Quantitative Catalyst-Substrate Association Relationships between Metathesis Molybdenum or Ruthenium Carbene Complexes and Their Substrates

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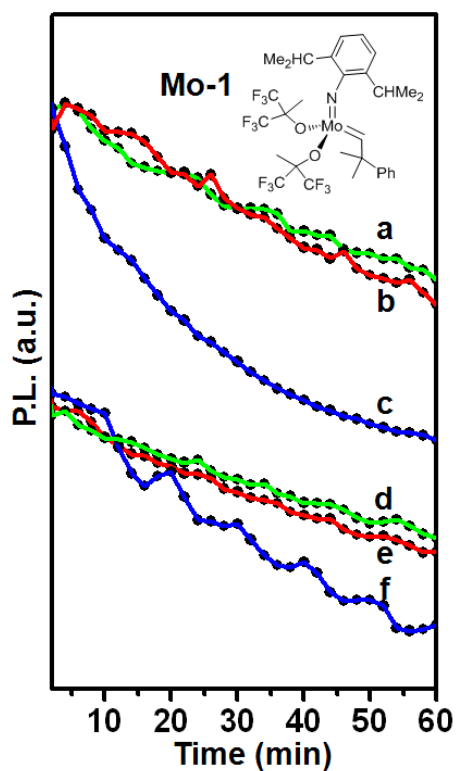
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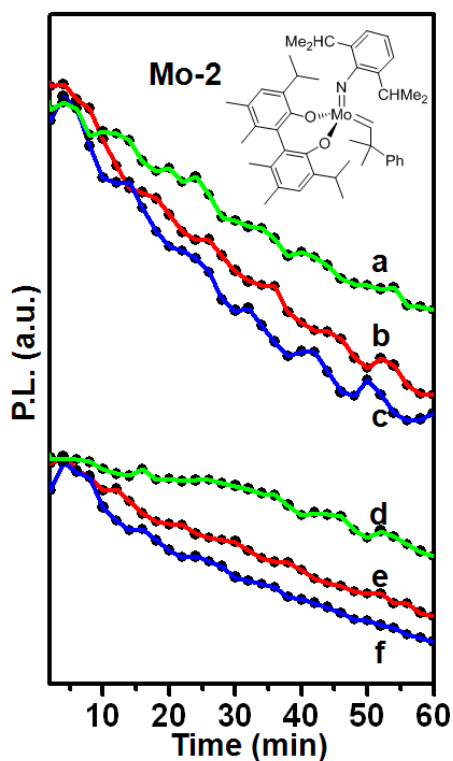*E-mail: sohnjh@ip-korea.org, hyotcherl.ihee@kaist.ac.kr*

CONTENTS

1) All time-dependent quenching traces and their conditions

Index	Condition
a	**3** (20 μM) + **Mo-1** (20 μM), 20 °C
b	**1** (20 μM) + **Mo-1** (20 μM), 20 °C
c	**2** (20 μM) + **Mo-1** (20 μM), 20 °C
d	**3** (10 μM) + **Mo-1** (20 μM), 20 °C
e	**1** (10 μM) + **Mo-1** (20 μM), 20 °C
f	**2** (10 μM) + **Mo-1** (20 μM), 20 °C

Figure S1. All time-dependent fluorescence quenching traces for the **Mo-1** catalyst are shown in the left pannel. Red, blue and green curves represent the cases with alkene, alkyne and allene substrates repectively. Experimental conditions for each trace are listed in the table.

Index	Condition
a	**3** (20 μM) + **Mo-2** (20 μM), 20 °C
b	**1** (20 μM) + **Mo-2** (20 μM), 20 °C
c	**2** (20 μM) + **Mo-2** (20 μM), 20 °C
d	**3** (10 μM) + **Mo-2** (20 μM), 20 °C
e	**1** (10 μM) + **Mo-2** (20 μM), 20 °C
f	**2** (10 μM) + **Mo-2** (20 μM), 20 °C

Figure S2. All time-dependent fluorescence quenching traces for the **Mo-2** catalyst are shown in the left pannel. Red, blue and green curves represent the cases with alkene, alkyne and allene substrates repectively. Experimental conditions for each trace are listed in the table.
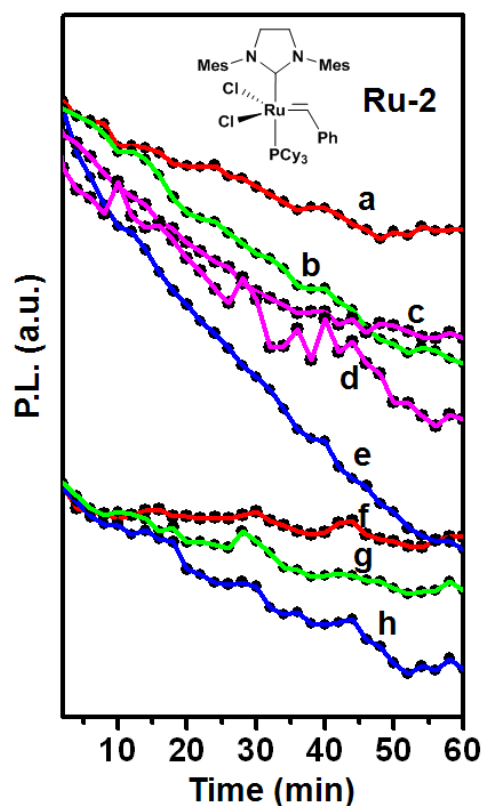
S3

Index	Condition
a	**6** (20 μM) + **Ru-1** (20 μM), 20 °C
b	**5** (10 μM) + **6** (10 μM) + **Ru-1** (20 μM), 20 °C
c	**5** (20 μM) + **Ru-1** (20 μM), 20 °C
d	**6** (10 μM) + **Ru-1** (20 μM), 20 °C
e	**5** (10 μM) + **Ru-1** (20 μM), 20 °C
f	**7** (20 μM) + **Ru-1** (20 μM), 20 °C
g	**7** (10 μM) + **Ru-1**(20 μM), 20 °C

Figure S3. All time-dependent fluorescence quenching traces for the **Ru-1** catalyst are shown in the left pannel. Red, blue, green and magenta curves represent the cases with alkene, alkyne, allene substrates, and a mixture of alkene and alkyne, repectively. Experimental conditions for each trace are listed in the table.

Index	Condition
a	**5** (20 μM) + **Ru-2** (20 μM), 30 °C
b	**7** (20 μM) + **Ru-2** (20 μM), 30 °C
c	**5** (10 μM) + **6** (10 μM) + **Ru-2** (20 μM), 30 °C
d	**6** (10 μM) + **7** (10 μM) + **Ru-2** (20 μM), 30 °C
e	**6** (20 μM) + **Ru-2** (20 μM), 30 °C
f	**5** (10 μM) + **Ru-2** (20 μM), 30 °C
g	**7** (10 μM) + **Ru-2** (20 μM), 30 °C
h	**6** (10 μM) + **Ru-2** (20 μM), 30 °C

Figure S4. All time-dependent fluorescence quenching traces by **Ru-2** catalyst are shown in the left pannel. Red, blue, green and magenta curves represent the cases with alkene, alkyne, allene substrates and a mixture of them, repectively. Experimental conditions for each trace are listed in the table.

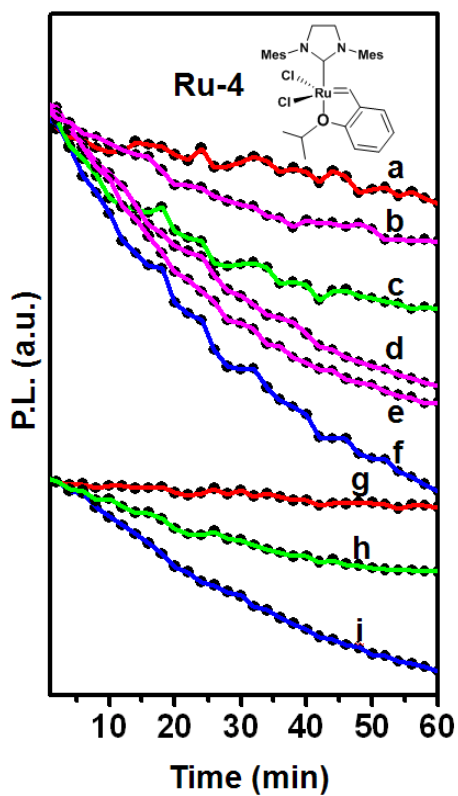| Index	Condition
a	**6** (20 μM) + **Ru-3** (20 μM), 30 °C
b	**5** (20 μM) + **Ru-3** (20 μM), 30 °C
c	**6** (10 μM) + **7** (10 μM) + **Ru-3** (20 μM), 30 °C
d	**5** (10 μM) + **7** (10 μM) + **Ru-3** (20 μM), 30 °C
e	**7** (20 μM) + **Ru-3** (20 μM), 30 °C
f	**6** (10 μM) + **Ru-3** (20 μM), 30 °C
g	**5** (10 μM) + **Ru-3** (20 μM), 30 °C
h	**7** (10 μM) + **Ru-3** (20 μM), 30 °C

Figure S5. All time-dependent fluorescence quenching traces by **Ru-3** catalyst are shown in the left pannel. Red, blue, green and magenta curves represent the cases with alkene, alkyne, allene substrates and a mixture of them, repectively. Experimental conditions for each trace are listed in the table.

| Index	Condition
a	**5** (20 μM) + **Ru-4** (20 μM), 30 °C
b	**5** (10 μM) + **7** (10 μM) + **Ru-4** (20 μM), 30 °C
c	**7** (20 μM) + **Ru-4** (20 μM), 30 °C
d	**5** (10 μM) + **6** (10 μM) + **Ru-4** (20 μM), 30 °C
e	**6** (10 μM) + **7** (10 μM) + **Ru-4** (20 μM), 30 °C
f	**6** (20 μM) + **Ru-4** (20 μM), 30 °C
g	**5** (10 μM) + **Ru-4** (20 μM), 30 °C
h	**7** (10 μM) + **Ru-4** (20 μM), 30 °C
i	**6** (10 μM) + **Ru-4** (20 μM), 30 °C

Figure S6. All time-dependent fluorescence quenching trasces by **Ru-4** catalyst are shown in the left pannel. Red, blue, green and magenta curves represent the cases with alkene, alkyne, allene substrates and a mixture of them repectively. Experimental conditions for each trace are listed in the table.

2) Substrate absorbance change during fluorescence quenching
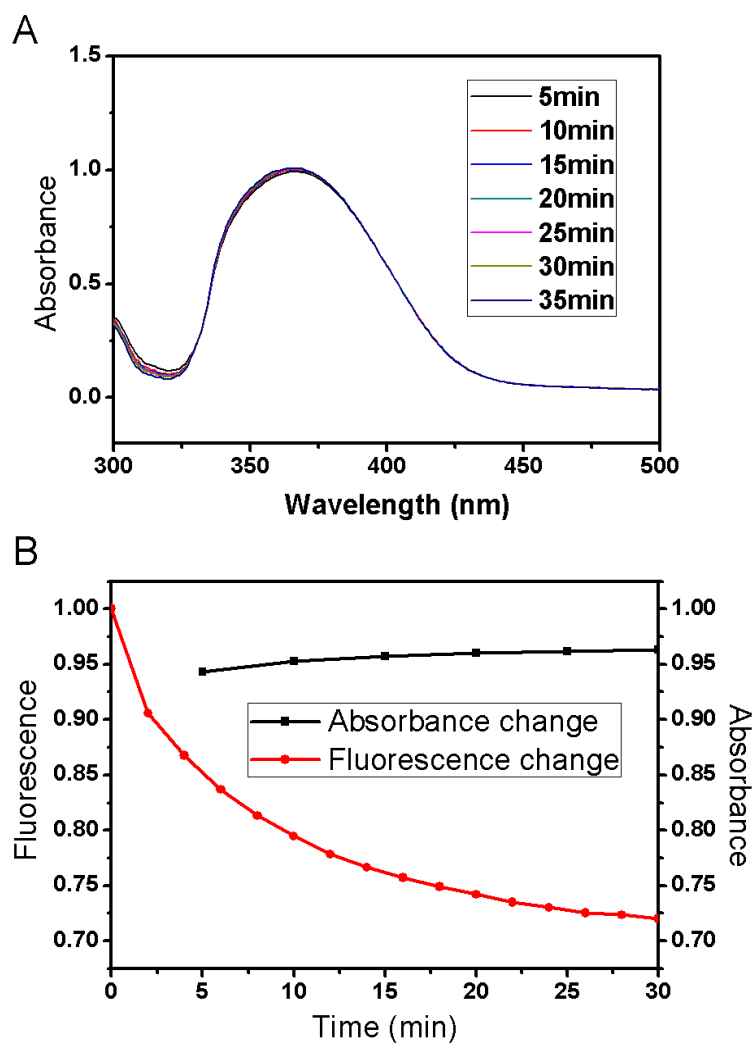
Figure S7. No significant absorbance change of the substrate during the fluorescence quenching. This guarantees that most of the changes in the fluorescence spectrum come from the fluorescence quenching effect rather than some reaction of the dye part itself. **(A)** Time-dependent absorbance change of the dye-alkene conjugate during the fluorescence quenching. **(B)** Absorbance change as a function of time and comparison with the fluorescence change.

3) Time-dependent fluorescence quenching of the substrates by Ru-1 in *n*-hexane
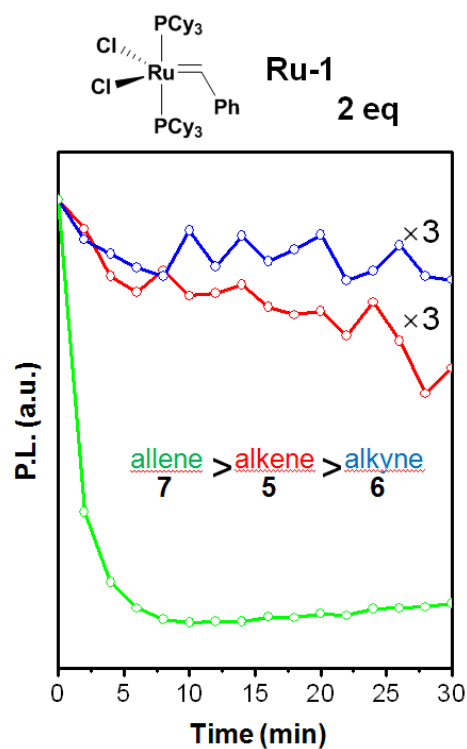
Figure S8. Time-dependent fluorescence quenching of dye-conjugated substrates (alkene **5**, alkyne **6** and allene **7**) by **Ru-1** in *n*-hexane. Quenching signal from alkene and alkyne are magnified by 3 times to obtain a clear view. It also shows a clear preference pattern that is the same as that in the CH_2Cl_2 solvent (main text Figure 2A).

4) Global fitting analysis of FRET data

In the following pages, we describe how we quantitatively analyzed FRET data.

Control subtraction

In order to obtain PL intensity change purely from the fruorecence quenching caused by the substrate-catalyst association reaction, the raw PL data were subtracted by the data from a control experiment. An example is displayed in Figure S9:
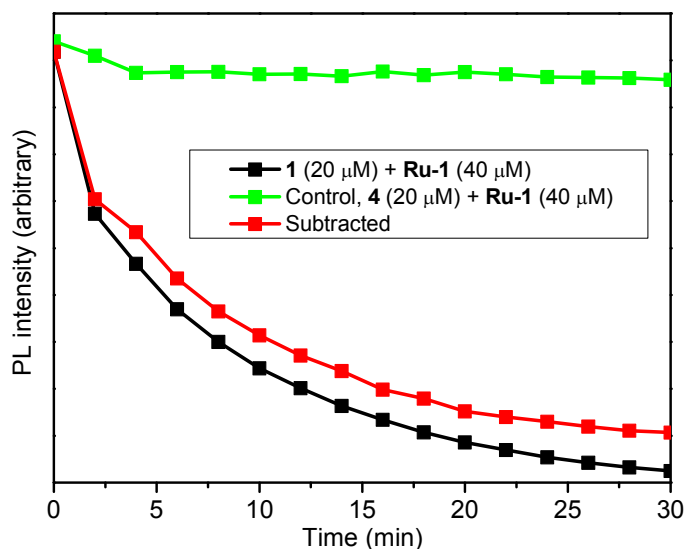

Figure S9. The green squares are from a control experiment with the control dye-ane substrate **8** and correspond to the fluorescence quenching due to non-specific binding, not from the specific substrate-catalyst association reaction. The black squares are raw data with the dye-alkene substrate **5**. The red squares are obtained by substracting the control data (green) from the raw data (black), and account for fluorescence quenching only from the association reaction.

Reaction scheme

We assumed the substrate-catalyst association and dissociation steps in Grubbs' Ru catalyzed enyne metathesis have the following reaction schemes.

$$5 + \textbf{Ru-1} \underset{k_{-1,\,1}}{\overset{k_1}{\rightleftharpoons}} \textbf{1:Ru-1}$$

$$6 + \textbf{Ru-1} \underset{k_{-1,\,2}}{\overset{k_2}{\rightleftharpoons}} \textbf{2:Ru-1}$$

$$7 + \textbf{Ru-1} \underset{k_{-1,\,3}}{\overset{k_3}{\rightleftharpoons}} \textbf{3:Ru-1}$$
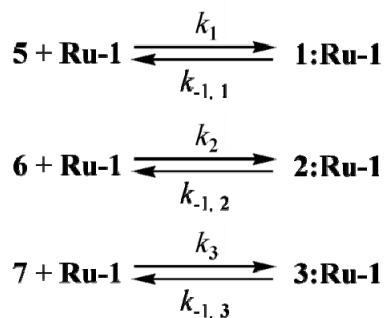
5, **6** and **7** represent dye-alkene, dye-alkyne, dye-allene substrates respectively. **5:Ru-1, 6**:**Ru-1** and **7:Ru-1** represent associated complexes formed by the substrate-catalyst association in the metathesis reactions. Same reaction schemes were used for **Mo-1**, **Mo-2**, **Ru-2**, **Ru-3** and **Ru-4**.

Fitting parameters

The fitting parameters are defined as follows:

Parameter	Definition
k_5	Binding rate constant between **5** and **Ru-1**
$k_{-1,5}$	Dissociation rate constant of **5:Ru-1**
k_6	Binding rate constant between **6** and **Ru-1**
$k_{-1,6}$	Dissociation rate constant of **6:Ru-1**
k_7	Binding rate constant between **7** and **Ru-1**
$k_{-1,7}$	Dissociation rate constant of **7:Ru-1**
A	PL intensity of **5, 6** or **7** per unit mole.
B	PL intensity of **5:Ru-1**, **6:Ru-1** or **7:Ru-1** per unit mole.
C	Background PL intensity.

Rate equations

We set up the rate equations as follows:

$$\frac{d[\mathbf{5}]}{dt} = -k_5[\mathbf{5}][\mathbf{Ru\text{-}1}] + k_{-1,5}[\mathbf{5{:}Ru\text{-}1}]$$

$$\frac{d[\mathbf{6}]}{dt} = -k_6[\mathbf{6}][\mathbf{Ru\text{-}1}] + k_{-1,6}[\mathbf{6{:}Ru\text{-}1}]$$

$$\frac{d[7]}{dt} = -k_7[7][\mathbf{Ru\text{-}1}] + k_{-1,7}[\mathbf{7\text{:}Ru\text{-}1}]$$

Although these equations have an analytical solution, numerical approximation has been employed for convenience. Δt is 0.01 minute.

$$\frac{\Delta[5]}{\Delta t} = -k_5[5][\mathbf{Ru\text{-}1}] + k_{-1,5}[\mathbf{5:Ru\text{-}1}]$$

$$\frac{\Delta[6]}{\Delta t} = -k_6[6][\mathbf{Ru\text{-}1}] + k_{-1,6}[\mathbf{6:Ru\text{-}1}]$$

$$\frac{\Delta[7]}{\Delta t} = -k_7[7][\mathbf{Ru\text{-}1}] + k_{-1,7}[\mathbf{7:Ru\text{-}1}]$$

Concentrations of each species at a certain time, $t + \Delta t$, are,

$$[5]_{t+\Delta t} = [5]_t + (-k_5[5]_t[\mathbf{Ru\text{-}1}]_t + k_{-1,5}[\mathbf{5:Ru\text{-}1}]_t)\Delta t$$

$$[6]_{t+\Delta t} = [6]_t + (-k_6[6]_t[\mathbf{Ru\text{-}1}]_t + k_{-1,6}[\mathbf{6:Ru\text{-}1}]_t)\Delta t$$

$$[7]_{t+\Delta t} = [7]_t + (-k_7[7]_t[\mathbf{Ru\text{-}1}]_t + k_{-1,7}[\mathbf{7:Ru\text{-}1}]_t)\Delta t$$

$$[\mathbf{5:Ru\text{-}1}]_{t+\Delta t} = [\mathbf{5:Ru\text{-}1}]_t + (k_5[5]_t[\mathbf{Ru\text{-}1}]_t - k_{-1,5}[\mathbf{5:Ru\text{-}1}]_t)\Delta t$$

$$[\mathbf{6:Ru\text{-}1}]_{t+\Delta t} = [\mathbf{6:Ru\text{-}1}]_t + (k_6[6]_t[\mathbf{Ru\text{-}1}]_t - k_{-1,6}[\mathbf{6:Ru\text{-}1}]_t)\Delta t$$

$$[\mathbf{7:Ru\text{-}1}]_{t+\Delta t} = [\mathbf{7:Ru\text{-}1}]_t + (k_7[7]_t[\mathbf{Ru\text{-}1}]_t - k_{-1,7}[\mathbf{7:Ru\text{-}1}]_t)\Delta t$$

$$[\mathbf{Ru\text{-}1}]_{t+\Delta t} = [\mathbf{Ru\text{-}1}]_t + (-k_5[5]_t[\mathbf{Ru\text{-}1}]_t + k_{-1,5}[\mathbf{5:Ru\text{-}1}]_t)\Delta t$$
$$+ (-k_6[6]_t[\mathbf{Ru\text{-}1}]_t + k_{-1,6}[\mathbf{6:Ru\text{-}1}]_t)\Delta t + (-k_7[7]_t[\mathbf{Ru\text{-}1}]_t + k_{-1,7}[\mathbf{7:Ru\text{-}1}]_t)\Delta t$$

Theoretical curves

Theoretical fluorescence intensity, I_{theory} has been calculated by the following expressions:

$$I_{theory,\,\mathbf{5}} = A[\mathbf{5}] + B[\mathbf{5}:\mathbf{Ru\text{-}1}] + C$$

$$I_{theory,\,\mathbf{6}} = A[\mathbf{6}] + B[\mathbf{6}:\mathbf{Ru\text{-}1}] + C$$

$$I_{theory,\,\mathbf{7}} = A[\mathbf{7}] + B[\mathbf{7}:\mathbf{Ru\text{-}1}] + C$$

The values of A, B and C of **5** are assumed to be the same as those of **6** and **7** because **5**, **6** and **7** have the same dye and other conditions such as amount of solvent or sample cell are exactly same.

A and C values

In order to reduce the number of parameters to be optimized together, the values of A and C were determined experimentally. If we measure fluorescence intensity at $t=0$, [**5**:**G-I**] should be zero and above equations are reduced to:

$$I_{theory,\,\mathbf{5},\,t\,=\,0} = A[\mathbf{5}]_{t\,=\,0} + C$$

By plotting [**5**]$_t$=0 vs. fluorescence intensity, A and C have been determined to be 9.32 and 1.19 respectively as shown in Figure S11.
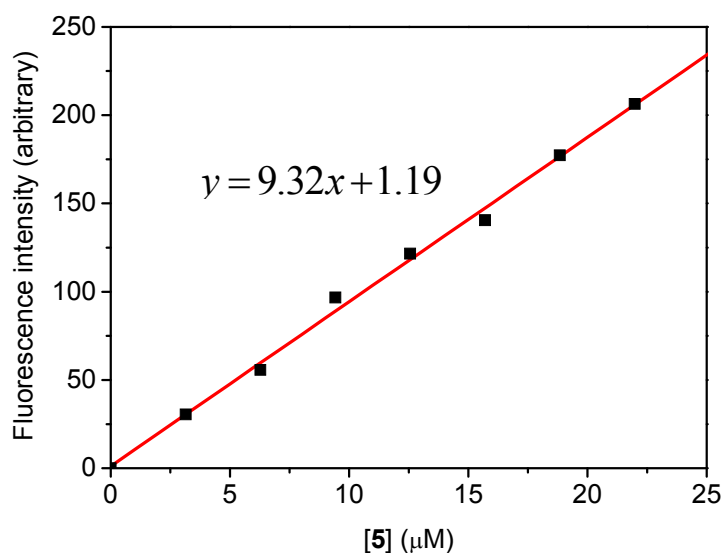
Figure S10. A plot of fluorescence intensity at $t=0$ versus [**5**]$_{t=0}$. Black squares are experimental points and the red line is a linear fit.

Definition of χ^2

The definition of the χ^2 is as follows:

$$\chi^2 = \sum_{i=\text{curve \#}} \chi_i^2$$

$$\chi_i^2 = \sum_j \left(I_{exp}(t_j, i) - I_{theory}(t_j, i) \right)^2$$

Least-squares fit

The least-squares fit of the model against the experimental data was done using the minimization package MINUIT written at CERN.[6] The quantity minimized is χ^2. The errors of the fitted parameters have been calculated by MINUIT and they represent one standard deviation. In total 7 parameters (6 rate constants and B value) have been optimized with 6 to 9 experimental curves. Since each experimental curve contains many data points, the data-to-parameter ration is sufficiently high enough.

Gibbs free energy change

The Gibbs free energy change was obtained using the following standard equations.

$$\Delta G = -RT \ln K$$
$$K = k / k_{-1}$$

5) References

1. CERN, Minuit; Function Minimization and Error Analysis,
http://wwwasdoc.web.cern.ch/wwwasdoc/minuit/minmain.html.

6) ^{1}H and ^{13}C NMR spectra of 1-4 and 7
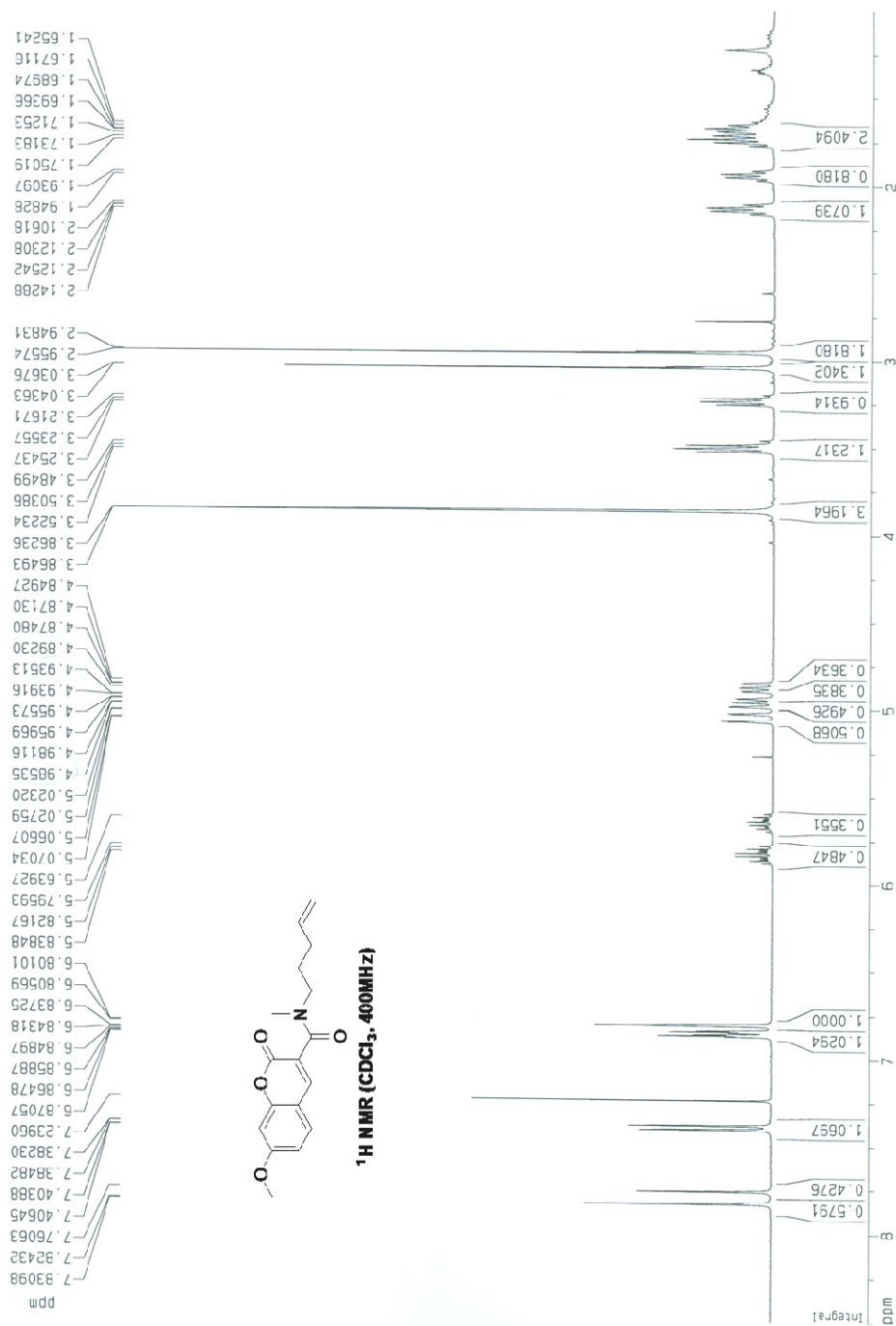
Figure S11. ^{1}H NMR spectrum of alkene **1**.

26.083
27.145
30.427
30.835
32.722
36.345

47.263
50.417

55.861

76.683
77.001
77.204
77.319

100.600
100.637

111.913
111.983
113.518
115.076
115.504
122.033
122.263
129.419
129.491

137.081
137.812
142.673
143.152

156.018
156.134
158.032
158.375
163.601
165.314
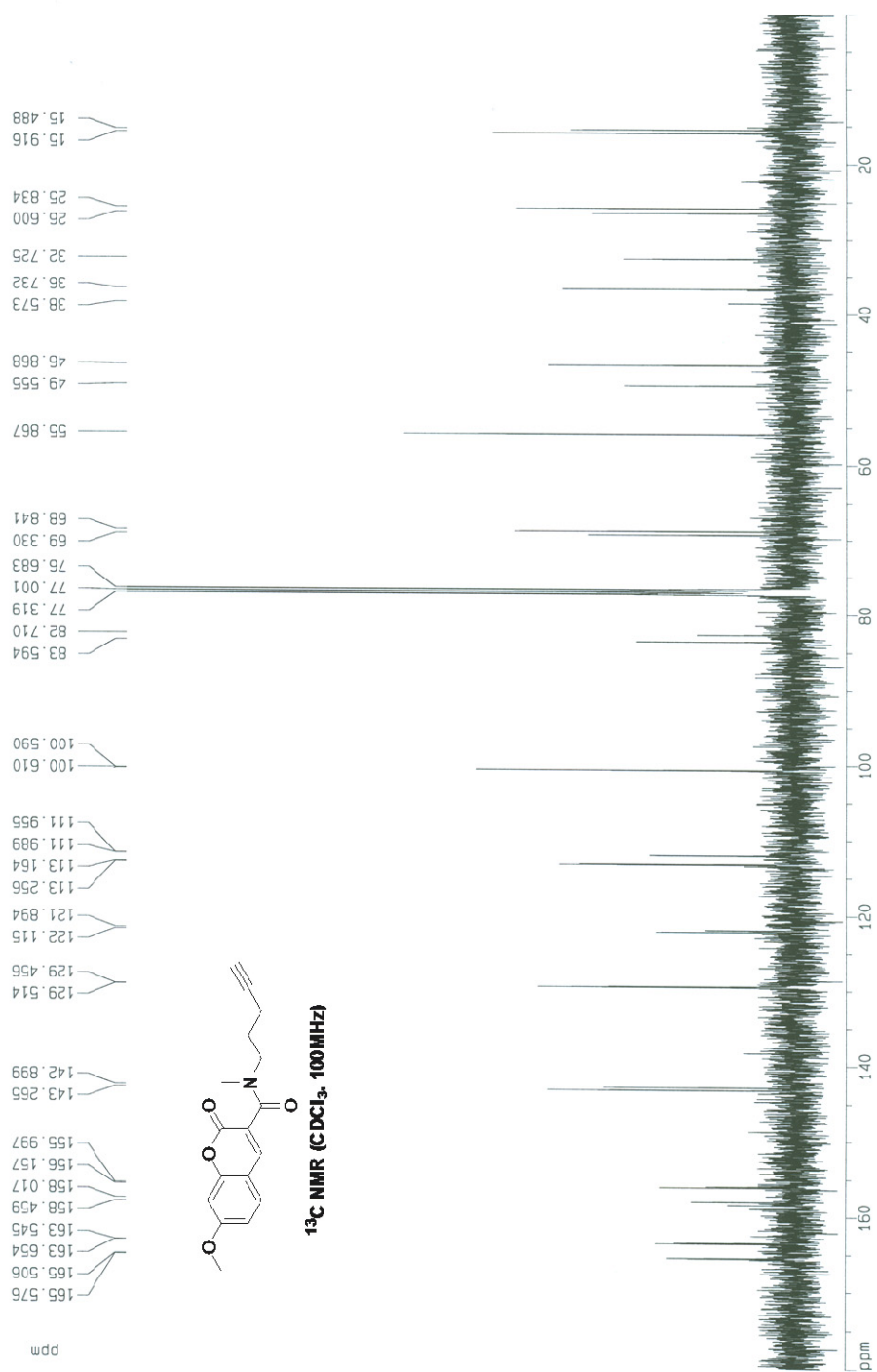165.374

ppm

^{13}C NMR (CDCl$_3$, 100MHz)
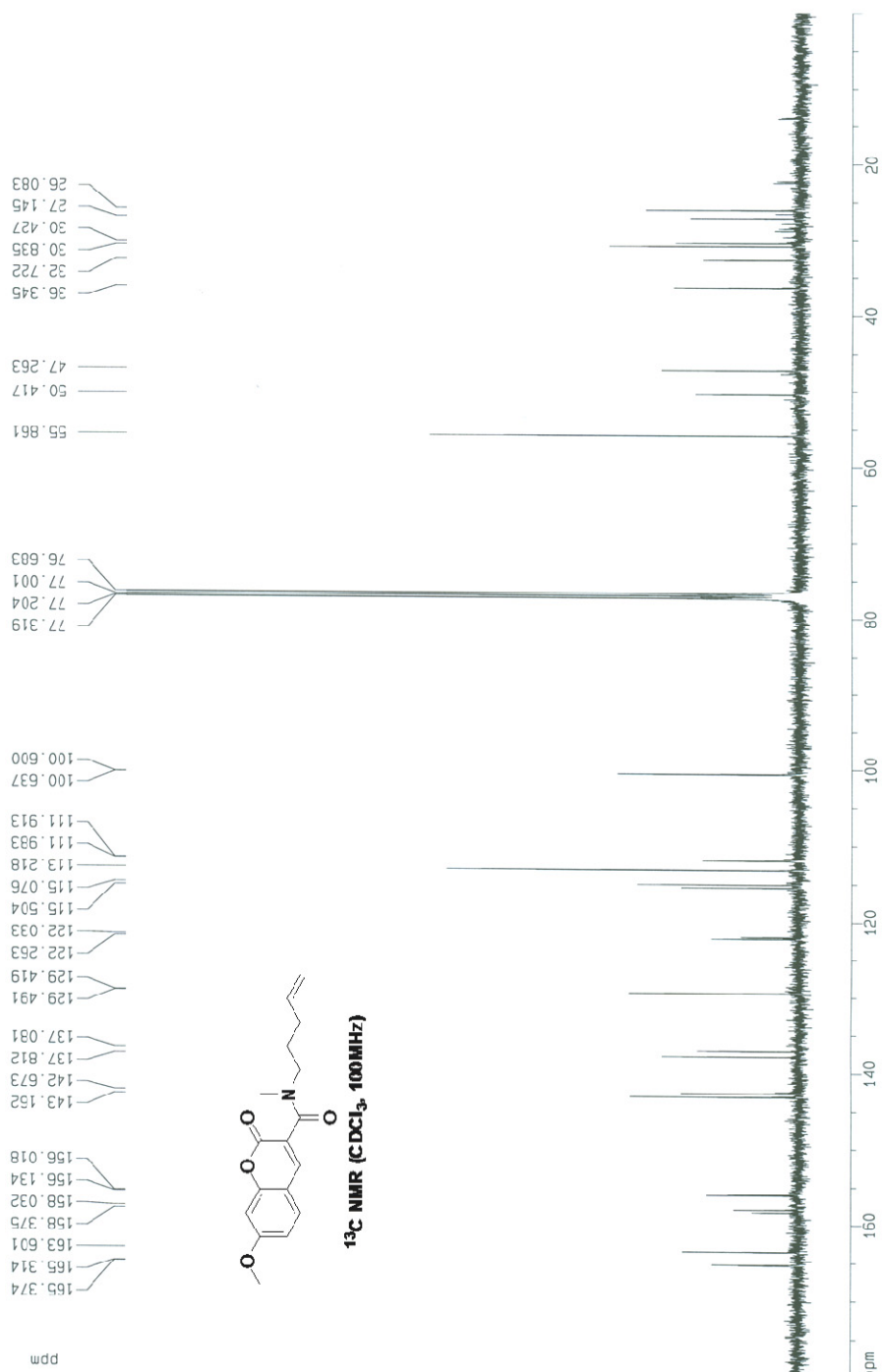
Figure S12. ^{13}C NMR spectrum of alkene **1**.
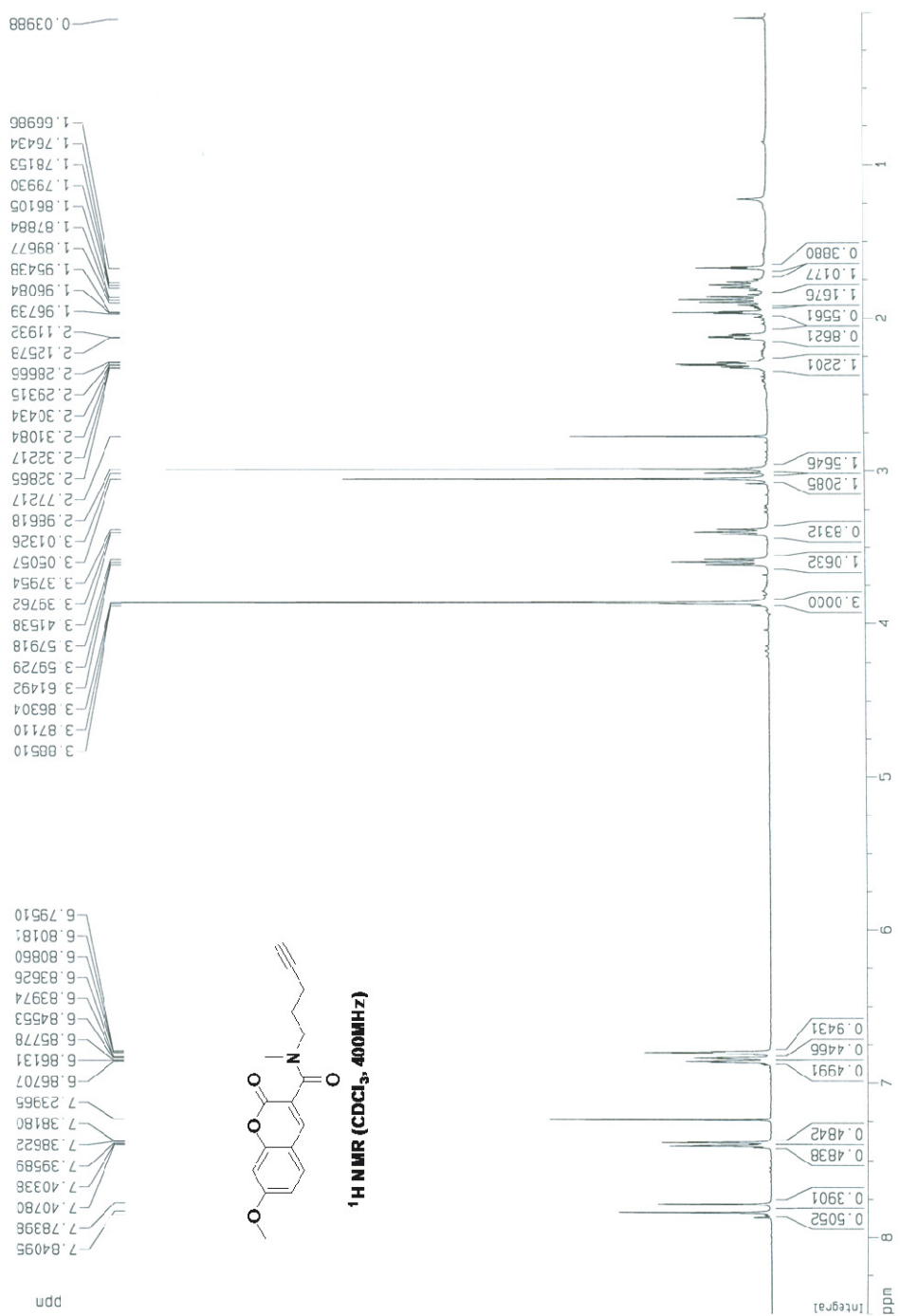
Figure S13. ¹H NMR spectrum of alkyne **2**.

The spectrum displays the following peaks (ppm):

15.488
15.916
25.834
26.600
32.725
36.732
38.573
46.868
49.555
55.867
68.841
69.330
76.683
77.001
77.319
82.710
83.594
100.590
100.610
111.955
111.989
113.164
113.256
121.894
122.115
129.456
129.514
142.899
143.255
155.997
156.157
158.017
158.459
163.545
163.654
165.506
165.576

¹³C NMR (CDCl₃, 100MHz)

Figure S14. ¹³C NMR spectrum of alkyne **2**.

S18

1.64158
1.68298
1.70129
1.71989
1.74578
1.76445
1.78275
1.87637
1.88493
1.89326
1.90612
2.06670
2.07521
2.08525
2.09408
2.10264
2.95993
3.04800
3.25210
3.28107
3.29948
3.51463
3.53330
3.55154
3.86378
4.50706
4.51557
4.52402
4.53230
4.54084
4.65810
4.66526
4.67472
4.68297
4.69134
4.96549
4.98191
5.12836
5.14495
5.16155
6.80274
6.80861
6.83825
6.84357
6.84888
6.85582
6.86519
6.87049
7.23961
7.38331
7.40485
7.76319
7.82985

ppm

2.7178
0.9688
1.2221

1.7879
1.3871

0.9392

1.2188

3.2098

0.8369
1.0836

0.4099
0.5309

1.0093
1.0452

1.0547

1.0000

ppm

¹H NMR (CDCl₃, 400MHz)

Figure S15. ¹H NMR spectrum of allene **3**.

S19
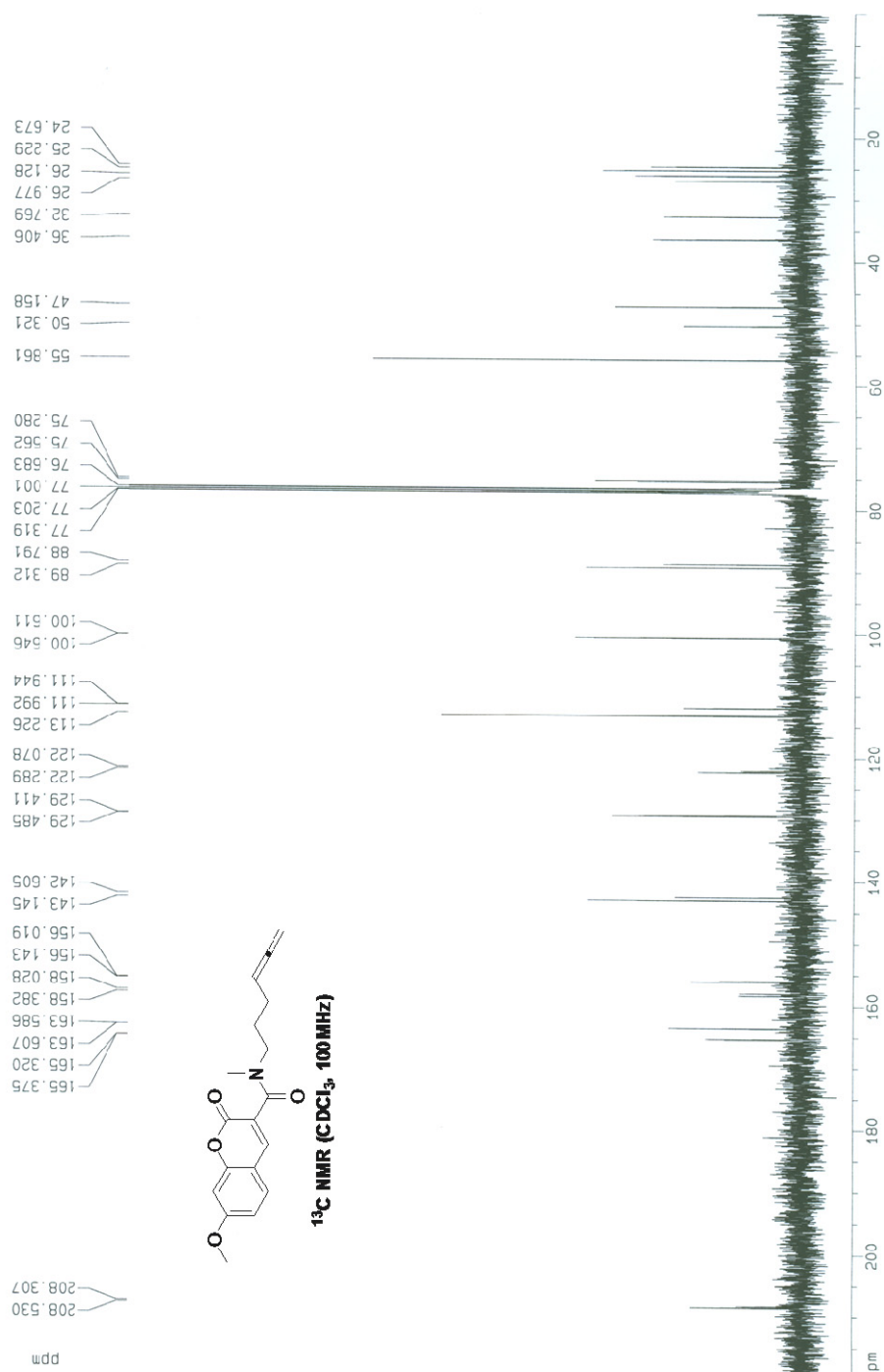
Figure S16. ^{13}C NMR spectrum of allene **3**.

^{13}C NMR (CDCl$_3$, 100 MHz)

24.673
26.229
26.828
26.977
32.769
36.406
47.158
50.321
55.861
75.280
75.562
76.683
77.001
77.203
77.319
88.791
89.312
100.511
100.546
111.944
111.992
113.226
122.078
122.289
129.411
129.486
142.605
143.145
156.019
156.143
158.028
158.382
163.586
163.607
165.320
165.375
208.307
208.530

ppm
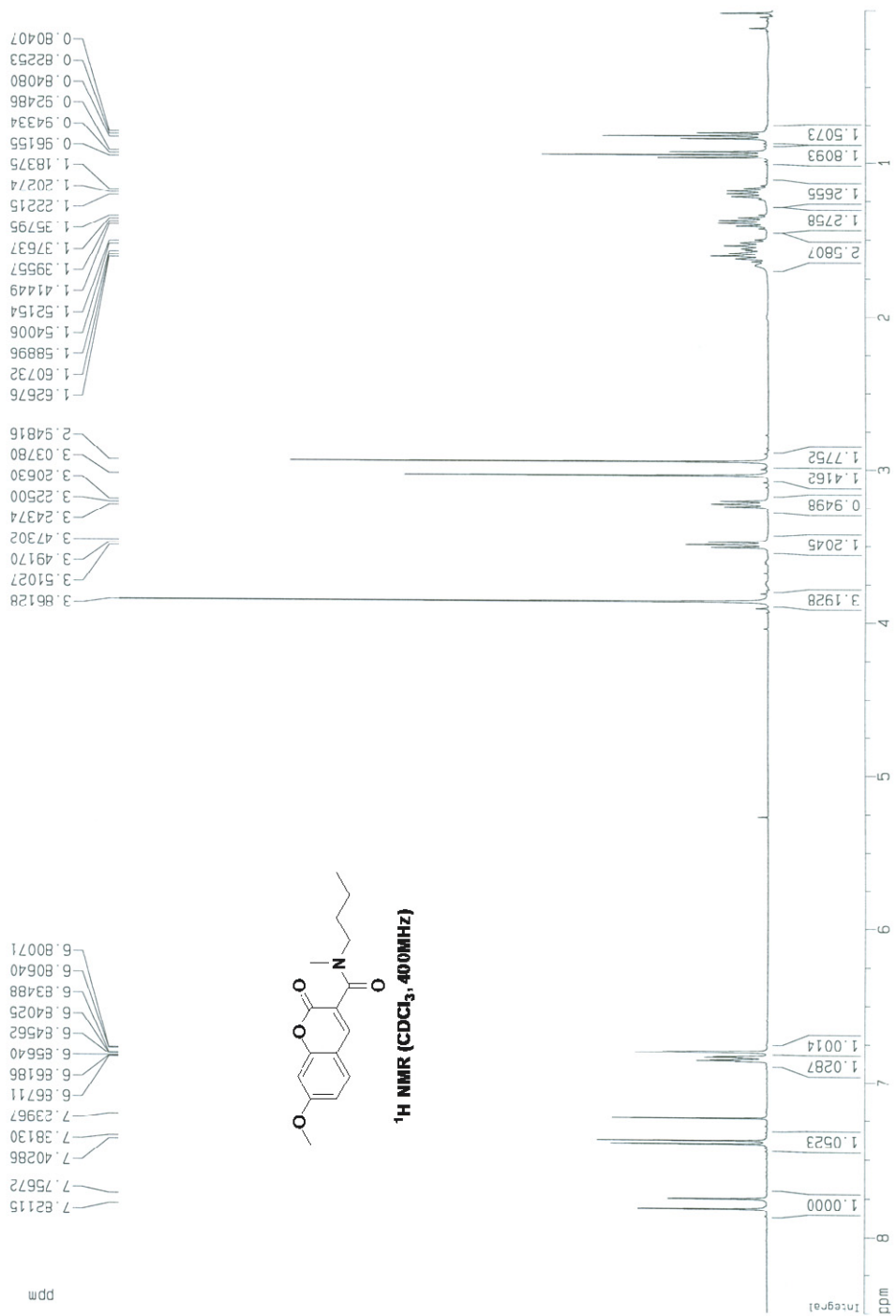
Figure S17. ¹H NMR spectrum of control **4**.
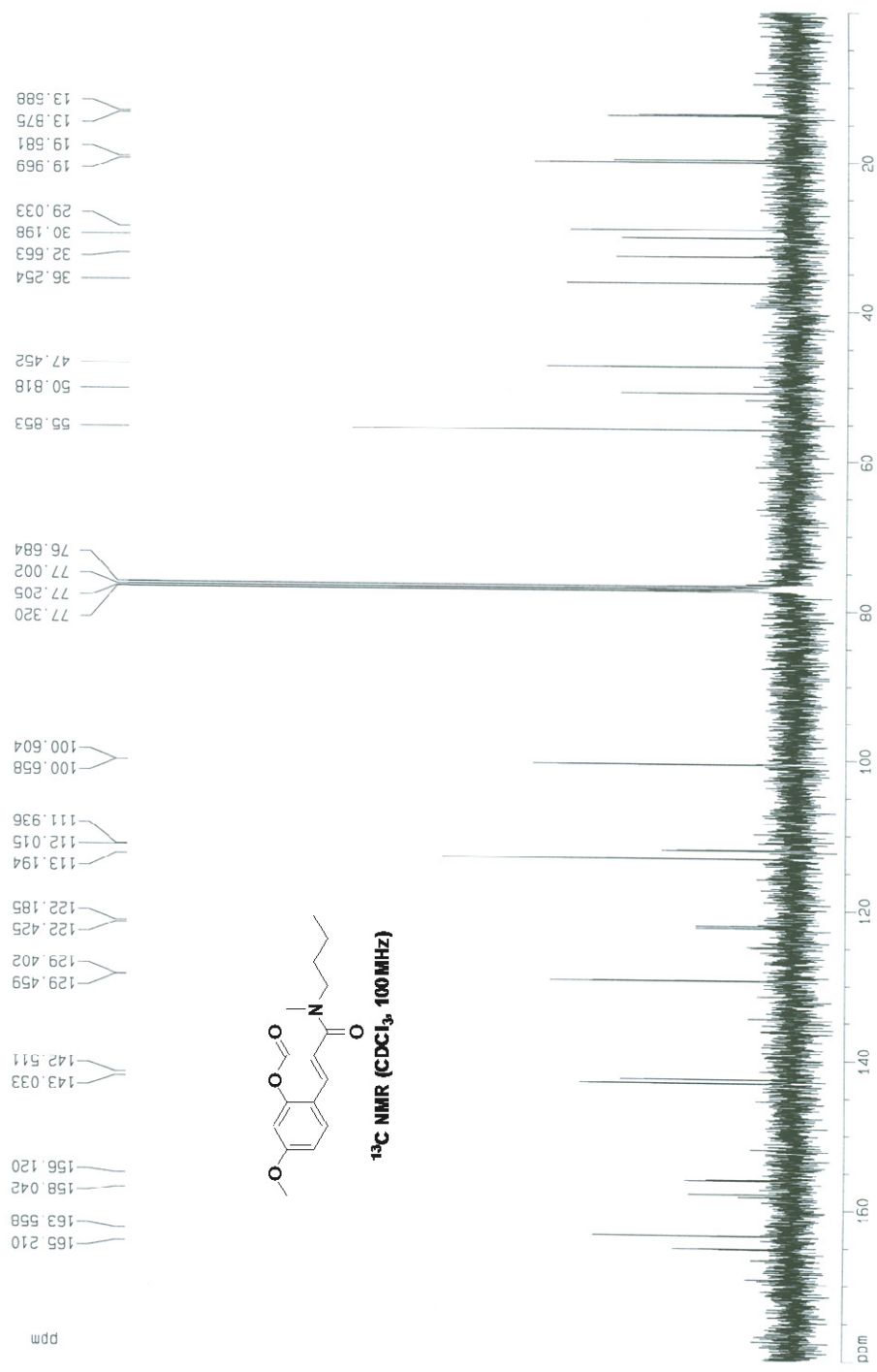
Figure S18. ^{13}C NMR spectrum of control **4**.
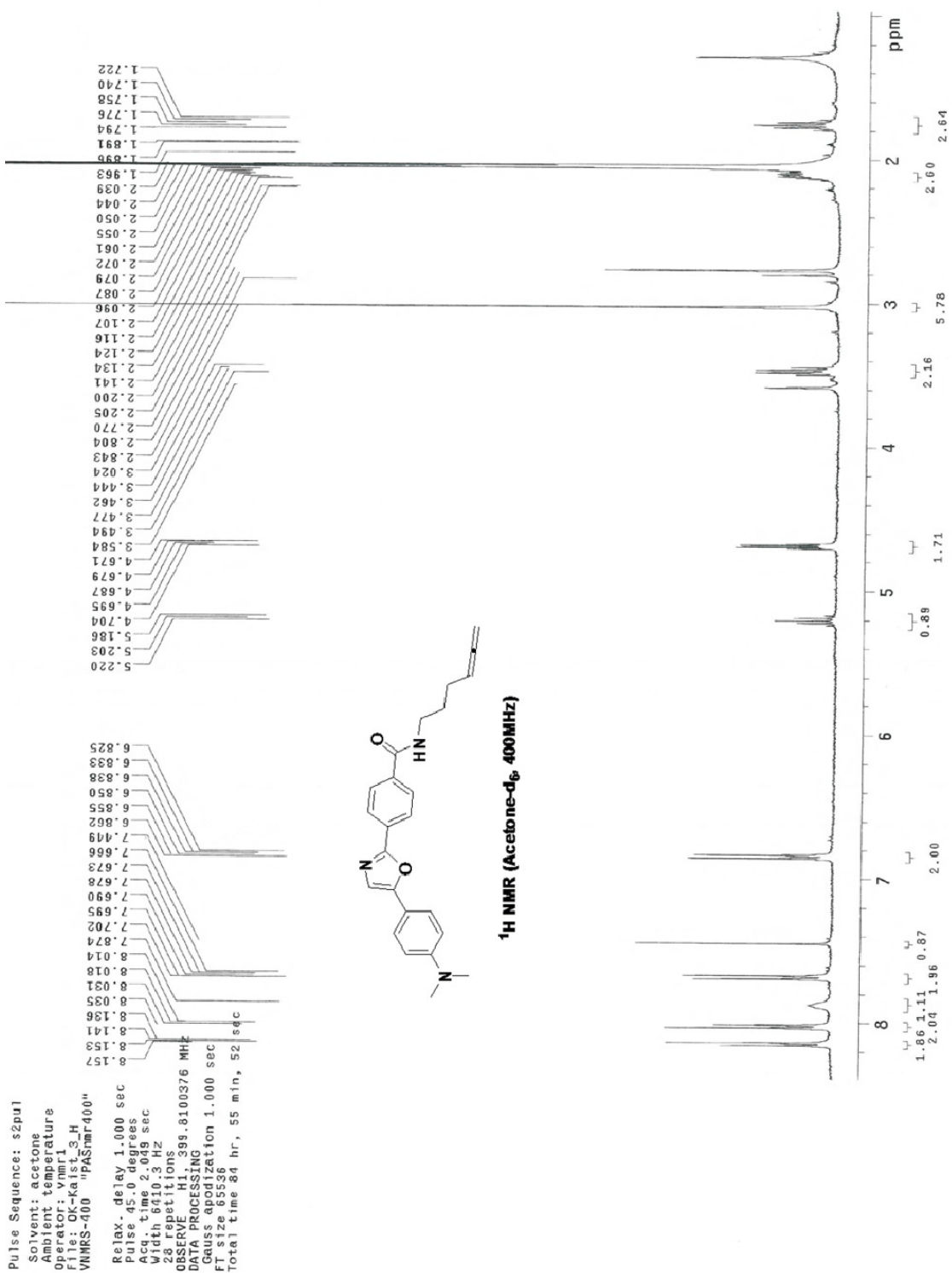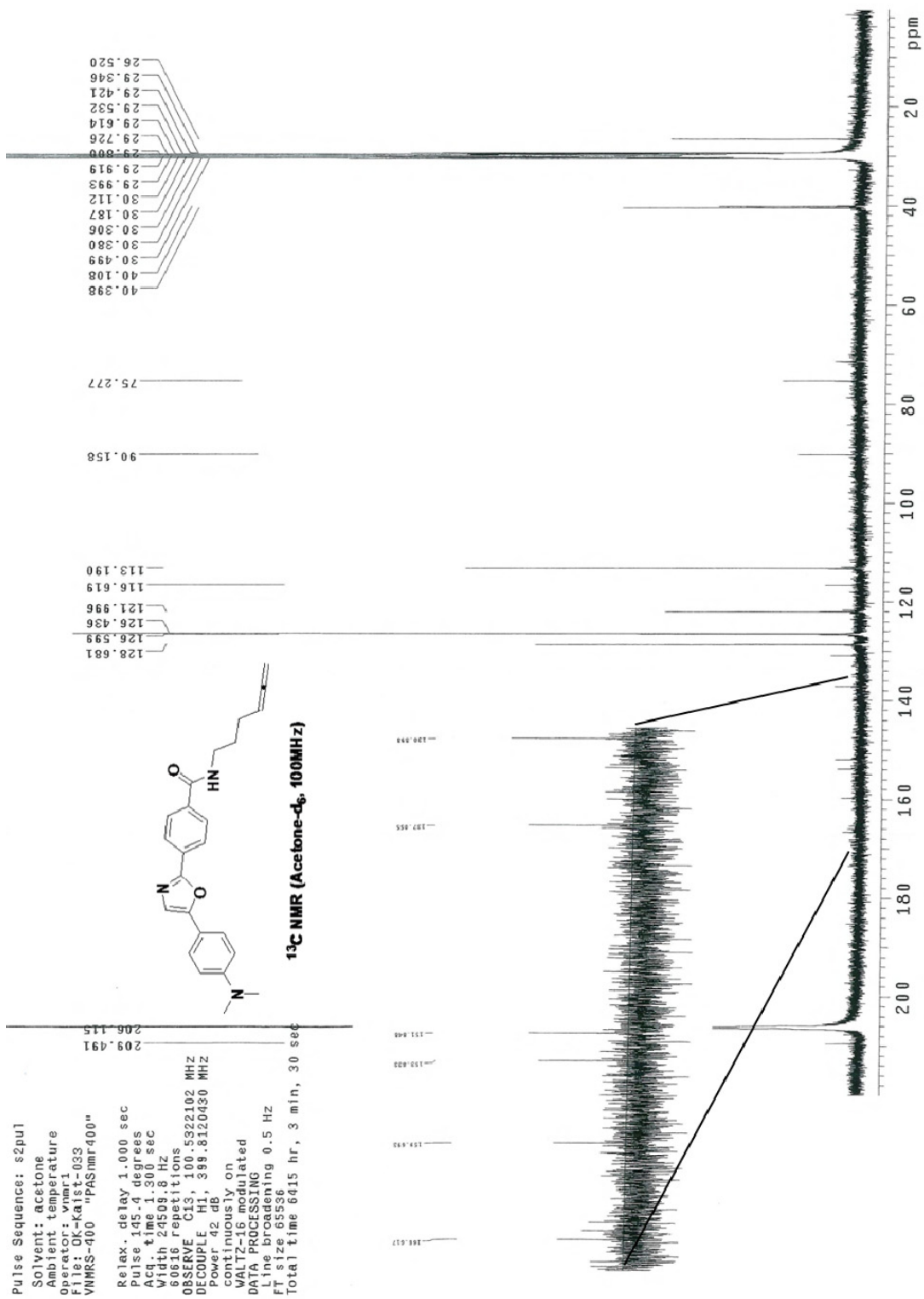
Figure S19. ^{1}H NMR spectrum of allene **7**.

Figure S20. ^{13}C NMR spectrum of allene **7**.