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## Lepadins I–K, 3-O-(3'-Methylthio)acryloyloxy-decahydroquinoline Esters from a Bahamian Ascidian *Didemnum* sp. Assignment of Absolute Stereostructures

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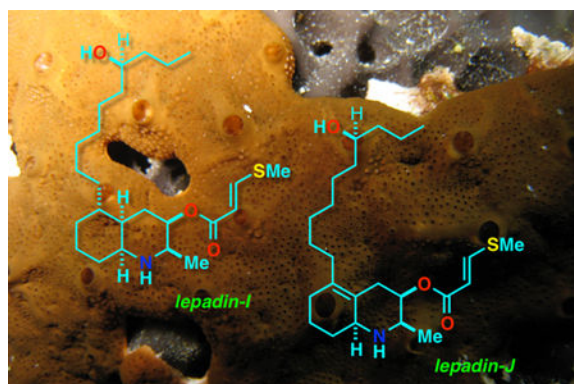
### Abstract

Three decahydroisoquinoline alkaloids, lepadins I–K, were isolated from a specimen of *Didemnum* sp. collected in the Bahamas. The structures of the new compounds were assigned by an integrated analysis of MS, IR<sup>1</sup>H, <sup>13</sup>C NMR and 2D NMR spectra. Like previously reported lepadins, the structures of the new compounds contain a decahydroquinoline heterocyclic core in lepadin I, and a new variation, an octahydroquinoline in lepadin J, but differ from earlier reported compounds by acylation of the 3-hydroxyl group by a rare 3'-methylthioacrylate. The absolute configuration of lepadin I was solved by interpretation of NOE measurements, and exciton coupled circular dichroism (ECCD) of the corresponding *N*-*p*-bromobenzoyl derivative. The latter constitutes a general method for determination of absolute configuration of the entire lepadin family. The configuration of the remote side-chain secondary carbinol was solved by the modified Mosher's esters method. Lepadin I inhibited butyrylcholineesterase (BuChE, IC<sub>50</sub> 3.1 μM), but only weakly inhibited acetylcholineesterase (AChE) (10% at 100 μM).

### Graphical Abstract

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Supporting Information Available. <sup>1</sup>H NMR for 1-3, <sup>13</sup>C NMR and APT spectra for 1, 1D and 2D NMR data for 6-7a,b and B3LP/6-31+G(d) calculations for 6 are available free of charge via Internet at <http://pubs.acs.org>.

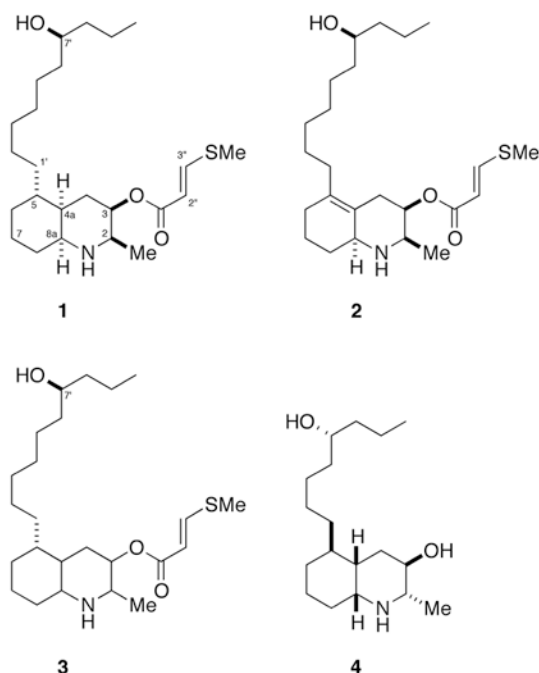


## Introduction

Ascidians express numerous secondary metabolites, and specialize in alkaloids, peptides and other nitrogenous natural products.<sup>1</sup> The lepadins are 5-alkyl-3-hydroxydehydroquinoline derivatives found exclusively in ascidians or their predators. Lepadins A-H have been described from various sources including the ascidian species *Clavelina lepadiformis* from temperate waters, its predator, the flatworm, *Prostheceraeus villatus*, and other ascidian species within the genera *Didemnum* and *Aplidium*.<sup>2</sup> Lepadins B and D are free secondary alcohols, but the other members are acylated at the 3-OH group as glycolates or short-chain alkenoate carboxylate esters. Bioactivities of some lepadins have been reported. Lepadins A and B were shown to have moderate cytotoxic effect on cancer cells<sup>2b</sup> and lepadins D (4), E and F possess antiplasmodial and antitrypanosomal activity.<sup>2c</sup> Additionally, (-)-lepadin B was shown to be a potent blocker of  $\alpha 7\alpha\beta 2$  and  $\alpha 7$  neuronal nicotinic acetylcholine receptors expressed in *Xenopus* oocytes,<sup>3</sup> but the broader potential of the alkaloid for nicotinic based therapies has not been evaluated. We describe here, the isolation and structure elucidation of lepadins I (1), J (2) and K (3) and absolute stereoassignment of 1 through development of a general method based on circular dichroism (CD). The new compounds are variants of the decahydroquinoline (azadecalin) core, common to other members of the series, but differ by the presence of a rare 3-methylthioacrylate ester at C-3.

## Results and Discussion

The colonial ascidian *Didemnum* sp., collected at Stirrup Cay in the Bahamas in 2008, was left standing in MeOH (~5 years, -20 °C). The MeOH extract was partitioned against solvents of increasing polarity (hexane, CH<sub>2</sub>Cl<sub>2</sub> and n-BuOH). The non-polar CH<sub>2</sub>Cl<sub>2</sub> fraction obtained by solvent partitioning was further separated on a silica column using a stepwise gradient of CH<sub>2</sub>Cl<sub>2</sub> and MeOH. Samples of pure lepadin I (1) and a mixture of lepadin J (2) and lepadin K (3) were obtained after purification by semi-preparative HPLC (aminopropyl column, 10:90 *i*-PrOH-hexanes, 1% Et<sub>2</sub>NH) conversion to their corresponding TFA salts and final elution from C<sub>18</sub> SPE cartridges (MeOH: H<sub>2</sub>O).



Lepadin I (**1**) is an optically active alkaloid (free base:  $[\alpha]_D +4.1 \pm 0.6$  ( $c$  0.24, MeOH), TFA salt:  $[\alpha]_D +15.0 \pm 0.9$  ( $c$  0.25, MeOH)) which gave a positive Dragendorff's test for basic nitrogen and a positive ninhydrin test (pale yellow), suggesting the N atom was associated with a secondary amine. The formula of lepadin I (**1**),  $C_{24}H_{43}NO_3S$ , and lepadin J (**2**),  $C_{24}H_{41}NO_3S$ , obtained from HRMS measurements, reveals four and five double bond equivalents, respectively and the presence of a sulfur atom. Evidence for the latter was obtained from MS and NMR data (see below). The ESIMS spectrum displayed an unusually intense  $M+2$  ion that was attributed to an isotopomer containing one sulfur atom ( $^{34}S$ ), and confirmed by matching the experimental and calculated isotopomer patterns of the quasimolecular ion.

Analysis of the  $^1H$  and  $^{13}C$  NMR data for **1** (Table 1) showed the presence of an ester ( $\delta_C$  164.6, s), an acyclic secondary alcohol ( $\delta_H$  3.57, m), an axial esterified cyclic secondary carbinol ( $\delta_H$  5.04, q,  $J = 2.8$  Hz;  $\delta_C$  67.8, CH), a secondary aliphatic methyl group ( $\delta_H$  1.31, d;  $\delta_C$  14.8,  $CH_3$ ) and a linear long-chain alkyl group (terminal Me;  $\delta_H$  0.91, t,  $J = 6.8$  Hz;  $\delta_C$  14.3,  $CH_3$ ). The key feature that distinguished the  $^1H$  NMR spectrum ( $CDCl_3$ ) of **1** from those of known lepadins was the presence of a highly polarized *E*-double bond ( $\delta_H$  8.07, d,  $J = 14.9$  Hz;  $\delta_H$  5.74, d,  $J = 14.9$  Hz) associated with the  $\beta$ -methylthioacrylate ester side chain (see below). Comparison of the key NMR chemical data of **1** and **2** with those of other alkaloids from ascidians (e.g. lepadin D, **42**<sup>c</sup>) narrowed the possibilities to new 2,3,5-trisubstituted *cis*-decahydroquinolines (*cis*-azadecalins). The substituted side-chain located at C-5 is two carbons longer ( $C_{10}$ ) than in other lepadins. TOCSY and HMBC experiments revealed a hydroxymethine signal due to a secondary alcohol at C-3' (CH-OH,  $\delta_H$  3.57, m;  $\delta_C$  71.8, CH) three carbons removed from the chain terminus.

The sulfur-containing functional group in **1–3** was identified as a 3-methylthioacrylate by a process of deduction, including observation of a large red-shifted UV-vis band ( $\lambda$  270 nm), evaluation of  $^1J_{\text{CH}}$  heteronuclear coupling constants, and matching the  $^1\text{H}$  NMR chemical shifts to those of known compounds. A three-proton singlet ( $\delta$  2.34, 3H, s) suggested a methyl group attached to a heteroatom – either S or N – based on the relatively large  $\text{sp}^3$  one-bond heteronuclear coupling ( $^1J_{\text{CH}} = 140$  Hz) observed in the  $^1\text{H}$  NMR spectrum of **1** and **2** ( $^{13}\text{C}$ -satellites') and in coupled  $^1\text{H}$ - $^{13}\text{C}$  HSQC spectra. The characteristically high-field  $^{13}\text{C}$  NMR chemical shift of C-5'' in **1** ( $\delta$  14.2, q) firmly established the identity of this methyl signal as SMe. The AB vinyl spin-system of **1** and **2** is associated with highly polarized  $^1\text{H}$  NMR chemical shifts ( $\delta$  2.33 ppm); far larger than the differences expected for vinyl  $^1\text{H}$  signals in a simple  $\alpha,\beta$ -unsaturated alkenoate ester. Furthermore, the polarization is in the opposite direction to the latter compounds, consistent with a  $\beta$ -methylthioacrylate ester.<sup>4</sup>

The bicyclic ring conformation of **1** was established from NOESY experiment (Figure 1,  $t_m = 300$  ms, 600 MHz). Of the two possible chair-chair conformers for the *cis*-azadecalin core of **1**, the one depicted – with a C-8a–N bond axial to the cyclohexane chair and an equatorial side chain subtended from C-5 – is supported by cross peaks between signals for the paired 1,3-*syn* diaxial protons – H-2<sub>ax</sub>–H-4<sub>ax</sub>, H-4<sub>ax</sub>–H-6<sub>ax</sub> – and is also the favored conformation of *cis*-azadecalin ring systems in other lepadins.

The  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectrum of lepadin J (**2**) (Table 2) showed similar features to those of **1**, except for the absence of two  $\text{sp}^3$  CH signals (DEPT) which were replaced by two quaternary C signals, ( $^{13}\text{C}$  NMR) corresponding to a tetrasubstituted C=C double bond.<sup>5</sup> This double bond was located at C-4a–C-5 based on HMBC evidence; correlations were observed from H-3, H-4, H-8 to C-4a and H-4 to C-5. Additional evidence for double bond placement at C-4a–C-5 was provided by the  $^1\text{H}$  NMR spectrum of **2**; the H-4 and H-1' signals were deshielded compared to **1**, consistent with allylic protons.

The  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectrum of lepadin K (**3**) (Table 3) supported a stereoisomer of **1**. The major differences in the  $^1\text{H}$  NMR spectra of **3** and **1** were the signals due to H-4 and those of the cyclohexane ring. Absence of a COSY correlation between H-2 and H-3 ( $^3J \sim 0$  Hz) supported the same C-2 and C-3 configurations and a *cis* azadecalin configuration as **1**. Attempted purification of **3** led to material decomposition, therefore the relative stereochemistry of the remaining stereocenters remain ambiguous.

The relative configurations of **1** and **2** were derived from a combination of  $J$  coupling analysis and 2D NOESY experiments (Figure 1). Scalar coupling ( $J = 4.8$  Hz) and an NOE cross peak between  $^1\text{H}$  NMR signals for H-4a and H-8a indicated a *cis*-decalin structure for **1**. ( $2R^*,3R^*,4aR^*,5S^*,8aS^*$ ) (C-2,3 *cis*; C4a5-*cis*; C-4a,8a *cis*). NOESY correlations between H-2 and H-8a showed these hydrogens adopted a diaxial disposition. The small coupling constant ( $^3J = 2.8$  Hz) between H-2 and H-3 revealed that they adopted a *syn* configuration and H-3 occupied the equatorial position. By considering the information above, the protons H-2, H-3, H-4a and H-8a were determined to be all *syn*-configured.<sup>6</sup>

Assignment of the geometry of the C-2''–C-3'' double bond of **1** is equivocal because the magnitude of the vicinal coupling constant lies close to the edge of the ranges for both *E* and *Z* double bonds. To securely assign the geometry by <sup>1</sup>H NMR comparison with a simple model compound, a mixture of geometrical isomers of β-methylthioacrylic acid (**5**, *E*:*Z* 40:1) was prepared by addition of NaSMe to freshly distilled propiolic acid (Figure 2).

The chemical shifts and coupling constants of **1** showed an excellent match with those of the major isomer *E*-**5** (δ 7.87, d, *J* = 14.7 Hz, H-3; 5.64, d, *J* = 14.7 Hz; 2.35, s, <sup>1</sup>*J*<sub>CH</sub> = 161.5 Hz), while the minor isomer *Z*-**5** exhibited a smaller vicinal coupling constant (δ 7.46 (d, 1H, *J* = 10.2 Hz, H-3); 5.85 (d, 1H, *J* = 10.2 Hz, H-2). Therefore the C-2''–C-3'' double bond of **1** has an *E*-configuration.

The reported lepadins exhibit heterogeneous relative and absolute configurations. The ring junction of the decahydroquinoline core in **1** has the same configuration as (+)-lepadin G<sup>2d</sup>, but is antipodal to (–)-lepadins A<sup>2a</sup> and B,<sup>2b,7,8</sup> and diastereomeric to those of other lepadins. No conclusive relational assignments of configuration can be made by comparisons of [α]<sub>D</sub>, rendering the assignment of absolute configuration a non-trivial problem. Not all lepadins – compounds **1** and **2** included – are crystalline; consequently, X-ray is of limited help, and no generally useful chiroptical property resides in these alkaloid structures for the independent assignment of their absolute stereostructures. The only definitive configurational assignments in the lepadin series have been made by total synthesis. For example, asymmetric syntheses of lepadins B,<sup>7</sup> D, E and H<sup>8</sup> and also of F (**3**)<sup>9,10</sup> and G<sup>9</sup> allowed assignment of absolute configurations by comparisons of optical rotations of synthetic and natural products.

We reasoned that the absolute configuration of **1** could be assigned by exciton coupled circular dichroism (ECCD), exploiting the pendant methythioacrylate at C-3 – a red-shifted, relatively strong chromophore (λ<sub>max</sub> 270 nm) – and introduction of a second chromophore, specifically an *N*-*p*-bromobenzoyl group. *N*-Acylation of **1** (Scheme 1, *p*-Br-C<sub>6</sub>H<sub>4</sub>COCl, Et<sub>3</sub>N, DMAP, CH<sub>2</sub>Cl<sub>2</sub>) gave the corresponding *p*-bromobenzamide **6**. Most of the ring <sup>1</sup>H NMR signals of tertiary amide **6** (600 MHz, CDCl<sub>3</sub>) were doubled (4:3 mixture of rotamers). Further examination of the <sup>1</sup>H NMR and NOESY data (Figure 1b) of **6** indicated that the azadecalin ring system had flipped to the alternate chair-chair conformation, most likely to relieve allylic strain about the tertiary amide. The <sup>1</sup>H NMR spectrum of **6** shows a broader multiplet structure for H-3 (δ 4.91, m, *w*<sub>1/2</sub> = 20.8 Hz), compared to the narrower H-3 multiplet of **1** (δ 5.04, q, *J* = 2.8 Hz), as a consequence of the larger H-3<sub>ax</sub>-H-4<sub>ax</sub> coupling in addition to *ax*-*eq* couplings. The NOESY correlations of **6** (Figure 1b) were fully consistent with flipped azadecalin chair-chair conformation, including a prominent H-4<sub>ax</sub>-H<sub>3</sub>-9 cross peak. Molecular modeling of **6** (Figure 3) clearly supported the alternate chair-chair conformation with the C-8<sub>a</sub>-N bond disposed equatorially to the cyclohexane ring.<sup>11</sup> This conclusion was upheld by DFT calculations (B3LYP/6–31+G(d)) of the minimized energy and geometry of **6** (Figure 1). Four low-lying conformations were discovered for **6**, each with essentially the same conformation of the heterocyclic ring, and differing only in the rotational conformers about the amide group and the relatively free rotating 3-methylthioacrylate group.

Unlike **1**, which exhibited only a weak simple Cotton effect, CE,  $\lambda$  270 nm ( $\epsilon$  +0.56), the CD spectrum of **6** (Figure 4) showed a strongly negative bisignate CE ( $\lambda$  239 nm ( $\epsilon$  +4.7), 252 (0), 272 (−9.4)) characteristic of exciton coupling between the benzamide and the methylthioacrylate chromophores. The electronic transition dipole moments of methylthioacrylate and *p*-bromobenzamide chromophores lie approximately parallel to the N-(C=O) and C-3-O bond vectors, respectively, and subtend an angle of  $\pm 66.5^\circ$ , the sign being dependent upon absolute helicity (Figure 3). The observed negative split Cotton effect is consistent with a helicity of  $-66.5^\circ$ ; consequently, the absolute configuration of the heterocyclic core of natural **1** is (2*R*,3*R*,4*aR*,5*S*,8*aS*) as depicted.

The above-described assignment can be generalized method for assignments of other stereochemically heterogeneous lepadines, all of which share a C-3 secondary hydroxyl or acyloxy group (the latter can be converted to the former by saponification). Simple derivatization of the core 3-hydroxyperhydroquinoline structure as a bis-*N,O*-diarenoyl derivative (e.g. by dibenzoylation) will give rise to ECCD that can be interpreted in a manner similar to that of **6**, assuming the *N*-benzamide ‘anchors’ the azadecalin in the preferred conformer (viz. Figure 5). Interpretation of the sign of the helicity for *N*-Bz and *O*-Bz electronic transition dipole moments in the idealized model will predict the sign of the split-Cotton effect as illustrated for the four possible C-2–C-3 diastereomers (Figure 6).

The remaining asymmetric center, at the secondary carbinol C-7', was solved independently by application of the advanced Mosher's ester method.<sup>12</sup> Acylation of **5** (Scheme 1, *R*- or *S*-MTPA-Cl, Et<sub>3</sub>N, DMAP, CH<sub>2</sub>Cl<sub>2</sub>) gave, after HPLC purification, the corresponding Mosher's esters, (*S*)-**7a** and (*R*)-**7b**, respectively. The anisotropic <sup>1</sup>H NMR shifts ( $\delta$ ) about the C-7' MTPA group in **7a,b** (Scheme 1) conformed to the Mosher's ester model<sup>12</sup> and therefore, the configuration is 7*S* in **1**. Therefore, the complete configuration of **1** is 2*R*,3*R*,4*aR*,5*S*,8*aS*,7' *S*. Given that lepadins I–K were obtained from the same ascidian sample, it is likely **1–3** share the 7*S* configuration.

The free acid **5** was first reported as a secondary metabolite from fermentations of *Streptomyces lincolnensis* var. *lincolnensis*,<sup>13</sup> and derivatives have been described since from other *Streptomyces* (e.g. the modified cytosine nucleoside, cytosaminomycin A (**8**)<sup>14</sup>, Figure 6). Derivatives of **5** are less common in plants. The first reported natural product 3-methylthioacrylate derivatives are petasol esters B and C, isolated from extracts of butterbur, *Petasites officinalis*.<sup>15</sup> Subsequently, 3-methylthioacrylate esters *S*-petasin<sup>16b</sup> and related sesquiterpenes<sup>16a</sup> were isolated from other *Petasites* spp. Entadamides A (**9**) and B (**10**), from the seeds of the tropical legume, *Entada phaseoloides*, are 3-methylthioacrylamides of ethanolamine.<sup>17</sup>

The pyrrolizidine alkaloid, planchonelline (**11**), was purified from *Planchonella thyrsoides* collected in Papua New Guinea.<sup>18</sup> Compounds **1–3** constitute the second finding of methylthioacrylate esters from a marine invertebrate; Cimino and coworkers earlier reported the 1,2-diacyl-*sn*-glycerol, umbraculumin C (**12**), from the skin glands of the Mediterranean opisthobranch, *Umbraculum mediterraneum*.<sup>19</sup>

The origin of **5** for biosynthesis **1–3**, **8–12** – which conceivably may proceed through either oxidative deamination and *S*-methylation of L-cysteine (Scheme 2), or more likely, oxidative degradation of L-methionine – has been studied only in *Streptomyces*.

Visser and Meyer showed, in-time dependent methionine-supplemented fermentation of *S. lincolnensis*, the appearance of **5** in the culture tracked with the concentration of methionine and production of lincomycin; therefore, **5** may be a shunt metabolite of secondary metabolite production. Surette and Vining demonstrated that cultures of *S. lincolnensis*, supplemented with either *S*-[<sup>14</sup>C<sub>3</sub>]-methionine or *S*-[<sup>14</sup>C<sub>3</sub>]-3-methylthiopropionic acid (but not *S*-[<sup>14</sup>C<sub>3</sub>]-3-methylthiopropionamide) delivered [<sup>14</sup>C]-**5**.<sup>20</sup>

The acrylate ester present in **1–3** appears to be a strong electrophile; a consequence of a polarized double bond, made more so by the presence of the 3-methylthio group, and evidenced by the large differences in the <sup>1</sup>H and <sup>13</sup>C NMR chemical shifts for the α and β CH signals. Consequently, **1–3** may be competent Michael acceptors and possible alkylating agents. For example, natural products **9** and **10** are potent inhibitors of 5-lipoxygenase of RBL-1 cells (IC<sub>50</sub> ~0.1 μg.mL<sup>-1</sup>), a property suggestive of the electrophilic nature of derivatives of **5**, and touted as the core of “a new type of anti-inflammatory drug”.<sup>21,22</sup> The activity of **1** was briefly examined in an anti-cholinesterase reporter assay using a modification of Ellman’s photometric method<sup>23</sup> with physostigmine as a positive control (IC<sub>50</sub> = 1.48 μM). Compound **1** showed no significant activity (IC<sub>50</sub> >100 μM) toward acetylcholinesterase (AChE), but modest inhibition of butyrylcholinesterase activity (BuChE, IC<sub>50</sub> = 3.1 μM) which is indicative of non-specific activity.

In conclusion, three new decahydroquinolines, lepadins I (**1**), J (**2**) and K (**3**), embodying a rare 3-methylthioacrylate, were isolated from a Bahamian *Didemnum* sp. A general method was developed for assignment of absolute configuration of 3-hydroxylated perhydroquinolines – the core heterocycle of the lepadin family – based on exciton coupled CD. Compound **1** showed modest inhibitory activity against butyrylcholinesterase, and essentially no activity against acetylcholinesterase.

## Experimental Section

### General Experimental Procedures.

General experimental procedures are described elsewhere.<sup>24</sup> Sub-milligram reaction % yields were determined by the <sup>1</sup>H NMR method of quantitation by solvent <sup>13</sup>C satellite (QSCS)<sup>25</sup> and external calibration against 3α-bromocholestane recorded under the same conditions. <sup>13</sup>C NMR signal multiplicities (CH<sub>3</sub>, CH<sub>2</sub>, CH, Cq) were determined from edited HSQC or APT experiments. Optical rotations were measured in a Jasco P-2000 digital polarimeter using a 1.00 cm quartz cell (fill volume = 125 μL) at the emission D line of Na°. HRMS measurements were made on an Agilent 6350 TOFMS instrument using ESI in positive ion mode.

### Animal Material.

The red-orange encrusting ascidian *Didemnum* sp. (08–13–072) was collected, in June of 2008, by hand using scuba at Stirrup Cay at a depth of approximately –10 m. A type sample



is archived at UC San Diego. The material was stored in MeOH at  $-20\text{ }^{\circ}\text{C}$  (five years) and the MeOH-extract concentrated and solvent-partitioned in the following manner.

### Extraction and Isolation of Lepadins I–K (1–3).

An aliquot of the total MeOH extract of the ascidian (30 mL) was adjusted to 9:1 MeOH/H<sub>2</sub>O and partitioned with hexanes (30 mL). The aqueous-MeOH layer was adjusted to 40% H<sub>2</sub>O and further extracted with CHCl<sub>2</sub> (2 × 35 mL). The CHCl<sub>2</sub>-MeOH fraction (99 mg) was concentrated and partially purified on silica column (1.5 × 9 cm) using stepwise gradient of CHCl<sub>2</sub>/MeOH (100:0–0:100 v/v). The vanillin positive bands were combined, resulting in five fractions. Fraction five (34.0 mg) was subjected to semi-preparative HPLC (Microsorb NH<sub>2</sub> column, 4.6 mm × 250 mm with an isocratic solvent hexane:*i*-PrOH:Et<sub>2</sub>NH, 90:10:1, v/v/v). Lepadin I (**1**) was obtained as the TFA salt together with Et<sub>2</sub>NH•TFA (25.4 mg) (r.t. = 7.3 min) and a mixture (1.2 mg) of lepadin J (**2**), K (**3**) and Et<sub>2</sub>NH•TFA. Further purification of the latter mixture by HPLC (Microsorb NH<sub>2</sub> column, 4.6 mm × 250 mm; gradient: 0–2 min: 9% *i*-PrOH-hexane (1% *i*-Pr<sub>2</sub>NH), gradient to 15 min: 10.5% *i*-PrOH/hexane (1% *i*-Pr<sub>2</sub>NH); 1 mL/min) gave additional lepadin J (**2**) (0.2 mg, r.t. = 10.3 min), however, lepadin K (**3**) could not be recovered.

**Lepadin I (1).**—Colorless oil. Free base:  $[\alpha]_{\text{D}}^{23} +4.1 \pm 0.6$  (c 0.24, MeOH), TFA salt:  $[\alpha]_{\text{D}}^{23} +15.0 \pm 0.9$  (c 0.25, MeOH). UV (MeOH)  $\lambda_{\text{max}}$  275 ( $\epsilon$  3500). CD (MeOH)  $\lambda$  269 ( $\epsilon$  +0.56). FTIR (ATR, ZnSe). 3300–2900 (bs), 1672 (s),  $\text{cm}^{-1}$ . <sup>1</sup>H NMR, <sup>13</sup>C NMR (see Table 1). HRESIMS  $m/z$  426.3037 [M+H]<sup>+</sup> (calcd for C<sub>24</sub>H<sub>44</sub>NO<sub>3</sub>S, 426.3036).

**Lepadin J (2).**—Colorless oil. <sup>1</sup>H NMR, <sup>13</sup>C NMR (see Table 2). FTIR (ATR, ZnSe). 3800–2900 (bs), 1672 (s),  $\text{cm}^{-1}$ . (see Table 2). HRESIMS  $m/z$  424.2886 [M+H]<sup>+</sup> (calcd for C<sub>24</sub>H<sub>42</sub>NO<sub>3</sub>S, 424.2880).

**Lepadin K (3).**—<sup>1</sup>H NMR, <sup>13</sup>C NMR (see Table 3). ESIMS  $m/z$  426.3 [M+H]<sup>+</sup> (calcd for C<sub>24</sub>H<sub>44</sub>NO<sub>3</sub>S, 426.3).

### 3-Methylthioacrylic acid (5)

Sodium thiomethoxide (123.2 mg, 1.74 mmol) was added to stirred, freshly distilled propiolic acid (123.0 mg, 1.74 mmol). After 15 minutes, methanol was added and stirring continued for 15 h during which time a flocculent white precipitate formed. Aqueous citric acid (10% w/v, 0.2 mL) was added and the mixture extracted with a mixture of diethyl ether and hexane (1:1, 2 × 1 mL). The organic layer was dried (MgSO<sub>4</sub>) and concentrated to give a pale yellow solid which was shown by <sup>1</sup>H NMR to be exclusively 3-methylthioacrylic acid (80.0 mg, 39%, **5**, *E:Z* = 40:1). <sup>1</sup>H NMR (CDCl<sub>3</sub>) *major* isomer *E*-**5**.  $\delta$  7.87 (d,  $J$  = 14.7 Hz, <sup>1</sup> $J_{\text{CH}}$  = 168.5 Hz, H-3); 5.64 (d,  $J$  = 14.7 Hz, <sup>1</sup> $J_{\text{CH}}$  = 165.8 Hz, H-2); 2.35 (s, 3H, <sup>1</sup> $J_{\text{CH}}$  = 136 Hz, SMe), consistent with literature values. <sup>1813</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  170.2 (s, C-1), 150.3 (CH), 112.2 (CH), 14.51 (CH<sub>3</sub>).

When the addition reaction was repeated with CH<sub>3</sub>SH (NaSMe and 0.86 equ AcOH in MeOH), the major product was *Z*-**5** (*E:Z* = 1:10), albeit isolated in low yield (5%).

*minor* isomer **Z-5**.  $\delta$  7.46 (d, 1H,  $J$  = 10.2 Hz, H-3); 5.85 (d, 1H,  $J$  = 10.2 Hz, H-2) consistent with literature values.<sup>17a</sup>

### Lepadin I *p*-bromobenzamide (**6**)

A solution of *p*-bromobenzoyl chloride in CH<sub>2</sub>Cl<sub>2</sub> (1M, 25  $\mu$ L, 25  $\mu$ mol) was added to a stirred solution of **1** (1 mg, 2.3  $\mu$ mol) in CH<sub>2</sub>Cl<sub>2</sub> (*ca.* 0.2 mL) containing a crystal of DMAP and triethylamine (10  $\mu$ L, 69  $\mu$ mol). The mixture was stirred for 6 h at room temperature then concentrated under a stream of N<sub>2</sub>. The residue was applied to a ‘pencil column’ packed with silica (8.5  $\times$  50 mm) and eluted with 1:19 MeOH-CH<sub>2</sub>Cl<sub>2</sub> followed by 1:4 MeOH-CH<sub>2</sub>Cl<sub>2</sub>. The fraction containing **6** eluted after 9 column volumes and was further purified by HPLC (silica, 250  $\times$  10 mm, 40:60 EtOAc-hexanes, 4.0 mL.min<sup>-1</sup>, UV detection  $\lambda$  = 250 nm) to give pure benzamide **6** (396  $\mu$ g, 28%).  $[\alpha]_D^{23}$  -3.2 (*c* 1.58, MeOH); UV (MeOH)  $\lambda_{\max}$  272 nm (log<sub>10</sub> $\epsilon$  4.50). CD (MeOH, 23 °C)  $\lambda$  239 nm ( $\epsilon$  +4.7), 252 (0), 272 (-9.4). <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>, 4:3 mixture of amide rotamers)  $\delta$  major [minor] 7.68 (d, 1H,  $J$  = 14.9 Hz, H-3’’, [7.77]), 7.53 (d,  $J$  = 8.7 Hz, 2H, *p*-Br-Bz), 7.22 (d,  $J$  = 8.7 Hz, 2H, *p*-Br-Bz), 5.54 (d,  $J$  = 14.9 Hz, H-2’’, [5.64]), 4.91 (m, 1H, H-3, [5.02]), 4.72 (m, 1H, H-8a, [3.71]), 4.15 (pent, 1H,  $J$  = 6.8 Hz, H-2, [5.02]), 3.61 (m, 1H, H-7’), 2.30 (s, 3H, SMe, [2.35]), 2.12 (m, 1H, H-4ax, [2.12]), 1.87 (m, 1H, H-8, [1.86]), 1.83 (m, 1H, H-4a, [1.54]), 1.68 (m, 1H, H-8, [1.39]), 1.64 (m, 1H, H-4eq, [1.69]), 1.13 (d, 3H,  $J$  = 6.8 Hz, C-2 Me, [1.33]), 0.93 (t, 3H,  $J$  = 7.3 Hz, H-10’). HRESIMS  $m/z$  630.2222, 632.2207 [M+Na<sup>+</sup>] (calcd for C<sub>31</sub>H<sub>46</sub><sup>79</sup>BrNO<sub>4</sub>SNa, 630.2223).

### Mosher’s Esters **7a,b** – Assignment of the Absolute Configuration at C-7’

(*R*)- and (*S*)- $\alpha$ -Methoxyl- $\alpha$ -(trifluoromethyl)phenylacetyl chloride (3  $\mu$ L, 16  $\mu$ mol) was added to separate solutions of **6** (100  $\mu$ g, 0.16  $\mu$ mol) and DMAP (0.1 mg, 0.8  $\mu$ mol) in pyridine-*d*<sub>5</sub> (30  $\mu$ L). The reactions, monitored by <sup>1</sup>H NMR, were complete in 1 h. The mixtures were dried under reduced pressure, re-dissolved in EtOAc, and the solutions sequentially washed with 0.5 M HCl, saturated NaHCO<sub>3</sub> and brine, then dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. After removal of solvent, the residues were separately purified by HPLC (silica, 250  $\times$  4.6 mm, 20:80 EtOAc-hexanes, 1.5 mL.min<sup>-1</sup>, UV detection  $\lambda$  = 250 nm) to give pure (*S*)-**7a** (35  $\mu$ g) or (*R*)-**7b** (38  $\mu$ g), respectively.

**7a**: (*S*)-Mosher ester: <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>, 4:3 mixture of amide rotamers), partial side-chain assignments:  $\delta$  0.85 (t,  $J$  = 6.8 Hz, H-10’); 1.26, m and 1.19, m (H-9’); 1.58, m and 1.53, m, H-8’); 5.11 (m, H-7’); 1.66, m and 1.60, m (H-6’); 1.28, m, 1.20, m; 1.31, m; 1.27, m, 1.29 (H-5’ to H-3’). The remainder of the <sup>1</sup>H NMR signals of **7a**, exclusive of the MTPA group, were identical to those of **6**. HRESIMS  $m/z$  846.2622 [M+Na<sup>+</sup>] (calcd for C<sub>41</sub>H<sub>53</sub><sup>79</sup>BrF<sub>3</sub>NO<sub>6</sub>SNa, 846.2621).

**7b**: (*R*)-Mosher ester: <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>, 4:3 mixture of amide rotamers)  $\delta$  0.92 (t,  $J$  = 6.8 Hz, H-10’); 1.35, m and 1.19, m (H-9’); 1.67, m and 1.58, m (H-8’); 5.11, m (H-7’); 1.57 (m, H-6’); 1.21, m; 1.15, m; 1.17, m; 1.23, m, 1.14, m (H-5’ to H-3’). The remainder of the <sup>1</sup>H NMR signals of **7b**, exclusive of the MTPA group, were identical to those of **6**. HRESIMS  $m/z$  846.2625 [M+Na<sup>+</sup>] (calcd for C<sub>41</sub>H<sub>53</sub><sup>79</sup>BrF<sub>3</sub>NO<sub>6</sub>SNa, 846.2621).

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

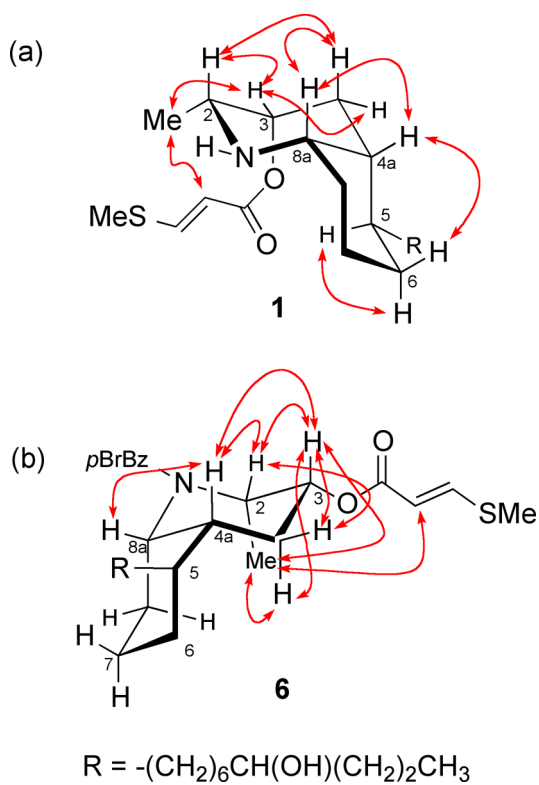
## Acknowledgement.

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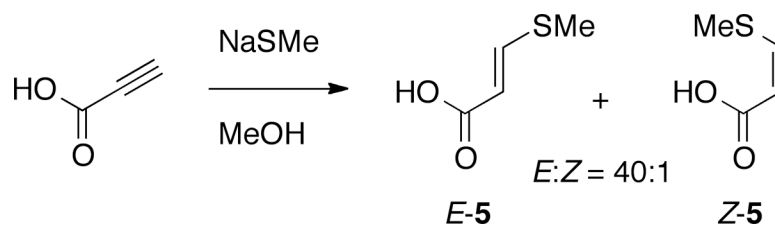
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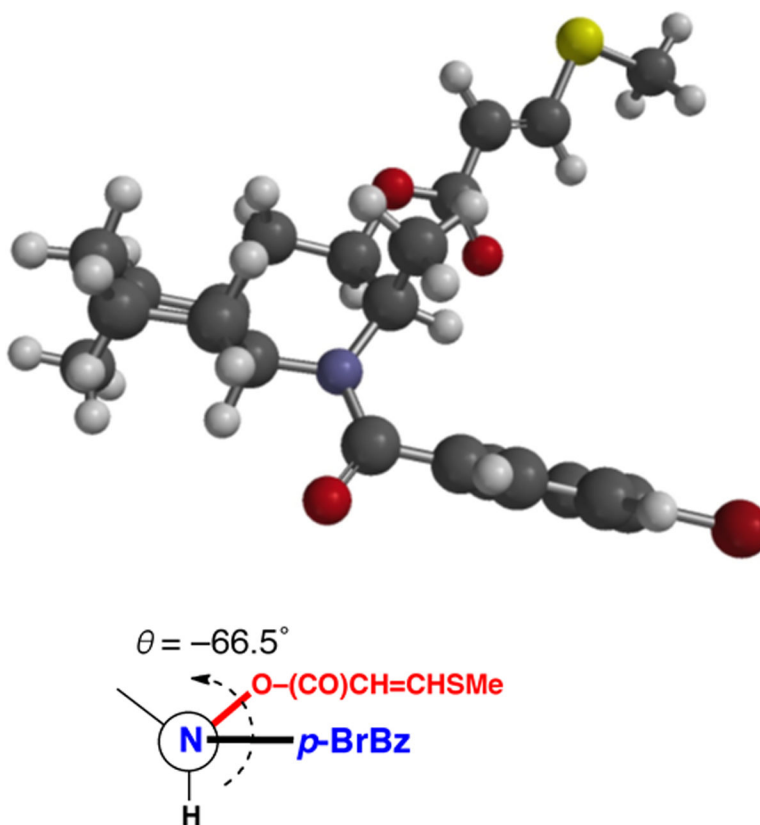
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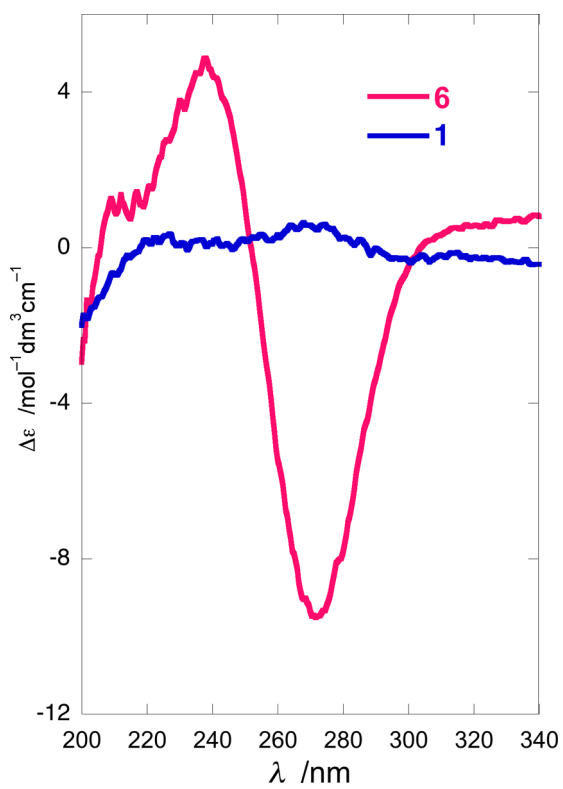
**Figure 1.** NOESY cross peaks (600 MHz,  $\text{CDCl}_3$ ) measured for (a) lepadin I (**1**),  $t_m = 300$  ms, and (b), *N*-*p*-bromobenzoyl-lepadin I (**6**),  $t_m = 400$  ms.



**Figure 2.**  
Preparation of geometrical isomers of 3-methylthioacrylic acid (5).

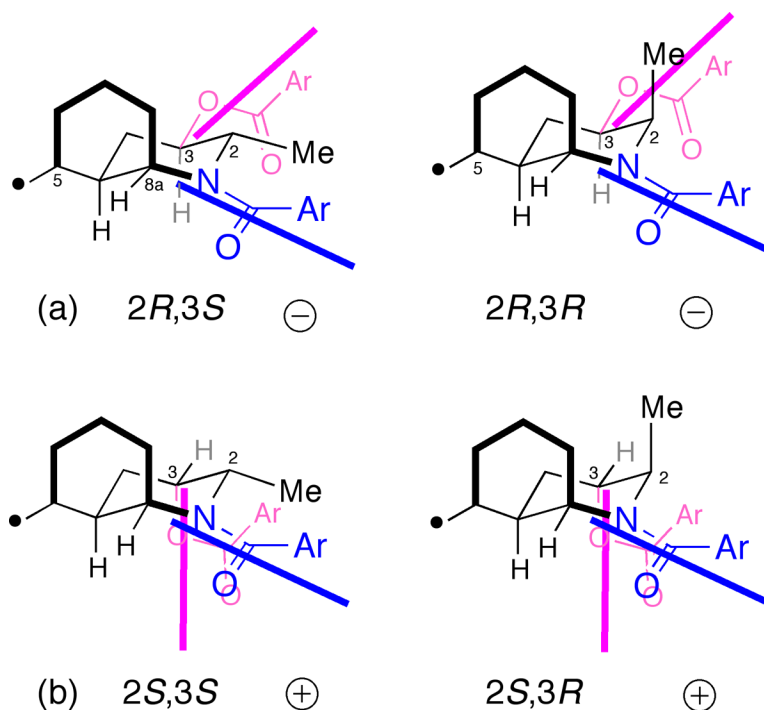


**Figure 3.** (a) Energy minimized molecular model of **6** (Gaussian, B3LYP/6–31+G(d), See Supporting Information). (b) Helicity of the N–(C=O)–Ar and C–3–O bond vectors subtends a negative angle (approximately  $\theta = -66.5^\circ$ ) in **6** corresponding to the  $2R,3R,4aR,5S,8aS$  configuration of natural **1**.

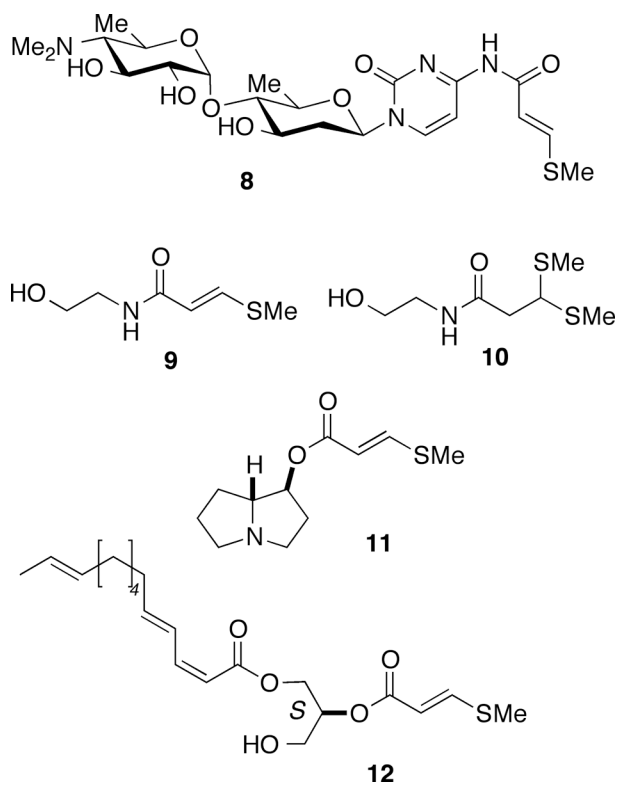


**Figure 4.** CD spectra of lepadin I (**1**), and its *p*-bromobenzamide **6** (MeOH, 23 °C).

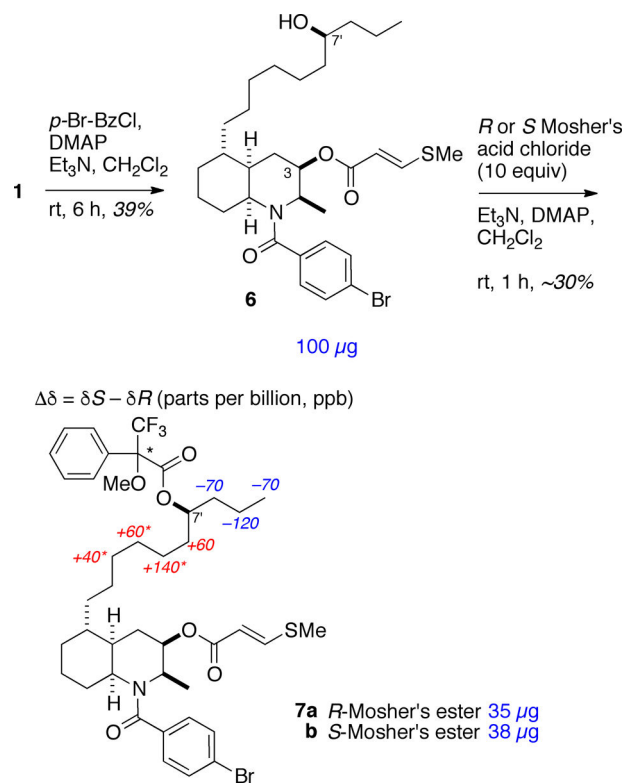




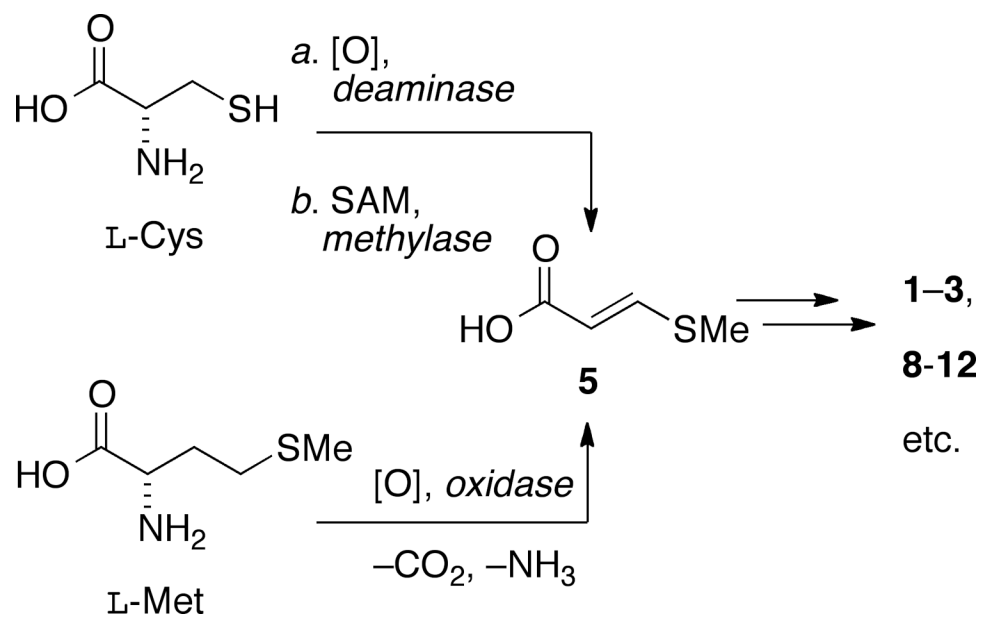
**Figure 5.** Model for prediction of absolute configurations of *N,O*-diarenoyl lepadin derivatives from ECCD. (a)  $2R,3S$  and  $2R,3R$  – both negative split Cotton effects, and (b)  $2S,3S$  and  $2S,3R$  – both positive split Cotton effects.



**Figure 6.** Structures of cytosaminomycin A (**8**), entadamides A (**9**) and B (**10**), planchonelline (**11**) and umbraculum C (**12**).

**Scheme 1.**

Conversion of lepadin I (**1**) to the corresponding benzamide, **6**, and Mosher's esters **7a,b** (*S* and *R* configurations at C\*, respectively), and anisotropic  $^1\text{H}$  NMR shifts ( $\Delta\delta = \delta_S - \delta_R$  in parts per-billion, ppb. See Ref. 12). Due to  $^1\text{H}$  signal overlap, signal assignments labeled \* (confirmed by TOCSY, see SI) are interchangeable.

**Scheme 2.**

Possible origin of 3-methylthioacrylic acid (5) in the biosynthesis of 1-3 and 8-12. The ordering of reactions is arbitrary.

Table 1.

 $^1\text{H}$  and  $^{13}\text{C}$  NMR data for lepadin I (1) (TFA salt,  $\text{CDCl}_3$ ,  $23^\circ\text{C}$ )

	$^1\text{H}^a$ , $\delta$ , mult (J, Hz)	$^{13}\text{C}^b$ $\delta$ (mult)	COSY <sup>a</sup>	NOESY <sup>a</sup>	HMBC <sup>a</sup>
1					
2	3.49, m	55.8 (CH)	9, 3	3, 4 <sub>ax</sub>	9
3	5.04, q (2.8)	67.8 (CH)	2, 4 <sub>ax</sub> , 4 <sub>eq</sub>	2, 4 <sub>ax</sub> , 4 <sub>eq</sub> , 9	1'', 4, 5, 9
4 <sub>ax</sub>	2.48, d (15.6)	28.9 (CH <sub>2</sub> )	3, 4 <sub>eq</sub> , 5	3, 4 <sub>eq</sub> , 4a, 1'	2, 3, 8a, 4a, 5
4 <sub>eq</sub>	1.63, m <sup>c</sup>		3, 4 <sub>ax</sub>	3, 4 <sub>ax</sub> , 4a,	8a, 4a, 5
4a	1.54, m <sup>d</sup>	37.5 (CH)	4 <sub>eq</sub> , 5	4 <sub>ax</sub> , 4 <sub>eq</sub> , 8a, 5, 6 <sub>ax</sub>	3, 8a, 5,
5	1.95, m	34.5 (CH)	4a, 6 <sub>ax</sub> ,	4a, 1', 2', 6 <sub>ax</sub>	4a, 6, 1',
6 <sub>ax</sub>	0.81, qd (12.6, 3.3)	31.2 (CH <sub>2</sub> )	5, 6 <sub>eq</sub> , 7 <sub>eq</sub> , 7 <sub>ax</sub>	4a, 5, 6 <sub>eq</sub> , 7 <sub>ax</sub> , 7 <sub>eq</sub> , 8 <sub>ax</sub>	4a, 5, 7, 8
6 <sub>eq</sub>	1.87, brd (12.6)		6 <sub>ax</sub> , 7 <sub>ax</sub> , 7 <sub>eq</sub>	5, 6 <sub>ax</sub> , 1', 2'	4a, 8,
7 <sub>eq</sub>	1.54, m <sup>d</sup>	19.9 (CH <sub>2</sub> )	6 <sub>eq</sub> , 7 <sub>ax</sub> ,	6 <sub>ax</sub> , 8 <sub>eq</sub> , 7 <sub>ax</sub>	8a, 5
7 <sub>ax</sub>	1.74, qt (13.5, 3.3)		6 <sub>eq</sub> , 6 <sub>ax</sub> , 7 <sub>eq</sub> , 8 <sub>eq</sub> , 8 <sub>ax</sub>	6 <sub>ax</sub> , 8 <sub>eq</sub> , 7 <sub>eq</sub> ,	6, 8
8 <sub>ax</sub>	1.64, m <sup>c</sup>	29.3 (CH <sub>2</sub> )	8a, 8 <sub>eq</sub> , 7 <sub>ax</sub>	6 <sub>ax</sub> , 8a, 8 <sub>eq</sub> ,	8a, 4a, 6, 7
8 <sub>eq</sub>	2.01, d (14.5)		8a, 8 <sub>ax</sub> , 7 <sub>eq</sub>	7 <sub>ax</sub> , 7 <sub>eq</sub> , 8 <sub>ax</sub>	8a, 4a, 6, 7
8a	3.46, m	57.4 (CH)	4a, 8 <sub>ax</sub> , 8 <sub>eq</sub>	4 <sub>ax</sub> , 4a, 8 <sub>ax</sub> , 8 <sub>eq</sub>	7
9	1.31, m <sup>h</sup>	14.8 (CH <sub>3</sub> )	2	3, 2''	2, 3
1''		164.6 (Cq)			
2''	5.74, d (14.9)	111.7 (CH)	4''	4''	2, 1'', 3''
3''	8.07, d (14.9)	150.0 (CH)	3''	3''	1'', 2'', 5''
4''					
5''	2.33, s, 3H	14.2 (CH <sub>3</sub> )			3''
1'	0.92, m <sup>f</sup> 1.36, m <sup>e</sup>	33.1 (CH <sub>2</sub> )	2'	4 <sub>eq</sub> , 5, 6 <sub>eq</sub>	5, 3', 2'
2'	1.07, m 1.31, m <sup>e</sup>	26.1 (CH <sub>2</sub> )	1', 3'	5, 6 <sub>eq</sub>	1', 3'
3'	1.20, m <sup>g</sup>	30.3 (CH <sub>2</sub> )	2', 4'		1', 4', 5'
4'	1.24, m <sup>g</sup>	30.0 (CH <sub>2</sub> )	3'		6', 5', 3',
5'	1.26, m <sup>g</sup> 1.36, m <sup>e</sup>	25.8 (CH <sub>2</sub> )			7',
6'	1.39, m <sup>e</sup>	37.7 (CH <sub>2</sub> )	7'		
7'	3.57, m	71.8 (CH)	6', 8'		5', 9'
8'	1.39, m <sup>e</sup>	39.9 (CH <sub>2</sub> )	7', 9'		
9'	1.35, m <sup>e</sup> 1.44, m <sup>e</sup>	19.3 (CH <sub>2</sub> )	8', 10'		7', 8'
10'	0.91, t (6.8) <sup>f</sup>	14.3 (CH <sub>3</sub> )	9'		8', 9'

	$^1\text{H}^a$ , $\delta$ , mult ( <i>J</i> , Hz)	$^{13}\text{C}^b$ $\delta$ (mult)	COSY <sup>a</sup>	NOESY <sup>a</sup>	HMBC <sup>a</sup>
NH	8.23, bs				

<sup>a</sup> 600 MHz, in the presence of Et<sub>2</sub>NH•TFA buffer (~20 mM).

<sup>b</sup> , 150 MHz, multiplicities assigned by APT and edited HSQC.

<sup>c-h</sup> overlapped signals. *i* signal overlapped.

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**Table 2.**<sup>1</sup>H and <sup>13</sup>C NMR<sup>a</sup> data for lepadin J (**2**) (TFA salt, CDCl<sub>3</sub>, 23° C)

Position	<sup>1</sup> H (mult, <i>J</i> ) <sup>a</sup>	<sup>13</sup> C (mult) <sup>b</sup>	COSY	HMBC
1	8.03, brs		2, 8a	
2	3.53, m	54.0 (CH)	1, 9	
3	5.07, m	68.4 (CH)	2, 4	4a
4	3.12, m; 2.12, m	31.1 (CH <sub>2</sub> )		4a, 5
4a		117.3 (C)		
5		142.4 (C)		
6	2.01, m; 1.85, m	32.8 (CH <sub>2</sub> )	7	
7	1.30, m	22.4 (CH <sub>2</sub> )	6	
8	2.05, m; 1.77, m	26.4 (CH <sub>2</sub> )	8a	4a
8a	3.73, m	55.3 (CH)	8	
9	1.29, m	14.6 (CH <sub>3</sub> )	1, 2	
1''		164.3 (C)		
2''	5.74, d (14.8)	111.3 (CH)	3''	1''
3''	8.08, dd (14.8, 1.5)	149.8 (CH)	2''	1'', 2'', 5''
4''				
5''	2.33, d (1.5)	13.6 (CH <sub>3</sub> )		
1'	2.02, m; 1.85, m	27.1 (CH <sub>2</sub> )		
2'	1.31, m; 1.20, m	27.9 (CH <sub>2</sub> )		
3'	1.24, m <sup>c</sup>	29.3 (CH <sub>2</sub> ) <sup>c</sup>		
4'	1.24, m <sup>c</sup>	29.3 (CH <sub>2</sub> ) <sup>c</sup>		
5'	1.38, m; 1.27, m <sup>c</sup>	25.2 (CH <sub>2</sub> ) <sup>c</sup>		
6'	1.40, m	37.1 (CH <sub>2</sub> )	7'	
7'	3.57, m	71.2 (CH)	6', 8'	
8'	1.41, m	39.3 (CH <sub>2</sub> )	7'	
9'	1.43, m; 1.33, m	18.5 (CH <sub>2</sub> )	10'	
10'	0.91, t (6.7)	13.6 (CH <sub>3</sub> )	9'	8', 9'
NH	8.23, bs			

<sup>a</sup>, 600 MHz.<sup>b</sup>, 150 MHz, multiplicities assigned by edited HSQC.<sup>c</sup>, interchangeable assignments.

**Table 3.**<sup>1</sup>H and <sup>13</sup>C NMR<sup>a</sup> data for lepadin K (**3**) (TFA salt, CDCl<sub>3</sub>, 23° C)

Position	<sup>1</sup> H (mult, <i>J</i> ) <sup>a</sup>	<sup>13</sup> C (mult) <sup>b</sup>	COSY	HMBC
1	8.22, brs		2, 8a	
2	3.35, m	53.1 (CH)	1	
3	5.04, m	67.2 (CH)	2, 4	4a
4	2.24, m; 1.89, m	25.8 (CH <sub>2</sub> )	4a	2, 3, 8a
4a	1.58, m	41.1 (CH)	4, 5, 8a	
5	2.24	39.9 (CH)	4a, 6	
6	2.05, m; 1.10, m	28.9 (CH <sub>2</sub> )	5	
7	1.77, m; 1.47, m	19.3 (CH <sub>2</sub> )	8	
8	2.13, m; 1.92, m	29.3 (CH <sub>2</sub> )	7	4a
8a	3.68, m	59.3 (CH)	4a	
9	1.28, m	14.6 (CH <sub>3</sub> )	2	
1''		164.3 (C)		
2''	5.72, d (14.9)	111.3 (CH)	3''	1''
3''	8.00, dd (14.9)	149.5 (CH)	2''	1'', 2'', 5''
4''				
5''	2.32, s	13.6 (CH <sub>3</sub> )		
1'	1.47, m; 0.96, m	33.0 (CH <sub>2</sub> )		
2'	1.31, m; 1.20, m	27.9 (CH <sub>2</sub> )		
3'	1.24, m <sup>c</sup>	29.3 (CH <sub>2</sub> ) <sup>c</sup>		
4'	1.24, m <sup>c</sup>	29.3 (CH <sub>2</sub> ) <sup>c</sup>		
5'	1.38, m; 1.27, m <sup>c</sup>	25.2 (CH <sub>2</sub> ) <sup>c</sup>		
6'	1.40, m	37.1 (CH <sub>2</sub> )	7'	
7'	3.57, m	71.2 (CH)	6', 8'	
8'	1.41, m	39.3 (CH <sub>2</sub> )	7'	
9'	1.43, m; 1.33, m	18.5 (CH <sub>2</sub> )	10'	
10'	0.91, t (6.7)	13.6 (CH <sub>3</sub> )	9'	8', 9'

<sup>a</sup>, 600 MHz.<sup>b</sup>, 150 MHz, multiplicities assigned by edited HSQC.<sup>c</sup>, interchangeable assignments.