Supporting Information

The Initial Catalyst-Substrate Association Step in the Enyne Metathesis Catalyzed by Grubbs Ruthenium Complex Probed by Time-Dependent Fluorescence Quenching

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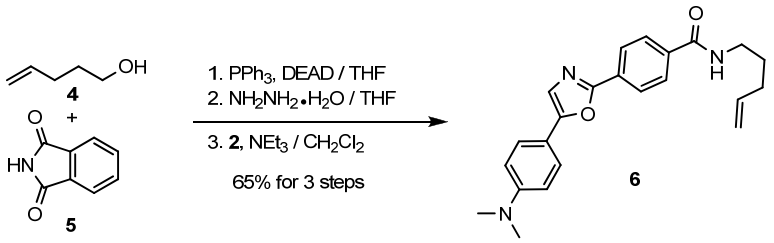
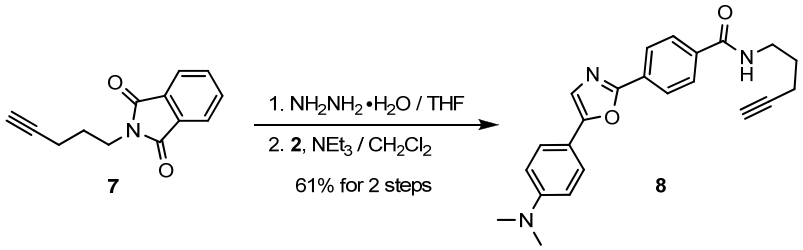
1) Experimental section

General Procedures—Common solvents were purified before use. Tetrahydrofuran (THF) and dichloromethane (CH_2Cl_2) were purified by distillation from sodium-benzophenone ketyl and calcium hydride respectively. All reagents were reagent grade and purified where necessary. 'water' refers to distilled water. Reactions were monitored by thin layer chromatography (TLC) using Whatman precoated silica gel plates. Flash column chromatography was performed over ultra pure silica gel (230-400 mesh) from Merck. ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian 400-MR spectrometer using residual solvent peaks as an internal standard ($CHCl_3$: δ 7.24 ppm for proton and δ 77.0 ppm for carbon, acetone: δ 2.05 ppm for proton and δ 29.9 ppm for carbon, CH_3OH: δ 3.30 ppm for proton and δ 49.0 ppm for carbon). Multiplicities for ^{1}H NMR are designated as: s = singlet, d = doublet, dd = doublet of doublets, ddd = doublet of dd, dt = doulblet of triplets, dq = doublet of quartets, td = triplet of doublets, t = triplet, q = quartet, quint = quintet, sext =sextet, m = multiplet, br = broad. Infrared spectra (IR) were recorded on Varian 3100 FT-IR spectrometer and are reported in reciprocal centimeter (cm^{-1}). Mass Spectra were obtained from the Korea Basic Science Institute (Daegu) by using FAB or EI method.

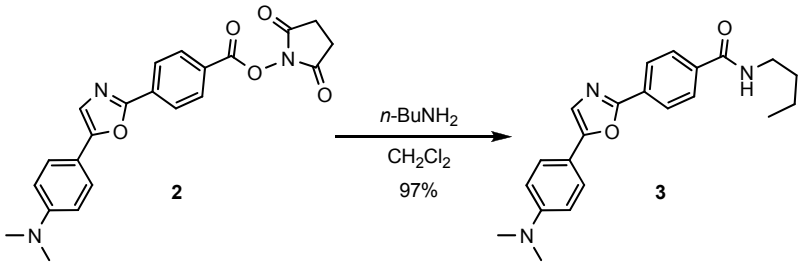


Synthesis of dye-ene 6. To a mixture of alcohol **4** (1.00 mL, 9.82 mmol), isoindolinedione **5** (1.445 g, 9.82 mmol), and PPh_3 (2.834 g, 10.81 mmol) in THF (15 mL) was added dropwise DEAD (40% in toluene, 4.91 mL, 11.79 mmol) at 0 °C, and the resulting mixture was allowed to warm to room temperature and stir overnight. After addition of sat. $NaHCO_3$ (20 mL), the mixture was extracted with EtOAc (25 mL x 3). The combined organic layer was washed with brine (15 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography to afford the desired 2-(pent-4-enyl)isoindoline-1,3-dione[1] (1.796 g, 85%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (dd, *J* = 3..0 Hz, 2H), 7.67 (dd, *J* = 2.9 Hz, 2H), 5.78 (m, 1H), 5.02 (dq, *J* = 1.6, 17.1 Hz, 1H), 4.94 (ddd, *J* = 1.2, 3.0, 10.1 Hz, 1H), 3.66 (t, *J* = 7.3 Hz, 2H), 2.08 (m, 2H), 1.75 (quint, *J* = 7.3 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.4, 137.3, 133.8, 132.1, 123.1, 115.2, 37.5, 30.9, 27.6. A mixture of 2-(pent-4-enyl)isoindoline-1,3-dione (1.795 g, 8.339 mmol) and H_2NNH_2•H_2O (80% in H_2O, 2.50 mL) in THF (10 mL) was allowed to stir overnight at 40 °C and for 6 h at room temperature. The reaction mixture was filtered and rinsed with EtOAc (50 mL). The filtrate was washed with 1 N NaOH (10 mL) and brine (10 mL), dried over $MgSO_4$, filtered, acidified with 2 M HCl in Et_2O (5 mL), and concentrated under reduced pressure. The solid crude product was washed with EtOAc to give 4-pentenyl-1-amine hydrochloride as colorless solid[2] (770 mg, 76%): ^{1}H NMR (400 MHz, CD_3OD) δ 5.83 (m, 1H), 5.10 (dq, *J* = 1.6, 17.2 Hz, 1H), 5.04 (dd, *J* = 1.5, 10.2 Hz, 1H), 2.93 (t, *J* = 7.6 Hz, 2H), 2.17 (q, *J* = 7.0 Hz, 2H), 1.76 (quint, *J* = 7.7 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 138.1, 116.5, 40.4, 31.6, 27.8. To a mixture of the 4-pentenyl-1-amine hydrochloride (1.1 mg, 9.05 μmol) and activated dye **2** (2.5 mg, 6.17 μmol) in CH_2Cl_2 (0.2 mL) was added Et_3N (3.8 μL,

27.3 μmol), and the resulting mixture was stirred for 20 min at 0 °C. The mixture was diluted with EtOAc (3 mL), washed with sat. NH_4Cl (0.5 mL) and brine (0.5 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by prep. TLC to give dye-ene **6** (2.2 mg, 95%) as pale green solid: ^{1}H NMR (400 MHz, acetone-d_6) δ 8.15 (d, J = 8.8 Hz, 2H), 8.02 (d, J = 8.8 Hz, 2H), 7.89 (br s, 1H), 7.68 (d, J = 9.0 Hz, 2H), 7.45 (s, 1H), 6.84 (d, J = 8.8 Hz, 2H), 5.88 (m, 1H), 5.06 (dq, J = 1.6, 17.2 Hz, 1H), 4.96 (m, 1H), 3.44 (m, 2H), 3.02 (s, 6H), 2.16 (m, 2H), 1.73 (quint, J = 7.2 Hz, 2H); ^{13}C NMR (100 MHz, acetone-d_6) δ 166.7, 159.7, 153.8, 151.8, 139.3, 137.0, 130.9, 128.72, 128.71, 126.4, 122.0, 116.6, 115.2, 113.2, 40.6, 40.4, 40.2, 32.0; IR (film) cm^{-1} 3333, 2920, 2853, 1730, 1630, 1541, 1513, 1453, 1358, 1285, 1225, 1196, 1147, 854, 811; HRMS calculated for $C_{23}H_{25}N_3O_2$ (M^+) 375.1947, found 375.1949.



Synthesis of dye-yne 8. A mixture of compound **7** (1.080 g, 5.065 mmol) and H_2NNH_2•H_2O (80% in H_2O, 1.5 mL) in THF (6 mL) was allowed to stir overnight at 40 °C and for 5 h at room temperature. The reaction mixture was filtered and rinsed with Et_2O (40 mL). The filtrate was washed with 1 N NaOH (8 mL) and brine (8 mL), dried over $MgSO_4$, filtered, acidified with 2 M HCl in Et_2O (5 mL), and concentrated under reduced pressure. The solid crude product was washed with EtOAc and CH_2Cl_2 to give 4-pentynyl-1-amine hydrochloride[3] as colorless solid (370 mg, 61%): ^{1}H NMR (400 MHz, CD_3OD) δ 3.05 (t, J = 7.6 Hz, 2H), 2.36 (m, 3H), 1.87 (quint, J = 7.4 Hz, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 83.0, 71.3, 39.9, 27.6, 16.4. To a mixture of the 4-pentynyl-1-amine (1.1 mg, 9.05 μmol) and activated dye **2** (2.5 mg, 6.17 μmol) in CH_2Cl_2 (0.2 mL) was added Et_3N (3.8 μL, 27.3 μmol), and the resulting mixture was stirred for 20 min at 0 °C. The mixture was diluted with EtOAc (3 mL), washed with sat. NH_4Cl (0.5 mL) and brine (0.5 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by prep. TLC to give dye-yne **8** (2.2 mg, 95%) as pale green solid: ^{1}H NMR (400 MHz, acetone-d_6) δ 8.14 (d, J = 8.8 Hz, 2H), 8.03 (d, J = 8.8 Hz, 2H), 7.93 (br s, 1H), 7.67 (d, J = 9.0 Hz, 2H), 7.45 (s, 1H), 6.83 (d, J = 8.8 Hz, 2H), 3.51 (q, J = 6.6 Hz, 2H), 3.01 (s, 6H), 2.37 (t, J = 2.6 Hz, 1H), 2.29 (td, J = 2.8, 7.2 Hz, 2H), 1.85 (quint, J = 7.0 Hz, 2H); ^{13}C NMR (100 MHz, acetone-d_6) δ 166.8, 159.7, 153.9, 151.8, 136.9, 130.9, 128.74, 128.73, 126.4, 122.0, 116.6, 113.2, 84.6, 70.3, 40.4, 39.8, 29.5,16.6; IR (film) cm^{-1} 3296, 2919, 2852, 2357, 1729, 1633, 1512, 1449, 1361, 1301, 1193, 1149, 1062, 947, 854, 811; HRMS calculated for $C_{23}H_{23}N_3O_2$ (M^+) 373.1790, found 373.1789.



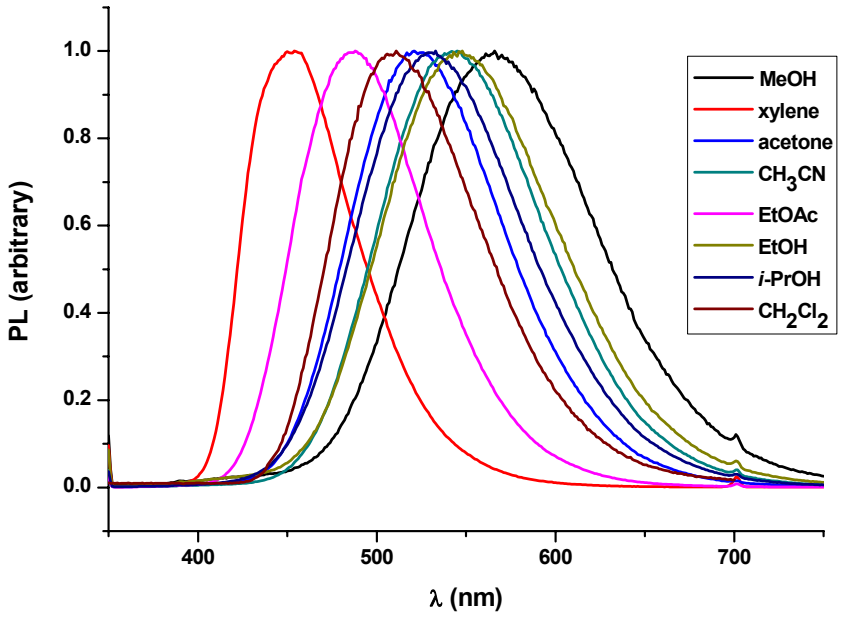
Synthesis of control 3. To a solution of activated dye **2** (2.3 mg, 5.67 μmol) in CH_2Cl_2 (0.2 mL) was added *n*-$BuNH_2$ (3 μL, 30 μmol), and the resulting mixture was stirred for 20 min at 0 °C. The mixture was diluted with EtOAc (3 mL), washed with sat. NH_4Cl (0.5 mL) and brine (0.5 mL), dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by prep. TLC to give dye-ane **3** (2.0 mg, 97%) as pale green solid: ^{1}H NMR (400 MHz, acetone-d_6) δ 8.14 (d, *J* = 8.6 Hz, 2H), 8.02 (d, *J* = 8.6 Hz, 2H), 7.84 (br s, 1H), 7.68 (d, *J* = 8.8 Hz, 2H), 7.45 (s, 1H), 6.84 (d, *J* = 8.8 Hz, 2H), 3.42 (q, *J* = 6.7 Hz, 2H), 3.02 (s, 6H), 1.61 (quint, *J* = 7.2 Hz, 2H), 1.41 (sext, *J* = 7.4 Hz, 2H), 0.94 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, acetone-d_6) δ 166.6, 159.7, 153.8, 151.9, 137.1, 130.9, 128.71, 128.70, 126.4, 122.0, 116.6, 113.2, 40.4, 40.3, 32.6, 20.9, 14.2; IR (film) cm^{-1} 3347, 2920, 2853, 1629, 1537, 1464, 1357, 1301, 1223, 1059, 948, 855, 810; HRMS calculated for $C_{22}H_{25}N_3O_2$ (M^+) 363.1947, found 363.1947.

General procedure for the measurement of PL.

Photoluminacence (PL) was measured by a Fluorolog®-3 spectrofluorometer (HORIBA, Jobin Yvon, UK) with excitation at 410 nm at an integration time of 0.2 sec and an excitation and emission slit width of 2 nm. A solution of a substrate in CH_2Cl_2 (3 mL) in 10 by 10 mm quartz cuvette was put in the fluorometer and fluorescence spectrum at time zero was acquired. To the solution was added a Ru catalyst solution in CH_2Cl_2 (10.3 mM solution) using 25 μL syringe and fluorescence spectra were obtained over time. All spectra were normalized by the maximum height for 18 μM of dye-ene **6** at time zero and the area of PL as the fluorescence intensity was calculated between 425 and 650 nm of wavelength using Origin software.

2) PL spectra of 3 in varying solvents

The fluorescence emission wavelength was varied with solvents.



solvent	emission (nm)	dielectic constant[4]	solvent	emission (nm)	dielectic constant[4]
xylene	454	2.4 (20 °C)	*i*-PrOH	530	18.3 (20 °C)
EtOAc	488	6.0 (25 °C)	CH_3CN	545	37.5 (21 °C)
CH_2Cl_2	508	9.1 (20 °C)	EtOH	548	24.3 (25 °C)
acetone	521	20.7 (25 °C)	MeOH	566	32.6 (25 °C)

- Ru complexes have been applied as FRET donor or acceptor in bioanalysis such as measurement of the rotational dynamics of proteins,[5a] immunoassays for human serum albumin[5b] and CO_2 detection.[5c]

3) FRET data at 20 °C with substrate concentration of 18 μM

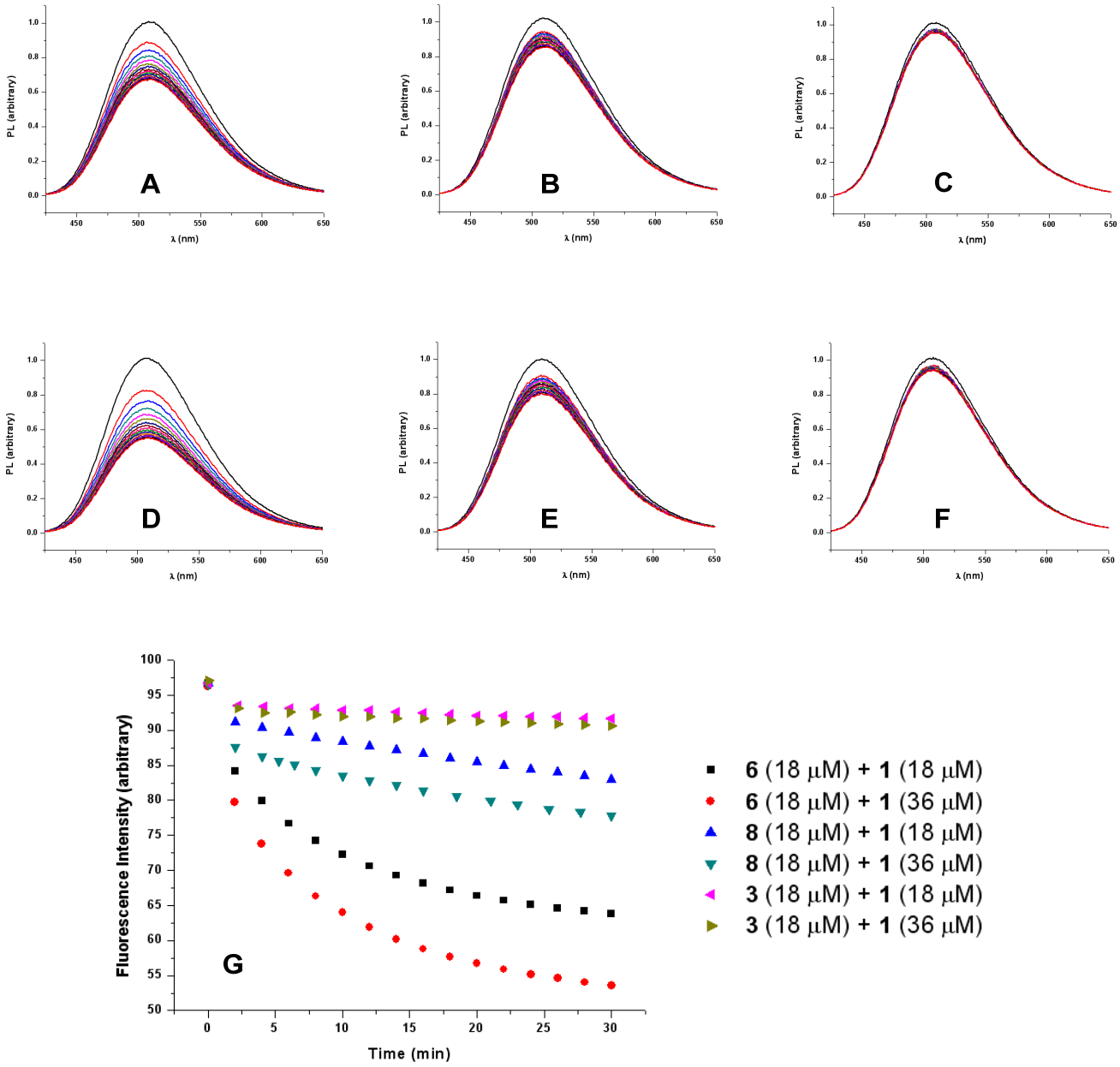
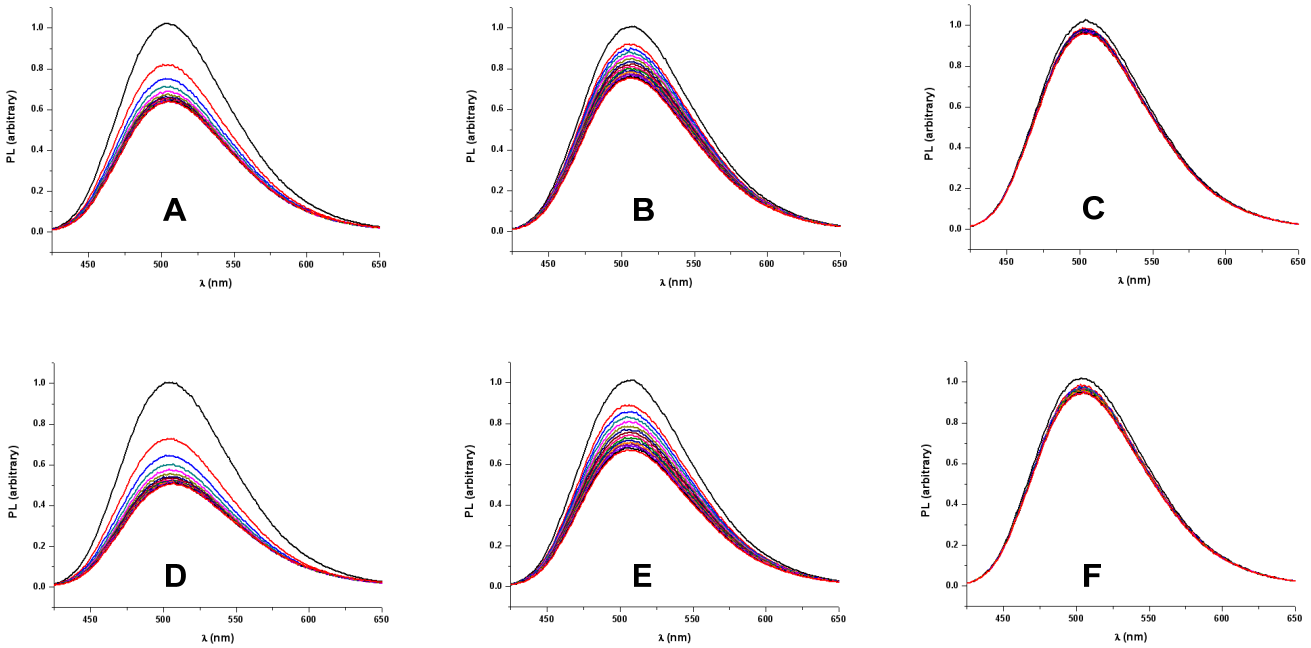


Figure S1. FRET data at 20 °C with substrate concentration of 18 μM. (**A**) **6** (18 μM) + **1** (18 μM), (**B**) **8** (18 μM) + **1** (18 μM), (**C**) **3** (18 μM) + **1** (18 μM), (**D**) **6** (18 μM) + **1** (36 μM), (**E**) **8** (18 μM) + **1** (36 μM), (**F**) **3** (18 μM) + **1** (36 μM), (**G**) Plot of fluorescence intensity *vs* time.

4) FRET data at 34 °C with substrate concentration of 18 μM



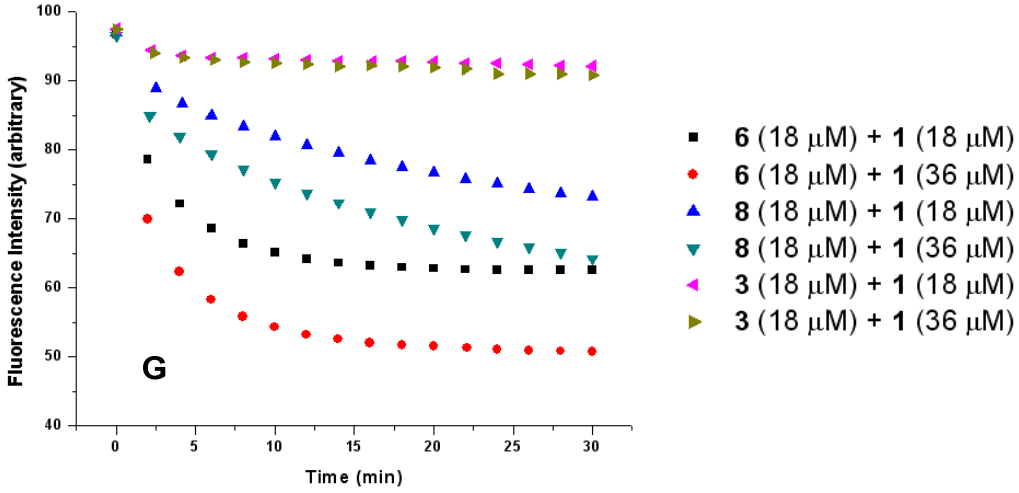


Figure S2. FRET data at 34 °C with substrate concentration of 18 μM. (**A**) **6** (18 μM) + **1** (18 μM), (**B**) **8** (18 μM) + **1** (18 μM), (**C**) **3** (18 μM) + **1** (18 μM), (**D**) **6** (18 μM) + **1** (36 μM), (**E**) **8** (18 μM) + **1** (36 μM), (**F**) **3** (18 μM) + **1** (36 μM), (**G**) Plot of fluorescence intensity *vs* time.

5) FRET data at 10 °C with substrate concentration of 18 µM

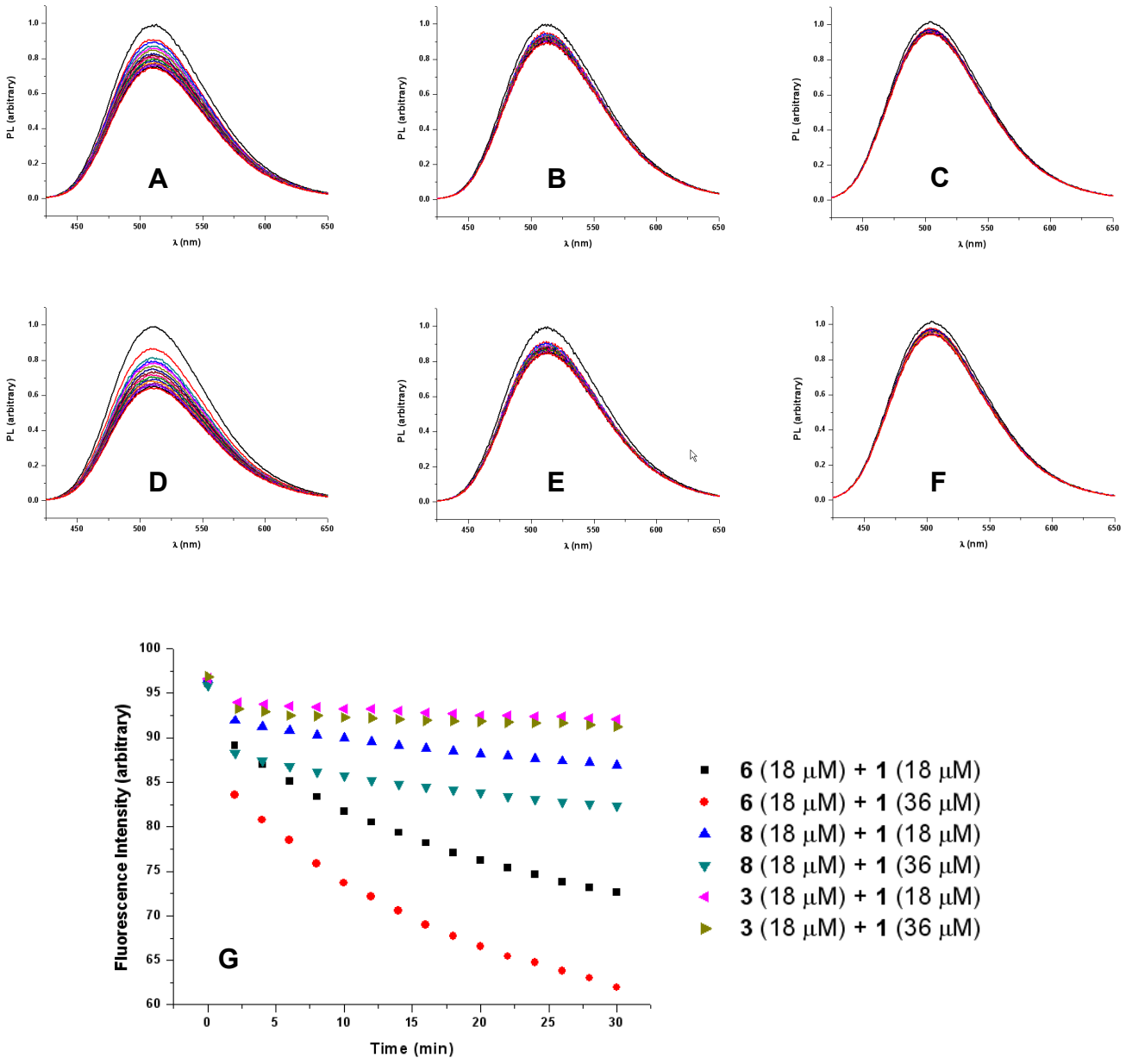


Figure S3. FRET data at 10 °C with substrate concentration of 18 µM. (**A**) **6** (18 µM) + **1** (18 µM), (**B**) **8** (18 µM) + **1** (18 µM), (**C**) **3** (18 µM) + **1** (18 µM), (**D**) **6** (18 µM) + **1** (36 µM), (**E**) **8** (18 µM) + **1** (36 µM), (**F**) **3** (18 µM) + **1** (36 µM), (**G**) Plot of fluorescence intensity *vs* time.

6) FRET data at 20 °C with other substrate concentrations

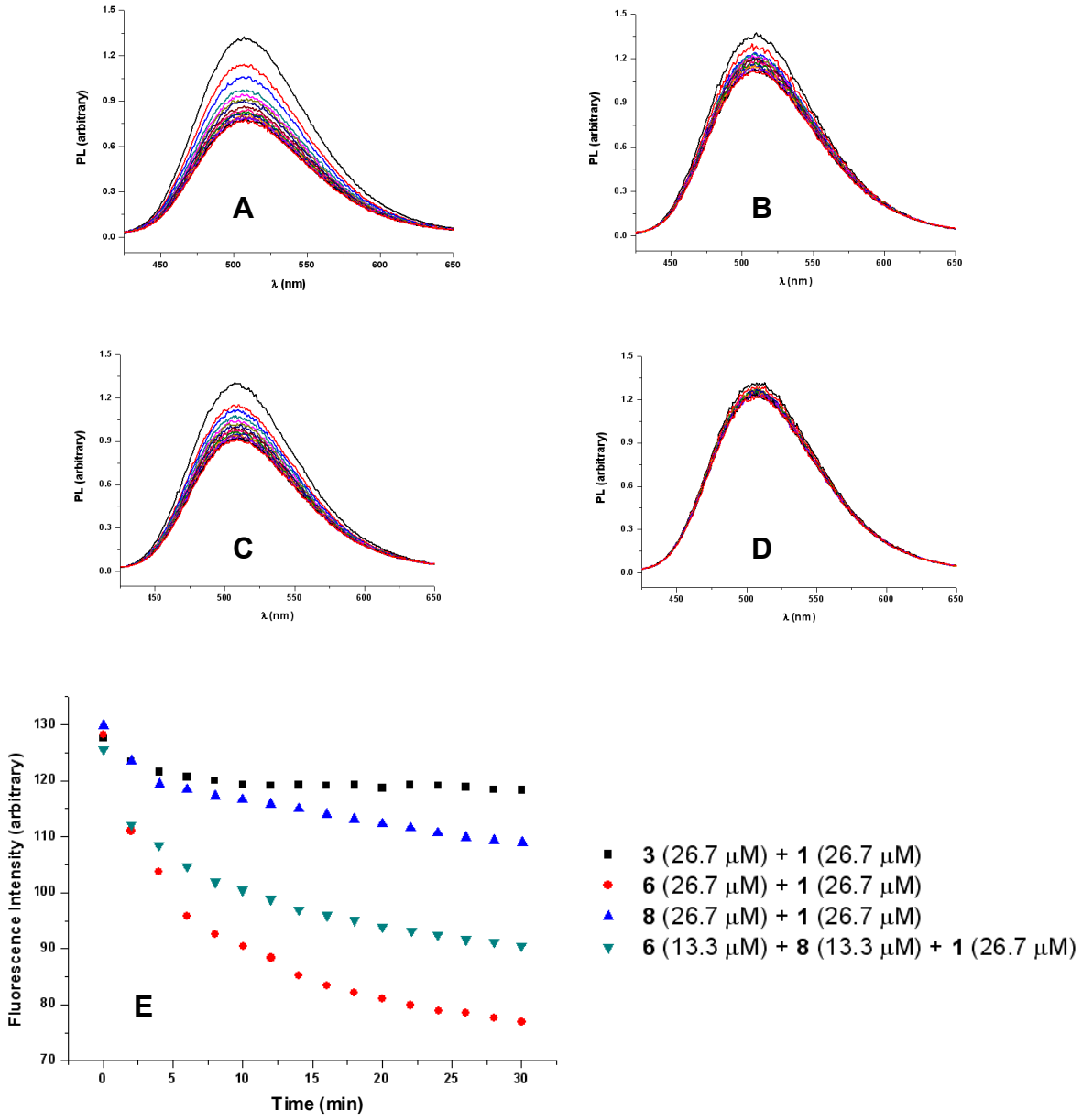


Figure S4. FRET data at 20 °C with other substrate concentrations. (**A**) **6** (26.7 μM) + **1** (26.7 μM), (**B**) **8** (26.7 μM) + **1** (26.7 μM), (**C**) **6** (13.3 μM) + **8** (13.3 μM) + **1** (26.7 μM), (**D**) **3** (26.7 μM) + **1** (26.7 μM), (**E**) Plot of fluorescence intensity *vs* time.

7) The reaction conditions for 14 quenching traces

Index	Condition
a	**8** (26.7 μM) + **1** (26.7 μM), 20 ^{o}C
b	**6** (13.3 μM) + **8** (13.3 μM) + **1** (26.7 μM), 20 ^{o}C
c	**6** (26.7 μM) + **1** (26.7 μM), 20 ^{o}C
d	**8** (18 μM) + **1** (18 μM), 10 ^{o}C
e	**8** (18 μM) + **1** (18 μM), 20 ^{o}C
f	**8** (18 μM) + **1** (36 μM), 10 ^{o}C
g	**8** (18 μM) + **1** (36 μM), 20 ^{o}C
h	**6** (18 μM) + **1** (18 μM), 10 ^{o}C
i	**8** (18 μM) + **1** (18 μM), 34 ^{o}C
j	**6** (18 μM) + **1** (18 μM), 20 ^{o}C
k	**6** (18 μM) + **1** (36 μM), 10 ^{o}C
l	**8** (18 μM) + **1** (36 μM), 34 ^{o}C
m	**6** (18 μM) + **1** (18 μM), 34 ^{o}C
n	**6** (18 μM) + **1** (36 μM), 20 ^{o}C
o	**6** (18 μM) + **1** (36 μM), 34 ^{o}C

8) Global fitting analysis of FRET data

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

Control subtraction

In order to get PL intensity change purely from fruorecence quenching caused by the substrate-catalyst association step, the raw PL data were subtracted by the data from a control experiment. An example is displayed in Figure S5:

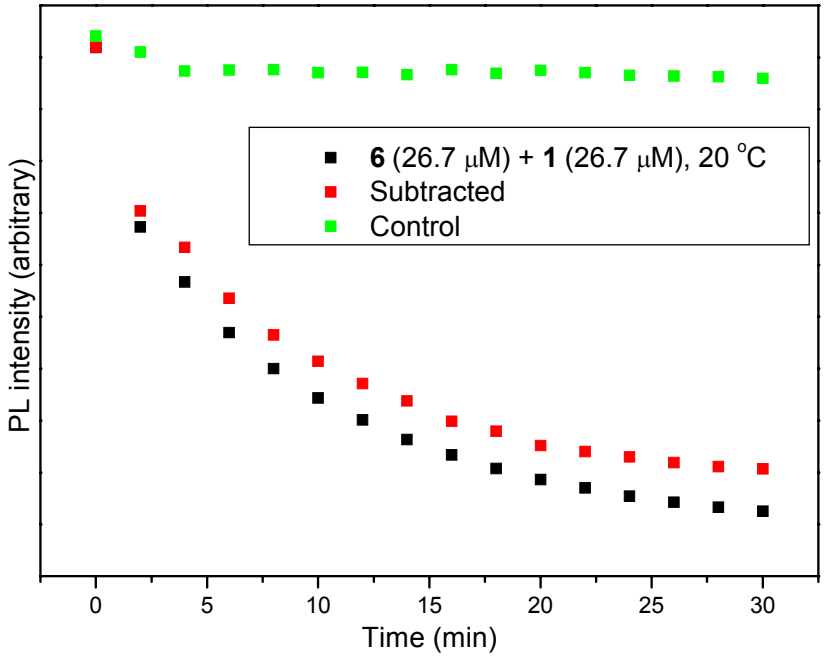


Figure S5. The green squares are from a control experiment with the control dye-ane substrate (**3**) and correspond to the fluorescence quenching due to non-specific binding, not from the specific substrate-catalyst association reaction. The black squares are raw data with the dye-ene substrate **6**. 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 has the following reaction schemes.

$$\mathbf{6} + \mathbf{1} \underset{k_{-1,\,\mathbf{6}}}{\overset{k_{\mathbf{6}}}{\rightleftharpoons}} \mathbf{6{:}1}$$

$$\mathbf{8} + \mathbf{1} \underset{k_{-1,\mathbf{8}}}{\overset{k_{\mathbf{8}}}{\rightleftharpoons}} \mathbf{8{:}1}$$

1, **6** and **8** represent dye-ane, dye-ene, and dye-yne substrates respectively. **6**:**1** and **8**:**1** represent associated complexes formed by the substrate-catalyste association step of the enyne metathesis.

Fitting parameters

The fitting parameters are as follows:

Parameter	Definition
$k_{\mathbf{6}}$	Binding rate constant between **6** and **1**.
$k_{-1,\,\mathbf{6}}$	Dissociation rate constant of **6:1**.
$k_{\mathbf{8}}$	Binding rate constant between **8** and **1**.
$k_{-1,\,\mathbf{8}}$	Dissociation rate constant of **8:1**.
A	PL intensity of **6** or **8** per unit mole.
B	PL intensity of **6:1** or **8:1** per unit mole.
C	Background PL intensity.

Rate equations

We set up the rate equations as follows:

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

$$\frac{d[8]}{dt} = -k_8[8][1] + k_{-1,8}[8:1]$$

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

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

$$\frac{\Delta[8]}{\Delta t} = -k_8[8][1] + k_{-1,8}[8:1]$$

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

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

$$[8]_{t+\Delta t} = [8]_t + (-k_8[8]_t[1]_t + k_{-1,8}[8:1]_t)\Delta t$$

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

$$[8:1]_{t+\Delta t} = [8:1]_t + (-k_8[8]_t[1]_t + k_{-1,8}[8:1]_t)\Delta t$$

$$[1]_{t+\Delta t} = [1]_t + (-k_6[6]_t[1]_t + k_{-1,6}[6:1]_t)\Delta t + (-k_8[8]_t[1]_t + k_{-1,8}[8:1]_t)\Delta t$$

Theoretical curves

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

$$I_{theory,6} = A[6] + B[6:1] + C$$

$$I_{theory,8} = A[8] + B[8:1] + C$$

The values of A, B and C of **6** are assumed to be the same as those of **8** because **6** and **8** are almost the same in terms of the role as a dye and other conditions such as amount of solvent or sample cell are exactly same.

A, 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 I_{FI} at t=0, [**6**:**1**] or [**8**:**1**] should be zero and above equations are reduced to:

$$I_{theory,6,t=0} = A[6]_{t=0} + C$$

By plotting $[\mathbf{6}]_{t=0}$ vs. I_{FI}, A and C have been determined to be 4.18 and 18.4 respectively as shown in the following plot.

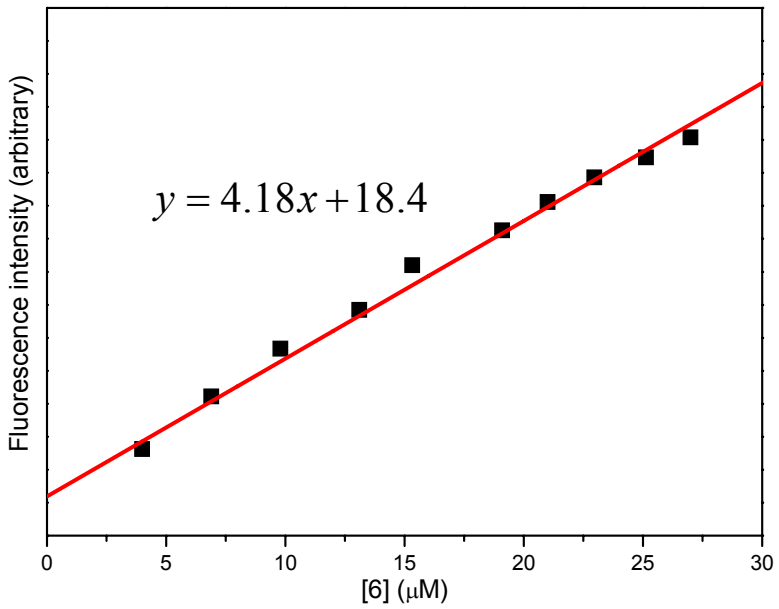


Figure S6. A plot of fluorescence intensity at t=0 versus $[6]_{t=0}$. Black squares are experimental points and the red line is a linear fit.

χ^2 definition

The definition of the χ^2 of the model is following:

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

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

Least-square fit

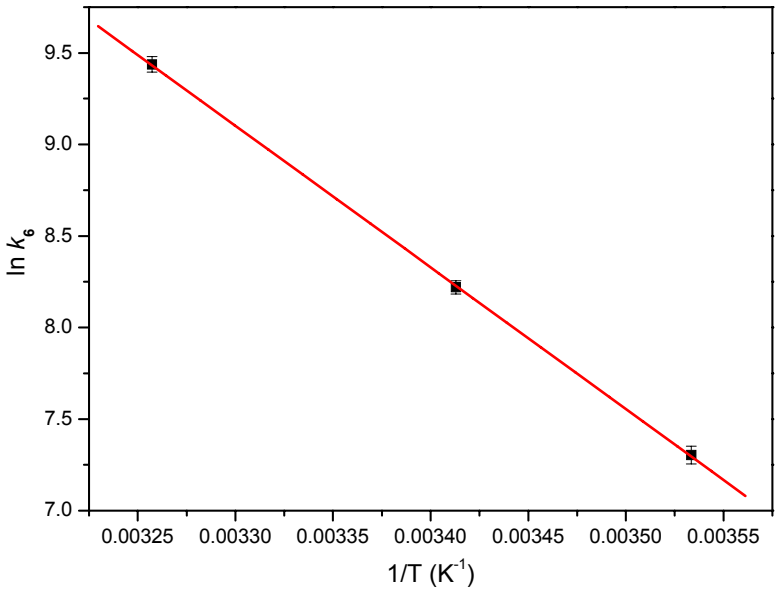
The least-square fit of the model against the experimental data was done using the minimization package MINUIT written at CERN.[6] The quantity minimized is χ^2. The errors of the fitted parameters have been calculated by MINUIT and they represent 1 standard deviation. In total 13 parameters (12 rate constants and B value) have been optimized with 15 experimental curves.

Activation energy

The activation energy (E_a) for substrate binding was determined using linear fits of ln*k* vs. 1/T gave E_a value based on the Arrhenius equation.

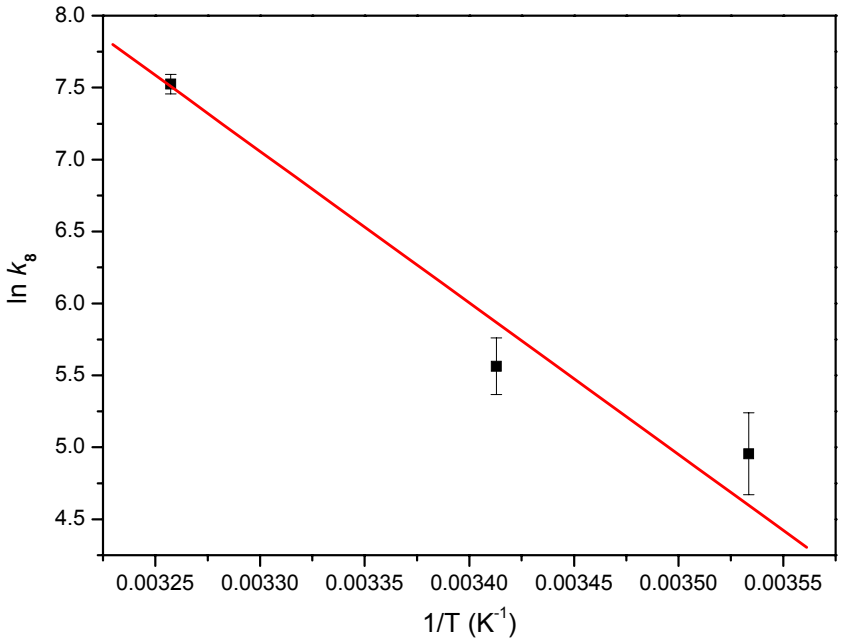
$$k = A\exp(-E_a / RT)$$

1) Activation energy for the k_6



$$-\frac{E_a}{R} = -7740 \pm 228,\ E_a = 64.2 \pm 1.90 \text{ kJ/mol}$$

2) Activation energy for the k_8



$$-\frac{E_a}{R} = -10800 \pm 734,\ E_a = 84.7 \pm 6.10 \text{ kJ/mol}$$

Gibbs free energy change

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

$$\Delta G = -RT \ln K$$

$$K = k / k_{-1}$$

9) Förster distance Ro and FRET efficiency analysis

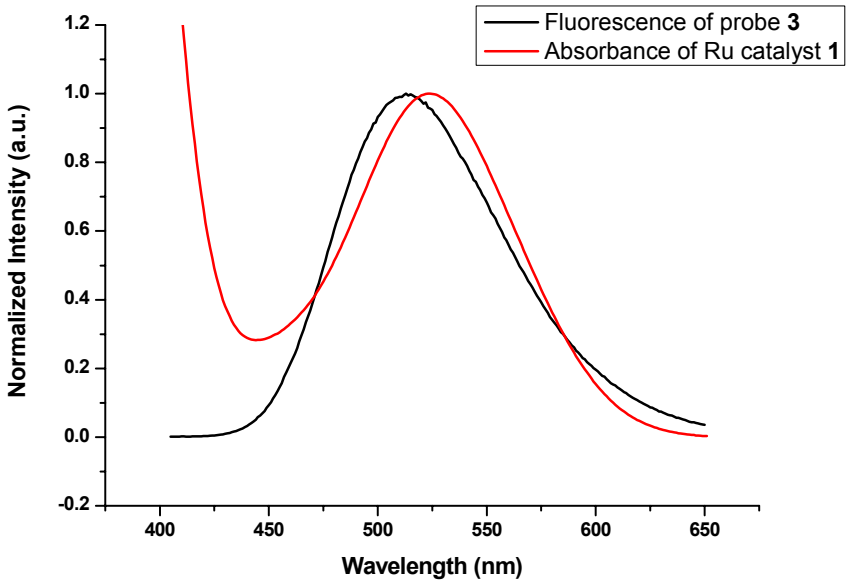


Figure S7. Spectral overlap of the fluorescence donor **3** and quencher Ru catalyst **1**.

(a) Calculation of Förster distance Ro (using Photochem CAD 2.1)

Donor	
Acceptor	
Solvent	
Refractive index (n)	1.420
Orientation factor	0.810
Quantum yield	0.780
Actual distance	0.100
Epsilon	375.800
Wavelength for epsilon (nm)	524.000
Low wavelength (nm)	405.000
High wavelength (nm)	650.000
J value (cm(6)/mmol)	1.960e-015
Forster distance	25.384

Forster distance Ro = 2.54 nm

(b) Actual average donor-quencher separation distance

The quenching of fluorescence donor by the Ru catalyst reached maximum at the case of probe **6** (18 μM) and Ru catalyst **1** (2 eq) at 34 ^{o}C, giving FRET efficiency E = 0.47.

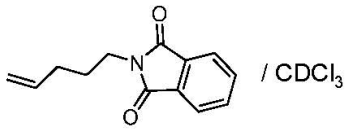
From the equation below

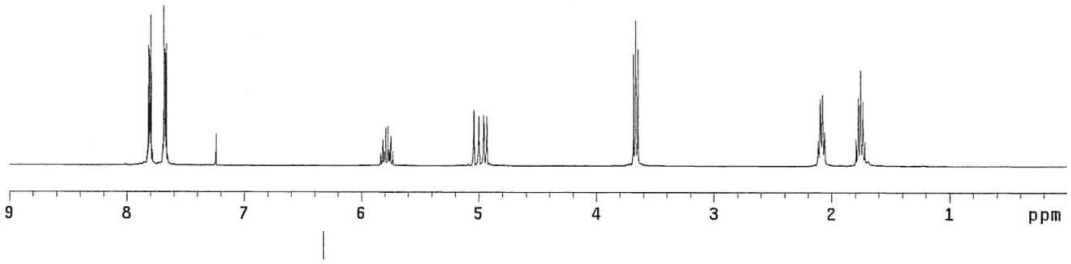
$$E = \frac{nR_0^{\ 6}}{(nR_0^{\ 6} + r^6)}$$

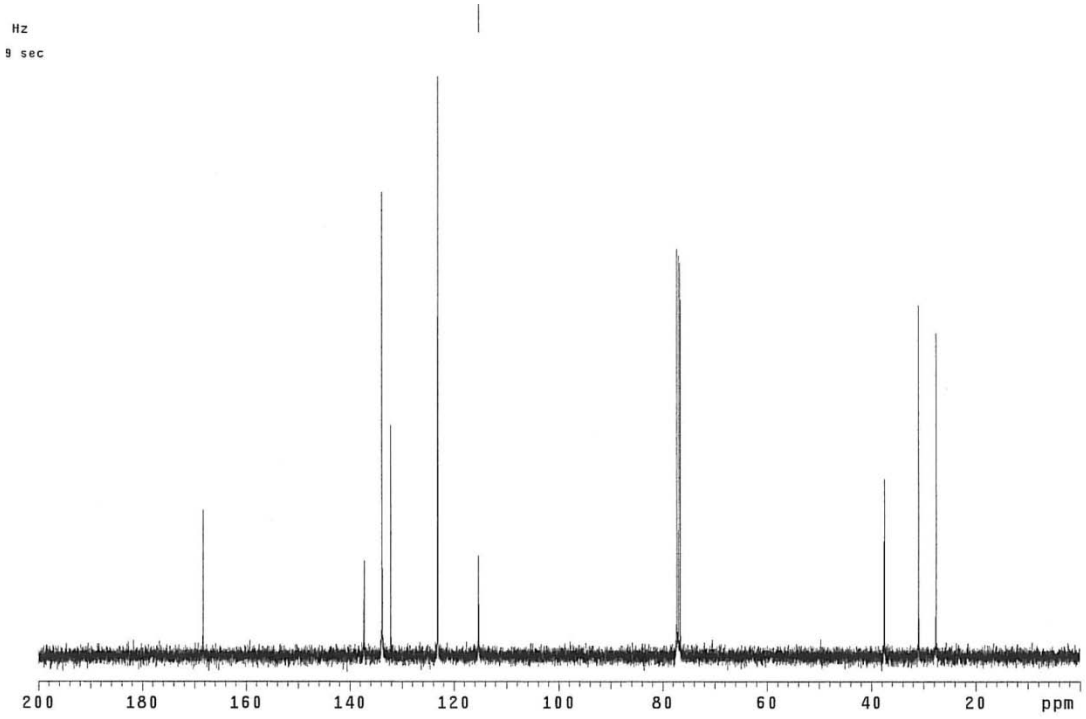
E = 0.47
n = 2
Ro = 2.54

the actual average donor-quencher separation distance r = 2.91 nm.

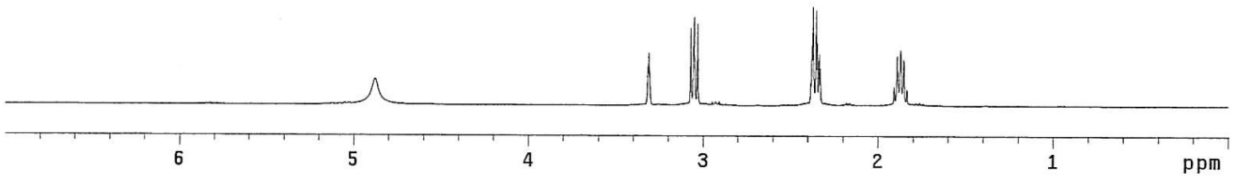
10) ^{1}H and ^{13}C NMR spectra of compounds








NH2•HCl
/ CD3OD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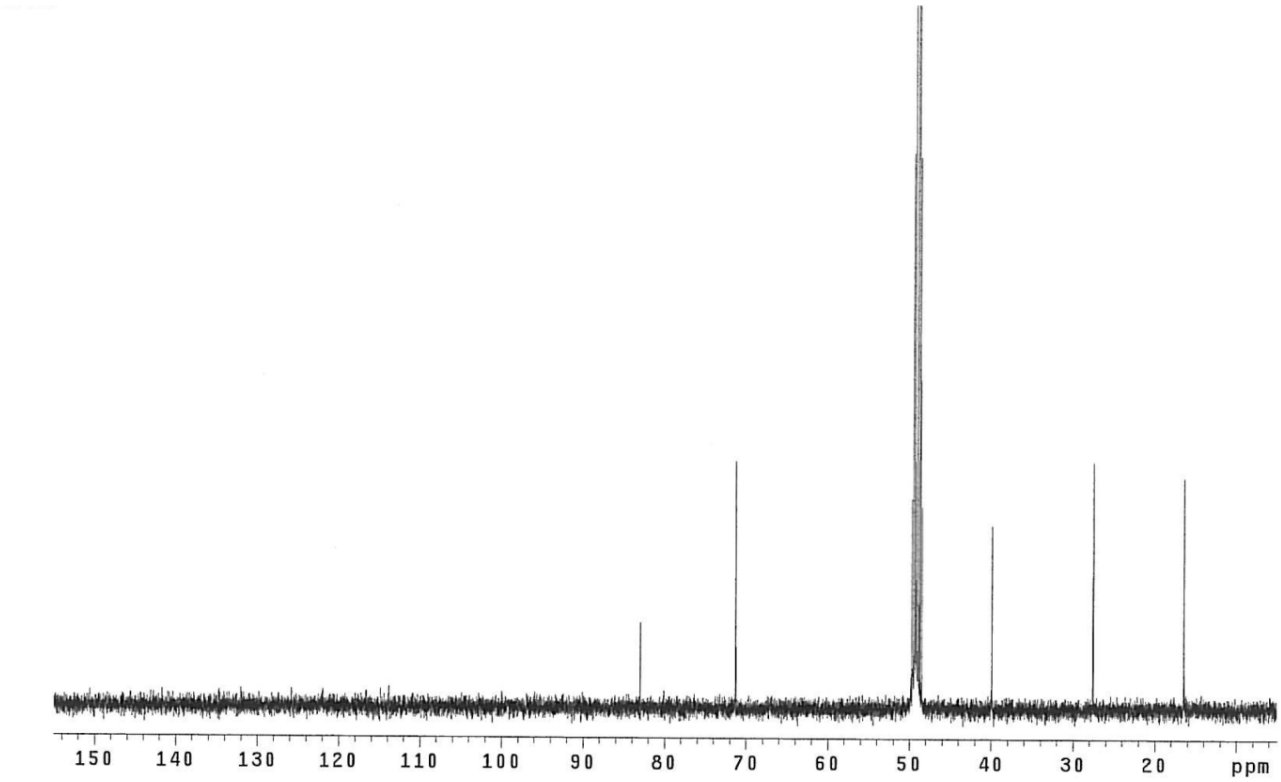
5
4
3
2
1
ppm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140
130
120
110
100
90
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70
60
50
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30
20
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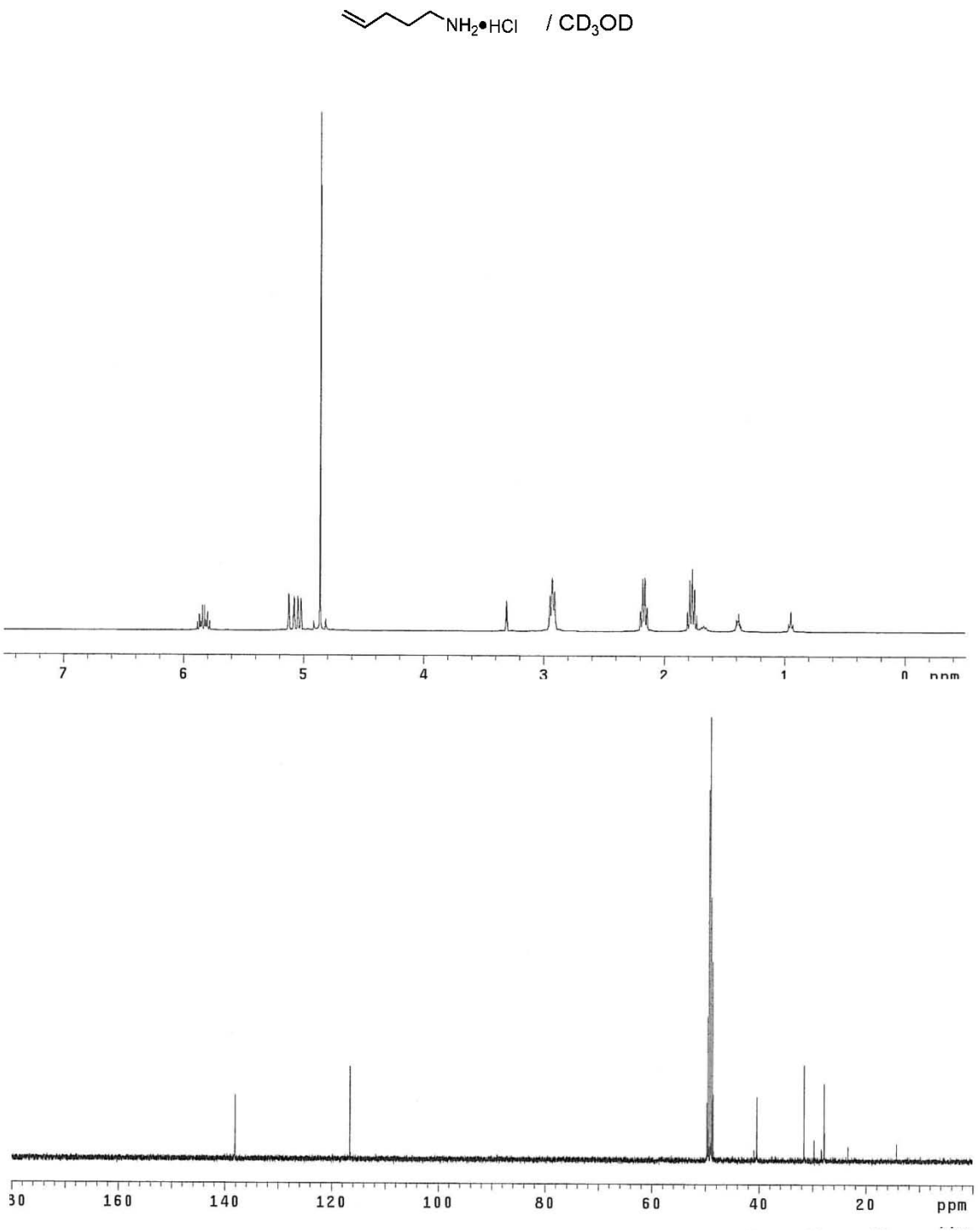
NH2•HCl / CD3OD
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4
3
2
1
0
ppm
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140
120
100
80
60
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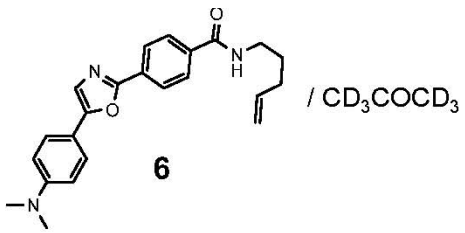
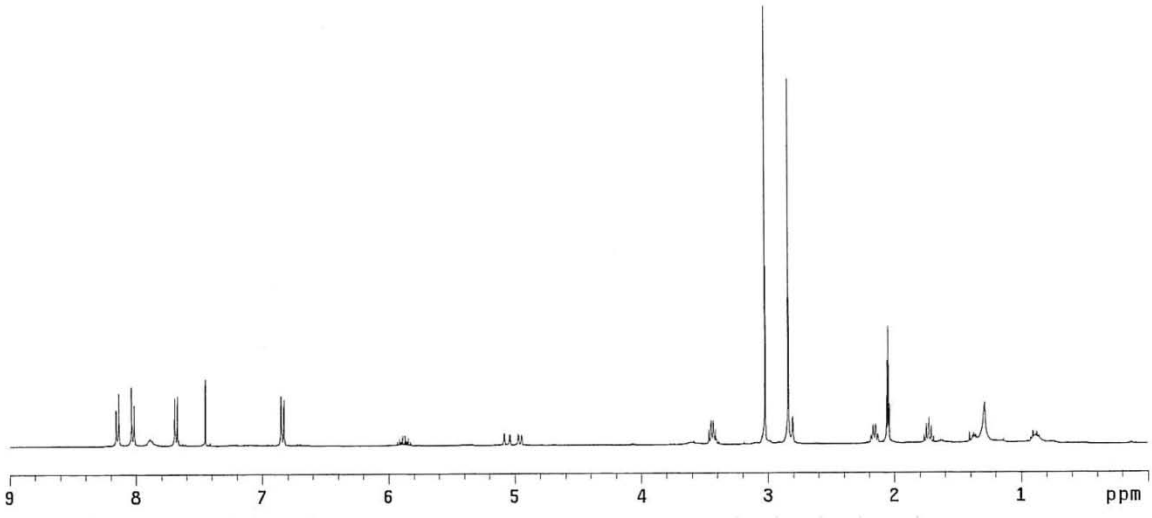
20
ppm


O
N
H
N
O
/ CD_3COCD_3

6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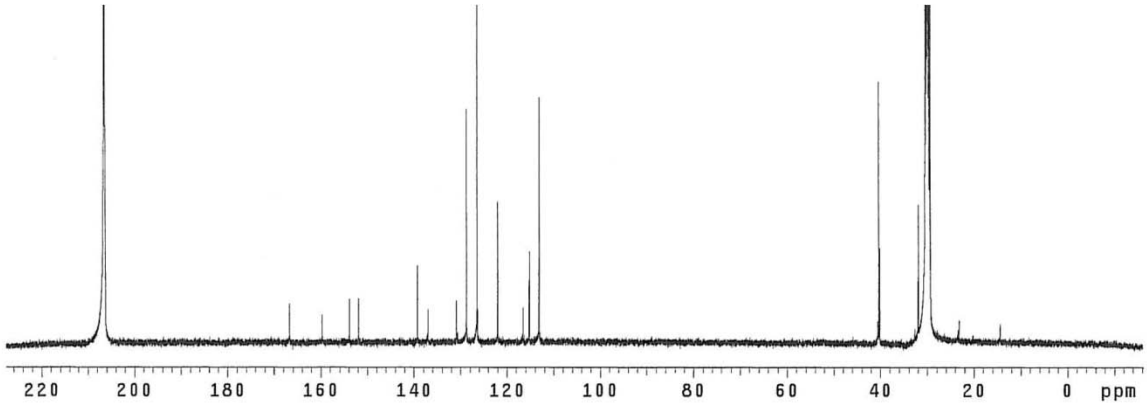
5
4
3
2
1
ppm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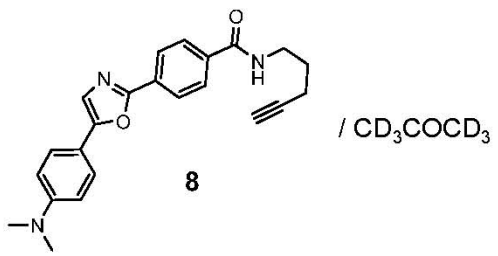
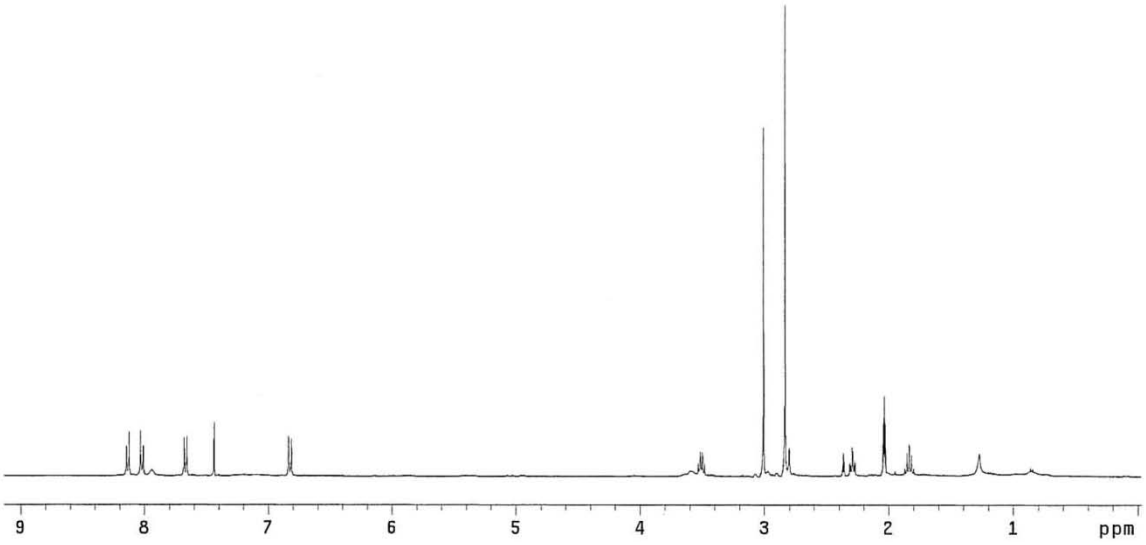
200
180
160
140
120
100
80
60
40
20
0
ppm


O
N
H
N
O
N

8 / CD_3COCD_3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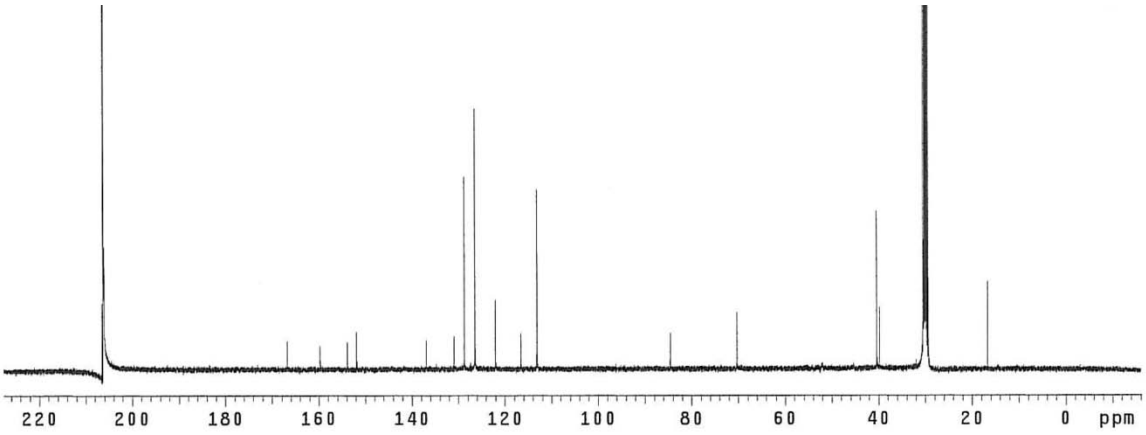
7
6
5
4
3
2
1
ppm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100
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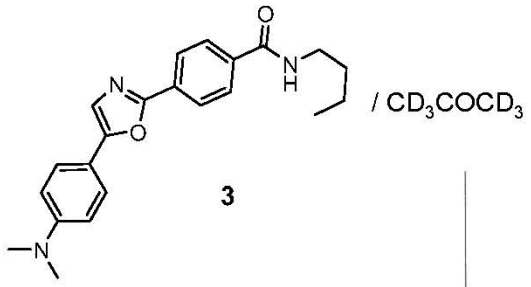
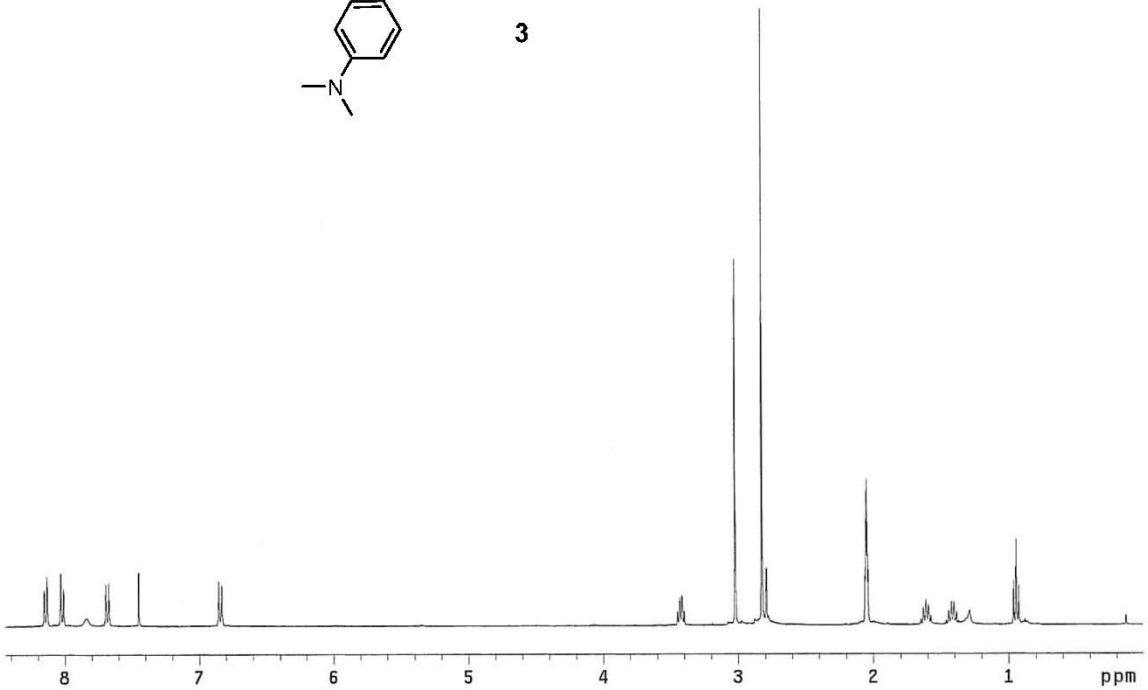
40
20
0
ppm


O
N
H
N
O
/ CD3COCD3

3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7
6
5
4
3
2
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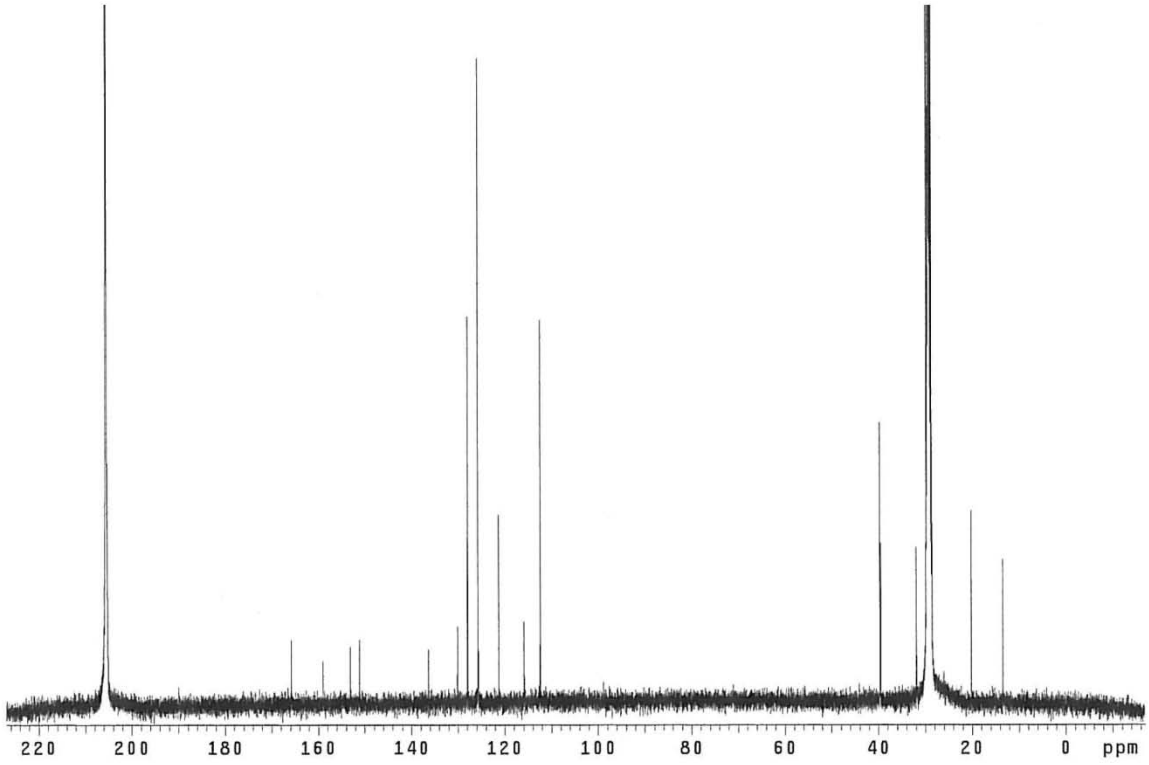
ppm


220
200
180
160
140
120
100
80
60
40
20
0
ppm

11) References

1. Adam, S. Bioorg. & Med. Chem. Lett. **1992**, 2, 53.
2. Michael, F. E.; Cochran, B. M. *J. Am. Chem. Soc*. **2006**, *128*, 4246.
3. Saito, Y.; Matsumoto, K; Bag, S. S.; Ogasawara, S.; Fujimoto, K.; Hanawa, K.; Saito, I. *Tetrahedron* **2008**, *64*, 3578.
4. http://www.asiinstruments.com/technical/Dielectric%20Constants.htm.
5. (a) Terpetschnig, E.; Szmacinski, H.; Malak, H.; Lakowicz, J. R. *Biophys. J.* **1995**, *68*, 342-350. (b) Youn, H. J.; Terpetschnig, E.; Szmacinski, H.; Lakowicz, J. R. *Anal. Biochem*. **1995**, *232*, 24-30. (c) von Bültzingslöwen, C.; McEvoy, A. K.; McDonagh, C.; MacCraith, B. D. *Anal. Chim. Acta* **2003**, *480*, 275-283.
6. CERN, Minuit; Function Minimization and Error Analysis, http://wwwasdoc.web.cern.ch/wwwasdoc/minuit/minmain.html.