ORGANIC LETTERS

2011
Vol. 13, No. 5
1052–1055

Elaiomycins B and C: Alkylhydrazide Antibiotics from *Streptomyces* sp. BK 190

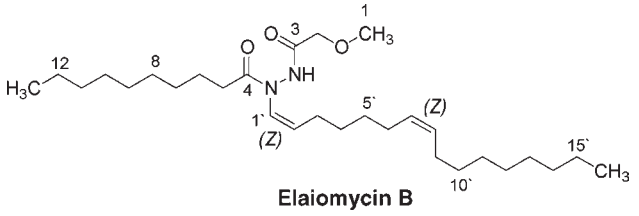
Soleiman E. Helaly,[†,§] Alexander Pesic,[†] Hans-Peter Fiedler,[*,‡] and Roderich D. Süssmuth[*,†]

Institut für Chemie, Technische Universität Berlin, 10623 Berlin, Germany, Mikrobiologisches Institut, Universität Tübingen, 72076 Tübingen, Germany, and Department of Chemistry, Faculty of Science, South Valley University, Aswan 81528, Egypt

suessmuth@chem.tu-berlin.de; hans-peter.fiedler@uni-tuebingen.de

Received December 22, 2010

ABSTRACT



Elaiomycin B

Two novel alkyhydrazides, elaiomycins B and C, together with the azoxy antibiotic elaiomycin were isolated from *Streptomyces* sp. BK 190. The structures were established by 1D- and 2D-NMR spectroscopy including ^{15}N NMR studies and high-resolution orbitrap-ESI-mass spectrometry.

Elaiomycin [[(2*S*,3*S*)-3-hydroxy-1-methoxybutan-2-yl]-imino-oct-1-enyloxidoazanium] is a natural compound described in 1954 from submerged culture filtrates of *Streptomyces gelaticus*. The gross structure of elaiomycin was elucidated by Stevens et al.[1] containing the chemically unique aliphatic α,β-unsaturated azoxy group. Elaiomycin exhibits an unusual bioactivity as it exhibits a strong *in vitro* inhibition of virulent and avirulent forms of the bovine and human strains of *Mycobacterium tuberculosis*.[2] However, the compound has been found to induce tumors in rats[3] and to be therapeutically ineffective at a subtoxic dosage against experimental tuberculosis infections of mice and rats.[2] Elaiomycin is the most prominent representative of a small number of naturally occurring azoxy compounds, all of which exhibit biological activity.

Herein we report on the structural determination of two novel alkylhydrazides, named elaiomycins B and C, produced by *Streptomyces* sp. BK 190 and the full two-dimensional NMR data set of elaiomycin including ^{15}N NMR spectroscopy. The taxonomy of the producing strain, its fermentation, isolation, and biological activity of elaiomycins B and C will be reported in a further publication; a brief description of the fermentation and the purification is given in the Supporting Information.

Compound **1** was obtained as a colorless oil. The molecular ions of *m*/*z* 259.1 $[M + H]^+$ and *m*/*z* 281.1 $[M + Na]^+$ in the ESI-MS spectrum of **1** revealed a molecular mass of 258.1 g/mol. The molecular formula of **1** was established as $C_{13}H_{26}N_2O_3$ by determining the exact molecular mass (*m*/*z* 259.20077, $[M + H]^+$) derived from the high-resolution orbitrap-ESI-MS spectrum (*m*/*z* calcd 259.20217, Δ*m* −3.31 ppm). A database search for the molecular mass was in accordance with the previously reported antibiotic elaiomycin,[1] upon which, however, to our knowledge no two-dimensional NMR spectroscopic data were available.[4,5] Subsequent ^1H NMR and ^{13}C NMR spectroscopy, including DEPT and $^1H-^{13}C$-HSQC spectra,

[†] Technische Universität Berlin.
[§] South Valley University.
[‡] Universität Tübingen.

(1) (a) Haskell, T. H.; Ryder, A.; Bartz, Q. R. *Antibiot. Chemother.* **1954**, *4*, 141–144. (b) Stevens, C. L.; Gillis, B. T.; French, J. C.; Haskell, T. H. *J. Am. Chem. Soc.* **1956**, *78*, 3229–3230. (c) Stevens, C. L.; Gillis, B. T.; French, J. C.; Haskell, T. H. *J. Am. Chem. Soc.* **1958**, *80*, 6088–6092. (d) Stevens, C. L.; Gillis, B. T.; Haskell, T. H. *J. Am. Chem. Soc.* **1959**, *81*, 1435–1437. (e) Taylor, K. G.; Riehl, T. *J. Am. Chem. Soc.* **1972**, *94*, 250–255.

(2) (a) Ehrlich, J.; Anderson, L. E.; Coffey, G. L.; Feldman, W. H.; Fisher, M. W.; Hillegas, A. B.; Karlson, A. G.; Kaudsen, M. P.; Weston, J. K.; Youmans, A. S.; Youmans, G. P. *Antibiot. Chemother.* **1954**, *4*, 338–342. (b) Karlson, A. G. *Antibiot. Chemother.* **1962**, *12*, 446–449.

(3) Schoental, R. *Nature* **1969**, *221*, 765–766.

(4) Parry, R. J.; Mueller, J. V. *J. Am. Chem. Soc.* **1984**, *106*, 5764–5765.

(5) Moss, R. A.; Matsuo, M. *J. Am. Chem. Soc.* **1977**, *99*, 1643–1645.

10.1021/ol1031014
Published on Web 02/10/2011

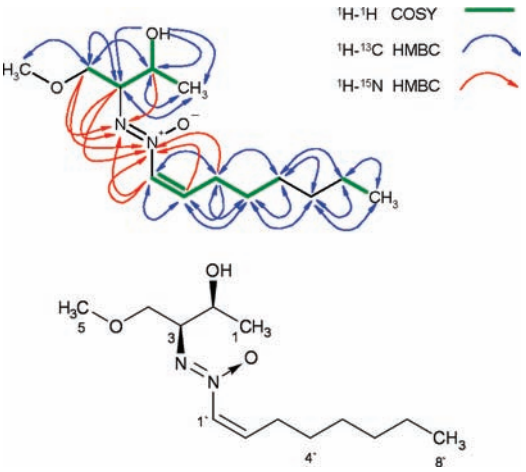


Figure 1. Elaiomycin (**1**) structure and 2D-NMR connectivities.

of **1** confirmed the presence of 26 protons and 13 carbon atoms (Supporting Information, Table S2). Signals were assigned to three methyl groups including a methoxy group at δ_H 3.19 (3H, s), δ_C 58.5; six methylene groups including one oxygen-bound methylene at δ_H 3.50, 3.58 (2H, dd, J = 3.9, 7.2, 3.9, 7.2 Hz), δ_C 70.3; two olefinic protons at δ_H 6.85 (1H, d, J = 9.0 Hz), δ_C 136.4, δ_H 5.78 (1H, dt, J = 7.9, 8.5 Hz), δ_C 132.8; two methine protons at δ_H 4.27 (1H, m), δ_C 64.5, δ_H 3.86 (1H, m), δ_C 64.9, and one OH group at δ_H 4.79. Detailed analysis of ^{1}H−^{1}H-COSY and ^{1}H−^{13}C-HMBC spectra rendered two structural moieties (C-1−C-5 and C-1′−C-8′, Figure 1). Since neither ^{1}H−^{1}H-COSY nor ^{1}H−^{13}C-HMBC connectivities were observed from C-3 with the olefinic chain C-1′−C-8′, we expected that the two moieties were connected by two nitrogen atoms, which is also implied by the molecular formula. A characteristic band at 1467 cm^{-1} in the IR spectrum of **1** (Supporting Information, Figure S10) suggests an azoxy group. The ^{1}H−^{15}N-HMBC experiment confirmed the presence of two nitrogen atoms at δ_N 336.2 ppm (N-2, N(O)) and δ_N 360.3 ppm (N-1, N), respectively. Furthermore, the ^{1}H−^{15}N-HMBC correlations from the olefinic protons H-1′ to both nitrogens as well as correlations from H-2′ to N-2 and the correlations from H-2, H-3, H-4 to N-1 as well as from H-3 to N-2 clearly confirmed a diimine-type structure. Hence, N-2 is directly connected to the olefinic chain and N-1 is connected to position 3 of a 4-methoxybutan-2-ol moiety (C-1−C-5). Consequently our NMR data confirm the previous structural assignment of **1** as elaiomycin (Figure 1). The (2*S*,3*S*) configuration of the stereocenters of compound **1** was deduced from the optical rotation values $[\alpha]^{23}_D$ +33.3 (*c* 2.8, EtOH), which were in good accordance with that determined previously for elaiomycin {$[\alpha]^{26}_D$ +38.4 (*c* 2.8, EtOH)}.[1a] Furthermore, The IR spectrum as well as the UV maximum of **1** was identical with those of elaiomycin.[1a,b]

Examination of the literature showed that the azoxy configuration of elaiomycin is tentative;[6] the NOESY spectrum of **1** revealed the azoxy group rather suggests a cis-configuration, since no NOEs correlations were observed between H-1′/H-2′ and H-2/H-3 or H-4/H-5, which would likely be expected in a trans-configuration (Supporting Information, Figure S8).

Compound **2** was obtained as a colorless oil. ESI-MS of **2** showed signals at m/z 479.5 $[M + H]^+$ and at m/z 501.5 $[M + Na]^+$. The exact molecular mass of **2** derived from the high-resolution orbitrap-ESI-MS spectrum m/z 479.42053 $[M + H]^+$ (m/z calcd 479.41344, Δm −0.35 ppm) gave a molecular formula of $C_{29}H_{54}N_2O_3$. The four degrees of unsaturation implied by the molecular formula were accounted for by two carbonyl groups and two olefinic double bonds, whereas the ^{13}C NMR including DEPT revealed the presence of 29 carbon atoms including two carbonyl carbons at δ_C 167.8 (C-3), δ_C 173.4 (C-4), four olefinic carbons at δ_C 124.4 (C-1′), δ_C 117.4 (C-2′), δ_C 129.7 (C-7′), and δ_C 129.8 (C-8′), 20 methylene groups including oxygen-bound methylene at δ_C 70.9 (C-2), as well as three methyl groups including a methoxy group at δ_C 58.5 (C-1). HSQC-NMR data established all ^1J ^{1}H−^{13}C connectivities (Supporting Information, Table S3). The key structural features revealed by ^{1}H NMR were a doublet at δ_H 6.39 (H-1′, d, J = 9.5 Hz) and a doublet of triplet at δ_H 4.75 (H-2′, dt, J = 9.2, 7.2 Hz), which both were assigned to one olefinic double bond (H-1′/H-2′). A triplet at δ_H 5.31 (H-7′, H-8′, m, J = 4.8 Hz) was assigned to two protons of another olefinic double bond (H-7′/H-8′). ^{1}H−^{1}H-COSY and ^{1}H−^{13}C-HMBC revealed a set of four methylene groups located between the above-mentioned double bonds which was assigned as δ_H 2.02 (H-3′, m), δ_H 1.26 (H-4′, m), δ_H 1.27 (H-5′, m), δ_H 1.96 (H-6′, dt, J = 5.8, 6.4 Hz). Finally, a singlet at δ_H 3.96 (H-2, s) was assigned to the oxygen-bound methylene group (C-2), a characteristic singlet at δ_H 3.34 (H-1, s) was assigned to the methoxy group C-1, and a group of prominent though overlapping signals between 1.20 and 1.27 ppm was assigned to the aliphatic methylene chains (C-7−C-12) and (C-10′−C-15′), respectively.

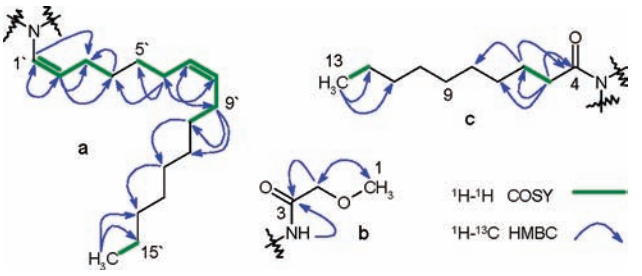


Figure 2. Substructures of elaiomycin B (**2**) and selective 2D-NMR connectivities.

(6) (a) McGahren, W. J.; Kunstmann, M. P. *J. Am. Chem. Soc.* **1970**, *92*, 1587–1590. (b) McGahren, W. J.; Kunstmann, M. P. *J. Org. Chem.* **1972**, *37*, 902–906.

Moreover $^{1}H-^{1}H$-COSY connectivities (Figure 2) from H-8′ to H-9′, H-9′ to H-10′ and $^{1}H-^{13}C$-HMBC connectivities from H-7′ to C-9′, H-9′ to C-10′/C-11′, H-10′ to C-12′, H-12′ to C-14′ and from the terminal methyl group H-16′ to C-14′/C-15′ allowed the construction of the olefinic chain C-1′−C-16′ (Figure 2a). Furthermore, $^{1}H-^{13}C$-HMBC connectivities from the methoxy methyl H-1 to the oxygen-bound methylene C-2, from H-2 to C-1/C-3, and from N*H* (δ_H 10.65, s, NH) to C-3 suggested the presence of a 2-methoxyacetamide fragment C-1−NH (Figure 2b). Additionally, HMBC connectivities were from H-5 (δ_H 2.33, t, J = 7.5 Hz) to C-4/C-6 (supported by $^{1}H-^{1}H$-COSY)/C-7, H-6 to C-4/C-8, and from H-13 (δ_H 0.84, t, J = 7.4 Hz) to C-11/C-12 referring to an aliphatic chain (C-4−C-13) (Figure 2c). In this context, orbitrap-MS-MS spectra of elaiomycin B (**2**) revealed characteristic fragment peaks at m/z 155.154 and 183.148 assigned to a decanoyl ion and a 1-oxo-decane-1-diazonium ion, respectively, which confirmed the length of the decanoyl chain in compound **2** (Supporting Information, Figure S28).

^{15}N NMR experiments were established to determine the positions of the nitrogen atoms as well as the connectivities of the three parts of the molecule. The lack of the characteristic azoxy band at 1467 cm^{-1} in the IR spectrum of **2** (Supporting Information, Figure S20) and the appearance of a band at 1692 cm^{-1} suggested a hydrazide group. $^{1}H-^{15}N$-HSQC-NMR data of **2** indicate the presence of two nitrogen atoms at δ_N 140.9 (N-1, NH) and δ_N 144.1 (N-2, N). Furthermore, $^{1}H-^{15}N$-HMBC correlations from the olefinic proton H-1′ to both nitrogens and from the H-2′ to N-2 strongly support the presence of a N−N-bond as well as the attachment of the olefinic chain C-1′−C-16′ to the tertiary nitrogen N-2 (Figure 3). Correlation from methylene protons (H-5) to N-2 showed that the tertiary nitrogen N-2 is attached to the aliphatic chain C-4−C-13 as well (Figure 3). Moreover a correlation from the oxygen-bound methylene H-2 to the secondary nitrogen N-1 and $^{1}H-^{13}C$-HMBC connectivities from the H-2/N*H* to C-3 indicates that the 2-methoxyacetamide fragment C-1−NH (Figure 2b) is bound to N-2 through a N−N-bond. Consequently, the overall analysis of the 1D-NMR (^{1}H, ^{13}C, DEPT) and 2D-NMR (COSY, TOCSY, HSQC, HMBC, $^{1}H-^{15}N$-HSQC and $^{1}H-^{15}N$-HMBC) data indicated that **2** although structurally related to elaiomycin (**1**) is a novel hydrazide derivative (Figure 3). The geometry of all double bonds was assigned to be *Z*, based on the coupling constants which were less than 12 Hz, as mentioned previously.

Compound **3** was obtained as a colorless oil. The ESI-MS in positive ionization mode of **3** showed ion peaks at m/z 477.5 $[M + H]^+$ and at m/z 499.5 $[M + Na]^+$. The exact molecular mass of **3** derived from high-resolution orbitrap-ESI-MS spectrum m/z 477.40483 $[M + H]^+$ (m/z calcd 477.39779, Δm = −0.50 ppm) gave a molecular formula of $C_{29}H_{52}N_2O_3$. At first glance, the molecular formula of **3** showed a difference of 2 Da compared to compound **2**. This suggested the presence of an additional double bond in compound **3**. ^{1}H and ^{13}C NMR data of compound **3** revealed a high similarity with those of **2**. Likewise ^{13}C NMR spectra of **3** showed the presence of 29 carbon atoms; nevertheless, DEPT and HSQC spectra revealed slight differences, particularly the lack of two methylene signals in the area between 22 and 32 ppm. Furthermore, a signal at δ_C 127.9 was assigned to two olefinic carbons corroborating the presence of one additional double bond in compound **3**. $^{1}H-^{1}H$-COSY correlations from H-9′ to H-8′/H-10′, from H-11′ to H-12′, and from H-12′ to H-13′ as well as $^{1}H-^{13}C$-HMBC correlations from H-9′ to C-7′/C-11′, from H-11′ to C-9′, from H-10′ to C-12′, from H-12′ to C-13′/C-14′, from H-13′ to C-15′, and from H-16′ to C-14′/C-15′ clearly pinpoint the position of the double bond at C-10′/C-11′. Consequently the length of the carbon chain C-1′/C-16′ in compound **3** was clearly defined by NMR spectral data and likewise in analogy to orbitrap-MS-MS spectra of **2** MS-MS data of **3** (Supporting Information, Figure S28) also support the presence of a decanoyl chain. As shown in Figure 4, the structure of compound **3** was

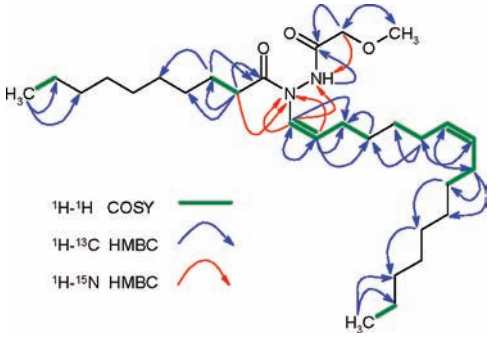


Figure 3. Elaiomycin B (**2**) structure and selective 2D-NMR connectivities including $^{1}H-^{15}N$-HMBC correlations.

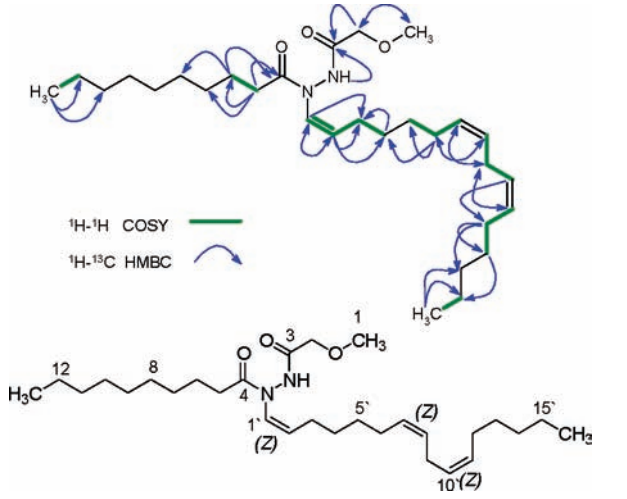


Figure 4. Elaiomycin C (**3**) structure and selective 2D-NMR connectivities.

deduced to be identical with that of compound **2** with one additional degree of unsaturation constituting a new member of the naturally occurring alkylhydrazides.

In general, hydrazines, hydrazones, and hydrazides impart a wide spectrum of biological activities and have various applications.[7] Interestingly, isonicotinic acid hydrazide has been used as an antituberculosis drug,[8,9] and maleic hydrazide has been used as a plant growth inhibitor and herbicide.[10] In long-term animal studies, however, hydrazide compounds have been shown to cause lung and lymphoreticular tissue tumors in mice,[11,12] and breast tumors in rats.[13] Nitrogen−nitrogen bond containing compounds are rarely found among natural products. Azoxy antibiotics reported so far include elaiomycin,[1] which is effective against *Mycobacterium tuberculosis*; LL-BH872α[14] displaying antifungal activity; the antitumor compound valanimycin[15] also active against Gram-positive and Gram-negative bacteria; the jietacins A and B,[16] which exhibit nematocidal activity; lyophyllin,[17] alkylazoxycarbamide showing tumor-inhibiting properties; a proximal *cis*-α,*β*-unsaturated azoxyalkene,[18] showing a weak antibiotic activity against *Rohdotorula* sp.; as well as the antifungal maniwamycins A and B[19] which possess a *trans*-α,*β*-unsaturated azoxy chromophore.

In this context, elaiomycins B and C represent new members of secondary metabolites with a hydrazide subunit as a characteristic structural feature. An Investigation on elaiomycin biosynthesis revealed that its carbon and nitrogen skeleton are derived from *n*-octylamine, L-serine, and acetate (C2-unit).[4,20] Previous biosynthesis studies of valanimycin,[15] a related azoxy antibiotic, showed L-serine and L-valine as biosynthesis precursors in which valine is transformed via isobutylamine into the intermediate isobutylhydroxylamine.[21] Recent investigations on valanimycin biosynthesis[22−25] revealed further mechanstic details, particularly on the formation of the unusual azoxy-group. Since the strain *Streptomyces* sp. BK 190 is capable of synthesizing elaiomycin and elaiomycins B and C, we assume related biosynthesis pathways for the assembly of these metabolites. Our current efforts are dedicated to the elucidation of elaiomycins biosynthesis.

Acknowledgment. Dr. Peter Schmieder (FMP Leibniz Institute for Molecular Pharmacology, Berlin) is kindly acknowledged for performing ^{15}N NMR experiments. Soleiman E. Helaly would like to thank the Egyptian government for a mission at TU Berlin. This research was supported by the Cluster of Excellence "Unifying Concepts in Catalysis" coordinated by the TU Berlin.

Supporting Information Available. The Experimental Section, physicochemical properties, 1D- and 2D-NMR spetroscopic data, IR data, and high-resolution orbitrap-ESI-MS. This material is available free of charge via the Internet at http://pubs.acs.org.

(7) Toth, B. *J. Cancer Res. Clin. Oncol.* **1980**, *97*, 97–108.
(8) *Synthetic organic chemicals*, US production and sales 1970, Tariff Commission publication no. 479, 1972, 262 pp.
(9) Some aromatic amines, hydrazine, and related substances, N-nitroso compounds and miscellaneous alkylating agents. In *International Agency for Research on Cancer (IARC) monographs on the evaluation of carcinogenic risk of chemicals to man*, 1974; Vol. 4, p 286.
(10) *The Merck Index: An encyclopedia of chemicals and drugs*, 9th ed.; Merck & Co.: Whitehouse Station, NJ, 1976, p 1937.
(11) Juhász, J.; Baló, J.; Kendrey, G. *Tuberkulózis* **1957**, *34*, 49–54.
(12) Epstein, S. S.; Andrea, J.; Jaffe, H.; Joshi, S.; Falk, H.; Mantel, N. *Nature* **1967**, *215*, 1388–1390.
(13) Severi, L.; Biancifiori, C. *J. Natl. Cancer Inst.* **1968**, *41*, 331–349.
(14) McGahren, W. J.; Kunstmann, M. P. *J. Am. Chem. Soc.* **1969**, *91*, 2808–2810.
(15) Yamato, M.; Iinuma, H.; Naganawa, H.; Yamagishi, Y.; Hamada, M.; Masuda, T.; Umezawa, H.; Abe, Y.; Hori, M. *J. Antibiot.* **1986**, *39*, 184–191.
(16) Omura, S.; Otoguro, K.; Imamura, N.; Kuga, H.; Takahashi, Y.; Masuma, R.; Tanaka, Y.; Tanaka, H.; Xue-hui, S.; En-tai, Y. *J. Antibiot.* **1987**, *40*, 623–629.
(17) (a) Fugmann, B.; Steglich, W. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 72–73. (b) Ye, Y.; Aulinger, K.; Arnold, N.; Spahl, W.; Steglich, W. *Tetrahedron Lett.* **1997**, *46*, 8013–8016.
(18) Bianchi, G.; Dallavalle, S.; Merlini, L.; Nasini, G.; Quaroni, S. *Planta Med.* **2003**, *69*, 574–576.
(19) (a) Nakayama, M.; Takahashi, Y.; Itoh, H.; Kamiya, K.; Shiratsuchi, M.; Otani, G. *J. Antibiot.* **1989**, *42*, 1535–1540. (b) Takahashi, Y.; Nakayama, M.; Watanabe, I.; Deushi, T.; Ishiwata, H.; Shiratsuchi, M.; Otani, G. *J. Antibiot.* **1989**, *42*, 1541–1546.
(20) Parry, R. J.; Rao, H. S. P.; Mueller, J. *J. Am. Chem. Soc.* **1982**, *104*, 339–340.
(21) Parry, R. J.; Li, Y.; Lii, F.-L. *J. Am. Chem. Soc.* **1992**, *114*, 10062–10064.
(22) Tao, T.; Alemany, L. B.; Parry, R. J. *Org. Lett.* **2003**, *5*, 1213–1215.
(23) Garg, R. P.; Gonzalez, J. M.; Parry, R. J. *Biol. Chem.* **2006**, *281*, 26785–26791.
(24) Garg, R. P.; Qian, X. L.; Alemany, L. B.; Moran, S.; Parry, R. J. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 6543–6547.
(25) Garg, R. P.; Alemany, L. B.; Moran, S.; Parry, R. J. *J. Am. Chem. Soc.* **2009**, *131*, 9608–9609.