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Absolute Configuration of the Solenopsins, Venom Alkaloids of the Fire Ants.

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Abstract. An effective and practical procedure has been developed that allows the assignment of the absolute configuration of solenopsins from diverse origins using only small amounts of material. The method is based on the transformation of the natural secondary amines into diastereoisomeric amides by reaction with (R)-2-methoxy-2-phenyl-2-(trifluoromethyl)acetic acid chloride (MTPA-Cl), followed by comparison of their chromatographic behaviour with those of standards of established absolute configuration. This procedure has been applied to three samples of ants: ***Solenopsis geminata*** workers, ***S. invicta*** workers and ***S. invicta*** alates. It has been found that the absolute configuration of the trans alkaloids is always (2R,6R) while that of the cis alkaloids is (2R,6S). Moreover, a new synthesis of enantiomerically pure solenopsin A (4) and isosolenopsin A (3) starting from L-alanine is presented.

Ants of the genus *Solenopsis* (Myrmicinae) secrete a venom consisting of a complex mixture of 2-methyl-6-alkylpiperidines accompanied in some cases by N-methylated, Δ^1 or side chain unsaturated derivatives[1-3]. These piperidine alkaloids have been assigned the trivial name solenopsins [1 to 12] and differ from each other by the relative configuration of their substituents, the length and unsaturation of the alkyl chain. Their relative proportions in the venom may differ between castes within a species, as well as between individuals of a particular caste[4]. The biological significance of these differences is still conjectural. However, the venom composition of pooled samples from populations of the same species from separated areas are uniform. These pooled samples show considerable interspecific variations[5,6]. It follows that the venom composition of this group of ants can be useful taxonomically and when considered with morphological characters might help clarify the taxonomic situation in this important genus[7,8].

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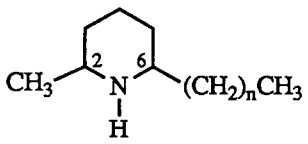
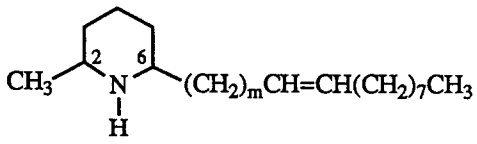




1 n = 8, cis
2 n = 8, trans
3 n = 10, cis (isosolenopsin A)
4 n = 10, trans (solenopsin A)
5 n = 12, cis (isosolenopsin B)
6 n = 12, trans (solenopsin B)
7 n = 14, cis (isosolenopsin C)
8 n = 14, trans (solenopsin C)

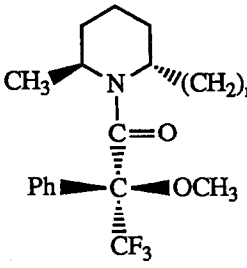
9 m = 3, cis
10 m = 3, trans
11 m = 5, cis
12 m = 5, trans

In this context, it is interesting to compare and determine the intra- and/or interspecific variation of the absolute configuration of solenopsins from different species as well as from different castes within a species. Moreover, it would be of great biogenetic significance to know at which carbon atom (C-2 or C-6) the configuration is reversed, when passing from the trans series to the cis series or the converse. Until now, the absolute configuration of only one natural solenopsin [(R,R)-solenopsin B from *S. invicta*[9]] has been determined. Thus, we have developed an effective and practical procedure allowing the assignment of the absolute configuration of solenopsins from diverse origins from only small amounts of material. The method is based on the transformation of the natural secondary amines into diastereoisomeric amides by reaction with (R)-2-methoxy-2-phenyl-2-(trifluoromethyl)acetic acid chloride [(R)-MTPA-Cl], followed by comparison of their chromatographic behaviour with those of standards with established absolute configuration.

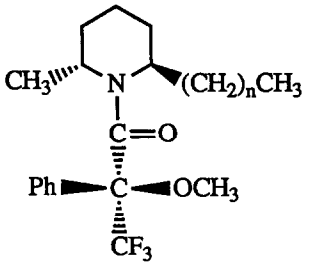
Samples of racemic solenopsin A [**4**] and B [**6**] as well as of isosolenopsin A [**3**] and B [**5**] were prepared according to the procedure described by MacConnell *et al*[10], starting from 2,6-dimethylpyridine. Alkylation of the lithium salt of 2,6-dimethylpyridine with 1-bromodecane or 1-bromododecane followed by reduction of the pyridine ring with Na in absolute ethanol yielded a 70/30 mixture of **3** and **4** and of **5** and **6**, respectively. The cis and trans derivatives were readily separated by chromatography on alumina and identified on the basis of the chemical shift of the carbon atoms α to the nitrogen[11]. Next, the four racemic compounds were separately N-acylated with an excess of (R)-MTPA-Cl prepared from the corresponding (S)-acid as reported by Ward and Rhee[12]. The eight amides (**13-20**) could be cleanly separated from the resulting diastereoisomeric mixtures by HPLC and were fully characterized (see experimental section).

In the ^{1}H NMR spectra of these amides, most of the protons appeared as either two signals or a single broad signal, indicating that they exist as a mixture of two slowly interconverting conformers resulting from restriction of the rotation around the amide bond. In particular, two doublets (J=7 Hz), accounting together for 3H, and attributable to the secondary methyl group of each conformer, could be easily identified in the spectrum of all amides (table 1). In the ^{1}H NMR spectra of compounds **14**, **16**, **18** and **20**, one of these

doublets appears at unusual high field (δ ~ 0.2), leading to chemical-shift differences of about 1 ppm between the two doublets. This must reflect a crucial differential interaction of the anisotropic phenyl group of the MTPA moiety with the methyl groups of the two conformers.



13 n = 10
15 n = 12



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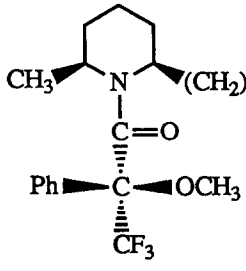
16 n = 12



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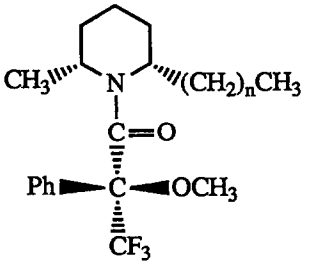
19 n = 12



18 n = 10
20 n = 12

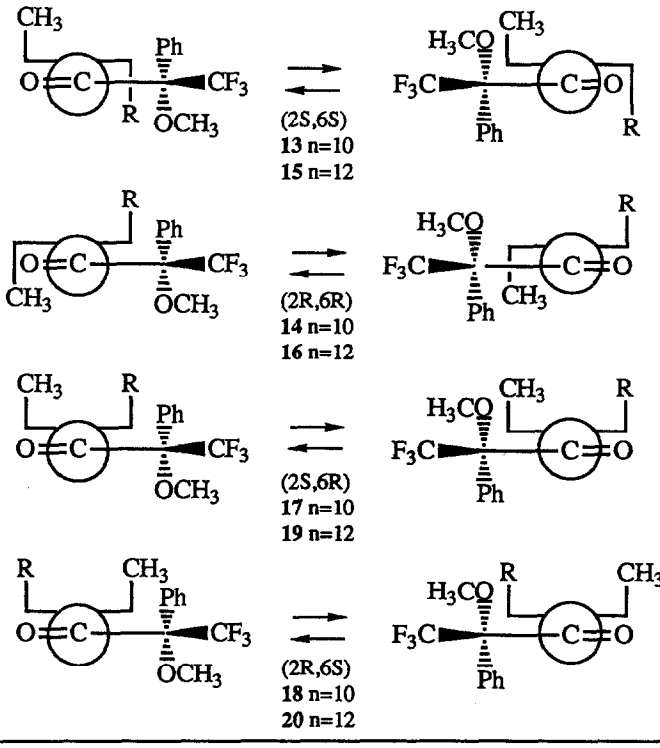
Amide	δ_1	δ_2	$\Delta\delta = \delta_1 - \delta_2$	Absolute configuration assignments
13	1.45	1.12	0.33	(2S, 6S)
14	1.43	0.19	1.24	(2R, 6R)
15	1.53	1.20	0.33	(2S, 6S)
16	1.44	0.19	1.25	(2R, 6R)
17	1.17	1.14	0.03	(2S, 6R)
18	1.17	0.19	0.98	(2R, 6S)
19	1.24	1.22	0.02	(2S, 6R)
20	1.21	0.26	0.95	(2R, 6S)

Table 1 : Chemical shifts of the secondary methyl group in the two conformers of amides **13** to **20**.

It has been proposed by Dale and Mosher[13] and later confirmed by X-ray studies[14] that the preferred conformation of the MTPA moiety in such derivatives is the one where the trifluoromethyl group lies in the same plane and is s-cis to the carbonyl. Moreover, cis-2,6-disubstituted N-acylpiperidines exhibit a flattened chair conformation in which the substituents are axial to avoid contact between the planar amide group and the C-2 and C-6 substituents which would occur if the latter were equatorial ($A^{1,3}$ strain)[15].

Since the atoms C-2, C-6 and N, as well as C_α, the carbonyl and the CF_3 group of the MTPA are in the same plane, the four diastereoisomers of each homologous group of amides can be represented using Newman projections as depicted in figure 1. This representation also takes into account the conformational

Figure 1 : Newman projections of amides **13** to **20** [R = $(CH_2)_nCH_3$].



equilibrium resulting from rotation restrictions around the amide bond. From these drawings it is clear that only amides having the (2R,6S) or the (2R,6R) configuration have one of the amide conformations where the secondary methyl group is juxtaposed with the phenyl group. This allowed us to propose the (2R,6R)

configuration for **14** and **16** and the (2R,6S) configuration for **18** and **20**. Further NMR data are in agreement with these assignments. For example, strongly shielded signals around 0 ppm, attributable to the H_2C-7 protons, are exclusively found in the (2S,6R) and (2R,6R) amides where this methylene is precisely juxtaposed with the phenyl group in one of the conformations.

These absolute configuration assignments have been confirmed by direct comparison with synthetic samples of (2S,6S)-solenopsin A [**4**] and (2S,6R)-isosolenopsin A [**3**] which have been prepared using the original synthesis described in this paper. Their corresponding (S)-MTPA amides present chromatographic behaviours and spectral data (MS, IR, ^1H NMR) identical to those of **13** and **17**, respectively.

The (S)-MTPA amides of the solenopsins can be cleanly separated by reverse-phase HPLC (RP18 - CH_3CN) according to the length of their alkyl chain. Moreover, for a given alkyl chain length, the four diastereoisomers can be cleanly resolved by adsorption HPLC (SiO_2 - hexane/THF 95:5). The relative retention volumes of the eight amides were measured using (-)-menthol-(S)-MTPA as internal reference and are reported in the experimental section. It has been shown for several series of homologous organic compounds that the logarithm of the retention volume versus the number of carbon atoms yields a good linear regression[16]. Taking this into account, four straight lines corresponding to the four series of homologous stereoisomers of the (S)-MTPA solenopsins could be drawn. The resulting diagram can be used to determine both relative and absolute configurations of any natural solenopsin isolated and transformed into its (S)-MTPA amide. To this end, the following general procedure was developed. About 500 ants were exhaustively extracted with methanol / dichloromethane. Silica gel flash chromatography of the extract, monitored by tlc (Dragendorff reagent), furnished a crude alkaloid fraction that was catalytically hydrogenated to reduce possible unsaturated derivatives into the corresponding saturated compounds. The alkaloids were precipitated from diethylether as their hydrochloride, then recrystallized from acetone and transformed into the (S)-MTPA amides. The latter were resolved by semi preparative reverse-phase HPLC according to the length of the alkyl chain. Thereafter, each isolated homologous group of amides was analysed by HPLC on a silica gel column and the diastereoisomers present in the mixtures identified using the diagram obtained as described above.

This procedure was applied to three samples of ants: *S. geminata* workers, *S. invicta* workers and *S. invicta* alates. Their venom alkaloid content is reported in table 2. Following these data the absolute configuration of the trans alkaloids is always (2R,6R) while that of the cis alkaloids is (2R,6S). Consequently, the absolute configuration at C-2 is maintained when going from one series of derivatives to the other. This could be a consequence of the way the alkaloids are biosynthesized. For example, if an intermediate such as the (2R)-amino alkanone pictured in figure 2 is involved, its cyclization might lead to the corresponding imine that can be reduced to either cis - or trans - 2,6-dialkylpiperidines. This supposes that the stereospecificity of the enzyme performing the amination step remains constant. Incorporation experiments of labeled compounds are currently underway in our laboratory to solve these problems.

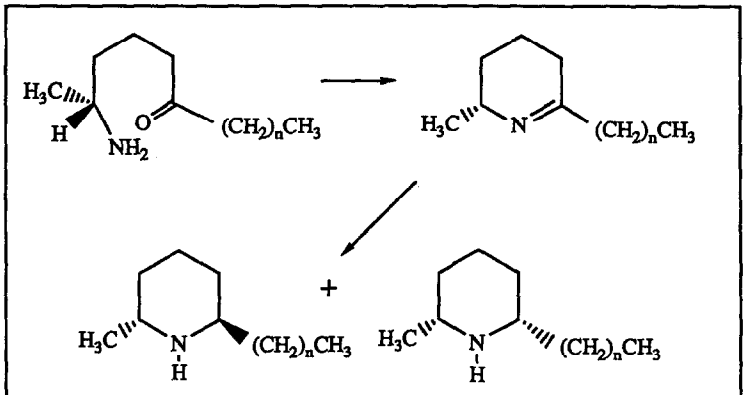


Figure 2 : Biogenetic hypothesis for the formation of the solenopsins

Ant samples	Alkaloids identified	Relative percentage
S. geminata workers	(2R,6R)-solenopsin A (4)	67%
	(2R,6S)-isosolenopsin A (3)	33%
S. invicta workers	(2R,6R)-solenopsin B (6)	43%
	(2R,6S)-isosolenopsin B (5)	2%
	(2R,6R)-solenopsin C (8)	54%
	(2R,6S)-isosolenopsin C (7)	1%
S. invicta alates	(2R,6R)-solenopsin A (4)	26%
	(2R,6S)-isosolenopsin A (3)	62%
	(2R,6R)-solenopsin B (6)	5%
	(2R,6S)-isosolenopsin B (5)	7%

Table 2 : Alkaloid contents of some *Solenopsis* venoms.

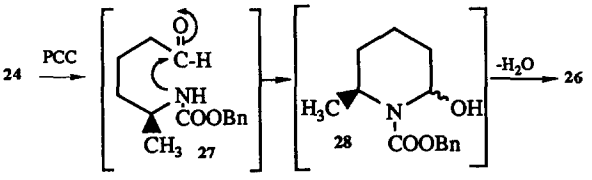
So far, six asymmetric syntheses of solenopsins have been reported[9,17-21]. We have developed a further synthesis of enantiomerically pure solenopsins by exploiting the innate chirality of the readily available amino acid L-alanine. The synthetic sequence is summarized in scheme 1.

L-alanine was first transformed in four steps into the iodocarbamate **21** using the reaction sequence reported by Schlessinger and Iwanowicz[22]. It should be pointed out that despite several trials we were unable to reproduce the high overall yield (78%) reported by the authors. In our hands, overall yields between 50 and 55% were repetitively obtained. The coupling of **21** with the organomagnesium bromide **22**, prepared from 3-bromopropan-1-ol, gave carbamate **23** in 55% yield. The tetrahydropyranyl group was cleaved with

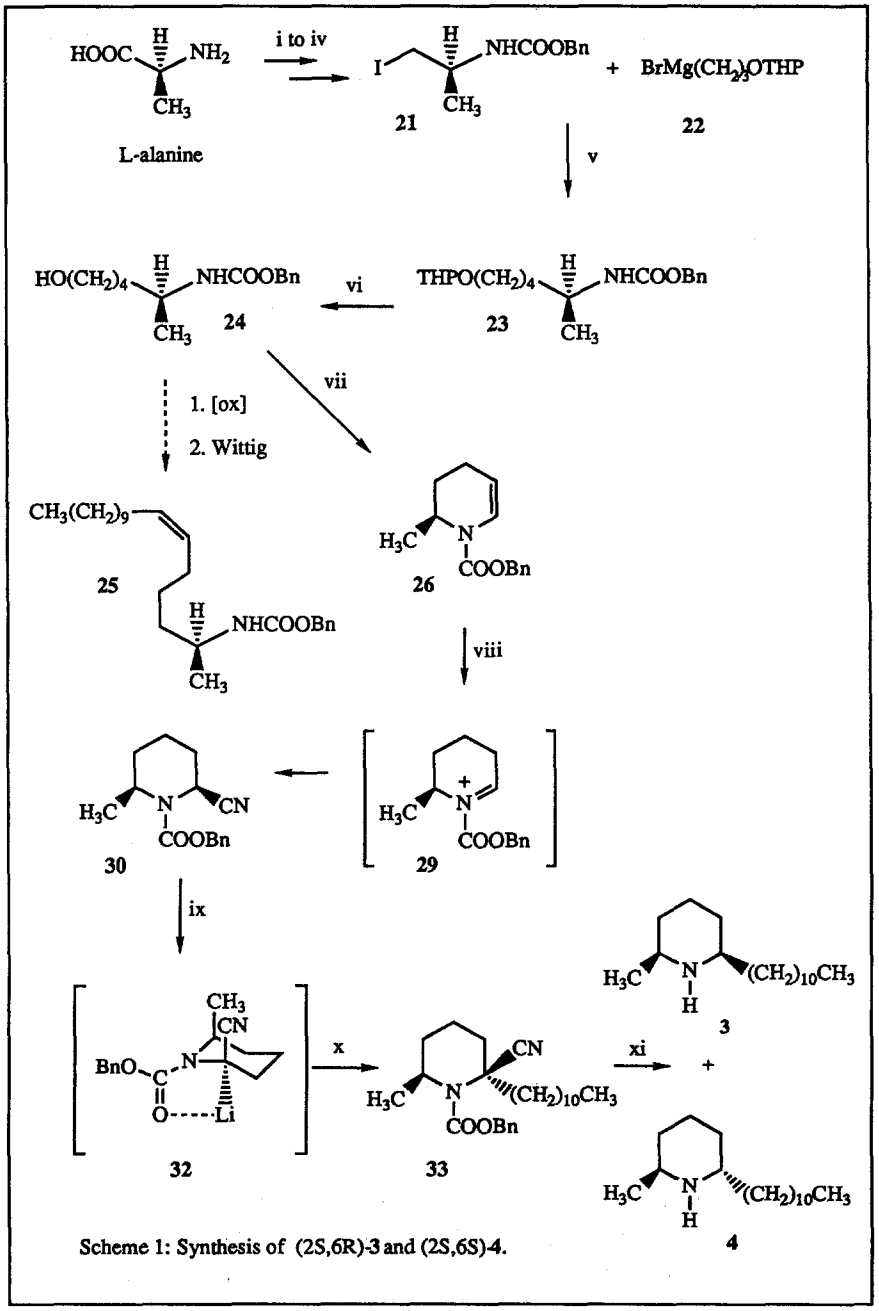
2N HCl/MeOH affording alcohol **24** in 85% yield. Initially, we planned to oxidize the primary alcohol into the aldehyde **27** and to introduce the 11-carbon alkyl chain through a Wittig reaction. The resulting Δ^5 olefinic carbamate **25** could then undergo cyclization to the piperidine ring[23,24].

However, oxidation of **24** with PCC did not yield the expected aldehyde **27** but rather the enecarbamate **26** in 75% yield. This can be rationalized by assuming that the intermediate aldehyde **27** spontaneously undergoes an intramolecular nucleophilic attack by the nitrogen atom, leading to the unstable α-hydroxylated carbamate **28** that might lose water (figure 3). Only few examples of such a cyclization under oxidative conditions have been reported in the literature[25-27]. Following this fortuitous result, we decided to utilize the enecarbamate **26** to introduce the 11-carbon side chain of the solenopsins via the α-cyanocarbamate **30**[18]. Thus, **26** was treated with $CF_3COOH/TiCl_4$ to give the iminium salt **29** and addition of trimethylsilyl cyanide furnished the α-cyanocarbamate **30**. Addition of cyanide to the iminium salt was highly stereoselective and only the cis isomer could be detected. The assignment of the cis configuration was inferred from comparison of the ^1H NMR data of **30** with those of reference compound **31** (figure 4). Moreover, the width at half-height of the H-2 (eq) signal in compound **30** is about 17 Hz. This value is significantly different from that of the corresponding proton in isosolenopsin A (**3**; $w_{1/2}$ = 22 Hz) in which H-2 is axial (δ 2.64; ddq; J = 10.7, 6.2 and 2.4 Hz). Reaction of α-cyanocarbamate **30** with LDA afforded

Figure 3 : Formation of enecarbamate **26**.



the lithium carbanion at C-6 (**32**). Addition of HMPT followed by 1-bromoundecane yielded the alkylated derivative **33** in 70% yield. As for the nucleophilic attack of cyanide, the alkylation was highly stereoselective. No trace of the C-6 epimer of **33** could be detected. The assignment of the configuration at C-6 was based on the results of Beak and Lee[28] who showed that lithiation of N-Boc-2-methylpiperidine with BuLi/TMEDA and reaction with electrophiles provides trans-2,6-dialkylpiperidines. Simultaneous deprotection of the secondary amino group and reductive decyanation of **33** with sodium in liquid ammonia provided a mixture of (2S,6S)-solenopsin A (**4**) and (2S,6R)-isosolenopsin A (**3**) in the ratio 77:23 (yield 80%). The two compounds were cleanly separated by chromatography on alumina and transformed into their corresponding hydrochlorides. The spectral properties (MS, ^1H and ^{13}C NMR) of the free bases are identical to those reported[21]. The specific optical rotations of the free bases and of their hydrochlorides are

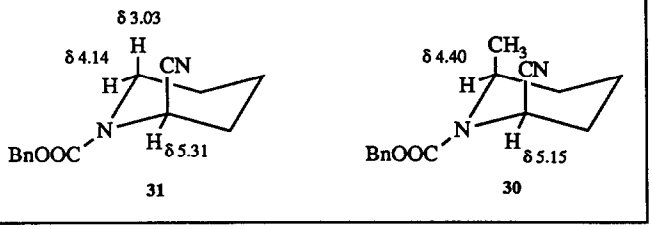


Scheme 1: Synthesis of (2S,6R)-3 and (2S,6S)-4.

i. H_4LiAl / THF, ii. ClCOOBn / Na_2CO_3, iii. TosylCl / pyridine, iv. NaI / acetone, v. THF / room temperature, vi. 2N HCl / MeOH, vii. PCC on SiO_2 / CH_2Cl_2, viii. TFA / $TiCl_4$ / TMSCN / CH_2Cl_2, ix. LDA / diglyme, x. n-$C_{11}H_{23}Br$, xi. Na / NH_3 / THF.

compatible with those reported for the corresponding natural and synthetic samples (see experimental section).

Figure 4 : ^{1}H NMR ($CDCl_3$) of **30** and **31**.



31 **30**

EXPERIMENTAL

^{1}H NMR spectra were recorded on a BRUKER WM 250 spectrometer or an AMX 400 spectrometer (working at 360 MHz) and are reported in ppm from internal TMS on the δ scale ($CDCl_3$). Data are reported as follows: chemical shift [multiplicity (s: singlet, bs: broad singlet, d: doublet, bd: broad doublet, t: triplet, q: quartet, m: multiplet), coupling constants in Hertz]. Infrared spectra were taken with a BRUKER IFS 25 instrument. EIMS were recorded on a VG Micromass 7070 spectrometer and HREIMS on a VG AutoSpec 6F spectrometer. Optical rotations were measured on a PERKIN-ELMER 141 polarimeter at 589 nm (sodium D line), in a 10 cm cell. Thin layer chromatography analyses were performed on POLYGRAM silica gel SILG/UV254 precoated plates (0.25mm). Unless otherwise stated, column chromatographies were performed over MACHEREY-NAGEL silica gel (0.04-0.063 mm), using the flash technique. All reactions were run under a nitrogen atmosphere. During work up, organic solutions were dried over $MgSO_4$. HPLC analyses were performed on a WATERS LC module 1 apparatus equipped with i) a RP 18 column (MERCK) (25 cm, 5μ, CH_3CN, 1 ml/min); ii) a Si 60 column (MERCK) (25 cm, 5μ, hexane/THF 95:5, 0.3 ml/min). In both cases, a PYE-UNICAM 4020 UV detector (211 nm) was used. Relative retention times (R'_f) were determined using (1R,2S,5R)-menthol-(S)-MTPA as internal standard. GC analyses were performed on a Varian 3700 apparatus equipped with an OV 1701 column (Rescom, 25m, 0.32 mm i. d.) isothermal at 250°C; relative retention times (R'_f) were determined using n-octacosane as internal standard.

Preparation and separation of the (S) MTPA amides 13-20. Freshly distilled oxalyl chloride (113 μl; 1.28 mmol) was added to a solution of (S)-MTPA 98% (0.048 g; 0.21 mmol) and DMF (19 μl; 0.22 mmol) in 6 ml of dry hexane at room temperature. A white precipitate formed immediately. After 90 min the hexane phase was concentrated in vacuo and IR analysis showed that the formation of (R)-MTPA-Cl was complete. To a solution of (±)-cis- and (±)-trans-solenopsin B (0.005 g; 0.018 mmol) in freshly distilled dry pyridine (400 μl) were added Et_3N (30 μl; 0.20 mmol) and (R)-MTPA-Cl (0.05 g; 0.20 mmol). After 5 h of refluxing, an additional 0.05 g of (R)-MTPA-Cl and 30μl of Et_3N were added. After refluxing for 24 h, the mixture was cooled and 3 ml of 10% Na_2CO_3 were added. The aqueous phase was extracted with Et_2O (4x3 ml), the combined organic extracts were dried and concentrated in vacuo to afford a dark residue. The separation of the four resulting diastereoisomeric amides **15, 16, 19 and 20** was effected by column chromatography (SiO_2, toluene), followed by preparative HPLC (hexane/THF 95:5). The yield was nearly

quantitative. The same procedure applied to (±)-cis- and (±)-trans-solenopsin A afforded the four corresponding MTPA amides **13**, **14**, **17** and **18**, in nearly quantitative yield.

13. ^{1}H NMR (360 MHz): 7.55, m, 2H; 7.30, m, 3H; 4.00, m, 0.6H, H_2 or H_6; 3.67, q, 2.0 Hz, 1.8H, OCH_3; 3.64, q, 2.0 Hz, 1.2H, OCH_3; 3.50, m, 0.8H, H_2+H_6; 3.16, m, 0.6H, H_6 or H_2; 1.45, d, 6.9 Hz, 1.2H, CH_3 at C-2; 1.12, d, 6.7 Hz, 1.8H, CH_3 at C-2; 0.82, t, 6.3 Hz, 3H, CH_3. IR (film): 1661, 1182, 1157 cm^{-1}. MS: m/z 470 [<1, $(M+H)^+$], 469 (<1, $M^{+\bullet}$), 468 (<1), 454 (<1, $M^{+\bullet}$ - $CH_3^\bullet$), 438 (<1, $M^{+\bullet}$ - $OCH_3^\bullet$), 314 (19), 280 (100, $M^{+\bullet}$ - $C_9H_8F_3O^\bullet$), 189 (34). HPLC: Rt=17.38 min; R'$_f$=1.27. GC: Rt=6.17 min; R'$_f$=1.42.

14. ^{1}H NMR (360 MHz): 7.59, m, 2H; 7.36, m, 3H; 4.30, m, 0.5H, H_2; 4.03 m, 1H, H_2+H_6; 3.88, m, 0.5H, H_6; 3.75, q, 2.0 Hz, 3H, OCH_3; 1.43, d, 6.5 Hz, 1.5H, CH_3 at C-2; 0.89, t, 7.0 Hz, 3H, CH_3; 0.19, d, 6.6 Hz, 1.5H, CH_3 at C-2. IR and MS identical to those of **13**. HPLC: Rt=21.83 min; R'$_f$=1.60. GC: Rt=6.87 min; R'$_f$=1.59.

17. ^{1}H NMR (360 MHz): 7.47, m, 2H; 7.30, m, 3H; 4.72, m, 1H, H_2 or H_6; 4.03, m, 1H, H_6 or H_2; 3.68, q, 2.0 Hz, 3H, OCH_3; 1.17, d, 7.2 Hz, integr. not measurable, CH_3 at C-2; 1.14, d, 7.0 Hz, integr. not measurable, CH_3 at C-2; 0.82, t, 6.8 Hz, 3H, CH_3. IR and MS identical to those of **13**. HPLC: Rt=25.44 min; R'$_f$=1.86. GC: Rt=7.53 min; R'$_f$=1.73.

18. ^{1}H NMR (360 MHz): 7.51, m, 2H; 7.30, m, 3H; 4.95, m, 0.27H, H_2 or H_6; 4.56, m, 0.73H, H_2 or H_6; 4.37, m, 0.73H, H_6 or H_2; 3.69, q, 2.0 Hz, 2.1H, OCH_3; 3.63, q, 2.0 Hz, 0.9H, OCH_3; 1.17, d, 6.6 H z 0.9H CH_3 at C-2; 0.82, t, 6.6 Hz, 3H, CH_3; 0.19, d, 7.0 Hz, 2.1H, CH_3 at C-2. IR and MS identical to those of **13**. HPLC: Rt=22.83 min; R'$_f$=1.67. GC: Rt=7.30 min; R'$_f$=1.69.

15. ^{1}H NMR (360 MHz): 7.63, m, 2H; 7.38, m, 3H; 4.06, m, 0.55H, H_2 or H_6; 3.75, q, 2.0 Hz, 1.8H, OCH_3; 3.72, q, 2.0 Hz, 1.2H, OCH_3; 3.58, m, 0.9H, H_2+H_6; 3.24, m, 0.55H, H_6 or H_2; 1.53, d, 6.9 Hz, 1.1H, CH_3 at C-2; 1.20, d, 6.7 Hz, 1.9H, CH_3 at C-2; 0.89, t, 6.3 Hz, 3H, CH_3. IR (film): 1661, 1182, 1157 cm^{-1}. MS: m/z 498 [<1, $(M+H)^+$], 497 (<1, $M^{+\bullet}$), 482 (<1, $M^{+\bullet}$ - $CH_3^\bullet$), 466 (<1, $M^{+\bullet}$ - $OCH_3^\bullet$), 314 (19), 308 (100, $M^{+\bullet}$ - $C_9H_8F_3O^\bullet$), 189.(34). HPLC: Rt=16.65 min; R'$_f$=1.22. GC: Rt=10.17 min; R'$_f$=2.29.

16. ^{1}H NMR (360 MHz): 7.59, m, 2H; 7.34, m, 3H; 4.37, m, 0.5H, H_2; 4.04, m, 1H, H_2+H_6; 3.89, m, 0.5H, H_6; 3.75, q, 2.0 Hz, 3H, OCH_3; 1.44, d, 6.5 Hz, 1.5H, CH_3 at C-2; 0.89, t, 7.0 Hz, 3H, CH_3; 0.19, d, 6.6 Hz,.5H, CH_3 at C-2. IR and MS identical to those of **15**. HPLC: Rt=20.77 min; R'$_f$=1.52. GC: Rt=11.60 min; R'$_f$=2.61.

19. ^{1}H NMR (360 MHz): 7.55, m, 2H; 7.38, m, 3H; 4.82, m, 1H, H_2 or H_6; 4.06, m, 1H, H_6 or H_2; 3.75, q, 2.0 Hz, 3H, OCH_3; 1.24, d, 7.2 Hz, integr. not measurable, CH_3 at C-2; 1.22, d, 7.0 Hz, integr. not measurable, CH_3 at C-2; 0.89, t, 6.8 Hz, 3H, CH_3. IR and MS identical to those of **15**. HPLC: Rt=24.22 min; R'$_f$=1.77. GC: Rt=11.83 min; R'$_f$=2.75.

20. ^{1}H NMR (360 MHz): 7.57, m, 2H; 7.39, m, 3H; 5.05, m, 0.3H; H_2 or H_6, 4.64, m, 0.7H, H_2 or H_6; 4.44, m, 0.7H, H_6 or H_2; 3.77, q, 2.0 Hz, 2.1H, OCH_3; 3.71, q, 2.0 Hz, 0.9H, OCH_3, 1.21, superimposed to the CH_2 signal, CH_3 at C-2 (integr. not measurable); 0.89, t, 6.6 Hz, 3H, CH_3; 0.26, d, 7.0 Hz, 2.1H, CH_3 at C-2. IR and MS identical to those of **15**. HPLC: Rt=21.74 min; R'$_f$=1.59. GC: Rt=11.80 min; R'$_f$=2.74.

Synthesis of iodide 21. Compound **21** was synthesized in 4 steps in an overall 55% yield from L-alanine following the procedure of Schlessinger and Iwanowicz[22]. **21** ($C_{11}H_{14}INO_2$); m. p.: 76-78°C (lit[22] 75-77.5°C); $[\alpha]^{20}$ -11.2° (c=3.0, CH_2Cl_2); lit.[22] $[\alpha]^{20}$ +11.0° (c=3.0, CH_2Cl_2, D-enantiomer). IR (KBr): 3340 (ν_{NH}), 3110-3022, 2982-2854, 1686 ($\nu_{C=O}$), 1522, 1450, 1254 (ν_{C-O}) cm^{-1} . MS: m/z 319 (100, $M^{+\bullet}$), 228 (37, $M^{+\bullet}$ - $C_6H_5CH_2^\bullet$), 192 (13, $M^{+\bullet}$- $I^\bullet$), 185 (18, $M^{+\bullet}$ - $C_6H_5CH_2OCO^\bullet$ + $H^\bullet$), 178 (100, $M^{+\bullet}$ - $ICH_2^\bullet$), 169 (63, $M^{+\bullet}$ - $C_6H_5CH_2OCONH^\bullet$), 134 (100), 108 (100, $M^{+\bullet}$ - $ICH_2CH(CH_3)NHCO^\bullet$ + $H^\bullet$), 91 (100, $C_6H_5CH_2^+$). ^{1}H NMR: 7.37-7.31, m, 5H; 5.11, bs, 2H; 4.81, bs, 1H; 3.61, m, 1H; 3.42 and 3.23, AB part of an ABCX3 system, J_{AB}=10.0 Hz, 2H; 1.23, d, 6.6 Hz, 3H.

Coupling of 21 with Grignard reagent 22. To 0.524 g (21.6 mmol) of Mg (Aldrich) in a 25 ml three-necked round bottomed flask were added 2 ml of dry THF, 15 µl (0.17 mmol) of 1,2-dibromoethane in 1 ml of dry THF, and finally, 180 µl of 3-bromopropyl tetrahydropyran-2-yl ether[29] in 2 ml of dry THF. The resulting mixture was briefly warmed on a water bath. When the reaction had started, the remaining of 3-bromopropyl tetrahydropyran-2-yl ether (1.6 g, 7.17 mmol) in 11 ml of dry THF was added over 1 h under stirring. The mixture was stirred at room temperature for a further 3 h. The resulting clear solution was titrated following Gilman's method[30]. The yield of organomagnesium reagent **22** was 92%. In a 100 ml three-necked round bottomed flask, 0.586 g (3.08 mmol) of purified[31] CuI were covered with 2 ml of dry THF. To this mixture at -45°C was added over 25 min the THF solution (0.45 M) containing **22**, using a double-tipped needle. Then, the temperature was allowed to rise to about -25°C for 1 to 2 min, after which it was again cooled to -45°C. Iodide **21** (0.654 g, 2.05 mmol) in 4 ml of dry THF was added at once and the mixture maintained at -40°C for 2 h. The mixture was allowed to warm to room temperature overnight. After 16 h the reaction was quenched by addition of aqueous saturated NH_4Cl and 10 ml of Et_2O. The Et_2O and aqueous phases were separated, the remaining salts carefully washed with Et_2O and the aqueous phase extracted with Et_2O (3x10 ml). The combined organic extracts were washed with 0.1N $Na_2S_2O_3$, with brine, dried and concentrated in vacuo. This material was purified by flash chromatography (hexane/AcOEt 8:2), to afford **23** as an oil (0.375 g, 55%, based on **21**). 17% of unchanged **21** were also recovered. **23**: ($C_{19}H_{29}NO_4$); $[\alpha]^{20}$ +5.3° (c=3.05, CH_2Cl_2); IR (film): 3328 (ν_{NH}), 3085-3034, 2941-2868, 1699 ($\nu_{C=O}$), 1538, 1455, 1246, 1090, 1029 cm^{-1}. MS: m/z 335 (5, $M^{+\bullet}$), 270 (16), 262 (17), 252 (99), 251 (99), 250 (99, $M^{+\bullet}$ - THP$^\bullet$), 234 (97, $M^{+\bullet}$ - THPO$^\bullet$), 233 (99), 221 (46, $M^{+\bullet}$ - $THPOCH_2^\bullet$ + H$^\bullet$), 208 (62), 206 (75, $M^{+\bullet}$ - $THPO(CH_2)_2^\bullet$), 200 (38, $M^{+\bullet}$ - $C_6H_5CH_2OCO^\bullet$), 192 (34, $M^{+\bullet}$ - $THPO(CH_2)_3^\bullet$), 190 (53), 178 (100, $M^{+\bullet}$ - $THPO(CH_2)_4^\bullet$), 160 (58), 157 (57, $M^{+\bullet}$ - $C_6H_5CH_2OCONHCHCH_3^\bullet$), 152 (87), 144 (100), 134 (100), 130 (69), 126 (83), 108 (100, $M^{+\bullet}$ - $THPO(CH_2)_4CH(CH_3)NHCO^\bullet$ + H$^\bullet$), 92 (100, $C_6H_5CH_3^+$), 91 (100, $C_6H_5CH_2^+$). ^1H NMR: 7.37-7.28, m, 5H; 5.09, bs, 2H; 4.56, bdd, J not measurable, 2H; 3.85, m, 1H; 3.77-3.67, dt, 9.6; 6.5 Hz, 2H; 3.49, m, 1H; 3.37, dt, 9.7, 6.4 Hz, 1H; 1.83-1.43, m, 12H; 1.14, d, 6.6 Hz, 3H.

Deprotection of 23 into 24. Compound **23** (0.1 g, 0.298 mmol) was dissolved in redistilled MeOH. Under stirring at room temperature, 4 ml of 2N HCl were added and the mixture stirred for 20 h. Then, 10 ml of Et_2O were added and the mixture was neutralized with 20 ml of saturated aqueous $NaHCO_3$. Extraction with Et_2O, washing of the combined organic extracts with H_2O, drying and evaporation in vacuo afforded a residue, which was purified by flash chromatography (hexane/AcOEt 9:1) to afford 0.064 g (85%) of **24**, as a white solid. **24** ($C_{14}H_{21}NO_3$): m. p.: 75-77°C; $[\alpha]^{20}$ +6.4° (c=3.0, CH_2Cl_2); IR (KBr): 3424 (ν_{OH}), 3313 (ν_{NH}), 3060-3040, 2971-2857, 1683 ($\nu_{C=O}$), 1538, 1455, 1271, 1091, 1054 cm^{-1}. MS: m/z 251 (65, $M^{+\bullet}$), 233 (13, $M^{+\bullet}$ - H_2O), 221 (10, $M^{+\bullet}$ - CH_2O), 192 (30, $M^{+\bullet}$ - $HO(CH_2)_3^\bullet$), 178 (100, $M^{+\bullet}$ - $HO(CH_2)_4^\bullet$), 160 (51, $M^{+\bullet}$ - $C_6H_5CH_2^\bullet$), 149 (100, $C_6H_5CH_2OCON^+$), 146 (100), 144 (38, $M^{+\bullet}$ - $C_6H_5CH_2O^\bullet$), 142 (100), 134 (100), 130 (100), 108 (100), 107 (100, $M^{+\bullet}$ - $HO(CH_2)_4CH(CH_3)NHCO^\bullet$), 91 (100, $C_6H_5CH_2^+$). ^1H NMR: 7.37-7.28, m, 5H; 5.09, bs, 2H; 4.53, bs, 1H; 3.73, m, 1H; 3.63, t, 6.3 Hz, 2H; 1.62-1.40, m, 6H; 1.14, d, 6.5 Hz, 3H.

Formation of enecarbamate 26. PCC (0.026 g, 0.12 mmol) and silica gel (0.026 g) dried for 24 h under vacuo were mixed and ground in a mortar. The resulting powder was suspended in 2 ml of dry CH_2Cl_2, alcohol **24** (0.02 g, 0.08 mmol) in 1 ml of dry CH_2Cl_2 was added at once, and the reaction mixture was sonicated for 20-30 min. The reaction was quenched by addition of 10 ml of Et_2O, the black precipitate washed with 5 ml of Et_2O and the combined organic extracts concentrated in vacuo. Filtration on a column

was sonicated for 20-30 min. The reaction was quenched by addition of 10 ml of Et_2O, the black precipitate washed with 5 ml of Et_2O and the combined organic extracts concentrated in vacuo. Filtration on a column of Florisil using Et_2O allowed us to isolate 0.014 g of **26** (74%), as a colorless oil, which must be stored under argon at -30°C. **26**: ($C_{14}H_{17}NO_2$); oil; $[\alpha]^{20}$ +68.4° (c=3.08, CH_2Cl_2); IR (film): 3064-3030, 2970-2845, 1705 ($\nu_{C=O}$), 1653, 1456, 1233, 1103, 1076 cm^{-1}. MS: m/z 231 (72, $M^{+\bullet}$), 187 (44, $M^{+\bullet}$ - CO_2), 172 (100, $M^{+\bullet}$ - CO_2 - $CH_3^{\bullet}$), 108 (27), 107 (25, $C_6H_5CH_2O^+$), 92 (100), 91 (100, $C_6H_5CH_2^+$). ^1H NMR: 7.38-7.27, m, 5H; 6.76, bs, 1H; 5.18, ill-resolved AB, 2H; 4.87, bs, 1H; 4.43, bs, 1H; 2.16-1.91, m, 2H; 1.85-1.71, m, 2H; 1.14, d, 6.6 Hz, 3H.

Preparation of cyanocarbamate 30. To 0.1 g (0.433 mmol) of **26**, in 20 ml of dry CH_2Cl_2, in a 100 ml three-necked round bottomed flask was added at -78°C, 33 µl (0.433 mmol) of TFA and the mixture was briefly warmed to allow the TFA to dissolve. After stirring for 5 min, 1 ml (0.5 mmol) of a 0.5 M CH_2Cl_2 solution of $TiCl_4$ was added dropwise, during which the mixture became pink. The solution was again stirred for 5 min, then 300 µl of TMSCN (2.25 mmol) were added and the mixture was stirred between -78 and -70°C for an additional 3 h. Then it was slowly allowed to warm to room temperature over a 2.30 h period. After 18 h at room temperature, the clear orange solution thus obtained was neutralized with 2 ml of 2N Na_2CO_3, diluted with 10 ml H_2O, and the aqueous phase extracted with CH_2Cl_2 (3x10 ml). The combined organic extracts were dried, concentrated in vacuo, and the residue purified by flash chromatography (hexane/AcOEt 9:1) to afford 0.775 g (70%) of **30**, as a slightly yellow oil. In another reaction, run on 0.31 g of **26**, the yield of **30** was 76%. **30**: ($C_{15}H_{18}N_2O_2$); $[\alpha]^{20}$ -53.2° (c=3.09, CH_2Cl_2). IR (film): 3090-3034, 2960-2856, 2234 (ν_{CN}), 1712-1682 ($\nu_{C=O}$), 1455, 1329, 1271, 1251, 1152, 1095, 1075, 697 cm^{-1}. MS: m/z 258 (67, $M^{+\bullet}$), 243 (18, $M^{+\bullet}$ - $CH_3^{\bullet}$), 199 (100, $M^{+\bullet}$ - $CH_3^{\bullet}$ - CO_2), 167 (100, $M^{+\bullet}$ - $C_6H_5CH_2^{\bullet}$), 151 (60, $M^{+\bullet}$ - $C_6H_5CH_2O^{\bullet}$), 142 (45), 123 (86, $M^{+\bullet}$ - $C_6H_5CH_2OCO^{\bullet}$), 108 (28), 107 (30, $C_6H_5CH_2O^+$), 97 (84), 96 (79), 92 (100), 91 (100, $C_6H_5CH_2^+$). ^1H NMR: 7.37-7.31, m, 5H; 5.19, bs, 2H; 5.16, m, 1H; 4.45, m, 1H; 1.97, m, 2H; 1.76-1.64, m, 4H; 1.38, d, 7.0 Hz, 3H. ^{13}C NMR: 129.1-128.4, 5 aromatic CH; 68.4, O-CH_2-Ph; 47.8, N-CH-CN; 41.3, N-CH-CH_3; 29.7, N-C-CH_2; 28.9, N-C-CH_2; 18.0, CH_3; 15.5, N-C-C-CH_2.

Alkylation of 30. In a 10 ml two-necked round bottomed flask, were successively introduced at -50°C, diglyme (2.5 ml), i-Pr_2NH (160 µl, 1.14 mmol) and 709 µl of a 1.6 M hexane solution of n-BuLi (1.14 mmol). The mixture was allowed to stir at room temperature for 20 min; then 199 µl (1.14 mmol) of HMPT were added and after 15 min, 0.24 g (0.93 mmol) of **30** in 2 ml of diglyme. The solution became orange-red. After an additional 20 min, 317 µl (1.42 mmol) of n-$C_{11}H_{23}Br$ were added and the mixture kept between -40 and -50°C for 75 min. The reaction mixture was poured into 10 ml of saturated NH_4Cl and the aqueous phase extracted with Et_2O (3x10 ml). Drying and concentration of the combined organic extracts in vacuo afforded an oily residue which was purified by flash chromatography (hexane/AcOEt 95:5) to afford 0.267 g (70%) of **33**, as a colorless oil. **33**: ($C_{26}H_{40}N_2O_2$); $[\alpha]^{20}$ -22.5° (c=3.02, CH_2Cl_2); IR (film): 3094-3034, 2926-2854, 2230 (ν_{CN}), 1712,($\nu_{C=O}$), 1456, 1282, 1076, 698 cm^{-1}. MS: m/z 412 (13, $M^{+\bullet}$), 386 (14, $M^{+\bullet}$ - $CN^{\bullet}$), 353 (100), 340 (8), 321 (23, $M^{+\bullet}$ - $C_6H_5CH_2^{\bullet}$), 314 (19), 305 (44, $M^{+\bullet}$ - $C_6H_5CH_2O^{\bullet}$), 300 (39), 277 (100, $M^{+\bullet}$ - $C_6H_5CH_2OCO^{\bullet}$), 262 (37, $M^{+\bullet}$ - $C_6H_5CH_2OCO^{\bullet}$ - $CH_3^{\bullet}$), 257 (50, $M^{+\bullet}$ - $C_{11}H_{23}^{\bullet}$), 250 (99), 236 (100, $M^{+\bullet}$ - $C_6H_5CH_2OCO^{\bullet}$ - $CH_3^{\bullet}$ - $CN^{\bullet}$), 213 (100), 189 (39), 167 (60), 154 (100), 149 (100), 137 (100). ^1H NMR: 7.40-7.28, m, 5H; 5.23 and 5.17, AB, J_{AB}= 12.2 Hz, 2H; 4.40, m, 1H; 2.29-1.59, m, 8H; 1.29, d, 6.9 Hz, 3H; 1.31-1.23, m, 18H; 0.88, t, 6.6 Hz, 3H.

(2S,6S)-Solenopsin A (4) and (2S,6R)-isosolenopsin A (3). To 8 ml of liquid ammonia distilled from Na at -78°C were added 0.045 g (1.94 mmol) of Na, upon which the solution became blue. After 20 min of

stirring, 0.1 g (0.24 mmol) of **33** in 4 ml of dry THF were added, and the mixture was allowed to stir for 35 min. Then, the excess of Na was destroyed by addition of 1 ml of MeOH, and the ammonia was allowed to evaporate. After 2h30, water was added and the aqueous phase extracted with CH_2Cl_2 (3x5 ml). The combined organic extracts were dried, concentrated in vacuo, and the residue submitted to a chromatography on an column of neutral alumina (Macherey Nagel, activity 1, 15 cm length, 1 cm i.d., using successively hexane/Et_2O, from 6:4 to 4:6, then Et_2O, Et_2O/MeOH 9:1, and finally Et_2O/MeOH 9:1, saturated with NH_4OH) to afford (2S,6S)-solenopsin A (**4**) (0.0376 g, 0.149 mmol, 62%; HRMS: $M^{+\cdot}$ at m/z 253.2747; calc. for $C_{17}H_{35}N$: 253.2769; $M^{+\cdot}$-1 at m/z 252.2681; calc. for $C_{17}H_{34}N$: 252.2691) and (2S,6R)-isosolenopsin A (**3**) (0.011 g, 0.043 mmol, 18%; HRMS: $M^{+\cdot}$ at m/z 253.2775 and $M^{+\cdot}$-1 at m/z 252.2677), whose physical and spectral data were identical to those described in the literature[21]. (2S,6S)-Solenopsin A (**4**): $[\alpha]^{20}$ +2.5° (c=3.0, $CHCl_3$); (2R,6R)-solenopsin A: lit[21] $[\alpha]^{20}$ -1.3° (c=1.3, MeOH); lit[20] $[\alpha]^{20}$ -2.2° (c=0.8, MeOH). (2S,6R)-Isosolenopsin A (**3**): $[\alpha]^{20}$ +5.8° (c=3.0, $CHCl_3$).

Formation of the hydrochlorides of 3 and 4. To the amine dissolved in a minimum amount of dry Et_2O were added a few ml of dry Et_2O saturated with gaseous HCl. The resulting white precipitate was dried in vacuo and recrystallized in the mixture CH_2Cl_2/Et_2O. (2S,6S)-Solenopsin A (**4**).HCl: $[\alpha]^{20}$ +7.5° (c=1.3, $CHCl_3$); lit[18] $[\alpha]^{20}$ +7.5° (c=1.3, $CHCl_3$); lit[18] $[\alpha]^{20}$ -1.0° (c=1.7, MeOH); ^{13}C NMR (62.8 MHz, $CDCl_3$): 52.0; 48.2; 32.1; 31.0; 29.8 (2 C); 29.75; 29.7; 29.6; 29.5; 29.2; 26.5; 26.1; 22.9; 17.6; 17.1; 14.3. (2R,6R)-solenopsin A.HCl: lit[21] $[\alpha]^{20}$ -7.7° (c=1.3, MeOH); lit[20] $[\alpha]^{20}$ -7.6° (c=0.7, $CHCl_3$). (2S,6R)-Isosolenopsin A (**3**).HCl: $[\alpha]^{20}$ -10.3° (c=1.3, $CHCl_3$); lit[21] $[\alpha]^{20}$ -10.6° (c=0.3, $CHCl_3$); ^{13}C NMR (62.8 MHz, $CDCl_3$): 59.2; 55.1; 33.8; 32.4; 31.3; 30.1 (3 C); 30.0; 29.9; 29.8; 28.1; 26.2; 23.5; 23.1; 20.0; 14.5.

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