Oxyhomologues of Anandamide and Related Endolipids: Chemoselective Synthesis and Biological Activity

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Received December 13, 2005

The three amide oxyhomologues of the endolipids *N*-arachidonoylethanolamine (anandamide, AEA, **1a**), *N*-oleoylethanolamine (OEA, **2a**), and *N*-palmitoylethanolamine (PEA, **3a**) have been prepared in a chemoselective way, capitalizing on the easy availability of *O*-[2-(triisopropylsilyoxy)ethyl]hydroxylamine (**6**) and the surprising complementary selectivity observed in the acylation of *N*-[2-(*tert*-butyldiphenylsilyloxy)-ethyl]hydroxylamine (**7**) with the PPAA and the DCC/HOBT protocols. Reversal of the cannabinoid CB_1/CB_2 receptor affinity ratio was observed for the first time in a derivative of anandamide (the *O*-alkyl-*N*-acyl hydroxylamine **1b**), while the other oxyhomologues (**1c** and **1d**) showed only marginal cannabimimetic activity. Compounds with unsaturated acyl chains generally retained vanilloid activity and showed an increased stability toward FAAH compared to their corresponding ethanolamides. Taken together, these observation show that oxyhomologation has a pronounced effect on both the pharmacodynamic and the pharmacokinetic properties of endogenous ethanolamides, suggesting a general relevance of this maneuver for the modification of amide pharmacophores.

Introduction

Amide conjugates of fatty acids with endogenous amines (ethanolamine, dopamine) and amino acids are ubiquitous in mammalian tissues.[1] These compounds have long been considered as a mere curiosity, but the discovery that *N*-arachidonoylethanolamine (anandamide, AEA, **1a**, Figure 1) acts as an endogenous mimic of Δ^9-THC, the psychotropic active principle of marijuana,[2] has led to a resurgence of interest for fatty acid amides, setting the stage for what is now referred to as lipidomics.[3] Over the past few years, further endolipids have been deorphanized in terms of molecular targets and functional activity. Thus, *N*-arachidonoyldopamine (NADA) activates cannabinoid (CB_1) and vanilloid (TRPV1) receptors,[4] *N*-oleylethanolamine (OEA, **2a**) binds to both TRPV1[5] and PPAR-α,[6] *N*-palmitoylethanolamine (**3a**) modulates pain and inflammation via several mechanisms including PPAR-α activation,[7] and oleamide behaves as a sleep-inducing factor.[8] The biosynthesis, release, and disposition of some of these compounds is still poorly understood, but the modulation of these processes has relevance for a variety of conditions that includes life-style-related diseases (obesity, addition), diabetes, chronic pain, and atherosclerosis.[9] Most, but not all, amide and ester conjugates of fatty acids are rapidly hydrolyzed by the enzyme fatty acid amide hydrolase (FAAH), a serine protease whose ubiquitous distribution precludes the direct clinical use of these compounds.[10] Furthermore, endolipids are inherently pleiotropic agents, acting, often with opposite effects, on more than one molecular target.[11] While several strategies have been pursued to solve the stability issue,[12] much less is known on the molecular details of the interaction of endolipids with their receptors and on the possibility to focus their activity on a specific target. The ethanolamide pharmacophore is structurally simple, and, at least for AEA, has been extensively investigated in terms of alkyl substitution and isosteric modification.[13,14] These studies provided important clues to the interaction of the AEA headgroup with cannabinoid receptors. Thus, the amide moiety could not be replaced by an ester, a thioester, or a thioamide group, suggesting that the acidic amide proton is involved in hydrogen bonding with a complementary site in the receptor pocket while the amide oxygen carbonyl acts as a hydrogen-bonding acceptor toward the headgroup hydroxyl.[15] Isosteric modification of the amide moiety might be further complemented by oxyhomologation, that is the insertion of an oxygen atom on each of the three bonds centered on the amide nitrogen. In peptides, oxyhomologation is associated with well-defined secondary structures (foldamers),[16] but the biological relevance of this maneuver is still largely unknown, mainly because of the difficulties associated with the synthesis of hydroxylamino acids and their coupling.[17] The pleoiotropic activity of *N*-acylethanolamines and their quick enzymatic degradation provided a rationale to assess the pharmacodynamic and pharmacokinetic effects of amide oxyhomologation in this simple but highly relevant biological context.

Chemistry

The silylated *O*-alkylhydroxylamine (**6**) precursor of the oxyhomologues **1b**−**3b** was prepared by Mitsunobu reaction of 2-(triisopropylsilyl)oxyethanol (**4b**) with *N*-hydroxyphthalimide (**5**) (Scheme 1) followed by hydrazinolysis.[18] The acylation of **6** was straightforward, and after deprotection with buffered TBAF, the oxyhomologues **1b**−**3b** were obtained.

The remaining compounds were prepared from the silylated *N*-alkylhydroxylamine **7**,[19] exploiting the surprising discovery that the chemoselectivity of its acylation could be governed by

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10.1021/jm051240y CCC: $33.50
Published on Web 02/22/2006

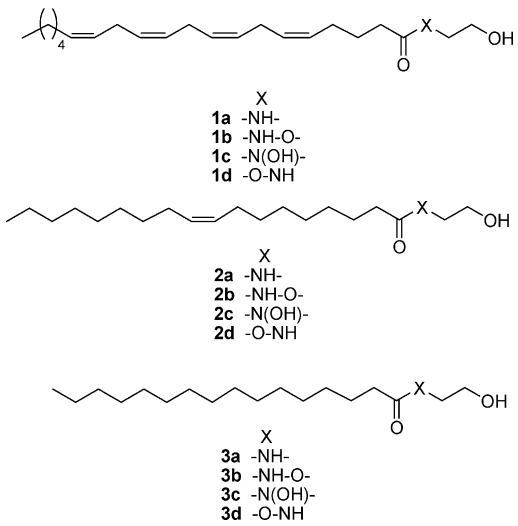
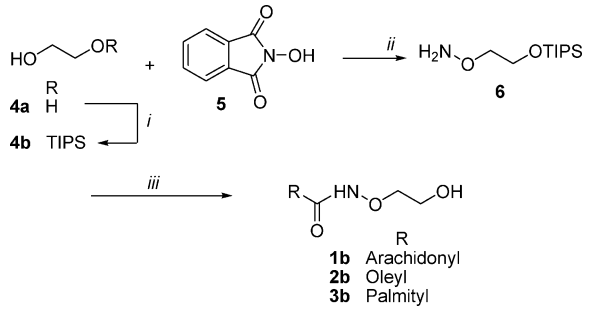


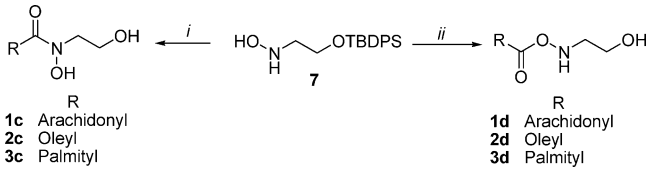
Figure 1. Endolipids anandamide (AEA, **1a**), OLEA (**2a**), and PALEA (**3a**) and their oxyhomologues **1b**−**d**, **2b**−**d**, and **3b**−**d**.

Scheme 1. Synthesis of the Oxyhomolgs **1b**−**3b** from the *O*-Alkylhydroxylamine **6**[a]



[a] (*i*) TIPSCl, imidazole, CH_2Cl_2 (80%). (*ii*) (a) TPP, DIAD, THF (26%), (b) NH_2-NH_2 (90%). (*iii*) (a) RCOOH, DCC, DMAP, CH_2Cl_2; (b) TBAF, HOAc (overall 20% for **1b**, 47% for **2b,** and 45% for **3b**). TIPS = triisopropylsilyl.

Scheme 2. Synthesis of the Oxyhomologues **1c**−**3c** and **1d**−**3d** from the *N*-Alkylhydroxylamine **7**[a]



[a] (*i*) (a) RCOOH, PPAA, TEA, CH_2Cl_2; (b) TBAF, HOAc (overall 36% for **1c**, 52% for **2c,** and 61% for **3c**). (*ii*) (a) RCOOH, EDC, HOBT, CH_2Cl_2; (b) TBAF, HOAc (overall 39% for **1d**, 47% for **2d,** and 53% for **3d**). TBDPS = *tert*-butyldiphenylsilyl.

the promoter employed. Thus, while acyl activation via a mixed phosphonic anhydride (PPAA, phenylpropionic acid anhydride)[20] afforded only the products of *N*-acylation, then deprotected to **1c**−**3c**, exclusively *O*-acylation occurred when the reaction was carried out with the EDC−HOBT protocol, leading, after deprotection, to the isomeric acylhydroxylamines **1d**−**3d** (Scheme 2). In both cases, chemoselectivity was unprecedented, with the alternative isomer being undetectable by ^1H NMR of the crude reaction mixture. The two isomeric acyl derivatives showed similar ^1H and ^{13}C NMR spectra but could be distinguished because compounds of the more polar *N*-hydroxyamide series (**1c**−**3c**) showed a HMBC correlation between the carbonyl carbon (δ ca. 169) and the methylene protons on the nitrogen-bearing carbon of the ethanolamine head (δ ca. 3.80). The *N*-hydroxyamides **1c**−**3c** showed broad NMR spectra at room temperature, diagnostic of a slow conformational equilibration, while their isomeric *O*-acylated analogues **1d**−**3d** showed sharp NMR spectra.

Biological Evaluation

Agonist activity at TRPV1 receptors was evaluated in human embryonic kidney (HEK) 293 cells overexpressing human recombinant TRPV1, measuring the effect of compounds on the intracellular calcium concentration. Affinity for cannabinoid CB_1 and CB_2 receptors was evaluated by means of binding assays carried out with membranes from COS cells overexpressing human recombinant CB_1 or CB_2 receptors.[22] Metabolic stability was evaluated by testing the capability of a compound to inhibit AEA hydrolysis by rat brain membranes, where a fatty acid amide hydrolase (FAAH) catalyzing this reaction is very abundant, or by studying the effect of amidase/esterase inhibition with phenylmethylsulfonyl fluoride (PMSF) on the affinity constants for CB_2 receptors. The interference with AEA cellular reuptake was assessed in intact RBL-2H3 cells, where a putative membrane transporter for endocannabinoids has been pharmacologically characterized.

Results and Discussion

The synthesis of amide oxyhomologues relies on the acylation of *N*- and *O*-alkylhydroxylamines, a reaction showing poor chemoselectivity with *N*-alkylhydroxylamines.[17] Nevertheless, capitalizing on the unexpected discovery of a complementary chemoselectivity for the PPAA and the EDC−HOBT acylation protocols, all three series of oxyhomologues could be prepared in a selective way from the alkylhydroxylamines **6** and **7**. The cyclic phosphonic anhydride PPAA has been shown to exhibit excellent selectivity for *N*- vs *O*-acylation with ambident nucleophiles such as phenolic amines and hydroxylamine,[21] and so obtaining exclusive *N*-acylation with the ambident nucleophile **7** is not totally surprising. Nevertheless, the amazing chemoselectivity swap when going from promotion with PPAA to promotion with the EDC−HOBT pair is puzzling because mixtures of acyl derivatives were obtained with other coupling conditions (carboxyl activation by EDC or DCC or via chlorides). Studies are underway to investigate the generality of these results and their application to the synthesis of peptidomimetics.

With the notable exception of the *O*-arachidonoyl- and *O*-oleoyl *N*-hydroxylamines **1d** and **2d**, which were significantly less active than their parent compounds **1a** and **2a**, oxyhomologation of the amide bond had only a marginal effect on the vanilloid activity (or inactivity) of fatty acid ethanolamides (Table 1). This observation highlights the relevance of an acidic N−H or N−OH bond adjacent to the amide carbonyl for the interaction of this type of compounds with TRPV1.[22] Conversely, oxyhomologation produced striking effects on CB_1 and CB_2 affinities. Thus, all three oxyhomologues of OEA (**2b**−**d**) were inactive in CB_1 binding assays but exhibited enhanced affinity for CB_2 (Table 1). This trend was even more striking for the oxyhomologues of the arachidonoyl series (**1b**−**d**) and especially for the *N*-acyl-*O*-alkylhydroxylamine **1b**. Compared to AEA (**1a**), **1b** exhibited an over 6-fold decrease of affinity for CB_1 (K_i = 470 nM for **1b** and 70 nM for **1a**) coupled to a more than 2-fold increase of affinity for CB_2 receptors (K_i = 81 nM for **1b** and 180 nM for **1a**). The oxyhomologue **1b** was inactive as an inhibitor of AEA hydrolysis in rat brain membranes as well as an inhibitor of AEA reuptake from intact RBL-2H3 cells. The lack of a significant affinity for FAAH and the putative AEA transporter should translate into a high

Table 1. Biological Profile of Endolipid Oxyhomologues toward Various End Points of the Endocannabinoid/Endovanilloid System[a]

compound	hCB_1 (K_i, μM)[b]	hCB_2 (K_i, μM)[b]	AEA hydrolysis (IC_{50}, μM)[c]	AEA uptake (IC_{50}, μM)[d]	hTRPV1 (EC_{50}, μM)[e]
anandamide (AEA, **1a**)	0.07 ± 0.01	0.18 ± 0.02	19.0 ± 1.8	14.5 ± 1.3	0.49 ± 0.05 *54.7 ± 4.5*
1b	0.47 ± 0.11	0.081 ± 0.01	25.0 ± 2.1	>25	0.25 ± 0.04 *58.5 ± 3.5*
1c	3.9 ± 0.5	1.9 ± 0.2	>50	>25	0.26 ± 0.04 *55.3 ± 3.8*
1d	3.5 ± 0.4	2.4 ± 0.3	>50	>25	N.D. *28.0 ± 2.6*
N-oleoylethanolamine (OEA, **2a**)	1.1 ± 0.2	>10	23.0 ± 2.2	20.0 ± 1.7	0.89 ± 0.09 *50.1 ± 4.2*
2b	>10	3.8 ± 0.4	>50	22.5 ± 1.9	0.32 ± 0.05 *50.0 ± 4.0*
2c	>10	5.4 ± 0.5	>50	20.0 ± 1.7	0.40 ± 0.04 *53.5 ± 4.1*
2d	>10	8.6 ± 0.5	>50	>25	N.D. *14.3 ± 2.1*
3b	>10	>10	>50	>25	N.D. *11.2 ± 1.8*
3c	>10	>10	>50	>25	0.60 ± 0.07 *37.0 ± 4.0*
3d	>10	>10	>50	>25	N.D. *1.8 ± 1.1*

[a] Under the conditions used for these assays, *N*-palmitoylethanolamine (**3a**) was inactive in all experiments. [b] Affinity constants for the human recombinant cannabinoid CB_1 and CB_2 receptors. [c] Concentrations eliciting 50% inhibition of the hydrolysis of anandamide (AEA) by rat brain membranes. [d] Uptake of AEA by rat basophilic leukaemia cells. [e] EC_{50} = Half-maximal concentrations eliciting a TRPV1-mediated enhancement of intracellular Ca^{2+} concentration in HEK-293 overexpressing the human recombinant TRPV1 receptor. The maximal effect on TRPV1-mediated intracellular Ca^{2+} concentration, exerted by a 10 μM concentration of the compounds and expressed as percent of the effect evoked by ionomycin (4 μM), is shown in italics below the EC_{50} values.

metabolic stability, as supported by the observation that the K_i of **1b** for the human CB_2 receptors was not significantly affected by the presence of the amidase/esterase inhibitor PMSF (data not shown). The two other oxyhomologues of AEA (compounds **1c** and **1d**) were much less active than **1b** at CB_2 receptors and almost inactive at CB_1 receptors. Taken together, these data identify **1b** as the first fatty acid amide with a reversed CB_1/CB_2 affinity ratio, qualifying it as a useful tool for pharmacological studies of CB_2 receptors.

Unlike the oxyhomologues of AEA, those of OEA (compounds **2b**−**d**) retained a certain capability to inhibit AEA cellular uptake. This finding may be relevant in light of the clinical potential of metabolically stable pleiotropic agents capable of amplifying endocannabinoid responses in a FAAH-independent way and endowed with TRPV1-activating properties.[23] The *N*-hydroxy derivative of PEA (**3c**) showed a potent but inefficacious TRPV1 activity, while the two other oxyhomologues of PEA (**3b** and **3d**) shared with their parent compound the total lack of direct activity at all end points considered (TRPV1 and cannabinoid CB_1/CB_2 receptors, AEA hydrolysis, AEA cellular uptake). These data indicate that while oxyhomologation may represent a useful chemical manipulation to modulate the activity of long-chain monounsaturated and polyunsaturated *N*-acylethanolamines toward a series of targets from the endocannabinoid−endovanilloid system (CB_1/CB_2 receptors, TRPV1 receptors, FAAH, the putative endocannabinoid transporter), this maneuver is per se insufficient to induce activity in compounds with a saturated acyl moiety such as PEA.

Conclusions

The amide bond is present in many bioactive natural products. Its isosteric modification with an ester bond has been explored extensively, while oxyhomologation, though recognized as potentially useful,[17] is still a largely unexplored maneuver. Apart from specific pharmacodynamic effects due to the insertion of an oxygen atom in the amide group, oxyhomologation also has the potential to increase stability toward amidases and esterases, and the biological profile of the oxyanandamide **1b** fully supports these expectations.

Experimental Section

Materials. Column chromatography: Merck Silica Gel. IR: Shimadzu DR 8001 spectrophotometer. NMR: JEOL Eclipse (300 and 75 MHz for ^1H and ^{13}C, respectively). For ^1H NMR, $CDCl_3$ as solvent, $CHCl_3$ at $\delta = 7.26$ as reference. For ^{13}C NMR, $CDCl_3$ as solvent, $CDCl_3$ at $\delta = 77.0$ as reference. CH_2Cl_2 and toluene were dried by distillation from CaH_2 and THF by distillation from Na/benzophenone. Reactions were monitored by TLC on Merck 60 F_{254} (0.25 mm) plates that were visualized by UV inspection and/or staining with 5% H_2SO_4 in ethanol and heating. High-purity (>98%) arachidonic acid was purchased from Alexis Biochemicals. Organic phases were dried with Na_2SO_4 before evaporation. Satisfactory elemental analyses (±0.4% of the theoretical value) were obtained for the final compounds of the palmitoyl and oleyl type. The purity (>95%) of the arachidonates **1b**−**d** was assed by HPLC on a Water microPorasil column (0.8 × 30 cm) with detection by a Waters differential refractometer 340 using petroleum ether−EtOAc 8:2 (system A) and chloroform−acetone 4:1 (system B).

Biological Evaluation. Human embryonic kidney (HEK) 293 cells overexpressing hTRPV1 were kindly donated by Dr. John Davis at GlaxoSmithKline. Cells were grown as monolayers in minimum essential medium supplemented with nonessential amino acids, 10% fetal calf serum, and 0.2 mM glutamine and maintained under 95%/0/5% O_2/CO_2 at 37 °C. One day prior to experiments, cells were transferred into six-well dishes coated with poly-L-lysine (Sigma) and grown in the culture medium described before. On the day of the experiment, the cells (50−60 000 per well) were loaded for 2 h at 25 °C with 4 μM Fluo-3-methylester (Molecular Probes) in DMSO containing 0.04% Pluoronic. After the loading, the cells were washed with Tyrode (pH = 7.4), trypsinized, resuspended in Tyrode, and transferred to the cuvette of the fluorescence detector (Perkin-Elmer LS50B) under continuous stirring. Experiments were carried out by measuring the fluorescence at 25 °C (λ_{EX} = 488 nm, λ_{EM} = 540 nm) before and after addition of the test compounds at various concentrations. Data were expressed as the concentration exerting a half-maximal stimulation (EC_{50}) calculated by GraphPad. For CB_1 and CB_2 receptor binding assays, the new compounds were analyzed by using P_2 membranes from COS cells transfected with either the human CB_1 or CB_2 receptor and [^{3}H]-(−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol ([^{3}H]CP-55,940) as the

high-affinity ligand as described by the manufacturer (Perkin-Elmer, Italia). Displacement curves were generated by incubating drugs with 0.5 nM of [^{3}H]CP-55,940. In all cases, K_i values were calculated by applying the Cheng−Prusoff equation to the IC_{50} values (obtained by GraphPad) for the displacement of the bound radioligand by increasing concentrations of the test compounds in the presence or absence of phenylmethylsulfonylfluoride (200 μM). The effect of compounds on the enzymatic hydrolysis of [^{14}C]-anandamide (6 μM) was studied by using membranes prepared from rat brain incubated with increasing concentrations of compounds in 50 mM Tris-HCl, pH 9, for 30 min at 37 °C. These conditions are optimal for fatty acid amide hydrolase (FAAH), the enzyme that catalyzes the hydrolysis of both anandamide and 2-arachidonoylglycerol. [^{14}C]Ethanolamine produced from [^{14}C]anandamide hydrolysis was measured by scintillation counting of the aqueous phase after extraction of the incubation mixture with 2 volumes of $CHCl_3$/CH_3OH (2:1 by volume). In most cases, only the most potent compounds in the binding assays were subjected to this assay. The effect of compounds on the uptake of [^{14}C]AEA by rat basophilic leukemia (RBL-2H3) cells was studied by using 2.4 μM (10 000 cpm) of [^{14}C]AEA as described previously.[4] Cells were incubated with [^{14}C]AEA for 5 min at 37 °C in the presence or absence of varying concentrations of the inhibitors. Residual [^{14}C]AEA in the incubation medium after extraction with $CHCl_3$/CH_3OH 2:1 (by volume), determined by scintillation counting of the lyophilized organic phase, was used as a measure of the AEA that was taken up by cells. Data are expressed as the concentration exerting 50% inhibition of AEA uptake (IC_{50}) calculated by GraphPad.

***O*-(2-Triisopropylsilyloxy)ethanol (4b).** To a stirred solution of anhydrous ethylene glycol (**4a**, 449 μL, 500 mg, 8.05 mmol) in dry CH_2Cl_2 (5 mL), imidazole (545 mg, 8.05 mmol, 1 mol equiv) and triisopropylsilyl chloride (1.72 mL, 1.55 g, 8.05 mmol, 1 mol equiv) were added. After stirring 5 h at room temperature, the reaction was worked up by acidification with 2 N H_2SO_4 and extraction with EtOAc. After washing with brine and evaporation, the organic phase was purified by gravity column chromatography on silica gel (10 g, petroleum ether−EtOAc 9:1 as eluant) to afford 1.40 g (80%) of **4b** as a colorless oil. IR (liquid film) ν_{max} 3380, 1435, 1279, 1200, 1174, 890 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 3.79 (t, J = 5.1 Hz, 2H), 3.65 (t, J = 5.1 Hz, 2H), ca. 1.00 (br s, 21H). CI MS (70 eV) m/z 219 $[M + H]^+$ $[C_{11}H_{26}O_2Si + H]^+$.

***O*-[2-(Triisopropylsilyloxy)ethyl]hydroxylamine (6).** To a solution of 2-(triisopropylsilyloxy)ethanol (**4b**, 600 mg, 2.75 mmol) in dry THF (6 mL), *N*-hydroxyphthalimide (448 mg, 2.75 mol, 1 mol equiv), triphenylphosphine (721 mg, 2.75 mmol, 1 mol equiv), and diisopropylazodicarboxylate (DIAD, 564 μL, 556 mg, 2.75 mmol, 1 mol equiv) were sequentially added. After stirring overnight at room temperature, the reaction was worked up by evaporation, and the residue was dissolved in toluene (5 mL) and cooled overnight at −18 °C. After filtration of the copious precipitate of the dihydroDIAD−triphenylphosphine oxide adduct,[24] the filtrate (386 mg) was evaporated, dissolved in CH_2Cl_2 (3.5 mL), and treated with an excess of hydrazine hydrate (411 μL). After stirring at room temperature for 1 h, the reaction was worked up by filtration and evaporation. The residue was purified by gravity column chromatography (8 g of silica gel, petroleum ether−EtOAc 9:1 as eluant) to afford 167 mg (26%) of **6** as a colorless oil. IR (liquid film) ν_{max} 3380, 1427, 1275, 1199, 1178, 890 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 5.47 (br s, 2H), 3.87 (m, 2H), 3.76 (m, 2H), ca. 1.00 (br s, 21H). CI MS (70 eV) m/z 234 $[M + H]^+$ $[C_{11}H_{27}NO_2Si + H]^+$.

Acylation of *O*-[2-(Triisopropylsilyloxy)ethyl]hydroxylamine (6) and Desilylation. Reaction with palmitic acid as an example: To a solution of **6** (200 mg, 0.86 mmol) in toluene (2.5 mL), palmitic acid (336 mg, 1.29 mmol, 1.5 mol equiv), DCC (295 mg, 1.29 mmol., 1.5 mol equiv), and DMAP (cat., 5 mg) were added. After stirring 4 h at room temperature, the reaction was worked up by filtration and evaporation. The residue was dissolved in THF (4 mL) and treated with 1 M TBAF (*N*-tetrabutylammonium fluoride, 2.28 mL, 2.28 mmol) and glacial acetic acid (150 μL, 157 mg, 2.28 mmol). After stirring overnight at 0 °C, the reaction was worked up by dilution with brine and extraction with EtOAc. The organic phase was evaporated, and the residue was purified by gravity column chromatography on silica gel (5 g, petroleum ether−EtOAc 5:5 and then 3:7 as eluant) to afford 122 mg (45%) of **3b**.

***N*-Arachidonoyl-*O*-(2-hydroxyethyl)hydroxylamine (1b).** Colorless oil, IR (liquid film) ν_{max} 3380, 1692, 1677, 1645, 1380, 1368, 1111, 1098, 920 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 8.11 (br s, 1H) 5.37 (m, 8H), 3.93 (t, J = 4.2 Hz, 2H), 3.67 (br t, J = 4.2 Hz, 2H), 2.84 (m, 6 H), 2.34 (t, J = 6.1 Hz, 2H), ca. 2.05 (m, 4H), 1.73 (m, 2 H), ca. 1.24 (m, 6 H), 0.89 (br t, J = 6.0 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 174.0 (s), 130.8 (d), 129.6 (d), 129.0 (d), 128.9 (d), 128.6 (d), 128.3 (d), 127.8 (d), 78.7 (t), 59.5 (t), 32.1 (t), 31.8 (t), 29.6 (t), 27.5 (t), 26.5 (t), 25.6 (t), 24.8 (t), 22.7 (t), 14.2 (q). CI MS (70 eV) m/z 364 $[M + H]^+$ $[C_{22}H_{37}NO_3 + H]^+$.

***N*-Oleoyl-*O*-(2-hydroxyethyl)hydroxylamine (2b).** Colorless oil, IR (liquid film) ν_{max} 3370, 1697, 1679, 1629, 1367, 1329, 1105, 1070, 930 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 8.24 (br s, 1H) 5.33 (m, 2H), 3.93 (t, J = 4.2 Hz, 2H), 3.68 (br t, J = 4.2 Hz, 2H), 2.09 (t, J = 6.1 Hz, 2H), 2.03 (m, 4 H), 1.64 (m, 2 H), ca. 1.27 (m, 20 H), 0.85 (br t, J = 6.0 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 174.4 (s), 130.2 (d), 129.8 (d), 78.6 (t), 59.4 (d), 29.9 (t), 29.8 (t), 29.6 (t), 29.4 (t), 29.2 (t), 29.1 (t), 27.3 (t), 27.2 (t), 14.2 (q). CI MS (70 eV) m/z 342 $[M + H]^+$ $[C_{20}H_{39}NO_3 + H]^+$. Anal. ($C_{20}H_{39}NO_3$) C, H, N.

***N*-Palmitoyl-*O*-(2-hydroxyethyl)hydroxylamine (3b).** White powder, mp 79 °C; IR (KBr) ν_{max} 3380, 1690, 1680, 1390, 1375, 1021, 1001, 920 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 8.09 (br s, 1H), 3.89 (t, J = 5.1 Hz, 2H), 3.69 (t, J = 5.1 Hz, 2H), 3.50 (br s, 1H), 2.36 (t, J = 6.8 Hz, 2H), 1.62 (m, 2H), ca. 1.24 (br s, 24H), 0.86 (br t, J = 6.8 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 174.0 (s), 78.5 (t), 59.7 (t), 32.1 (t), 29.9 (t), 29.8 (t), 29.6 (t), 29.1 (t), 27.3 (t), 22.8 (t), 14.2 (q). CI MS (70 eV) m/z 316 $[M + H]^+$ $[C_{18}H_{37}NO_3 + H]^+$. Anal. ($C_{18}H_{37}NO_3$) C, H, N.

Acylation of *N*-[2-(*tert*-Butyldiphenylsilyloxy)ethyl]hydroxylamine (7) with the PPAA Protocol and Deprotection. Reaction with oleic acid as an example: To a solution of oleic acid (90 mg, 0.32 mmol) in CH_2Cl_2 (3.5 mL), triethylamine (227 μL, 165 mg, 1.63 mmol, 4 mol equiv) and PPAA (122 mg, 0.384 mmol, 1.2 mol equiv) were added. After stirring at room temperature for 30 min, a solution of *N*-[2-(*tert*-butyldiphenylsilyloxy)ethyl]hydroxylamine (7) (100 mg, 0.32 mmol) in CH_2Cl_2 (1 mL) was added, and stirring was continued for 1 h. The reaction was next worked up by evaporation, and the residue was dissolved in THF (4 mL) and treated with 1 M TBAF (1.14 mL, 1.14 mmol) and glacial acetic acid (75 μL, 78 mg, 1.14 mmol). After stirring 2 h at 0 °C, the reaction was worked up by dilution with brine and extraction with EtOAc. The organic phase was evaporated, and the residue was purified by gravity column chromatography on silica gel (5 g, petroleum ether−EtOAc 4:6 and then 2:8 as eluant) to afford 57 mg (52%) of 2c.

***N*-Arachidonoyl-*N*-(2-hydroxyethyl)hydroxylamine (1c).** Colorless oil, IR (liquid film) ν_{max} 3470, 1701, 1668, 1421, 1366, 1098, 1011, 914 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 5.36 (m, 8H), 3.89 (br s, 1H), 3.80 (m, 2H), 3.78 (m, 2H), 2.80 (m, 6 H), 2.39 (t, J = 6.1 Hz, 2H), ca. 2.05 (m, 4H), 1.75 (m, 2 H), ca. 1.24 (m, 6 H), 0.88 (br t, J = 6.0 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 168.4 (s), 130.9 (d), 129.7 (d), 129.0 (d), 128.8 (d), 128.4 (d), 128.3 (d), 127.8 (d), 59.7 (t), 50.7 (t), 32.0 (t), 31.5 (t), 29.3 (t), 27.7 (t), 26.5 (t), 25.7 (t), 24.8 (t), 22.9 (t), 14.2 (q). CI MS (70 eV) m/z 364 $[M + H]^+$ $[C_{22}H_{37}NO_3 + H]^+$.

***N*-Oleoyl-*N*-(2-hydroxyethyl)hydroxylamine (2c).** Colorless oil, IR (liquid film) ν_{max} 3476, 1700, 1661, 1421, 1366, 1098, 1011, 918 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 5.33 (m, 2H), 3.87 (br s, 1H), 3.85 (m, 2H), 3.74 (m, 2H), 2.37 (t, J = 6.1 Hz, 2H), ca. 2.0 (m, 4H), 1.61 (m, 2 H), ca. 1.26 (m, 20 H), 0.88 (br t, J = 6.0 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 168.5 (s), 130.8 (d), 129.6 (d), 59.8 (t), 50.9 (t), 32.0 (t), 29.8 (t), 29.6 (t), 29.4 (t), 29.2 (t), 26.8 (t), 25.4 (t), 22.8 (t), 14.2 (q). CI MS (70 eV) m/z 342 $[M + H]^+$ $[C_{20}H_{39}NO_3 + H]^+$. Anal. ($C_{20}H_{39}NO_3$) C, H, N.

***N*-Palmitoyl-*N*-(2-hydroxyethyl)hydroxylamine (3c).** Amorphous foam; IR (KBr) ν_{max} 3390, 1690, 1680, 1382, 1365, 1105,

1087, 917 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.85 (br m, 3H), 3.74 (br m, 1H), 2.36 (t, $J = 6.8$ Hz, 2H), 1.62 (m, 2H), *ca.* 1.26 (br s, 24H), 0.87 (br t, $J = 6.8$ Hz, 3H). ^{13}C NMR ($CDCl_3$/DMSO-d_6, 3:1): δ 168.2 (s), 60.4 (t), 52.6 (t), 34.3 (t), 32.0 (t), 31.7 (t), 29.8 (t), 29.6 (t), 29.4 (t), 29.2 (t), 25.0 (t), 22.8 (t), 14.2 (q). CI MS (70 eV) *m/z* 316 $[M + H]^+$ $[C_{18}H_{37}NO_3 + H]^+$. Anal. ($C_{18}H_{37}NO_3$) C, H, N.

Acylation of *N*-[2-(*tert*-Butyldiphenylsilyloxy)ethyl]hydroxylamine (7) with the EDC−HOBt Protocol and Deprotection. Reaction with oleic acid as an example: To a solution of *N*-[2-(*tert*-butyldiphenylsilyl)oxyethyl]hydroxylamine (7) (100 mg, 0.32 mmol) in CH_2Cl_2 (2 mL), oleic acid (103 mg, 0.4 mmol, 1.2 mol equiv), EDC (154 mg, 0.8 mmol, 2.0 mol equiv), HOBT (108 mg, 0.8 mmol, 2 mol equiv), and cat. DMAP (5 mg) were sequentially added. After stirring at room temperature for 2 h, the reaction was worked up by evaporation, and the residue was dissolved in THF (1.5 mL) and then treated with 1 M TBAF in THF (805 μL, 0.80 mmol) and glacial acetic acid (45 μL, 46 mg, 0.80 mmol). After stirring at room temperature for 30 min, the reaction was worked up by dilution with brine and extraction with EtOAc. After removal of the solvent, the residue was purified by gravity column chromatography on silica gel (5 g, petroleum ether−EtOAc 8:2 as eluant) to afford 52 mg of 2d (47%).

***O*-Arachidonoyl-*N*-(2-hydroxyethyl)hydroxylamine (1d).** Colorless oil, IR (liquid film) ν_{max} 3220, 1708, 1681, 1421, 1366, 1098, 1011, 914 cm^{-1}. ^1H NMR ($CDCl_3$): δ 5.36 (m, 8H), 3.70 (t, $J =$ 6.2 Hz, 2H), 3.14 (t, $J = 6.2$ Hz, 2H), 2.81 (m, 6 H), 2.38 (t, $J =$ 6.1 Hz, 2H), ca. 2.05 (m, 4H), 1.73 (m, 2 H), ca. 1.24 (m, 6 H), 0.88 (br t, $J = 6.0$ Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 174.1 (s), 130.8 (d), 129.6 (d), 128.6 (d), 128.8 (d), 128.5 (d), 128.3 (d), 127.9 (d) 127.8 (d), 58.8 (t), 54.2 (t), 32.0 (t), 31.6 (t), 29.4 (t), 27.3 (t), 26.5 (t), 25.7 (t), 24.8 (t), 22.7 (t), 14.2 (q). CI MS (70 eV) *m/z* 364 $[M + H]^+$ $[C_{22}H_{37}NO_3 + H]^+$.

***O*-Oleoyl-*N*-(2-hydroxyethyl)hydroxylamine (2d).** Colorless oil, IR (liquid film) ν_{max} 3470, 1708, 1681, 1424, 1376, 1101, 1067, 916 cm^{-1}. ^1H NMR ($CDCl_3$): δ 5.32 (m, 2H), 3.69 (t, $J = 5.3$ Hz, 2H), 3.14 (t, $J = 5.3$ Hz, 2H), 2.33 (t, $J = 6.1$ Hz, 2H), ca. 2.0 (m, 4H), 1.64 (m, 2 H), ca. 1.25 (m, 20 H), 0.86 (br t, $J = 6.0$ Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 174.1 (s), 130.4 (d), 129.3 (d), 58.9 (t), 54.0 (t), 32.0 (t), 29.7 (t), 29.5 (t), 29.3 (t), 29.1 (t), 26.8 (t), 25.6 (t), 22.5 (t), 14.1 (q). CI MS (70 eV) *m/z* 342 $[M + H]^+$ $[C_{20}H_{39}NO_3 + H]^+$. Anal. ($C_{20}H_{39}NO_3$) C, H, N.

***O*-Palmitoyl-*N*-(2-hydroxyethyl)hydroxylamine (3d).** Amorphous foam; IR (KBr) ν_{max} 3386, 1699, 1387, 1345, 1121, 1088, 921 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.66 (br s, 1H), 3.70 (t, $J = 5.3$ Hz, 2H), 3.15 (t, $J = 5.3$ Hz, 2H), 2.34 (t, $J = 6.8$ Hz, 2H), 1.62 (m, 2H), ca. 1.25 (br s, 24H), 0.87 (br t, $J = 6.8$ Hz, 3H). ^{13}C NMR ($CDCl_3$/DMSO-d_6, 3:1): δ 174.4 (s), 58.5 (t), 54.2 (t), 32.0 (t), 29.7 (t), 29.5 (t), 29.3 (t), 29.1 (t), 26.8 (t), 25.6 (t), 22.1 (t), 14.1 (q). CI MS (70 eV) *m/z* 316 $[M + H]^+$ $[C_{18}H_{37}NO_3 + H]^+$. Anal. ($C_{18}H_{37}NO_3$) C, H, N.

Acknowledgment. This research was supported by MIUR (Program: Chemorecezione Gustativa. Sintesi e Relazioni Attività-Struttura di Molecole Attive sul Sapore). We are grateful to Dr. Paolo Dambruoso for the HMBC measurements.

Supporting Information Available: Elemental analysis results for compounds **2b**−**d** and **3b**−**d**. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM051240Y