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The Synthesis of *N*-Vanillyl-arachidonoyl-amide (Arvanil) and its Analogs: An Improved Procedure for the Synthesis of the Key Synthon Methyl 14-Hydroxy-(all-*cis*)-5,8,11-tetradecatrienoate

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Abstract—Several arvanil analogs were synthesized where the end *n*-pentyl chain was branched and carried substituents at the terminal end of the chain. A high yielding total synthesis of these analogs was developed from methyl hex-5-ynoate, which was converted to the synthon **6** in a facile five step sequence (overall yield, 33%). The pharmacological profile of these novel analogs suggests that they may be acting through a novel site of action for anandamide (arachidonylethanolamide, AEA).

Introduction

Since the discovery of anandamide (arachidonylethanolamide, AEA) as one of the endogenous ligands which bind to the G-protein coupled receptor (CB1) for cannabinoids, some progress has been made in understanding their mechanism of action. It appears that a family of fatty acid derivatives interacts in different degrees with the cannabinoid receptors.[1–8] AEA exhibits cannabimimetic effects similar to those of Δ^9-tetrahydrocannabinol (Δ^9-THC), the active constituent of marijuana but there are some pharmacological differences between AEA and Δ^9-THC, for example the onset of action of AEA is faster than that of Δ^9-THC but the duration of action is much shorter.[4] This is attributed to the rapid enzymatic hydrolysis of the amide bond in AEA by amidases.[9] However, several analogs of AEA have been developed which are metabolically stable and have much greater binding affinities than AEA. Their pharmacological activity has substantiated the similarities between Δ^9-THC and AEA.[8] It is noteworthy, that several other fatty acid ethanolamides besides AEA are present in the brain, two of which, gamma-linolenyl ethanolamide and docosatetraenyl ethanolamide have comparable binding affinities to AEA but the others do not bind to the CB1 receptor.[10] Another peripherally expressed cannabinoid receptor subtype CB2 was identified from macrophages in the spleen, canine gut and pancreas and it has been shown that 2-arachidonyl glycerol (2-Ara-Gl) acts at this receptor.[8c,11–13] It has since been found in the brain and binds to both CB1 and CB2 receptors.[13] Like AEA, 2-Ara-Gl is found together with a family of related fatty acid glycerol esters. Furthermore, the potency of 2-Ara-Gl was increased in various tests in the presence of these related 2-acyl-glycerols which when tested alone do not show any significant activity. This effect[8c] has been referred to as the 'entourage effect' which may represent a route for molecular regulation of endogenous cannabinoid activity. In summary both AEA and 2-Ara-Gl interact with the cannabinoid receptors, in the brain as well as the periphery, to produce a myriad of pharmacological effects.

In an effort to further examine the interactions of cannabinoid receptors (endocannabinoids) with other receptors in the brain, Di Marzo et al. reported on the interactions with vanilloid receptors which are present in sensory neurons[14] and some peripheral tissues.[15] Capsaicin [*N*-(3-methoxy-4-hydroxy)-benzyl-8-methyl-6-*trans*-nonenamide] which is the pungent ingredient of chili pepper exerts its vasodilatory, analgesic and anti-inflammatory properties by activating the vanilloid receptor (VR1).[15,16] Another fatty acyl chain analog of capsaicin called olvanil, [*N*-(3-methoxy-4-hydroxy)-benzyl-*cis*-9-octadecenoamide] was also found to exhibit vasodilatory, analgesic and anti-inflammatory properties but to a lesser extent than capsaicin.[17–19] Based on the differences in their pharmacological behavior it was postulated that this synthetic analog might be activating a different vanilloid receptor subtype.[19]

Keywords: cannabinoid receptor; vanilloid receptors; anandamide; arvanil analogs.
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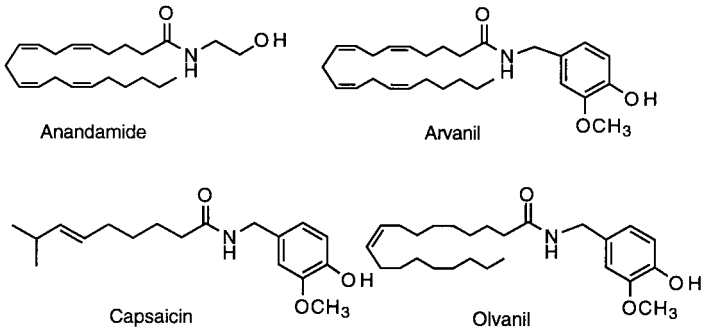


Figure 1. Structures of anandamide, arvanil, capsaicin and olvanil.

Di Marzo et al. reported[14] that olvanil is a potent inhibitor of AEA facilitated transport, blocking the uptake but not the hydrolysis of AEA. Olvanil was also found to be a weak agonist of CB1, but not CB2 receptors. Further modification of olvanil, by Di Marzo et al. led to the development of arvanil (*N*-Vanillyl-arachidonyl-amide)[20] (Fig. 1). Arvanil is a unique vanilloid/cannabinoid hybrid, which is metabolically stable, a novel ligand of vanilloid receptors and cannabinoid CB1, but not CB2, receptors and it inhibits the uptake of the proposed endogenous ligand of CB1 receptor, AEA. It was proposed that this compound may have therapeutic potential for use as analgesics, vasodilators, and anti-inflammatory or anti-tumor agents, and due to its interaction with the endogenous cannabinoid system may lead to novel class of cannabinoids with potential therapeutic applications.[14,20]

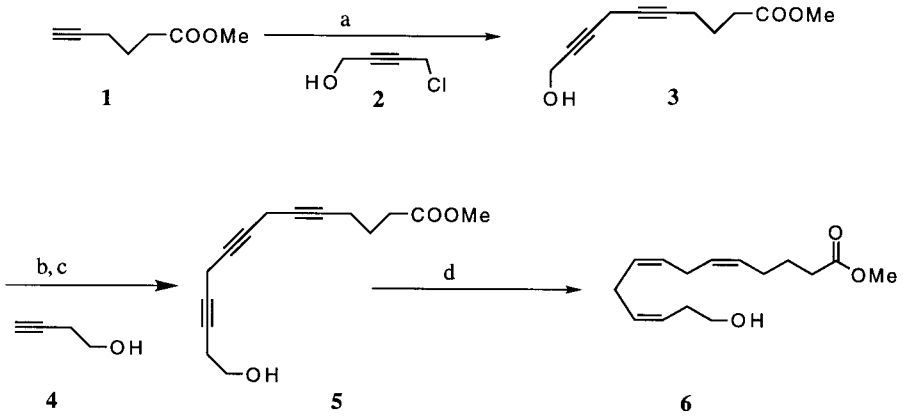
In order to optimize the activity of arvanil, we synthesized analogs **10**, **13**, **22** and **23** where the end *n*-pentyl chain in arvanil was branched and carried substituents at the terminal end of the chain. Similar structural changes in THCs and AEA series have led to analogs with potent cannabimimetic activity.[21–23]

The biological evaluation of analogs **10**, **13**, **22** and **23** was carried out and in summary they exhibit a pharmacological profile that is more similar to AEA than capsaicin. In binding studies,[8c,22] in the displacement of [^{3}H]CP 55,940 from rat whole brain P_2 membrane preparations (CB1), compounds **10**, **13**, **22** and **23** had Ki's=262, 790, 33 and 67 nM, respectively. The CB1 binding affinity of AEA was 89 nM[22] and arvanil was reported to be almost 4-fold more potent than AEA in the displacement of [^{3}H]SR 141716A from membrane preparations from N18TG2 cells.[20] However, in in vivo potency (tetrad tests)[22] the arvanil dimethylheptyl analog **10** (arvanil DMH) was very potent (~100-fold more potent than AEA), and was equiactive with arvanil but its binding affinity to CB1 receptors was one third that of AEA. Analog **22** was more potent than arvanil whereas analogs **13** and **23** were less active than arvanil. Their in vivo potency was much greater than what would be expected based on their binding affinity and efficacy at CB1 and VR1 receptors, thus suggesting that they may be acting through a novel site of action for AEA. The details of their pharmacological evaluation will be published elsewhere.

This series of arvanil analogs is important as it may lead to a novel class of cannabimimetics with potential therapeutic applications, and in this paper the details of the synthetic route to these analogs are described.

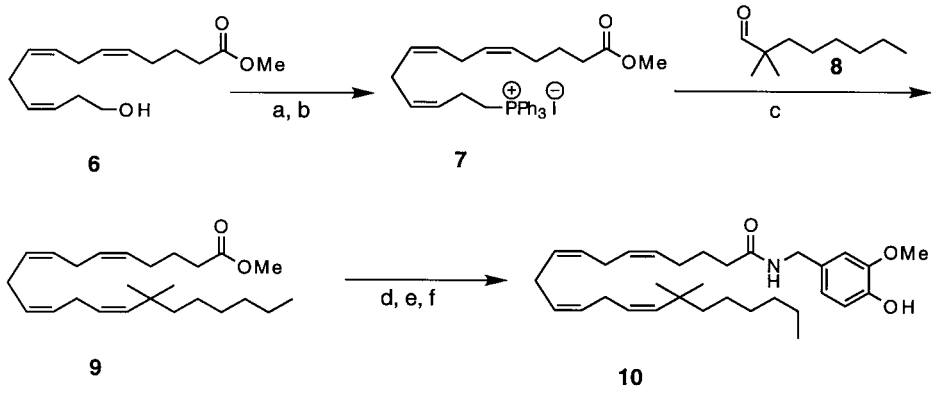
Results

In the synthesis of the arvanil analogs the alcohol, methyl 14-hydroxy-(all-*cis*)-5,8,11-tetradecatrienoate **6**, was the key intermediate (Scheme 1). In general our strategy involves



(a) CuI, NaI, K_2CO_3, **2**, DMF, 18 h, 23 °C, 87%; (b) CBr_4, PPh_3, CH_2Cl_2, -20 °C to 23 °C, 1 h, 92%; (c) CuI, NaI, K_2CO_3, **4**, DMF, 18 h, 23 °C, 85%; (d) P-2 Ni, Ethanol, 3 h, 23 °C, 50%.

Scheme 1.



(a) I_2, PPh_3, Imidazole, Ether/CH_3CN, 0 °C to 23 °C, 1 h, 98%; (b) PPh_3, CH_3CN, reflux, 18 h, 90%; (c) NHMDS, THF/HMPA, **9**, -78 °C to 23 °C, 2 h, 61%; (d) LiOH, MeOH/H_2O, 23 °C, 18 h, 94%; (e) Oxalyl chloride, CH_2Cl_2, 0 °C, 2 h, 100%; (f) Vanillylamine, CH_2Cl_2, 0 °C to 23 °C, 18 h, 51%

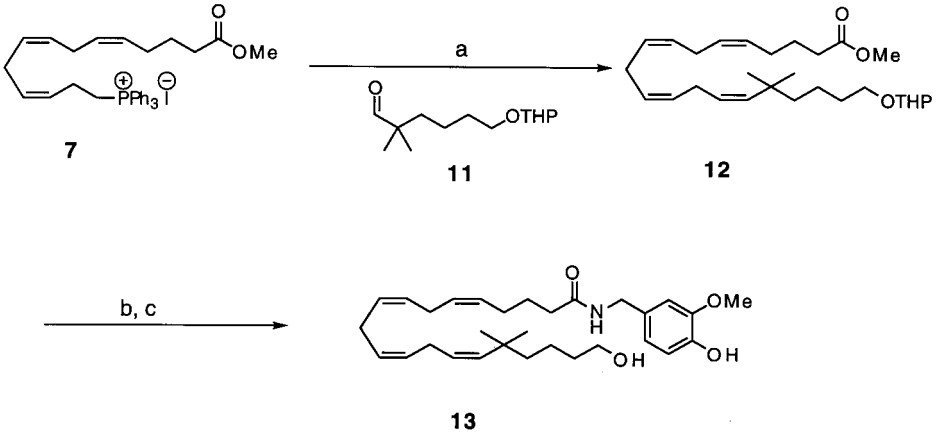
Scheme 2.

conversion of **6** to the corresponding phosphonium iodide (Scheme 2), the ylide of which was treated with the desired aldehyde in a Wittig reaction, to give the required ester. Hydrolysis of the ester followed by treatment with oxalyl chloride and vanillylamine gave the desired arvanil analogs.

Several procedures have been reported for the synthesis of the alcohol **6** from arachidonic acid.[24] Our previously reported synthesis of this synthon was a 11 step sequence (overall yield, 14%),[25] but here we report a much more flexible and shorter route to **6**, a five step sequence (overall yield, 33%), which allows us to efficiently synthesize different AEA derivatives.

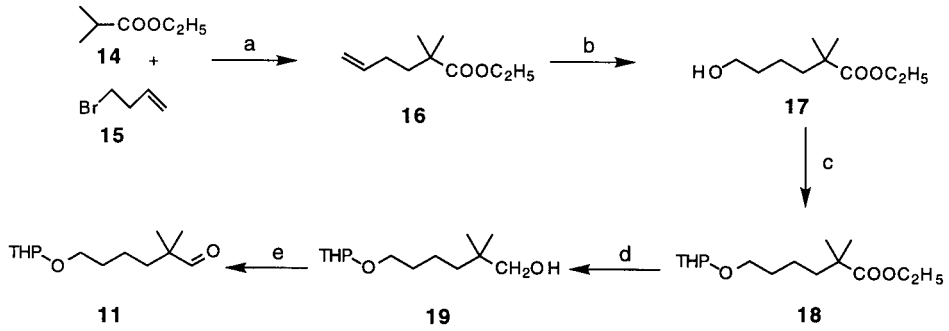
Methyl 14-hydroxy-(all-*cis*)-5,8,11-tetradecatrienoate **6** was synthesized (Scheme 1) starting from hex-5-ynoic acid which was converted into its methyl ester **1** by treatment with *p*-TSA in MeOH. The ester **1** was then coupled with the known 4-chloro-but-2-yn-1-ol **2**[26] in the presence of CuI as a catalyst to give 10-hydroxy-deca-5,8-diynoic acid methyl ester **3**. Bromination with CBr_4/Ph_3P gave the propargylic bromide which was then coupled with but-3-yn-1-ol in the presence of CuI as a catalyst to provide the triynoic acid methyl ester **5**.[27] Partial reduction of the triyne **5** over 'nickel boride' catalyst provided the key intermediate **6**.[28]

Alcohol **6** was converted to the iodide quantitatively by treatment with triphenyl phosphine/I_2.[29] The iodide was then converted to the corresponding phosphonium iodide **7** by refluxing with triphenyl phosphine. Treatment of the phosphonium iodide **7** with NaHMDS converted it into the ylide which was then allowed to react with the aldehyde **8** (which we had previously synthesized from ethyl isobutyrate)[22] in a Wittig reaction to give the ester derivative **9**.[22] Hydrolysis of the ester **9** with LiOH followed by treatment with oxalyl chloride[22] and vanillylamine formed the target analog **10** (Scheme 2). Similarly, the ylide of **7** on treatment with aldehyde **11** gave the ester derivative **12** (Scheme 3).



(a) NHMDS, THF/HMPA, **11**, -78 °C to 23 °C, 2 h, 50%; (b) LiOH, MeOH/H_2O, 23 °C, 18 h, 94%; (c) EDCI, DMAP, Vanillylamine, CH_2Cl_2, 0 °C to 23 °C, 18 h, 26%.

Scheme 3.

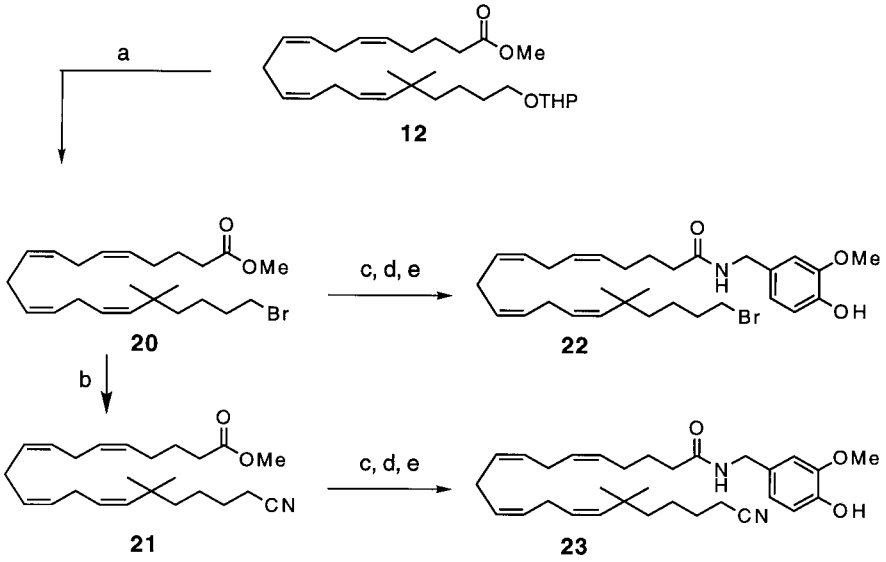


Scheme 4.

The synthesis of the aldehyde **11** was achieved following a modification of the sequence we developed for the synthesis of **8** (Scheme 4) from ethyl isobutyrate (**14**)[22] by alkylation with 4-bromobut-1-ene (**15**) to give **16**. Hydroboration of the ester **16** formed **17**,[30] the hydroxyl group of which was protected as the THP derivative **18**.[31] Reduction of the carboxyester group[32] in **18** with LAH formed the alcohol **19** which was oxidized with PCC[33] to the desired aldehyde **11** in an overall yield of 50%.

Hydrolysis of the ester **12** (Scheme 3) with LiOH followed by coupling with vanillylamine in the presence of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDCI) formed the target analog **13**.[22] Interestingly the THP protecting group was also removed during this coupling reaction. Ester derivative **12** was converted into the corresponding bromo derivative **20** (Scheme 5) by treatment with Ph_3P/Br_2.[34] The bromide **20** was then transformed into the cyano derivative **21** by treatment with KCN.[35] Hydrolysis of the esters **20** and **21** with LiOH followed by treatment with oxalyl chloride and vanillylamine formed the target analogs **22** and **23**, respectively.

In summary, we have developed a facile five step sequence for the synthesis of the key synthon, methyl 14-hydroxy-(all-*cis*)-5,8,11-tetradecatrienoate (**6**) from commercially available hex-5-ynoic acid (overall yield, 33%). This synthon (**6**) was used to synthesize novel arvanil analogs which are important for the study of 'endocannabinoids' and may lead to a novel class of cannabinoids with potential therapeutic use. It is noteworthy that synthon **6** could also provide an entry to the synthesis of novel analogs in other fields where arachidonic acid plays an important role.



Scheme 5.

Experimental

All reagents were of commercial quality, reagent grade, and used without further purification. Anhydrous solvents were purchased from Aldrich and used without further purification. All reactions were carried out under N_2 atmosphere. ^{1}H NMR spectra were recorded on a JEOL Eclipse 300 spectrophotometer using $CDCl_3$ as the solvent with tetramethylsilane as an internal standard. Thin-layer chromatography (TLC) was carried out on Baker Si 250F plates and was developed upon treatment with phosphomolybdic acid (PMA). Flash column chromatography was carried out on EM Science silica gel 60. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA, and were found to be within ±0.4% of calculated values for the elements shown, unless otherwise noted.

Methyl hex-5-ynoate-1. A stirred solution of hex-5-ynoic acid (5 g, 44.6 mmol), *p*-TSA (58 mg, 0.3 mmol) in MeOH (8 mL) and CH_2Cl_2 (17 mL) was refluxed for 24 h. The mixture was quenched with saturated $NaHCO_3$ and the organic layer was separated. The aqueous layer was extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and evaporated under reduced pressure to yield the methyl ester (5.46 g, 97%). ^{1}H NMR δ 1.84 (quint, 2H, J=7.2 Hz), 1.96 (t, 1H, J=2.75 Hz), 2.25 (dt, 2H, J=7.2, 2.75 Hz), 2.45 (t, 2H, J=7.2 Hz), 3.67 (s, 3H).

4-Chloro-but-2-yn-1-ol 2.[26] To a stirred solution of but-2-yn-1,4-diol (86 g, 1 mol) and pyridine (89 mL, 1.1 mol) in benzene (100 mL) was added thionyl chloride (80.23 mL, 1.1 mol) dropwise over a period of 6 h, while the temperature was maintained between 10–20°C. The reaction mixture was then stirred overnight at 25°C. The mixture was poured into ice water (250 mL) and the benzene layer was separated. The aqueous layer was extracted with ether (4×100 mL) and the combined organic layers were washed with saturated $NaHCO_3$, followed by water. The ether extract was dried over $MgSO_4$ and the solvent was removed under vacuum. Purification of the residual oil by distillation (80°C, 5 mmHg) provided the title compound as a colorless liquid (34.5 g, 33%). ^{1}H NMR δ 4.18 (t, 2H, J=1.9 Hz), 4.33 (dt, 2H, J=6.3, 1.9 Hz).

10-Hydroxy-deca-5,8-diynoic acid methyl ester 3.[27] A mixture of K_2CO_3 (5.94 g, 43 mmol), CuI (4.1 g, 22 mmol), 4-chloro-but-2-yn-1-ol (**2**; 4.47 g, 43 mmol), NaI (6.44 g, 43 mmol) and methyl hex-5-ynoate (5.46 g, 43 mmol) in DMF (86 mL) was stirred overnight at 25°C. The mixture was diluted with ethyl acetate and plugged through a pad of celite. It was washed with saturated NH_4Cl followed by brine. The solution was dried over $MgSO_4$ and the solvent was evaporated under vacuum. The oily residue was dissolved in hexanes/ethyl acetate (1/1) and plugged through a pad of silica gel to provide a yellowish oil (7.2 g, 87%) which was used in the subsequent step without further purification since some of the diyne decomposed upon flash chromatography. ^{1}H NMR δ 1.81 (quint, 2H, J=6.7 Hz), 2.23 (tt, 2H, J=6.7, 2.2 Hz), 2.43 (t, 2H, J=7.4 Hz), 3.17 (quint, 2H, J=2.2 Hz), 3.67 (s, 3H), 4.25 (dt, 2H, J=6, 2.2 Hz).

14-Hydroxy-tetradeca-5,8,11-triynoic acid methyl ester 5. To a stirred solution of diyne **3** (8.65 g, 44.6 mmol) and CBr_4 (17.74 g, 53.5 mmol) in CH_2Cl_2 (80 mL) cooled to −20°C, a solution of triphenylphosphine (14.6 g, 55.7 mmol) in CH_2Cl_2 (40 mL) was added dropwise. After the addition the cooling bath was removed and the mixture was stirred for an additional 1 h. Hexanes/ethyl acetate (4/1) was then added until triphenylphosphine oxide precipitated. The mixture was plugged through a pad of silica gel to yield methyl 10-bromo-deca-5,8-diynoate as a colorless oil (10.54 g, 92%). Attempts to further purify it by flash chromatography resulted in partial decomposition of the bromide. Hence, it was used as such in the subsequent reaction. ^{1}H NMR δ 1.81 (quint, 2H, J=7.1 Hz), 2.23 (tt, 2H, J=7.1, 2.5 Hz), 2.43 (t, 2H, J=7.1 Hz), 3.20 (quint, 2H, J=2.5 Hz), 3.67 (s, 3H), 3.90 (t, 2H, J=2.5 Hz).

A mixture of K_2CO_3 (9.67 g, 70 mmol), CuI (6.66 g, 35 mmol), but-3-yn-1-ol (**4**; 5.3 mL, 70 mmol), NaI (10.50 g, 70 mmol) and methyl 10-bromo-deca-5,8-diynoate (17.99 g, 70 mmol) in DMF (140 mL) was stirred overnight at 25°C. The mixture was diluted with ethyl acetate and plugged through a pad of celite. It was washed with saturated NH_4Cl followed by brine. The solution was dried over $MgSO_4$ and the solvent was evaporated under vacuum. The oily residue was dissolved in hexanes/ethyl acetate (1/1) and plugged through a pad of silica gel to provide compound **5** as a yellowish oil (14.63 g, 85%) which was used in the subsequent step without further purification. Attempts to purify by flash chromatography resulted in partial decomposition of the triyne. ^{1}H NMR δ 1.80 (quint, 2H, J=6.7 Hz), 2.23 (tt, 2H, J=6.9, 2.2 Hz), 2.42 (t, 2H, J=7.1 Hz), 2.42–2.48 (m, 2H), 3.14 (dq, 4H, J=6.3, 2 Hz), 3.67 (s, 3H), 3.69 (t, 2H, J=6 Hz).

14-Hydroxy-tetradeca-all-*cis*-5,8,11-trienoic acid methyl ester 6.[28] To a stirred solution of $Ni(OAc)_2$ (14.93 g, 60 mmol) in EtOH (450 mL) was added ethylenediamine (4 mL, 60 mmol) followed by a 1 M solution of $NaBH_4$ (60 mL). The mixture was stirred at 25°C for 0.5 h. The triyne **5** (6.6 g, 26.8 mmol) was added to the reaction mixture and a H_2 atmosphere (balloon) was kept over the reaction mixture. It was stirred for 3 h at 25°C and the solvent was removed in vacuo. The residue was dissolved in hexanes/ethyl acetate (1/1) and plugged through a pad of silica gel. Purification by flash chromatography (eluting with hexanes/ethyl acetate 2/1) provided the desired triene **6** as a colorless oil (3.38 g, 50%). ^{1}H NMR δ 1.70 (quint, 2H, J=7.1 Hz), 1.98–2.14 (m, 2H), 2.27–2.39 (m, 2H), 2.32 (t, 2H, J=7.1 Hz), 2.79 (dd, 2H, J=5.2, 5.2 Hz), 2.84 (dd, 2H, J=14.0, 5.8 Hz), 3.65 (t, 2H, J=6.6 Hz), 3.66 (s, 3H), 5.29–5.58 (m, 6H).

Methyl 14-triphenylphosphonio-tetradeca-all-*cis*-5,8,11-trienoate iodide 7. To a stirred solution of triphenylphosphine (456 mg, 1.74 mmol) and imidazole (118 mg, 1.74 mmol) in Et_2O/CH_3CN (5/1.7 mL) cooled to 0°C, I_2 was added (441 mg, 1.74 mmol) in several portions. The resulting slurry was warmed to 25°C and stirred for 20 min. It was again cooled to 0°C and the alcohol **6** was added slowly. The mixture was warmed to 25°C after addition and stirred for 1 h. It was diluted with pentane/ether (4/1) and plugged through a pad of silica gel to yield the iodide as a colorless oil (562 mg, 98%). ^{1}H NMR δ 1.70 (quint, 2H, J=7.4 Hz), 1.95–2.17 (m, 4H), 2.32 (t, 2H,

J=7.4 Hz), 2.66 (q, 2H, J=7.4 Hz), 2.80 (m, 2H), 3.15 (t, 2H, J=7.2 Hz), 3.66 (s, 3H), 5.29–5.58 (m, 6H).

A solution of triphenylphosphine (2.25 g, 8.6 mmol) and the above iodide (2.83 g, 7.82 mmol) in acetonitrile (50 mL) was refluxed overnight. The solvent was removed under reduced pressure and the oily residue was purified by washing and decanting with hexanes/benzene (1/1; 80 mL). The solvent was removed and the oily residue was heated in a vacuum oven overnight at 60°C to yield **7** as a yellow gum (90%) which was used in the subsequent step without further purification. ^{1}H NMR δ 1.66 (quint, 2H, J=7.4 Hz), 2.04 (q, 2H, J=7.4 Hz), 2.29 (t, 2H, J=7.4 Hz), 2.39–2.52 (m, 2H), 2.58 (t, 2H, J=7.2 Hz), 2.63 (t, 2H, J=6.6 Hz), 3.64 (s, 3H), 3.80–3.90 (m, 2H), 5.14–5.67 (m, 6H), 7.64–7.73 (m, 6H), 7.78–7.88 (m, 9H).

16,16-Dimethyl-docosa-5,8,11,14-all-*cis*-tetraenoic acid methyl ester 9.[22] To a stirred solution of the phosphonium salt **7** (686 mg, 1.1 mmol) in THF/HMPA (6/1 mL) cooled to −10°C was added dropwise a 1 M solution of NHMDS in THF (1.1 mL). The mixture was stirred at 0°C for 30 min and cooled to −78°C. The aldehyde **8** (171 mg, 1.1 mmol)[22] was added dropwise in THF (1.5 mL) to the reaction mixture. The cooling bath was removed and it was left to warm to 25°C over 2 h. It was quenched with hexanes and the mixture was plugged through a pad of silica gel (eluting with ethyl acetate/hexanes 1/4). The filtrate was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The oily residue was purified by flash chromatography (3% EtOAc/97% hexanes) to yield the tetraene **9** as a colorless oil (0.25 g, 61%). ^{1}H NMR δ 0.87 (t, 3H, J=6.6 Hz), 1.09 (s, 6H), 1.18–1.35 (m, 10H), 1.70 (quint, 2H, J=7.4 Hz), 2.00–2.11 (m, 2H), 2.32 (t, 2H, J=7.4 Hz), 2.70–2.91 (m, 6H), 3.66 (s, 3H), 5.12–5.25 (m, 2H), 5.30–5.41 (m, 6H).

16,16-Dimethyl-docosa-5,8,11,14-all-*cis*-tetraenoic acid (4-hydroxy-3-methoxy-benzyl) amide 10.[22] To a stirred solution of ester **9** (250 mg, 0.67 mmol) in MeOH (33 mL) and water (11 mL) was added $LiOH{\cdot}H_2O$ (190 mg, 4.8 mmol) and the reaction mixture was stirred at 55°C overnight. It was diluted with ether and acidified with 10% HCl. The layers were separated and the aqueous layer was extracted with ether. The combined organic layers were dried over $MgSO_4$ and evaporated to yield the crude acid (224 mg, 93%) which was used directly. The acid (224 mg, 0.62 mmol) was dissolved in CH_2Cl_2 (7 mL) and cooled to 0°C. A 2 M solution of oxalyl chloride in CH_2Cl_2 (0.67 mL) was added dropwise followed by 2 drops of DMF. The ice bath was removed and the mixture was stirred at 25°C for 2 h. The solvent was evaporated under vacuum. A solution of the acid chloride in CH_2Cl_2 (3 mL) was added to a solution of 4-hydroxy-3-methoxy benzylamine (1 g, 5.2 mmol) in CH_2Cl_2 (5 mL) at 0°C. The ice bath was removed and the mixture was stirred at 25°C overnight. It was diluted with CH_2Cl_2 and washed with brine. The organic layer was dried over $MgSO_4$ and then concentrated in vacuo. The crude product was purified by flash chromatography (hexanes/EtOAc 2/1) to yield the amide **10** (170 mg, 55%). ^{1}H NMR δ 0.87 (t, 3H, J=6.9 Hz), 1.08 (s, 6H), 1.22–1.35 (m, 10H), 1.73 (quint, 2H, J=7.4 Hz), 2.00–2.12 (m, 2H), 2.20 (t, 2H, J=7.4 Hz), 2.77 (t, 2H, J=5.5 Hz), 2.81 (t, 2H, J=5.5 Hz), 2.92 (t, 2H, J=5.5 Hz), 3.87 (s, 3H), 4.34 (d, 2H, J=5.8 Hz), 5.12–5.25 (m, 2H), 5.30–5.41 (m, 6H), 5.60 (s, 1H), 5.63 (br s, 1H), 6.74–6.87 (m, 3H). Anal. Calcd for $C_{31}H_{44}O_3N_20.6H_2O$: C, 75.87; H, 9.99. Found: C, 75.89; H, 9.89.

2,2-Dimethyl-(tetrahydro-pyran-6-yloxy)-hexanal 11. *Ethyl 2,2-dimethyl-hex-5-enoate **16**:*[22] To a stirred solution of diisopropylamine (5.7 mL, 41 mmol) in THF cooled to −78°C was added dropwise a 2.5 M solution of *n*-BuLi in hexanes (16.4, 41 mL). The reaction mixture was stirred for 30 min and ethyl isobutyrate **14** (5.1 mL, 37 mmol) was added dropwise at −78°C. The mixture was stirred for 30 min and 4-bromo-but-1-ene (**15**; 5.0 g, 37 mmol) in HMPA (7.4 mL) was added dropwise. Upon addition, the mixture was left to warm to 25°C and was stirred for 2 h. It was quenched with water and extracted with ethyl acetate. The organic extract was then washed with 10% HCl, saturated $NaHCO_3$ and dried over $MgSO_4$. The residual oil was plugged through a pad of silica gel (4/1 hexanes/EtOAc) to yield a slightly yellow oil (5.54 g, 88%). ^{1}H NMR δ 1.17 (s, 6H), 1.24 (t, 3H, J=7.2 Hz), 1.56–1.63 (m, 2H), 1.94–2.02 (m, 2H), 4.10 (q, 2H, J=7.2 Hz), 4.92 (ddt, 1H, J=10.2, 1.9, 1.4 Hz), 4.99 (ddt, 1H, J=17, 1.9, 1.9 Hz), 5.78 (ddt, 1H, J=17, 10.2, 6.6 Hz).

*Ethyl 2,2-dimethyl-6-hydroxy-hexanoate **17**:*[30] To a stirred 0.5 M solution of 9-BBN in THF (65.2 mL, 32.6 mmol) was added ethyl 2,2-dimethyl-hex-5-enoate (**16**; 5.54 g, 32.6 mmol) in THF (16.2 mL). After 2 h at 25°C, the reaction mixture was cooled to 0°C. Ethanol (20 mL) was added followed by 6N NaOH (6.5 mL) and 30% H_2O_2 (13 mL). The mixture was then heated to 50°C and stirred for 1 h. After cooling to 25°C, it was diluted with brine and extracted with EtOAc. The organic layer was dried and evaporated under vacuum. Purification of the oil by flash chromatography (1/1 hexanes/EtOAc) yielded the alcohol as a colorless oil (5.1 g, 83%). ^{1}H NMR δ 1.17 (s, 6H), 1.21–1.34 (m, 4H), 1.24 (t, 3H, J=7.2 Hz), 1.49–1.55 (m, 2H), 3.63 (br s, 2H), 4.10 (q, 2H, J=7.2 Hz).

*Ethyl 2,2-dimethyl-6-(tetrahydro-pyran-2-yloxy)-hexanoate **18**:*[31] A mixture of alcohol **17** (5.1 g, 27 mmol), PPTS (200 mg, 0.8 mmol) and DHP (2.96 mL, 32.4 mmol) in CH_2Cl_2 was stirred at 25°C for 4 h. The mixture was diluted with water and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and evaporated to yield the product as a colorless oil (6.75 g, 92%). ^{1}H NMR δ 1.16 (s, 6H), 1.23–1.32 (m, 4H), 1.24 (t, 3H, J=7.2 Hz), 1.49–1.90 (m, 8H), 3.36 (dt, 1H, J=9.4, 6.6 Hz), 3.48–3.52 (m, 1H), 3.70 (dt, 1H, J=9.4, 6.6 Hz), 3.83–3.90 (m, 1H), 4.10 (q, 2H, J=7.2 Hz), 4.56 (br t, 1H, J=2.5 Hz).

*2,2-Dimethyl-(tetrahydro-pyran-6-yloxy)-hexan-1-ol **19**:*[32] To a suspension of LAH (943 mg, 24.8 mmol) in ether (120 mL) cooled to 0°C was added the ester **18** (6.75 g, 24.8 mmol) in ether (10 mL) dropwise. After stirring for 30 min the reaction was quenched by the careful addition of water. The aqueous layer was extracted with ether and the combined organic layers were dried and evaporated to provide the alcohol as a colorless oil (5.34 g, 95%). ^{1}H NMR δ 0.86 (s, 6H), 1.23–1.32 (m, 4H), 1.49–1.90 (m, 8H), 3.30 (dd, 2H, J=6.3, 1.9 Hz), 3.41 (dt, 1H,

J=9.4, 6.6 Hz), 3.48–3.52 (m, 1H), 3.75 (dt, 1H, J=9.4, 6.6 Hz), 3.83–3.90 (m, 1H), 4.56 (br t, 1H, J=2.5 Hz).

2,2-Dimethyl-(tetrahydro-pyran-6-yloxy)-hexanal ***11***:[33] To a stirred suspension of PCC (4.55 g, 21.45 mmol) and celite in CH_2Cl_2 (40 mL) was added a solution of the alcohol **19** (3.3 g, 14.3 mmol) in CH_2Cl_2 (10 mL). The mixture was stirred at 25°C for 2 h. It was then filtered through a pad of silica gel (hexanes/EtOAc 3/1) and the solution was evaporated to yield the aldehyde as a colorless oil (3.6 g, 79%). ^{1}H NMR δ 1.04 (s, 6H), 1.23–1.32 (m, 4H), 1.45–1.85 (m, 8H), 3.36 (dt, 1H, J=9.4, 6.6 Hz), 3.48–3.52 (m, 1H), 3.70 (dt, 1H, J=9.4, 6.6 Hz), 3.83–3.90 (m, 1H), 4.56 (br t, 1H, J=2.5 Hz), 9.44 (s, 1H).

20-(Tetrahydro-pyran-2-yloxy)-16,16-dimethyl-eicosa-5,8,11,14-all-*cis*-tetraenoic acid methyl ester 12.[22] To a stirred solution of the phosphonium salt **7** (4.36 g, 6.98 mmol) in THF/HMPA (40/5 mL) cooled to −10°C was added dropwise a 1 M solution of NHMDS in THF (6.98 mL). The mixture was stirred at 0°C for 30 min and cooled to −78°C. The aldehyde **11** (1.58 g, 6.98 mmol) was added dropwise in THF (8 mL) to the reaction mixture. The cooling bath was removed and it was left to warm to 25°C over 2 h. It was quenched with hexanes and the mixture was plugged through a pad of silica gel (eluting with ethyl acetate/hexanes 1/4). The filtrate was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The oily residue was purified by flash chromatography (EtOAc/hexanes 1/4) to yield the tetraene **12** as a colorless oil (1.56 g, 50%). ^{1}H NMR δ 1.10 (s, 6H), 1.25–1.40 (m, 4H), 1.45–1.63 (m, 8H), 1.70 (quint, 2H, J=7.4 Hz), 1.97–2.13 (m, 2H), 2.31 (t, 2H, J=7.4 Hz), 2.75–2.95 (m, 6H), 3.37 (dt, 1H, J=9.6, 6.6 Hz), 3.50 (br dt, 1H, J=11.3, 5.2 Hz), 3.66 (s, 3H), 3.72 (dt, 1H, J=9.6, 6.6 Hz), 3.86 (br dt, 1H, J=11.3, 5.2 Hz), 4.57 (br t, 1H, J=4.4 Hz), 5.12–5.41 (m, 8H).

16,16-Dimethyl-20-hydroxy-eicosa-5,8,11,14-all-*cis*-tetraenoic acid (4-hydroxy-3-methoxy-benzyl) amide 13.[22] To a stirred solution of tetraene **12** (300 mg, 0.67 mmol) in MeOH (33 mL) and water (11 mL) was added $LiOH{\cdot}H_2O$ (190 mg, 4.8 mmol) and the mixture was stirred at 25°C overnight. It was diluted with ether and acidified with 10% HCl. The layers were separated and the aqueous layer was extracted with ether. The combined organic layers were dried over $MgSO_4$ and evaporated to yield the crude acid (258 mg, 90%) which was used directly in the next step. The acid was added to a stirred solution of 4-hydroxy-3-methoxy benzylamine (358 mg, 1.89 mmol) pretreated with Et_3N, DMAP (93 mg, 0.76 mmol) and EDCI (146 mg, 0.76 mmol) in CH_2Cl_2 (5 mL) cooled to 0°C. The mixture was stirred for 30 min and the ice bath was removed. It was left to stir overnight at 25°C. The reaction mixture was diluted with CH_2Cl_2 and plugged through a pad of celite. Purification by flash chromatography (hexanes/EtOAc 4/1) provided the amide (80 mg, 26%). ^{1}H NMR δ 1.09 (s, 6H), 1.28–1.40 (m, 4H), 1.52 (quint, 2H, J=6.3 Hz), 1.73 (quint, 2H, J=7.4 Hz), 2.00–2.12 (m, 2H), 2.20 (t, 2H, J=7.4 Hz), 2.77 (t, 2H, J=5.5 Hz), 2.80 (t, 2H, J= 5.5 Hz), 2.92 (t, 2H, J=5.5 Hz), 3.62 (dt, 2H, J=6.3, 5.5 Hz), 3.87 (s, 3H), 4.33 (d, 2H, J=5.8 Hz), 5.12–5.25 (m, 2H), 5.30–5.41 (m, 6H), 5.72 (br s, 1H), 6.74–6.87 (m, 3H). Anal. Calcd for $C_{30}H_{45}O_4N{\cdot}0.6H_2O$: C, 72.87; H, 9.41. Found: C, 72.77; H, 9.34.

20-Bromo-16,16-dimethyl-eicosa-5,8,11,14-all-*cis*-tetraenoic acid methyl ester 20.[34] To a stirred solution of triphenylphosphine (524 mg, 2 mmol) in CH_2Cl_2 (4 mL) cooled to 0°C was added bromine (0.1 mL, 1.95 mmol). The tetraene **12** (850 mg, 1.9 mmol) was then added dropwise and the ice bath was removed. The reaction mixture was stirred overnight at 25°C. The solvent was removed under vacuum and the residue was dissolved in hexanes/EtOAc (9/1) and plugged through a pad of silica gel. Further purification by flash chromatography (hexanes/EtOAc 9/1) provided the desired bromide **20** (404 mg, 50%). ^{1}H NMR δ 1.10 (s, 6H), 1.25–1.40 (m, 4H), 1.70 (quint, 2H, J=7.2 Hz), 1.82 (quint, 2H, J=7.2 Hz), 2.10 (m, 2H), 2.31 (t, 2H, J=7.2 Hz), 2.75–2.95 (m, 6H), 3.40 (t, 2H, J=6.6 Hz), 3.66 (s, 3H), 5.12–5.41 (m, 8H).

20-Cyano-16,16-dimethyl-eicosa-5,8,11,14-all-*cis*-tetraenoic acid methyl ester 21.[35] A mixture of bromide **20** (318 mg, 0.68 mmol) and KCN (90 mg, 1.36 mmol) in DMSO (3.4 mL) was heated at 50°C for 5 h. After cooling, the reaction mixture was diluted with hexanes/EtOAc (4/1) and plugged through a pad of silica gel. Further purification by flash chromatography (hexanes/EtOAc 9/1) afforded the cyano derivative **21** (195 mg, 78%). ^{1}H NMR δ 1.11 (s, 6H), 1.30–1.49 (m, 4H), 1.63 (quint, 2H, J=7.6 Hz), 1.70 (quint, 2H, J=7.2 Hz), 2.00–2.12 (m, 2H), 2.32 (t, 4H, J=7.6 Hz), 2.75–2.95 (m, 6H), 3.66 (s, 3H), 5.17–5.25 (m, 2H), 5.31–5.43 (m, 6H).

20-Bromo-16,16-dimethyl-eicosa-5,8,11,14-all-*cis*-tetraenoic acid (4-hydroxy-3-methoxy-benzyl) amide 22.[22] To a stirred solution of bromide **20** (404 mg, 0.97 mmol) in MeOH (48 mL) and water (16 mL) was added $LiOH{\cdot}H_2O$ (267 mg, 6.8 mmol) and the mixture was stirred at 25°C overnight. It was diluted with ether and acidified with 10% HCl. The layers were separated and the aqueous layer was extracted with ether. The combined organic layers were dried over $MgSO_4$ and evaporated to yield the crude acid (375 mg, 94%) which was used directly in the next step. The acid (200 mg, 0.49 mmol) was dissolved in CH_2Cl_2 (5 mL) and cooled to 0°C. A 2 M solution of oxalyl chloride in CH_2Cl_2 (0.49 mL) was added dropwise followed by 2 drops of DMF. The ice bath was removed and the mixture was stirred at 25°C for 2 h. The solvent was evaporated under vacuum. A solution of the acid chloride in CH_2Cl_2 (3 mL) was added to a solution of 4-hydroxy-3-methoxy benzylamine (0.4 mL, 5 mmol) in CH_2Cl_2 (5 mL) at 0°C. The ice bath was removed and the mixture was stirred at 25°C overnight. It was diluted with CH_2Cl_2 and washed with brine. The organic layer was dried over $MgSO_4$ and then concentrated in vacuo. The crude product was purified by flash chromatography (hexanes/EtOAc 1/1) to yield the amide **22** (85 mg, 32%). ^{1}H NMR δ 1.10 (s, 6H), 1.31–1.45 (m, 4H), 1.73 (quint, 2H, J=7.4 Hz), 1.82 (quint, 2H, J=6.9 Hz), 2.00–2.12 (m, 2H), 2.20 (t, 2H, J=7.4 Hz), 2.77 (t, 2H, J=5.5 Hz), 2.81 (t, 2H, J=5.5 Hz), 2.91 (t, 2H, J=5.5 Hz), 3.40 (t, 2H, J=6.9 Hz), 3.86 (s, 3H), 4.34 (d, 2H, J=5.8 Hz), 5.12–5.25 (m, 2H), 5.30–5.41 (m, 6H), 5.59 (s, 1H), 5.62 (br s, 1H), 6.74–6.87 (m, 3H). Anal. Calcd

for $C_{30}H_{44}O_3NBr·0.4H_2O$: C, 65.06; H, 8.15. Found: C, 65.06; H, 7.97.

20-Cyano-16,16-dimethyl-eicosa-5,8,11,14-all-*cis*-tetraenoic acid (4-hydroxy-3-methoxy-benzyl) amide 23. Prepared as described for 20-Bromo-16,16-dimethyl-eicosa-5,8,11,14-*all-cis*-tetraenoic acid (4-hydroxy-3-methoxy-benzyl) amide **22** (28%). ^{1}H NMR δ 1.10 (s, 6H), 1.31–1.45 (m, 4H), 1.62 (quint, 2H, J=7.1 Hz), 1.73 (quint, 2H, J=7.4 Hz), 2.00–2.12 (m, 2H), 2.20 (t, 2H, J=7.4 Hz), 2.33 (t, 2H, J=7.2), 2.77 (t, 2H, J=5.5 Hz), 2.81 (t, 2H, J=5.5 Hz), 2.91 (t, 2H, J=5.5 Hz), 3.86 (s, 3H), 4.34 (d, 2H, J=5.8 Hz), 5.12–5.25 (m, 2H), 5.30–5.41 (m, 6H), 5.59 (s, 1H), 5.62 (br s, 1H), 6.74–6.87 (m, 3H). Anal. Calcd for $C_{31}H_{44}O_3N_20.4H_2O$: C, 75.29; H, 9.01. Found: C, 74.99; H, 8.87.

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