Efficient Chemoenzymatic Synthesis of Phenylacetylrinvanil: An Ultrapotent Capsaicinoid

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Abstract: The straightforward synthesis of phenylacetylrinvanyl (PhAR), an ultrapotent capsaicinoid is described. The process starts with the quantitative synthesis of methyl ricinoleate (MeRic) by castor oil methanolysis. Afterwards, two alternative routes are possible: a) chemoselective vanillylamine aminolysis of MeRic catalyzed by *Candida antarctica* lipase-B (CaLB) to yield rinvanil, which after reaction with phenylacetic acid and DCC–DMAP followed by a regioselectively pyrrolidine deacylation results in PhAR with a 51% global yield, b) methyl 12-phenylacetylricinoleate synthesis by reaction of MeRic with phenylacetic acid and DCC–DMAP, followed by a chemoselective vanillylamine aminolysis catalyzed by CaLB to obtain PhAR with a 70% global yield.

Key words: chemoselective synthesis, solvent effects, enzymes, *Candida antarctica B*, ultrapotent capsaicinoid

Transient receptor potential vanilloid type 1 (TRPV1) is a vanilloid receptor highly expressed in sensory neurons.[1] This protein acts as a rapid and direct detector of potentially harmful thermal and chemical stimuli, which are perceived as painful. Activation of this receptor, potentiated by endogenous pronociceptive mediators such as prostaglandins, bradykinin, and ATP, results in a release of neurotransmitters from peripheral and central nerve terminals, inducing pain and neurogenic inflammation, thus playing an important role in neuropatic pain, intestinal inflammation, and other disorders.[2,3]

Capsaicin (*E*)-*N*-[(4-hydroxy-3-methoxyphenyl)methyl]-8-methyl-6-nonenamide (**1**, Figure 1) is a compound found in the fruit of plants of the Capsicum genus and is responsible for the pungency of chilli peppers. Due to its exogenous effect as agonist of TRPV1 channels, capsaicin has been widely used to study pain mechanisms and to evaluate the analgesic efficacy of various drugs. While initial exposure to capsaicin results in pain and hyperalgesia, repeated or prolonged treatment with capsaicin can desensitize nociceptive neurons, leading to a progressive decrease in subsequent responses to capsaicin or to other stimuli.[4] In spite of its pungency, this functional desensititation is the basis of the topical analgesic and anti-inflamatory effect of capsaicin. The discovery of new analogues that could bind the TRPV1 receptor with high specificity but with reduced pungency would lead to more effective drugs for the treatment of health disorders such as inflammatory hyperalgesia, bladder hyperactivity, emesis, cancer growth, neuronal excitotoxicity, pain, and dysmotility.[5] In fact, it has been shown that analogues of capsaicin, which do not irritate the skin, such as capsiate (**2**) and olvanil (**3**) (Figure 1), strongly activate TRPV1 in vitro and have in vivo antihyperalgesic effects.[6,7]

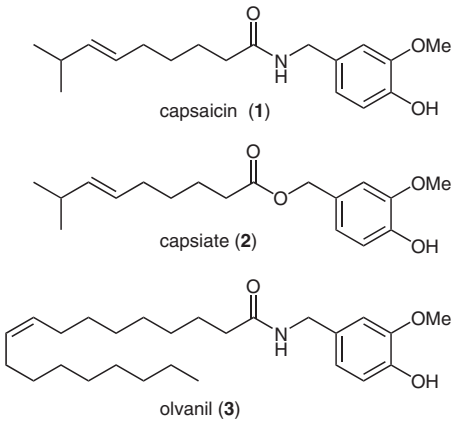

Figure 1 Chemical structures of capsainoids TRPV1 agonists

Recently, the synthesis of phenylacetylrinvanil (**4**, PhAR, IDN 5890, Figure 2), an ultrapotent TRPV1 agonist with moderate affinity for human cannabinoid CB_2 receptors, has been described.[5] In terms of its affinity for the receptor, PhAR was found similar to resiniferatoxin (**5**) (Figure 2), a natural product isolated from certain succulent African euphorbias, about 1000 times more potent as a TRPV1 agonist than capsaicin,[8] that has been successfully applied in the treatment of urinary incontinence.[9] It is interesting to observe that the high affinity of PhAR results from the simple phenylacetylation of 12-hydroxyl group in rinvanil (**6**) (Scheme 1).

Appendino et al. (2005) have proposed two strategies for the chemical synthesis of PhAR: starting from expensive pure ricinoleic acid, the protection of the carboxyl group with trichloroethanol (DCC–DMAP), followed by acylation with phenylacetic acid–DCC–DMAP at 12-hydroxy position of trichloroethyl ester, deprotection of trichloro-

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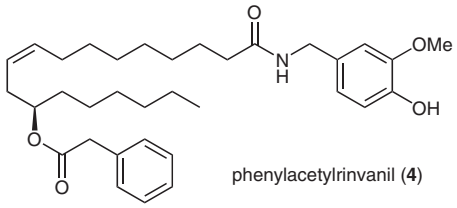


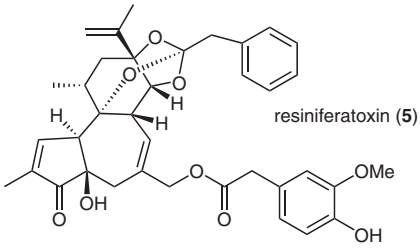
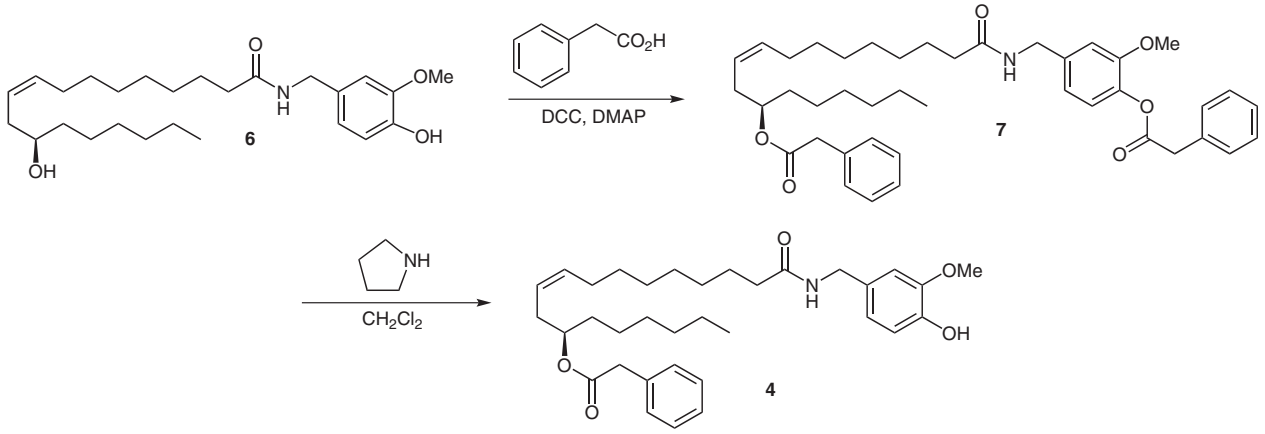


Figure 2 Chemical structures of resiniferatoxin and phenylacetylrinvanil

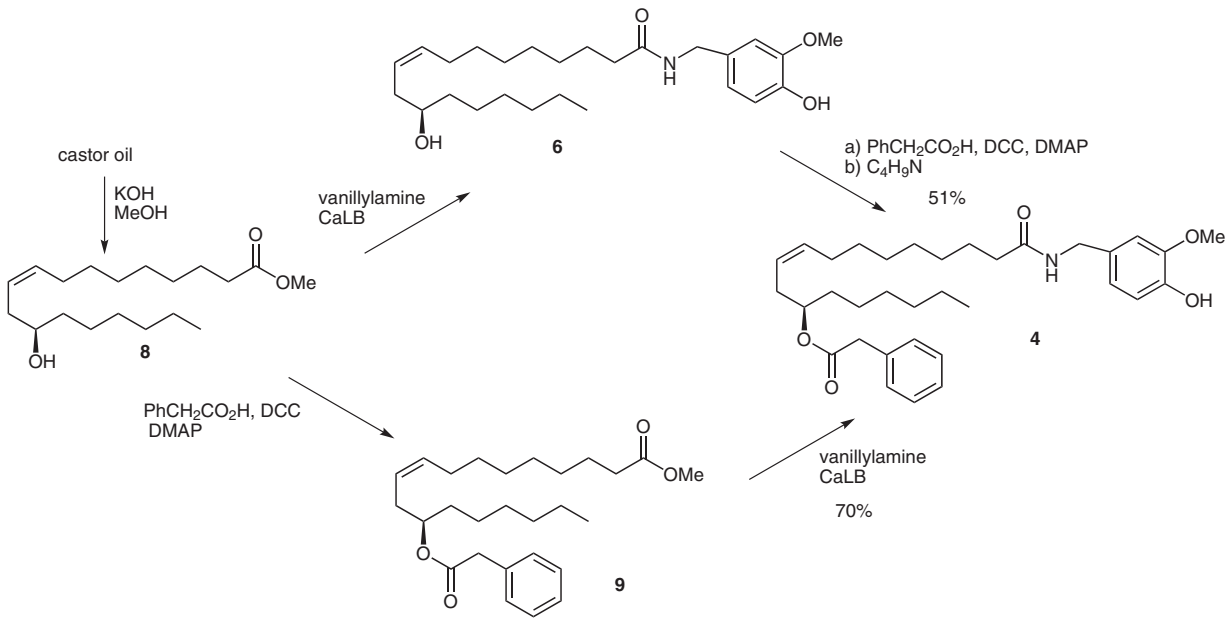
ethyl ester, and amide formation with vanillylamine using a cyclic anhydride protocol, to obtain PhAR with a global yield of 18.5%.[5] Their alternative proposal (Scheme 1) consists in the esterification of rinvanil (**6**) with phenylacetic acid using DCC–DMAP in toluene, to obtain **7**, the 4′,12-diester of rinvanil, which can be regioselectively hydrolyzed (pyrrolidine, CH_2Cl_2) to give PhAR (**4**).[10]

In the present work, a chemoenzymatic strategy for the synthesis of PhAR from commercially available castor oil is described (Scheme 2). This regio- and chemoselective strategy not only allows higher global PhAR yields but also results in a more economical process as it involves the use of castor oil as a not expensive source of ricinoleoyl moiety in the form of easily purified methyl ester, therefore, avoiding the difficult task of ricinoleic acid purification (Scheme 2).

Indeed, the production of methyl ricinoleate (**8**), the first step of PhAR synthesis, was actually explored from a medium containing castor oil (20 g) in MeOH (155 mL) by



Scheme 1 Chemical synthesis of PhAR from rinvanil (**6**)[5]



Scheme 2 Alternative syntheses of PhAR from castor oil using a chemoenzymatic approach

two alternative catalytic methods of alcoholysis: an enzymatic reaction with 10 mg/mL of *Candida antarctica* lipase B (CaLB) and a chemically catalyzed reaction with KOH (Scheme 2). Although an 80% yield was obtained through the CaLB reaction after 24 hours of reaction, only 30 minutes under reflux was required with the KOH reaction to obtain a quantitative mixture of methyl esters, which, after column chromatography, resulted in a 85% yield of **8** (16.67 g), equivalent to 95% yield of methyl ricinoleate on the basis of available ricinoleic acid on castor oil (87%). The KOH-catalyzed alcoholysis was therefore the best option. Furthermore, while optimizing the required amount of KOH for the reaction, it was possible to reduce it to 0.28% w/v retaining the same yield and productivity. This is, to our knowledge, the lowest KOH concentration reported for the preparation of fatty acid methyl esters.[11]

The complexity of synthesis of vanillylamides relies on the low chemoselectivity of the acylation process, due to the presence of both an amino and a phenolic hydroxyl group on the vanillylamine molecule. For instance, equimolar amounts of vanillylamine and oleic acid in the presence of DCC and DMAP result in a mixture of the amide (olvanil) and the ester (*O*-oleylolvanil), as well as unreacted vanillylamine.[12] Therefore, protection of the phenolic group of vanillylamine is required when the acid is activated as a hydroxysuccinimide derivative or as a mixed anhydride[13] and although Schotten–Baumann conditions are usually selective, acid chlorides are needed for this strategy. An interesting alternative proposed for the synthesis of PhAR that does not require the phenolic protection of vanillylamine is the use of propylphosphoric anhydride; however, this process results in a low PhAR yield (35%).[12] In order to overcome these limitations, we developed two chemoenzymatic approaches taking advantage of the high regio- and chemoselectivity of the lipase-catalyzed aminolysis. The first approach consists in the chemoselective enzymatic amidation of **8** (2 g, 6.4 mmol) with CaLB (9 mg/mL) and vanillylamine (1.21 g, 6.4 mmol) at 37 °C, to obtain rinvanil (**6**, 1.94 g, 70% yield) (Scheme 2). The enzymatic amidation proceeds with high regio- and chemoselective specificity as the amide **6** is the only product observed in the synthesis. In the next step, **6** (0.330 g, 0.76 mmol) is acylated with 2.2 equivalents of phenylacetic acid–DCC–DMAP to produce 12,4′-diphenylacetyl rinvanil (**7**, 0.390 g, 76.5% yield). Finally, the phenolic group of **7** (0.360 g, 0.5 mmol) is deacylated with pyrrolidine and CH_2Cl_2 (186 mL, 2.29 mmol) to produce PhAR (**4**, 0.285 g, 96.28% yield) with a global yield of 51% from **8** (Scheme 2).

In our second approach, the synthesis is now started with the phenylacetylation of **8** (13.5 g, 43 mmol) with phenylacetic acid–DCC–DMAP (11.66 g, 85.7 mmol) to yield methyl 12-phenylacetylricinoleate (**9**, 16.37 g, 88% yield), followed by the enzymatic amidation of **9** (2 g, 4.6 mmol) with vanillylamine (0.190 g, 1 mmol) by CaLB (35 mg/mL) to produce PhAR (**4**, 434 mg, 79% yield) with a global yield of 70% from **8** (Scheme 2). In this simplified approach, the acid function in the ricinoleic acid is protected as a methyl ester, allowing in a first step a straight and efficient esterification with phenylacetic acid at the 12-hydroxy position; subsequently, the chemoselective lipase-catalyzed amidation of the methylated carboxyl group of **9** is highly favored by the specificity of CaLB and the higher nucleophilicity of primary amino group than that of phenol of vanillylamine. Actually, we have previously demonstrated that the acylation of phenolic group of vanillylamine does not take place at all in this kind of reactions.[14] Finally, it is also important to underline that based on thermodynamic assumptions, the use of a polar solvent such as dry 2-methyl-2-butanol and an excess of diester **9** favors a high yield of amide synthesis.[15] The spare diester was easily recovered during PhAR purification. To the best of our knowledge, this is the first example of a chemo- and regioselective enzymatic aminolysis of hydroxylated fatty acid diesters.

In summary, the chemoenzymatic strategy here described results in a valuable alternative for the synthesis of the ultrapotent capsaicinoid phenylacetylrinvanil (**4**), allowing to envisage a general method for the synthesis of these valuable compounds. Indeed, in contrast to chemical methods, the chemo- and regioselective properties of lipase-catalyzed reactions allowed higher yields of **4**, confirming the high potential of enzymatic processes to impact traditional chemical synthetic routes. In addition, the use of castor oil, a renewable and widely available source of ricinoleoyl moiety, renders this process feasible for large-scale application of PhAR synthesis and its derivatives.

Typical Procedure of the Chemical Aminolysis for the Synthesis of 12-Phenylacetylrinvanil (4)[17]

To a solution of 12,4′-diphenylacetylrinvanil (**7**, 360 mg, 0.5 mmol) in CH_2Cl_2 (25 mL), pyrrolidine (186 mL, 2.29 mmol, 5 mol equiv) was added. The reaction mixture was stirred at r.t. for 6 h (TLC, hexane–EtOAc, 6:4); 2 N H_2SO_4 (2.29 mL) was added, the organic layer separated, and the aqueous layer (pH = 4–5) extracted (2 × 5 mL) with CH_2Cl_2. The organic extract was washed with H_2O (2 × 5 mL) and brine (5 mL), dried with Na_2SO_4, and concentrated to dryness to yield 550 mg of raw product, purified by column chromatography (70–230 mesh; 9 g silica gel; hexane–EtOAc, 7:3) to yield 285 mg (96.28%) of pure **4**. Due to the recognized ultrapotent activity of this compound in case of skin contact (very irritant), it is highly recommended the use of splash goggles and gloves when handling this material.

^{1}H NMR (300 MHz, $CDCl_3$): d = 0.86 (t, *J* = 7.2 Hz, 3 H), 1.19–1.32 (m, 16 H), 1.46–1.54 (m, 2 H), 1.63 (q, *J* = 7.2 Hz, 2 H), 1.94 (c, *J* = 6.3 Hz, 2 H), 2.18 (t, *J* = 7.8 Hz, 2 H), 2.23–2.32 (m, 2 H), 3.57 (s, 2 H), 3.84 (s, 3 H), 4.32 (d, *J* = 5.4 Hz, 2 H), 4.86 (q, *J* = 6.6 Hz, 1 H), 5.22–5.31 (m, 1 H), 5.38–5.47 (m, 1 H), 5.83 (br t, *J* = 4.5 Hz, 1 H), 6.73 (dd, J_1 = 7.8 Hz, J_2 = 2.1 Hz, 1 H), 6.78 (d, *J* = 1.8 Hz, 1 H), 6.86 (d, *J* = 8.1 Hz, 1 H), 7.20–7.33 (m, 5 H). ^{13}C NMR (75 MHz, $CDCl_3$): d = 14.0, 22.4, 25.1, 25.6, 27.2, 29.01, 29.04, 29.13, 29.16, 29.4, 31.6, 31.8, 33.4, 36.7, 41.7, 43.4, 55.8, 74.5, 110.6, 114.3, 120.7, 124.0, 126.9, 127.0, 128.4, 128.5, 129.1, 129.2, 130.2, 132.5, 145.0, 146.6, 171.3, 173.0. MS (EI): *m/z* (%) = 551 (30) [M$^+$], 415 (41), 277 (13), 152 (64), 137 (100), 91 (22). IR (film): n_{max} = 3362, 3306, 2928, 2856, 1729, 1646, 1517, 1274,

1034, 721, 700 cm^{-1}. [a]$_D^{20}$ +15.2 (*c* 1, MeOH); lit.[5] [a]$_D^{20}$ +35.6 (*c* 1.1, MeOH).

Typical Procedure of the Enzymatic Aminolysis for the Preparation of 12-Phenylacetylrinvanil (4)

Vanillylamine·HCl (190 mg, 1 mmol) was suspended in dry 2-methyl-2-butanol (20 mL) containing 4 Å MS (500 mg) and Et_3N (840 mL). The mixture was agitated 30 min at 30 °C and 250 rpm. Then, methyl 12-phenylacetylricinoleate (2 g, 4.6 mmol) and CaLB (750 mg) was added, and the mixture was incubated at 30 °C. The reaction was monitored by TLC (hexane–EtOAc, 1:1; ninhydrine as visualizing reagent). Filtration over Celite and vacuum concentration afforded 434 mg of **4** (78.7% yield based on the vanillylamine·HCl) and 1.55 g of pure methyl 12-phenylacetylricinoleate after silica gel chromatography.

[a]$_D^{20}$ +15.2 (*c* 1, MeOH); Lit.[5] [a]$_D^{20}$ +35.6 (*c* 1.1, MeOH). IR (film): 3362, 3306, 2928, 2856, 1729, 1646, 1517, 1274, 1034, 721, 700 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): d = 0.86 (t, 3 H, *J* = 7.2 Hz), 1.19–1.32 (m, 16 H), 1.46–1.54 (m, 2 H), 1.63 (q, 2 H, *J* = 7.2 Hz), 1.94 (c, 2 H, *J* = 6.3 Hz), 2.18 (t, *J* = 7.8 Hz, 2 H), 2.23–2.32 (m, 2 H), 3.57 (s, 2 H), 3.84 (s, 3 H), 4.32 (d, *J* = 5.4 Hz, 2 H), 4.86 (q, *J* = 6.6 Hz, 1 H), 5.22–5.31 (m, 1 H), 5.38–5.47 (m, 1 H), 5.83 (br t, *J* = 4.5 Hz, 1 H), 6.73 (dd, J_1 = 7.8 Hz, J_2 = 2.1 Hz, 1 H), 6.78 (d, *J* = 1.8 Hz, 1 H), 6.86 (d, *J* = 8.1 Hz, 1 H), 7.20–7.33 (m, 5 H). ^{13}C NMR (75 MHz, $CDCl_3$): d = 14.0, 22.4, 25.1, 25.6, 27.2, 29.01, 29.04, 29.13, 29.16, 29.4, 31.6, 31.8, 33.4, 36.7, 41.7, 43.4, 55.8, 74.5, 110.6, 114.3, 120.7, 124.0, 126.9, 127.0, 128.4, 128.5, 129.1, 129.2, 130.2, 132.5, 145.0, 146.6, 171.3, 173.0. MS (EI): *m/z* (%) = 551 (30) [M$^+$], 415 (41), 277 (13), 152 (64), 137 (100), 91 (22).

Rinvanil (6)

To a suspension of vanillylamine·HCl (1.21 g, 6.4 mmol) in 2-methyl-2-butanol (130 mL), Et_3N (6 mL, 42.7 mmol, 6.67 mol equiv) was added. The mixture was incubated for 30 min at 37 °C and 250 rpm, and reaction started by addition of MS (1.2 g), methyl ricinoleate (2 g, 6.4 mmol), and CaLB (1.2 g). The reaction was monitored by TLC (hexane–EtOAc, 85:15). Thereafter, enzyme and MS were filtered over Celite, a mixture of hexane and EtOAc (1:1, 15 mL) added, and the solid formed was filtered over Celite. The filtrate was concentrated under vacuum yielding 3.1 g of raw product, which after column chromatography (45 g silica gel; 70–230 mesh; hexane–EtOAc, 7:3) afforded 1.94 g of pure **6** (70.2% yield).

[a]$_D^{20}$ +2.9 (*c* 1, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): d = 0.88 (t, *J* = 6.9 Hz, 3 H), 1.25–1.35 (m, 16 H), 1.44–1.49 (m, 2 H), 1.63 (q, *J* = 6.0 Hz, 2 H), 1.99 (c, *J* = 6.6 Hz, 2 H), 2.16–2.22 (m, 4 H), 3.56 (q, *J* = 5.4 Hz, 1 H), 3.85 (s, 3 H), 4.32 (d, *J* = 5.7 Hz, 2 H), 5.34–5.43 (m, 1 H), 5.49–5.58 (m, 1 H), 5.91 (br t, *J* = 5.1 Hz, 1 H), 6.72 (dd, J_1 = 7.8 Hz, J_2 = 1.8 Hz, 1 H), 6.79 (d, *J* = 2.1 Hz, 1 H), 6.83 (d, *J* = 8.1 Hz, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$): d = 14.0, 22.5, 25.6, 27.2, 28.9, 29.01, 29.8, 29.1, 29.2, 29.4, 31.7, 35.2, 36.6, 36.7, 43.4, 55.8, 71.4, 110.7, 114.4, 120.6, 125.2, 130.2, 133.1, 145.0, 146.7, 173.0.

12,4′-Diphenylacetylrinvanil (7)

In a three-necked flask rinvanil (**6**, 330 mg, 0.76 mmol) and phenylacetic acid (227 mg, 1.67 mmol, 2.2 mol equiv) were disolved in CH_2Cl_2 (5 mL). The solution was cooled to 0 °C and then added DCC (312 mg, 1.52 mmol, 2 mol equiv) and DMAP (92 mg, 0.76 mmol). After stirring for 20 min at 0 °C and 2 h at r.t. (TLC; hexane–EtOAc, 1:1), the reaction mixture was filtered over Celite and washed with CH_2Cl_2. The amount of 550 mg of concentrated raw product yielded 390 mg of **7** (76.5% yield, yellow oil) after column chromatography (hexane–EtOAc, 7:3).

^{1}H NMR (300 MHz, $CDCl_3$): d = 0.86 (t, *J* = 7.1 Hz, 3 H), 1.21–1.28 (m, 16 H), 1.50–1.52 (m, 2 H), 1.63 (q, *J* = 6.2 Hz, 2 H), 1.96 (c, *J* = 7.4 Hz, 2 H), 2.16 (t, *J* = 7.7 Hz, 2 H), 2.21–2.35 (m, 2 H), 3.57 (s, 2 H), 3.71 (s, 3 H), 3.87 (s, 2 H), 4.34 (d, *J* = 5.8 Hz, 2 H), 4.86 (q, *J* = 6.0 Hz, 1 H), 5.24–5.32 (m, 1 H), 5.40–5.49 (m, 1 H), 5.93 (br t, *J* = 4.2 Hz, 1 H), 6.76 (dd, J_1 = 7.9 Hz, J_2 = 1.9 Hz, 1 H), 6.84 (d, *J* = 1.7 Hz, 1 H), 6.91 (d, *J* = 7.9 Hz, 1 H). ^{13}C NMR (75 MHz, $CDCl_3$): d = 14.1, 22.6, 25.2, 25.8, 27.3, 29.1, 29.2, 29.3, 29.4, 29.6, 31.7, 32.0, 33.6, 36.7, 41.0, 41.85, 43.4, 55.9, 74.6, 112.2, 120.0, 122.7, 124.2, 127.0, 127.3, 128.5, 128.6, 129.3, 129.4, 132.7, 133.6, 134.4, 137.6, 139.2, 151.2, 169.7, 171.4, 173.1.

Methyl ricinoleate (8)

Castor oil (20 g) was dissolved in MeOH (155 mL) and heated under reflux for 30 min in the presence of solid KOH (58 mg, 0.28%). At the end of the reaction (TLC, hexane–EtOAc, 85:15) AcOH (80 mL) was added, and the mixture was evaporated to dryness under reduced pressure. The residue was redissolved in hexane (30 mL) and washed with H_2O (2 × 25 mL) and with sat. NaCl solution (1 × 25 mL). The organic phase was dried with anhyd Na_2SO_4 and concentrated under reduced pressure, hence, 20.3 g of crude product afforded 16.67 g of **8** (85% yield) after column chromatography (400 g silica gel, hexane–EtOAc, 9.5:5 to 9:1 gradient). Purity of **8** (99% pure) was established by HPLC in a Waters HPLC system equipped with a Waters Symmetry C18 4.6 × 250 mm, 5 mm column (Milford, MA, USA) eluted with 1.5 mL/min of 85:15 MeCN–H_2O and using a refractive index detector at 35 °C; t_R = 6.45 min.

[a]$_D^{20}$ +7.4 (*c* 1, MeOH), +3.33 (*c* 1.5, $CHCl_3$); Lit.[16] [a]$_D^{20}$ +3.4 (*c* 1.5, $CHCl_3$). IR (film): 3435, 2928, 2856, 1742, 1439, 1173, 859, 725 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): d = 0.89 (t, *J* = 6.8 Hz, 3 H), 1.29–1.33 (m, 16 H), 1.45–1.50 (m, 2 H), 1.62 (q, *J* = 7.2 Hz, 2 H), 2.04 (c, *J* = 6.4 Hz, 2 H), 2.21 (t, *J* = 6.4 Hz, 2 H), 2.30 (t, *J* = 7.6 Hz, 2 H), 3.61 (q, *J* = 5.6 Hz, 1 H), 3.67 (s, 3 H), 5.37–5.44 (m, 1 H), 5.52–5.59 (m, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$): d = 14.365, 22.873, 25.149, 25.969, 27.609, 29.316, 39.362, 29.605, 29.810, 32.079, 34.295, 35.570, 37.058, 51.675, 71.644, 125.341, 133.379, 174.371.

Methyl 12-Phenylacetylricinoleate (9)

In a three-necked round-bottom flask **8** (13.5 g, 43 mmol) and phenylacetic acid (11.66 g, 85.7 mmol) were suspended in CH_2Cl_2 (150 mL). The reaction mixture was cooled to 0 °C in an ice bath and DCC (17.6 g, 85.6 mmol) was added. Stirring was continued for 10 min, DMAP (6.3 g, 30 mmol) was added and stirred for an additional 15 min at 0 °C. Thereafter, stirring was continued for 1 h at r.t. The reaction mixture was then refluxed until the reaction was complete (TLC, hexane–EtOAc, 85:15), filtered over Celite and concentrated in a rotatory evaporator. The residue was suspended in hexane–EtOAc (9:1, 50 mL), vacuum filtered over Celite, and washed with hexane–EtOAc (9:1, 100 mL); it was then dried over anhyd Na_2SO_4 and concentrated in rotatory evaporator (24.68 g of a crude product). After column chromatography 16.37 g of **9** were obtained (88.5% yield).

[a]$_D^{20}$ +29.6 (*c* 1, MeOH). IR (film): 2929, 2856, 1737, 1459, 1437, 1253, 1164, 1023, 722, 700 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): d = 0.86 (t, *J* = 6.0 Hz, 3 H), 1.21–1.29 (m, 16 H), 1.50–1.54 (m, 2 H), 1.61 (q, *J* = 7.2 Hz, 2 H), 1.95 (c, *J* = 6.6 Hz, 2 H), 2.21–2.32 (m, 4 H), 3.58 (s, 2 H), 3.65 (s, 3 H), 4.87 (q, *J* = 6.6 Hz, 1 H), 5.22–5.32 (m, 1 H), 5.39–5.48 (m, 1 H), 7.21–7.33 (m, 5 H). ^{13}C NMR (75 MHz, $CDCl_3$): d = 14.0, 22.4, 25.1, 27.2, 29.0, 29.1, 29.4, 31.6, 31.8, 33.5, 34.0, 41.7, 51.3, 74.4, 124.1, 126.8, 128.4, 129.1, 132.5, 134.3, 171.2, 174.2. HRMS–FAB$^+$: *m/z* calcd for $C_{27}H_{43}O_4$ [M + H]$^+$: 431.3161; found: 431.3151.

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References and Notes

(1) Caterina, M. J.; Schumacher, M. A.; Tominaga, M.; Rosen, T. A.; Levine, J. D.; Julius, D. *Nature (London)* **1997**, *389*, 816.

(2) (a) Walker, K. M.; Urban, L.; Medhurst, S. J.; Patel, S.; Panesar, M.; Fox, A. J.; McIntyre, P. *J. Pharmacol. Exper. Ther.* **2003**, *304*, 56. (b) Voets, T.; Droogmans, G.; Wissenbach, U.; Janssens, A.; Flockerzi, V.; Nilius, B. *Nature (London)* **2004**, *430*, 748.

(3) Yiangou, Y.; Facer, P.; Dyer, N. H. C.; Chan, C. L. H.; Knowles, C.; Williams, N. S.; Anand, P. *Lancet* **2001**, *357*, 1338.

(4) Winter, J.; Bevan, S.; Campbell, E. A. *Br. J. Anaesth.* **1995**, *75*, 157.

(5) (a) Appendino, G.; De Petrocellis, L.; Trevisani, M.; Minassi, A.; Daddario, N.; Moriello, A. S.; Gazzieri, D.; Ligresti, A.; Campi, B.; Fontana, G.; Pinna, C.; Geppetti, P.; Di Marzo, V. *J. Pharmacol. Exp. Ther.* **2005**, *312*, 561. (b) Appendino, G.; Cascio, M. G.; Bacchiega, S.; Moriello, A. S.; Minassi, A.; Thomas, A.; Ross, R.; Pertwee, R.; De Petrocellis, L.; Di Marzo, V. *FEBS Lett.* **2006**, *580*, 568.

(6) Iida, T.; Moriyama, T.; Kobata, K.; Morita, A.; Murayama, N.; Hashizume, S.; Fushiki, T.; Yazawa, S.; Watanabe, T.; Tominaga, M. *Neuropharmacology* **2003**, *44*, 958.

(7) Brand, L.; Berman, E.; Schwen, R.; Loomans, M.; Janusz, J.; Bohne, R.; Maddin, C.; Gardne, J.; Lahann, T.; Farmer, R.; Jones, L.; Chiabrando, C.; Fanneli, R. *Drug Exp. Clin. Res.* **1987**, *13*, 259.

(8) Voets, T.; Droogmans, G.; Wissenbach, U.; Janssens, A.; Flockerzi, V.; Nilius, B. *Nature (London)* **2004**, *430*, 748.

(9) Andersson, K. E. *Urology* **2004**, *63*, 32.

(10) Morazzoni, P.; Riva, A.; Fontana, G.; Appendino, G.; Di Marzo, V. WO 2006010445, **2006**.

(11) Dorado, M. P.; Ballesteros, E.; Mittelbach, M.; López, F. J. *Energy Fuels* **2004**, *18*, 1457.

(12) Appendino, G.; Minassi, A. N. *J. Med. Chem.* **2002**, *45*, 3739.

(13) Walpole, C. S.; Wrigglesworth, R.; Bevan, S.; Campbell, E. A.; Dray, A.; James, I. F.; Masdin, K. J.; Perkins, M. N.; Winter, J. *J. Med. Chem.* **1993**, *36*, 2381.

(14) Reyes-Duarte, D.; Castillo, E.; Martínez, R.; López-Munguía, A. *Biotechnol. Lett.* **2002**, *24*, 2057.

(15) (a) Castillo, E.; López-González, I.; de Regil-Hernández, R.; Reyes-Duarte, D.; Sánchez-Herrera, D.; López-Munguía, A.; Darszon, A. *Biochem. Biophys. Res. Commun.* **2007**, *356*, 424. (b) Torres-Gavilán, A.; Escalante, J.; Regla, I.; López-Munguía, A.; Castillo, E. *Tetrahedron: Asymmetry* **2007**, 2621.

(16) Borsotti, G.; Guglielmetti, G.; Speras, S.; Battistel, E. *Tetrahedron* **2001**, *57*, 10219.

(17) ^{1}H and ^{13}C NMR spectroscopy was carried out on a JEOL Eclipse instrument at 300 and 400 MHz (^{1}H NMR operating frequency), with TMS as an internal standard and $CDCl_3$ as solvent. The IR spectra were carried out on a Bruker spectrophotometer Tensor 27. The $[a]_D^{20}$ values were determined on a 341 Perkin Elmer polarimeter, at 1 dm cell length. HRMS was determined on a JEOL JMS-SX102A instrument. Silica gel chromatography: 70–230 mesh. Multiplicity keys: s = singlet, d = doublet, t = triplet, c = quartet, q = quintet, m = multiplet, br = broad, dd = doublet of doublets.