Umpolung Amide Synthesis: Discovery and Characterization of Anaerobic and Aerobic Pathways to Amide, Introducing an Opportunity for Straightforward ^{18}O-Amide Synthesis

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General Experimental Details

All reagents and solvents were commercial grade and purified prior to use when necessary. Tetrahydrofuran (THF) was dried by passage through a column of activated alumina as described by Grubbs.[1] This was done to accurately quantitate the amount of water in each reaction. NIS was recrystallized from dioxane/CCl_4.

Thin layer chromatography (TLC) was performed using glass-backed silica gel (250 μm) plates, and flash chromatography utilized 230-400 mesh silica gel from Scientific Adsorbents. Products were visualized by UV light, iodine, and/or the use of ninhydrin solution.

IR spectra were recorded on a Thermo Nicolet IR100 spectrophotometer and are reported in wavenumbers (cm^{-1}). Compounds were analyzed as neat films on a NaCl plate (transmission). Nuclear magnetic resonance spectra (NMR) were acquired on a Bruker DRX-400 (400 MHz) or a Bruker AVIII-600 (600 MHz) spectrometer. Chemical shifts are measured relative to residual solvent peaks as an internal standard set to 7.26 and 77.1 for $CDCl_3$. Mass spectra were recorded on a Thermo Electron Corporation MAT 95XP-Trap mass spectrometer by use of chemical ionization (CI), electron impact ionization (EI) or electrospray ionization (ESI) by the Indiana University Mass Spectrometry Facility, or on a Synapt hybrid quadrupole/oa-TOF mass spectrometer equipped with a dual chemical ionization/electrospray (ESCI) source by Vanderbilt University Mass Spectrometry Facility. A post-acquisition gain correction was applied using sodium formate or sodium iodide as the lock mass. Optical rotations were measured on a Perkin Elmer-341 polarimeter.

^{18}O Percentage Mass Spectrometry Calculation

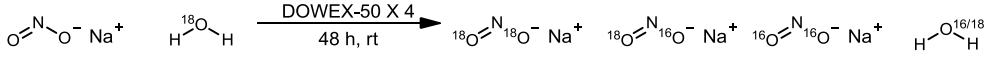
Contributions to the [M+2] mass peak include M(^{18}O) and the natural abundance of M($^{13}C_2$) and M(^{15}N). Their contribution is removed from the final ^{18}O percentage by the following calculation:

(^{16}O ion intensity) x (predicted ^{18}O ion natural abundance in the unlabeled compound) / 100 = ^{18}O ion intensity expected in the unlabeled compound

[1]Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996,** *15*, 1518-1520.

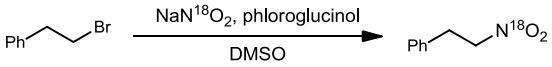
(^{18}O ion intensity) – (^{18}O ion intensity expected in the unlabeled compound) = corrected ^{18}O ion intensity

(Corrected ^{18}O ion intensity) / (Corrected ^{18}O ion intensity + ^{16}O ion intensity) x 100 = XX% ^{18}O



Preparation of $^{18}O_2$-Labeled Sodium Nitrite

Following the procedure developed by Yang and Goldberg,[2] a round bottomed flask was charged with $H_2{}^{18}O$ (0.5 mL, 27.8 mmol) via syringe and cooled to 0 °C. Sodium nitrite (109 mg, 1.58 mmol) and dry, activated Dowex-50X4 (30 mg) were added, and the flask was sealed with a glass stopper and parafilm. The reaction mixture was allowed to stir at room temperature for 48 h, using pH paper to confirm reaction completion (pH less than 4). The reaction mixture was filtered to remove Dowex-50X4 and brought to pH 11.8 with NaOH powder. H_2O was removed via short path distillation under reduced pressure to give $^{18}O_2$-labeled sodium nitrite as a solid (79.5 mg, 69%). The ^{18}O enrichment in $NaN^{18}O_2$ was not determined.

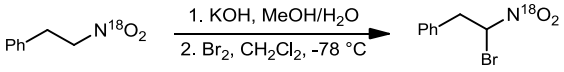


^{18}O-(2-Nitroethyl)benzene

$NaN^{18}O_2$ (110 mg, 1.51 mmol) was added to a solution of the bromide (155 mg, 837 µmol) in DMSO (2.8 ml, 0.3 M), followed by phloroglucinol (116 mg, 921 µmol). The mixture was stirred at room temperature for 2 d. The resulting solution was poured into ice water and extracted with Et_2O. The organic layer was dried, filtered, and concentrated. The residue was purified by flash column chromatography on silica gel (5% ethyl acetate in hexanes) to give the nitroalkane as a colorless oil (82.7mg, 64%). R_f= 0.40 (20% EtOAc/hexanes); IR (neat) 3065, 3032, 2920, 1527, 1340 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35-7.31 (m, 2H), 7.30-7.27 (m, 1H), 7.22-7.20 (m, 2H), 4.62 (t, *J* = 7.6 Hz, 2H), 3.33 (t, *J* = 7.6 Hz, 2H); ^{13}C NMR (100 MHz,

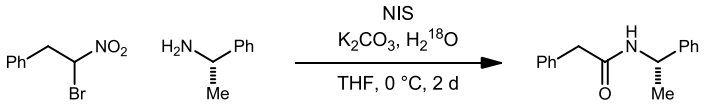
[2] Yang, C. C.; Goldberg, I. H. *J. Labelled Compd. Radiopharm.* **1989**, *27*, 423.

$CDCl_3$) ppm 135.7, 128.9, 128.6, 127.4, 76.2, 33.4; HRMS (CI): Exact mass calcd for $C_8H_{13}N_2{}^{16}O_2$ $[M+NH_4]^+$ 169.0976, found 169.0964: 3.1%; $C_8H_{13}N_2{}^{16}O^{18}O$ $[M+NH_4]^+$ 171.1014, found 171.1017: 26.9%; $C_8H_{13}N_2{}^{18}O_2$ $[M+NH_4]^+$ 173.1056, found 173.1051: 70.1%. Overall 83.6% ^{18}O incorporation (=1/2(26.9)+70.1).



^{18}O-(2-Bromo-2-nitroethyl)benzene (^{18}O-Labeled 1)

The nitroalkane (78.8 mg, 508 µmol) was added to a solution of KOH (28.5 mg, 508 µmol) in 25% MeOH:H_2O (1.2 mL) and allowed to stir until the nitroalkane completely dissolved. The reaction was cooled to -22 °C and transferred to a separatory funnel. A solution of bromine (81.1 mg, 508 µmol) in DCM (2.5 mL) was cooled to -78 °C and quickly added to the separatory funnel, which was shaken vigorously until the orange color disappeared. The reaction mixture was extracted with DCM, dried over $MgSO_4$, filtered, and concentrated.[3] The residue was purified via flash column chromatography (2-3% ethyl acetate in hexanes) to afford the α-bromonitroalkane as a colorless oil (95.0 mg, 80%). IR (neat) 3066, 3032, 2927, 1538, 1323 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37-7.32 (m, 3H), 7.22-7.19 (m, 2H), 6.04 (dd, *J* = 8.2, 6.1 Hz, 1H), 3.76 (dd, *J* = 14.6, 8.2 Hz, 1H), 3.51 (dd, *J* = 14.6, 6.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) ppm 133.3, 129.2, 129.1, 128.4, 79.2, 43.5; HRMS (CI): Exact mass calcd for $C_8H_{12}BrN_2{}^{16}O_2$ $[M+NH_4]^+$ 247.0082, found 247.0322, 3.3%; $C_8H_{12}BrN_2{}^{16}O^{18}O$ $[M+NH_4]^+$ 249.0125, found 249.0195, 29.1%; $C_8H_{12}BrN_2{}^{18}O_2$ $[M+NH_4]^+$ 251.0167, found 251.0220, 67.7%. Overall 82.2% ^{18}O incorporation (=1/2(29.1)+67.7).[4]



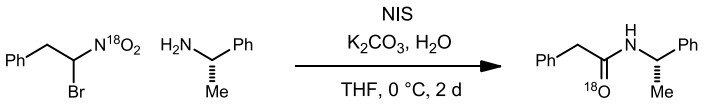
Amide 4a Prepared Using >99% $H_2{}^{18}O$ (Table 1, Entry 1)

A 15 mL round-bottom was charged with a solution of α-bromonitroalkane (30.8 mg, 134 µmol) dissolved in THF (0.67 mL). (*S*)-α-Methylbenzylamine (20.7 µL, 161 µmol) was added

[3]Procedure adapted from Erickson, A. S.; Kornblum, N. *J. Org. Chem.* **1977**, *42*, 3764.

[4]Only the nominal masses match with the calculated values. The NH_3 data are good for the relatives ratios. They are not internally calibrated since NH_3 gas will not ionize perfluorokerosene. CH_4 is too aggressive to make a stable M^+ ion for nitro compounds. HNO_2 comes off as a neutral loss easily.

via microsyringe, and the reaction was cooled to 0°C. Solid potassium carbonate (37.0 mg, 267 μmol) and NIS (30.1 mg, 134 μmol) were added, followed by $H_2{}^{18}O$ (13.4 μL, 669 μmol). The reaction mixture was allowed to stir for 48 h at 0°C. Anhydrous $MgSO_4$ was added, and the reaction mixture was diluted with dichloromethane, filtered, and concentrated. The residue was purified via flash column chromatography (20% ethyl acetate in hexanes) to afford the amide as a yellow solid (24.5 mg, 76%). R_f=0.35 (40% EtOAc/hexanes); spectroscopic data (IR, ^{1}H NMR and ^{13}C NMR) was in complete accord with that previously reported.[5] HRMS (ES): Exact mass calcd for $C_{16}H_{18}NO$ $[M+H]^+$ 240.1388, found 240.1389. The labeled product was not observed by mass spectrometry, indicating <1% ^{18}O incorporation.



Amide 4a Prepared Using 82% ^{18}O-Labeled α-Bromonitroalkane (Table 1, Entry 2)

A 15 mL round bottom was charged with a solution of ^{18}O-labeled α-bromonitroalkane (20.0 mg, 85 μmol) dissolved in THF (430 μL). (*S*)-α-Methylbenzylamine (13.2 μL, 103 μmol) was added via microsyringe, and the reaction was cooled to 0°C. Solid potassium carbonate (23.6 mg, 171 μmol) and NIS (19.2 mg, 85 μmol) were added, followed by distilled H_2O (7.7 μL, 427 μmol). The reaction mixture was allowed to stir for 48 h at 0°C. Anhydrous $MgSO_4$ was added, and the reaction mixture was diluted with dichloromethane, filtered, and concentrated. The residue was purified via flash column chromatography (20% ethyl acetate in hexanes) to afford the amide as a yellow solid (15.8 mg, 76%). R_f=0.35 (40% EtOAc/hexanes); spectroscopic data (IR, ^{1}H NMR and ^{13}C NMR) was in complete accord with that previously reported for ^{16}O-amide,[5] but two IR carbonyl peaks are present at 1644 and 1629 cm^{-1}. The ^{13}C NMR also showed the presence of ^{16}O and ^{18}O carbonyl peaks with the ^{18}O peak shifted upfield approximately 0.03 ppm. Integration of these two peaks indicated around a 22% ^{18}O incorporation. HRMS (CI): Exact mass calcd for $C_{16}H_{18}NO$ $[M+H]^+$ 240.1383 and $C_{16}H_{18}N^{18}O$ $[M+H]^+$ 242.1425, found 240.1605 and 242.1664. The relative intensities of these two peaks and their natural abundances were used to determine a 17% ^{18}O incorporation.

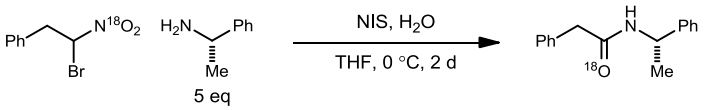
[5]Nordstrøm, L. U.; Vogt, H.; Madsen, R. *J. Am. Chem. Soc.* **2008**, *130*, 17672.

^{18}O Percentage Mass Spectrometry Calculation:

(834168 X 1.62) / 100 = 13514

179346 − 13514 = 165832

(165832/(165832 + 834168)) X 100 = 17% ^{18}O



Amide 4a Prepared Using ^{18}O-Labeled α-Bromonitroalkane and Excess Amine (Table 1, Entry 3)

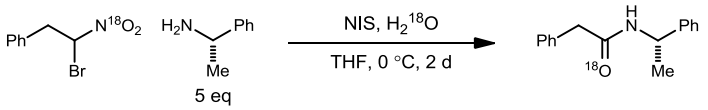
A 15 mL round bottomed flask was charged with a solution of ^{18}O-labeled α-bromonitroalkane (20 mg, 85 μmol) dissolved in THF (430 μL). (*S*)-α-Methylbenzylamine (55 μL, 427 μmol) was added via microsyringe, and the reaction was cooled to 0°C. NIS (19.2 mg, 85 μmol) was added, followed by distilled H_2O (7.7 μL, 427 μmol). The reaction mixture was allowed to stir for 48 h at 0°C. Anhydrous $MgSO_4$ was added, and the reaction mixture was diluted with dichloromethane, filtered, and concentrated. The residue was purified via flash column chromatography (20% ethyl acetate in hexanes) to afford the amide as a yellow solid (14.2 mg, 70%). R_f=0.35 (40% EtOAc/hexanes); spectroscopic data (IR, ^{1}H NMR and ^{13}C NMR) was in complete accord with that previously reported,[5] but two IR carbonyl peaks are present at 1648 and 1627 cm^{-1}. The ^{13}C NMR also showed the presence of ^{16}O and ^{18}O carbonyl peaks with the ^{18}O peak shifted upfield approximately 0.03 ppm. Integration of these two peaks indicated a 49% ^{18}O incorporation. HRMS (CI): Exact mass calcd for $C_{16}H_{18}NO$ $[M+H]^+$ 240.1383 and $C_{16}H_{18}N^{18}O$ $[M+H]^+$ 242.1425, found 240.1592 and 242.1637. The relative intensities of these two peaks and their natural abundances were used to determine a 49% ^{18}O incorporation.

^{18}O Percentage Mass Spectrometry Calculation:

(510360 X 1.62) / 100 = 8268

497908 − 8268 = 489640

(489640/ (489640 + 510360)) X 100 = 49% ^{18}O



Amide 4a Prepared Using ^{18}O-Labeled α-Bromonitroalkane/$H_2{}^{18}O$ and Excess Amine (Table 1, Entry 4)

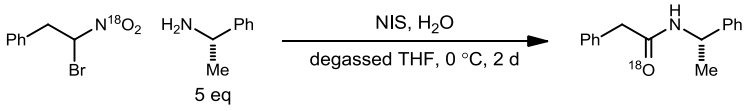
A 15 mL round bottom was charged with a solution of ^{18}O-labeled α-bromonitroalkane (20 mg, 85 μmol) dissolved in THF (430 μL). (*S*)-α-Methylbenzylamine (55 μL, 427 μmol) was added via microsyringe, and the reaction was cooled to 0°C. NIS (19.2 mg, 85 μmol) was added, followed by $H_2{}^{18}O$ (7.7 μL, 427 μmol). The reaction mixture was allowed to stir for 48 h at 0°C. Anhydrous $MgSO_4$ was added, and the reaction mixture was diluted with dichloromethane, filtered, and concentrated. The residue was purified via flash column chromatography (20% ethyl acetate in hexanes) to afford the amide as a yellow solid (14.2 mg, 70%). R_f=0.35 (40% EtOAc/hexanes); spectroscopic data (IR, ^{1}H NMR and ^{13}C NMR) was in complete accord with that previously reported,[5] but two IR carbonyl peaks are present at 1649 and 1629 cm^{-1}. The ^{13}C NMR also showed the presence of ^{16}O and ^{18}O carbonyl peaks with the ^{18}O peak shifted upfield approximately 0.03 ppm. Integration of these two peaks indicated around a 46% ^{18}O incorporation. HRMS (CI): Exact mass calcd for $C_{16}H_{18}NO$ $[M+H]^+$ 240.1383 and $C_{16}H_{18}N^{18}O$ $[M+H]^+$ 242.1425, found 240.1592 and 242.1637. The relative intensities of these two peaks and their natural abundances were used to determine a 49% ^{18}O incorporation.

^{18}O Percentage Mass Spectrometry Calculation:

(510334 X 1.62) / 100 = 8267

497933 − 8267 = 489666

(489666/ (489666 + 510334)) X 100 = 49% ^{18}O



Amide 4a Prepared Using ^{18}O-Labeled α-Bromonitroalkane, Excess Amine, and Degassed Solvent (Table 1, Entry 5)

A 15 mL round bottom was charged with a solution of ^{18}O-labeled α-bromonitroalkane (9 mg, 39 μmol) dissolved in degassed THF (190 μL). (*S*)-α-Methylbenzylamine (24.8 μL, 192 μmol)

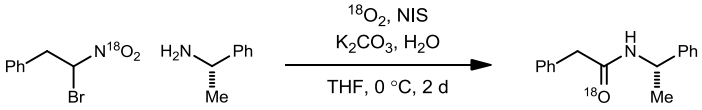
was added via microsyringe, and the reaction was cooled to 0°C. NIS (8.7 mg, 39 µmol) was added, followed by distilled H_2O (3.5 µL, 192 µmol). The reaction mixture was allowed to stir under argon for 48 h at 0°C. Anhydrous $MgSO_4$ was added, and the reaction mixture was diluted with dichloromethane, filtered, and concentrated. The residue was purified via flash column chromatography (20% ethyl acetate in hexanes) to afford the amide as a yellow solid (6.9 mg, 70%). R_f=0.35 (40% EtOAc/hexanes); spectroscopic data (IR, ^{1}H NMR and ^{13}C NMR) was in complete accord with that previously reported,[5] but two IR carbonyl peaks are present at 1654 and 1629 cm^{-1}. The ^{13}C NMR also showed the presence of ^{16}O and ^{18}O carbonyl peaks with the ^{18}O peak shifted upfield approximately 0.03 ppm. Integration of these two peaks indicated around a 58% ^{18}O incorporation. HRMS (EI): Exact mass calcd for $C_{16}H_{17}NO$ $[M]^+$ 239.1310 and $C_{16}H_{17}N^{18}O$ $[M]^+$ 241.1353, found 239.1287 and 241.1328. The relative intensities of these two peaks and their natural abundances were used to determine a 66% ^{18}O incorporation.

^{18}O Percentage Mass Spectrometry Calculation:

(10660544 X 1.62) / 100 = 172701

20970944 − 172701 = 20798243

(20798243 / (20798243 + 10660544)) X 100 = 66% ^{18}O



Amide 4a Prepared Using Degassing and $^{18}O_2$ Gas (Table 1, Entry 6)

The α-bromonitroalkane (28.5 mg, 124 µmol) and amine (19 µl, 149 µmol) were added to a 10 mL round-bottomed flask (flask A) and sealed with a septum wrapped with parafilm. K_2CO_3 (34.3 mg, 124 µmol), NIS (27.9 mg, 124 µmol), and H_2O (11.2 µl, 620 µmol) were added to a second 10 mL round-bottomed flask (flask B) in a solution of THF (620 µL), which was subsequently sealed with a septum and wrapped with parafilm. Both flasks were degassed using three 80 minute freeze-pump-thaw cycles. A balloon (that had been evacuated and purged 3 times with nitrogen) was added to flask B, and $^{18}O_2$ gas was added directly to flask B through the septum until the balloon was fully inflated. The contents of flask A were transferred to flask B via dry microsyringe in one portion. The reaction was allowed to stir at 0 °C for 16 h. Anhydrous

$MgSO_4$ was added, and the reaction mixture was diluted with dichloromethane, filtered, and concentrated. The residue was purified via flash column chromatography (20% ethyl acetate in hexanes) to afford the amide as a yellow solid (20.1 mg, 68%). R_f=0.35 (40% EtOAc/hexanes); spectroscopic data (IR, ^{1}H NMR and ^{13}C NMR) was in complete accord with that previously reported,[5] but two IR carbonyl peaks are present at 1654 and 1624 cm^{-1}. The ^{13}C NMR also showed the presence of ^{16}O and ^{18}O carbonyl peaks with the ^{18}O peak shifted upfield approximately 0.03 ppm. Integration of these two peaks indicated around a 79% ^{18}O incorporation. LRMS (EI): Exact mass calcd for $C_{16}H_{17}NO$ $[M]^+$ 239.1305 and $C_{16}H_{17}N^{18}O$ $[M]^+$ 241.1347, found 239.20 and 241.20. The relative intensities of these two peaks and their natural abundances were used to determine a 83% ^{18}O incorporation.

^{18}O Percentage Mass Spectrometry Calculation:

(180492 X 1.62) / 100 = 2924

904618 − 2924 = 901694

(901694 / (901694 + 180492)) X 100 = 83% ^{18}O

General Procedure: Amide Synthesis Using an Ammonium Salt

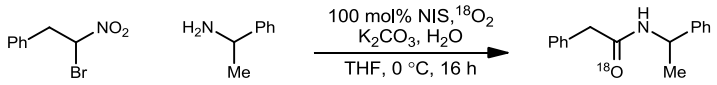
K_2CO_3 (3.2 equiv) was added to the suspension of the ammonium salt (1.2 equiv) and the α-bromonitroalkane (1.0 equiv, 0.2 M) in THF and H_2O (5.0 equiv) at 0 °C, followed by NIS (1.0 equiv). The reaction mixture was stirred at 0 °C for 2 d. The resulting mixture was diluted with dichloromethane and filtered to remove K_2CO_3. The filtrate was concentrated and subjected to purification by flash column chromatography on silica gel.

Procedure for Optimized Amide ^{18}O-Labelling using a 1 mL Vial

The α-bromonitroalkane (1 equiv) and amine (1.2 equiv) were added to a 1 mL glass screw cap HPLC vial (vial A), followed by addition of H_2O (5 equiv) and K_2CO_3 (2.0 equiv with free amines and 3.2 equiv with ammonium salts). THF (200 μL) was added to the vial, which was subsequently sealed with the screw cap (containing a silicone septum) and parafilm. NIS (1 equiv) in THF (200 μL) was added to a second 1 mL glass screw cap HPLC vial (vial B) and sealed with the silicone septum screw cap and parafilm. Both flasks were degassed using three 80 minute freeze-pump-thaw cycles (Figure 1). Once degassing was complete, vial A was

Figure 1. $^{18}O_2$ Gas Addition Experimental Setup: Freeze-Pump-Thaw Cycles for Substrates and NIS Solution (left), and Back-Fill with $^{18}O_2$ Following Final Thaw Cycle

refrozen in liquid nitrogen. The NIS solution in vial B was transferred to vial A via dry micro-syringe in one portion. Once the transferred solution had frozen, the $^{18}O_2$ regulator needle was inserted through the septum, and the entire system was placed under high vacuum (0.3 Torr). The vacuum was turned off, and the $^{18}O_2$ gas regulator was opened to allow its entry to the system under static vacuum, and until the regulator pressure gauge remained steady. The reaction was warmed to 0 °C and allowed to stir overnight. If the pressure gauge needle dropped while the reaction was warming, additional $^{18}O_2$ gas was added to allow the needle to return to its previously stable state. Following a 16 to 44 h reaction time, anhydrous $MgSO_4$ was added. The reaction mixture was diluted with dichloromethane, filtered, and concentrated. The residue was purified via flash column chromatography.



^{18}O-Labeled-2-Phenyl-*N*-(1-phenylethyl)acetamide (11a)

Following the procedure, the α-bromonitroalkane (26.1 mg, 113 μmol) and amine (17.3 μl, 136 μmol) provided the amide after flash column chromatography (20% ethyl acetate in hexanes) as a white solid (21.7 mg, 79%). R_f=0.35 (40% EtOAc/hexanes); spectroscopic data (IR, ^{1}H NMR and ^{13}C NMR) was in complete accord with that previously reported,[5] but a shift in the IR carbonyl peak from 1645 to 1624 cm^{-1} was observed. The ^{13}C NMR also showed the presence of ^{16}O and ^{18}O carbonyl peak with the ^{18}O peak shifted upfield approximately 0.03 ppm. Integration of these two peaks indicated around a 71% ^{18}O incorporation. LRMS (EI):

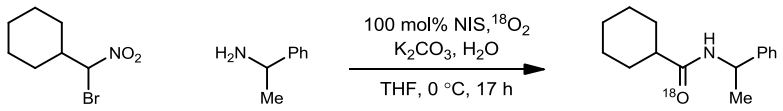
Exact mass calcd for $C_{16}H_{17}NO$ $[M]^+$ 239.13 and $C_{16}H_{17}N^{18}O$ $[M]^+$ 241.13, found 239.20 and 241.20. The relative intensities of these two peaks and their natural abundances were used to determine a 79% ^{18}O incorporation.

^{18}O Percentage Mass Spectrometry Calculation:

(489962 X 1.62) / 100 = 7937

1807239 − 7937 = 1799302

(1799302 / (1799302 + 489962)) X 100 = 79% ^{18}O



^{18}O-Labeled-*N*-(1-Phenylethyl)cyclohexanecarboxamide (11b)

Following the procedure, the α-bromonitroalkane (28.7 mg, 129 µmol) and amine (19.7 µl, 155 µmol) provided the amide after flash column chromatography (10% ethyl acetate in hexanes) as an off-white solid (24.5 mg, 81%). R_f=0.25 (20% EtOAc/hexanes); spectroscopic data (^1H NMR) was in complete accord with that previously reported.[6] A shift in the IR carbonyl peak from 1644 cm^{-1} to 1623 cm^{-1} was observed. The ^{13}C NMR also showed the presence of ^{16}O and ^{18}O carbonyl peak with the ^{18}O peak shifted upfield approximately 0.03 ppm. Integration of these two peaks indicated around a 75% ^{18}O incorporation. LRMS (EI): Exact mass calcd for $C_{15}H_{21}NO$ $[M]^+$ 231.16 and $C_{15}H_{21}N^{18}O$ $[M]^+$ 233.17, found 231.25 and 233.30. The relative intensities of these two peaks and their natural abundances were used to determine a 65% ^{18}O incorporation.

^{18}O Percentage Mass Spectrometry Calculation:

(95132 X 1.44) / 100 = 1370

180814 − 1370 = 179444

(179444 / (179444 + 95132)) X 100 = 65% ^{18}O

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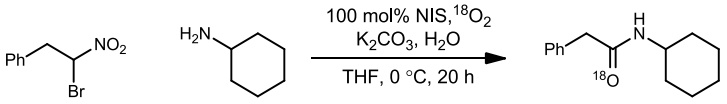
[6] Vora, H. U.; Rovis, T.*J. Am. Chem. Soc.***2007**, *129*, 13796.



^{18}O-Labeled *N*-Cyclohexyl-2-phenylacetamide (11c)

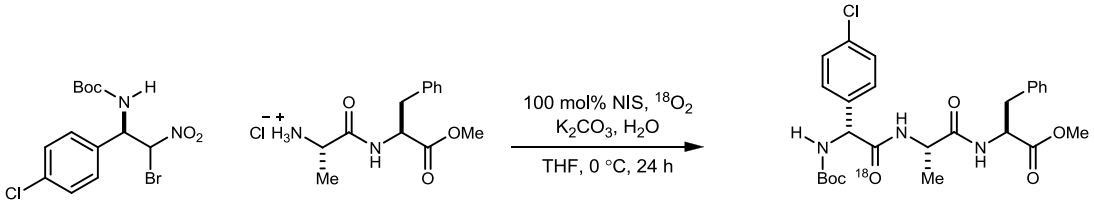
Following the procedure, the α-bromonitroalkane (30.1 mg, 131 μmol) and amine (18 μl, 157 μmol) provided the amide after flash column chromatography (20% ethyl acetate in hexanes) as a white solid (22.5 mg, 78%). R_f= 0.11 (20% EtOAc/hexanes); spectroscopic data (^{1}H NMR) was in complete accord with that previously reported.[7] A shift in the IR carbonyl peak from 1638 cm^{-1} to 1620 cm^{-1} was observed. The ^{13}C NMR also showed the presence of ^{16}O and ^{18}O carbonyl peaks with the ^{18}O peak shifted upfield approximately 0.03 ppm. Integration of these two peaks indicated approximately 72% ^{18}O incorporation. LRMS (EI): Exact mass calcd for $C_{14}H_{19}NO$ $[M]^+$ 217.15 and $C_{14}H_{19}N^{18}O$ $[M]^+$ 219.15, found 217.20 and 219.20. The relative intensities of these two peaks and their natural abundances were used to determine a 79% ^{18}O incorporation.

^{18}O Percentage Mass Spectrometry Calculation:

(163531 X 1.28) / 100 = 2093

601428 − 2093 = 599335

(599335 / (599335 + 163531)) X 100 = 79% ^{18}O



^{18}O-Labeled *N*-Boc-4-Cl-Phenylglycine-Ala-Phe-OMe (11d)

Following the procedure, with an additional base wash (satd aq K_2CO_3) after chromatography, the α-bromonitroalkane (39.9 mg, 105 μmol) and ammonium salt (36.2 mg, 126 μmol) provided the amide after flash column chromatography (30-40% ethyl acetate in hexanes) as a white solid

[7]Chan, W.-K.; Ho, C.-M.; Wong, M.-K.; Che, C.-M.*J. Am. Chem. Soc.***2006**, *128*, 14796.

(31.4 mg, 58%). R_f = 0.16 (40% EtOAc/hexanes); spectroscopic data (^{1}H NMR, ^{13}C NMR, and IR) was in complete accord with that previously reported.[8] A shift in the IR carbonyl peak from 1643 cm^{-1} to 1625 cm^{-1} was observed. The ^{13}C NMR ^{16}O and ^{18}O carbonyl peaks were too close together to approximate the ^{18}O incorporation. HMBC NMR analysis confirmed the identity of the ^{18}O-labeled carbonyl at 169.5 ppm. HRMS (ESI): Exact mass calcd for $C_{26}H_{32}ClN_3NaO_6$ $[M+Na]^+$ 540.1877 and $C_{26}H_{32}ClN_3NaO_5{}^{18}O$ $[M+Na]^+$ 542.1862, found 540.1837 and 542.1928. The relative intensities of these two peaks and their natural abundances were used to determine a 93% ^{18}O incorporation.

^{18}O Percentage Mass Spectrometry Calculation:

(12293 X 32.27) / 100 = 3967

156806 − 3967 = 152839

(152839 / (152839 + 12293)) X 100 = 93% ^{18}O

HMBC Analysis (Carbonyl Assignments):

Shown in Figure 2, C8 correlates to H6 and H9 indicating it is the ester carbonyl. C5 correlates to H3 and H4 indicating it is likely the unlabeled amide carbonyl, as the labeled amide carbonyl should not correlate to H4. C2 is likely to be the labeled amide carbonyl peak as it weakly correlates to H3 but not H4. As shown in Figure 3, the carbamate carbonyl of the Boc group only shows one potential correlation to H1 when the spetrum is highly magnified. This weak signal is likely due to the broad nature of both the carbon and proton shifts. A correlation between the labeled amide carbonyl (C2) and H1 cannot be clearly identified, which is also likely due to the broad nature of both the carbon and proton shifts. The shift of the carbamate carbonyl of the Boc group is also much further upfield than the ester and amide carbonyl peaks, which is to be expected.

[8] Shen, B.; Makley, D. ; Johnston, J. N. *Nature* **2010**, *465*, 1027.

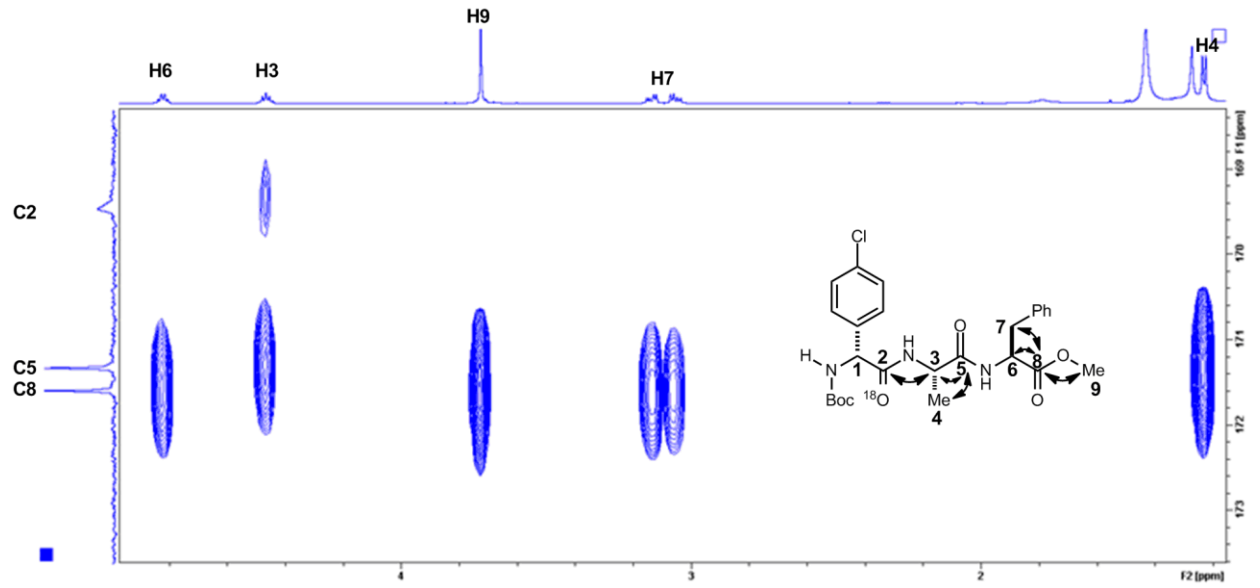


Figure 2. HMBC Expansion for Key Carbonyl Region

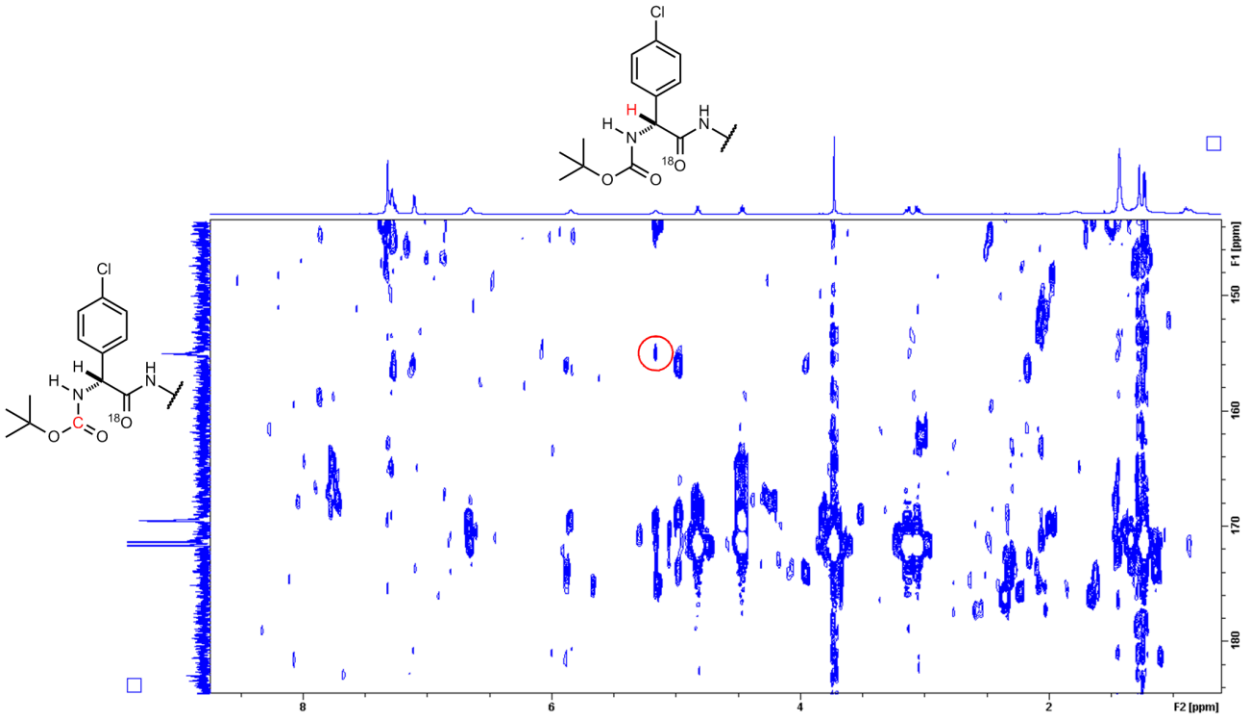
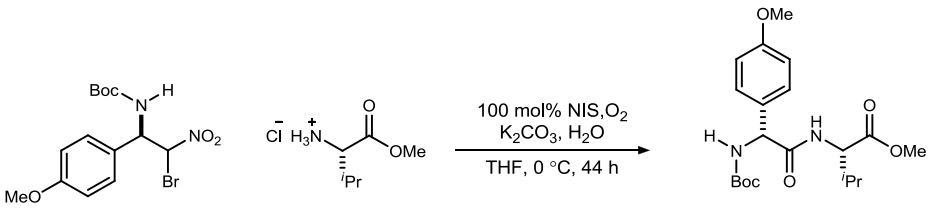
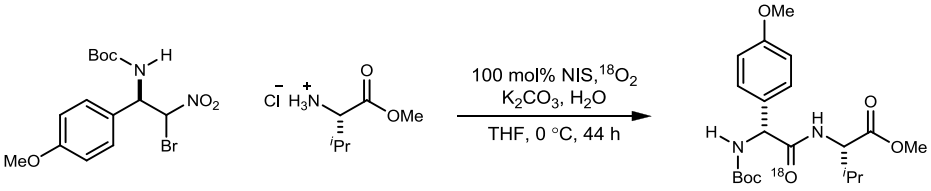


Figure 3. HMBC Magnification Containing the Boc Group Carbonyl



_N_-Boc-4-OMe-Phenylglycine-Val-OMe (Unlabeled 11e)

Following the General Procedure, the α-bromonitroalkane (23 mg, 61 μmol) and the ammonium salt of valine (12.3 mg, 73.2μmol) provided the dipeptide (single diastereomer) after flash column chromatography (20% ethyl acetate in hexanes) as a viscous oil (17.8 mg, 74%). $[\alpha]_D^{20}$ -37 (*c* 1.0, $CHCl_3$); R_f=0.32 (30% EtOAc/hexanes); IR (film) 3321, 2965, 2930, 2362, 1740, 1664, 1612, 1511 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.30 (d, *J* = 8.0 Hz, 2H), 6.87 (d, *J* = 8.4 Hz, 2H), 6.30 (br s, 1H), 5.67 (br s, 1H),5.13 (br s, 1H), 4.54 (dd, *J* = 8.8, 4.8 Hz, 1H), 3.79 (s, 3H),3.73 (s, 3H), 2.06 (qqd, *J* = 6.8, 6.8, 5.0 Hz,1H), 1.41 (s, 9H), 0.76 (d, *J* = 6.8 Hz, 3H), 0.71 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) ppm 172.1, 170.2, 159.5, 128.4, 114.3, 58.0, 56.93, 55.2, 52.2, 31.3, 31.1, 29.6, 28.2, 18.7, 17.3; HRMS (ESI): Exact mass calcd for $C_{20}H_{30}N_2NaO_6$ $[M+Na]^+$ 417.2002, found 417.2001.



^{18}O-Labeled-_N_-Boc-4-OMe-Phenylglycine-Val-OMe (11e)

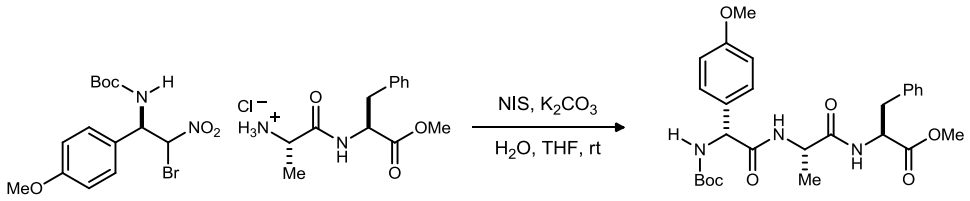
Following the procedure, the α-bromonitroalkane (40.6 mg, 108 μmol) and ammonium salt of valine (21.7 mg, 130 μmol) provided the dipeptide (single diastereomer) after flash column chromatography (20% ethyl acetate in hexanes) as a viscous oil (27.6 mg, 65%). R_f=0.32 (30% EtOAc/hexanes); spectroscopic data (^1H NMR, ^{13}C NMR, and IR) was in complete accord with that previously reported. A shift in the IR carbonyl peak from 1664 cm^{-1} to 1645 cm^{-1} was observed. The ^{13}C NMR ^{16}O and ^{18}O carbonyl peaks were too close to approximate the ^{18}O incorporation. HRMS (ESI): Exact mass calcd for $C_{20}H_{30}N_2NaO_6$ $[M+Na]^+$ 417.2002 and $C_{20}H_{30}N_2NaO_5{}^{18}O$ $[M+Na]^+$ 419.2059, found 417.1995and 419.2036. The relative intensities of these two peaks and their natural abundances were used to determine a 76% ^{18}O incorporation.

^{18}O Percentage Mass Spectrometry Calculation:

(46161 X 3.62) / 100 = 1671.03

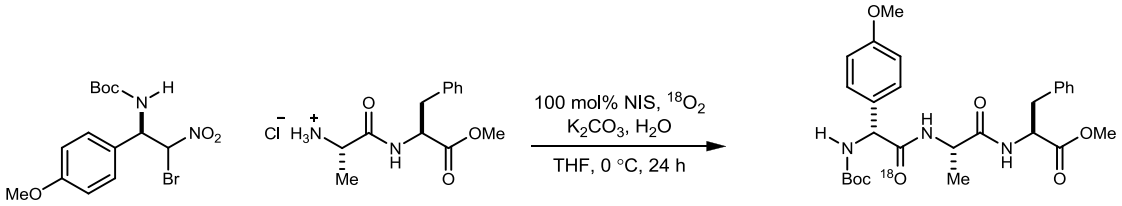
145922 − 1671 = 144251

(144251 / (144251 + 46161)) X 100 = 76% ^{18}O



***N*-Boc-4-MeO-Phenylglycine-Ala-Phe-OMe (Unlabeled 11f)**

Following the General Procedure, the α-bromonitroalkane (20.0 mg, 50.0 μmol) and the ammonium salt of the Ala-Phe dipeptide (18.0 mg, 60.0 μmol) provided the tripeptide (single diastereomer), after flash column chromatography (40% ethyl acetate in hexanes), as a white solid (19.7 mg, 72%). $[\alpha]_D^{20}$ -38.1 (*c* 2.2, $CHCl_3$); R_f=0.17 (40% EtOAc/hexanes); mp =179-180 °C; IR (film) 3282, 2927, 1651, 1512, 1247, 1169 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.25 (m, 5H), 7.09 (d, *J* = 6.6 Hz, 2H), 6.85 (d, *J* = 9.0 Hz, 2H), 6.60 (br s, 1H), 6.36 (d, *J* = 7.8 Hz, 1H), 5.66 (br s, 1H), 5.06 (br s, 1H), 4.80 (ddd, *J* = 6.6, 6.6, 6.6 Hz, 1H), 4.45 (dq, *J* = 6.6, 6.6 Hz, 1H), 3.78 (s, 3H), 3.70 (s, 3H), 3.12 (dd, *J* = 13.8, 6.0 Hz, 1H), 3.03 (dd, *J* = 13.8, 6.6 Hz, 1H), 1.42 (s, 9H), 1.22 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) ppm 171.6, 171.4, 170.2, 159.6, 155.1, 135.7, 130.0, 129.2, 128.6, 128.5, 127.2, 114.4, 80.1, 58.2, 55.3, 53.3, 52.3, 48.8, 37.7, 28.3, 17.9; HRMS (ESI): Exact mass calcd for $C_{27}H_{36}N_3O_7$ $[M+H]^+$ 514.2548, found 514.2552.



^{18}O-Labeled-*N*-Boc-4-OMe-Phenylglycine-Ala-Phe-OMe (11f)

Following the procedure, with an additional base wash (satd aq K_2CO_3) after chromatography, the α-bromonitroalkane (41.6 mg, 111 μmol) and ammonium salt (38.2 mg, 133 μmol) provided

the amide after flash column chromatography (40% ethyl acetate in hexanes) as a white solid (27.7 mg, 49%). R_f=0.17 (40% EtOAc/hexanes); spectroscopic data (^{1}H NMR, ^{13}C NMR, and IR) was in complete accord with that previously reported. A shift in the IR carbonyl peak from 1647 cm^{-1} to 1630 cm^{-1} was observed. The ^{13}C NMR ^{16}O and ^{18}O carbonyl peaks were too close to approximate the ^{18}O incorporation. HMBC NMR analysis confirmed the identity of the ^{18}O-labeled carbonyl at 170.2 ppm. HRMS (ESI): Exact mass calcd for $C_{27}H_{35}N_3NaO_7$ $[M+Na]^+$ 536.2372 and $C_{27}H_{35}N_3NaO_6{}^{18}O$ $[M+Na]^+$ 538.2432, found 536.2352 and 538.2407. The relative intensities of these two peaks and their natural abundances were used to determine a 88% ^{18}O incorporation.

^{18}O Percentage Mass Spectrometry Calculation:

(6168 X 5.99) / 100 = 370

45097 − 370 = 44728

(44728 / (44728 + 6168)) X 100 = 88% ^{18}O

HMBC Analysis (Carbonyl Assignments):

Shown in Figure 4, C8 correlates to H6 and H9 indicating it is the ester carbonyl. C5 correlates to H3 and H4 indicating it is likely the unlabeled amide carbonyl, as the labeled amide carbonyl should not correlate to H4. C2 and the carbamate carbonyl shift of the Boc group (not shown) show no correlations, which is likely due to the broad nature of the carbon and proton shifts. C2 is likely to be the labeled amide carbonyl due to the other carbonyl shift being much higher upfield (155 ppm), which would be expected for the carbamate carbonyl of the Boc group and not the amide.

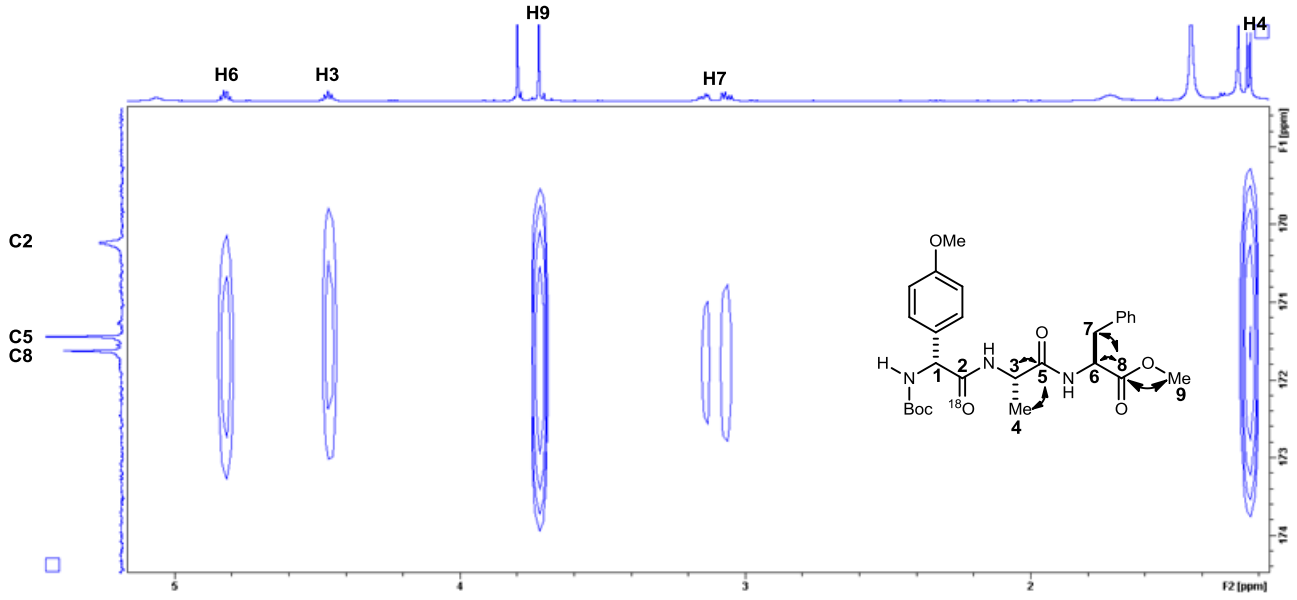


Figure 4. HMBC Expansion for Key Carbonyl Region

Umpolung Amide Synthesis: Discovery and Characterization of Anaerobic and Aerobic Pathways to Amide, Introducing an Opportunity for Straightforward ^{18}O-Amide Synthesis

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Vanderbilt University
2301 Vanderbilt Place, Nashville, TN 37235-1822

SI-II-X

Figure S1. ^{13}C NMR ($CDCl_3$) *of* ^{18}O-(2-Nitroethyl)benzene

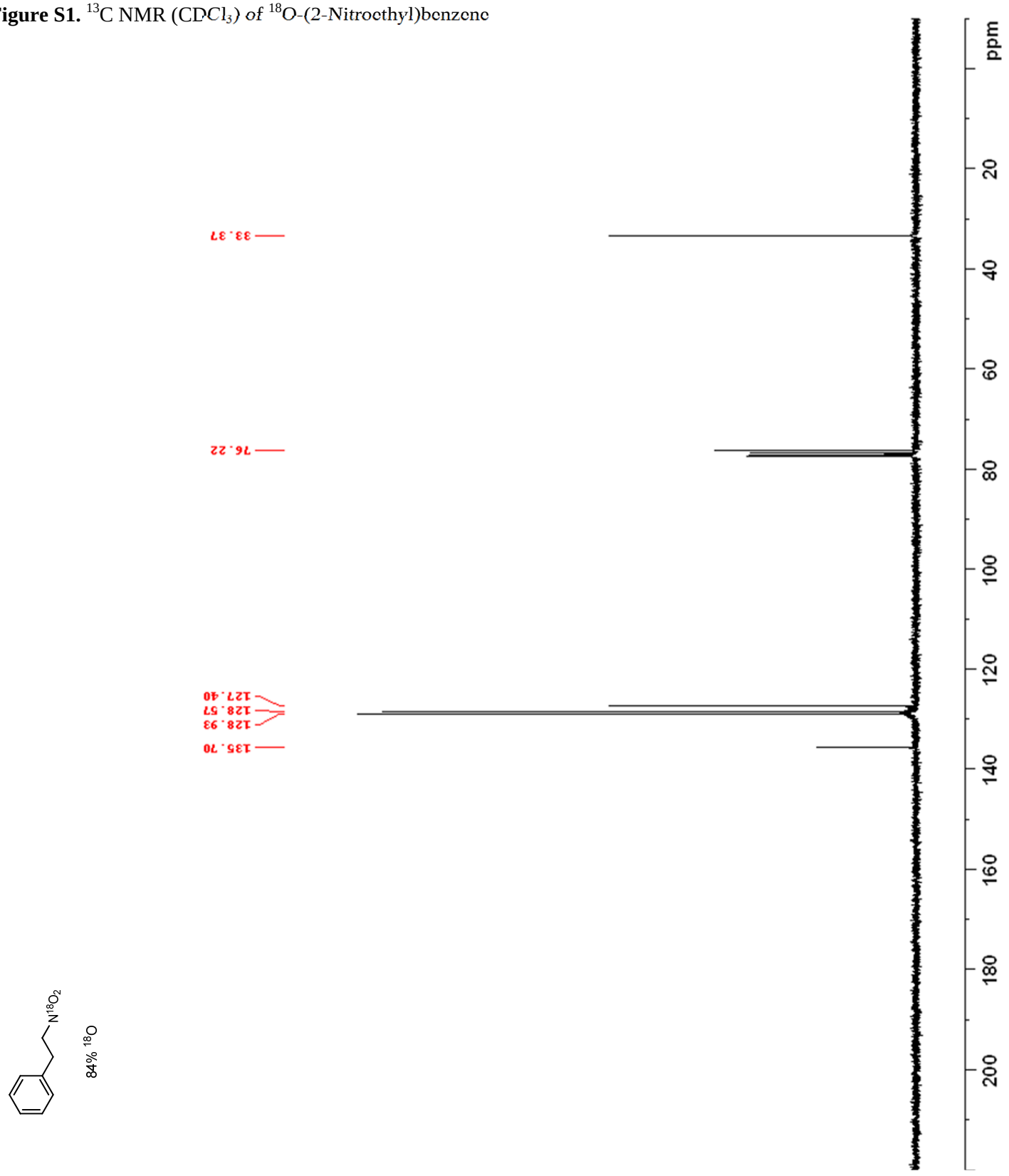

Figure S2. IR of ^{18}O-(2-Nitroethyl)benzene

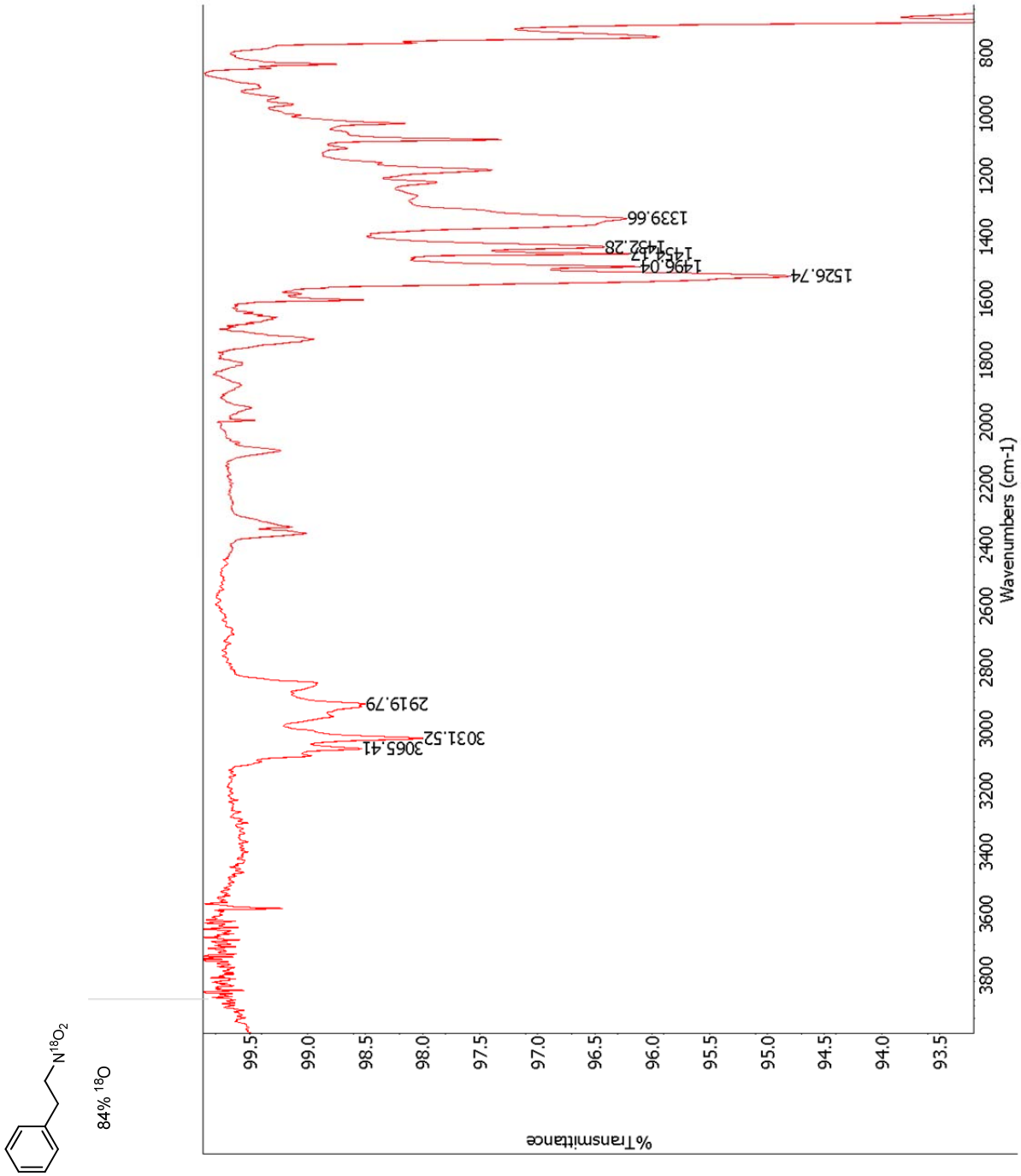

Figure S3. ^{13}C NMR ($CDCl_3$) of **^{18}O-Labeled 1**

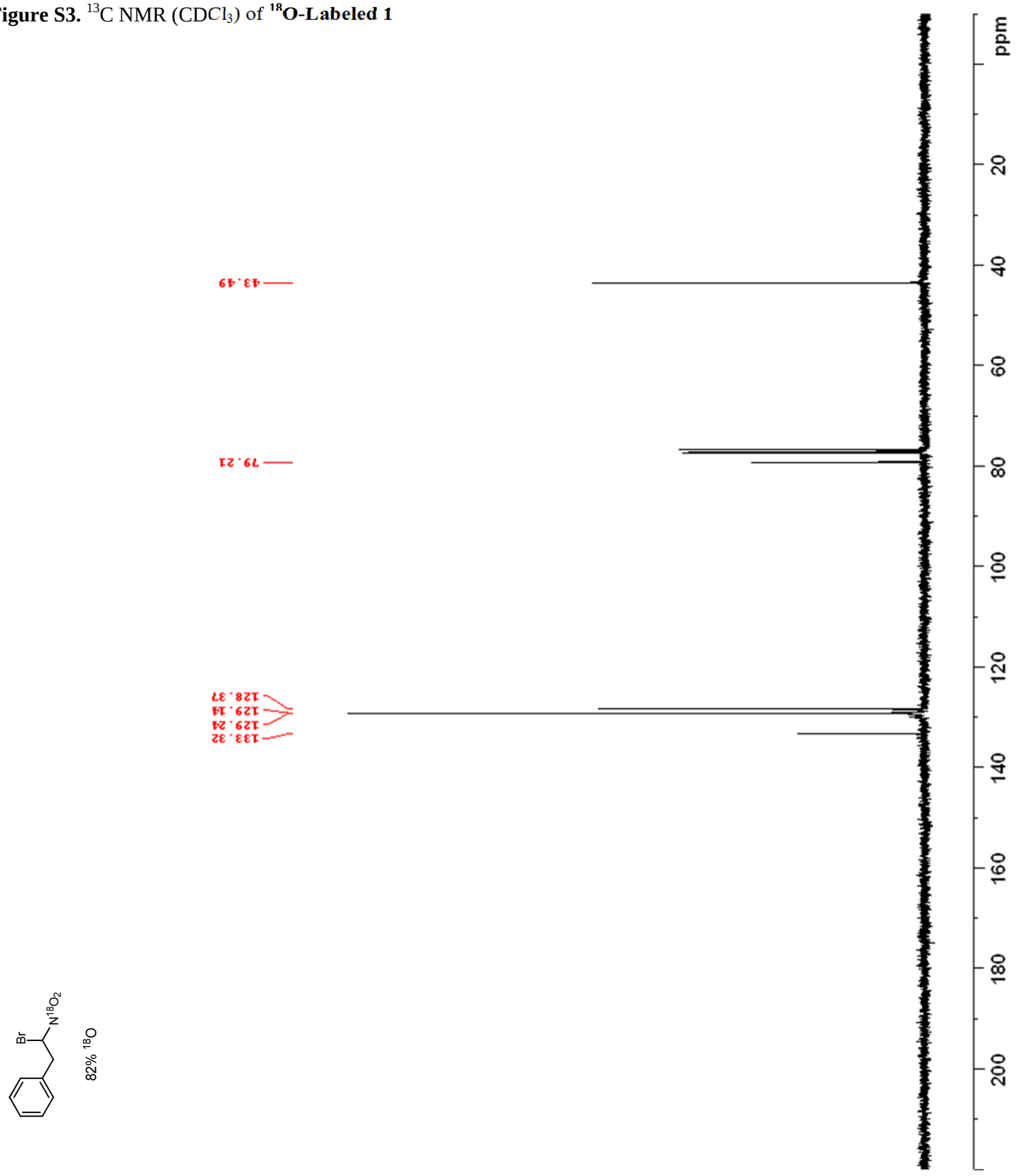

Figure S4. IR of ^{8}**O-Labeled 1**

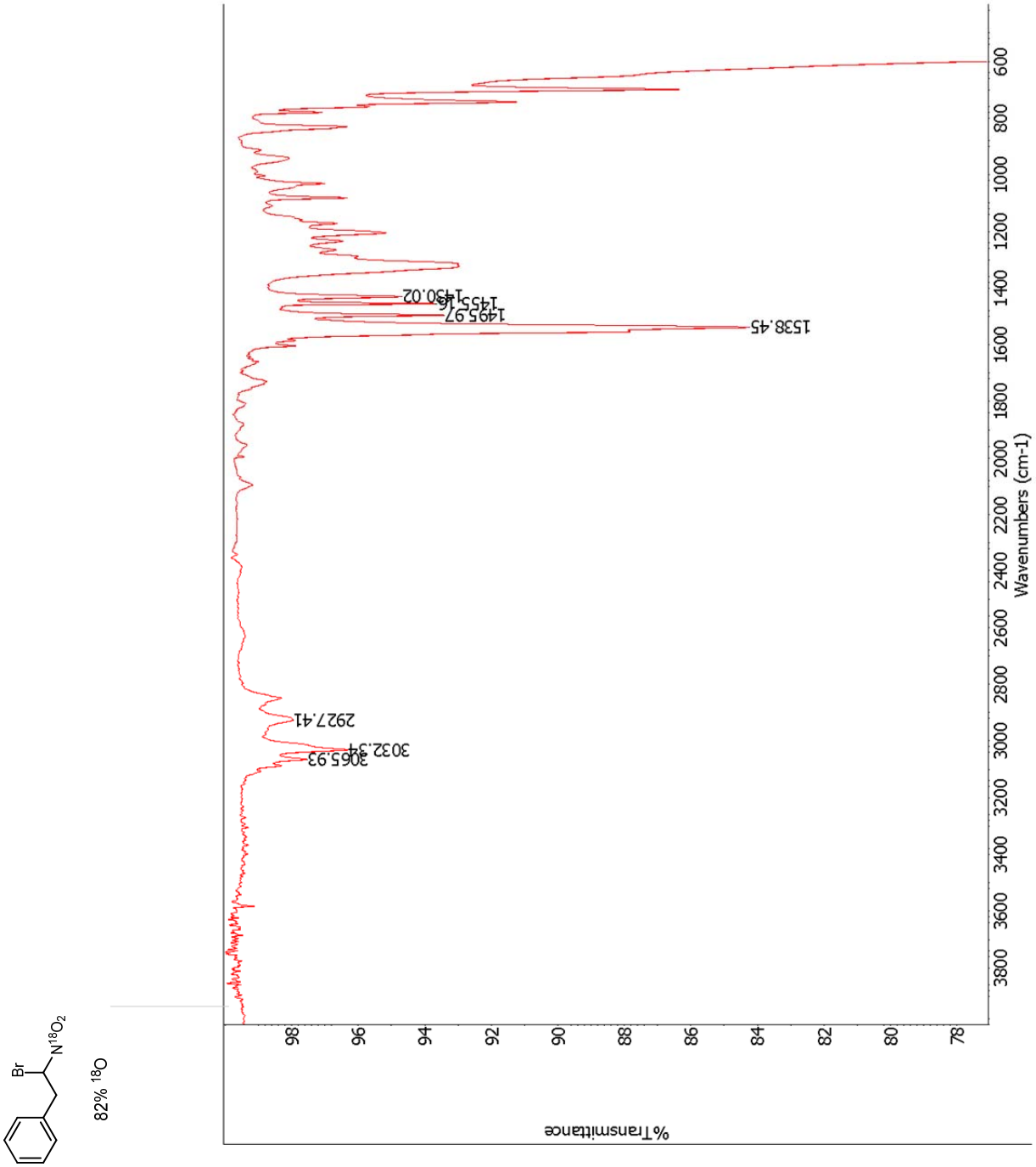

Figure S5. ^{13}C NMR ($CDCl_3$) of **4a, Table 1, Entry 1**

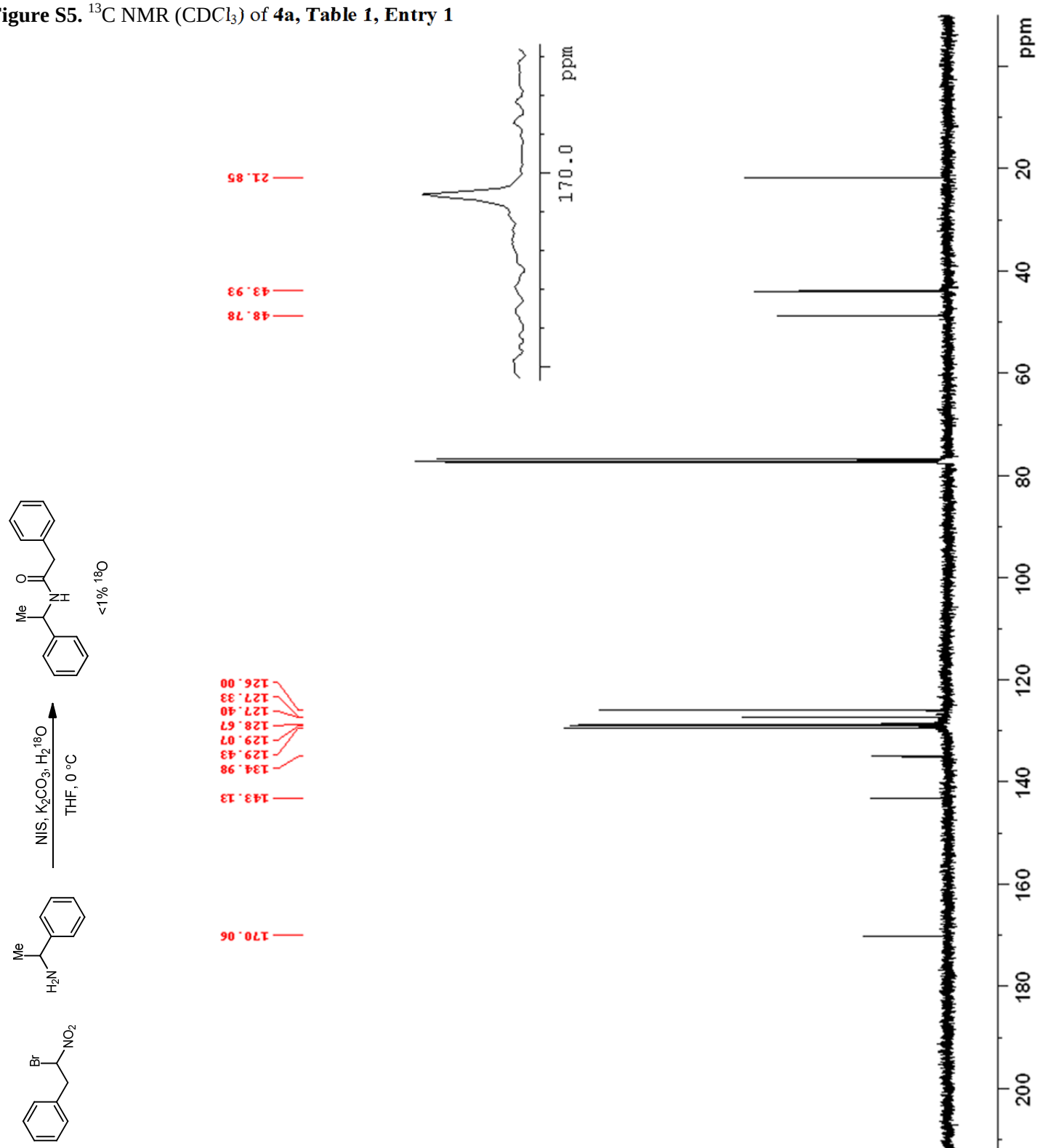

Figure S6. IR of **4a, Table 1, Entry 1**

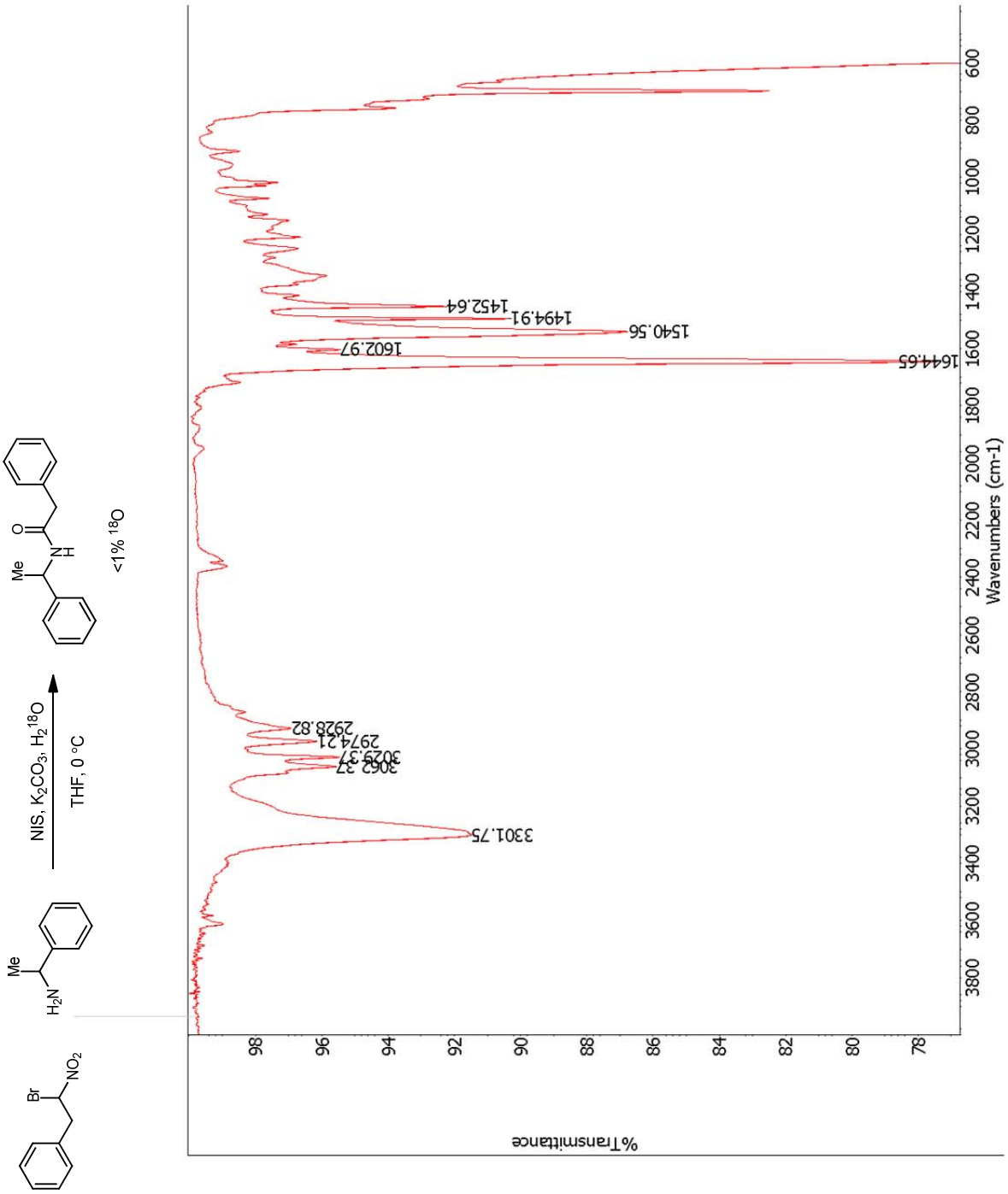

Figure S7. ^{13}C NMR ($CDCl_3$) of **4a, Table 1, Entry 2**

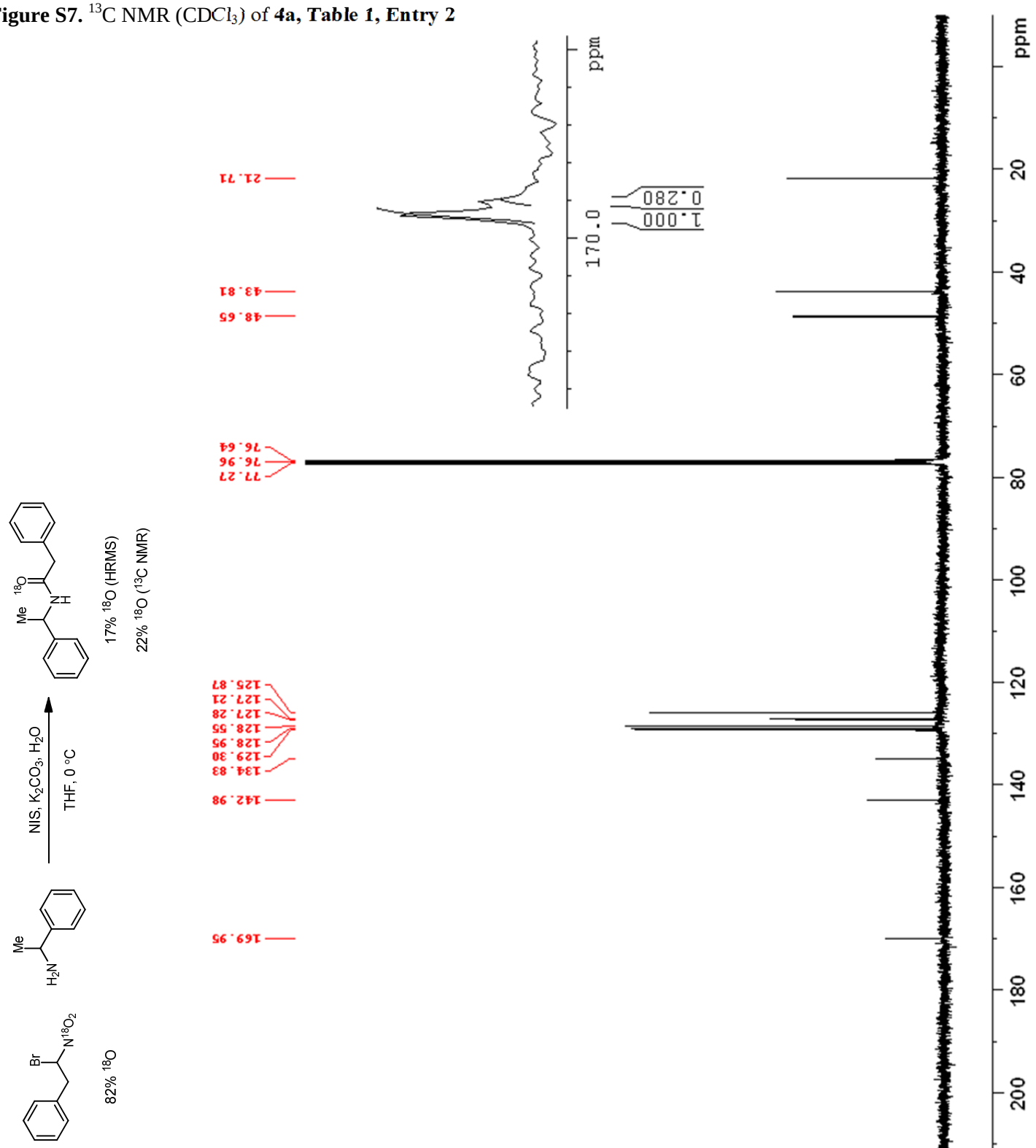

Figure S8. IR of **4a, Table 1, Entry 2**

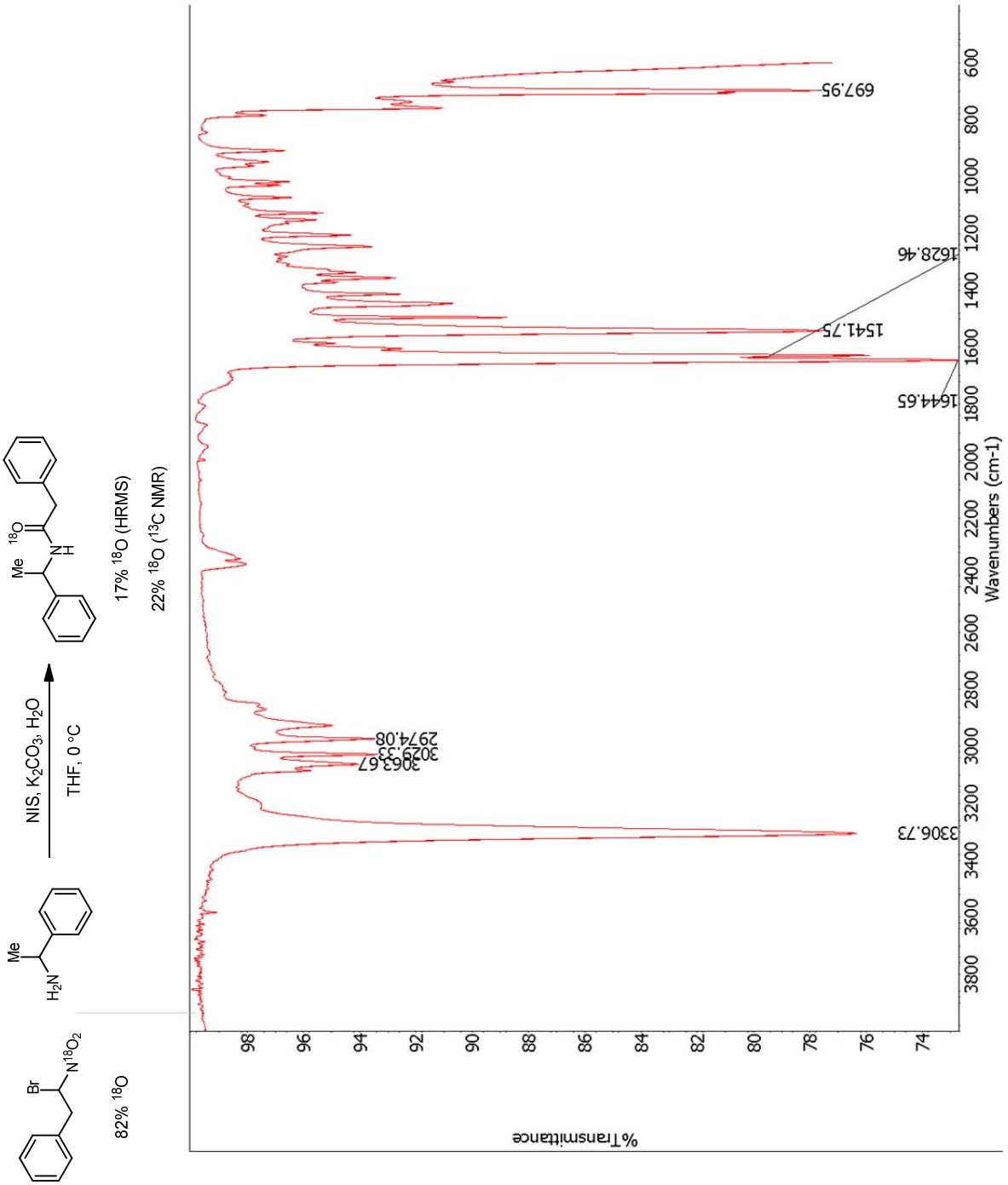

Figure S9. ^{13}C NMR ($CDCl_3$) of **4a, Table 1, Entry 3**

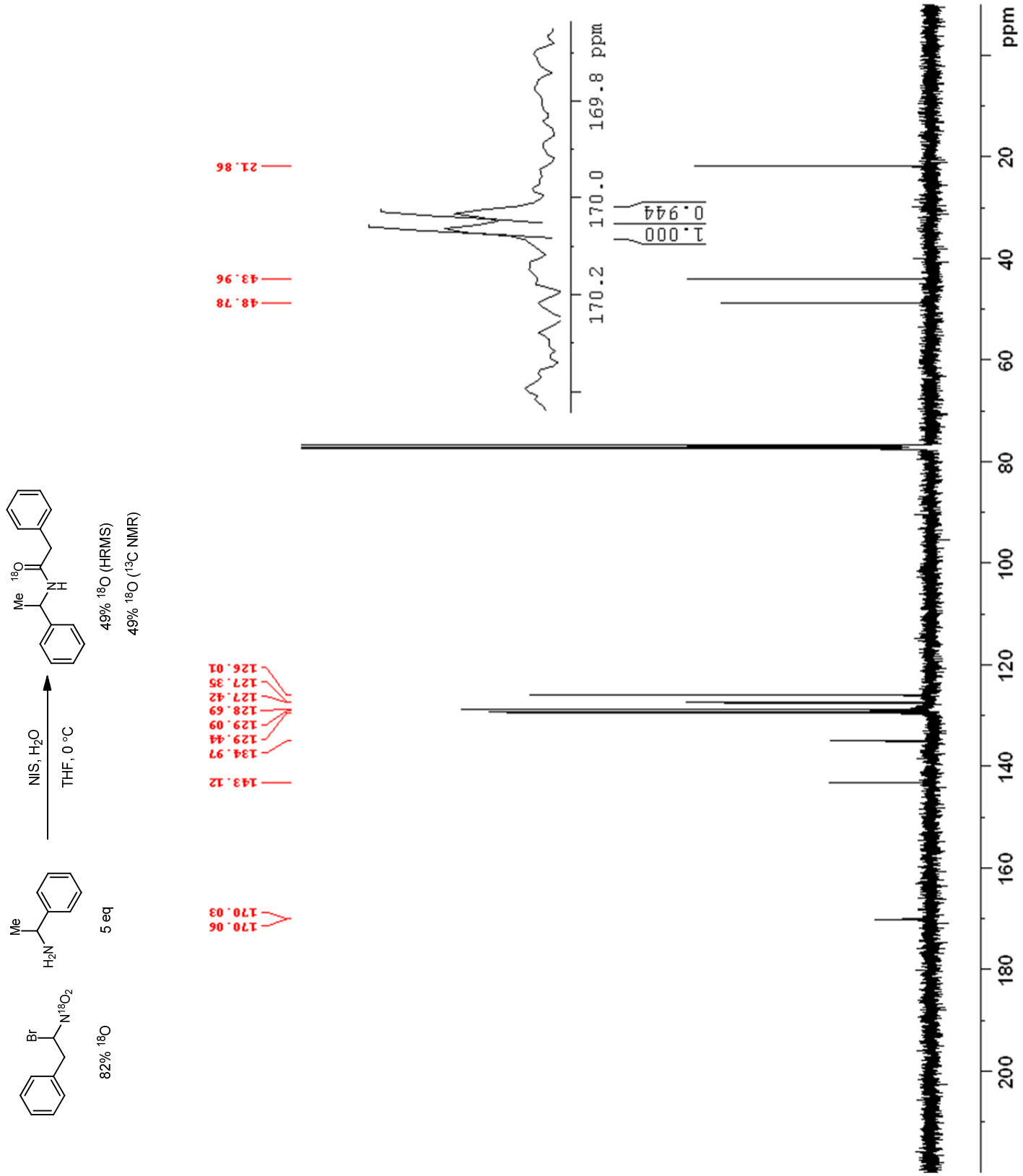

Figure S10. IR of **4a, Table 1, Entry 3**

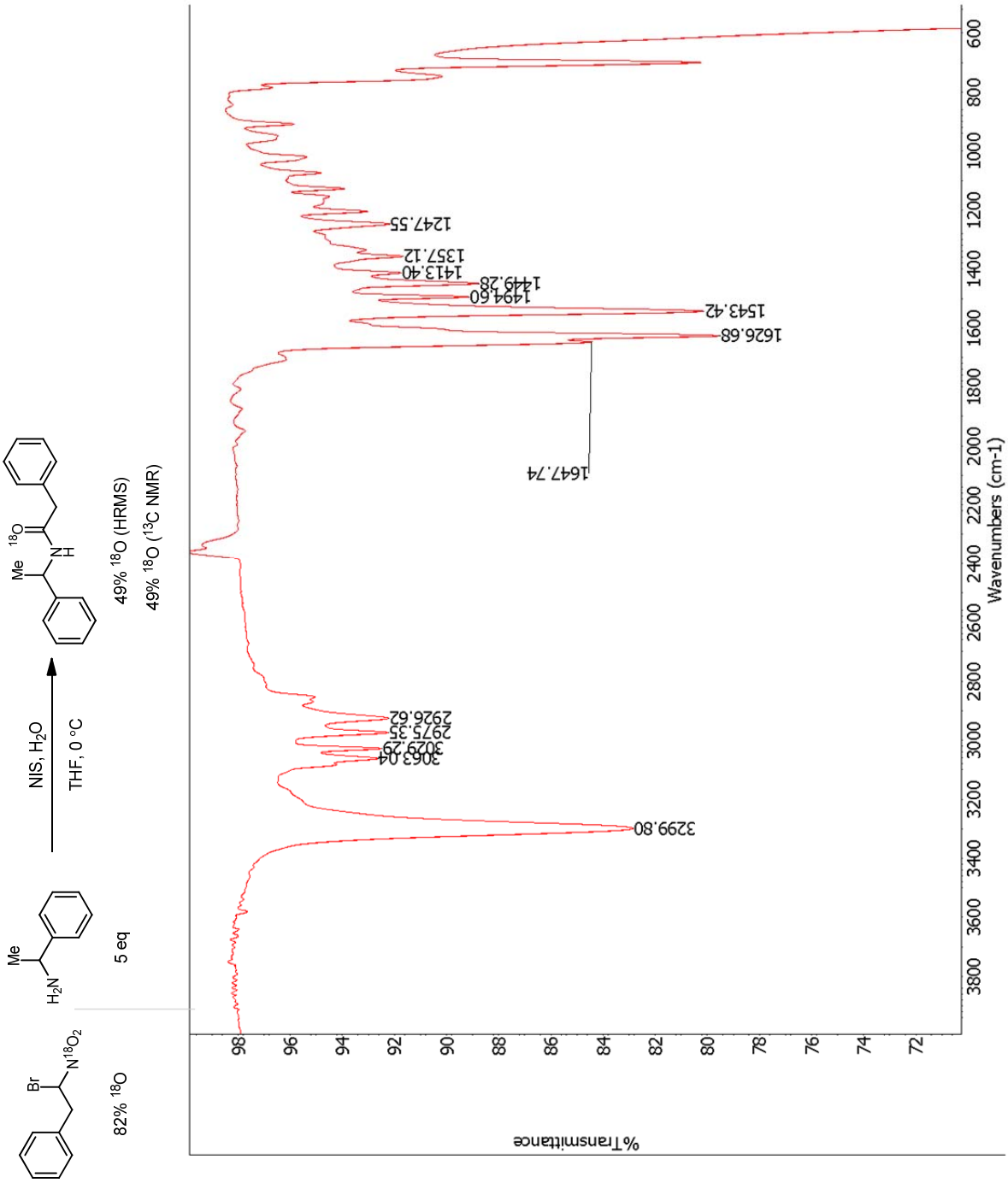

Figure S11. ^{13}C NMR ($CDCl_3$) of **4a, Table 1, Entry 4**

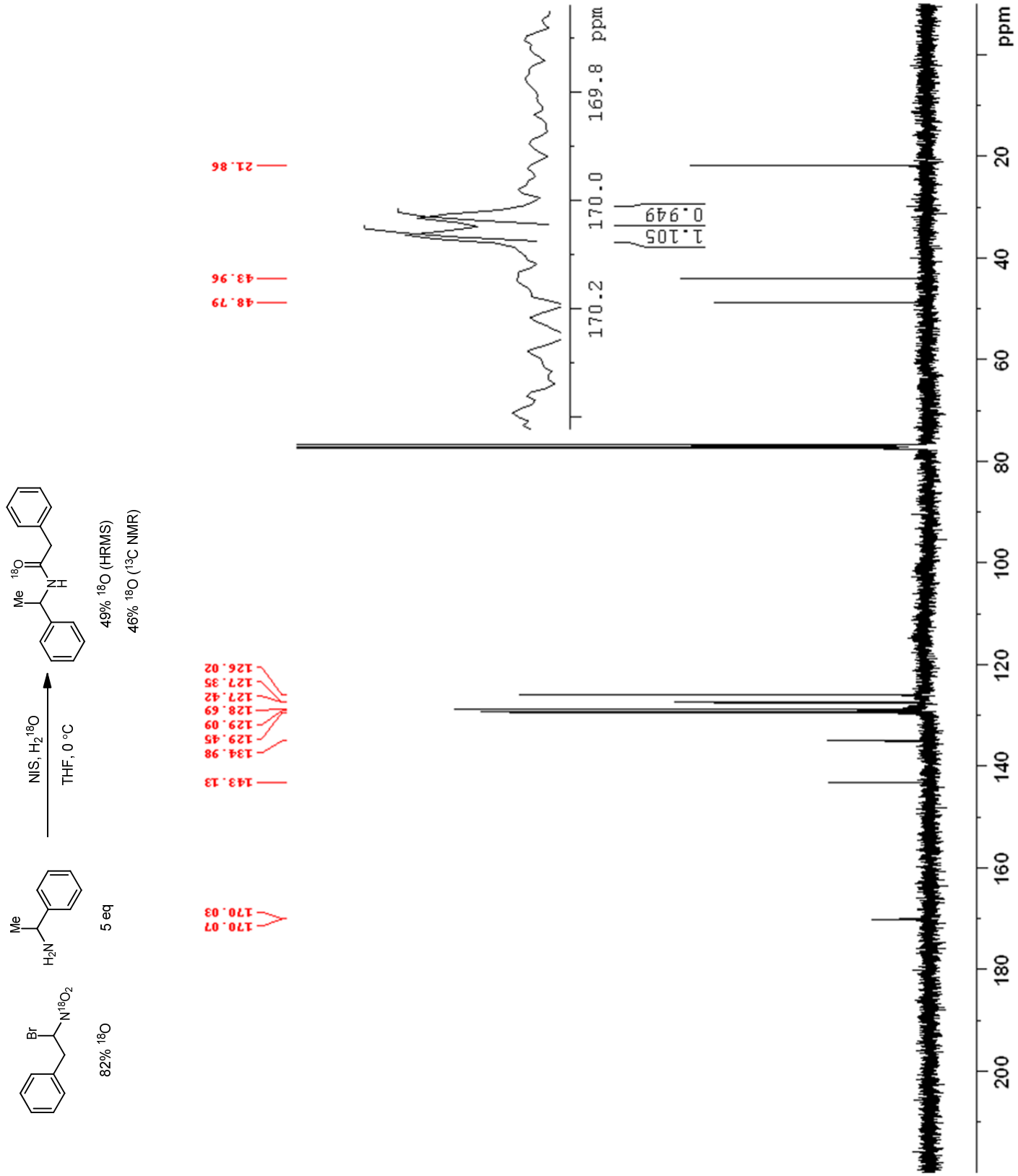

Figure S12. IR of **4a, Table 1, Entry 4**

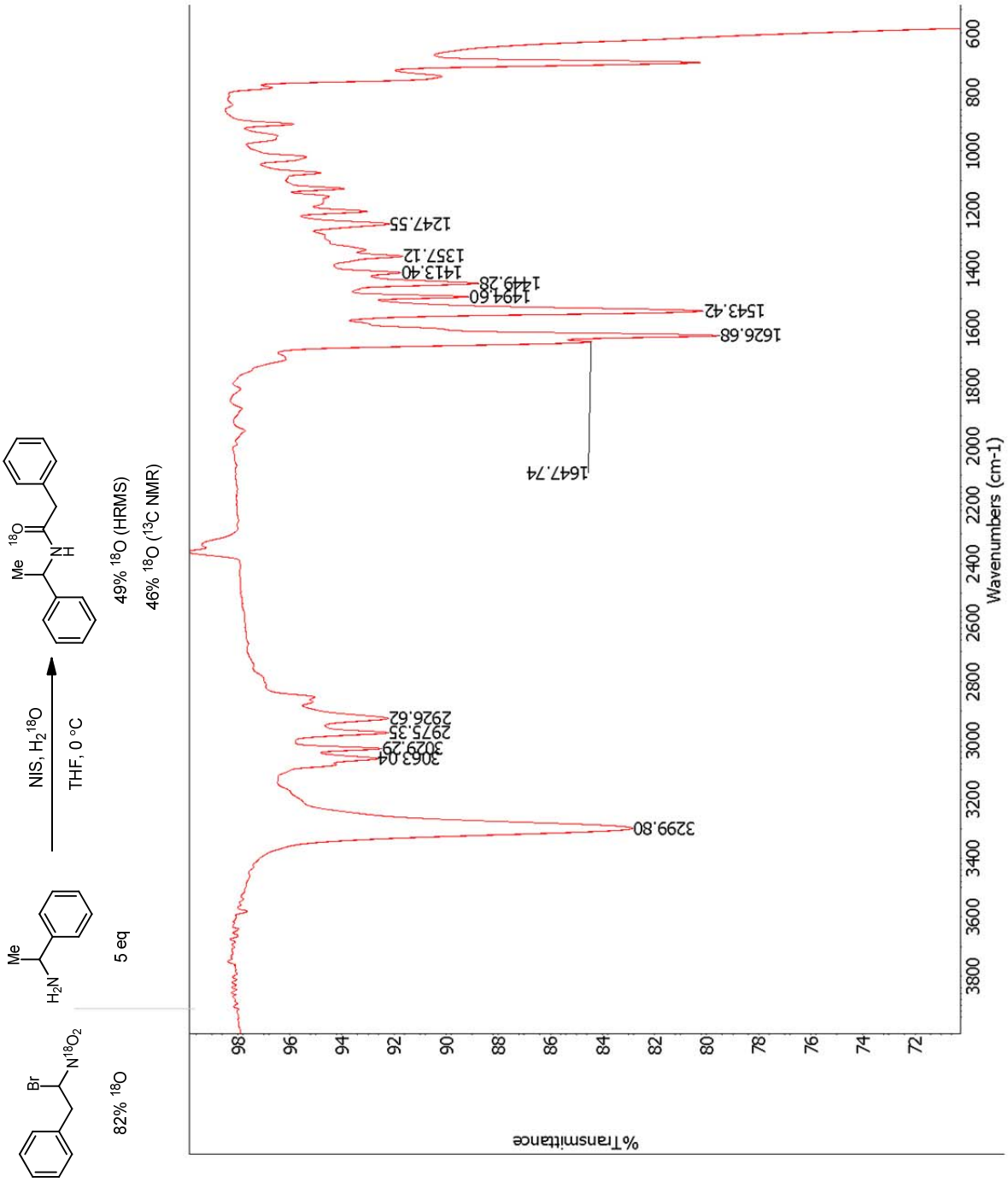

Figure S13. ^{13}C NMR ($CDCl_3$) of **4a, Table 1, Entry 5**

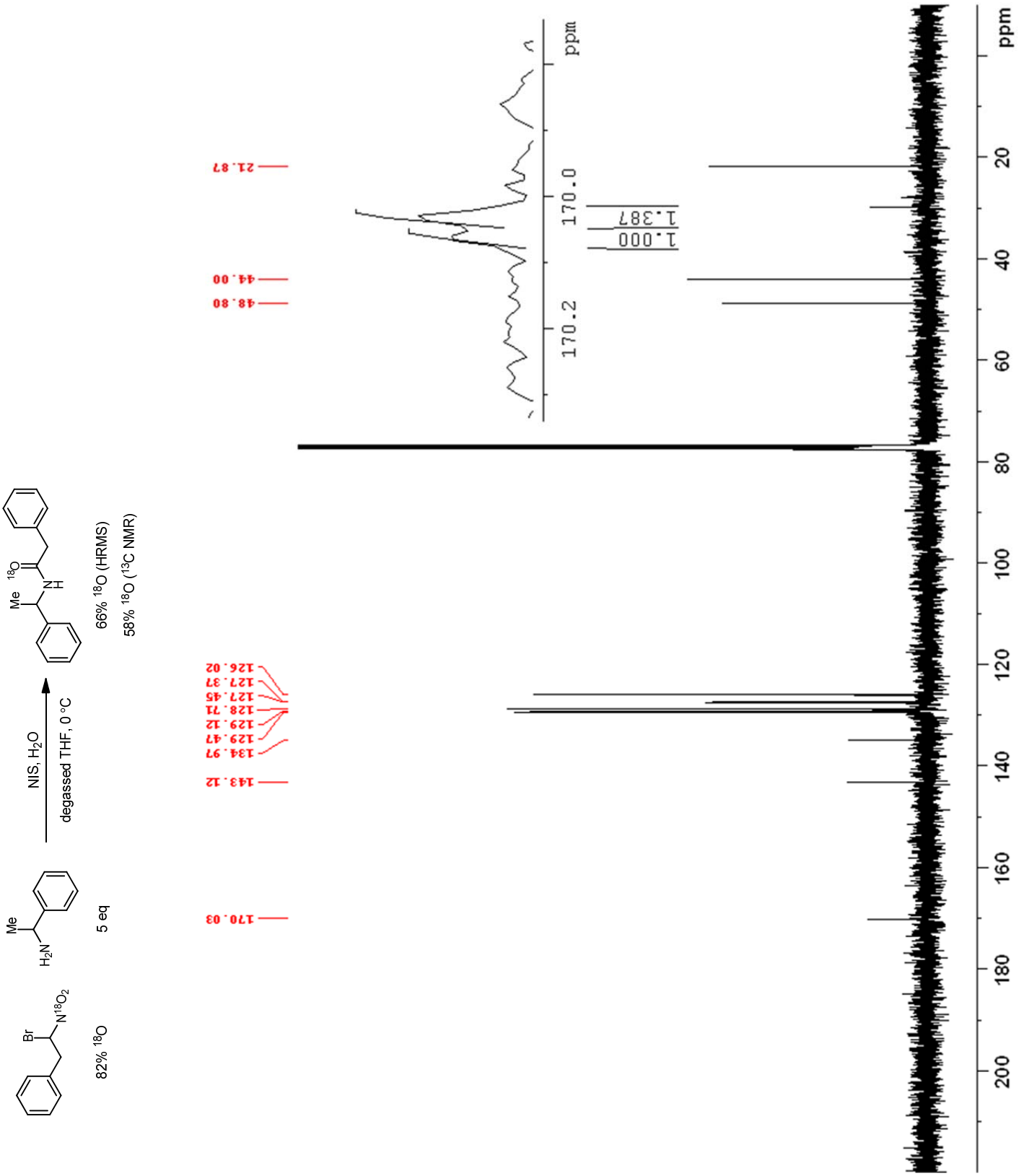

Figure S14. IR of **4a, Table 1, Entry 5**

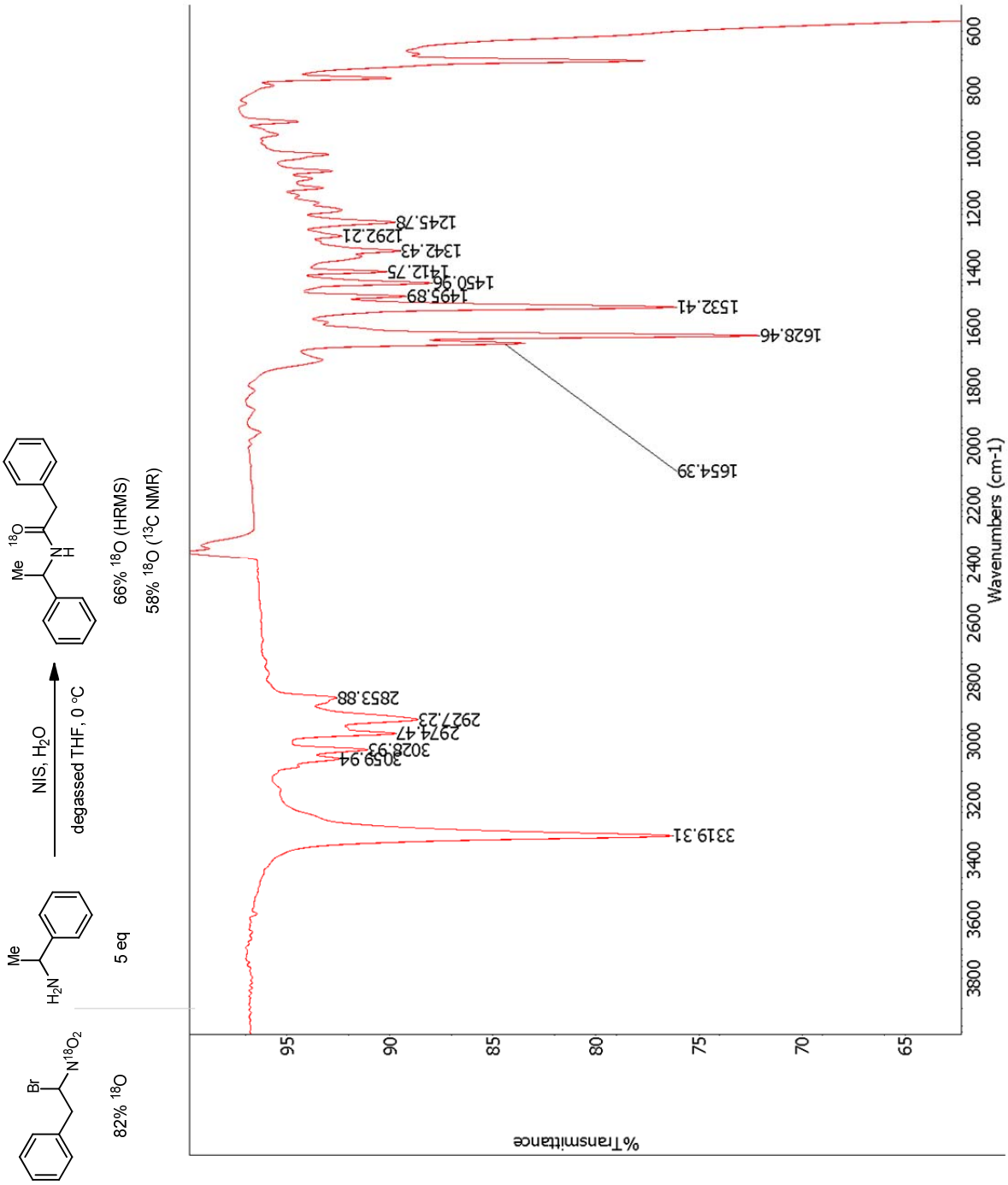

Figure S15. ^{13}C NMR ($CDCl_3$) of **4a, Table 1, Entry 6**

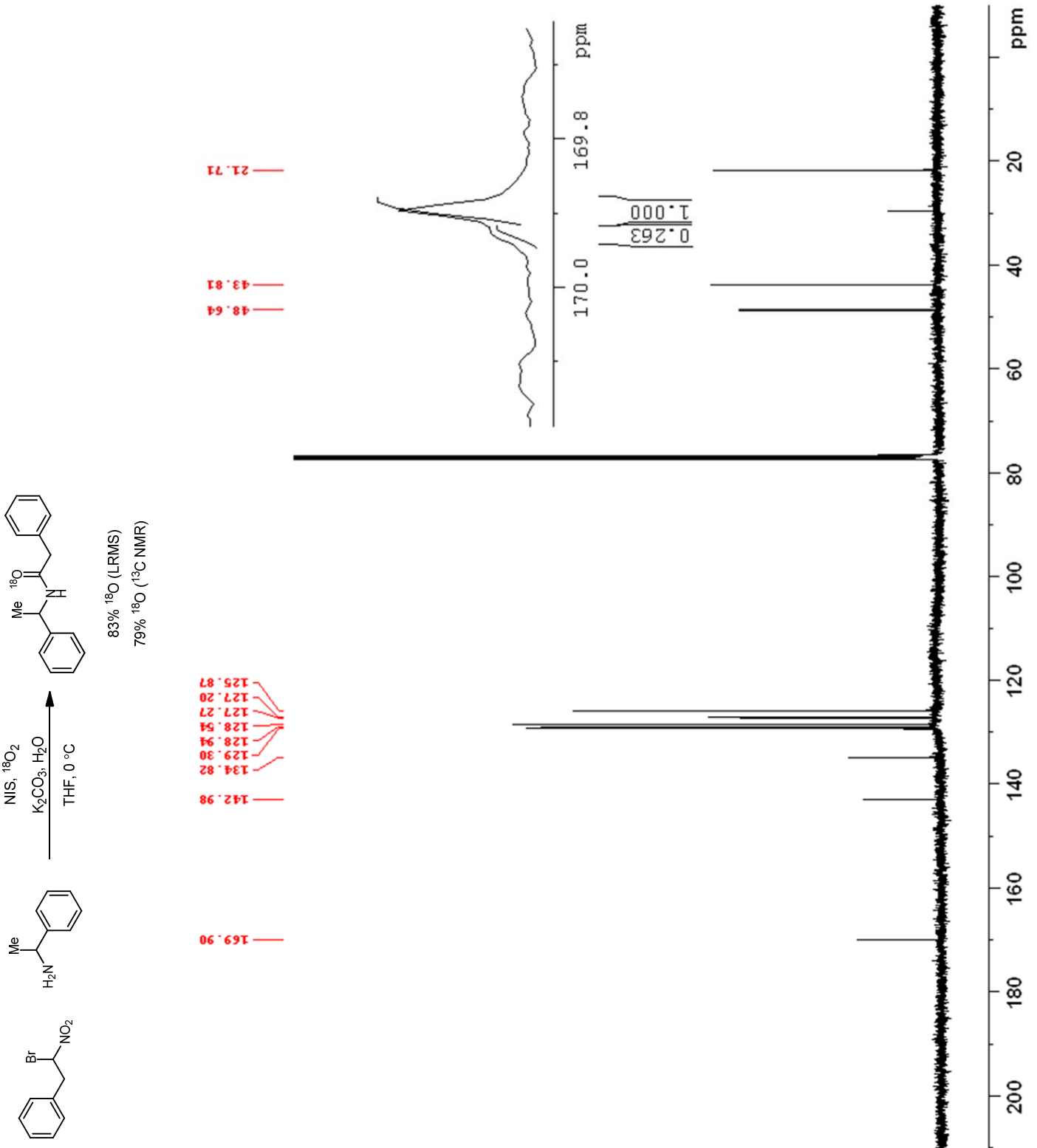

Figure S16. IR of **4a, Table 1, Entry 6**

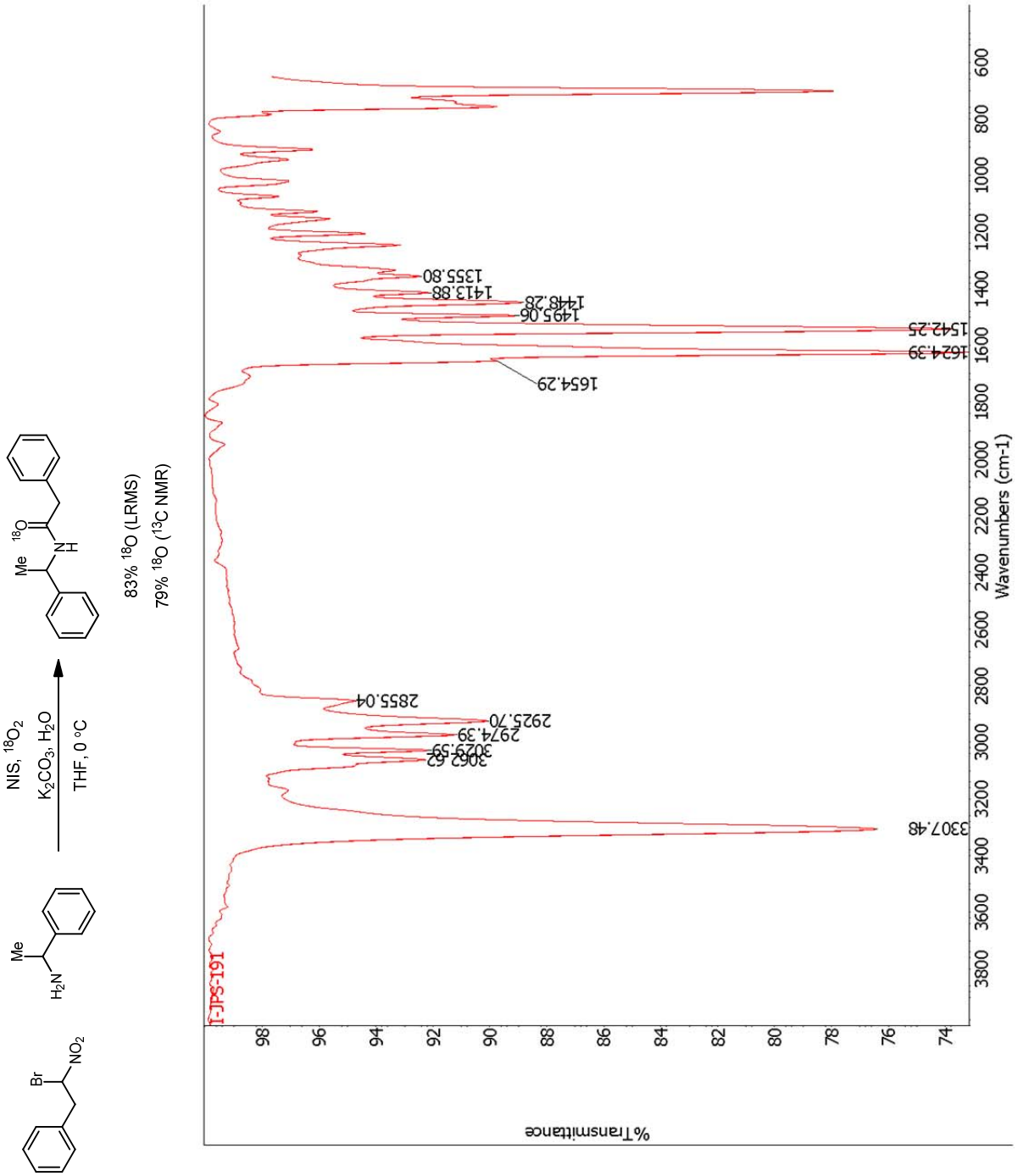

Figure S17. ^{13}C NMR ($CDCl_3$) of **11a**

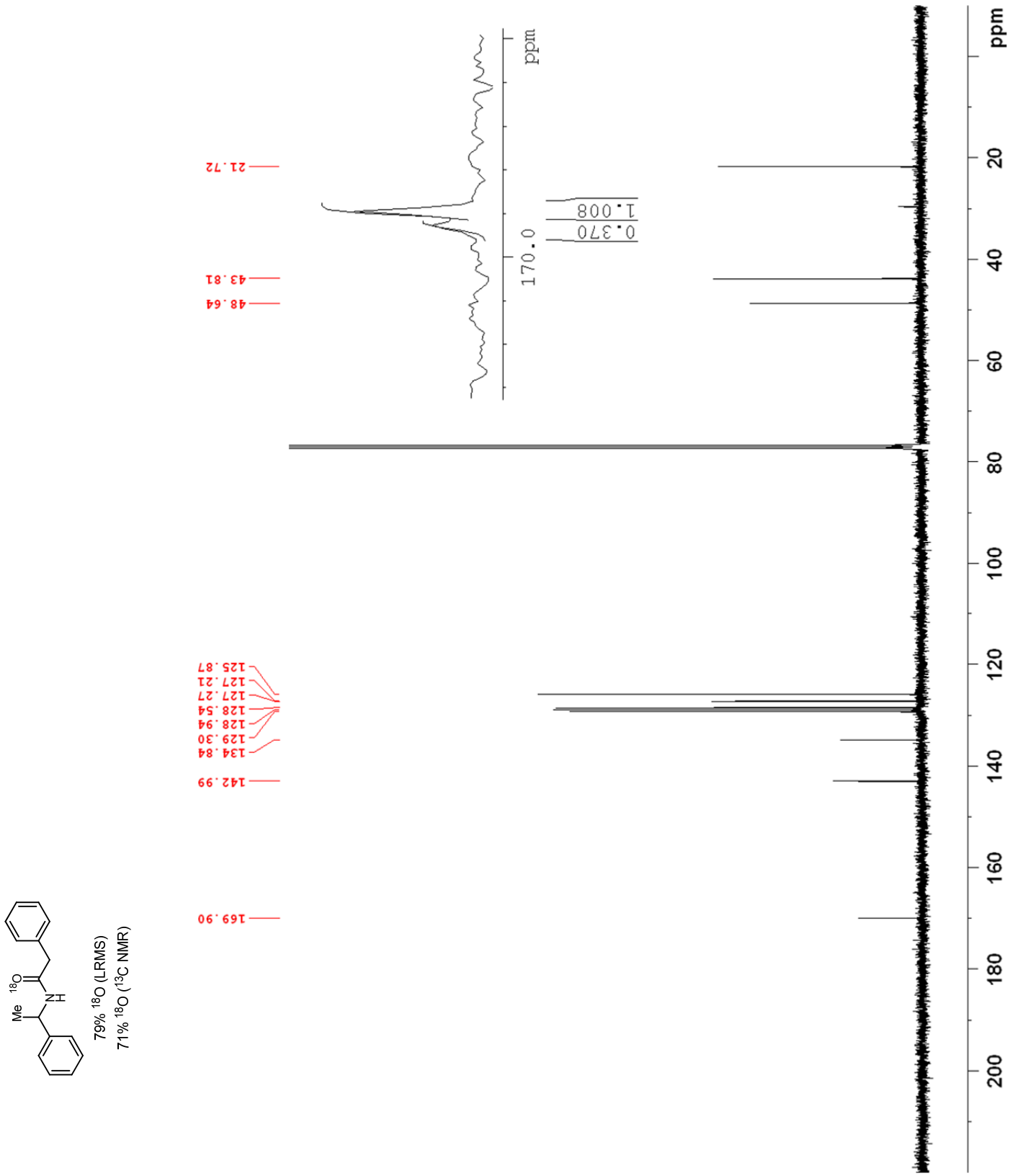

Figure S18. IR of **11a** compared to its non-^{18}O-labeled form

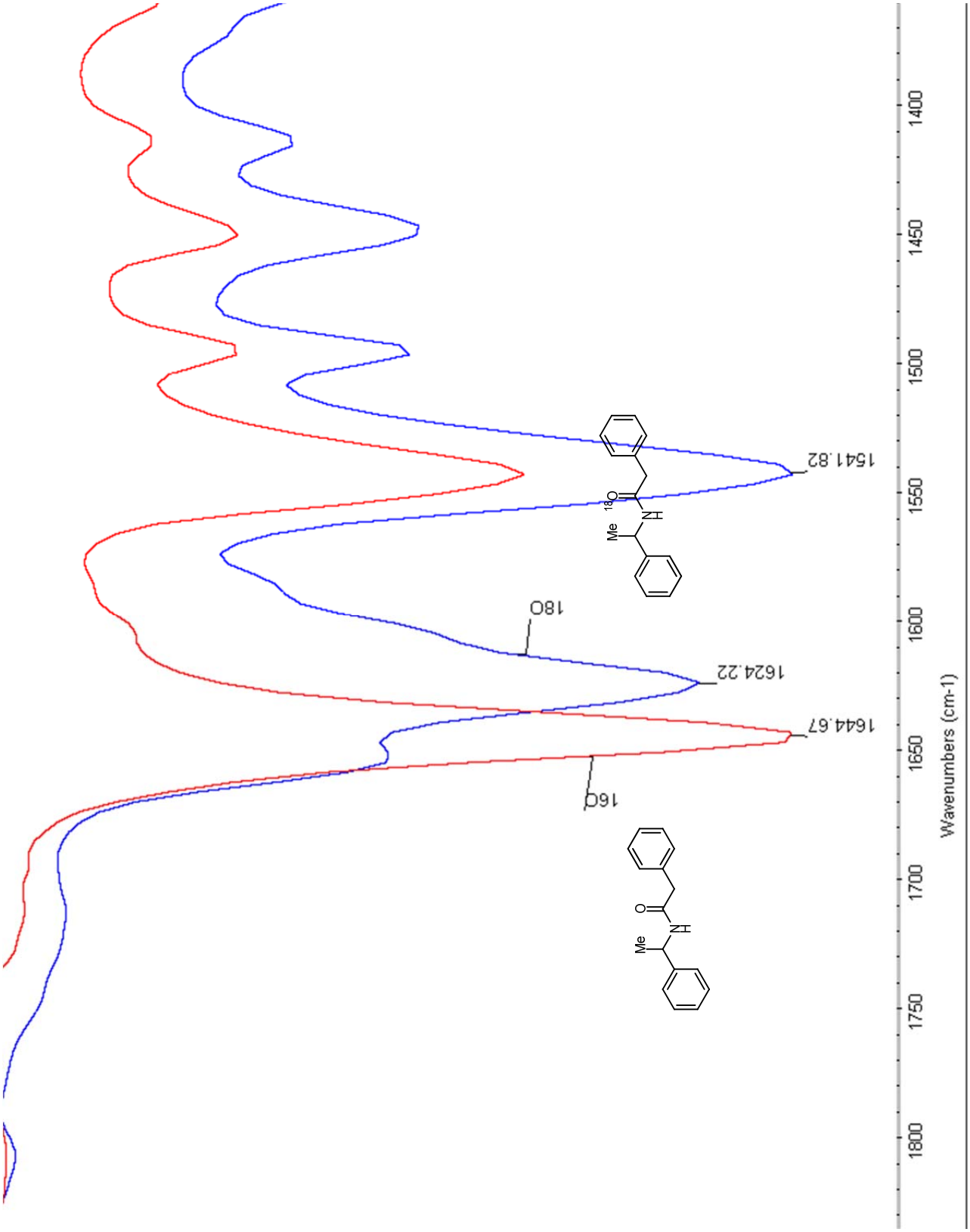

Figure S19. ^{13}C NMR ($CDCl_3$) of **11b**

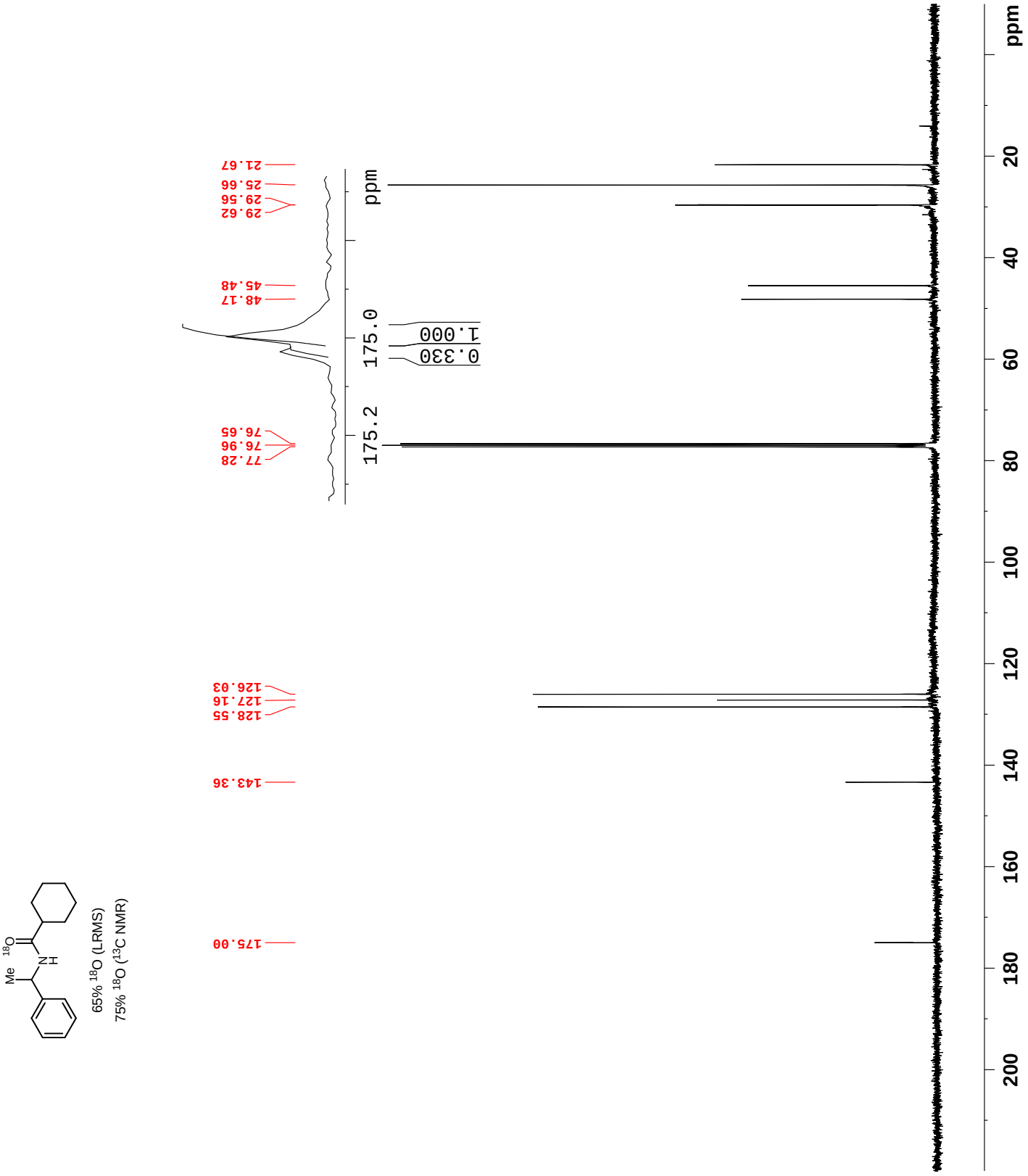

Figure S20. IR of **11b** compared to its non-^{18}O-labeled form

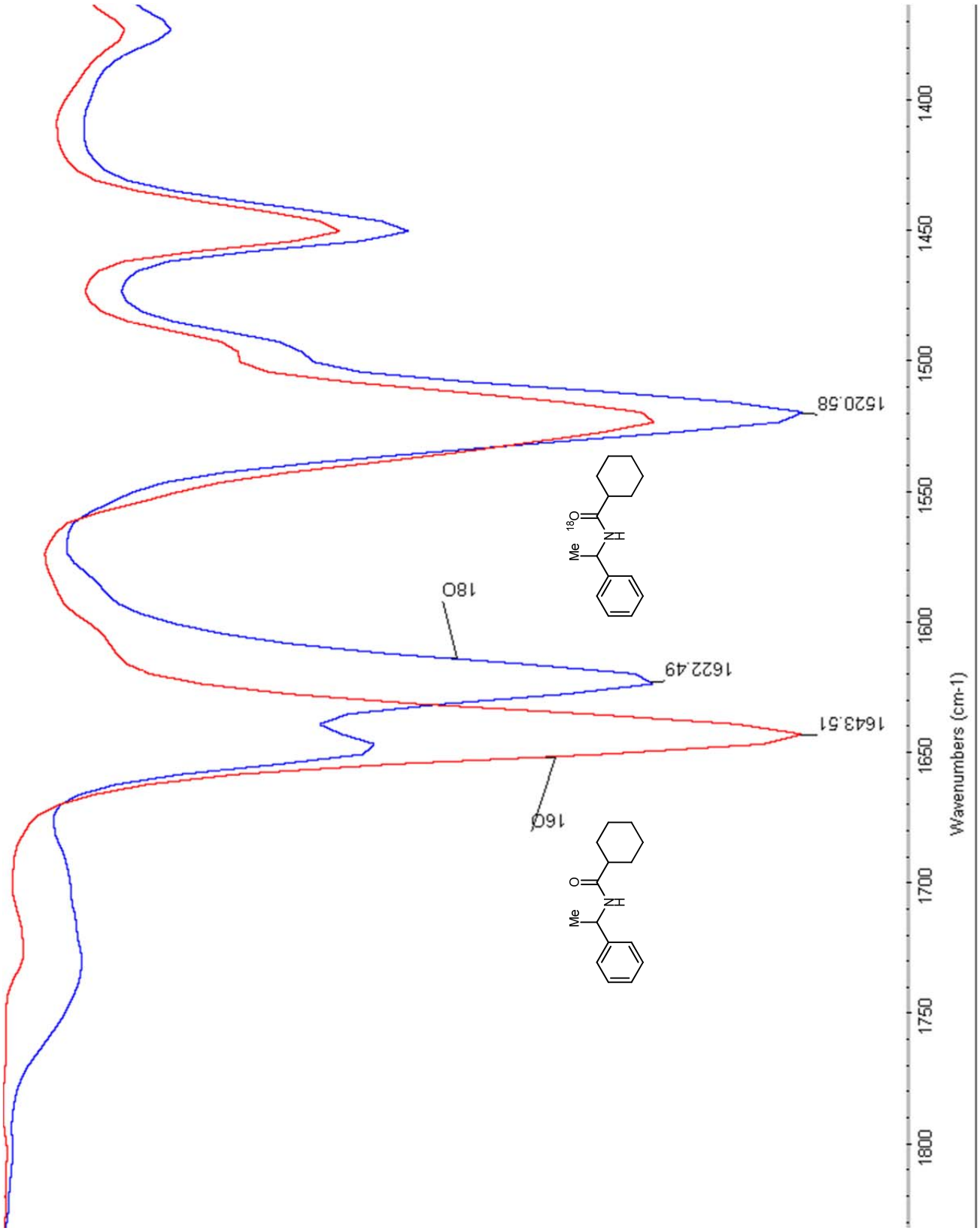

Figure S21. ^{13}C NMR ($CDCl_3$) of **11c**

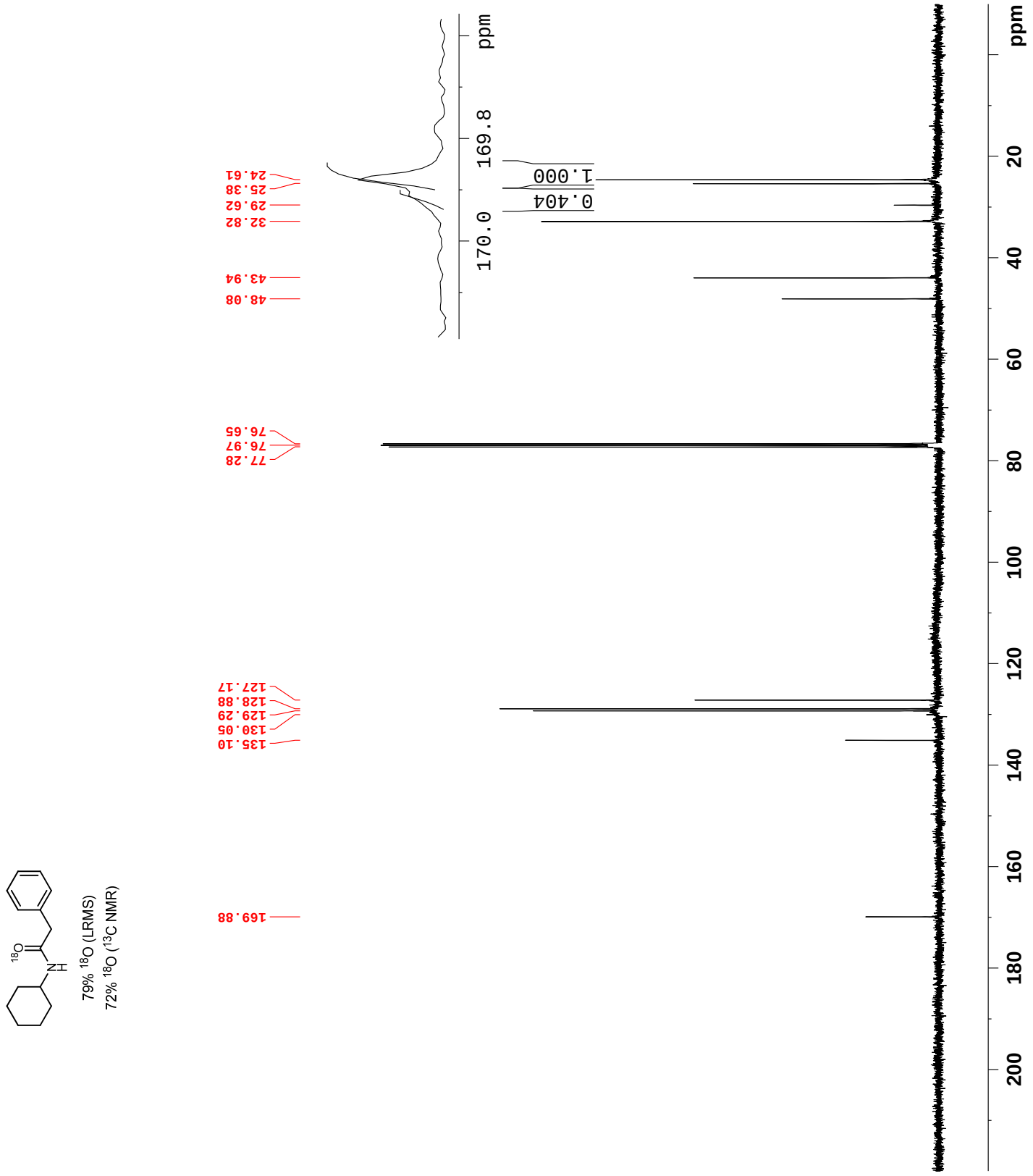

Figure S22. IR of **11c** compared to its non-^{18}O-labeled form

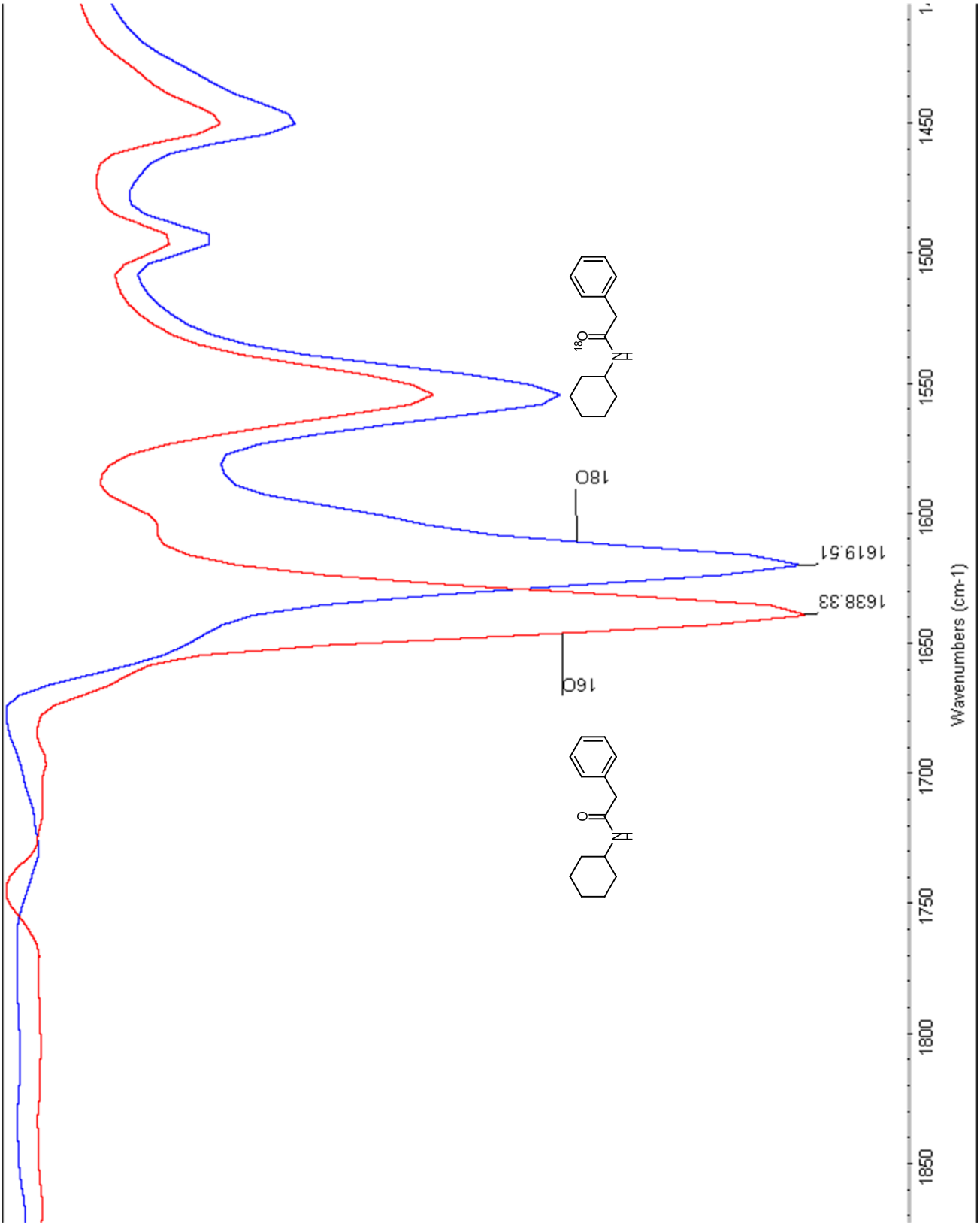

Figure S23. ^{13}C NMR ($CDCl_3$) of **11d**

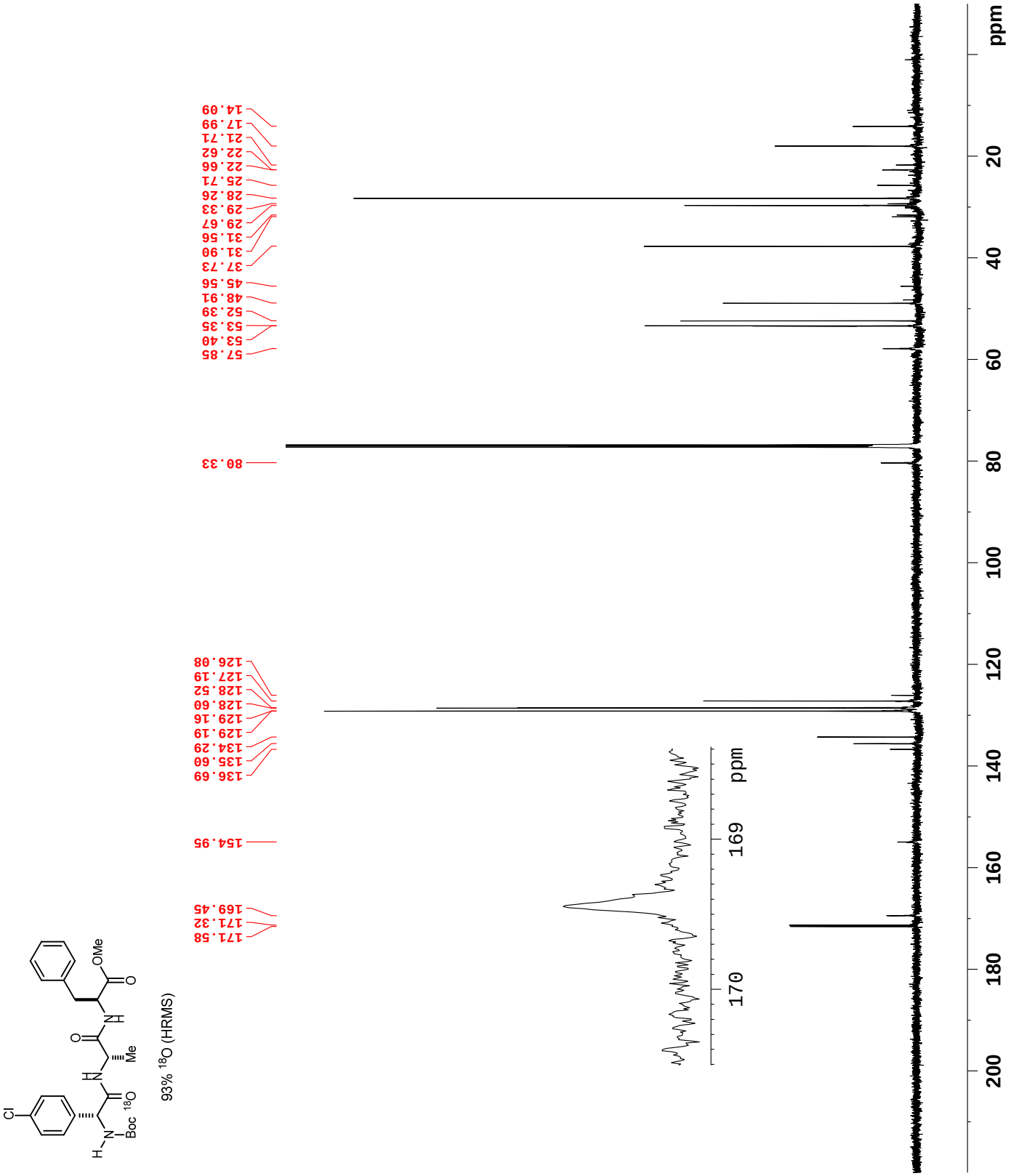

Figure S24.HSQC NMR ($CDCl_3$) of **11d**

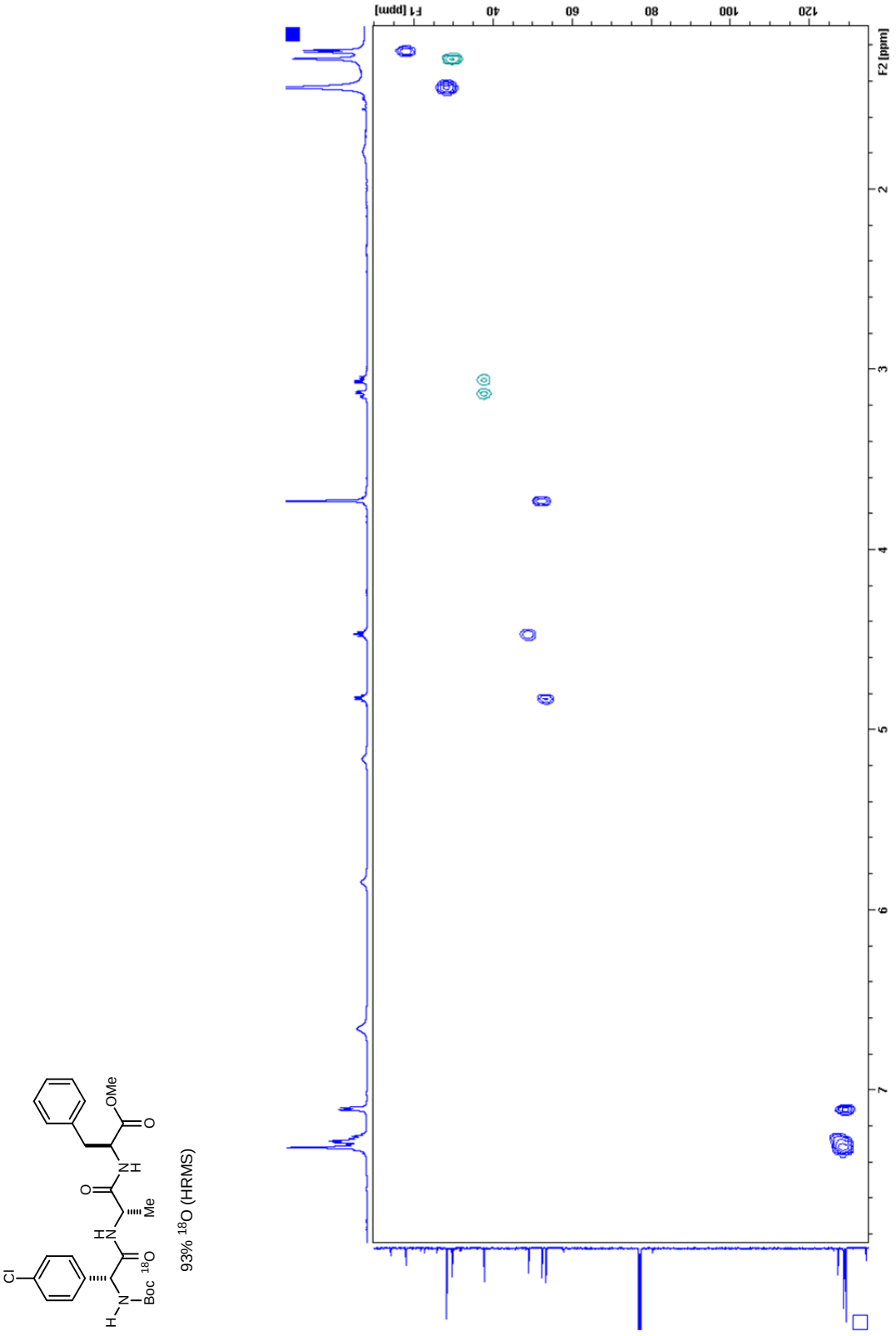

Figure S25.HMBC NMR ($CDCl_3$) of **11d**

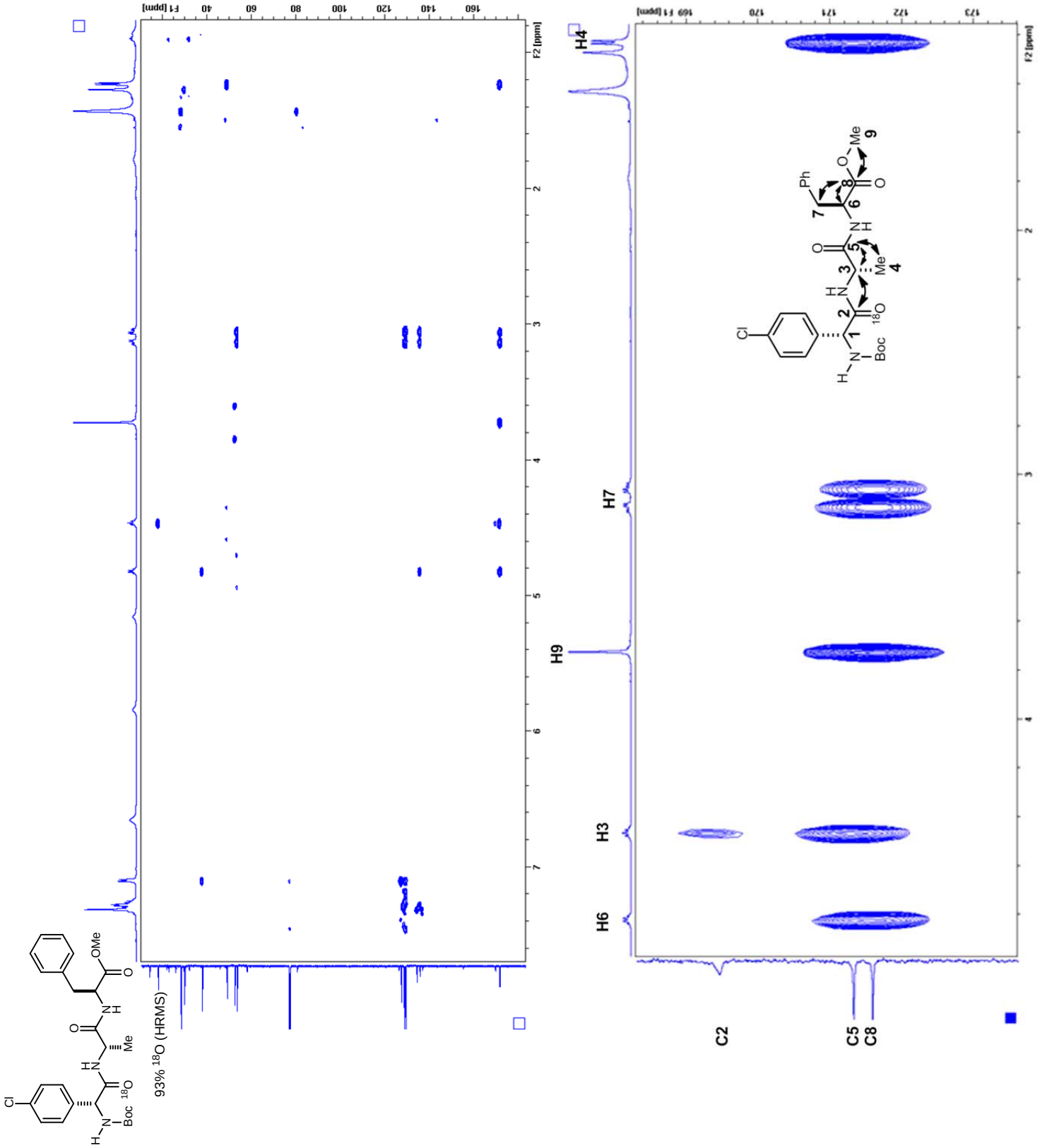

Figure S26. IR of **11d** compared to its non-^{18}O-labeled form

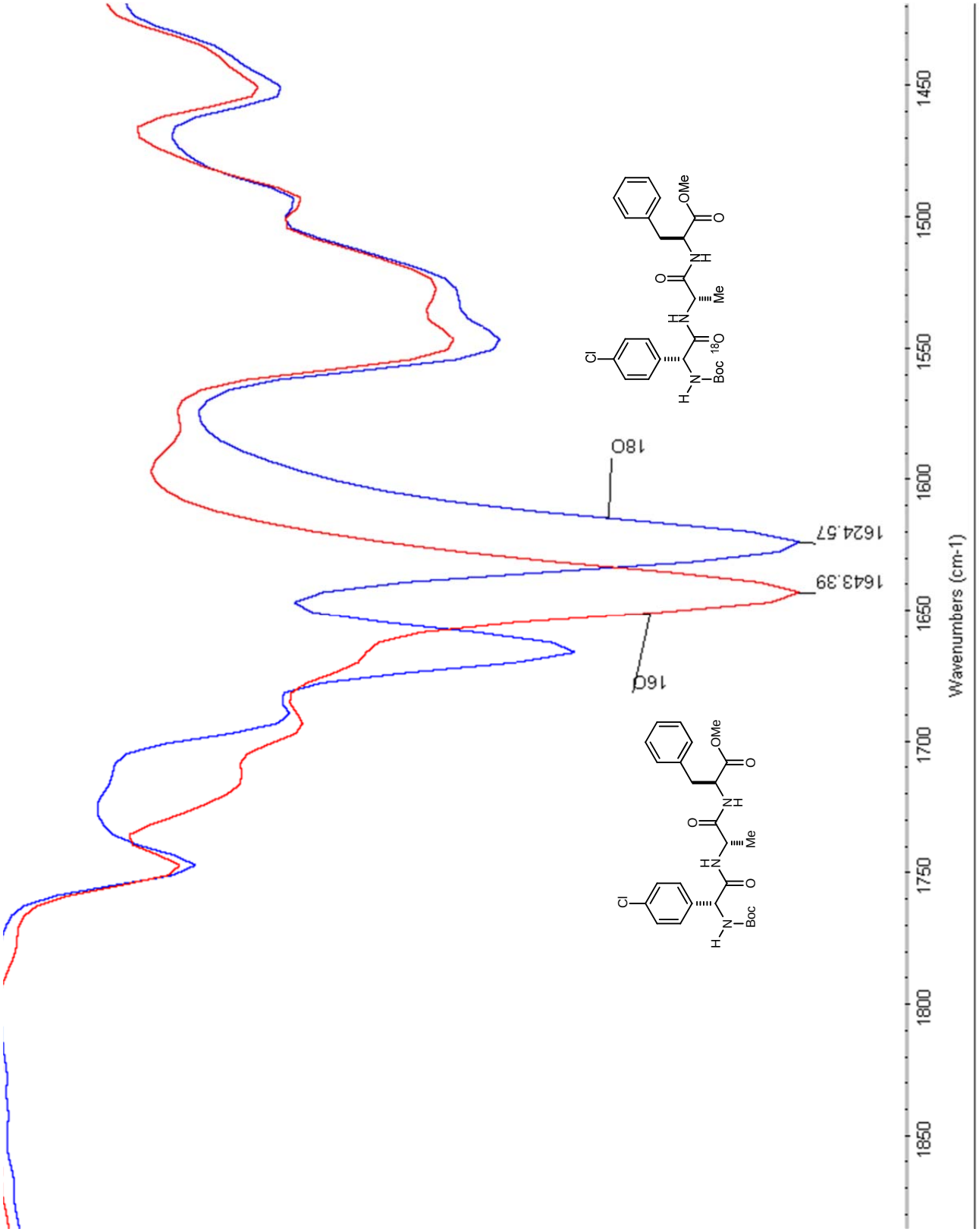

Figure S27. ^{13}C NMR ($CDCl_3$) Comparison of **11d** at various levels of ^{18}O Enrichment

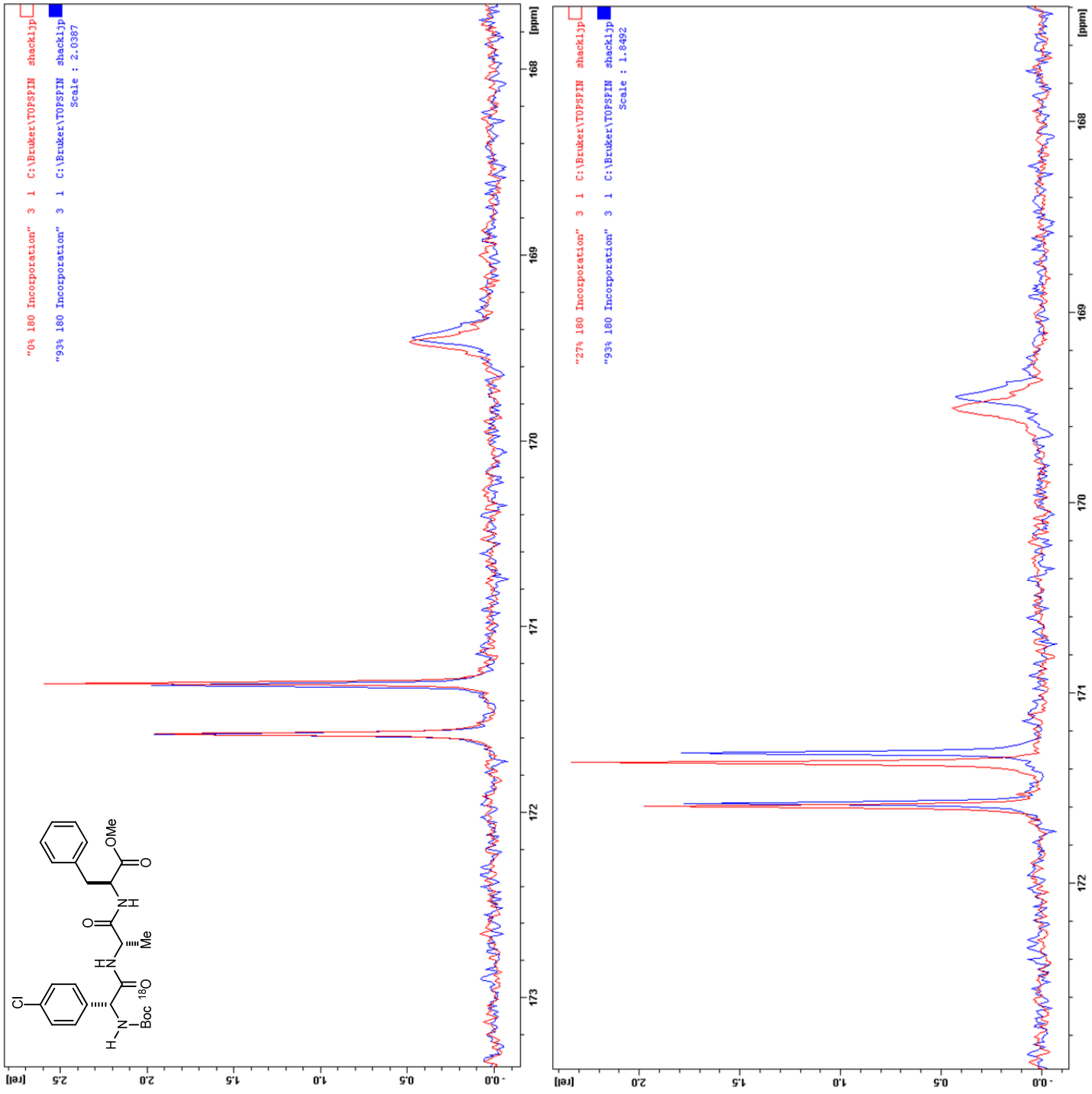

Figure S28. ^{1}H NMR ($CDCl_3$) of **11e**

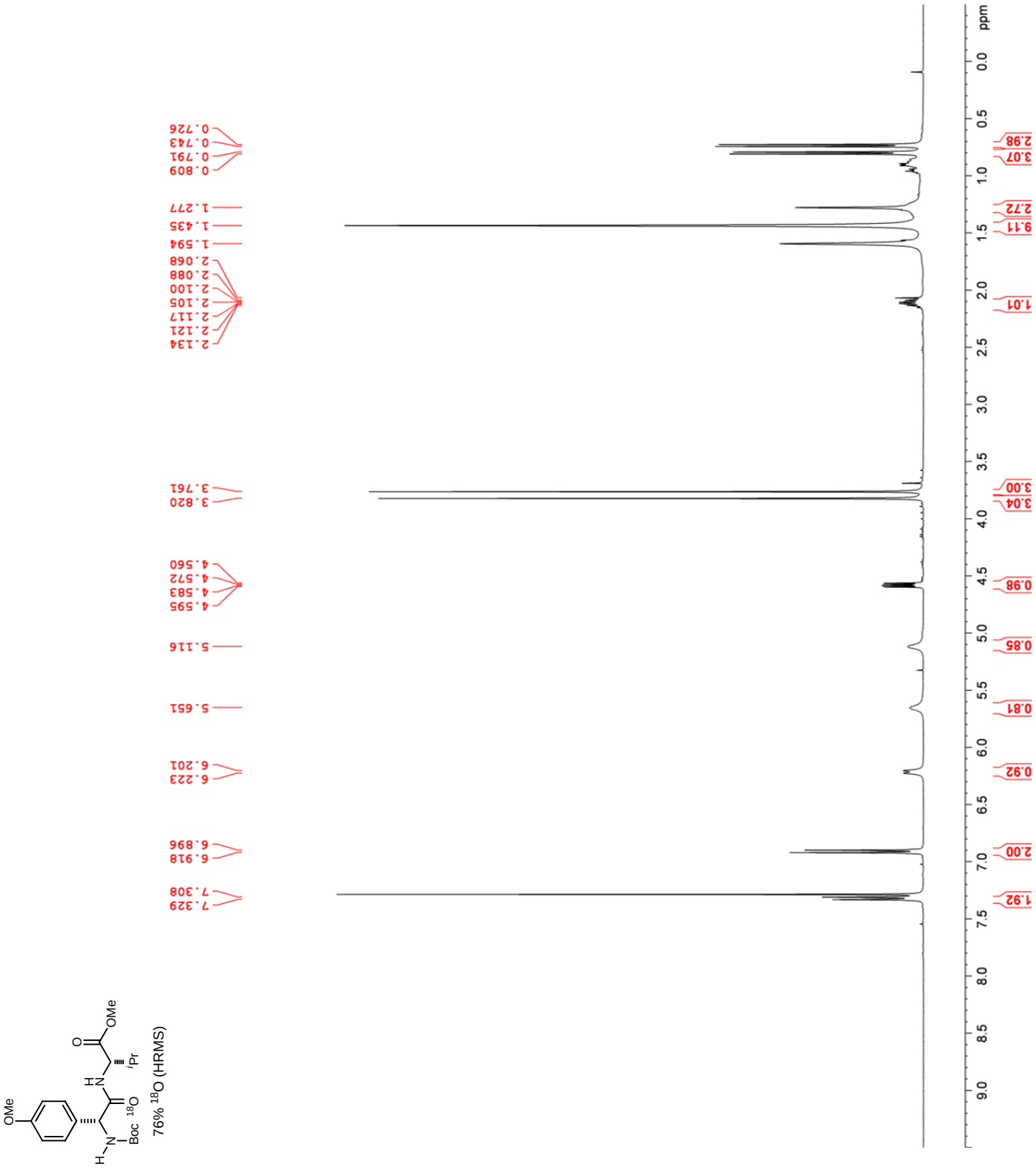

Figure S29. ^{13}C NMR ($CDCl_3$) of **11e**

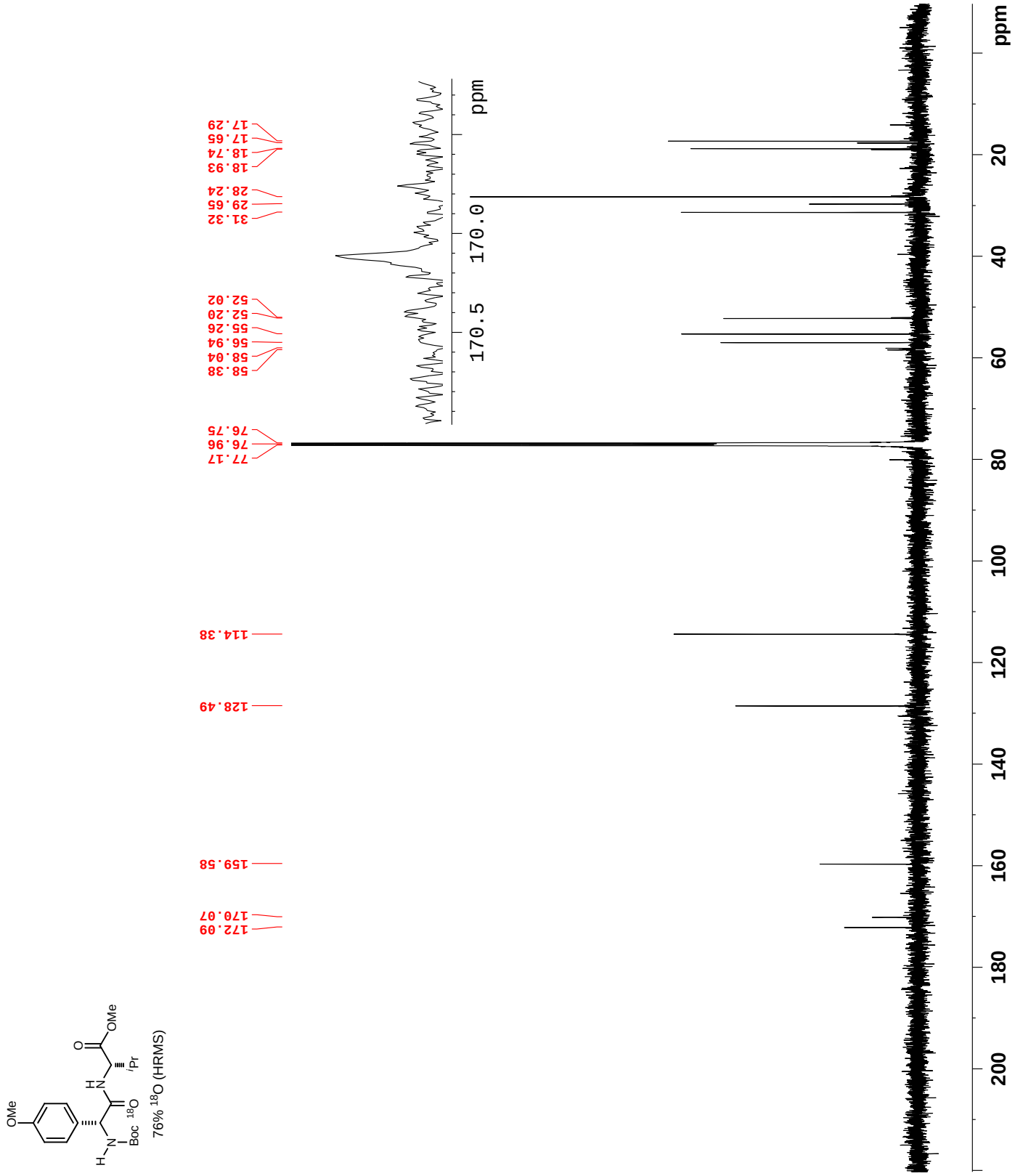

Figure S30. IR of **11e** compared to its non-^{18}O-labeled form

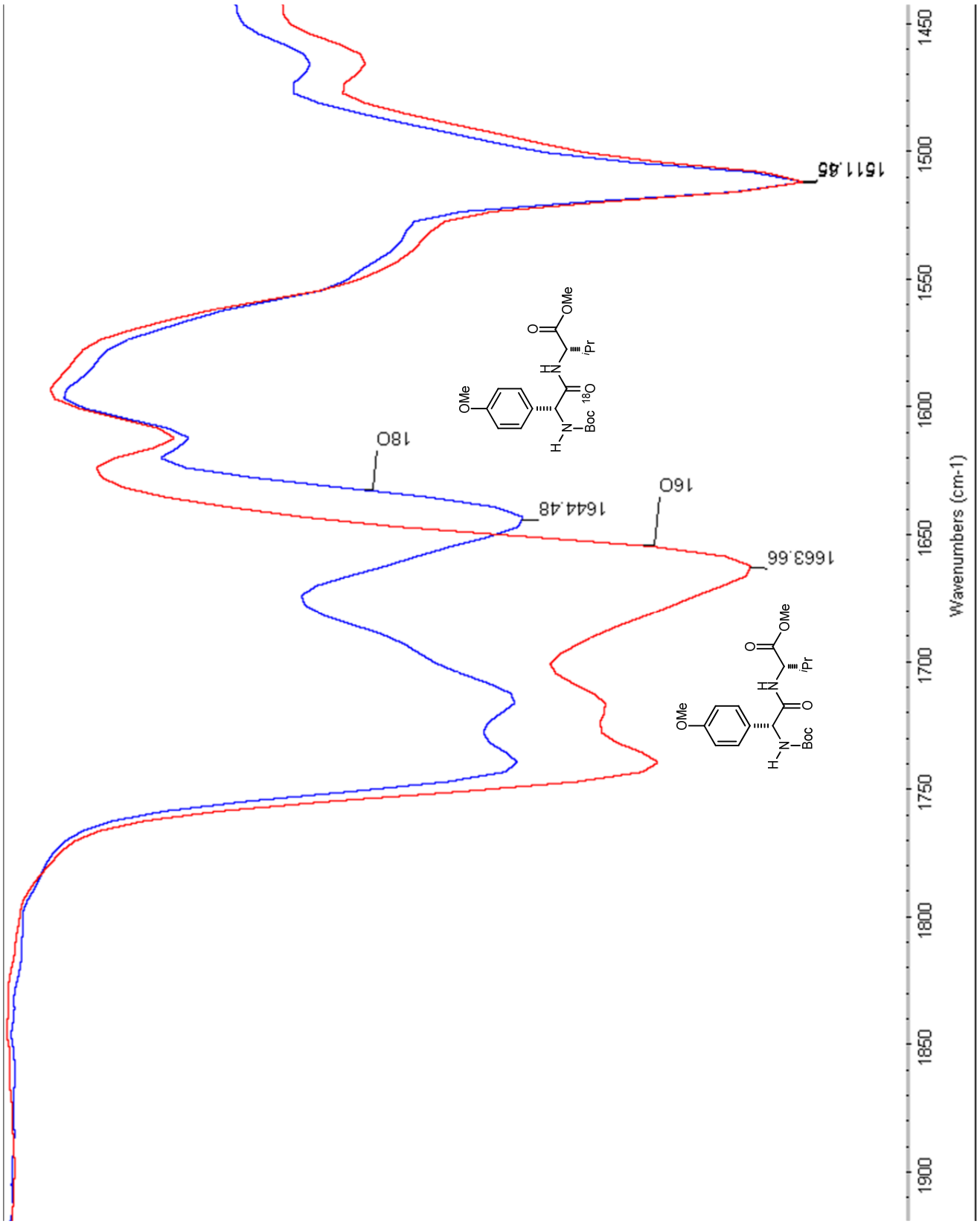

Figure S31. ^{13}C NMR ($CDCl_3$) Comparison of **11e** at various levels of ^{18}O Enrichment

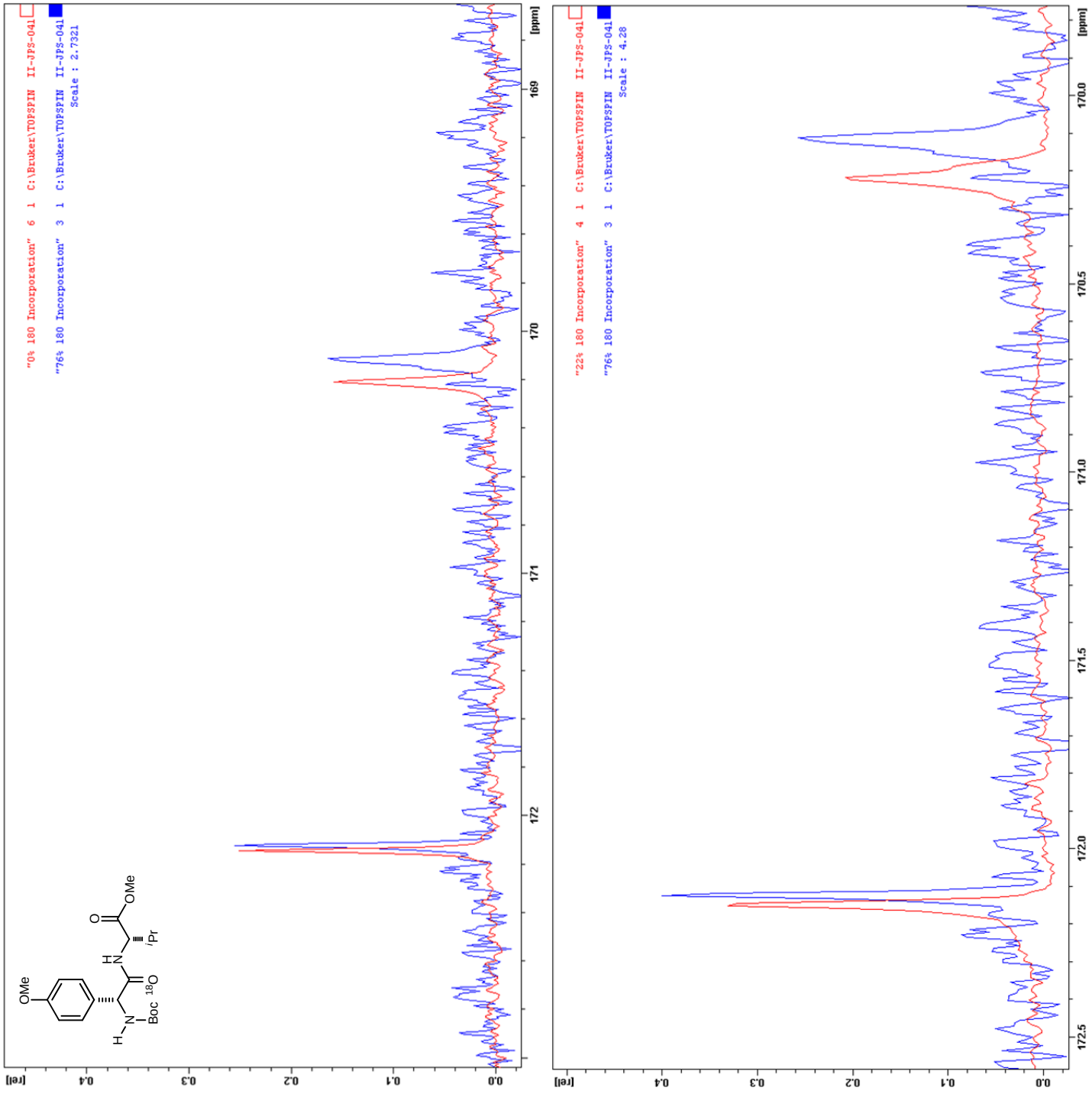

Figure S32. ^{1}H NMR ($CDCl_3$) of **11f**

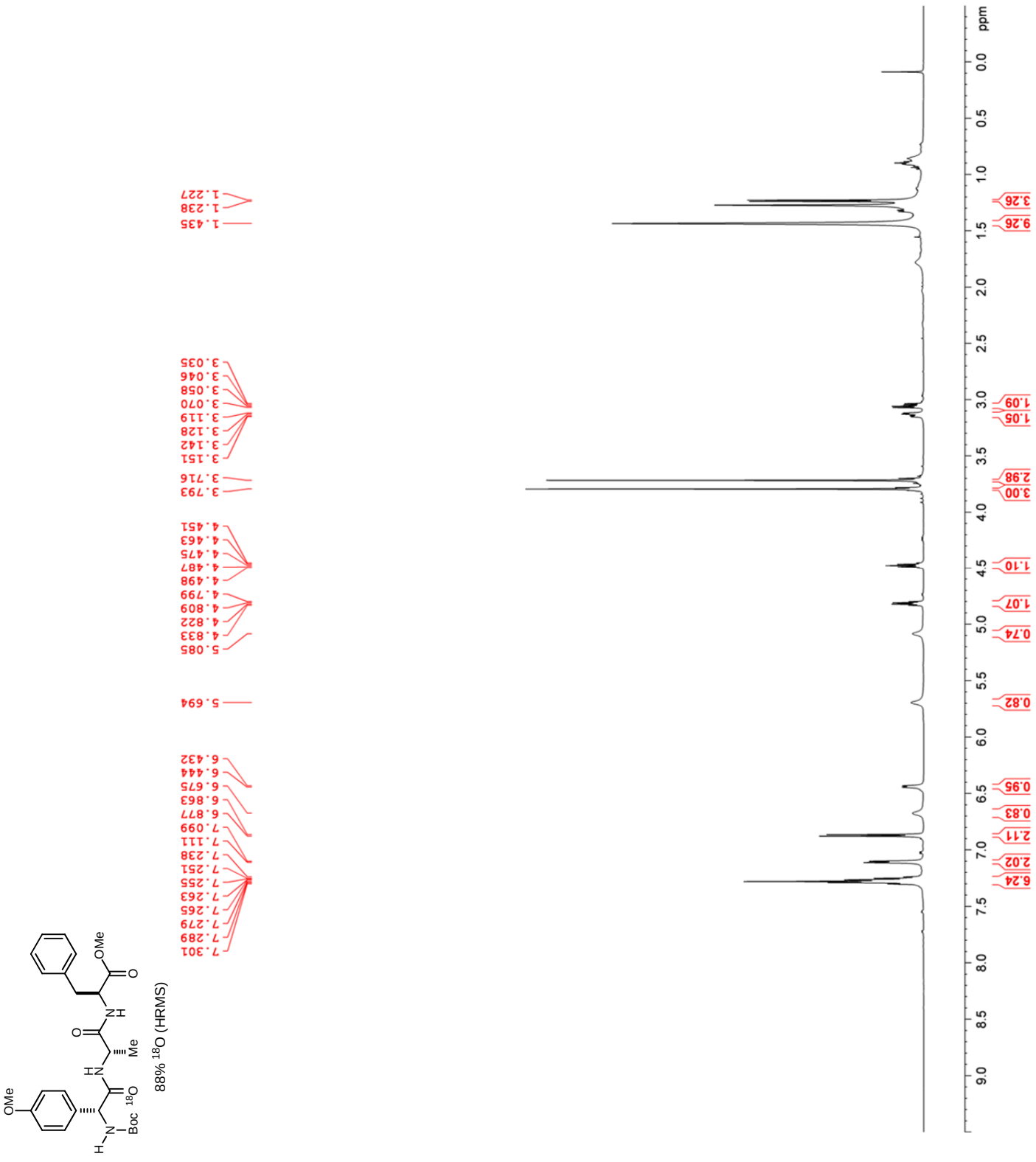

Figure S33. ^{13}C NMR ($CDCl_3$) of **11f**

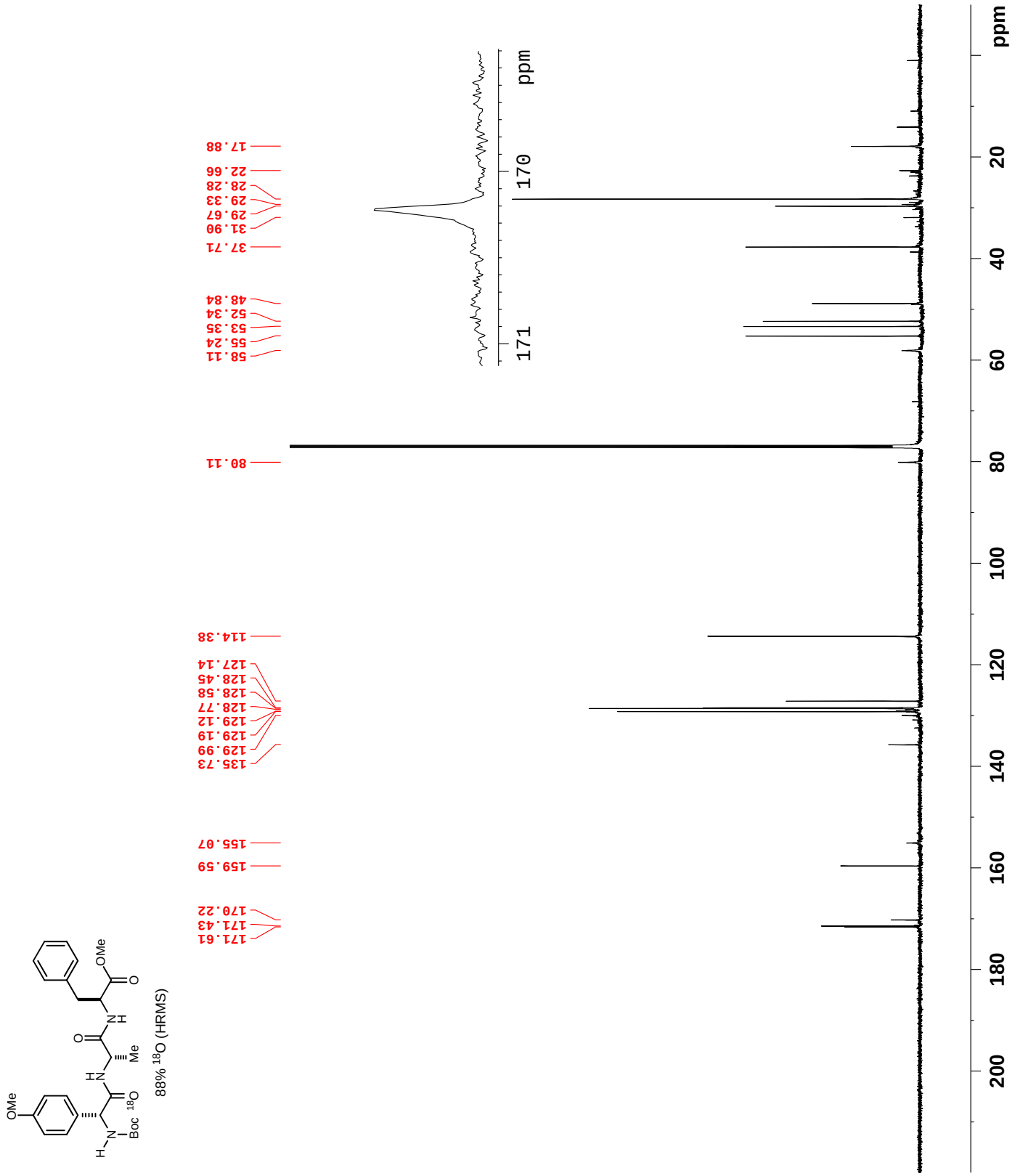

Figure S34.HSQC NMR ($CDCl_3$) of **11f**

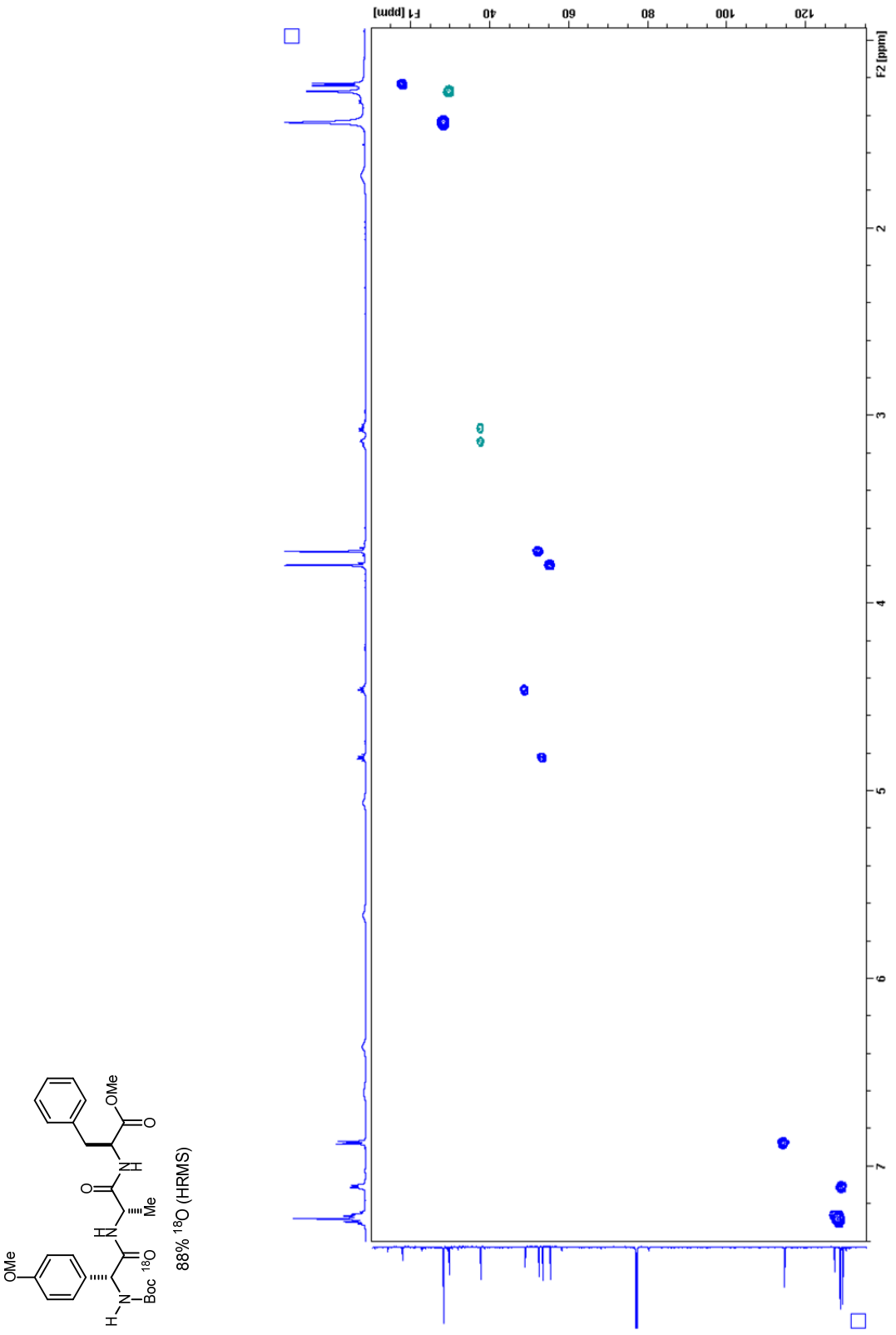

Figure S35.HMBC NMR ($CDCl_3$) of **11f**

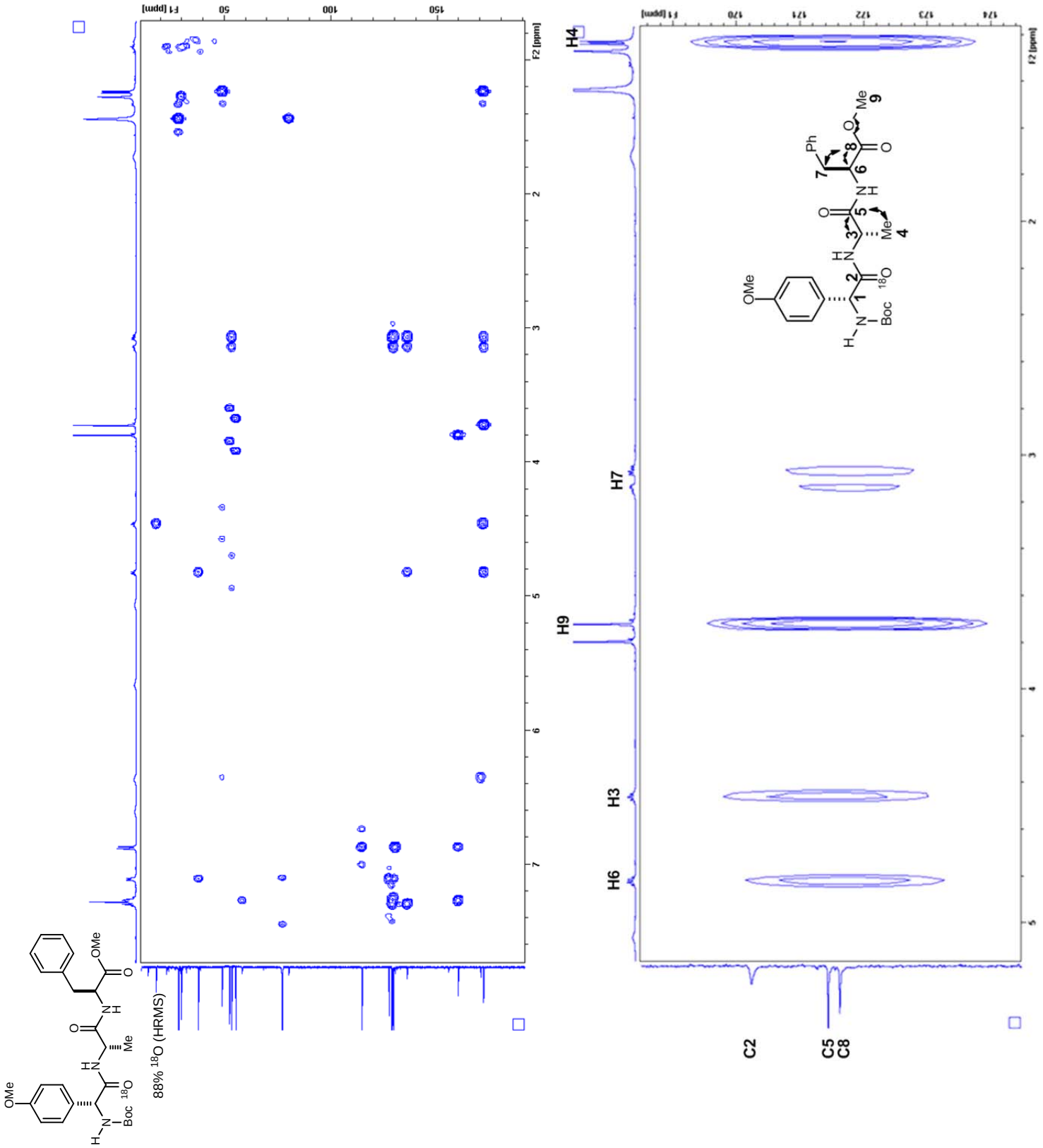

Figure S36. IR of **11f** compared to its non-^{18}O-labeled form

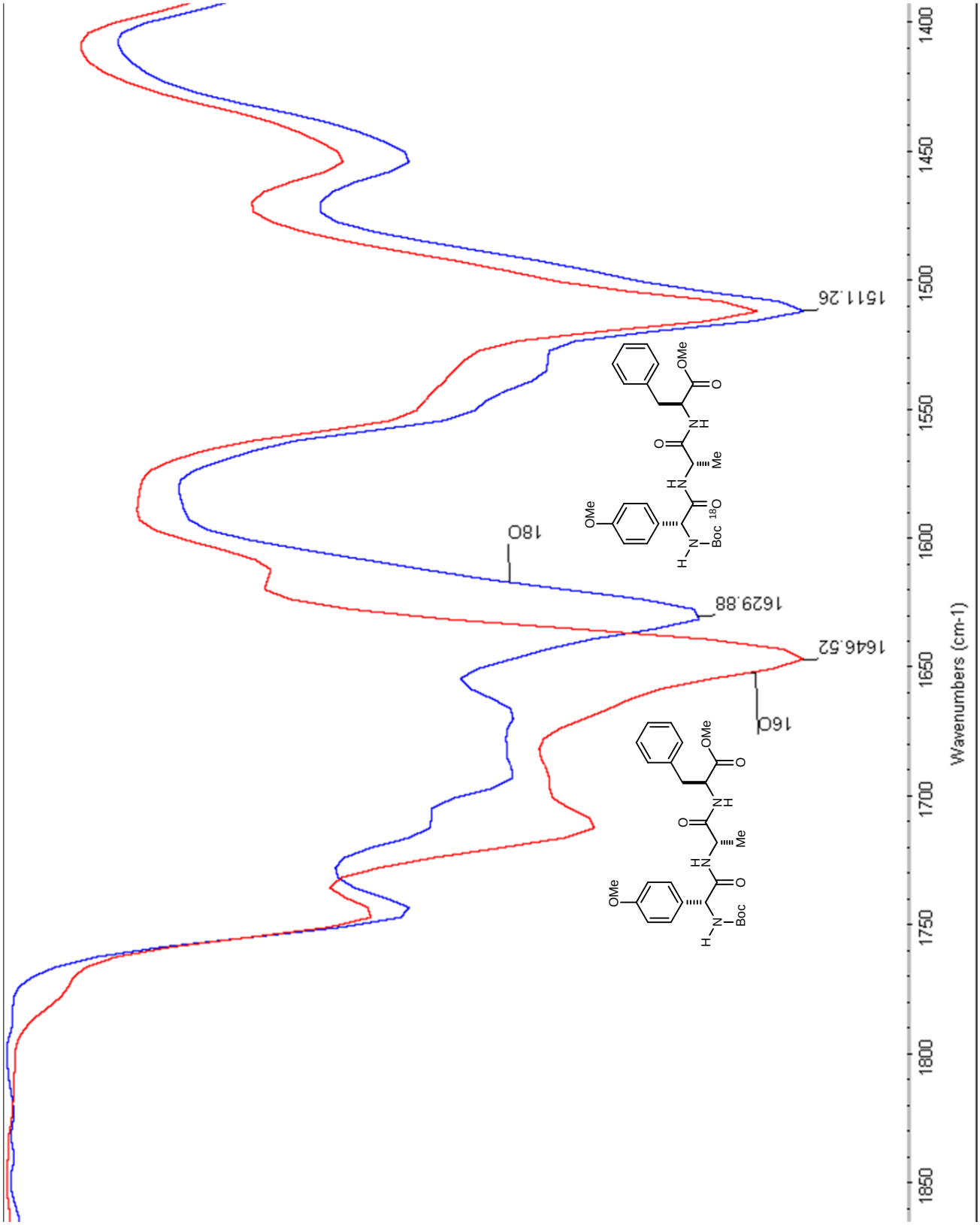

Figure S37. ^{13}C NMR ($CDCl_3$) Comparison of **11f** at various levels of ^{18}O Enrichment