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Mollenyne A, a Long-Chain Chlorodibromohydrin Amide from the Sponge *Spirastrella mollis*[§]

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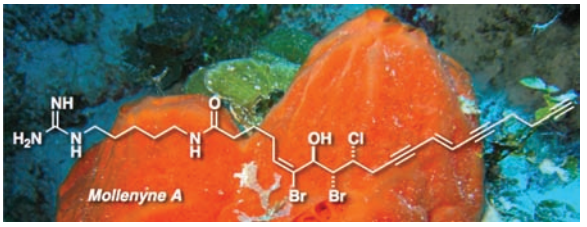
ABSTRACT



The structure of mollenyne A, a cytotoxic nitrogenous halogenated long-chain carboxamide from the sponge *Spirastrella mollis*, was elucidated by integrated spectroscopic analysis, including CD, and chemical conversion.

Brominated lipids commonly occur in several genera of marine sponges including *Petrosia*, *Xestospongia* and *Oceanapia*.[1] Unlike the Rhodophyta (red algae), which are replete with halogenated terpenes and lipids containing myriad combinations of Br, Cl, and sometimes I, Porifera (sponges), suprisingly, rarely contain compounds with *both* Br and Cl. We describe here mollenyne A (**1**) from the sponge *Spirastrella mollis* Verrill, 1907,[2] collected from the Bahamas (Plana Cays) (Figure 1). Mollenyne A (**1**) is a chiral trihalogenated cytotoxic C_{20} carboxamide, embodying three units: a triyne-ene terminus, an allylic alcohol flanked by halogenated carbons, and homoagmatine (decarboxyhomoarginine).[3]

The only other bromochloro natural products from sponges are axinellamine A[4] (**2**) from *Axinella* sp. and tetrabromostyloguanidine (**3**)[5] from *Stylissa caribica* (Figure 2), long-chain terminal vinyl bromochloro azirines (4*E*-**4** and 4*Z*-**5**)[6] from *Dysidea fragilis*, and halogenated cyclohexenones from *Aplysina cavernicola* (e.g., 3-bromo-5-chloroverongiaquinol, **6**),[7] but none resemble **1**. Screening of crude extracts from a collection of Bahamian sponges and tunicates (n = 180) in assays with cultured cancer cells (human colon tumor, HCT-116) identified a highly cytotoxic extract from the red encrusting sponge *Spirastrella mollis* ($IC_{50} \leq 0.1\ \mu g/mL$). Further purification of the CH_2Cl_2 partition from *S. mollis* by C_{18} flash chromatography and reversed-phase HPLC gave a new halogenated lipid, mollenyne A (**1**).

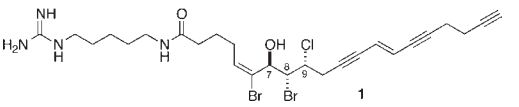


Figure 1. Mollenyne A (**1**) from *Spirastrella mollis*.

[§] Dedicated to Chris M. Ireland on the occasion of his 60th birthday.
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(1) (a) Gribble, G. W. *J. Chem. Educ.* **2004**, *81*, 1441. (b) Gribble, G. W. *Acc. Chem. Res.* **1998**, *31*, 141. (c) Hartung, J. *Angew. Chem., Int. Ed.* **1999**, *38*, 1209.

(2) *S. mollis* may be conspecific with *S. hartmani*. Boury-Esnault, N.; Klautau, M.; Bézac, C.; Wulff, J.; Solé-Cava, A. M. *J. Mar. Biol. Ass. U.K.* **1999**, *79*, 39.

(3) Ramakrishna, S.; Adiga, P. R. *Phytochemistry* **1973**, *12*, 2691.

(4) Urban, S.; Leone, P.; de, A.; Carroll, A. R.; Fechner, G. A.; Smith, J.; Hooper, J. N. A.; Quinn, R. J. *J. Org. Chem.* **1999**, *64*, 731.

(5) Grube, A; Köck, M. *Angew. Chem., Int. Ed.* **2007**, *46*, 2320.

(6) Skepper, C. K.; Molinski, T. F. *J. Org. Chem.* **2008**, *73*, 2592.

(7) D'Ambrosio, M.; Guerriero, A; Pietra, F. *Helv. Chim. Acta* **1984**, *67*, 1484.

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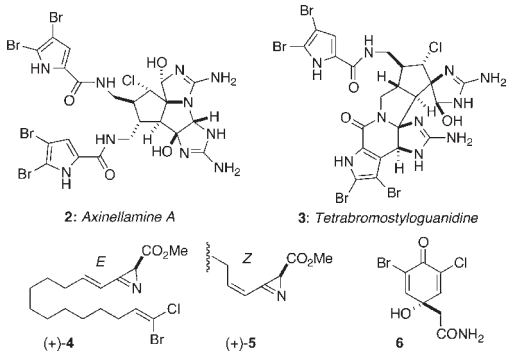


Figure 2. Bromochloro-natural products from sponges.

HRESIMS analysis of **1** revealed the molecular formula of $C_{26}H_{36}O_2N_4Br_2Cl$ (*m*/*z* 629.0888 $[M+H]^+$) with 10 degrees of unsaturation and six exchangable hydrogens (LRESIMS in CD_3OD, *m*/*z* at 635 $[M+H]^+$). Interpretation of 1D and 2D NMR experiments allowed the assignment of five substructures (*A*−*E*, Figure 3; Table 1). Substructure *A* contained a ^1H spin system of three contiguous methylene groups H2−H4 (δ 2.23, t, J = 7.4 Hz, δ 1.79, and δ 2.23, respectively) and terminated with a vinyl proton H5 (δ 6.10, t, J = 7.7 Hz).

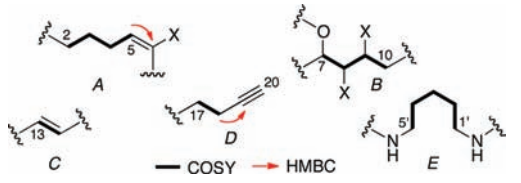


Figure 3. Substructures of **1** (*A*−*E*) assigned from 2D NMR.

C5 (δ 137.3) was connected to a quaternary sp^2 carbon C6 (δ 128.5) by an HMBC cross peak from H5 to C6. COSY and TOCSY correlations showed the oxygenated methine H7 (δ 4.73, d, J = 9.7 Hz) was coupled to methine H8 (δ 4.57, m), and H9 (δ 4.57, m) was coupled to H10 (δ, 2.97, m) leading to substructure *B*.

Substructure *C* of **1** is a *trans* disubstituted alkene (δ 5.91, dt, J = 16.1, 1.9 Hz, H13; 5.97, dt, J = 16.1, 2.0 Hz, H14). Substructure *D* contained the C19−C20 acetylene terminus (δ_H 2.31, t, J = 2.6 Hz, H2O; δ_C 70.5, C2O, CH; 94.6, Cq, C19) linked to a 1,2-disubstituted ethane, H17 (δ 2.54, td, J = 7.2, 2.0 Hz) and H18 (δ 2.39, td, J = 7.2, 2.6 Hz). The final substructure *E* was assigned as a 1,5-diamine unit.

Substructures *A*−*E* were assembled by further analysis of HMBC spectra (Figure 4). *A* and *E* were connected through an amide carbonyl group (δ 175.6, Cl) which showed HMBC correlations to H2 and H1′. Hydroxymethine H7 showed a correlation to C5, establishing the link between substructures *A* and *B*. The C5−C6 double bond was assigned the *E* geometry based on the NOESY

Table 1. ^1H and ^{13}C NMR Data for **1** (CD_3OD, 600 MHz); see Supporting Information for NMR Data in CD_3CN

No.	δ_H, m (*J* in Hz)	δ_C[a]		DQF-COSY	HMBC[b]
1		175.6	C		
2	2.23, t (7.4)	36.4	CH_2	3	1, 3, 4
3	1.79 (m)	26.3	CH_2	2, 4	1, 2, 4, 5
4	2.23 (m)	30.3	CH_2	3, 5	2, 3, 5, 6
5	6.10, t (7.7)	137.3	CH	4	3, 4, 6, 7
6		128.5	C		
7	4.73, d (9.7)	70.8	CH	8	5, 6, 8, 9
8	4.57, m[d]	59.3[c]	CH	7	6, 7, 9, 10
9	4.57, m[d]	59.4[c]	CH	10	7, 8, 10, 11
10	2.97, m	29.6	CH_2	9, 13	8, 9, 11, 12, 13
11		89.9	C		
12		82.6	C		
13	5.91, dt (16.1, 1.9)	122.6	CH	10, 14	14
14	5.97, dt (16.1, 2.0)	120.7	CH	13, 17	13
15		80.4	C		
16		94.6	C		
17	2.54, td (7.2, 2.0)	20.4	CH_2	14, 18	15, 16, 18, 19
18	2.39, td (7.2, 2.6)	19.2	CH_2	17, 20	16, 17, 19, 20
19		83.3	C		
20	2.31, t (2.6)	70.5	CH	18	18
1′	3.18, t (7.2)	40.1	CH_2	2′	1, 2′, 3′
2′	1.54, p (7.2)	30.1	CH_2	1′, 3′	1′, 3′, 4′
3′	1.38, p (7.2)	24.9	CH_2	2′, 4′	1′, 2′, 4′, 5′
4′	1.61, p (7.2)	29.4	CH_2	3′, 5′	2′, 3′, 5′
5′	3.16, t (7.2)	42.4	CH_2	4′	3′, 4′, 6′
6′		158.6	C		

[a] Inferred from DEPT and HSQC, 600 MHz. [b] HMBC correlations, optimized for 8 Hz. Correlations are from H→C. [c] May be interchanged. [d] *J* obscured by overlapping multiplets.

correlation between H4 and H7. H10 and H13 showed correlations to sp carbons C11 (δ 89.9) and C12 (δ 82.6).

Substructure *D* was attached to *C* by a third C−C triple bond [C15 (δ 80.4) and C16 (δ 94.6)]. The left *N*-terminal fragment of **1** was identified as a guanidine group based on the balance of the molecular formula and HMBC correlation from H5′ to C6′ with a characteristic C=N chemical shift (δ 158.6).

Placement of the halogens (two Br and one Cl) was supported by NMR and chemical conversion.[8] A vinyl bromide was favored over a vinyl chloride based on comparison of ^{13}C chemical shifts with known synthetic compounds.[9] The quaternary sp^2 carbon bearing a Br (∼δ 125 ppm) is typically observed upfield of the protonated sp^2 carbon (∼δ 133 ppm) in trisubstituted vinyl bromides ('heavy atom' effect). In vinyl chlorides, the opposite trend is observed; the quaternary sp^2 carbon (∼δ 135 ppm) is observed downfield of the protonated sp^2 carbon (∼δ 125 ppm). Based on ^{13}C chemical shift and chemical transformation (see below) the remaining Br and Cl were placed at C8 and C9, respectively.

(8) In favorable cases, Cl atoms can also be located upon observation of ^{35}Cl,^{37}Cl isotope shifts in ^{13}C NMR. Sergeyev, N. M.; Sandor, P.; Sergeyeva, N. D.; Raynes, W. *J. Magn. Reson., Ser. A* **1995**, *115*, 174.

(9) For synthesis of naturally occurring vinyl bromides and chlorides, see: (a) Trost, B. M.; Pinkerton, A. B. *J. Am. Chem. Soc.* **2002**, *124*, 7376. (b) Lütjens, H.; Wahl, G.; Möller, F.; Knochel, P.; Sundermeyer, J. *Organometallics* **1997**, *16*, 5869. (c) Hodgson, D. M.; Arif, T. *J. Am. Chem. Soc.* **2008**, *130*, 6500.

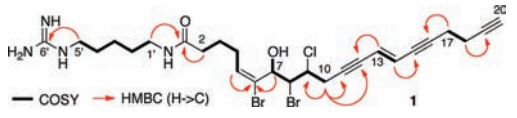


Figure 4. Assembly of substructures *A*–*E* of **1** through COSY and HMBC correlations.

To assign the relative configuration of mollenyne A (**1**), we turned to *J*-based configurational analysis (JBCA), established by Murata et al. for hydroxylated polyketides.[10] The stereotriad of **1** resembles those found in chlorosulfolipids[11] isolated from Adriatic shellfish,[12] freshwater algae,[13] and marine cyanobacteria.[14] Recently, the Carreira group completed a meticulous investigation of the homo- and heteronuclear two- and three-bond coupling constants in a series of chlorosulfolipid models[15] and demonstrated that subtle differences should be considered when analyzing spin systems of polychlorinated compounds compared to those of polyhydroxylated ketides. The former more reliable basis sets were used as a starting point for JBCA analysis of **1**.

The C8–C9 *threo* configuration was assigned by JBCA in a straightforward manner (Figure 5, conformer ***c***). H8 and H9 were oriented gauche based on a small vicinal homonuclear coupling (J = 1.6 Hz); however the low magnitude lead to inefficient magnetization transfer and we were unable to observe $^2J_{HC}$ and $^3J_{HC}$ coupling constants by HETLOC or HSQC-HECADE experiments. Consequently, we turned to *J*-resolved HMBC that revealed the magnitudes of all heteronuclear couplings were small (Figure 5). The vicinal Br and Cl were oriented *anti* to H9 and H8, respectively, and C7 and C10 were assigned *gauche* to H9 and H8, respectively. H7 and H8 were oriented *anti* based on a large H–H vicinal coupling (J = 9.0 Hz), and HSQC-HECADE revealed that both $^2J_{HC}$ H7–C8 and H8–C7 have large couplings. These data are accommodated by either diastereomer *threo* ***a*** or *erythro* ***b*** (Figure 5), an ambiguity that was resolved by chemical conversion as follows.

Mollenyne A (**1**) underwent hydrogenation (H_2, Pd/C, MeOH, 3 days, Scheme 1), with concomitant hydrogenolysis of the vinyl bromide to give **7**. Exposure of the product **7** to base (K_2CO_3, MeOH) gave epoxide **8**[16] after neutralization

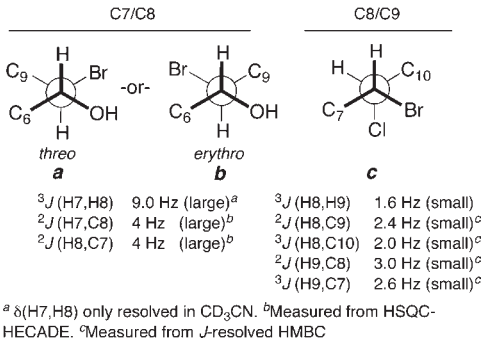
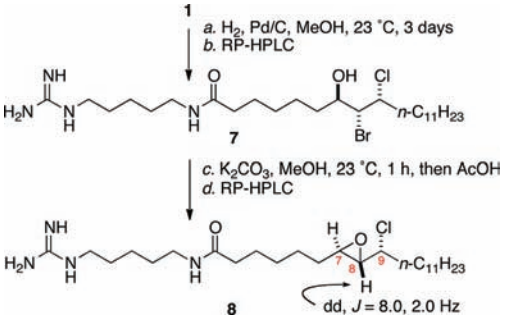


Figure 5. $^3J_{HH}$, $^2J_{HC}$, and $^3J_{HC}$ coupling constants for C7–C9 of **1** (600 MHz, CD_3CN).

(AcOH) and HPLC purification. The vicinal H7–H8 coupling constant (J = 2 Hz) confirmed a *trans* epoxide. Accounting for inversion at C8 during epoxide ring closure by S_N2 substitution, the relative configuration of the C7–C8 stereocenters in **1** was revealed as *erythro* ***b*** (Figure 5).

Scheme 1. Conversion of **1** to Chloroepoxide **8**



Assignment of the carbinol center C7 and completion of the absolute stereostructure of **1** were achieved by conversion of the latter to chromophoric derivatives and application of the exciton chirality CD (ECCD) method. Nakanishi reported[17] that cyclic and acyclic allylic alcohols can be assigned by simple interpretation of ECCD of their corresponding benzoate derivatives; the latter arises from exciton coupling between π–π^* transitions of the *O*-benzoate and C=C double bond.

In principle, this analysis is applicable to an *O*-benzoyl derivative of **1**; however, additional contributions were anticipated from long-range EC with a second chromophore: the C11–C16 conjugated yne-ene-yne.

Mollenyne A (**1**) was benzoylated under standard conditions (Bz-Cl, pyridine), and the CD spectrum of the

(10) Matsumori, N.; Kaneno, D.; Murata, M.; Nakamura, H.; Tachibana, K. *J. Org. Chem.* **1999**, *64*, 866.

(11) Bedke, D. K.; Vanderwal, C. D. *Nat. Prod. Rep.* **2010**, *28*, 15.

(12) (a) Ciminiello, P.; Fattorusso, E.; Forino, M.; Di Rosa, M.; Ianaro, A.; Poletti, A. *J. Org. Chem.* **2001**, *66*, 578. (b) Ciminiello, P.; Dell'Aversano, C.; Fattorusso, E.; Forino, M.; Magno, S.; Di Rosa, M.; Ianaro, A.; Poletti, R. *J. Am. Chem. Soc.* **2002**, *124*, 13114. (c) Ciminiello, P.; Dell'Aversano, C.; Fattorusso, E.; Forino, M.; Magno, S.; Di Meglio, P.; Ianaro, A.; Poletti, R. *Tetrahedron* **2004**, *60*, 7093.

(13) (a) Mercer, E. I.; Davies, C. L. *Phytochemistry* **1974**, *13*, 1607. (b) Mercer, E. I.; Davies, C. L. *Phytochemistry* **1975**, *14*, 1545. (c) Mercer, E. I.; Davies, C. L. *Phytochemistry* **1979**, *18*, 457.

(14) (a) Chen, J. L.; Proteau, P. J.; Roberts, M. A.; Gerwick, W. H.; Slate, D. L.; Lee, R. H. *J. Nat. Prod.* **1994**, *57*, 524. (b) Bedke, D. K.; Shibuya, G. M.; Pereira, A. R.; Gerwick, W. H.; Vanderwal, C. D. *J. Am. Chem. Soc.* **2010**, *132*, 2542.

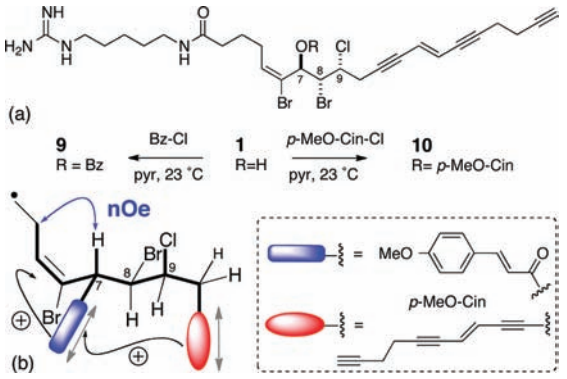
(15) Nilewski, C.; Geisser, R. W.; Ebert, M.-O.; Carreira, E. M. *J. Am. Chem. Soc.* **2009**, *131*, 15866.

(16) HRESIMS *m*/*z* 487.3776 $[M+H]^+$ (calcd for $C_{26}H_{52}ClN_4O_2$ 487.3773). Sequential elimination of two molecules of HBr upon conversion of **1** to **8** confirmed the location of the halogens in **1**.

(17) (a) Harada, N.; Iwabuchi, J.; Yokota, Y.; Uda, H.; Nakanishi, K. *J. Am. Chem. Soc.* **1981**, *103*, 5590. (b) Gonnella, N. C.; Nakanishi, K.; Martin, V. S.; Sharpless, K. B. *J. Am. Chem. Soc.* **1982**, *104*, 3775.

(18) CD and UV spectra were normalized using literature ε values for an ene–yne–ene chromophore. λ_{max} 261 nm (ε 32 000), 276 nm (ε 30 500). Crombie, L.; Jacklin, A. G. *J. Chem. Soc.* **1957**, 1632.

Scheme 2. Conversion of **1** to the Corresponding Benzoate (**9**) and *p*-Methoxycinnamate (**10**) Esters, and the Major Conformer Accounting for the Observed ECCD Spectra



HPLC-purified benzoate ester **9** was acquired in MeOH (Scheme 2 and Figure 6). Two positive Cotton effects (CEs) associated with the benzoate chromophore [λ 227 nm ($\Delta\varepsilon$ +6.1)] and yne-ene-yne chromophore[18] [λ 260 nm ($\Delta\varepsilon$ +7.5), 275 nm ($\Delta\varepsilon$ +6.5)] were observed and ascribed to separate EC effects. The positive CE at λ 227 nm was assigned as the high-energy component of the exciton couplet arising from positive helicity between the double bond (λ ~190 nm) and the benzoate chromophore (λ = 227 nm) (Scheme 2). The negative CE was expected from the C=C π–π* contribution but was obscured by solvent (MeOH, cutoff ~200 nm). A positive split CE between the yne-ene-yne chromophore and the benzoate was assigned to a positive helicity; however, the expected negative CE at λ = 227 nm is canceled by the positive component of the allylic benzoate EC couplet.

To remove the ambiguity and 'red-shift' the ECCD interactions, mollenyne A (**1**) was derivatized with *para*-methoxycinnamoyl chloride to give **10** (Scheme 2). Gratifyingly, a clear bisignate CE emerged [Figure 6, λ 262 nm ($\Delta\varepsilon$ −6.6); ~296 nm ($\Delta\varepsilon$ +10.8)] that was assigned to a positive helicity of the allylic *O*-cinnamate ester. Consequently, **10** and **1** have the 7*S*,8*R*,9*R* configuration. The latter assignment was verified by calculation of mimimized structures of a truncated benzoate model of **9** (C4 to C12, Spartan, MMFF). The lowest energy conformer (65% of Boltzmann-weighted population) of **1** was consistent with the solution structure predicted from NMR and CD studies and fully supports the 7*S*,8*R*,9*R* assignment.

Few compounds have been reported from the genus *Spirastrella*,[20] including cytotoxic macrolides, spirastrellolides A–G,[20a–c] sphingosine sulfates,[20d] and halogenated hydrocarbons.[20e] An account of the biosynthesis of **1**, the

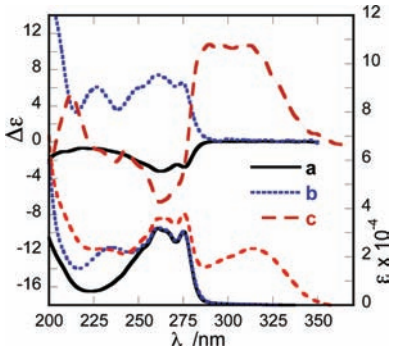


Figure 6. UV (lower) and CD (upper) spectra (MeOH, 23 °C) for (a) **1**, (b) **9**, and (c) **10**. $\Delta\varepsilon$ for **1** and **9** were normalized to an ene–yne–ene chromophore[18] and that for **10** was normalized to *p*-methoxycinnamate.[19]

first natural products reported from *S. mollis*, must explain two uncommon features: the rare homoagmatine (1-amino-5-*N*-guanidino) group,[21] which likely arises from decarboxylation of arginine,[3] and the chlorodibromohydrin in the C_{20} carboxamide segment that is without precedent among sponge metabolites. Compound **1** exhibited significant cytotoxicity against human colon tumor cells (HCT-116; IC_{50} = 1.3 μg/mL; etoposide = 0.55 μg/mL).

In conclusion an unusual optically active chlorodibromohydrin, mollenyne A (**1**), was obtained from the marine sponge *Spirastrella mollis*, and its complete stereostructure was solved by an integrated approach employing NMR, MS, CD, and chemical synthesis.

Acknowledgment. We thank S. Zea (INVAMAR, University of Columbia) for identification of the sponge; J. Pawlik (University of North Carolina, Wilmington) and the crew of the *RV* Seward Johnson for logistics of sponge collection, Y. Su for HRMS measurements; T. Handel, A. Jansma, and X. Huang (all UCSD) for assistance with NMR experiments; and D. Dalisay for assistance with biological assays. We also thank an anonymous reviewer for the reference to $^{37}Cl/^{35}Cl$ isotope effects on ^{13}C NMR (ref 8). The 500 MHz NMR spectrometers were purchased with a grant from the NSF (CRIF, CHE0741968). B.I.M. is grateful for a Ruth L. Kirstein National Research Service Award NIH/NCI (T32 CA009523). This work was supported by the NIH (AI039987, CA122256).

Supporting Information Available. Experimental procedures and full spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

(19) λ_{max} 311 nm (ε 24 000).Wiesler, W. T.; Nakanishi, K. *J. Am. Chem. Soc.* **1990**, *112*, 5574.

(20) (a) Williams, D. E.; Keyzers, R. A.; Warabi, K.; Desjardine, K.; Riffell, J. L.; Roberge, M.; Andersen, R. J. *J. Org. Chem.* **2007**, *72*, 9842. (b) Williams, D. E.; Lapawa, M.; Feng, X.; Tarling, T.; Roberge, M.; Andersen, R. J. *Org. Lett.* **2004**, *6*, 2607. (c) Williams, D. E.; Roberge, M.; Van Soest, R.; Andersen, R. J. *J. Am. Chem. Soc.* **2003**, *125*, 5296. (d) Alam, N.; Wang, W.; Hong, J.; Lee, C.-O.; Im, K. S.; Jung, J. H. *J. Nat. Prod.* **2002**, *65*, 944. (e) Martín, M. J.; Berrué, F.; Amade, P.; Fernández, R.; Francesch, A.; Reyes, F.; Cuevas, C. *J. Nat. Prod.* **2005**, *68*, 1554.

(21) Homoagmatine has also been observed in the sponge metabolite clavatadine from a species of *Suberea*. Buchanan, M. S.; Carroll, A. R.; Wessling, D.; Jobling, M.; Avery, V. M.; Davis, R. A.; Feng, Y.; Hooper, J. N.; Quinn, R. J. *J. Nat. Prod.* **2009**, *72*, 973.