The Asymmetric Synthesis of the [D_8]-Labeled (−)-(*S*)-Dihydroxyverbacine, the Terminal Precursor in the Biogenesis of the Macrobicyclic Spermine Alkaloids Aphelandrine and Orantine

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Dedicated to Professor *Jürgen O. Metzger* on the occasion of his 60th birthday

The asymmetric synthesis of the unlabeled and [D_8]-labeled terminal precursors, **4** ((−)-(*S*)-dihydroxyverbacine) and **19**, respectively, in the biogenesis of the spermine alkaloids aphelandrine (**5**) and orantine (**6**), respectively, is described. A partial synthesis of the alkaloid (−)-(*S*)-[(*E*)-4-methoxycinnamoyl]buchnerine (**10**) is also presented.

Introduction. – The macrobicyclic spermine alkaloid aphelandrine (**5**) is found in the roots of *Encephalosphaera lasiandra* and, together with its diastereoisomer orantine (**6**), in several *Aphelandra* species. In addition, orantine was isolated from *Chaenorhinum minus*, *Schweinfurthia papilionacea* (Scrophulariaceae), and from *Ephedra* sp. (Ephedraceae) [1]. Both alkaloids contain a 13-membered and a 17-membered ring, with spermine and two *para*-coumaric acids as precursor moieties. The two hypotheses proposed for the biogenesis of these alkaloids are shown in *Scheme 1*. The first involves the *N*(1),*N*(5)-di(*p*-coumaroyl)spermine (**1**), which first undergoes cyclization by *Michael* addition to the compound (*S*)-**4** and subsequently yields, *via* phenol coupling, aphelandrine (**5**) [2]. The second possibility is the transformation of monoacylated spermine derivative **2** by *Michael* addition to the macrocycle (*S*)-**3**, which is further acylated to (*S*)-**4**. Subsequent phenol coupling leads to aphelandrine (**5**) or orantine (**6**).

Compound **3** was recently detected in the roots of *A. squarrosa* and named prelandrine [3]. The presence of **3** in *Aphelandra* plants supports its possible key role in the biogenetic pathway of aphelandrine (**5**) and strongly favors the second pathway mentioned above [3]. Although the acylated derivative **4** of prelandrine (**3**) was not detected in the plant material, it seems to represent the terminal biogenetic precursor of aphelandrine (**5**) and orantine (**6**).

The last step in the biosynthesis of this type of alkaloids is proposed to be a diastereoselective intramolecular oxidative phenol coupling that leads to the substituted benzofuran ring of aphelandrine (**5** (11*S*,17*S*,18*S*)) or orantine (**6** (11*S*,17*R*,18*R*)). The presumable substrate for this diastereoselective intramolecular coupling is compound (*S*)-**4**, the dihydroxy derivative of the alkaloid (*S*)-verbacine, which was isolated earlier from different *Verbascum* species [4]. The (*Z*)-isomer of its (*S*)-di-*O*-methyl derivative has been isolated from *Clerodendrum buchneri* [5].

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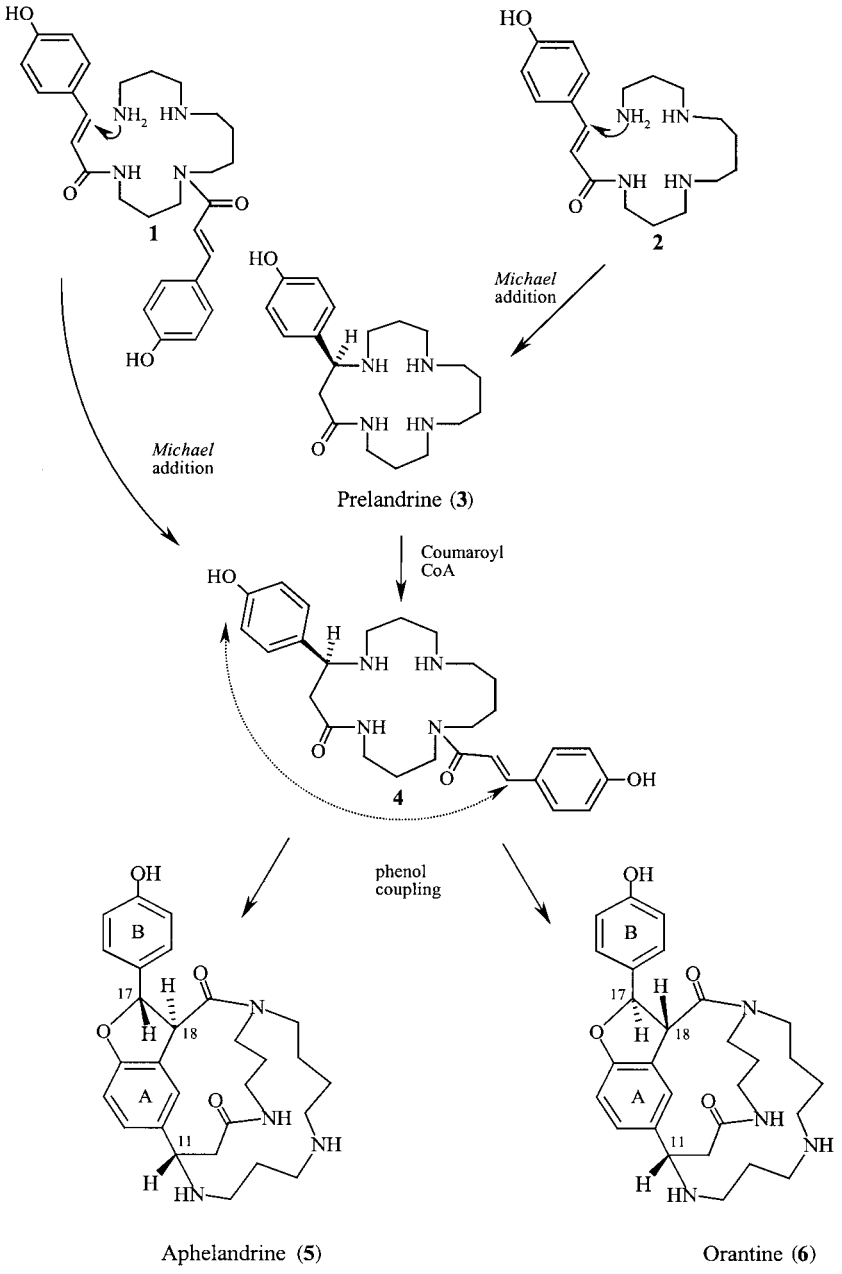
Scheme 1



Since microsomal-bound cytochrome P-450 enzymes are known to participate in highly regio- and stereoselective phenol-coupling reactions in benzylisoquinoline alkaloids [6][7], it was expected that an enzyme of this type might be responsible for

the phenol coupling of the compound (*S*)-**4** in the biosynthesis of aphelandrine (**5**) and orantine (**6**). To prove whether this type of enzyme, indeed, is responsible for the formation of the benzofuran ring of aphelandrine, a method for the isolation of a biochemically active microsomal fraction from the roots of *A. squarrosa* was developed. Additionally, highly sensitive analytical methods for the *in vitro* enzymatic studies were introduced [8].

To validate the hypothesis that (*S*)-dihydroxyverbacine (**4**) is the substrate for the enzymatic phenol coupling and thus the terminal precursor in the biosynthesis of aphelandrine (**5**) and orantine (**6**), the unlabeled and [D_8]-labeled derivatives, **4** and **19**, respectively, were synthesized. In the present paper, the syntheses of these two compounds for the *in vitro* enzymatic studies, and the synthesis of the alkaloid (−)-(*S*)-[(*E*)-4-methoxycinnamoyl]buchnerine (**10**) are described.

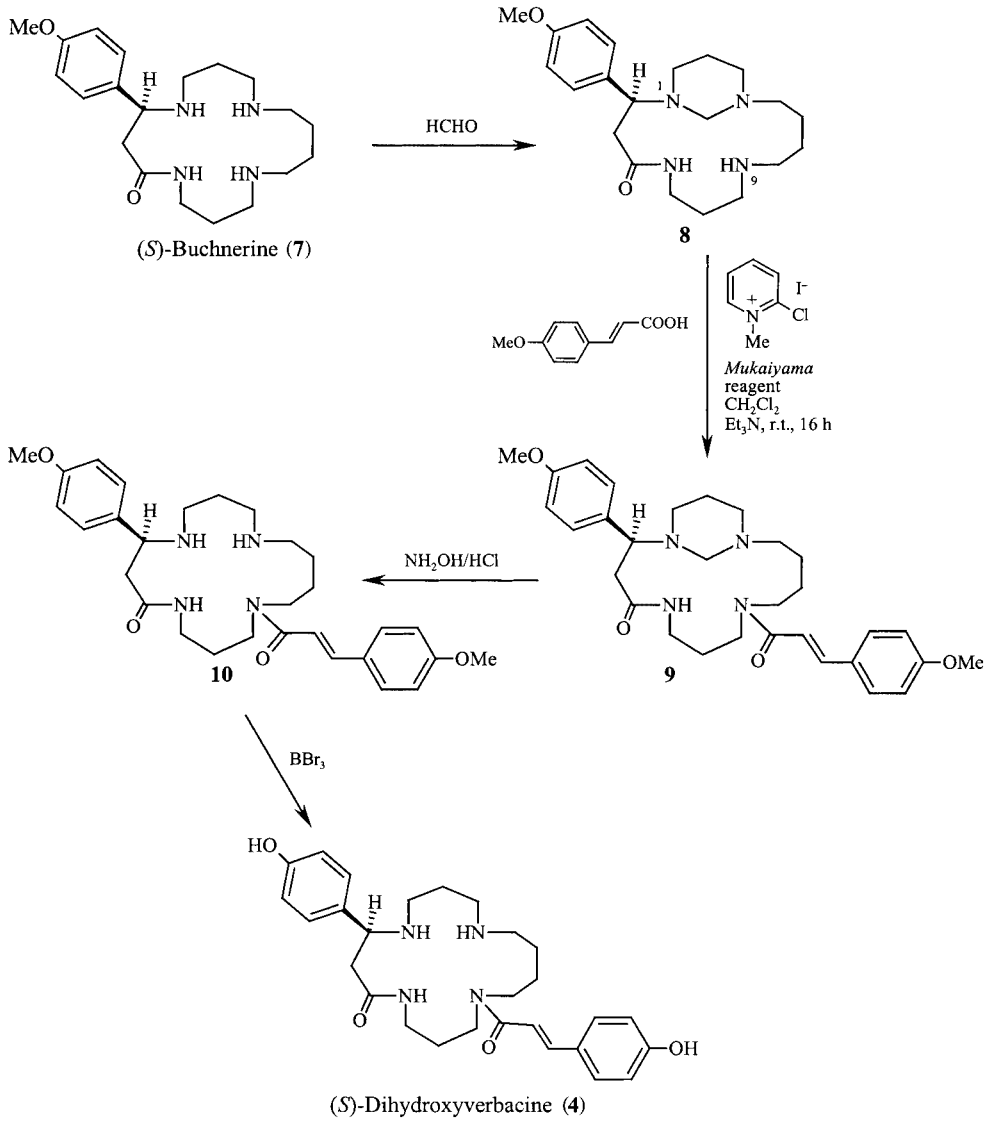
Results and Discussion. – Unlabeled (*S*)-dihydroxyverbacine (**4**) was synthesized in four steps starting from the synthetically prepared naturally occurring alkaloid (*S*)-buchnerine (**7**) [9] (*Scheme 2*). The bicyclic compound (*S*)-**8** was quantitatively obtained from (*S*)-**7** with 1 equiv. of HCHO. Additional acylation of (*S*)-**8** at N(9) gave the macrobicycle (*S*)-**9**, which was further hydrolyzed in the presence of NH_2OH (a HCHO interceptor) to compound (*S*)-**10**. The (*Z*)-isomer of (*S*)-**10**, (−)-(*S*)-[(*Z*)-4-methoxycinnamoyl]buchnerine, is a naturally occurring alkaloid detected earlier in *Clerodendrum buchneri* (Verbenaceae) [5]. *O*-Demethylation of (*S*)-**10** with BBr_3 led to (*S*)-dihydroxyverbacine (**4**).

The enzymatic assay with (*S*)-dihydroxyverbacine (**4**) showed the presence of aphelandrine (**5**) in the reaction medium, but it was impossible to distinguish between aphelandrine formed by the intramolecular cyclization of (*S*)-**4** and the endogenous aphelandrine present in small amounts in the plant material (cell-microsomal fraction). To answer the question of whether (*S*)-dihydroxyverbacine ((*S*)-**4**) is indeed the precursor for the terminal diastereospecific macrocyclization, it was necessary to conduct experiments with labeled material.

The synthesis of the [D_8]-(*S*)-dihydroxyverbacine (**19**) is depicted in *Scheme 3*. Compound (*S*)-**11** was prepared according to the procedure for the asymmetric synthesis of the alkaloid (*S*)-buchnerine (**7**) [9]. The cyclization of (*S*)-**11** was performed according to a modified *Richman-Atkins* protocol in DMF in presence of Cs_2CO_3 [10]: the [D_8]-labeled butane-1,4-diyl moiety was introduced to (*S*)-**11** (→ (*S*)-**14**), using (1,1,2,2,3,3,4,4-D_8)butane-1,4-diyl bis(methanesulfonate) (**13**), which was obtained from the commercially available [1,1,2,2,3,3,4,4-D_8]butane-1,4-diol (**12**). The electrochemical detosylation [11] of compound (*S*)-**14** provided [D_8]-(*S*)-buchnerine (**15**) in excellent yield. With 1 equiv. HCHO in MeOH, compound (*S*)-**15** led quantitatively to the CH_2-bridged compound (*S*)-**16**, which was additionally acylated at N(9) with (*E*)-3-(4-methoxyphenyl)prop-2-enoyl chloride to give (*S*)-**17**. By mild acid hydrolysis in the presence of NH_2OH, compound (*S*)-**17** was converted quantitatively to (*S*)-*N*(1)-[(*E*)-4-methoxycinnamoyl][D_8]buchnerine ((*S*)-**18**). O-Demethylation of (*S*)-**18** with BBr_3 gave the [D_8]-labeled precursor (*S*)-**19**.

The [D_8]-labeled precursor (*S*)-**19** can now be used as a substrate for the enzymatic oxidative phenol-coupling experiments with microsomal fractions from the roots of *A. squarrosa.* These biochemical experiments will pave the way to answer the question of

Scheme 2



whether the proposed terminal precursor (*S*)-**4** and its labeled analog (*S*)-**19** are converted to aphelandrine or orantine, respectively, by a cytochrome P-450.

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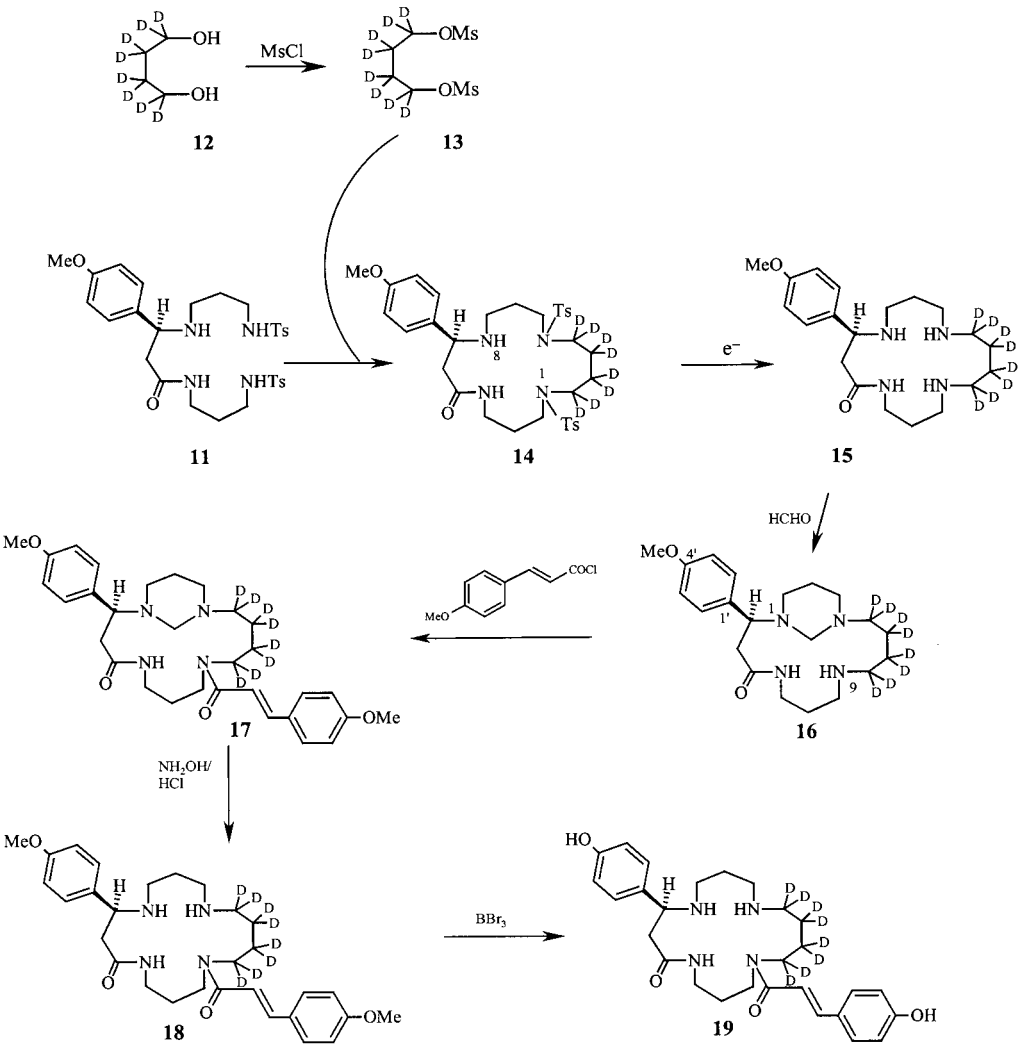
Scheme 3



Experimental Part

General. [1,1,2,2,3,3,4,4-$^2H_8$]butane-1,4-diol (98 atom-% D) was purchased from *Aldrich*. TLC: *Merck* precoated plates *Kieselgel 60* F_{254} were used, detection by UV (254 or 366 nm), *Schlittler* [12], and *Dragendorff* reagents. CC: *Kieselgel 60* (70–230 mesh) from *Merck*. M.p.: *Büchi 510* melting-point apparatus; uncorrected. Optical rotation: *Perkin-Elmer 241* polarimeter. NMR: *Bruker AC-300*, *ARX-300*, or *AMX-600* (^1H) and *Bruker ARX-300* (75 MHz) or *AMX-600* (150 MHz; ^{13}C; only selected, well-defined signals are given); chemical shifts δ in ppm rel. to Me_4Si as internal standard. ESI-MS: *Finnigan TSQ 700* mass spectrometer.

(+)-(2S)-2-(4-Methoxyphenyl)-1,5,9,14-tetraazabicyclo[12.3.1]octadecan-4-one (**8**). To a soln. of *(−)-(S)-buchnerine* (**7**; 85 mg, 0.23 mmol) in 5 ml MeOH was added 37% aq. HCHO soln. (19 µl, 0.23 mmol). After several min, the mixture was evaporated, and the residue was dissolved in $CHCl_3$. The soln. was washed with dil. aq. NH_3 soln. and aq. sat. NaCl soln., dried (Na_2SO_4), and evaporated to give (*S*)-**8** quantitatively. Colorless

glass-like solid. TLC ($CHCl_3$/MeOH/25% aq. NH_3 soln. 7:3:1): R_f 0.74. $[\alpha]_D^{20} = +6.27$ ($c = 1.7$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 8.95 (br. *s*, CONH···N); 7.04, 7.03 (2*d*, $J = 9$, 2 arom. H); 6.84 (*d*, $J = 9$, 2 arom. H); 4.16–3.97 (br. *m*, PhC*H*N); 3.79 (*s*, MeO); 3.57–3.1 (*m*, 3 H); 3.07–1.82 (*m*, 14 H); 1.82–1.17 (*m*, 9 H). ^{13}C-NMR ($CDCl_3$): 171.9 (C=O); 158.8 (arom. quat. C); 129.0 (arom. quat. C); 128.9, 113.3 (CH=CH); 63.5 (Ph*C*N); 55.1 (MeO). ESI-MS: 374 ($[M+H]^+$).

(+)-(2S)-9-[(E)-3-(4-Methoxyphenyl)prop-2-enoyl]-2-(4-methoxyphenyl)-1,5,9,14-tetraazabicyclo[12.3.1]octadecan-4-one (**9**). To a suspension of 74 mg (0.29 mmol) of 1-methyl-2-chloropyridinium iodide [13] and 43 mg (0.24 mmol) of (*E*)-4-methoxycinnamic acid in a mixture of 2 ml of CH_2Cl_2 and 0.15 ml of Et_3N, cooled to 0°, was added dropwise a soln. of 90 mg (0.24 mmol) of (*S*)-**8**. The mixture was stirred in the dark at r.t. for 16 h. The mixture was diluted with $CHCl_3$, washed with 10% aq. soln. of K_2CO_3 and H_2O, dried (Na_2SO_4), and evaporated. The residue was dissolved in $CHCl_3$ and purified by CC (alumina, consecutively $CHCl_3$, AcOEt/MeOH 9:1) to obtain (*S*)-**9** as colorless glass-like solid (109 mg, 85%). TLC (AcOEt/MeOH 4:1): R_f 0.45, $[\alpha]_D^{20} = +5$ ($c = 2$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 9.55, 9.35 (2 br, *s*, CONH···N); 7.68, 7.67 (2*d*, $J = 15$, PhCH=CHCO); 7.46 (*d*, $J = 9$, 2 arom. H); 6.97 (*d*, $J = 9$, 2 arom. H); 6.92–6.8 (*m*, 4 arom. H); 6.69, 6.67 (2*d*, $J = 15$, PhCH=CHCO); 3.97 (br. *d*, PhC*H*N); 3.83 (*s*, MeO); 3.8 (*s*, MeO); 3.75–2.1 (*m*, 16 H); 1.98–1.3 (*m*, 8 H). ^{13}C-NMR ($CDCl_3$): 172, 166 (2 C=O); 160.6, 159.1 (arom. quat. C); 142.2, 141.9, 129.2, 129, 128.1, 114.9, 114.1, 113.4 (C=C); 63.4 (Ph*C*N); 55.2, 55.1 (2 MeO). ESI-MS: 535 ($[M+H]^+$).

(−)-(8S)-1-[(E)-3-(4-Methoxyphenyl)prop-2-enoyl]-8-(4-methoxyphenyl)-1,5,9,13-tetraazacycloheptadecan-6-one (**10**). A mixture of 80 mg (0.15 mmol) of (*S*)-**9** and 200 mg of $NH_2OH \cdot HCl$ in 5 ml of 1% aq. HCl soln. was heated at 70° for 1 h. The mixture was washed with $CHCl_3$, alkalinized with 25% aq. NH_3 soln., and extracted with $CHCl_3$. The org. extract was washed with H_2O, dried (Na_2SO_4), and evaporated to give 60 mg (77%) of (*S*)-(**10**). Colorless, glass-like solid. TLC ($CHCl_3$/MeOH/25% aq. NH_3 soln. 8:2:0.5): R_f 0.45. $[\alpha]_D^{20} = -12.6$ ($c = 1.5$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, mixture of conformers): 8.46 (br. *s*, 0.5 H, CONH···N); 7.67 (*d*, $J = 15$, PhC*H*=CHCO); 7.48 (*d*, $J = 8$, 2 H); 7.35 (br. *s*, 0.5 H, CONH); 7.26–7.09 (*m*, 2 arom. H); 6.93–6.85 (*m*, 3 arom. H, H–C(8′)); 6.76–6.56 (*m*, 2 arom. H); 3.94–3.91 (br. *d*, PhC*H*N); 3.84–3.13 (*m*, 12 H, including 2 MeO at 3.84, 3.8, 3.79); 2.76–2.32 (*m*, 8 H); 1.94–1.43 (*m*, 10 H). ^{13}C-NMR ($CDCl_3$): 172, 166 (2 C=O); 161, 159, 142.4, 129.3, 127.9, 127.6, 115, 114.1, 113.8 (C=C); 58.9 (Ph*C*N); 55.2, 55.1 (MeO). ESI-MS: 523 ($[M+H]^+$).

(−)-(8S)-1-[(E)-3-(4-Hydroxyphenyl)prop-2-enoyl]-8-(4-hydroxyphenyl)-1,5,9,13-tetraazacycloheptadecan-6-one Dihydrochloride (**4** · 2 HCl). To a soln. of (*S*)-**10** (60 mg, 0.11 mmol) in dry CH_2Cl_2 (5 ml), cooled to −78° (dry ice/acetone bath), 1.5 ml (1.5 mmol) of 1M CH_2Cl_2 soln. of BBr_3 were added. The mixture was kept at −78° for 1 h, then stirred for 16 h at r.t. The reaction was quenched by adding of 3 ml of 90% aq. MeOH soln. and evaporated to dryness *in vacuo* (50°). The excess BBr_3 was removed by repetitive addition of MeOH saturated with gas. HCl and evaporation. The solid residue was washed several times with $CHCl_3$, dissolved in $CHCl_3$/MeOH/25% aq. NH_3 soln. 7:3:0.3 and purified by CC (consecutively $CHCl_3$, $CHCl_3$/MeOH/25% aq. NH_3 soln. 7:3:0.3). The eluate was evaporated to dryness. The residual free base of (*S*)-**4** was transformed immediately to its dihydrochloride by addition of MeOH saturated with gas. HCl and evaporated to dryness: 50 mg (77%) of (*S*)-**4** · 2 HCl. Colorless solid. All steps were performed under red light to prevent (*E*) → (*Z*) photoisomerization. TLC ($CHCl_3$/MeOH/25% aq. NH_3 soln. 7:3:1): R_f 0.5. $[\alpha]_D^{20} = -1.5$ ($c = 1.3$, MeOH). ^{1}H-NMR ((D$_4$)MeOH): 7.6–7.43 (*m*, including 2 arom. H and PhC*H*=CHCO, *d*, $J = 15$, at 7.55); 7.37, 7.36 (2*d*, $J = 8$, 2 arom. H); 6.93–6.84 (*m*, 3 H, including PhCH=C*H*CO, *d*, $J = 15$, at 6.89); 6.84–6.78 (2*d*, $J = 8$, 2 arom. H); 4.84–4.74 (*m*, PhC*H*N); 3.8–3.1 (*m*, 13 H); 2.85 (*d*, $J = 17$, 1 H); 2.45–2.35 (*m*, 1 H); 2.18–2.07 (*m*, 1 H); 2.08–1.83 (*m*, 6 H). ESI-MS: 495 ($[M+H]^+$).

[1,1,2,2,3,3,4,4-$^2H_8$]Butane-1,4-diyl Bis(methanesulfonate) (**13**). According to [14], to a soln. of 0.857 g (8.7 mmol) of *[1,1,2,2,3,3,4,4-$^2H_8$]butane-1,4-diol* (**12**) in CH_2Cl_2 (30 ml) and Et_3N (3 ml, 22 mmol), MsCl (2.27 g, 20 mmol) was added dropwise at 0° over a period of 5 min. The mixture was stirred for 1.5 h at r.t. and then evaporated to dryness *in vacuo* (50°). The crystalline residue was washed with H_2O and then with MeOH. Recrystallization from EtOH gave 1.6 g (100%) of colorless crystalline **13**. M.p. 113°. TLC (AcOEt): R_f 0.8. ^{1}H-NMR ((D$_6$)acetone): 3.1 (*s*, 6 H).

(−)-(8S)-8-(4-Methoxyphenyl)-1,13-(4-tolylsulfonyl)[14,14,15,15,16,16,17,17-$^2H_8$]-1,5,9,13-tetraazacycloheptadecan-6-one (**14**). To a soln. of (*S*)-**11** (600 mg, 0.9 mmol) in DMF (95 ml), Cs_2CO_3 (764 mg, 2.4 mmol) was added. The mixture was heated at 62° for 30 min. The soln. was cooled to r.t., and a soln. of **13** (248 mg, 1.3 mmol) in DMF (19 ml) was added dropwise. The mixture was stirred for 48 h at 55° and evaporated *in vacuo*. The residue was dissolved in 10 ml of H_2O and extracted with $CHCl_3$ (5 ×), the org. layer was washed with H_2O, dried (Na_2SO_4), and evaporated. Compound (*S*)-**14** was purified by CC (consecutively AcOEt, $CHCl_3$/MeOH 20:1, $CHCl_3$/MeOH 10:1): (*S*)-**14** (353 mg, 54%). Colorless, glass-like solid. TLC ($CHCl_3$/MeOH 10:1):

R_f 0.61. $[\alpha]_D^{20} = -15.4$ ($c = 3.25$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 8.24 (t, CONH); 7.68, 7.63 ($2d$, $J = 8$, 4 arom. H); 7.3 (t, $J = 8$, 4 arom. H); 7.15 (d, $J = 9$, 2 arom. H); 6.88 (d, $J = 9$, 2 arom. H); 3.89, 3.87 ($2d$, $J = 9$, PhC*H*N); 3.8 (s, MeO); 3.49–3.43 (m, 1 H); 3.39–3.01 (m, 5 H); 2.61–2.42 (m, 10 H, including 2 Me at 2.43, 2.42); 2.04–1.74 (m, 3 H); 1.72–1.65 (m, 2 H). ^{13}C-NMR ($CDCl_3$): 172.8 (C=O); 162.4, 158.6, 143.2, 143.1, 135.7, 134.8, 129.6, 129.5, 127.3, 126.9, 113.9 (C=C); 58.8 (Ph*C*N); 55.1 (MeO); 47.8, 47.3, 44.1, 43.8, 37.2, 36.6 (Me), 36.3, 31.2, 29.9, 29.5, 21.3 (CH_2). ESI-MS: 679 ($[M+H]^+$).

*(−)-(8*S*)-(4-Methoxyphenyl)[14,14,15,15,16,16,17,17-$^2H_8$]-1,5,9,13-tetraazacycloheptadecan-6-one* (**15**). The detosylation of (*S*)-**14** was achieved by electrolysis according to [11]. The mixture was evaporated *in vacuo*. The residue was dissolved in 10 ml of H_2O, and the soln. was saturated with K_2CO_3 and extracted with a mixture of $CHCl_3$/i-PrOH 4:1. The combined org. extracts were evaporated. The residue was dissolved in $CHCl_3$ and purified by CC (consecutively $CHCl_3$, $CHCl_3$/MeOH 7:3, $CHCl_3$/MeOH/25% aq. NH_3 soln. 7:3:1): (*S*)-**15** (129.7 mg, 80%). Colorless, glass-like solid. TLC ($CHCl_3$/MeOH/25% aq. NH_3 soln. 7:3:1): R_f 0.54. $[\alpha]_D^{20} = -33.4$ ($c = 3.5$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 8.38 (br. *s*, CONH···N); 7.2 (d, $J = 9$, 2 arom. H); 6.86 (d, $J = 9$, 2 arom. H); 3.95 (m, PhC*H*N); 3.8 (s, MeO); 3.5–3.44 (m, 1 H); 3.33–3.28 (m, 1 H); 2.84–2.8 (m, 1 H); 2.76–2.67 (m, 2 H); 2.61–2.4 (m, 8 H); 1.75–1.49 (m, 4 H). ^{13}C-NMR ($CDCl_3$): 171.4 (C=O); 158.5 (C(4′)); 135.2 (C(1′)); 127.6 (C(3′), C(5′)); 113.8 (C(2′), C(6′)); 59.5 (Ph*C*N); 55.1 (MeO); 48.9, 47.8, 46.7, 45.5, 38.7, 28.3, 28.0 (CH_2). ESI-MS: 371 ($[M+H]^+$).

*(+)-(2*S*)-2-(4-Methoxyphenyl)[10,10,11,11,12,12,13,13-$^2H_8$]-1,5,9,14-tetraazabicyclo[12.3.1]octadecan-4-one* (**16**). To a soln. of (*S*)-**15** (130 mg, 0.35 mmol) in 5 ml of MeOH was added 37% aq. HCHO soln. (32 µl, 0.4 mmol). After several min, the mixture was evaporated. The residue was purified by CC (consecutively, MeOH, $CHCl_3$/MeOH/25% aq. NH_3 soln. 7:3:1): (*S*)-**16** (123.5 mg, 92%). Colorless, glass-like solid. TLC ($CHCl_3$/MeOH/25% aq. NH_3 soln. 7:3:1): R_f 0.7. $[\alpha]_D^{20} = +2.48$ ($c = 1.295$, $CHCl_3$).^{1}H-NMR ($CDCl_3$): 8.95 (br. *s*, CONH···N); 7.06 (d, $J = 8$, 2 arom. H); 6.85 (d, $J = 8$, 2 arom. H); 3.94 (br. d, $J = 10$, PhC*H*N); 3.73 (s, MeO); 3.62–3.29 (m, 3 H); 2.97–1.94 (m, 10 H); 1.78–1.39 (m, 4 H). ^{13}C-NMR ($CDCl_3$): 171.8 (C=O); 158.8 C(4′)); 129.1 (C(3′), C(5′)); 128.7 (C(1′)); 113.2 (C(2′), C(6′)); 63.8 (Ph*C*N); 55.1 (MeO); 52.5, 48.2, 38.2, 24.6 (CH_2). ESI-MS: 383 ($[M+H]^+$).

*(+)-(2*S*)-2-(4-Methoxyphenyl)-9-[(*E*)-3-(4-methoxyphenyl)prop-2-enoyl][10,10,11,11,12,12,13,13-$^2H_8$]-1,5,9,14-tetraazabicyclo[12.3.1]octadecan-4-one* (**17**). Compound (*S*)-**16** (110 mg, 0.29 mmol) was dissolved in CH_2Cl_2 (2 ml), and the soln. was cooled to 0°. A soln. of (*E*)-3-(4-methoxyphenyl)propenoyl chloride (prepared according to [15]; 75 mg, 0.38 mmol) in 4 ml of CH_2Cl_2 and Et_3N (300 µl) were then added dropwise. The mixture was stirred at 0° for 2 h. Then, the reaction was quenched by adding 5 ml of H_2O and allowed to warm to r.t. The aq. layer was extracted with $CHCl_3$ (4×). The combined org. layers were washed with H_2O and evaporated. The residue was purified by CC (consecutively, AcOEt, AcOEt/MeOH 9:1): (*S*)-**17** (101 mg, 65%). Colorless solid. TLC (AcOEt/MeOH 4:1: R_f 0.45. $[\alpha]_D^{20} = +1.67$ ($c = 2.30$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 9.45 (br. *s*, CONH···N); 7.68 (d, $J = 15$, PhC*H*=CHCO); 7.48–7.46 (m, 2 arom. H); 7.03–6.91 (m, 2 arom. H); 6.88–6.85 (m, 4 arom. H); 6.68 (d, $J = 15$, PhCH=C*H*CO); 3.98 (br. d, $J = 12$, PhC*H*N); 3.84, 3.8 ($2s$, 2 MeO); 3.66–2.36 (m, 9 H); 2.04–1.43 (m, 7 H). ESI-MS: 543 ($[M+H]^+$).

*(−)-(8*S*)-8-(4-Methoxyphenyl)-1-[(*E*)-3-(4-methoxyphenyl)prop-2-enoyl][14,14,15,15,16,16,17,17-$^2H_8$]-1,5,9,13-tetraazacycloheptadecan-6-one* (**18**). Compound (*S*)-**17** (46.1 mg, 0.085 mmol) was hydrolyzed in 1% aq. HCl soln. (2.5 ml) at 70° for 1 h in the presence of $NH_2OH \cdot HCl$ (124 mg, 1.8 mmol). The mixture was extracted $CHCl_3$ (2×), the H_2O layer was alkalinized with 25% aq. NH_3 soln. and extracted with $CHCl_3$ (4×). The org. extract was washed with H_2O, dried (Na_2SO_4), and evaporated to give (*S*)-**18** (44 mg, 98%). Colorless, glass-like solid. $[\alpha]_D^{20} = -8.06$ ($c = 2.27$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$, mixture of conformers): 8.37 (br. *s*, 0.5 H, CONH···N); 7.68 (d, $J = 15$, PhC*H*=CHCO); 7.52–7.49 (m, 2 arom. H); 7.32 (br. *s*, 0.5 H, CONH); 7.11–7.08 (m, 2 arom. H); 6.95–6.84 (m, 3 arom. H, including PhCH=C*H*CO); 6.74–6.72 (m, 2 arom. H); 3.94–3.01 (m, 11 H, including PhC*H*N, br. d at 3.93; $2s$, 2 MeO at 3.85, 3.75); 2.85–2.34 (m, 8 CH); 2.08–1.4 (m, 4 CH). ^{13}C-NMR ($CDCl_3$): 172, 166 (2 C=O); 161, 159, 142.4, 129.3, 127.9, 127.6, 115, 114.1, 113.8 (C=C); 58.9 (Ph*C*N); 55.2, 55.1 (MeO). ESI-MS: 531 ($[M+H]^+$).

*(+)-(8*S*)-8-(4-Hydroxyphenyl)-1-[(*E*)-3-(4-hydroxyphenyl)prop-2-enoyl][14,14,15,15,16,16,17,17-$^2H_8$]-1,5,9,13-tetraazacycloheptadecan-6-one Dihydrochloride* (**19**; 2 HCl). All steps were performed under red light to avoid (*E*) → (*Z*) photoisomerization. A soln. of (*S*)-**18** (44 mg, 0.08 mmol) in dry CH_2Cl_2 (2 ml) was cooled in a dry ice/acetone bath to −78°. A 1M CH_2Cl_2 soln. of BBr_3 (1.5 ml, 1.5 mmol) was added. The mixture was kept at −78° for 1 h, then the bath was removed, and the mixture was stirred for 16 h at r.t. The reaction was quenched by adding 3 ml of 90% aq. MeOH soln. and evaporated to dryness *in vacuo* (50°). The excess BBr_3 was removed by repetitive addition of MeOH sat. with gas. HCl and evaporation. The solid residue was washed several times with $CHCl_3$. The residue was then dissolved in $CHCl_3$/MeOH/25% aq. NH_3 soln. 7:3:0.3 and purified by CC

(consecutively, $CHCl_3$, $CHCl_3$/MeOH/25% aq. NH_3 soln. 7:3:0.3). The eluate was evaporated to dryness. The residual free base of compound (*S*)-**19** was transformed immediately to its dihydrochloride by addition of MeOH sat. with gas. HCl and evaporated to dryness: (*S*)-**19** · 2 HCl (46 mg, 92%). Colorless solid. TLC ($CHCl_3$/MeOH/ 25% aq. NH_3 soln. 7:3:1): R_f 0.5. $[\alpha]_D^{20} = +5.19$ ($c = 1.79$, MeOH). ^{1}H-NMR ((D_4)MeOH, mixture of conformers): 7.56–7.53 (*m*, 3 H, including 2*d*, PhC*H*=CHCO, at 7.55, 7.54, $J = 15$); 7.43–7.37 (*m*, 2 arom. H); 6.96, 6.92 (2*d*, PhCH=C*H*CO); 6.88–6.84 (*m*, 2 arom. H); 6.83–6.79 (*m*, 2 arom. H); 4.9, 4.89, 4.86, 4.85 (4*d*, $J = 10$, PhC*H*N); 3.82–3.6 (*m*, 1.5 H); 3.57–3.38 (*m*, 2.5 H); 3.29–3.05 (*m*, 5 H); 2.86–2.8 (*m*, 1 H); 2.52–2.4 (*m*, 1 H); 2.14–2.04 (*m*, 1 H); 2.04–1.88 (*m*, 2 H). ^{2}H-NMR (MeOH): 3.5 (br. *s*, $2\,^2$H); 3.18 (br. *s*, $2\,^2$H); 1.8 (br. *s*, $4\,^2$H). ^{13}C-NMR ((D_4)MeOH): 173.4, 169.7 (2 C=O); 160.5, 159.4, 144.5, 131.3, 131.1, 128.0, 125.9, 117.2, 116.6, 115.2 (C=C); 58.9 (Ph*C*N). ESI-MS: 503 ($[M+H]^+$).

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