**Supporting Information**

# 3-Cyano-3-aza-β-amino Acid Derivatives as Inhibitors of Human Cysteine Cathepsins

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## Table of Contents

## General methods and materials

Melting points were determined on a Büchi 510 oil bath apparatus and are uncorrected. Thin layer chromatography was performed on Merck aluminum sheets. $^{13}$C NMR (125 MHz) and $^{1}$H NMR (500 MHz) spectra were recorded on a BrukerAvanceDRX500 spectrometer. LC-DAD chromatograms and ESI-MS spectra were recorded on an Agilent 1100HPLC system with Applied Biosystems API-2000 mass spectrometer. *tert*-Butyl carbazate (**6**) was obtained from Acros, Geel, Belgium. Balicatib was obtained from Hoelzel Diagnostika GmbH, Cologne, Germany

## Synthetic procedures

***tert*-Butyl 1-methylhydrazinecarboxylate (7).**[1] Di-*tert*-butyl dicarbonate (10.00 g, 45.8 mmol) was dissolved in MeOH (50 mL) and added dropwise to a solution of methyl hydrazine (2.74 g, 3.12 mL, 59.6 mmol) and MeOH (50 mL) over a period of 30 min at room temperature. Stirring was continued for 40 min and the mixture was concentrated in vacuo. The oily residue was taken up in EtOAc (100 mL) and washed with sat. $NaHCO_3$ (2 × 50 mL) and brine (1 × 30 mL). After drying ($Na_2SO_4$) the solvent was removed under reduced pressure to obtain *tert*-butyl methylhydrazinecarboxylate as a colorless oil (5.18 g, 77%) $^{1}$H NMR (500 MHz, DMSO-$d_6$) δ 1.39 (s, 9H, $C(CH_3)_3$), 2.91 (s, 3H, $NCH_3$), 4.46 (s, 2H, $NH_2$); $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ 28.22 ($C(CH_3)_3$), 38.36 ($NCH_3$), 78.91 ($C(CH_3)_3$), 156.20 (CO); LC-MS (ESI) (90% $H_2O$ to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 200-400 nm), $\tau_r$ = 6.97 min, 100% purity, m/z 147.21 ($[M + H]^+$), $C_6H_{14}N_2O_2$ (146.19).

**2-[2-(*tert*-Butoxycarbonyl)hydrazono]acetic acid (8).**[2] *tert*-Butyl carbazate **6** (529 mg, 4.0 mmol) and glyoxylic acid monohydrate (368 mg, 4.0 mmol) were dissolved in EtOH 95% (10 mL). The mixture was stirred for 19 h at room temperature. Evaporation gave a crude powder that was filtrated after trituration with $Et_2O$ (10 mL) (356 g, 47 %). mp. 139-141 °C; $^{1}$H NMR

(500 MHz, DMSO-$d_6$) δ 1.45 (s, 9H, $C(CH_3)_3$), 7.30 (s, 1H, CH), 11.31 (s, 1H, NH), 12.82 (s, 1H, COOH); $^{13}$C NMR δ (125 MHz, DMSO-$d_6$) δ 28.02 ($C(CH_3)_3$), 80.74 ($C(CH_3)_3$), 134.43 (N*C*HC), 151.88 (OCON), 164.64 ($CO_2H$); LC-MS (ESI) (90% $H_2O$ to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), $\tau_r$ = 1.39 min, 100% purity, m/z 206.17 ($[M + NH_4]^+$), m/z 187.16 ($[M - H]^-$), $C_7H_{12}N_2O_4$ (188.18).

**2-[2-(*tert*-Butoxycarbonyl)-2-methylhydrazono]acetic acid (9).** *tert*-Butyl 1-methylhydrazinecarboxylate **7** (585 mg, 0.59 mL, 4.0 mmol) and glyoxylic acid monohydrate (368 mg, 4.0 mmol) were dissolved in 95% EtOH (10 mL). The mixture was stirred for 19 h at room temperature. Evaporation gave an oily residue. After trituration with $Et_2O$ (10 mL) and cooling, a white powder was formed which was filtered off (660 mg, 82%). mp 100-102 °C; $^1$H NMR (500 MHz, DMSO-$d_6$) δ 1.47 (s, 9H, $C(CH_3)_3$), 3.20 (s, 3H, $NCH_3$), 6.99 (s, 1H, CH); $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ 27.87 ($C(CH_3)_3$), 31.51 ($NCH_3$), 82.12 ($C(CH_3)_3$), 130.77 (N*C*HCOOH), 152.06 (O*C*ON), 164.72 ($CO_2H$); LC-MS (ESI) (90% $H_2O$ to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), $\tau_r$ = 4.4 min, 100% purity, m/z 220.18 ($[M + NH_4]^+$), m/z 201.29 ($[M - H]^-$), $C_8H_{14}N_2O_4$ (202.21).

***tert*-Butyl 2-(2-morpholino-2-oxoethylidene)hydrazinecarboxylate (10).** Compound **8** (565 mg, 3.0 mmol) was dissolved in dry THF (20 mL) and cooled to -25°C. *N*-methylmorpholine (303 mg, 3.0 mmol, 0.33 mL) and isobutyl chloroformate (410 mg, 3.0 mmol, 0.39 mL) were given to the stirred solution. Morpholine (261 mg, 3.0 mmol, 0.26 mL) was added to the reaction mixture when the precipitation of *N*-methylmorpholinum chloride occurred. The mixture was allowed to warm to room temperature within 30 min, and stirred for additional 18 h. The solvent was evaporated, and the resulting white solid was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with 10% $KHSO_4$ (30 mL) and brine (30 mL). The solvent was dried ($Na_2SO_4$) and evaporated. The crude product was purified by column chromatography using dichloromethane/methanol (9:1) to obtain **10** as a white solid (450 mg, 58%). mp. 151-158 °C; $^1$H NMR (500 MHz, DMSO-$d_6$) δ 1.44 (s, 9H, $C(CH_3)_3$), 3.48-3.50 (m, 2H, $NCH_2CH_2CH_2O$ ), 3.60-3.62 (m, 4H, $NCH_2CH_2CH_2O$ ), 3.68-3.70 (m, 2H, $NCH_2CH_2$ $CH_2O$), 7.37 (s, 1H, NCH), 12.49 (s, 1H, NH); $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ 27.88 ($C(CH_3)_3$), 41.40, 45.81 ($NCH_2CH_2$), 65.92, 66.16 ($OCH_2CH_2$), 80.90 ($C(CH_3)_3$), 127.40 (N*C*HC), 151.65 (OCON), 160.93 (CH*C*ON); LC-MS (ESI) (90% $H_2O$ to

100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), $\tau_r$ = 9.24 min, 98% purity, m/z 258.30 ($[M + H]^+$), m/z 256.04 ($[M - H]^-$), $C_{11}H_{19}N_3O_4$ (257.29).

***tert*-Butyl 2-[2-(benzyl(methyl)amino)-2-oxoethylidene]hydrazinecarboxylate (11).** Compound **8** (565 mg, 3.0 mmol) was dissolved in dry THF (20 mL) and cooled to -25 °C. *N*-methylmorpholine (303 mg, 3.0 mmol, 0.33 mL) and isobutyl chloroformate (410 mg, 3.0 mmol, 0.39 mL) were given to the stirred solution. *N*-Methylbenzylamine (364 mg, 3.00 mmol, 0.39 mL) was dissolved in dry THF and added to the reaction mixture when the precipitation of *N*-methylmorpholinum chloride occurred. The mixture was allowed to warm to room temperature within 30 min, and stirred for additional 19 h. The solvent was evaporated, and the resulting oily residue was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with 10% $KHSO_4$ (30 mL) and brine (30 mL). The solvent was dried ($Na_2SO_4$) and evaporated. The oily residue was purified by column chromatography on silica gel using dichloromethane/methanol (9:1) as eluent to obtain **11** as a mixture of isomers (565 mg, 65%). $^1$H NMR (500 MHz, DMSO-$d_6$) δ 1.41 (s, 9H, $C(CH_3)_3$, minor), 1.44 (s, 9H, $C(CH_3)_3$, major), 2.82 (s, 3H, $NCH_3$, minor), 3.04 (s, 3H, $NCH_3$, major), 4.56 (s, 2H, $CH_2C_6H_5$, major), 4.75 (s, 2H, $CH_2C_6H_5$, minor), 7.24-7.37 (m, 6H, $C_6H_5$, NCH, major & minor), 7.67 (s, 1H each, NH, major & minor); $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ 28.08 ($C(CH_3)_3$), 33.48, 35.44 ($NNCH_3$), 50.50, 52.63 ($CH_2C_6H_5$), 80.29 ($C(CH_3)_3$), 127.30 (C2', C3', C4'), 127.40 (C2', C3', C4'), 127.77 (C2', C3', C4'), 128.63 (C2', C3', C4'), 128.73 (C2', C3', C4'), 136.99 (NCH), 137.29, 137.37 (C1'), 152.14 (OCON), 163.50 (CHCON), six signals for $C(CH_3)_3$, $C(CH_3)_3$, C2', C3', C4', NCH, OCON, CHCON were not observed; LC-MS (ESI) (90% $H_2O$ to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), $\tau_r$ = 10.25 min, 97% purity, m/z 292.26 ($[M + H]^+$), m/z 290.30 ([M - H]), $C_{15}H_{21}N_3O_3$ (291.35).

***tert*-Butyl 2-[2-(dibenzylamino)-2-oxoethylidene]hydrazinecarboxylate (12).** Compound **8** (941 mg, 5.0 mmol) was dissolved in dry THF (30 mL) and cooled to -25 °C. *N*-methylmorpholine (506 mg, 5.0 mmol, 0.55 mL) and isobutyl chloroformate (683 mg , 5.0 mmol, 0.65 mL) were given to the strirred solution. Dibenzylamine (986 mg, 5.0 mmol, 0.96 mL) was added to the reaction mixture when the precipitation of *N*-methylmorpholinum chloride occurred. The mixture was allowed to warm to room temperature within 30 min, and stirred for additional 18 h. The solvent was evaporated, and the resulting white solid was

extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with 10% $KHSO_4$ (30 mL) and brine (30 mL). The solvent was dried ($Na_2SO_4$) and evaporated. The oily residue was purified by column chromatography on silica gel using ethyl acetate/petroleum ether (1:2) as eluent to obtain **12** as an oily product (630 mg, 44%). $^1$H NMR (500 MHz, DMSO-$d_6$) δ 1.41 (s, 9H, C$(CH_3)_3$), 4.49 (s, 2H, C$H_2C_6H_5$), 4.69 (s, 2H, C$H_2C_6H_5$), 7.22-7.28 (m, 6H, $C_6H_5$, NCH) 7.29-7.36 (m, 5H, $C_6H_5$); $^{13}$C NMR (500 MHz, DMSO-$d_6$) δ 28.05 (C($CH_3)_3$), 48.31 ($CH_2C_6H_5$), 50.22 ($CH_2C_6H_5$), 80.35 ($C(CH_3)_3$), 127.41 (C2', C3', C4', C2'', C3'', C4''), 127.90 (C2', C3', C4', C2'', C3'', C4''), 128.59 (C2', C3', C4', C2'', C3'', C4''), 128.75 (NCHCO), 137.18 (C1', C1''), 152.11 (OCON), 163.71 (CHCON); LC-MS (ESI) (90% $H_2O$ to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), $\tau_r$ = 11.42 min, 89% purity, m/z 368.01 ($[M + H]^+$), m/z 366.08 ($[M - H]^-$), $C_{21}H_{25}N_3O_3$ (367.44).

***tert*-Butyl 1-methyl-2-(2-morpholino-2-oxoethylidene)hydrazinecarboxylate (13).** Compound **9** (303 mg, 1.5 mmol) was dissolved in dry THF (10 mL) and cooled to -25 °C. *N*-methylmorpholine (152 mg, 1.5 mmol, 0.17 mL) and isobutyl chloroformate (205 mg, 1.5 mmol, 0.20 mL) were given to the stirred solution. Morpholine (131 mg, 1.5 mmol, 0.13 mL) was added to the reaction mixture when the precipitation of *N*-methylmorpholinum chloride occurred. The mixture was allowed to warm to room temperature within 30 min, and stirred for additional 70 h. The solvent was evaporated, and the resulting oily residue was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with 10% $KHSO_4$ (30 mL) and brine (30 mL). The solvent was dried ($Na_2SO_4$) and evaporated to obtain **13** as a colorless oil (240 mg, 59%). $^1$H NMR (500 MHz, DMSO-$d_6$) δ 1.45 (s, 9H, C$(CH_3)_3$), 3.18 (s, 3H, $NCH_3$), 3.53-3.55 (m, 2H, $NCH_2CH_2O$), 3.59-3.61 (m, 4H, $NCH_2CH_2O$), 3.78-3.81(m, 2H, -$NCH_2CH_2O$ ), 7.20 (1H, NCH); $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ 27.89 (C($CH_3)_3$), 30.40 ($NCH_3$), 42.40 46.50 ($NCH_2$), 66.26 66.53 ($CH_2O$), 81.54 ($C(CH_3)_3$), 133.09 (NCHC), 152.36 (OCON), 162.53 (CHCON); LC-MS (ESI) (90% $H_2O$ to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), $\tau_r$ = 8.53 min, 99% purity, m/z 272.15 ($[M + H]^+$), m/z 270.13 ($[M - H]^-$), $C_{12}H_{21}N_3O_4$ (271.31).

***tert*-Butyl 2-[2-(benzyl(methyl)amino)-2-oxoethylidene]-1-methylhydrazinecarboxylate (14).** Compound **9** (606 mg, 3.0 mmol) was dissolved in dry THF (20 mL) and cooled to -25 °C. *N*-methylmorpholine (303 mg, 3.0 mmol, 0.33 mL) and isobutyl chloroformate (410 mg,

3.0 mmol, 0.39 mL) were given to the stirred solution. *N*-Methylbenzylamine (364 mg, 3.0 mmol, 0.39 mL) was dissolved in dry THF and added to the reaction mixture when the precipitation of *N*-methylmorpholinum chloride occurred. The mixture was allowed to warm to room temperature within 30 min, and stirred for additional 5 h. The solvent was evaporated, and the oily residue was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with 10% $KHSO_4$ (30 mL) and brine (30 mL). The solvent was dried ($Na_2SO_4$) and evaporated. The oily residue was purified by column chromatography on silica gel using dichloromethane/methanol (9:1) as eluent to obtain **14** as a mixture of isomers (420 mg, 46%). $^1$H NMR (500 MHz, DMSO-$d_6$) δ 1.36 (s, 9H, $C(CH_3)_3$, minor), 1.45 (s, 9H, $C(CH_3)_3$, major), 2.81 (s, 3H, $NCH_3$, minor), 3.12 (s, 3H, $NCH_3$, major), 3.17 (s, 3H, $NCH_3$, minor), 3.21 (s, 3H, $NCH_3$, major), 4.59 (s, 2H, $CH_2C_6H_5$, major), 4.90 (s, 2H, $CH_2C_6H_5$, minor), 7.25-7.30 (m, 6H each, $C_6H_5$, NCH, major & minor) 7.31-7.35 (m, 5H each, $C_6H_5$, major & minor ) $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ 27.82, 27.89 ($C(CH_3)_3$), 30.44, 30.54 ($NNCH_3$), 33.53, 35.80 ($CH_2NCH_3$), 50.74, 52.72 ($CH_2C_6H_5$), 81.50 ($C(CH_3)_3$), 127.27 (C2', C3', C4'), 127.45 (C2', C3', C4'), 127.60 (C2', C3', C4'), 127.73 (C2', C3', C4'), 128.61 (C2', C3', C4'), 133.51, 133.89 (NCH), 137.43, 137.69 (C1'), 152.41 (OCON), 163.87, 163.91 (CHCON), three signals for $C(CH_3)_3$, C2', C3', C4', OCON were not observed; LC-MS (ESI) (90% $H_2O$ to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), $\tau_r$ = 10.54 min, 97% purity, m/z 306.43 ($[M + H]^+$), $C_{16}H_{23}N_3O_3$ (305.37).

***tert*-Butyl 2-[2-(dibenzylamino)-2-oxoethylidene]-1-methylhydrazinecarboxylate (15).** Compound **9** (606 mg, 3.0 mmol) was dissolved in dry THF (20 mL) and cooled to -25 °C. *N*-methylmorpholine (303 mg, 3.0 mmol, 0.33 mL) and isobutyl chloroformate (410 mg ,3.0 mmol, 0.39 mL) were given to the stirred solution. Dibenzylamine (592 mg, 3.0 mmol, 0.58 mL) was added to the reaction mixture when the precipitation of *N*-methylmorpholinum chloride occurred. The mixture was allowed to warm to room temperature within 30 min, and stirred for additional 28 h. The solvent was evaporated, and the resulting white solid was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with 10% $KHSO_4$ (30 mL) and brine (30mL). The solvent was dried ($Na_2SO_4$) and evaporated. The crude product was purified by column chromatography on silica gel using ethyl acetate/petroleum ether (1:2) as eluent to obtain **15** as a white solid (383 mg, 34%). mp 114-116 °C; $^1$H NMR (500 MHz, DMSO-$d_6$) δ 1.36 (s, 9H, $C(CH_3)_3$), 3.16 (s, 3H, $NCH_3$), 4.49 (s, 2H, $CH_2C_6H_5$), 4.85 (s, 2H, $CH_2C_6H_5$), 7.23-7.29 (m, 6H, $C_6H_5$, NCH), 7.31-7.35 (m, 5H,

$C_6H_5$); $^{13}C$ NMR (125 MHz, DMSO-$d_6$) δ 27.81 (C($CH_3$)$_3$), 30.51 ($NCH_3$), 48.28 ($CH_2C_6H_5$), 50.36 ($CH_2C_6H_5$), 81.58 ($C$($CH_3$)$_3$), 127.30 (C2', C3', C4', C2'', C3'', C4''), 127.51 (C2', C3', C4', C2'', C3'', C4''), 127.69 (C2', C3', C4', C2'', C3'', C4''), 127.88 (C2', C3', C4', C2'', C3'', C4''), 128.57 (C2', C3', C4', C2'', C3'', C4''), 128.65 (C2', C3', C4', C2'', C3'', C4''), 133.64 (NCH), 137.23 (C1', C1''), 137.54 (C1', C1''), 152.38 (OCON), 164.05 (CH$C$ON); LC-MS (ESI) (90% $H_2O$ to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), $\tau_r$ = 11.78 min, 100% purity, m/z 381.88 ($[M + H]^+$), $C_{22}H_{27}N_3O_3$ (381.47).

***tert*-Butyl 2-(2-morpholino-2-oxoethyl)hydrazinecarboxylate (16).** To a solution of compound **10** (420 mg, 1.6 mmol) in MeOH (10 mL) was added 10% Pd/C (50 mg). After 4 h of stirring under $H_2$ atmosphere and 3.3 bar, the solution was filtered and evaporated to obtain **16** as an oily residue (360 mg, 85%). $^1H$ NMR (500 MHz, DMSO-$d_6$) δ 1.37 (s, 9H, C($CH_3$)$_3$), 3.40-3.41 (m, 4H, $NCH_2CH_2O$), 3.51-3.55 (m, 6H, $NCH_2CH_2O$, $NCH_2CO$), 4.60 (1H, q, $^3J$ = 8.8 Hz, N$H$$CH_2$), 8.06 (broad, s, 1H, OCNH); $^{13}C$ NMR (125 MHz, DMSO-$d_6$) δ 28.32 (C($CH_3$)$_3$), 41.55, 44.89 (N$C$$H_2CH_2O$), 66.12 ($NCH_2C$$H_2O$), 78.61 ($C$($CH_3$)$_3$), 156.13 (OCON), 168.18 ($CH_2C$ON); LC-MS (ESI) (90% $H_2O$ to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 200-400 nm), $\tau_r$ = 5.50 min, 91% purity, m/z 260.35 ($[M + H]^+$), m/z 258.38 ($[M - H]^-$), $C_{11}H_{21}N_3O_4$ (259.30).

***tert*-Butyl 2-[2-(benzyl(methyl)amino)-2-oxoethyl]hydrazinecarboxylate (17).** To a solution of compound **11** (565 mg, 1.9 mmol) in MeOH (20 mL) was added 10% Pd/C (60 mg). After 8 h of stirring under $H_2$ atmosphere and 3.3 bar, the solution was filtered and evaporated to obtain **17** as an oily product and as a mixture of isomers. (0.501 mg, 88%). $^1H$ NMR (500 MHz, DMSO-$d_6$) δ 1.35 (s, 9H, C($CH_3$)$_3$, minor), 1.38 (s, 9H, C($CH_3$)$_3$, major), 2.80 (s, 3H, $NCH_3$, minor), 2.85 (s, 3H, $NCH_3$, major), 3.55 (d, $^3J$ = 5.7 Hz, 2H, $NCH_2CO$, minor), 3.59 (d, $^3J$ = 6.0 Hz, 2H, $NCH_2CO$, major) 4.49 (s, 2H, $CH_2C_6H_5$, major), 4.52 (s, 2H, $CH_2C_6H_5$, minor), 4.60 (m, 2H, NHN$H$$CH_2$, major & minor), 7.18-7.32 (m, 10H, $C_6H_5$, major & minor), 8.06 (s, 2H, N$H$NH$CH_2$, major & minor); $^{13}C$ NMR (125 MHz, DMSO-$d_6$) δ 28.31 (C($C$$H_3$)$_3$), 33.41, 33.89 (NN$C$$H_3$), 50.00, 51.65, 52.31 ($C$$H_2C_6H_5$, $C$$H_2C_6H_5$), 78.62 ($C$($CH_3$)$_3$), 126.70 (C2', C3', C4'), 127.14 (C2', C3', C4'), 127.37 (C2', C3', C4'), 127.64 (C2', C3', C4'), 128.54 (C2', C3', C4'), 128.80 (C2', C3', C4'), 137.64 (C1'), 156.13 ($CH_2$OCON), 169.65 (CH$C$ON), six signals for C($C$$H_3$)$_3$, $C$$H_2C_6H_5$ or NH$C$$H_2$CO, $C$($CH_3$)$_3$,

C1', OCON, CH*C*ON were not observed; LC-MS (ESI) (90% $H_2O$ to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), $\tau_r$ = 10.17 min, 93% purity, m/z 294.26 ($[M + H]^+$), $C_{15}H_{23}N_3O_3$ (293.36).

***tert*-Butyl 2-[2-(dibenzylamino)-2-oxoethyl]hydrazinecarboxylate (18).** To a solution of compound **12** (630 mg, 1.7 mmol) in MeOH (20 mL) was added 10% Pd/C (90 mg). After 8 h of stirring under $H_2$ atmosphere and 3.3 bar, the solution was filtered and evaporated. The oily product was purified by column chromatography on silica gel using dichloromethane/methanol (9:1) as eluent to obtain compound **18** as a white solid (480 mg, 76%). mp. 94-96 °C; $^1$H NMR (500 MHz, DMSO-$d_6$) δ 1.35 (s, 9H, $C(CH_3)_3$), 3.61 (d, $^3J$ = 5.4 Hz, 2H, $NCH_2CO$), 4.49 (s, 2H, $CH_2C_6H_5$), 4.50 (s, 2H, $CH_2C_6H_5$), 4.67 (q , $^3J$ = 5.1 Hz, 1H, CNHN*H*$CH_2$), 7.16-7.29 (m, 10H, $C_6H_5$), 8.07 (s, 1H, N*H*N$HCH_2$); $^{13}$C NMR (500 MHz, DMSO-$d_6$) δ 28.26 ($C(CH_3)_3$), 48.21 ($NCH_2CO$, $CH_2C_6H_5$), 49.27 ($NCH_2CO$, $CH_2C_6H_5$), 52.37 ($NCH_2CO$, $CH_2C_6H_5$), 78.64 ($C(CH_3)_3$), 126.64 (C2', C3', C4', C2'', C3'', C4''), 127.19 (C2', C3', C4', C2'', C3'', C4''), 127.39 (C2', C3', C4', C2'', C3'', C4'') , 127.80 (C2', C3', C4', C2'', C3'', C4''), 128.52 (C2', C3', C4', C2'', C3'', C4''), 128.80 (C2', C3', C4', C2'', C3'', C4''), 137.08 (C1', C1''), 137.49 (C1', C1''), 156.10 (OCON), 170.09 ($CH_2CON$); LC-MS (ESI) (90% $H_2O$ to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220-400 nm), $\tau_r$ = 11.43 min, 98% purity, m/z 370.33 ($[M + H]^+$), $C_{21}H_{27}N_3O_3$ (369.46).

**Preparation of *tert*-butyl 1-methyl-2-(2-morpholino-2-oxoethyl)hydrazinecarboxylate (19) from *tert*-butyl 1-methyl-2-(2-morpholino-2-oxoethylidene)hydrazinecarboxylate (13).** To a solution of compound **13** (200 mg, 0.75 mmol) in MeOH (10 mL) was added 10% Pd/C (30 mg). After 90 min of stirring under $H_2$ atmosphere and 3.3 bar, the solution was filtered and evaporated. The oily product was purified by column chromatography on silica gel using dichloromethane/methanol (9:1) as eluent to obtain compound **19** as a yellow oil (70 mg, 35%). $^1$H NMR (500 MHz, DMSO-$d_6$) δ 1.39 (s, 9H, $C(CH_3)_3$), 2.92 (s, 3H, $NCH_3$), 3.41-3.58 (m, 4H, $NCH_2CH_2O$ ), 3.53-3.58 (m, 6H, $NCH_2CH_2O$, $NCH_2CO$), 5.12 (1H, NH); $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ 28.16 ($C(CH_3)_3$), 36.67 ($NCH_3$), 41.52, 44.95 ($NCH_2CH_2O$), 50.28 ($NCH_2CO$), 66.13 ($NCH_2CH_2O$), 79.55 ($C(CH_3)_3$), 155.28 (OCON), 167.81 ($CH_2CON$); LC-MS (ESI) (90% $H_2O$ to 100% MeOH in 10 min, then 100% MeOH to

20 min, DAD 200-300 nm), $\tau_r$ = 8.11 min, 94% purity, m/z 274.43 ($[M + H]^+$), $C_{12}H_{23}N_3O_4$ (273.33).

**Preparation of *tert*-butyl 1-methyl-2-(2-morpholino-2-oxoethyl)hydrazinecarboxylate (19) from 2-[2-(*tert*-butoxycarbonyl)-2-methylhydrazinyl]acetic acid (29).** Compound **29** (320 g, 1.57 mmol) was dissolved in dry THF (20 mL) and cooled to -25 °C. *N*-methylmorpholine (159 mg, 1.57 mmol, 0.17 mL) and isobutyl chloroformate (214 mg, 1.57 mmol, 0.21 mL). Morpholine (137 mg, 1.57 mmol) was added to the reaction mixture when the precipitation of *N*-methylmorpholinum chloride occurred. The mixture was allowed to warm to room temperature within 30 min, and stirred for additional 24 h. The solvent was evaporated, and the resulting solid was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with 10% $KHSO_4$ (30 mL) and brine (30 mL). The solvent was dried ($Na_2SO_4$) and evaporated. The resulting residue was purified by column chromatography using dichloromethane/methanol (9:1) to obtain **19** (0.27 g, 63%).

***tert*-Butyl 2-[2-(benzyl(methyl)amino)-2-oxoethyl]-1-methylhydrazinecarboxylate (20).** To a solution of compound **14** (611 mg, 2.0 mmol) in MeOH (20 mL) was added 10% Pd/C (70 mg). After 8 h of stirring under $H_2$ atmosphere and 3.3 bar, the solution was filtered and evaporated. The oily product was purified by column chromatography on silica gel using dichloromethane/methanol (9:1) as eluent to obtain compound **20** as a yellow oil and a mixture of isomers (233 mg, 38%). $^1$H NMR (500 MHz, DMSO-$d_6$) δ 1.35 (s, 9H, $C(CH_3)_3$ minor), 1.40 (s, 9H, $C(CH_3)_3$, major), 2.81 (s, 3H, $NCH_3$, minor), 2.89 (s, 3H, $NCH_3$, major), 2.89 (s, 3H, $NCH_3$, minor), 2.91 (s, 3H, $NCH_3$, major), 3.62 (d, $^3J$ = 5.4 Hz, 2H, $NCH_2CO$, minor), 3.65 (d, $^3J$ = 5.4 Hz, 2H, $NCH_2CO$, major), 4.49 (s, 2H, $CH_2C_6H_5$, major), 4.55 (s, 2H, $CH_2C_6H_5$, minor), 5.12 (s, 1H each, NH, major & minor ), 7.18-7.32 (m, 5H each, $C_6H_5$, major & minor); $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ 27.98 ($C(CH_3)_3$), 33.26, 33.79 ($NNCH_3$), 36.44 ($CH_2NCH_3$), 49.82, 50.15, 51.54 ($CH_2C_6H_5$, $NHCH_2CO$), 79.35 ($C(CH_3)_3$), 126.49 (C2', C3', C4'), 126.99 (C2', C3', C4') , 127.50 (C2', C3', C4'), 128.35 (C2', C3', C4'), 128.63 (C2', C3', C4'), 137.17, 137.45 (C1'), 155.10 (OCON), 168.98, 169.03 ($CH_2CON$), six signals for $C(CH_3)_3$, $CH_2NCH_3$, $CH_2C_6H_5$ or $NHCH_2CO$, $C(CH_3)_3$, C2', C3', C4', OCON were not observed ; LC-MS (ESI) (90% $H_2O$ to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 200-400 nm), $\tau_r$ = 10.46 min, 79% purity, m/z 308.30 ($[M + H]^+$), $C_{16}H_{25}N_3O_3$ (307.39).

***tert*-Butyl 2-[2-(dibenzylamino)-2-oxoethyl]-1-methylhydrazinecarboxylate (21).** To a solution of compound **15** (730 mg, 1.9 mmol) in MeOH (20 mL) was added 10% Pd/C (90 mg). After 8 h of stirring under $H_2$ atmosphere and 3.3 bar, the solution was filtered and evaporated. The oily product was purified by column chromatography on silica gel using ethyl acetate/petroleum ether (1:1) as eluent to obtain compound **21** as a yellow oil (292 mg, 40%) $^1$H NMR (500 MHz, DMSO-$d_6$) δ 1.34 (s, 9H, C($CH_3$)$_3$), 2.89 (s, 3H, $NCH_3$), 3.68 (s, 2H, $NCH_2CO$), 4.50 (s, 2H, C$H_2C_6H_5$), 4.51 (s, 2H, C$H_2C_6H_5$), 7.17-7.37 (m, 10H, $C_6H_5$,); $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ 28.09 (C($CH_3$)$_3$), 48.31 ($CH_2C_6H_5$, $NCH_2CO$), 49.39 ($CH_2C_6H_5$, $NCH_2CO$), 50.45 ($CH_2C_6H_5$, $NCH_2CO$), 79.57 ($C$($CH_3$)$_3$), 126.66 (C2', C3', C4', C2'', C3'', C4''), 127.26 (C2', C3', C4', C2'', C3'', C4''), 127.44 (C2', C3', C4', C2'', C3'', C4'') , 127.89 (C2', C3', C4', C2'', C3'', C4'') , 128.54 (C2', C3', C4', C2'', C3'', C4''), 128.84 (C2', C3', C4', C2'', C3'', C4'') , 137.13 (C1', C1''), 137.53 (C1', C1''), 155.23 (OCON), 169.66 ($CH_2CON$); LC-MS (ESI) (90% $H_2O$ to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 200-400 nm), $\tau_r$ = 11.62 min, 89% purity, m/z 384.15 ($[M + H]^+$), $C_{22}H_{29}N_3O_3$ (383.48).

***tert*-Butyl 2-cyano-2-(2-morpholino-2-oxoethyl)hydrazinecarboxylate (22).** Sodium acetate (227 mg, 2.8 mmol) and cyanogen bromide (196 mg, 1.9 mmol) were added to a solution of compound **16** (240 mg, 0.9 mmol) in MeOH (15 mL). The mixture was stirred at room temperature for 17 h, and the solvent was removed under reduced pressure. The oily residue was purified by column chromatography on silica gel using dichloromethane/methanol (9:1) as eluent to obtain **22** as a white solid (74 mg, 28%). mp. 154-155 °C; $^1$H NMR (500 MHz, DMSO-$d_6$) δ 1.41 (s, 9H, C($CH_3$)$_3$), 3.32-3.34 (m, 2H, $OCH_2CH_2N$), 3.43-3.44 (m, 2H, $OCH_2CH_2N$), 3.55-3.57 (4H, m, $OCH_2CH_2N$), 4.25 (s, 2H, $NCH_2CO$); $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ 28.03 (C($CH_3$)$_3$), 41.81, 44.80 ($NCH_2CH_2O$), 55.50 ($NCH_2CO$), 65.90, 65.99 ($NCH_2CH_2O$), 81.07 ($C$($CH_3$)$_3$), 115.43 (NCN), 154.13 (OCON), 164.66 ($CH_2CON$); LC-MS (ESI) (90% $H_2O$ to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 200-400 nm), $\tau_r$ = 7.79 min, 97% purity, m/z 285.07 ($[M + H]^+$), m/z 283.27 ($[M - H]^-$), $C_{12}H_{20}N_4O_4$ (284.31).

***tert*-Butyl 2-[2-(benzyl(methyl)amino)-2-oxoethyl]-2-cyanohydrazinecarboxylate (23).** Sodium acetate (286 mg, 3.5 mmol) and cyanogen bromide (246 mg, 2.3 mmol) were added to a solution of compound **17** (341 mg, 1.2 mmol) in MeOH (15 mL). The mixture was stirred

at room temperature for 17 h, and the solvent was removed under reduced pressure. The oily residue was purified by column chromatography on silica gel using dichloromethane/methanol (9:1) as eluent to obtain **23** as a white solid (48 mg, 13%). mp. 118-120 C°; $^{1}$H NMR (500 MHz, DMSO-$d_6$) δ 1.37 (s, 9H, C($CH_3$)$_3$ minor), 1.42 (s, 9H, C($CH_3$)$_3$, major), 2.83 (s, 3H, $NCH_3$, minor), 2.85 (s, 3H, $NCH_3$, major), 4.25 (s, 2H, $NCH_2CO$, minor), 4.33 (s, 2H, $NCH_2CO$, major), 4.49 (s, 2H, C$H_2C_6H_5$), 4.51 (s, 2H, C$H_2C_6H_5$), 7.18-7.30 (m, 5H each, $C_6H_5$, major & minor), 9.68 (s, 1H each, major & minor); $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ 27.98, 28.06 (C(*C*$H_3$)$_3$), 33.88 ($CH_2NCH_3$), 50.45, 51.59, 54.47, 55.67 (*C*$H_2C_6H_5$, NH*C*$H_2CO$), 81.06 (*C*($CH_3$)$_3$), 115.46, 115.53 (NCN), 126.74 (C2', C3', C4'), 127.28 (C2', C3', C4') , 127.56 (C2', C3', C4'), 127.74 (C2', C3', C4'), 128.58 (C2', C3', C4'), 128.89 (C2', C3', C4'), 136.75, 137.30 (C1'), 154.19 (OCON), 166.00, 166.20 ($CH_2$*C*ON), two signals for C(*C*$H_3$)$_3$, OCON were not observed ; LC-MS (ESI) (90% $H_2O$ to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 200-400 nm), $\tau_r$ = 10.03 min, 94% purity, m/z 319.22 ($[M + H]^+$), m/z 317.06 ($[M - H]^-$), $C_{16}H_{22}N_4O_3$ (318.37).

***tert*-Butyl 2-cyano-2-[2-(dibenzylamino)-2-oxoethyl]hydrazinecarboxylate (24).** Sodium acetate (320 mg, 3.9 mmol) and cyanogen bromide (275 mg, 2.6 mmol) were added to a solution of compound **18** (480 mg, 1.3 mmol) in MeOH (15 mL). The mixture was stirred at room temperature for 17 h, and the solvent was removed under reduced pressure. The oily residue was purified by column chromatography on silica gel using dichloromethane/methanol (40:1) as eluent to obtain 208 mg as the crude product. 100 mg of the crude product were purified again by column chromatography to obtain **24** as a white solid. (39 mg, 37%). mp. 92-93 °C; $^{1}$H NMR (500 MHz, DMSO-$d_6$) δ 1.36 (s, 9H, C($CH_3$)$_3$), 4.31 (s, 2H, $NCH_2CO$), 4.46 (s, 2H, $CH_2C_6H_5$), 4.52 (s, 2H, $CH_2C_6H_5$), 7.15-7.36 (m, 10H, $C_6H_5$), 9.79 (s, 1H, NH); $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ 27.97 (C(*C*$H_3$)$_3$), 48.79 (N*C*$H_2CO$, *C*$H_2C_6H_5$), 49.20 (N*C*$H_2CO$, *C*$H_2C_6H_5$), 55.50 (N*C*$H_2CO$, *C*$H_2C_6H_5$), 81.09 (*C*($CH_3$)$_3$), 115.48 (NCN), 126.59 (C2', C3', C4', C2'', C3'', C4''), 127.36 (C2', C3', C4', C2'', C3'', C4''), 127.59 (C2', C3', C4', C2'', C3'', C4'') , 127.91 (C2', C3', C4', C2'', C3'', C4''), 128.58 (C2', C3', C4', C2'', C3'', C4''), 128.91 (C2', C3', C4', C2'', C3'', C4''), 136.52 (C1', C1''), 137.14 (C1', C1''), 154.08 (OCON), 166.56 ($CH_2$*C*ON); LC-MS (ESI) (90% $H_2O$ to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 200-400 nm), $\tau_r$ = 11.07 min, 90% purity, m/z 395.41 ($[M + H]^+$), m/z 393.30 ($[M - H]^-$), $C_{22}H_{26}N_4O_3$ (394.47).

***tert*-Butyl 2-cyano-1-methyl-2-(2-morpholino-2-oxoethyl)hydrazinecarboxylate (25).** Sodium acetate (243 mg, 3.0 mmol) and cyanogen bromide (209 mg, 2.0 mmol) were added to a solution of compound **19** (270 mg, 1.0 mmol) in MeOH (15 mL). The mixture was stirred at room temperature for 5 h, and the solvent was removed under reduced pressure. The oily residue was purified by column chromatography on silica gel using ethyl acetate/ethanol (9:1) as eluent to obtain **25** as a white solid (41 mg, 14%). mp. 93-95 °C; $^{1}$H NMR (500 MHz, DMSO-$d_6$) δ 1.45 (s, 9H, C($CH_3$)$_3$), 3.08 (s, 3H, $NCH_3$), 3.33-3.35 (m, 2H, $OCH_2CH_2N$), 3.43-3.44 (m, 2H, $OCH_2CH_2N$), 3.56 (4H, m, $OCH_2CH_2N$), 4.35 (s, 2H, $NCH_2CO$); $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ 27.91 (C(*C*$H_3$)$_3$), 36.13 ($NCH_3$), 41.84, 44.80 (N*C*$H_2CH_2O$), 54.74 (N*C*$H_2CO$), 65.88, 66.00 ($NCH_2$*C*$H_2O$), 82.16 (*C*($CH_3$)$_3$), 114.05 (NCN), 153.78 (OCON), 164.82 ($CH_2$*C*ON); LC-MS (ESI) (90% $H_2O$ to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 200-400 nm), $\tau_r$ = 8.39 min, 95% purity, m/z 299.30 ($[M + H]^+$), m/z 297.33 ($[M - H]^-$), $C_{13}H_{22}N_4O_4$ (298.34).

***tert*-Butyl 2-[2-(benzyl(methyl)amino)-2-oxoethyl]-2-cyano-1-methylhydrazine-carboxylate (26).** Sodium acetate (187 mg, 2.3 mmol) and cyanogen bromide (161 mg, 1.5 mmol) were added to a solution of compound **20** (233 mg, 0.8 mmol) in MeOH (15 mL). The mixture was stirred at room temperature for 18 h, and the solvent was removed under reduced pressure. The oily residue was purified by column chromatography on silica gel using dichloromethane/methanol (30:1) as eluent to obtain **26** as a colorless oil (32 mg, 13%). $^{1}$H NMR (500 MHz, DMSO-$d_6$) δ 1.36 (s, 9H, C($CH_3$)$_3$ minor), 1.45 (s, 9H, C($CH_3$)$_3$, major), 2.86 (s, 3H, $NCH_3$, minor), 2.87 (s, 3H, $NCH_3$, major), 3.08 (s, 3H, $NCH_3$, minor), 3.10 (s, 3H, $NCH_3$, major), 4.34 (s, 2H, $NCH_2CO$, minor), 3.43 (s, 2H, $NCH_2CO$, major), 4.52 (s, 2H each, C*H*$_2C_6H_5$, major & minor), 7.18-7.37 (m, 5H each, $C_6H_5$, major & minor); $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ 27.80, 27.93 (C(*C*$H_3$)$_3$), 33.95, 34.12, 36.14 (NN*C*$H_3$, $CH_2$N*C*$H_3$), 50.54, 51.72, 54.86, 54.90 (*C*$H_2C_6H_5$, NH*C*$H_2$CO), 82.14 (*C*($CH_3$)$_3$), 114.02, 114.14 (NCN), 126.63 (C2', C3', C4'), 127.28 (C2', C3', C4') , 127.72 (C2', C3', C4'), 128.59 (C2', C3', C4'), 128.92 (C2', C3', C4'), 136.88, 137.31 (C1'), 153.63, 153.82 (OCON), 166.09, 166.31 ($CH_2$*C*ON), two signals for C(*C*$H_3$)$_3$, (C2', C3', C4') were not observed; LC-MS (ESI) (90% $H_2O$ to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 200-400 nm), $\tau_r$ = 10.15 min, 90% purity, m/z 333.06 ($[M + H]^+$), m/z 331.41 ($[M - H]^-$), $C_{17}H_{24}N_4O_3$ (332.40).

***tert*-Butyl 2-cyano-2-[2-(dibenzylamino)-2-oxoethyl]-1-methylhydrazinecarboxylate (27).** Sodium acetate (187 mg, 2.3 mmol) and cyanogen bromide (161 mg, 1.5 mmol) were added to a solution of compound **21** (292 mg, 0.8 mmol) in MeOH (15 mL). The mixture was stirred at room temperature for 18 h, and the solvent was removed under reduced pressure. The oily residue was purified by column chromatography on silica gel using dichloromethane/methanol (40:1) as eluent to obtain **27** as a yellow oil (18 mg, 6%). $^{1}$H NMR (500 MHz, DMSO-$d_6$) δ 1.33 (s, 9H, $C(CH_3)_3$), 3.08 (s, 3H, $NCH_3$), 4.39 (s, 2H, $NCH_2CO$), 4.49 (s, 2H, $CH_2C_6H_5$), 4.55 (s, 2H, $CH_2C_6H_5$), 7.15-7.33 (m, 10H, $C_6H_5$); $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ 27.79 ($C(CH_3)_3$), 36.26 ($NCH_3$), 49.20 ($NCH_2CO$, $CH_2C_6H_5$), 49.46 ($NCH_2CO$, $CH_2C_6H_5$), 54.98 ($NCH_2CO$, $CH_2C_6H_5$), 82.20 ($C(CH_3)_3$), 114.02 (NCN), 126.50 (C2', C3', C4', C2'', C3'', C4''), 127.39 (C2', C3', C4', C2'', C3'', C4''), 127.61 (C2', C3', C4', C2'', C3'', C4'') , 127.93 (C2', C3', C4', C2'', C3'', C4''), 128.61 (C2', C3', C4', C2'', C3'', C4''), 128.97 (C2', C3', C4', C2'', C3'', C4''), 136.69 (C1', C1''), 137.20 (C1', C1''), 153.62 (OCON), 166.69 ($CH_2CON$); LC-MS (ESI) (90% $H_2O$ to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 200-400 nm), $\tau_r$ = 11.35 min, 75% purity, m/z 409.46 ($[M + H]^+$), m/z 407.27 ($[M - H]^-$), $C_{23}H_{28}N_4O_3$ (408.49).

**2-[2-(*tert*-Butoxycarbonyl)hydrazinyl]acetic acid (28).** To a solution of compound **8** (200 mg, 1.1 mmol) in MeOH (10 mL) was added 10% Pd/C (30 mg). After 90 min of stirring under $H_2$ atmosphere and 3.3 bar, the solution was filtered and evaporated. The residue was suspended in dichloromethane (5 mL) and a precipitate was filtered off to afford **28** (160 mg, 78%). mp. 144-145 °C; lit. mp. 144 °C;[2] $^{1}$H NMR (500 MHz, DMSO-$d_6$) δ 1.37 (s, 9H, C(CH3)3), 3.40 (s, 2H, CH2), 8.12 (br s, 1H NH), one NH signal was not observed; $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ 28.27 ($C(CH_3)_3$), 52.47 ($CH_2$), 78.79 ($C(CH_3)_3$), 155.57 (OCON), 171.88 ($CO_2H$); LC-MS (ESI) (90% $H_2O$ to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 200-400 nm), $\tau_r$ = 1.37 min, 97% purity, m/z 208.18 ($[M + NH_4]^+$), m/z 189.14 ($[M - H]^-$), $C_7H_{14}N_2O_4$ (190.20).

**2-[2-(*tert*-Butoxycarbonyl)-2-methylhydrazinyl]acetic acid (29).** To a solution of compound **9** (300 mg, 1.5 mmol) in *i*-PrOH (10 mL) was added 10% Pd/C (30 mg). After 20 h of stirring under $H_2$ atmosphere, the solution was filtered and evaporated. The residue precipitated by addition of dichloromethane to give a yellow solid which was filtered off to afford **29** (173 mg, 57%). mp. 86-88 °C; $^{1}$H NMR (500 MHz, DMSO-$d_6$) δ 1.40 (s, 9H,

$C(CH_3)_3$), 2.93 (s, 3H, $NCH_3$), 3.47 (s, 2H, $CH_2$); $^{13}C$ NMR (125 MHz, DMSO-$d_6$) δ 28.13 ($C(CH_3)_3$), 50.95 ($CH_2$), 79.67 ($C(CH_3)_3$), 155.57 (OCON), 171.88 ($CO_2H$) a signal for the $NCH_3$ was not observed; LC-MS (ESI) (90% $H_2O$ to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 200-400 nm), $\tau_r$ = 2.28 min, 83% purity, m/z 205.17 ($[M + H]^+$), m/z 203.18 ($[M - H]^-$), $C_8H_{16}N_2O_4$ (204.22).

**Enzyme inhibition assays**

**Cathepsin K inhibition assay**.[3] Cathepsin K was assayed fluorimetrically on a Monaco Safas spektrofluorometer flx. The wavelenght for excitation was 360 nm and for emission 440 nm. The reactions were followed at 25 °C over 60 min. A human recombinant cathepsin K (Enzo Life Sciences, Lörrach, Germany) stock solution of 23 μg/mL in 50 mM sodium acetate pH 5.5, 50 mM NaCl, 0.5 mM EDTA, 5 mM DTT was diluted 1:100 with assay buffer (100 mM sodium citrate pH 5.0, 100 mM NaCl, 1 mM EDTA, 0.01% CHAPS) containing 5 mM DTT and incubated for 30 min at 37 ºC. Inhibitor stock solutions were prepared in DMSO. A 10 mM stock solution of the fluorogenic substrate Z-Leu-Arg-AMC was prepared with DMSO. The final concentration of DMSO was 2%, and the final concentration of the substrate was 40 μM (= 13.3 $K_m$). Assays were performed with a final concentration of 5 ng/mL of cathepsin K. Into a cuvette containing 960 μL assay buffer, inhibitor solution and DMSO in a total volume of 16 μL, and 4 μL of the substrate solution were added and thoroughly mixed. The reaction was initiated by adding 20 μL of the cathepsin K solution. Experiments were performed in duplicate with five different inhibitor concentrations.

**Cathepsin S inhibition assay.**[4] Human recombinant cathepsin S (Enzo Life Sciences, Lörrach, Germany) was assayed fluorometrically on a Monaco Safas spektrofluorometer flx. The wavelength for excitation was 360 nm and for emission 440 nm. The reactions were followed at 25 °C over 60 min. Assay buffer was 100 mM sodium phosphate buffer pH 6.0, 100 mM NaCl, 5 mM EDTA, and 0.01% Brij 35. An enzyme stock solution of 70 μg/mL in 100 mM MES buffer, pH 6.5, 1 mM EDTA, 50 mM L-cysteine, 10 mM dithiothreitol (DTT), 0.5% Triton X-100 and 30% glycerol was diluted 1:100 with a 50 mM sodium phosphate buffer pH 6.5, 50 mM NaCl, 2 mM EDTA, 0.01% Triton X-100 and 5 mM DTT and incubated for 60 min at 37 °C. A 10 mM stock solution of the fluorogenic substrate Z-Phe-Arg-AMC was prepared in DMSO. The assay was performed with a final substrate

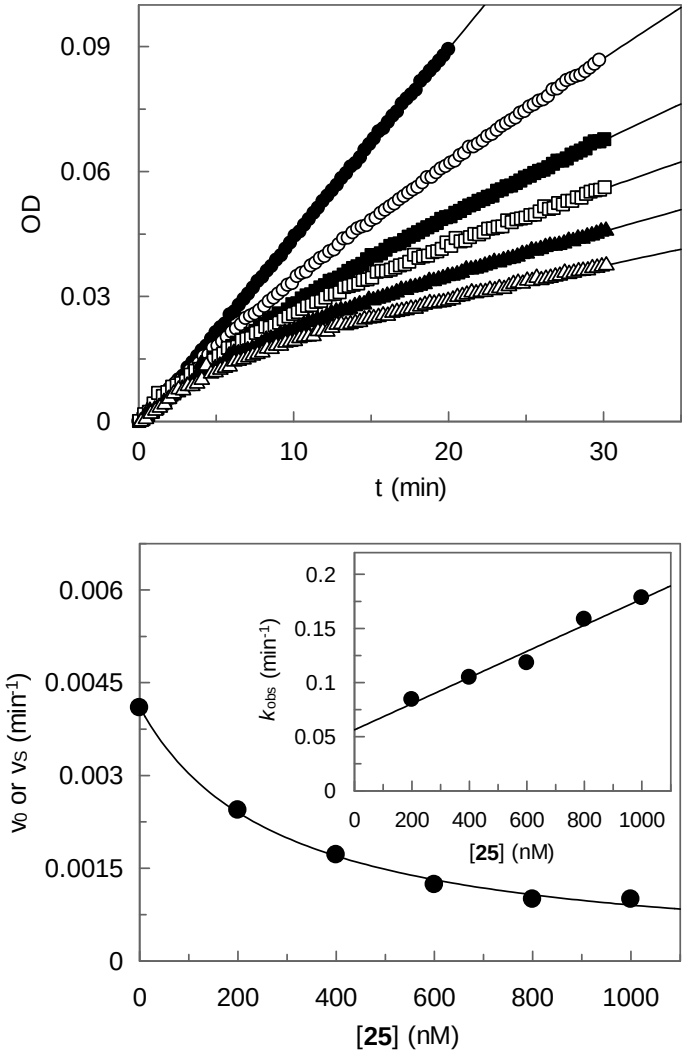
concentration of 40 μM (= 0.74 $K_m$), a final concentration of 42 ng/mL of cathepsin S, and a final DMSO concentration of 2%. Into a cuvette containing 920 μL assay buffer, inhibitor solution and DMSO in a total volume of 16 μL, and 4 μL of the substrate solution were added and thoroughly mixed. The reaction was initiated by adding 60 μL of the cathepsin S solution. Experiments were performed in duplicate with five different inhibitor concentrations.

**Cathepsin B inhibition assay.**[3] Human isolated cathepsin B (Calbiochem, Darmstadt, Germany) was assayed spectrophotometrically (Cary 50 Bio, Varian) at 405 nm and at 37 ºC. The reactions were followed over 30 min. Assay buffer was 100 mM sodium phosphate buffer pH 6.0, 100 mM NaCl, 5 mM EDTA, 0.01% Brij 35. An enzyme stock solution of 1.81 mg/mL in 20 mM sodium acetate buffer pH 5.0, 1 mM EDTA was diluted 1:500 with assay buffer containing 5 mM DTT and incubated for 30 min at 37 ºC. Inhibitor stock solutions were prepared in DMSO. A 100 mM stock solution of the chromogenic substrate Z-Arg-Arg-pNA was prepared with DMSO. The final concentration of DMSO was 2%, and the final concentration of the substrate was 500 μM (0.45 $K_m$). Assays were performed with a final concentration of 72 ng/mL of cathepsin B. Into a cuvette containing 960 μL assay buffer, inhibitor solution and DMSO in a total volume of 15 μL, and 5 μL of the substrate solution were added and thoroughly mixed. The reaction was initiated by adding 20 μL of the cathepsin B solution. Experiments were performed in duplicate with five different inhibitor concentrations.

**Cathepsin L inhibition assay.**[3] Human isolated cathepsin L (Enzo Life Sciences, Lörrach, Germany) was assayed spectrophotometrically (Cary 50 Bio, Varian) at 405 nm and at 37 ºC. The reactions were followed over 30 min. Assay buffer was 100 mM sodium phosphate buffer pH 6.0, 100 mM NaCl, 5 mM EDTA, and 0.01% Brij 35. An enzyme stock solution of 135 μg/mL in 20 mM malonate buffer pH 5.5, 400 mM NaCl, and 1 mM EDTA was diluted 1:100 with assay buffer containing 5 mM DTT and incubated for 30 min at 37 ºC. Inhibitor stock solutions were prepared in DMSO. A 10 mM stock solution of the chromogenic substrate Z-Phe-Arg-pNA was prepared with DMSO. The final concentration of DMSO was 2%, and the final concentration of the substrate was 100 μM (= 5.88 $K_m$). Assays were performed with a final concentration of 54 ng/mL of cathepsin L. Into a cuvette containing 940 μL assay buffer, inhibitor solution and DMSO in a total volume of 10 μL, and 10 μL of the substrate solution were added and thoroughly mixed. The reaction was initiated by adding

40 µL of the cathepsin L solution. Experiments were performed in duplicate with five different inhibitor concentrations.

**Inhibition of human cathepsin B by 25**




Top: Monitoring of the cathepsin B-catalyzed hydrolysis of Z-Arg-Arg-pNA in the presence of increasing concentrations of compound **25** (closed circles, 0 pM; open circles 200 nM; closed squares 400 nM; open squares 600 nM; closed triangles 800 nM; open triangles 1000 nM. The absorption at 405 nm was measured. Bottom: Plot of the rates obtained in duplicate measurements *versus* concentrations of **25**. Nonlinear regression gave an $IC_{50}$ value of 282 (± 14) nM. Inset: Plot of the first-order rate constants $k_{obs}$ *versus* concentrations of **25**. Linear regression gave a value $k_{on}/(1+[S]/K_m)$ of 121 (± 12) × $10^{-6}$ $nM^{-1}min^{-1}$.

## References

(1) Ottersbach, P. A.; Schnakenburg, G.; Gütschow, M. Induction of chirality: experimental evidence of atropisomerism in azapeptides. *Chem. Commun.* **2012***, 48,* 5772-5774.

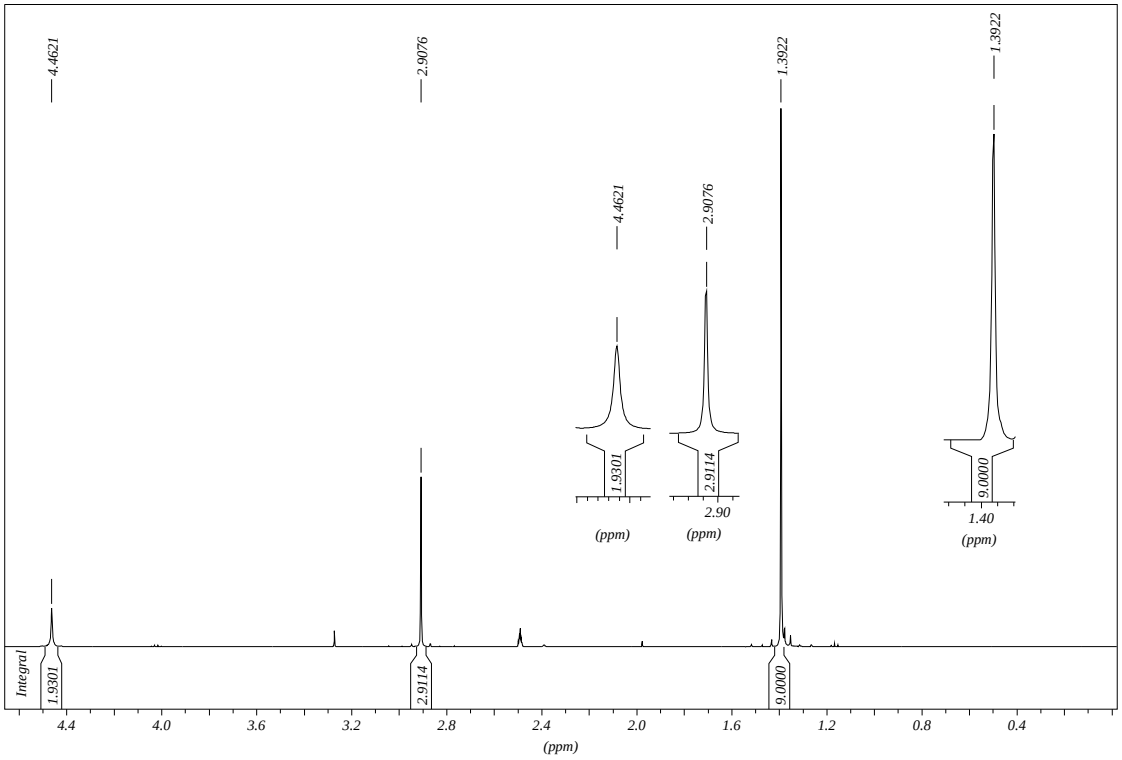
(2) Salaün, A.; Mocquet, C.; Perochon, R.; Lecorgne, A.; Le Grel, B.; Potel, M.; Le Grel, P. Aza-$\beta^3$-cyclotetrapeptides. *J. Org. Chem.* **2008**, *73*, 8579-8582.

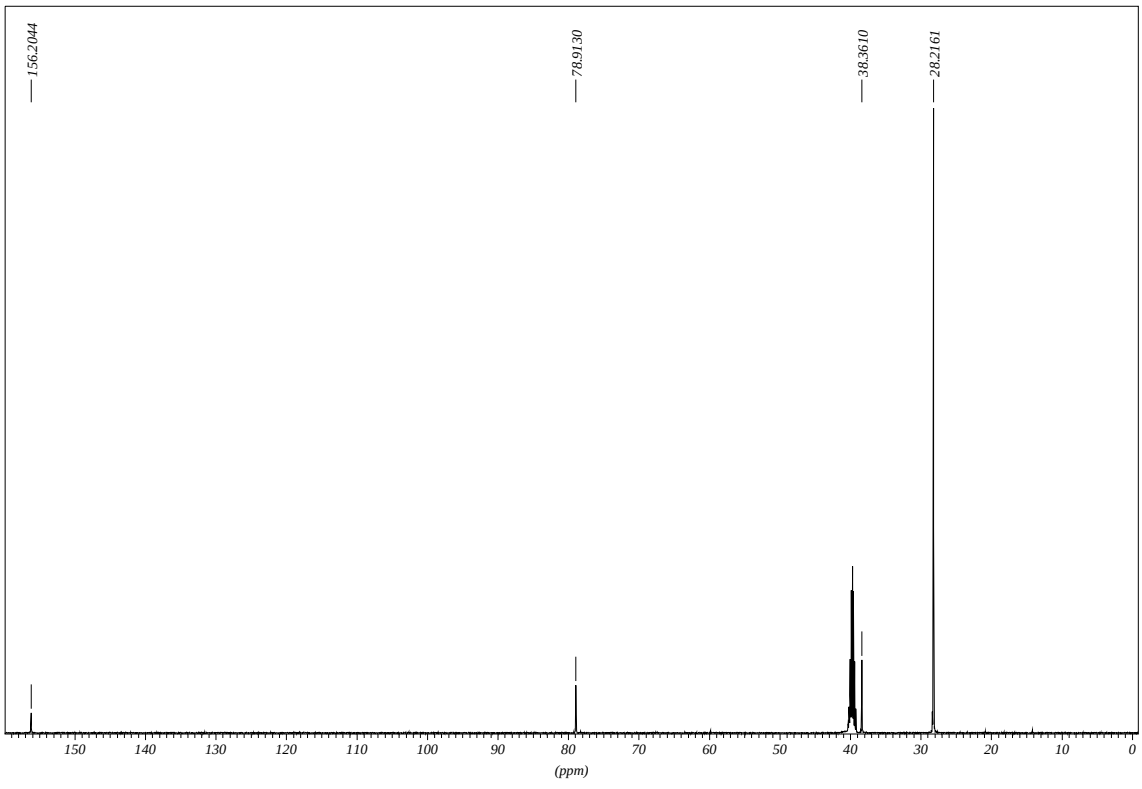
(3) Frizler, M.; Lohr, F.; Furtmann, N.; Kläs, J.; Gütschow, M. Structural optimization of azadipeptide nitriles strongly increases association rates and allows the development of selective cathepsin inhibitors. *J. Med. Chem.* **2011**, *54*, 396-400.

(4) Mertens, M. D.; Schmitz, J., Horn, M.; Furtmann, N.; Bajorath, J.; Mareš, M.; Gütschow, M. A coumarin-labeled vinyl sulfone as tripeptidomimetic activity-based probe for cysteine cathepsins. *ChemBioChem* **2014**, *15*, 955-959.

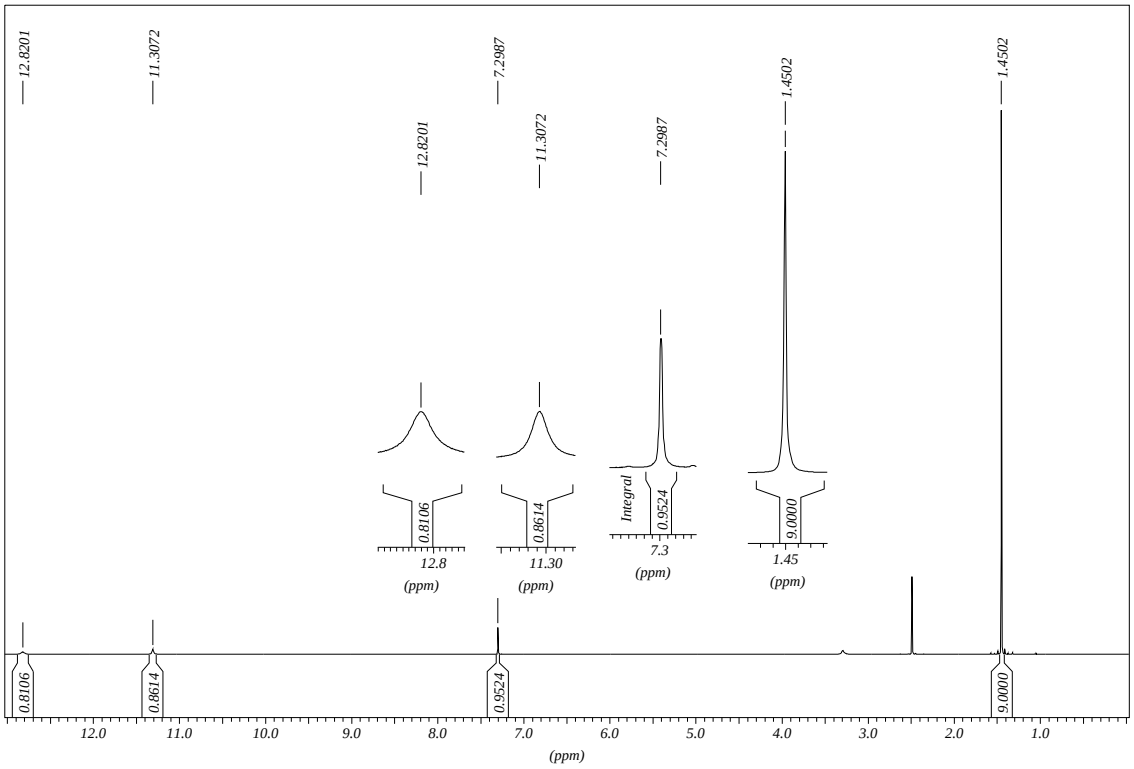
## $^{1}$H and $^{13}$C NMR spectra

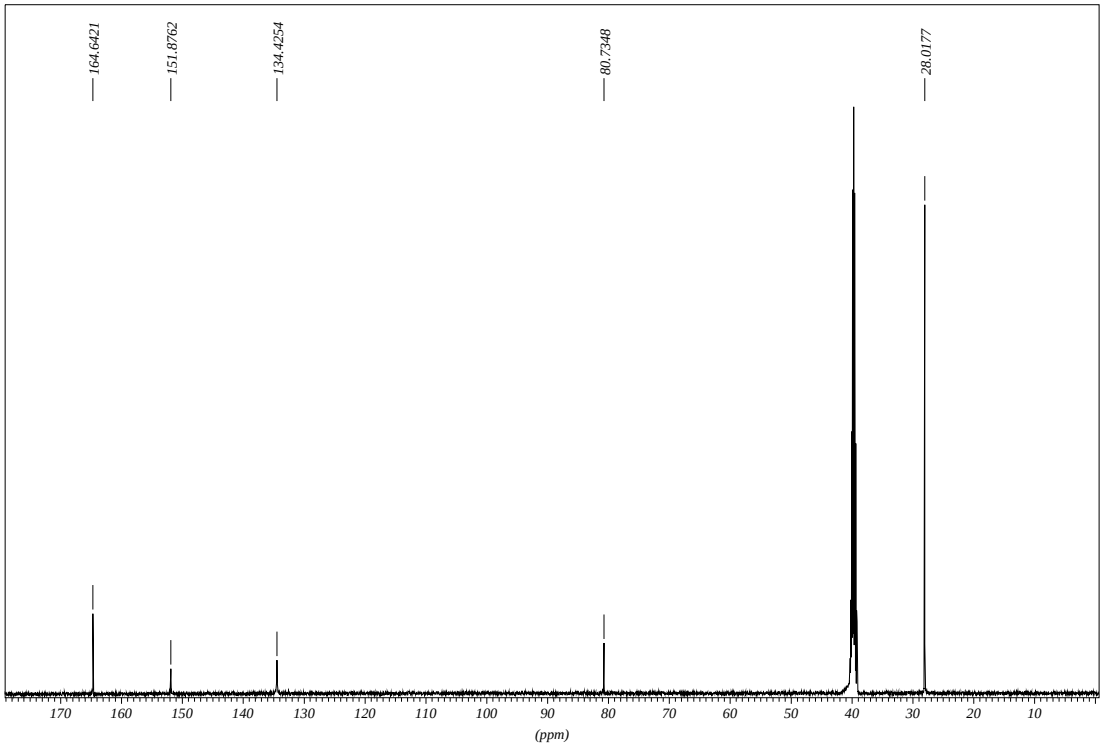
### *tert*-Butyl 1-methylhydrazinecarboxylate (7)



## 2-[2-(tert-Butoxycarbonyl)hydrazono]acetic acid (8)



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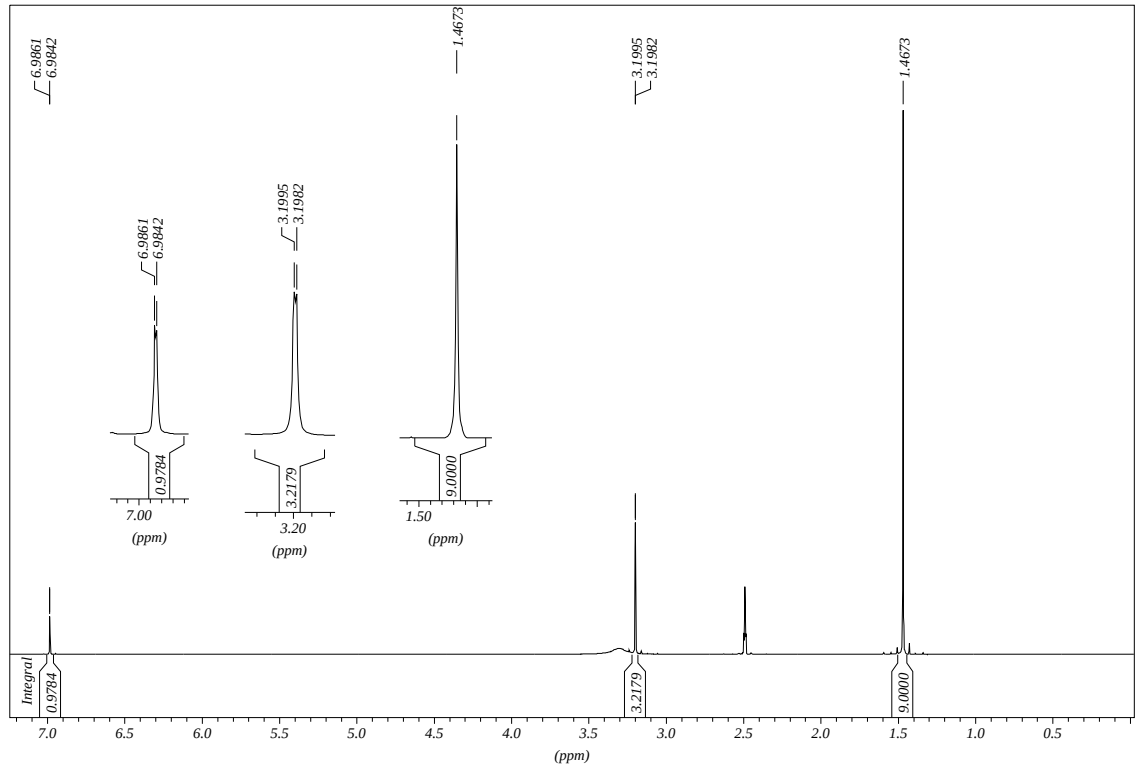


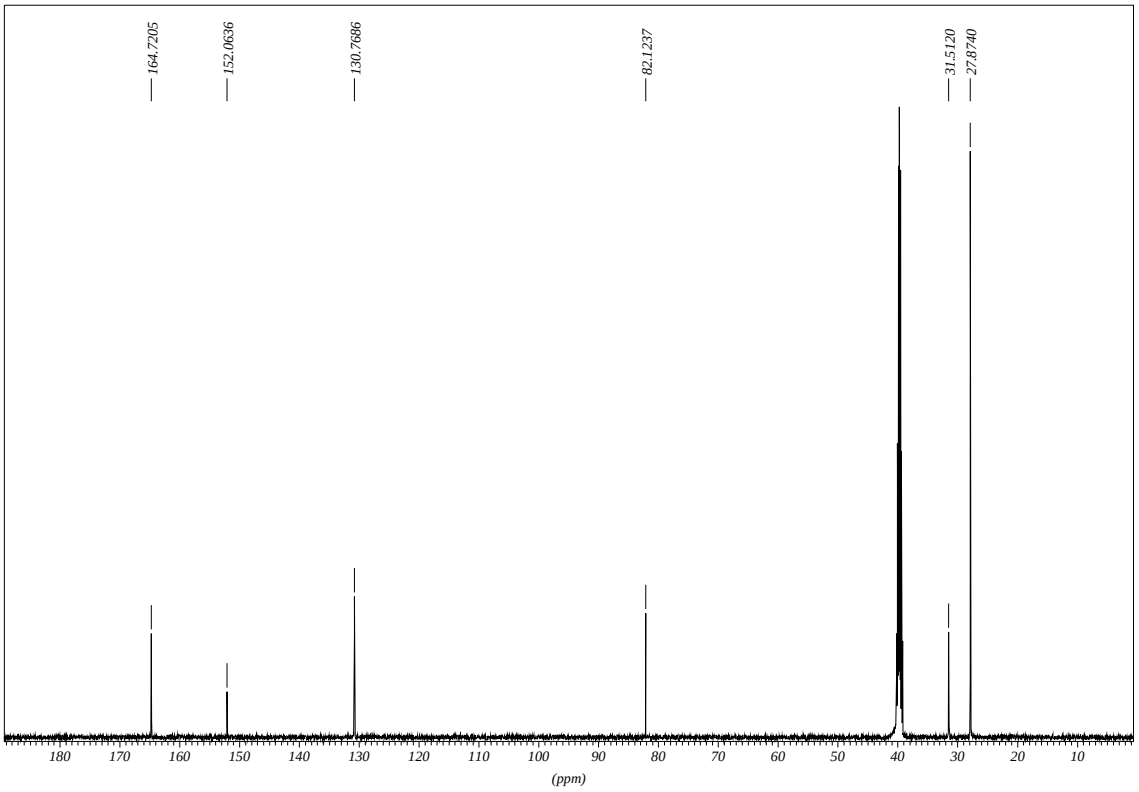

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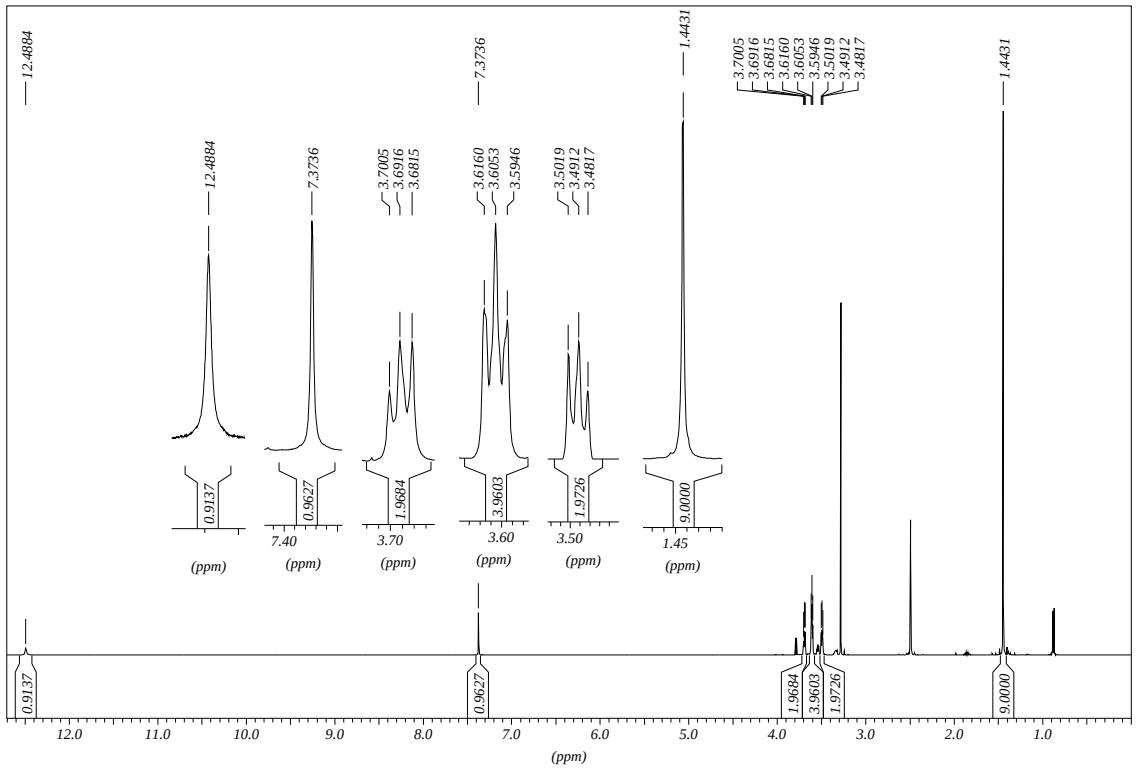


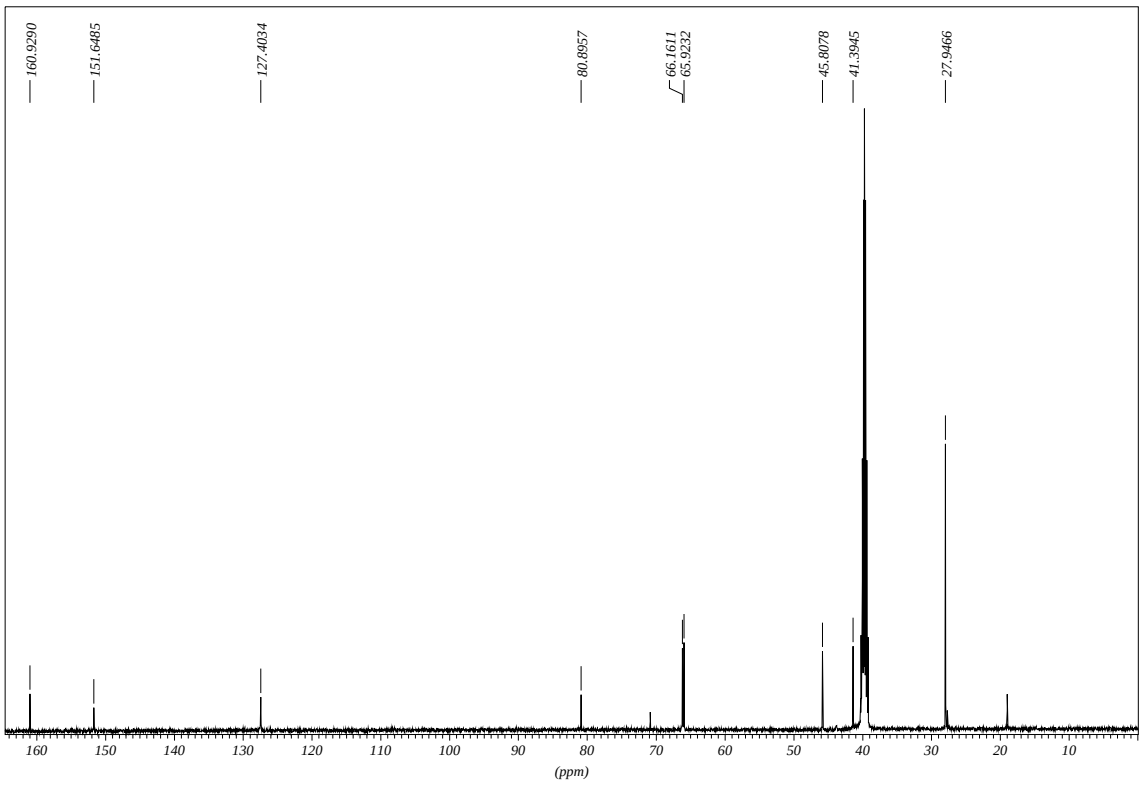

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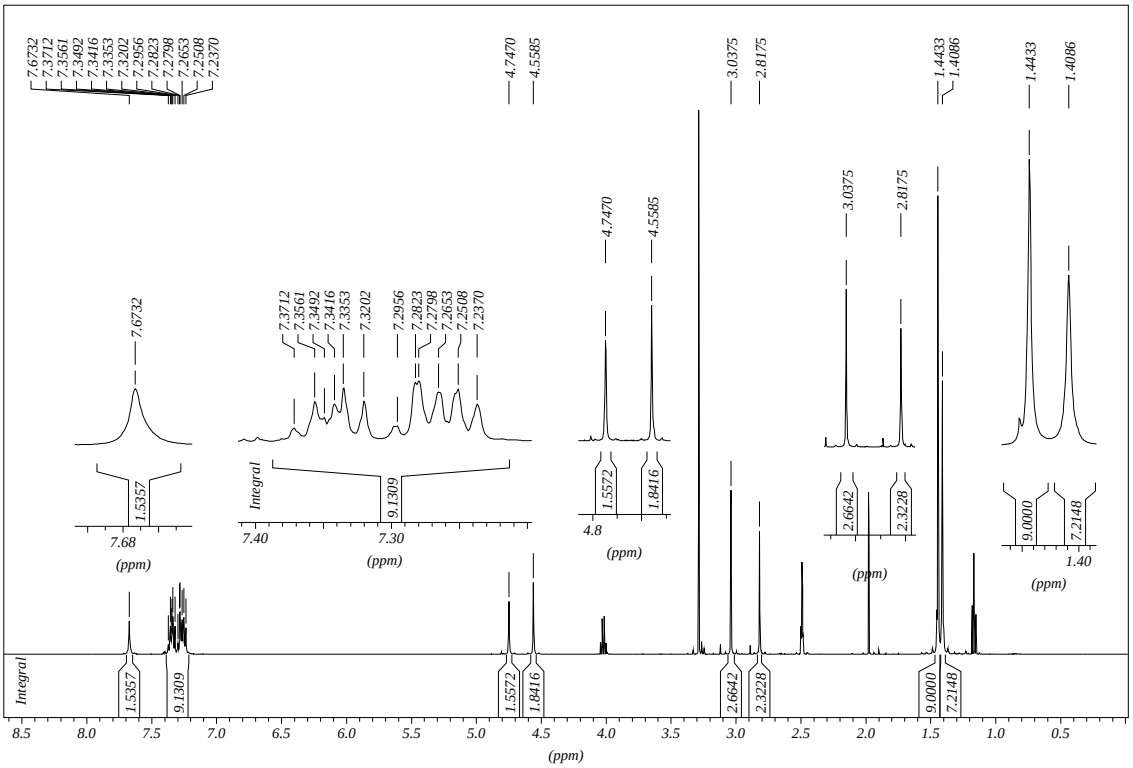


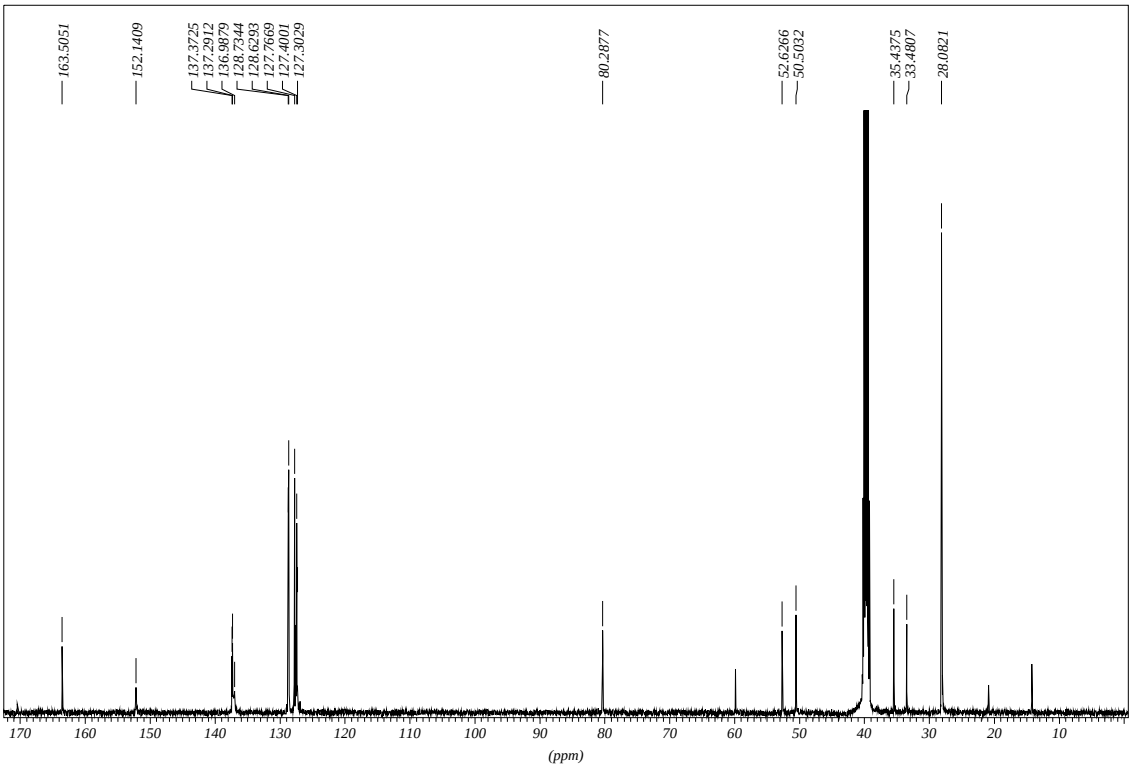

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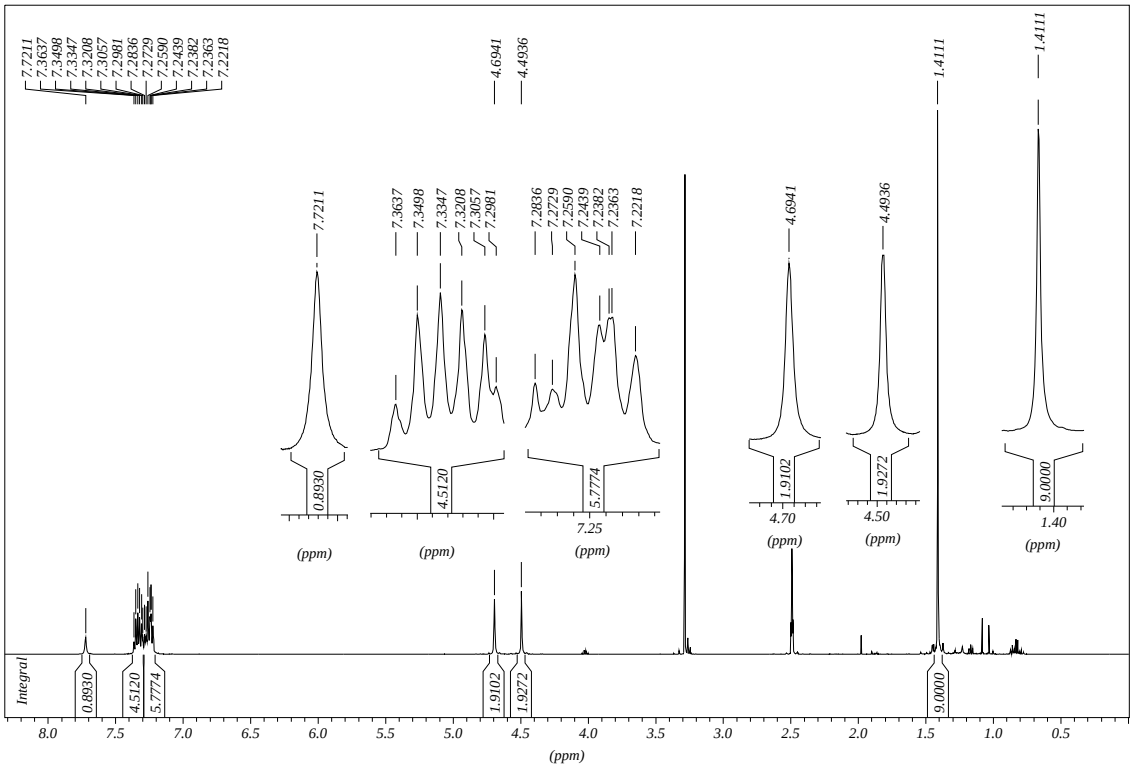


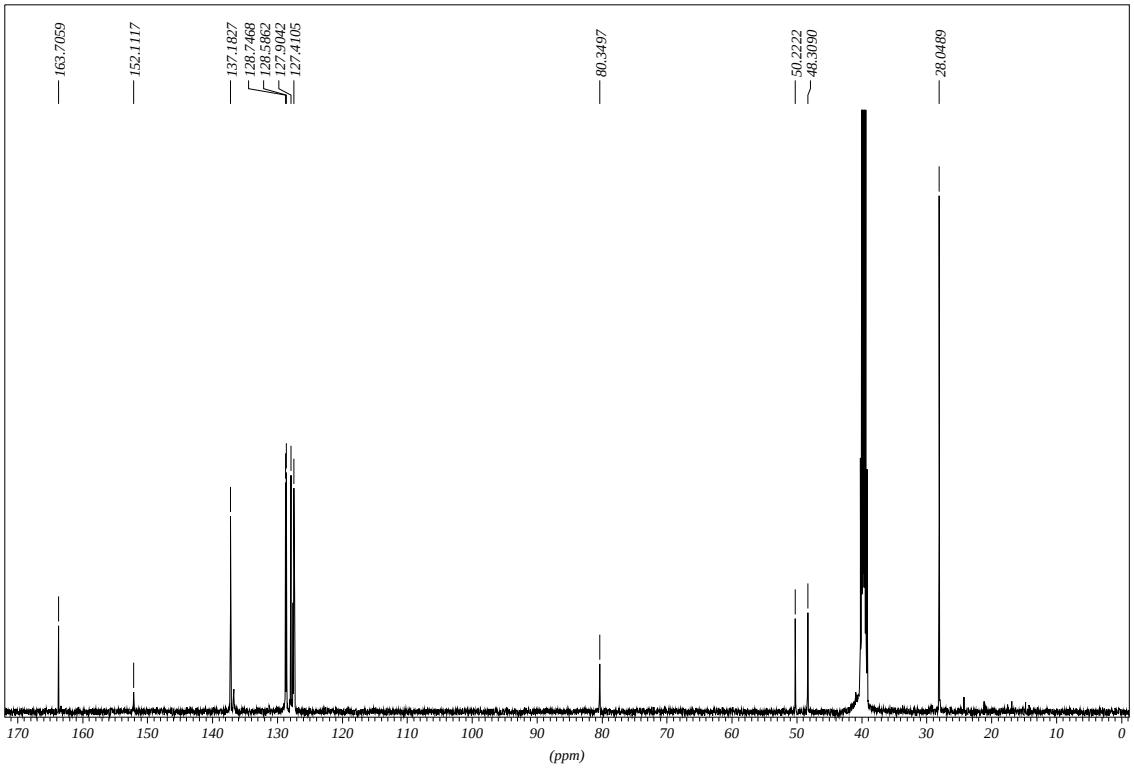

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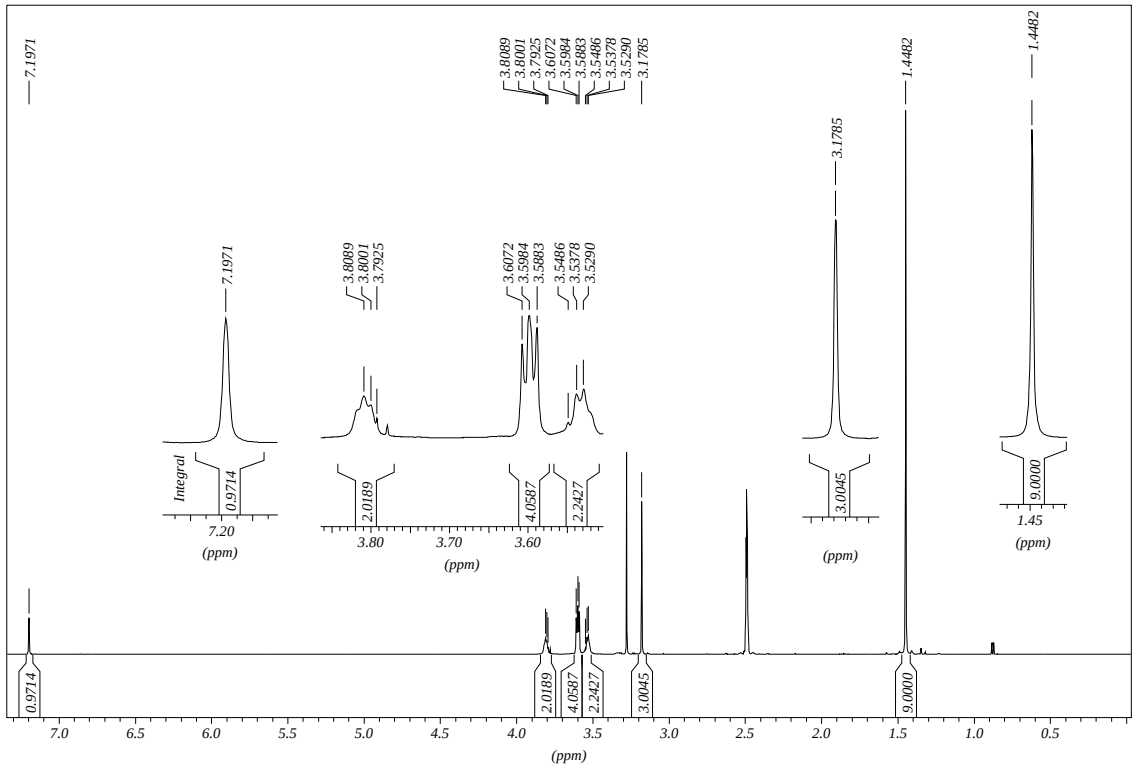


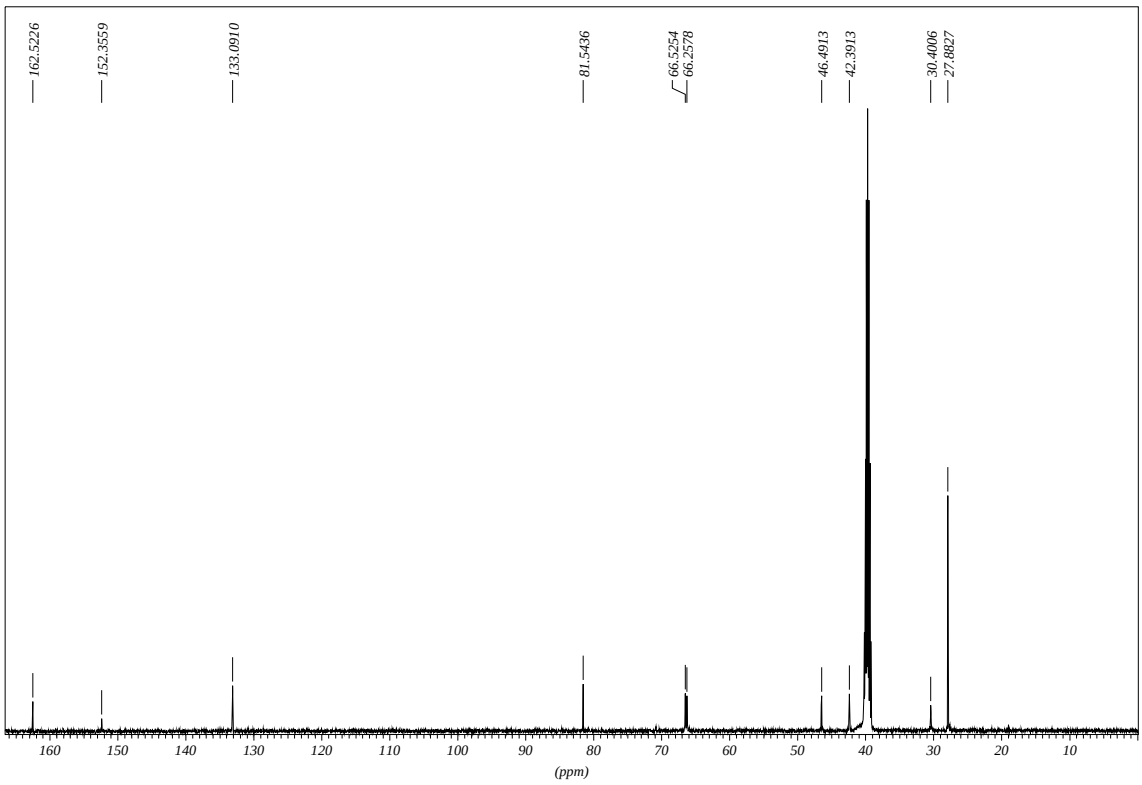

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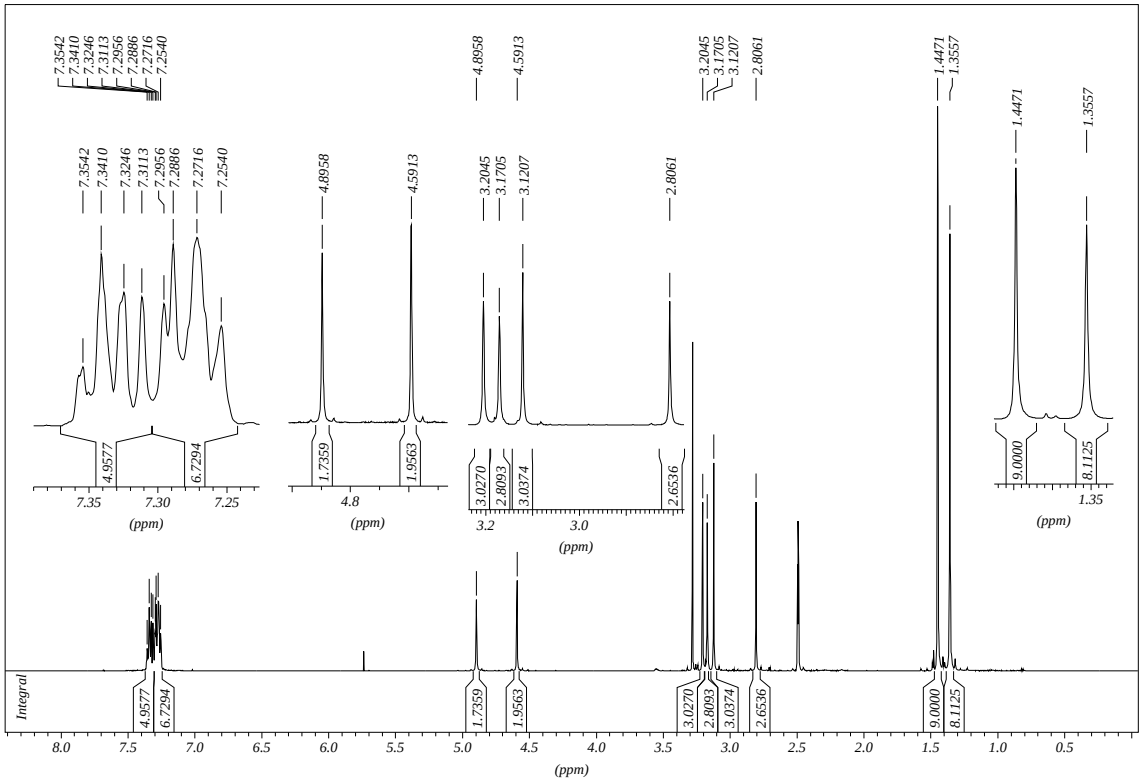


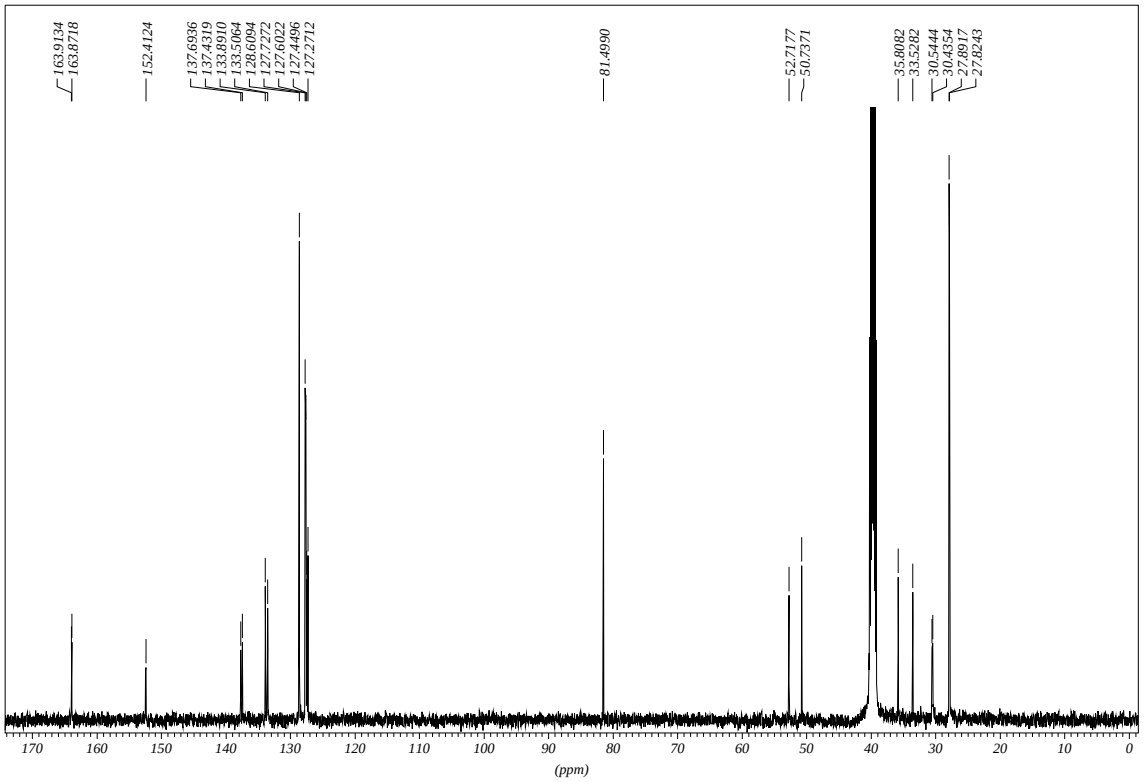

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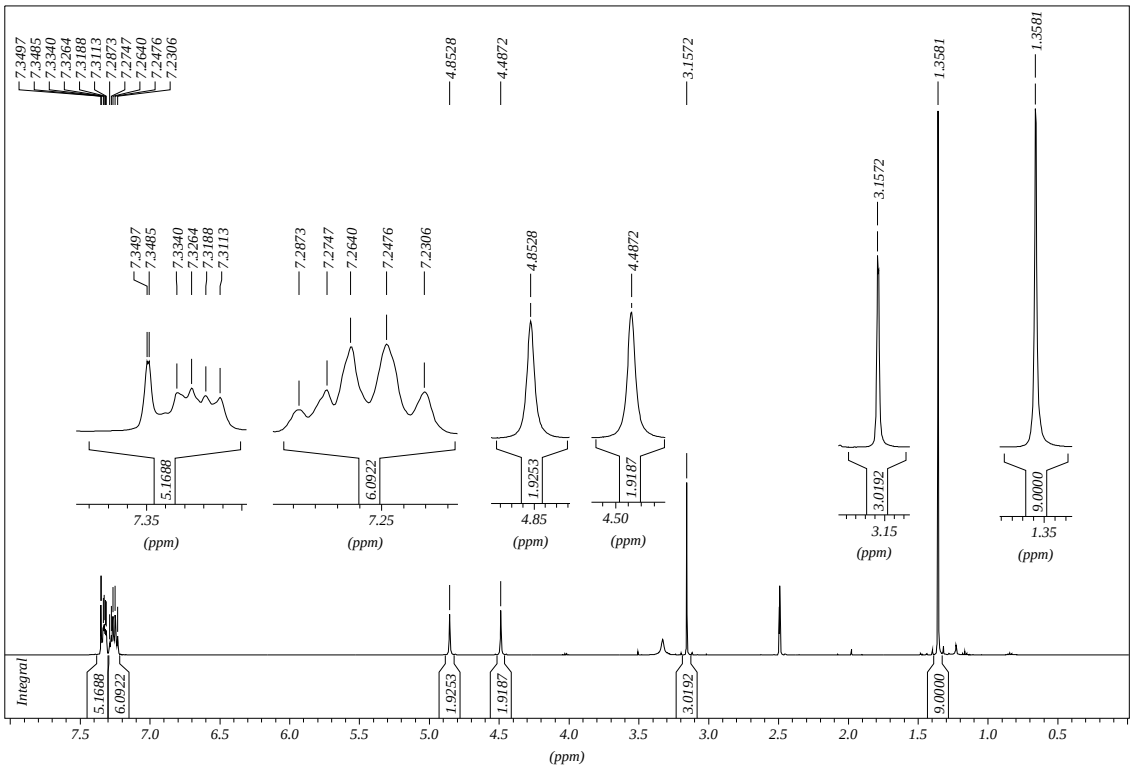


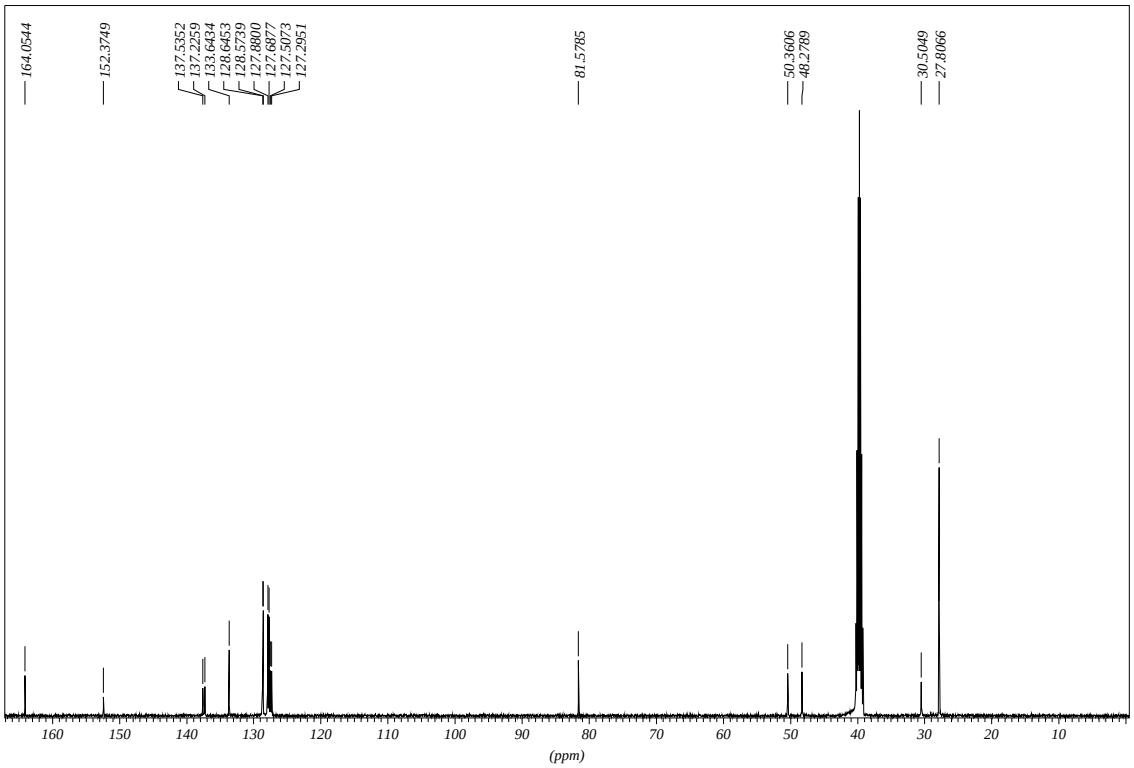

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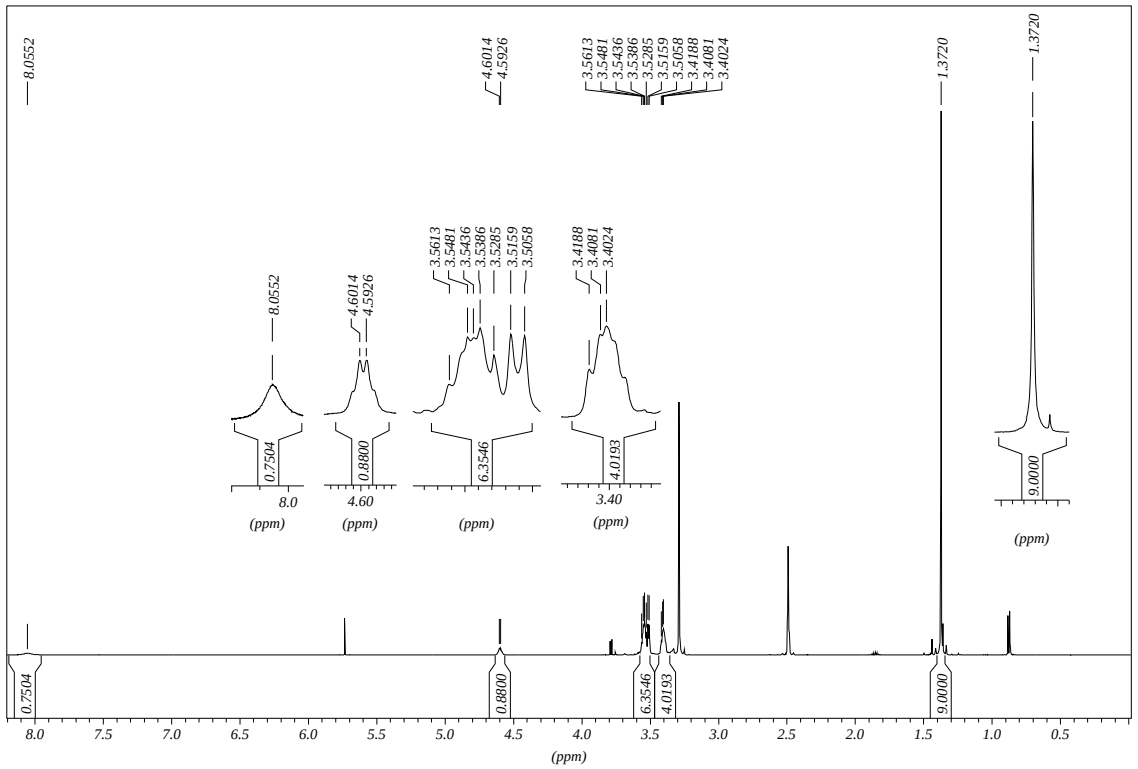


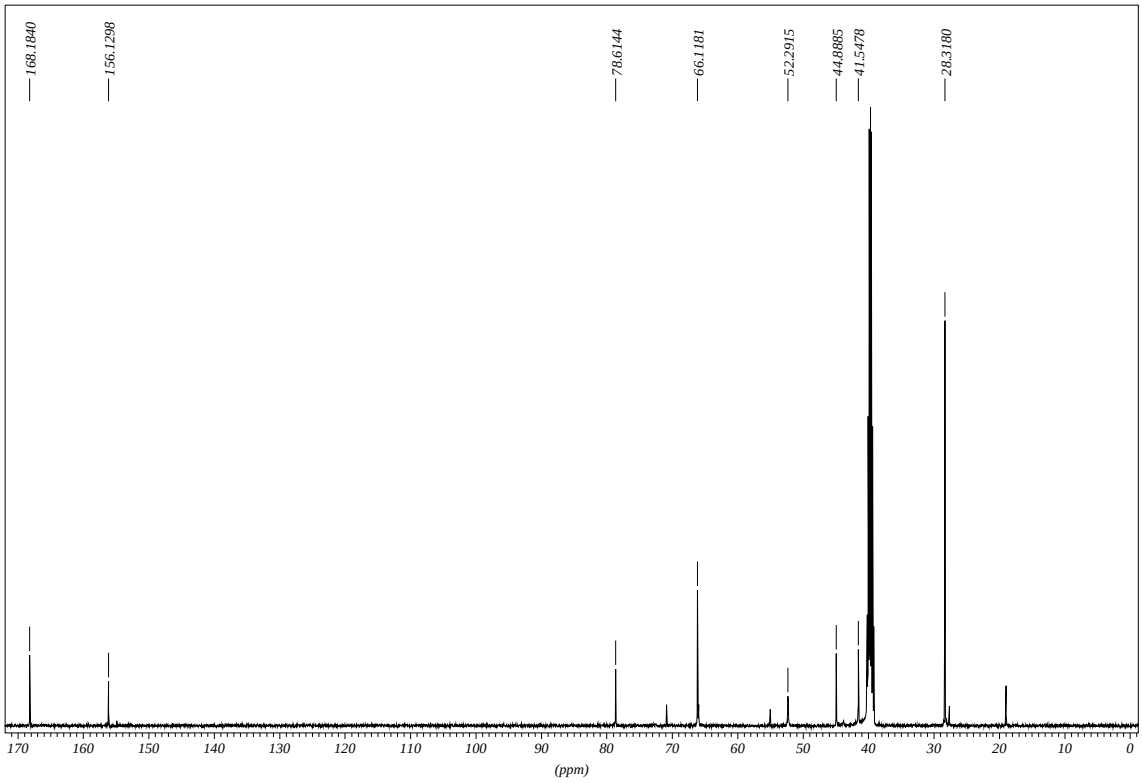

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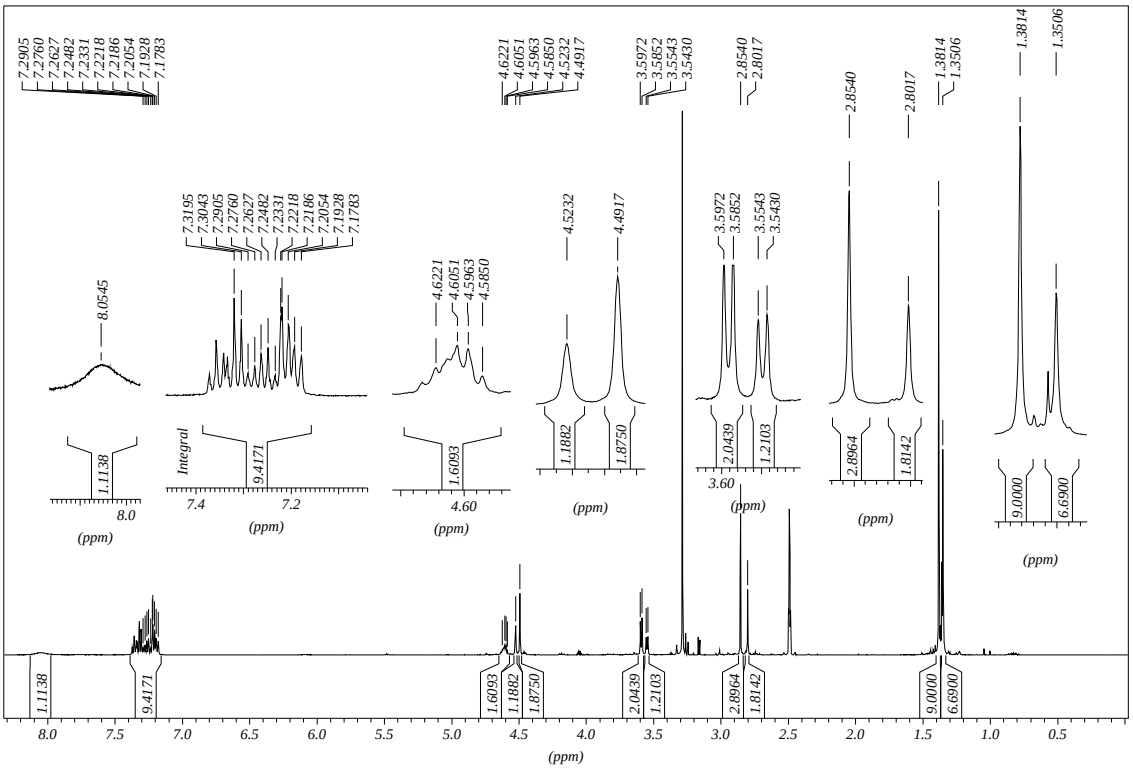

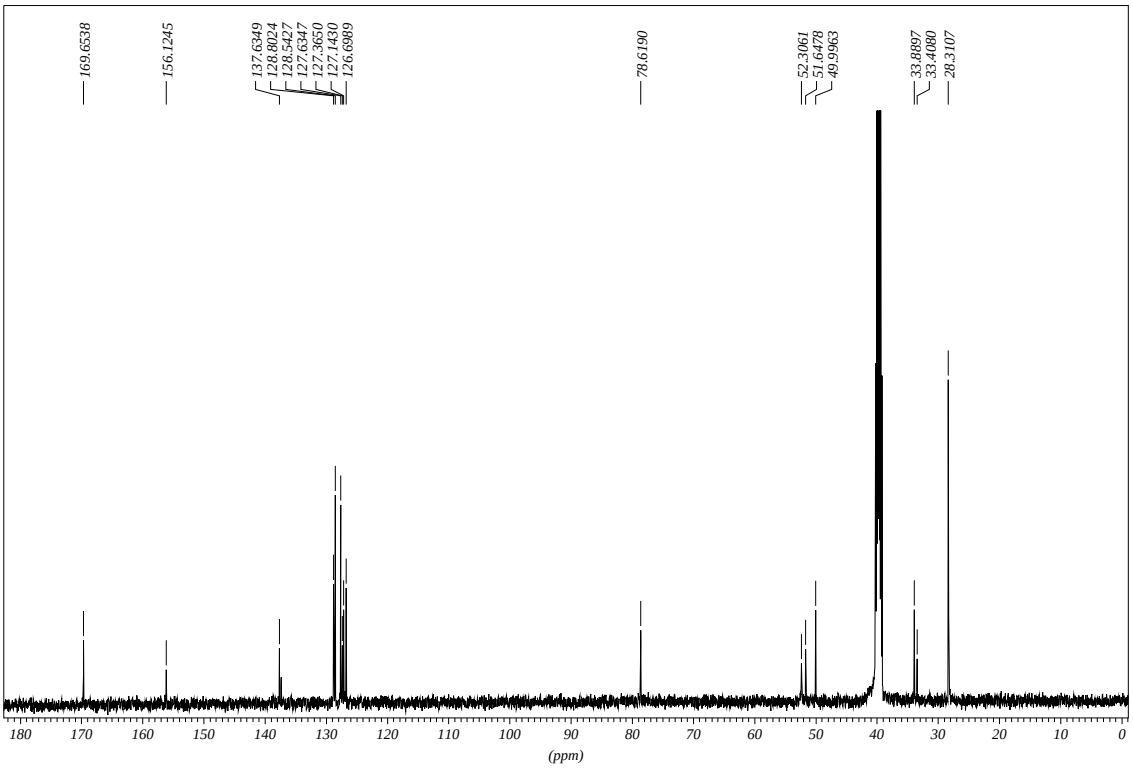

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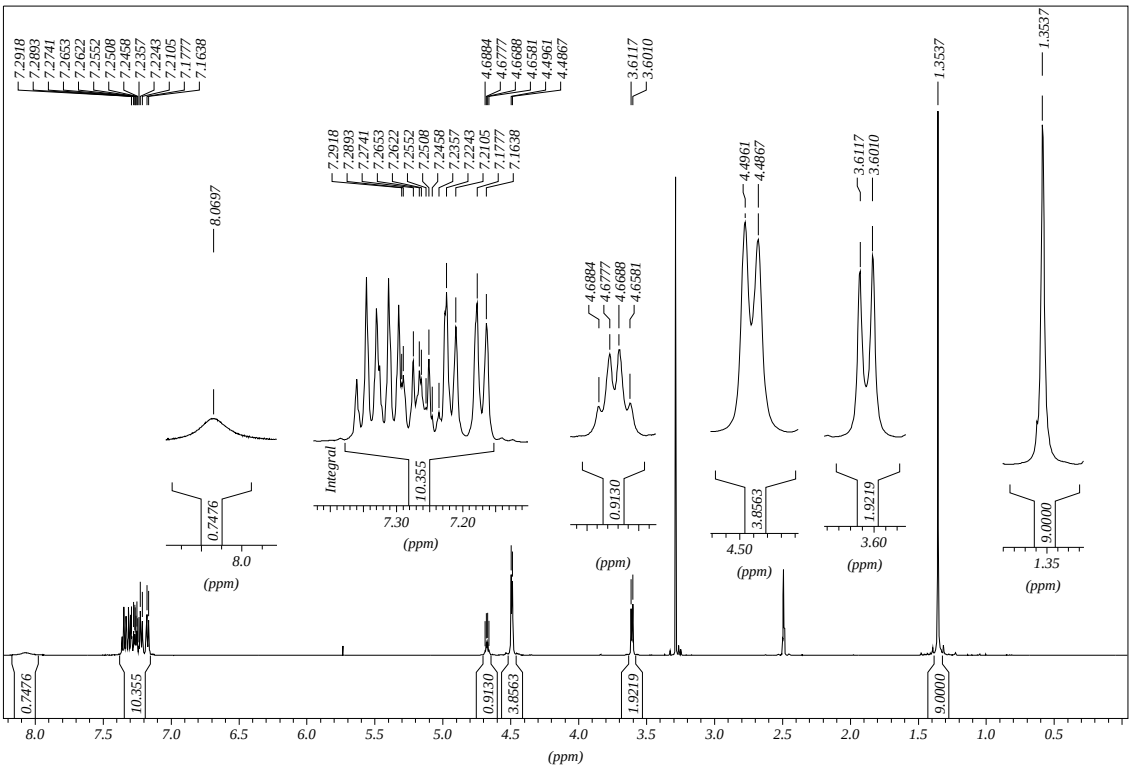
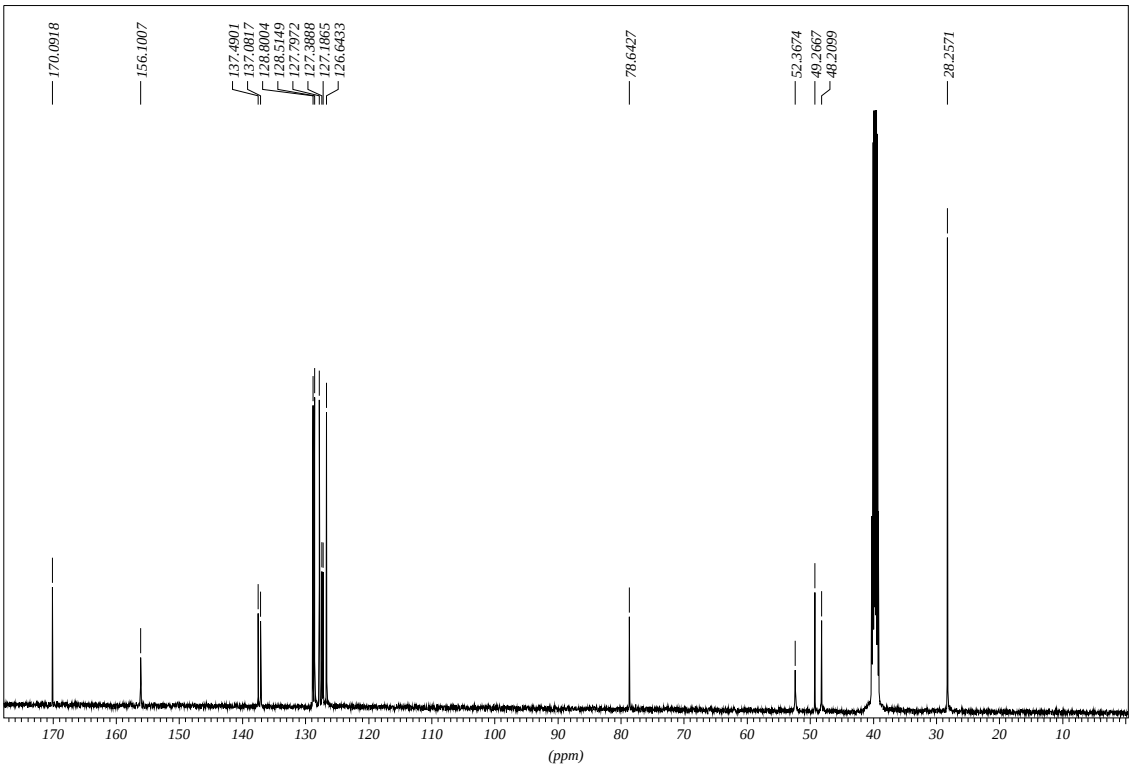
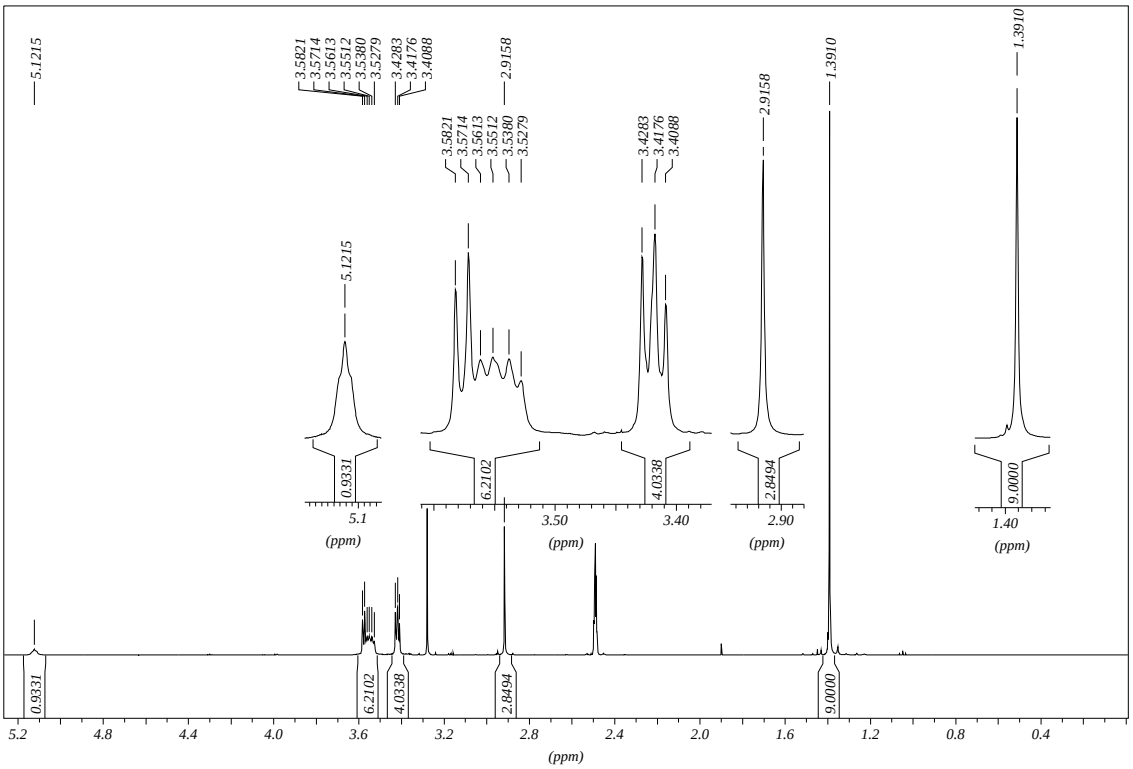
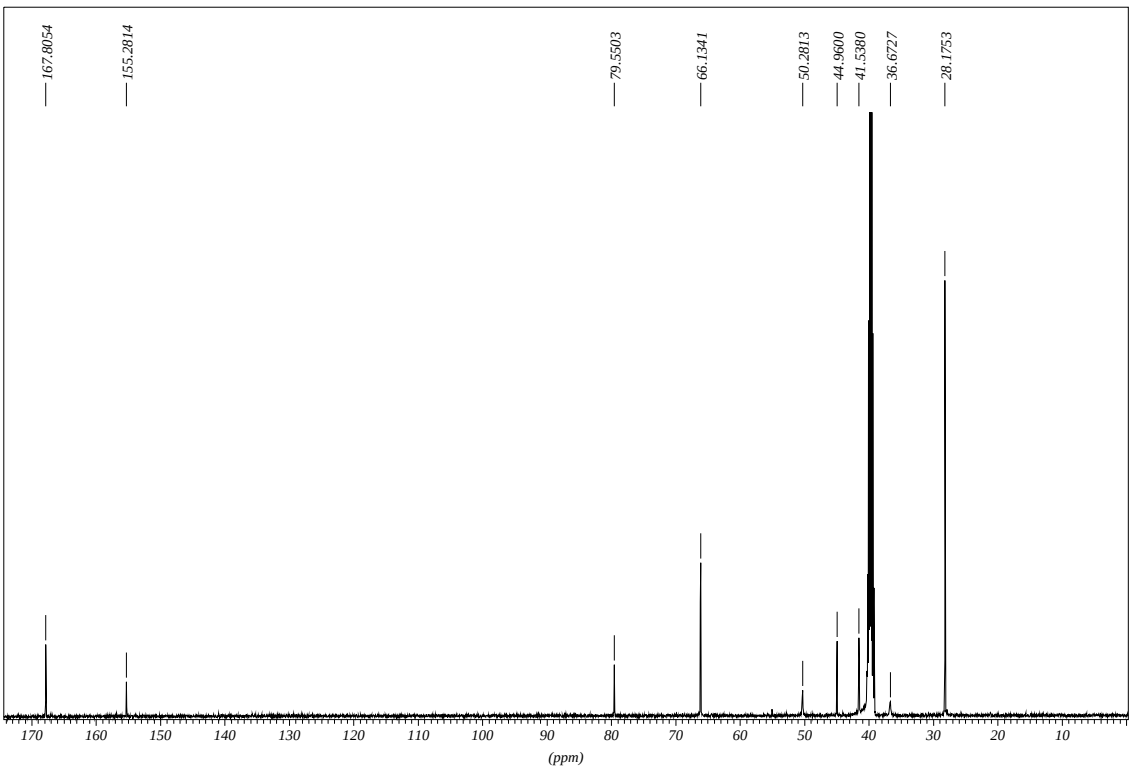


## *tert*-Butyl 1-methyl-2-(2-morpholino-2-oxoethyl)hydrazinecarboxylate (19)



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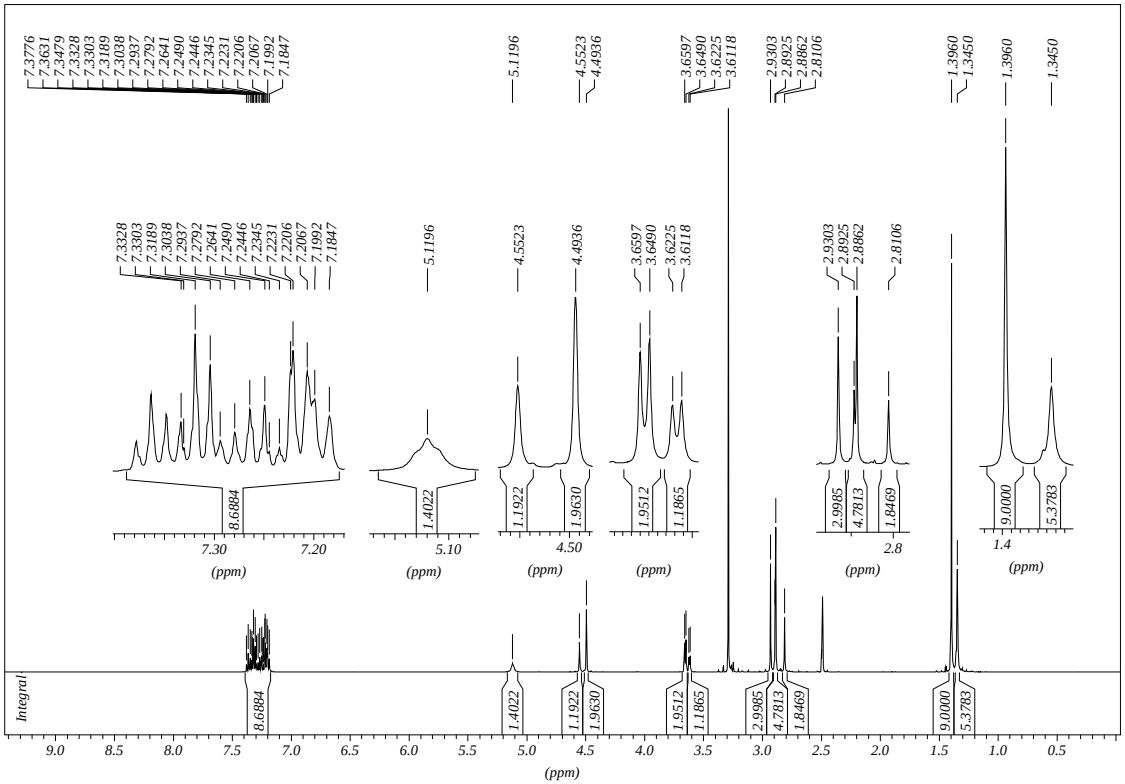


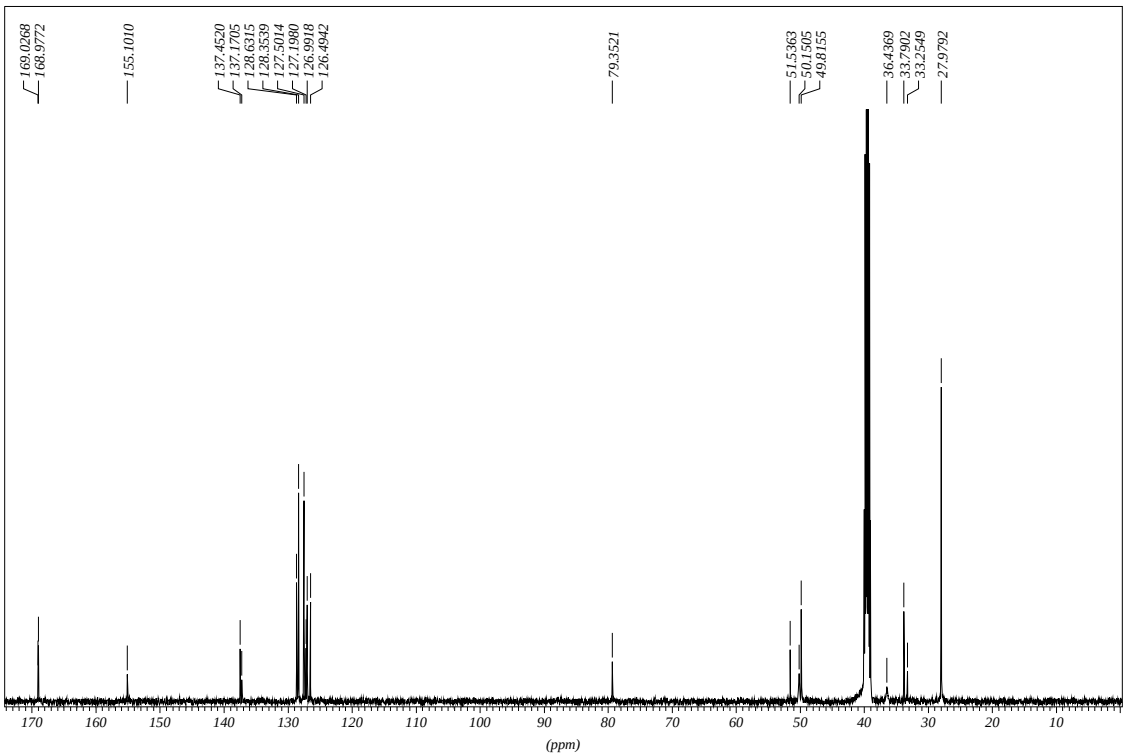

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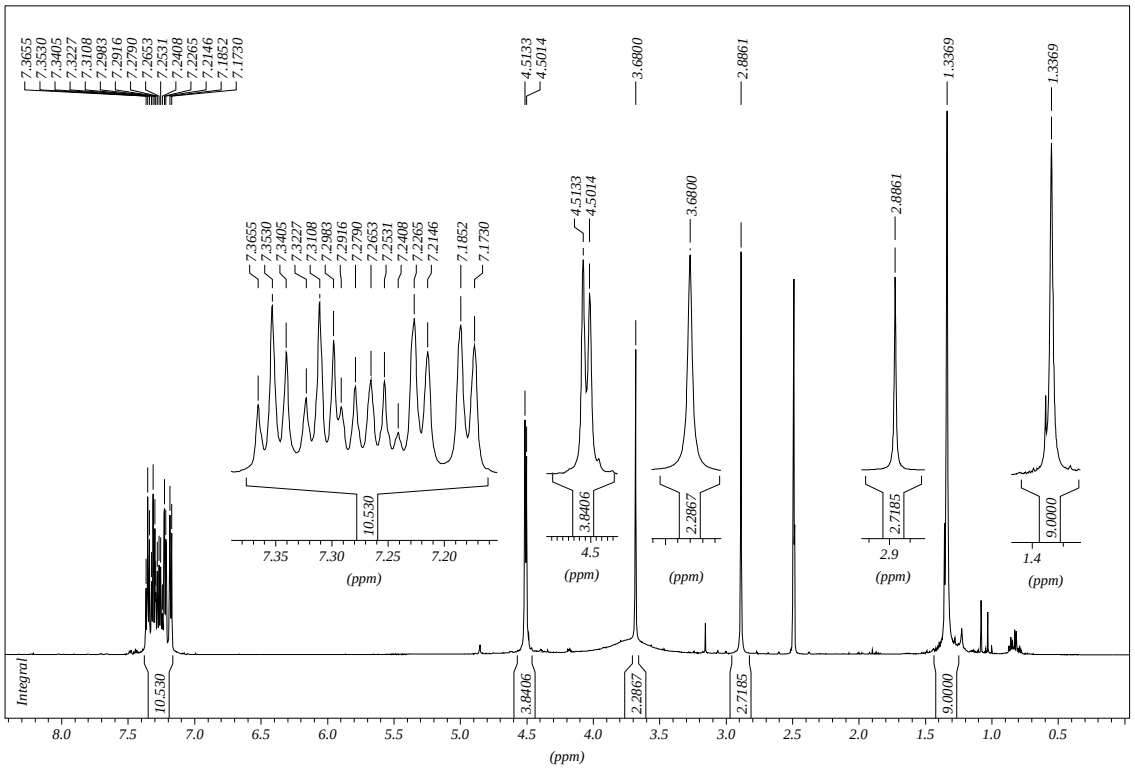
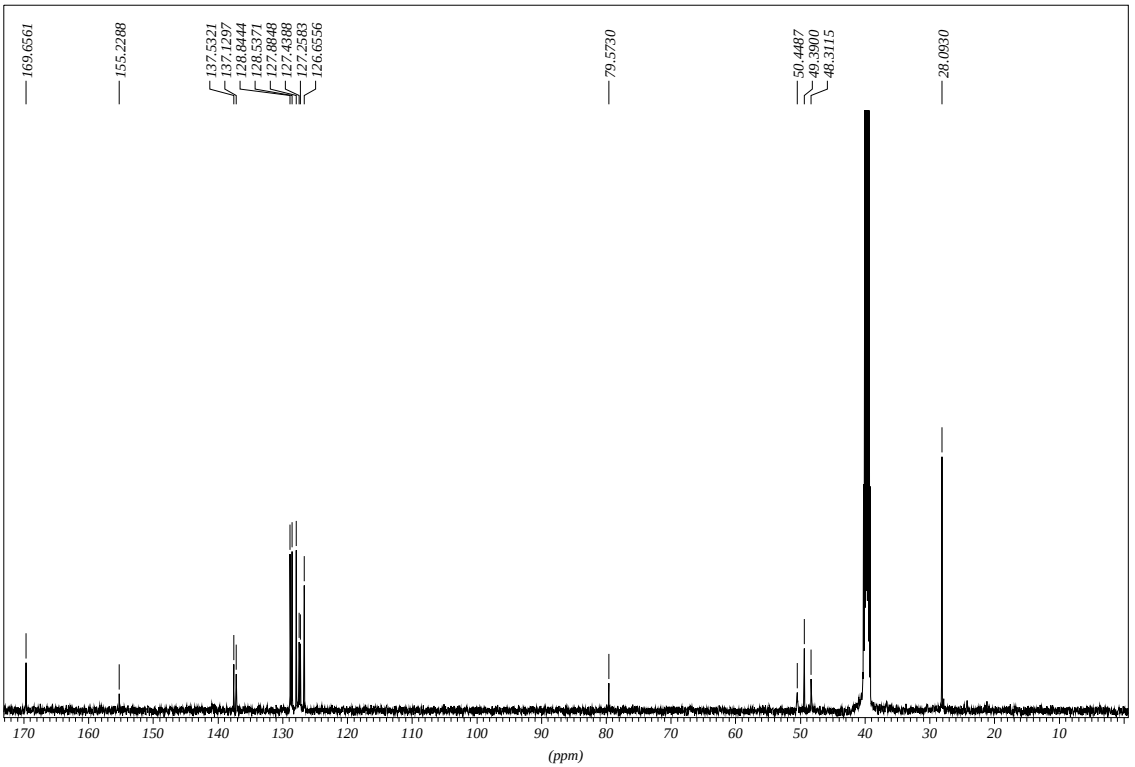
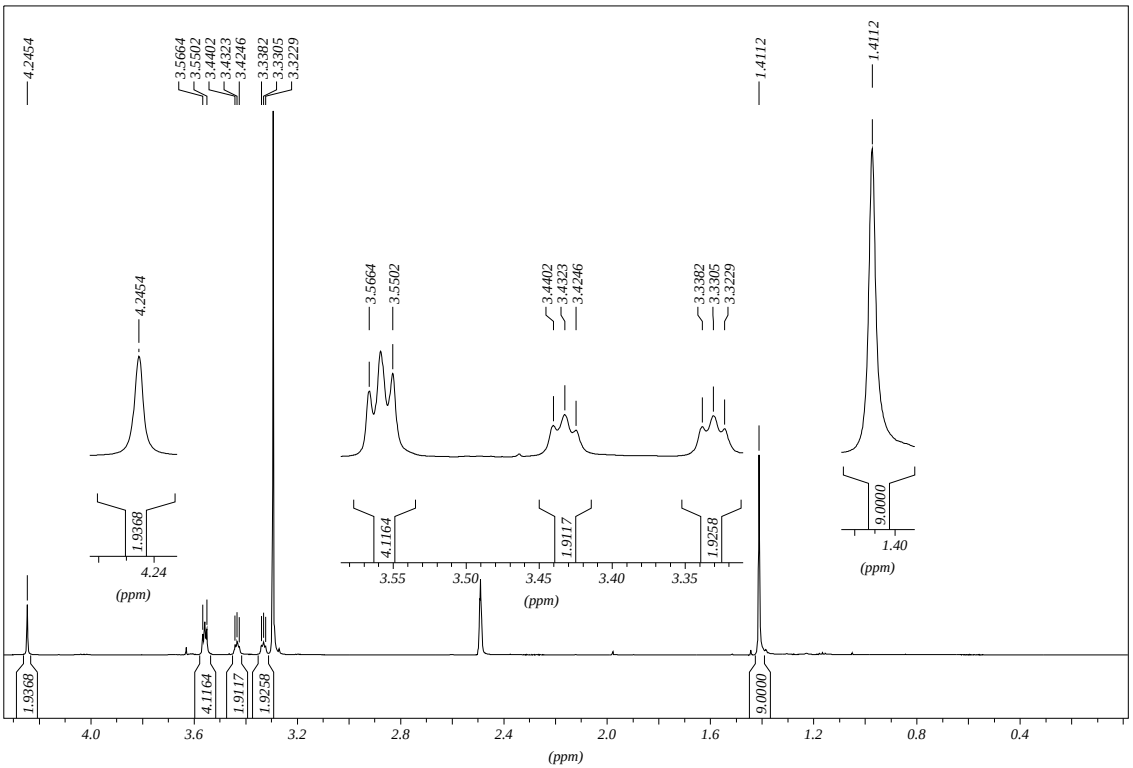
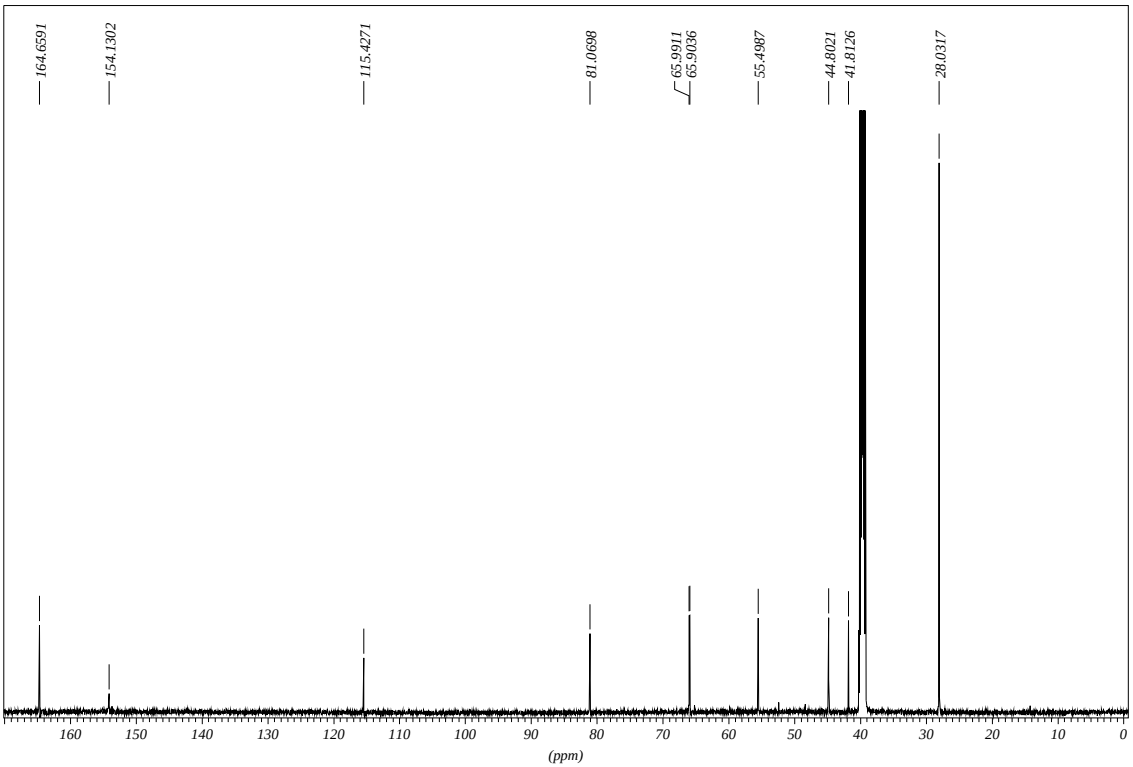


## *tert*-Butyl 2-cyano-2-(2-morpholino-2-oxoethyl)hydrazinecarboxylate (22)



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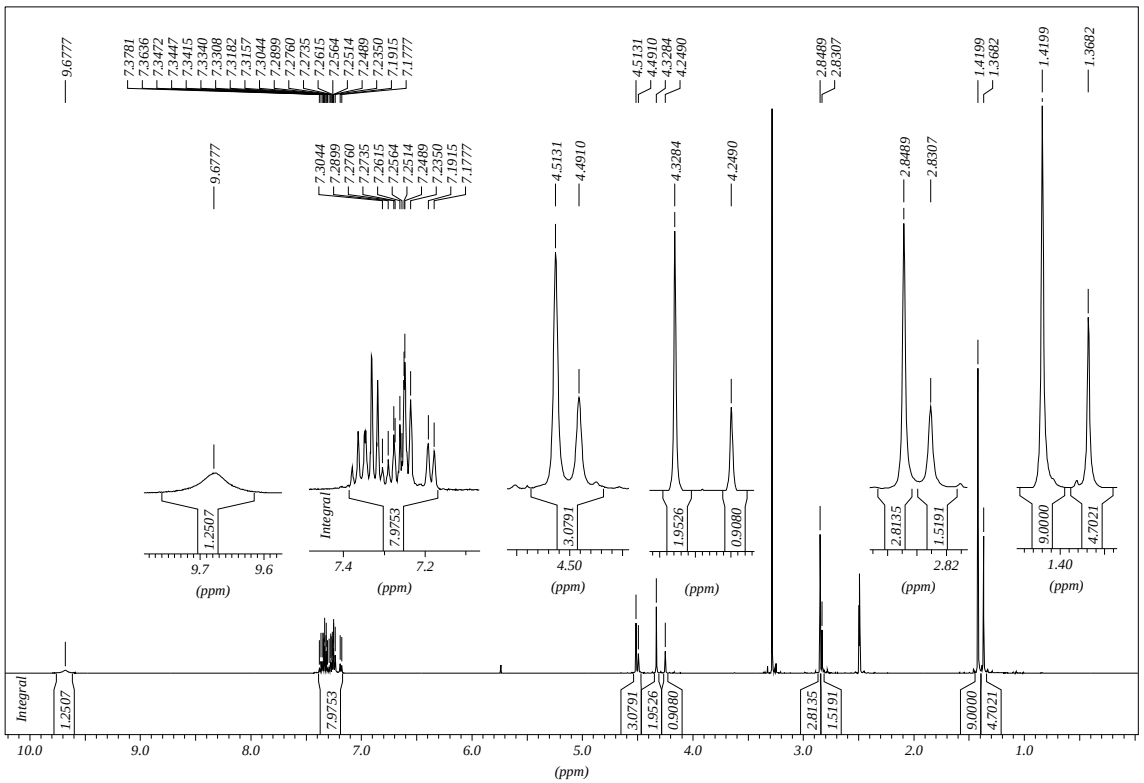
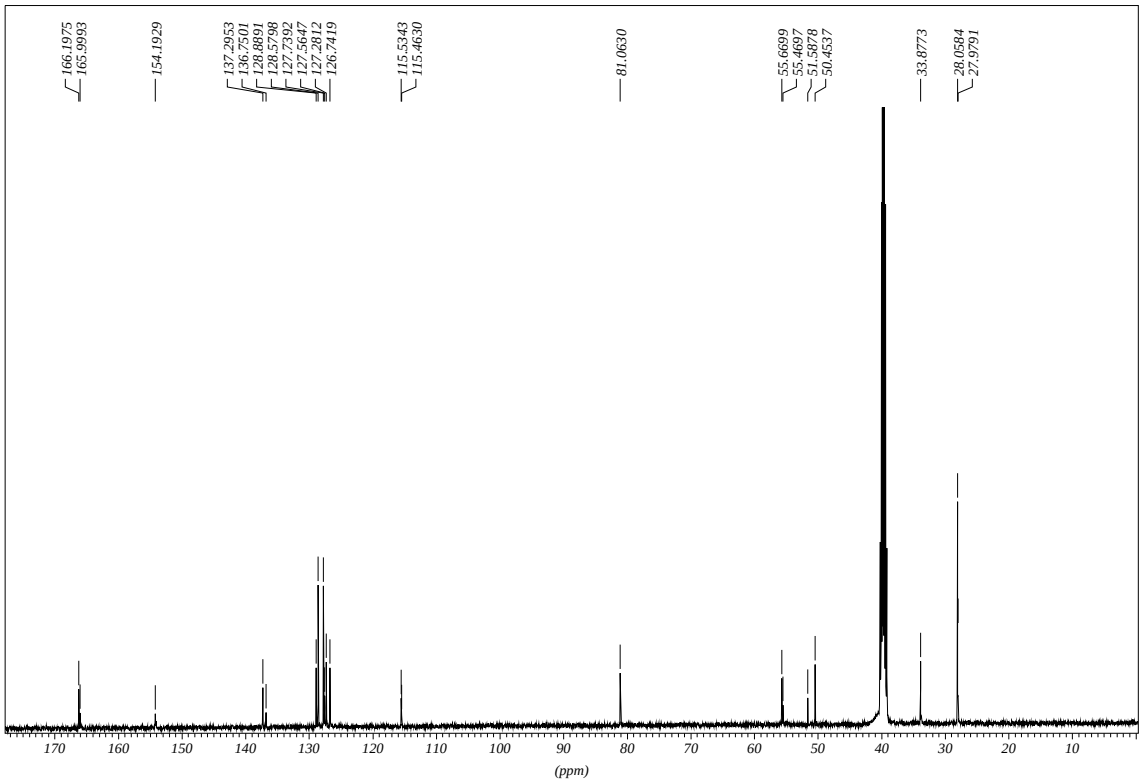
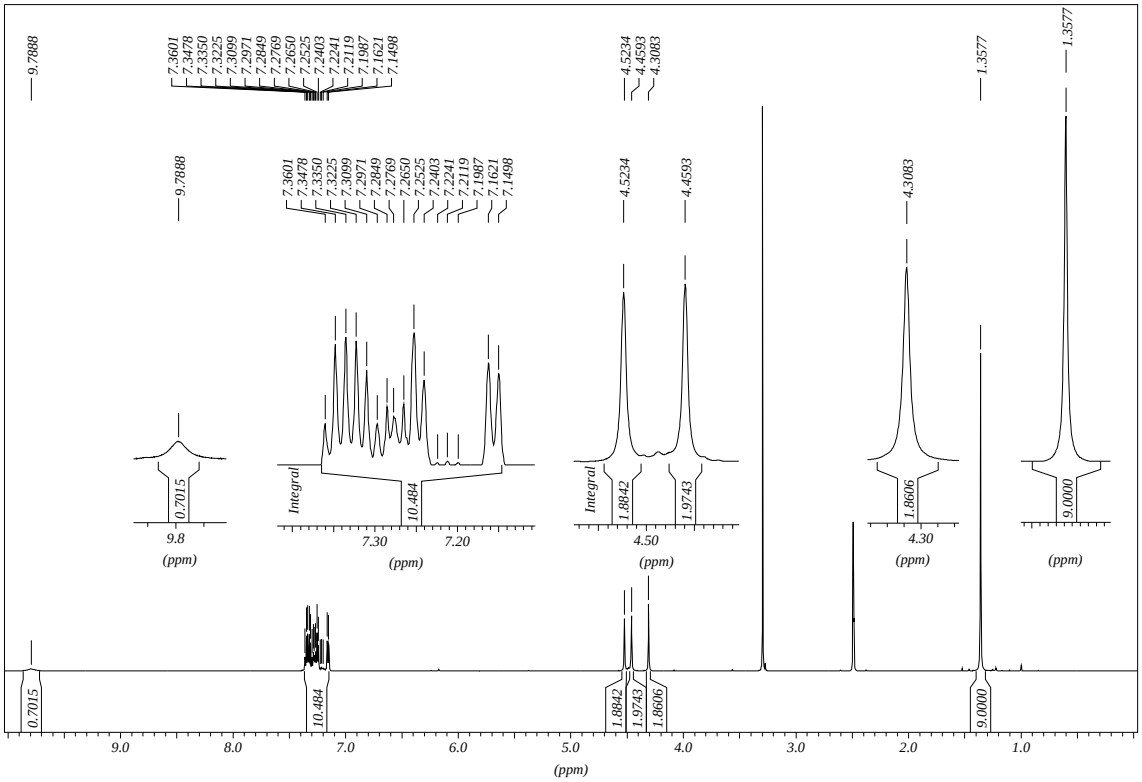
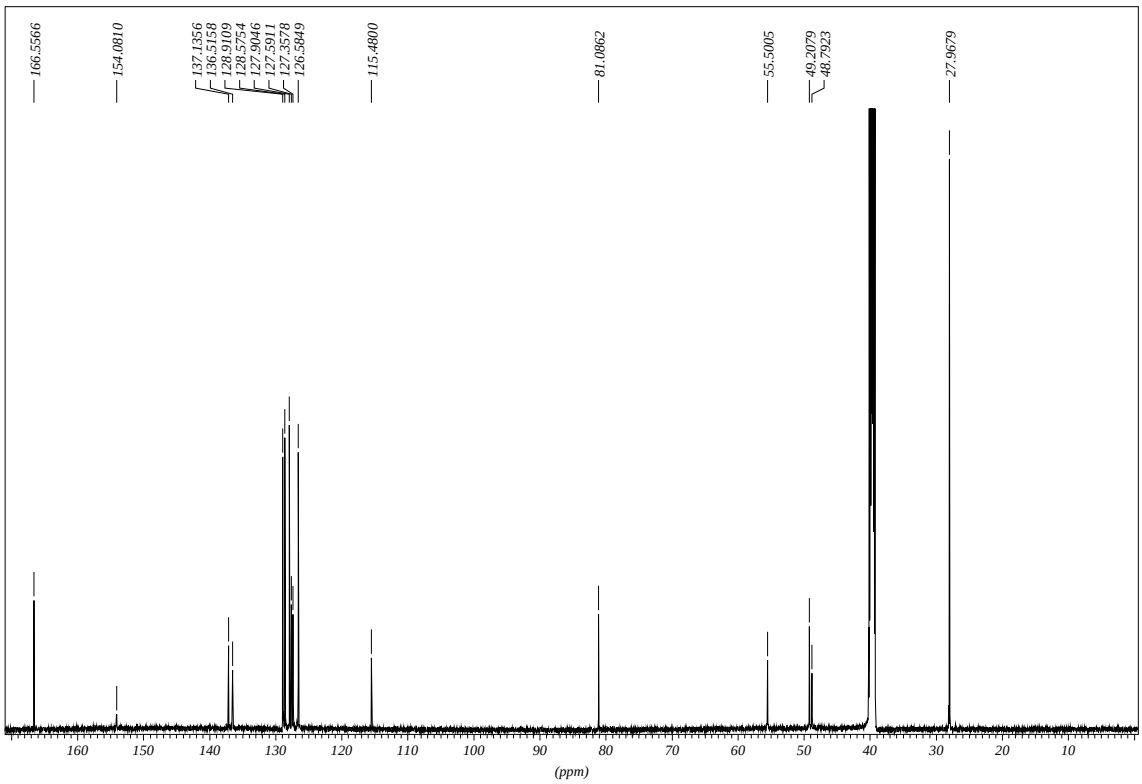


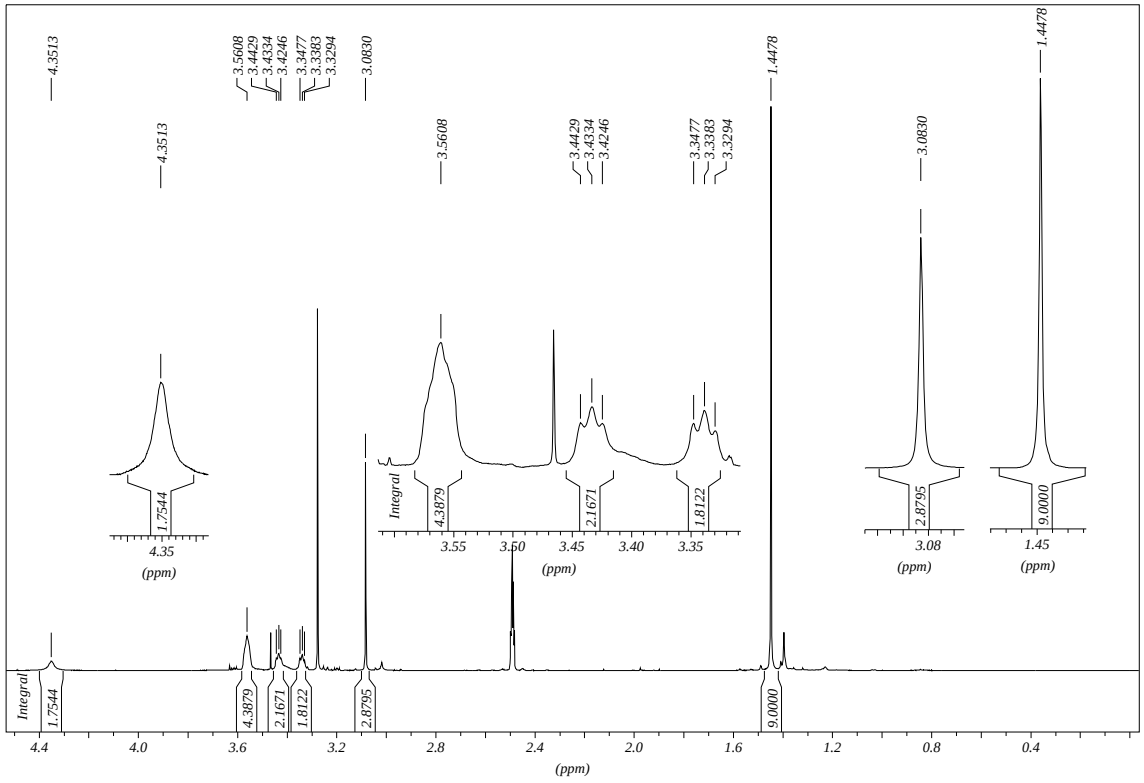



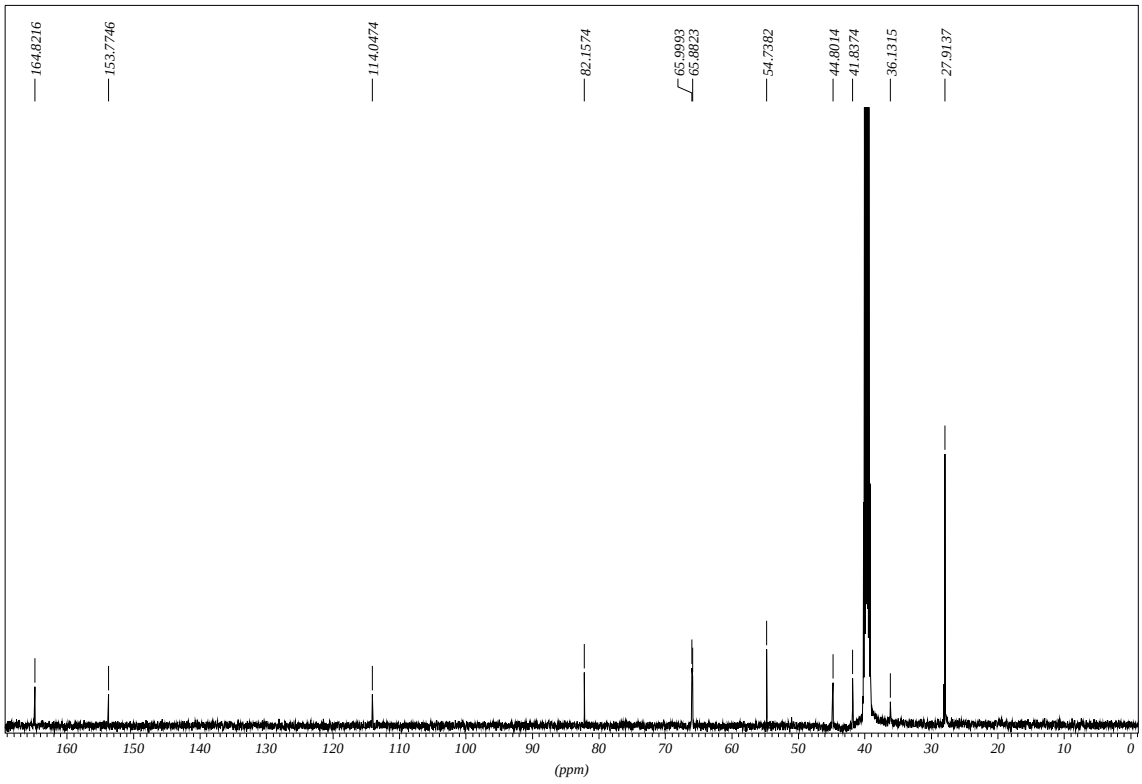
## *tert*-Butyl 2-cyano-2-[2-(dibenzylamino)-2-oxoethyl]hydrazinecarboxylate (24)



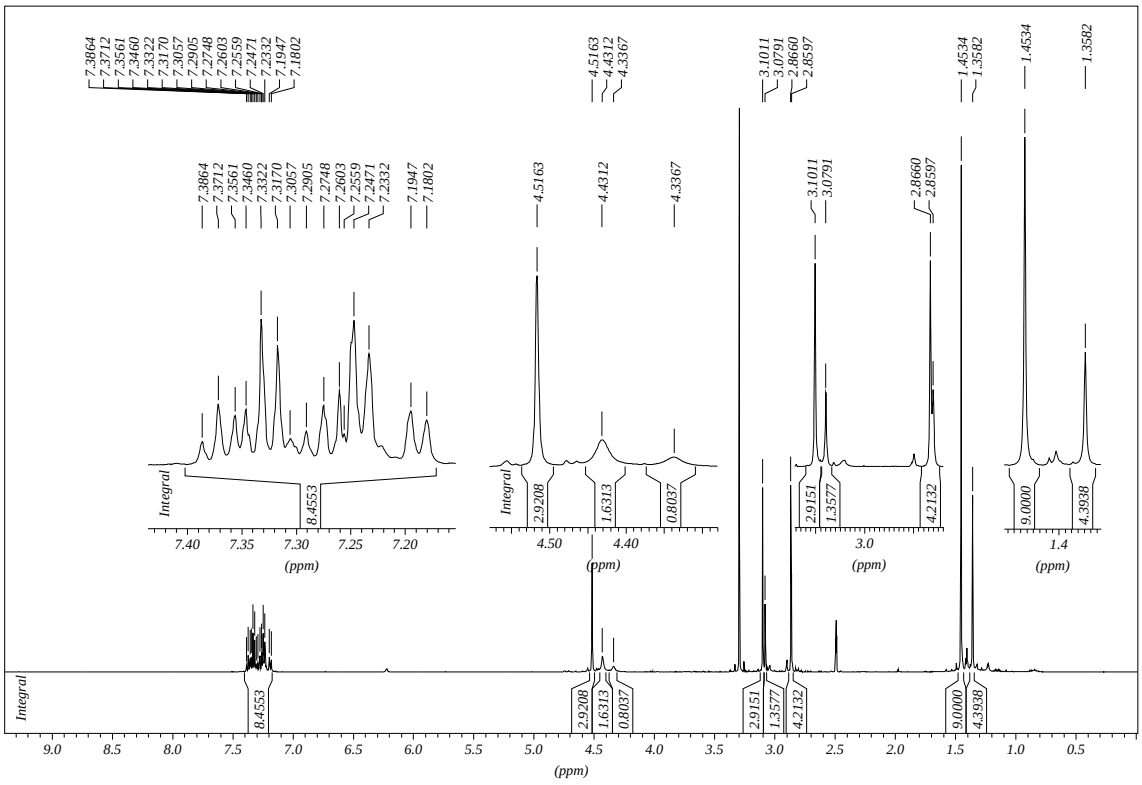



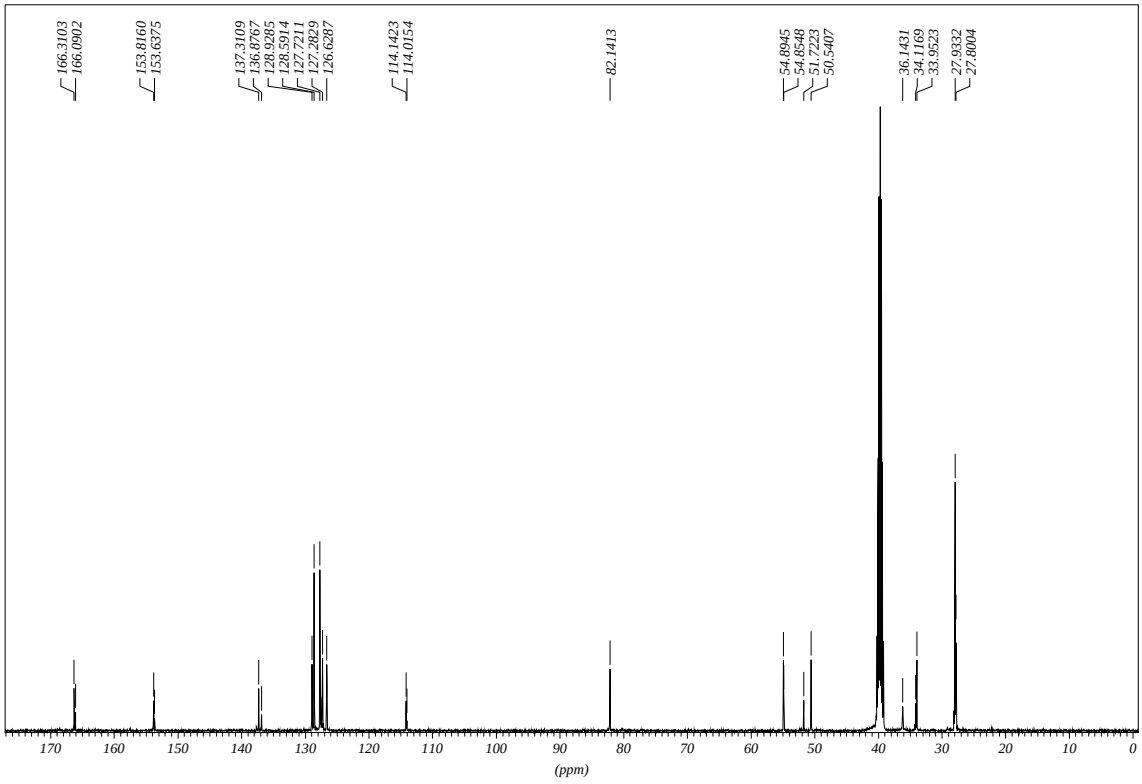
## *tert*-Butyl 2-cyano-1-methyl-2-(2-morpholino-2-oxoethyl)hydrazinecarboxylate (25)



## *tert*-Butyl 2-[2-(benzyl(methyl)amino)-2-oxoethyl]-2-cyano-1-methylhydrazinecarboxylate (26)



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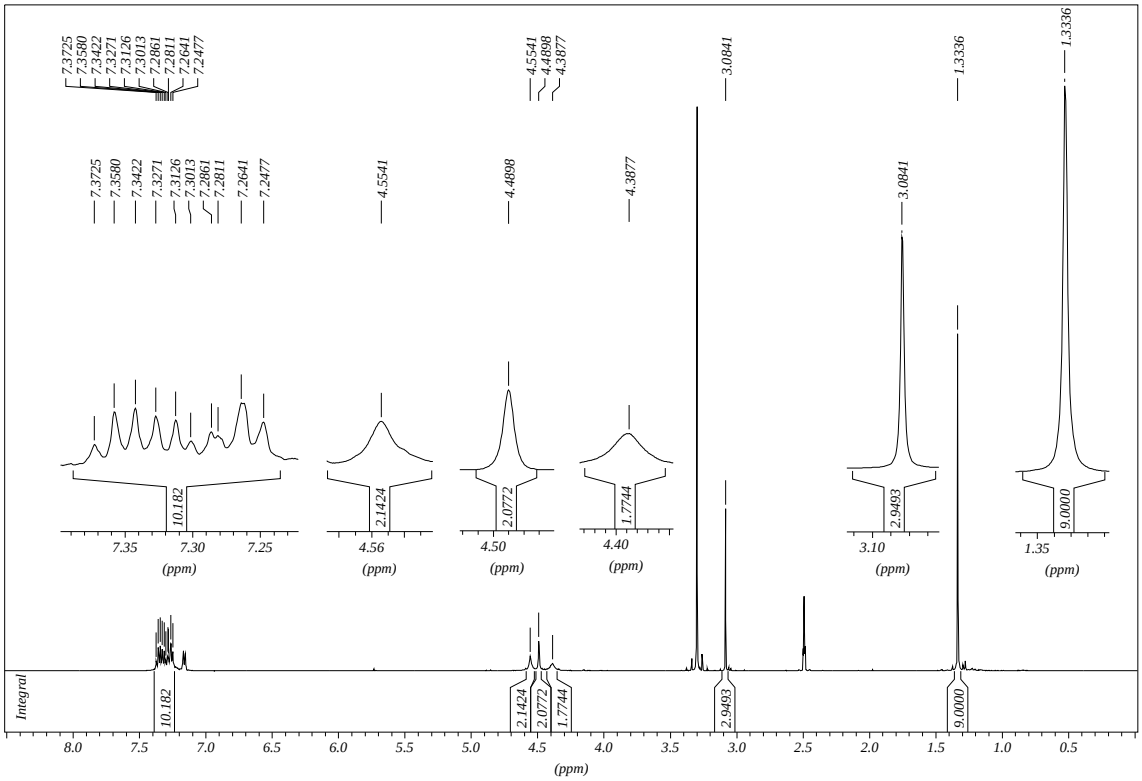
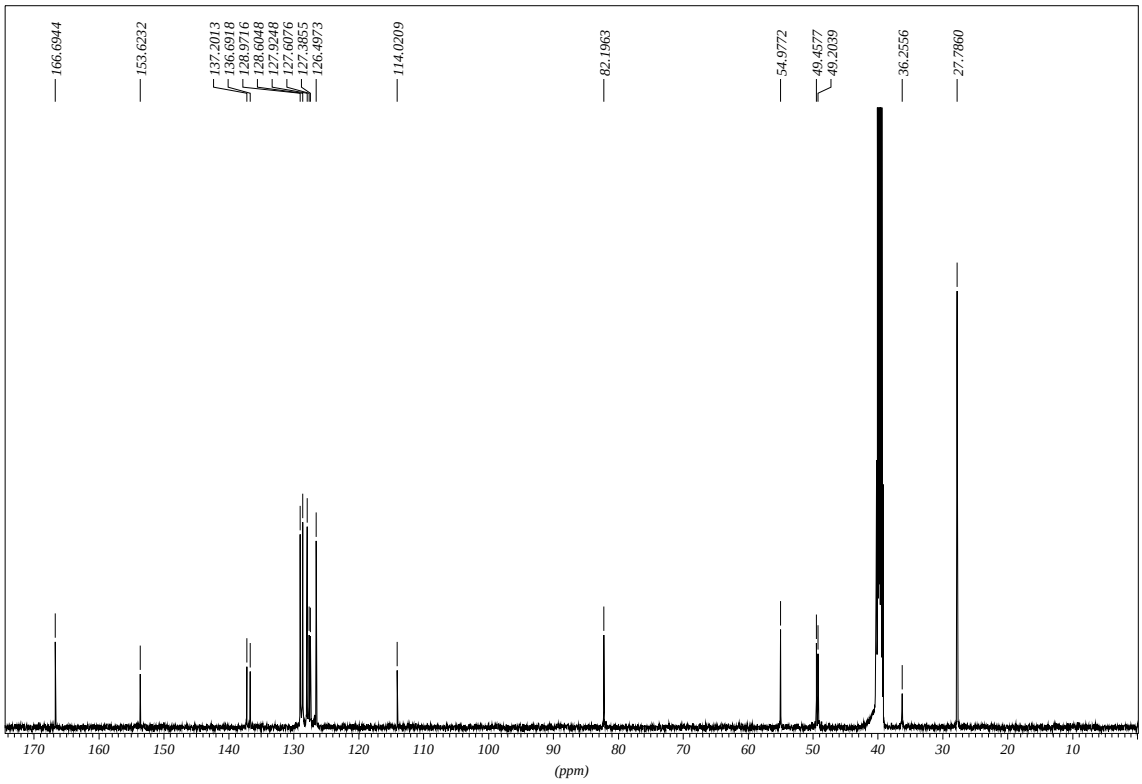
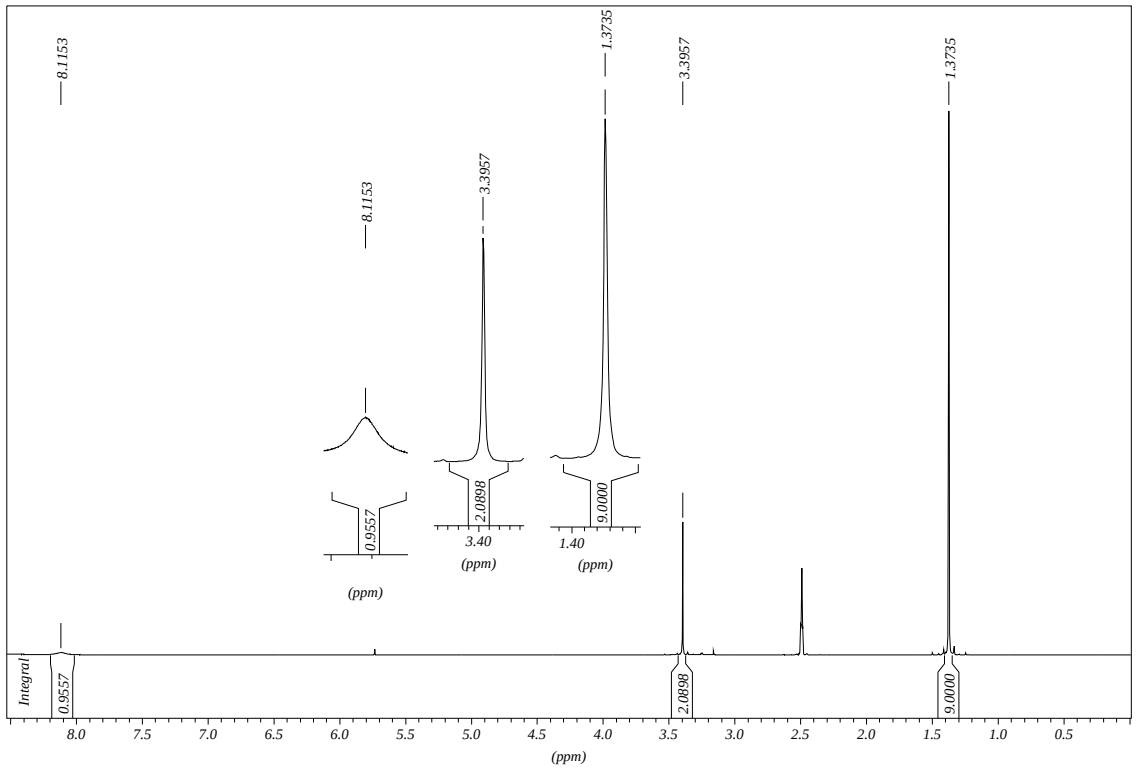
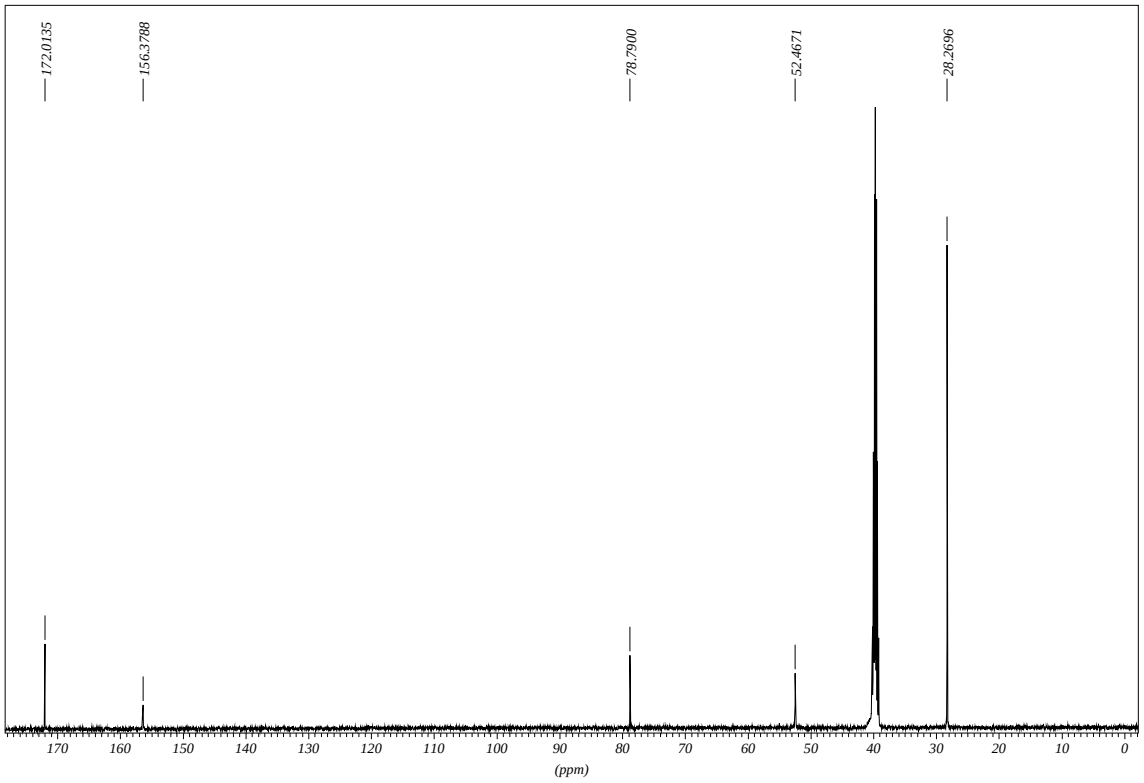


## 2-[2-(*tert*-Butoxycarbonyl)hydrazinyl]acetic acid (28)



## 2-[2-(*tert*-Butoxycarbonyl)-2-methylhydrazinyl]acetic acid (29)