Electronic Supplementary Material (ESI) for Catalysis Science & Technology


# Old Yellow Enzyme – Mediated Reduction of β-Cyano-α,β-Unsaturated Esters for the Synthesis of Chiral Building Blocks: Stereochemical Analysis of the Reaction

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## Electronic Supplementary Information

### Contents

## General methods

TLC analyses were performed on Merck Kieselgel 60 F254 plates. All the chromatographic separations were carried out on silica gel columns. $^{1}H$ and $^{13}C$ NMR spectra were recorded on a 400 or 500 MHz spectrometer. The chemical shift scale was based on internal tetramethylsilane. $^{2}H$ spectra were recorded on a 400 MHz spectrometer with proton broad band decoupling, in $CHCl_3$ solutions, using $CDCl_3$ as internal reference for chemical shift scale. GC-MS analyses were performed using a HP-5MS column (30 m × 0.25 mm × 0.25 μm, Agilent). The following temperature program was employed: 60°C (1 min) / 6°C $min^{-1}$ / 150°C (1 min) / 12°C $min^{-1}$ / 280°C (5 min). The enantiomeric excess values were determined by GC analysis, performed using a DAcTBSil.BetaCDX (0.25 μm × 0.25 mm × 25 m column, Mega, Italy), according to the following conditions: **6a**: 80°C (2 min) / 1.5°C $min^{-1}$ / 110°C / 30°C$min^{-1}$ / 220°C (10 min); (*R*)-**6a** $t_R$ = 18.9 min, (*S*)-**6a** $t_R$ = 19.9 min; dimethyl 2-methylsuccinate: (*S*)-enantiomer $t_R$ = 5.33 min, (*R*)-enantiomer $t_R$ = 5.48 min; (*R*)-**7a** $t_R$ = 17.3 min, (*S*)-**7a** $t_R$ = 18.4 min; ii) **6b**: 65°C (2 min) / 1°C $min^{-1}$ / 105°C / 30°C$min^{-1}$ / 220°C (10 min); (*S*)-**6b** $t_R$ = 32.7 min, (*R*)-**6b** $t_R$ = 33.8 min; iii) **6c** and **6e**: 70°C (2 min) / 1°C $min^{-1}$ / 115°C / 30°C$min^{-1}$ / 220°C (10 min); (*S*)-**6c** $t_R$ = 31.1 min, (*R*)-**6c** $t_R$ = 31.9 min; (*S*)-**6e** $t_R$ = 39.5 min, (*R*)-**6e** $t_R$ = 40.2 min; (*R*)-**7b** $t_R$ = 41.8 min, (*S*)-**7b** $t_R$ = 43.3 min iv) **6h**: 80°C (2 min) / 2°C $min^{-1}$ / 160°C / 30°C$min^{-1}$ / 220°C (10 min); (*R*)-**6h** $t_R$ = 32.9 min, (*S*)-**6h** $t_R$ = 33.2 min; v) **6g:** 70°C (3 min) / 3°C $min^{-1}$ / 180°C / 30°C$min^{-1}$ / 220°C (10 min); (*S*)-**6g** $t_R$ = 31.9 min, (*R*)-**6g** $t_R$ = 32.1 min; vi) 90°C / 2°C $min^{-1}$ / 115°C / 30°C$min^{-1}$ / 220°C (10 min); (*R*)-**7c** $t_R$ = 17.7 min, (*S*)-**7c** $t_R$ = 18.2 min; vii) **8**: 80°C / 0.8°C $min^{-1}$ / 125°C / 30°C$min^{-1}$ / 220°C (10 min); (*R*)-**8** $t_R$ = 51.0 min, (*S*)-**8** $t_R$ = 51.3min

## Strains and enzymes

### Sources

Fresh baker's yeast from Lesaffre Italia was employed. Old yellow enzymes (OYE1-3) and glucose dehydrogenase (GDH) were overexpressed in *Escherichia coli* BL21 (DE3) strains harboring a specific plasmid prepared according to standard molecular biology techniques: pET30a-OYE1 from the original plasmid provided by Neil C. Bruce,[1] pET30a-OYE2 and pET30a-OYE3 from *Saccharomyces cerevisiae* BY4741 and pKTS-GDH from *Bacillus megaterium* DSM509 (detailed steps reported in reference [2]). Alcohol dehydrogenase from *Thermoanaerobium brockii* (TBADH) was obtained from Sigma-Aldrich.

### Overexpression of the enzymes in *E. coli* BL21 (DE3)

LB medium (5 mL) containing the appropriate antibiotic (50 μg $mL^{-1}$ kanamycin for pET-30a, 100 μg $mL^{-1}$ ampicillin for pKTS) was inoculated with a single colony from a fresh plate and grown for 8 h at 37°C and 220 rpm. This starter culture was used to inoculate 200 mL medium, which was incubated for 8 h at the same conditions and used to inoculate 1.5 L medium. The latter culture was shaken at 37°C and 220 rpm until $OD_{600}$ reached 0.4-0.5, then enzyme expression was induced by the addition of 0.1 mM IPTG (50 ng $mL^{-1}$ anhydrotetracycline was also added in the case of the pKTS-GDH plasmid). After 5-6 h the cells were harvested by centrifugation (5000 *g*, 20 min, 4°C), resuspended in 50 mL of lysis buffer (20 mM $KP_i$ buffer $KH_2PO_4/K_2HPO_4$ pH 7.0, 300 mM NaCl, 10 mM imidazole) and disrupted by sonication (Omni Ruptor 250 ultrasonic homogeniser, five sonication cycles, 15 s each, 50% duty). The cell-free extract, after centrifugation (20000 *g*, 20 min, 4°C), was chromatographed on IMAC stationary phase (Ni-Sepharose Fast Flow, GE Healthcare) with a mobile phase composed of 20 mM $KP_i$ buffer pH 7.0, 300 mM NaCl and a 10-300 mM imidazole gradient. Protein elution was monitored at 280 nm, the fractions were collected according to the chromatogram and dialysed twice against 1.0 L of 50 mM phosphate buffer pH 7.0 (12 h each, 4°C) to remove imidazole and salts. Purified protein aliquots were stored frozen at –80°C.

## Synthesis and characterization data of the substrates

### General procedure for the synthesis of methyl cyanoacrylates (*E*)- and (*Z*)-4

A solution of the suitable 2-oxoalkanoate (0.200 mol) and 2-(triphenylphosphoranylidene)-acetonitrile (0.300 mol) in toluene (300 ml) was refluxed for 4 h. After the usual work up, the residue was purified by silica gel column chromatography, using *n*-hexane with increasing percentage of AcOEt as an eluent, in order to separate (*E*)- and (*Z*)-stereoisomers.

The (*E*) and (*Z*) configurational assignment was based on the more downfield chemical shift of the vinylic hydrogen of (*E*)-isomer, and on the more upfield chemical shift of the first carbon atom of the alkyl substituent in position 2 of the (*E*)-isomer (*syn* upfield γ effect of $^{13}$C NMR).

### (*E*)- and (*Z*)-Methyl 3-cyano-2-methylacrylate [(*E*)- and (*Z*)-4a]

From methyl pyruvate (20.4 g, 0.200 mol) (*E*)- (14.1 g, 52 %) and (*Z*)-**4a** (6.50 g, 26 %) were obtained.

*Data of (E)-**4a**:* $^{1}$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 6.32 (q, *J*=1.4 Hz, 1H, C*H*CN), 3.83 (s, 3H, COOC*H*$_3$), 2.24 (d, *J*=1.4 Hz, 3H, *CH*$_3$); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$ = 165.1, 149.7, 115.3, 107.7, 52.9, 16.9; GC-MS (EI) $t_R$ = 6.28 min: *m*/*z* (%) = 125 ($M^+$, 40), 94 (100), 66 (55).

*Data of (Z)-**4a**:* $^{1}$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 5.73 (q, *J*=1.8 Hz, 1H, C*H*CN), 3.89 (s, 3H, COOC*H*$_3$), 2.13 (d, *J* = 1.8 Hz, 3H, *CH*$_3$); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$ = 164.5, 149.2, 114.9, 105.3, 52.6, 19.9; GC-MS (EI) $t_R$ = 7.54 min: *m*/*z* (%) = 125 ($M^+$, 33), 94 (100), 66 (27).

### (*E*)- and (*Z*)-Methyl 2-(cyanomethylene)butanoate [(*E*)- and (*Z*)-4b]

From methyl 2-oxobutanoate (20.0 g, 0.172 mol) (*E*)- (10.3 g, 43 %) and (*Z*)-**4b** (7.65 g, 32 %) were obtained.

*Data of (E)-**4b**:* $^{1}$H NMR ($CDCl_3$, 500 MHz): $\delta$ = 6.28 (s, 1H, C*H*CN, 3.83 (s, 3H, COOC*H*$_3$), 2.64 (q, 2H, *J*= 7.5 Hz, C*H*$_2$), 1.17 (t, 3H, *J* = 7.5 Hz, $CH_2CH_3$); $^{13}$C NMR (125.7 MHz,

$CDCl_3$): $\delta$ = 164.6, 155.2, 114.9, 106.7, 52.5, 24.3, 12.9; GC-MS (EI) $t_R$ = 7.70 min: *m*/*z* (%) = 139 ($M^+$, 20), 124 (100), 108 (95), 94 (60).

*Data of (Z)-**4b**:* $^1$H NMR ($CDCl_3$, 500 MHz): $\delta$ = 5.73 (t, *J*=1.5 Hz, 1H, C*H*CN), 3.88 (s, 3H, COOC$H_3$), 2.50 (dq, 2H, *J* = 1.5 and 7.5 Hz, C$H_2$), 1.13 (t, 3H, *J* = 7.5 Hz, C$H_3$); $^{13}$C NMR ($CDCl_3$, 125.7 MHz): $\delta$ =164.3, 154.8, 115.0, 103.3, 52.2, 26.3, 11.6; GC-MS (EI) $t_R$ = 9.29 min: *m*/*z* (%) 139 ($M^+$, 8), 124 (100), 108 (100), 94 (31).

**(*E*)- and (Z)-Methyl 2-(cyanomethylene)pentanoate [(*E*)- and (*Z*)-4c**]

From methyl 2-oxopentanoate (20.0 g, 0.154 mol) (*E*)- (10.8 g, 46 %) and (*Z*)-**4c** (6.32 g, 27 %) were obtained.

*Data of (E)-**4c**:* $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 6.31 (s, 1H, C*H*CN), 3.83 (s, 3H, COOC$H_3$), 2.61 (t, 2H, *J* = 7.4 Hz, C$H_2$C=C), 1.56 (m, 2H, C$H_2$), 0.98 (t, 3H, *J* = 7.4 Hz, C$H_3$); $^{13}$C NMR (100.6 MHz, $CDCl_3$), $\delta$ = 164.9, 153.9, 115.3, 107.4, 52.8, 32.9, 22.0, 13.5; GC-MS (EI) $t_R$ = 9.59 min: *m*/*z* (%) = 152 ($M^+$-1, 3), 138 (80), 122 (53), 94 (100).

*Data of (Z)-**4c**:* $^1$H NMR ($CDCl_3$, 500 MHz): $\delta$ = 5.66 (t, *J*=1.4 Hz, 1H, C*H*CN), 3.89 (s, 3H, COOC$H_3$), 2.43 (dt, 2H, *J* = 1.7, 7.5 Hz, C$H_2$C=C), 1.52 (m, 2H, C$H_2$), 0.95 (t, 3H, *J* = 7.5 Hz, C$H_3$); $^{13}$C NMR ($CDCl_3$, 125.7 MHz): $\delta$ =164.9, 153.8, 115.2, 104.2, 52.6, 35.5, 21.1, 13.4; GC-MS (EI) $t_R$ = 11.18 min: *m*/*z* (%) 152 ($M^+$ - 1, 1), 138 (92), 122 (75), 94 (100).

**(*E*)- and (*Z*)-Methyl 2-(cyanomethylene)-3-methylbutanoate [(*E*)- and (*Z*)-4d**]

From 3-methyl-2-oxobutanoate (20.0 g, 0.154 mol) (*E*)- (9.71 g, 41 %) and (*Z*)-**4d** (4.40 g, 19 %) were obtained.

*Data of (E)-**4d**:* $^1$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 6.18 (s, 1H, C*H*CN), 3.81 (s, 3H, COOC$H_3$), 3.22 (septuplet, 1H, *J* = 6.9 Hz, C*H*($CH_3$)$_2$), 1.29 (d, 6H, *J* = 6.9 Hz, ($CH_3$)$_2$CH); $^{13}$C NMR (100.6 MHz, $CDCl_3$), $\delta$ = 164.9, 159.3, 115.1, 105.5, 52.5, 31.9, 20.4; GC-MS (EI) $t_R$ = 8.90 min: *m*/*z* (%) = 153 ($M^+$, 2), 138 (31), 122 (56), 108 (73), 94 (100).

*Data of (Z)-**4d**:* $^{1}$H NMR ($CDCl_3$, 500 MHz): $\delta$ = 5.60 (d, 1H, $J$ = 1.1 Hz, C*H*CN), 3.91 (s, 3H, COOC$H_3$), 2.94 (d septuplet, 1H, $J$ = 1.1, and 7.1 Hz, C$H$($CH_3$)$_2$), 1.14 (d, 6H, $J$ = 7.1 Hz, ($CH_3$)$_2$CH); $^{13}$C NMR ($CDCl_3$, 125.7 MHz): $\delta$ = 165.1, 160.3, 115.4, 101.4, 52.5, 31.7, 20.3; GC-MS (EI) $t_R$ = 10.24 min: *m*/*z* (%) = 153 ($M^+$, 2), 138 (22), 122 (72), 108 (75), 94 (100).

**(*E*)- and (*Z*)-Methyl 2-(cyanomethylene)hexanoate [(*E*)- and (*Z*)-4e]**

From methyl 2-oxohexanoate (20.0 g, 0.139 mol) (*E*)- (10.2 g, 44 %) and (*Z*)-**4e** (5.31 g, 23 %) were obtained.

*Data of (E)-**4e**:* $^{1}$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 6.28 (s, 1H, C*H*CN), 3.83 (s, 3H, COOC$H_3$), 2.63 (m, 2H, C$H_2$C=C), 1.53 (m, 2H, $CH_2$$CH_2$C=C), 1.40 (m, 2H, $CH_3CH_2$), 0.93 (t, 3H, $J$=7.2 Hz, C$H_3$]; $^{13}$C NMR (100.6 MHz, $CDCl_3$), $\delta$ = 165.0, 154.2, 115.3, 107.2, 52.7, 30.8, 30.7, 22.3, 13.6; GC-MS (EI) $t_R$ = 11.75 min: *m*/*z* (%) = 166 ($M^+$-1, 3), 152 (20), 139 (25), 125 (100).

*Data of (Z)-**4e**:* $^{1}$H NMR ($CDCl_3$, 400 MHz): $\delta$ 5.67 (t, $J$ = 1.4 Hz, 1H, C*H*CN), 3.88 (s, 3H, COOC$H_3$), 2.45 (m, 2H, C$H_2$C=C) 1.47 (m, 2H, $CH_2$$CH_2$C=C), 1.36 (m, 2H, $CH_3CH_2$), 0.92 (t, 3H, $J$ = 7.3 Hz, C$H_3$); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$ = 164.6, 154.0, 115.1, 103.9, 52.5, 33.2, 29.8, 22.0, 13.5; GC-MS (EI) $t_R$ = 13.22 min: *m*/*z* (%)=166 ($M^+$-1, 3), 152 (38), 139 (41), 111 (100).

**(*E*)- and (*Z*)-Methyl 2-(cyanomethylene)-3-methylpentanoate [(*E*)- and (*Z*)-4f]**

From methyl 3-methyl-2-oxopentanoate (20.0 g, 0.139 mol) (*E*)- (9.05 g, 39 %) and (*Z*)-**4f** (4.87 g, 21 %) were obtained.

*Data of (E)-**4f**:* $^{1}$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 6.24 (s, 1H, C*H*CN), 3.81 (s, 3H, COOC$H_3$), 2.97 (m, 1H, C*H*C=C), 1.76 (m, 1H, *CH*HCHC=C), 1.66 (m, 1H, *CH*HCHC=C), 1.27 (d, $J$ = 6.6 Hz, 3H, $CH_3$CHC=C), 0.90 (t, $J$ = 7.4 Hz, 3H,C$H_3$CH$_2$); $^{13}$C NMR (100.6 MHz, $CDCl_3$), $\delta$ = 164.8, 158.1, 115.3, 106.7, 52.5, 39.3, 27.7, 18.6, 12.3; GC-MS (EI) $t_R$ = 10.76 min: *m*/*z* (%) = 167 ($M^+$, 3), 152 (70), 136 (45), 124 (75), 108 (100).

*Data of (Z)-**4f**:* $^{1}$H NMR ($CDCl_3$, 400 MHz): $\delta$ 5.57 (d, $J$ = 1.1 Hz, 1H, C*H*CN), 3.89 (s, 3H, COOC$H_3$), 2.70 (m, 1H, C*H*C=C), 1.55 (m, 1H, *CH*HCHC=C), 1.43 (m, 1H, *CH*HCHC=C), 1.11 (d, $J$ = 6.6 Hz, 3H,$CH_3$CHC=C), 0.89 (t, $J$ = 7.4 Hz, 3H, C$H_3$); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$ = 165.2, 159.4, 115.3, 102.2, 52.5, 38.7, 28.1, 18.3, 11.3; GC-MS (EI) $t_R$ = 12.13 min: *m*/*z* (%)=167 ($M^+$, 1), 152 (27), 136 (54), 124 (42), 108 (100).

**(*E*)- and (*Z*)-Dimethyl 2-(cyanomethylene)pentanedioate [(*E*)- and (*Z*)-4g]**

From dimethyl 2-oxopentanedioate (20.0 g, 0.115 mol) (*E*)- (11.8 g, 52 %) and (*Z*)-**4g** (3.62 g, 16 %) were obtained.

*Data of (E)-**4g**:* $^{1}$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 6.37 (s, 1H, C*H*CN), 3.84 (s, 3H, COOC$H_3$), 3.70 (s, 3H, COOC$H_3$), 2.95 (t, $J$ = 7.5 Hz, $CH_2$COOCH$_3$), 2.61 (t, $J$ = 7.5 Hz, $CH_2$C=); $^{13}$C NMR (100.6 MHz, $CDCl_3$), $\delta$ = 171.8, 164.5, 151.7, 114.8, 108.9, 53.0, 51.9, 32.3, 26.4; GC-MS (EI) $t_R$ = 16.80 min: *m*/*z* (%) = 197 ($M^+$, 3), 165 (81), 138 (100), 106 (57).

*Data of (Z)-**4g**:* $^{1}$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 5.82 (s, 1H, C*H*CN), 3.90 (s, 3H, COOC$H_3$), 3.69 (s, 3H, COOC$H_3$), 2.77 (t, $J$ = 7.2 Hz, $CH_2$COOCH$_3$), 2.56 (t, $J$ = 7.2 Hz, $CH_2$C=); $^{13}$C NMR (100.6 MHz, $CDCl_3$), $\delta$ = 171.0, 164.4, 151.0, 114.8, 106.3, 52.7, 51.8, 31.9, 28.6; GC-MS (EI) $t_R$ = 18.02 min: *m*/*z* (%) =165 ($M^+$ - 32, 70), 138 (100), 106 (50).

**(*E*)- and (*Z*)-Methyl 3-cyano-2-phenylacrylate [(*E*)- and (*Z*)-4h]**

From methyl 2-oxo-2-phenylacetate (20.0 g, 0.122 mol) (*E*)- (11.2 g, 49 %) and (*Z*)-**4h** (6.16 g, 27 %) were obtained.

*Data of (E)-**4h**:* $^{1}$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 7.50 – 7.40 (m, 5H, aromatic hydrogens), 6.51 (s, 1H, C*H*CN), 3.85 (s, 3H, COOC$H_3$); $^{13}$C NMR (100.6 MHz, $CDCl_3$), $\delta$ = 165.0, 151.5, 132.1, 130.3, 128.9, 128.4, 115.6, 107.3, 53.2; GC-MS (EI) $t_R$ = 18.26 min: *m*/*z* (%) = 187 ($M^+$, 100), 172 (43), 156 (28), 128 (100).

*Data of (Z)-**4h**:* $^{1}$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 7.50 – 7.40 (m, 5H, aromatic hydrogens), 5.91 (s, 1H, C*H*CN), 3.95 (s, 3H, COOC$H_3$); $^{13}$C NMR (100.6 MHz, $CDCl_3$), $\delta$ = 164.9, 152.6, 133.1, 130.9, 128.9, 127.4, 115.3, 101.2, 52.9; GC-MS (EI) $t_R$ = 19.39 min: *m*/*z* (%)=187 ($M^+$, 85), 172 (45), 156 (18) 128 (100).

**(*E*)- and (*Z*)-Methyl 3-cyano-2-(furan-2-yl)acrylate [(*E*)- and (*Z*)-4i**]

From methyl 2-(furan-2-yl)-2-oxoacetate (20.0 g, 0.130 mol), after column chromatography, a 50/50 mixture of (*E*)- and (*Z*)-**4i** (15.6 g, 68 %) was obtained: $^{1}$H NMR ($CDCl_3$, 400 MHz) for the two diastereoisomers: $\delta$ = 7.63 (d, *J* = 1.5 Hz, H of the furan ring), 7.49 (d, *J* = 1.5 Hz, H of the furane ring), 7.21 (d, *J* = 3.5 Hz, H of the furan ring), 7.01 (d, *J* = 3.5 Hz, H of the furan ring), 6.53 (dd, *J* = 1.5 and 3.5 Hz, H of the furan ring), 6.52 (dd, *J* = 1.5 and 3.5 Hz, H of the furan ring), 6.18 (s, 1H, C*H*CN), 6.14 (s, 1H, C*H*CN), 3.96 (s, 3H, COOC$H_3$), 3.88 (s, 3H, COOC$H_3$); GC-MS (EI) (*E*)-**4i** $t_R$ = 16.28 min: *m*/*z* (%) = 177 ($M^+$, 100), 148 (18), 118 (56); (*Z*)-**4i** $t_R$ = 16.67 min: *m*/*z* (%) = 177 ($M^+$, 100), 148 (22), 118 (50).

**(*E*)- and (*Z*)-Ethyl 3-cyano-2-methylacrylate [(*E*)- and (*Z*)-5a**]

From ethyl pyruvate (20.0 g, 0.172 mol) (*E*)- (9.80 g, 41 %) and (*Z*)-**5a** (6.93 g, 29 %) were obtained.

*Data of (E)-**5a**:* $^{1}$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 6.31 (q, *J*=1.4 Hz, 1H, C*H*CN), 4.27 (q, *J* = 7.1 Hz, 2H, COOC$H_2$), 2.23 (d, *J* = 1.4 Hz, 3H, $CH_3$C=), 1.32 (t, *J* = 7.1 Hz, $CH_2CH_3$); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$ = 164.6, 150.1, 115.3, 107.4, 62.1, 16.9, 14.0; GC-MS (EI) $t_R$ = 7.81 min: *m*/*z* (%) = 139 ($M^+$, 6), 124 (28), 94 (100).

*Data of (Z)-**5a**:* $^{1}$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 5.78 (q, *J* = 1.7 Hz, 1H, C*H*CN), 4.34 (q, *J* = 7.1 Hz, 2H, COOC$H_2$), 2.15 (d, *J* = 1.7 Hz, 3H, $CH_3$C=), 1.37 (t, *J* = 7.1 Hz, $CH_2CH_3$); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$ = 163.8, 149.2, 114.8, 104.7, 61.8, 19.5, 13.6; GC-MS (EI) $t_R$ = 8.88 min: *m*/*z* (%) = 139 ($M^+$, 4), 124 (11), 112 (42), 94 (100).

**(*E*)- and (*Z*)-Butyl 3-cyano-2-methylacrylate [(*E*)- and (*Z*)-5b]**

From *n*-butyl pyruvate (20.0 g, 0.139 mol) (*E*)- (12.1 g, 52 %) and (*Z*)-**5b** (3.71 g, 16 %) were obtained.

*Data of (E)-**5b**:* $^{1}$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 6.31 (s, 1H, C*H*CN), 4.23 (t, $J$ = 6.6 Hz, 2H, COOC$H_2$), 2.24 (s, 3H, $CH_3$C=), 1.69 (m, 2H, $CH_2$), 1.42 (m, 2H, $CH_2$), 0.96 (t, $J$ = 7.6 Hz, $CH_2CH_3$); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$ = 164.7, 150.1, 115.3, 107.4, 66.0, 30.1, 19.1, 16.9, 13.5; GC-MS (EI) $t_R$ = 12.49 min: *m*/*z* (%) = 166 ($M^+$-1, 1), 112 (25), 94 (70), 56 (100).

*Data of (Z)-**5b**:* $^{1}$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 5.72 (q, $J$ = 1.7 Hz, 1H, C*H*CN), 4.29 (t, $J$ = 6.6 Hz, 2H, COOC$H_2$), 2.12 (d, $J$ = 1.7 Hz, 3H, $CH_3$C=), 1.73 (m, 2H, $CH_2$), 1.46 (m, 2H, $CH_2$), 0.96 (t, $J$ = 7.4 Hz, $CH_2CH_3$); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$ = 164.1, 149.5, 115.0, 104.9, 66.1, 30.3, 19.9, 19.1, 13.5; GC-MS (EI) $t_R$ = 13.52 min: *m*/*z* (%) = 166 ($M^+$-1, 1), 112 (80), 94 (100), 56 (73).

**(*E*)- and (Z)-Hexyl 3-cyano-2-methylacrylate [(*E*)- and (*Z*)-5c]**

From *n*-hexyl pyruvate (20.0 g, 0.116 mol) (*E*)- (12.2 g, 54 %) and (*Z*)-**5c** (3.62 g, 16 %) were obtained.

*Data of (E)-**5c**:* $^{1}$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 6.30 (s, 1H, C*H*CN), 4.21 (t, $J$ = 6.6 Hz, 2H, COOC$H_2$), 2.23 (s, 3H, $CH_3$C=), 1.68 (m, 2H, $CH_2$), 1.42 – 1.24 (m, 6H, 3$CH_2$), 0.90 (t, $J$ = 6.4 Hz, $CH_2CH_3$); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$ = 164.7, 150.2, 115.4, 107.4, 66.3, 31.3, 28.4, 25.5, 22.4, 16.9, 13.9; GC-MS (EI) $t_R$ = 17.20 min: *m*/*z* (%) = 194 ($M^+$-1, 1), 112 (35), 94 (71), 56 (100).

*Data of (Z)-**5c**: :* $^{1}$H NMR ($CDCl_3$, 400 MHz): $\delta$ = 5.72 (q, $J$ = 1.7 Hz, 1H, C*H*CN), 4.27 (t, $J$ = 6.8 Hz, 2H, COOC$H_2$), 2.12 (d, $J$ = 1.7 Hz, 3H, $CH_3$C=), 1.74 (m, 2H, $CH_2$), 1.48 – 1.28 (m, 6H, 3$CH_2$), 0.90 (t, $J$ = 6.8 Hz, $CH_2CH_3$); $^{13}$C NMR ($CDCl_3$, 100.6 MHz): $\delta$ = 164.1, 149.5, 115.0,

104.9, 66.3, 31.3, 28.2, 25.5, 22.4, 19.9, 13.8; GC-MS (EI) $t_R$ = 18.20 min: *m*/*z* (%) = 194 ($M^+$-1, 1), 112 (100), 94 (64), 56 (50).

**Characterization data of the compounds obtained upon bioreduction**

**(*R*)-Methyl 3-cyano-2-methylpropanoate [(*R*)-6a]**

From (*Z*)-**4a** (3.0 g, 0.024 mol) compound (*R*)-**6a** (2.38 g, 78 %) was obtained: ee = 67 %, $[\alpha]_D^{20}$ = + 4.0 (*c* 1.89, $CHCl_3$); $^1$H NMR ($CDCl_3$, 400 MHz,) $\delta$ = 3.74 (s, 3H, COOC*H*$_3$), 2.82 (m, C*H*COOCH$_3$), 2.67 (dd, *J* = 6.0, and 17 Hz, 1H, C*H*$_a$CN), 2.54 (dd, *J* = 7.5, and 17 Hz, 1H, C*H*$_b$CN), 1.37 (d, *J* = 7.2 Hz, 3H, CHC*H*$_3$); $^{13}$C NMR ($CDCl_3$, 100.6 MHz) $\delta$ = 173.4, 117.7, 52.4, 36.1, 21.0, 16.6; GC-MS (EI) $t_R$ = 7.02 min: *m*/*z* (%) = 127 ($M^+$, 1), 96 (41), 68 (100).

By reaction with refluxing methanol in the presence of a catalytic quantity of sulphuric acid, (+)-**6a** was converted into (*R*)-dimethyl 2-methylsuccinate, showing with ee = 70%: the assignment was established by comparison by GC analysis on a chiral stationary phase with a commercial sample of (*R*)-dimethyl 2-methylsuccinate (Sigma-Aldrich).

**(*S*)-Methyl 2-(cyanomethyl)butanoate [(*S*)-6b]**

From (*E*)-**4b** (3.0 g, 0.022 mol) compound (*S*)-**6b** (2.19 g, 72 %) was obtained: ee = 99 %, $[\alpha]_D^{25}$ = -16.8 (*c* 1.02, $CHCl_3$) $^1$H NMR ($CDCl_3$, 500 MHz,) $\delta$ = 3.75 (s, 3H, COOC*H*$_3$), 2.70 (m, 1H, C*H*COOCH$_3$), 2.66 (dd, *J* = 6.8, and 16.5 Hz, 1H, C*H*$_a$CN), 2.56 (dd, *J* = 6.5, and 16.5 Hz, 1H, C*H*$_b$CN), 1.80 (m, 2H, C*H*$_2$), 0.96 (t, *J* = 7.5 Hz, 3H, C*H*$_3$); $^{13}$C NMR ($CDCl_3$, 100.6 MHz) $\delta$ = 172.8, 117.6, 52.0, 42.6, 24.2, 18.6, 10.6; GC-MS (EI) $t_R$ = 8.92 min: *m*/*z* (%) = 141 ($M^+$, 1), 113 (54), 98 (58), 82 (100).

By reaction with refluxing methanol in the presence of a catalytic quantity of sulphuric acid, (-)-**6b** was converted into (*S*)-dimethyl 2-ethylsuccinate : $[\alpha]_D^{20}$ = -12.7 (*c* 1.1, $CHCl_3$) (lit.[3] for the (*R*)-enantiomer $[\alpha]_D^{22}$= + 13 (*c* 1.23, $CHCl_3$).

**(*S*)-Methyl 2-(cyanomethyl)pentanoate [(*S*)-6c]**

From (*E*)-**4c** (3.0 g, 0.020 mol) compound (*S*)-**6c** (1.02 g, 33 %) was obtained: ee = 99 %, $[\alpha]_D^{25}$ = - 11.8 (*c* 2.5, $CHCl_3$) $^1$H NMR ($CDCl_3$, 400 MHz,) $\delta$ 3.74 (s, 3H, COOC$H_3$), 2.75 (quintuplet, *J* = 6.7, 1H, C*H*COOCH$_3$), 2.64 (dd, *J* = 6.7, 16.8 Hz, 1H, C$H_a$CN), 2.54 (dd, *J* = 6.7, 16.8 Hz, 1H, C$H_b$CN), 1.65 - 1.55 (m, 2H), 1.40 – 1.25 (m, 2H), 0.95 (t, *J* = 7.4 Hz, 3H, C$H_3$); $^{13}$C NMR ($CDCl_3$, 100.6 MHz) $\delta$ = 173.2, 117.7, 52.2, 41.3, 33.4, 19.8, 19.3, 13.6; GC-MS (EI) $t_R$ = 10.91 min: *m*/*z* (%) = 154 ($M^+$ - 1, 0.5), 113 (86), 98 (100), 82.

By reaction with refluxing methanol in the presence of a catalytic quantity of sulphuric acid, (-)-**6c** was converted into (*S*)-dimethyl 2-propylsuccinate : $[\alpha]_D^{20}$ = - 20.7 (*c* 1.1, $CHCl_3$) (lit.[4] for the (*R*)-enantiomer $[\alpha]_D^{20}$ = + 20.1 (neat).

**(*S*)-Methyl 2-(cyanomethyl)hexanoate [(*S*)-6e]**

From (*E*)-**4e** (3.0 g, 0.018 mol) compound (*S*)-**6e** (0.91 g, 30 %) was obtained: ee = 94 %, $[\alpha]_D^{25}$ = - 10.5 (*c* 1.1, $CHCl_3$) $^1$H NMR ($CDCl_3$, 400 MHz)[5] $\delta$ 3.75 (s, 3H, COOC$H_3$), 2.75 (quintuplet, *J* = 6.9, 1H, C*H*COOCH$_3$), 2.65 (dd, *J* = 6.9, 16.8 Hz, 1H, C$H_a$CN), 2.56 (dd, *J* = 6.9, 16.8 Hz, 1H, C$H_b$CN), 1.80 - 1.60 (m, 2H), 1.35 – 1.25 (m, 4H), 0.95 (t, *J* = 7.4 Hz, 3H, C$H_3$); $^{13}$C NMR ($CDCl_3$, 100.6 MHz) $\delta$ = 173.2, 117.8, 52.2, 41.4, 31.0, 28.6, 22.3, 19.2, 13.7; GC-MS (EI) $t_R$ = 13.11 min: *m*/*z* (%) = 168 ($M^+$ -1, 0.5), 138 (20), 129 (22), 113 (100), 98 (85).

By reaction with refluxing concentrated HCl, (-)-**6e** was converted into (*S*)-2-butylsuccinic acid : $[\alpha]_D^{20}$ = - 23.7 (*c* 3.4, EtOH) (lit.[6] for the (*R*)-enantiomer $[\alpha]_D^{25}$ = + 26.5 (*c* 5.2, EtOH).

**(*S*)-Dimethyl 2-(cyanomethyl)pentanedioate [(*S*)-6g]**

From (*E*)-**4g** (3.0 g, 0.015 mol) compound (*S*)-**6g** (1.67 g, 55 %) was obtained: ee = 78 %, $[\alpha]_D^{20}$ = - 8.2 (*c* 3.5, $CHCl_3$) $^1$H NMR ($CDCl_3$, 400 MHz,) $\delta$ 3.76 (s, 3H, COOC$H_3$), 3.69 (s, *3*H,

COOC$H_3$), 2.84 (m, 1H, C*H*COOMe), 2.68 (dd, *J* = 6.8, 16.9 Hz, 1H, C$H_a$CN), 2.60 (dd, *J* = 6.8, 16.9 Hz, 1H, C$H_b$CN), 2.43 (m, 2H, $CH_2$), 2.20 -1.90 (m, 2H, $CH_2$); $^{13}$C NMR ($CDCl_3$, 100.6 MHz) $\delta$ 172.5, 172.4, 117.3, 52.5, 51.8, 40.6, 30.9, 26.3, 19.6; GC-MS (EI) $t_R$ = 17.67 min: *m*/*z* (%) = 168 ($M^+$ - 31, 90), 139 (100), 127 (30)

By reaction with refluxing methanol in the presence of a catalytic quantity of sulphuric acid, (-)-**6g** was converted into (*S*)-trimethyl butane-1,2,4-tricarboxylate: $[\alpha]_D^{25}$ = - 11.9 (*c* 3.5, acetone ) (lit.[7] for the (*R*)-enantiomer $[\alpha]_D^{20}$ = + 16.2 (*c* 0.1368, acetone).

**(*R*)-Methyl 3-cyano-2-phenylpropanoate [(*R*)-6h]**

From (*E*)-**4h** (3.0 g, 0.016 mol) compound (*R*)-**6h** (0.544 g, 18 %) was obtained: ee = 28 %, $[\alpha]_D^{20}$ = - 36.1 (*c* 3.9, $CHCl_3$) $^1$H NMR ($CDCl_3$, 400 MHz)[8] $\delta$ = 7.40 – 7.20 (m, 5H, aromatic hydrogens), 3.94 (t, *J* = 7.5 Hz, C*H*COOMe), 3.72 (s, 3H, COOC$H_3$), 3.02 (dd, *J* = 7.5, 16.8 Hz, 1H, C$H_a$CN), 2.80 (dd, *J* = 7.5, 16.8 Hz, 1H, C$H_b$CN); $^{13}$C NMR ($CDCl_3$, 100.6 MHz) $\delta$ 171.4, 135.7, 129.2, 128.5, 127.5, 117.4, 52.7, 47.6, 21.7; GC-MS (EI) $t_R$ = 18.54 min: *m*/*z* (%) = 189 ($M^+$, 35), 130 (100), 104 (95)

By reaction with refluxing methanol in the presence of a catalytic quantity of sulphuric acid, (-)-**6h** was converted into (*R*)-dimethyl 2-phenylsuccinate: $[\alpha]_D^{25}$ = - 26.9 (*c* 3.0, MeOH) (lit.[9] for the (*S*)-enantiomer with ee = 92.3 % $[\alpha]_D^{20}$ = + 80 (*c* 0.12, MeOH).

**(*R*)-Ethyl 3-cyano-2-methylpropanoate [(*R*)-7a]**

From (*Z*)-**5a** (3.0 g, 0.022 mol) compound (*R*)-**7a** (1.67 g, 54 %) was obtained: ee = 99 %, $[\alpha]_D^{20}$ = + 10.9 (*c* 2.7, $CHCl_3$); lit.[10] for (*S*)-**7a** %, $[\alpha]_D^{17}$ = - 11 (*c* 0.9, $CHCl_3$) $^1$H NMR ($CDCl_3$, 400 MHz)[10] $\delta$ = 4.20 (q, *J* = 7.1 Hz, COO$CH_2$), 2.81 (m, C*H*COOCH$_3$), 2.68 (dd, *J* = 6.1, and 16.9 Hz, 1H, C$H_a$CN), 2.55 (dd, *J* = 7.5, and 16.8 Hz, 1H, C$H_b$CN), 1.36 (d, *J* = 7.1 Hz, 3H, CHC$H_3$), 1.29 (t, *J* = 7.1 Hz, 3H, COOCH$_2$C$H_3$); $^{13}$C NMR[10] ($CDCl_3$, 100.6 MHz) $\delta$ = 172.9, 117.8, 61.3,

36.1, 20.9, 16.5, 14.0; GC-MS (EI) $t_R$ = 8.56 min: *m*/*z* (%) = 141 ($M^+$, 9), 114 (27), 96 (91), 68 (100).

**(*R*)-Butyl 3-cyano-2-methylpropanoate [(*R*)-7b]**

From (*Z*)-**5b** (3.0 g, 0.018 mol) compound (*R*)-**7b** (0.76 g, 25 %) was obtained: ee = 96 %, $[\alpha]_D^{20}$ = + 13.2 (*c* 2.2, $CHCl_3$); $^1$H NMR ($CDCl_3$, 400 MHz,) $\delta$ = 4.14 (t, *J* = 6.7 Hz, 2H, COO*CH*$_2$), 2.81 (m, 1H, C*H*COOCH$_2$), 2.67 (dd, *J* = 6.1, and 16.8 Hz, 1H, C*H*$_a$CN), 2.53 (dd, *J* = 7.6, and 16.8 Hz, 1H, C*H*$_b$CN), 1.70 – 1.57 (m, 2H, *CH*$_2$), 1.45 -1.34 (m + d, *J* = 7.1 Hz, 5H, *CH*$_2$ + CH*CH*$_3$), 0.94 (t, *J* = 7.4 Hz, 3H, COO(CH$_2$)$_3$C*H*$_3$); $^{13}$C NMR ($CDCl_3$, 100.6 MHz) $\delta$ = 173.0, 117.7, 65.3, 36.3, 30.5, 21.0, 19.1, 16.7, 13.7; GC-MS (EI) $t_R$ = 12.96 min: *m*/*z* (%) = 168 ($M^+$ - 1, 1), 114 (34), 96 (100), 68 (79).

By reaction with refluxing methanol in the presence of a catalytic quantity of sulphuric acid, (+)-**7b** was converted into (*R*)-dimethyl 2-methylsuccinate, showing with ee = 96%: the assignment was established by comparison by GC analysis on a chiral stationary phase with a commercial sample of (*R*)-dimethyl 2-methylsuccinate (Sigma-Aldrich).

**(*R*)-Hexyl 3-cyano-2-methylpropanoate [(*R*)-7c]**

From (*Z*)-**5c** (3.0 g, 0.015 mol) compound (*R*)-**7c** (0.561 g, 19 %) was obtained: ee = 99 %, $[\alpha]_D^{20}$ = + 7.3 (*c* 1.5, $CHCl_3$); $^1$H NMR ($CDCl_3$, 400 MHz,) $\delta$ = 4.12 (t, *J* = 7.1 Hz, COO*CH*$_2$), 2.81 (m, C*H*COOCH$_3$), 2.67 (dd, *J* = 6.1, and 16.8 Hz, 1H, C*H*$_a$CN), 2.54 (dd, *J* = 7.6, and 16.8 Hz, 1H, C*H*$_b$CN), 1.70 – 1.58 (m, 2H, *CH*$_2$), 1.40 -1.26 (m + d, *J* = 7.1 Hz, 9H, 3*CH*$_2$ + CH*CH*$_3$), 0.94 (t, *J* = 6.9 Hz, 3H, COO(CH$_2$)$_5$C*H*$_3$); $^{13}$C NMR ($CDCl_3$, 100.6 MHz) $\delta$ = 173.0, 117.7, 65.3, 36.2, , 31.2, 28.4, 25.4, 22.4, 21.0, 16.6, 13.9; GC-MS (EI) $t_R$ = 17.39 min: *m*/*z* (%) = 196 ($M^+$ - 1, 1), 114 (79), 96 (67), 68 (75), 56 (100).

By reaction with refluxing methanol in the presence of a catalytic quantity of sulphuric acid, (+)-**7c** was converted into (*R*)-dimethyl 2-methylsuccinate, showing with ee = 99%: the assignment was

established by comparison by GC analysis on a chiral stationary phase with a commercial sample of (*R*)-dimethyl 2-methylsuccinate (Sigma-Aldrich).

## Synthesis of the $\gamma^2$-aminoacid derivative

### (*S*)-Methyl 4-(*t*-butoxycarbonylamino)-2-ethylbutanoate [(*S*)-8]

To a stirring solution of (*S*)-**6b** (ee = 99%, 0.340 g, 2.4 mmol) in MeOH (30 ml) $Boc_2O$ (1.05 g, 4.8 mmol) and a catalytic amount of $NiCl_2 \cdot 6H_2O$ were first added; then, at 0°C, $NaBH_4$ (0.64 g, 16.8 mmol) was added portionwise over 30 min. The reaction mixture was stirred for 24 h. After the usual workup, column chromatography eluting with hexane and increasing amount of ethyl acetate gave compound (*S*)-**8** (0.41 g, 70%): ee = 99 %, $[\alpha]_D^{20}$ = + 16.5 (*c* 1.96, $CHCl_3$); $^1$H NMR ($CDCl_3$, 400 MHz,) $\delta$ = 3.66 (s, 3H, COOC$H_3$), 3.09 (m, 2H, $CH_2$NH), 2.31 (m, 1H, C*H*COOCH$_3$), 1.80 – 1.45 (m, 4H, 2C$H_2$), 1.41 (s, 9H, ($CH_3$)$_3$C), 0.86 (t, *J* = 7.4 Hz, 3H, C$H_3$); $^{13}$C NMR ($CDCl_3$, 100.6 MHz) $\delta$ = 176.1, 155.8, 79.1, 51.4, 44.6, 38.8, 32.0, 28.4, 25.3, 11.5; GC-MS (EI) $t_R$ = 20.0 min: *m*/*z* (%) = 189 ($M^+$ - 56, 7), 172 (13), 144 (27), 57 (100).

## Data of deuterated compounds

Chemical doubly deuterated compounds were prepared by reduction of the (*Z*)-stereosiomer of **4a**, **4b**, **4e** and **5b** with Pt/C in benzene solution in the presence of deuterium gas.

The relevant chemical shifts of the $^{1}$H NMR spectra of monodeuterated and doubly deuterated compounds are reported in Table S1 and S2. The chemical shifts of the $^{2}$H NMR spectra of doubly deuterated compounds are reported in Table S3.

**Table S1.** Chemical shifts ($^{1}$H NMR) of the AB system due to the methylene hydrogens (C*H*$_2$CN) of monodeuterated (*S*)-**6a**-$d_1$, (*S*)-**6b**-$d_1$, and (*S*)-**7b**-$d_1$, and of the quartet due to C*H*COOR of monodeuterated (2*R*,1'*S*)-**6b**-$d_1$.

| **Compound** | ***δ (ppm)* C*H*$_2$CN** |
|---|---|
| (*S*)-**6a**-$d_1$ | 2.68 and 2.55 ($J$ = 16.8 Hz) |
| (*S*)-**6b**-$d_1$ | 2.65 and 2.55 ($J$ = 16.6 Hz) |
| (*S*)-**6e**-$d_1$ | 2.64 and 2.54 ($J$ = 16.9 Hz) |
| (*S*)-**7b**-$d_1$ | 2.67 and 2.53 ($J$ = 16.8 Hz) |
| **Compound** | ***δ (ppm)* C*H*COOR** |
| (2*R*,1'*S*)-**6b**-$d_1$ | 2.74 ($J$ = 6.8 Hz) |

**Table S2.** Chemical shifts ($^{1}$H NMR) of the survived hydrogen atom (C*H*DCN) of doubly deuterated compounds.

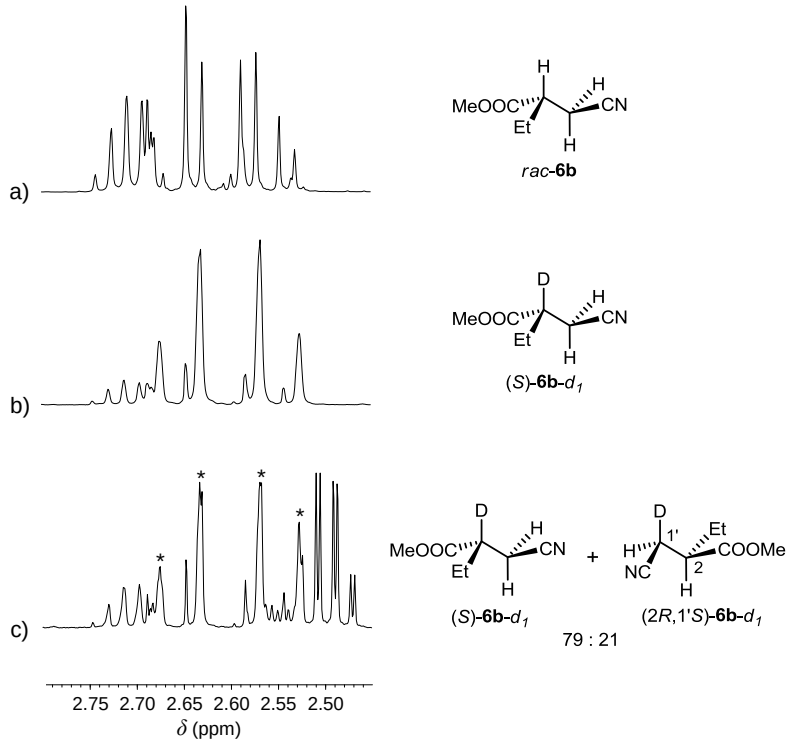
| **Compound** | ***δ (ppm)* C*H*DCN** |
|---|---|
| (2*S*,1'*S*)-**6a**-$d_2$ | 2.66 (m) |
| (2*R*,1'*S*)-**6a**-$d_2$ | 2.53 (m) |
| (2*S*,1'*S*)-**6b**-$d_2$ | 2.64 (m) |
| (2*S*,1'*R*)-**6b**-$d_2$ | 2.54 (m) |
| (2*S*,1'*S*)-**6e**-$d_2$ | 2.64 (m) |
| (2*R*,1'*S*)-**6e**-$d_2$ | 2.54 (m) |
| (2*R*,1'*R*)-**7b**-$d_2$ | 2.65 (m) |
| (2*R*,1'*S*)-**7b**-$d_2$ | 2.53 (m) |

**Table S3.** Chemical shifts ($^2$H NMR) of doubly deuterated compounds.

| **Compound** | ***δ (ppm)* C*D*COOR** | ***δ (ppm)* CH*D*CN** |
|---|---|---|
| (2*S*,1'*S*)-**6a**-$d_2$ | 2.81 | 2.54 |
| (2*R*,1'*S*)-**6a**-$d_2$ | 2.82 | 2.67 |
| (2*S*,1'*S*)-**6b**-$d_2$ | 2.70 | 2.55 |
| (2*S*,1'*R*)-**6b**-$d_2$ | 2.70 | 2.64 |
| (2*S*,1'*S*)-**6e**-$d_2$ | 2.74 | 2.55 |
| (2*R*,1'*S*)-**6e**-$d_2$ | 2.74 | 2.64 |
| (2*R*,1'*R*)-**7b**-$d_2$ | 2.80 | 2.53 |
| (2*R*,1'*S*)-**7b**-$d_2$ | 2.80 | 2.66 |

## $^1$H NMR Spectra of monodeuterated compounds

The $^1$H NMR spectra of (*S*)-**6b**-$d_1$ (Fig. S1b) and (*S*)-**6e**-$d_1$ (Fig. S2b), obtained upon reduction of (*E*)-**4b** (c = 100%, ee = 99%) and (*E*)-**4e** (c = 67%, ee = 99%), respectively, are reported, and compared to those of the hydrogenated counterparts *rac*-**6b** and **6e** (Fig. S1a and S2a). In Fig. S1c and S2b the lines of the AB system of the $CH_2CN$ group are marked with asterisks for the sake of clarity. In Figure S2c the appearance of the signal at 2.55 ppm due to CHDCN was made more complex by traces of hydrogenated **6e**, and by the geminal coupling with the deuterium on the same carbon atom.




*rac*-**6b** (S)-**6b**-$d_1$ (S)-**6b**-$d_1$ + (2*R*,1'*S*)-**6b**-$d_1$

**Figure S1.** a) $^1$H NMR spectrum of *rac*-**6b**; b) $^1$H NMR spectrum of (*S*)-**6b**-$d_1$ obtained from (*E*)-**4b**; c) $^1$H NMR spectrum of the 79/21 mixture (determined by GC analysis on a chiral stationary phase) of (*S*)-**6b**-$d_1$ and (2*R*,1'*S*)-**6b**-$d_1$ obtained from (*Z*)-**4b**.

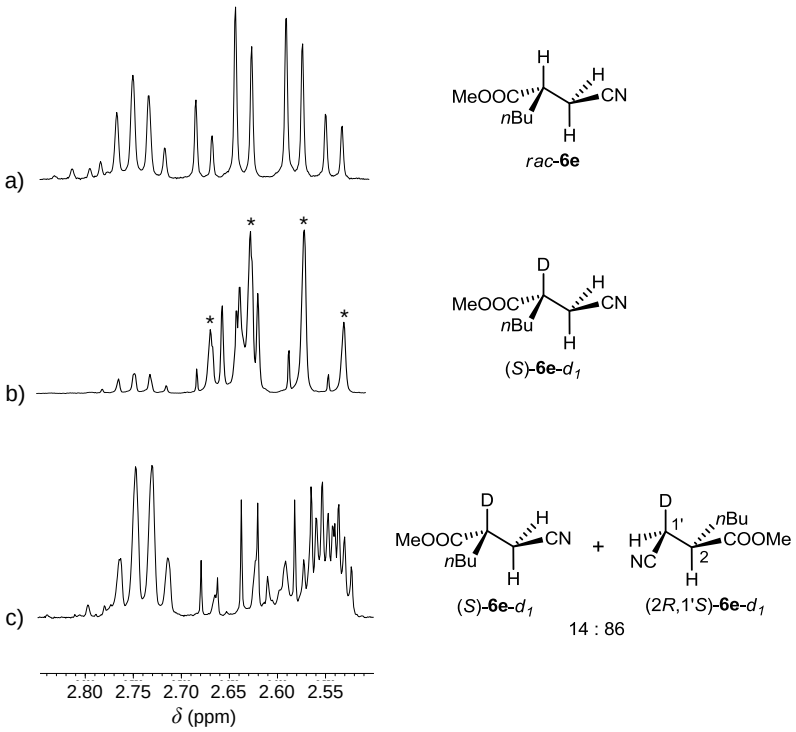



**Figure S2**: a) $^1$H NMR spectrum of *rac*-**6e**; b) and d) $^1$H NMR spectrum of (*S*)-**6e**-$d_1$ obtained from (*E*)-**4e**; c) and e) $^1$H NMR spectrum of the 14/86 mixture ( determined by GC analysis on a chiral stationary phase) of (*S*)-**6e**-$d_1$ and (2*R*,1'*S*)-**6e**-$d_1$ obtained from (*Z*)-**4e**.

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