Supporting Information

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SI Methods

Materials. Chemicals were obtained from Sigma–Aldrich, Merck, Applichem, Roth, or Gerbu. Materials for cloning and expression were purchased from MBI Fermentas, Novagen, Genaxxon Bioscience, biomers.net, or Qiagen. Materials and equipment for protein purification were obtained from GE Healthcare or Millipore. $NaH^{14}CO_3$ was from Hartmann Analytic. 2-Propanol-d_8 (99.5 atom%), D-glucose-*1-d* (97 atom%), $[^2H]_2O$ (99.98 atom%), glucose-6-phosphate dehydrogenase from baker's yeast (type VII), alcohol dehydrogenase from *Thermoanaerobium brockii*, and carbonic anhydrase from bovine erythrocytes were purchased from Sigma-Aldrich.

Preparation of Enzymes. Recombinant crotonyl-CoA carboxylase/reductase (Ccr) was produced in 200-L scale and purified from cell extracts (15 mL, 1.6 g of protein) as described (16).

Methylmalonyl-CoA epimerase (Epi) and (2*R*)-methylmalonyl-CoA mutase (Mcm) were prepared as recombinant histidine-tagged (his-tagged) promiscuous ethylmalonyl-CoA/methylmalonyl-CoA epimerase and his-tagged methylmalonyl-CoA mutase as described previously (20).

A his-tagged version of Ccr(Ccr_{his}) was produced by amplifying the gene encoding Ccr by PCR from *R. sphaeroides* chromosomal DNA using the forward primer (5′-GGA GGC AA*C CAT GG*C CCT CGA CGT GCA GAG-3′) introducing a NcoI site (italicized) at the initiation codon and reverse primer (5′-GAG ACT TGC *GGA TCC* CTC CGA TCA GGC CTT GC-3′) introducing a BamHI site (italicized) after the stop codon. The PCR product was isolated and cloned into the expression vector pRSET-B (Invitrogen), generating pTE42. Recombinant Ccr_{his} was produced in *E. coli* BL21(DE3) or Rosetta 2 (DE3) that had been transformed with pTE42. The cells were grown at 37 °C in LB medium with 100 μg mL^{-1} ampicillin. Expression was induced at an OD_{578} = 0.6–0.9 with 0.5 mM isopropyl-thiogalacto-pyranoside, the temperature was lowered to 30 °C, and the cells were harvested after additional growth for 3 h. Cells (1 g) were suspended in 2 mL of 20 mM Tris(hydroxymethyl)aminomethane Tris·HCl (pH 7.8) containing 0.1 mg μL^{-1} of DNase I, the suspension was passed twice through a chilled French pressure cell at 137 Mpa, and the cell lysate was centrifuged (100,000 g) at 4 °C for 1 h. An aliquot of the supernatant (2 mL, ≈60 mg of protein) was applied at a flow rate of 1 mL min^{-1} onto a 1-mL Ni-Sepharose Fast Flow Column (HisTrap FF; Amersham) that had been equilibrated with 10 volumes of buffer A containing 20 mM Tris·HCl (pH 7.8) and 200 mM KCl. The column was washed with buffer A and buffer A containing 75 mM imidazole. Ccr_{his} was eluted with buffer A containing 500 mM imidazole. The enzyme was desalted and concentrated by ultrafiltration (Amicon YM 10 membrane; Millipore). The protein (3 mg) was stored at −20 °C in 10 mM Tris·HCl (pH 7.8) with 50% glycerol.

Butyryl-CoA dehydrogenase was prepared from pig liver as described (21, 22) yielding 30.5 g of mitochondria (wet weight) from 500 g of liver. Isolated mitochondria (6.5 g) were sonicated, and the crude extract was fractionated with $(NH_4)_2SO_4$. Protein precipitated between 40% and 57% $(NH_4)_2SO_4$ saturation was resuspended in 0.5 mL of 20 mM potassium phosphate buffer (pH 6.5) and dialyzed 2 times for 1.5 h against 2 L of the same buffer. One third of the nondiffusible material was applied onto a Sephadex A-50 column (16 mL) that had been equilibrated by the passage of 10 volumes of buffer B containing 25 mM potassium phosphate (pH 7.6) at a flow rate of 1 mL min^{-1}. The column was washed with 30 mL of buffer B, followed by 20 mL of buffer B with 50 mM KCl, a linear gradient from 50 mM to 250 mM KCl in buffer B over 40 mL, and a linear gradient from 250 to 500 mM KCl in buffer B over 10 mL. Fractions were collected and tested for the oxidation of butyryl-CoA (4). Active fractions that eluted at 200–400 mM KCl were pooled, desalted, and concentrated to a final volume of 1 mL by ultrafiltration (YM 10 membrane; Amicon). The protein (2 mg) was stored at −20 °C in 10 mM Tris·HCl (pH 7.8) containing 50% glycerol.

Syntheses. Crotonyl-CoA was synthesized from its anhydride (23) and acryloyl-CoA was synthesized from the free acid by the method of Stadtman (24). Both CoA-esters were quantified by absorption at 260 nm (ε = 22,000 M^{-1} cm^{-1}) (25), and the purity was analyzed by a previously described HPLC method (16).

[^{2}H]-(4*R*)- and [2H]-(4*S*)-NADPH were synthesized according to Pollock and Barber (26) with some modifications. [^{2}H]-(4*R*) NADPH was synthesized from 18 mg $NADP^+$, 0.6 mL of 2-propanol-d_8, and 38 units of alcohol dehydrogenase (*Thermoanaerobium brockii*), dissolved in 7.5 mL 25 mM Tris·HCl buffer (pH 9) at 42 °C. [^{2}H]-(4*S*)-NADPH was synthesized from 18 mg of $NADP^+$, 10 mg of D-glucose-*1-d*, 0.85 mL of dimethyl sulfoxide and 50 units of glucose-6-phosphate dehydrogenase (*Saccharomyces cerevisiae*), dissolved in 83 mM potassium phosphate buffer (pH 8) at 30 °C. Both reactions were followed spectrophotometrically at 340 nm until no further increase in absorbance was observed. The respective [^{2}H]-NADPH stereoisomers were purified individually. To precipitate the reduced nucleotide, 12 volumes of ice-cold ethanol were added, and the solution was incubated for 20 min at −20 °C. After centrifugation for 30 min at 27,000 × *g*, the supernatant was discarded, and the yellowish pellet was dissolved in 2 mL of H_2O. The [^{2}H]-NADPH solution was applied onto a Whatman DE23 column (5 mL) that had been equilibrated with 10 volumes of H_2O at a flow rate of 2 mL min^{-1}. The column was washed with 40 mL of H_2O, followed by a linear gradient from 0 to 0.5 M NH_4HCO_3 over 120 mL, and the reduced nucleotide eluted at 0.16–0.33 M NH_4HCO_3. The eluate was evaporated to remove NH_4HCO_3, the aqueous solution was lyophilized, and the product was stored at −20 °C. To follow the synthesis and purification, each step was controlled by recording a spectrum of the respective solution and determining the absorbance ratio of A_{260}/A_{340}. The purified products were also characterized by NMR (Fig. S3).

Determination of the Carboxylating Species. The active species of CO_2 was determined spectrophotometrically after a modified method (27) in a cuvette (*d* = 0.1 cm) at 15 °C, following the rate of NADPH oxidation at 360 nm (ε_{NADPH} = 3.4 mM^{-1} cm^{-1}). All solutions were prepared freshly and stored on ice until they were used. The carboxylation reaction with "dissolved CO_2" was measured in a reaction mixture (0.182 mL) containing 180 mM Tris·HCl (pH 7.9), 4.9 mM NADPH, 1.7 mM crotonyl-CoA, and 38 μg of Ccr. To start the carboxylation reaction, 20 μL of 50 mM $KHCO_3$ were mixed with 6 μL of 1 M acetic acid to dissolve CO_2 from bicarbonate by acidification, and this "CO_2 solution" was immediately added to the reaction mixture in the cuvette. To follow the carboxylation reaction with "bicarbonate," the reaction mixture in the cuvette contained 173 mM instead of 180 mM Tris·HCl (pH 7.9) and 33 mM acetic acid. The "bicarbonate" solution was prepared by mixing 20 μL of 50 mM $KHCO_3$ with 6 μL of 200 mM Tris·HCl (pH 7.9) and was added immediately to the reaction mixture in the cuvette. As control, both reactions

were also performed in the presence of 0.3 mg of carbonic anhydrase in the reaction mixture.

Determination of the Stereochemistry of the Carboxylation Product. Radioactive-labeled methylmalonyl-CoA was synthesized from acryloyl-CoA and $H^{14}CO_3$ by Ccr and subsequently used as substrate for different combinations of Epi and/or Mcm (20). The substrate mixture (495 μL) contained 80 mM Tris·HCl (pH7.8), 3.7 mM NADPH, 1.9 mM acryloyl-CoA, 7.9 mM $NaHCO_3$, 0.4 MBq mL^{-1} $NaH^{14}CO_3$, and 270 μg of Ccr. After incubation at 30 °C for 5 min, a sample of 100 μL was removed from the substrate mixture and added to 10 μL of 20% formic acid (t_0 sample). Aliquots of 106 μL were added to "protein solutions" containing 0.10 μmol of Co^{2+}, 0.07 μmol of coenzyme B_{12}, and either 0.3 μg of Epi, 6.3 μg of Mcm, or a combination of both proteins. Samples were withdrawn after 1 min of incubation at 30 °C and added to 10 μL of 20% formic acid (t_1 samples). All samples were centrifuged to remove denatured protein and analyzed subsequently by HPLC and radioactive monitoring (see below).

Determination of the Stereospecificity of the Hydrogen Transfer from NADPH (Carboxylase Reaction). All reactions were performed in a cuvette (d = 0.1 cm) at 30 °C and followed spectrophotometrically at 360 nm. Crotonyl-CoA (1.8 mM) was incubated for 5 min in 0.335 mL of 90 mM Tris·HCl buffer (pH 8) containing 30 mM $NaHCO_3$ and 4.5 mM [^{2}H]-(4*R*)-NADPH, [^{2}H]-(4*S*)-NADPH, or unlabeled NADPH (16). A sample of 100 μL was withdrawn, and 400 μL of methanol were added ("crotonyl-CoA-t_0 sample"). The carboxylation reaction was started by adding 6 μg of Ccr [in 5 μL of 10 mM Tris·HCl (pH 7.8) containing 5% glycerol] to the solution in the cuvette. A sample of 100 μL was withdrawn after 10 min, and 400 μL of methanol were added ("ethylmalonyl-CoA-CCR sample"). Methanol was evaporated in a Speedvac concentrator, and the samples were analyzed subsequently by HPLC-MS (see below).

Determination of the Stereospecificity of the Hydrogen Transfer from NADPH (Reductase Reaction). All reactions were performed in a cuvette (d = 0.1 cm) at 30 °C and monitored by a spectrophotometer at 360 nm. Crotonyl-CoA (2 mM) was incubated for 5 min in 0.3 mL of 40 mM Tris·HCl buffer (pH 7.6) containing 4 mM [^{2}H]-(4*R*)-NADPH, [2H]-(4*S*)-NADPH, or unlabelled NADPH (16). A sample of 100 μL was withdrawn, and 10 μL of 20% HCOOH were added ("crotonyl-CoA-t_0 sample"). The reduction reaction was started by adding 12 μg of Ccr_{his} [in 12 μL of 10 mM Tris·HCl (pH 7.8) containing 50% glycerol] to the solution in the cuvette. When no further decrease in the absorption was observed, 20 μL of 20% HCOOH were added to the reaction mixture ("butyryl-CoA-CCR sample"). All samples were centrifuged to remove denatured protein and analyzed subsequently by HPLC-MS (see below).

Determination of the Cryptic Stereochemistry at C3 (Reductase Reaction). [^{2}H]-C_3-butyryl-CoA (synthesized from [^{2}H]-(4*R*)-NADPH) and butyryl-CoA (synthesized from NADPH) were purified by preparative HPLC from 100 μL of a "butyryl-CoA sample" (see above), followed by lyophilization. The CoA esters isolated were each dissolved in 194 μL of 60 mM Tris·HCl (pH 7.8) containing 2.7 mM ferrocenium hexafluorophosphate (30). The solutions were transferred into a cuvette (d = 0.1 cm), and all reactions were followed spectrophotometrically at 300 nm and 30 °C. The dehydrogenase reaction was started by adding 20–40 μg of protein of the butyryl-CoA dehydrogenase preparation (see above) to the cuvette and 20 μL of 20% HCOOH were added after 15–20 min ("crotonyl-CoA-BDH sample") to stop the reaction. The samples were centrifuged to remove denatured protein and subsequently analyzed by HPLC-MS (see below).

Determination of the Stereoselectivity of the Solvent Hydrogen Addition (Reductase Reaction). Reduction of crotonyl-CoA by Ccr was performed as described using $[^2H]_2O$ instead of water. [^{2}H]-C_2-butyryl-CoA (synthesized from NADPH in $[^2H]_2O$) and [$^2H_2$]-C2,C3-butyryl-CoA (synthesized from [^{2}H]-(4*R*)-NADPH in $[^2H]_2O$) were isolated and subjected to butyryl-CoA dehydrogenase as described above.

Analysis of CoA Esters by HPLC and HPLC-MS. All CoA esters were separated by reversed-phase HPLC on a C_{18} column (LiChrospher 100, end-capped, 5 μm, 125 × 4 mm; Merck). Reaction products and standard compounds were detected by UV absorbance with a Waters 996 photodiode array detector. Radioactivity of eluting compounds was monitored by a Ramona 2000 radioactive monitor (Raytest) connected in series. For the separation of methylmalonyl-CoA and succinyl-CoA, the column was developed at a flow rate of 1 mL min^{-1} for 7 min under isocratic conditions with 100 mM NaH_2PO4 (pH 4.0) in 7.5% methanol (vol/vol), followed by a linear 10-min gradient from 0 to 60% 100 mM sodium acetate (pH 4.2) in 90% methanol (vol/vol) (retention times: methylmalonyl-CoA, 11.7 min; succinyl-CoA, 12.3 min) (20). For the separation of crotonyl-CoA and ethylmalonyl-CoA, the column was developed at a flow rate of 1 mL min^{-1} by a linear gradient from 2% acetonitrile (CH_3CN) in 50 mM ammonium acetate (pH 6.8) to 10% CH_3CN within 30 min, followed by a linear gradient from 10% CH_3CN to 45% CH_3CN within 5 min (retention times: crotonyl-CoA, 10.1 min; ethylmalonyl-CoA, 22.0 min) (16). Crotonyl-CoA and butyryl-CoA were separated at a flow rate of 1 mL min^{-1} for 2 min under isocratic conditions with 6% CH_3CN in 50 mM ammonium acetate (pH 6.8), followed by a 17-min linear gradient from 6% CH_3CN to 17% CH_3CN and a 3-min linear gradient from 17% CH_3CN to 45% (retention times: crotonyl-CoA, 10.7 min; butyryl-CoA, 13.6 min). HPLC-MS was performed on an Agilent 1100 system (Agilent Technologies) interfaced with an Applied Biosystems API 2000 triple-quadrupole spectrometer. The temperature of the Turbo-Ionspray auxiliary gas was 400 °C, and the ionization voltage was −4,500 V. The samples were analyzed with a mass range of 100–1,600 Da.

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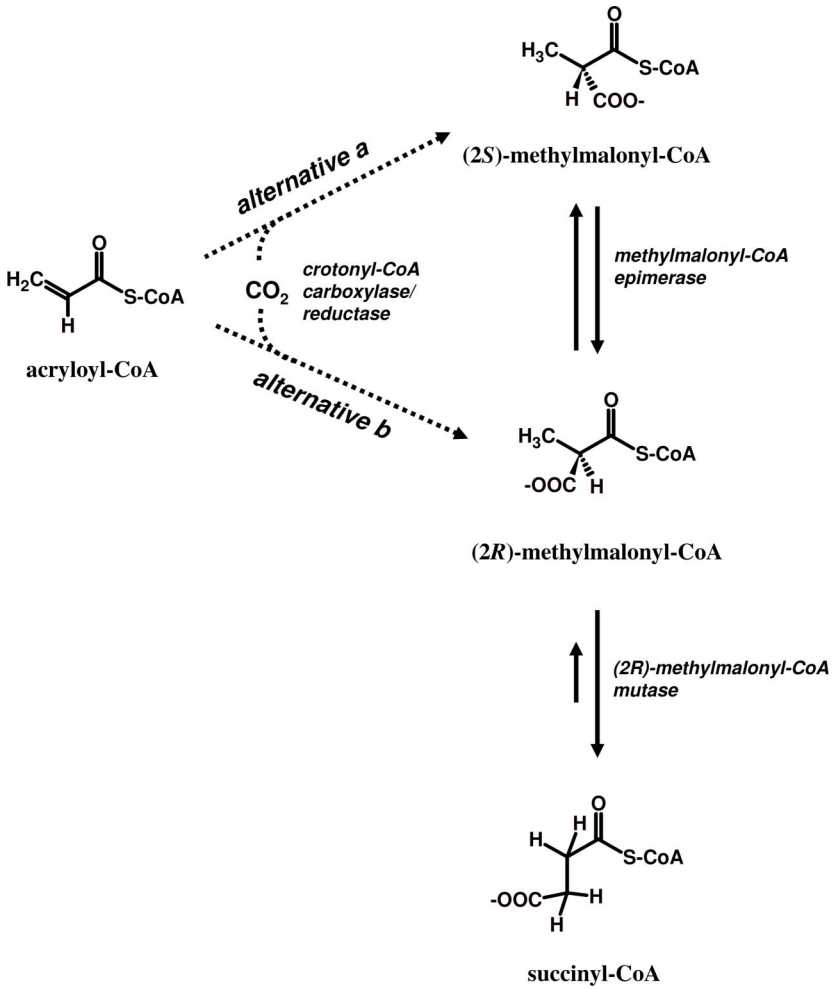

Fig. S1. Enzymatic analysis of the stereochemistry of acryloyl-CoA carboxylation by using methylmalonyl-CoA epimerase and (2*R*)-methylmalonyl-CoA mutase. Reductive carboxylation of acryloyl-CoA by Ccr yields methylmalonyl-CoA that is further transformed into succinyl-CoA. In case of (2*S*)-methylmalonyl-CoA as reaction product (alternative a), methylmalonyl-CoA epimerase and (2*R*)-methylmalonyl-CoA mutase are required. In case of (2*R*)-methylmalonyl-CoA as reaction product (alternative b), (2*R*)-methylmalonyl-CoA mutase alone is sufficient for the formation of succinyl-CoA. For *R. sphaeroides* Ccr, reductive carboxylation was shown to follow alternative a.

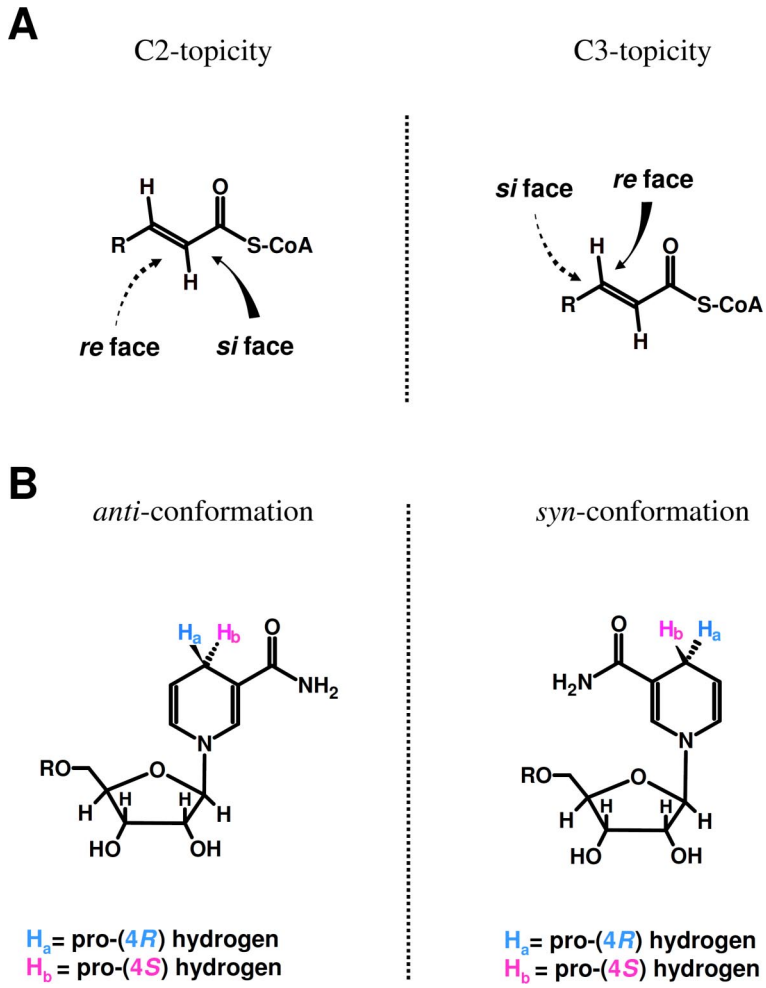

Fig. S2. Topicity, stereochemistry and conformations of crotonyl-CoA and NADPH. (*A*) *re* face and *si* face at C2 and C3 of crotonyl-CoA. (B) Conformations of the reduced nicotinamide adenine dinucleotide phosphate (NADPH) cofactor and stereochemistry of the hydrogen atoms at C4 of the nicotinamide ring.

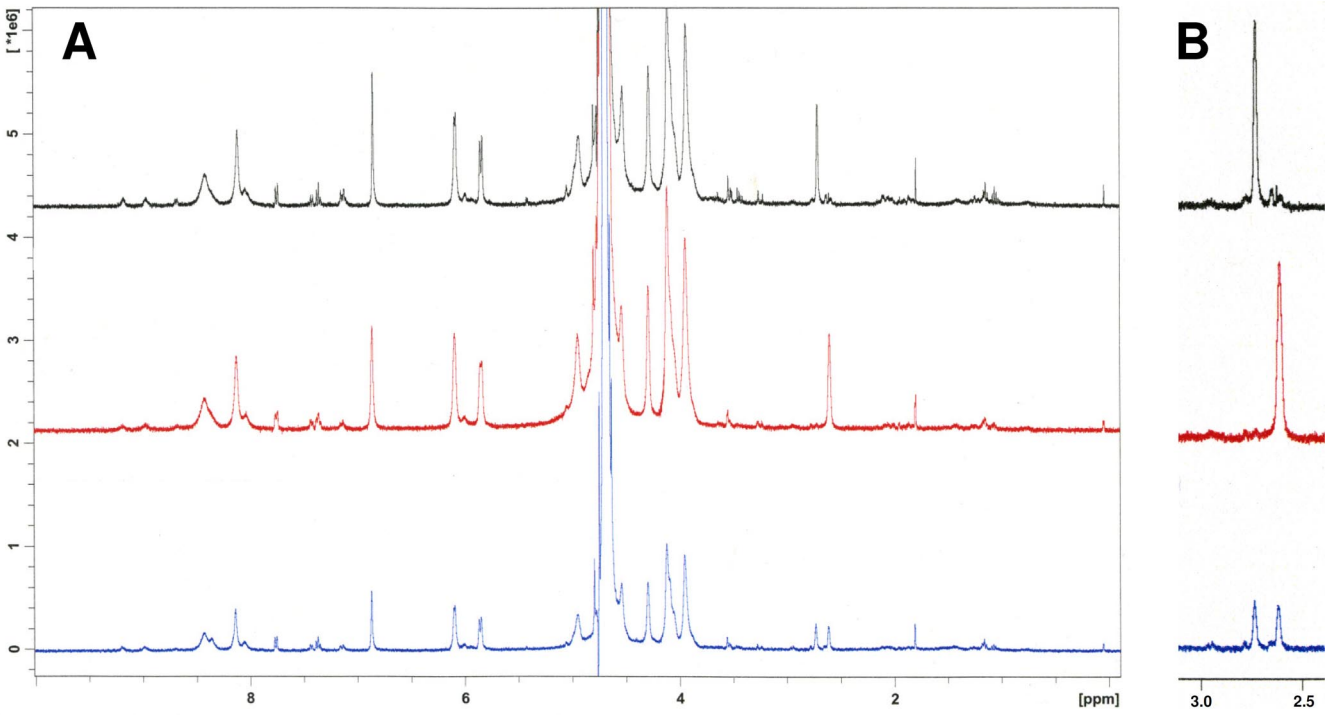

Fig. S3. ^{1}H-NMR spectra of enzymatically synthesized and purified [^{2}H]-(4*R*)-NADPH and [^{2}H]-(4*S*)-NADPH. (*A*) Overlay of the ^{1}H-NMR spectra of [^{2}H]-(4*S*)-NADPH (black), [^{2}H]-(4*R*)-NADPH (red) and a mixture of [^{2}H]-(4*S*)- and [^{2}H]-(4*R*)-NADPH (blue). (*B*) ^{1}H-NMR spectra of the methylene protons at C4 of the nicotinamide ring in detail. For reference spectra and chemical shifts, see refs.erences 17–19.

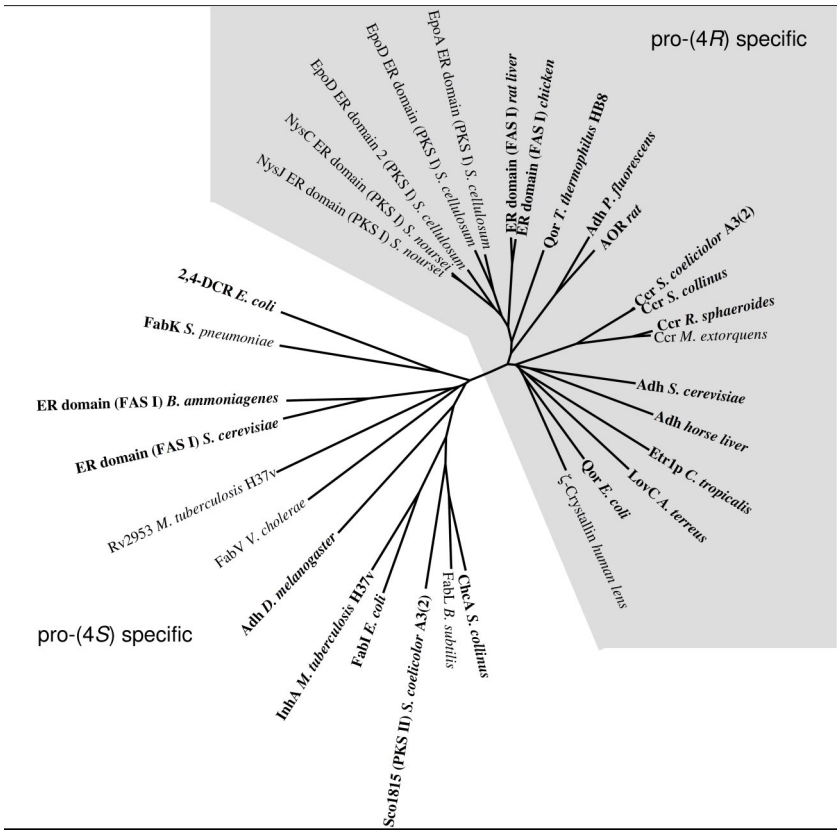

Fig. S4. Analysis of amino acid sequences and stereospecificity of enoyl(-thioester) reductases (ERs) and related proteins. Neighbor- joining tree of ERs and related proteins, listed in Table S2. The stereospecificity of proteins shown in bold type has been experimentally verified or suggested from crystal structures. The tree was constructed by using ClustalW as implemented in the BioEdit 7.0.9.0 software package and neighbor-joining algorithms as implemented in the Tree-ConW1.3b software package. The accession numbers for the proteins and the corresponding sequences are listed in Tables S3 and S4, respectively.

Table S1. Enzymes in biological carboxylation reactions

Enzyme	Substrate	Source of carbon	Cofactor(s)	Metals	Product
Ribulose-1,5- bisphosphate carboxylase/oxygenase (RuBisCO) (1)	Ribulose-1,5-phosphate	CO_2	–	Mg^{2+}	3-Phosphoglycerate
Phosphoenolpyruvate carboxylase (2)	Phosphoenolpyruvate	HCO_3^-	–	Mg^{2+}	Oxaloacetate
Phosphoenolpyruvate carboxykinase (3)	Phosphoenolpyruvate	CO_2	ATP (GTP)	Mg^{2+}	Oxaloacetate
″Methylketone carboxylases″ (4,5)	Acetone	CO_2	ATP*	Mn^{2+}	Acetoacetate
	Acetophenone	CO_2 (?)	2 ATP§	Mg^{2+}	Benzoylacetate
″2-Oxoacid carboxylases″ (6,7)	Pyruvic acid 2-Oxoglutaric acid	HCO_3-	ATP, biotin	Mg^{2+}	Oxaloacetate Oxalosuccinate
″Acyl-CoA carboxylases″ (8, 9)	Acetyl-CoA Propionyl-CoA 3-Methylcrotonyl-CoA Geranoyl-CoA	HCO_3-	ATP, biotin	Mg^{2+}	Malonyl-CoA Methylmalonyl-CoA 3-Methylglutaconyl-CoA Isohexenyl-glutaconyl-CoA
Vitamin K-dependent carboxylase (10)	Glutamic acid resiudes of proteins	CO_2	Vitamin K	Mn^{2+}	g-Carboxyglutamic acid
Pyrrole-2-carboxylate decarboxylase (11)	Pyrrole	HCO_3-	Organic acid	?	Pyrrole-2-carboxylate
″2-Oxoacid synthases″ (12,13)	Acetyl-CoA	CO_2	Reduced ferredoxin, thiamine diphosphate	[FeS]-cluster	Pyruvate
	Propionyl-CoA				2-Oxobutyrate
	Succinyl-CoA				2-Oxoglutarate
	S-(2-methylpropionyl)-CoA				3-Methyl-2-oxobutanoate
	S-2-(indol-3-yl)acetyl-CoA				Indolepyruvate
2-Ketopropyl-CoM carboxylase (14)	2-Ketopropyl-CoM	CO_2	NADPH, FAD^+	–	Acetoacetate
Phenylphosphate carboxylase (15)	Phenylphosphate	CO_2	–	K^+, Mn^{2+}	4-Hydroxybenzoate
Crotonyl-CoA carboxylase/reductase (6)	Crotonyl-CoA, (Acryloyl-CoA)	CO_2	NADPH	–	Ethylmalonyl-CoA, (methyl-malonyl-CoA)

?, A role of iron in catalysis cannot be clearly ruled out.
*Hydrolyzed into AMP and 2 inorganic phosphates.
§Hydrolyzed into 2 ADP and 2 inorganic phosphates.

Table S2. Domain architecture and stereochemistry of various ER and alcohol dehydrogenases

Stereochemistry of reduction				Conserved domains (NCBI database) and amino acid region		
NAD(P)H	C3	C2	Protein(fragment) and organism	ADH_zinc_N superfamily pfam00107	AdoHcyase superfamily cl09931	TIM_phosphate-binding super-family cl09108
4R	*re*	*si*	ER domain (FAS I) rat liver	50–200		
4R	*re*	*si*	ER domain (FAS I) chicken	50–185		
4R	*re*	*re*	Ccr *Rhodobacter sphaeroides*	190–320		
4R†	*re*	*re*	Ccr *Streptomyces collinus*	225–380		
4R	–	–	AOR *Rattus norvegicus*	165–290		
4R	–	–	Adh horse liver	210–375		
4R	–	–	Adh *Saccharomyces cervisiae*	190–310		
4R	–	–	Adh *Pseudomonas fluorescens*	170–285		
4R*	nd	nd	Qor *Escherichia coli*	140–280		
4R*	nd	nd	Qor *Thermus thermophilus* HB8	190–245		
4R*	nd	nd	ζ-Crystallin *human lens*	170–310		
4R*	nd	nd	Etr1p *Candida tropicalis*	170–300		
4R§	nd	nd	Ccr *Methylobacterium extorquens*	210–375		
4R§	nd	nd	Ccr *Streptomyces coelicolor* A3(2)	225–385		
nd	nd	nd	LovC *Aspergillus terreus*	165–260		
nd	nd	nd	NysC ER domain (PKS I) *Streptomyces noursei*	110–235		
nd	nd	nd	NysJ ER domain (PKS I) *Streptomyces noursei*	110–250		
nd	nd	nd	EpoA ER domain (PKS I) *Sorangium cellulosum*	115–260		
nd	nd	nd	EpoD ER domain 1 (PKS I) *Sorangium cellulosum*	80–220		
nd	nd	nd	EpoD ER domain 2 (PKS I) *Sorangium cellulosum*	115–250		
4S	*si*	*si*	ChcA *Streptomyces collinus*		15–255	
4S	*si*	*re*	FabI (FAS II) *Escherichia coli* K12		1–262	
4S	–	–	InhA (FAS II) *Mycobacterim tuberculosis*		1–269	
4S	–	–	Adh *Drosophila melanogaster*		1–190	
4S*	–	–	Sco1815 (PKS II) *Streptomyces coelicolor* A3(2)		20–240	
nd	nd	nd	FabV *Vibrio cholerae*		1–401	
nd	nd	nd	FabL *Bacillus subtilis*		1–250	
4S	*si*	*si*	ER domain (FAS I) *Saccharomyces cerevisiae*			10–285
4S	*si*	*si?*	ER domain (FAS I) *Breibacterium ammoniagenes*			100–250
4S	–	–	2,4-DCR *Escherichia coli* K12			1–360
nd	nd	nd	FabK *Streptococcus pneumoniae*			10–230
nd	nd	nd	Rv2953 *Mycobacterium tuberculosis*			

nd, not determined.
*Suggested from crystal structure.
†Mistakenly described as 4S in the original publication (see text).
§Suggested because of very high sequence similarity to Ccr of *R. sphaeroides.*

Table S3. Accession numbers of the proteins analyzed in this study

Accession number	Name	Function	Organism
1YB5	–	*z*-Crystallin	*Homo sapiens* (lens)
1PS9	2,4-DCR	2,4-Dienoyl-CoA reductase	*Escherichia coli*
1MG5	Adh	Alcohol dehydrogenase	*Drosophila melanogaster*
1HLD	Adh	Alcohol dehydrogenase	*Equus caballus* (liver)
Q4KFF8	Adh	Alcohol dehydrogenase	*Pseudomonas fluorescens*
P00330	Adh	Alcohol dehydrogenase	*Saccharomyces cerevisiae*
NP_620218.1	Aor	Alkenal/alkenone oxidoreductase (Leukotriene B4 12-hydroxydehydrogenase)	*Rattus norvegicus*
ZP_02056035.1	Ccr	Crotonyl-CoA carboxylase/reductase	*Methylobacterium extorquens* AM1
YP_354044.1	Ccr	Crotonyl-CoA carboxylase/reductase	*Rhodobacter sphaeroides* 2.4.1.
NP_630556.1	Ccr	Crotonyl-CoA carboxylase/reductase	*Streptomyces coelicolor* A3(2)
AAA92890.1	Ccr	Crotonyl-CoA reductase (carboxylase?)	*Streptomyces collinus*
AAC44655.1	ChcA	1-Cyclohexenylcarbonyl CoA reductase	*Streptomyces collinus*
AAF62880.1	EpoA	Epothilone polyketide synthase module 0*	*Sorangium (Polyangium) cellulosum*
AAF62883.1	EpoD	Epothilone polyketide synthase module 3, 4, 5 and 6§	*Sorangium (Polyangium) cellulosum*
Q8WZM3.1	Etr1p	Trans-2 enoyl-(acyl-carrier-protein) reductase 1 (mitochondrial)	*Candida tropicalis*
P0AEK4.2	FabI	Enoyl-(acyl-carrier-protein) reductase	*Escherichia coli*
AAF98273.1	FabK	Trans-2-enoyl-(acyl-carrier-protein) reductase II	*Streptococcus pneumoniae*
NP_388745.1	FabL	Enoyl-(acyl carrier protein) reductase	*Bacillus subtilis*
ABX38717.1	FabV	Enoyl-(acyl-carrier-protein) reductase IV	*Vibrio cholerae*
CAA46024.1	FAS	Fatty acid synthase*	*Brevibacterium (Corynebacterium) ammoniagenes*
P12276.5	FAS	Fatty acid synthase*	*Gallus gallus*
P12785.3	FAS	Fatty acid synthase*	*Rattus norvegicus* (liver)
NP_012739.1	FAS	Fatty acid synthase, b-subunit*	*Saccharomyces cerevisiae*
CAB02034.1	InhA	Enoyl-(acyl-carrier-protein) reductase	*Mycobacterium tuberculosis* H37v
3B6Z	LovC	Lovastatin polyketide enoyl reductase	*Aspergillus terreus*
AAF71776.1	NysC	Nystatin polyketide synthase, module 5 (steps 3–8)*	*Streptomyces noursei*
AAF71767.1	NysJ	Nystatin polyketide synthase, module 15 (steps 15–17)*	*Streptomyces noursei*
1QOR	Qor	Quinone oxidoreductase	*Escherichia coli*
1IYZ	Qor	Quinone oxidoreductase	*Thermus thermophilus* HB8
P95139.1	Rv2953	Trans-acting enoyl reductase	*Mycobacterium tuberculosis* H37v
2NMO	Sco1815	b-Ketoacyl-(acyl-carrier-protein) reductase	*Streptomyces coelicolor* A3(2)

*Contains 1 enoyl-thioester reductase domain that was identified and selected for amino acid sequence analysis.
§Contains 2 enoyl-CoA reductase domains that were both identified and selected for amino acid sequence analysis.

Table S4. Amino acid sequences of the proteins analyzed in this study (FASTA format)

>zeta-CRYSTALLIN_HOMO
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KGDRVFTSSTISGGYAEYALAADHTVYKLPEKLDFKQGAAIGIPYFTAYRALIHSACVKAGESVLVHGASGGVGLAACQIARAYGLKILGTAGTEEGQKIVLQNGAHEVFN
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NIIHGSGATGKMILLL
>2,4-DCR_ESCHERICHIA
SYPSLFAPLDLGFTTLKNRVLMGSMHTGLEEYPDGAERLAAFYAERARHGVALIVSGGIAPDLTGVGMEGGAMLNDASQIPHHRTITEAVHQEGGKIALQILHTGRYSYQP
HLVAPSALQAPINRFVPHELSHEEILQLIDNFARCAQLAREAGYDGVEVMGSEGYLINEFLTLRTNQRSDQWGGDYRNRMRFAVEVVRAVRERVGNDFIIIYRLSMLDLVE
DGGTFAETVELAQAIEAAGATIINTGIGWHEARIPTIATPVPRGAFSWVTRKLKGHVSLPLVTTNRINDPQVADDILSRGDADMVSMARPFLADAELLSKAQSGRADEINTCI
GCNQACLDQIFVGKVTSCLVNPRACHETKMPILPAVQKKNLAVVGAGPAGLAFAINAAARGHQVTLFDAHSEIGGQFNIAKQIPGKEEFYETLRYYRRMIEVTGVTLKLN
HTVTADQLQAFDETILASGIVPRTPPIDGIDHPKVLSYLDVLRDKAPVGNKVAIIGCGGIGFDTAMYLSQPGESTSQNIAGFCNEWGIDSSLQQAGGLSPQGMQIPRSPRQIV
MLQRKASKPGQGLGKTTGWIHRTTLLSRGVKMIPGVSYQKIDDDGLHVVINGETQVLAVDNVVICAGQEPNRALAQPLIDSGKTVHLIGGCDVAMELDARRAIAQGTRL
ALEI
>ADH_DROSOPHILA
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NQNGAIWKLDLGTLEAIQWTKHWDSGI
>ADH_EQUUS
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>CCR_Rhodobacter
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GGMVVICAGTTGFNCTFDVRYMWMHQKRLQGSHFANLKQASAANQLMIERRLDPCMSEVFPWAEIPAAHTKMYRNQHKPGNMAVLVQAPRTGLRTFADVLEAGRKA
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NRFRNV
>ChcA_STREPTOMYCES_collinus
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>FAS_BREVIbacterium
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>rv2953_mycobacterium

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