Supporting Information for:
Exploiting an Inherent Neighboring Group Effect of α-Amino acids to Synthesize Extremely Hindered Dipeptides

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General Methods.

HFIP (1,1,1,3,3,3-Hexafluoro-2-propanol), DAST (diethylaminosulfurtrifluoride), anhydrous Dichloromethane (DCM), anhydrous Dimethylformamide (DMF), anhydrous Methanol (MeOH) and redistilled Diisopropylethylamine (DIPEA) were obtained from Sigma-Aldrich and used without purification. All Fmoc-amino acids and unprotected amino acids were obtained from either Novabiochem or Bachem. All other chemicals were purchased from Sigma-Aldrich and used without purification.
HPLC-MS analysis was performed on a Hewlett-Packard Series 1200 with a Waters Xterra MS C18 column (3.5μm packing, 4.6 mm x 100mm) with a solvent system of H_2O/acetonitrile with 0.1% formic acid at a flow rate of 0.8mL/min.
NMR experiments were performed on a Bruker 500mHz NMR with chemical shifts (δ) reported relative to DMSO-d_6 residual solvent peaks. Analysis of 2D NMR data was performed using Sparky 3, T. D. Goddard and D. G. Kneller, University of California, San Francisco.
Preparatory Scale HPLC purification was performed on a Varian Prostar Prep HPLC with a Waters Xterra column (5 μm packing, 19 mm x 100mm) with a solvent system of H_2O/acetonitrile with 0.1% formic acid at a flow rate of 18 mL/min.
HRMS analysis was performed by either the University of Pittsburgh (Waters LC/Q-Tof) or Ohio State University (ToF/ES).

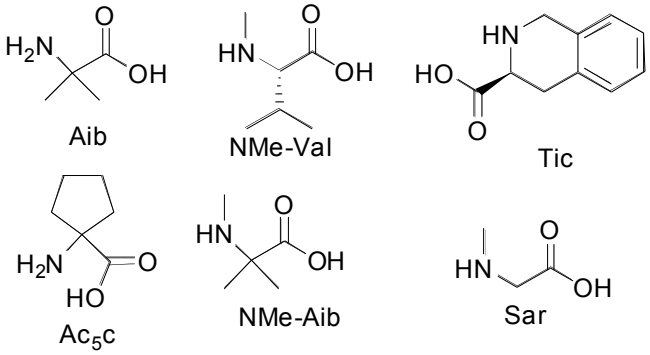
General procedure for acid fluoride formation:

Fmoc protected amino acid fluorides were synthesized with the reagent DAST by the method of Carpino[1]. In a polypropylene tube, the Fmoc-amino acid (0.92 mmole) was suspended in 4mL anhydrous dichloromethane and a few drops of DMF were added to give complete dissolution. Then 145 μL (1.1 mmole) of DAST was added in a single portion. After 1 hr, the mixture was washed with ice water, the organic layer was dried over sodium sulfate, filtered through a cotton plug and the solvent was removed *in vacuo*. An esterification test was performed to assure quantitative acid fluoride formation by dissolving 5 mg of Fmoc-amino acid fluoride in 0.3 mL of anhydrous Methanol with 10% DIPEA and allowing to react for 15 min at room temperature. An aliquot was then removed and analyzed by HPLC-MS showing less then 2% of residual Fmoc-amino acid present.

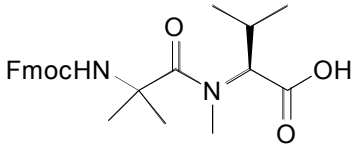
General procedure for dipeptide synthesis:

In a typical experiment the Fmoc amino acid fluoride (0.3 mmole, 1eq) was weighed into a polypropylene tube. The amino acid (1.2 mmole, 4eq) was dissolved in HFIP (6 mL) to a concentration of 0.2M. For reactions run at 55^{o}C, the amino acid solution was placed in the oven for 15 mintues to allow the solution to come to temperature. The amino acid solution was then added to the Fmoc-acid fluoride and the vessel placed in an oven for the specified amount of time. An aliquot was then removed, dissolved in H_2O/acetonitrile with 0.1% formic acid and the results analyzed by HPLC-MS.

[1]Kaduk, C., Wenschuh, H., Beyermann, M., Forner, K., Carpino, L., Bienert, M., *Letters in Peptide Science*, **1995**, 2, 285-288



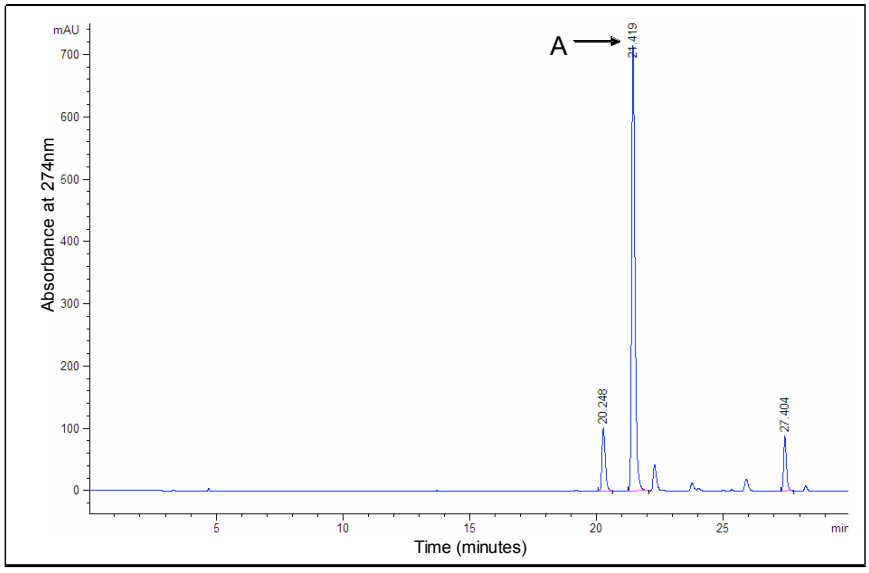
Supplementary Table 1. The structures of the amino acids used in this study. Abbreviations: Aib: α-amino-isobutryic acid, NMeVal: N-Methyl-Valine, Tic: L-1,2,3,4-Tetrahydroisoquinoline-3-carboxylic acid, NMeAib: N-Methyl-α-amino-isobutryic acid, Ac_5c: 1-amino-cyclopentanecarboxylic acid, Sar: Sarcosine (NMe-Glycine)



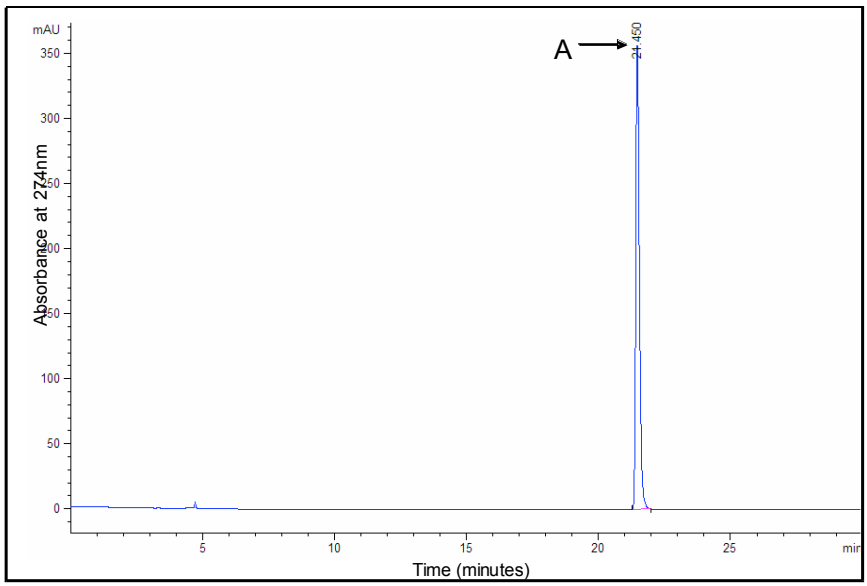
Fmoc-Aib-(S)-NMeVal-OH (sc1)

Fmoc-Aib-(S)-NMeVal-OH (sc1):

Fmoc-Aib-F was synthesized via the general procedure for acid fluoride formation, and the dipeptide was synthesized by the general procedure for dipeptide synthesis. (S)-NMe-Val-OH (106mg, 808 μmole, 4 eq) was dissolved in HFIP (4.03mL, concentration of 0.2 M) in a polypropylene tube. Fmoc-Aib-F (66 mg, 202 μmole) was then added and the reaction placed in a conventional oven held at 55°C. After 1hr, an aliquot of the reaction mixture was removed, dissolved in H_2O/acetonitrile with 0.1% formic acid and the results analyzed by HPLC-MS. The crude yield (80%) is estimated from HPLC area integration, monitoring at 274nm. HPLC-MS analysis (see Supplementary Figure 1): calced for Fmoc-Aib-(S)-NMeVal-OH + H^+: 439.2, found 439.2. The product was purified by RP purification. Anal. Calced for $C_{25}H_{30}N_2O_5Na$: 461.2052 (difference 3.5 ppm), Found: 461.2036. ^{1}H NMR (500 MHz, DMSO-d_6 with 1% trifluoroacetic acid), δ 7.88 (d, J = 7.6 Hz, 2H), 7.79 (s, 1H, -N*H*), 7.69 (d, J = 7.4 Hz, 2H), 7.41 (t, J = 7.6 Hz, 2H), 7.32 (m, 2H), 4.47 (d, J = 9.6 Hz, 1H, -C*H*CH(CH_3,CH_3)), 4.32 (m, 2H), 4.19 (t, J = 6.5 Hz, 1H), 2.93 (s, 3H, -N(C*H*$_3$)), 2.05 (m, 1H, -CHC*H*(CH_3)$_2$), 1.36 (s, 3H, -C(C*H*$_3$,CH_3)), 1.33 (s, 3H, -C(CH_3,C*H*$_3$)), 0.92 (d, J = 6.5 Hz, 3H, -CHCH(*CH*$_3$,CH_3)), 0.73 (d, J = 6.8 Hz, 3H, -CHCH(CH_3,*CH*$_3$)); ^{13}C NMR (from HMQC and HMBC, 500 MHz, DMSO-d_6 with 1% trifluoroacetic acid), δ 172.5, 172.0, 154.2, 143.6 (2C), 140.6 (2C), 127.4 (2C), 126.7 (2C), 125.1 (2C), 120.1 (2C), 64.7, 63.3, 56.2, 46.5, 32.1, 26.2, 25.5 (2C), 19.8, 18.8

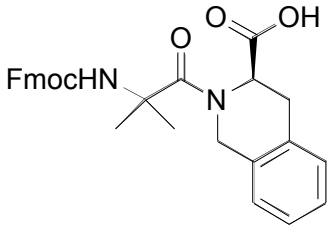


Supplemental Figure 1. Crude HPLC trace of the reaction of Fmoc-Aib-F with (S)-NMeVal-OH, monitoring at a wavelength of 274nm with a gradient of 5-95% ACN/H_2O with 0.1% formic acid over 30 minutes. The peak marked "A" has a m/z = 439.2 (calced for Fmoc-Aib-NMeVal-OH + H^+: 439.2).



Supplemental Figure 2. Reverse-Phase chromatogram of purified Fmoc-Aib-NMeVal-OH (sc1) monitoring at a wavelength of 274nm with a gradient of 5-95% ACN/H_2O with

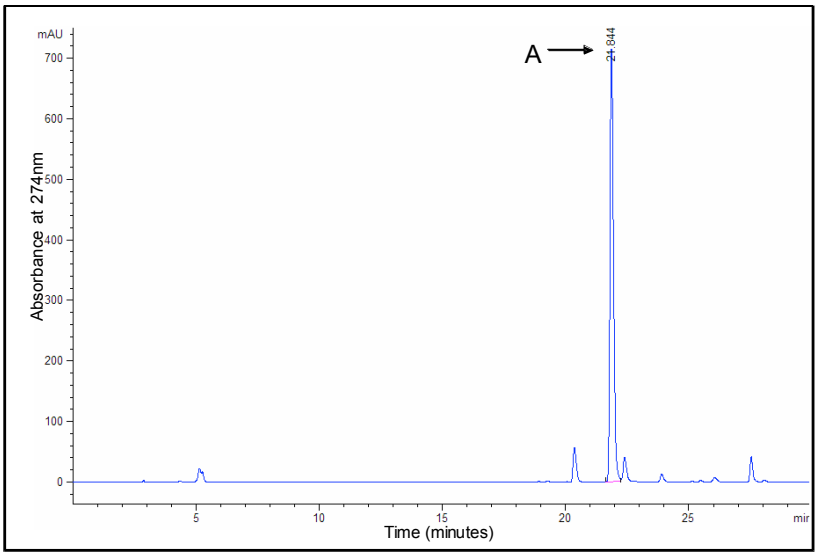
0.1% formic acid over 30 minutes. The peak marked "A" has a m/z = 439.2 (calced for Fmoc-Aib-NMeVal-OH + H^+: 439.2).



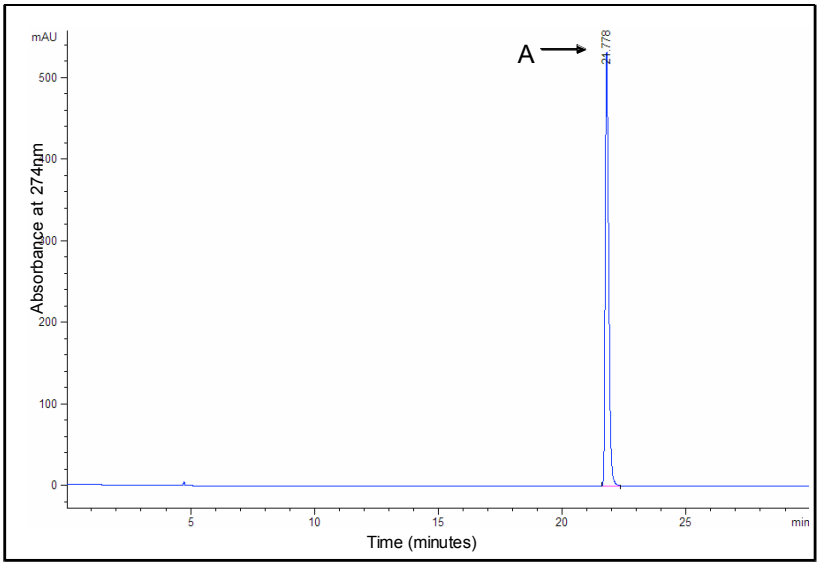
Fmoc-Aib-(S)-Tic-OH (sc2)

Fmoc-Aib-(S)-Tic-OH (sc2)

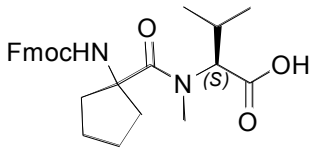
Fmoc-Aib-F was synthesized via the general procedure for acid fluoride formation, and the dipeptide was synthesized by the general procedure for dipeptide synthesis. H-Tic-OH (88 mg, 497 μmole, 4 eq) was dissolved in HFIP (2.49 mL, concentration of 0.2 M) in a polypropylene tube. Fmoc-Aib-F (41 mg, 124 μmole, 1eq) was then added and the reaction placed in a conventional oven held at 55°C. After 1hr, an aliquot of the reaction mixture was removed, dissolved in H_2O/acetonitrile with 0.1% formic acid and the results analyzed by HPLC-MS (See Supplementary Figure 3). The crude yield (86%) is estimated from HPLC area integration, monitoring at 274nm. HPLC-MS analysis: calced for Fmoc-Aib-Tic-OH + H^+: 485.2, found 485.2. The product was purified by RP purification. Anal. Calced for $C_{29}H_{28}N_2O_5Na$: 507.1896, Found: 507.1875 (diff 4.1 ppm). ^1H NMR consistent with two rotamers, including multiple signal overlap.



Supplemental Figure 3. Crude HPLC trace of the reaction of Fmoc-Aib-F with H-Tic-OH, monitoring at a wavelength of 274nm with a gradient of 5-95% ACN/H_2O with 0.1% formic acid over 30 minutes. The peak marked "A" has a m/z = 485.2 (calced for Fmoc-Aib-Tic-OH + H^+: 485.2).



Supplemental Figure 4. Reverse-Phase chromatogram of purified Fmoc-Aib-Tic-OH (sc2) monitoring at a wavelength of 274nm with a gradient of 5-95% ACN/H_2O with 0.1% formic acid over 30 minutes. The peak marked "A" has a m/z = 485.2 (calced for Fmoc-Aib-Tic-OH + H^+: 485.2).

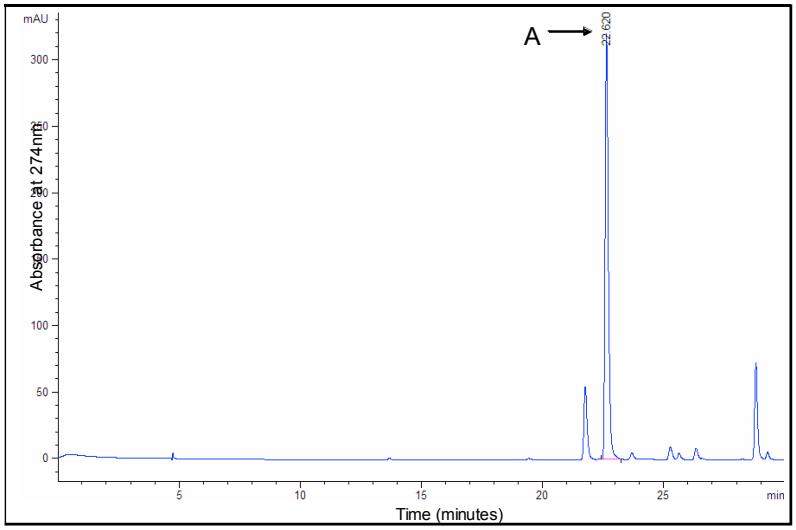


Fmoc-Ac_5c-(S)-NMe-Val-OH (sc3)

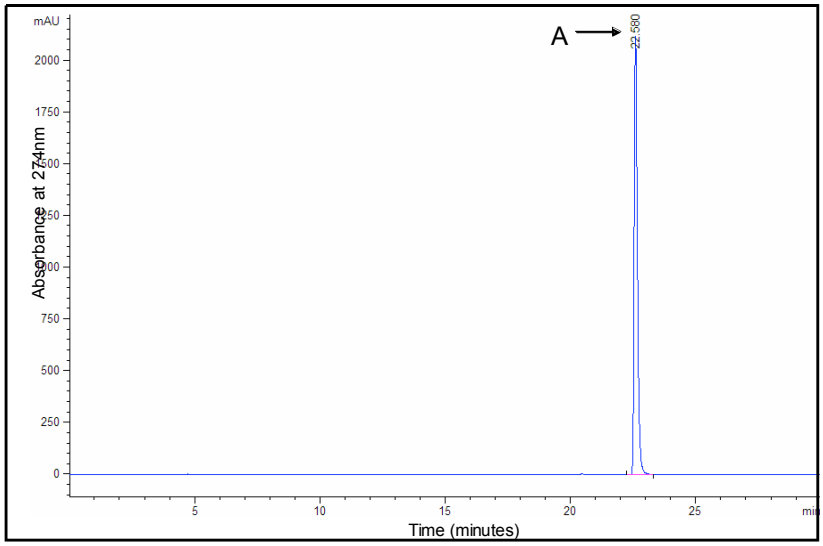
Fmoc-Ac_5c-(S)-NMe-Val-OH (sc3)

Fmoc-Ac_5c-F was synthesized via the general procedure for acid fluoride formation, and the dipeptide was synthesized by the general procedure for dipeptide synthesis. (S)-NMeVal-OH (86 mg, 657 μmole, 4 eq) was dissolved in HFIP (3.29 mL, concentration of 0.2 M) in a polypropylene tube. Fmoc-Ac_5c-F (58 mg, 164 μmole, 1eq) was then added and the reaction placed in a conventional oven held at 55°C. After 45 min, an aliquot of the reaction mixture was removed, dissolved in H_2O/acetonitrile with 0.1% formic acid and the results analyzed by HPLC-MS. The crude yield (74%) is estimated from HPLC area integration, monitoring at 274nm (See Supplemental Figure 5). HPLC-MS analysis calced for Fmoc-Ac5c-(S)-NMe-Val-OH + H^+: 465.2, found 465.2. The product was purified by RP purification. Anal Calced for $C_{27}H_{32}N_2O_5Na$: 487.2209, Found: 487.2177 (diff 6.5 ppm). ^{1}H NMR (500 MHz, DMSO-d_6 with 1% trifluoroacetic acid), δ 7.87 (d, J = 7.6 Hz, 2H), 7.84 (s, 1H, -N*H*, overlap with δ 7.87), 7.67 (m, 2H), 7.41 (t, J = 7.4 Hz, 2H), 7.32 (t, J = 7.4 Hz, 2H), 4.39 (d, J = 10.2 Hz, 1H, -

C*H*CH(CH_3,CH_3)), 4.32 (m, 1H), 4.25 (m, 1H), 4.19 (m, 1H), 2.93 (s, 3H, -N(C*H*$_3$)), 2.23 (m, 1H,), 2.04 (m, 1H, -CHC*H*(CH_3)$_2$), 1.96 (m, 2H), 1.87 (m, 1H), 1.55 (m, 4H,), 0.90 (d, *J* = 6.3 Hz, 3H, -CHCH(*CH*$_3$,CH_3)), 0.67 (d, *J* = 6.3 Hz, 3H, -CHCH(CH_3,*CH*$_3$)); ^{13}C NMR (from HMQC and HMBC, 500 MHz, DMSO-d_6 with 1% trifluoroacetic acid), δ 172.3, 172.1, 153.9, 143.7 (2C), 140.4 (2C), 127.4 (2C), 126.7 (2C), 124.9 (2C), 120.0 (2C), 65.9, 64.7, 62.9, 46.7, 39.6, 35.9, 35.8, 32.3, 26.2, 23.7, 19.8, 18.8



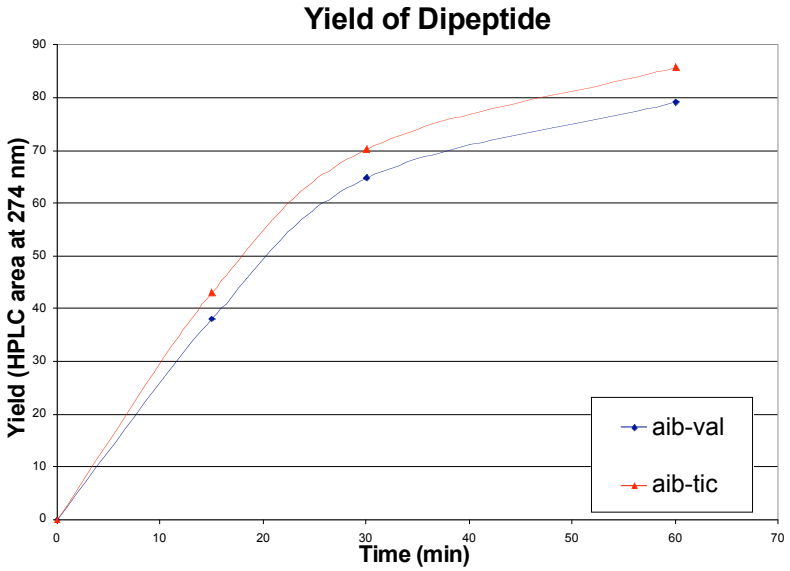
Supplemental Figure 5. Crude HPLC trace of the reaction of Fmoc-Ac$_5$c-F with (S)-NMe-Val-OH (sc3), monitoring at a wavelength of 274nm with a gradient of 5-95% ACN/H_2O with 0.1% formic acid over 30 minutes. The peak marked "A" has a m/z = 465.2 (calced for Fmoc-Ac5c-(S)-NMe-Val-OH+ H^+: 465.2).



Supplemental Figure 6. Reverse-Phase chromatogram of purified Fmoc-Ac$_5$c-F with (S)-NMe-Val-OH (sc3) monitoring at a wavelength of 274nm with a gradient of 5-95% ACN/H_2O with 0.1% formic acid over 30 minutes. The peak marked "A" has a m/z = 465.2 (calced for Fmoc-Ac5c-(S)-NMe-Val-OH + H^+: 465.2).

Time Course Data:

Time course data was undertaken to verify the time scale of the reaction. Dipeptides were synthesized according to the procedures here in the Supporting Information. At the appropriate time points, an aliquot of the reaction mixture was withdrawn, dissolved in H_2O/acetonitrile with 0.1% formic acid and the results analyzed by HPLC-MS. The results are shown in Supplemental Figure 7 below.



Supplemental Figure 7. Time course data for the formation of dipeptides Fmoc-Aib-(S)-NMeVal-OH (marked "aib-val") and Fmoc-Aib-(S)-Tic-OH (marked "aib-tic"). The yield is shown as the integrated area of each chromatogram at 274 nm.

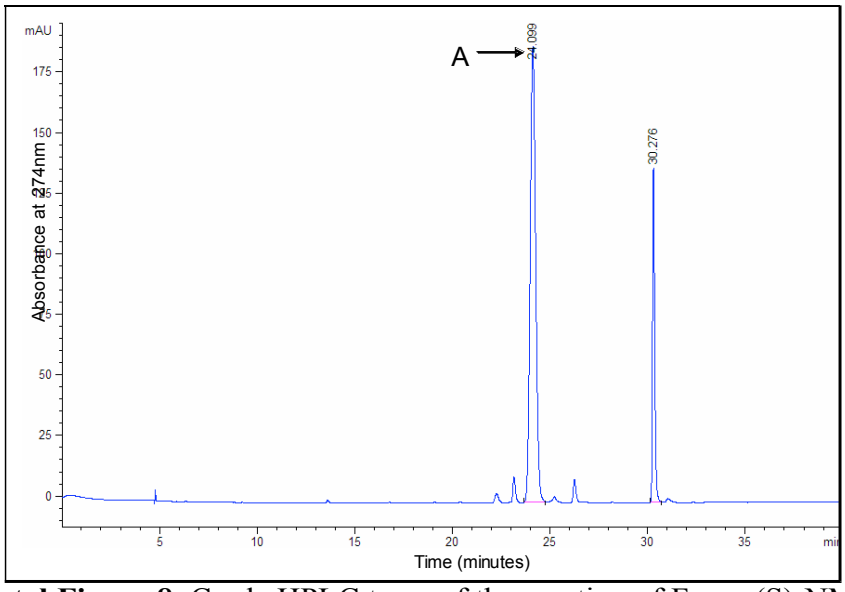
Racemization Trials:

To assess potential racemization at either stereocenter of the resulting dipeptide, a diastereomeric mixture was synthesized with Fmoc-(S)-NMeVal and either (S)-NMeVal-OH or a racemic mixture of (S)-NMeVal-OH and (R)-NMeVal-OH.

A.) Fmoc-(S)-NMeVal-(S)-NMeVal-OH

Fmoc-(S)-NMeVal-F was synthesized via the general procedure for acid fluoride formation, and the dipeptide was synthesized by the general procedure for dipeptide synthesis. (S)-NMeVal-OH (27 mg, 207 μmole, 4 eq) was dissolved in HFIP (1.03 mL, concentration of 0.2 M) in a polypropylene tube. Fmoc-(S)-NMeVal-F (25 mg, 52 μmole, 1eq) was then added and the reaction placed in a conventional oven held at 55°C. After 5 min, an aliquot of the reaction mixture was removed, dissolved in H_2O/acetonitrile with

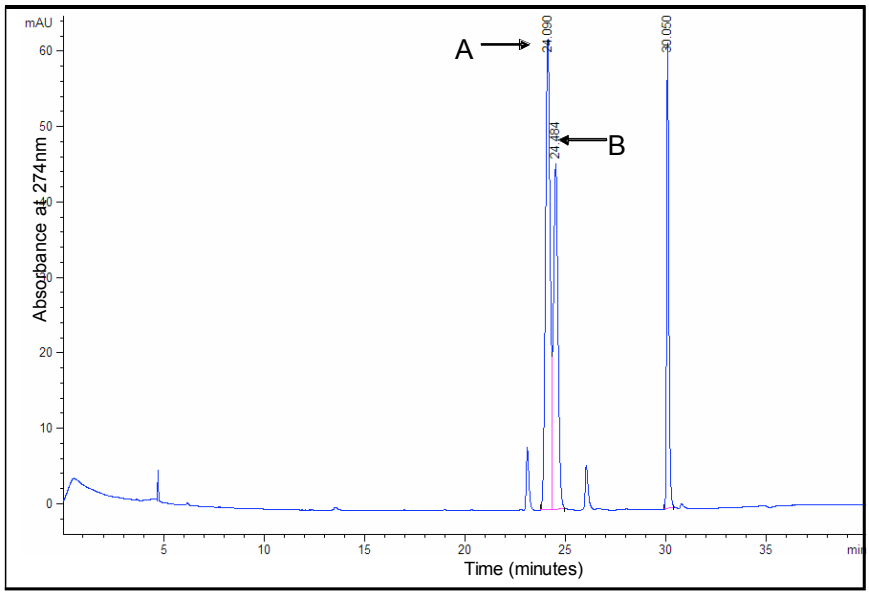
0.1% formic acid and the results analyzed by HPLC-MS (See Supplemental Figure 8).



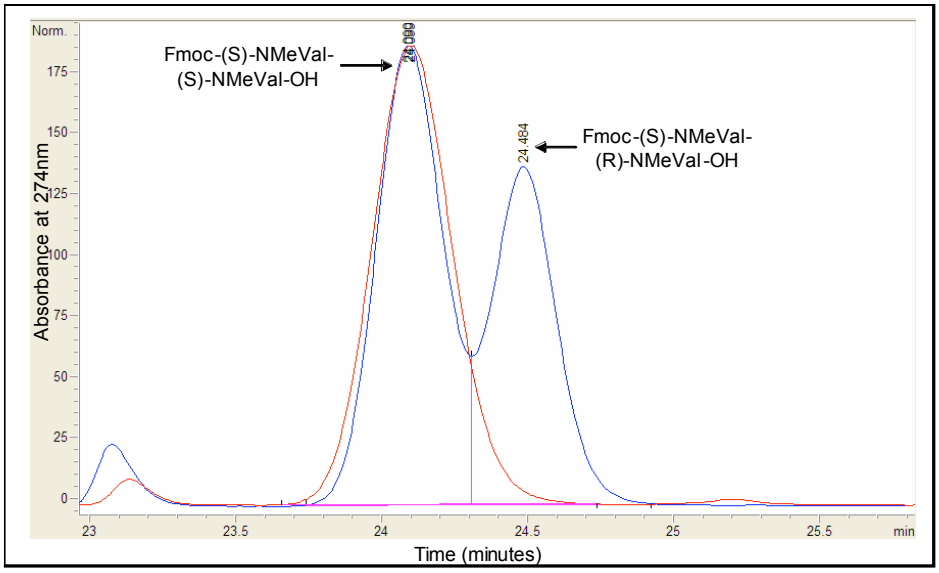
Supplemental Figure 8. Crude HPLC trace of the reaction of Fmoc-(S)-NMeVal-F with H-(S)-NMeVal-OH, monitoring at a wavelength of 274nm with a gradient of 5-95% ACN/H_2O with 0.1% formic acid over 40 minutes. The peak marked "A" has a m/z = 467.2 (calced for Fmoc-(S)-NMeVal-(S)-NMeVal-OH + H^+: 467.2).

B.) Fmoc-(S)-NMeVal-(S,R)-NMeVal-OH

Fmoc-(S)-NMeVal-F was synthesized via the general procedure for acid fluoride formation, and the dipeptide was synthesized by the general procedure for dipeptide synthesis. H-(S,R)-NMeVal-OH (22 mg, 166 μmole, 4 eq) was dissolved in HFIP (0.83 mL, concentration of 0.2 M) in a polypropylene tube. Fmoc-(S)-NMeVal-F (20 mg, 41 μmole, 1eq) was then added and the reaction placed in a conventional oven held at 55°C. After 5 min, an aliquot of the reaction mixture was removed, dissolved in H_2O/acetonitrile with 0.1% formic acid and the results analyzed by HPLC-MS (See Supplemental 9).



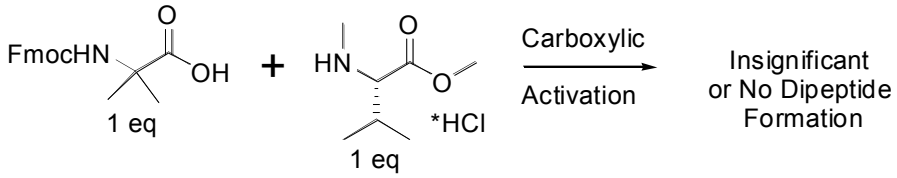
Supplemental Figure 9. Crude HPLC trace of the reaction of Fmoc-(S)-NMeVal-F with (S,R)-NMeVal-OH, monitoring at a wavelength of 274nm with a gradient of 5-95% ACN/H_2O with 0.1% formic acid over 40 minutes. Both peaks marked "A" and "B" have a m/z = 467.2 (calced for Fmoc-(S)-NMeVal-(S)-NMeVal-OH + H^+: 467.2), indicating that the diastereomeric dipeptides resolve in the present chromatography conditions.



Supplemental Figure 10. Overlay of the crude chromatograms of the racemization trials: the trace in red in the coupling of Fmoc-(S)-NMeVal-F with (S)-NMeVal-OH, while the trace in blue is the product from the coupling of Fmoc-(S)-NMeVal-F and (S,R)-NMeVal-OH. The peaks of each of the diastereomers are annotated and have ES-MS consistent with the expected product.

Control Acylations:

A number of conventional carboxylic activating agents were also tried for control acylations using a methyl ester protected amino acid. The schematic is shown below.



Supplemental Figure 11 . Schematic of the control acylations using Fmoc-Aib-OH and (S)-NMe-Val-OMe*HCl. The various activating agents and conditions are shown in Table 2 .

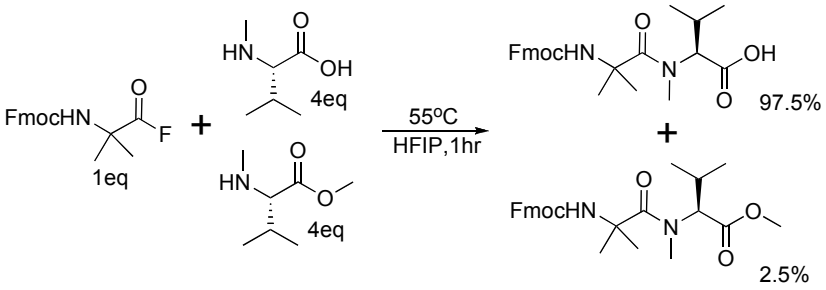
Entry	Coupling Agent[a]	Eq of DIPEA
1	HCTU	3
2	PyBrop	3
3	HATU	3
4	BTFFH	3
5	Preformed acid fluroide[b]	2

Supplemental Table 2 . The activating agents and the equivalents of DIPEA used for the reactions. [a] All reactions performed as described in the text below. [b] Preformed acid fluoride used the DAST procedure as outlined in the general synthesis of amino acid fluorides.

In a typical experiment, 32 mg (99 μmole, 1eq), 1eq activating agent (See Supplemental Table 2), and 18 mg of (S)-NMeVal-OMe*HCl (99 μmole, 1eq) were dissolved in 493 μL of DMF (concentration of reagents 0.2M) in a polypropylene tube. After complete dissolution of all materials, the appropriate amount of base was added. The reaction was stirred overnight at room temperature after which an aliquot of the reaction mixture was removed, dissolved in H_2O/acetonitrile with 0.1% formic acid and the results analyzed by HPLC-MS. No significant amount of dipeptide (<5%) was seen for any of the reactions. Significant amounts of premature deblocking of the Fmoc group occurred for most acylation trials as evidenced by the appearance of a dibenzofulvene peak in HPLC-MS analysis as well as other side reactions not readily identifiable by LC-MS analysis.

Competition Experiments:

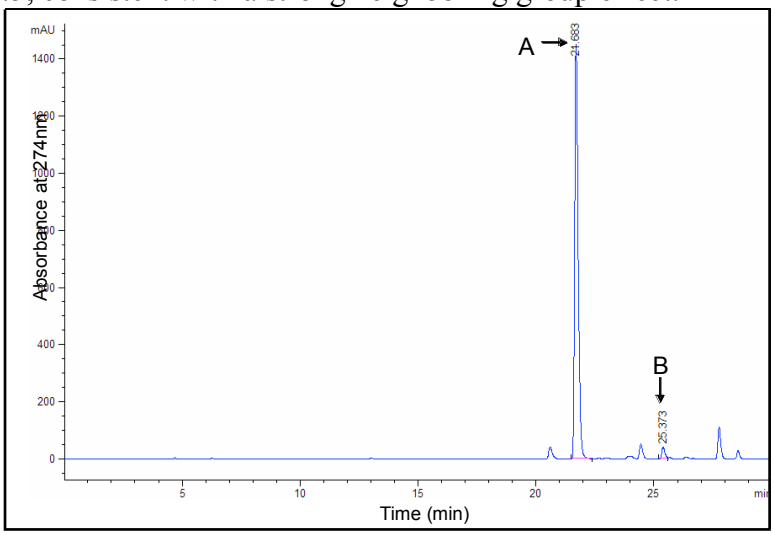
To test the hypothesis that a neighboring group effect is involved in the dipeptide formation reaction we carried out a competition experiment in which we combined NMeVal-OH and NMeVal-OMe.HCl in one pot as potential coupling partners with Fmoc-Aib-F (Supplemental Figure 12). The relative amounts of the two possible dipeptide products: Fmoc-Aib-NMeVal-OH and Fmoc-Aib-NMeVal-OMe were quantified and identified by HPLC-MS. If no neighboring group effect is involved then we would expect roughly equal amounts of the two dipeptide products.



Supplemental Figure 12 . Schematic of the competition acylations using Fmoc-Aib-F, (S)-NMe-Val-OH and (S)-NMe-Val-OMe*HCl and the two dipeptidyl products.

Fmoc-Aib-F was synthesized via the general procedure for acid fluoride formation, and the dipeptide was synthesized by the general procedure for dipeptide synthesis. H-(S)-NMeVal-OH (53 mg, 404 μmole, 4 eq) and H-(S)-NMeVal-OMe*HCl (73mg, 404 μmole, 4eq) was dissolved in HFIP (2.02 mL, concentration of 0.2 M) in a polypropylene tube. Fmoc-Aib-F (33 mg, 101 μmole, 1eq) was then added and the reaction placed in a conventional oven held at 55°C. After 60 min, an aliquot of the reaction mixture was removed, dissolved in H_2O/acetonitrile with 0.1% formic acid and the results analyzed by HPLC-MS (See Supplemental 13).

The HPLC-MS results showed a ratio of Fmoc-Aib-NMeVal-OH to Fmoc-Aib-NMeVal-OMe of 97.5:2.5, consistent with a strong neighboring group effect.

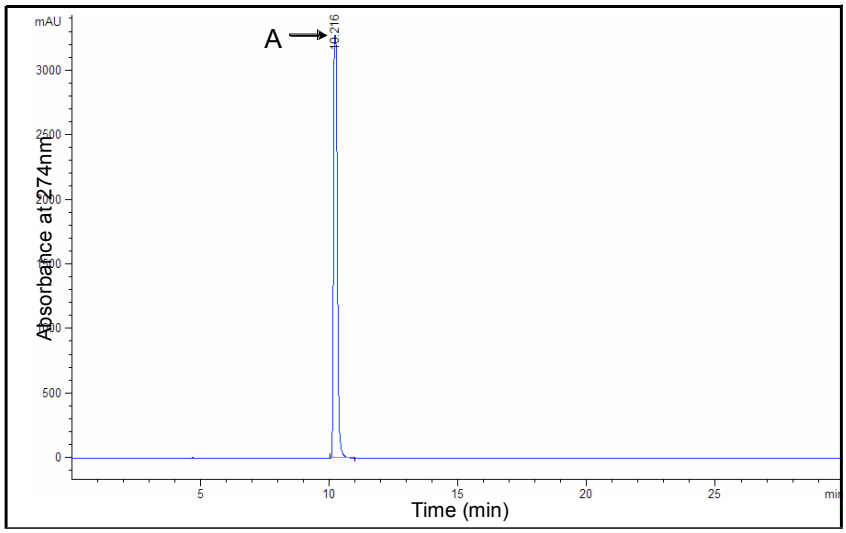


Supplemental Figure 13. Crude HPLC trace of the reaction of Fmoc-Aib-F with (S)-NMeVal-OH and (S)-NMeVal-OMe*HCl , monitoring at a wavelength of 274nm with a gradient of 5-95% ACN/H_2O with 0.1% formic acid over 30 minutes. The peak marked "A" has a m/z = 439.0 (calced for Fmoc-Aib-(S)-NMeVal-OH + H^+: 439.2) and the peak marked "B" has a m/z = 475.0 (calced for Fmoc-Aib-(S)-NMeVal-OMe + Na^+: 475.2)

***N*- (2-methylnaphthylene)-Sarcosine-OH (compound 16)**

Sarcosine-OtBu*HCl (150 mg, 830 μmole, 1eq) and 2-(Bromomethyl)-naphthalene (830 μmole, 183 mg, 1eq) were placed in a 25 mL round bottom flask and the vessel was

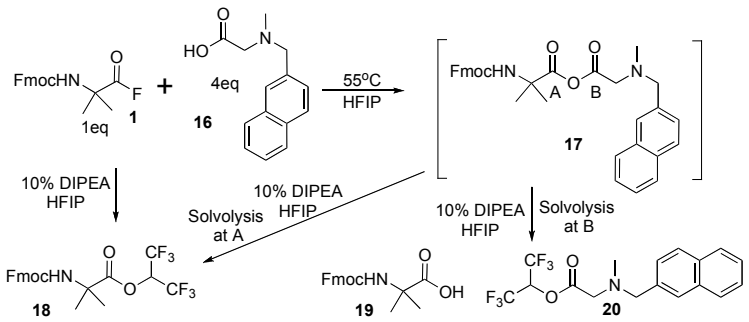
charged with 8 mL of tetrahydrofuran. DIPEA (290 μL, 1.6 mmole, 2 eq) was then added and the reaction left to stir overnight. The crude product was concentrated *in vacuo* and used without further purification. The residue was dissolved 5 mL DCM, an equal volume of trifluoroacetic acid was added, and the reaction was allowed to stir at room temperature for 2 hrs. The product was then purified by RP purification and lyophilized from the ACN/H_2O with 0.1% formic acid solvent mixture. HPLC-MS analysis (See Supplemental Figure 14) for the product found m/z = 230.0 (calced for *N*-Napthyl-Sarcosine-OH + H^+ = 230.1). ^{1}H NMR (500 MHz, DMSO-d_6 with 1% trifluoroacetic acid), δ 8.07 (bs, 1H), 8.02 (d, 1H), 7.97 (m, 2H), 7.64 (dd, 1H), 7.6 (m, 2H), 4.52 (bs, 2H), 4.13 (s, 2H), 2.83 (s, 3H). ^{13}C NMR (500 MHz, DMSO-d_6 with 1% trifluoroacetic acid),δ 167.8, 133.8, 133.1, 131.7, 129.0, 128.6, 128.5, 128.1, 127.6, 127.4, 127.1, 59.8, 55.1, 41.0



Supplemental Figure 14. HPLC trace of the purified product *N*-(2-methylnaphthylene)-Sarcosine-OH, monitoring at a wavelength of 274 nm with a gradient of 5-95% ACN/H_2O with 0.1% formic acid over 30 minutes. The peak marked "A" has a m/z = 230.0 (calced for *N*-(2-methylnaphthylene)-Sarcosine-OH + H^+: 230.1).

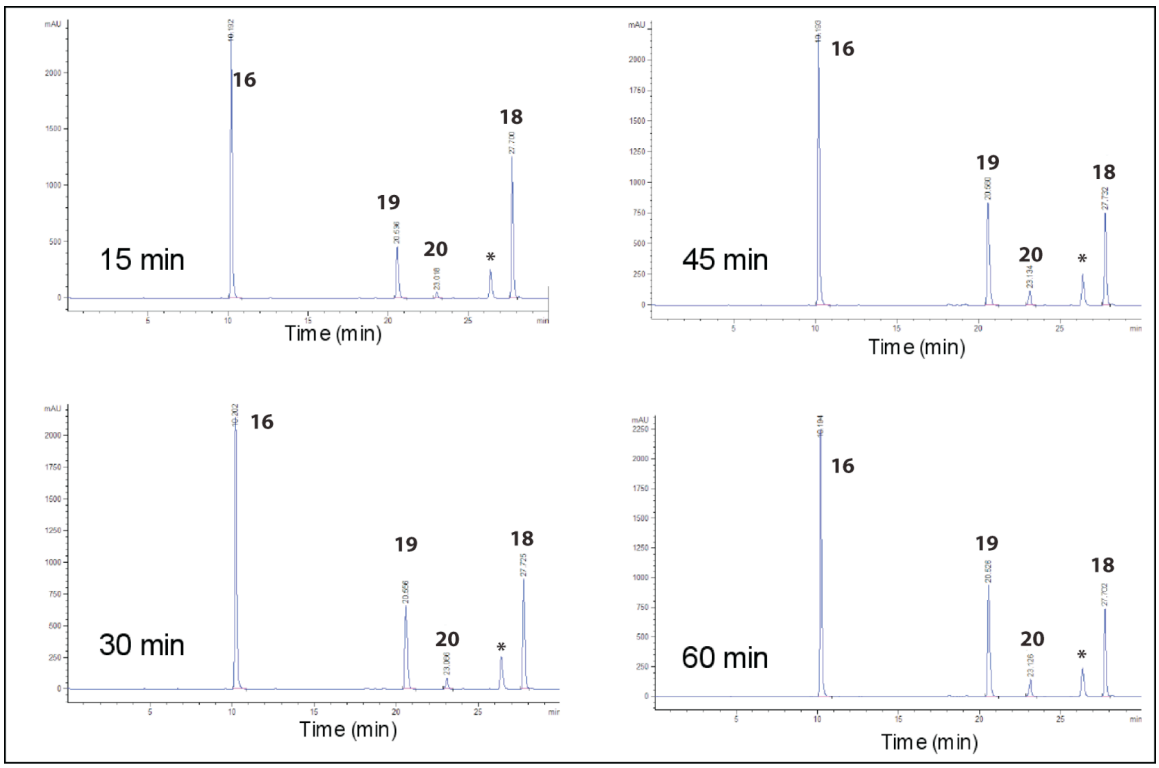
Anhydride Trapping Experiment:

To investigate the hypothesis that a transient amino anhydride is formed during the reaction, *N*-(2-methylnaphthylene)-Sarcosine-OH **16** was combined with Fmoc-Aib-F **1**. The carboxylate of the amino acid **16** can still attack the acid fluoride **1** forming the anhydride **17**, but this intermediate would not be able to undergo the hypothesized acyl transfer necessary for amidation. Quenching this reaction with 10% DIPEA would lead to solvolysis of any active acylating species (either acid fluoride or anhydride), and anhydride solvolysis should occur preferentially at the less hindered side of the mixed anhydride (here the tertiary amino acid side, See Supplemental Figure 15).



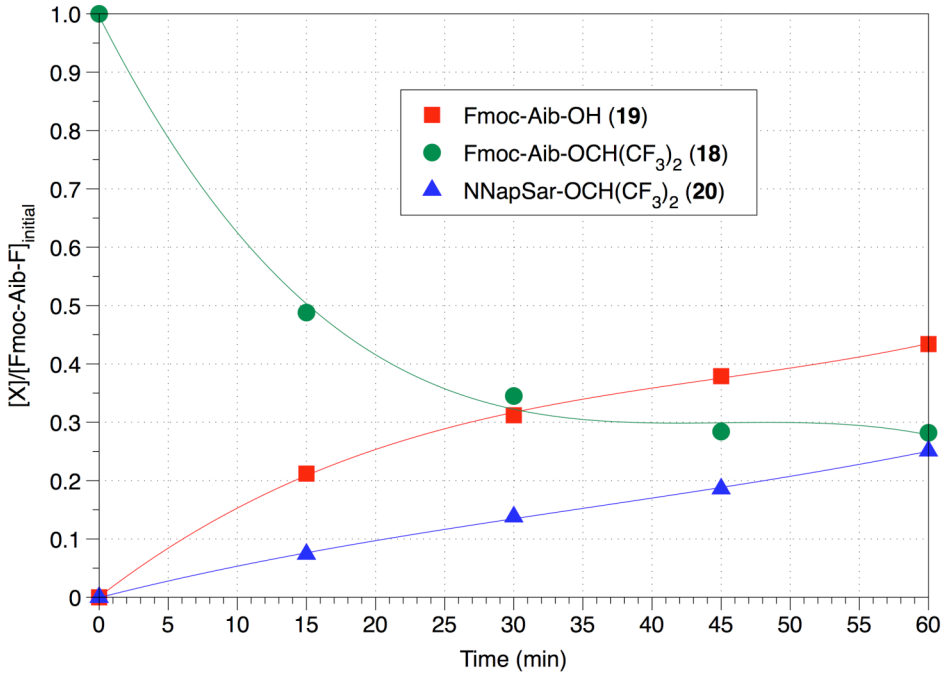
Supplemental Figure 15. Reaction scheme of the amino-anhydride trapping experiments showing the various solvolytic pathways.

Fmoc-Aib-F was synthesized via the general procedure for acid fluoride formation, and the trapping experiment was performed using the general procedure for dipeptide synthesis. *N*-(2-methylnaphthylene)-Sarcosine-OH (30 mg, 131 μmole, 1 eq) was dissolved in 670 μL of HFIP (conc of 200mM) and Fmoc-Aib-F (11 mg, 34 μmole, 1 eq) was added. At 15 minute intervals, an aliquot of the reaction was withdrawn, quenched with 10% DIPEA, and allowed to stand for at least 20 minutes at room temperature. 75% ACN/H_2O with 0.1% formic acid was then added and the mixture analyzed by LC-MS (See Supplemental Figure 16).



Supplemental Figure 16. HPLC trace of the crude products of the time course experiments with Fmoc-Aib-F and *N*-(2-methylnaphthylene)-Sarcosine-OH, monitoring at a wavelength of 274 nm with a gradient of 5-95% ACN/H_2O with 0.1% formic acid

over 30 minutes. The peaks labeled **18**, **19** and **20** have m/z values consistent with those compound numbers: compound **19** m/z = 326.1 (calc. for Fmoc-Aib-OH + H^+ = 326.1); the peak marked **20** has a m/z = 380.1 (calc; for *N*-(2-methylnaphthylene)-Sarcosine-OCH$(CF_3)_2$ + H^+: 380.1); the peak marked **18** has a m/z = 498.0 (calc; for Fmoc-Aib-OCH$(CF_3)_2$ + Na^+ = 498.1); the peak marked **16** has a retention time and UV/VIS spectrum consistent with compound **16** but for this run we did extend the mass-spectrometry window down to the mass of **16**; the peak marked "*" had no mass spectrum but the UV/VIS spectrum and retention time is consistent with dibenzofulvene (Fmoc deprotection).



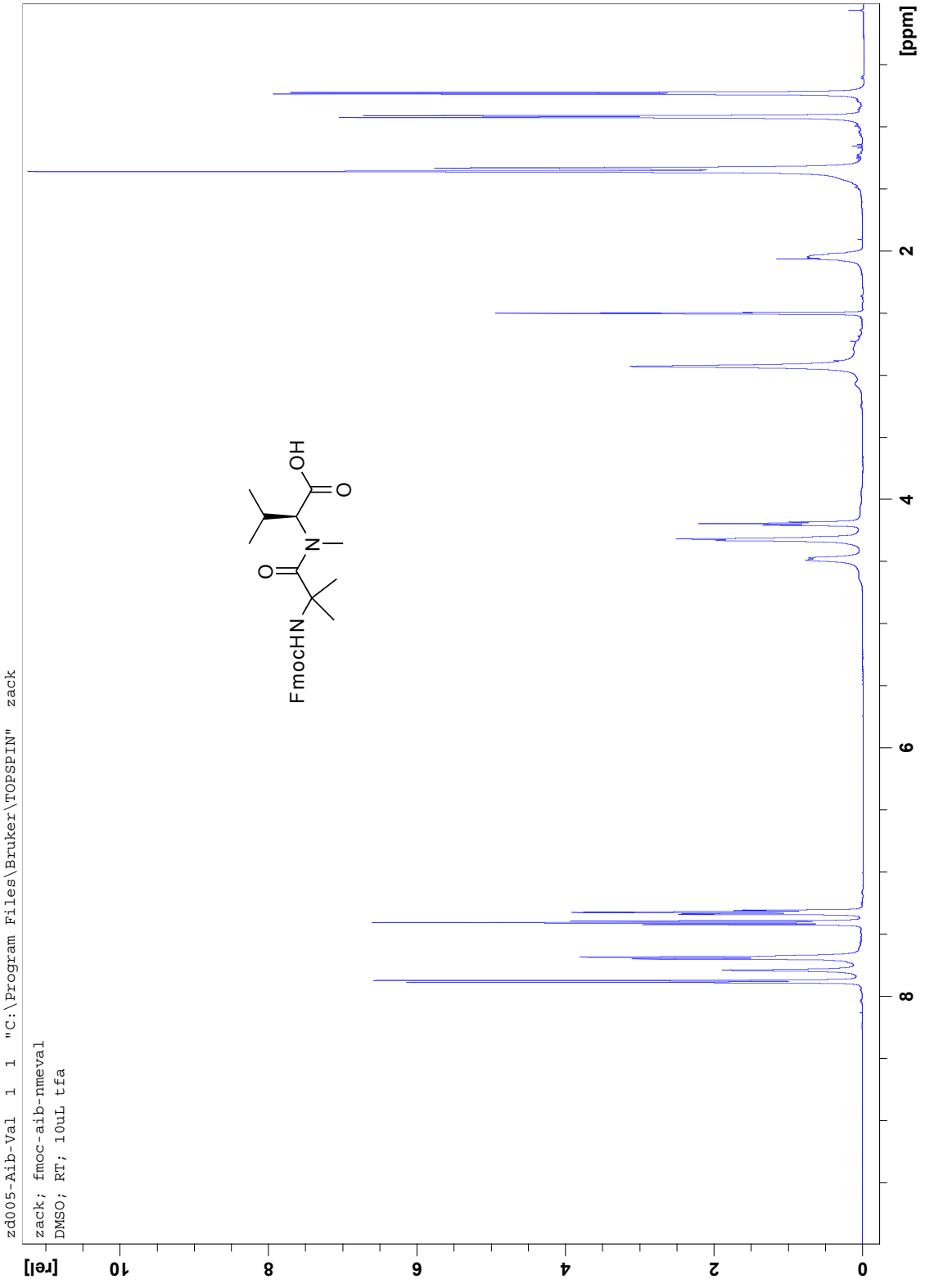
Supplemental Figure 17. Mole fractions of compounds **18**, **19** and **20** calculated from integrals of peaks from Supplemental Figure 16. The concentrations of these species were calculated using previously determined calibration curves and the concentrations were divided by the initial concentration of Fmoc-Aib-F to obtain a mole fraction.

To rule out the possibility that *N*-(2-methylnaphthylene)-Sarcosine-OCH$(CF_3)_2$ is due to background transesterification in HFIP under the dipeptide reaction conditions, a control reaction with only the *N*-(2-methylnaphthylene)-Sarcosine-OH dissolved in HFIP was carried out. *N*-(2-methylnaphthylene)-Sarcosine-OH (10 mg, 44 μmole) was dissolved in 220 μL of HFIP and placed in an oven at 55°C for one hour. No esterification product was found by LC-MS of the solution.

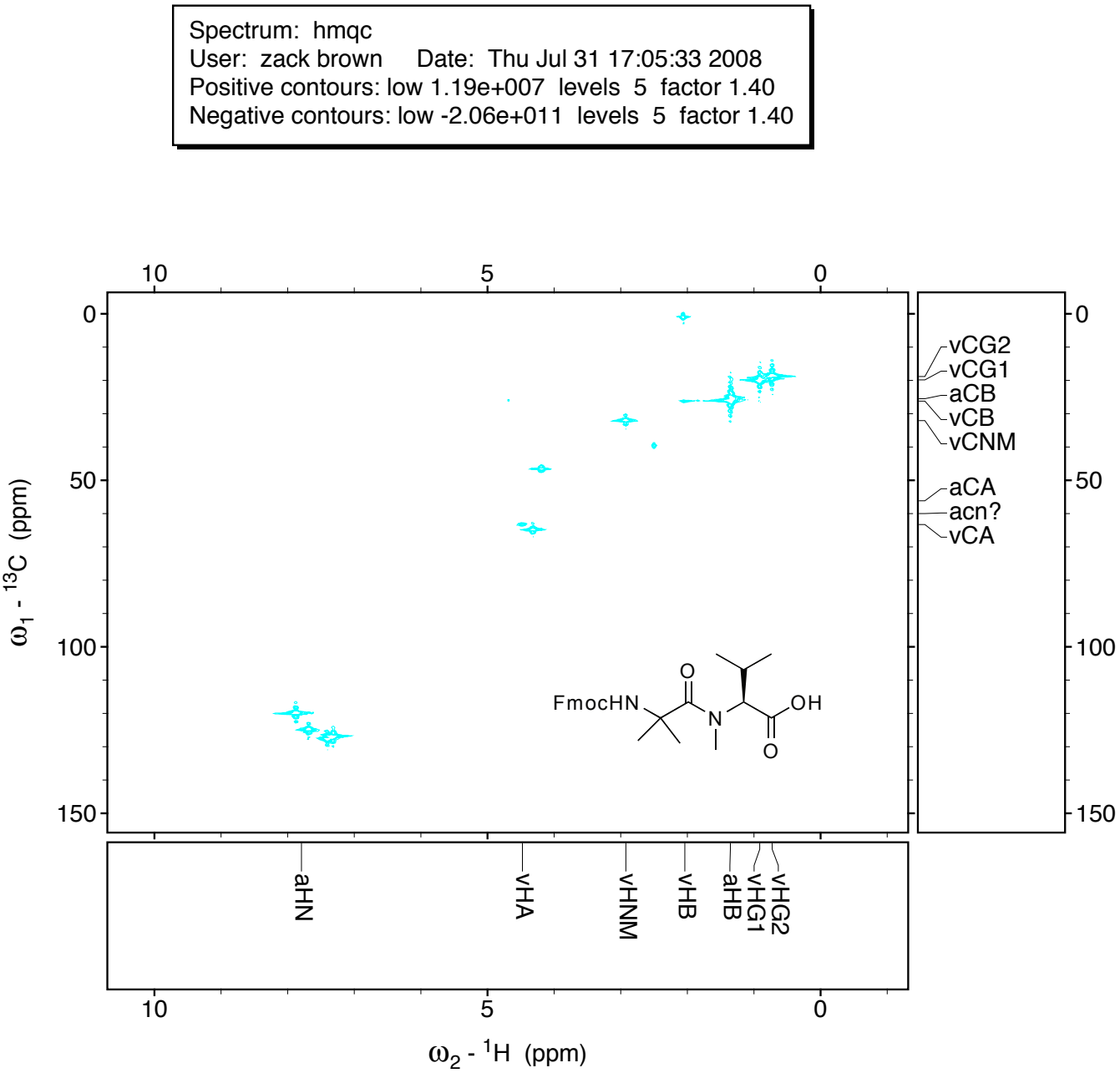
Structural Characterization:

Supplemental Figure 18. ^{1}H NMR of Fmoc-Aib-(S)-NMeVal-OH (sc1), (50mM, 500 MHz, DMSO-d_6 with 1% trifluoroacetic acid)
Supplemental Figure 19. HMQC of Fmoc-Aib-(S)-NMeVal-OH (sc1), (25mM, 500 MHz, DMSO-d_6 with 1% trifluoroacetic acid)
Supplemental Figure 20. HMBC of Fmoc-Aib-(S)-NMeVal-OH (sc1), (25mM, 500 MHz, DMSO-d_6 with 1% trifluoroacetic acid)
Supplemental Figure 21. ^{1}H NMR Temperature coalescence profile of Fmoc-Aib-(S)-NMeVal-OH (sc1), (50mM, 500 MHz, DMSO-d_6 with 1% trifluoroacetic acid, RT to 360K)
Supplemental Figure 22. ^{1}H NMR of Fmoc- Ac5c-(S)-NMeVal-OH (sc3), (25mM, 500 MHz, DMSO-d_6 with 1% trifluoroacetic acid)
Supplemental Figure 23. HMQC of Fmoc- Ac5c-(S)-NMeVal-OH (sc3), (25mM, 500 MHz, DMSO-d_6 with 1% trifluoroacetic acid)
Supplemental Figure 24. HMBC of Fmoc- Ac5c-(S)-NMeVal-OH (sc3), (25mM, 500 MHz, DMSO-d_6 with 1% trifluoroacetic acid)
Supplemental Figure 25. ^{1}H NMR of Fmoc-Aib-(S)-Tic-OH (sc2), (25mM, 500 MHz, DMSO-d_6 with 1% trifluoroacetic acid)
Supplemental Figure 26. HMQC of Fmoc-Aib-(S)-Tic-OH (sc2), (25mM, 500 MHz, DMSO-d_6 with 1% trifluoroacetic acid)
Supplemental Figure 27. HMBC of Fmoc-Aib-(S)-Tic-OH (sc2), (25mM, 500 MHz, DMSO-d_6 with 1% trifluoroacetic acid)

Supplemental Figure 18



Supplemental Figure 19



Supplemental Figure 20

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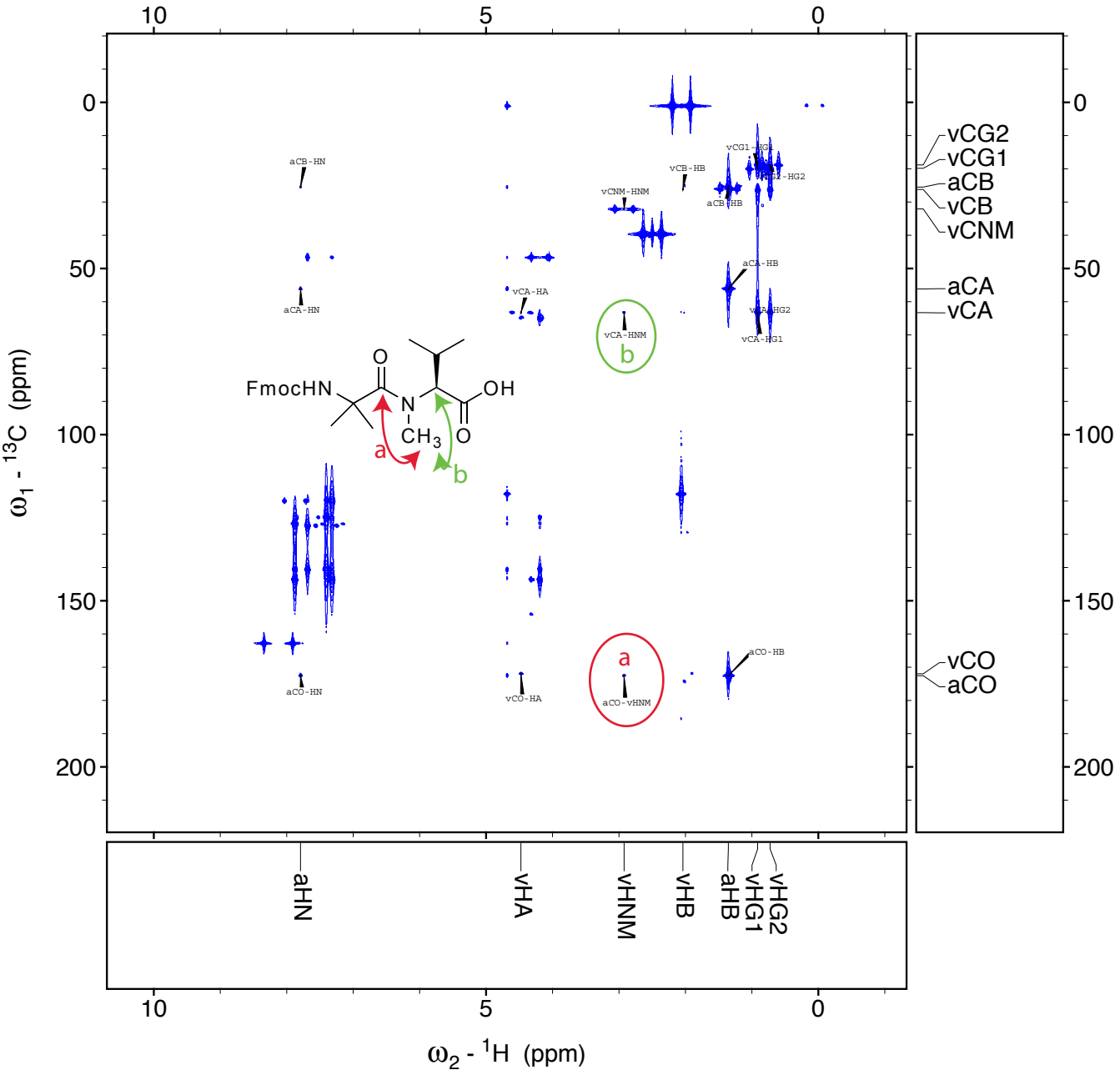
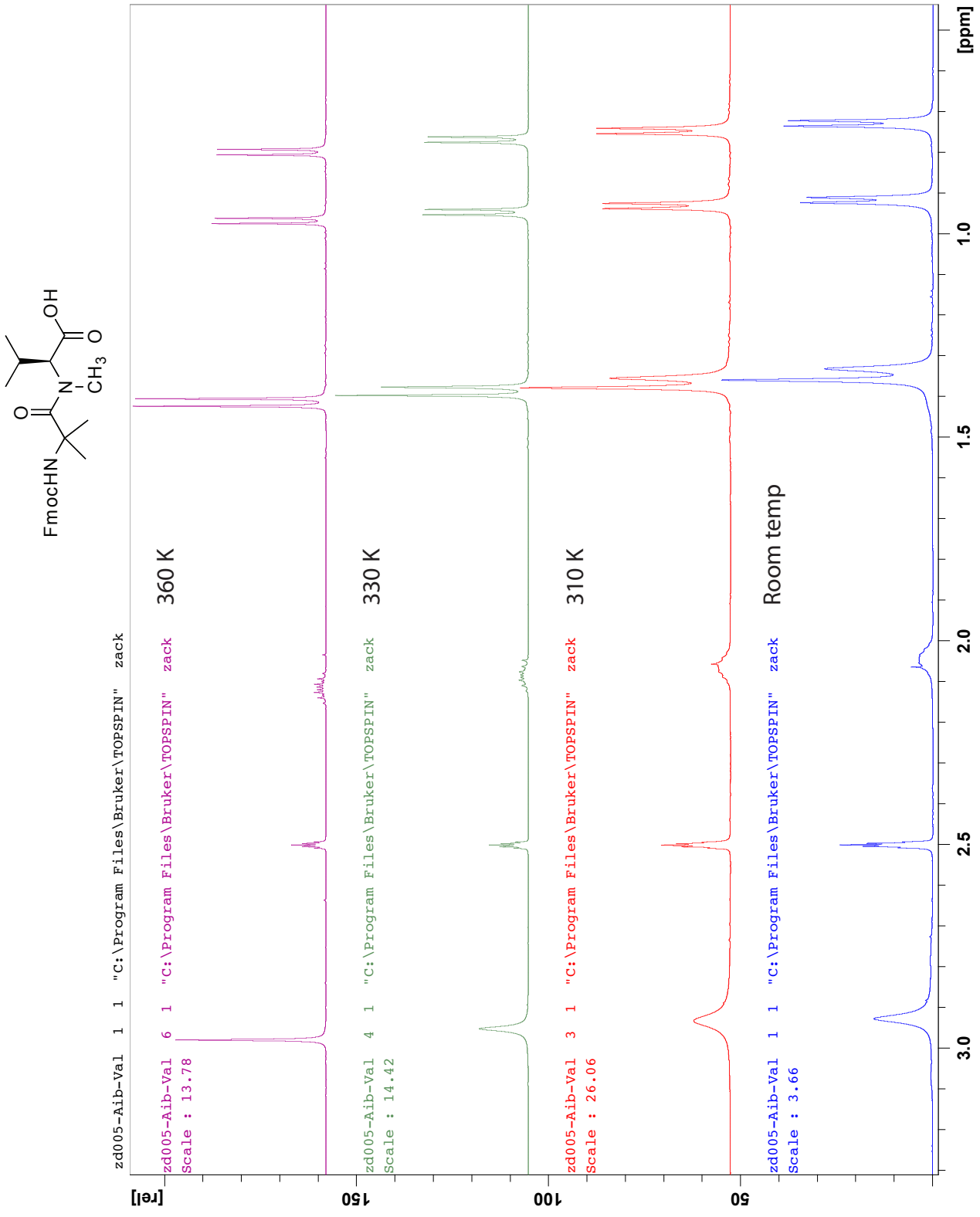
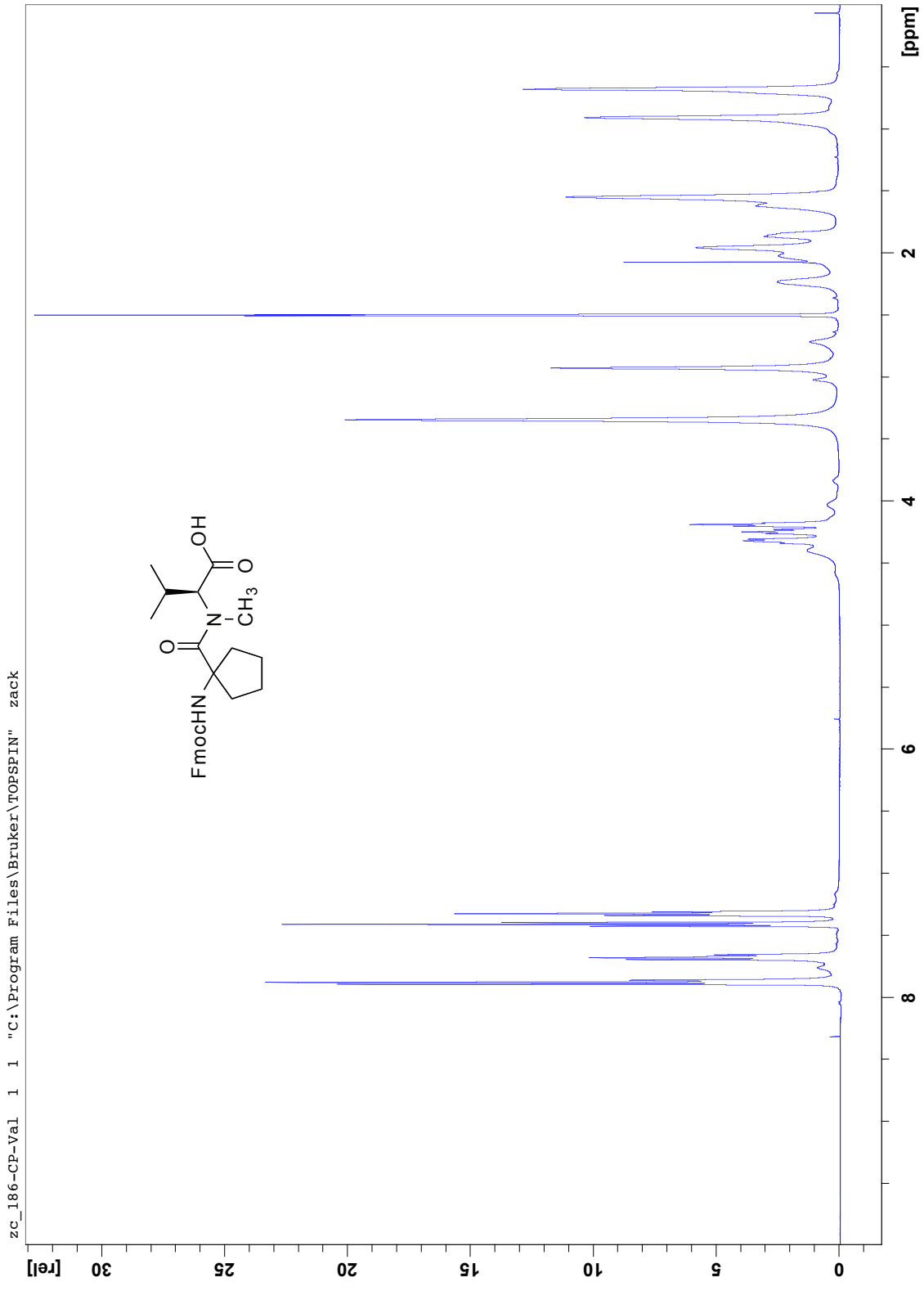


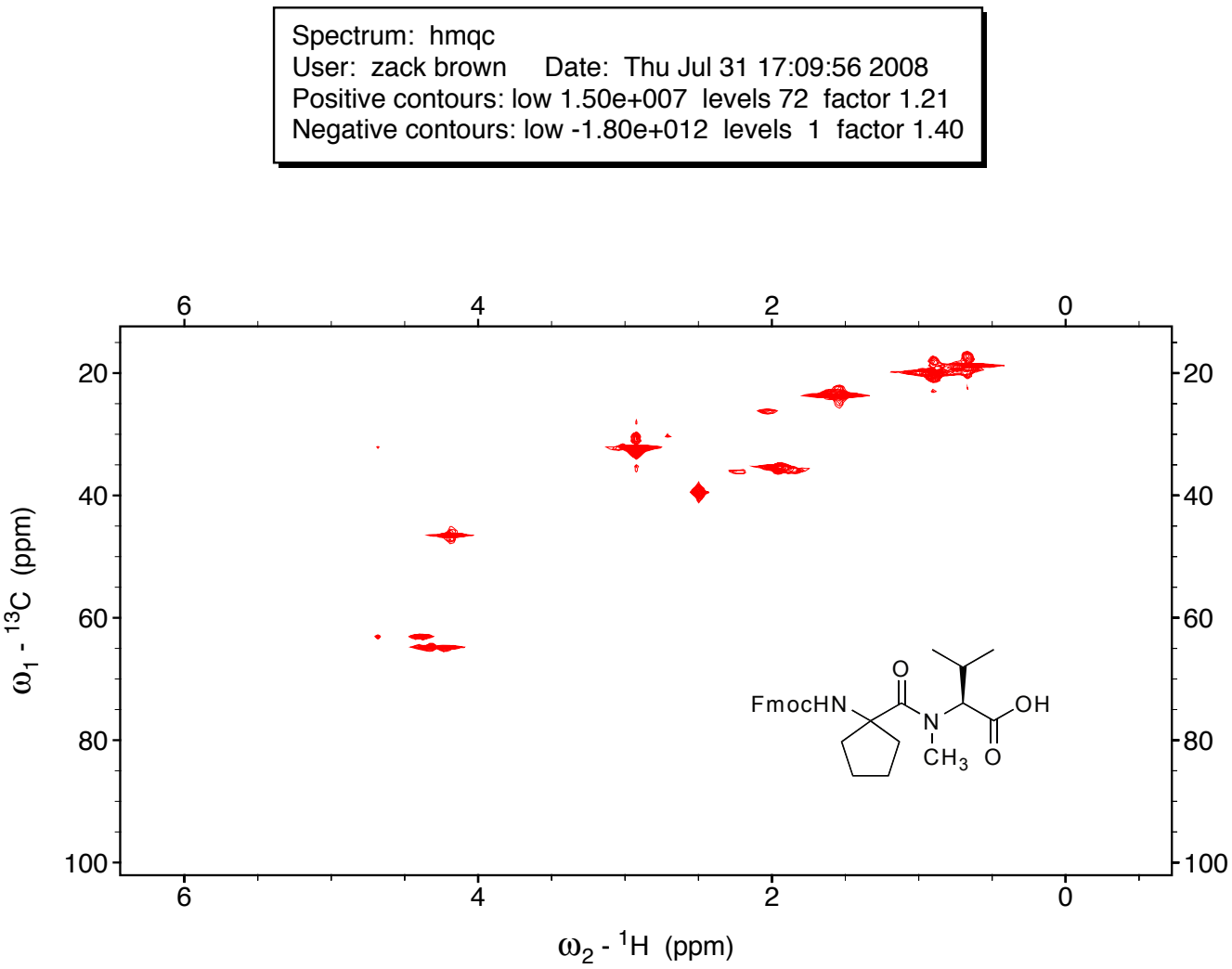
Supplemental Figure 21



Supplemental Figure 22



Supplemental Figure 23



Supplemental Figure 24

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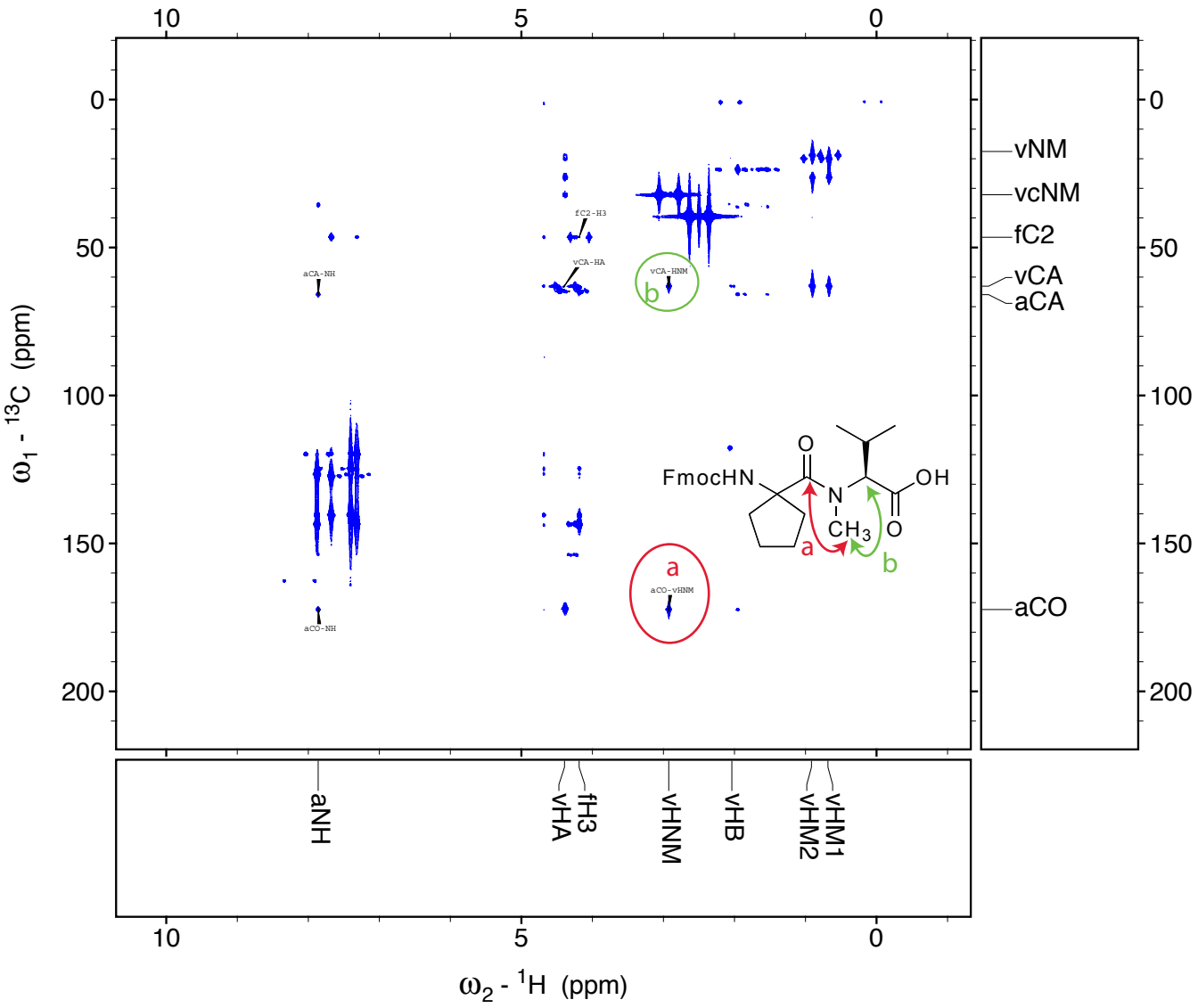
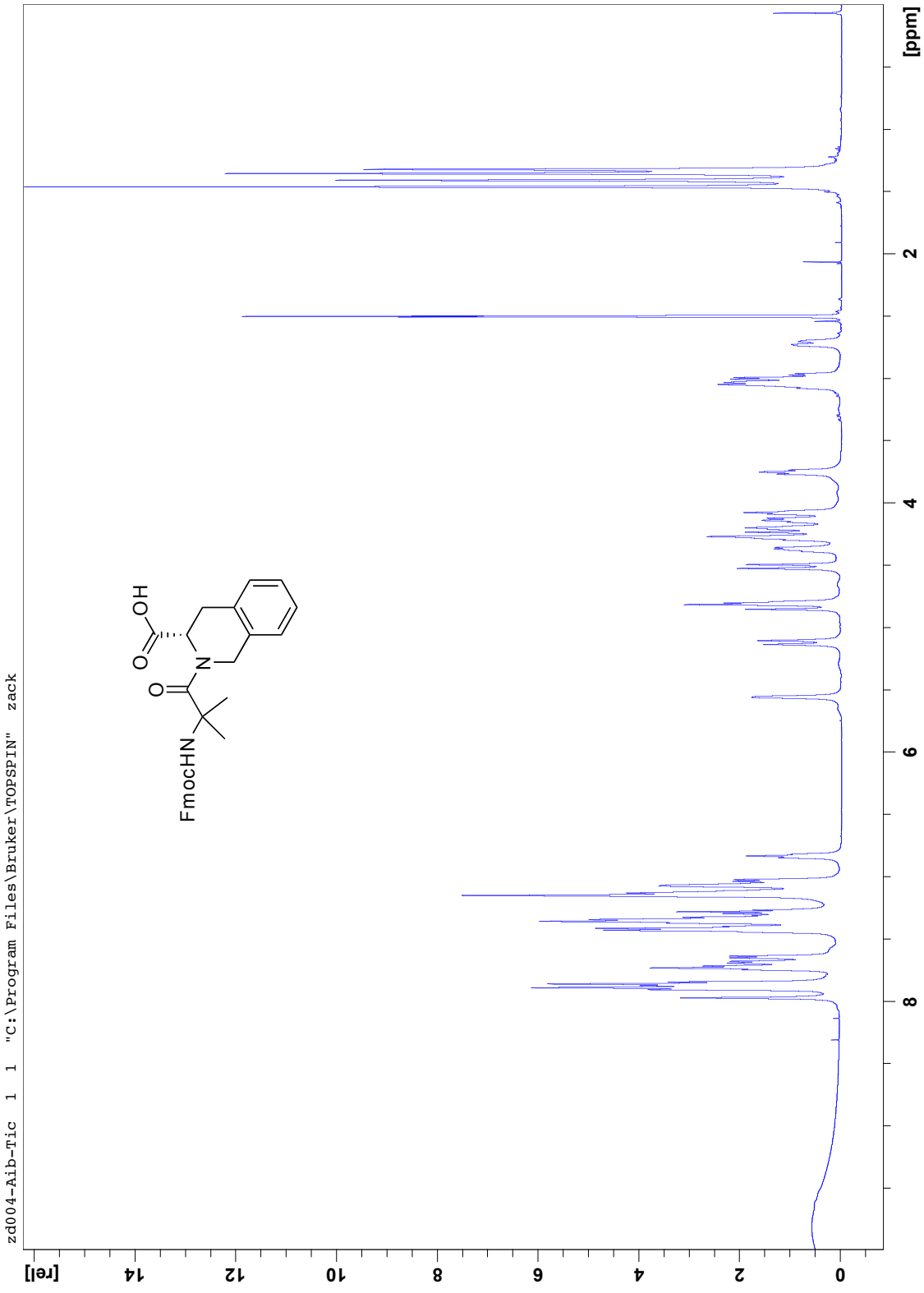
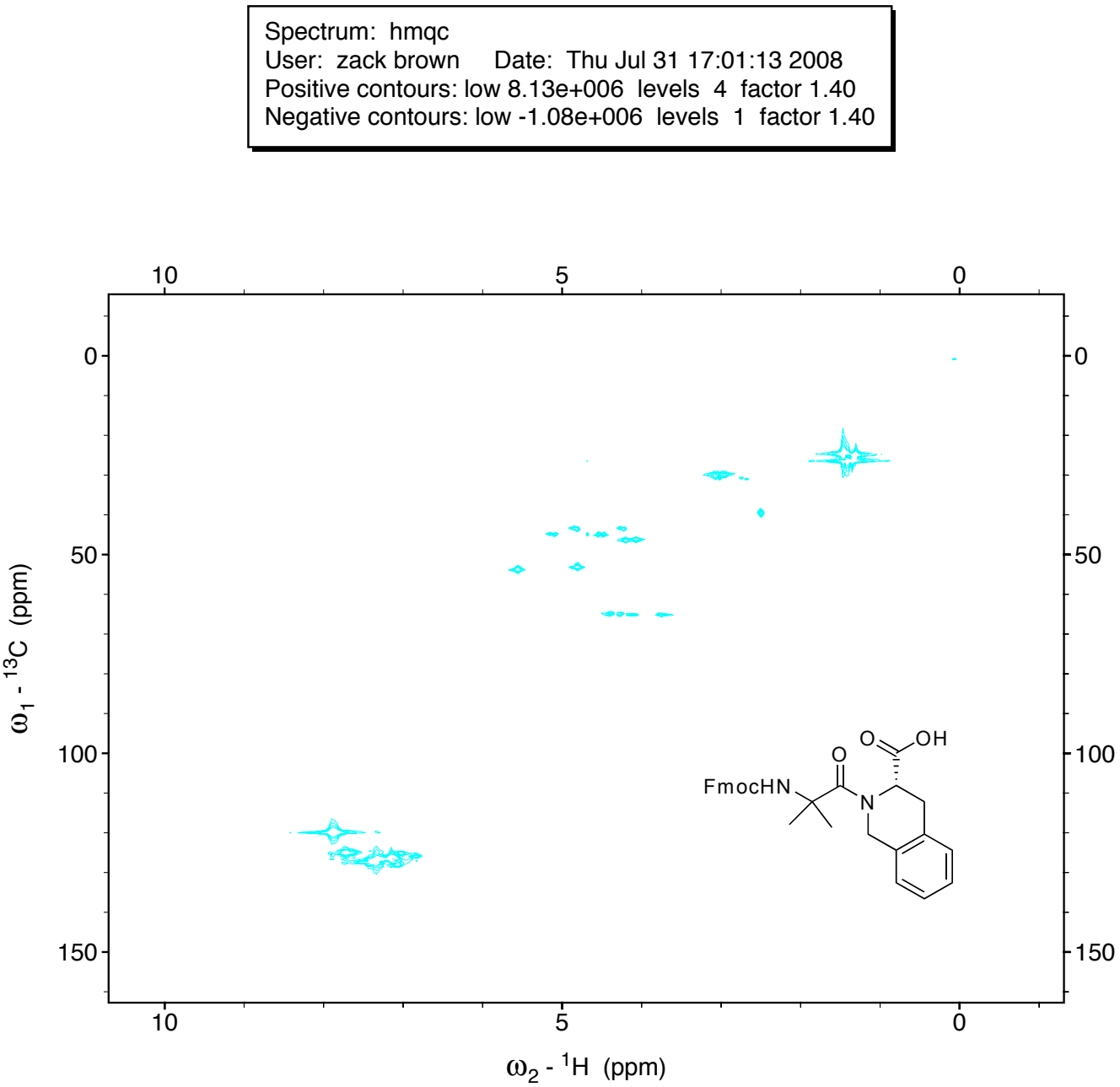


Supplemental Figure 25



Supplemental Figure 26



Supplemental Figure 27

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