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Design and Synthesis of (13*S*)-Methyl-Substituted Arachidonic Acid Analogues: Templates for Novel Endocannabinoids

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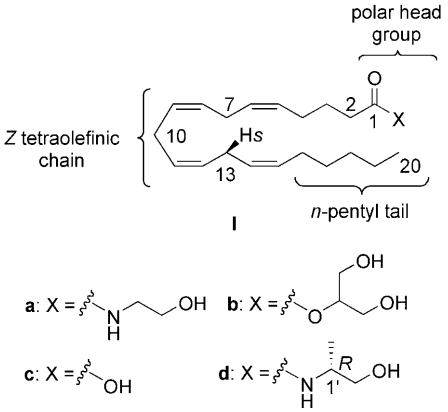
Abstract: Two novel methyl-substituted arachidonic acid derivatives were prepared in an enantioselective manner from commercially available chiral building blocks, and were found to be excellent templates for the development of (13*S*)-methyl-substituted anandamide analogues. One of the compounds synthesized, namely, (13*S*,5*Z*,8*Z*,11*Z*,14*Z*)-13-methyl-eicosa-5,8,11,14-tetraenoic acid *N*-(2-hydroxyethyl)amide, is an endocannabinoid analogue with remarkably high affinity for the CB1 cannabinoid receptor.

Keywords: chirality · endocannabinoids · lipids · olefination · structure–activity relationships

Introduction

By the mid 1990s, the first two key endocannabinoids *N*-arachidonoylethanolamine (anandamide, **Ia**) and 2-arachidonoyl glycerol (**Ib**) were isolated and characterized as derivatives of the noncannabinergic arachidonic acid (**Ic**).[1–3] Compounds **Ia** and **Ib** act at the CB1 and CB2 receptors, two $G_{i/o}$-protein-coupled cannabinoid receptors (CBs)[4–8] known to modulate physiological and pathological processes, including nociception,[9–11] inflammation,[12] neuroprotection,[13–15] feeding,[16,17] memory,[18] anxiety,[19] and cell proliferation.[20,21] The biological actions of **Ia** and **Ib** are terminated by a transport mechanism and enzymatic deactivation. In most tissues, compound **Ia** is metabolized hydrolytically by fatty acid amide hydrolase (FAAH),[22,23] and **Ib** is metabolized by monoacylglycerol lipase (MAGL).[24,25] However, recent investigations have demonstrated that oxidative enzymes of the arachidonate cascade, including lipoxygenases (LOX),[26–29] cytochrome P450,[28–31] and cyclooxygenase-2 (COX-2),[28,29,32–34] can transform **Ia** and **Ib** into eicosanoid-related bioactive products.



In addition to the hydrolytic metabolism of **Ia** and **Ib**, the alternative COX-2 metabolic route becomes important when FAAH or MAGL are inhibited and when endocannabinoid biosynthesis is activated following tissue damage.[35,36] The first step in the COX-2 oxidative metabolism of **Ic** has been proposed to involve abstraction of the *pro*-(*S*) hydrogen from the C13 position by a tyrosyl radical.[37,38] It has also been reported that mutation of Tyr385 prevents **Ia** oxygenation by COX-2, suggesting that, as in the case of **Ic**, metabolism of **Ia** is initiated by Tyr385-mediated hydrogen abstraction.[39]

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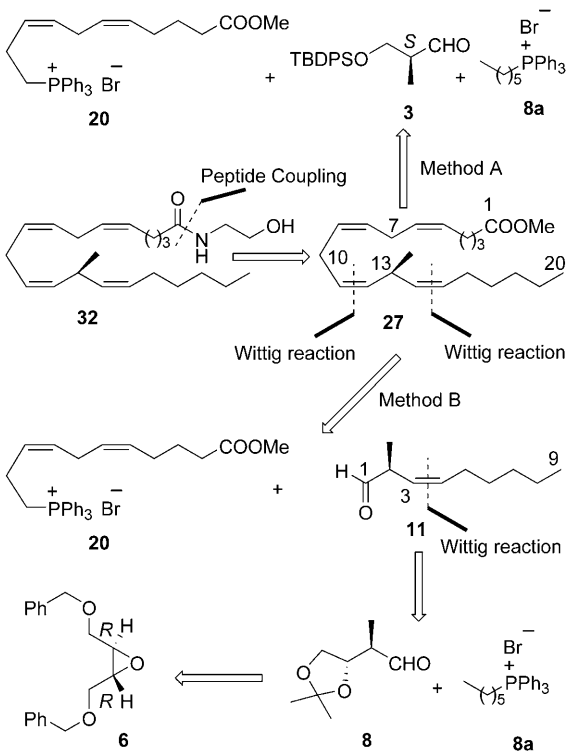
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.200902880. It contains experimental procedures, physical properties, and spectroscopic data for compounds **2**–**4**, **8**–**11**, **13**–**23**, and **30**, as well as ^{1}H NMR, COSY, HSQC, HMBC, and NOESY spectra of compound **27**, and a ^{1}H NMR spectrum of compound **24**.

The chemical structure of **Ia** can be divided into two major molecular fragments: 1) a polar head group and 2) a hydrophobic fragment comprising a nonconjugated tetraolefinic chain (with *Z*-configured double bonds) and an *n*-pentyl tail. Extensive structure–activity relationship (SAR) studies aimed at developing potent and metabolically stable analogues of **Ia** have focused on the polar head group and on the *n*-pentyl tail.[40–42] In this regard, earlier work from our laboratories has led to the synthesis of a high-affinity and hydrolytically stable analogue of **Ia**, (*R*)-methanandamide (**Id**).[43–47]

Unlike the polar head group and the *n*-pentyl tail, the nonconjugated tetraolefinic chain is essentially unexplored, and SAR studies seeking to probe unsaturation requirements have suggested that it plays a pivotal role in determining the bioactive conformation(s) of **Ia** and its congeners.[45,49] Earlier computational and biophysical work by us[50] and others[51–54] on the conformational properties of anandamide has shown that the hydrophobic fragment is capable of assuming a variety of conformations in solution, which are generally characterized as hairpin (U shaped), partially extended (J shaped) and fully extended. Of these, the U- and the J-shaped conformations are believed to be responsible for CB receptor recognition.[50–54] Additionally, in the crystal structure of **Ic** bound in the COX active site of prostaglandin synthase-1, compound **Ic** adopts a partially extended L-shaped conformation.[55]

As a part of our ongoing program in cannabinoid medicinal chemistry, we focus on the development of novel endocannabinoid templates that possess high CB receptor binding affinity as well as metabolic stability to the action of the COX-2 enzyme. The novel analogues can provide additional insight into the stereoelectronic requirements for interaction with CB receptors, and aid in the discovery of more potent and selective cannabinergic drug candidates and COX-2 inhibitors. They can also serve as pharmacological tools towards understanding the connection between the endocannabinoid and COX-2 systems.

We report herein the design, synthesis, and preliminary biological data of two novel endocannabinoid molecular probes referred to as (13*S*)-methyl-substituted anandamide analogues (**32** and **39**, Schemes 1 and 6, respectively). Our synthetic strategy involves two approaches (methods A and B, Scheme 1) for the enantioselective construction of the cannabinergic skeleton. The first approach (method A) yields the final products in fewer synthetic steps, whereas the second approach (method B) facilitates the enantioselective introduction of different substituents at the C13 position. Biological testing results demonstrate that both analogues **32** and **39** are well recognized by the CB1 receptor. It is especially worthy of note that **32** is among the endocannabinoid analogues with the highest CB1 binding affinities known to date. A detailed SAR study as well as a full biological evaluation of the novel endocannabinoid analogues reported herein is underway.



Scheme 1. Retrosynthetic analysis of (13*S*)-methylanandamide **32**. TBDPS = *tert*-butyldiphenylsilyl.

Results and Discussion

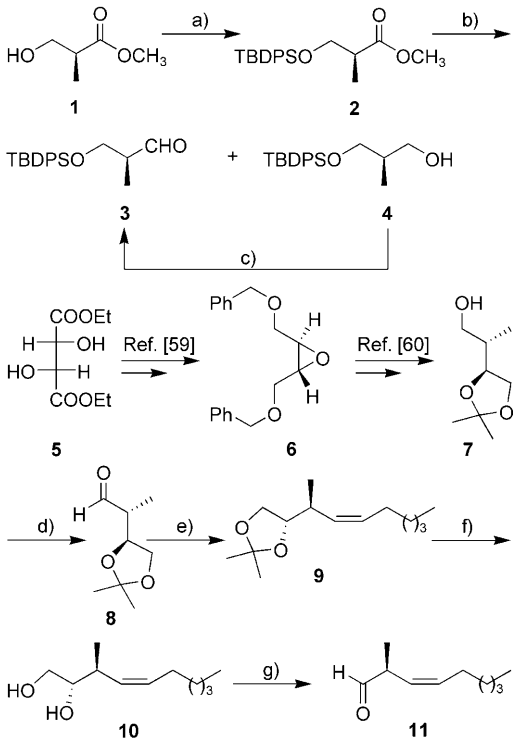
Analogue design: Our approach for the design of the novel endocannabinoid analogues involved addition of a methyl substituent at position 13*S* of the lipophilic fragment (analogue **32**, Scheme 1). It was hypothesized that the presence of a methyl substituent at C13 would not significantly affect the ability of the nonconjugated tetraolefinic chain to assume U- and/or J-shaped conformations, and thus would not significantly affect its bioactivity for CB1. In contrast, it seemed plausible that endocannabinoid analogues substituted at the C13*S* position might "shut off" the COX-2-mediated metabolism due to the absence of a hydrogen atom in the respective position. We also extended our design to include analogue **39** (Scheme 6), which can be considered to mimic the J- and/or L-shaped conformers of **Ia** and **Ic**, because of the conformational restriction imposed by the two triple bonds. The head group of the endogenous anandamide was incorporated in our templates.

Synthesis: Our retrosynthetic analysis identifies methyl ester **27** as the key intermediate from which (13*S*)-methylanandamide (**32**) would be generated through peptide coupling (Scheme 1).

Retrosynthetic disconnection (method A) of both the C11=C12 and C14=C15 double bonds in **27** generated three fragments: the phosphonium salts **20** and **8a**, and the chiral aldehyde **3**, which possesses the *S* configuration correspond-

ing to the C13 stereogenic center of **27**. In the synthetic direction, fragments **20**, **8a**, and **3** are joined by Wittig reactions. An alternative approach (method B) involves cleavage of the C11=C12 double bond, yielding phosphonium salt **20** and chiral aldehyde **11**. Further disconnection at the C3=C4 double bond of **11** led to **8a** and chiral methyl-substituted aldehyde **8**, which can in turn be derived from the enantiomerically pure (*R*,*R*)-epoxide **6**. The synthetic direction could thus be completed through Wittig reactions. Although method B involves more steps, it facilitates the enantioselective introduction of various substituents at the C13 position of **Ia**, through S_N2-type reactions on the epoxide **6**. Because of the similarity in the structures, a closely related retrosynthetic analysis could be envisaged for the conformationally partially extended (13*S*)-methyl analogue **39**.

The syntheses of the required chiral aldehydes **3** and **11** are summarized in Scheme 2. Protection of the primary hydroxyl group in **1** as the TBDPS ether (**2**)[56] was followed

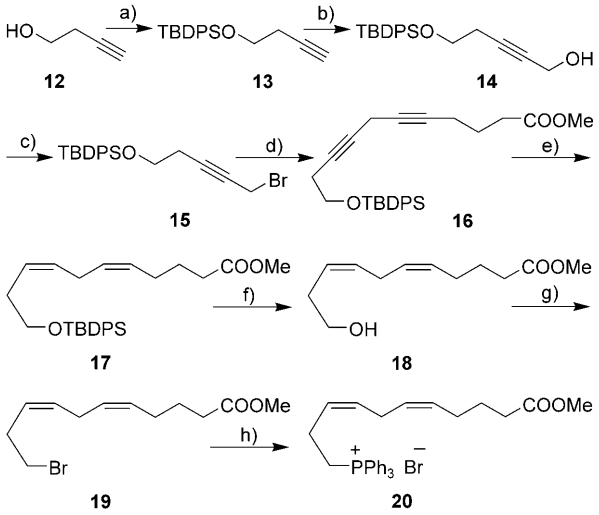


Scheme 2. Synthesis of chiral aldehydes **3** and **11**. Reagents and conditions: a) TBDPSCl, imidazole, CH_2Cl_2, 0°C to RT, 1.5 h, 98%; b) DIBAL-H, CH_2Cl_2, −110–−90°C, 20 min, 61% for **3** and 37% for **4**; c) Dess–Martin periodinane, 0°C to RT, 45 min, 90%; d) PCC, CH_2Cl_2, 4 Å molecular sieves, RT, 2.5 h, 93%; e) $CH_3(CH_2)_5P^+Ph_3Br^-$, KHMDS, THF, 10°C, 50 min, then **8**, −98°C, 1 h, 50%; f) $BF_3{\cdot}CH_3COOH$, MeOH, 0°C, 1 h, 84%; g) $Pb(OAc)_4$, CH_2Cl_2, −78°C, 1.5 h, 97%. PCC = pyridinium chlorochromate, KHMDS = potassium bis(trimethylsilyl)amide.

by diisobutylaluminum hydride (DIBAL-H) reduction to give aldehyde **3**[57] (61% yield) and alcohol **4**[58] (37% yield). Conversion of **4** to **3** was carried out through Dess–Martin periodinane oxidation in 90% yield.[56] Enantiomerically pure (*R*,*R*)-epoxide **6** was synthesized in six steps starting from commercially available (−)-diethyl tartrate (**5**) by following our recently reported procedures.[59] Conversion of **6** to acetonide **7** involved the following steps:[60] 1) methylation of **6** with Me_2CuLi, 2) benzyl ether cleavage by hydrogenolysis, and 3) treatment of the resulting triol with acetone, in the presence of *p*-toluenesulfonic acid.

Oxidation of alcohol **7** with PCC in dry CH_2Cl_2 gave aldehyde **8** in excellent yield (93%).[61,62] Combination of aldehyde **8** and the ylide derived from hexyltriphenylphosphonium bromide and KHMDS, at −98°C, resulted in the formation of exclusively the *Z* isomer **9** in 50% yield (J(3H,4H) = 10.4 Hz, see the Supporting Information). Mild deprotection of the ketal **9** by using the $BF_3{\cdot}CH_3COOH$ complex (84% yield), and lead tetraacetate mediated cleavage of the resulting 1,2-diol **10** at −78°C provided aldehyde **11** (97% yield), which was used immediately in a Wittig reaction with phosphonium salts **20** and **23**.

The construction of the required alkenyl phosphonium salt **20** proceeded as shown in Scheme 3. Commercially available 3-butyn-1-ol (**12**) was converted into alcohol **18**



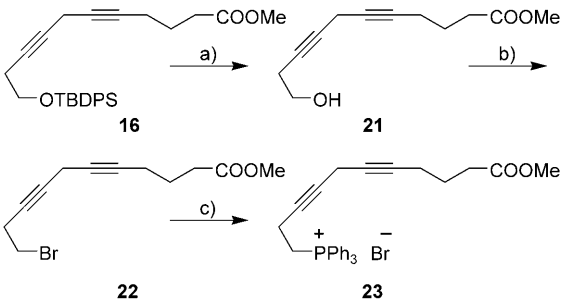
Scheme 3. Synthesis of alkenyl phosphonium salt **20**. Reagents and conditions: a) TBDPSCl, imidazole, THF, 0°C, 1.5 h, 99%; b) *n*BuLi, THF, 0°C, 1.5 h, then $(CH_2O)_n$, −50°C to RT, 1.5 h, 73%; c) CBr_4, Ph_3P, CH_2Cl_2, 0°C to RT, 3 h, 85%; d) CH≡C-$(CH_2)_3$-COOMe, Cs_2CO_3, NaI, CuI, DMF, RT, 2.5 h, 95%; e) $Ni(OAc)_2$, $NaBH_4$, ethylenediamine, H_2, MeOH, RT, 2 h, 85%; f) TBAF, THF, 0°C to RT, 1.5 h, 93%; g) CBr_4, Ph_3P, CH_2Cl_2, −25–0°C, 1.5 h, 96%; h) Ph_3P, CH_3CN, 72–75°C, 8 days, 95%. TBAF = tetrabutylammonium fluoride.

through several modifications of a known sequence,[63a] in improved overall yield. Thus, TBDPS protection of **12** led to alkyne **13** (99% yield), which was subsequently treated with *n*BuLi and quenched with paraformaldehyde to give alcohol **14** in 73% yield. Conversion of **14** to bromide **15** was carried out by using the PPh_3/CBr_4 method (85% yield).

Copper-mediated cross-coupling of **15** with methyl 5-hexynoate in the presence of Cs_2CO_3 and NaI afforded diyne **16** (95% yield), which was partially hydrogenated over P-2 nickel catalyst to give the corresponding skipped *Z* diene **17**

in 85% yield.[63] Deprotection with TBAF at 0°C and exposure of the resulting alcohol **18** to the PPh_3/CBr_4 system gave bromide **19** in 89% overall yield. Heating **19** (72–75°C) with PPh_3 in dry CH_3CN for 8 days afforded the target phosphonium salt **20** in 95% yield after purification. In the ^1H NMR spectrum of **20**, all four double bond protons are well separated with coupling constants J(5H,6H) and J(8H,9H) of less than 10.8 Hz (see the Supporting Information), which suggests a *Z* relationship between the hydrogen atoms in the 5H–6H and 8H–9H spin systems.

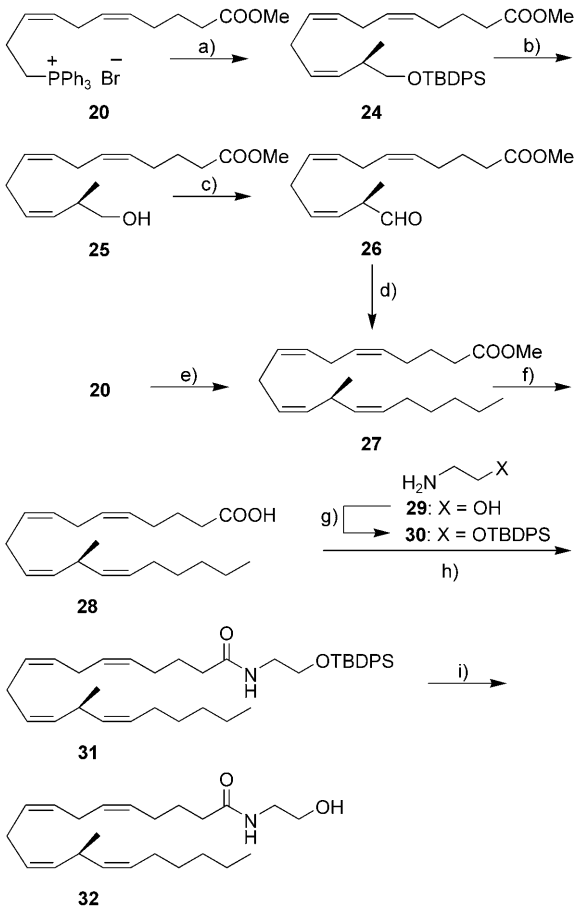
Similarly, the alkynylphosphonium salt **23** was produced in three steps (72% overall yield) from diyne **16** through desilylation, conversion of alcohol **21** to the bromide **22**, and reaction with PPh_3 (Scheme 4).



Scheme 4. Synthesis of alkynyl phosphonium salt **23**. Reagents and conditions: a) TBAF, THF, 0°C, 2 h, 84%; b) CBr_4, Ph_3P, CH_2Cl_2, −16°C, 2 h, 98%; c) Ph_3P, CH_3CN, 70–72°C, 7 days, 88%.

The assembly of the synthesized aldehydes **3** and **11** with phosphonium salt **20** into the (13*S*)-methyl anandamide (**32**) is shown in Scheme 5. Thus, treatment of **20** with KHMDS and coupling of the resulting ylide with aldehyde **3** at −115°C produced ester **24** (61% yield). Based on ^1H NMR analysis, this Wittig olefination reaction afforded exclusively the *Z* olefin with J(11H,12H) = 10.8 Hz (the ^1H NMR spectrum of **24** is available in the Supporting Information). Desilylation of **24** with TBAF gave alcohol **25** in 85% yield.

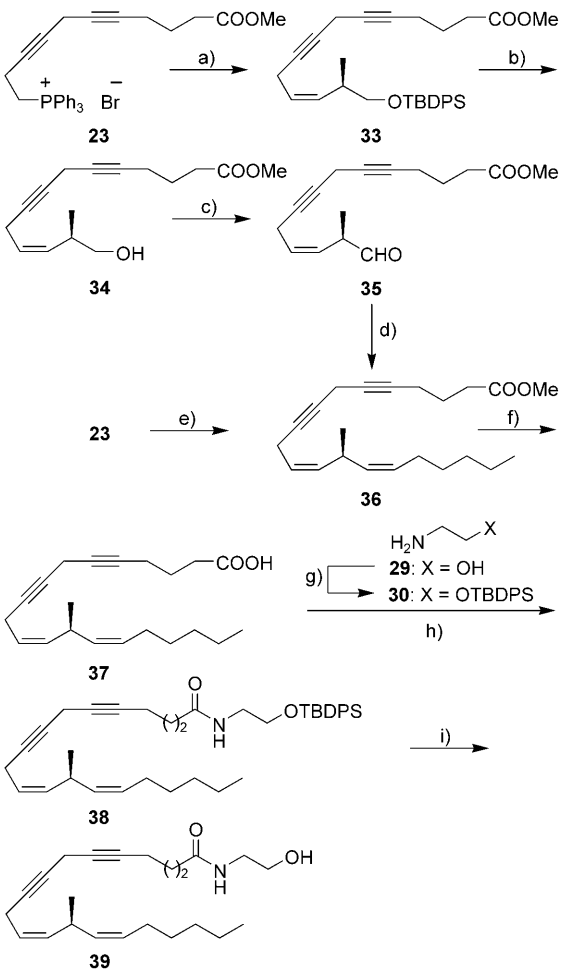
The coupling constant between 11H and 12H of **25** (J(11H,12H) = 10.4 Hz) correlates well with *Z* stereochemistry of the C11=C12 double bond. Dess–Martin periodinane oxidation of **25** led to aldehyde **26**, which was used immediately, without purification, in a Wittig reaction with hexyltriphenylphosphonium bromide, under salt-free conditions, to give (13*S*)-methyl arachidonate **27** in 65% yield from **25**. By using our second approach, phosphonium bromide **20** was treated with KHMDS at −78°C, and the resulting phosphorane was coupled with aldehyde **11** at −98°C to give **27** in 22% yield. The structure of **27** was established by using 1D and 2D NMR methods (COSY, HSQC, HMBC, and NOESY; data are available in the Supporting Information). NOESY interactions between 13H and 16H confirm the *Z* stereochemistry for the newly formed double bond at the C14 position. Saponification of ester **27** with lithium hydroxide in THF/H_2O led to acid **28** (86% yield), which was coupled with 2-(*tert*-butyldiphenylsilyloxy)ethanamine (**30**) to



Scheme 5. Synthesis of **32**. Reagents and conditions: a) KHMDS, THF, −78–−60°C, 20 min, then **3**, −115–0°C, 3 h, 61%; b) TBAF, THF, 0°C to RT, 1.5 h, 85%; c) Dess–Martin periodinane, 0°C to RT, 75 min; d) $CH_3(CH_2)_5P^+Ph_3Br^-$, KHMDS, THF, 0°C, 40 min, then addition of **26**, −115–−100°C, 50 min, 65% from alcohol **25**; e) KHMDS, THF, −78–−60°C, 20 min, then **11**, −98–0°C, 2.5 h, 22%; f) LiOH, THF/H_2O, RT, 24 h, 86%; g) TBDPSCl, imidazole, CH_3CN, 0°C, 30 min, 97%; h) carbonyldiimidazole, THF, RT, 2 h, then **30**, RT, 1 h, 91%; i) TBAF, THF, 0°C to RT, 1 h, 88%.

give amide **31** (91% yield) by using the carbonyldiimidazole activation procedure. Removal of the silyl protecting group was carried out by using TBAF in THF, and gave (13*S*)-methylanandamide (**32**) in 88% yield.

Conformationally partially extended (13*S*)-methyl analogue **39** was synthesized in a similar fashion, as depicted in Scheme 6. Thus, Wittig reaction of aldehyde **3** with phosphonium salt **23** in the presence of KHMDS gave exclusively the *Z* olefin **33** in 58% yield (J(11H,12H) = 10.8 Hz). Deprotection with TBAF under neutral conditions (to suppress the formation of byproducts) gave **34** (76% yield). Aldehyde **35** was obtained from **34** through Dess–Martin periodinane oxidation, and was used immediately in the next step. Wittig reaction of aldehyde **35** with hexyltriphenylphosphonium bromide produced ester **36** in 58% yield from **34**. Alternatively, ester **36** could also be obtained in 21% yield through Wittig reaction of aldehyde **11** and alkynyl phosphonium salt **23**. These Wittig olefination reactions led ex-



Scheme 6. Synthesis of the conformationally partially extended (13*S*)-methyl analogue **39**. Reagents and conditions: a) KHMDS, THF, −78 °C, 50 min, then **3**, −98–0 °C, 2.5 h, 58 %; b) TBAF, CH_3COOH, THF, 0 °C to RT, 20 h, 76 %; c) Dess–Martin periodinane, 0 °C, 2 h; d) $CH_3(CH_2)_5P^+Ph_3Br^-$, KHMDS, THF, 10 °C, 40 min, then addition of **35**, −98 °C, 1 h, 58 % from alcohol **34**; e) KHMDS, THF, −78 °C, 50 min, then **11**, −98–0 °C, 2.5 h, 21 %; f) LiOH, THF/H_2O, RT, 7 h, 82 %; g) TBDPSCl, imidazole, CH_3CN, 0 °C, 30 min, 91 %; h) Carbonyldiimidazole, THF, RT, 2 h, then **30**, RT, 1 h, 97 %; i) TBAF, CH_3COOH, THF, 0 °C to RT, 20 h, 75 %.

clusively to the required *Z* geometry at the newly generated double bonds under the experimental conditions used. Subsequent saponification, coupling with protected ethanolamine **30** and deprotection by using the TBAF/CH_3COOH system led to the target amide **39** in 60 % overall yield from **36**.

Receptor binding studies: Binding affinities of the newly synthesized analogues for CB1 and CB2 cannabinoid receptors were determined as described in the Experimental Section.[4,44,45,47,64,65] For the CB1 receptor, binding data were obtained by using a rat brain membrane in the presence of phenylmethanesulfonyl fluoride (PMSF),[66,67] a general serine protease inhibitor that is used to protect the analogues from the hydrolytic activity of fatty acid amide hydrolase (FAAH).[43,67] Rat brain membranes have a high concentration of CB1 receptors without significant CB2 receptors present. CB2 receptor affinity was measured by using mouse spleen membranes, in which FAAH activity is absent and does not require pretreatment with PMSF. [^{3}H]CP-55,940 was chosen as a competing ligand for the assays, because it has high affinity for both CB1 and CB2 receptors and is nonselective. It is one of the most widely used radioligands for characterizing both CB1 and CB2 cannabinoid receptors.[40–42] SAR, mutation, and computer modeling study results indicate that nonclassical cannabinoids (e.g., CP-55,940), classical cannabinoids, and anandamide share key binding motifs, whereas other classes of cannabinergic compounds, such as the aminoalkylindole WIN55212-2, might have different binding features.[53] The binding affinities (K_i values) of the novel endocannabinoid analogues are summarized in Table 1, in which the endogenous anandamide is included for comparison.

Table 1. Affinities (K_i) of endocannabinoid analogues for CB1 and CB2 cannabinoid receptors (95 % confidence limits).

Compound	CB1 K_i [nM][a] with PMSF	CB2 K_i [nM][a]
anandamide	61[b]	1930[b]
32	4.8 ± 1.3	137 ± 22
39	139 ± 25	967 ± 321

[a] Affinities for CB1 and CB2 were determined by using rat brain (CB1) or mouse spleen (CB2) membranes and [^{3}H]CP-55,940 as the radioligand following previously described procedures.[4,44,45,47,64,65] Data were analyzed by using nonlinear regression analysis. K_i values were obtained from three independent experiments run in duplicate and are expressed as the mean of the three values. [b] Reported previously.[40]

It is apparent from the CB2 affinities reported herein that these analogues show selectivity for the CB1 receptor. Additionally, the backbone of the two ligands is well recognized by the CB1 receptor. In this regard, compound **32** is the most interesting, with a 13-fold higher affinity for CB1 over anandamide, a feature which places it among the endocannabinoid analogues with the highest CB1 binding affinities known to date. Furthermore, preliminary functional characterization of **32** using the cannabinoid-stimulated guanosine-5′-O-(3-[^{35}S]thio)triphosphate ([^{35}S]GTPγS) binding assay[68] indicates that **32** is a full agonist for the CB1 cannabinoid receptor.

Conclusion

We have designed and synthesized two novel endocannabinoid templates with potential resistance to oxidative metabolism by COX-2. The synthetic strategy developed involves two approaches for the enantioselective construction of the cannabinergic (13*S*)-methyl-substituted skeleton. The first approach yields the final products in 18–19 synthetic steps starting from commercially available **1**. The second approach provides the targeted compounds in 19–20 synthetic steps from the key intermediate **6**, which can in turn be pre-

pared in six steps from commercially available **5**. Although this synthetic route involves more steps, it facilitates the introduction of different substituents at the 13*S* position. Both approaches use Wittig reactions and peptide coupling as key steps.

Biological testing results show that the synthesized analogues are well recognized by the CB1 receptor, and **32** is one of the endocannabinoid analogues with the highest CB1-binding affinity known to date ($K_i = 4.8$ nM for CB1). In addition, preliminary functional characterization of **32** indicates that this analogue is a full agonist for the CB1 cannabinoid receptor. A detailed SAR study and a full biological evaluation of the two novel endocannabinoids described are currently underway.

Experimental Section

General: All reagents and solvents were purchased from Aldrich Chemical Company, unless otherwise specified, and used without further purification. All anhydrous reactions were performed under a static argon or nitrogen atmosphere in flame-dried glassware using scrupulously dry solvents. Flash column chromatography was carried out by using silica gel 60 (230–400 mesh). All compounds were demonstrated to be homogeneous by analytical TLC on precoated silica gel TLC plates (Merck, 60 F_{245} on glass, layer thickness 250 mm), and chromatograms were visualized by staining with phosphomolybdic acid. Melting points were determined on a micromelting point apparatus and are uncorrected. Optical rotations were recorded on a Perkin–Elmer 341 digital polarimeter. NMR spectra were recorded in $CDCl_3$, unless otherwise stated, on Varian 300 (^{1}H at 300 MHz, ^{13}C at 75 MHz) and Varian 600 (^{1}H at 600 MHz, ^{13}C at 150 MHz) NMR spectrometers and chemical shifts are reported in units of δ relative to internal TMS. Multiplicities are indicated as br (broadened), s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and coupling constants (J) are reported in hertz (Hz). Low- and high-resolution mass spectra were obtained in the School of Chemical Sciences, University of Illinois at Urbana-Champaign. Mass spectral data are reported in the form of m/z (intensity relative to base = 100). Elemental analyses were obtained in Baron Consulting, Milford, CT.

Compound 24: KHMDS (384 mg, 1.93 mmol) was added to a solution of **20** (1.09 g, 2.03 mmol) in dry THF (8 mL) at −78 °C under an argon atmosphere. The mixture was stirred and allowed to warm from −78 to −60 °C over 20 min to ensure complete formation of the orange ylide, then the resulting mixture was cooled to −115 °C. A solution of aldehyde **3** (331 mg, 1.02 mmol) in dry THF (2 mL) was added dropwise. The reaction mixture was stirred for 35 min at −115 °C, then warmed to 0 °C over 1.5 h, and stirring was continued at that temperature for 1 h. The reaction mixture was cooled to −115 °C and quenched with a saturated aqueous solution of sodium bicarbonate. The mixture was warmed to room temperature, extracted with diethyl ether, and the combined organic extracts were washed with brine, dried (Na_2SO_4), and concentrated in vacuo. Purification by flash column chromatography on silica gel (5–7% diethyl ether in hexane) gave **24** as a colorless oil (312 mg, 61% yield). $[\alpha]_D^{20} = -33.59$ ($c = 0.00521$ g mL^{-1} in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz): $\delta =$ 1.00 (d, $J = 7.2$ Hz, 3H; CH-CH_3), 1.05 (s, 9H; C$(CH_3)_3$), 1.70 (quintet, $J = 7.8$ Hz, 2H; 3-H), 2.09 (dt, $J = 6.6$, 6.6 Hz, 2H; 4-H), 2.31 (t, $J =$ 7.8 Hz, 2H; 2-H), 2.67–2.74 (m, 2H; 10-H), 2.75–2.84 (m and t overlapping, 3H; 13-H, 7-H, including 2.77, t, $J = 6.0$ Hz; 7-H), 3.46 (dd, $J = 10.2$, 6.8 Hz, 1H; 14-H), 3.50 (dd, $J = 10.2$, 6.3 Hz, 1H; 14-H), 3.66 (s, 3H; $COOCH_3$), 5.21 (tdd, $J = 10.8$, 9.6, 1.2 Hz, 1H; 12-H), 5.28–5.42 (m, 5H; 5-H, 6-H, 8-H, 9-H, 11-H), 7.34–7.38 (m, 4H; 3-H, 5-H, ArH), 7.39–7.44 (m, 2H; 4-H, ArH), 7.66 ppm (d, $J = 7.0$ Hz, 4H; 2-H, 6-H, ArH); ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta = 17.4$, 19.3, 24.8, 25.6, 25.9, 26.5, 26.8, 33.4, 34.7, 51.5 (OMe), 68.5 (CH_2O), 127.5, 127.6, 128.0, 128.5, 128.9, 129.5, 133.1, 133.9, 135.6, 174.1 ppm (C=O); MS (ESI): m/z (%): 527.9 $[M+Na]^+$ (24), 427.7 (12), 249.4 (100), 217.3 (47), 123.2 (23); MS (EI): m/z (%): 447 $[M-C(CH_3)_3]^+$ (39), 213 (45), 199 (100), 183 (19), 133 (39), 91 (27), 77 (18); MS (ESI): m/z (%): 527 $[M+Na]^+$ (100); HRMS (ESI): m/z calcd for $C_{32}H_{44}O_3NaSi + Na^+$: 527.2957 $[M+Na]^+$; found: 527.2954.

Compound 25: TBAF (0.8 mL, 0.8 mmol, 1 M solution in THF) was added dropwise to a stirred solution of **24** (290 mg, 0.575 mmol) in dry THF (12 mL), at 0 °C under an argon atmosphere. Stirring was continued for 10 min at 0 °C and for 1.5 h at room temperature. The reaction mixture was quenched with a saturated aqueous solution of NH_4Cl at 0 °C and extracted with AcOEt. The combined organic extracts were washed with brine, dried (Na_2SO_4), and concentrated in vacuo at 37 °C. The crude oil was purified by flash column chromatography on silica gel (40% ethyl acetate in hexane) to afford **25** as a colorless viscous liquid (130 mg, 85% yield). $[\alpha]_D^{20} = +7.46$ ($c = 0.00067$ g mL^{-1} in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz): $\delta = 0.90$ (d, $J = 6.6$ Hz, 3H; CH-CH_3), 1.54 (br s, 1H; OH), 1.64 (quintet, $J = 7.8$ Hz, 2H; 3-H), 2.04 (dt, $J = 6.6$, 6.6 Hz, 2H; 4-H), 2.26 (t, $J = 7.8$ Hz, 2H; 2-H), 2.67 (m as septet, $J =$ 7.8 Hz, 1H; 13-H), 2.73 (t, $J = 5.4$ Hz, 2H; 7-H), 2.75–2.85 (m, 2H; 10-H), 3.29 (dd, $J = 10.2$, 7.8 Hz, 1H; 14-H), 3.42 (dd, $J = 10.2$, 6.0 Hz, 1H; 14-H), 3.60 (s, 3H; $COOCH_3$), 5.10 (tdd, $J = 10.4$, 9.6, 1.8 Hz, 1H; 12-H), 5.26–5.36 (m, 4H; 5-H, 6-H, 8-H, 9-H), 5.43 ppm (dtd, $J = 10.4$, 7.8, 1.2 Hz, 1H; 11-H; ^{13}C NMR ($CDCl_3$, 150 MHz): $\delta = 16.95$, 24.75, 25.62, 26.04, 26.56, 33.43, 34.95, 51.55 (OCH_3), 67.64 (CH_2OH), 128.05, 128.32, 128.77, 128.93, 129.94, 132.53, 174.12 ppm (C=O); MS (EI): m/z (%): 266 $[M]^+$ (2), 248 (7), 236 (16), 175 (9), 161 (17), 147 (18), 133 (37), 119 (32), 107 (58), 93 (89), 79 (100); MS (ESI): m/z (%): 289 $[M+Na]^+$ (100), 267 $[M+H]^+$ (38), 249 (41), 217 (39); HRMS (EI): m/z calcd for $C_{16}H_{26}O_3$: 266.1882 $[M]^+$; found: 266.1890.

Compound 26: Dess–Martin periodiane (DMP; 271 mg, 0.639 mmol) was added to a solution of alcohol **25** (100 mg, 0.376 mmol) in dry CH_2Cl_2 (8 mL) at 0 °C under an argon atmosphere. The resulting suspension was warmed to room temperature and stirred for 45 min. An additional amount of DMP (112 mg, 0.263 mmol) was added at 0 °C and stirring was continued for 30 min at room temperature to ensure total consumption of alcohol **25**. The reaction mixture was quenched by adding a 1:1 (v/v) mixture of a 10% aqueous solution of $Na_2S_2O_3$ and a saturated aqueous solution of $NaHCO_3$, and diluted with diethyl ether. The slurry was filtered through Celite, the layers were separated and the aqueous layer was extracted with diethyl ether. The combined organic extracts were washed with a saturated aqueous solution of $NaHCO_3$, brine, and dried (Na_2SO_4). Concentration in vacuo at 37–40 °C provided the sensitive crude product **26** as a colorless oil, which was used in the next step immediately. ^{1}H NMR ($CDCl_3$, 600 MHz): $\delta = 1.18$ (d, $J = 6.6$ Hz, 3H; CH-(CH_3)), 1.69 (quintet, $J = 7.8$ Hz, 2H; CH_2-CH_2-CH_2-COO), 2.10 (dt, $J =$ 6.6, 6.6 Hz, 2H; CH_2-CH_2-CH_2-COO), 2.31 (t, $J = 7.8$ Hz, 2H; CH_2-COO), 2.78 (t, $J = 5.8$ Hz, 2H; CH=CH-CH_2-CH=CH), 2.83 (ddd, $J =$ 16.0, 8.1, 7.3 Hz, 1H; CH_2-CH=CH-CH(CH_3)), 2.89 (ddd, $J = 16.0$, 8.1, 7.2 Hz, 1H; CH_2-CH=CH-CH(CH_3)), 3.37 (m as quintet, $J = 8.4$ Hz, 1H; C$H(CH_3)$), 3.65 (s, 3H; $COOCH_3$), 5.24 (tdd, $J = 10.5$, 9.5, 1.8 Hz, 1H; CH=CH-CH(CH_3)), 5.31–5.44 (m, 4H; CH=CH-CH_2-CH=CH), 5.64 (dtd, $J = 10.5$, 7.8, 1.2 Hz, 1H; CH=CH-CH(CH_3)), 9.52 ppm (d, $J =$ 1.2 Hz, 1H; CHO).

Compound 27

*Method A (from aldehyde **26**)*: KHMDS (291 mg, 1.46 mmol) was added to a stirred solution of hexyltriphenylphosphonium bromide (641 mg, 1.50 mmol) in dry THF (21 mL) at 0 °C under an argon atmosphere. The mixture was stirred for 40 min at 0 °C to ensure complete formation of the orange ylide, then cooled to −115 °C. A solution of crude aldehyde **26** in dry THF (3 mL) was added dropwise, the reaction mixture was stirred for 50 min at −115–100 °C, and then quenched by the addition of a saturated aqueous solution of sodium bicarbonate. The mixture was warmed to room temperature, extracted with diethyl ether, and the combined organic extracts were washed with brine, dried (Na_2SO_4), and concentrated in vacuo. Purification by flash column chromatography on silica gel (2–4% diethyl ether in hexane) gave ester **27** as a colorless oil (81 mg, 65% yield from alcohol **25**). $[\alpha]_D^{20} = -4.89$ ($c = 0.00225$ g mL^{-1} in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz): $\delta = 0.89$ (t, $J = 7.2$ Hz, 3H; 20-H), 1.01 (d, $J = 6.6$ Hz, 3H; CHCH_3), 1.24–1.33 (m, 4H; 18-H, 19-H), 1.36

(sextet, $J=7.2$ Hz, 2H; 17-H), 1.71 (quintet, $J=7.8$ Hz, 2H; 3-H), 2.02–2.09 (m, 2H; 16-H), 2.11 (dt, $J=6.8$, 6.8 Hz, 2H; 4-H), 2.32 (t, $J=7.8$ Hz, 2H; 2-H), 2.75–2.84 (ddd and t overlapping, 3H; 10-H and 7-H; including 2.80, t, $J=6.6$ Hz, 2H; 7-H), 2.87 (ddd, $J=15.6$, 7.5, 6.5 Hz, 1H; 10-H), 3.46 (ddq as sextet, $J=7.5$ Hz, 1H; 13-H), 3.67 (s, 3H; $COOCH_3$), 5.20–5.30 (m, 4H; 11-H, 12-H, 14-H, 15-H), 5.43–5.32 ppm (m, 4H; 5-H, 6-H, 8-H, 9-H); ^{13}C NMR ($CDCl_3$, 150 MHz): $\delta=14.03$ (C-20), 22.01 (C-13-CH_3), 22.56 (C-19), 24.76 (C-3), 25.59 (C-7), 25.81 (C-10), 26.53 (C-4), 27.48 (C-16), 29.44 (C-17), 30.50 (C-13), 31.55 (C-18), 33.42 (C-2), 51.48 (-OCH_3), 125.61 (C-11), 128.07 (C-15), 128.24 (C-5), 128.35 (C-9), 128.84 (C-8 or C-6), 128.88 (C-6 or C-8), 134.12 (C-14), 135.02 (C-12), 174.00 ppm (C=O); MS (EI): m/z (%): 332 $[M]^+$ (9), 248 (16), 217 (12), 164 (13), 147 (11), 133 (23), 119 (29), 107 (43), 93 (100), 91 (44), 81 (58); MS (ESI): m/z (%): 355 $[M+Na]^+$ (100), 333 $[M+H]^+$ (64), 217 (48), 199 (43), 170 (36); HRMS (EI): m/z calcd for $C_{22}H_{36}O_2$: 332.2715 $[M]^+$; found: 332.2722.

*Method B (from aldehyde **11**)*: The synthesis was carried out as described for **24**, using phosphonium salt **20** (167 mg, 0.31 mmol), KHMDS (58 mg, 0.29 mmol), and aldehyde **11** (53 mg, 0.34 mmol). The reaction was completed in 2.5 h at −98–0 °C to give **27** (25 mg, 22% yield).

Compound 28: A 1 M aqueous solution of LiOH (0.2 mL) was added to a stirred solution of **27** (34 mg, 0.102 mmol) in dry THF (1 mL) at room temperature, under an argon atmosphere. Stirring was continued for 24 h, then the reaction mixture was acidified with a 5% aqueous solution of HCl to pH 3, and lipophilic products were extracted with Et_2O. The combined organic extracts were washed with brine, and dried (Na_2SO_4). Concentration in vacuo at 37–39 °C gave acid **28** as a colorless oil (28 mg, 86% yield), which was used in the next step without further purification. ^{1}H NMR ($CDCl_3$, 600 MHz): $\delta=0.89$ (t, $J=6.6$ Hz, 3H; 20-H), 1.01 (d, $J=7.2$ Hz, 3H; CH-CH_3), 1.24–1.33 (m, 4H; 18-H, 19-H), 1.36 (sextet, $J=7.2$ Hz, 2H; 17-H), 1.72 (quintet, $J=7.8$ Hz, 2H; 3-H), 2.01–210 (m, 2H; 16-H), 2.14 (dt, $J=7.2$, 7.2 Hz, 2H; 4-H), 2.37 (t, $J=7.8$ Hz, 2H; 2-H), 2.76–2.85 (ddd and t overlapping, 3H; 10-H and 7-H; including 2.81, t, $J=6.3$ Hz, 2H; 7-H), 2.86 (ddd, $J=15.7$, 7.5, 6.5 Hz, 1H; 10-H), 3.46 (ddq as sextet, $J=7.2$ Hz, 1H; 13-H), 5.20–5.31 (m, 4H; 11-H, 12-H, 14-H, 15-H), 5.32–5.45 (m, 4H; 5-H, 6-H, 8-H, 9-H), 10.55 ppm (brs, 1H; COOH); MS (EI): m/z (%): 318 $[M]^+$ (4), 248 (6), 220 (15), 191 (9), 164 (12), 133 (23), 119 (38), 107 (46), 93 (100), 81 (67); HRMS (EI): m/z calcd for $C_{21}H_{34}O_2$: 318.2559 $[M]^+$; found: 318.2551.

Compound 31: A mixture of acid **28** (22 mg, 0.069 mmol), and fresh carbonyldiimidazole (23 mg, 0.138 mmol) in dry THF (1 mL) was stirred for 2 h at room temperature under an argon atmosphere. A solution of protected ethanolamine **30** (62 mg, 0.208 mmol) in THF (0.5 mL) was added. The reaction mixture was stirred for 1 h and then diluted with water and ethyl acetate. The organic layer was separated and the aqueous layer was extracted with AcOEt. The combined organic extracts were washed with brine, dried (Na_2SO_4), and concentrated in vacuo. The crude product was purified by flash column chromatography on silica gel (25% acetone in hexane) to give **31** as a colorless oil (37 mg, 91% yield). ^{1}H NMR ($CDCl_3$, 600 MHz): $\delta=0.88$ (t, $J=6.9$ Hz, 3H; 20-H), 1.02 (d, $J=6.6$ Hz, 3H; CH-CH_3), 1.07 (s, 9H; $C(CH_3)_3$), 1.24–1.31 (m, 4H; 18-H, 19-H), 1.35 (sextet, $J=7.2$ Hz, 2H; 17-H), 1.69 (quintet, $J=7.2$ Hz, 2H; 3-H), 2.02–2.09 (m, 2H; 16-H), 2.12 (dt, $J=7.2$, 7.2 Hz, 2H; 4-H), 2.13 (t, $J=7.6$ Hz, 2H; 2-H), 2.75–2.84 (ddd and t overlapping, 3H; 10-H and 7-H; including 2.81, t, $J=6.0$ Hz, 2H; 7-H), 2.86 (ddd, $J=15.7$, 7.5, 6.5 Hz, 1H; 10-H), 3.40 (dt, $J=5.4$, 5.4 Hz, 2H; CH_2-NH), 3.45 (sextet, $J=7.5$ Hz, 1H; 13-H), 3.75 (t, $J=5.4$ Hz, 2H; CH_2-OTBDPS), 5.18–5.31 (m, 4H; 11-H, 12-H, 14-H, 15-H), 5.32–5.44 (m, 4H; 5-H, 6-H, 8-H, 9-H), 5.73 (brs, 1H; NH), 7.39 (t, $J=7.8$ Hz, 4H; 3-H, 5-H, ArH), 7.44 (t, $J=7.8$ Hz, 2H; 4-H, ArH), 7.64 ppm (d, $J=7.8$ Hz, 4H; 2-H, 6-H, ArH); MS (EI): m/z (%): 599 $[M]^+$ (3), 542 (100), 296 (15), 276 (9), 242 (38), 199 (89), 164 (35), 91 (24); HRMS (EI): m/z calcd for $C_{39}H_{57}NO_2Si$: 599.4159 $[M]^+$; found: 599.4165.

Compound 32: The synthesis was carried out as described for **18**, using **31** (32 mg, 0.053 mmol) and TBAF (0.07 mL, 0.07 mmol, 1 M solution in THF) in dry THF (2 mL). The reaction was completed in 1 h and the resulting crude oil was purified by flash column chromatography on silica gel (57:40:3 ethyl acetate/hexane/MeOH) to afford **32** (17 mg, 88% yield) as a colorless oil. $[\alpha]_D^{20}=-8.89$ ($c=0.0009$ g mL^{-1} in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 600 MHz): $\delta=0.89$ (t, $J=6.9$ Hz, 3H; 20-H), 1.02 (d, $J=7.2$ Hz, 3H; CH-CH_3), 1.24–1.33 (m, 4H; 18-H, 19-H), 1.35 (sextet, $J=7.2$ Hz, 2H; 17-H), 1.73 (quintet, $J=7.2$ Hz, 2H; 3-H), 2.06 (nonet, $J=6.3$ Hz, 2H; 16-H), 2.12 (dt, $J=7.2$, 7.2 Hz, 2H; 4-H), 2.22 (t, $J=7.6$ Hz, 2H; 2-H), 2.54 (brs, 1H; OH), 2.76–2.84 (ddd and t, overlapping, 3H; 10-H and 7-H, including 2.81, t, $J=6.0$ Hz, 2H; 7-H), 2.87 (ddd, $J=15.0$, 7.5, 6.3 Hz, 1H; 10-H), 3.42 (dt, $J=5.2$, 5.2 Hz, 2H, CH_2N), 3.46 (sextet, $J=7.5$ Hz, 1H; 13-H), 3.73 (t, $J=5.2$ Hz, 2H; CH_2O), 5.20–5.31 (m, 4H; 11-H, 12-H, 14-H, 15-H), 5.44–5.32 (m, 4H; 5-H, 6-H, 8-H, 9-H), 5.88 ppm (brs, 1H; NH); ^{13}C NMR ($CDCl_3$, 150 MHz): $\delta=14.09$ (C-20), 22.04 (C-13-CH_3), 22.59 (C-19), 25.44 (C-3), 25.61 (C-7), 25.84 (C-10), 26.61 (C-4), 27.51 (C-16), 29.46 (C-17), 30.50 (C-13), 31.57 (C-18), 35.92 (C-2), 42.48 (NH-CH_2), 62.70 (-CH_2-OH), 125.61 (C-11), 128.10 (C-9), 128.26 (C-15), 128.43 (C-6), 128.84 (C-5), 129.02 (C-8), 134.12 (C-14), 135.10 (C-12), 174.16 ppm (C=O); MS (EI): m/z (%): 361 $[M]^+$ (2), 328 (12), 218 (9), 178 (14), 125 (20), 103 (33), 85 (100); HRMS (EI): m/z calcd for $C_{23}H_{39}NO_2$: 361.2981 $[M]^+$; found: 361.2973; elemental analysis calcd (%) for $C_{23}H_{39}NO_2$: C 76.40, H 10.87, N 3.87; found: C 76.15, H 10.59, N 4.16.

Compound 33: The synthesis was carried out as described for **24**, using phosphonium salt **23** (217 mg, 0.41 mmol), KHMDS (75 mg, 0.38 mmol), and aldehyde **3** (94 mg, 0.29 mmol) in anhydrous THF (0.8 mL). The reaction was completed in 2.5 h at −98–0 °C to give **33** (84 mg, 58% yield). $[\alpha]_D^{20}=-29.6$ ($c=0.00550$ g mL^{-1} in $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta=0.98$ (d, $J=6.6$ Hz, 3H; CH-CH_3), 1.04 (s, 9H; $Si(Ph)_2C(CH_3)_3$), 1.80 (quintet, $J=7.2$ Hz, 2H; 3-H), 2.23 (tt, $J=6.9$, 2.4 Hz, 2H; 4-H), 2.42 (t, $J=7.5$ Hz, 2H; 2-H), 2.63 (m, 1H; 13-H), 2.74–2.86 (m, 1H; 10-H), 2.86–2.98 (m, 1H; 10-H), 3.08 (quintet, $J=2.4$ Hz, 2H; 7-H), 3.45 (d, $J=6.3$ Hz, 2H; 14-H), 3.67 (s, 3H; $COOCH_3$), 5.24 (tdd, $J=10.8$, 9.6, 0.9 Hz, 1H; 12-H), 5.41 (dtd, $J=10.8$, 6.3, 0.5 Hz, 1H; 11-H), 7.33–7.46 (m, 6H; 3-H, 4-H, 5-H, ArH), 7.68–7.62 ppm (m, 4H; 2-H, 6-H, ArH); ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta=9.7$, 17.2, 17.4, 18.2, 19.3, 23.9, 26.8, 26.9, 32.8, 34.7, 51.5 (OCH_3), 68.3 (CH_2O), 74.2 (C≡C), 75.3 (C≡C), 78.8 (C≡C), 79.1 (C≡C), 124.7, 127.6, 129.5, 133.8, 134.3, 135.6, 173.6 ppm (C=O); MS (ESI): m/z (%): 523 $[M+Na]^+$ (100), 423 (6), 270 (14).

Compound 34: Acetic acid (0.1 mL) and TBAF (0.73 mL, 0.73 mmol, 1 M solution in THF) were added sequentially to a stirred solution of **33** (81 mg, 0.16 mmol) in dry THF (3.2 mL), at 0 °C under an argon atmosphere. Stirring was continued for 10 min at 0 °C and for 20 h at room temperature. The reaction mixture was quenched by the addition of a saturated aqueous solution of NH_4Cl at 0 °C, then extracted with AcOEt. The combined organic extracts were washed with a saturated aqueous solution of $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated in vacuo at 37 °C. Purification by flash column chromatography on silica gel (40% ethyl acetate in hexane) gave **34** as a colorless viscous liquid (32 mg, 76% yield). $[\alpha]_D^{20}=+10.57$ ($c=0.00265$ g mL^{-1} in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta=0.96$ (d, $J=6.9$ Hz, 3H; CH-CH_3), 1.80 (quintet, $J=7.2$ Hz, 2H; 3-H), 2.22 (tt, $J=6.9$, 2.4 Hz, 2H; 4-H), 2.42 (t, $J=7.2$ Hz, 2H; 2-H), 2.70 (m, 1H; 13-H), 2.96 (m as d, $J=7.2$ Hz, 2H; 10-H), 3.10 (quintet, $J=2.4$ Hz, 2H; 7-H), 3.36 (dd, $J=10.5$, 8.1 Hz, 1H; 14-H), 3.50 (dd, $J=10.5$, 5.7 Hz, 1H; 14-H), 3.67 (s, 3H; $COOCH_3$), 5.23 (tdd, $J=10.9$, 9.5, 0.9 Hz, 1H; 12-H), 5.57 ppm (dtd, $J=10.9$, 6.3, 0.5 Hz, 1H; 11-H); ^{13}C NMR ($CDCl_3$, 75 MHz): $\delta=9.7$, 16.7, 17.5, 18.2, 23.8, 32.8, 34.9, 51.5 (OCH_3), 67.5 (CH_2O), 74.5 (C≡C), 75.2 (C≡C), 78.5 (C≡C), 79.2 (C≡C), 126.3 (CH=CH), 133.9 (CH=CH), 173.7 ppm (C=O); MS (ESI): m/z (%): 285 $[M+Na]^+$ (40), 263 $[M+H]^+$ (12), 202 (100), 186 (24).

Compound 35: The synthesis was carried out as described for **26**, using alcohol **34** (32 mg, 0.12 mmol) and Dess–Martin periodinane (155 mg, 0.37 mmol) in anhydrous CH_2Cl_2 (2.4 mL). The reaction was completed in 2 h at 0 °C, and the sensitive aldehyde **35** was used in the next step without further purification. ^{1}H NMR ($CDCl_3$, 300 MHz): $\delta=1.18$ (d, $J=6.6$ Hz, 3H; CH-CH_3), 1.80 (quintet, $J=7.2$ Hz, 2H; 3-H), 2.22 (tt, $J=6.9$, 2.4 Hz, 2H; 4-H), 2.42 (t, $J=7.2$ Hz, 2H; 2-H), 2.82 (m, 1H; 13-H), 2.98 (m as d, $J=7.1$ Hz, 2H; 10-H), 3.10 (quintet, $J=2.4$ Hz, 2H; 7-H), 3.25–3.52 (m, 2H; 14-H), 3.67 (s, 3H; $COOCH_3$), 5.32 (dd, $J=10.8$,

10.0 Hz, 1H; 12-H), 5.71 (td, J=10.8, 6.3 Hz, 1H; 11-H), 9.55 ppm (s, 1H; CHO).

Compound 36

*Method A (from aldehyde **35**)*: The synthesis was carried out as described for **27** (method A), using hexyltriphenylphosphonium bromide (156 mg, 0.37 mmol), KHMDS (70 mg, 0.35 mmol), and aldehyde **35** (31 mg, 0.12 mmol). The reaction was completed in 1 h at −98 °C to give **36** as a viscous oil (23 mg, 58% yield). $[\alpha]_D^{20}=-6.16$ (c=0.00185 g mL^{-1} in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ=0.88 (t, J=6.6 Hz, 3H; 20-H), 1.00 (d, J=6.9 Hz, 3H; CH-CH_3), 1.22–1.38 (m, 6H; 17-H, 18-H, 19-H), 1.80 (quintet, J=7.5 Hz, 2H; 3-H), 2.03 (dt, J=6.9, 6.9 Hz, 2H; 16-H), 2.22 (tt, J=6.9, 2.4 Hz, 2H; 4-H), 2.42 (t, J=7.5 Hz, 2H; 2-H), 2.94 (m, 2H; 10-H), 3.10 (quintet, J=2.4 Hz, 2H; 7-H), 3.39 (sextet, J=7.2 Hz, 1H; 13-H), 3.66 (s, 3H; $COOCH_3$), 5.35–5.15 ppm (m, 4H; 11-H, 12-H, 14-H, 15-H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ=9.7, 14.1, 17.3, 18.2, 21.8, 22.6, 23.9, 27.4, 29.4, 30.4, 31.5, 32.8, 51.5 (OCH_3), 74.2 (C≡C), 75.3 (C≡C), 78.7 (C≡C), 79.1 (C≡C), 122.3 (CH=CH), 128.6 (CH=CH), 133.6 (CH=CH), 136.1 (CH=CH), 173.6 ppm (C=O).

*Method B (from aldehyde **11**)*: The synthesis was carried out as described for **24**, using phosphonium salt **23** (425 mg, 0.77 mmol), KHMDS (144 mg, 0.72 mmol), and aldehyde **11** (131 mg, 0.85 mmol). The reaction was completed in 2.5 h at −98–0 °C to give **36** (50 mg, 21% yield).

Compound 37: The synthesis was carried out as described for **28**, using **36** (23 mg, 0.07 mmol) and a 1 M aqueous solution of LiOH (0.14 mL, 0.14 mmol) in THF (0.25 mL). The reaction was completed in 7 h at room temperature to give **37** as a viscous oil (18 mg, 82% yield). $[\alpha]_D^{20}=-7.08$ (c=0.00226 g mL^{-1} in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ=0.88 (t, J=6.6 Hz, 3H; 20-H), 1.01 (d, J=6.9 Hz, 3H; CH-CH_3), 1.23–1.38 (m, 6H; 17-H, 18-H, 19-H), 1.82 (quintet, J=7.5 Hz, 2H; 3-H), 2.04 (dt, J=6.9, 6.9 Hz, 2H; 16-H), 2.25 (tt, J=6.9, 2.4 Hz, 2H; 4-H), 2.48 (t, J=7.5 Hz, 2H; 2-H), 2.95 (m, 2H; 10-H), 3.11 (quintet, J=2.4 Hz, 2H; 7-H), 3.39 (m as sextet, J=7.0 Hz, 1H; 13-H), 5.15–5.36 ppm (m, 4H; 11-H, 12-H, 14-H, 15-H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ=9.7, 14.1, 17.3, 18.1, 21.8, 22.6, 23.5, 27.5, 29.4, 30.4, 31.5, 32.7, 74.2 (C≡C), 75.5 (C≡C), 78.7 (C≡C), 79.0 (C≡C), 122.3 (CH=CH), 128.6 (CH=CH), 133.6 (CH=CH), 136.1 (CH=CH), 179.1 ppm (C=O); MS (ESI): m/z (%): 337 $[M+Na]^+$ (100), 315 $[M+H]^+$ (4), 271 (16), 239 (44).

Compound 38: The synthesis was carried out as described for **31**, using **37** (6 mg, 0.02 mmol), carbonyldiimidazole (6 mg, 0.04 mmol), and protected ethanolamine **30** (11 mg, 0.04 mmol) in dry THF (0.45 mL) to give **38** as a viscous oil (11 mg, 97% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ=0.88 (t, J=6.9 Hz, 3H; 20-H), 1.01 (d, J=6.6 Hz, 3H; CH-CH_3), 1.07 (s, 9H; $Si(Ph)_2C(CH_3)_3$), 1.22–1.38 (m, 6H; 17-H, 18-H, 19-H), 1.79 (quintet, J=7.2 Hz, 2H; 3-H), 2.04 (dt, J=6.7, 6.7 Hz, 2H; 16-H), 2.17–2.28 (t and tt overlapping, 4H; 2-H, 4-H; including 2.23, t, J=7.5 Hz; 2-H), 2.94 (m, 2H; 10-H), 3.11 (quintet, J=2.4 Hz, 2H; 7-H), 3.34–3.45 (dt and sextet overlapping, 3H; 13-H, CH_2-NH; including 3.39, dt, J=5.1, 5.1 Hz; CH_2-NH), 3.74 (t, J=5.1 Hz, 2H; CH_2OTBDPS), 5.12–5.34 (m, 4H; 11-H, 12-H, 14-H, 15-H), 5.76 (br s, 1H; NH), 7.34–7.46 (m, 6H; 3-H, 4-H, 5-H, ArH), 7.60–7.68 (m, 4H; 2-H, 6-H, ArH); MS (ESI): m/z (%): 618 $[M+Na]^+$ (36), 596 $[M+H]^+$ (38), 518 (100), 505 (44), 287 (96), 253 (52), 219 (44), 69 (38).

Compound 39: The synthesis was carried out as described for **34**, using **38** (11 mg, 0.02 mmol), acetic acid (0.4 mL) and TBAF (0.1 mL, 0.1 mmol, 1 M solution in THF) in dry THF (0.4 mL). The reaction was completed in 20 h and the crude oil was purified by flash column chromatography on silica gel (ethyl acetate) to give **39** (5 mg, 75% yield) as a viscous oil. $[\alpha]_D^{20}=-3.98$ (c=0.00201 g mL^{-1} in $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz): δ=0.88 (t, J=6.9 Hz, 3H; 20-H), 1.01 (d, J=6.9 Hz, 3H; CH-CH_3), 1.21–1.39 (m, 6H; 17-H, 18-H, 19-H), 1.82 (quintet, J=7.2 Hz, 2H; 3-H), 2.05 (dt, J=6.9, 6.9 Hz, 2H; 16-H), 2.23 (tt, J=6.9, 2.4 Hz, 2H; 4-H), 2.33 (t, J=7.5 Hz, 2H; 2-H), 2.95 (m, 2H; 10-H), 3.11 (quintet, J=2.4 Hz, 2H; 7-H), 3.35–3.48 (dt and sextet overlapping, 3H; 13-H, CH_2-NH; including 3.42, dt, J=5.1, 5.1 Hz; CH_2-NH), 3.72 (t, J=5.1 Hz, 2H; CH_2O), 5.14–5.40 (m, 4H; 11-H, 12-H, 14-H, 15-H), 5.99 (br s, 1H; NH); ^{13}C NMR ($CDCl_3$ 75 MHz): δ=9.7, 14.1, 17.3, 18.0, 21.8, 22.6, 24.3, 27.4, 29.4, 30.4, 31.5, 35.1, 42.5 (CH_2NH), 62.6 (CH_2O), 74.3 (C≡C), 75.5 (C≡C), 78.8 (C≡C), 79.3 (C≡C), 122.2 (CH=CH), 128.7 (CH=CH), 133.5 (CH=CH), 136.2 (CH=CH), 173.7 ppm (C=O); MS (ESI): m/z (%): 358 $[M+H]^+$, (100), 255 (9), 198 (12); HRMS (ESI): m/z calcd for $C_{23}H_{36}NO_2$ $[M+H]^+$: 358.2746; found: 358.2751; elemental analysis calcd (%) for $C_{23}H_{35}NO_2$: C 77.27, H 9.87, N 3.92; found: C 77.62, H 10.09, N 4.11.

Radioligand binding assay: For CB1, rat forebrain membranes were prepared according to the procedure of Dodd et al.[69] The binding of the novel anandamide analogues to the cannabinoid receptor was assessed as previously described,[4,44,45,47,64,65] except that the membranes were pretreated with PMSF. Membranes, previously frozen at −80 °C, were thawed on ice. Three volumes of TME buffer (25 mM Tris-HCl buffer, 5 mM $MgCl_2$, and 1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.4) containing 150 μM PMSF (freshly prepared in 2-propanol as a 100 mM stock solution) were added to the stirred suspension. The suspension was incubated at 4 °C, then after 15 min, a second addition of PMSF stock brought the concentration to 300 μM PMSF, and the mixture was incubated for another 15 min. At the end of the second 15 min incubation, the membranes were pelleted and washed three times with TME to remove unreacted PMSF. The pretreated membranes were subsequently used in the binding assay described next. The resulting PMSF-treated membrane (approximately 30 μg) was incubated in a silanized 96-well microtiter plate with TME containing 0.1% essentially fatty acid-free bovine serum albumin (BSA), 0.76 nM [^{3}H]CP-55,940, and various concentrations of anandamide analogues in a final volume of 200 μL. The binding assay was performed at 30 °C for 1 h with gentle agitation. The resultant material was transferred to Unifilter GF/B filter plates, and unbound ligand was removed by using a Packard Filtermate-96 Cell Harvester (Perkin–Elmer Packard, Shelton, CT). Filter plates were washed four times with ice-cold wash buffer (50 mM Tris, 5 mM $MgCl_2$ containing 0.5% BSA, pH 7.4). Radioactivity was determined by using a Packard Top-Count. Data collected from three independent experiments performed with duplicate determinations were normalized between 100 and 0% specific binding for [^{3}H]CP-55,940, determined using buffer and 100 nM CP-55,940, respectively. The normalized data were analyzed using nonlinear regression to yield IC_{50} values. Data from at least three independent experiments performed in duplicate were used to calculate IC_{50} values, which were converted to K_i values by using the assumptions of Cheng and Prusoff.[70]

For CB2 receptor binding studies, membranes were prepared from frozen mouse spleen essentially according to the procedure of Dodd et al.[69] Silanized centrifuge tubes were used throughout to minimize receptor loss due to absorption. The CB2 binding assay was conducted in the same manner as for CB1.

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[1] W. A. Devane, L. Hanus, A. Breuer, R. G. Pertwee, L. A. Stevenson, G. Griffin, D. Gibson, A. Mandelbaum, A. Etinger, R. Mechoulam, *Science* **1992**, *258*, 1946–1949.
[2] T. Sugiura, S. Kondo, A. Sukagawa, S. Nakane, A. Shinoda, K. Itoh, A. Yamashita, K. Waku, *Biochem. Biophys. Res. Commun.* **1995**, *215*, 89–97.
[3] R. Mechoulam, S. Ben-Shabat, L. Hanus, M. Ligumsky, N. E. Kaminski, A. R. Schatz, A. Gopher, S. Almog, B. R. Martin, D. R. Compton, R. G. Pertwee, G. Griffin, M. Bayewitch, J. Barg, Z. Vogel, *Biochem. Pharmacol.* **1995**, *50*, 83–90.
[4] W. A. Devane, F. A. Dysarz, M. R. Johnson, L. S. Melvin, A. C. Howlett, *Mol. Pharmacol.* **1988**, *34*, 605–613.
[5] S. Munro, K. L. Thomas, M. Abu-Shaar, *Nature* **1993**, *365*, 61–65.
[6] L. A. Matsuda, S. J. Lolait, M. J. Brownstein, A. C. Young, T. I. Bonner, *Nature* **1990**, *346*, 561–564.
[7] A. C. Howlett, *Mol. Pharmacol.* **1985**, *27*, 429–436.
[8] R. G. Pertwee, *Pharmacol. Ther.* **1997**, *74*, 129–180.

[9] A. Calignano, G. La Rana, A. Giuffrida, D. Piomelli, *Nature* **1998**, *394*, 277–281.
[10] J. M. Walker, S. M. Huang, N. M. Strangman, K. Tsou, M. C. Sanudo-Pena, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 12198–12203.
[11] B. F. Cravatt, A. H. Lichtman, *J. Neurobiol.* **2004**, *61*, 149–160.
[12] F. Massa, G. Marsicano, H. Hermann, A. Cannich, K. Monory, B. F. Cravatt, G. L. Ferri, A. Sibaev, M. Storr, B. Lutz, *J. Clin. Invest.* **2004**, *113*, 1202–1209.
[13] C. J. Fowler, *Brain Res. Rev.* **2003**, *41*, 26–43.
[14] D. A. Karanian, Q. B. Brown, A. Makriyannis, T. A. Kosten, B. A. Bahr, *J. Neurosci.* **2005**, *25*, 7813–7820.
[15] D. Panikashvili, C. Simeonidou, S. Ben-Shabat, L. Hanus, A. Breuer, R. Mechoulam, E. Shohami, *Nature* **2001**, *413*, 527–531.
[16] R. Gomez, M. Navarro, B. Ferrer, J. M. Trigo, A. Bilbao, I. Del Arco, A. Cippitelli, F. Nava, D. Piomelli, F. Rodriguez de Fonseca, *J. Neurosci.* **2002**, *22*, 9612–9617.
[17] C. M. Williams, T. C. Kirkham, *Physiol. Behav.* **2002**, *76*, 241–250.
[18] P. E. Mallet, R. J. Beninger, *Psychopharmacology* **1998**, *140*, 11–19.
[19] S. Kathuria, S. Gaetani, D. Fegley, F. Valino, A. Duranti, A. Tontini, M. Mor, G. Tarzia, G. La Rana, A. Calignano, A. Giustino, M. Tattoli, M. Palmery, V. Cuomo, D. Piomelli, *Nat. Med.* **2003**, *9*, 76–81.
[20] D. Melck, D. Rueda, I. Galve-Roberh, L. De Petrocellis, M. Guzman, V. Di Marzo, *FEBS Lett.* **1999**, *463*, 235–240.
[21] K. Yamaji, K. P. Sarker, K. Kawahara, S. Iino, M. Yamakuchi, K. Abeyama, T. Hashiguchi, I. Maruyama, *Thromb. Haemost.* **2003**, *89*, 875–884.
[22] B. F. Cravatt, D. K. Giang, S. P. Mayfield, D. L. Boger, R. A. Lerner, N. B. Gilula, *Nature* **1996**, *384*, 83–87.
[23] M. H. Bracey, M. A. Hanson, K. R. Masuda, R. C. Stevens, B. F. Cravatt, *Science* **2002**, *298*, 1793–1796.
[24] T. P. Dinh, D. Carpenter, F. M. Leslie, T. F. Freund, I. Katona, S. L. Sensi, S. Kathuria, D. Piomelli, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 10819–10824.
[25] S. Vandevoorde, D. M. Lambert, *Curr. Pharm. Des.* **2005**, *11*, 2647–2668.
[26] N. Ueda, K. Yamamoto, S. Yamamoto, T. Tokunaga, E. Shirakawa, H. Shinkai, M. Ogawa, T. Sato, I. Kudo, K. Inoue, *Biochim. Biophys. Acta* **1995**, *1254*, 127–134.
[27] A. J. Hampson, W. A. Hill, M. Zan-Phillips, A. Makriyannis, E. Leung, R. M. Eglen, L. M. Bornheim, *Biochim. Biophys. Acta* **1995**, *1259*, 173–179.
[28] K. R. Kozak, L. J. Marnett, *Prostaglandins Leukotrienes Essent. Fatty Acids* **2002**, *66*, 211–220.
[29] V. Di Marzo, *Pharmacol. Res.* **2009**, *60*, 77–84.
[30] N. T. Snider, M. J. Sikora, C. Sridar, T. J. Feuerstein, J. M. Rae, P. F. Hollenberg, *J. Pharmacol. Exp. Ther.* **2008**, *327*, 538–545.
[31] L. M. Bornheim, K. Y. Kim, B. Chen, M. A. Correira, *Biochem. Pharmacol.* **1995**, *50*, 677–686.
[32] M. Yu, D. Ives, C. S. Ramesha, *J. Biol. Chem.* **1997**, *272*, 21181–21186.
[33] K. R. Kozak, S. W. Rowlinson, L. J. Marnet, *J. Biol. Chem.* **2000**, *275*, 33744–33749.
[34] K. R. Kozak, B. C. Crews, J. D. Morrow, L-H. Wang, Y. H. Ma, R. Weinander, P-J. Jakobsson, L. J. Marnett, *J. Biol. Chem.* **2002**, *277*, 44877–44885.
[35] M. D. Jhaveri, D. Richardson, V. Chapman, *Br. J. Pharmacol.* **2007**, *152*, 624–632.
[36] C. J. Fowler, *Br. J. Pharmacol.* **2007**, *152*, 594–601.
[37] M. Hamberg, B. Samuelsson, *J. Biol. Chem.* **1967**, *242*, 5336–5343.
[38] S. Peng, N. M. Okeley, A-L. Tsai, G. Wu, R. J. Kulmacz, W. A. van der Donk, *J. Am. Chem. Soc.* **2002**, *124*, 10785–10796.
[39] K. R. Kozak, J. J. Prusakiewicz, S. W. Rowlinson, D. R. Prudhomme, L. J. Marnett, *Biochemistry* **2003**, *42*, 9041–9049.
[40] G. A. Thakur, S. P. Nikas, C. Li, A. Makriyannis, In *Handbook of Experimental Pharmacology*, (Ed.: R. G.Pertwee), Springer, New York, **2005**, pp. 209–246.
[41] G. A. Thakur, S. P. Nikas, A. Makriyannis, *Mini-Rev. Med. Chem.* **2005**, *5*, 631–640.
[42] S. Pavlopoulos, G. A. Thakur, S. P. Nikas, A. Makriyannis, *Curr. Pharm. Des.* **2006**, *12*, 1751–1769.
[43] V. Abadji, S. Lin, G. Taha, G. Griffin, L. A. Stevenson, R. G. Pertwee, A. Makriyannis, *J. Med. Chem.* **1994**, *37*, 1889–1893.
[44] A. D. Khanolkar, V. Abadji, S. Lin, W. A. G. Hill, G. Taha, K. Abouzid, Z. Meng, P. Fan, A. Makriyannis, *J. Med. Chem.* **1996**, *39*, 4515–4519.
[45] S. Lin, A. D. Khanolkar, P. Fan, A. Goutopoulos, C. Qin, D. Papahadjis, A. Makriyannis, *J. Med. Chem.* **1998**, *41*, 5353–5361.
[46] A. Goutopoulos, P. Fan, A. D. Khanolkar, X. Q. Xie, S. Lin, A. Makriyannis, *Bioorg. Med. Chem.* **2001**, *9*, 1673–1684.
[47] C. Li, W. Xu, S. K. Vadivel, P. Fan, A. Makriyannis, *J. Med. Chem.* **2005**, *48*, 6423–6429.
[48] F. Yao, C. Li, S. K. Vadivel, A. L. Bowman, A. Makriyannis, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5912–5915.
[49] T. Sheskin, L. Hanus, J. Slager, Z. Vogel, R. Mechoulam, *J. Med. Chem.* **1997**, *40*, 659–667.
[50] X. Tian, J. Guo, F. Yao, D.-P. Yang, A. Makriyannis, *J. Biol. Chem.* **2005**, *280*, 29788–29795.
[51] J. Barnett-Norris, D. P. Hurst, D. L. Lynch, F. Guarnieri, A. Makriyannis, P. H. Reggio, *J. Med. Chem.* **2002**, *45*, 3649–3659.
[52] M. van der Stelt, J. A. van Kuik, M. Bari, G. van Zadelhoff, B. R. Leeflang, G. A. Veldink, A. Finazzi-Agro, J. F. G. Vliegenthart, M. Maccarrone, *J. Med. Chem.* **2002**, *45*, 3709–3720.
[53] P. H. Reggio, *Curr. Pharm. Des.* **2003**, *9*, 1607–1633.
[54] J. Barnett-Norris, F. Guarnieri, D. P. Hurst, P. H. Reggio, *J. Med. Chem.* **1998**, *41*, 4861–4872.
[55] M. G. Malkowski, S. L. Ginell, W. L. Smith, R. M. Garavito, *Science* **2000**, *289*, 1933–1937.
[56] E. J. Zylstra, M. W.-L. She, W. A. Salamant, J. W. Leahy, *Synlett* **2007**, 623–627.
[57] J. Cossy, D. Bauer, V. Bellosta, *Tetrahedron* **2002**, *58*, 5909–5922.
[58] Y. Guan, J. Wu, L. Sun, W.-M. Dai, *J. Org. Chem.* **2007**, *72*, 4953–4960.
[59] D. P. Papahatjis, T. Kourouli, V. R. Nahmias, *Lett. Org. Chem.* **2006**, *3*, 45–47.
[60] K. C. Nicolaou, D. P. Papahatjis, D. A. Claremon, R. E. Dolle III, *J. Am. Chem. Soc.* **1981**, *103*, 6967–6969.
[61] E. J. Corey, J. W. Suggs, *Tetrahedron Lett.* **1975**, *16*, 2647–2650.
[62] K. C. Nicolaou, R. A. Daines, J. Uenishi, W. S. Li, D. P. Papahatjis, T. K. Chakraborty, *J. Am. Chem. Soc.* **1988**, *110*, 4672–4685.
[63] a) C. Bourne, S. Roy, J. L. Wiley, B. R. Martin, B. F. Thomas, A. Mahadevan, R. K. Razdan, *Bioorg. Med. Chem.* **2007**, *15*, 7850–7864; b) C. A. Brown, V. K. Ahuja, *J. Org. Chem.* **1973**, *38*, 2226–2230; c) C. A. Brown, V. K. Ahuja, *J. Chem. Soc. Chem. Commun.* **1973**, *15*, 553–554.
[64] D. P. Papahatjis, S. P. Nikas, T. Kourouli, R. Chari, W. Xu, R. G. Pertwee, A. Makriyannis, *J. Med. Chem.* **2003**, *46*, 3221–3229.
[65] D. P. Papahatjis, V. R. Nahmias, S. P. Nikas, T. Andreou, S. O. Alapafuja, A. Tsotinis, J. Guo, P. Fan, A. Makriyannis, *J. Med. Chem.* **2007**, *50*, 4048–4060.
[66] D. G. Deutsch, S. A. Chin, *Biochem. Pharmacol.* **1993**, *46*, 791–796.
[67] F. Desarnaud, H. Cadas, D. Piomelli, *J. Biol. Chem.* **1995**, *270*, 6030–6035.
[68] C. S. Breivogel, D. E. Selley, S. R. Childers, *J. Biol. Chem.* **1998**, *273*, 16865–16873.
[69] P. R. Dodd, J. A. Hardy, A. E. Oakley, J. A. Edwardson, E. K. Perry, J.-P. Delaunoy, *Brain Res.* **1981**, *226*, 107–118.
[70] Y. C. Cheng, W. H. Prusoff, *Biochem. Pharmacol.* **1973**, *22*, 3099–3102.

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