# Protoverbine, the Parent Member of a Class of Macrocyclic Spermine Alkaloids

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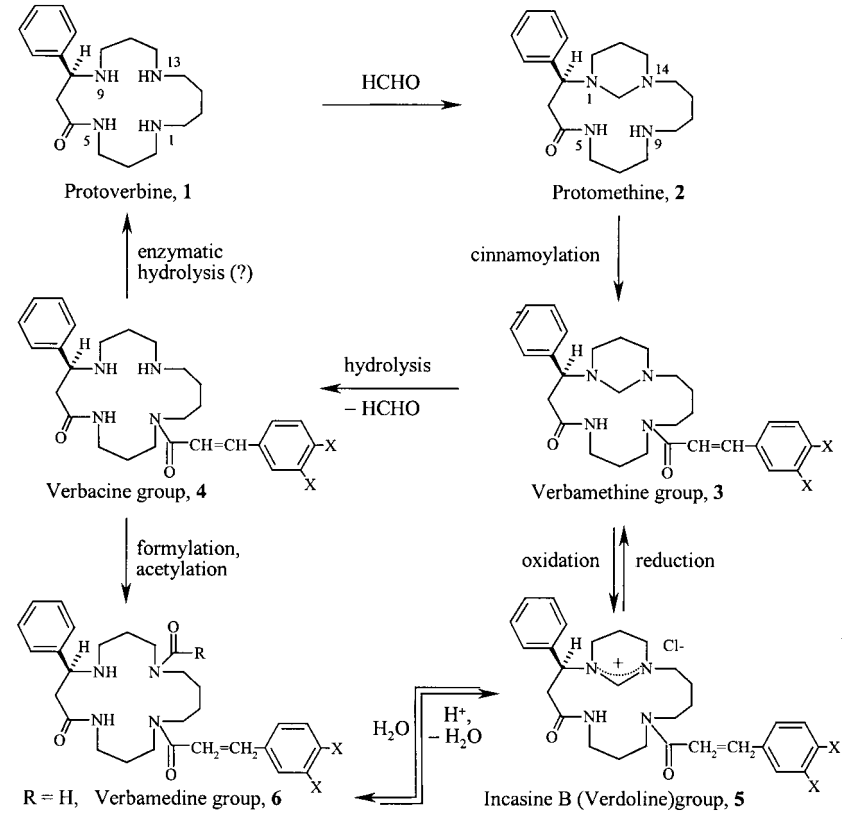
The 17-membered macrocyclic spermine alkaloids protoverbine ((8*S*)-8-phenyl-1,5,9,13-tetraazacycloheptadecan-6-one, **1**) and its *N*(9),*N*(13)-methylene-bridged derivative protomethine ((2*S*)-2-phenyl-1,5,9,14-tetraazabicyclo[12.3.1]octadecan-4-one, **2**) were isolated from *Verbascum pseudonobile* STOJ. *et* STEF. (Scrophulariaceae) and characterized. The synthesis of their racemates is described. The possible role of (*S*)-protoverbine (**1**) and (*S*)-protomethine (**2**) as precursors in the biogenesis of the whole class of *N*(1),*N*(9)- and/or *N*(13)-substituted alkaloids, the groups of verbamethine ((*S*)-**3a**–**f**), verbacine ((*S*)-**4a**–**f**), incasine B′/verdoline ((*S*)-**5a**–**f**), verbamedine ((*S*)-**6a**–**f**), and verbascenine ((*S*)-**7a**–**f**), is discussed.

**Introduction.** – The 17-membered macrocyclic spermine alkaloids (–)-(*S*)-verbacine (**4a**, *Scheme 1*), (–)-(*S*)-verballocine (**4b**), and their mono- and dimethoxy analogues (*S*)-**4c**–**f** have been isolated from the leaves of *Verbascum pseudonobile* STOJ. *et* STEF. (Scrophulariaceae) together with their *N*(9),*N*(13)-methylene-bridged derivatives (+)-(*S*)-verbamethine (**3a**), (+)-(*S*)-isoverbamethine (**3b**), and their MeO analogues (*S*)-**3c**–**f** [1–3]. In the same plant material were found also the *N*(13)-formyl ((*S*)-**6a**–**f**) and *N*(13)-acetyl ((*S*)-**7a**–**f**) derivatives of (*S*)-**4a**–**f**, and the amidinium salts (*S*)-**5a**–**f** [4]. (*S*)-Verbascenine (**7a**), (*S*)-verballoscenine (**7b**), (*S*)-verbacine (**4a**), and (*S*)-verballocine (**4b**) have been isolated also from *V. phoeniceum* L. [5][6]. (*S*)-Isoverbamethine **3b** (recently republished with the incorrect (*R*)-configuration under the trivial name incasine C [7]), (*S*)-verballocine (**4b**), and the amidinium salts (*S*)-incasine B′ (**5a**), and (*S*)-incasine B (**5b**) have been isolated also from *Incavilea sinensis* LAM. (Bignoniaceae) [7].

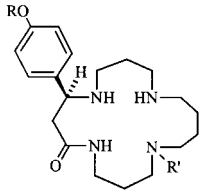
All of these 30 alkaloids are derived from the (*S*)-configured macrolactam **1** (*Scheme 1*). A similar precedent is already known, namely the closely related alkaloids (*S*)-buchnerine (**9**) (MeO derivative of **1**) and its (*S*)-*N*(1)-[(*Z*)-4-methoxycinnamoyl] derivative **10** (dimethoxy derivative of (*S*)-verballocine, **4b**), which have been isolated together from the leaves of *Clerodendrum buchneri* GÜRKE (Verbenaceae) [8]. Therefore, it was likely that compound **1** is also present as natural product in the alkaloid extract from *V. pseudonobile*. The verification of this inference required a synthetically prepared sample of **1** as a reference compound for a more detailed investigation of the minor alkaloids from *V. pseudonobile*.

**Results and Discussion.** – The macrocyclic compound (±)-**1** has been synthesized in the course of the total synthesis of (±)-verbacine (**4a**) and (±)-verbascenine (**7a**) by Sb-template macrolactamization of the (±)-amino ester **15** (*Scheme 2*) [9]. The starting (±)-amino ester **15** was prepared by *Michael* addition of spermine (**13**) to ethyl

Scheme 1. *Proposed Biogenesis of the Protoverbine Class of Macrocyclic Spermine Alkaloids*




| Group (Number) | | |
|---|---|---|
| Compound | X | CH=CH bond |
| **a** | H | (*E*) |
| **b** | H | (*Z*) |
| **c** | 4'-MeO | (*E*) |
| **d** | 4'-MeO | (*Z*) |
| **e** | 3',4'-(MeO)$_2$ | (*E*) |
| **f** | 3',4'-(MeO)$_2$ | (*Z*) |



**8** R = R' = H, Prelandrine

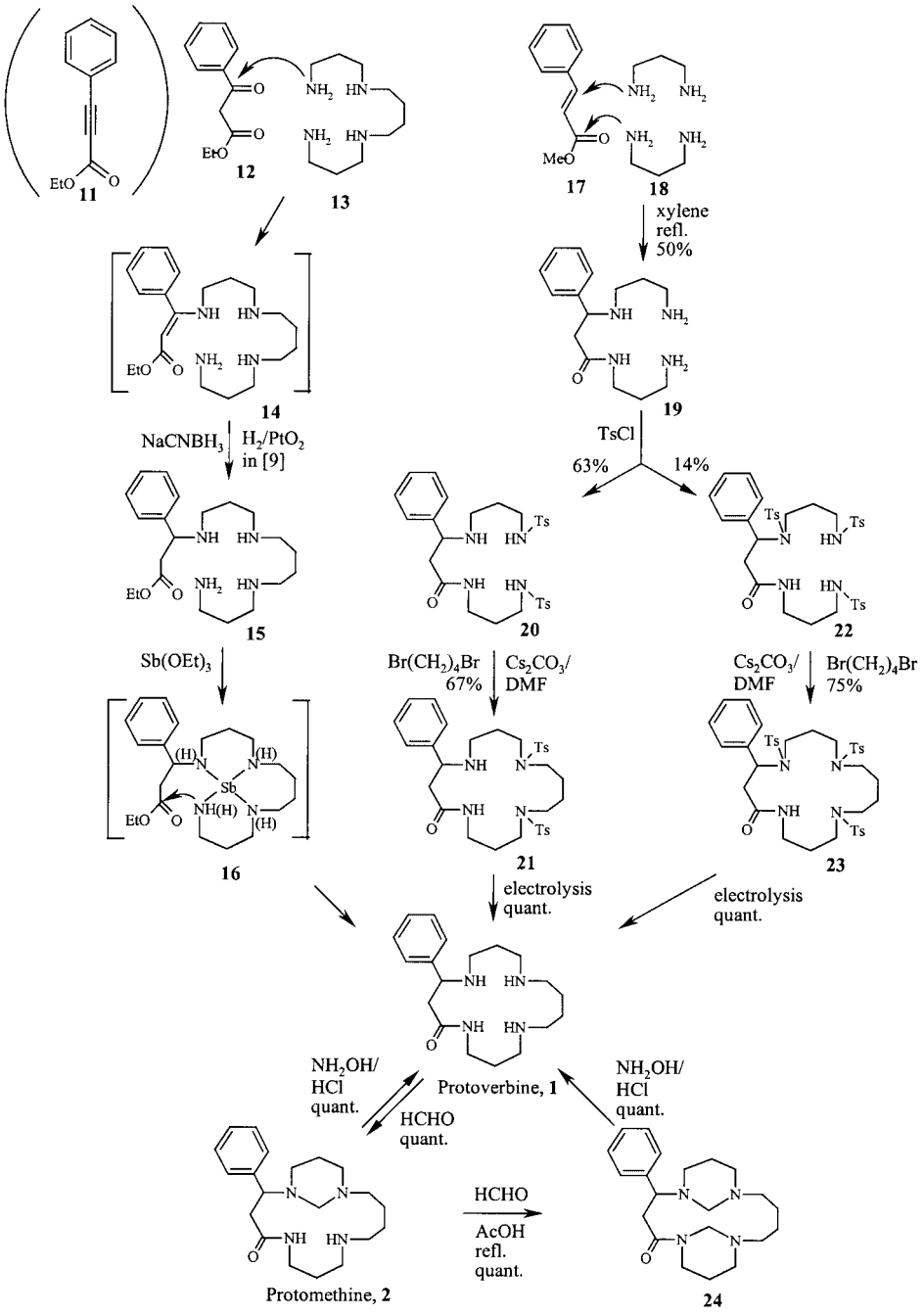
**9** R = Me, R' = H, Buchnerine

**10** R = Me, R' = (*Z*)-4-MeO–C$_6$H$_4$CH=CHCO

phenylpropiolate (**11**). The enamino ester **14** formed was isolated by column chromatography and catalytically hydrogenated over $PtO_2$ to yield **15** [9].

Here, we report a one-pot procedure for a large-scale preparation of (±)-amino ester **15**. Ethyl phenylpropiolate (**11**) (or ethyl 3-oxo-3-phenylpropanoate (**12**)) and

*Scheme 2*


11
12
13
17
18
xylene
refl.
50%
14
19
NaCNBH3
H2/PtO2
in [9]
TsCl
63%
14%
15
20
22
Sb(OEt)3
Br(CH2)4Br
67%
Cs2CO3/
DMF
Cs2CO3/
DMF
Br(CH2)4Br
75%
16
21
23
electrolysis
quant.
electrolysis
quant.
NH2OH/
HCl
quant.
Protoverbine, 1
HCHO
quant.
NH2OH/
HCl
quant.
HCHO
AcOH
refl.
quant.
Protomethine, 2
24

spermine (**13**) were refluxed in EtOH in the presence of AcOH to yield the enamino ester **14**, which, without isolation, was reduced by $NaCNBH_3$ (analogously as reported in [10]) to amino ester (±)-**15**. Compound (±)-**15** was isolated as tetrahydrochloride in 54% overall yield. The free base of (±)-**15** was successively transformed to compound (±)-**1** according to [9].

An alternative method for synthesis of macrolactam (±)-**1**, mentioned earlier in [11], is reported here in more detail (*Scheme 2*). Methyl (*E*)-3-phenylprop-2-enoate (**17**) was readily transformed to the polyamine (±)-**19** by consecutive *Michael* addition and aminolysis in the presence of propane-1,3-diamine (**18**) in refluxing xylene. The further protection/activation of compound (±)-**19** by tosylation gave the disubstituted ((±)-**20**; 63%) and the trisubstituted ((±)-**22**; 14%) derivatives. With 1,4-dibromobutane in DMF, by a modified *Richman-Atkins* procedure [12] in the presence of $Cs_2CO_3$, both (±)-**20** and (±)-**22** were smoothly cyclized to compounds (±)-**21** and (±)-**23**, respectively. By the routinely used, well-established, mild electrolytic detosylation method [13], both (±)-**21** and (±)-**23** were almost quantitatively transformed to the target macrolactam (±)-**1**.

The TLC analysis of the extract from *V. pseudonobile*, compared to the synthetically prepared compound (±)-**1** as a standard, confirmed the presence of **1** in the plant material. It was isolated by preparative TLC in 0.35% yield from the total alkaloid mixture and given the name protoverbine (**1**). Apart from the chiroptical properties, both synthetic and natural protoverbine (**1**) are identical in all physical aspects.

In the same plant extract, the *N*(9),*N*(13)-methylene-bridged alkaloids (*S*)-**3a**–**f** are present (verbamethine group; *Scheme 1*) [1–3]. With 1 equiv. HCHO at room temperature, protoverbine (**1**) forms quantitatively the corresponding aminal, which was also detected and isolated from the alkaloid mixture by prep. TLC in 0.03% yield and given the name protomethine (**2**).

In the presence of AcOH and a molar excess of HCHO after 30 min reflux in EtOH, protomethine (**2**) reacts with a second equiv. of HCHO to give the corresponding tricyclic derivative **24** (*Scheme 2*). Compound **24**, however, was not detected as a natural product in the plant material.

Similarly to compounds (*S*)-**3a**–**f**, protomethine (**2**) and the tricyclic compound **24** are quantitatively converted to protoverbine (**1**) by mild acid hydrolysis, in the presence of $NH_2OH$ as a HCHO interceptor [1].

By comparison with the dihydro derivatives of (*S*)-verbacine (**4a**), (*S*)-verbascenine (**7a**), and a large number of their isosteric (*S*)-analogues, the same (*S*)-configuration at the chiral benzylic C-atom in both (−)-protoverbine (**1**) and (+)-protomethine (**2**) was unambiguously established [14].

Refluxing compounds (*S*)-**4a**–**f** in 6N aqueous HCl soln. for 2 h causes quantitative opening of the macrocycle by hydrolytic fusion of the lactam bond. The tertiary amide functionality is stable under these conditions. Thus, eventual formation of (*S*)-protoverbine (**1**) from (*S*)-verbacine (**4a**) or its derivatives (*S*)-**4b**–**f** under the mild acidic isolation conditions seems unlikely. Nevertheless, a regioselective enzymatic hydrolysis of the alkaloids (*S*)-**4a**–**f** to yield protoverbine (**1**; *Scheme 1*), at present, could not be excluded.

The presence of the simplest, prototypic macrolactam (−)-(*S*)-protoverbine (**1**) in the alkaloid mixture from *V. pseudonobile* suggests its potential role in the biogenesis of

the whole class of these alkaloids. A possible biosynthetic pathway is shown in *Scheme 1*. By transannular condensation with endogenous HCHO, (*S*)-protoverbine (**1**) gives (*S*)-protomethine (**2**). An additional acyl group ((*E*)-cinnamoyl, (*E*)-4-methoxycinnamoyl, or (*E*)-3,4-dimethoxycinnamoyl) could be introduced at N(9)), to the *N*(1),*N*(13)-protected (*S*)-protomethine (**2**), yielding the alkaloids of the verbamethine group ((*S*)-**3a**–**f**). Such a reaction sequence explains the specific location of the additional cinnamoyl residue (at N(9) of protomethine), established so far for all alkaloids of this type [1–8]. The hydrolysis of the aminals (*S*)-**3a**–**f** leads to the verbacine group of alkaloids ((*S*)-**4a**–**f**), which could be further acylated enzymatically (formylated or acetylated) at N(13) to the corresponding members of the verbamedine ((*S*)-**6a**–**f**) and verbascenine ((*S*)-**7a**–**f**) groups of alkaloids. The oxidation of the aminals (*S*)-**3a**–**f**, or the acid-catalyzed transannular cyclization of the *N*-formyl derivatives (*S*)-**6a**–**f** leads also to the amidinium salts (*S*)-**5a**–**f** [4]. In addition, the amidinium salts (*S*)-**5a**–**f** could be reduced to the aminals (*S*)-**3a**–**f** [4], or hydrolyzed to the *N*(13)-formyl derivatives (*S*)-**6a**–**f**. All these alkaloids are present in the plant material as (*E*/*Z*)-isomeric pairs. The enzymatically catalyzed cinnamoylation leads exclusively to the (*E*)-cinnamamides, but further sunlight-mediated (*E*) → (*Z*) photoisomerization in the plant leaves leads to the appearance of the corresponding (*Z*)-isomers [2][3].

Recently, a derivative of protoverbine (**1**), hydroxylated on the aromatic ring, prelandrine (**8**), was detected in extracts from the roots of *Aphelandra squarrosa* NEES (Acanthaceae). Its potential biogenetic role, similar to that of protoverbine (**1**), in the biogenesis of the macrobicyclic spermine alkaloid aphelandrine was underlined [15].

We thank the *Swiss National Science Foundation* for generous support. *K. D.* thanks the *Dr. Helmut Legerlotz Foundation* for financial support.

## Experimental Part

*General*. TLC: *Merck* precoated plates *Kieselgel 60 $F_{254}$*; detection by *Schlittler*'s (potassium iodoplatinate) [16] and *Dragendorff*'s (No. D 156a in [17]) reagents (for more details on TLC retention of the (*E*/*Z*)-isomers of macrocyclic spermine alkaloids, their dihydro derivatives, and simpler cinnamamides, see [18]). CC: *Kieselgel 60* (70–230 mesh) from *Merck*, and alumina *N ICN Biomedicals* were used. M.p.: *Mettler FP-5*. Optical rotations: *Perkin-Elmer 241* polarimeter. NMR Spectra: *Bruker ARX-300* ($^1$H); *Bruker ARX-300* ($^{13}$C; 75 MHz); chemical shifts $\delta$ in ppm rel. to $Me_4Si$ as internal standard; $CDCl_3$ solns. at r.t. ESI-MS: *Finnigan TSQ-700* mass spectrometer.

*Plant Material and Extraction Procedure*. Air-dried leaves of *V. pseudonobile*, cultivated and collected in summer in south-west Bulgaria, were used. The plant material was extracted twice overnight with 3% aq. $H_3PO_4$ soln. The combined extracts were alkalinized (25% aq. $NH_3$ soln.) and extracted twice with $CHCl_3$. The $CHCl_3$ extracts were concentrated and re-extracted with 3% aq. $H_3PO_4$ soln. The acidic aq. re-extract was alkalinized (25% aq. $NH_3$ soln.), extracted with $CHCl_3$ to yield, after evaporation, the total alkaloid extract. From 1.5 kg dry leaves, 9 g (0.6%) of an alkaloid mixture were obtained.

*Isolation of an Enriched Mixture of Protoverbine* (**1**) *and Protomethine* (**2**). The total alkaloid mixture (9 g) was separated into groups by CC (silica gel). The alkaloids from the groups **3**–**7** were removed from the column by consecutive elution with $CHCl_3$ and MeOH/25% aq. $NH_3$ soln. 9:1. Afterwards, the column was washed with $CHCl_3$/MeOH/25% aq. $NH_3$ soln. 4:3:1. The eluate was evaporated to yield an enriched mixture of (*S*)-protoverbine (**1**) and (*S*)-protomethine (**2**).

*(−)-(8*S*)-8-Phenyl-1,5,9,13-tetraazacycloheptadecan-6-one* (*=(−)-(*S*)-Protoverbine*; **1**). Alkaloid **1** was isolated from the enriched mixture by prep. TLC (silica gel; $CHCl_3$/MeOH/25% aq. $NH_3$ soln. 7:3:1). From the scraped chromatographic zone, **1** was recovered with $CHCl_3$/MeOH/25% aq. $NH_3$ soln. 4:3:1. The eluate was

evaporated, and the residue was heated for 30 min at 60° with 20 mg of $NH_2OH \cdot HCl$ in 2 ml of 1% aq. HCl soln. The mixture was diluted twice with $H_2O$, washed twice with $CHCl_3$, alkalinized (sat.) with solid $K_2CO_3$, extracted 5 times with equal volumes of $CHCl_3$/i-PrOH 4:1. The extract was evaporated, the residue dissolved in $CHCl_3$, dried ($Na_2SO_4$), and evaporated to yield 28 mg (0.35% from the total alkaloid mixture) of **1**. Colorless glasslike solid. TLC (silica gel; $CHCl_3$/MeOH/25% aq. $NH_3$ soln. 7:3:1): $R_f$ 0.43. $[\alpha]_D = -28.6$ ($c = 1.47$, $CHCl_3$). $^1$H-NMR: 8.36 (br. *t*, CONH); 7.45–7.15 (*m*, 5 arom. H); 4.0 (*q*, PhCHN); 3.6–3.2 (*m*, 2 H); 2.95–2.15 (*m*, 15 H, incl. 3 NH); 1.9–1.4 (*m*, 4 $CH_2$). $^{13}$C-NMR: 171.24 (C=O); 143.32 (arom. quat. C): 128.48, 127.06, 126.6 (arom. C); 60.27 (Ph*C*N); 49.03, 48.73, 48.24, 48.04, 46.96, 45.59, 38.88, 28.42, 28.04, 27.21, 27.07 ($CH_2$). ESI-MS: 333 ($[M+H]^+$).

*(+)-(2*S*)-2-Phenyl-1,5,9,14-tetraazabicyclo[12.3.1]octadecan-4-one (=(+)-(*S*)-Protomethine*; **2**). Alkaloid **2** was isolated from the enriched mixture by prep. TLC (silica gel; $CHCl_3$/MeOH/25% aq. $NH_3$ soln. 7:3:1). From the scraped chromatographic zone, **2** was recovered with $CHCl_3$/MeOH/25% aq. $NH_3$ soln. 4:3:1. The eluate was evaporated. The residue was dissolved in $CHCl_3$, washed with conc. aq. $K_2CO_3$ soln., and evaporated. The residue was dissolved in $CHCl_3$, dried ($Na_2SO_4$), and evaporated to give 2.5 mg (0.03% from the total alkaloid mixture) of **2**. Colorless glasslike solid[1]). TLC (silica gel; $CHCl_3$/MeOH/25% aq. $NH_3$ soln. 7:3:1): $R_f$ 0.7. $[\alpha]_D = +4.9$ ($c = 1.39$, $CHCl_3$). $^1$H-NMR: 8.89 (br. *s*, CONH); 7.35–7.25 (*m*, 3 arom. H); 7.15–7.1 (*m*, 2 arom. H); 4.11 (br. *d*, PhC*H*N); 3.8–2.2 (*m*, 16 H); 2.0–1.3 (*m*, 8 H). $^{13}$C-NMR: 171.83 (C=O); 136.39 (arom. quat. C); 128.11, 127.98, 127.47 (arom. C); 77.27 (NCN); 64.67 (Ph*C*N); 53.72, 52.67, 49.05, 48.33, 38.17, 28.72, 27.31, 24.78, 24.48 ($CH_2$). ESI-MS: 345 ($[M+H]^+$).

*Ethyl (±)-3-{[3-({4-[(3-Aminopropyl)amino]butyl}amino)propyl]amino}-3-phenylpropanoate* (**15**). A mixture of 4 g (20 mmol) *spermine* (**13**), 7.3 g (120 mmol) AcOH and 4 g (23 mmol) *ethyl 3-phenylprop-2-ynoate* (**11**) (or *ethyl 3-oxo-3-phenylpropanoate* (**12**)) in 30 ml of EtOH was refluxed 2 h. After cooling to r.t., 20 ml of MeOH, 10 g of AcOH, and soln. of 3 g (20.6 mmol) of $NaCNBH_3$ in 4 ml of MeOH were added. The mixture was stirred at r.t. overnight. After evaporation of the solvent, 30 ml of $H_2O$ were added, and the mixture was extracted 3 times with $CHCl_3$. The $H_2O$ phase was alkalinized (saturated) with solid $K_2CO_3$, extracted once with $CHCl_3$ and five times with $CHCl_3$/i-PrOH 4:1. After evaporation of the $CHCl_3$/i-PrOH extract at $\leq 45°$ the residual pale yellow oil was dissolved in 100 ml of EtOH, and the soln. was acidified with 32% HCl. The tetrahydrochloride of compound **15** crystallized. After filtration, washing with EtOH and $Et_2O$, the product was recrystallized from 35 ml of AcOH. After slow crystallization at r.t. overnight, filtration, washing with EtOH and $Et_2O$, the yield of compound **15** · 4 HCl was 5.68 g (54.7%). For the preparation of the free base, 1.8 g of **15** · 4 HCl were dissolved in 30 ml of $H_2O$ soln., alkalinized by saturation with solid $K_2CO_3$, and extracted 5 times with 25 ml of $CHCl_3$/i-PrOH 4:1. After evaporation of the extract at $\leq 45°$, the residue was dissolved in $CHCl_3$, filtered, and evaporated to give 1.1 g (84%) of the free base **15** as colorless oil.

*Data of* **15** · 4 HCl: M.p. >300° (dec.). TLC (silica gel; $CHCl_3$/MeOH/25% aq. $NH_3$ soln. 7:3:1): $R_f$ 0.35. $^1$H-NMR (free base **15**, $CDCl_3$): 7.40–7.20 (*m*, 5 arom. H); 4.13–4.01 (*m*, therein 4.09 (*q*, MeC$H_2$) and 4.05 (*m*, H–C(3)); 2.9–2.35 (*m*, 14 H); 2.19 (br. *s*, 5 NH); 1.8–1.4 (*m*, 8 H); 1.19 (*t*, Me). $^{13}$C-NMR: 171.69 (C=O); 142.69 (arom. quat. C); 128.38, 127.24, 126.86 (arom. C); 60.29 (Me*C*$H_2$); 59.61 (Ph*C*N); 49.51, 48.05, 47.63, 45.8, 42.87, 40.41, 32.99, 29.76, 27.57 ($CH_2$); 14.01 (Me). ESI-MS: 379 ($[M+H]^+$).

*(±)-*N*-(3-Aminopropyl)-3-[(3-aminopropyl)amino]-3-phenylpropanamide* (**19**). To 20 ml (0.24 mol) of boiling propane-1,3-diamine, a soln. of 3.24 g (20 mmol) of methyl (*E*)-3-phenylprop-2-enoate in 20 ml of xylene was added dropwise under Ar during 1 h. After additional 4 h reflux, the mixture was concentrated under reduced pressure, and the oily residue (5 g) was separated by CC (silica gel; $CHCl_3$/MeOH/25% aq. $NH_3$ 7:3:1) to yield 2.8 g (50%) of **19**. Colorless oil. TLC (silica gel; $CHCl_3$/MeOH/25% aq. $NH_3$ soln. 7:3:1): $R_f$ 0.13. $^1$H-NMR: 7.56 (br. *t*, CONH); 7.35–7.2 (*m*, 5 arom. H); 3.96 (*q*, PhC*H*N); 3.35–3.2 (*m*, 2 H), 2.8–2.3 (*m*, 8 H), 1.8–1.4 (*m*, incl. 5 NH and 2 $CH_2$). $^{13}$C-NMR: 171.34 (C=O); 143.03 (arom. quat. C); 128.63, 127.36, 126.72 (arom. C); 60.27 (Ph*C*N); 45.18, 44.49, 40.42, 39.86, 37.23, 33.77, 32.71 (Me). ESI-MS: 279 ($[M+H]^+$).

*(±)-3-Phenyl-*N*-{3-[(toluene-4-sulfonyl)amino]propyl}-3-({3-[(toluene-4-sulfonyl)amino]propyl}amino)-propanamide* (**20**) *and (±)-3-Phenyl-*N*-{3-[(toluene-4-sulfonyl)amino]propyl}-3-((toluene-4-sulfonyl){3-[(toluene-4-sulfonyl)amino]propyl}amino)propanamide* (**22**). To a soln. of 3.27 g (11.76 mmol) of **19** in 50 ml $CH_2Cl_2$, in the presence of 15 ml of $Et_3N$, a soln. of 5 g (26.23 mmol) of TsCl in 24 ml of $CH_2Cl_2$ was added dropwise under stirring and cooling to 0° during 15 min. After 20-h stirring at r.t., the solvent was evaporated

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[1]) Because of scarcity of material, the $[\alpha]_D$, values, and $^1$H- and $^{13}$C-NMR spectra were measured with (+)-(*S*)-protomethine (**2**), semisynthetically prepared from (−)-(*S*)-protoverbine (**1**) and HCHO as described below.

under reduced pressure. The residue was dissolved in 200 ml of $CH_2Cl_2$, the soln. was washed consecutively with 50 ml of sat. aq. $NaHCO_3$ soln. and brine, dried ($Na_2SO_4$), and evaporated. The resulting crude mixture (6.9 g) of **20** and **22** was separated by CC (silica gel; $CHCl_3$/MeOH 19:1) to yield 4.3 g (62.4%) of **20** and 1.26 g (14%) of **22**.

*Data of* **20**: TLC (silica gel, $CHCl_3$/MeOH 9:1): $R_f$ 0.11. $^1$H-NMR: 7.73 (*d*, *J* = 8, 4 arom. H); 7.30–7.15 (*m*, 9 arom. H); 7.08 (br. *t*, CONH); 6.5–5.5 (br. *ca.* 1 H, $NHSO_2$); 3.93 (*q*, PhC*H*N); 3.35–3.15 (*m*, 2 H), 2.98–2.7 (*m*, 8 H), 2.65–2.35 (*m*, 10 H, incl. 2 Me); 1.65–1.45 (*m*, 2 $CH_2$). $^{13}$C-NMR: 171.92 (C=O); 143.26, 142.11, 137.08, 36.83 (arom. quat. C); 129.72, 128.69, 127.52, 127.01, 127.03, 128.82 (arom. C); 60.02 (Ph*C*N); 43.60, 44.12, 42.7, 40.21, 35.16, 29.23, 28.47 ($CH_2$); 21.49 (Me). ESI-MS: 587 ($[M+H]^+$).

*Data of* **22**: TLC (silica gel; $CHCl_3$/MeOH 9:1): $R_f$ 0.44. $^1$H-NMR: 7.70, 7.68 (2*d*, *J* = 8, 4 arom. H); 7.56 (*d*, *J* = 8, 2 arom. H); 7.3–7.1 (*m*, 11 arom. H); 6.54 (br. *t*, CONH); 5.68 (br. *t*, PhC*H*N); 5.35 (br. *t*, 2 $NHSO_2$); 3.3–2.6 (*m*, 10 H); 2.4 (*s*, 2 Me); 2.39 (*s*, Me); 1.75–1.4 (*m*, 2 $CH_2$). $^{13}$C-NMR: 170.24 (C=O); 143.5, 143.43, 143.31, 137.4, 137.15, 137.06, 136.7 (arom. quat. C); 129.73, 128.53, 126.18, 127.96, 127.23, 126.99 (arom. C); 58.35 (Ph*C*N); 43.02, 40.29, 39.4, 39.21, 36.16, 30.13, 29.33 ($CH_2$); 21.51 (Me). ESI-MS: 764 ($[M+Na]^+$).

*(±)-8-Phenyl-1,13-bis(toluene-4-sulfonyl)-1,5,9,13-tetraazacycloheptadecan-6-one* (**21**). A mixture of 1.45 g (2.48 mmol) of (±)-**20**, 1.68 g (5.15 mmol) of $Cs_2CO_3$, and 250 ml of abs. DMF was stirred at 60° for 15 min. After cooling to r.t., a soln. of 600 mg (2.8 mmol) of 1,4-dibromobutane in 50 ml of abs. DMF was introduced to the mixture dropwise during 15 min. After additional 18 h stirring at r.t. and 5 h at 40°, the mixture was concentrated *i.v.* The residue was splashed with ice-$H_2O$, the insoluble product was suspended, filtered, washed with ice-$H_2O$, and dried to yield 2.13 g of a solid, purification of which by CC (alumina, activity II–III, $CHCl_3$) gave 1.046 g (67%) of pure **21**. TLC (silica gel; $CHCl_3$/MeOH 9:1): $R_f$ 0.44. $^1$H-NMR: 8.19 (*t*, CONH; 7.67, 7.62 (2*d*, *J* = 8, 4 arom. H); 7.4–7.2 (*m*, 9 arom. H); 3.93 (*q*, PhC*H*N); 3.6–2.9 (*m*, 10 H); 2.7–2.3 (*m*, 10 H, incl. 2 Me at 2.43 and 2.41); 2.0–1.5 (*m*, 4 $CH_2$). $^{13}$C-NMR: 171.8 (C=O); 143.38, 143.29, 142.5, 135.64, 135.59 (arom. quat. C); 129.71, 129.62, 128.66, 127.33, 127.07, 126.27 (arom. C); 59.59 (Ph*C*N); 49.95, 49.56, 47.99, 47.57, 44.23, 43.65, 36.74, 30.06, 29.63, 26.61, 26.34 ($CH_2$); 21.39, 21.37 (Me). ESI-MS: 641 ($[M+H]^+$).

*(±)-8-Phenyl-1,9,13-tris(toluene-4-sulfonyl)-1,5,9,13-tetraazacycloheptadecan-6-one* (**23**). A mixture of 1.09 g (1.48 mmol) of (±)-**22** and 1.01 g (3 mmol) of $Cs_2CO_3$ in 200 ml of abs. DMF was stirred at 60° for 15 min. After cooling to r.t., a soln. of 320 mg (1.48 mmol) 1,4-dibromobutane in 25 ml of abs. DMF was introduced to the mixture dropwise during 15 min. After additional 18-h stirring at r.t. and 5 h at 40°, the mixture was concentrated under vacuum. The residue was splashed with ice-$H_2O$, the insoluble product was suspended, filtered, washed with ice-$H_2O$, and dried. The crude product was purified by CC (silica gel; $CHCl_3$/MeCN 9:1) to give 0.88 g (75%) of pure **23**. Colorless crystalline mass. M.p. 229.5–231° ($CHCl_3$/EtOH). TLC (silica gel; $CHCl_3$/MeOH 9:1): $R_f$ 0.63. $^1$H-NMR: 7.68, 7.62 (2*d*, *J* = 8, 4 arom. H); 7.38 (*d*, *J* = 8, 2 arom. H); 7.35–7.2 (*m*, 9 arom. H); 7.19 (*d*, *J* = 8, 2 arom. H); 6.42 (br. *t*, CONH); 5.35 (2 br. *d*, PhC*H*N); 3.8–2.65 (*m*, 14 H); 2.41 (*s*, 2 Me); 2.33 (*s*, $CH_3$); 2.1–1.4 (*m*, 4 $CH_2$). $^{13}$C-NMR: 170.13 (C=O); 143.44, 143.13, 138.34, 137.31, 136.19, 135.41 (arom. quat. C); 129.7, 129.62, 120.16, 128.36, 127.83, 127.74, 127.35, 127.06, 127.02 (arom. C); 59.7 (Ph*C*N); 49.3, 48.95, 47.17, 46.69, 45.42, 40.65, 37.42, 30.43, 29.93, 25.86, 25.58 ($CH_2$); 21.36, 21.28 (Me). ESI-MS: 817 ($[M+Na]^+$).

*(±)-8-Phenyl-1,5,9,13-tetraazacycloheptadecan-6-one* (=*(±)-Protoverbine*; **1**). The electrochemical detosylation of both (±)-**21** and (±)-**23** was performed according to [13b]. Instead of $Bu_4N^+Br^-$, 0.1M soln. of $Me_4N^+Cl^-$ in 94% aq. EtOH soln. as catholyte and anolyte was used. The reaction was carried out under Ar at 5°. After evaporation of the catholyte, the residue was dissolved in $H_2O$, saturated with solid $K_2CO_3$, and extracted 5 times with $CHCl_3$/i-PrOH 4:1. The extract was evaporated, the residue was dissolved in $CHCl_3$ and purified by CC (silica gel; $CHCl_3$/MeOH/25% aq. $NH_3$ soln. 7:3:1; $R_f$ 0.43). From 300 mg of (±)-**21** and 150 mg of (±)-**23**, 151 mg (89%) and 55 mg (81%) of (±)-**1**, respectively, were obtained. The $^1$H- and $^{13}$C-NMR spectra of (±)-**1** were identical to those of the natural (−)-(*S*)-protoverbine (**1**). ESI-MS: 333 ($[M+H]^+$).

*(±)-2-Phenyl-1,5,9,14-tetraazabicyclo[12.3.1]octadecan-4-one* (=*(±)-Protomethine*; **2**). A mixture of 10 µl of 37% aq. HCHO (3.7 mg, 0.12 mmol) soln. and 1 ml of MeOH was added to a soln. of 38 mg (0.11 mmol) of (±)-**1** in 3 ml of MeOH. The mixture was stirred for 15 min, and the solvent was evaporated. The residue was dissolved in $CHCl_3$, washed with dil. aq. $NH_3$ soln. and a few times with $H_2O$, dried ($Na_2SO_4$), and evaporated. The residue was dissolved in $CHCl_3$ and purified by CC (silica gel; $CHCl_3$/MeOH/25% aq. $NH_3$ soln. 7:3:1; $R_f$ 0.7) to give 36 mg (91%) of (±)-**2** as colorless glasslike solid. The $^1$H- and $^{13}$C-NMR spectra of (±)-**2** are identical to those of the natural (−)-(*S*)-protomethine (**2**). ESI-MS: 345 ($[M+H]^+$).

*(±)-4-Phenyl-1,5,9,14-tetraazatricyclo[12.3.1.1$^{5,9}$]nonadecan-2-one* (**24**). A mixture of 48 mg (0.14 mmol) of (±)-**1**, 0.1 ml of 37% aq. HCHO (37 mg, 1.2 mmol) soln. 0.1 ml of AcOH, and 3 ml of EtOH was refluxed for 30 min. The solvent was evaporated. The residue was dissolved in $CHCl_3$, the soln. was washed with dil. aq. $NH_3$ soln., and evaporated. The residue was purified by CC (silica gel; $CHCl_3$/MeOH/25% aq. $NH_3$ soln. 9:1:0.2) to

give 42 mg (82%) of (±)-**24**. Colorless glasslike solid. TLC (silica gel; $CHCl_3$/MeOH/25% aq. $NH_3$ soln. 40:10:1): $R_f$ 0.85. $^1$H-NMR 7.45–7.2 (*m*, 5 arom. H); 4.8–3.9 (*m*, 3 H); 3.9–2.9 (*m*, 6 H); 2.9–2.1 (*m*, 9 H); 2.1–1.2 (*m*, 8 H). $^{13}$C-NMR: 170.55 (C=O); 141.25 (arom. quat. C); 128.88, 128.21, 127.98, 127.32, 126.57 (arom. C); 72.63 (N*C*N); 71.2 (Ph*C*N); 65.96 (N*C*N); 60.09, 55.59, 55.08, 53.57, 53.47, 53.36, 48.54, 42.76, 39.55, 24.89, 24.42 ($CH_2$). ESI-MS: 357 ($[M+H]^+$).

*Hydrolysis of (±)-***2** *and (±)-***24**. A mixture of 20 mg (0.06 mmol) of (±)-**2** or (±)-**24**, and 20 mg of $NH_2OH \cdot HCl$ (0.3 mmol) in 3 ml of 1% aq. HCl was heated at 60° for 1 h, alkalinized with sat. aq. $K_2CO_3$ soln., and extracted 5 times with $CHCl_3$/i-PrOH 4:1. The org. extract was evaporated. The residue was dissolved in $CHCl_3$, dried ($Na_2SO_4$), and evaporated to give (±)-**1** almost quantitatively. ESI-MS: 333 ($[M+H]^+$).

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*Received July 26, 2000*