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Revision of the structure of haliclorensin to (*S*)-7-methyl-1,5-diazacyclotetradecane and confirmation of the new structure by synthesis

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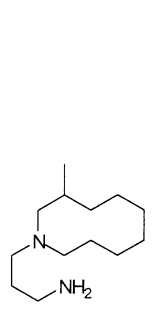
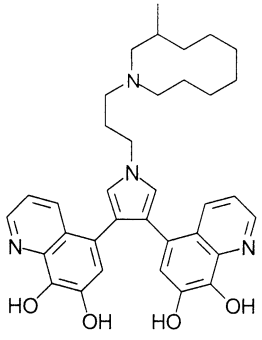
Abstract—A reinvestigation of the marine alkaloid haliclorensin led to a revision of the proposed structure to 7-methyl-1,5-diazacyclotetradecane. The new structure was confirmed by total synthesis of both optical forms. According to chiroptical measurements and GC–MS investigations, natural haliclorensin consists of a 3:1 mixture of the (*S*)- and (*R*)-enantiomers. © 2001 Published by Elsevier Science Ltd.

1. Introduction

Recently, Kashman et al. reported the isolation of two unique marine alkaloids from the South African sponge *Haliclona tulearensis*. For haliclorensin the diamine structure **1** was proposed,[1] which also forms part of the more complex pyrrole alkaloid halitulin (**2**).[2] In this communication we report on the revision of the structure of haliclorensin to that of the isomeric diamine **3**.



1



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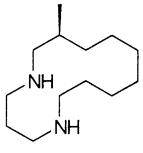

3

Recent syntheses of (*R*)- and (*S*)-**1** by Heinrich and Steglich[3] and of (±)-**1** by Banwell et al.[4] raised doubts about the proposed structure. Major discrepancies include the presence of an intense $M^+ - CH_2CH_2NH_2$ ion in the mass spectrum of synthetic **1** as well as strong deviations in the ^{13}C NMR data and the optical rotation from those of natural haliclorensin.

2. Results and discussion

Re-isolation of haliclorensin and a careful re-investigation of its HMBC spectrum led to the H–C correlations shown in Fig. 1 and Table 1. As a result, the structure of haliclorensin has to be revised to 7-methyl-1,5-diazacyclotetradecane (**3**). Structure **3** explains the pronounced solvent and pH dependency of the ^{13}C NMR data of this alkaloid[1] by the formation of (bridged) mono- and di-cations. Furthermore, on treatment with acetic anhydride, haliclorensin yields a diacetyl derivative, in accordance with structure **3**.

Structure **3** was confirmed by syntheses of the optically pure enantiomers as shown in Scheme 1. In this synthesis, a similar strategy was applied as for isomer **1**,[3] for which the name isohaliclorensin is proposed.

The synthesis of **3** starts with bis-sulfonamide **4** that was obtained in 82% yield by reaction of 1,3-diaminopropane with *o*-nitrobenzenesulfonyl chloride. Two successive couplings of **4** under Mitsunobu conditions[5] with 4-penten-1-ol and (*S*)-2-methyl-5-hexen-1-ol afforded the protected secondary diamines **5** and **6**, respectively.

Keywords: haliclorensin; alkaloids; marine metabolites; metathesis; stereochemistry.

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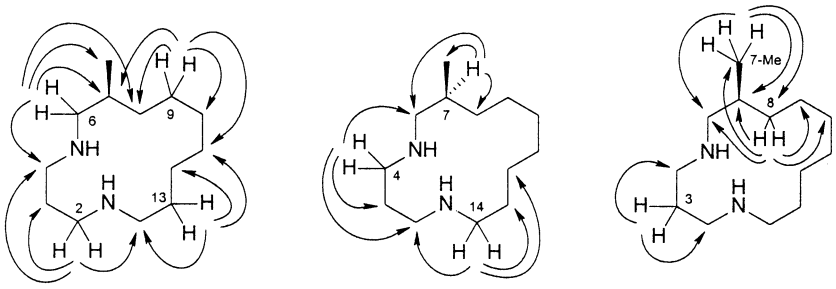


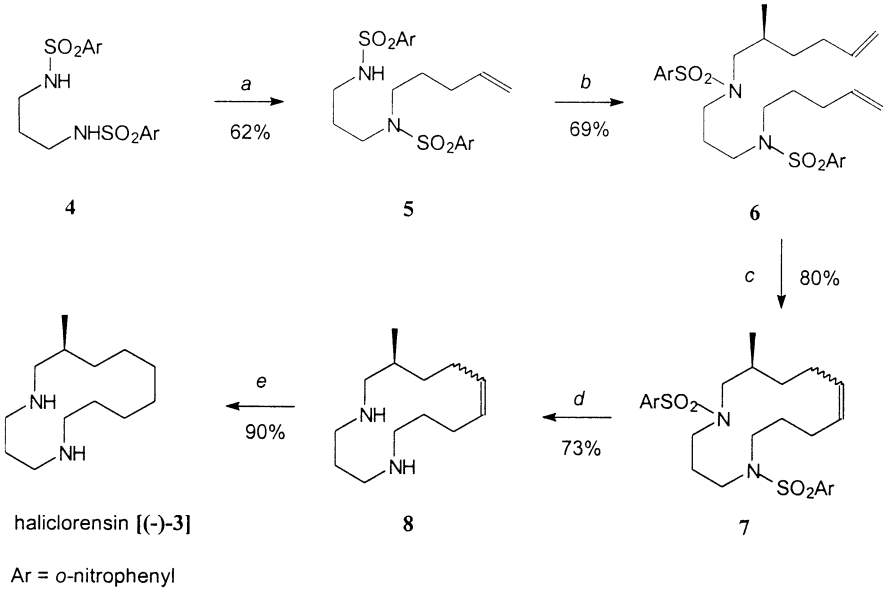
Figure 1. HMBC correlations of haliclorensin (**3**).

Table 1. NMR data of natural and synthetic haliclorensin (600 MHz, 10 mg **3** in CD_3OD+20 μL CF_3CO_2D, 286 K)

	δ_C (natural)	δ_H	Multiplicity	J (Hz)	HMBC correlations	δ_C (synthetic)
2	42.03	3.23	dd	6.6, 6.6	C-3, C-4, C-14	42.02
3	20.64	2.08	ddm	14.4, 7.0	C-2, C-4	20.82
		2.24	ddm	14.4, 7.2	C-2, C-4	
4	42.54	3.20–3.25	m		C-2, C-3, C-6	42.62
6	50.16	2.87	dd	13.0, 7.8	C-4, C-7, C-8, 7-Me	50.36
		3.13	dd	13.0, 5.5	C-4, C-7, C-8, 7-Me	
7	28.13	2.04	qm	6.6	C-6, C-8, 7-Me	28.35
8	31.96	1.31	ddm	13.5, 6.8	C-6, C-7, C-9, C-10, 7-Me	32.26
		1.52–1.56	m		C-6, C-7, C-9, C-10, 7-Me	
9	24.15	1.46–1.49	m		C-7, C-8, C-10, C-11	24.48
10	25.37	1.45–1.55	m		Assignment not possible	25.61
11	25.78	1.45–1.55	m		Assignment not possible	26.09
12	23.64	1.45–1.55	m		Assignment not possible	23.88
13	22.70	1.67	ddm	13.3, 6.6	C-11, C-12, C-14	22.88
		1.83	ddm	13.3, 6.7	C-11, C-12, C-14	
14	44.35	3.08–3.12	m		C-2, C-13, C-12	44.54
		3.17–3.20	m		C-2, C-13, C-12	
7-Me	18.12	1.05	d	6.5	C-6, C-7, C-8	18.35

(*S*)-2-Methyl-5-hexen-1-ol was synthesized by established methods[6] in five steps from commercially available methyl (*R*)-3-hydroxy-2-methylpropanoate (73% overall yield).

Ring closing metathesis of diene **6** with Grubbs' catalyst afforded diazacyclotetradecene **7** without significant formation of dimers and oligomers. In contrast, such by-products were observed during the azacyclodecene ring formation associated with the synthesis of **1**.[3] Analysis of the NMR integrals of **7** revealed a 7:3 ratio of the *E*/*Z*-isomers. Deprotection with PhSH and K_2CO_3 according to Fukuyama[7] afforded the *E*/*Z*-mixture of diazacyclotetradecene **8**. The stereochemistry of the diastereomers was determined from the ^1H NMR signals of the olefinic protons ($J_{(E)}$=15 Hz, $J_{(Z)}$=10 Hz).



Scheme 1. *Reagents and conditions*: (a) 4-penten-1-ol (1 equiv.), PPh_3 (1+0.33 equiv.), DEAD (1+0.33 equiv.), dry THF, 0°C→rt, 24 h; (b) (*S*)-2-methyl-5-hexen-1-ol (1.5 equiv.), PPh_3 (1+0.33 equiv.), DEAD (1+0.33 equiv.), dry THF, 0°C→rt, 48 h; (c) Grubbs' catalyst (0.1 equiv.), CH_2Cl_2, reflux, 24 h; (d) PhSH (2.5 equiv.), K_2CO_3 (7.5 equiv.), dry DMF, rt, 48 h; (e) Pd/C (10% Pd), 40 bar (580 psi) H_2, dry MeOH, rt, 18 h.

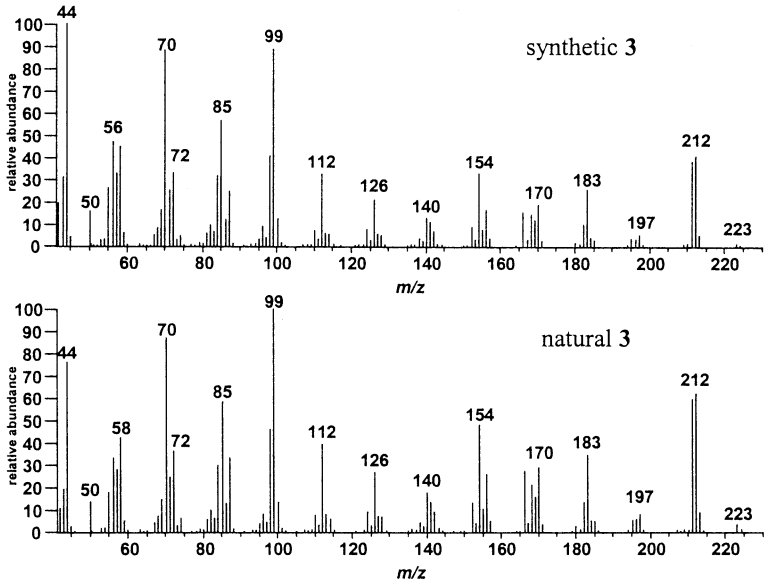
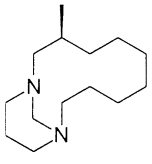


Figure 2. EIMS spectra of natural and synthetic haliclorensin (**3**).

Catalytic hydrogenation of olefin **8** over Pd/C yielded (*S*)-haliclorensin (**3**), identical with the natural product. The (*R*)-enantiomer of **3** was synthesized in the same manner from (*R*)-2-methyl-5-hexen-1-ol. The comparison of the EI-MS spectra of natural and synthetic haliclorensin (**3**) is shown in Fig. 2.

The optical rotation of haliclorensin depends strongly on the pH value. Thus, synthetic (*S*)-haliclorensin exhibits an $[\alpha]_D$-value of −18.5 (*c* 0.5, in MeOH), the (*R*)-compound +20 (*c* 2.0, in MeOH). For the natural compound a much lower rotation of −8.5 was observed. Nevertheless, this comparison indicates that the natural product has the (*S*)-configuration. In 1 M HCl the optical rotation of synthetically derived (*S*)-haliclorensin changes to +7.0. Because of the lower rotation of the natural product, additional investigations were carried out.

In order to determine the optical purity, natural haliclorensin was treated with formaldehyde to yield the methylene derivative **9**.[8] In contrast to diamine **3**, the enantiomers of **9** can be separated by GC–MS on a chiral β-cyclodextrin column. The comparison with optically pure samples of (*S*)- and (*R*)-**9** obtained from the synthetic haliclorensins, revealed the presence of a 3:1-mixture of the (*S*)- and (*R*)-enantiomers. This result is in good agreement with the lower $[\alpha]_D$-value of −8.5 observed for the natural product.



(*S*)-9

Haliclorensin (**3**) can be easily converted to its bis-dinitrophenyl derivative **10** by treatment with an excess of 2,4-dinitrofluorobenzene (Sanger's reagent).[9] The CD-spectrum of the resulting yellow oil shows an exciton coupling between the two chromophores. A comparison of the CD-spectrum of the derivative derived from natural haliclorensin with that of (*R*)-**10** prepared from synthetic (*R*)-3 supported the predominant (*S*)-configuration of the natural product (Fig. 3).

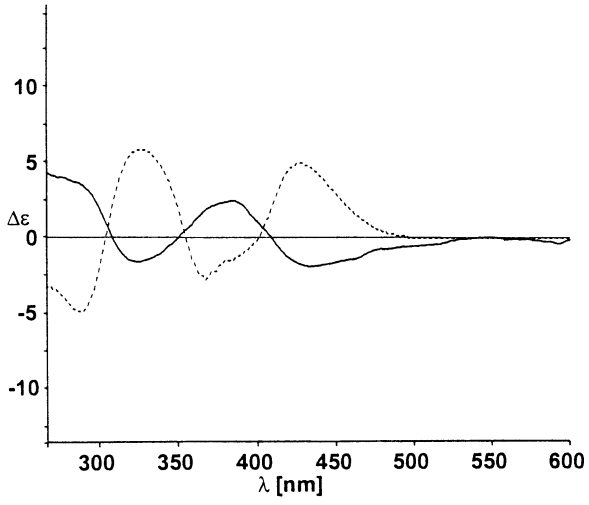
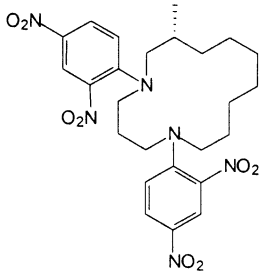
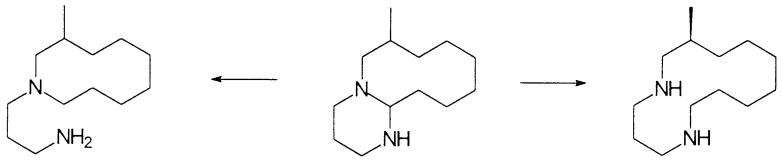


Figure 3. CD spectra of (*R*)-**10** (- - -) and the same derivative derived from natural **3** (—) (in acetonitrile).



(*R*)-10



1 11 3

Scheme 2. Proposed common intermediate **11** for the biosynthesis of **1** and **3**.

Haliclorensin (**3**) and its synthetic isomer **1** (isohaliclorensin) can be derived hypothetically from the same intermediate **11**, which might explain the common occurrence of **3** and halitulin (**2**) in *H. tulearensis* (Scheme 2). The synthesis of **11** is under active investigation.

3. Experimental

3.1. General

Silica gel 60 230–400 mesh (Merck) and Sephadex LH-20 (Pharmacia) were used for chromatography. R_f values were determined on silica gel 60 F_{254} TLC plates (Merck). Petroleum ether refers to the fractions with bp 40–60°C. ^{1}H and ^{13}C NMR spectra were recorded on Bruker ARX 300 and Bruker AMX 600 instruments. ^{1}H and ^{13}C chemical shifts are given with respect to the solvent as internal standard. IR spectra were measured on a Perkin–Elmer FT 1000. C, H, N and S analyses were performed by the microanalytical laboratory of the Chemistry Department, LMU, München. Mass spectra were recorded with a Finnigan MAT 90 sector field mass spectrometer at 70 eV. For GC–MS a Varian GC 3400 gas chromatograph with a fused silica DB-5ms (Macherey–Nagel) capillary column (30 m×0.25 mm, coated with a 0.25 μm layer of liquid phase) or a β-DEX 120 (Supelco) capillary column (25 m× 0.25 mm, coated with a 0.25 μm layer of liquid phase) and helium as carrier gas was used for sample separation. The injector temperature was kept at 300°C (column DB-5ms) 220°C (column β-DEX 120), respectively, injection volumes were 0.1–0.2 μL of a 1–2% (m/v) solution. Temperature programmes: Column DB-5ms: 2 min isothermal at 50°C, then 10 K/min up to 300°C, finally 10 min isothermal at 300°C; Column β-DEX 120: 2 min isothermal at 150°C, then 2 K/min up to 200°C, finally 10 min isothermal at 200°C. Retention indices[10] R_i were determined by co-injection of a 0.2 μL sample of a standard mixture of saturated straight chain alkanes (C_{10}–C_{36}). Optical rotations were determined on a Perkin–Elmer 241 polarimeter. For removal of oxygen, nitrogen was bubbled through the dichloromethane prior to its use in the RCM reaction.

3.1.1. *N*-[3-(2-Nitrobenzenesulfonyl)amino-propyl]-2-nitrobenzenesulfonamide (4). To a stirred suspension of 2-nitrobenzenesulfonyl chloride (4.16 g, 18.7 mmol) and potassium carbonate (2.59 g, 18.7 mmol) in dry THF (40 mL) was added, dropwise at 0°C, 1,3-diaminopropane (0.78 mL, 9.35 mmol). The resultant mixture was heated under reflux for 24 h, and after evaporation of the solvent, the residue was partitioned between CH_2Cl_2 (50 mL) and 2 M HCl (50 mL). The organic phase was washed with saturated aqueous sodium bicarbonate and water. Evaporation of the solvent and trituration of the resulting crude oil with Et_2O yielded pure **4** (3.40 g, 82%) as a colorless solid: mp 117–119°C; R_f 0.3 (EtOAc–petroleum ether, 1:1 v/v); ^{1}H NMR (300 MHz, [D_6]acetone): δ 1.83 (tt, *J*=6.7, 6.7 Hz, 2H), 3.21 (dt, *J*=6.7, 6.7 Hz, 4H), 6.66 (t, *J*=6.7 Hz, 2NH), 7.86–7.98 (m, 6H), 8.08–8.14 (m, 2H); ^{13}C NMR (75.5 MHz, [D_6]acetone): δ 30.3 (CH_2), 41.0 (2×CH_2), 125.3 (2×CH), 130.8 (2×CH), 133.0 (2×CH), 133.8 (2×C_q), 134.3 (2×CH), 148.6 (2×C_q); MS (ESI) *m/z*: 445.1 $[M+H]^+$; HRMS (ESI) calcd for $C_{15}H_{16}N_4O_8S_2$+Na $[M^+$+Na] 467.0307, found 467.0333; Anal. calcd C, 40.54; H, 3.63; N, 12.61; S, 14.43; found C, 40.44; H, 3.63; N, 12.54; S, 14.50.

3.1.2. *N*-{3-[(2-Nitrobenzenesulfonyl)pent-4-enylamino]-propyl}-2-nitrobenzenesulfonamide (5). A solution of 4-penten-1-ol (0.78 mL, 7.65 mmol), **4** (3.40 g, 7.65 mmol), and triphenylphosphine (2.01 g, 7.65 mmol) in dry THF (60 mL) was cooled to 0°C under an argon atmosphere. After the dropwise addition of diethyl azodicarboxylate (1.19 mL, 7.65 mmol), the ice-bath was removed and the mixture stirred at room temperature. After 8 h, additional triphenylphosphine (0.67 g, 2.55 mmol) and diethyl azodicarboxylate (0.44 g, 2.55 mmol) were added, and the stirring was continued for 16 h. The solvent was removed under reduced pressure and the residue chromatographed on silica gel with CH_2Cl_2–acetone, 20:1 v/v, to yield **5** (2.43 g, 62%) as a clear yellow oil: R_f 0.7 (CH_2Cl_2–acetone, 20:1 v/v); ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.57 (tt, *J*=7.4, 7.4 Hz, 2H), 1.81 (tt, *J*=6.7, 6.7 Hz, 2H), 1.97 (dt, *J*=7.4, 7.4 Hz, 2H), 3.14 (dt, *J*=6.7, 6.7 Hz, 2H), 3.23 (t, *J*=7.4 Hz, 2H), 3.36 (t, *J*=6.7 Hz, 2H), 4.91–4.99 (m, 2H), 5.61–5.75 (m, 1H); 5.68–5.73 (m, NH), 7.58–7.62 (m, 1H), 7.66–7.74 (m, 4H), 7.81–7.85 (m, 1H), 7.95–7.99 (m, 1H), 8.06–8.10 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$): 27.3 (CH_2), 28.8 (CH_2), 30.5 (CH_2), 40.6 (CH_2), 44.8 (CH_2), 47.3 (CH_2), 115.5 (CH_2), 124.2 (CH), 125.3 (CH), 130.7 (CH), 130.8 (CH), 131.8 (CH), 132.8 (CH), 133.0 (C_q), 133.5 (C_q), 133.59 (CH), 133.64 (CH), 136.9 (CH), 147.9 (2×C_q); MS (EI) *m/z* (rel. int.): 457 (2, $M^+-C_4H_7$), 326 (37, $M^+-C_6H_4NO_4S$), 186 (100, $C_6H_4NO_4S^+$); MS (FAB) *m/z*: 513 $[M^+$+H]; HRMS (ESI) calcd for $C_{20}H_{24}N_4O_8S_2$+H $[M^+$+H] 513.1114, found 513.1108; Anal. calcd C, 46.87; H, 4.72; N, 10.93; S, 12.51; found C, 47.36; H, 5.06; N, 10.59; S, 12,05.

3.1.3. (*S*)-*N*-{3-[(2-Nitrobenzenesulfonyl)-(2-methylhex-5-enyl)amino]propyl}-*N*-(pent-4-enyl)-2-nitrobenzenesulfonamide (6). A solution of (*S*)-2-methylhex-5-en-1-ol[6] (115 mg, 1.01 mmol), **5** (345 mg, 0.67 mmol) and triphenylphosphine (177 mg, 0.67 mmol) in dry THF (10 mL) was cooled to 0°C. After the dropwise addition of diethyl azodicarboxylate (105 μL, 0.67 mmol) the ice-bath was

removed and the mixture stirred at room temperature for 48 h. After ca. 24 h, additional triphenylphosphine (60 mg, 0.23 mmol) and diethyl azodicarboxylate (40 mg, 0.23 mmol) were added. The solvent was then removed under reduced pressure and the residue chromatographed on silica gel (EtOAc–petroleum ether, 1:1 v/v) to afford **6** (280 mg, 69%) as a light yellow foam: R_f 0.8 (EtOAc–petroleum ether, 1:1 v/v); $[\alpha]_D=-5.6$ (*c* 0.8, MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.82 (d, J=6.6 Hz, 3H), 1.05–1.17 (m, 1H), 1.35–1.47 (m, 1H), 1.57 (tt, J=7.6, 7.6 Hz, 2H), 1.64–1.75 (m, 1H), 1.76–1.86 (m, 2H), 1.93–2.02 (m, 3H), 2.05–2.15 (m, 1H), 3.05–3.18 (m, 2H), 3.20–3.27 (m, 6H), 4.89–5.01 (m, 4H), 5.62–5.78 (m, 2H), 7.57–7.61 (m, 2H), 7.65–7.71 (m, 4H), 7.95–7.99 (m, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$): 16.8 (CH_3), 27.0 (CH_2), 27.1 (CH_2), 30.47 (CH_2), 30.49 (CH), 30.8 (CH_2), 32.9 (CH_2), 44.9 (2×CH_2), 47.1 (CH_2), 53.8 (CH_2), 114.8 (CH_2), 115.5 (CH_2), 124.1 (2×CH), 130.7 (CH), 130.8 (CH), 131.69 (CH), 131.72 (CH), 133.0 (2×C_q), 133.51 (CH), 133.55 (CH), 137.0 (CH), 138.2 (CH), 147.9 (2×C_q); MS (EI) *m/z* (rel. int.): 608 (0.3, M^+), 525 (32, $M^+-C_6H_{11}$), 422 (37, $M^+-C_6H_4NO_4S$), 229 (72), 186 (100, $C_6H_4NO_4S^+$); HRMS (ESI) calcd for $C_{27}H_{36}N_4O_8S_2$ [M^+] 608.1974, found 608.1964; Anal. calcd C, 53.27; H, 5.96; N, 9.20; S, 10.54; found C, 53.07; H, 6.03; N, 9.06; S, 10.43.

3.1.4. (*S*)-1,5-Bis(2-nitrobenzenesulfonyl)-13-methyl-1,5-diazacyclotetradec-9-ene (7) (mixture of *E*/*Z*-isomers). To dry refluxing CH_2Cl_2 (500 mL) under argon were added Grubbs' catalyst (24 mg, 0.029 mmol) and a solution of **6** (175 mg, 0.29 mmol) in dry CH_2Cl_2 (5 mL). The resulting mixture was refluxed for 24 h, then cooled to room temperature and filtered over Celite. After evaporation of the solvent, the residue was purified by chromatography (EtOAc–petroleum ether, 1:1 v/v) to afford **7** (34 mg, 80%) as a 7:3 mixture of the *E*/*Z* isomers (NMR analysis): colorless oil, R_f 0.65 (EtOAc–petroleum ether, 1:1 v/v); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.77^E and 0.87^Z (2×d, J=6.5, 6.6 Hz, 3H), 1.32–1.57 (m, 4H), 1.57–1.97 (m, 3H), 2.00–2.15 (m, 4H), 2.80–3.56 (m, 8H), 5.34–5.38 and 5.42–5.47^E (2×m, 2H), 7.56–7.60 and 7.65–7.70 (2×m, 6H), 7.86–7.92 and 7.96–8.00 (2×m, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$): 17.2^E (CH_3), 18.2^Z (CH_3), 24.0^Z (CH_2), 24.8^E (CH_2), 24.9^E (CH), 25.1^Z (CH_2), 28.0^E (CH), 28.6^E (CH_2), 28.9^E (CH_2), 29.1^Z (CH_2), 29.5^Z (CH_2), 31.6^Z (CH), 33.0^E (CH_2), 33.9^Z (CH_2), 42.6^E (CH_2), 43.4^E (CH_2), 44.3^E (CH_2), 46.5^Z (CH_2), 46.7^Z (CH_2), 49.5^Z (CH_2), 53.2^E (CH_2), 56.0^Z (CH_2), 124.02^Z (CH), 124.06^E (CH), 124.10^Z (CH), 124.15^E (CH), 128.9^Z (CH), 130.18^Z (CH), 130.22^E (CH), 130.50^E (CH), 130.63^Z (CH), 130.67^E (CH), 130.7^Z (CH), 131.57^Z (CH), 131.61^Z (CH), 131.66^E (CH), 131.73^Z (CH), 131.78^E (CH), 131.85^Z (C_q), 132.3^Z (C_q), 132.4^E (CH), 132.8^E (C_q), 132.9^E (C_q), 133.5^E (CH), 133.6^Z (CH), 133.7^E (CH), 147.7^E (C_q), 147.9^E (C_q), 148.2^Z (C_q), 148.3^Z (C_q); MS (EI) *m/z* (rel. int.): 580 (0.02, M^+), 563 (4), 395 (22), 394 (100, $M^+-C_6H_4NO_4S$), 209 (31), 208 (5, $M^+-2\times C_6H_4NO_4S$), 207 (23), 186 (23, $C_6H_4NO_4S^+$); HRMS (EI) calcd for $C_{25}H_{33}N_4O_8S_2$ [M^++H] 581.1739, found 581.1785; Anal. calcd ($C_{25}H_{32}N_4O_8S_2\times H_2O$) C, 50.15; H, 5.72; N, 9.36; S, 10.71; found C, 50.01; H, 5.45; N, 9.19; S, 10.43.

3.1.5. (*S*)-13-Methyl-1,5-diazacyclotetradec-9-ene (8) (mixture of *E*/*Z* isomers). A magnetically stirred solution of **7** (150 mg, 0.26 mmol) in dry DMF (5 mL), maintained under argon, was treated with potassium carbonate (270 mg, 1.96 mmol) and thiophenol (66 μL, 0.65 mmol). The resulting reaction mixture was stirred at room temperature for 48 h. After addition of water (15 mL) and 2 M NaOH (5 mL), the aqueous phase was extracted with $CHCl_3$ (3×15 mL). The combined organic layers were extracted with 2 M HCl (3×5 mL) and the aqueous phase adjusted to alkaline pH by adding 2 M NaOH which resulted in partial precipitation of the product. It was extracted with $CHCl_3$, washed with 2 M NaOH (2×10 mL) and dried over sodium sulfate. Evaporation of the solvent yielded pure **8** (40 mg, 73%) as a 7:3 mixture of *E*/*Z* isomers as judged by NMR analysis: colorless oil; ^{1}H NMR (600 MHz, $CDCl_3$): δ 0.85^E and 0.87^Z (2×d, J=6.7, 6.9 Hz, 3H), 1.16–1.55 (m, 3H), 1.56–1.71 (m, 4H), 1.72–1.94 (m, 3H), 1.94–2.16 (m, 3H), 2.22–2.75 (m, 8H), 5.23^Z (dt, br., J=10, 8 Hz, 0.3H), 5.40^E (dt, J=15, 7 Hz, 0.7H), 5.47^Z (dt, br., J=10, 8 Hz, 0.3H), 5.52^E (dt, J=15, 7 Hz, 0.7H); ^{13}C NMR (151 MHz, $CDCl_3$): 18.3^Z (CH_3), 18.5^E (CH_3), 22.1^Z (CH_2), 23.2^Z (CH_2), 27.87^E (CH_2), 27.88^E (CH_2), 28.3^Z (CH_2), 28.9^E (CH_2), 29.1^Z (CH_2), 29.2^E (CH), 30.4^E (CH_2), 30.8^Z (CH), 33.1^Z (CH_2), 33.4^E (CH_2), 45.1^E (CH_2), 47.25^Z (CH_2), 47.31^E (CH_2), 47.4^E (CH_2), 48.7^Z (CH_2) 49.3^Z (CH_2), 53.7^Z (CH_2), 54.5^E (CH_2), 129.0^Z (CH), 130.4^E (CH) 130.8^Z (CH) 131.7^E (CH); GC–MS (EI) *m/z* (rel. int.): 211 (16, M^++H), 210 (5, M^+), 181 (12), 167 (25), 152 (33), 139 (32), 124 (24), 112 (26), 110 (33), 99 (38), 84 (40), 70 (69), 58 (43), 44 (100); HRMS (EI) calcd for $C_{13}H_{26}N_2$ [M^+] 210.2096, found 210.2092.

3.1.6. (*S*)-7-Methyl-1,5-diazacyclotetradecane, (*S*)-(−)-haliclorensin (3). Palladium on activated charcoal (20 mg, 10% Pd) was added to a solution of **8** (25 mg, 0.12 mmol) in dry MeOH (5 mL). Hydrogenation was carried out at 40 bar (580 psi) hydrogen pressure for 18 h. The catalyst was removed by filtration and the solvent evaporated under reduced pressure to yield pure (−)-haliclorensin [(−)-**3**] (23 mg, 90%) as a colorless oil: $[\alpha]_D=-18.5$ (*c* 0.6, MeOH), after removal of traces of acids by washing the probe with 2 M NaOH; IR (film): 3306w, 2927s, 2860m, 2804m, 1678w, 1460m, 1129w; ^{1}H NMR (600 MHz, CD_3OD): δ 0.94 (d, J=7.0 Hz, 3H), 1.25–1.31 (m, 1H), 1.32–1.55 (m, 9H), 1.56–1.63 (m, 2H), 1.71–1.80 (m, 3H), 2.42 (dd, J=11.8, 9.7 Hz, 1H), 2.57 (dd, J=11.8, 3.8 Hz, 1H), 2.64 (ddd, J=11.1, 7.6, 3.5 Hz, 1H), 2.66–2.70 (m, 2H), 2.72–2.75 (m, 2H), 2.84 (ddd, J=11.1, 7.0, 3.5 Hz, 1H); ^{13}C NMR (100 MHz, CD_3OD): 19.1 (CH_3), 22.3 (CH_2), 23.5 (CH_2), 24.9 (CH_2), 27.6 (CH_2), 28.0 (CH_2), 29.6 (CH_2), 30.8 (CH_2), 33.0 (CH), 47.8 (CH_2), 50.1 (CH_2), 50.8 (CH_2), 55.9 (CH_2); GC–MS (column DB-5ms): R_i 1711; (EI) *m/z* (rel. int.): see Fig. 1. HRMS (EI) calcd for $C_{13}H_{28}N_2$ [M^+] 212.2253, found 212.2245.

3.1.7. (*R*)-7-Methyl-1,5-diazacyclotetradecane, (*R*)-(+)-haliclorensin (3). The compound was prepared by the same synthetic steps by using (*R*)-2-methylhex-5-en-1-ol[6] instead of the (*S*)-isomer: $[\alpha]_D=+20.0$ (*c* 2.0, MeOH).

3.1.8. Natural haliclorensin (3). NMR, see data for (*S*)-(−)-haliclorensin (**3**); GC–MS (column DB-5ms): R_i 1711; (EI) *m/z* (rel. int.): see Fig. 1; HRMS (EI) calcd for $C_{13}H_{28}N_2$ [M^+] 212.2253, found 212.2248.

3.2. Determination of the (*R*)/(*S*)-ratio of natural haliclorensin after conversion into the 3-methyl-1,11-diazabicyclo[9.3.1]pentadecane derivative (9)

Formaldehyde (20 μL of a 37% aq. solution) was added to a solution of natural **3** (100 μg) in $CHCl_3$ (100 μL) and incubated for 30 min at 25°C before injection (0.1 μL) of this solution in the GC–MS. GC–MS (column: β-DEX 120): (*S*)-**9**: R_i 1800 (rel. int.: 75%); (*R*)-**9**: R_i 1803 (rel int.: 25%); (EI) *m*/*z* (rel. int.): 224 (42), 223 (100), 209 (5), 182 (5), 180 (14), 168 (7), 166 (10), 139 (7), 138 (7), 124 (7), 112 (7), 110 (7), 99 (13), 98 (9), 96 (5), 84 (6), 82 (5), 70 (15), 58 (8), 56 (5), 44 (24).

3.2.1. (*R*)-1,5-Bis(2,4-dinitrophenyl)-7-methyl-1,5-diazacyclotetradecane (10). To a solution of (*R*)-**3** (10 mg, 0.047 mmol) in dry CH_2Cl_2 (2 mL) under argon were added 2,4-dinitrofluorobenzene (15 μL, 22 mg, 0.12 mmol) and triethylamine (20 μL, 0.14 mmol). After the resulting mixture had been stirred at room temperature for 18 h, the solvent was evaporated. The residue was purified by chromatography (EtOAc–petroleum ether, 1:3 v/v) to yield **10** (20 mg, 0.037 mmol, 78%) as a clear yellow oil. R_f 0.3 (EtOAc–petroleum ether, 1:3 v/v); UV λ_{max} (CH_3CN) nm (log ε): 363 (4.31), 389 (4.28); CD λ (CH_3CN) nm ($\Delta\varepsilon$): 286 (−4.68), 304 (0.00), 326 (6.01), 355 (0.00), 366 (−2.31), 400 (0.00), 428 (5.03); ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.88 (d, J=6.6 Hz, 3H), 1.30–1.55 (m, 10H), 1.64–1.74 (m, 2H), 1.98–2.12 (m, 2H), 2.17–2.33 (m, 1H), 2.88 (dd, J=13.9, 8.2 Hz, 1H), 3.07–3.30 (m, 4H), 3.32–3.46 (m, 3H), 7.10 (d, J=9.4 Hz, 1H), 7.13 (d, J=9.4 Hz, 1H), 8.20 (dd, J=9.4, 2.8 Hz, 1H), 8.22 (dd, J=9.4, 2.8 Hz, 1H), 8.59 (d, J=2.8 Hz, 1H), 8.65 (d, J=2.8 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$): 17.9 (CH_3), 22.8 (CH_2), 23.8 (CH_2), 24.4 (CH_2), 25.0 (CH_2), 25.2 (CH_2), 25.6 (CH_2), 28.8 (CH), 31.8 (CH_2), 49.7 (CH_2), 49.9 (CH_2), 51.1 (CH_2), 56.2 (CH_2), 119.2 (CH), 120.0 (CH), 123.5 (CH), 123.8 (CH), 127.7 (CH), 127.9 (CH), 137.7 (C_q), 137.9 (C_q), 138.2 (C_q), 138.3 (C_q), 148.7 (C_q), 150.2 (C_q); MS (EI) *m*/*z* (rel. int.): 544 (8, M^+), 527 (9), 497 (8), 288 (29), 210 (57), 184 (30), 180 (100), 164 (38), 149 (62), 55 (48); HRMS (EI) calcd for $C_{25}H_{32}N_6O_8$ [M^+] 544.2282, found 544.2273.

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