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Mechanistic Insight into the Lability of Benzyloxycarbonyl (Z) Group in N-Protected Peptides under Mild Basic Conditions

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The unexpected lability of Z protecting group under mild basic conditions at room temperature is explained by a mechanism based on anchimeric assistance. It is found that the vicinal amide group stabilizes the tetrahedral intermediate formed after the nucleophilic addition of hydroxide to the carbonyl of the Z group. This effect operates in N-protected tripeptides and tetrapeptides but Z-protected dipeptides are stable under the same conditions due to the blockage of the vicinal amide NH by intramolecular H-bonding with terminal carboxylate moiety.

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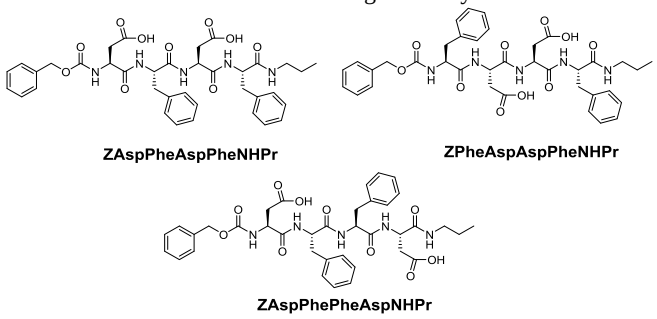
Introduction

Anchimeric assistance or neighbouring group participation constitutes a classic topic in organic chemistry and in particular in organic reaction mechanisms. Widespread examples of unusual reactivity associated to anchimeric assistance have been reported in the last decades and the relevance of this effect is still often highlighted nowadays in different areas of organic chemistry such as saccharides,[1] synthesis of natural products,[2] reactive intermediates,[3] biochemical reactions,[4] nucleotide chemistry[5] or the reactivity of mustard reagents.[6] Here we report on the lability of benzyloxycarbonyl (Z thereafter) N-protecting group in diluted aqueous NaOH at room temperature and on a mechanistic rationale for this behaviour based on anchimeric assistance of a vicinal amide group. The lability of Z group in cold, diluted sodium hydroxide solutions is unexpected if one considers that the literature on this protecting group in peptide and organic chemistry does not report such property. It is well known that the Z group can be cleaved by catalytic hydrogenolysis, acidolysis or, less commonly, by dissolving metal reduction.[7,8] However, Z group is described to tolerate basic conditions[9] and only a few reports are available of its removal under rather harsh conditions such as, refluxing with $Ba(OH)_2$ in glyme/H_2O for 40 h or using 40% NaOH in MeOH/H_2O.[10,11]

Results and Discussion

Following our previous work on supramolecular hydrogels,[12,13] three isomeric ionizable Z-protected tetrapeptides derived from aspartic acid and phenylalanine were prepared to be used as hydrogelators (Scheme 1). Unexpectedly, we noticed that upon dissolving the peptides in basic water (0.1 M NaOH) the Z groups were rapidly depleted from these molecules at room temperature.[14] ^{1}H NMR spectra showed that the signal corresponding to the benzyl protons of the Z group progressively disappeared giving place to a new signal at at 4.57 ppm which was assigned unambiguously to the benzylic protons of benzyl alcohol (Figure 1; notice that bottom spectrum is poorly resolved due to peptide aggregation. Well resolved spectra were obtained for smaller compounds shown in Table 1. See an example in SI.). A set of experiments was then designed to find out which structural factors of the peptides are important in this behavior and to propose a reasonable mechanism for this striking reactivity.



Scheme 1. Structure of the tetrapeptide derivatives studied.

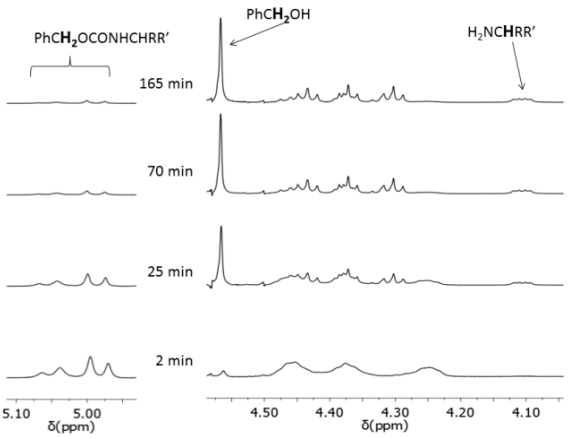
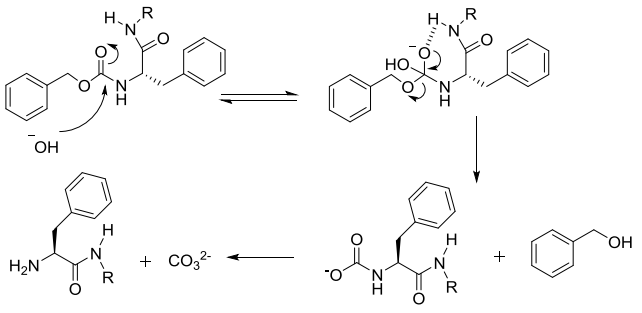


Figure 1. ^{1}H NMR spectra recorded for the basic hydrolysis of ZAspPhePheAspNHPr (15 mM) in D_2O ([NaOH] = 0.1 M).

First, a kinetic study was carried out in D_2O and half-life times associated to pseudo first order kinetics were calculated for the tetrapeptides. Half-life times of 1-2 h were obtained in all the cases (entries 11-13, table 1) revealing a fast removal of the Z group under the diluted basic conditions. We hypothesized that Z lability in those substrates could be explained considering that the tetrahedral intermediate formed upon nucleophilic attack of the hydroxide anion to the carbamate carbonyl is stabilized by intramolecular H-bonding with the vicinal amide group as shown in Scheme 2.



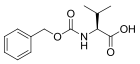
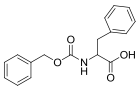
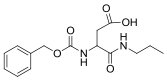
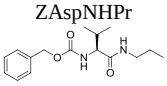
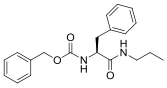
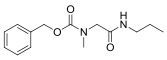
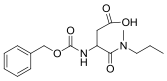
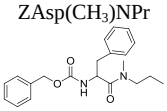
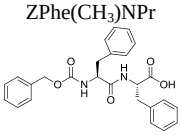
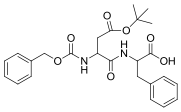
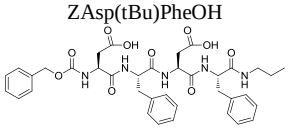
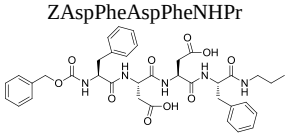
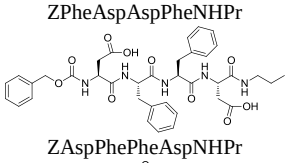
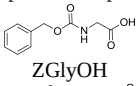
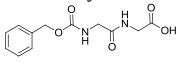
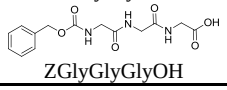
Scheme 2. Proposed mechanism for the basic hydrolysis of the carbamate group.

To prove the feasibility of this mechanism different Z-protected amino acids were studied (entries 1-8, Table 1). In D_2O no hydrolysis was observed for Z-protected amino acids (ZValOH and ZPheOH) but hydrolysis took place readily when the carboxylic acid terminal unit was converted to amide as is the case of ZAspNHPr. Due to the low solubility of some of the compounds in water, we found very convenient to analyze the results obtained in methanol. As shown in the table, in this solvent no hydrolysis was observed for the carboxylic acid terminated amino acids (entries 1-2) but the Z-removal was rather fast when a terminal amide was present (entries 3-5). These results support the mechanism proposed in Scheme 2 which requires anchimeric assistance by a vicinal amide to stabilize the tetrahedral intermediate.

The hydrolysis of carbamates under basic conditions can take place via a BAc2 mechanism as depicted in scheme 2 or via an E1cB mechanism based on the deprotonation of the carbamate and formation of an intermediate isocyanate.[15-18] To clarify this point, ZSarNHPr (Sar = sarcosine or N-methylglycine) was used as substrate. This amino acid derivative contains a N,N-disubstituted carbamate and therefore cannot participate in an E1cB mechanism due to the lack of a deprotonable NH unit. The fact that ZSarNHPr is also reacting in basic methanol at a rate even faster than the other amino acid derivatives (entry 6) agrees with a BAc2 mechanism taking place in this reaction. Furthermore, no reaction was observed for a ZAspN(CH_3)Pr or ZPhe(CH_3)NPr (entries 7 and 8), which are N-methylated analogues of ZAspNHPr and ZPheNHPr respectively, confirming the key role of the primary amide NH unit in the hydrolysis.

Next, to have results in a homopeptidic series the compounds ZGlyOH, ZGlyGlyOH and ZGlyGlyGlyOH were studied (entries 14-16, table 1). The assumption made was that the presence of the amide unit in a vicinal position to the carbamate unit would favor the hydrolytic Z-removal as was the case in the tetrapeptides reported above. So, under basic conditions no hydrolysis was expected to take place for ZGlyOH but Z-removal should occur for ZGlyGlyGlyOH and ZGlyGlyGlyOH.

Table 1. Half-life times measured in basic media for different Z-protected amino acids and peptides at 30 ºC

Entry	Compound	$t_{½}$ in D_2O / h	$t_{½}$ in methanol / h
1	 ZValOH	n. r.	n. r.
2	 ZPheOH	n. r.	n. r.
3	 ZAspNHPr	1.8	36
4	 ZValNHPr	ins.	26
5	 ZPheNHPr	ins.	8.3
6	 ZSarNHPr	ins.	4.3
7	 ZAsp(CH_3)NPr	n. r.	n. r.
8	 ZPhe(CH_3)NPr	ins	n. r.
9	 ZPhePheOH	59	n. r.
10	 ZAsp(tBu)PheOH	>72	n. r.
11	 ZAspPheAspPheNHPr	1.4	17
12	 ZPheAspAspPheNHPr	1.0	-
13	 ZAspPhePheAspNHPr	2.0	-
14	 ZGlyOH	>72	>72
15	 ZGlyGlyOH	1.4	48
16	 ZGlyGlyGlyOH	0.04	2.0

[a] n. r. = no reaction; ins. = insoluble. [Z-protected substrate] = 15 mM; [NaOH] = 0.1 M. Half-life times were calculated considering a pseudo first order kinetics

Sursprisingly, it was found that Z hydrolysis took place in all these compounds. Presumably the rather hydrophilic nature of Gly derivatives favors this reaction. However, very significant differences were found in the reactivity of these compounds. For the triglycine derivative in D_2O a rather fast hydrolysis rate was measured with a half-life time of only 0.04 h but for ZGlyOH the reaction was much slower presenting a the half-life larger than 72 h. It is noteworthy that the diglycine derivative ZGlyGlyOH reacted considerably slower that the corresponding tripeptide ZGlyGlyGlyOH, being this effect more pronounced in the methanolic basic solution with half-life times of 48 h and 2 h respectively. To confirm the hinted resistance to hydrolysis of the Z-protected dipeptides, two Z-dipeptides available in the laboratory, ZPhePheOH and ZAsp(tBu)PheOH, were studied (entries 9 and 10, table 1). Noticeably, in basic methanolic solutions no hydrolysis was observed at all for these compounds and in water, only a quite slow hydrolysis process was detected with half-life times larger than 59 h for both dipeptides. This puzzling difference found for Z-protected tripeptides or tetrapeptides when compared to Z-dipeptides can be explained considering the intramolecular hydrogen bond between the terminal carboxylate unit in the deprotonated peptides and the NH of the vicinal peptidic bond (figure 2B). It should be noted that this intramolecular bond blocks the assistance of the amide NH in the stabilization of the tetrahedral intermediate required for carbamate hydrolysis. Intramolecular H-bonding between terminal carboxylate and vicinal amide in peptides has been reported previously and clearly demonstrated by the NMR shift experienced by the amide NH resonance.[12] Interestingly carbamate hydrolysis is not blocked for ZAspNHPr (entry 3, Table 1) despite the fact that this compound presents a pendant carboxylic acid moietiy. In this case an intramolecular H-bond between carboxylate and amide NH would form a 7-membered which is not thermodynamically as favorable as the case described in figure 2B (5-membered ring).

Molecular models with computational chemistry (DFT-B3LYP/3-21 G) agree well with the experimental results described. Minimum energy models could be obtained with the negatively charged tetrahedral intermediate stabilized with intramolecular H-bonding from the adjacent amide bond (figure 2A). Furthermore, computational models also point out to the formation of a stable intramolecular H-bond between terminal carboxylate unit and vicinal peptide (figure 2B).

Finally, due the widespread use of the related protecting group BOC (tertbutoxycarbonyl) the stability of BOCPheNHPr in the presence of 0.1 M NaOH in MeOH was also assesed. No hydrolysis was observed after 24 h in difference to ZPheNHPr (entry 5, Table 1). The resistance of BOCPheNHPr towards hydrolysis is in accordance to the well known resistance of tertbutyl esters to basic hydrolysis as a result of the steric hindrance provided by the tertbutyl unit.

Conclusions

In summary, anchimeric assistance by intramolecular H-bonding affords the stabilization of the tetrahedral intermediate associated to carbamate hydrolysis in a related way to that observed in enzymatic catalysis, in particular to the so called oxyanion holes.[19,20] A subtle interplay among different H-bonds is key for the observed reactivity: intramolecular H-bond between carboxylate terminal group and the adjacent amide explains the hydrolytic stability of Z-dipeptides but switching to larger Z-peptides results in the emergence of a intramolecular catalytic mechanism responsible of the observed hydrolysis. The labile nature of Z group under diluted base and room temperature conditions provides with new, unreported, incompatibilities for this widely used protecting group but is not synthetically useful because of the associated epimerization.

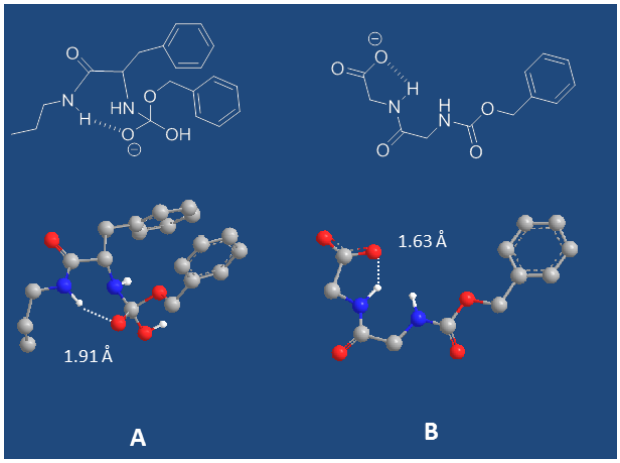


Figure 2. DFT-B3LYP/3-21 G energy minimized conformations (solvent model: PCM, H2O). The distances shown refer to the H-bond distances. A): Tetrahedral intermediate from the addition of hydroxide to ZPheNHPr. B): $ZGlyGlyO^-$.

Experimental Section

4.1 General Considerations.

NMR spectra were recorded at 500 MHz, 300 MHz (^{1}H NMR) and 125 MHz, 75 MHz (^{13}C NMR) in different solvents at 30 ºC with the solvent signals as internal reference. Mass spectra were run in the electrospray (ESMS) mode. Optical rotations were measured at 25 ºC. Reactions which required an inert atmosphere were carried out under dry N_2 with flame-dried glassware. Commercial reagents were used as received. THF and DME were used as commercial available solvents.

4.2 Synthesis.

ZPheNHPr: The N-hydroxysuccinimide ester, ZPheOSu, (3.21 g, 8.7 mmol) was dissolved in DME (50 mL). 1-Aminopropane (0.79 mL, 9.6 mmol) dissolved in DME (15 mL) was added drop wise and the resulting solution was stirred at room temperature for 18 hours and then was warmed for 2 hours at 40-50ºC. The solvent was evaporated under vacuum. The resulting solid was dissolved in dichloromethane (25 mL) and washed three times with HCl 0.1 M (3 x 25 mL) and water (3 x 25 mL). The organic phase was dried with anhydrous magnesium sulfate and the solvent was evaporated under vacuum. A white solid was obtained (1.7 g, 4.99 mmol, 93%). 1H NMR (500 MHz, 30 ºC, d6-DMSO) δ 7.92 (t, J = 5.5 Hz, 1H), 7.45 (d, J = 15.6 Hz, 1H), 7.38 – 7.10 (m, 10H), 4.99 – 4.89 (s, 2H), 4.21 (m, 1H), 3.10 – 2.86 (m, 3H), 2.77 (dd, *J* = 13.6, 10.1 Hz, 1H), 1.45 – 1.30 (m, 2H), 0.80 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, 30 ºC, d_6-DMSO) δ 171.6, 156.2, 138.5, 137.5, 129.6, 128.7, 128.5, 128.1, 127.9, 126.7, 65.6, 56.7, 40.8, 38.3, 22.7, 11.8. (ESI-TOF, positive mode) m/z exp $[M + H]^+$ calcd for $C_{20}H_{25}N_2O_3^+$ 341.1865 ; found, 341.1860 $[M + H]^+$, (Δ = 1.5 ppm).

ZValNHPr: A similar procedure to that described for ZPheNHPr was used starting with ZValOSu (3.48 g, 10 mmol) and 1-aminopropane (0.90 mL, 11 mmol). A white solid was obtained (2.23 g, 7.62 mmol, 85 %). Compound ZValNHPr was previously described in literature and both ^{1}H and ^{13}C NMR spectra were in good agreement with the literature spectra.[21]

ZSarNHPr: A similar procedure to that described for ZPheNHPr was used starting with ZSarOSu (577 mg, 1.8 mmol) and 1-aminopropane (0.90 mL, 1.9 mmol). A white solid was obtained (0.36 g, 1.4 mmol, 76 %). ^{1}H NMR (500 MHz, 30 ºC, d_6-DMSO) δ 7.86 (s, 1H), 7.43 – 7.24 (m, 5H), 5.05 (ds, 2H), 3.82 (ds, 2H), 3.01 (m, 2H), 2.87 (ds, 3H), 1.39 (m, 2H), 0.92 – 0.72 (dt, *J* = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, 30 ºC, d_6-DMSO) δ 168.9, 168.4, 156.4, 156.1, 137.4, 128.8, 128.7, 128.2, 128.4, 127.9, 127.5, 66.7, 66.5, 51.9, 51.6, 40.7, 36.4, 35.6, 22.8, 11.8. HRMS (ESI-TOF, positive mode) m/z exp $[M + H]^+$ calcd for $C_{14}H_{21}N_2O_3^+$ 265.1548 ; found, 265.1552 $[M + H]^+$, (Δ = 1.5 ppm).

ZPheN(CH$_3$)Pr: This compound was obtained by following the same synthesis described for the compound ZPheNHPr. The starting materials in this case were ZPheOsu (369 mg, 1 mmol) and N-methylpropan-1-amine (0.11 mL, 1.1 mmol). A transparent oil was obtained (0.27 g, 0.76 mmol, 76 %). ^{1}H NMR (300 MHz, 30 ºC, d_6-DMSO) δ 7.63 (dd, *J* = 8.3 Hz, 1H), 7.40 – 7.05 (m, 10H), 5.05 – 4.78 (m, 2H), 4.59 (m, 1H), 3.10 – 3.30 (m, 4H), 2.95 – 2.62 (m, 5H), 1.61 – 1.22 (m, 2H), 0.74 (dt, *J* = 7.6 Hz, 3H). ^{13}C NMR (75 MHz, 30 ºC, d_6-DMSO) δ 171.3, 156.1, 138.2, 138.0, 137.48, 129.7, 129.7, 128.7, 128.7, 128.5, 128.1, 127.9, 126.8, 65.7, 52.7, 52.6, 50.8, 49.3, 38.2, 37.7, 35.1, 33.8, 21.6, 20.2, 11.5, 11.3. HRMS (ESI-TOF, positive mode) m/z exp $[M + H]^+$ calcd for $C21H_{27}N_2O_3^+$ 355.2022 ; found, 355.2022 $[M + H]^+$, (Δ = 0.0 ppm).

ZAspNHPr: The reaction coupling between the N-hydroxysuccinimidyl ester, ZAsp(tBu)OSu (1.35 g, 3.2 mmol) , and the corresponding amine (3.5 mmol) was carried out in a similar way described above for ZPheNHPr. After that, an extra deprotection step was needed. Removal of tBu protecting group was carried based on the procedure reported in the literature.22 A solution t-butyl ester ZAsp(tBu)NHPr (1.09 g, 3.0 mmol) in a mixture of TFA (12 mL) (trifluroacetic), dichloromethane (12 mL) and water (0.9 mL) was stirred for 40 min at room temperature. Dichloromethane and TFA were evaporated under reduced pressure and the residue was co-distilled three times with eter. The obtained product was dissolved in 20 mL of dichloromethane and washed with HCl 0.1 M (3x25 ml) and water (3x25 ml). The organic phase was dried with magnesium sulfate anhydrous and the solvent was evaporated under vacuum. A white solid was obtained (0.83 g, 2.69 mmol, Global Yield = 85 %). 1H NMR (500 MHz, 30 ºC, d6-DMSO) δ 7.81 (t, J = 5.1 Hz, 1H), 7.46 (d, J = 8.2 Hz, 1H), 7.41 – 7.22 (m, 5H), 5.10 – 4.97 (m, 2H), 4.37 – 4.26 (m, 1H), 3.08 – 2.90 (m, 2H), 2.71 – 2.56 (m, 1H), 2.56 – 2.42 (m, 1H), 1.44 – 1.33 (m, 2H), 0.81 (t, J = 7.4 Hz, 3H). 13C NMR (126 MHz, 30 ºC, d6-DMSO) δ 172.2, 170.9, 156.2, 137.4, 128.8, 128.2, 128.1, 65.9, 52.0, 40.9, 37.0, 22.7, 11.7. (ESI-TOF, negative mode) m/z exp [M -H]- calcd for C15H19N2O5- 307.1294 ; found, 307.1295 [M -H]-, (Δ = 0.3 ppm).

ZAspN(CH$_3$)Pr: A similar procedure to that described for ZAspNHPr was used. In this case the reaction coupling was between ZAsp(tBu)OSu (1.35 g, 3.2 mmol) and N-methylpropan-1-amine (0.36 mL, 3.5 mmol). The deprotection with TFA and CH_2Cl_2 produced a transparent oil (0.73 g, 2.26 mmol, Global Yield = 71 %). 1H NMR (300 MHz, 30 ºC, d6-DMSO) δ 7.69 (dd, J = 11.9, 9.1 Hz, 1H), 7.43 – 7.17 (m, 5H), 5.03 (s, 2H), 4.72 (m, 1H), 3.27 (m, 2H), 3.00- 2.77 (ds, 3H), 2.76 – 2.20 (m, 2H), 1.47 (dt, *J* = 29.1, 14.1, 7.1 Hz, 13H), 0.78 (dt, *J* 7.4 Hz, 3H). ^{13}C NMR (75 MHz, 30 ºC, d_6-DMSO) δ 172.2, 170.5, 170.2, 156.0, 156.0, 137.4, 137.3, 128.8, 128.2, 128.1, 128.1, 66.0, 66.0, 55.3, 50.7, 49.3, 49.0, 48.0, 37.3, 36.9, 35.0, 33.7, 21.5, 20.2, 11.4, 11.3. (ESI-TOF, negative mode) m/z exp $[M -H]^-$ calcd for $C_{16}H_{21}N_2O_5^-$ 321.1450 ; found, 321.1451 $[M -H]^-$, (Δ = 0.3 ppm).

ZPhePheOH: In a first step the corresponding methyl ester of the dipeptide was obtained following a described procedure.[23] The L-Phenylalanine methyl ester hydrochloride (518 mg, 2.4 mmol) was dissolved in 30 mL of DME (dimethoxyethane). Then triethylamine were added (0.36 mL, 2.6 mmol) and immediately a white precipitate appeared. A solution of ZPheOSu (887 mg, 2.4 mmol) in DME (30 mL) were added drop wise and the reaction mixture was stirred at room temperature 2 hours, filtered and evaporated. The residue was dissolved in dichloromethane (25 mL) and washed four times with water (25 mL). The organic phase was dried with magnesium sulfate anhydrous and the solvent evaporated under vacuum.

Then, following a reported procedure,[24] the corresponding methyl ester (875 mg, 1.9 mmol) was dissolved in THF (20 mL) and cooled at 0 ºC. A solution of lithium hydroxide (62.4 mg, 2.6 mmol) in water (10 mL) was added drop wise. The reaction mixture was stirred for 0.5 hour. Then this mixture was washed with ether (20 mL). The aqueous layer was acidified with with 1 M HCl to adjust to pH 2 and extracted with ether (3 x 30 mL). The organic extracts were combined, dried with magnesium sulfate anhydrous powder and evaporated.

A white solid was obtain (0.92 g, 2.06 mmol, Global Yield 82 %). ^{1}H NMR (300 MHz, 30 ºC, d_6-DMSO) δ 8.23 (d, *J* = 7.8 Hz, 1H), 7.42 (d, *J* = 8.8 Hz, 1H), 7.36 – 6.94 (m, 15H), 5.03 – 4.81 (s, 2H), 4.57 – 4.38 (m, 1H), 4.29 (m, 1H), 3.43 (dd, *J* = 41.4, 34.4 Hz, 2H), 3.15 – 2.81 (m, 3H), 2.70 (dd, *J* = 13.7, 10.9 Hz, 1H). ^{13}C NMR (75 MHz, 30 ºC, d_6-DMSO) δ 173.2, 172.0, 156.2, 138.5, 137.8, 137.4, 129.6, 128.7, 128.6, 128.4, 128.1, 127.8, 127.2, 126.9, 126.7, 65.6, 56.4, 53.9, 37.9, 37.2. (ESI-TOF, positive mode) m/z exp $[M + Na]^+$ calcd for $C_{26}H_{26}N_2NaO_5^+$ 469.1739 ; found, 369.1737 $[M + Na]^+$, (Δ = 0.4 ppm).

ZAsp(tBu)PheOH: A similar procedure to that described for ZPhePheOH was used. The starting materials in this case were L-Phenylalanine methyl ester hydrochloride (518 mg, 2.4 mmol) triethylamine (0.36 mL, 2.6 mmol) and ZAsp(tBu)OSu (1.00 g, 2.4 mmol). A white solid was obtained (0.89g, 1.89 mmol, Global Yield 75 %). ^{1}H NMR (300 MHz, 30 ºC, d_6-DMSO) δ 7.98 (d, *J* = 7.4 Hz, 1H), 7.51 (d, *J* = 8.4 Hz, 1H), 7.42 – 7.12 (m, 10H), 5.12 – 4.91 (m, 2H), 4.49 – 4.25 (m, 2H), 3.07 – 2.85 (m, 2H), 2.71 – 2.30 (m, 2H), 1.36 (s, 9H). ^{13}C NMR (75 MHz, 30 ºC, d_6-DMSO) δ 173.0, 171.3, 171.0, 169.6, 156.1, 137.7, 137.3, 129.6, 128.8, 128.6, 128.2, 128.1, 126.9, 80.6, 65.9, 53.9, 51.8, 38.0, 37.0, 28.1. (ESI-TOF, positive mode) m/z exp $[M + Na]^+$ calcd for $C_{25}H_{30}N_2NaO_7^+$ 493.1951 ; found, 493.1947 $[M + Na]^+$, (Δ = 0.8 ppm).

The following peptides were obtained using conventional solution peptide chemistry. See supporting information for synthetic scheme. General procedures are shown below.

General procedure for peptide coupling. The N-hydroxysuccinimide ester of the Z-protected amino acid (8.7 mmol) was dissolved in DME (50 mL). Then corresponding peptide or amino acid with the C-terminal group as propylamide (8.7 mmol) was dissolved in DME (15 mL) and added drop wise. The resulting solution was stirred at room temperature for 18 hours and then was warmed for 2 hours at 40-50ºC. The solvent was evaporated under vacuum. The resulting solid was washed with sodium hydrogencarbonate saturated solution, HCl 0.1 M aqueous solution and water. The final product was dried at 40ºC under vacuum.

General procedure for N-benzyloxycarbonyl deprotection. The corresponding N-benzyloxycarbonyl protected peptide or amino acide derivative (7.8 mmol) and catalytic amount of Pd over activated carbon (5-10% w/w) were placed in a two necked round bottom flask and suspended in MeOH (50 mL). The system was purged to remove the air with N_2 and connected to H_2 atmosphere.

The pasty grey suspension was stirred for several hours until it turned completely black (also checked with TLC, MeOH: CH_2Cl_2 (2:8) and revealed with ninhydrin). The black suspension was filtered over celite and the solvent was evaporated under reduced pressure. The resulting oil was dried further in vacuum pump for 24 hours.

General procedure t-butyl ester group deprotection. A solution of t-butyl ester (3.0 mmol) in a mixture of TFA (12 mL) (trifluroacetic), dichloromethane (12 mL) and water (0.9 mL) was stirred for 40 min at room temperature. Dichloromethane and TFA were evaporated under reduced pressure and the residue was co-distilled three times with eter. The solid was washed with water.

ZPheAspAspPheNHPr: A white solid was obtained (2.8 g, 3.9 mmol, Global Yield 51 %) ^{1}H NMR (500 MHz, 30 ºC, δ_6-DMSO) δ 8.40 (d, *J* = 7.5 Hz, 1H), 8.15 (d, *J* = 7.5 Hz, 1H), 7.77 (d, *J* = 8.2 Hz, 1H), 7.62 (t, *J* = 5.5 Hz, 1H), 7.46 (d, *J* = 8.5 Hz, 1H), 7.39 – 6.96 (m, 15H), 4.97–4.87 (m, 2H), 4.58–4.54 (m, 1H), 4.50–4.56 (m, 1H), 4.38–4.34 (m, 1H), 4.32 – 4.21 (m, 1H), 3.08 – 2.88 (m, 4H), 2.86–2.81 (m, 1H), 2.78 – 2.39 (m, 6H), 1.43 – 1.27 (m, 2H), 0.76 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, 30 ºC, d_6-DMSO) δ 172.3, 172.2, 172.2, 171.2, 170.6, 170.4, 156.3, 138.5, 138.2, 137.4, 129.7, 129.5, 128.7, 128.5, 128.4, 128.1, 127.9, 126.7, 65.7, 56.4, 54.7, 50.2, 50.0, 37.8, 36.5, 36.2, 22.6, 11.7. (ESI-TOF, negative mode) m/z exp [M -H]$^-$ calcd for $C_{37}H_{42}N_5O_{10}^-$ 716.2932 ; found, 716.2924 [M -H]$^-$, (Δ = 1.1 ppm).

ZAspPhePheAspNHPr: A White solid was obtained (1.5 g, 2.09 mmol, Global Yield 45 %). ^{1}H NMR (300 MHz, 30 ºC, d_6-DMSO) δ 8.16 (dd, *J* = 7.4 Hz, 2H), 7.78 (d, *J* = 7.6 Hz, 1H), 7.50 (m, 2H), 7.36–7.10 (m, 15H), 5.12 – 4.87 (m, 2H), 4.62 – 4.16 (m, 4H), 3.10 – 2.16 (m, 11H), 1.51 – 1.27 (m, 2H), 0.80 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, 30 ºC, d_6-DMSO) δ 172.2, 172.2, 171.2, 171.1, 171.1, 170.3, 156.2, 137.9, 137.8, 137.3, 129.7, 129.6, 128.8, 128.5, 128.4, 128.2, 128.1, 126.8, 126.6, 66.0, 54.5, 54.1, 51.8, 50.1, 40.9, 37.8, 36.7, 36.5, 22.6, 11.7. (ESI-TOF, negative mode) m/z exp [M -H]$^-$ calcd for $C_{37}H_{42}N_5O_{10}^-$ 716.2932 ; found, 716.2926 [M -H]$^-$, (Δ = 0.8 ppm).

ZAspPheAspPheNHPr: A white solid was obtain (1.46 g, 2.03 mmol, Global Yield 52 %).^{1}H NMR (300 MHz, 30 ºC, d_6-DMSO) δ 8.28 (d, *J* = 7.2 Hz, 1H), 7.85 (dd, *J* = 8.2 Hz, 2H), 7.70 (t, *J* = 5.8 Hz, 1H), 7.51 (d, *J* = 7.8 Hz, 1H), 7.42 – 7.03 (m, 15H), 5.09 – 4.91 (m,2H), 4.58 – 4.23 (m, 4H), 2.48 (m, 11H), 1.31 (m, 2H), 0.75 (t, *J* = 7.4 Hz, 3H).^{13}C NMR (75 MHz, 30 ºC, d_6-DMSO) δ 172.3, 172.2, 171.3, 171.2, 170.6, 170.5, 156.2, 138.1, 137.9, 137.3, 130.5, 129.7, 129.5, 128.8, 128.5, 128.4, 128.2, 128.1, 126.7, 66.0, 54.6, 54.2, 51.8, 50.1, 38.0, 37.9, 36.6, 36.4, 22.6, 11.7. (ESI-TOF, negative mode) m/z exp [M -H]$^-$ calcd for $C_{37}H_{42}N_5O_{10}^-$ 716.2932 ; found, 716.2930 [M -H]$^-$, (Δ = 0.3 ppm).

ZGlyGlyOH: GlyGly (304 mg, 2.3 mmol) was dissolved in a 4 N aqueous solution of sodium hydroxide (0.7 mL). The solution was cooled at 0ºC and a 4 N aqueous solution of sodium hydroxide (1.4 mL) and benzyloxycarbonylchloride (0.40 mL, 2.8 mmol) were added drop wise. Then the mixture was stirred overnight at 20 ºC. The reaction mixture was washed with ether and the aqueous layer was cooled at 0 ºC. To the aqueous layer was added 5 M HCl to adjust its pH to 3 which was left standing overnight at a cool place. Resulting crystalline precipitates were collected by filtration, washed with cool water and dried. White crystals were obtain (0.43 g, 1.61 mmol, 70 %). ^{1}H NMR (500 MHz, 30 ºC, d_6-DMSO) δ 8.12 (t, *J* = 5.5 Hz, 1H), 7.51 – 7.42 (t, *J* = 6.2 Hz 1H), 7.41 – 7.25 (m, 5H), 5.04 (s, 2H), 3.76 (t, *J* = 6.2 Hz, 2H), 3.68 (d, *J* =6.4 Hz, 2H). ^{13}C NMR (126 MHz, 30 ºC, d_6-DMSO) δ 171.1, 169.5, 156.5, 137.0, 128.4, 127.8, 127.7, 65.5, 43.4, 40.6. (ESI-TOF, negative mode) m/z exp [M -H]$^-$ calcd for $C_{12}H_{13}N_2O_5^-$ 265.0824 ; found, 265.0819 [M -H]$^-$, (Δ = 1.9 ppm).

ZGlyGlyGlyOH: A similar procedure to that described for ZGlyGlyOH was used. The starting material in this case was GlyGlyGly (435 mg, 2.3 mmol). White crystals were obtain (0.72 g, 2.23 mmol, 97 %) ^{1}H NMR (500 MHz, 30 ºC, d_6-DMSO) δ 8.12 (m, 2H), 7.45 (t, *J* = 5.4 Hz, 1H), 7.33 (m, 4H), 5.03 (s, 2H), 3.75 (dd, *J* = 5.4 Hz, 3H), 3.66 (d, *J* = 6.0 Hz, 2H).^{13}C NMR (75 MHz, 30 ºC, d_6-DMSO) δ 171.5, 169.8, 169.6, 156.9, 137.4, 128.8, 128.2, 128.2, 66.0, 44.0, 42.2, 41.0 . (ESI-TOF, negative mode) m/z exp [M -H]$^-$ calcd for $C_{14}H_{16}N_3O_6^-$ 322.1039 ; found, 322.1034 [M -H]$^-$, (Δ = 1.6 ppm).

4.3 Kinetic studies.

All the experiments were carried out at 30ºC. [Substrate] = 15 mM, [NaOH] = 0.1 M. The hydrolysis was followed by ^{1}H NMR (500 MHz) monitoring the disappearance of the benzylic protons of the carbamate moiety. The data obtained by NMR at different times were adjusted to pseudo first order kinetics considering the concentration of hydroxide approximately constant in the studied time interval. Half-life times were calculated from the kinetic constant ($t_{1/2}$ = ln2/k).

4.4 Racemization study

PheNHPr was prepared by Z-removal with hydrogenolysis and by treatment with NaOH in methanol and the optical rotation compared.

PheNHPr obtained by basic treatment: Compound **ZPheNHPr** (10.2 mg, 0.03 mmol) was dissolved in CD_3OD 0.1 M of NaOH. The reaction was monitorized by ^{1}H NMR. When the reaction was completed, the solvent was removed under reduced pressure. The crude was dissolved in CH2Cl2 (10 mL) and washed with water (2x10 mL) and brine (10 mL). Organic layer was dried with MgSO4 anhidrous and the solvent was removed under reduced pressure (6 mg, 0.029 mmol, 95 %, $[\alpha]_D$ = 0, c 0.1, CHCl3).

PheNHPr obtained by hydrogenolysis. Palladium catalyst (10% w/w, Pd/C, 30 mg) was suspended in MeOH (10 mL) and stirred under H_2 at room temperature for 10 min. Subsequently, a solution of compound ZPheNHPr, (300 mg, 0.88 mmol) in MeOH (20 mL) was added via syringe, followed by stirring under H_2 at room temperature for 4 h. The reaction mixture was then filtered through Celite, and the solvent was removed under reduced pressure (172 mg, 0.83 mmol, 95%, $[\alpha]_D$ = -86, *c* 0.3, $CHCl_3$).

^{1}H NMR (500 MHz, 30 ºC, d_6-DMSO) δ 7.71 (t, *J* = 5.0 Hz, 2H), 7.27 – 7.21 (m, 2H), 7.20 – 7.14 (m, 3H), 3.33 (dd, *J* = 7.9, 5.4 Hz, 1H), 3.06 – 2.91 (m, 2H), 2.91 – 2.80 (m, 2H), 2.65 – 2.52 (m, 2H), 1.42 – 1.24 (m, 2H), 0.76 (t, *J* = 7.4 Hz, 3H).^{13}C NMR (75 MHz, $CDCl_3$) δ 173.3, 137.2, 128.5, 127.8, 125.9, 55.7, 40.3, 40.0, 22.0, 10.6. (ESI-TOF, positive mode) m/z exp $[M+H]^+$ calcd for $C_{12}H_{19}N_2O^+$ 207.1497; found, 207.1497 $[M+H]^+$, (Δ = 0.0 ppm).

((Experimental Details))

Supporting Information (see footnote on the first page of this article): ^{1}H and ^{13}C NMR spectra, synthetic schemes and details of kinetic experiments

Acknowledgments. We thank Spanish Ministry of Science and Innovation (grant CTQ2012-37735) and Universitat Jaume I (grant P1.1B2012-25) for financial support. M.T.-S. thanks Spanish Ministry of Education for a FPU fellowship.

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Layout 1:

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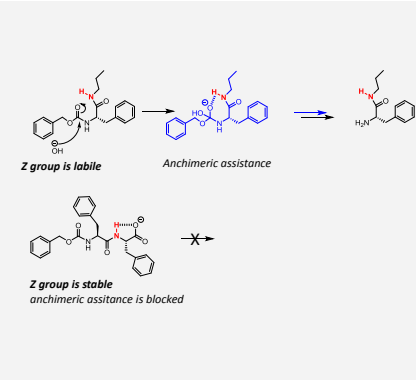
Z group from N-protected peptides is found to be extremely labile in diluted aqueous sodium hydroxide. The key for this unexpected behaviour is the anchimeric assistance by the vicinal amide NH. Amazingly, this assistance is blocked in dipeptides due to an intramolecular H-bonding with the terminal carboxylate.



Marta Tena-Solsona, César A. Angulo-Pachón, Beatriu Escuder* and Juan F. Miravet*.

Mechanistic Insight into the Lability of Benzyloxycarbonyl (Z) Group in N-Protected Peptides under Mild Basic Conditions

Keywords: Anchimeric assistance / neighbouring group participation /

Layout 2:

((Key Topic))

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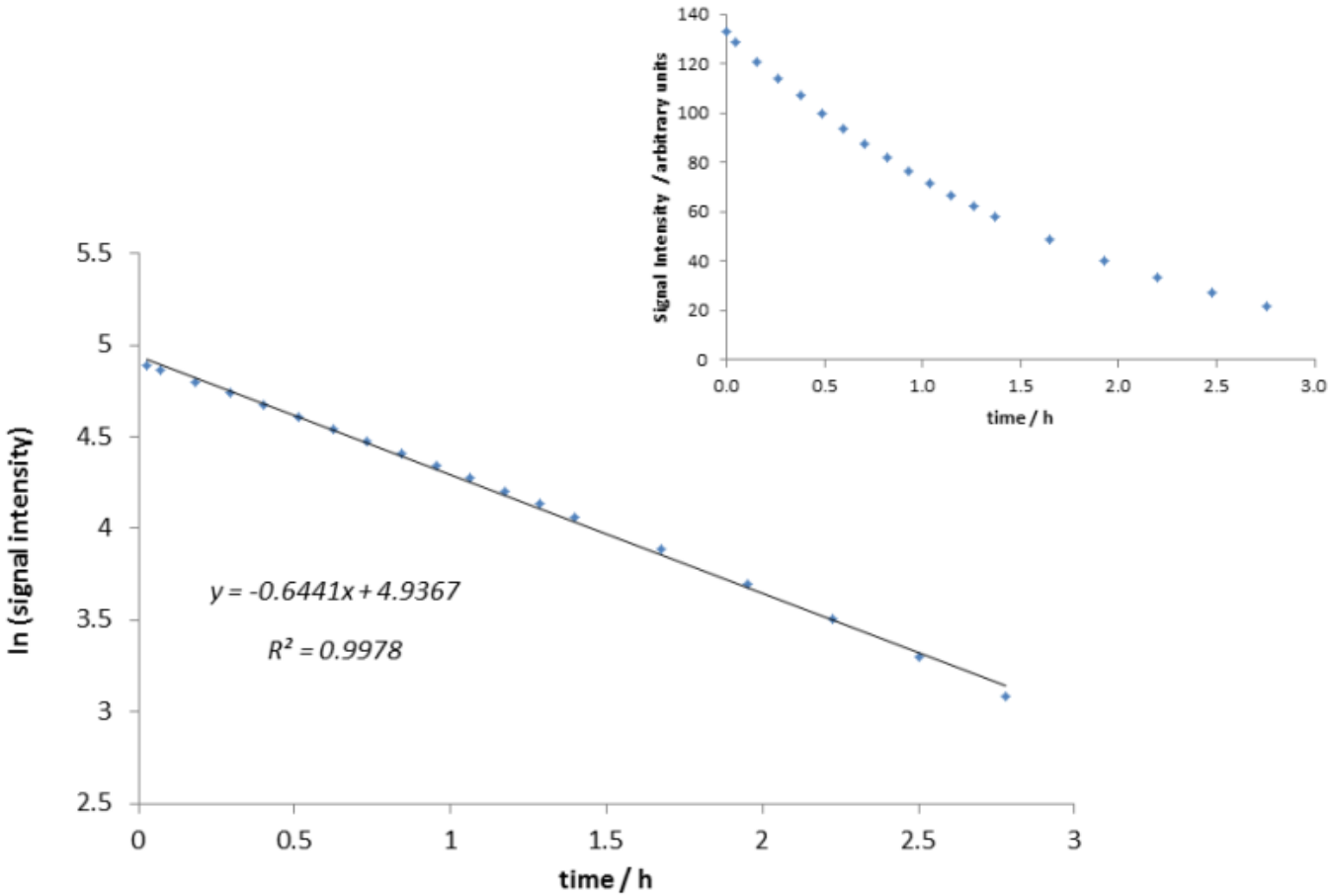
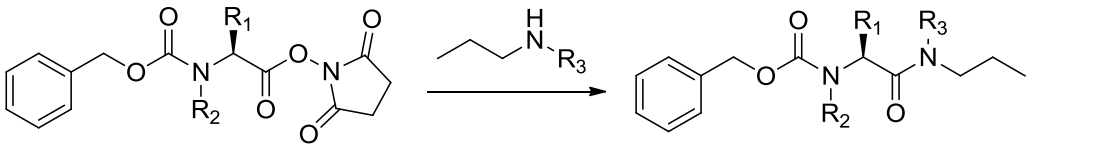


Figure S1. Graphical representation of the kinetic data obtained from NMR for the hydrolysis of **ZDFFDNHPr** in D_2O.

Compounds, **ZPheNHPr**, **ZValNHPr**, **ZSarNHPr** and **ZPheN(CH_3)Pr** were prepared following the steps shown in scheme S1.

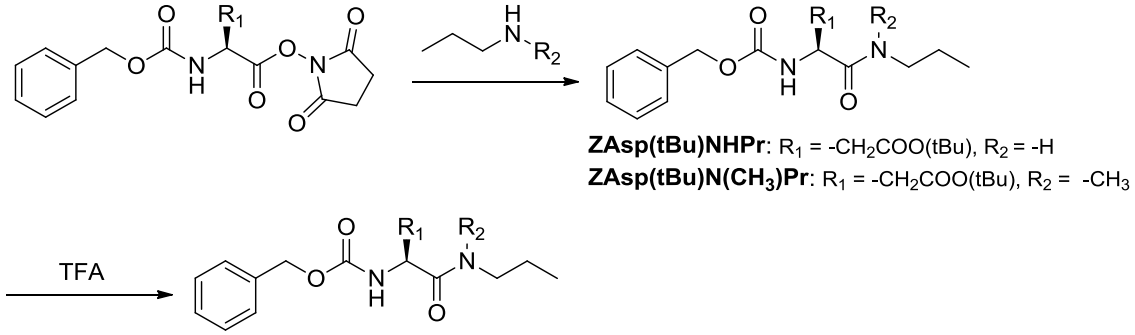
SCHEME S1



ZPheNHPr: R_1 = -CH_2Ph, R_2 = -H, R_3 = -H
ZValNHPr: R_1 = -$CH(CH_3)_2$, R_2 = -H, R_3 = -H
ZSarNHPr: R_1 = -H, R_2 = -CH_3, R_3 = -H
ZPheN(CH_3)Pr: R_1 = -CH_2Ph, R_2 = -H,R_3 = -CH_3

Compounds, **ZAspNHPr**, **ZAspN(CH_3)Pr** were prepared following the steps shown in scheme S2.

SCHEME S2

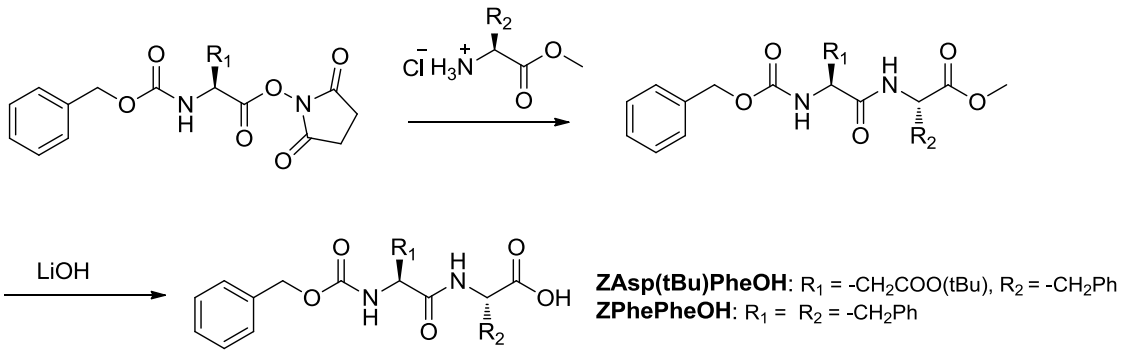


ZAsp(tBu)NHPr: R_1 = -CH_2COO(tBu), R_2 = -H
ZAsp(tBu)N(CH_3)Pr: R_1 = -CH_2COO(tBu), R_2 = -CH_3

ZAspNHPr: R_1 = -CH_2COOH, R_2 = -H
ZAspN(CH_3)Pr: R_1 = -CH_2COOH, R_2 = -CH_3

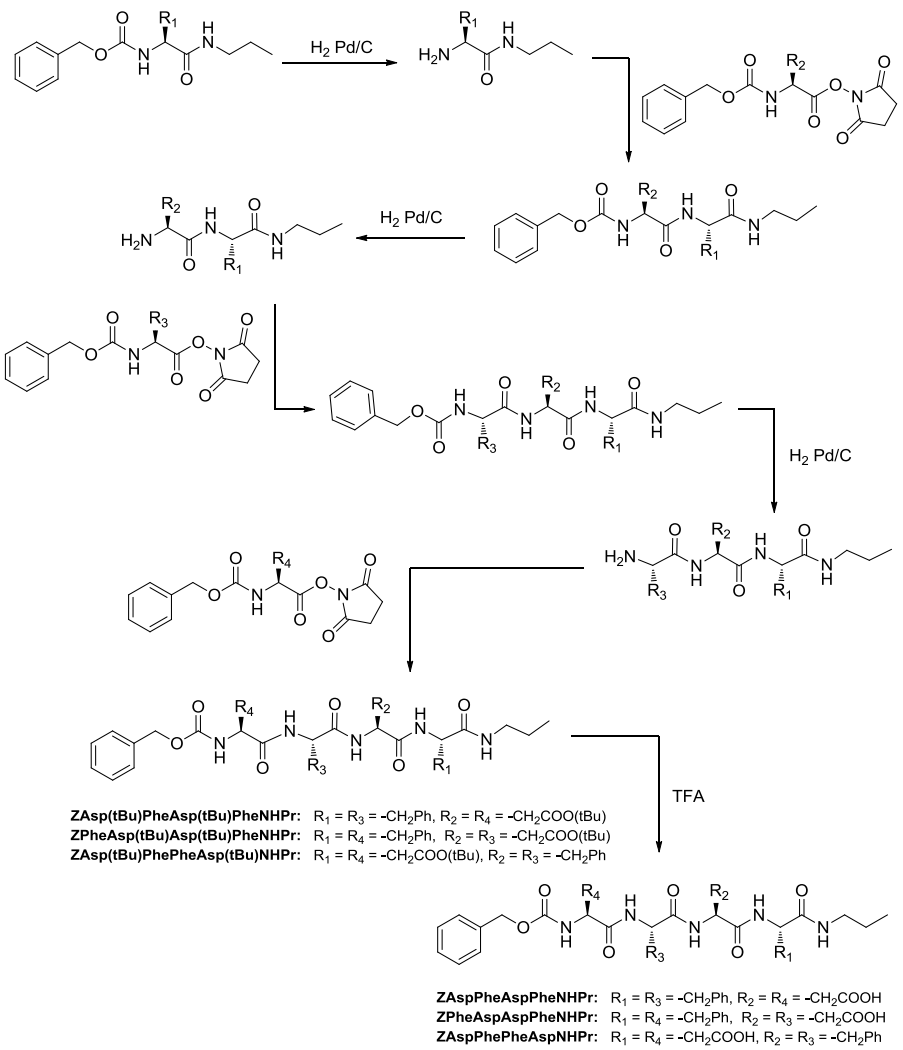
Compounds, **ZPhePheOH** and **ZAsp(tBu)PheOH** were prepared following the steps shown in scheme S3.

SCHEME S3

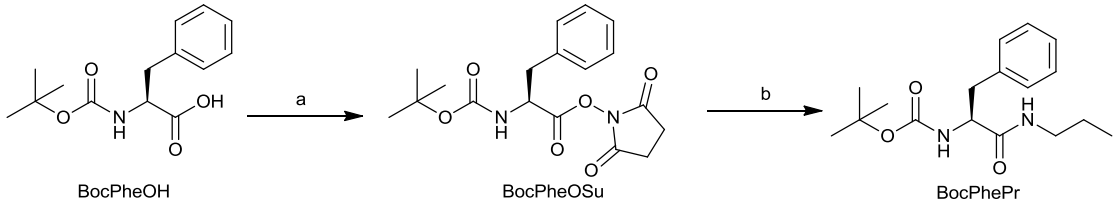


Compounds, **ZPheAspAspPheNHPr** and **ZAspPheAspPheNHPr** and **ZAspPhePheAspNHPr** were prepared following the steps shown in scheme S4.

SCHEME S4



Synthesis of compound BocPhePr



Scheme S5. Reagents and conditions: a) DCC, *N*-Hydroxisuccinimide, DME, 0 ºC, 1 h, 98%; b) *n*-Propylamine, THF, room temperature, 16 h, 96%.

Synthesis of compound (*S*)-2,5-dioxopyrrolidin-1-yl 2-((*tert*-butoxycarbonyl)amino)-3-phenylpropanoate (BocPheOSu): A solution of commercial available *tert*-butoxycarbonyl-*L*-phenylalanine **BocPheOH** (2.0 g, 7.53 mmol) and *N*-hydroxysuccinimide (867 mg, 7.53 mmol, 1.0 eq.) in DME (30 mL) was added dropwise under N_2 at 0 ºC with a dropping funnel to a solution of *N*,*N*′-Dicyclohexylcarbodiimide (1.7 g, 8.28 mmol, 1.01 eq.) in dry DME (15 mL). The mixture was further stirred for 1 h at 0 ºC. The solution was then allowed to stand into refrigerator for 16 h, which caused precipitation of *N*,*N*′-Dicyclohexylurea. After this time, the mixture was filtered under vacuum, and the filtrate was removed under reduced pressure and the crude residue was purified by crystallization in isopropanol to yield **BocPheOSu** (2.7 g, 7.34 mmol, 98%) as a white solid. The NMR spectra were consistent with those described in the literature (N. I., Howard; T. D. H., Bugg. *Bioorg. Med. Chem.* **2003**, *11*, 3083)

^{1}H NMR (300 MHz, 30ºC, $CDCl_3$): δ 7.20 – 7.38 (m, 5H), 4.96 (br s, 2H), 3.21 (dd, 2H, *J* = 4.1, 11.8 Hz), 2.82 (s, 4H), 1.40 (s, 9H).

Synthesis of compound (*S*)-*tert*-butyl (1-oxo-3-phenyl-1-(propylamino)propan-2-yl)carbamate (BocPhePr): A solution of compound **BocPheOSu** (2.0 g, 5.52 mmol) in THF (40 mL) was added dropwise under N_2 at 25 ºC with a dropping funnel to a solution of commercial available *n*-Propylamine (499 µL, 6.07 mmol, 1.1 eq.) in THF (10 mL). The mixture was further stirred for 5 h at 50 ºC. After this time, the solvent was removed under reduced pressure and the residue was poured into dissolution aq. HCl 0.1 and worked up (extraction with CH_3Cl), removal of all volatiles under reduced pressure to yield **BocPhePr** (1.62 g, 5.30 mmol, 96%) as a white solid . The compound was used in crude form for the next reaction. The NMR spectra were consistent with those described in the literature. (D., Tessaro; L., Cerioli; S., Servi; F., Viani; P., D'Arrigo. *Adv. Synth. Catal*. **2011**, *353*, 233.)

^{1}H NMR (500 MHz, 30ºC, $CDCl_3$): δ 7.19 – 7.32 (m, 5H), 5.69 (br s, 1H), 5.08 (br s, 1H), 4.26 (d, 1H, *J* = 6.8 Hz), 3.18 – 2.97 (m, 4H), 1.44 – 1.33 (m, 11H), 0.80 (t, 9H, *J* = 7.3 Hz).

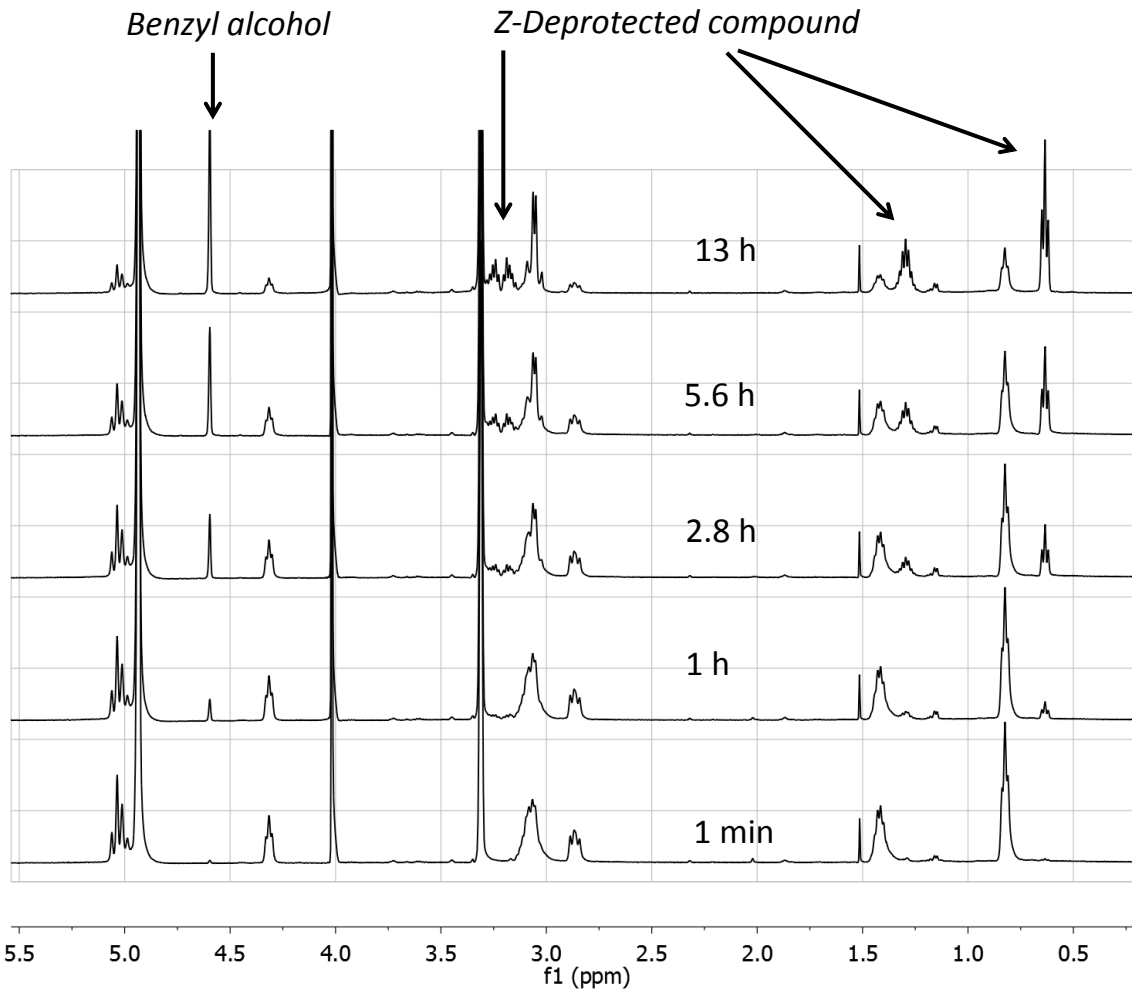
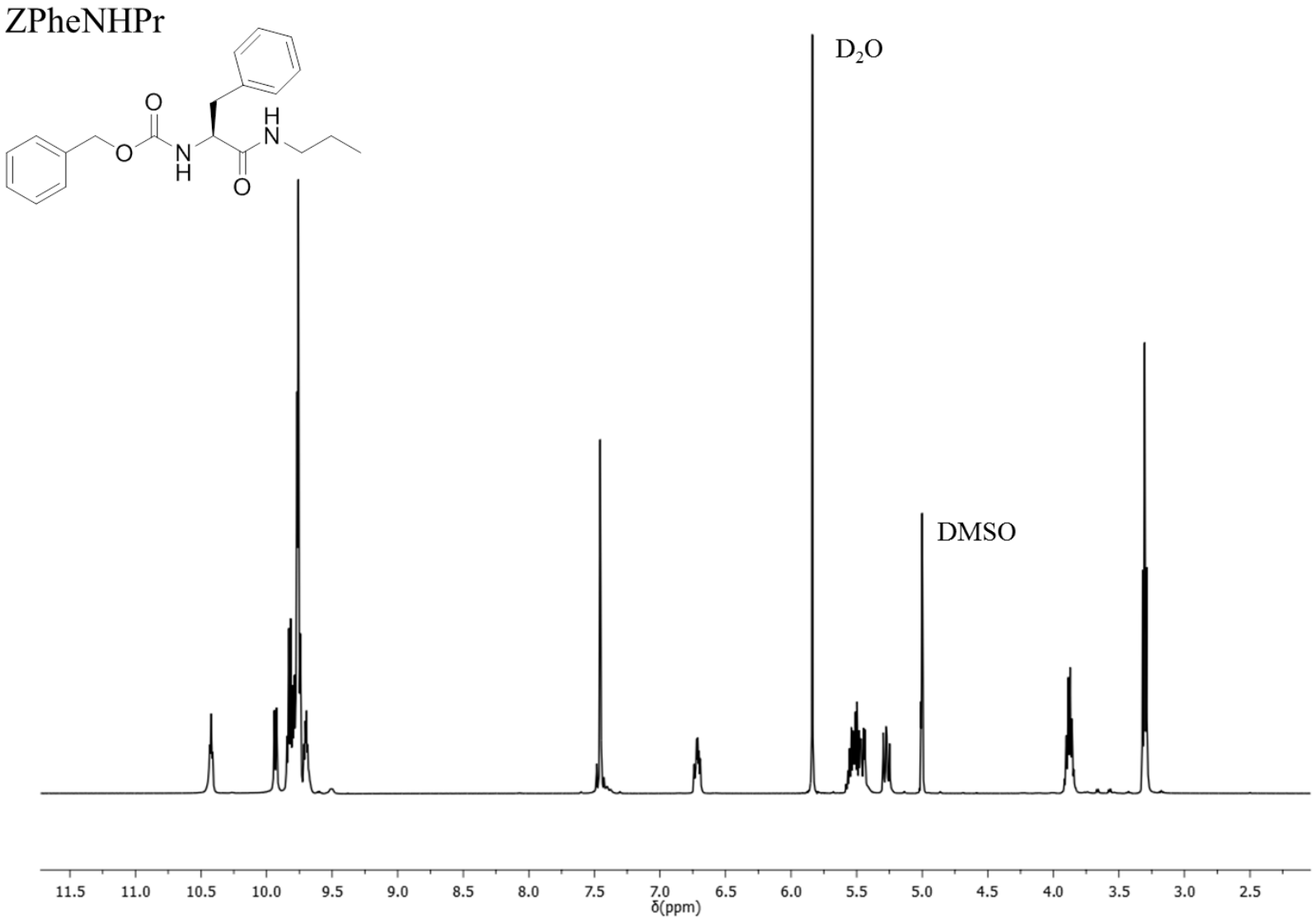
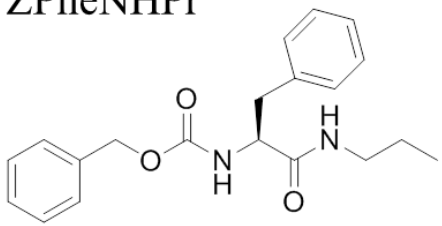


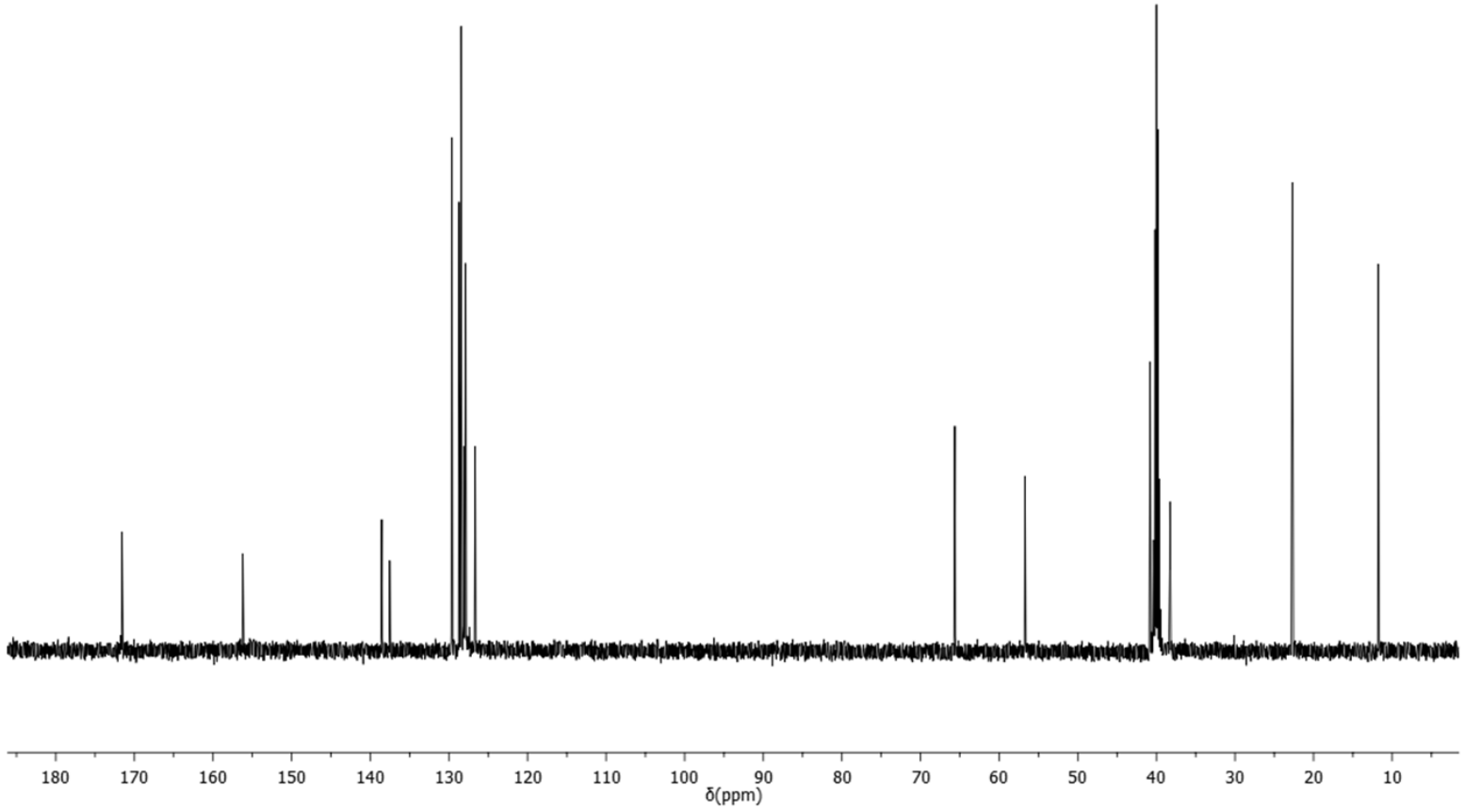
Figure S2. ^{1}H NMR spectra recorded for the basic hydrolysis of ZPheNHPr (15 mM) in CD_3OD ([NaOH] = 0.1 M).

^{1}H and ^{13}C NMR spectra for characterization purposes

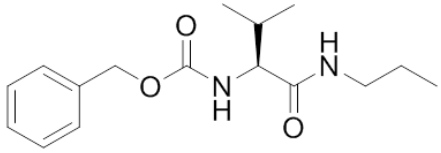

ZPheNHPr
O
O
N
H
H
N
O
D2O
DMSO
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
δ(ppm)

ZPheNHPr


O
O
N
H
H
N
O

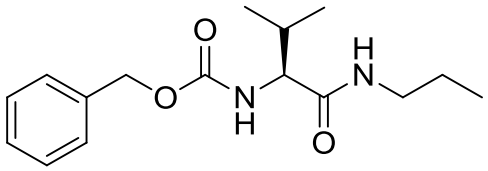

180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
δ(ppm)

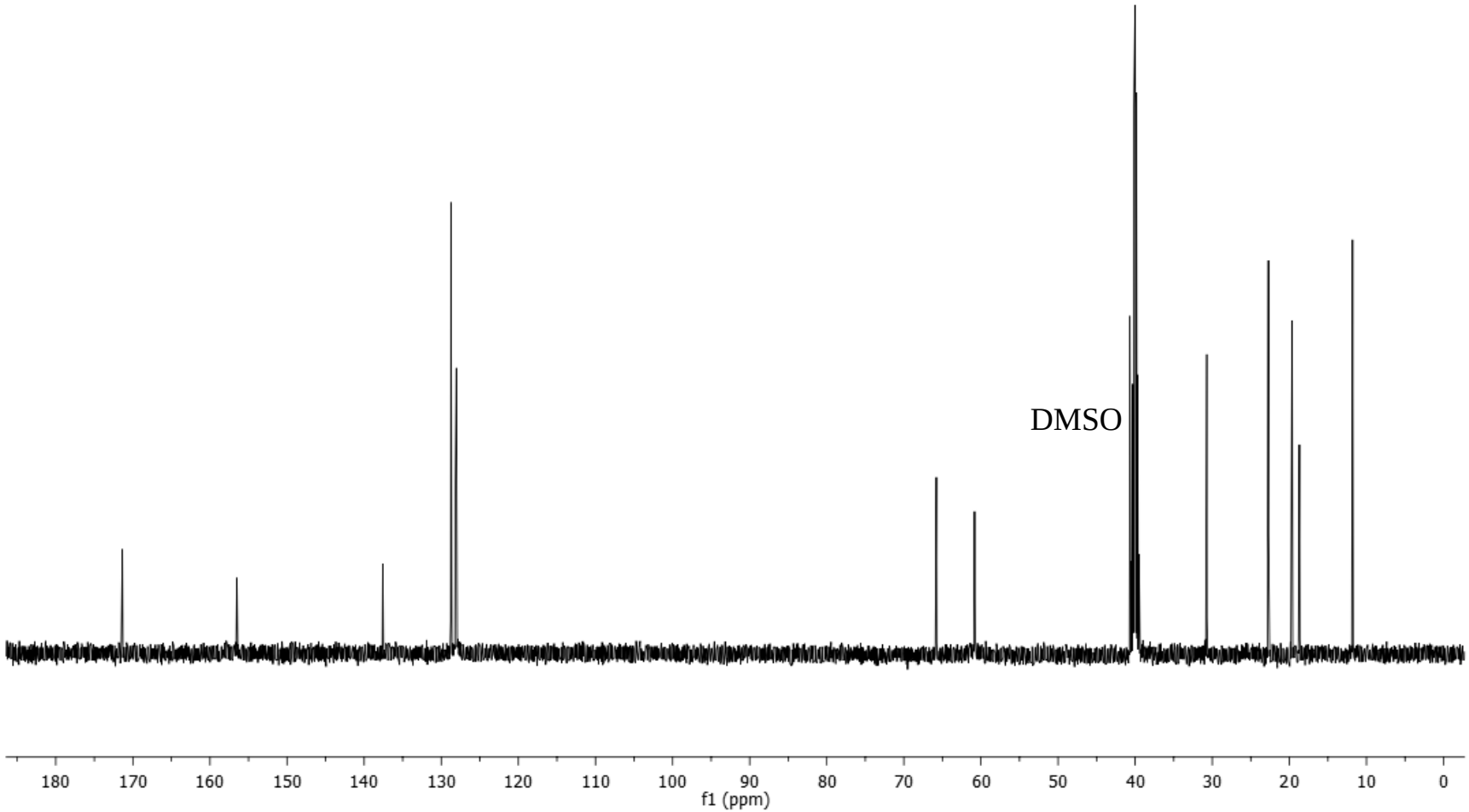
ZValNHPr


O
O
N
H
H
N
O

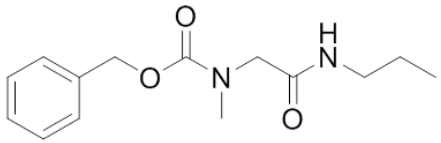
D2O
DMSO
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

ZValNHPr


O
O
N
H
H
N
O

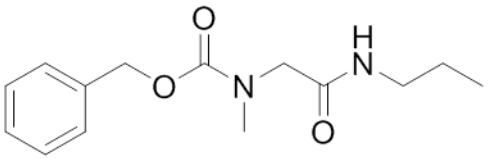

DMSO
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

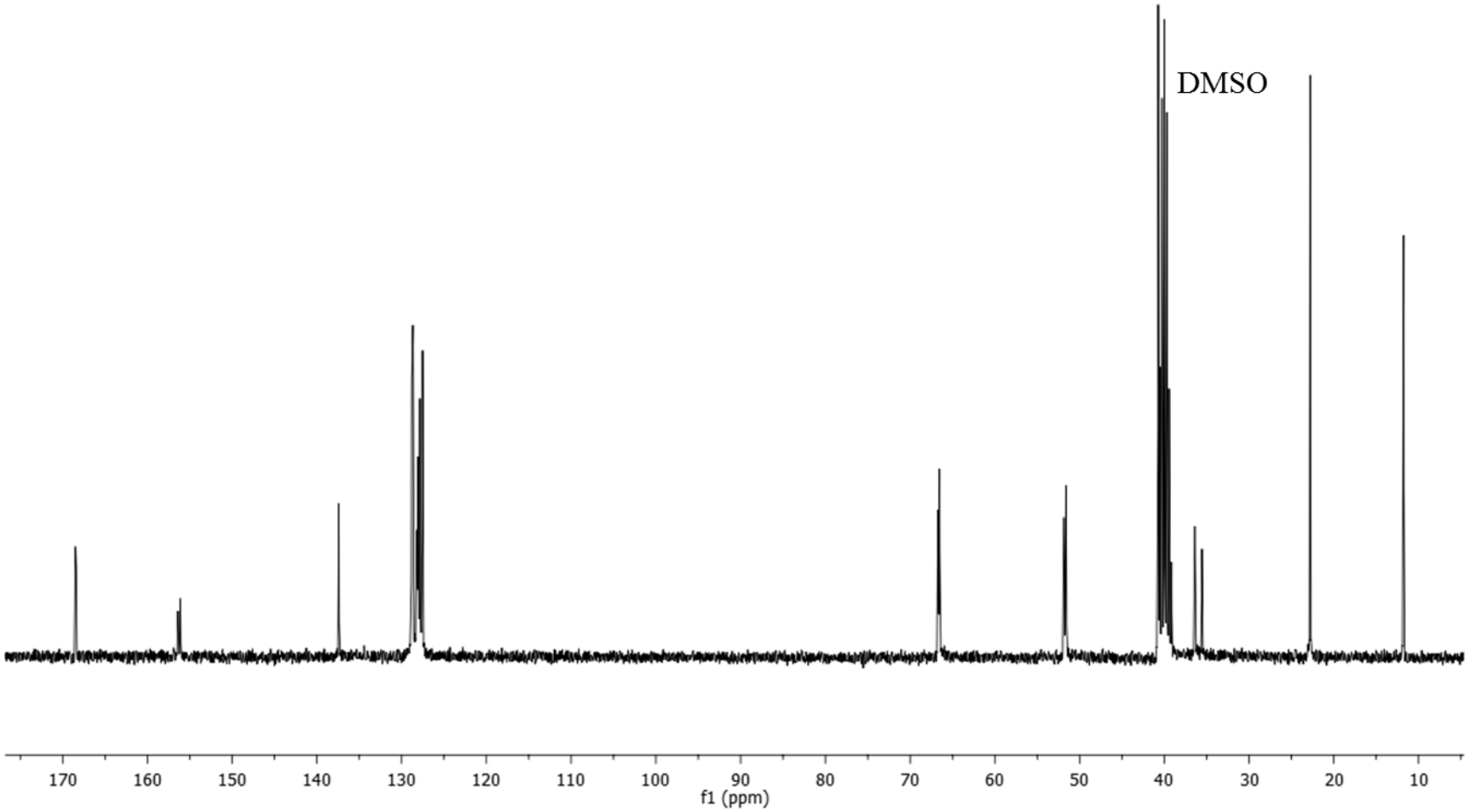
ZSarNHPr

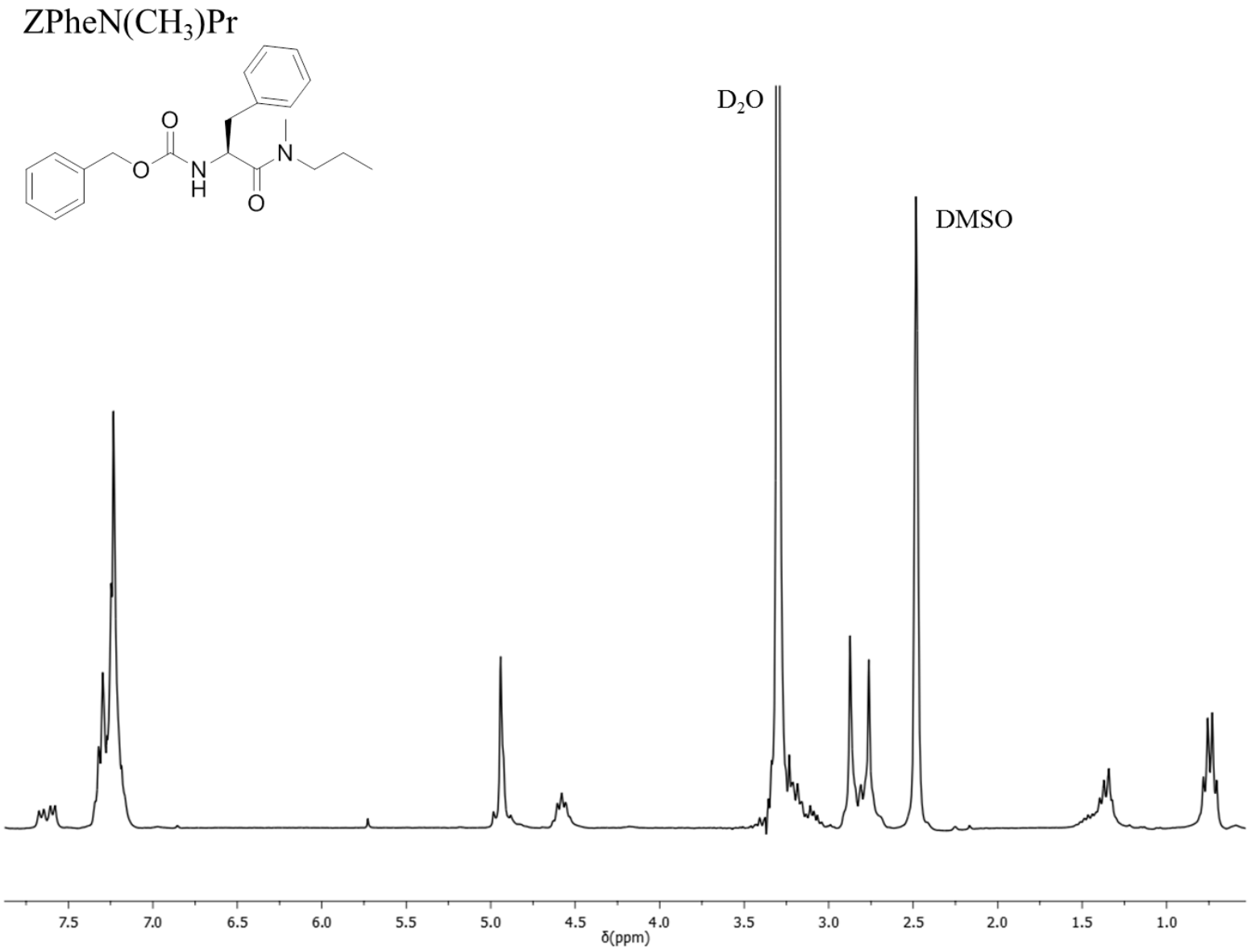

O
O
N
H
N
O

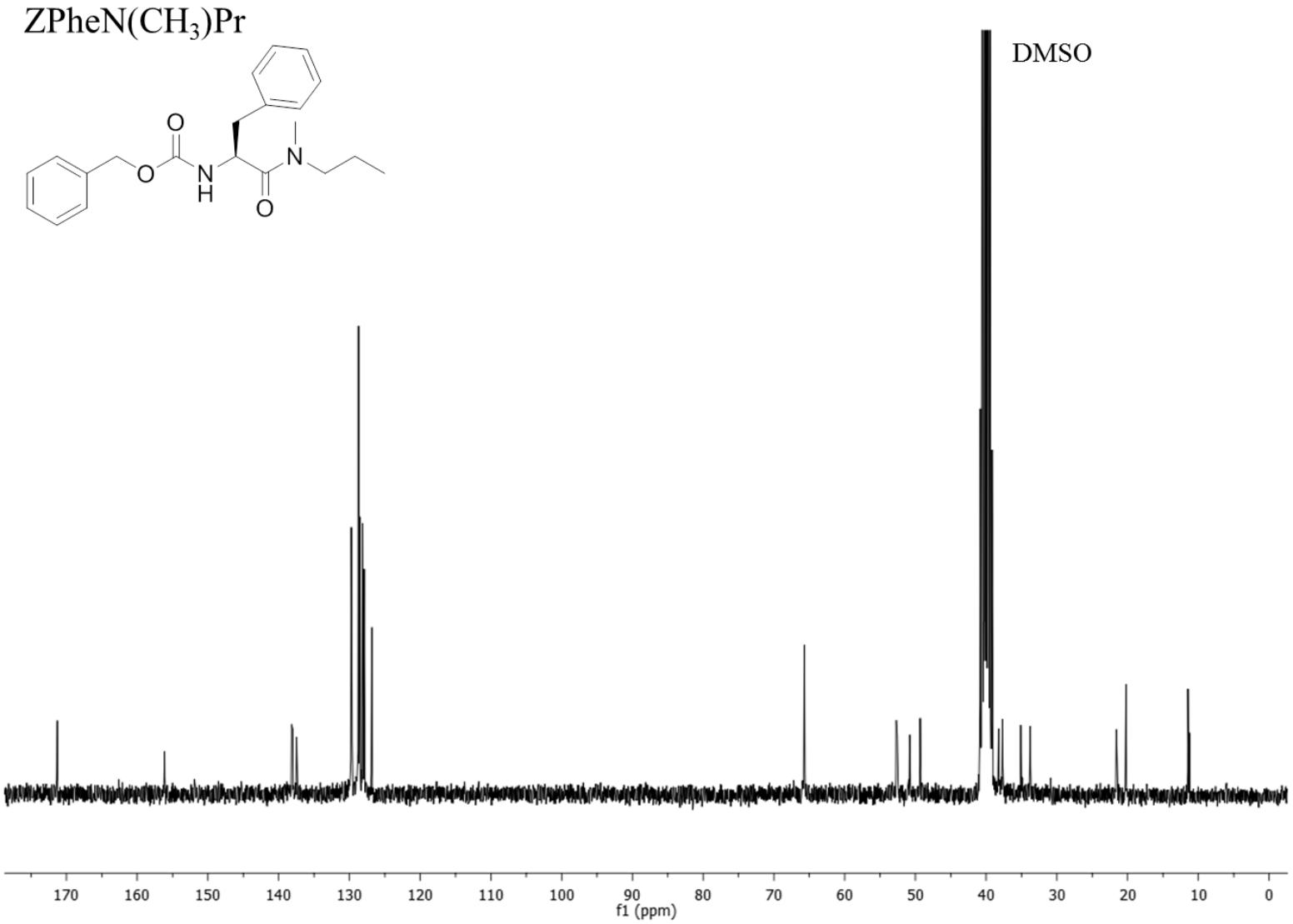
D2O
DMSO
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

ZSarNHPr

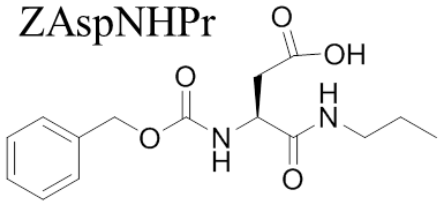

O
O
N
H
N
O

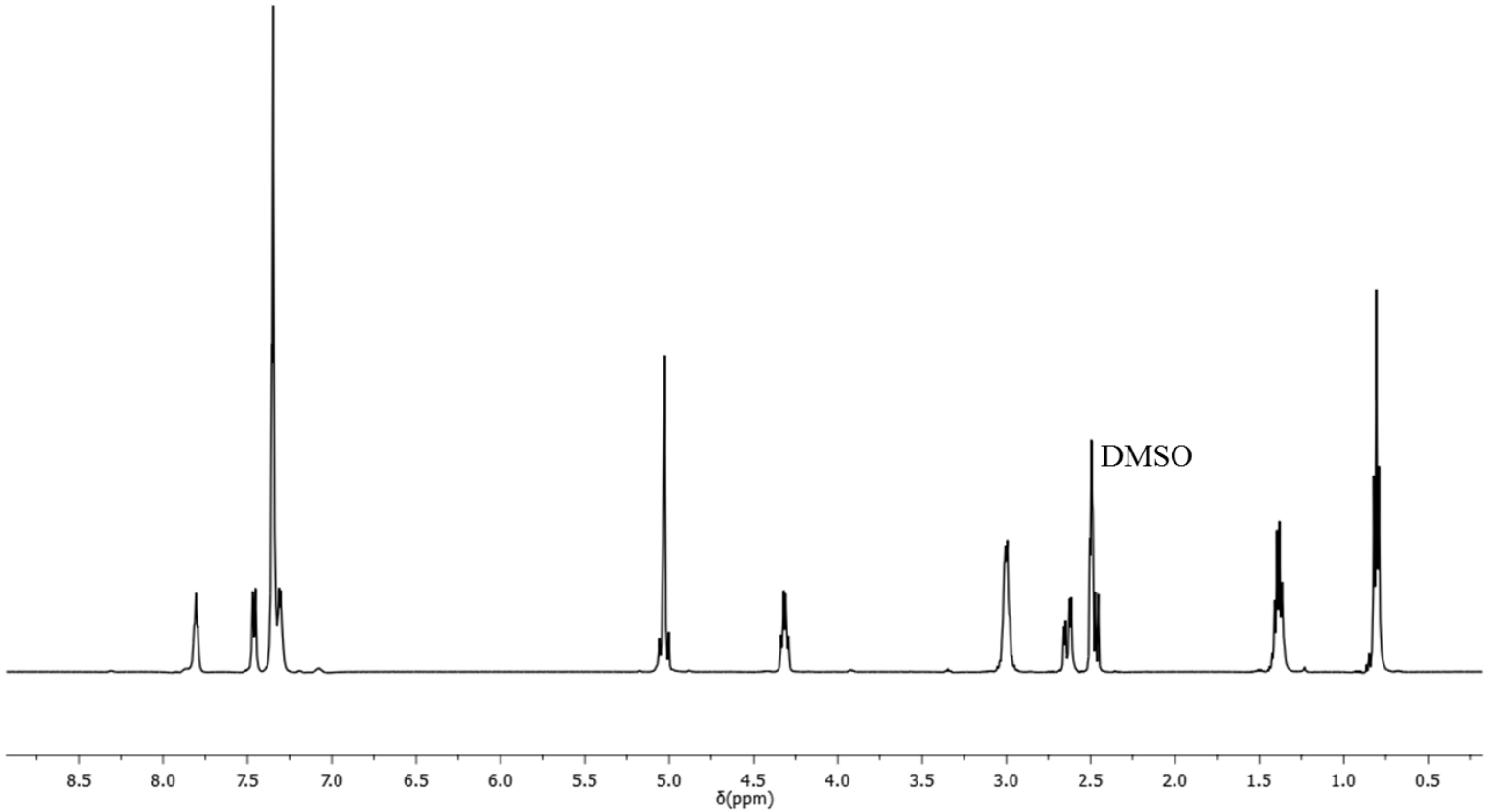

DMSO
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)


ZPheN(CH3)Pr
D2O
DMSO
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
δ(ppm)

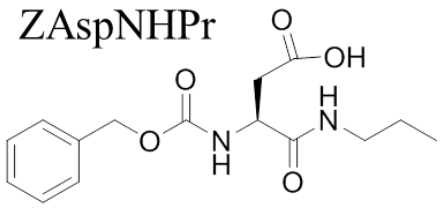

ZPheN(CH3)Pr
DMSO
O
O
N
H
N
O
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

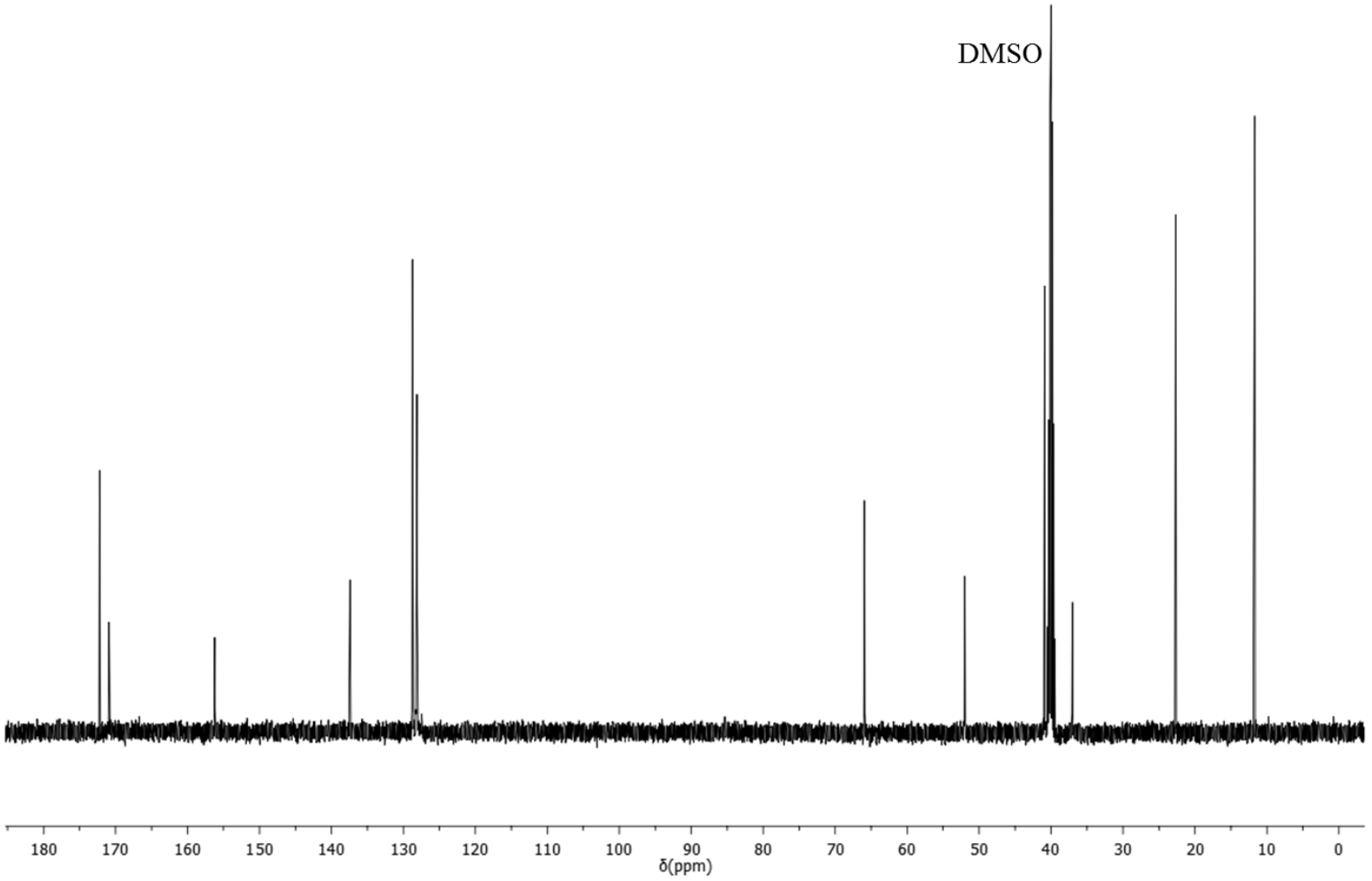
ZAspNHPr


O
OH
O
H
N
O
N
H
O

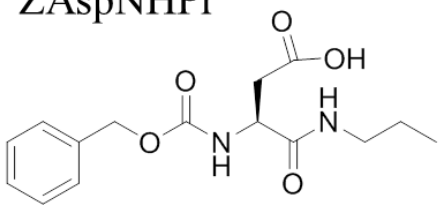

DMSO
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
δ(ppm)

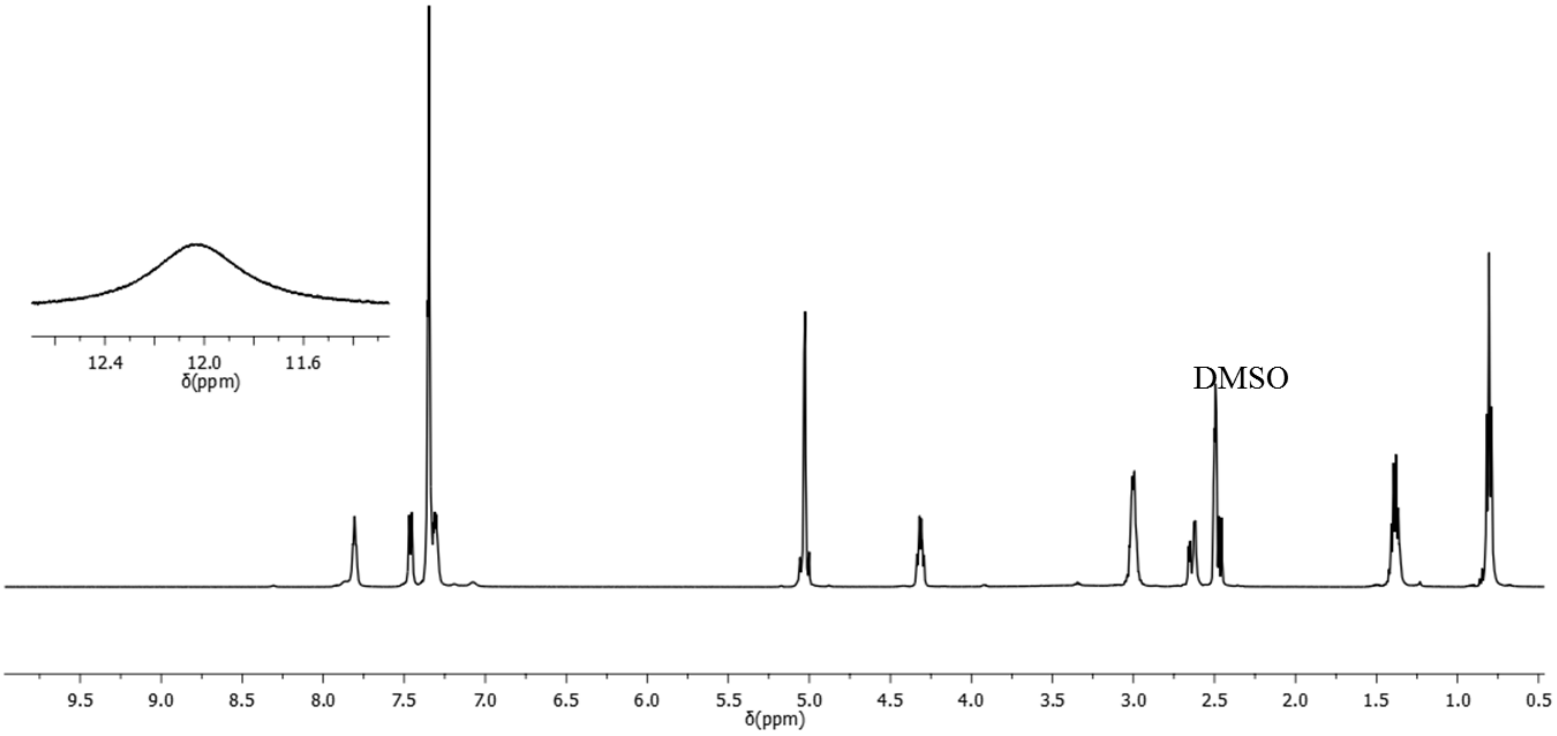
ZAspNHPr


O
OH
O
H
N
O
N
H
O

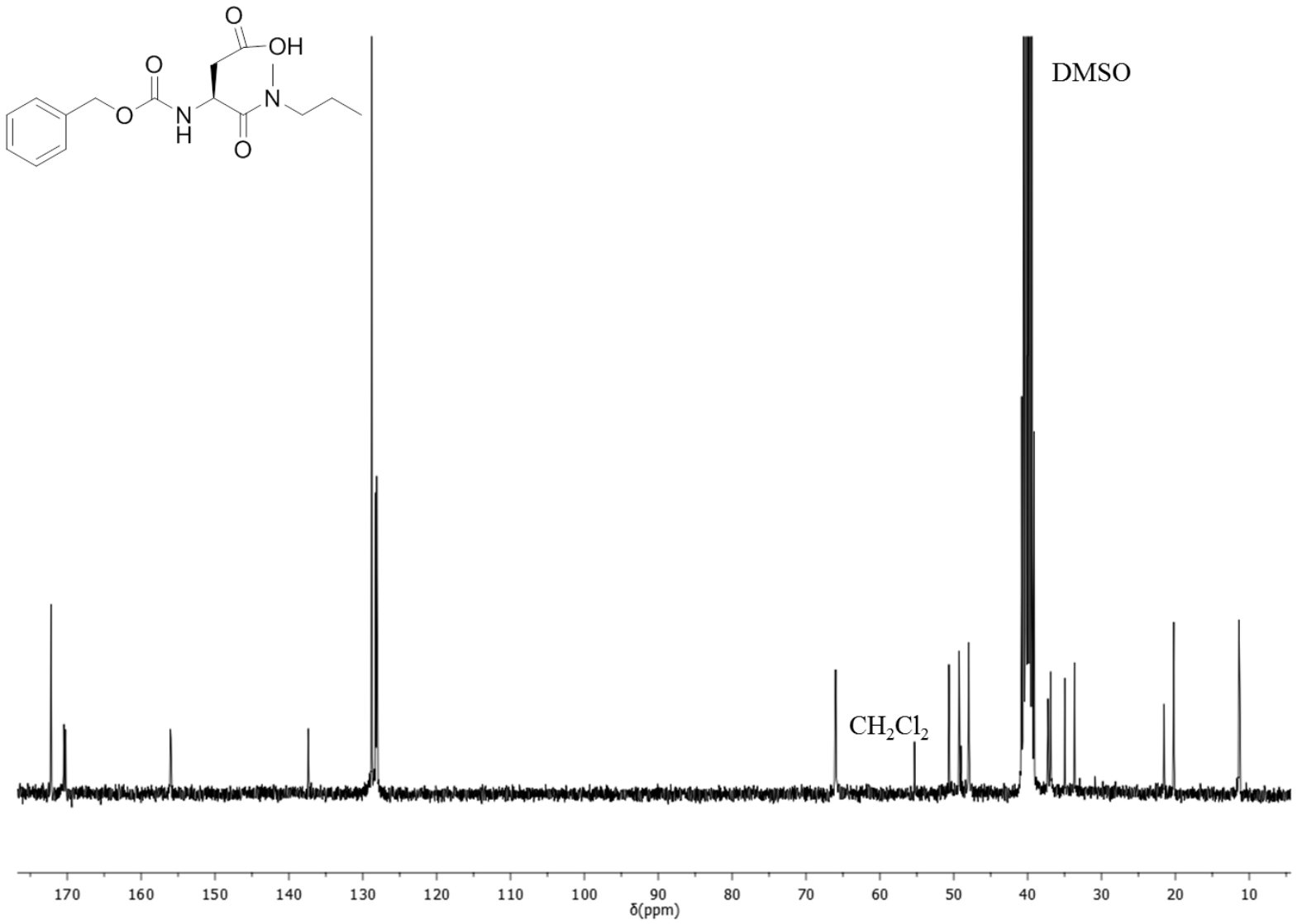

DMSO
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
δ(ppm)

ZAspNHPr

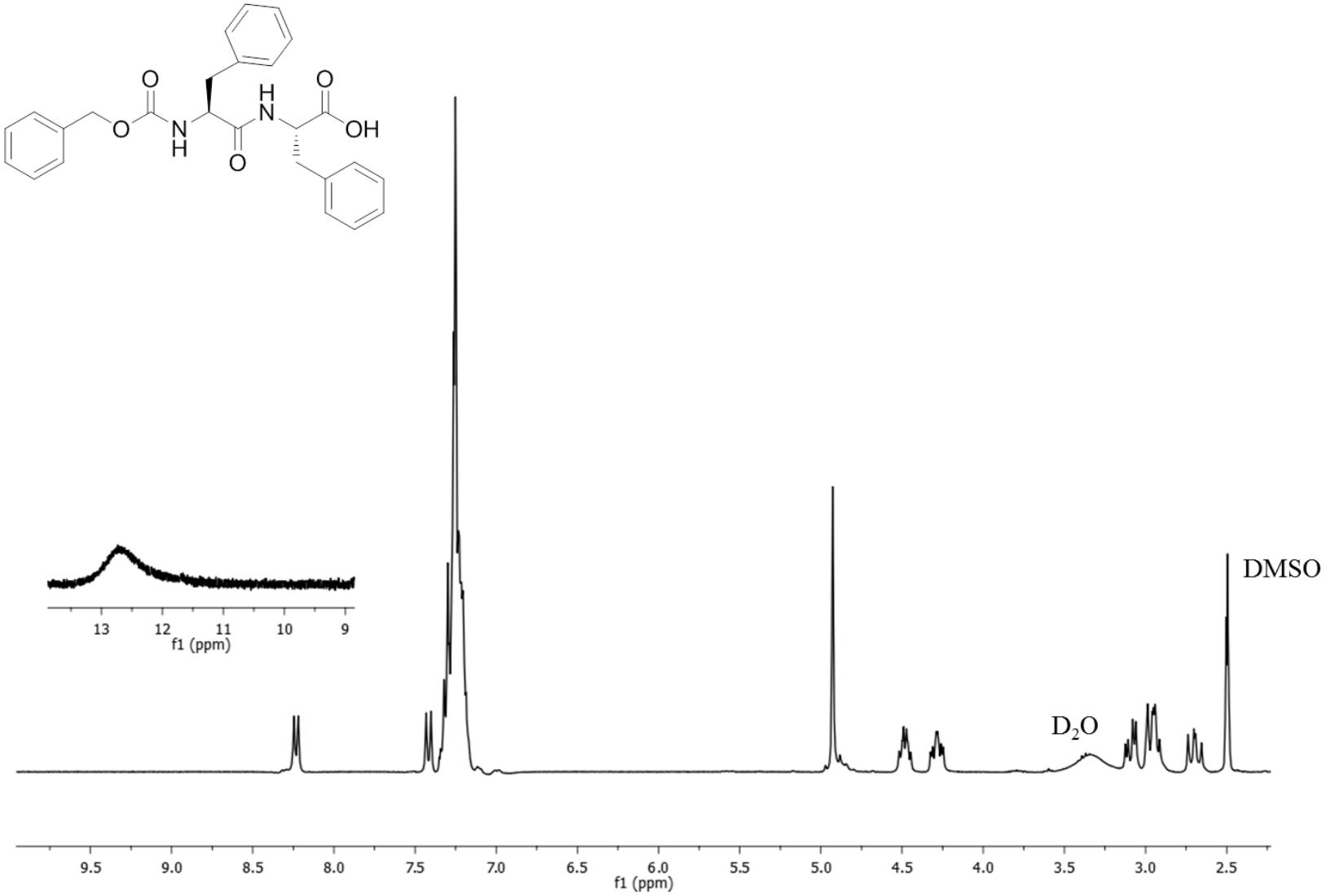

O
OH
O
H
N
O
N
H
O


12.4
12.0
11.6
δ(ppm)
DMSO
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
δ(ppm)

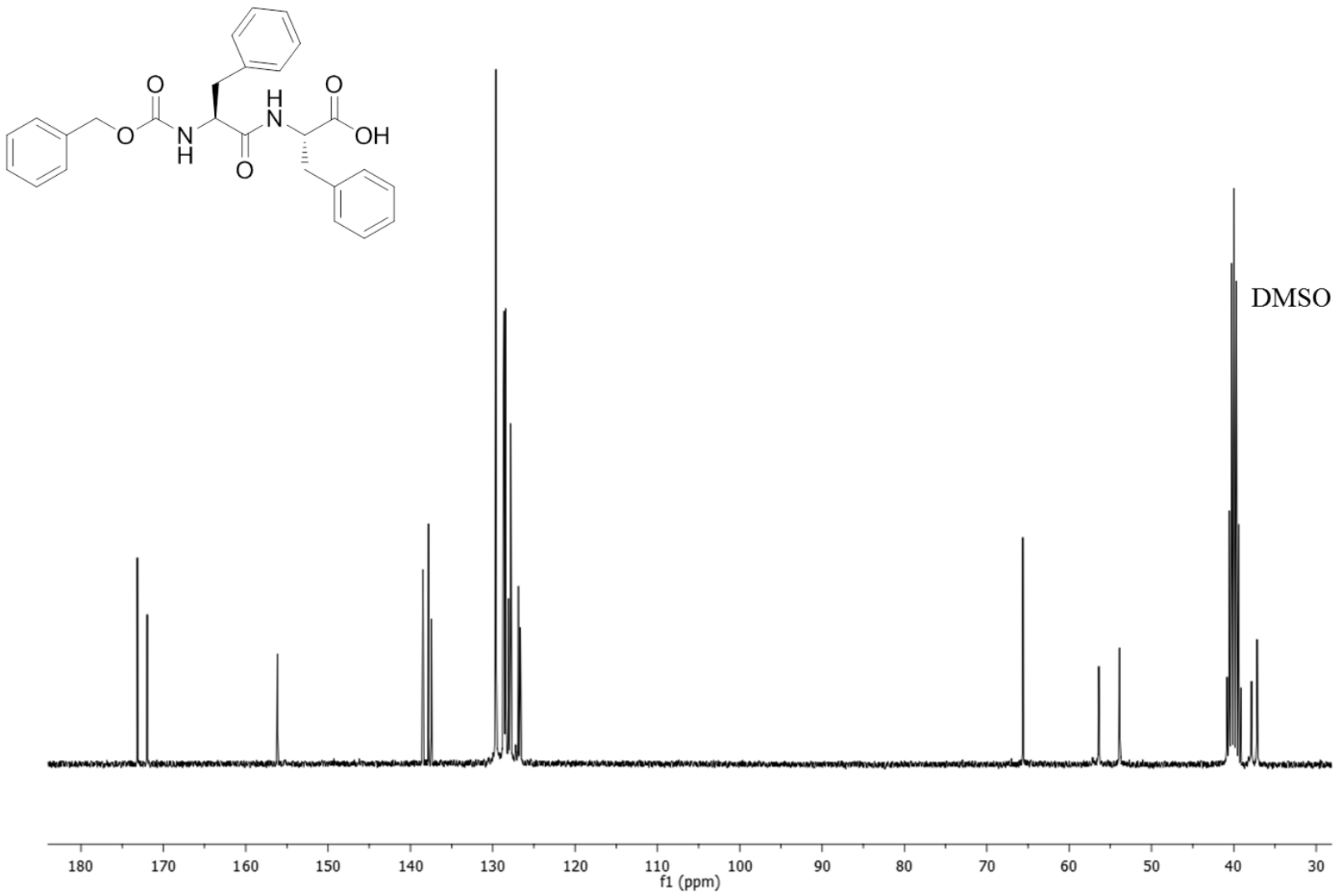
ZAspN(CH_3)Pr


O
OH
O
O
N
H
N
O
DMSO
CH_2Cl_2
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
δ(ppm)

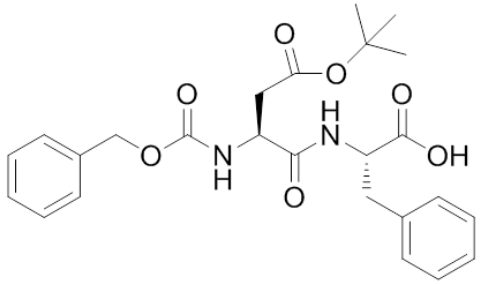
ZPhePheOH


O
O
N
H
H
N
O
O
OH
13
12
11
10
9
f1 (ppm)
DMSO
D2O
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
f1 (ppm)

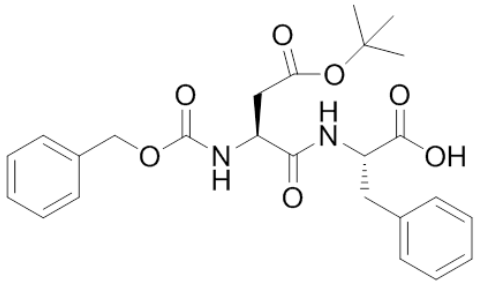
ZPhePheOH

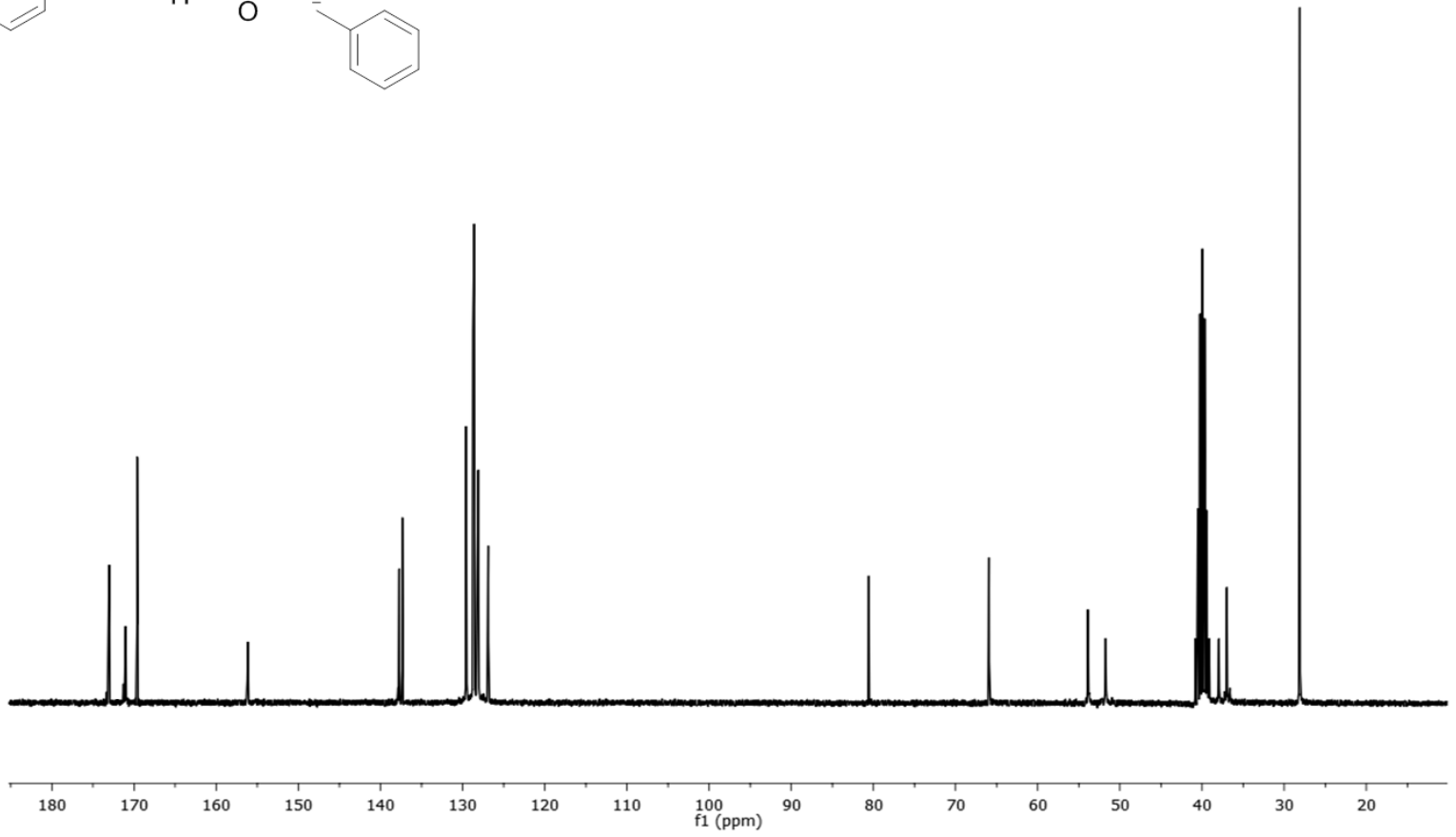

O
O
N
H
H
N
O
O
OH
DMSO
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
f1 (ppm)

ZAsp(tBu)PheOH

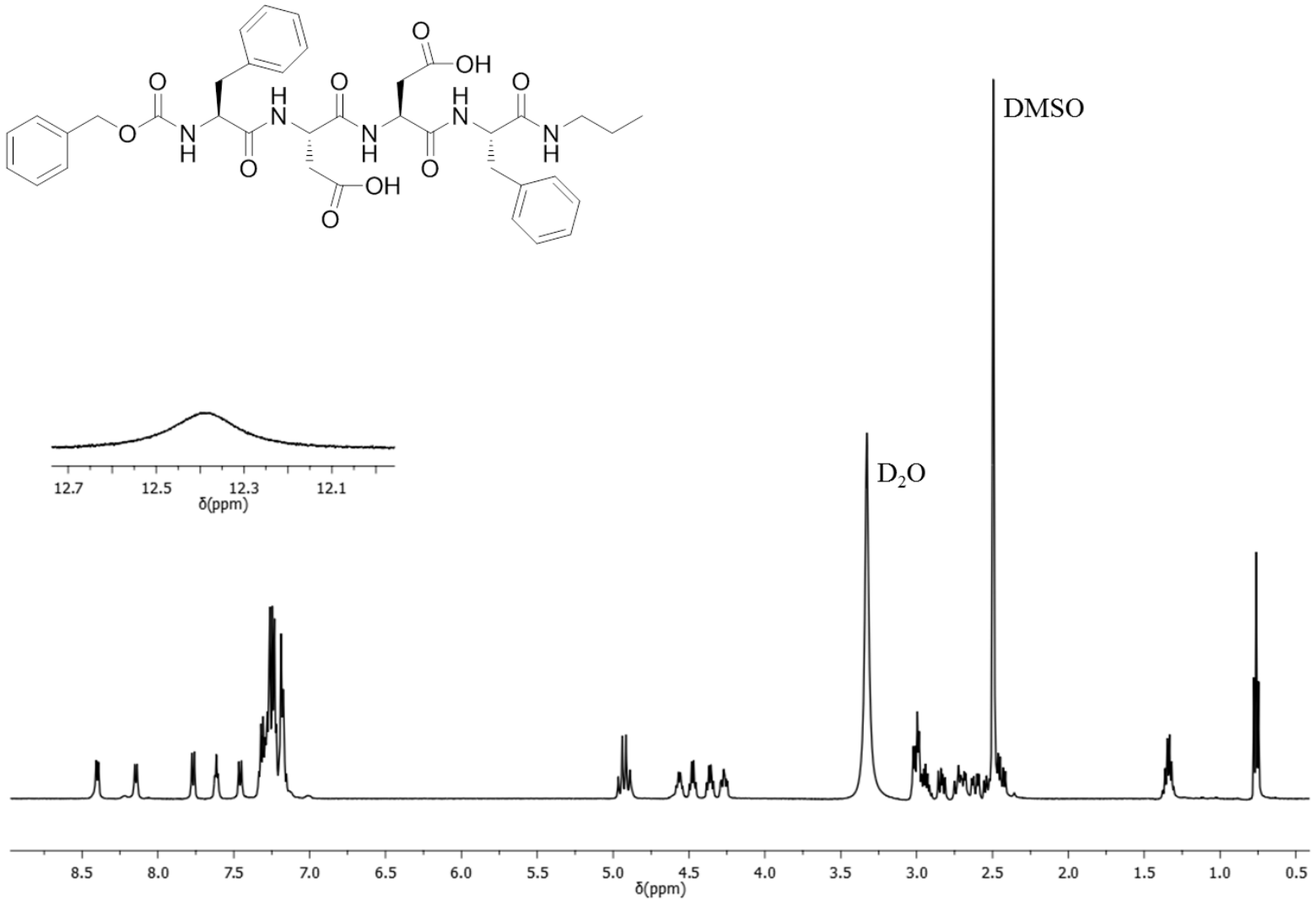


ZAsp(tBu)PheOH

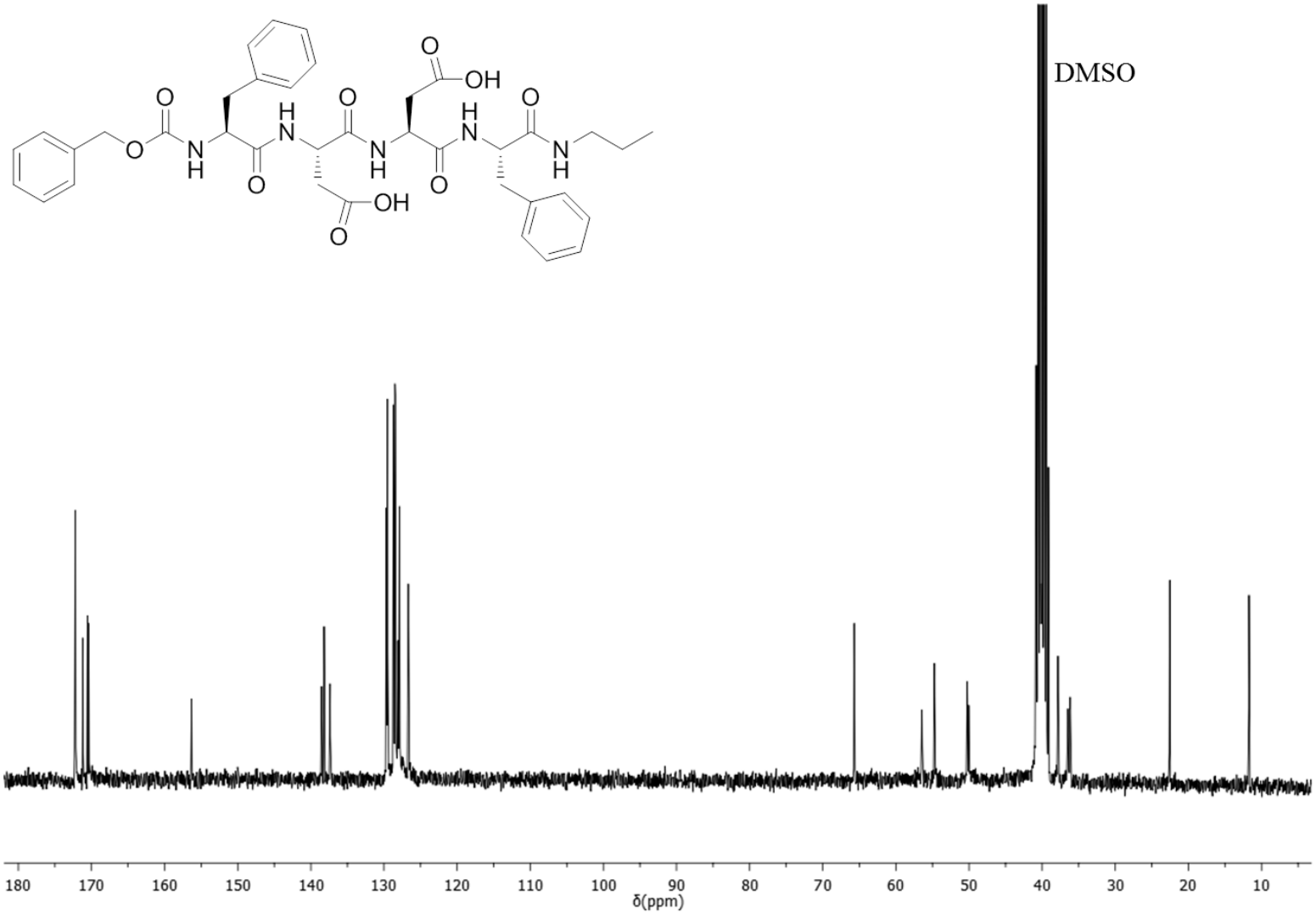




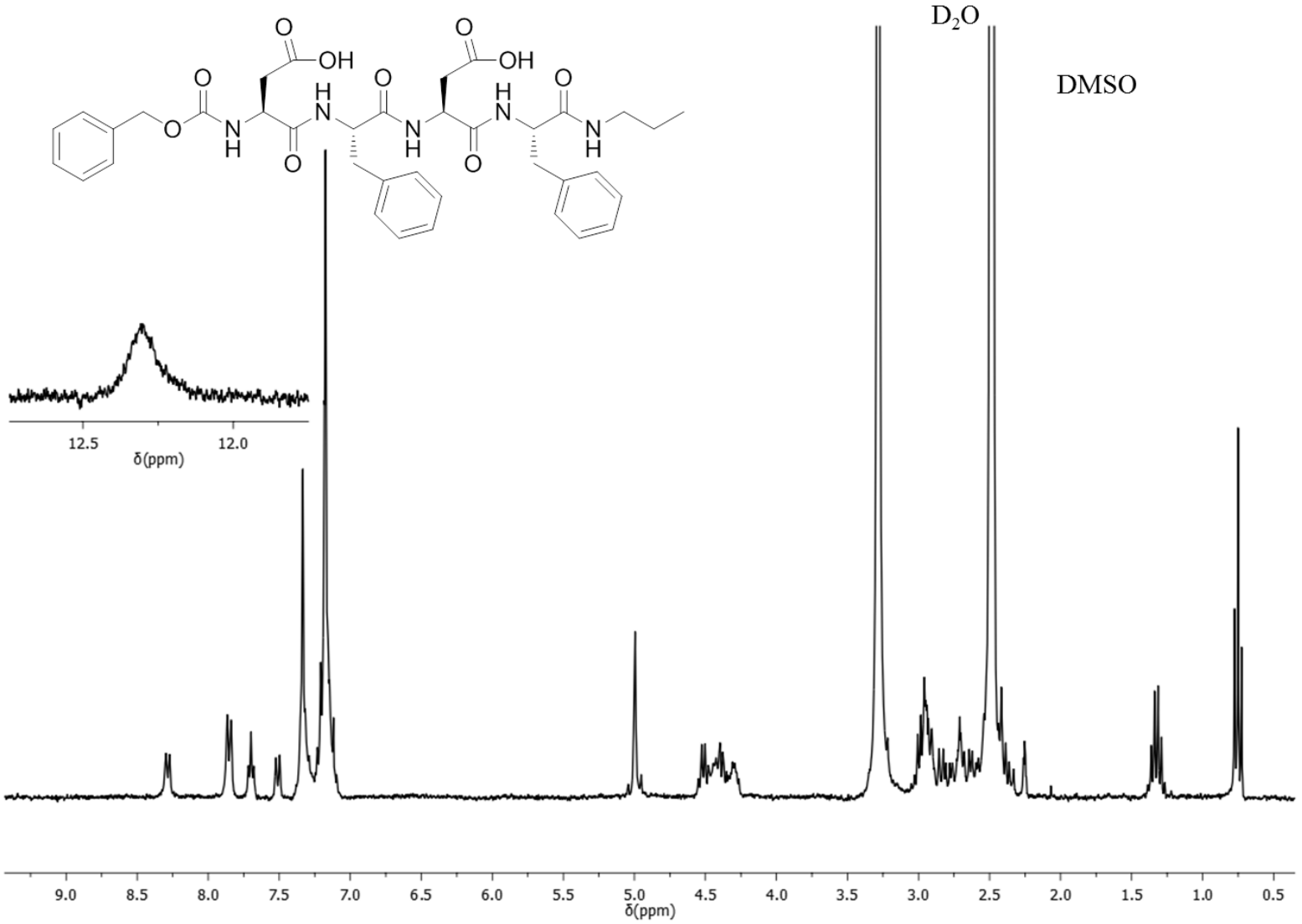
ZPheAspAspPheNHPr



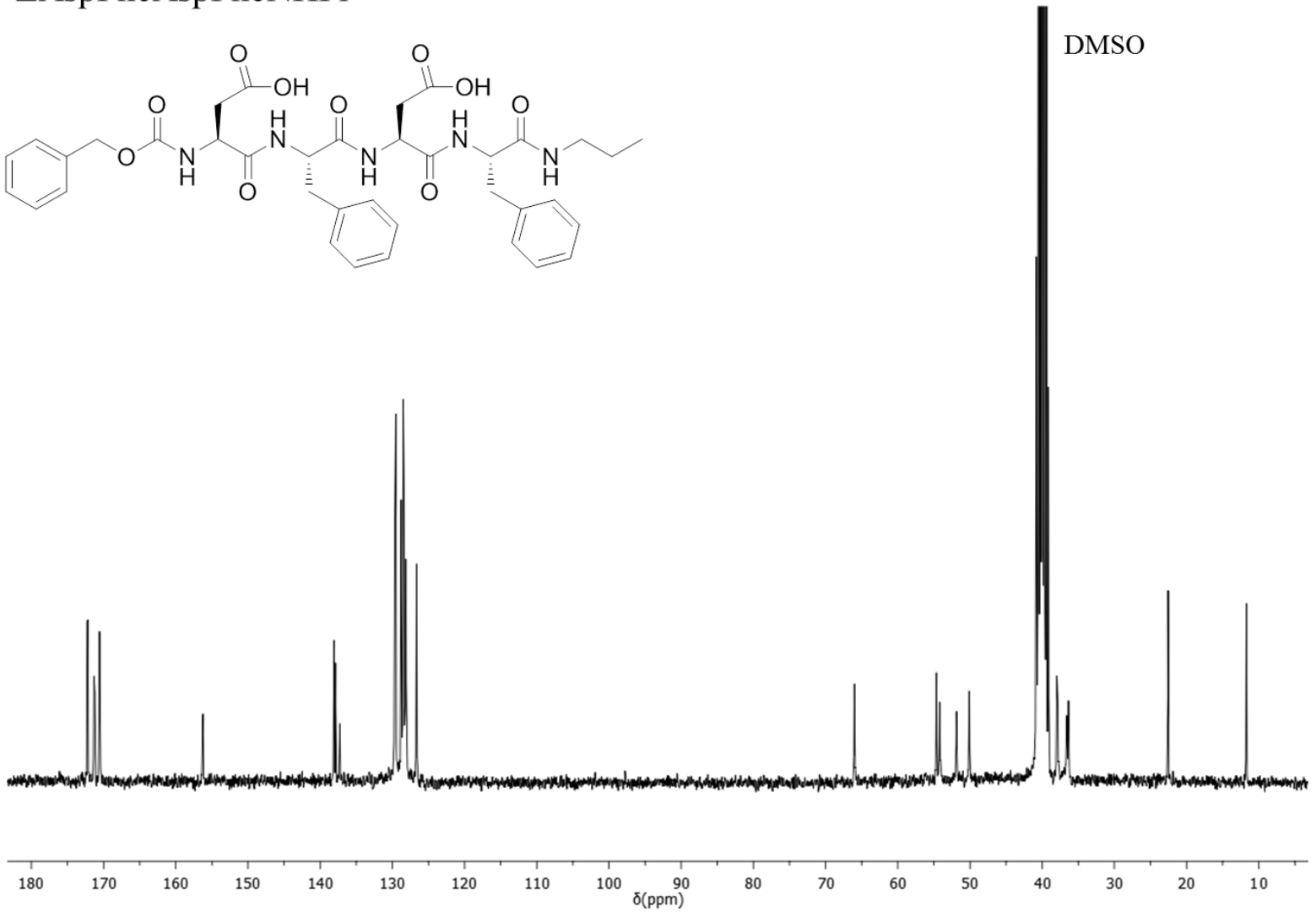
ZPheAspAspPheNHPr



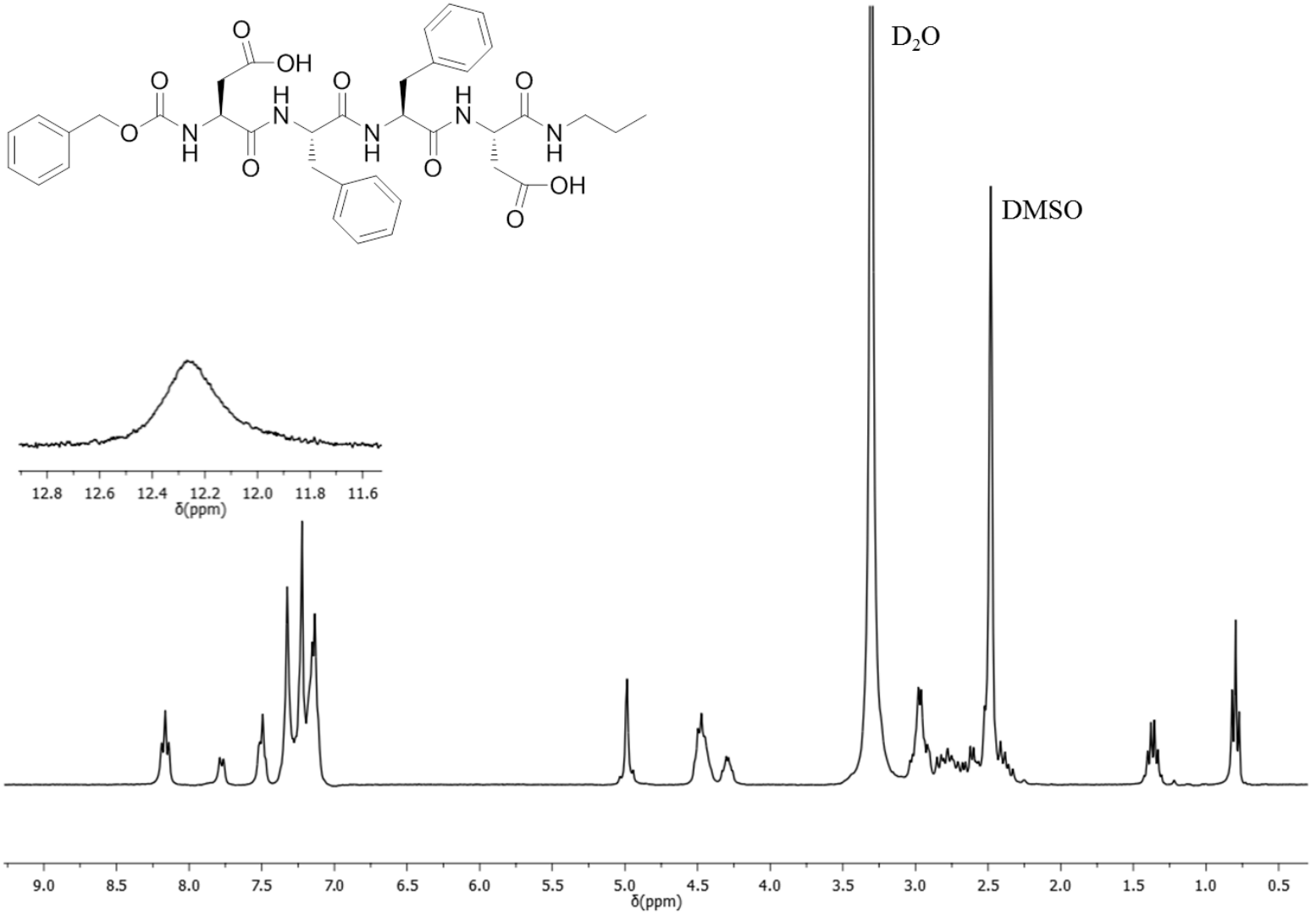
ZAspPheAspPheNHPr



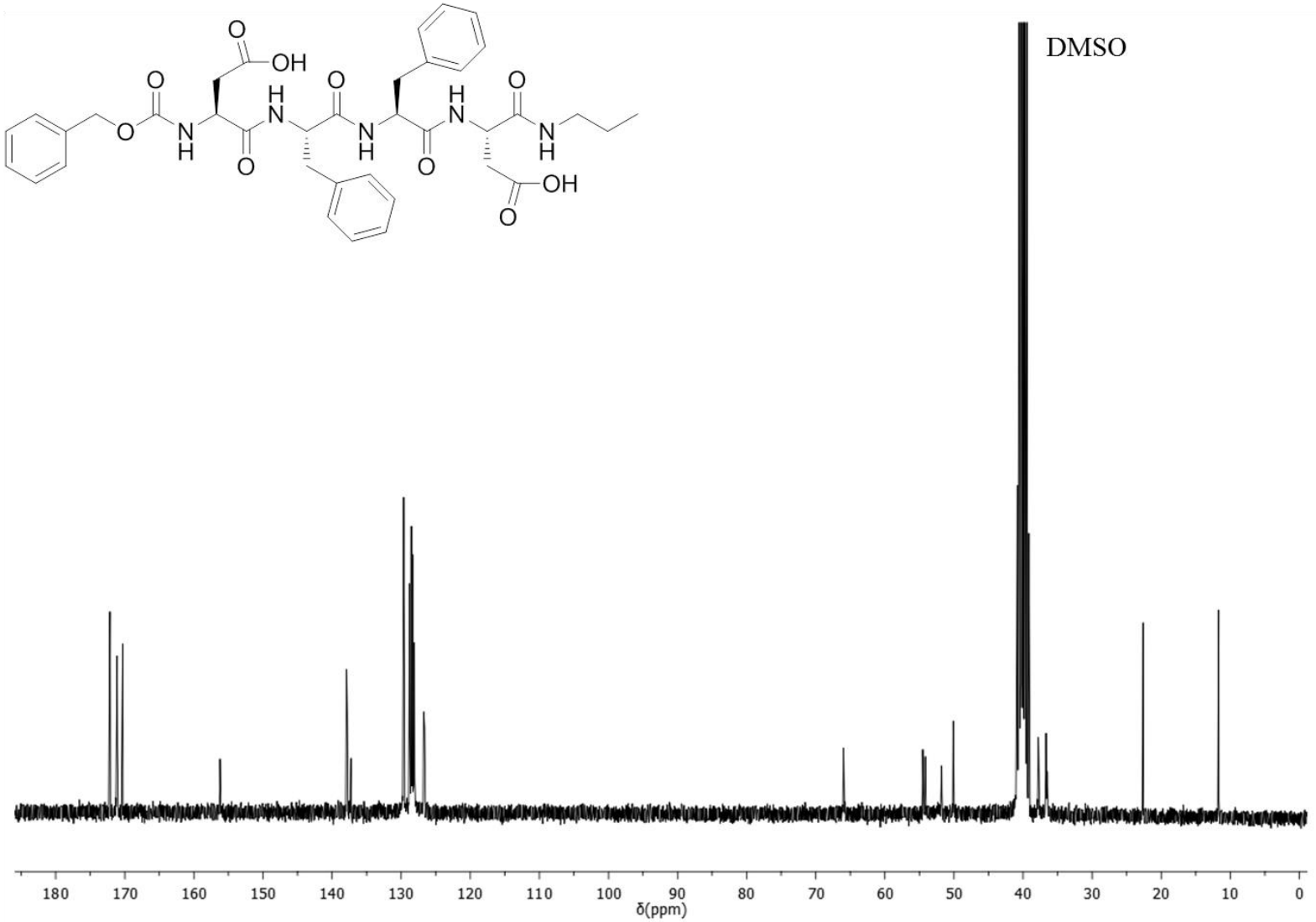
ZAspPheAspPheNHPr



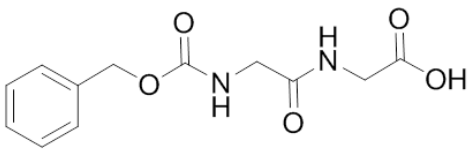
ZAspPhePheAspNHPr

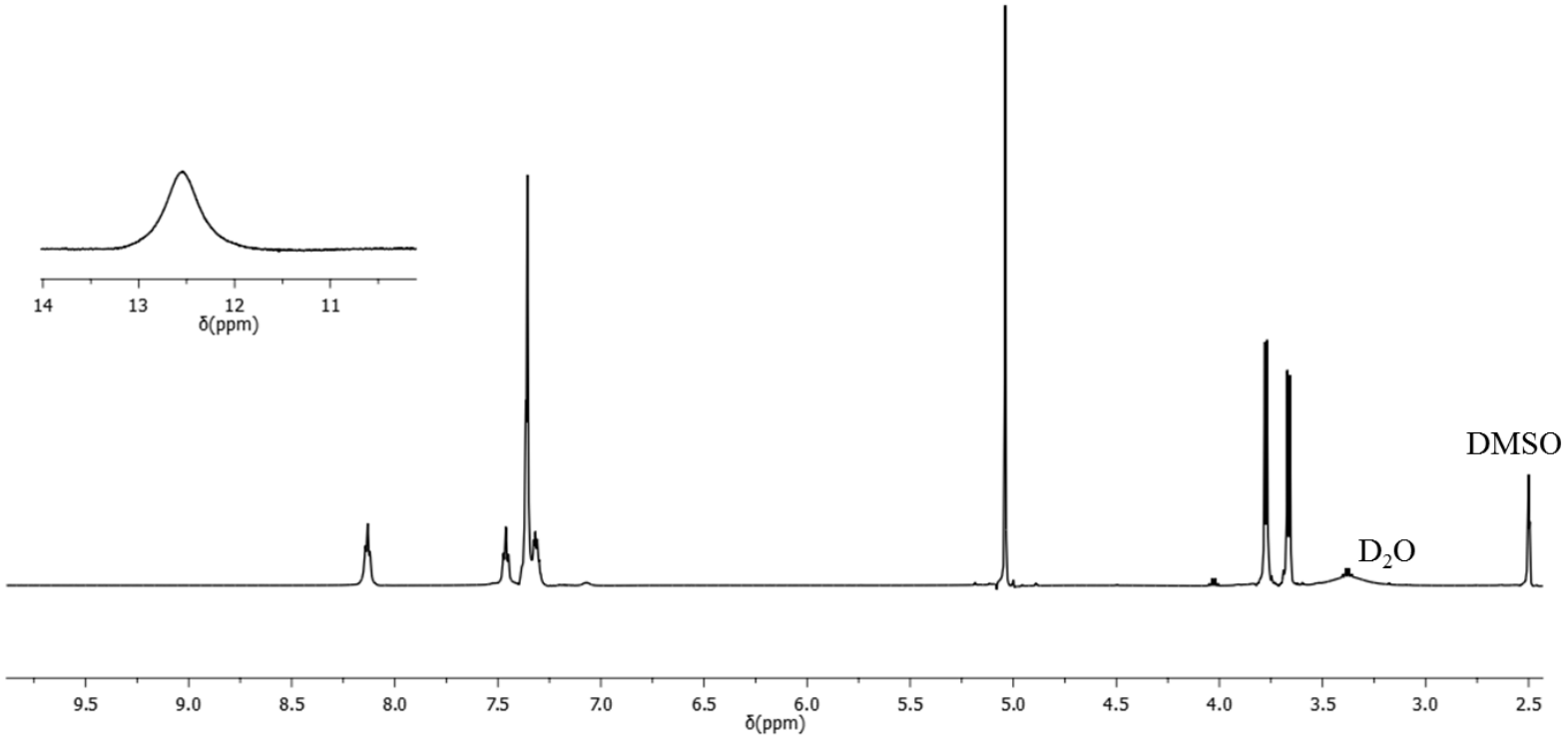


ZAspPhePheAspNHPr



ZGlyGlyOH


O
O
N
H
H
N
O
O
OH


14
13
12
11
δ(ppm)
DMSO
D2O
9.5
9.0
8.5
8.0
7.5
7.0
6.5
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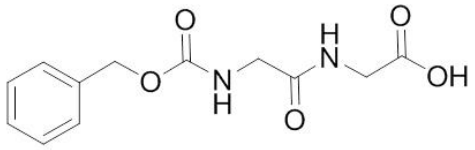
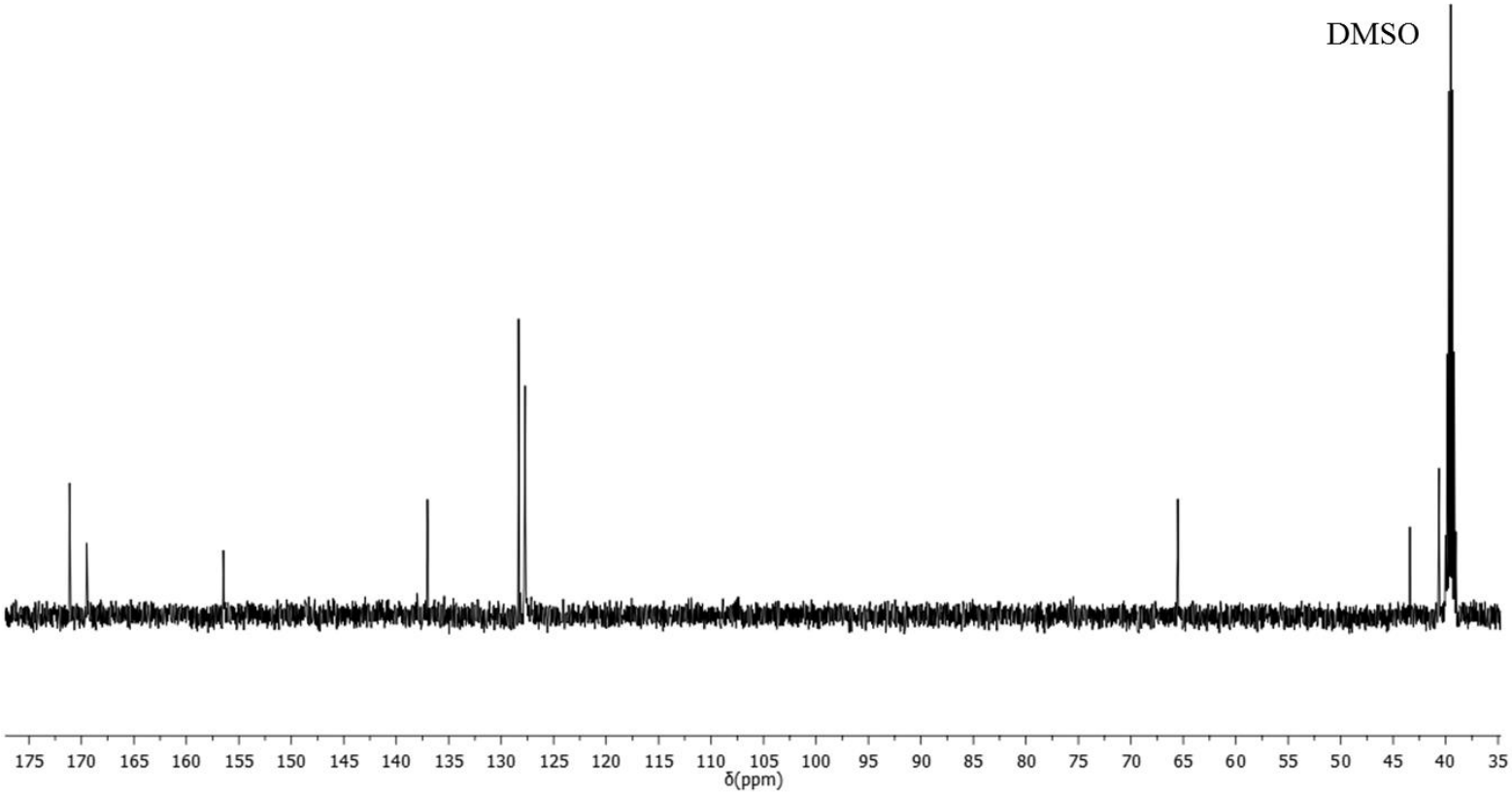
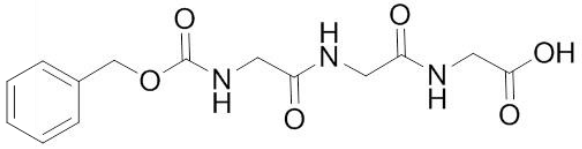
4.0
3.5
3.0
2.5
δ(ppm)

ZGlyGlyOH

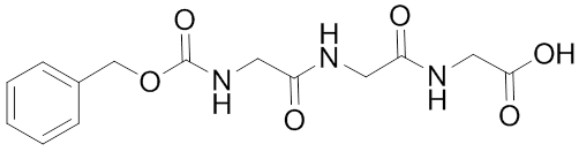

O
O
N
H
H
N
O
OH
O

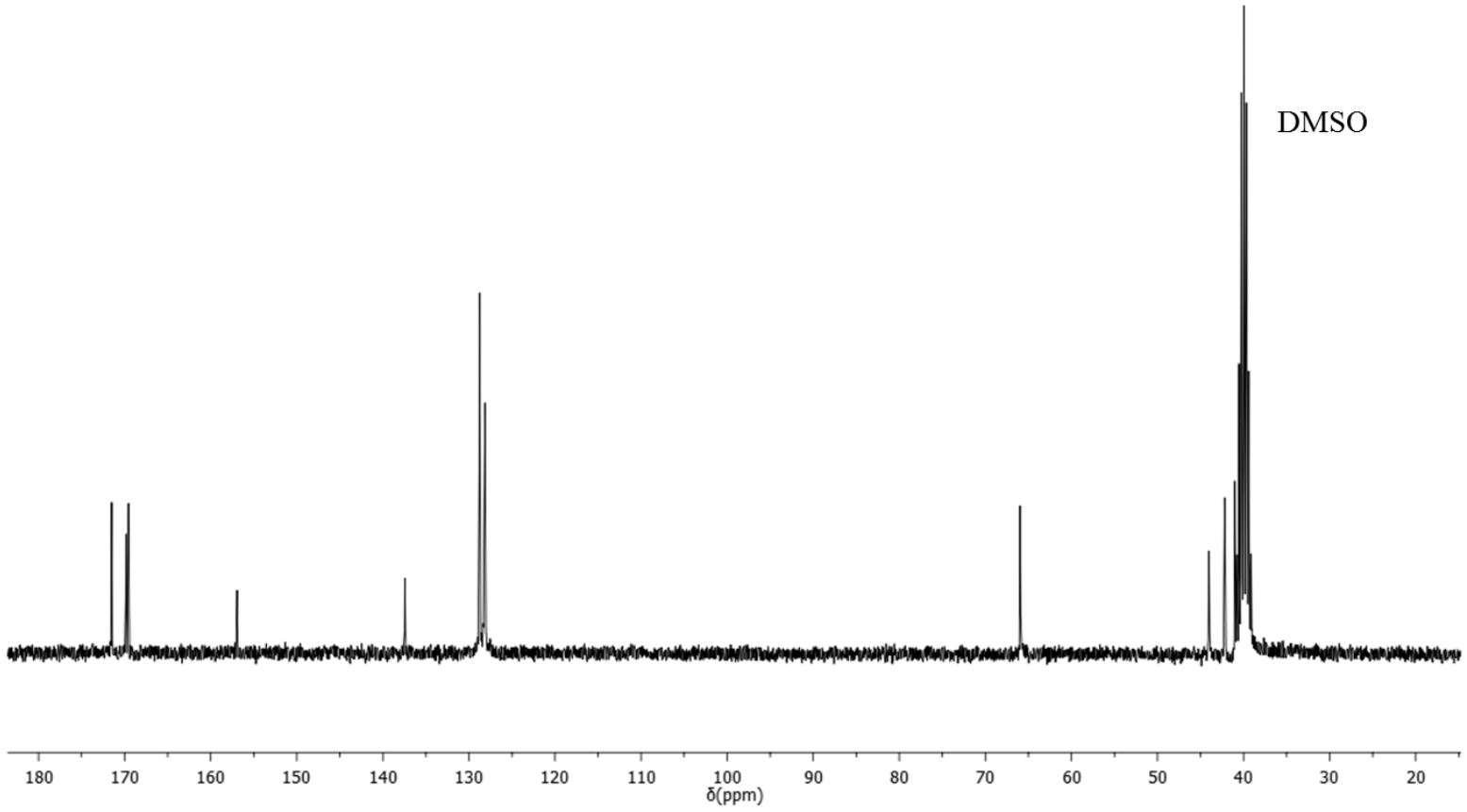

DMSO
175 170 165 160 155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35
δ(ppm)

ZGlyGlyGlyOH



ZGlyGlyGlyOH





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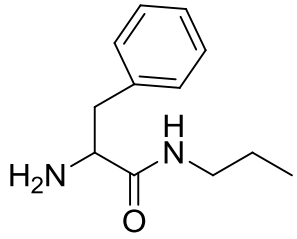

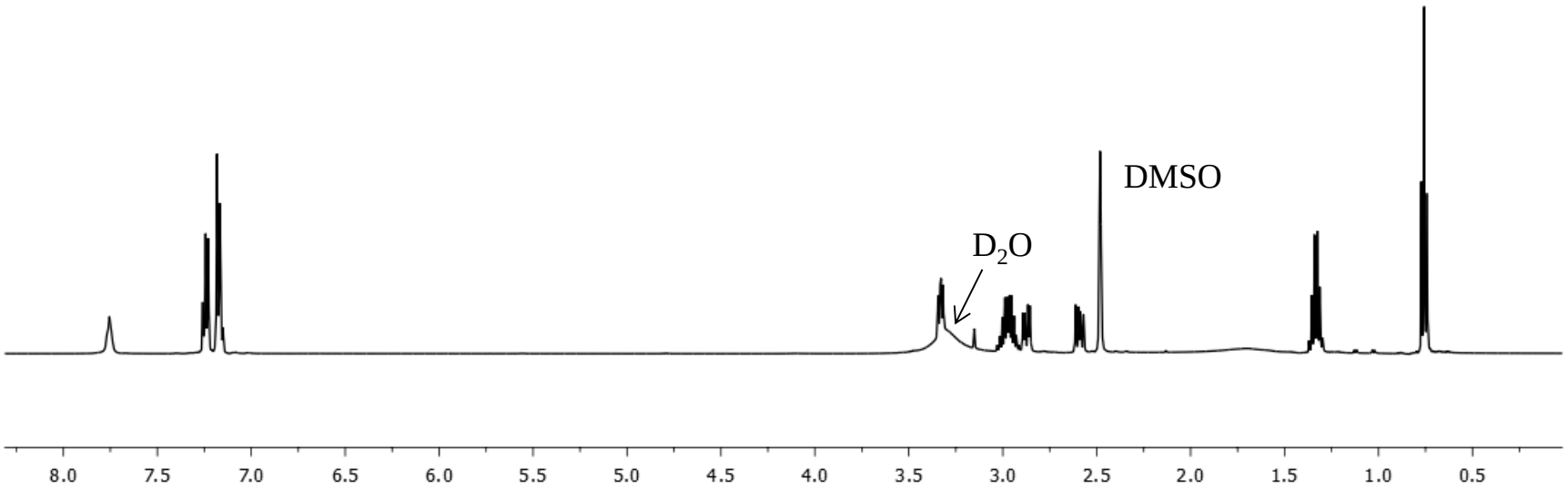

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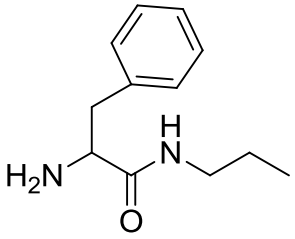
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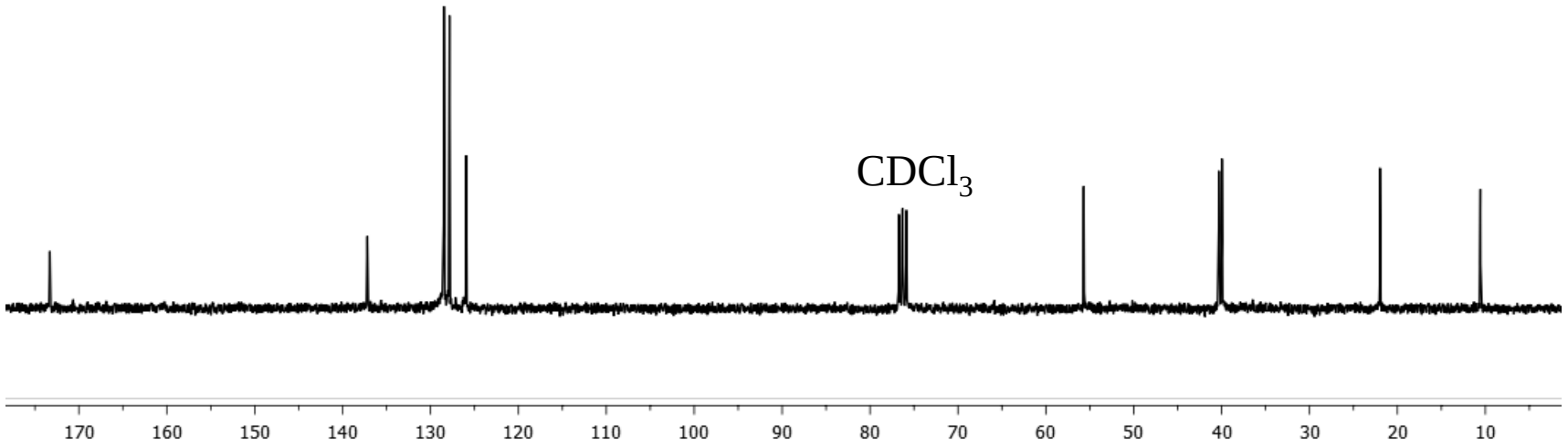
O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