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Lipophilic amines as potent inhibitors of *N*-acylethanolamine-hydrolyzing acid amidase

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ABSTRACT

N-Acylethanolamines (NAEs) including *N*-arachidonoylethanolamine (anandamide) and *N*-palmitoylethanolamine are endogenous lipid mediators. These molecules are degraded to the corresponding fatty acids and ethanolamine by fatty acid amide hydrolase (FAAH) or NAE-hydrolyzing acid amidase (NAAA). Lipophilic amines, especially pentadecylamine (**2c**) and tridecyl 2-aminoacetate (**11b**), were found to exhibit potent NAAA inhibitory activities (IC_{50} = 5.7 and 11.8 μM), with much weaker effects on FAAH. These simple structures would provide a scaffold for further improvement in NAAA inhibitory activity.
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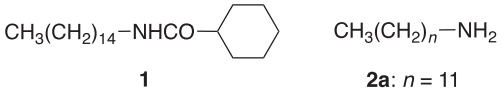
1. Introduction

N-Acylethanolamines (NAEs) are ethanolamides of long-chain fatty acids and are found in animal tissues.[1–3] Following the discovery of *N*-arachidonoylethanolamine (anandamide),[4] endogenous NAEs have been the subject of growing interest in pharmacology. Anandamide acts on cannabinoid and vanilloid receptors, and thus displays a number of interesting pharmacological effects on nociception, memory processes, lung function, spasticity, appetite, and cell proliferation.[5] On the other hand, endogenous saturated and monounsaturated NAEs, such as *N*-palmitoylethanolamine (palmitoylethanolamide, PEA) and *N*-oleoylethanolamine (oleoylethanolamide, OEA), induce pharmacological responses without significant binding to these receptors. PEA has attracted considerable attention, because it exerts potent anti-inflammatory and analgesic effects[6,7] that mainly result from the activation of the nuclear receptor peroxisome proliferator-activated receptor-α.[8] OEA is known as an appetite suppressant.[9]

NAEs are not stored in the cell, but are made on demand, and their endogenous levels appear to be regulated directly by enzymes responsible for their formation and degradation.[10,11] Therefore, these enzymes are potential targets for drug therapy, and their selective inhibitors are expected to exert therapeutic effects.[12] The major pathway for the degradation of NAEs is their hydrolysis to free fatty acids and ethanolamine. Fatty acid amide hydrolase (FAAH)[13–15] plays a central role in this reaction in vivo. NAE-hydrolyzing acid amidase (NAAA)[11,16–18] can also catalyze the same reaction and may be another attractive drug target. NAAA is a cysteine hydrolase that belongs to the N-terminal nucleophile family of enzymes. NAAA preferentially hydrolyzes PEA over other NAEs and is localized in lysosomes. Although potent FAAH inhibitors have been used to determine the function of the preferred FAAH substrate, anandamide,[19] the search for potent and selective NAAA inhibitors is in its infancy.[20–24]

Recently, Solorzano et al. reported[22,23] that some *N*-(2-oxo-3-oxetanyl)amides were potent, selective inhibitors of NAAA and that they increased PEA levels in activated leukocytes. They proposed that these inhibitors, which contain a β-lactone ring, acylated the catalytic cysteine in NAAA. Previously, Tsuboi et al.



1

2a: *n* = 11
2b: *n* = 13
2c: *n* = 14
2d: *n* = 15

Figure 1. Chemical structures of the previously reported NAAA inhibitor and the long-chain alkylamines tested for NAAA inhibition.

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(co-authors of this study) reported[21] *N*-pentadecylcyclohexanecarboxamide (**1**) (Fig. 1) as the first potent and selective NAAA inhibitor. However, when amide **1** was resynthesized it did not inhibit NAAA, whereas, pentadecylamine (**2c**), which we detected as a contaminant in the first batch of amide **1**,[21] showed strong inhibitory activity. This finding led us to investigate long-chain alkylamines **2a–d** and other long-chain amines for NAAA inhibition.

2. Results and discussion

2.1. Chemistry

Commercially available *N-tert*-butoxycarbonyl (boc)-2-aminoethanol (**3**) and *N*-boc-3-aminopropanol (**6**) were acylated using various fatty acid chlorides, and then deprotected using trifluoroacetic acid (TFA) to obtain esters of ethanolamine **5a–d** and propanolamine **8a–d**, according to a previously reported method for preparing 2-aminoethyl octanoate[25] (Scheme 1).

Esters of glycine **11a–e** and β-alanine **14a–c** were also prepared by the condensation of commercially available *N*-boc-glycine (**9**) and *N*-boc-β-alanine (**12**) with various long-chain fatty alcohols under Mukaiyama's conditions,[26] followed by deprotection (Scheme 2).

Long-chain alkyl ethers of ethanolamine **18a–c** and propanolamine **22a–c** were prepared by using a modified literature method (Scheme 3).[27] Ethylene glycol (**15**) and 1,3-propanediol (**19**) were heated with dodecyl, tetradecyl, and hexadecyl bromides in the presence of sodium hydride and potassium iodide in *N,N*-dimethylformamide (DMF) to provide their corresponding monoalkylated alcohols **16a–c** and **20a–c**. These alcohols were converted into azides **17a–c** and **21a–c** by using diphenyl phosphorazidate (DPPA) and 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU)[28] which were then reduced with triphenylphosphine to give the desired amines **18a–c** and **22a–c**.

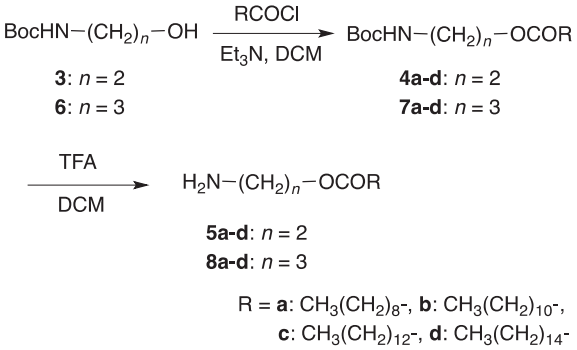
2.2. Biological evaluation

Tsuboi et al. previously reported[21] that *N*-pentadecylcyclohexanecarboxamide (**1**) exhibited potent inhibition of NAAA, with IC_{50} value of 4.5 μM. Subsequently, ^1H NMR revealed that the previous sample of amide **1** was contaminated with ~50% pentadecylamine (**2c**). Thus, amide **1** was resynthesized and its inhibitory effect on rat lung NAAA reexamined along with that of commercially available long-chain alkylamines **2a–d** (Fig. 1), according to a previously reported assay method.[21]

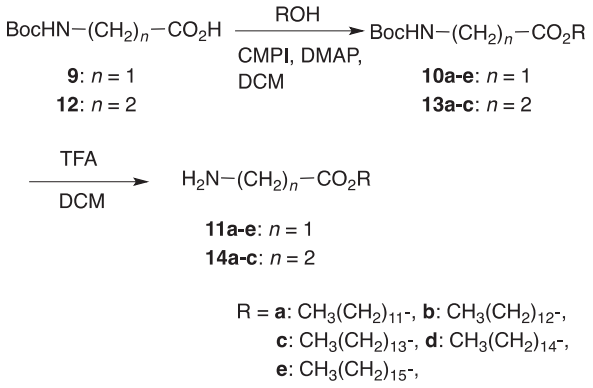
As a result, amide **1** showed no inhibitory effect at 100 μM, whereas, pentadecylamine (**2c**) and amines **2a, 2b,** and **2d** all inhibited NAAA at the same concentration (Fig. 2); amine **2c** was the most potent of the compounds. These amines exist as ammonium salts under the assay conditions (pH 5); therefore the inhibitory activity of the commercially available hexadecyltrimethylammonium chloride (**23**) was also tested. Ammonium salt **23** showed similar activity to that of hexadecylamine (**2d**) (Fig. 2).

To improve the inhibitory effect, we designed to add hydrophilic moieties, such as an ester (**5a–d, 8a–d, 11a–d, 14a–c**) or an ether (**18a–c, 22a–c**) to the amine alkyl chains.

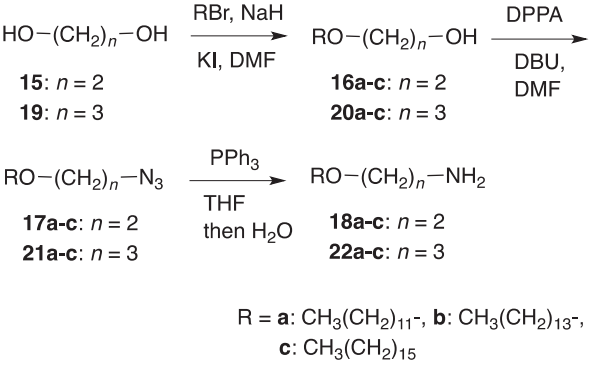
The inhibition of NAAA by these amines is shown in Table 1. Amino acid esters **11** and **14** showed more potent inhibition compared with the esters of ethanolamine **5** and propanolamine **8**.



Scheme 1. Synthesis of ethanolamine and propanolamine esters.



Scheme 2. Synthesis of glycine and β-alanine esters.



Scheme 3. Synthesis of ethanolamine and propanolamine ethers.

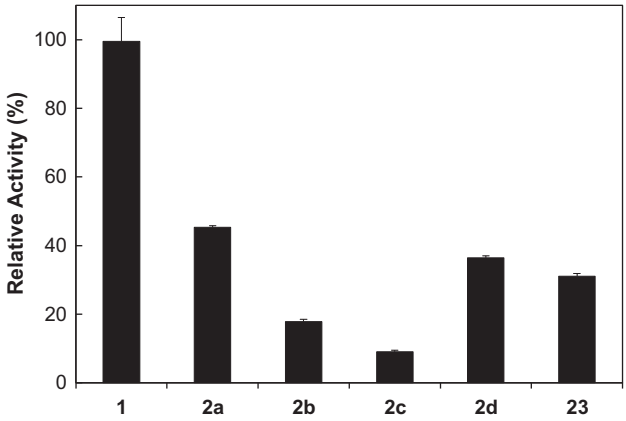


Figure 2. Inhibitory effect of *N*-pentadecylcyclohexanecarboxamide (**1**), long-chain alkyl amines **2a–d** and hexadecyltrimethylammonium chloride (**23**) on NAAA. The compounds were tested at 100 μM. PEA hydrolysis in the absence of the test compounds was normalized to 100%, and the relative values are shown (mean values ± S.D., $n = 3$).

Table 1
Inhibition of NAAA by various amines

Compound	% Activity	Compound	% Activity
*(a) Esters of ethanolamine **5a–d** and propanolamine **8a–d***			
5a	89 ± 5.2	**8a**	55 ± 1.9
5b	74 ± 0.3	**8b**	63 ± 1.6
5c	46 ± 1.9	**8c**	46 ± 0.9
5d	50 ± 1.0	**8d**	48 ± 2.3
*(b) Esters of glycine **11a–e** and β-alanine **14a–c***			
11a	34 ± 2.7	**14a**	51 ± 1.3
11b	11 ± 0.3	**14b**	29 ± 0.6
11c	16 ± 1.3	**14c**	30 ± 0.9
11d	42 ± 1.3		
11e	71 ± 1.9		
*(c) Ethers of ethanolamine **18a–c** and propanolamine **22a–c***			
18a	40 ± 1.8	**22a**	73 ± 2.2
18b	48 ± 1.5	**22b**	71 ± 0.8
18c	91 ± 1.0	**22c**	84 ± 2.1

The compounds were tested at 100 μM. PEA hydrolysis in the absence of the test compounds was normalized to 100%, and the relative values are shown (mean values ± S.D., $n = 3$).

Ethers **18** and **22** showed moderate inhibition of NAAA activity. The tridecyl ester of glycine (**11b**) exhibited the most potent activity (89% inhibition at 100 μM), which was similar to that of pentadecylamine (**2c**) (91% inhibition at 100 μM). The potency of the glycine esters **11a–d** (Table 1(b)) and the alkylamines **2a–d** (Fig. 2) were dependent upon alkyl chain length.

The dose-dependent effects of amines **2c** and **11b** on rat lung NAAA were examined. Both amines inhibited the enzyme in a dose-dependent manner (Fig. 3). Their IC_{50} values were calculated as 5.7 μM for **2c** and 11.8 μM for **11b**. As examined by Lineweaver–Burk plot, the inhibitions by **2c** and **11b** were of competitive type (Fig. 4).

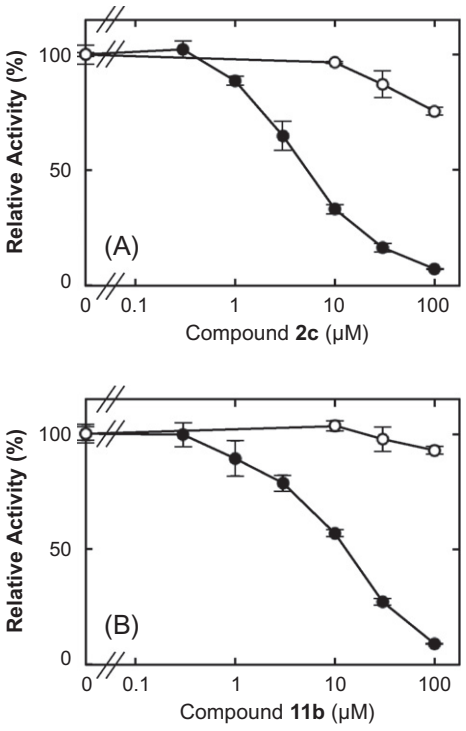


Figure 3. Inhibitory effects of pentadecylamine (**2c**) and tridecyl 2-aminoacetate (**11b**) on NAAA and FAAH. Compounds **2c** (A) and **11b** (B) were tested for their inhibitory effects on NAAA (closed circles) or FAAH (open circles). PEA hydrolysis in the absence of the test compounds was normalized to 100%, and the relative values are shown (mean values ± S.D., $n = 3$).

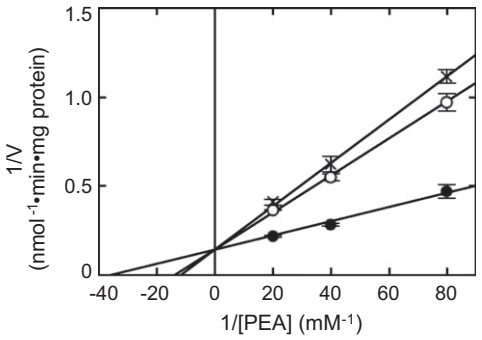


Figure 4. NAAA was allowed to react with the indicated concentrations of PEA in the presence of 5 μM compound **2c** (open circles) or 10 μM compound **11b** (crosses) or in their absence (close circles). The values (means ± S.D., $n = 3$) are shown in a Lineweaver–Burk plot.

Previously, *N*-arachidonoylglycine was reported to inhibit FAAH.[29,30] Therefore, we purchased *N*-arachidonoylglycine and *N*-palmitoylglycine from Cayman Chemical (Ann Arbor, MI) and tested their possible inhibitory effects on NAAA. However, no inhibition was observed with 100 μM *N*-arachidonoylglycine or *N*-palmitoylglycine (data not shown).

FAAH has high esterase activity with fatty acid esters such as 2-arachidonoylglycerol and methyl arachidonate in addition to its amidase activity with NAEs.[16] It has been reported that several ester compounds showed weak inhibition of FAAH.[20] Thus, compounds **2c** and **11b** were also tested for inhibition of rat liver FAAH (Fig. 3). Although the optimal pH range for FAAH is 8.5–10,[18] we performed the present FAAH assay at pH 5 (the optimal pH of NAAA) to avoid possible structural changes in the amines at alkaline pH. The compounds showed weak inhibition of FAAH; at 100 μM, pentadecylamine (**2c**) and the tridecyl ester of glycine (**11b**) decreased the activity by only 25% and 7%, respectively. When pH was adjusted to 8.5 with 50 mM Tris–HCl, both compounds at 100 μM did not inhibit FAAH (data not shown).

NAAA is a cysteine hydrolase that belongs to the N-terminal nucleophile family of enzymes, which are characterized by highly conserved sequences in the catalytic N-terminal region.[17,18] Recently, Solorzano et al. investigated[22] the properties of the NAAA active site by using the crystallographic coordinates of conjugated bile acid hydrolase, which is another cysteine hydrolase in the same enzyme family. In this model, the PEA acyl chain remains in the hydrophobic pocket of the enzyme and the PEA carbonyl group is attacked by the active cysteine. The resulting tetrahedral intermediate is stabilized by an electrostatic interaction in the enzyme oxyanion hole.

In our study, amines with an alkyl chain of suitable length exhibited potent inhibition of NAAA. Simple alkyl amines with a chain length of 14 (**2b**) or 15 (**2c**) atoms showed strong inhibition. In contrast, the glycine esters required a chain length of 16 (**11b**) or 17 (**11c**) atoms to obtain inhibition similar to that of **2b** and **2c**. The lipophilicity of the alkyl chain may be important for the interaction with the hydrophobic pocket of the N-terminal region of NAAA, to allow it to compete with PEA. These amines may inhibit NAAA as a result of the interaction between the amino group and an acidic amino acid residue in the active site.

In summary, lipophilic amines, particularly pentadecylamine (**2c**) and tridecyl 2-aminoacetate (**11b**), exhibited potent NAAA inhibition (IC_{50} = 5.7 and 11.8 μM), with much weaker FAAH inhibition. These simple structures provide a scaffold for further improvement of the NAAA inhibitory activity. Research into their inhibitory mechanism is underway.

3. Experimental

3.1. Chemistry

IR spectra were measured on a Perkin–Elmer FT-IR spectrometer, spectrum 100. ^{1}H and ^{13}C NMR spectra were determined on a Varian Gemini-300 superconducting FT-NMR spectrometer and the chemical shifts were referenced to tetramethylsilane. Mass spectra were taken on a Thermo Fisher Scientific Exactive spectrometer. Column chromatography (CC) was performed on using Kanto Silica Gel 60 N or silica gel (No. 37563-79) or Merck aluminiumoxide 90 (No. 1.01097.5000).

3.1.1. Preparation of *N*-boc-2-aminoethyl esters 4a–d

To a solution of *N*-boc-2-aminoethanol (**3**) (1.61 g, 10 mmol) and Et_3N (4.17 mL, 30 mmol) in CH_2Cl_2 (DCM, 20 mL) was added dropwise decanoyl chloride (2.23 mL, 11 mmol) at 0 °C. After being stirred at room temperature for 30 min, the reaction mixture was poured into ice–water and extracted with AcOEt. The extracts were washed with brine, dried over Na_2SO_4, and evaporated. The resulting crude product was purified by flash CC (silica gel; AcOEt/hexane, 1:3) to provide the decanoate **4a** (2.83 g, 90%). Dodecanoate **4b** (86%), tetradecanoate **4c** (82%), and hexadecanoate **4d** (96%) were similarly prepared from *N*-boc-2-aminoethanol (**3**).

2-((*tert*-Butoxycarbonyl)amino)ethyl decanoate (**4a**): Colorless oil; ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 7 Hz), 1.27 (12H, br s), 1.45 (9H, s), 1.62 (2H, quint, J = 7.5 Hz), 2.32 (2H, t, J = 7.5 Hz), 3.39 (2H, q, J = 5 Hz), 4.13 (2H, t, J = 5 Hz), 4.76 (1H, br s). ^{13}C NMR ($CDCl_3$) δ: 14.05, 22.61, 24.84, 28.30 (C × 3), 29.09, 29.21 (C × 2), 29.36, 31.79, 34.12, 39.67, 63.38, 79.49, 155.70, 173.74. IR ν_{max}($CHCl_3$) cm^{-1}: 3458, 1733 (sh), 1713. HR-SIMS *m/z*: 338.2299 (MNa^+, $C_{17}H_{33}O_4NNa$ requires 338.2302).

2-((*tert*-Butoxycarbonyl)amino)ethyl dodecanoate (**4b**): Colorless oil; ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 7 Hz), 1.26 (16H, br s), 1.45 (9H, s), 1.62 (2H, quint, J = 7.5 Hz), 2.32 (2H, t, J = 7.5 Hz), 3.39 (2H, q, J = 5.5 Hz), 4.13 (2H, t, J = 5.5 Hz), 4.84 (1H, br s). ^{13}C NMR ($CDCl_3$) δ: 14.06, 22.61, 24.83, 28.29 (C × 3), 29.08, 29.20, 29.27, 29.39, 29.54 (C × 2), 31.84, 34.11, 39.64, 63.36, 79.46, 155.69, 173.73. IR ν_{max}($CHCl_3$) cm^{-1}: 3459, 1734 (sh), 1713. HRSIMS *m/z*: 366.2610 (MNa^+, $C_{19}H_{37}O_4NNa$ requires 366.2615).

2-((*tert*-Butoxycarbonyl)amino)ethyl tetradecanoate (**4c**): Colorless solid; ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 7 Hz,), 1.26 (20H, br s), 1.45 (9H, s), 1.62 (2H, quint, J = 7.5 Hz), 2.32 (2H, t, J = 7.5 Hz), 3.39 (2H, q, J = 5.5 Hz), 4.13 (2H, t, J = 5.5 Hz), 4.81 (1H, br s). ^{13}C NMR ($CDCl_3$) δ: 14.08, 22.64, 24.84, 28.30 (C × 3), 29.09, 29.22, 29.31, 29.41, 29.56, 29.60 (C × 2), 29.63, 31.87, 34.12, 39.66, 63.39, 79.49, 155.70, 173.75. IR ν_{max}($CHCl_3$) cm^{-1}: 3459, 1734 (sh), 1713. HR-SIMS *m/z*: 394.2926 (MNa^+, $C_{21}H_{41}O_4NNa$ requires 394.2928).

2-((*tert*-Butoxycarbonyl)amino)ethyl hexadecanoate (**4d**): Colorless solid; ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 7 Hz), 1.26 (24H, br s), 1.45 (9H, s), 1.62 (2H, quint, J = 7.5 Hz), 2.32 (2H, t, J = 7.5 Hz), 3.39 (2H, q, J = 5.5 Hz), 4.13 (2H, t, J = 5.5 Hz), 4.79 (1H, br s, NH). ^{13}C NMR ($CDCl_3$) δ: 14.10, 22.66, 24.86, 28.31 (C × 4), 29.11, 29.23, 29.33, 29.43, 29.57, 29.62 (C × 2), 29.66 (C × 2), 31.89, 34.14, 39.67, 63.40, 79.51, 155.71, 173.77. IR ν_{max}($CHCl_3$) cm^{-1}: 3460, 1735 (sh), 1713 (CO). HR-SIMS *m/z*: 422.3241 (MNa^+, $C_{23}H_{45}O_4NNa$ requires 422.3241).

3.1.2. Preparation of 2-aminoethyl esters 5a–d

To a solution of *N*-boc-2-aminoethyl decanoate (**4a**) (1.00 g, 3.2 mmol) in DCM (4 mL) was added TFA (4 mL) and the mixture was stirred at room temperature for 30 min. The reaction mixture was slowly poured into aqueous $NaHCO_3$ and extracted with AcOEt. The extracts were washed with brine, dried over Na_2SO_4, and evaporated. The resulting crude product was purified by flash CC (silica gel; MeOH/DCM, 1:4) to provide the amine **5a** (500 mg, 73%). *N*-Boc-2-aminoethyl esters **4b–d** were similarly treated with TFA. In these case, precipitates appeared during extraction were collected. The obtained solids were washed with water and then with mixed solution of AcOEt and hexane, and dried to give amines **5b** (26%), **5c** (32%) and **5d** (62%), respectively.

2-Aminoethyl decanoate (**5a**): Colorless solid; ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 7 Hz), 1.26 (12H, br s), 1.59 (2H, quint, J = 7.5 Hz), 2.37 (2H, t, J = 7.5 Hz), 3.27 (2H, t, J = 5.5 Hz), 4.37 (2H, t, J = 5.5 Hz), 8.10 (2H, br s). ^{13}C NMR (75 MHz) δ: 14.07, 22.64, 24.61, 29.09, 29.26, 29.30, 29.44, 31.84, 33.82, 39.15, 60.21, 174.20. IR ν_{max}($CHCl_3$) cm^{-1}: 3689, 3398, 1739, 1681. HR-SIMS *m/z*: 216.1959 (MH^+, $C_{12}H_{26}O_2N$ requires 216.1958).

2-Aminoethyl dodecanoate (**5b**): Colorless solid; ^{1}H NMR ($CDCl_3$ + CD_3OD) δ: 0.88 (3H, t, J = 7 Hz), 1.26 (16H, br s), 1.62 (2H, quint, J = 7.5 Hz), 2.40 (2H, t, J = 7.5 Hz), 3.25 (2H, t, J = 5.5 Hz), 4.37 (2H, t, J = 5.5 Hz). ^{13}C NMR ($CDCl_3$ + CD_3OD) δ: 13.82, 22.46, 24.45, 28.93, 29.11, 29.12, 29.27, 29.40 (C × 2), 31.70, 33.68, 38.68, 59.94, 173.77. IR ν_{max}(KBr) cm^{-1}: 3465, 1741. HR-SIMS *m/z*: 244.2270 (MH^+, $C_{14}H_{30}O_2N$ requires 244.2272).

2-Aminoethyl tetradecanoate (**5c**): Colorless solid; ^{1}H NMR ($CDCl_3$ + CD_3OD) δ: 0.88 (3H, t, J = 7 Hz), 1.26 (20H, br s), 1.63 (2H, quint, J = 7.5 Hz), 2.40 (2H, t, J = 7.5 Hz), 3.24 (2H, t, J = 5.5 Hz), 4.36 (2H, t, J = 5.5 Hz). ^{13}C NMR ($CDCl_3$ + CD_3OD) δ: 13.80, 22.43, 24.42, 28.90, 29.07, 29.11, 29.24, 29.38, 29.40 (C × 2), 29.43, 31.67, 33.60, 38.60, 59.93, 173.74. IR ν_{max}(KBr) cm^{-1}: 3465, 1741. HR-SIMS *m/z*: 272.2582 (MH^+, $C_{16}H_{34}O_2N$ requires 272.2584).

2-Aminoethyl hexadecanoate (**5d**): Colorless solid; ^{1}H NMR ($CDCl_3$ + CD_3OD) δ: 0.88 (3H, t, J = 7 Hz), 1.26 (24H, br s), 1.63 (2H, quint, J = 7.5 Hz), 2.40 (2H, t, J = 7.5 Hz), 3.23 (2H, t, J = 5.5 Hz), 4.35 (2H, t, J = 5.5 Hz). ^{13}C NMR ($CDCl_3$ + CD_3OD) δ: 13.76, 22.41, 24.39, 28.87, 29.04, 29.09, 29.22, 29.35, 29.41 (C × 5), 31.65, 33.54, 38.55, 59.91, 173.72. IR ν_{max}(KBr) cm^{-1}: 3466, 1740. HR-SIMS *m/z*: 300.2896 (MH^+, $C_{18}H_{38}O_2N$ requires 300.2897).

3.1.3. Preparation of *N*-boc-3-aminopropyl esters 7a–d

In a manner similar to that for the preparation of *N*-boc-2-aminoethyl esters **4a–d** from *N*-boc-2-aminoethanol (**3**), *N*-boc-3-aminopropyl esters **7a** (97%), **7b** (77%), **7c** (77%) and **7d** (76%) were prepared from *N*-boc-3-aminopropanol (**6**).

3-((*tert*-Butoxycarbonyl)amino)propyl decanoate (**7a**): Colorless solid; ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 7 Hz), 1.27 (12H, br s), 1.44 (9H, s), 1.62 (2H, quint, J = 7.5 Hz), 1.82 (2H, quint, J = 6.5), 2.30 (2H, t, J = 7.5 Hz), 3.19 (2H, q, J = 6.5 Hz), 4.14 (2H, t, J = 6.5 Hz), 4.76 (1H, br s). ^{13}C NMR ($CDCl_3$) δ: 14.07, 22.62, 24.92, 28.36 (C × 3), 29.12, 29.22 (C × 2), 29.38, 31.82, 34.26, 37.41, 61.71, 63.22, 79.21, 155.86, 173.96. IR ν_{max}($CHCl_3$) cm^{-1}: 3458, 1729 (sh), 1712. HR-SIMS *m/z*: 330.2638 (MH^+, $C_{18}H_{36}O_4N$ requires 330.2639).

3-((*tert*-Butoxycarbonyl)amino)propyl dodecanoate (**7b**): Colorless solid; ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 7 Hz), 1.26 (16H, br s), 1.44 (9H, s), 1.64 (2H, quint, J = 7.5 Hz), 1.81 (2H, quint, J = 6.5), 2.30 (2H, t, J = 7.5 Hz), 3.19 (2H, q, J = 6.5 Hz), 4.14 (2H, t, J = 6.5 Hz), 4.73 (1H, br s). ^{13}C NMR ($CDCl_3$) δ: 14.09, 22.64, 24.93, 28.36 (C × 4), 29.13, 29.22, 29.30, 29.43, 29.56 (C × 2), 31.87, 34.27, 37.41, 61.71, 79.20, 155.86, 173.96. IR ν_{max}($CHCl_3$) cm^{-1}: 3457, 1730 (sh), 1712. HR-SIMS *m/z*: 358.2949 (MH^+, $C_{20}H_{40}O_4N$ requires 358.2952).

3-((*tert*-Butoxycarbonyl)amino)propyl tetradecanoate (**7c**): Colorless solid; ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 7 Hz), 1.26 (20H, br s), 1.41 (9H, s), 1.61 (2H, quint, J = 7.5 Hz), 1.81 (2H, quint, J = 6.5), 2.30 (2H, t, J = 7.5 Hz), 3.19 (2H, q, J = 6.5 Hz), 4.14 (2H, t,

J = 6.5 Hz), 4.73 (1H, br s). ^{13}C NMR ($CDCl_3$) δ: 14.09, 22.65, 24.92, 28.36 (C × 4), 29.12, 29.23, 29.31, 29.43, 29.57, 29.60 (C × 2), 29.63, 31.89, 34.26, 37.40, 61.70, 79.21, 155.86, 173.96. IR ν_{max}($CHCl_3$) cm^{-1}: 3458, 1732 (sh), 1712. HR-SIMS m/z: 408.3082 (MNa^+, $C_{22}H_{43}O_4NNa$ requires 408.3084).

3-((*tert*-Butoxycarbonyl)amino)propyl hexadecanoate (**7d**): Colorless solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.88 (3H, t, J = 7 Hz), 1.25 (24H, br s), 1.44 (9H, s), 1.61 (2H, quint, J = 7.5 Hz), 1.81 (2H, quint, J = 6.5), 2.30 (2H, t, J = 7.5 Hz), 3.19 (2H, q, J = 6.5 Hz), 4.14 (2H, t, J = 6.5 Hz), 4.73 (1H, br s). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 14.10, 22.67, 24.93, 28.37 (C × 4), 29.15, 29.25, 29.34, 29.44, 29.58, 29.63 (C × 2), 29.66 (C × 3), 31.90, 34.27, 37.42, 61.71, 79.23 155.88, 173.98. IR ν_{max}($CHCl_3$) cm^{-1}: 3458, 1729 (sh), 1712. HR-SIMS m/z: 436.3393 (MNa^+, $C_{24}H_{47}O_4NNa$ requires 436.3397).

3.1.4. Preparation of 3-aminopropyl esters 8a–d

In a manner similar to that for the preparation of 2-aminoethyl esters **5a–d** from *N*-boc-2-aminoethyl esters **4a–d**, *N*-boc-3-aminopropyl esters **7b–d** were treated with TFA to provide amines **8a** (quant), **8b** (71%), **8c** (47%) and **8d** (42%).

3-Aaminopropyl decanoate (**8a**): Colorless solid; ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 7 Hz), 1.27 (12H, br s), 1.60 (2H, quint, J = 7.5 Hz), 2.07 (2H, quint, J = 6.5), 2.31 (2H, t, J = 7.5 Hz), 3.07 (2H, t, J = 6.5 Hz), 4.19 (2H, t, J = 6.5 Hz), 7.68 (2H, br s). ^{13}C NMR ($CDCl_3$) δ: 14.08, 22.64, 24.80, 26.75, 29.12, 29.25 (C × 2), 29.40, 31.83, 34.06, 36.95, 60.66, 174.30. IR ν_{max}($CHCl_3$) cm^{-1}: 3550-2450 (br), 1734, 1680. HR-SIMS m/z: 230.2115 (MH^+, $C_{13}H_{28}O_2N$ requires 230.2115).

3-Aaminopropyl dodecanoate (**8b**): Colorless solid; ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 7 Hz), 1.27 (16H, br s), 1.59 (2H, quint, J = 7.5 Hz), 2.04 (2H, quint, J = 6.5), 2.30 (2H, t, J = 7.5 Hz), 3.05 (2H, t, J = 6.5 Hz), 4.17 (2H, t, J = 6.5 Hz), 7.98 (2H, br s). ^{13}C NMR ($CDCl_3$) δ: 14.09, 22.66, 24.79, 26.69, 29.12, 29.25, 29.32, 29.45, 29.60 (C × 2), 31.89, 34.01, 36.85, 60.59, 174.35. IR ν_{max}($CHCl_3$) cm^{-1}: 3550-2450 (br), 1733, 1682. HR-SIMS m/z: 258.2426 (MH^+, $C_{15}H_{32}O_2N$ requires 258.2426).

3-Aaminopropyl tetradecanoate (**8c**): Colorless solid; ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 7 Hz), 1.26 (20H, br s), 1.60 (2H, quint, J = 7.5 Hz), 2.16 (2H, quint, J = 6.5), 2.32 (2H, t, J = 7.5 Hz), 3.14 (2H, t, J = 6.5 Hz), 4.22 (2H, t, J = 6.5 Hz), 8.32 (2H, br s). ^{13}C NMR ($CDCl_3$) δ: 14.09, 22.66, 24.83, 26.76, 29.17, 29.29, 29.33, 29.48, 29.63 (C × 4), 31.89, 34.14, 37.07, 60.77, 174.03. IR ν_{max}($CHCl_3$) cm^{-1}: 3550-2450 (br), 1734, 1682. HR-SIMS m/z: 286.2741 (MH^+, $C_{17}H_{36}O_2N$ requires 286.2741).

3-Aaminopropyl hexadecanoate (**8d**): Colorless solid; ^{1}H NMR ($CDCl_3$ + CD_3OD) δ: 0.88 (3H, t, J = 7 Hz), 1.26 (24H, br s), 1.60 (2H, quint, J = 7.5 Hz), 2.09 (2H, quint, J = 6.5), 2.33 (2H, t, J = 7.5 Hz), 3.04 (2H, t, J = 6.5 Hz), 4.18 (2H, t, J = 6.5 Hz). ^{13}C NMR ($CDCl_3$ + CD_3OD) δ: 13.90, 22.51, 24.67, 26.42, 28.99, 29.11, 29.19, 29.31, 29.44, 29.48 (C × 2), 29.51 (C × 3), 31.75, 33.93, 36.81, 60.83, 174.13. IR ν_{max}(KBr) cm^{-1}: 3461, 1742. HR-SIMS m/z: 314.3053 (MH^+, $C_{19}H_{40}O_2N$ requires 314.3054).

3.1.5. Preparation of *N*-boc-aminoacetates 10a–e

2-Chloro-1-methylpyridinium iodide (CMPI, 3.32 g, 13 mmol) and *N*,*N*-dimethylaminopyridine (DMAP, 3.18 g, 26 mmol) were added to a solution of *N*-boc-glycine (**9**) (1.75 g, 10 mmol) and dodecanol (2.47 mL, 10 mmol) in dry DCM (20 mL) at 0 °C and the mixture was stirred at 0 °C for 30 min. After the mixture was diluted with AcOEt, the resulting precipitates were filtered off. The filtrates were successively washed with aqueous 5% HCl, saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4, and evaporated. The resulting crude product was purified by flash CC (silica gel; AcOEt/DCM, 5:95) to provide the dodecyl ester **10a** (2.78 g, 81%). Tridecyl ester **10b** (86%), tetradecyl ester **10c** (82%), pentadecyl ester **10d** (74%) and hexadecyl ester **11e** (79%) were similarly prepared from *N*-boc-glycine (**9**).

Dodecyl 2-((*tert*-butoxycarbonyl)amino)acetate (**10a**): Colorless oil; ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 7 Hz), 1.26 (18H, br s), 1.45 (9H, s), 1.64 (2H, quint, J = 7 Hz), 3.90 (2H, d, J = 5.5 Hz), 4.14 (2H, t, J = 7 Hz), 5.08 (1H, br s). ^{13}C NMR ($CDCl_3$) δ: 14.05, 22.62, 25.75, 28.23 (C × 3), 28.47, 29.15, 29.28, 29.43, 29.50, 29.55, 29.57, 31.85, 42.36, 65.43, 79.81, 155.63, 170.40. IR ν_{max}($CHCl_3$) cm^{-1}: 3449, 1742, 1712. HR-SIMS m/z: 344.2790 (MH^+, $C_{19}H_{38}O_4N$ requires 344.2795).

Tridecyl 2-((*tert*-butoxycarbonyl)amino)acetate (**10b**): Colorless oil; ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 7 Hz), 1.26 (20H, br s), 1.45 (9H, s), 1.64 (2H, quint, J = 7 Hz), 3.90 (2H, d, J = 5.5 Hz), 4.14 (2H, t, J = 7 Hz), 5.04 (1H, br s). ^{13}C NMR ($CDCl_3$) δ: 14.09, 22.66, 25.79, 28.28 (C × 3), 28.50, 29.19, 29.32, 29.46, 29.54, 29.61 (C × 2), 29.63, 31.89 42.41, 65.49, 79.90, 155.66, 170.41. IR ν_{max}($CHCl_3$) cm^{-1}: 3448, 1742, 1713. HR-SIMS m/z: 358.2943 (MH^+, $C_{20}H_{40}O_4N$ requires 358.2952).

Tetradecyl 2-((*tert*-butoxycarbonyl)amino)acetate (**10c**): Colorless oil; ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 7 Hz), 1.26 (22H, br s), 1.45 (9H, s), 1.65 (2H, quint, J = 7 Hz), 3.90 (2H, d, J = 5.5 Hz), 4.14 (2H, t, J = 7 Hz), 5.01 (1H, br s). ^{13}C NMR ($CDCl_3$) δ: 14.10, 22.67, 25.79, 28.28 (C × 3), 28.50, 29.19, 29.33, 29.47, 29.54, 29.63 (C × 3), 29.66, 31.90, 42.41, 65.51, 79.91, 155.65, 170.44. IR ν_{max}($CHCl_3$) cm^{-1}: 3449, 1741, 1712. HR-SIMS m/z: 372.3103 (MH^+, $C_{21}H_{42}O_4N$ requires 372.3108).

Pentadecyl 2-((*tert*-butoxycarbonyl)amino)acetate (**10d**): Colorless solid; ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 7 Hz), 1.26 (24H, br s), 1.45 (9H, s), 1.63 (2H, quint, J = 7 Hz), 3.90 (2H, d, J = 5.5 Hz), 4.14 (2H, t, J = 7 Hz), 5.06 (1H, br s). ^{13}C NMR ($CDCl_3$) δ: 14.07, 22.64, 25.77, 28.26 (C × 3), 28.49, 29.18, 29.31, 29.45, 29.52, 29.61 (C × 3), 29.64 (C × 2), 31.88, 42.39, 65.46, 79.84, 155.64, 170.39. IR ν_{max}($CHCl_3$) cm^{-1}: 3448, 1741, 1713. HR-SIMS m/z: 386.3209 (MH^+, $C_{22}H_{44}O_4N$ requires 386.3265).

Hexadecyl 2-((*tert*-butoxycarbonyl)amino)acetate (**10e**); Colorless solid; ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 7 Hz), 1.26 (26H, br s), 1.45 (9H, s), 1.63 (2H, quint, J = 7 Hz), 3.90 (2H, d, J = 5.5 Hz), 4.14 (2H, t, J = 7 Hz), 5.04 (1H, br s). ^{13}C NMR ($CDCl_3$) δ: 14.07, 22.65, 25.78, 28.26 (C × 3), 28.50, 29.19, 29.32, 29.46, 29.53, 29.62 (C × 3), 29.66 (C × 3), 31.89, 42.40, 65.47, 79.86, 155.66, 170.40. IR ν_{max}($CHCl_3$) cm^{-1}: 3450, 1743, 1713. HR-SIMS m/z: 400.3415 (MH^+, $C_{23}H_{46}O_4N$ requires 400.3421).

3.1.6. Preparation of aminoacetates 11a–d

In a manner similar to that for the preparation of 2-aminoethyl esters **5a–d** from *N*-boc-2-aminoethyl esters **4a–d**, *N*-boc-aminoacetates **10a–e** were treated with TFA to provide amines **11a** (46%), **11b** (99%), **11c** (46%), **11d** (84%) and **11e** (77%).

Dodecyl 2-aminoacetate (**11a**): Colorless solid; ^{1}H NMR ($CDCl_3$ + CD_3OD) δ: 0.88 (3H, t, J = 7 Hz), 1.26 (18H, br s), 1.62 (2H, quint, J = 7 Hz), 3.57 (2H, br s), 4.13 (2H, t, J = 7 Hz). ^{13}C NMR ($CDCl_3$ + CD_3OD) δ: 13.92, 22.54, 25.62, 28.30, 29.06, 29.20, 29.34, 29.42, 29.47 (C × 2), 31.77, 41.46, 65.93, 170.77. IR ν_{max}($CHCl_3$) cm^{-1}: 3392, 1734 (sh), 1680. HR-SIMS m/z: 244.2267 (MH^+, $C_{14}H_{30}O_2N$ requires 244.2271).

Tridecyl 2-aminoacetate (**11b**): Colorless solid; ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 7 Hz), 1.26 (20H, br s), 1.47 (2H, br s), 1.64 (2H, quint, J = 7 Hz), 3.42 (2H, s), 4.13 (2H, t, J = 7 Hz). ^{13}C NMR ($CDCl_3$) δ: 14.04, 22.62, 25.81, 28.56, 29.17, 29.28, 29.44, 29.50, 29.57 (C × 2), 29.60, 31.85, 43.94, 65.01, 174.33. IR ν_{max}($CHCl_3$) cm^{-1}: 3398, 1734. HR-SIMS m/z: 258.2422 (MH^+, $C_{15}H_{32}O_2N$ requires 258.2428).

Tetradecyl 2-aminoacetate (**11c**): Colorless solid; ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 7 Hz), 1.26 (22H, br s), 1.44 (2H, br s), 1.63 (2H, quint, J = 7 Hz), 3.42 (2H, s), 4.12 (2H, t, J = 7 Hz). ^{13}C NMR ($CDCl_3$) δ: 14.08, 22.65, 25.84, 28.58, 29.20, 29.32, 29.47,

29.53, 29.61 (C × 3), 29.65, 31.89, 43.98, 65.04, 174.38. IR ν_{max}($CHCl_3$) cm^{-1}: 3391, 1735. HR-SIMS *m/z*: 272.2580 (MH^+, $C_{16}H_{34}O_2N$ requires 272.2584).

Pentadecyl 2-aminoacetate (**11d**): Colorless solid; ^1H NMR ($CDCl_3$) δ: 0.88 (3H, t, *J* = 7 Hz), 1.26 (24H, br s), 1.49 (2H, br s), 1.63 (2H, quint, *J* = 7 Hz), 3.42 (2H, s), 4.12 (2H, t, *J* = 7 Hz). ^{13}C NMR ($CDCl_3$) δ: 14.09, 22.66, 25.85, 28.59, 29.21, 29.33, 29.47, 29.54, 29.63 (C × 3), 29.66 (C × 2), 31.89, 43.96, 65.06, 174.34. IR ν_{max}($CHCl_3$) cm^{-1}: 3393, 1734. HR-SIMS *m/z*: 286.2734 (MH^+, $C_{17}H_{36}O_2N$ requires 286.2741).

Hexadecyl 2-aminoacetate (**11e**): Colorless solid; ^1H NMR ($CDCl_3$ + CD_3OD) δ: 0.88 (3H, t, *J* = 7 Hz), 1.26 (26H, br s), 1.66 (2H, quint, *J* = 7 Hz), 3.60 (2H, s), 4.18 (2H, t, *J* = 7 Hz). ^{13}C NMR (7$CDCl_3$ + CD_3OD) δ: 13.84, 22.48, 25.56, 28.25, 29.01, 29.15, 29.29, 29.37, 29.45 (C × 3), 29.49 (C × 3), 31.73, 41.12, 65.93, 170.11. IR ν_{max}($CHCl_3$) cm^{-1}: 3387, 1755. HR-SIMS *m/z*: 300.2891 (MH^+, $C_{18}H_{40}O_2N$ requires 300.2997).

3.1.7. Preparation of *N*-boc-3-aminopropanoates 13a–e

In a manner similar to that for the preparation of *N*-boc-amonoacetates **10a–d** from *N*-boc-gclycine (**9**), *N*-boc-3-amino- propanoates **13a** (72%), **13b** (79%) and **13c** (80%) were prepared from *N*-boc-β-alanine (**12**).

Dodecyl 3-((*tert*-butoxycarbonyl)amino)propanoate (**13a**): Colorless oil; ^1H NMR ($CDCl_3$) δ: 0.88 (3H, t, *J* = 7 Hz), 1.26 (18H, br s), 1.44 (9H, s), 1.62 (2H, quint, *J* = 7.5 Hz), 2.52 (2H, t, *J* = 6), 3.39 (2H, q, *J* = 6 Hz), 4.08 (2H, t, *J* = 7 Hz), 5.03 (1H, br s). ^{13}C NMR ($CDCl_3$) δ: 14.07, 22.65, 25.87, 28.35 (C × 3), 28.54, 29.21, 29.31, 29.47, 29.54, 29.58 (C × 2), 31.88, 34.59, 36.09, 64.83, 79.28, 155.75, 172.56. IR ν_{max}($CHCl_3$) cm^{-1}: 3455, 1725 (sh), 1711. HR-SIMS *m/z*: 358.29481 (MH^+, $C_{20}H_{39}O_4N$ requires 358.29518).

Tridecyl 3-((*tert*-butoxycarbonyl)amino)propanoate (**13b**): Colorless oil; ^1H NMR ($CDCl_3$) δ: 0.86 (3H, t, *J* = 7 Hz), 1.24 (20H, br s), 1.41 (9H, s), 1.60 (2H, quint, *J* = 7.5 Hz), 2.49 (2H, t, *J* = 6), 3.37 (2H, q, *J* = 6 Hz), 4.06 (2H, t, *J* = 7 Hz), 5.02 (1H, br s). ^{13}C NMR ($CDCl_3$) δ: 14.06, 22.64, 25.86, 28.33 (C × 3), 28.53, 29.19, 29.30, 29.46, 29.52, 29.59 (C × 2), 29.61, 31.87, 34.57, 36.08, 64.80, 79.24, 155.74, 172.53. IR ν_{max}($CHCl_3$) cm^{-1}: 3460, 1724, 1710. HR-SIMS *m/z*: 372.30999 (MH^+, $C_{21}H_{42}O_4N$ requires 372.31084).

Tetradecyl 3-((*tert*-butoxycarbonyl)amino)propanoate (**13c**): Colorless oil; ^1H NMR ($CDCl_3$) δ: 0.88 (3H, t, *J* = 7 Hz), 1.26 (22H, br s), 1.44 (9H, s), 1.62 (2H, quint, *J* = 7.5 Hz), 2.52 (2H, t, *J* = 6), 3.39 (2H, q, *J* = 6 Hz), 4.08 (2H, t, *J* = 7 Hz), 5.09 (1H, br s). ^{13}C NMR ($CDCl_3$) δ: 14.00, 22.59, 25.82, 28.28 (C × 3), 28.49, 29.15, 29.26, 29.42, 29.48, 29.55 (C × 3), 29.60, 31.83, 34.52, 36.04, 64.73, 79.15, 155.69, 172.45. IR ν_{max}($CHCl_3$) cm^{-1}: 3456, 1724, 1711. HR-SIMS *m/z*: 386.32592 (MH^+, $C_{22}H_{44}O_4N$ requires 386.32649).

3.1.8. Preparation of 3-aminopropanoates 14a–c

In a manner similar to that for the preparation of 2-aminoethyl esters **5a–d** from *N*-boc-2-aminoethyl esters **4a–d**, *N*-boc-3-aminopropanoates **13a–c** were treated with TFA to provide amines **14a** (quant.), **14b** (quant.) and **14c** (quant.).

Dodecyl 3-aminopropanoate (**14a**): Colorless solid; ^1H NMR ($CDCl_3$) δ: 0.88 (3H, t, *J* = 7 Hz), 1.26 (18H, br s), 1.62 (2H, quint, *J* = 7.5 Hz), 2.72 (2H, t, *J* = 6), 3.21 (2H, t, *J* = 6 Hz), 4.09 (2H, t, *J* = 7 Hz), 6.91 (2H, br s). ^{13}C NMR ($CDCl_3$) δ: 14.08, 22.67, 25.79, 28.38, 29.23, 29.34, 29.50, 29.58, 29.62 (C × 2), 31.44, 31.90, 35.71, 65.61, 171.81. IR ν_{max}($CHCl_3$) cm^{-1}: 3550–2450 (br), 1721, 1680. HR-SIMS *m/z*: 258.24192 (MH^+, $C_{15}H_{32}O_2N$ requires 258.24276).

Tridecyl 3-aminopropanoate (**14b**): Colorless solid; ^1H NMR ($CDCl_3$) δ: 0.88 (3H, t, *J* = 7 Hz), 1.26 (20H, br s), 1.62 (2H, quint, *J* = 7.5 Hz), 2.71 (2H, t, *J* = 6), 3.21 (2H, t, *J* = 6 Hz), 4.09 (2H, t, *J* = 7 Hz), 6.56 (2H, br s). ^{13}C NMR ($CDCl_3$) δ: 14.09, 22.67, 25.79, 28.38, 29.24, 29.34, 29.50, 29.58, 29.64 (C × 3), 31.43, 31.91, 35.76, 65.64, 171.89. IR ν_{max}($CHCl_3$) cm^{-1}: 3550–2450 (br), 1723, 1680. HR-SIMS *m/z*: 272.25754 (MH^+, $C_{16}H_{34}O_2N$ requires 272.25840).

Tridecyl 3-aminopropanoate (**14c**): Colorless solid; ^1H NMR ($CDCl_3$) δ: 0.88 (3H, t, *J* = 7 Hz), 1.26 (22H, br s), 1.62 (2H, quint, *J* = 7.5 Hz), 2.72 (2H, t, *J* = 6), 3.22 (2H, t, *J* = 6 Hz), 4.09 (2H, t, *J* = 7 Hz), 6.44 (2H, br s). ^{13}C NMR ($CDCl_3$) δ: 14.09, 22.67, 25.79, 28.38, 29.25, 29.34, 29.50, 29.59, 29.65 (C × 4), 31.26, 31.91, 35.65, 65.65, 171.78. IR ν_{max}($CHCl_3$) cm^{-1}: 3550–2450 (br), 1721, 1680. HR-SIMS *m/z*: 286.27363 (MH^+, $C_{17}H_{36}O_2N$ requires 286.27405).

3.1.9. Preparation of alkyloxyethanamines 18a–c

According to the preparation of alkyloxypropanamines **22a–c** from 1,3-propanediol (**19**) as described bellow, ethylene glycol (**15**) was etherified (**16a**: 60%, **16b**: 62%, **16c**: 69%), transformed into azides **17a** (78%), **17b** (86%) and **17c** (75%), and then reduced to provide alkyloxyethanamines **18a** (76%), **18b** (95%) and **18c** (75%). These spectral data were in accordance with those reported.[27]

3.1.10. Preparation of alkyloxypropaol 20a–c

To a solution of 1,3-propanediol (**19**) (6.51 mL, 90 mmol) in dry DMF (40 mL) was added NaH (60% oil dispersion; 1.20 g, 30 mmol) in installments at 0 °C and the mixture was stirred at room temperature for 10 min. Dodecyl bromide (4.80 mL, 20 mmol) and KI (3.32 g, 20 mmol) were added and the mixture was heated at 95 °C for 4 h. After cooling, the mixture was poured into ice–water and extracted with AcOEt. The extracts were washed with brine, dried over Na_2SO_4 and evaporated. The resulting residue was purified by flash CC (silica gel; AcOEt/hexane, 1:2) to provide dodecyl ether **20a** (3.38 g, 69%). Tetradecyl ether **20b** (79%) and hexadecyl ether **20c** (77%) were similarly prepared from 1,3-propanediol (**19**).

3-Dodecyloxy-1-propanol (**20a**): Colorless solid; ^1H NMR ($CDCl_3$) δ: 0.88 (3H, t, *J* = 7 Hz), 1.26 (18H, br s), 1.57 (2H, quint, *J* = 7 Hz), 1.83 (2H, quint, *J* = 5.5 Hz), 2.60 (1H, t, *J* = 5.5 Hz), 3.43 (2H, t, *J* = 7 Hz), 3.61 (2H, t, *J* = 5.5 Hz), 3.78 (2H, q, *J* = 5.5 Hz). ^{13}C NMR ($CDCl_3$) δ: 14.08, 22.66, 26.12, 29.32, 29.44, 29.55, 29.58, 29.60, 26.63, 29.66, 31.89 (C × 2), 62.40, 74.40, 71.47. IR ν_{max}($CHCl_3$) cm^{-1}: 3631, 3497 (br). HR-SIMS *m/z*: 245.2468 (MH^+, $C_{15}H_{33}O_2$ requires 245.2475).

3-Tetradecyloxy-1-propanol (**20b**): Colorless solid; ^1H NMR ($CDCl_3$) δ: 0.88 (3H, t, *J* = 7 Hz), 1.26 (22H, br s), 1.57 (2H, quint, *J* = 7 Hz), 1.83 (2H, quint, *J* = 5.5 Hz), 2.60 (1H, t, *J* = 5.5 Hz), 3.43 (2H, t, *J* = 7 Hz), 3.61 (2H, t, *J* = 5.5 Hz), 3.78 (2H, q, *J* = 5.5 Hz). ^{13}C NMR ($CDCl_3$) δ: 14.10, 22.67, 26.13, 29.34, 29.45, 29.56, 29.59, 29.63 (C × 3), 29.67 (C × 2), 31.90 (C × 2), 62.46, 70.45, 71.49. IR ν_{max}($CHCl_3$) cm^{-1}: 3632, 3498 (br). HR-SIMS *m/z*: 273.2782 (MH^+, $C_{17}H_{37}O_2$ requires 273.2788).

3-Hexadecyloxy-1-propanol (**20c**): Colorless solid; ^1H NMR ($CDCl_3$) δ: 0.88 (3H, t, *J* = 7 Hz), 1.26 (26H, br s), 1.57 (2H, quint, *J* = 7 Hz), 1.83 (2H, quint, *J* = 5.5 Hz), 2.56 (1H, t, *J* = 5.5 Hz), 3.43 (2H, t, *J* = 7 Hz), 3.62 (2H, t, *J* = 5.5 Hz), 3.76 (2H, q, *J* = 5.5 Hz). ^{13}C NMR ($CDCl_3$) δ: 14.10, 22.68, 26.14, 29.35, 29.46, 29.57, 29.60, 29.65 (C × 2), 29.69 (C × 5), 31.92 (C × 2), 62.47, 70.47, 71.50. IR ν_{max}($CHCl_3$) cm^{-1}: 3631, 3497 (br). HR-SIMS *m/z*: 301.3094 (MH^+, $C_{19}H_{41}O_2$ requires 301.3101).

3.1.11. Preparation of azides 21a–c

To a solution of the alcohol **20a** (2.44 g, 10 mmol) and DPPA (2.59 mL, 12 mmol) in dry DMF (20 mL) was added DBU (1.79 mL, 12 mmol) and the mixture was heated at 90 °C for 3 h. After cooling, the mixture was diluted with AcOEt and washed with brine, dried over Na_2SO_4 and evaporated. The resulting residue was purified by flash CC (silica gel; AcOEt/hexane, 8:92)

to provide the azide **21a** (2.39 g, 89%). Azides **21b** (94%) and **21c** (81%) were similarly prepared from alcohols **20b** and **20c**.

1-(3-Azidopropoxy)dodecane (**21a**); Colorless solid; ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 7 Hz), 1.26 (18H, br s), 1.56 (2H, quint, J = 7 Hz), 1.84 (2H, quint, J = 6.5 Hz), 3.38 (2H, t, J = 6.5 Hz), 3.41 (2H, t, J = 7 Hz), 3.48 (2H, t, J = 6.5 Hz). ^{13}C NMR ($CDCl_3$) δ: 14.10, 22.67, 26.14, 29.24, 29.34, 29.47, 29.59 (C × 2), 29.62, 29.65 (C × 2), 31.90, 48.53, 67.22, 71.21. IR ν_{max}($CHCl_3$) cm^{-1}: 2100. HR-SIMS m/z: 292.2352 (MNa^+, $C_{15}H_{31}ON_3Na$ requires 292.2359).

1-(3-Azidopropoxy)tetradecane (**21b**): Colorless solid; ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 7 Hz), 1.26 (22H, br s), 1.56 (2H, quint, J = 7 Hz), 1.84 (2H, quint, J = 6.5 Hz), 3.39 (2H, t, J = 6.5 Hz), 3.41 (2H, t, J = 7 Hz), 3.49 (2H, t, J = 6.5 Hz). ^{13}C NMR ($CDCl_3$) δ: 14.11, 22.68, 26.15, 29.25, 29.35, 29.47, 29.60 (C × 2), 29.66 (C × 5), 31.92, 48.53, 67.23, 71.21. IR ν_{max}($CHCl_3$) cm^{-1}: 2100. HR-SIMS m/z: 320.2664 (MNa^+, $C_{17}H_{35}ON_3Na$ requires 320.2672).

1-(3-Azidopropoxy)hexadecane (**21c**): Colorless solid; ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 7 Hz), 1.26 (26H, br s), 1.56 (2H, quint, J = 7 Hz), 1.84 (2H, quint, J = 6.5 Hz), 3.39 (2H, t, J = 6.5 Hz), 3.41 (2H, t, J = 7 Hz), 3.48 (2H, t, J = 6.5 Hz). ^{13}C NMR ($CDCl_3$) δ: 14.11, 22.68, 26.15, 29.25, 29.36, 29.48, 29.60 (C × 2), 29.69 (C × 7), 31.92, 48.53, 67.23, 71.21. IR ν_{max}($CHCl_3$) cm^{-1}: 2100. HR-SIMS m/z: 348.2977 (MNa^+, $C_{19}H_{39}ON_3Na$ requires 348.2985).

3.1.12. Preparation of alkyloxypropanamines 22a–c

To a solution of the azide **21a** (807 mg, 3 mmol) in tetrahydrofuran (THF, 20 mL) was added PPh_3 (943 mg, 3.6 mmol). After being stirred at room temperature for 10 min, water was added and the reaction mixture was kept at room temperature under stirring for 28 h. The mixture was then concentrated to give a residue, which was purified by CC (aluminiumoxide; MeOH/DCM, 5:95 to 1:9) to provide the amine **22a** (413 mg, 57%). Amines **22b** (67%) and **22c** (98%) were similarly prepared from azides **21b** and **21c**.

3-Dodecyloxy-1-propanamine (**22a**): Colorless solid; ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 7 Hz), 1.26 (18H, br s), 1.56 (2H, quint, J = 7 Hz), 1.71 (2H, quint, J = = 6.5 Hz), 2.80 (2H, t, J = 6.5 Hz), 3.40 (2H, t, J = 7 Hz), 3.49 (2H, t, J = 6.5 Hz). ^{13}C NMR ($CDCl_3$) δ: 14.07, 22.64, 26.16, 29.31, 29.47, 29.57 (C × 2), 29.60, 29.63, 29.72, 31.88, 33.58, 39.71, 68.89, 71.10. IR ν_{max}($CHCl_3$) cm^{-1}: 3380, 3197 (br). HR-SIMS m/z: 244.2628 (MH^+, $C_{15}H_{34}ON$ requires 244.2635).

3-Tetradecyloxy-1-propanamine (**22b**): Colorless solid; ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 7 Hz), 1.26 (22H, br s), 1.56 (2H, quint, J = 7 Hz), 1.71 (2H, quint, J = 6.5 Hz), 2.80 (2H, t, J = 6.5 Hz), 3.40 (2H, t, J = 7 Hz), 3.49 (2H, t, J = 6.5 Hz). ^{13}C NMR ($CDCl_3$) δ: 14.07, 22.65, 26.16, 29.31, 29.46, 29.57 (C × 2), 29.62 (C × 4), 29.72, 31.88, 33.59, 39.72, 68.89, 71.09. IR ν_{max}($CHCl_3$) cm^{-1}: 3380, 3194 (br). HR-SIMS m/z: 272.2939 (MH^+, $C_{17}H_{38}ON$ requires 272.2948).

3-Hexadecyloxy-1-propanamine (**22c**): Colorless solid; ^{1}H NMR ($CDCl_3$) δ: 0.88 (3H, t, J = 7 Hz), 1.26 (26H, br s), 1.56 (2H, quint, J = 7 Hz), 1.71 (2H, quint, J = 6.5 Hz), 2.80 (2H, t, J = 6.5 Hz), 3.40 (2H, t, J = 7 Hz), 3.49 (2H, t, J = 6.5 Hz). ^{13}C NMR ($CDCl_3$) δ: 14.09, 22.66, 26.17, 29.34, 29.48, 29.59 (C × 2), 29.66 (C × 6), 29.73, 31.89, 33.63, 39.73, 68.90, 71.11. IR ν_{max}($CHCl_3$) cm^{-1}: 3380, 3172 (br). HR-SIMS m/z: 300.3253 (MH^+, $C_{19}H_{42}ON$ requires 300.3261).

3.2. Biology

3.2.1. Enzyme preparation

As described previously,[21] we used rat lung and liver as sources of NAAA and FAAH, respectively. Male Wistar/ST rats (300–500 g body weight; Japan SLC, Hamamatsu, Japan) were anesthetized with diethyl ether and sacrificed by decapitation. Lung and liver were removed from the rats and homogenized in 9 or 5 volume of ice-cold 20 mM Tris–HCl (pH 7.4) containing 0.32 M sucrose, respectively, with a polytron homogenizer. The lung homogenate was centrifuged at 800 g for 15 min and the resulting supernatant was further centrifuged at 12,000 g for 30 min. The 12,000 g pellet was then subjected to two cycles of freezing and thawing in phosphate-buffered saline, and followed by further centrifugation at 105,000 g for 50 min. The obtained supernatant was used for NAAA assay. The liver homogenate was centrifuged at 800 g for 15 min and the obtained supernatant was further centrifuged at 105,000 g for 50 min. The 105,000 g pellet was then treated with 1% Triton X-100 and followed by further centrifugation at 105,000 g for 50 min. The obtained supernatant was used for FAAH assay. Protein concentration was determined by the method of Bradford[31] with bovine serum albumin as standard.

3.2.2. Enzyme assay

N-[^{14}C]Palmitoylethanolamine ([^{14}C]PEA) was synthesized from [1-^{14}C]palmitic acid (Perkin–Elmer Life Science; Boston, MA, USA) and ethanolamine as described previously.[32] Rat lung NAAA (17.8 μg protein) or rat liver FAAH (110 μg protein) was allowed to react at 37 °C for 30 min with 100 μM [^{14}C]PEA (10000 cpm, dissolved in 5 μL of DMSO) in 100 μL of 50 mM citrate–sodium phosphate buffer at pH 5 containing the test compound (dissolved in 5 μL of DMSO/EtOH, 9:1), 0.1% Triton X-100, and 3 mM dithiothreitol. The reaction was terminated by the addition of 0.32 mL of Et_2O–MeOH-1 M citric acid (30:4:1). The resultant ether extract (100 μL) was spotted on a silica gel thin-layer plate (10 cm height; Merck, No. 1.05554.0009) and then developed at 4 °C for 20 min in $CHCl_3$–MeOH-28% ammonium hydroxide (80:20:2). The produced [^{14}C]palmitic acid and remaining [^{14}C]PEA were quantified with a Fujix bioimaging analyzer BAS1500 and the conversion rate from [^{14}C]PEA to [^{14}C]palmitic acid was calculated. Assays were performed in triplicate. The NAAA and FAAH activities in the absence of test compounds (4.3–6.7 and 0.68 nmol/min/mg of protein, respectively) were normalized to 100%, and relative enzyme activities were shown (mean values ± S.D.).

References and notes

1. Hansen, H. S.; Moesgaard, B.; Hansen, H. H.; Petersen, G. *Chem. Phys. Lipids* **2000**, *108*, 135.
2. Schmid, H. H. O.; Schmid, P. C.; Berdyshev, E. V. *Chem. Phys. Lipids* **2002**, *121*, 111.
3. Sugiura, T.; Kobayashi, Y.; Oka, S.; Waku, K. *Prostaglandins Leukot. Essent. Fatty Acid* **2002**, *66*, 173.
4. Devane, W. A.; Hanus, L.; Breuer, A.; Pertwee, R. G.; Stevenson, L. A.; Griffin, G.; Gibson, D.; Mandelbaum, A.; Etinger, A.; Mechoulam, R. *Science* **1992**, *258*, 1946.
5. Di Marzo, V.; Bisogno, T.; De Petrocellis, L.; Melck, D.; Martin, B. R. *Curr. Med. Chem.* **1999**, *6*, 721.
6. Calignano, A.; La Rana, G.; Gluffrida, A.; Piomelli, D. *Nature* **1998**, *394*, 277.
7. Lambert, D. M.; Vandevoorde, S.; Jonsson, K.-O.; Fowler, C. J. *Curr. Med. Chem.* **2002**, *9*, 663.
8. Fu, J.; Gaetani, S.; Oveisi, F.; Lo Veme, J.; Serrano, A.; Rodriguez de Fonseca, F.; Rosengarth, A.; Leucke, H.; Di Giacomo, B.; Tazia, G.; Piomelli, D. *Nature* **2003**, *425*, 90.
9. Rodriguez de Fonseca, F.; Navarro, M.; Gómez, R.; Escuredo, L.; Nava, F.; Fu, J.; Murillo-Rodríguez, E.; Giuffrida, A.; LoVerme, J.; Gaetani, S.; Kathuria, S.; Gall, C.; Piomelli, D. *Nature* **2001**, *414*, 209.
10. Ueda, N.; Tsuboi, K.; Uyama, T. *Biochim. Biophys. Acta* **2010**, *1801*, 1274.
11. Ueda, N.; Tsuboi, K.; Uyama, T. *Prog. Lipid Res.* **2010**, *49*, 299 .
12. Di Marzo, V. *Nat. Rev. Drug Disc.* **2008**, *7*, 438.
13. Ueda, N.; Kurahashi, Y.; Yamamoto, S.; Tokunaga, T. *J. Biol. Chem.* **1995**, *270*, 23823.
14. Cravatt, B. F.; Giang, D. K.; Mayfield, S. P.; Boger, D. L.; Lerner, R. A.; Gilula, N. B. *Nature* **1996**, *384*, 83.
15. McKinney, M. K.; Cravatt, B. F. *Annu. Rev. Biochem.* **2005**, *74*, 411.
16. Ueda, N.; Yamanaka, K.; Yamamoto, S. *J. Biol. Chem.* **2001**, *276*, 35552.
17. Tsuboi, K.; Sun, Y.-X.; Okamoto, Y.; Araki, N.; Tonai, T.; Ueda, N. *J. Biol. Chem.* **2005**, *280*, 11082.
18. Tsuboi, K.; Takezaki, N.; Ueda, N. *Chem. Biodivers.* **2007**, *4*, 1914.
19. Kathuria, S.; Gaetani, S.; Fegley, D.; Valino, F.; Duranti, A.; Tontini, A.; Mor, M.; Tarzia, G.; La Rana, G.; Calignano, A.; Giustino, A.; Tattoli, M.; Palmery, M.; Cuomo, V.; Piomelli, D. *Nat. Med.* **2003**, *9*, 76.

20. Vandevoorde, S.; Tsuboi, K.; Ueda, N.; Jonsson, K.-O.; Fowler, C. J.; Lambert, D. M. *J. Med. Chem.* **2003**, *46*, 4373.
21. Tsuboi, K.; Hilligsmann, C.; Vandevoorde, S.; Lambert, D. M.; Ueda, N. *Biochem. J.* **2004**, *379*, 99.
22. Solorzano, C.; Zhu, C.; Battista, N.; Astarita, G.; Lodola, A.; Rivara, S.; Mor, M.; Russo, R.; Maccarrone, M.; Antonietti, F.; Duranti, A.; Tontini, A.; Cuzzocrea, S.; Tarzia, G.; Piomelli, D. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 20966.
23. Solorzano, C.; Antonietti, F.; Duranti, A.; Tontini, A.; Rivara, S.; Lodola, A.; Vacondio, F.; Tarzia, G.; Piomelli, D.; Mor, M. *J. Med. Chem.* **2010**, *53*, 5770.
24. Saturnino, C.; Petrosino, S.; Ligreti, A.; Palladino, C.; De Martino, G.; Di Marzo, V. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1210.
25. Boros, E.; Häfeli, U. O.; Partrick, B. O.; Adam, M. J.; Orvig, C. *Bioconjugate Chem.* **2009**, *20*, 1002.
26. Mukaiyama, T.; Usui, M.; Saigo, K. *Chem. Lett.* **1975**, 1163.
27. Singh, R. S.; Mukherjee, K.; Banerjee, R.; Chaudhuri, A.; Hait, S. K.; Moulik, S. P.; Ramadas, Y.; Vijayalakshmi, A.; Rao, N. M. *Chem. Eur. J.* **2002**, *8*, 900.
28. Thompson, A. S.; Humphrey, G. R.; DeMarco, A. M.; Mathre, D. J.; Grabowski, E. J. J. *J. Org. Chem.* **1993**, *58*, 5886.
29. Huang, S. M.; Bisogno, T.; Petros, T. J.; Chang, S. Y.; Zavitsanos, P. A.; Zipkin, R. E.; Sivakumar, R.; Coop, A.; Maeda, D. Y.; De Petrocellis, L.; Burstein, S.; Di Marzo, V.; Walker, J. M. *J. Biol. Chem.* **2001**, *276*, 42639.
30. Cascio, M. G.; Minassi, A.; Ligresti, A.; Appendino, G.; Burstein, S.; Di Marzo, V. *Biochem. Biophys. Res. Commun.* **2004**, *314*, 192.
31. Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248.
32. Ueda, N.; Yamamoto, K.; Yamamoto, S.; Tokunaga, T.; Shirakawa, E.; Shinkai, H.; Ogawa, M.; Sato, T.; Kudo, I.; Inoue, K.; Takizawa, H.; Nagano, T.; Hirobe, M.; Matsuki, N.; Saito, H. *Biochim. Biophys. Acta* **1995**, *1254*, 127.