Efficient synthesis of α-alkyl-β-amino amides by transaminase-mediated dynamic kinetic resolutions

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Electronic Supplementary Information

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1. General information

Chemical reagents were purchased from different commercial sources and used without further purification. β-Keto amides **2a-g** were synthesized following two procedures previously described in the literature.[1]

Commercial transaminases were purchased from Codexis, and transaminases from *Bacillus megaterium* and its mutant *Bm*TA S119G overexpressed on *E. coli* cells were obtained as previously described in the literature.[2]

The solvents employed, dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), diethyl ether (Et_2O), ethanol (EtOH), dimethylsulfoxide (DMSO) and acetonitrile (MeCN) were employed without previous drying. Methanol and acetone were previously dried over calcium hydride or calcium sulfate, respectively, to be later distilled under nitrogen atmosphere.

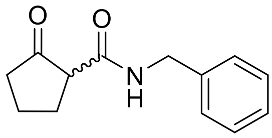
Column chromatographies were performed using silica gel 60 (230-240 mesh). Melting points were taken on samples in open capillary tubes and are uncorrected. IR spectra were recorded on using NaCl plates or KBr pellets; ν_{max} is given for the main absorption bands. ^{1}H, ^{13}C and DEPT NMRs were obtained using different spectrometers (^{1}H, 300.13 MHz and ^{13}C, 75.5 MHz). The chemical shifts are given in delta (δ) values and the coupling constants (*J*) in Hertz (Hz). High resolution mass spectra (HRMS) experiments were carried out by ESI^{+} using a Micro Tof Q spectrometer.

[1] (*a*) M. J. García, F. Rebolledo and V. Gotor, *Tetrahedron Lett.*, 1993, **38**, 6141; (*b*) B. Štefane and S. Polanc, *Tetrahedron*, 2007, **63**, 10902.
[2] (*a*) R. L. Hanson, B. L. Davis, Y. Chen, S. L. Goldberg, W. L. Parker, T. P. Tully, M. A. Montana and R. N. Patel, *Adv. Synth. Catal.*, 2008, **350**, 1367; (*b*) D. Koszelewski, M. Göritzer, D. Clay, B. Seisser and W. Kroutil, *ChemCatChem*, 2010, **2**, 73; (*c*) N. van Oosterwijk, S. Willies, J. Hekelaar, A. C. Terwisscha van Scheltinga, N. J. Turner and B. W. Dijkstra, *Biochemistry*, 2016, **55**, 4422; (*d*) B. Z. Costa, J. L. Galman, I. Slabu, S. P. France, A. J. Marsaioli and N. J. Turner, *ChemCatChem*, 2018, **10**, 4733.

2. Chemical syntheses

*2.1. Synthesis of β-keto amide **2h***

Benzyl amine (1.1 mmol) was added to a suspension containing β-keto ester **1c** (1 mmol, 0.1 M), CAL-B (50 mg) and dry 1,4-dioxane (10 mL) under inert atmosphere. The reaction was shaken at 30 °C and 250 rpm for 48 h. After this time, the enzyme was filtered off, washed with CH_2Cl_2 (3 x 10 mL) and the solvent evaporated under reduced pressure. The crude reaction was purified by column chromatography on silica gel (30% EtOAc/Hexane) leading to the corresponding β-keto amide **2h** (48%).

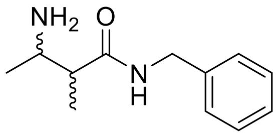


2h

***N*-benzyl-2-oxocyclopentane-1-carboxamide (2h).** Yellowish solid (48% yield). R_f (50% EtOAc/Hexane): 0.30. Mp: 64-65 °C. IR (KBr): ν 3299, 3053, 2961, 1731, 1659, 1540, 1453, 1265, 738, 703 cm^{-1}. ^1H RMN (300.13 MHz, $CDCl_3$): δ 1.78-2.02 (m, 1H), 2.04-2.21 (m, 1H), 2.24-2.42 (m, 4H), 3.01 (t, J_{HH} = 9.3 Hz, 1H), 4.41 (dd, J_{HH} = 14.9, 5.7 Hz, 1H), 4.52 (dd, J_{HH} = 14.9, 5.7 Hz, 1H), 7.12 (br s, 1H), 7.24-7.37 (m, 5H) ppm. ^{13}C RMN (75.5 MHz, $CDCl_3$): δ 20.4 (CH_2), 25.9 (CH_2), 38.9 (CH_2), 43.6 (CH_2), 54.2 (CH), 127.4 (CH), 127.6 (2CH), 128.6 (2CH), 138.0 (C), 166.7 (C), 216.5 (C) ppm. HRMS (ESI^+, *m/z*): calcd for $(C_{13}H_{15}NO_2Na)^+$ $(M+Na)^+$ 240.0995; found 240.1006.

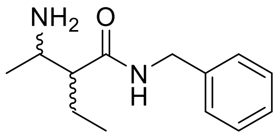
*2.2. General procedure for the synthesis of racemic α-substituted β-amino amides **3a-h***

Ammonium acetate (770 mg, 10 mmol) and sodium cyanoborohydride (128 mg, 2 mmol) were successively added to a solution of the corresponding keto amide **2a-h** (1 mmol, 0.3 M) in dry MeOH (3.3 mL) under inert atmosphere. The mixture was stirred at room temperature during 16 h and, after this time, H_2O (5 mL) was added to quench the reaction. The solution was acidified using an aqueous HCl 3 M solution up to pH ~ 3 and extracted with Et_2O (4 x 10 mL). The resulting aqueous layer was basified by adding 2-3 pellets of NaOH up to pH ~ 13 and extracted with Et_2O (4 x 10 mL). The organic layers were combined, dried over sodium sulfate, filtered and the solvent was distilled under reduced pressure, obtaining the corresponding amines **3a-h** (36-70%).



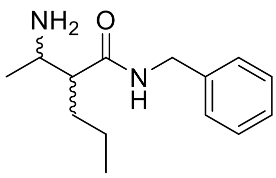
3a

3-Amino-*N*-benzyl-2-methylbutanamide (3a). Yellowish oil (36% yield). Mixture of *syn* and *anti* isomers. R_f (10% MeOH/CH_2Cl_2): 0.30. IR (NaCl): ν 3301, 3053, 2975, 2930, 2877, 1655, 1547, 1454, 1265, 737, 703 cm^{-1}. ^1H RMN (300.13 MHz, $CDCl_3$): δ 1.07-1.22 (m, 6H), 1.75 (br s, 2H), 2.17 (m, 0.7H), 2.28 (m, 0.3H), 3.12 (m, 0.6H), 3.21 (m, 0.4H), 4.44 (d, J_{HH} = 7.1 Hz, 2H), 7.23-7.39 (m, 5H), 7.48 (br s, 0.7H), 7.86 (br s, 0.3H) ppm. ^{13}C RMN (75.5 MHz, $CDCl_3$): δ 12.4 (CH_3), 16.0 (CH_3), 20.7 (CH_3), 21.3 (CH_3), 43.1 (CH_2), 46.2 (CH), 48.4 (CH), 48.8 (CH), 49.3 (CH), 127.2 (CH), 127.2 (2CH), 127.6 (2CH), 138.7 (C), 138.9 (C), 175.3 (C), 175.4 (C) ppm. HRMS (ESI^+, *m/z*): calcd for $(C_{12}H_{19}N_2O)^+$ $(M+H)^+$ 207.1492; found 207.1491.



3b

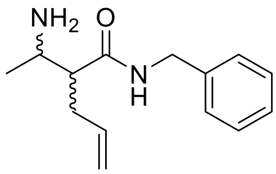
3-Amino-*N*-benzyl-2-ethylbutanamide (3b). Yellowish oil (58% yield). Mixture of *syn* and *anti* isomers. R_f (10% MeOH/CH_2Cl_2): 0.30. IR (NaCl): ν 3289, 3054 2966, 1650, 1585, 1454, 1265, 1029, 738, 702 cm^{-1}. ^1H RMN (300.13 MHz, $CDCl_3$): δ 0.92 (t, J_{HH} = 7.4 Hz, 3H), 1.07 (d, J_{HH} = 6.6 Hz, 2H), 1.12 (d, J_{HH} = 6.6 Hz, 1H), 1.49-1.60 (m, 3H), 1.65-1.80 (m, 1H), 1.95 (m, 1H), 3.18 (m, 1H), 4.43 (d, J_{HH} = 5.3 Hz, 2H), 7.25-7.35 (m, 5.5H), 7.52 (br s, 0.2H) ppm. ^{13}C RMN (75.5 MHz, $CDCl_3$): δ 12.1 (CH_3), 12.5 (CH_3), 20.9 (CH_2), 21.4 (CH_3), 21.5 (CH_3), 23.9 (CH_2), 43.0 (CH_2), 43.2 (CH_2), 47.6 (CH), 48.4 (CH), 55.5 (CH), 56.0 (CH), 127.2 (CH), 127.7 (2CH), 128.6 (2CH), 138.8 (C), 174.5 (C), 174.8 (C) ppm. HRMS (ESI^+, *m/z*): calcd for $(C_{13}H_{21}N_2O)^+$ $(M+H)^+$ 221.1648; found 221.1647.



3c

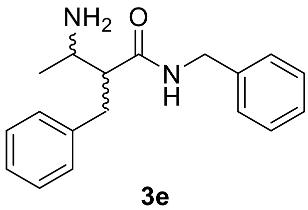
2-(1-Aminoethyl)-*N*-benzylpentanamide (3c). Yellowish oil (70% yield). Mixture of *syn* and *anti* isomers. R_f (10% MeOH/CH_2Cl_2): 0.30. IR (NaCl): ν 3289, 3054, 2963, 1658, 1546, 1422, 1265, 1156, 738, 705 cm^{-1}. ^1H RMN (300.13 MHz, $CDCl_3$): δ 0.93 (t, J_{HH} = 6.8 Hz, 3H), 1.09 (d, J_{HH} = 6.5 Hz, 1.5H), 1.14 (d, J_{HH} = 6.5 Hz, 1.5H), 1.25-1.50 (m, 4H), 1.54 (s, 2H), 1.63-1.75

(m, 1H), 2.06 (m, 1H), 3.17 (m, 1H), 4.46 (m, 2H), 7.23-7.35 (m, 5.7H), 7.45 (br s, 0.3H) ppm. ^{13}C RMN (75.5 MHz, $CDCl_3$): δ 14.1 (CH_3), 14.2 (CH_3), 20.8 (CH_2), 21.2 (CH_2), 21.4 (CH_3), 21.5 (CH_3), 29.9 (CH_2), 33.0 (CH_2), 43.0 (CH_2), 43.2 (CH_2), 47.9 (CH), 48.6 (CH), 53.5 (CH), 54.2 (CH), 127.2 (CH), 127.7 (2CH), 128.6 (2CH), 138.8 (C), 174.6 (C), 174.9 (C) ppm. HRMS (ESI^+, *m/z*): calcd for $(C_{14}H_{23}N_2O)^+$ $(M+H)^+$ 235.1805; found 235.1806.



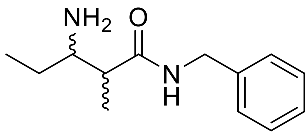
3d

2-(1-Aminoethyl)-*N*-benzylpent-4-enamide (3d). Yellowish oil (53% yield). Mixture of *syn* and *anti* isomers. R_f (10% MeOH/CH_2Cl_2): 0.30. IR (NaCl): ν 3054, 2982, 1643, 1546, 1497, 1454, 1423, 1265, 1167, 1080, 737, 704 cm^{-1}. ^{1}H RMN (300.13 MHz, $CDCl_3$): δ 1.09 (d, J_{HH} = 6.5 Hz, 1.5H), 1.15 (d, J_{HH} = 6.5 Hz, 1.5H), 1.63 (br s, 2H), 2.14-2.34 (m, 1H), 2.44-2.58 (m, 1H), 3.23 (m, 1H), 4.44 (m, 2H), 5.00-5.12 (m, 2H), 5.75-5.86 (m, 1H), 7.24-7.42 (m, 5.6H), 7.63 (br s, 0.4H) ppm. ^{13}C RMN (75.5 MHz, $CDCl_3$): δ 20.5 (CH_3), 21.4 (CH_3), 32.4 (CH_2), 35.3 (CH_2), 43.1 (CH_2), 43.2 (CH_2), 47.0 (CH), 47.9 (CH), 52.7 (CH), 53.7 (CH), 116.6 (CH_2), 116.8 (CH_2), 127.2 (CH), 127.8 (2CH), 128.6 (2CH), 135.7 (CH), 136.5 (CH), 138.7 (C), 173.6 (C), 174.1 (C) ppm. HRMS (ESI^+, *m/z*): calcd for $(C_{14}H_{21}N_2O)^+$ $(M+H)^+$ 233.1648; found 233.1646.



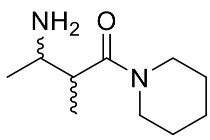
3e

3-Amino-*N*,2-dibenzylbutanamide (3e). Yellowish oil (60% yield). Mixture of *syn* and *anti* isomers. R_f (10% MeOH/CH_2Cl_2): 0.10. IR (NaCl): ν 3292, 3054, 2985, 1647, 1545, 1422, 1265, 1136, 1030, 738, 704 cm^{-1}. ^{1}H RMN (300.13 MHz, $CDCl_3$): δ 1.15 (d, J_{HH} = 6.5 Hz, 2H), 1.19 (d, J_{HH} = 6.5 Hz, 1H), 1.68 (br s, 2H), 2.33 (m, 1H), 2.83 (m, 1H), 3.06-3.26 (m, 2H), 4.25-4.53 (m, 2H), 6.79 (br s, 0.6H), 6.92 (br s, 0.3H), 7.05 (dd, J_{HH} = 7.4, 2.1 Hz, 2H), 7.24 (m, 8H) ppm. ^{13}C RMN (75.5 MHz, $CDCl_3$): δ 20.5 (CH_3), 21.6 (CH_3), 34.4 (CH_2), 36.9 (CH_2), 43.1 (CH_2), 43.2 (CH_2), 47.4 (CH), 48.0 (CH), 55.9 (CH), 56.8 (CH), 126.2 (CH), 126.4 (CH), 127.2 (CH), 127.6 (CH), 128.5 (CH), 128.6 (CH), 128.8 (CH), 128.9 (CH), 138.3 (C), 138.4 (C), 139.4 (C), 140.2 (C), 173.4 (C), 173.8 (C) ppm. HRMS (ESI^+, *m/z*): calcd for $(C_{18}H_{23}N_2O)^+$ $(M+H)^+$ 283.1805; found 283.1805.



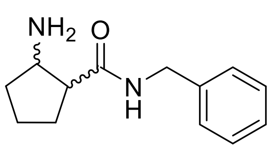
3f

3-Amino-*N*-benzyl-2-methylpentanamide (3f). Yellowish oil (52% yield). Mixture of *syn* and *anti* isomers. R_f (10% MeOH/CH_2Cl_2): 0.30. IR (NaCl): ν 3285, 3054, 2969, 1655, 1514, 1422, 1265, 1156, 739, 705 cm^{-1}. ^1H RMN (300.13 MHz, $CDCl_3$): δ 0.93 (m, 3H), 1.13 (d, J_{HH} = 7.2 Hz, 1H), 1.21 (d, J_{HH} = 7.2 Hz, 2H), 1.26-1.56 (m, 2H), 1.59 (s, 2H), 2.28 (m, 0.6H), 2.36 (m, 0.4H), 2.88 (m, 1H), 4.44 (d, J_{HH} = 5.7 Hz, 2H), 7.33 (m, 5H), 7.70 (s, 0.7H), 8.00 (s, 0.3H) ppm. ^{13}C RMN (75.5 MHz, $CDCl_3$): δ 10.6 (CH_3), 11.1 (CH_3), 11.7 (CH_3), 16.2 (CH_3), 27.7 (CH_2), 28.3 (CH_2), 43.0 (CH_2), 44.8 (CH), 46.1 (CH), 54.4 (CH), 55.2 (CH), 127.1 (CH), 127.6 (2CH), 128.6 (2CH), 138.9 (C), 175.7 (C), 176.0 (C) ppm. HRMS (ESI^+, *m/z*): calcd for $(C_{13}H_{21}N_2O)^+$ $(M+H)^+$ 221.1648; found 221.1652.



3g

3-Amino-2-methyl-1-(piperidin-1-yl)butan-1-one (3g). Yellowish oil (57% yield). Mixture of *syn* and *anti* isomers. R_f (10% MeOH/CH_2Cl_2): 0.10. IR (NaCl): ν 3313, 3053, 2924, 1624, 1442, 1265, 1026, 739, 705 cm^{-1}. ^1H RMN (300.13 MHz, $CDCl_3$): δ 1.07 (d, J_{HH} = 6.4 Hz, 3H), 1.27 (d, J_{HH} = 6.4 Hz, 3H), 1.50-1.66 (m, 6H), 1.71 (s, 2H), 2.45-2.73 (m, 1H), 3.12-3.23 (m, 0.8H), 3.39-3.63 (m, 4.2H) ppm. ^{13}C RMN (75.5 MHz, $CDCl_3$): δ 12.6 (CH_3), 15.1 (CH_3), 20.5 (CH_3), 21.8 (CH_3), 24.6 (CH_2), 25.7 (CH_2), 26.8 (CH_2), 41.9 (CH), 42.7 (CH_2), 43.7 (CH), 46.7 (CH_2), 48.9 (CH), 49.5 (CH), 173.9 (C), 174.2 (C) ppm. HRMS (ESI^+, *m/z*): calcd for $(C_{10}H_{21}N_2O)^+$ $(M+H)^+$ 185.1648; found 185.1654.



3h

2-Amino-*N*-benzylcyclopentane-1-carboxamide (3h). Yellowish oil (52% yield). Mixture of *cis* and *trans* isomers. R_f (10% MeOH/CH_2Cl_2): 0.20. IR (KBr): ν 3299, 3053, 2961, 1659, 1544, 1453, 1265, 1081, 738, 703 cm^{-1}. ^1H RMN (300.13 MHz, $CDCl_3$): δ 1.26-1.39 (m, 2H), 1.58-1.72 (m, 3H), 1.90-2.07 (m, 3H), 2.25 (m, 0.8H), 2.58 (m, 0.2H), 3.25 (m, 0.8H), 3.50 (m, 0.2H), 4.47 (d, J_{HH} = 5.7Hz, 2H), 7.22-7.34 (m, 5H), 7.41 (br s, 0.2H), 7.54 (br s, 0.8H) ppm.

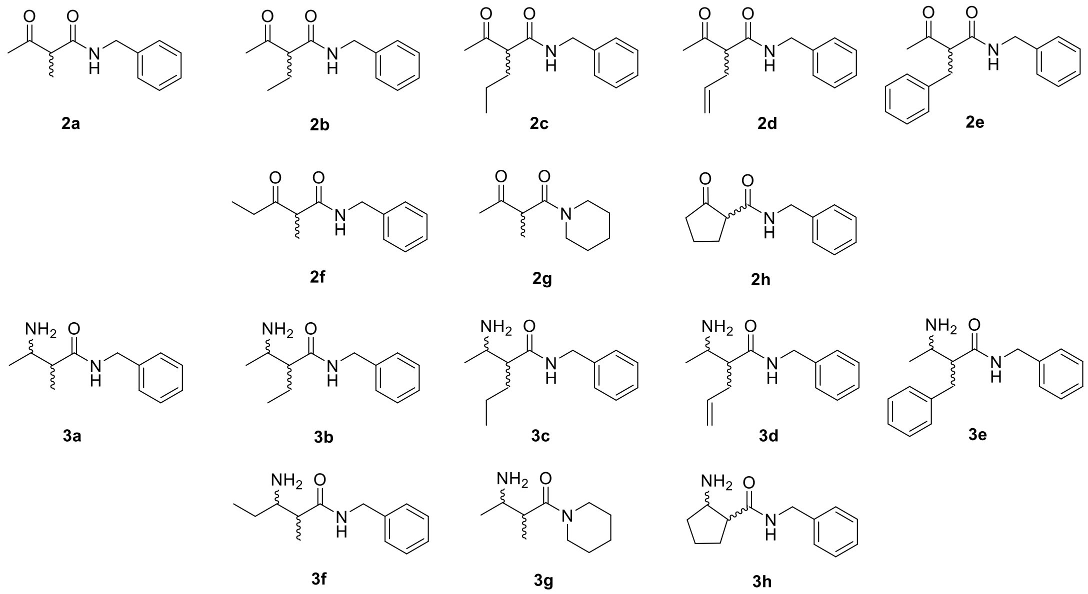
^{13}C RMN (75.5 MHz, $CDCl_3$): δ 21.7 (CH_2), 21.9 (CH_2), 26.7 (CH_2), 27.0 (CH_2), 29.7 (CH_2), 35.1 (CH_2), 37.1 (CH_2), 43.2 (CH_2), 50.4 (CH), 52.8 (CH), 54.7 (CH), 57.3 (CH), 127.2 (CH), 127.6 (2CH), 128.7 (2CH), 138.8 (C), 173.8 (C), 174.5 (C) ppm. HRMS (ESI^+, *m/z*): calcd for $(C_{13}H_{19}N_2O)^+$ $(M+H)^+$ 219.1419; found 219.1420.

3. Analytics

3.1. GC retention times

GC analyses were carried out with an Agilent 7890A GC-system and an achiral stationary phase Agilent HP-1 column (30 m x 0.25 mm, 0.25 μm) for conversion measurements.

Table S1 Analytical separation by GC for conversion measurements in the transamination of keto amides **2a-h**



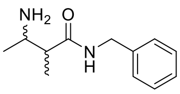
Compound	Temperature program[a]	Time (min)
(±)-**2a**	90/2/10/160/0/15/240/2	10.9
(±)-**2b**	90/2/10/160/0/15/240/2	11.5
(±)-**2c**	90/2/10/160/0/15/240/2	12.1
(±)-**2d**	90/2/10/160/0/15/240/2	12.0
(±)-**2e**	110/2/10/160/0/10/200/0/5/240/2	16.7
(±)-**2f**	90/2/10/160/0/15/240/2	11.9
(±)-**2g**	90/2/10/160/0/15/240/2	8.5
(±)-**2h**	90/2/10/160/0/15/240/2	13.0
(±)-**3a**	90/2/10/160/0/15/240/2	11.7, 11.8[b]
(±)-**3b**	90/2/10/160/0/15/240/2	12.4
(±)-**3c**	90/2/10/160/0/15/240/2	12.8, 12.9[b]
(±)-**3d**	90/2/10/160/0/15/240/2	12.9, 13.0[b]
(±)-**3e**	110/2/10/160/0/10/200/0/5/240/2	17.1, 17.3[b]
(±)-**3f**	90/2/10/160/0/15/240/2	12.7, 12.9[b]
(±)-**3g**	90/2/10/160/0/15/240/2	9.2, 9.4[b]
(±)-**3h**	90/2/10/160/0/15/240/2	13.9

[a] Initial temperature (ºC)/time (min)/slope (ºC/min)/final temperature (ºC)/time (min). [b] Both diastereoisomers can be (at least partially) separated.

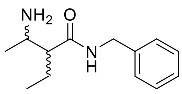
3.2. HPLC separation

High performance liquid chromatography (HPLC) analyses were carried out at 210 nm using a Chiralpak AD-H (25 cm × 4.6 mm), Chiralcel OJ-H (25 cm x 4.6 nm) or Chiralpak IC (25 cm × 4.6 mm).

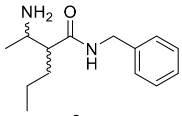
Table S2 Analytical separation by HPLC for derivatized β-amino amides (±)-**3a-h**



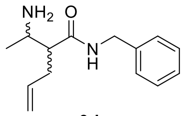
3a



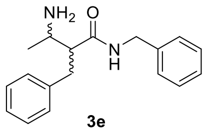
3b



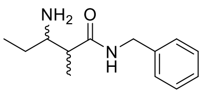
3c



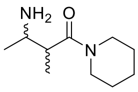
3d



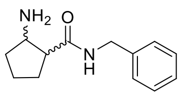
3e



3f



3g



3h

Compound[a]	**Column**	**Eluent**[b]	**Time (min)**	
			syn	***anti***
(±)-**3a**	AD-H	97:3	21.0 (2*R*,3*S*), 34.4 (2*S*,3*R*)	25.4 (2*R*,3*R*), 28.5 (2*S*,3*S*)
(±)-**3b**	OJ-H	97:3	9.1 (2*R*,3*S*), 13.3 (2*S*,3*R*)	16.4 (2*R*,3*R*), 26.1 (2*S*,3*S*)
(±)-**3c**	OJ-H	98:2	12.7 (2*R*,3*S*), 16.4 (2*S*,3*R*)	24.6 (2*R*,3*R*), 31.7 (2*S*,3*S*)
(±)-**3d**	OJ-H	97:3	9.9 (2*R*,3*S*), 15.0 (2*S*,3*R*)	20.0 (2*R*,3*R*), 27.6 (2*S*,3*S*)
(±)-**3e**	OJ-H	95:5	18.5 (2*S*,3*R*), 20.7 (2*R*,3*S*)	10.3 (2*R*,3*R*), 15.7 (2*S*,3*S*)
(±)-**3f**	IC	80:20	8.2 (2*R*,3*S*), 11.8 (2*S*,3*R*)	9.6 (2*S*,3*S*), 18.6 (2*R*,3*R*)
(±)-**3g**	OJ-H	95:5	9.8 (2*S*,3*R*), 11.4 (2*R*,3*S*)	12.2 (2*S*,3*S*), 13.1 (2*R*,3*R*)
(±)-**3h**	AD-H	95:5	10.2 (1*R*,2*S*), 16.1 (1*S*,2*R*)	21.0 (1*R*,2*R*), 28.9 (1*S*,2*S*)

[a] Amines **3a**-**f**,**h** were derivatized using acetic anhydride and potassium carbonate; amine **3g** was derivatized by the addition of benzoyl chloride and potassium carbonate. [b] Mixtures of *n*-hexane/2-propanol, and a flow of 0.8 mL/min were used in all cases; the temperature was set at 30 ºC for amines **3a**-**f**,**h** and room temperature for **3g**.

4. Screening of transaminases

*4.1. General procedure for the biotransamination of α-substituted β-keto amides **2a-h** using commercial transaminases*

In a 1.5 mL Eppendorf tube, transaminase (2 mg) and α-substituted β-keto amides (**2a-h**, 25 mM) were added in phosphate buffer 100 mM pH 7.5, 9.0 or 10.0 (final volume: 500 µL, 1 mM PLP, 1 M isopropylamine), and DMSO (12.5 µL, 2.5% v/v). The reaction was shaken at 10 or 30 °C and 250 rpm for 24 h and stopped by addition of an aqueous saturated solution of Na_2CO_3 (200 µL). Then the mixture was extracted with EtOAc (2 x 500 µL), the organic layers separated by centrifugation (2 min, 13000 rpm), combined and finally dried over Na_2SO_4. Conversions of α-substituted β-amino amides **3a-h** were determined by GC and *ee* were measured by HPLC.

*4.1. Biotransamination of model substrate N-benzyl-2-methyl-3-oxobutanamide (**2a**) using commercial transaminases*

Entry	Transaminase[a]	Conv. (%)[b]	Ratio *anti*:*syn*[c]	*ee anti* (%)[c]	*ee syn* (%)[c]
1	ATA-217 (*S*)	96	35:65	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)
2	ATA-234 (*S*)	98	46:54	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)
3	ATA-237 (*S*)	95	33:67	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)
4	ATA-238 (*S*)	76	37:63	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)
5	ATA-251 (*S*)	98	65:35	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)
6	ATA-254 (*S*)	89	85:15	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)
7	ATA-256 (*S*)	68	36:64	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)
8	ATA-260 (*S*)	96	31:69	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)
9	TA-P1-A06 (*S*)	89	59:41	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)
10	TA-P1-F03 (*S*)	84	44:56	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)
11	TA-P2-G06 (*S*)	97	66:34	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)
12	ATA-013 (*R*)	98	62:38	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
13	ATA-024 (*R*)	97	50:50	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
14	ATA-025 (*R*)	98	52:48	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
15	ATA-033 (*R*)	98	53:47	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
16	ATA-415 (*R*)	97	60:40	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)

[a] The selectivity of the transaminase appears in parentheses. [b] Conversion values measured by GC analysis. [c] Diastereomeric ratios and enantiomeric excess values were measured by chiral HPLC analysis. The major diastereoisomer is shown in parentheses.

*4.2. Biotransamination of N-benzyl-2-ethyl-3-oxobutanamide (**2b**) using commercial transaminases*

Entry	Transaminase[a]	Conv. (%)[b]	Ratio *anti*:*syn*[c]	*ee anti* (%)[c]	*ee syn* (%)[c]
1	ATA-234 (*S*)	64	97:3	95 (2*S*,3*S*)	n.d.
2	ATA-237 (*S*)	93	69:31	50 (2*S*,3*S*)	50 (2*R*,3*S*)
3	ATA-238 (*S*)	55	76:24	72 (2*S*,3*S*)	75 (2*R*,3*S*)
4	ATA-251 (*S*)	98	80:20	17 (2*S*,3*S*)	15 (2*R*,3*S*)
5	ATA-254 (*S*)	93	82:12	4 (2*S*,3*S*)	10 (2*R*,3*S*)
6	ATA-256 (*S*)	97	82:12	20 (2*S*,3*S*)	18 (2*R*,3*S*)
7	ATA-260 (*S*)	97	81:19	26 (2*S*,3*S*)	25 (2*R*,3*S*)
8	TA-P1-F03 (*S*)	85	72:28	31 (2*S*,3*S*)	30 (2*R*,3*S*)
9	ATA-013 (*R*)	96	80:20	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
10	ATA-024 (*R*)	97	78:22	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
11	ATA-025 (*R*)	98	78:22	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
12	ATA-033 (*R*)	97	78:22	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
13	ATA-415 (*R*)	96	80:20	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)

[a] The selectivity of the transaminase appears in parentheses. [b] Conversion values measured by GC analysis. [c] Diastereomeric ratios and enantiomeric excess values were measured by chiral HPLC analysis. The major diastereoisomer is shown in parentheses. n.d. not determined.

*4.3. Biotransamination of 2-acetyl-N-benzylpentanamide (**2c**) using commercial transaminases*

Entry	Transaminase[a]	Conv. (%)[b]	Ratio *anti*:*syn*[c]	*ee anti* (%)[c]	*ee syn* (%)[c]
1	ATA-234 (*S*)	97	10:90	45 (2*S*,3*S*)	42 (2*R*,3*S*)
2	ATA-237 (*S*)	95	14:86	45 (2*S*,3*S*)	40 (2*R*,3*S*)
3	ATA-251 (*S*)	97	21:79	12 (2*S*,3*S*)	11 (2*R*,3*S*)
4	ATA-254 (*S*)	96	22:88	10 (2*S*,3*S*)	1 (2*R*,3*S*)
5	ATA-256 (*S*)	68	15:85	45 (2*S*,3*S*)	44 (2*R*,3*S*)
6	ATA-260 (*S*)	98	15:85	10 (2*S*,3*S*)	7 (2*R*,3*S*)
7	TA-P1-F03 (*S*)	53	20:80	20 (2*S*,3*S*)	9 (2*R*,3*S*)
8	ATA-013 (*R*)	67	83:17	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
9	ATA-024 (*R*)	97	89:11	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
10	ATA-025 (*R*)	97	82:18	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
11	ATA-033 (*R*)	98	73:27	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
12	ATA-415 (*R*)	96	81:19	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)

[a] The selectivity of the transaminase appears in parentheses. [b] Conversion values measured by GC analysis. [c] Diastereomeric ratios and enantiomeric excess values were measured by chiral HPLC analysis. The major diastereoisomer is shown in parentheses.

*4.4. Biotransamination of 2-acetyl-N-benzylpent-4-enamide (**2d**) using commercial transaminases*

Entry	Transaminase[a]	Conv. (%)[b]	Ratio *anti*:*syn*[c]	*ee anti* (%)[c]	*ee syn* (%)[c]
1	ATA-234 (*S*)	96	40:60	45 (2*S*,3*S*)	50 (2*R*,3*S*)
2	ATA-013 (*R*)	97	74:26	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
3	ATA-024 (*R*)	97	70:30	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
4	ATA-025 (*R*)	97	70:30	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
5	ATA-033 (*R*)	97	65:35	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
6	ATA-415 (*R*)	95	60:40	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)

[a] The selectivity of the transaminase appears in parentheses. [b] Conversion values measured by GC analysis. [c] Diastereomeric ratios and enantiomeric excess values were measured by chiral HPLC analysis. The major diastereoisomer is shown in parentheses.

*4.5. Biotransamination of N,2-dibenzyl-3-oxobutanamide (**2e**) using commercial transaminases*

Entry	Transaminase[a]	Conv. (%)[b]	Ratio *anti*:*syn*[c]	*ee anti* (%)[c]	*ee syn* (%)[c]
1	ATA-234 (*S*)	29	10:90	42 (2*S*,3*S*)	44 (2*R*,3*S*)
2	ATA-013 (*R*)	90	22:78	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
3	ATA-024 (*R*)	88	11:89	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
4	ATA-025 (*R*)	99	31:69	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
5	ATA-033 (*R*)	99	28:72	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
6	ATA-415 (*R*)	80	19:81	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)

[a] The selectivity of the transaminase appears in parentheses. [b] Conversion values measured by GC analysis. [c] Diastereomeric ratios and enantiomeric excess values were measured by chiral HPLC analysis. The major diastereoisomer is shown in parentheses.

*4.6. Biotransamination of N-benzyl-2-methyl-3-oxopentanamide (**2f**) using commercial transaminases*

Entry	Transaminase[a]	Conv. (%)[b]	Ratio *anti*:*syn*[c]	*ee anti* (%)[c]	*ee syn* (%)[c]
1	ATA-234 (*S*)	15	99:1	>99 (2*S*,3*S*)	n.d.
2	ATA-013 (*R*)	38	82:18	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
3	ATA-024 (*R*)	84	79:21	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
4	ATA-025 (*R*)	85	78:22	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
5	ATA-033 (*R*)	89	72:28	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
6	ATA-415 (*R*)	54	75:25	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)

[a] The selectivity of the transaminase appears in parentheses. [b] Conversion values measured by GC analysis. [c] Diastereomeric ratios and enantiomeric excess values were measured by chiral HPLC analysis. The major diastereoisomer is shown in parentheses.

*4.7. Biotransamination of 2-methyl-1-(piperidin-1-yl)butane-1,3-dione (**2g**) using commercial transaminases*

Entry	Transaminase[a]	Conv. (%)[b]	Ratio *anti*:*syn*[c]	*ee anti* (%)[c]	*ee syn* (%)[c]
1	ATA-217 (*S*)	47	14:86	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)
2	ATA-234 (*S*)	66	10:90	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)
3	ATA-237 (*S*)	71	33:67	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)
4	ATA-251 (*S*)	84	32:68	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)
5	ATA-256 (*S*)	85	2:98	n.d.	>99 (2*R*,3*S*)
6	ATA-260 (*S*)	77	14:86	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)
7	ATA-013 (*R*)	74	62:38	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
8	ATA-024 (*R*)	92	65:35	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
9	ATA-025 (*R*)	95	64:36	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
10	ATA-033 (*R*)	92	66:34	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
11	ATA-412 (*R*)	62	36:64	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
12	ATA-415 (*R*)	82	63:37	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)

[a] The selectivity of the transaminase appears in parentheses. [b] Conversion values measured by GC analysis. [c] Diastereomeric ratios and enantiomeric excess values were measured by chiral HPLC analysis. The major diastereoisomer is shown in parentheses. n.d. not determined.

*4.8. Biotransamination of N-benzyl-2-oxocyclopentane-1-carboxamide (**2h**) using commercial transaminases*

Entry	Transaminase[a]	Conv. (%)[b]	Ratio *anti*:*syn*[c]	*ee trans* (%)[c]	*ee cis* (%)[c]
1	ATA-013 (*R*)	91	82:18	>99 (1*R*,2*R*)	>99 (1*S*,2*R*)
2	ATA-024 (*R*)	92	86:14	>99 (1*R*,2*R*)	>99 (1*S*,2*R*)
3	ATA-025 (*R*)	89	80:20	>99 (1*R*,2*R*)	>99 (1*S*,2*R*)
4	ATA-033 (*R*)	93	83:17	>99 (1*R*,2*R*)	>99 (1*S*,2*R*)
5	ATA-415 (*R*)	82	90:10	>99 (1*R*,2*R*)	>99 (1*S*,2*R*)

[a] The selectivity of the transaminase appears in parentheses. [b] Conversion values measured by GC analysis. [c] Diastereomeric ratios and enantiomeric excess values were measured by chiral HPLC analysis. The major diastereoisomer is shown in parentheses.

5. Influence of temperature and amount of transaminase in the biotransamination of the model substrate 2a

Entry	Transaminase	T (°C)	TA (mg)	Conv. (%)[a]	Ratio *anti*:*syn*[b]	*ee anti* (%)[b]	*ee syn* (%)[b]
1	ATA-251 (*S*)	30	2	98	62:38	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)
2	ATA-251 (*S*)	30	1	94	65:35	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)
3	ATA-251 (*S*)	10	2	65	63:37	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)
4	ATA-013 (*R*)	30	2	98	65:35	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
5	ATA-013 (*R*)	30	1	74	70:30	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
6	ATA-013 (*R*)	10	2	11	n.d.	n.d.	n.d.

[a] Conversion values measured by GC analysis. [b] Diastereomeric ratios and enantiomeric excess values were measured by chiral HPLC analysis. The major diastereoisomer is shown in parentheses. n.d. not determined.

6. Biotransamination of substrates 2a-h at pH 10.0 using (*R*)-selective commercial transaminases

*6.1. Biotransamination of model substrate N-benzyl-2-methyl-3-oxobutanamide (**2a**)*

Entry	Transaminase	Conv. (%)[a]	Ratio *anti*:*syn*[b]	*ee anti* (%)[b]	*ee syn* (%)[b]
1	ATA-013 (*R*)	>99	80:20	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
2	ATA-024 (*R*)	>99	80:20	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
3	ATA-025 (*R*)	>99	80:20	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
4	ATA-033 (*R*)	>99	80:20	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
5	ATA-415 (*R*)	>99	70:30	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)

[a] Conversion values measured by GC analysis. [b] Diastereomeric ratios and enantiomeric excess values were measured by chiral HPLC analysis. The major diastereoisomer is shown in parentheses.

*6.2. Biotransamination of N-benzyl-2-ethyl-3-oxobutanamide (**2b**)*

Entry	Transaminase	Conv. (%)[a]	Ratio *anti*:*syn*[b]	*ee anti* (%)[b]	*ee syn* (%)[b]
1	ATA-013 (*R*)	>99	47:53	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
2	ATA-024 (*R*)	>99	45:55	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
3	ATA-025 (*R*)	98	78:22	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
4	ATA-033 (*R*)	>99	50:50	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
5	ATA-415 (*R*)	98	80:20	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)

[a] Conversion values measured by GC analysis. [b] Diastereomeric ratios and enantiomeric excess values were measured by chiral HPLC analysis. The major diastereoisomer is shown in parentheses.

*6.3. Biotransamination of 2-acetyl-N-benzylpentanamide (**2c**)*

Entry	Transaminase	Conv. (%)[a]	Ratio *anti*:*syn*[b]	*ee anti* (%)[b]	*ee syn* (%)[b]
1	ATA-013 (*R*)	>99	87:13	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
2	ATA-024 (*R*)	>99	88:12	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
3	ATA-025 (*R*)	>99	85:15	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
4	ATA-033 (*R*)	>99	85:15	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
5	ATA-415 (*R*)	>99	87:13	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)

[a] Conversion values measured by GC analysis. [b] Diastereomeric ratios and enantiomeric excess values were measured by chiral HPLC analysis. The major diastereoisomer is shown in parentheses.

*6.4. Biotransamination of 2-acetyl-N-benzylpent-4-enamide (**2d**)*

Entry	Transaminase	Conv. (%)[a]	Ratio *anti*:*syn*[b]	*ee anti* (%)[b]	*ee syn* (%)[b]
1	ATA-013 (*R*)	>99	80:20	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
2	ATA-024 (*R*)	>99	73:26	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
3	ATA-025 (*R*)	>99	75:25	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
4	ATA-033 (*R*)	>99	78:22	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
5	ATA-415 (*R*)	>99	80:20	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)

[a] Conversion values measured by GC analysis. [b] Diastereomeric ratios and enantiomeric excess values were measured by chiral HPLC analysis. The major diastereoisomer is shown in parentheses.

*6.5. Biotransamination of N,2-dibenzyl-3-oxobutanamide (**2e**)*

Entry	Transaminase	Conv. (%)[a]	Ratio *anti*:*syn*[b]	*ee anti* (%)[b]	*ee syn* (%)[b]
1	ATA-013 (*R*)	>99	30:70	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
2	ATA-024 (*R*)	>99	27:73	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
3	ATA-025 (*R*)	99	25:75	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
4	ATA-033 (*R*)	99	13:87	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
5	ATA-415 (*R*)	95	20:80	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)

[a] Conversion values measured by GC analysis. [b] Diastereomeric ratios and enantiomeric excess values were measured by chiral HPLC analysis. The major diastereoisomer is shown in parentheses.

*6.6. Biotransamination of N-benzyl-2-methyl-3-oxopentanamide (**2f**)*

Entry	Transaminase	Conv. (%)[a]	Ratio *anti*:*syn*[b]	*ee anti* (%)[b]	*ee syn* (%)[b]
1	ATA-013 (*R*)	22	80:20	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
2	ATA-024 (*R*)	97	72:28	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
3	ATA-025 (*R*)	91	73:27	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
4	ATA-033 (*R*)	94	74:26	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
5	ATA-415 (*R*)	50	74:26	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)

[a] Conversion values measured by GC analysis. [b] Diastereomeric ratios and enantiomeric excess values were measured by chiral HPLC analysis. The major diastereoisomer is shown in parentheses.

*6.7. Biotransamination of 2-methyl-1-(piperidin-1-yl)butane-1,3-dione (**2g**)*

Entry	Transaminase	Conv. (%)[a]	Ratio *anti*:*syn*[b]	*ee anti* (%)[b]	*ee syn* (%)[b]
1	ATA-013 (*R*)	70	88:12	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
2	ATA-024 (*R*)	>99	92:8	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
3	ATA-025 (*R*)	>99	91:9	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
4	ATA-033 (*R*)	>99	91:9	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)
5	ATA-415 (*R*)	98	89:11	>99 (2*R*,3*R*)	>99 (2*S*,3*R*)

[a] Conversion values measured by GC analysis. [b] Diastereomeric ratios and enantiomeric excess values were measured by chiral HPLC analysis. The major diastereoisomer is shown in parentheses.

*6.8. Biotransamination of N-benzyl-2-oxocyclopentane-1-carboxamide (**2h**)*

Entry	Transaminase	Conv. (%)[a]	Ratio *anti*:*syn*[b]	*ee trans* (%)[b]	*ee cis* (%)[b]
1	ATA-013 (*R*)	65[c]	n.d.	n.d.	n.d.
2	ATA-024 (*R*)	>99	83:17	>99 (1*R*,2*R*)	>99 (1*S*,2*R*)
3	ATA-025 (*R*)	97	75:25	>99 (1*R*,2*R*)	>99 (1*S*,2*R*)
4	ATA-033 (*R*)	98	82:19	>99 (1*R*,2*R*)	>99 (1*S*,2*R*)
5	ATA-415 (*R*)	97	92:8	>99 (1*R*,2*R*)	>99 (1*S*,2*R*)

[a] Conversion values measured by GC analysis. [b] Diastereomeric ratios and enantiomeric excess values were measured by chiral HPLC analysis. The major diastereoisomer is shown in parentheses. [c] The enamine formed by the addition of one molecule of isopropylamine to one molecule of the substrate was detected. n.d. not determined.

7. *Bm*TA and *Bm*TA S119G-catalyzed biotransaminations

7.1. Protein expression protocols

The transaminase gene from *Bacillus megaterium*[2c] was cloned in a pET21a expression vector containing a C-terminal His_6 tag and the variant *Bacillus megaterium* S119G[2d] were transformed into *E. coli* BL21 (DE3) cells. A single colony was selected and grown overnight at 37 ºC and 220 rpm. The starter culture was used to inoculate 600 mL of LB medium supplemented with 50 µL/mg ampicillin in 2-L Erlenmeyer flasks. The cultures were incubated at a rotary shaking rate of 220 rpm at 37 ºC. The recombinant protein expression was induced by adding IPTG (0.2 mM, final) when A_{600} reached 0.6-0.8. The cell cultures were incubated at 18 ºC for 16 h. The cells were then harvested by centrifugation.

7.2. Protein purification

For purification purposes, cell pellets were resuspended (1 g of wet cell paste/10 mL) in HEPES buffer 100 mM pH 8.0 containing PLP (1 mM) and imidazole (5 mM). The cell pellets were lysed in an iced bath by ultrasonication (20 cycles of 20s on/20s off). After centrifugation (4 ºC, 16.000 x *g*, 20 min) the supernatant was loaded into a 5 mL HisTrap column containing Ni-NTA agarose resin. The column was initially washed with HEPES buffer 100 mM pH 8.0 containing PLP (1 mM) and imidazole (30 mM). Afterwards, the protein was eluted and collected with HEPES 100 mM buffer pH 8.0 containing PLP (1 mM) and imidazole (300 mM). The protein solutions were concentrated using a 20-mL 10K MWCO Vivaspin® centrifugal ultrafiltration unit. The concentration of the purified protein was determined using the Bradford assay using bovine serum albumin (BSA) as the protein standard.

7.3. General procedure for the biotransamination of α-substituted β-keto amides ***2a-h*** *using transaminases from Bacillus megaterium and from Bacillus megaterium S119G*

In a 1.5 mL Eppendorf tube, transaminase (final concentration: 2 mg/mL) and α-substituted β-keto amides (**2a-h**, 5 mM) were added in HEPES buffer 50 mM pH 9.0 (500 µL, 1 mM PLP, 250 mM isopropylamine), and DMSO (5.0 µL). The reaction was shaken at 37 °C and 250 rpm for 24 h and stopped by addition of an aqueous saturated solution of Na_2CO_3 (200 µL). Then the mixture was extracted with EtOAc (2 x 500 µL), the organic layers separated by centrifugation (2 min, 13000 rpm), combined and finally dried over Na_2SO_4. Conversions of α-substituted β-amino amides **3a-h** were determined by GC and *ee* and *dr* were measured by HPLC.

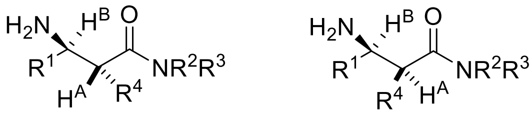
7.4. Effect of the substrate concentration in the transaminations catalyzed by Bm and Bm S119G

Entry	Transaminase	[**2a**] (mM)	Conv. (%)[a]	Ratio *anti*:*syn*[b]	*ee anti* (%)[b]	*ee syn* (%)[b]
1	*Bm*	5	74	51:49	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)
2	*Bm*	10	65	51:49	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)
3	*Bm*	15	65	51:49	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)
4	*Bm*	20	64	52:48	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)
5	*Bm*	25	64	51:49	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)
6	*Bm* S119G	5	70	30:70	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)
7	*Bm* S119G	10	44	30:70	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)
8	*Bm* S119G	15	45	30:70	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)
9	*Bm* S119G	20	43	30:70	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)
10	*Bm* S119G	25	43	30:70	>99 (2*S*,3*S*)	>99 (2*R*,3*S*)

[a] Conversion values measured by GC analysis. [b] Diastereomeric ratios and enantiomeric excess values were measured by chiral HPLC analysis. The major diastereoisomer is shown in parentheses.

8. Relative configuration assignment

NMR homonuclear decoupling experiments were performed with the obtained racemic α-alkyl-β-amino amides in order to obtain the coupling constants between H^A (proton at carbonyl α position) and H^B (proton at α position of the amine moiety). For this, the other hydrogens coupled to H^A (in R^4) or to H^B (in R^1) were irradiated, so $^3J_{HAHB}$ could be obtained, as shown in the following table:



	3a	**3b**	**3c**	**3d**	**3e**	**3f**	**3g**	**3h**
J_{anti} (Hz)	5.6	5.6	5.5	5.3	5.7	5.6	8.0	5.9
J_{syn} (Hz)	3.9	4.8	4.7	4.2	4.8	3.4	5.5	3.6

As can be seen, J_{syn} was always clearly lower than J_{anti}. This is in accordance with previous observations in α-substituted β-substituted carbonylic compounds[3] and also as observed by Gotor and co-workers when they obtained similar α-alkyl-β-amino esters.[4]

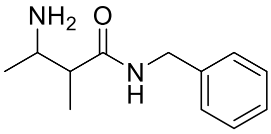
Comparing all these facts with the NMR obtained from the enantioenriched amines derived from our transaminations, we assigned the relative configuration as *anti* for the major diastereoisomer obtained, except for **3e**, where the *syn* isomer was usually mainly achieved.

The absolute configuration was based on the known stereospecificity of these stereocomplementary TAs.[4]

[3] (*a*) J. Limanto, S. W. Krska, B. T. Dorner, E. Vazquez, N. Yoshikawa and L. Tan, *Org. Lett.*, 2010, **12**, 512; (*b*) A. Cuetos, A. Rioz-Martínez, F. R. Bisogno, B. Grischek, I. Lavandera, G. de Gonzalo, W. Kroutil and V. Gotor, *Adv. Synth. Catal.*, 2012, **354**, 1743.
[4] A. Cuetos, I. Lavandera and V. Gotor, *Chem. Commun.*, 2013, **49**, 10688.

9. HPLC chromatograms of optically active β-amino amides

3-Amino-*N*-benzyl-2-methylbutanamide (3a)



Analytical data for acetylated β-amino amide 3a

Column: Chiralpak AD-H

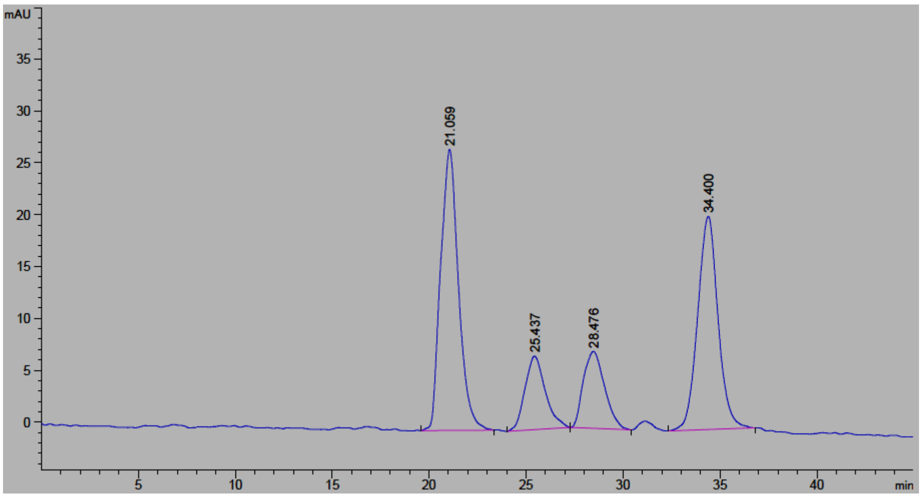
Eluent: *n*-hexane/2-propanol 97:3

Flow: 0.8 mL/min

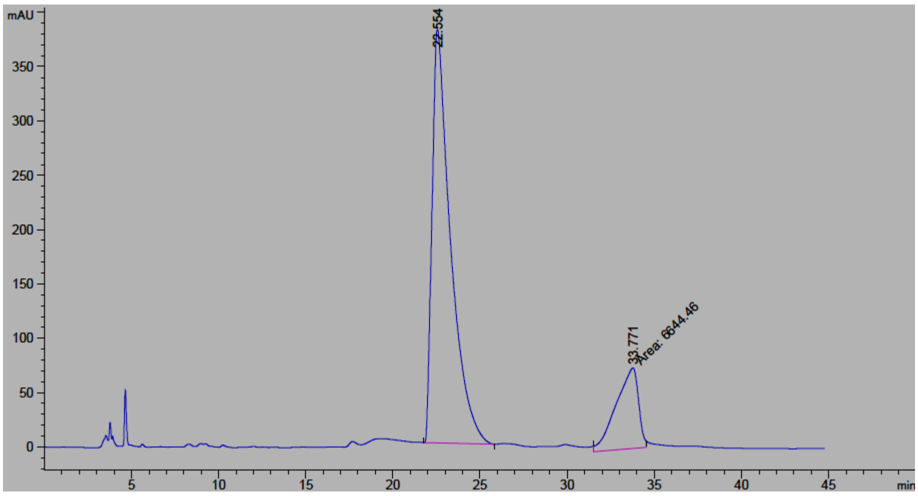
Temperature: 30 °C

Retention times: 21.0 (2*R*,3*S*), 25.4 (2*R*,3*R*), 28.5 (2*S*,3*S*), 34.4 (2*S*,3*R*)

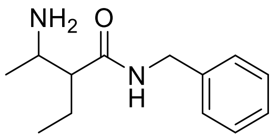
HPLC of (±)-3a in *anti*:*syn* 30:70 obtained by chemical reduction of 2a



HPLC of (2*R*,3*R*)-3a in *anti*:*syn* 80:20 obtained by biotransamination of 2a using ATA-025



3-Amino-*N*-benzyl-2-ethylbutanamide (3b)



Analytical data for acetylated β-amino amide 3b

Column: Chiralpak OJ-H

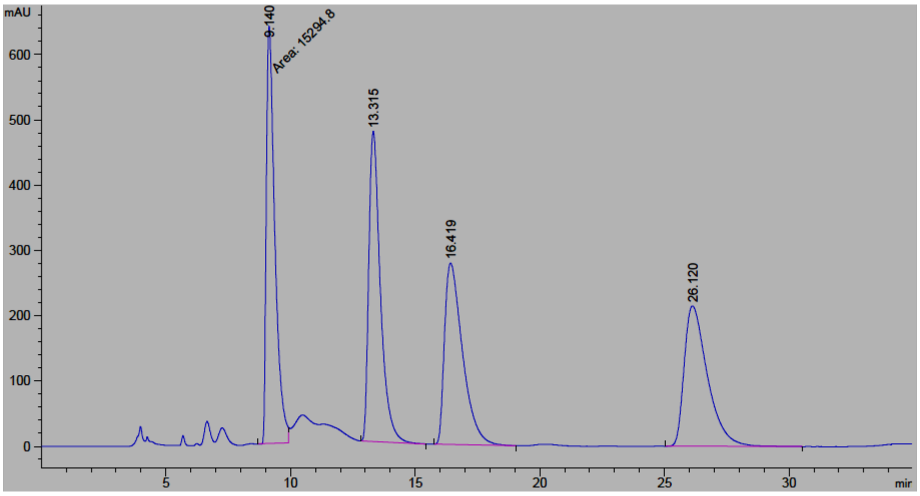
Eluent: *n*-hexane/2-propanol 97:3

Flow: 0.8 mL/min

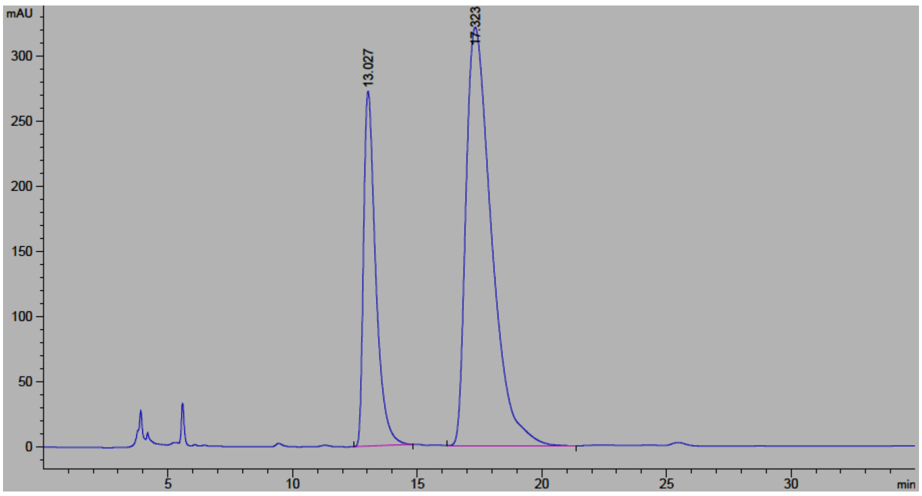
Temperature: 30 °C

Retention times: 9.1 (2*R*,3*S*), 13.3 (2*S*,3*R*), 16.4 (2*R*,3*R*), 26.1 (2*S*,3*S*)

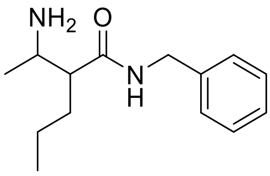
HPLC of (±)-3b in *anti*:*syn* 30:70 obtained by chemical reduction of 2b



HPLC of (2*R*,3*R*)-3b in *anti*:*syn* 76:24 obtained by biotransamination of 2b using ATA-025



2-(1-Aminoethyl)-*N*-benzylpentanamide (3c)



Analytical data for acetylated β-amino amide 3c

Column: Chiralpak OJ-H

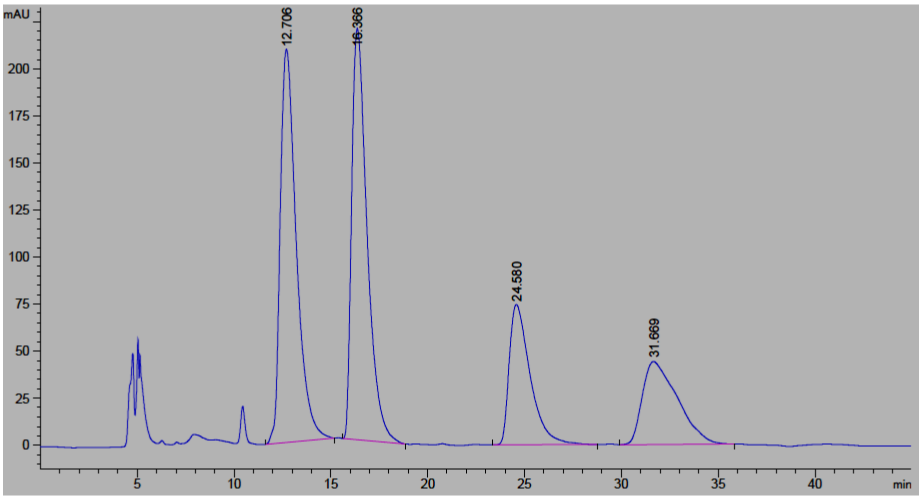
Eluent: *n*-hexane/2-propanol 98:2

Flow: 0.8 mL/min

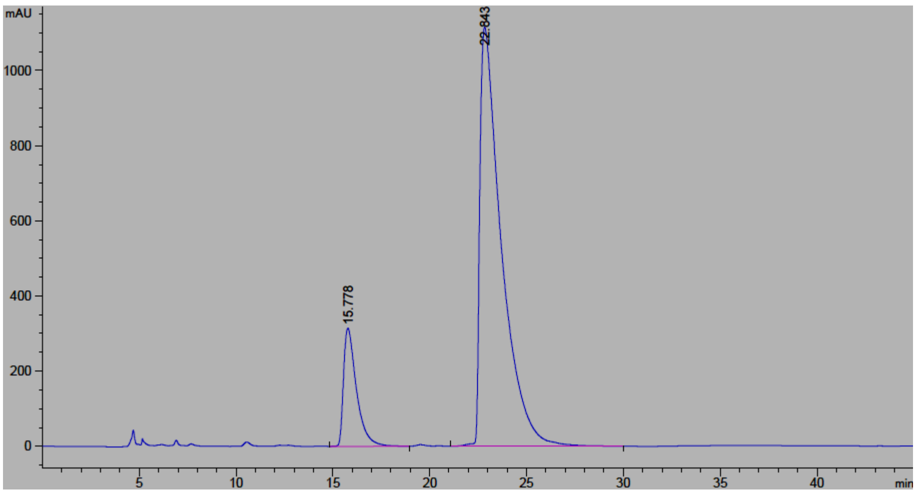
Temperature: 30 °C

Retention times: 12.7 (2*R*,3*S*), 16.4 (2*S*,3*R*), 24.6 (2*R*,3*R*), 31.7 (2*S*,3*S*)

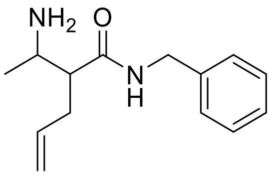
HPLC of (±)-3c in *anti*:*syn* 30:70 obtained by chemical reduction of 2c



HPLC of (2*R*,3*R*)-3c in *anti*:*syn* 85:15 obtained by biotransamination of 2c using ATA-025



2-(1-Aminoethyl)-*N*-benzylpent-4-enamide (3d)



Analytical data for acetylated β-amino amide 3d

Column: Chiralpak OJ-H

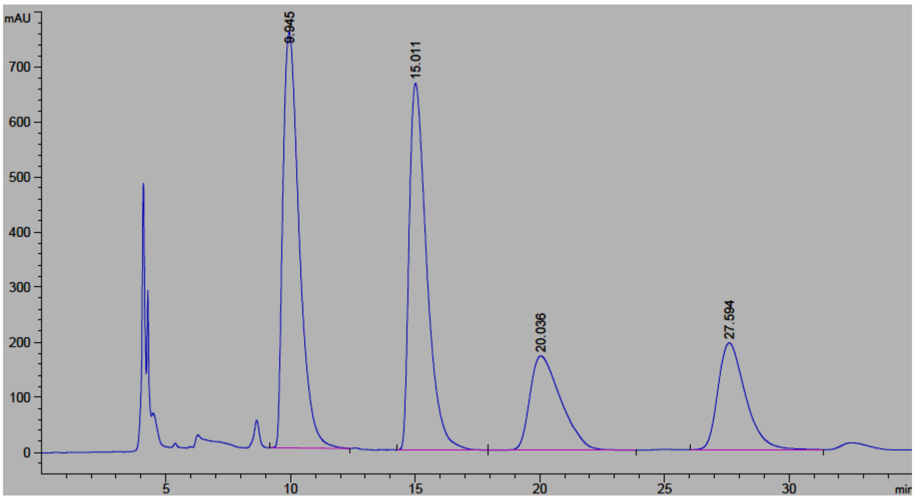
Eluent: *n*-hexane/2-propanol 97:3

Flow: 0.8 mL/min

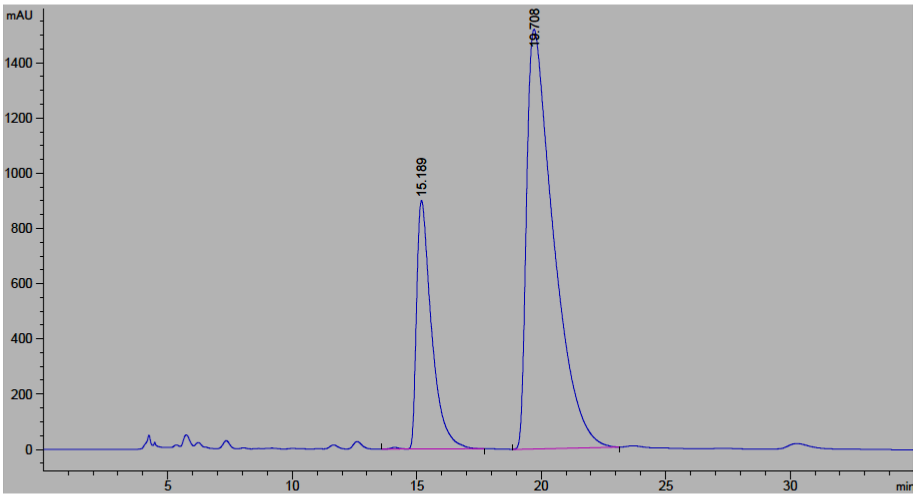
Temperature: 30 °C

Retention times: 9.9 (2*R*,3*S*), 15.0 (2*S*,3*R*), 20.0 (2*R*,3*R*), 27.6 (2*S*,3*S*)

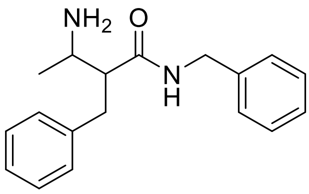
HPLC of (±)-3d in *anti*:*syn* 30:70 obtained by chemical reduction of 2d



HPLC of (2*R*,3*R*)-3d in *anti*:*syn* 75:25 obtained by biotransamination of 2d using ATA-025



3-Amino-*N*,2-dibenzylbutanamide (3e)



Analytical data for acetylated β-amino amide 3e

Column: Chiralpak OJ-H

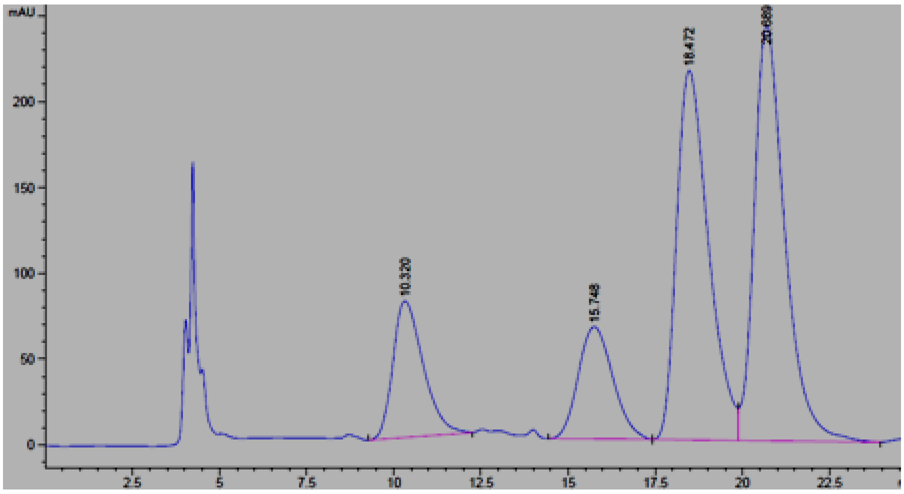
Eluent: *n*-hexane/2-propanol 95:5

Flow: 0.8 mL/min

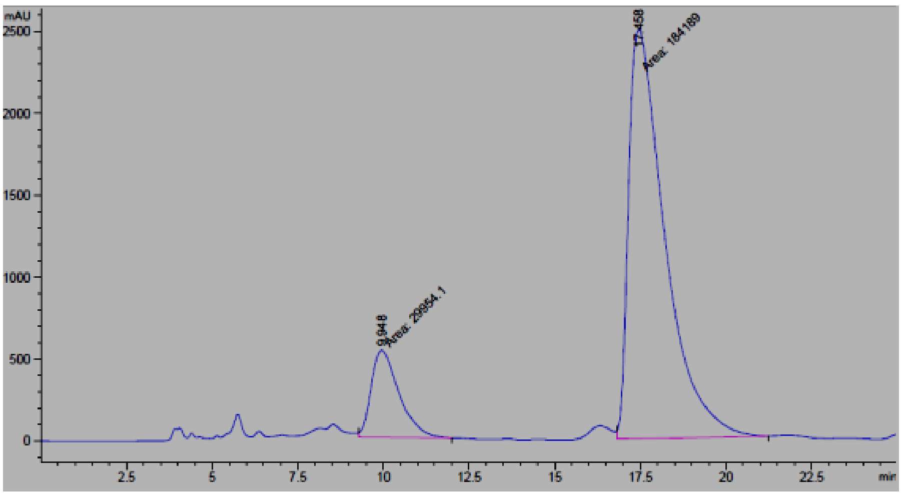
Temperature: 30 °C

Retention times: 10.3 (2*R*,3*R*), 15.7 (2*S*,3*S*), 18.5 (2*S*,3*R*), 20.7 (2*R*,3*S*)

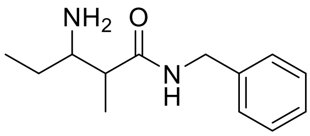
HPLC of (±)-3e in *anti*:*syn* 30:70 obtained by chemical reduction of 2e



HPLC of (2*R*,3*R*)-3e in *anti*:*syn* 15:85 obtained by biotransamination of 2e using ATA-025



3-Amino-*N*-benzyl-2-methylpentanamide (3f)



Analytical data for acetylated β-amino amide 3f

Column: Chiralpak IC

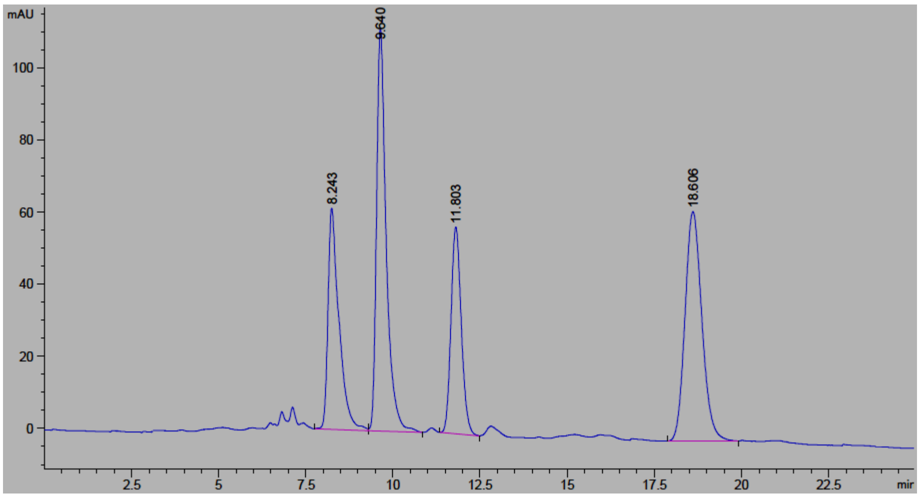
Eluent: *n*-hexane/2-propanol 80:20

Flow: 0.8 mL/min

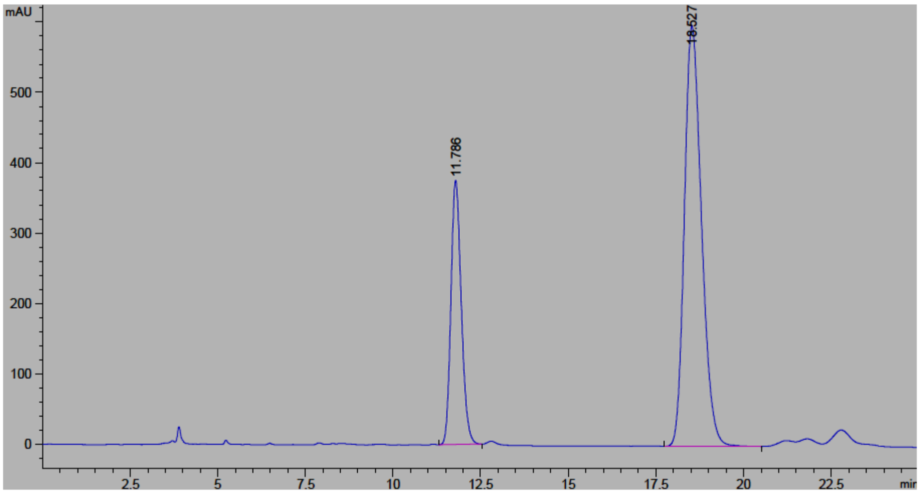
Temperature: 30 °C

Retention times: 8.2 (2*R*,3*S*), 9.6 (2*S*,3*S*), 11.8 (2*S*,3*R*), 18.6 (2*R*,3*R*)

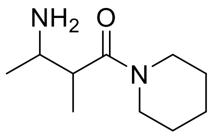
HPLC of (±)-3f in *anti*:*syn* 70:30 obtained by chemical reduction of 2f



HPLC of (2*R*,3*R*)-3f in *anti*:*syn* 73:27 obtained by biotransamination of 2f using ATA-025



3-Amino-2-methyl-1-(piperidin-1-yl)butan-1-one (3g)



Analytical data for benzoylated β-amino amide 3g

Column: Chiralpak OJ-H

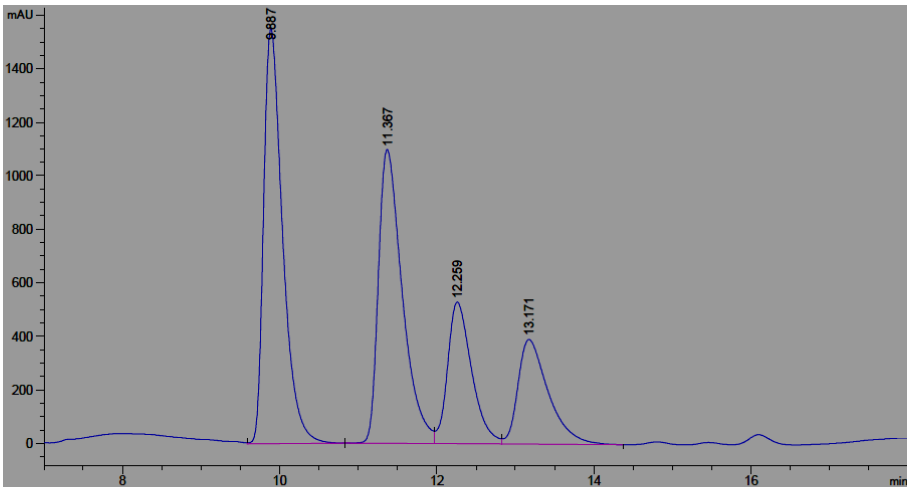
Eluent: *n*-hexane/2-propanol 95:5

Flow: 0.8 mL/min

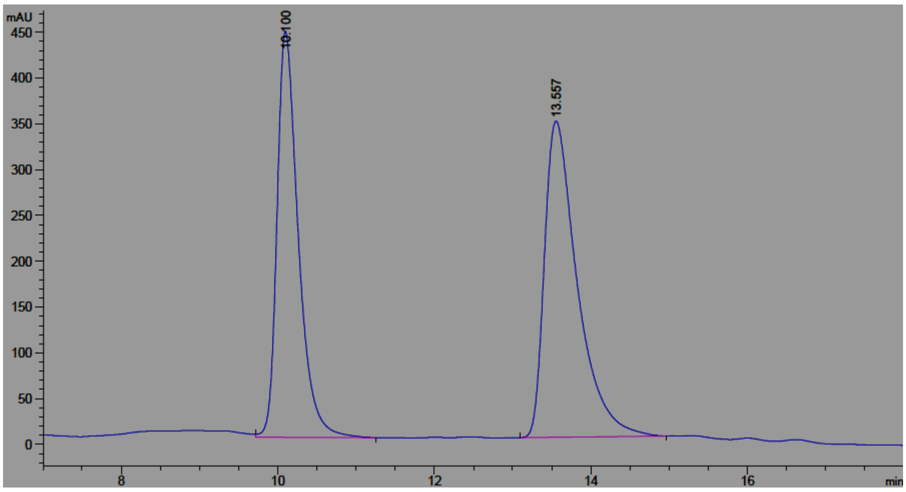
Temperature: room temperature

Retention times: 9.8 (2*S*,3*R*), 11.4 (2*R*,3*S*), 12.2 (2*S*,3*S*), 13.1 (2*R*,3*R*)

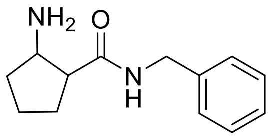
HPLC of (±)-3g in *anti*:*syn* 40:60 obtained by chemical reduction of 2g



HPLC of (2*R*,3*R*)-3g in *anti*:*syn* 68:32 obtained by biotransamination of 2g using ATA-025



2-Amino-*N*-benzylcyclopentane-1-carboxamide (3h)



Analytical data for acetylated β-amino amide 3h

Column: Chiralpak AD-H

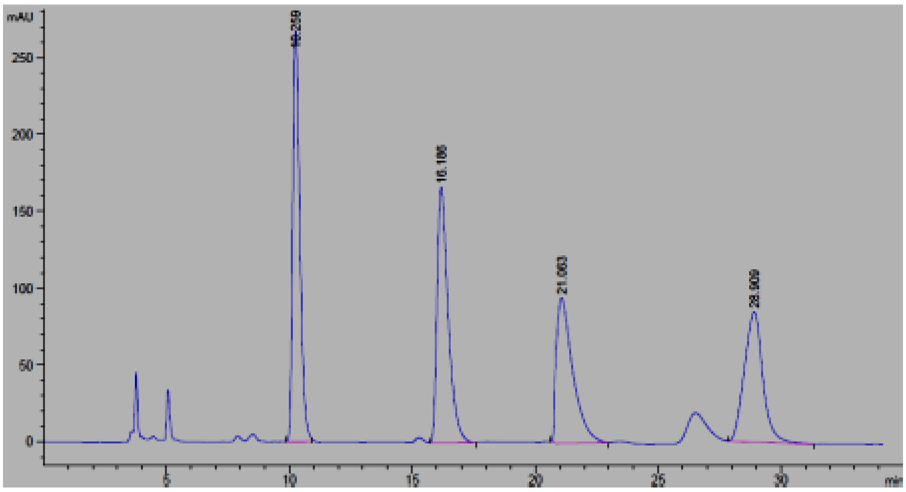
Eluent: *n*-hexane/2-propanol 95:5

Flow: 0.8 mL/min

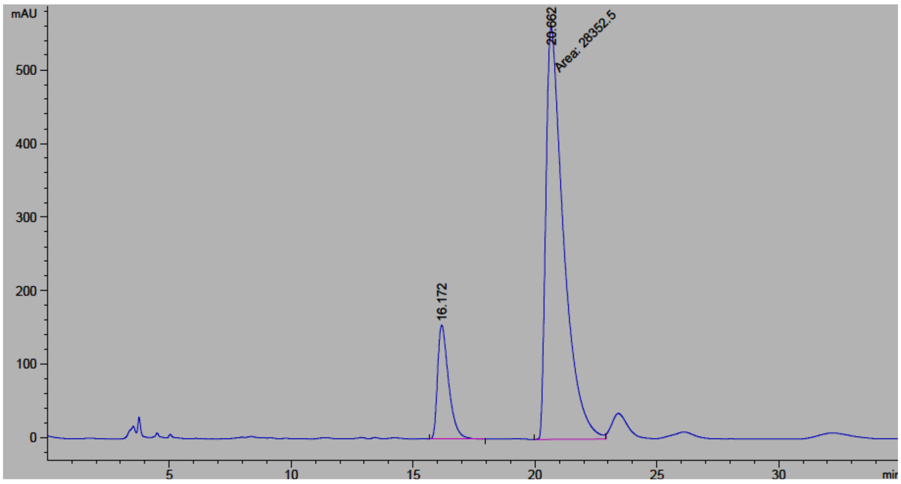
Temperature: 30 °C

Retention times: 10.2 (1*R*,2*S*), 16.1 (1*S*,2*R*), 21.0 (1*R*,2*R*), 28.9 (1*S*,2*S*)

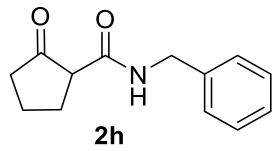
HPLC of (±)-3h in *trans*:*cis* 40:60 obtained by chemical reduction of 2h

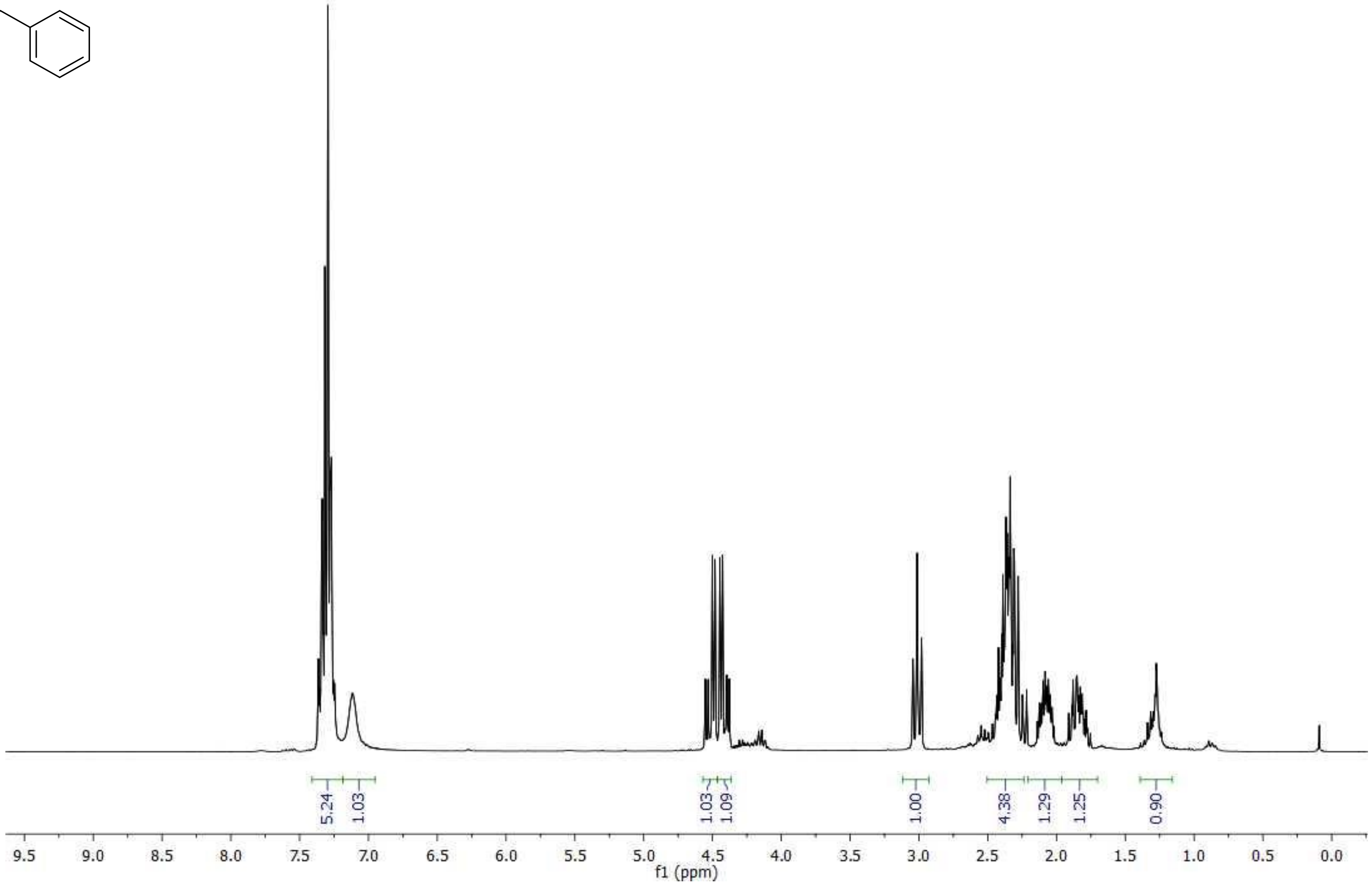


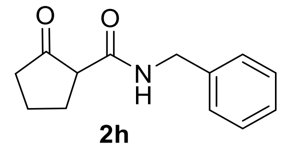
HPLC of (1*R*,2*R*)-3h in *trans*:*cis* 85:15 obtained by biotransamination of 2h using ATA-025



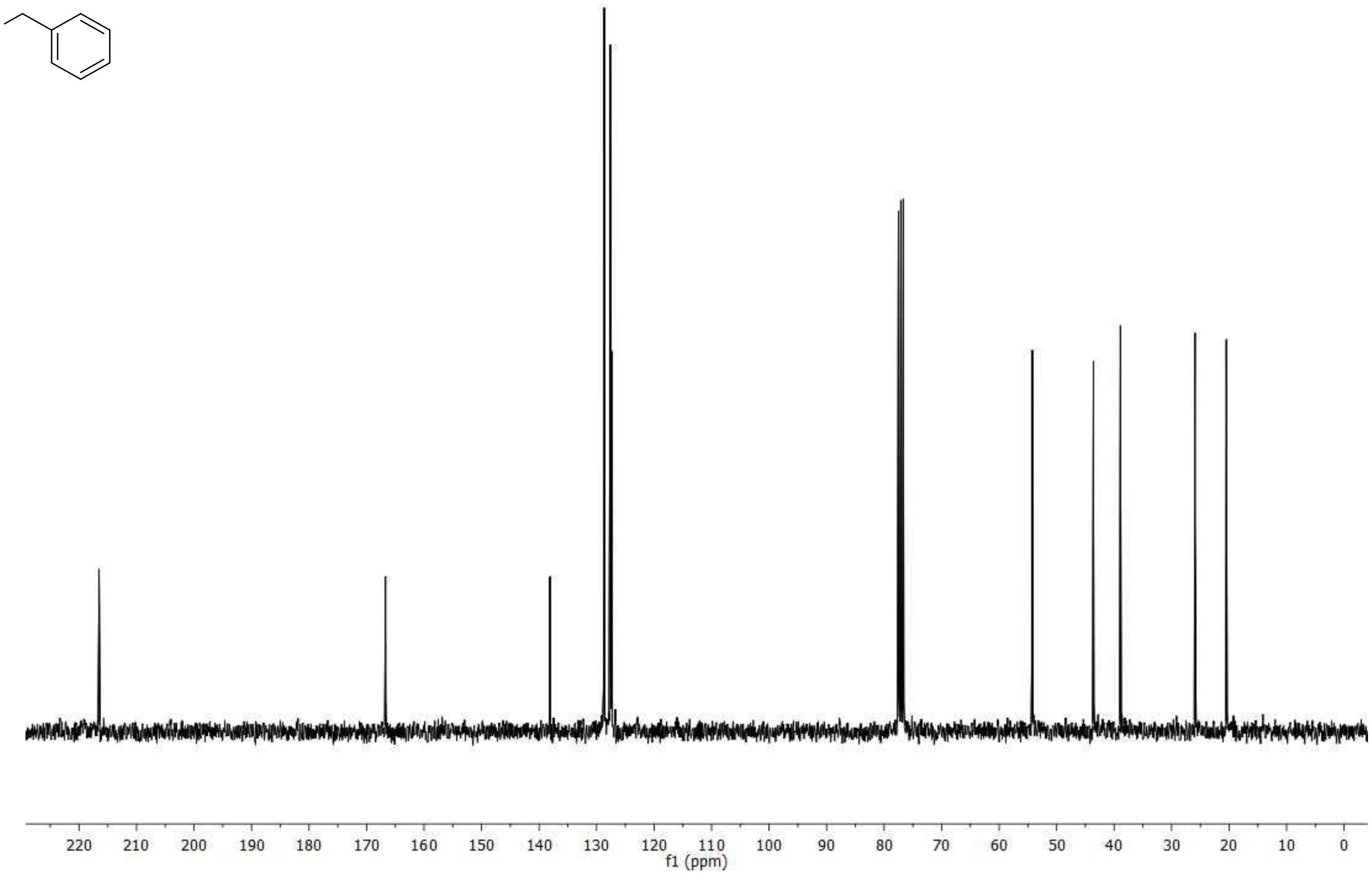
10. NMR spectra

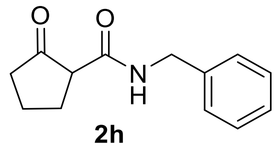




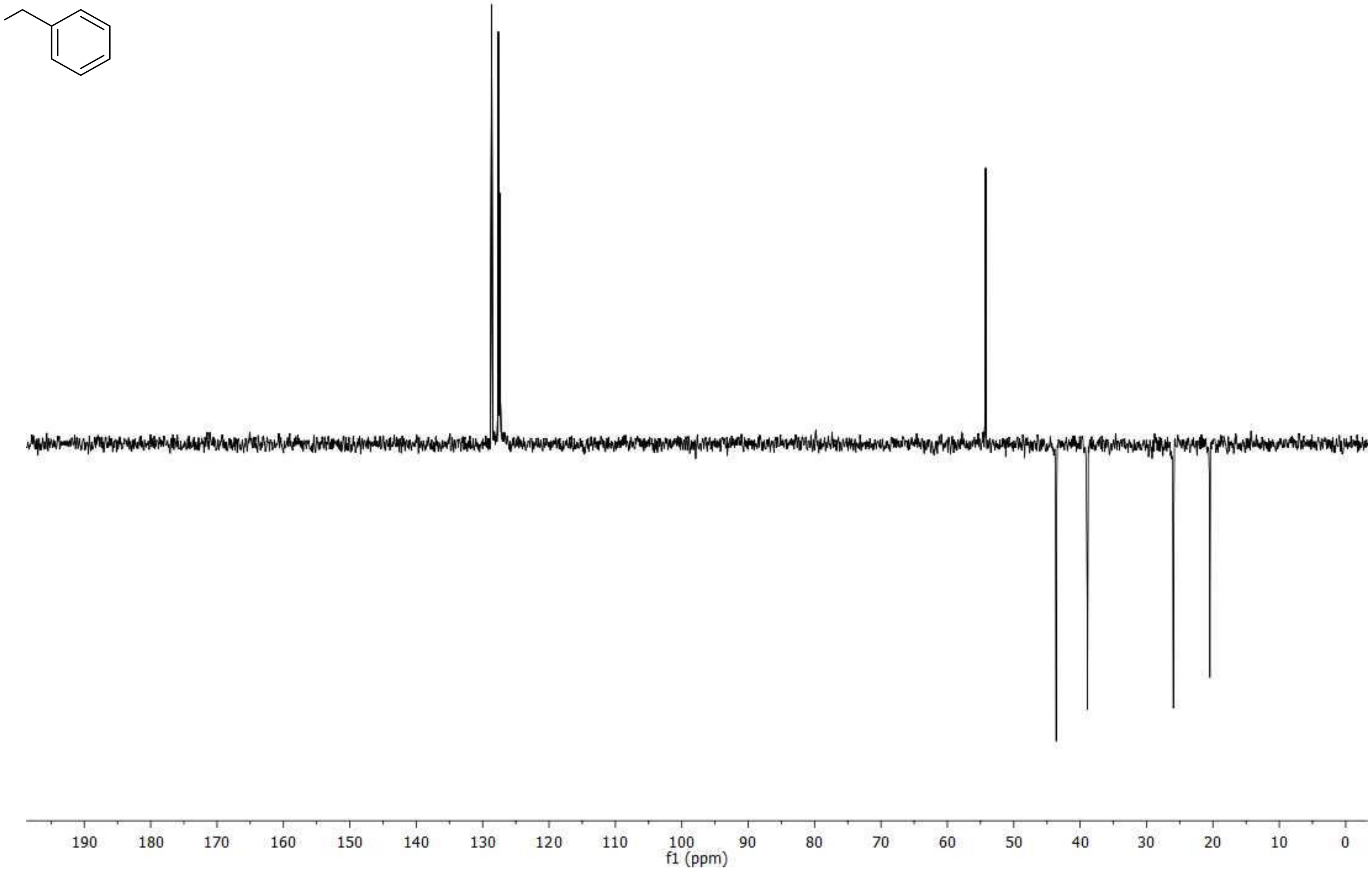


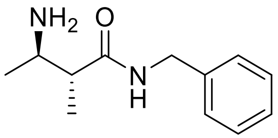
2h


220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


O
O
N
H

2h


190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)



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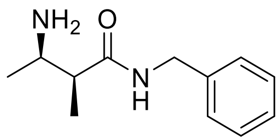
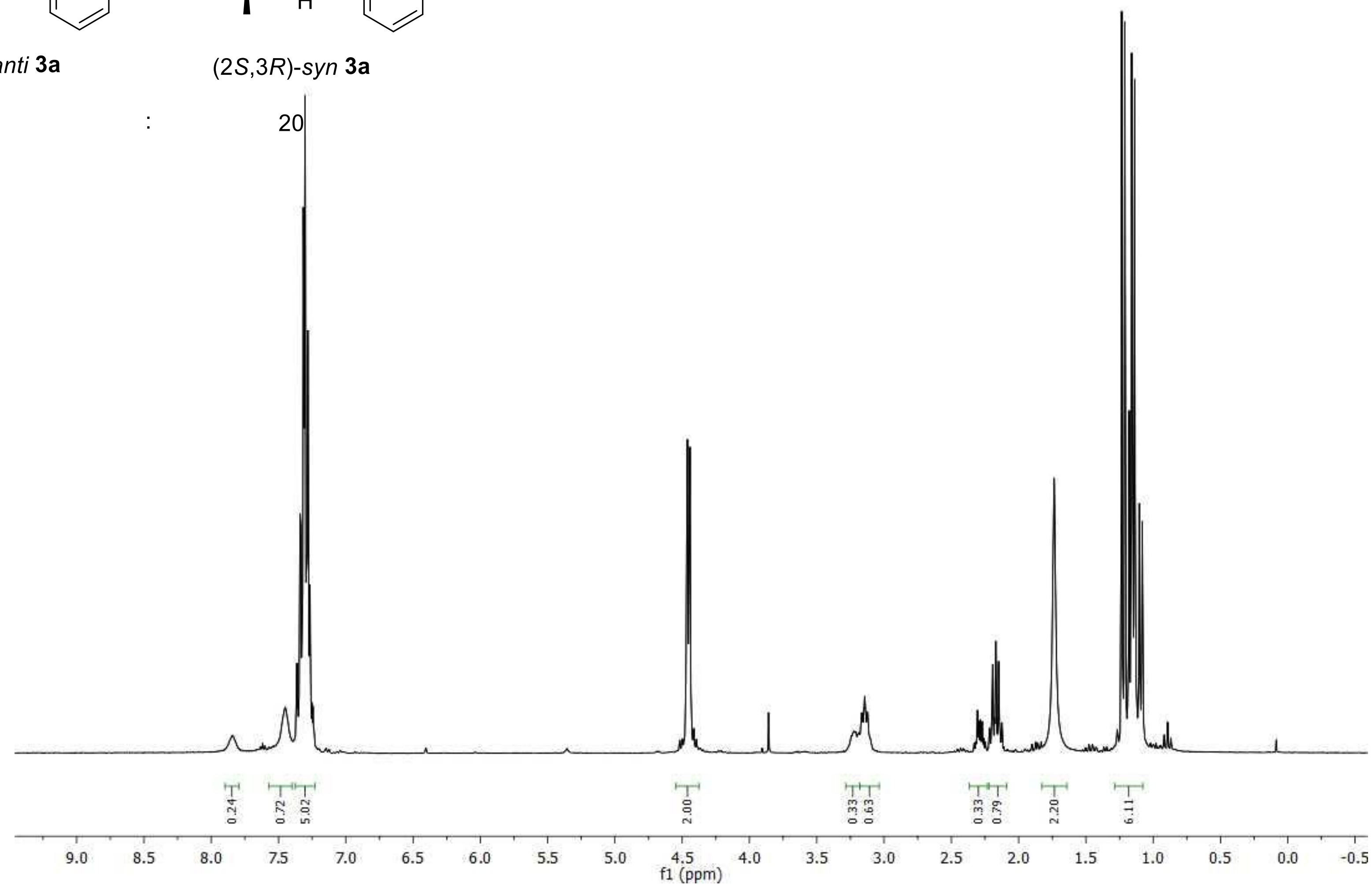
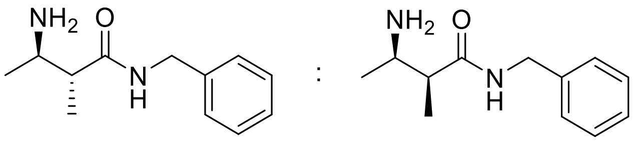


(2*R*,3*R*)-*anti* **3a** (2*S*,3*R*)-*syn* **3a**

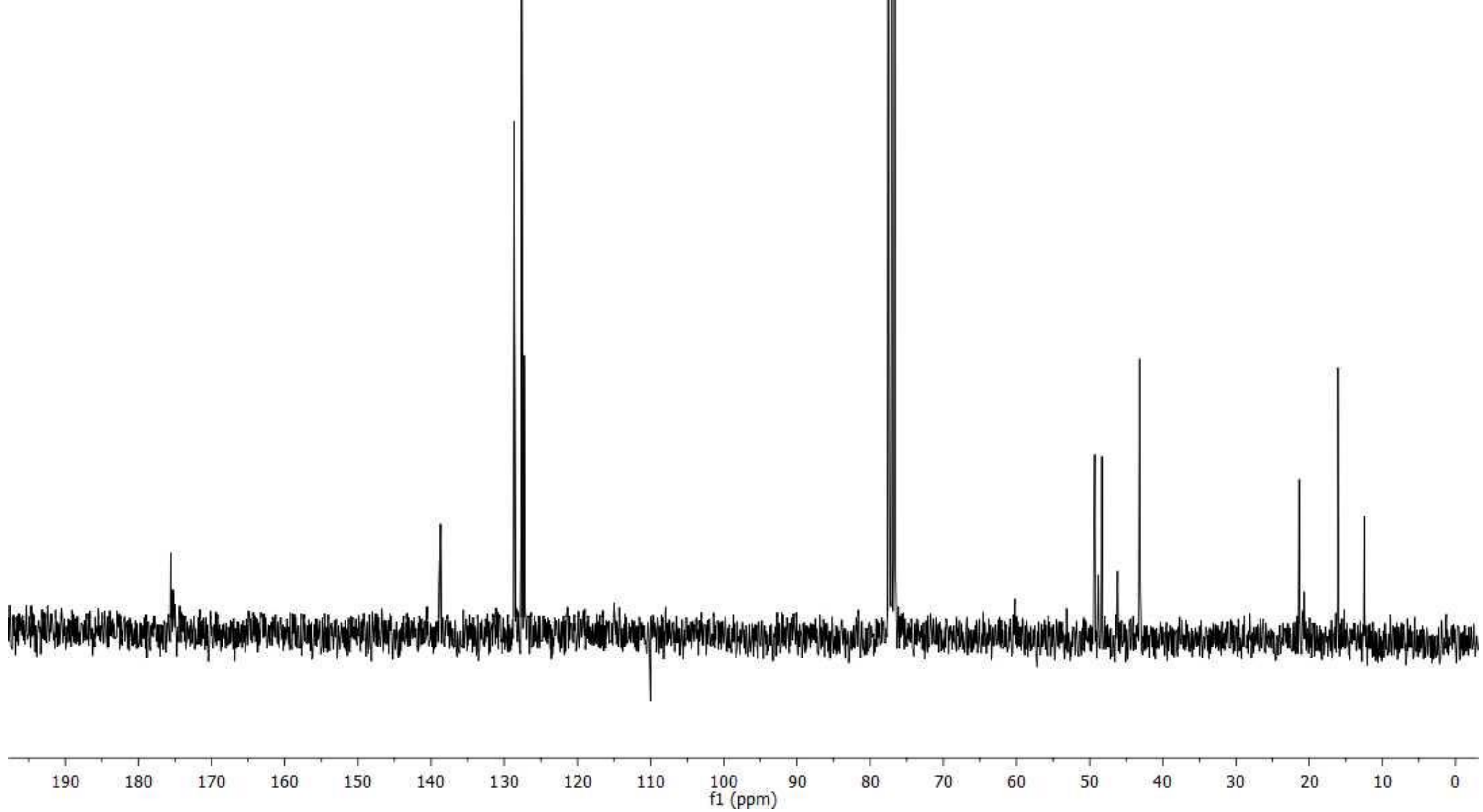
80 : 20

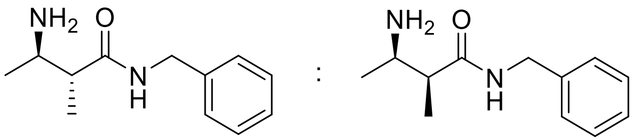




(2*R*,3*R*)-*anti* **3a** : (2*S*,3*R*)-*syn* **3a**

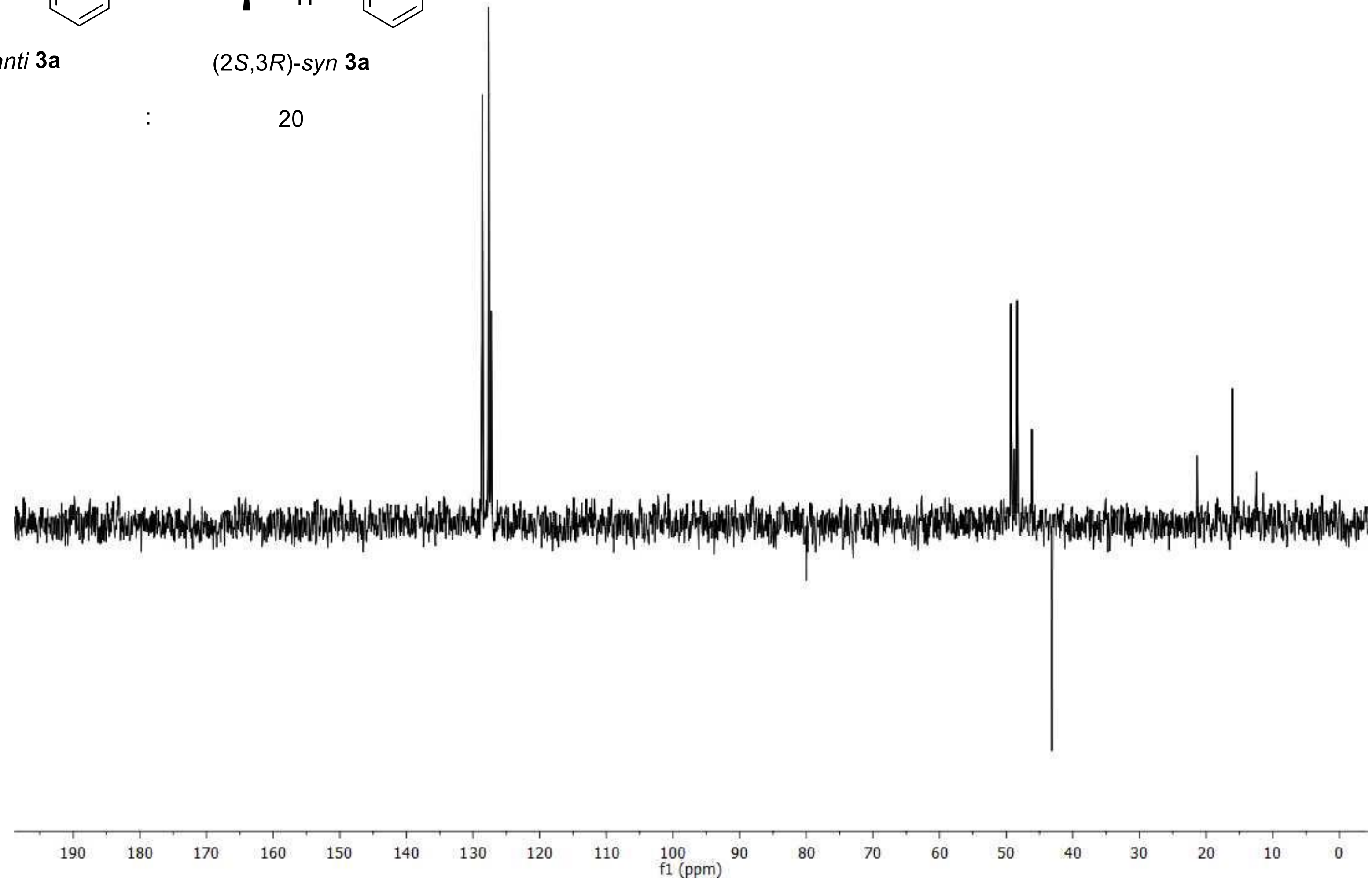
80 : 20

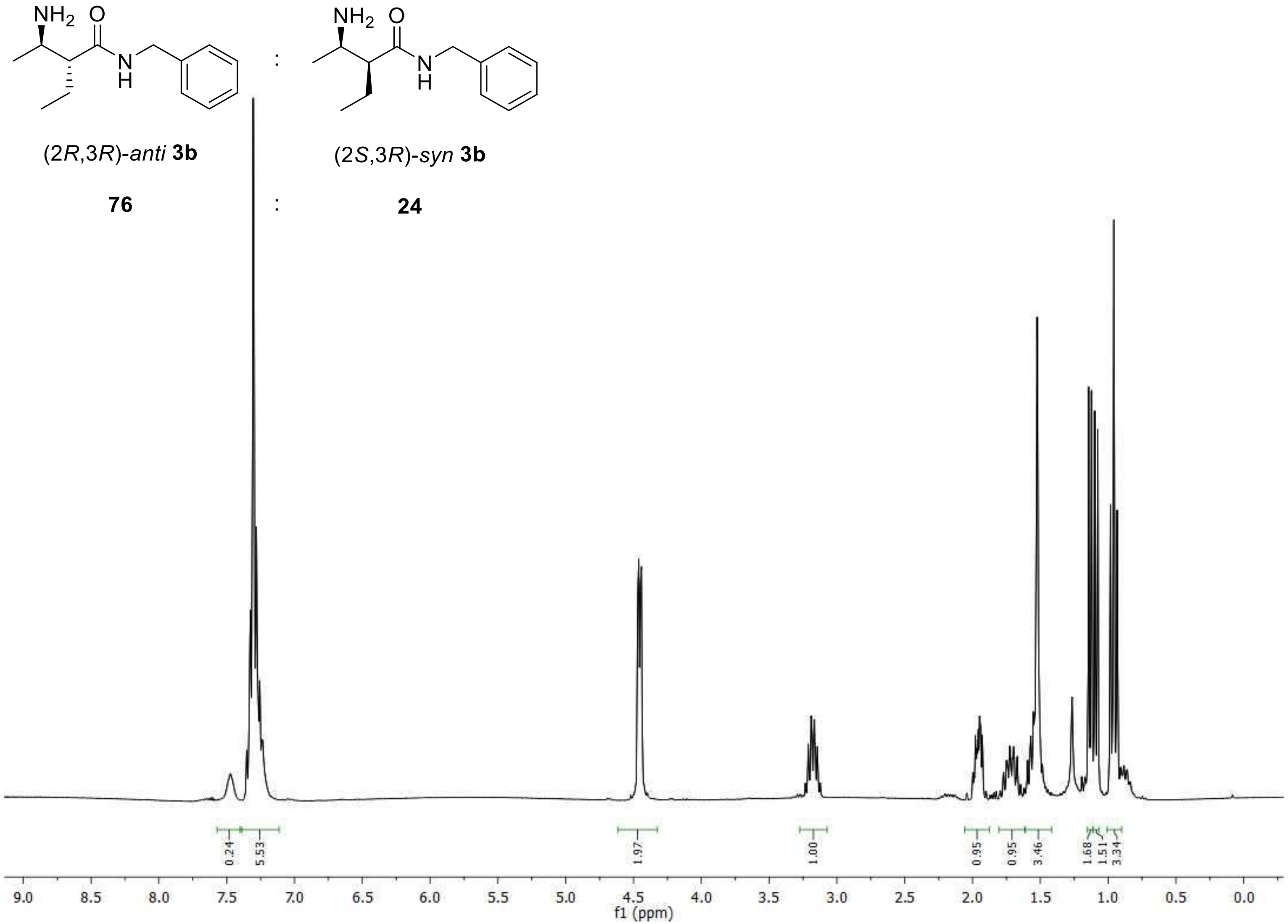




(2*R*,3*R*)-*anti* **3a** (2*S*,3*R*)-*syn* **3a**

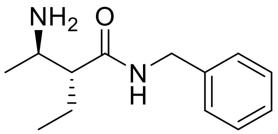
80 : 20





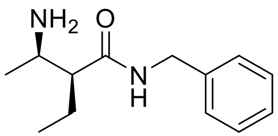
(2*R*,3*R*)-*anti* **3b** : (2*S*,3*R*)-*syn* **3b**

76 : **24**



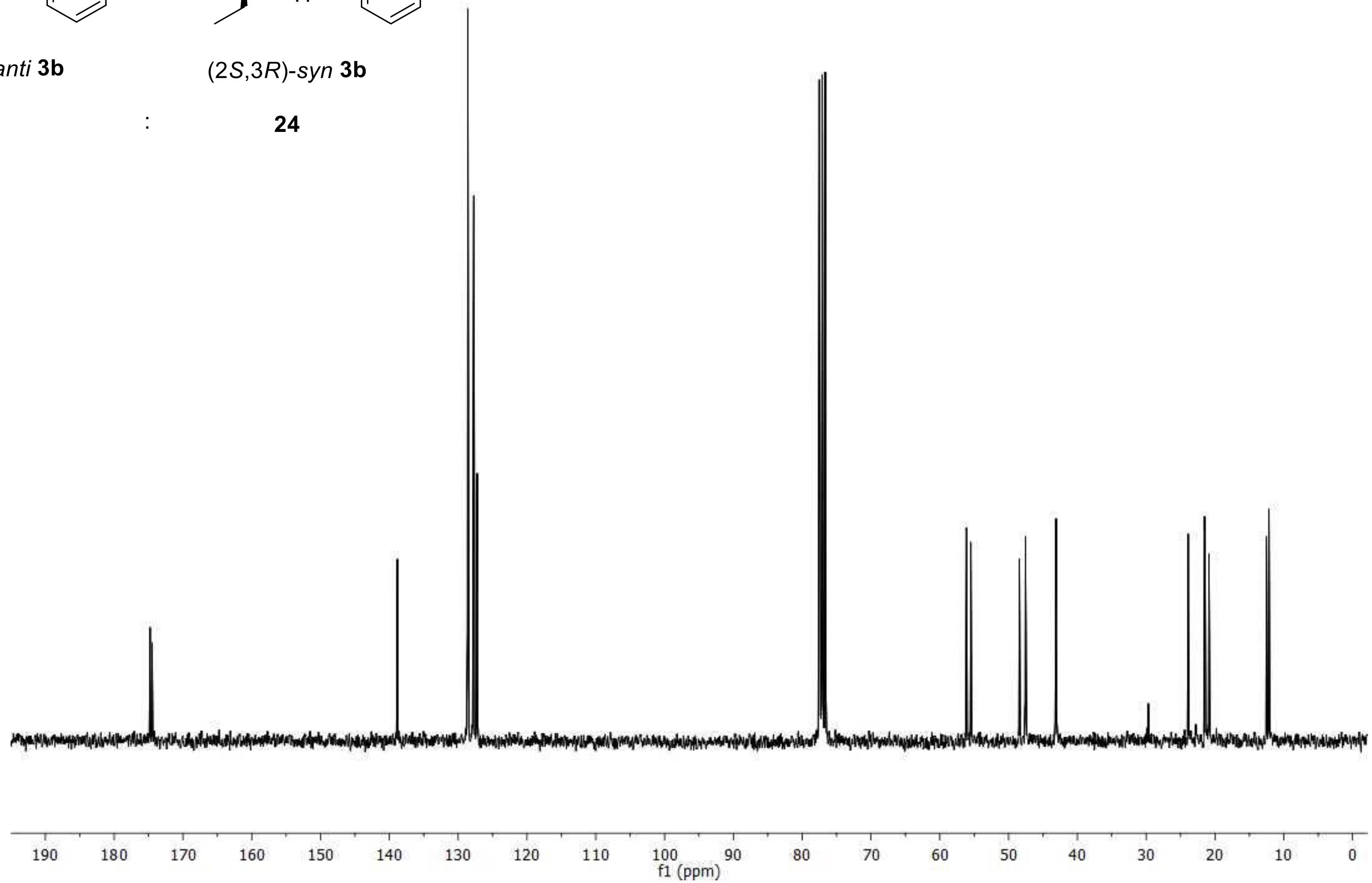
(2*R*,3*R*)-*anti* **3b**

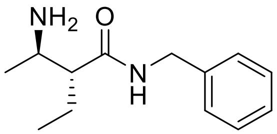
:



(2*S*,3*R*)-*syn* **3b**

76 : **24**





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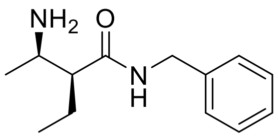
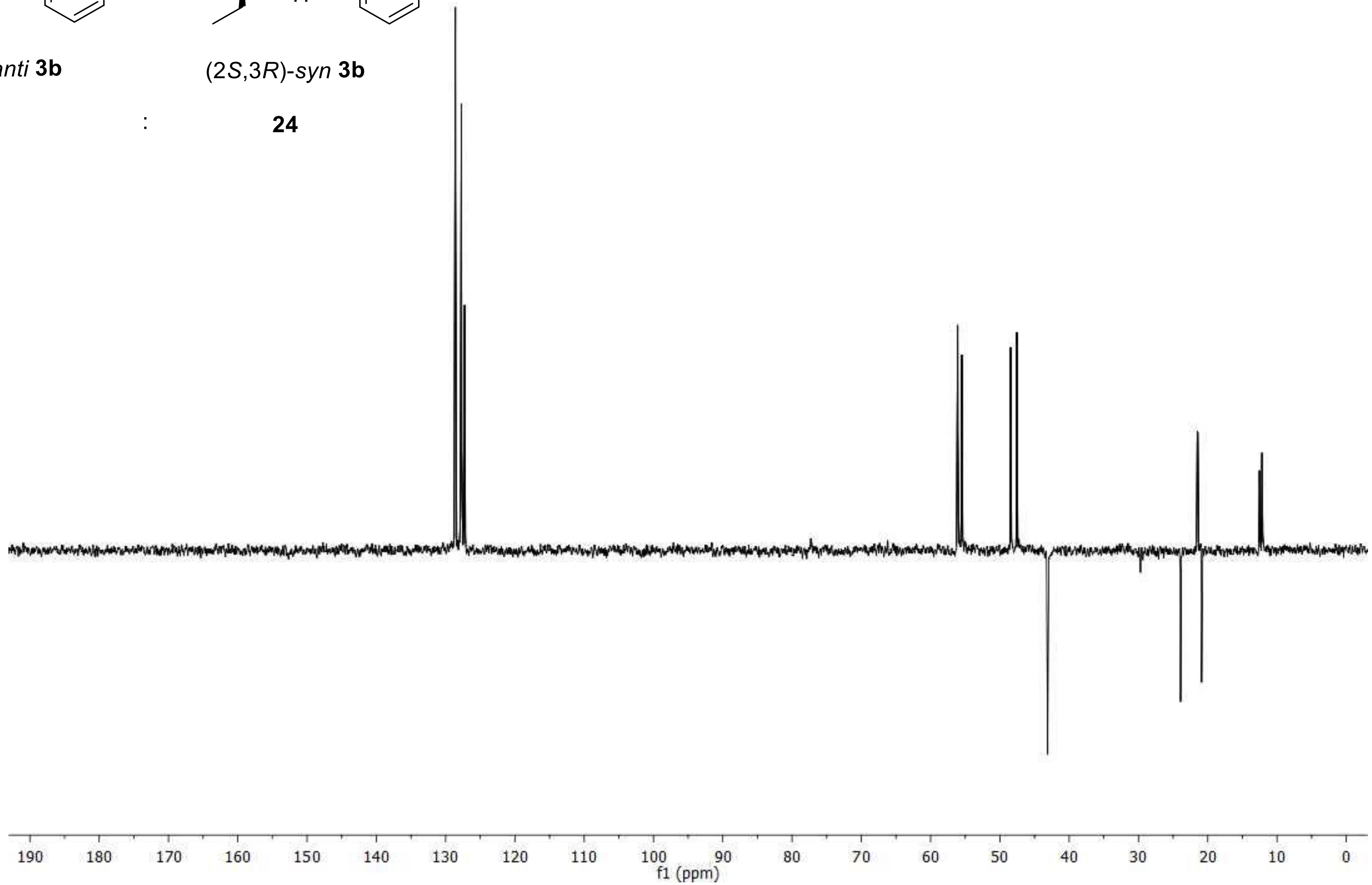
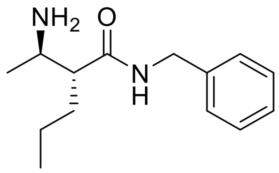


(2*R*,3*R*)-*anti* **3b** (2*S*,3*R*)-*syn* **3b**

76 : **24**





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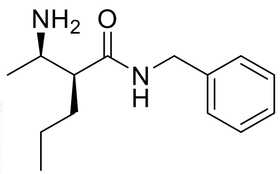
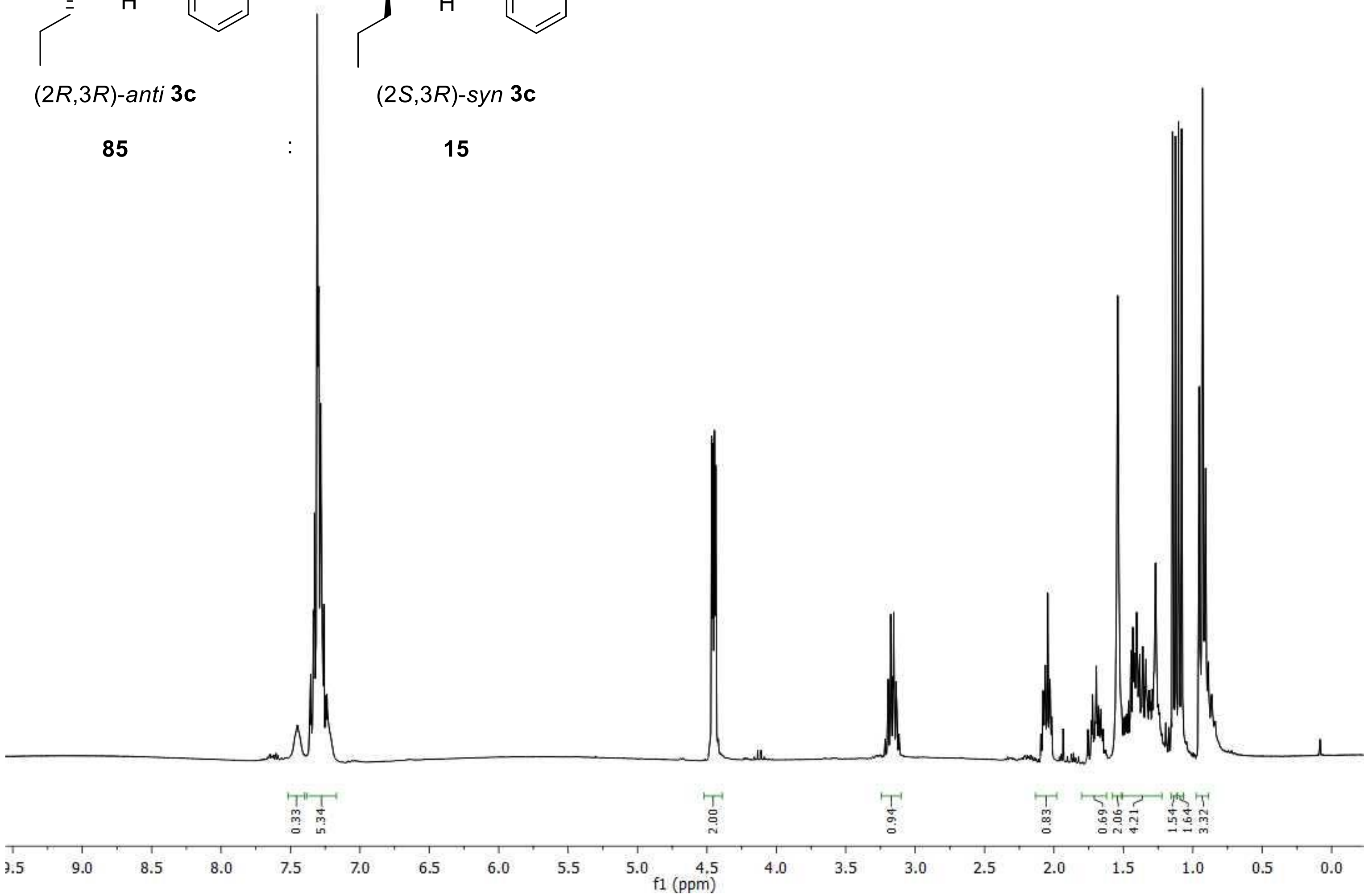
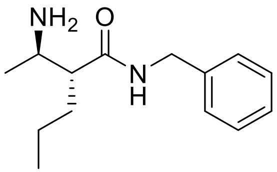


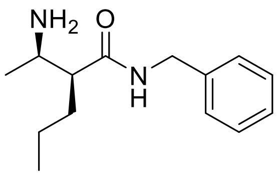
(2*R*,3*R*)-*anti* **3c** : (2*S*,3*R*)-*syn* **3c**

85 : **15**



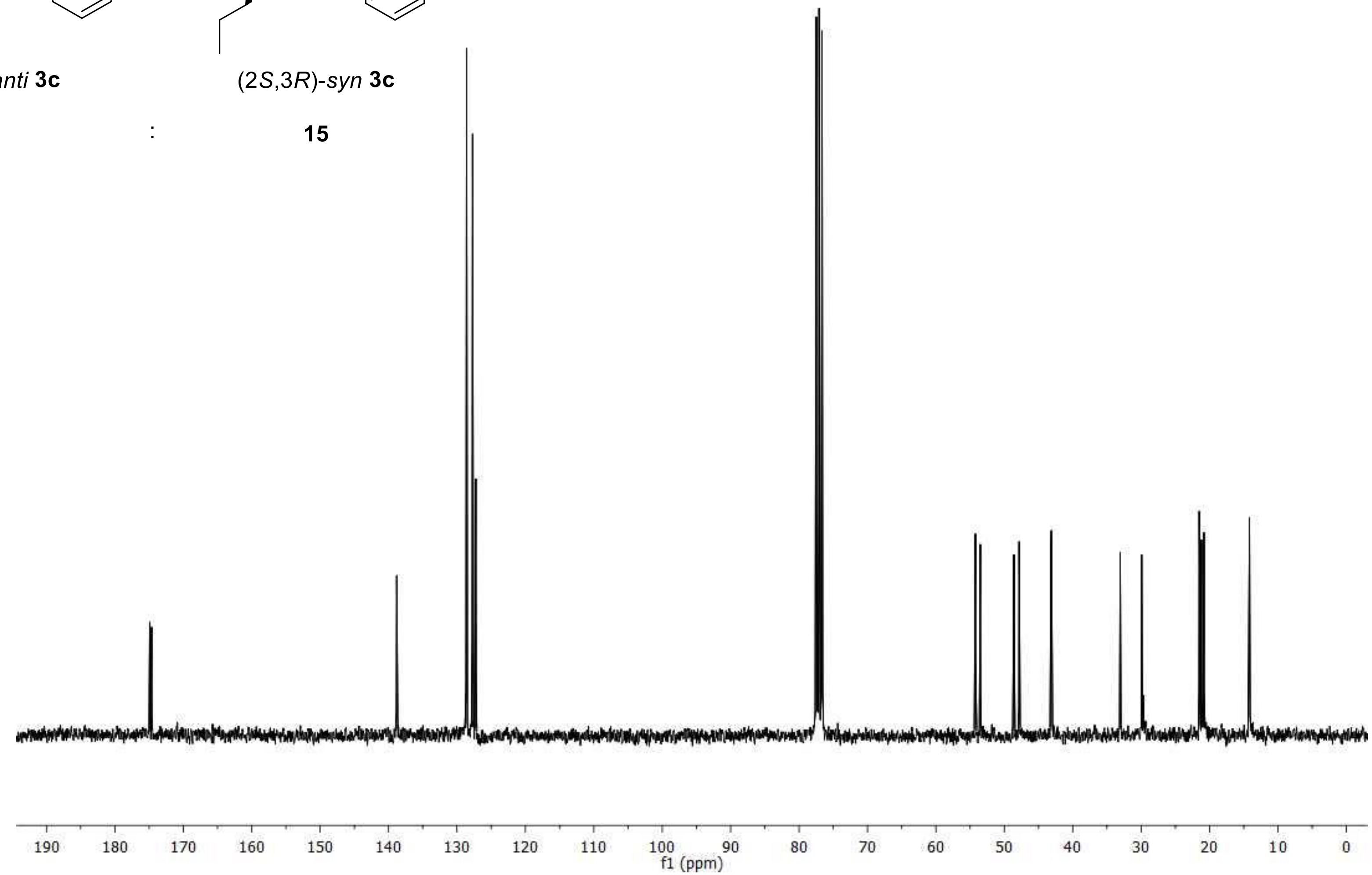


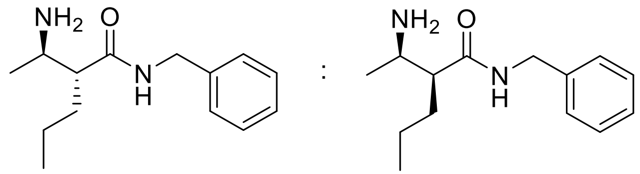
(2*R*,3*R*)-*anti* **3c**



(2*S*,3*R*)-*syn* **3c**

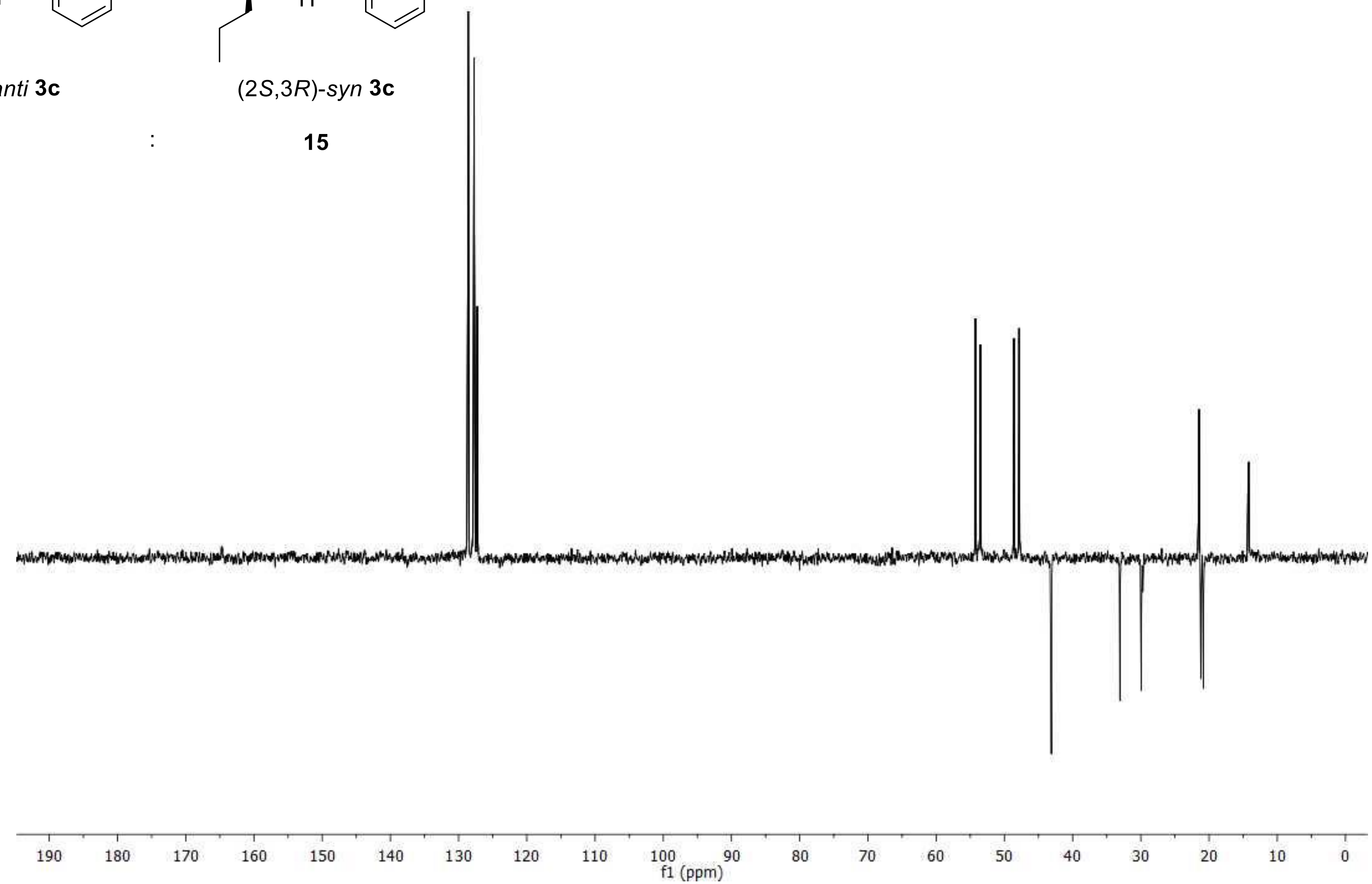
85 : **15**

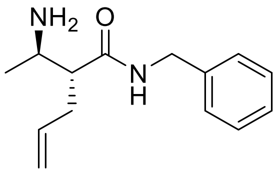




(2*R*,3*R*)-*anti* **3c** : (2*S*,3*R*)-*syn* **3c**

85 : **15**





(2*R*,3*R*)-*anti* **3d**

75

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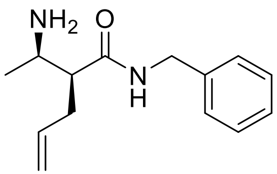
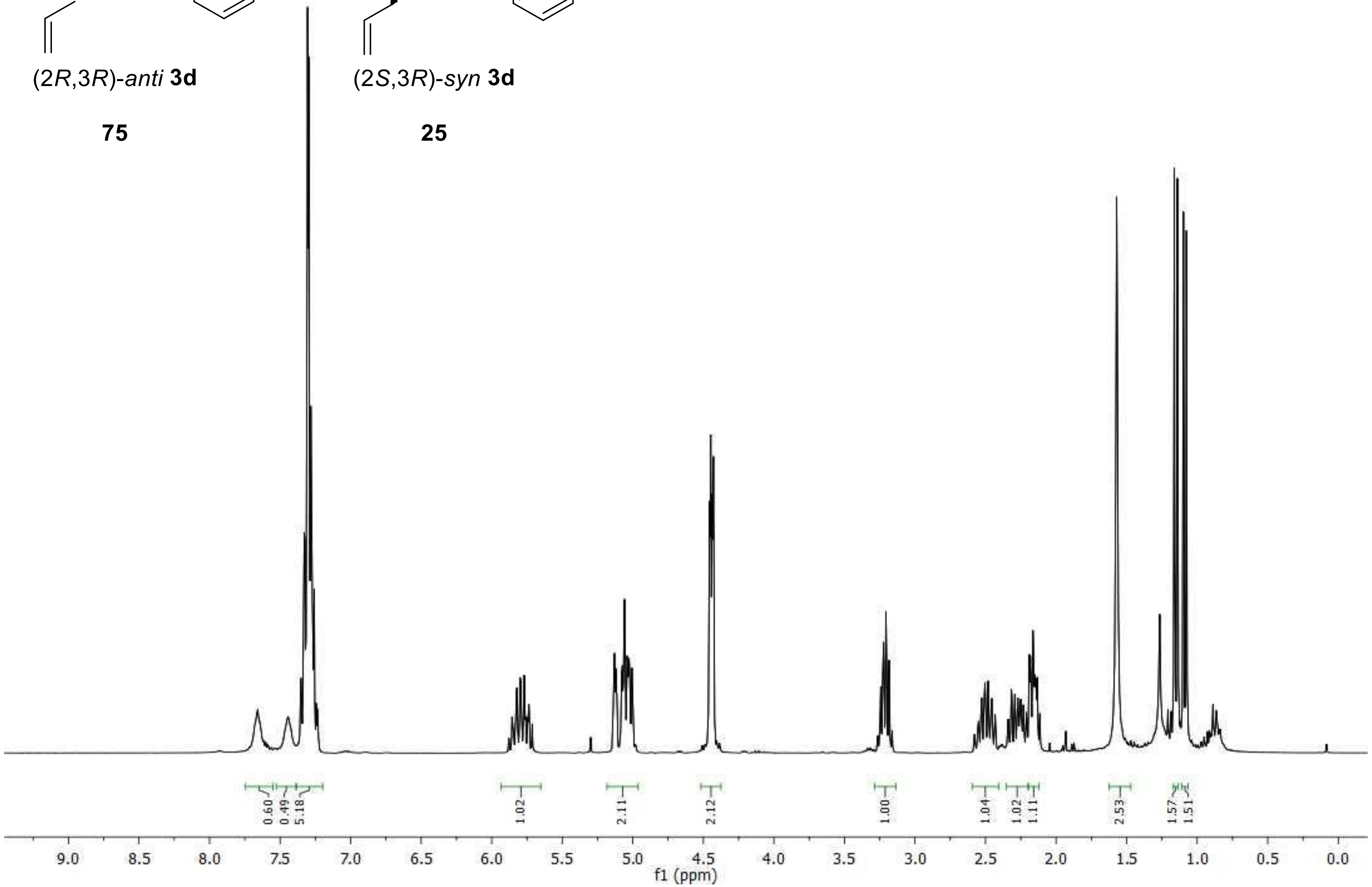
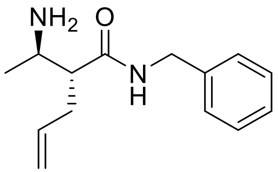


(2*S*,3*R*)-*syn* **3d**

25

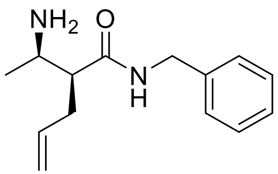




(2*R*,3*R*)-*anti* **3d**

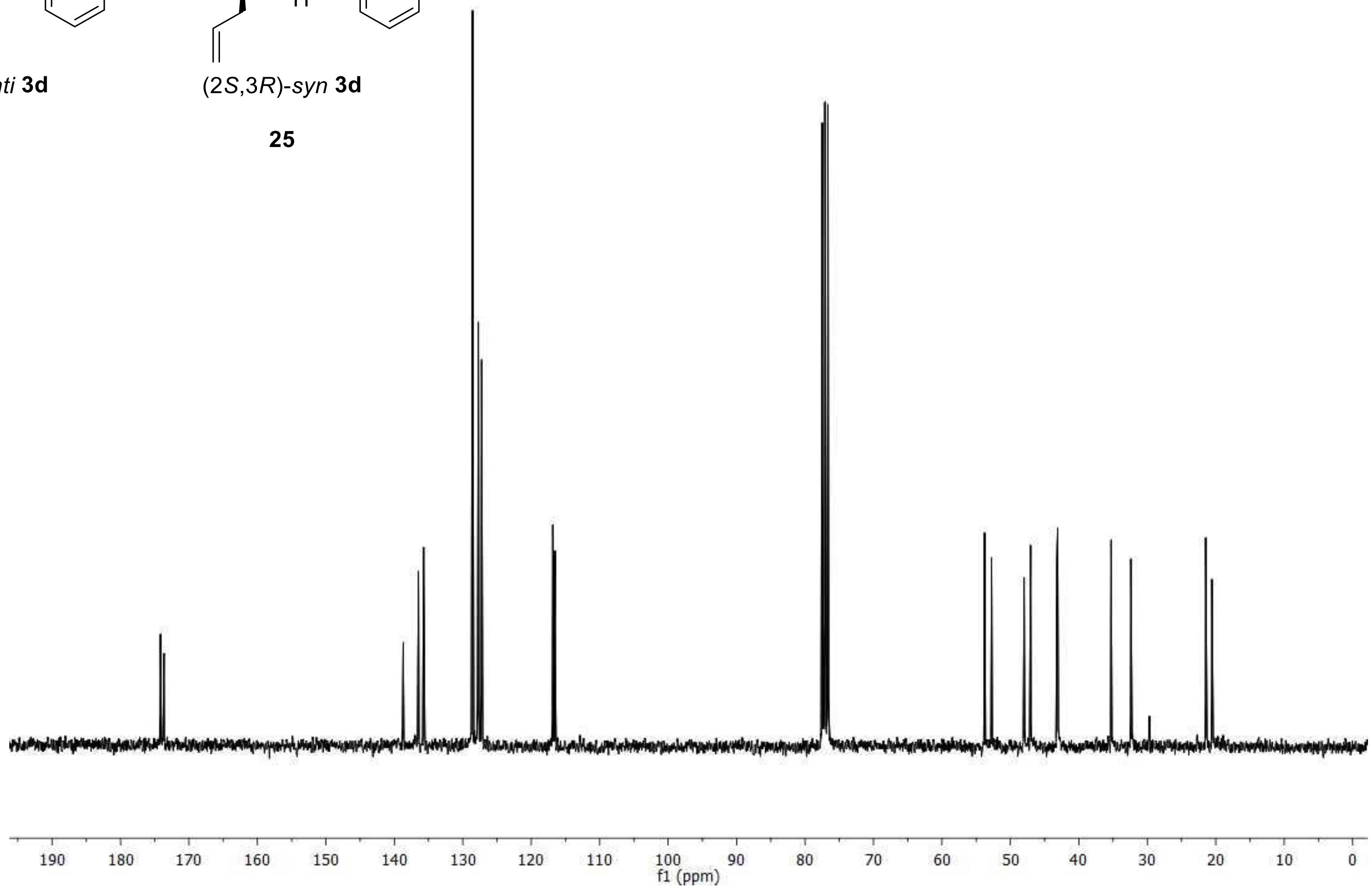
75

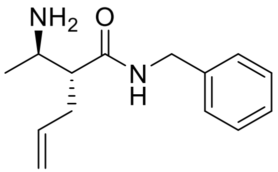
:



(2*S*,3*R*)-*syn* **3d**

25





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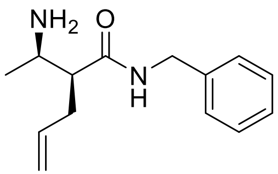
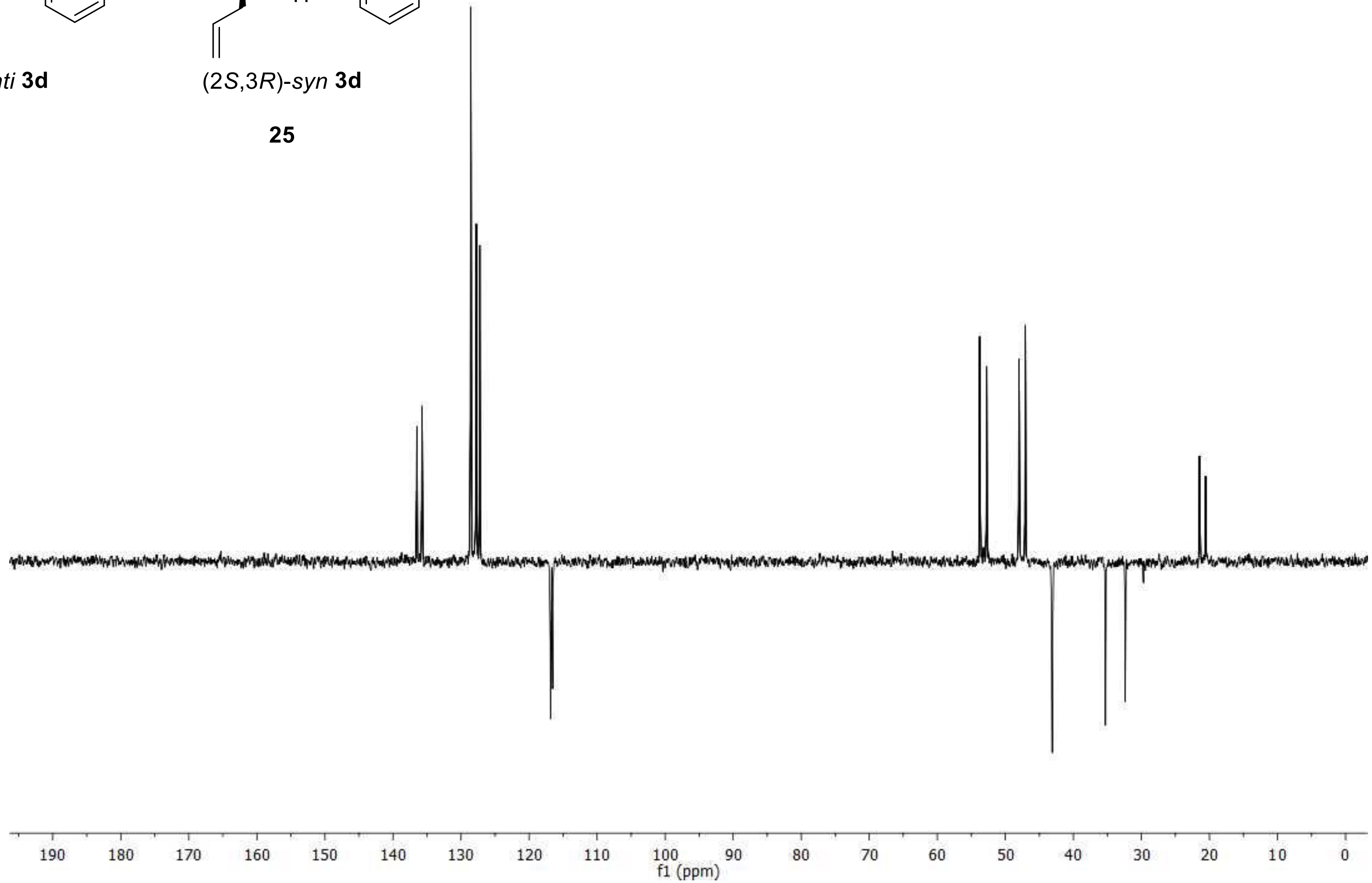
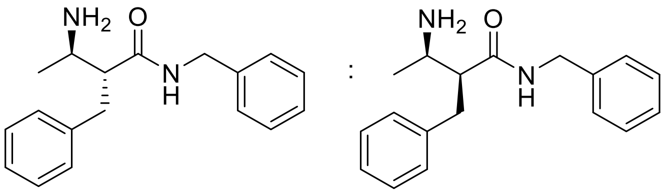


(2*R*,3*R*)-*anti* **3d** (2*S*,3*R*)-*syn* **3d**

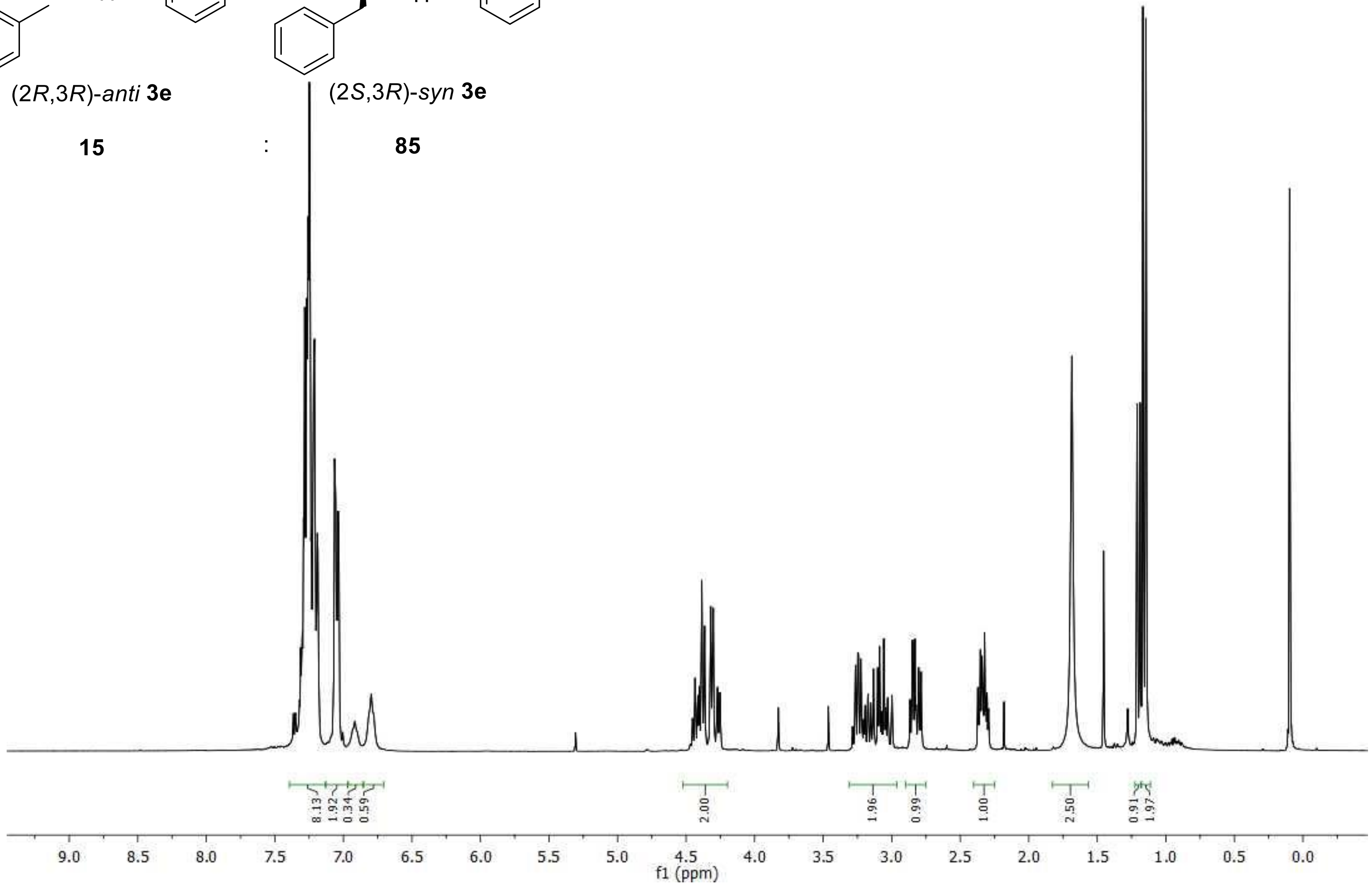
75 **25**

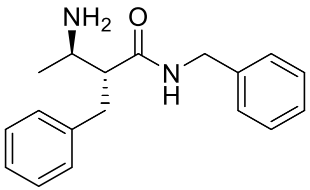




(2*R*,3*R*)-*anti* **3e** (2*S*,3*R*)-*syn* **3e**

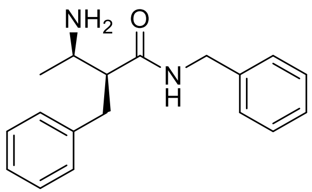
15 : **85**





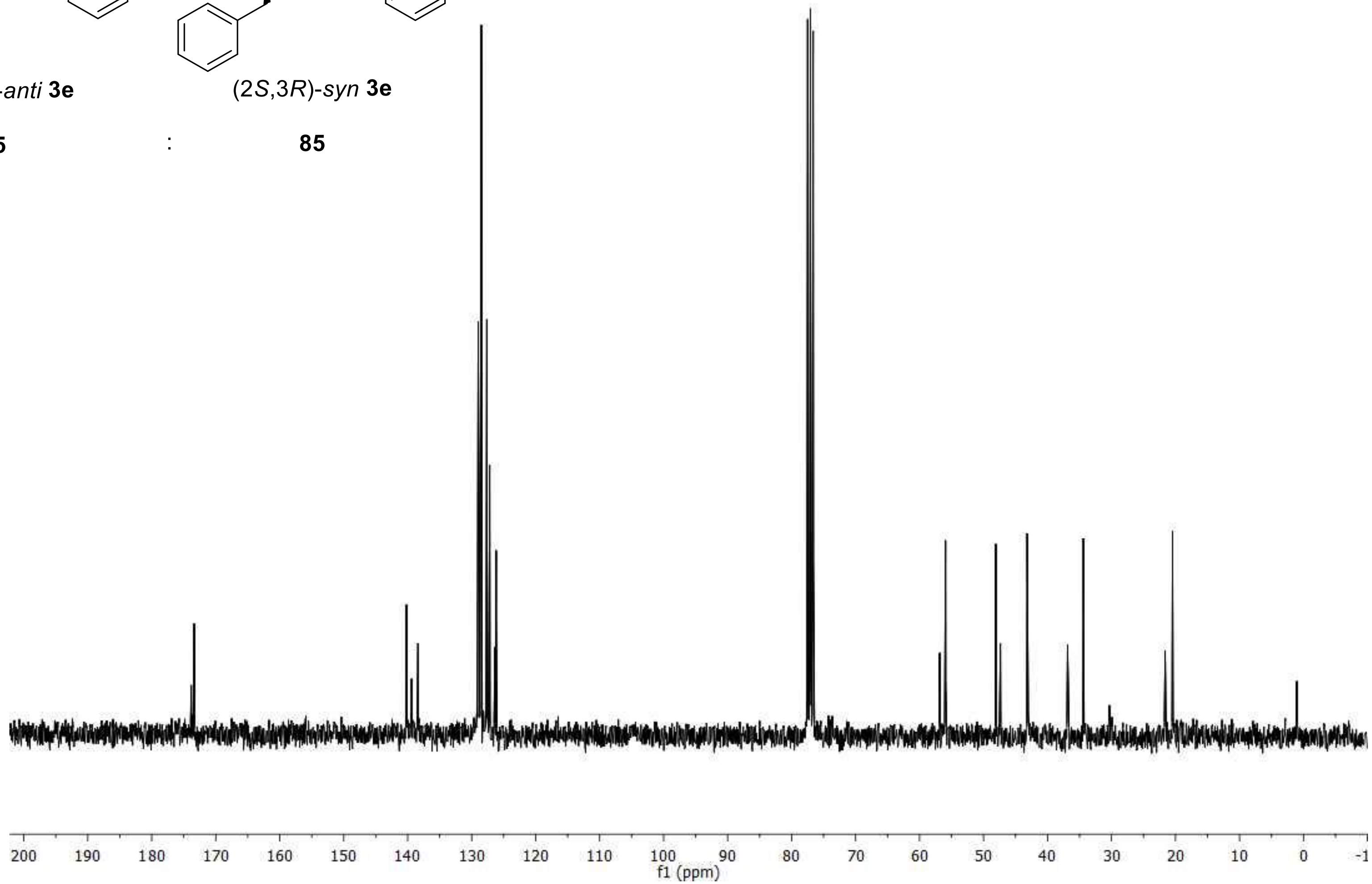
(2*R*,3*R*)-*anti* **3e**

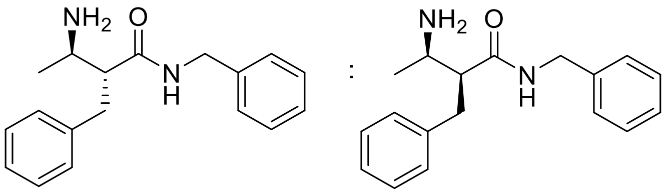
:



(2*S*,3*R*)-*syn* **3e**

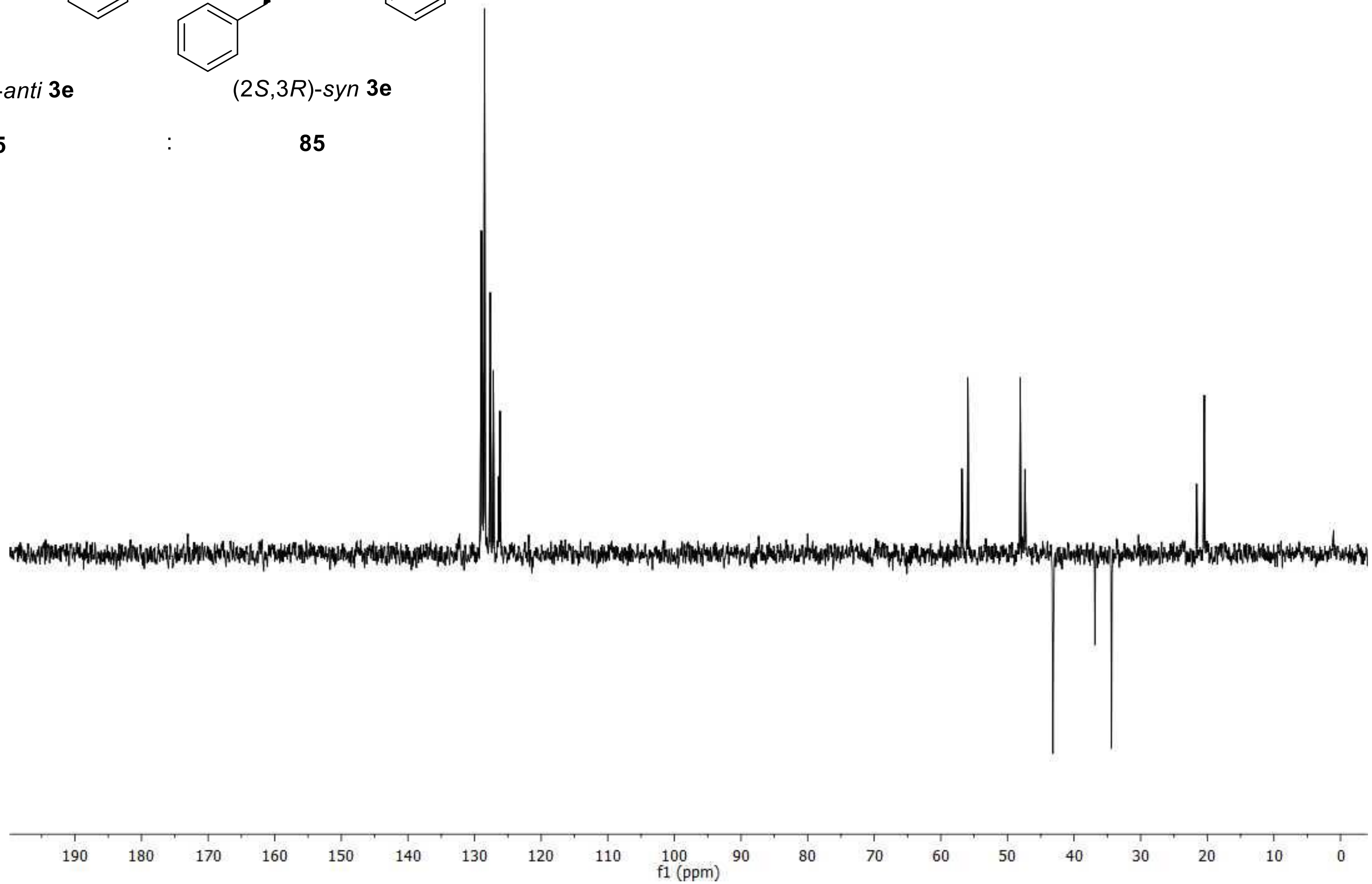
15 : **85**

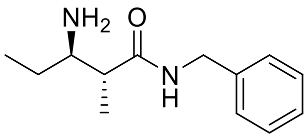




(2*R*,3*R*)-*anti* **3e** : (2*S*,3*R*)-*syn* **3e**

15 : **85**





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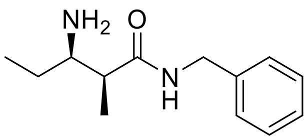
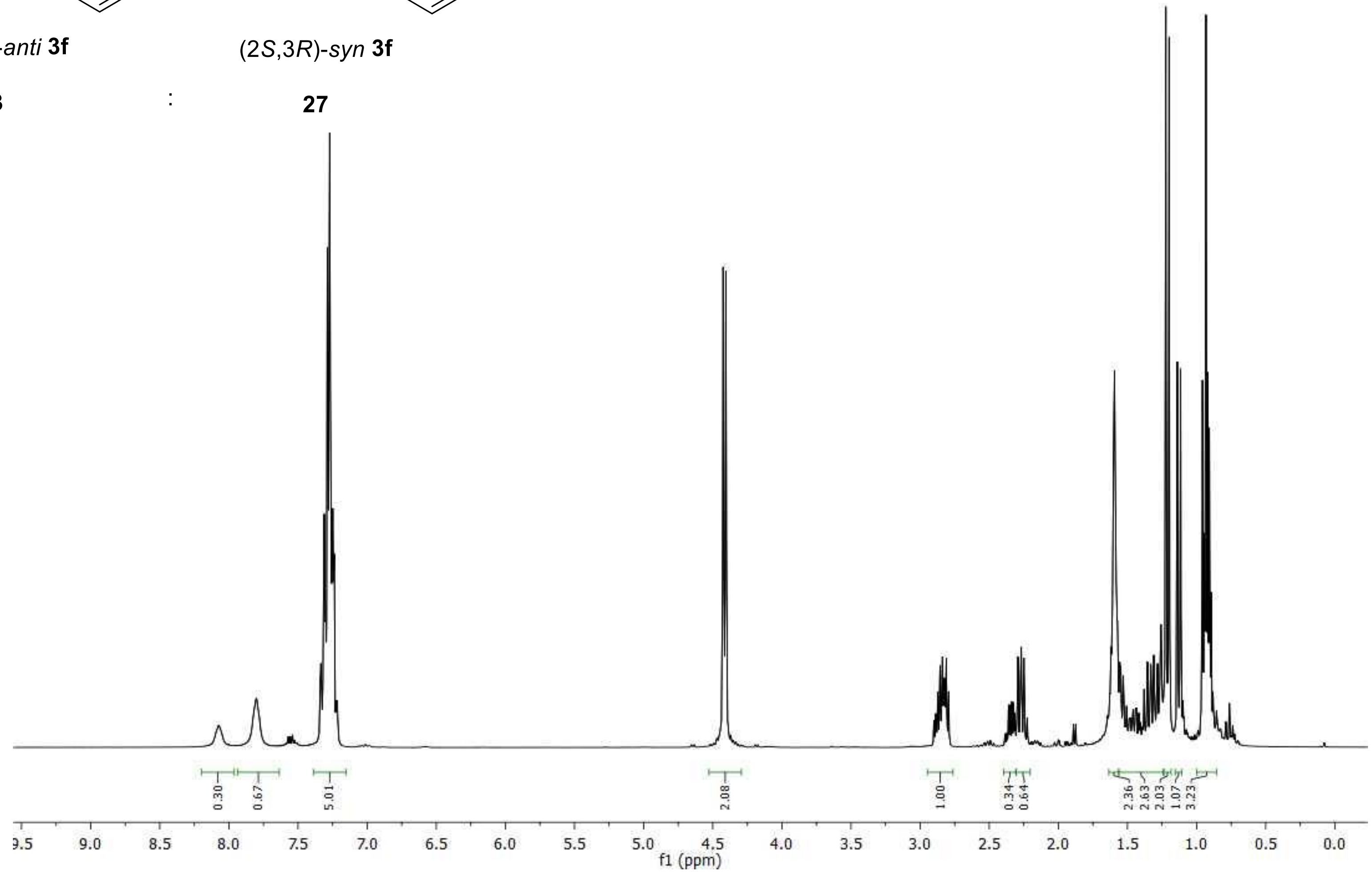
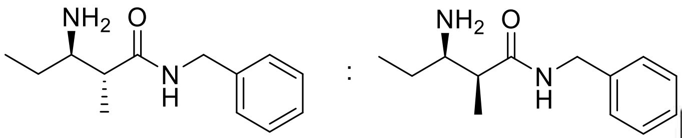


(2*R*,3*R*)-*anti* **3f** (2*S*,3*R*)-*syn* **3f**

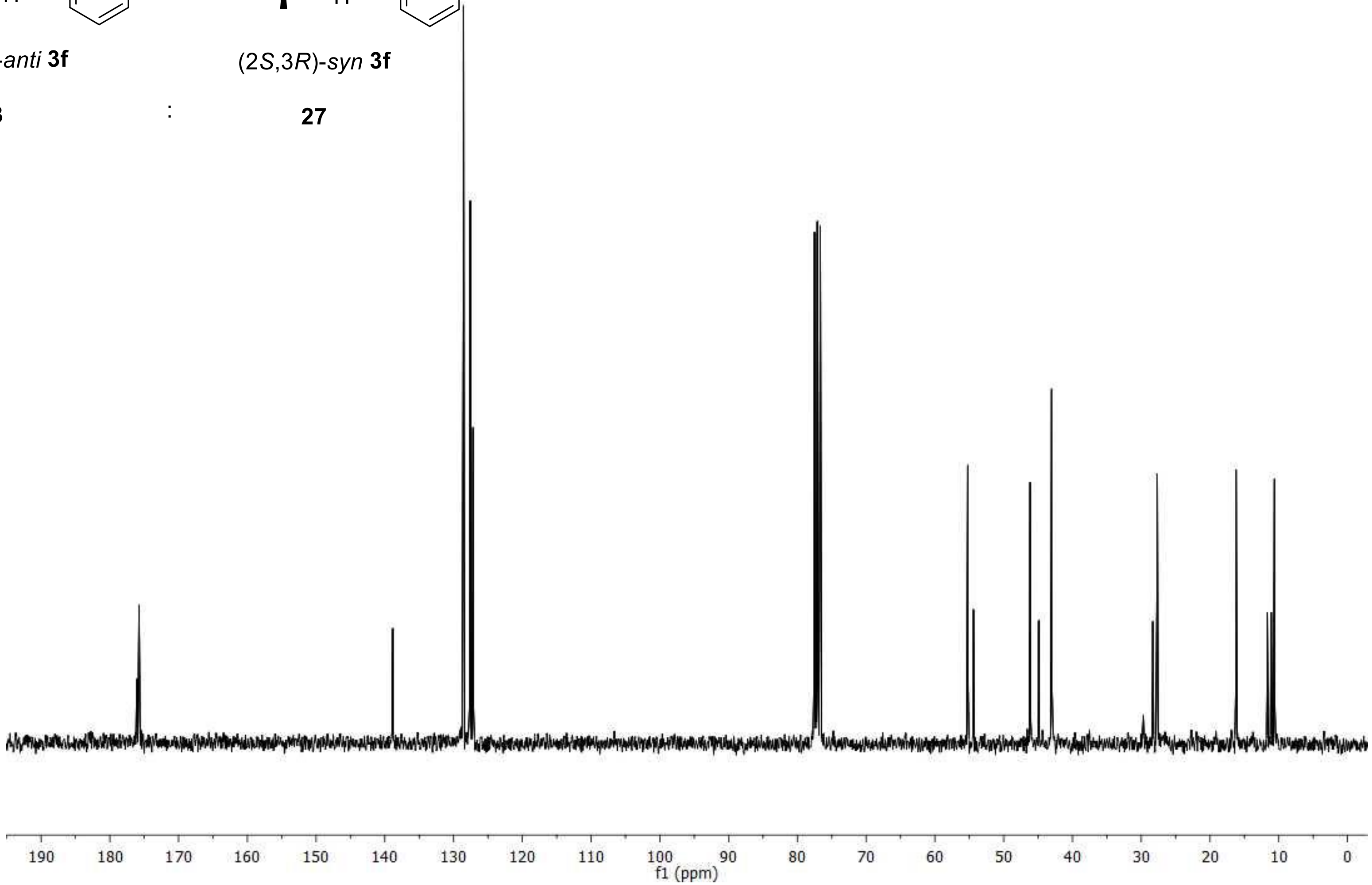
73 : **27**

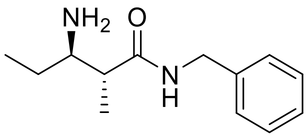




(2*R*,3*R*)-*anti* **3f** : (2*S*,3*R*)-*syn* **3f**

73 : **27**





(2*R*,3*R*)-*anti* **3f**

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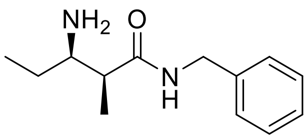
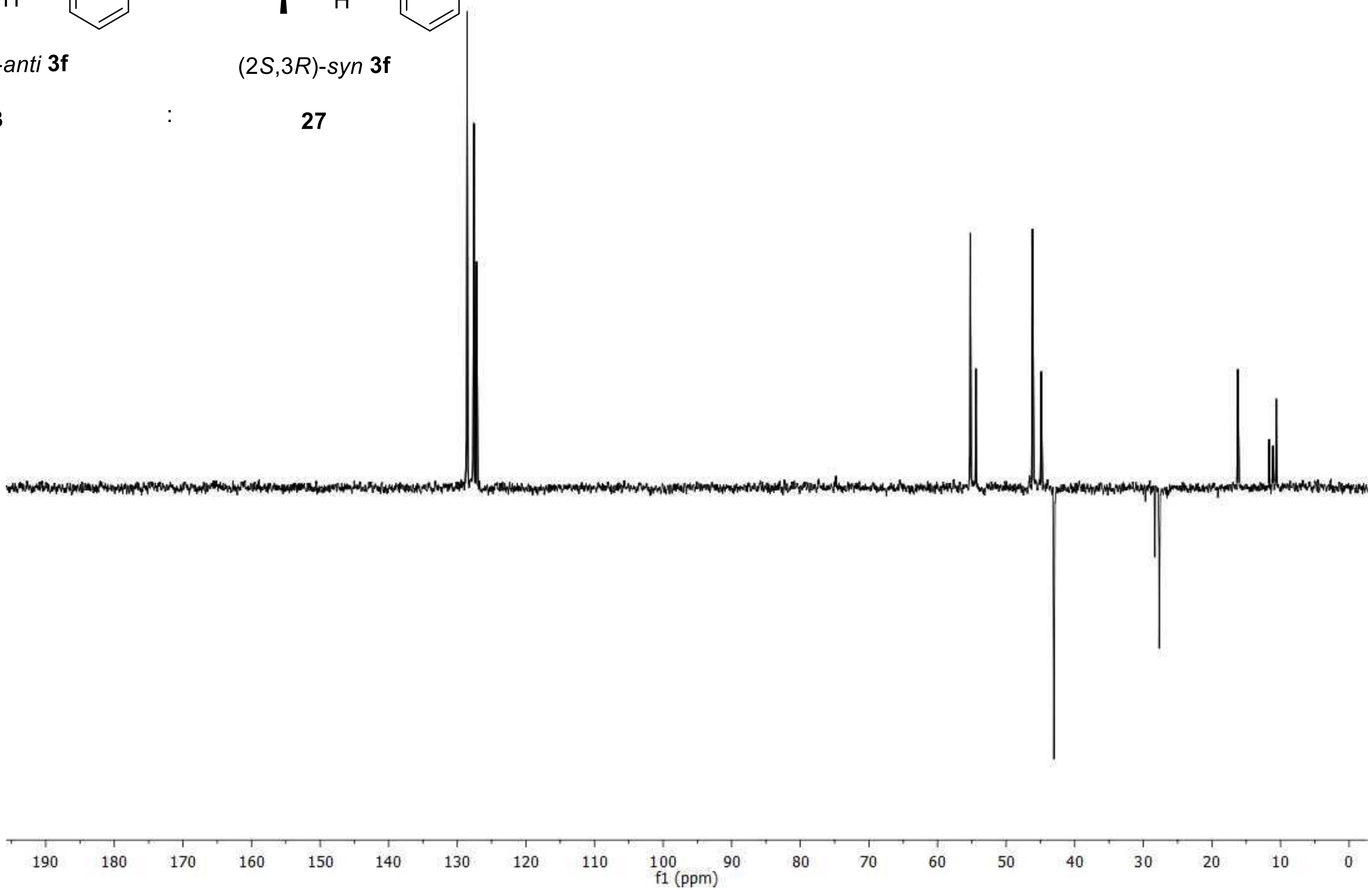
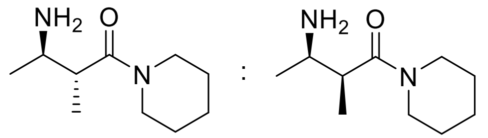


(2*S*,3*R*)-*syn* **3f**

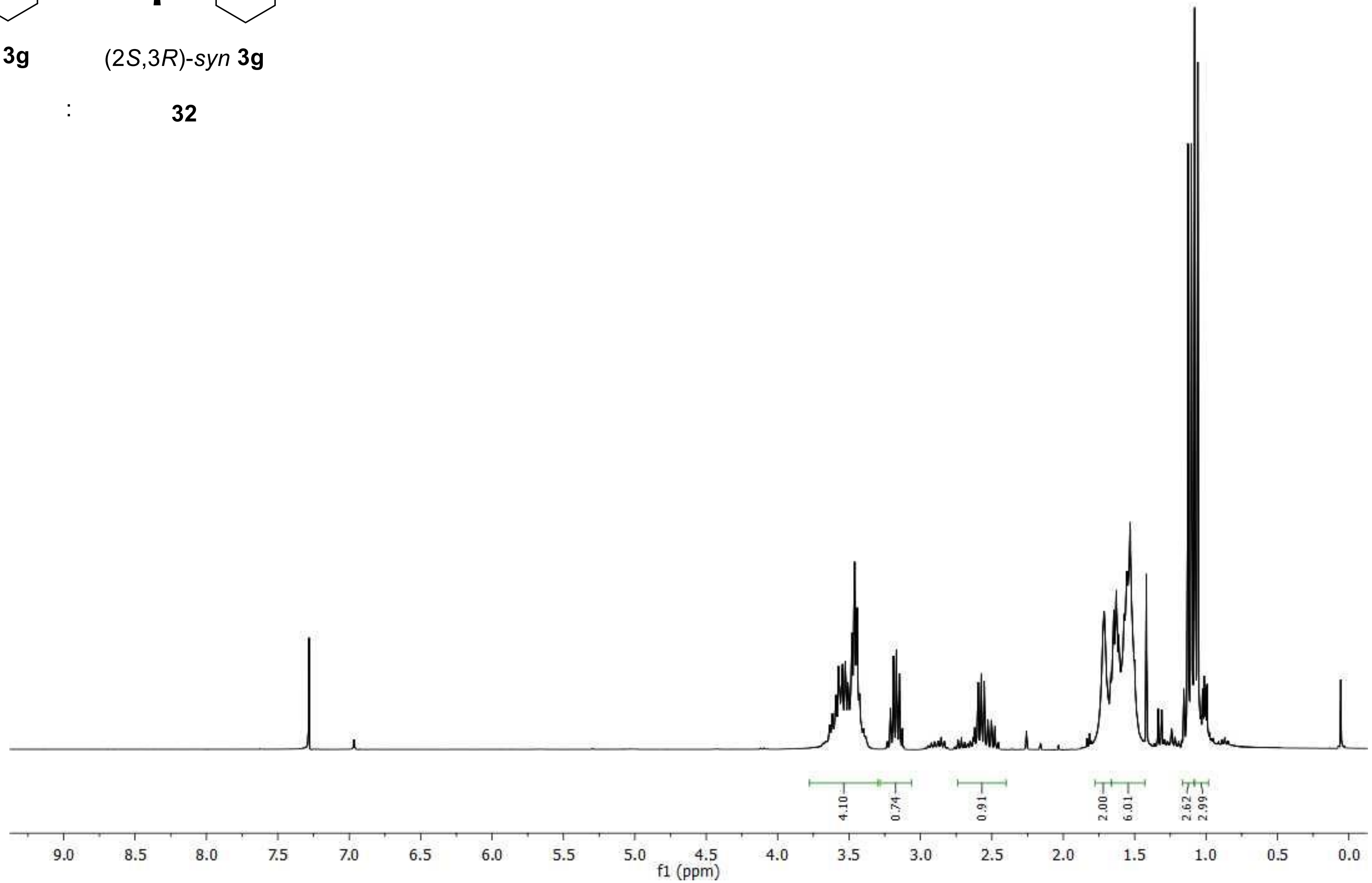
73 : **27**

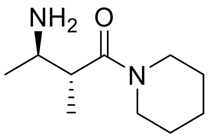




(2*R*,3*R*)-*anti* **3g** (2*S*,3*R*)-*syn* **3g**

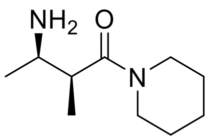
68 : **32**





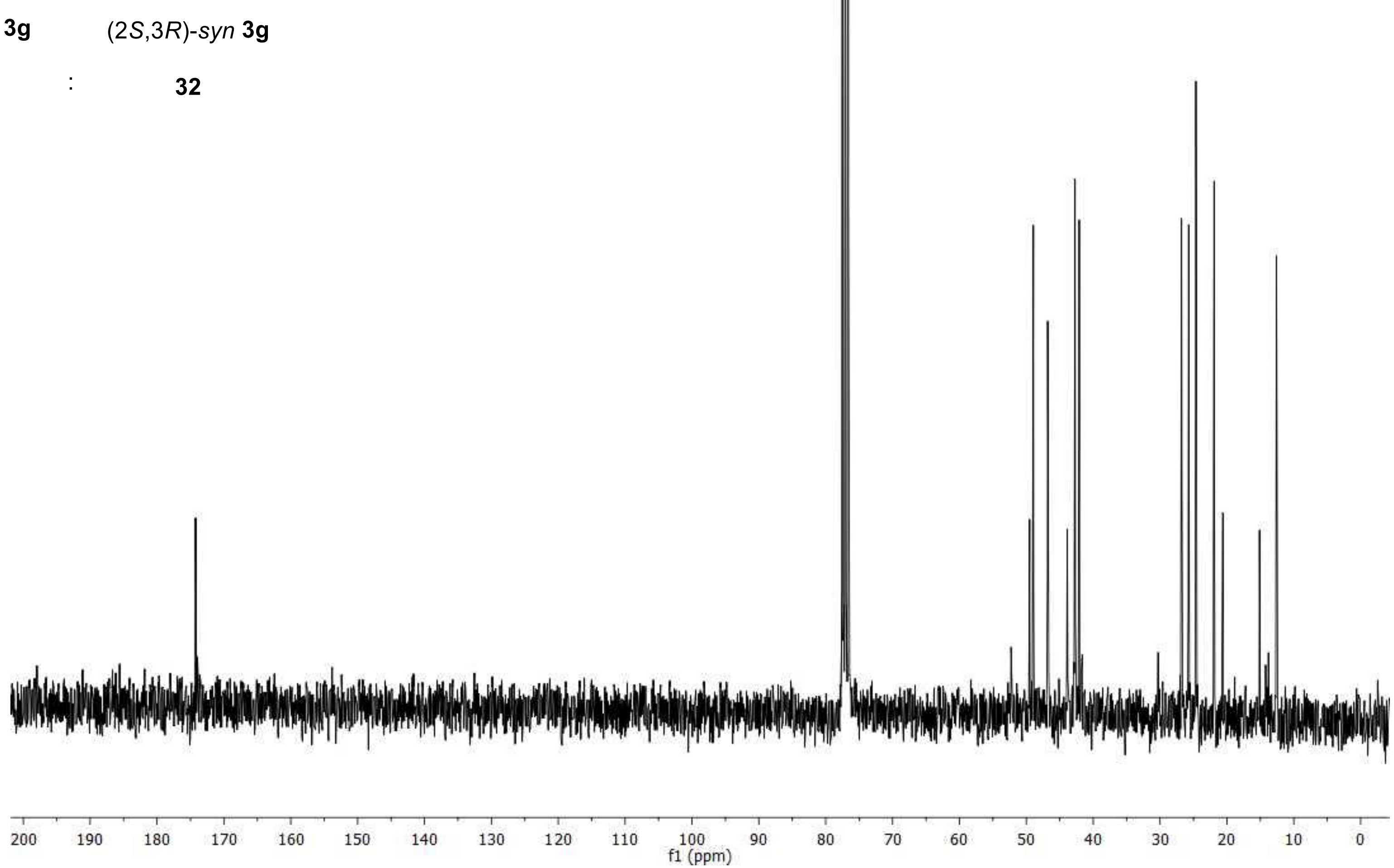
(2*R*,3*R*)-*anti* **3g**

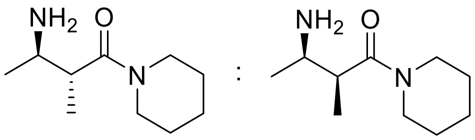
:



(2*S*,3*R*)-*syn* **3g**

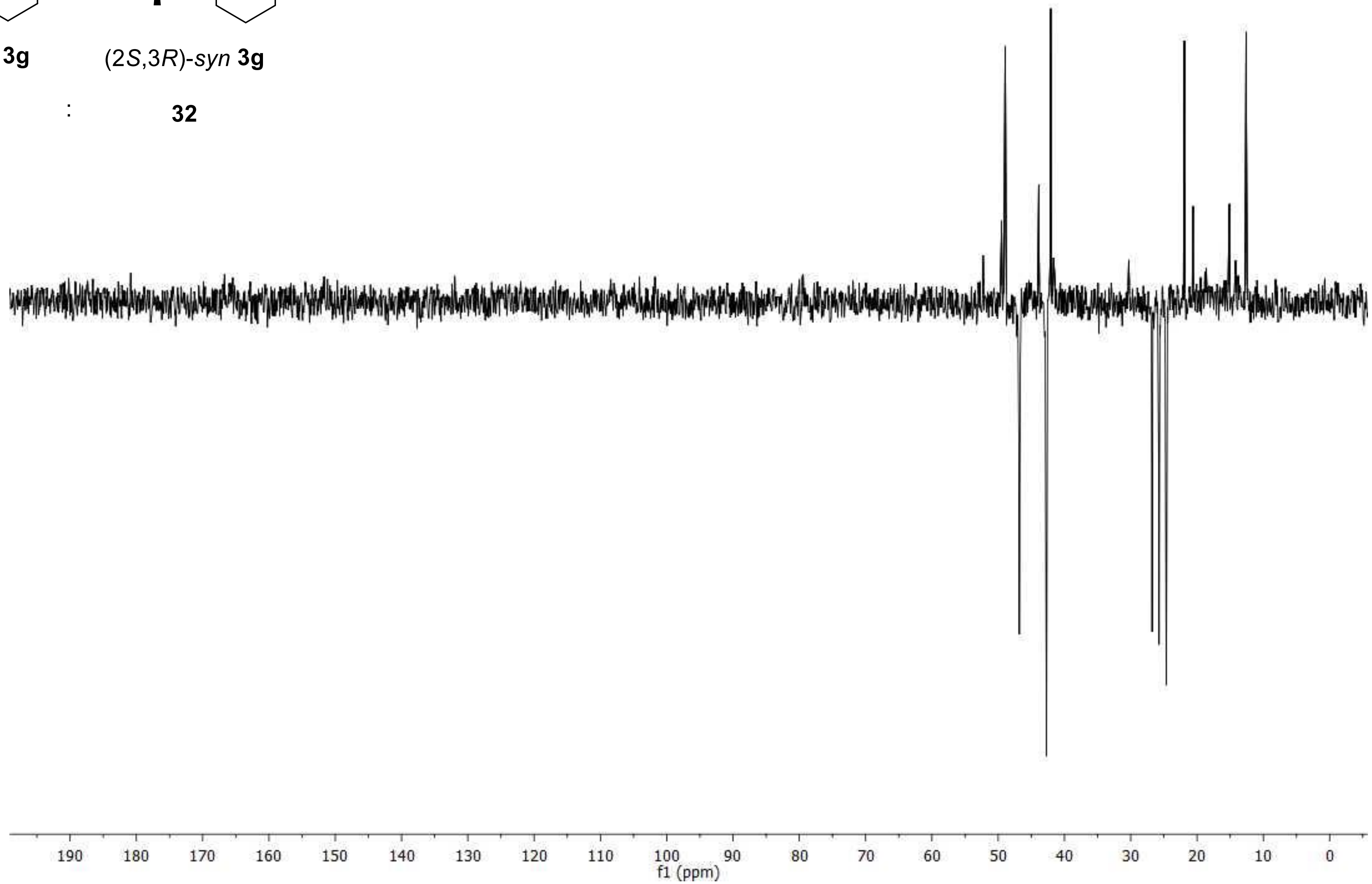
68 : **32**

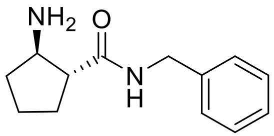




(2*R*,3*R*)-*anti* **3g** : (2*S*,3*R*)-*syn* **3g**

68 : **32**





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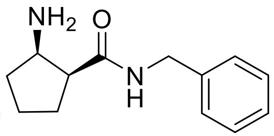
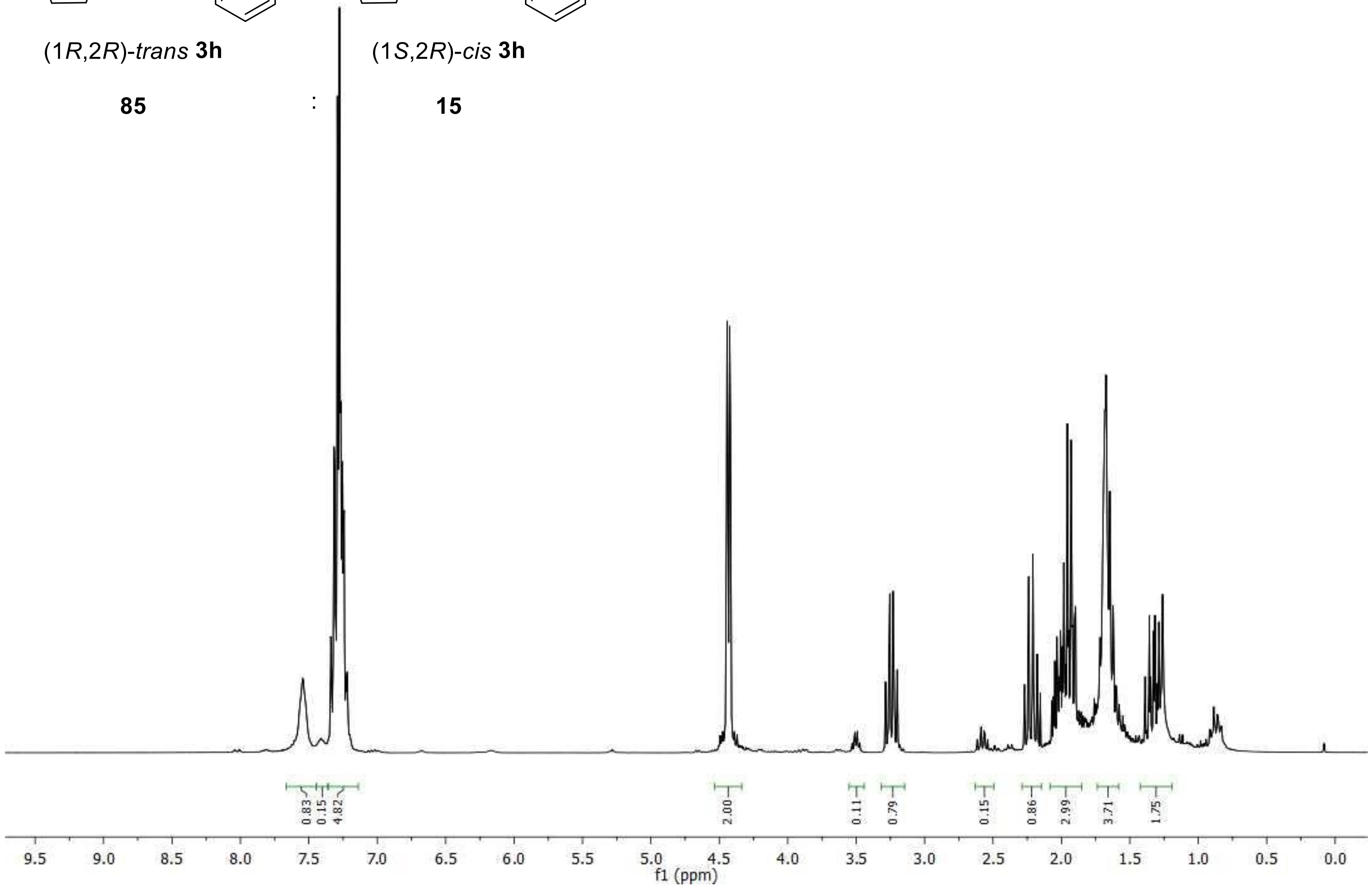
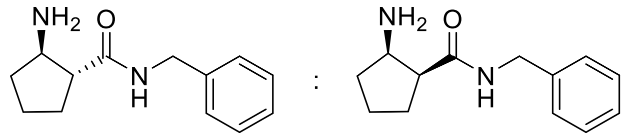


(1*R*,2*R*)-*trans* **3h** : (1*S*,2*R*)-*cis* **3h**

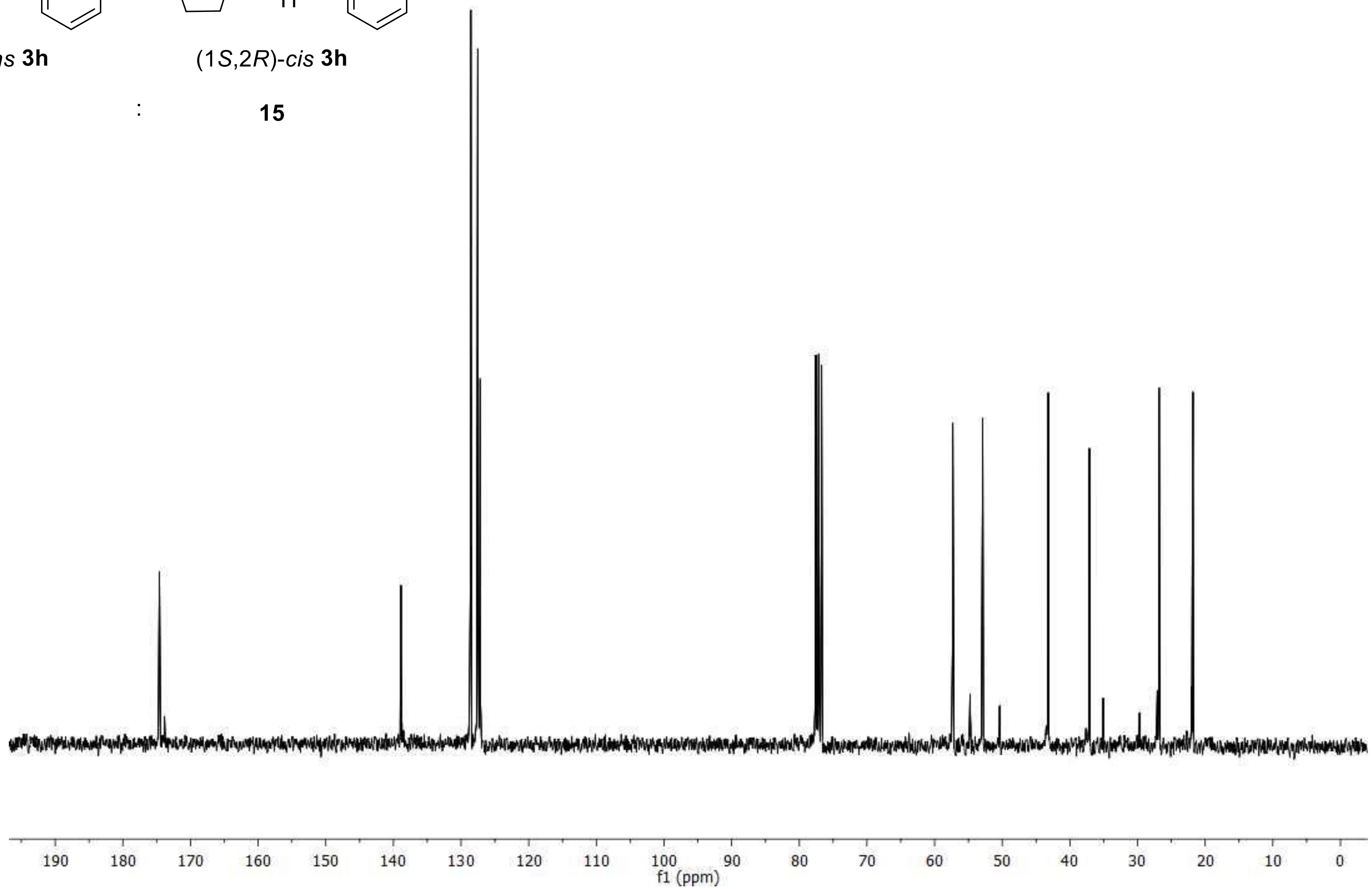
85 : **15**

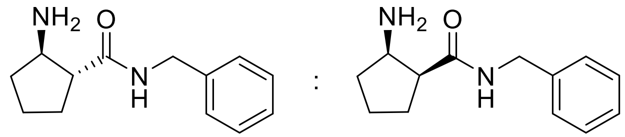




(1*R*,2*R*)-*trans* **3h** (1*S*,2*R*)-*cis* **3h**

85 : **15**





(1*R*,2*R*)-*trans* **3h** : (1*S*,2*R*)-*cis* **3h**

85 : **15**