Olefin Metathesis in Homogeneous Aqueous Media Catalyzed by Conventional Ruthenium Catalysts

Joseph B. Binder, Jacqueline J. Blank, and Ronald T. Raines

Department of Chemistry, University of Wisconsin–Madison, 1101 University Avenue, Madison, WI 53706-1322, USA, and Department of Biochemistry, University of Wisconsin–Madison, 433 Babcock Drive, Madison, WI 53706-1544, USA.

raines@biochem.wisc.edu

Page	Contents
S1	Table of Contents
S2	General Experimental
S2	Synthesis of Methyl (D,L)-*N*-(allyl)allylglycinate
S2	Protein Solubility Measurements
S2–S3	Representative Procedures for Metathesis Reactions
S4	^{1}H NMR Spectrum of Methyl (D,L)-*N*-(allyl)allylglycinate
S5	^{13}C NMR Spectrum of Methyl (D,L)-*N*-(allyl)allylglycinate
S6	^{1}H NMR Spectrum of **16**

General Experimental. Commercial chemicals were of reagent grade or better, and were used without further purification. The term "concentrated under reduced pressure" refers to the removal of solvents and other volatile materials using a rotary evaporator at water aspirator pressure (<20 torr) while maintaining the water-bath temperature below 40 °C. NMR spectra were acquired with a Bruker DMX-400 Avance spectrometer (^{1}H, 400 MHz; ^{13}C, 100.6 MHz) or a Bruker Avance DMX-500 spectrometer (^{1}H, 500 MHz; ^{13}C, 125.7 MHz) at the National Magnetic Resonance Facility at Madison (NMRFAM). NMR spectra were obtained at ambient temperature unless indicated otherwise. Coupling constants *J* are given in Hertz. Mass spectrometry was performed with a Micromass LCT (electrospray ionization, ESI) in the Mass Spectrometry Facility in the Department of Chemistry. Absorption spectra were recorded in 1-cm path length cuvettes on a Cary model 50 spectrometer from Varian.

Complexes **1–4** were obtained from Aldrich (Milwaukee, WI) and used without further purification. Bovine pancreatic ribonuclease A (RNase A type III-A, >85%) was obtained from Sigma (St. Louis, MO). Phosphate-buffered saline (PBS), pH 7.4, contained (in 1.00 L) KCl (0.20 g), KH_2PO4 (0.20 g), NaCl (8.0 g), and $Na_2HPO_4{\cdot}7H_2O$ (2.16 g). The following metathesis substrates were obtained from commercial sources and used without further purification: diethyl diallylmalonate (**9**), *N*,*N*-diallyl-2,2,2-trifluoroacetamide (**10**), diallyl ether (**13**), 1,7-octadiene (**15**), and allyl alcohol (**18**) from Aldrich (Milwaukee, WI); *N*,*N*-diallyl-*N*,*N*-dimethylammonium chloride (**12**) from Fluka (Buchs, Switzerland); and diallyldiphenylsilane (**14**) from Acros Organics (Geel, Belgium). *N*,*N*-Diallyl-4-methylbenzenesulfonamide (**8**) was prepared by the method of Lamaty and co-workers.[1] Diallylamine hydrochloride (**11**) was prepared from the corresponding amine (Aldrich) by treatment with ethereal HCl. *N*,*N*-Di-3-butenyl-2-nitrobenzenesulfonamide (**17**) was prepared as previously reported.[2] Methyl (D,L)-allylglycinate hydrochloride was prepared by the method of Creighton and coworkers.[3]

Methyl (D,L)-*N*-(Allyl)allylglycinate. Methyl (D,L)-allylglycinate hydrochloride (1.35 g, 8.15 mmol) and triethylamine (3.41 mL, 24.5 mmol) were dissolved in CH_2Cl_2 (50 mL), and 2-nitrobenzenesulfonylchloride (1.84 g, 8.31 mmol) was added. After stirring overnight, the resulting solution was washed twice with 1 M aqueous HCl (50 mL), twice with saturated aqueous $NaHCO_3$ (50 mL), and once with brine (50 mL). The organic layer was dried over $MgSO_4$(s) and concentrated under reduced pressure. The yellow residue was taken up in DMF (10 mL), and combined with allyl bromide (0.848 mL, 9.78 mmol) and potassium carbonate (2.53 g, 18.3 mmol). After the reaction mixture was stirred for 24 h, mercaptoacetic acid (1.25 mL, 17.9 mmol) was added, followed after 30 min by DBU (9.75 mL, 65.2 mmol). After stirring for 3 h, the resulting mixture was diluted with EtOAc (75 mL) and mixed with saturated aqueous $NaHCO_3$ (50 mL). The organic layer was separated and washed twice with saturated aqueous $NaHCO_3$ (50 mL). The aqueous washes were combined and extracted with with EtOAc (100 mL). The organic extracts were combined, washed with brine (50 mL), and concentrated under reduced pressure. The crude product was purified by flash chromatography (10% EtOAc v/v in hexane with 0.1% triethylamine to elute byprouct followed by 15-20% EtOAc v/v in hexane with 0.1% triethylamine) to afford methyl (D,L)-*N*-(allyl)allylglycinate (0.477 g, 2.82 mmol, 35%) as a colorless oil. R_f = 0.30 (20% EtOAc in hexanes with 1% triethylamine). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.92–5.69 (m, 2H), 5.22–5.02 (m, 4H), 3.72 (s, 3H), 3.37 (X of ABX, *J* = 6.2, 5.8 Hz, 1H), 3.28 (A of ABX, *J* = 13.8, 5.8 Hz, 1H), 3.13 (B of ABX, *J* = 13.8, 6.2 Hz, 1H), 2.42 (t, *J* = 6.7 Hz, 2H), 1.84 (br. s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.9, 136.1, 133.5, 118.0, 116.4, 60.0, 51.5, 50.6, 37.5; HRMS–ESI (*m*/*z*): $[M+H]^+$ calcd for $C_9H_{16}NO_2$, 170.1181; found 170.1179. Methyl (D,L)-*N*-(allyl)allylglycinate hydrochloride (**16**) was prepared from methyl (D,L)-*N*-(allyl)allylglycinate by treatment with ethereal HCl. ^{1}H NMR (400 MHz, CD_3OD) δ 5.99–5.87 (m, 1H), 5.82–5.69 (m, 1H), 5.59–5.50 (m, 2H), 5.34–5.25 (m, 2H), 4.19 (t, *J* = 5.8, 1H), 3.86 (s, 3H), 3.72 (d, *J* = 6.9 Hz, 2H), 2.85–2.66 (m, 2H).

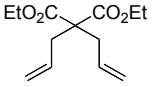
Protein Solubility Measurements. Ribonuclease A (RNase A; 4 mg) was dissolved in PBS (4 mL). To prepare solutions with four different DME concentrations, this solution was mixed in the following proportions with DME: 0:1, 1:1, 6:4, and 2:1 DME/PBS. After thorough mixing, the solutions were subjected to centrifugation (13,000 rpm, 10 min) and their absorbances at 277.5 nm were measured. A sharp decrease in absorbance was apparent between the 6:4 and 2:1 DME:PBS solutions, indicating that RNase A is soluble at 0.4 mg/mL in 6:4 DME:PBS. Observation of RNase A precipitation at DME concentrations above this threshold corroborated this result.

Representative Procedures for Metathesis Reactions. *Non-deuterated Solvents*. The ruthenium complex **2** (1.0 mg, 1.2 μmol) was dissolved in DME (0.67 g), and deionized water (0.33 g) was added to this solution, followed by *N*-tosyl diallylamine **8** (5.0 μL, 24 μmol). The reaction mixture was shaken at room temperature for 1

[1] Varray, S.; Lazaro, R.; Martinez, J.; Lamaty, F. *Organometallics* **2003,** *22*, 2426–2435.
[2] Binder, J. B.; Guzei, I., A.; Raines, R. T. *Adv. Synth. Catal.* **2007,** *349*, 395–404.
[3] Creighton, C. J.; Leo, G. C.; Du, Y.; Reitz, A. B. *Bioorg. Med. Chem.* **2004,** *12*, 4375–4385.

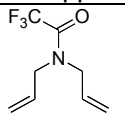
day before it was quenched by addition of ethyl vinyl ether (1 mL). This mixture was concentrated under reduced pressure, and the residue was analyzed by ^{1}H NMR spectroscopy of its $CDCl_3$ or D_2O solution. Conversion was determined by the ratio of the integrals of the substrate and product signals (*vide infra*).

*Acetone-d_6/D_2O solvent***.** Diallylamine hydrochloride (**11**) (1.3 mg, 10 μmol) was dissolved in acetone-d_6 (0.617 mL) and D_2O (0.333 mL) in an NMR tube. The ruthenium complex **4** (0.63 mg, 1 μmol) was added as a solution in acetone-d_6 (50 μL), and the NMR tube was capped. The reaction mixture was shaken at room temperature, and the reaction was monitored by ^{1}H NMR spectroscopy. Conversion was determined by the ratio of the integrals of the signals described below.



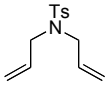
9

substrate methylene doublet at 2.6 ppm *versus* product singlet at 3.0 ppm



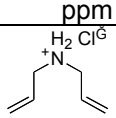
10

substrate methylene signals at 4.0 ppm *versus* product signals at 4.4 and 4.5 ppm



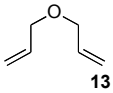
8

substrate methylene doublet at 3.8 ppm *versus* product singlet at 4.1 ppm



11

substrate methylene doublet at 3.6 ppm *versus* product singlet at 4.0 ppm

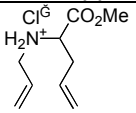


13

substrate methylene doublet at 4.0 ppm *versus* product singlet at 4.3 ppm

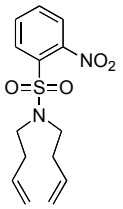
15

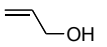
substrate vinylic signals at 5.0 ppm *versus* product olefin singlet at 5.6 ppm

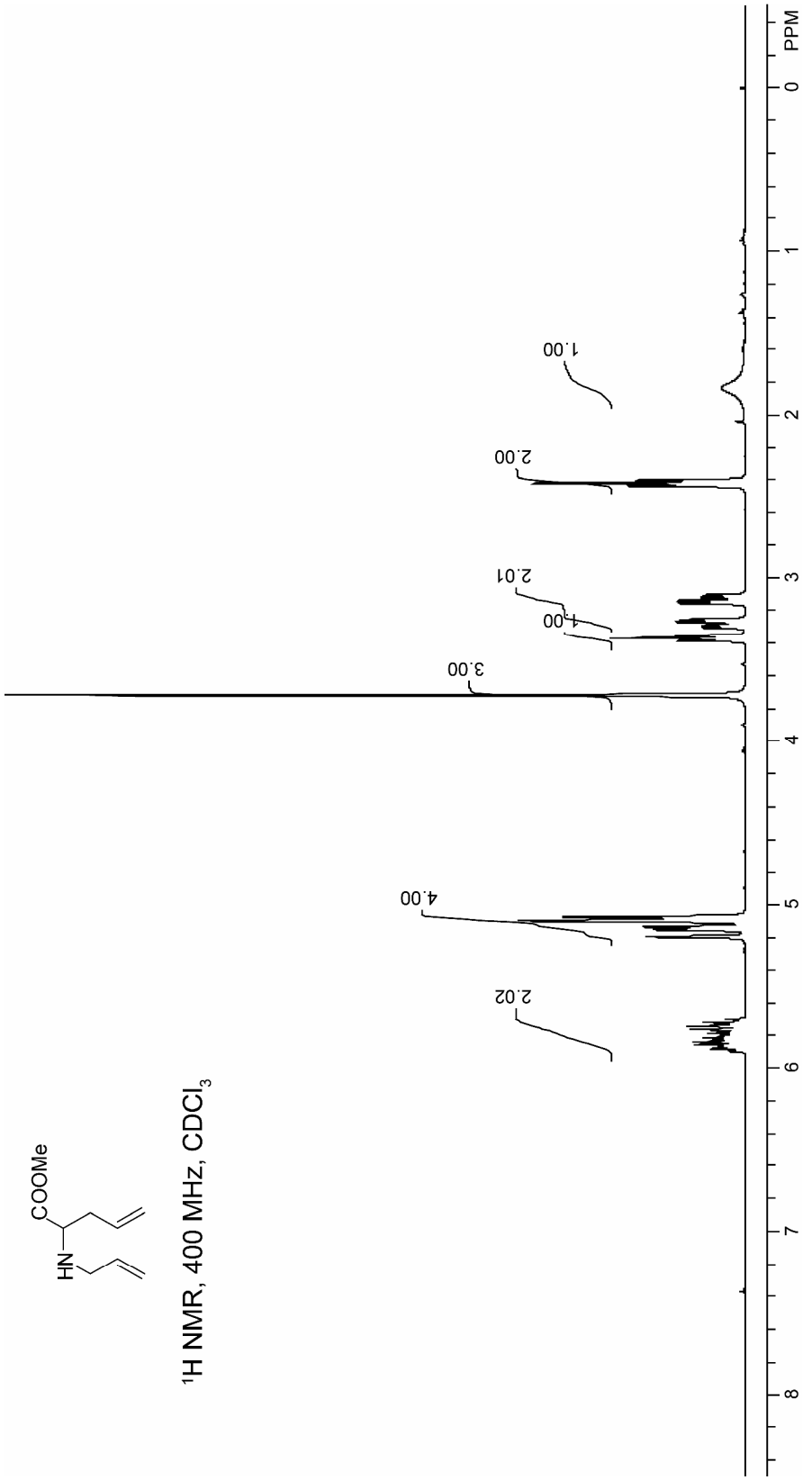


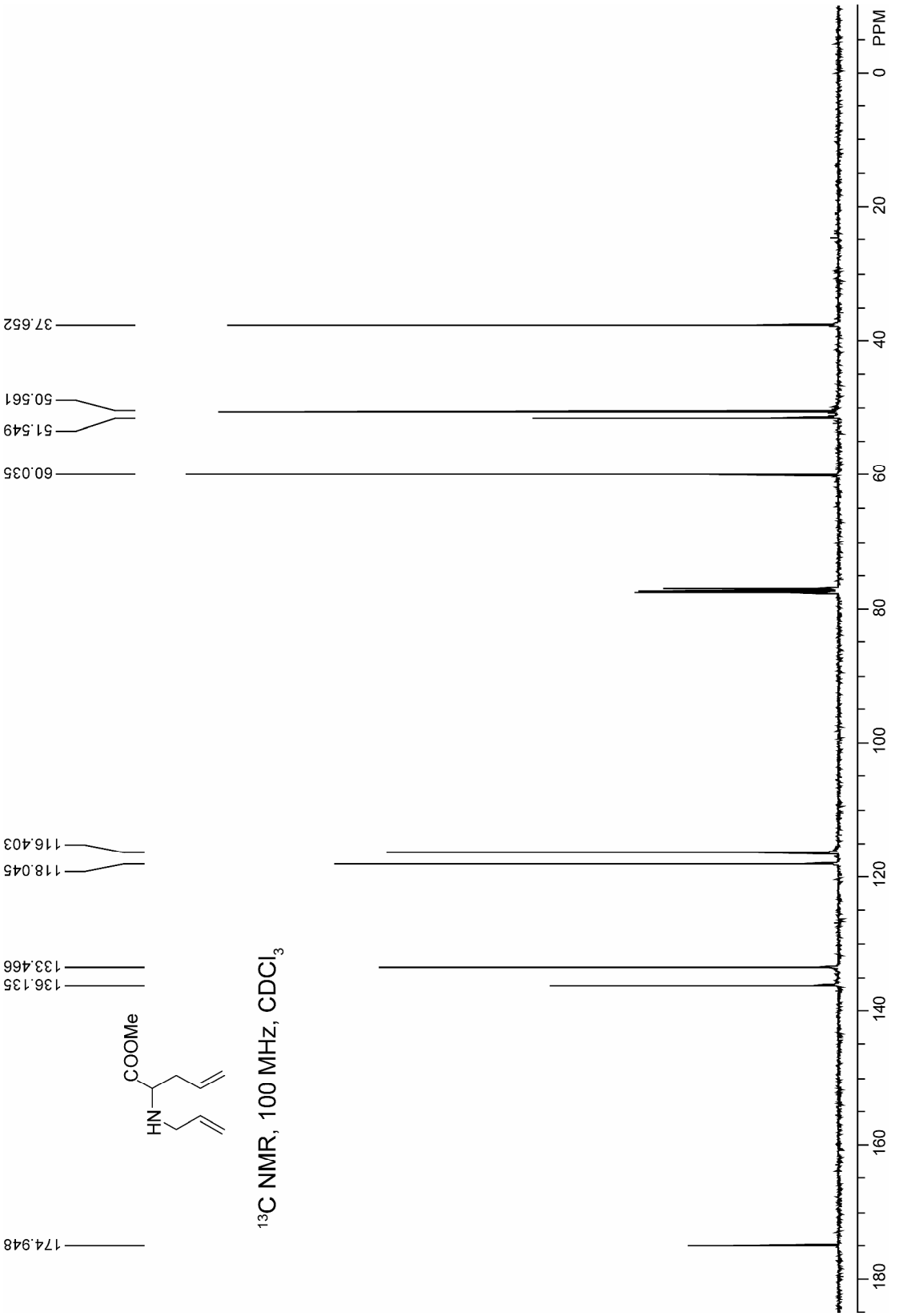
16

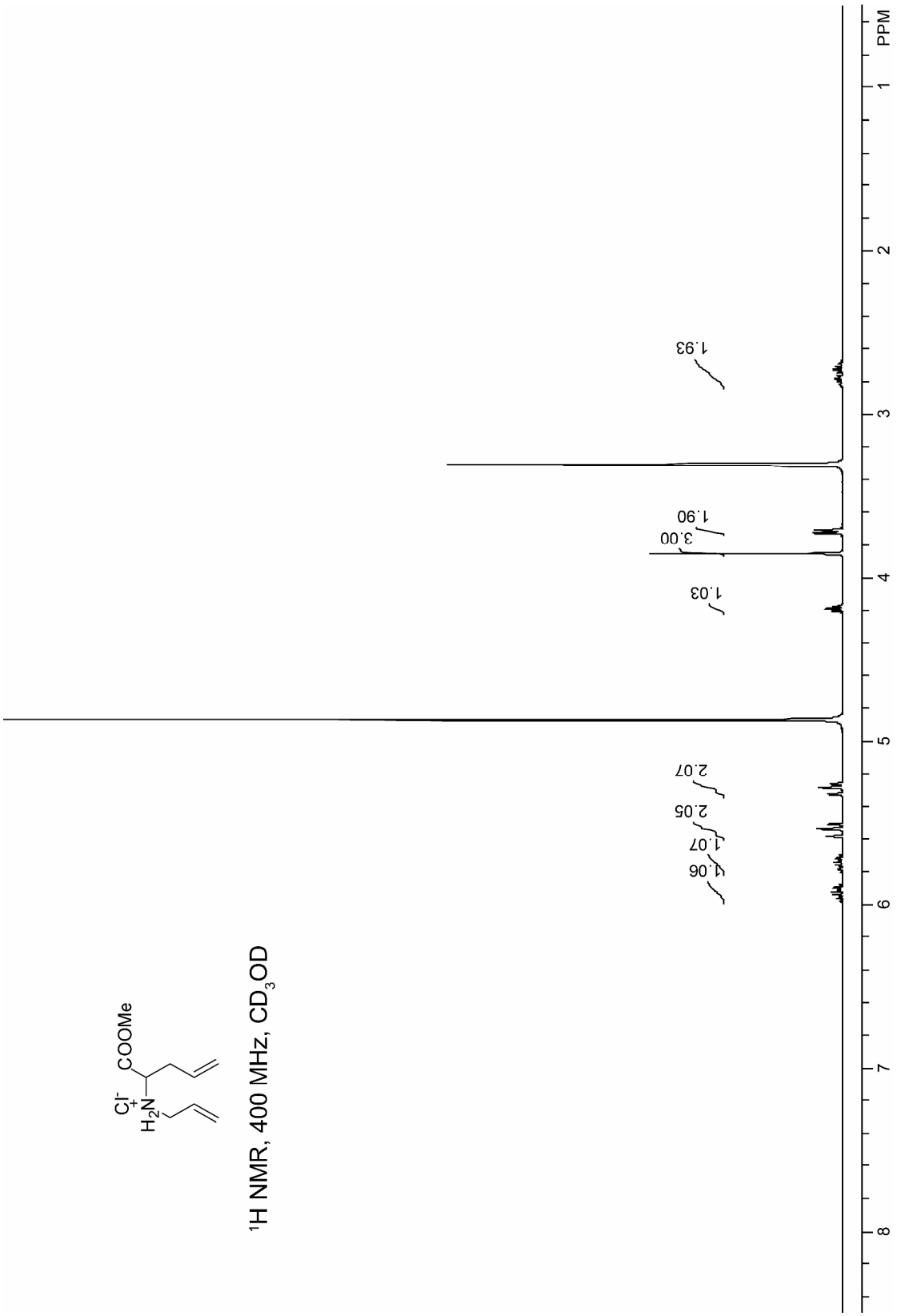
substrate vinylic signals at 5.2 and 5.4 ppm *versus* product olefinic signals at 5.7 and 5.9 ppm



17 substrate vinylic signals at 5.0 and 5.7 ppm	*versus*	product singlet at 5.8 ppm
		
18 substrate vinylic signals at 5.0 and 5.9 ppm	*versus*	product olefinic signal at 5.8 ppm


^{1}H NMR, 400 MHz, $CDCl_3$
COOMe
HN
2.02
4.00
3.00
4.00
2.01
2.00
1.00
PPM
0
1
2
3
4
5
6
7
8


174.948
133.466
136.135
118.045
116.403
60.035
51.549
50.561
37.652
COOMe
HN
^{13}C NMR, 100 MHz, CDCl$_3$
PPM
0
20
40
60
80
100
120
140
160
180


^{1}H NMR, 400 MHz, CD_3OD
Cl^-
H_2N^+
COOMe
1.93
3.00
1.90
1.03
2.07
2.05
1.07
1.06
PPM
1
2
3
4
5
6
7
8