Supporting Information for

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

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Table of Contents

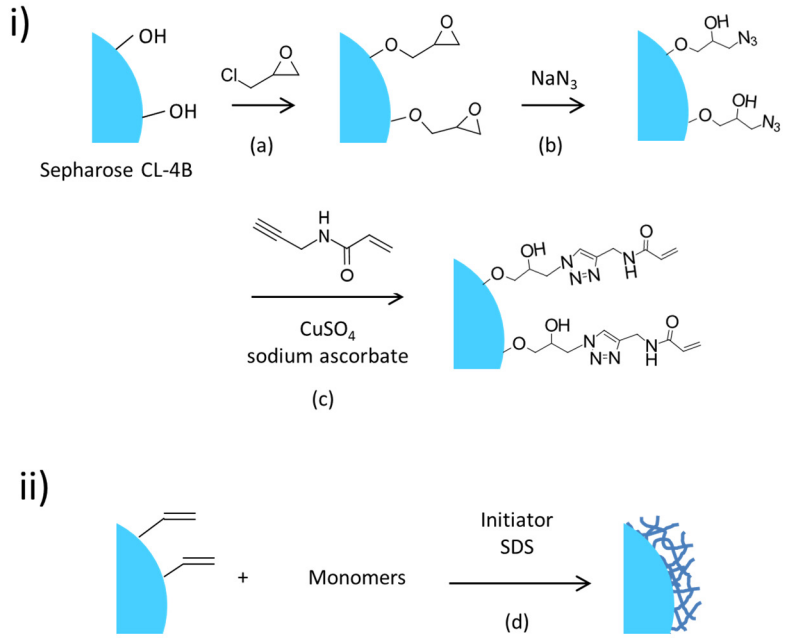
Supporting Methods

NMR Measurements

All ^{1}H 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 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 H_2O 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

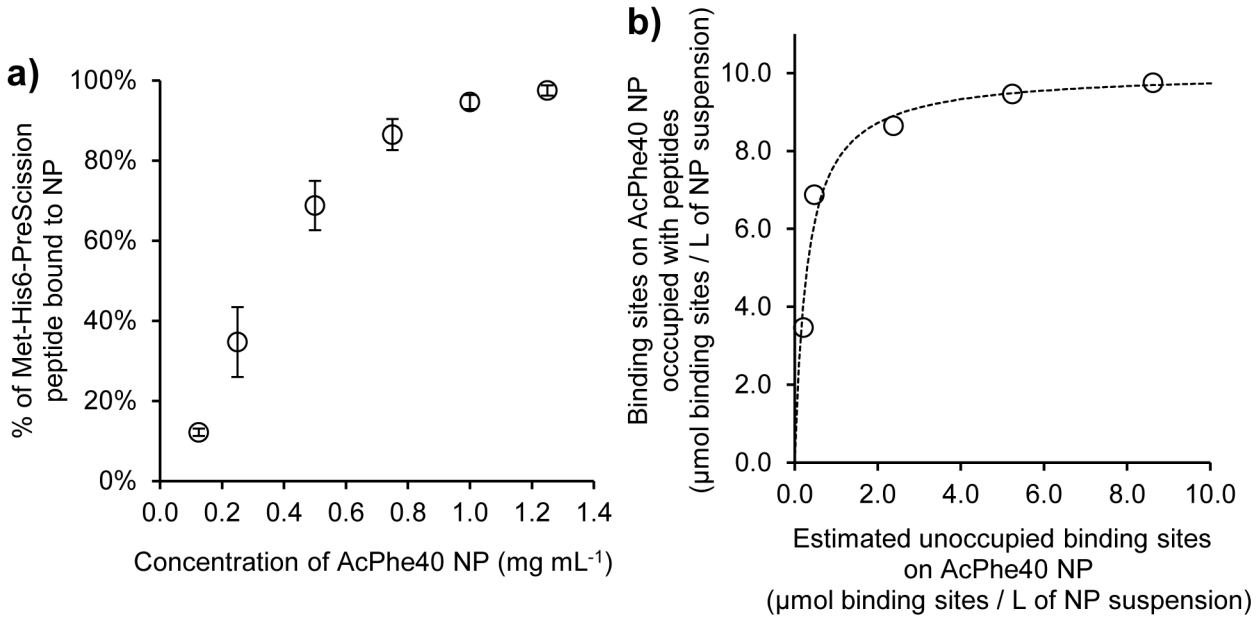


Figure S1. a) Equilibrium binding curve of AcPhe40 NP for Met-His6-PreScission peptide in 15 mM Tris-HCl buffer (pH 7.8) containing 0.1% Tween 20 (w/v). Concentration of peptide = 10 µM (18.55 µg/mL). b) Binding isotherm of AcPhe40 NP for Met-His6-PreScission peptide. The plot was established based on 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.

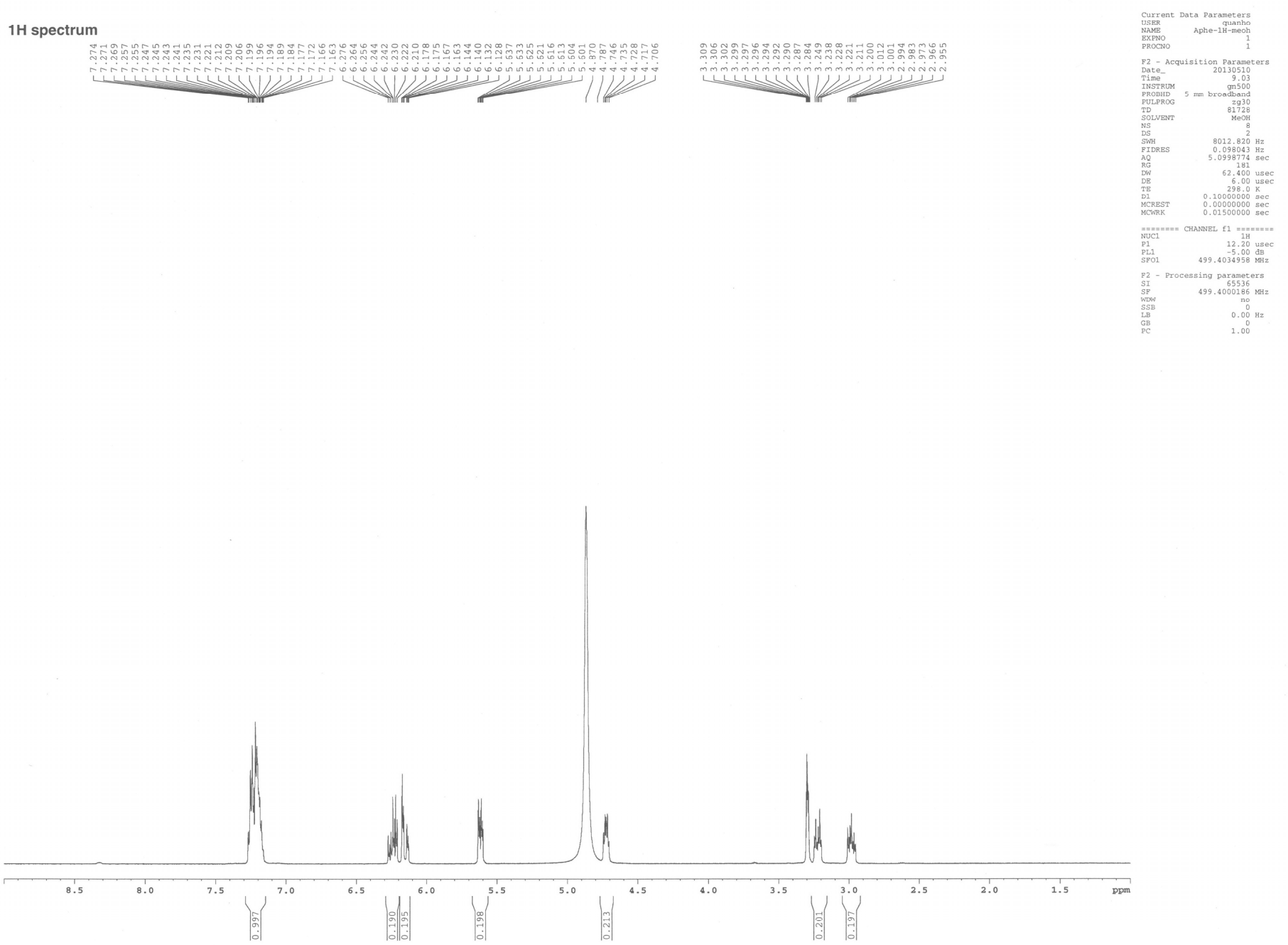


Figure S2. ^{1}H NMR spectrum of *N*-acryloyl *L*-phenylalanine (CD_3OD, 500 MHz)

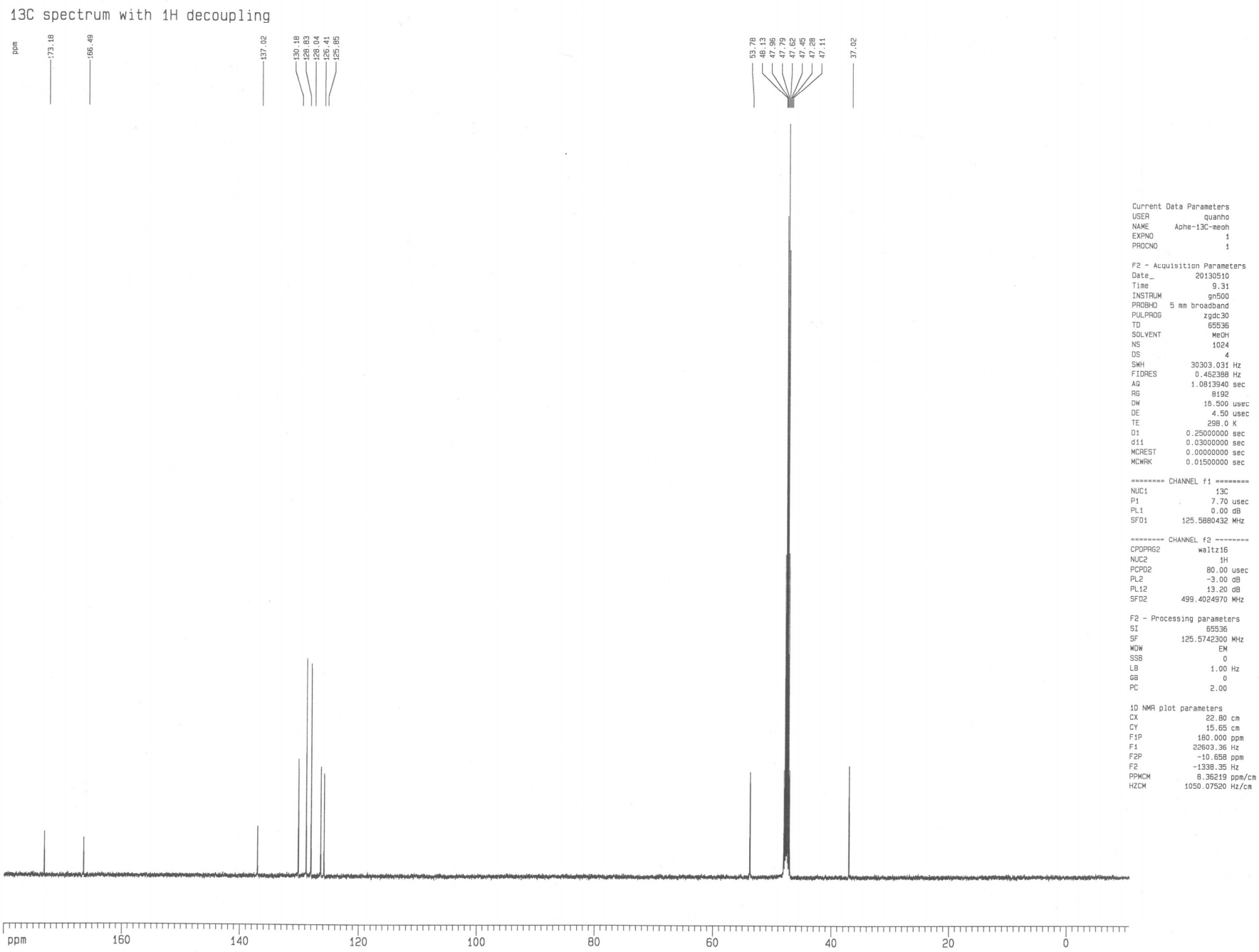


Figure S3. ^{13}C NMR of *N*-acryloyl *L*-phenylalanine (CD_3OD, 125 MHz)

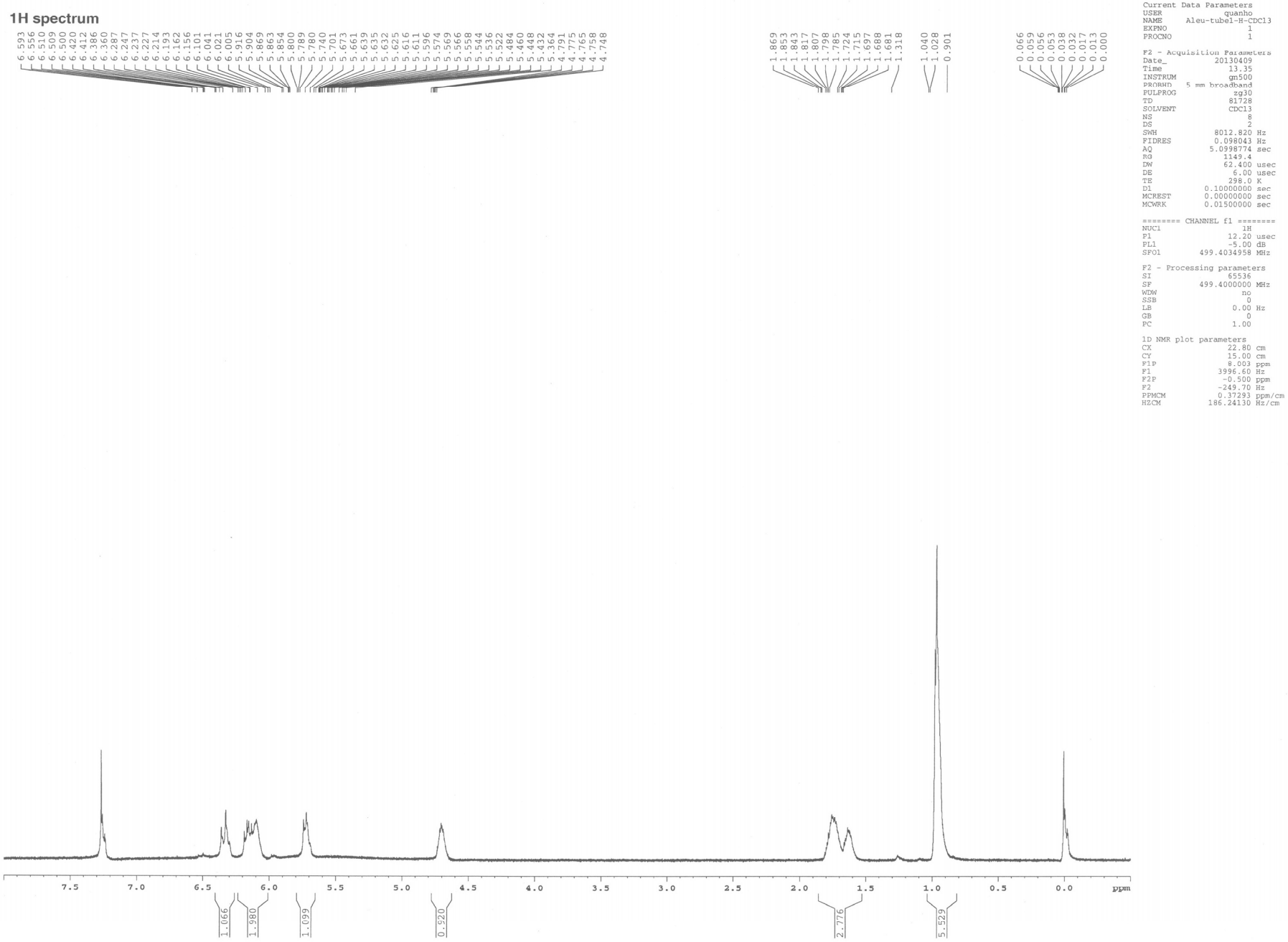


Figure S4. ^{1}H NMR spectrum of *N*-acryloyl *L*-leucine ($CDCl_3$, 500 MHz)

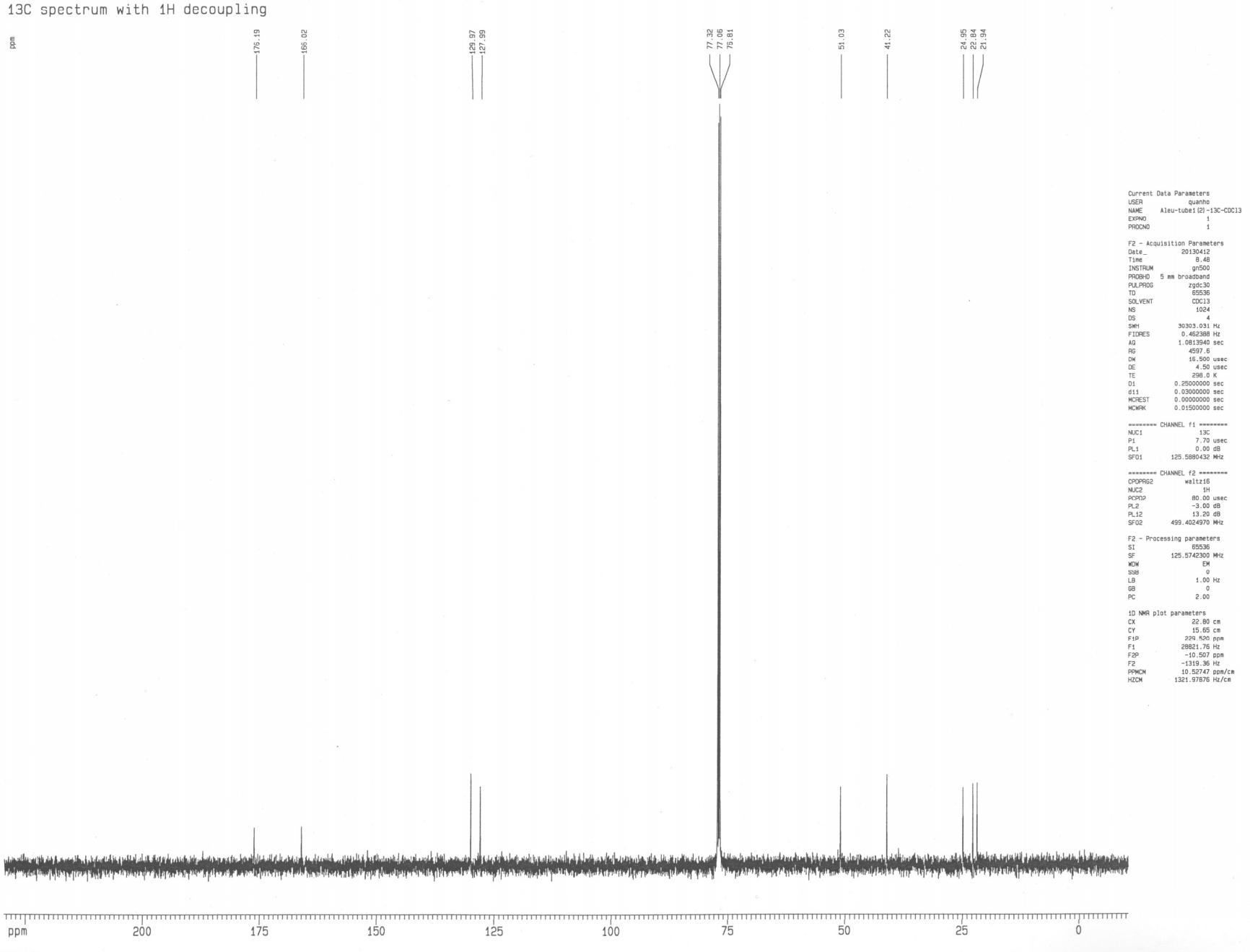


Figure S5. ^{13}C NMR spectrum of *N*-acryloyl *L*-leucine ($CDCl_3$, 125 MHz)

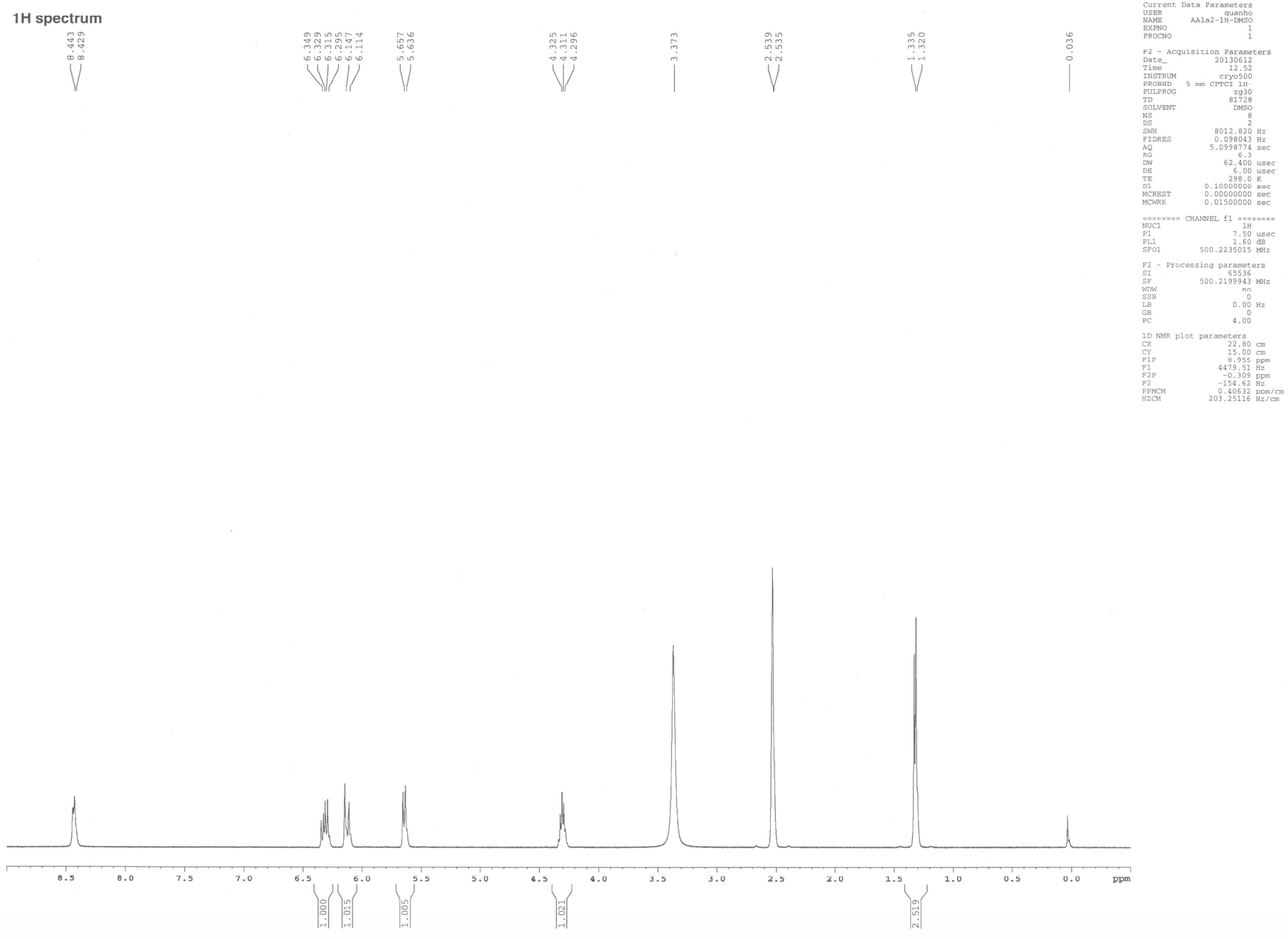
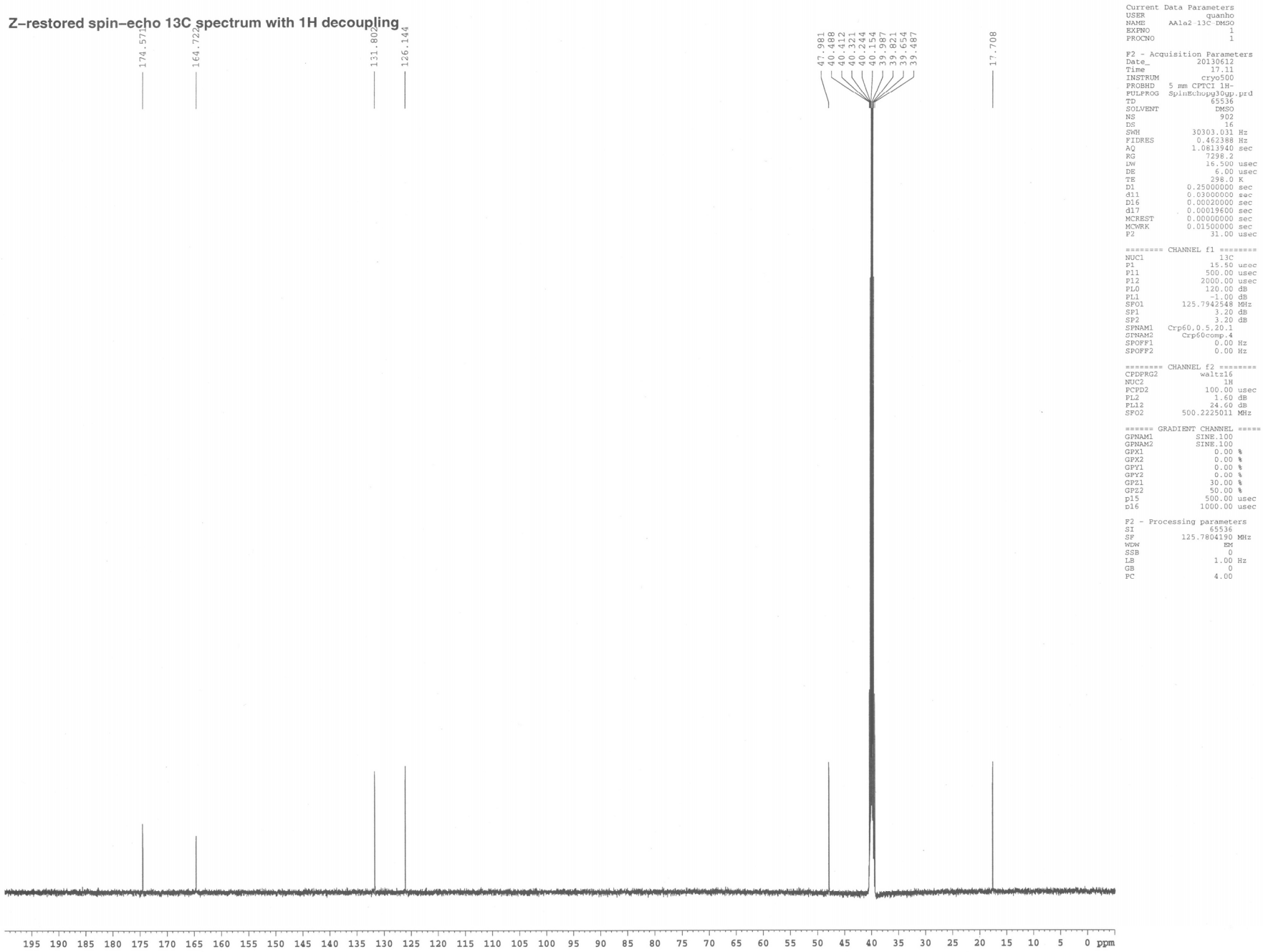


Figure S6. ^{1}H NMR spectrum of *N*-acryloyl *L*-alanine (DMSO-*d*$_6$, 500 MHz)



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 SpinEchopg30gp.prd
TD 65536
SOLVENT DMSO
NS 902
DS 16
SWH 30303.031 Hz
FIDRES 0.462388 Hz
AQ 1.0813940 sec
RG 7298.2
DW 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
MCREST 0.00000000 sec
MCWRK 0.01500000 sec
P2 31.00 usec

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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
SP1 3.20 dB
SP2 3.20 dB
SPNAM1 Crp60,0.5,20.1
SPNAM2 Crp60comp.4
SPOFF1 0.00 Hz
SPOFF2 0.00 Hz

======== CHANNEL f2 ========
CPDPRG2 waltz16
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)

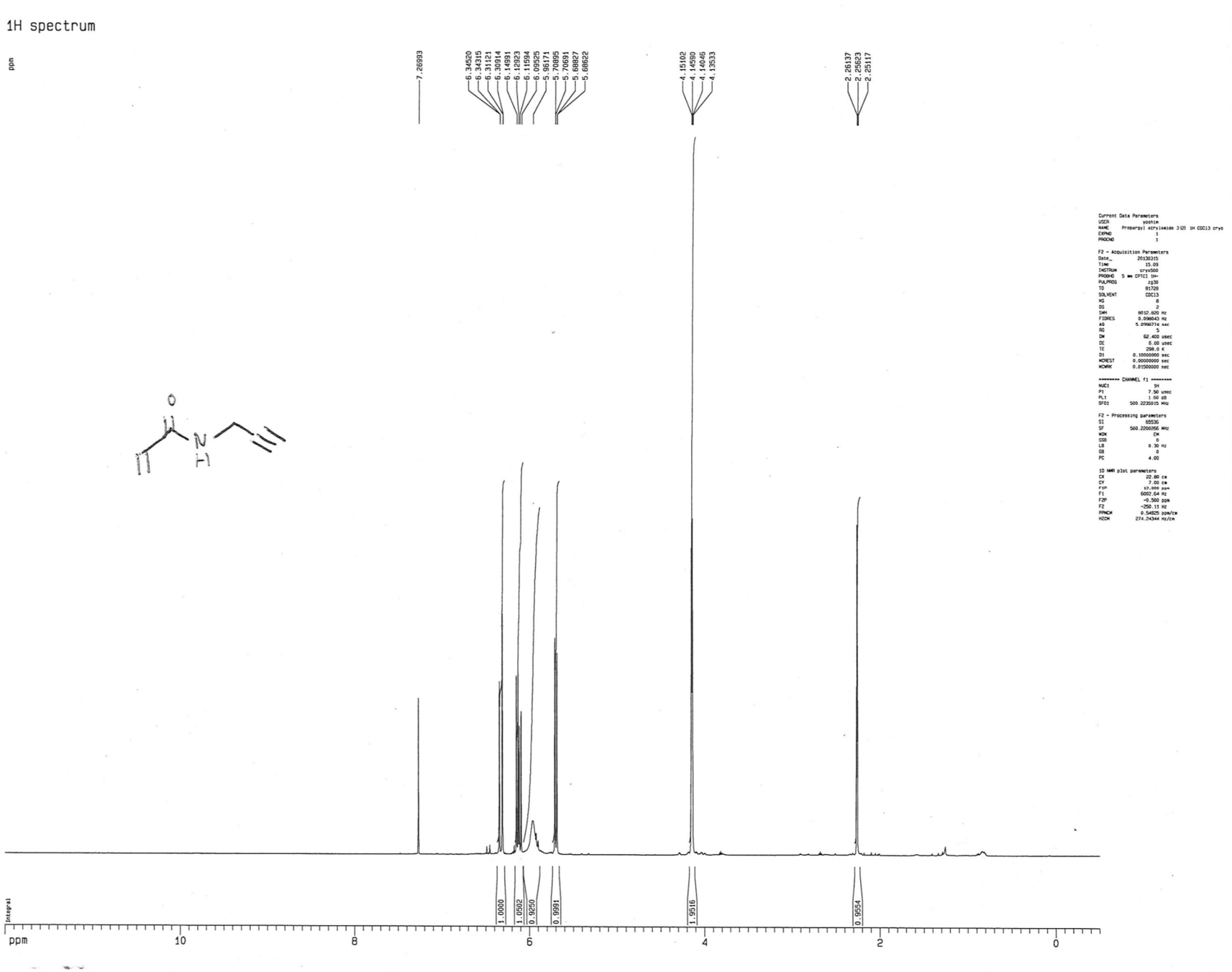

Figure S8. ^{1}H NMR spectrum of *N*-propargyl acrylamide ($CDCl_3$, 500 MHz)

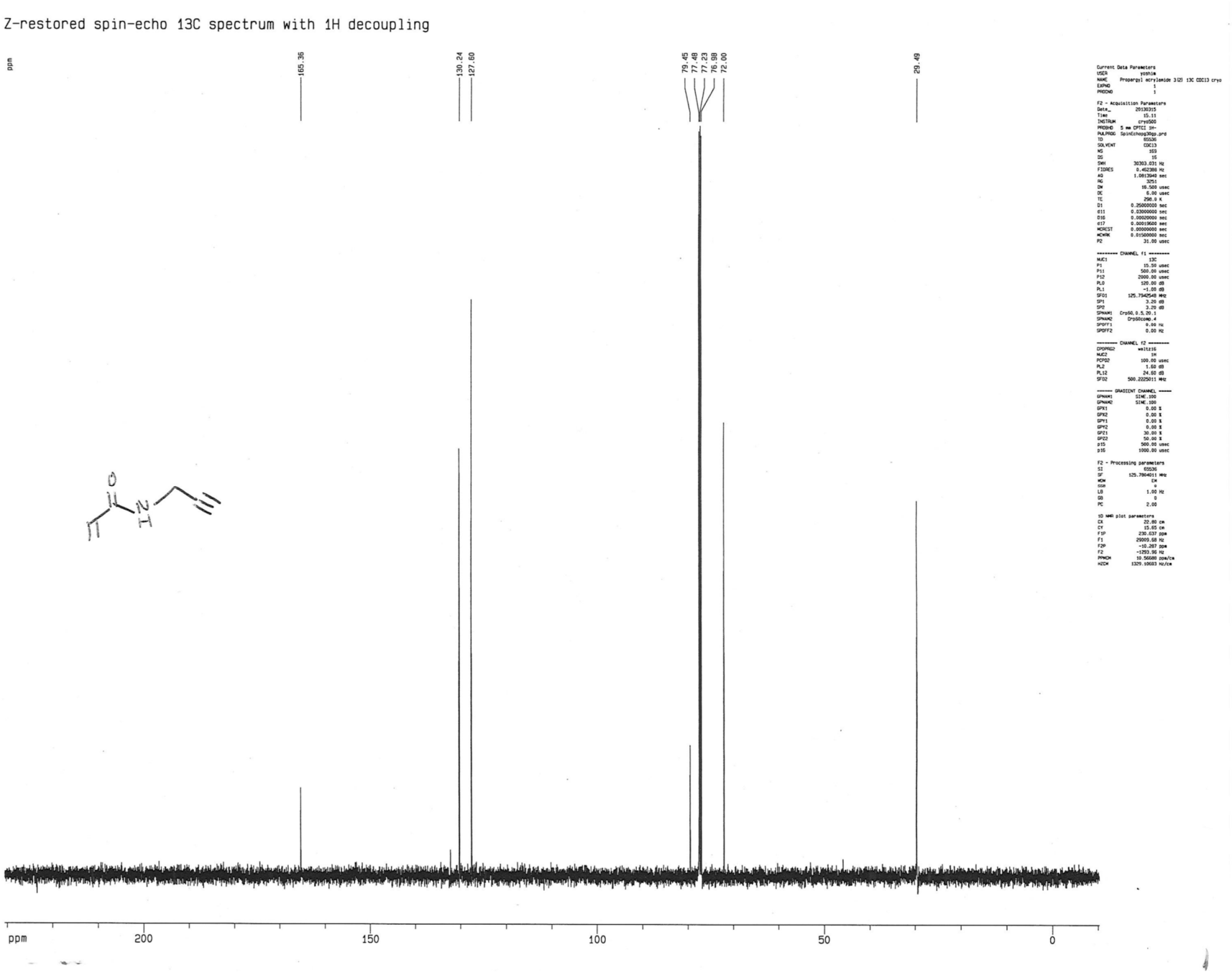

Figure S9. ^{13}C NMR spectrum of *N*-propargyl acrylamide ($CDCl_3$, 125 MHz)