Organic LETTERS

Letter

pubs.acs.org/OrgLett

Stereochemical Study of Sphingosine by Vibrational Circular Dichroism

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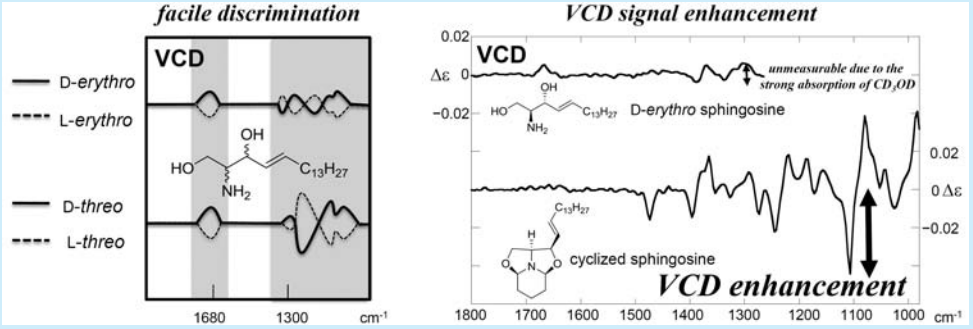
Supporting Information



ABSTRACT: Vibrational circular dichroism (VCD) was first applied to the stereochemical analysis of sphingosine. VCD patterns derived from the C═C stretch as well as other mid-infrared (IR) regions were practical markers to discriminate all the stereoisomers of intact sphingosine. Glutaraldehyde was found as an excellent derivatizing reagent for sphingosine which improves its solubility in VCD-friendly nonpolar solvents such as chloroform and enhances the VCD intensities by forming a rigid cyclized structure.

Sphingolipids were initially isolated as chemical constituents of brain in 1884 by German physician J. L. W. Thudichum,[1] and were named after the mythological Sphinx owing to their enigmatic natures. They are found in essentially all animals, plants, and fungi as well as some prokaryotic organism and viruses. While sphingolipids exist mostly in membranes, they have recently emerged as extracellular signals[2] in addition to being major constituents of lipoproteins and the multilamellar water barrier of skin. Their chemical structures consist of a sphingoid base as a backbone structure, amide linked fatty acids, and various moieties at the primary hydroxyl group whose variations generate at least 300 different sphingolipids in mammalian (Figure 1).[3] The most common sphingoid base in mammalian cells is an amino alcohol D-*erythro* sphingosine [(2*S*, 3*R*, 4*E*)-2-aminooctadec-4-ene-1,3-diol] (**1**) that is synthesized *de novo* from serine and palmitoyl-CoA (Figure 2). However, the stereochemistry of most sphingolipids remains unassigned due to a lack of facile method for determining the relative and absolute configurations of such an amphiphilic long-chain base. The absolute configuration of sphingosine was initially assigned as D-*erythro* via chemical relay reactions in the 1950s.[4] In the 1990s, Nakanishi and Berova et al. developed stereochemical analysis methods for sphingolipids utilizing HPLC[5] and circular dichroism (CD)[6] techniques. Although these methods are excellent both in sensitivity and reliability, they still have several drawbacks. The former needs troublesome two-step analyses:

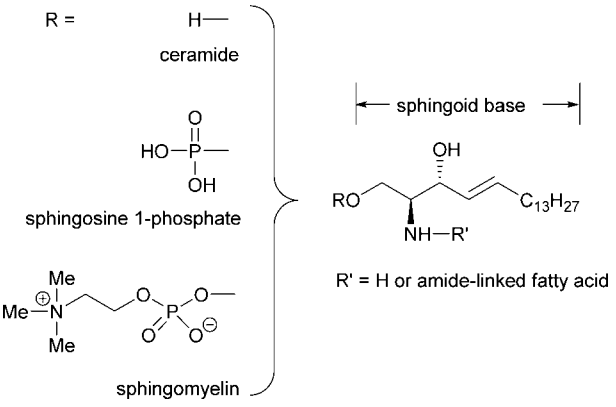


Figure 1. Structures of representative sphingolipids.

namely, after a fluorescence derivatization, *erythro* and *threo* isomers are separated on a first normal phase HPLC column, and then each collected diastereomer is further analyzed on a second chiral HPLC. Meanwhile, the latter demands two-step derivatization reactions to apply the bichromophoric exciton coupled CD method. In the past two decades, pioneering works

Received: February 19, 2016
Published: May 2, 2016

DOI: 10.1021/acs.orglett.6b00477
Org. Lett. 2016, 18, 2327−2330

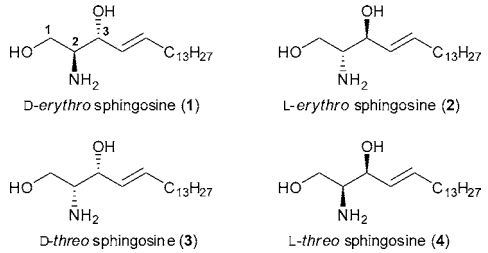


Figure 2. Stereoisomers of sphingosine.

have reported *threo*- and *erythro*-isomers exhibit different activities.[7]

In addition, naturally rare *threo*-isomers have recently been found in drug discovery research[8] as well as in natural product chemistry.[9] Keeping in mind that sphingosine in itself is an important signaling molecule,[10] besides a backbone moiety of the other essential sphingolipids such as ceramide and sphingosine1-phosphate, convenient and reliable methods to determine the stereochemistry of the sphingoid base are required. We recently reported that the vibrational circular dichroism (VCD) technique is a powerful tool for stereochemical analysis of glycoconjugates, including carbohydrates and glycopeptides.[11]

VCD measures the differential absorption of left versus right circularly polarized infrared (IR) radiation by molecular vibrational transitions, having the advantages of both CD and IR features. Herein, we report the first application of VCD to the stereochemical analysis of sphingosine.[12]

All four stereoisomers of sphingosine were prepared according to a well established method[13] to ensure their stereochemistries at each C2 and C3 carbons (Figure 2). To verify the practicality of the VCD method, VCD studies of intact sphingosine were first conducted. IR and VCD spectra were measured at 8 cm^{-1} resolution with a Bomem/BioTools ChiralIR spectrometer equipped with the dual PEM system.[14] All of the spectra were obtained in CD_3OD with a CaF_2 cell of 100 μm path length at a concentration of 0.20 M, after a process of sample dissolution in CD_3OD followed by its evaporation was repeated twice to avoid the noise associated with H−D exchange. The IR and VCD spectra of intact sphingosine **1** (D-*erythro*), **2** (L-*erythro*), **3** (D-*threo*), and **4** (L-*threo*) are shown in Figure 3. The IR spectra of the four stereoisomers are quite similar, whereas their VCD spectra are completely dissimilar reflecting their absolute configurations. These characteristic VCD patterns obviously show their potential for an efficient differentiation methodology toward all the stereoisomers of sphingosine. Their IR spectra exhibit characteristic absorption bands around 1670 cm^{-1}. They were ascribed to C=C stretch of the allylic alcohol moieties by observing no absorption in sphinganine, a saturated sphingosine analogue. Utilizing VCD patterns in this region provides a notable advantage for discriminating stereochemistry at the C3 carbon. The VCD spectra of the D-isomers (**1** and **3**) show positive signals (**1**, 1670 cm^{-1}, $\Delta\varepsilon$ = 0.0050; **3**, 1670 cm^{-1}, $\Delta\varepsilon$ = 0.0041), while those of the L-isomers (**2** and **4**) show negative signals (**2**, 1670 cm^{-1}, $\Delta\varepsilon$ = −0.0033; **4**, 1670 cm^{-1}, $\Delta\varepsilon$ = −0.0033). Since these VCD signals are relatively strong for their small IR intensities (e.g., *g*-values of **1** and **3** are 4.2×10^{-4} and 3.8×10^{-4}, respectively), they would be practical markers to distinguish between D- and L-isomers. Complete stereochemical assignment for sphingosine can be achieved by observing VCD spectral patterns in the fingerprint region in addition to the C=C stretching region. The D-*erythro* isomer (**1**) shows negative (1388 cm^{-1}, $\Delta\varepsilon$ = −0.0037), positive (1369 cm^{-1}, $\Delta\varepsilon$ = 0.0051), negative (1338 cm^{-1}, $\Delta\varepsilon$ = −0.0026), and positive (1303 cm^{-1}, $\Delta\varepsilon$ = 0.0061) signals, whereas the D-*threo* isomer (**3**) shows positive (1393 cm^{-1}, $\Delta\varepsilon$ = 0.0045), negative (1369 cm^{-1}, $\Delta\varepsilon$ = −0.0061), positive (1330 cm^{-1}, $\Delta\varepsilon$ = 0.0090), and positive (1310 cm^{-1}, $\Delta\varepsilon$ = 0.0065) signals. In an opposite manner, the L-*erythro* isomer (**2**) shows positive (1392 cm^{-1}, $\Delta\varepsilon$ = 0.0046), negative (1369 cm^{-1}, $\Delta\varepsilon$ = −0.0016), positive (1334 cm^{-1}, $\Delta\varepsilon$ = 0.0040), and negative (1297 cm^{-1}, $\Delta\varepsilon$ = −0.0026) patterns, while the L-*threo* isomer (**4**) shows negative (1393 cm^{-1}, $\Delta\varepsilon$ = −0.0040), positive (1369 cm^{-1}, $\Delta\varepsilon$ = 0.0069), negative (1330 cm^{-1}, $\Delta\varepsilon$ = −0.0087), and negative (1306 cm^{-1}, $\Delta\varepsilon$ = −0.0065) patterns. These results clearly exhibit VCD is a powerful tool for the discrimination of all the stereoisomers of intact sphingosine which has been impossible by conventional analytical methods. Since several milligrams of the samples are enough for VCD measurements, this straightforward method is convenient for the stereochemical analysis of synthesized sphingosine.

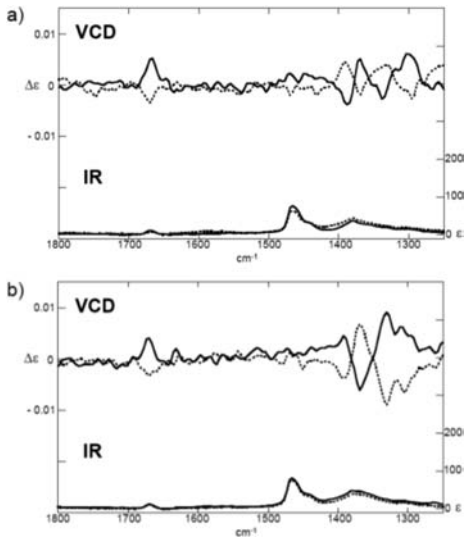
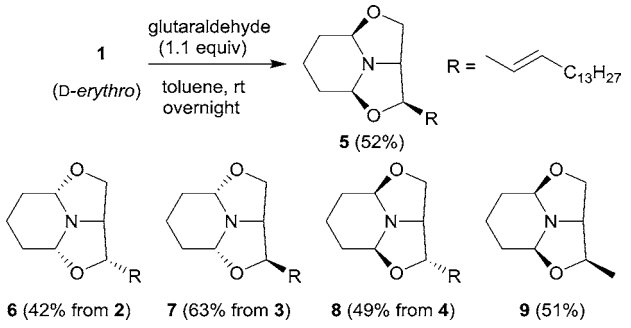


Figure 3. (a) VCD and IR spectra in CD_3OD (c = 0.20 M, l = 100 μm) of D-*erythro* sphingosine **1** (solid line) and L-*erythro* sphingosine **2** (dotted line). (b) VCD and IR spectra in CD_3OD (c = 0.20 M, l = 100 μm) of D-*threo* sphingosine **3** (solid line) and L-*threo* sphingosine **4** (dotted line). Data collection time and resolution were 3 h and 8 cm^{-1}, respectively.

On the other hand, cyclized derivatives of sphingosine are expected to have more rigid structures which reduce the number of conformations and would enhance the VCD intensities. Reactions of glutaraldehyde with all stereoisomers of sphingosine occurred in toluene at ambient temperature without the presence of any catalyst, to give intriguing tricyclic products in moderate yields (Scheme 1). Structural assignments for these compounds **5** ($[\alpha]_D^{25}$ = −64.4 (*c* 1.0, $CHCl_3$)), **6** ($[\alpha]_D^{25}$ = +62.7 (*c* 1.0, $CHCl_3$)), **7** ($[\alpha]_D^{25}$ = −36.9 (*c* 1.0, $CHCl_3$)), and **8** ($[\alpha]_D^{25}$ = +37.6 (*c* 1.0, $CHCl_3$)) were made on the basis of the NMR spectra.[15] Clear NOE signals (e.g., H1−H3, H7−H5α in **5**) in each NOESY spectrum strongly suggested each derivative has *syn*-form structure in which the lone pair of the nitrogen is *syn* to the hydrogens of methine groups at C1 and C7 carbons. These stereochemical assignments are also supported by the preceding study where *syn*-form of 4-methyl 2,6-dioxa-11-azatricyclo[5. 3. 1. 0 4, 11]

DOI: 10.1021/acs.orglett.6b00477
Org. Lett. 2016, 18, 2327−2330

Scheme 1. Synthesis of Cyclized Sphingosine 5−9



undecane was estimated to be 26 kcal/mol more stable than the *anti*-form.[16]

The IR and VCD spectra of cyclized derivatives are shown in Figure 4. Their VCD spectra completely differ from one another, and their intensities were obviously enhanced when compared to those of intact sphingosine. The absolute value of $\Delta\varepsilon$ maximum of **5** was 0.044, whereas the corresponding value of **1** was 0.0061. It is also noteworthy that they can be measured in $CDCl_3$ owing to the simultaneous protection of highly polar amino group and two hydroxyl groups of sphingosine. Measurements in $CDCl_3$ exhibit several valuable VCD bands in the 1000−1250 cm^{-1} region where strong CD_3OD absorption precludes the measurements. For instance, VCD spectra of **5** and **6** derived from *erythro* sphingosine showed intense bisignate type signals around 1100 cm^{-1}. Meanwhile, those of *threo* derivatives (**7** and **8**) had no such bisignate type Cotton effect, probably due to the conformation effect of the R side chain. These distinctive features as well as clear VCD signals in the other regions are quite helpful for discriminating the four stereoisomers of sphingosine more clearly.

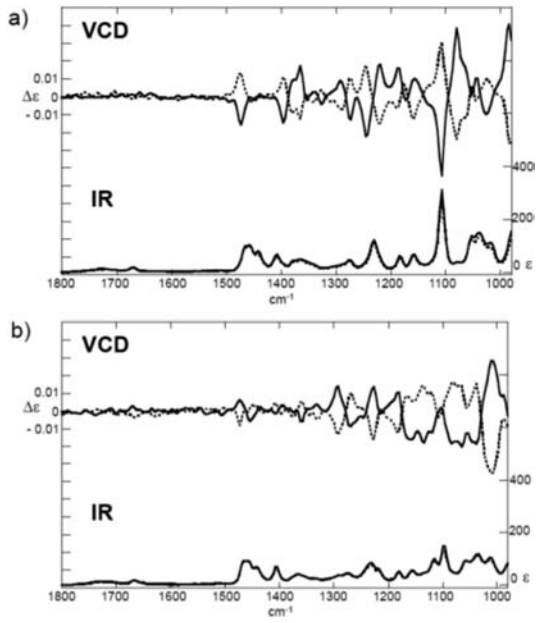


Figure 4. (a) VCD and IR spectra in $CDCl_3$ (c = 0.15 M, l = 100 μm) of cyclized D-*erythro* sphingosine **5** (solid line) and cyclized L-*erythro* sphingosine **6** (dotted line). (b) VCD and IR spectra in $CDCl_3$ (c = 0.15 M, l = 100 μm) of cyclized D-*threo* sphingosine **7** (solid line) and cyclized L-*threo* sphingosine **8** (dotted line). Data collection time and resolution were 3 h and 8 cm^{-1}, respectively.

VCD theoretical calculation of a model compound (**9**) ($[\alpha]_D^{25}$ = −36.6 (c 1.0, $CHCl_3$)) was conducted using density functional theory (DFT) at the B3LYP/6-311+g(2d,p) level (Figure 5). The observed VCD spectra of **5** and **9** showed similar spectral patterns, even though **9** lacks the flexible aliphatic hydrocarbon chain. This indicates the entire VCD spectra of the cyclic derivatives could be dominated by tricyclic moiety features because the VCD signals of the hydrocarbon chain are generally silent. Between the observed and calculated spectra of **9**, the major IR and VCD absorptions show good agreement. This result demonstrates the usefulness of VCD simulation toward the stereochemical analysis of sphingosine, which has been hampered by its sheer number of conformations.

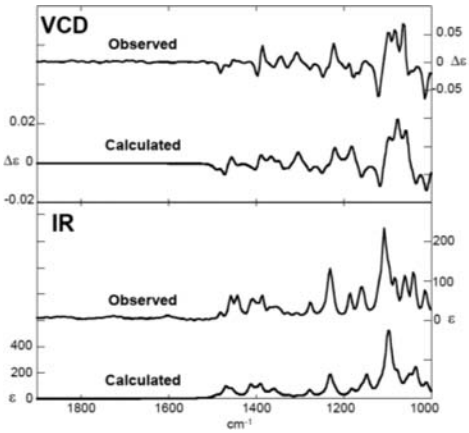


Figure 5. Comparison of IR (lower frame) and VCD (upper frame) spectra observed for **9** in $CDCl_3$ (c = 0.15 M, l = 100 μm) with those calculated for **9**. Data collection time and resolution were 3 h and 8 cm^{-1}, respectively.

In summary, we found that the VCD patterns derived from the C=C stretch as well as other mid-IR regions would be practical markers to discriminate all the stereoisomers of intact sphingosine. Glutaraldehyde was found as an excellent derivatizing agent for sphingosine which improves its solubility in VCD-friendly solvent $CDCl_3$ by protecting its highly polar groups and enhances the VCD intensities through the formation of rigid tricyclic derivative. These results verify the practicality of VCD method for the stereochemical analysis of sphingosine, and suggest VCD would be a powerful tool for the analysis of other important sphingoid base such as sphinganine and phytosphingosine.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b00477.

Synthetic procedures and spectral data of **5**−**9**, and details of VCD spectroscopy measurements and VCD calculation (PDF)

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DOI: 10.1021/acs.orglett.6b00477
Org. Lett. 2016, 18, 2327−2330

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was partially supported by the Hoansha Foundation, Innovation COE project for Future Medicine and Medicinal Research' and a grant-in-aid for scientific research (Grant 23651211, 24310151, 15H03111) from the MEXT of Japan. A.N. gratefully acknowledges a fellowship from the Japan Society of the promotion of Science. A.K.C.S. thanks International Research Fellow of the Japan Society for the Promotion of Science.

■ REFERENCES

(1) Thudichum, J. L. W. *A Treatise on the Chemical Constitution of the Brain*; Bailliere, Tindall and Cox: London, 1884.
(2) (a) Jana, A.; Pahan, K. *NeuroMol. Med.* **2010**, *12*, 351−361. (b) Alewijnse, A. E.; Peters, S. L. M. *Eur. J. Pharmacol.* **2008**, *585*, 292−302. (c) Mari, M.; Fernandez-Checa, J. C. *Liver Int.* **2007**, *27*, 440−450. (d) Dobrowsky, R. T. *Cell. Signalling* **2000**, *12*, 81−90. (e) Ohanian, J.; Ohanian, V. *Cell. Mol. Life Sci.* **2001**, *58*, 2053−2068.
(3) (a) Merrill, A. H., Jr.; Wang, M. D.; Park, M.; Sullards, M. C. *Trends Biochem. Sci.* **2007**, *32*, 457−468. (b) Pruett, S. T.; Bushnev, A.; Hagedorn, K.; Adiga, M.; Haynes, C. A.; Sullards, M. C.; Liotta, D. C.; Merrill, A. H., Jr. *J. Lipid Res.* **2008**, *49*, 1621−1639.
(4) Kiss, J.; Fodor, G.; Banfi, D. *Helv. Chim. Acta* **1954**, *37*, 1471−1478.
(5) Kawamura, A.; Berova, N.; Dirsch, V.; Mangoni, A.; Nakanishi, K.; Schwartz, G.; Bielawska, A.; Hannun, Y. A.; Kitagawa, I. *Bioorg. Med. Chem.* **1996**, *4*, 1035−1043.
(6) (a) Dirsch, V.; Frederico, J.; Zhao, N.; Cai, G.; Chen, Y.; Vunnam, S.; Odingo, J.; Pu, H.; Nakanishi, K.; Berova, N. *Tetrahedron Lett.* **1995**, *36*, 4959−4962. (b) Jiang, H.; Huang, X.; Nakanishi, K.; Berova, N. *Tetrahedron Lett.* **1999**, *40*, 7645−7649.
(7) (a) Thayyullathil, F.; Chathoth, S.; Shahin, A.; Kizhakkayil, J.; Hago, A.; Patel, M.; Galadari, S. *J. Cell. Biochem.* **2011**, *112*, 1138−1153. (b) Ramachandran, S.; Chu, U. B.; Mavlyutov, T. A.; Pal, A.; Pyne, S.; Ruoho, A. E. *Eur. J. Pharmacol.* **2009**, *609*, 19−26. (c) Megidish, T.; Takio, K.; Titani, K.; Iwabuchi, K.; Hamaguchi, A.; Igarashi, Y.; Hakomori, S. *Biochemistry* **1999**, *38*, 3369−3378. (d) Chao, R.; Khan, W.; Hannun, Y. A. *J. Biol. Chem.* **1992**, *267*, 23459−23462. (e) Buehrer, B. M.; Bell, R. M. *J. Biol. Chem.* **1992**, *267*, 3154−3159. (f) Mullmann, T. J.; Siegel, M. I.; Egan, R. W.; Billah, M. M. *J. Biol. Chem.* **1991**, *266*, 2013−2016. (g) Igarashi, Y.; Hakomori, S.; Toyokuni, T.; Dean, B.; Fujita, S.; Sugimoto, M.; Ogawa, T.; El-Ghendy, K.; Racker, E. *Biochemistry* **1989**, *28*, 6796−6800.
(8) (a) Schwartz, G. K.; Ward, D.; Saltz, L.; Casper, E. S.; Spiess, T.; Mullen, E.; Woodworth, J.; Venuti, R.; Zervos, P.; Storniolo, A.-M.; Kelsen, D. P. *Clin. Cancer Res.* **1997**, *3*, 537−543. (b) Schwartz, G. K.; Haimovitz-Friedman, A.; Dhupar, S. K.; Ehleiter, D.; Maslak, P.; Lai, L.; Loganzo, F., Jr.; Kelsen, D. P.; Fuks, Z.; Albino, A. P. *J. Natl. Cancer Inst.* **1995**, *87*, 1394−1399. (c) Maurer, B. J.; Melton, L.; Billups, C.; Cabot, M. C.; Reynolds, C. P. *J. Natl. Cancer Inst.* **2000**, *92*, 1897−1909.
(9) Makarieva, T. N.; Dmitrenok, P. S.; Zakharenko, A. M.; Denisenko, V. A.; Guzii, A. G.; Li, R.; Skepper, C. K.; Molinski, T. F.; Stonik, V. A. *J. Nat. Prod.* **2007**, *70*, 1991−1998.
(10) (a) Hannun, Y. A.; Bell, R. M. *Science* **1989**, *243*, 500−507. (b) Hannun, Y. A.; Loomis, C. R.; Merrill, A. H., Jr.; Bell, R. M. *J. Biol. Chem.* **1986**, *261*, 12604−12609.
(11) (a) Taniguchi, T.; Miura, N.; Nishimura, S.-I.; Monde, K. *Mol. Nutr. Food Res.* **2004**, *48*, 246−254. (b) Nakahashi, A.; Taniguchi, T.; Miura, N.; Monde, K. *Org. Lett.* **2007**, *9*, 4741−4744.
(12) (a) Nafie, L. A. *Vibrational Optical Activity: Principles and Applications*; Wiley: Chichester, U.K., 2011. (b) Stephens, P. J.; Devlin, F. J.; Cheeseman, J. R. *VCD: Spectrocopy for Organic Chemists*; CRC Press: Boca Raton, FL, 2012. (c) Keiderling, T. A.; Lakhani, A. In *Comprehensive Chiroptical Spectroscopy*; Berova, N., Polavarapu, P. L., Nakanishi, K., Woody, R. W., Eds.; Wiley: Hoboken, NJ, 2012; Vol. 2, p 707. (d) Poopari, M. R.; Dezhahang, Z.; Shen, K.; Wang, L.; Lowary, T. L.; Xu, Y. *J. Org. Chem.* **2015**, *80*, 428. (e) Longhi, G.; Tommasini, M.; Abbate, S.; Polavarapu, P. L. *Chem. Phys. Lett.* **2016**, *648*, 208. (f) Taniguchi, T.; Manai, D.; Shibata, M.; Itabashi, Y.; Monde, K. *J. Am. Chem. Soc.* **2015**, *137*, 12191.
(13) Koskinen, P. M.; Koskinen, A. M. P. In *Methods in Enzymology*; Merrill, A. H., Jr., Hannun, Y. A., Eds.; Academic Press: San Diego, CA, 2000; Vol. *311*, pp458−479.
(14) Nafie, L. A. *Appl. Spectrosc.* **2000**, *54*, 1634−1645.
(15) See Supporting Information.
(16) Okawara, T.; Ehara, S.; Hakui, N.; Furukawa, M. *Chem. Express* **1992**, *7*, 621−624.

■ NOTE ADDED AFTER ASAP PUBLICATION

Scheme 1 was corrected on May 5, 2016 due to a production error.

DOI: 10.1021/acs.orglett.6b00477
Org. Lett. 2016, 18, 2327−2330