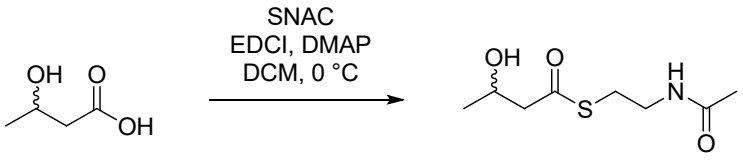
Synthetic procedures



(*R*)-**S1**
(*S*)-**S1**

(*R*)-**1** (53%)
(*S*)-**1** (57%)

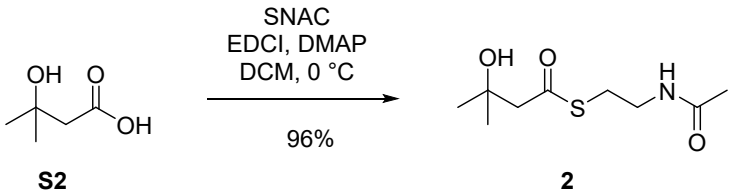
Scheme S1. Synthesis of both enantiomers of **1**.

Synthesis of S-(2-acetamidoethyl) (*R*)-3-hydroxybutanethioate ((*R*)-1).[1]

The alcohol (*R*)-**S1** (100 mg, 0.96 mmol) was dissolved in CH_2Cl_2 (10 mL). DMAP (23 mg, 0.19 mmol), EDC·HCl (203 mg, 1.06 mmol) and N-acetylcysteamine (114 mg, 0.96 mmol) were added to this solution. The mixture was stirred overnight at room temperature and then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel (EtOAc) to yield (*R*)-**1** as a colourless oil (105 mg, 0.51 mmol, 53%).[2] ^{1}H NMR (500 MHz, C_6D_6): δ_H 4.52 (br s, NH), 4.07 (dqt, $^3J_{H,H}$ = 9.8 Hz, $^3J_{H,H}$ = 6.4 Hz, $^3J_{H,H}$ = 3.5 Hz, 1H), 3.12 (m, 2H), 2.68 (m, 2H), 2.53 (br d, $^3J_{H,H}$ = 4.4 Hz, OH), 2.39 (dd, $^2J_{H,H}$ = 14.9 Hz, $^3J_{H,H}$ = 8.6 Hz, 1H), 2.26 (dd, $^2J_{H,H}$ = 14.9 Hz, $^3J_{H,H}$ = 3.5 Hz, 1H), 1.45 (s, 3H), 0.94 (d, $^3J_{H,H}$ = 6.3 Hz, 3H) ppm; ^{13}C NMR (126 MHz, C_6D_6): δ_C 198.5 (C_q), 169.3 (C_q), 65.1 (CH), 53.1 (CH_2), 39.0 (CH_2), 29.2 (CH_2), 22.9 (CH_3), 22.7 (CH_3) ppm. Optical rotation: [α]25 D = –28.3 (*c* 0.40, CH_2Cl_2), lit. [α]25 D = –23.2 (*c* 1.0, $CHCl_3$).[2]

Synthesis of S-(2-acetamidoethyl) (*S*)-3-hydroxybutanethioate ((*S*)-1).

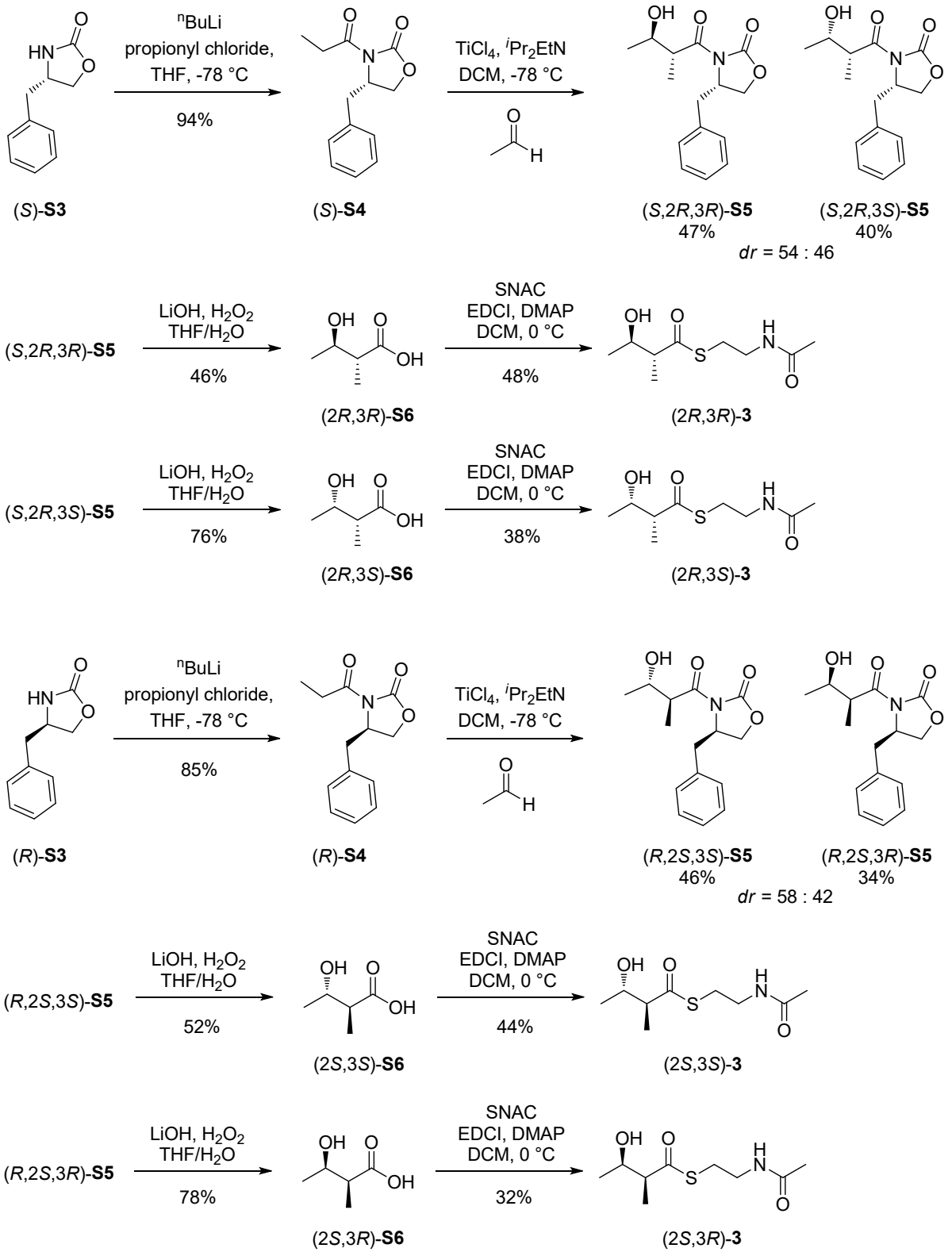
Following the same procedure as for (*R*)-**1**, (*S*)-**S1** (100 mg, 0.96 mmol) was converted into (*S*)-**1** that was obtained as a colourless oil (112 mg, 0.55 mmol, 57%).[2] Spectroscopic data were identical to those of (*R*)-**1**. Optical rotation: [α]25 D = +26.1 (*c* 0.50, CH_2Cl_2), lit. [α]25 D = +27.9 (*c* 1.0, $CHCl_3$).[2]



Scheme S2. Synthesis of **2**.

Synthesis of S-(2-acetamidoethyl) 3-hydroxy-3-methylbutanethioate (2).

Following the same procedure as for (*R*)-**1**, **S2** (50 mg, 0.42 mmol) was converted into **2** that was obtained as a colourless oil (89 mg, 0.41 mmol, 96%). ^{1}H NMR (500 MHz, C_6D_6): δ_H 4.69 (br s, NH), 3.13 (q, $^{3}J_{H,H}$ = 6.5 Hz, 2H), 3.09 (br s, OH), 2.69 (t, $^{3}J_{H,H}$ = 6.6 Hz, 2H), 2.42 (s, 2H), 1.48 (s, 3H)), 1.15 (s, 6H) ppm; ^{13}C NMR (126 MHz, C_6D_6): δ_C 199.3 (C_q), 169.2 (C_q), 69.8 (C_q), 56.1 (CH_2), 39.1 (CH_2), 29.4 (CH_3), 29.2 (CH_2), 22.8 (CH_3) ppm. HRMS (ESI): $[M+Na]^+$ calculated for $C_9H_{17}NO_3SNa^+$ *m*/*z* 242.0821; found *m*/*z* 242.0820.



Scheme S3. Synthesis of all four stereoisomers of **3**.

Synthesis of (*S*)-4-benzyl-3-propionyloxazolidin-2-one ((*S*)-S4).

To a solution of (*S*)-4-benzyl-2-oxazolidinone ((*S*)-**S3**, 6.50 g, 36.7 mmol) in THF (60 mL) was added ^{n}BuLi (23.4 mL, 1.6 M in hexane, 37.4 mmol) dropwise at –78 °C under

Ar. After 20 min, propionyl chloride (3.90 g, 42.2 mmol) was added dropwise. The reaction mixture was allowed to stir at –78 °C for 2.5 h. The mixture was poured into a saturated aqueous solution of NH_4Cl (60 mL). After removal of THF under reduced pressure, the aqueous layer was extracted with CH_2Cl_2 (3 x 60 mL). The combined organic layers were washed with 10% NaOH solution and dried with $MgSO_4$, filtered and concentrated to dryness. The residue was puridied through silica gel column chromatography (cyclohexane/EtOAc, 5:1) to afford (*S*)-4-benzyl-3-propionyloxazolidin-2-one ((*S*)-**S4**) as a white solid (8.00 g, 34.3 mmol, 94%). ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 7.28 (m, 5H), 4.68 (ddt, $^3J_{H,H}$ = 9.5 Hz, $^3J_{H,H}$ = 7.5 Hz, $^3J_{H,H}$ = 3.2 Hz, 1H), 4.18 (m, 2H), 3.31 (dd, $^2J_{H,H}$ = 13.3 Hz, $^3J_{H,H}$ = 3.3 Hz, 1H), 2.95 (m, 2H), 2.77 (dd, $^2J_{H,H}$ = 13.3 Hz, $^3J_{H,H}$ = 9.6 Hz, 1H), 1.21 (t, $^3J_{H,H}$ = 7.3 Hz, 3H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ_C 174.2 (C_q), 153.7 (C_q), 135.5 (C_q), 129.6 (2xCH), 129.1 (2xCH), 127.5 (CH), 66.4 (CH_2), 55.3 (CH), 38.1 (CH_2), 29.4 (CH_2), 8.4 (CH_3) ppm.

Synthesis of (*R*)-4-benzyl-3-propionyloxazolidin-2-one ((*R*)-S4).

Following the same procedure as for (*S*)-**S4**, (*R*)-4-benzyloxazolidin-2-one ((*R*)-**S3**, 2.00 g, 11.3 mmol) was converted into (*R*)-**S4** that was obtained as a white solid (2.25 g, 9.60 mmol, 85%). Spectroscopic data were identical to those of (*S*)-**S4**.

Synthesis of (*S*)-4-benzyl-3-((2*R*,3*R*)-3-hydroxy-2-methylbutanoyl)oxazolidin-2-one ((*S*,2*R*,3*R*)-S5) and (*S*)-4-benzyl-3-((2*R*,3*S*)-3-hydroxy-2-methylbutanoyl)-oxazolidin-2-one ((*S*,2*R*,3*S*)-S5).[3]

(*S*)-3-Acetyl-4-benzyloxazolidin-2-one ((*S*)-**S4**) (2.10 g, 9.00 mmol) was dissolved in CH_2Cl_2 (60 mL, –78 °C) under Ar. $TiCl_4$ (3.46 g, 18.2 mmol) was added dropwise, followed by the addition of diisopropylethylamine (2.35 g, 18.2 mmol). The resulting mixture was allowed to stir at –78 °C for 1 h, and then a solution of crotonaldehyde (1.28 g, 18.2 mmol) was added dropwise. Stirring was continued at –78 °C for 5 h, and then the reaction mixture was allowed to warm to room temperature with continued stirring overnight. The mixture was poured onto a saturated aqueous solution of NH_4Cl (50 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 100 mL). The combined organic layers were washed with brine, dried with $MgSO_4$, filtered and concentrated to dryness. Purification of the crude product by column chromatography on silica gel (cyclohexane/EtOAc, 3:1) gave (*S*,2*R*,3*R*)-**S5** (1.18 g, 4.24 mmol, 47 %) and (*S*,2*R*,3*S*)-**S5** (1.01 g, 3.60 mmol, 40 %) as white solids.[4]

(*S*)-4-Benzyl-3-((2*R*,3*R*)-3-hydroxy-2-methylbutanoyl)oxazolidin-2-one ((*S*,2*R*,3*R*)-S5). ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 7.28 (m, 5H), 4.69 (dddd, $^{3}J_{H,H}$ = 9.5 Hz, $^{3}J_{H,H}$ = 7.3 Hz, $^{3}J_{H,H}$ = 3.5 Hz, $^{3}J_{H,H}$ = 2.9 Hz, 1H), 4.18 (m, 2H), 3.95 (m, 1H), 3.82 (qd, $^{3}J_{H,H}$ = 7.0 Hz, $^{3}J_{H,H}$ = 7.0 Hz, 1H), 3.33 (dd, $^{2}J_{H,H}$ = 13.5 Hz, $^{3}J_{H,H}$ = 3.5 Hz, 1H), 2.79 (dd, $^{2}J_{H,H}$ = 13.5 Hz, $^{3}J_{H,H}$ = 9.5 Hz, 1H), 1.31 (d, $^{3}J_{H,H}$ = 6.3 Hz, 3H), 1.20 (d, $^{3}J_{H,H}$ = 6.9 Hz, 3H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ_C 176.8 (C_q), 153.7 (C_q), 135.4 (C_q), 129.6 (2xCH), 129.1 (2xCH), 127.5 (CH), 70.9 (CH), 66.2 (CH_2), 55.7 (CH), 45.2 (CH), 38.0 (CH_2), 21.4 (CH_3), 14.7 (CH_3) ppm. Optical rotation: [*α*]25 D = +32.2 (*c* 0.37, CH_2Cl_2).[5]

(*S*)-4-Benzyl-3-((2*R*,3*S*)-3-hydroxy-2-methylbutanoyl)oxazolidin-2-one ((*S*,2*R*,3*S*)-S5). ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 7.28 (m, 5H), 4.70 (m, 1H), 4.20 (m, 3H), 3.85 (qd, $^{3}J_{H,H}$ = 7.0 Hz, $^{3}J_{H,H}$ = 3.2 Hz, 1H), 3.31 (dd, $^{2}J_{H,H}$ = 13.4 Hz, $^{3}J_{H,H}$ = 3.5 Hz, 1H), 2.78 (dd, $^{2}J_{H,H}$ = 13.4 Hz, $^{3}J_{H,H}$ = 9.6 Hz, 1H), 1.24 (d, $^{3}J_{H,H}$ = 6.4 Hz, 3H), 1.21 (d, $^{3}J_{H,H}$ = 7.0 Hz, 3H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ_C 176.9 (C_q), 153.6 (C_q), 135.3 (C_q), 129.6 (2xCH), 129.1 (2xCH), 127.6 (CH), 68.3 (CH), 66.3 (CH_2), 55.6 (CH), 43.1 (CH), 38.2 (CH_2), 19.6 (CH_3), 10.7 (CH_3) ppm. Optical rotation: [*α*]25 D = +61.5 (*c* 0.34, CH_2Cl_2), lit. [*α*]30 D = +42.0 (*c* 0.50, $CHCl_3$).[4]

Synthesis of (*R*)-4-benzyl-3-((2*S*,3*S*)-3-hydroxy-2-methylbutanoyl)oxazolidin-2-one ((*R*,2*S*,3*S*)-S5) and (*R*)-4-benzyl-3-((2*S*,3*R*)-3-hydroxy-2-methylbutanoyl)-oxazolidin-2-one ((*R*,2*S*,3*R*)-S5).[4]

Following the same procedure as for (*S*,2*R*,3*R*)-**S5** and (*S*,2*R*,3*S*)-**S5**, (*R*)-**S4** (2.10 g, 9.00 mmol) was converted into (*R*,2*S*,3*S*)-**S5** (1.14 g, 4.11 mmol, 46%) and (*R*,2*S*,3*R*)-**S5** (0.85 g, 3.08 mmol, 34%) that were obtained as white solids.

(*R*)-4-Benzyl-3-((2*S*,3*S*)-3-hydroxy-2-methylbutanoyl)oxazolidin-2-one ((*R*,2*S*,3*S*)-S5). Spectroscopic data were identical to those of (*S*,2*R*,3*R*)-**S5**. Optical rotation: [*α*]25 D = –31.5 (*c* 0.20, CH_2Cl_2), lit. [*α*]30 D = –19.8 (*c* 0.50, $CHCl_3$).[4]

(*R*)-4-Benzyl-3-((2*S*,3*R*)-3-hydroxy-2-methylbutanoyl)oxazolidin-2-one ((*R*,2*S*,3*R*)-S5). Spectroscopic data were identical to those of (*S*,2*R*,3*S*)-**S5**. Optical rotation: [*α*]25 D = –55.0 (*c* 0.20, CH_2Cl_2).

Synthesis of (2*R*,3*R*)-3-hydroxy-2-methylbutanoic acid ((2*R*,3*R*)-S6).[4]

(*S*)-4-Benzyl-3-((2*R*,3*R*)-3-hydroxy-2-methylbutanoyl)oxazolidin-2-one ((*S*,2*R*,3*R*)-**S5**, 86 mg, 0.31 mmol) was dissolved in THF (2 mL) and H_2O (0.5 mL). The solution was

cooled to 0 °C and a solution of H_2O_2 (35%, 0.2 mL, 18.2 mmol) was added dropwise. LiOH (36 mg, 1.50 mmol) was added and the resulting mixture was allowed to stir for 2 h and then quenched by the addition of an aqueous solution of Na_2SO_3 (270 mg in 1.6 mL H_2O). After removal of THF under reduced pressure the aqueous phase was extracted with CH_2Cl_2 (3 x 10 mL), then acidified by the addition of 2 N HCl solution to pH 1, and extracted with Et_2O (3 x 10 mL). The combined organic layers were dried with $MgSO_4$, filtered and concentrated to yield (2*R*,3*R*)-3-hydroxy-2-methylbutanoic acid ((2*R*,3*R*)-**S6**) as a colourless oil (17 mg, 0.14 mmol, 46 %). ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 3.92 (dq, $^3J_{H,H}$ = 7.2 Hz, $^3J_{H,H}$ = 6.4 Hz, 1H), 2.49 (qd, $^3J_{H,H}$ = 7.2 Hz, $^3J_{H,H}$ = 7.2 Hz, 1H), 1.26 (d, $^3J_{H,H}$ = 6.4 Hz, 3H), 1.22 (d, $^3J_{H,H}$ = 7.2 Hz, 3H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ_C 180.7 (C_q), 69.6 (CH), 47.0 (CH), 20.9 (CH_3), 14.1 (CH_3) ppm. Optical rotation: [*α*]25 D = –23.2 (*c* 0.20, CH_2Cl_2), lit. [*α*]24 D = –29.0 (*c* 1.00, $CHCl_3$).[6]

Synthesis of (2*R*,3*S*)-3-hydroxy-2-methylbutanoic acid ((2*R*,3*S*)-S6).

Following the same procedure as for (2*R*,3*R*)-**S6**, (*S*,2*R*,3*S*)-**S5** (86 mg, 0.31 mmol) was converted into (2*R*,3*S*)-**S6** that was obtained as a colourless oil (28 mg, 0.24 mmol, 76%). ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 4.14 (qd, $^3J_{H,H}$ = 6.5 Hz, $^3J_{H,H}$ = 3.8 Hz, 1H), 2.59 (qd, $^3J_{H,H}$ = 7.2 Hz, $^3J_{H,H}$ = 3.8 Hz, 1H), 1.23 (d, $^3J_{H,H}$ = 6.5 Hz, 3H), 1.22 (d, $^3J_{H,H}$ = 7.2 Hz, 3H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ_C 180.6 (C_q), 68.1 (CH), 45.3 (CH), 19.8 (CH_3), 11.0 (CH_3) ppm. Optical rotation: [*α*]25 D = +4.9 (*c* 0.20, CH_2Cl_2), lit. [*α*]30 D = +6.9 (*c* 1.02, $CHCl_3$).[4]

Synthesis of (2*S*,3*S*)-3-hydroxy-2-methylbutanoic acid ((2*S*,3*S*)-S6).

Following the same procedure as for (2*R*,3*R*)-**S6**, (*R*,2*S*,3*S*)-**S5** (90 mg, 0.32 mmol) was converted into (2*S*,3*S*)-**S6** that was obtained as a colourless oil (20 mg, 0.17 mmol, 52%). Spectroscopic data were identical to those of (2*R*,3*R*)-**S6**. Optical rotation: [*α*]25 D = +16.9 (*c* 0.40, CH_2Cl_2).[7]

Synthesis of (2*S*,3*R*)-3-hydroxy-2-methylbutanoic acid ((2*S*,3*R*)-S6).

Following the same procedure as for (2*R*,3*R*)-**S6**, (2*S*,3*R*)-**S5** (120 mg, 0.43 mmol) was converted into (2*S*,3*R*)-**S6** that was obtained as a colourless oil (40 mg, 0.34 mmol, 78%). Spectroscopic data were identical to those of (2*R*,3*S*)-**S6**. Optical rotation: [*α*]25 D = –3.8 (*c* 0.5, CH_2Cl_2), lit. [*α*]30 D = –6.8 (*c* 1.02, $CHCl_3$).[4]

Synthesis of S-(2-acetamidoethyl) (2*R*,3*R*)-3-hydroxy-2-methylbutanethioate ((2*R*,3*R*)-3).

Following the same procedure as for (*R*)-**1**, (2*R*,3*R*)-**S6** (17 mg, 0.14 mmol) was converted into (2*R*,3*R*)-**3** that was obtained as a colourless oil (15 mg, 0.07 mmol, 48%). ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 5.88 (br s, NH), 3.94 (dq, $^{3}J_{H,H}$ = 7.0 Hz, $^{3}J_{H,H}$ = 6.4 Hz, 1H), 3.47 (m, 2H), 3.06 (m, 2H), 2.69 (m, 1H), 1.97 (s, 3H), 1.24 (d, $^{3}J_{H,H}$ = 6.4 Hz, 3H), 1.20 (d, $^{3}J_{H,H}$ = 7.1 Hz, 3H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ_C 204.1 (C_q), 170.7 (C_q), 70.2 (CH), 56.0 (CH), 39.6 (CH_2), 28.8 (CH_2), 23.3 (CH_3), 21.3 (CH_3), 15.1 (CH_3) ppm. Optical rotation: [*α*]25 D = –18.7 (*c* 0.10, CH_2Cl_2), lit. [*α*] D = –32.8 (*c* 0.33, $CHCl_3$).[2]

Synthesis of S-(2-acetamidoethyl) (2*R*,3*S*)-3-hydroxy-2-methylbutanethioate ((2*R*,3*S*)-3).

Following the same procedure as for (*R*)-**1**, (2*R*,3*S*)-**S6** (28 mg, 0.24 mmol) was converted into (2*R*,3*S*)-**3** that was obtained as a colourless oil (20 mg, 0.09 mmol, 38%). ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 5.88 (br s, NH), 4.11 (qd, $^{3}J_{H,H}$ = 6.4 Hz, $^{3}J_{H,H}$ = 4.0 Hz, 1H), 3.46 (m, 2H), 3.03 (m, 2H), 2.70 (qd, $^{3}J_{H,H}$ = 7.1 Hz, $^{3}J_{H,H}$ = 4.0 Hz, 1H), 1.98 (s, 3H), 1.23 (d, $^{3}J_{H,H}$ = 7.1 Hz, 3H), 1.20 (d, $^{3}J_{H,H}$ = 6.4 Hz, 3H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ_C 204.2 (C_q), 170.7 (C_q), 68.5 (CH), 54.6 (CH), 39.6 (CH_2), 28.7 (CH_2), 23.3 (CH_3), 20.3 (CH_3), 11.6 (CH_3) ppm. Optical rotation: [*α*]25 D = –3.7 (*c* 0.07, $CHCl_3$), lit. [*α*]30 D = –4.9 (*c* 1.0, $CHCl_3$).[2]

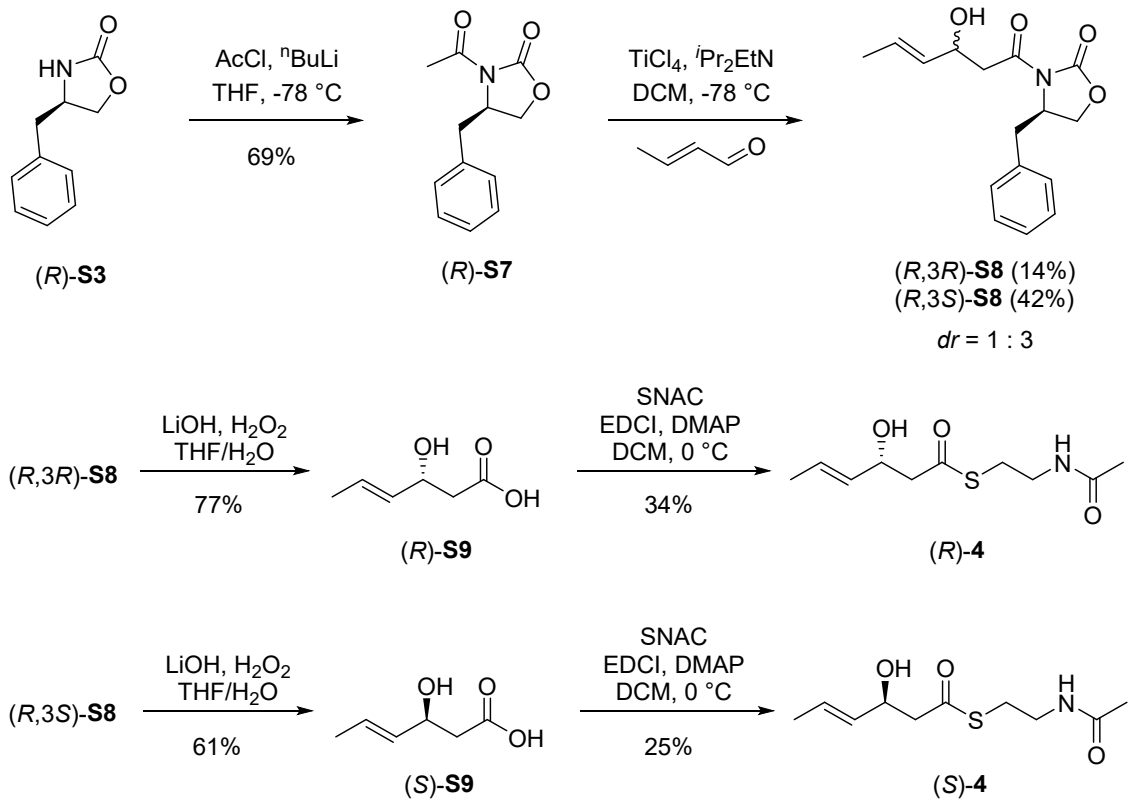
Synthesis of S-(2-acetamidoethyl) (2*S*,3*S*)-3-hydroxy-2-methylbutanethioate ((2*S*,3*S*)-3).

Following the same procedure as for (*R*)-**1**, (2*S*,3*S*)-**S6** (17 mg, 0.14 mmol) was converted into (2*S*,3*S*)-**3** that was obtained as a colourless oil (14 mg, 0.06 mmol, 44%). Spectroscopic data were identical to those of (2*R*,3*R*)-**3**. Optical rotation: [*α*]25 D = +17.3 (*c* 0.20, $CHCl_3$), lit. [*α*] D = +36.8 (*c* 0.64, $CHCl_3$).[2]

Synthesis of S-(2-acetamidoethyl) (2*S*,3*R*)-3-hydroxy-2-methylbutanethioate ((2*S*,3*R*)-3).

Following the same procedure as for (*R*)-**1**, (2*S*,3*R*)-**S6** (40 mg, 0.34 mmol) was converted into (2*S*,3*R*)-**3** that was obtained as a colourless oil (24 mg, 0.11 mmol, 32%). Spectroscopic data were identical to those of (2*R*,3*S*)-**3**. Optical rotation: [*α*]25

D = +2.7 (*c* 0.10, $CHCl_3$), lit. [*α*]30 D = +3.0 (*c* 1.0, $CHCl_3$).[2]



Scheme S4. Preparation of both enantiomers of **4**.

Synthesis of (*R*)-3-acetyl-4-benzyloxazolidin-2-one ((*R*)-S7).
Following the same procedure as for (*S*)-**S4**, (*R*)-**S3** (3.30 g, 18.5 mmol) was converted into (*R*)-**S7** that was obtained as a white solid (2.80 g, 12.8 mmol, 69%). ^{1}H NMR (500 MHz, C_6D_6): δ_H 7.03 (m, 3H), 6.84 (m, 2H), 4.08 (ddt, $^{3}J_{H,H}$ = 9.5 Hz, $^{3}J_{H,H}$ = 8.0 Hz, $^{3}J_{H,H}$ = 3.0 Hz, 1H), 3.45 (dd, $^{2}J_{H,H}$ = 8.9 Hz, $^{3}J_{H,H}$ = 2.8 Hz, 1H), 3.17 (dd, $^{2}J_{H,H}$ = 8.8 Hz, $^{3}J_{H,H}$ = 8.0 Hz, 1H), 2.95 (dd, $^{2}J_{H,H}$ = 13.3 Hz, $^{3}J_{H,H}$ = 3.3 Hz, 1H), 2.56 (s, 3H), 2.26 (dd, $^{2}J_{H,H}$ = 13.4 Hz, $^{3}J_{H,H}$ = 9.5 Hz, 1H) ppm; ^{13}C NMR (126 MHz, C_6D_6): δ_C 169.6 (C_q), 153.5 (C_q), 136.0 (C_q), 129.6 (2xCH), 129.0 (2xCH), 127.3 (CH), 65.6 (CH_2), 54.9 (CH_3), 37.7 (CH_2), 23.6 (CH_3) ppm. HRMS (ESI): $[M+H]^+$ calculated for $C_{12}H_{13}NO_3H^+$ *m*/*z* 220.0968; found *m*/*z* 220.0971. Optical rotation: [*α*]25 D = –77.2 (*c* 0.30, CH_2Cl_2).

Synthesis of (*R*)-4-benzyl-3-((*R*,*E*)-3-hydroxyhex-4-enoyl)oxazolidin-2-one ((*R*,3*R*)-S8) and (*R*)-4-benzyl-3-((*S*,*E*)-3-hydroxyhex-4-enoyl)oxazolidin-2-one ((*R*,3*S*)-S8). Following the same procedure as for (2*R*,3*R*)-**S5**, (*R*)-**S7** (2.50 g, 11.4 mmol) was converted into the minor diastereomer (*R*)-4-benzyl-3-((*R*,*E*)-3-hydroxyhex-4-enoyl)oxazolidin-2-one ((*R*,3*R*)-**S8**, 450 mg, 1.56 mmol, 14%) and the major diastereomer (*R*)-4-benzyl-3-((*S*,*E*)-3-hydroxyhex-4-enoyl)oxazolidin-2-one

((*R*,3*S*)-**S8**) (1.40 g, 4.84 mmol, 42%), *dr* = 1 : 3, that were separable by column chromatography on silica gel (petroleum ether/EtOAc, 3:1). The compounds were obtained as white solids.

(*R*)-4-Benzyl-3-((*R*,*E*)-3-hydroxyhex-4-enoyl)oxazolidin-2-one ((*R*,3*R*)-S8). ^{1}H NMR (500 MHz, C_6D_6): δ_H 7.04 (m, 3H), 6.85 (m, 2H), 5.72 (dqd, $^{3}J_{H,H}$ = 15.3 Hz, $^{3}J_{H,H}$ = 6.5 Hz, $^{4}J_{H,H}$ = 1.3 Hz, 1H), 5.55 (ddq, $^{3}J_{H,H}$ = 15.3 Hz, $^{3}J_{H,H}$ = 5.9 Hz, $^{4}J_{H,H}$ = 1.7 Hz, 1H), 4.68 (m, 1H), 4.07 (m, 1H), 3.41 (dd, $^{2}J_{H,H}$ = 9.0 Hz, $^{3}J_{H,H}$ = 2.8 Hz, 1H), 3.34 (dd, $^{2}J_{H,H}$ = 16.8 Hz, $^{3}J_{H,H}$ = 8.9 Hz, 1H), 3.13 (dd, $^{2}J_{H,H}$ = 8.9 Hz, $^{3}J_{H,H}$ = 8.0 Hz, 1H), 3.07 (dd, $^{2}J_{H,H}$ = 16.8 Hz, $^{3}J_{H,H}$ = 3.5 Hz, 1H), 2.87 (dd, $^{2}J_{H,H}$ = 13.5 Hz, $^{3}J_{H,H}$ = 3.4 Hz, 1H), 2.83 (m, OH), 2.23 (dd, $^{2}J_{H,H}$ = 13.4 Hz, $^{3}J_{H,H}$ = 9.4 Hz, 1H), 1.53 (ddd, $^{3}J_{H,H}$ = 6.5 Hz, $^{4}J_{H,H}$ = 1.7 Hz, $^{5}J_{H,H}$ = 1.1 Hz, 3H) ppm; ^{13}C NMR (125 MHz, C_6D_6): δ_C 172.1 (C_q), 153.3 (C_q), 135.8 (C_q), 133.2 (CH), 129.7 (2xCH), 129.0 (2xCH), 127.4 (CH), 126.3 (CH), 69.0 (CH), 65.7 (CH_2), 54.9 (CH), 43.3 (CH_2), 37.6 (CH_2), 17.7 (CH_3) ppm. HRMS (ESI): $[M+Na]^+$ calculated for $C_{16}H_{19}NO_4Na^+$ *m*/*z* 312.1206; found *m*/*z* 312.1206. Optical rotation: [*α*]25 D = –51.7 (*c* 0.30, CH_2Cl_2), lit. [*α*]25 D = –35.0 (*c* 1.35, $CHCl_3$).[8]

(*R*)-4-Benzyl-3-((*S*,*E*)-3-hydroxyhex-4-enoyl)oxazolidin-2-one ((*R*,3*S*)-S8). ^{1}H NMR (500 MHz, C_6D_6): δ_H 7.04 (m, 3H), 6.85 (m, 2H), 5.71 (dqd, $^{3}J_{H,H}$ = 15.4 Hz, $^{3}J_{H,H}$ = 6.5 Hz, $^{4}J_{H,H}$ = 1.3 Hz, 1H), 5.54 (ddq, $^{3}J_{H,H}$ = 15.3 Hz, $^{3}J_{H,H}$ = 6.0 Hz, $^{4}J_{H,H}$ = 1.6 Hz, 1H), 4.70 (m, 1H), 4.05 (m, 1H), 3.40 (dd, $^{2}J_{H,H}$ = 8.9 Hz, $^{3}J_{H,H}$ = 2.8 Hz, 1H), 3.23 (d, $^{3}J_{H,H}$ = 2.7 Hz, 1H), 3.22 (d, $^{3}J_{H,H}$ = 0.6 Hz, 1H), 3.10 (dd, $^{2}J_{H,H}$ = 8.8 Hz, $^{3}J_{H,H}$ = 8.0 Hz, 1H), 2.92 (dd, $^{2}J_{H,H}$ = 13.4 Hz, $^{3}J_{H,H}$ = 3.3 Hz, 1H), 2.73 (br s, OH), 2.26 (dd, $^{2}J_{H,H}$ = 13.4 Hz, $^{3}J_{H,H}$ = 9.5 Hz, 1H), 1.53 (ddd, $^{3}J_{H,H}$ = 6.5 Hz, $^{4}J_{H,H}$ = 1.7 Hz, $^{5}J_{H,H}$ = 1.1 Hz, 3H) ppm; ^{13}C NMR (126 MHz, C_6D_6): δ_C 172.0 (C_q), 153.3 (C_q), 135.8 (C_q), 133.1 (CH), 129.6 (2xCH), 129.0 (2xCH), 127.4 (CH), 126.4 (CH), 68.9 (CH), 65.7 (CH_2), 54.9 (CH), 43.4 (CH_2), 37.8 (CH_2), 17.7 (CH_3) ppm. HRMS (ESI): $[M+Na]^+$ calculated for $C_{16}H_{19}NO_4Na^+$ *m*/*z* 312.1206; found *m*/*z* 312.1209. Optical rotation: [*α*]25 D = –93.0 (*c* 0.30, CH_2Cl_2), lit. [*α*] D = –76.3 (*c* 0.085, $CHCl_3$).[8]

Synthesis of (*R*,*E*)-3-hydroxyhex-4-enoic acid ((*R*)-S9).

Following the same procedure as for (2*R*,3*R*)-**S6**, (*R*,3*R*)-**S8** (145 mg, 0.50 mmol) was converted into (*R*,*E*)-3-hydroxyhex-4-enoic acid ((*R*)-**S9**) that was obtained as a colourless oil (50 mg, 0.38 mmol, 77%). ^{1}H NMR (500 MHz, C_6D_6): δ_H 5.46 (dqd, $^{3}J_{H,H}$ = 15.4 Hz, $^{3}J_{H,H}$ = 6.5 Hz, $^{4}J_{H,H}$ = 1.2 Hz, 1H), 5.26 (ddq, $^{3}J_{H,H}$ = 15.3 Hz, $^{3}J_{H,H}$ = 6.3 Hz, $^{4}J_{H,H}$ = 1.7 Hz, 1H), 4.29 (m, 1H), 2.32 (dd, $^{2}J_{H,H}$ = 16.0 Hz, $^{3}J_{H,H}$ = 8.5 Hz, 1H),

2.23 (dd, $^{2}J_{H,H}$ = 16.0 Hz, $^{3}J_{H,H}$ = 4.1 Hz, 1H), 1.43 (ddd, $^{3}J_{H,H}$ = 6.5 Hz, $^{4}J_{H,H}$ = 1.4 Hz, $^{5}J_{H,H}$ = 1.4 Hz, 3H) ppm; ^{13}C NMR (125 MHz, C_6D_6): δ_C 177.1 (C_q), 132.5 (CH), 126.8 (CH), 68.9 (CH), 41.7 (CH_2), 17.6 (CH_3) ppm. Optical rotation: [α]25 D = +12.5 (*c* 0.40, CH_2Cl_2), lit. [α]25 D = +22.5 (*c* 0.4, EtOH).[9]

Synthesis of (*S*,*E*)-3-hydroxyhex-4-enoic acid ((*S*)-S9).

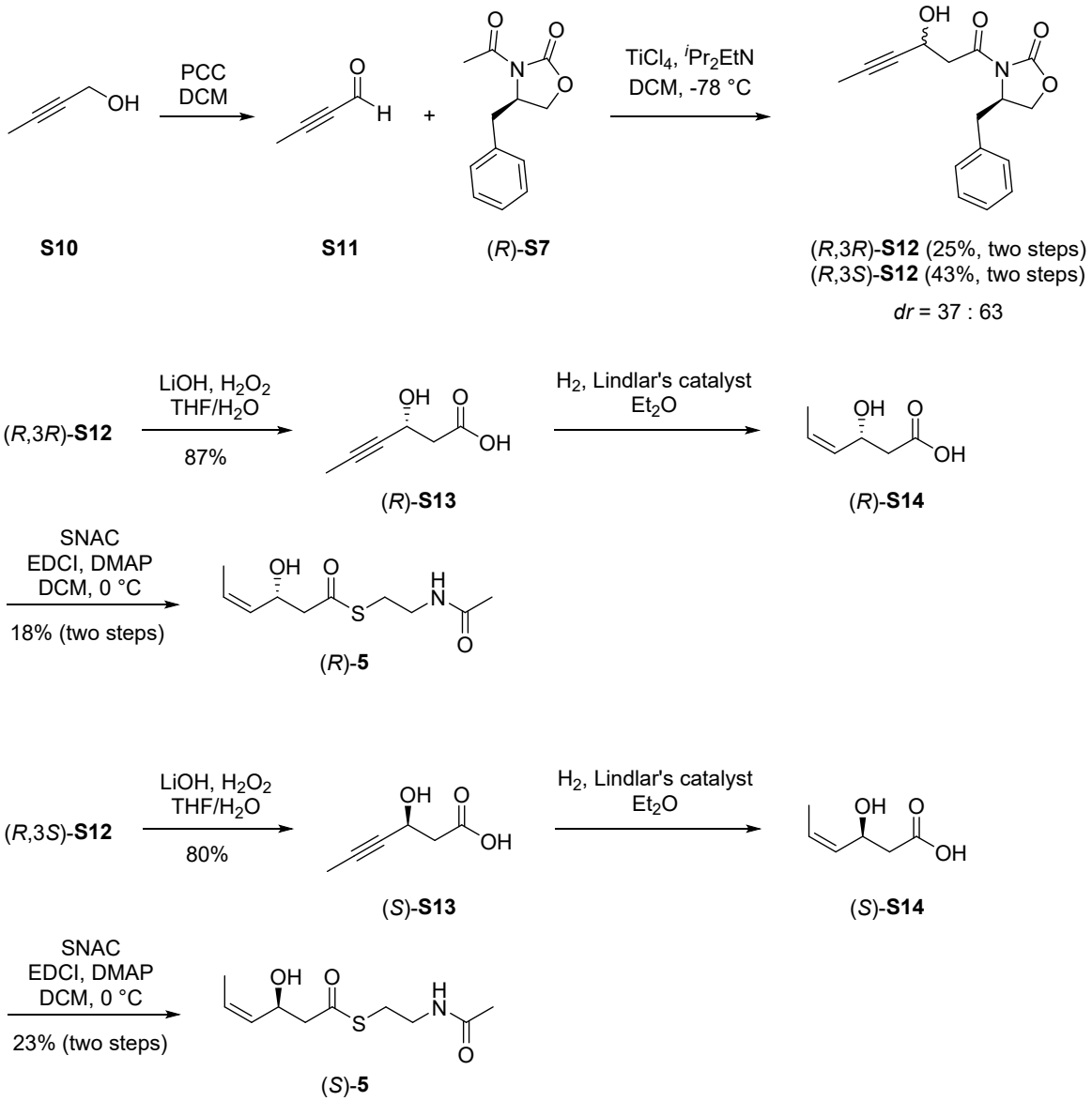
Following the same procedure as for (2*R*,3*R*)-**S6**, (*R*,3*S*)-**S8** (145 mg, 0.50 mmol) was converted into (*S*,*E*)-3-hydroxyhex-4-enoic acid ((*S*)-**S9**) that was obtained as a colourless oil (40 mg, 0.31 mmol, 61%). Spectroscopic data were identical to those of (*R*)-**S9**. Optical rotation: [α]25 D = –15.5 (*c* 0.30, CH_2Cl_2), lit. [α]25 D = –22.2 (*c* 0.4, EtOH).[9]

Synthesis of S-(2-acetamidoethyl) (*R*,*E*)-3-hydroxyhex-4-enethioate ((*R*)-4).

Following the same procedure as for (*R*)-**1**, (*R*)-**S9** (20 mg, 0.15 mmol) was converted into (*R*)-**4** that was obtained as a colourless oil (12 mg, 0.05 mmol, 34%). ^{1}H NMR (500 MHz, C_6D_6): δ_H 5.53 (dqd, $^{3}J_{H,H}$ = 15.3 Hz, $^{3}J_{H,H}$ = 6.5 Hz, $^{4}J_{H,H}$ = 1.3 Hz, 1H), 5.33 (ddq, $^{3}J_{H,H}$ = 15.3 Hz, $^{3}J_{H,H}$ = 6.1 Hz, $^{4}J_{H,H}$ = 1.6 Hz, 1H), 4.91 (br s, NH), 4.52 (m, 1H), 3.17 (m, 2H), 2.74 (m, 2H), 2.61 (dd, $^{2}J_{H,H}$ = 14.8 Hz, $^{3}J_{H,H}$ = 8.7 Hz, 1H), 2.47 (dd, $^{2}J_{H,H}$ = 14.7 Hz, $^{3}J_{H,H}$ = 3.9 Hz, 1H), 1.52 (s, 3H), 1.47 (ddd, $^{3}J_{H,H}$ = 6.5 Hz, $^{4}J_{H,H}$ = 1.7 Hz, $^{5}J_{H,H}$ = 1.1 Hz, 3H) ppm; ^{13}C NMR (126 MHz, C_6D_6): δ_C 197.8 (C_q), 169.6 (C_q), 132.9 (CH), 126.4 (CH), 69.6 (CH), 51.8 (CH_2), 39.2 (CH_2), 29.1 (CH_2), 22.7 (CH_3), 17.6 (CH_3) ppm. Optical rotation: [α]25 D = +19.0 (*c* 0.10, CH_2Cl_2), lit. [α]25 D = +16.7 (*c* 0.42, CH_2Cl_2).[10]

Synthesis of S-(2-acetamidoethyl) (*S*,*E*)-3-hydroxyhex-4-enethioate ((*S*)-4).

Following the same procedure as for (*R*)-**1**, (*S*)-**S9** (20 mg, 0.15 mmol) was converted into (*S*,*E*)-3-hydroxyhex-4-enoic acid ((*S*)-**4**) that was obtained as a colourless oil (9 mg, 0.04 mmol, 25%). Spectroscopic data were identical to those of (*R*)-**4**. Optical rotation: [α]25 D = –18.0 (*c* 0.15, CH_2Cl_2), lit. [α]25 D = –18.6 (*c* 0.44, CH_2Cl_2).[10]



Scheme S5. Synthesis of both enantiomers of **5**.

Synthesis of (*R*)-4-benzyl-3-((*R*)-3-hydroxyhex-4-ynoyl)oxazolidin-2-one ((*R*,3*R*)-S12) and (*R*)-4-benzyl-3-((*S*)-3-hydroxyhex-4-ynoyl)oxazolidin-2-one ((*R*,3*S*)-S12).

A mixture of **S10** (1.10 g, 15.7 mmol), silica gel and PCC (5.07 g, 23.5 mmol) in CH_2Cl_2 (60 mL) was stirred for 3 h with ice cooling. At the end of the reaction the solids were removed by filtration through a pad of silica gel. The solvents were evaporated to obtain the crude product **S11** that was used for the next step without purification. Following the same procedure as for (2*R*,3*R*)-**S5**, (*R*)-**S7** (300 mg, 1.37 mmol) was converted into the minor diastereomer (*R*,3*R*)-**S12** (99 mg, 0.34 mmol, 25%) and the major diastereomer (*R*,3*S*)-**S12** (169 mg, 0.59 mmol, 43%), *dr* = 37 : 63, that were separable by column chromatography on silica gel (petroleum ether/EtOAc, 3:1). The compounds

were obtained as colourless solids.

(*R*)-4-Benzyl-3-((*R*)-3-hydroxyhex-4-ynoyl)oxazolidin-2-one ((*R*,3*R*)-S12). ^{1}H NMR (500 MHz, C_6D_6): δ_H 7.02 (m, 3H), 6.83 (m, 2H), 4.95 (m, 1H), 3.99 (m, 1H), 3.67 (ddd, $^{2}J_{H,H}$ = 16.9 Hz, $^{3}J_{H,H}$ = 7.7 Hz, $^{4}J_{H,H}$ = 1.8 Hz, 1H), 3.37 (m, 1H), 3.21 (ddd, $^{2}J_{H,H}$ = 17.1 Hz, $^{3}J_{H,H}$ = 4.3 Hz, $^{4}J_{H,H}$ = 1.8 Hz, 1H), 3.07 (ddt, $^{2}J_{H,H}$ = 11.1 Hz, $^{3}J_{H,H}$ = 8.5 Hz, $^{4}J_{H,H}$ = 2.2 Hz, 1H), 2.88 (dt, $^{3}J_{H,H}$ = 6.7 Hz, $^{4}J_{H,H}$ = 1.9 Hz, OH), 2.83 (dd, $^{2}J_{H,H}$ = 13.4 Hz, $^{3}J_{H,H}$ = 3.6 Hz, 1H), 2.22 (ddt, $^{2}J_{H,H}$ = 13.9 Hz, $^{3}J_{H,H}$ = 9.3 Hz, $^{4}J_{H,H}$ = 2.3 Hz, 1H), 1.46 (m, 3H) ppm; ^{13}C NMR (126 MHz, C_6D_6): δ_C 171.1 (C_q), 153.1 (C_q), 135.7 (C_q), 129.6 (2xCH), 129.0 (2xCH), 127.4 (CH), 81.1 (C_q), 80.1 (C_q), 65.7 (CH_2), 59.4 (CH), 54.8 (CH), 44.0 (CH_2), 37.5 (CH_2), 3.3 (CH_3) ppm. HRMS (ESI): $[M+H]^+$ calculated for $C_{16}H_{17}NO_4H^+$ *m*/*z* 288.1230; found *m*/*z* 288.1229. Optical rotation: [*α*]25 D = –51.3 (*c* 0.55, CH_2Cl_2).

(*R*)-4-Benzyl-3-((*S*)-3-hydroxyhex-4-ynoyl)oxazolidin-2-one ((*R*,3*S*)-S12). ^{1}H NMR (500 MHz, C_6D_6): δ_H 7.02 (m, 3H), 6.83 (m, 2H), 4.98 (m, 1H), 4.01 (ddt, $^{3}J_{H,H}$ = 9.3 Hz, $^{3}J_{H,H}$ = 7.9 Hz, $^{3}J_{H,H}$ = 3.1 Hz, 1H), 3.59 (dd, $^{2}J_{H,H}$ = 17.3 Hz, $^{3}J_{H,H}$ = 7.8 Hz, 1H), 3.37 (dd, $^{2}J_{H,H}$ = 9.0 Hz, $^{3}J_{H,H}$ = 2.8 Hz, 1H), 3.34 (dd, $^{2}J_{H,H}$ = 17.3 Hz, $^{3}J_{H,H}$ = 4.0 Hz, 1H), 3.05 (ddt, $^{2}J_{H,H}$ = 8.8 Hz, $^{3}J_{H,H}$ = 8.0 Hz, $^{4}J_{H,H}$ = 0.8 Hz, 1H), 2.92 (d, $^{3}J_{H,H}$ = 6.5 Hz, OH), 2.86 (dd, $^{2}J_{H,H}$ = 13.4 Hz, $^{3}J_{H,H}$ = 3.3 Hz, 1H), 2.21 (dd, $^{2}J_{H,H}$ = 13.4 Hz, $^{3}J_{H,H}$ = 9.5 Hz, 1H), 1.47 (d, $^{5}J_{H,H}$ = 2.2 Hz, 3H) ppm; ^{13}C NMR (126 MHz, C_6D_6): δ_C 171.1 (C_q), 153.1 (C_q), 135.8 (C_q), 129.6 (2xCH), 129.0 (2xCH), 127.4 (CH), 81.1 (C_q), 80.1 (C_q), 65.7 (CH_2), 59.2 (CH), 54.8 (CH), 44.1 (CH_2), 37.6 (CH_2), 3.3 (CH_3) ppm. HRMS (ESI): $[M+H]^+$ calculated for $C_{16}H_{17}NO_4H^+$ *m*/*z* 288.1230; found *m*/*z* 288.1226. Optical rotation: [*α*]25 D = –93.5 (*c* 0.20, CH_2Cl_2).

Synthesis of (*R*)-3-hydroxyhex-4-ynoic acid ((*R*)-S13).

Following the same procedure as for (2*R*,3*R*)-**S6**, (*R*,3*R*)-**S12** (88 mg, 0.31 mmol) was converted into (*R*)-3-hydroxyhex-4-ynoic acid ((*R*)-**S13**) that was obtained as a colourless oil (34 mg, 0.27 mmol, 87%). ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 4.75 (tq, $^{3}J_{H,H}$ = 6.1 Hz, $^{5}J_{H,H}$ = 2.2 Hz, 1H), 2.78 (d, $^{3}J_{H,H}$ = 6.0 Hz, 2H), 1.84 (d, $^{5}J_{H,H}$ = 2.1 Hz, 3H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ_C 176.2 (C_q), 82.2 (C_q), 78.2 (C_q), 58.9 (CH), 42.1 (CH_2), 3.7 (CH_3) ppm. HRMS (ESI): $[M-H]^-$ calculated for $C_6H_7O_3^-$ *m*/*z* 127.0401; found *m*/*z* 127.0402. Optical rotation: [*α*]25 D = +14.7 (*c* 0.40, CH_2Cl_2).

Synthesis of (*S*)-3-hydroxyhex-4-ynoic acid ((*S*)-S13).

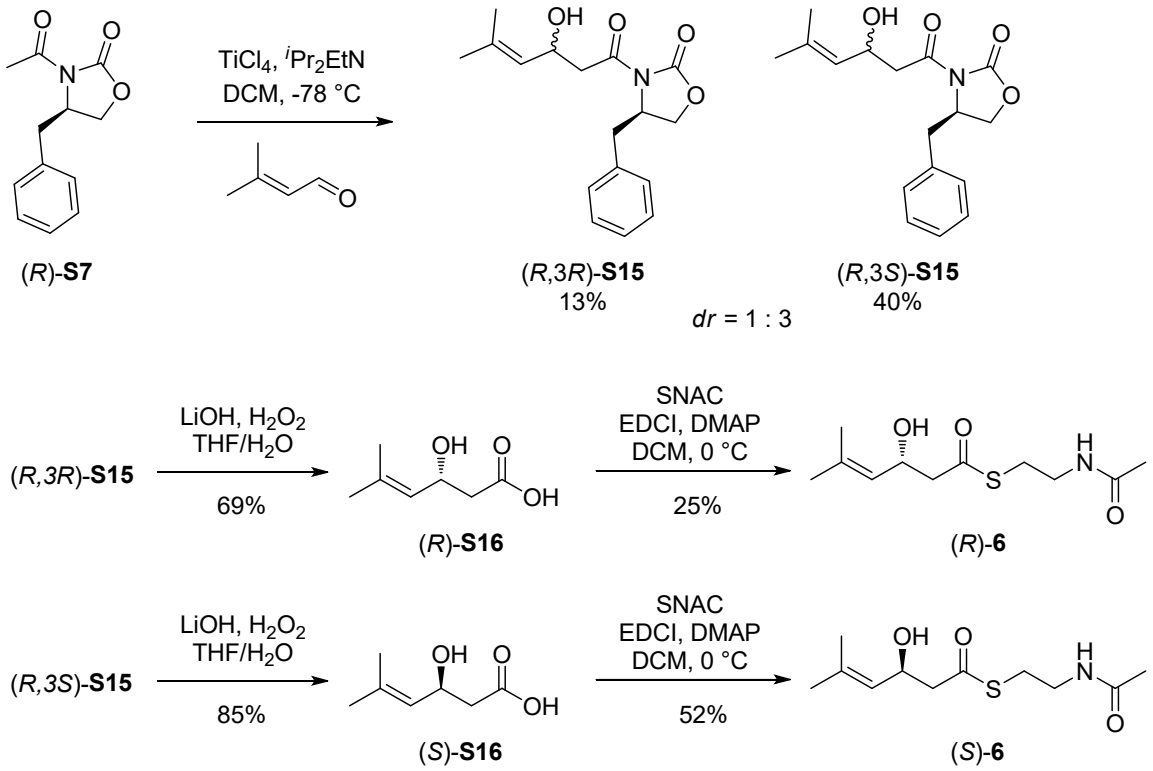
Following the same procedure as for (2*R*,3*R*)-**S6**, (*R*,3*S*)-**S12** (169 mg, 0.59 mmol) was converted into (*S*)-3-hydroxyhex-4-ynoic acid ((*S*)-**S13**) that was obtained as a colourless oil (60 mg, 0.47 mmol, 80%). Spectroscopic data were identical to those of (*R*)-**S13**. HRMS (ESI): $[M-H]^-$ calculated for $C_6H_7O_3^-$ *m*/*z* 127.0401; found *m*/*z* 127.0401. Optical rotation: [*α*]25 D = –16.6 (*c* 0.50, CH_2Cl_2).

Synthesis of S-(2-acetamidoethyl) (*R*,*Z*)-3-hydroxyhex-4-enethioate ((*R*)-5).

A mixture of (*R*)-3-hydroxyhex-4-ynoic acid ((*R*)-**S13**) (30 mg, 0.23 mmol), Lindlar's catalyst (5 mg) and quinoline (3 mg, 0.02 mmol) in Et_2O (10 mL) was stirred in a H_2 atmosphere (10 bar) for 1 h. At the end of the reaction the catalyst was removed by filtration and the solvents were evaporated to obtain the crude product (*R*)-**S14** that was used for the next step without purification. Then following the same procedure as for (*R*)-**1**, (*R*)-**S14** was converted into S-(2-acetamidoethyl) (*R*,*Z*)-3-hydroxyhex-4-enethioate ((*R*)-**5**) that was obtained as a colourless oil (10 mg, 0.04 mmol, 18% over two steps). ^{1}H NMR (500 MHz, C_6D_6): 5.39 (m, 1H), 5.32 (m, 1H), 4.94 (tdd, $^3J_{H,H}$ = 8.5 Hz, $^3J_{H,H}$ = 4.0 Hz, $^4J_{H,H}$ = 1.0 Hz, 1H), 4.80 (br s, NH), 3.17 (m, 2H), 2.76 (m, 1H), 2.68 (m, 1H), 2.66 (dd, $^2J_{H,H}$ = 14.8 Hz, $^3J_{H,H}$ = 4.0 Hz, 1H), 2.46 (dd, $^2J_{H,H}$ = 14.8 Hz, $^3J_{H,H}$ = 8.6 Hz, 1H), 1.50 (s, 3H), 1.43 (dd, $^3J_{H,H}$ = 6.7 Hz, $^4J_{H,H}$ = 1.5 Hz, 3H) ppm; ^{13}C NMR (126 MHz, C_6D_6): δ_C 197.7 (C_q), 169.5 (C_q), 132.5 (CH), 126.2 (CH), 65.0 (CH), 51.7 (CH_2), 39.1 (CH_2), 29.2 (CH_2), 22.8 (CH_3), 13.2 (CH_3) ppm. HRMS (ESI): $[M+Na]^+$ calculated for $C_{10}H_{17}NO_3SNa^+$ *m*/*z* 254.0821; found *m*/*z* 254.0815. Optical rotation: [*α*]25 D = +15.7 (*c* 0.17, CH_2Cl_2).

Synthesis of S-(2-acetamidoethyl) (*S*,*Z*)-3-hydroxyhex-4-enethioate ((*S*)-5).

Following the same procedure as for (*R*)-**5**, (*S*)-**S13** (50 mg, 0.39 mmol) was converted into S-(2-acetamidoethyl) (*S*,*Z*)-3-hydroxyhex-4-enethioate ((*S*)-**5**) that was obtained as a colourless oil (21 mg, 0.09 mmol, 23% over two steps). Spectroscopic data were identical to those of (*R*)-**5**. HRMS (ESI): $[M+Na]^+$ calculated for $C_{11}H_{19}NO_3SNa^+$ *m*/*z* 254.0821; found *m*/*z* 254.0823. Optical rotation: [*α*]25 D = –14.3 (*c* 0.20, CH_2Cl_2).



Scheme S6. Synthesis of both enantiomers of **6**.

Synthesis of (*R*)-4-benzyl-3-((*R*)-3-hydroxy-5-methylhex-4-enoyl)oxazolidin-2-one ((*R*,3*R*)-S15) and (*R*)-4-benzyl-3-((*S*)-3-hydroxy-5-methylhex-4-enoyl)oxazolidin-2-one ((*R*,3*S*)-S15). Following the same procedure as for (2*R*,3*R*)-**S5**, (*R*)-**S7** (1.50 g, 6.84 mmol) was converted into the minor diastereomer (*R*)-4-benzyl-3-((*R*)-3-hydroxy-5-methylhex-4-enoyl)oxazolidin-2-one ((*R*,3*R*)-**S15**, 272 mg, 0.90 mmol, 13%) and the major diastereomer (*R*)-4-benzyl-3-((*S*)-3-hydroxy-5-methylhex-4-enoyl)oxazolidin-2-one ((*R*,3*S*)-**S15**, 825 mg, 2.72 mmol, 40%), *dr* = 1 : 3, that were separable by column chromatography on silica gel (petroleum ether/EtOAc, 3:1). The compounds were obtained as colourless solids.

(*R*)-4-Benzyl-3-((*R*)-3-hydroxy-5-methylhex-4-enoyl)oxazolidin-2-one ((*R*,3*R*)-S15). ^{1}H NMR (700 MHz, $CDCl_3$): δ_H 7.34 (m, 2H), 7.28 (m, 1H), 7.21 (m, 2H), 5.29 (dhept, $^{3}J_{H,H}$ = 8.7 Hz, $^{4}J_{H,H}$ = 1.4 Hz, 1H), 4.89 (ddd, $^{3}J_{H,H}$ = 8.8 Hz, $^{3}J_{H,H}$ = 8.8 Hz, $^{3}J_{H,H}$ = 3.2 Hz, 1H), 4.71 (m, 1H), 4.22 (ddd, $^{2}J_{H,H}$ = 9.1 Hz, $^{3}J_{H,H}$ = 7.7 Hz, $^{4}J_{H,H}$ = 0.5 Hz, 1H), 4.19 (dd, $^{2}J_{H,H}$ = 9.1 Hz, $^{3}J_{H,H}$ = 2.8 Hz, 1H), 3.30 (dd, $^{2}J_{H,H}$ = 13.5 Hz, $^{3}J_{H,H}$ = 3.5 Hz, 1H), 3.20 (dd, $^{2}J_{H,H}$ = 17.3 Hz, $^{3}J_{H,H}$ = 9.1 Hz, 1H), 3.08 (dd, $^{2}J_{H,H}$ = 17.3 Hz, $^{3}J_{H,H}$ = 3.2 Hz, 1H), 2.80 (dd, $^{2}J_{H,H}$ = 13.5 Hz, $^{3}J_{H,H}$ = 9.4 Hz, 1H), 1.74 (d, $^{4}J_{H,H}$ = 1.4 Hz, 3H), 1.72 (d, $^{4}J_{H,H}$ = 1.4 Hz, 3H) ppm; ^{13}C NMR (176 MHz, $CDCl_3$): δ_C 172.6 (C_q), 153.5 (C_q), 136.2 (C_q), 135.2 (C_q), 129.6 (2xCH), 129.2 (2xCH), 127.6 (CH), 125.9

(CH), 66.5 (CH_2), 65.2 (CH), 55.2 (CH), 43.1 (CH_2), 38.0 (CH_2), 25.9 (CH_3), 18.4 (CH_3) ppm. HRMS (ESI): $[M+Na]^+$ calculated for $C_{17}H_{21}NO_4Na^+$ *m*/*z* 326.1363; found *m*/*z* 326.1357. Optical rotation: [*α*]25 D = –51.0 (*c* 0.20, CH_2Cl_2).

(*R*)-4-Benzyl-3-((*S*)-3-hydroxy-5-methylhex-4-enoyl)oxazolidin-2-one ((*R*,3*S*)-S15). ^1H NMR (500 MHz, $CDCl_3$): δ_H 7.34 (m, 2H), 7.28 (m, 1H), 7.21 (m, 2H), 5.28 (dhept, $^3J_{H,H}$ = 8.7 Hz, $^4J_{H,H}$ = 1.4 Hz, 1H), 4.91 (ddd, $^3J_{H,H}$ = 8.7 Hz, $^3J_{H,H}$ = 7.5 Hz, $^3J_{H,H}$ = 4.6 Hz, 1H), 4.70 (m, 1H), 4.22 (ddd, $^2J_{H,H}$ = 9.3 Hz, $^3J_{H,H}$ = 7.6 Hz, $^4J_{H,H}$ = 0.5 Hz, 1H), 4.19 (dd, $^2J_{H,H}$ = 9.2 Hz, $^3J_{H,H}$ = 3.0 Hz, 1H), 3.31 (dd, $^2J_{H,H}$ = 13.4 Hz, $^3J_{H,H}$ = 3.4 Hz, 1H), 3.15 (dd, $^2J_{H,H}$ = 17.4 Hz, $^3J_{H,H}$ = 4.7 Hz, 1H), 3.11 (dd, $^2J_{H,H}$ = 17.4 Hz, $^3J_{H,H}$ = 7.7 Hz, 1H), 2.79 (dd, $^2J_{H,H}$ = 13.5 Hz, $^3J_{H,H}$ = 9.6 Hz, 1H), 1.74 (br s, OH), 1.74 (d, $^4J_{H,H}$ = 1.5 Hz, 3H), 1.74 (d, $^4J_{H,H}$ = 1.4 Hz, 3H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ_C 172.4 (C_q), 153.5 (C_q), 136.2 (C_q), 135.3 (C_q), 129.5 (2xCH), 129.1 (2xCH), 127.6 (CH), 125.8 (CH), 66.5 (CH_2), 65.1 (CH), 55.2 (CH), 43.0 (CH_2), 38.1 (CH_2), 25.9 (CH_3), 18.4 (CH_3) ppm. HRMS (ESI): $[M+Na]^+$ calculated for $C_{17}H_{21}NO_4Na^+$ *m*/*z* 326.1363; found *m*/*z* 326.1358. Optical rotation: [*α*]25 D = –90.0 (*c* 0.10, CH_2Cl_2).

Synthesis of (*R*)-3-hydroxy-5-methylhex-4-enoic acid ((*R*)-S16).

Following the same procedure as for (2*R*,3*R*)-**S6**, (*R*,3*R*)-**S15** (122 mg, 0.40 mmol) was converted into (*R*)-3-hydroxy-5-methylhex-4-enoic acid ((*R*)-**S16**) that was obtained as a colourless oil (40 mg, 0.28 mmol, 69%). ^1H NMR (500 MHz, $CDCl_3$): 5.22 (dhept, $^3J_{H,H}$ = 8.6 Hz, $^4J_{H,H}$ = 1.4 Hz, 1H), 4.80 (ddd, $^3J_{H,H}$ = 8.5 Hz, $^3J_{H,H}$ = 8.5 Hz, $^3J_{H,H}$ = 4.0 Hz, 1H), 2.60 (dd, $^2J_{H,H}$ = 16.4 Hz, $^3J_{H,H}$ = 8.4 Hz, 1H), 2.54 (dd, $^2J_{H,H}$ = 16.4 Hz, $^3J_{H,H}$ = 4.0 Hz, 1H), 1.73 (d, $^4J_{H,H}$ = 1.4 Hz, 3H), 1.71 (d, $^4J_{H,H}$ = 1.4 Hz, 3H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ_C 176.8 (C_q), 137.1 (C_q), 125.4 (CH), 65.3 (CH), 41.5 (CH_2), 25.9 (CH_3), 18.4 (CH_3) ppm. HRMS (ESI): $[M-H]^-$ calculated for $C_7H_{11}O_3^-$ *m*/*z* 143.0714; found *m*/*z* 143.0714. Optical rotation: [*α*]25 D = +12.0 (*c* 0.15, CH_2Cl_2).

Synthesis of (*S*)-3-hydroxy-5-methylhex-4-enoic acid ((*S*)-S16).

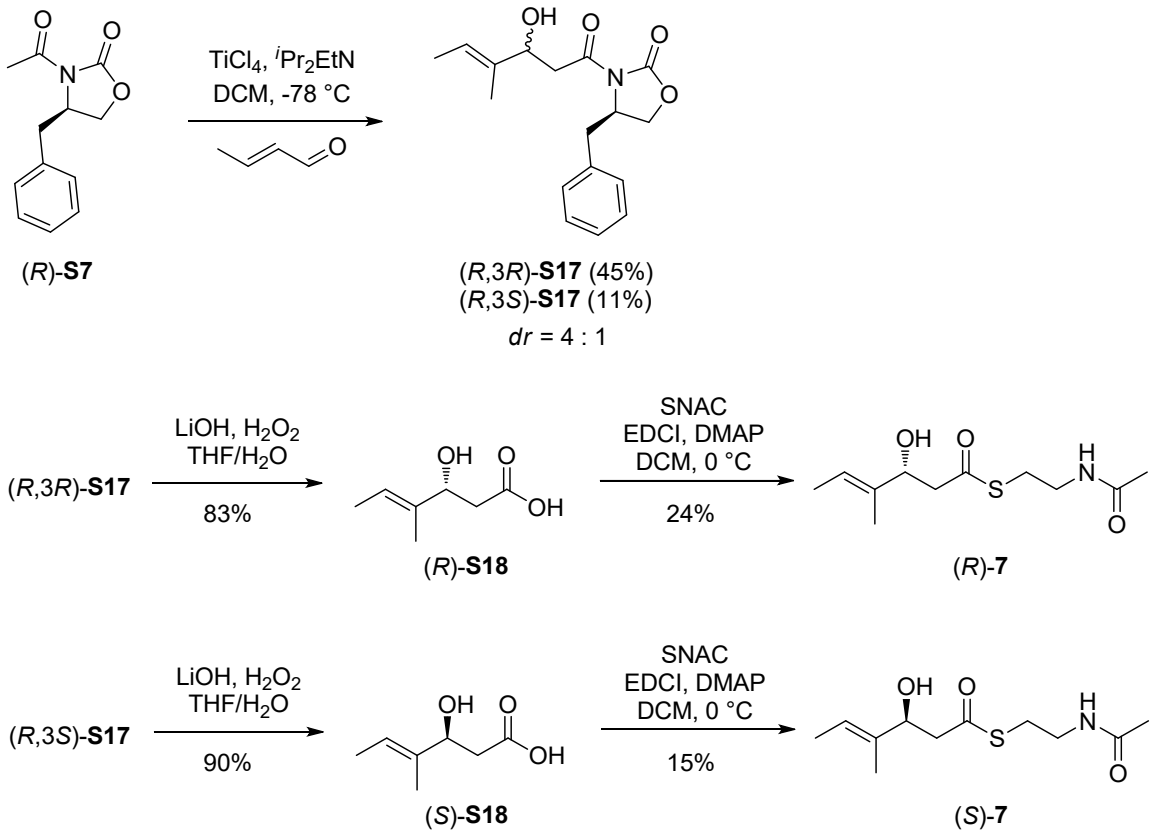
Following the same procedure as for (2*R*,3*R*)-**S6**, (*R*,3*S*)-**S15** (350 mg, 1.15 mmol) was converted into (*S*)-3-hydroxy-5-methylhex-4-enoic acid ((*S*)-**S16**) that was obtained as a colourless oil (142 mg, 0.98 mmol, 85%). Spectroscopic data were identical to those of (*R*)-**S16**. HRMS (ESI): $[M-H]^-$ calculated for $C_7H_{11}O_3^-$ *m*/*z* 143.0714; found *m*/*z* 143.0714. Optical rotation: [*α*]25 D = –14.0 (*c* 0.10, CH_2Cl_2).

Synthesis of S-(2-acetamidoethyl) (*R*)-3-hydroxy-5-methylhex-4-enethioate ((*R*)-6).

Following the same procedure as for (*R*)-**1**, (*R*)-**S16** (35 mg, 0.24 mmol) was converted into S-(2-acetamidoethyl) (*R*)-3-hydroxy-5-methylhex-4-enethioate ((*R*)-**6**) that was obtained as a colourless oil (15 mg, 0.06 mmol, 25%). ^{1}H NMR (500 MHz, C_6D_6): 5.14 (dhept, $^{3}J_{H,H}$ = 8.5 Hz, $^{4}J_{H,H}$ = 1.4 Hz, 1H), 4.85 (m, 1H), 4.79 (br s, NH), 3.17 (m, 2H), 2.77 (m, 1H), 2.69 (m, 1H), 2.67 (dd, $^{2}J_{H,H}$ = 14.7 Hz, $^{3}J_{H,H}$ = 8.5 Hz, 1H), 2.48 (dd, $^{2}J_{H,H}$ = 14.7 Hz, $^{3}J_{H,H}$ = 4.1 Hz, 1H), 2.37 (d, $^{3}J_{H,H}$ = 3.3 Hz, OH), 1.50 (d, $^{4}J_{H,H}$ = 1.6 Hz, 3H),1.49 (s, 3H), 1.45 (d, $^{4}J_{H,H}$ = 1.4 Hz, 3H) ppm; ^{13}C NMR (126 MHz, C_6D_6): δ_C 197.8 (C_q), 169.4 (C_q), 134.9 (C_q), 127.2 (CH), 66.2 (CH), 52.0 (CH_2), 39.2 (CH_2), 29.1 (CH_2), 25.6 (CH_3), 22.8 (CH_3), 18.1 (CH_3) ppm. HRMS (ESI): $[M+Na]^+$ calculated for $C_{11}H_{19}NO_3SNa^+$ *m*/*z* 268.0978; found *m*/*z* 268.0978. Optical rotation: [*α*]25 D = +16.5 (*c* 0.15, CH_2Cl_2).

Synthesis of S-(2-acetamidoethyl) (*S*)-3-hydroxy-5-methylhex-4-enethioate ((*S*)-6).

Following the same procedure of (*R*)-**1**, (*S*)-**S16** (65 mg, 0.45 mmol) was converted into S-(2-acetamidoethyl) (*S*)-3-hydroxy-5-methylhex-4-enethioate ((*S*)-**6**) that was obtained as a colourless oil (58 mg, 0.24 mmol, 52%). Spectroscopic data were identical to those of (*R*)-**6**. HRMS (ESI): $[M+Na]^+$ calculated for $C_{11}H_{19}NO_3SNa^+$ *m*/*z* 268.0978; found *m*/*z* 268.0976. Optical rotation: [*α*]25 D = –15.7 (*c* 0.12, CH_2Cl_2).



Scheme S7. Synthesis of both enantiomers of **7**.

Synthesis of (*R*)-4-benzyl-3-((*R*,*E*)-3-hydroxy-4-methylhex-4-enoyl)oxazolidin-2-one ((*R*,3*R*)-S17) and (*R*)-4-benzyl-3-((*S*,*E*)-3-hydroxy-4-methylhex-4-enoyl)oxazolidin-2-one ((*R*,3*S*)-S17).

Following the same procedure as for (2*R*,3*R*)-**S5**, (*R*)-**S7** (1.00 g, 4.56 mmol) was converted into the major diastereomer (*R*)-4-benzyl-3-((*R*,*E*)-3-hydroxy-4-methylhex-4-enoyl)oxazolidin-2-one ((*R,3R*)-**S17**, 625 mg, 2.06 mmol, 45%) and the minor diastereomer (*R*)-4-benzyl-3-((*S*,*E*)-3-hydroxy-4-methylhex-4-enoyl)oxazolidin-2-one ((*R*,3*S*)-**S17**, 147 mg, 0.48 mmol, 11%), *dr* = 4 : 1, that were separable by column chromatography on silica gel (petroleum ether/EtOAc, 3:1). The compounds were obtained as colourless solids.

(*R*)-4-Benzyl-3-((*R*,*E*)-3-hydroxy-4-methylhex-4-enoyl)oxazolidin-2-one ((*R*,3*R*)-S17). ^{1}H NMR (700 MHz, C_6D_6): δ_H 7.04 (m, 2H), 6.99 (m, 1H), 6.86 (m, 2H), 5.61 (qquin, $^3J_{H,H}$ = 6.6 Hz, $^4J_{H,H}$ = 1.2 Hz, 1H), 4.67 (m, 1H), 4.08 (ddt, $^3J_{H,H}$ = 9.3 Hz, $^3J_{H,H}$ = 8.0 Hz, $^3J_{H,H}$ = 3.1 Hz, 1H), 3.46 (dd, $^2J_{H,H}$ = 16.3 Hz, $^3J_{H,H}$ = 9.6 Hz, 1H), 3.41 (dd, $^2J_{H,H}$ = 9.0 Hz, $^3J_{H,H}$ = 2.8 Hz, 1H), 3.13 (dd, $^2J_{H,H}$ = 9.0 Hz, $^3J_{H,H}$ = 8.0 Hz, 1H), 3.08 (dd, $^2J_{H,H}$ = 16.3 Hz, $^3J_{H,H}$ = 3.2 Hz, 1H), 2.89 (dd, $^2J_{H,H}$ = 13.5 Hz, $^3J_{H,H}$ = 3.4 Hz, 1H), 2.79 (d, $^3J_{H,H}$ = 4.3 Hz, OH), 2.25 (dd, $^2J_{H,H}$ = 13.5 Hz, $^3J_{H,H}$ = 9.4 Hz, 1H), 1.65 (s, 3H),

1.49 (d, $^{3}J_{H,H}$ = 6.8, Hz, 3H) ppm; ^{13}C NMR (176 MHz, C_6D_6): δ_C 172.4 (C_q), 153.4 (C_q), 137.2 (C_q), 135.8 (C_q), 129.7 (2xCH), 129.0 (2xCH), 127.4 (CH), 120.4 (CH), 73.7 (CH), 65.7 (CH_2), 55.0 (CH), 41.9 (CH_2), 37.6 (CH_2), 13.1 (CH_3), 11.9 (CH_3) ppm. HRMS (ESI): $[M+Na]^+$ calculated for $C_{17}H_{21}NO_4Na^+$ *m*/*z* 326.1363; found *m*/*z* 326.1359. Optical rotation: [*α*]25 D = –48.9 (*c* 0.10, CH_2Cl_2).

(*R*)-4-Benzyl-3-((*S*,*E*)-3-hydroxy-4-methylhex-4-enoyl)oxazolidin-2-one ((*R*,3*S*)-S17). ^{1}H NMR (700 MHz, C_6D_6): δ_H 7.04 (m, 2H), 6.99 (m, 1H), 6.85 (m, 2H), 5.61 (qquin, $^{3}J_{H,H}$ = 6.7 Hz, $^{4}J_{H,H}$ = 1.3 Hz, 1H), 4.69 (m, 1H), 4.07 (ddt, $^{3}J_{H,H}$ = 9.5 Hz, $^{3}J_{H,H}$ = 8.0 Hz, $^{3}J_{H,H}$ = 3.1 Hz, 1H), 3.41 (dd, $^{2}J_{H,H}$ = 8.9 Hz, $^{3}J_{H,H}$ = 2.8 Hz, 1H), 3.34 (dd, $^{2}J_{H,H}$ = 16.5 Hz, $^{3}J_{H,H}$ = 9.7 Hz, 1H), 3.24 (dd, $^{2}J_{H,H}$ = 16.5 Hz, $^{3}J_{H,H}$ = 3.1 Hz, 1H), 3.10 (dd, $^{2}J_{H,H}$ = 8.9 Hz, $^{3}J_{H,H}$ = 8.1 Hz, 1H), 2.94 (dd, $^{2}J_{H,H}$ = 13.4 Hz, $^{3}J_{H,H}$ = 3.4 Hz, 1H), 2.63 (d, $^{3}J_{H,H}$ = 3.9 Hz, OH), 2.27 (dd, $^{2}J_{H,H}$ = 13.4 Hz, $^{3}J_{H,H}$ = 9.5 Hz, 1H), 1.65 (s, 3H), 1.49 (d, $^{3}J_{H,H}$ = 6.8, Hz, 3H) ppm; ^{13}C NMR (176 MHz, C_6D_6): δ_C 172.4 (C_q), 153.3 (C_q), 137.2 (C_q), 135.8 (C_q), 129.6 (2xCH), 129.0 (2xCH), 127.4 (CH), 120.4 (CH), 73.5 (CH), 65.7 (CH_2), 55.0 (CH), 41.9 (CH_2), 37.8 (CH_2), 13.1 (CH_3), 11.9 (CH_3) ppm. HRMS (ESI): $[M+Na]^+$ calculated for $C_{17}H_{21}NO_4Na^+$ *m*/*z* 326.1363; found *m*/*z* 326.1359. Optical rotation: [*α*]25 D = –100.0 (*c* 0.16, CH_2Cl_2).

Synthesis of (*R*,*E*)-3-hydroxy-4-methylhex-4-enoic acid ((*R*)-S18).

Following the same procedure as for (2*R*,3*R*)-**S6**, (*R*)-**S17** (300 mg, 0.99 mmol) was converted into (*R*,*E*)-3-hydroxy-4-methylhex-4-enoic acid ((*R*)-**S18**) that was obtained as a colourless oil (118 mg, 0.82 mmol, 83%). ^{1}H NMR (500 MHz, $CDCl_3$): 5.58 (qquin, $^{3}J_{H,H}$ = 6.7 Hz, $^{4}J_{H,H}$ = 1.1 Hz, 1H), 4.47 (dd, $^{3}J_{H,H}$ = 9.4 Hz, $^{3}J_{H,H}$ = 3.5 Hz, 1H), 2.64 (dd, $^{2}J_{H,H}$ = 16.1 Hz, $^{3}J_{H,H}$ = 9.4 Hz, 1H), 2.55 (dd, $^{2}J_{H,H}$ = 16.1 Hz, $^{3}J_{H,H}$ = 3.5 Hz, 1H), 1.64 (m, 3H), 1.62 (d, $^{3}J_{H,H}$ = 6.8 Hz, 3H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ_C 177.6 (C_q), 135.8 (C_q), 121.8 (CH), 73.5 (CH), 40.0 (CH_2), 13.2 (CH_3), 11.6 (CH_3) ppm. HRMS (ESI): $[M-H]^-$ calculated for $C_7H_{11}O_3^-$ *m*/*z* 143.0714; found *m*/*z* 143.0714. Optical rotation: [*α*]25 D = +18.5 (*c* 0.40, CH_2Cl_2).

Synthesis of (*S*,*E*)-3-hydroxy-4-methylhex-4-enoic acid ((*S*)-S18).

Following the same procedure as for (2*R*,3*R*)-**S6**, (*S*)-**S17** (147 mg, 0.48 mmol) was converted into (*S*,*E*)-3-hydroxy-4-methylhex-4-enoic acid ((*S*)-**S18**) that was obtained as a colourless oil (63 mg, 0.44 mmol, 90%). Spectroscopic data were identical to those of (*R*)-**S18**. HRMS (ESI): $[M-H]^-$ calculated for $C_7H_{11}O_3^-$ *m*/*z* 143.0714; found *m*/*z*

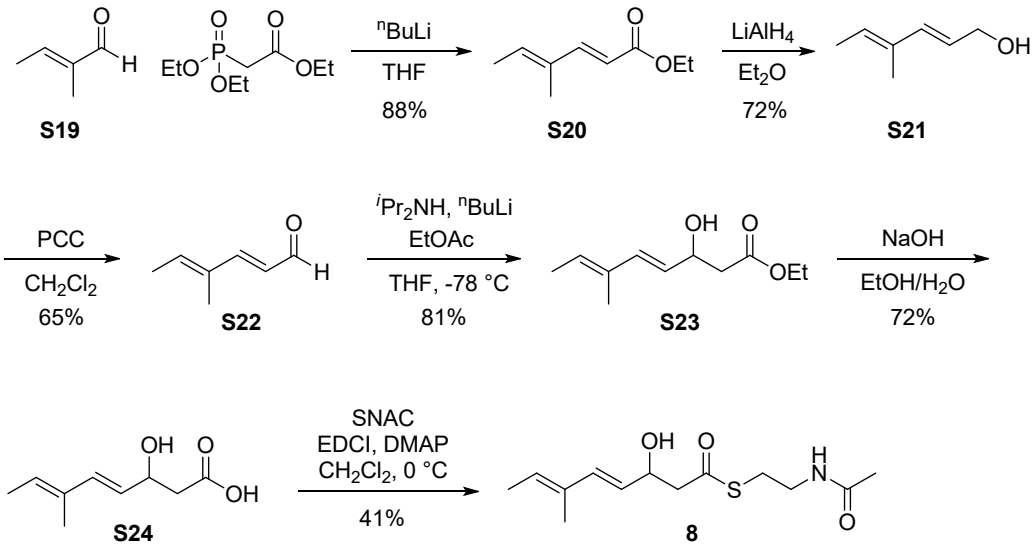
143.0714. Optical rotation: [α]25 D = –18.9 (*c* 0.50, CH_2Cl_2).

Synthesis of S-(2-acetamidoethyl) (*R*,*E*)-3-hydroxy-4-methylhex-4-enethioate ((*R*)-7).

Following the same procedure as for (*R*)-**1**, (*R*)-**S18** (118 mg, 0.82 mmol) was converted into S-(2-acetamidoethyl) (*R*,*E*)-3-hydroxy-4-methylhex-4-enethioate ((*R*)-**7**) that was obtained as a colourless oil (48 mg, 0.20 mmol, 24%). ^{1}H NMR (500 MHz, $CDCl_3$): 6.06 (br s, NH), 5.56 (qquin, $^3J_{H,H}$ = 6.7 Hz, $^4J_{H,H}$ = 1.1 Hz, 1H), 4.49 (dd, $^3J_{H,H}$ = 9.2 Hz, $^3J_{H,H}$ = 3.5 Hz, 1H), 3.45 (m, 2H), 3.06 (m, 2H), 2.83 (dd, $^2J_{H,H}$ = 15.0 Hz, $^3J_{H,H}$ = 9.2 Hz, 1H), 2.73 (dd, $^2J_{H,H}$ = 15.1 Hz, $^3J_{H,H}$ = 3.2 Hz, 1H), 2.35 (br s, OH), 1.98 (s, 3H), 1.63 (s, 3H), 1.61 (d, $^3J_{H,H}$ = 6.7 Hz, 3H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ_C 199.2 (C_q), 170.8 (C_q), 136.1 (C_q), 121.7 (CH), 74.2 (CH), 49.7 (CH_2), 39.6 (CH_2), 28.9 (CH_2), 23.2 (CH_3), 13.2 (CH_3), 11.7 (CH_3) ppm. HRMS (ESI): $[M+Na]^+$ calculated for $C_{11}H_{19}NO_3SNa^+$ *m*/*z* 268.0978; found *m*/*z* 268.0971. Optical rotation: [α]25 D = +16.0 (*c* 0.10, CH_2Cl_2).

Synthesis of S-(2-acetamidoethyl) (*S*,*E*)-3-hydroxy-4-methylhex-4-enethioate ((*S*)-7).

Following the same procedure as for (*R*)-**1**, (*S*)-**S18** (50 mg, 0.35 mmol) was converted into S-(2-acetamidoethyl) (*S*,*E*)-3-hydroxy-4-methylhex-4-enethioate ((*S*)-**7**) that was obtained as a colourless oil (13 mg, 0.05 mmol, 15%). Spectroscopic data were identical to those of (*R*)-**7**. HRMS (ESI): $[M+Na]^+$ calculated for $C_{11}H_{19}NO_3SNa^+$ *m*/*z* 268.0978; found *m*/*z* 268.0979. Optical rotation: [α]25 D = –15.7 (*c* 0.10, CH_2Cl_2).



Scheme S8. Synthesis of (*rac*)-**8**.

Synthesis of ethyl (2*E*,4*E*)-4-methylhexa-2,4-dienoate (S20).

To a solution of triethyl phosphonoacetate (3.46 g, 15.4 mmol) in THF (18 mL) was added ^{n}BuLi (6.2 mL, 2.5 M in hexane, 15.4 mmol) dropwise at 0 °C under Ar. After 0.5 h, (*E*)-2-methylbut-2-enal (1.30 g, 15.4 mmol) was added dropwise. Stirring was continued at 0 °C for 1.5 h. The mixture was quenched by the addition of sat.NH_4Cl solution (20 mL). The aqueous layer was extracted with Et_2O (4 x 20 mL). The combined organic layers were dried with $MgSO_4$ and concentrated to dryness. The residue was purified through silica gel column chromatography (petroleum ether/EtOAc, 20:1 – 5:1) to afford ethyl (2*E*,4*E*)-4-methylhexa-2,4-dienoate (**S20**) as a colourless solid (2.10 g, 13.6 mmol, 88%). ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 7.31 (d, $^{3}J_{H,H}$ = 15.7 Hz, 1H), 5.98 (q, $^{3}J_{H,H}$ = 7.2 Hz, 1H), 5.78 (d, $^{3}J_{H,H}$ = 15.7 Hz, 1H), 4.20 (q, $^{3}J_{H,H}$ = 7.1 Hz, 2H), 1.81 (d, $^{3}J_{H,H}$ = 7.1 Hz, 3H), 1.76 (m, 3H), 1.29 (t, $^{3}J_{H,H}$ = 7.1 Hz, 3H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ_C 167.8 (C_q), 149.6 (CH), 136.4 (CH), 133.9 (C_q), 115.4 (CH), 60.3 (CH_2), 14.7 (CH_3), 14.5 (CH_3), 11.9 (CH_3) ppm.[11]

Synthesis of (2*E*,4*E*)-4-methylhexa-2,4-dien-1-ol (S21).

To a solution of ethyl (2*E*,4*E*)-4-methylhexa-2,4-dienoate (**S20**) (2.10 g, 13.6 mmol) in Et_2O (30 mL) was added $LiAlH_4$ (517 mg, 13.6 mmol) in small portions at 0 °C under Ar. The reaction mixture was stirred for 30 min, then saturated NH_4Cl solution (30 mL) was added and stirring was continued for 10 min. The aqueous layer was extracted with Et_2O (4 x 50 mL). The combined organic layers were dried with $MgSO_4$ and concentrated to dryness. Purification of the crude product by column chromatography

on silica gel (petroleum ether/Et_2O, 10:1) gave (2*E*,4*E*)-4-methylhexa-2,4-dien-1-ol (**S21**) as a colourless oil (1.10 g, 9.81 mmol, 72%). ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 6.25 (d, $^3J_{H,H}$ = 15.6 Hz, 1H), 5.71 (dt, $^3J_{H,H}$ = 15.6 Hz, $^3J_{H,H}$ = 6.1 Hz, 1H), 5.57 (q, $^3J_{H,H}$ = 6.8 Hz, 1H), 4.19 (d, $^3J_{H,H}$ = 6.2 Hz, 2H), 1.74 (m, 3H), 1.72 (d, $^3J_{H,H}$ = 7.4 Hz) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ_C 136.9 (CH), 134.0 (C_q), 127.7 (CH), 124.9 (CH), 64.1 (CH_2), 14.0 (CH_3), 12.2 (CH_3) ppm.[12]

Synthesis of (2*E*,4*E*)-4-methylhexa-2,4-dienal (S22).

A mixture of **S21** (1.10 g, 9.81 mmol), silica gel and PCC (3.17 g, 14.7 mmol) in CH_2Cl_2 (40 mL) was stirred for 3 h with ice cooling. At the end of the reaction the silica gel was removed by silica gel column and the solvents were evaporated to obtain the crude product. Purification by column chromatography on silica gel (pentane/ Et_2O, 20:1) yielded **S22** (705 mg, 6.40 mmol, 65%) as a colourless oil. The NMR spectra indicated the presence of two conformers. ^{1}H NMR (500 MHz, C_6D_6): δ_H 9.47 (d, $^3J_{H,H}$ = 7.6 Hz, 1H), 6.52 and 6.53 (d, $^3J_{H,H}$ = 15.7 Hz, 1H), 6.00 (ddquin, $^3J_{H,H}$ = 15.7 Hz, $^3J_{H,H}$ = 7.6 Hz, $^5J_{H,H}$ = 0.5 Hz, 1H), 5.47 (m, 1H), 1.34 (br d, $^3J_{H,H}$ = 7.0 Hz, 1H), 1.31 (m, 1H) ppm; ^{13}C NMR (126 MHz, C_6D_6): δ_C 192.80 and 192.76 (CH), 156.14 and 156.07 (CH), 137.34 and 137.26 (CH), 134.46 (C_q), 127.15 and 127.13 (CH), 14.34 (CH_3), 11.56 (CH_3) ppm.[13]

Synthesis of ethyl (4*E*,6*E*)-3-hydroxy-6-methylocta-4,6-dienoate (S23).

To a solution of ^{i}Pr$_2$NH (0.9 mL, 6.42 mmol) in THF (12 mL) was added ^{n}BuLi (3.5 mL, 1.6 M in hexane, 5.60 mmol) dropwise at –78 °C under N_2. EtOAc (0.5 mL, 5.50 mmol) was then added dropwise. The resulting mixture was allowed to stir at –78 °C for 30 min, and then (2*E*,4*E*)-4-methylhexa-2,4-dienal (**S22**) (500 mg, 4.54 mmol) was added dropwise. After 2 h at –78 °C, the solution was poured onto an ice-cold solution of NH_4Cl. Ether was added and the resulting mixture was stirred vigorously for a few minutes. The layers were separated and the aqueous layer was extracted with Et_2O (3 x 30 mL). The combined organic layers were dried with $MgSO_4$, filtered and concentrated to dryness. Pure ethyl (4*E*,6*E*)-3-hydroxy-6-methylocta-4,6-dienoate (**S23**, 730 mg, 3.68 mmol, 81%) was obtained by column chromatography on silica gel (petroleum ether/EtOAc, 5:1 – 3:1). ^{1}H NMR (700 MHz, C_6D_6): δ_H 6.33 (d, $^3J_{H,H}$ = 15.7 Hz, 1H), 5.53 (ddquin, $^3J_{H,H}$ = 15.7 Hz, $^3J_{H,H}$ = 6.2 Hz, $^5J_{H,H}$ = 0.5 Hz, 1H), 5.45 (q, $^3J_{H,H}$ = 6.8 Hz, 1H), 4.58 (m, 1H), 3.89 (q, $^3J_{H,H}$ = 7.1 Hz, 2H), 2.66 (br s, OH), 2.44 (dd, $^2J_{H,H}$

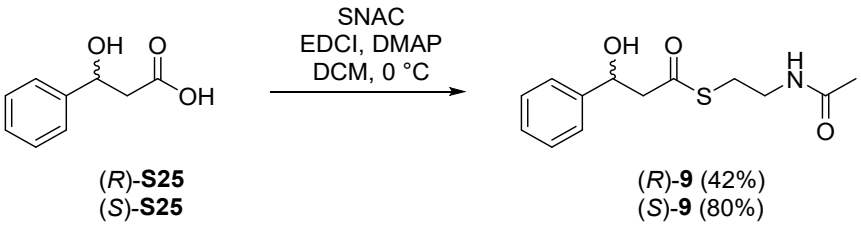
= 15.8 Hz, $^{3}J_{H,H}$ = 8.4 Hz, 1H), 2.35 (dd, $^{2}J_{H,H}$ = 15.8 Hz, $^{3}J_{H,H}$ = 4.2 Hz, 1H), 1.60 (m, 3H), 1.53 (d, $^{3}J_{H,H}$ = 7.0 Hz, 3H), 0.90 (t, $^{3}J_{H,H}$ = 7.1 Hz, 3H) ppm; ^{13}C NMR (176 MHz, C_6D_6): δ_C 172.0 (C_q), 135.6 (CH), 134.1 (C_q), 127.7 (CH), 127.3 (CH), 69.3 (CH_2), 60.4 (CH), 42.3 (CH_2), 14.2 (CH_3), 13.8 (CH_3), 12.1 (CH_3) ppm.

Synthesis of (4*E*,6*E*)-3-hydroxy-6-methylocta-4,6-dienoic acid (S24).

To a solution of **S23** (200 mg, 1.01 mmol) in EtOH/H_2O (4/1 mL), NaOH (100 mg, 2.50 mmol) was added and the mixture was stirred for 0.5 h. The mixture was acidified by adding HCl (1 N, 3 mL), followed by extraction with Et_2O (3 x 20 mL). The combined extracts were dried with $MgSO_4$ and concentrated to yield the product (4*E*,6*E*)-3-hydroxy-6-methylocta-4,6-dienoic acid (**S24**) (124 mg, 0.73 mmol, 72%). ^{1}H NMR (700 MHz, C_6D_6): δ_H 6.24 (d, $^{3}J_{H,H}$ = 15.8 Hz, 1H), 5.45 (q, $^{3}J_{H,H}$ = 6.8 Hz, 1H), 5.41 (dd, $^{3}J_{H,H}$ = 15.7 Hz, $^{3}J_{H,H}$ = 6.4 Hz, 1H), 4.45 (m, 1H), 2.39 (dd, $^{2}J_{H,H}$ = 16.0 Hz, $^{3}J_{H,H}$ = 8.7 Hz, 1H), 2.28 (dd, $^{2}J_{H,H}$ = 16.0 Hz, $^{3}J_{H,H}$ = 4.0 Hz, 1H), 1.57 (m, 3H), 1.53 (d, $^{3}J_{H,H}$ = 6.8 Hz, 3H) ppm; ^{13}C NMR (176 MHz, C_6D_6): δ_C 177.2 (C_q), 136.0 (CH), 134.0 (C_q), 127.7 (CH), 127.0 (CH), 69.2 (CH), 42.0 (CH_2), 13.8 (CH_3), 12.1 (CH_3) ppm.

Synthesis of S-(2-acetamidoethyl) (4*E*,6*E*)-3-hydroxy-6-methylocta-4,6-dienethioate ((*rac*)-8).

Following the same procedure as for (*R*)-**1**, **S24** (100 mg, 0.59 mmol) was converted into S-(2-acetamidoethyl) (4*E*,6*E*)-3-hydroxy-6-methylocta-4,6-dienethioate ((*rac*)-**8**) that was obtained as a colourless oil (65 mg, 0.24 mmol, 41%). ^{1}H NMR (700 MHz, C_6D_6): δ_H 6.29 (d, $^{3}J_{H,H}$ = 15.8 Hz, 1H), 5.44 (m, 2H), 4.71 (br s, NH), 4.63 (m, 1H), 3.16 (m, 2H), 2.72 (m, 2H), 2.64 (dd, $^{2}J_{H,H}$ = 14.7 Hz, $^{3}J_{H,H}$ = 8.6 Hz, 1H), 2.50 (dd, $^{2}J_{H,H}$ = 14.7 Hz, $^{3}J_{H,H}$ = 3.9 Hz, 1H), 1.60 (m, 3H), 1.53 (d, $^{3}J_{H,H}$ = 6.7 Hz, 3H), 1.49 (s, 3H) ppm; ^{13}C NMR (176 MHz, C_6D_6): δ_C 197.7 (C_q), 169.4 (C_q), 135.6 (CH), 134.1 (C_q), 127.6 (CH), 127.5 (CH), 69.9 (CH), 52.0 (CH_2), 39.2 (CH_2), 29.2 (CH_2), 22.8 (CH_3), 13.9 (CH_3), 12.1 (CH_3) ppm. HRMS (ESI): $[M+Na]^+$ calculated for $C_{13}H_{21}NO_3SNa^+$ *m*/*z* 294.1134; found *m*/*z* 294.1133.



(*R*)-**S25**
(*S*)-**S25**

(*R*)-**9** (42%)
(*S*)-**9** (80%)

Scheme S9. Synthesis of both enantiomers of **9**.

Synthesis of S-(2-acetamidoethyl) (*R*)-3-hydroxy-3-phenylpropanethioate ((*R*)-9). Following the same procedure as for (*R*)-**1**, (*R*)-**S25** (50 mg, 0.30 mmol) was converted into S-(2-acetamidoethyl) (*R*)-3-hydroxy-3-phenylpropanethioate ((*R*)-**9**) that was obtained as a colourless oil (34 mg, 0.13 mmol, 42%). ^{1}H NMR (500 MHz, C_6D_6): δ_H 7.20 (m, 2H), 7.11 (m, 2H), 7.05 (m, 1H), 5.12 (ddd, $^{3}J_{H,H}$ = 9.4 Hz, $^{4}J_{H,H}$ = 3.6 Hz, $^{4}J_{H,H}$ = 3.6 Hz, 1H), 4.61 (br s, NH), 3.18 (m, 1H), 3.08 (m, 2H), 2.81 (dd, $^{2}J_{H,H}$ = 15.0 Hz, $^{3}J_{H,H}$ = 9.5 Hz, 1H), 2.75 (dt, $^{2}J_{H,H}$ = 13.7 Hz, $^{3}J_{H,H}$ = 6.3 Hz, 1H), 2.64 (m, 1H), 2.60 (dd, $^{2}J_{H,H}$ = 15.1 Hz, $^{3}J_{H,H}$ = 3.4 Hz, 1H), 1.46 (s, 3H) ppm; ^{13}C NMR (126 MHz, C_6D_6): δ_C 197.9 (C_q), 169.4 (C_q), 143.5 (C_q), 128.6 (2xCH), 127.8 (CH), 126.0 (2xCH), 71.1 (CH), 53.6 (CH_2), 39.0 (CH_2), 29.2 (CH_3), 22.7 (CH_3) ppm. HRMS (ESI): $[M+Na]^+$ calculated for $C_{13}H_{17}NO_3SNa^+$ *m*/*z* 290.0821; found *m*/*z* 290.0825. Optical rotation: [*α*]25 D = +21.2 (*c* 0.20, $CHCl_3$).

Synthesis of S-(2-acetamidoethyl) (*S*)-3-hydroxy-3-phenylpropanethioate ((*S*)-9). Following the same procedure as for (*R*)-**1**, (*S*)-**S25** (50 mg, 0.30 mmol) was converted into S-(2-acetamidoethyl) (*S*)-3-hydroxy-3-phenylpropanethioate ((*S*)-**9**) that was obtained as a colourless oil (64 mg, 0.24 mmol, 80%). Spectroscopic data were identical to those of (*R*)-**9**. HRMS (ESI): $[M+Na]^+$ calculated for $C_{13}H_{17}NO_3SNa^+$ *m*/*z* 290.0821; found *m*/*z* 290.0825. Optical rotation: [*α*]25 D = –18.2 (*c* 0.50, $CHCl_3$).

Gene cloning

Cloning of the coding genes for BorDH2, BorDH3, BorDH5, FosDH2, FosDH2, RifDH10, ShawDH1, ShawDH2, Cpz and FabZ into the pYE-Express[17] or pET28 expression vectors was reported previously.[15]

Amplification of *cpz2* from cosmid cpzLK09 was performed using primer pair Cpz2-pET28_FW (AAAAAAGAATTCATGAGCATCACCGTCAACGGC) and Cpz2-pET28_RV (AAAAAAAAGCTTTCAGGCGTAGAACCGCGACAG).[18] The resulting PCR product was cloned into the EcoRI and HindIII sites of expression vector pET28 and the obtained plasmid was verified by PCR and sequencing.

Gene expression and protein purification

E. coli BL21(DE3) cells harboring the corresponding pYE-Express or pET28 derived plasmids were used to inoculate a preculture in LB medium (10 mL) supplied with kanamycin (50 µg/mL final concentration), which was grown with shaking at 37 °C overnight. The precultures were used to inoculate main cultures (1/100) in LB medium with kanamycin (50 µg/mL final concentration) and the cells were grown with shaking at 37 °C until OD_{600} = 0.4 – 0.6 was reached. The cultures were cooled down to 18 °C, before IPTG (0.4 mM final concentration) was added to induce expression. The cultures were shaken at the same temperature overnight and then centrifuged (3500 x g, 40 min, 4 °C). The medium was discarded and the cell pellet was resuspended in binding buffer (10 mL/L culture; 40 mM Tris-HCl, 100 mM NaCl, pH 7.8, 4 °C). The cells were lysied by ultrasonication (10 x 1 min). The cell debris was spun down (14600 x g, 10 min, 4 °C) and the soluble protein fraction was filtrated and loaded onto a Ni^{2+}-NTA affinity chromatography column (Ni-NTA superflow, Qiagen, Venlo, Netherlands). The bound target protein was washed with wash buffer (2 x 10 mL/L culture; 40 mM Tris-HCl, 100 mM NaCl, 50 mM imidazole, pH 7.8, 4 °C) and desorbed from the stationary phase with elution buffer (1 x 10 mL/L culture; 40 mM Tris-HCl, 100 mM NaCl, 500 mM imidazole, pH 7.8, 4 °C) with fractionation. The fractions were analysed by SDS-PAGE and fractions containing pure protein were pooled and used for incubation experiments (Figure S1). Finally, the eluate was concentrated, the buffer was replaced by incubation buffer (25 mM HEPES, 100 mM NaCl, pH 7.5). For ShawDH1 expression, the strain was *E. coli* BL21(DE3) transformed with plasmid pGro7, additionally supplemented with arabinose (500 mg/L) to induce expression of the GroEL/ES chaperone.[15]

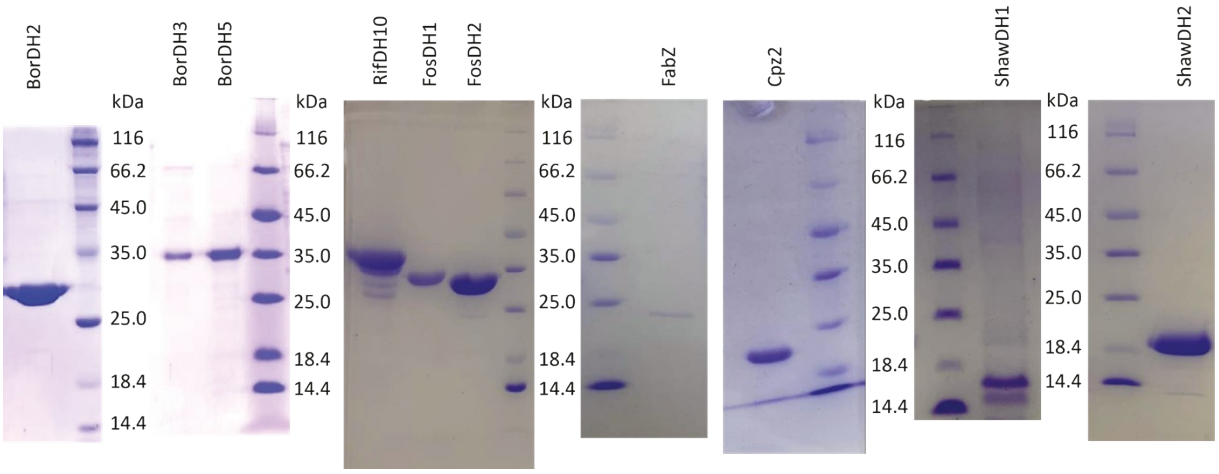
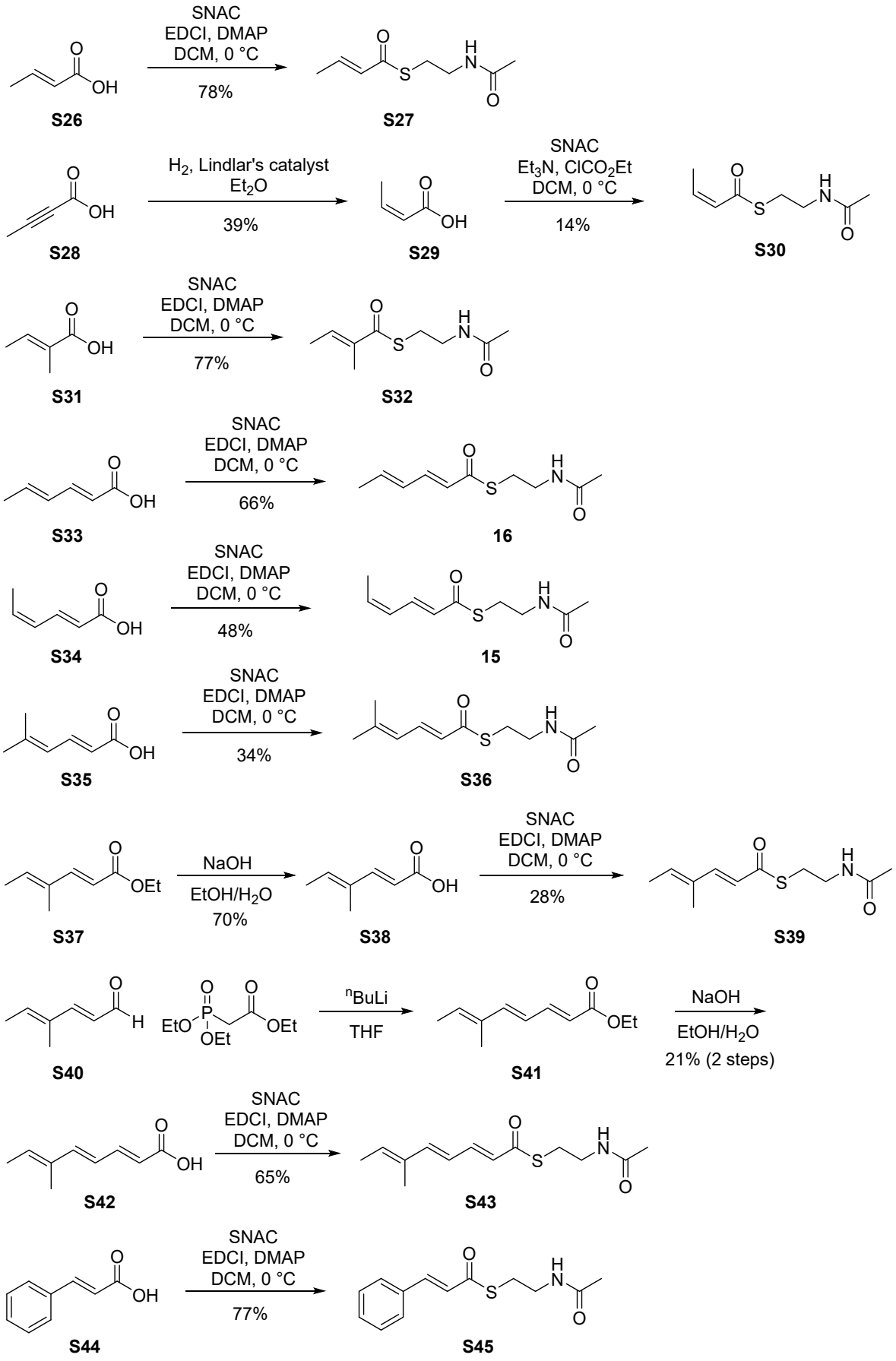


Figure S1. SDS-PAGE analysis of all recombinant enzymes used in this study. The theoretical molecular weights of target proteins are 34.6 kDa (BorDH2), 34.0 kDa (BorDH3), 34.2 kDa (BorDH5), 34.1 kDa (RifDH10), 33.3 kDa (FosDH1), 33.1 kDa (FosDH2), 19.8 kDa (FabZ), 20.3 kDa (Cpz2), 15.2 kDa (ShawDH1) and 16.5 kDa (ShawDH2).

Activity assays

Activity assays were carried out for all combinations of substrate and enzyme listed in Table S1. The reactions were performed in HEPES buffer (25 mM HEPES, 100 mm NaCl, pH 7.5). A solution of enzyme in HEPES buffer (100 µL, enzyme concentration adjusted to 6 mg/mL) was added into SNAC thioesters (1 mg dissolved in 5 µL DMSO). The reaction mixtures were incubated at 30 °C for 16 h and then extracted with C_6D_6 (0.6 mL). After extraction the samples were directly analysed by ^{1}H NMR spectroscopy (Figures S2–S9). For the most active enzyme BorDH2 the reactions were repeated in triplicates and conversions (in %) were quantified by ^{1}H NMR peak integrations (Table S2).



Scheme S10. Synthesis of reference standards for DH products.

Synthesis of S-(2-acetamidoethyl) (*E*)-but-2-enethioate (S27).

Following the same procedure as for (*R*)-**1**, **S26** (100 mg, 1.16 mmol) was converted into S-(2-acetamidoethyl) (*E*)-but-2-enethioate (**S27**) that was obtained as a colourless oil (170 mg, 0.91 mmol, 78%). ^{1}H NMR (700 MHz, C_6D_6): δ_H 6.73 (dq, $^{3}J_{H,H}$ = 15.4 Hz, $^{3}J_{H,H}$ = 6.9 Hz, 1H), 5.91 (dq, $^{3}J_{H,H}$ = 15.4 Hz, $^{4}J_{H,H}$ = 1.7 Hz, 1H), 3.24 (dt, $^{3}J_{H,H}$ = 5.8 Hz, $^{3}J_{H,H}$ = 6.8 Hz, 2H), 2.88 (t, $^{3}J_{H,H}$ = 6.8 Hz, 2H), 1.48 (s, 3H), 1.22 (dd, $^{3}J_{H,H}$ = 6.9 Hz, $^{4}J_{H,H}$ = 1.7 Hz, 3H) ppm; ^{13}C NMR (176 MHz, C_6D_6): δ_C 189.3 (C_q), 168.9 (C_q), 141.1 (CH), 130.2 (CH), 39.8 (CH_2), 28.5 (CH_2), 22.7 (CH_3), 17.4 (CH_3) ppm. HRMS (ESI): $[M+H]^+$ calculated for $C_8H_{13}NO_2SH^+$ *m*/*z* 188.0740; found *m*/*z* 188.0737.[15]

Synthesis of S-(2-acetamidoethyl) (*Z*)-but-2-enethioate (S30).

A mixture of but-2-ynoic acid (100 mg, 1.19 mmol), quinoline (5 mg, 0.04 mmol) and Lindlar's catalyst (23 mg) in Et_2O (10 mL) was stirred in a H_2 atmosphere (10 bar) for 1 h at room temperature. The catalyst was removed by filtration and the solvents were evaporated. The product (*Z*)-but-2-enoic acid (40 mg, 0.46 mmol, 39%) was obtained by column chromatography on silica gel (pentane / Et_2O, 2:1). ^{1}H NMR (700 MHz, $CDCl_3$): δ_H 6.47 (dq, $^{3}J_{H,H}$ = 11.5 Hz, $^{3}J_{H,H}$ = 7.3 Hz, 1H), 5.83 (dq, $^{3}J_{H,H}$ = 11.6 Hz, $^{4}J_{H,H}$ = 1.8 Hz, 1H), 2.16 (dd, $^{3}J_{H,H}$ = 7.3 Hz, $^{3}J_{H,H}$ = 1.8 Hz, 3H) ppm; ^{13}C NMR (176 MHz, $CDCl_3$): δ_C 172.0 (C_q), 148.0 (CH), 120.2 (CH), 15.8 (CH_3) ppm.

A mixture of (*Z*)-but-2-enoic acid (20 mg, 0.23 mmol) and triethylamine (47 mg, 0.46 mmol) in CH_2Cl_2 (2 mL) was stirred for 10 mins with ice cooling. $ClCO_2Et$ (50 mg, 0.46 mmol) was added to this solution. After 2 h, N-acetylcysteamine (29 mg, 0.23 mmol) was added. The mixture was stirred for 3 h at room temperature and then concentrated under reduced pressure. The residue was purified by HPLC to yield **S30** (6 mg, 0.03 mmol, 14%). ^{1}H NMR (500 MHz, C_6D_6): δ_H 5.86 (dq, $^{3}J_{H,H}$ = 11.2 Hz, $^{4}J_{H,H}$ = 1.7 Hz, 1H), 5.51 (dq, $^{3}J_{H,H}$ = 11.3 Hz, $^{3}J_{H,H}$ = 7.3 Hz, 1H), 3.21 (dt, $^{3}J_{H,H}$ = 5.8 Hz, $^{3}J_{H,H}$ = 6.8 Hz, 2H), 2.84 (t, $^{3}J_{H,H}$ = 6.7 Hz, 2H), 1.92 (dd, $^{3}J_{H,H}$ = 7.3 Hz, $^{4}J_{H,H}$ = 1.8 Hz, 3H), 1.47 (s, 3H) ppm; ^{13}C NMR (126 MHz, C_6D_6): δ_C 189.4 (C_q), 168.9 (C_q), 141.8 (CH), 127.2 (CH), 39.7 (CH_2), 28.8 (CH_2), 22.7 (CH_3), 16.2 (CH_3) ppm. HRMS (ESI): $[M+H]^+$ calculated for $C_8H_{13}NO_2SH^+$ *m*/*z* 188.0740; found *m*/*z* 188.0738.[15]

Synthesis of S-(2-acetamidoethyl) (*E*)-2-methylbut-2-enethioate (S32).

Following the same procedure as for (*R*)-**1**, **S31** (50 mg, 0.50 mmol) was converted into S-(2-acetamidoethyl) (*E*)-2-methylbut-2-enethioate (**S32**) that was obtained as a

colourless oil (77 mg, 0.38 mmol, 77%). ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 6.87 (qq, $^{3}J_{H,H}$ = 6.9 Hz, $^{4}J_{H,H}$ = 1.3 Hz, 1H), 6.04 (br s, NH), 3.44 (q, $^{3}J_{H,H}$ = 6.1 Hz, 2H), 3.07 (t, $^{3}J_{H,H}$ = 6.6 Hz, 2H), 1.98 (s, 3H), 1.87 (quin, $^{4}J_{H,H}$ = 1.1 Hz, 3H), 1.84 (dq, $^{3}J_{H,H}$ = 6.9 Hz, $^{4}J_{H,H}$ = 1.0 Hz, 3H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ_C 194.0 (C_q), 170.6 (C_q), 137.0 (CH), 137.0 (C_q), 40.1 (CH_2), 28.4 (CH_2), 23.3 (CH_3), 14.6 (CH_3), 12.3 (CH_3) ppm.[2] HRMS (APCI): $[M+H]^+$ calculated for $C_9H_{15}NO_2SH^+$ *m*/*z* 202.0896; found *m*/*z* 202.0895.

Synthesis of S-(2-acetamidoethyl) (2*E*,4*E*)-hexa-2,4-dienethioate (16).

Following the same procedure as for (*R*)-**1**, **S33** (50 mg, 0.45 mmol) was converted into S-(2-acetamidoethyl) (2*E*,4*E*)-hexa-2,4-dienethioate (**16**) that was obtained as a pale yellow oil (63 mg, 0.30 mmol, 66%). ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 7.27 (dd, $^{3}J_{H,H}$ = 15.2 Hz, $^{3}J_{H,H}$ = 10.8 Hz, 1H), 5.98 (dd, $^{3}J_{H,H}$ = 15.2 Hz, $^{4}J_{H,H}$ = 0.7 Hz, 1H), 5.68 (dddq, $^{3}J_{H,H}$ = 15.1 Hz, $^{3}J_{H,H}$ = 10.8 Hz, $^{4}J_{H,H}$ = 1.5 Hz, $^{4}J_{H,H}$ = 0.6 Hz, 1H), 5.59 (dq, $^{3}J_{H,H}$ = 15.1 Hz, $^{3}J_{H,H}$ = 6.7 Hz, 1H), 4.90 (br s, NH), 3.27 (dt, $^{3}J_{H,H}$ = 5.9 Hz, $^{3}J_{H,H}$ = 6.7 Hz, 2H), 2.93 (t, $^{3}J_{H,H}$ = 6.7 Hz, 2H), 1.49 (s, 3H), 1.34 (d, $^{3}J_{H,H}$ = 6.8, 3H) ppm; ^{13}C NMR (126 MHz, C_6D_6): δ_C 189.5 (C_q), 168.9 (C_q), 141.4 (CH), 141.1 (CH), 129.8 (CH), 126.4 (CH), 39.9 (CH_2), 28.7 (CH_2), 22.8 (CH_3), 18.5 (CH_3) ppm.[16]

Synthesis of S-(2-acetamidoethyl) (2*E*,4*Z*)-hexa-2,4-dienethioate (15).

Following the same procedure as for (*R*)-**1**, **S34** (52 mg, 0.46 mmol) was converted into **15** that was obtained as a pale yellow oil (47 mg, 0.22 mmol, 48%). ^{1}H NMR (500 MHz, C_6D_6): δ_H 7.69 (ddd, $^{3}J_{H,H}$ = 15.0 Hz, $^{3}J_{H,H}$ = 11.6 Hz, $^{4}J_{H,H}$ = 1.1 Hz, 1H), 6.04 (d, $^{3}J_{H,H}$ = 15.0 Hz, 1H), 5.75 (dddq, $^{3}J_{H,H}$ = 11.9 Hz, $^{3}J_{H,H}$ = 10.7 Hz, $^{4}J_{H,H}$ = 1.7 Hz, $^{4}J_{H,H}$ = 0.6 Hz, 1H), 5.53 (dq, $^{3}J_{H,H}$ = 10.7 Hz, $^{3}J_{H,H}$ = 7.3 Hz, 1H), 4.96 (br s, NH), 3.26 (q, $^{3}J_{H,H}$ = 6.4 Hz, 2H), 2.93 (t, $^{3}J_{H,H}$ = 6.7 Hz, 2H), 1.50 (br s, 3H), 1.35 (dd, $^{3}J_{H,H}$ = 7.3 Hz, $^{4}J_{H,H}$ = 1.7 Hz, 3H) ppm; ^{13}C NMR (126 MHz, C_6D_6): δ_C 189.8 (C_q), 169.1 (C_q), 137.7 (CH), 135.4 (CH), 128.2 (CH), 127.5 (CH), 39.9 (CH_2), 28.8 (CH_2), 22.9 (CH_3), 13.8 (CH_3) ppm. HRMS (APCI): $[M+H]^+$ calculated for $C_{10}H_{15}NO_2SH^+$ *m*/*z* 214.0896; found *m*/*z* 214.0895.

Synthesis of S-(2-acetamidoethyl) (*E*)-5-methylhexa-2,4-dienethioate (S36).

Following the same procedure as for (*R*)-**1**, **S35** (100 mg, 0.79 mmol) was converted into S-(2-acetamidoethyl) (*E*)-5-methylhexa-2,4-dienethioate (**S36**) that was obtained

as a pale yellow oil (62 mg, 0.27 mmol, 34%). ^{1}H NMR (500 MHz, C_6D_6): δ_H 7.66 (dd, $^{3}J_{H,H}$ = 14.9 Hz, $^{3}J_{H,H}$ = 11.6 Hz, 1H), 6.06 (d, $^{3}J_{H,H}$ = 14.9 Hz, 1H), 5.81 (br s, NH), 5.63 (doct, $^{3}J_{H,H}$ = 11.6 Hz, $^{4}J_{H,H}$ = 0.7 Hz, 1H), 3.37 (dt, $^{3}J_{H,H}$ = 5.9 Hz, $^{3}J_{H,H}$ = 6.8 Hz, 2H), 3.03 (t, $^{3}J_{H,H}$ = 6.7 Hz, 2H), 1.64 (s, 3H), 1.44 (br s, 3H), 1.39 (br s, 3H) ppm; ^{13}C NMR (126 MHz, C_6D_6): δ_C 189.6 (C_q), 169.5 (C_q), 148.4 (C_q), 137.3 (CH), 126.1 (CH), 124.0 (CH), 40.0 (CH_2), 28.7 (CH_2), 26.4 (CH_3), 22.8 (CH_3), 18.6 (CH_3) ppm. HRMS (APCI): $[M+H]^+$ calculated for $C_{11}H_{17}NO_2SH^+$ *m*/*z* 228.1053; found *m*/*z* 228.1048.

Synthesis of (2*E*,4*E*)-4-methylhexa-2,4-dienoic acid (S38).

Following the same procedure as for **S24**, **S37** (1.40 g, 9.08 mmol) was converted into (2*E*,4*E*)-4-methylhexa-2,4-dienoic acid (**S38**) that was obtained as a pale yellow oil (800 mg, 6.34 mmol, 70%). ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 10.26 (br s, 1H), 7.40 (d, $^{3}J_{H,H}$ = 15.6 Hz, 1H), 6.04 (q, $^{3}J_{H,H}$ = 7.0 Hz, 1H), 5.78 (d, $^{3}J_{H,H}$ = 15.6 Hz, 1H), 1.83 (d, $^{3}J_{H,H}$ = 7.2 Hz, 3H), 1.79 (m, 3H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ_C 173.4 (C_q), 152.0 (CH), 138.0 (CH), 134.0 (C_q), 114.6 (CH), 14.8 (CH_3), 11.9 (CH_3) ppm.

Synthesis of S-(2-acetamidoethyl) (2*E*,4*E*)-4-methylhexa-2,4-dienethioate (S39).

Following the same procedure as for (*R*)-**1**, **S38** (50 mg, 0.40 mmol) was converted into S-(2-acetamidoethyl) (2*E*,4*E*)-4-methylhexa-2,4-dienethioate (**S39**) that was obtained as a pale yellow oil (25 mg, 0.11 mmol, 28%). ^{1}H NMR (500 MHz, C_6D_6): δ_H 7.41 (dd, $^{3}J_{H,H}$ = 15.5 Hz, $^{4}J_{H,H}$ = 0.8 Hz, 1H), 6.13 (d, $^{3}J_{H,H}$ = 15.5 Hz, 1H), 5.55 (q, $^{3}J_{H,H}$ = 6.9 Hz, 1H), 5.27 (br s, NH), 3.34 (dt, $^{3}J_{H,H}$ = 5.7 Hz, $^{3}J_{H,H}$ = 6.7 Hz, 2H), 2.99 (t, $^{3}J_{H,H}$ = 6.7 Hz, 2H), 1.56 (s, 3H), 1.33 (m, 3H), 1.32 (d, $^{3}J_{H,H}$ = 7.4 Hz, 3H) ppm; ^{13}C NMR (126 MHz, C_6D_6): δ_C 189.7 (C_q), 169.2 (C_q), 146.0 (CH), 138.5 (CH), 133.8 (C_q), 122.8 (CH), 40.0 (CH_2), 28.7 (CH_2), 22.8 (CH_3), 14.5 (CH_3), 11.4 (CH_3) ppm. HRMS (APCI): $[M+H]^+$ calculated for $C_{11}H_{17}NO_2SH^+$ *m*/*z* 228.1053; found *m*/*z* 228.1051.

Synthesis of (2*E*,4*E*,6*E*)-6-methylocta-2,4,6-trienoic acid (S42).

Following the same procedure as for **S20**, **S40** (900 mg, 8.17 mmol) was converted into ethyl (2*E*,4*E*,6*E*)-6-methylocta-2,4,6-trienoate (**S41**) that was obtained as a pale yellow oil (856 mg, 4.75 mmol). Following the same procedure as for **S24**, **S41** (200 mg, 1.11 mmol) was further converted into (2*E*,4*E*,6*E*)-6-methylocta-2,4,6-trienoic acid (**S42**) that was obtained as a pale yellow oil (260 mg, 1.69 mmol, 21% over two steps). ^{1}H NMR (700 MHz, C_6D_6): δ_H 7.54 (dd, $^{3}J_{H,H}$ = 15.2 Hz, $^{3}J_{H,H}$ = 11.2 Hz, 1H), 6.22 (d,

$^{3}J_{H,H}$ = 15.2 Hz, 1H), 5.95 (dd, $^{3}J_{H,H}$ = 15.2 Hz, $^{3}J_{H,H}$ = 11.2 Hz, 1H), 5.86 (d, $^{3}J_{H,H}$ = 15.2 Hz, 1H), 5.41 (q, $^{3}J_{H,H}$ = 6.8 Hz, 1H), 1.44 (d, $^{3}J_{H,H}$ = 6.8 Hz, 3H), 1.43 (br s, 3H) ppm; ^{13}C NMR (176 MHz, C_6D_6): δ_C 173.5 (C_q), 148.1 (CH), 146.9 (CH), 134.9 (C_q), 133.0 (CH), 123.7 (CH), 119.5 (CH), 14.2 (CH_3), 11.7 (CH_3) ppm. HRMS (ESI): $[M-H]^-$ calculated for $C_9H_{11}O_2^-$ *m*/*z* 151.0765; found *m*/*z* 151.0763.

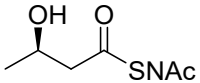
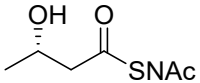
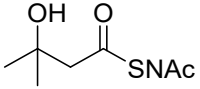
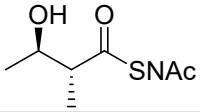
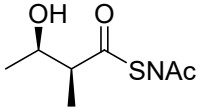
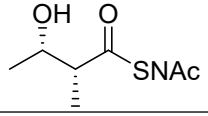
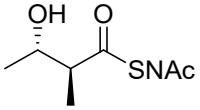
Synthesis of S-(2-acetamidoethyl) (2*E*,4*E*,6*E*)-6-methylocta-2,4,6-trienethioate (S43).

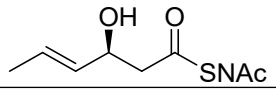
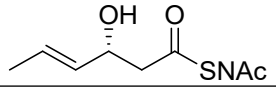
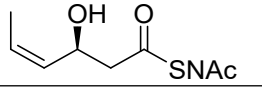
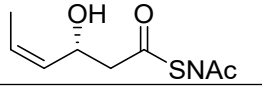
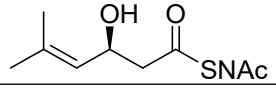
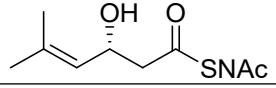
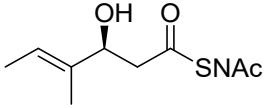
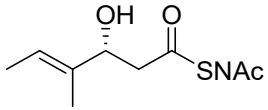
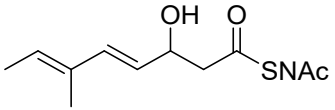
Following the same procedure as for (*R*)-**1**, **S42** (47 mg, 0.31 mmol) was converted into S-(2-acetamidoethyl) (2*E*,4*E*,6*E*)-6-methylocta-2,4,6-trienethioate (**S43**) that was obtained as a pale yellow solid (51 mg, 0.20 mmol, 65%). ^{1}H NMR (700 MHz, C_6D_6): δ_H 7.43 (ddd, $^{3}J_{H,H}$ = 15.1 Hz, $^{3}J_{H,H}$ = 11.1 Hz, $^{4}J_{H,H}$ = 0.8 Hz, 1H), 6.27 (d, $^{3}J_{H,H}$ = 15.2 Hz, 1H), 6.08 (d, $^{3}J_{H,H}$ = 15.0 Hz, 1H), 5.92 (dd, $^{3}J_{H,H}$ = 15.1 Hz, $^{3}J_{H,H}$ = 11.1 Hz, 1H), 5.43 (q, $^{3}J_{H,H}$ = 7.0 Hz, 1H), 4.99 (br s, NH), 3.31 (dt, $^{3}J_{H,H}$ = 6.0 Hz, $^{3}J_{H,H}$ = 6.7 Hz, 2H), 2.97 (t, $^{3}J_{H,H}$ = 6.7 Hz, 2H), 1.52 (br s, 3H), 1.47 (m, 3H), 1.45 (d, $^{3}J_{H,H}$ = 7.2 Hz, 1H) ppm; ^{13}C NMR (176 MHz, C_6D_6): δ_C 189.2 (C_q), 169.0 (C_q), 147.7 (CH), 142.0 (CH), 135.1 (C_q), 133.3 (CH), 127.1 (CH), 123.6 (CH), 40.0 (CH_2), 28.7 (CH_2), 22.8 (CH_3), 14.2 (CH_3), 11.7 (CH_3) ppm. HRMS (ESI): $[M+Na]^+$ calculated for $C_{13}H_{19}NO_2SNa^+$ *m*/*z* 276.1029; found *m*/*z* 276.1034.

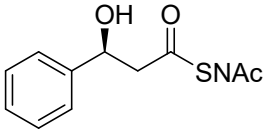
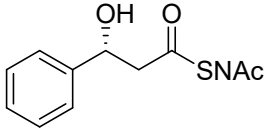
Synthesis of S-(2-acetamidoethyl) (*E*)-3-phenylprop-2-enethioate (S45).

Following the same procedure as for (*R*)-**1**, **S44** (62 mg, 0.42 mmol) was converted into S-(2-acetamidoethyl) (*E*)-3-phenylprop-2-enethioate (**S45**) that was obtained as a pale yellow oil (80 mg, 0.32 mmol, 77%). ^{1}H NMR (700 MHz, $CDCl_3$): δ_H 7.62 (d, $^{3}J_{H,H}$ = 15.8 Hz, 1H), 7.53 (m, 2H), 7.39 (m, 3H), 6.72 (d, $^{3}J_{H,H}$ = 15.8 Hz, 1H), 6.20 (br s, NH), 3.50 (q, $^{3}J_{H,H}$ = 6.1 Hz, 2H), 3.16 (t, $^{3}J_{H,H}$ = 6.7 Hz, 2H), 1.99 (s, 3H) ppm; ^{13}C NMR (176 MHz, $CDCl_3$): δ_C 190.3 (C_q), 170.6 (C_q), 141.4 (CH), 134.0 (C_q), 130.9 (CH), 129.1 (2xCH), 128.6 (2xCH), 124.7 (CH), 39.9 (CH_2), 28.6 (CH_2), 23.3 (CH_3) ppm. HRMS (APCI): $[M+H]^+$ calculated for $C_{13}H_{15}NO_2SH^+$ *m*/*z* 250.0896; found *m*/*z* 250.0892.

Table S1. Activity screening of dehydratases.

substrate	no.	BorDH2[a]	BorDH3	BorDH5	FosDH1	FosDH2	RifDH10	ShawDH1	ShawDH2	Cpz2	FabZ
short chain compounds											
	(*R*)-**1**	89%		11%	57%	53%			14%		36%
	(*S*)-**1**										
	**2**										
compounds with α-methyl branch											
	(2*R*,3*R*)-**3**	100%		47%	81%	40%					22%
	(2*S*,3*R*)-**3**										
	(2*R*,3*S*)-**3**										
	(2*S*,3*S*)-**3**										

structure	no.	BorDH2	BorDH3	BorDH5	FosDH1	FosDH2	RifDH10	ShawDH1	ShawDH2	Cpz2	FabZ
γ,δ-unsaturated compounds											
	(*S*)-**4**	100%	41%	36%	100%	100%	68%	28%	24%	69%	100%
	(*R*)-**4**										
	(*S*)-**5**	100%	46%	54%	100%	100%	58%	40%	6%	43%	42%
	(*R*)-**5**										
	(*S*)-**6**	100%	76%	83%	100%	100%	98%	8%	42%	16%	97%
	(*R*)-**6**										
	(*S*)-**7**	100%	47%	16%	100%	100%	99%	3%	4%	18%	75%
	(*R*)-**7**										
	(*rac*)-**8**	45%	33%	32%	15%	13%	29%			47%	15%

structure	no.	BorDH2	BorDH3	BorDH5	FosDH1	FosDH2	RifDH10	ShawDH1	ShawDH2	Cpz2	FabZ
aromatic compounds											
	(*S*)-**9**	100%	42%	25%	29%	100%	13%			23%	39%
	(*R*)-**9**										

[a] The efficiency of enzymatic conversions is indicated by colour code (dark green = full conversion (100%), green = partial conversion (99 – 50%), light green = partial conversion (49 – 1%), grey = no conversion (0%), based on peak integrations for the peaks highlighted in Figures S2 – S9).

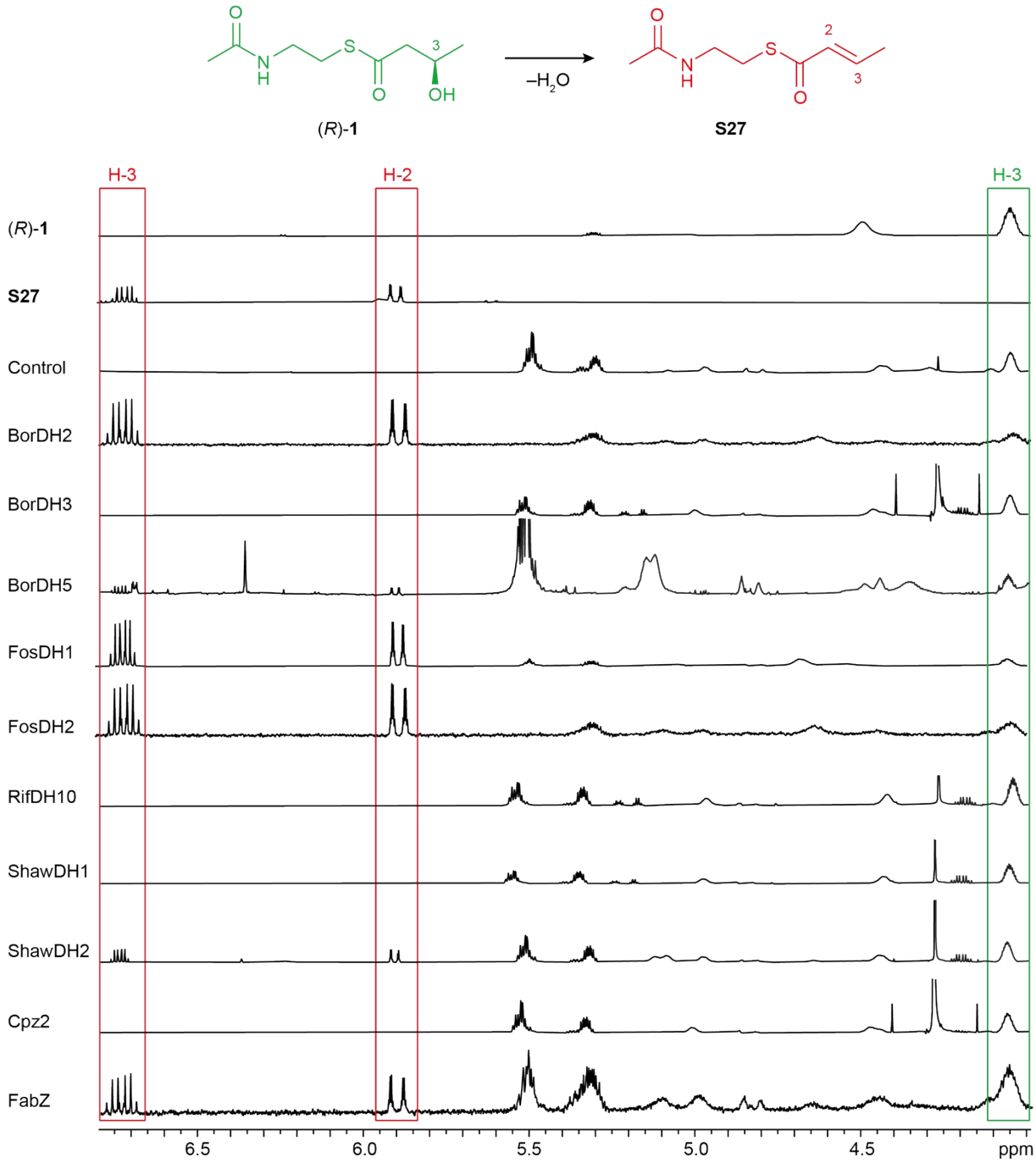


Figure S2. Enzymatic conversions of (*R*)-**1**.

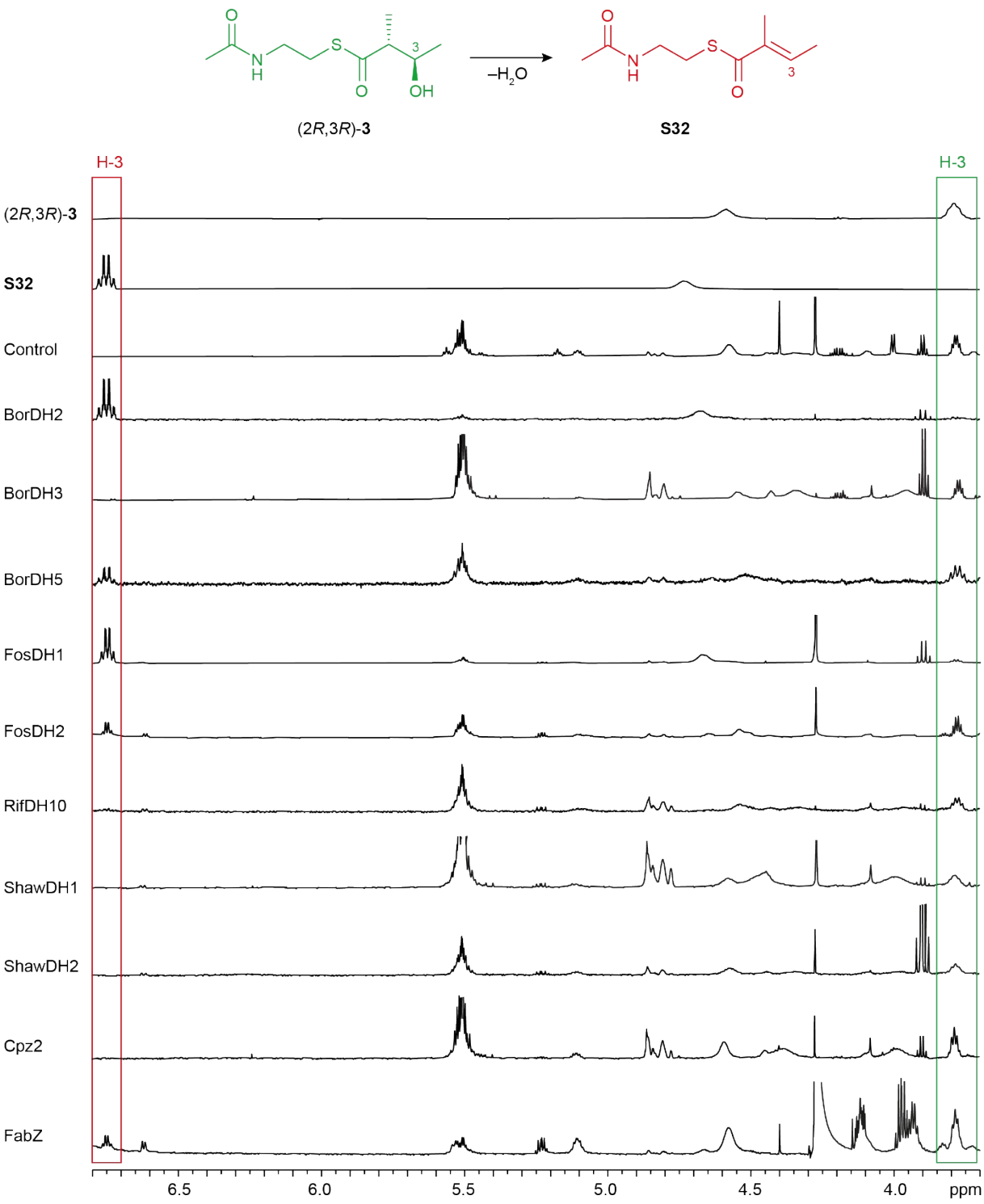


Figure S3. Enzymatic conversions of (2*R*,3*R*)-**3**.

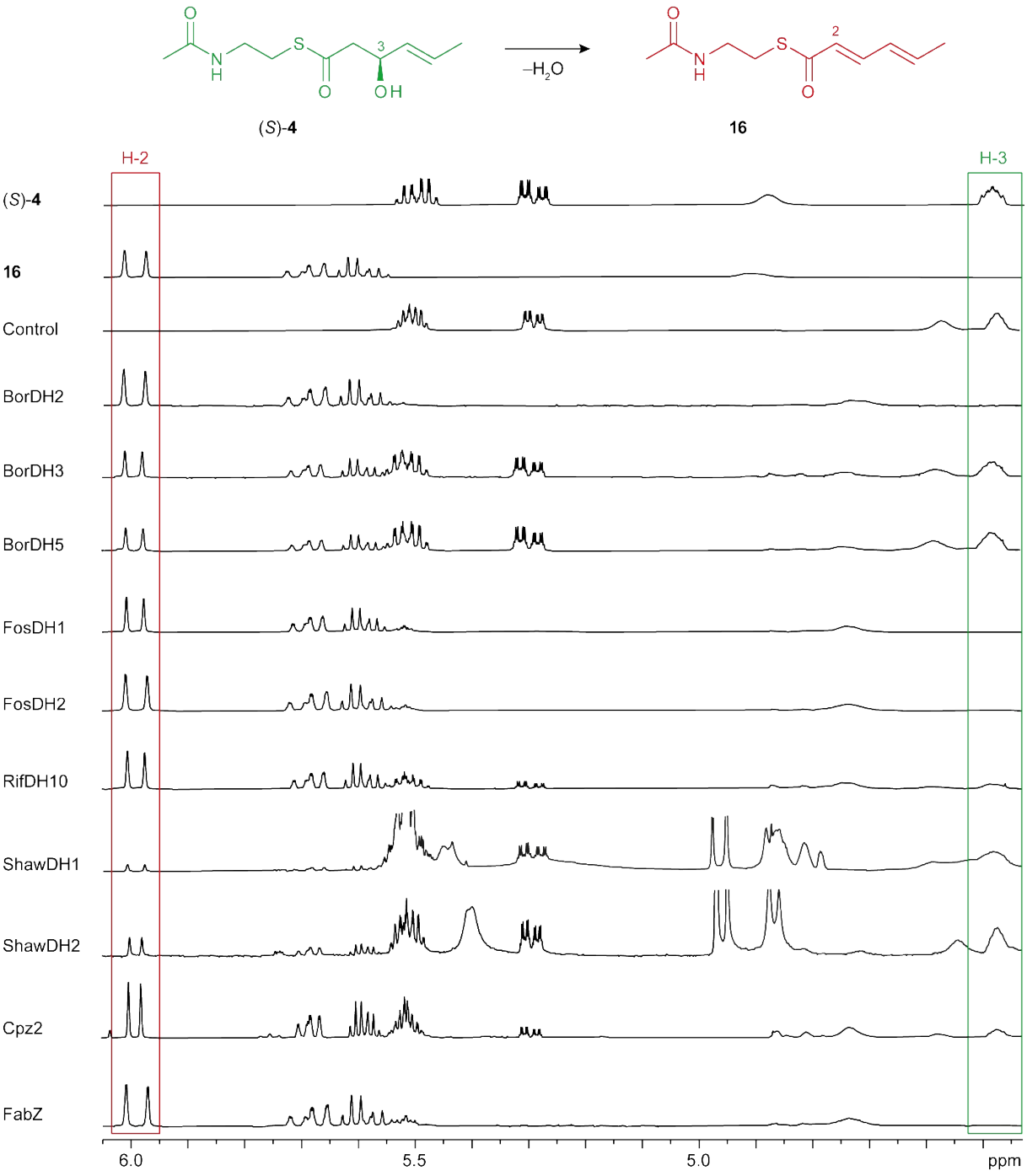


Figure S4. Enzymatic conversions of (*S*)-**4**.

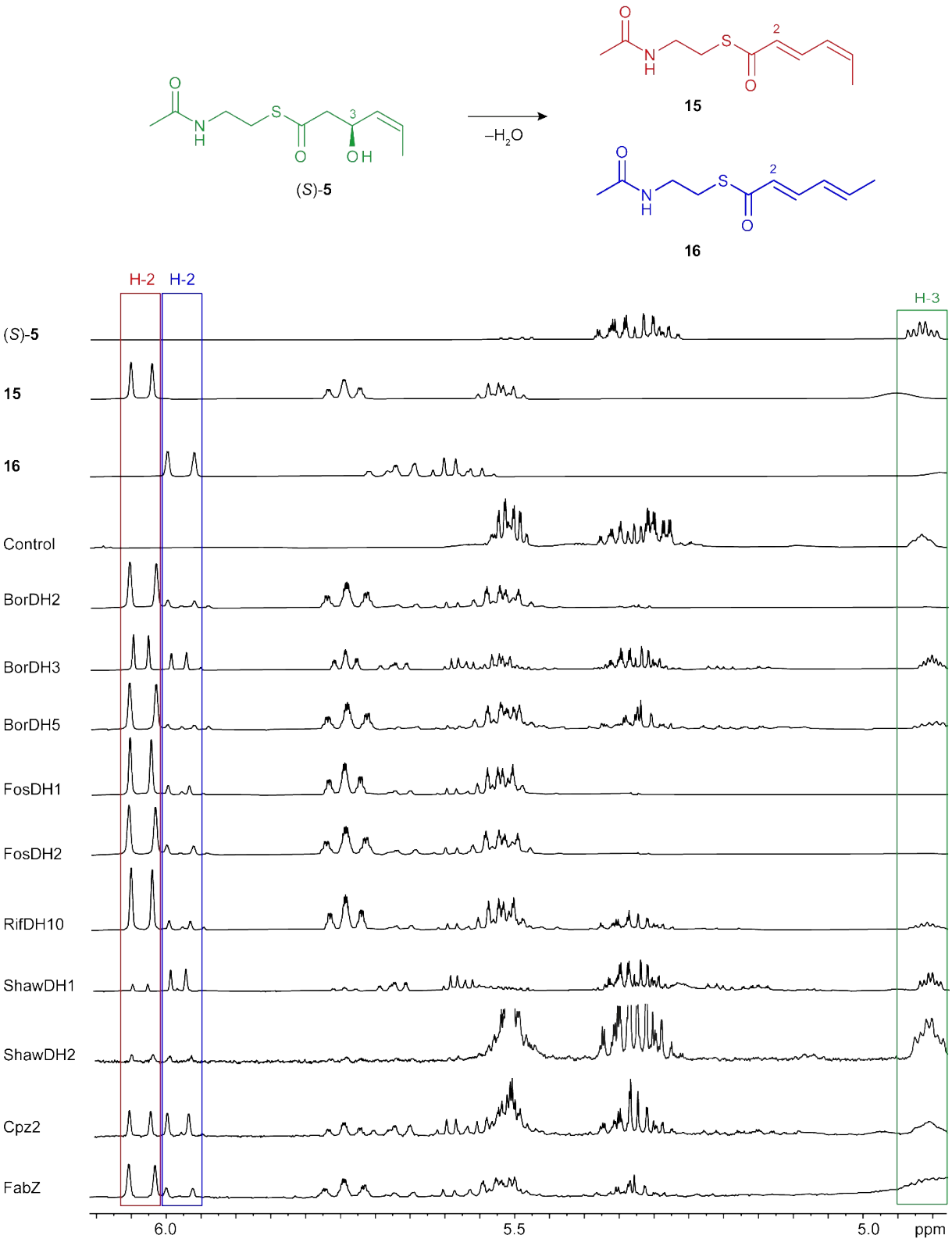


Figure S5. Enzymatic conversions of (*S*)-**5**.

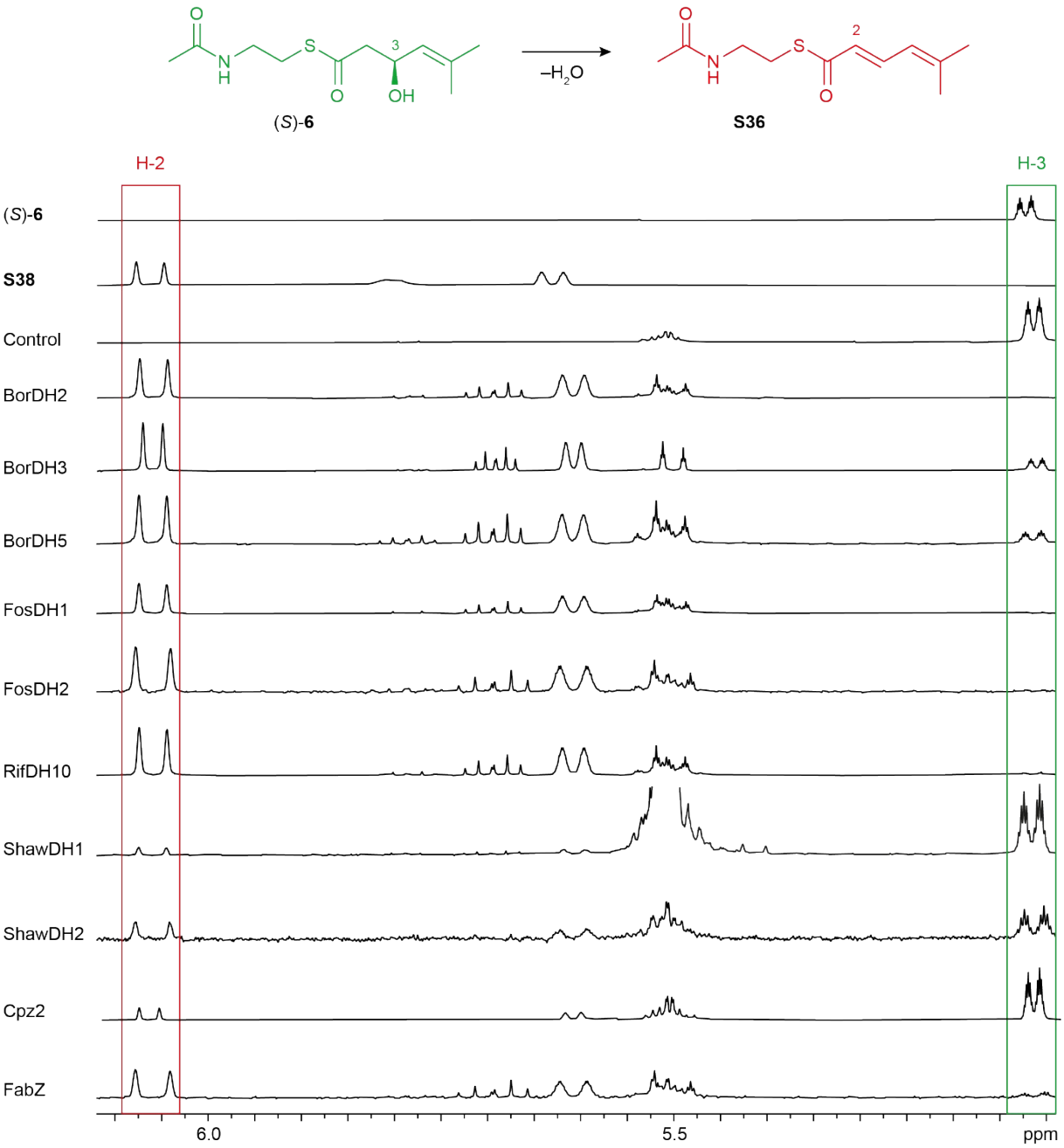


Figure S6. Enzymatic conversions of (*S*)-**6**.

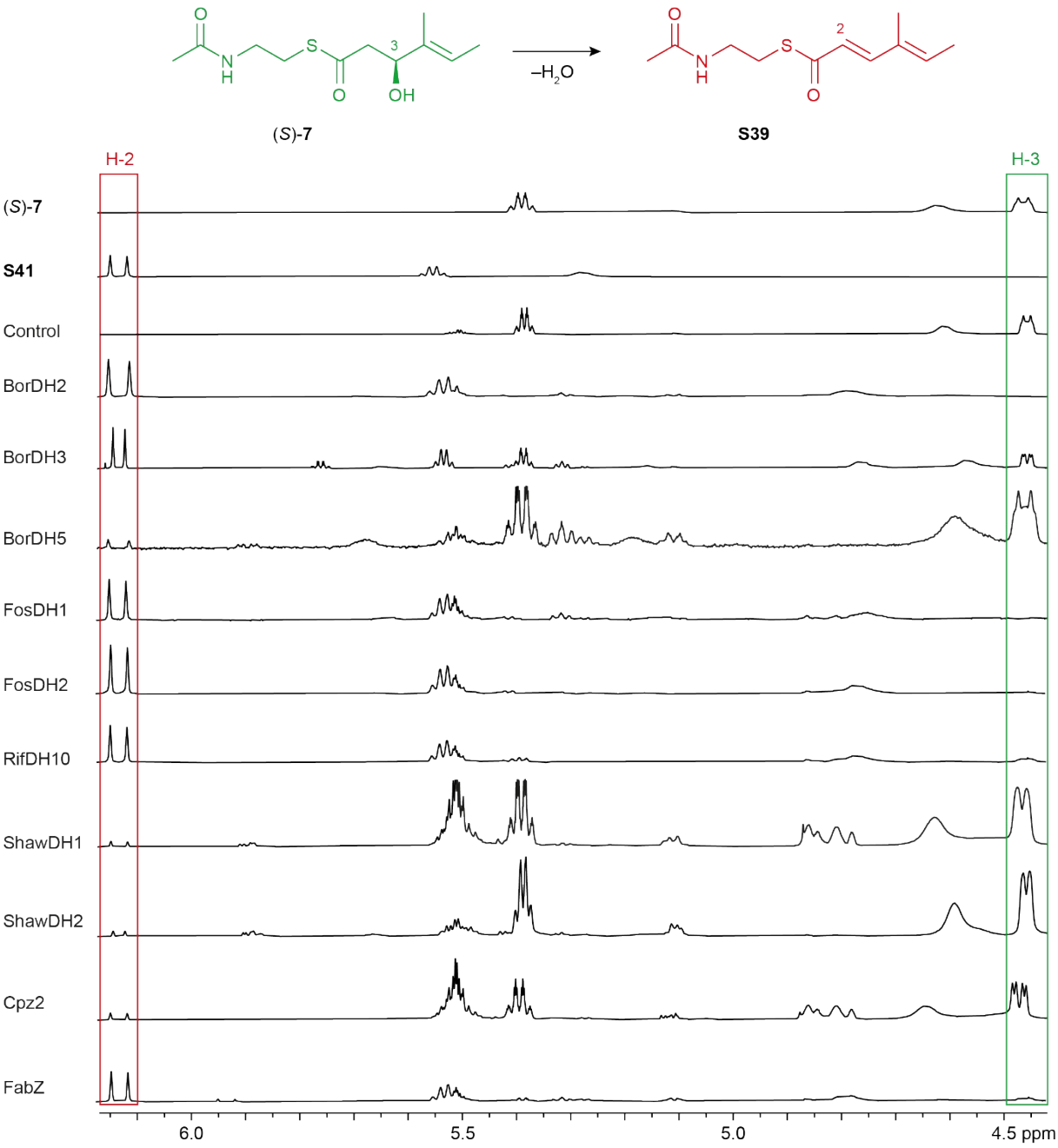


Figure S7. Enzymatic conversions of (*S*)-**7**.

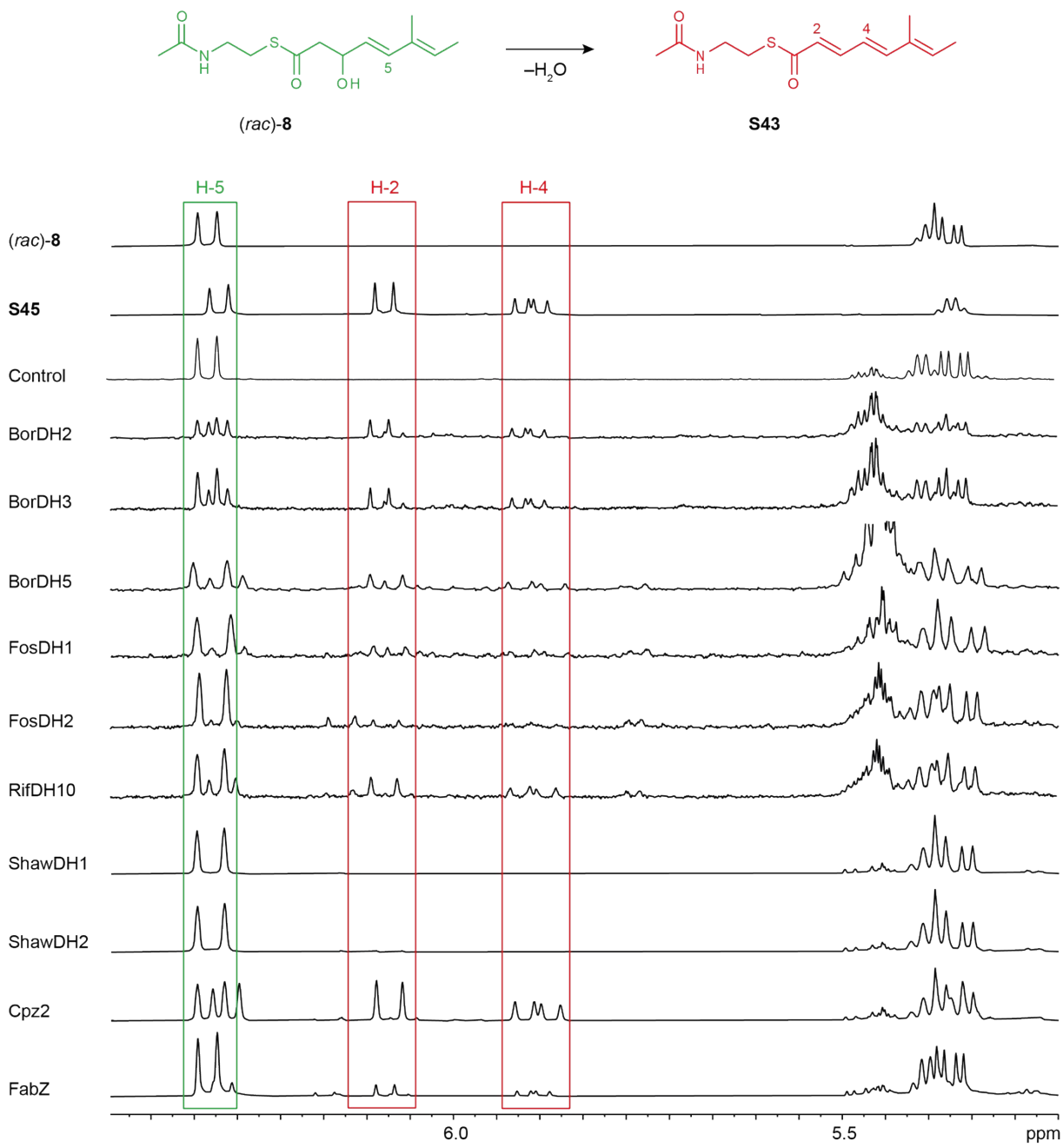


Figure S8. Enzymatic conversions of (*rac*)-**8**.

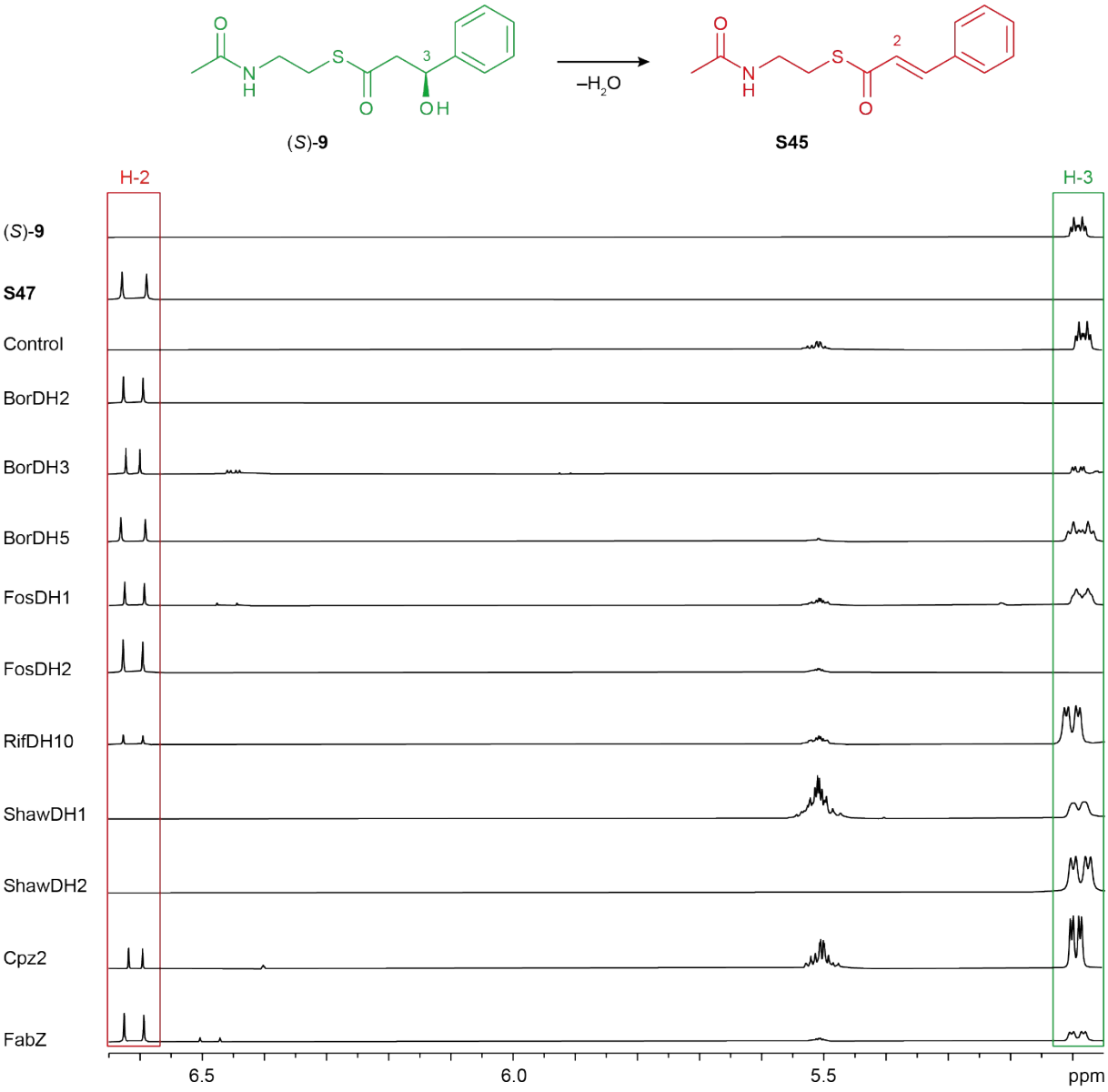
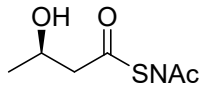
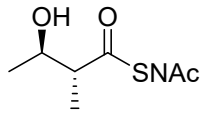
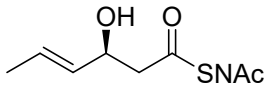
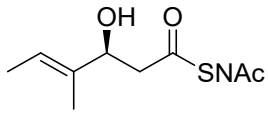
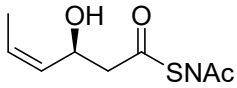
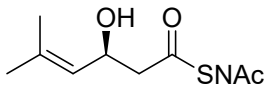
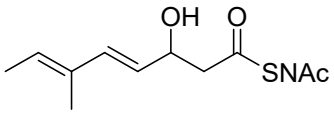
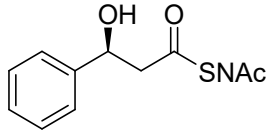
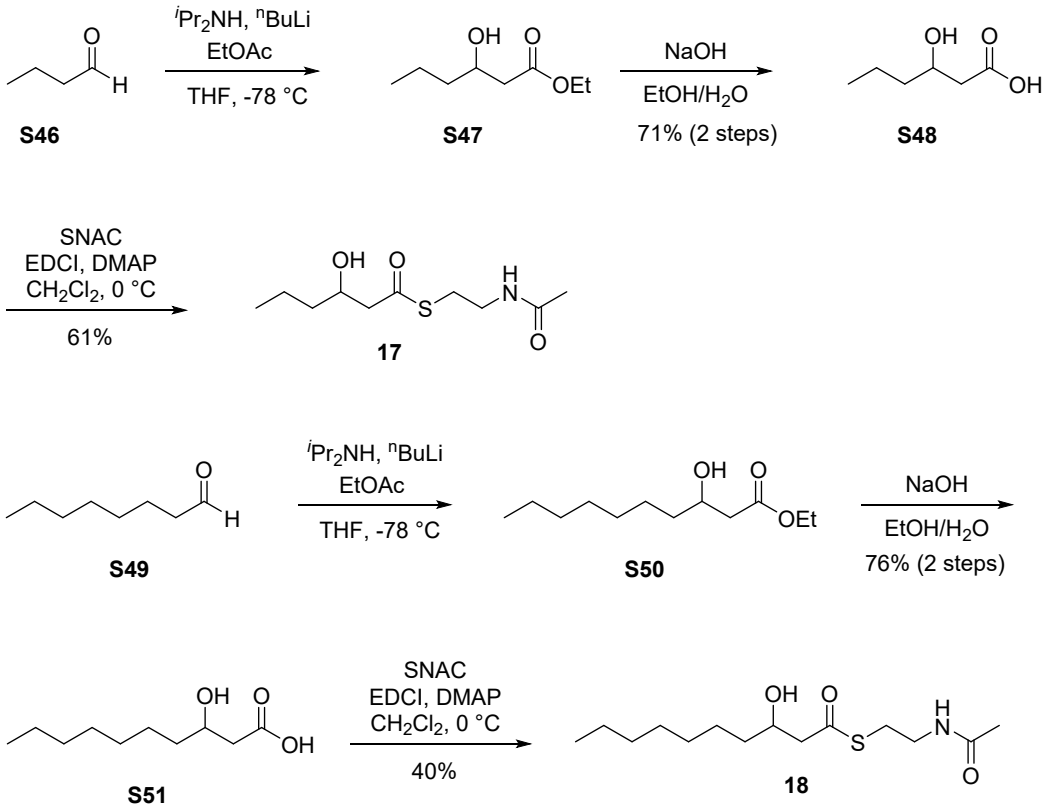


Figure S9. Enzymatic conversions of (*S*)-**9**.

Table S2. Conversion efficiencies of BorDH2 enzyme reactions.

compound	conversion[a]			
	experiment 1	**experiment 2**	**experiment 3**	**mean ± SD**
 (*R*)-**1**	88%	93%	95%	92 ± 4%
 (2*R*,3*R*)-**3**	100%	97%	100%	99 ± 1%
 (*S*)-**4**	100%	100%	100%	100 ± 0%
 (*S*)-**5**	100%	100%	100%	100 ± 0%
 (*S*)-**6**	100%	100%	100%	100 ± 0%
 (*S*)-**7**	100%	100%	100%	100 ± 0%
 (*rac*)-**8**	31%	35%	32%	33 ± 5%
 (*S*)-**9**	100%	100%	100%	100 ± 0%

[a] Determined by peak integration in the ^{1}H NMR spectra of crude extracts from enzyme reactions.



Scheme S11. Synthesis of (*rac*)-**17** and (*rac*)-**18**.

Synthesis of 3-hydroxyhexanoic acid (S48).

Following the same procedure as for **S23**, **S46** (720 mg, 10.0 mmol) was converted into ethyl 3-hydroxyhexanoate (**S47**) that was obtained as a pale yellow oil (1.42 g, 8.86 mmol). Following the same procedure as for **S24**, **S47** (300 mg, 1.87 mmol) was further converted into 3-hydroxyhexanoic acid (**S48**) that was obtained as a pale yellow oil (200 mg, 1.51 mmol, 71% over two steps). ^{1}H NMR (500 MHz, C_6D_6): δ_H 6.11 (br s, COOH), 3.92 (m, 1H), 2.30 (dd, $^{3}J_{H,H}$ = 16.1 Hz, $^{3}J_{H,H}$ = 9.1 Hz, 1H), 2.22 (dd, $^{3}J_{H,H}$ = 16.1 Hz, $^{3}J_{H,H}$ = 3.3 Hz, 1H), 1.36 (m, 2H), 1.22 (m, 1H), 1.14 (m, 1H), 0.82 (t, $^{3}J_{H,H}$ = 7.2 Hz, 3H) ppm; ^{13}C NMR (126 MHz, C_6D_6): δ_C 177.5 (C_q), 68.1 (CH), 41.7 (CH_2), 38.9 (CH_2), 19.0 (CH_2),14.1 (CH_3) ppm.

Synthesis of S-(2-acetamidoethyl) 3-hydroxyhexanethioate ((*rac*)-17).

Following the same procedure as for (*R*)-**1**, **S48** (100 mg, 0.76 mmol) was converted into S-(2-acetamidoethyl) 3-hydroxyhexanethioate ((*rac*)-**17**) that was obtained as a colourless oil (108 mg, 0.46 mmol, 61%). ^{1}H NMR (500 MHz, C_6D_6): δ_H 5.35 (br s, NH), 4.03 (m, 1H), 3.23 (m, 2H), 2.82 (m, 1H), 2.76 (m, 1H), 2.52 (m, 1H), 2.41 (m, 1H), 1.60 (s, 3H), 1.37 (m, 2H), 1.26 (m, 1H), 1.17 (m, 1H), 0.83 (t, $^{3}J_{H,H}$ = 7.2 Hz, 3H) ppm;

^{13}C NMR (126 MHz, C_6D_6): δ_C 198.6 (C_q), 170.0 (C_q), 68.7 (CH), 52.0 (CH_2), 39.4 (CH_2), 39.2 (CH_2), 29.2 (CH_2), 22.8 (CH_3), 19.0 (CH_2), 14.1 (CH_3) ppm.

Synthesis of 3-hydroxydecanoic acid (S51).

Following the same procedure as for **S23**, **S49** (1.28 g, 10.0 mmol) was converted into ethyl 3-hydroxydecanoate (**S50**) that was obtained as a pale yellow solid (1.92 g, 8.88 mmol). Following the same procedure as for **S24**, **S50** (500 mg, 2.31 mmol) was further converted into 3-hydroxydecanoic acid (**S51**) that was obtained as a colourless solid (372 mg, 1.98 mmol, 76% over two steps). ^{1}H NMR (500 MHz, C_6D_6): δ_H 3.89 (m, 1H), 2.29 (dd, $^3J_{H,H}$ = 16.2 Hz, $^3J_{H,H}$ = 8.7 Hz, 1H), 2.22 (dd, $^3J_{H,H}$ = 16.2 Hz, $^3J_{H,H}$ = 3.5 Hz, 1H), 1.24 (m, 12H), 0.92 (t, $^3J_{H,H}$ = 7.0 Hz, 3H) ppm; ^{13}C NMR (126 MHz, C_6D_6): δ_C 177.6 (C_q), 68.2 (CH), 41.7 (CH_2), 36.9 (CH_2), 32.2 (CH_2), 29.9 (CH_2), 29.7 (CH_2), 25.8 (CH_2), 23.1 (CH_2),14.4 (CH_3) ppm.

Synthesis of S-(2-acetamidoethyl) 3-hydroxydecanethioate ((*rac*)-18).

Following the same procedure as for (*R*)-**1**, **S51** (100 mg, 0.53 mmol) was converted into S-(2-acetamidoethyl) 3-hydroxydecanethioate ((*rac*)-**18**) that was obtained as a colourless solid (61 mg, 0.21 mmol, 40%). ^{1}H NMR (500 MHz, C_6D_6): δ_H 4.77 (br s, NH), 4.02 (m, 1H), 3.18 (m, 2H), 2.74 (m, 2H), 2.50 (dd, $^2J_{H,H}$ = 14.8 Hz, $^3J_{H,H}$ = 8.8 Hz, 1H), 2.42 (dd, $^2J_{H,H}$ = 14.8 Hz, $^3J_{H,H}$ = 3.3 Hz, 1H), 1.50 (s, 3H), 1.38 (m, 2H), 1.25 (m, 10H), 0.91 (t, $^3J_{H,H}$ = 7.0 Hz, 3H) ppm; ^{13}C NMR (126 MHz, C_6D_6): δ_C 198.7 (C_q), 169.6 (C_q), 69.0 (CH), 52.0 (CH_2), 39.1 (CH_2), 37.3 (CH_2), 32.2 (CH_2), 29.9 (CH_2), 29.7 (CH_2), 29.3 (CH_2), 25.9 (CH_2), 23.1 (CH_2), 22.8 (CH_3), 14.4 (CH_3) ppm.

Compounds (*rac*)-**4**, (*rac*)-**6**, (*rac*)-**7** and (*rac*)-**9** were synthesised analogously through aldol addition of the ester enolate of ethyl acetate to the corresponding aldehyde, saponification and esterification with N-acetylcysteamine. Spectroscopic data matched those reported above for the enantiomerically pure compounds.

Kinetic resolutions with BorDH2

S-(2-Acetamidoethyl) (*E*)-3-hydroxyhex-4-enethioate ((*rac*)-**4**, 35 mg, 0.15 mmol) was dissolved in DMSO (50 µL) and BorDH2 dissolved in incubation buffer (6.9 mg/mL, 3.5 mL) was added. The reaction was incubated at 30 °C for 16 h and then extracted with C_6H_6 (3 x 5 mL). After evaporation of the solvents the product was isolated through silica gel chromatography to obtain S-(2-acetamidoethyl) (*R*,*E*)-3-hydroxyhex-4-enethioate ((*R*)-**4**) as a colourless oil (12 mg, 34%, 96% *ee* determined by HPLC analysis on a chiral stationary phase, Figure S10). Optical rotation: [*α*]25 D = +17.4 (*c* 0.10, CH_2Cl_2).

The same procedure was applied for the following transformations:

Compound (*rac*)-**6** (25 mg, 0.10 mmol) was converted into S-(2-acetamidoethyl) (*R*)-3-hydroxy-5-methylhex-4-enethioate ((*R*)-**6**) that was obtained as a colourless oil (9 mg, 0.04 mmol, 36%, 89% *ee* determined by HPLC analysis on a chiral stationary phase, Figure S11). Optical rotation: [*α*]25 D = +16.3 (*c* 0.13, CH_2Cl_2).

Compound (*rac*)-**7** (38 mg, 0.15 mmol) was converted into S-(2-acetamidoethyl) (*R*,*E*)-3-hydroxy-4-methylhex-4-enethioate ((*R*)-**7**) that was obtained as a colourless oil (13 mg, 0.05 mmol, 34%, >99% *ee* determined by HPLC analysis on a chiral stationary phase, Figure S12). Optical rotation: [*α*]25 D = +15.6 (*c* 0.20, CH_2Cl_2).

Compound (*rac*)-**9** (50 mg, 0.19 mmol) was converted into S-(2-acetamidoethyl) (*R*)-3-hydroxy-3-phenylpropanethioate ((*R*)-**9**) that was obtained as a colourless oil (22 mg, 0.08 mmol, 44%, 99% *ee*). Optical rotation: [*α*]25 D = +19.5 (*c* 0.30, CH_2Cl_2). For determination of the enantiomeric excess the enzyme product and a racemic sample were converted into 2,2-dimethyl-4-phenyl-1,3-dioxane through reduction with $LiAlH_4$ and subsequent treatment with *p*-TsOH (10 mol-%) in 2,2-dimethoxypropane for 20 h at room temperature (Scheme S12).[19] The enantiomeric excess of the enzyme product was then determined to be 99% *ee* by GC analysis on a chiral stationary phase (Figure S13).

Compound (*rac*)-**17** (22 mg, 0.09 mmol) was converted into S-(2-acetamidoethyl) (*S*)-3-hydroxyhexanethioate ((*S*)-**17**) that was obtained as a colourless oil (13 mg, 0.06 mmol, 59%, 19% *ee* determined by HPLC analysis on a chiral stationary phase, Figure S14).

Compound (*rac*)-**18** (20 mg, 0.07 mmol) was converted into S-(2-acetamidoethyl) (*S*)-3-hydroxydecanethioate ((*S*)-**18**) that was obtained as a white solid (14 mg, 0.05 mmol, 70%, 3% *ee* determined by HPLC analysis on a chiral stationary phase, Figure S15).

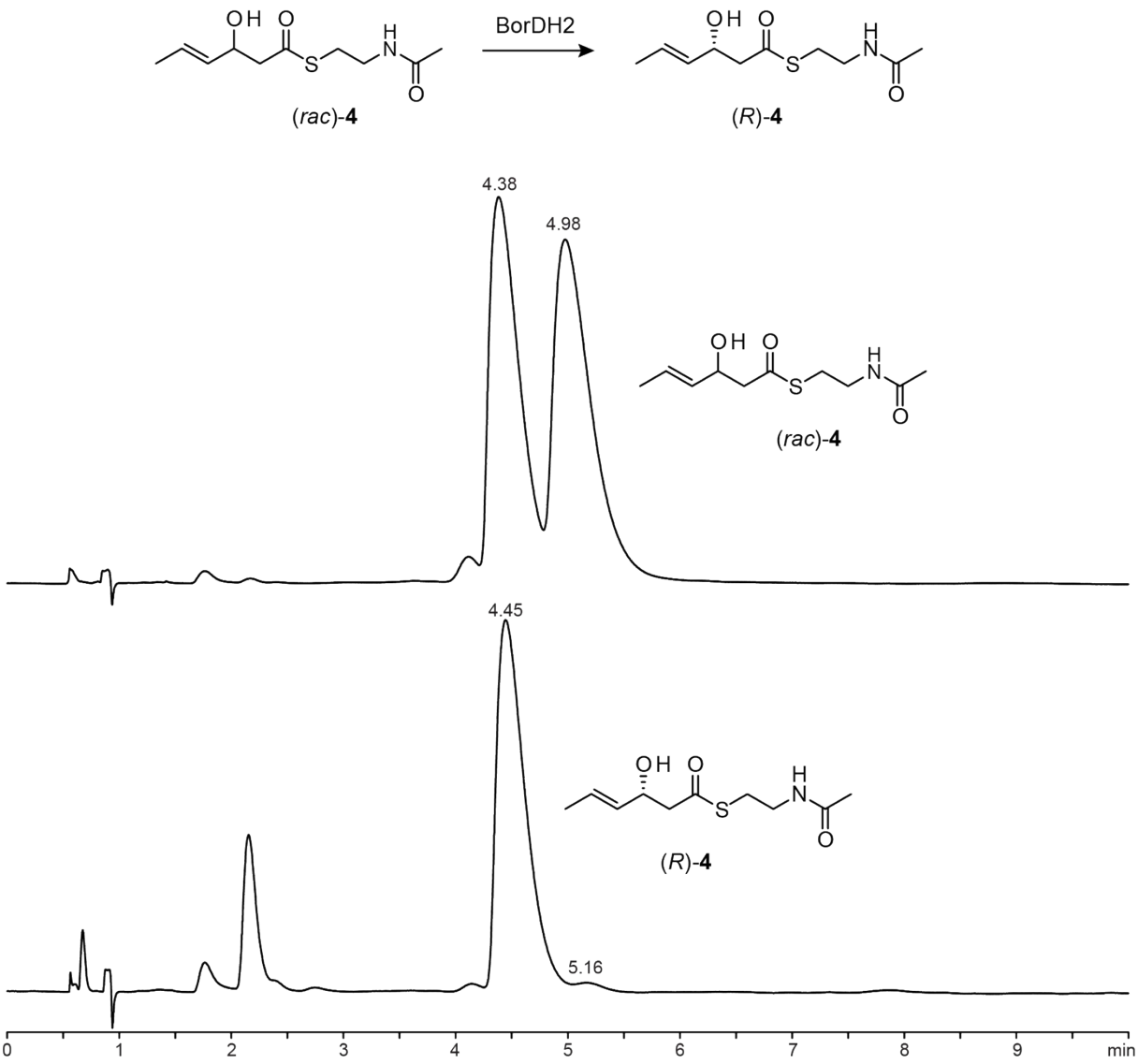


Figure S10. HPLC chromatograms of synthetic (*rac*)-**4** (top) and (*R*)-**4** (bottom) obtained by kinetic resolution with BorDH2.

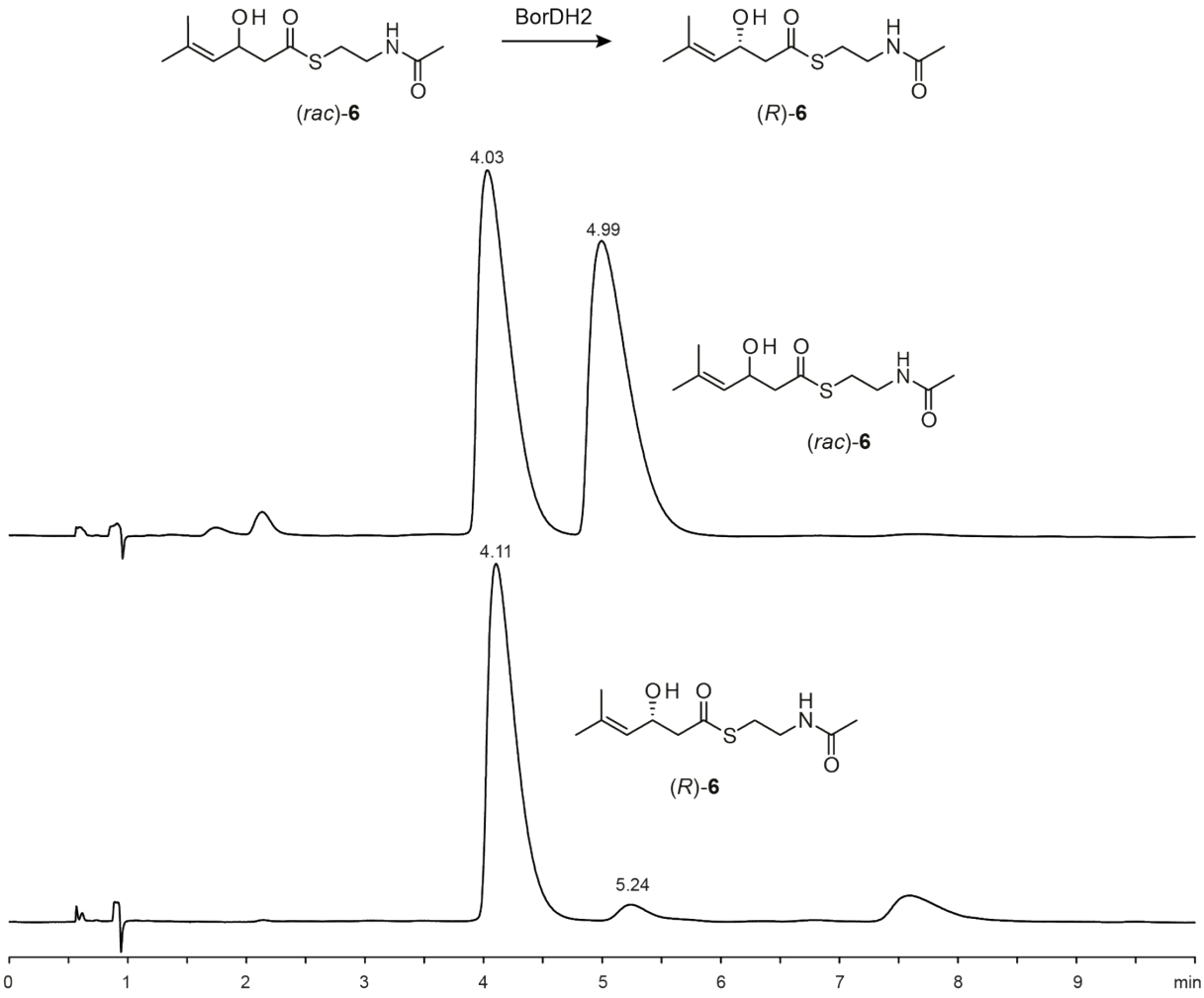


Figure S11. HPLC chromatograms of synthetic (*rac*)-**6** (top) and (*R*)-**6** (bottom) obtained by kinetic resolution with BorDH2.

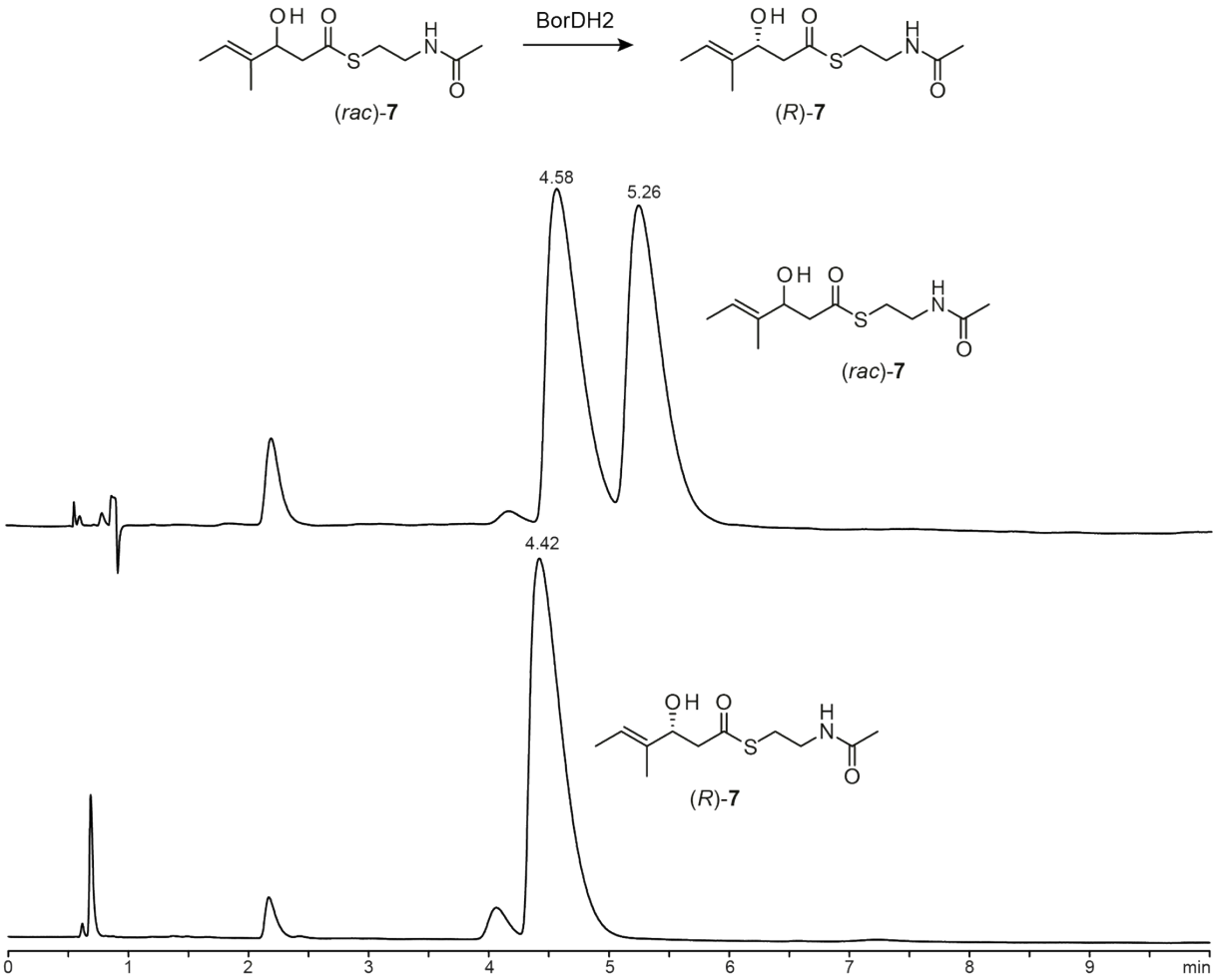
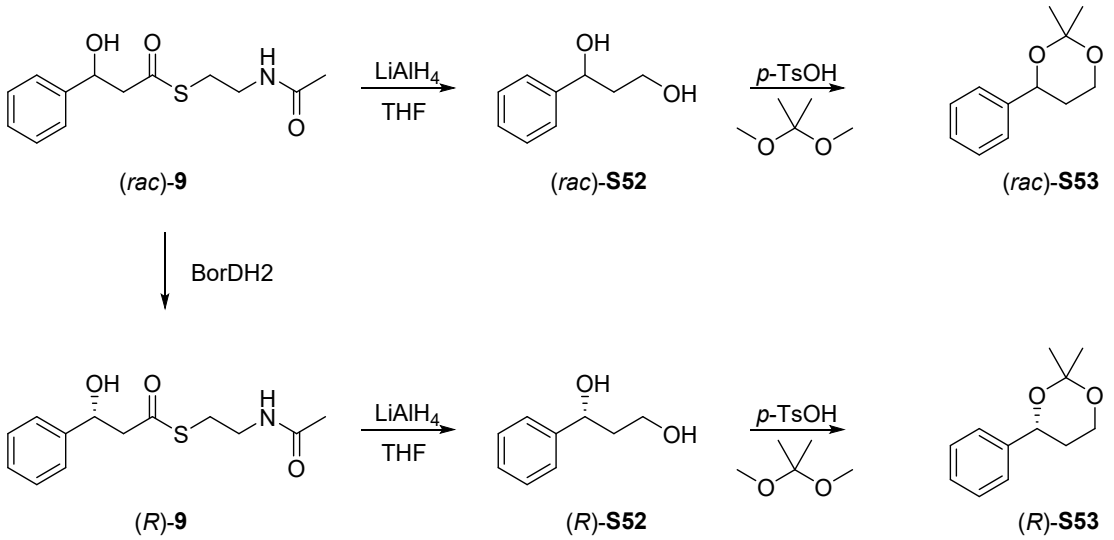


Figure S12. HPLC chromatograms of synthetic (*rac*)-**7** (top) and (*R*)-**7** (bottom) obtained by kinetic resolution with BorDH2.



Scheme S12. Kinetic resolution of (*rac*)-**9** and synthesis of (rac)- and (*R*)-**S53** for GC analysis.

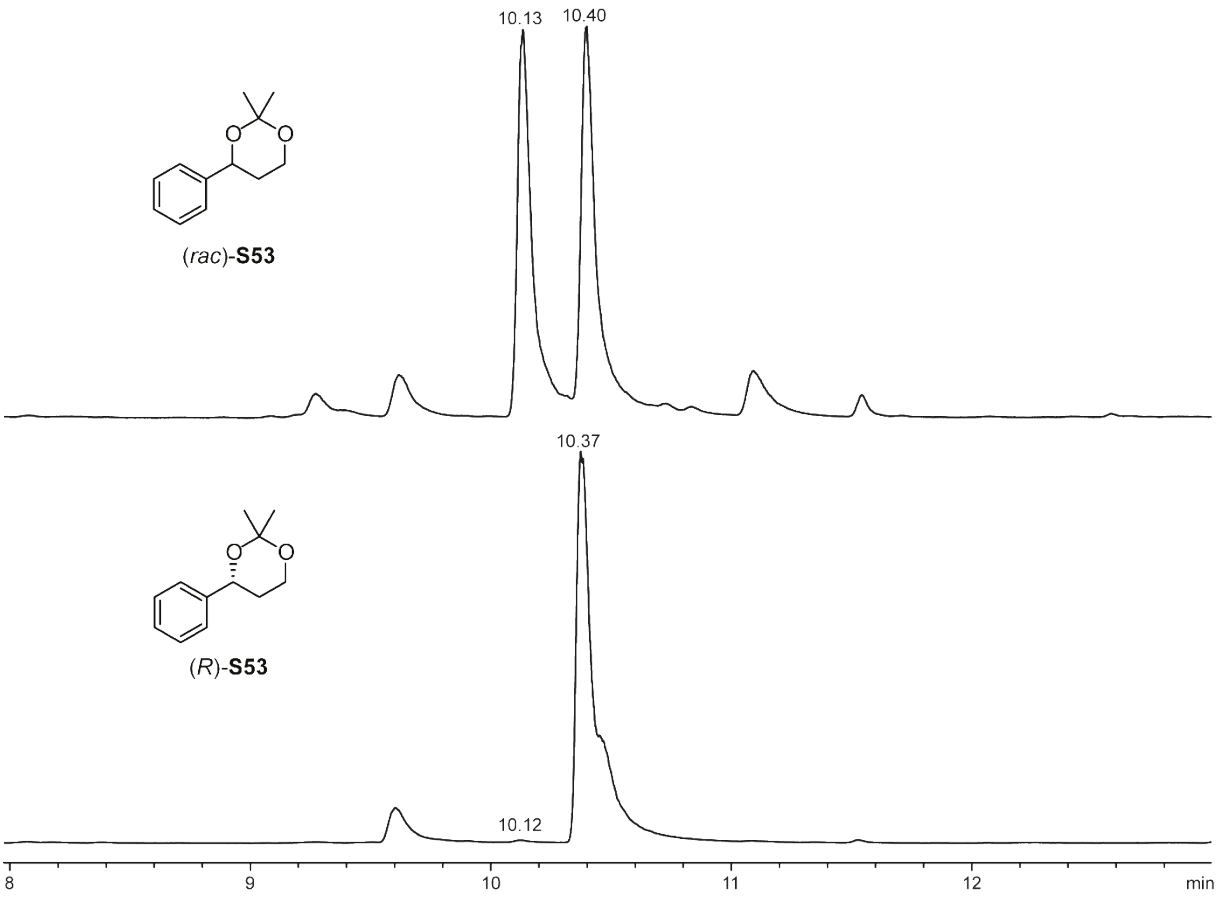


Figure S13. Gas chromatograms of synthetic (*rac*)-**S53** (top) and (*R*)-**S53** (bottom) obtained by kinetic resolution with BorDH2.

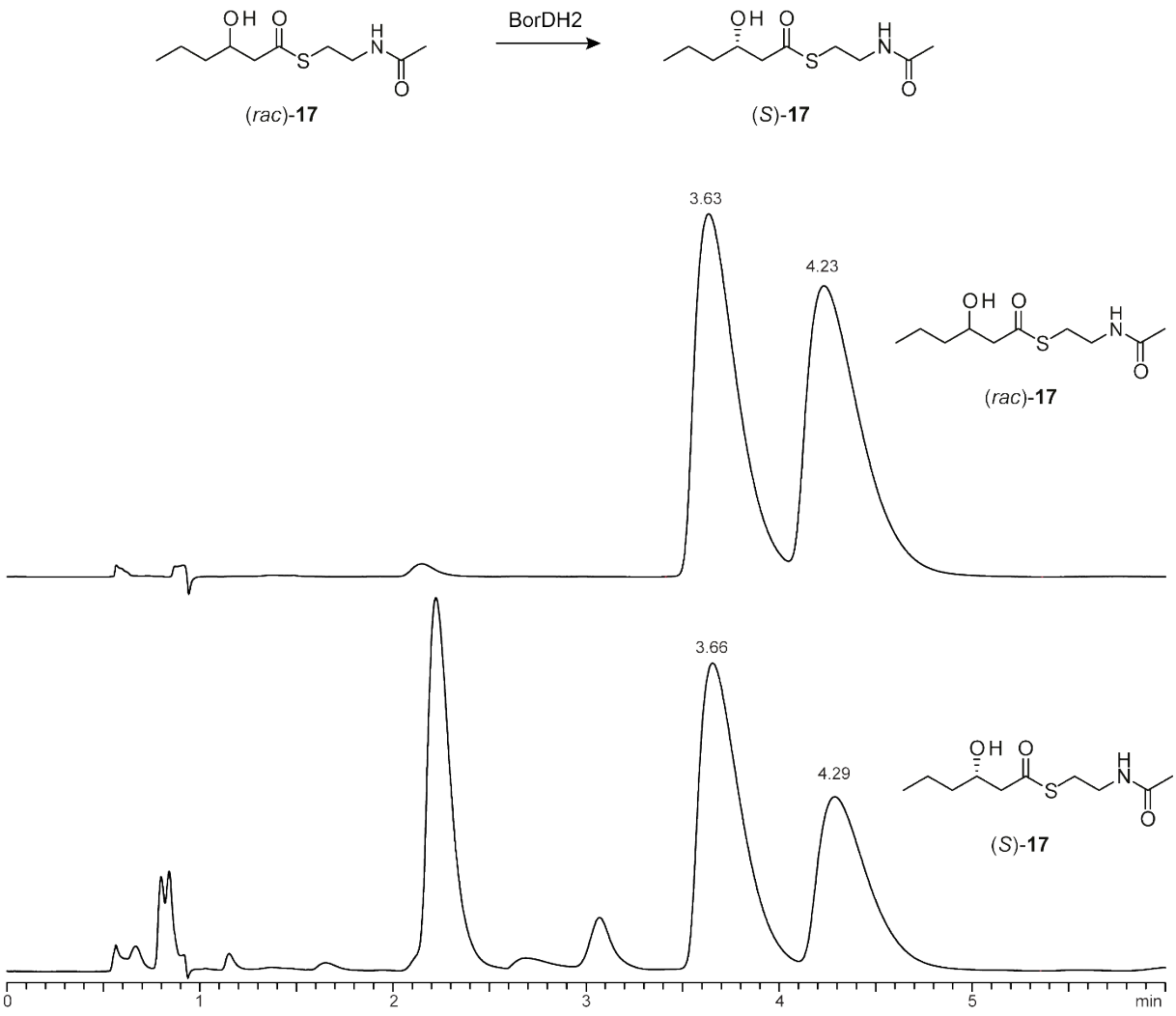


Figure S14. HPLC chromatograms of synthetic (*rac*)-**17** (top) and (*S*)-**17** (bottom) obtained by kinetic resolution with BorDH2.

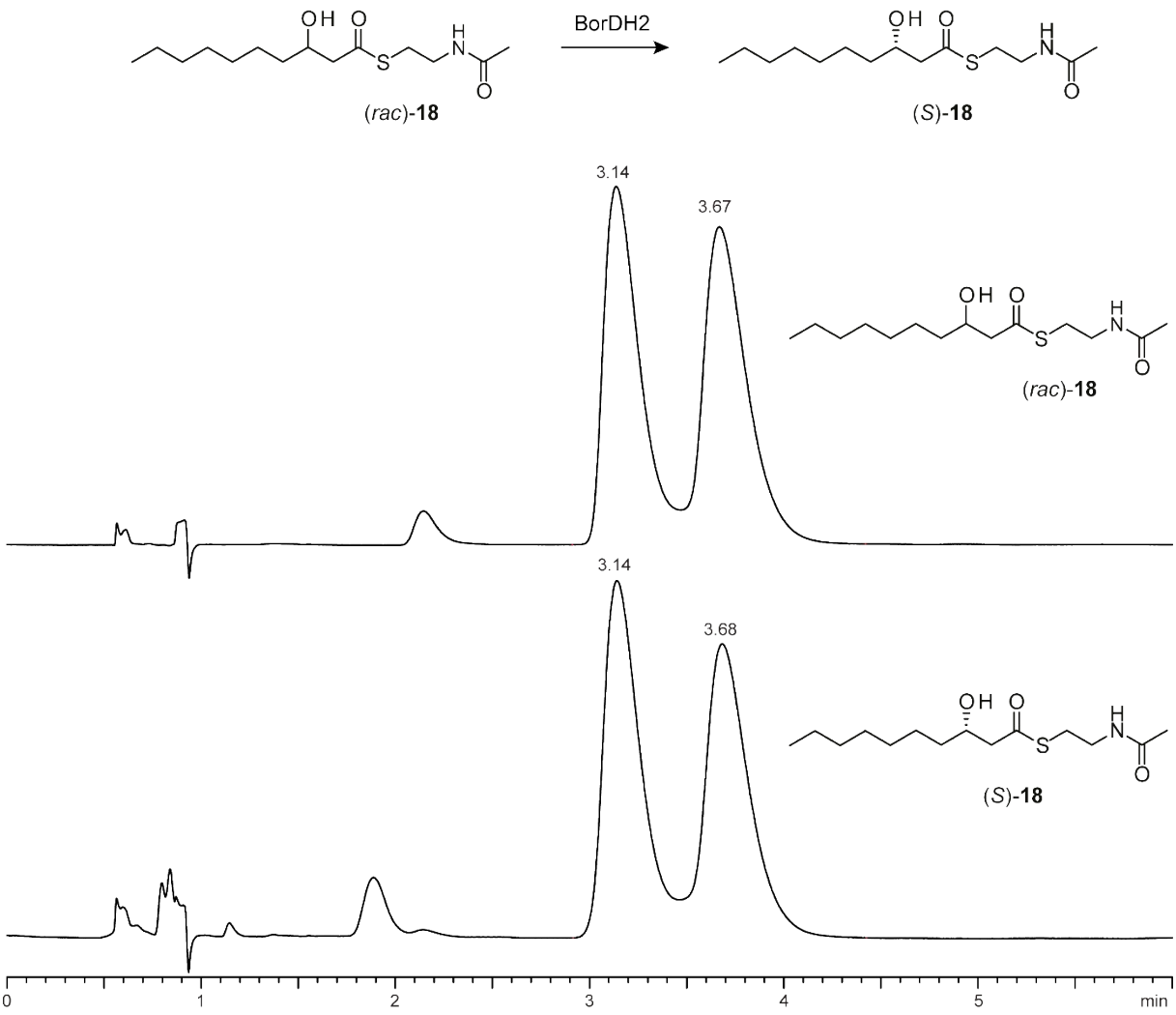


Figure S15. HPLC chromatograms of synthetic (*rac*)-**18** (top) and (*S*)-**18** (bottom) obtained by kinetic resolution with BorDH2.

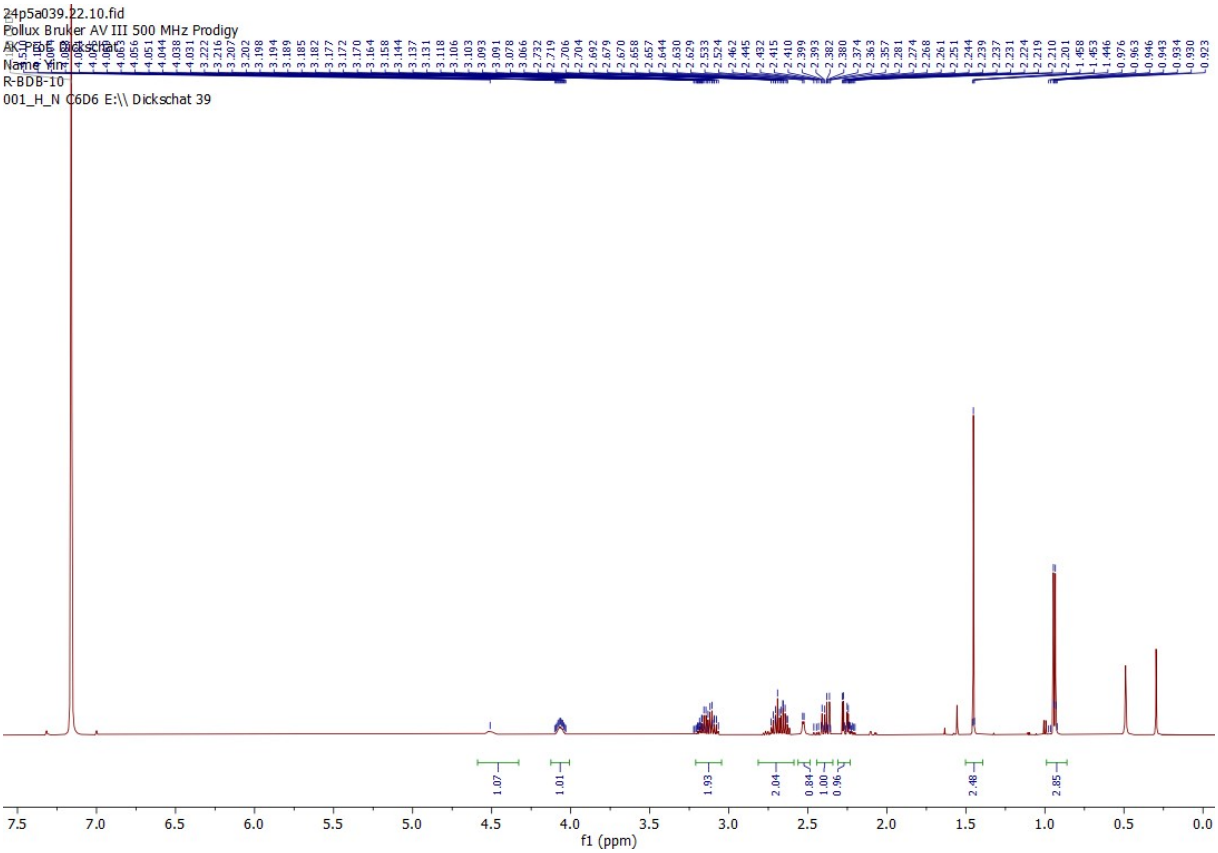


Figure S16. ^{1}H NMR (500 MHz, C_6D_6) of (*R*)-**1**.

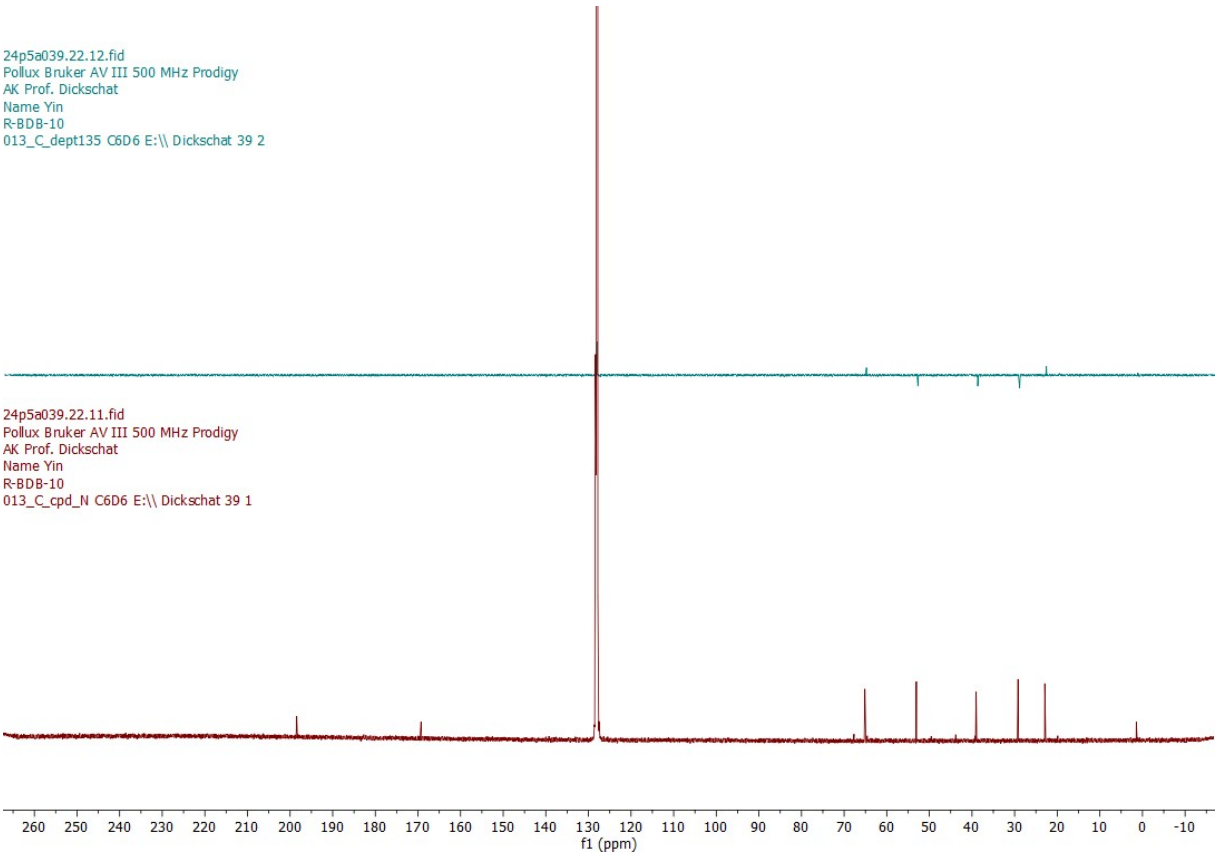


Figure S17. ^{13}C NMR (126 MHz, C_6D_6) of (*R*)-**1**.

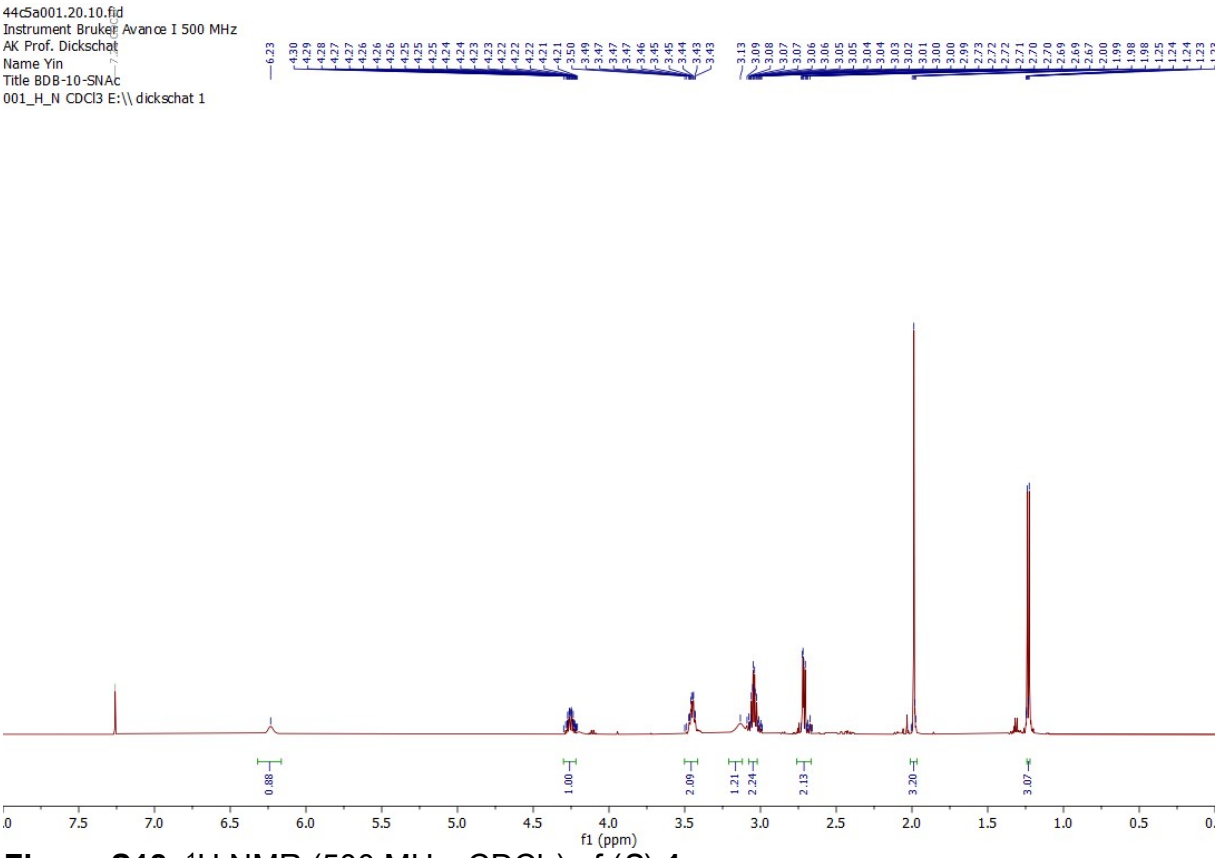


Figure S18. ^{1}H NMR (500 MHz, $CDCl_3$) of (*S*)-**1**.

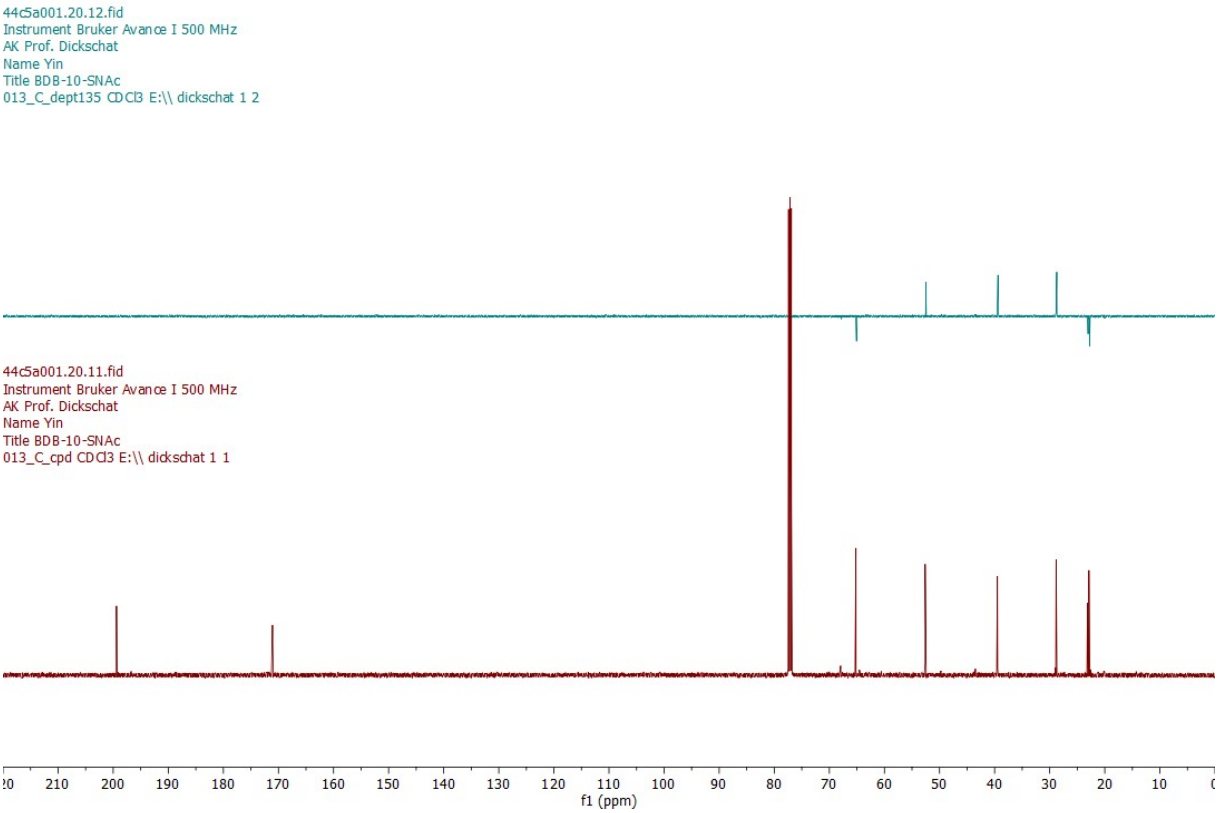


Figure S19. ^{13}C NMR (126 MHz, $CDCl_3$) of (*S*)-**1**.

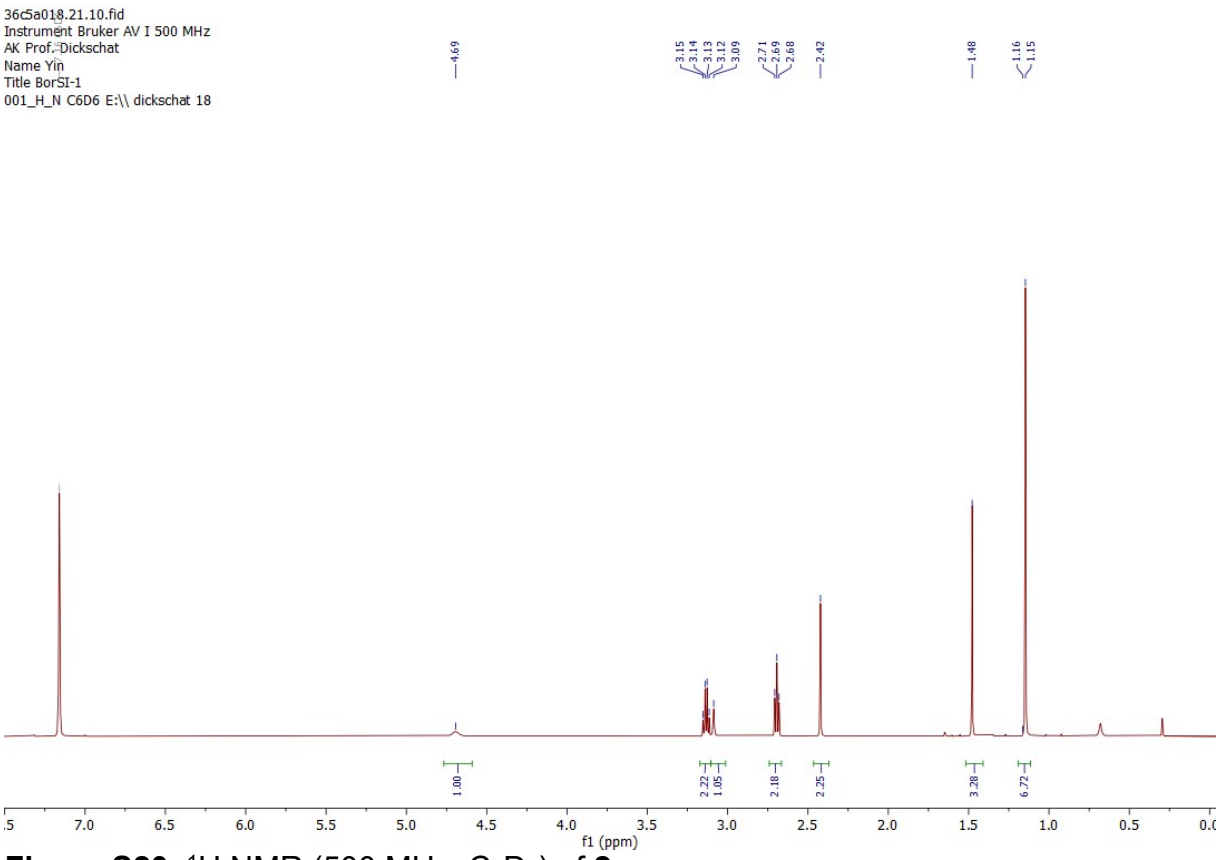


Figure S20. ^{1}H NMR (500 MHz, C_6D_6) of **2**.

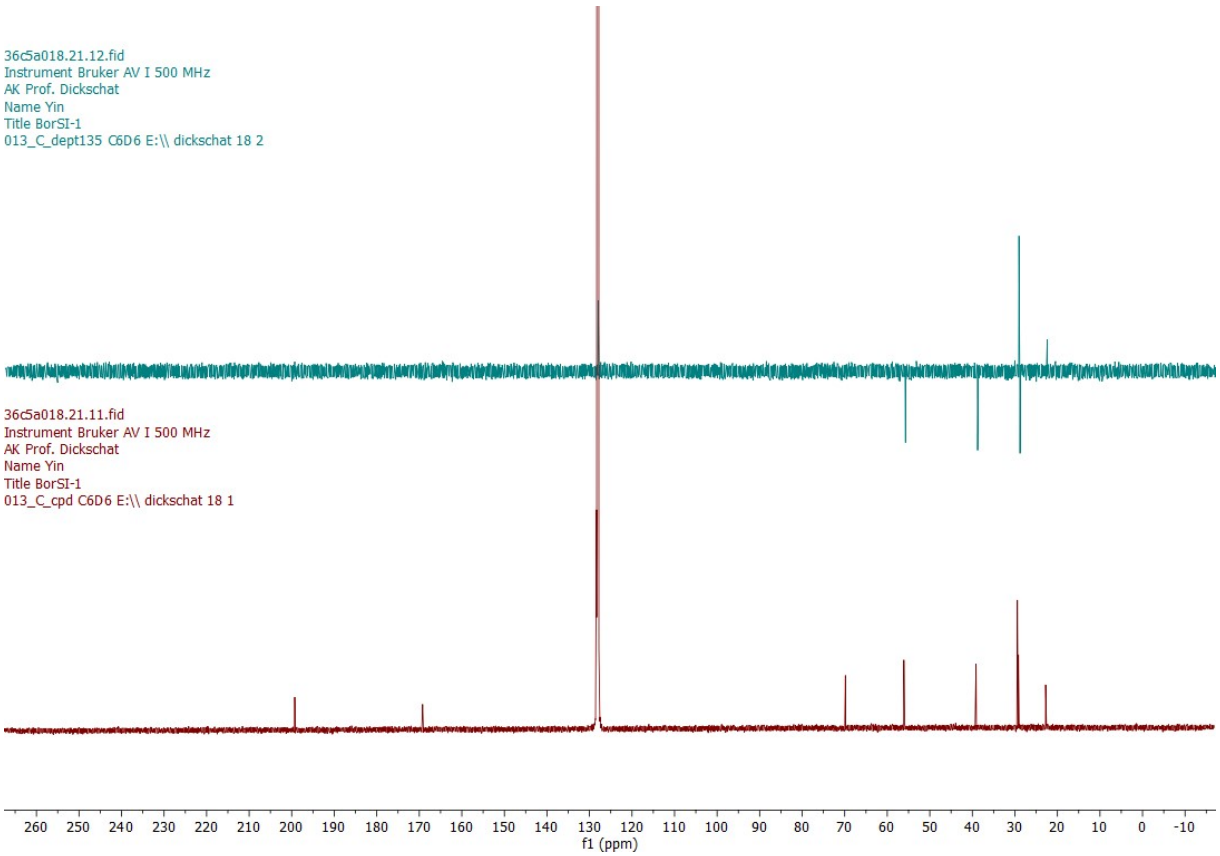


Figure S21. ^{13}C NMR (126 MHz, C_6D_6) of **2**.

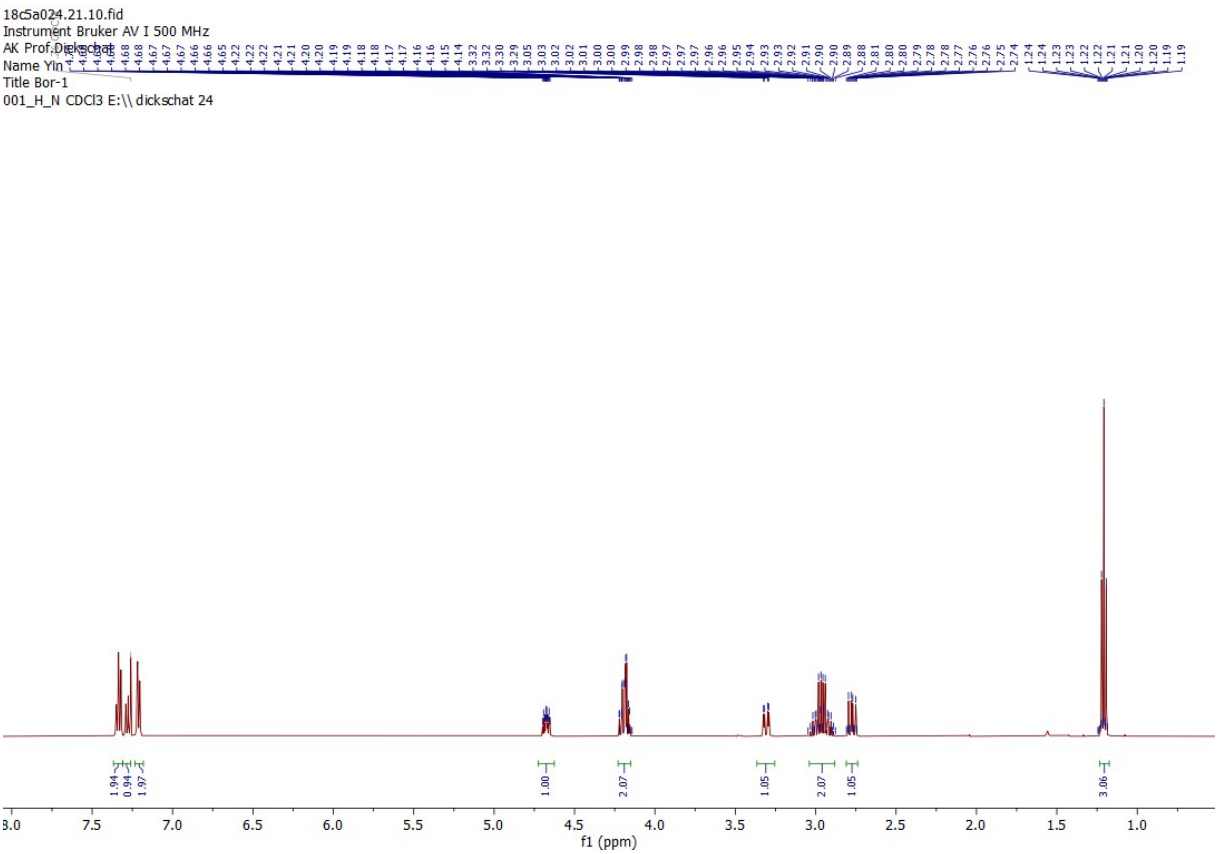


Figure S22. ^{1}H NMR (500 MHz, $CDCl_3$) of (*S*)-**S4**.

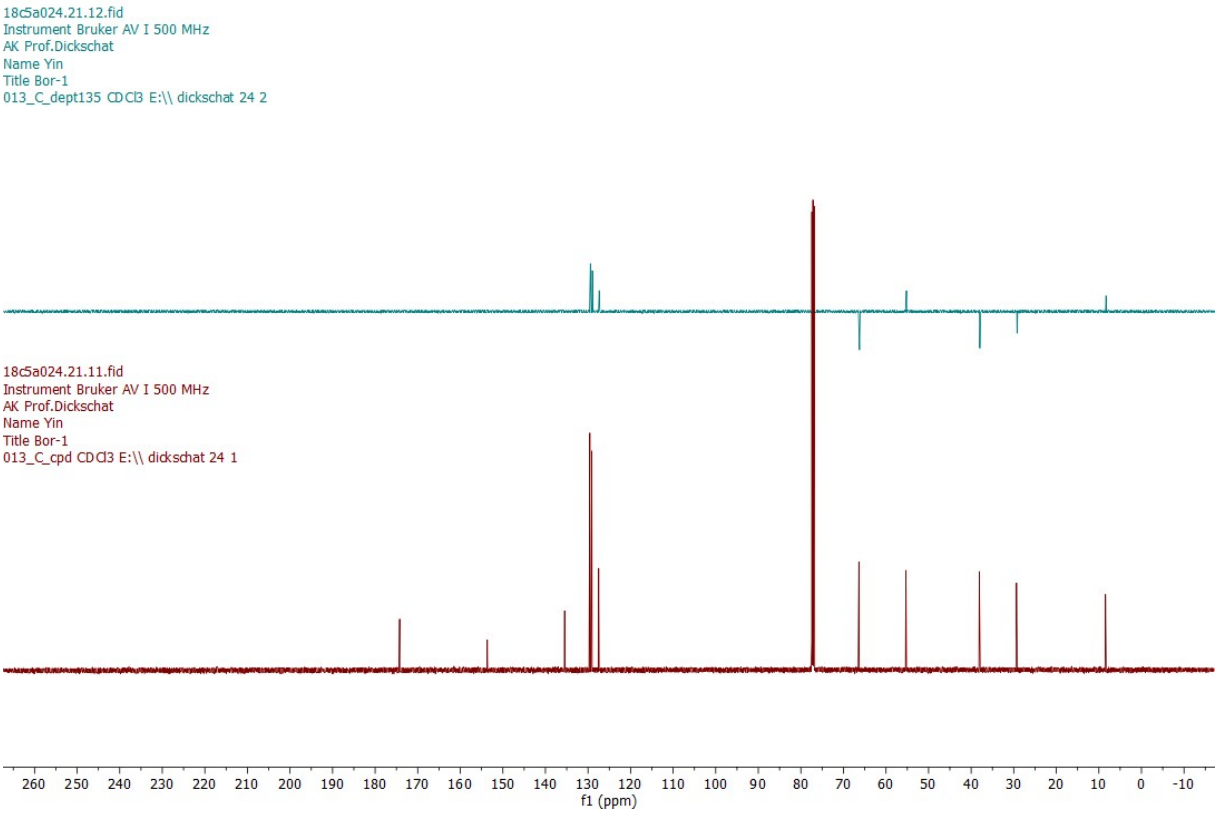


Figure S23. ^{13}C NMR (126 MHz, $CDCl_3$) of (*S*)-**S4**.

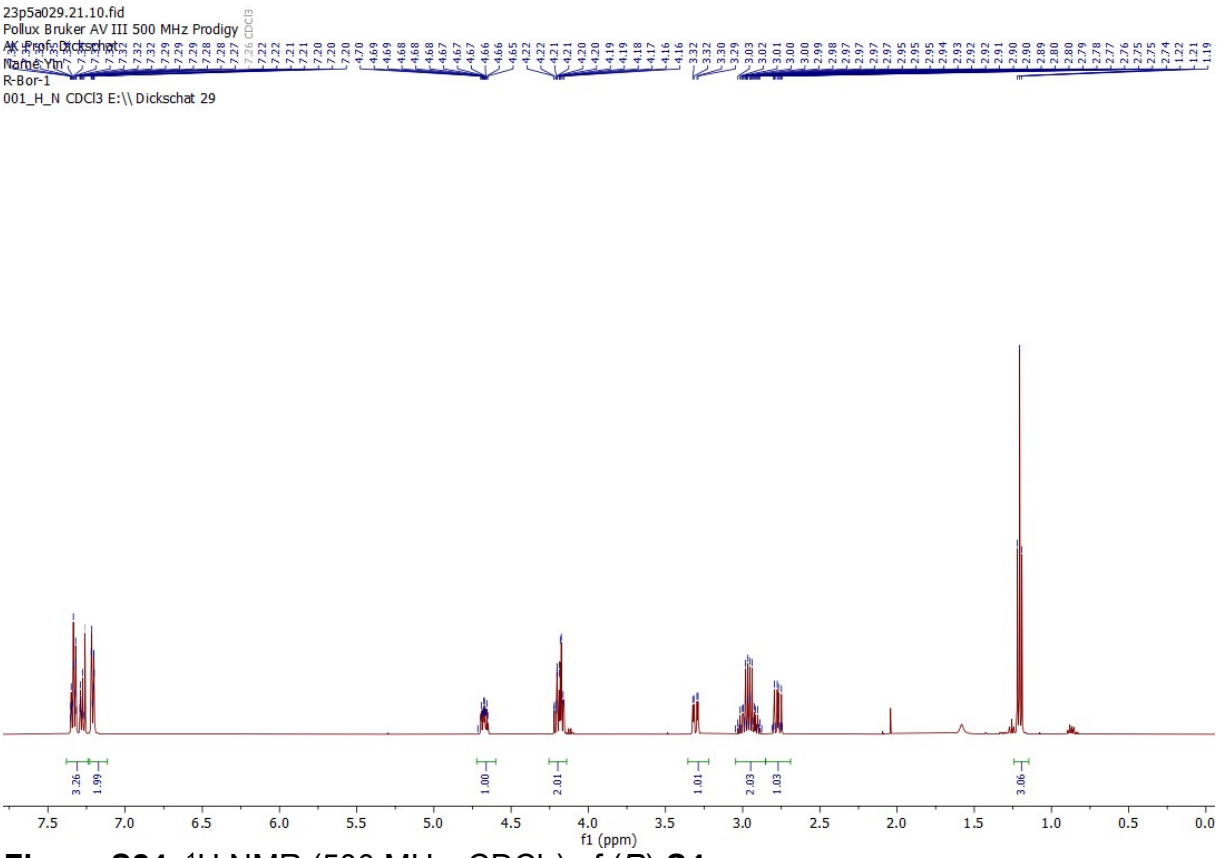


Figure S24. ^{1}H NMR (500 MHz, $CDCl_3$) of (*R*)-**S4**.

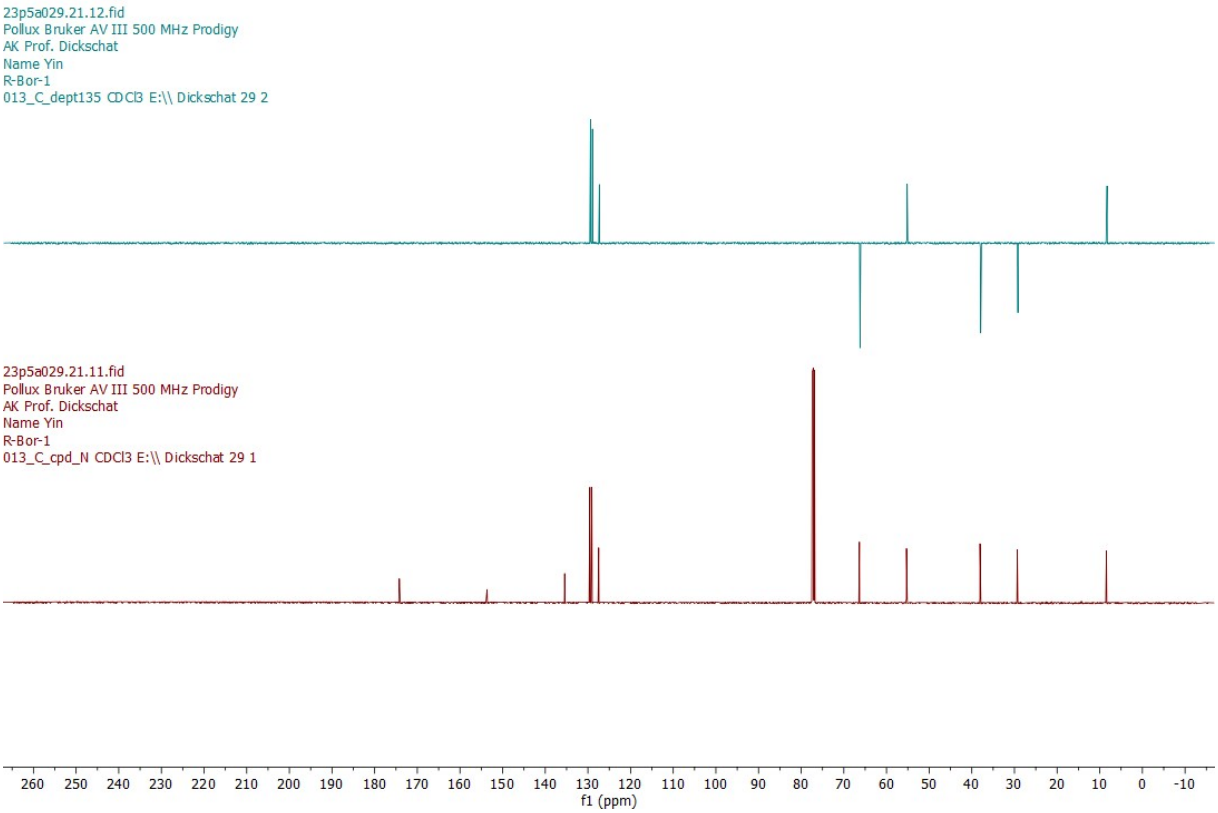


Figure S25. ^{13}C NMR (126 MHz, $CDCl_3$) of (*R*)-**S4**.

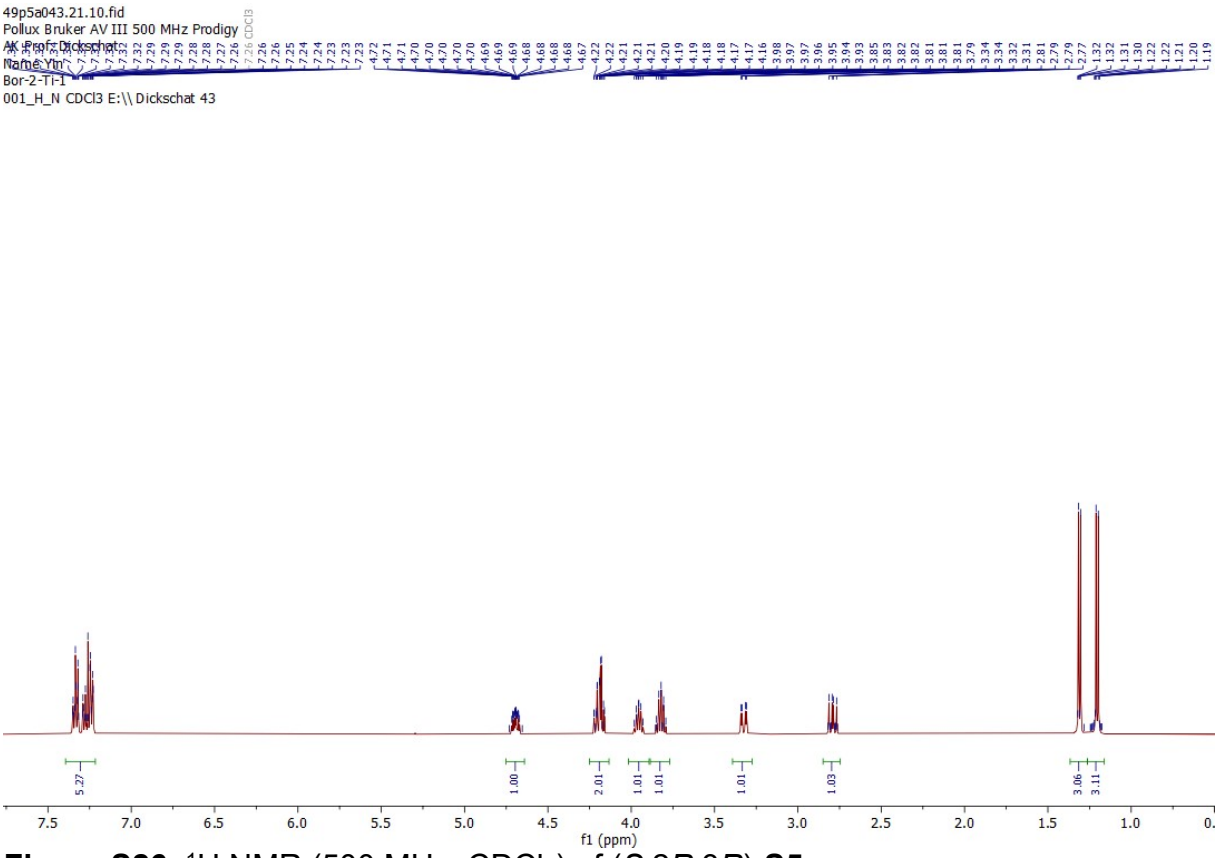


Figure S26. ^{1}H NMR (500 MHz, $CDCl_3$) of (*S*,2*R*,3*R*)-**S5**.

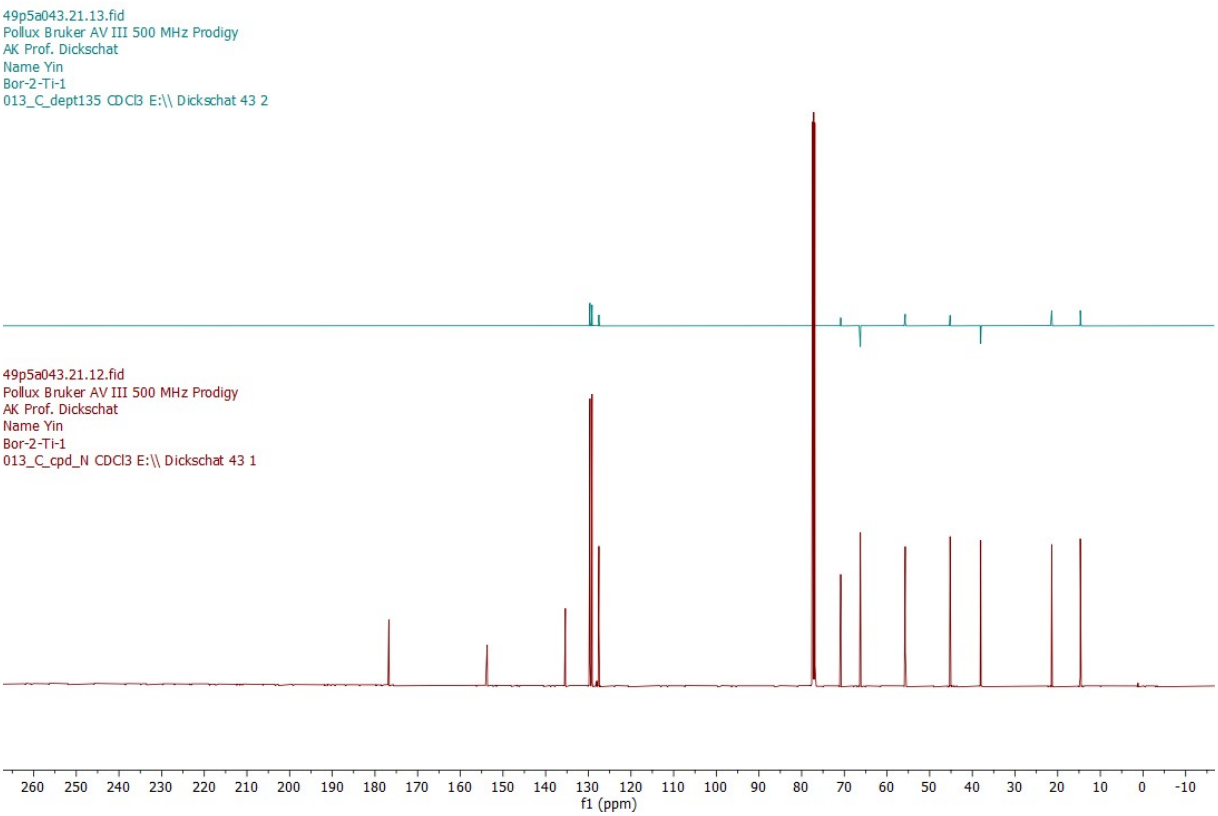


Figure S27. ^{13}C NMR (126 MHz, $CDCl_3$) of (*S*,2*R*,3*R*)-**S5**.

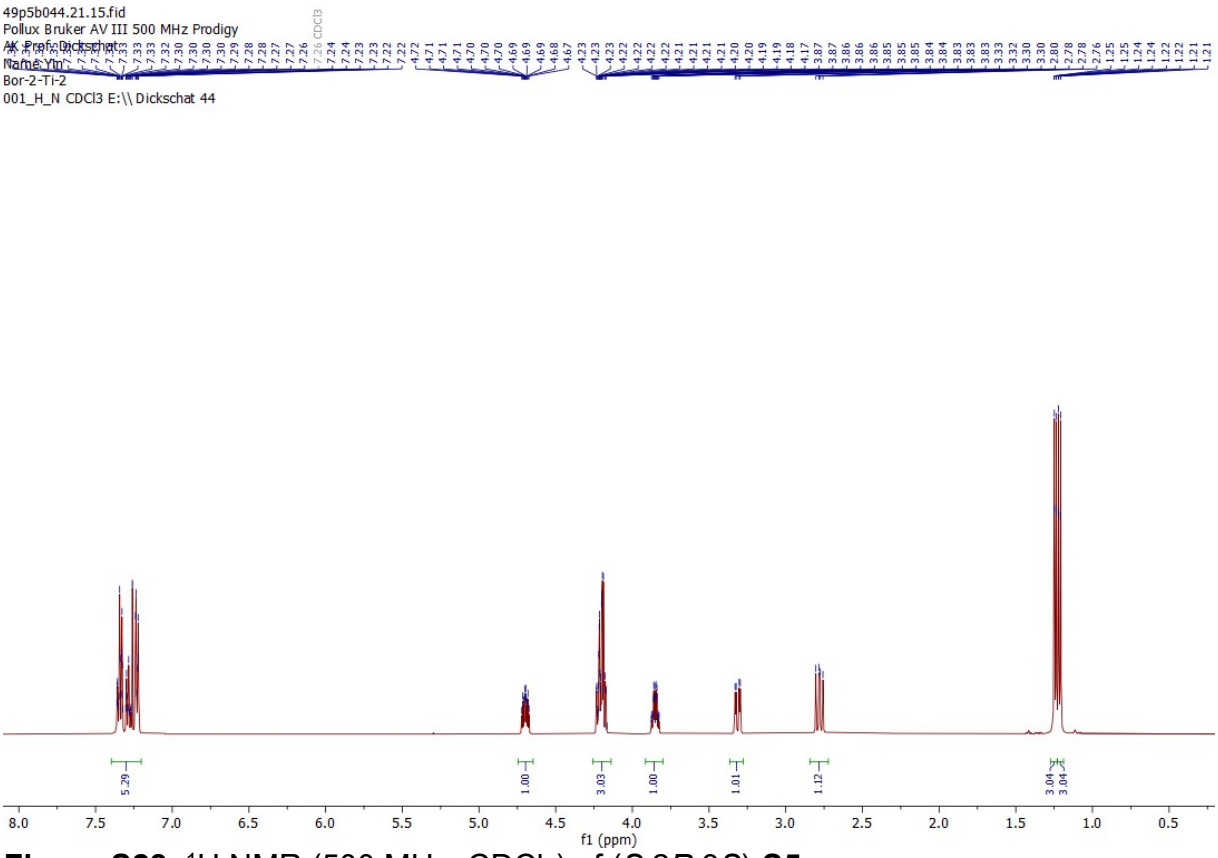


Figure S28. ^{1}H NMR (500 MHz, $CDCl_3$) of (*S*,2*R*,3*S*)-**S5**.

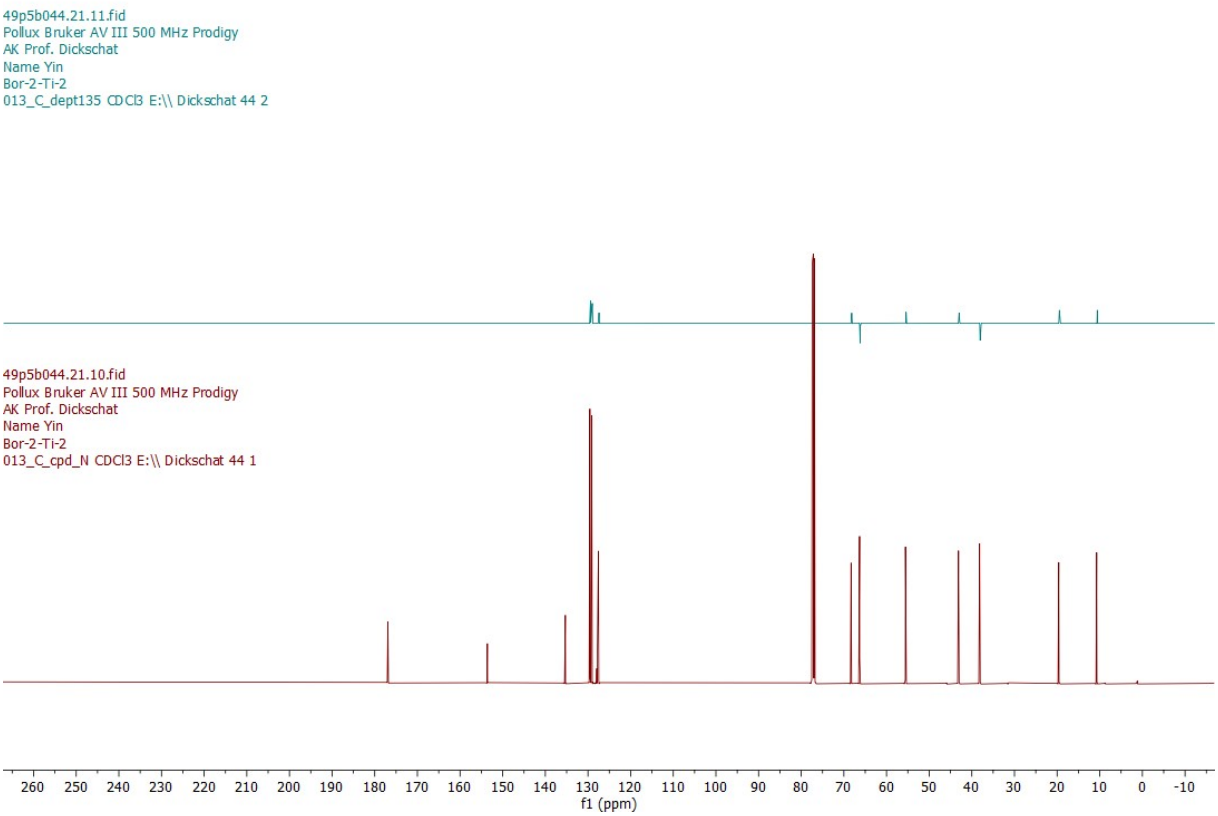


Figure S29. ^{13}C NMR (126 MHz, $CDCl_3$) of (*S*,2*R*,3*S*)-**S5**.

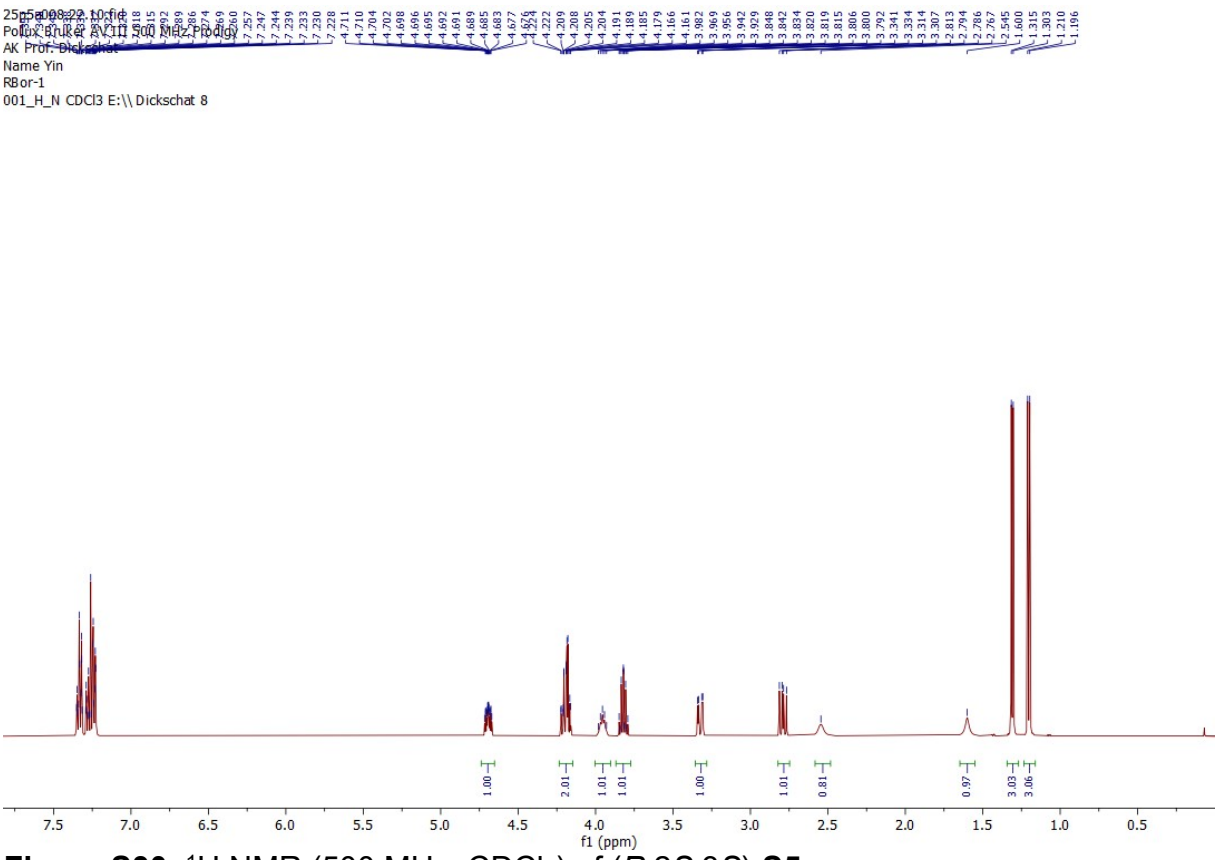


Figure S30. ^{1}H NMR (500 MHz, $CDCl_3$) of (*R*,2*S*,3*S*)-**S5**.

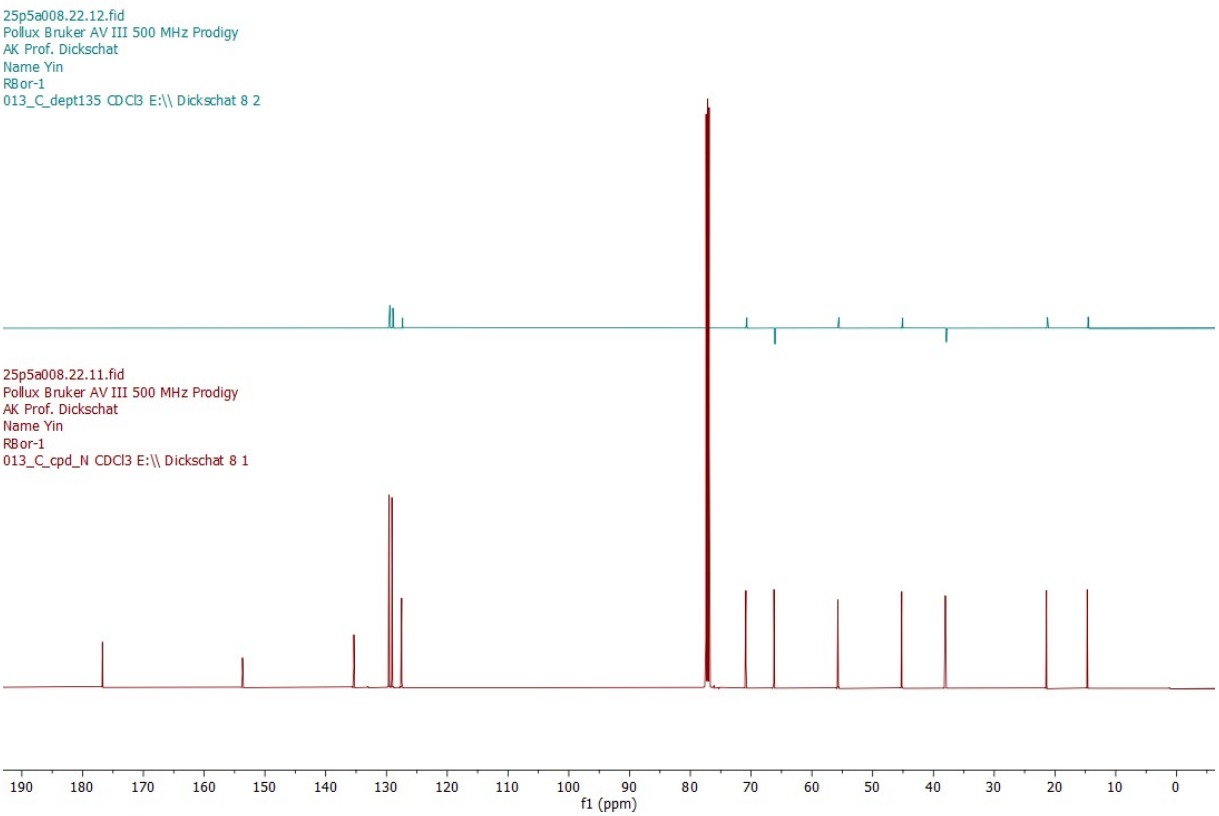


Figure S31. ^{13}C NMR (126 MHz, $CDCl_3$) of (*R*,2*S*,3*S*)-**S5**.

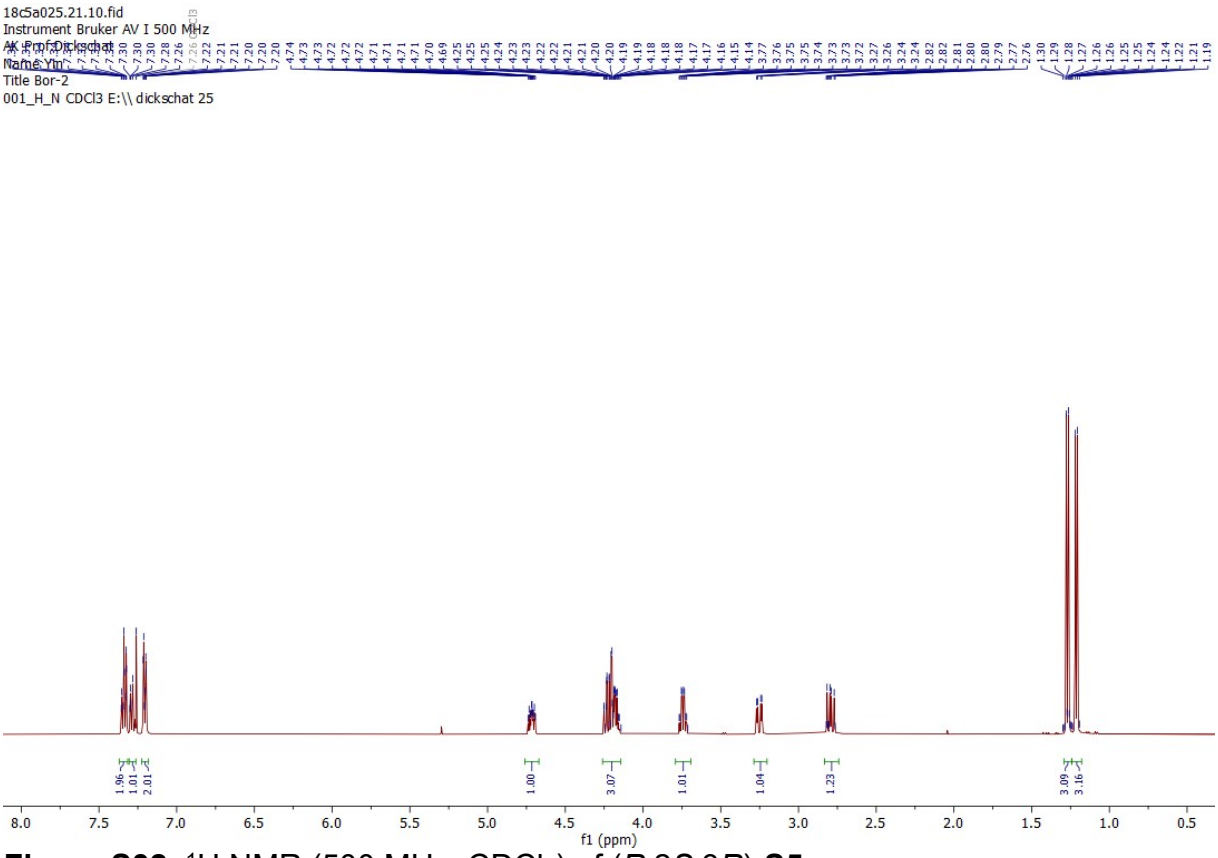


Figure S32. ^{1}H NMR (500 MHz, $CDCl_3$) of (*R*,2*S*,3*R*)-**S5**.

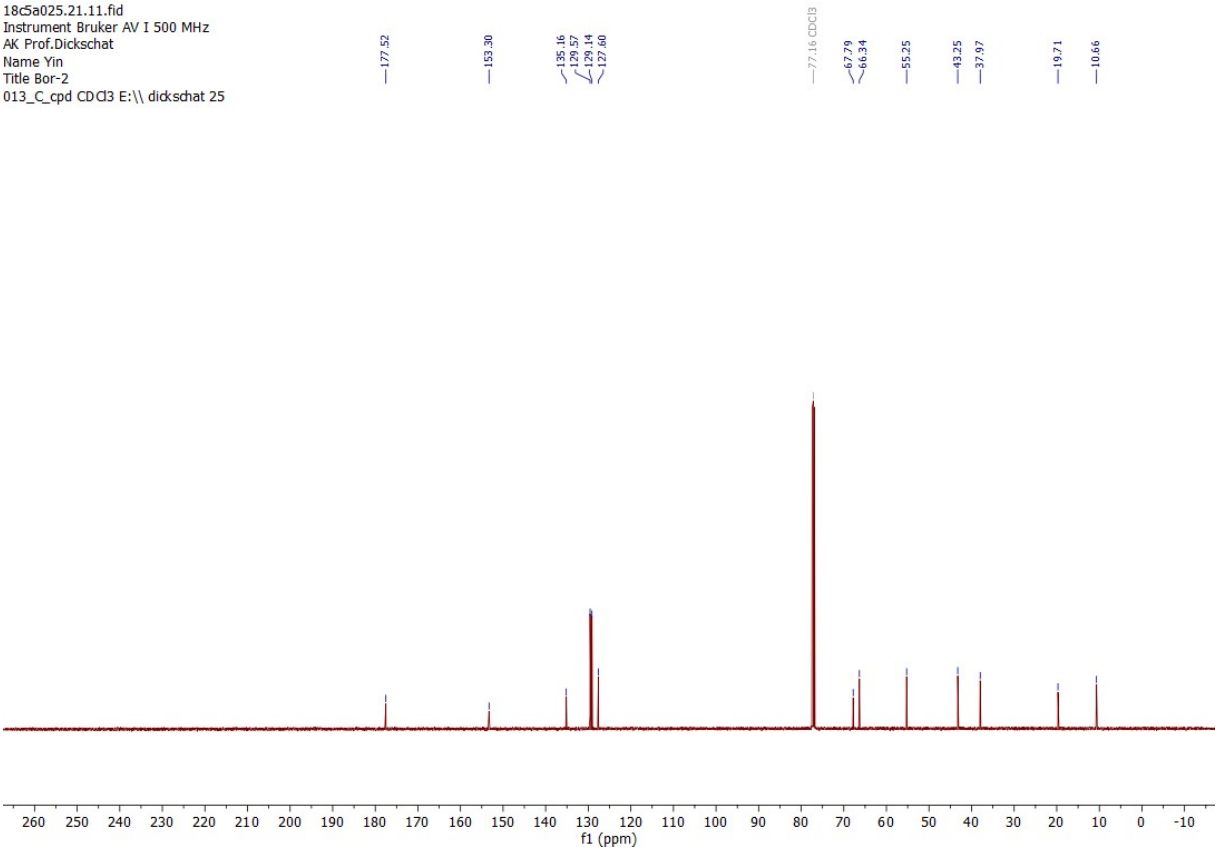


Figure S33. ^{13}C NMR (126 MHz, $CDCl_3$) of (*R*,2*S*,3*R*)-**S5**.

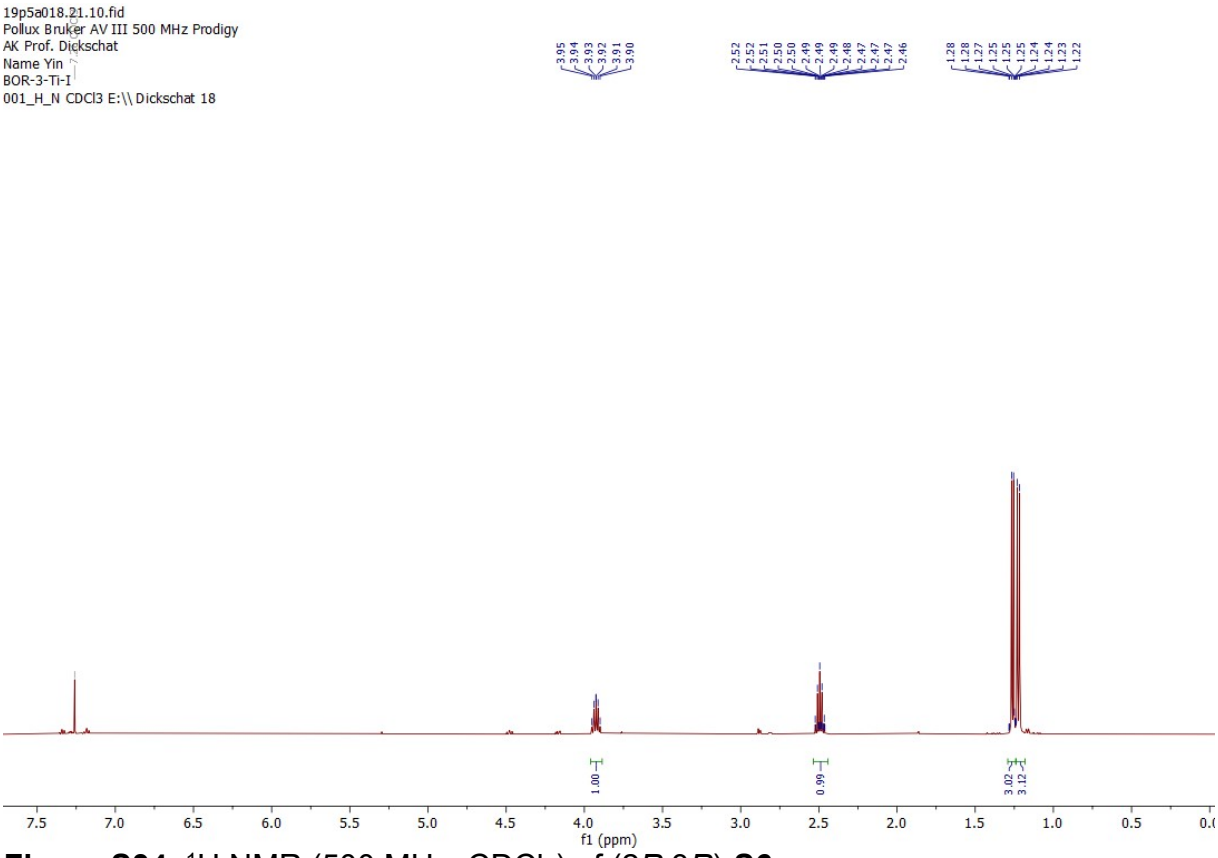


Figure S34. ^{1}H NMR (500 MHz, $CDCl_3$) of (2*R*,3*R*)-**S6**.

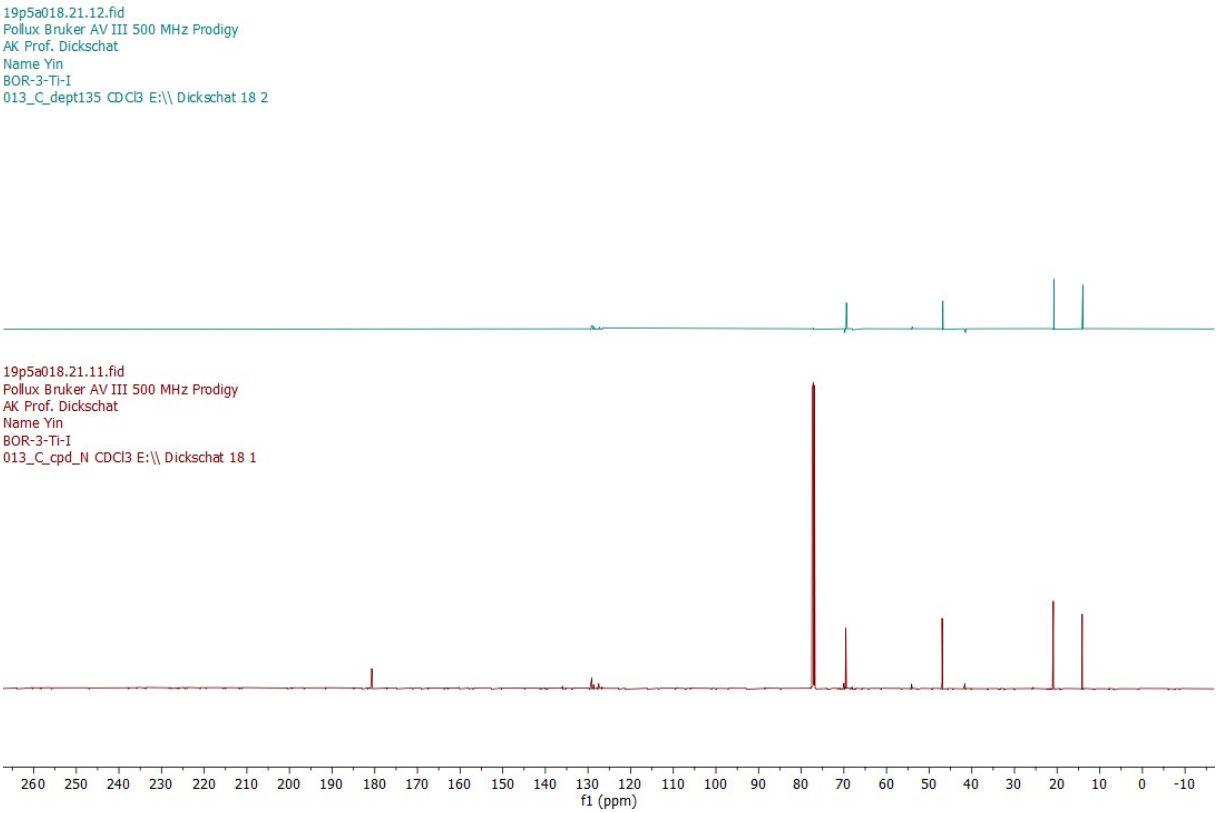


Figure S35. ^{13}C NMR (126 MHz, $CDCl_3$) of (2*R*,3*R*)-**S6**.

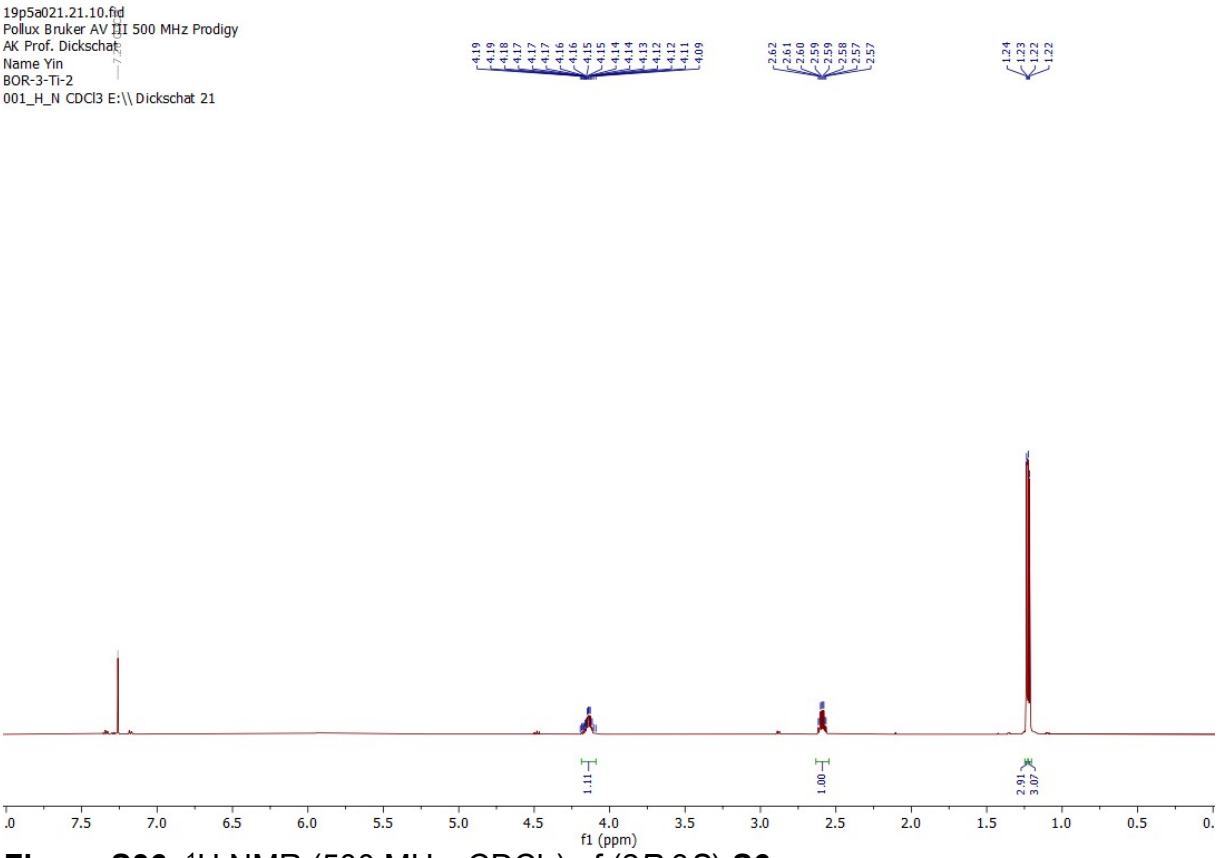


Figure S36. ^{1}H NMR (500 MHz, $CDCl_3$) of (2*R*,3*S*)-**S6**.

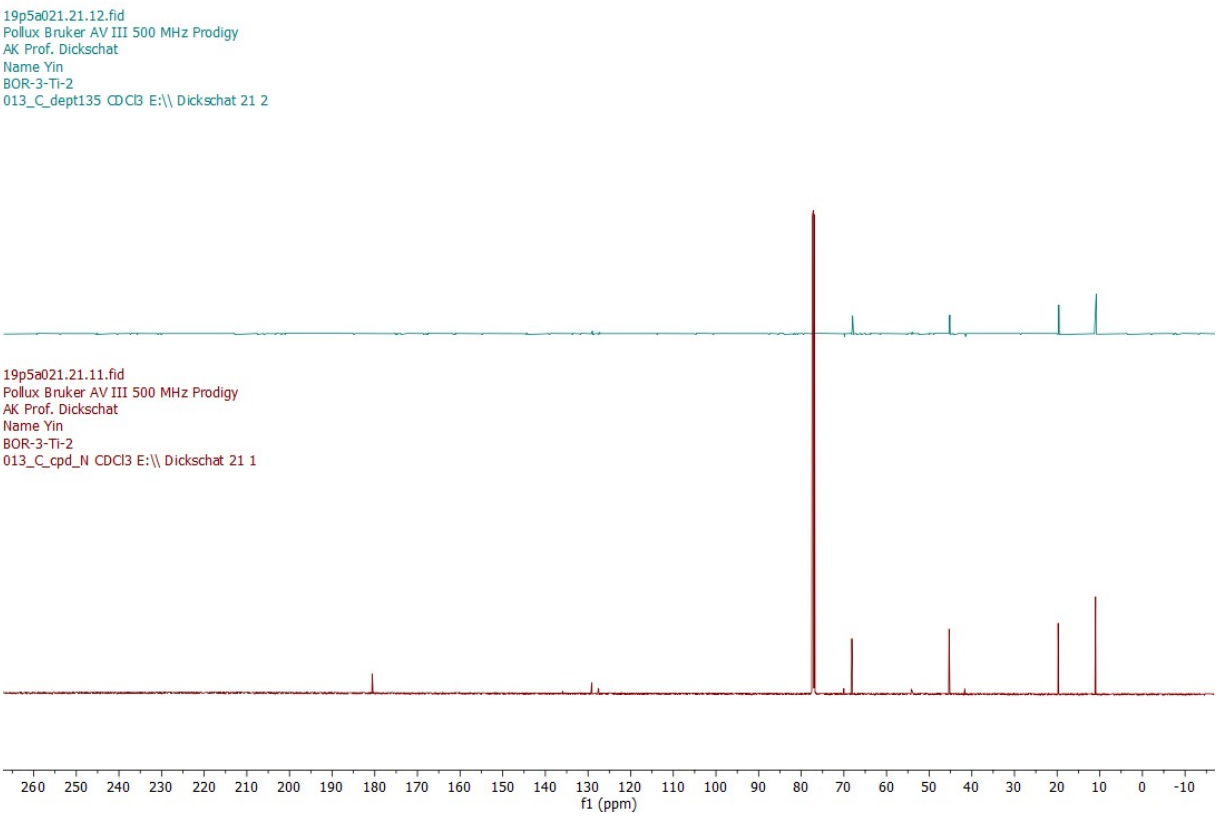


Figure S37. ^{13}C NMR (126 MHz, $CDCl_3$) of (2*R*,3*S*)-**S6**.

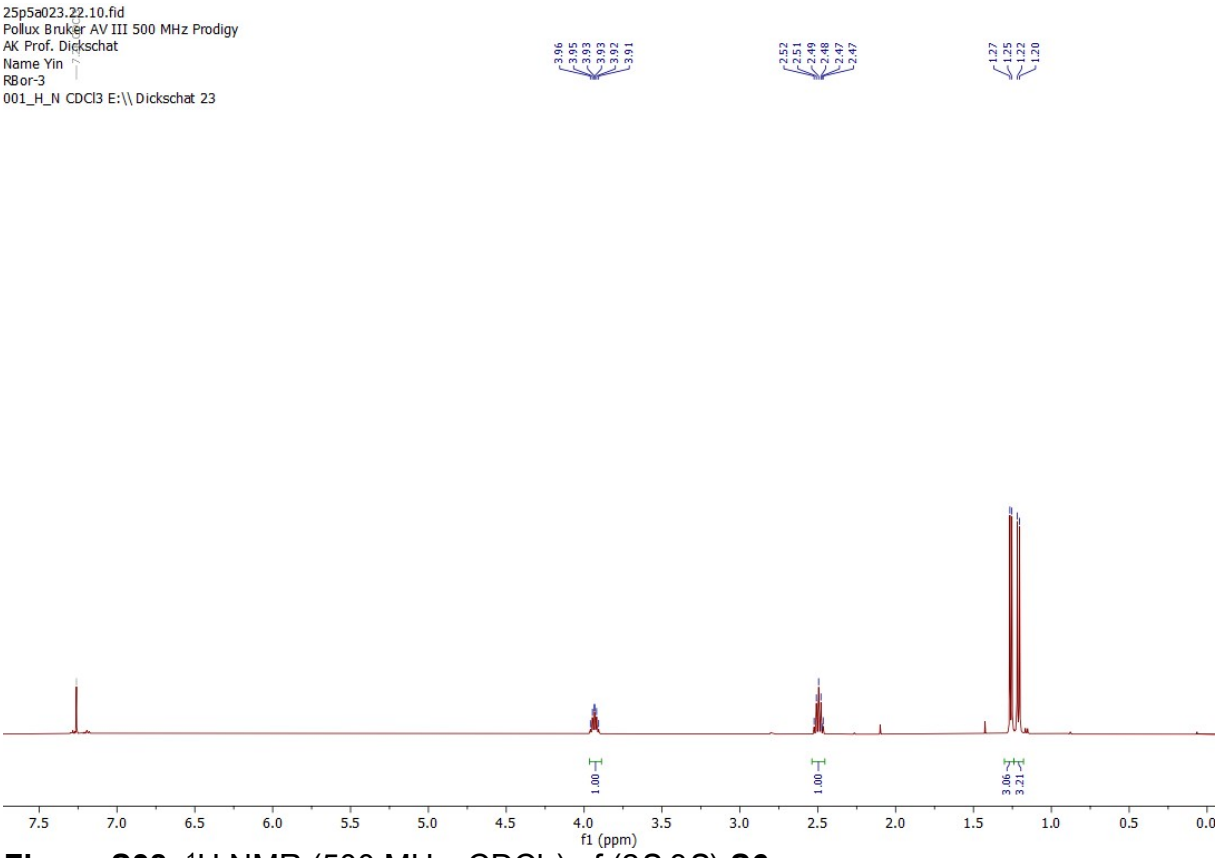


Figure S38. ^{1}H NMR (500 MHz, $CDCl_3$) of (2*S*,3*S*)-**S6**.

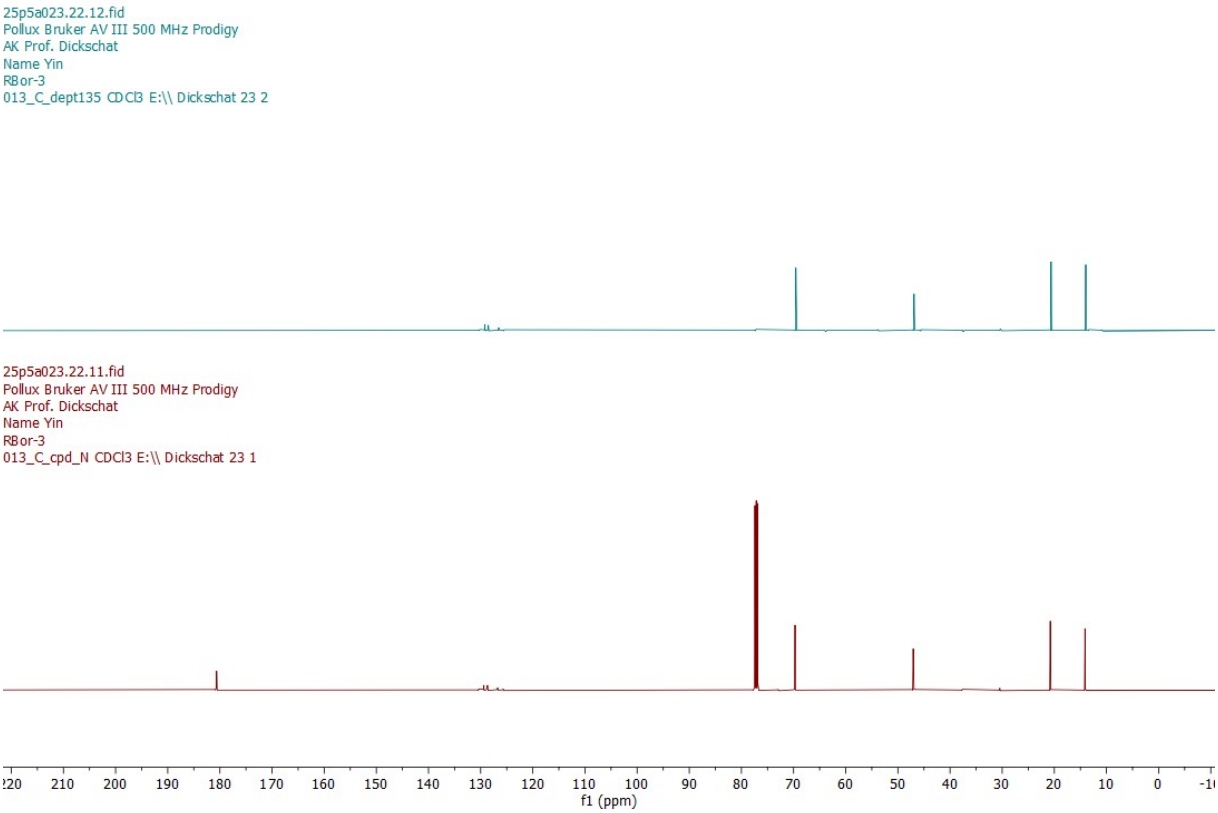


Figure S39. ^{13}C NMR (126 MHz, $CDCl_3$) of (2*S*,3*S*)-**S6**.

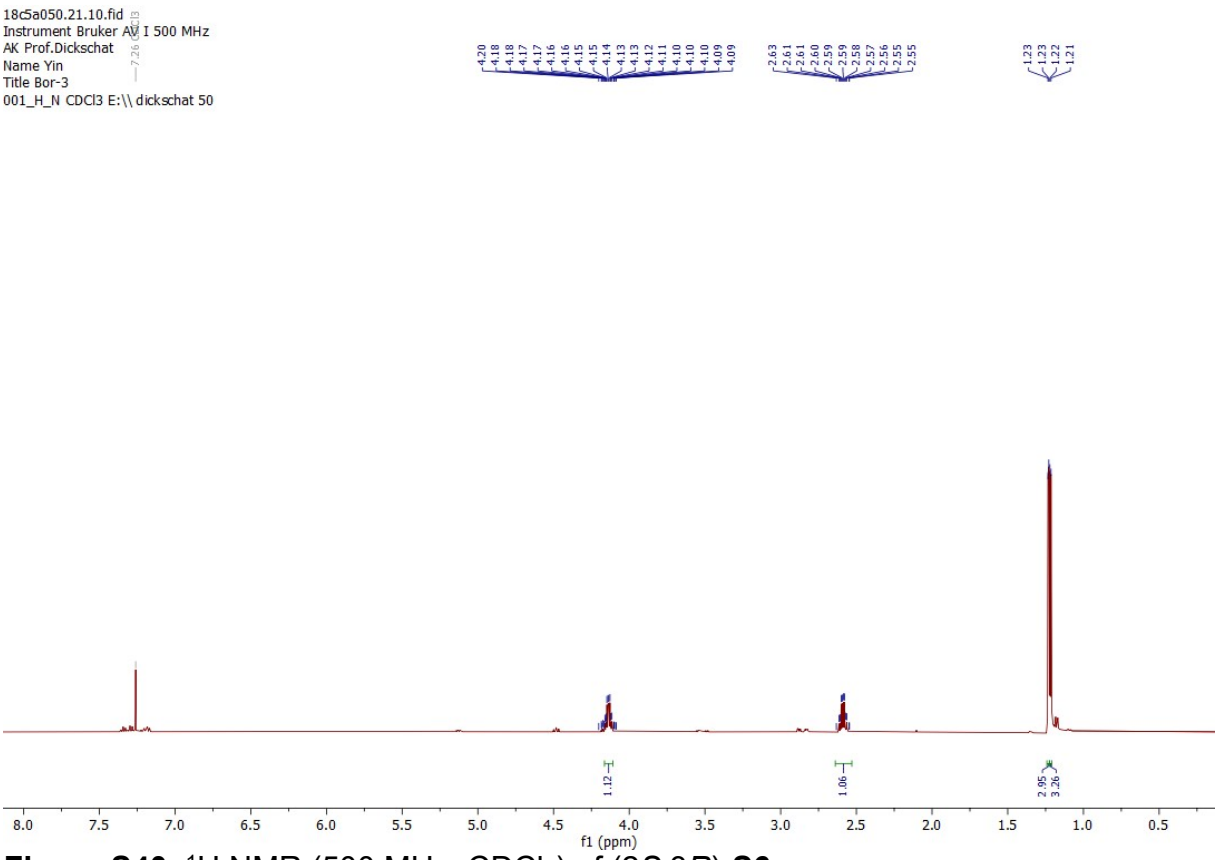


Figure S40. ^{1}H NMR (500 MHz, $CDCl_3$) of (2*S*,3*R*)-**S6**.

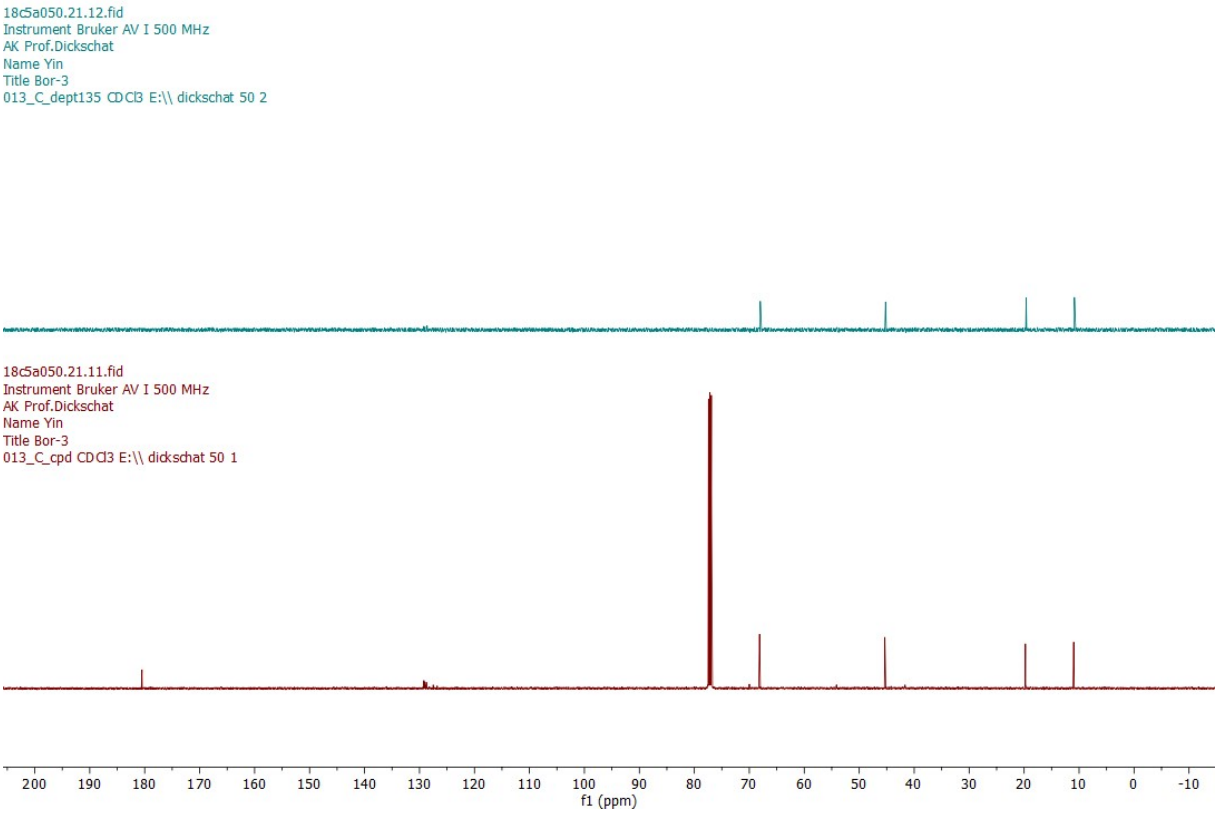


Figure S41. ^{13}C NMR (126 MHz, $CDCl_3$) of (2*S*,3*R*)-**S6**.

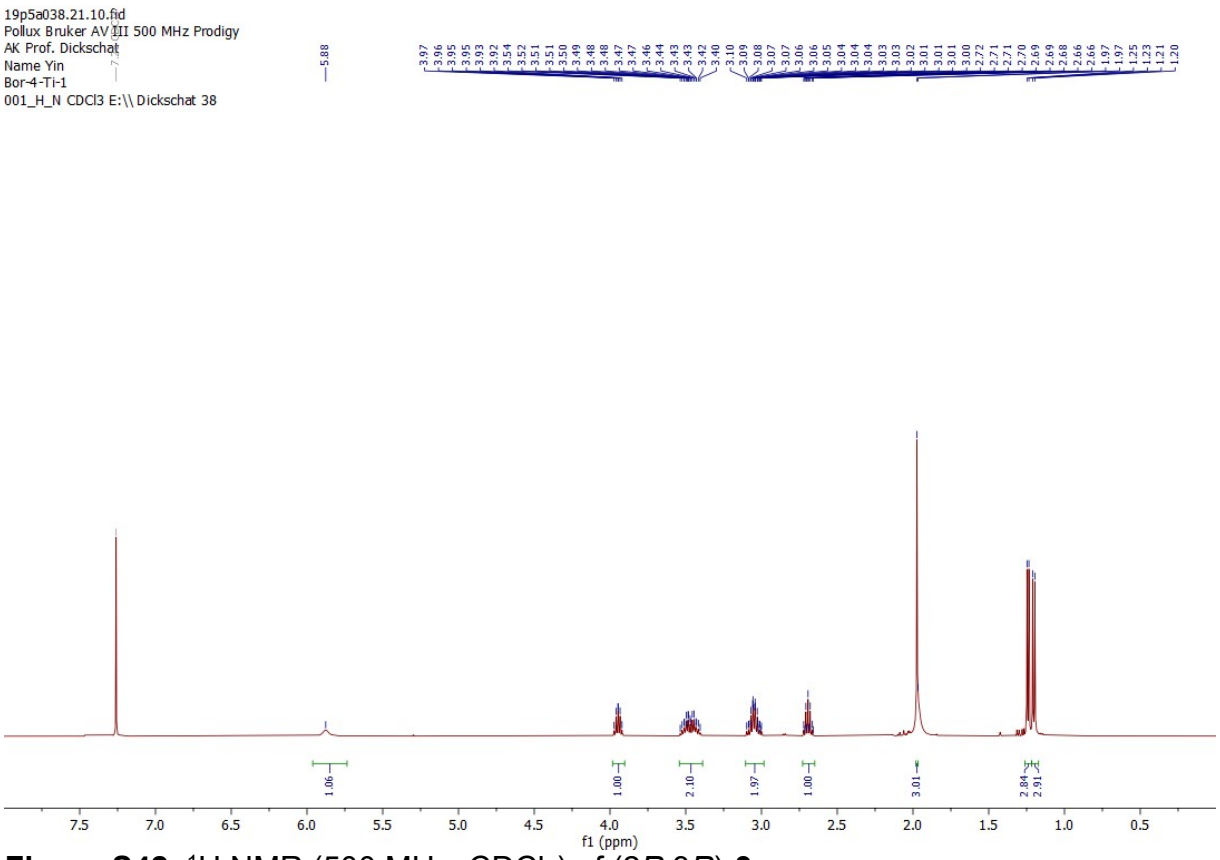


Figure S42. ^{1}H NMR (500 MHz, $CDCl_3$) of (2*R*,3*R*)-**3**.

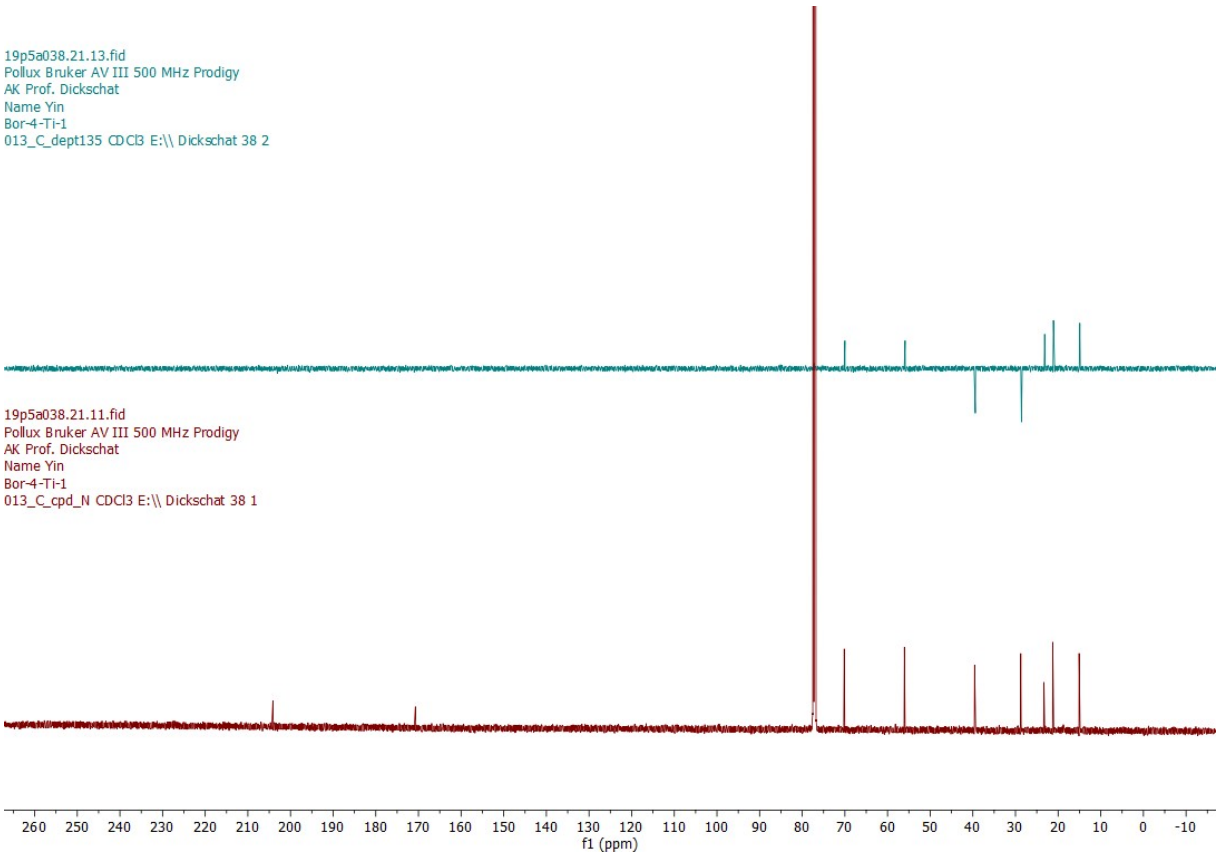


Figure S43. ^{13}C NMR (126 MHz, $CDCl_3$) of (2*R*,3*R*)-**3**.

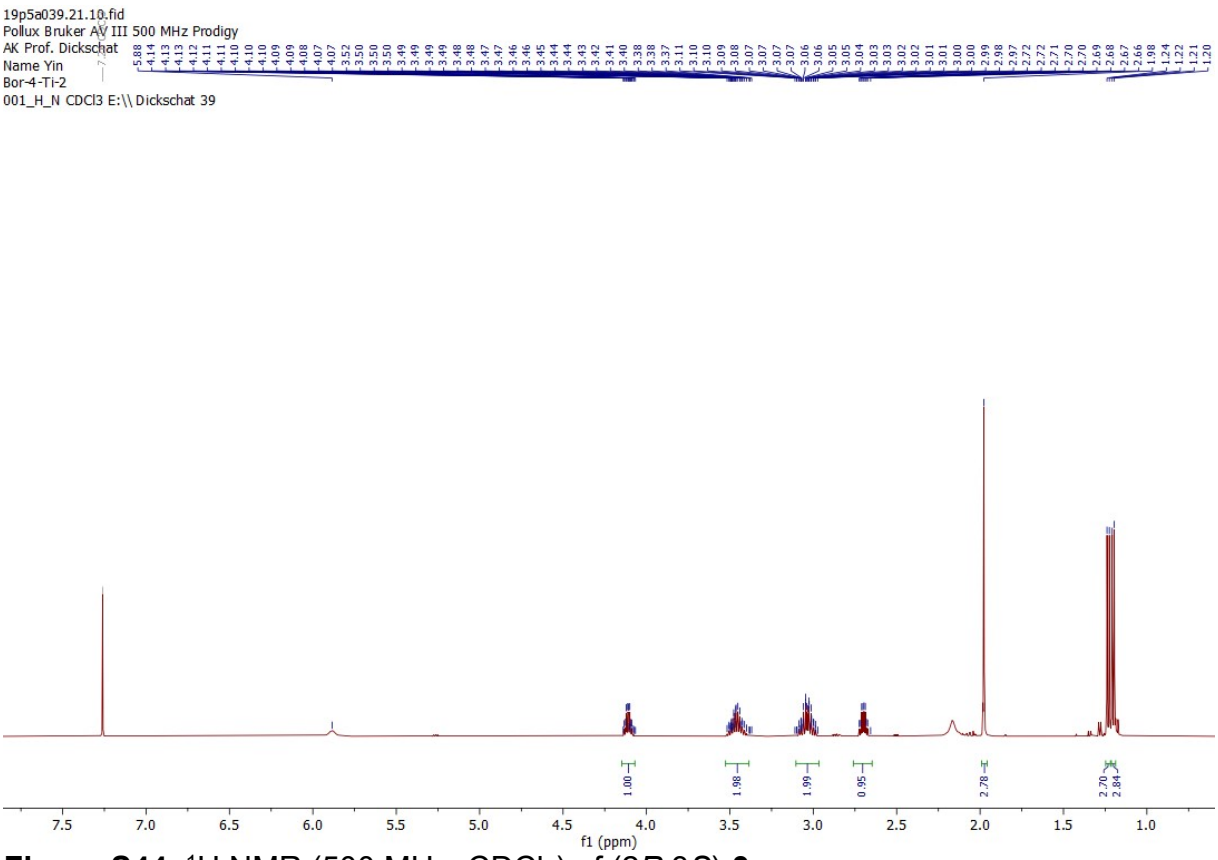


Figure S44. ^{1}H NMR (500 MHz, $CDCl_3$) of (2*R*,3*S*)-**3**.

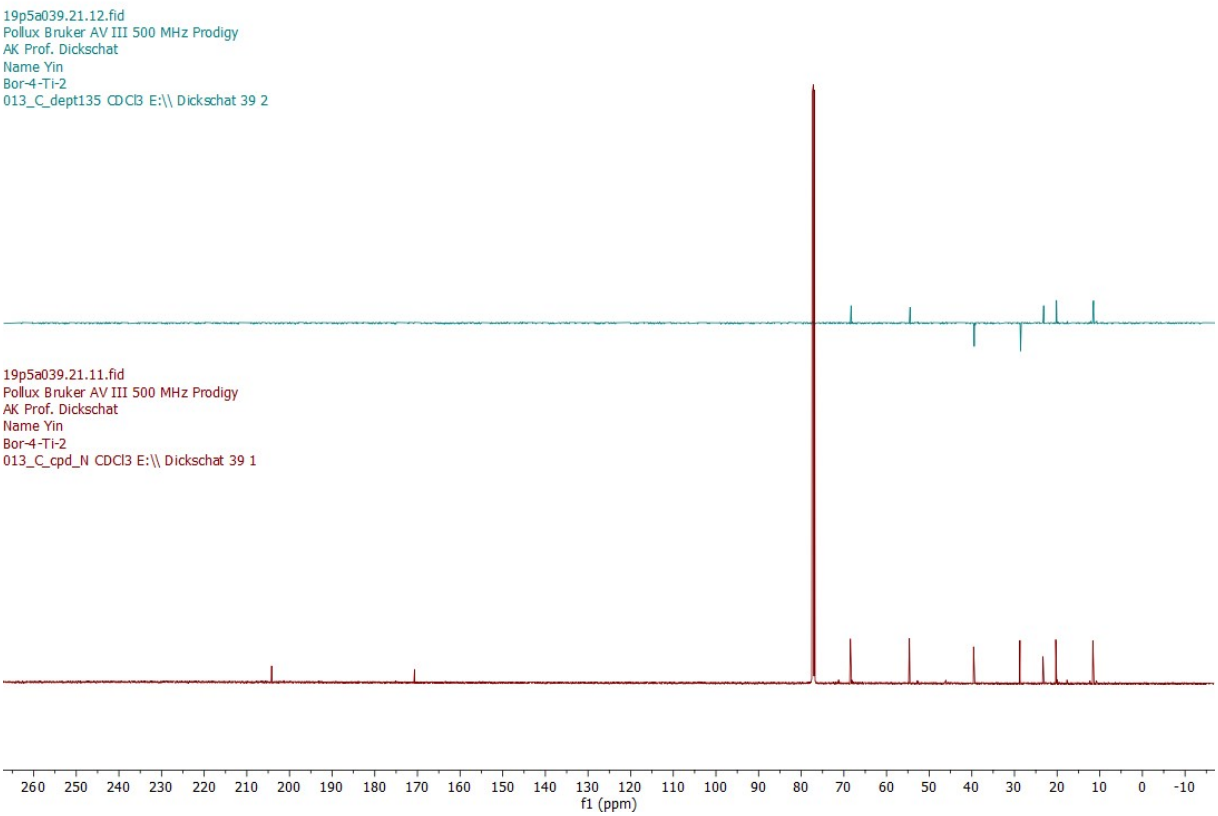


Figure S45. ^{13}C NMR (126 MHz, $CDCl_3$) of (2*R*,3*S*)-**3**.

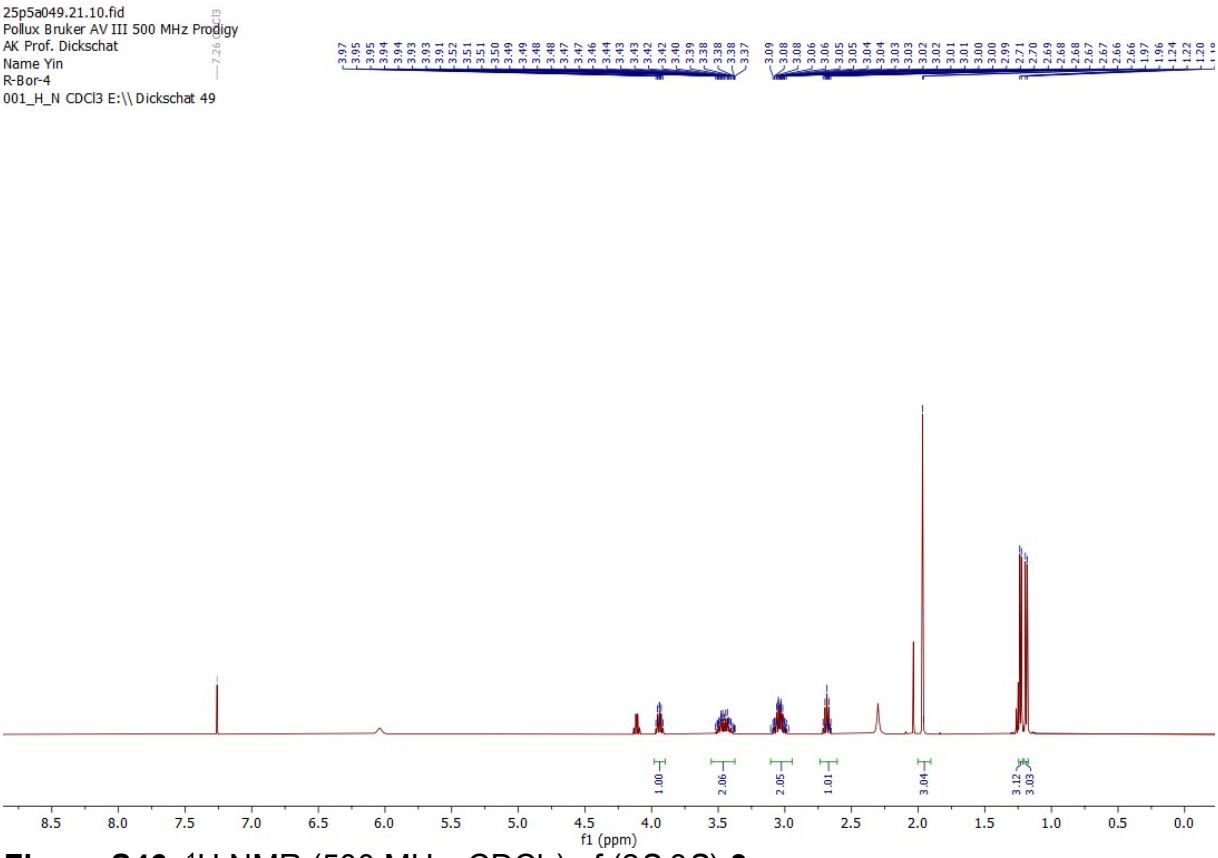


Figure S46. ^{1}H NMR (500 MHz, $CDCl_3$) of (2*S*,3*S*)-**3**.

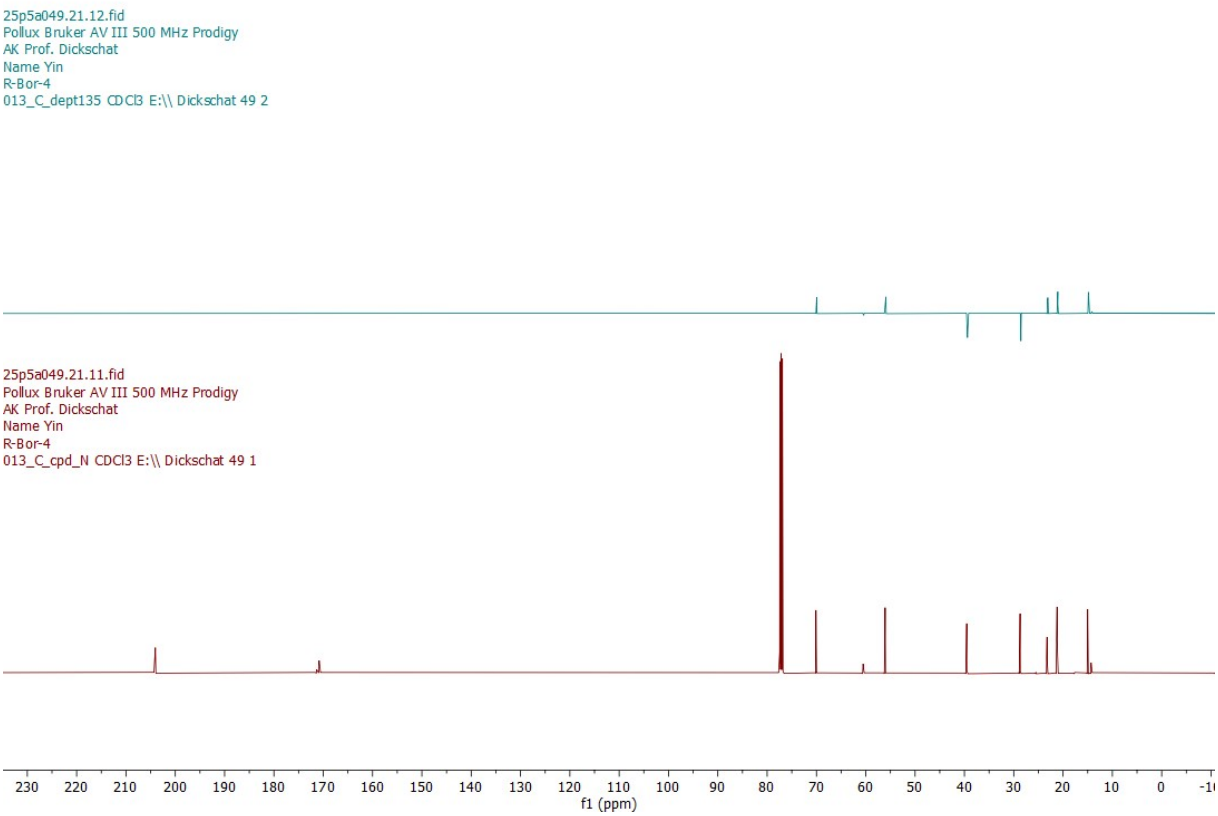


Figure S47. ^{13}C NMR (126 MHz, $CDCl_3$) of (2*S*,3*S*)-**3**.

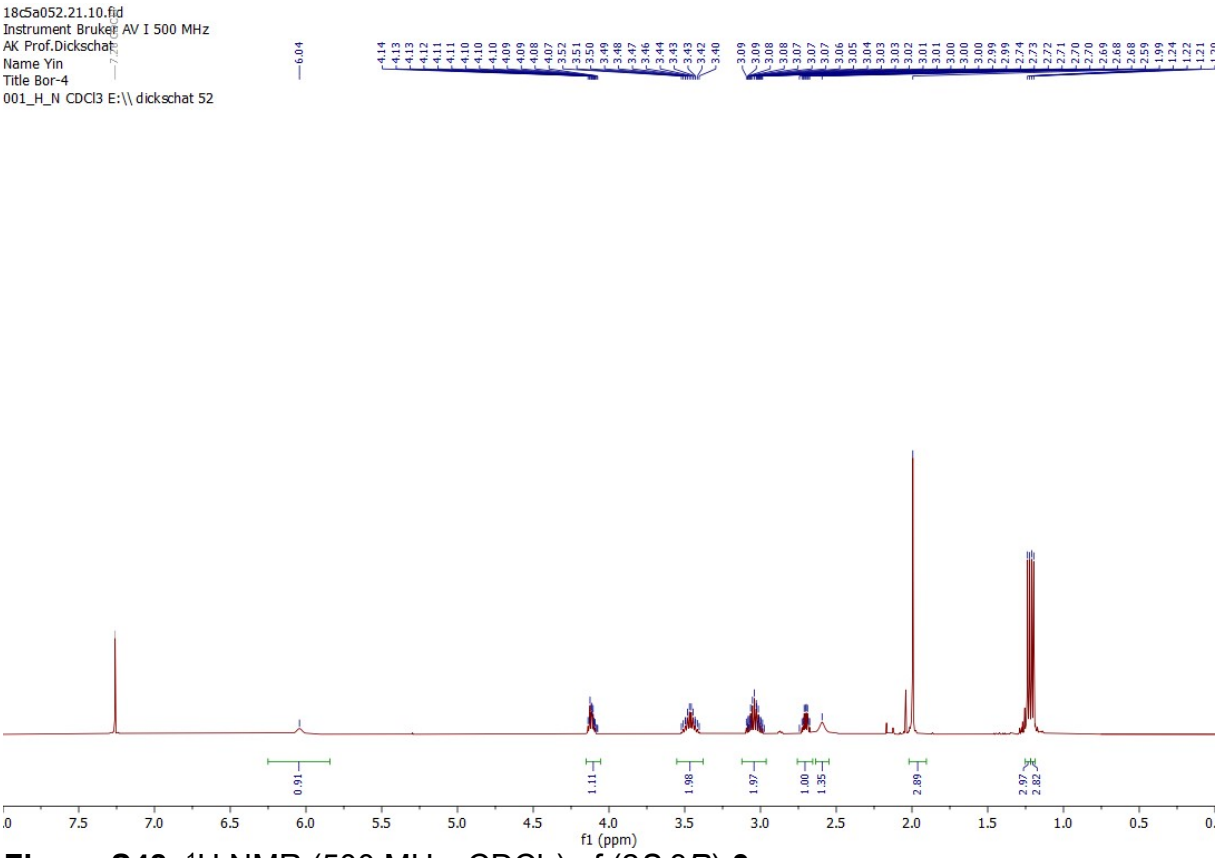


Figure S48. ^{1}H NMR (500 MHz, $CDCl_3$) of (2*S*,3*R*)-**3**.

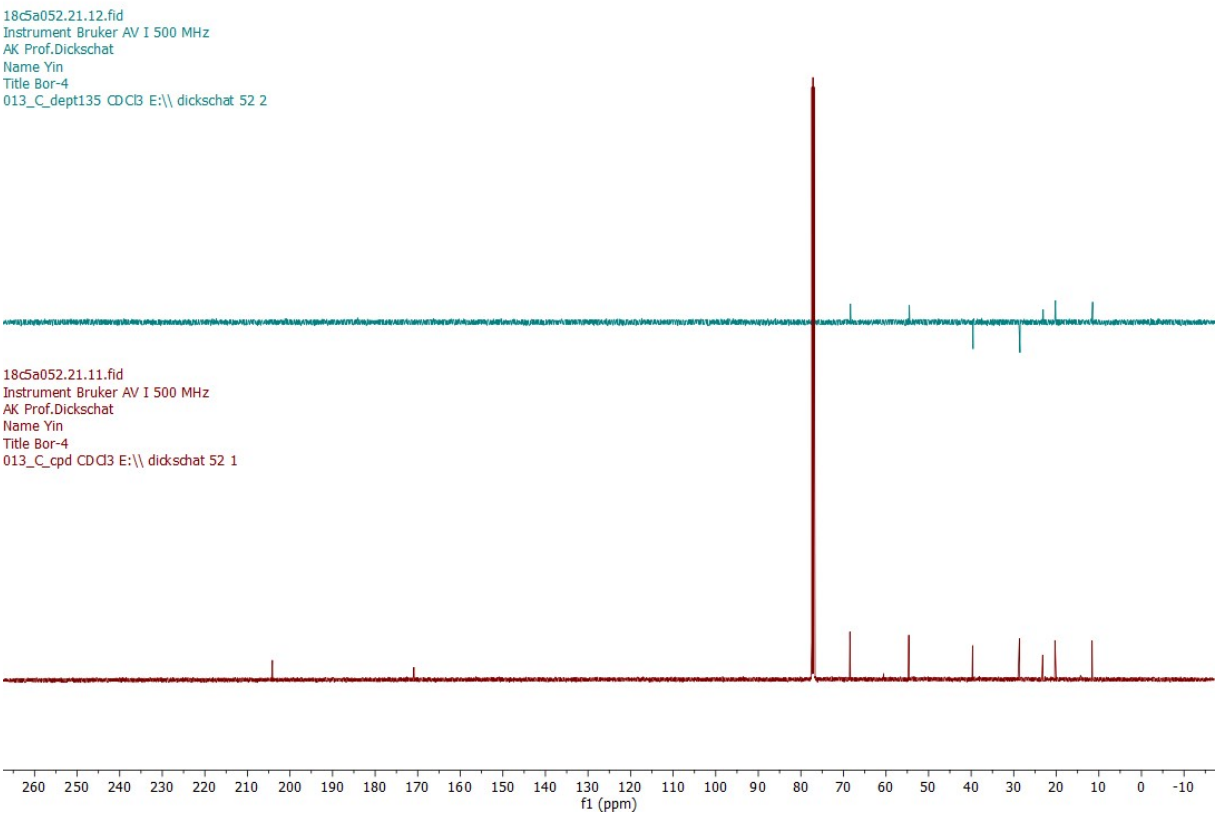


Figure S49. ^{13}C NMR (126 MHz, $CDCl_3$) of (2*S*,3*R*)-**3**.

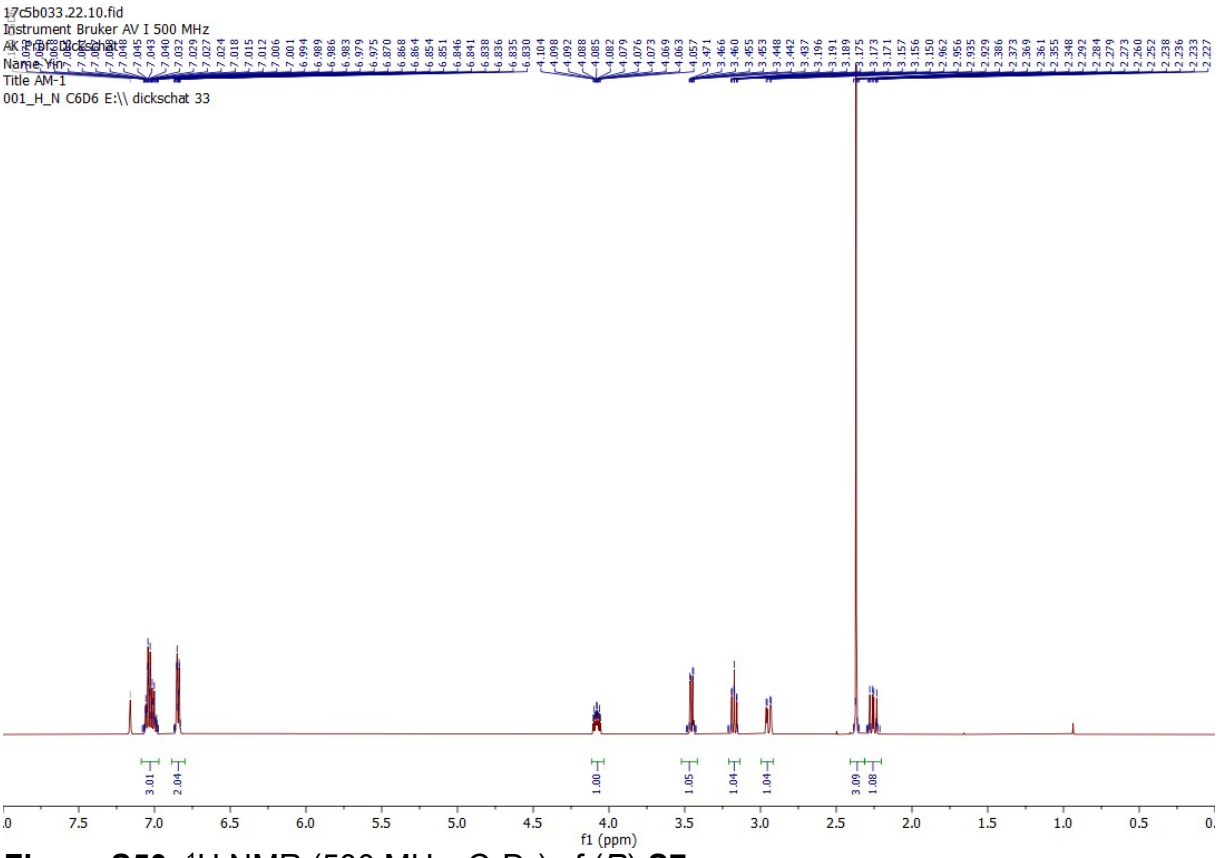


Figure S50. ^{1}H NMR (500 MHz, C_6D_6) of (*R*)-**S7**.

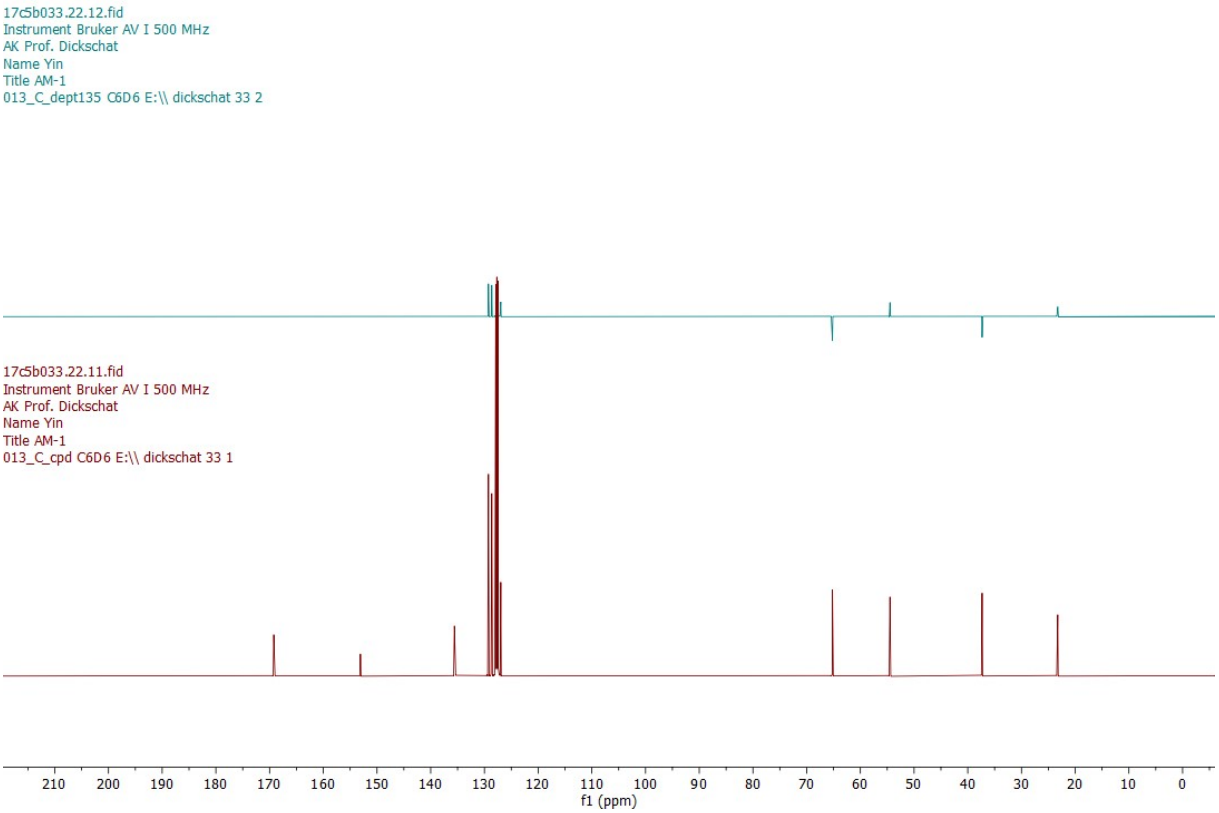


Figure S51. ^{13}C NMR (126 MHz, C_6D_6) of (*R*)-**S7**.

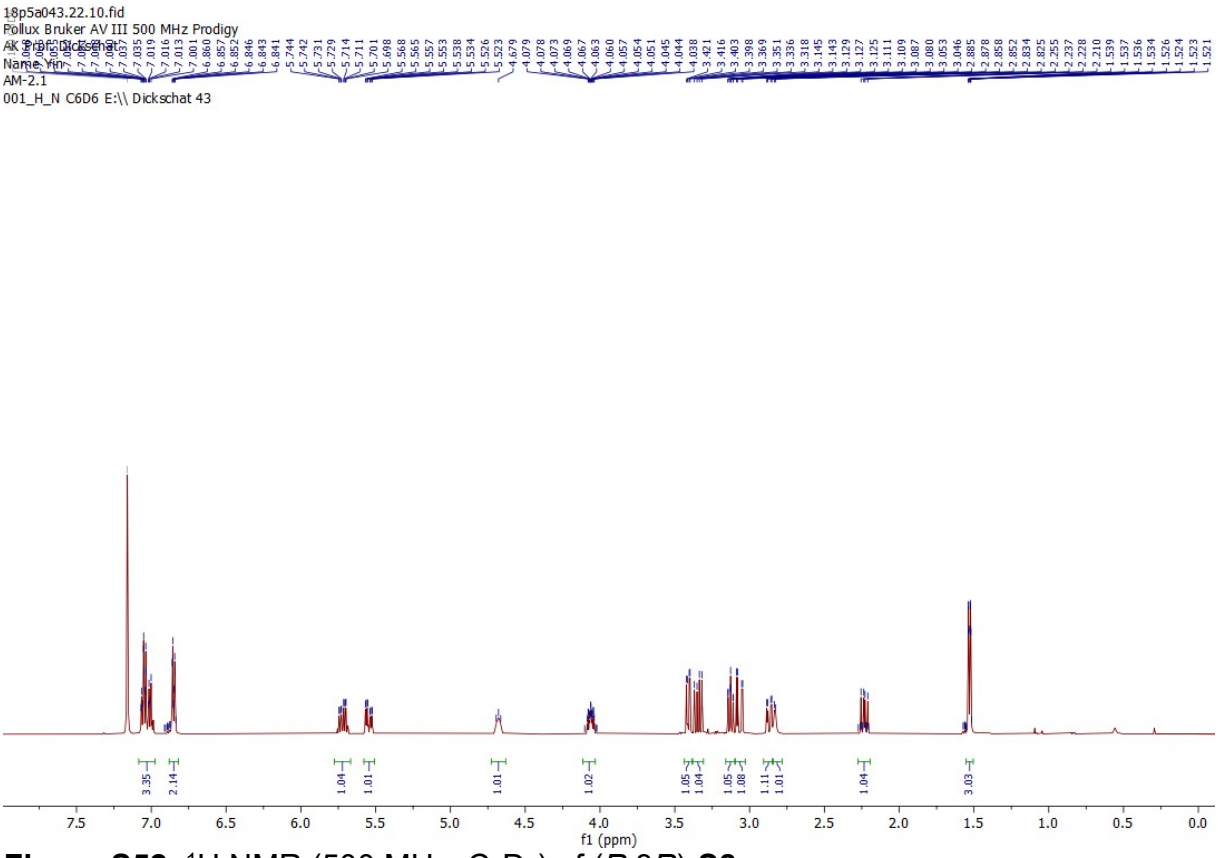


Figure S52. ^{1}H NMR (500 MHz, C_6D_6) of (*R*,3*R*)-**S8**.

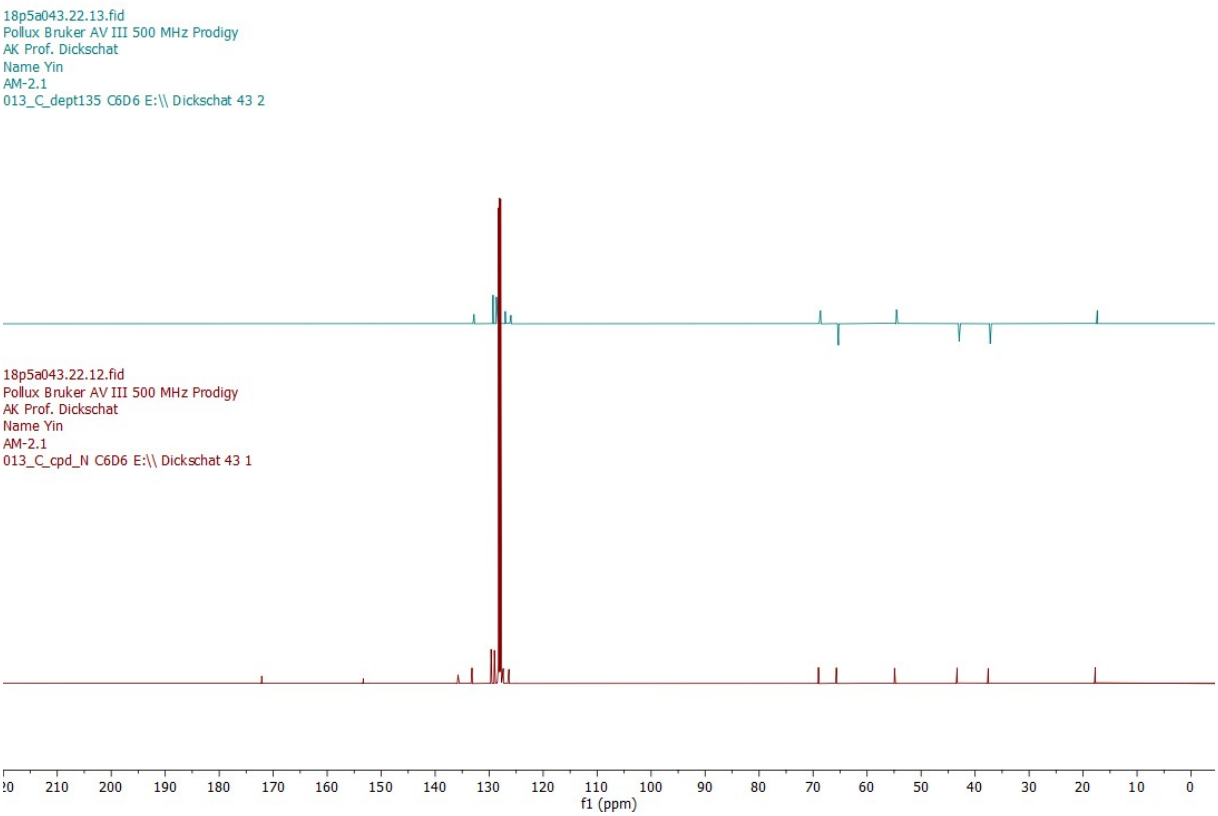


Figure S53. ^{13}C NMR (126 MHz, C_6D_6) of (*R*,3*R*)-**S8**.

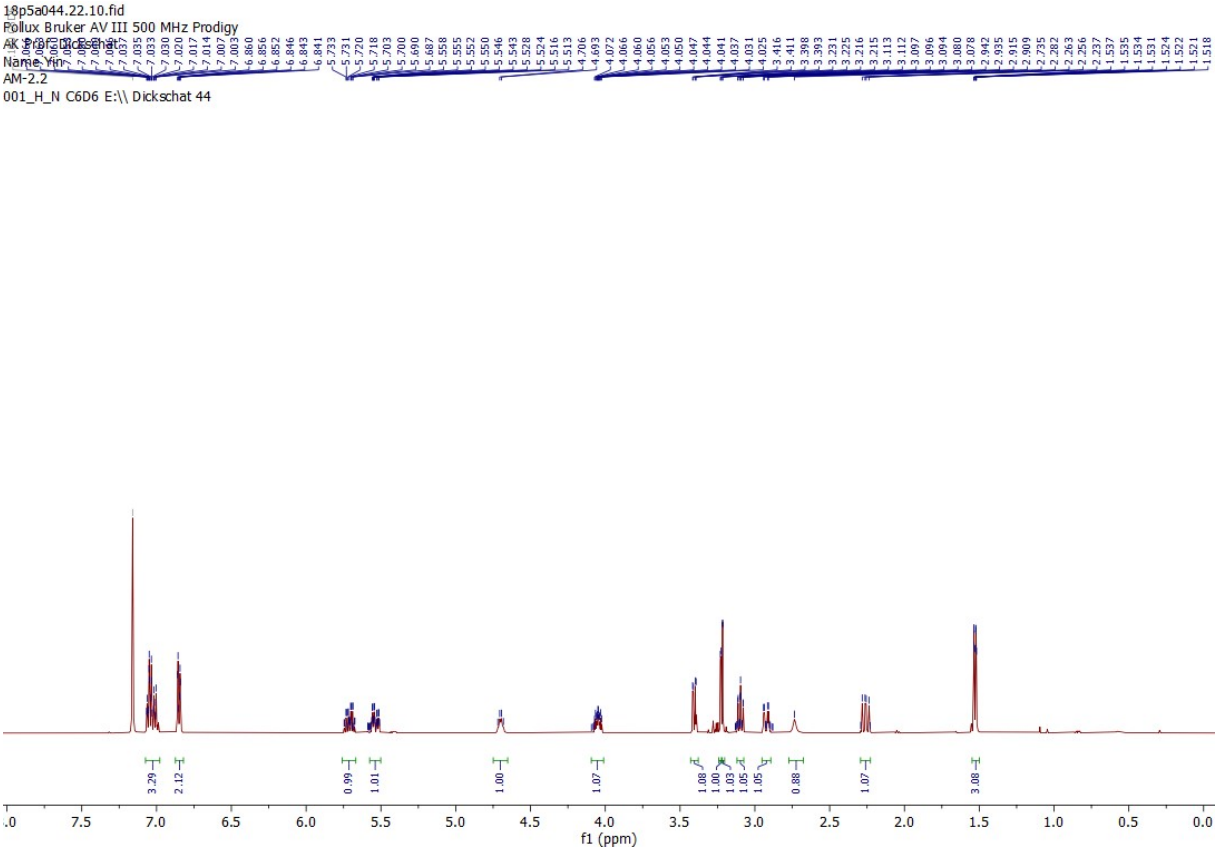


Figure S54. ^{1}H NMR (500 MHz, C_6D_6) of (*R*,3*S*)-**S8**.

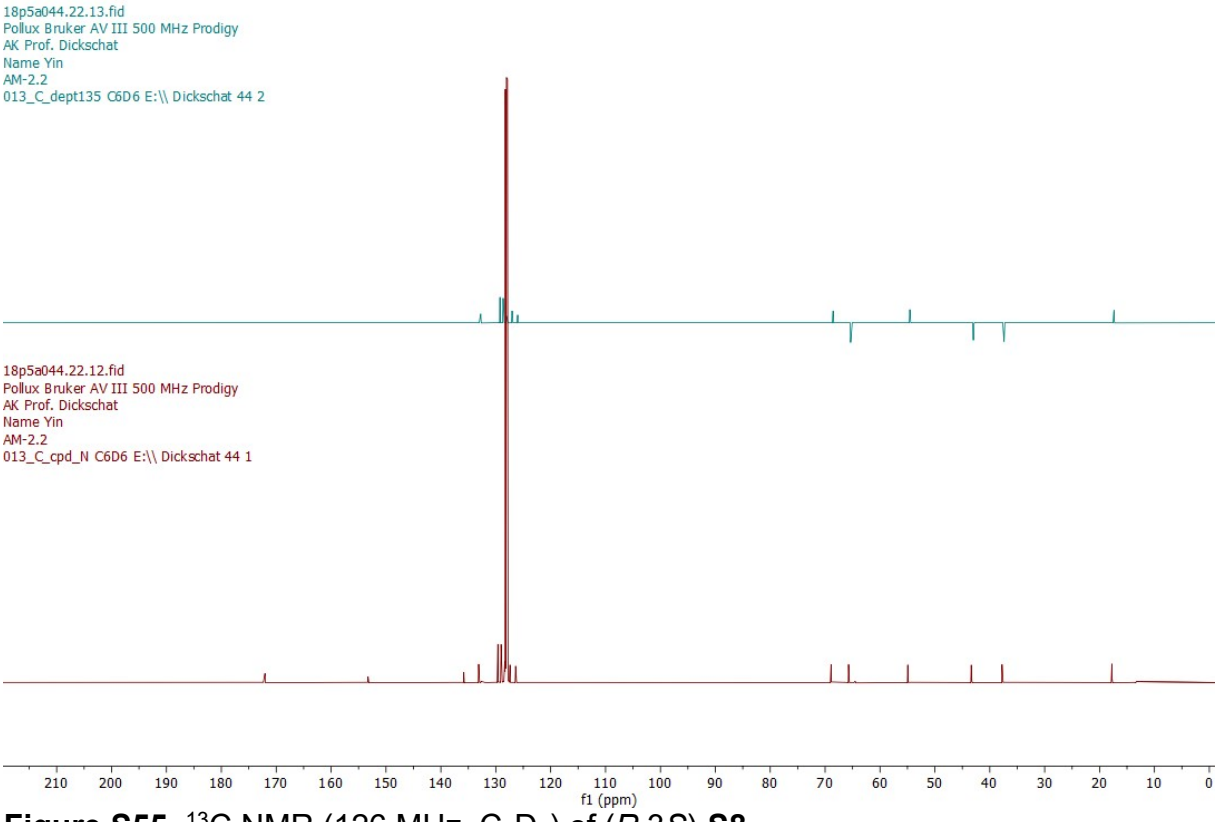


Figure S55. ^{13}C NMR (126 MHz, C_6D_6) of (*R*,3*S*)-**S8**.

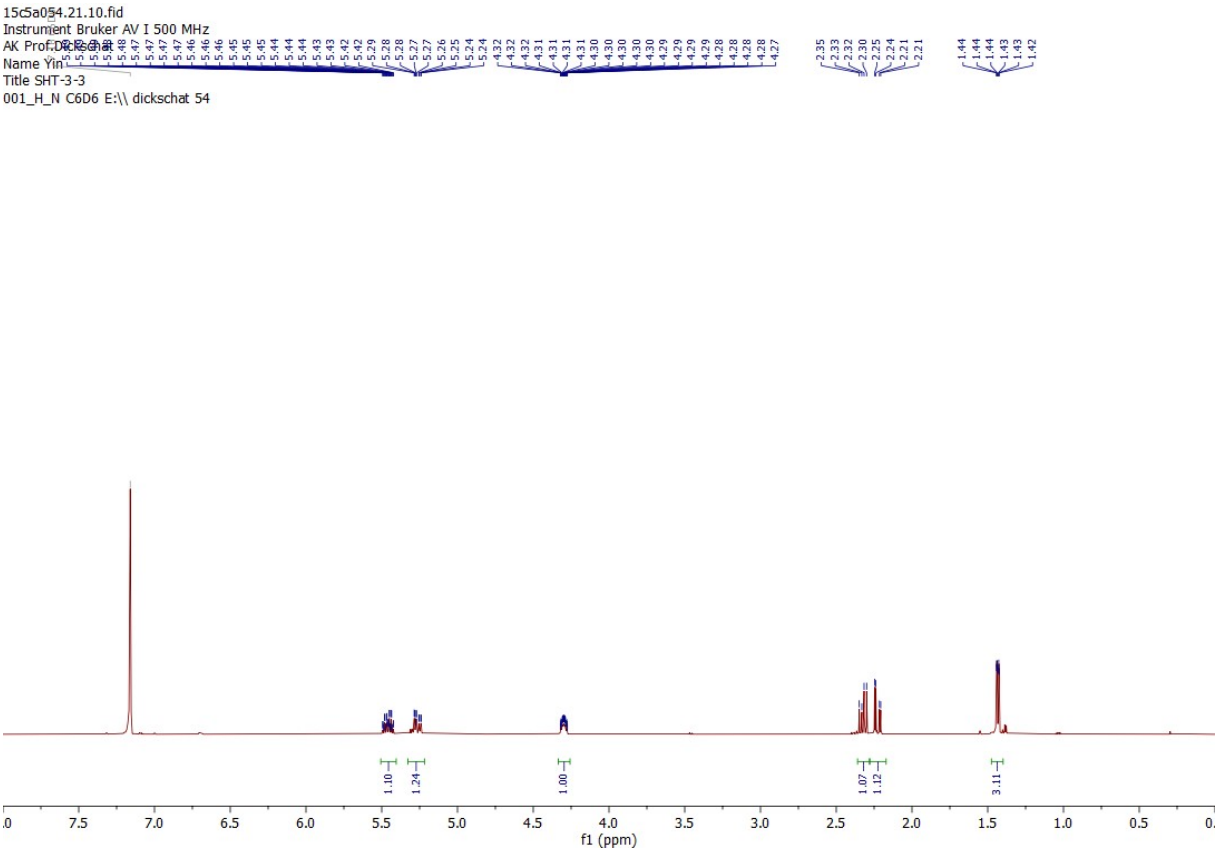


Figure S56. ^{1}H NMR (500 MHz, C_6D_6) of (*R*)-**S9**.

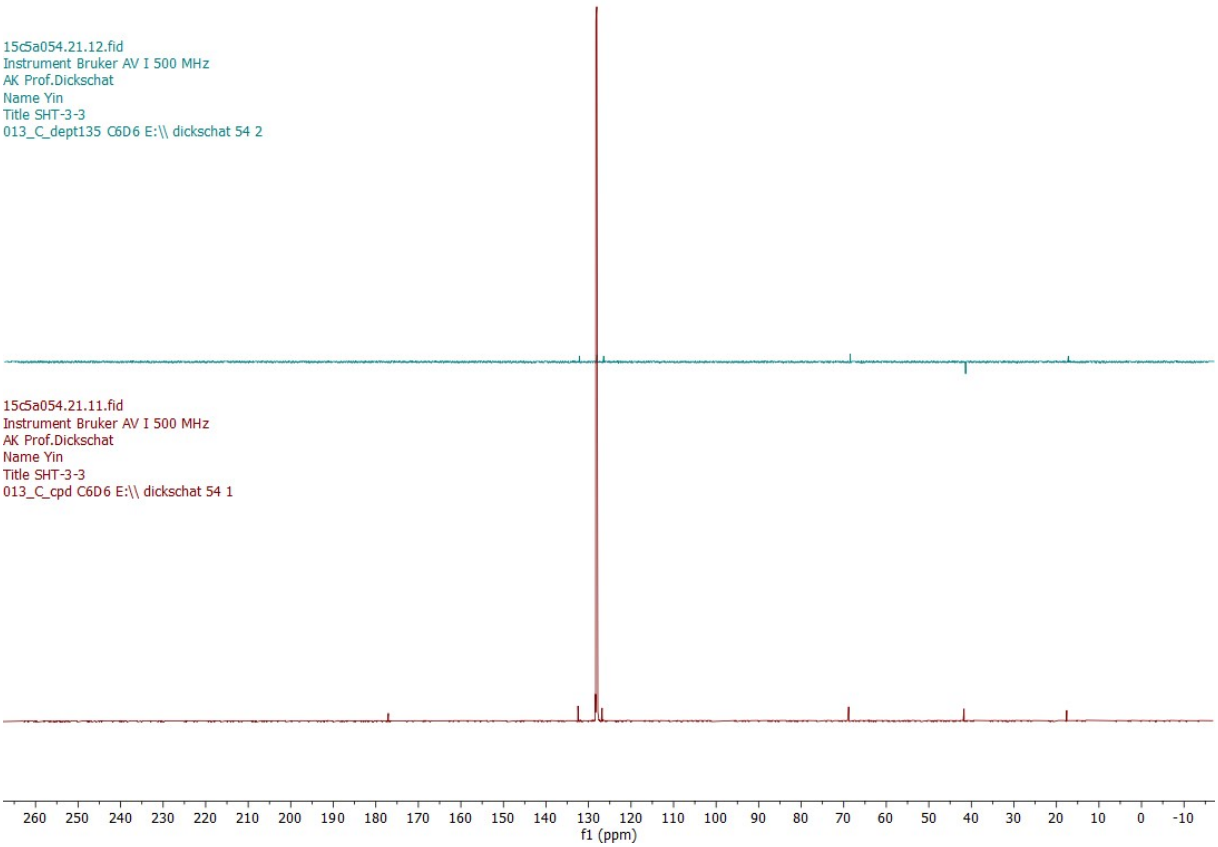


Figure S57. ^{13}C NMR (126 MHz, C_6D_6) of (*R*)-**S9**.

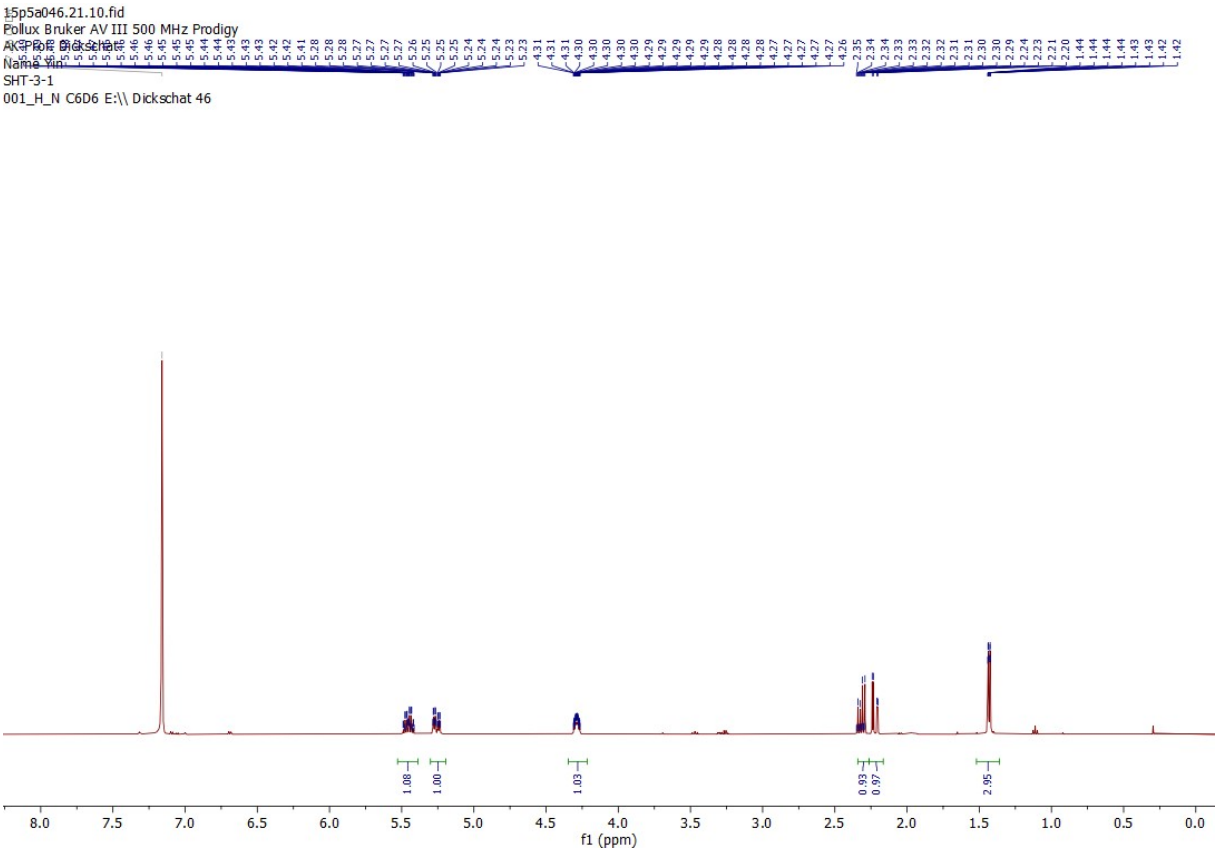


Figure S58. ^{1}H NMR (500 MHz, C_6D_6) of (*S*)-**S9**.

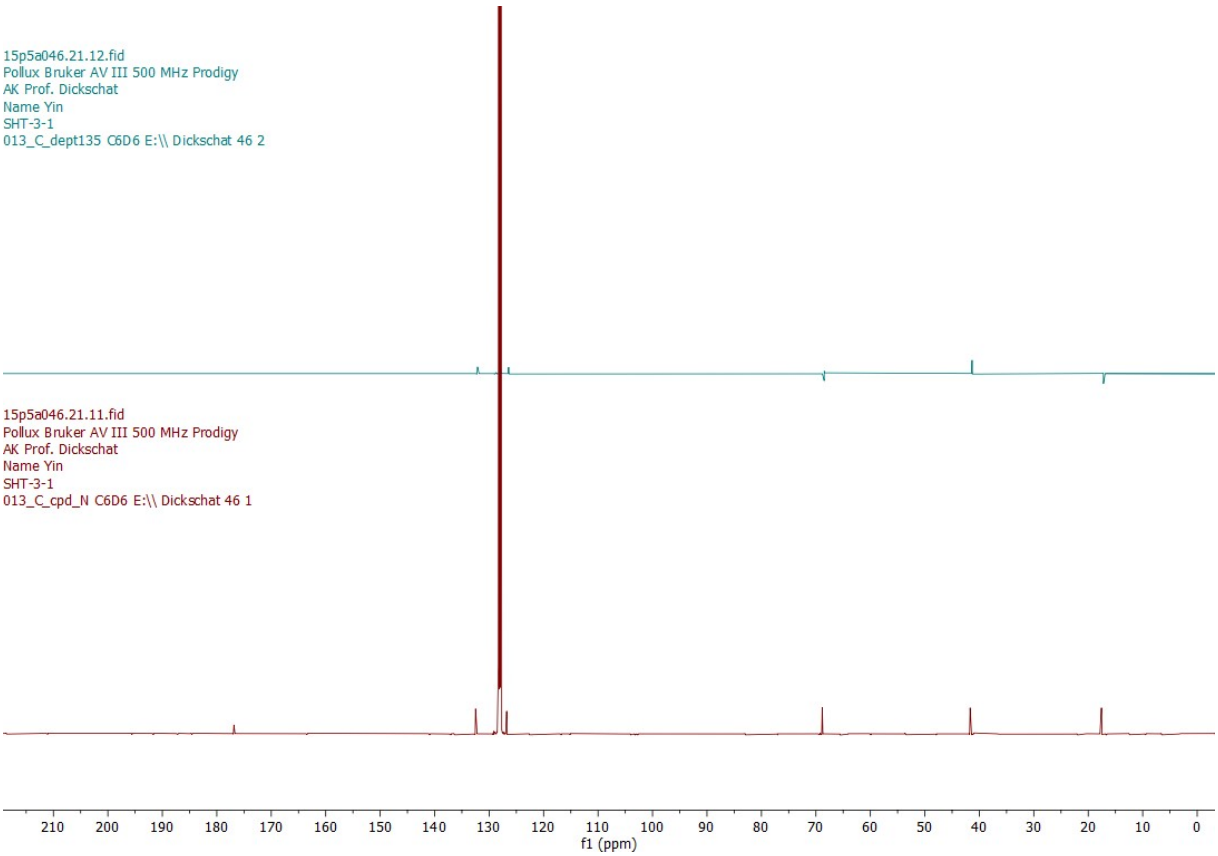


Figure S59. ^{13}C NMR (126 MHz, C_6D_6) of (*S*)-**S9**.

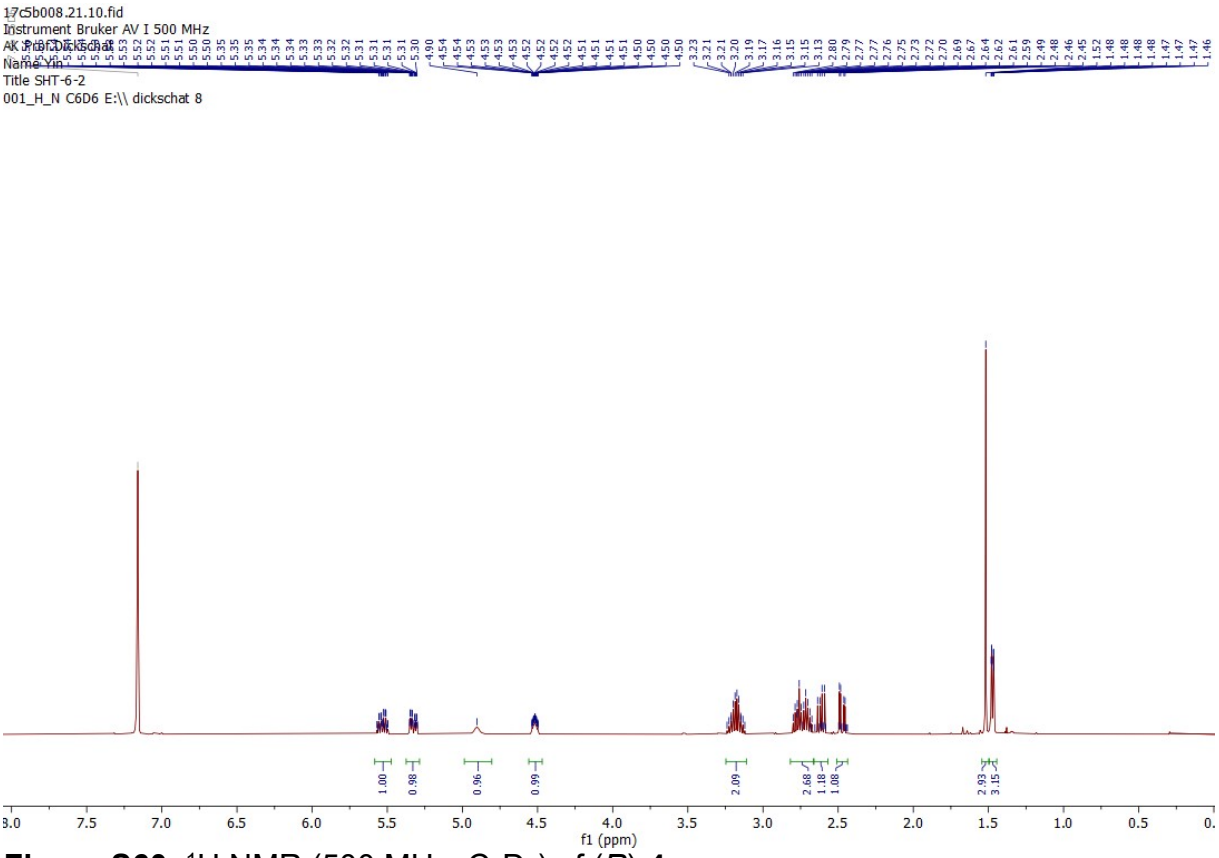


Figure S60. ^{1}H NMR (500 MHz, C_6D_6) of (*R*)-**4**.

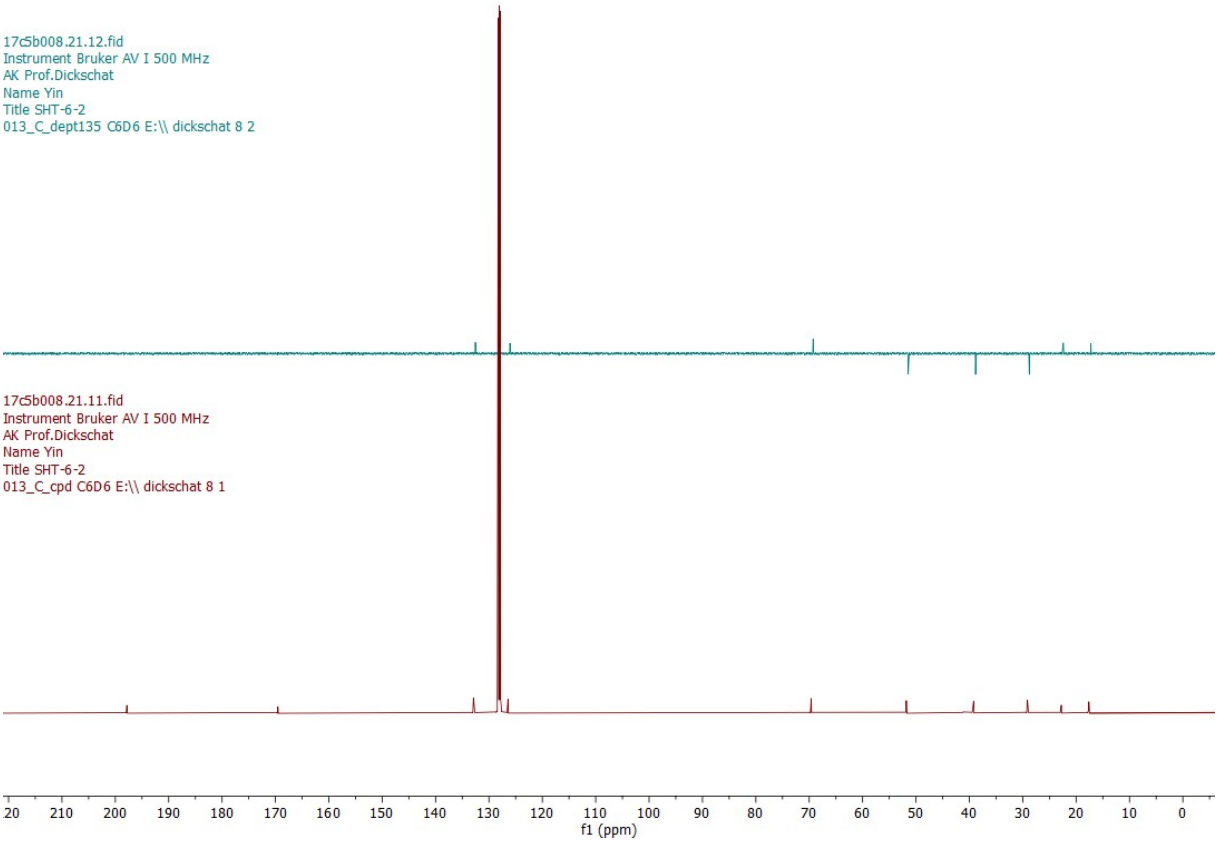


Figure S61. ^{13}C NMR (126 MHz, C_6D_6) of (*R*)-**4**.

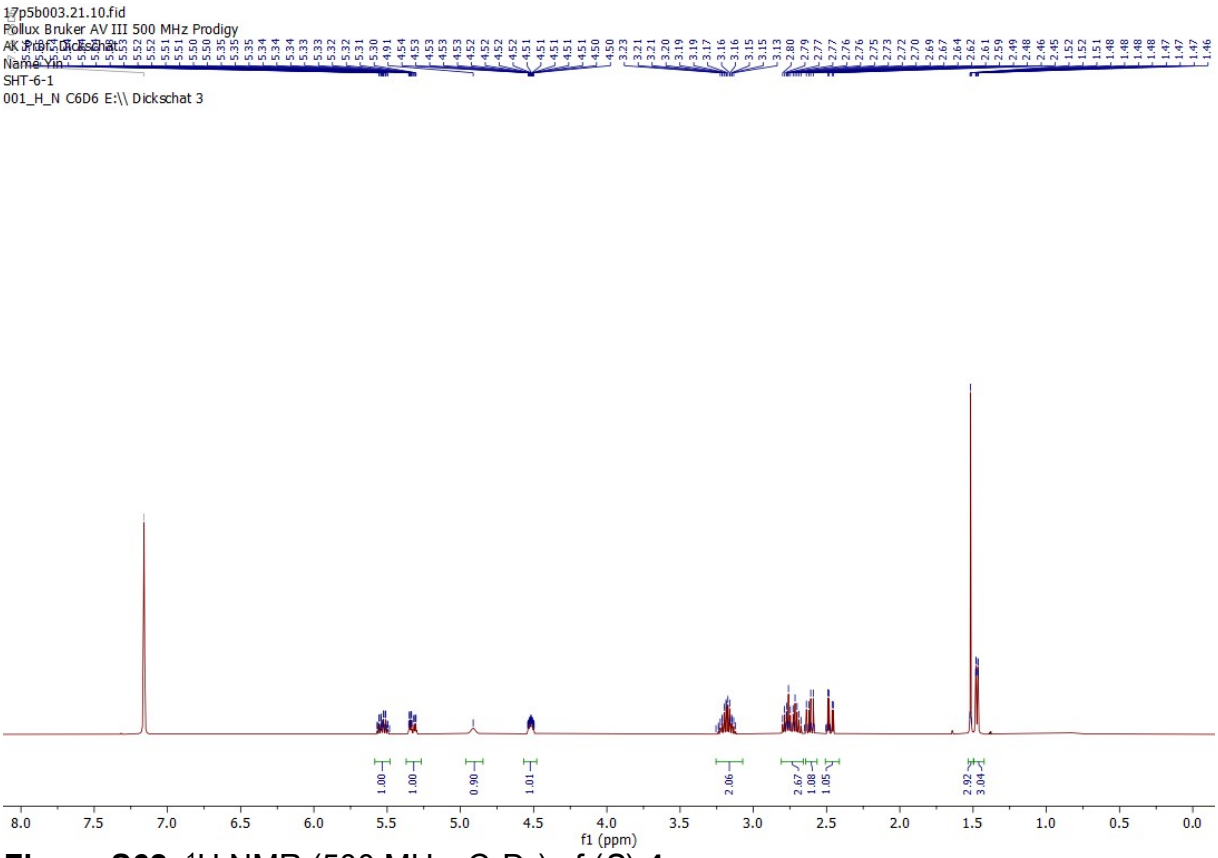


Figure S62. ^{1}H NMR (500 MHz, C_6D_6) of (*S*)-**4**.

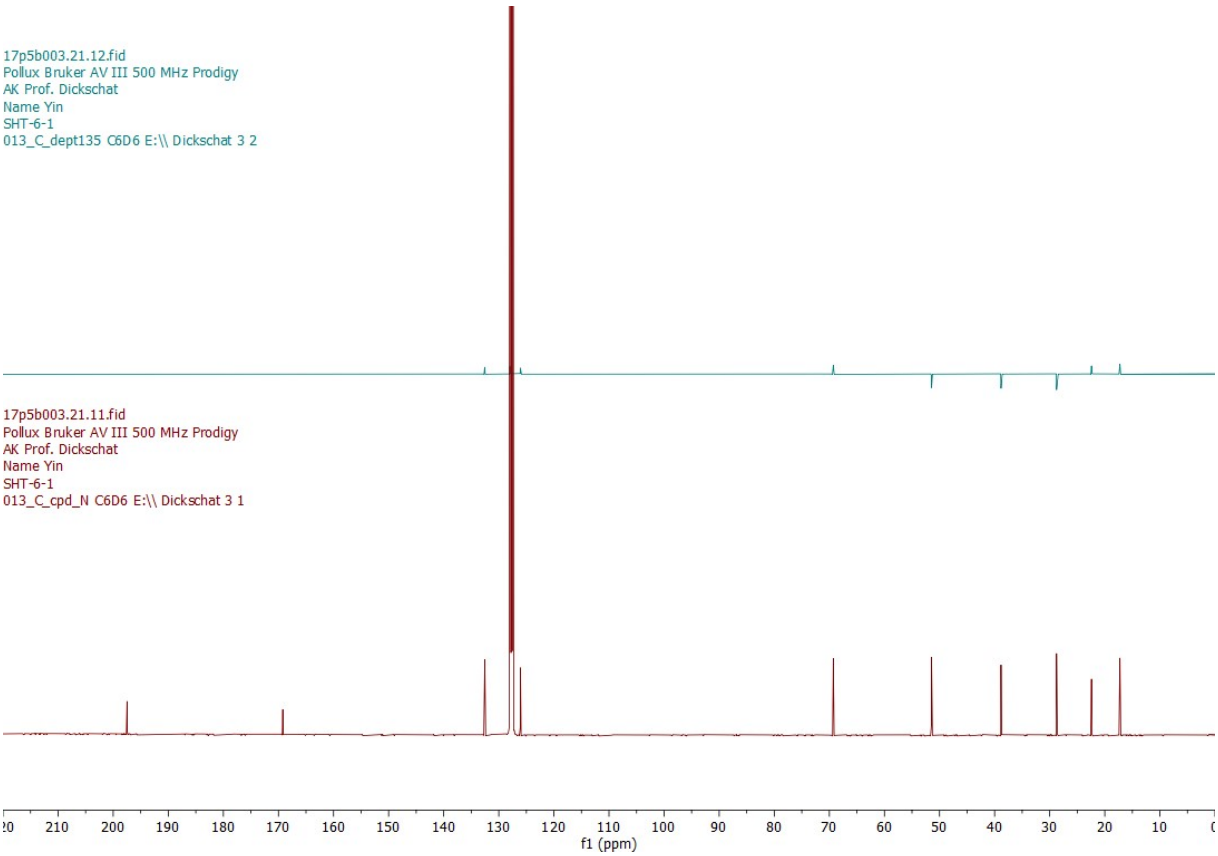


Figure S63. ^{13}C NMR (126 MHz, C_6D_6) of (*S*)-**4**.

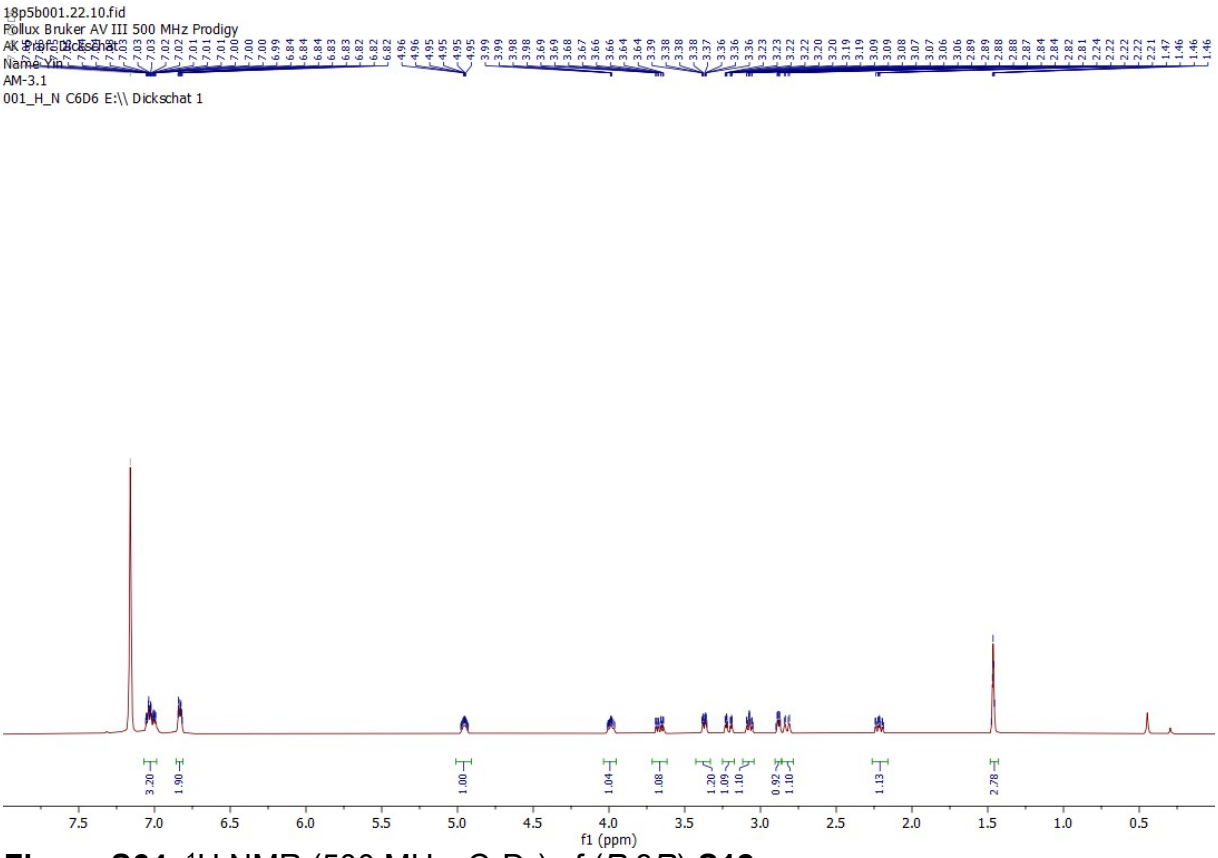


Figure S64. ^{1}H NMR (500 MHz, C_6D_6) of (*R*,3*R*)-**S12**.

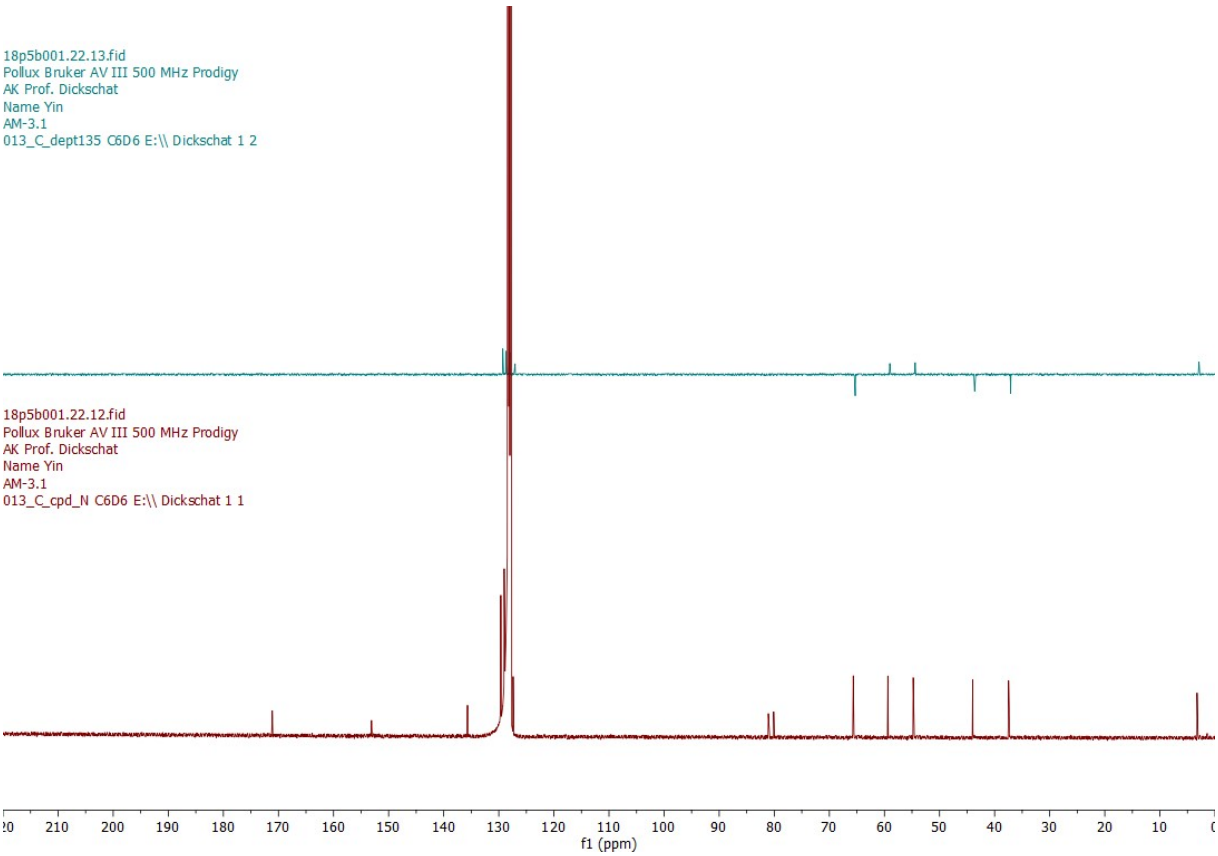


Figure S65. ^{13}C NMR (126 MHz, C_6D_6) of (*R*,3*R*)-**S12**.

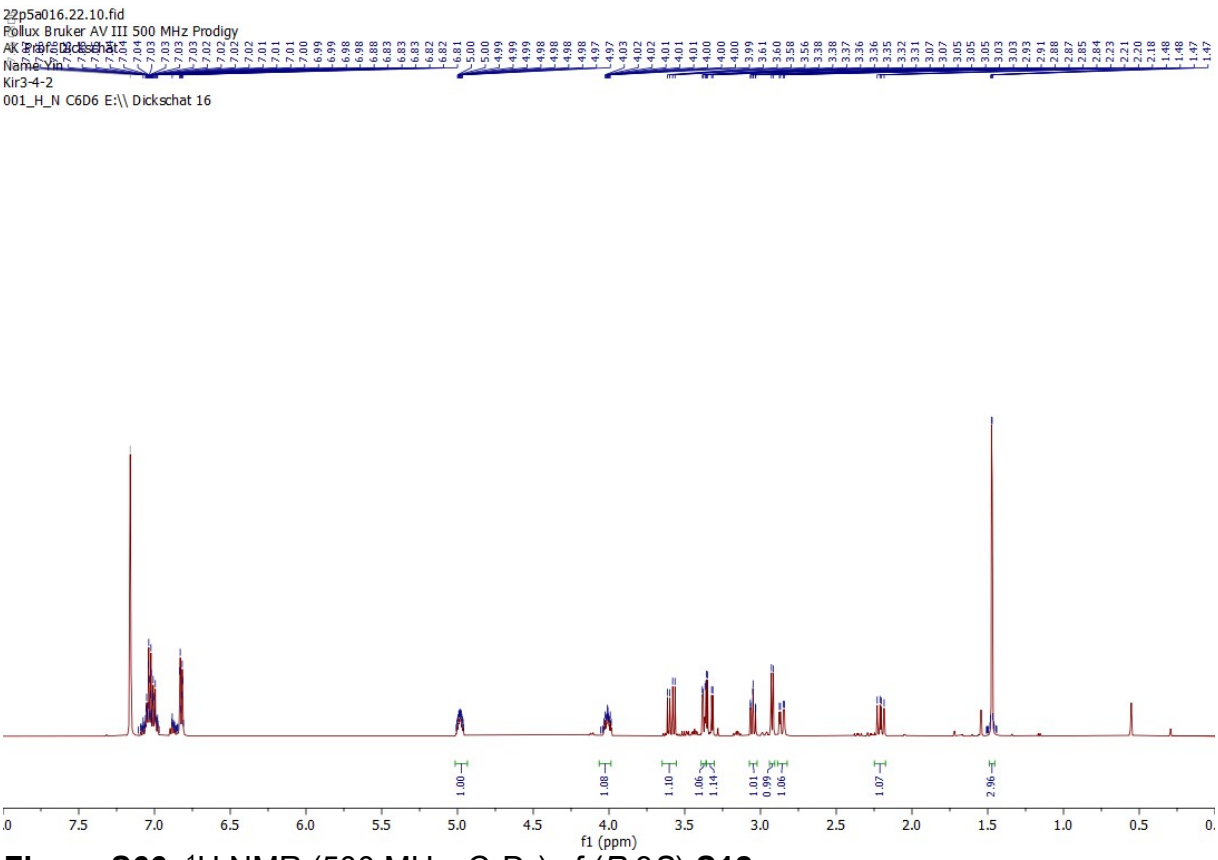


Figure S66. ^{1}H NMR (500 MHz, C_6D_6) of (*R*,3*S*)-**S12**.

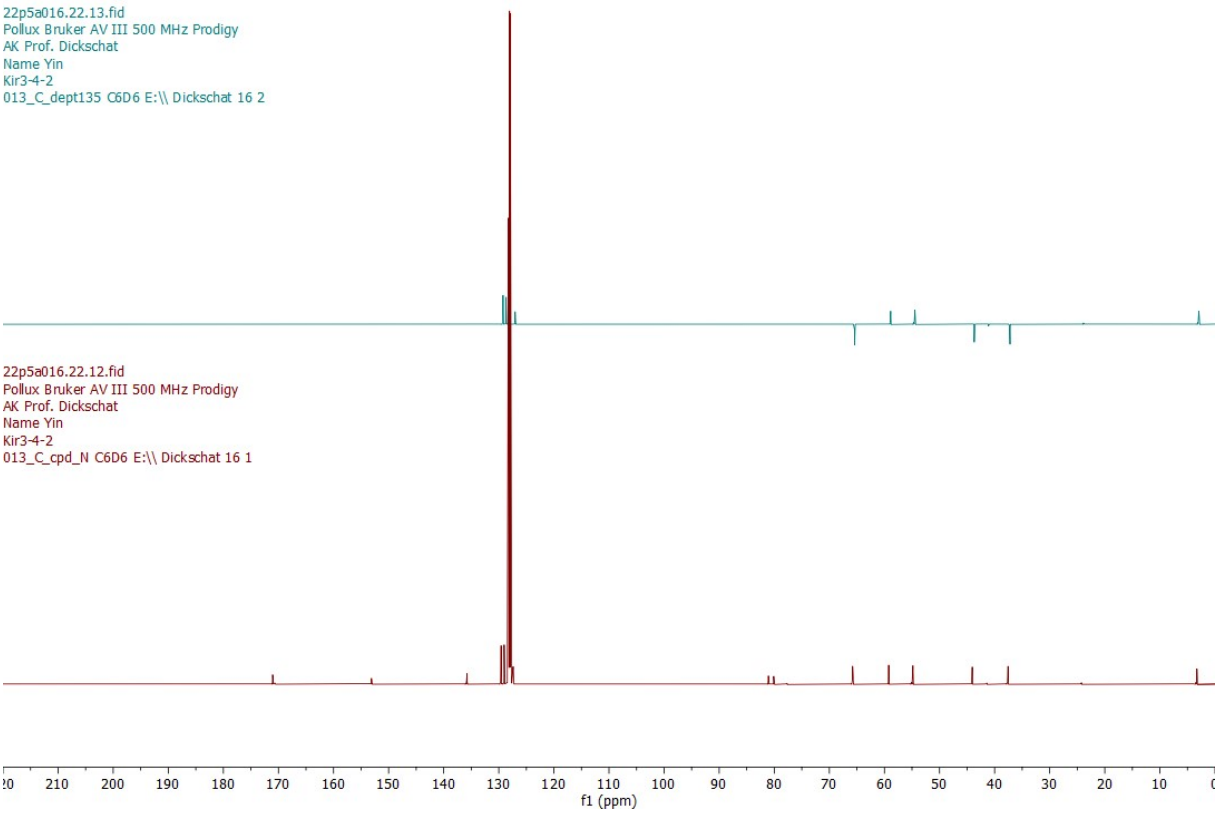


Figure S67. ^{13}C NMR (126 MHz, C_6D_6) of (*R*,3*S*)-**S12**.

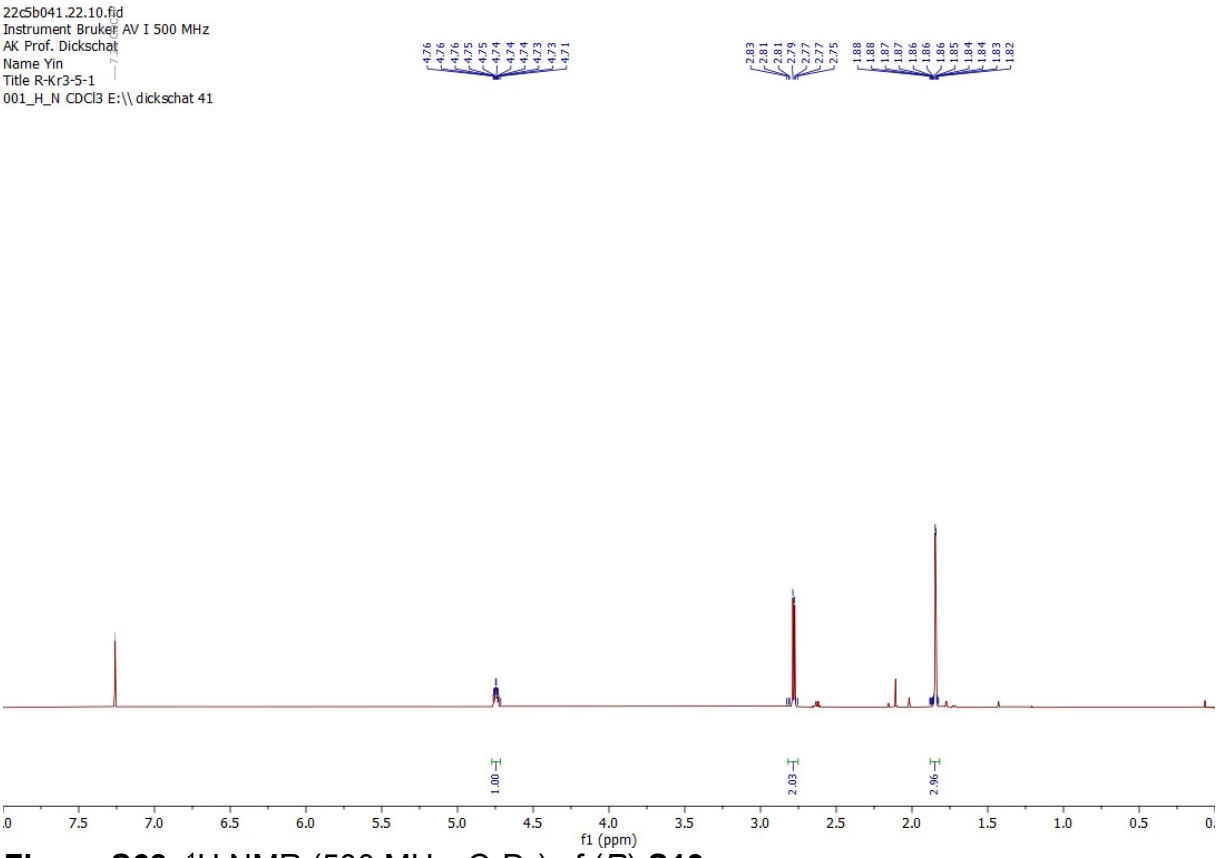


Figure S68. ^{1}H NMR (500 MHz, C_6D_6) of (*R*)-**S13**.

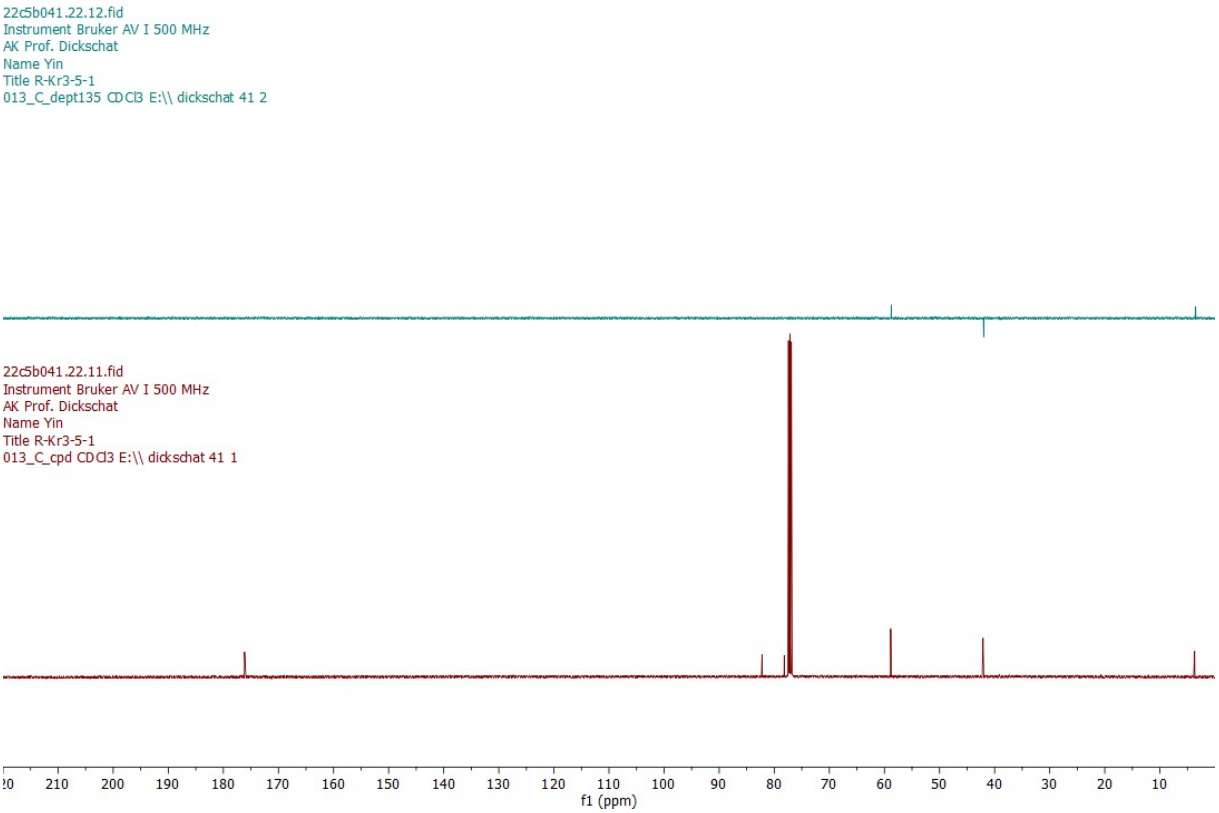


Figure S69. ^{13}C NMR (126 MHz, C_6D_6) of (*R*)-**S13**.

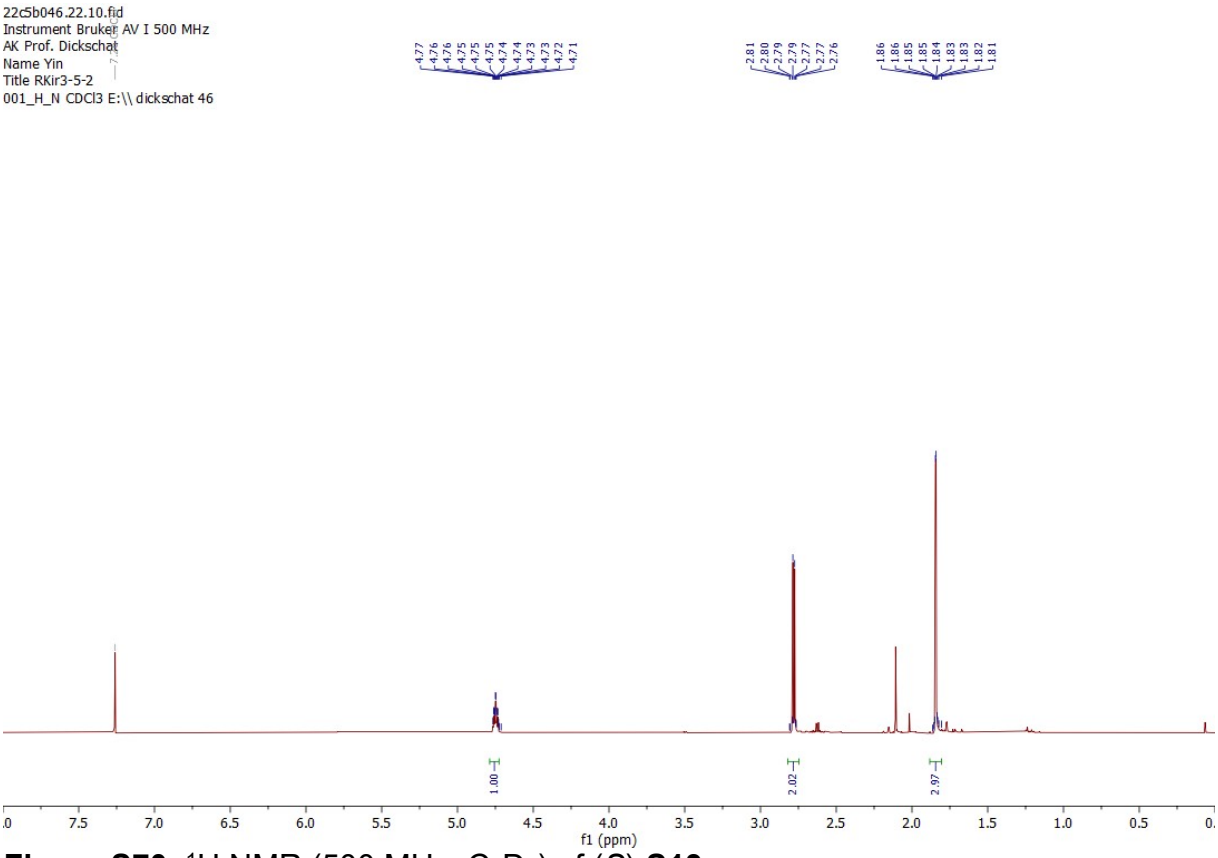


Figure S70. ^{1}H NMR (500 MHz, C_6D_6) of (*S*)-**S13**.

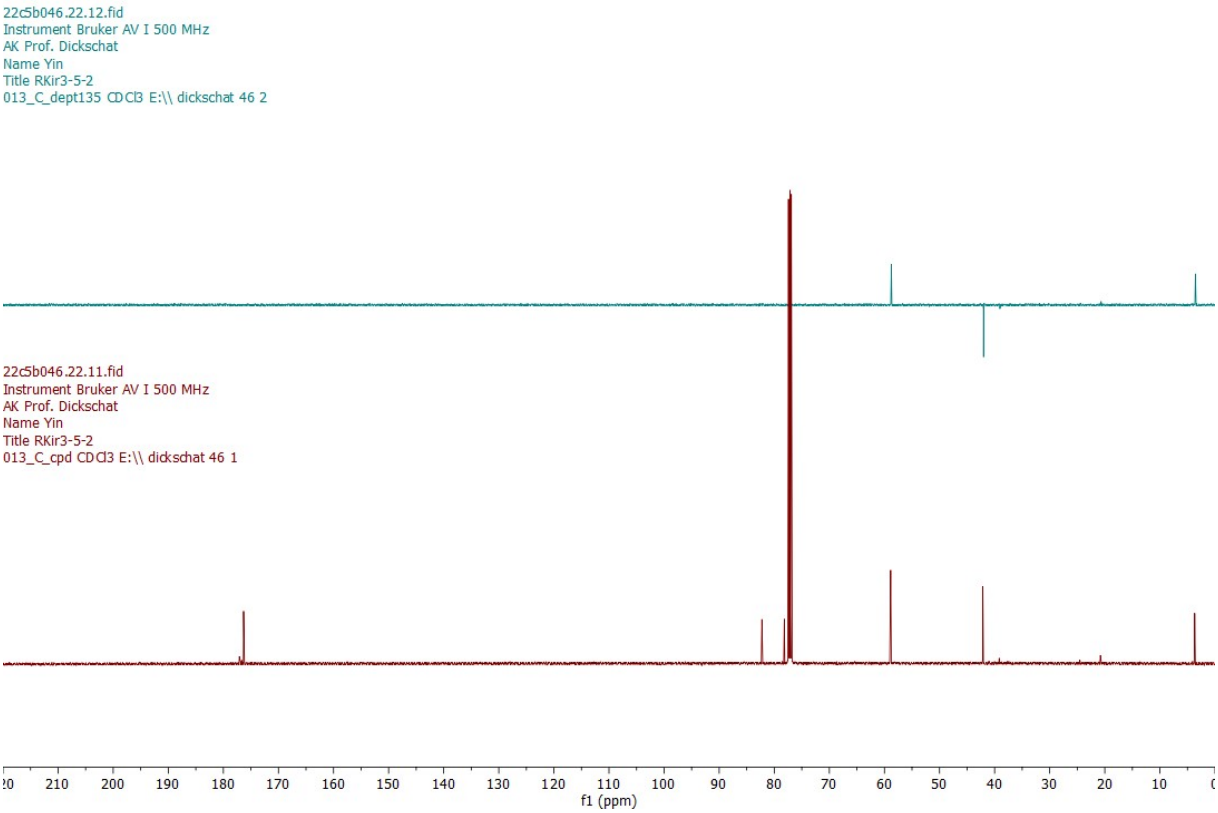


Figure S71. ^{13}C NMR (126 MHz, C_6D_6) of (*S*)-**S13**.

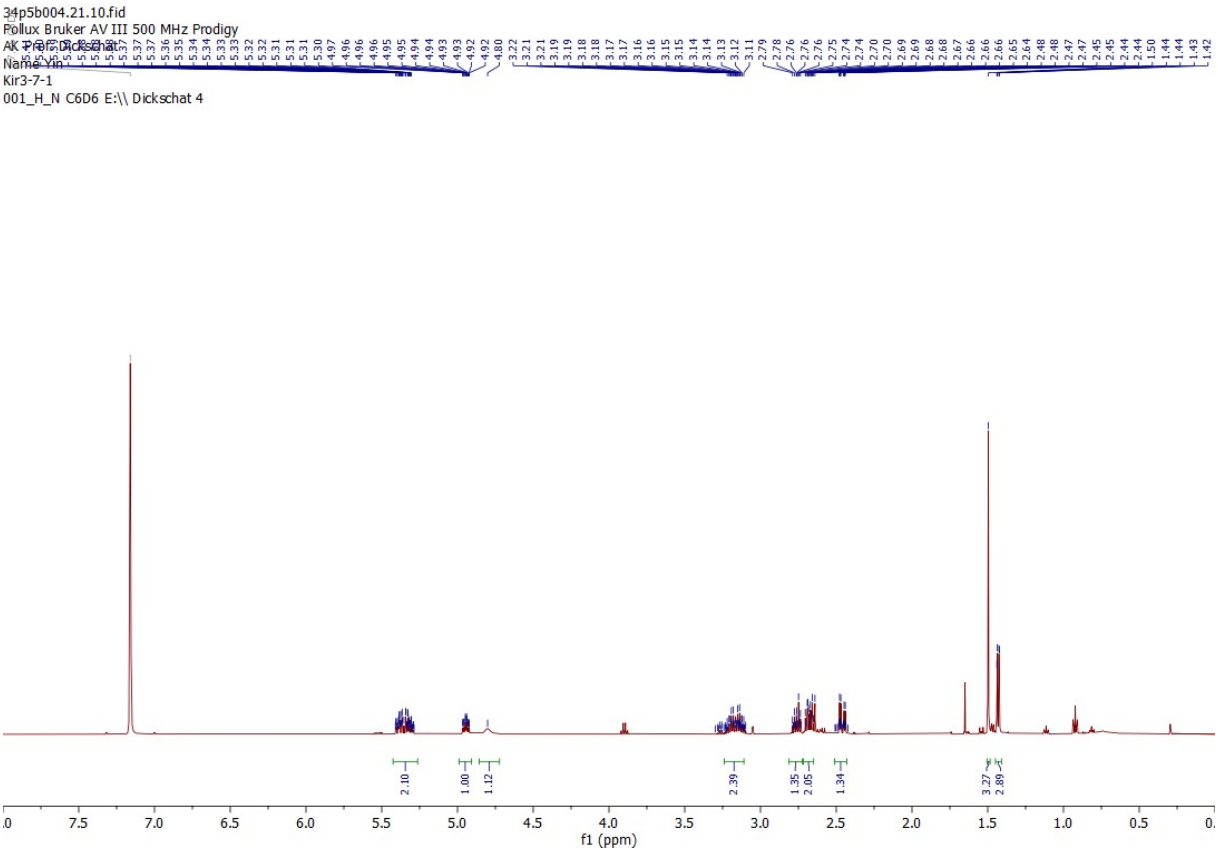


Figure S72. ^{1}H NMR (500 MHz, C_6D_6) of (*R*)-**5**.

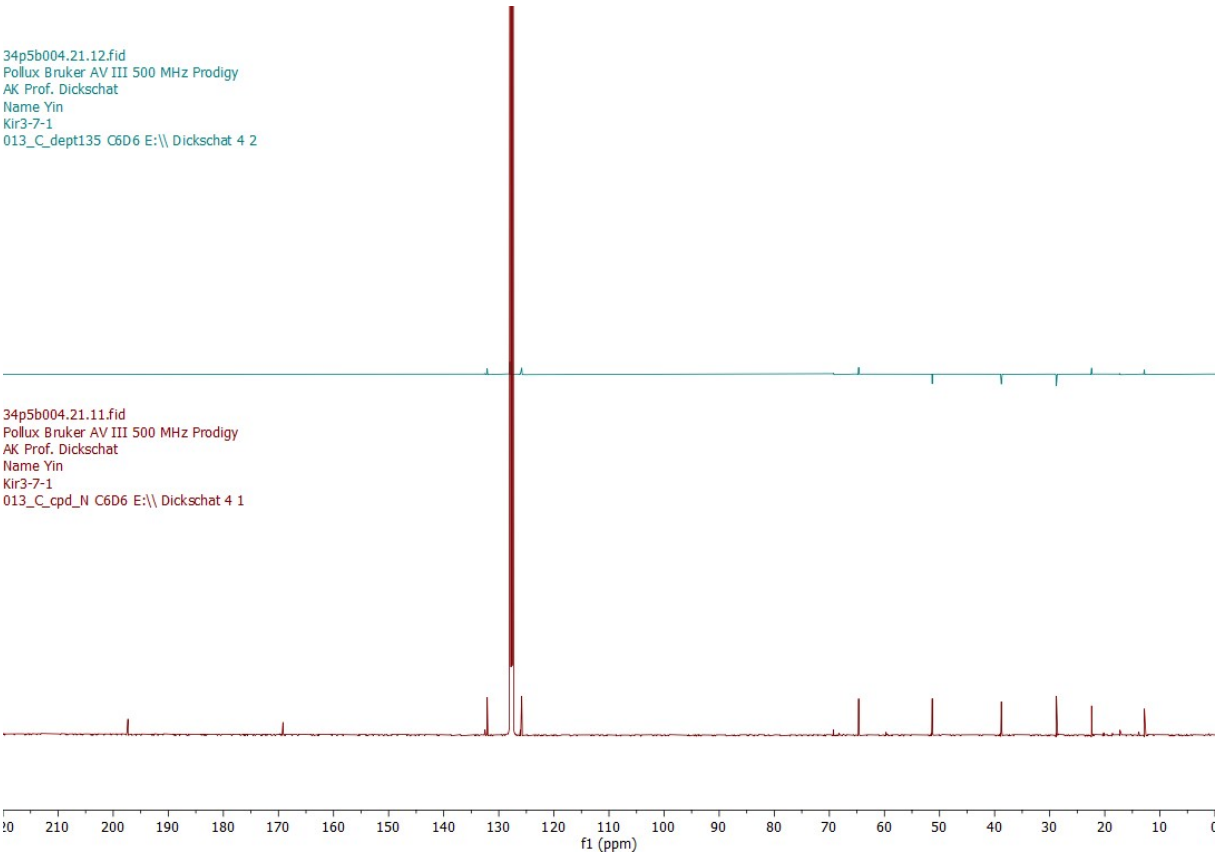


Figure S73. ^{13}C NMR (126 MHz, C_6D_6) of (*R*)-**5**.

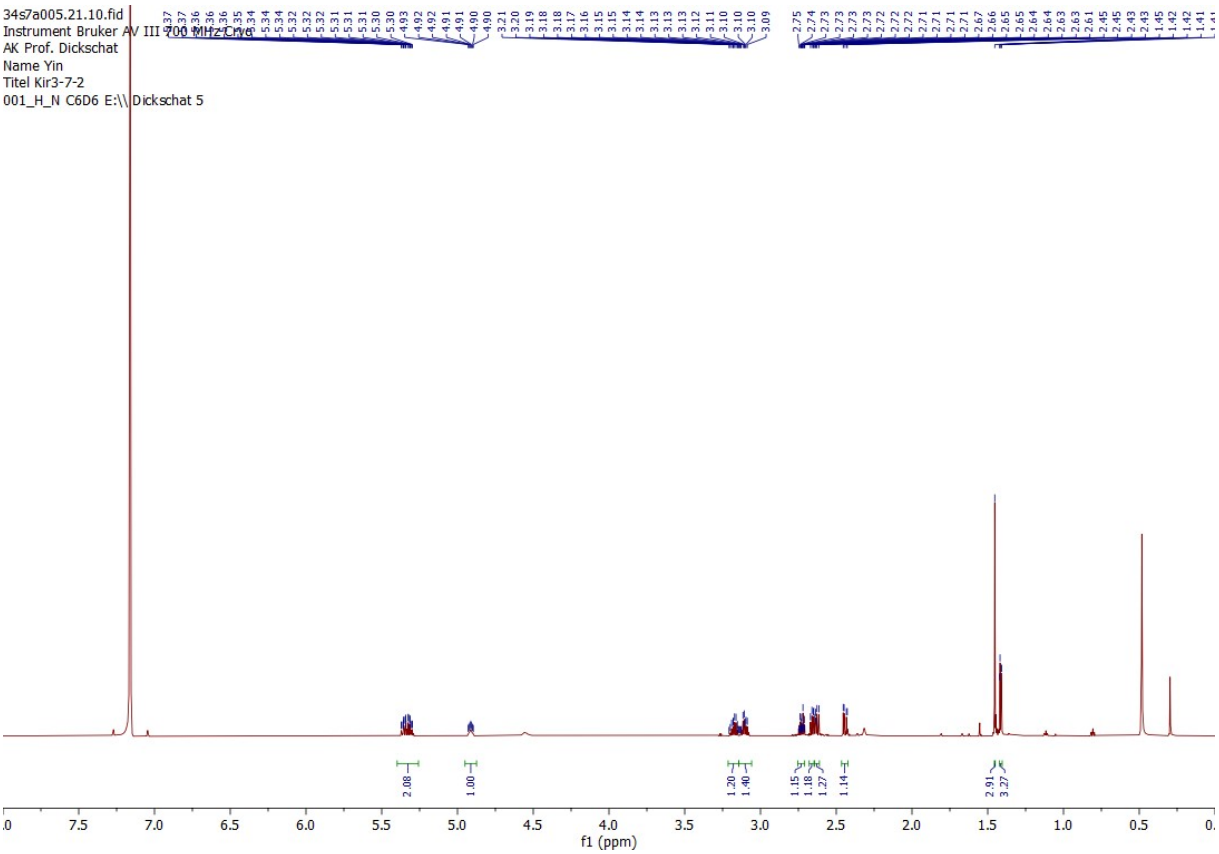


Figure S74. ^{1}H NMR (700 MHz, C_6D_6) of (*S*)-**5**.

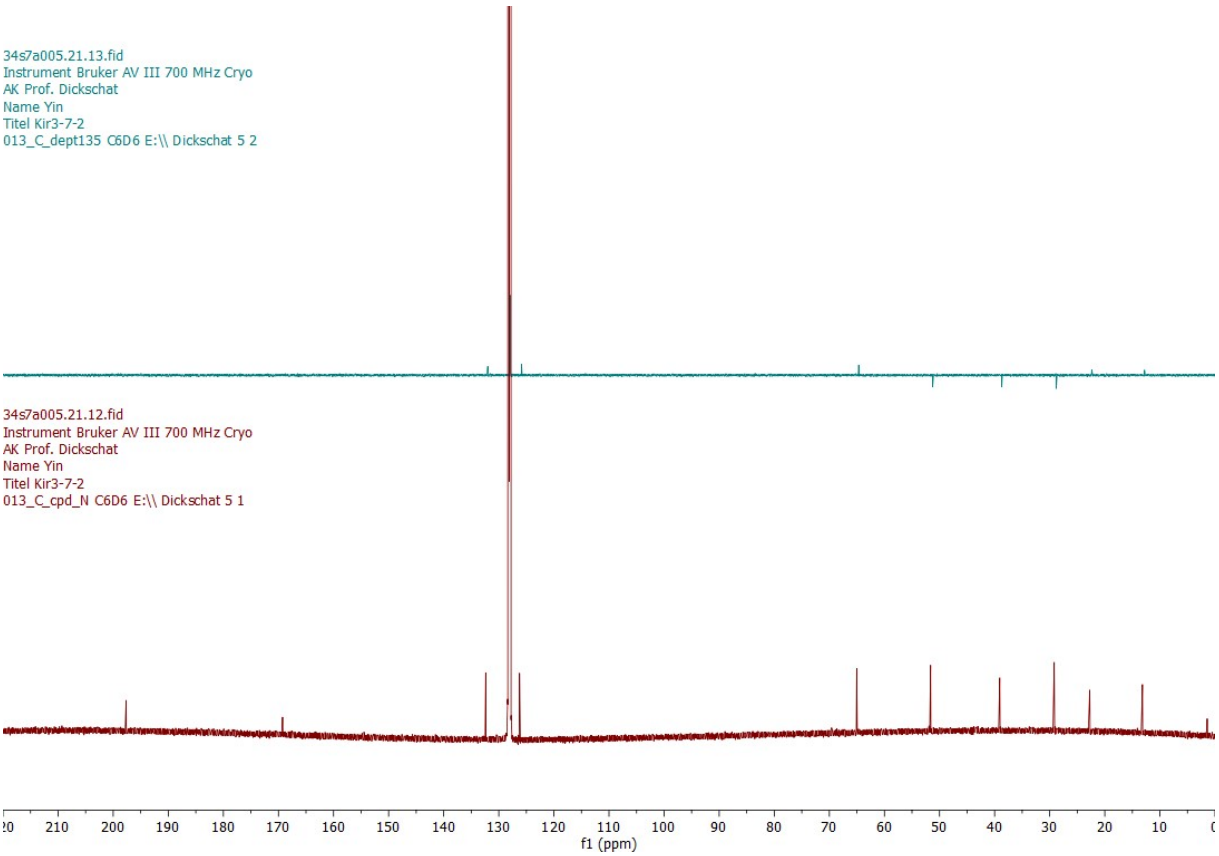


Figure S75. ^{13}C NMR (176 MHz, C_6D_6) of (*S*)-**5**.

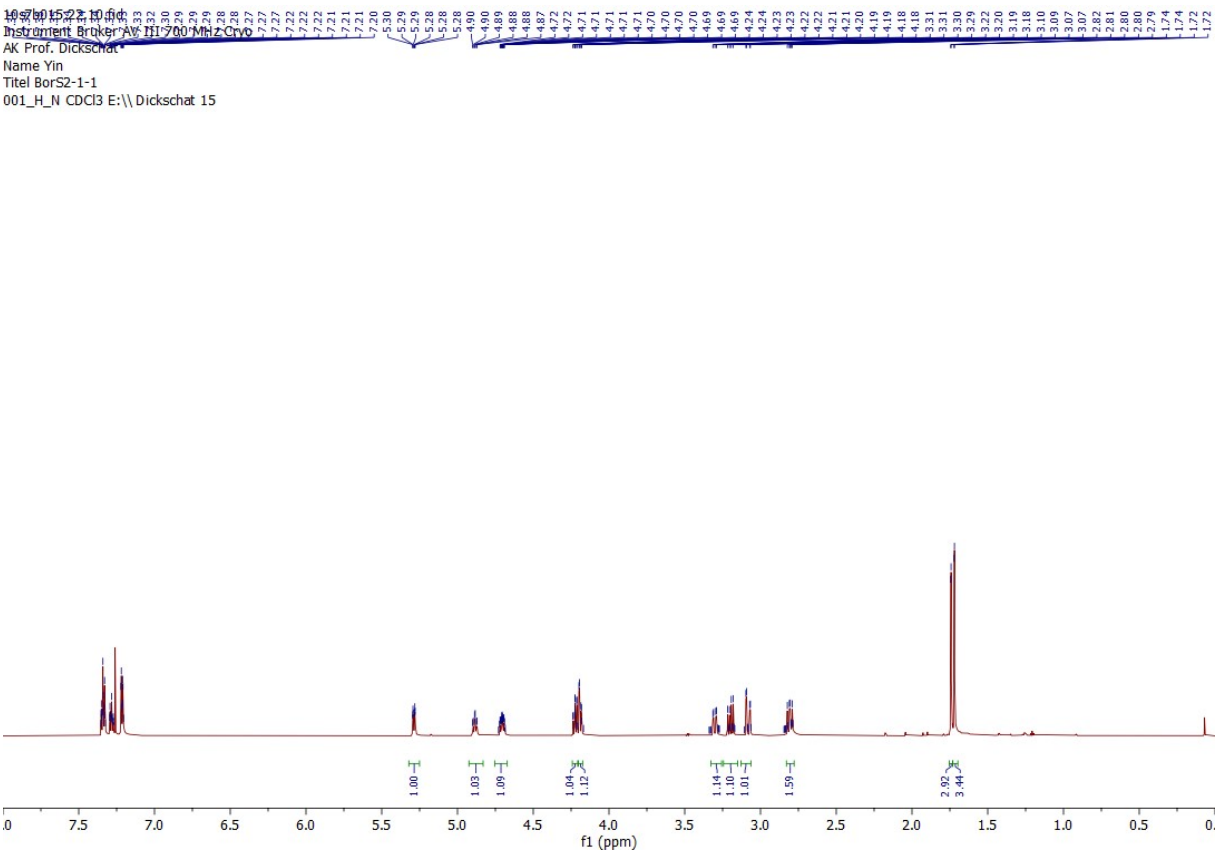


Figure S76. ^{1}H NMR (700 MHz, $CDCl_3$) of (*R*,3*R*)-**S15**.

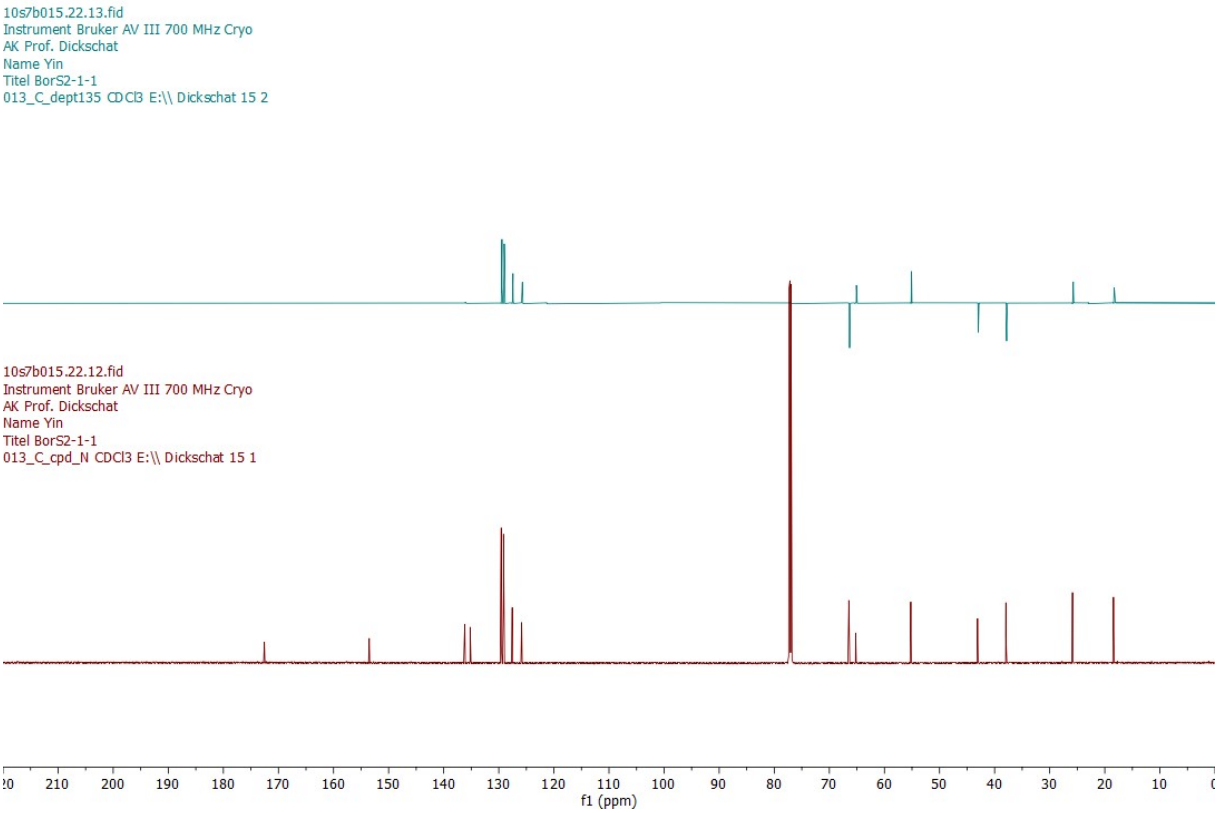


Figure S77. ^{13}C NMR (176 MHz, $CDCl_3$) of (*R*,3*R*)-**S15**.

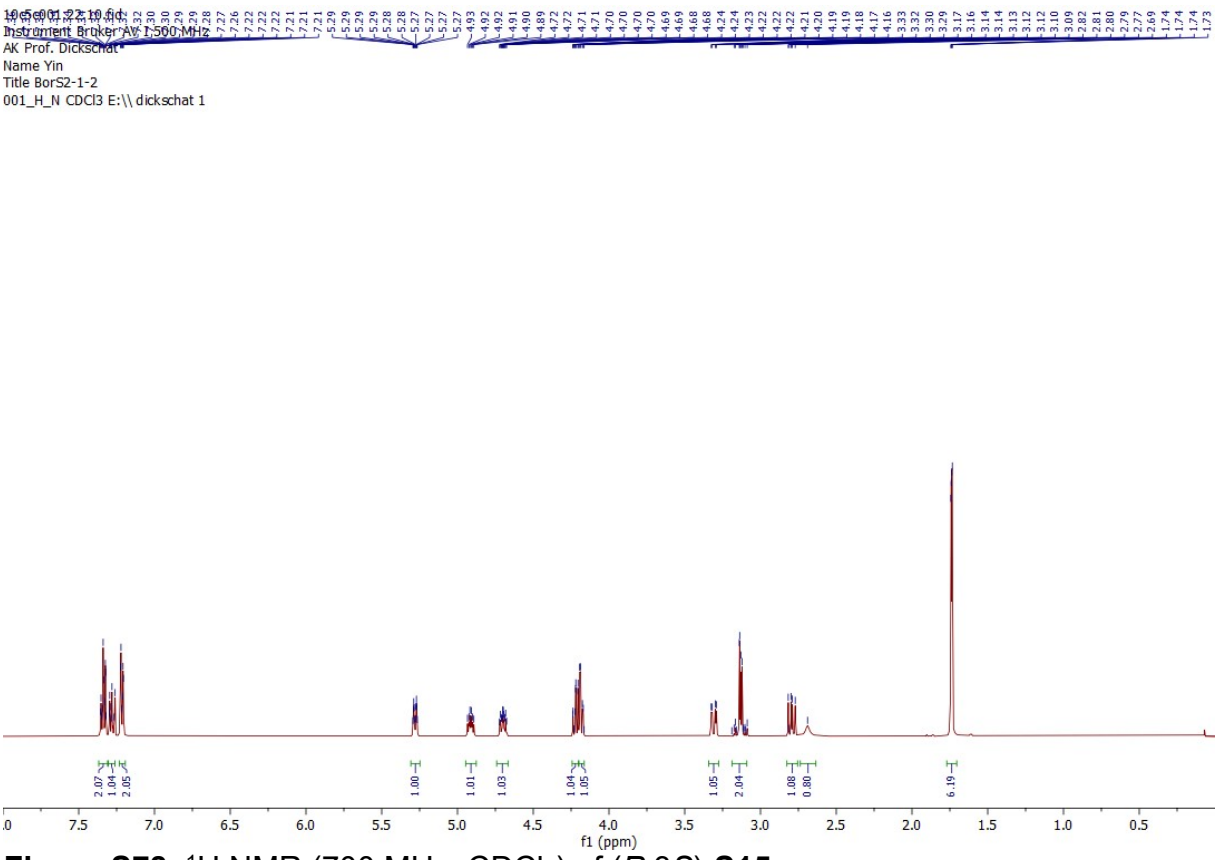


Figure S78. ^{1}H NMR (700 MHz, $CDCl_3$) of (*R*,3*S*)-**S15**.

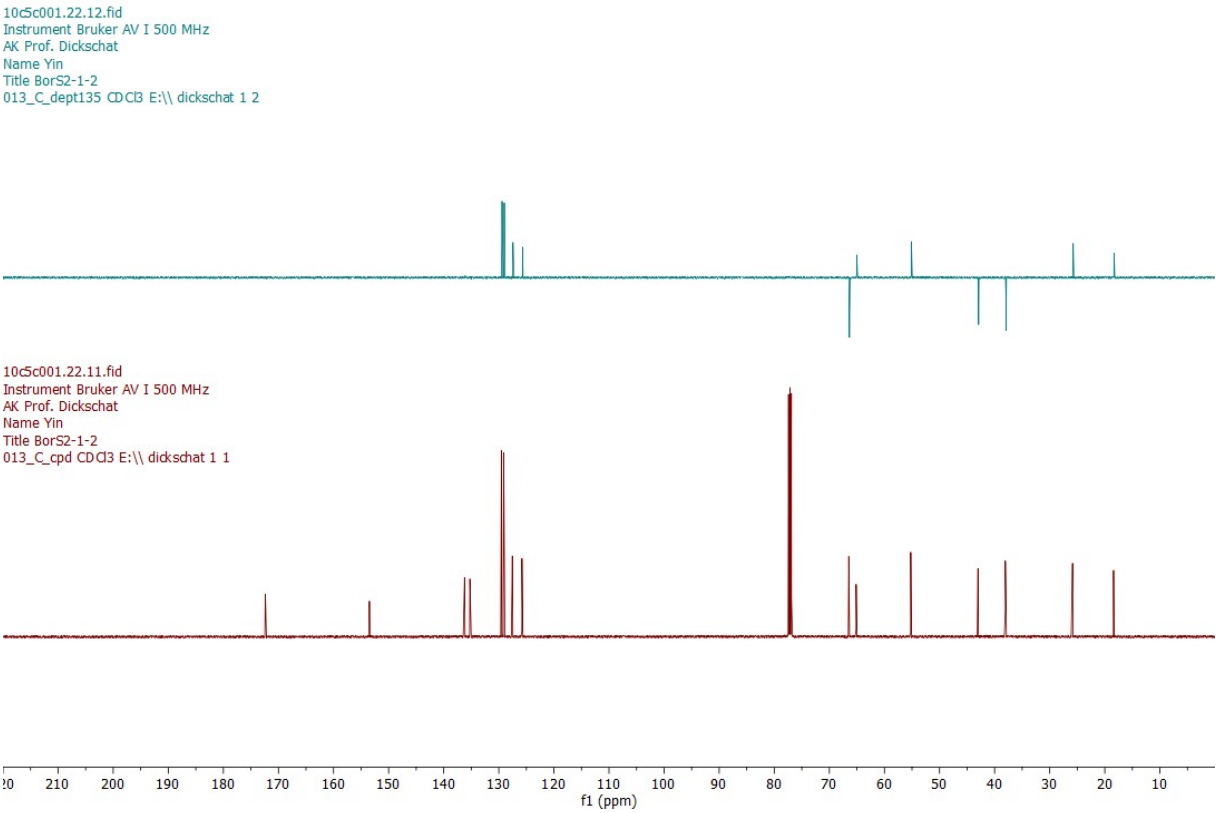


Figure S79. ^{13}C NMR (176 MHz, $CDCl_3$) of (*R*,3*S*)-**S15**.

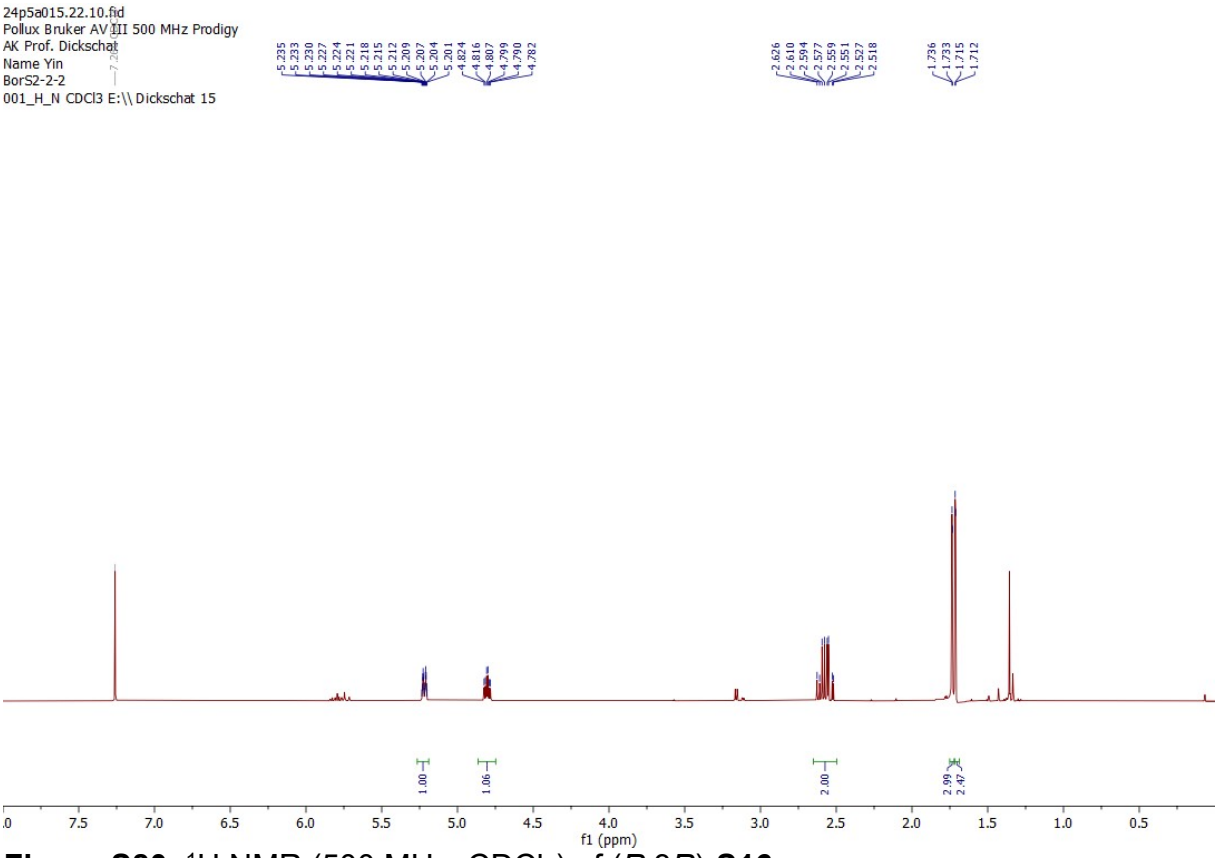


Figure S80. ^{1}H NMR (500 MHz, $CDCl_3$) of (*R*,3*R*)-**S16**.

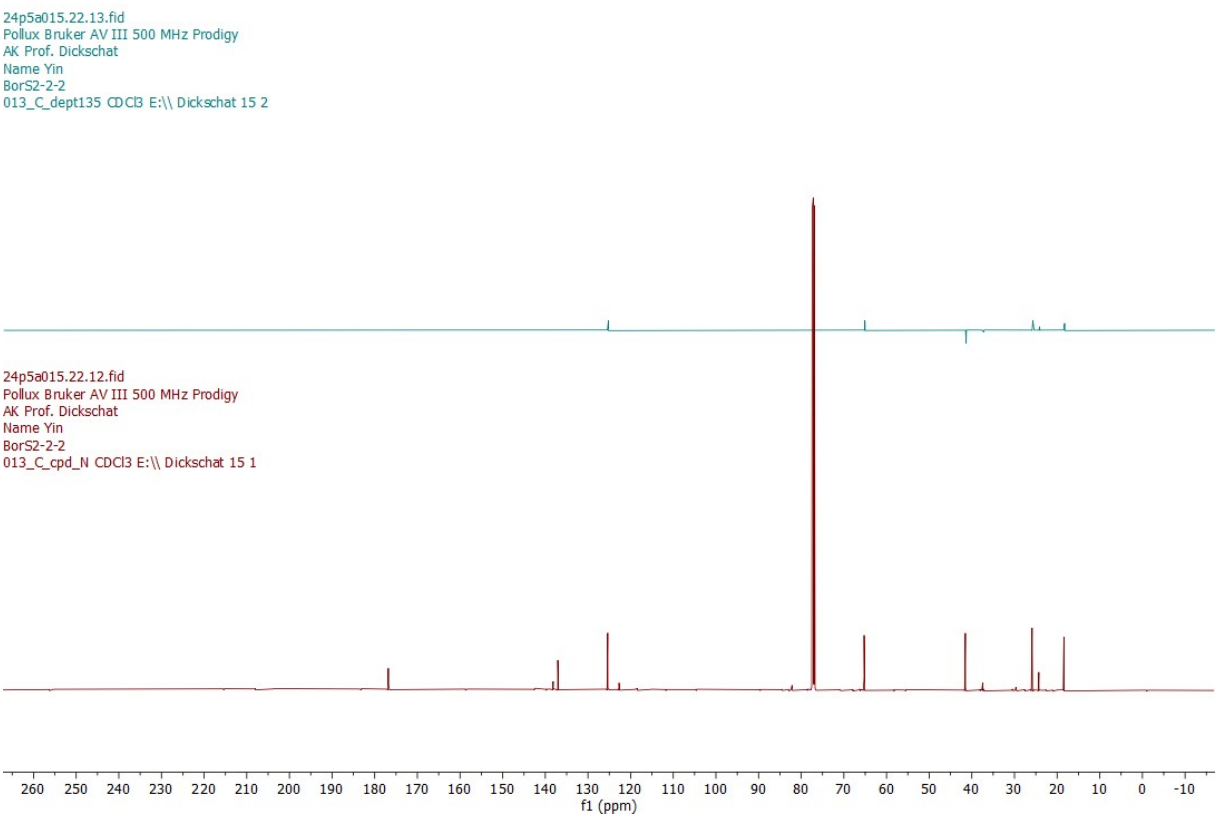


Figure S81. ^{13}C NMR (126 MHz, $CDCl_3$) of (*R*,3*R*)-**S16**.

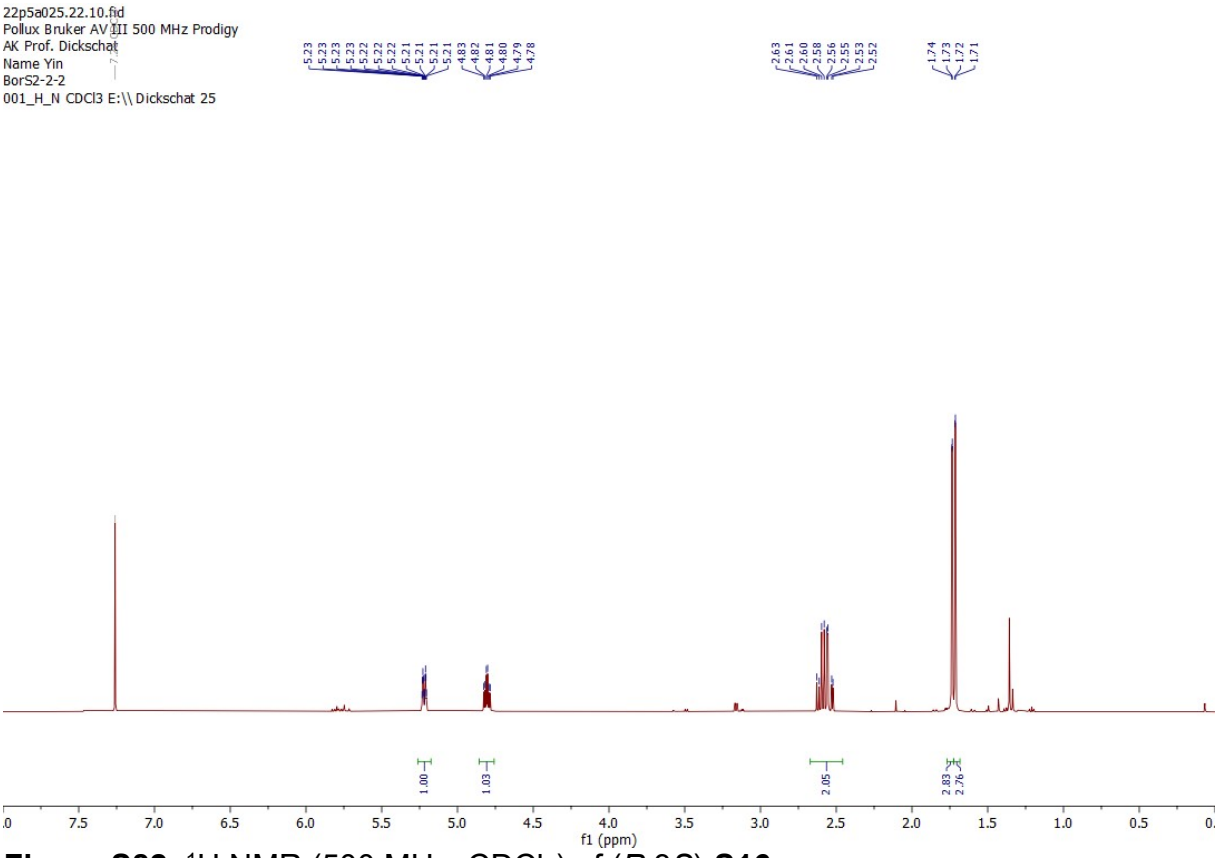


Figure S82. ^{1}H NMR (500 MHz, $CDCl_3$) of (*R*,3*S*)-**S16**.

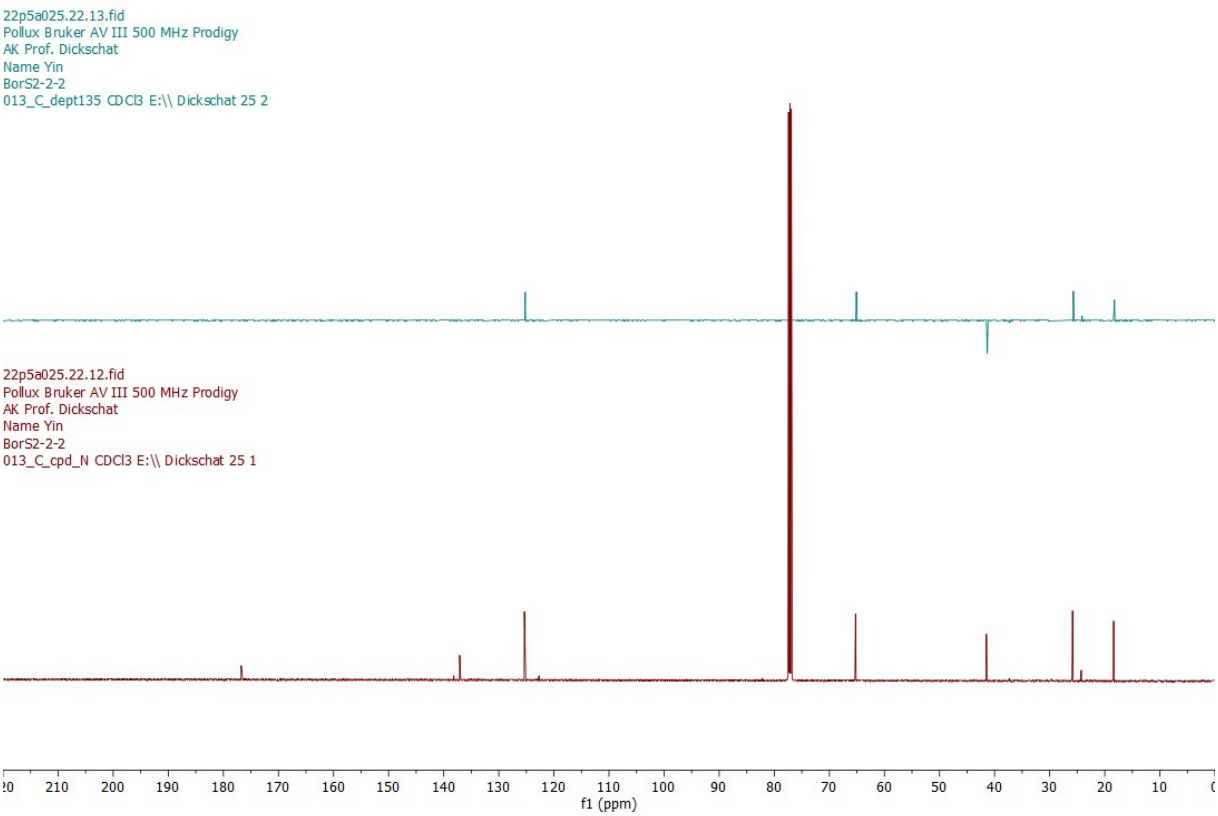


Figure S83. ^{13}C NMR (126 MHz, $CDCl_3$) of (*R*,3*S*)-**S16**.

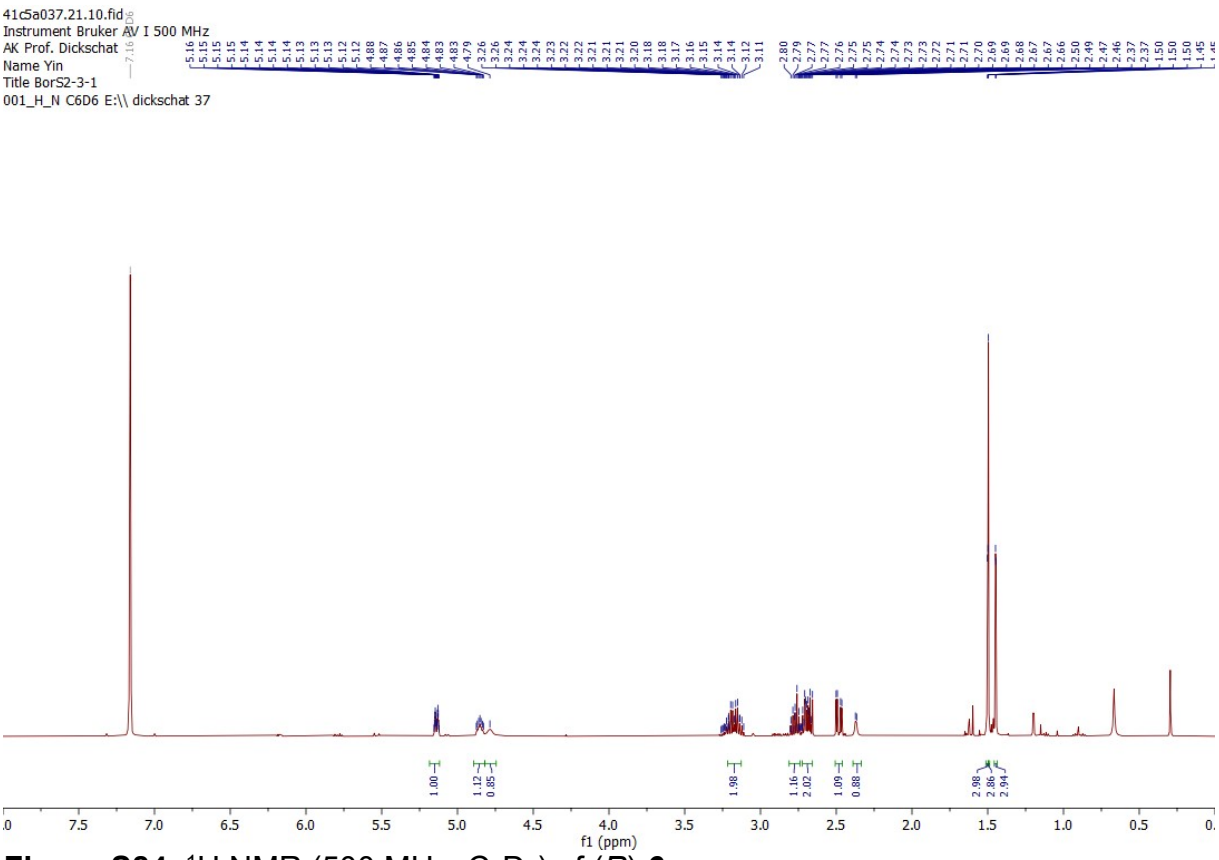


Figure S84. ^{1}H NMR (500 MHz, C_6D_6) of (*R*)-**6**.

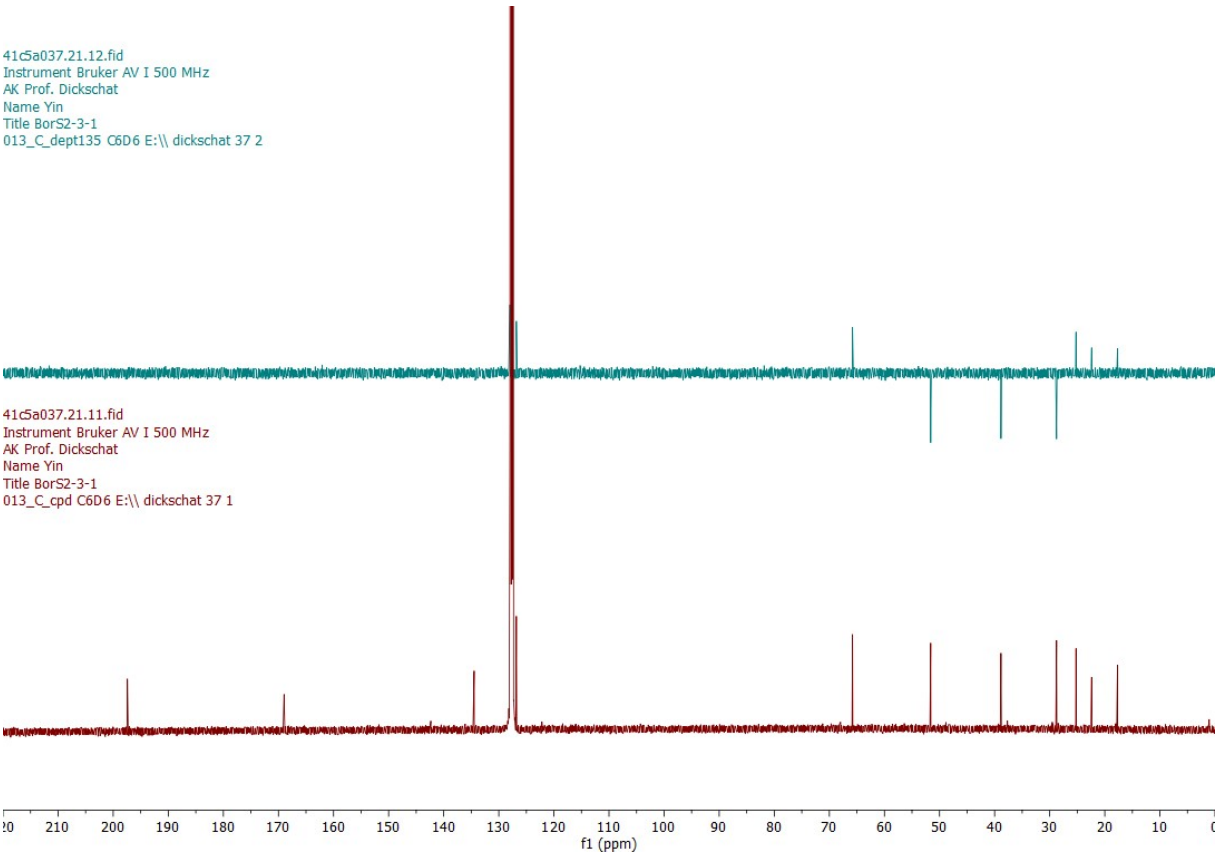


Figure S85. ^{13}C NMR (126 MHz, C_6D_6) of (*R*)-**6**.

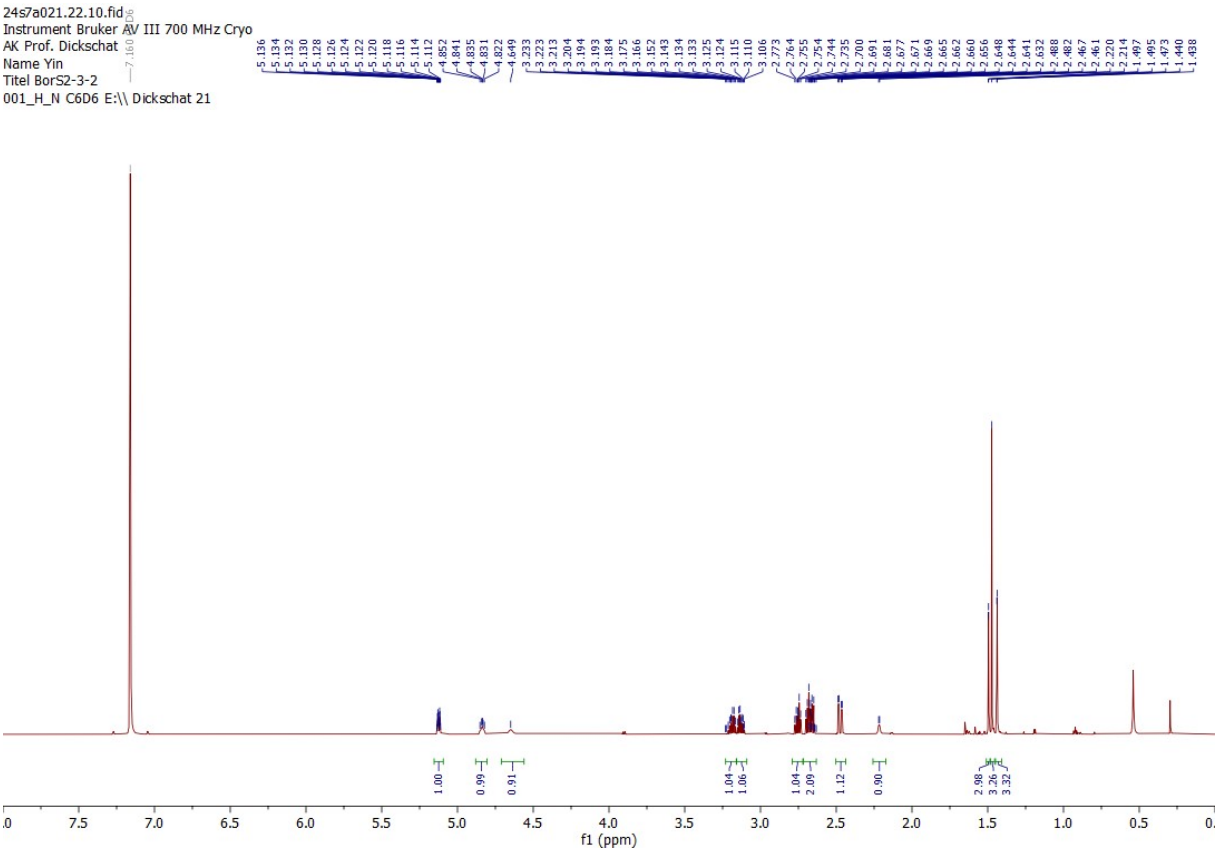


Figure S86. ^{1}H NMR (700 MHz, C_6D_6) of (*S*)-**6**.

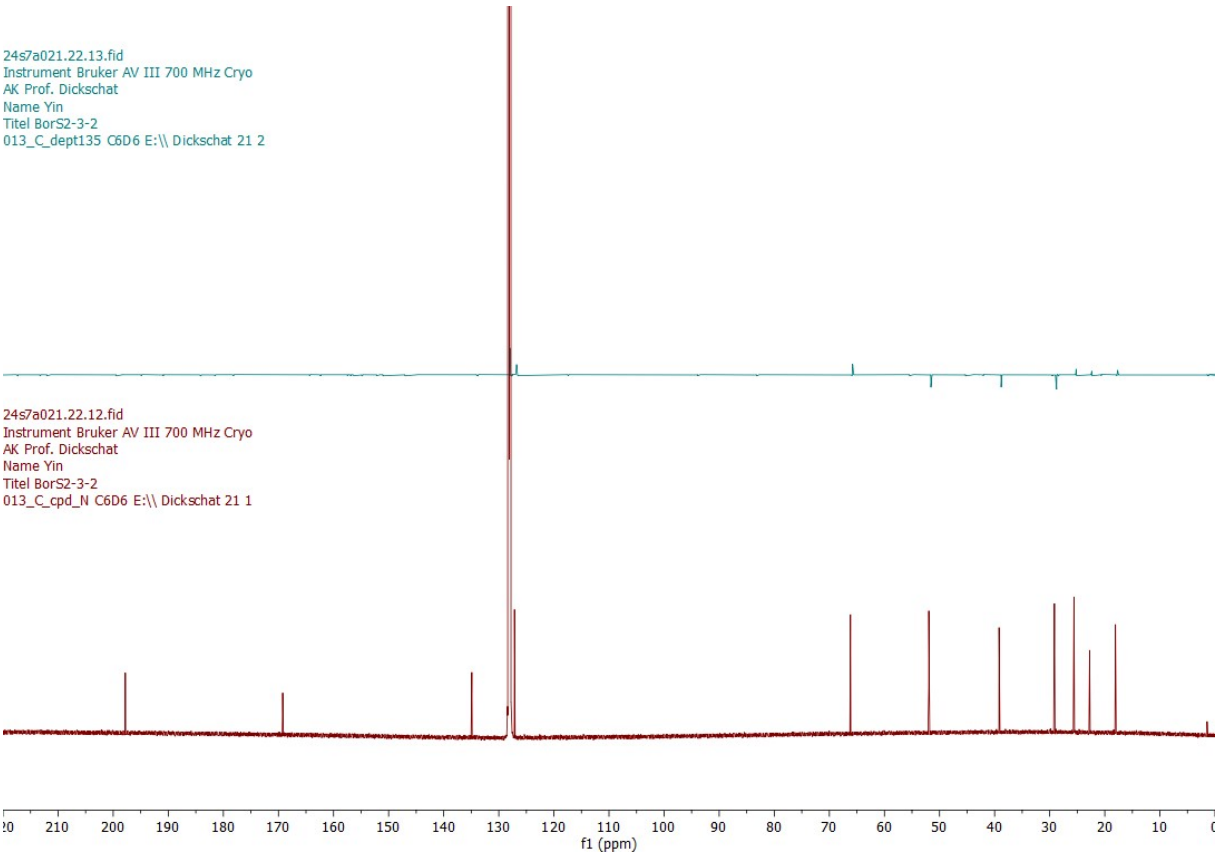


Figure S87. ^{13}C NMR (176 MHz, C_6D_6) of (*S*)-**6**.

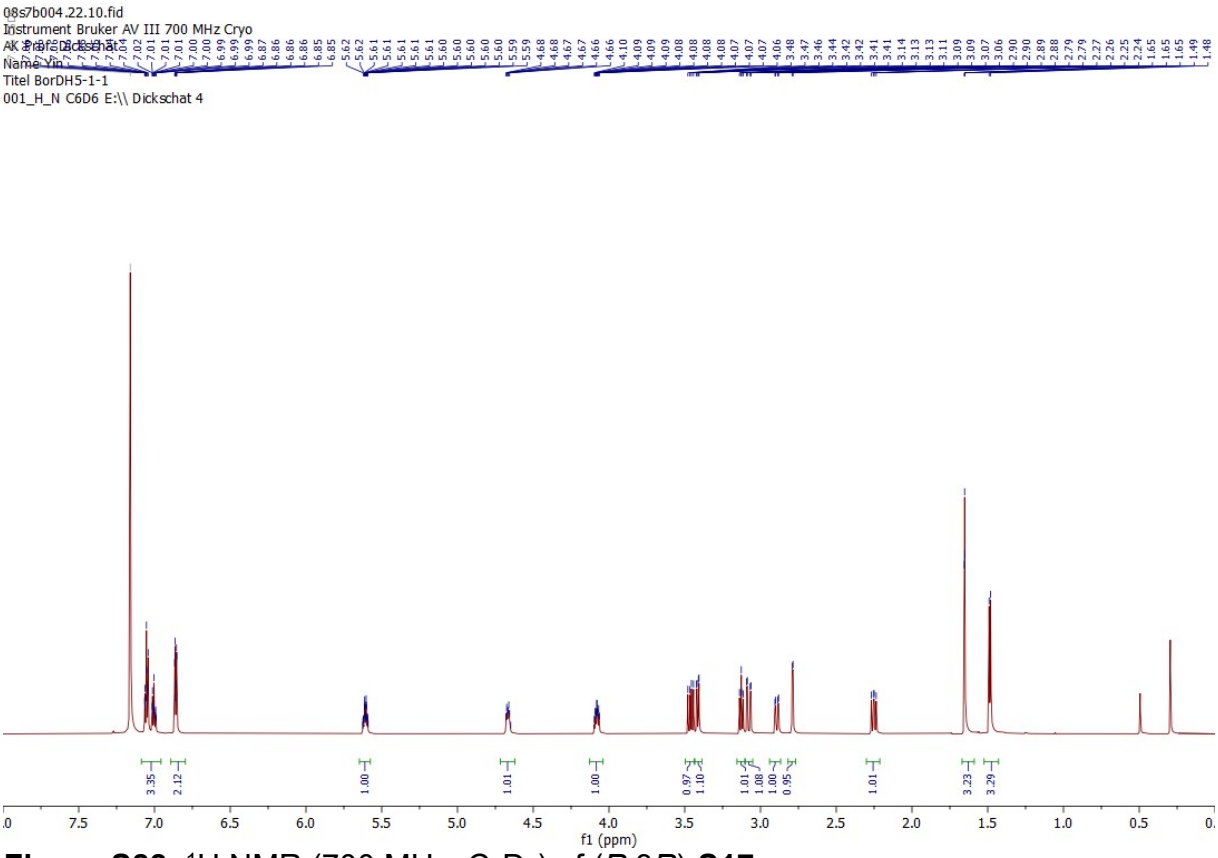


Figure S88. ^{1}H NMR (700 MHz, C_6D_6) of (*R*,3*R*)-**S17**.

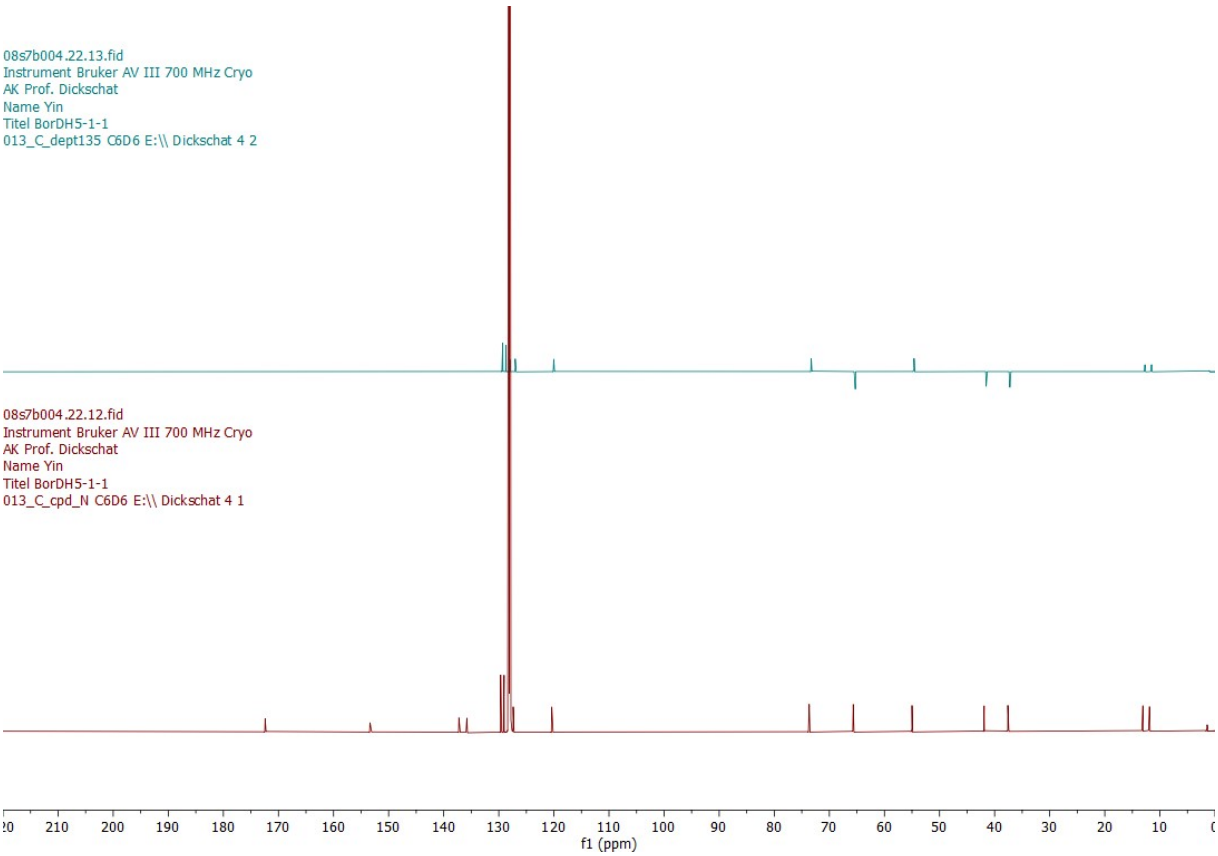


Figure S89. ^{13}C NMR (176 MHz, C_6D_6) of (*R*,3*R*)-**S17**.

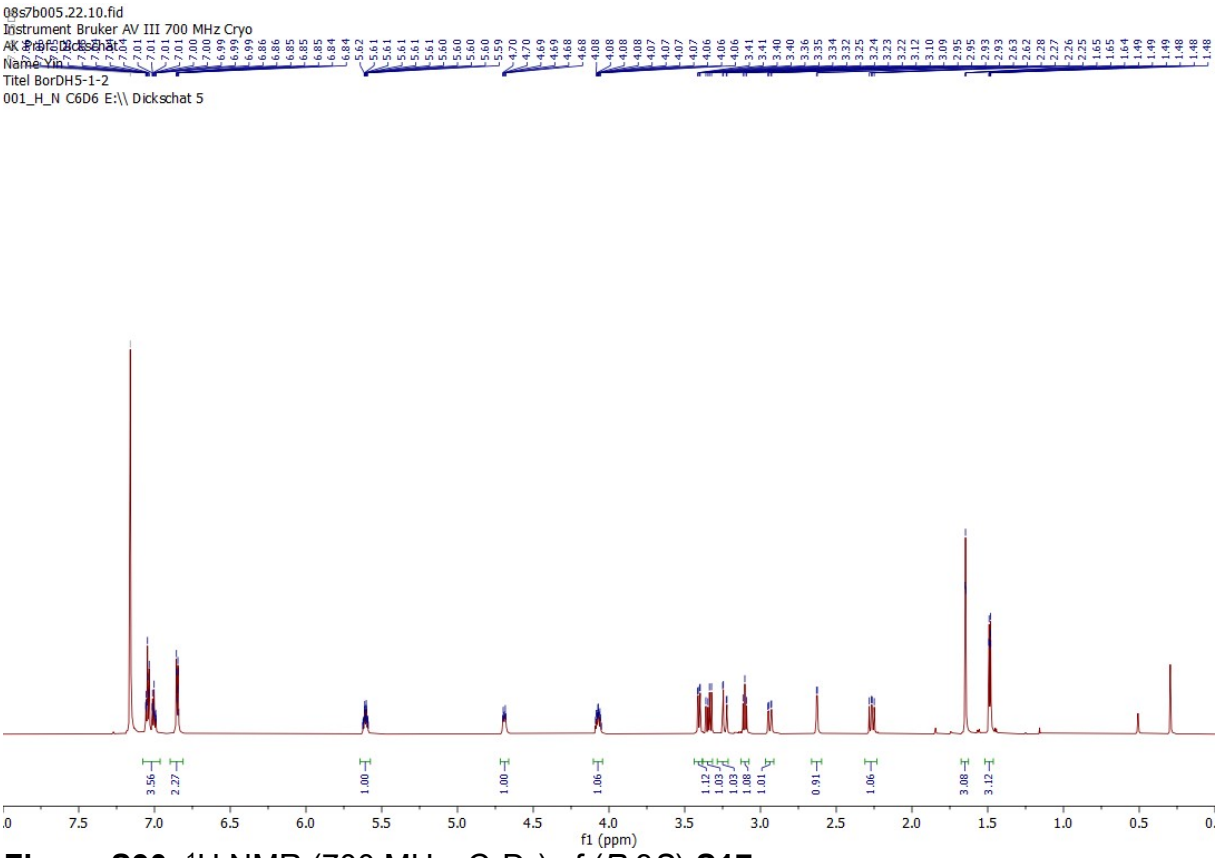


Figure S90. ^{1}H NMR (700 MHz, C_6D_6) of (*R*,3*S*)-**S17**.

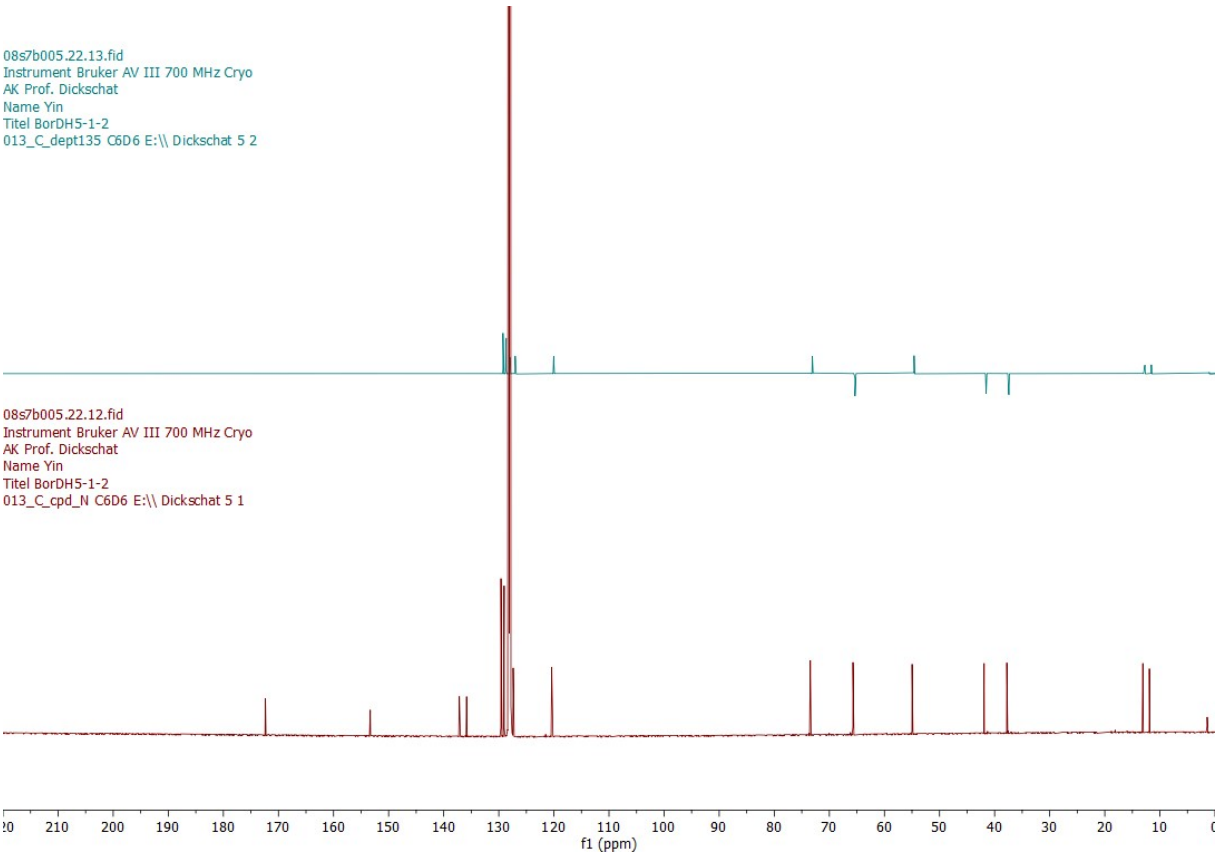


Figure S91. ^{13}C NMR (176 MHz, C_6D_6) of (*R*,3*S*)-**S17**.

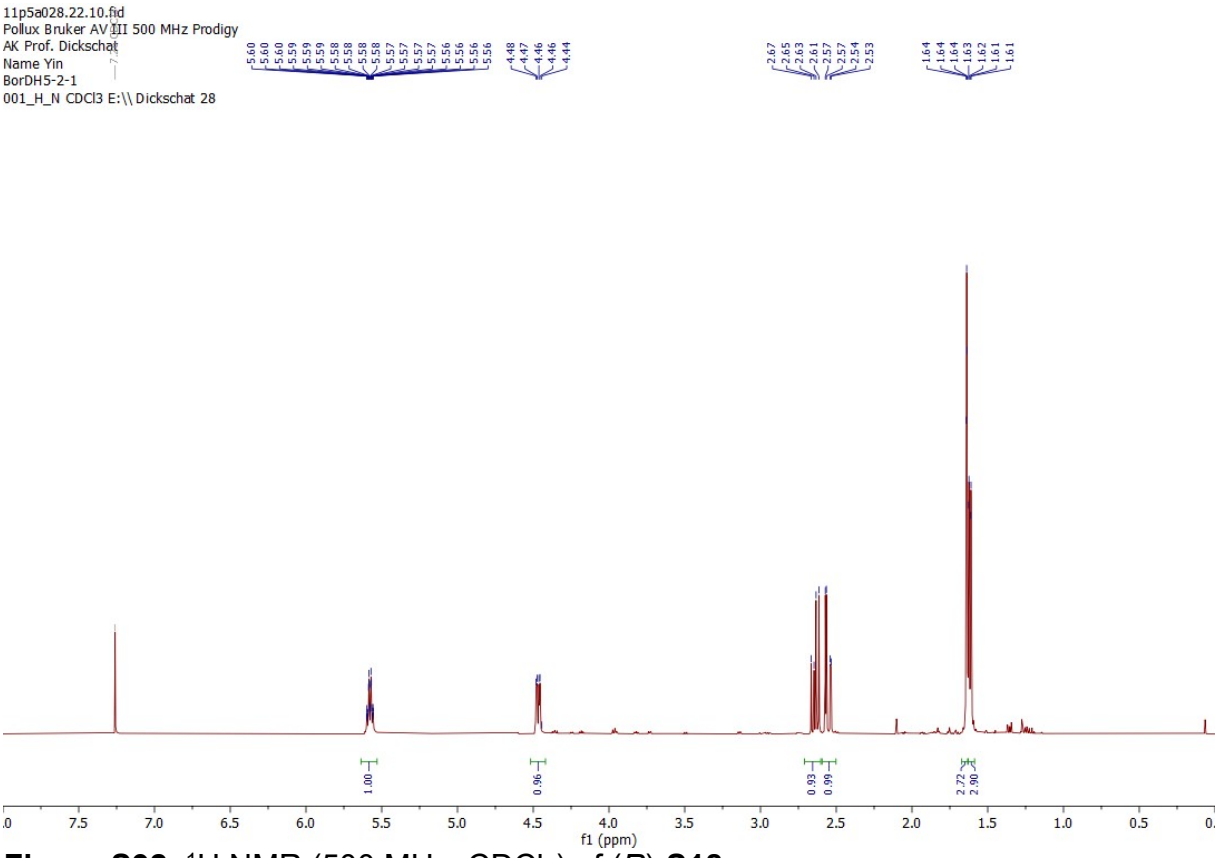


Figure S92. ^{1}H NMR (500 MHz, $CDCl_3$) of (*R*)-**S18**.

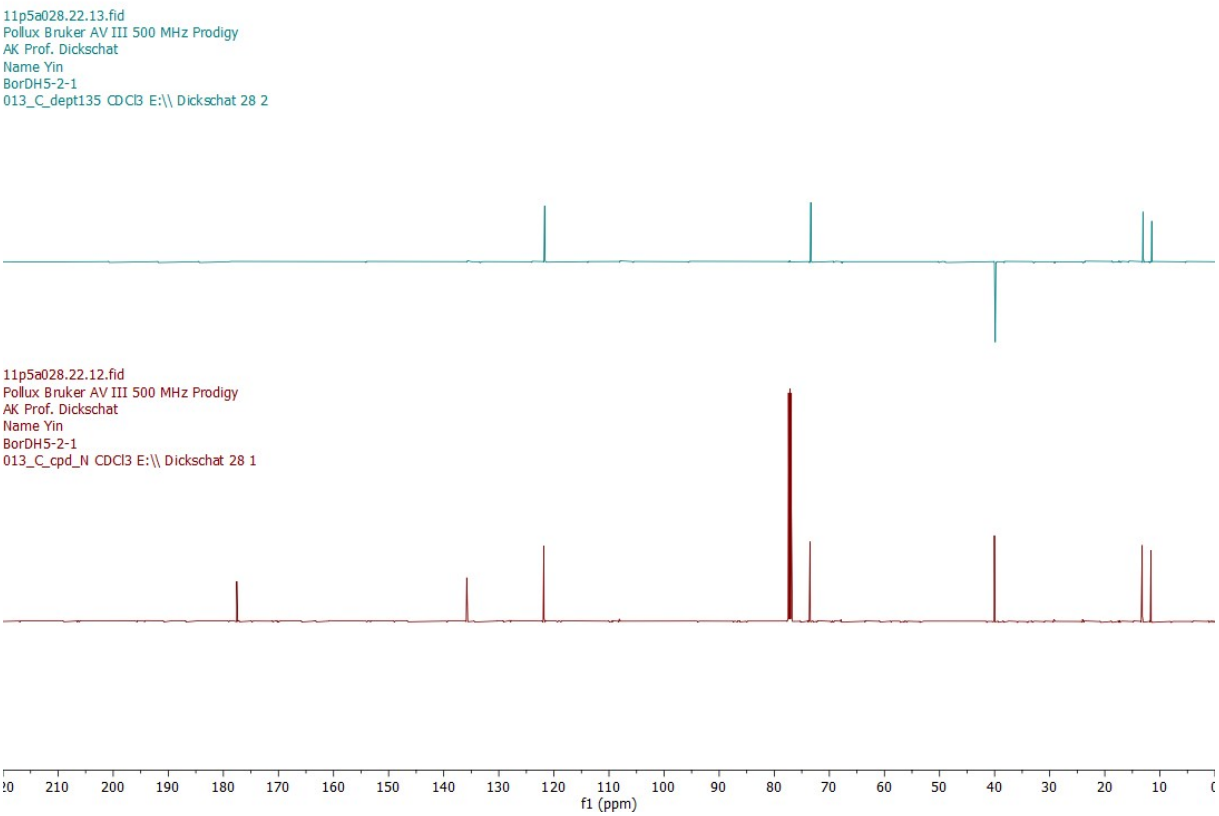


Figure S93. ^{13}C NMR (126 MHz, $CDCl_3$) of (*R*)-**S18**.

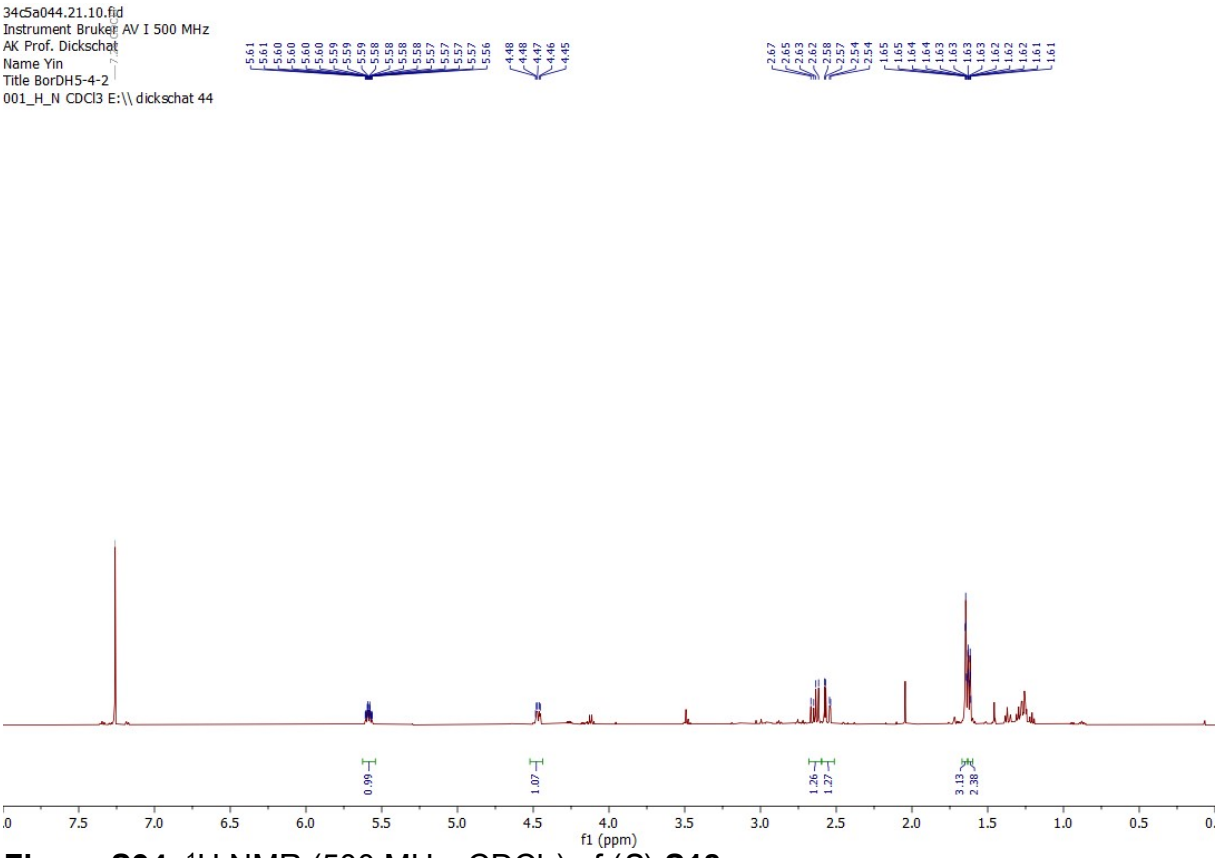


Figure S94. ^{1}H NMR (500 MHz, $CDCl_3$) of (*S*)-**S18**.

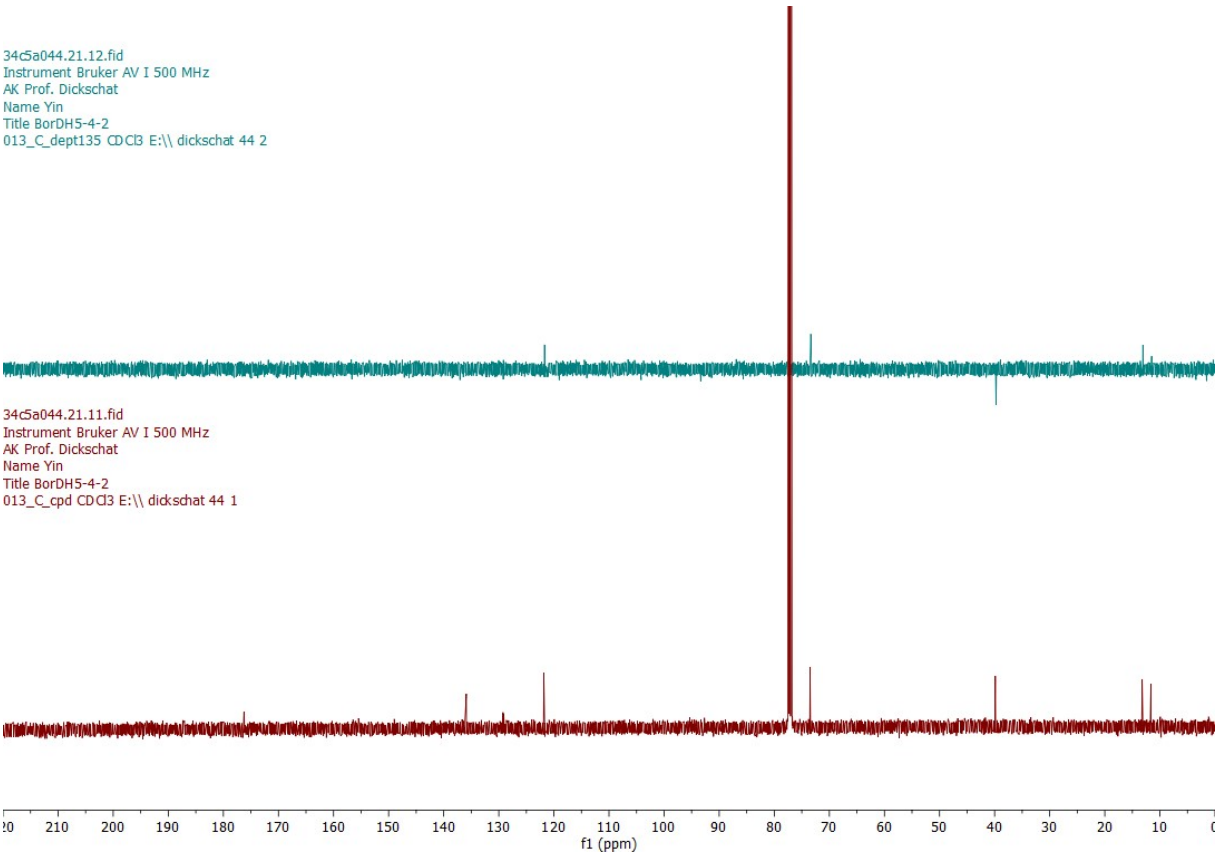


Figure S95. ^{13}C NMR (126 MHz, $CDCl_3$) of (*S*)-**S18**.

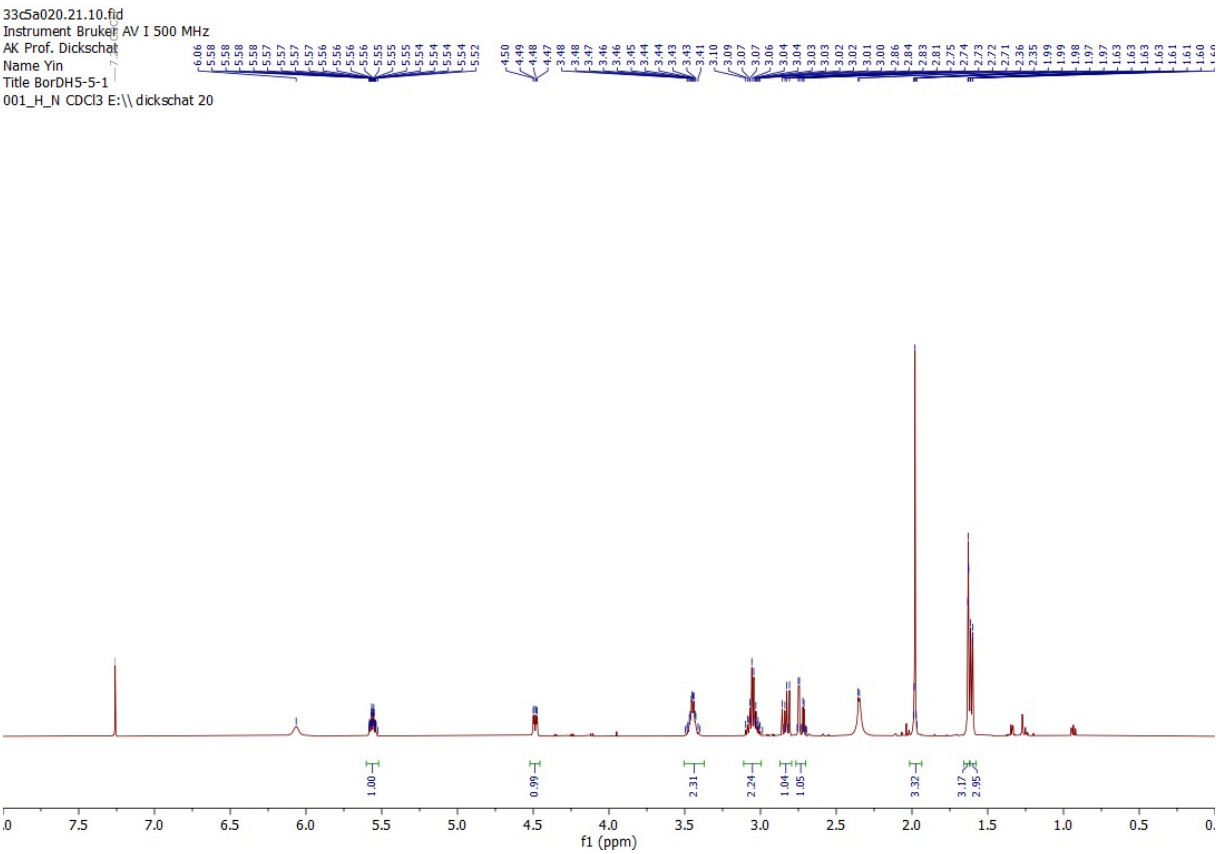


Figure S96. ^{1}H NMR (500 MHz, C_6D_6) of (*R*)-**7**.

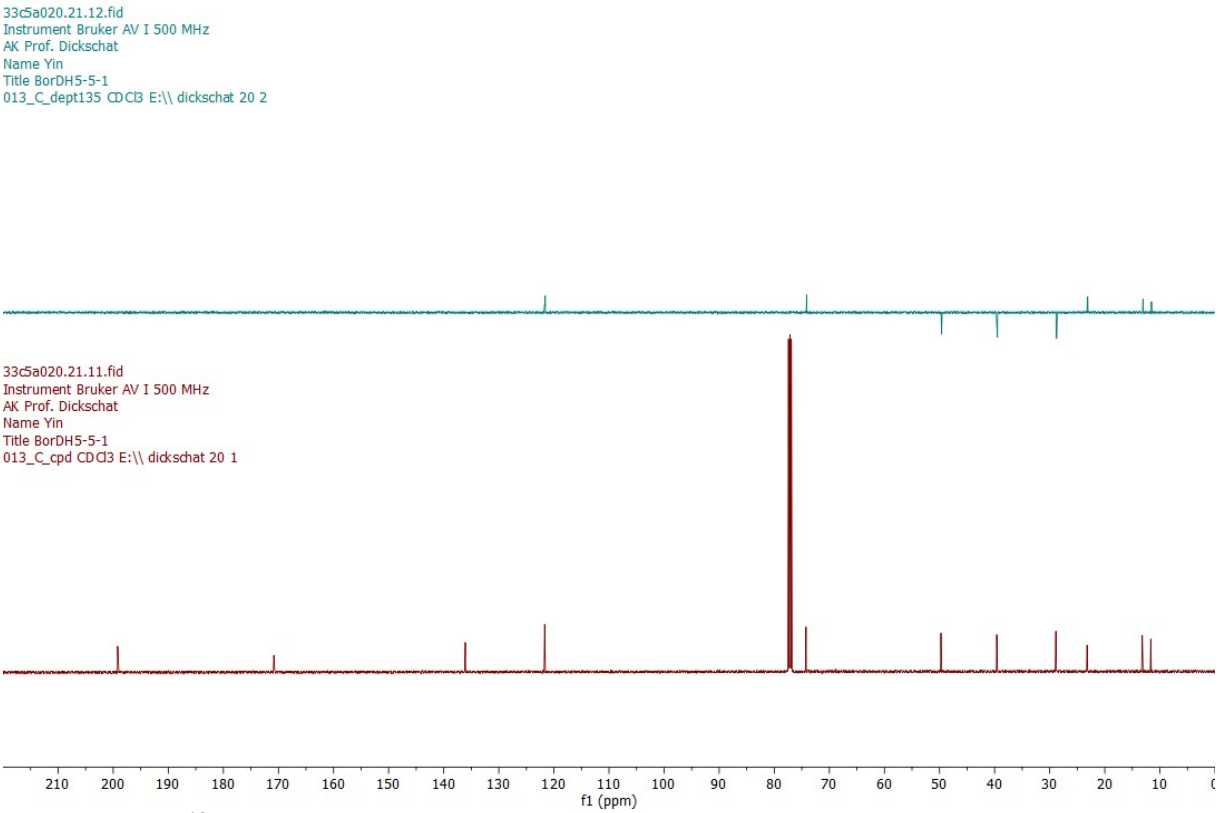


Figure S97. ^{13}C NMR (126 MHz, C_6D_6) of (*R*)-**7**.

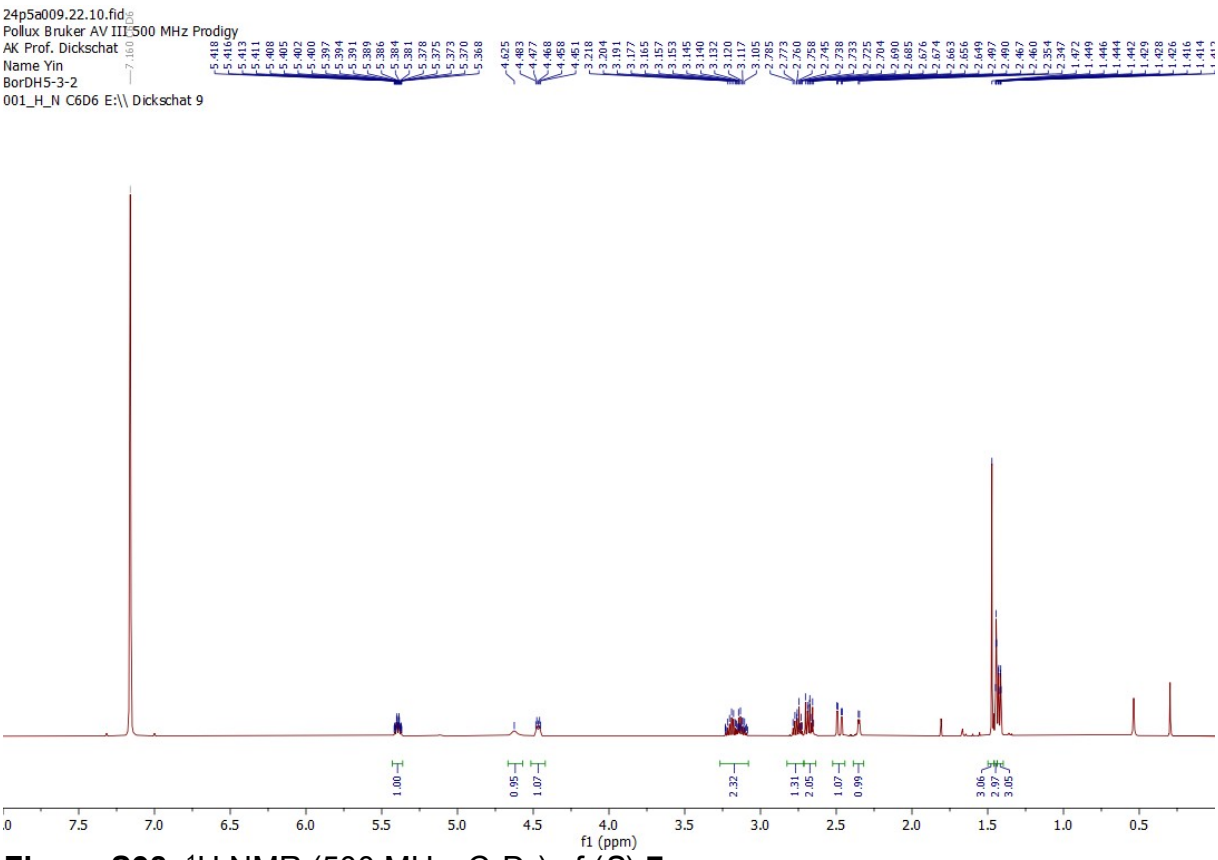


Figure S98. ^{1}H NMR (500 MHz, C_6D_6) of (*S*)-**7**.

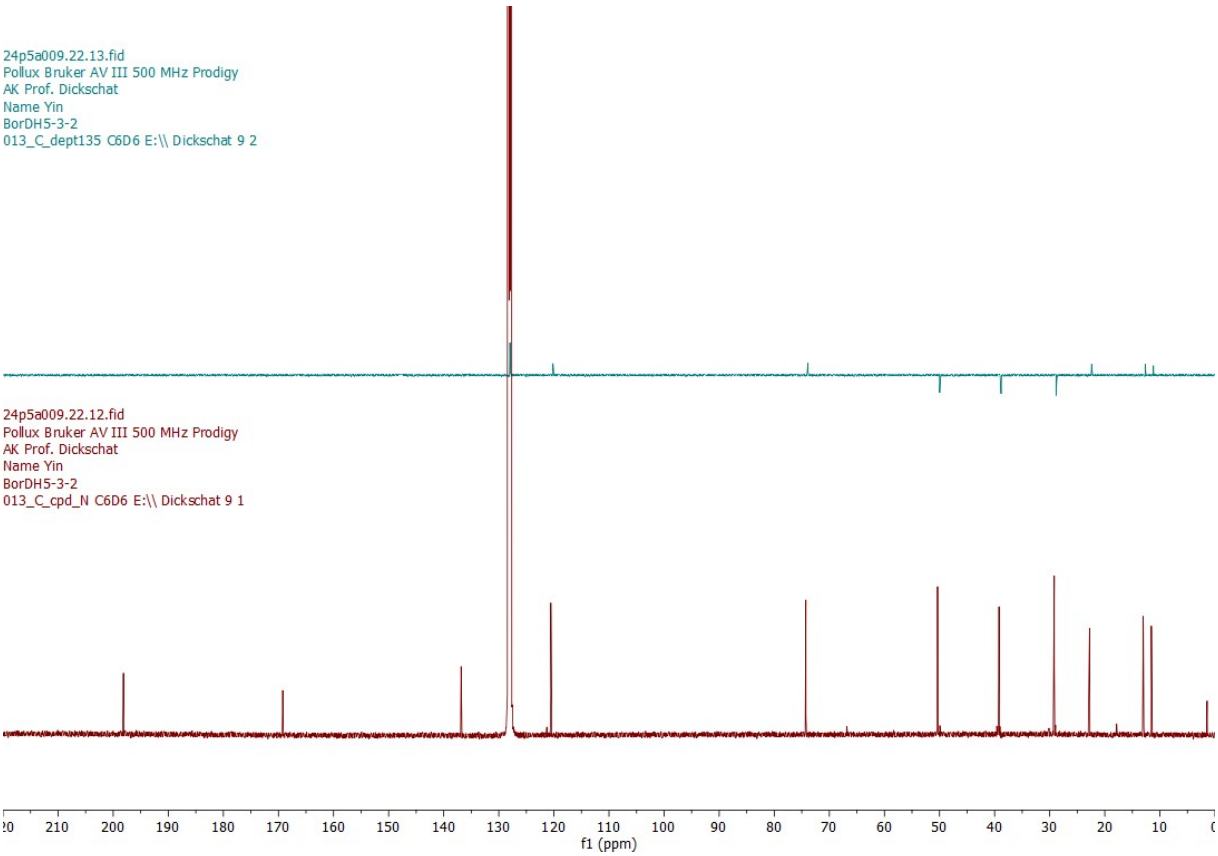


Figure S99. ^{13}C NMR (126 MHz, C_6D_6) of (*S*)-**7**.

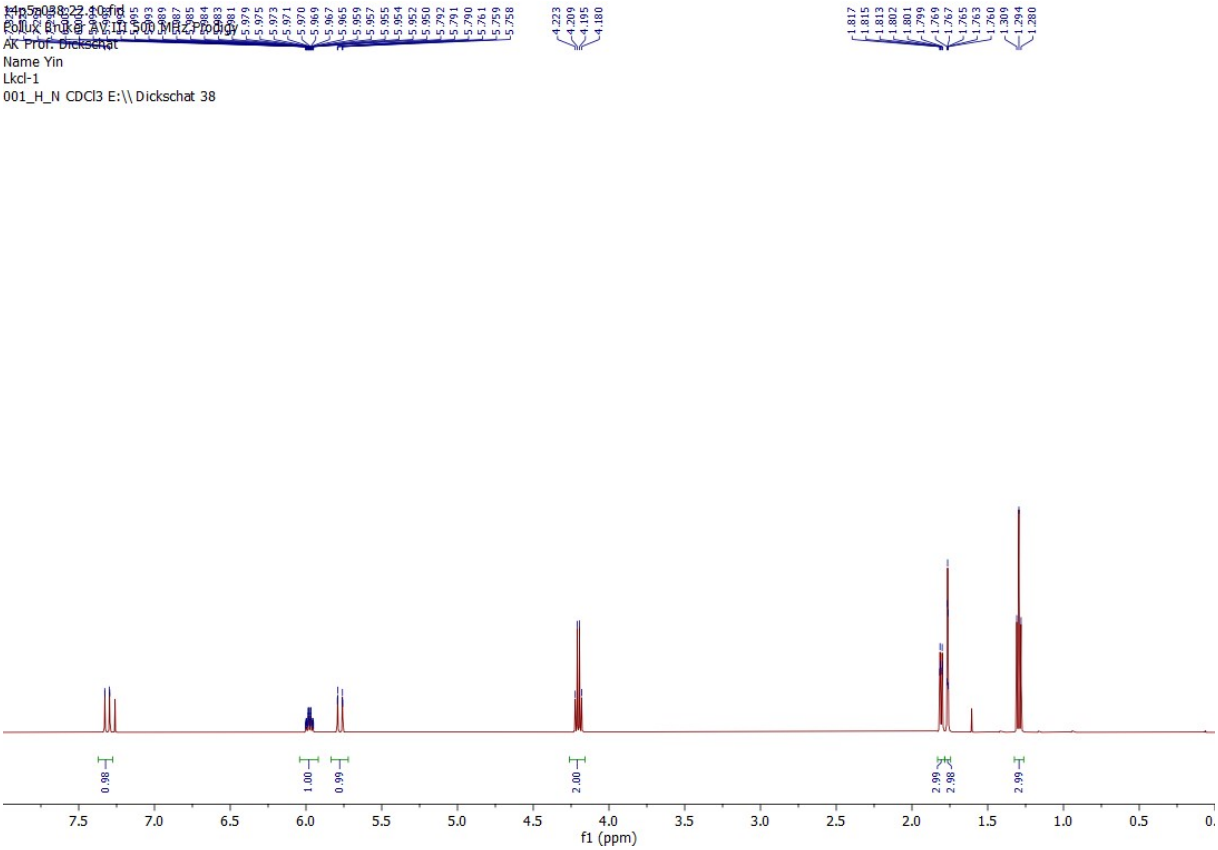


Figure S100. ^{1}H NMR (500 MHz, $CDCl_3$) of **S20**.

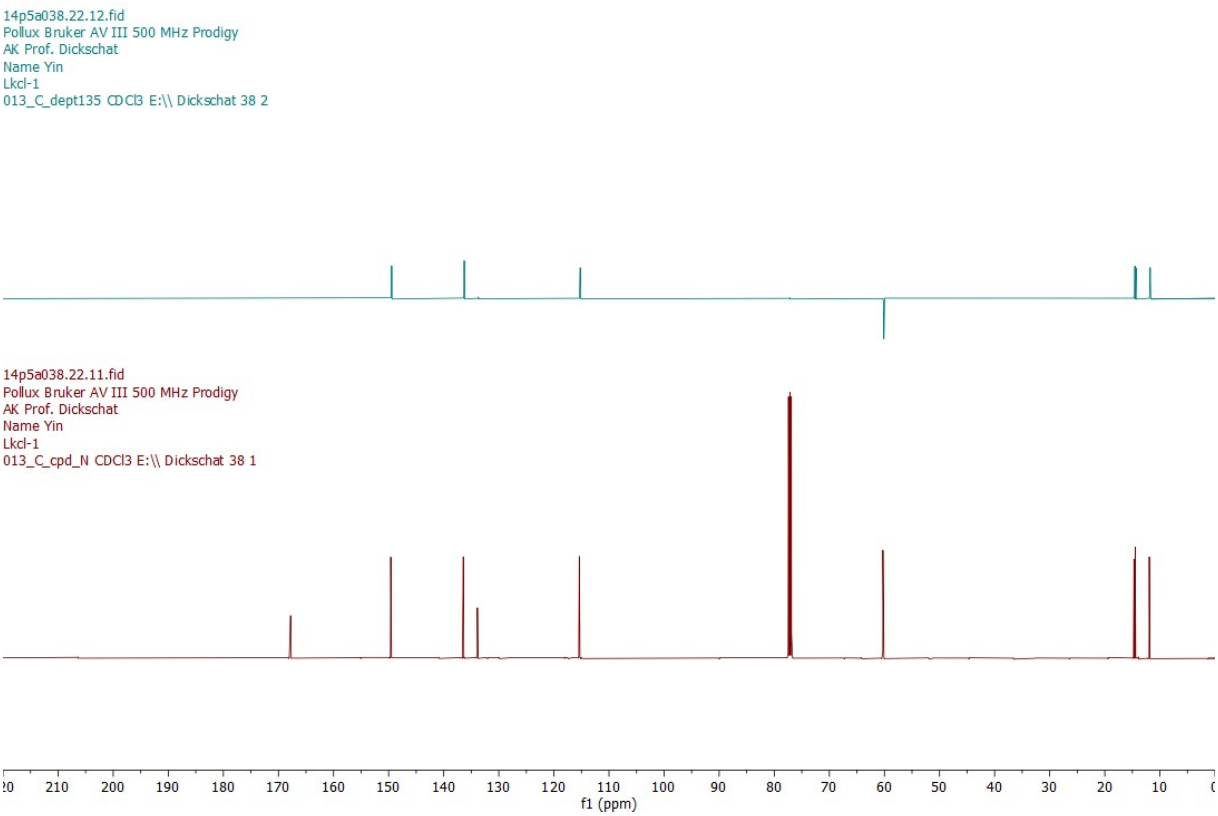


Figure S101. ^{13}C NMR (126 MHz, $CDCl_3$) of **S20**.

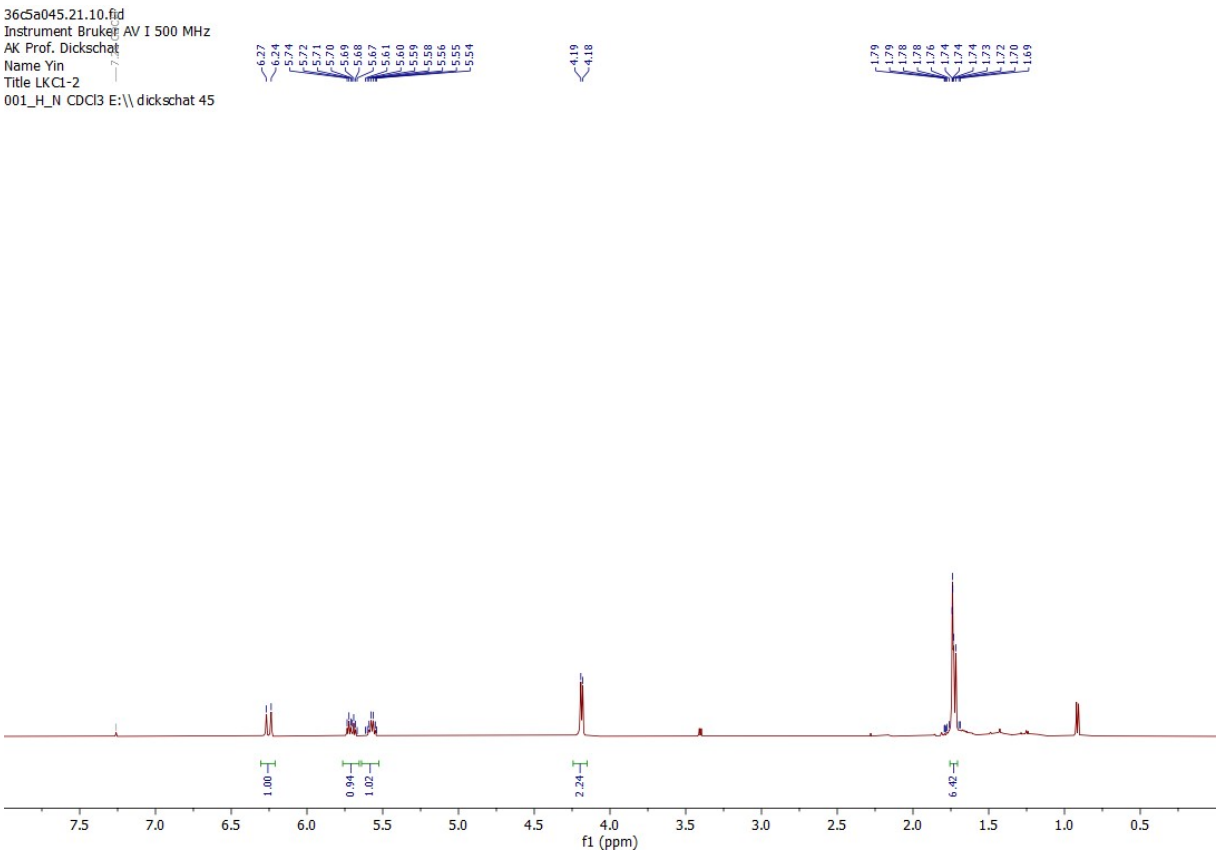


Figure S102. ^{1}H NMR (500 MHz, $CDCl_3$) of **S21**.

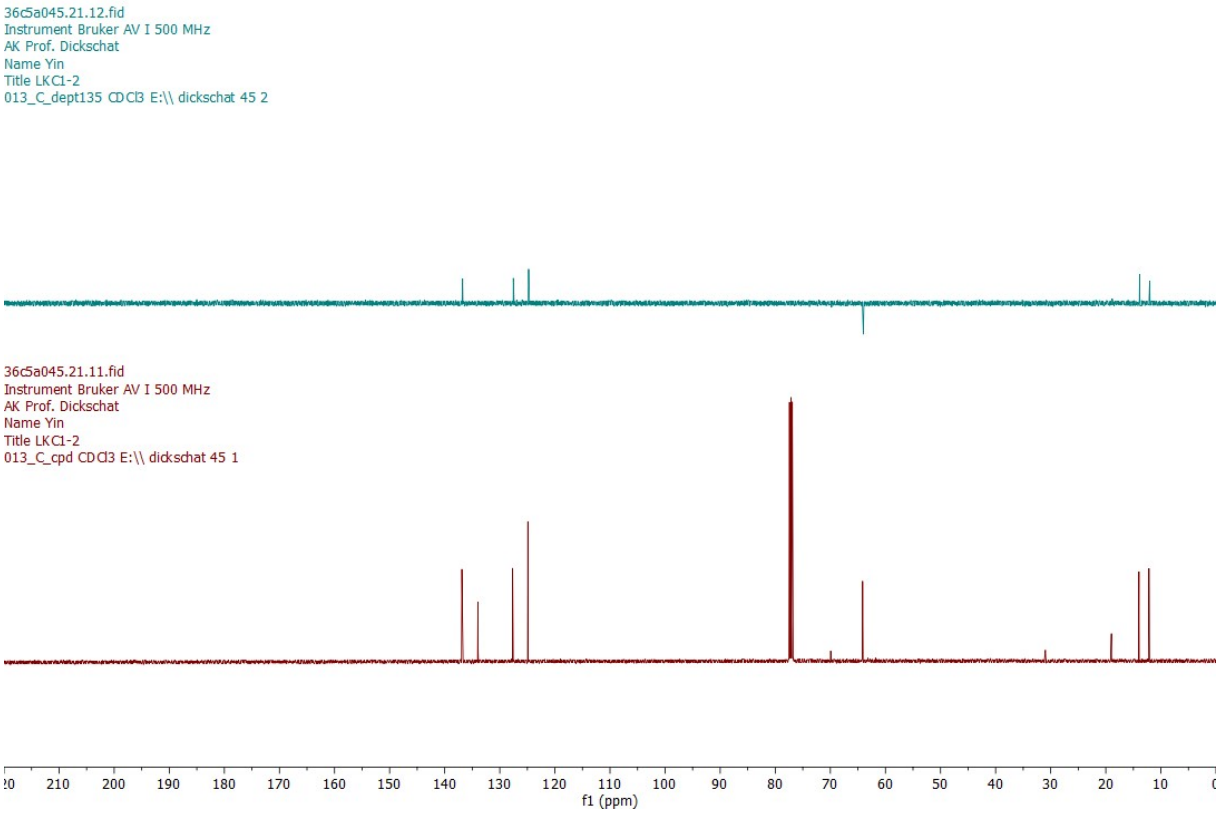


Figure S103. ^{13}C NMR (126 MHz, $CDCl_3$) of **S21**.

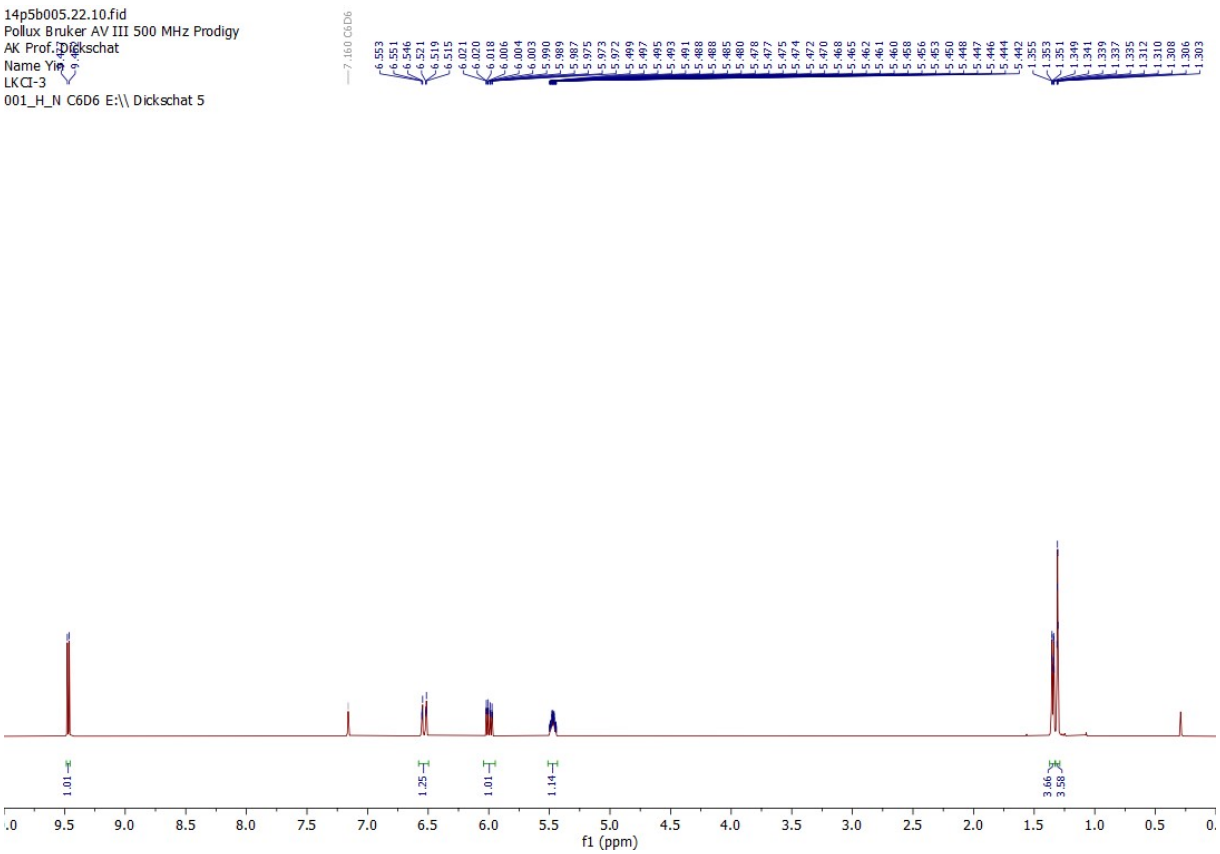


Figure S104. ^{1}H NMR (500 MHz, C_6D_6) of **S22**.

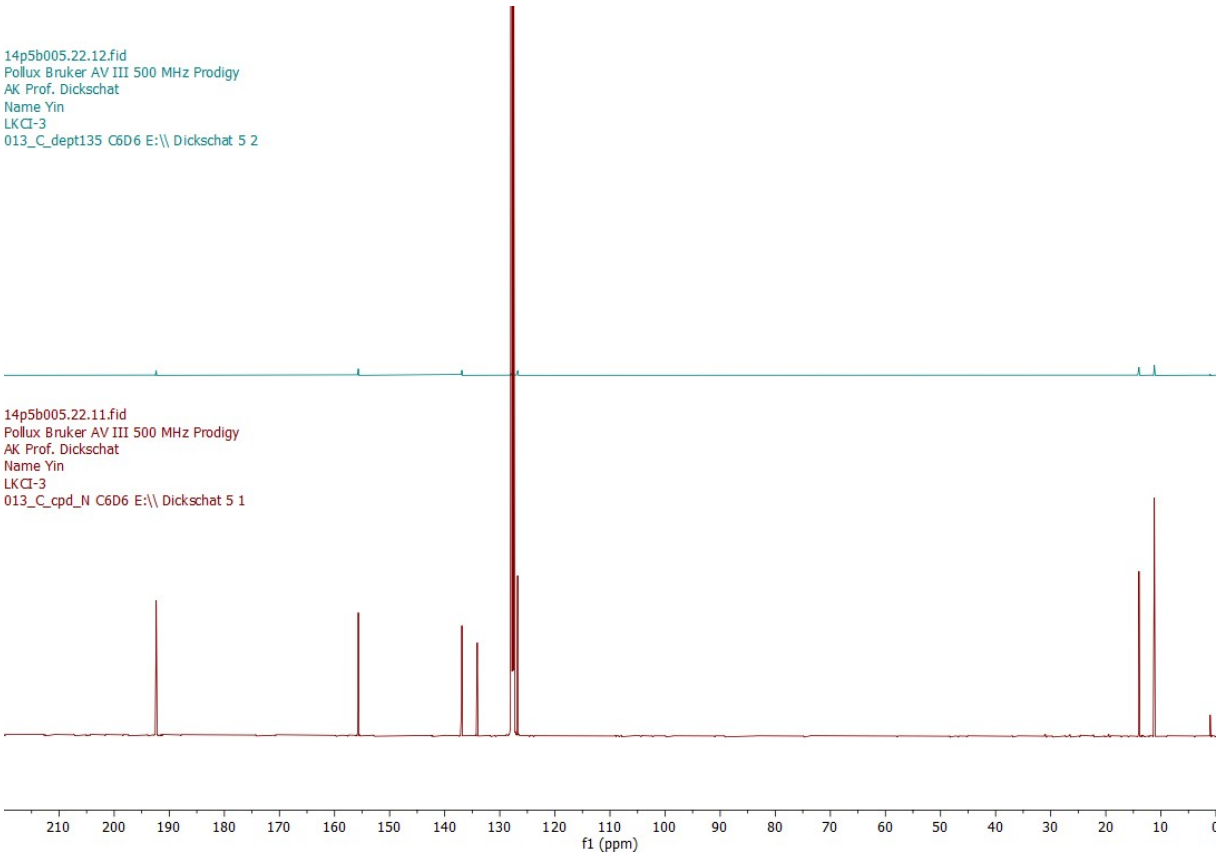


Figure S105. ^{13}C NMR (126 MHz, C_6D_6) of **S22**.

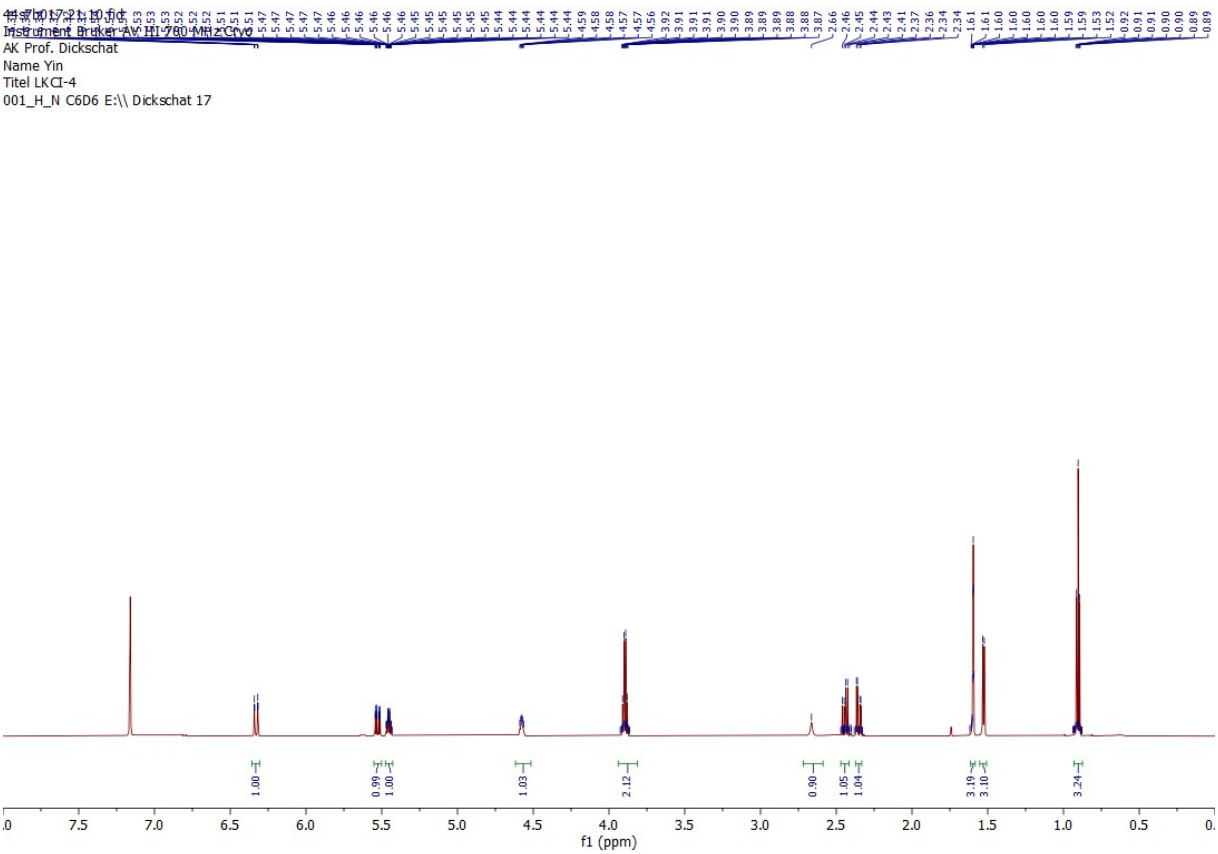


Figure S106. ^{1}H NMR (700 MHz, C_6D_6) of **S23**.

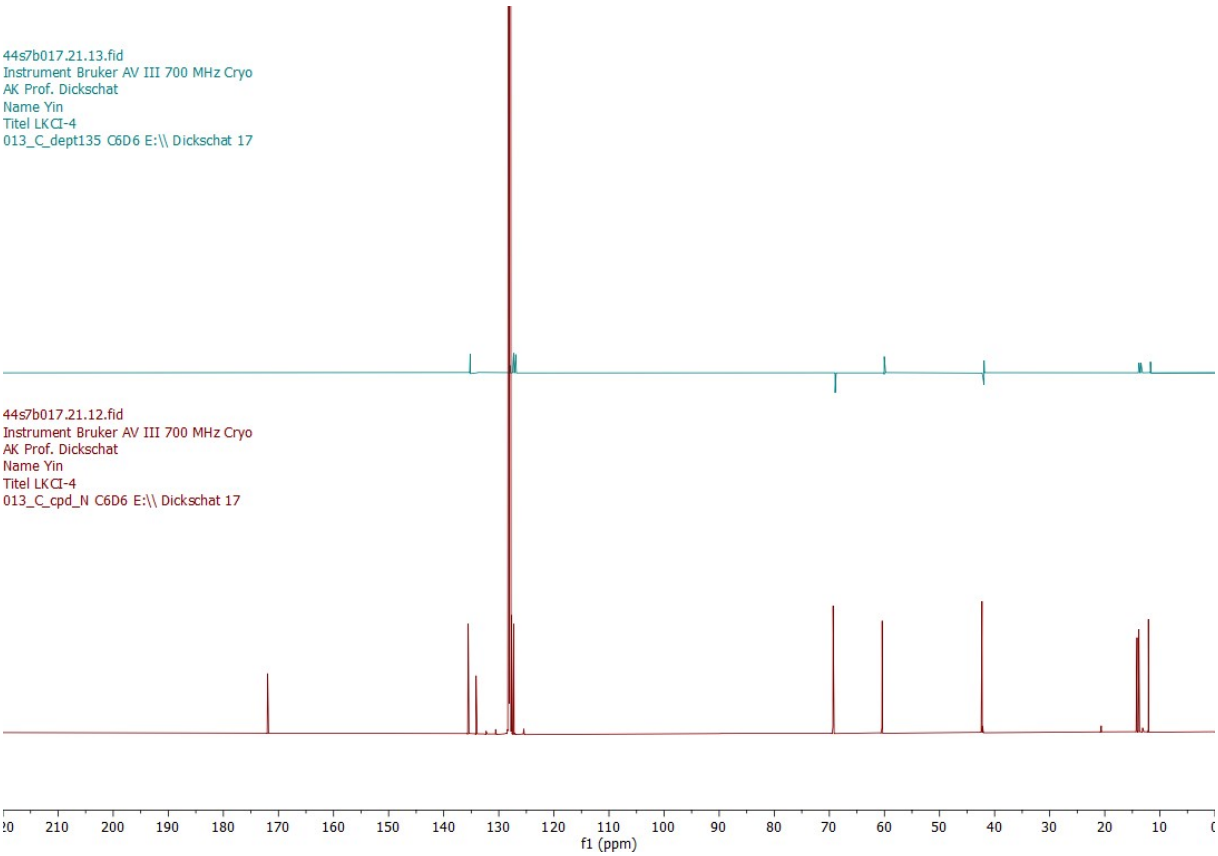


Figure S107. ^{13}C NMR (176 MHz, C_6D_6) of **S23**.

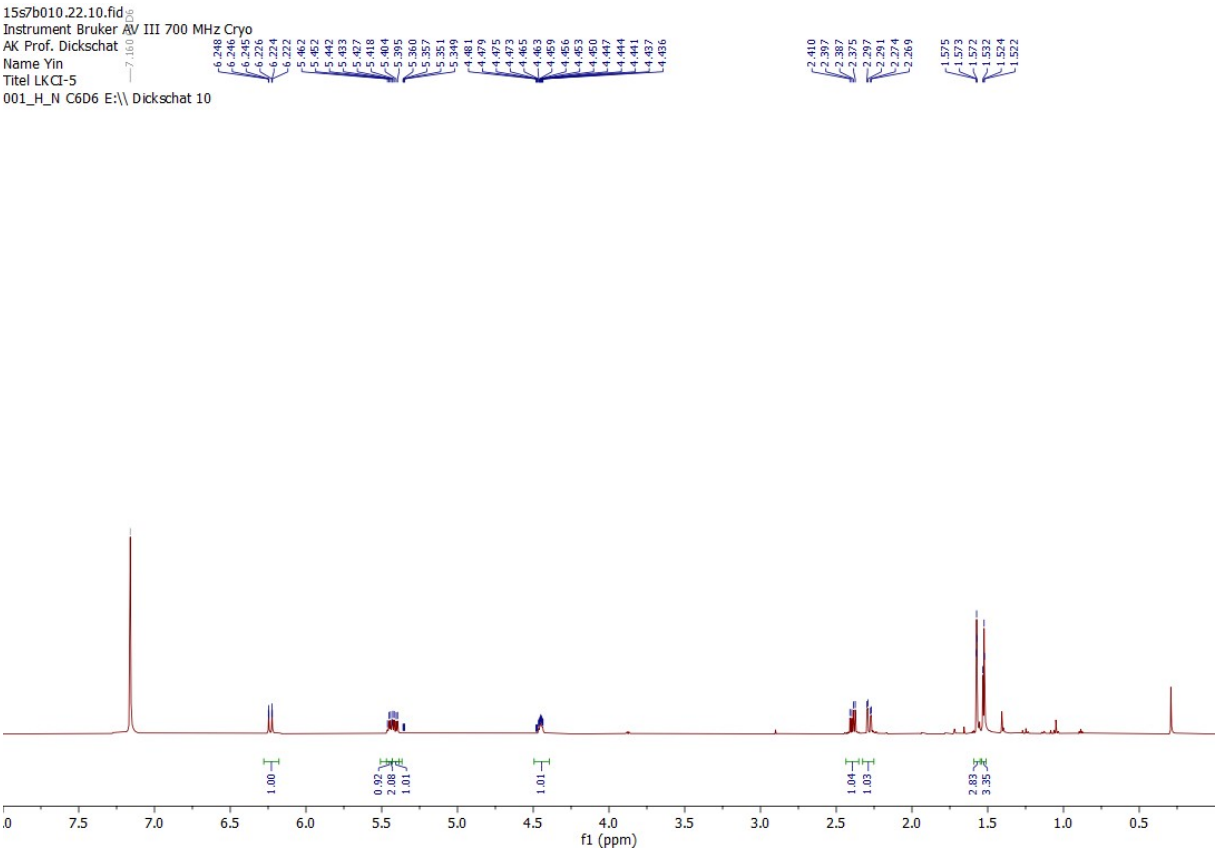


Figure S108. ^{1}H NMR (700 MHz, C_6D_6) of **S24**.

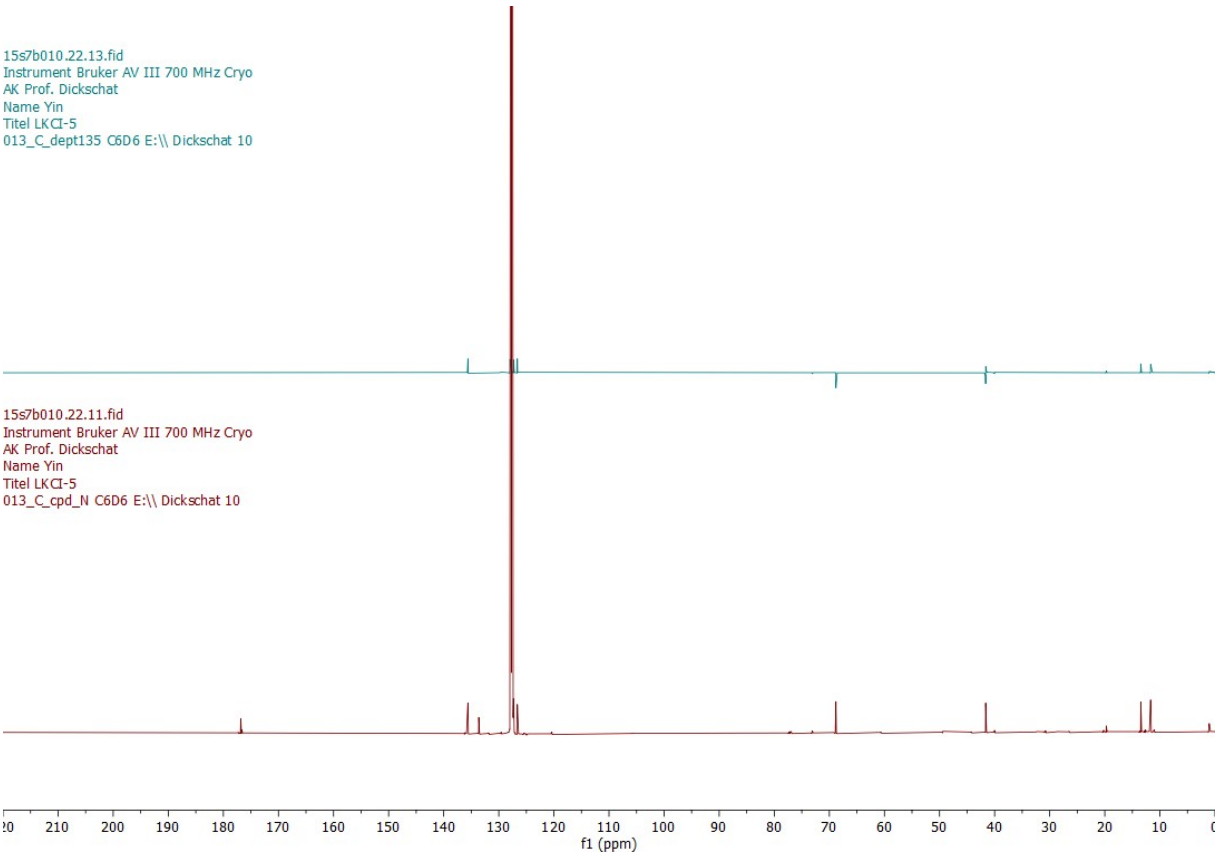


Figure S109. ^{13}C NMR (176 MHz, C_6D_6) of **S24**.

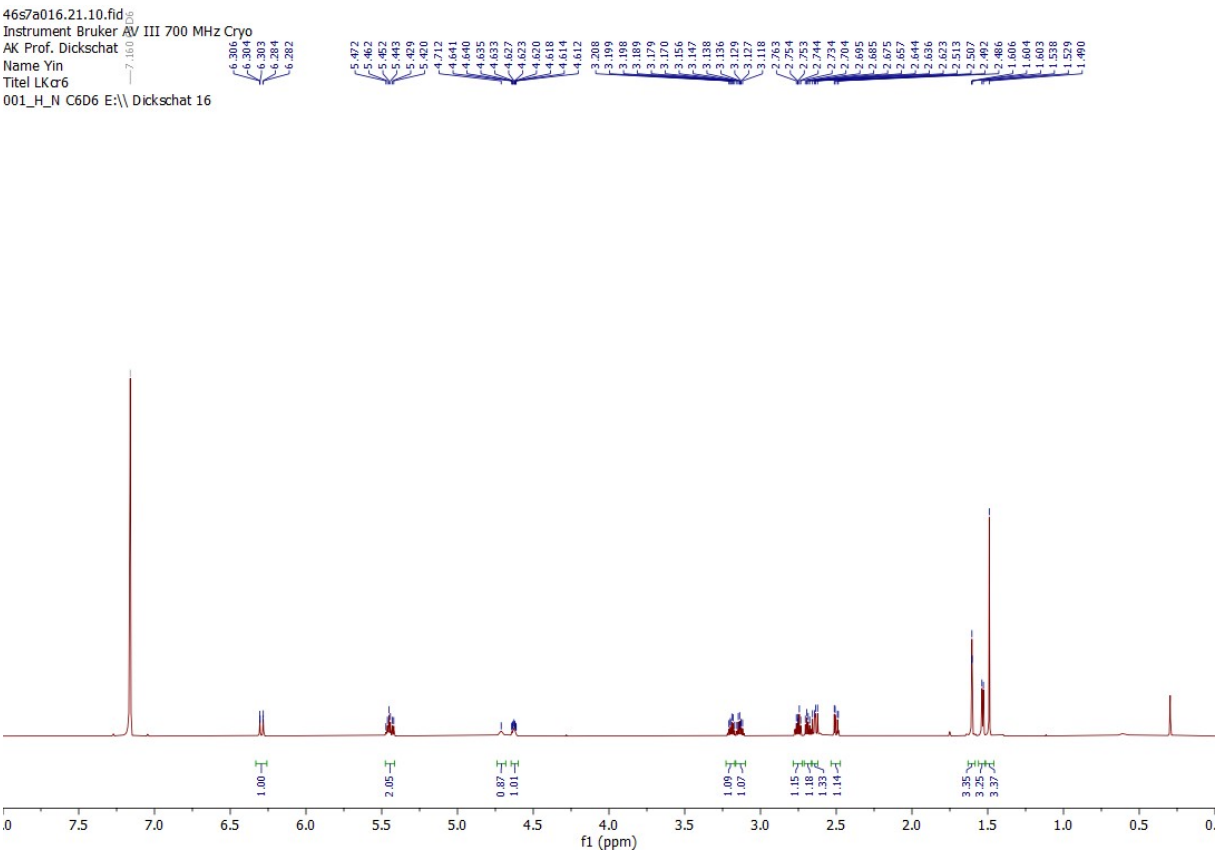


Figure S110. ^{1}H NMR (700 MHz, C_6D_6) of (*rac*)-**8**.

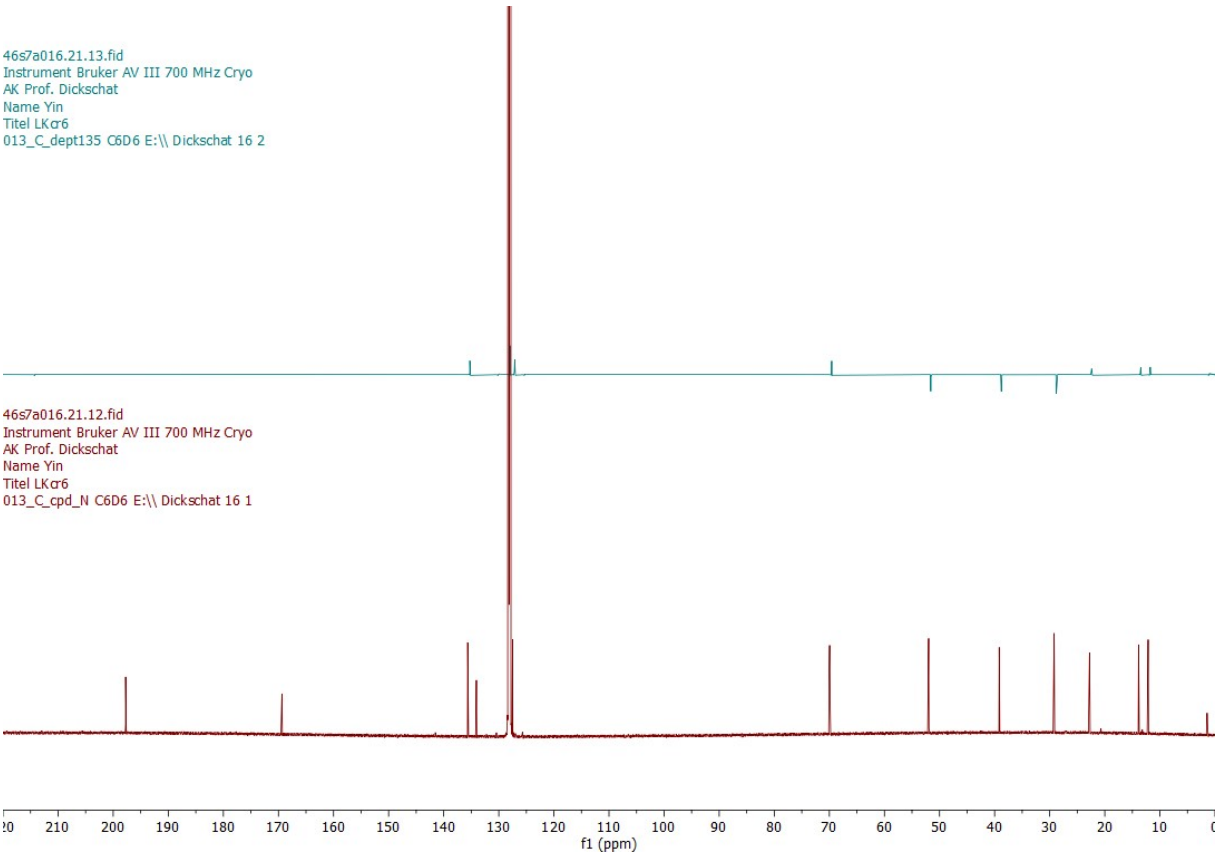


Figure S111. ^{13}C NMR (176 MHz, C_6D_6) of (*rac*)-**8**.

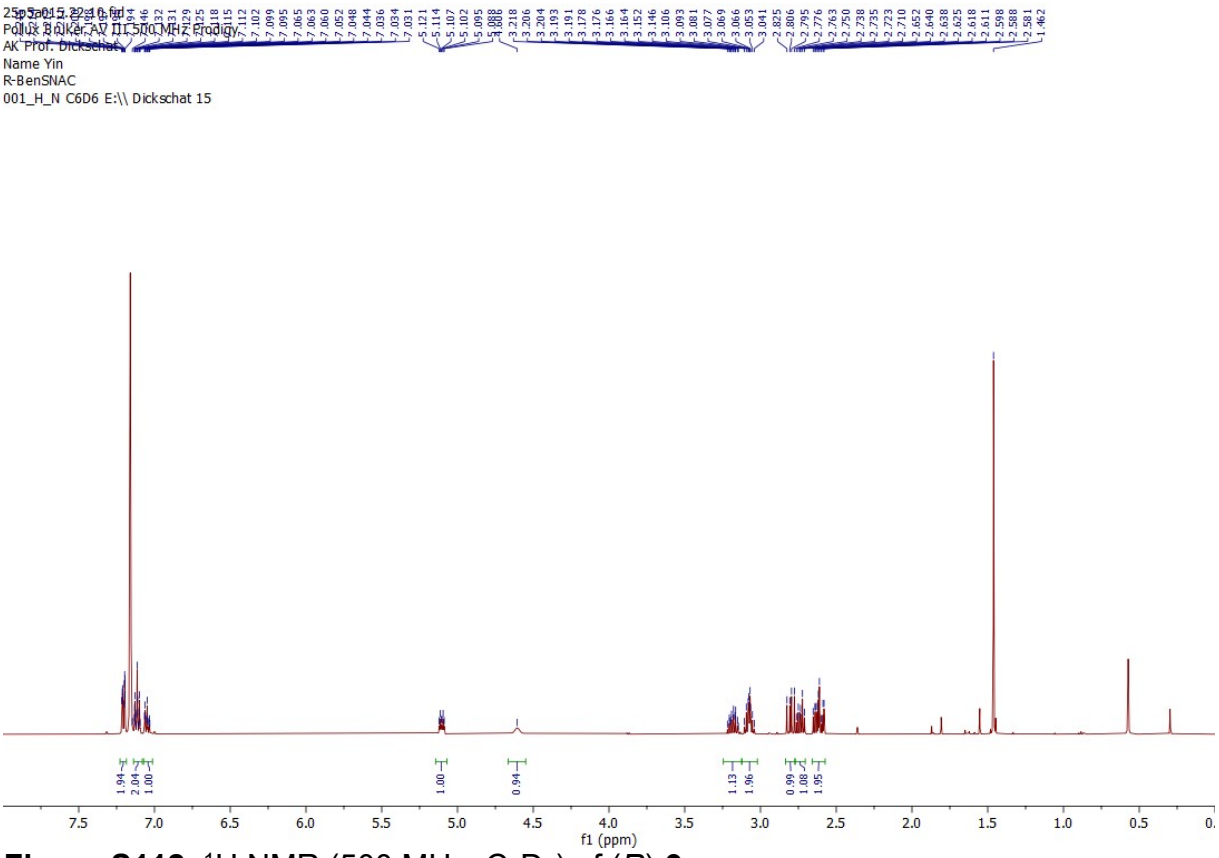


Figure S112. ^{1}H NMR (500 MHz, C_6D_6) of (*R*)-**9**.

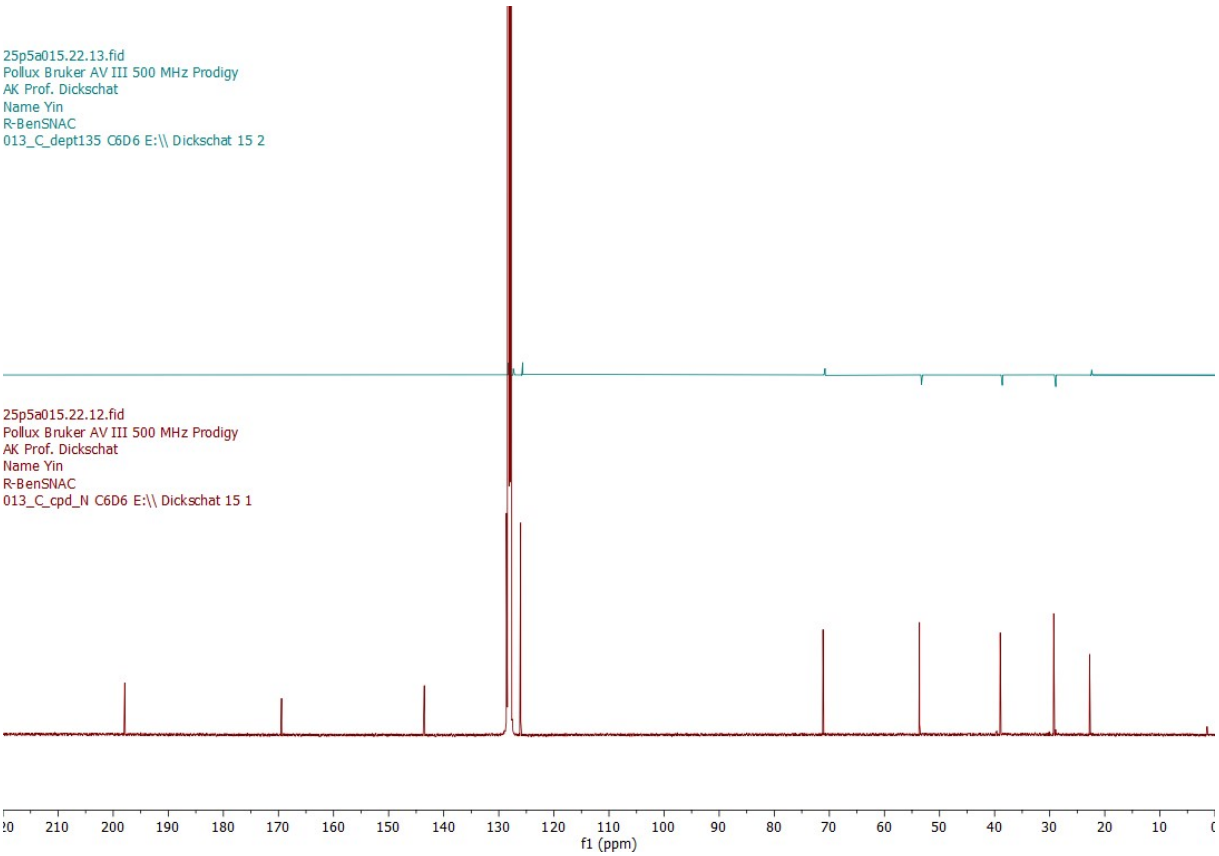


Figure S113. ^{13}C NMR (126 MHz, C_6D_6) of (*R*)-**9**.

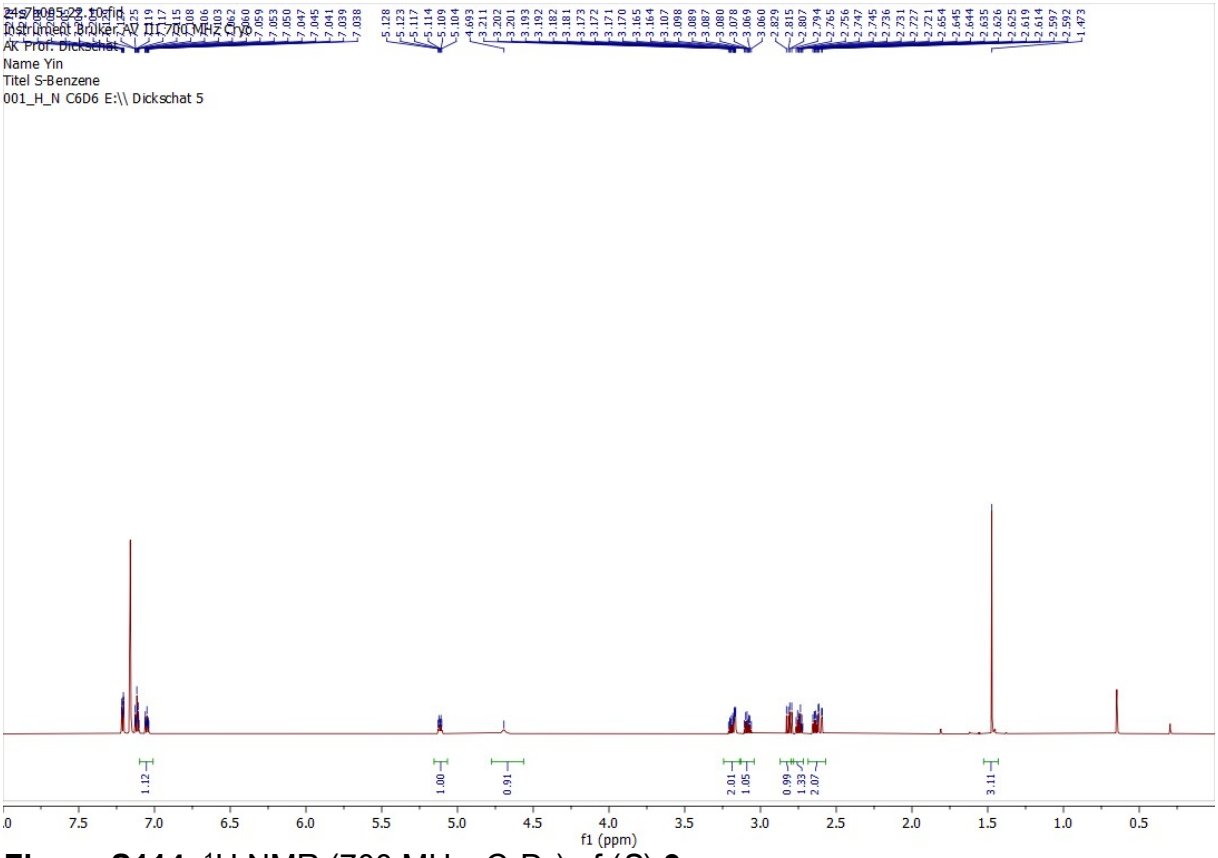


Figure S114. ^{1}H NMR (700 MHz, C_6D_6) of (*S*)-**9**.

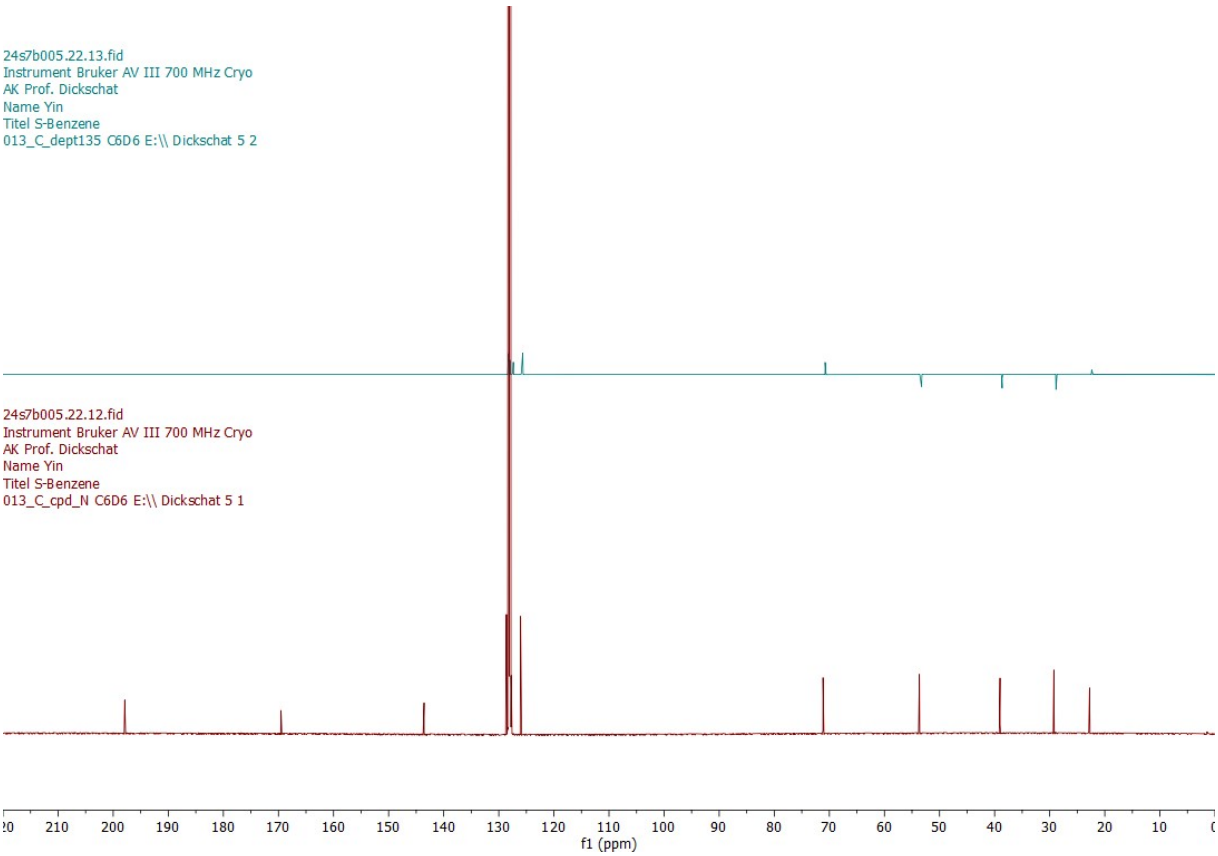


Figure S115. ^{13}C NMR (176 MHz, C_6D_6) of (*S*)-**9**.

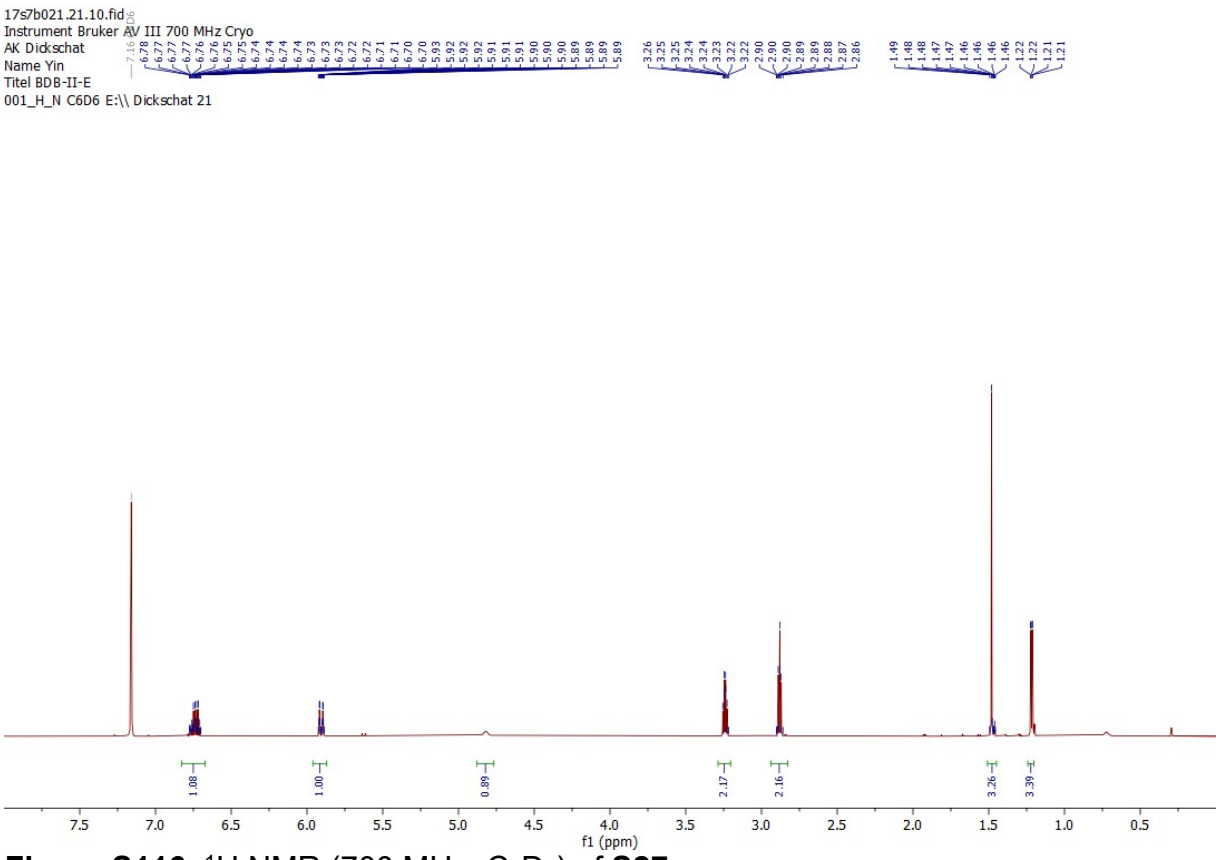


Figure S116. ^{1}H NMR (700 MHz, C_6D_6) of **S27**.

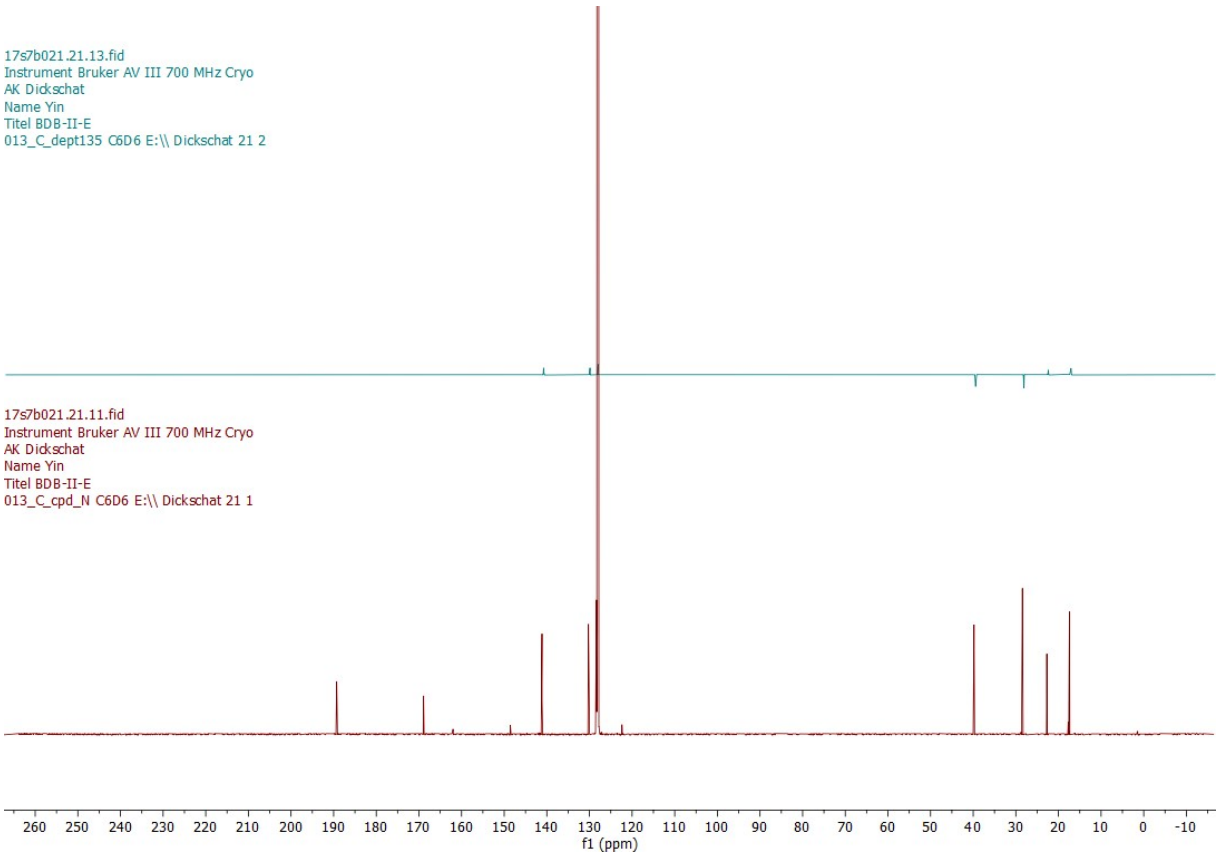


Figure S117. ^{13}C NMR (176 MHz, C_6D_6) of **S27**.

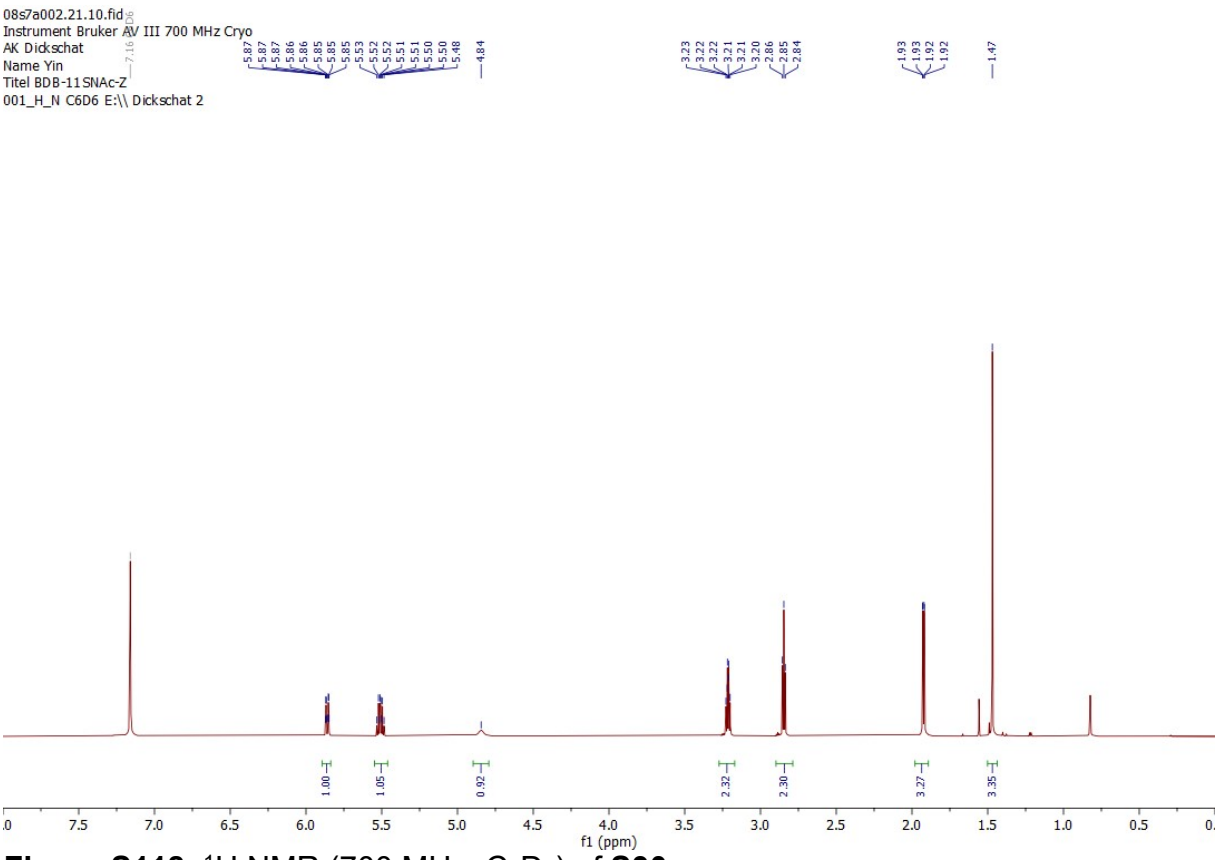


Figure S118. ^{1}H NMR (700 MHz, C_6D_6) of **S30**.

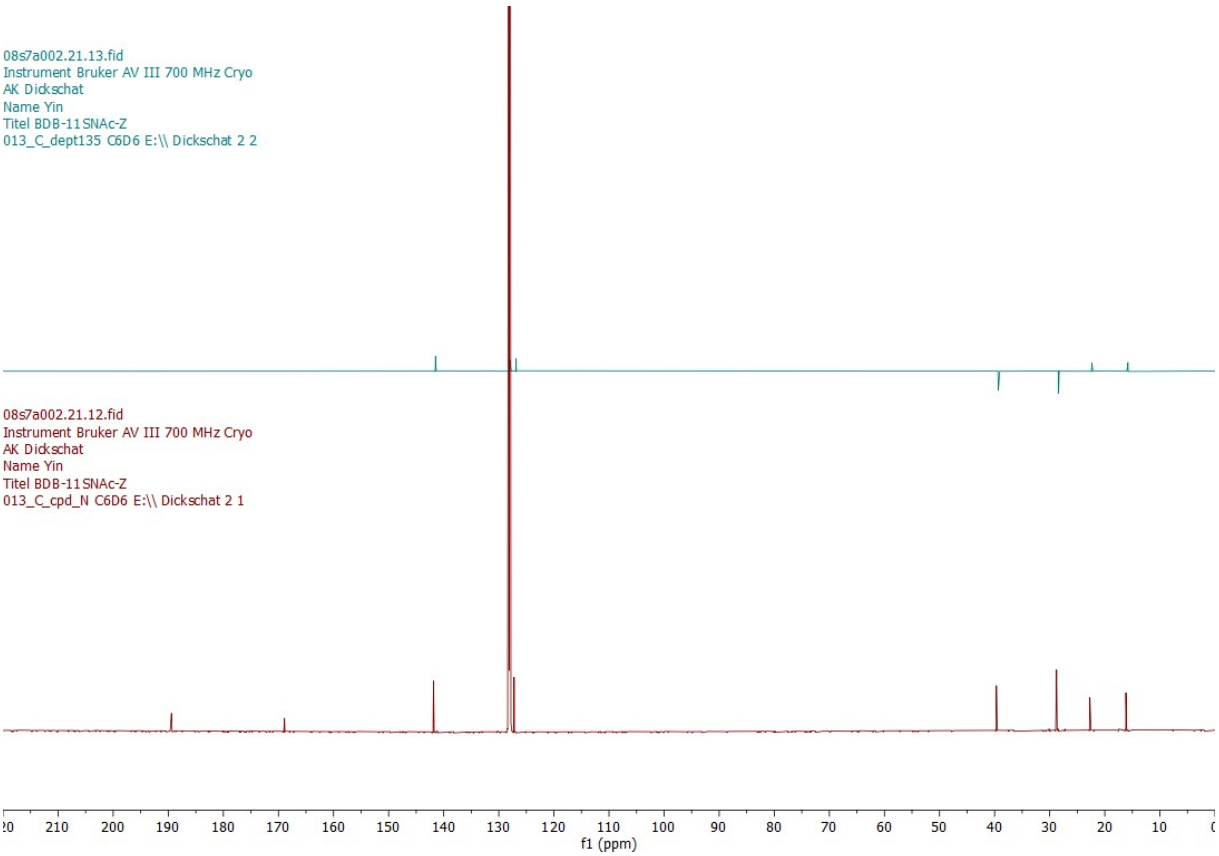


Figure S119. ^{13}C NMR (176 MHz, C_6D_6) of **S30**.

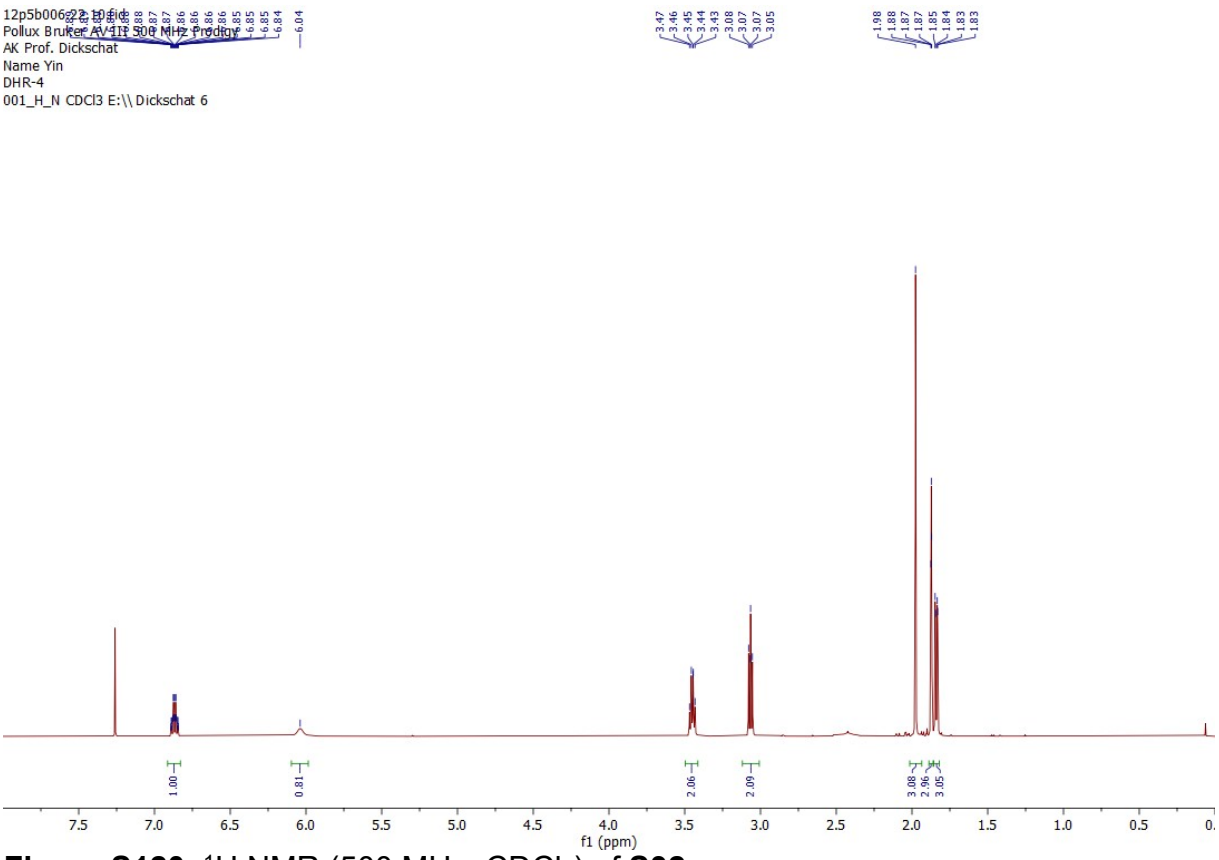


Figure S120. ^{1}H NMR (500 MHz, $CDCl_3$) of **S32**.

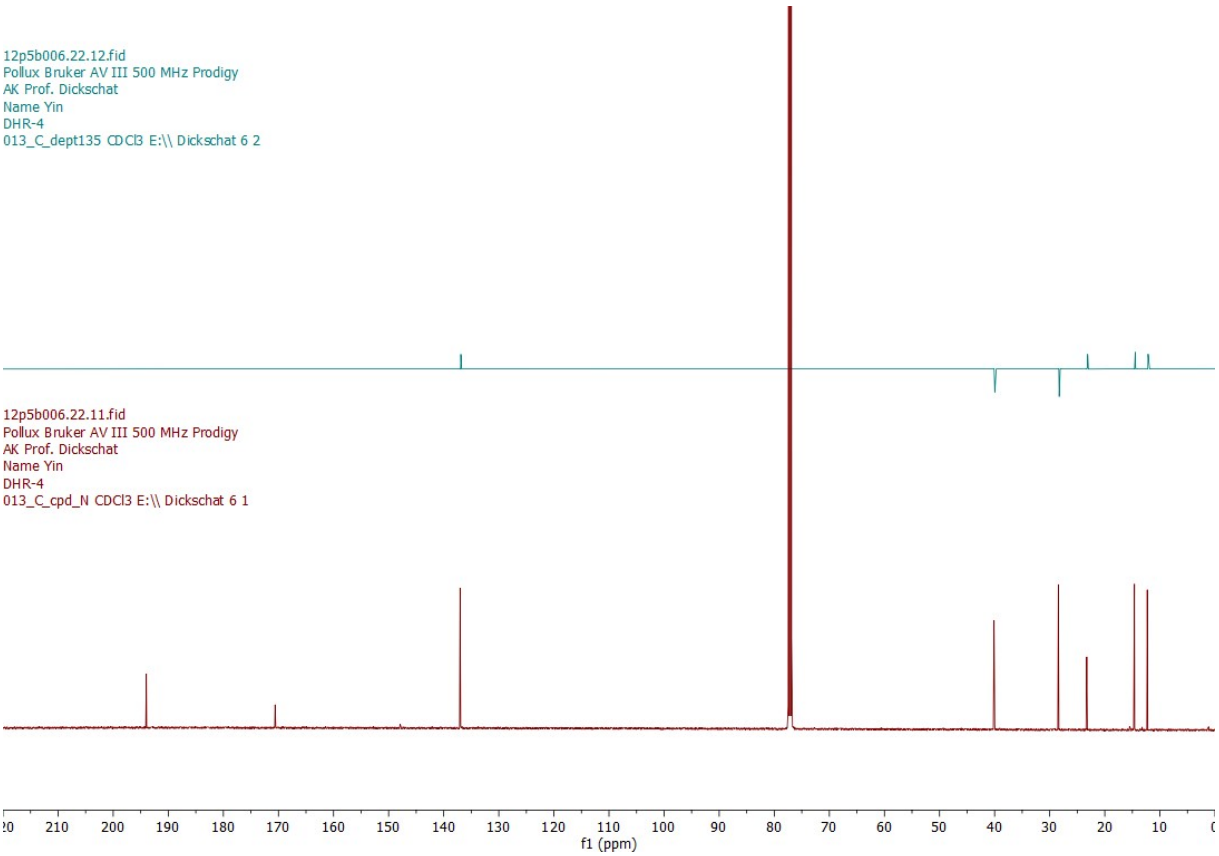


Figure S121. ^{13}C NMR (126 MHz, $CDCl_3$) of **S32**.

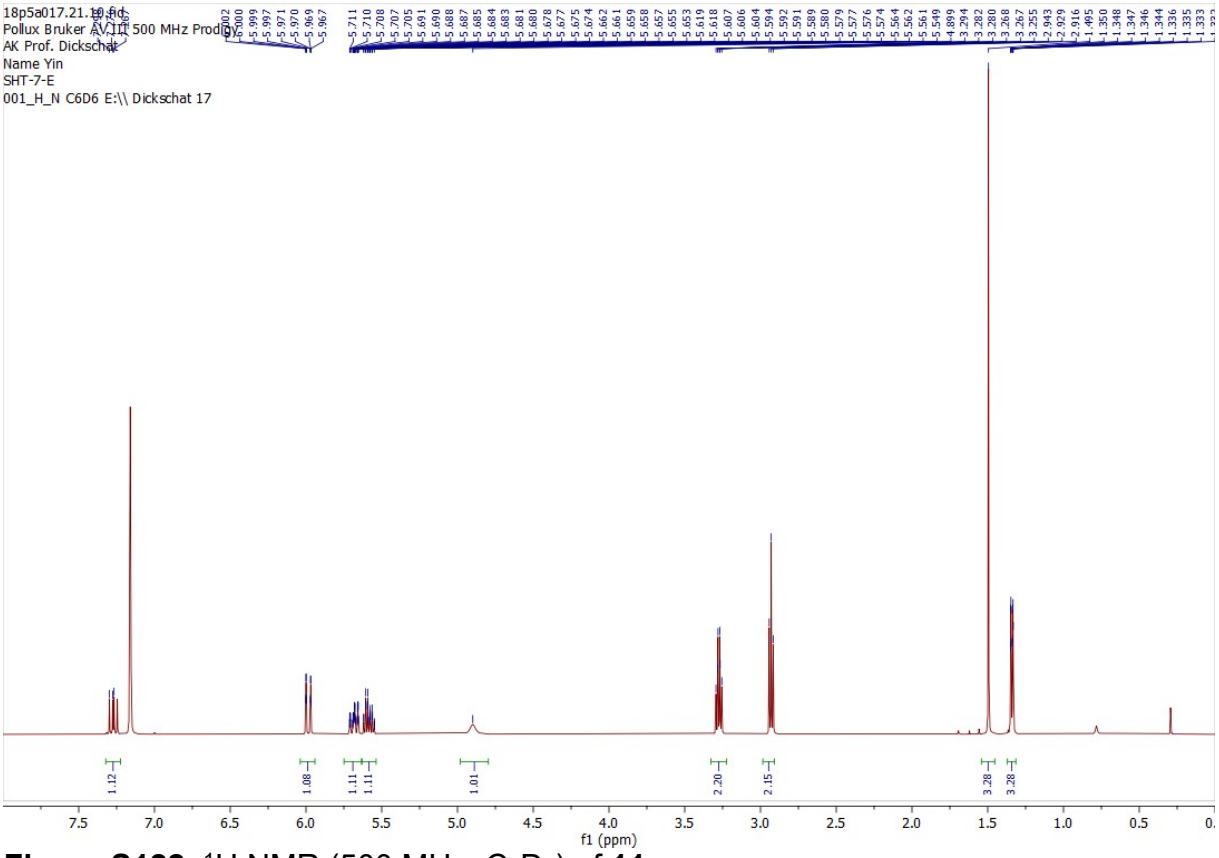


Figure S122. ^{1}H NMR (500 MHz, C_6D_6) of **11**.

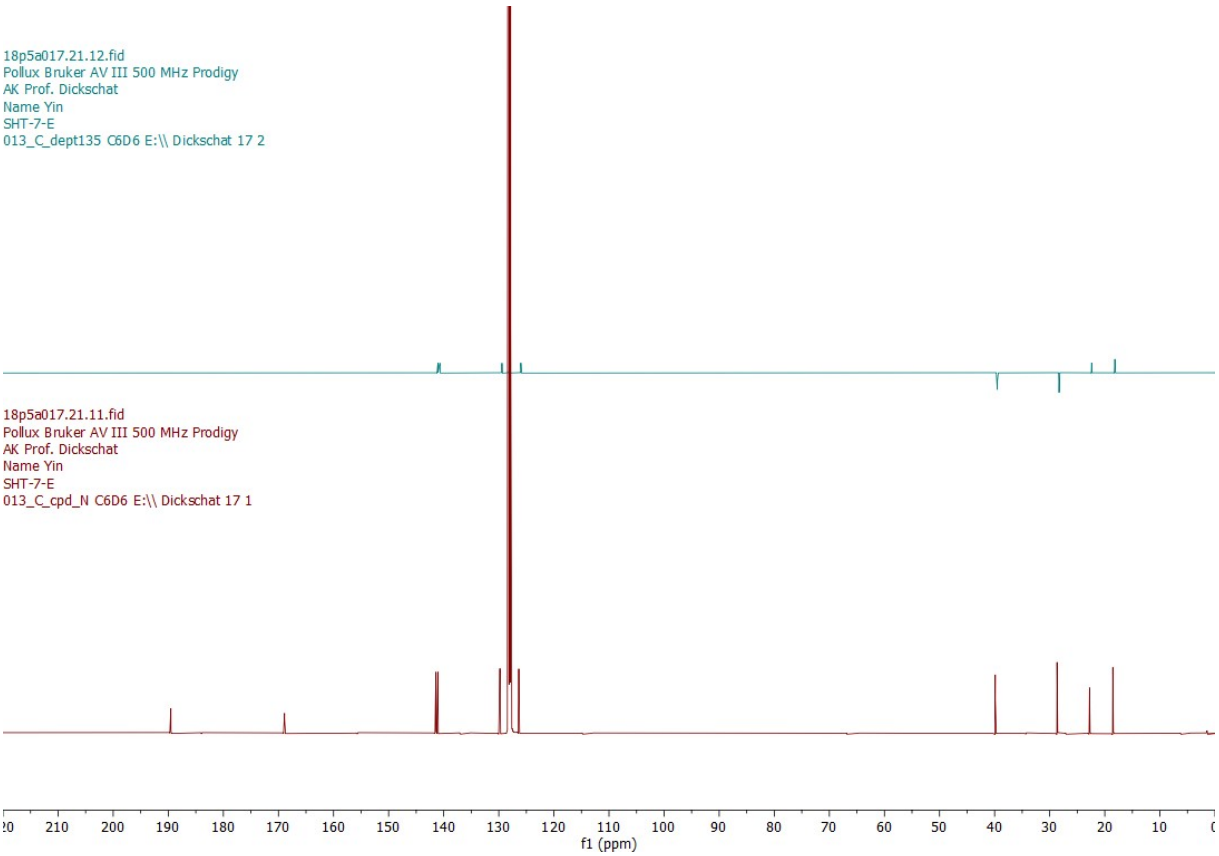


Figure S123. ^{13}C NMR (126 MHz, C_6D_6) of **11**.

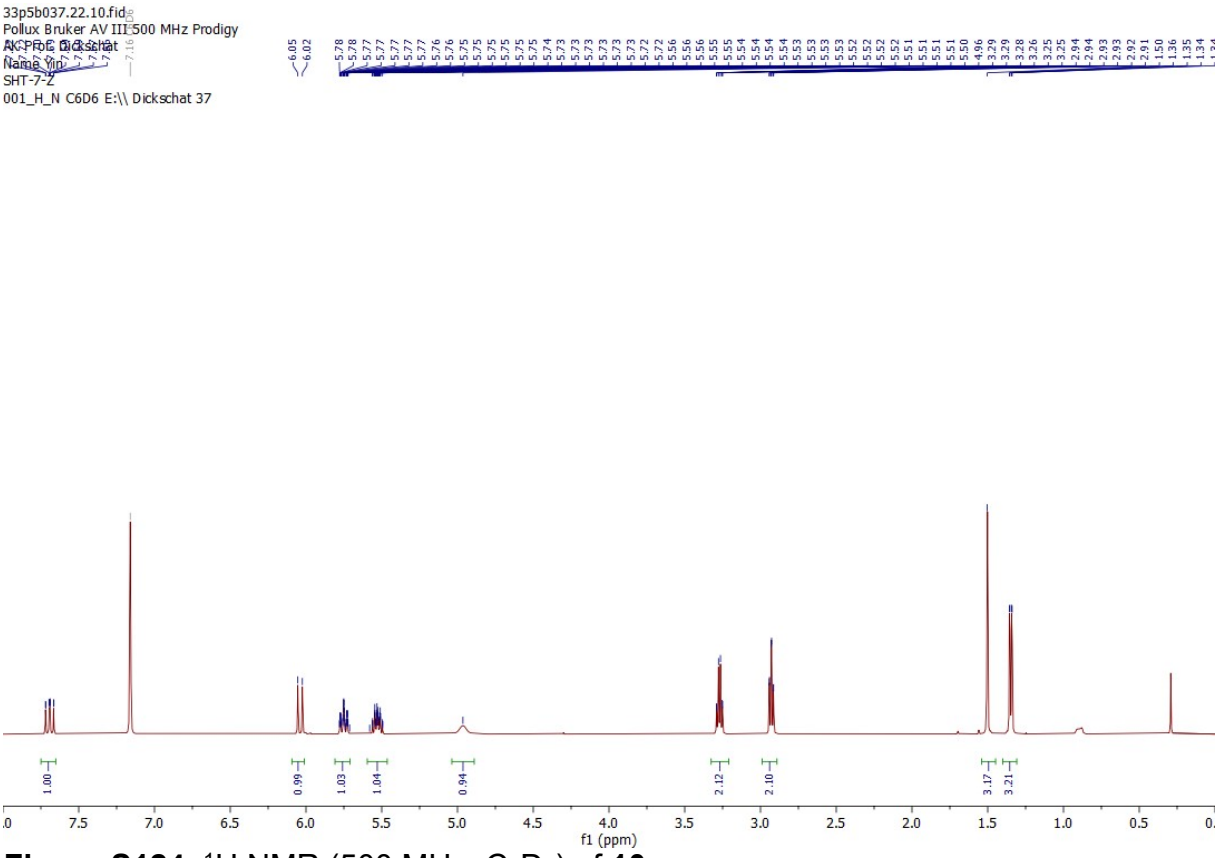


Figure S124. ^{1}H NMR (500 MHz, C_6D_6) of **10**.

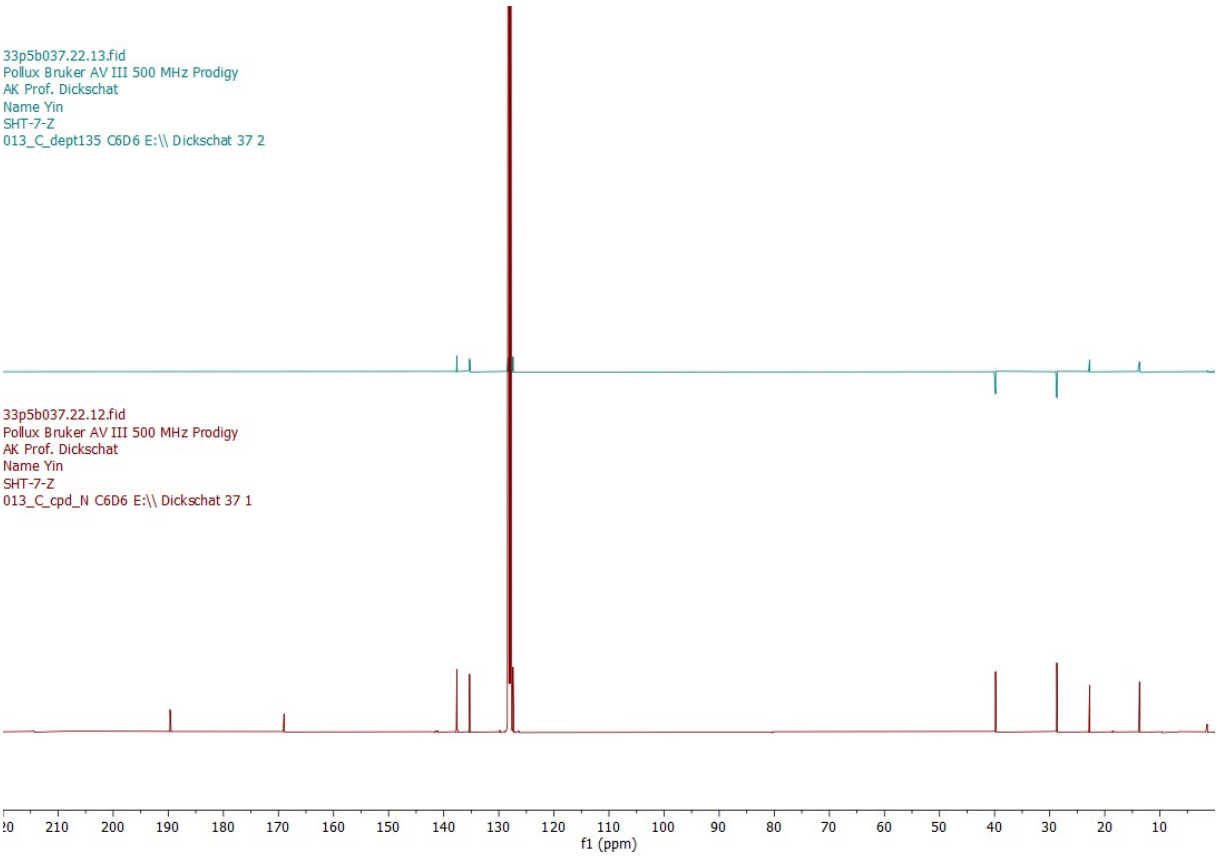


Figure S125. ^{13}C NMR (126 MHz, C_6D_6) of **10**.

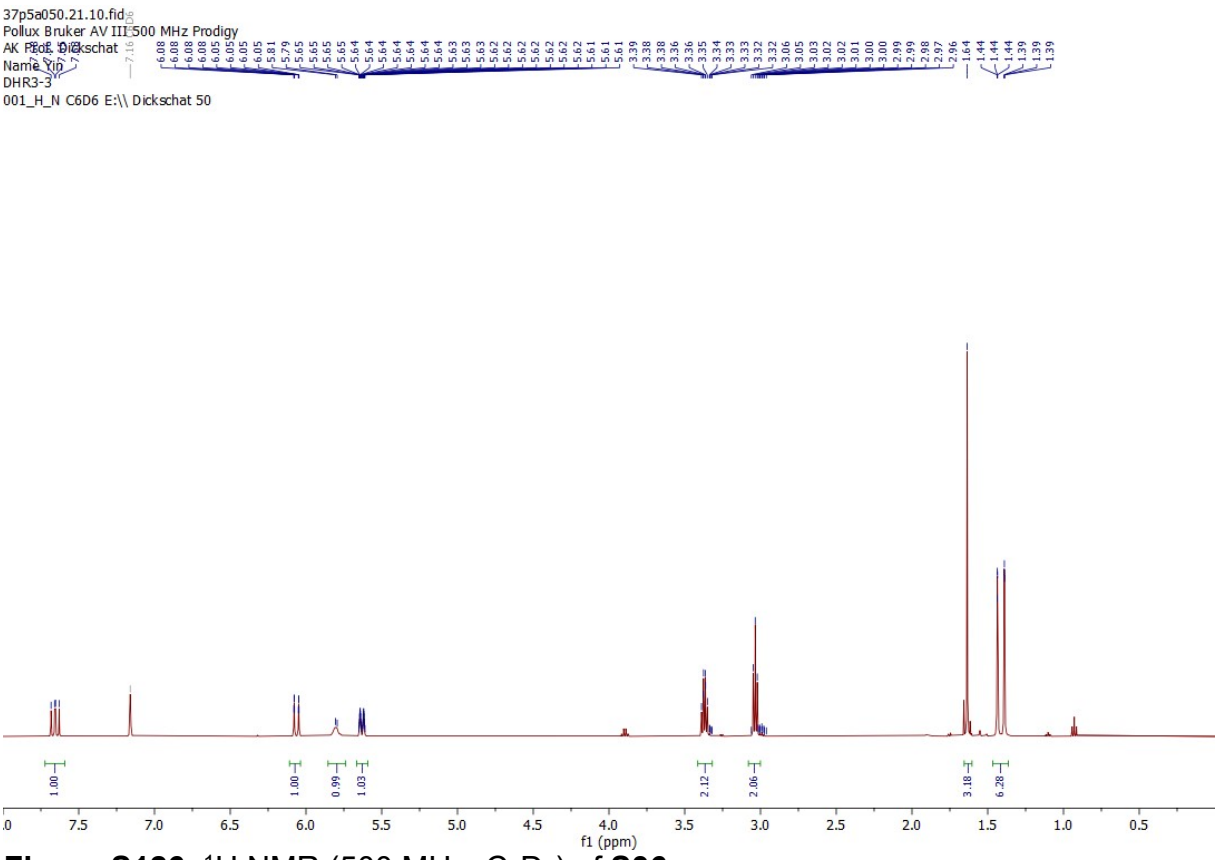


Figure S126. ^{1}H NMR (500 MHz, C_6D_6) of **S36**.

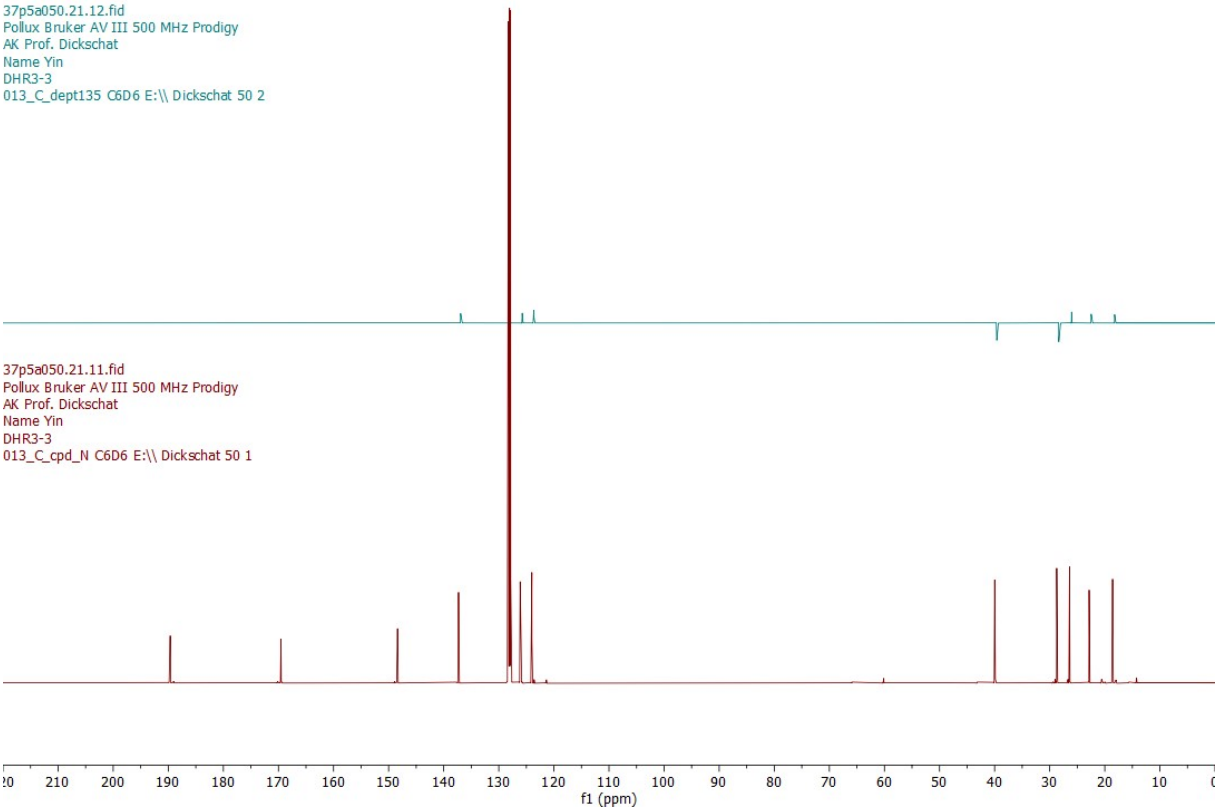


Figure S127. ^{13}C NMR (126 MHz, C_6D_6) of **S36**.

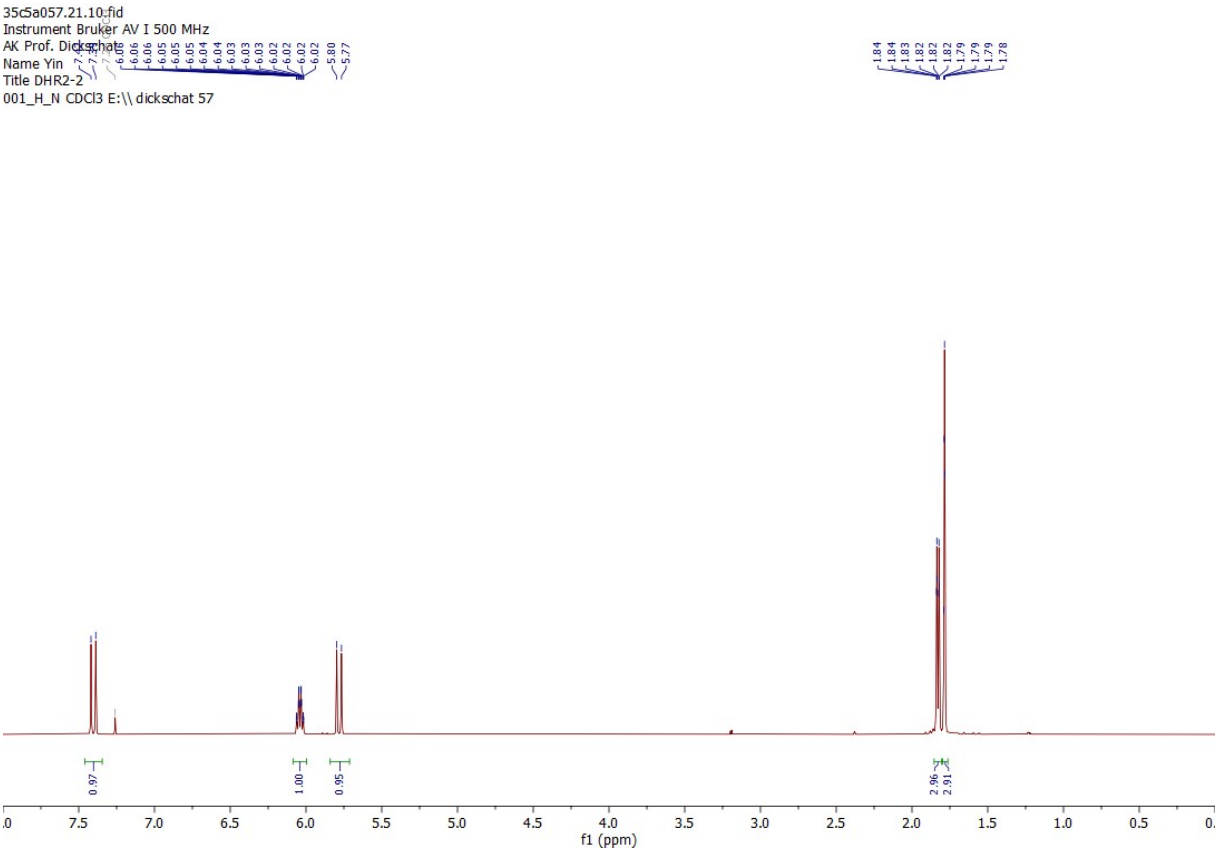


Figure S128. ^{1}H NMR (500 MHz, $CDCl_3$) of **S38**.

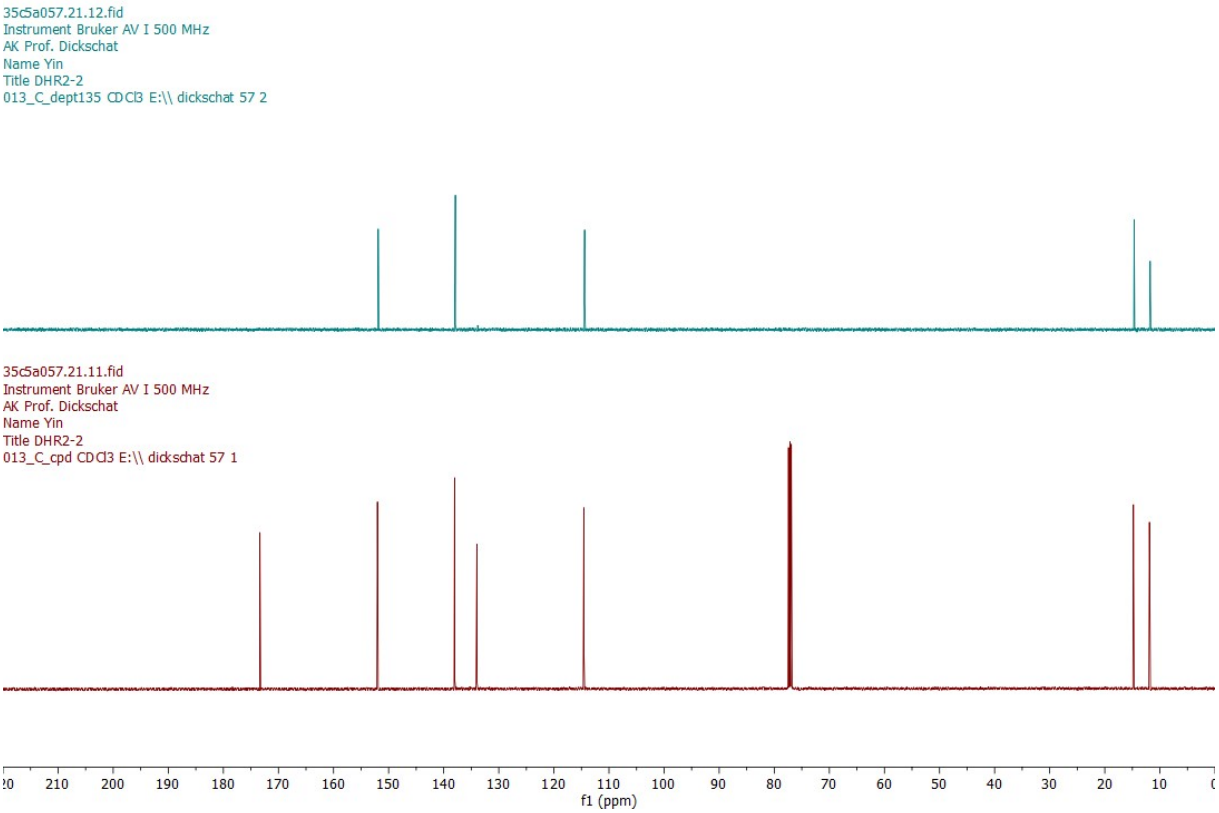


Figure S129. ^{13}C NMR (126 MHz, $CDCl_3$) of **S38**.

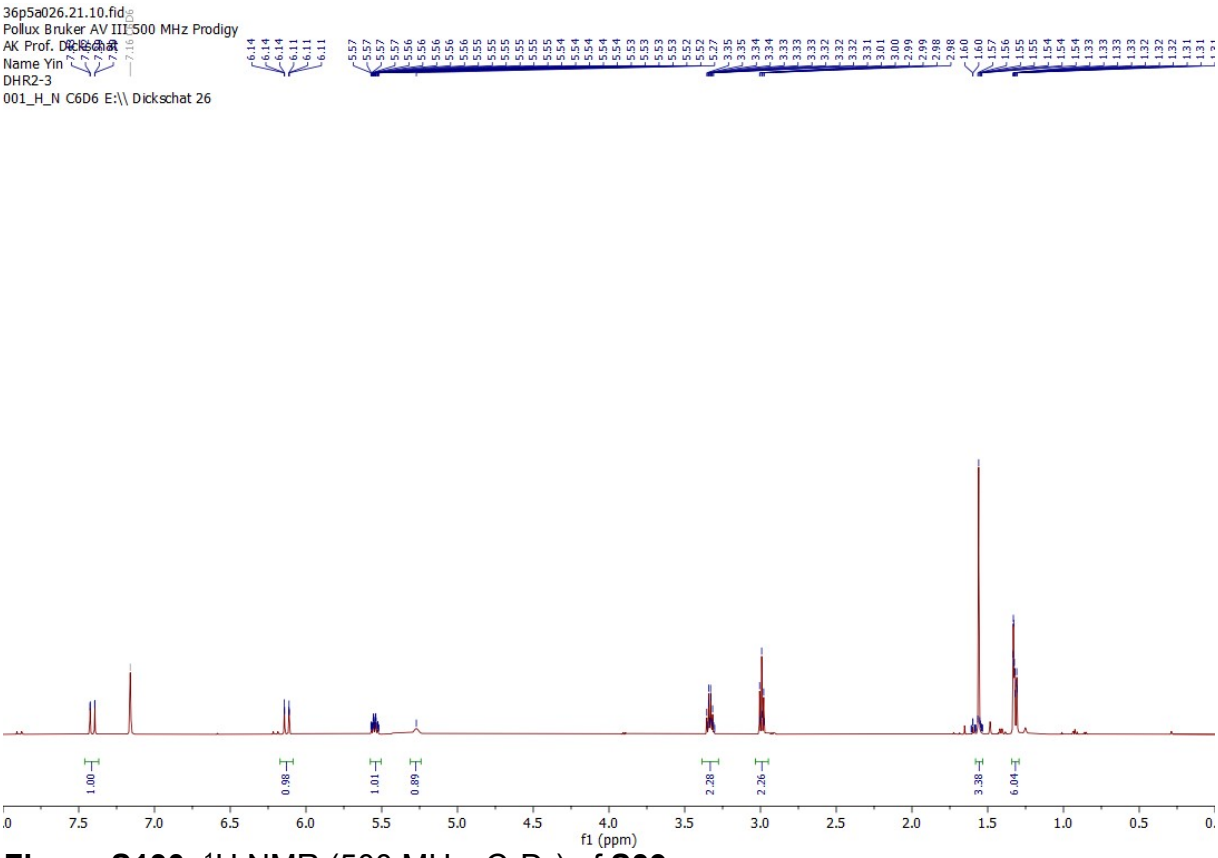


Figure S130. ^{1}H NMR (500 MHz, C_6D_6) of **S39**.

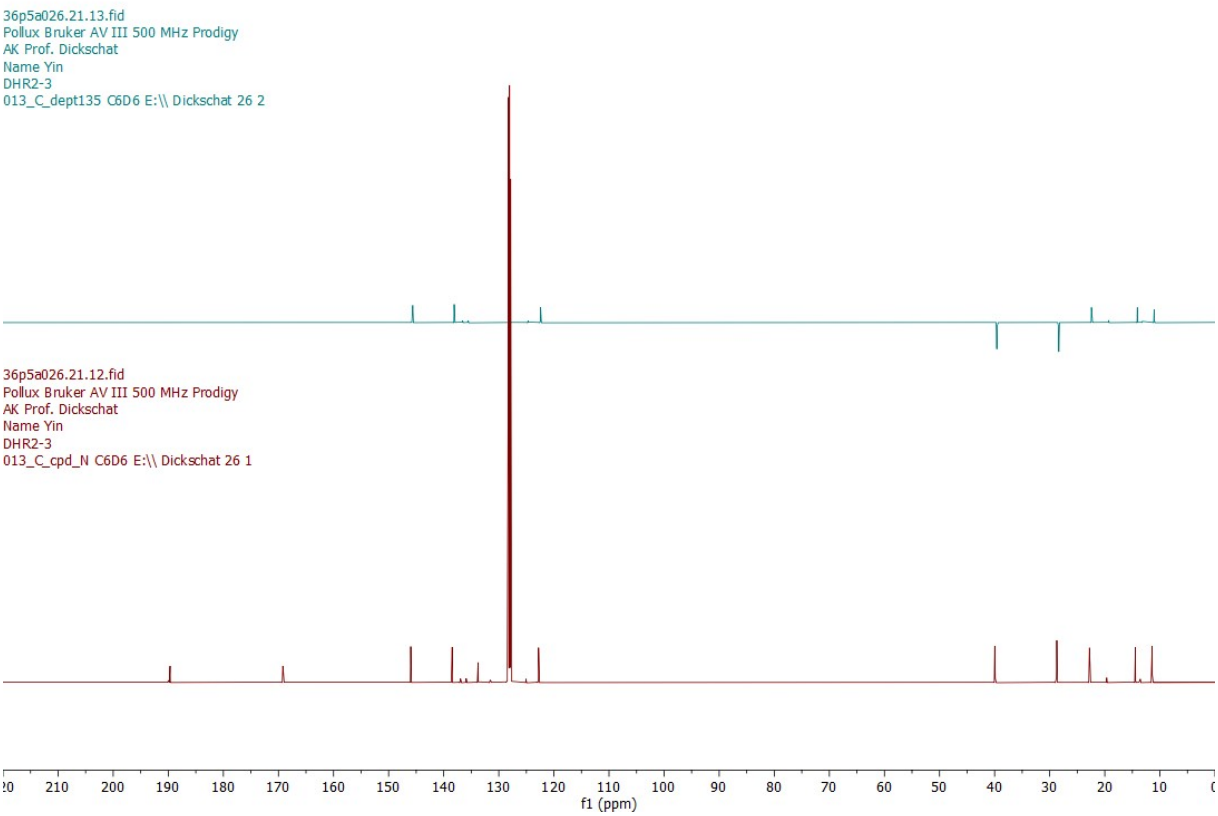


Figure S131. ^{13}C NMR (126 MHz, C_6D_6) of **S39**.

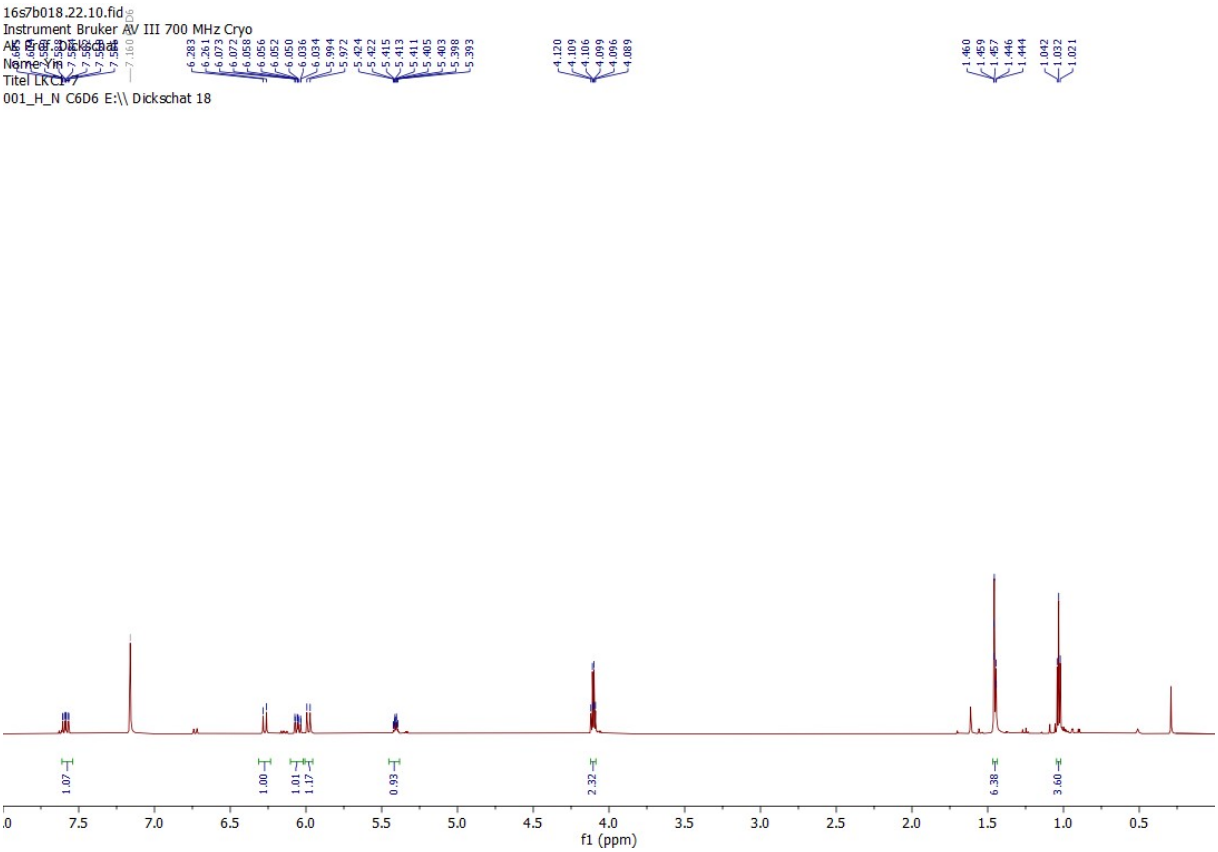


Figure S132. ^{1}H NMR (700 MHz, C_6D_6) of **S41**.

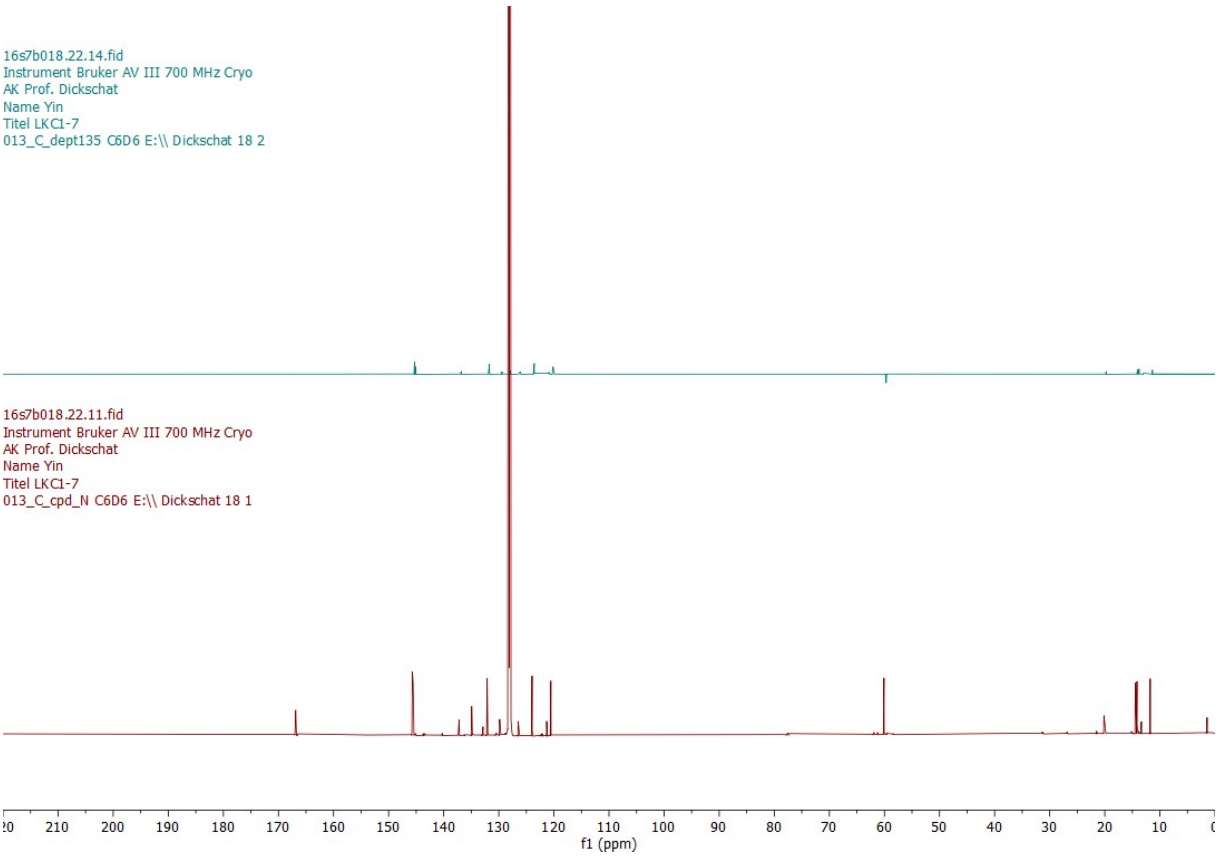


Figure S133. ^{13}C NMR (176 MHz, C_6D_6) of **S41**.

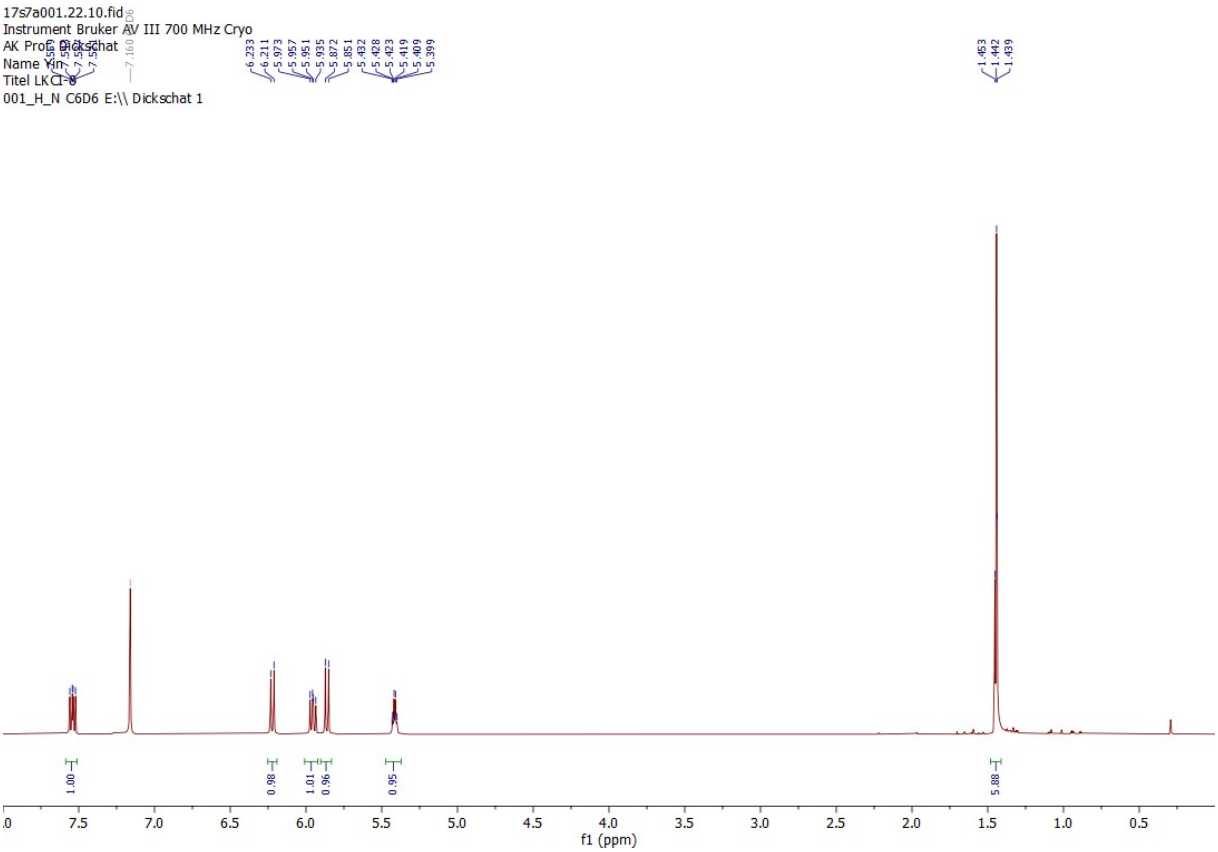


Figure S134. ^{1}H NMR (700 MHz, C_6D_6) of **S42**.

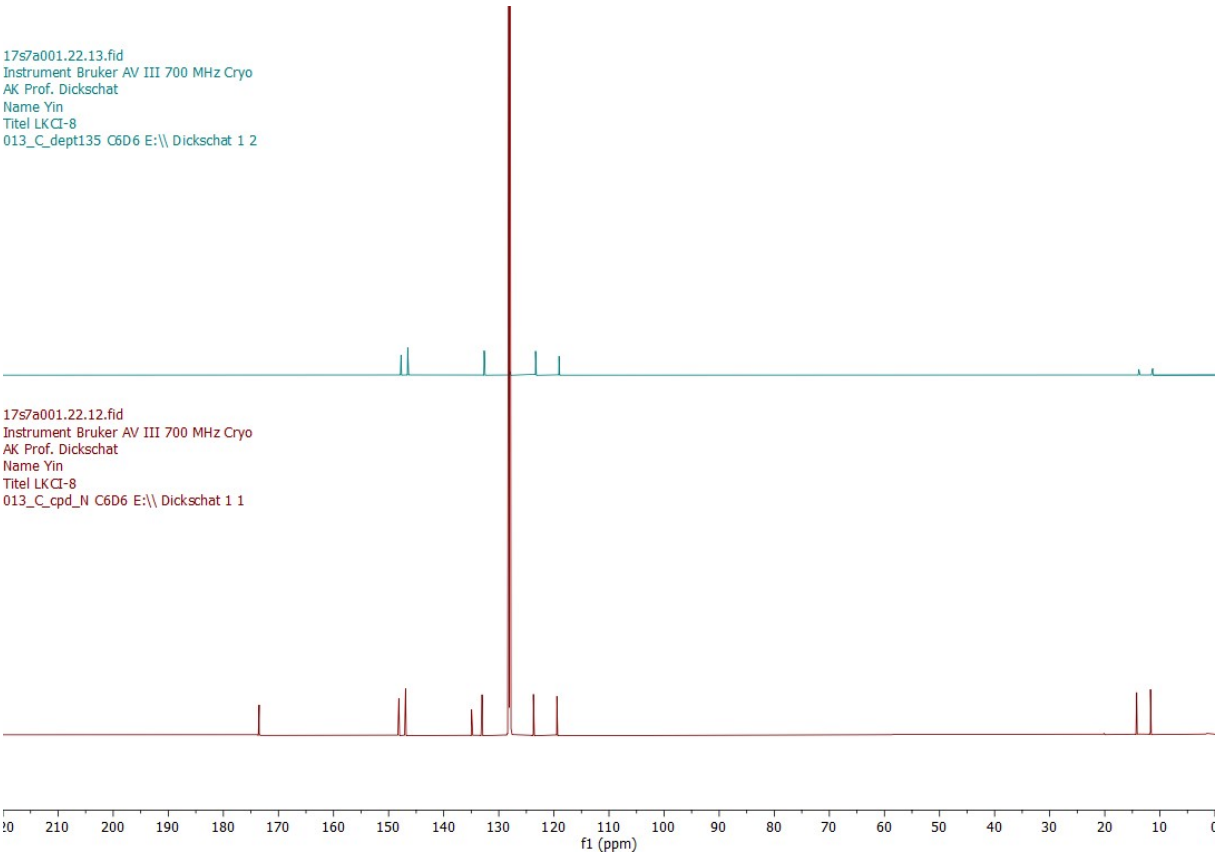


Figure S135. ^{13}C NMR (176 MHz, C_6D_6) of **S42**.

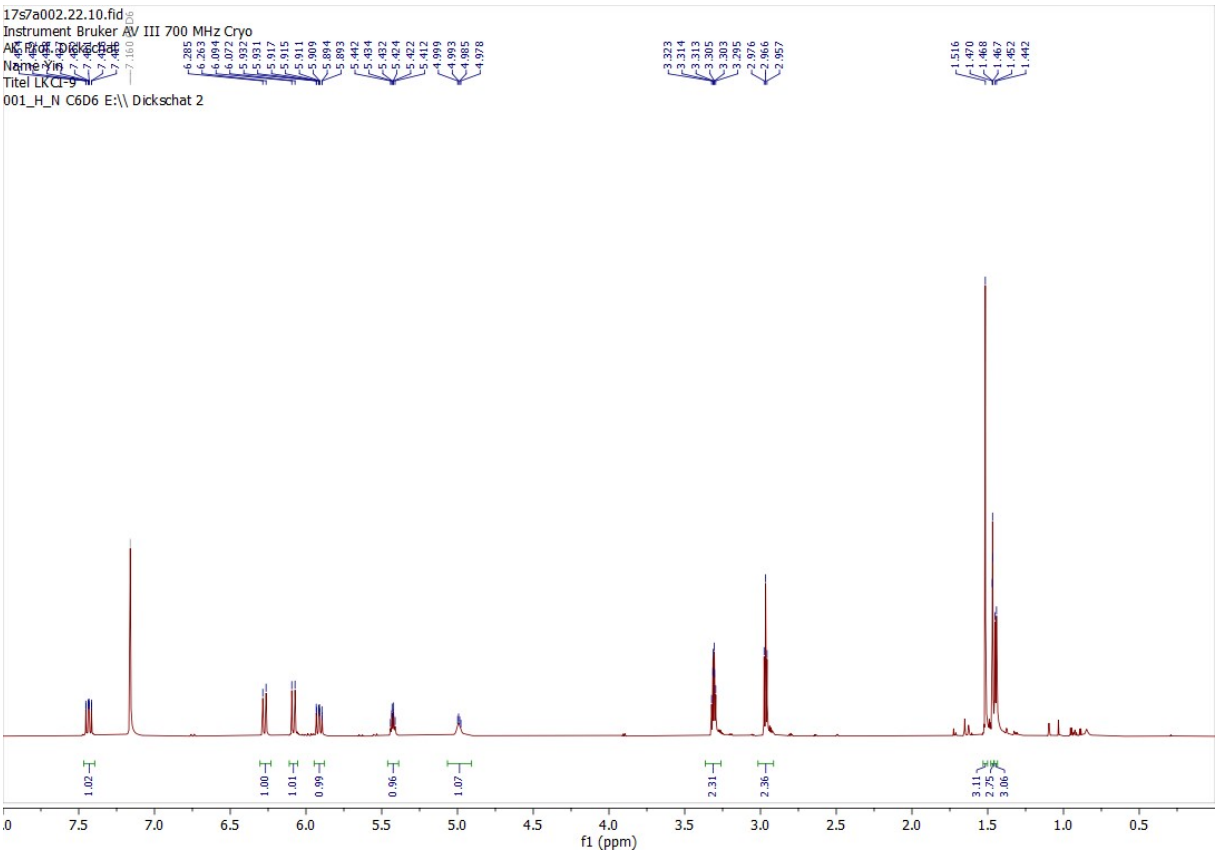


Figure S136. ^{1}H NMR (700 MHz, C_6D_6) of **S43**.

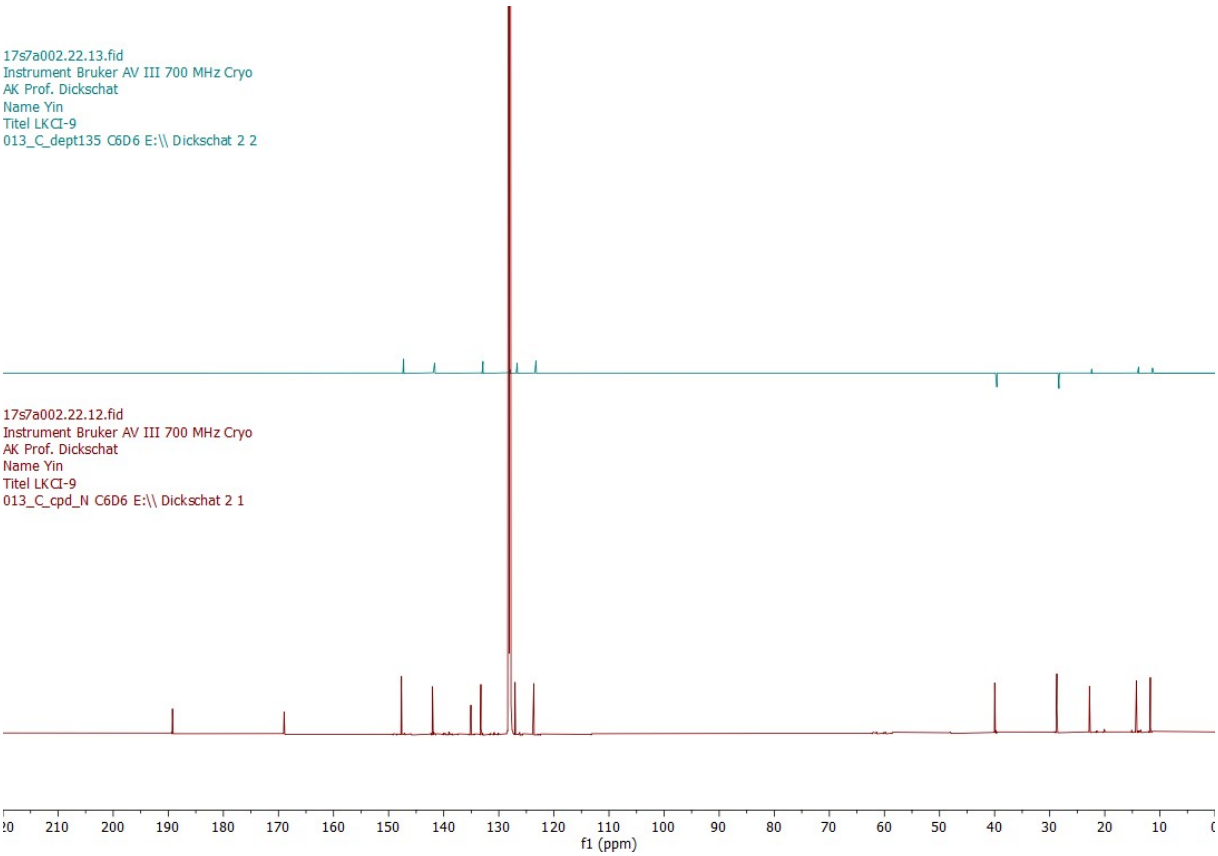


Figure S137. ^{13}C NMR (176 MHz, C_6D_6) of **S43**.

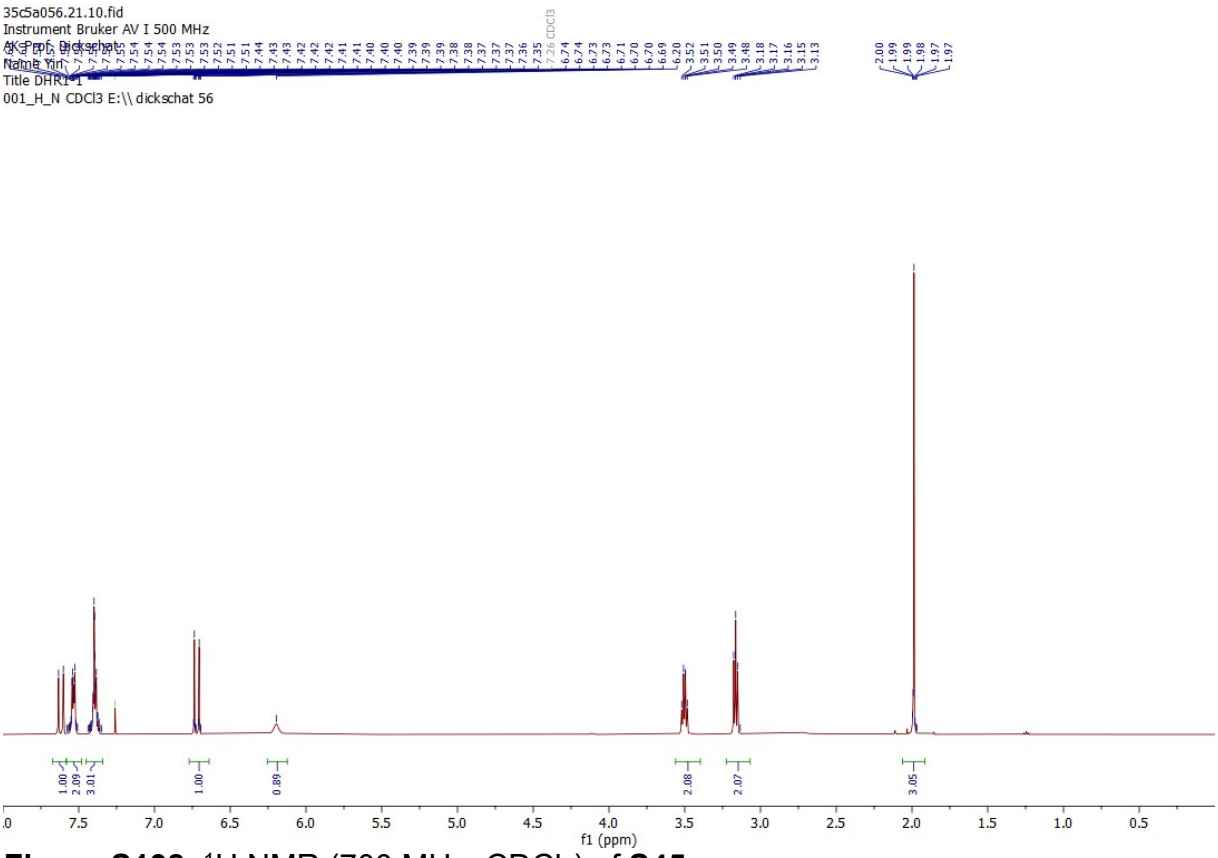


Figure S138. ^{1}H NMR (700 MHz, $CDCl_3$) of **S45**.

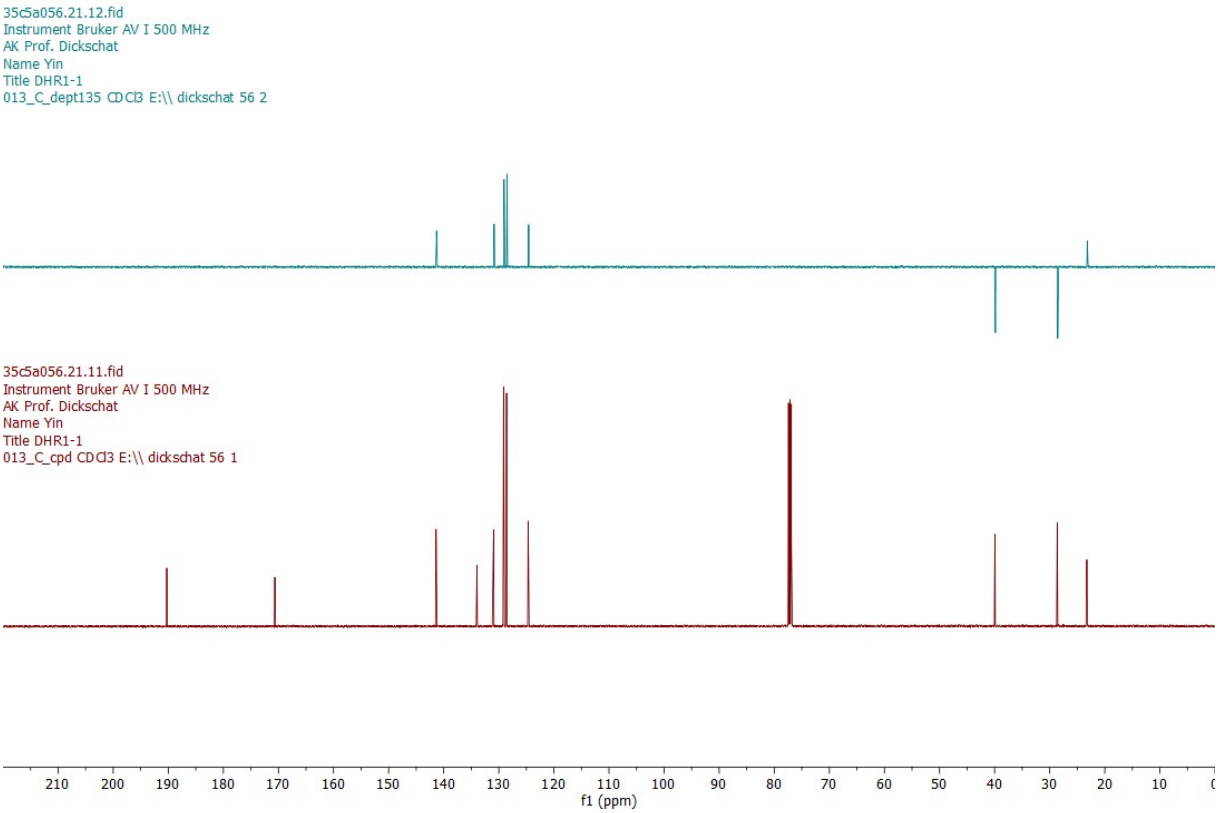


Figure S139. ^{13}C NMR (176 MHz, $CDCl_3$) of **S45**.

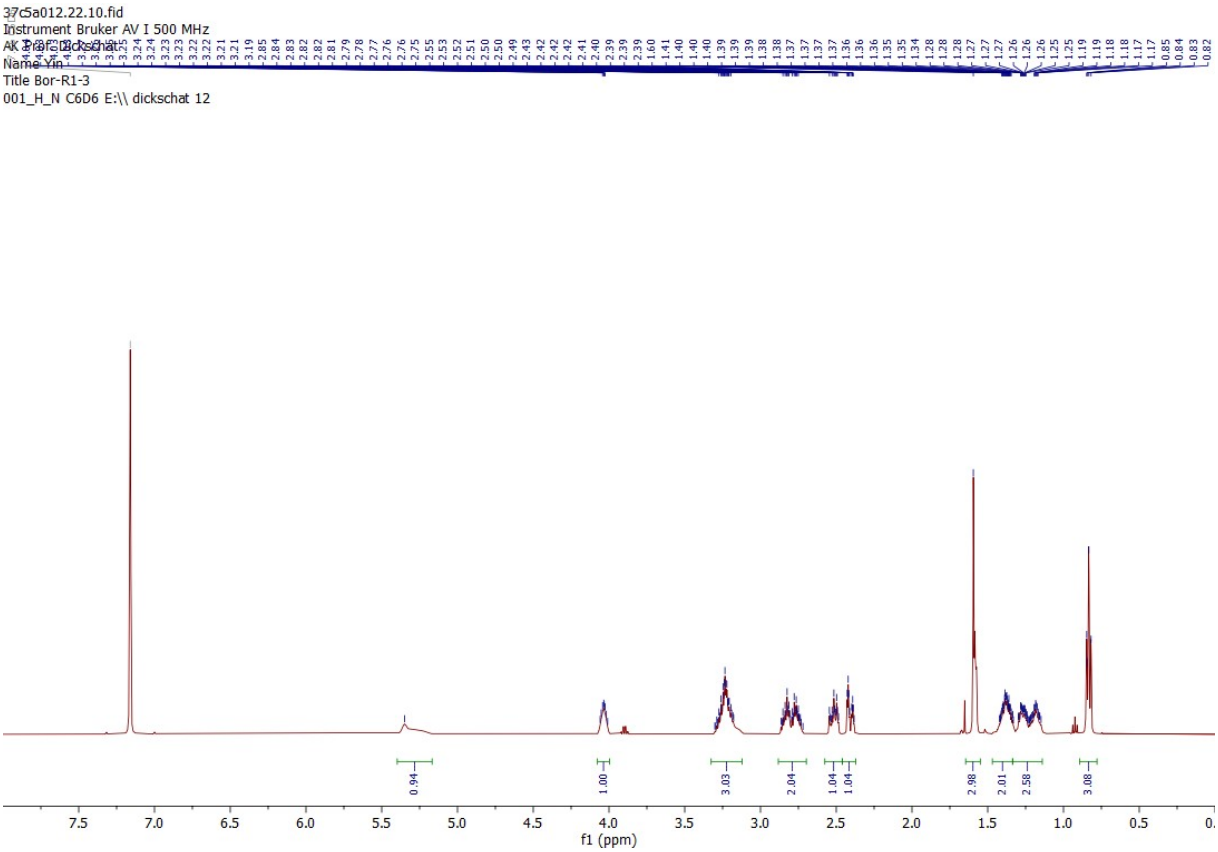


Figure S140. ^{1}H NMR (500 MHz, C_6D_6) of **12**.

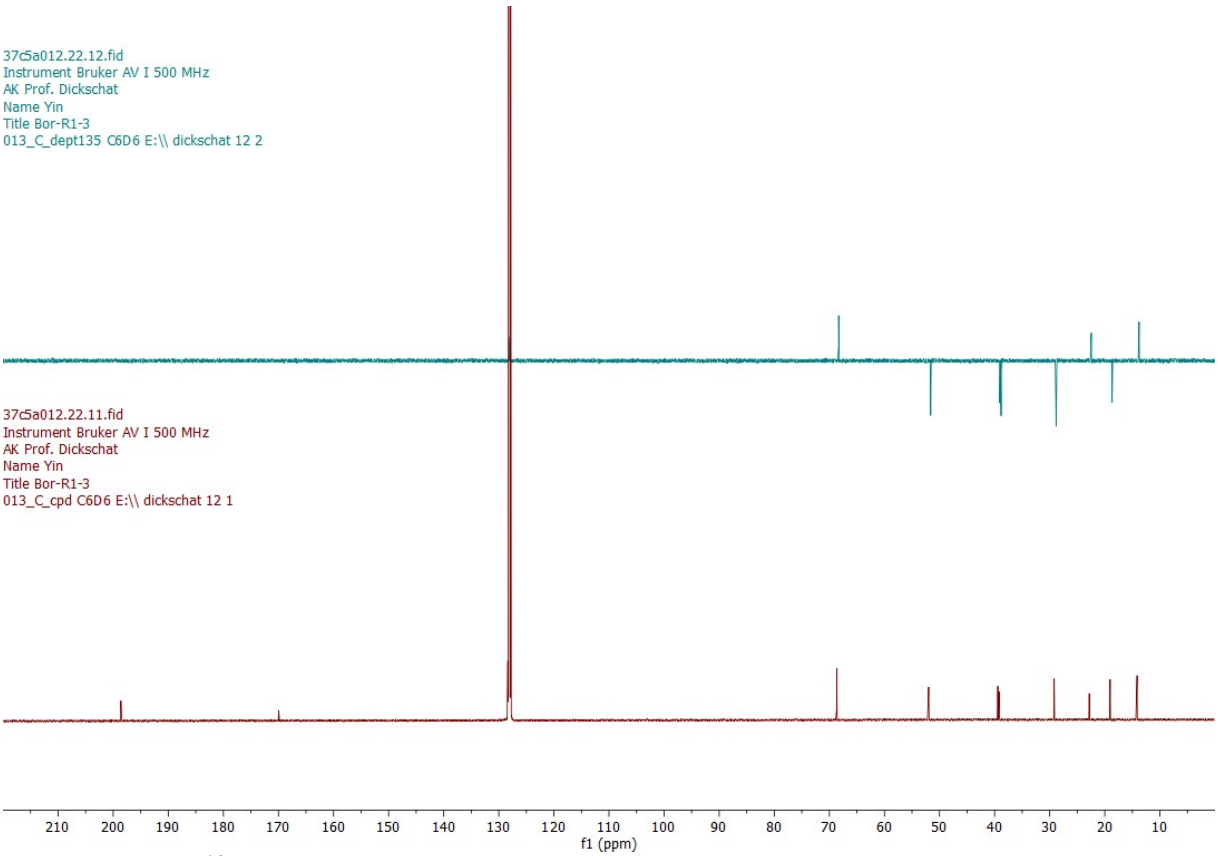


Figure S141. ^{13}C NMR (126 MHz, C_6D_6) of **12**.

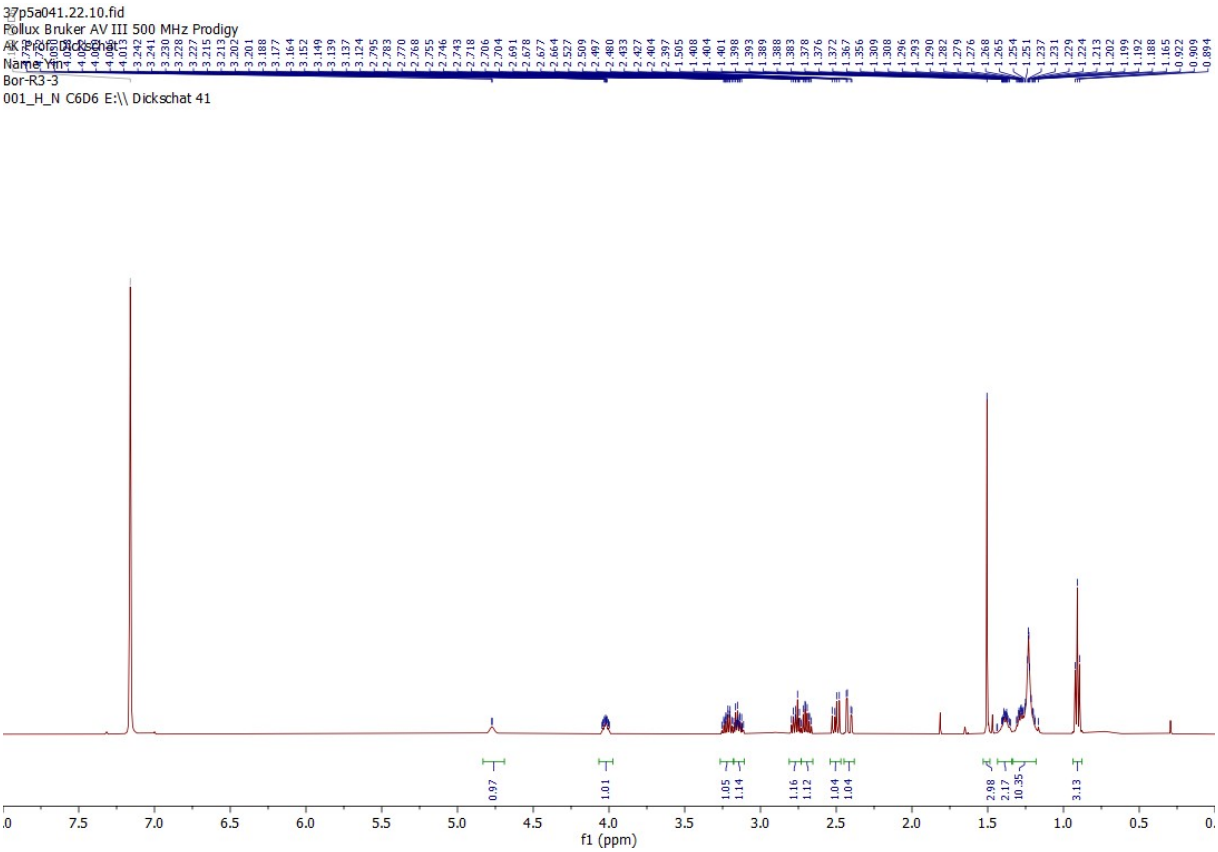


Figure S142. ^{1}H NMR (500 MHz, C_6D_6) of **13**.

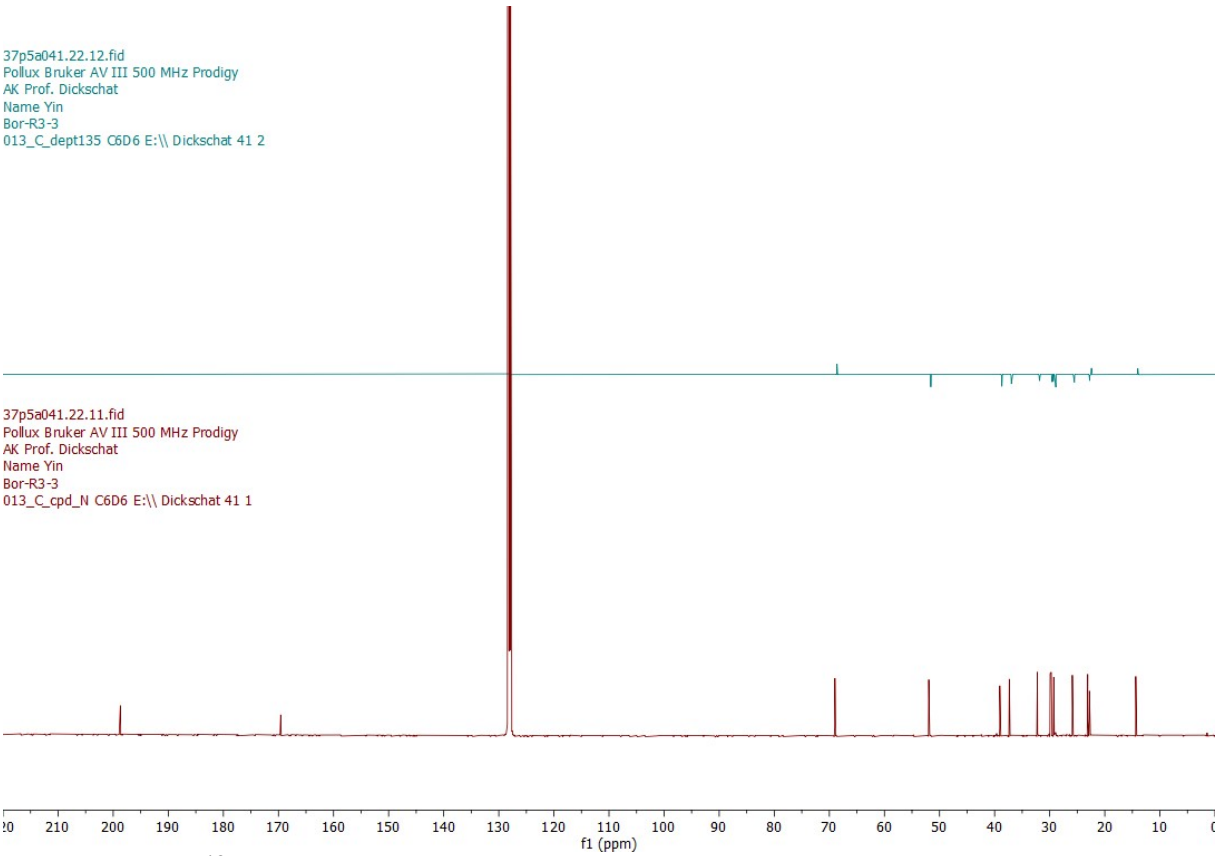


Figure S143. ^{13}C NMR (176 MHz, C_6D_6) of **13**.

References

1 T. Lin, L. S. Borketey, G. Prasad, S. A. Waters and N. A. Schnarr, *ACS Synth. Biol.*, 2013, **2**, 635-642.

2 E. Liddle, A. Scott, L. C. Han, D. Ivison, T. S. Simpson, C. L. Willis and R. J. Cox, *Chem. Commun.*, 2017, **53**, 1727-1730.

3 A. May, P. Willoughby and T. Hoye, *J. Org. Chem.*, 2008, **73**, 3292-3294.

4 N. Claudia and J. M. J. Williams, *Adv. Synth. Catal.*, 2003, **345**, 835-848.

5 S. K. Piasecki, C. A. Taylor, J. F. Detelich, J. Liu, J. Zheng, A. Komsoukaniants, D. R. Siegel and A. T. Keatinge-Clay, *Chem. Biol.*, 2011, **18**, 1331-1340.

6 J. A. Davies, F. M. Bull, P. D. Walker, A. N. Weir, R. Lavigne, J. Masschelein, T. J. Simpson, P. R. Race, M. P. Crump and V. L. Willis, *Org. Lett.*, 2020, **22**, 6349-6353.

7 B. C. Raimundo and C. H. Heathcock, *Synlett*, 1995, **12**, 1213-1214.

8 C. Bauder, *Eur. J. Org. Chem.*, 2010, 16207-16216.

9 T. Yan, A. Hung, H. Lee, C. Chang and W. Liu, *J. Org. Chem.*, 1995, **60**, 3301-3306.

10 H. M. Ge, T. Huang, J. D. Rudolf, J. R. Lohman, S. X. Huang, X. Guo and B. Shen, *Org. Lett.*, 2014, **16**, 3958-3961.

11 M. M. Ahmed, M. S. Mortensen and G. A. O'Doherty, *J. Org. Chem.*, 2006, **71**, 7741-7746.

12 B. DeBoef, W. R. Counts and S. R. Gilbertson, *J. Org. Chem.*, 2007, **72**, 799-804.

13 Z. J. Jia, Q. Zhou, Q. Q. Zhou, P. Q. Chen and Y. C. Chen, *Angew. Chem. Int. Ed.*, 2011, **123**, 8797-8800.

14 C. Le Sann, D. M. Muñoz, N. Saunders, T. J. Simpson, D. I. Smith, F. Soulas, P. Watts and C. L. Willis, *Org. Biomol. Chem.*, 2005, **3**, 1719-1728.

15 Z. Yin, E. Liebhart, E. Stegmann, H. Brötz-Oesterhelt and J. S. Dickschat, *Org. Chem. Front.*, 2022, **9**, 2714-2720.

16 B. D. Ames, C. Nguyen, J. Bruegger, P. Smith, W. Xu, S. Ma, E. Wong, S. Wong, X. Xie, J. W. H. Li and J. C. Vederas, *Proc. Natl. Acad. Sci.*, 2012, **109**, 11144-11149.

17 J. S. Dickschat, K. A. K. Pahirulzaman, P. Rabe and T. A. Klapschinski, *ChemBioChem*, 2014, **15**, 810-814.

18 L. Kaysser, L. Lutsch, S. Siebenberg, E. Wemakor, B. Kammerer and B. Gust, *J. Biol. Chem.*, 2009, **284**, 14987-14996.

19 K. J. Berger, J. L. Driscoll, M. Yuan, B. D. Dherange, O. Gutierrez and M. D. Levin, *J. Am. Chem. Soc.*, 2021, **143**, 17366-17373.