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From protopines to berbines: synthesis of 1-methoxystylopine and its *N*-metho salts from coulteropine

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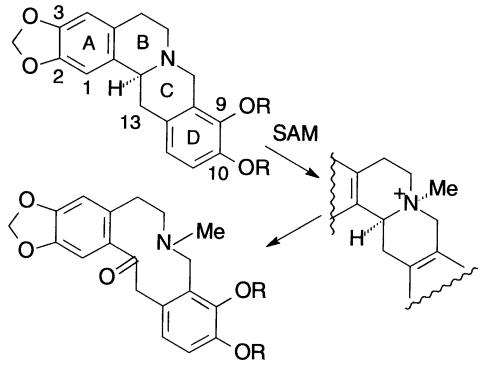
Abstract—The transformation of protopines into berbines under improved conditions has been used to synthesize 1-methoxystylopine. Coulteropine, the main alkaloid from *Romneya coulteri*, was used as the starting protopine to accomplish the stereocontrolled synthesis of both *cis* and *trans N*-methyl-1-methoxystylopinium salts. The results of ab initio calculations (B3LYP/6-31G**) which are consistent with experimental data, sustain the influence of the C-1 substituent on both the conformational equilibrium of berbines and the rate of *N*-methylation.

1. Introduction

Berbines (tetrahydroprotoberberines) play a crucial role in the metabolism of isoquinoline alkaloids given that they are the origin of other types of alkaloids such as protopines, benzophenanthridines, and spirobenzylisoquinolines.[1] The oxygenation pattern on ring A gives rise to two main types of naturally occurring berbines, the most frequent of which are 2,3-disubstituted, (*S*)-reticuline being the biogenetic precursor, and the 1,2-disubstituted, (*S*)-crassifoline being the benzylisoquinoline precursor.[2] A third type of ring A substitution pattern is the result of the C-1 hydroxylation of the 2,3-disubstituted members and leads to 1,2,3-trisubstituted berbines.

2,3-Dioxygenated (*S*)-tetrahydroprotoberberines are metabolised to the corresponding protopines through their *cis*-*N*-methosalts formed in a stereospecific manner by methylation with *S*-adenosyl-L-methionine and the enzyme (*S*)-tetrahydroprotoberberine-*cis*-*N*-methyltransferase (Scheme 1). This enzyme is especially active with substrates possessing a methylenedioxy group at positions 2 and 3 and this may explain why this functional group is present at ring D in most benzophenanthridine alkaloids.[3]

However, little is known about the metabolism of berbines oxygenated at C-1. For 1-hydroxy-2-methoxyberbines, no biogenetically related alkaloids, with this substitution pattern have so far been isolated.[4] The situation is quite similar for 1-hydroxy-2,3-dimethoxyberbines although some *N*-methosalts in the *cis* configuration have recently been isolated,[5] no 1-hydroxyprotopines are known from natural sources.



Scheme 1. Biosynthetic pathway leading to protopine.

In contrast, no simple berbine with 1-methoxy-2,3-methylenedioxy as substituents at ring A have ever been isolated although quaternized berbine with additional oxygenation at position 13 of ring C such as (−)-papaverberbine-*N*-metho salt from *Papaver pseudoorientale*[6] or (−)-coulteroberbinone isolated from *Romneya coulteri* are known,[7] being likely precursors of 13-oxoprotopines.

Interestingly, the protopines with this substitution model at ring A and bearing at positions 9,10 a methylenedioxy or dimethoxy group, coulteropine (**1a**) from *R. coulteri*,[7,8] and oreonone from *Papaver curviscapum*,[9] respectively, are known in spite of the fact that their biogenetic precursors have never been isolated (Fig. 1).

In order to obtain more insight into the metabolic pathways of this type of C-1 oxygenated alkaloids, we sought to prepare 1-methoxystylopine (**2a**) and both the isomer

Keywords: protopines; berbines; coulteropine; 1-methoxystylopine; tetrahydroprotoberberinium salts.

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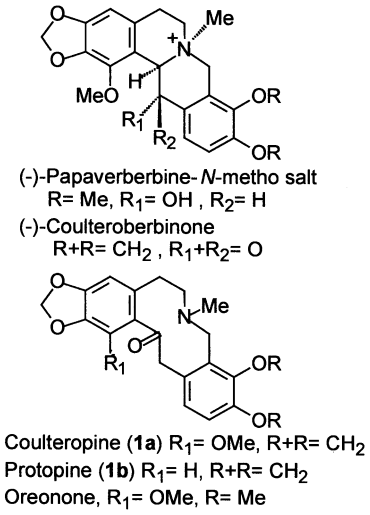
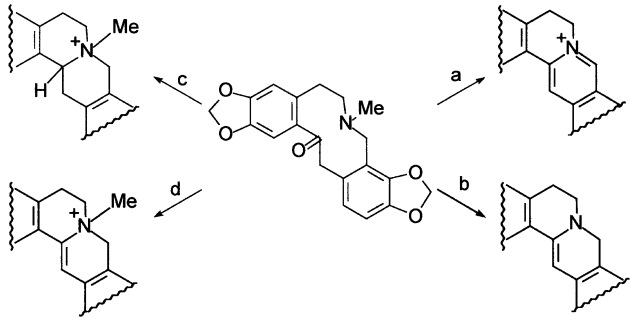


Figure 1. Structure of selected alkaloids.



Scheme 2. (a) $h\nu$, EtOH; (b) $ClCO_2Et$, benzene, reflux; (c) Na/Hg followed by $POCl_3$; (d) $POCl_3$.

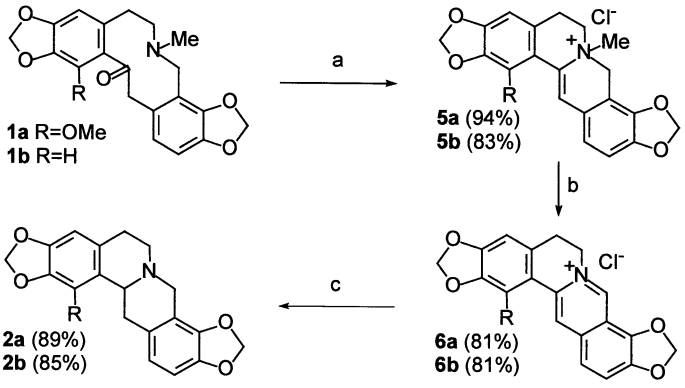
cis-(**3a**) and *trans*-(**4a**) of its *N*-metho salts. In this paper, we report the stereocontrolled synthesis of these compounds from coulteropine (**1a**) as the most suitable starting material. The *cis*-*N*-metho salt was prepared by direct methylation of the berbine, while the *trans* isomer was the result of cyclization of dihydrocoulteropine. Reaction conditions were investigated in parallel for protopine (**1b**), which exhibits a differential reactivity that is ascribed to the absence of the methoxy substituent at C-1.

2. Results and discussion

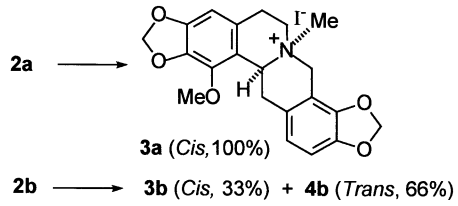
In protopines, the presence of two complementary functionalities (viz. carbonyl and amino groups) in a 10-membered ring generates a strong intramolecular interaction. This interaction has been used to accomplish the cyclization to the protoberberine skeleton using various approaches (Scheme 2). The most direct approach involves a single photochemical reaction from protopines to berberinium salts with moderate yield (path a).[10] A high yielding method has been reported that converts protopine (**1b**) into 13,14-dehydrostylopine by reaction with ethyl chloroformate (path b).[11] One of the earlier conversions of protopine system to a tetrahydroprotoberberine-*N*-metho salt used the reduction (Na/Hg) and cyclization ($POCl_3$) sequence (path c).[12] Another approach implements the cyclization–dehydration sequence ($POCl_3$) to the 13,14-dehydroberbines-*N*-metho chloride (path d), followed by demethylation–reduction to the corresponding berbines.[13]

The coulteropine (**1a**) employed in this work was isolated from *R. coulteri* (Papaveraceae). The alkaloid, isolated from plant roots was reported by Stermitz[8] for the first time; subsequently we found[7] that it was more concentrated and easily isolated from the leaves. Protopine (**1b**), a ubiquitous alkaloid among Papaveraceae, is usually the main alkaloid in Fumariaceae and was isolated from a mixture of several species of *Fumaria* genera from the Mediterranean area.

The photochemical transformation of coulteropine (**1a**) or protopine (**1b**) to the corresponding berberinium salt was unsuccessfully attempted. As expected, the reaction of protopine (**1b**) with ethyl chloroformate yielded 13,14-dehydrostylopine in excellent yield. Under identical conditions, however, coulteropine (**1a**) led to a mixture from which the expected cyclized enammonium salt could not be isolated. Next, the reaction of coulteropine (**1a**) with electrophiles was examined. With the most extensively used reagent $POCl_3$ the outcome of the reaction was unclear, so other electrophiles (Cl_2SO, $ClSO_2Me$, $ClSiMe_3$ and $(COCl)_2$) were tested. The reaction was monitored by ^1H NMR ($CDCl_3$); in all cases, formation of the cyclized intermediates was observed, but evolution to the dehydroderivatives was faster and cleaner with Cl_2SO and $(COCl)_2$ (Scheme 3). On a preparative scale, the reaction of **1a** with oxalyl chloride was found to be more effective and



Scheme 3. (a) $(COCl)_2$, $CHCl_3$, reflux, 1 h; (b) DMSO, 115°C, 90 min; (c) $NaBH_4$ (4 equiv.), MeOH, room temperature, 24 h.



Scheme 4. CH_3I (10 equiv.), acetone, room temperature, 90 min.

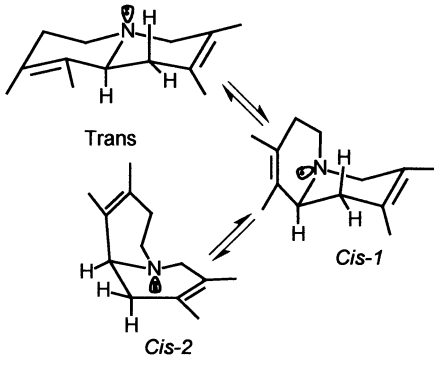
the quaternary salt, **5a**, was isolated in a 94% yield. Under identical conditions protopine (**1b**) yielded 83% of **5b**.

Demethylation of **5b** has been reported to take place under pyrolytic conditions (10^{-3} mm Hg/270°C).[13] An alternative procedure, heating a DMSO solution of **5b** at 115°C was found superior. The solution acquired a deep yellow colour and the ^{1}H NMR spectrum exhibited a low-field singlet consistent with aromatisation of ring C. After the starting product disappeared, coptysine chloride (**6b**) was isolated in good yield. Under these conditions, 1-methoxycoptysine chloride (**6a**) was obtained in a 76% overall yield from coulteropine. The $NaBH_4$ reduction of **6b** and **6a** afforded stylopine (**2b**) and the new berbine 1-methoxystylopine (**2a**), both in good yields.

The reaction of **2a** with methyl iodide resulted in a single product that was characterized as the *cis*-*N*-methyl-1-methoxystylopinium salt (**3a**). Methylation of stylopine (**2b**) gave a mixture of *cis* (**3b**) and *trans* (**4b**) *N*-methylstylopinium salts in a 1:2 ratio (Scheme 4).

The observed differential behaviour of the 1,2,3-trisubstituted and 2,3-disusbstituted berbines in their reaction with methyl iodide can be ascribed to the C-1 substituent and its influence on the conformation of the quinolizidine nucleus.

The quinolizidine moiety of berbine alkaloids exhibits pyramidal inversion at the N bridgehead. The B/C rings can be present in three different conformations namely: *trans*, *cis*-1 and *cis*-2, which are in equilibrium in solution (Scheme 5).[14a]



Scheme 5. Conformational equilibrium in quinolizidines.

Table 1. Relevant ^{1}H and ^{13}C NMR data for **2a** and **2b**

	^{13}C NMR			^{1}H NMR		
	C-6	C-13	C-14	H-14	$J_{13ax,14}$	$J_{13eq,14}$
1-Methoxystylopine (**2a**)	47.2	31.8	54.8	4.0	11.1	3.8
Stylopine (**2b**)	51.2	36.4	59.7	3.52	11.3	3.6

δ (ppm), J (Hz), $CDCl_3$.

Berbines have been found to occur as mixtures of the *trans* and *cis*-1 conformers from spectroscopic studies in solution (IR, ^{1}H and ^{13}C NMR).[4,14] The contribution of the *cis*-2 structure to the equilibrium is negligible, as inferred from the magnitude of the vicinal coupling constant between H-14 and H-13 (Table 1).

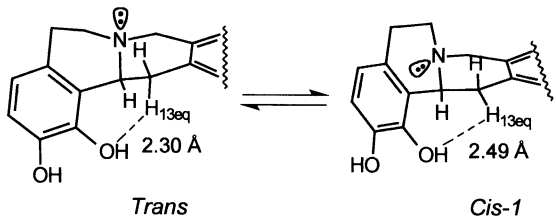
Stylopine (**2b**) and other 2,3-disubstituted berbines occur almost exclusively in the *trans* B/C quinolizidine conformation. For 1-methoxystylopine (**2a**) and other 1,2-disubstituted berbines, the absence of Bohlmann's bands, the high-field chemical shifts for carbons C-14, C-6 and C-13, as well as the deshielding in H-14 suggest a major contribution of the *cis*-1 conformation to the equilibrium.

In order to complement this conformational analysis, we performed ab initio calculations at the B3LYP/6-31G** level for dibenzo[*a*,*g*]quinolizidines with variable oxy substituents at the A and D rings. The energy differences relative to the *trans* conformation for tetrahydroxysubstituted tetrahydroprotoberberines are shown in Table 2. Irrespective of the substitution model, the *trans* and *cis*-1 systems exhibit a half-chair conformation in rings B and C. The *cis*-2 structure, however, exhibits a half-chair/half-boat conformation and is the less favoured. The energy differences between *trans* and *cis*-1 are strongly influenced by the substitution model, the energy difference decreases when a substituent is introduced at C-1.

The *trans* conformation is the more stable irrespective of the substitution pattern. However, the energy difference between the *trans* and *cis*-1 conformations is unimportant if a substituent is present at C-1, in which case the two are virtually isoenergetic (ΔE +0.53 and +0.36 kcal/mol). The new non-bonding interaction involving the HO- substituent at C-1 and H-13$_{eq}$ may account for this behaviour. The distance between these two sites in 1,2-disubstituted berbines increases by around 0.2 Å from the *trans* to *cis*-1 conformation (Scheme 6). The energy difference increases

Table 2. Energy differences referred to the *trans* conformation (kcal/mol)

Substitution model	*cis*-1	*cis*-2
1,2,9,10-	0.53	6.15
1,2,10,11-	0.36	5.75
2,3,9,10-	1.35	6.16
2,3,10,11-	1.18	5.73



Scheme 6. Non-bonding interaction in C-1 substituted berbines.

when no substituent is present at C-1; under these conditions, the *trans* conformation is the major compound in the equilibrium mixture. This equilibrium is faster than the time scale of ^{1}H NMR spectroscopy at −88°C, so an average spectrum for the two non-isolable conformations is obtained as a result.[14a,b]

The isoenergetic *trans* and *cis*-1 conformations of 1,2-disubstituted berbines can be rapidly equilibrated in solution. However, the rate of *N*-methylation of the former is lower because of the more hindered nitrogen lone pair, so the *cis*-*N*-metho salt is the sole product of the iodomethylation of C-1 substituted berbines. The *cis*-1 and *trans* conformations of the 2,3-disubstituted berbines exhibit larger energy differences, the *trans* conformation being the more stable, which allows both the *trans* and *cis*-*N*-metho salts to be isolated.

In order to obtain the *trans*-*N*-metho salt (**4a**) of 1-methoxystylopine, two different approaches were used, namely: (1) reduction of coulteropine to the dihydrocompound followed by cyclization, and (2) catalytic hydrogenation of the corresponding 13,14-dehydro-1-methoxystylopinium salt (**5a**). The former reaction was previously used for dihydrocryptopine, heating of which with acetyl chloride or $POCl_3$ yielded a mixture of the *cis* and *trans* diastereomeric *N*-methosalts. We found the reaction to develop under mild conditions; thus, treatment of dihydrocoulteropine (**7a**) with TFA at room temperature gave the *trans*-*N*-methosalt (**4a**) as the sole product in a 75% yield (Scheme 7).

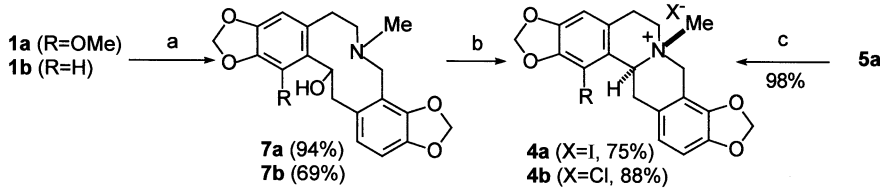
From the molecular model, it can be seen that the steric interactions in the transition state strongly favour the *trans* configuration. Irrespective of the mechanism, the transition state leading to the *cis*-*N*-metho salts involves strong steric hindrance of H-13 from H-6 and H-5. Since the effect of the substituent on the cyclization is apparently negligible, under the above described conditions, dihydroprotopine (**7b**) also gives the *trans*-*N*-metho salt (**4b**) as a single diastereoisomer.

In general, the *trans* salts have their C-13 and NMe shifted upfield due to a γ-*gauche* steric effect. On the other hand, the C-6 carbons are found around δ 61 ppm for the *trans*-fused tetrahydroprotoberberine *N*-metho salts and around δ 52 ppm for the *cis*-1 (Table 3). These data confirm the *trans* B/C quinolizidine junction for the products obtained by ring closure of the dihydroprotopines **7a** and **7b**, probably as a result of the 10-membered ring conformations that control the stereoselectivity of the ring closure. This assignment of a *trans*-B/C fusion is also supported by the ^{1}H NMR values for NMe, where the *trans* form exhibits a lower-field chemical shift (δ∼3.0 ppm) than the *cis* one (δ∼3.3 ppm). Finally, the value of the vicinal coupling constant for *cis* **3a** between H-14 and H-13$_{ax}$, H-13$_{eq}$ suggest a *cis*-1 preferred conformation.

A second alternative to the preparation of **4a**, is catalytic hydrogenation of the 13,14-dehydro-1-methoxystylopinium salt (**5a**). As expected, hydrogenation from the less hindered face yielded **4a** in a 93% overall yield from coulteropine.

3. Conclusions

Oxalyl chloride has proved to be a superior reagent for the cyclization of protopines to their 13,14-dehydroberbines-*N*-metho salts. This methodology has been used for the synthesis of 1-methoxystylopine (**2a**), a berbine never isolated from natural sources, even though it probably plays an important role in the biosynthesis of other isoquinoline alkaloids. Experimental data and theoretical calculations sustain that, in solution, C-1 substituted dibenzo[*a*,*g*]quinolizidine exists in almost equimolar equilibrium between the *cis*-1 and *trans* conformations; on the other hand, the absence of this substituent drastically diminishes the contribution of the *cis*-1 conformer. As expected, the rate of *N*-methylation of the *cis*-1 conformer is much higher than that of the *trans* conformer, so the stereoselective preparation of the *cis*-*N*-methosalt (**3a**) is accomplished. Intermolecular cyclization of dihydroprotopines takes



Scheme 7. (a) $LiAlH_4$ (4 equiv.), Et_2O, room temperature, 1 h, for **7a**; $NaBH_4$ (4 equiv.), MeOH, room temperature, 4 h, for **7b**; (b) CF_3CO_2H, $CHCl_3$, room temperature, 30 min, then IK, MeOH for **4a**; CF_3CO_2H, $CHCl_3$, room temperature, 90 min, then HCl, MeOH for **4b**; (c) H_2 (1 atm), PtO_2 (5%), MeOH, room temperature, 20 h, then IK, MeOH.

Table 3. Relevant ^{1}H and ^{13}C NMR data for *cis* **3a**,**b** and *trans* **4a**,**b**

	^{13}C NMR			^{1}H NMR			
	C-6	C-13	NMe	NMe	H-14	$J_{13ax,14}$	$J_{13eq,14}$
cis-*N*-Methyl-1-methoxystylopinium I^- (**3a**)	51.3	33.1	51.0	3.31	4.88	11.2	5.3
cis-*N*-Methylstylopinium I^- (**3b**)	53.4	33.8	51.2	3.43	4.70	10.0	4.8
trans-*N*-Methyl-1-methoxystylopinium I^- (**4a**)	61.0	28.1	39.8	3.11	4.96	12.4	3.8
trans-*N*-Methylstylopinium Cl^- (**4b**)	61.3	29.1	39.4	2.97	4.81	11.0	5.0

δ (ppm), *J* (Hz), $CDCl_3$/TFA (10:1).

place stereoselectively to the thermodinamically more favourable *trans-N*-metho salt (**4a**). The differential stereoselectivity obtained by ring closure vs *N*-methylation in the 1-substituted system is being further investigated in our laboratory.

4. Experimental

4.1. General

Melting points were determined on a Gallenkamp instrument and are given uncorrected. UV spectra were recorded on a Hewlett–Packard 8452A spectrophotometer and IR spectra on a Perkin–Elmer 883 spectrophotometer. EIMS were recorded on a HP-MS 5988A spectrometer operating at 70 eV. HRMS were recorded on a VG Autospec spectrometer. FAB-HRMS were recorded using *m*-nitrobenzyl alcohol as the matrix. NMR spectra were obtained on a Bruker WP-200 SY instrument at 200 MHz for ^{1}H and 50.3 MHz for ^{13}C. ^{1}H Chemical shifts are given relative to residual $CHCl_3$ (δ 7.24 ppm) in deuteriochloroform or to residual CHD_2CN in CD_3CN (δ 1.95 ppm). *J* values are in Hertz. ^{13}C chemical shifts are given relative to $CDCl_3$ (δ 77.0 ppm) in deuteriochloroform.

Protopine alkaloids were isolated from natural source. Coulteropine (**1a**) from *R. coulteri*[7] and protopine (**1b**) was isolated from *Fumaria* sps.

4.1.1. *N*-Methyl-13,14-dehydro-1-methoxystylopinium chloride (5a). To a solution of coulteropine (**1a**, 383 mg, 1 mmol) in chloroform (15 mL), oxalyl chloride (0.8 mL) was added and the mixture refluxed for 1 h. After this period, the solvent was removed in vacuo. The reaction crude was dissolved in $CHCl_3$/MeOH (10:1) and diethyl ether was added until a precipitate was observed. The solid was filtered and dried to obtain **5a** as a yellow solid (375 mg, 94%), mp 158–160°C. [Found: C, 57.20; H, 5.22; N, 3.41. $C_{21}H_{20}NO_5Cl\times 2H_2O$ requires C, 57.60; H, 5.52; N, 3.20%]; IR (KBr) ν_{max} 3400, 1615 cm^{-1}; UV λ_{max} (log ε) (MeOH) 224 (4.19), 262 (3.97), 350 (4.27), 366 (4.06); ^{1}H NMR ($CDCl_3$+CD_3OD) δ 7.63 (s, 1H, H-13), 6.82 (s, 2H, H-11, H-12), 6.49 (s, 1H, H-4), 6.10 (s, 2H, OCH_2O), 5.98, 5.97 (two d, 1H each, *J*=1.0 Hz, OCH_2O), 5.59 (d, 1H, *J*=14.7 Hz, H-8), 4.60 (d, 1H, *J*=14.7 Hz, H-8′), 4.4–4.2 (m, 1H, H-6), 4.2-4.0 (m, 1H, H-6′), 4.04 (s, 3H, OCH_3), 3.4–3.3 (m, 1H, H-5), 3.27 (s, 3H, NMe), 3.1–2.9 (m, 1H, H-5′); ^{13}C NMR ($CDCl_3$+TFA) δ 150.5, 150.0, 145.3, 141.9 (C-1, C-3, C-9, C-10), 137.1, 132.3 (C-2, C-14), 128.7, 128.6, 111.5, 104.8 (C-4a, C-12a, C-14a, C-8a), 122.5, 121.8 (C-12, C-13), 109.6 (C-11), 103.1 (C-4), 102.8, 101.9 (2×OCH_2O), 62.9, 59.6 (C-6, C-8), 60.0 (OMe), 48.5 (NMe), 25.5 (C-5); MS (*m*/*z*) 351 (M−15, 92), 350 (100%); FAB-HRMS calcd for $C_{21}H_{20}NO_5$ 366.1341, found 366.1323.

4.1.2. *N*-Methyl-13,14-dehydrostylopinium chloride (5b). In a manner similar to that described above, protopine (**1b**, 353 mg, 1 mmol) gave compound **5b** (307 mg, 83%), as an orange solid, mp 162–163°C [lit.[13] mp 193–195°C]. [Found: C, 59.11; H, 5.06; N, 3.56. $C_{20}H_{18}NO_4Cl\times 2H_2O$ requires C, 58.90; H, 5.44; N, 3.43%]; IR (KBr) ν_{max} 3450, 1613 cm^{-1}; UV λ_{max} (log ε) (MeOH) 220 (4.06), 258 (3.70), 358 (4.21), 374 (4.15); ^{1}H NMR (CD_3CN) δ 7.36, 7.27 (two s, 1H each, H-1, H-13), 7.01, 6.92 (two d, 1H each, *J*=8.1 Hz, H-11, H-12), 6.76 (s, 1H, H-4), 6.12, 6.08 (two bs, 1H each, OCH_2O), 6.01 (s, 2H, OCH_2O), 4.87 (d, 1H, *J*=14.7 Hz, H-8), 4.75 (d, 1H, *J*=14.7 Hz, H-8′), 4.06 (dd, 1H, *J*=12.0, 5.0 Hz, H-6), 3.92 (ddd, 1H, *J*=12.4, 12.0, 4.2 Hz, H-6′), 3.41 (ddd, 1H, *J*=17.8, 12.4, 5.0 Hz, H-5), 3.10 (m, 1H, H-5′), 3.09 (s, 3H, NMe); ^{13}C NMR ($CDCl_3$+TFA) δ 150.2, 150.0, 148.8, 145.4 (C-2, C-3, C-9, C-10), 134.8 (C-14), 125.1, 122.0, 117.6, 104.4 (C-4a, C-12a, C-14a, C-8a), 122.2, (C-12), 115.7 (C-13), 109.8, 108.7, 102.8 (C-1, C-11, C-4), 102.9, 102.1 (2×OCH_2O), 62.2, 61.0 (C-6, C-8), 45.9 (NMe), 24.2 (C-5); MS (*m*/*z*) 321 (M−15, 74), 320 (100%); FAB-HRMS calcd for $C_{20}H_{18}NO_4$ 336.1236, found 336.1241.

4.1.3. 1-Methoxycoptysine chloride (6a). A solution of compound **5a** (200 mg, 0.5 mmol) in DMSO (10 mL) was heated at 115°C for 90 min. The solvent was removed in vacuo at 80°C for 24 h. The residue was dissolved in chloroform/methanol (10:1) and diethyl ether was added until a precipitate was observed. The solid was filtered and dried in vacuo at 80°C to give the chloride **6a** (156 mg, 81%) as an orange solid, mp >300°C; IR (KBr) ν_{max} 3030, 1615 cm^{-1}; ^{1}H NMR ($CDCl_3$+TFA) δ 9.53 (s, 1H, H-8), 8.84 (s, 1H, H-13), 7.69, 7.60 (two d, 1H each, *J*=8.8 Hz, H-11, H-12), 6.57 (s, 1H, H-4), 6.37, 6.06 (two s, 2H each, 2×OCH_2O), 4.80 (t, 2H, *J*=5.2 Hz, H-6), 4.11 (s, 3H, OMe), 3.13 (t, 2H, *J*=5.2 Hz, H-5); ^{13}C NMR ($CDCl_3$+TFA) δ 151.8, 148.0, 144.5, 142.1 (C-1, C-3, C-9, C-10), 143.3 (C-8), 136.8, 134.9 (C-2, C-14), 132.4, 131.9 (C4a, C-12a), 112.4, 111.9 (C-14a, C-8a), 125.5, 121.7, 121.1 (C-12, C-11, C-13), 104.7, 102.1 (2×OCH_2O), 103.3 (C-4), 60.4 (OMe), 56.5 (C-6), 28.4 (C-5); MS (*m*/*z*) 350 (M^+, 100), 142 (72%); FAB-HRMS calcd for $C_{20}H_{16}NO_5$ 350.1028, found 350.1025.

4.1.4. Coptysine chloride (6b). In a manner similar to that described above for compound **6a**, compound **5b** (186 mg, 0.5 mmol) gave coptysine chloride (**6b**, 143 mg, 81%) as an orange solid, mp >300°C; ^{1}H NMR ($CDCl_3$+TFA) δ 9.54 (s, 1H, H-8), 8.30 (s, 1H, H-13), 7.75, 7.68 (two d, 1H each, *J*=8.8 Hz, H-11, H-12), 7.36 (s, 1H, H-1), 6.84 (s, 1H, H-4), 6.40, 6.09 (two s, 2H each, 2×OCH_2O), 4.90 (m, 2H, H-6), 3.26 (m, 2H, H-5). This compound (**6b**) was dissolved in methanol and converted to the iodide[15] with potassium iodide.

4.1.5. 1-Methoxystylopine (2a). To a solution of compound **6a** (128 mg, 0.33 mmol) in methanol (10 mL), $NaBH_4$ (50 mg, 1.3 mmol) was added over a period of 15 min and the mixture stirred for 24 h. The solvent was evaporated in vacuo, water was added (1 mL) to the residue and the mixture extracted with chloroform (2×10 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo, and the residue was purified by column chromatography (SiO_2, $CHCl_3$) to give **2a** (104 mg, 89%) as a white solid, mp 160–161°C; IR ($CHCl_3$) ν_{max} 3030, 2950–2870, 1600, 1580, 1470, 1450 cm^{-1}; UV λ_{max} (log ε) (MeOH) 244 (4.03), 286 (3.83); ^{1}H NMR ($CDCl_3$) δ 6.65, 6.55 (two d, 1H each, *J*=8.0 Hz, H-11, H-12), 6.33 (s, 1H, H-4), 5.92, 5.90 (two d, 1H each, *J*=1.2 Hz, OCH_2O), 5.88, 5.86 (two d, 1H

each, J=1.2 Hz, OCH_2O), 4.1–3.9 (m, 3H, H-8, H-8′, H-14), 3.96 (s, 3H, OMe), 3.28 (dd, 1H, J=16.4, 3.8 Hz, H-13), 3.05 (ddd, 1H, J=11.0, 6.0, 4.5 Hz, H-6), 2.9–2.8 (m, 2H, H-5, H-6′), 2.65 (dd, 1H, J=16.4, 11.1 Hz, H-13′), 2.60 (m, 1H, H-5′); ^{13}C NMR ($CDCl_3$) δ 147.8, 144.8, 143.6, 140.3 (C-1, C-3, C-9, C-10), 134.5 (C-2), 128.9, 128.5, 123.2 (C-4a, C-12a, C-14a), 116.0 (C-8a), 121.1 (C-12), 106.7 (C-11), 103.0 (C-4), 101.0, 100.6 (2×OCH_2O), 59.3 (OMe), 54.8 (C-14), 52.0 (C-8), 47.2 (C-6), 31.8 (C-13), 30.0 (C-5); MS (*m*/*z*) 353 (M^+, 14), 352 (16), 204 (29), 148 (100%); EI-HRMS calcd for $C_{20}H_{19}NO_5$ 353.1263, found 353.1251.

4.1.6. Stylopine (2b). In a manner similar to that described above for compound **2a**, compound **6b** (118 mg, 0.33 mmol) gave **2b** (91 mg, 85%) as white solid, mp 193–194°C [lit.[16] mp 194–195°C (EtOH)].

4.1.7. *cis*-*N*-Methyl-1-methoxystylopinium iodide (3a). To a solution of 1-methoxystylopine (**2a**, 25 mg, 0.06 mmol) in acetone (6 mL), methyl iodide was added. After stirring at room temperature for 90 min, the mixture was filtered. The solid was dried in vacuo and *cis*-*N*-methyl-1-methoxystylopinium iodide (**3a**) was obtained (34 mg, 97%) as a yellow solid, mp 254–255°C. [Found C, 51.17; H, 4.38; N, 2.90. $C_{21}H_{22}NO_5I$ requires C, 50.92; H, 4.48; N, 2.83%]; IR (KBr) ν_{max} 1615, 1495, 1465, 1270 cm^{-1}; UV λ_{max} (log ε) (MeOH) 212 (4.58), 246 (4.03), 278 (4.13); ^{1}H NMR ($CDCl_3$+TFA) δ 6.81, 6.62 (two d, 1H each, J=8.0 Hz, H-11, H-12), 6.43 (s, 1H, H-4), 6.03, 5.99, 5.97, 5.96 (4s, 1H each, 2×OCH_2O), 4.88 (d, 1H, J=16.0 Hz, H-8), 4.86 (dd, 1H, J=11.2, 5.3 Hz, H-14), 4.69 (d, 1H, J=16.0 Hz, H-8′), 4.09 (s, 3H, OMe), 3.8–3.5 (m, 2H, H-6, H-5), 3.47 (dd, 1H, J=18.5, 5.3 Hz, H-13), 3.31 (s, 3H, NMe), 3.30 (m, 1H, H-6′), 3.15 (dd, 1H, J=18.5, 6.2 Hz, H-5′), 2.85 (dd, 1H, J=18.5, 11.2 Hz, H-13′); ^{13}C NMR ($CDCl_3$+TFA) δ 150.6, 146.9, 144.5, 139.2 (C-1, C-3, C-9, C-10), 134.6 (C-2), 121.4, 121.0, 115.5, 106.5 (C-4a, C-12a, C-14a, C-8a), 120.9 (C-12), 109.7 (C-11), 102.7 (C-4), 102.4, 101.6 (2×OCH_2O), 62.7 (C-14), 61.2 (C-8), 59.9 (OMe), 51.3 (C-6), 51.0 (NMe), 33.1 (C-13), 23.5 (C-5); MS (*m*/*z*) 353 (M−15, 29), 205 (15), 204 (12), 148 (100%).

4.1.8. *cis*-*N*-Methylstylopinium iodide (3b). In a manner similar to that described above, compound **2b** (25 mg, 0.06 mmol) gave a mixture of *cis*/*trans* isomers **3b/4b**, (1:2) as a white solid (28 mg, 97%). Preparative TLC (SiO_2, $CHCl_3$/MeOH, 10:1) yielded pure isomer **4b** (physical and spectroscopic data are given below) and a **3b/4b** mixture enriched in **3b**. See Table 3 for spectral data of **3b** obtained from a mixture of isomers.

4.1.9. Dihydrocoulteropine (7a). To a solution of coulteropine **1a** (150 mg, 0.39 mmol) in dry C_6H_6 (15 mL), a suspension of $LiAlH_4$ (15 mg, 0.39 mmol) in dry Et_2O (15 mL) was added and the mixture stirred for 1 h at room temperature. The solvent was removed in vacuo, the reaction crude being washed with 5% HCl (2 mL) and extracted with chloroform (2×20 mL). The organic layer was dried ($MgSO_4$), concentrated in vacuo and crystallized from C_6H_6/hexane to give compound **7a** (142 mg, 94%) as a white solid, mp 189–190°C [lit.[8] 193–194°C (C_6H_6/petroleum ether)]; IR (KBr) ν_{max} 3560, 1615, 1480, 1445, 1235, 1225, 1055 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.66, 6.58 (two d, 1H each, J=7.8 Hz, H-11, H-12), 6.32 (s, 1H, H-4), 5.88, 5.87 (two s, 2H each, 2×OCH_2O), 5.17 (t, 1H, J=7.3 Hz, H-14), 4.11 (s, 3H, OMe), 3.96 (d, 1H, J=15.2 Hz, H-8), 3.54 (d, 1H, J=14.4 Hz, H-13), 3.5–3.4 (bs, OH), 3.44 (d, 1H, J=15.2 Hz, H-8′), 2.96 (dd, 1H, J=14.4, 7.3 Hz, H-13′), 3.0–2.9 (m, 1H, H-5), 2.71 (dt, 1H, J=12.5, 3.6, 3.6 Hz, H6), 2.5–2.4 (m, 2H, H-5′, H-6′), 2.11 (s, 3H, NMe); ^{13}C NMR ($CDCl_3$) δ 147.7, 146.6, 145.2, 141.4 (C-1, C-3, C-9, C-10), 134.8, 133.4, 133.2, 129.2 (C-2, C-4a, C-12a, C-14a), 124.0 (C-12), 118.7 (C-8a), 106.2 (C-11), 104.9 (C-4), 100.7, 100.5 (2×OCH_2O), 71.9 (C-14), 59.6 (OMe), 59.5 (C-6), 52.2 (C-8), 46.1 (C-13), 43.0 (NMe), 33.3 (C-5); MS (*m*/*z*) 385 (M^+, 10), 367 (1), 220 (13), 148 (100%).

4.1.10. Dihydroprotopine (7b). To a solution of protopine (**1b**, 75 mg, 0.21 mmol) in methanol (7 mL), $NaBH_4$ (30 mg, 0.8 mmol) was added and the mixture stirred for 4 h at room temperature. The solvent was removed in vacuo, water (1 mL) was added to the residue and the mixture was extracted with $CHCl_3$ (2×15 mL). The organic phase was dried over $MgSO_4$, concentrated in vacuo and purified by chromatography ($CHCl_3$/CH_3OH, 8:1) to give compound **7b** (52 mg, 69%). White crystals, mp 147–148°C [lit.[17] 143–144°C (ethanol)]; IR (KBr) ν_{max} 3530–3480, 1617, 1480, 1445, 1250, 1235, 1040 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.06 (s, 1H, H-1), 6.68 and 6.61 (two d, 1H each, J=7.9 Hz, H-11, H-12), 6.58 (s, 1H, H-4), 5.90–5.85 (m, 4H, 2×OCH_2O), 5.26 (d, 1H, J=7.5 Hz, H-14), 3.98 (d, 1H, J=15.1 Hz, H-8), 3.5 (bs, OH), 3.48 (d, 1H, J=14.0 Hz, H-13), 3.42 (d, 1H, J=15.1 Hz, H-8′), 2.99 (ddd, 1H, J=14.0, 12.0, 4.0 Hz, H-5), 2.81 (ddd, 1H, J=12.0, 4.0, 3.0 Hz, H-6), 2.66 (dd, 1H, J=14.0, 7.5 Hz, H-13′), 2.6–2.4 (m, 2H, H-5′, H-6′), 2.09 (s, 3H, NMe); ^{13}C NMR ($CDCl_3$) δ 146.6, 146.4, 146.2, 145.5 (C-2, C-3, C-9, C-10), 139.1, 133.2, 131.6 (C-12a, C-14a, C-4a), 119.1 (C-8a), 123.7 (C-12), 110.2, 106.3, 105.6 (C-1, C-11, C-4), 100.8, 100.6 (2×OCH_2O), 71.0 (C-14), 59.6 (C-6), 52.3 (C-8), 46.8 (C-13), 42.6 (NMe), 33.5 (C-5); MS (*m*/*z*) 355 (M^+, 1), 337 (M−18, 21), 188 (24), 148 (100%).

4.1.11. *trans*-*N*-Methyl-1-methoxystylopinium iodide (4a). To a solution of dihydrocoulteropine (**7a**, 64 mg, 0.17 mmol) in chloroform (15 mL), a few drops of TFA were added and the mixture was stirred for 30 min. The solvent was removed in vacuo and the water by azeotropic distillation. The residue was dissolved in methanol and saturated methanolic potassium iodide was added. The solid was filtered to obtain the iodide **4a** (62 mg, 75%) as a yellow solid, mp 268–269°C; IR (KBr) ν_{max} 1620, 1590, 1500, 1450, 1280 cm^{-1}; UV λ_{max} (log ε) (MeOH) 208 (4.48), 250 (4.06), 274 (4.15); ^{1}H NMR ($CDCl_3$+TFA) δ 6.83, 6.73 (two d, 1H each, J=8.1 Hz, H-11, H-12), 6.46 (s, 1H, H-4), 6.01 (d, 1H, J=1.0 Hz, OCH_2O), 5.99 (s, 2H, OCH_2O), 5.95 (d, 1H, J=1.0 Hz, OCH_2O), 4.96 (dd, 1H, J=12.4, 3.8 Hz, H-14), 4.88 (d, 1H, J=15.8 Hz, H-8), 4.74 (d, 1H, J=15.8 Hz, H-8′), 4.53 (dd, 1H, J=18.5, 3.8 Hz, H-13), 4.29 (dd, 1H, J=11.9, 5.2 Hz, H-6), 4.00 (s, 3H, OMe), 3.81 (ddd, 1H, J=11.9, 4.5, 12.0 Hz, H-6′), 3.43 (ddd, 1H, J=18.2, 12.0, 5.2 Hz, H-5), 3.11 (s, 3H, NMe), 3.05 (dd, 1H, J=18.2, 4.5 Hz, H-5′), 2.84 (dd, 1H,

J=18.5, 12.4 Hz, H-13′); ^{13}C NMR ($CDCl_3$+TFA) δ 150.0, 146.7, 144.6, 141.5 (C-1, C-3, C-9, C-10), 136.8 (C-2), 124.8, 123.4, 113.8, 107.4 (C-4a, C-12a, C-14a, C-8a), 121.6 (C-12), 109.2 (C-11), 103.3 (C-4), 102.2, 101.6 (2×OCH_2O), 67.7 (C-14), 61.5, 61.0 (C-8, C-6), 59.8 (OMe), 39.8 (NMe), 28.1 (C-13), 24.1 (C-5); MS (*m/z*) 368 (M^+, 4), 353 (M−15, 17), 205 (14), 204 (9), 148 (100%); FAB-HRMS calcd for $C_{21}H_{22}NO_5$ 368.1498, found 368.1507.

4.1.12. *trans*-*N*-Methylstylopinium chloride (4b). To a solution of dihydroprotopine (**7b**, 39 mg, 0.11 mmol) in $CHCl_3$ (7 mL), a few drops of trifluoroacetic acid were added and the mixture was stirred for 90 min. The solvent was removed in vacuo and the water removed by azeotropic distillation. The residue was dissolved in methanol saturated with hydrogen chloride. The solid was filtered and washed with diethyl ether to give **4b** (36 mg, 88%) as a white solid, mp 283–284°C. [Found C, 61.10; H, 5.56; N, 3.54. $C_{20}H_{20}NO_4Cl{\times}H_2O$ requires C, 61.30; H, 5.66; N, 3.57%]; IR (KBr) ν_{max} 3300, 1620, 1490, 1462, 1270 cm^{-1}; UV λ_{max} (log ε) (MeOH) 208 (4.60), 246 (3.91), 288 (3.95); ^{1}H NMR ($CDCl_3$+TFA) δ 6.86, 6.79 (two d, 1H each, J=8.0 Hz, H-11, H-12), 6.74, 6.70 (two s, 1H each, H-1, H-4), 6.02 (m, 4H, 2×OCH_2O), 4.81 (dd, 1H, J=11.0, 5.0 Hz, H-14), 4.75 (d, 1H, J=15.7 Hz, H-8), 4.53 (d, 1H, J=15.7 Hz, H-8′), 4.07 (dd, 1H, J=12.5, 5.5 Hz, H-6), 3.77 (m, 2H, H-6′, H-13), 3.34 (ddd, 1H, J=18.0, 12.5, 5.5 Hz, H-5), 3.10 (dd, 1H, J=18.0, 5.0 Hz, H-5′), 3.01 (dd, 1H, J=18.0, 11.0 Hz, H-13′), 2.97 (s, 3H, NMe); ^{13}C NMR ($CDCl_3$+TFA) δ 148.9, 148.4, 147.2, 144.8 (C-2, C-3, C-9, C-10), 121.9, 121.2, 121.1, 106.8 (C-4a, C-12a, C-14a, C-8a), 121.8 (C-12), 109.8, 108.7, 105.0 (C-1, C-11, C-4), 102.5, 102.0 (2×OCH_2O), 67.4 (C-14), 62.5, 61.3 (C-8, C-6), 39.4 (NMe), 29.1 (C-13), 23.7 (C-5); MS (*m/z*) 323 (M−15, 17), 322 (11), 174 (12), 148 (100%).

4.1.13. Hydrogenation of *N*-methyl-13,14-dehydro-1-methoxystylopinium chloride (5a). A solution containing **5a** (170 mg) in MeOH (6 mL) was hydrogenated under atmospheric pressure in the presence of PtO_2 (10 mg) for 20 h. The solution was filtered and the filtrate was concentrated under vacuum affording quantitatively *trans*-*N*-methyl-1-methoxystylopinium chloride as a yellow solid.

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