Natural Products

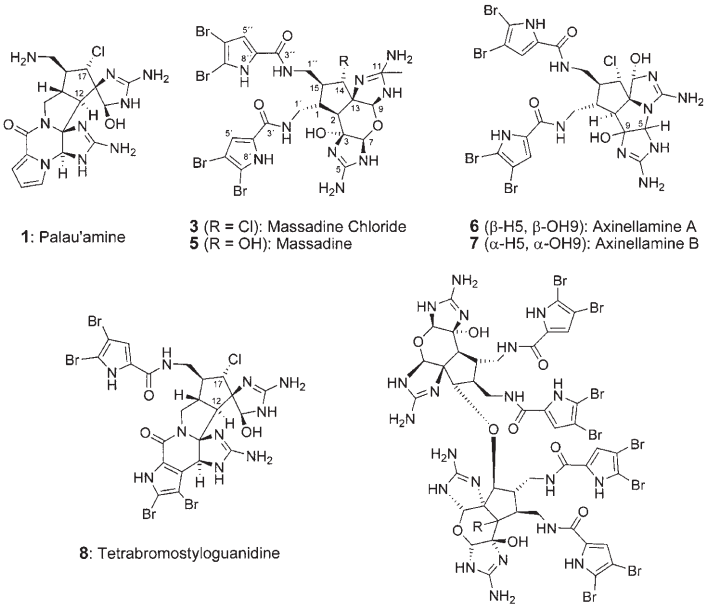
DOI: 10.1002/anie.200701935

Massadine Chloride: A Biosynthetic Precursor of Massadine and Stylissadine**

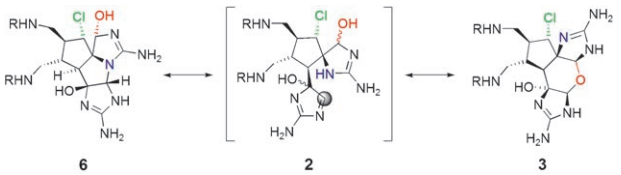
Achim Grube, Stefan Immel, Phil S. Baran, and Matthias Köck**

To date about 30 dimeric pyrrole–imidazole alkaloids (PIAs) are known from the marine-sponge families Agelasidae, Axinellidae, Dictyonellidae, and Hymeniacidonidae.[1] Their structures have encouraged an array of synthetic studies.[1b,2] While their biosynthesis remains a mystery, some interesting proposals have been put forward.[3] Inspired by the reassignment of the relative configuration of palau′amine (**1**),[4] we recently proposed a unified biosynthetic hypothesis which provides a logical rationalization for the origin of all the complex PIAs that contain five-membered rings.[1b] In that proposal, three new entities were described: "preaxinellamine" (**2**), massadine chloride (**3**), and "massadine aziridine" (**4**). Herein, we provide indirect evidence for the existence of both the hypothetical structures **2** and **4**, as well as the first isolation and structural characterization of **3**.

The dimeric PIA massadine (**5**) was isolated in 2003 from the marine sponge *Stylissa* aff. *massa*.[5] This alkaloid is unique among the dimeric PIAs, in that it contains a hydroxy group at C14 compared to a chlorine atom that is common in many PIAs such as in axinellamines A (**6**) and B (**7**)[6] (C13), palau'amine (**1**)[7] (C17), and tetrabromostyloguanidine (**8**)[4a] (C17). Our study began with the goal of determining if massadine (**5**) is actually derived from massadine chloride (**3**) in sponges of the genus *Stylissa*. The isolation of **3** would have two implications for the biosynthesis of the dimeric PIAs. First, it would provide substantial evidence that the biosynthesis of the axinellamines, palau'amines, and styloguanidines occurs via "preaxinellamine" (**2**), where **2** is simply a ring-chain tautomer of **3** and **6** (see Scheme 1). Second, it would explain the existence of both the unusual hydroxy group at C14 of **3** and the most-complex known PIAs stylissadines A (**9**) and B (**10**).



The Caribbean sponge *Stylissa caribica* has emerged as a rich source of monomeric, dimeric, and tetrameric pyrrole–imidazole alkaloids.[4a,8] Very recently the dimeric massadine derivatives stylissadines A (**9**) and B (**10**, tetrameric PIAs), as

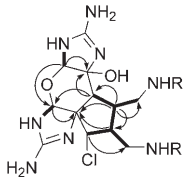


Scheme 1. "Preaxinellamine" (**2**) is the ring-chain tautomer of massadine chloride (**3**) and axinellamine A (**6**).

[*] A. Grube, Priv.-Doz. Dr. M. Köck
Alfred-Wegener-Institut für Polar- und Meeresforschung in der Helmholtz-Gemeinschaft
Am Handelshafen 12, 27570 Bremerhaven (Deutschland)
Fax: (+49) 471-4831-1425
E-mail: mkoeck@awi.de
Priv.-Doz. Dr. S. Immel
Institut für Organische Chemie und Biochemie
Technische Universität Darmstadt
Petersenstrasse 22, 64287 Darmstadt (Deutschland)
Prof. Dr. P. S. Baran
Department of Chemistry
The Scripps Research Institute
10550 North Torrey Pines Road, La Jolla, CA 92037 (USA)
Fax: (+1) 858-784-7375
E-mail: pbaran@scripps.edu

[**] We thank Dr. Michael Assmann for sponge collection and Ellen Lichte for performing preparative HPLC analysis. We gratefully acknowledge support by the Frankfurt Center for Scientific Computing. P.S.B. is grateful to the NIH for financial support (GM-073949).

well as massadine (**5**) were isolated from this sponge.[8c] To answer the question if massadine chloride (**3**) can be found in *Stylissa caribica*, the organism was examined by an HPLC-HRMS screening.[8d] This approach led to the discovery of a compound with m/z 842.8393 ($[M+H]^+$). This value corresponds to the molecular formula $C_{22}H_{24}Br_4ClN_{10}O_4$, identical to that of the axinellamines A (**6**) and B (**7**). The isolated compound was investigated by NMR spectroscopy and the 1D ^{1}H NMR spectrum clearly showed a different chemical structure to that of **6** and **7**. Furthermore, 2D NMR experiments revealed the structure to be that of **3**. The most important ^{1}H,^{1}H-COSY and ^{1}H,^{13}C-HMBC correlations, which were essential for the structure elucidation of **3**, are given in Scheme 2.



Scheme 2. Section of the structure of massadine chloride (**3**); the bold bonds denote ^{1}H,^{1}H-COSY correlations observed for **3**; the arrows indicate selected ^{1}H,^{13}C-HMBC correlations.

The relative configuration of massadine chloride (**3**) was elucidated by comparison of ^{13}C NMR shifts of the eight stereogenic centers with massadine (**5**), which revealed very similar values except for that of C14 (Figure 1). This difference can be explained by the exchange of the hydroxy group with a chlorine atom,

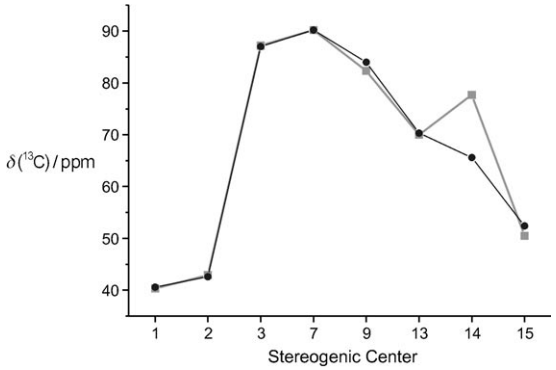


Figure 1. Comparison of $\delta(^{13}C)$ values of the eight stereogenic centers of **3** (black circles) and **5** (gray squares) in [D_6]DMSO. The coherent lines do not represent a mathematical function but give the best overview for the comparison of different compounds.

and was confirmed by increment calculations for aliphatic hydroxy and chloride moieties.[9a] In addition, $^3J_{HH}$ coupling constants[9b] and ROE-derived interproton distances (see the Supporting Information for details) support an identical relative configuration of massadine chloride (**3**) and massadine (**5**). An identical absolute configuration for both compounds is indicated by very similar values for the optical rotation for **3** ($[\alpha]_D^{20} = -14.9$ ($c = 0.49$, MeOH)) and **5** ($[\alpha]_D^{20} = -18.5$ ($c = 0.45$, MeOH[8c])). The absolute configuration of **5** depicted herein is based on the studies of Fusetani and coworkers.[5]

During our NMR investigations of massadine chloride (**3**) a degradation of the compound was observed even in anhydrous [D_6]DMSO. To investigate the chemical stability of **3** in aqueous solutions, various experiments were performed. In a first approach **3** was dissolved in 500 μL of [D_6]DMSO and 10 μL of H_2O, the sample was stored at 40 °C in an NMR tube, and the degradation progress was followed by 1D ^{1}H NMR spectra. Comparison of the ^{1}H and ^{13}C NMR shifts revealed that massadine (**5**) was the product of the reaction between **3** and H_2O (Figure 2).

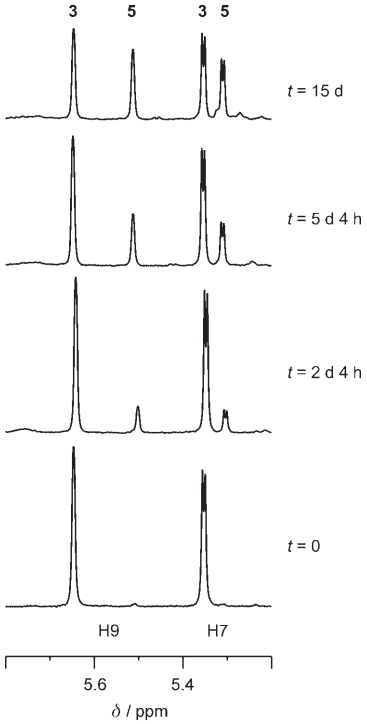


Figure 2. Expanded region (H7 and H9) of the 1D ^{1}H NMR spectra of massadine chloride (**3**) in H_2O/[D_6]DMSO. At $t = 0$ only **3** is observed. The signals that arise result from formation of massadine (**5**).

In a second experiment **3** was dissolved in H_2O and stored at 60 °C. The solution was analyzed by HPLC-HRMS (Figure 3). After 4 h, **3** was completely converted into **5**, which was proven by the change in the isotopic pattern, the retention time, and the accurate mass of massadine (**5**).

Since the relative configuration of the cyclopentane ring in massadine (**5**) and massadine chloride (**3**) is identical, the mechanism of nucleophilic substitution must be considered.

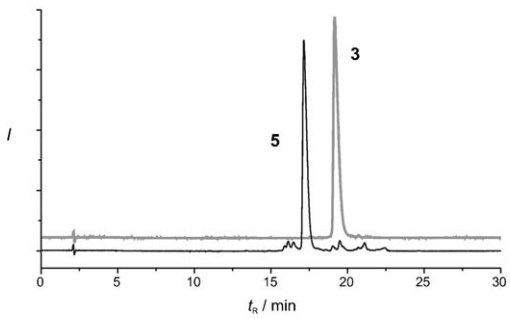
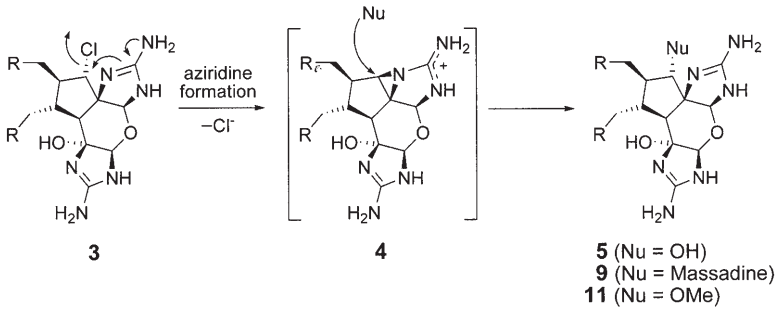


Figure 3. HPLC chromatograms for the reaction of massadine chloride (**3**) in H_2O, for $t = 0$ (gray line) and $t = 4$ h at 60 °C (black line).

We previously put forward a mechanism which hinges upon the formation of an aziridine intermediate and a double inversion of the configuration at C14 (Scheme 3).[1b] In the first step position the nitrogen atom at position 12 attacks the



Scheme 3. Proposed mechanism for the solvolysis of massadine chloride (**3**) in H_2O.

carbon atom at C14 to form an aziridine ring with expulsion of chloride anion. Then, H_2O attacks the carbon atom at C14 from the same face, which results in retention of configuration. Such neighboring-group participation is well precedented. The same behavior is observed for the reaction of **3** with MeOH. The solvolysis product observed by HPLC-HRMS is 14-methoxymassadine (**11**). It seems likely that **3** is converted into **11** via "massadine aziridine" (**4**).

If it is possible to observe such a fast and efficient conversion of **3** into **5**, why do the other dimeric PIAs such as tetrabromostyloguanidine (**8**)[4] or the palau'amines[7] not exhibit a similar reactivity? Indeed, the chemical structures of **8** and **3** are very similar and therefore a related reaction of **8** in H_2O might be expected. In reality, **8** is indefinitely stable at room temperature in [D_6]DMSO and H_2O. In a solution of H_2O at 60 °C, decomposition sets in rapidly. Several unidentified compounds were observed from HPLC-HRMS analysis after short reaction times (4 h) in addition to a signal that corresponded to a hydroxy-substituted product (**8**−Cl + OH, $C_{22}H_{23}Br_4N_{10}O_4$, $\Delta m = 0.1$ ppm). After a longer reaction time, a formal elimination product prevailed that was characterized by its accurate mass (**8**−HCl, $C_{22}H_{21}Br_4N_{10}O_3$, $\Delta m = 1.4$ ppm) and contained 16 instead of 15 degrees of unsaturation. Unfortunately, isolation and structure elucidation by NMR of these products failed because of their low quantities.

Molecular modeling can explain why massadine chloride (**3**) reacts rapidly with H_2O while tetrabromostyloguanidine (**8**) is stable. The molecular structures of **3**, axinellamine A (**6**), and tetrabromostyloguanidine (**8**) were examined based on model compounds optimized from DFT calculations. In these models the dibromopyrrole side chains were replaced by *N*-acetyl residues, whereas the substitution pattern of the central rings was retained in all cases.

The different reactivities of massadine chloride (**3**) and tetrabromostyloguanidine (**8**) are explained in Figure 4. The geometrical disposition and dihedral angle between the nitrogen atom of the imidazole (N12) and the chlorine atom (atoms a, b, c, and d) in massadine chloride (152°) allows an intramolecular displacement via back-side attack of the free electron pair of the nitrogen atom at N12 to the carbon atom at C14 to occur. The structures of axinellamine,[6] and styloguanidine[10]/palau'amine[7] display dihedral angles of 79° and 99° and an almost perpendicular arrangement of the N−C and C−Cl bonds, which prevents chloride displacement. Thus, these compounds retain their characteristic chlorine atoms.

In summary, the novel PIA massadine chloride (**3**) has been isolated and characterized for the first time. Experiments have unambiguously demonstrated that **3** is the biosynthetic precursor of massadine (**5**), most probably via a remarkable aziridine intermediate **4**. Optimized molecular models and experimental evidence (with **8**) provide a clear mechanistic rationale for the existence of a hydroxy group at C14 of massadine (**5**) rather than the characteristic chlorine atom found in other complex PIAs. Taken together, these results strongly implicate "preaxinellamine" (**2**)[1b] as a key intermediate that leads to many of the complex PIAs. Any total synthesis program in this area that is influenced by biosynthetic considerations is likely to benefit from these findings. Efforts to convert massadine chloride (**3**) into the structurally unique tetrameric stylissadines (**9** and **10**) are underway.

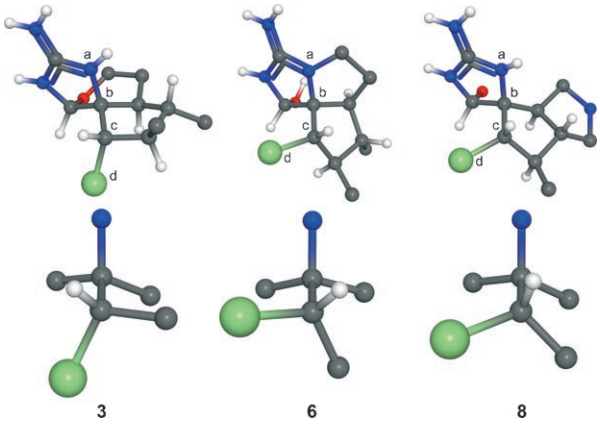


Figure 4. DFT-optimized structures of the model compounds for massadine chloride (**3**), axinellamine A (**6**), and tetrabromostyloguanidine (**8**); The complete structures are shown in the Supporting Information. Top: the central cyclopentane rings with substituents. The dihedral angle of interest is indicated by the atoms a, b, c, and d. Bottom: the dihedral angle of interest presented as Newman projections. The obtained values are: 152° for **3**, 79° for **6**, and 99° for **8**. C black, H white, O red, N blue, Cl green.

Experimental Section

Sponge collection, extraction, and isolation were performed as previously reported.[8a,b] The crude extract and each fraction obtained from Sephadex LH-20 chromatography (eluent: MeOH) were analyzed by HPLC-MS to follow the unknown compound. The isolated compounds were purified by preparative reversed-phase HPLC (Kromasil RP_{18} column (16 × 250 mm, 10 μm), MeCN/H_2O/trifluoroacetic acid gradient) to afford **5** (31.8 mg, 0.03 % of dry weight) and **3** (104.1 mg, 0.11 % of dry weight).

^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AV 400 and AV 600 NMR spectrometers at 25 °C. The ^{1}H,^{1}H-DQF-COSY, ^{1}H,^{13}C-HSQC, ^{1}H,^{13}C-HMBC, and ^{1}H,^{15}N-HSQC experiments were carried out using standard parameters. HPLC-MS analyses were performed with an Agilent 1100 HPLC system and a Bruker Daltonics microTOF$_{LC}$ mass spectrometer. Separation was achieved by a Waters XTerra RP$_{18}$ column (3.0 × 150 mm, 3.5 μm) applying a MeCN/H$_2$O/HCOOH gradient (0 min: 10 % MeCN/90 % HCOOH (0.01 %); 30 min: 60 % MeCN/40 % HCOOH (0.01 %)) with a flow rate of 0.4 mL min^{-1}. UV spectra were recorded during HPLC analysis with a DAD (Agilent). HRMS data were acquired using a Bruker Daltonics microTOF$_{LC}$ mass spectrometer.

3: colorless powder; $[\alpha]_D^{20} = -14.9$ ($c = 0.49$, MeOH); complete NMR data may be found in the Supporting Information; ^{1}H NMR (400.14 MHz, [D$_6$]DMSO, 25 °C): $\delta = 12.77$ (d, $J = 2.4$ Hz, 1 H, H8′), 12.73 (d, $J = 2.5$ Hz, 1 H, H8″), 9.64 (s, 1 H, H4), 9.62 (s, 1 H, H12), 9.57 (s, 1 H, H10), 9.15 (s, 1 H, H6), 8.42 (t, $J = 5.6$ Hz, 1 H, H2″), 8.39 (s, 2 H, 5-NH$_2$), 8.39 (s, 2 H, 11-NH$_2$), 8.08 (t, $J = 5.8$ Hz, 1 H, H2′), 7.53 (s, 1 H, 3-OH), 6.97 (d, $J = 2.7$ Hz, 1 H, H5′), 6.94 (d, $J = 2.7$ Hz, 1 H, H5″), 5.65 (s, 1 H, H9), 5.35 (d, $J = 2.4$ Hz, 1 H, H7), 4.32 (d, $J = 1.5$ Hz, 1 H, H14), 3.71 (m, 1 H, H1′), 3.57 (m, 1 H, H1′), 3.47 (m, 2 H, H1″), 2.46 (m, 1 H, H15), 2.44 (d, $J = 12.3$ Hz, 1 H, H2), 2.20 ppm (m, 1 H, H1); ^{13}C NMR (150.30 MHz, [D$_6$]DMSO, 25 °C): $\delta = 159.6$ (C3′), 159.2 (C3″), 157.2 (C11), 157.1 (C5), 127.8 (C4′), 127.7 (C4″), 113.2 (C5′), 112.8 (C5″), 105.0 (C7′), 104.9 (C7″), 98.1 (C6′), 97.9 (C6″), 90.2 (C7), 87.0 (C3), 84.0 (C9), 70.3 (C13), 65.6 (C14), 52.4 (C5), 42.6 (C2), 41.4 (C1″), 40.6 (C1), 40.4 ppm (C1′); ^{15}N-NMR (40.56 MHz, [D$_6$]DMSO, 25 °C): $\delta = 166$ (N8′), 166 (N8″), 106 (N2″), 105 (N4), 104 (N2′), 100 (N10), 95 (N6), 93 (N12), 76 (5-NH$_2$), 76 ppm (11-NH$_2$); UV (DAD) λ_{max} 280 nm; HPLC/HR(+)ESI-MS: $t_R = 19.8$ min, m/z 842.8393 $[M+H]^+$, calcd for $C_{22}H_{24}{}^{79}Br_4{}^{35}ClN_{10}O_4$, m/z 842.8399, $\Delta m = 0.6$ ppm.

Stability assay for **3** and **8**: each compound (1 mg) was dissolved in H$_2$O (500 μL) in a HPLC vial. Compound **8** was first dissolved in MeOH (50 μL). After dissolution, each sample was measured by HPLC-HRMS. The samples were stored at 60 °C and analyzed by HPLC-HRMS after varying times.

Reaction of **3** with H$_2$O: **3** (5 mg) was dissolved in dry [D$_6$]DMSO (500 μL) in an NMR tube. The sample was stored at 30 °C in an NMR spectrometer. After 48 h, H$_2$O (10 μL) was added and the sample was stored at 40 °C. The reaction was followed by the acquisition of 1D ^{1}H NMR experiments at varying times.

DFT calculations were used to fully optimize the molecular geometries of compounds **3**, **6**, and **8** (with *N*-acetyl side chains). All computations were carried out using the Gaussian03[11] program and applying the Becke–Lee–Young–Parr parameters (B3LYP)[12,13] in combination with the 6-311G + (d,p) basis set.[14] The polarizable continuum (PCM) model for water as a solvent was included in the optimizations of all doubly charged cations. Molecular graphics were prepared using the MolArch$^+$ program.[15]

Received: May 2, 2007
Published online: August 2, 2007

Keywords: natural product · NMR spectroscopy · structure elucidation

[1] a) D. J. Faulkner, *Nat. Prod. Rep.* **2002**, *19*, 1 – 48; b) M. Köck, A. Grube, I. B. Seiple, P. S. Baran, *Angew. Chem.*, DOI: 10.1002/ange.200701798; *Angew. Chem. Int. Ed.*, DOI: 10.1002/anie.200701798.##

[2] a) H. Hoffmann, T. Lindel, *Synthesis* **2003**, 1753 – 1783; b) D. E. N. Jacquot, T. Lindel, *Curr. Org. Chem.* **2005**, *9*, 1551 – 1565.

[3] a) A. Al Mourabit, P. Potier, *Eur. J. Org. Chem.* **2001**, 237 – 243; b) P. S. Baran, D. P. O'Malley, A. L. Zografos, *Angew. Chem.* **2004**, *116*, 2728 – 2731; *Angew. Chem. Int. Ed.* **2004**, *43*, 2674 – 2677; c) B. H. Northrop, D. P. O'Malley, A. L. Zografos, P. S. Baran, K. N. Houk, *Angew. Chem.* **2006**, *118*, 4232 – 4236; *Angew. Chem. Int. Ed.* **2006**, *45*, 4126 – 4130; d) D. P. O'Malley, K. Li, M. Maue, A. L. Zografos, P. S. Baran, *J. Am. Chem. Soc.* **2007**, *129*, 4762 – 4775.

[4] a) A. Grube, M. Köck, *Angew. Chem.* **2007**, *119*, 2372 – 2376; *Angew. Chem. Int. Ed.* **2007**, *46*, 2320 – 2324; b) M. S. Buchanan, A. R. Carroll, R. Addepalli, V. M. Avery, J. N. A. Hooper, R. J. Quinn, *J. Org. Chem.* **2007**, *72*, 2309 – 2317; c) H. Kobayashi, K. Kitamura, K. Nagai, Y. Nakao, N. Fusetani, R. W. M. van Soest, S. Matsunaga, *Tetrahedron Lett.* **2007**, *48*, 2127 – 2129.

[5] S. Nishimura, S. Matsunaga, N. Fusetani, M. Shibazaki, K. Suzuki, K. Furihata, R. W. M. van Soest, *Org. Lett.* **2003**, *5*, 2255 – 2257.

[6] S. Urban, P. de Almeida Leone, A. R. Carroll, G. A. Fechner, J. Smith, R. J. Quinn, J. N. A. Hooper, *J. Org. Chem.* **1999**, *64*, 731 – 735.

[7] R. B. Kinnel, R. Swali, G. Skoropowski, H.-P. Gehrken, P. J. Scheuer, *J. Org. Chem.* **1998**, *63*, 3281 – 3286.

[8] a) A. Grube, E. Lichte, M. Köck, *J. Nat. Prod.* **2006**, *69*, 125 – 127; b) A. Grube, M. Köck, *J. Nat. Prod.* **2006**, *69*, 1212 – 1214; c) A. Grube, M. Köck, *Org. Lett.* **2006**, *8*, 4675 – 4678; d) A. Grube, T. Maier, M. Kostrzewa, M. Köck, *Z. Naturforsch. B* **2007**, *62b*, 600 – 604.

[9] a) Increment calculations show a difference in $\delta(^{13}C)$ values of 13.3 ppm for a secondary alcohol group and a chloride atom which is in agreement with the experimental values for C14 (**3**: 65.6 ppm, **5**: 77.7 ppm). b) The coupling constant between H14 and H15 for the Gaussian-optimized structure of massadine chloride (**3**) is very close to the experimental value (1.6 versus 1.5 Hz), whereas for the structure of 14-epi-massadine chloride, a calculated coupling constant of 9.2 Hz was obtained.

[10] T. Kato, Y. Shizuri, H. Izumida, A. Yokoyama, M. Endo, *Tetrahedron Lett.* **1995**, *36*, 2133 – 2136.

[11] J. A. Pople et al., Gaussian03 (Revision C.02)—Electronic Structure Program, Gaussian, Inc., Wallingford CT, **2004**.

[12] a) A. D. Becke, *Phys. Rev. A* **1988**, *38*, 3098 – 3100; b) A. D. Becke, *J. Chem. Phys.* **1993**, *98*, 5648 – 5652.

[13] a) C. Lee, W. Yang, R. G. Parr, *Phys. Rev. B* **1988**, *37*, 785 – 789; b) B. Miehlich, A. Savin, H. Stoll, H. Preuss, *Chem. Phys. Lett.* **1989**, *157*, 200 – 206.

[14] R. Krishnan, J. S. Binkley, R. Seeger, J. A. Pople, *J. Chem. Phys.* **1980**, *72*, 650 – 654.

[15] S. Immel, MolArch$^+$—Molecular Architecture Modeling Program v9.00, Technical University of Darmstadt, Germany, **2006**.

