Supporting information of

Prebiotic Access to Enantioenriched Glyceraldehyde Controlled by Peptides

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Table of content

Table of content 2
1. General information 3
2. General procedures of kinetic resolution of *rac*-glyceraldehyde by peptides 4
Resolution of rac-glyceraldehyde by peptides 4
Measurement of concentration of remaining glyceraldehyde 4
Measurement of enantiomeric excess of remaining glyceraldehyde 5
Measurement of concentration of DHA 6
Calculation of selectivity factor 7
3. Measurement of glyceraldehyde concentrations and ee's from kinetic resolution 8
3.1 *L*-Dipeptide-mediated kinetic resolution of *rac*-glyceraldehyde 8
3.2 Ratios of *LL*-PV to *rac*-glyceraldehyde 9
3.3 L-Prolinamide and *N*-terminal *L*-Proline tri- and tetra- peptide-mediated kinetic resolution of *rac*-glyceraldehyde 9
3.4 Comparison of Homo- and Hetero-chiral Peptides 10
4. Mechanistic studies 11
4.1 Monitoring the kinetic profile using ^{13}C-labeled material 11
4.2 Independent Reactions with *D*- and *L*-glyceraldehyde 12
4.2.1 Reaction protocol 12
4.2.2 Measurement of concentrations of glyceraldehyde and DHA 12
4.2.3 Estimation of ARP Concentration in Independent *D*-/*L*-Glyceraldehyde Reactions 13
4.2.4 Determination of Mass Balance 14
4.3 Control experiment with *DD*-PV 15
5. Solid Phase enrichment of *LL*-PV from *LL*/*DL*-PV mixtures 16
Measurement of saturation concentration of LL-PV and DL-PV 16
Solid phase enrichment of LL-PV 16
6. Characterization of Amadori Rearrangement Product 18
7. Preparation of peptides 23
7.1 Synthesis of peptides 23
7.2 NMR spectra for new peptides 26

1. General information

Materials: Commercial reagents were purchased from Sigma Aldrich, AlfaAesar, Acros, Combi-Block and Oakwood and used as received. Water and acetonitrile for HPLC were purchased from Fischer Scientific.

Analysis: NMR spectra were recorded on Bruker, DRX-500 and AMX-400 instruments. Calibration methods of NMR spectra could be found in related sections. The following abbreviations were used to explain multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. High resolution mass spectrometry information was collected on Waters G2-XS time of flight (TOF) mass spectrometry. The aliquots were analyzed by HPLC-MS and SFC. Details could be found in related sections.

2. General procedures of kinetic resolution of *rac*-glyceraldehyde by peptides

Resolution of rac-glyceraldehyde by peptides

Rac-glyceraldehyde (9.0 mg, 1.0 mmol), *LL*-PV (21.4 mg, 1.0 mmol) and 3,5-*N*-dinitrophenyl-*L*-alanine (DNB-Ala, internal standard, 2~3 mg) were dissolved in 1.0 mL phosphate buffer (4.0 M, pH=9.5). The sample was let to stand at room temperature for 6 hrs.

Measurement of concentration of remaining glyceraldehyde

Aliquots (20 μL) were removed from reaction mixture at fixed time points and quenched with 30 μL aqueous solution of TFA (TFA/water: 1v/9v). A saturated solution of dinitrophenylhydrazine (DNPH) in ACN (950 μL) was added and the samples were let to react at room temperature for 30 minutes. The aliquots were analyzed by LC-MS using a Dionex Ultimate 3000 HPLC system coupled to a Thermo-Fisher MSQ mass spectrometer. HPLC analysis of the DNPH derivatized glyceraldehyde (DNPH-glyc) was performed using Waters Xbridge BEH C18 column (4.6 x 150 mm, 5.0 μm) under gradient conditions (1.2 mL/min, 30-70% B, 15 mins; A = H_2O + 0.1% FA, B = ACN; glyceraldehyde: 3.5 min; DNB-Ala: 5.8 min; UV detection @ 254 nm).

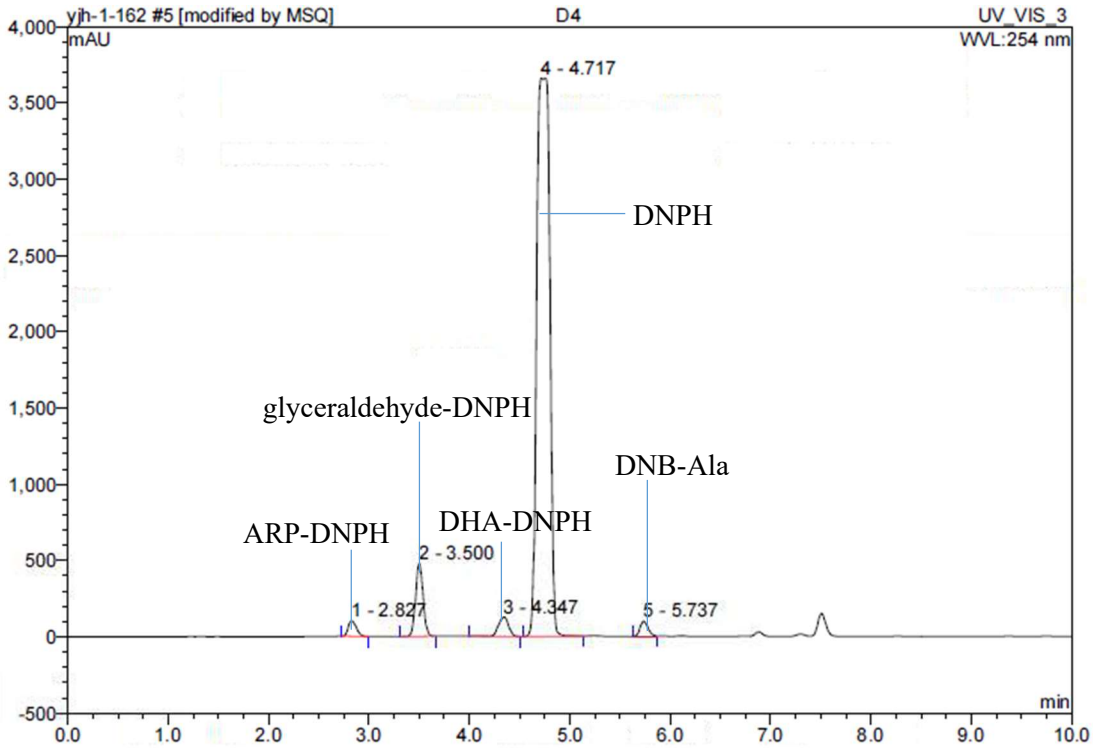


Figure 2. 1 Reaction aliquot sample of kinetic resolution by *LL*-PV and *rac*-glyceraldehyde

entry	retention time (min)	compound
1	2.827	ARP-DNPH
2	3.500	glyceraldehyde-DNPH
3	4.347	DHA-DNPH
4	4.717	DNPH
5	5.737	DNB-Ala

Compound 1 was identified by MS. Compound 2-5 were identified by comparison of retention time to real samples.

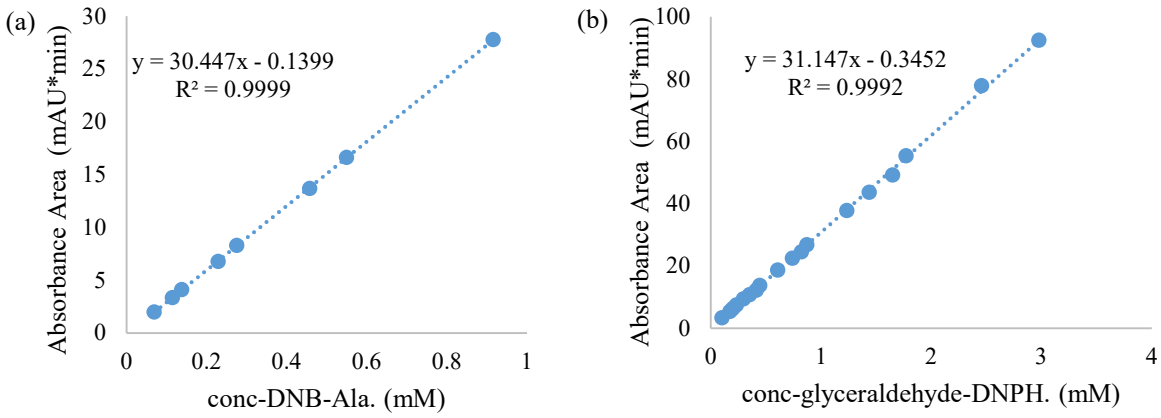
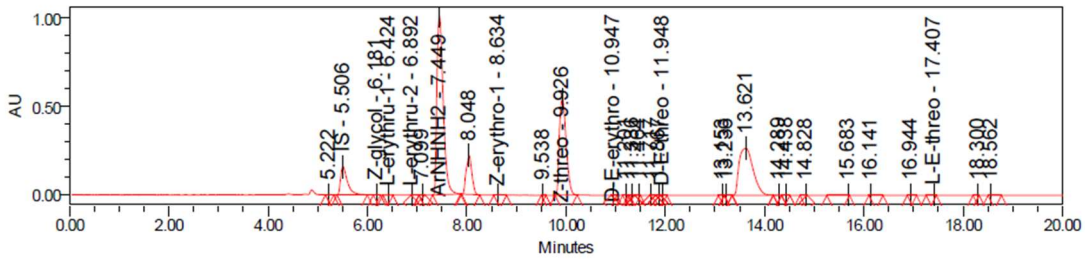


Figure 2. 2 UV calibration curves of DNB-Ala (a) and glyceraldehyde-DNPH (b)

Measurement of enantiomeric excess of remaining glyceraldehyde

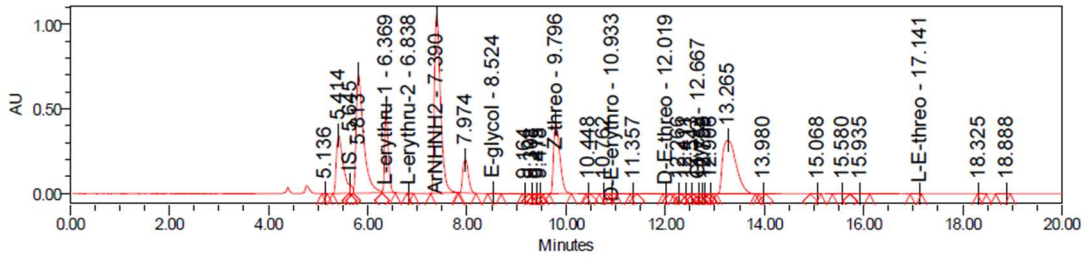
Aliquots (40 µL) were removed from the reaction mixture at fixed time points and quenched with 60 µL aqueous solution of TFA (TFA/water: 1v/9v). A saturated solution of DNPH in ACN (250 µL) was added and the aliquots were let to react at room temperature for 30 minutes. Chiral SFC analysis of glyceraldehyde-DNPH was performed using a Waters UPC2 SFC with a Daicel IA column (4.6x250 mm, 3 µm) under gradient conditions (3 mL/min, 10-60% B over 10 minutes, then hold 60% B; A = CO_2, B = MeOH containing 0.5% 7N NH_3 in MeOH; 1600 psi backpressure; 30 °C; *L*-glyceraldehyde: 9.93 min; *D*-glyceraldehyde: 13.6 min; UV detection @ 350 nm).

(a)



time (min)	compound	area (µV*sec)	area percentage (%)
9.837	*L*-glyceraldehyde	7673244	49.9
13.482	*D*-glyceraldehyde	7714965	50.1

(b)



time (min)	specie	area (μV*sec)	area percentage (%)
9.796	*L*-glyceraldehyde	3648898	38.0
13.265	*D*-glyceraldehyde	5954276	62.0

Figure 2. 3 SFC Chromatogram of kinetic resolution of *rac*-glyceraldehyde by *LL*-PV. (a) t=0 hr; (b) t=6 hr

Measurement of concentration of DHA

Aliquots (20 μL) were taken at fixed time points and quenched into 1.0 mL PMP solution. The samples were heated to 55 °C for 40 min and submitted to LC-MS analysis. HPLC analysis of the PMP derivatized DHA was performed using Waters Xbridge BEH C18 column (4.6 x 150 mm, 5.0 μm) under gradient conditions (1.0 mL/min, 10-60% B, 30 mins; A = H_2O + 0.1% FA, B = ACN; DHA: 12.3 min; UV detection @ 254 nm).

PMP solution: 1-phenyl-3-methyl-5-pyrazolone (PMP) 50 mM in MeOH/borate buffer pH=8.4, 4 M (1v/1v).

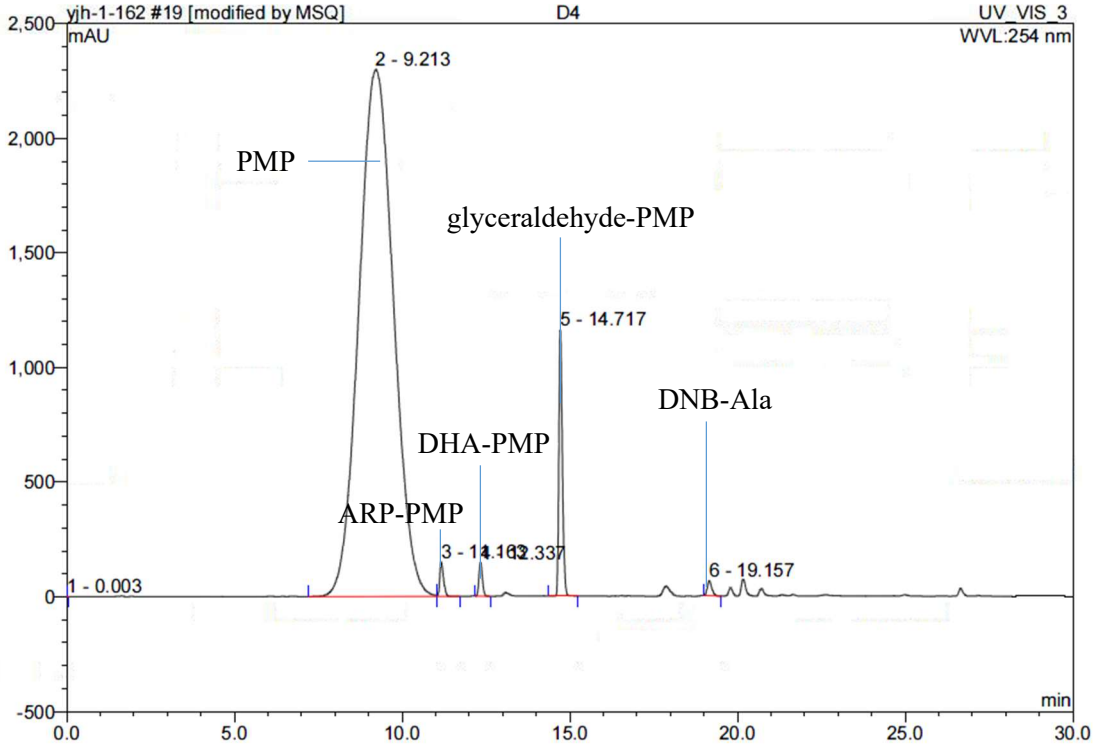


Figure 2. 4 Reaction aliquot sample of kinetic resolution by *LL*-PV and *rac*-glyceraldehyde

entry	retention time (min)	compound
1	9.217	PMP
2	11.167	ARP-PMP
3	12.337	glyceraldehyde-PMP
4	14.727	DHA-PMP
5	19.157	DNB-Ala

Compound 2 was identified by MS. Compound 1, 3-5 were identified by comparison of retention time to real samples.

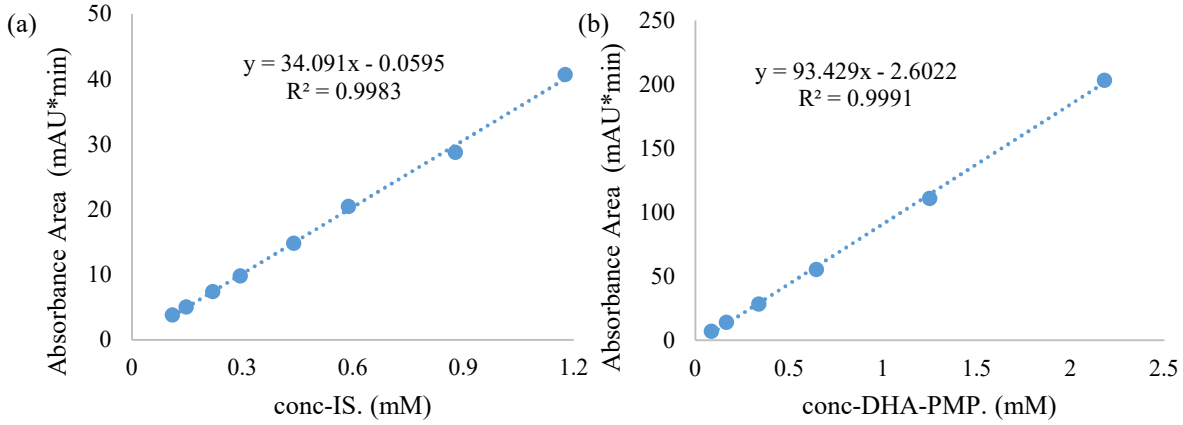


Figure 2. 5 UV calibration curves of DNB-Ala (a) and DHA-PMP (b)

Calculation of selectivity factor

In the kinetic resolution of *rac*-glyceraldehyde by *L*-peptides if *L*-glyceraldehyde reacts faster than *D*-glyceraldehyde ($k_L > k_D$):

Selectivity factor (s-factor) $= \frac{k_L}{k_D}$

If first-order kinetics in substrate are assumed, s-factor can be expressed in terms of ee of the recovered starting material and conversion (c):

$$s - \text{factor} = \frac{\log[1 - c(1 + ee)]}{\log[1 - c(1 - ee)]}$$

S-factor of a kinetic resolution was calculated by taking the average of each aliquot.

3. Measurement of glyceraldehyde concentrations and ee's from kinetic resolution

3.1 *L*-Dipeptide-mediated kinetic resolution of *rac*-glyceraldehyde

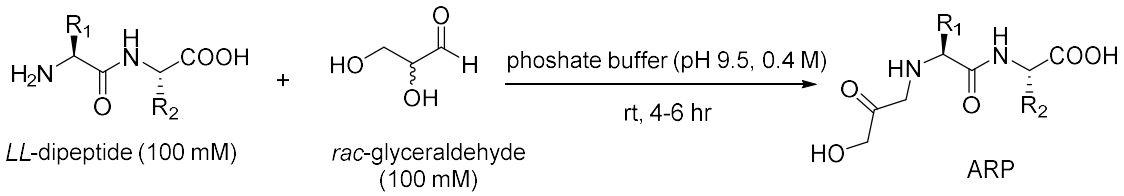


Table 3. 1 screening of different *L*-peptides.

time (hrs)	*LL*-PV	*LL*-PA	*LL*-PF*	*LL*-AA
0	98.2 (0.3)	99.5 (0.2)	11.8 (0.1)	128.6 (0.1)
1	80.9 (5.9)			76.1 (1.2)
2	69.8 (10.7)	87.4 (4.7)		48.1 (2.9)
3	61.8 (14.6)			29.4 (5.3)
4	54.4 (18.3)	74.4 (8.6)		18.2 (7.1)
5	49.6 (21.3)			
6	44.9 (24.1)	64.1 (12.4)	5.7 (22.0)	

time (hrs)	*LL*-VL	*LL*-VV	*LL*-LV	*LL*-VD
0	98.3 (0.1)	97.1 (0.1)	92.8 (-0.1)	98.4 (0.2)
2	80.9 (-6.4)	34.8 (-6.5)	36.4 (0.3)	41.7 (-5.8)
4	69.8 (-8.7)	11.5 (-11.0)	14.8 (-2.2)	15.5 (-9.6)

Time course analysis of glyceraldehyde concentration (mM) and %*ee* (in parenthesis) in the presence of *LL*-dipeptides.* *LL*-PF was saturated, the concentration of the peptide was around 10 mM

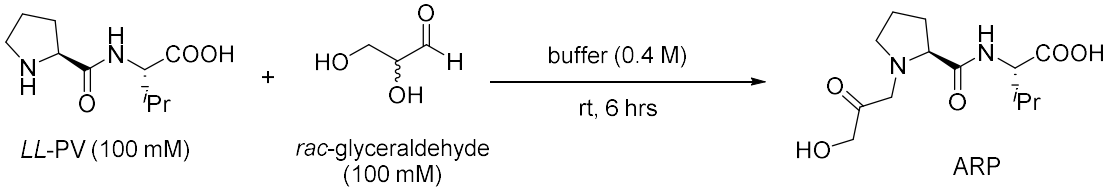
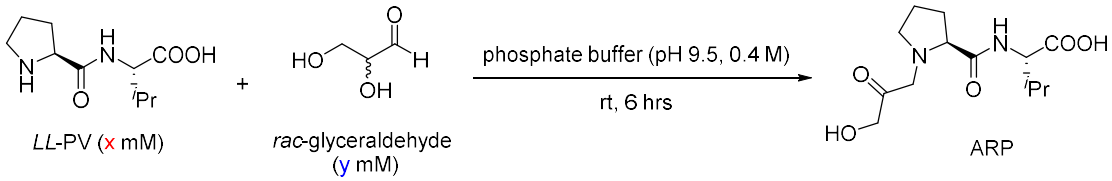


Table 3. 2 The influence of buffer in the kinetic resolution.

time (hrs)	Carbonate (9.6)*	Borate (8.4)	Acetate (3.8)	Phosphate (7.1)
0	105.7 (0.7)	100.1 (0.0)	108.1 (--)	117.6 (0.0)
3	88.9 (3.2)	95.8 (2.4)	106.8 (--)	72.7 (7.8)
6	72.3 (5.3)	93.5 (4.5)	108.1 (--)	67.3 (13.8)

Time course analysis of glyceraldehyde concentration (mM) and %ee (in parenthesis) in the presence of LL-PV and different buffers;*buffer (pH)

3.2 Ratios of *LL*-PV to *rac*-glyceraldehyde



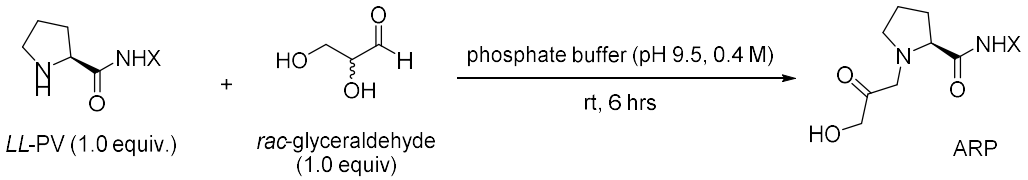
LL-PV (x mM) + *rac*-glyceraldehyde (y mM)

Table 3. 3 The effect of peptide/sugar ratios in the kinetic resolution.

time (hrs)	50: 100*	100: 50	100: 20	100:10
0	94.0 (0.0)	50.1 (0.3)	29.1 (0.0)	10.7 (0.0)
1			22.2 (11.1)	7.9 (12.2)
2	86.1 (5.1)	38.3 (11.2)	18.2 (18.6)	6.3 (21.1)
3			14.2 (26.1)	5.0 (30.3)
4	76.0 (8.6)	29.8 (20.5)	12.1 (32.4)	4.0 (37.5)
5			10.0 (38.9)	3.2 (44.7)
6	65.1 (12.0)	22.8 (29.4)		

Time course analysis of glyceraldehyde concentration (mM) and %ee (in parenthesis) for reactions at different initial LL-PV: glyceraldehyde (x:y) ratios; *peptide conc. (mM): glyceraldehyde conc. (mM)

3.3 L-Prolinamide and *N*-terminal *L*-Proline tri- and tetra- peptide-mediated kinetic resolution of *rac*-glyceraldehyde



LL-PV (1.0 equiv.) + *rac*-glyceraldehyde (1.0 equiv)

Table 3. 4 The effect of the peptide sequence in the kinetic resolution.

time (hrs)	*L*-Pro-NH_2	*LLL*-PFV	*LLLL*-PVVV
0	103 (0.5)	11.6 (0.1)	12.0 (0.0)
6	49.6 (13.0)	5.1 (28.1)	6.4 (21.0)

time (hrs)	*LLLL*-PVV	*LLLL*-PVFV	*LLLL*-PFVV
0	25.3 (0.4)	11.8 (0.1)	11.9 (0.2)
6	13.8 (22.0)	5.7 (22.7)	5.4 (30.1)

Initial and end point analysis of glyceraldehyde concentration (mM) and %*ee* (in parenthesis) for reactions with different *N*-terminal-*L*-Proline peptides and *L*-prolinamide.

3.4 Comparison of Homo- and Hetero-chiral Peptides

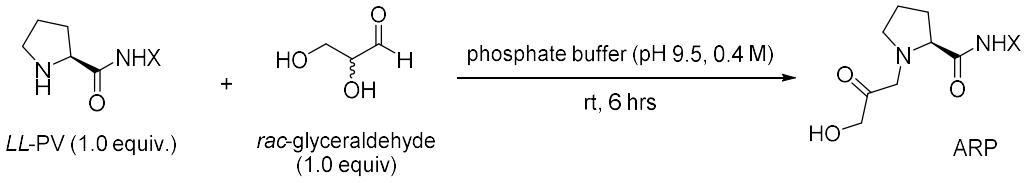


Table 3. 5 Reaction monitoring of proline-based peptides diastereomers.

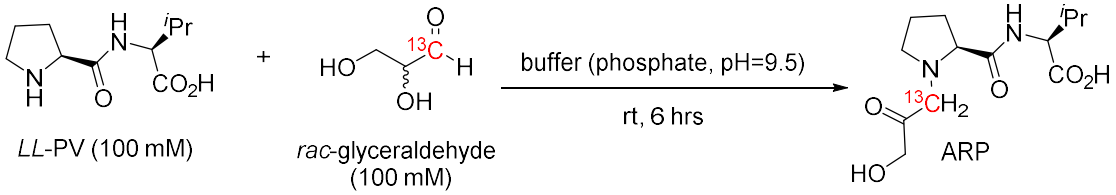
time (hrs)	*LL*-PV-OMe	*LD*-PV	*DL*-PV
0	105 (0.7)	101 (0.3)	102 (0.2)
2	75.6 (11.9)	84.2 (2.4)	96.0 (-1.0)
4	58.8 (19.7)	71.0 (4.0)	83.9 (-2.2)
6	46.7 (26.6)	59.4 (5.9)	75.4 (-3.2)

time (hrs)	*LDL*-PVV	*LLD*-PVV	*LDD*-PVV
0	25.3 (0.2)	20.9 (1.2)	21.4 (0.6)
2	22.0 (2.5)	15.3 (10.7)	17.5 (2.3)
4	19.6 (4.5)	12.5 (17.5)	14.5 (4.4)
6	17.2 (6.4)	10.0 (23.2)	12.9 (6.6)

Initial and end point analysis of glyceraldehyde concentration (mM) and %*ee* (in parenthesis) for reactions with different *N*-terminal Proline peptide diastereomers.

4. Mechanistic studies

4.1 Monitoring the kinetic profile using ^{13}C-labeled material



To a 900 µL solution of ^{13}C-labeled *D, L*-glyceraldehyde, *LL*-PV (21.4 mg, 1.0 mmol) and 100 µL internal standard solution were added Na_2HPO_4 $7H_2O$ (96 mg) and K_3PO_4 (4 mg) to buffer the mixture. The sample was submitted to quantitative ^{13}C NMR analysis at fixed time points.

Stock solution of internal standard: sodium benzoate, 2.2 M in D_2O.

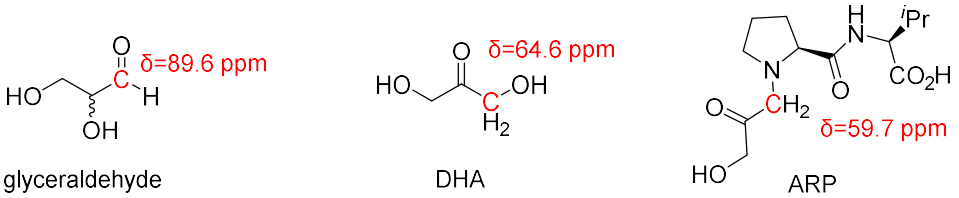


Figure 4. 1 chemical shift of carbons in different molecules

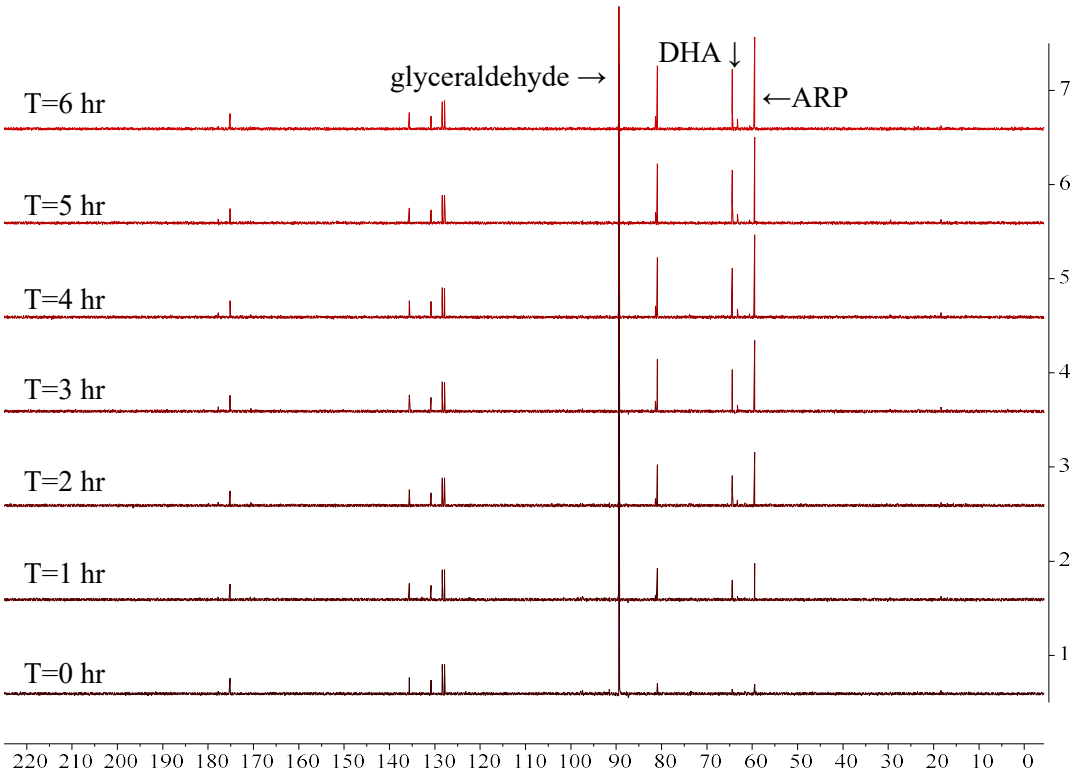


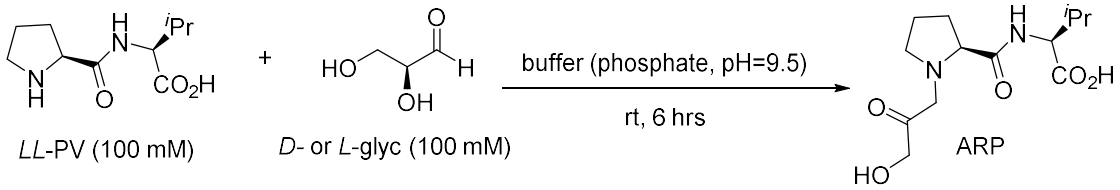
Figure 4. 2 ^{13}C spectra of reaction mixture overtime.

Table 4. 1 Changes of concentration of each component in the reaction mixture overtime.

time (hrs)	glyceraldehyde (mM)	ARP (mM)	DHA (mM)	glyceraldehyde +ARP+DHA (mM)
0	93.28	3.02	0.81	97.11
1	84.21	10.06	3.15	97.42
2	77.86	15.42	4.95	98.23
3	72.16	19.26	6.56	97.98
4	65.41	22.68	7.62	95.71
5	59.40	24.59	8.72	92.71
6	55.03	26.12	9.72	90.87

4.2 Independent Reactions with *D*- and *L*-glyceraldehyde

4.2.1 Reaction protocol



LL-PV (21.4 mg, 1 mmol) was reacted separately with *L*-/*D*-glyceraldehyde (9.0 mg, 1.0 mmol). To each reaction, 800 μL phosphate buffer (pH=9.5, 4 M) and 200 uL stock solution of DNB-Ala were added. The two reactions were let to stand at room temperature for 6 hrs. Aliquots were removed at fixed time points.

Stock solution of internal standard: DNB-Ala, 80.8 mM in phosphate buffer (pH=9.5, 4 M).

4.2.2 Measurement of concentrations of glyceraldehyde and DHA

The concentration of remaining glyceraldehyde was measured by DNPH derivatization protocol combined with LC-MS analysis as described in section 2. The concentration of DHA was measured by PMP derivatization protocol and analyzed by corresponding LC-MS method.

Table 4. 2 concentrations of glyceraldehyde and DHA in separated reactions

time (hrs)	[*L*-glyceraldehyde] (mM)	[*D*-glyceraldehyde] (mM)	[DHA_L] (mM)*	[DHA_D] (mM)*
0	96.12	93.04	2.39	1.75
1	80.08	87.05	4.65	3.67
2	66.27	81.67	6.80	5.73
3	57.01	74.96	8.79	7.31
4	49.39	70.05	10.69	8.55
5	43.22	64.04	12.45	9.64
6	38.64	60.24	13.54	10.46

* DHA_L/DHA_D: DHA generated from L/D-glyceraldehyde.

4.2.3 Estimation of ARP Concentration in Independent *D*-/*L*-Glyceraldehyde Reactions

ARP could be derivatized by DNPH and ARP-DNPH could be detected by LC-UV (@254 nm) using the same analyzing protocol of glyceraldehyde (See section **2**). Unfortunately, we were not able to obtain a standard of ARP for calibration. However, the concentration of ARP could be estimated as follows:

In a reaction of *LL*-PV and ^{13}C-rac-glyceraldehyde (see section 4.1), concentrations of ARP could be measured by quantitative ^{13}C NMR. By submitting the aliquots to DMPH derivatization and HPLC analysis, the correlation between UV absorption areas of ARP-DMPH and corresponding concentrations (known from NMR) could be developed.

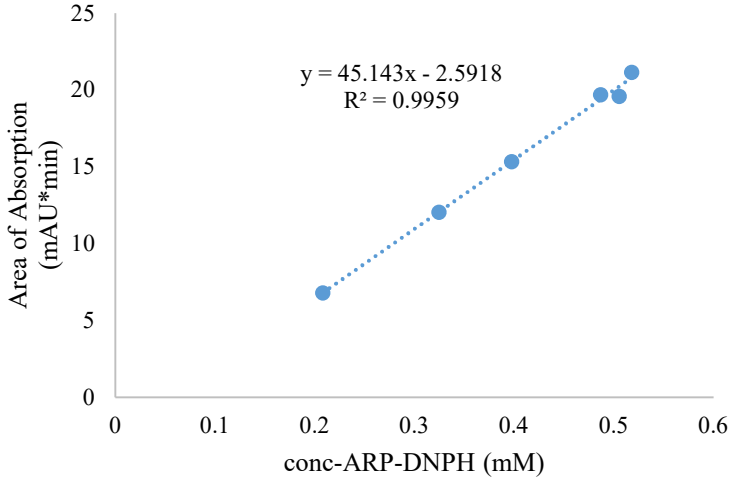


Figure 4. 3 Calibration curve of ARP-DNPH

Table 4. 3 Concentrations of ARP in separated reactions.

time (hrs)	**[ARP$_L$]* (mM)**	**[ARP$_D$]* (mM)**
0	6.04	0.00
1	15.17	4.95
2	21.79	9.05
3	26.26	12.26
4	29.90	15.46
5	32.44	16.74
6	34.66	17.58

* ARP$_L$/ARP$_D$: ARP generated from L/D-glyceraldehyde.

4.2.4 Determination of Mass Balance

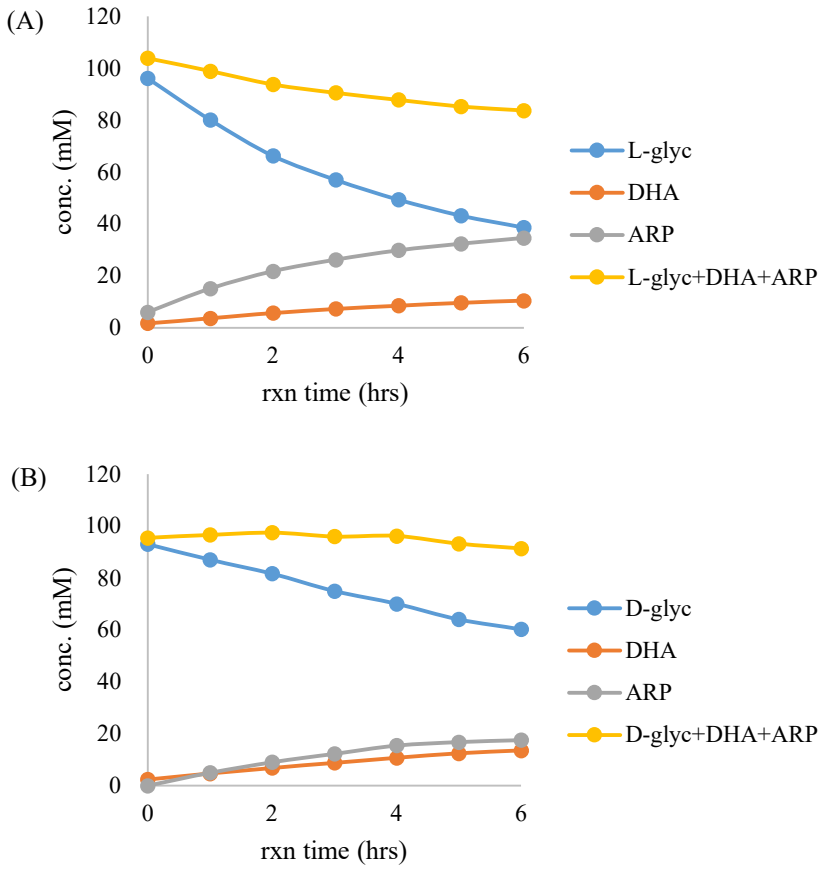


Figure 4. 4 Concentrations of reaction components and mass balance (yellow) in separated reactions.

4.3 Control experiment with *DD*-PV

DD-PV was reacted with *rac*-glyceraldehyde under the optimized conditions. Equal and opposite e.e. was achieved compared to the reaction with *LL*-PV and *rac*-glyceraldehyde.

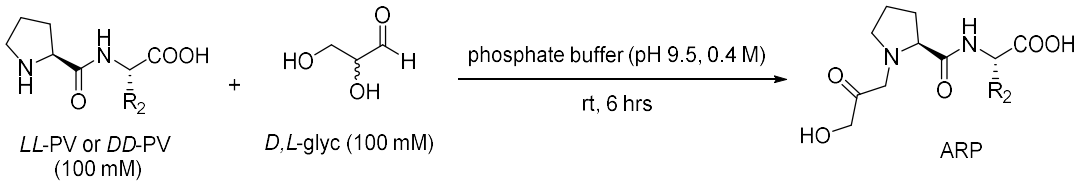


Table 4. 4 Time course of the kinetic resolution by *LL*-PV and *DD*-PV.

	LL-PV		*DD*-PV	
time (hrs)	conc. (mM)	ee (%)	conc. (mM)	ee (%)
0	98.3	0.3	106.6	-0.0
1	80.9	5.8	94.6	-3.1
2	69.8	10.7	85.6	-6.4
3	61.8	14.6	77.6	-8.8
4	54.4	18.3	71.2	-11.0
5	49.6	21.3	65.6	-13.5
6	44.9	24.0		

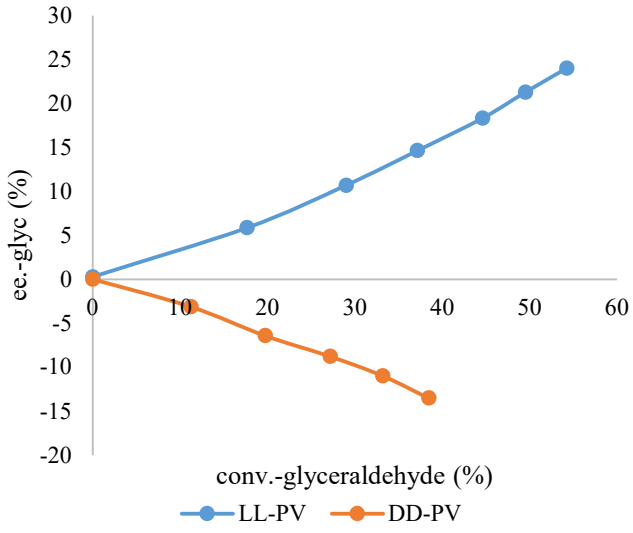


Figure 4. 5 Enantiomeric excess as a function of % conversion of *rac*-glyceraldehyde mediated by *LL*-/*DD*-PV

5. Solid Phase enrichment of *LL*-PV from *LL*/*DL*-PV mixtures

Measurement of saturation concentration of LL-PV and DL-PV

2 mL of peptide solution was subjected to a nitrogen flow. Aliquots (200 µL) were removed upon observation of peptide precipitation and filtered. The filtrate (80 µL) was dissolved in D_2O (320 µL) and sodium benzoate solution (80 µL, 203 mM in D_2O). The mixture was analyzed by quantitative ^1H NMR.

Peptide solution: Equal amount of *LL*-PV (100 mM) and *DL*-PV (100 mM) were dissolved in deuterated phosphate buffer (pH=9.5, 4 M).

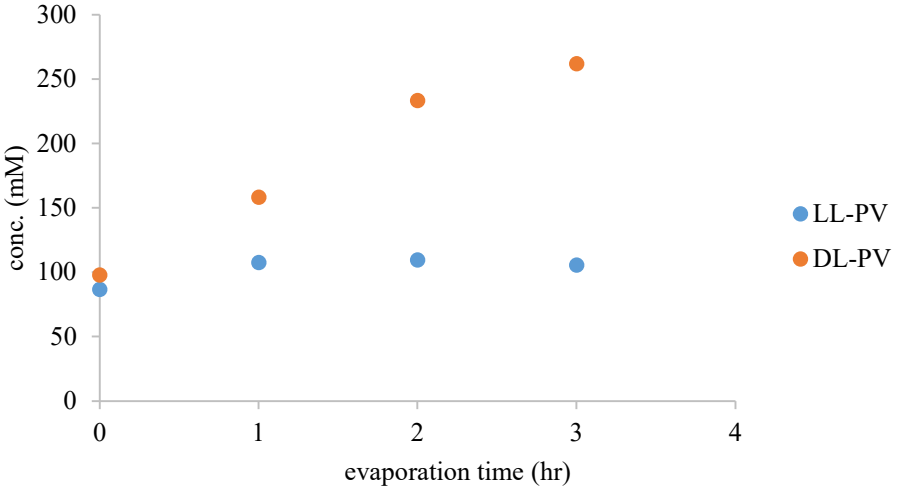


Figure 5. 1 Concentration of *LL*-PV and *DL*-PV in solution phase as the sample evaporated.

The saturation concentration of *LL*-PV was found to be around 100~110 mM, while saturation concentration of *DL*-PV was higher than 250 mM.

Solid phase enrichment of LL-PV

4 mL peptide solution was subjected to a nitrogen flow and evaporated to around 2 mL. The remaining suspension was then filtered. The solid was washed with D_2O. Quantitative ^1H NMR analysis indicated a 20:1 ratio of *LL*-PV to *DL*-PV in precipitation.

Peptide solution: Equal amount of *LL*-PV (100 mM) and *DL*-PV (100 mM) were dissolved in deuterated phosphate buffer (pH=9.5, 4 M).

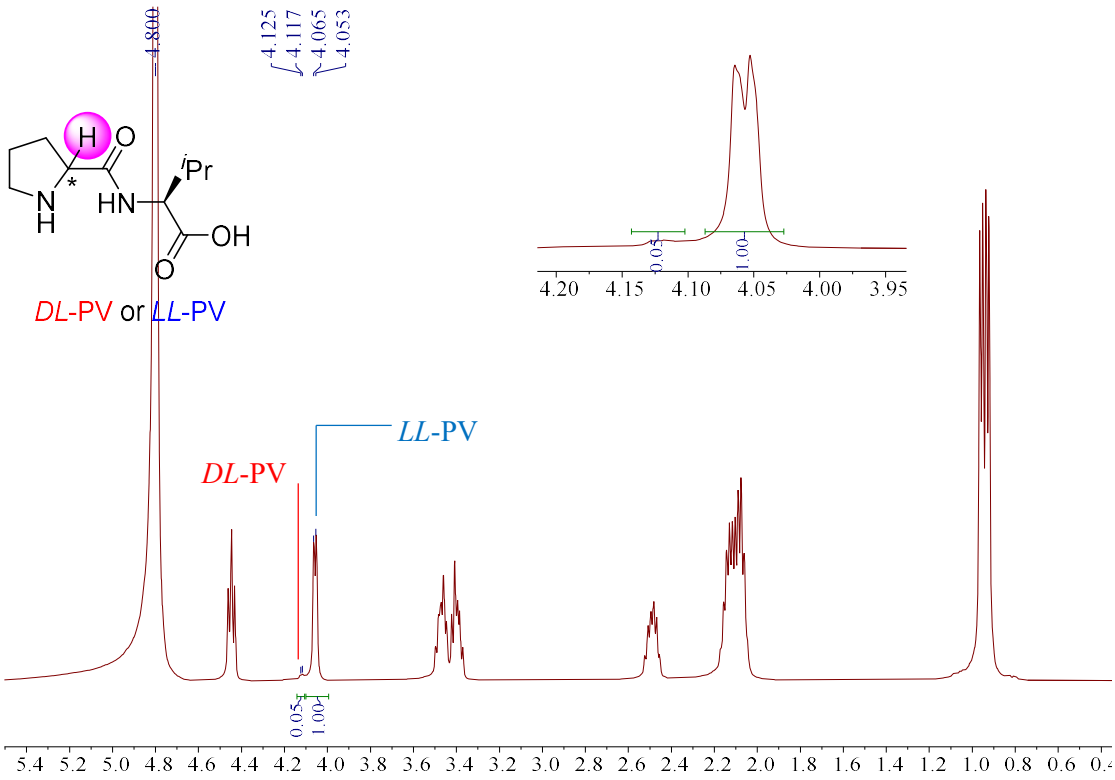
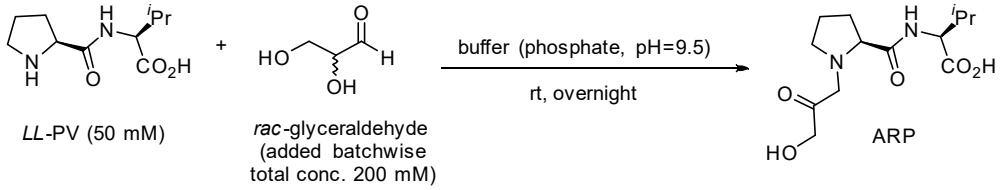


Figure 5. 2 Components of the precipitant in saturated solution.

6. Characterization of Amadori Rearrangement Product



The Amadori Rearrangement Product (ARP) was prepared by reacting *LL*-PV with *rac*-glyceraldehyde and was characterized directly in the reaction mixture.

LL-PV (53 mg, 2.5 mmol) and *rac*-glyceraldehyde (45 mg, 5.0 mmol) were dissolved in a mixture of phosphate buffer (pH=9.5, 4.0 M, 4.5 mL) and D_2O (0.5 mL) The solution was let to stand at room temperature for 6 hrs. Another batch of *rac*-glyceraldehyde (45 mg, 5.0 mmol) was added as solid into the solution, dissolved and reacted overnight under room temperature.

The reaction mixture was submitted to HRMS and NMR analysis.

HRMS:
Calculated [ARP+H]+: 287.1607
Found [ARP+H]+: 287.1611

Crude NMR analysis:
NMR spectra were recorded on Bruker AMX-500 instrument. The ^{1}H NMR spectrum was referenced by H_2O and ^{13}C NMR spectrum by remaining glyceraldehyde (δ 89.6 for carbonyl carbon).

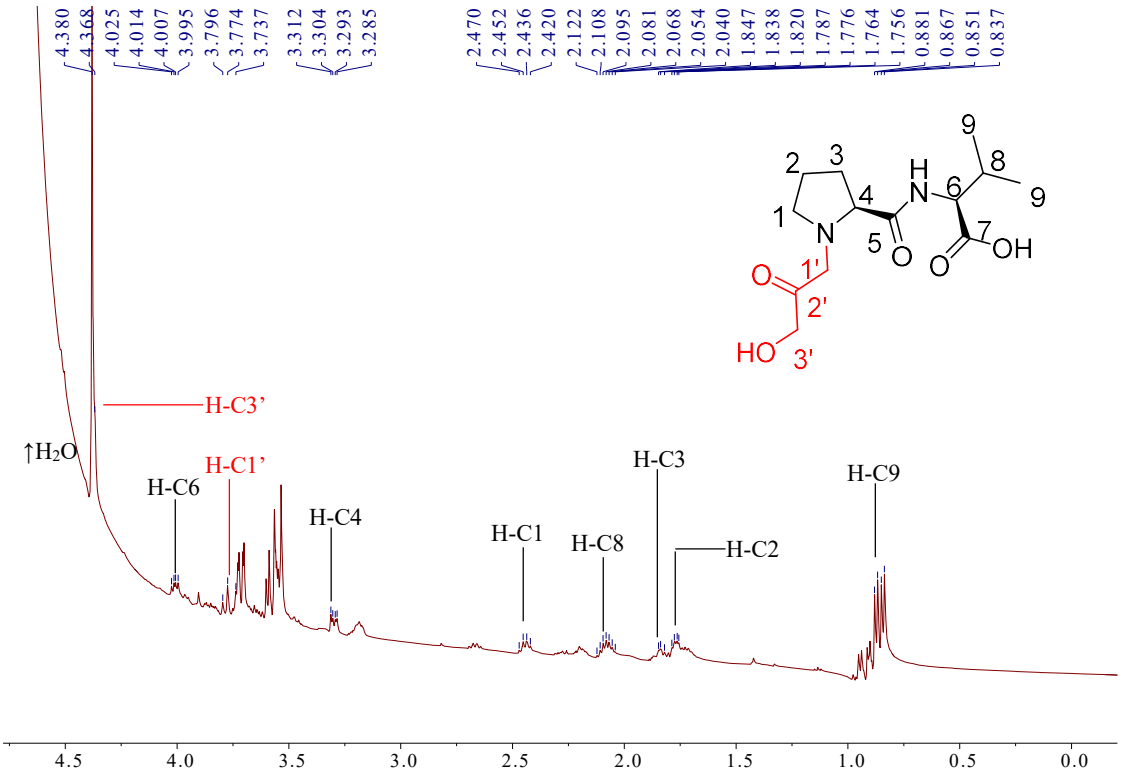


Figure 6. 1 [1]H NMR of reaction mixture and assignment of ARP peaks

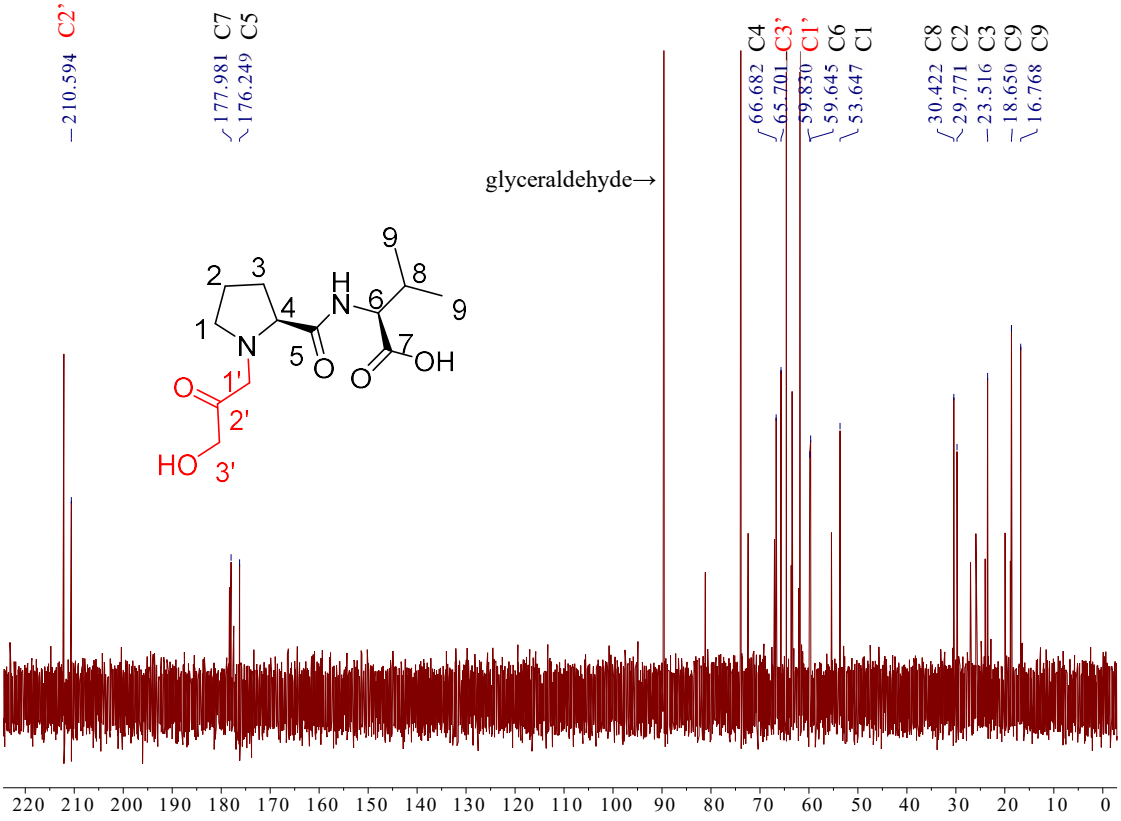


Figure 6. 2 13C NMR of reaction mixture and assignment of ARP peaks

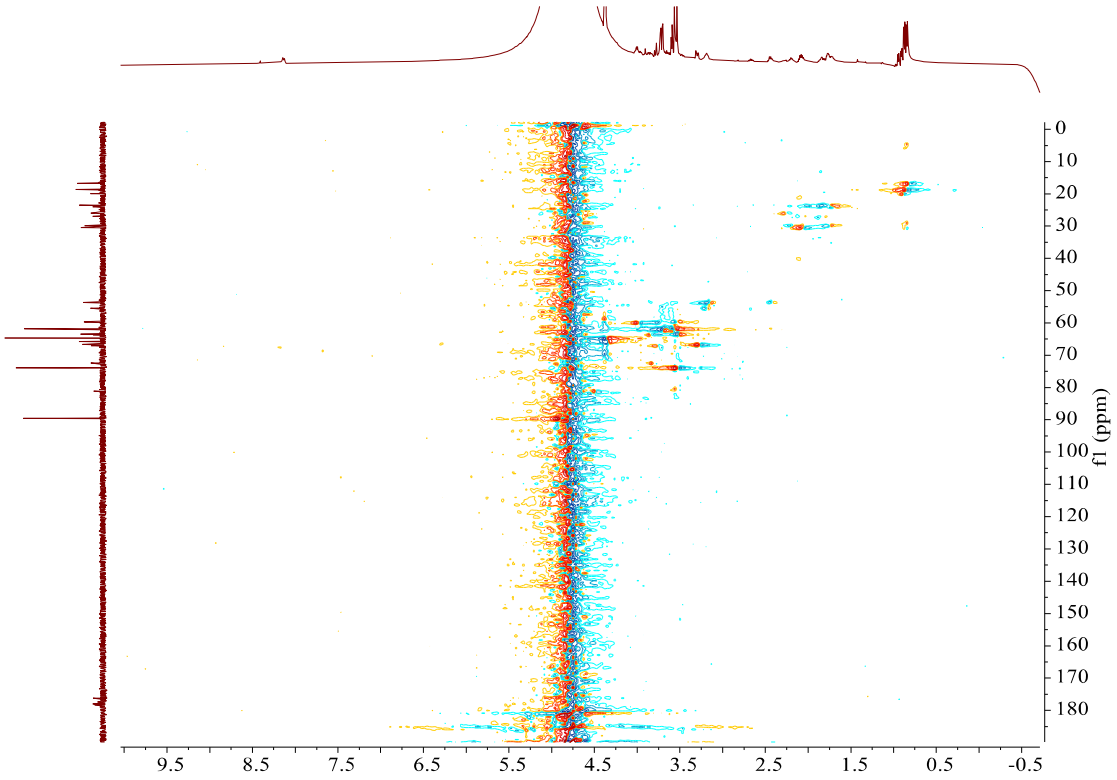


Figure 6. 3 ^{1}H-^{13}C HSQC of reaction mixture

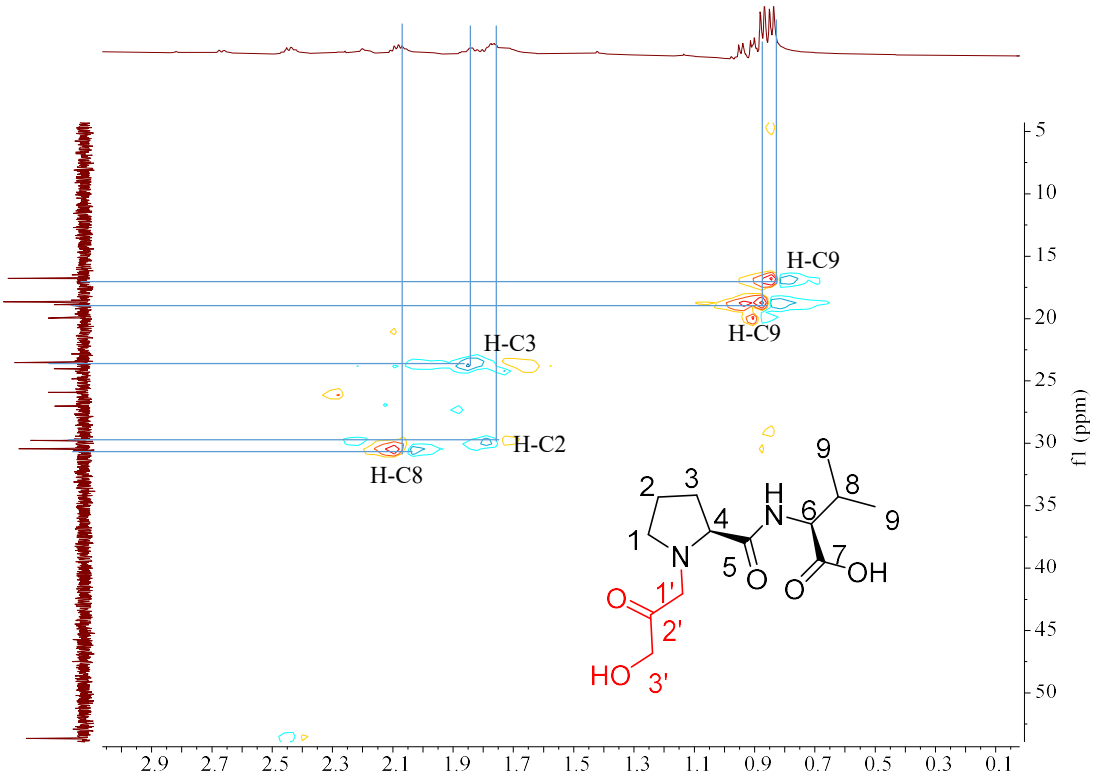


Figure 6. 4 HSQC signals of ARP

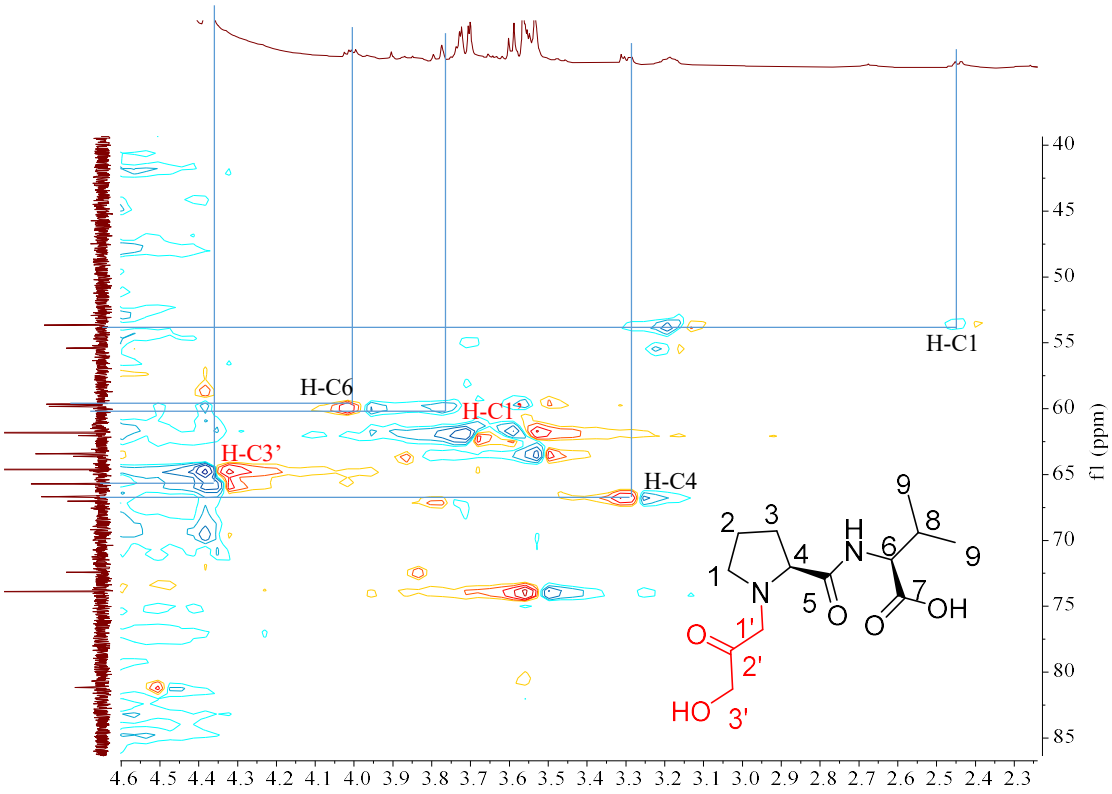


Figure 6. 5 HSQC signals of ARP

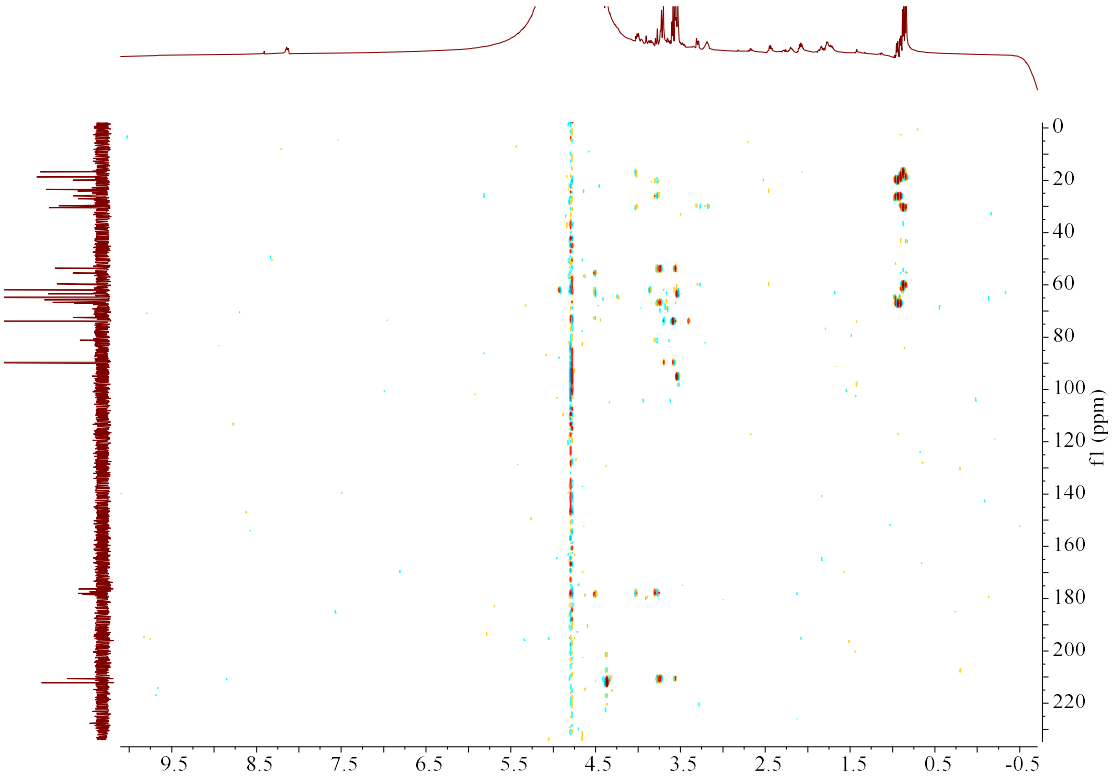


Figure 6. 6 ^{1}H-^{13}C HMBC of reaction mixture

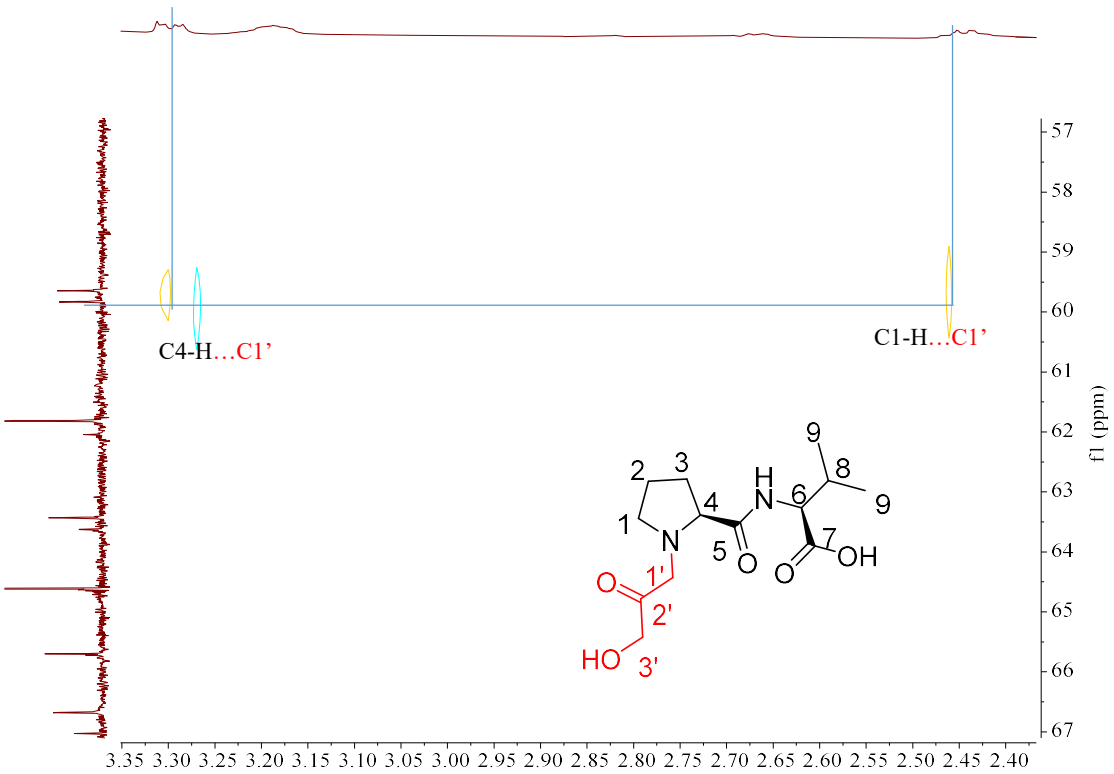


Figure 6. 7 HMBC signals of ARP

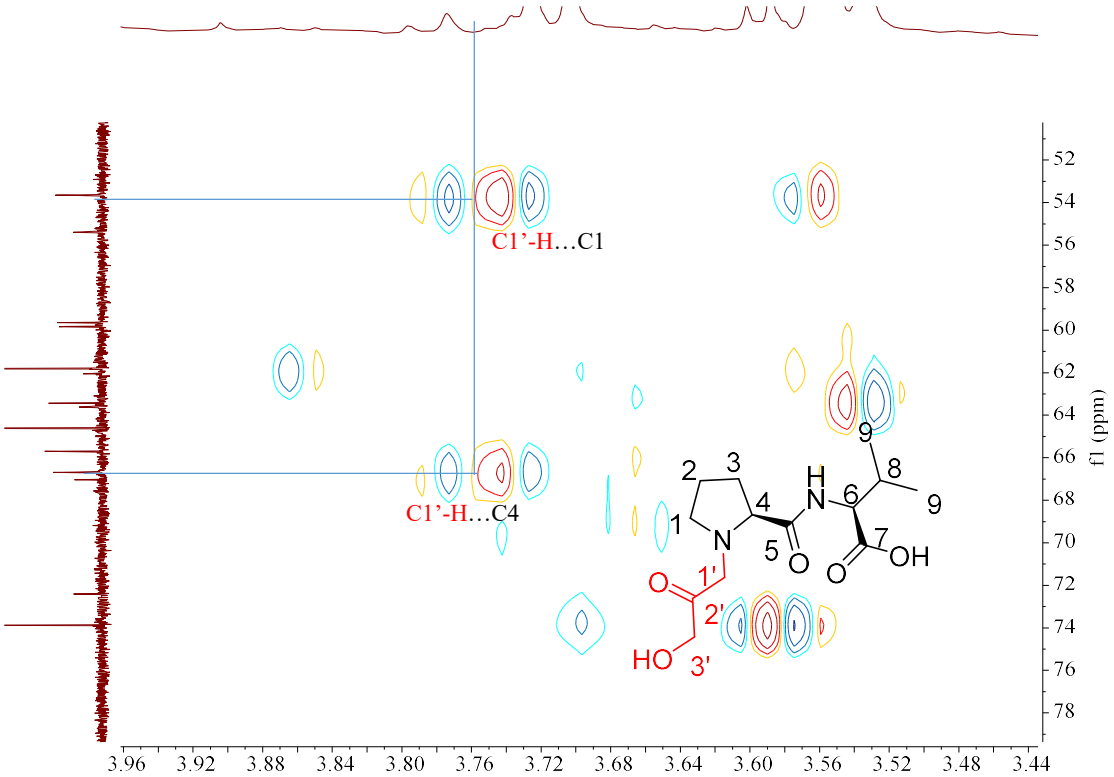


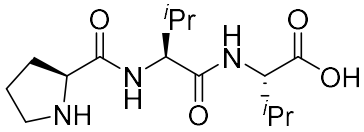
Figure 6. 8 HMBC signals of reaction ARP

7. Preparation of peptides

7.1 Synthesis of peptides

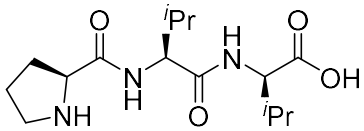
Peptides that were not commercially available were synthesized by an automated solid-phase synthesizer.

***LLL*-PVV**



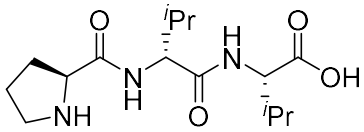
^{1}H NMR (600 MHz, D2O) δ 4.47 (dd, J = 8.2, 5.8 Hz, 1H), 4.19 (d, J = 7.8 Hz, 1H), 4.08 (d, J = 6.2 Hz, 1H), 3.44 (ddt, J = 38.8, 12.1, 6.8 Hz, 2H), 2.54 – 2.44 (m, 1H), 2.16 – 1.99 (m, 5H), 1.04 – 0.95 (m, 6H), 0.92 (dd, J = 12.1, 6.8 Hz, 6H).
^{13}C NMR (150 MHz, D_2O) δ 172.0, 169.1, 59.9, 59.1, 46.1, 30.2, 29.4, 29.4, 23.20, 18.3, 17.9, 17.3, 17.0.

***LLD*-PVV**



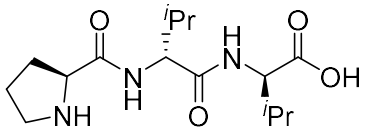
^{1}H NMR (600 MHz, D2O) δ 4.51 – 4.45 (m, 1H), 4.28 (d, J = 6.9 Hz, 1H), 4.10 (d, J = 5.5 Hz, 1H), 3.50 – 3.38 (m, 2H), 2.49 (dd, J = 8.3, 6.2 Hz, 1H), 2.20 – 2.01 (m, 5H), 1.09 – 0.83 (m, 12H).
^{13}C NMR (150 MHz, D2O) δ 177.8, 172.0, 169.1, 60.3, 59.6, 59.1, 46.0, 30.1, 29.8, 29.4, 23.2, 18.5, 18.1, 16.9, 16.9.

***LDL*-PVV**



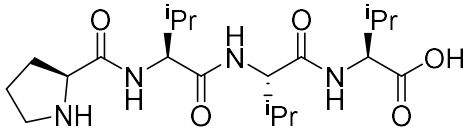
^{1}H NMR (600 MHz, D2O) δ 4.49 – 4.42 (m, 1H), 4.34 (d, J = 7.1 Hz, 1H), 4.08 (d, J = 5.9 Hz, 1H), 3.52 – 3.37 (m, 2H), 2.52 (td, J = 8.8, 8.1, 5.4 Hz, 1H), 2.20 – 2.02 (m, 5H), 0.96 (ddd, J = 20.3, 16.4, 6.8 Hz, 12H).
^{13}C NMR (150 MHz, D2O) δ 177.7, 172.0, 169.2, 60.5, 59.3, 59.0, 46.1, 30.1, 29.8, 29.6, 23.3, 18.5, 18.1, 17.0, 16.7.

***LDD*-PVV**



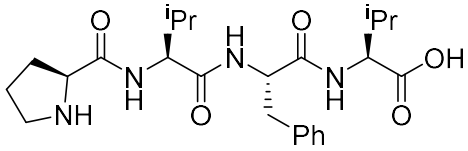
^{1}H NMR (600 MHz, D2O) δ 4.49 – 4.43 (m, 1H), 4.25 (d, J = 7.2 Hz, 1H), 4.09 (d, J = 6.3 Hz, 1H), 3.51 – 3.39 (m, 2H), 2.57 – 2.47 (m, 1H), 2.22 – 2.02 (m, 5H), 0.98 (dd, J = 6.4, 3.9 Hz, 6H), 0.93 (dd, J = 12.7, 7.0 Hz, 6H).
^{13}C NMR (150 MHz, D2O) δ 177.5, 172.2, 169.3, 60.4, 59.5, 59.3, 46.0, 30.1, 29.7, 29.6, 23.3, 18.3, 18.0, 17.0, 16.9.

***LLLL*-PVVV**



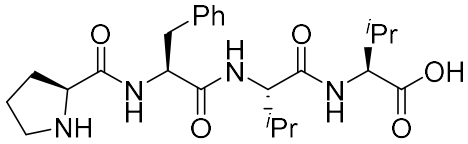
^{1}H NMR (600 MHz, D2O) δ 4.49 – 4.43 (m, 1H), 4.18 (t, J = 8.6 Hz, 2H), 4.09 (dd, J = 6.2, 2.1 Hz, 1H), 3.49 – 3.38 (m, 2H), 2.49 (q, J = 6.9, 6.4 Hz, 1H), 2.15 – 1.98 (m, 6H), 1.01 – 0.87 (m, 18H).
^{13}C NMR (150 MHz, D2O) δ 177.0, 172.5, 172.1, 169.0, 60.0, 59.6, 59.2, 59.0, 46.1, 29.4, 29.7, 29.5, 29.4, 23.2, 18.2, 17.9, 17.4, 17.0.

***LLLL*-PVFV**



^{1}H NMR (600 MHz, D2O) δ 7.36 (t, J = 7.5 Hz, 2H), 7.30 (t, J = 8.4 Hz, 3H), 4.43 – 4.35 (m, 1H), 4.09 (dd, J = 15.5, 7.1 Hz, 2H), 3.42 (q, J = 7.1 Hz, 2H), 3.20 (dd, J = 13.9, 5.9 Hz, 1H), 2.99 (dd, J = 13.9, 9.3 Hz, 1H), 2.39 (dq, J = 14.5, 7.3 Hz, 1H), 2.13 – 1.94 (m, 4H), 1.83 (dq, J = 14.0, 7.1 Hz, 1H), 0.95 – 0.83 (m, 12H).
^{13}C NMR (150 MHz, D2O) δ 176.8, 172.1, 171.5, 168.7, 136.0, 128.7, 128.2, 126.5, 59.9, 59.3, 58.9, 54.3, 46.1, 36.6, 30.2, 29.7, 29.4, 23.3, 18.0, 17.9, 17.3, 16.9.

***LLLL*-PFVV**

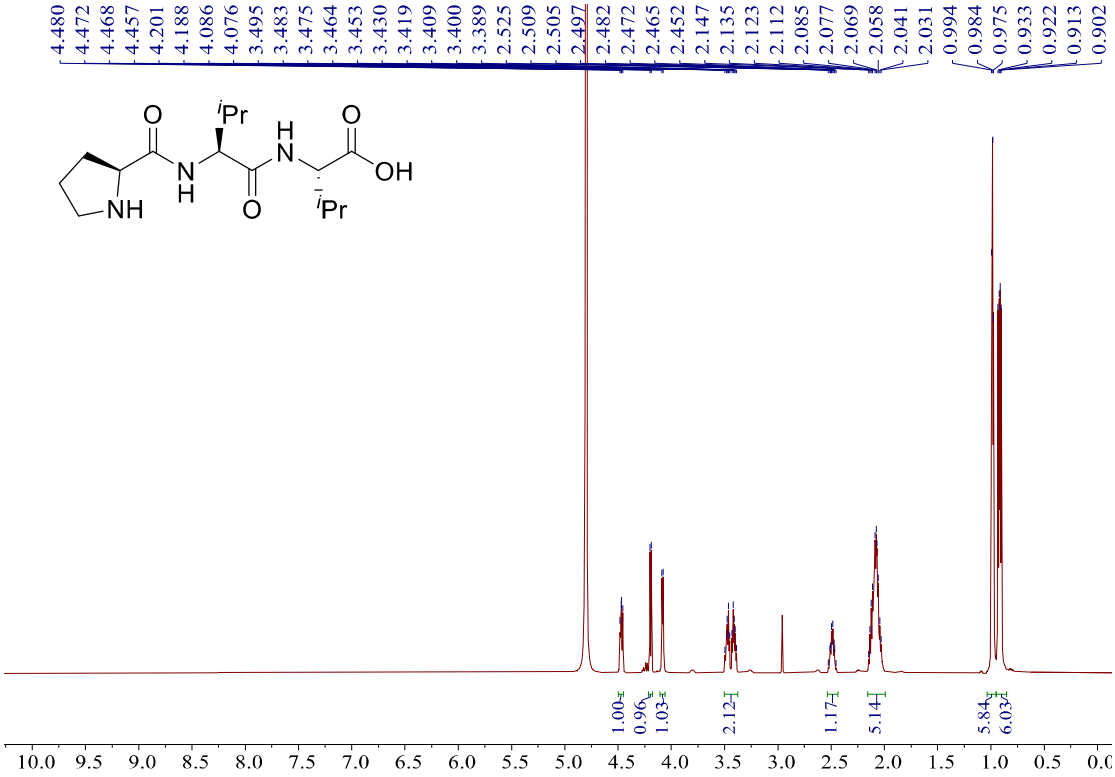


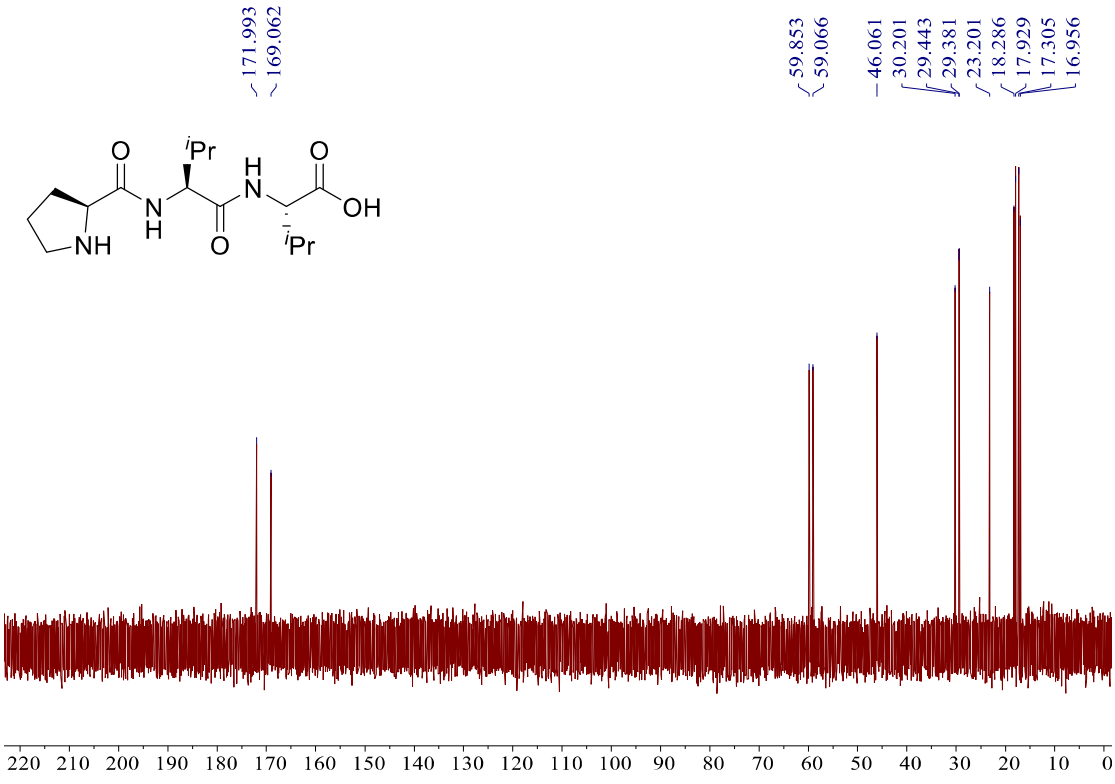
^{1}H NMR (600 MHz, D_2O) δ 7.38 (t, *J* = 7.5 Hz, 2H), 7.33 (t, *J* = 7.4 Hz, 1H), 7.28 (d, *J* = 6.8 Hz, 2H), 4.73 (t, *J* = 7.7 Hz, 1H), 4.38 – 4.32 (m, 1H), 4.14 (d, *J* = 8.4 Hz, 1H), 4.05 (d, *J* = 6.3 Hz, 1H), 3.47 – 3.34 (m, 2H), 3.15 (dd, *J* = 13.9, 7.2 Hz, 1H), 3.05 (dd, *J* = 13.9, 8.2 Hz, 1H), 2.49 – 2.40 (m, 1H), 2.06 (ddq, *J* = 45.7, 13.6, 6.9 Hz, 5H), 0.94 (ddd, *J* = 16.6, 6.8, 2.5 Hz, 12H).

^{13}C NMR (150 MHz, D2O) δ 176.6, 171.8, 171.8, 168.7, 135.5, 128.6, 128.3, 126.8, 59.7, 59.0, 58.9, 54.8, 46.0, 36.4, 30.1, 29.8, 23.2, 18.1, 17.8, 17.4, 17.2.

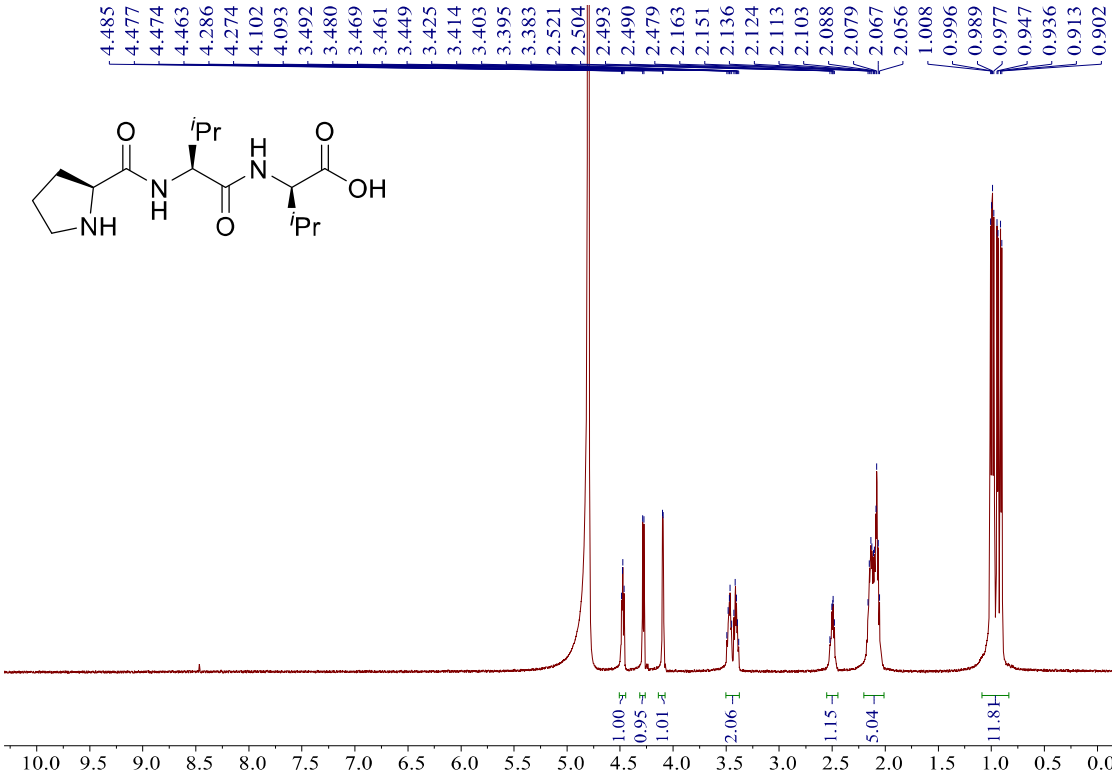
7.2 NMR spectra for new peptides

LLL-PVV





***LLD*-PVV**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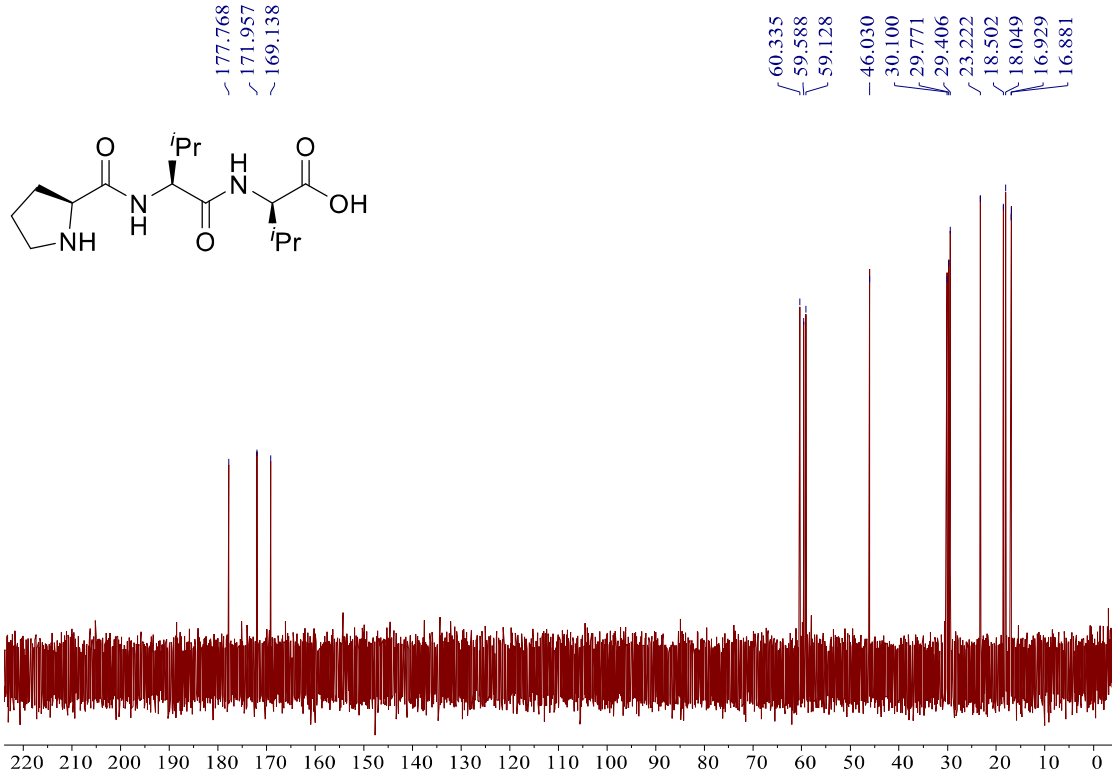
4.474
4.463
4.286
4.274
4.102
4.093
3.492
3.480
3.469
3.461
3.449
3.425
3.414
3.403
3.395
3.383
2.521
2.504
2.493
2.490
2.479
2.163
2.151
2.136
2.124
2.113
2.103
2.088
2.079
2.067
2.056
1.008
0.996
0.989
0.977
0.947
0.936
0.913
0.902
1.00
0.95
1.01
2.06
1.15
5.04
11.81
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0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59.128
46.030
30.100
29.771
29.406
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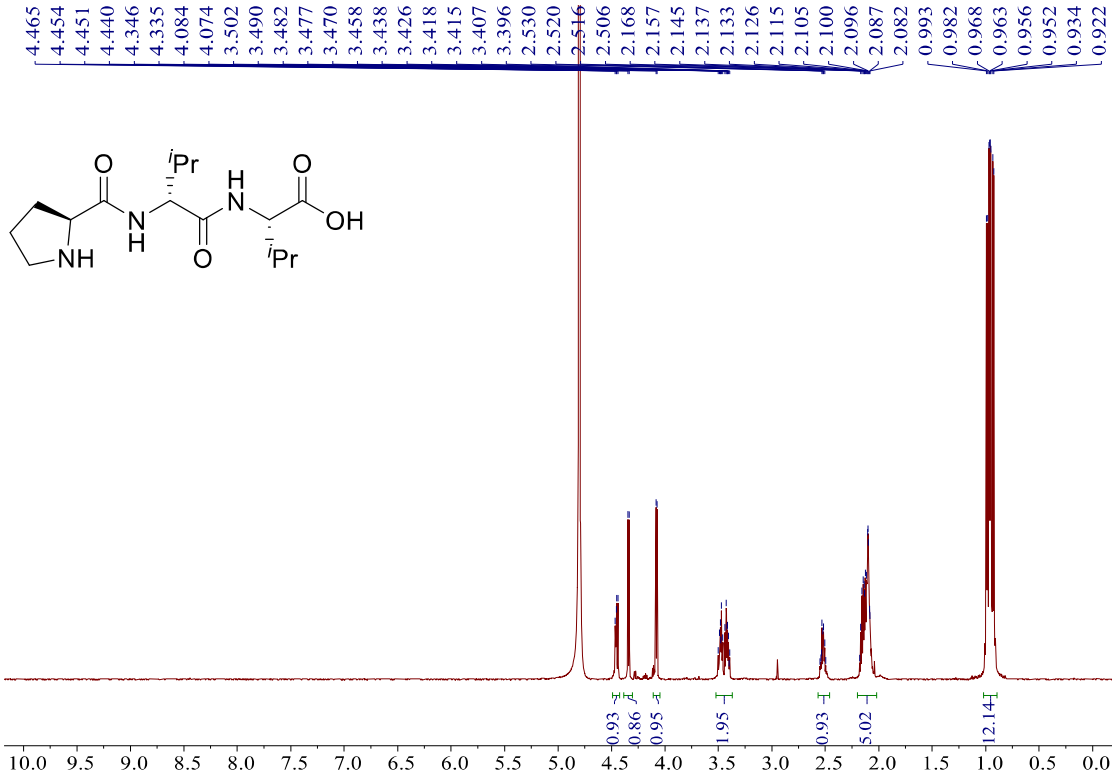
18.049
16.929
16.881
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

***LDL*-PVV**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2.530
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2.145
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2.133
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0.952
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H
N
O
OH
NH
N
H
O
iPr
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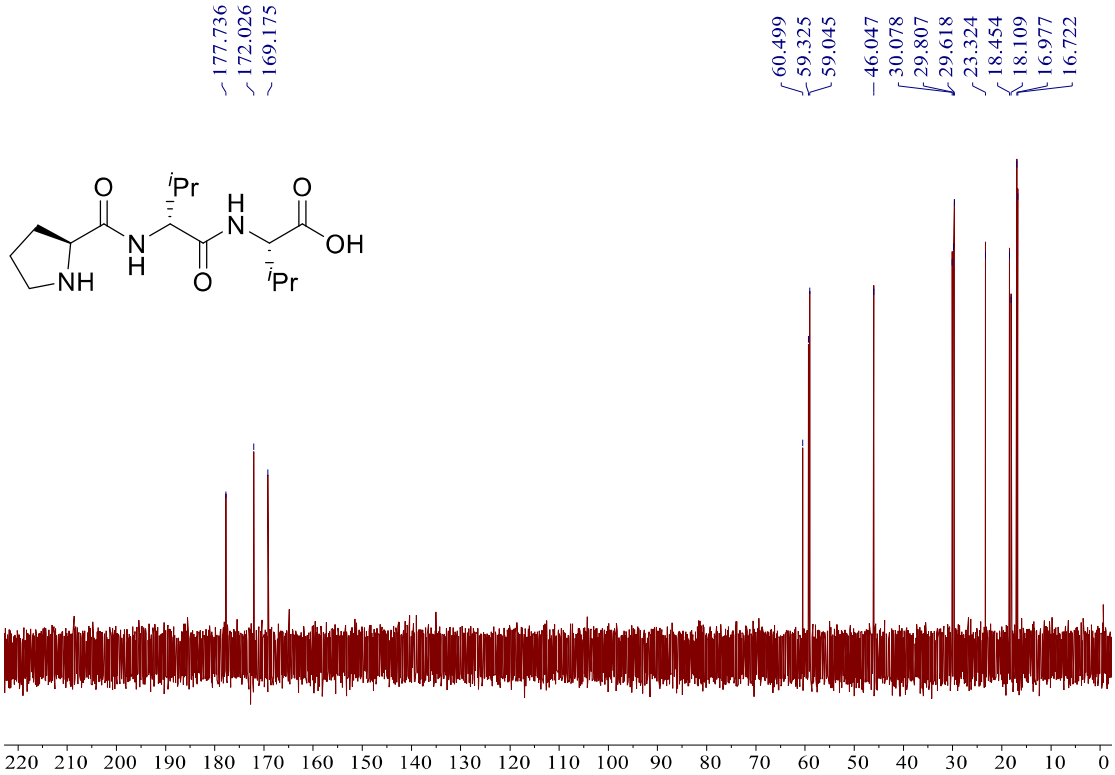
0.86
0.95
1.95
0.93
5.02
12.14
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0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18.109
16.977
16.722
O
iPr
H
N
O
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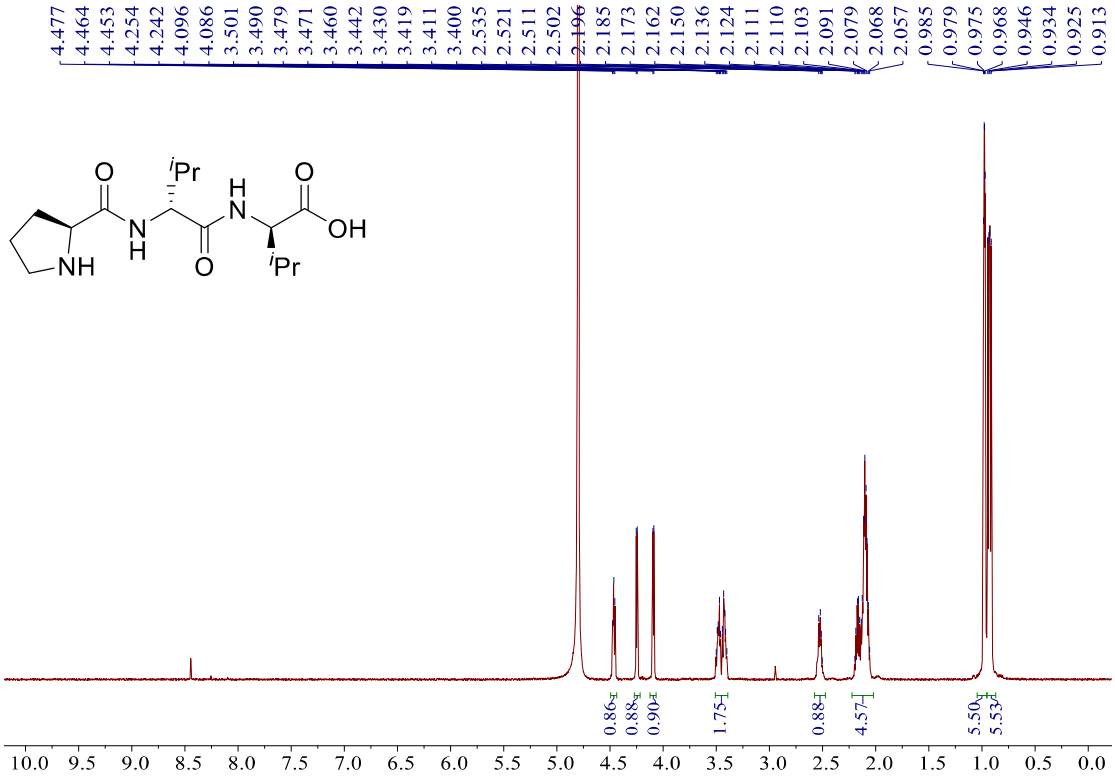
NH
N
H
O
iPr
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

***LDD*-PVV**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2.502
2.196
2.185
2.173
2.162
2.150
2.136
2.124
2.111
2.110
2.103
2.091
2.079
2.068
2.057
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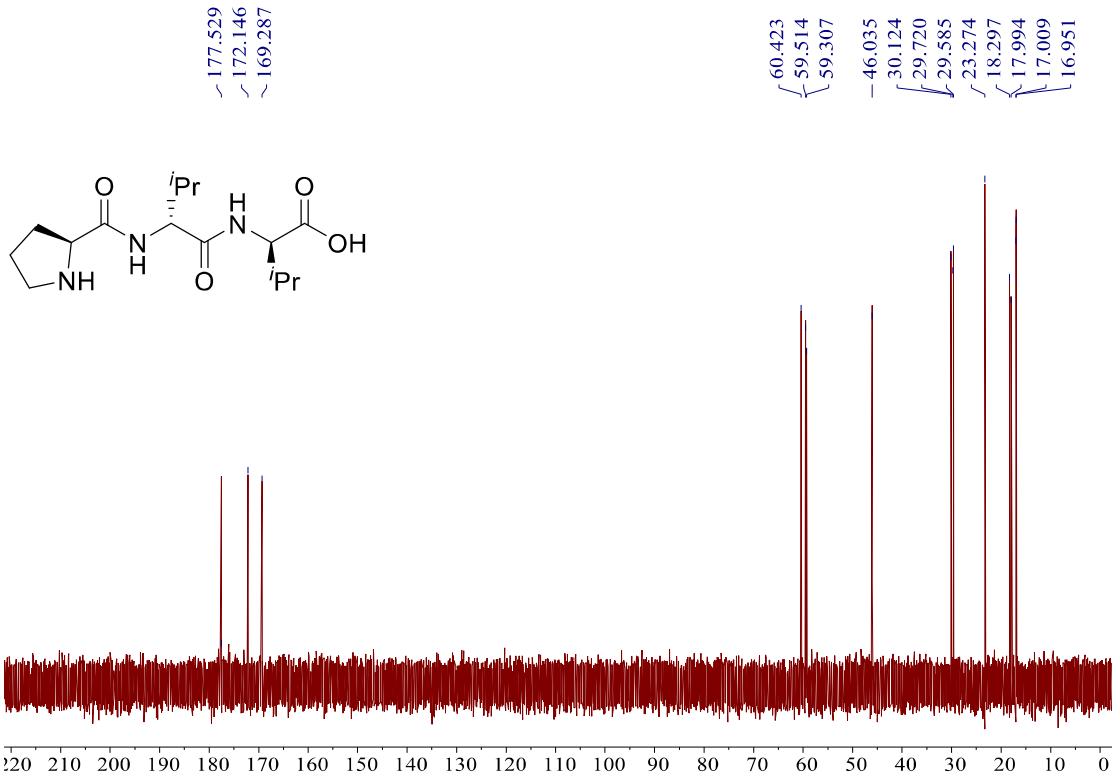
0.979
0.975
0.968
0.946
0.934
0.925
0.913
0.86
0.88
0.90
1.75
0.88
4.57
5.50
5.53
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0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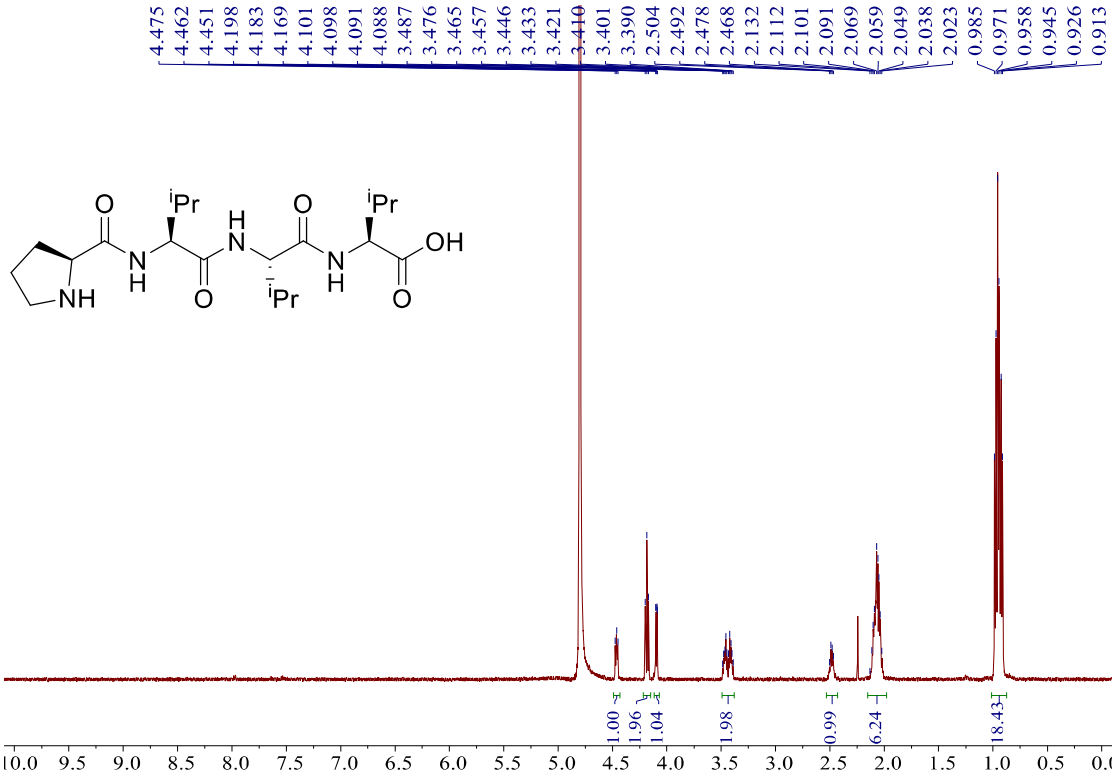
***LLLL*-PVVV**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2.069
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2.023
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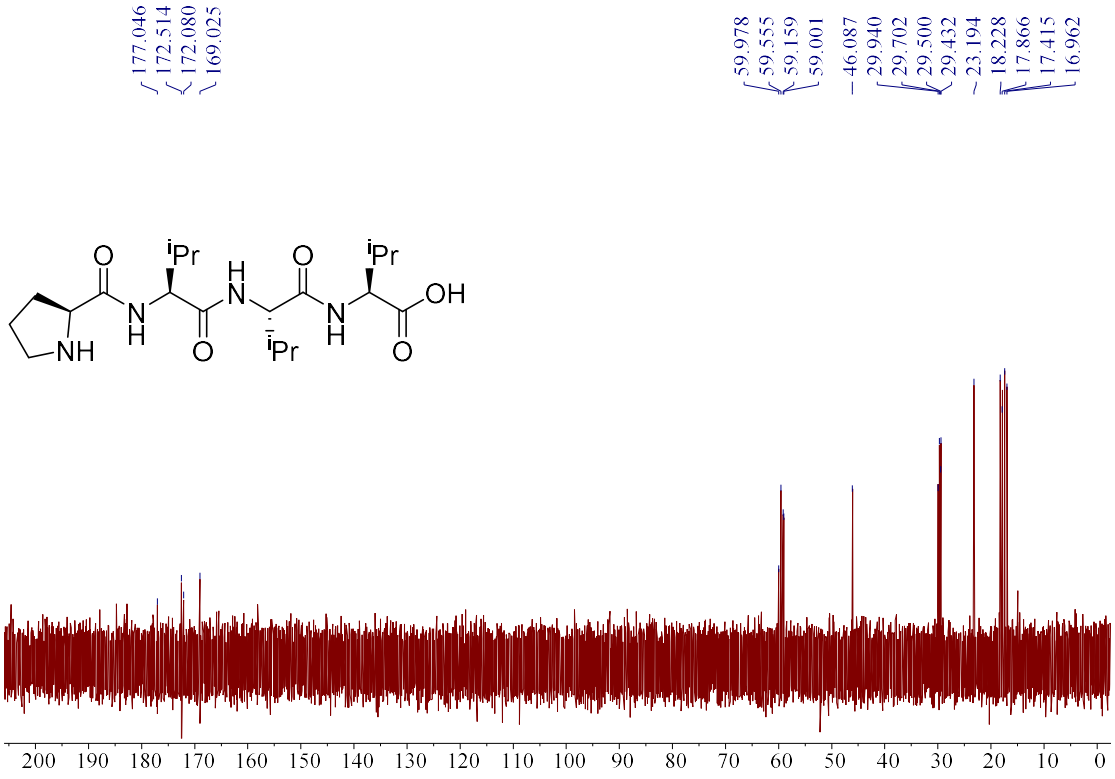
0.971
0.958
0.945
0.926
0.913
1.00
1.96
1.04
1.98
0.99
6.24
18.43
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0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172.080
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29.702
29.500
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17.866
17.415
16.962
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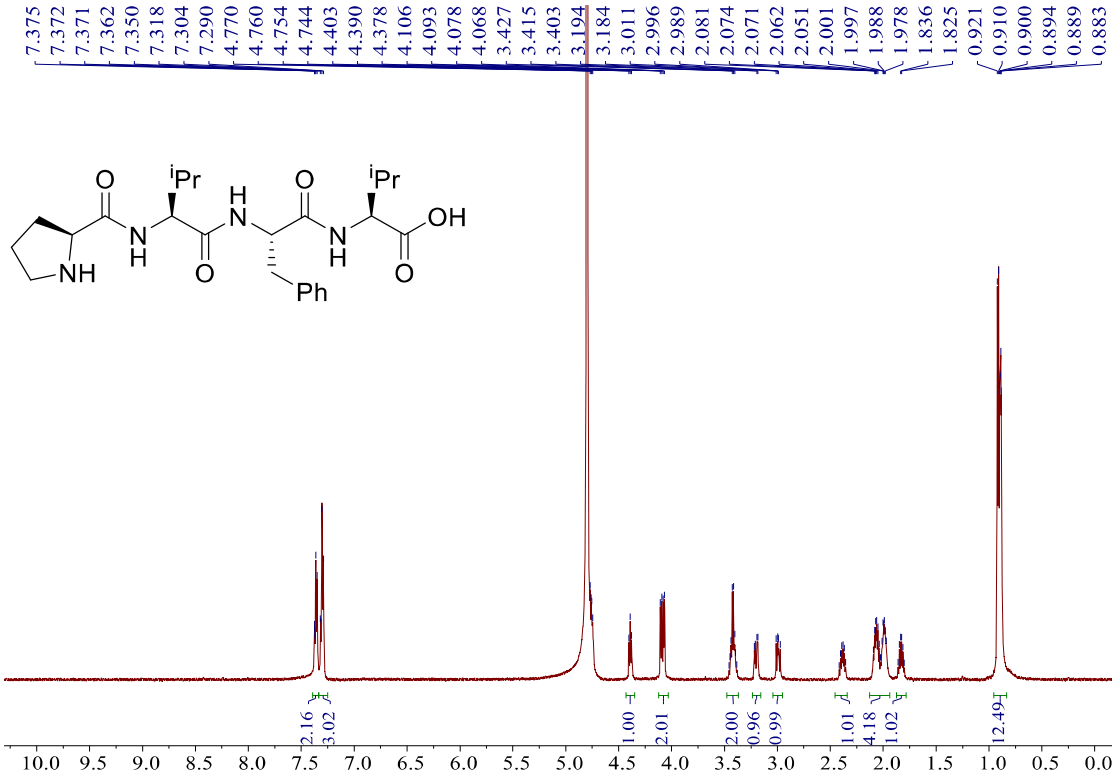
***LLLL*-PVFV**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4.093
4.078
4.068
3.427
3.415
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3.194
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3.011
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2.081
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2.071
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1.997
1.988
1.978
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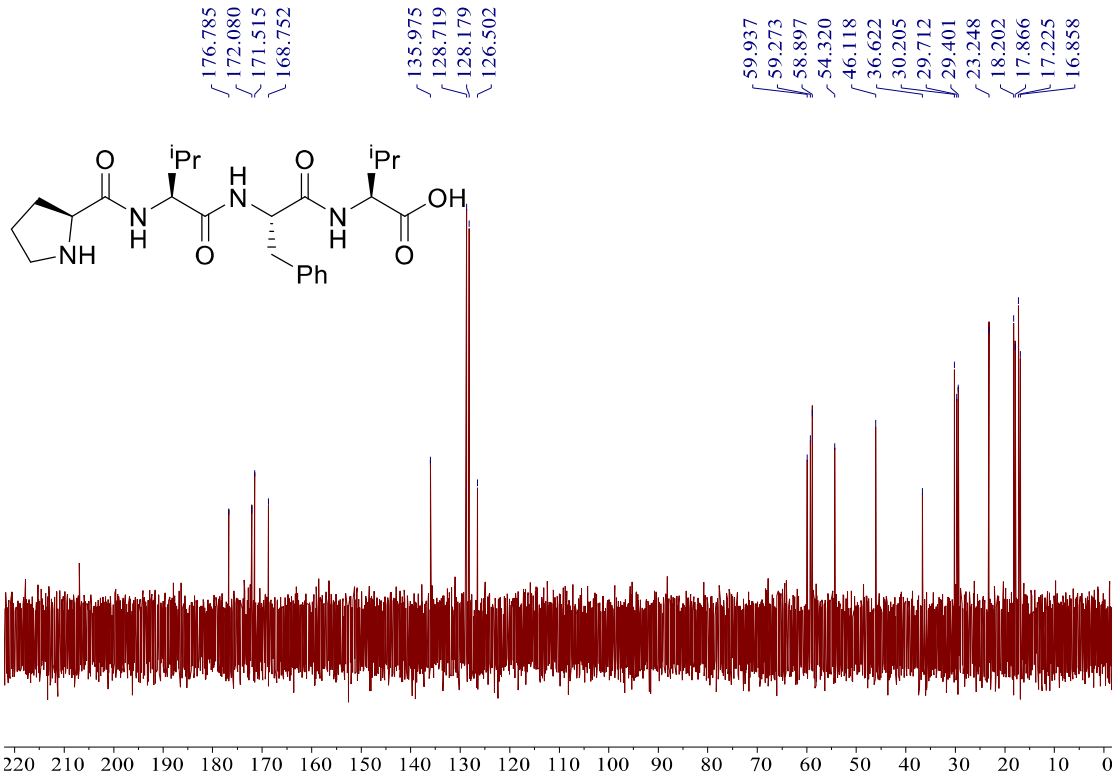
1.825
0.921
0.910
0.900
0.894
0.889
0.883
2.16
3.02
1.00
2.01
2.00
0.96
0.99
1.01
4.18
1.02
12.49
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0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36.622
30.205
29.712
29.401
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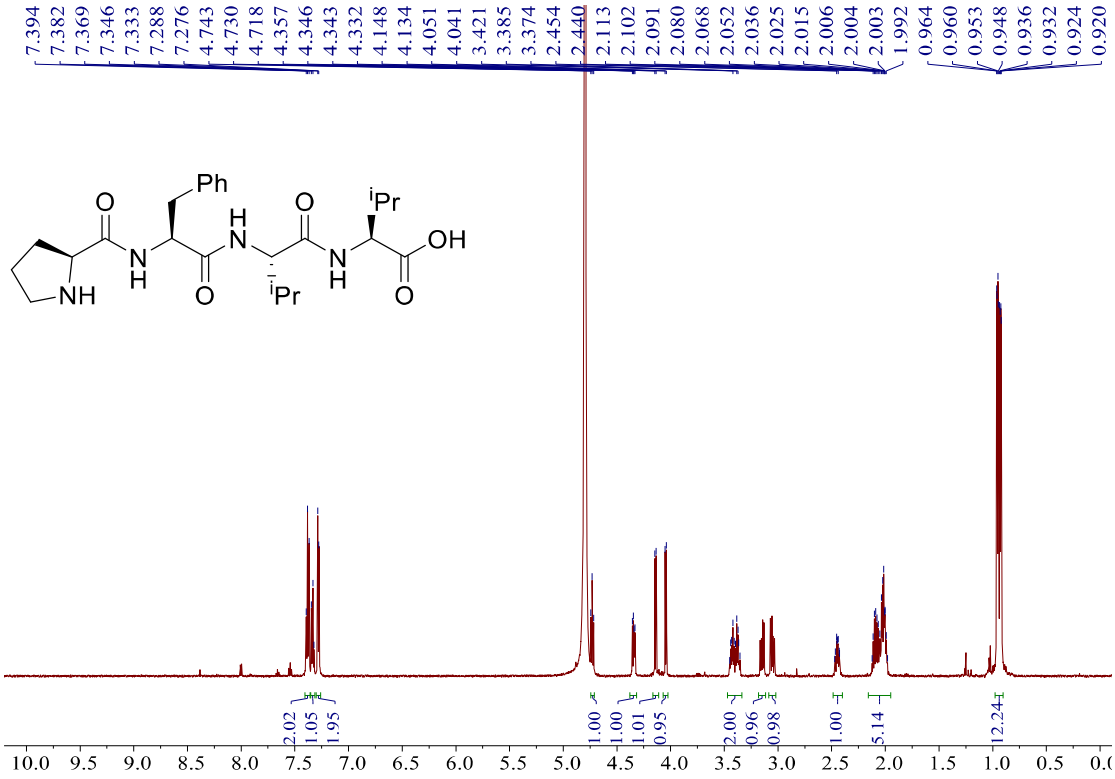
17.866
17.225
16.858
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0

***LLLL*-PFVV**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3.374
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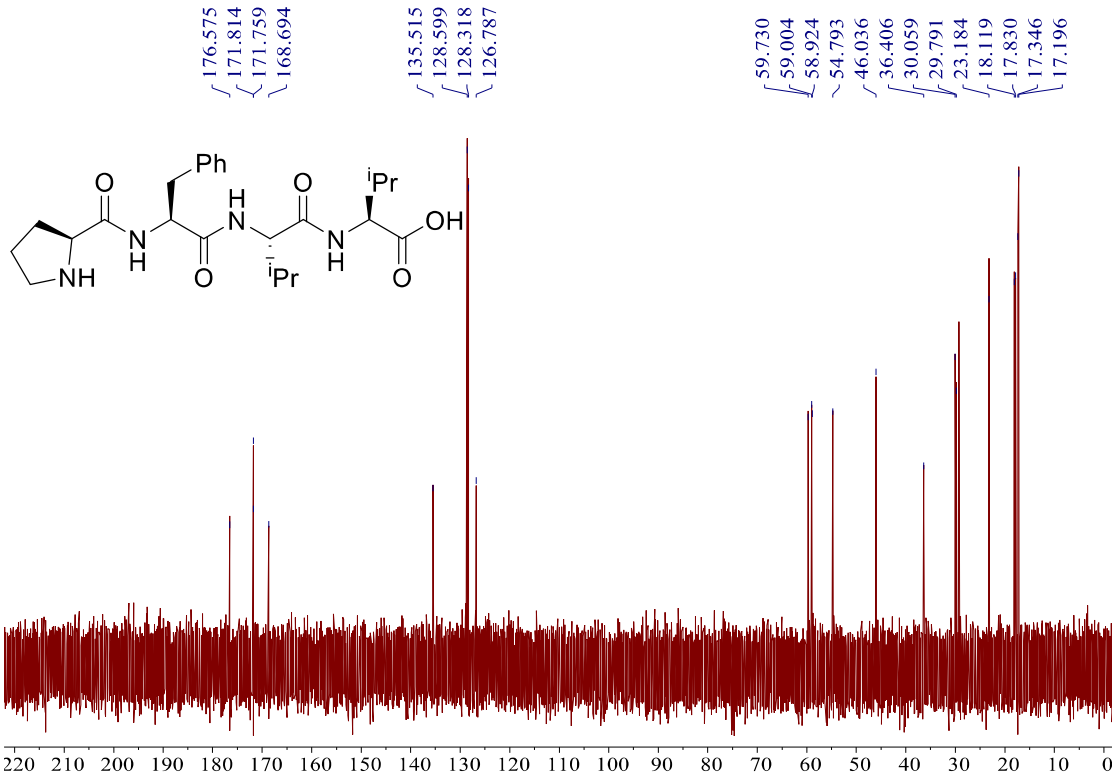
2.068
2.052
2.036
2.025
2.015
2.006
2.004
2.003
1.992
0.964
0.960
0.953
0.948
0.936
0.932
0.924
0.920
Ph
O
H
N
O
iPr
OH
NH
N
H
O
iPr
O
2.02
1.05
1.95
1.00
1.00
1.01
0.95
2.00
0.96
0.98
1.00
5.14
12.24
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0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O
H
N
O
iPr
OH
NH
N
H
O
iPr
O
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0