Potent Anandamide Analogs: The Effect of Changing the Length and Branching of the End Pentyl Chain

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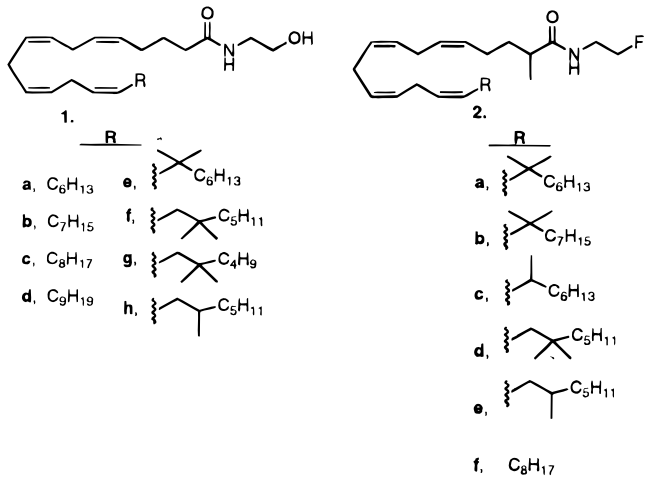
To examine the effect of changing the length and branching of the end pentyl chain (C_5H_{11}) of anandamide (AN), various analogs **1a**−**h** and **2a**−**f** were synthesized from either the known aldehyde ester **6a** or from the alcohol **6b** and tested for their pharmacological activity. A reproducible procedure was developed for the conversion of arachidonic acid to **6a** or **6b** in gram quantities (overall yield 15%). The appropriate tetraene esters **7** were prepared by carrying out a Wittig reaction, between **6a** and the ylide generated from the phosphonium salt of the appropriate alkyl halide or between the ylide of **6d** (prepared from **6a** → **6b** → **6c** → **6d**) and the appropriate alkyl aldehydes. They were then hydrolyzed to the corresponding acids and transformed into AN analogs **1** *via* their acid chlorides then treated with excess ethanolamine. α-Alkylation of esters **7** gave compounds **8** which were hydrolyzed to the corresponding acids. These acids *via* their acid chlorides and subsequent treatment with excess fluoroethylamine gave the target compounds **2**. In this way analogs **1e** and **2a**−**c** were synthesized from **6d** while all the remaining analogs were prepared from **6a**. In order to assess the optimal length of the alkyl terminus, analogs **1a**−**d** were prepared and showed moderately high affinities (18−55 nM). However analogs **1a**−**c** failed to produce significant pharmacological effects at doses up to 30 mg/kg. Analog **1d** was found to be a weak partial agonist. The reason for the lack of activity in **1a**−**c** is presently not clear. Like the THCs, the branching of the end pentyl chain in AN (**1e**−**h**) increased potency both in *in vitro* and *in vivo* activities; the dimethylheptyl (DMH) analog **1e** was the most potent in the series. Similar alkyl substitutions were carried out in the fluoro-2-methylanandamide series (**2a**−**f**), and all of these analogs had high receptor affinities (1−14 nM), the DMH analog **2a** being the most potent. With a few exceptions they showed robust pharmacological effects, and AN-like profiles. It was shown that the SAR of the end pentyl chain in AN is very similar to that of THCs. However, the magnitude of enhanced potency observed when the side chain of THC was changed from straight to branched was not observed when the end chain of AN was similarly changed.

Introduction

Since the discovery of anandamide (AN; **1**, R = C_5H_{11}), a putative endogenous ligand for the cannabinoid receptor (CB1) in the brain,[1] several groups including our own[2−11] have initiated the synthesis and biological evaluation of AN analogs. AN shares biological activities exhibited by Δ^9-THC, including decreased spontaneous motor activity, immobility, and production of hypothermia and analgesia. However, it has a faster onset and shorter duration of action than Δ^9-THC.[6] Binding studies have to be carried out in the presence of the enzyme inhibitor phenylmethylsulfonyl fluoride (PMSF) since AN is susceptible to metabolic degradation due to an amidase.[5,12,13] Since the discovery of AN, two additional brain constituents (homo-γ-linolenylethanolamide and docosatetraenylethanolamide) that are structurally related to AN and exhibit cannabinoid activity have been reported.[14] Although several analogs have been reported with a variation in the ethanolamine part of AN, only limited work has been carried out in the arachidonic acid part of AN, indicating that the presence of three double bonds or two more methylene groups had little effect on activity.[3,5] However, the activity was greatly reduced with the introduction of an additional double bond in the end pentyl chain (C17−C18).[3,5,10] This observation led Felder et al.[3] to suggest that "the end pentyl chain may play a role similar to the pentyl side chain of THC". Results from our group[9,10] have revealed that the introduction of substituents on the carbon in the 2-position of the arachidonic acid part of AN enhances metabolic stability, presumably by sterically hindering the amidase(s) responsible for AN degradation. Of this series the methyl substituent was found to be optimal, and it was found that (±)-2-methylarachidonyl-2′-fluoroethylamide (**2**, R = C_5H_{11};



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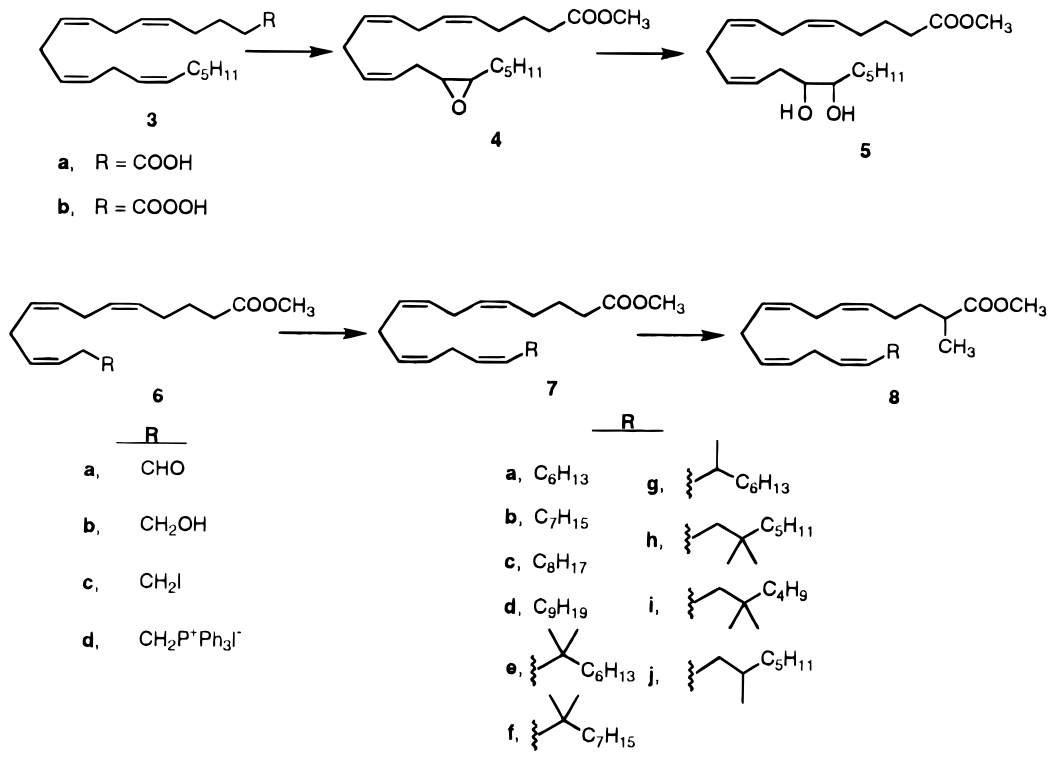
Scheme 1



O-689) was the most potent both in binding ($K_i = 5.7 \pm 2.1$ nM) and in *in vivo* activity. Additionally, our molecular modeling studies also suggested that the end pentyl chain of AN is mimicking the side chain in THCs.[15] Our studies further showed that in various AN analogs, the tetraene analogs, had an optimal pharmacophore overlay with Δ^9-THC, and the potencies were predictable by QSAR with a reasonable degree of accuracy.[15] With this background we designed compounds **1e** and **2a** as they both carry a dimethylheptyl (DMH) chain which is known to impart very potent agonist activity in THCs.[16] To test this hypothesis and lend support to our alignment strategy for AN and THCs, we synthesized compounds **1a**−**h** and **2a**−**f** and examined the effect of changing the length and branching of the end pentyl chain (C_5H_{11}) of AN.

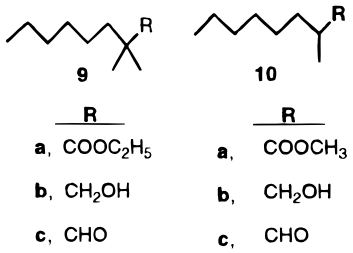
Chemistry

Our general strategy for the synthesis of compounds **1a**−**h** and **2a**−**f** is based on the conversion of the known aldehyde ester **6a**[17,18] (Scheme 1) to the appropriate tetraene esters **7**. This was achieved by carrying out a Wittig reaction between **6a** and the ylide generated from the phosphonium salt of the appropriate alkyl halide or between **6d** (prepared from **6a** by reduction → mesylation/sodium iodide → phosphonium salt) and the appropriate alkyl aldehyde. The tetraene esters **7** were then hydrolyzed to the corresponding acids[9] and transformed into AN analogs **1** via their acid chlorides and treated with excess ethanolamine as in the synthesis of AN.[1,9] The racemic fluorinated analogs **2** were synthesized from **7** by methylation in the 2-position using LDA and CH_3I in THF at −40 °C to give the esters **8** and then hydrolyzed to give the appropriate acids. These acids *via* their acid chlorides and subsequent treatment with excess fluoroethylamine gave target compounds **2**.[9,10] Thus compounds **1e**, and **2a**−**c** were synthesized from **6d** while all the remaining target compounds were prepared from **6a**. It is noteworthy that the Wittig reaction between **6a** and the ylide of the phosphonium salts to form **7e**−**g** failed under a variety of conditions.

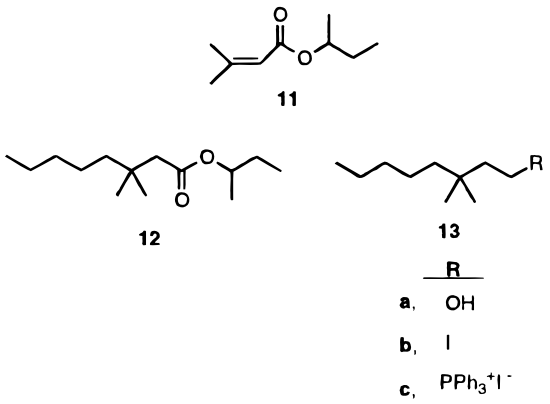
To synthesize the above compounds we required a large supply of the aldehyde **6a**. Its synthesis was reported by Corey et al.[17] and Manna et al.[18] who both used essentially the same procedure. In 1979 Corey et al.[19] reported a procedure using 90% H_2O_2 for the selective epoxidation of arachidonic acid **3a** to give **4** via the peroxy acid **3b**. As 90% hydrogen peroxide is hazardous to use and no longer commercially available, we developed a modified procedure for the synthesis of **4** using 50% hydrogen peroxide. We also tried the modified procedure described by Perrier et al.[20] using 30% aqueous H_2O_2 but in our hands it could not be scaled up successfully.

In our modified procedure, the acid chloride of arachidonic acid (oxalyl chloride in benzene at 0 °C → 30 °C, 24 h) was treated with 0.5 equiv of pyridine in dry THF at 0 °C, followed by a solution of $LiOH \cdot H_2O$ in 50% H_2O_2. After stirring for 20 min, the reaction was quenched with water and then extracted with CH_2Cl_2. The resulting solution was left standing over Na_2SO_4 for 1.5 to 3 h while the peracid **3b** rearranged to the epoxide acid (followed by TLC). It was isolated as the methyl ester **4** (diazomethane/ether at 0 °C) after chromatography. This procedure gave the epoxide **4** in reproducible yields of 30−35% from **3a** in addition to 50% recovered methyl arachidonate. Conversion of **4** to the diol **5** (53%) was achieved with 1.2 M solution of $HClO_4$ in THF by stirring at 30 °C for 16 h. Oxidation with lead tetraacetate/CH_2Cl_2 at −18 °C for 0.5 h gave the unstable aldehyde **6a**, which was quickly filtered through a thin pad of Celite/silica gel and washed with hexanes. After removal of the solvent and drying *in*

vacuo the aldehyde **6a** was either used as such for the Wittig reaction or reduced to the alcohol with $NaBH_4$ in methanol to form **6b** (43%).



For the synthesis of **7a**−**d**, the appropriate alkyl bromides (commercially available) were refluxed in xylene with triphenylphosphine to form the phosphonium salts[21] which on treatment with butyllithium/hexane in THF at −78 °C formed the ylide. Further treatment with the aldehyde **6a** in a Wittig reaction[22] gave the tetraenes **7**. For the synthesis of **7h** the phosphonium salt **13c** was prepared from **11** which was synthesized from 3,3-dimethylacrylic acid and *sec*-butanol.[23] A Grignard reaction of **11** in the presence of CuCl and trimethylsilyl chloride at 0 °C with *n*-pentylmagnesium chloride gave the ester **12** (97%).[24] Reduction with LAH formed the alcohol **13a** which on treatment with triethylamine/mesyl chloride[25] followed by treatment with tetrabutylammonium iodide gave **13b**.



The phosphonium salt **13c** was similarly prepared as described above and then condensed as the ylide with aldehyde **6a** to form **7h**. Compound **7i** was synthesized in the same fashion. For the synthesis of **7j**, the same sequence as described for **7h** was used except that 3-methylacrylic acid was used in place of 3,3-dimethylacrylic acid.

Compounds **7e** to **7g** were synthesized by treatment of the ylide from **6d** and the appropriate aldehydes. The phosphonium salt **6d** was prepared from **6b** via **6c** following the same sequence as described for **13c**. For the synthesis of aldehyde **9c**, ethyl isobutyrate was treated with LDA followed by *n*-bromohexane to form the ester **9a**. Reduction with LAH converted **9a** to **9b**, which on oxidation with pyridinium chlorochromate (PCC)[26] gave the aldehyde **9c**. Wittig reaction with this aldehyde and the ylide of **6d** furnished **7e**. The homolog **7f** was similarly prepared from 2,2-dimethylnonanal. The synthesis of **7g** was achieved in a similar way. Methyl caprylate was monoalkylated to give **10a** which was then transformed to the aldehyde **10c** as in the case of **9c**. A Wittig reaction with the ylide of **6d** furnished **7g**. Improved yields (~70%) were obtained in the Wittig reaction when lithiohexamethyldisilazane (LiHMDS) was used in a 3:2 HMPA/THF mixture at −78 °C and a solution of **6d** in THF was added at −78 °C to form the ylide[22] (see Experimental Section).

In summary, arachidonic acid was converted to the aldehyde ester **6a** or the alcohol **6b** in an overall yield of 15% (based on recovered starting material). The yields in the Wittig reactions varied from 30−70%.

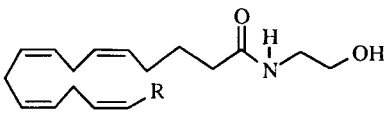
Pharmacology and Discussion of Results

In previous studies,[6,10] we have demonstrated that Δ^9-THC binds to the CB1 receptor with a K_d of 41 nM and is very effective in the four behavioral tests presented in Table 1. Anandamide (AN) is not effective in competing for binding to the CB1 receptor unless the enzyme inhibitor, PMSF, is added. In the presence of PMSF, AN binds to the receptor with an affinity approximately half that of Δ^9-THC. AN is also effective in all four mouse pharmacological assays, although it is considerably less potent than Δ^9-THC. In an effort to retard the enzymatic hydrolysis, a methyl group was added at C-2 in a fluoro derivative of AN to form fluoro-2-methylanandamide (F-2-Me-AN) as depicted in structure **2** (Table 1). As shown in Table 1, F-2-Me-AN was capable of binding to the CB1 receptor either in the presence or absence of PMSF.[10] Despite the dramatic increase in binding affinity that occurred with the addition of this C-2 methyl, pharmacological potency between AN and F-2-Me-AN was similar for antinociceptive activity and only fourfold different for spontaneous activity. However it showed the expected increase in potency for hypothermia (9-fold) and ring immobility (12-fold).

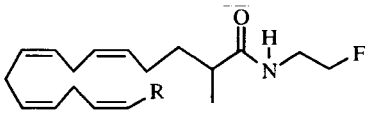
In order to assess the optimal length of the alkyl terminus to AN, analogs **1a**−**d** were prepared. Additions of one and two methylenes to AN resulted in **1a** and **1b**, respectively, which failed to bind effectively in the absence of PMSF. On the other hand, additions of three and four methylenes produced analogs **1c** and **1d**, respectively, that exhibited much greater affinity and metabolic stability as evidenced by their binding affinities with and without PMSF. In the presence of PMSF, all four of these analogs bound with moderately high affinities in the range of 18−55 nM. Based upon these affinities, it was anticipated that all four analogs would be pharmacologically more potent than AN. Analogs **1a**−**c** failed to produce significant pharmacological effects at doses up to 30 mg/kg, with the exception of analog **1b** that was a partial agonist in decreasing spontaneous activity. Analog **1d** was reasonably potent in reducing spontaneous activity and in producing antinociception and ring immobility; however, it acted only as a partial agonist with maximal effects of 57, 50, and 42% in the respective assays. It was also unique in that it failed to produce hypothermia at doses up to 30 mg/kg.

The addition of a dimethylheptyl (DMH) side chain to Δ^9-THC dramatically enhanced pharmacological activity from 10−75 fold, depending upon the pharmacological assay.[27] A similar structural change in AN resulted in analog **1e** that had a receptor affinity 12 times greater than that of AN. It was also more potent than AN in producing hypoactivity (8 times), antinociception (3 times), hypothermia (3 times) and ring

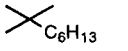
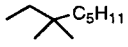
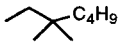
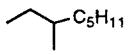
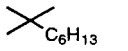
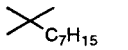
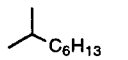
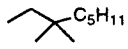
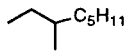
Table 1. Receptor Affinity and Pharmacological Effects of Analogs[a]



1



2

#	Structure	Ki	Ki (PMSF)	SA	TF	RT	RI
Δ^9-THC	C_5H_{11}	41±2[b]	NT[c]	1.0[d]	1.4[d]	1.4[d]	1.5[d]
AN (**1**)	C_5H_{11}	5400±1600[c]	89±10[e]	17.9[d]	6.20[d]	26.5[d]	19.1[d]
F-2-Me-AN(**2**)	C_5H_{11}	15±6[e]	5.7±2.1[e]	4.78[e]	6.99[e]	2.73	1.60
1a	C_6H_{13}	3060	24±1.4	IA[f]	IA	IA	IA
1b	C_7H_{15}	1780±650	55±6.0	20.4[g]	IA	IA	IA
1c	C_8H_{17}	156±59	18±1.7	IA	IA	NT[c]	NT[c]
1d	C_9H_{19}	79±21	25±2.3	7.35[g]	14.4[g]	IA	5.13[g]
1e		ND	7.0±0.6	2.17	2.16	7.49	1.77
1f		39±2.1	19±2.4	3.36	57.3[g]	9.38	5.05
1g		382±39	48±4	44.1[g]	IA	IA	IA
1h		51±19	11±0.6	6.22	5.48	12.2	5.29
2a		ND	1.0±0.1	3.48	0.86	3.60	3.11
2b		ND	14±3.1	20.3[g]	12.8[g]	IA	33.3[g]
2c		ND	4.8±1.7	1.15	1.30	0.08	2.22
2d		ND	7.7±1.1	3.14	4.12	76.2	11.4[g]
2e		ND	3.3±0.5	2.09	4.08	0.24	2.57
2f	C_8H_{17}	ND	8.2±0.2	3.52	15.3	IA	6.37

[a] K_i's (nM) were determined in the presence and absence of PMSF and are expressed as means ± SE for at least three experiments. The pharmacological measures included inhibition of spontaneous activity (SA), antinociception as measured by the tail-flick response (TF), hypothermia as changes in rectal temperature (RT), and ring immobility (RI). The pharmacological data are expressed as the ED_{50} (mg/kg). Inactive (IA); not determined (ND). [b] Reported previously.[32] [c] Not tested. [d] Reported previously.[6] [e] Reported previously.[10] [f] Inactive because maximal effects at 30 mg/kg were either less than 30% or, in the case of hypothermic, less than 1 °C. [g] Partial agonist. Maximum effect of 40−60% (30−40% for immobility) at 30 mg/kg.

immobility (11 times). **1e** is also unique in that it produced 84% immobility making it more efficacious than Δ^9-THC (60% maximal effect). Moving the dimethyl groups to the adjacent C atom to form **1f** had relatively little effect on activity since both **1e** and **1f** were active in all four tests. The fact that subtle changes in the terminal alkyl position can be influential is amply illustrated by reducing the side chain of **1f** by one methylene to produce **1g**. Analog **1g** was a weak partial agonist with regard to reduction of spontaneous activity and was inactive in the other three tests. We were able to evaluate a dose of 100 mg/kg in SA and TF and no greater effects were obtained, supporting our contention of partial agonistic effects. Insufficient quantities precluded testing this higher dose in the other two pharmacological measures. **1h**, the monomethyl derivative of **1f**, has a profile quite similar to that of **1f**.

Alkyl substitutions similar to those described above were also prepared in the fluoro-2-methylanandamide series, and all of these analogs had high receptor affinity. The highest receptor affinity was exhibited by the dimethylheptyl derivative **2a** and the least affinity by the dimethyloctyl analog **2b**. All of these compounds produced robust pharmacological effects with a few exceptions. **2b** was only a partial agonist and in analog **2d**, where the *gem*-dimethyl is moved to the adjacent carbon, a similar profile (i.e. active in all four tests) was obtained in pharmacological measures as found in **1f** except that partial agonism was found in the ring immobility test rather than in the antinociceptive test. This is interesting, since it could provide a lead for separation of pharmacological activities. These analogs were similar to AN in that they failed to produce hypothermia greater than 3.0 °C. However, their potencies were not always consistent with potencies in the other pharmacological assays. For example, analogs **2c** and **2e**, which are diastereomeric mixtures, were considerably more potent in reducing rectal temperature than in producing the other effects, whereas the degree of hypothermia caused by **2d** and **2f** was considerably less than their other effects.

These findings provide additional evidence that AN and Δ^9-THC share common pharmacological properties

in that these analogs bound to the cannabinoid receptor and produced pharmacological effects in all four mouse pharmacological procedures. These data also reveal some important pharmacological differences between AN and Δ^9-THC. It is unclear why several of the analogs in the **1** series failed to produce pharmacological effects despite relatively good receptor affinity. The receptor binding studies demonstrate that these analogs are metabolized by brain tissue, as it is for anandamide. While it is possible that they are degraded to such an extent *in vivo* that they are inactive, it seems unlikely that metabolism would differ between the potent dimethylheptyl analog **1e** and the other analogs. In addition, the pharmacological evaluations were made rapidly after iv administration which has been shown to effectively measure AN's effects as well as a wide range of AN analogs that are not metabolically stable.[10] We cannot rule out the possibility that the inactive analogs in series **1** lack intrinsic activity or they may have some antagonist activity. On the other hand, analogs in the metabolically stable **2** series were quite potent in most of the pharmacological assays. It is intriguing that the alterations in the putative "side-chain" did not have the anticipated impact on pharmacological potency. These findings reinforce the notion that AN's interactions with the cannabinoid receptor is not identical to that of Δ^9-THC, in consideration of the weak hypothermic effects of the anandamides.

Conclusions

We conclude that the SAR of the end pentyl chain in AN is very similar to that of THCs with certain exceptions. In anandamides like the THCs, increasing the length of the end pentyl chain increases the binding affinity; however, unlike the THCs, the *in vivo* activity does not parallel the binding affinities. Additionally, increasing the chain length in anandamides appears to impart some metabolic stability toward amidases. Branching the chain enhances both binding affinity and *in vivo* activity as in THCs. However, by branching the chain, the effect in pharmacological measures is not as dramatic in the AN series as in THCs. This dissimilarity might be a reflection of the differences in chemical structure and in the biodisposition of THCs and anandamides.

Experimental Section

^{1}H NMR spectra were recorded on either a Bruker 100 or a Varian XL400 spectrophotometer using $CDCl_3$ as the solvent with trimethylsilane as an internal standard. Thin layer chromatography (TLC) was carried out on Baker Si 250F plates. Visualization was accomplished with either iodine vapor, UV exposure, or treatment with phosphomolybdic acid (PMA). Flash chromatography was carried out on EM Science Silica Gel 60. Elemental analyses were performed by Atlantic Microlab, Atlanta, GA. The purity of the products on which the high-resolution mass spectral data are reported were determined by GC, TLC, and ^{1}H NMR analysis. The analyses by gas chromatography (GC) were performed on a Perkin Elmer 8500 instrument equipped with a 25 m fused silica capillary column, 0.53 mm i.d. with 0.25 μm film thickness (007 Methyl Phenyl (5%) Silicone; Quadrex Corp.). For anandamide analogs **1a**−**h** the column temperature was programmed to increase from 60 to 280 °C at a rate of 20 °C/min. For analogs in the **2** series, the temperature ranged from 140 to 280 °C at a rate of 20 °C/min. The injector and detector temperatures were set at 300 °C for both. The GC purity is indicated in parenthesis of samples kept as stock solutions (ethanol) at −20 °C and analyzed after about 2 years in the **1** series and 1 year in the **2** series. All reactions were carried out under nitrogen.

Methyl 14,15-Epoxyarachidonate (4). To a solution of arachidonic acid (6.59 g, 21.6 mmol) in anhydrous benzene (60 mL) cooled to 0 °C was added oxalyl chloride (3.78 mL, 43.3 mmol). The mixture was stirred overnight allowing it to come to room temperature. Evidence of the formation of the acid chloride was determined by IR (C═O, 1790 cm^{-1}). The solvent was removed *in vacuo* followed by addition of benzene and evaporation on a rotary evaporator to remove traces of oxalyl chloride. To a solution of this acid chloride in anhyd THF (90 mL) was added pyridine (0.87 mL, 10.8 mmol), and the mixture was stirred for 10 min at 0 °C. A solution of $LiOH\cdot H_2O$ (0.91 g, 21.6 mmol) in 50% H_2O_2 (29.4 mL) was added to the mixture in one portion at 0 °C, and the mixture was stirred for 20 min. The reaction was quenched with pH 7 buffer (50 mL) and brine (50 mL) and then extracted with CH_2Cl_2 (50 mL). The aqueous layer was saturated with NaCl and extracted further with CH_2Cl_2 (4 × 50 mL). The combined organic extracts were washed with brine (50 mL) and dried (Na_2SO_4). The organic extracts were dried for 1.5 to 3 h while following the formation of the epoxy acid by TLC (30% ethyl acetate/hexanes; $R_f = 0.40$ for epoxy acid). The solvent was decanted, and the drying agent was washed with CH_2Cl_2. After removal of the solvent *in vacuo*, the resultant oil was taken up in anhyd ether (100 mL) and cooled to 0 °C. The solution was then treated with an excess of diazomethane solution in ether and stirred for 15 min. After evaporation of the unreacted diazomethane in the fume hood at room temperature, the solvent was removed *in vacuo* to yield a yellow oil which was purified by flash chromatography (225 g SiO_2, 5% ethyl acetate/hexanes): Yield 2.30 g (32%); NMR δ 0.89 (t, J = 6.6 Hz, 3H), 1.27−1.58 (m, 8H), 1.63−1.85 (m, 2H), 2.02−2.43 (m, 6H), 2.75−3.00 (m, 6H), 3.68 (s, 3H), 5.33−5.58 (m, 6H).

Methyl 14,15-Dihydroxyarachidonate (5). To a solution of epoxide **4** (1.22 g, 3.66 mmol) in THF (60 mL) were added H_2O (30 mL) and 1.2 M $HClO_4$ (15 mL). The reaction was stirred overnight at room temperature and quenched with pH 7 buffer (50 mL). Ethyl acetate (30 mL) was added, and the layers were separated. The aqueous layer was saturated with NaCl and extracted with ethyl acetate (3 × 30 mL). The combined organic extracts were washed with brine (30 mL) and dried ($MgSO_4$). Removal of the solvent *in vacuo* gave a brown oil which was purified by flash chromatography (100 g SiO_2, 20% ethyl acetate/hexanes) to yield 0.68 g (53%) of a colorless oil: NMR δ 0.89 (t, J = 6.6 Hz, 3H), 1.20−1.60 (m, 8H), 1.63−1.85 (m, 2H), 2.05−2.40 (m, 6H), 2.75−2.92 (m, 4H), 3.45−3.58 (m, 2H), 3.68 (s, 3H), 5.21−5.71 (m, 6H).

Methyl 14-Hydroxy-5,8,11-*all-cis*-tetradecatrienoate (6b). To a stirred solution of **5** (1.12 g, 3.17 mmol) in CH_2Cl_2 (20 mL) at −18 °C (ice/salt bath) was added a solution of lead(IV) acetate (1.42 g, 3.21 mmol) in CH_2Cl_2 (20 mL). The solution was stirred for 30 min at −18 °C. The reaction was filtered through a thin pad of Celite/silica and the filter cake was washed with anhyd hexanes (5 × 10 mL). The solvent was removed *in vacuo*, and the unstable aldehyde **6a** was dissolved in MeOH (12 mL) and cooled to 0 °C. $NaBH_4$ (0.14 g, 3.8 mmol) was added in one portion, and the reaction was stirred for 10 min. The reaction was quenched with H_2O (20 mL), and most of the MeOH was removed *in vacuo*. The aqueous layer was saturated with NaCl and extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with brine (15 mL) and dried ($MgSO_4$). Removal of the solvent *in vacuo* gave a cloudy oil which was purified by flash chromatography (120 g SiO_2, 15% ethyl acetate/hexanes) to yield 0.34 g (43%) of alchol **6b** as a yellow oil: NMR δ 1.62−1.82 (m, 3H), 2.02−2.50 (m, 6H), 2.75−2.90 (m, 4H), 3.59−3.72 (m, 5H), 5.32−5.56 (m, 6H).

Methyl 14-Iodo-5,8,11-*all-cis*-tetradecatrienoate (6c).[22] To a stirred solution of alcohol **6b** (0.19 g, 0.75 mmol) and triethylamine (0.17 mL, 1.2 mmol) in CH_2Cl_2 (1.5 mL) cooled to −20 °C was added methanesulfonyl chloride (0.08 mL, 0.97 mmol). The reaction was stirred for 15 min at −20 °C, and then H_2O (1.25 mL) was added and the reaction was allowed to warm to room temperature. The reaction was diluted with

$CHCl_3$ (10 mL), and the layers were separated. The organic phase was dried ($MgSO_4$), and the solvent was removed *in vacuo*. A solution of the crude mesylate and sodium iodide (0.45 g, 3.0 mmol) dissolved in acetone (5 mL) was refluxed under nitrogen for 1.5 h. The cooled reaction was diluted with hexanes (20 mL) and filtered through a pad of Celite. The solvent was removed *in vacuo*. The resultant yellow oil was dissolved in 10% ethyl acetate/hexanes and filtered through a bed of silica gel. The solvent was removed *in vacuo* to yield the product (0.26 g, 95%) as a colorless oil which was used without further purification: NMR δ 1.61−1.88 (m, 2H), 2.03−2.40 (m, 6H), 2.56−2.85 (m, 4H), 3.15 (m, 2H), 3.67 (s, 3H), 5.29−5.48 (m, 6H).

Methyl 5,8,11-*all-cis*-Tetradecatrienoate-14-triphenylphosphonium Iodide (6d).[22] A solution of **6c** (0.26 g, 0.71 mmol) and triphenylphosphine (0.21 g, 0.82 mmol) in acetonitrile (3 mL) was heated at reflux overnight. While the reaction was still warm, the condenser was removed and a stream of N_2 was blown over the reaction until the solvent evaporated. After cooling, the excess Ph_3P was removed by repeated washing and decanting with hexanes/benzene (1/1, 5 mL). The solvent was decanted, and the procedure was repeated. The remaining solvent was removed *in vacuo*, and the orange gum was heated in a vacuum oven overnight at 90 °C and used directly in the Wittig reaction: Yield 0.42 g (95%).

Methyl 16,16-Dimethyl-5,8,11,14-*all-cis*-docosatetraenoate (7e).[22] To a solution of HMDS (0.14 mL, 0.68 mmol) and HMPA (0.47 mL, 2.7 mmol) in THF (1.9 mL) at −78 °C was added *n*-butyllithium (2.2 M in hexanes, 0.29 mL, 0.64 mmol), and the reaction was warmed for 3 min to 0 °C followed by cooling back to −78 °C. A solution of phosphonium iodide **6d** (0.42 g, 0.68 mmol) in THF (2.5 mL) was added dropwise to the LiHMDS solution, and the reaction was stirred for 30 min at −78 °C. Then a solution of **9c** (0.15 g, 0.95 mmol) in THF (0.75 mL) was added to the ylide and the dry ice/acetone bath was removed. The reaction was allowed to warm to room temperature and stirred for 1 h. The reaction was diluted with hexanes (12 mL), and the precipitate was allowed to settle. The solvent was decanted, and the residue was extracted with another portion of hexanes (6 mL). The combined organic extracts were evaporated to give the crude product which was purified by flash chromatography (60 g SiO_2, gradient 0% to 2% ethyl acetate/hexanes): Yield 0.18 g (70%); NMR δ 0.88 (t, J = 5.5 Hz, 3H), 1.09 (s, 6H), 1.26 (br s, 10H), 1.60−1.85 (m, 2H), 2.02−2.45 (m, 6H), 2.75−2.98 (m, 6H), 3.66 (s, 3H), 5.13−5.41 (m, 8H).

Ethyl 2,2-Dimethyloctanoate (9a). To a solution of LDA (2.0 M in THF/ethylbenzene/heptane, 13.0 mL, 26 mmol) in THF (60 mL) at −45 °C was added ethyl isobutyrate (1.01 g, 8.7 mmol), and the reaction was maintained at −45 °C for 45 min with stirring. The reaction was then cooled to −60 °C, and *n*-bromohexane (14.38 g, 87 mmol) was added in one batch. The reaction was stirred for 3.5 h, slowly coming to room temperature, followed by quenching with H_2O (50 mL) and diluting with ether (100 mL). The layers were separated, the aqueous layer was saturated with NaCl and extracted with 3 × 25 mL of ether, and the combined ether extracts were washed with 1 N HCl (15 mL) and brine (2 × 20 mL), and dried ($MgSO_4$). Removal of the solvent *in vacuo* gave an oil which was fractionally distilled at reduced pressure to give the product as a colorless oil: Yield 1.09 g (63%); bp 64−66 °C /1.3 mm; NMR δ 0.88 (t, J = 6.2 Hz, 3H), 1.15 (s, 6H), 1.17−1.49 (m, 13H), 4.11 (q, J = 7.1 Hz, 3H).

2,2-Dimethyloctanol (9b). To a suspension of LAH (0.62 g, 16.3 mmol) in THF (15 mL) cooled to 0 °C was added dropwise a solution of ester **9a** in THF (5 mL). The reaction was stirred overnight allowing it to come slowly to room temperature. The reaction was quenched by the sequential addition of water (0.61 mL), 15% sodium hydroxide solution (0.61 mL), and water (1.83 mL). The white suspension was filtered through a pad of Celite, and the solid was washed with ether. The filtrate was washed with H_2O (2 × 20 mL), brine (10 mL), and dried ($MgSO_4$). The solvent was removed *in vacuo* to give the product as a colorless oil which was used directly: Yield 0.78 g (91%); NMR δ 0.85−0.93 (m, 9H), 1.23−1.54 (m, 11H), 3.30 (s, 2H).

2,2-Dimethyloctanal (9c).[26] To a suspension of PCC (1.54 g, 7.1 mmol) in CH_2Cl_2 (10 mL) was added dropwise **9b** (0.75 g, 4.8 mmol) in CH_2Cl_2 (5 mL). The reaction was stirred at room temperature for 2 h followed by diluting with ether (35 mL) and decanting the solvent. The black residue was washed with ether (3 × 10 mL) followed by decanting the ether. The combined ether washings were filtered through a bed of Florisil, and the solvent was removed *in vacuo* to give the product as a yellow/green oil which was used directly: Yield 0.65 g (87%); NMR δ 0.88 (t, J = 6.1 Hz, 3H), 1.04 (s, 6H), 1.20−1.50 (m, 10H), 9.44 (s, 1H).

Methyl 2-Methylcaprylate (10a). The product was prepared according to the method described for **9a** and was used without futher purification: Yield 97%; NMR δ 0.88 (t, J = 6.0 Hz, 3H), 1.16 (d, J = 7.2 Hz, 3H), 1.20−1.72 (m, 10H), 3.67 (s, 3H).

2-Methyloctanol (10b). The product was prepared according to the method described for **9b**: Yield 83%; NMR δ 0.75−0.99 (m, 6H), 1.15−1.70 (m, 12H), 3.30−3.60 (m, 2H).

2-Methyloctanal (10c). The product was prepared according to the method described for **9c**: Yield 83%; NMR δ 0.89 (t, J = 5.5 Hz, 3H), 1.09 (d, J = 7.0 Hz, 3H), 1.16−1.70 (m, 10H), 2.25−2.45 (m, 1H), 9.61 (d, J = 1.9 Hz, 1H).

2,2-Dimethylnonanal. The product was prepared according to the method described for **9c**: Yield 56%; NMR δ 0.88 (t, J = 6.0 Hz, 3H), 1.05 (s, 6H), 1.15−1.45 (m, 12H), 9.44 (s, 1H).

(±)-Methyl 2,16,16-Trimethyl-5,8,11,14-*all-cis*-docosatetraenoate (8e). The product was prepared according to the alkylation method described for **9a** using MeI in the place of *n*-bromohexane. The product was purified by flash chromatography (0.5% ethyl acetate/hexanes): Yield 66%; NMR δ 0.88 (t, J = 6.4 Hz, 3H), 1.09 (s, 6H), 1.16 (d, J = 7.0 Hz, 3H), 1.27 (br s, 10H), 1.40−2.20 (m, 4H), 2.32 (m, 1H), 2.80−2.98 (m, 6H), 3.67 (s, 3H), 5.13−5.42 (m, 8H).

(±)-2,16,16-Trimethyl-5,8,11,14-*all-cis*-docosatetraenoic Acid. To a solution of **8e** (0.07 g, 0.18 mmol) in MeOH (9 mL) and H_2O (3 mL) was added $LiOH·H_2O$ (0.05 g, 1.27 mmol), and the reaction was heated to 55 °C overnight. After cooling, the reaction was diluted with H_2O (10 mL) and ether (10 mL). The layers were separated, and the aqueous layer was acidified with 1 N HCl and saturated with NaCl. The aqueous layer was extracted with 3 × 10 mL ether, and the combined organic extracts were washed with brine (5 mL) and dried ($MgSO_4$). The solvent was removed *in vacuo*, and the resultant oil was dried *in vacuo* and used directly: Yield 0.06 g (94%); NMR δ 0.88 (t, J = 6.4 Hz, 3H), 1.09 (s, 6H), 1.16 (d, J = 7.0 Hz, 3H), 1.26 (br s, 10H), 1.37−2.55 (m, 5H), 2.81−2.98 (m, 6H), 5.13−5.42 (m, 8H), 10.11 (br s, 1H).

(±)-2,16,16-Trimethyl-5,8,11,14-*all-cis*-docosatetraenoyl-2′-fluoroethylamide (2a). To a solution of 2,16,16-trimethyl-5,8,11,14-*all-cis*-docosatetraenoic acid (0.06 g, 0.17 mmol) in benzene (5 mL) cooled to 0 °C were added oxalyl chloride (0.03 mL, 0.34 mmol) and a drop of DMF. The reaction was slowly brought to room temperature while stirring for 2 h. The solvent was removed *in vacuo*, and the oil was treated twice with fresh benzene (10 mL) and evaporated to remove moisture. A solution of the acid chloride in CH_2Cl_2 (3 mL) was added to a solution of fluoroethylamine hydrochloride (0.17 g, 1.71 mmol) in CH_2Cl_2 (10 mL) pretreated (stirred for 15 min at 0 °C to make the free base) with triethylamine (0.24 mL, 1.73 mmol) at 0 °C. The reaction was stirred for 30 min at 0 °C followed by quenching with brine (10 mL). The reaction was diluted with CH_2Cl_2 (10 mL), and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL), the combined organic extracts were dried ($MgSO_4$), and the solvent was removed *in vacuo*. The crude product was purified by flash chromatography (15 g of SiO_2, 15% ethyl acetate/hexanes) to give the product as a yellow oil: Yield 0.05 g (65%); NMR δ 0.88 (t, J = 4.0 Hz, 3H), 1.09 (s, 6H), 1.16 (d, J = 6.9 Hz, 3H), 1.26 (br s, 10H), 1.39−2.34 (m, 5H), 2.72−2.98 (m, 6H), 3.58 (ddt, J = 29.0, 5.0, 4.5 Hz, 2H), 4.49 (dt, J = 47.5, 4.6 Hz, 2H), 5.13−5.53 (m, 8H), 5.82 (br s, 1H); HRMS calcd for $C_{27}H_{47}FNO$ (M + 1), 420.3641; found 420.3591; GC, t_R 6.13 min (97%).

Hydrolysis of 7e. 16,16-Dimethyl-5,8,11,14-*all-cis*-docosatetraenoic Acid. The product was prepared according to the method described for 2,16,16-trimethyl-5,8,11,14-*all-cis*-docosatetraenoic acid: Yield 99%; NMR δ 0.88 (t, J = 6.4 Hz, 3H), 1.09 (s, 6H), 1.26 (br s, 10H), 1.63−1.86 (m, 2H), 2.04−2.44 (m, 4H), 2.81−2.98 (m, 6H), 5.19−5.53 (m, 8H), 10.20 (br s, 1H).

16,16-Dimethyl-5,8,11,14-*all-cis*-docosatetraenoylethanolamide (1e). To a solution of 16,16-dimethyl-5,8,11,14-*all-cis*-docosatetraenoic acid (0.14 g, 0.38 mmol) in benzene (4 mL) cooled to 0 °C were added oxalyl chloride (0.07 mL, 0.75 mmol) and DMF (0.0029 mL, 0.04 mmol). The reaction was slowly brought to room temperature while stirring for 2 h. The solvent was removed *in vacuo*, and the oil was treated twice with fresh benzene (10 mL) and evaporated to remove moisture. A solution of the acid chloride in CH_2Cl_2 (2 mL) was added to a solution of ethanolamine (0.27 mL, 3.77 mmol) in CH_2Cl_2 (2 mL) at 0 °C. The reaction was stirred for 10 min and poured into H_2O (10 mL). The layers were separated, and the aqueous layer was saturated with NaCl followed by further extractions with CH_2Cl_2 (3 × 10 mL). The combined organic extracts were washed with brine (10 mL) and dried (Na_2SO_4). After removal of the solvent *in vacuo*, the crude product was dissolved in 10% MeOH/$CHCl_3$ and passed through a pad of silica gel. The solvent was removed *in vacuo*, and the oil was taken up in pentane and filtered through a tightly packed cotton plug. Removal of the solvent gave pure product as a yellow oil: Yield 0.14 g (93%); NMR δ 0.88 (t, J = 5.4 Hz, 3H), 1.09 (s, 6H), 1.27 (br s, 10H), 1.62−1.86 (m, 2H), 2.03−2.30 (m, 4H), 2.68−2.98 (m, 6H), 3.40 (dt, J = 4.0, 6.3 Hz, 2H), 3.71 (t, J = 5.0 Hz, 2H), 5.13−5.41 (m, 8H), 6.19 (br s, 1H). Anal. ($C_{26}H_{45}NO_2 \cdot 0.2H_2O$) C, H, N.

***sec*-Butyl 3,3-Dimethylheptanoate (12).**[24] *sec*-Butyl 3,3-dimethylacrylate (2.0 g, 12.80 mmol), prepared from 3,3-dimethylacrylic acid and 2-methylpropyl alcohol according to a literature procedure,[23] was dissolved in THF (5 mL) and treated with solid CuCl (0.039 g, 0.39 mmol, 0.031 equiv). Trimethylsilyl chloride (1.95 mL, 15.4 mmol, 1.2 equiv) was added dropwise *via* a syringe and the reaction mixture cooled to 0 °C in an ice bath.

n-Pentylmagnesium chloride (6.7 mL of a 2.0 M solution in ether; 13.4 mmol, 1.05 equiv) was added dropwise *via* a syringe, and the reaction was stirred, allowing it to warm to room temperature over 3 h. The reaction was quenched by the addition of sat. NH_4Cl solution (5 mL) and filtered, and the product was extracted with ether. After drying, the ether was evaporated to yield the product as an oil (2.83 g, 97%); NMR δ 0.85−1.70 (m, 19H), 1.00 (s, 6H), 2.20 (s, 2H), 4.75−4.95 (m, 1H).

3,3-Dimethyloctanol (13a). Lithium aluminum hydride (7.30 g, 192 mmol, 3.0 equiv) was placed in a dry flask cooled in an ice bath. Dry THF (80 mL) was added via a cannula followed by the dropwise addition of a solution of **12** in THF (40 mL, 64 mmol). The reaction mixture was allowed to stir overnight, and after cooling it was quenched cautiously by the sequential addition of water (16 mL), 15% sodium hydroxide solution (16 mL), and water (32 mL). After stirring for 1 h, the mixture was filtered, and the precipitate was triturated with warm THF. The THF washings were combined with the filtrate and then concentrated. The product was partitioned between brine and ether, and after separating the layers, the organic layer was dried. After evaporation the product was isolated as an oil (9.77 g, 96%); NMR δ 0.90 (s, 6H), 0.90 (t, J = 5.0 Hz, 3H), 1.20 (br s, 8H), 1.40−1.60 (m, 3H), 3.70 (t, J = 8.5 Hz, 2H).

3,3-Dimethyl-1-iodooctane (13b).[25] Compound **13a** (9.77 g, 61.7 mmol) was dissolved in CH_2Cl_2 (150 mL), cooled to 0 °C, and treated dropwise with an excess of triethylamine (1.2 mL, 92.6 mmol). A solution of methanesulfonyl chloride (5.27 mL, 68.0 mmol, 1.1 equiv) in CH_2Cl_2 (150 mL) was added via a syringe, and the reaction mixture was stirred 1 h, allowing it to warm to room temprature. The mesylate was isolated by quenching with brine and extracting into CH_2Cl_2. It was filtered and evaporated to an oil. The product was taken into hexanes, filtered through Celite, and evaporated, and the residue was treated with dry benzene and again evaporated to remove water. The residual oil was then dissolved in benzene and refluxed with excess tetra-*n*-butylammonium iodide (45.0 g, 123 mmol, 2.0 equiv) for 1.5 h. The reaction mixture was then filtered and evaporated, and the residue was taken up in pentane. It was passed through a plug of silica and evaporated to leave the product as an oil (10.32 g, 62%); NMR δ 1.86 (s, 6H), 1.90 (t, J = 7.5 Hz, 3H), 1.30 (br s, 8H), 1.80−2.00 (m, 2H), 3.05−3.25 (m, 2H).

3,3-Dimethyloctyltriphenylphosphonium Iodide (13c).[21] Triphenylphosphine (13.8 g, 52.7 mmol, 1.4 equiv) was dissolved in xylene (150 mL) with **13b** (10.2 g, 38.1 mmol) and refluxed overnight. The xylene was decanted from the oil and the residue was covered with ether and the flask was put in a sonic bath for 1 h. After decanting the ether to remove triphenylphosphine oxide, the residue was similarly treated again. The residue was the desired product isolated as a hygroscopic solid (4.28 g, 21%); mp 66.1−68.5 °C; NMR δ 0.85 (t, J = 6.9 Hz, 3H), 0.97 (s, 6H), 1.10−1.70 (m, 10 H), 3.20−3.60 (m, 2H), 7.65−7.95 (m, 15H).

Methyl 17,17-Dimethyl-5,8,11,14-*all-cis*-docosatetraenoate (7h). A solution of the Wittig reagent was prepared by the dropwise addition of a solution of *n*-butyllithium in hexanes (1.0 mL of 2.5 M solution, 2.5 mmol, 1.15 equiv) to a solution of **13c** (1.20 g, 2.26 mmol, 1.04 equiv) in THF at −78 °C and stirred at that temperature for 0.5 h. The aldehyde **6a** (prepared from 0.766 g, 2.17 mmol of **5**) was dissolved in dry THF (10 mL) and added dropwise to the solution of the Wittig reagent. The reaction mixture was stirred, allowing it to warm to room temperature over 2.5 h. The reaction was quenched with brine, extracted with ethyl acetate, dried, evaporated, and then chromatographed on silica gel eluting with 2.5% ethyl acetate/hexanes to yield the product as an oil (0.814 g, 30%); NMR δ 0.85 (s, 6H), 0.95 (t, J = 7.5 Hz, 3H), 1.20−2.45 (m, 16H), 2.70−2.90 (m, 6H), 3.65 (s, 3H), 5.20−5.50 (m, 8H).

Compounds **7a**−**d**, **7i**, and **7j** were similarly prepared from **6a** and the appropriate ylide of the phosphonium salts as described for the preparation of **7h**. They were then hydrolyzed and transformed into **1a**−**d** and **1f**−**h** using the same procedure as described for the conversion of **7e** to **1e**.

5,8,11,14-*all-cis*-Heneicosatetraenoylethanolamide (1a). Obtained as an oil (76% from **7a**); NMR δ 0.88 (t, J = 7.5 Hz, 3H), 1.3 (br s, 8H), 1.60−2.30.(m, 8H), 2.82 (br s, 7H), 3.30−3.50 (m, 2H), 3.65−3.80 (m, 2H), 5.20−5.55 (m, 8H), 6.05 (br s, 1H); MS m/z 362 (M + 1) (100), 322 (83), 282 (81); HRMS calcd for $C_{23}H_{40}NO_2$ (M + 1), 362.3059; found 362.3077; GC, t_R 10.99 min (86%).

5,8,11,14-*all-cis*-Docosatetraenoylethanolamide (1b). Obtained as an oil (35% from **7b**); NMR δ 0.88 (t, J =7.5 Hz, 3H), 1.29 (br s, 10H), 1.60−1.90 (m, 2H), 2.0−2.30 (m, 6H), 2.65−2.95 (m, 7H), 3.30−3.55 (m, 2H), 3.65−3.80 (m, 2H), 5.20−5.55 (m, 8H), 5.9 (br s, 1H); MS m/z 376 (M + 1) (100), 336 (40), 296 (23); HRMS calcd for $C_{24}H_{42}NO_2$ (M + 1), 376.3215; found 376.3200; GC, t_R 11.24 min (57%).

5,8,11,14-*all-cis*-Tricosatetraenoylethanolamide (1c). Obtained as an oil (57% from **7c**); NMR δ 0.89 (t, J = 7.5 Hz, 3H), 1.29 (br s, 12H), 1.60−1.90 (m, 2H), 2.05−3.50 (m, 6H), 2.65−2.95 (m, 7H), 3.30−3.50 (m, 2H), 3.60−3.85 (m, 2H), 5.20−5.55 (m, 8H), 5.95 (br s, 1H); MS m/z 390 (M + 1) (100), 372 (10); HRMS calcd for $C_{25}H_{44}NO_2$ (M + 1), 390.3372; found 390.3339; GC, t_R 11.70 min (66%).

5,8,11,14-*all-cis*-Tetracosanoylethanolamide (1d). Obtained as an oil (66% from **7d**); NMR δ 0.88 (t, J = 7.5 Hz, 3H), 1.28 (br s, 14 H), 1.60−1.90 (m, 2H), 2.05−2.30 (m, 6H), 2.75−2.95 (m, 7H), 3.35−3.55 (m, 2H), 3.65−3.80 (m, 2H), 5.20−5.55 (m, 8H), 5.85 (br s, 1H); MS m/z 404 (M + 1) (100), 364 (70), 324 (57); HRMS calcd for $C_{26}H_{46}NO_2$ (M + 1), 404.3528; found 404.3523; GC, t_R 12.10 min (87%).

17,17-Dimethyl-5,8,11,14-*all-cis*-docosatetraenoylethanolamide (1f). Obtained as an oil (61% from **7h**); NMR δ 0.88 (s, 6H), 0.90 (t, J = 7.5 Hz, 3H), 1.20 (br s, 8H), 1.60−2.30 (m, 8H), 2.70−2.95 (m, 7H), 3.35−3.55 (m, 2H), 3.65−3.80 (m, 2H), 5.20−5.60 (m, 8H), 6.05 (br s, 1H); MS m/z 404 (M + 1) (97), 364 (72), 324 (100), 294 (14); HRMS calcd for $C_{26}H_{46}NO_2$ (M + 1), 404.3528; found 404.3498; GC, t_R 11.70 min (61%).

17,17-Dimethyl-5,8,11,14-*all-cis*-heneicosanoylethanolamide (1g). Obtained as an oil (40% from **7i**); NMR δ 0.85 (s, 6H), 0.90 (t, J = 7.5 Hz, 3H), 1.25 (br s, 6H), 1.65−2.30 (m, 8H), 2.65−2.95 (m, 7H), 3.30−3.50 (m, 2H), 3.65−3.80 (m, 2H), 5.20−5.50 (m, 8H), 6.05 (br s, 1H); MS m/z 390 (M + 1) (98), 290 (42), 210 (27); HRMS calcd for $C_{25}H_{44}NO_2$ (M + 1), 390.3372; found 390.3335; GC, t_R 11.42 min (79%).

17-Methyl-5,8,11,14-*all-cis*-docosatetraenoylethanolamide (1h). Obtained as an oil (64% from **7j**); NMR δ 0.90 (m, 6H), 1.28 (br s, 9H), 1.60−2.35 (m, 8H), 2.65−2.95 (m, 7H), 3.30−3.50 (m, 2H), 3.65−3.85 (m, 2H), 5.20−5.60 (m, 8H), 5.95 (br s, 1H); MS m/z 390 (M + 1) (38), 350 (91), 310 (100); HRMS calcd for $C_{25}H_{44}NO_2$ (M + 1), 390.3372; found 390.3365; GC, t_R 11.53 min (71%).

(±)-2,16,16-Trimethyl-5,8,11,14-*all-cis*-tricosatetraenoyl-2′-fluoroethylamide (2b). The product was prepared according to the method described for **2a**: Yield 57%; NMR δ 0.88 (t, J = 6.0 Hz, 3H), 1.09 (s, 6H), 1.16 (d, J = 6.8 Hz, 3H), 1.25 (br s, 10H), 1.39−2.40 (m, 5H), 2.81−2.97 (m, 6H), 3.57 (ddt, J = 28.5, 5.2, 4.5 Hz, 2H), 4.49 (dt, J = 47.4, 4.6 Hz, 2H), 5.13−5.52 (m, 8H), 5.86 (br s, 1H). Anal. ($C_{28}H_{48}NOF\cdot 0.4CHCl_3$) C, H, N. The presence of chloroform was confirmed by NMR in DMSO-d_6.

(±)-2,16-Dimethyl-5,8,11,14-*all-cis*-docosatetraenoyl-2′-fluoroethylamide (2c). The product was prepared according to the method described for **2a**: Yield 37%; NMR δ 0.88 (t, J = 6.0 Hz, 3H), 0.94 (d, J = 6.2 Hz, 2H), 1.16 (d, J = 6.8 Hz, 3H), 1.25 (br s, 10H), 1.36−2.60 (m, 6H), 2.70−2.90 (m, 6H), 3.60 (ddt, J = 28.5, 5.1, 4.6 Hz, 2H), 4.49 (dt, J = 47.4, 4.8 Hz, 2H), 5.15−5.60 (m, 8H), 5.81 (br s, 1H); HRMS calcd for $C_{26}H_{45}FNO$ (M + 1), 406.3485; found 406.3480; GC, t_R 9.79 min (73%).

(±)-2,17,17-Trimethyl-5,8,11,14-*all-cis*-docosatetraenoyl-2′-fluoroethylamide (2d). It was obtained as an oil (72% from **7h**); NMR δ 0.85 (s, 6H), 0.90 (t, J = 6.0 Hz, 3H), 1.15 (d, J = 7.0 Hz, 3H), 1.25 (br s, 8H), 1.40−2.40 (m, 7H), 2.70−2.95 (m, 6H), 3.58 (ddt, J = 28.5, 5.2, 4.5 Hz, 2H), 4.50 (dt, J = 47.4, 4.6, Hz, 2H), 5.20−5.55 (m, 8H), 5.85 (br s, 1H); MS m/z (M + 1) (100), 272 (37), 2328 (19); HRMS calcd for $C_{27}H_{47}FNO$ (M + 1), 420.3641; found 420.3608. Anal. ($C_{27}H_{46}NOF\cdot 0.9H_2O$) C, H, N.

(±)-2,17-Dimethyl-5,8,11,14-*all-cis*-docosatetraenoyl-2′-fluoroethylamide (2e). It was obtained as an oil (69% from **7j**); NMR δ 0.80−0.98 (m, 6H), 1.15 (d, J = 6.5 Hz, 3H), 1.25 (br s, 8H), 1.38−2.40 (m, 8H), 2.65−3.0 (m, 6H), 3.60 (ddt, J = 28.5, 5.2, 4.6 Hz, 2H), 4.50 (dt, J = 47.5, 4.8 Hz, 2H), 5.20−5.55 (m, 8H), 5.80 (br s, 1H). Anal. ($C_{26}H_{44}FNO\cdot 0.15CHCl_3$) C, H, N. The presence of chloroform was confirmed by NMR in DMSO-d_6.

(±)-2-Methyl-5,8,11,14-*all-cis*-tricosatetraenoyl-2′-fluoroethylamide (2f). It was obtained as an oil (72% from **7c**); NMR δ 0.88 (t, J = 6.0 Hz, 3H), 1.13 (d, J = 7.5 Hz, 3H), 1.25 (br s, 12H), 1.38−2.40 (m, 7H), 2.70−2.95 (m, 6H), 3.60 (ddt, J = 28.5, 5.2, 4.5 Hz, 2H), 4.50 (dt, J = 47.5, 4.5 Hz, 2H), 5.20−5.60 (m, 8H), 5.80 (br s, 1H); MS m/z 406 (M + 1) (100), 326 (27), 119 (96); HRMS calcd for $C_{26}H_{45}FNO$ (M + 1), 406.3485; found 406.3459; GC, t_R 6.10 min (81%).

Pharmacology. Drug Preparation and Administration. For binding assays, compounds were prepared as 1 mg/mL stock solutions in absolute ethanol and were stored at −20 °C. For behavioral assays, drugs were dissolved in a 1:1:18 mixture of ethanol, emulphor (GAF Corporation, Linden, NJ), and saline (0.9% NaCl) and were administered intravenously (iv) in the mouse tail vein in volumes of 0.1 mL/10 g of body weight.

Binding Assays. Radioligand binding to P_2 membrane preparations were performed as described elsewhere.[10] Ethanolic 1 mg/mL stock solutions of anandamide analogs were diluted in buffer (50 mM Tris-HCl, 1 mM EDTA, 3 mM $MgCl_2$, and 5 mg/mL bovine serum albumin) without evaporation of the ethanol (final concentration not exceeding 0.4%). Analog concentrations ranging from 1 nM to 10 μM and a 1 nM concentration of [^{3}H]CP-55,940 were used. Binding was initiated with the addition of 150 μg of membrane preparation protein. Nonspecific binding was determined in the presence of 1 μM CP-55,940. The binding experiments were performed either with or without the amidase inhibitor phenylmethylsulfonyl fluoride (PMSF, 50 μM). After a 1-h incubation at 30 °C, the reaction was terminated with the addition of 2 mL of ice-cold buffer (50 mM Tris-HCl, 1 mg/mL bovine serum albumin) followed by rapid filtration through PEI-treated filters. The assays were performed in triplicate, and the results represent the combined data from three to six independent experiments.

Behavioral Evaluations. Mice were acclimated to the laboratory overnight. Depression of locomotor activity and antinociception, as determined by the tail-flick (TF) response to a heat stimulus,[28] were measured in the same animal. Control tail-flick latencies of 2 to 4 s were measured for each animal with a standard tail-flick apparatus prior to drug or vehicle administration. Four minutes following an iv injection of either vehicle or drug, mice were tested for tail-flick response. Immediately thereafter, the mice were placed into individual photocell activity cages (11 × 6.5 in.) for assessment of spontaneous activity. For the next 10 min the total number of beam interruptions in the 16 photocell beams per cage were recorded using a Digiscan Animal Activity Monitor (Omnitech Electronics Inc., Columbus, OH). Spontaneous activity was expressed as percent of control activity. Antinociception was expressed as the % maximum possible effect (MPE) using a 10-s maximum test latency as described earlier.[28] Each dose tested in the antinociception and hypomotility assays represents one group of animals (six mice per group). Cannabinoid-induced hypothermia and immobility were determined in a separate group of animals. Prior to vehicle or drug administration, rectal temperature was determined by a thermistor probe (inserted 25 mm) and a telethermometer (Yellow Springs Instrument Co., Yellow Springs, OH). Four minutes after the iv injection of the drug, mice were tested for body temperature. The difference between pre- and postinjection rectal temperatures were calculated. Immediately after measure of body temperature, the mice were placed on a 5.5 cm ring attached at a height of 16 cm to a ring stand, and amount of time the animals remained motionless during a 5-min period was recorded.[29] The time that each animal remained motionless on the ring was divided by 300 s and multiplied by 100 to obtain a % immobility rating.

Data Analysis. IC_{50} values were converted to K_i values.[30] Statistical evaluation of parallelism between displacement curves generated in the presence and absence of PMSF were performed using ALLFIT.[31] Dose−response relationships were determined for each analog in the pharmacological assays. Percent effect was determined based upon the maximal effects that are produced by Δ^9-THC and anandamide which are 90, 100, and 60% for spontaneous activity, antinociception and ring immobility, respectively. The percent effect for hypothermia was based upon the maximal effect produced by anandamide (−3.0 °C) rather than that produced by Δ^9-THC (−6.0 °C). Antinociception, hypomotility, and immobility data were converted to probit values, and ED_{50}'s were calculated by unweighted least-squares linear regression analysis of the log dose versus the probit values. Several analogs were classified as having partial agonist effects because they produced dose−responsive effects that failed to exceed 60% of maximal effect. Analogs producing effects less than 30% or hypothermia less than 1 °C at the highest doses tested were considered to be inactive.

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