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Synthesis and Vanilloid Receptor (TRPV1) Activity of the Enantiomers of α-Fluorinated Capsaicin

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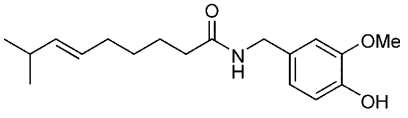
Capsaicin (**1**) is the major component responsible for the pungency of chilli peppers. The compound was isolated as early as 1876, and synthesised in 1919.[1] Its full structure was unambiguously determined in 1955.[2] The strong interest in this compound emerged from the use of chilli peppers in traditional medicine treatments. Nowadays, capsaicin is available under different brand names, mainly for pain treatment. Despite a long standing history of capsaicin in medicine, the relevant pain receptor was isolated and cloned only a decade ago and has been named the *t*ransient *r*eceptor *p*otential *v*anilloid subtype 1 (TRPV1).[3] TRPV1 is a nonselective cation channel that also responds to heat and low pH. In the course of numerous studies,[4] several natural and synthetic agonists[5] as well as synthetic antagonists[6] of the capsaicin receptor have been investigated and a structure–activity relationship has been defined. In these studies the aromatic residue is much more sensitive to modification than the lipophilic side chain. The nature of capsaicin binding to the TRPV1 receptor is not yet known in full detail, although a comparison of receptor sequences and site-directed mutations from species sensitive (human, rat) and less sensitive (rabbit, chicken) to capsaicin, have identified specific amino acid residues in the transmembrane region of the receptor that are important for capsaicin binding.[7,8] Models arising from these studies suggest that the side chain of capsaicin locates linearly and in an extended chain form between two transmembrane helices, embedded within, and running parallel to, the lipid chains of the membrane.[7] In this study we have explored the agonist activity of two enantiomers of α-fluorocapsaicin, which were designed to probe the enantiomeric location of the side chain in capsaicin. The study reinforces the models proposed so far and also suggests a linear and extended binding mode of the capsaicin side chain.



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Fluorine is only a little larger than hydrogen, however, it is much more polar. When fluorine is placed α to an amide the resulting dipoles/electrostatics dictate that there is a very strong preference for the C–F bond to align antiparallel to the amide carbonyl, and *syn* to the N–H bond.[9–11] This is illustrated in Figure 1 for the previously reported[9] rotational energy profile of *N*-methyl-2-fluoropropionamide, as measured by ab initio calculations (B3LYP/6-31G*9(d)).

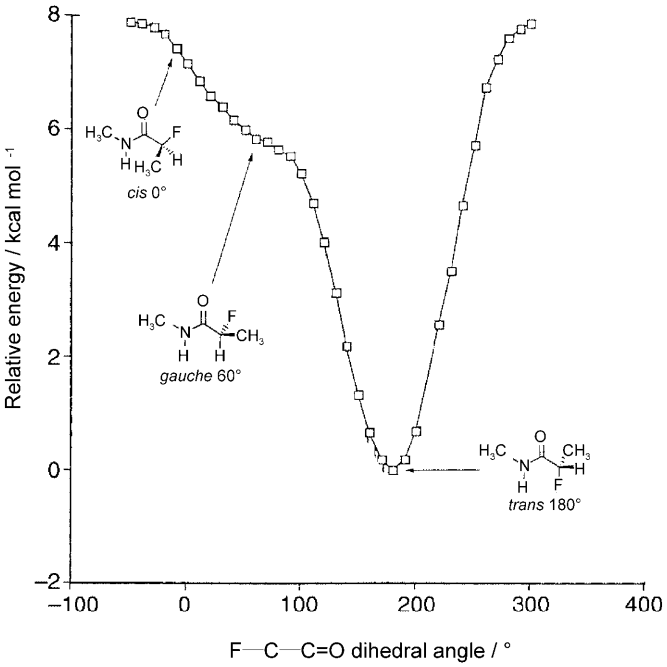

Figure 1. Rotational energy profile determined by ab initio calculations (B3LP/6-31G*9(d)) of *N*-methyl-2-fluoropropionamide, demonstrating a steep energy well of up to 8.0 kcal mol^{-1}. Taken from ref. [9], reproduced by permission of The Royal Society of Chemistry.

The *trans* conformer was calculated to fall into an 8 kcal mol^{-1} energy well where stabilisation results from dipolar relaxation between the C–F bond and the amide, as well as a favourable CF···HN electrostatic interaction. In the rotational energy profile (Figure 1) there is a plateau at the *gauche* (anticlinal) conformation—a full 6.0 kcal mol^{-1} above the *trans* structure. Mathad et al.[12] have subsequently developed an orbital symmetry argument suggesting that the *gauche* conformer cannot support a stabilising π(amide) to σ*(C–F) HOMO–LUMO interaction. The *trans* amide structure emerges as the only minimum on the rotational energy profile.

Capsaicin (**1**) is nonchiral, however, for the fluoro-enantiomers (*S*)-**2** and (*R*)-**2** this has the consequence that the side chains might orientate enantiomerically. Thus, their relative efficacies might be able to report an enantiomeric preference of

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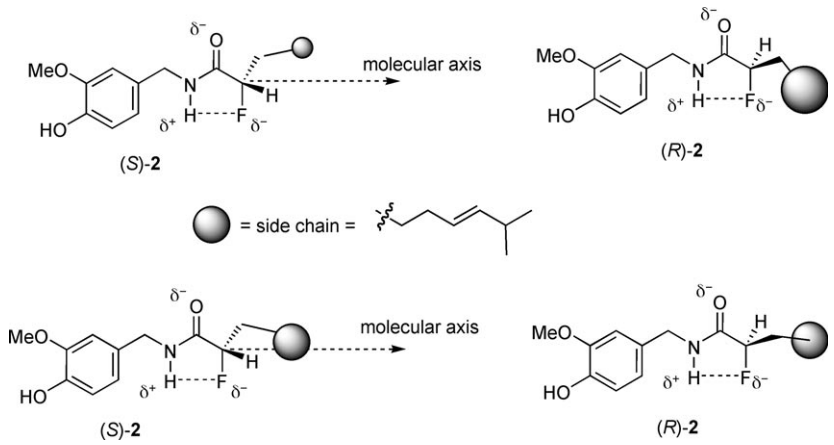
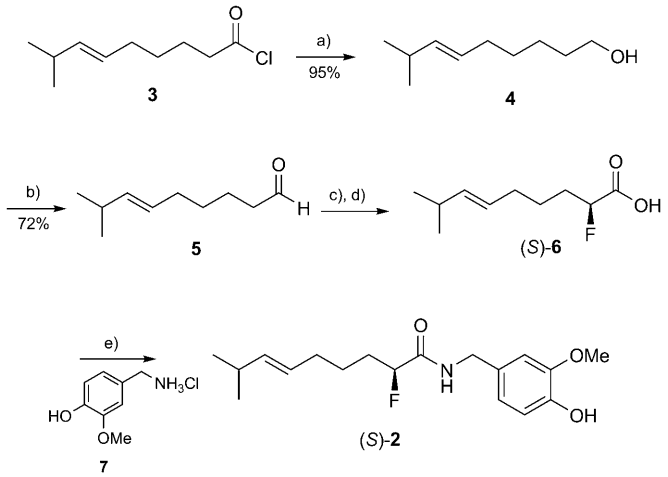


Figure 2. Putative enantiomeric binding orientations of α-fluorinated capsaicins either away from the molecular axis in different enantiomeric directions (above) or along the molecular axis (below). If the binding mode deviates from the molecular axis as shown (above), then the enantiomer response should differ. If it is along the molecular axis, then the enantiomer response should be similar.

side-chain binding to the receptor, particularly if the side chain deviates significantly from the molecular axis of binding as illustrated in Figure 2. If, however, the side chains orientate along the molecular axis, then both enantiomers can equally access that conformation, and elicit a similar response.

A synthetic strategy to (*S*)-**2** and (*R*)-**2** was developed, which utilised an asymmetric organocatalytic eletrophilic fluorination as the key step for introducing the fluorine. The synthetic route is outlined in Scheme 1 for (*S*)-**2** and starts from (*E*)-8-methylnon-6-enoyl chloride (**3**). The manufacturer indicates that this material is "predominantly *trans*" and GC–MS determined that the *E*/*Z* ratio was 89:11. This ratio was carried through all steps of the synthesis and thus the final products had approximately 10% of the *Z* isomer. The difference in the biological activity of (*E*)- and (*Z*)-capsaicin has been investigated earlier and there is no significant difference in activity.[5c]



Scheme 1. Synthetic route to α-fluorinated capsaicin (*S*)-**2**; a) $LiAlH_4$, THF, room temperature; b) PCC, DCM, room temperature; c) NFSI, (*S*)-5-benzyl-2,2,3-trimethylimidazolidin-4-one dichloroacetic acid salt, THF, *i*PrOH, −10 °C; d) CrO_3/H_2SO_4, Et_2O, acetone, H_2O, THF; E) TBTU, Et_3N, DMF, room temperature. Combined yield of steps c)–e) = 12%.

After a few attempts to reduce the acid chloride **3** directly to aldehyde **5** it was found more practical to adopt a two-step route via alcohol **4**.[13] The enantioselective organocatalytic introduction of fluorine α to the aldehyde comprised the key step of the synthesis.[14]

By using both enantiomers of the imidazolidinone catalysts and *N*-fluorobenzenesulfonimide (NFSI) as the electrophilic source of fluorine, the respective α-fluoroaldehydes were prepared in high *ee* values. Notably, a typical formation of 10–15% of α,α-difluorinated aldehyde could not be avoided.[15] Due to their volatility, the aldehydes were oxidised[16] to the respective acids without prior purification. The α,α-difluorinated aldehyde did not oxidise under the reaction conditions and was readily separated from the fluorinated carboxylic acids (*S*)-**6** and (*R*)-**6** by extraction. The resultant carboxylic acids were then directly coupled with vanillinamine[17] (**7**) by TBTU mediated amide bond formation[18] to give the desired capsaicinoids (*S*)-**2** and (*R*)-**2**. The enantiomeric purity of the fluorinated capsaicins after recrystallisation from hexane was determined by chiral HPLC (96% *ee* and 95% *ee*, respectively). The resultant (*S*)-**2** crystallised in long colourless needles and X-ray crystallography further confirmed the structure and absolute stereochemistry. The X-ray structure also revealed the expected orientation of the C–F bond relative to the amide moiety (Figure 3).

The synthetic enantiomers were then investigated as capsaicin agonist analogues in rat vanilloid receptors. Dorsal root ganglia (DRG) neurones in vivo and in culture are a heterogeneous population of cells and as previously reported[19,20] only a subpopulation of neurones express TRPV1 receptors and thus respond to capsaicin. In this study capsaicin (1 μM)

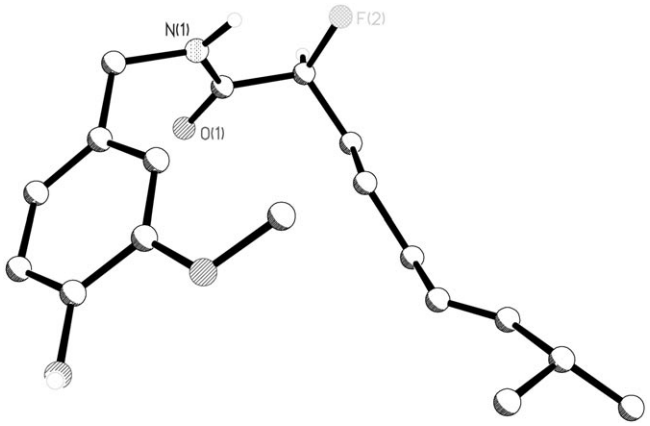


Figure 3. Crystal structure of (*S*)-**2** confirming the *anti*-planar orientation of the C–F bond and the amide carbonyl.

evoked transient increases in intracellular Ca^{2+} in a high proportion of DRG neurones (73 of 80 neurones). This ~90% response rate to capsaicin reflected selection of neurones with smaller diameter cell somas. Both synthetic isomers of fluorinated capsaicin, (*S*)-**2** and (*R*)-**2**, evoked increases in intracellular Ca^{2+}. The amplitudes of these responses were not significantly different from the responses to an equal concentration of capsaicin (1 μM; Figures 4A–D). Also consistent with an agonist action of the fluorinated capsaicin isomers at TRPV1 receptors was the fact that the 14 neurones that did not respond to capsaicin also failed to respond to (*R*)-**2** (Figure 4E). Although all the cells that responded to the fluorinated capsaicin isomers also responded to capsaicin, 3 out of 28 neurones that responded to capsaicin failed to respond to (*R*)-**2** (Figure 4F). We did not find similar neurones that responded to capsaicin but not (*S*)-**2**.

This apparent anomaly with (*R*)-**2** could reflect a sensitisation phenomenon rather than a distinct pharmacology of the *R* isomer. To investigate this further we used the TRPV1 receptor antagonist capsazepine (1 μM) to assess predicted inhibition of (*R*)-**2** and (*S*)-**2** (1 μM) responses. Pretreatment of neurones for 5 min with capsazepine greatly inhibited the responses to both (*R*)-**2** and (*S*)-**2** in neurones that after removal of the antagonist, were subsequently shown to respond to either capsaicin or one of the synthetic agonist isomers alone. Capsazepine completely abolished the responses to (*R*)-**2** (32 out of 38) and (*S*)-**2** (five out of eight neurones). The mean population response to (*R*)-**2** in the presence of capsazepine was 0.016 ± 0.008 fluorescence ratio units ($n = 28$), a value significantly lower than the mean responses to capsaicin (0.77 ± 0.14 fluorescence ratio units; $n = 28$, $P < 0.001$; Figure 2F). The mean population response to (*R*)-**2** in the presence of capsazepine was inhibited by (98 ± 2)% ($n = 15$) and (98 ± 2)% ($n = 26$) compared with responses to (*R*)-**2** and capsaicin alone, respectively. The mean population response to (*S*)-**2** in the presence of capsazepine was inhibited by (64 ± 13)% ($n = 8$) compared with responses to (*S*)-**2** alone.

To determine the lower threshold of response, experiments were also conducted at 250 and 330 nM (*R*)-**2**, but at these concentrations responses were 20% or less than the maximum

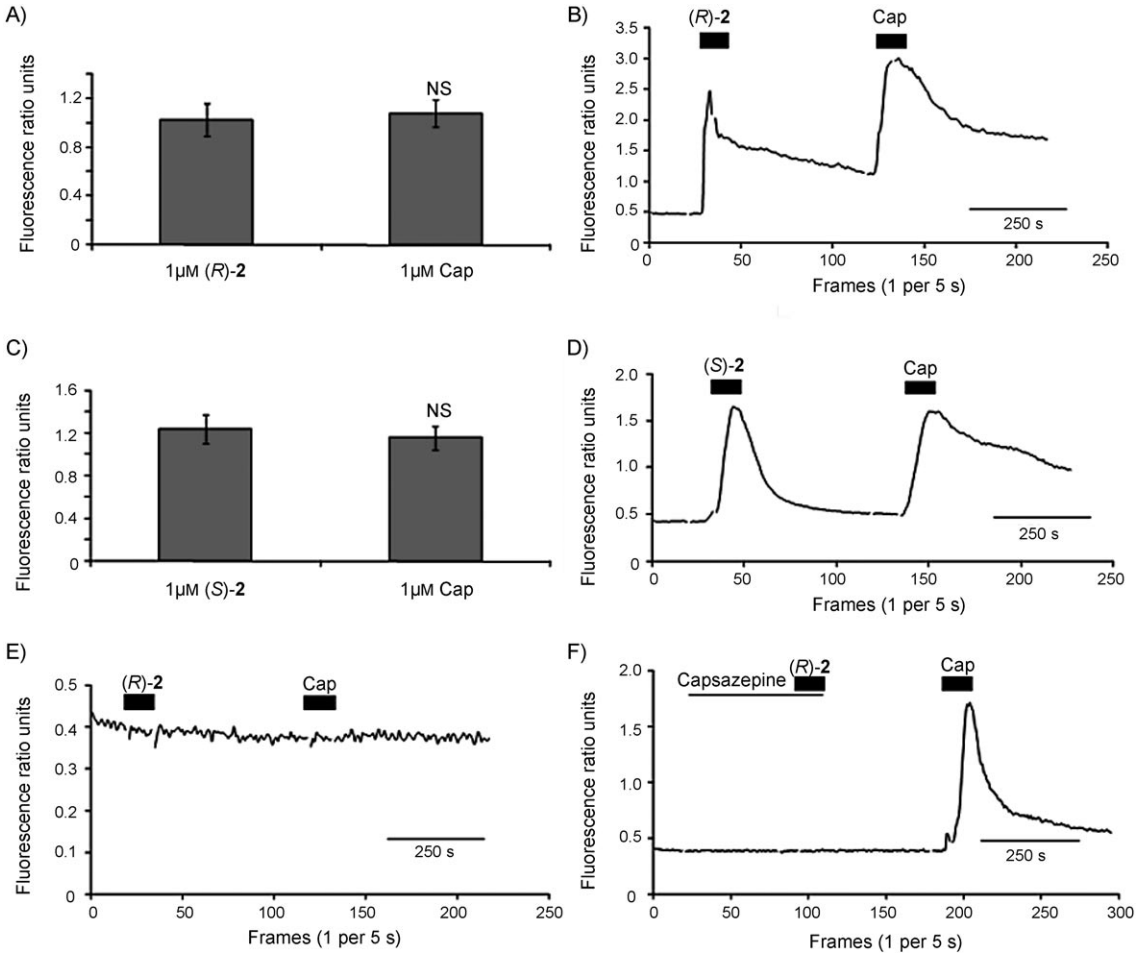


Figure 4. Synthetic fluorinated capsaicin isomers evoked transient increases in intracellular Ca^{2+} that were similar to capsaicin (Cap) responses in cultured DRG neurones. A) Bar chart showing the mean peak responses to 1 μM (*R*)-**2** and 1 μM Cap ($n = 25$; NS: not significantly different). B) An example record showing increases in intracellular Ca^{2+} evoked in a single DRG neurone exposed to (*R*)-**2** and capsaicin. C) Bar chart showing mean peak responses to 1 μM (*S*)-**2** and 1 μM Cap ($n = 19$; NS: not significantly different). D) An example record showing increases in intracellular Ca^{2+} evoked in a single DRG neurone exposed to (*S*)-**2** and Cap. E) An example record from a neurone that failed to respond to both 1 μM (*R*)-**2** and 1 μM Cap. F) An example record showing the complete block of 1 μM (*R*)-**2** response by 1 μM capsazepine. A robust response to capsaicin could be seen after the antagonist had been washed away; this demonstrates the expression of TRPV1 receptors in this neurone.

response. Also, 70% of DRG neurones that responded to (*R*)-**2** (1 μM) failed to respond to the lower concentration of the same synthetic capsaicin analogue. However, raising the concentration to 500 nM resulted in clear intracellular Ca^{2+} transients to both capsaicin analogues. Both (*R*)-**2** and (*S*)-**2** gave similar responses—the peak amplitudes relative to the maximum responses produced by the same analogue applied at 1 μM were (50 ± 5)% ($n = 19$) and (51 ± 7)% ($n = 8$; NS: not significantly different), respectively. Therefore, the apparent EC_{50} values for the two synthetic capsaicin analogues were not significantly different.

In conclusion we have synthesised the enantiomers of α-fluorocapsaicin in high enantiopurity. The enantiomers gave similar agonist responses to each other and to capsaicin itself in rat TRPV1 receptors. The agonist response of the α-fluorocapsaicins was successfully inhibited by the antagonist capsazepine; this indicates that they indeed located at the capsaicin binding site. If we assume that the fluorines adopt the anticipated *trans* conformation then the similar efficacy of the enantiomers suggests that the binding mode of the capsaicin side chain is not enantiomerically biased, and reinforces the developing models of capsaicin binding from mutagenesis and structural studies, which indicate that the side chain binds in an extended conformation from the amide bond directly along a molecular axis as illustrated in Figure 5.

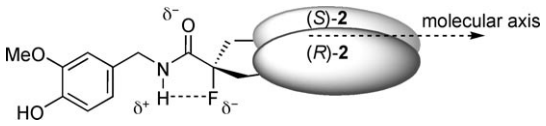


Figure 5. Suggested binding model of the enantiomers of **2** with the TRPV1 receptor, where both side chains can access a similar conformation along an extended molecular axis and maintain a *trans* amide conformation.

The theoretical study[9] suggests that there is a deep energy well favouring the *anti* conformer in α-fluoroamides, with *synclinal* and *anticlinal* (*gauche*) conformers lying ~3.0 and ~6.0 kcal mol^{-1}, respectively, above the minimum (Figure 1). However, if both fluorinated enantiomers were forced into, for example, equal and opposite *syn–clinal* conformer enantipodes to maintain a common orientation of the alkyl chain, then it would be difficult to distinguish this from the anticipated *anti* conformer mode of binding. However this would constitute an enantiomeric bias on binding and therefore appears less likely in view of the comparable agonistic (EC_{50}) efficacy displayed by each of the fluorinated enantiomers. Interestingly, these are the only fluorinated analogues of capsaicin that have been reported so far and they also emerge as potential ^{19}F NMR probes for magnetic resonance brain imaging in small animal models.[21] In view of recent developments where radiolabelled [^{18}F]-NFSI has been prepared as a new reagent for positron emission tomography (PET) synthesis,[22] the synthetic route described in this report has prospects for the fluorine-18 PET labelling of capsaicin for brain imaging by PET, particularly as the fluorinated analogues show similar efficacy to capsaicin itself.

Experimental Section

(*R*)-2-Phenylethylamine was purchased from Acros Organics and NFSI from Fluorochem Ltd. (Derbyshire, UK). (*E*)-8-Methylnon-6-enoyl chloride, fura-2 pentakis(acetoxymethyl)ester (fura-2-AM) and all other chemicals were purchased from Sigma–Aldrich. Ham's F14 medium was obtained from Imperial Laboratories (Hampshire, UK) and horse serum from Gibco (Paisley, Scotland). For GC–MS analysis a Supelco MDN-35 column (30.0 m × 250 μm × 0.25 μm) on an Agilent 6890 Series GC coupled to an Agilent 5973Network MSD and 7683 Series Autosampler was used (method: 1 mL min^{-1} He; 1 min 50 °C, 10 °C min^{-1} to 260 °C, 18 min 260 °C). NMR spectra were recorded by using a Bruker Avance II 400 MHz with 400.14 MHz (^1H), 100.62 MHz (^{13}C) and 376.41 MHz (^{19}F). Chiral analysis was carried out with a Daicel Chiralcel OD-H column (5 μm, 250 × 4.6 mm) by using a VARIAN Prostar equipped with a Model 410 Autosampler.

Synthesis

8-Methylnon-6-enol (***4***): (*E*)-8-Methylnon-6-enoyl chloride is specified as "predominantly *trans*" (Sigma–Aldrich). The *E*:*Z* ratio was determined by GC–MS to be 89:11 and this ratio was carried through all following steps. NMR spectra of the *Z* isomers are given for selected compounds. LAH (1.14 g, 30 mmol) was suspended in dry THF (15 mL) and cooled to 0 °C. (*E*)-8-Methylnon-6-enoyl chloride (4.26 g, 22.6 mmol) was dissolved in dry THF (5 mL) and added dropwise. The cooling was removed, the reaction was stirred for 1 h at room temperature and quenched under ice-cooling with HCl (2 N; 20 mL). The mixture was diluted with sat. NaCl to 100 mL and the product was extracted into Et_2O (3 × 50 mL). The organic layer was dried ($MgSO_4$) and the solvent was evaporated to yield alcohol **4** (3.36 g, 95%) as a colourless oil. (*E*)-**4**: ^1H NMR (400 MHz, $CDCl_3$, room temperature): $\delta = 0.89$ (d, $^3J(H,H) = 6.7$ Hz, 6H; CH_3), 1.25–1.35 (m, 4H; $2CH_2$), 1.50 (m, 2H; CH_2), 1.89–1.94 (m, 2H; CH_2), 2.16 (m, 1H; CH), 3.57 (t, $^3J(H,H) = 6.6$ Hz, 2H; CH_2), 5.23–5.34 (m, 2H; 2CH); ^{13}C NMR (125 MHz, $CDCl_3$, room temperature): $\delta = 22.7$, 25.2, 29.4, 31.0, 32.5, 32.7, 63.1, 126.9, 137.8; MS (EI; 69.9 eV): *m/z* (%): 156 (2) [M^+], 138 (2), 123 (13), 95 (79), 69 (100), 55 (91); (*Z*)-**4**: ^1H NMR (400 MHz, $CDCl_3$, room temperature): $\delta = 0.87$ (d, $^3J(H,H) = 6.8$ Hz, 6H; CH_3), 1.25–1.35 (m, 4H; $2CH_2$), 1.49 (m, 2H; CH_2), 1.95–2.01 (m, 2H; CH_2), 2.52 (m, 1H; CH), 3.57 (t, $^3J(H,H) = 6.6$ Hz, 2H; CH_2), 5.09–5.19 (m, 2H; 2CH); ^{13}C NMR (125 MHz, $CDCl_3$, room temperature): $\delta = 23.2$, 25.3, 29.4, 31.0, 32.5, 32.7, 63.1, 126.9, 137.8; MS (EI) (69.9 eV): *m/z* (%): 156 (1) [M^+], 138 (2), 123 (12), 95 (80), 69 (100), 55 (99).

8-Methylnon-6-enal (***5***): Alcohol **4** (1.56 g, 10 mmol) was dissolved in dry DCM (20 mL) and oxidised by addition of PCC (3.23 g, 15 mmol). After being stirred for 2 h at room temperature, the heterogeneous mixture was transfused and the residue was washed with Et_2O. The combined organic extracts were adsorbed onto Celite and purified by silica gel chromatography. Aldehyde **2** was obtained as a pungent colourless oil (1.13 g, 72%) and stored at −18 °C. (*E*)-**5**: ^1H NMR (400 MHz, $CDCl_3$, room temperature): $\delta =$ 0.88 (d, $^3J(H,H) = 6.8$ Hz, 6H; $2CH_3$), 1.25–1.35 (m, 2H; CH_2), 1.56 (m, 2H; CH_2), 1.90–1.95 (m, 2H; CH_2), 2.11–2.20 (qd, $^3J(H,H)$ 6.7, 13.4 Hz, 1H; CH), 2.35 (dt, $^3J(H,H) = 1.8$, 7.4 Hz, 2H; CH_2), 5.21–5.34 (m, 2H, 2CH), 9.69 (t, $^3J(H,H) = 1.8$ Hz, 1H; CHO); ^{13}C NMR (125 MHz, $CDCl_3$, room temperature): $\delta = 21.5$, 22.6, 29.0, 31.0, 32.1, 43.7, 126.3, 138.2, 202.8; MS (EI; 69.9 eV): *m/z* (%): 154 (0.2) [M^+], 136 (25), 121 (26), 95 (37), 69 (100), 55 (85); (*Z*)-**5**: ^1H NMR (400 MHz, $CDCl_3$, room temperature): $\delta = 0.87$ (d, $^3J(H,H) = 6.9$ Hz, 6H; $2CH_3$), 1.25–1.35 (m, 2H; CH_2), 1.56 (m, 2H; CH_2), 1.97–2.02 (m, 2H; CH_2), 2.36 (dt, $^3J(H,H) = 1.8$, 7.4 Hz, 2H; CH_2), 2.46–2.55 (m, 1H; CH), 5.09–5.17 (m, 2H; 2CH), 9.69 (t, $^3J(H,H) = 1.8$ Hz, 1H; CHO);

^{13}C NMR (125 MHz, $CDCl_3$, room temperature): $\delta = 21.6$, 23.2, 26.5, 29.0, 32.1, 43.7, 126.5, 138.1, 202.7; MS (EI; 69.9 eV): *m/z* (%): 136 (30), 121 (28), 95 (46), 69 (100), 55 (95).

*(S,E)-2-Fluoro-8-methylnon-6-enoic acid, (S)-**6***: A solution of NFSI (1.36 g, 3.92 mmol) and (*S*)-5-benzyl-2,2,3-trimethylimidazolidin-4-one dichloroacetic acid salt (70 mg, 0.2 mmol) in THF:*i*-propanol (10 mL, 9:1) was cooled to $-15\,^{\circ}C$ and then aldehyde **5** (206 mg, 1.34 mmol) was added. The reaction proceeded at $-10\,^{\circ}C$ and was monitored by GC–MS. After 12 h the reaction mixture was filtered over a pad of silica gel and eluted with Et_2O to a final volume of 25 mL. After reductive amination of an aliquot with (*R*)-2-phenylethylamine, the *ee* (90%) was determined by using ^{19}F NMR spectroscopy. For the oxidation of the aldehyde to the carboxylic acid, the mixture was diluted with acetone (25 mL) and water (5 mL) and treated dropwise with a solution of CrO_3 (1 M in 30% H_2SO_4) until complete consumption of the α-fluorinated aldehyde. Water (50 mL) was added and the aqueous phase was extracted into Et_2O (3×30 mL). The fluorinated carboxylic acid (*S*)-**6** was extracted into sat. $NaHCO_3$ solution and was subsequently acidified by addition of conc. HCl, and then re-extracted into Et_2O. The organic layer was dried with $MgSO_4$ and the solvent was evaporated to yield (*S*)-**6** (255 mg, 101%) as a pale yellow oil, which was directly used without further purification. After amide formation of an aliquot with (*R*)-2-phenylethylamine, the *ee* (87%) was determined by using ^{19}F NMR spectroscopy.

*(R,E)-2-Fluoro-8-methylnon-6-enoic acid, (R)-**6***: This was prepared in the same way as (*S*)-**6**, but (*R*)-5-benzyl-2,2,3-trimethylimidazolidin-4-one dichloroacetic acid salt (70 mg, 0.2 mmol) was used as the catalyst.

*4-Hydroxy-3-methoxybenzylamine hydrochloride (**7**)*: A solution of hydroxylamine hydrochloride (2.37 g, 34.0 mmol) and sodium acetate trihydrate (4.48 g, 32.9 mmol) in water (10 mL) was added to an aqueous solution of vanillin (5.00 g, 32.9 mmol, 20 mL) and the mixture was stirred at 80 °C for 2 h. The resultant oxime was extracted into ethyl acetate (3×50 mL), the organic layer was dried ($MgSO_4$) and the solvent was evaporated. The residue was dissolved in EtOH (150 mL) and then Pd/C (10% wt, dry basis) in H_2O (1.05 g) and HCl (36%, 20 mL) were added. The reaction was vigorously stirred under H_2 at atmospheric pressure and room temperature for 18 h. The catalyst was removed by filtration over Celite and the solvent volume was reduced, the residue was taken up in EtOH and crystallised from EtOH/ethyl acetate. The product was obtained as a pale yellow solid (5.52 g, 89% over the two steps). ^{1}H NMR (400 MHz, [D$_6$]DMSO, room temperature): $\delta = 3.76$ (s, 3H; CH_3), 3.88 (m, 2H; CH_2), 6.77 (d, ^3J(H,H) = 8.0 Hz, 1H; CH), 6.83 (dd, ^3J(H,H) = 1.9, 8.0 Hz, 1H; CH), 7.09 (d, ^3J(H,H) = 1.9 Hz, 1H; CH), 8.14 (br s, 3H; NH_3), 9.18 (s, 1H; OH).

*(S,E)-2-Fluoro-N-(4-hydroxy-3-methoxybenzyl)-8-methylnon-6-enamide, (S)-**2***: Amine **7** (208 mg, 1.1 mmol) was added to a solution of (*S*)-**6** in DMF (2 mL), Et_3N (306 μL, 2.2 mmol) and *O*-(benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium tetrafluoroborate (321 mg, 1.0 mmol) and the reaction was stirred at room temperature for 20 min. The mixture was then taken up in brine (15 mL) and extracted into ethyl acetate (2×10 mL). The organic extract was washed sequentially with HCl (1 N, 15 mL), saturated $NaHCO_3$ (15 mL) and brine (15 mL) and was then dried ($MgSO_4$) and the solvent was evaporated. Purification by silica gel chromatography gave capsaicinoid (*S*)-**2** (52 mg, 12% over three steps) as colourless needles after recrystallisation from hexane. The *ee* was determined by chiral HPLC by using an OD-H column with hexane/*i*-propanol (9:1) as solvent (flow: 1 mL min^{-1}, room temperature). The *ee* was calculated to be 96%; m.p. 74–75 °C. ^{1}H NMR (400 MHz, $CDCl_3$, room temperature): $\delta = 0.95$ (d, ^3J(H,H) = 6.8 Hz, 6H; 2CH_3), 1.51 (m, 2H; CH_2), 1.77–1.97 (m, 2H; CH_2), 2.02 (m, 2H; CH_2), 2.22 (qd, ^3J(H,H) = 6.8, 13.5 Hz, 1H; CH), 3.89 (s, 3H; OCH_3), 4.38 (dd, ^2J(H,H) = 14.2 Hz, ^3J(H,H) = 5.6 Hz, 1H; CH_aH_b), 4.43 (dd, ^2J(H,H) = 14.2 Hz, ^3J(H,H) = 5.8 Hz, 1H; CH_aH_b), 4.93 (ddd, ^2J(H,F) = 49.9 Hz, ^3J(H,H) = 3.7, 7.6 Hz, 1H; CHF), 5.27–5.42 (m, 2H; 2CH), 5.64 (s, 1H; OH), 6.57 (m, 1H; NH), 6.79 (m, 2H; 2CH), 6.88 (d, ^3J(H,H) = 7.9 Hz, 1H; CH); ^{13}C NMR (125 MHz, $CDCl_3$, room temperature): $\delta = 22.6$, 24.4 (^2J(C,F) = 2.2 Hz), 31.0, 31.8, 32.0, 43.0, 55.9, 92.1 (^1J(C,F) = 185.6 Hz), 110.6, 114.5, 120.9, 126.0, 129.6, 138.6, 145.3, 146.7, 169.9 (^2J(C,F) = 19.1 Hz); MS (EI; 69.9 eV): *m/z* (%): 323 (4) [M^+], 280 (2), 137 (100); ^{19}F NMR (376 MHz, $CDCl_3$, room temperature): $\delta = -190.71$ (dddd, ^1J(H,F) = 49.3 Hz, ^2J(H,F) = 23.0, 27.0 Hz, ^3J(H,F) = 4.4 Hz); HRMS $C_{18}H_{26}NO_3FNa$ [M+Na^+] calcd 346.1794, found 346.1793.

CCDC 706393 (**2**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/cgi-bin/catreq.cgi

*(R,E)-2-Fluoro-N-(4-hydroxy-3-methoxybenzyl)-8-methylnon-6-enamide, (R)-**2***: The capsaicinoid (*R*)-**2** (52 mg, 12% over three steps, *ee* 95%) was prepared and characterised from (*R*)-**6** as described for (*S*)-**2** and all spectral data were identical.

DRG neurone culture: All cultures were prepared by using DRG from decapitated 2-day-old Spargue–Dawley rats. The animals were killed under a Home Office licence and after local ethical review. The isolated ganglia were treated enzymatically (collagenase 0.125% for 13 min at 37 °C, trypsin 0.25% for 6 min) and mechanically. Dissociated sensory neurons were plated on to laminin-polyornithine coated coverslips and bathed in F14/HS culture media containing Ham's F14, heat inactivated horse serum (10%), penicillin (50 U mL^{-1}), streptomycin (5000 ng mL^{-1}) and $NaHCO_3$ (14 mM). Culture media were supplemented with nerve growth factor (NGF-2.5S; 20 ng mL^{-1}). DRG sensory neurones were used in experiments after being incubated at 37 °C in a humidified atmosphere with 5% CO_2 for between one and five days.

Fura-2 calcium imaging: DRG neurones were loaded with acetoxymethyl (AM) derivative of fura-2 (stock 1 mM in DMF for 1 h in NaCl-based extracellular medium containing fura-2-AM (10 μM). The extracellular bathing solution used contained NaCl (130 mM), KCl (3 mM), $MgCl_2$ (0.6 mM), $CaCl_2$ (2 mM), $NaHCO_3$ (1 mM), HEPES (10 mM) and glucose (5 mM). The pH and osmolarity of the extracellular bathing solutions were corrected to 7.4 and 320 mOsm L^{-1} with NaOH and sucrose, respectively. After loading with fura-2, the neurones were washed in extracellular medium and then preincubated in medium that contained DMSO (0.01%). Experiments and fura-2 loading were conducted at room temperature (20–25 °C). During the Ca^{2+} imaging experiments the cultured neurones were continuously perfused (3 mL min^{-1}) with NaCl-based extracellular medium containing DMSO (0.01%). Capsaicin, synthetic (*S*)-**2** and (*R*)-**2** isomers of fluorinated capsaicin and the TRPV1 receptor antagonist capsazepine were bath applied to the neurones at a concentration of 1 μM. The protocol used in this study was similar to that used previously.[16] Briefly, after equilibration, an isomer of fluorinated capsaicin was applied to the neurones for 1 min, the neurones were then washed with extracellular bathing solution for 7 min to allow recovery, and then capsicin was applied to the same neurones for 1 min before a final wash with extracellular bathing solution. Fluorescence ratio imaging was done at one frame captured every 5 s. Images were viewed and analysed by using the Ultraview

software (version 3). The mean peak amplitudes of Ca^{2+} transients ± standard errors of the means are given, and statistical analysis was conducted by using a paired Student's t-test.

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[1] E. K. Nelson, *J. Am. Chem. Soc.* **1919**, *41*, 1115–1121.
[2] L. Crombie, S. H. Dandegaonker, K. B. Simpson, *J. Chem. Soc.* **1955**, 1025–1027.
[3] M. J. Caterina, M. A. Schumacher, M. Tominaga, T. A. Rosen, J. D. Levine, D. Julius, *Nature* **1997**, *389*, 816–824.
[4] S. J. Conway, *Chem. Soc. Rev.* **2008**, *37*, 1530–1545 and references therein.
[5] a) C. S. J. Walpole, R. Wrigglesworth, S. Bevan, E. A. Campbell, A. Dray, I. F. James, M. N. Perkins, D. J. Reid, J. Winter, *J. Med. Chem.* **1993**, *36*, 2362–2372; b) C. S. J. Walpole, R. Wrigglesworth, S. Bevan, E. A. Campbell, A. Dray, I. F. James, K. J. Masdin, M. N. Perkins, J. Winter, *J. Med. Chem.* **1993**, *36*, 2373–2380; c) C. S. J. Walpole, R. Wrigglesworth, S. Bevan, E. A. Campbell, A. Dray, I. F. James, K. J. Masdin, M. N. Perkins, J. Winter, *J. Med. Chem.* **1993**, *36*, 2381–2389.
[6] C. S. J. Walpole, S. Bevan, G. Bovermann, J. J. Boelsterli, R. Breckenridge, J. W. Davies, G. A. Hughes, I. James, L. Oberer, J. Winter, R. Wrigglesworth, *J. Med. Chem.* **1994**, *37*, 1942–1954.
[7] S.-E. Jordt, D. Julius, *Cell* **2002**, *108*, 421–430.
[8] N. R. Gavva, L. Klionsky, Y. Qu, L. Shi, R. Tamir, S. Edenson, T. J. Zhang, V. N. Viswanadhan, A. Toth, L. V. Pearce, T. W. Vanderah, F. Porreca, P. M. Blumberg, J. Lile, Y. Sun, K. Wild, J.-C. Louis, J. J. S. Treanor, *J. Biol. Chem.* **2004**, *279*, 20283–20295.
[9] J. W. Banks, A. S. Batsanov, J. A. K. Howard, D. O'Hagan, H. S. Rzepa, S. Martin-Santamaria, *J. Chem. Soc. Perkin Trans. 2* **1999**, 2409–2411.
[10] C. R. S. Briggs, D. O'Hagan, J. A. K. Howard, D. S. Yufit, *J. Fluorine Chem.* **2003**, *119*, 9–13.
[11] D. O'Hagan, *Chem. Soc. Rev.* **2008**, *37*, 308–319.
[12] R. I. Mathad, B. Juan, O. Flogel, J. Gardiner, M. Loweneck, J. D. C. Codee, P. H. Seeberger, D. Seebach, M. K. Edmonds, F. H. M. Graichen, A. D. Abell, *Helv. Chim. Acta* **2007**, *90*, 2251–2273.
[13] J. R. Bull, R. Gordon, R. Hunter, *J. Chem. Soc. Perkin Trans. 1* **2000**, 3129–3139.
[14] T. D. Beeson, D. W. C. MacMillan, *J. Am. Chem. Soc.* **2005**, *127*, 8826–8828.
[15] J. Franzén, M. Marigo, D. Fielenbach, T. C. Wabnitz, A. Kjaersgaard, K. A. Jorgensen, *J. Am. Chem. Soc.* **2005**, *127*, 18296–18304.
[16] J.-L. Abad, S. Rodríguez, F. Camps, G. Fabriàs, *J. Org. Chem.* **2006**, *71*, 7558–7564.
[17] a) J. P. Ley, H.-J. Bertram, *Bioorg. Med. Chem.* **2001**, *9*, 1879–1885; b) P. M. Gannett, D. L. Nagel, P. J. Reilly, T. Lawson, J. Sharpe, B. Toth, *J. Org. Chem.* **1988**, *53*, 1064–1071.
[18] I. Abdelmoty, F. Albericio, L. A. Carpino, B. M. Foxman, S. A. Kates, *Lett. Pept. Sci.* **1994**, *1*, 57–67.
[19] R. M. Evans, R. H. Scott, R. Ross, *Br. J. Pharmacol.* **2004**, *141*, 1223–1233.
[20] R. M. Evans, R. H. Scott, R. Ross, *Br. J. Pharmacol.* **2007**, *152*, 404–413.
[21] J. W. M. Bulte, *Nat. Biotechnol.* **2005**, *23*, 945–946.
[22] H. Teare, E. G. Robins, E. Åstrad, S. K. Luthra, V. Gouverneur, *Chem. Commun.* **2007**, 2330–2332.

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