Structure Determination of an Endogenous Sleep-Inducing Lipid, *cis*-9-Octadecenamide (Oleamide): A Synthetic Approach to the Chemical Analysis of Trace Quantities of a Natural Product

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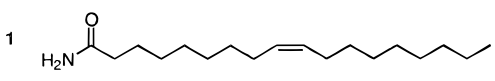
Abstract: The pursuit of endogenous sleep-inducing substances has been the focus of an extensive, complicated body of research. Several compounds, including Δ-sleep-inducing peptide and prostaglandin D_2, have been suggested to play a role in sleep induction, and yet, the molecular mechanisms of this physiological process remain largely unknown. In recent efforts, the cerebrospinal fluid of sleep-deprived cats was analyzed in search of compounds that accumulated during sleep deprivation. An agent with the chemical formula $C_{18}H_{35}NO$ was found to cycle with sleep−wake patterns, increasing in concentration with sleep deprivation and decreasing in amount upon recovery sleep. Since the material was generated in minute quantities and only under the special conditions of sleep deprivation, efforts to isolate sufficient material for adequate characterization, structure identification, and subsequent detailed evaluation of its properties proved unrealistic. With the trace amounts of the impure endogenous compound available, extensive MS studies on the agent were completed, revealing key structural features of the molecule including two degrees of unsaturation, a long alkyl chain, and a nitrogen substituent capable of fragmenting as ammonia. Additionally, HPLC traces suggested a weak UV absorbance for the unknown material. With this data in hand and encouraged by the relatively small size of the molecule, MW = 281, a synthetic approach toward the structural identification of the natural compound was initiated. Herein, we report the full details of the synthesis and comparative characterization of candidate structures for this endogenous agent that led to the unambiguous structural correlation with synthetic *cis*-9-octadecenamide.

The investigation of endogenous sleep-inducing substances has been the focus of extensive efforts.[1] The first evidence for the presence of endogenous sleep factors was obtained as early as 1913 when Pieron and his colleagues transfused cerebrospinal fluid from sleep-deprived dogs to the cerebrospinal system of normal dogs and induced 6−8 h of sleep.[2] At the same time, dogs receiving cerebrospinal fluid from alert dogs remained awake. The validity of the studies was reconfirmed in 1939[3] and more recently extended by Pappenheimer.[4] In these latter studies, perfusate from the ventricular system of sleep-deprived goats induced sleep lasting up to 18 h in cats and mice. Although the substance was not identified, fractionation revealed its molecular weight to be less than 500 g/mol and it increased sleep by 50% in rats and rabbits following intraventricular infusion. Monnier and co-workers also confirmed the existence of sleep-inducing factors.[5] They ultimately isolated, characterized, and prepared Δ-sleep-inducing peptide (Trp-Ala-Gly-Gly-Asp-Ala-Ser-Gly-Glu), a nonapeptide which induces slow-wave (Δ) sleep observed in the EEG of rabbits. This material differs from the low molecular weight sleep factor of Pappenheimer[4] and from prostaglandin D_2, which has been more recently identified as a sleep-promoting material by Nagasaki and Inoue.[6]

In recent efforts, the cerebrospinal fluid of cats was analyzed in efforts to detect and identify compounds that accumulated during sleep deprivation.[7] An agent with the chemical formula $C_{18}H_{35}NO$ was isolated from the cerebrospinal fluid of sleep-deprived cats and was determined to accumulate and disappear under conditions of sleep deprivation and resting, respectively. Since the material was generated in such trace quantities and is only detected in sleep-deprived animals, efforts to isolate sufficient amounts for adequate characterization, structure identification, and subsequent detailed evaluation of its properties proved unrealistic. Although insufficient endogenous material was available for structural characterization, extensive MS studies of the agent revealed key structural features including two degrees of unsaturation, a long alkyl chain, and a nitrogen substituent capable of fragmenting as ammonia. These features were compatible with a conjugated or nonconjugated diene in which a primary amine was allylic or a monounsaturated alkane chain terminating in a primary amide.[8] Herein, we report full details of the synthesis and comparative characterization of candidate structures for this endogenous agent that led to the unambiguous structural correlation with synthetic *cis*-9-octadecenamide (**1**, oleamide).[9]



Sphingosine-Derived Candidate Structures. HRFABMS (NBA−NaI) of the natural lipid provided an exact mass of *m/z* 304.2614 ($M + Na^+$) and the best fit for the molecular formula

[⊗] Abstract published in *Advance ACS Abstracts,* January 1, 1996.

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(4) Pappenheimer, J. R.; Miller, T. B.; Goodrich, C. A. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *58*, 513. Pappenheimer, J. R. *Sci. Am.* **1976**, *235*, 24. Fencl, V.; Koski, G.; Pappenheimer, J. R. *J. Physiol. (London)* **1971**, *261*, 565. Pappenheimer, J. R.; Koski, G.; Fencl, V.; Karnovsky, M. L.; Kruger, J. *J. Neurophys.* **1975**, *38*, 1299.
(5) Monnier, M.; Koller, T.; Graver, S. *Exp. Neurol.* **1963**, *8*, 264. Monnier, M. *Actual. Neurophysiol.* **1964**, *5*, 203. Monnier, M.; Hosli, L. *Science* **1964**, *146*, 796. Monnier, M.; Hosli, L. *Prog. Brain Res.* **1965**, *18*, 118. Monnier, M.; Schoenenberger, G. A.; Glatt, A.; Dudler, L.; Gachter, R. *Exp. Brain Res.* **1975**, *23*, 144. Schoenenberger, G. A.; Monnier, M. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 1282. Schoenenberger, G. A. *Eur. Neurol.* **1984**, *23*, 321.

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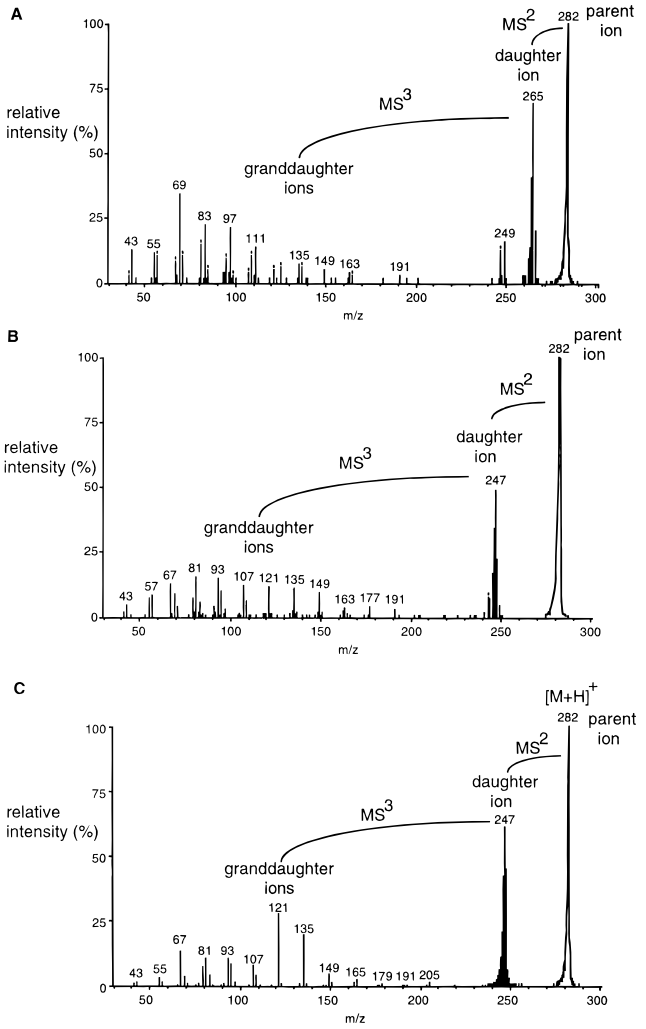

Figure 1. MS^3 data on (A) *m/z* 265 daughter ion of the natural compound, (B) *m/z* 247 daughter ion of the natural compound, and (C) *m/z* 247 daughter ion of 2(*R*)-amino-1-hydroxy-3(*Z*),12(*Z*)-octadecadiene (**19**).

$C_{18}H_{35}NO$ ($M + Na^+$ requires 304.2616). Tandem electrospray MS revealed a distinctive set of two primary fragments and a subsequent fragmentation pattern in the low molecular weight range consistent with a long-chain alkane (Figure 1). The primary and sequential loss of 17 and 35 mass units from the parent ion indicated a loss of NH_3 followed by loss of H_2O. Additional electrospray MS experiments on the daughter ions *m/z* 265 and 247 revealed a distinctive pattern that we felt would permit detailed comparisons with candidate structures.

An initial and attractive series of candidate agents included a new class of lipids derived from sphingosine (**2**) through the loss of water with incorporation of an additional degree of unsaturation. Such sphingosine-derived conjugated dienes, which have not been previously characterized, have been detected in the acid-catalyzed degradation of sphingosine and its congeners[10] although no implications for their biological role(s) have been detailed. Nonconjugated sphingosine-derived dienes also seemed reasonable candidate structures since several

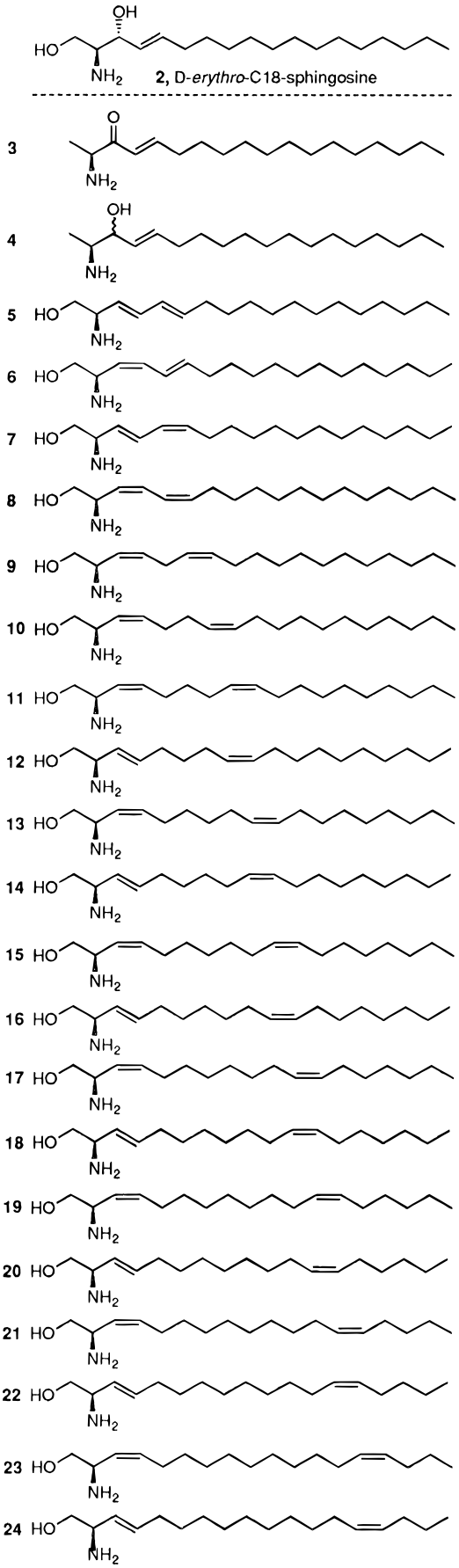

Figure 2. Candidate structures prepared and comparatively characterized.

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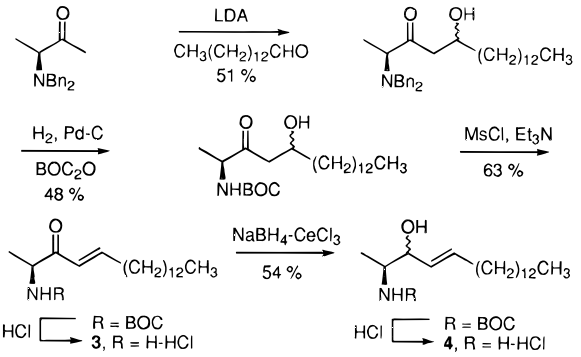
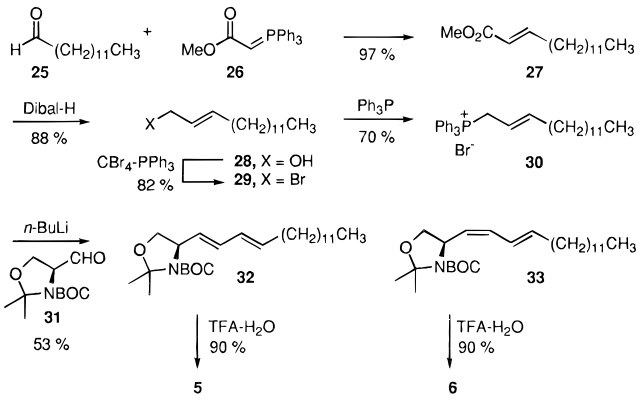
Scheme 1



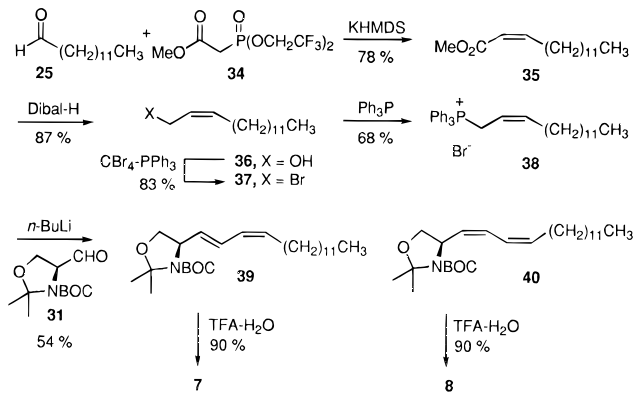
sphingoid bases have been identified with double bonds at various positions along their alkane chains.[11] Since the trace quantities and purity of the endogenous material precluded characterization efforts to distinguish between the potential candidate structures, the chemical synthesis of **3**−**24** (Figure 2) which includes the full set of conjugated and nonconjugated sphingosine-derived dienes, their characterization, and potential correlation with the endogenous agent was conducted.

At the onset of our efforts, the structures **3** and **4** were prepared, of which the former fits the molecular formula while the latter lacks one of the required degrees of unsaturation. The agent **3** was prepared in efforts to determine whether such structures would mimic the MS fragmentation pattern of the natural agent which exhibited a primary loss of ammonia followed by loss of water. Additionally, the conjugated system of **3** was used to address concerns over an ambiguous and weak 280 nm UV absorbance potentially associated with the endogenous material. The agent **3** was prepared by an aldol condensation of 3-(*N*,*N*-dibenzylamino)-2-butanone[12] with tetradecylaldehyde to provide the corresponding α-hydroxyketone followed by exchange of the dibenzyl protecting groups for a BOC protecting group, elimination of the hydroxy substituent upon treatment with Et_3N and MsCl, and finally deprotection of the amine with 4 N HCl−EtOAc to provide **3** as the hydrochloride salt (Scheme 1). The aminoketone **3** proved to be quite unstable, and therefore, MS analysis of the molecule was carried out immediately following its generation. Contrary to the natural agent, **3** fragmented first to lose water rather than ammonia, disqualifying such α-aminoketones as candidate structures for the endogenous substance. To obtain more information on the location of a putative hydroxy substituent of the natural agent, *N*-BOC-**3** was reduced with $CeCl_3$−$NaBH_4$ in CH_3OH, deprotected with 4 N HCl−EtOAc to provide **4** as a 3:1 mixture of diastereomers, and subjected to electrospray MS fragmentation analysis. The agent **4**, like sphingosine, generated a strong fragment at *m/z* 30 indicative of a secondary hydroxy substituent fragmenting as CHOH.[13] The natural agent showed no such fragmentation, suggesting that a hydroxy substituent was either not present in the molecule or present as a primary alcohol.

Scheme 2



Scheme 3



Sphingosine-Derived Candidate Structures: Conjugated Dienes. The first set of serious candidate structures prepared and examined constitute the four stereoisomers of the conjugated diene derived from elimination of the D-*erythro*-C18-sphingosine secondary hydroxyl group. The agents **5**−**8** were especially attractive since they possess a UV chromophore with a potential absorbance as high as 280 nm and an allylic amine derived from a chemically reasonable dehydration reaction and they could be expected to sequentially undergo MS fragmentation with loss of ammonia and water. The 3*E*,5*E*- and 3*Z*,5*E*-dienes **5** and **6** were prepared as detailed in Scheme 2. Wittig reaction of methyl (triphenylphosphoranylidene)acetate (**26**) with tridecanal (**25**, THF, 0−25 °C, 10 h, 97%) provided methyl *trans*-2-pentadecenoate (**27**), which was reduced to 1-hydroxy-2(*E*)-pentadecene (**28**) with Dibal-H (THF, −78 °C, 1 h, 88%). Treatment of **28** with CBr_4−Ph_3P (CH_2Cl_2, 25 °C, 6 h, 82%) to provide 1-bromo-2(*E*)-pentadecene (**29**) preceded conversion to the phosphonium salt **30** (Ph_3P, THF−benzene 1:1, 70 °C, 12 h, 70%). Wittig reaction of the *in situ* generated phosphorane (PhLi, THF, 25 °C, 10 min) with (*S*)-**31**[14] (THF, −78 to 25 °C, 0.5 h, 53%) provided a near 1:1 mixture of separable *E*,*E*- and *Z*,*E*-dienes **32** and **33**. Independent hydrolysis of both **32** and **33** (3:1 TFA−H_2O, 25 °C, 10 min, 90%) cleanly provided (*E*,*E*)-**5** and (*Z*,*E*)-**6**, respectively.

Although the configuration of the Δ^5 double bond was established as *E* on the basis of its original formation through a Wittig reaction with a stabilized phosphorane, the determination of the stereochemistry of the newly formed Δ^3 double bond was less straightforward. The Δ^3 double bond configurations of **5** and **6** were assigned primarily on the basis of comparison of the chemical shifts of their C-2 allylic protons

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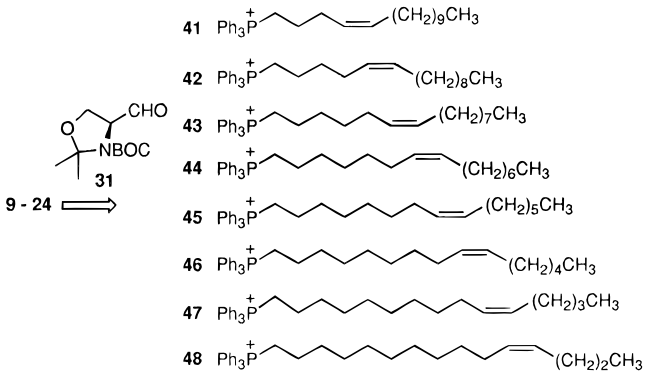
to those of the nonconjugated dienes **10**−**24**. Because the nonconjugated dienes **10**−**24** were prepared from Wittig reactions with unstabilized phosphoranes, they are generated dependably and predominately with the 3*Z* stereochemistry (*ca.* 10:1 *Z*:*E*). The C-2 allylic proton of the 3*Z*,*ωZ*-dienes displays a chemical shift of 3.93 ppm, significantly further downfield than that of the 3*E*,*ωZ*-dienes which is found at 3.53 ppm. Analogously, the chemical shift of the C-2 allylic proton of **6** is 3.93 ppm, while the shift of C-2 allylic proton of **5** is 3.54 ppm, leading to the assignment of **6** as the *Z*,*E*-diene and **5** as the *E*,*E*-diene. These assignments are further supported by the IR spectra of **5** and **6**, in which **5** displays a much stronger 984 cm^{-1} absorbance attributable to disubstituted trans olefin absorption.

By following an analogous approach, the 3*E*,5*Z*- and 3*Z*,5*Z*-dienes **7** and **8** were prepared as detailed in Scheme 3. Modified Wadsworth−Horner−Emmons condensation of **34** with tridecanal (**25**) under the conditions detailed by Still−Gennari[15] (1.1 equiv of KHMDS, 5.0 equiv of 18-crown-6, THF, −78 °C, 1 h, 78%) cleanly provided methyl 2(*Z*)-pentadecenoate (**35**, ≥10:1 *Z*:*E*). Subsequent conversion of **35** to the phosphonium salt **38** followed by Wittig reaction of the *in situ* generated phosphorane (PhLi, THF, 25 °C, 10 min) with (*S*)-**31**[14] (THF, −78 to 25 °C, 0.5 h, 54%) cleanly provided a near 1:1 mixture of a separable dienes **39** and **40** gratifyingly with the detection of only a trace of a contaminating Δ^5-trans isomer in **40**. Hydrolysis of **39** and **40** (3:1 TFA−H_2O, 25 °C, 10 min, 90%) cleanly provided (*E*,*Z*)-**7** and (*Z*,*Z*)-**8**, respectively. The stereochemistry of the Δ^3 double bond of **7** and **8** was assigned as detailed for **5** and **6**.

Electrospray mass analysis of **5**−**8** revealed fragmentation patterns nearly identical to that of the natural agent. Like the endogenous substance, **5**−**8** each underwent sequential loss of ammonia and water from the parent ion and displayed low molecular weight fragments entailing the repeated loss of 14 mass units consistent with the presence of a long-chain alkane. The only discernible differences between the mass spectra of **5**−**8** and the natural agent were the relative intensities of particular low molecular weight fragments. Since these subtle distinguishing features demonstrated the natural agent to be distinct from **5**−**8**, we focused on the set of closely related nonconjugated dienes **9**−**24**. Two additional pieces of evidence surfaced at this time that further directed the efforts toward this family of molecules. The weak 280 nm absorbance originally attributed to the natural agent proved to be the result of HPLC coelution of the endogenous substance with a UV active impurity. Although the purity of the samples of the endogenous material precluded IR assessment of the functional groups present, very crude NMR data on the trace amounts of the natural substance then available were obtained but with difficulty (Figure 2, supporting information). Because of the sample purity, benzene-d_6 was the only solvent from which meaningful information could be extracted. The methanol-d_4 and chloroform spectra were too complicated to analyze due to cosolublization of benzene-insoluble impurities. Although quite unrefined, the NMR spectrum of the impure natural agent in benzene-d_6 demonstrated the presence of a methyl group (0.9 ppm), a long alkyl chain (broad singlet, 1.2−1.3 ppm), allylic protons (multiplet, 2.1 ppm), and alkene protons (multiplet, 5.5 ppm). Additional, unassigned signals resided at 1.6−1.7 ppm (multiplet), from 3.5 to 4.1 ppm (set of broad unrefined resonances), and at 5.2 ppm (broad singlet). Of particular significance was the chemical shift of the olefinic protons as compared to those of the conjugated dienes **5**−**8**. Whereas the olefinic protons of **5**−**8** consisted of a series of four individual proton resonances well spread over the 6.4−5.1 ppm region, the olefinic protons of the natural agent resided as one broad peak centered at 5.5 ppm. Since the wide chemical shift range of the olefinic protons of **5**−**8** was due to their incorporation in a conjugated diene, the nonconjugated dienes would more likely match the NMR pattern of the natural agent. Aware that **9**−**24** should also display protons resonances in the 3.5−4.0 ppm range due to the CH_2OH and C*H*NH protons, as well as resonances from exchangeable protons, we undertook the synthesis of the full set of nonconjugated dienes. With the protocols established for the synthesis with the efforts on **5**−**8**, the preparation of the full set of agents **9**−**24** took approximately 3 months and proved more effective than the continued isolation and purification efforts on the endogenous agent.

Scheme 4



Nonconjugated Diene Candidate Structures. The full set of nonconjugated dienes **9**−**24** were sequentially prepared for examination. Each of the dienes maintained the sphingosine-derived allylic amine thought to be potentially characteristic of the endogenous agent but placed the remaining double bond at various distances from the Δ^3 double bond. We anticipated that the comparative examination of **9**−**24**, if not providing the endogenous agent itself, would permit a detailed interpretation of the alkyl chain MS fragmentation pattern sufficient to narrow down or pinpoint the location of the second distal site of unsaturation. Given the propensity for cis versus trans olefins found in naturally occurring lipids, the distal double bond was selected to be cis in the candidate structures and both the cis and trans Δ^3 isomers of each were targeted for synthesis. This proved most accessible through Wittig reaction of aldehyde **31**[14] with the appropriate phosphoranes derived from **41**−**48**, in which the stereochemistry of the newly introduced Δ^3 double bond could be controlled by choice of reaction conditions (Scheme 4).[16]

The required phosphonium salts **41**−**48** were prepared from the corresponding ω-bromoalcohols as outlined in Scheme 5 for **46**.[17−23] Notably, the distal double bond in each of the phosphonium salts was installed cleanly with the *Z* stereochemistry (≥20:1) resulting from the inherent preference of the Wittig reaction of a nonstabilized ylide and no *E*-olefin was detected by ^{1}H NMR spectra.

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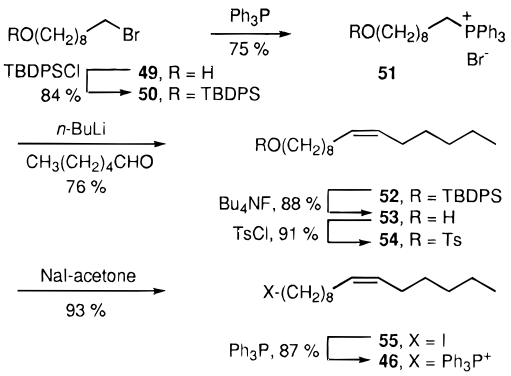
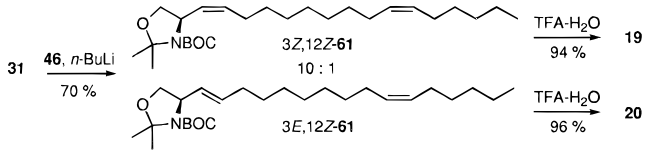
(16) Only **9** was prepared by a different route. The synthesis of **9** was conducted prior to the development of the route detailed in Scheme 4. Briefly, **9** was prepared as follows: aldehyde **31** was converted to the corresponding vinyl dibromide (2 equiv of Zn, 2 equiv of Ph_3P, 2 equiv of CBr_4, 24 h, 25 °C; 1 equiv of **31**, 1 h, 25 °C, 65%) which was treated with *n*-BuLi (2.2 equiv, THF, −78 °C, 1 h) followed by 1-bromo-2-tetradecene (2 equiv, prepared as outlined for **29**) to give 3-(*tert*-butoxycarbonyl)-2,2-dimethyl-4(*R*)-[4(*E*)-hexadecen-1-ynyl]oxazolidine (10%). Lindlar reduction of the alkyne (Pd/$CaCO_3$ with lead (10% weight), quinoline, hexanes, H_2 atmosphere, 3 h, 25 °C, 90%) followed by deprotection of the diene (TFA−H_2O, 3:1, 10 min, 25 °C, 90%) provided 2(*S*)-amino-1-hydroxy-3(*Z*),6(*E*)-octadecadiene.

Scheme 5



Scheme 6



As illustrated in Scheme 6 for **19**−**20**, Wittig reaction of the aldehyde **31**[14] with the *in situ* generated phosphoranes derived from the phosphonium salts **41**−**48** (THF, −78 to 25 °C, 30 min, 68−82%) provided cleanly the protected 3*Z*-alkenes **56**−**63** (≥10:1 *Z*:*E*) containing small amounts of the readily separable and isolable 3*E*-alkenes from which the dienes **10**−**24** were obtained by acid-catalyzed deprotection (TFA−H_2O, 3:1, 25 °C, 10 min, 90−95%).

As with the conjugated dienes **5**−**8**, the nonconjugated dienes **9**−**24** exhibited MS fragmentation patterns nearly identical to that of the natural substance, differing only in the relative intensities of lower molecular weight fragments (*cf.* Figure 1). Interestingly, the MS fragmentation patterns of the *m/z* 247 daughter ions of **9**−**24** fell into two categories on the basis of the position of the varied double bond. In the low molecular weight region of the *m/z* 247 fragmentation spectrum, **9**−**12** exhibited a 79 mass unit fragment greater in intensity than the neighboring 81 fragment, while **13**−**24** showed the reversed relative intensities. The latter set matched more closely the fragmentation pattern of the *m/z* 247 daughter ion of the natural agent, suggesting that the endogenous substance contained a double bond several methylenes away from the carbon bearing the released amino substituent (more than five methylenes away).

The ^{1}H NMR spectra of **9**−**24** were taken in benzene-d_6 to compare directly to the ^{1}H NMR of the crude natural agent. Like the natural substance, **9**−**24** exhibited resonances at 0.9 ppm (methyl protons), 1.2−1.3 ppm (alkyl methylene protons), 2.1 ppm (allylic protons), and 5.2 ppm (double-bond protons). The agent **9** could be eliminated since its bisallylic protons appeared at 2.8−3.0 ppm and a corresponding signal was not present in the endogenous material. The candidate structures **10**−**24** also showed resonances in the 3.8−3.4 ppm range corresponding to the C*H*NH and CH_2OH protons. While these particular resonances did not match exactly those of the natural substance, their chemical shifts also showed a significant dependence on the state of the neighboring amino group. When in the form of either an HCl or TFA salt, **10**−**24** exhibited a downfield shift in their C*H*NH and CH_2OH proton resonances comparable to that of the natural agent in the 3.5−4.1 ppm range. The single reproducible mismatch between the NMR spectra of **10**−**24** and the natural agent was the unassigned multiplet resonance at 1.6−1.7 ppm in the spectrum of the endogenous substance. While the benzene-d_6 ^{1}H NMR spectra of **10**−**24** did demonstrate a significant degree of sample concentration dependence with the C*H*NH and CH_2OH proton resonances shifting downfield and the allylic proton resonances shifting upfield upon dilution, the allylic proton resonances never exhibited a chemical shift further upfield than 1.85 ppm leaving the potential nature of the 1.6−1.7 ppm multiplet unresolved. Given the highly impure state of the natural agent, it was not even clear whether this was part of the authentic spectrum (*cf.* Figure 2, supporting information).

At this stage and still uncertain whether the natural substance was something other than **13**−**24**, we chose to derivatize **13**−**24** in attempts to establish protocols for future chemical degradation studies of the natural agent. Upon acetylating the amine of **23** and acquiring ^{1}H NMR spectrum in benzene-d_6, we observed the acetyl methyl resonance at 1.55 ppm, quite close in chemical shift to the unassigned multiplet of the natural agent. This unanticipated observation of the acetyl protons so far upfield in the spectrum was surprising since they appear at 1.94 ppm in CD_3OD. The prospect of the endogenous substance being a monounsaturated primary amide became clear with the 1.6−1.7 ppm multiplet being attributed to the resonance of the methylene protons adjacent to the amide.

Fatty Acid Primary Amide Candidate Structures and Related Agents. 9(*Z*)-Octadecenamide: Correlation with the Authentic Lipid. Aware that oleic acid was the most common

(17) The following sequence was employed for **41**: 4-bromo-1-butanol (1 equiv of TBDPSCl, 1.2 equiv of Et_3N, 0.33 equiv of DMAP, CH_2Cl_2, 25 °C, 12 h, 72%); Ph_3P (1.5 equiv, toluene, reflux, 12 h, 69%); *n*-BuLi (1.1 equiv, −78 to 25 °C, 30 min; $CH_3(CH_2)_9CHO$, −78 to 25 °C, 1 h, 73%); Bu_4NF (2 equiv, THF, 25 °C, 2 h, 90%); *p*TsCl (1.1 equiv, pyridine, 0 °C, 12 h, 87%); NaI (2 equiv, acetone, reflux, 2 h, 80%); Ph_3P (1.5 equiv, toluene, reflux, 12 h, 86%).

(18) The following sequence was employed for **42**: 5-bromo-1-pentanol (1 equiv of TBDPSCl, 1.2 equiv of Et_3N, 0.33 equiv of DMAP, CH_2Cl_2, 25 °C, 12 h, 78%); Ph_3P (1.5 equiv, toluene, reflux, 12 h, 63%); *n*-BuLi (1.1 equiv, −78 °C to 25 °C, 30 min; $CH_3(CH_2)_8CHO$, −78 to 25 °C, 1 h, 81%); Bu_4NF (2 equiv, THF, 25 °C, 2 h, 97%); *p*TsCl (1.1 equiv, pyridine, 0 °C, 12 h, 81%); NaI (2 equiv, acetone, reflux, 2 h, 82%); Ph_3P (1.5 equiv, toluene, reflux, 12 h, 83%).

(19) The following sequence was employed for **43**: 6-bromo-1-hexanol (1 equiv of TBDPSCl, 1.2 equiv of Et_3N, 0.33 equiv of DMAP, CH_2Cl_2, 25 °C, 12 h, 82%); Ph_3P (1.5 equiv, toluene, reflux, 12 h, 73%); *n*-BuLi (1.1 equiv, −78 to 25 °C, 30 min; $CH_3(CH_2)_7CHO$, −78 to 25 °C, 1 h, 73%); Bu_4NF (2 equiv, THF, 25 °C, 2 h, 90%); *p*TsCl (1.1 equiv, pyridine, 0 °C, 12 h, 90%); NaI (2 equiv, acetone, reflux, 2 h, 89%); Ph_3P (1.5 equiv, toluene, reflux, 12 h, 88%).

(20) The following sequence was employed for **44**: 7-bromo-1-heptanol (1 equiv of TBDPSCl, 1.2 equiv of Et_3N, 0.33 equiv of DMAP, CH_2Cl_2, 25 °C, 12 h, 75%); Ph_3P (1.5 equiv, toluene, reflux, 12 h, 72%); *n*-BuLi (1.1 equiv, −78 to 25 °C, 30 min; $CH_3(CH_2)_6CHO$, −78 to 25 °C, 1 h, 68%); Bu_4NF (2 equiv, THF, 25 °C, 2 h, 87%); *p*TsCl (1.1 equiv, pyridine, 0 °C, 12 h, 86%); NaI (2 equiv, acetone, reflux, 2 h, 93%); Ph_3P (1.5 equiv, toluene, reflux, 12 h, 86%).

(21) The following sequence was employed for **45**: 8-bromo-1-octanol (1 equiv of TBDPSCl, 1.2 equiv of Et_3N, 0.33 equiv of DMAP, CH_2Cl_2, 25 °C, 12 h, 81%); Ph_3P (1.5 equiv, toluene, reflux, 12 h, 76%); *n*-BuLi (1.1 equiv, −78 to 25 °C, 30 min; $CH_3(CH_2)_5CHO$, −78 to 25 °C, 1 h, 70%); Bu_4NF (2 equiv, THF, 25 °C, 2 h, 95%); *p*TsCl (1.1 equiv, pyridine, 0 °C, 12 h, 89%); NaI (2 equiv, acetone, reflux, 2 h, 95%); Ph_3P (1.5 equiv, toluene, reflux, 12 h, 88%).

(22) The following sequence was employed for **47**: 10-bromo-1-decanol (1 equiv of TBDPSCl, 1.2 equiv of Et_3N, 0.33 equiv of DMAP, CH_2Cl_2, 25 °C, 12 h, 83%); Ph_3P (1.5 equiv, toluene, reflux, 12 h, 65%); *n*-BuLi (1.1 equiv, −78 to 25 °C, 30 min; $CH_3(CH_2)_3CHO$, −78 to 25 °C, 1 h, 70%); Bu_4NF (2 equiv, THF, 25 °C, 2 h, 95%); *p*TsCl (1.1 equiv, pyridine, 0 °C, 12 h, 88%); NaI (2 equiv, acetone, reflux, 2 h, 83%) identical in all respects with material previously disclosed;[31] Ph_3P (1.5 equiv, toluene, reflux, 12 h, 90%).

(23) The following sequence was employed for **48**: 11-bromo-1-undecanol (1 equiv of TBDPSCl, 1.2 equiv of Et_3N, 0.33 equiv of DMAP, CH_2Cl_2, 25 °C, 12 h, 84%); Ph_3P (1.5 equiv, toluene, reflux, 12 h, 50%); *n*-BuLi (1.1 equiv, −78 to 25 °C, 30 min; $CH_3(CH_2)_2CHO$, −78 to 25 °C, 1 h, 80%); Bu_4NF (2 equiv, THF, 25 °C, 2 h, 87%); *p*TsCl (1.1 equiv, pyridine, 0 °C, 12 h, 94%); NaI (2 equiv, acetone, reflux, 2 h, 88%); Ph_3P (1.5 equiv, toluene, reflux, 12 h, 89%).

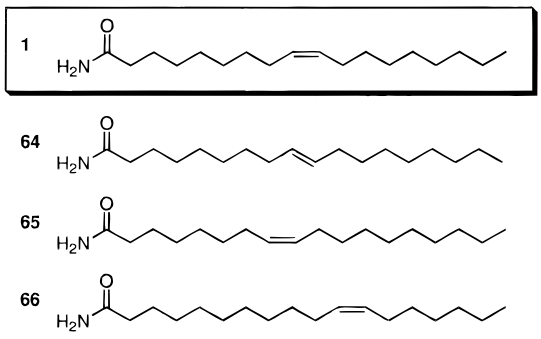


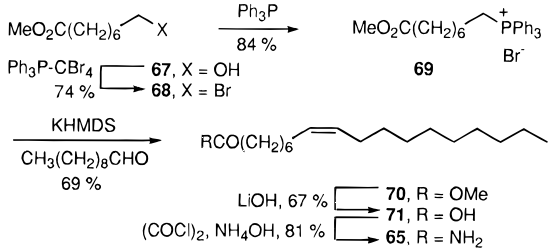
Figure 3.

natural C18 fatty acid and confident that the double bond of the natural agent was several methylenes away from its heteroatom-bearing carbon based on the MS work with **9**−**24**, we prepared **1** and subjected it to MS and benzene-d_6 ^{1}H NMR analysis.

The agent **1** matched the MS profile of the endogenous substance exactly. The fragmentation patterns of the parent ion, *m/z* 282, and the two daughter ions, *m/z* 265 (parent $-NH_3$) and *m/z* 247 (parent $-NH_3$ and H_2O) of **1** were identical to those of the natural agent. Of special interest was the unexpected electrospray MS fragmentation of NH_3 followed by H_2O from **1**. While in retrospect the fatty acid primary amides may appear more obvious candidate structures, the MS behavior of the conjugated and nonconjugated sphingosine-derived dienes was so close to and nearly indistinguishable from that of the authentic lipid and the projected MS behavior of such amides less obvious that their consideration was delayed. The benzene-d_6 ^{1}H NMR spectra of **1** exhibited the required resonances at 0.9, 1.2−1.3, 2.1, and 5.5 ppm. Most importantly, the CH_2-CONH_2 protons of **1** demonstrated a concentration-dependent chemical shift in benzene-d_6 that upon serial dilution moved upfield from 1.8 to 1.6−1.7 ppm. No other resonances except those of the amide protons showed this concentration dependence on their chemical shift. Interestingly, the protons of **1** are found much further downfield (2.1−2.2 ppm) in other NMR solvents (e.g., CD_3OD and $CDCl_3$), indicating a unique effect of benzene on the chemical shift of these protons. Finally, the unrefined resonances in the 3.5−4.1 ppm range of the natural agent were now likely attributable to either amide proton resonances or impurities in the sample of the endogenous substance.

While encouraged by the common MS and NMR characteristics shared by **1** and the natural agent, we were also aware that several fatty acid primary amides in addition to **1** exhibited these properties. To unambiguously determine the structure of the endogenous substance, 9(*E*)-octadecenamide (**64**), 8(*Z*)-octadecenamide (**65**), and 11(*Z*)-octadecenamide (**66**) were prepared for direct comparison to the natural agent (Figure 3). The agents **64** and **66** were prepared from their corresponding commercially available carboxylic acids, while **65** was synthesized as outlined in Scheme 7.[24] Additionally, sufficient amounts of the endogenous substance were now capable of being secured through extensive isolation[25] and refined purification efforts to perform the required chemical analyses for comparison with the synthetic fatty acid primary amides. Previous attempts to accumulate the endogenous substance had

Scheme 7



been hampered by continual loss of the agent.[25] The disappearance of the natural agent was originally attributed to chemical instability. Once the chemical features of the agent were elucidated, the apparent instability of the natural agent could be attributed to loss by a combination of solubility properties and absorption to the sides of plastic tubes. This facilitated both the isolation and, more importantly, the purification of the endogenous lipid.[9]

Both synthetic **1** and the natural lipid exhibited identical TLC (R_f = 0.3, 75% EtOAc−hexane), HPLC, and GC−MS[26] behavior. However, these techniques proved insensitive to the fatty acid amide double-bond position and stereochemistry and **64**−**66** also exhibited indistinguishable chromatographic properties.[27] The position of the double bond in the endogenous lipid was unambiguously determined by ozonolysis. GC−MS analysis of the ozonolysis reaction products derived from the natural lipid alongside those derived from synthetic **1**, **65**, and **66** revealed the release of nonal as the only CH_3-terminal aldehyde present in the endogenous sample which corresponds to a Δ^9 double bond.

The cis stereochemistry of the double bond was tentatively established on the basis of IR spectroscopy. With one exception, both 9(*Z*)- and 9(*E*)-octadecenamide exhibited FTIR spectra which were not distinguishable from that of the natural lipid. The trans isomer exhibited an additional strong and characteristic absorption peak at 960 cm^{-1} attributable to a trans disubstituted alkene that neither synthetic nor natural 9(*Z*)-octadecenamide displayed.

Ultimately, ^{1}H NMR was used to unambiguously distinguish **1** and **64**. Given the new appreciation for the solubility characteristics and chromatographic properties of **1**, approximately 300 μg of the pure endogenous lipid was obtained and used in ^{1}H NMR analysis and for comparison with synthetic **1** and **64**. Illustrations of these comparisons have been previously disclosed and reproductions of the ^{1}H NMR comparisons may be found in the supporting information.[9] The natural lipid and synthetic **1** proved identical and readily distinguishable from the 9*E*-isomer **64**. Both the olefinic and allylic regions of the ^{1}H NMR (CD_3OD, 400 MHz) exhibited readily distinguishable signals. In the double-bond region, both the chemical shift and the peak shape of the overlapping signals for the two protons were considerably different with that of **1** and the authentic lipid appearing at δ 5.24 as a rough apparent tt and that of **64** appearing as a set of two apparent overlapping doublet split

(24) In subsequent efforts, the full range of fatty acid amides have been prepared and subjected to comparison analysis: Patterson, J. E. Unpublished studies. Potent inhibitors of oleamide hydrolase: Patterson, J. E.; Ollmann, I. R.; Cravatt, B. F.; Lerner, R. A.; Wong, C.-H.; Boger, D. L. Submitted for publication.

(25) We thank Dr. O. Prospero-Garcia, Dr. S. J. Henriksen, and Dr. L. Bibbs for these efforts.

(26) GC−MS analyses were carried out on a 5890 Hewlett Packard GC with a 5971A Hewlett Packard mass selective detector. Separations were performed on a DB-5 (0.25 μM film) capillary column that was 30 m in length and had an internal diameter of 0.25 mm. The column temperature was programmed to increase from 50 to 290 °C at a rate of 20 °C/min. The column was maintained at the final temperature of 290 °C for an additional 10 min. The injector temperature was 250 °C and the detector temperature was 290 °C. The compounds were injected into the GC in CH_2Cl_2. The electron energy in EI measurements was 70 eV with 1 scan/s. Retention times: synthetic *cis*-9-octadecenamide, 16.95 min; natural compound, 16.95 min.

(27) The natural compound and synthetic *cis*-13-docosenamide also exhibited identical elution propeties on TLC.

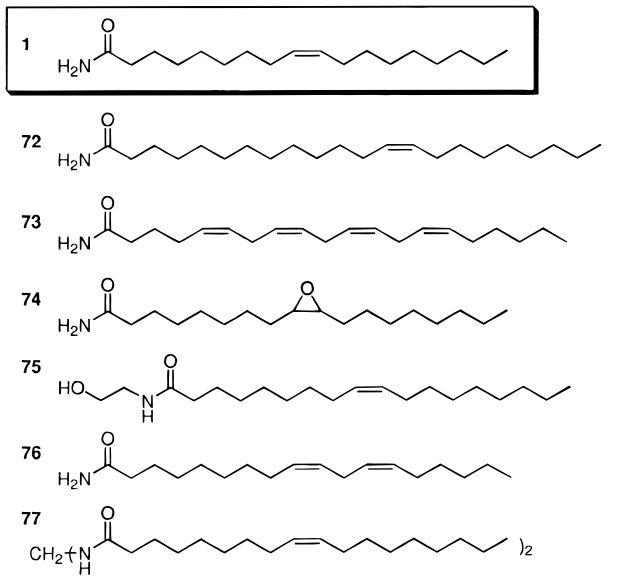


Figure 4.

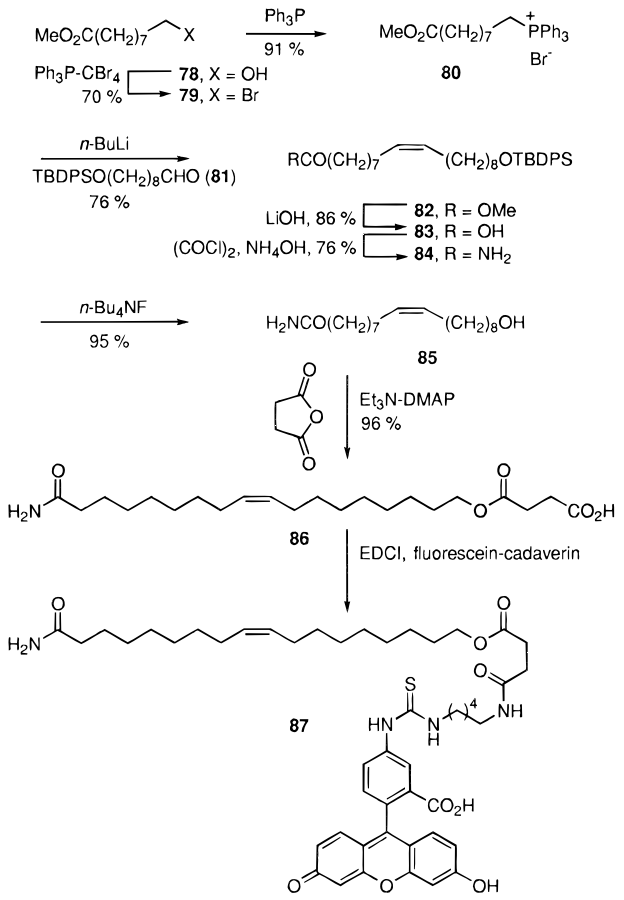
triplets at δ 5.28. In the allylic region, the 4H multiplet for synthetic 9*Z* and natural lipid appears at δ 1.94−1.91 while that of the 9*E* isomer is found at δ 1.88−1.86.

Related Fatty Acid Amides for *in Vivo* Testing. Several agents related to **1** have been prepared in order to assess the structural specificity of the sleep-inducing effect (Figure 4). In addition to **1** and **64**−**66**, 13(*Z*)-docosenamide (**72**), and arachidonyl amide (**73**) have been tested in rats for their sleep-inducing potential. Of these agents, only **64** exhibited similar, albeit much less potent, pharmacological effects to **1**.[9] Thus, the sleep-inducing efficacy of **1** appears structurally specific, as changes in the position or configuration of the Δ^9 double bond, the degree of unsaturation of the molecule, and the length of its alkyl chain either greatly reduce or eliminate the pharmacological effect. Additional agents, including *cis*-9,10-epoxyoctadecanamide (**74**), 9(*Z*)-octadecenoylthanolamide (**75**), 9(*Z*),12(*Z*)-octadecadienamide (**76**), and the dimer **77**, have been prepared and are presently being tested for their sleep-inducing potential.

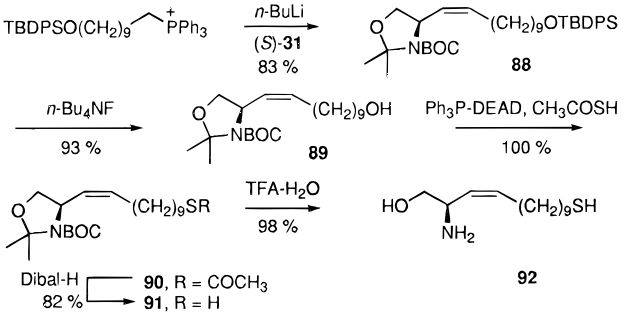
Affinity Chromatography and Fluorescent Probe Reagents. Subsequent to the identification of **1** and in conjunction with efforts to isolate and identify binding proteins associated with the natural lipid, the affinity agent **86** was prepared for linkage to Affi-gel 102 affinity chromatography beads (Bio-Rad). Conversion of methyl 9-hydroxynonanoate to the phosphonium salt **80** followed by Wittig reaction of its *in situ* generated phosphorane with aldehyde **81** cleanly provided (*Z*)-**82** (Scheme 8). Two-step conversion of **82** to the corresponding primary amide **84**, TBDPS ether deprotection, and esterification of the resulting primary alcohol **85** with succinic anhydride provided the hemisuccinate ester **86**. Studies mixing the **86**-linked beads with rat brain protein extracts are presently underway, and **86** linked to carrier protein KLH has been injected into mice for antibody generation. Additionally, **86** was coupled to fluorescein−cadaverin (Molecular Probes) effected by EDCI (4 equiv, DMF, 25 °C), providing **87** [FABHRMS (NBA−CsI) *m/z* 1003.3341 (M^+ + Cs, $C_{48}H_{62}N_4O_9S$ requires 1003.3292)] as a fluorescent ligand for cellular studies.

Similarly, at a time when the most likely candidate structure was thought to be a C18-sphingosine-derived nonconjugated diene, we elected to prepare a generic affinity chromatography reagent incorporating the intact allylic 2-amino-1-hydroxy terminus of the candidate structures. Agent **92** which incorporates a distal thiol for ultimate linkage through a heterobifunctional linker to Affi-gel 102 was selected for synthesis (Scheme 9) since the requisite phosphonium salt was available from the preceding studies. Wittig reaction of the corresponding phosphorane (*n*-BuLi, THF, 25 °C, 10 min) with (*S*)-**31** (THF, −78 to 25 °C, 30 min, 83%) cleanly provided (*Z*)-**88** (12:1 *Z*:*E*), and the minor amount of contaminating trans isomer was readily removed chromatographically. Deprotection of the TBDPS ether, thiolacetic acid displacement of the primary alcohol upon Mitsunobu activation, and sequential removal of the thiol and aminoalcohol protecting groups provided **92**.[28]

Scheme 8



Scheme 9



Conclusion. Herein, we have disclosed the experimental details of the structure determination of an endogenous sleep-inducing lipid, *cis*-9-octadecenamide. Initially confronted with insufficient quantities of the natural agent for adequate chemical characterization, we elected to pursue a synthetic approach to the identification of the unknown compound in tandem with the ongoing isolation efforts. The molecular formula and electrospray MS fragmentation pattern of the endogenous substance provided clues regarding the general chemical features

of the endogenous substance, and the MS fragmentation pattern served as a molecular fingerprint for the natural agent. The MS fragmentation patterns generated by synthetic candidate structures were compared with that of the natural agent, allowing identification of incorrect (aminoketone **3**, conjugated dienes **5**−**8**, and nonconjugated dienes **9**−**12**) and potentially correct (nonconjugated dienes **13**−**24** and primary amides **1** and **64**−**66**) structures. Further correlation of the acetylated derivative of **23** with data provided by a benzene-d_6 NMR spectrum of the crude natural agent directed the efforts to the family of fatty acid primary amides. Once the general structural features of the natural agent were determined, improved efforts toward the isolation of the endogenous compound in conjunction with a greater appreciation for its subsequent handling and storage provided sufficient quantities of the agent for its identification as structure **1**. In total and with the assistance of the synthetic studies, the structure identification of **1** required a little less than 5 months and ultimately *ca*. 300 μg of the endogenous material for unambiguous correlation. Moreover, initial studies with much smaller and highly impure samples of the natural agent provided sufficient information (HPLC/MS) to direct the synthetic correlations. Since disclosed, synthetic **1** has been found to effect a number of biological actions including sleep induction in rats in a structurally specific manner at nanomolar amounts when injected intraventricularly.[9] Additionally, the rapid hydrolysis of **1** to oleic acid was observed upon mixing ^{14}C-radiolabeled **1** with rat brain membrane extracts and potent inhibitors of the enzyme responsible for this hydrolysis are now available.[24] The biological mode of action of **1** as well as its biosynthesis and metabolism are topics under present investigation.

Just as importantly, *cis*-9-octadecenamide may serve as the prototypical member of a class of fatty acid primary amides that function as biological signaling molecules. In this regard, *cis*-13-docosenamide was identified as a natural constituent of the cerebrospinal fluid of cats, rats, and humans.[9] The diversity and selectivity of function of the fatty acid primary amides would be derived from the length of the alkane chain, as well as the position, stereochemistry, and number of double bonds. While simplistic, such apparently small structural changes can be expected to significantly restrict the accessible or preferred conformations, especially through employing cis versus trans olefins, and are perhaps suggestive of the coevolution of the fatty acid primary amides along with basal physiological functions like sleep.

Experimental Section[29]

3-(*tert*-Butyloxycarbonyl)-2,2-dimethyl-4(*R*)-[1(*E*),3(*E*)-hexadecadienyl]oxazolidine (32) and 3-(*tert*-Butyloxycarbonyl)-2,2-dimethyl-4(*R*)-[1(*Z*),3(*E*)-hexadecadienyl]oxazolidine (33). A solution of **30**[29] (0.100 g, 0.180 mmol, 1.0 equiv) in THF (2.0 mL, 0.09 M) at −78 °C was treated with PhLi (0.100 mL, 1.8 M solution in cyclohexane−ether, 0.18 mmol, 1.0 equiv), and the mixture was warmed to 25 °C and stirred for 10 min. The bright orange-red reaction mixture was cooled to −78 °C, 1,1-dimethylethyl (*S*)-4-formyl-2,2-dimethyl-3-oxazolidinecarboxylate (**31**,[14] 0.053 g, 0.234 mmol, 1.3 equiv) was added, and the reaction mixture was warmed to 25 °C. After being stirred at 25 °C for 30 min, the reaction mixture was treated with saturated aqueous NH_4Cl and partitioned between EtOAc (50 mL) and H_2O (50 mL). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography (SiO_2, 3 × 15 cm, 0−2% EtOAc−hexane gradient elution) afforded **32** (0.015 g), **33** (0.012 g), and a 1:1 mixture of **32** and **33** (0.013 g, 0.040 g total yield, 0.076 g theoretical, 53%) as colorless oils. A second chromatography (SiO_2, 3 × 15 cm, 0−1% EtOAc−hexane gradient elution) on the remaining mixture of **32** and **33** provided the isomers cleanly separated from one another.

32: ^{1}H NMR (CD_3OD, 250 MHz) δ 6.14 (m, 2H, CH═CH), 5.70 (m, 1H, C*H*═CH), 5.48 (dd, J = 8.1 and 15.0 Hz, 1H, CH═C*H*), 4.32 (m, 1H, RNC*H*), 4.05 (dd, 1H, J = 6.0, 8.8 Hz, OC*H*H), 3.70 (dd, 1H, J = 2.3, 8.9 Hz, OCH*H*), 2.09 (q, 2H, J = 6.7 Hz, CH_2CH═CH), 1.64−1.15 (m, 35H), 0.89 (t, 3H, J = 6.7 Hz, CH_3); IR (neat) ν_{max} 2933, 2851, 1702, 1384, 1364, 1251, 1174, 1097, 1082, 1056, 984 cm^{-1} (strong, trans absorption); electrospray MS *m/z* 422 ($C_{26}H_{47}NO_3 + H^+$ requires 422).

33: ^{1}H NMR (CD_3OD, 250 MHz) δ 6.41 (m, 1H, C*H*═CH), 6.02 (t, 1H, J = 10.9 Hz, CH═C*H*), 5.71 (m, 1H, C*H*═CH), 5.25 (t, 1H, J = 10.2 Hz, CH═C*H*), 4.79 (m, 1H, RNC*H*), 4.09 (dd, 1H, J = 6.0, 8.9 Hz, OC*H*H), 3.62 (dd, 1H, J = 2.6, 8.8 Hz, OCH*H*), 2.11 (q, 2H, J = 6.7 Hz, CH_2CH═CH), 1.64−1.15 (m, 35H), 0.89 (t, 3H, J = 6.7 Hz, CH_3); IR (neat) ν_{max} 2923, 2851, 1702, 1384, 1369, 1251, 1174, 1087, 1051, 984, 948, 851 cm^{-1}; electrospray MS *m/z* 422, 444 ($C_{26}H_{47}NO_3 + H^+$ for 422, $C_{26}H_{47}NO_3 + Na^+$ requires 444).

3-(*tert*-Butyloxycarbonyl-2,2-dimethyl-4(*R*)-[1(*E*),3(*Z*)-hexadecadienyl]oxazolidine (39) and 3-(*tert*-Butyloxycarbonyl)-2,2-dimethyl-4(*R*)-[1(*Z*),3(*Z*)-hexadecadienyl]oxazolidine (40). A solution of **38**[29] (0.080 g, 0.143 mmol, 1.0 equiv) in THF (1.4 mL, 0.1 M) at −78 °C was treated with PhLi (0.080 mL, 1.8 M solution in cyclohexane−ether, 0.143 mmol, 1.0 equiv). The bright orange-red reaction mixture was warmed to 25 °C and stirred for 10 min. The reaction mixture was then cooled to −78 °C, 1,1-dimethylethyl (*S*)-4-formyl-2,2-dimethyl-3-oxazolidinecarboxylate (**31**,[14] 0.049 g, 0.215 mmol, 1.3 equiv) was added, and the reaction mixture was warmed to 25 °C. After being stirred at 25 °C for 30 min, the reaction mixture was treated with saturated aqueous NH_4Cl and partitioned between EtOAc (50 mL) and H_2O (50 mL). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography (SiO_2, 3 × 15 cm, 0−2% EtOAc−hexane gradient elution) afforded **39** (0.011 g), **40** (0.008 g), and a 1:1 mixture of **39** and **40** (0.013 g, 0.032 g total yield, 0.060 g theoretical, 54%) as colorless oils. A second chromatography (SiO_2 3 × 15 cm, 0−1% EtOAc−hexane gradient elution) on the remaining mixture of **39** and **40** provided the isomers cleanly separated from one another.

39: ^{1}H NMR (CD_3OD, 250 MHz) δ 6.46 (m, 1H, C*H*═CH), 5.98 (t, 1H, J = 10.9 Hz, CH═C*H*), 5.60 (m, 1H, C*H*═CH), 5.46 (q, 1H, J = 7.5 Hz, CH═C*H*), 4.37 (m, 1H, RNC*H*), 4.05 (dd, 1H, J = 6.4, 8.9 Hz, OC*H*H), 3.53 (dd, 1H, J = 2.3, 9.0 Hz, OCH*H*), 2.18 (q, 2H, J = 6.5 Hz, CH_2CH═CH), 1.64−1.15 (m, 35H), 0.89 (t, 3H, J = 6.7 Hz, CH_3); IR (neat) ν_{max} 2923, 2851, 1702, 1384, 1369, 1251, 1174, 1097, 1051, 984, 948, 856 cm^{-1}; electrospray MS *m/z* 422, 444 ($C_{26}H_{47}NO_3 + H^+$ requires 422, $C_{26}H_{47}NO_3 + Na^+$ requires 444).

40: ^{1}H NMR (CD_3OD, 250 MHz) δ 6.34 (m, 2H, C*H*═C*H*), 5.54 (q, 1H, J = 9.2 Hz, C*H*═CH), 5.40 (t, 1H, J = 8.8 Hz, CH═C*H*), 4.81 (m, 1H, RNC*H*), 4.10 (dd, 1H, J = 6.4, 8.9 Hz, OC*H*H), 3.63 (dd, 1H, J = 2.8, 8.9 Hz, OCH*H*), 2.18 (q, 2H, J = 6.5 Hz, CH_2-CH═CH), 1.64−1.15 (m, 35H), 0.89 (t, 3H, J = 6.7 Hz, CH_3); IR (neat) ν_{max} 2923, 2851, 1702, 1384, 1369, 1251, 1174, 1097, 1051, 851 cm^{-1}; electrospray MS *m/z* 422, 444 ($C_{26}H_{47}NO_3 + H^+$ requires 422, $C_{26}H_{47}NO_3 + Na^+$ requires 444).

General Procedure for the Deprotection of 32−33 and 39−40. 2(*R*)-Amino-1-hydroxy-3(*E*),5(*E*)-octadecadiene (**5**). A solution of **32** (0.010 g, 0.036 mmol) in trifluoroacetic acid−H_2O (3:1, 0.20 mL, 0.18 M) was stirred at 25 °C for 10 min. The reaction mixture was treated dropwise with saturated aqueous $NaHCO_3$ and partitioned between EtOAc (50 mL) and H_2O (50 mL). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to provide **5** as a colorless oil (0.0060 g, 0.0067 g theoretical, 90%): ^{1}H NMR (CD_3OD, 400 MHz) δ 6.21 (dd, 1H, J = 10.3, 15.3 Hz, C*H*═CH), 6.02 (m, 1H, CH═C*H*), 5.69 (m, 1H, C*H*═CH), 5.49 (dd, 1H, J = 6.6, 15.4 Hz, CH═C*H*), 3.64 (m, 1H, H_2NC*H*), 3.53 (dd, 1H, J = 3.6, 9.5 Hz, C*H*HOH), 3.38

(28) The same reaction sequence was also carried out on (*E*)-**88** to provide 3-(*tert*-butyloxycarbonyl)-2,2-dimethyl-4(*R*)-(11-(acetylthio)-1(*E*)-undecenyl)oxazolidine: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 5.71−5.25 (m, 4H, C*H*═C*H*), 4.22 (m, 1H, RNCH), 3.99 (dd, J = 6.0, 8.7 Hz, 1H, OC*H*H), 3.70 (dd, J = 3.1, 8.7 Hz, 1H, OCH*H*), 2.86 (t, 2H, J = 7.5 Hz, CH_2-SCOCH$_3$), 2.33 (s, 3H, CH_3COS), 2.04 (q, J = 6.7 Hz, 2H, CH_2CH═CH), 1.69−1.20 (m, 29H).

(29) Detailed experimentals for **27**−**30**, **35**−**38**, **41**−**48**, **50**−**63**, **64**−**66**, **68**−**71**, **73**−**74**, **76**, **78**−**81**, and **88**−**92** may be found in the supporting inormation.

(m, 1H, CH*H*OH), 2.06 (q, 2H, J = 6.8 Hz, CH_2CH=CH), 1.28 (m, 20H), 0.89 (t, 3H, J = 6.7 Hz, CH_3); IR (neat) ν_{max} 3435, 2923, 2848, 1682, 1677, 1205, 1184, 1137, 984, 802 cm^{-1}; UV (CH_3OH) λ_{max} 228−230 nm; FABHRMS (NBA−NaI) *m/z* 304.2624 ($C_{18}H_{35}NO + Na^+$ requires 304.2616).

2(*R*)-Amino-1-hydroxy-3(*Z*),5(*E*)-octadecadiene (6): ^{1}H NMR (CD_3OD, 400 MHz) δ 6.36 (m, 1H, C*H*=CH), 6.18 (t, 1H, J = 11.3 Hz, CH=C*H*), 5.83 (p, 1H, J = 7.5 Hz, C*H*=CH), 5.15 (t, 1H, J = 10.0 Hz, CH=C*H*), 4.04 (m, 1H, H_2NC*H*), 3.55 (dd, 1H, J = 3.6, 9.5 Hz, C*H*HOH), 3.30 (m, 1H, CH*H*OH), 2.13 (q, 2H, J = 6.7 Hz, CH_2-CH=CH), 1.30 (m, 20H), 0.89 (t, 3H, J = 6.7 Hz, CH_3); IR (neat) ν_{max} 3343, 2923, 2851, 1682, 1205, 1184, 1138, 984, 948, 805 cm^{-1}; UV (CH_3OH) λ_{max} 228−230 nm; FABHRMS (NBA−CsI) *m/z* 414.1758 ($C_{18}H_{35}NO + Cs^+$ requires 414.1773).

2(*R*)-Amino-1-hydroxy-3(*E*),5(*Z*)-octadecadiene (7): ^{1}H NMR (CD_3OD, 400 MHz) δ 6.64 (dd, 1H, J = 4.4, 11.1 Hz, C*H*=CH), 5.98 (t, 1H, J = 10.9 Hz, CH=C*H*), 5.59 (m, 1H, C*H*=CH), 5.47 (q, 1H, J = 8.8 Hz, CH=C*H*), 3.65−3.40 (m, 3H, H_2NC*H* and CH_2OH), 2.20 (q, 2H, J = 7.2 Hz, CH_2CH=CH), 1.30 (m, 20H), 0.89 (t, 3H, J = 6.7 Hz, CH_3); IR (neat) ν_{max} 3353, 2923, 2851, 1676, 1205, 1179, 1133, 984, 948, 800 cm^{-1}; UV (CH_3OH) λ_{max} 232−234 nm; FABHRMS (NBA−CsI) *m/z* 414.1760 ($C_{18}H_{35}NO + Cs^+$ requires 414.1773).

2(*R*)-Amino-1-hydroxy-3(*Z*),5(*Z*)-octadecadiene (8): ^{1}H NMR (CD_3OD, 400 MHz) δ 6.47 (t, 1H, J = 11.4 Hz, C*H*=CH), 6.29 (t, 1H, J = 11.4 Hz, CH=C*H*), 5.61 (m, 1H, C*H*=CH), 5.29 (t, 1H, J = 10.2 Hz, CH=C*H*), 3.98 (m, 1H, H_2NC*H*), 3.55 (dd, 1H, J = 4.3, 10.8 Hz, C*H*HOH), 3.39 (m, 1H, CH*H*OH), 2.20 (q, 2H, J = 7.2 Hz, CH_2-CH=CH), 1.30 (m, 20H), 0.89 (t, 3H, J = 6.7 Hz, CH_3); IR (neat) ν_{max} 3353, 2923, 2851, 1682, 1205, 1179, 1138, 800 cm^{-1}; UV (CH_3OH) λ_{max} 232−234 nm; FABHRMS (NBA−CsI) *m/z* 414.1761 ($C_{18}H_{35}NO + Cs^+$ requires 414.1773).

3-(*tert*-Butyloxycarbonyl)-2,2-dimethyl-4(*R*)-[1(*Z*),10(*Z*)-hexadecadienyl]oxazolidine [(2*R*,3*Z*,12*Z*)-61] and 3-(*tert*-Butyloxycarbonyl)-2,2-dimethyl-4(*R*)-[1(*E*),10(*Z*)-hexadecadienyl]oxazolidine [(2*R*,3*E*,12*Z*)-61]. A solution of **46**[29] (0.235 g, 0.39 mmol, 1.0 equiv) in THF (2.0 mL, 0.2 M) at −78 °C was treated with *n*-BuLi (0.173 mL, 2.5 M solution in hexanes, 0.43 mmol, 1.1 equiv). The bright orange-red reaction mixture was warmed to 25 °C and stirred for 10 min. The reaction mixture was recooled to −78 °C, (*S*)-**31** (0.107 g, 0.47 mmol, 1.2 equiv) was added, and the reaction mixture was warmed to 25 °C. After being stirred at 25 °C for 30 min, the reaction mixture was treated with saturated aqueous NH_4Cl and partitioned between EtOAc (50 mL) and H_2O (50 mL). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography (SiO_2, 3 × 15 cm, 0−2% EtOAc−hexane gradient elution) afforded (3*Z*,12*Z*)-**61** (0.095 g) and a 1:1 mixture of (3*Z*,12*Z*)- and (3*E*,12*Z*)-**61** (0.0021 g, 0.116 g total yield, 0.165 g theoretical, 70%) as colorless oils. A second chromatography (SiO_2, 3 × 15 cm, 0−1% EtOAc−hexane gradient elution) on the remaining mixture provided the olefin isomers cleanly separated from one another.

(2*R*,3*Z*,12*Z*)-61: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 5.57−5.25 (m, 4H, C*H*=C*H*), 4.62 (m, 1H, RNC*H*), 4.05 (dd, J = 6.2, 8.6 Hz, 1H, OC*H*H), 3.64 (dd, J = 3.3, 8.7 Hz, 1H, OCH*H*), 2.23−1.88 (m, 6H, CH_2-CH=CH), 1.70−1.18 (m, 31H), 0.89 (t, J = 6.7 Hz, 3H, CH_3); $[\alpha]^{25}_D$ +60 (*c* 1.15, THF); IR (neat) ν_{max} 2925, 2854, 1701, 1383, 1250, 1175, 1089, 850 cm^{-1}; FABHRMS (NBA−CsI) *m/z* 554.2635 ($C_{26}H_{47}NO_3 + Cs^+$ requires 554.2610).

(2*R*,3*E*,12*Z*)-61: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 5.71−5.25 (m, 4H, C*H*=C*H*), 4.22 (m, 1H, RNC*H*), 3.99 (dd, J = 6.0, 8.7 Hz, 1H, OC*H*H), 3.70 (dd, J = 2.1, 8.7 Hz, 1H, OCH*H*), 2.16−1.90 (m, 6H, CH_2-CH=CH), 1.70−1.18 (m, 31H), 0.89 (t, J = 6.7 Hz, 3H, CH_3); IR (neat) ν_{max} 2925, 2864, 1701, 1383, 1250, 1175, 1089, 965, 850 cm^{-1}; FABHRMS (NBA−NaI) *m/z* 444.3465 ($C_{26}H_{47}NO_3 + Na^+$ requires 444.3454).

Intermediates **56**−**63**[29] were obtained by the procedure detailed for **61**.

2(*R*)-Amino-1-hydroxy-3(*Z*),12(*Z*)-octadecadiene (19). A solution of (2*R*,3*Z*,12*Z*)-**61** (0.015 g, 0.036 mmol) in TFA−H_2O (3:1, 0.200 mL, 0.18 M) was stirred at 25 °C for 10 min. The reaction mixture was treated dropwise with saturated aqueous $NaHCO_3$ and partitioned between EtOAc (50 mL) and H_2O (50 mL). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to provide **19** as a colorless oil (0.0095 g, 0.0101 g theoretical, 94%): ^{1}H NMR (CD_3OD, 400 MHz) δ 5.66 (m, 1H, C*H*=CH), 5.41−5.24 (m, 3H, C*H*=C*H*), 3.89 (m, 1H, H_2NC*H*), 3.57−3.34 (m, 2H, HOCH_2), 2.18−1.94 (m, 6H, CH_2CH=CH), 1.49−1.22 (m, 16H), 0.89 (t, 3H, J = 6.7 Hz, CH_3); FABHRMS (NBA) *m/z* 282.2810 ($C_{18}H_{35}NO + H^+$ requires 282.2797).

2(*R*)-Amino-1-hydroxy-3(*E*),12(*Z*)-octadecadiene (20). A solution of (2*R*,3*Z*,12*Z*)-**61** (0.010 g, 0.036 mmol) in TFA−H_2O (3:1 ratio, 0.200 mL, 0.18 M) was stirred at 25 °C for 10 min. The reaction mixture was treated dropwise with saturated aqueous $NaHCO_3$ and partitioned between EtOAc (50 mL) and H_2O (50 mL). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to provide **20** as a colorless oil (0.0064 g, 0.0067 g theoretical, 96%): ^{1}H NMR (CD_3OD, 250 MHz) δ 5.69 (m, 1H, C*H*=CH), 5.43−5.30 (m, 3H, C*H*=C*H*), 3.53 (m, 1H, H_2NC*H*), 3.36 (m, 2H, HOCH_2), 2.07−1.94 (m, 6H, CH_2-CH=CH), 1.49−1.22 (m, 16H), 0.89 (t, 3H, J = 6.7 Hz, CH_3); FABHRMS (NBA−NaI) *m/z* 304.2609 ($C_{18}H_{35}NO + Na^+$ requires 304.2616).

The deprotections to provide **9**−**24** were conducted following the procedures for **19**−**20**.

2(*R*)-Amino-1-hydroxy-3(*Z*),6(*E*)-octadecadiene (9): ^{1}H NMR (CD_3OD, 250 MHz) δ 5.76−5.25 (m, 4H, C*H*=C*H*), 3.91 (m, 1H, H_2-NC*H*), 3.59−3.32 (m, 2H, HOCH_2), 2.84 (m, 2H, CHCH_2CH), 2.04 (m, 2H, CHCH_2CH$_2$), 1.49−1.22 (m, 16H), 0.89 (t, J = 6.7 Hz, 3H, CH_3); electrospray MS *m/z* 282, 304 ($C_{18}H_{35}NO + H^+$ requires 282, $C_{18}H_{35}NO + Na^+$ requires 304).

2(*R*)-Amino-1-hydroxy-3(*Z*),7(*Z*)-octadecadiene (10): ^{1}H NMR (CD_3OD, 400 MHz) δ 5.66 (m, 1H, C*H*=CH), 5.41−5.24 (m, 3H, C*H*=C*H*), 3.89 (m, 1H, H_2NC*H*), 3.57−3.34 (m, 2H, HOCH_2), 2.18−1.94 (m, 6H, CH_2CH=CH), 1.49−1.22 (m, 16H), 0.89 (t, J = 6.7 Hz, 3H, CH_3); IR (neat) ν_{max} 3364, 2923, 2851, 1677, 1205, 1138, 1056, 800 cm^{-1}; FABHRMS (NBA) *m/z* 282.2784 ($C_{18}H_{35}NO + H^+$ requires 282.2797).

2(*R*)-Amino-1-hydroxy-3(*Z*),8(*Z*)-octadecadiene (11): ^{1}H NMR (CD_3OD, 400 MHz) δ 5.66 (m, 1H, C*H*=CH), 5.41−5.24 (m, 3H, C*H*=C*H*), 3.89 (m, 1H, H_2NC*H*), 3.57−3.34 (m, 2H, HOCH_2), 2.18−1.94 (m, 6H, CH_2CH=CH), 1.49−1.22 (m, 16H), 0.89 (t, J = 6.7 Hz, 3H, CH_3); IR (neat) ν_{max} 3364, 2923, 2851, 1677, 1205, 1138, 1056, 800 cm^{-1}; FABHRMS (NBA−CsI) *m/z* 414.1786 ($C_{18}H_{35}NO + Cs^+$ requires 414.1773).

2(*R*)-Amino-1-hydroxy-3(*E*),8(*Z*)-octadecadiene (12): ^{1}H NMR (CD_3OD, 400 MHz) δ 5.69 (m, 1H, C*H*=CH), 5.43−5.30 (m, 3H, C*H*=C*H*), 3.53 (m, 1H, H_2NC*H*), 3.36 (m, 2H, HOCH_2), 2.07−1.94 (m, 6H, CH_2CH=CH), 1.49−1.22 (m, 16H), 0.89 (t, J = 6.7 Hz, 3H, CH_3); IR (neat) ν_{max} 3364, 2923, 2851, 1677, 1205, 1138, 1056, 969, 800 cm^{-1}; electrospray MS *m/z* 282, 304 ($C_{18}H_{35}NO + H^+$ requires 282, $C_{18}H_{35}NO + Na^+$ requires 304).

2(*R*)-Amino-1-hydroxy-3(*Z*),9(*Z*)-octadecadiene (13): ^{1}H NMR (CD_3OD, 400 MHz) δ 5.66 (m, 1H, C*H*=CH), 5.41−5.24 (m, 3H, C*H*=C*H*), 3.89 (m, 1H, H_2NC*H*), 3.57−3.34 (m, 2H, HOCH_2), 2.18−1.94 (m, 6H, CH_2CH=CH), 1.49−1.22 (m, 16H), 0.89 (t, J = 6.7 Hz, 3H, CH_3); IR (neat) ν_{max} 3364, 2923, 2851, 1677, 1205, 1138, 1056, 800 cm^{-1}; FABHRMS (NBA−CsI) *m/z* 414.1760 ($C_{18}H_{35}NO + Cs^+$ requires 414.1773).

2(*R*)-Amino-1-hydroxy-3(*E*),9(*Z*)-octadecadiene (14): ^{1}H NMR (CD_3OD, 400 MHz) δ 5.69 (m, 1H, C*H*=CH), 5.43−5.30 (m, 3H, C*H*=C*H*), 3.53 (m, 1H, H_2NC*H*), 3.36 (m, 2H, HOCH_2), 2.07−1.94 (m, 6H, CH_2CH=CH), 1.49−1.22 (m, 16H), 0.89 (t, J = 6.7 Hz, 3H, CH_3); IR (neat) ν_{max} 3364, 2923, 2851, 1677, 1205, 1138, 1056, 969, 800 cm^{-1}; FABHRMS (NBA−CsI) *m/z* 414.1756 ($C_{18}H_{35}NO + Cs^+$ requires 414.1773).

2(*R*)-Amino-1-hydroxy-3(*Z*),10(*Z*)-octadecadiene (15): ^{1}H NMR (CD_3OD, 400 MHz) δ 5.66 (m, 1H, C*H*=CH), 5.41−5.24 (m, 3H, C*H*=C*H*), 3.89 (m, 1H, H_2NC*H*), 3.57−3.34 (m, 2H, HOCH_2), 2.18−1.94 (m, 6H, CH_2CH=CH), 1.49−1.22 (m, 16H), 0.89 (t, J = 6.7 Hz, 3H, CH_3); IR (neat) ν_{max} 3364, 2923, 2851, 1677, 1205, 1138, 1056, 800 cm^{-1}; FABHRMS (NBA) *m/z* 282.2806 ($C_{18}H_{35}NO + H^+$ requires 282.2797).

2(*R*)-Amino-1-hydroxy-3(*E*),10(*Z*)-octadecadiene (16): ^{1}H NMR (CD_3OD, 400 MHz) δ 5.69 (m, 1H, C*H*=CH), 5.43−5.30 (m, 3H, C*H*=C*H*), 3.53 (m, 1H, H_2NC*H*), 3.36 (m, 2H, HOCH_2), 2.07−1.94 (m, 6H, CH_2CH=CH), 1.49−1.22 (m, 16H), 0.89 (t, J = 6.7 Hz, 3H,

CH_3); IR (neat) ν_{max} 3364, 2923, 2851, 1677, 1205, 1138, 1056, 969, 800 cm^{-1}; FABHRMS (NBA) *m/z* 282.2787 ($C_{18}H_{35}NO + H^+$ requires 282.2797).

2(*R*)-Amino-1-hydroxy-3(*Z*),11(*Z*)-octadecadiene (17): ^1H NMR (CD_3OD, 400 MHz) δ 5.66 (m, 1H, C*H*═CH), 5.41−5.24 (m, 3H, C*H*═C*H*), 3.89 (m, 1H, H_2NCH), 3.57−3.34 (m, 2H, $HOCH_2$), 2.18−1.94 (m, 6H, CH_2CH═CH), 1.49−1.22 (m, 16H), 0.89 (t, J = 6.7 Hz, 3H, CH_3); IR (neat) ν_{max} 3364, 2923, 2851, 1677, 1205, 1138, 1056, 800 cm^{-1}; FABHRMS (NBA) *m/z* 282.2803 ($C_{18}H_{35}NO + H^+$ requires 282.2797).

2(*R*)-Amino-1-hydroxy-3(*E*),11(*Z*)-octadecadiene (18): ^1H NMR (CD_3OD, 400 MHz) δ 5.69 (m, 1H, C*H*═CH), 5.43−5.30 (m, 3H, C*H*═C*H*), 3.53 (m, 1H, H_2NCH), 3.36 (m, 2H, $HOCH_2$), 2.07−1.94 (m, 6H, CH_2CH═CH), 1.49−1.22 (m, 16H), 0.89 (t, J = 6.7 Hz, 3H, CH_3); IR (neat) ν_{max} 3364, 2923, 2851, 1677, 1205, 1138, 1056, 969, 800 cm^{-1}; FABHRMS (NBA) *m/z* 282.2788 ($C_{18}H_{35}NO + H^+$ requires 282.2797).

2(*R*)-Amino-1-hydroxy-3(*Z*),13(*Z*)-octadecadiene (21): ^1H NMR (CD_3OD, 400 MHz) δ 5.66 (m, 1H, C*H*═CH), 5.41−5.24 (m, 3H, C*H*═C*H*), 3.89 (m, 1H, H_2NCH), 3.57−3.34 (m, 2H, $HOCH_2$), 2.18−1.94 (m, 6H, CH_2CH═CH), 1.49−1.22 (m, 16H), 0.89 (t, J = 6.7 Hz, 3H, CH_3); IR (neat) ν_{max} 3364, 2923, 2851, 1677, 1205, 1138, 1056, 800 cm^{-1}; FABHRMS (NBA) *m/z* 282.2790 ($C_{18}H_{35}NO + H^+$ requires 282.2797).

2(*R*)-Amino-1-hydroxy-3(*E*),13(*Z*)-octadecadiene (22): ^1H NMR (CD_3OD, 400 MHz) δ 5.69 (m, 1H, C*H*═CH), 5.43−5.30 (m, 3H, C*H*═C*H*), 3.53 (m, 1H, H_2NCH), 3.36 (m, 2H, $HOCH_2$), 2.07−1.94 (m, 6H, CH_2CH═CH), 1.49−1.22 (m, 16H), 0.89 (t, J = 6.7 Hz, 3H, CH_3); IR (neat) ν_{max} 3364, 2923, 2851, 1677, 1205, 1138, 1056, 969, 800 cm^{-1}; electrospray MS *m/z* 282, 304 ($C_{18}H_{35}NO + H^+$ requires 282, $C_{18}H_{35}NO + Na^+$ requires 304).

2(*R*)-Amino-1-hydroxy-3(*Z*),14(*Z*)-octadecadiene (23): ^1H NMR (CD_3OD, 400 MHz) δ 5.66 (m, 1H, C*H*═CH), 5.41−5.24 (m, 3H, C*H*═C*H*), 3.89 (m, 1H, H_2NCH), 3.57−3.34 (m, 2H, $HOCH_2$), 2.18−1.94 (m, 6H, CH_2CH═CH), 1.49−1.22 (m, 16H), 0.89 (t, J = 6.7 Hz, 3H, CH_3); IR (neat) ν_{max} 3364, 2923, 2851, 1677, 1205, 1138, 1056, 800 cm^{-1}; FABHRMS (NBA) *m/z* 282.2789 ($C_{18}H_{35}NO + H^+$ requires 282.2797).

2(*R*)-Amino-1-hydroxy-3(*E*),14(*Z*)-octadecadiene (24): ^1H NMR (CD_3OD, 400 MHz) δ 5.69 (m, 1H, C*H*═CH), 5.43−5.30 (m, 3H, C*H*═C*H*), 3.53 (m, 1H, H_2NCH), 3.36 (m, 2H, $HOCH_2$), 2.07−1.94 (m, 6H, CH_2CH═CH), 1.49−1.22 (m, 16H), 0.89 (t, J = 6.7 Hz, 3H, CH_3); IR (neat) ν_{max} 3364, 2923, 2851, 1677, 1205, 1138, 1056, 969, 800 cm^{-1}; FABHRMS (NBA) *m/z* 282.2786 ($C_{18}H_{35}NO + H^+$ requires 282.2797).

2(*R*)-(*N*-Acetylamino)-1-hydroxy-3(*Z*),14(*Z*)-octadecadiene. A sample of **23** (0.013 g, 0.046 mmol, 1.0 equiv) was dissolved in CH_3OH-Ac_2O (5:1, 1.1 mL, 0.05 M), and the reaction mixture was stirred at 25 °C for 10 h. The reaction mixture was then partitioned between EtOAc (40 mL) and H_2O (40 mL), and the organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to provide the product as a colorless oil (0.013 g, 0.015 g theoretical, 87%): ^1H NMR (CD_3OD, 250 MHz) δ 5.58 (m, 1H, C*H*═CH), 5.42−5.22 (m, 3H, C*H*═C*H*), 4.70 (m, 1H, C*H*$NHCOCH_3$), 3.45 (m, 2H, CH_2OH), 2.19−1.94 (m, 6H, CH_2CH═CH), 1.94 (s, 3H, $COCH_3$), 1.59−1.20 (m, 31H, alkyl protons), 0.89 (t, 3H, J = 6.7 Hz, CH_3); FABHRMS (NBA) *m/z* 324.2913 ($C_{20}H_{37}NO_2 + H^+$ requires 324.2903).

9(*Z*)-Octadecenamide (1, Oleamide). A solution of oleic acid (1.0 g, 3.55 mmol, 1.0 equiv) at 0 °C was treated dropwise with oxalyl chloride (5.32 mL, 2 M solution in CH_2Cl_2, 10.64 mmol, 3.0 equiv). The reaction mixture was stirred at 25 °C for 4 h, concentrated under reduced pressure, cooled to 0 °C, and treated with saturated aqueous NH_4OH (2.0 mL). After 5 min, the reaction mixture was patitioned between EtOAc (100 mL) and H_2O (100 mL), and the organic layer was dried (Na_2SO_4) and concentrated under pressure. Chromatography (SiO_2, 5 × 15 cm, 40−100% EtOAc−hexanes gradient elution) afforded **1** as a white solid (0.81 g, 0.996 g theoretical, 81%) identical in all respects with material previously disclosed:[30] mp 73−74 °C; ^1H NMR (CD_3OD, 400 MHz) δ 5.24 (m, 2H, C*H*═C*H*), 2.09 (t, J = 7.7 Hz, 2H, CH_2CONH_2), 1.92 (m, 4H, CH_2CH═CH), 1.50 (m, 2H, $CH_2CH_2CONH_2$), 1.35−1.13 (m, 14H), 0.81 (t, J = 6.7 Hz, 3H, CH_3); IR (neat) ν_{max} 3354, 3320, 2923, 2851, 1656, 1630, 1466, 1410 cm^{-1}; FABHRMS (NBA) *m/z* 282.2804 ($C_{18}H_{35}NO + H^+$ requires 282.2797).

Ozonolysis of the Natural Agent. Approximately 100 μg of the natural lipid was dissolved in CH_2Cl_2 (300 μL) and cooled to −78 °C. Ozone-saturated CH_2Cl_2 (600 μL, generated at −78 °C) was added to the mixture and stirring was continued at −78 °C for 30 min. Excess ozone was removed by purging the reaction mixture with Ar, and the ozonide products were reduced with the addition of dimethyl sulfide (excess). The reaction mixture was warmed to 0 °C and stirred for 1 h and 25 °C for 1 h. The solvent was evaporated, and the residue was redissolved in 50 μL of Et_2O and directly analyzed by GC−MS.

GC−MS analyses of the products were carried out as described previously.[26] The GC program used to analyze the aldehyde product(s) was as follows: After 4 min at an initial temperature of 50 °C, the column temperature was increased at a rate of 10 °C/min until a final temperature of 200 °C was reached. The column temperature was maintained at 200 °C for an additional 5 min. Retention times: nonyl aldehyde, 10.78 min; ozonolysis reaction product of the natural compound, 10.76 min; pentyl aldehyde, 2.50 min; hexyl aldehyde, 4.22 min; heptyl aldehyde, 6.52 min; octyl aldehyde, 8.81 min; decyl aldehyde, 12.65 min.

9(*Z*)-Octadecenoylethanolamine (75). A solution of oleic acid (0.500 g, 0.177 mmol, 1.0 equiv) in CH_2Cl_2 (6.0 mL, 0.3 M) at 0 °C was treated dropwise with oxalyl chloride (2.7 mL, 2 M solution in CH_2Cl_2, 5.4 mmol, 3.0 equiv). The reaction mixture was stirred at 25 °C for 4 h, concentrated under reduced pressure, cooled to 0 °C, and treated with ethanolamine (1.07 mL, 17.7 mmol, 10 equiv). The reaction mixture was stirred for 15 min at 25 °C and then partitioned between EtOAc (100 mL) and H_2O (100 mL), and the organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography (SiO_2, 5 × 15 cm, 40−100% EtOAc−hexane gradient elution) afforded **75** as a white solid (0.470 g, 0.578 g theoretical, 81%) identical in all respects with material previously disclosed:[30] mp 62−63 °C; ^1H NMR ($CDCl_3$, 250 MHz) δ 5.90 (br s, 1H, NH), 5.34 (m, 2H, C*H*═C*H*), 3.73 (q, J = 7 Hz, 2H, CH_2OH), 3.44 (q, J = 7 Hz, 2H, CH_2NHCO), 2.63 (t, J = 5.1 Hz, 1H, OH), 2.20 (t, J = 7 Hz, CH_2CONH_2), 2.00 (m, 4H, CH_2CH═$CHCH_2$), 1.63 (m, 2H, $CH_2CH_2CONH_2$), 1.50−1.30 (m, 20H), 0.89 (t, J = 6.7 Hz, 3H, CH_3); FABHRMS (NBA) *m/z* 326.3048 ($C_{20}H_{39}NO_2 + H^+$ requires 326.3059).

***N,N′*-Bis[9(*Z*)-octadecenoylamino]methane (77).** Cu powder (0.135 g, 2.16 mmol, 4.5 equiv) in toluene (1.0 mL, 2.16 M) was treated with I_2 (0.006 g, 0.024 mmol, 0.05 equiv), and the reaction mixture was stirred at 25 °C until the brown color disappeared (1−2 h). A sample of **1** (0.135 g, 0.48 mmol, 1.0 equiv) and CH_2I_2 (0.078 μL, 0.96 mmol, 2 equiv) were sequentially added to the reaction mixture, and the solution was warmed at reflux for 48 h. The reaction mixture was patitioned between EtOAc (50 mL) and H_2O (50 mL), and the organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography (SiO_2, 5 × 15 cm, 20−60% EtOAc−hexanes) afforded **77** as a white solid (0.048 g, 0.138 g theoretical, 35%, 22% recovered **1**): mp 111−113 °C; ^1H NMR ($CDCl_3$, 400 MHz) δ 6.74 (br s, 2H, NH), 5.33 (m, 4H, CH═CH), 4.57 (t, 2H, J = 6.0 Hz, $HNCH_2NH$), 2.15 (t, J = 7.4 Hz, 4H, CH_2CO), 1.99 (m, 8H, CH_2CH═CH), 1.59−1.10 (m, 44H), 0.87 (t, J = 6.8 Hz, 6H, CH_3); FABHRMS (NBA) *m/z* 575.5500 ($C_{37}H_{70}N_2O_2 + H^+$ requires 575.5516).

Methyl 18-[(*tert*-Butyldiphenysilyl)oxy]-9(*Z*)-octadecenoate (82). A solution of **80**[29] (1.0 g, 1.95 mmol, 1.0 equiv) in THF (6.5 mL, 0.3 M) at 25 °C was treated with KHMDS (3.9 mL, 0.5 M solution in THF, 1.95 mmol, 1.0 equiv), and the reaction mixture was stirred at reflux for 1 h. The reaction mixture was cooled to −78 °C, treated with **81**[29] (0.93 g, 2.35 mmol, 1.2 equiv), warmed to 25 °C, and stirred for an additional 30 min. The reaction mixture was treated with saturated aqueous NH_4Cl and partitioned between EtOAc (100 mL) and H_2O (100 mL). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. Chromatography (SiO_2, 5 × 15 cm, 0−2% EtOAc−hexane gradient elution) afforded **82** as a colorless oil (0.82 g, 1.07 g theoretical, 76%): ^1H NMR ($CDCl_3$, 250 MHz) δ 7.67 (m, 4H, ArH), 7.41 (m, 6H, ArH), 5.34 (m, 2H, C*H*═C*H*), 3.66−3.62 (m, 2H, $CH_2OTBDPS$), 3.65 (s, 3H, CO_2CH_3), 2.29 (t, 2H, J =

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7.4 Hz, CH_2CO$_2$CH$_3$), 2.00 (m, 4H, CH_2CH═CHCH_2), 1.55 (m, 4H, CH_2CH$_2$CO$_2$CH$_3$ and CH_2CH$_2$OTBDPS), 1.29 (br s, 18H), 1.04 (s, 9H, C(CH$_3$)$_3$).

18-[(*tert*-Butyldiphenylsilyl)oxy]-9(*Z*)-octadecenoic Acid (83). A solution of **82** (0.81 g, 1.47 mmol, 1.0 equiv) in THF−CH$_3$OH−H$_2$O (3:1:1, 7.3 mL, 0.2 M) at 0 °C was treated with LiOH·H$_2$O (0.188 g, 4.48 mmol, 3.0 equiv). The reaction mixture was warmed to 25 °C, stirred for 8 h, and then partitioned between EtOAc (100 mL) and H$_2$O (100 mL). The organic layer was washed successively with 10% aqueous HCl (100 mL) and saturated aqueous NaCl (100 mL), dried, and concentrated under reduced pressure. Chromatography (SiO$_2$, 5 × 15 cm, 10−30% EtOAc−hexane gradient elution) afforded **83** as a colorless oil (0.700 g, 0.790 g theoretical, 89%): ^{1}H NMR (CDCl$_3$, 250 MHz) δ 7.67 (m, 4H, ArH), 7.41 (m, 6H, ArH), 5.34 (m, 2H, C*H*═C*H*), 3.65 (t, 3H, J = 6.5 Hz, CH$_2$OTBDPS), 2.34 (t, 2H, J = 7.4 Hz, CH_2CO$_2$H), 2.00 (m, 4H, CH_2CH═CHCH_2), 1.65−1.50 (m, 4H, CH_2CH$_2$CO$_2$H and CH_2CH$_2$OTBDPS), 1.47−1.23 (m, 18H), 1.05 (s, 9H, C(CH$_3$)$_3$); FABHRMS (NBA−CsI) *m/z* 669.2772 (C$_{34}$H$_{52}$O$_3$Si + Cs$^+$ requires 669.2740).

18-[(*tert*-Butyldiphenylsilyl)oxy]-9(*Z*)-octadecenamide (84). A solution of **83** (0.685 g, 1.28 mmol, 1.0 equiv) in CH$_2$Cl$_2$ (4.3 mL, 0.3 M) at 0 °C was treated dropwise with oxalyl chloride (1.92 mL, 2 M solution in CH$_2$Cl$_2$, 3.84 mmol, 3.0 equiv). The reaction mixture was stirred at 25 °C for 4 h, concentrated under reduced pressure, cooled to 0 °C, and treated with saturated aqueous NH$_4$OH (2.0 mL). After 5 min, The reaction mixture was partitioned between EtOAc (100 mL) and H$_2$O (100 mL), and the organic layer was dried (Na$_2$SO$_4$) and concentrated under reduced pressure. Chromatography (SiO$_2$, 5 × 15 cm, 40−100% EtOAc−hexane gradient elution) afforded **84** as a colorless oil (0.520 g, 0.684 g, 76%): ^{1}H NMR (CDCl$_3$, 250 MHz) δ 7.67 (m, 4H, ArH), 7.41 (m, 6H, ArH), 5.70−5.34 (m, 4H, NH$_2$ and C*H*═C*H*), 3.65 (t, 3H, J = 6.5 Hz, CH$_2$OTBDPS), 2.21 (t, 2H, J = 7.5 Hz, CH_2CONH$_2$), 2.00 (m, 4H, CH_2CH═CHCH_2), 1.65−1.50 (m, 4H, CH_2CH$_2$CONH$_2$ and CH_2CH$_2$OTBDPS), 1.47−1.23 (m, 18H), 1.05 (s, 9H, C(CH$_3$)$_3$); FABHRMS (NBA−CsI) *m/z* 668.2929 (C$_{34}$H$_{53}$O$_2$Si + Cs$^+$ requires 668.2900).

18-Hydroxy-9(*Z*)-octadecenamide (85). A solution of **84** (0.185 g, 0.345 mmol, 1.0 equiv) in THF (1.1 mL, 0.31 M) was treated with Bu$_4$NF (0.69 mL, 1.0 M solution in THF, 0.69 mmol, 2.0 equiv), and the reaction mixture was stirred at 25 °C for 2 h. The reaction mixture was partitioned between EtOAc (50 mL) and H$_2$O (50 mL), and the organic layer was dried (Na$_2$SO$_4$) and concentrated under reduced pressure. Chromatography (SiO$_2$, 3 × 15 cm, 0−5% CH$_3$OH−EtOAc gradient elution) afforded **85** as a white solid (0.097 g, 0.103 g theoretical, 95%): mp 68−69 °C; ^{1}H NMR (CDCl$_3$, 250 MHz) δ 5.65−5.34 (m, 4H, NH$_2$ and C*H*═C*H*), 3.62 (t, 3H, J = 6.5 Hz, CH_2OH), 2.21 (t, 2H, J = 7.5 Hz, CH_2CONH$_2$), 2.00 (m, 4H, CH_2CH═CHCH_2), 1.65−1.50 (m, 4H, CH_2CH$_2$CONH$_2$ and CH_2CH$_2$OH), 1.29 (br s, 18H); FABHRMS (NBA) *m/z* 298.2732 (C$_{18}$H$_{35}$NO$_2$ + H$^+$ requires 298.2746).

18-Hydroxy-9(*Z*)-octadecenamide Hemisuccinate (86). A solution of **85** (70 mg, 0.236 mmol, 1.0 equiv) in CH$_2$Cl$_2$−CHCl$_3$ (3:1, 2.3 mL, 0.1 M) was treated successively with Et$_3$N (0.066 mL, 0.471 mmol, 2.0 equiv), succinic anhydride (47 mg, 0.47 mmol, 2.0 equiv) and DMAP (3 mg, 0.024 mmol, 0.1 equiv), and the reaction mixture was stirred at 25 °C for 10 h. The reaction mixture was partitioned between CH$_2$Cl$_2$ (50 mL), dried (Na$_2$SO$_4$), and concentrated under reduced pressure. Chromatography (SiO$_2$, 3 × 15 cm, 0−10% CH$_3$OH−EtOAc) afforded **9** as a white solid (90 mg, 94 mg theoretical, 96%): mp 76−77 °C; ^{1}H NMR (CDCl$_3$, 250 MHz) δ 6.95 (br s, 1H, CONH*H*), 5.72 (br s, 1H, CONH*H*), 5.34 (m, 2H, C*H*═C*H*), 4.08 (t, 3H, J = 6.6 Hz, CH_2OCOR), 2.61 (m, 4H, RO$_2$CCH_2CH_2COOH), 2.21 (t, 2H, J = 7.5 Hz, CH_2CONH$_2$), 2.00 (m, 4H, CH_2CH═CHCH_2), 1.70−1.52 (m, 4H, CH_2CH$_2$CONH$_2$ and CH_2CH$_2$OH), 1.29 (br s, 18H); FABHRMS (NBA) *m/z* 398.2893 (C$_{22}$H$_{39}$NO$_5$ + H$^+$ requires 398.2906).

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Supporting Information Available: Comparison ^{1}H NMR spectra of endogenous **1**, synthetic **1**, and **64** (Figure 1), ^{1}H NMR of crude **1** initially available and synthetic **1** and **19** in C$_6$D$_6$ (Figure 2), and text describing experimental details for the preparation of **3**−**4**, **27**−**30**, **35**−**38**, **41**−**48**, **50**−**66**, **68**−**71**, **73**−**74**, **76**, **78**−**81**, and **88**−**92** (24 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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