Supplementary Information

The perils of rational design – unexpected irreversible elimination of fluoride from 3-fluoro-2-methylacyl-CoA esters catalysed by α-methylacyl-CoA racemase (AMACR; P504S)

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Table of contents

Sources of materials & general experimental p 2
Assignment of stereochemical configurations in compounds pp 2- 3
Description of the AMACR reaction and its role in metabolic pathways p 3
Synthesis of anti-3-fluoro-2-methyldecanoyl-CoA 2R pp 4- 7
Synthesis of syn-3-fluoro-2-methyldecanoyl-CoA 2S pp 7 - 11
Synthesis of S- and R- E-2-methyldec-3-enoyl-CoA esters 3S and 3R pp 11 - 15
Synthesis of E-2-methyldec-2-enoyl-CoA (4) pp 15 - 16
Biological experimental pp 16 - 17
References p 18
Time course of elimination reaction of 2R catalysed by AMACR p 19
Kinetic plots for 2R pp 20 - 22
Kinetic plots for 2S pp 23 - 25
NMR spectra of 3S and 3R incubated with AMACR p 26

Abbreviations used: AcOH, acetic (ethanoic) acid; CDI, N,N'-carbonyldiimidazole; CoA, coenzyme A; DAST, diethylaminosulfur trifluoride; DBQ, 2,6-dichloro-1,4-benzoquinone; DCM, dichloromethane; ESI-TOF, electrospray ionisation-time-of-flight; HRMS (ES), High resolution mass spectrometry (electrospray); IPTG, isopropyl-β-D-thiogalactopyranoside; Pe, petroleum ether; PMSF, phenylmethylsulfonl fluoride; THF, tetrahydrofuran; p.p.m., parts per million.
Sources of materials: All reactions that require anhydrous conditions were performed under an argon atmosphere. Anhydrous and general grade solvents were purchased from the Sigma-Aldrich Chemical Co. and used without further purification unless otherwise noted. Oasis HLB cartridges were obtained from Waters Corporation. Biochemical grade reagents were purchased from the Sigma-Aldrich Chemical Co. or Fisher Scientific Ltd. Water for aqueous solutions was obtained from a Nanopure Diamond system and was of 18.2 MΩ.cm⁻¹ quality. The Rosetta2 (DE3) expression strain was obtained from Novagen. Metal-chelate chromatography columns were from GE Healthcare. Construction of the expression plasmid for human AMACR has been previously described.¹ Fenoprofenoyl-CoA was synthesised as previously described.²

General experimental: Solvents were removed using Büchi rotary evaporators. Thin layer chromatography was performed on Merck silica aluminium plates 60 (F254) and UV light, potassium permanganate or phosphomolibdric acid were used for visualization. Column chromatography was performed using Fisher silica gel (particle size 35-70 micron). Purifications of acyl-CoA esters were performed by solid phase extraction using Oasis HLB 6cc (200 mg) extraction cartridges. Phosphate buffer was prepared from monobasic and dibasic potassium phosphates at the required proportion for 0.1 M pH 7.0 buffer. Optical rotations were recorded on an Optical Activity AA-10 Automatic polarimeter instrument. IR spectra were recorded on Perkin-Elmer RXI FTIR spectrometer instrument. NMR spectra were recorded on Bruker Avance III 400.04 MHz or 500.13 MHz spectrometers in D₂O or CDCl₃ and solvent was used as an internal standard. Shifts are given in ppm and J values reported to 0.1 Hz. Multiplicities of peaks are described as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Stock concentrations of acyl-CoA esters for assays were determined using ¹H NMR.² Mass spectra were recorded by ESI-TOF at the University of Bath Mass Spectrometry Service. High resolution mass spectra were recorded in ES mode. Optical rotations are reported in 10⁻¹ deg cm² g⁻¹. Aqueous solutions for biological experiments were prepared in 18.2 MΩ.cm⁻¹ Nanopure water and pH-adjusted with aq. HCl or NaOH as appropriate. Syntheses were carried out at ambient temperature, unless otherwise specified.

Assignment of stereochemical configurations
Configurations of chiral centres and double bonds are assigned based on the rules set forth by Cahn, Ingold and Prelog in 1966.³ Priorities of ligands in this paper are assigned in the following order:

1) Atomic number (mass) of atoms;
2) Atomic number (mass) of neighbouring atoms;
3) Presence of double and triple bonds.
In 3-fluoro-2-methylcarboxylic acids and their derivatives a change in the priority order at carbon 2 occurs upon substitution of fluorine for the hydroxy group. In the aldol products the carboxylate amide or ester takes priority over the side-chain substituent, whilst the reverse is true in the fluorine-containing compounds (rule 2). A second change in priority order occurs upon going from the carboxylic acid to the acyl-CoA ester. This is because fluorine takes priority over oxygen and nitrogen, whilst sulfur takes priority over fluorine in the acyl-CoA ester (according to rule 2) (Scheme S1). Hence the relative orientation of ligands around carbon-2 does not change in this latter reaction but the stereochemical assignment changes due to changes in ligand priority.

Scheme S1: Assignment of stereochemistry of compounds

Syn- and anti- designation of 3-fluoro-2-methyldecanoyl-CoAs refer to the relative orientations of the methyl group and fluorine atom, whilst syn- and anti- elimination refers to the relative orientation of the α-proton and fluorine.

Description of the AMACR reaction and its role in metabolic pathways

AMACR catalyses an in vitro reaction in which either an R- or S-2-methylacyl-CoA ester is converted into a near 1:1 mixture of the C-2 epimers. The reaction differs from that of 2-methylmalony-CoA epimerase, in which the product has the opposite configuration at the methyl centre to the substrate, i.e. the product is only one epimer. For convenience, the AMACR catalysed reaction is described as ‘racemisation’ to reflect the fact that both 2-methylacyl-CoA epimers are formed in the reaction.

In vivo metabolism of 2-methyl fatty acids (as their corresponding acyl-CoA esters) occurs with a net R- to S- conversion of the chiral centre with the methyl group (C-2). This is because the S-2-methylacyl-CoA ester is removed by β-oxidation, whilst the R-2-methylacyl-CoA ester is not a substrate for the branched-chain acyl-CoA oxidase. In the metabolism of Ibuprofen™ and related 2-APA drugs, the R-2-APA is specifically converted into the R-2-APA-CoA, which is acted on by AMACR. Hydrolysis of the ‘racemic’ (2R/S) product results in a mixture of R- and S-2-APA drug, but the R-2-APA undergoes further cycles of metabolism. Since the S-2-APA is not recycled in this way, a net R- to S- conversion occurs. This three enzyme metabolic pathway is generally referred to in the literature (reviewed in) as ‘chiral inversion’.
Synthesis of anti-3-fluoro-2-methyldecanoyl-CoA (2R)

anti-3-Fluoro-2-methyldecanoyl-CoA 2R was synthesised by modification of the route to anti-3-fluoro-2-methyldecanoic acid reported by Carnell et al.,17 using octanal in place of tetradecanal. Conversion of the acid to 2R was achieved using carbonyldiimidazole as previously reported.2

Scheme S2: Synthesis of anti-3-fluoro-2-methyldecanoyl-CoA 2R. Reagents and conditions: i. n-BuLi, THF, propanoyl chloride, -78°C, 99%; ii. Bu₂BOTf, Pr₂NEt, octanal, DCM, -78°C, 99%; iii. DAST, DCM, -78°C, 64%; iv. LiOH, H₂O₂, H₂O/THF, 0°C, 61%; v. CDI, DCM, rt; vi. CoA-SH-Li⁺³, 0.1 M NaHCO₃ aq./THF (1:1).

(R)-4-Benzyl-3-propanoyloxazolidin-2-one (11)

R-Evans’ auxiliary 10 (4.056 g, 23 mmol) in anhydrous THF (60 mL) was cooled to -78°C, then n-BuLi (1.6 M, 14.3 mL, 23 mmol) in THF was added dropwise and the resulting mixture was stirred at -78°C for 30 min. A solution of propanoyl chloride (2.0 mL, 23 mmol) in anhydrous THF (20 mL) was added dropwise and the reaction mixture was stirred at -78°C for 30 min and then allowed to reach ambient temperature over a period of 1 h. The reaction mixture was quenched by slow addition of saturated aqueous NH₄Cl (80 mL) and extracted with DCM (2 × 100 mL). The combined organic extracts were washed with saturated aq. NaHCO₃ and brine. Drying (MgSO₄), filtration, evaporation and column chromatography (Pe / EtOAc 4:1) to give 11 (5.31 g, 99 %) as a colourless solid. mp 44-46°C, lit.18 43-46°C. ¹H NMR (400.04 MHz, CDCl₃) δ 7.34-7.15 (5H, m), 4.69-4.61 (1H, m), 4.21-4.12 (2H, m), 3.28 (1H, dd, J = 13.4, 3.3 Hz), 3.04-2.85 (2H, m), 2.76 (1H, dd, J = 13.4, 9.5 Hz), 1.18 (3H, t, J = 7.3 Hz).19
(R)-4-Benzyl-3-[(2R,3S)-3-hydroxy-2-methyldecanoyl]oxazolidin-2-one (12)

Dibutylboron triflate in DCM (1.00 M, 1.30 mL, 1.29 mmol) and diisopropylethylamine (0.25 mL, 1.29 mmol) were added to a stirred solution of oxazolidinone 11 (300 mg, 2.14 mmol) in DCM (10 mL) cooled to -78°C and the solution was stirred for 30 min. Octanal (0.15 mL, 0.92 mmol) in DCM (3.0 mL) was added dropwise and the reaction mixture was stirred at -78°C for 30 min, then allowed to reach ambient temperature. The reaction was quenched by slow addition of phosphate buffer (0.1 M, pH = 7, 10 mL). The organic layer was then washed with aq. hydrochloric acid (1 M), saturated aq. NaHCO₃ and brine. Drying (MgSO₄), filtration, evaporation and column chromatography (Pe / EtOAc 10:1) gave 12 (330 mg, 99%) as a colourless oil: R₇r=0.25 Pe:EtO 1:1. [α]²¹_D = -59.3°(CHCl₃, c = 0.54); IR (liquid film, cm⁻¹) 3514, 1783, 1698; ¹H NMR (400.04 MHz, CDCl₃) δ 7.36-7.17 (5H, m), 4.75-4.65 (1H, m), 4.26-4.16 (2H, m), 3.98-3.89 (1H, m), 3.76 (1H, dq J = 7.0, 2.7 Hz), 3.25 (1H, dd, J = 13.4, 3.3 Hz), 2.84-2.74 (2H, m), 1.35-1.22 (12H, m), 1.25 (3H, d, J = 7.0 Hz), 0.87 (3H, t, J = 7.0 Hz); ¹³C NMR (100.59 MHz) δ 177.42, 152.98, 135.03, 129.36, 128.88, 127.33, 71.48, 67.85, 55.04, 42.13, 37.72, 33.88, 31.74, 29.47, 25.95, 22.56, 14.00, 10.40; HRMS (ES) ([M + Na]⁺) Calcd. for C₂₁H₃₁NNaO₄: 384.2151, Found: 384.2198.

(R)-4-Benzyl-3-[(2S,3R)-3-fluoro-2-methyldecanoyl]oxazolidin-2-one (13)

Compound 12 (156 mg, 0.43 mmol) in anhydrous DCM (3.0 mL) was cooled to -78°C, then DAST (57 µL, 0.43 mmol) in anhydrous DCM (2.0 mL) was added dropwise to the reaction mixture. The mixture was, stirred at -78°C for 2 h, then allowed to reach ambient temperature. The reaction mixture was quenched by slow addition of water (5.0 mL). The organic layer was washed with saturated aq. NaHCO₃ and brine. Drying (MgSO₄), filtration, evaporation and column chromatography (Pe / EtOAc 10:1) gave 13 (101 mg, 64%) as a colourless oil: R₇r=0.66 Pe:EtOAc 5:1. [α]²¹_D = -42.0°(CHCl₃, c=0.87). IR (liquid film, cm⁻¹): 1781, 1670, 1498. ¹H NMR (400.04
MHz, CDCl$_3$): δ 7.37-7.16 (5H, m), 4.87-4.64 (2H, m), 4.24-4.07 (3H, m), 3.26 (1H, dd, $J = 13.4$, 3.3 Hz), 2.87-2.75 (1H, m), 1.55-1.22 (12H, m), 1.18 (3H, d, $J = 7.0$ Hz), 0.88 (3H, t, $J = 6.8$ Hz).

$^{13}$C NMR (125.76 MHz) δ 174.31 (d, $J = 3.0$ Hz), 153.04, 135.15, 129.38, 128.86, 127.30, 95.51, 94.15, 66.11, 55.32, 41.95 (d, $J = 21.2$ Hz), 37.78, 31.97 (d, $J = 21.0$ Hz), 31.70, 29.18 (d, $J = 27.0$ Hz), 24.48 (d, $J = 2.8$ Hz), 22.55, 14.01, 13.54 (d, $J = 8.3$ Hz).

$^{19}$F NMR (470.52 MHz) δ -179.69.


(2S,3R)-3-Fluoro-2-methyldecanoic acid (5)

Oxazolidinone 13 (100 mg, 0.28 mmol) in THF (3.0 mL) was cooled to 0°C, then 30% (v/v) aq. H$_2$O$_2$ (0.14 mL, 1.7 mmol) and LiOH (13 mg, 0.55 mmol) were added and the reaction mixture was stirred for 12 h. The reaction mixture was quenched by addition of saturated aq. Na$_2$SO$_3$ (3.0 mL) and was extracted with DCM. The organic phase was washed with water and brine. Drying (MgSO$_4$), filtration, evaporation and column chromatography (Pe / EtOAc 5:1) gave 5 (34 mg, 61%) as a colourless solid. mp 63-65°C; [$\alpha$]$^1$$_D$ = +0.74° (CHCl$_3$, c = 0.35); $^1$H NMR (400.04 MHz, CDCl$_3$) δ 10.56 (1H, br s), 4.78-4.57 (1H, m), 2.85-2.71 (1H, m), 1.45-1.18 (12H, m), 1.19 (3H, d, $J = 7.2$ Hz), 0.87 (3H, t, $J = 7.0$ Hz). $^{13}$C NMR (125.76 MHz) δ 179.93 (d, $J = 172.1$ Hz), 44.36 (d, $J = 22.1$ Hz), 31.74 (d, $J = 21.2$ Hz), 31.74, 29.30, 29.12, 24.82 (d, $J = 2.8$ Hz), 22.61, 14.06, 12.56 (d, $J = 6.6$ Hz); $^{19}$F NMR (470.52 MHz) δ -181.96. HRMS (ES) ([M - H]$^-$) Calcd. for C$_{11}$H$_{20}$FO$_2$: 203.1447, Found: 203.1447.

(2R,3R)-3-Fluoro-2-methyldecanoyl-CoA (2R)

Acid 5 (10 mg, 0.05 mmol) in anhydrous DCM (1.0 mL) was treated with N,N’-carbonyldiimidazole (14 mg, 0.09 mmol) in one portion and the mixture was stirred for 1 h. The mixture was washed with water (5 × 2 mL) and brine. Drying (MgSO$_4$), filtration and evaporation gave the crude imidazolide. This material was dissolved in THF (1.0 mL) and CoA-Li$_3$ (17 mg, 0.02 mmol) was added, followed by aq. NaHCO$_3$ (0.1 M, 1.0 mL) and the mixture was stirred for 18 h.
The solution was acidified to pH ~3 with aq. HCl (1 M) and the solvents were partly removed under reduced pressure. Water (2.0 mL) was added and the mixture was washed with EtOAc (5 × 3 mL). Solid-phase extraction of the aqueous layer gave $2R$ (6.0 mg) as white powder: $^1$H NMR (500.13 MHz, D$_2$O) $\delta$ 8.47 (1H, s), 8.18 (1H, s), 6.07 (1H, d, $J = 7.1$ Hz), 4.18-4.11 (1H, m), 3.92 (1H, s), 3.71-3.68 (1H, d, $J = 4.6$ Hz), 3.57 (1H, d, $J = 4.6$ Hz), 3.49-3.43 (2H, m), 3.37 (1H, d, $J = 4.6$ Hz), 3.49-3.43 (2H, m), 3.37-3.33 (1H, m), 3.28-3.23 (1H, m), 3.17 (2H, t, $J = 6.5$ Hz), 2.99-2.91 (2H, m), 2.61 (2H, t, $J = 6.5$ Hz), 2.32 (2H, t, $J = 6.5$ Hz), 1.25-1.09 (10H, m), 1.04 (3H, d, $J = 7.1$ Hz), 0.79 (3H, s), 0.75 (3H, t, $J = 6.9$ Hz), 0.67 (3H, s); $^{19}$F NMR (470.52 MHz) $\delta$ -181.08; HRMS (ES) ([M - H$^+$]) Calcd. for C$_{32}$H$_{54}$FN$_7$SO$_7$P$_3$: 952.2494, Found: 952.2528.

Synthesis of syn-3-fluoro-2-methyldecanoyl-CoA (2S)

syn-Fluoro-2-methyldecanoyl-CoA 2S was synthesised (Scheme S3a) by condensation of 2-octenal with propanoyl-Evans’ auxiliary 11 to give the unsaturated aldol product 14. Hydrogenation over Pd/C gave the desired saturated compound 15. The Evan’s auxiliary was exchanged for the methyl ester 16, which was converted to the fluorinated compound 17 and deprotected under acid conditions to give 6. Conversion to the acyl-CoA ester 2S was accomplished using the general literature procedure. Direct treatment of 15 with DAST resulted in an unexpected rearrangement (Scheme S3b) via intermediate 18 to give product 19. This is believed to result from steric hindrance of the $\alpha$-methyl group to the nucleophilic attack of the fluoride on the derivatised hydroxy group. Instead fluoride performs a nucleophilic attack on the oxazolidin-2-one ring.

Scheme S3: a) Synthesis of syn-3-fluoro-2-methyldecanoyl-CoA 2S. Reagents and conditions: i. Bu$_2$BOTf, Pr$_2$Net, 2-octenal, Et$_3$O, -78°C, 30%; ii. H$_2$, Pd/C, MeOH, 87%; iii. NaOMe, MeOH, 0°C, 56%; iv. DAST, DCM, -78°C, 41%; v. aq. HCl, AcOH, 100°C, 50%; vi. CDI, DCM, rt; vii. CoA-SH-Li$^+$, 0.1 M NaHCO$_3$ aq. / THF (1:1); b) Unexpected rearrangement of 15 upon treatment with DAST. Reagents and conditions: iv. DAST, DCM, -78°C, 99%.
(R)-4-Benzyl-3-[(2S,3S,E)-3-hydroxy-2-methyldec-4-enoyl]oxazolidin-2-one (14)

A solution of dibutylboron triflate in DCM (1.0 M, 1.7 mL, 1.71 mmol) and diisopropylethylamine (0.17 mL, 0.99 mmol) were added to 11 (200 mg, 0.86 mmol) in Et₂O (5.0 mL) at 0 °C and the solution was stirred for 30 min. The reaction mixture was cooled to -78°C and 2-octenal (0.16 mL, 1.1 mmol) in Et₂O (1.0 mL) was added dropwise. The reaction mixture was stirred at -78°C for 30 min and then allowed to reach ambient temperature. The reaction was quenched by slow addition of aq. phosphate buffer (0.1 M, pH = 7, 7 mL) and the organic layer was washed with aq. HCl (1.0 M), saturated aq. NaHCO₃ and brine. Drying (MgSO₄), filtration, evaporation and column chromatography (Pe / EtOAc 10:1) gave 14 (92 mg, 30%) as colourless oil: Rₜ=0.37 Pe:EtOAc 5:1. [α]²₁⁺D = +49.8 (CHCl₃, c = 0.58). IR (liquid film, cm⁻¹): 3498, 1780, 1698; ¹H NMR (400.04 MHz, CDCl₃) δ 7.37-7.20 (5H, m), 5.83-5.69 (1H, m), 5.56-5.46 (1H, m), 4.74-4.64 (1H, m), 4.27-4.12 (3H, m), 3.98-3.89 (1H, m), 3.30 (1H, dd, J = 13.5, 3.4 Hz), 2.83-2.72 (1H, m), 2.51 (1H, d, J = 7.0 Hz), 2.10-2.01 (2H, m), 1.44-1.23 (6H, m), 1.16 (3H, d, J = 7.1 Hz), 0.87 (3H, t, J = 7.0 Hz); ¹³C NMR (125.76 MHz) δ 176.02, 153.35, 135.17, 133.74, 129.34, 128.91, 128.73, 127.32, 73.24, 66.07, 55.35, 42.75, 37.98. 32.18, 31.33, 28.73, 22.42, 13.93, 11.22. HRMS (ES) ([M + Na⁺]⁺) Calcd. for C₂₁H₃₁NNaO₄: 382.1994, Found: 382.2054.

(R)-4-Benzyl-3-[(2S,3S)-3-hydroxy-2-methyldecanoyl]oxazolidin-2-one (15)

Aldol 14 (150 mg, 0.42 mmol) in EtOAc (10 mL) was stirred vigorously under hydrogen in the presence of 10 % Pd/C (10 mass%, 15 mg) for 1 d. The mixture was filtered (Celite) and the solvent was evaporated. Column chromatography (Pe / EtOAc 5:1) gave 15 (132 mg, 87 %) as a colourless oil: Rₜ=0.37 Pe:EtOAc 5:1. [α]²¹⁺D = -40.0°(CHCl₃, c = 0.5). IR (liquid film, cm⁻¹) 3524, 1780, 1698; ¹H NMR (400.04 MHz, CDCl₃) δ 7.38-7.19 (5H, m), 4.74-4.63 (1H, m), 4.25-4.14 (2H, m), 3.94-3.84 (1H, m), 3.78-3.66 (1H, m), 3.33 (1H, dd, J = 13.4, 3.3 Hz), 2.81-2.72 (1H, m), 2.51 (1H,
\( d, J = 8.6 \text{ Hz}, 1.65-1.23 (12\text{H, m}), 1.21 (3\text{H, d, } J = 6.8 \text{ Hz}), 0.88 (3\text{H, t, } J = 7.0 \text{ Hz}). \) 

\( ^{13}\text{C NMR (100.59 MHz)} \delta 176.84, 153.49, 135.21, 129.38, 128.89, 127.28, 74.61, 65.98, 55.48, 43.22, 37.82, 34.95, 31.75, 29.48, 29.18, 25.40, 22.57, 14.55, 14.01; \) HRMS (ES) \([\text{M + Na}^+]\) Calcd. for \( \text{C}_{21}\text{H}_{31}\text{NNaO}_4 \): 384.2151, Found: 384.2198.

(2S,3S)-Methyl 3-hydroxy-2-methyldecanoate (16)

Sodium (48 mg, 2.1 mmol) was stirred with anhydrous MeOH (15 mL) until dissolved then cooled to 0 °C. Aldol 15 (470 mg, 1.30 mmol) in anhydrous MeOH (5.0 mL) was added and the mixture was stirred at 0 °C for 15 min. The reaction mixture was quenched by slow addition of aq. phosphate buffer (0.1 M, pH = 7, 20 mL) and extracted with DCM (4 × 20 mL). The combined organic layers were washed with brine and dried (MgSO\(_4\)). Filtration, evaporation and column chromatography (Pe / EtOAc 5:1) gave 16 (158 mg, 56%) as a colourless oil. \( R_f = 0.67 \) Pe:EtOAc 5:1. \([\alpha]_{D}^{21} = +5.9^\circ \) (CHCl\(_3\), c = 1.0). \(^1\text{H NMR (400.04 MHz, CDCl}_3\) \( \delta 3.70 (3\text{H, s}), 3.65 (1\text{H, br s}), 2.58-2.44 (2\text{H, m}), 1.60-1.22 (12\text{H, m}), 1.20 (3\text{H, d, } J = 7.2 \text{ Hz}), 0.87 (3\text{H, t, } J = 7.0 \text{ Hz}). \) \(^{13}\text{C NMR (100.59 MHz)} \delta 176.39, 73.32, 51.60, 45.11, 34.71, 31.73, 29.45, 29.16, 25.44, 22.56, 14.26, 13.99. \) HRMS (ES) \([\text{M + Na}^+]\) Calcd. for \( \text{C}_{12}\text{H}_{24}\text{NaO}_3 \): 239.1623, Found: 239.1634.

(2R,3R)-Methyl 3-fluoro-2-methyldecanoate (17)

Compound 17 was prepared from 16 (54 mg, 0.25 mmol), following the same procedure as for 13. The product was purified by column chromatography (Pe / EtOAc 20:1) to give 17 (42 mg, 78%) as a colourless oil: \( R_f = 0.41 \) Pe:EtOAc 20:1. \([\alpha]_{D}^{21} = +9.1^\circ \) (CHCl\(_3\), c = 0.55). \(^1\text{H NMR (400.04 MHz, CDCl}_3\) \( \delta 4.80-4.59 (1\text{H, m}), 3.70 (3\text{H, s}), 2.71-2.55 (1\text{H, m}), 1.59-1.20 (12\text{H, m}), 1.23 (3\text{H, d, } J = 7.1 \text{ Hz}), 0.87 (3\text{H, t, } J = 7.0 \text{ Hz}). \) \(^{13}\text{C NMR (100.59 MHz)} \delta 174.02 (d, \text{ } J = 7.8 \text{ Hz}), 94.06 (d, \text{ } J = 173.7 \text{ Hz}), 198.51, 44.19 (d, \text{ } J = 22.8 \text{ Hz}), 32.91 (d, \text{ } J = 21.0 \text{ Hz}), 31.73, 29.27, 29.11, 25.23 (d, \text{ } J = 3.8 \text{ Hz}), 22.61, 14.06, 11.59 (d, \text{ } J = 5.4 \text{ Hz}). \) \(^{19}\text{F NMR (470.52 MHz)} \delta -189.85. \)
Methyl ester 17 (19 mg, 0.09 mmol) in acetic acid (1.0 mL) and aq. HCl (12 M, 1.0 mL) was heated at 100°C for 4 h. The mixture was cooled to ambient temperature and the solvents were evaporated under reduced pressure. The residue, in DCM, was washed with water (twice) and brine. Drying (MgSO₄), filtration, evaporation and column chromatography (Pe / EtOAc 2:1) gave 6 (9 mg, 50%) as a colourless oil; Rₛ=0.61 Pe:EtOAc 2:1; [α]ᵢ²₁D = +7.5° (CHCl₃, c = 0.4); ¹H NMR (400.04 MHz, CDCl₃) δ 10.38 (1H, br s), 4.86-4.64 (1H, m), 2.73-2.59 (1H, m), 1.53-1.19 (15H, m), 0.88 (3H, t, J = 6.9 Hz); ¹⁹F NMR (470.52 MHz) δ -190.19. HRMS (ES) ([M - H⁻]) Calcd. for C₁₁H₂₀F₂O₂: 203.1447, Found: 203.1452.

(2S,3R)-3-Fluoro-2-methyldecanoyl-CoA (2S)

Compound 2S was prepared from 6 (7 mg), following the same procedure as for compound 2R to give 2 mg of 2S. ¹H NMR (500.13 MHz, D₂O) δ 8.71 (1H, s), 8.48 (1H, s), 6.30-6.20 (1H, m), 4.67-4.58 (1H, m), 4.36-4.24 (2H, m), 4.11-4.02 (1H, m), 3.96-3.85 (1H, m), 3.70-3.57 (1H, m), 3.55-3.34 (2H, m), 3.15-2.99 (2H, m), 2.59-2.40 (2H, m), 2.31-2.17 (1H, m), 1.84 (2H, s), 1.50-1.16 (10H, m), 0.97 (3H, d, J = 6.5 Hz), 0.86 (3H, s), 0.84 (3H, s); ¹⁹F NMR (470.52 MHz) δ -187.20.

(5R,6S)-3-[(S)-1-Fluoro-3-phenylpropan-2-yl]-6-heptyl-5-methyl-1,3-oxazinane-2,4-dione (19)

Compound 19 was prepared following the synthesis of 13 from 15 (115 mg, 0.32 mmol). Product was purified by column chromatography (Pe:EtOAc 20:1) to give 116 mg (99 %) of 19 as a colourless oil. Rₛ=0.72 Pe:EtOAc 5:1. [α]ᵢ²¹D = +66.7° (CHCl₃, c = 0.87). IR (neat, cm⁻¹): 1754.82, 1704.78. ¹H NMR (500.13 MHz, CDCl₃): δ 7.29-7.14 (5H, m), 5.49-5.31 (1H, m), 4.92 (1H, dt, J =
48.4, 9.1 Hz), 4.59 (1H, ddd, J = 45.4, 9.4, 5.1 Hz), 4.24-4.12 (1H, m), 3.21 (1H, dd, J = 13.6, 11.1 Hz), 2.99 (1H, dd, J = 13.6, 6.2 Hz), 2.61 (1H, dq, J = 7.2, 3.9 Hz), 1.40-1.14 (12H, m), 0.87 (3H, t, J = 7.1 Hz), 0.82 (3H, d, J = 7.2 Hz). 13C NMR (125.76 MHz) δ 172.53, 150.57, 136.27, 129.19, 128.44, 126.80, 81.79 (d, J = 171.1 Hz), 77.03, 53.66 (d, J = 17.7 Hz), 39.15, 33.44 (d, J = 5.9 Hz), 31.61, 29.13, 28.93 (d, J = 4.7 Hz), 24.96, 23.77, 22.51, 13.98, 9.10. 19F NMR (470.59 MHz) δ -221.77. HRMS (ES) ([M + Na]+) Calcd. for C21H30FNNaO3: 386.2102, Found: 386.2102.

Synthesis of S- and R-E-2-methyldec-3-enoyl-CoA esters (3S) and (3R)

Synthesis of S- and R-E-2-methyldec-3-enoyl-CoA esters 3S and 3R was accomplished by reaction of the Grignard reagent from crotyl chloride 8 with CO2 to form the key unsaturated intermediate 20. Derivatisation with R-Evan’s auxiliary 10 allowed separation of the diastereoisomers 21S and 21R, which reacted with oct-1-ene in a metathesis reaction to give 22S and 22R. Deprotection afforded the required acids 7S and 7R, which were esterified with CoA-SH as previously described.2

Scheme S4: Synthesis of E-2-methyldec-2-enoyl-CoA esters 3S and 3R. Reagents and conditions: i. Mg, I2, THF, reflux; ii. CO2, -78°C, H3O+, 55%; iii. n-BuLi, (COCl)2, R-Evan’s auxiliary 10, THF, -78°C, 13% (21S) and 19% (21R); iv. 1-Octene, Hoveyda-Grubbs 2nd Generation cat., DBQ, DCM, reflux, 80% (22S) and 65% (22R); v. LiOH, H2O2, H2O/THF (1:1), room temperature, 98% (7S) and 99% (7R); vi. CDI, DCM, rt; vii. CoA-SH-Li+3, 0.1M NaHCO3 aq./ THF (1:1).

(±)-2-Methylbut-3-enoic acid (20)

Under strictly dry conditions, Mg turnings (275 mg, 11.3 mmol) were stirred with iodine (2 mg) in anhydrous THF (10 mL) for 30 min. Crotyl chloride 8 (2 drops) (E-1-chlorobut-2-ene) was added
to initiate reflux and the remaining 8 (1.00 mL, 10.3 mmol) was added dropwise, maintaining gentle reflux. The reaction mixture was stirred at ambient temperature for 30 min, then cooled to -78°C. CO2 from dry ice was passed through concentrated sulfuric acid and bubbled through the mixture for 30 min. The reaction mixture was then allowed to reach ambient temperature under a flow of CO2. The reaction mixture was basified to pH 11 with aq. NaOH (4 M) and was washed with Et2O (3 × 30 mL). The aqueous layer was separated and acidified to pH 2 with aq. HCl (1 M) and extracted with Et2O (2 × 30 mL). The combined organic layers were washed with brine and dried (MgSO4). The solution was filtered and the solvent was evaporated to give 20 (560 mg, 55%) as a colourless liquid: 1H NMR (400.04 MHz, CDCl3) δ 11.01 (br s, 1H), 5.93 (1H, ddd, J = 17.4, 10.3, 7.4 Hz), 5.21-5.12 (1H, m), 3.23-3.12 (m, 1H), 1.30 (d, 3H, J = 7.1 Hz).

(R)-4-Benzyl-3-[((R)-2-methylbut-3-enoyl]oxazolidin-2-one (21R) and (R)-4-benzyl-3-[(S)-2-methylbut-3-enoyl]oxazolidin-2-one (21S)

Oxalyl chloride (1.60 mL, 18.7 mmol) was added dropwise to 20 (0.940 g, 9.40 mmol) in CHCl3 (15 mL) at 0°C. The reaction mixture was stirred at ambient temperature for 3 h. Evaporation of the solvent and excess reagent gave the corresponding acid chloride. n-BuLi (8.4 mL, 1.6 M, 13 mmol) in THF was added dropwise to 10 (1.165 g, 9.40 mmol) in anhydrous THF (20 mL) at -78°C and the mixture was stirred for 30 min. The acid chloride in anhydrous THF (5.0 mL) was added dropwise. The reaction mixture was stirred at -78°C for 1 h and was then allowed to reach ambient temperature. The reaction mixture was quenched by slow addition of saturated aq. NH4Cl (25 mL) and was extracted with DCM (2 × 50 mL). The combined organic layers were washed with saturated aq. NaHCO3 and brine. Drying (MgSO4), filtration, evaporation and column chromatography (Pe / EtOAc 10:1) gave 21R (467 mg, 19%) as a colourless oil. [α]21D = -84.4° (CHCl3, c = 1.2), lit.20 -87.3°. 1H NMR (400.04 MHz, CDCl3) δ 7.37-7.16 (5H, m), 5.98 (1H, ddd, J = 7.7, 10.3, 17.4 Hz), 5.19 (1H, ddd, J = 1.2, 1.2, 17.4 Hz), 5.13 (1H, ddd, J = 1.0, 1.2, 10.3 Hz), 4.70-4.61 (1H, m), 4.51-4.41 (1H, m), 4.22-4.14 (2H, m), 3.28 (1H, dd, J = 3.3, 13.4 Hz), 2.78 (1H, dd, J = 9.6, 13.4 Hz), 1.34 (d, 3H, J = 6.9 Hz).
Further elution gave $21S$ (311 mg, 13%) as a white solid: mp 69-71°C, lit.\textsuperscript{20} 72-74°C; $[\alpha]^{21}_D = -32.8^\circ$ (CHCl\textsubscript{3}, c = 0.43), lit.\textsuperscript{20} -28.9\textdegree. \textsuperscript{1}H NMR (400.04 MHz, CDCl\textsubscript{3}) $\delta$ 7.36-7.17 (5H, m), 6.02 (1H, ddd, $J = 7.6, 10.3, 17.5$ Hz), 5.26 (1H, ddd, $J = 1.1, 1.2, 17.5$ Hz), 5.19 (1H, ddd, $J = 1.0, 1.1, 10.3$ Hz), 4.73-4.65 (1H, m), 4.53-4.43 (1H, m), 4.24-4.12 (2H, m), 3.25 (1H, dd, $J = 3.3, 13.4$ Hz), 2.74 (1H, dd, $J = 9.5, 13.4$ Hz), 1.31 (3H, d, $J = 6.9$ Hz).

(R)-4-Benzyl-3-[(S,E)-2-methyldec-3-enoyl]oxazolidin-2-one ($22S$)

Grubbs’ catalyst (Hoveyda-Grubbs Catalyst, 2\textsuperscript{nd} Generation) (37 mg, 0.06 mmol) and DBQ (21 mg, 0.12 mmol) were stirred in anhydrous DCM (3.0 mL) for 5 min, then $21S$ (305 mg, 1.18 mmol) and octene (0.37 mL, 2.35 mmol) in anhydrous DCM (2.0 mL) were added. The reaction mixture was stirred at reflux for 40 h. Evaporation and column chromatography (Pe / EtOAc 20:1) gave $22S$ (324 mg, 80%) as a colourless oil: R\textsubscript{f}=0.57 Pe:EtOAc 5:1. $[\alpha]^{21}_D = -23.5^\circ$ (CHCl\textsubscript{3}, c = 1.53); IR (liquid film, cm\textsuperscript{-1}) 1782, 1700; \textsuperscript{1}H NMR (400.04 MHz, CDCl\textsubscript{3}) $\delta$ 7.34-7.16 (5H, m), 5.74-5.53 (2H, m), 4.73-4.64 (1H, m), 4.49-4.38 (1H, m), 4.22-4.10 (2H, m), 3.22 (1H, dd, $J = 13.4, 3.4$ Hz), 2.78-2.69 (1H, m), 2.08-2.00 (2H, m), 1.41-1.23 (11H, m), 0.87 (3H, t, $J = 7.0$ Hz); \textsuperscript{13}C NMR (100.59 MHz) $\delta$ 175.21, 152.91, 135.21, 133.32, 129.42, 128.83, 128.37, 127.24, 65.81, 55.09, 40.45, 37.68, 32.49, 31.62, 29.10, 28.72, 22.55, 17.23, 13.99. HRMS (ES) ([M + Na\textsuperscript{+}]) Calcd. for C\textsubscript{21}H\textsubscript{29}NNaO\textsubscript{3}: 366.2040, Found: 366.2077.

(R)-4-Benzyl-3-[(R,E)-2-methyldec-3-enoyl]oxazolidin-2-one ($22R$)

Compound $22R$ was prepared from $21R$ (460 mg, 1.77 mmol), following the same procedure as for compound $22S$. The product was purified by column chromatography (Pe / EtOAc 20:1) to give $22R$ (396 mg, 65 %) as a colourless oil: R\textsubscript{f}=0.70 Pe:EtOAc 5:1. $[\alpha]^{21}_D = -85.3^\circ$ (CHCl\textsubscript{3}, c = 1.98); IR (liquid film, cm\textsuperscript{-1}) 2957, 2927, 2872, 2856, 1783, 1699, 1455, 1382, 1355, 1210, 1104, 973, 703;
\(^1\)H NMR (400.04 MHz, CDCl\(_3\)) \(\delta\) 7.35-7.17 (5H, m), 5.65-5.50 (2H, m), 4.68-4.58 (1H, m), 4.45-4.36 (1H, m), 4.17-4.12 (2H, m), 3.27 (1H, dd, \(J = 13.3, 3.2\) Hz), 2.82-2.73 (1H, m), 2.04-1.95 (2H, m), 1.39-1.20 (11H, m), 0.87 (3H, t, \(J = 7.0\) Hz). \(^{13}\)C NMR (100.59 MHz) \(\delta\) 175.39, 152.88, 135.32, 133.10, 129.37, 128.86, 128.41, 127.26, 65.94, 55.48, 40.72, 37.92, 32.41, 31.65, 29.02, 28.68, 22.52, 18.74, 15.06. HRMS (ES) ([M + Na\(^+\)]\(^+\)) Calcd. for C\(_{21}\)H\(_{29}\)NNaO\(_3\): 366.2040, Found: 366.2063.

(2S,E)-2-Methyldec-3-enoic acid (7S)

Compound 7S was prepared from oxazolidinone 22S (200 mg, 0.58 mmol), following the same procedure as for compound 5. The product was purified by column chromatography (Pe / EtOAc 5:1) to give 7S (106 mg, 99 %) as a colourless oil: \(R_f = 0.45\) Pe:EtOAc 5:1. \([\alpha]^{21}_D = +32.0^\circ\) (CHCl\(_3\), c = 0.77) (lit.\(^{21}\) \([\alpha]^{20}_D = +42.2^\circ\) (DCM, c = 1.0); \(^1\)H NMR (400.04 MHz, CDCl\(_3\)) \(\delta\) 11.08 (1H, br s), 5.64-5.44 (2H, m), 3.11 (1H, dq, \(J = 7.1, 7.0\) Hz), 2.06-1.94 (2H, m), 1.41-1.20 (11H, m), 0.88 (3H, t, \(J = 6.9\) Hz); \(^{13}\)C NMR (100.59 MHz) \(\delta\) 181.35, 132.92, 127.93, 42.61, 32.32, 31.59, 29.02, 28.70, 22.51, 17.18, 13.96.

(2R,E)-2-Methyldec-3-enoic acid (7R)

Compound 7R was prepared from oxazolidinone 22R (200 mg, 0.58 mmol), following the same procedure as for compound 5. The product was purified by column chromatography (Pe / EtOAc 5:1) to give 7R (105 mg, 98 %) as a colourless oil: \(R_f = 0.45\) Pe:EtOAc 5:1. \([\alpha]^{21}_D = -45.9^\circ\) (CHCl\(_3\), c = 0.43) \(^1\)H NMR (500.13 MHz, CDCl\(_3\)) \(\delta\) 11.02 (1H, br s), 5.57-5.39 (2H, m), 3.05 (1H, dq, \(J = 7.1, 7.0\) Hz), 1.98-1.90 (2H, m), 1.34-1.14 (11H, m), 0.81 (3H, t, \(J = 6.9\) Hz). \(^{13}\)C NMR (125.76 MHz) \(\delta\) 181.55, 133.04, 127.95, 42.72, 32.43, 31.69, 29.10, 28.80, 22.62, 17.28, 14.10. HRMS (ES) ([M - H\(^+\)]\(^+\)) Calcd. for C\(_{11}\)H\(_{19}\)O\(_2\): 183.1385, Found: 183.1551.

(2S,E)-2-Methyldec-3-enoyl-CoA (3S)
Compound 3\text{S} was prepared from 7\text{S} (15 mg), following the same procedure as for compound 2\text{R} to give 4 mg of 3\text{S}. \textsuperscript{1}H NMR (500.13 MHz, D\textsubscript{2}O): \(\delta\) 8.47 (1H, s), 8.18 (1H, s), 6.08 (1H, d, \(J = 7.1\) Hz), 4.70-4.67 (1H, m), 4.20-4.08 (2H, m), 3.93 (1H, s), 3.77-3.70 (1H, m), 3.62 (1H, s), 3.48-3.42 (1H, m), 3.39-3.31 (2H, m), 3.27-3.20 (2H, m), 2.96-2.82 (1H, m), 2.32 (2H, t, \(J = 6.8\) Hz), 1.88 (1H, q, \(J = 6.9\) Hz), 1.26-1.04 (11H, m), 0.80 (3H, s), 0.73 (3H, t, \(J = 6.5\) Hz), 0.68 (3H, s).

(2\text{R, E})-2-Methyldec-3-enoyl-CoA (3\text{R})

Compound 3\text{R} was prepared from 7\text{R} (15 mg), following the same procedure as for compound 2\text{R} to give 6 mg of 3\text{R}. \textsuperscript{1}H NMR (500.13 MHz, D\textsubscript{2}O): \(\delta\) 8.47 (1H, s), 8.18 (1H, s), 6.07 (1H, d, \(J = 7.1\) Hz), 4.71-4.67 (1H, m), 4.20-4.08 (2H, m), 3.92 (1H, s), 3.78-3.71 (1H, m), 3.62 (1H, s), 3.48-3.42 (1H, m), 3.38-3.31 (2H, m), 3.27-3.20 (2H, m), 2.96-2.82 (1H, m), 2.32 (2H, t, \(J = 6.8\) Hz), 1.88 (1H, q, \(J = 6.9\) Hz), 1.31-1.03 (11H, m), 0.80 (3H, s), 0.74 (3H, t, \(J = 6.5\) Hz), 0.68 (3H, s).

Synthesis of \textit{E}-2-methyldec-2-enoyl-CoA (4)

Ylide 23 was prepared using the method of Baktharaman \textit{et al.}\textsuperscript{22} and the desired unsaturated ester 24 prepared by Wittig reaction between ylide 23 and octanal. Ester 24 was hydrolysed to the acid 9 under basic conditions, which was converted to 4 by the literature procedure.\textsuperscript{2}

Scheme S5: Synthesis of \textit{E}-2-methyldec-2-enoyl-CoA 4. \textit{Reagents and conditions:} i. Octanal, DCM, 0°C, 77%; ii. KOH, EtOH/H\textsubscript{2}O (2:1), rt, 97%; iii. CDI, DCM, rt; iv. CoA-SH-Li\textsuperscript{+3}, 0.1 M NaHCO\textsubscript{3} aq./THF (1:1).

Ethyl (\textit{E})-2-Methyldec-2-enoate (24)\textsuperscript{23}

Octanal (2.62 mL, 16.8 mmol) in anhydrous DCM (20 mL) was added dropwise to 23 (6.09 g, 16.8 mmol) in anhydrous DCM (50 mL) at 0°C and the reaction mixture was allowed to reach ambient temperature over 2 h. Evaporation and column chromatography (Pe / EtOAc 50:1) gave 24 (2.74 g,
77 %) as a colourless oil: \(^1\)H NMR (400.04 MHz, CDCl\(_3\)) \(\delta\) 6.75 (1H, tq, \(J = 7.5, 1.5\) Hz), 4.17 (2H, q, \(J = 7.1\) Hz), 2.19-2.11 (2H, m), 1.83-1.80 (3H, m), 1.49-1.37 (2H, m), 1.34-1.21 (11H, m), 0.90-0.83 (3H, m).

\((E)-2\)-Methyldec-2-enoic acid (9)\(^{24}\)

![Chemical structure of (E)-2-Methyldec-2-enoic acid](image)

Ethyl ester 24 (442 mg, 2.1 mmol) in EtOH (10 mL) was stirred with KOH (467 mg, 8.34 mmol) in H\(_2\)O (5.0 mL) for 20 h. The reaction mixture was acidified to pH ~3 with aq. HCl (3 M) and the solvents were partly evaporated under reduced pressure. DCM was added to the residue and the organic layer was washed with water and brine. Drying (MgSO\(_4\)), filtration, evaporation and column chromatography (DCM / MeOH 100:1) gave 9 (374 mg, 97%) as a colourless oil: \(^1\)H NMR (400.04 MHz, CDCl\(_3\)) \(\delta\) 11.70 (1H, br s), 6.91 (1H, tq, \(J = 7.5, 1.4\) Hz), 2.23-2.15 (2H, m), 1.84-1.81 (3H, m), 1.51-1.39 (2H, m), 1.36-1.20 (8H, m), 0.93-0.83 (3H, m).

\((E)-2\)-Methyldec-2-enoyl-CoA (4)

![Chemical structure of (E)-2-Methyldec-2-enoyl-CoA](image)

Compound 4 was prepared from 9 (10 mg), following the same procedure as for compound 2R to give 3 mg of 4. \(^1\)H NMR (500.13 MHz, D\(_2\)O): \(\delta\) 8.62 (1H, s), 8.37 (1H, s), 6.75 (1H, dt, \(J = 7.8, 1.0\) Hz), 6.15 (1H, d, \(J = 6.0\) Hz), 4.56 (1H, s), 4.18 (2H, s), 4.12-3.95 (2H, m), 3.84-3.75 (1H, m), 3.57-3.48 (1H, m), 3.37 (2H, t, \(J = 6.4\) Hz), 3.28 (2H, t, \(J = 6.4\) Hz), 2.95 (2H, t, \(J = 6.5\) Hz), 2.36 (2H, t, \(J = 6.9\) Hz), 2.18-2.11 (2H, m), 1.74 (3H, s), 1.40-1.05 (10H, m), 0.87 (3H, s), 0.76 (3H, t, \(J = 6.9\) Hz), 0.70 (3H, s).

**AMACR assays**\(^1\)\(^-\)\(^2\)

Human AMACR was expressed in *E. coli* Rosetta2 (DE3) at 22 °C overnight shaking at 220 r.p.m., inducting with 0.25 mM IPTG. Cells (~2 g) were lysed using the ‘one shot’ in ~30 mL 20 mM NaH\(_2\)PO\(_4\)-NaOH, 300 mM NaCl, 10 mM imidazole, pH 7.2 supplemented with 1 mM PMSF and 250 u benzonase (Novagen) and stirred with N-lauroyl-sarcosine at 4 °C for 1 hour. Following centrifugation, enzyme was purified by metal-chelate chromatography, dialysed into 10 mM NaH\(_2\)PO\(_4\)-NaOH, pH 7.4 and stored at -80 °C. Protein purity of pooled fractions was *ca.* 95 – 98 % by SDS-PAGE analyses. Protein concentrations were quantified using UV-visible absorbance\(^2\) at
280 nm ($\varepsilon_{280} = 35785 \text{ M}^{-1} \text{ cm}^{-1}$) and assumed a molecular mass of 47146.8 Da. for the His-tag protein.

Assays were conducted in 50 mM NaH$_2$PO$_4$-NaOH, pH 7.4 containing ca. 85% $^2$H$_2$O and 100 µM acyl-CoA substrate as previously described, with negative controls containing heat-inactivated enzyme. $\pm$-Fenoprofenoyl-CoA or $S$-2-methyldecanoyl-CoA with wild-type enzyme were used as positive controls.$^{1,2}$ Assays were quenched by heating to 50 °C for 10 minutes before $^1$H NMR analysis (500.13 MHz). Conversion of substrates was quantified by conversion of the 2-Me doublet at ca. 1.0 p.p.m. into a singlet at ca. 1.75 p.p.m., and were corrected for non-enzymatic conversion in heat-inactivated negative controls.$^{1,2}$ Kinetic assays contained 1.1 µg (0.023 nmol) protein per assay. Parameters were obtained using SigmaPlot 12. Data was analysed with the Direct Linear Plot$^{25,26}$ and non-linear fitting of data to the Michaelis-Menten equation. Error bars are ± SE.
References
**Figure S1:** Time course of the AMACR catalysed elimination reaction. The reaction was carried out as described in the experimental using 100 μM substrate and 2.5 μM enzyme. % conversion was calculated based on integration of the peaks at *ca.* 1.1 ppm (2*R* methyl group) and *ca.* 1.75 ppm (4 methyl group). A negative control containing heat-inactivated enzyme showed <5% conversion at all time points.
Kinetic analysis of anti-(3R,2R)-3-Fluoro-2-methyldecanoyl-CoA 2R

Direct Linear Plot

Apparent $K_m = 21.0300 \, \mu M$;
Apparent $V_{max} = 96.5300 \, \text{nmol.min.}^{-1}\text{mg.}^{-1}$

Michaelis-Menten

Apparent $K_m = 21.0300 \, \mu M$;
Apparent $V_{max} = 96.5300 \, \text{nmol.min.}^{-1}\text{mg.}^{-1}$
Enzyme Kinetics Nonlinear Fit Results

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Michaelis-Menten
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Data

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Number of replicates | 2
Total number of values | 10
Number of missing values | 0
Kinetic analysis of syn-(3R,2S)-3-Fluoro-2-methyldecanoyl-CoA 2S

**Direct Linear Plot**

![Direct Linear Plot]

Apparent $K_m = 39.9400 \mu M$;  
Apparent $V_{max} = 50.5600 \text{ nmol.min}^{-1}\text{mg}^{-1}$

**Michaelis-Menten**

![Michaelis-Menten]

**[Substrate] (μM)**

**Rate (nmol.m.min$^{-1}$mg$^{-1}$)**
Enzyme Kinetics Nonlinear Fit Results

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</table>
Unsaturated 2-methylacyl-CoA esters $3R$ and $3S$ as substrates for AMACR

Figure S1. $3R$ shows exchange of the $\alpha$-proton in with live AMACR in $^2$H$_2$O. This results in the doublet of the methyl group at 1.04 ppm becoming a singlet (outlined in green) and the concomitant conversion of the doublet of doublets for $H^a$ at 5.29 ppm into a doublet (outlined in red). As exchange is an obligatory step in chiral inversion, it is highly likely that racemization also occurs. An identical reaction occurred upon incubation of $3S$ with AMACR under identical conditions (data not shown).