Enantiopure *trans*-3-Arylaziridine-2-carboxamides: Preparation by Bacterial Hydrolysis and Ring Openings to (towards) Enantiopure, Unnatural D-α-Amino Acids

Roberto Morán-Ramallal, Ramón Liz, and Vicente Gotor

Departamento de Química Orgánica e Inorgánica and Instituto Universitario de Biotecnología de Asturias, Universidad de Oviedo, E-33071 Oviedo, Spain

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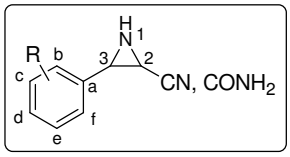
1. General

Organic extracts were dried over anhydrous Na_2SO_4. Thin-layer chromatography was performed on precoated TLC plates of silica gel, using potassium permanganate as developing reagent. For column chromatography, silica gel 60 (particle size, 40 - 63 μm) was used. Melting points are uncorrected. IR spectra were measured in CH_2Cl_2 (solids, except those of amino acids, for which Nujol was used) or as neat films (liquids). ^{1}H NMR and proton-decoupled ^{13}C NMR spectra (using $CDCl_3$ or CD_3OD generally) were obtained with two types of spectrometer: (1) 300.13 MHz for the ^{1}H and 75.48 MHz for the ^{13}C nuclei, and (2) 400.13 MHz for the ^{1}H and 100.63 MHz for the ^{13}C nuclei, using the δ scale (ppm) for chemical shifts; calibration was made on the $CDCl_3$ (^{13}C; 76.95 ppm) / CD_3OD (^{13}C; 49.0 ppm), the residual $CHCl_3$ (^{1}H; 7.26 ppm) / CHD_2OD (^{1}H; 3.35 ppm) signals or the appropriate solvent residual signal; ^{13}C NMR spectra were edited using DEPT techniques. Several unclear assignments of some ^{1}H and ^{13}C NMR spectra and the regioselectivity in the ring openings were deduced by analysis of the corresponding COSY, NOESY, HSQC and HMBC spectra. Enantiomeric excesses were determined with a high performance liquid chromatograph with UV detection.

Keys for NMR assignments.

- Aziridines



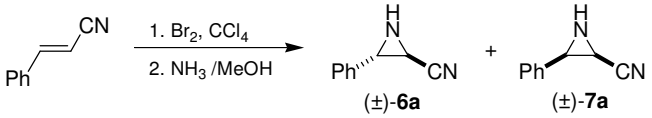
- Acyclic products. For the numbering of the chains, IUPAC rules are applied. Aromatic positions (*ipso, ortho, meta, para*) are referred to the point of union between the aromatic ring and the open chain.

2. Preparation of enantiopure 1-alkyl- and 1-arylaziridine-2-carboxamides 1

The preparations as racemates and further bacterial resolutions of 1-alkyl- and 1-arylaziridine-2-carboxamides, **1**, and -carbonitriles, **2**, were previously described by us.[1]

3. Preparation of racemic 3-phenylaziridine-2-carbonitriles *trans* (6a) and *cis* (7a)

Racemic aziridine-2-carbonitriles **6a** and **7a** were prepared starting from cinnamonitrile (predominantly *trans*) in two steps: bromination of the alkene followed by a Gabriel-Cromwell reaction (Scheme 1).



Scheme 1

(a) Bromination of cinnamonitrile (predominantly *trans*):[2] A solution of bromine (7.74 mmol, 0.40 mL) in CCl_4 (3 mL) was added dropwise over cinnamonitrile (7.74 mmol, 1.00 mL) in CCl_4 (3 mL) at 0 ºC and the resulting mixture was stirred overnight at room temperature. The mixture was then washed with saturated aqueous $Na_2S_2O_5$ (2 × 10 mL) and the aqueous layers were extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layers were dried and concentrated *in vacuo* to obtain (±)-2,3-dibromo-3-phenylpropanenitrile (2.20 g, 98%).

(b) Gabriel-Cromwell reaction. Gaseous ammonia was bubbled through MeOH (100 mL at 0º C) until saturation (15 min) in a well ventilated fumehood and the resulting solution was stored at −20 ºC. A sealable flask was charged with (±)-2,3-dibromo-3-phenylpropanenitrile (7.61 mmol, 2.20 g) and the above solution of NH_3 in MeOH (39 mL). After sealing the flask, stirring (−20 ºC to room temperature) was maintained for 3 days, and NH_3 an MeOH were then eliminated *in vacuo*. The crude material, which includes NH_4Cl, was extracted with CH_2Cl_2 (20 mL) and water (20 mL), the aqueous layer additionally extracted with CH_2Cl_2 (2 × 20 mL) and the combined organic layers then washed with brine and dried. After concentrating at reduced pressure and purifying by flash column chromatography [eluents: hexane/AcOEt (1) 5:1; (2) 4:1], *trans*-3-phenylaziridine-2-carbonitrile, (±)-**6a** (245 mg, 22% overall yield), and *cis*-3-phenylaziridine-2-carbonitrile, (±)-**7a** (245 mg, 22% overall yield) were obtained.

(±)-*trans*-3-Phenylaziridine-2-carbonitrile (6a). Cream-coloured solid; m.p. 61.0-62.5 ºC (lit.:[3] 58.0-60.0 ºC). IR (CH_2Cl_2): 3296, 2239 cm^{-1}. ^1H NMR ($CDCl_3$, 300.13 MHz): 7.52-7.08 (m, Ph), 3.56 (broad s, H^3), 2.32 (broad s, H^2), 1.98 (broad s, NH). ^{13}C NMR ($CDCl_3$, 75.48 MHz): 135.3 (C^a), 128.5, 125.6 (C^b + C^f, C^c + C^e), 128.2 (C^d), 118.6 (CN), 39.3 (C^3), 24.0 (C^2). ESI-MS (*m/z*, %): 289.1 [$(2M+H)^+$, 85], 145.1 [$(M+H)^+$, 100].

(±)-*cis*-3-Phenylaziridine-2-carbonitrile (7a). Cream-coloured solid; m.p. 102.2-103.5 ºC (lit.:[4] 105.0-106.0 ºC). IR (CH_2Cl_2): 3235, 2245 cm^{-1}. ^1H NMR ($CDCl_3$, 400.13 MHz): 7.47-7.30 (m, Ph), 3.48 (d, H^3, $^3J_{3,2}$ = 5.9 Hz), 2.91 (d, H^2, $^3J_{2,3}$ = 5.9 Hz), 1.71 (broad s, NH). ^{13}C NMR ($CDCl_3$, 100.63 MHz): 133.9 (C^a), 128.5, 126.8 (C^b + C^f, C^c + C^e), 128.4 (C^d), 117.3 (CN), 37.9 (C^3), 25.0 (C^2). ESI-MS (*m/z*, %): 289.1 [$(2M+H)^+$, 20], 145.1 [$(M+H)^+$, 100].

4. Synthesis of racemic 3-arylaziridine-2-carboxamides *trans* (8a-h) and *cis* (9a)

4.1. *trans*-3-Arylaziridine-2-carboxamides **8a-h**

Aziridine-2-carboxamides **8** were prepared according to Scheme 2. Two out of the eight *trans*-3-arylglycidates (±)-**10** [(±)-**10a** (R = Et) and (±)-**10h** (R = Me)] are commercially available.

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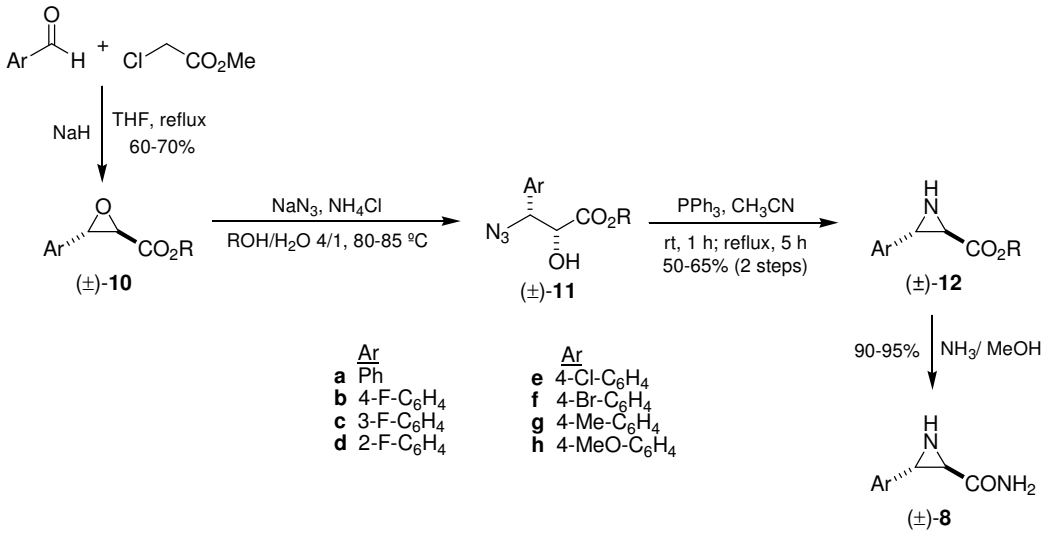

Scheme 2

(a) Darzens reaction.[5] Methyl chloroacetate (7.11 mmol, 0.62 mL) was added at 0 ºC under a nitrogen atmosphere to a stirred suspension of NaH (7.11 mmol, 171 mg) in anhydrous THF (13 mL). After 10 min, the corresponding aromatic aldehyde (7.11 mmol) was added and the reaction mixture was allowed to reach room temperature. When the evolution of hydrogen ceased, the mixture was refluxed for 24 h. After cooling again at 0 ºC, aqueous 0.5 M HCl (8 mL) was added, and the mixture then extracted with Et_2O (3 × 15 mL). The combined organic layers were dried and concentrated *in vacuo*, and the crude material thus obtained was purified by flash column chromatography [eluents: hexane/AcOEt (1) 15:1; (2) 10:1] to obtain the corresponding *trans*-3-arylglycidate, (±)-**10** (60-70% yield).

*(b) Ring opening of epoxides (±)-**10** with NaN_3*.[6] NH_4Cl (19.2 mmol, 1.02 g) and NaN_3 (7.65 mmol, 498 mg) were added to a solution of the corresponding epoxide (±)-**10** (3.83 mmol) in ROH/H_2O (7.5 mL; R = Me or Et, the same alkyl group present in **10**) and the resulting mixture was heated overnight at 80-85 ºC. The solvent was removed under reduced pressure, water (15 mL) was added, and the mixture was then extracted with AcOEt (3 × 15 mL). After drying the combined organic layers and removing the solvents *in vacuo*, the corresponding azido(hydroxy)ester, (±)-**11**, was obtained and used in the next step without further purification.

*(c) Staudinger reaction and further evolution to (±)-**12***.[7] PPh_3 (3.10 mmol, 814 mg) was added under a nitrogen atmosphere to a stirred solution of the corresponding azido(hydroxy)ester (±)-**11** (3.00 mmol) in anhydrous CH_3CN (12 mL) in the presence of activated molecular sieves (4 Å). The mixture was stirred at room temperature until the evolution of nitrogen ceased (*ca*. 1 h) and was then additionally refluxed for 5 h. After cooling at room temperature, the reaction mixture was filtered through a pad of diatomaceous earth, and this one washed with CH_2Cl_2 (2 × 5 mL). The removal of the solvents under reduced pressure gave a crude material which was further purified by flash column chromatography [eluents: hexane/AcOEt (1) 15:1; (2) 7:1] to obtain the corresponding alkyl (±)-*trans*-3-arylaziridine-2-carboxylate, (±)-**12** [50-65% overall yield for the steps (b) and (c)].

(d) Conventional ammonolysis. Each aziridine-2-carboxylate (±)-**12** (2.00 mmol) was treated (sealed flask, room temperature, 36 h) with a saturated solution of NH_3 in MeOH [10 mL; see the above protocol (2b)], to isolate, after removal of the solvent *in vacuo*, the corresponding *trans*-aziridineamide, (±)-**8** (*ca*. 95% yield).

4.2. *cis*-3-Phenylaziridine-2-carboxamide **9a**

A solution of *cis*-3-phenylaziridine-2-carbonitrile, (±)-**7a**, (0.347 mmol, 50 mg) in EtOH (500 μL) was added to a *R. rhodochrous* IFO 15564 bacterial suspension (A_{650} = 6.0) in fresh 0.10 M potassium phosphate buffer pH 7.0 (50 mL). After 5 h (28 ºC, 200 rpm), no nitrile was observed by TLC and the biotransformation was stopped by centrifugation. The supernatant liquid was filtered through a pad of diatomaceous earth and then continuously extracted (CH_2Cl_2, 6 h). After drying the organic layer, low pressure removal of the solvent yielded (±)-*cis*-3-phenylaziridine-2-carboxamide, (±)-**9a** (47 mg, 84%), the racemic nature of which was corroborated by HPLC.

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(±)-*cis*-3-Phenylaziridine-2-carboxamide (9a). White solid; m.p. 168.5-170.0 ºC (lit.:[8] 179.0-180.0 ºC). IR (CH_2Cl_2): 3396, 3318, 3241, 1656 cm^{-1}. ^1H NMR (CD_3OD, 300.13 MHz): 7.42 (d, H^b + H^f, ^3J = 6.8 Hz), 7.37-7.20 (m, H^c + H^e, H^d), 3.53 (d, H^3, $^3J_{3,2}$ = 7.0 Hz), 2.98 (d, H^2, $^3J_{2,3}$ = 7.0 Hz). ^{13}C NMR (CD_3OD, 75.48 MHz): 173.8 (CO), 137.1 (C^a), 129.0, 128.8 (C^b + C^f, C^c + C^e), 128.3 (C^d), 39.4 (C^3), 39.0 (C^2). ESI-MS (*m/z*, %): 325.1 [$(2M+H)^+$, 85], 163.0 [$(M+H)^+$, 100].

5. Bacterial resolution of the aziridine-2-carbonitriles 6a, 7a and -carboxamides 8a-h, 9a by *R. rhodochrous* catalyzed hydrolysis

5.1. Cultures of *Rhodococcus rhodochrous* IFO 15564

The bacterium was maintained at 4 ºC on Petri plates containing peptone (10 g/L), yeast extract (2 g/L), $MgSO_4·7H_2O$ (1 g/L) and agar (15 g/L). Sub-culturing was performed every three months. The bacterium was grown in a liquid culture medium[9] whose components were heat-sterilized (115 ºC, 20 min) in two separate groups. Group 1: glucose (15.0 g), yeast extract (1.0 g), KH_2PO_4 (0.50 g), K_2HPO_4 (0.50 g) and distilled water (950 mL), with pH adjusted at 7.2; group 2: ε-caprolactam (5.0 g), $MgSO_4·7H_2O$ (0.50 g) and distilled water (50 mL). A loop of solid culture of *R. rhodochrous* IFO 15564, from an agar plate, was sowed on 100 mL of the above sterilized medium in 250 mL sterilized erlenmeyer flasks. After growing (rotary shaker, 200 rpm, 28 ºC) for 36-42 hours, cells were harvested by centrifugation (5000 rpm, 3 min), washed with aqueous 0.10 M KH_2PO_4–K_2HPO_4 buffer pH 7.0 (20 mL), again collected by centrifugation and then resuspended in the same, fresh potassium phosphate buffer, adjusting the absorbance (A_{650}) of the bacterial suspension to the needed value. Absorbance values for the different substrates: 0.3 [(±)-**6a**], 3.0 [(±)-**8a-h**], 6.0 [(±)-**7a**, (±)-**9a**]. The standard absorbance A_{650} = 3.0 corresponded to 265 mg of bacterial dry weight (BDW) per 100 mL of bacterial suspension in phosphate buffer.

5.2. Biotransformations with *Rhodococcus rhodochrous* IFO 15564

See the Experimental Part. The bacterial activities in the hydrolysis of the *trans*-3-arylaziridine-2-carboxamides (±)-**8,** determined in the linear initial phase of the conversion curves, range from 1.4 to 4.7 nmol min^{-1} mg^{-1} of BDW.

(2*R*,3*S*)-3-Phenylaziridine-2-carboxamide [(2*R*,3*S*)-8a]. White solid; m.p. 136.5-137.5 ºC (lit.:[8] 134.5-136.0 ºC). $[\alpha]_D^{20}$ = −211.9 (*c* 1.0, MeOH) [lit.:[10] −260.0 (*c* 0.5, MeOH)]. *ee* > 99.5%. IR (CH_2Cl_2): 3385, 3300, 1667 cm^{-1}. ^1H NMR (CD_3OD, 300.13 MHz): 7.40-7.25 (m, Ph), 3.19 (broad s, H^3), 2.68 (broad s, H^2). ^{13}C NMR (CD_3OD, 75.48 MHz): 173.8 (CO), 139.4 (C^a), 129.5 (C^c + C^e), 128.7 (C^d), 127.3 (C^b + C^f), 40.6 (C^2), 40.2 (C^3). ESI-MS (*m/z*, %): 347.0 [$(2M+Na)^+$, 100], 185.1 [$(M+Na)^+$, 12]. ESI-TOF-HRMS calcd. for $C_9H_{10}N_2O$, 163.0866 $(M+H)^+$; found, 163.0871.

(2*R*,3*S*)-3-(*p*-Fluorophenyl)aziridine-2-carboxamide [(2*R*,3*S*)-8b]. White solid; m.p. 157.0-158.5 ºC. $[\alpha]_D^{20}$ = −214.0 (*c* 0.93, MeOH). *ee* > 99.5%. IR (CH_2Cl_2): 3500, 3365, 1666 cm^{-1}. ^1H NMR (CD_3OD, 300.13 MHz): 7.34 (dd, H^b + H^f, ^3J = 8.8, $^4J_{H-F}$ = 5.4 Hz), 7.08 (t, H^c + H^e, ^3J = 8.8 Hz), 3.19 (broad s, H^3), 2.66 (broad s, H^2). ^{13}C NMR (CD_3OD, 75.48 MHz): 173.7 (CO), 163.7 (d, C^d, $^1J_{C-F}$ = 242.5 Hz), 135.5 (C^a), 129.1 (d, C^b + C^f, $^3J_{C-F}$ = 8.1 Hz), 116.1 (d, C^c + C^e, $^2J_{C-F}$ = 22.0 Hz), 40.6 (C^2), 39.6 (C^3). ESI-MS (*m/z*, %): 361.1 [$(2M+H)^+$, 8], 181.0 [$(M+H)^+$, 100]. ESI-TOF-HRMS calcd. for $C_9H_9FN_2O$, 181.0772 $(M+H)^+$; found, 181.0773.

(2*R*,3*S*)-3-(*m*-Fluorophenyl)aziridine-2-carboxamide [(2*R*,3*S*)-8c]. White solid; m.p. 143.5-144.5 ºC. $[\alpha]_D^{20}$ = −219.1 (*c* 0.87, MeOH). *ee* > 99.5%. IR (CH_2Cl_2): 3500, 3384, 1669 cm^{-1}. ^1H NMR (CD_3OD, 300.13 MHz): 7.36 (c, H^e, ^3J = 7.8 Hz), 7.16 (d, H^f, ^3J = 7.8 Hz), 7.10-6.97 (m, H^b + H^d), 3.21 (broad s, H^3), 2.66 (broad s, H^2). ^{13}C NMR (CD_3OD, 75.48 MHz): 173.5 (CO), 164.4 (d, C^c, $^1J_{C-F}$ = 242.5 Hz), 142.6 (d, C^a, $^3J_{C-F}$ = 7.5 Hz), 131.2 (d, C^e, $^3J_{C-F}$ = 8.1 Hz), 123.4 (C^f), 115.4 (d, C^b or C^d, $^2J_{C-F}$ = 20.8 Hz), 113.8 (d, C^d or C^b, $^2J_{C-F}$ = 22.6 Hz), 40.9 (C^2), 39.6 (C^3). ESI-MS (*m/z*, %): 361.1 [$(2M+H)^+$, 5], 181.0 [$(M+H)^+$, 100].

(2*R*,3*S*)-3-(*o*-Fluorophenyl)aziridine-2-carboxamide [(2*R*,3*S*)-8d]. White solid; m.p. 139.4-140.3 ºC. $[\alpha]_D^{20}$ = −179.9 (*c* 1.0, MeOH). *ee* > 99.5%. IR (CH_2Cl_2): 3450, 3397, 1665 cm^{-1}. ^1H NMR (CD_3OD, 400.13 MHz): 7.28 (m, H^d + H^f), 7.15 (t, H^e, ^3J = 7.3 Hz), 7.09 (dd, H^c, $^3J_{H-F}$ = 10.2, ^3J = 8.6 Hz), 3.35 (broad s, H^3), 2.67 (broad s, H^2). ^{13}C NMR (CD_3OD, 100.63 MHz): 173.6 (CO), 163.1 (d, C^b, $^1J_{C-F}$ = 244.0 Hz), 130.2 (d, C^f, $^3J_{C-F}$ = 8.1 Hz), 128.1 (C^e), 126.5 (d, C^a, $^2J_{C-F}$ = 13.2 Hz), 125.4 (d, C^d, $^3J_{C-F}$ = 2.9 Hz), 116.0 (d, C^c, $^2J_{C-F}$ = 21.3 Hz), 39.5 (C^2), 34.3 (C^3). ESI-MS (*m/z*, %): 361.0 [$(2M+H)^+$, 8], 181.0 [$(M+H)^+$, 100].

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[9] Kakeya, H.; Sakai, N.; Sugai, T.; Ohta, H. *Tetrahedron Lett.* **1991**, *32*, 1343–1346.
[10] Wang, J.-Y.; Wang, D.-X.; Pan, J.; Huang, Z.-T.; Wang, M.-X. *J. Org. Chem.* **2007**, *72*, 9391–9394.

(2*R*,3*S*)-3-(*p*-Chlorophenyl)aziridine-2-carboxamide [(2*R*,3*S*)-8e]. See the Experimental Part.

(2*R*,3*S*)-3-(*p*-Bromophenyl)aziridine-2-carboxamide [(2*R*,3*S*)-8f]. White solid; m.p. 178.5-179.5 ºC. $[\alpha]_D^{20}$ = −200.0 (*c* 0.53, MeOH). *ee* > 99.5%. IR (CH_2Cl_2): 3460, 3368, 1665 cm^{-1}. ^1H NMR (CD_3OD, 300.13 MHz): 7.51 (d, H^c + H^e, ^3J = 8.4 Hz), 7.25 (d, H^b + H^f, ^3J = 8.4 Hz), 3.17 (broad s, H^3), 2.64 (broad s, H^2). ^{13}C NMR (CD_3OD, 75.48 MHz): 173.6 (CO), 139.0 (C^d), 132.6 (C^c + C^e), 129.2 (C^b + C^f), 122.3 (C^a), 40.8 (C^2), 39.6 (C^3). ESI-MS (*m/z*, %): 242.9 [$(M+H)^+$, 83], 241.9 [$(M+H)^+$, 10], 240.9 [$(M)^+$, 100]. EI-HRMS calcd. for $C_9H_9BrN_2O$, 240.9881; found, 240.9893.

(2*R*,3*S*)-3-(*p*-Tolyl)aziridine-2-carboxamide [(2*R*,3*S*)-8g]. White solid; m.p. 165.2-166.3 ºC. $[\alpha]_D^{20}$ = −225.4 (*c* 0.8, MeOH). *ee* > 99.5%. IR (CH_2Cl_2): 3349, 3245, 1666 cm^{-1}. ^1H NMR (CD_3OD, 300.13 MHz): 7.25-7.10 (m, Ar), 3.15 (broad s, H^3), 2.66 (broad s, H^2), 2.35 (s, CH_3). ^{13}C NMR (CD_3OD, 75.48 MHz): 173.8 (CO), 138.6 (C^d), 136.3 (C^a), 130.1 (C^c + C^e), 127.2 (C^b + C^f), 40.4 (C^2), 40.1 (C^3), 21.2 (CH_3). ESI-MS (*m/z*, %): 353.2 [$(2M+H)^+$, 5], 177.0 [$(M+H)^+$, 100].

(±)-*trans*-3-(*p*-Anisyl)aziridine-2-carboxamide (8h). White solid; m.p. 124.0-126.0 ºC (lit.:[8] 128.0-129.0 ºC). IR (CH_2Cl_2): 3372, 3190, 1664 cm^{-1}. ^1H NMR (CD_3OD, 300.13 MHz): 7.22 (d, H^b + H^f, ^3J = 8.7 Hz), 6.90 (d, H^c + H^e, ^3J = 8.7 Hz), 3.80 (s, OCH_3), 3.14 (broad s, H^3), 2.66 (broad s, H^2). ^{13}C NMR (CD_3OD, 75.48 MHz): 173.9 (CO), 160.8 (C^d), 131.2 (C^a), 128.4 (C^b + C^f), 114.9 (C^c + C^e), 55.7 (OCH_3), 40.3 (C^2), 40.0 (C^3). ESI-MS (*m/z*, %): 407.0 [$(2M+Na)^+$, 100], 215.0 [$(M+Na)^+$, 35], 193.0 [$(M+H)^+$, 15].

2-(*p*-Tolyl)ethanol (14g).[11] Colorless oil. IR (film): 3370 cm^{-1}. ^1H NMR ($CDCl_3$, 300.13 MHz): 7.17-7.05 (m, Ar), 3.84 [t, C(1)H_2, ^3J = 6.6 Hz], 2.84 [t, C(2)H_2, ^3J = 6.6 Hz], 2.34 (s, CH_3), 1.58 (broad s, OH). ^{13}C NMR ($CDCl_3$, 100.63 MHz): 135.9, 135.2 (C_{ipso}, C_{para}), 129.2, 128.8 ($2C_{ortho}$, $2C_{meta}$), 63.7 (C^1), 38.7 (C^2), 20.9 (CH_3). ESI-MS (*m/z*, %): 249.2 (40), 119.0 [$(M+H-H_2O)^+$, 100], 105 (65), 91.1 (40).

2-(*p*-Anisyl)ethanol (14h).[11] Colorless oil. IR (film): 3375 cm^{-1}. ^1H NMR (300.13 MHz): 7.15 (d, $2H_{ortho}$, ^3J = 8.7 Hz), 6.86 (d, $2H_{meta}$, ^3J = 8.7 Hz), 3.81 [t, C(1)H_2, ^3J = 6.6 Hz], 3.79 (s, OCH_3), [t, C(2)H_2, ^3J = 6.6 Hz], 1.64 (broad s, OH). ESI-MS (*m/z*, %): 135.0 [$(M+H-H_2O)^+$, 100], 65.0 (40).

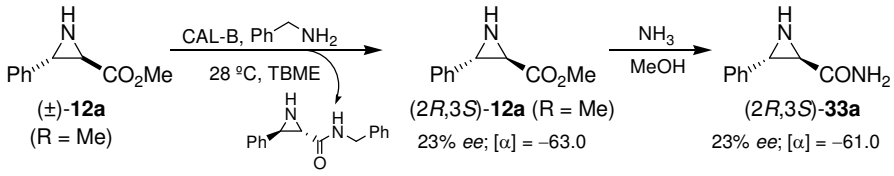
6. Bioreduction of phenylacetaldehyde, 16a, with *R. rhodochrous* IFO 15564

The procedure is similar to those describe above (see 5.2) and in the Experimental Part. Starting now from a solution of phenylacetaldehyde, **16a**, (0.833 mmol, 97 µL) in EtOH (1.0 mL), and using a bacterial suspension of A_{650} = 3.0, 2-phenylethanol, **14a**, was obtained (66 mg, 65%).

2-Phenylethanol (14a).[11] Colorless oil. IR (film): 3366 cm^{-1}. ^1H NMR ($CDCl_3$, 300.13 MHz): 7.41-7.20 (m, Ph), 3.85 [t, C(1)H_2, ^3J = 6.7 Hz], 2.89 [t, C(2)H_2, ^3J = 6.7 Hz], 2.24 (broad s, OH). ^{13}C NMR ($CDCl_3$, 75.48 MHz): 138.4 (C_{ipso}), 128.9, 128.4 ($2C_{ortho}$, $2C_{meta}$), 126.2 (C_{para}), 63.4 (C^1), 39.0 (C^2).

7. Assigment of the absolute configuration of the enantiopure *trans*-3-arylaziridine-2-carboxamides (2*R*,3*S*)-8a,g

7.1. (2*R*,3*S*)-3-Phenylaziridine-2-carboxamide, (2*R*,3*S*)-**8a** (Scheme 3)



Scheme 3

*(a) Preparation of methyl (±)-trans-3-phenylaziridine-2-carboxylate, (±)-**12a** (R = Me).* See the above Section 4.1, steps (a, b, c).

*(b) Enzymatic preparation of methyl (2R,3S)-(−)-3-phenylaziridine-2-carboxylate, (2R,3S)-(−)-**12a** (R = Me). tert*-Butyl methyl ether (1.4 mL) and benzylamine (0.176 mmol, 19 µL) were added under a nitrogen atmosphere to a mixture of the racemic ester (±)-**12a** (R = Me; 0.294 mmol, 52 mg), CAL-B (28 mg) and 4 Å molecular sieves (15 mg). After rotary shaking (28 ºC, 200 rpm) during 15 hours the aminolysis progressed until a conversion greater than 50%. The reaction mixture was then filtered through a pad of diatomaceous earth, and this one washed with CH_2Cl_2 (2 × 2 mL). After removal of the solvents under reduced pressure, the resulting residue was purified by flash column chromatography

[11] Gómez, C.; Maciá, B.; Lillo, V. J.; Yus, M. *Tetrahedron* **2006**, *62*, 9832–9839.

(hexane/AcOEt 8:1 as eluent) to obtain (2*R*,3*S*)-(−)-**12a** (R = Me) with 23% *ee* and [α] = −63.0 (*c* 1.0, $CHCl_3$) {lit.[12] for (2*S*,3*R*)-(+)-**12a** (R = Me): [α] = +210.0 (*c* 0.7, $CHCl_3$), 81% *ee*}.

*(c) Preparation of (2R,3S)-(−)-3-phenylaziridine-2-carboxamide, (2R,3S)-(−)-**8a**.* A conventional ammonolysis [see the above Section 4.1, step (d)] of (2*R*,3*S*)-(−)-**12a** (R = Me) led to the aziridineamide (2*R*,3*S*)-(−)-**8a** with 23% *ee* and [α] = −61.0 (*c* 0.31, MeOH).

7.2. (2*R*,3*S*)-3-(*p*-Tolyl)aziridine-2-carboxamide, (2*R*,3*S*)-**8g**

In this case, (2*R*,3*S*)-**8g** was transformed into β-(*p*-tolyl)-D-alanine·HCl, (*R*)-**35g**, [α] = +10.0 (*c* 1.0, H_2O) {lit.[13] for (*S*)-**35g**: [α] = −6.5 (*c* 0.1, 0.5 M HCl)}. The experimental procedure includes three successive steps (hydrogenolysis, *N-tert*-butoxycarbonylation, and hydrolysis) identical to those described in the Experimental Part for (2*R*,3*S*)-**8a**, (*R*)-**31a** and (*R*)-**34a**, respectively.

8. Chiral HPLC analyses of the compounds involved in the biotransformations

General analysis conditions (except otherwise established): Column, Chiralpak IA. Column temperature, 20 ºC. Eluent, hexane/2-propanol 90:10 (V/V). Eluent flow, 0.8 mL/min. Detector, UV, λ = 210, 215 nm. Substrate concentration, 1 mg/mL.

Compound	t_{R1} (min)[a]	t_{R2} (min)[a]	R_S[b]
(±)-**6a**	12.9 (2*S*,3*R*)	13.9 (2*R*,3*S*)	1.8
(±)-**7a**[c]	17.9	30.0	> 3.0
(±)-**8a**	14.3 (2*S*,3*R*)	17.6 (2*R*,3*S*)	> 3.0
(±)-**8b**	14.7 (2*S*,3*R*)	20.3 (2*R*,3*S*)	> 3.0
(±)-**8c**	13.4 (2*S*,3*R*)	15.2 (2*R*,3*S*)	2.6
(±)-**8d**	13.3 (2*S*,3*R*)	16.1 (2*R*,3*S*)	> 3.0
(±)-**8e**	16.1 (2*S*,3*R*)	22.6 (2*R*,3*S*)	> 3.0
(±)-**8f**	16.8 (2*S*,3*R*)	25.3 (2*R*,3*S*)	> 3.0
(±)-**8g**	14.7 (2*S*,3*R*)	20.0 (2*R*,3*S*)	> 3.0
(±)-**8h**	21.4	29.6	> 3.0
(±)-**9a**[c]	19.8	37.2	> 3.0
(±)-**12a** (R = Me)[d]	10.0 (2*R*,3*S*)	15.0 (2*S*,3*R*)	> 3.0

[a] Retention time. [b] Resolution factor. [c] Chiralcel OD; 1.0 ml/min. [d] Chiralcel OD

9. Hydrolysis of the *N*-Boc group in the azido(carbamoyl)carbamate (2*R*,3*R*)-23

A mixture of (3*R*)-β-azido-N^2-Boc-D-phenylalaninamide, (2*R*,3*R*)-**23** (0.0951 mmol, 29 mg), and aqueous 3 M HCl (3.5 mL) was stirred overnight at room temperature and was then basified with solid NaOH at 0 ºC. The resulting basic mixture was extracted with CH_2Cl_2 (3 × 10 mL) and the combined organic layers dried. After removing solvents *in vacuo*, (3*R*)-β-azido-D-phenylalaninamide [(2*R*,3*R*)-2-amino-3-azido-3-phenylpropanamide], (2*R*,3*R*)-**22** (58 mg, 92%), was isolated.

10. Assignment of the absolute configuration of the ring opening product (2*R*,3*R*)-22

(See printed text, Schemes 8 and 9).

*(a) Preparation of the methyl ester (2*R*,3*R*)-**27**.* Starting from methyl (±)-3-phenylaziridine-2-carboxylate, (±)-**12a** (R = Me), in four successive steps (printed text, Scheme 9): (i) enzymatic aminolysis [see the above Section 7.1, step (b)]; (ii) ring opening of the aziridine with $NaN_3/AlCl_3$[1]; (iii) *N-tert*-butoxycarbonylation [see an identical reaction for (*R*)-**31a** in the Experimental Part]; (iv) catalytic hydrogenolysis of the azido group [see an identical process for (2*R*,3*R*)-**23** in the Experimental Part].

[12] Yamagiwa, N.; Quin, H.; Matsunaga, S.; Shibasaki, M.; *J. Am. Chem. Soc.* **2005**, *127*, 13419–13427.
[13] Zhuze, A.; Lost, K.; Kasafirek, E.; Rudinger, J. *Coll. Czech. Chem. Commun.* **1964**, *29*, 2648–2662.

$[\alpha]_D^{20}$ found for (2*R*,3*R*)-**27**: −7.5 (*c* 0.6, $CHCl_3$), 20% *ee*; described[14] for (2*S*,3*S*)-**27,** +29.0 (*c* 0.9, $CHCl_3$).

*(b) Transformation of the methyl ester (2*R,*3*R*)-**27** into the amide (2*R,*3*R*)-**24***. It was carried out by conventional ammonolysis [see the above Section 4.1, step (d)]. $[\alpha]_D^{20}$ values for (2*R*,3*R*)-**24**: sample proceeding from this methodology, −2.5 (*c* 0.53, MeOH), 20% *ee*; sample coming from the ring opening of (2*R*,3*S*)-**8a** and further elaboration, −13.8 (*c* 1.0, MeOH).

11. Chiral HPLC analyses of the ring opening products (after *N-tert*-butoxycarbonylation)

General analyses conditions (except otherwise established). Column, Chiralcel OD. Column temperature, 20 ºC. Eluent flow, 0.8 ml/min. Detector, UV, λ = 210, 215 nm. Substrate concentration, 1 mg/mL.

Compound	Eluent (V/V)	t_{R1} (min)[a]	t_{R2} (min)[a]	R_S[b]
(±)-**18**[c]	H/IPA 90:10	11.7 (2*S*,3*S*)	13.3 (2*R*,3*R*)	2.1
(±)-**20**[d]	H/IPA 80:20	13.9(2*S*,3*S*)	22.8 (2*R*,3*R*)	> 3.0
(±)-**23**	H/IPA 90:10	8.6 (2*R*,3*R*)	10.7 (2*S*,3*S*)	2.0
(±)-**34a**	H/IPA 92:8	12.0 (*S*)	13.9 (*R*)	1.6
(±)-**34g**	H/IPA 95:5	17.1 (*S*)	18.9 (*R*)	1.5

[a] Retention time. [b] Resolution factor. [c] Chiralpak IA. [d] Chiralpak AS

12. Miscellaneous products

β-(*p*-Tolyl)-D-alaninamide [(R)-31g]. White solid; m.p. 128.0-129.5 ºC. $[\alpha]_D^{20}$ = −10.2 (*c* 1.0, MeOH). IR (CH_2Cl_2): 3434, 3356, 3292, 1682 cm^{-1}. ^1H NMR (CD_3OD, 300.13 MHz): 7.10-7.22 (m, Ar), 3.57 (t, H^2, ^3J = 6.7 Hz), 3.02 [dd, C(3)*H*H, ^2J = 13.4, ^3J = 5.8 Hz], 2.83 [dd, C(3)H*H*, ^2J = 13.4, ^3J = 7.6 Hz]. ^{13}C NMR (CD_3OD, 75.48 MHz): 179.7 (CO), 137.4, 135.7 (C_{ipso}, C_{para}), 130.3, 130.2 ($2C_{ortho}$, $2C_{meta}$), 57.4 (C^2), 42.1 (C^3), 21.1 (CH_3). ESI-MS (*m/z*, %): 357.2 [$(2M+H)^+$, 15], 179.1 [$(M+H)^+$, 100].

***N*^{2}-Boc-β-(*p*-tolyl)-D-alaninamide [(R)-34g]**. White solid; m.p. 172.5-174.0 ºC. $[\alpha]_D^{20}$ = −19.0 (*c* 1.0, $CHCl_3$). *ee* > 99.5%. IR (CH_2Cl_2): 3476, 3393, 3248, 1689, 1662 cm^{-1}. ^1H NMR ($CDCl_3$, 300.13 MHz): 7.18-7.00 (m, Ar), 6.00, 5.79 (broad s, $CONH_2$), 5.16 (d, N*H*Boc, ^3J = 7.9 Hz), 4.35 (m, H^2), 3.01 [d, C(3)H_2, ^3J = 6.3 Hz], 2.31 (s, CH_3), 1.40 [s, $(CH_3)_3C$]. ^{13}C NMR ($CDCl_3$, 75.48 MHz): 173.9 ($CONH_2$), 155.4 (NH*C*OOBu^t), 136.4, 133.4 (C_{ipso}, C_{para}), 129.3, 129.1 ($2C_{ortho}$, $2C_{meta}$), 80.1 (O*C*Me$_3$), 55.4 (C^2), 38.0 (C^3), 28.2 [C(*C*H$_3$)$_3$], 20.9 (CH_3). ESI-MS (*m/z*, %): 457.2 [$(2M+H-C_4H_8-CO_2)^+$, 85], 352.2 (100), 279.1 [$(M+H)^+$, 15], 223.0 [$(M+H-C_4H_8)^+$, 70], 179.2 [$(M+H-C_4H_8-CO_2)^+$, 55].

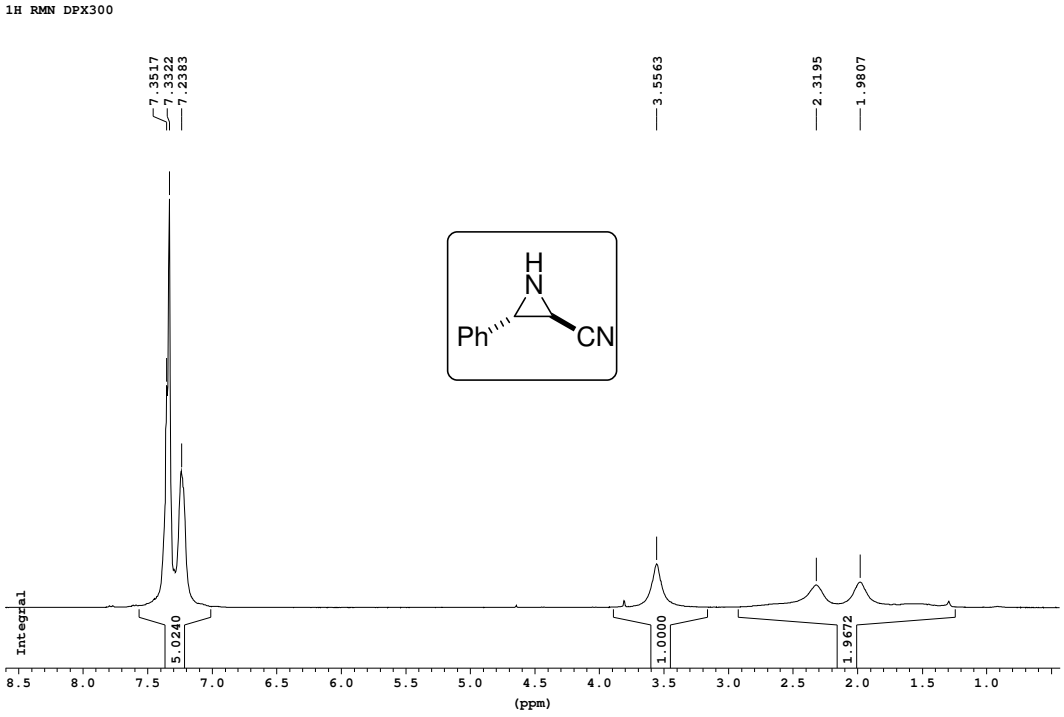
D-Phenylalanine hydrochloride [(R)-35a]. White solid; m.p. 220.0-221.0 ºC (dec.) [lit.:[15] 215.0-216.0 ºC (dec.) for (*S*)-**35a**]. $[\alpha]_D^{20}$ = +8.8 (*c* 1.1, H_2O) [lit.:[15] −8.1 (*c* 0.91, H_2O) for (*S*)-**35a**]. IR (nujol): 3132, 3019, 1732 cm^{-1}. ^1H NMR (D_2O, 300.13 MHz): 7.22-7.00 (m, Ph), 4.05 (m, H^2), 3.03 [m, C(3)H_2]. ^{13}C NMR (D_2O, 100.63 MHz): 171.8 (CO), 133.9 (C_{ipso}), 129.1, 129.0 ($2C_{ortho}$, $2C_{meta}$), 127.7 (C_{para}), 55.9 (C^2), 35.4 (C^3). ESI-MS (*m/z*, %): 166.0 (M^+, 100).

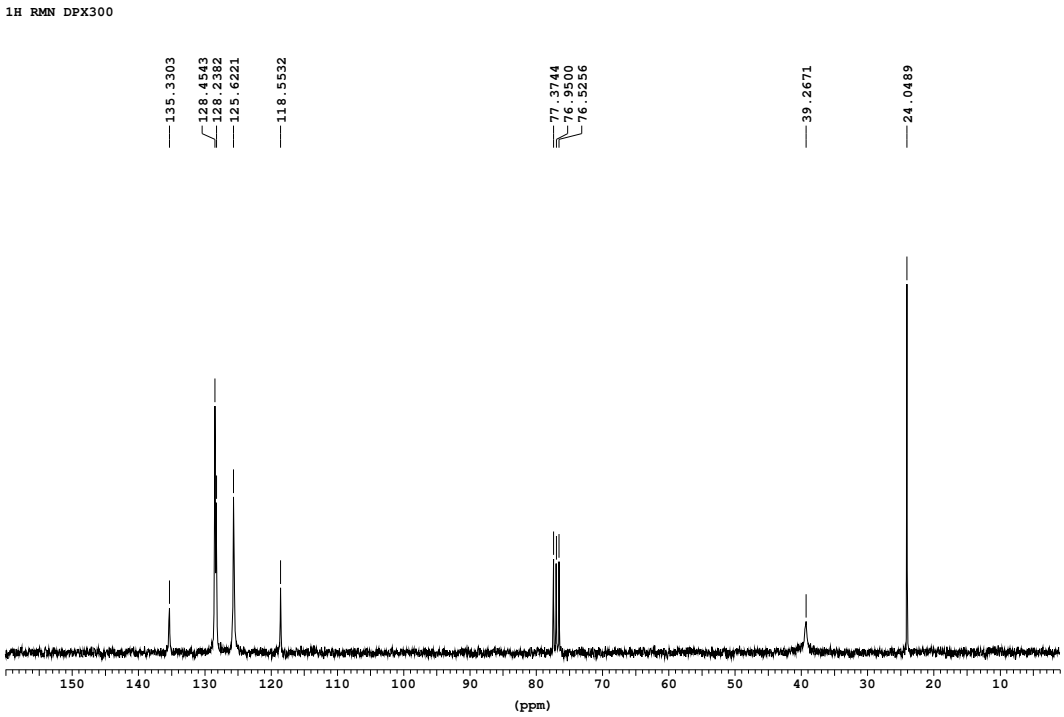
13. NMR section: ^1H, ^{13}C and HMBC spectra

See the following pages.

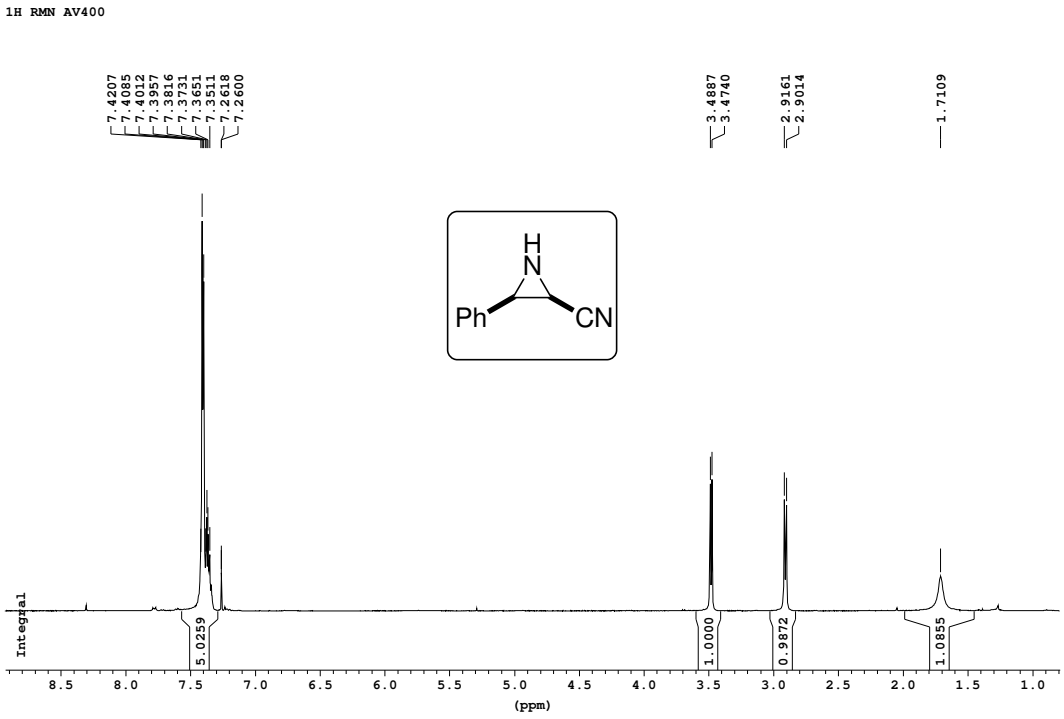
[14] Capone, S.; Guaragna, A.; Palumbo, G.; Pedatella, S. *Tetrahedron* **2005**, *61*, 6575–6579.
[15] Barfoot, C. W.; Harvey, J. E.; Kenworthy, M. N.; Kilburn, J. P.; Ahmed, M.; Taylor, R. J. K. *Tetrahedron* **2005**, *61*, 3403-3417.

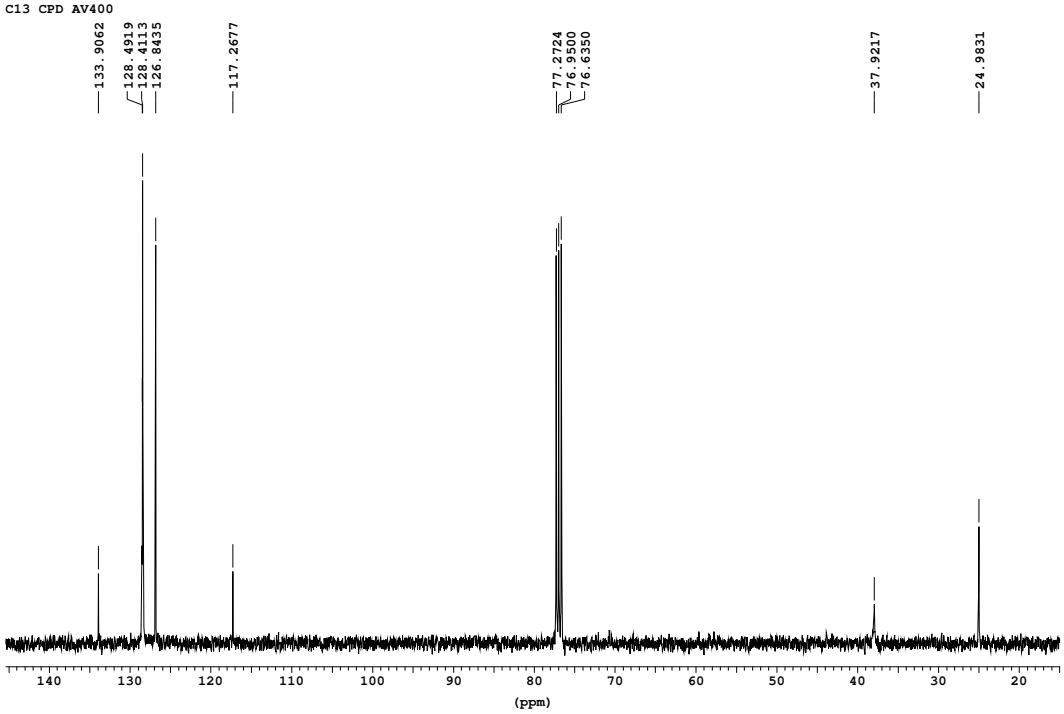
(±)-*trans*-3-Phenylaziridine-2-carbonitrile (6a)



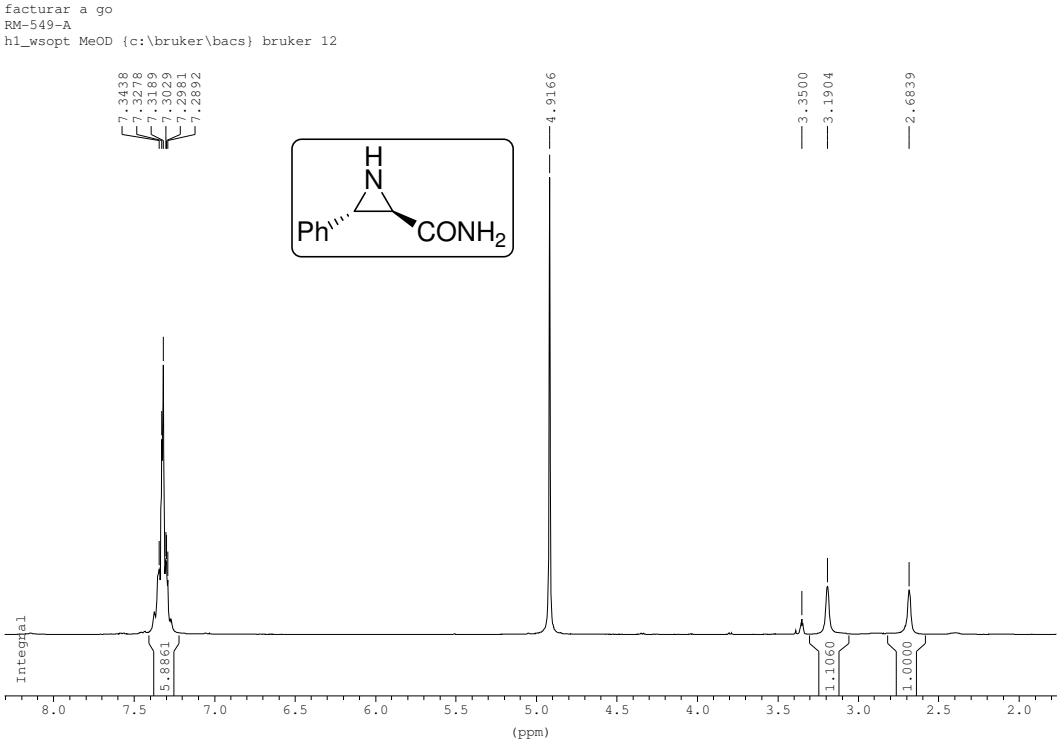


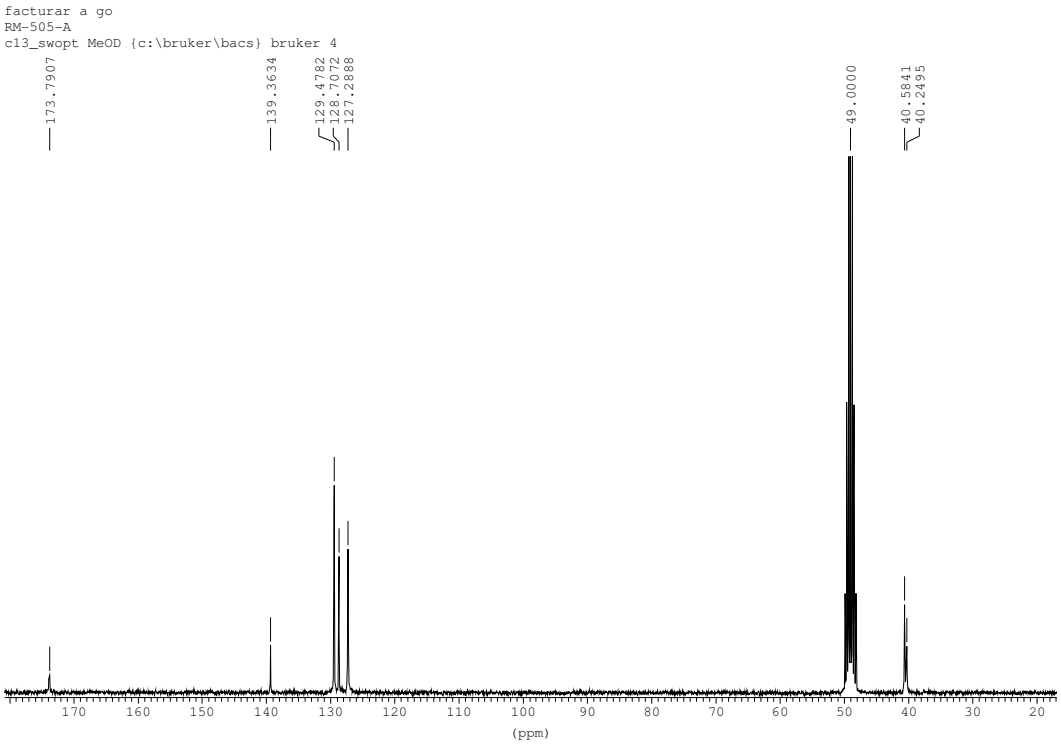
(±)-*cis*-3-Phenylaziridine-2-carbonitrile (7a)



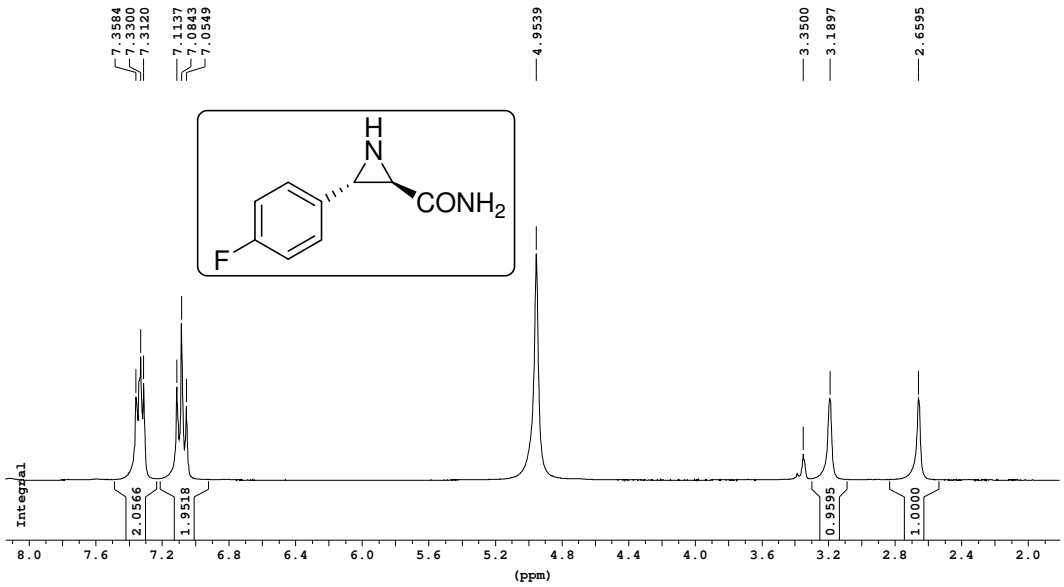


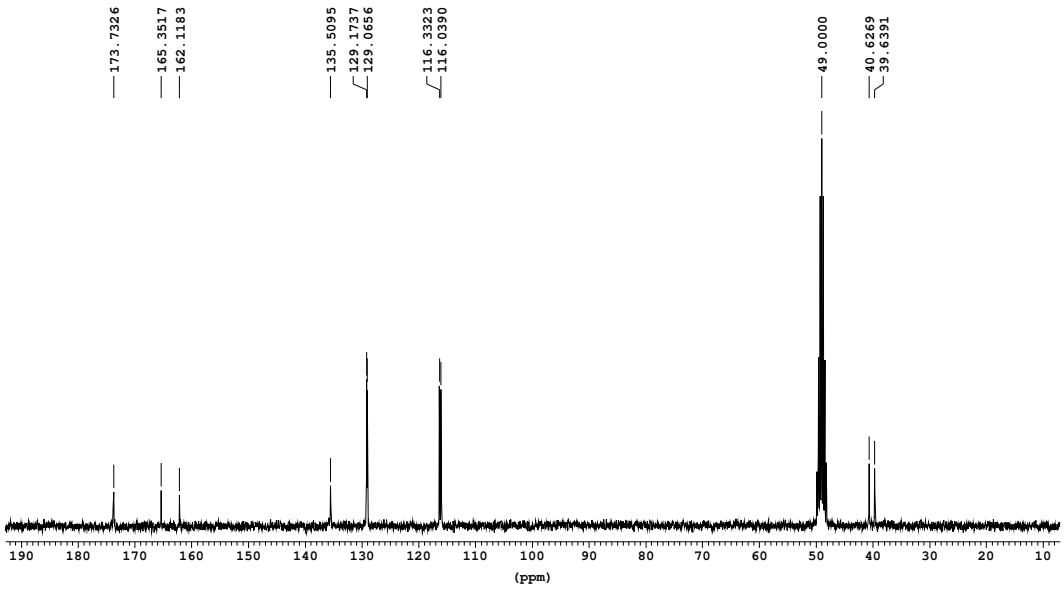
(2*R*,3*S*)-3-Phenylaziridine-2-carboxamide [(2*R*,3*S*)-8a]



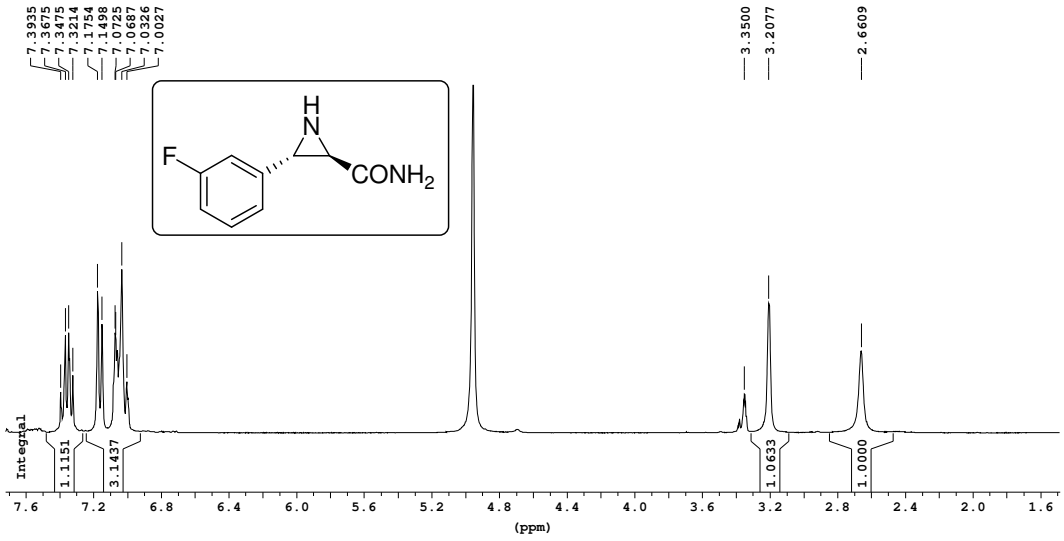


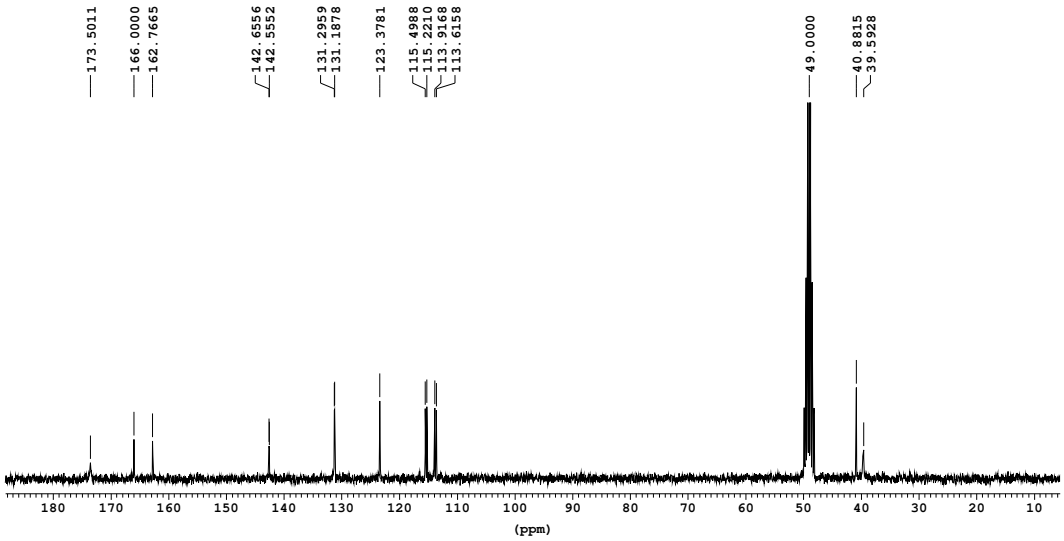
(2*R*,3*S*)-3-(*p*-Fluorophenyl)aziridine-2-carboxamide [(2*R*,3*S*)-8b]



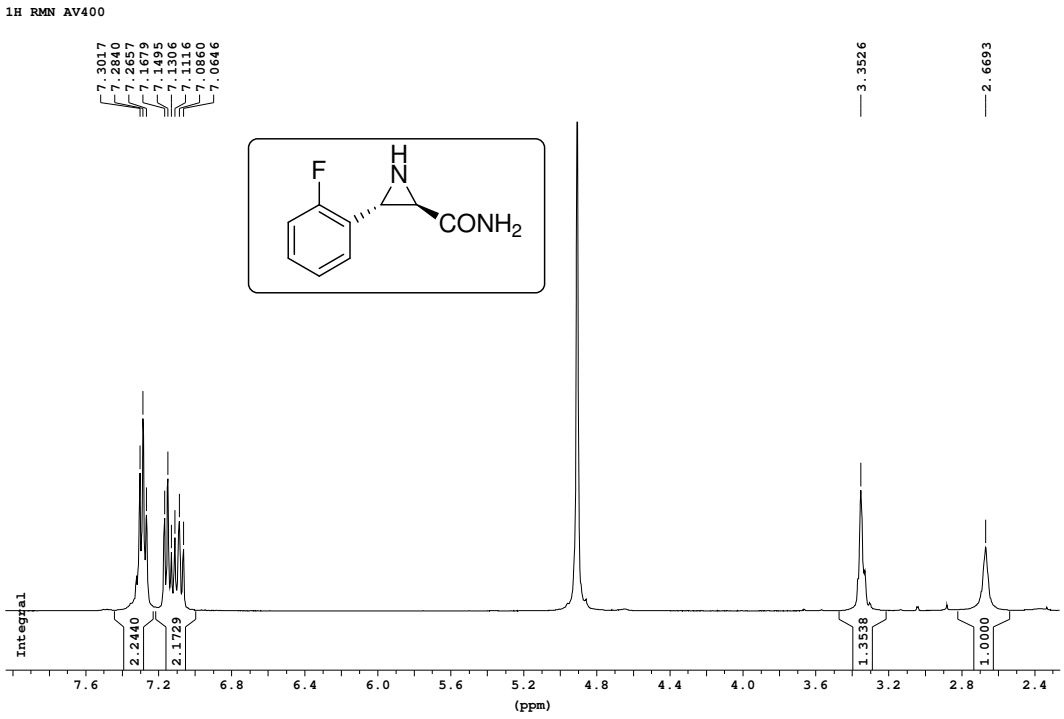


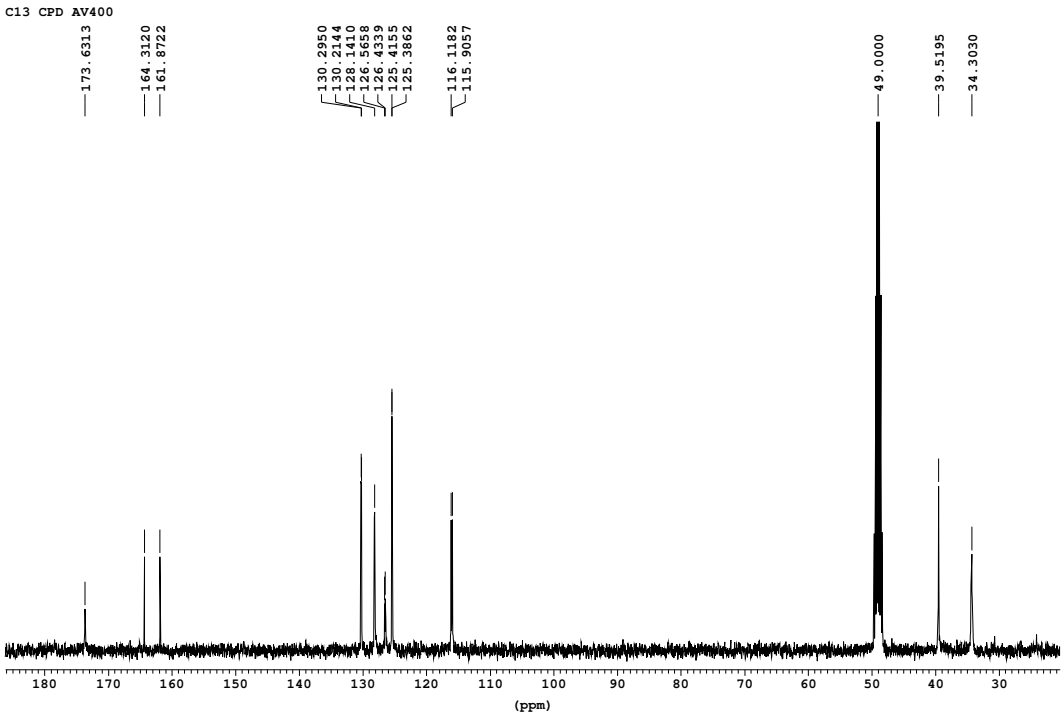
(2*R*,3*S*)-3-(*m*-Fluorophenyl)aziridine-2-carboxamide [(2*R*,3*S*)-8c]



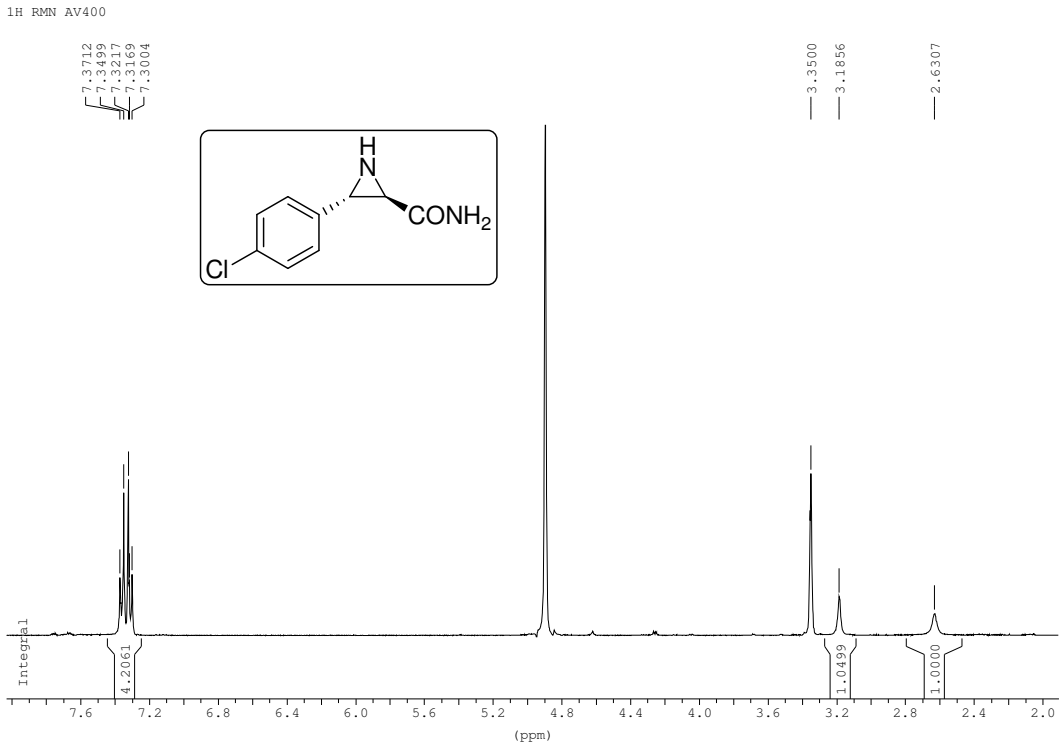


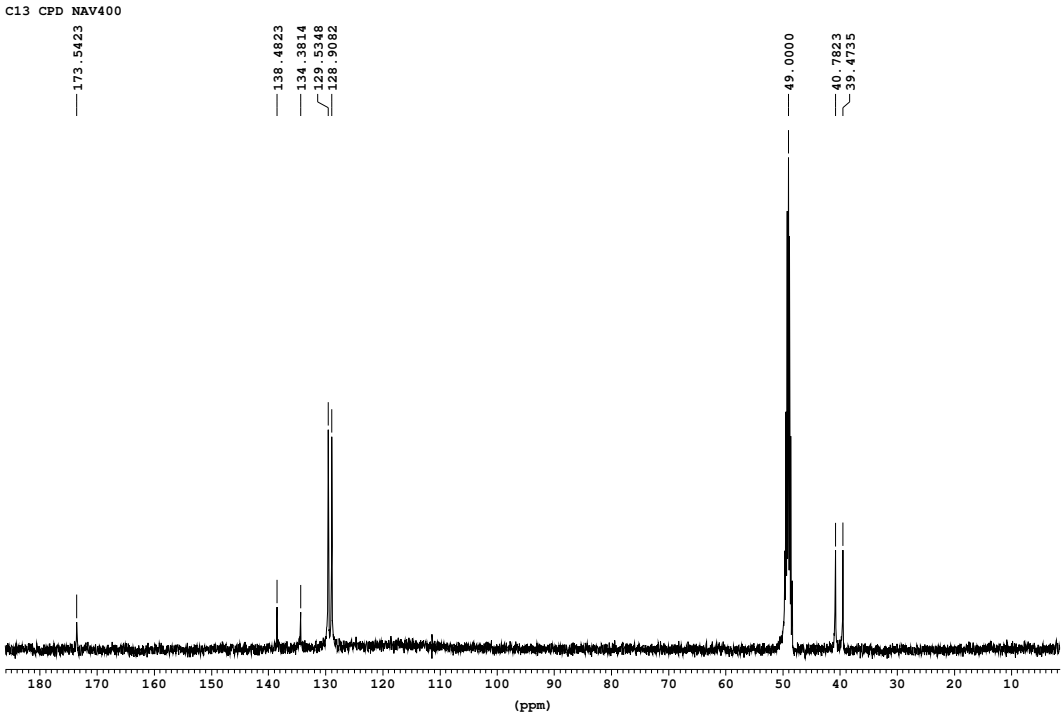
(2*R*,3*S*)-3-(*o*-Fluorophenyl)aziridine-2-carboxamide [(2*R*,3*S*)-8d]



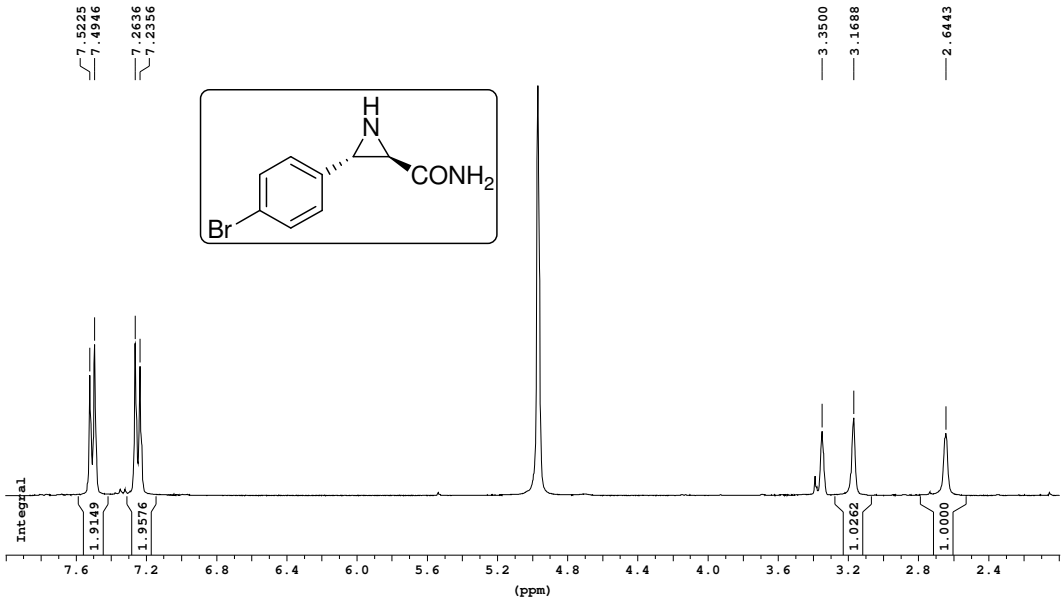


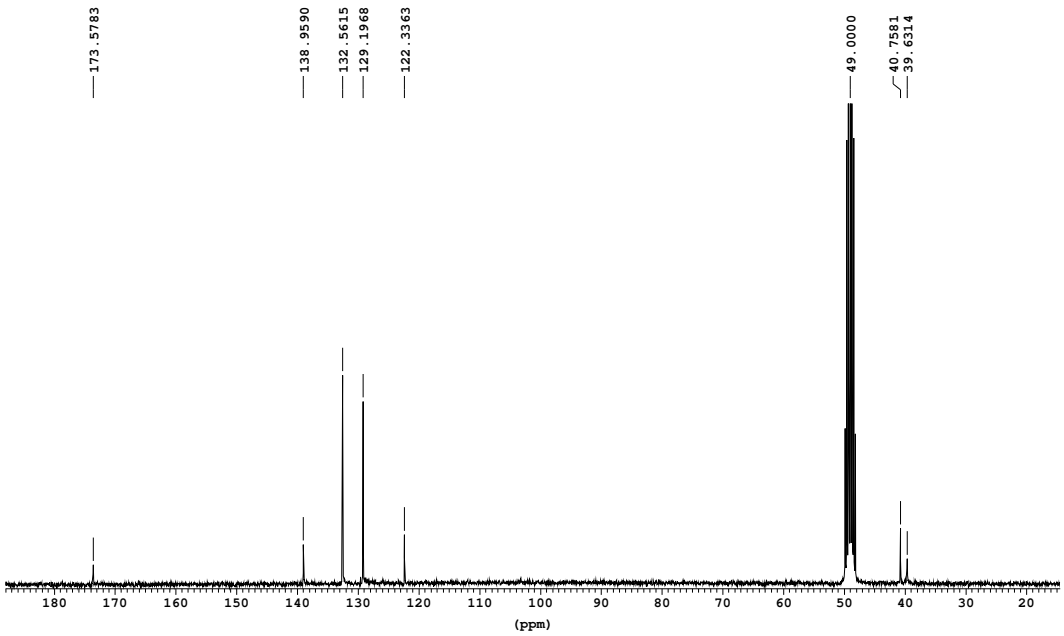
(2*R*,3*S*)-3-(*p*-Chlorophenyl)aziridine-2-carboxamide [(2*R*,3*S*)-8e]



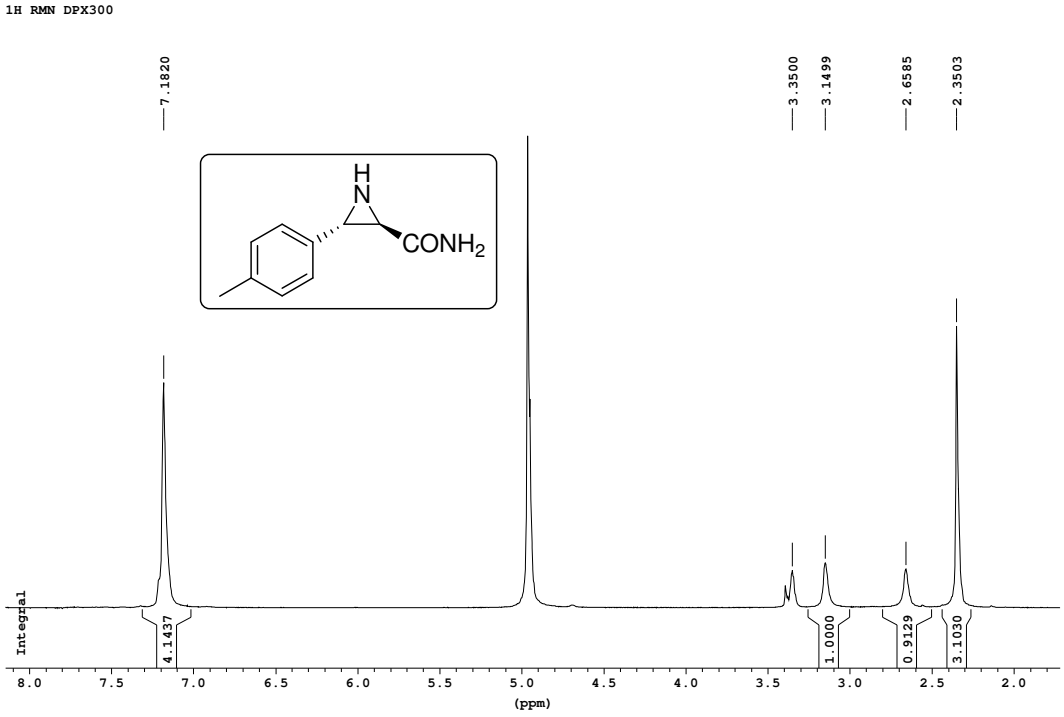


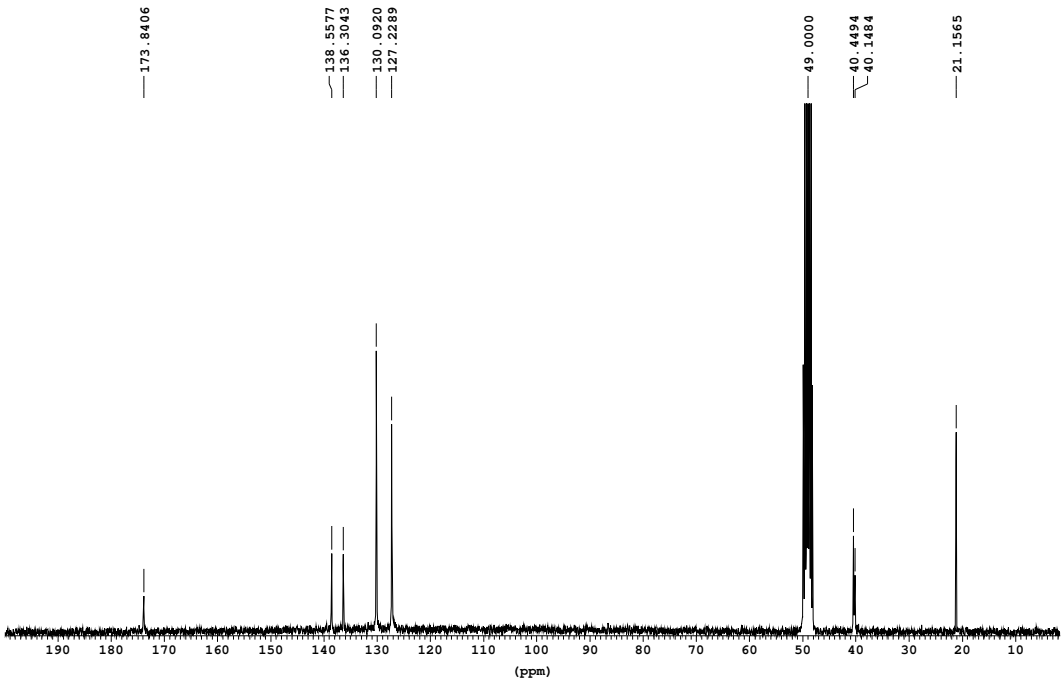
(2*R*,3*S*)-3-(*p*-Bromophenyl)aziridine-2-carboxamide [(2*R*,3*S*)-8f]



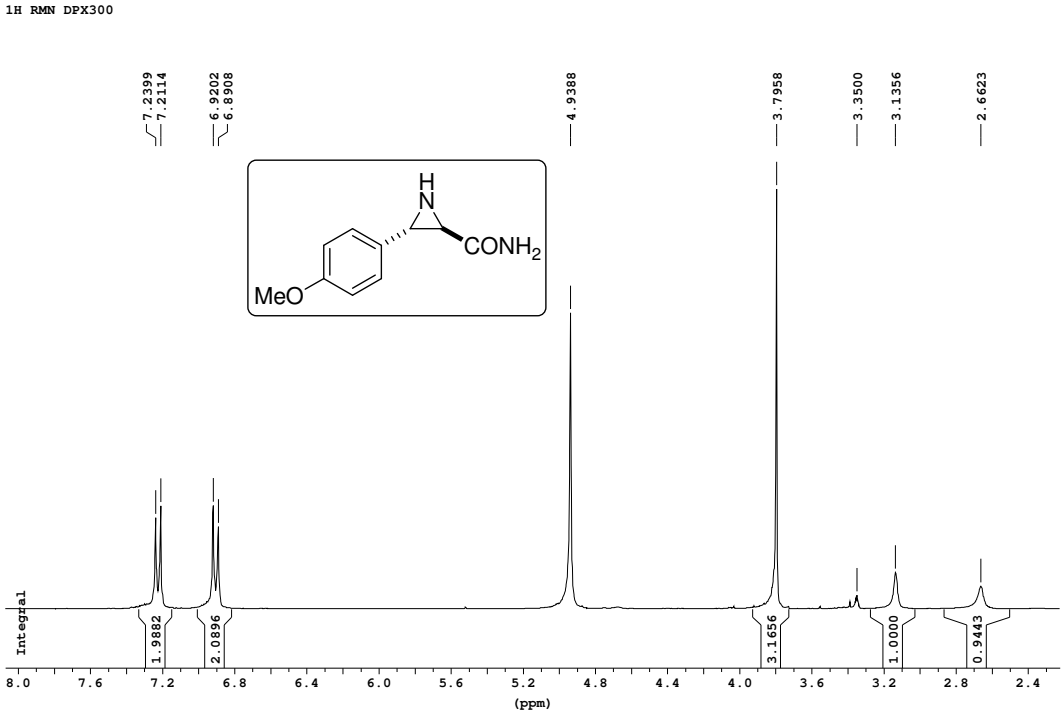


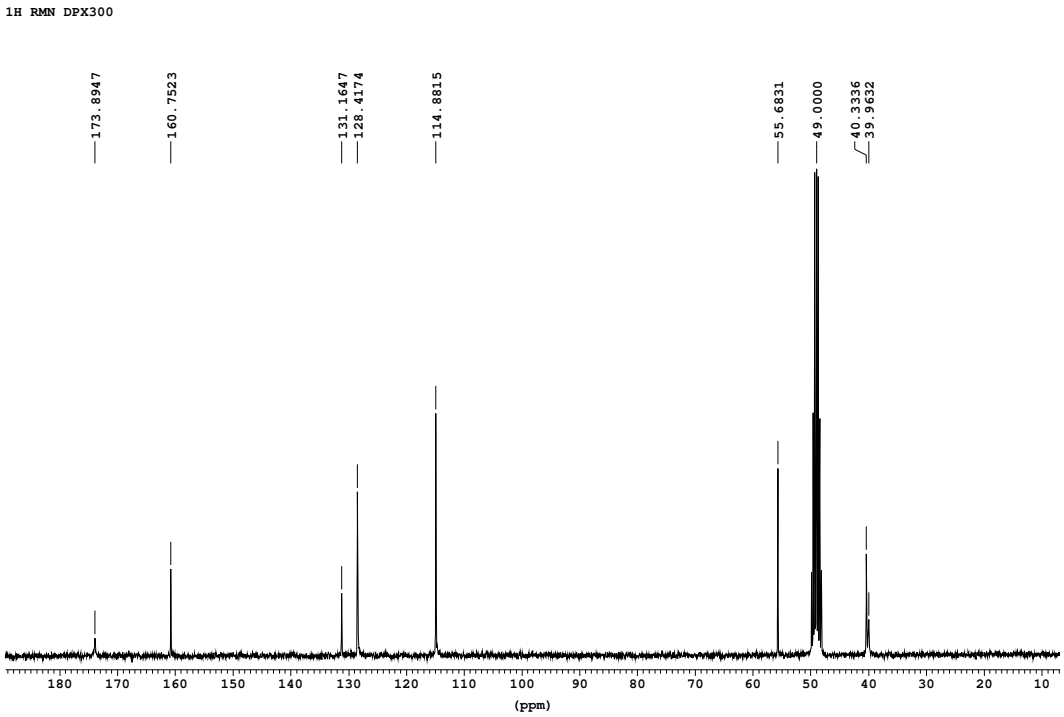
(2*R*,3*S*)-3-(*p*-Tolyl)aziridine-2-carboxamide [(2*R*,3*S*)-8g]



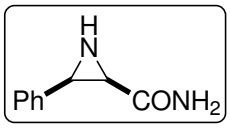


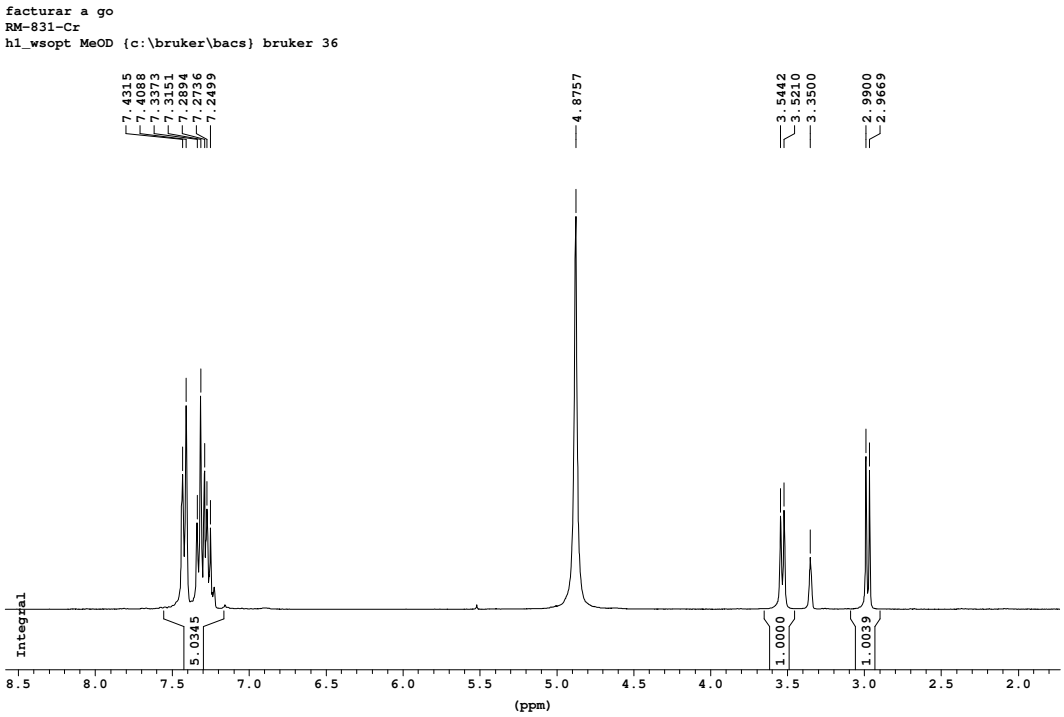
(±)-*trans*-3-(*p*-Anisyl)aziridine-2-carboxamide (8h)

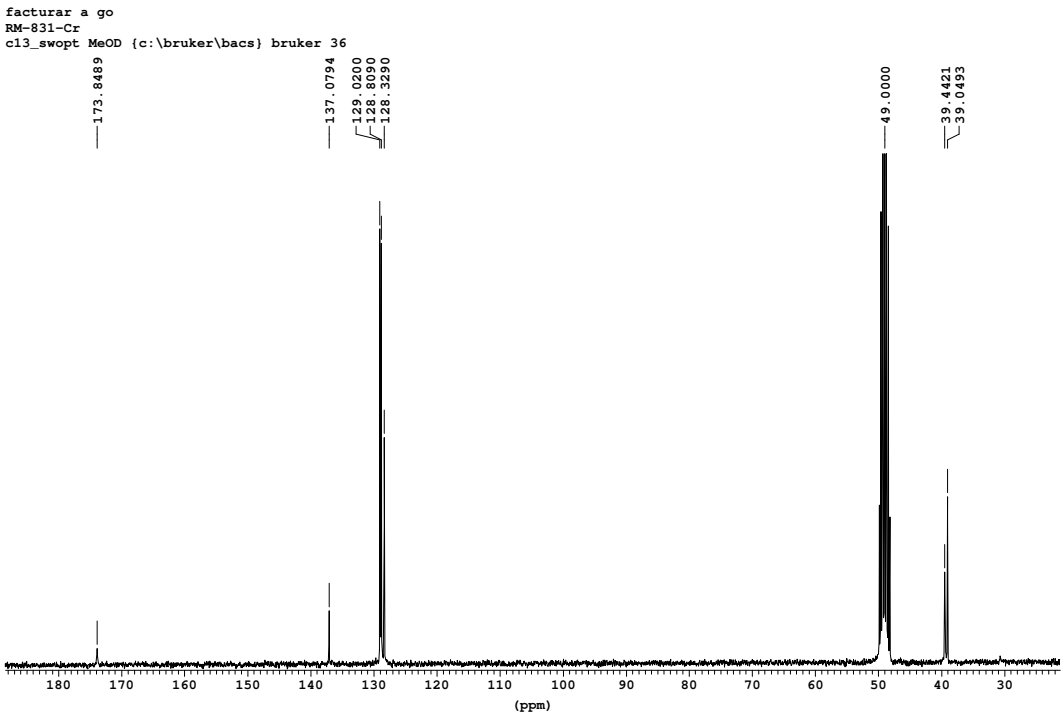




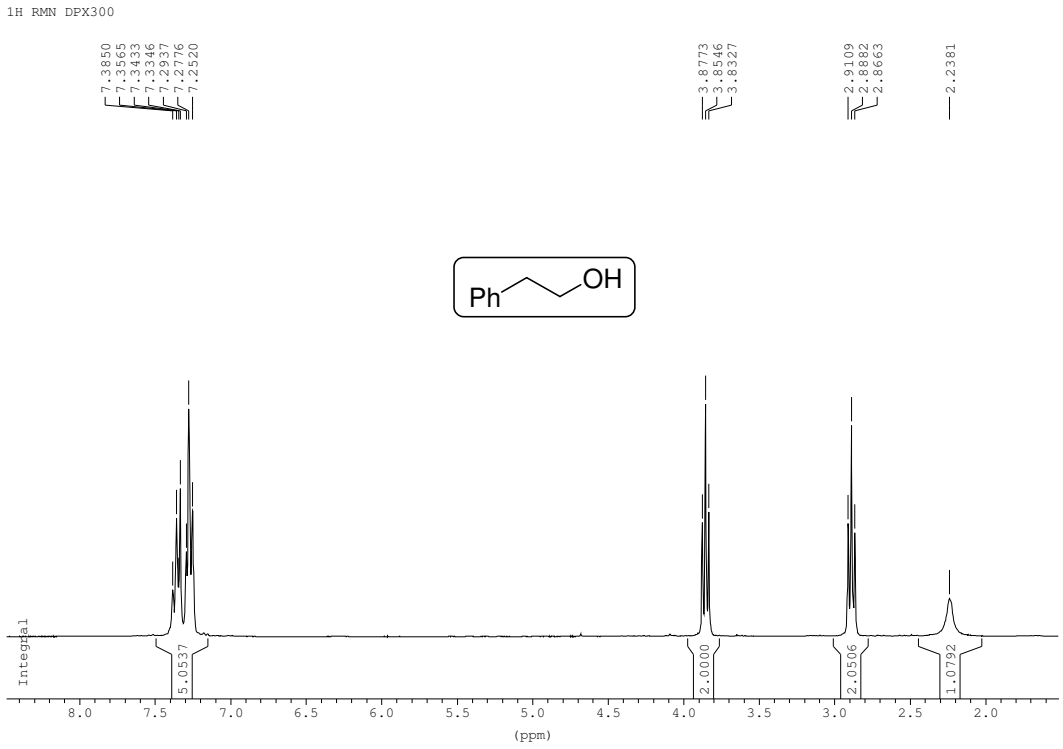
(±)-*cis*-3-Phenylaziridine-2-carboxamide (9a)

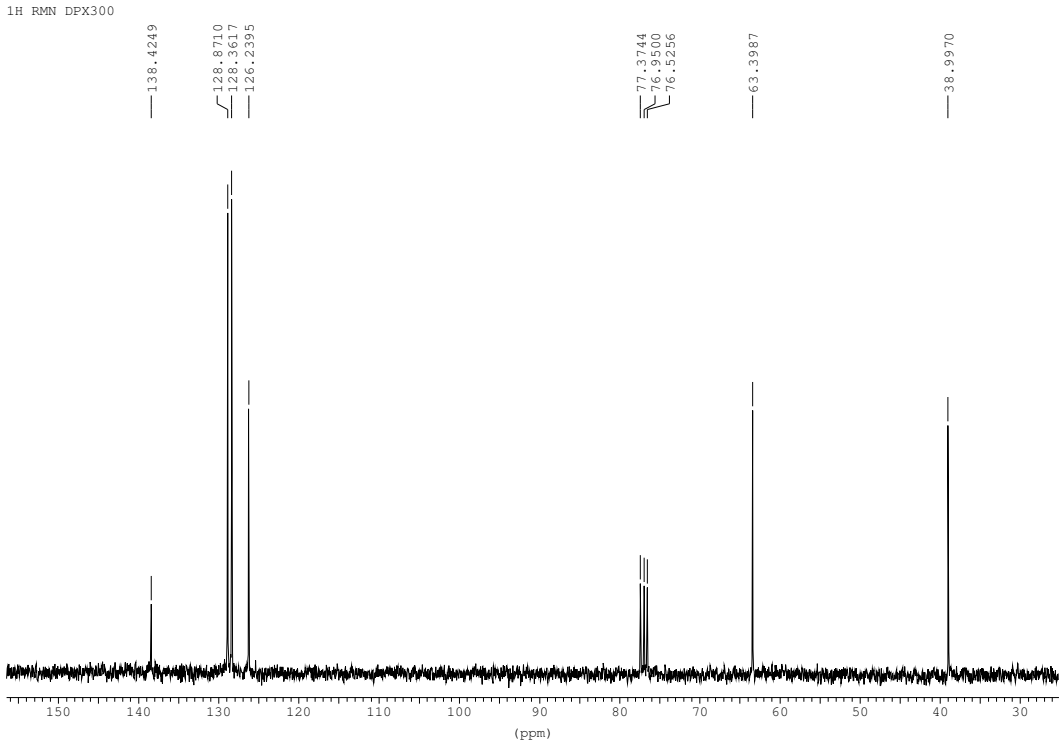




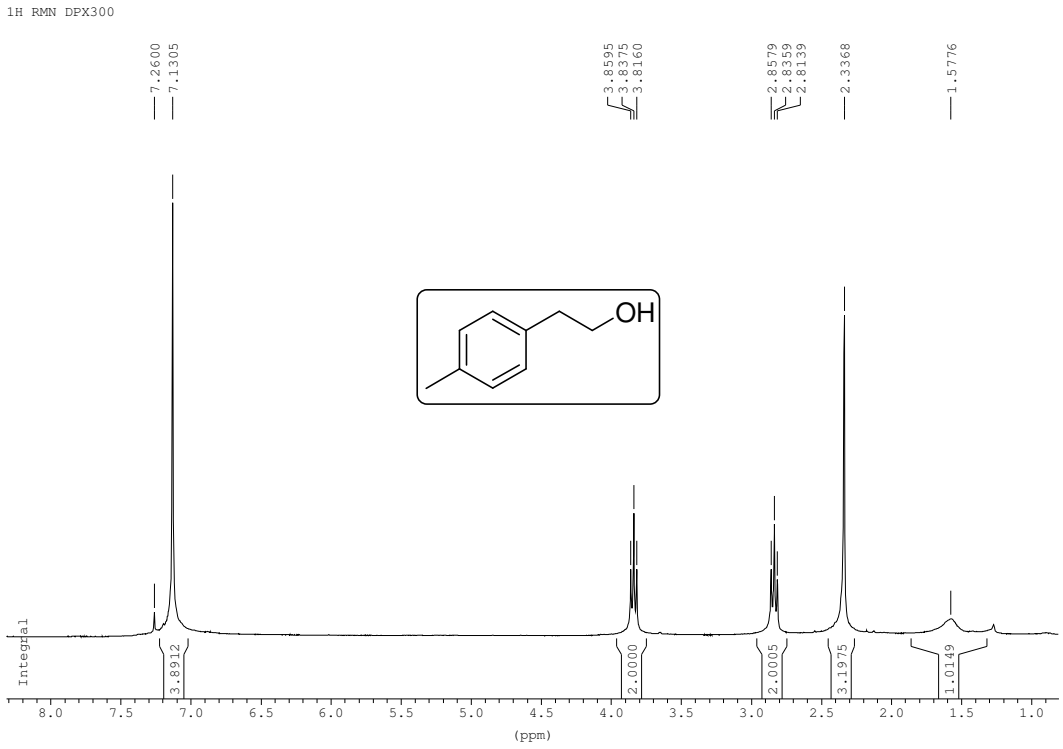


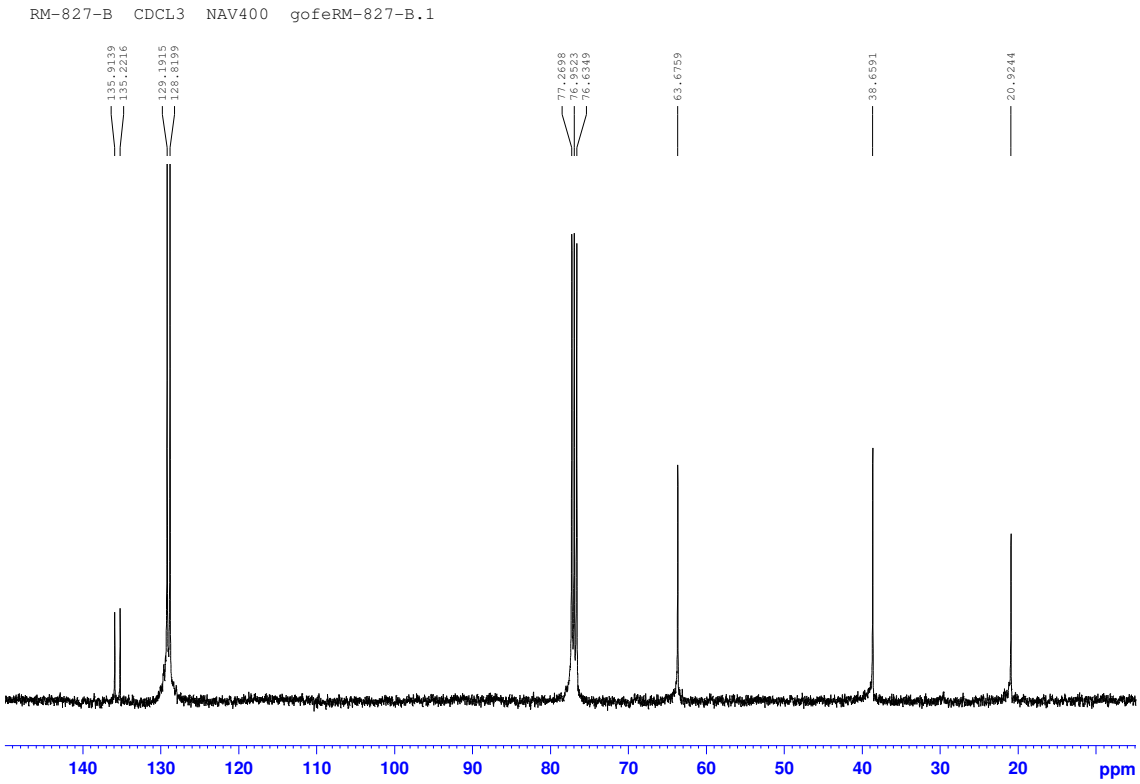
2-Phenylethanol (14a)





2-(*p*-Tolyl)ethanol (14g)





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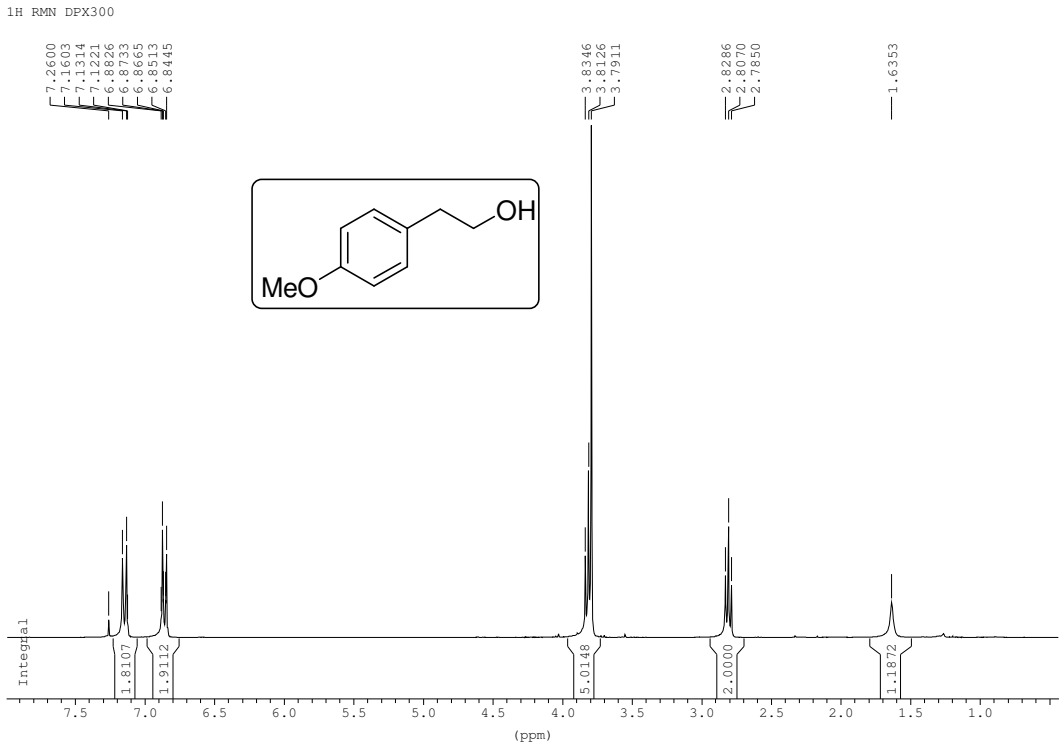
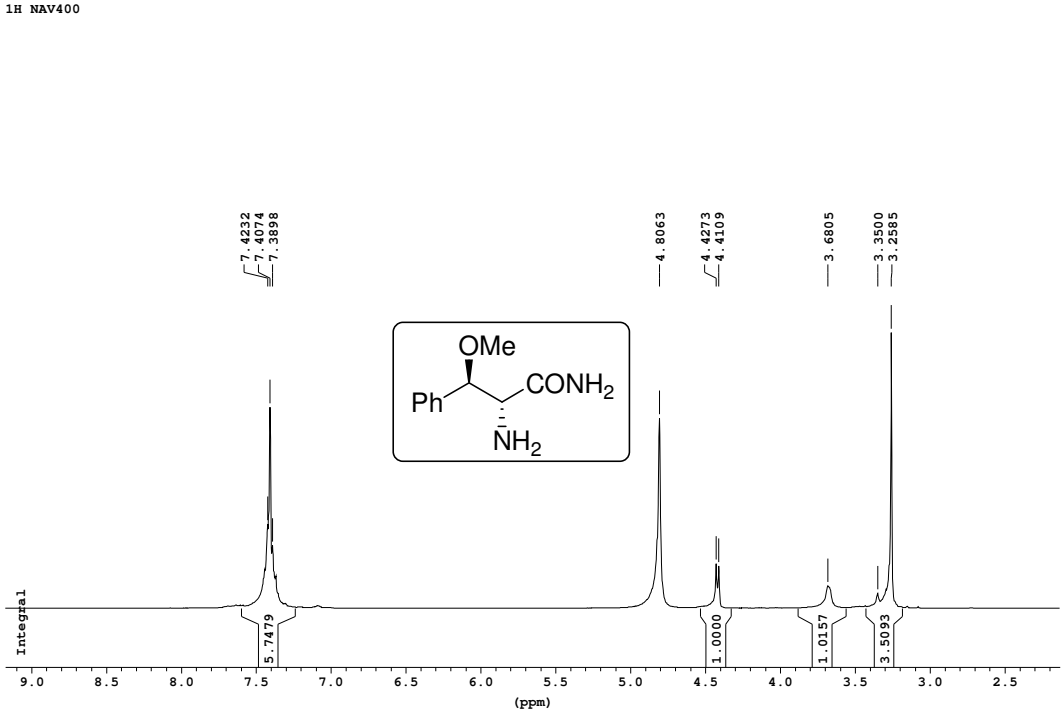
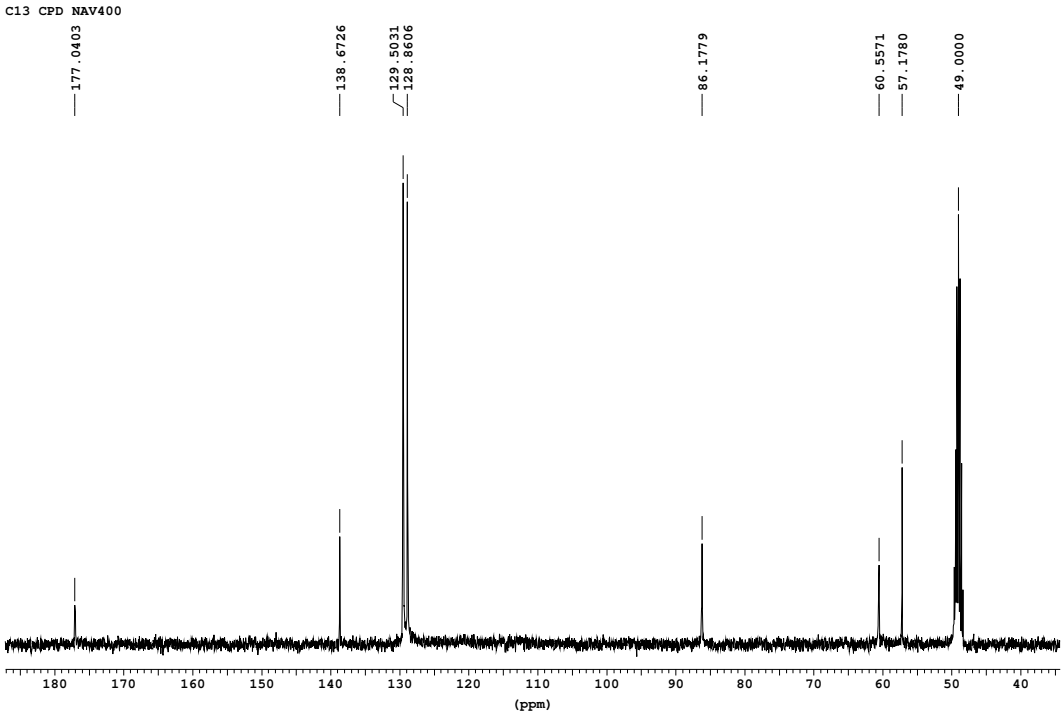
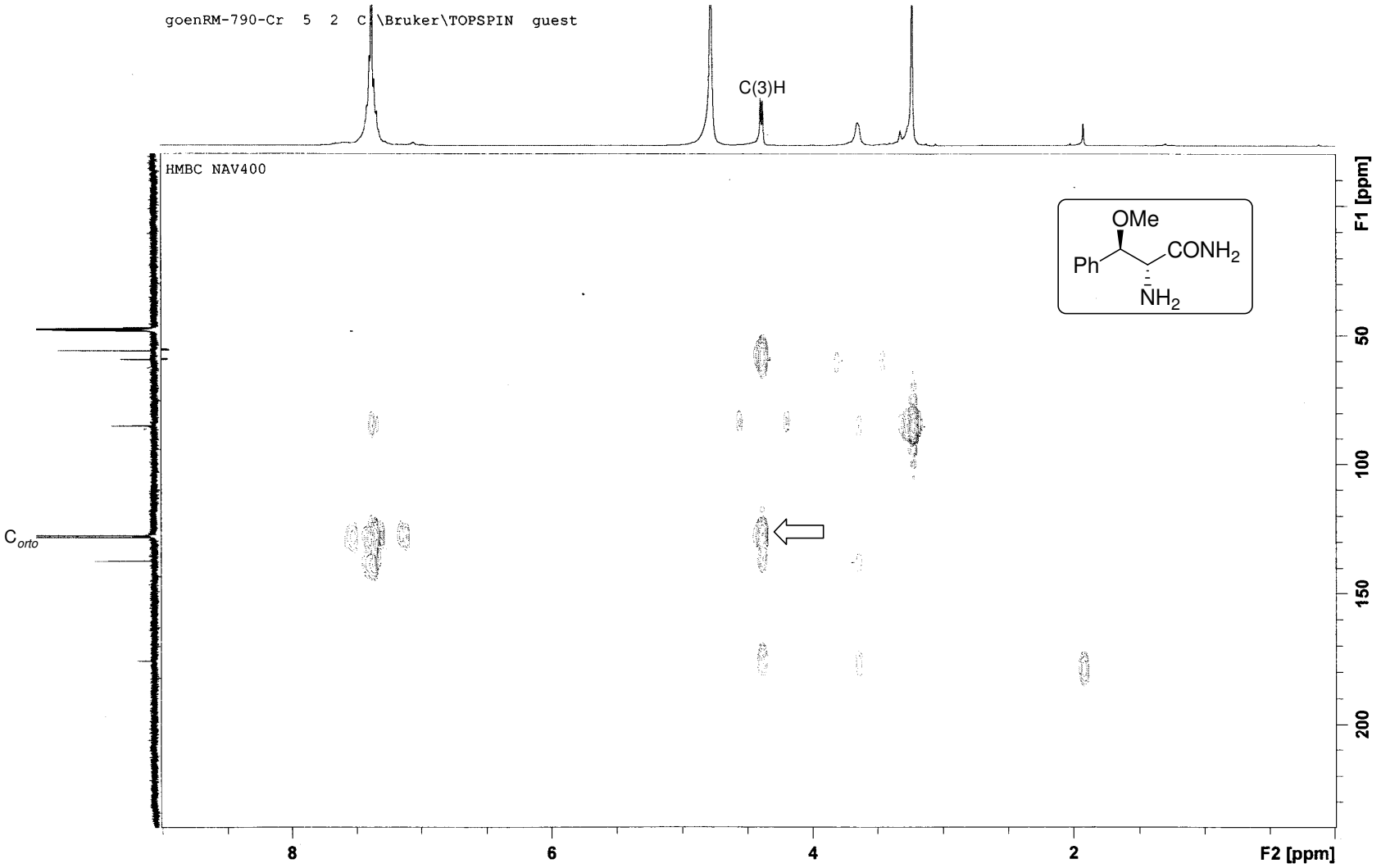


(3*R*)-β-Methoxy-D-phenylalaninamide [(2*R*,3*R*)-17]

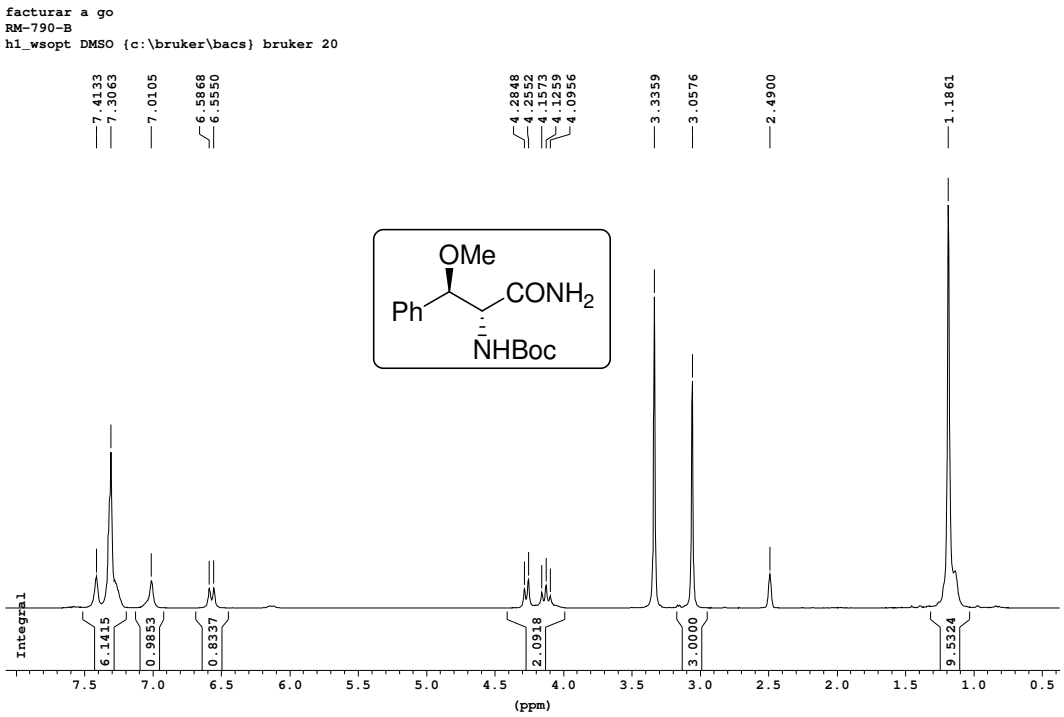


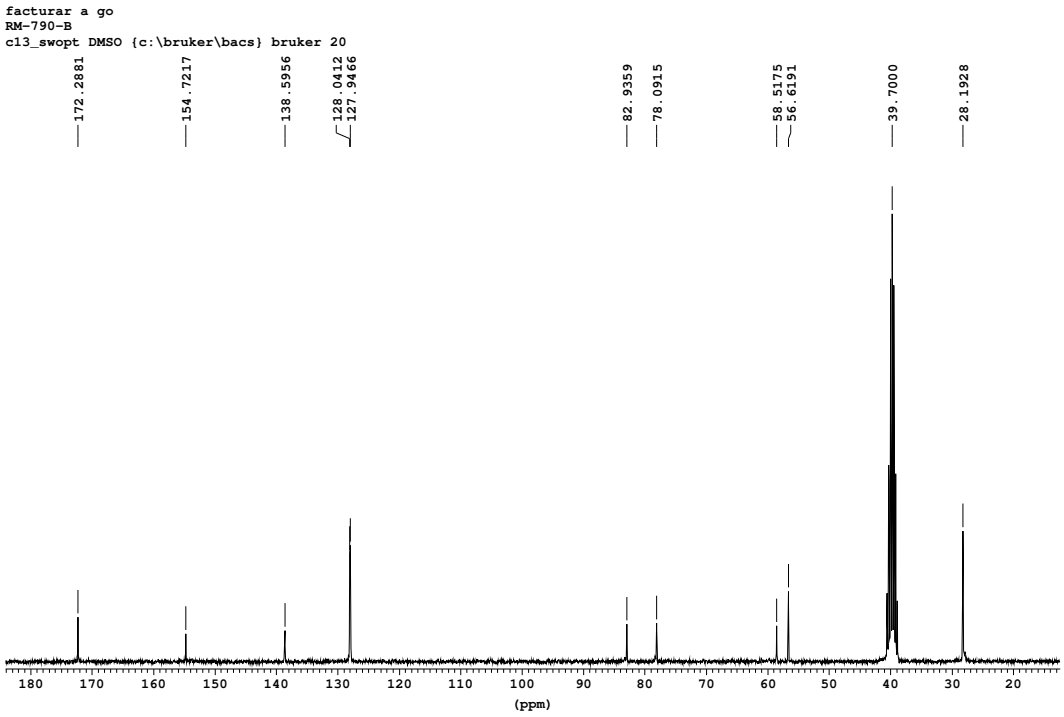


(3*R*)-β-Methoxy-D-phenylalaninamide [(2*R*,3*R*)-17] (HMBC)

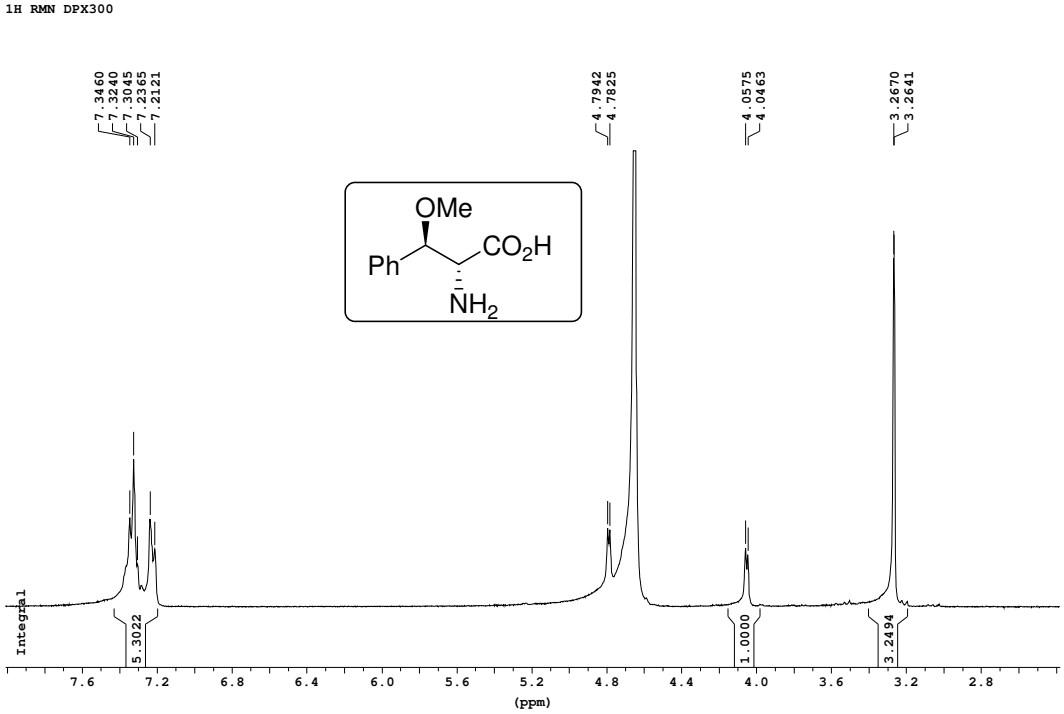


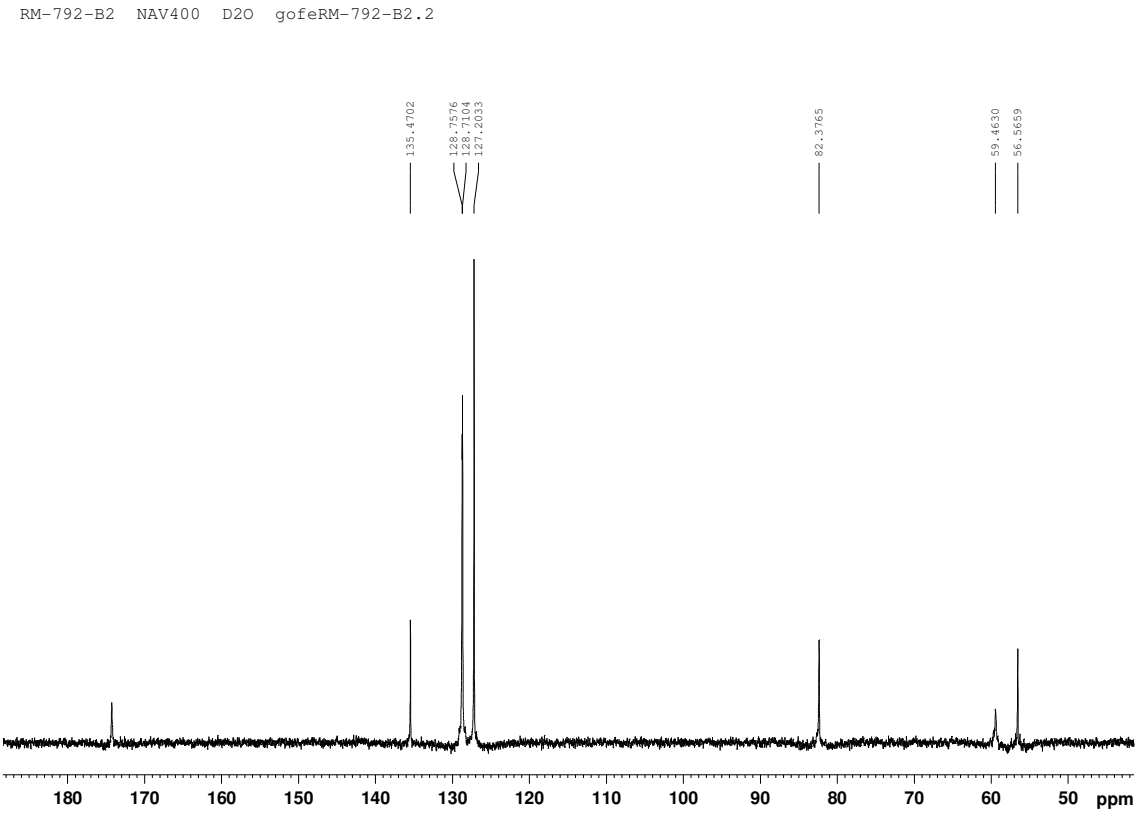
(3*R*)-N^2-Boc-β-methoxy-D-phenylalaninamide [(2*R*,3*R*)-18]



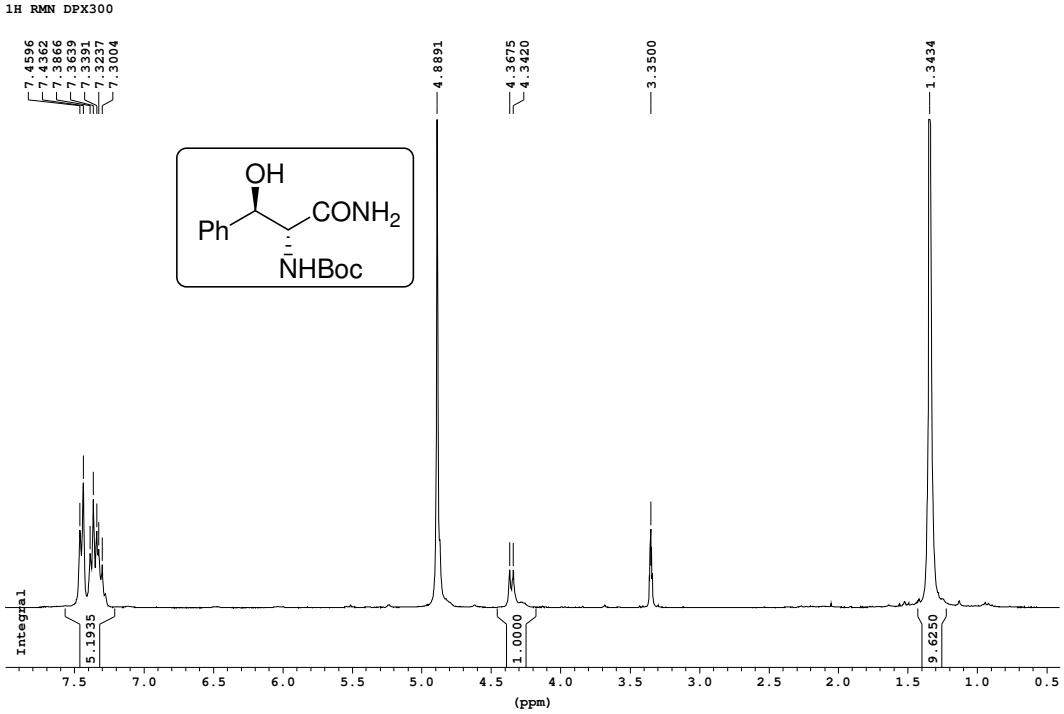


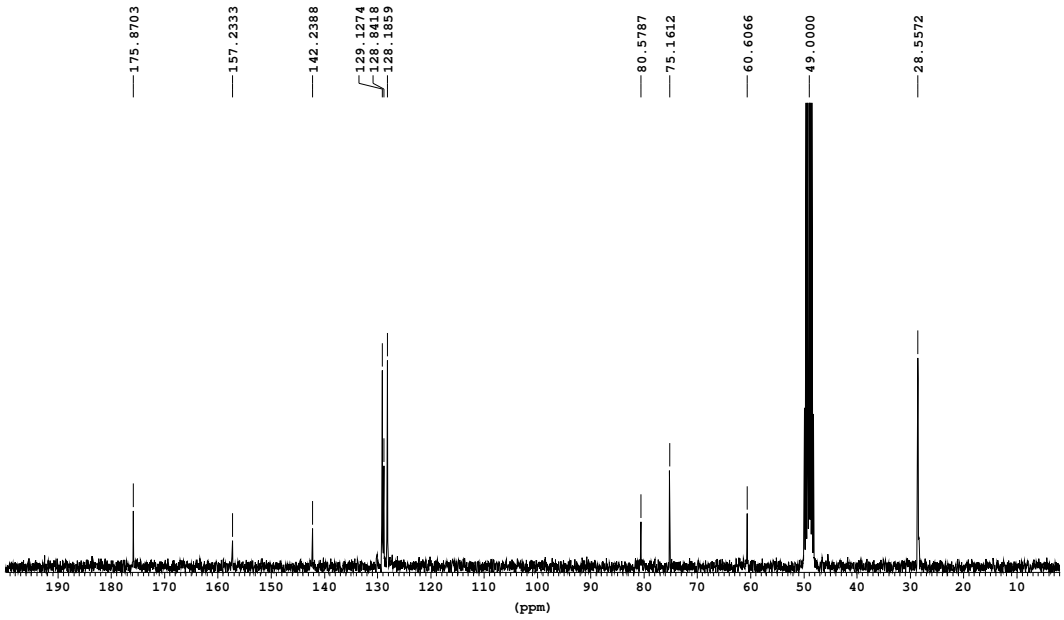
(3*R*)-β-Methoxy-D-phenylalanine [(2*R*,3*R*)-19]



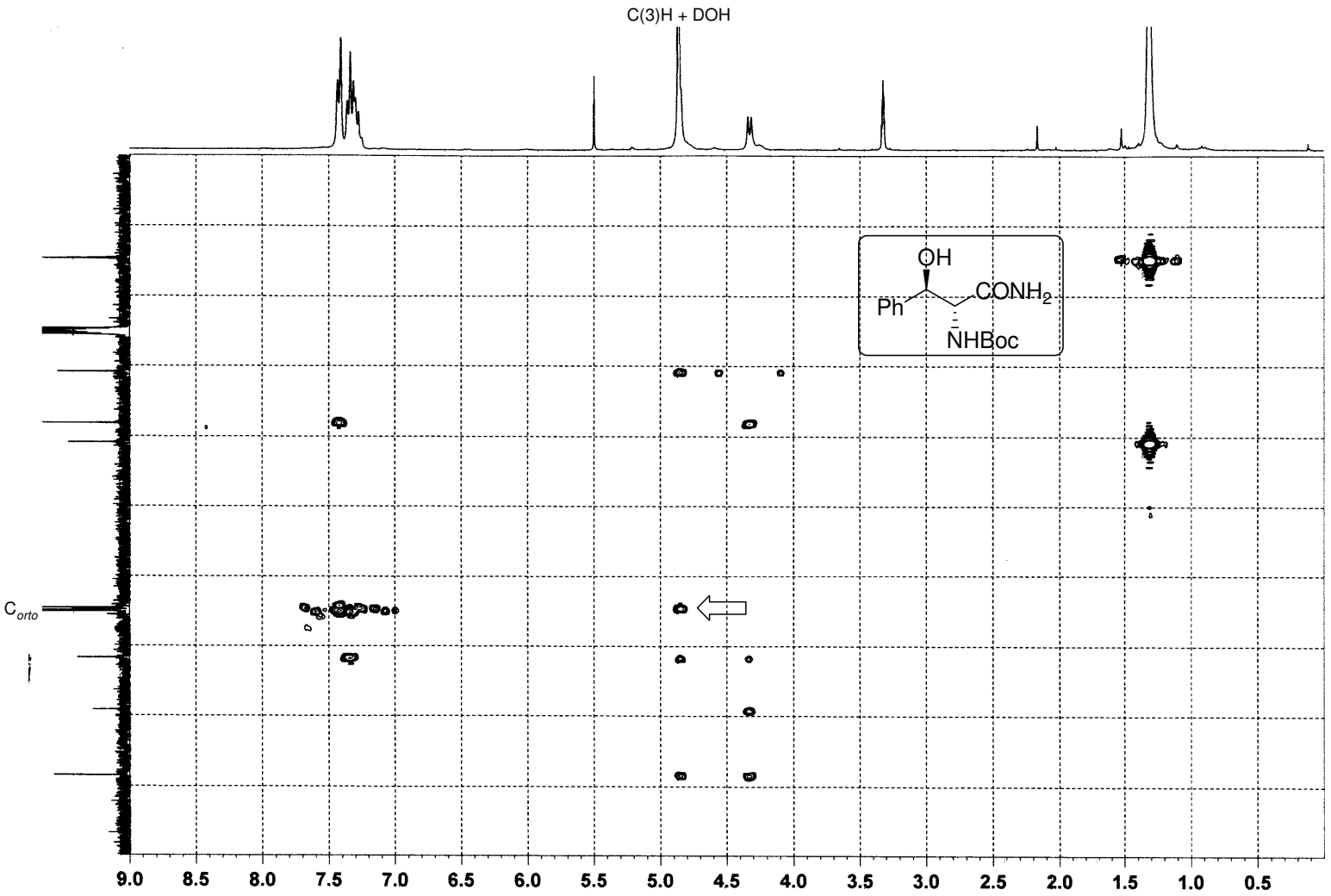


(3*R*)-N^2-Boc-β-hydroxy-D-phenylalaninamide [(2*R*,3*R*)-20]

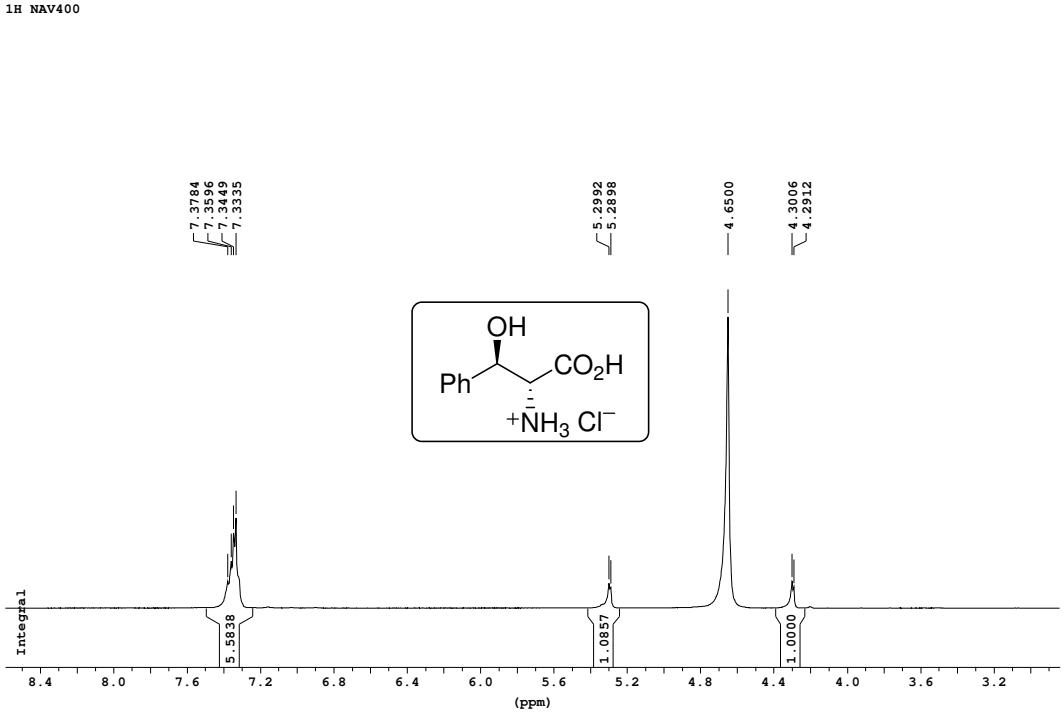


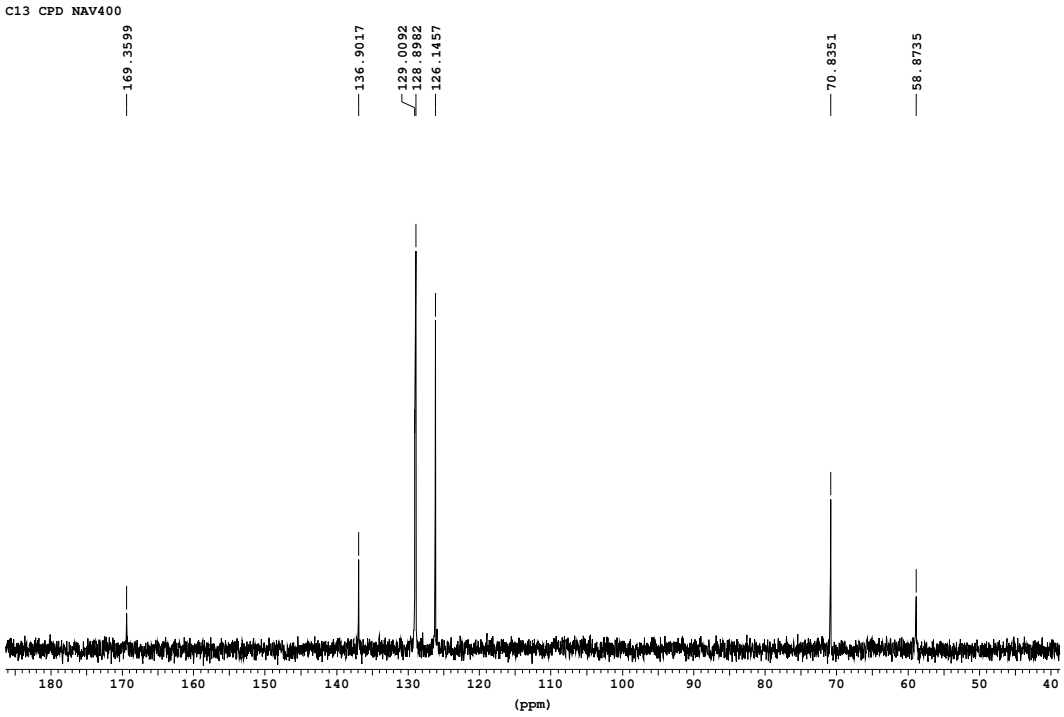


(3*R*)-N^2-Boc-β-hydroxy-D-phenylalaninamide [(2*R*,3*R*)-20] (HMBC)

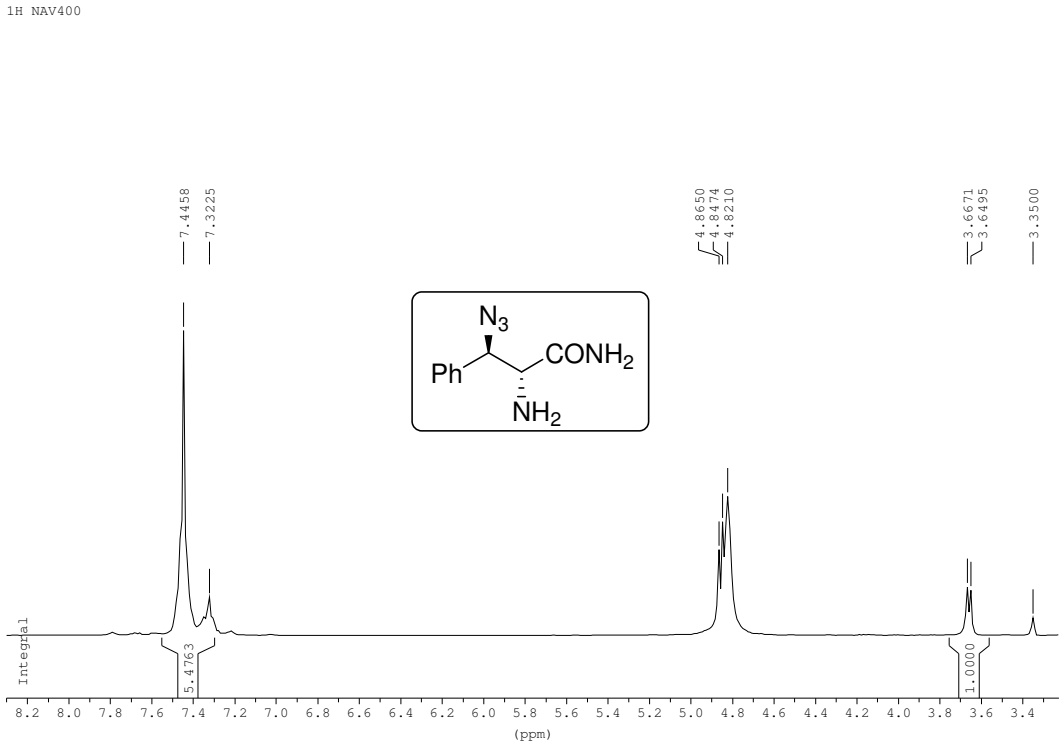


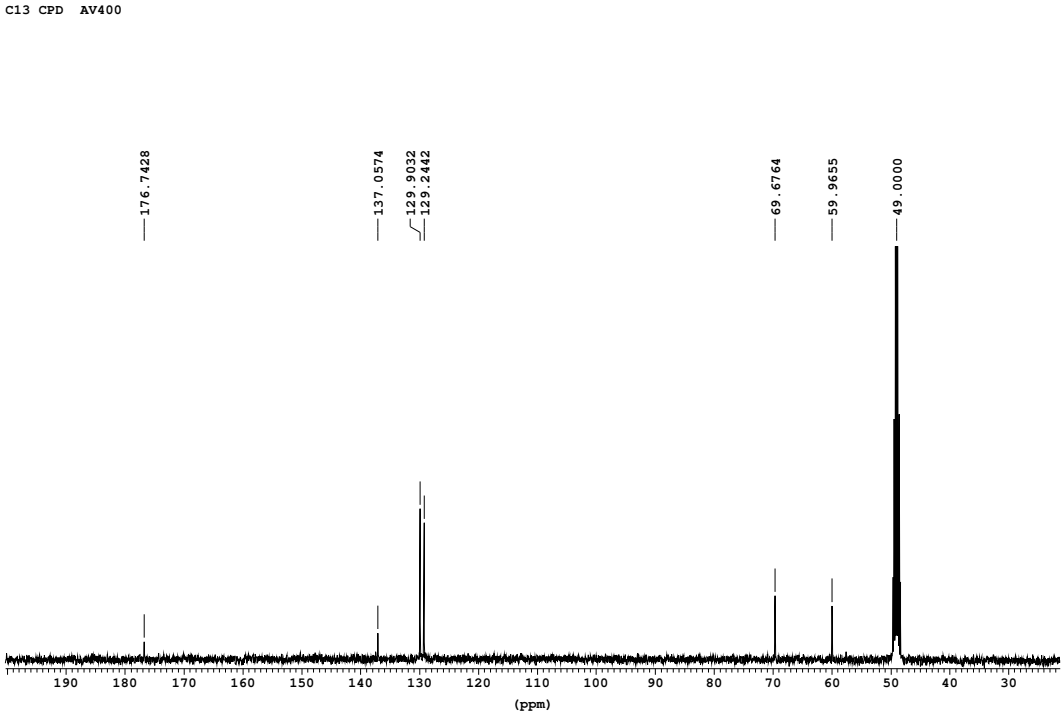
(3*R*)-β-Hydroxy-D-phenylalanine hydrochloride [(2*R*,3*R*)-21]



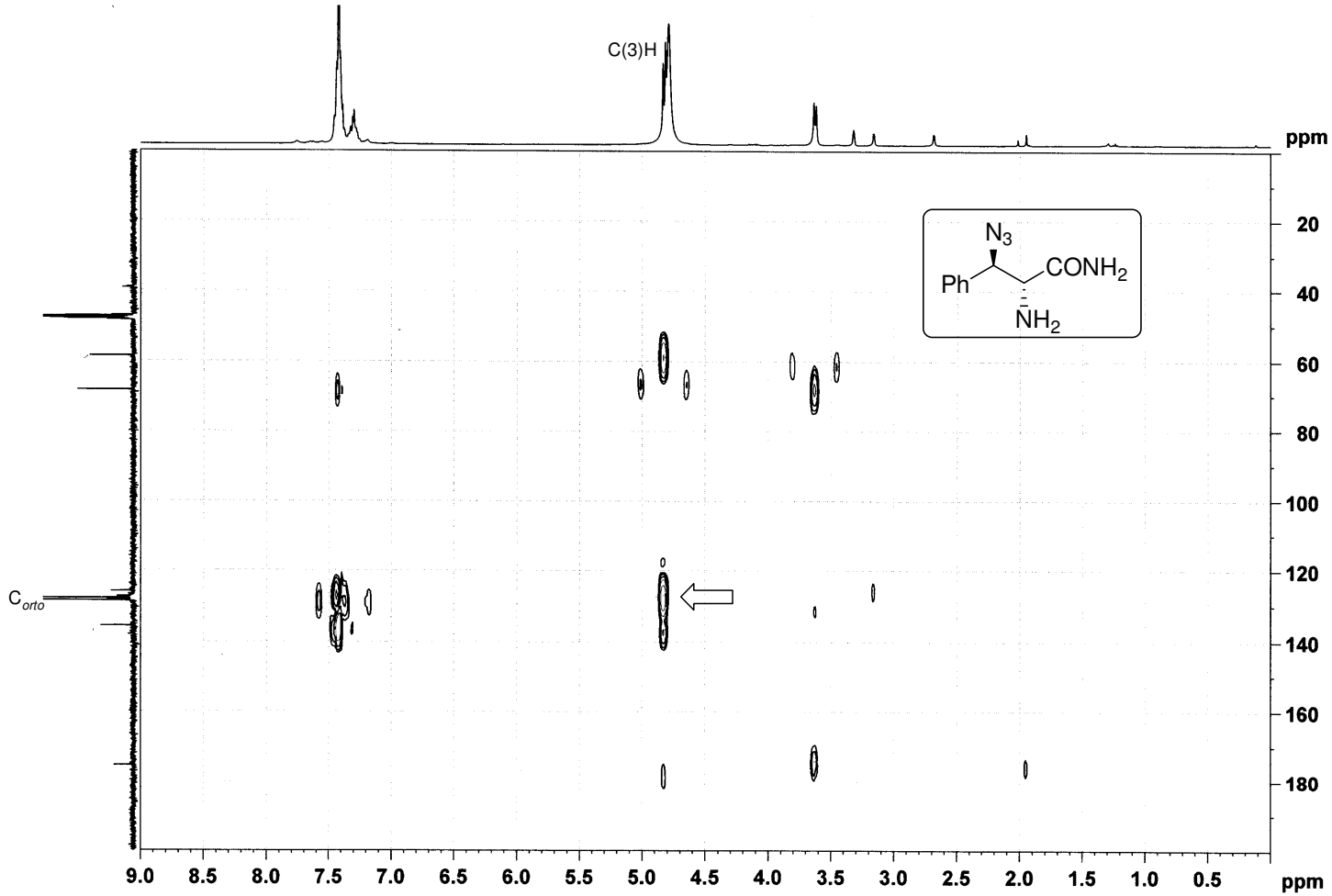


(3*R*)-β-Azido-D-phenylalaninamide [(2*R*,3*R*)-22]

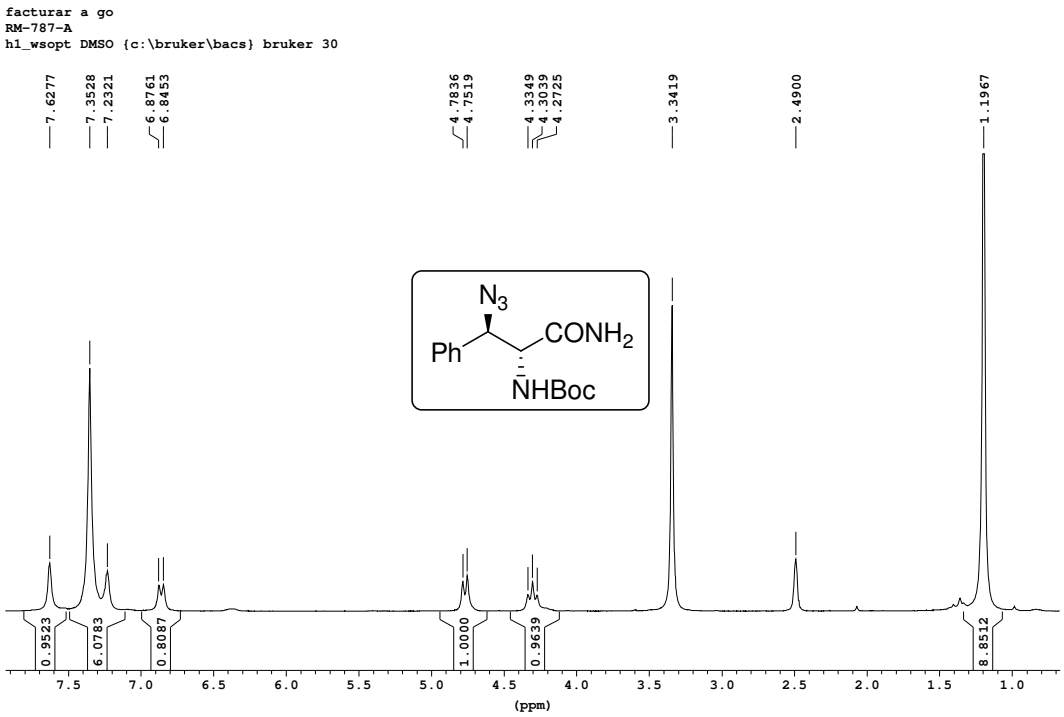


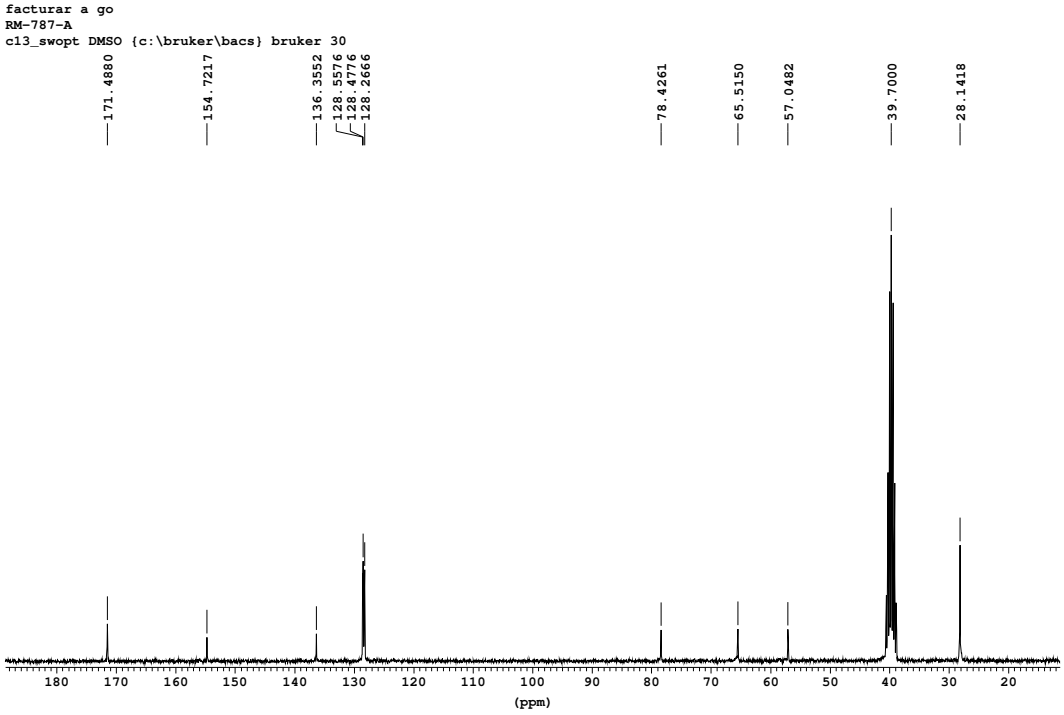


(3*R*)-β-Azido-D-phenylalaninamide [(2*R*,3*R*)-22] (HMBC)

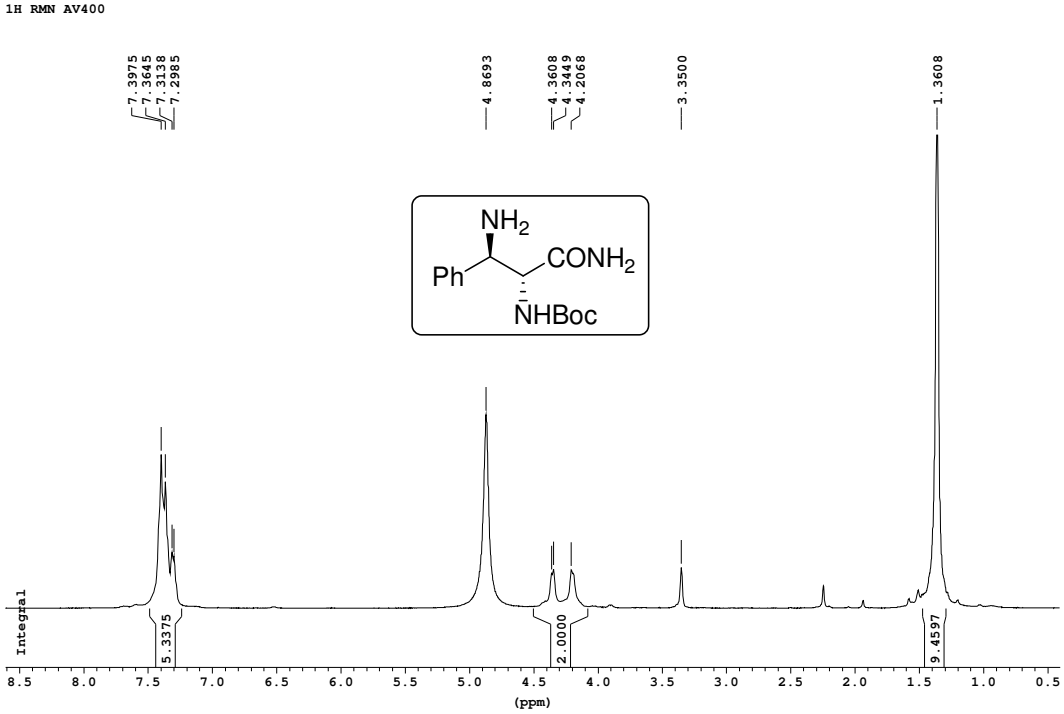


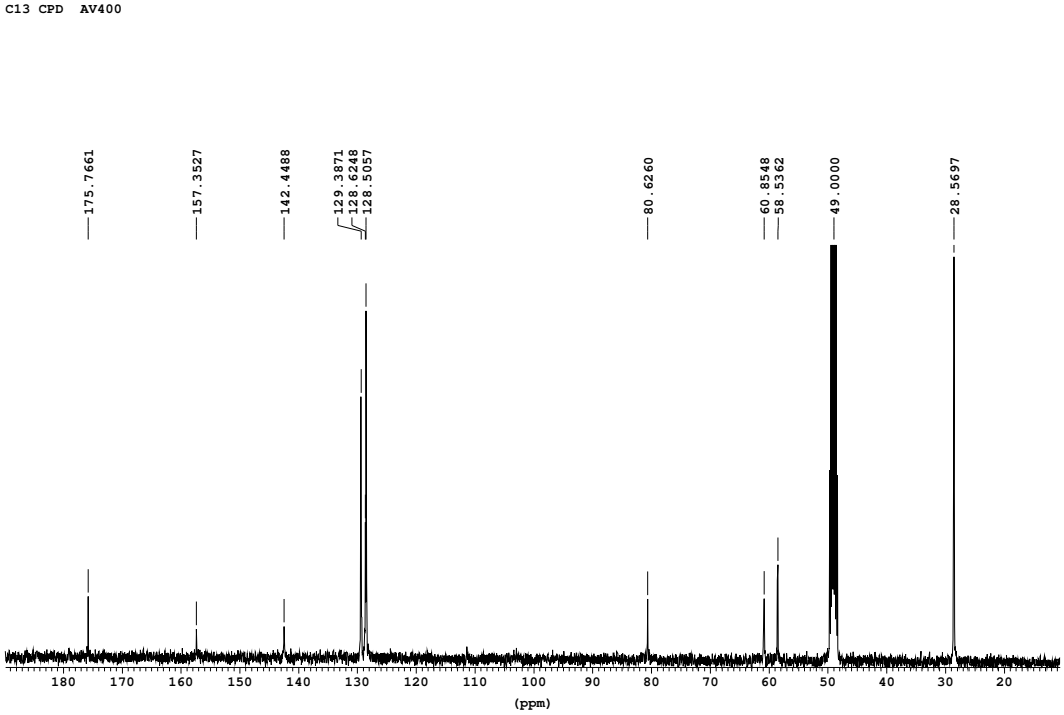
(3*R*)-β-Azido-N^2-Boc-D-phenylalaninamide [(2*R*,3*R*)-23]



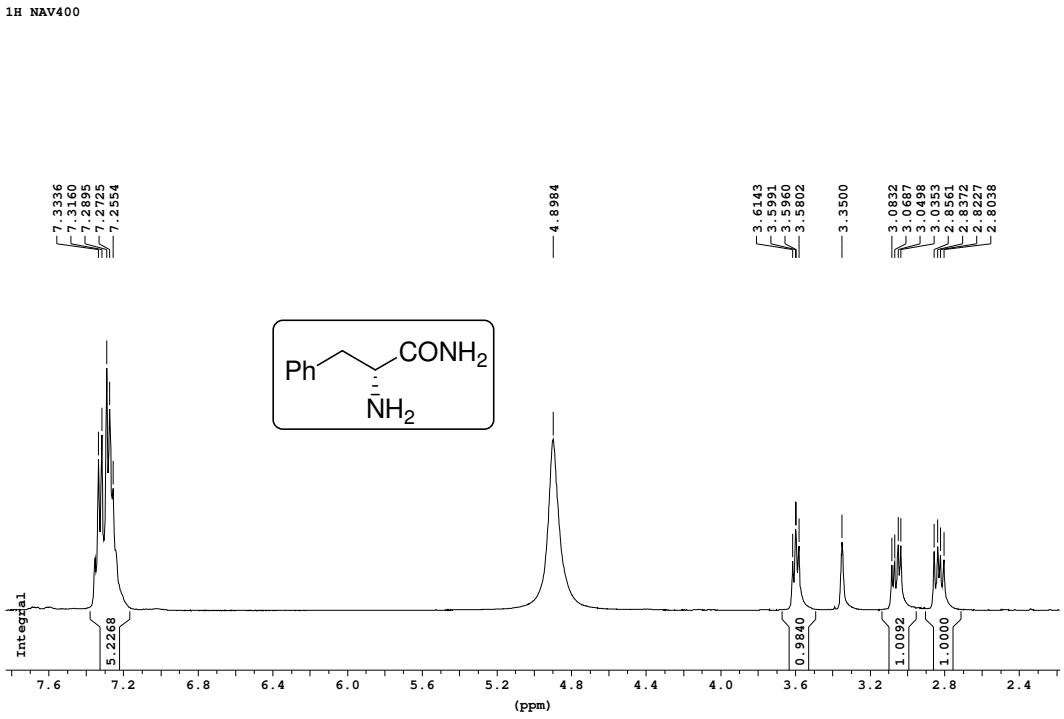


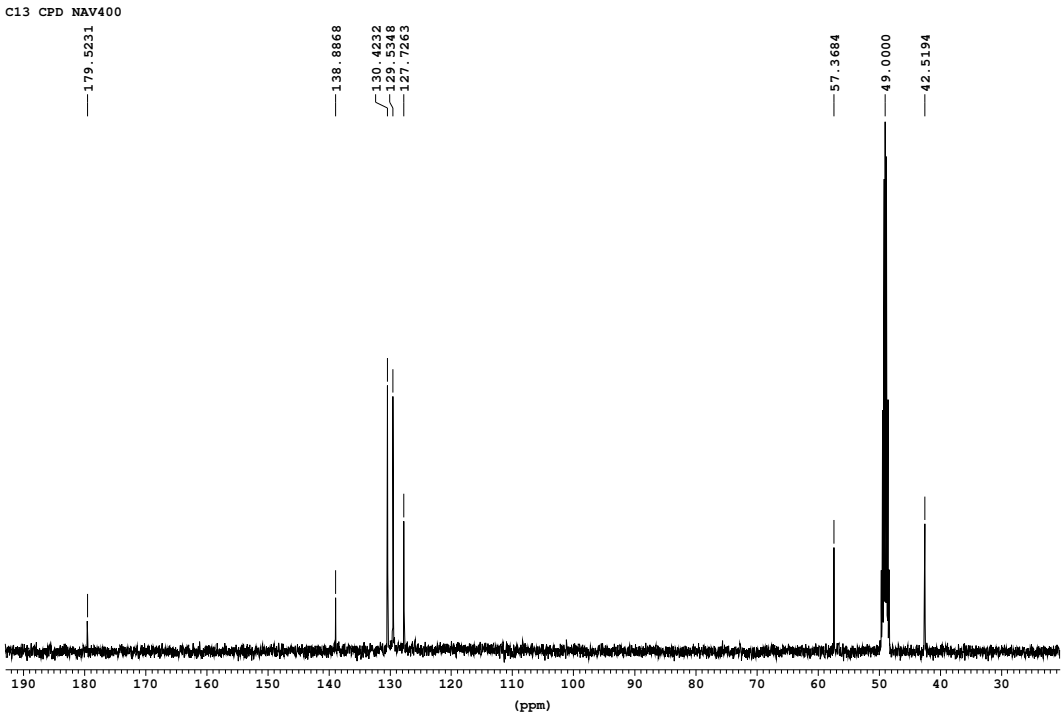
(3*R*)-β-Amino-N^2-Boc-D-phenylalaninamide [(2*R*,3*R*)-24]



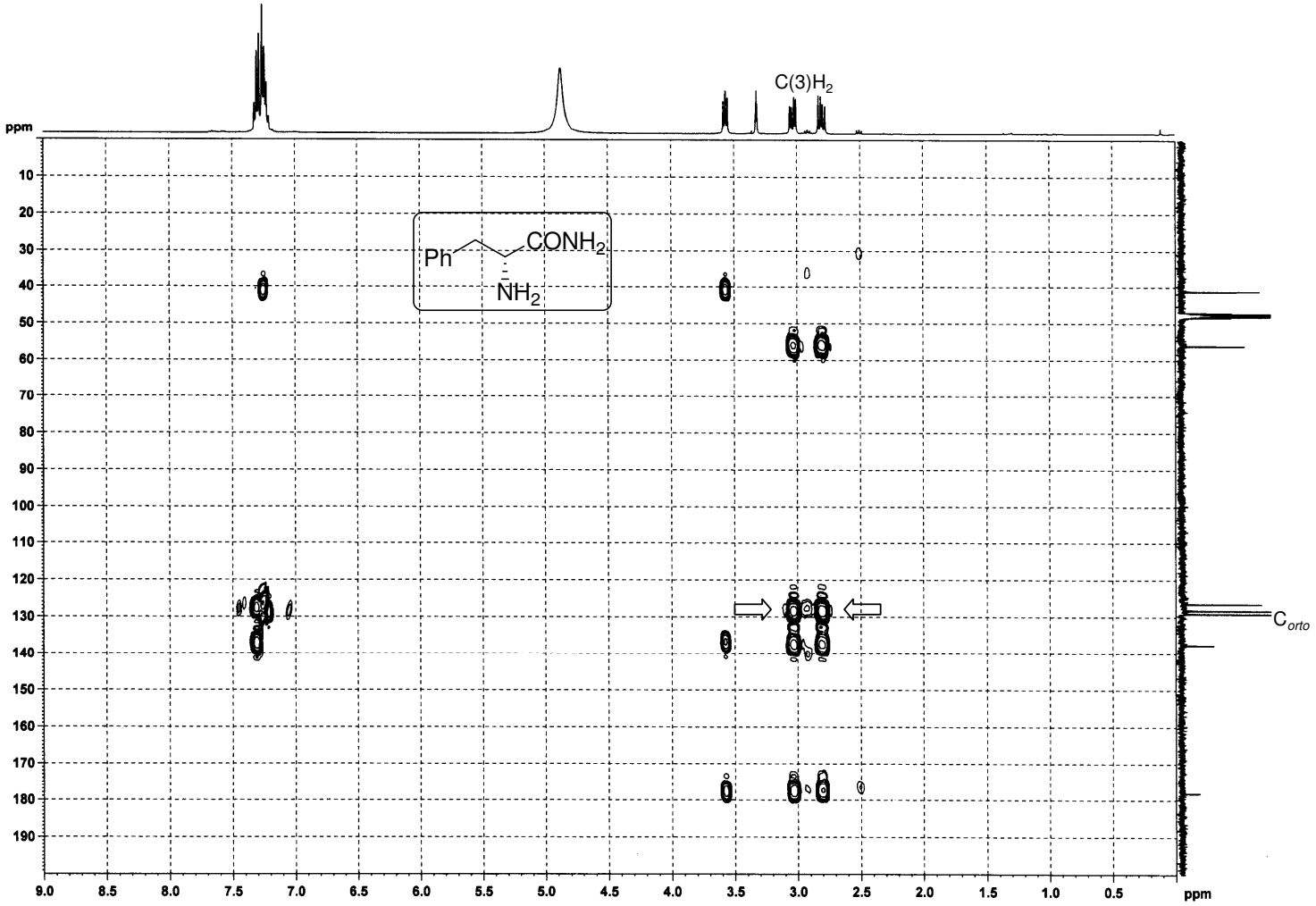


D-Phenylalaninamide [(*R*)-31a]

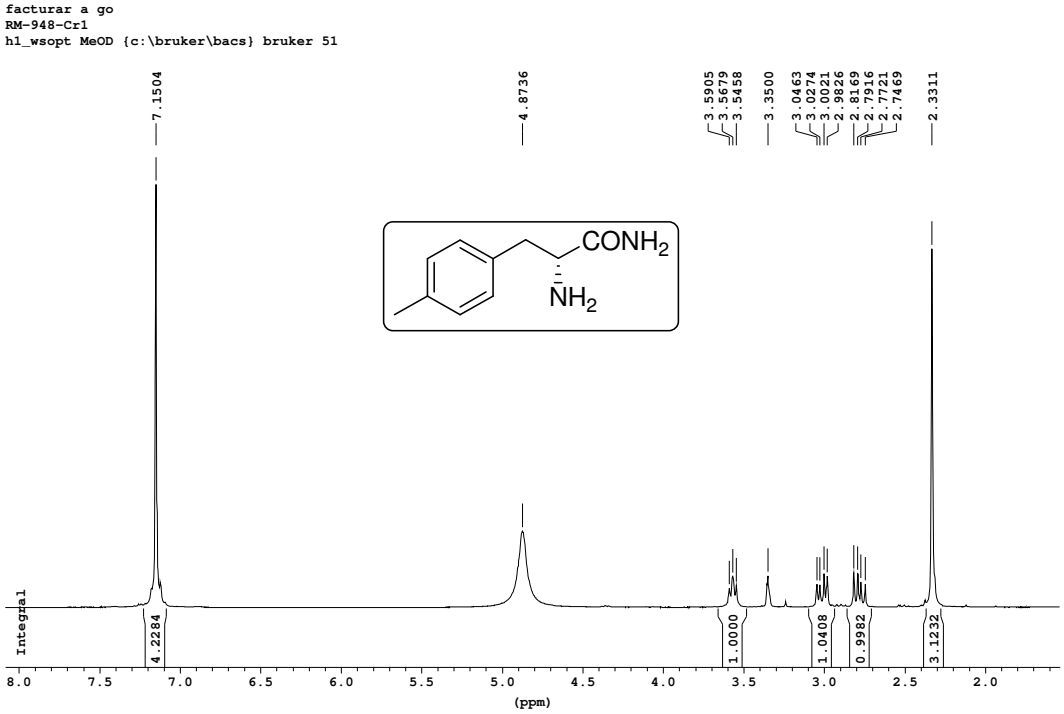


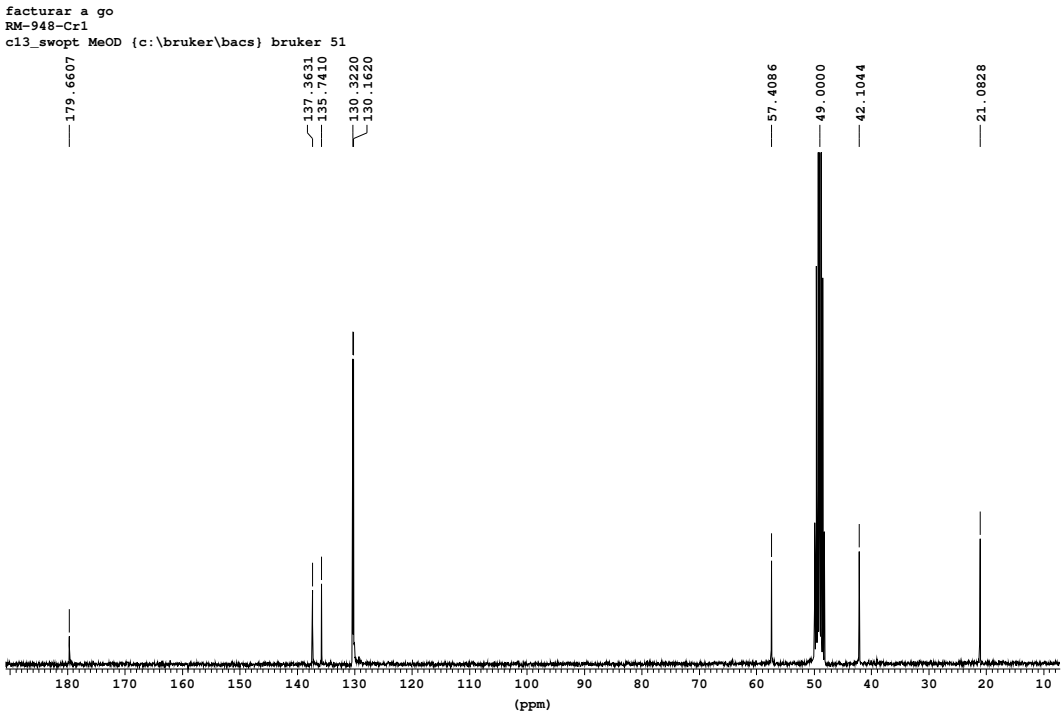


D-Phenylalaninamide [(*R*)-31a] (HMBC)

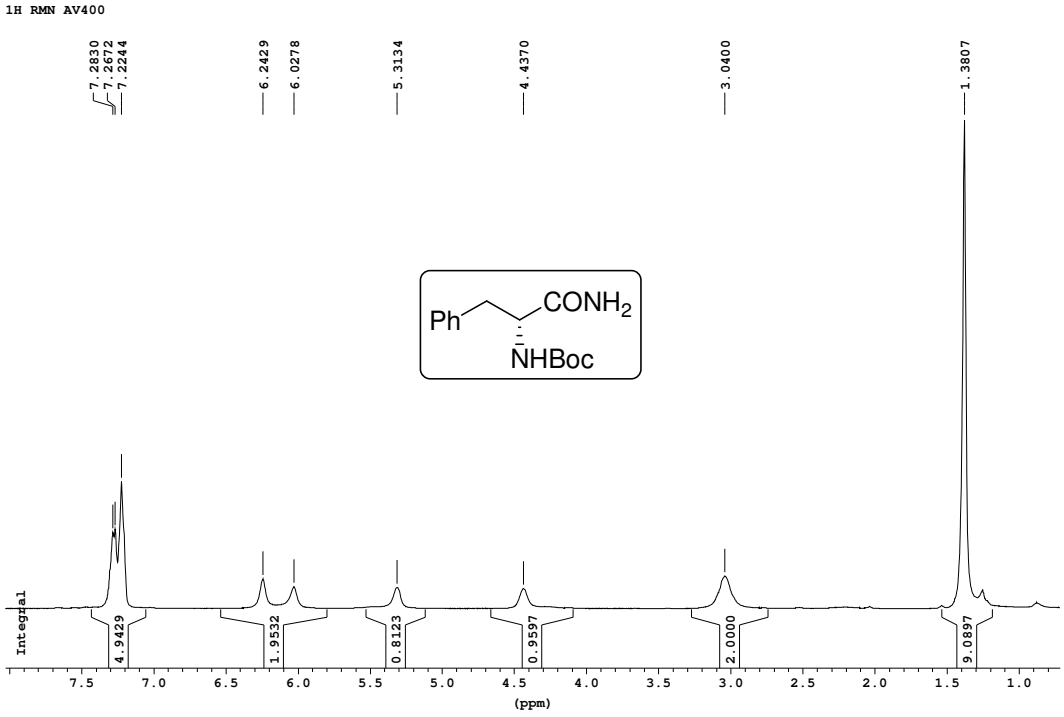


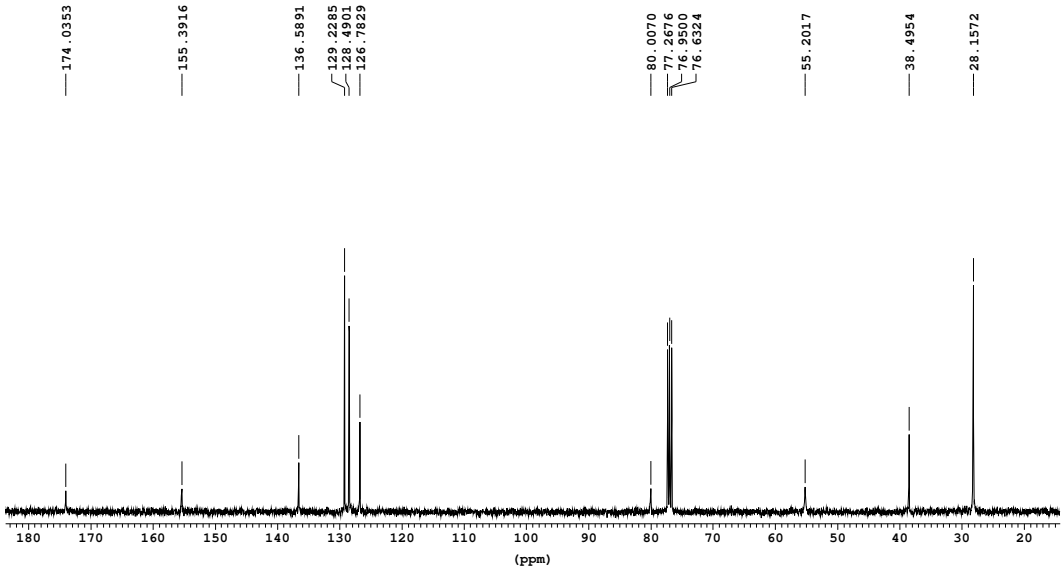
β-(*p*-Tolyl)-D-alaninamide [(*R*)-31g]



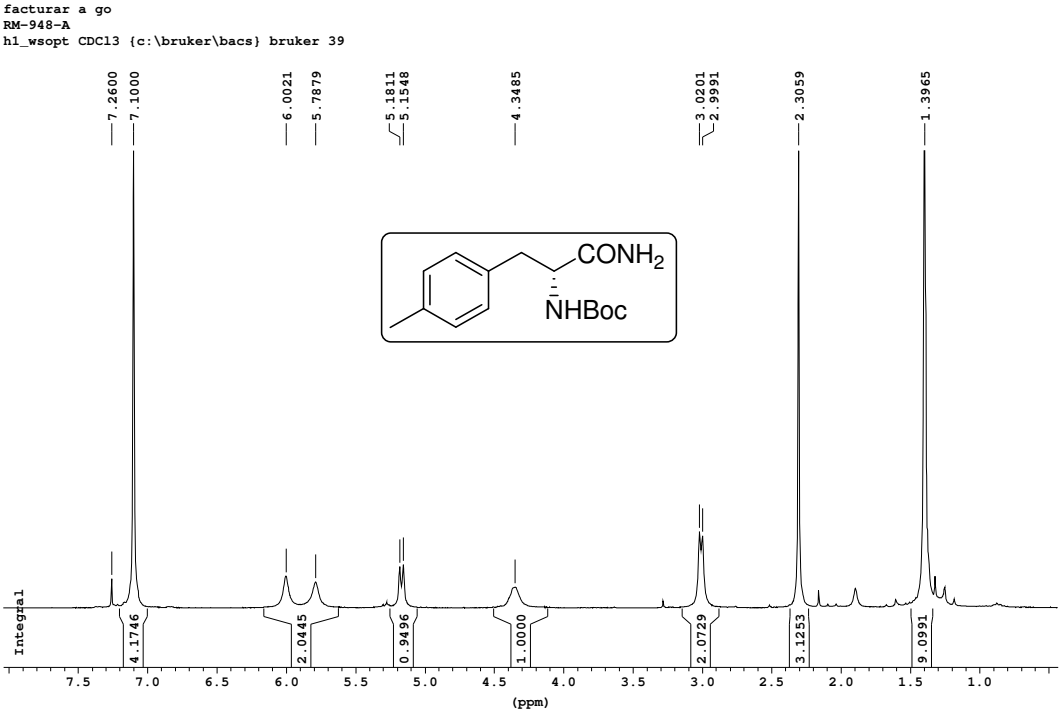


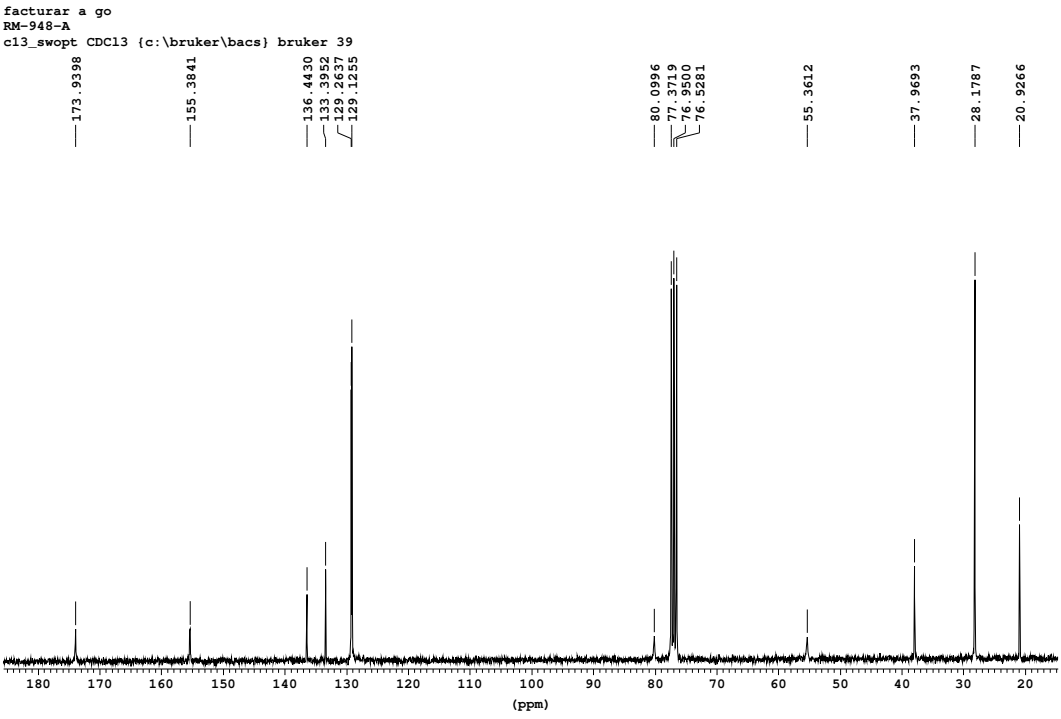
N^2-Boc-D-phenylalaninamide [(*R*)-34a]



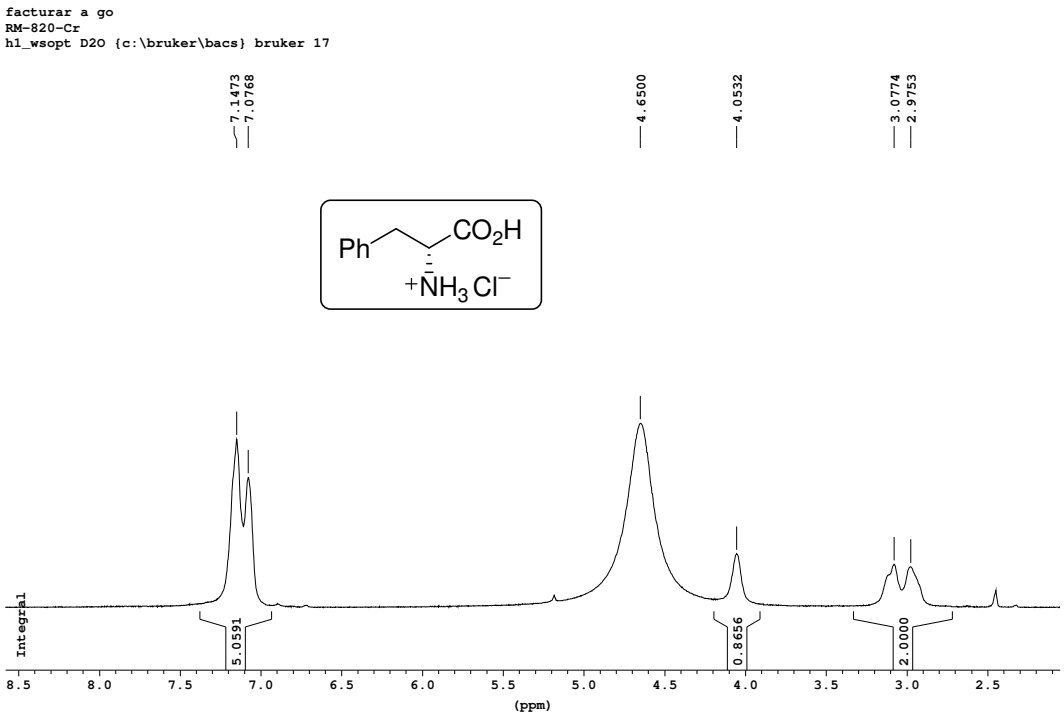


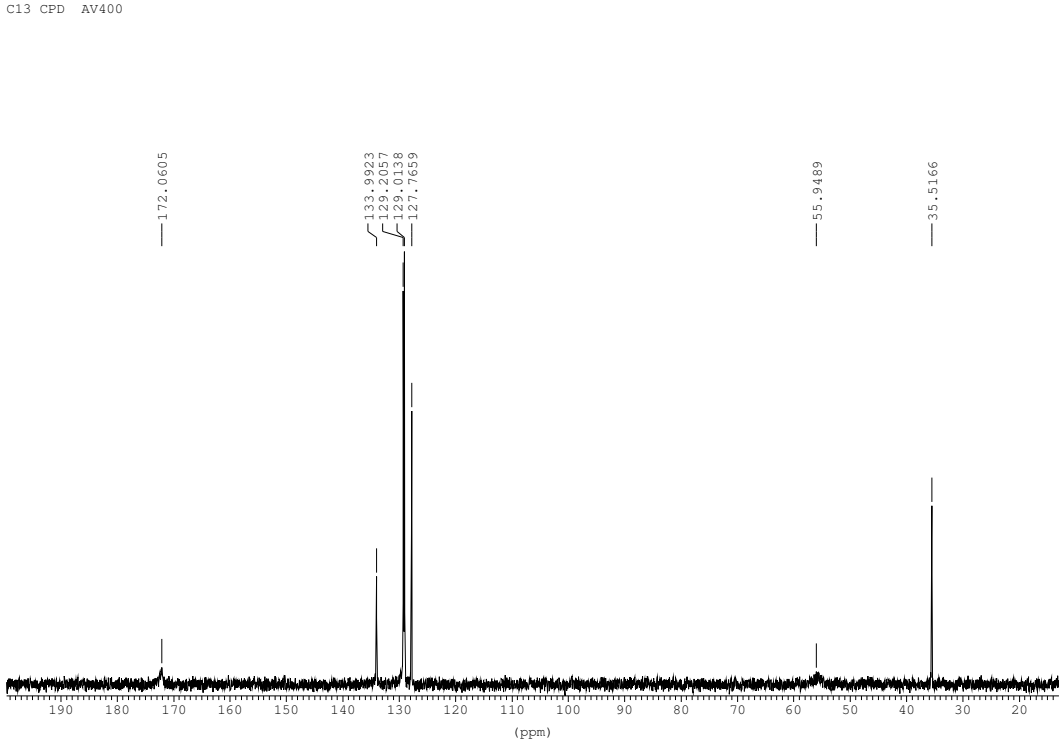
N^2-Boc-β-(*p*-tolyl)-D-alaninamide [(*R*)-34g]





D-Phenylalanine hydrochloride [(*R*)-35a]





β-(*p*-Tolyl)-D-alanine hydrochloride [(*R*)-35g]