

Metal-Free Polymer-Based Affinity Medium for Selective Purification of His6-Tagged Proteins

Keiichi Yoshimatsu,^{†,‡} Krista R. Fruehauf,[†] Quanhong Zhu,[†] Adam Weisman,[†] Jun Fan,[†]
Min Xue,[†] John M. Beierle,[†] Paul E. Rose,[§] Jennifer Aral,[§] Linda F. Epstein,[§] Philip Tagari,[§]
Les P. Miranda,[§] and Kenneth J. Shea^{*,†}

[†]*Department of Chemistry, University of California, Irvine, Irvine, California 92697, United States*

[§]*Department of Chemistry, Missouri State University, 901 South National Avenue, Springfield, Missouri, 65897, United States*

[#]*Therapeutic Discovery, Amgen Inc., One Amgen Center Drive, Thousand Oaks, California 91320, United States*

**e-mail: kjshea@uci.edu*

Table of Contents

Supporting Methods	2
--------------------	---

Scheme S1. Steps in the preparation of polymer-coated Sepharose CL-4B beads

Supporting Data	3-12
-----------------	------

Table S1. Yield and hydrodynamic diameter of NPs.

Table S2. Result of elemental analysis. The values are weight % per total mass of dried samples.

Figure S1. Binding curve of AcPhe40 for Met-His6-PreScission peptide

Figure S2. ¹H NMR spectrum of *N*-acryloyl *L*-phenylalanine (CD₃OD, 500 MHz)

Figure S3. ¹³C NMR spectrum of *N*-acryloyl *L*-phenylalanine (CD₃OD, 125 MHz)

Figure S4. ¹H NMR spectrum of *N*-acryloyl *L*-leucine (CDCl₃, 500 MHz)

Figure S5. ¹³C NMR spectrum of *N*-acryloyl *L*-leucine (CDCl₃, 125 MHz)

Figure S6. ¹H NMR spectrum of *N*-acryloyl *L*-alanine (DMSO-*d*₆, 500 MHz)

Figure S7. ¹³C NMR spectrum of *N*-acryloyl *L*-alanine (DMSO-*d*₆, 125 MHz)

Figure S8. ¹H NMR spectrum of *N*-propargyl acrylamide (CDCl₃, 500 MHz)

Figure S9. ¹³C NMR spectrum of *N*-propargyl acrylamide (CDCl₃, 125 MHz)

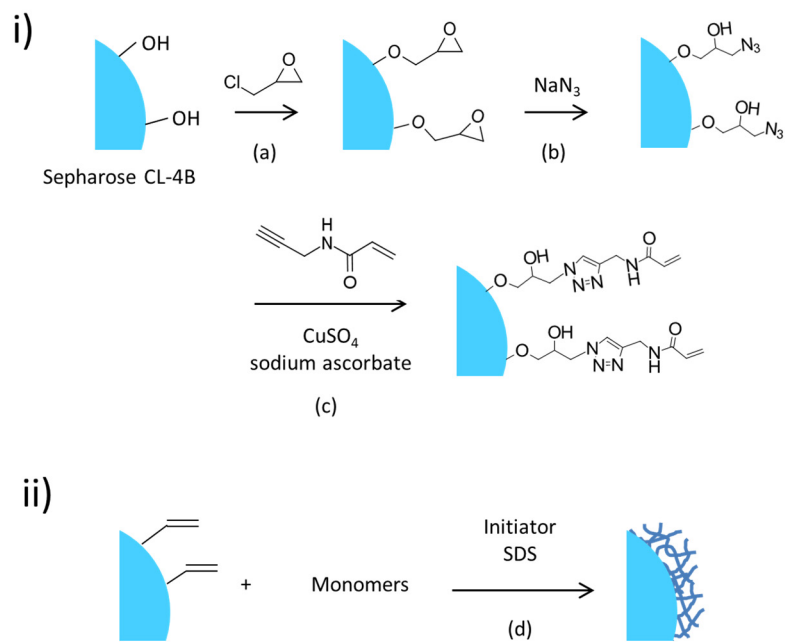
Supporting Methods

NMR Measurements

All ^1H NMR and ^{13}C NMR spectra were recorded at 500 MHz and 125 MHz, respectively, on a Bruker DRX500 spectrometer at room temperature with CDCl_3 (Cambridge Isotope Laboratories, Inc), CD_3OD (Acros Organic), or $\text{DMSO}-d_6$ (Acros Organic) as the solvent. The chemical shifts (δ) were referenced to as an internal standard, tetramethylsilane (TMS).

Elemental analyses

Elemental analyses of the unmodified and polymer-coated agarose beads were performed by Atlantic Microlab (Norcross, GA).



Scheme S1. Steps in the preparation of polymer-coated Sepharose CL-4B beads

Supporting Data

Table S1. Yield and hydrodynamic diameter of NPs.

Sample	Hydrodynamic diameter ^a		Yield ^b
	nm	PDI	%
AAc5/PAm20	127	0.071	85
AAc5/PAm40	108	0.087	57
AAc20/PAm20	100	0.063	80
AAc20/PAm40	74	0.064	58
AAc5/TBAm40	85	0.060	85
AAc20/TBAm40	97	0.055	80
PAm20	109	0.42	78
PAm40	85	0.42	65
TBAm40	75	0.087	88
NIPAm	431	0.166	75
AcPhe20	106	0.120	77
AcPhe40	94	0.106	67
AcLeu20	81	0.068	88
AcLeu40	76	0.077	75
AcAla20	817	0.113	69
AcAla40	1232	0.261	63

^a Intensity-based calculated mean value (Z-average) in H₂O at 25 °C by dynamic light scattering (DLS) instrument equipped with Zetasizer software Ver. 6.12 (Zetasizer Nano ZS, Malvern Instruments Ltd) ^b Determined by a gravimetric analysis of lyophilized polymer NPs.

Table S2. Result of elemental analysis. The values are weight % per total mass of dried samples.

Sample	C	H	N
Sepharose [®] CL-4B beads, before modification	46.5	6.4	0
Polymer-coated beads	48.1	6.67	3.57

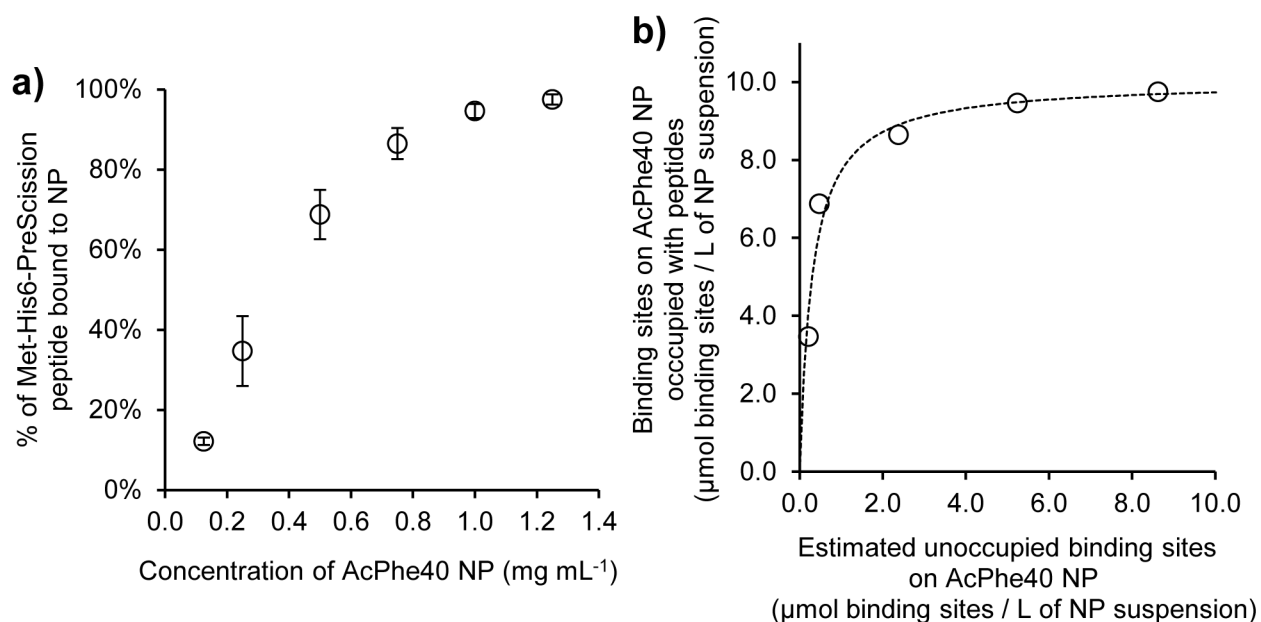


Figure S1. a) Equilibrium binding curve of AcPhe40 NP for Met-His6-PreScission peptide in 15 mM Tris-HCl buffer (pH 7.8) containing 0.1% Tween 20 (w/v). Concentration of peptide = 10 μM (18.55 μg/mL). b) Binding isotherm of AcPhe40 NP for Met-His6-PreScission peptide. The plot was established based on the assumption of 14.7 nmol of peptide binding sites being present in every mg of AcPhe40 NP. The dashed line is the best fitted curve obtained by using the one-site model with the parameters of $B_{\max} = 10.03$ μmol binding sites / L of NP suspension and $K_d = 300.2$ nM.

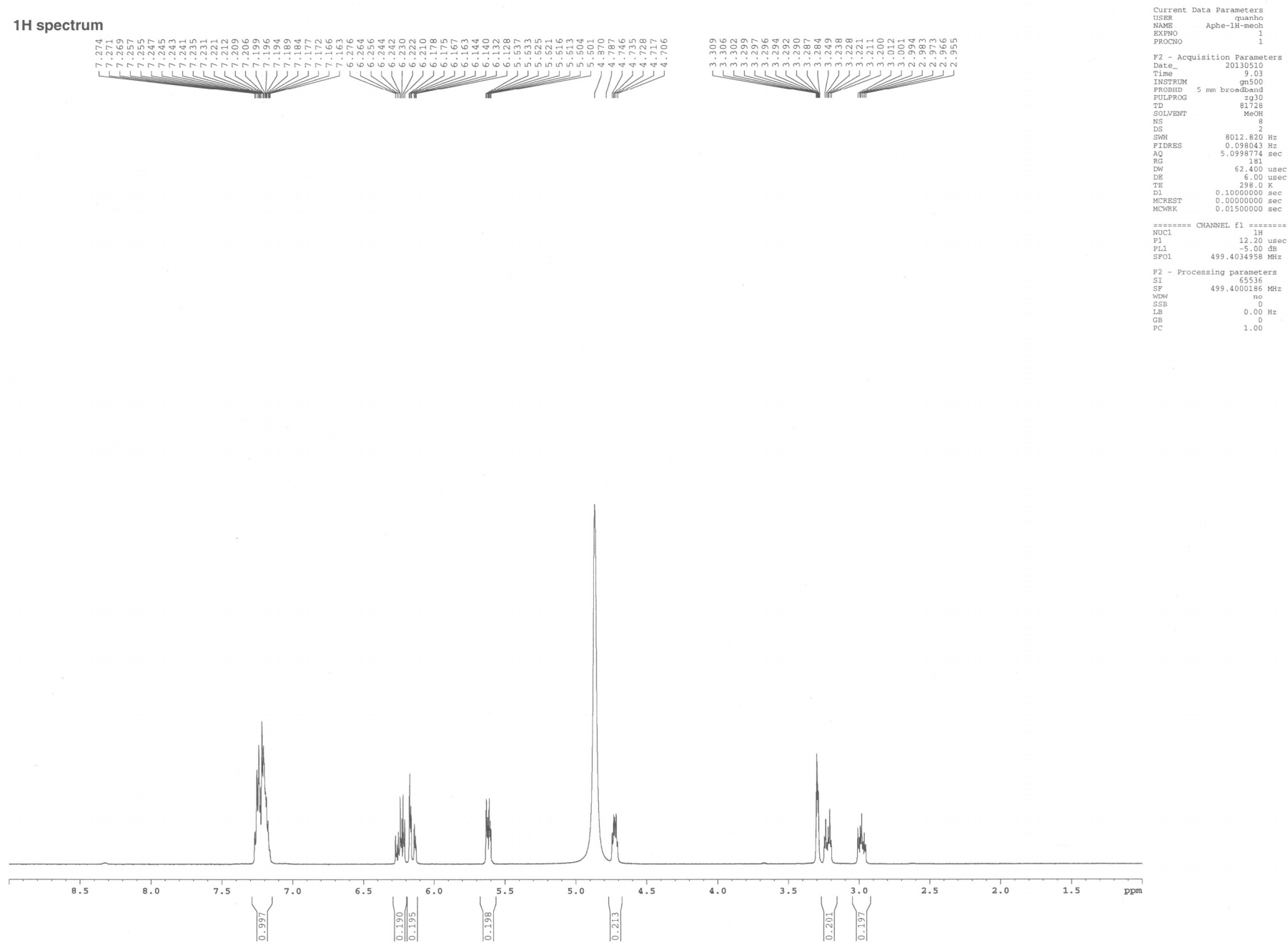
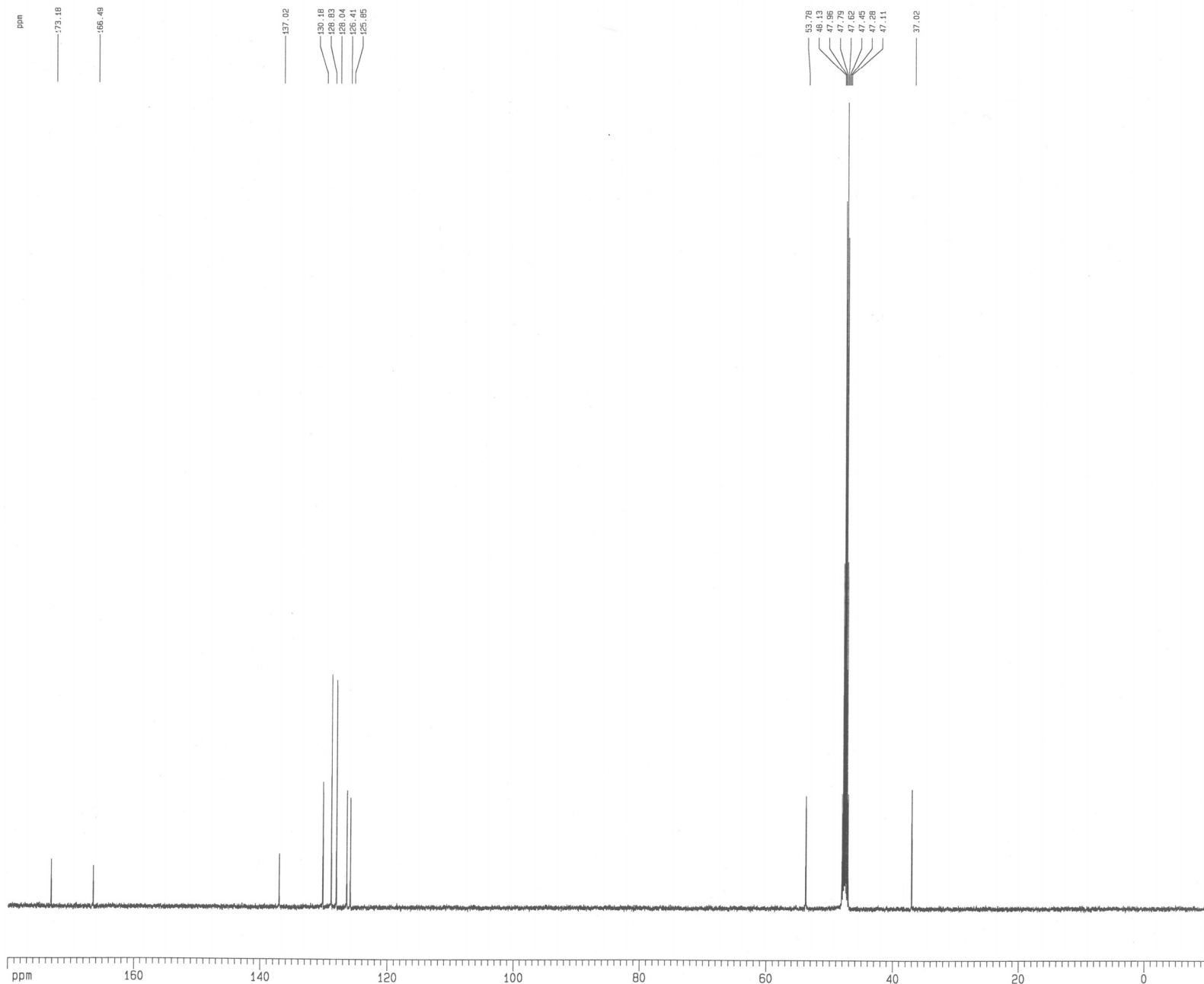


Figure S2. ^1H NMR spectrum of *N*-acryloyl *L*-phenylalanine (CD_3OD , 500 MHz)

¹³C spectrum with ¹H decoupling



```

Current Data Parameters
USER      quanho
NAME      Aphe-13C-meh
EXPNO     1
PROCNO    1

F2 - Acquisition Parameters
Date_     20130510
Time      9.31
INSTRUM   gnm500
PROBHD    5 mm broadband
PULPROG   zgdc30
TD         65536
SOLVENT   MeOH
NS         1024
DS         4
SWH        30303.034 Hz
FIDRES     0.462388 Hz
AQ         1.0813940 sec
RG          8192
DW         15.500 usec
DE         4.50 usec
TE         298.0 K
d1         0.25000000 sec
d11        0.03000000 sec
MCREST     0.00000000 sec
MCWRK      0.01500000 sec

===== CHANNEL f1 =====
NUC1       13C
P1         7.70 usec
PL1        0.00 dB
SFO1       125.5880432 MHz

===== CHANNEL f2 =====
CPDPRG2    waltz16
NUC2       1H
PCPD2      80.00 usec
PL2        -3.00 dB
PL12       13.20 dB
SFO2       499.4024970 MHz

F2 - Processing parameters
SI         65536
SF         125.5742300 MHz
WDW        EM
SSB        0
LB         1.00 Hz
GB         0
PC         2.00

1D NMR plot parameters
CX         22.80 cm
CY         15.65 cm
F1P        180.000 ppm
F1          22603.36 Hz
F2P        -10.658 ppm
F2         -1338.35 Hz
PPMCM      8.36219 ppm/cm
HZCM       1050.07520 Hz/cm

```

Figure S3. ¹³C NMR of *N*-acryloyl *L*-phenylalanine (CD₃OD, 125 MHz)

¹H spectrum

6.593
6.556
6.510
6.509
6.500
6.429
6.386
6.360
6.287
6.247
6.237
6.227
6.214
6.193
6.162
6.150
6.101
6.041
6.021
6.005
5.916
5.904
5.869
5.863
5.854
5.800
5.789
5.780
5.740
5.701
5.673
5.661
5.639
5.635
5.632
5.625
5.611
5.596
5.574
5.569
5.566
5.558
5.544
5.536
5.522
5.484
5.460
5.448
5.432
5.364
4.791
4.775
4.765
4.758
4.748

1.869
1.823
1.817
1.807
1.798
1.785
1.724
1.715
1.697
1.688
1.681
1.318
1.040
1.028
0.901

0.066
0.059
0.056
0.053
0.038
0.032
0.017
0.013
0.000

```

Current Data Parameters
USER      quanho
NAME      Aleu-tubel-H-CDCl3
EXPNO     1
PROCNO    1

F2 - Acquisition Parameters
Date_     20130409
Time      13.35
INSTRUM   gn500
PROBHD    5 mm broadband
PULPROG   zg30
TD         81728
SOLVENT   CDCl3
NS         8
DS         2
SWH        8012.820 Hz
FIDRES     0.098043 Hz
AQ         5.0998774 sec
RG         1149.4
RW         62.400 usec
DE         6.00 usec
TE         298.0 K
D1         0.10000000 sec
MCREST    0.00000000 sec
MCWRK     0.01500000 sec

===== CHANNEL f1 =====
NUC1       1H
P1         12.20 usec
PL1        -5.00 dB
SFO1       499.4034958 MHz

F2 - Processing parameters
SI         65536
SF         499.4000000 MHz
WDW        no
SSB        0
LB         0.00 Hz
GB         0
PC         1.00

1d NMR plot parameters
CX         22.80 cm
CY         15.00 cm
F1P        8.003 ppm
F1         3996.60 Hz
F2P        -0.500 ppm
F2         -249.70 Hz
PPMCM      0.37293 ppm/cm
HZCM       186.24130 Hz/cm
    
```

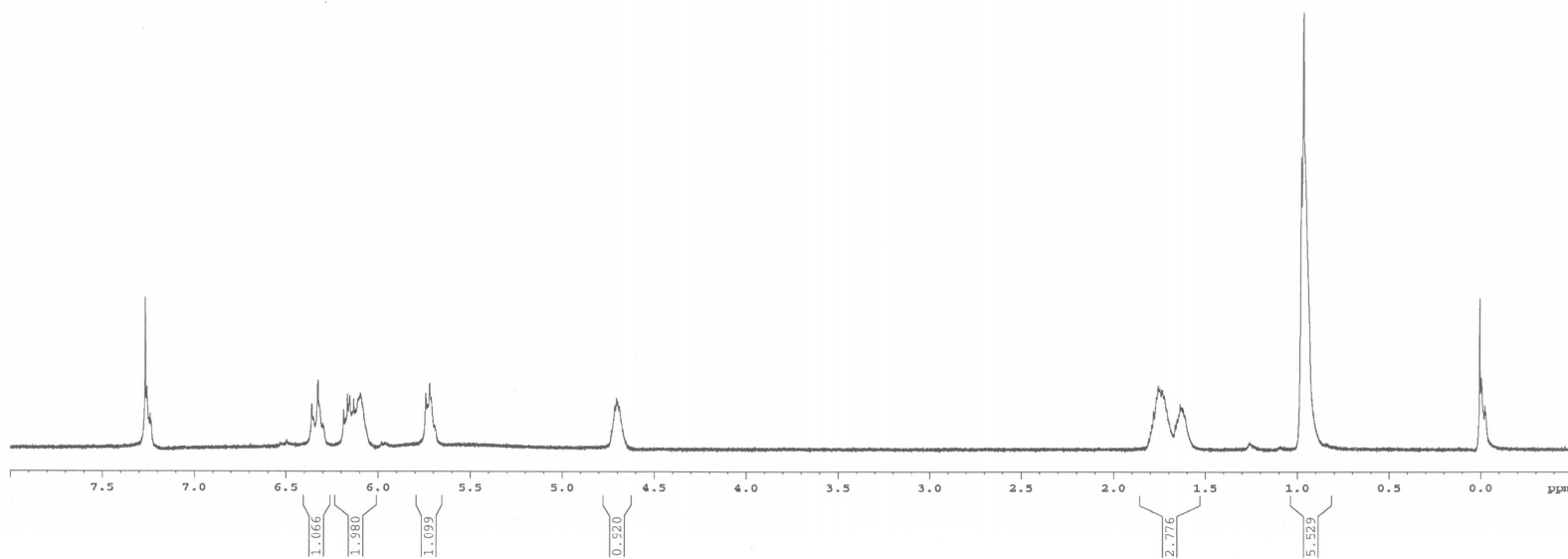
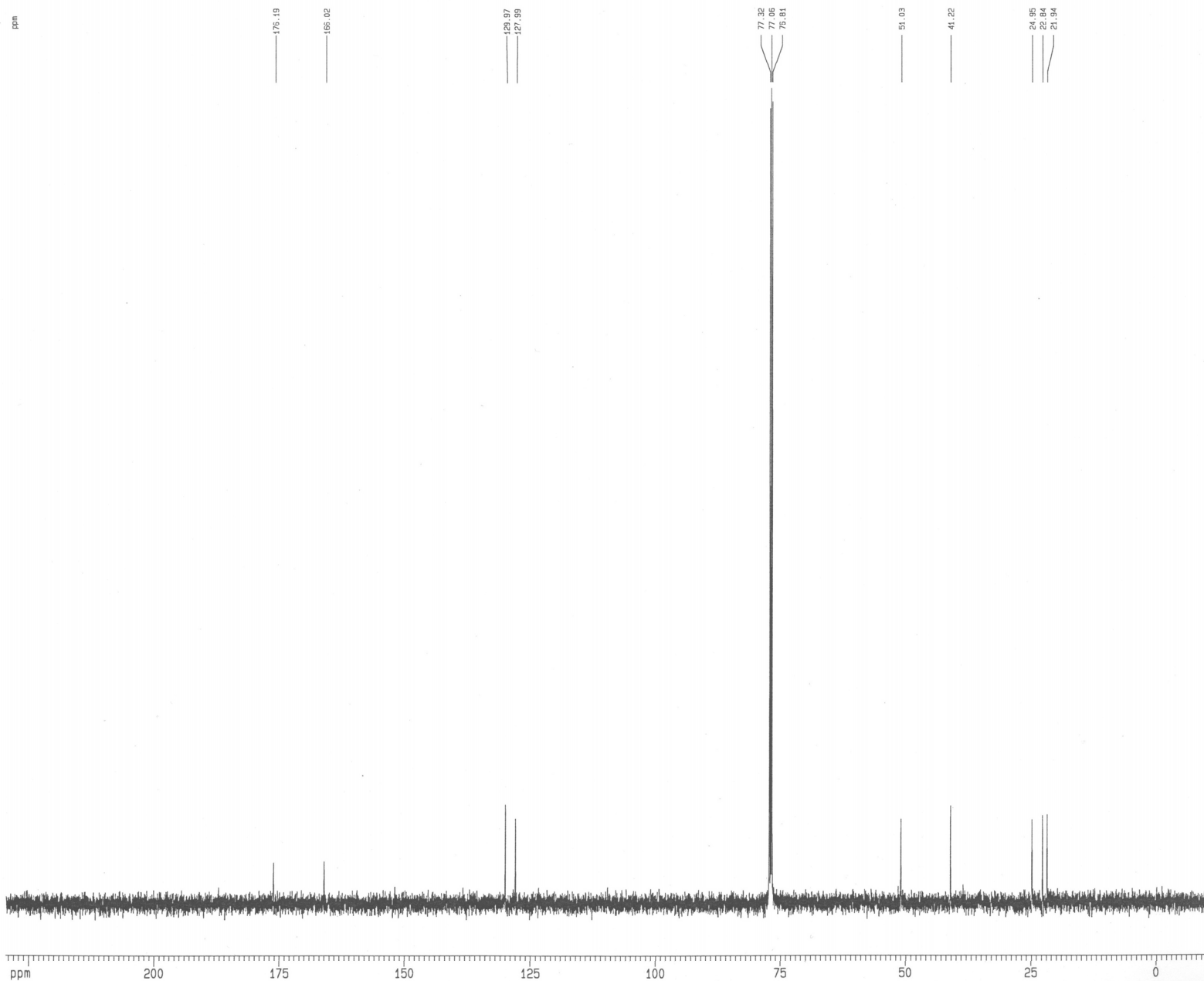


Figure S4. ¹H NMR spectrum of *N*-acryloyl *L*-leucine (CDCl₃, 500 MHz)

¹³C spectrum with ¹H decoupling



```

Current Data Parameters
USER      quanho
NAME      Aleu-tube1 (2) -13C-CDCl3
EXPNO     1
PROCNO    1

F2 - Acquisition Parameters
Data_     20130412
Time      8.48
INSTRUM    gn500
PROBHD     5 mm broadband
PULPROG    zgpg30
TD         65536
SOLVENT    CDCl3
NS         1024
DS         4
SWH         30303.031 Hz
FIDRES     0.462368 Hz
AQ         1.0813940 sec
RG         4597.6
DM         16.500 usec
DE         4.50 usec
TE         298.0 K
D1         0.25000000 sec
d11        0.03000000 sec
MCREST     0.00000000 sec
MCMK       0.01500000 sec

===== CHANNEL f1 =====
NUC1       13C
P1         7.70 usec
PL1        0.00 dB
SFO1       125.5880432 MHz

===== CHANNEL f2 =====
CPDPRG2    waltz16
NUC2       1H
PCPD2      80.00 usec
PL2        -1.00 dB
PL12       13.20 dB
SFO2       499.4024970 MHz

F2 - Processing parameters
SI         65536
SF         125.5742300 MHz
MCM        EM
SMB        0
LB         1.00 Hz
GB         0
PC         2.00

1D NMR plot parameters
CX         22.80 cm
CY         15.65 cm
FID        220.500 ppm
F1         28621.76 Hz
F2         -10.507 ppm
F2         -1319.36 Hz
PWCN       10.52747 ppm/cm
HZCN       1321.97876 Hz/cm
  
```

Figure S5. ¹³C NMR spectrum of *N*-acryloyl *L*-leucine (CDCl₃, 125 MHz)

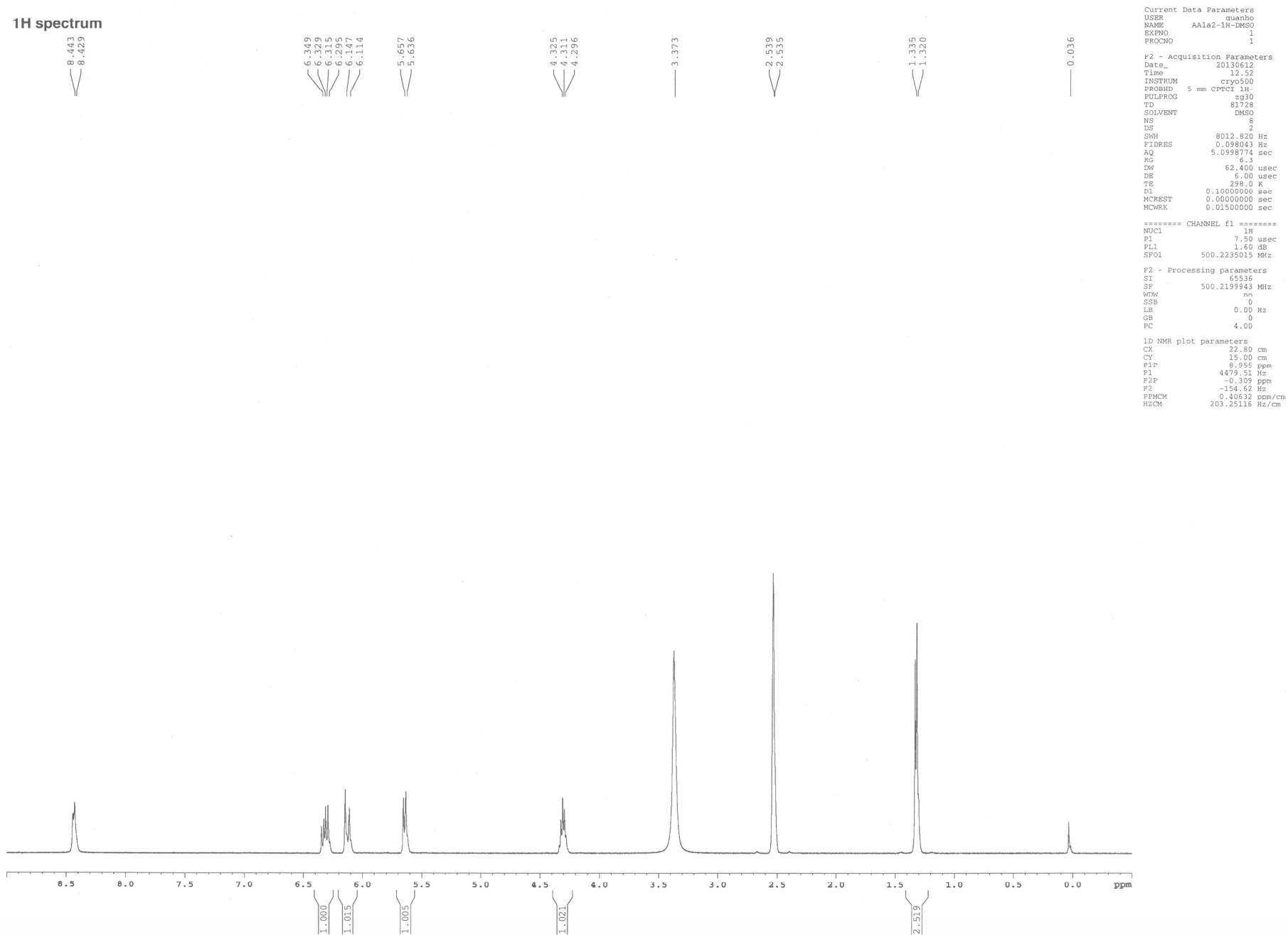
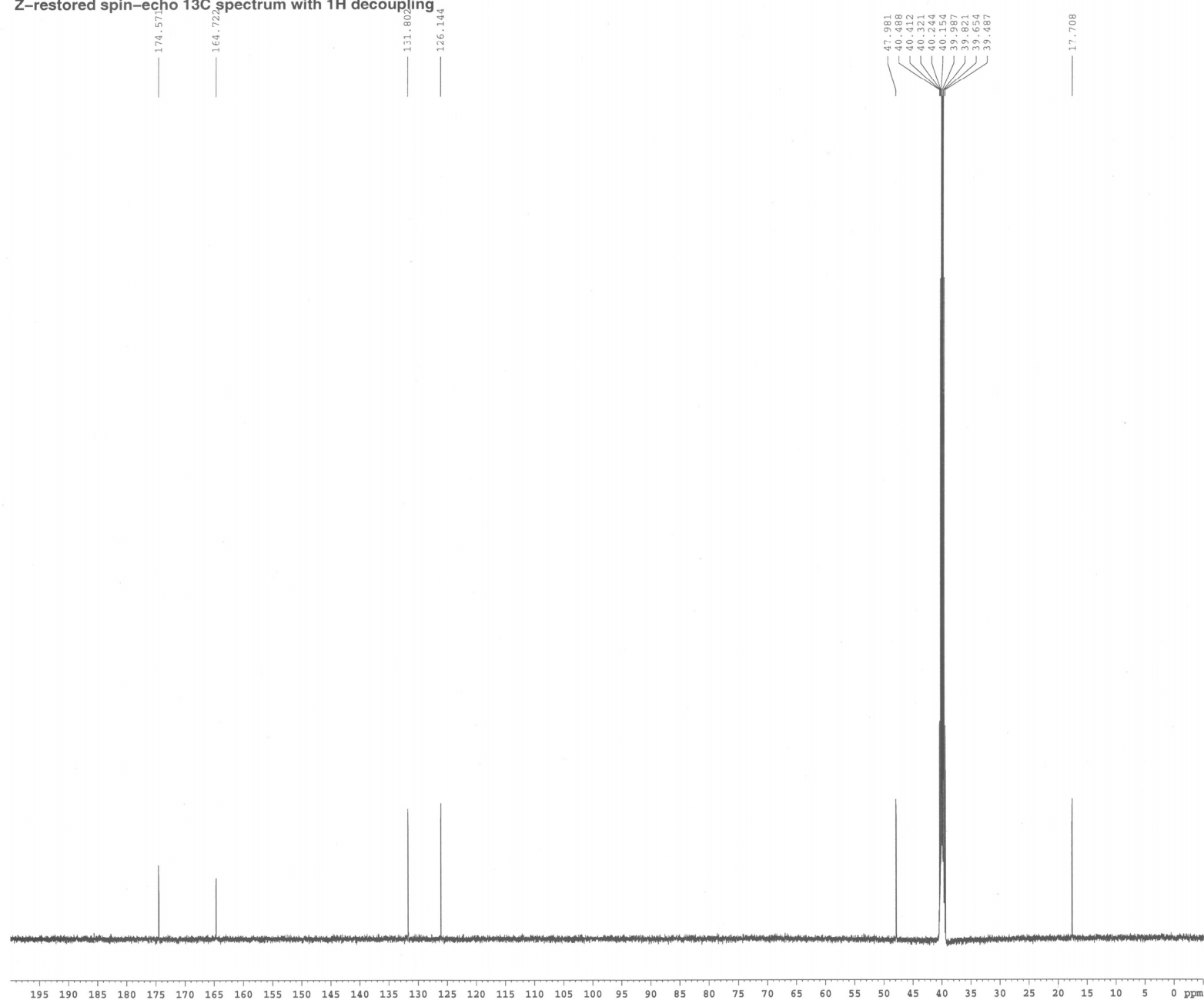


Figure S6. ¹H NMR spectrum of *N*-acryloyl *L*-alanine (DMSO-*d*₆, 500 MHz)

Z-restored spin-echo ^{13}C spectrum with ^1H decoupling



```

Current Data Parameters
USER      quanho
NAME      AAla2-13C DMSO
EXPNO     1
PROCNO    1

F2 - Acquisition Parameters
Date_     20130612
Time      17.11
INSTRUM   cryo500
PROBHD    5 mm CPTCI 1H-
PULPROG   SpinEcho300pp.prd
TD         65536
SOLVENT   DMSO
NS         902
DS         16
SWH        30303.031 Hz
FIDRES     0.462388 Hz
AQ         1.0813940 sec
RG         7298.2
LW         16.500 usec
DE         6.00 usec
TE         298.0 K
D1         0.25000000 sec
d11        0.03000000 sec
D16        0.00020000 sec
d17        0.00019600 sec
MCOREST    0.00000000 sec
MCNREK     0.01500000 sec
P2         31.00 usec

===== CHANNEL f1 =====
NUC1       13C
P1         15.50 usec
P11        500.00 usec
P12        2000.00 usec
PL0        120.00 dB
PL1        -1.00 dB
SFO1       125.7942548 MHz
SF1        3.20 dB
SF2        3.20 dB
SFOFF1     Crp60,0.5,20.1
SFOFF2     Crp60comp,4
SFOFF1     0.00 Hz
SFOFF2     0.00 Hz

===== CHANNEL f2 =====
CPDPRG2    waitz16
NUC2       1H
PCPD2      100.00 usec
PL2        1.60 dB
PL12       24.60 dB
SFO2       500.2225011 MHz

===== GRADIENT CHANNEL =====
GPNAM1     SINE.100
GPNAM2     SINE.100
GPX1       0.00 %
GPX2       0.00 %
GPY1       0.00 %
GPY2       0.00 %
GPZ1       30.00 %
GPZ2       50.00 %
p15        500.00 usec
p16        1000.00 usec

F2 - Processing parameters
SI         65536
SF         125.7804190 MHz
WDW        EM
SSB        0
LB         1.00 Hz
GB         0
PC         4.00

```

Figure S7. ^{13}C NMR spectrum of *N*-acryloyl *L*-alanine (DMSO- d_6 , 125 MHz)

¹H spectrum

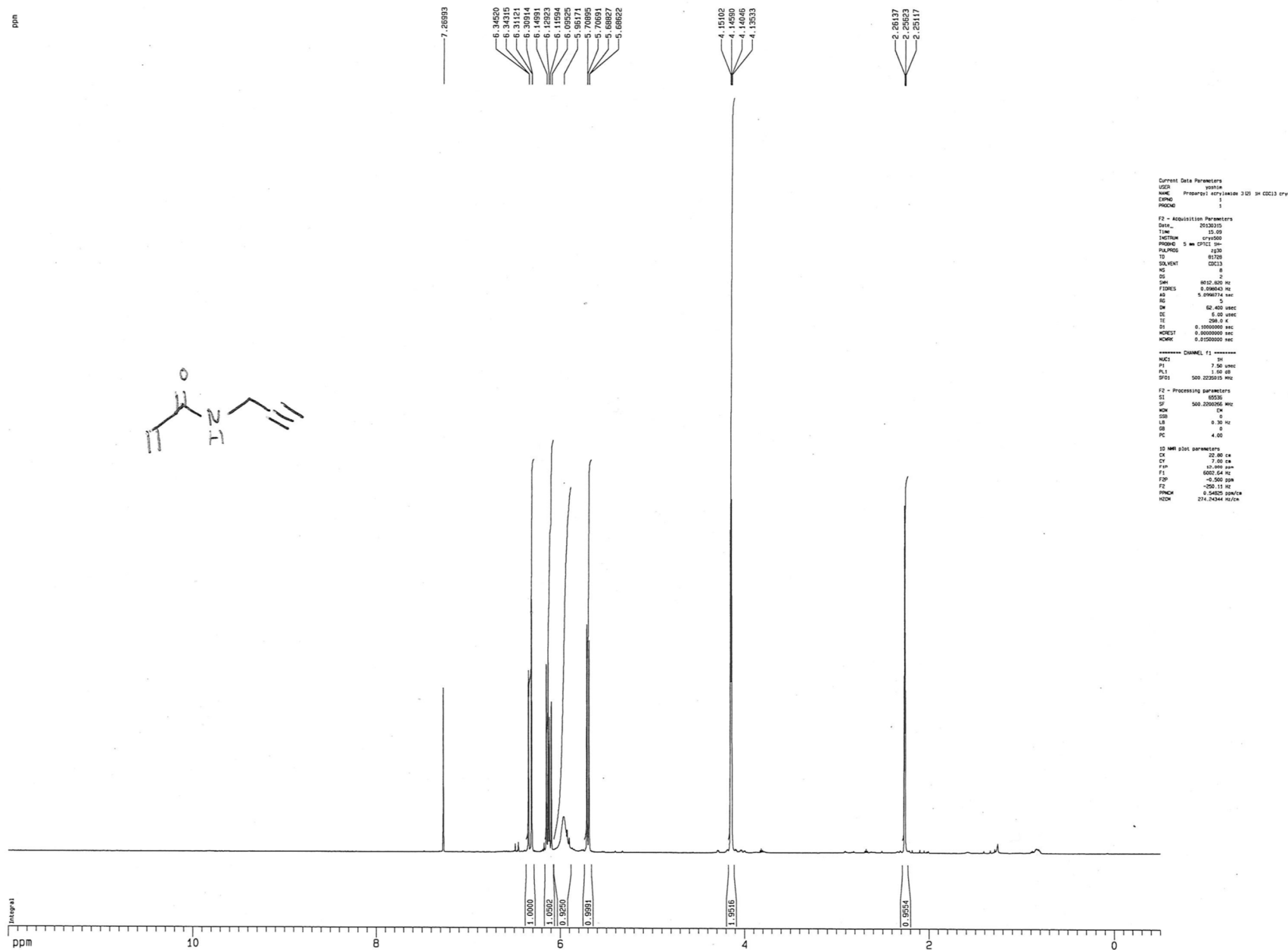


Figure S8. ¹H NMR spectrum of *N*-propargyl acrylamide (CDCl₃, 500 MHz)

Z-restored spin-echo ^{13}C spectrum with ^1H decoupling

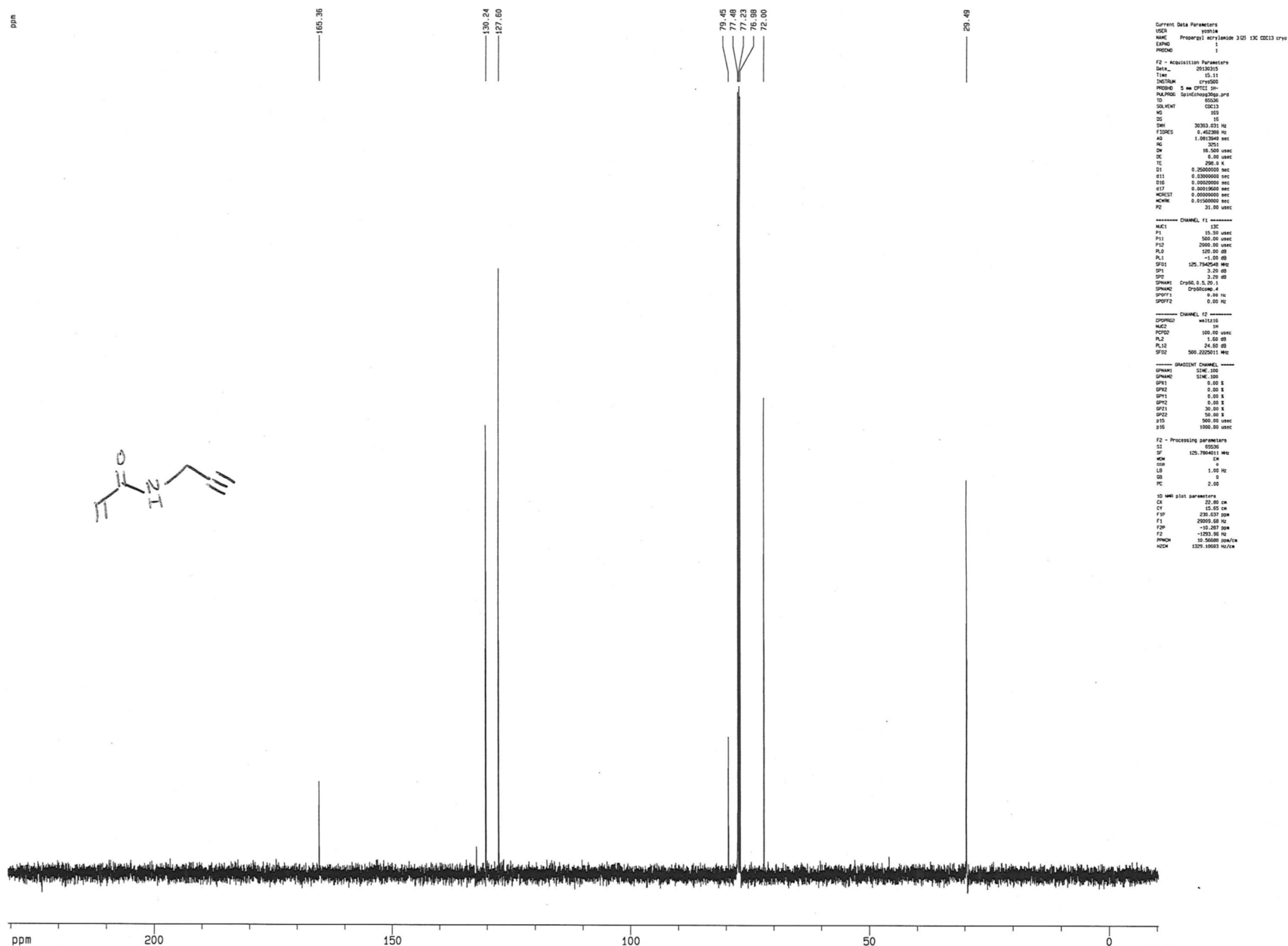


Figure S9. ^{13}C NMR spectrum of *N*-propargyl acrylamide (CDCl_3 , 125 MHz)
S12