloids have been shown to possess antiamoebic,[5] antibacterial and antifungal activities.[6] Recently, we also reported that phenanthroindolizidines, isolated from the leaves of *Ficus septica*, exhibited strong cytotoxic activity against gastric carcinoma (NUGC-3) and nasopharyngeal carcinoma (HONE-1) cell lines.[7]

The wide range of pharmacological properties that the phenanthroindolizidine alkaloids exhibit have drawn the attention of chemists. In the last three decades, for example, there were three types of methodologies that could give access to tylophorine. One was by a biogenetically patterned sequence *via* β-amino ketone intermediates **2**,[8] another was by a route involving an acid-catalyzed cyclization of amino-acids **3**,[8b,9] and the other was by the route to tylophorine where both rings of the indolizidine nucleus were assembled through a single cycloaddition reaction.[10] In this paper, we report a convenient synthetic approach to the pentacyclic alkaloids **1** in high yields. Subsequently, these alkaloids and synthetic intermediates undergo preliminary screening tests for their anticancer activities against human cancer cell lines including breast carcinoma (MCF-7), lung carcinoma (NCI-H460) and central nervous system carcinoma (SF-268). The structure–activity relations (SAR) are also discussed.

Results and discussion

Synthetic results

The synthetic strategy for constructing the phenanthroindolizidines **1a** and **1b** and phenanthroquinolizidine **1c** is shown in



1a: R = H, n = 1
1b: R = OCH_3, n = 1
1c: R= OCH_3, n = 2

2 **3**

Scheme 1. This method represented a new route for the preparation of the pentacyclic alkaloid in that the nitrogen-containing bicyclic heterocycles were constructed by double cyclizations. First, the isocyanate **7**, formed by Curtius rearrangement of acyl azide **8**, underwent an electrocyclic reaction followed by hydrogen shift to yield isoquinolinone **6**. Second, the *N*-haloalkylisoquinolinone **5** proceeded through radical cyclization to afford the pyrrolidine or piperidine rings **4**. Then, hydride reduction would give the target products **1**.

Unsubstituted phenanthroindolizidine **1a** was chosen as our initial target since its simplicity would allow us to test the feasibility of the approach (Scheme 2). The Wittig reaction between commercially available phenanthrene-9-carboxaldehyde **10a** and (carboethoxymethylene)triphenylphosphorane followed by hydrolysis directly yielded the desired acid **9a**, which upon purification was found to be exclusively the *trans* isomer. The acid **9a** was almost quantitatively transformed to the acyl azide **8a** on treatment with oxalyl chloride and sodium azide. Since the azide was unstable under heat, it was immediately used for the next reaction after purification using a short column. When a solution of **8a** in *o*-dichlorobenzene containing a catalytic amount of $Hg(OAc)_2$ was refluxed for 1 h, the Curtius rearrangement, electrocyclic reaction, and hydrogen rearrangement occurred to supply the isoquinolinone **6a** in 87% yield. Treatment of the compound **6a** with NaH in DMF provided an amide salt, and subsequent *N*-alkylation with 1-bromo-3-chloropropane at room temperature produced a mixture of chloride **5a1** and bromide **5a2** in a 20 : 1 ratio. Even at 0 °C, the ratio of the mixed *N*-haloalkylisoquinolinones **5a1** and **5a2** was lifted to 50 : 1 in a *ca.* 95% combined yield. The mixed halides were directly subjected to

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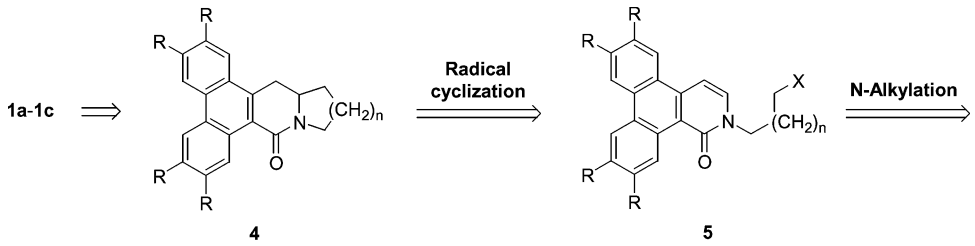
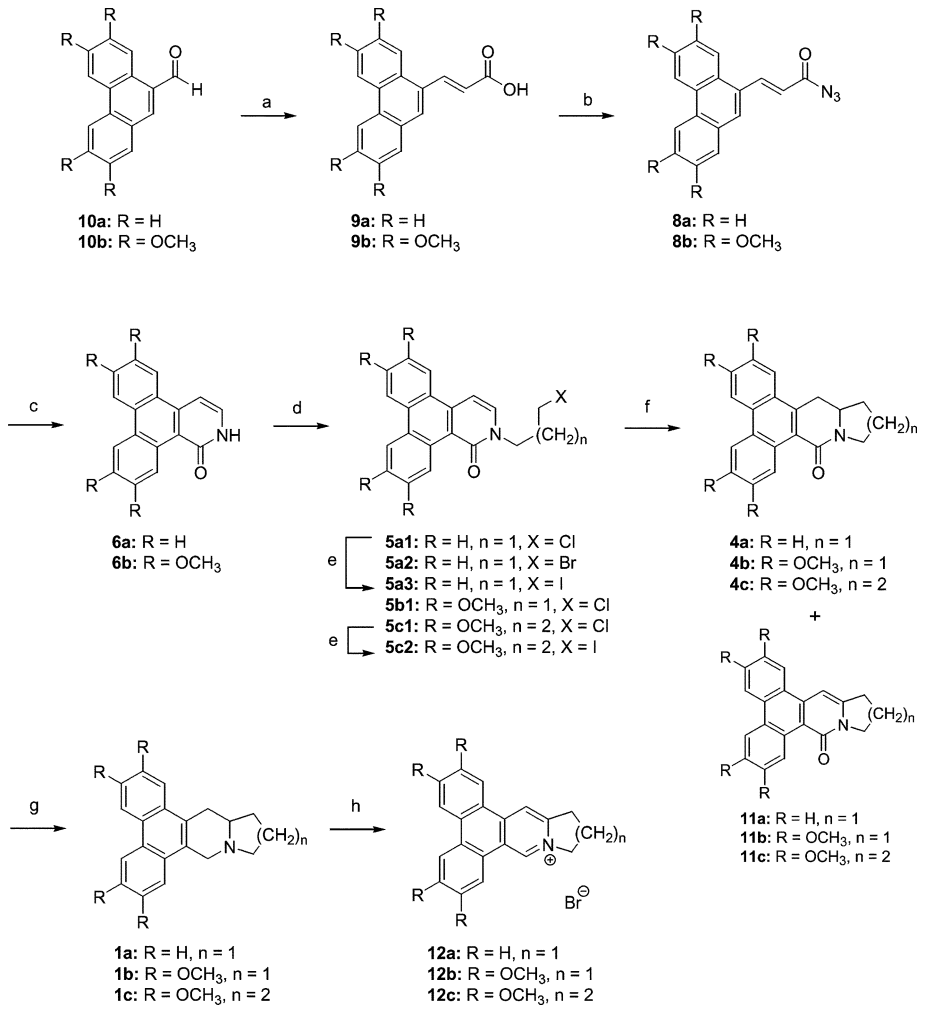


Scheme 1



Scheme 2 *Reagents and conditions:* (a) (i) $Ph_3P{=}CHCO_2Et$, toluene, reflux, 4 h; (ii) KOH, EtOH–H_2O, reflux, 3 h; (b) (i) $(COCl)_2$, toluene, 80 °C, 5 h; (ii) NaN_3, acetone, rt, 2 h; (c) cat. $Hg(OAc)_2$, *o*-dichlorobenzene, reflux, 1 h; (d) (i) NaH, DMF; (ii) $Br(CH_2)_nCl$, $n = 3$ or 4, DMF, rt, overnight; (e) NaI, CH_3CN, 125 °C, 12 h; (f) AIBN, Bu_3SnH, toluene, reflux, 6 h; (g) $NaAl(OCH_2CH_2OMe)_2H_2$, dioxane, reflux, 2 h; (h) NBS, $CHCl_3$, rt, 1 h.

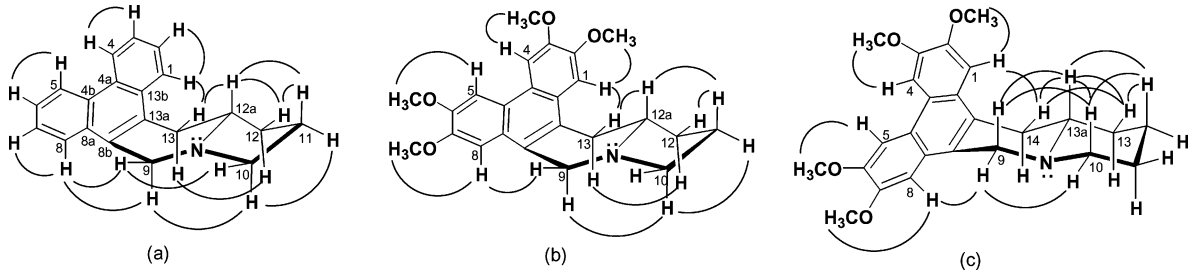


Fig. 1 The key NOESY correlations of **1a** (a), **1b** (b), and **1c** (c).

radical cyclization according to the method of Osornio *et al.*[11] to give the cyclization adduct **4a** in low yield, and there remained a considerable amount of the starting material **5a1**. Because of the low activity of chloride, the more active iodide was considered as the radical precursor in the radical cyclization. The mixed halides were successfully converted to the corresponding iodide **5a3** by halogen exchange with an excess of sodium iodide in CH_3CN. Without further purification, treatment of compound **5a3** and Bu_3SnH in boiling toluene, followed by addition of small incremental amounts of 2,2′-azobisisobutyronitrile (AIBN, 0.4 mol equiv.) for 6 h, gave phenanthroindolizidione **4a** (76%) and its dehydro derivative **11a** (6%). Attempts to avoid the formation of the undesired product **11a** by reducing the amount of AIBN caused incomplete consumption of the starting material. Reduction of the lactam **4a** with sodium bis(2-methoxyethoxy)aluminium hydride in refluxing dioxane afforded racemic phenanthroindolizidine **1a** in a 46% overall yield starting from the aldehyde **10a**.

On the basis of the above results, we anticipated that tylophorine **1b** and phenanthroquinolizidine **1c** would be readily available from 2,3,6,7-tetramethoxyphenanthrene-9-carboxaldehyde **10b**. The same protocol was followed for producing intermediates **9b** (76%), **8b** (92%), **6b** (85%), **5b1** (96%) and **5c1** (89%) with generally excellent yields. We were concerned about the intramolecular radical cyclization to afford the pyrrolidine and piperidine rings, respectively. Surprisingly, when chloride **5b1** was directly subjected to the *n*-Bu_3SnH–AIBN reaction condition, the starting material was completely consumed and generated phenanthroindolizidione **4b** (87%) and traces of the dehydro derivative **11b** (5%). Comparing the results of the 5-*exo* cyclizations of **5a1** and **5b1**, the OCH_3 groups on the phenanthrene ring might increase the reactivity of radical cyclization. In contrast to **5b1**, the chloride **5c1** showed inefficient intramolecular cyclization under the same conditions and led to incomplete consumption of the starting material. It was thought that this was mainly due to the higher activation energy for 6-*exo* ring closure.[12] Therefore, phenanthroquinolizidione **4c** (80%) and the oxidative side-product **11c** (12%) were obtained *via* halogen exchange followed by radical cyclization. Tylophorine **1b** and phenanthroquinolizidine **1c** could be afforded by reduction of the lactams **4b** and **4c** both in high 98% yields. The overall yields from the aldehyde **10b** to **1b** and **1c** were calculated to be 49%, and 42%, respectively.

It should be mentioned here that the phenanthroindolizidine and phenanthroquinolizidine alkaloids were known to be unstable when exposed to light.[13] We also observed that, after 1 day, compounds **1a–c** in $CHCl_3$ decomposed to a yellow crystalline solid. The NMR spectral properties showed agreement with dehydroiminium salts **12a–c** which might be obtained by treatment of **1a–c** with *N*-bromosuccinimide (NBS) in $CHCl_3$.[14]

The complete assignment of the ^1H and ^{13}C NMR signals of **1a–c** was obtained from 2D-NMR spectra, such as COSY, HMQC, HMBC and NOESY. In **1a**, the existence of NOEs of H-12a (δ 2.49) with H-11ax (δ 1.94) and H-12eq (δ 2.24) and the absence of NOEs of H-12a with H-9 (δ 3.74 and 4.74) and H-10 (δ 2.46 and 3.48) suggested that **1a** possessed the *cis*-fused indolizidine skeleton with a boat-like conformation in the six-membered ring and an envelope conformation in the five-membered ring (Fig. 1a).[15] Similarly, the absence of NOEs between H-12a (δ 2.51) and H-9 (δ 3.67 and 4.62) and H-10 (δ 2.45 and 3.47) indicated the *cis*-boat indolizidine ring in **1b** (Fig. 1b). In contrast to the *cis*-fused phenanthroindolizidine skeleton, the presence of NOEs between H-13a (δ 2.39) and H-9ax (δ 3.60), H-10ax (δ 2.32) and H-12ax (δ1.49) in **1c** suggested a *trans*-fused quinolizidine ring adopting a chair-like conformation (Fig. 1c). In addition, the triplet–quintet–triplet splitting pattern of three mutually coupled methylenes in **11a–b** and **12a–b** and the triplet–quintet–quintet–triplet pattern of four methylenes in **11c** and **12c** let us conclude that the pentacyclic rings in dehydrolactams **11a–c** and dehydroiminium salts **12a–c** established a planar conformation, in spite of the five- or six-membered saturated ring.

Biological results

We now describe the biological evaluation of the pentacyclic alkaloids **1** and some of their analogues **4**, **5**, **6**, **11**, and **12**, available by total synthesis. The cytotoxic activities of the synthesized compounds were tested against three human cancer cell lines, MCF-7, NCI-H460, and SF-268. The results are shown in Table 1. We started our SAR with tylophorine **1b** which showed pronounced cytotoxicity, with IC_{50} values of 489 to 1764 nM. A 6 to 20-fold decrease in cytotoxicity was observed for **1a** relative to **1b**, indicating that the methoxyl group on the phenanthrene ring was of importance. It should be noted that phenanthroquinolizidine **1c** exhibited the best activity in the three cell lines ranging from 104 to 130 nM. This revealed that the *trans*-fused quinolizidine ring seems to be more active than the *cis*-fused indolizidine ring. The lack of either an indolizidine or a quinolizidine ring led isoquinolinones **5a–c** and **6a–b** to show no significant activity in all cell lines. Moreover, comparison of the cytotoxic activities of lactams **4a–c** and the corresponding reduced compounds **1a–c** revealed that the presence of a carbonyl in the bicyclic lactam may dramatically diminish cytotoxicity. In addition, the almost planar dehydrolactams **11a–c** did not show any activity in our test. This

Table 1 Cytotoxicity of compounds toward several cancer cell lines[a]

Compound	IC_{50}/nM[b]		
	MCF-7	NCI-H460	SF-268
1a	9997 ± 408	10 231 ± 632	10 811 ± 1213
1b	489 ± 45	584 ± 39	1764 ± 105
1c	104 ± 7	109 ± 9	130 ± 3
4a	>50 000	>50 000	>50 000
4b	>50 000	>50 000	>50 000
4c	41 603 ± 2815	24 144 ± 471	34 070 ± 607
5a1	13 687 ± 1233	17 229 ± 975	17 849 ± 948
5a2	11 435 ± 222	17 462 ± 1818	7567 ± 627
5b1	>50 000	>50 000	>50 000
5c1	>50 000	>50 000	>50 000
6a	>50 000	>50 000	>50 000
6b	>50 000	>50 000	>50 000
11a	>50 000	>50 000	>50 000
11b	>50 000	>50 000	>50 000
11c	>50 000	>50 000	>50 000
12a	8275 ± 74	13 483 ± 2615	4440 ± 164
12b	>50 000	>50 000	>50 000
12c	2764 ± 296	23 038 ± 2435	11 878 ± 2212

[a] MCF-7 = human breast carcinoma; NCI-H460 = human lung carcinoma; SF-268 = human central nervous system carcinoma. [b] Values are means ± SD, where SD = standard deviation; all experiments were independently performed at least three times.

probably resulted from the rigid indolizidinone or quinolizidinone structure. Therefore, the planar dehydroiminium salts **12a–c** with no carbonyl group showed substantial loss of activity.

Conclusions

In conclusion, the indolizidine and quinolizidine rings were constructed by thermal and radical cyclizations. By the protocol outlined above, tylophorine **1b** and the modified analogues **1a** and **1c** were synthesized in high yields. The SAR of phenanthroindolizidine and phenanthroquinolizidine skeletons toward inhibition of three cancer lines, MCF-7, NCI-H460, and SF-268, revealed that the more rigid bicyclic heterocycles **11a–c** and **12a–c** showed a remarkable decrease in cytotoxicity. A potency improvement was observed by the introduction of a methoxyl functionality on the phenanthrene ring. A *trans*-fused quinolizidine ring could further enhance the cytotoxic activities. Compound **1c**, for instance, is clearly a promising anticancer agent for further study.

Experimental

General

Melting points were taken on a Buchi 535 melting-point apparatus and were not corrected. Infrared spectra were measured on a Nicolet Magna FT-IR spectrometer as either thin film or solid dispersion in KBr. Nuclear magnetic resonance spectra were recorded on Bruker Avance-300 and AMX-400 FT-NMR spectrometers; all chemical shifts were reported in ppm from tetramethylsilane as an internal standard. Mass spectra were obtained on a VG 70-250S spectrometer. Elemental analyses were performed on a Heraeus CHN-RAPID elemental analyzer. Column chromatography was carried out using 70–230 mesh silica gel.

3-(Phenanthren-9-yl)acrylic acid 9a. A mixture of phenanthrene-9-carboxaldehyde (1.03 g, 5 mmol) and (carboethoxymethylene)triphenylphosphorane (2.09 g, 6 mmol) in toluene (30 cm^3) was refluxed under N_2 for 4 h. After cooling, the resulting solution was directly purified by flash chromatography on silica gel with hexane–EtOAc (1 : 1) as eluent. Recrystallization from hexane–EtOAc afforded the ethyl ester of **9a** (1.21 g, 88%) as white needles: mp 119–120 °C (lit.,[16] mp 120–121 °C).

A solution of 1 N KOH (10 cm^3) was added to a solution of the ester (1.10 g, 4 mmol) in EtOH (20 cm^3) and the reaction mixture was heated to reflux for 3 h. After cooling, the solution was evaporated, and the residue was dissolved in water (25 cm^3), acidified with 10% HCl and extracted with EtOAc (5 × 20 cm^3). The combined extracts were dried with anhydrous $MgSO_4$, filtered, and evaporated under reduced pressure to give **9a** (0.96 g, 97%) as white crystals: mp 224–225 °C (from EtOAc) (lit.,[17] mp 230–233 °C); (found: C, 82.27; H, 4.88. Calcd for $C_{17}H_{12}O_2$: C, 82.24; H, 4.87%); ν_{max}(KBr)/cm^{-1} 3000 (br), 1694 and 1630; δ_H(300 MHz; DMSO-d_6; Me_4Si) 6.69 (1H, d, *J* 15.7), 7.72 (4H, m), 8.07 (1H, d, *J* 7.5), 8.23 (1H, m), 8.30 (1H, s), 8.40 (1H, d, *J* 15.7), 8.84 (1H, d, *J* 8.1), 8.91 (1H, m) and 12.57 (1H, br s); δ_C(75 MHz; DMSO-d_6; Me_4Si) 122.6, 122.9, 123.6, 124.0, 126.5, 127.2, 127.3, 127.5, 128.0, 129.3, 129.4, 129.9, 130.0, 130.4, 130.8, 140.8 and 167.4; *m*/*z* (EI) 248 (M^+, 31%), 203 (90), 202 (100) and 176 (17).

3-(2,3,6,7-Tetramethoxyphenanthren-9-yl)acrylic acid 9b. The analogous procedure for the preparation of acid **9a** was used. The aldehyde **10b**[18] (1.63 g, 5 mmol) gave **9b** (1.40 g, 76%) as pale yellow crystals: mp 282–284 °C (from *o*-dichlorobenzene); (found: C, 68.03; H, 5.43. Calcd for $C_{21}H_{20}O_6$: C, 68.47; H, 5.47%); ν_{max}(KBr)/cm^{-1} 3000 (br), 1686 and 1616; δ_H(300 MHz; DMSO-d_6; Me_4Si) 3.91 (3H, s), 3.97 (3H, s), 4.05 (6H, s), 6.59 (1H, d, *J* 15.6), 7.47 (2H, s), 8.00 (1H, s), 8.06 (1H, s), 8.08 (1H, s), 8.36 (1H, d, *J* 15.6) and 12.46 (1H, br s); δ_C(75 MHz; DMSO-d_6; Me_4Si) 55.4, 55.9, 103.5, 104.0, 104.3, 108.9, 120.8, 123.7, 123.9, 124.4, 125.2, 125.3, 127.0, 141.5, 148.7, 148.8, 149.1, 150.1 and 167.6; *m*/*z* (EI) 368 (M^+, 100%), 279 (13), 249 (13), 207 (14), 176 (20) and 163 (24).

3-(Phenanthren-9-yl)acryloyl azide 8a. A mixture of acid **9a** (1.24 g, 5 mmol) and oxalyl chloride (1.27 g, 10 mmol) in toluene (50 cm^3) was heated for 5 h at 80 °C. After cooling, the resulting mixture was concentrated under reduced pressure to afford the acyl chloride quantitatively. The acyl chloride was added immediately into a suspension of NaN_3 (0.98 g, 15 mmol) in dry acetone (30 cm^3) on an ice bath. The reaction mixture was stirred gently for 2 h at room temperature and filtered.[19] The solvent was evaporated *in vacuo*, and the residue was purified by short column chromatography over silica gel eluting with $CHCl_3$–hexane (2 : 1) to yield the pure acyl azide **8a** (1.27 g, 93%) as a pale yellow solid: ν_{max}(KBr)/cm^{-1} 2145 and 1682; δ_H(300 MHz; $CDCl_3$; Me_4Si) 6.62 (1H, d, *J* 15.5), 7.68 (4H, m), 7.93 (1H, d, *J* 7.7), 8.03 (1H, s), 8.20 (1H, d, *J* 7.2), 8.61 (1H, d, *J* 15.5), 8.68 (1H, d, *J* 8.2) and 8.76 (1H, d, *J* 7.5); δ_C(75 MHz; $CDCl_3$; Me_4Si) 121.9, 122.6, 123.3, 124.0, 127.0, 127.1, 127.2, 127.3, 128.1, 129.4, 129.8, 130.1, 130.4, 130.9, 131.4, 144.2 and 171.9; *m*/*z* (EI) 245.0841 ($M^+ - N_2$. $C_{17}H_{11}NO$ requires 245.0841), 202 (100%), 189 (33) and 176 (24).

3-(2,3,6,7-Tetramethoxyphenanthren-9-yl)acryloyl azide 8b. The analogous procedure for the preparation of azide **8a** was

used. The acid **9b** (1.84 g, 5 mmol) gave **8b** (1.80 g, 92%) as a pale yellow solid: ν_{max}(KBr)/cm^{-1} 2140 and 1677; δ_H(300 MHz; $CDCl_3$; Me_4Si) 4.04 (3H, s), 4.07 (3H, s), 4.13 (6H, s), 6.56 (1H, d, *J* 15.4), 7.20 (1H, s), 7.41 (1H, s), 7.72 (1H, s), 7.78 (1H, s), 7.84 (1H, s) and 8.50 (1H, d, *J* 15.4); δ_C(75 MHz; $CDCl_3$; Me_4Si) 56.0, 102.6, 103.2, 104.0, 108.7, 120.4, 124.4, 124.7, 125.5, 126.1, 127.2, 144.5, 149.2, 149.4, 150.6 and 172.0; *m*/*z* (EI) 365.1267 ($M^+ - N_2$. $C_{21}H_{19}NO_5$ requires 365.1263), 306 (15%) and 164 (10).

2*H*-2-Aza-triphenylen-1-one 6a. A mixture of azide **8a** (1.1 g, 4 mmol) and $Hg(OAc)_2$ (31.9 mg, 0.1 mmol) in *o*-dichlorobenzene (50 cm^3) was refluxed for 1 h. After cooling, the compound **6a** was isolated by precipitation and trituration with hexane to give **6a** (0.86 g, 87%) as pale yellow needles: mp >300 °C (from EtOH); (found: C, 83.05; H, 4.53; N, 5.71. Calcd for $C_{17}H_{11}NO$: C, 83.25; H, 4.52; N, 5.71%); ν_{max}(KBr)/cm^{-1} 3135 and 1630; δ_H(300 MHz; DMSO-d_6; Me_4Si) 7.56 (2H, m), 7.70 (2H, m), 7.76 (1H, t, *J* 7.8), 7.85 (1H, t, *J* 7.8), 8.70 (1H, d, *J* 7.8), 8.88 (2H, m), 10.32 (1H, m) and 11.93 (1H, br s); δ_C(75 MHz; DMSO-d_6; Me_4Si) 100.6, 118.1, 122.7, 123.5, 125.4, 126.8, 127.2, 127.4, 127.6, 128.8, 129.6, 131.6, 131.9, 138.8 and 162.6; *m*/*z* (EI) 245 (M^+, 64%), 216 (26) and 189 (100).

6,7,10,11-Tetramethoxy-2*H*-2-aza-triphenylen-1-one 6b. The analogous procedure for the preparation of **6a** was used. The azide **8b** (1.97 g, 5 mmol) gave **6b** (1.56 g, 85%) as tan needles: mp >300 °C; (found: C, 68.75; H, 5.30; N, 3.75. Calcd for $C_{21}H_{19}NO_5$: C, 69.03; H, 5.24; N, 3.83%.); ν_{max}(KBr)/cm^{-1} 3115 and 1631; δ_H(300 MHz; DMSO-d_6; Me_4Si) 3.92 (3H, s), 4.01 (3H, s), 4.05 (3H, s), 4.09 (3H, s), 7.48 (2H, m), 7.94 (1H, s), 8.05 (2H, s), 10.05 (1H, s) and 11.63 (1H, d, *J* 4.4); δ_C(75 MHz; DMSO-d_6; Me_4Si) 55.2, 55.7, 55.9, 101.1, 104.1, 104.2, 106.0, 108.7, 116.6, 120.9, 123.6, 124.0, 126.5, 129.9, 136.5, 148.3, 148.5, 148.8, 151.1 and 162.8; *m*/*z* (EI) 365 (M^+, 100%) and 332 (18).

2-(3-Chloropropyl)-2-aza-triphenylen-1-one 5a1 and 2-(3-bromopropyl)-2-aza-triphenylen-1-one 5a2. To a suspension of NaH (60% dispersion in oil, 80 mg, 2 mmol) in DMF (5 cm^3), cooled in an ice bath, a solution of **6a** (245 mg, 1 mmol) in DMF (15 cm^3) was added with stirring at a rate such as to maintain gentle evolution of hydrogen. After the addition was complete, the reaction mixture was stirred at room temperature for 30 min. This mixture was added dropwise to a solution of 1-bromo-3-chloropropane (628 mg, 4 mmol) and DMF (5 cm^3) with stirring. This mixture was stirred at room temperature overnight. The solvent was evaporated *in vacuo*, and water (10 cm^3) was then added. The mixture was extracted with $CHCl_3$, and the combined extracts were washed with water, dried with anhydrous $MgSO_4$, and filtered. The filtrate was concentrated, and the residue was purified by column chromatography over silica gel eluting with pure $CHCl_3$ to give a 20 : 1 mixture of chloride **5a1** and bromide **5a2** (95% combined yield). The mixture was rechromatographed with $CHCl_3$–hexane (5 : 1) to give a material enriched in **5a1** and a little of pure **5a2** for spectral and mass analysis. For **5a1**: ν_{max}(KBr)/cm^{-1} 1646; δ_H(300 MHz; $CDCl_3$; Me_4Si) 2.39 (2H, quintet, *J* 6.3), 3.62 (2H, t, *J* 6.3), 4.30 (2H, t, *J* 6.3), 7.32 (1H, d, *J* 7.4), 7.49 (1H, d, *J* 7.4), 7.71 (4H, m), 8.41 (1H, d, *J* 8.1), 8.69 (2H, t, *J* 8.1) and 10.31 (1H, d, *J* 8.1); δ_C(75 MHz; $CDCl_3$; Me_4Si) 31.1, 42.1, 48.0, 101.3, 119.2, 122.3, 123.3, 124.6, 126.9, 127.1, 127.3, 127.8, 127.9, 129.3, 129.6, 129.9, 132.4, 134.1, 138.0 and 162.3; *m*/*z* (EI) 323 ([M + 2]$^+$, 30%), 321.0921 (M^+. $C_{20}H_{16}ClNO$ requires 321.0920), 286 (100), 245 (60), 228 (77) and 202 (41); for **5a2**: white solid: mp 92–93 °C; ν_{max}(KBr)/cm^{-1} 1645; δ_H(300 MHz; $CDCl_3$; Me_4Si) 2.49 (2H, quintet, *J* 6.3), 3.49 (2H, t, *J* 6.3), 4.32 (2H, t, *J* 6.3), 7.36 (1H, d, *J* 7.5), 7.55 (1H, d, *J* 7.5), 7.72 (4H, m), 8.44 (1H, d, *J* 8.0), 8.72 (2H, t, *J* 8.0) and 8.72 (1H, dd, *J* 8.0 and 2.0); δ_C(75 MHz; $CDCl_3$; Me_4Si) 30.7, 31.2, 49.0, 101.3, 119.1, 122.2, 123.2, 124.5, 126.9, 127.0, 127.2, 127.7, 127.9, 129.2, 129.6, 129.9, 132.3, 134.1, 138.0 and 162.2; *m*/*z* (EI) 367 ([M + 2]$^+$, 39%), 365.0418 (M^+. $C_{20}H_{16}BrNO$ requires 365.0415), 286 (100), 259 (39), 245 (49), 228 (49), 202 (45) and 189 (50).

6,7,10,11-Tetramethoxy-2-(3-chloropropyl)-2-aza-triphenylen-1-one 5b1. The analogous procedure for the preparation of **5a1** was used. The compound **6b** (365 mg, 1mmol) gave pure **5b1** (423 mg, 96%) as pale yellow crystals: mp 211–212 °C (from $CHCl_3$–hexane); (found: C, 65.52; H, 5.75; N, 3.02. Calcd for $C_{24}H_{24}ClNO_5$: C, 65.23; H, 5.47; N, 3.17%); ν_{max}(KBr)/cm^{-1} 1645; δ_H(300 MHz; $CDCl_3$; Me_4Si) 2.41 (2H, quintet, *J* 6.1), 3.64 (2H, t, *J* 6.1), 4.09 (3H, s), 4.13 (3H, s), 4.14 (3H, s), 4.16 (3H, s), 4.32 (2H, t, *J* 6.1), 7.20 (1H, d, *J* 7.5), 7.46 (1H, d, *J* 7.5), 7.70 (1H, s), 7.79 (1H, s), 7.80 (1H, s) and 10.05 (1H, s); δ_C(75 MHz; $CDCl_3$; Me_4Si) 31.1, 42.0, 47.6, 55.7, 55.8, 55.9, 101.3, 102.6, 103.0, 104.8, 108.9, 117.3, 120.8, 124.0, 124.5, 126.9, 132.6, 135.6, 148.5, 148.6, 148.7, 150.8 and 162.4; *m*/*z* (EI) 443 ([M + 2]$^+$, 37%), 441 (M^+, 100) and 405 (23).

6,7,10,11-Tetramethoxy-2-(4-chlorobutyl)-2-aza-triphenylen-1-one 5c1. The analogous procedure for the preparation of **5a1** was used. The compound **6b** (365 mg, 1mmol) reacted with 1-bromo-4-chlorobutane (686 mg, 4 mmol) and gave pure **5c1** (405 g, 89%) as pale yellow crystals: mp 184–185 °C (from EtOAc); (found: C, 65.40; H, 5.82; N, 3.02. Calcd for $C_{25}H_{26}ClNO_5$: C, 65.86; H, 5.75; N, 3.07%); ν_{max}(KBr)/cm^{-1} 1645; δ_H(300 MHz; $CDCl_3$; Me_4Si) 1.91 (2H, quintet, *J* 6.1), 2.05 (2H, quintet, *J* 6.1), 3.61 (2H, t, *J* 6.1), 4.14 (14H, m), 7.20 (1H, d, *J* 7.3), 7.37 (1H, d, *J* 7.3), 7.70 (1H, s), 7.80 (2H, s) and 10.08 (1H, s); δ_C(75 MHz; $CDCl_3$; Me_4Si) 26.7, 29.7, 44.4, 49.3, 55.7, 55.8, 55.9, 101.5, 102.7, 103.1, 104.9, 109.1, 117.5, 120.9, 124.1, 124.7, 127.0, 132.0, 135.5, 148.5, 148.7, 148.8, 150.9 and 162.4; *m*/*z* (EI) 457 ([M + 2]$^+$, 38%), 455 (M^+, 100) and 421 (49).

11,12,12*a*,13-Tetrahydro-10*H*-9*a*-aza-cyclopenta[*b*]triphenylen-9-one 4a and 11,12-dihydro-10*H*-9*a*-aza-cyclopenta[*b*]triphenylen-9-one 11a. A solution of chloride **5a1** (161 mg, 0.5 mmol) and NaI (375 mg, 2.5 mmol) in dry CH_3CN (10 cm^3) was placed in a sealed tube and then heated at 125 °C for 12 h. After cooling, the reaction mixture was filtered, and concentrated *in vacuo* to yield iodide **5a3** quantitatively. Subsequently, a solution of AIBN (33 mg, 0.2 mmol) in toluene (2.4 cm^3) was added dropwise (syringe pump) to a degassed solution of iodide **5a3** (207 mg, 0.5 mmol) and *n*-Bu_3SnH (175 mg, 0.6 mmol) in refluxing toluene (25 cm^3) for 6 h. The reaction mixture was then cooled and the solvent removed under reduced pressure. The residue was triturated with hexane (3 × 2 cm^3) and purified by column chromatography over silica gel eluting with $CHCl_3$–hexane (5 : 1) to give **4a** (109 mg, 76%) as white crystals and **11a** (8 mg, 6%) as a white solid. For **4a**: mp 163–164 °C (from $CHCl_3$–hexane); (found: C, 83.39; H,

6.01; N, 4.85. Calcd for $C_{20}H_{17}NO$: C, 83.59; H, 5.96; N, 4.87%); ν_{max}(KBr)/cm^{-1} 1636; δ_H(300 MHz; $CDCl_3$; Me_4Si) 1.80 (1H, m), 1.85 (1H, m), 1.96 (1H, m), 2.31 (1H, m), 2.82 (1H, dd, *J* 15.6 and 13.8), 3.57 (1H, dd, *J* 15.6 and 3.8), 3.77 (2H, m), 3.79 (1H, m), 7.62 (4H, m), 7.98 (1H, d, *J* 8.1), 8.63 (1H, d, *J* 8.1), 8.66 (1H, d, *J* 8.1) and 9.30 (1H, d, *J* 8.1); δ_C(75 MHz; $CDCl_3$; Me_4Si) 23.5, 32.2, 33.6, 45.2, 55.0, 122.2, 123.1, 124.6, 125.3, 126.3, 126.8, 127.0, 127.7, 127.9, 128.9, 129.1, 129.9, 131.7, 135.6 and 163.8; *m*/*z* (EI) 287 (M$^+$, 82%), 218 (100) and 190 (84); for **11a**: mp 230–232 °C; ν_{max}(KBr)/cm^{-1} 1650; δ_H(300 MHz; $CDCl_3$; Me_4Si) 2.33 (2H, quintet, *J* 7.6), 3.31 (2H, t, *J* 7.6), 4.38 (2H, t, *J* 7.6), 7.34 (1H, s), 7.71 (4H, m), 8.46 (1H, d, *J* 8.2), 8.69 (1H, d, *J* 8.2), 8.73 (1H, d, *J* 8.2) and 10.39 (1H, d, *J* 8.2); δ_C(75 MHz; $CDCl_3$; Me_4Si) 21.4, 32.1, 49.2, 96.2, 116.8, 122.1, 123.3, 124.7, 126.4, 126.9, 127.7, 129.0, 130.4, 132.5, 139.0, 146.1 and 162.0; *m*/*z* (EI) 285.1152 (M$^+$. $C_{20}H_{15}NO$ requires 285.1154) and 189 (12%).

Tylophorin-9-one 4b and 2,3,6,7-tetramethoxy-11,12-dihydro-10*H*-9*a*-aza-cyclopenta[*b*]triphenylen-9-one 11b. By the analogous procedure for the radical cyclization of iodide **5a3**, chloride **5b1** (221 mg, 0.5 mmol) gave **4b** (177 mg, 87%) as white crystals and **11b** (10 mg, 5%) as a pale yellow solid. For **4b**: mp 284–286 °C (from $CHCl_3$–hexane) (lit.,[20] mp 280–281 °C); (found: C, 70.69; H, 6.40; N, 3.27. Calcd for $C_{24}H_{25}NO_5$: C, 70.74; H, 6.18; N, 3.44%); ν_{max}(KBr)/cm^{-1} 1622; δ_H(300 MHz; $CDCl_3$; Me_4Si) 1.93 (1H, m), 1.96 (1H, m), 2.17 (1H, m), 2.43 (1H, m), 2.96 (1H, dd, *J* 15.4 and 13.4), 3.61 (1H, dd, *J* 15.4 and 4.0), 3.84 (2H, m), 3.87 (1H, m), 4.06 (3H, s), 4.08 (3H, s), 4.12 (3H, s), 4.15 (3H, s), 7.36 (1H, s), 7.79 (1H, s), 7.84 (1H, s) and 9.03 (1H, s); δ_C(75 MHz; $CDCl_3$; Me_4Si) 23.4, 32.4, 33.8, 45.2, 55.1, 55.8, 102.2, 102.9, 104.7, 107.9, 122.3, 123.0, 124.2, 124.3, 126.5, 133.2, 148.5, 148.6, 148.8, 150.1 and 164.6; *m*/*z* (EI) 407 (M$^+$, 100%), 338 (24), 310 (20) and 295 (16); for **11b**: mp >300 °C; ν_{max}(KBr)/cm^{-1} 1647; δ_H(300 MHz; $CDCl_3$; Me_4Si) 2.32 (2H, quintet, *J* 7.5), 3.31 (2H, t, *J* 7.5), 4.10 (3H, s), 4.13 (3H, s), 4.15 (3H, s), 4.16 (3H, s), 4.37 (2H, t, *J* 7.5), 7.15 (1H, s), 7.71 (1H, s), 7.81 (2H, s) and 10.15 (1H, s); δ_C(75 MHz; $CDCl_3$; Me_4Si) 21.6, 31.9, 49.1, 55.9, 96.3, 103.0, 103.4, 105.3, 108.9, 115.4, 121.4, 123.5, 125.1, 127.2, 136.9, 144.6, 148.3, 148.7, 148.9, 151.0 and 162.3; *m*/*z* (EI) 405.1579 (M$^+$. $C_{24}H_{23}NO_5$ requires 405.1576), 362 (14%) and 304 (13).

2,3,6,7-Tetramethoxy-10,11,12,13,13*a*,14-hexahydro-9*a*-aza-benzo[*b*]triphenylen-9-one 4c and 2,3,6,7-tetramethoxy-10,11,12,13-tetrahydro-9*a*-aza-benzo[*b*]triphenylen-9-one 11c. The analogous procedure for the preparation of **4a** was used. The compound **5c1** (228 mg, 0.5 mmol) gave **4c** (168 mg, 80%) as white crystals and **11c** (25 mg, 12%) as a pale yellow solid. For **4c**: mp 190–191 °C (from $CHCl_3$–hexane); (found: C, 71.24; H, 6.46; N, 3.32. Calcd for $C_{25}H_{27}NO_5$: C, 70.92; H, 6.43; N, 3.32%); ν_{max}(KBr)/cm^{-1} 1621; δ_H(300 MHz; $CDCl_3$; Me_4Si) 1.52 (1H, m), 1.64 (2H, m), 1.92 (2H, m), 2.04 (1H, m), 2.90 (1H, td, *J* 12.6 and 1.5), 3.05 (1H, dd, *J* 16.3 and 10.9), 3.46 (1H, dd, *J* 16.3 and 5.0), 3.61 (1H, m), 4.06 (3H, s), 4.10 (3H, s), 4.12 (3H, s), 4.14 (3H, s), 4.72 (1H, br d, *J* 12.6), 7.34 (1H, s), 7.79 (1H, s), 7.82 (1H, s) and 9.39 (1H, s); δ_C(75 MHz; $CDCl_3$; Me_4Si) 23.0, 24.7, 33.0, 42.8, 52.8, 56.0, 102.5, 103.2, 104.7, 108.7, 120.2, 122.9, 124.6, 124.8, 126.9, 133.4, 148.5, 148.9, 149.0, 150.6 and 167.6; *m*/*z* (EI) 421 (M$^+$, 100%), 338 (36), 310 (19), 276 (19) and 203 (39); for **11c**: mp 122–123 °C; ν_{max}(KBr)/cm^{-1} 1636; δ_H(300 MHz; $CDCl_3$; Me_4Si) 1.92 (2H, quintet, *J* 6.4), 2.08 (2H, quintet, *J* 6.4), 3.03 (2H, t, *J* 6.4), 4.12 (3H, s), 4.13 (3H, s), 4.15 (3H, s), 4.16 (3H, s), 4.23 (2H, t, *J* 6.4), 7.02 (1H, s), 7.73 (1H, s), 7.84 (2H, s) and 10.09 (1H, s); δ_C(75 MHz; $CDCl_3$; Me_4Si) 19.2, 22.8, 29.4, 42.1, 56.0 (4 × C), 101.1, 103.1, 103.5, 105.2, 109.0, 115.0, 120.9, 123.6, 125.0, 127.3, 135.3, 141.9, 148.3, 148.7, 148.9, 151.0 and 163.4; *m*/*z* (EI) 419.1736 (M$^+$. $C_{25}H_{25}NO_5$ requires 419.1733), 404 (17%), 376 (18), 322 (14) and 318 (13).

9,10,11,12,12*a*,13-Hexahydro-9*a*-aza-cyclopenta[*b*]triphenylene 1a. To a solution of the lactam **4a** (29 mg, 0.1 mmol) in dry dioxane (5 cm^3) was added a 3.5 M solution of sodium bis(2-methoxyethoxy)aluminium hydride in toluene (0.4 cm^3, 1.4 mmol) and the mixture was refluxed for 2 h in the dark. After evaporation of the solvents, the residue was diluted with water (10 cm^3) and then basified with 10% aqueous NaOH. The mixture was extracted with $CHCl_3$ (5 × 15 cm^3), and the combined extracts were washed with water, dried with anhydrous $MgSO_4$, and filtered. The filtrate was concentrated, and the residue was purified by column chromatography over silica gel eluting with $CHCl_3$–MeOH (50 : 1) to give **1a** (25 mg, 92%) as white powder: mp 169–170 °C (decomp.) (lit.,[21] mp 170 °C); ν_{max}(KBr)/cm^{-1} 1606 and 1495; δ_H(300 MHz; $CDCl_3$; Me_4Si) 1.78 (1H, m, H-12ax), 1.94 (1H, m, H-11ax), 2.02 (1H, m, H-11eq), 2.24 (1H, m, H-12eq), 2.46 (1H, m, H-10ax), 2.49 (1H, m, H-12a), 2.99 (1H, dd, *J* 16.0 and 10.5, H-13ax), 3.48 (2H, m, H-10eq and H-13eq), 3.74 (1H, d, *J* 15.2, H-9ax), 4.74 (1H, d, *J* 15.2, H-9eq), 7.62 (4H, m, H-2, H-3, H-6, and H-7), 7.92 (1H, dd, *J* 7.8 and 1.5, H-8), 8.04 (1H, dd, *J* 6.0 and 3.3, H-1) and 8.70 (2H, m, H-4 and H-5); δ_C(75 MHz; $CDCl_3$; Me_4Si) 21.6 (C-11), 31.3 (C-12), 33.7 (C-13), 53.9 (C-9), 55.1 (C-10), 60.1 (C-12a), 122.6 (C-5), 122.8 (C-8), 122.9 (C-9), 123.4 (C-1), 125.8 (C-6), 125.9 (C-3), 126.7 (C-2 and C-7), 128.4 (C-8b), 128.8 (C-13a), 129.3 (C-4b), 129.5 (C-4a), 130.0 (C-8a) and 131.5 (C-13b); *m*/*z* (EI) 273.1517 (M$^+$. $C_{20}H_{19}N$ requires 273.1517), 245 (17%), 228 (27), 202 (100) and 189 (38).

Tylophorine 1b. The analogous procedure for the preparation of **1a** was used. The lactam **4b** (41 mg, 0.1 mmol) gave **1b** (39 mg, 98%) as white powder: mp 270 °C (decomp.) (lit.,[22] mp 282–284 °C); ν_{max}(KBr)/cm^{-1} 1618 and 1515; δ_H(300 MHz; $CDCl_3$; Me_4Si) 1.78 (1H, m, H-12ax), 1.94 (1H, m, H-11ax), 2.03 (1H, m, H-11eq), 2.29 (1H, m, H-12eq), 2.45 (1H, m, H-10eq), 2.51 (1H, m, H-12a), 2.92 (1H, dd, *J* 15.6 and 10.7, H-13ax), 3.36 (1H, dd, *J* 15.6 and 2.3, H-13eq), 3.47 (1H, t, *J* 8.4, H-10ax), 3.67 (1H, d, *J* 14.5, H-9eq), 4.05 (6H, s, 2-OCH_3 and 7-OCH_3), 4.11 (6H, s, 3-OCH_3 and 6-OCH_3), 4.62 (1H, d, *J* 14.5, H-9ax), 7.15 (1H, s, H-8), 7.31 (1H, s, H-1) and 7.82 (2H, s, H-4 and H-5); δ_C(75 MHz; $CDCl_3$; Me_4Si) 21.6 (C-11), 31.2 (C-12), 33.7 (C-13), 53.9 (C-9), 55.1 (C-10), 55.8 (2-OCH_3), 55.9 (7-OCH_3), 56.0 (3-OCH_3 and 6-OCH_3), 60.2 (C-12a), 103.1 (C-8), 103.3 (C-4), 103.4 (C-5), 104.0 (C-1), 123.4 (C-4b), 123.6 (C-4a), 124.3 (C-13a), 125.8 (C-8a), 125.9 (C-8b), 126.2 (C-13b), 148.4 (C-3), 148.5 (C-6) and 148.7 (C-2 and C-7); *m*/*z* (EI) 393.1943 (M$^+$. $C_{24}H_{27}NO_4$ requires 393.1940), 324 (100%) and 309 (12).

2,3,6,7-Tetramethoxy-10,11,12,13,13*a*,14-hexahydro-9*H*-9*a*-aza-benzo[*b*]triphenylene 1c. The analogous procedure for the preparation of **1a** was used. The lactam **4c** (42 mg, 0.1 mmol)

gave **1c** (40 mg, 98%) as white powder: mp 245–247 °C (decomp.); ν_{max}(KBr)/cm^{-1} 1615 and 1514; δ_H(300 MHz; $CDCl_3$; Me_4Si) 1.49 (1H, m, H-12ax), 1.55 (1H, m, H-13ax), 1.81 (2H, m, H-11), 1.88 (1H, m, H-12eq), 2.05 (1H, br d, *J* 11.2, H-13eq), 2.32 (1H, m, H-10ax), 2.39 (1H, m, H-13a), 2.92 (1H, dd, *J* 16.6 and 10.7, H-14ax), 3.12 (1H, br d, *J* 16.6, H-14eq), 3.29 (1H, br d, *J* 10.7, H-10eq), 3.60 (1H, br d, *J* 15.3, H-9ax), 4.04 (3H, s, 7-OCH_3), 4.05 (3H, s, 2-OCH_3), 4.11 (6H, s, 3-OCH_3 and 6-OCH_3), 4.36 (1H, br d, *J* 15.3, H-9eq), 7.13 (1H, s, H-8), 7.26 (1H, s, H-1) and 7.82 (2H, s, H-4 and H-5); δ_C(75 MHz; $CDCl_3$; Me_4Si) 24.3 (C-12), 25.9 (C-11), 33.7 (C-13), 34.8 (C-14), 56.0 (2-OCH_3, 3-OCH_3, 6-OCH_3 and 7-OCH_3), 56.2 (C-9), 56.3 (C-10), 57.6 (C-13a), 103.0 (C-8), 103.5 (C-4 and C-5), 103.9 (C-1), 123.3 (C-14b), 123.5 (C-8b), 123.9 (C-14a), 124.9 (C-4b), 125.2 (C-4a and C-8a), 148.4 (C-3 and C-6) and 148.7 (C-2 and C-7); *m*/*z* (EI) 407.2100 (M^+. $C_{25}H_{29}NO_4$ requires 407.2097), 324 (100%) and 294 (22).

11,12-Dihydro-10*H*-9*a*-azoniacyclopenta[*b*]triphenylene 12a. To a solution of phenanthroindolizidine **1a** (27 mg, 0.1 mmol) in $CHCl_3$ (10 cm^3) was added NBS (150 mg, 0.4 mmol) in small portions with stirring. The solution turned orange-red and began to deposit an orange crystalline solid. After 1 h following the addition, the solid was filtered and purified by column chromatography over Al_2O_3 eluting with $CHCl_3$–MeOH (10 : 1) to give **12a** (27 mg, 78%) as a yellow solid: mp 219 °C (decomp.); ν_{max}(KBr)/cm^{-1} 1640 and 1618; δ_H(300 MHz; DMSO-d_6; Me_4Si) 2.55 (2H, quintet, *J* 7.8), 3.64 (2H, t, *J* 7.8), 5.02 (2H, t, *J* 7.8), 7.93 (3H, m), 8.04 (1H, t, *J* 7.6), 8.97 (4H, m), 9.40 (1H, s) and 10.58 (1H, s); δ_C(75 MHz; DMSO-d_6; Me_4Si) 21.9, 31.4, 58.7, 117.4, 123.8, 124.2, 124.3, 124.9, 125.3, 125.8, 126.1, 128.8, 128.9, 129.8, 130.2, 132.4, 132.5, 139.5, 140.3 and 152.9; *m*/*z* (FAB) 270.1280 (M^+ − Br. $C_{20}H_{16}N$ requires 270.1283).

Dehydrotylophorine 12b. The analogous procedure for the preparation of **12a** was used. Phenanthroindolizidine **1b** (39 mg, 0.1 mmol) gave **12b** (24 mg, 51%) as a yellow solid: mp >300 °C (decomp.); ν_{max}(KBr)/cm^{-1} 1632 and 1611; δ_H(300 MHz; DMSO-d_6; Me_4Si) 2.50 (2H, quintet, *J* 7.3), 3.61 (2H, t, *J* 7.3), 4.07 (6H, s), 4.11 (3H, s), 4.14 (3H, s), 4.99 (2H, t, *J* 7.3), 8.09 (2H, s), 8.26 (1H, s), 8.29 (1H, s), 9.31 (1H, s) and 10.50 (1H, s); δ_C(75 MHz; DMSO-d_6; Me_4Si) 22.2, 31.3, 56.4, 58.3, 105.0, 105.2, 106.7, 116.6, 119.0, 119.8, 123.7, 124.5, 128.1, 138.6, 138.7, 149.6, 149.9, 150.4, 151.2 and 153.3; *m*/*z* (FAB) 390.1704 (M^+ − Br. $C_{24}H_{24}NO_4$ requires 390.1705).

2,3,6,7-Tetramethoxy-10,11,12,13-tetrahydro-9*a*-azoniabenzo[*b*]triphenylene 12c. The analogous procedure for the preparation of **12a** was used. Phenanthroquinolizidine **1c** (41 mg, 0.1 mmol) gave **12c** (30 mg, 62%) as a yellow solid: mp 268 °C (decomp.); ν_{max}(KBr)/cm^{-1} 1636 and 1610; δ_H(300 MHz; DMSO-d_6; Me_4Si) 2.03 (2H, quintet, *J* 6.3), 2.19 (2H, quintet, *J* 6.3), 3.38 (2H, t, *J* 6.3), 4.10 (6H, s), 4.11 (3H, s), 4.14 (3H, s), 4.81 (2H, t, *J* 6.3), 8.04 (2H, s), 8.24 (1H, s), 8.26 (1H, s), 9.15 (1H, s) and 10.24 (1H, s); δ_C(75 MHz; DMSO-d_6; Me_4Si) 17.6, 20.9, 27.0, 54.4, 56.1, 104.4, 104.5, 104.6, 106.2, 118.2, 119.0, 119.9, 122.6, 124.1, 127.7, 137.4, 141.2, 147.3, 149.2, 149.5, 150.7 and 152.9; *m*/*z* (FAB) 404.1862 (M^+ − Br. $C_{25}H_{26}NO_4$ requires 404.1862).

Cell growth inhibitory assay[23]. Carcinoma cells MCF-7 and SF-268 were maintained in DMEM medium (Hyclone) and NCI-H460 were maintained in RPMI medium (ICN) supplemented with 10% fetal bovine serum (Biological Industries Inc.) and were seeded in 96 well plates and incubated in a CO_2 incubator at 37 °C for 24 h. The seeding numbers were 6500, 7500, and 2500 per well, respectively. The cells were treated with at least ten different concentrations of test compounds in a CO_2 incubator for 72 h. The number of viable cells was estimated using the tetrazolium dye reduction assay (MTS assay), and the experiment was performed as the manufacturer recommended (Promega, Madison, WI). The absorbance was measured at 490 nm on a Wallac 1420 VICTOR2 Multilabel Counter (Perkin-Elmer, Boston, MA). The results of these assays were used to obtain the dose–response curves from which the IC_{50} values were determined. An IC_{50} value represents the concentration (nM) of the test compound at which a 50% cell growth inhibition after 3 days of incubation is produced. The values represent averages of three or more independent experiments, each with duplicate samples.

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