Synthesis of tri-functionalized MMP2 FRET probes using a chemo-selective and late-stage modification of unprotected peptides

–supporting information–

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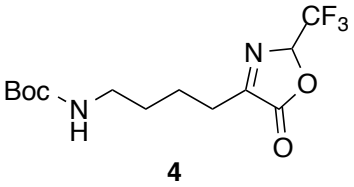
1. Synthesis of ketoacid derivative of methoxylcounarin and hydroxylamine Lys deriveative



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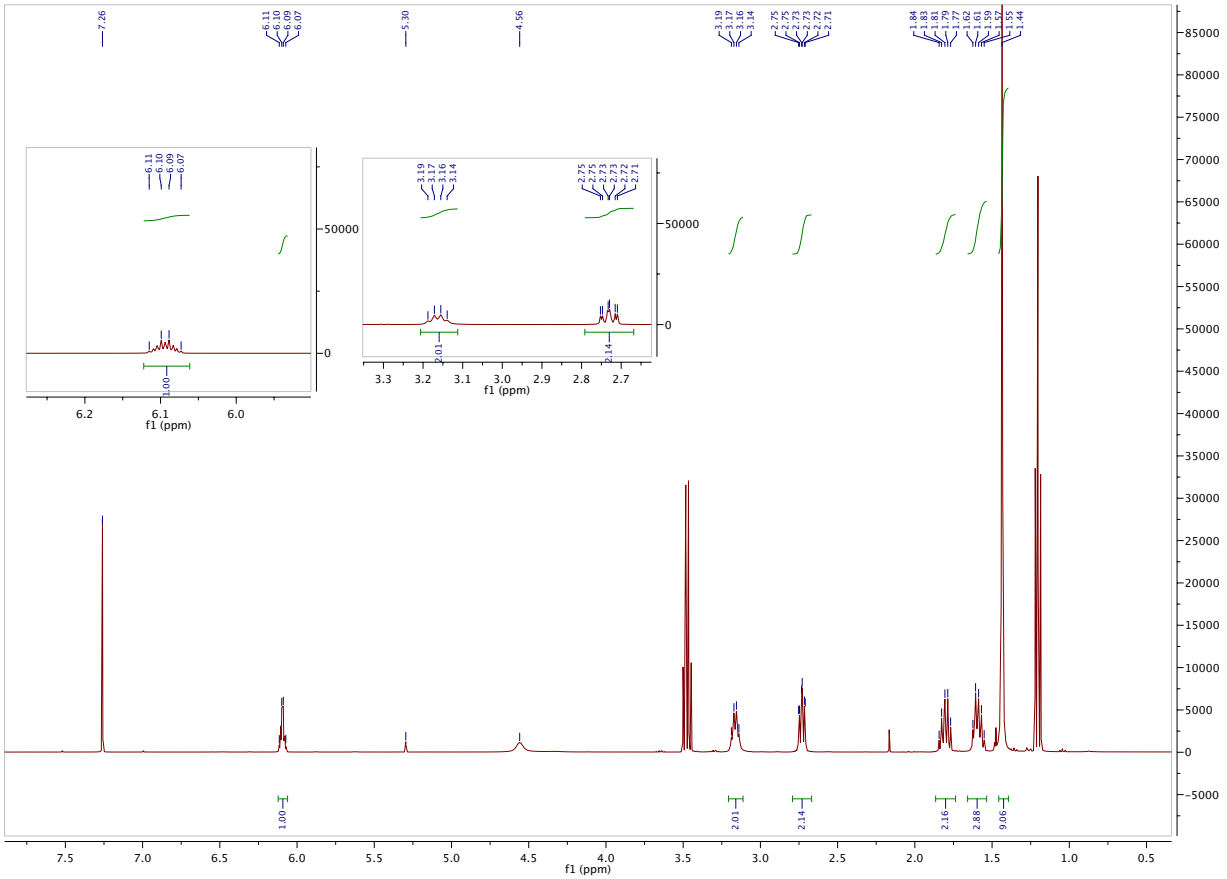


Fig. S1 ^{1}H-NMR spectrum of compound **4** (in $CDCl_3$, 400 MHz).

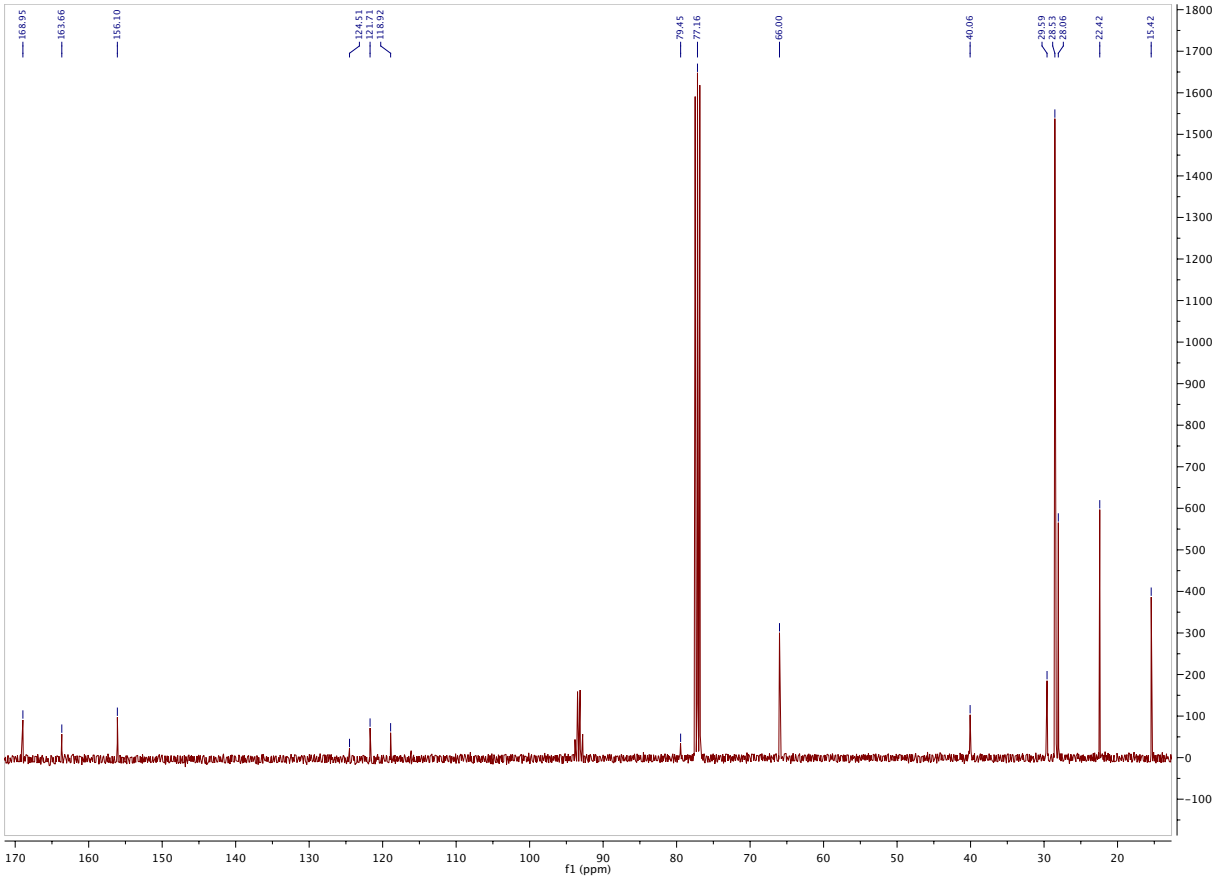


Fig. S2 ^{13}C-NMR spectrum of compound **4** (in $CDCl_3$, 100 MHz).

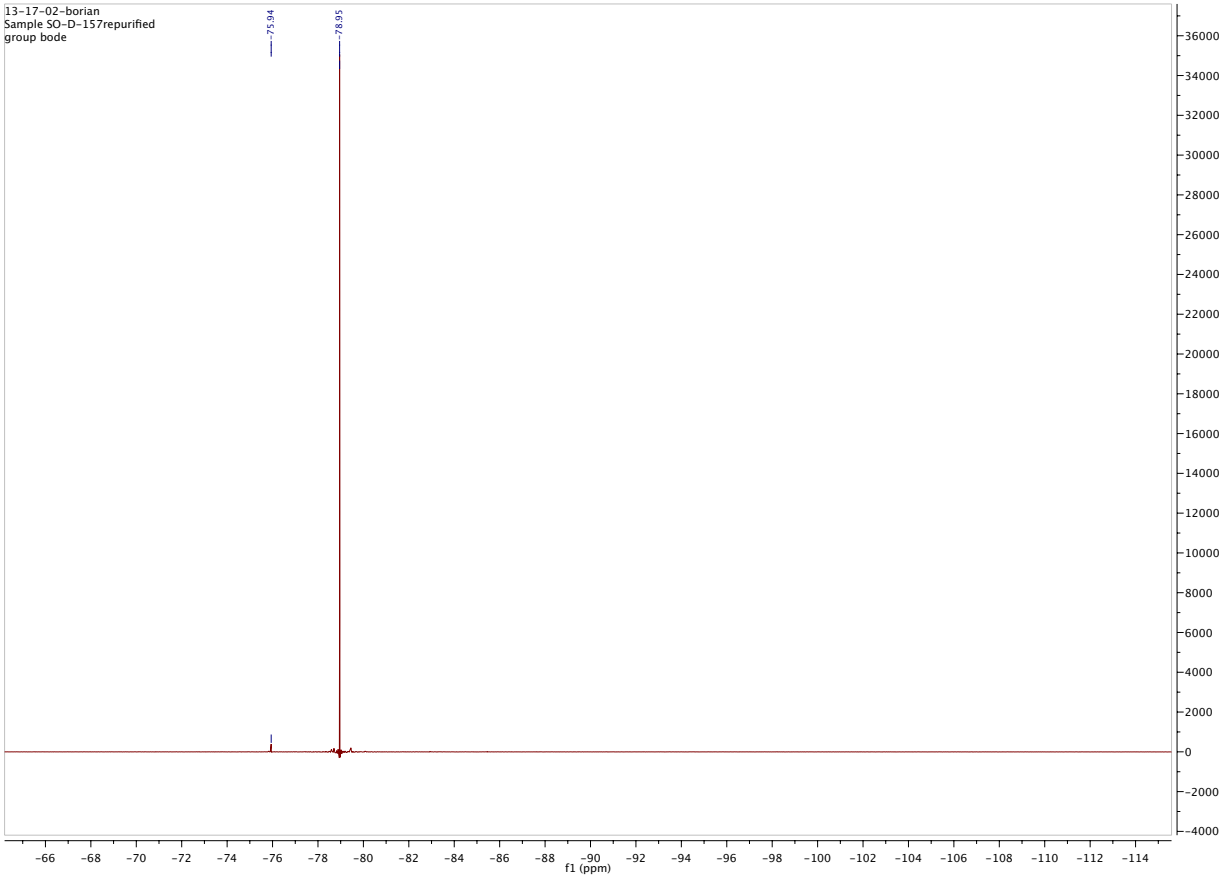


Fig. S3 ^{19}F-NMR spectrum of compound **4** (in $CDCl_3$, 377 MHz).

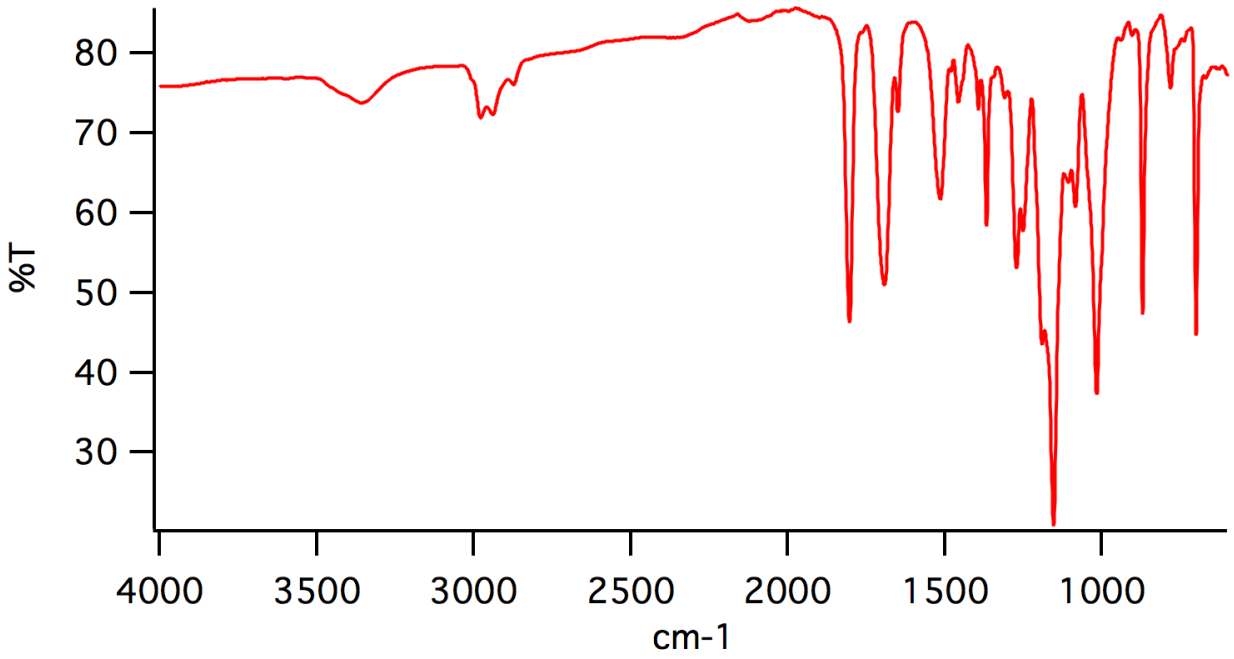
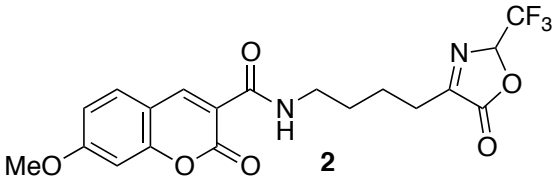


Fig. S4 IR spectrum of compound **4**



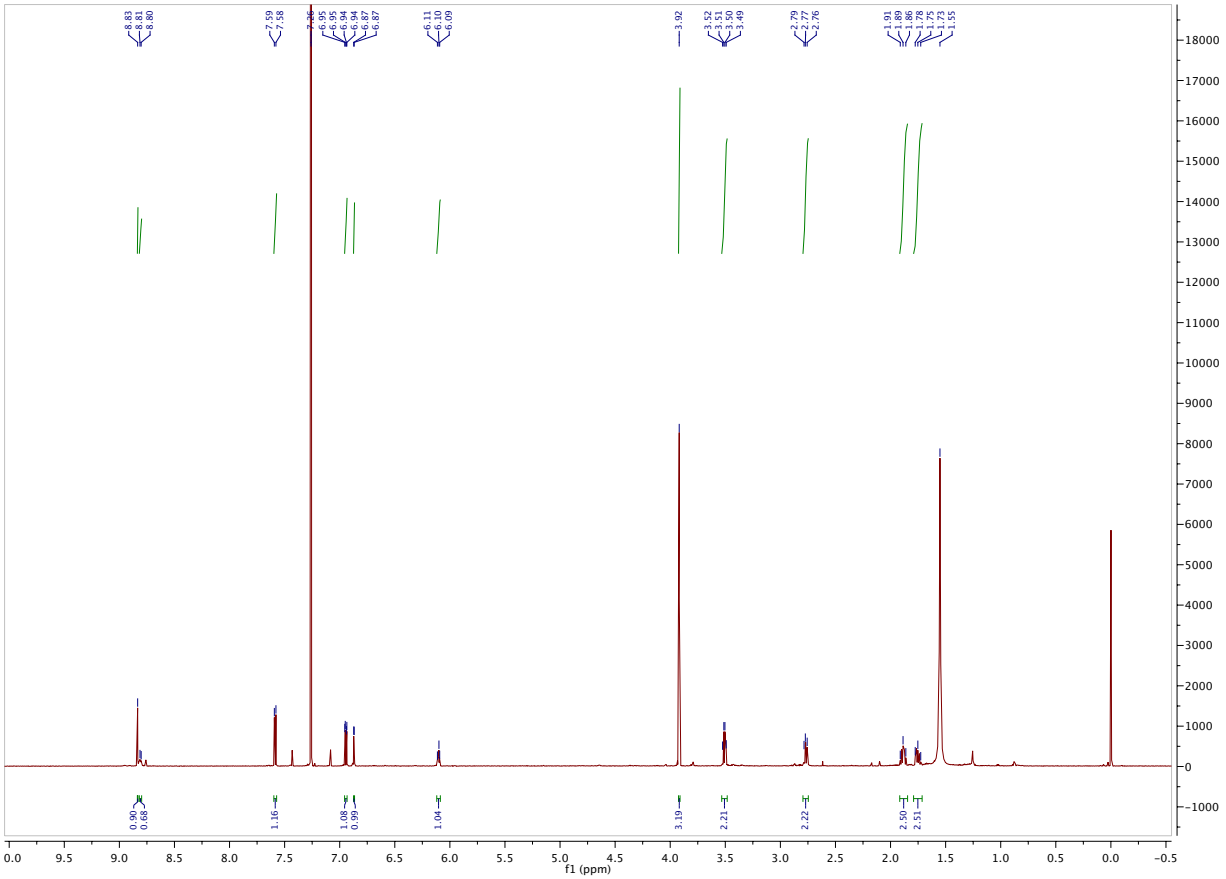


Fig. S5 ^{1}H NMR spectrum of compound **2** (in $CDCl_3$, 600 MHz).

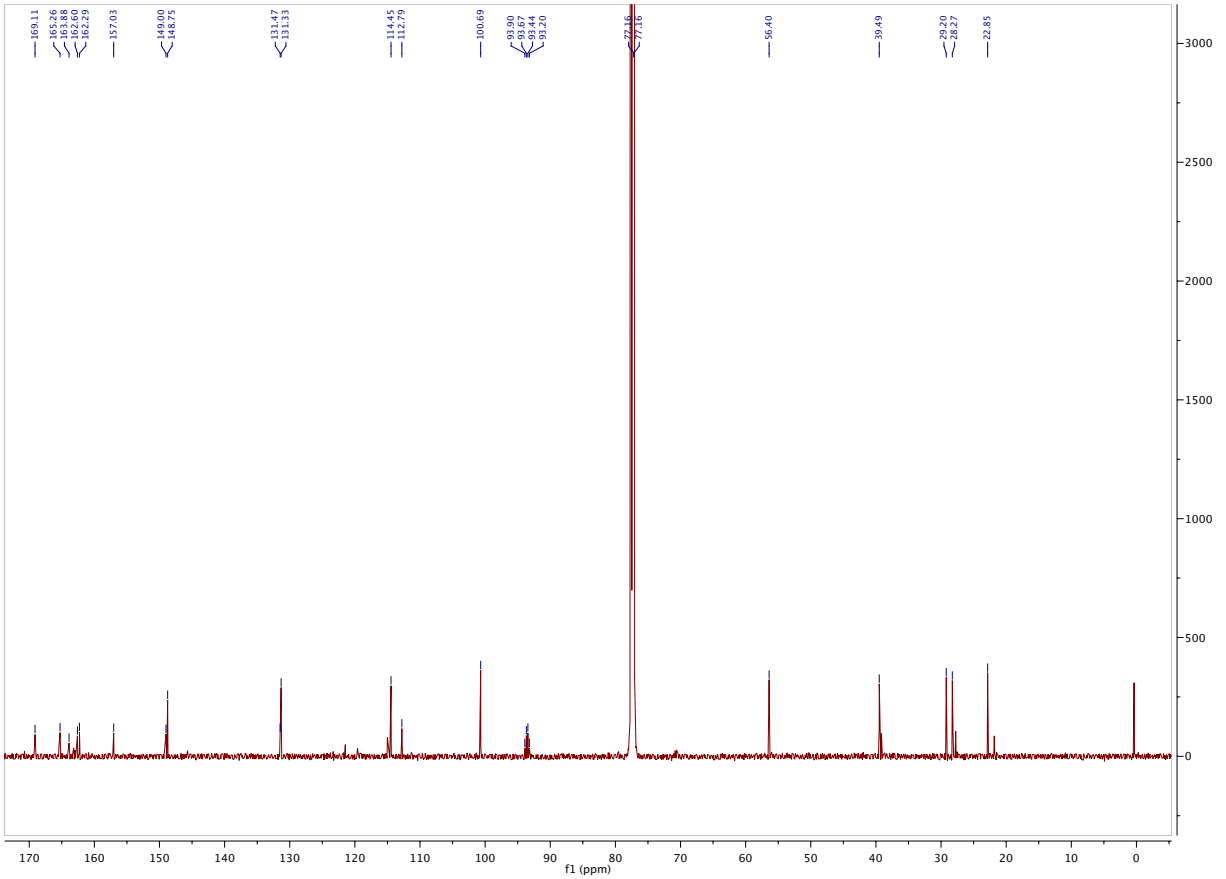


Fig. S6 ^{13}C NMR spectrum of compound **2** (in $CDCl_3$, 150 MHz).

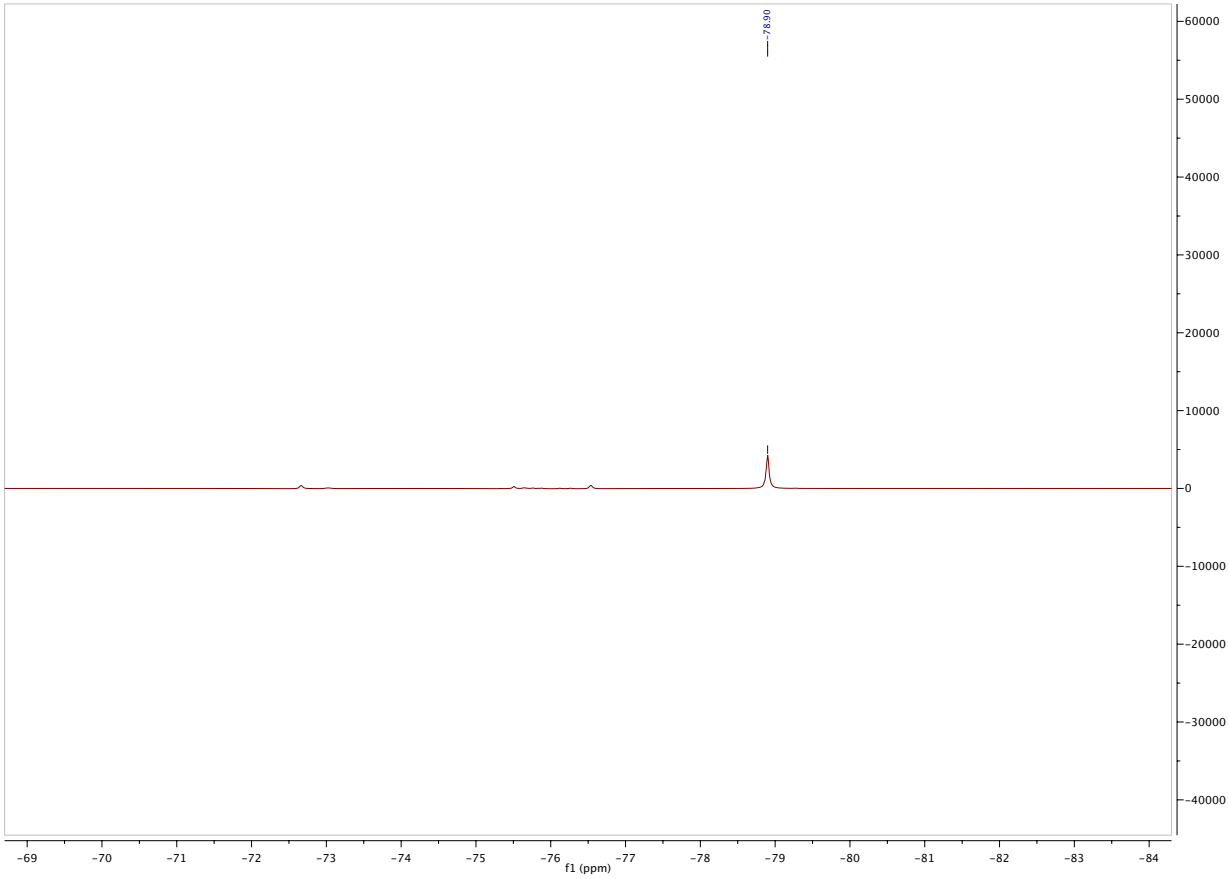


Fig. S7 ^{19}F NMR spectrum of compound **2** (in $CDCl_3$, 377 MHz).

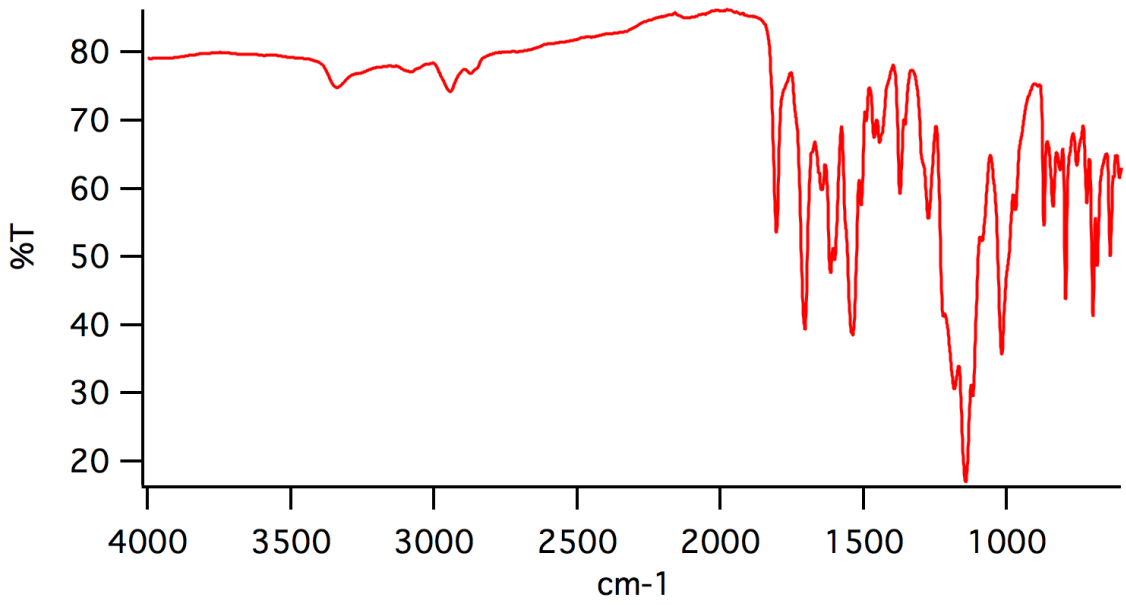
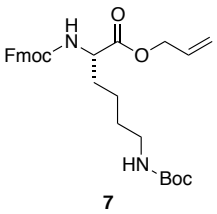


Fig. S8 IR spectrum of compound **2**



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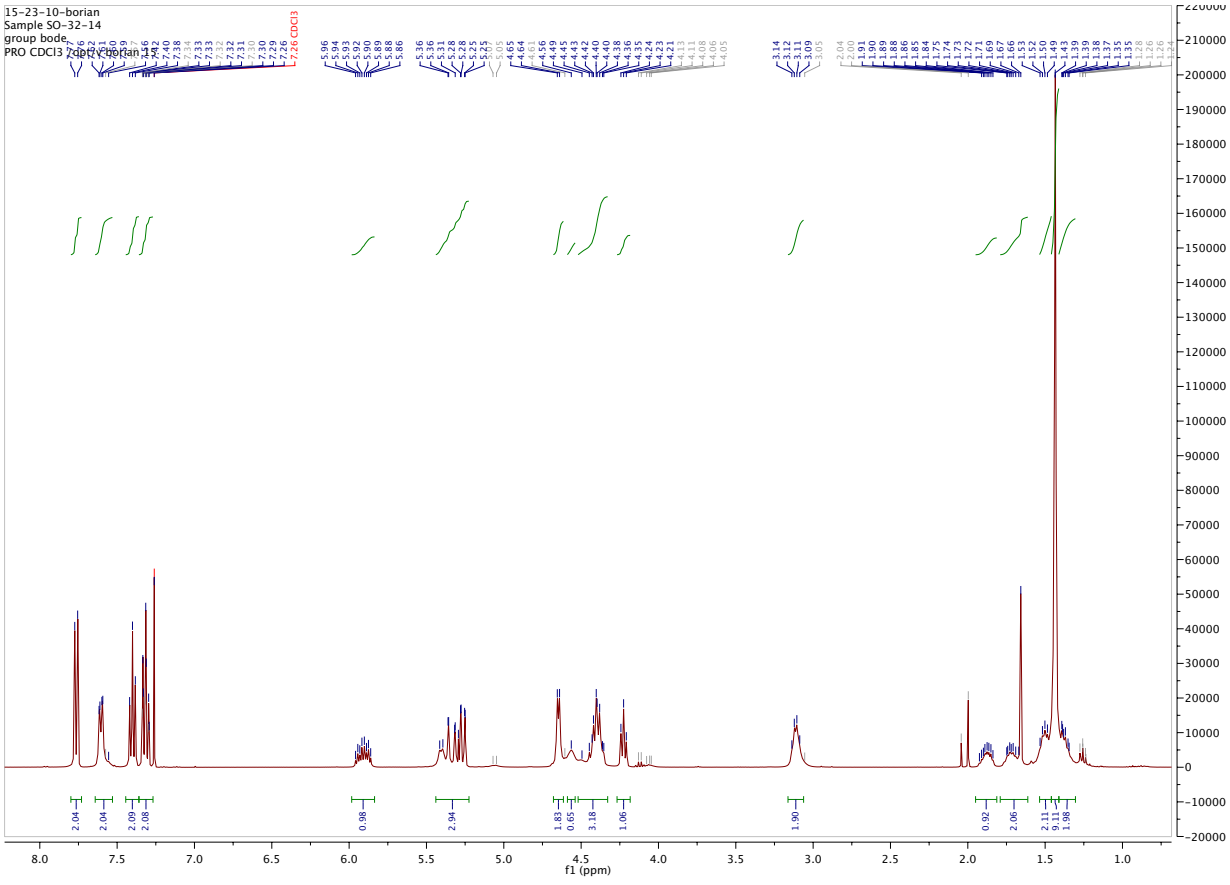


Fig. S9 ^{1}H-NMR spectrum of compound **7** (in $CDCl_3$, 400 MHz).

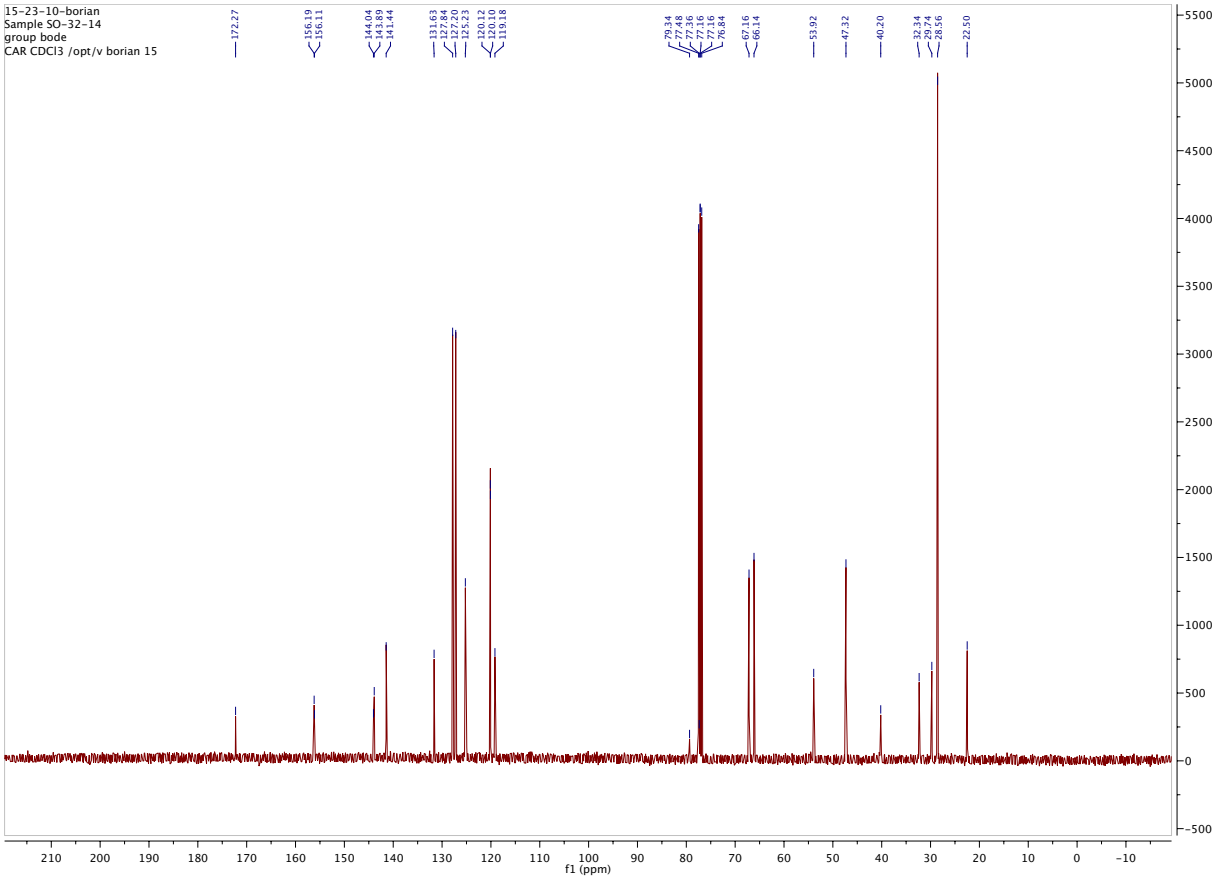


Fig. S10 ^{13}C-NMR spectrum of compound **7** (in $CDCl_3$, 100 MHz).

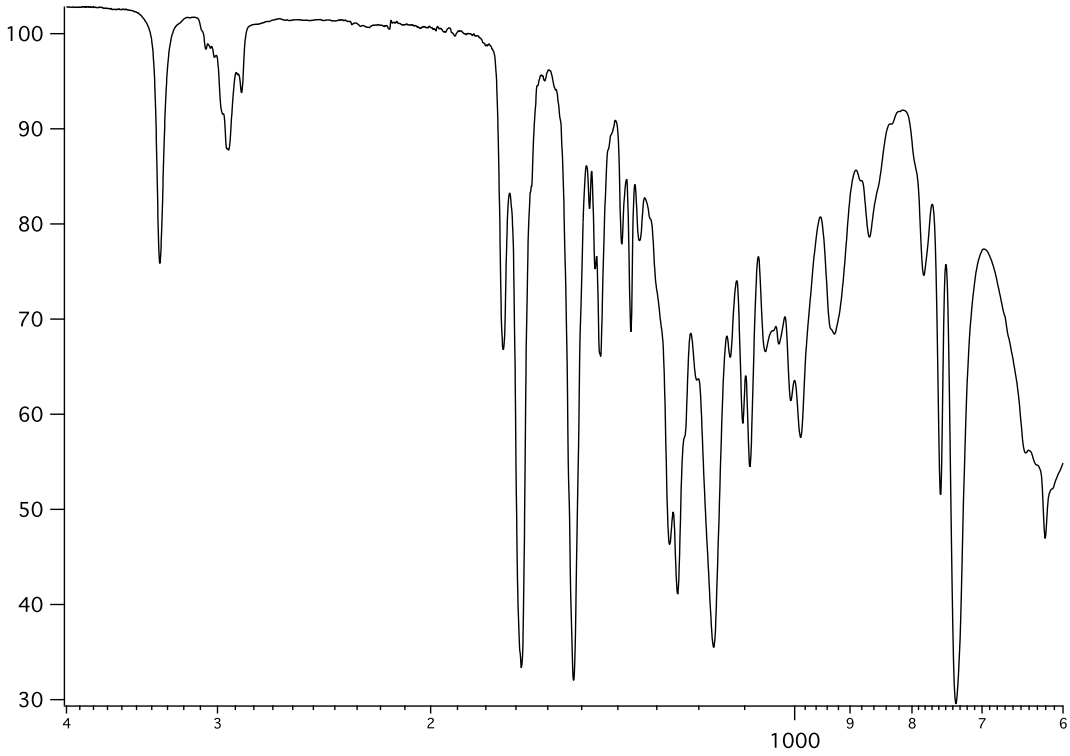
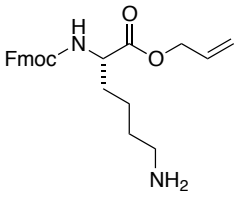


Fig. S11 IR spectrum of compound **7**.



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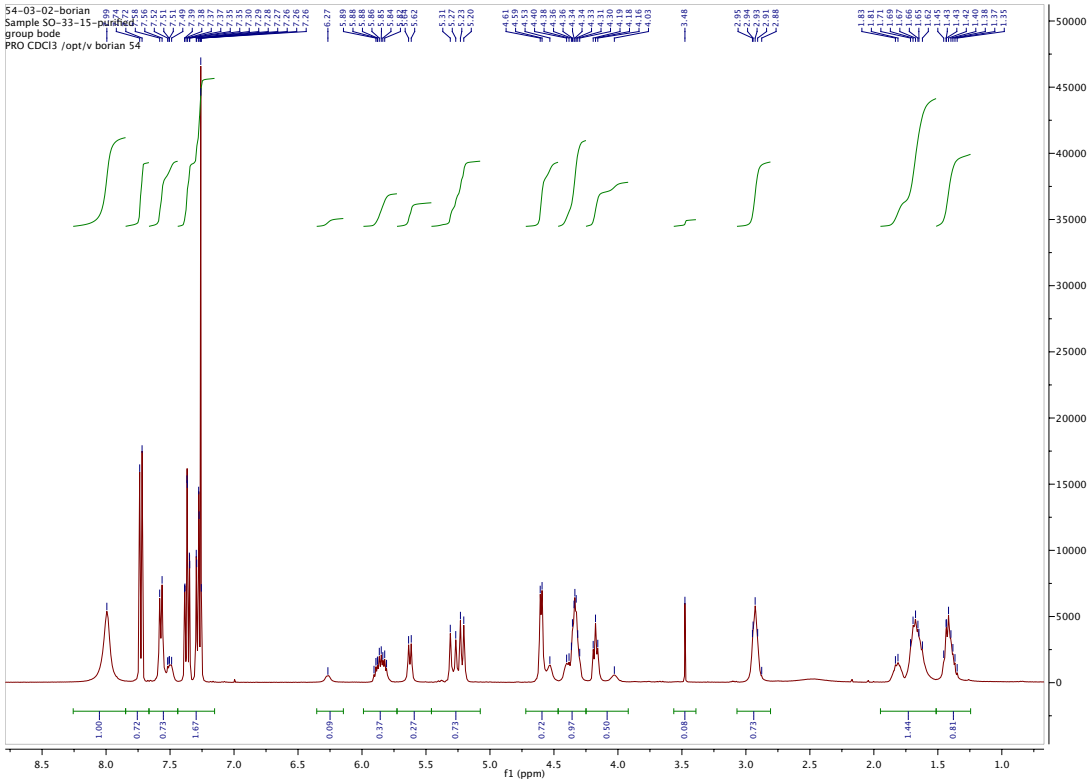


Fig. S12 ^{1}H-NMR spectrum of compound **8** (in $CDCl_3$, 400 MHz).

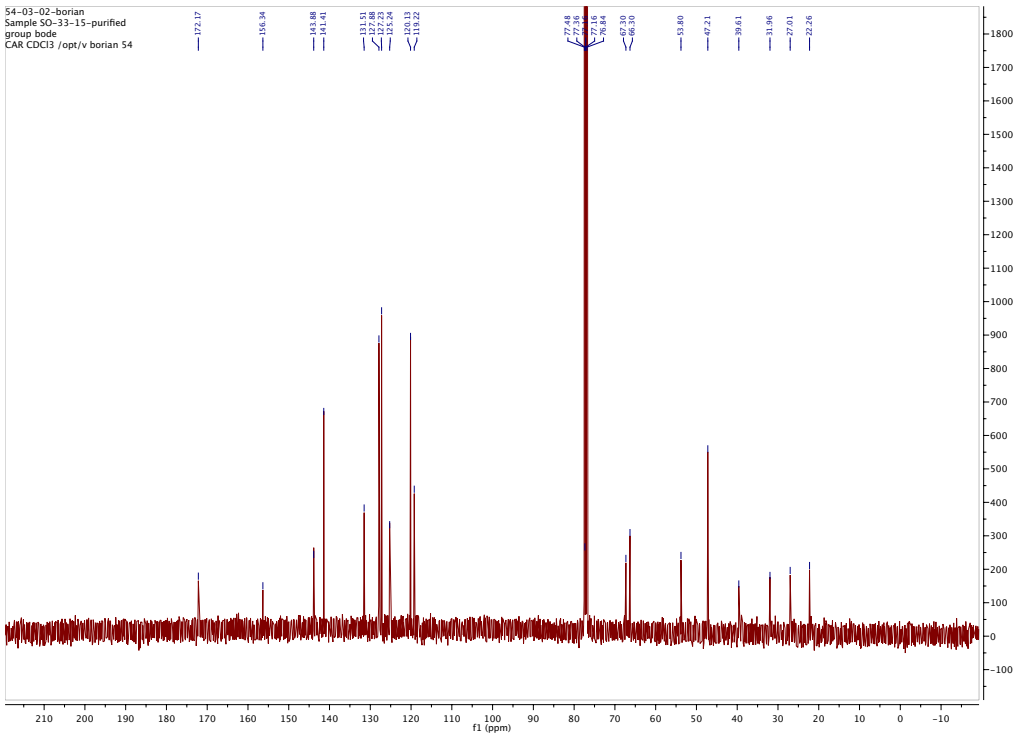


Fig. S13 ^{13}C-NMR spectrum of compound **8** (in $CDCl_3$, 100 MHz).

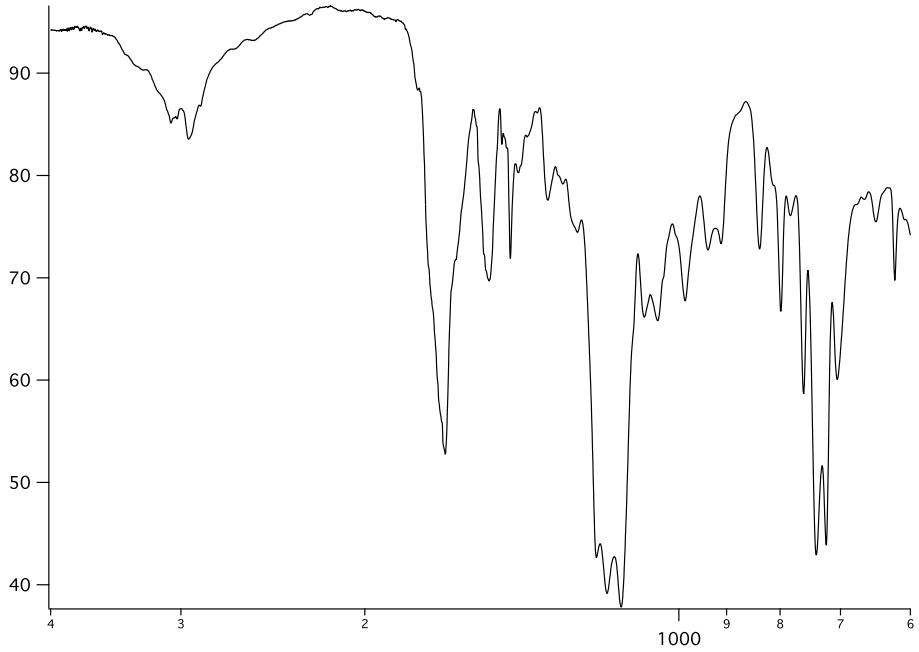
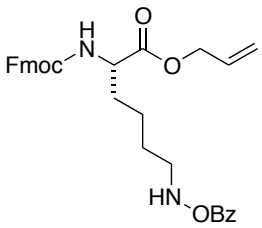


Fig. S14 IR spectrum of compound **8**.



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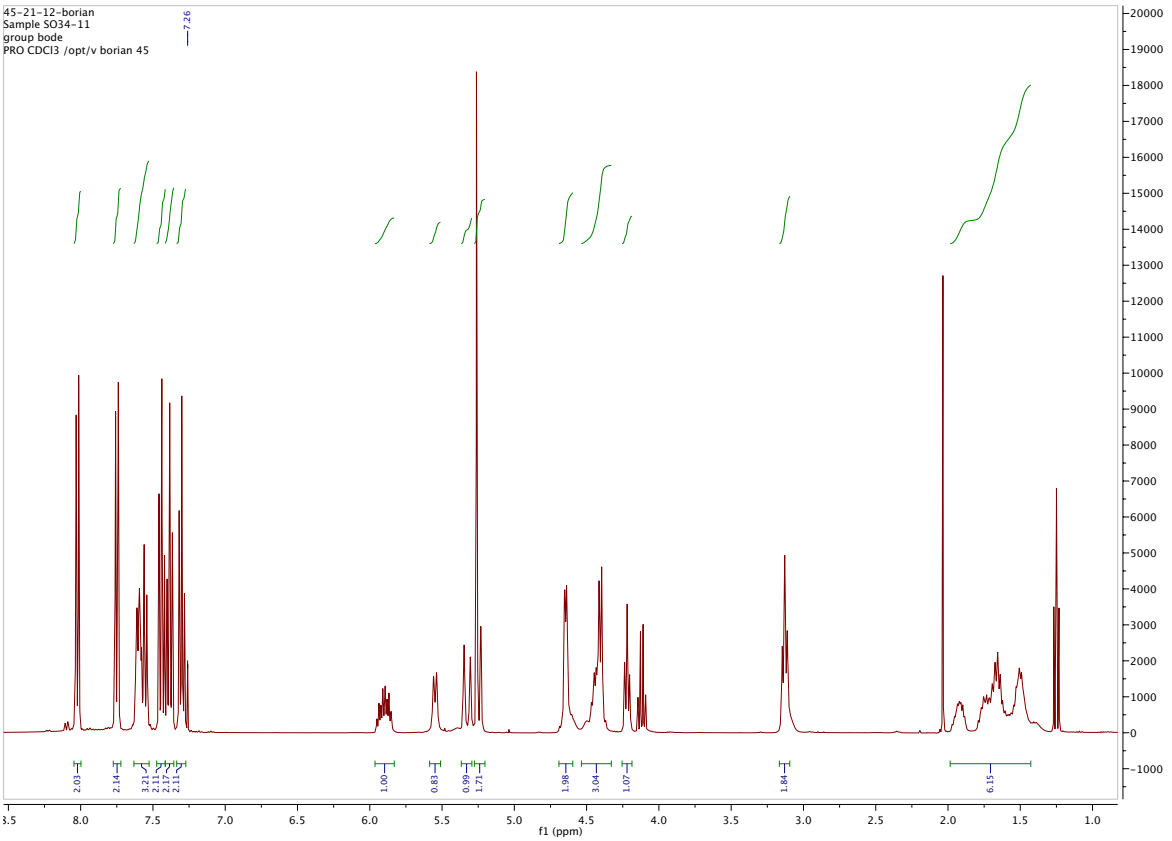


Fig. S15 ^{1}H-NMR spectrum of compound **9** (in $CDCl_3$, 400 MHz).

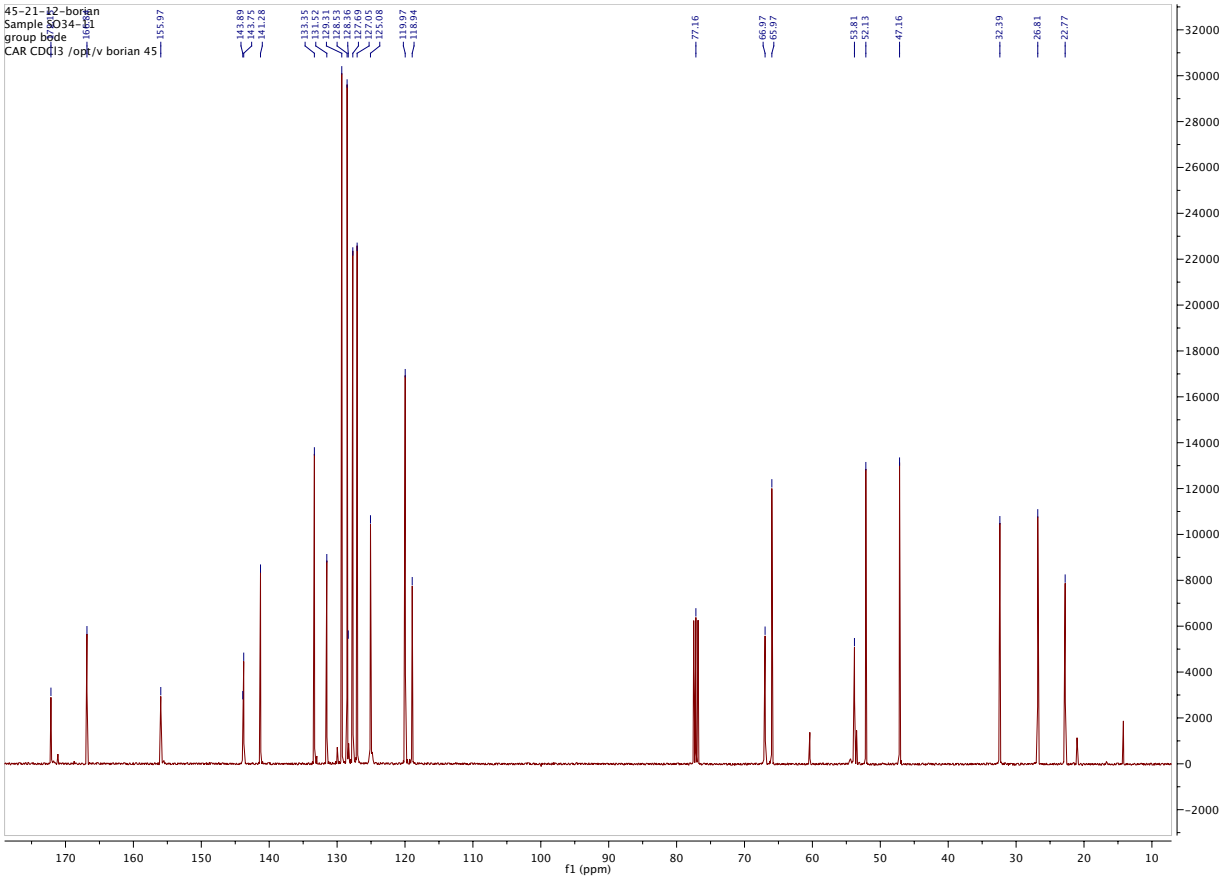


Fig. S16 ^{13}C-NMR spectrum of compound **9** (in $CDCl_3$, 100 MHz).

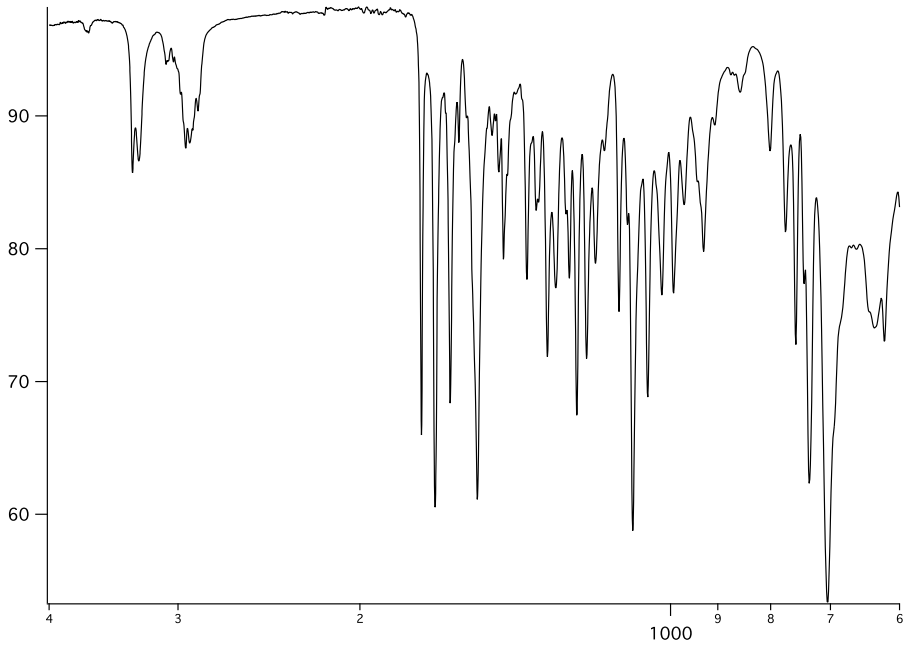
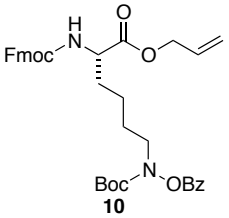


Fig. S17 IR spectrum of compound **9**



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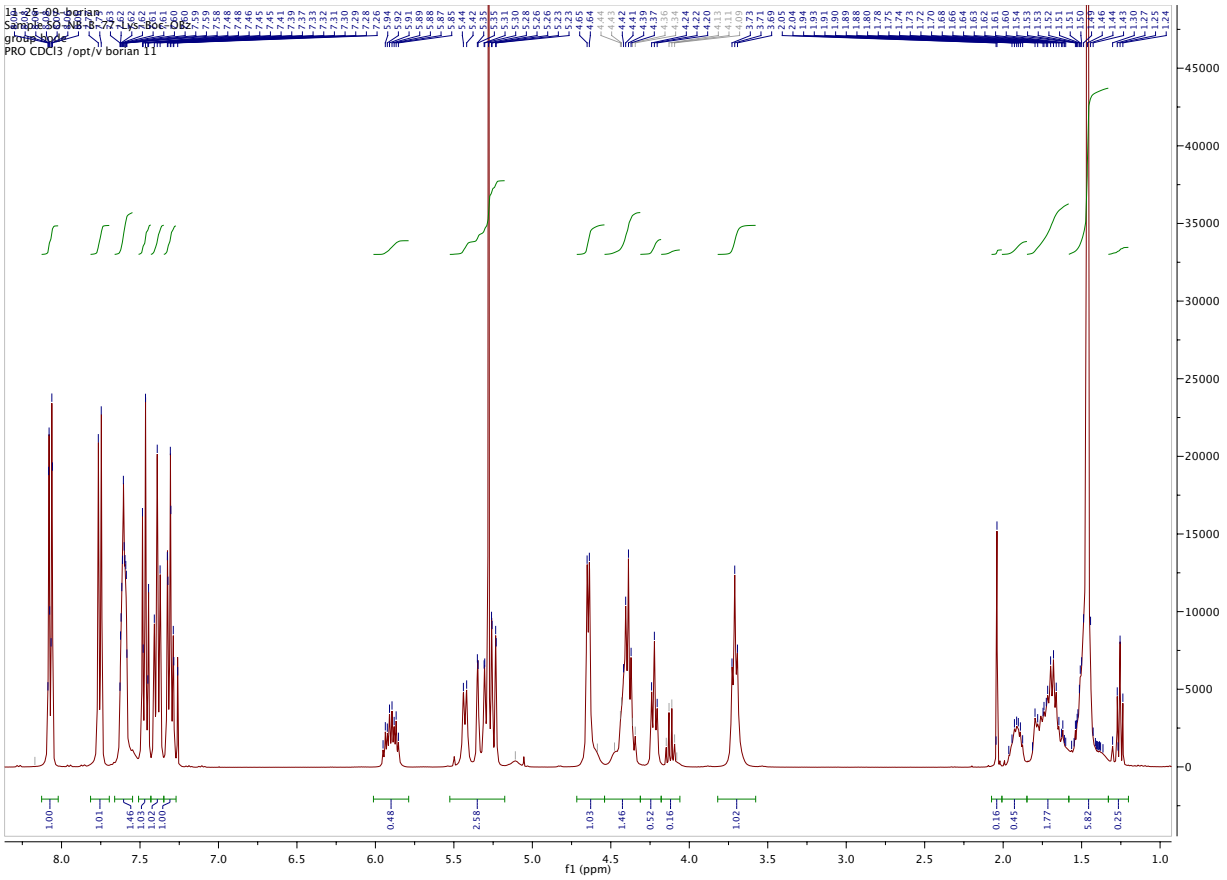


Fig. S18 ^{1}H-NMR spectrum of compound **10** (in $CDCl_3$, 400 MHz).

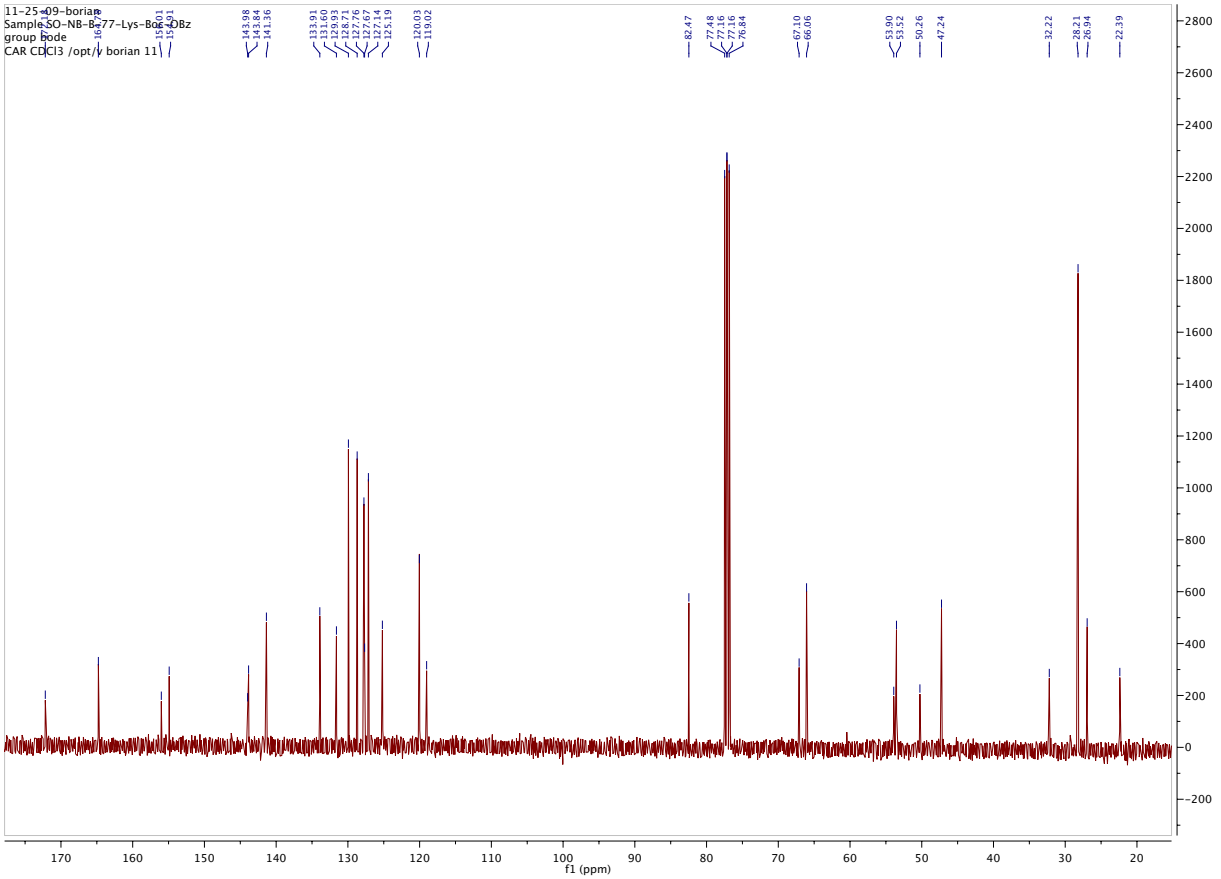


Fig. S19 ^{13}C-NMR spectrum of compound **10** (in $CDCl_3$, 100 MHz).

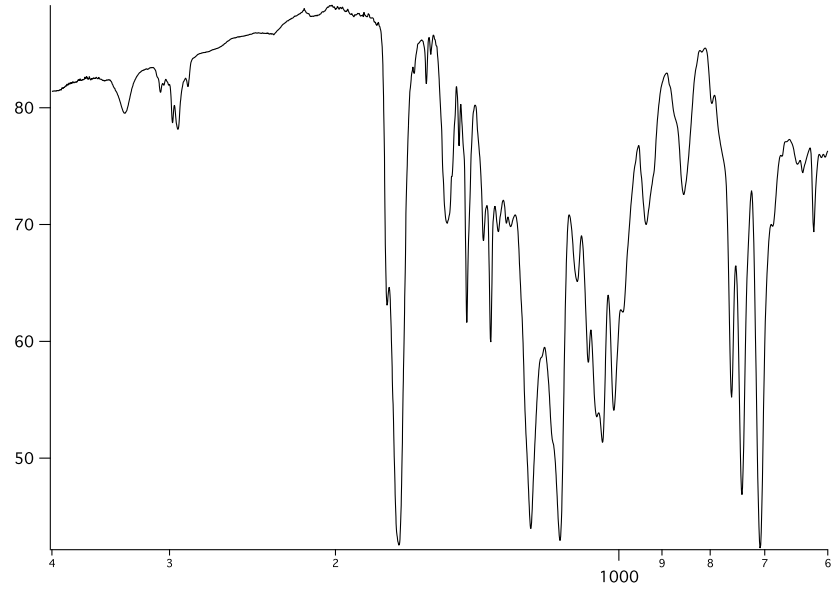
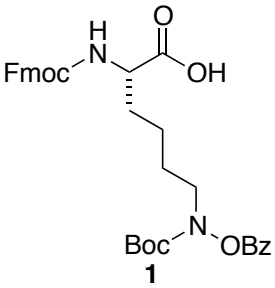


Fig. S20 IR spectrum of compound **10**.



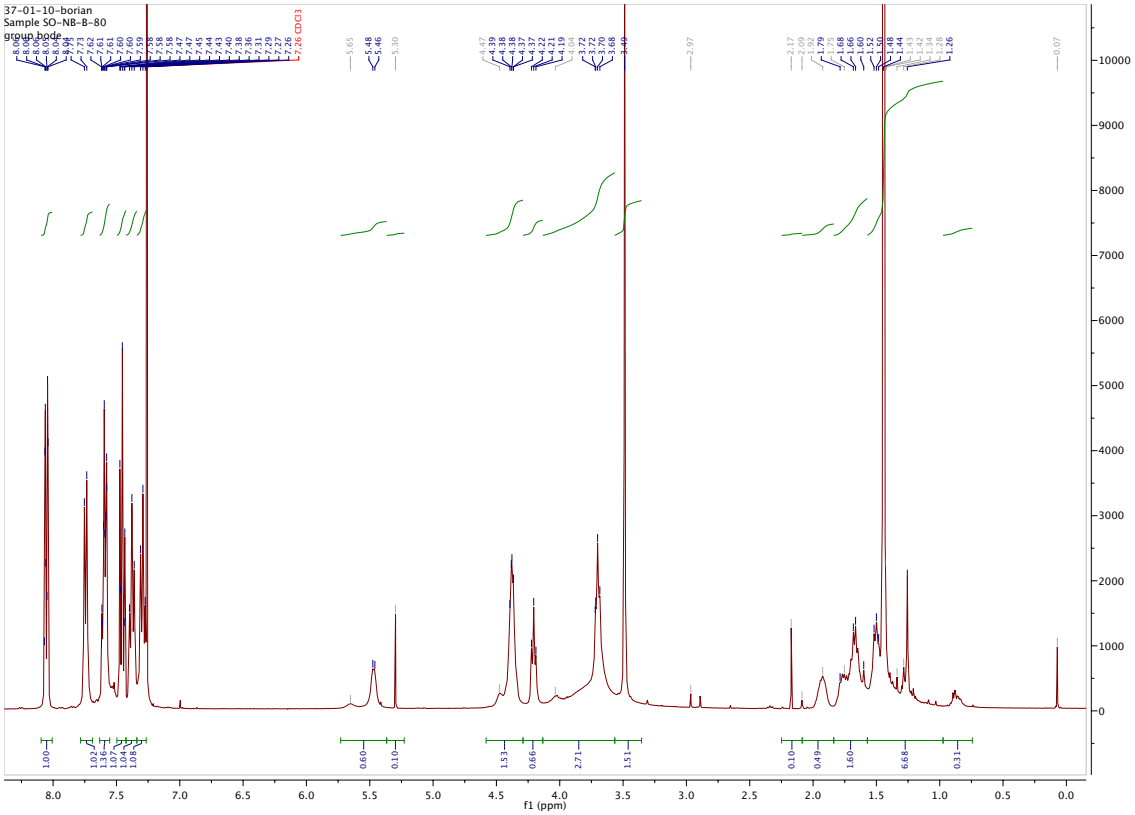


Fig. S21 ^{1}H-NMR spectrum of compound **1** (in $CDCl_3$, 400 MHz).

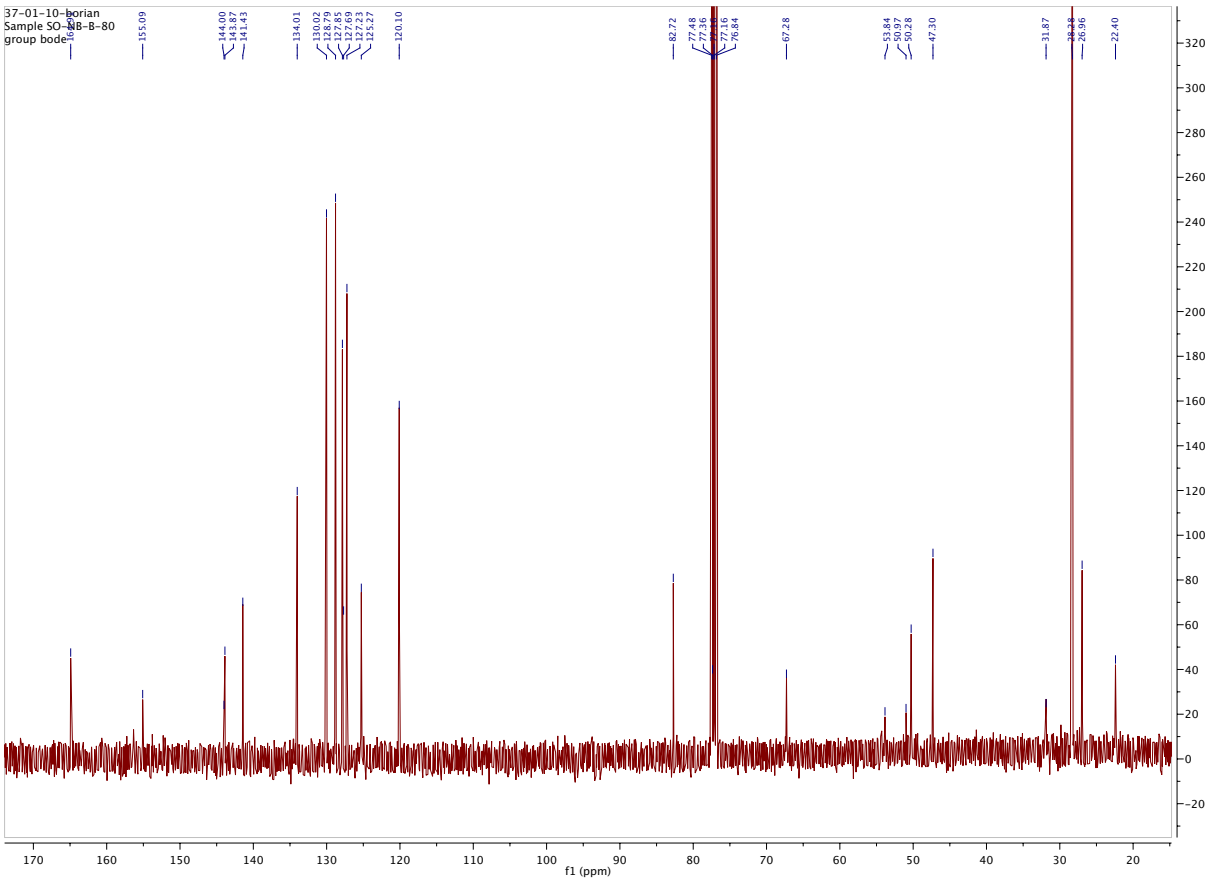


Fig. S22 Expanded ^{13}C-NMR spectrum of compound **1** (in $CDCl_3$, 100 MHz).

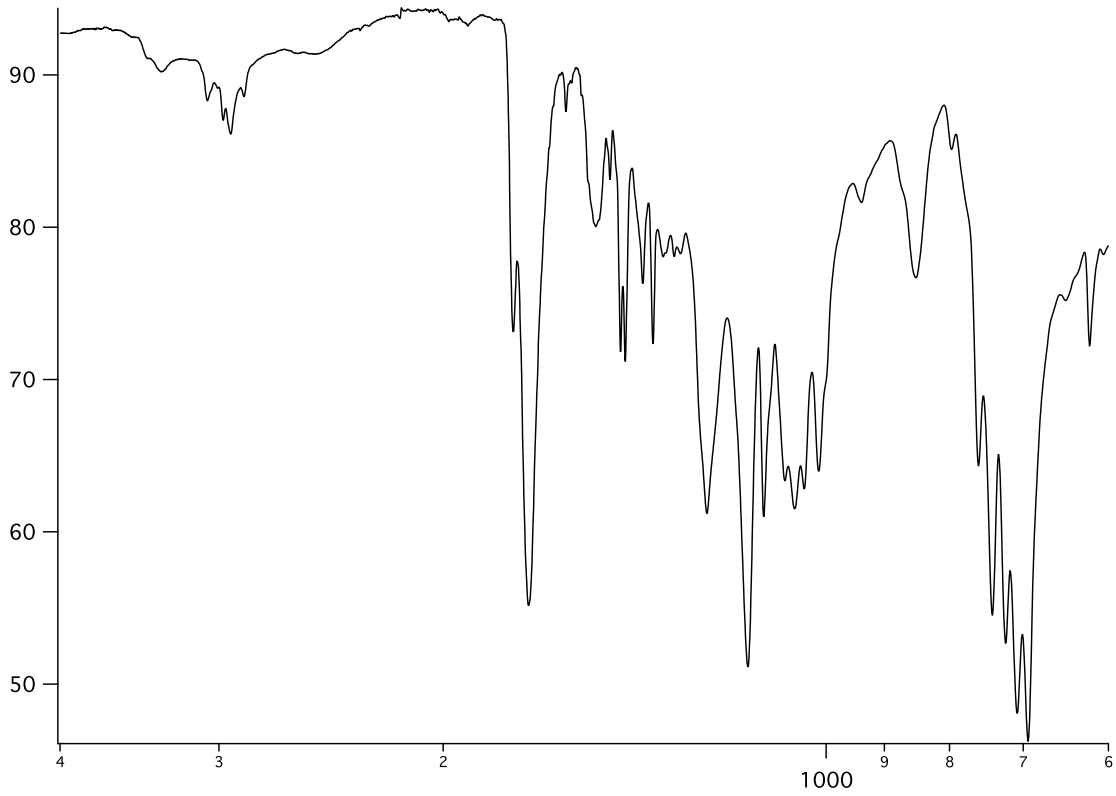
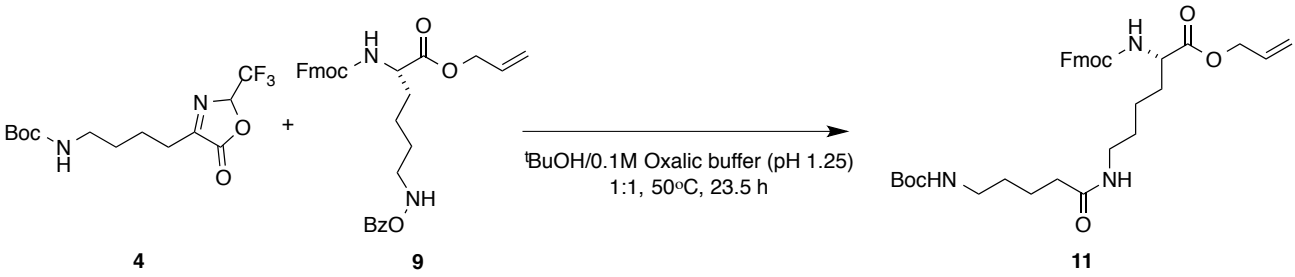


Fig. S23 IR spectrum of compound **1**

2. Test KAHA reaction of 4 and 9



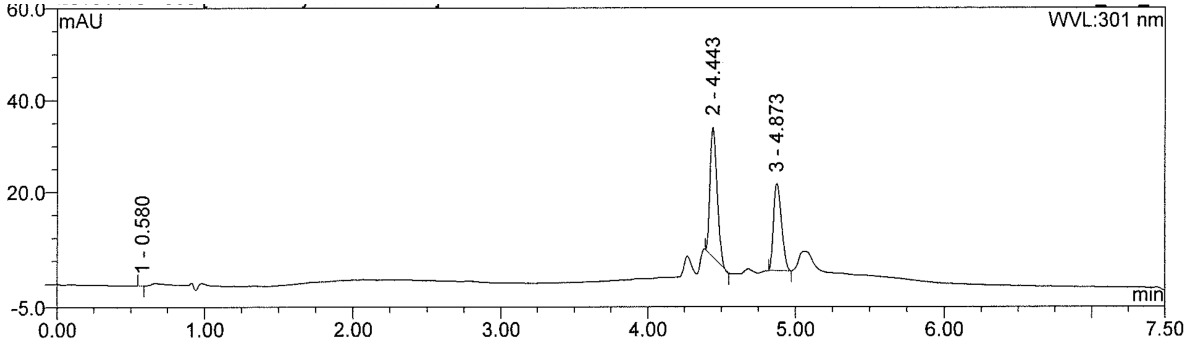


Fig. S24 LC trace of test KAHA ligation reaction mixture (23.5 hours).

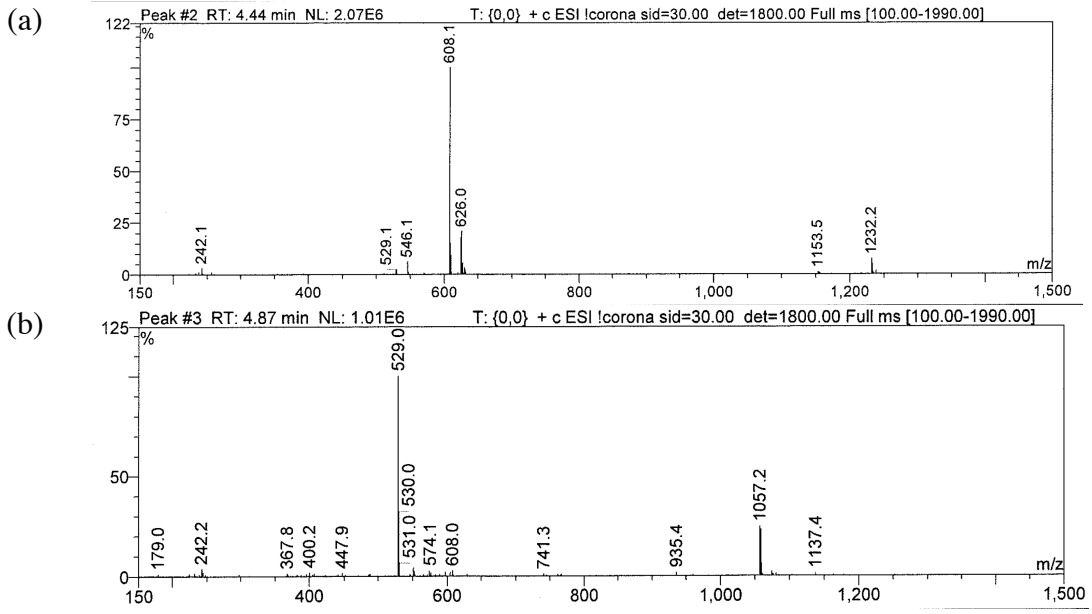
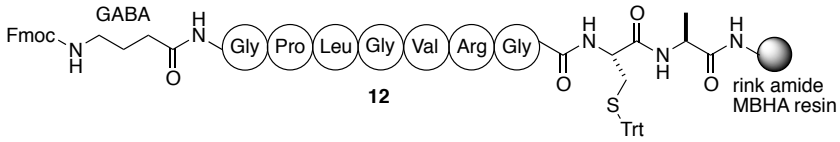


Fig. S25 ESI mass spectra of peaks with retention time at (a) 4.4 (m/z 608, corresponding to [M+H] of **11**), (b) 4.9 (m/z 529, corresponding to [M+H] of **9**.

3. Solid phase peptide synthesis (SPPS)



Fmoc-GABA-Gly-Pro-Leu-Gly-Val-Arg(Pbf)-Gly-Cys(Trt)-Ala-NH_2 on MBHA rink amide resin12.

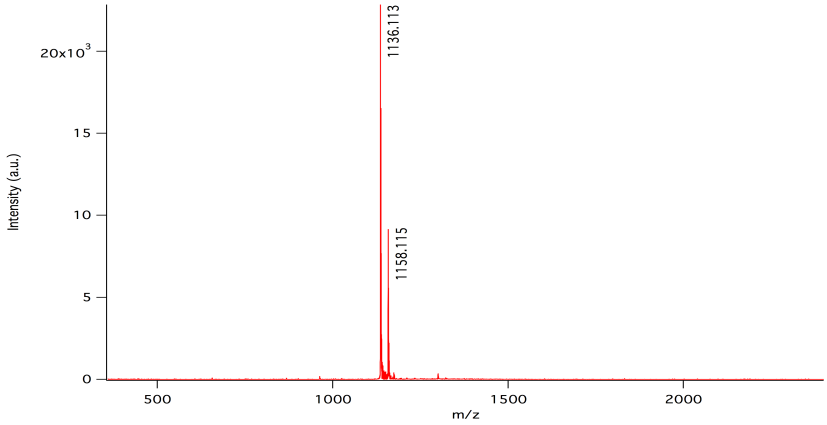
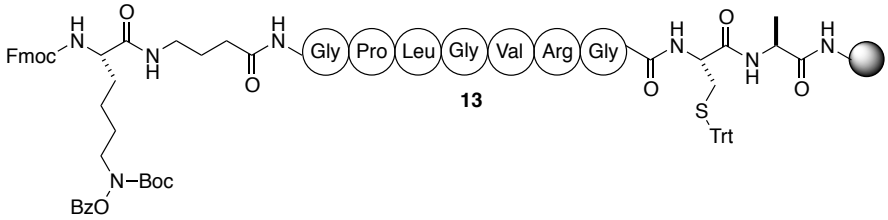


Fig. S26 MALDI spectrum of crude compound **12** (Matrix: HCCA)



Fmoc-Lys(OBz)-GABA-Gly-Pro-Leu-Gly-Val-Arg(Pbf)-Gly-Cys(Trt)-Ala-NH_2 on MBHA rink amide resin 13.

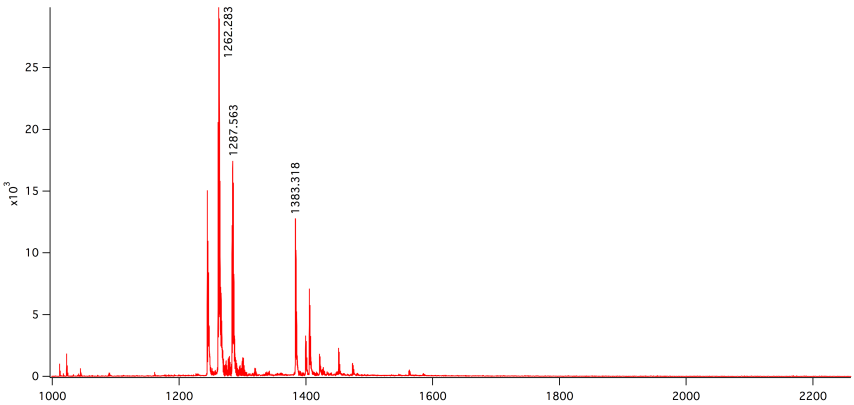
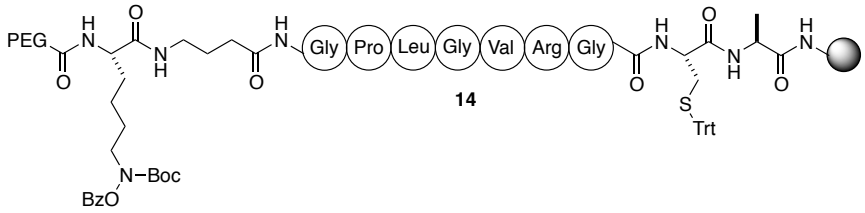


Fig. S27 MALDI spectrum of crude compound **13** (Matrix: HCCA)



MPEG$_{12}$-Fmoc-Lys(OBz)Boc-GABA-Gly-Pro-Leu-Gly-Val-Arg(Pbf)-Gly-Cys(Trt)-Ala-NH$_2$ on MBHA rink amide resin 14.

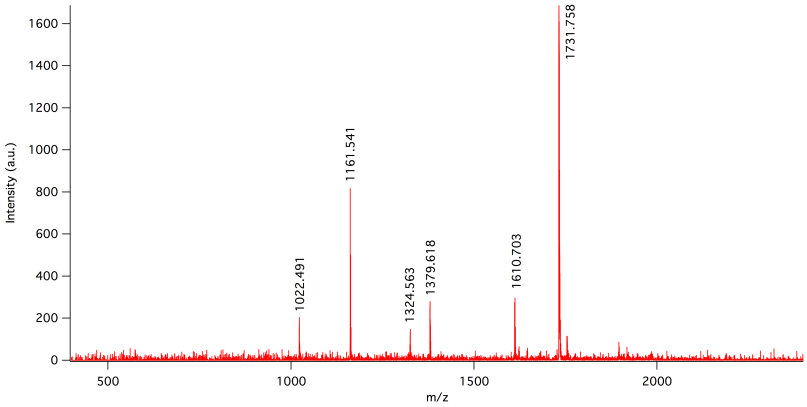
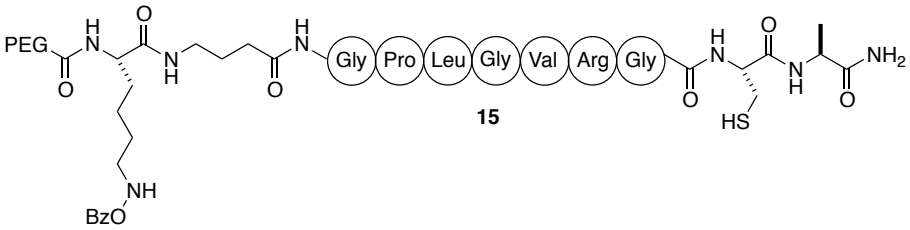


Fig. S28 MALDI spectrum of crude compound **14** (Matrix: HCCA)



MPEG$_{12}$-Fmoc-Lys(OBz)-GABA-Gly-Pro-Leu-Gly-Val-Arg-Gly-Cys-Ala-NH$_2$ 15.

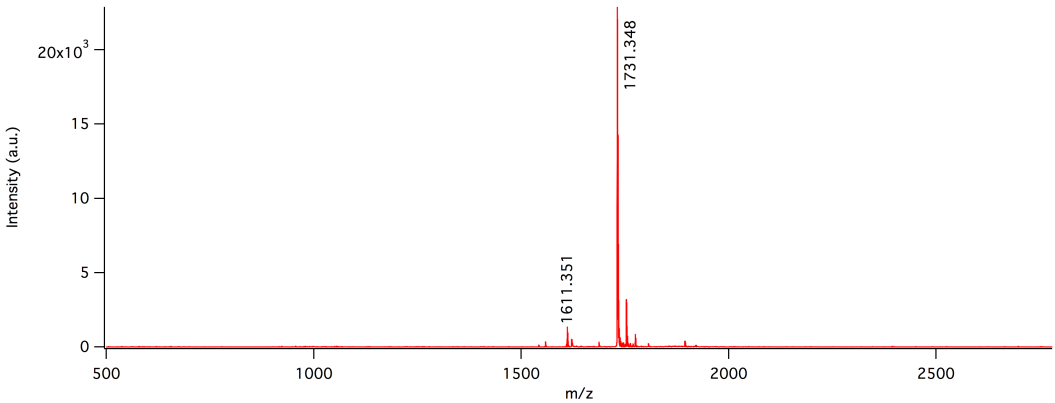
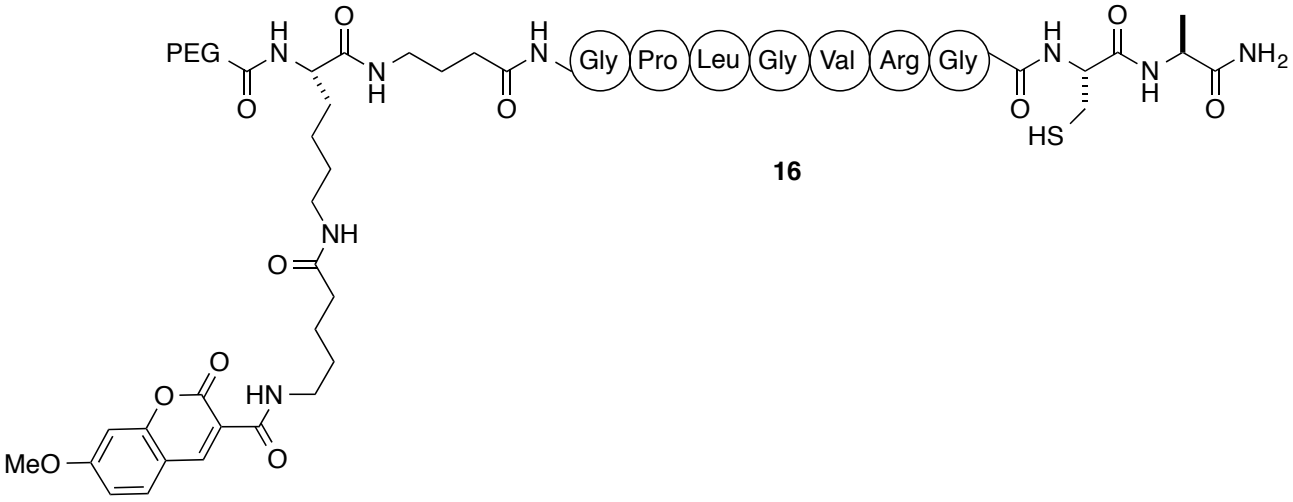


Fig. S29 MALDI-TOF-MS spectrum of compound **15** purified by HPLC (Matrix: HCCA)

4. Peptide modification with FRET acceptor, donor, and polymer



MPEG$_{12}$-Fmoc-Lys(MC)-GABA-Gly-Pro-Leu-Gly-Val-Arg-Gly-Cys-Ala-NH$_2$ 16.

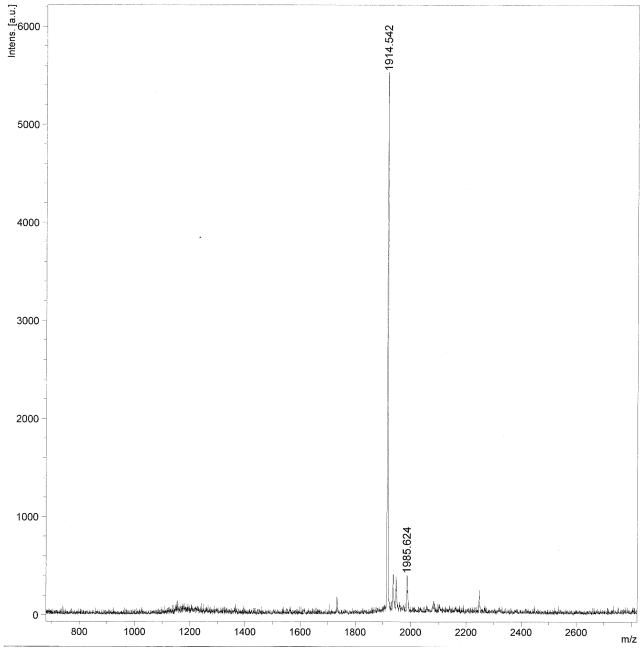
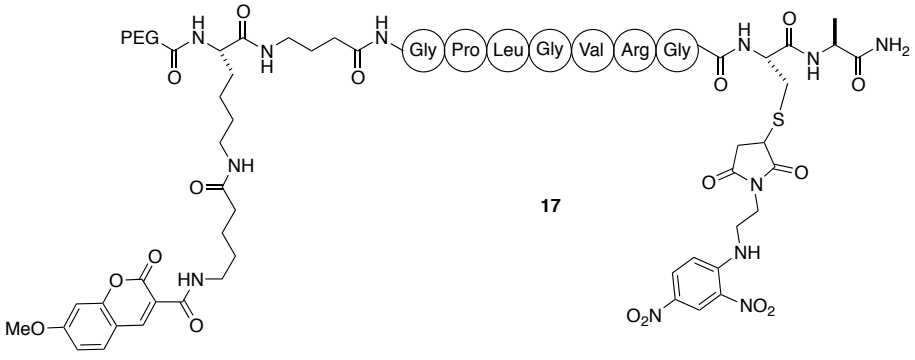


Fig. S30 MALDI spectrum of HPLC purified compound **16** (Matrix: HCCA)



MPEG$_{12}$-Fmoc-Lys(MC)-GABA-Gly-Pro-Leu-Gly-Val-Arg-Gly-Cys(DNP)-Ala-NH$_2$ Probe A 17.

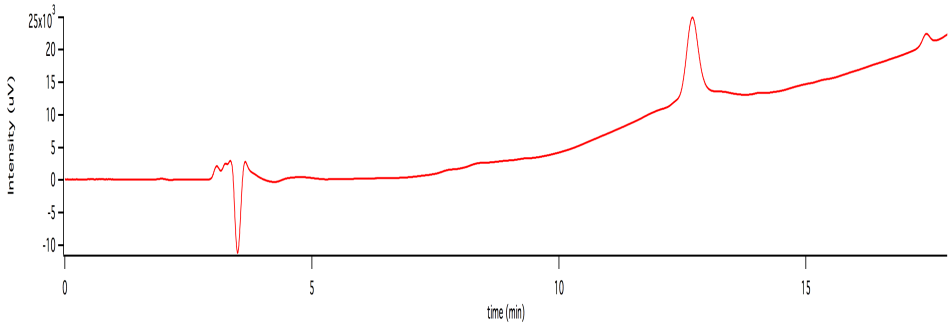


Fig. S31 Analytical HPLC trace of purified compound **17 (probe A)** at 220 nm.

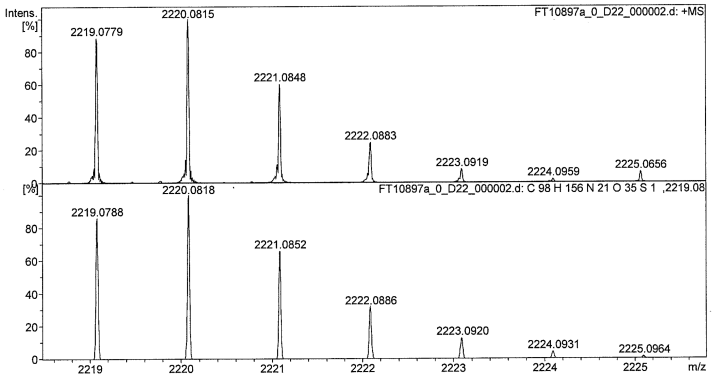


Fig. S32 High resolution MALDI spectrum of purified compound **17 (probe A).**

MPEG$_{12}$-Fmoc-Lys(MC)-GABA-Gly-Val-Arg-Leu-Gly-Pro-Gly-Cys(DNP)-Ala-NH$_2$ Probe B.

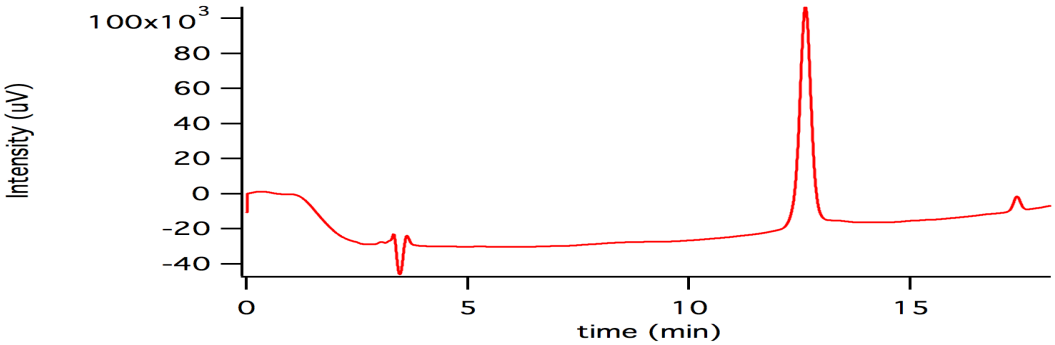


Fig. S33 Analytical HPLC trace of purified **probe B** at 220 nm.

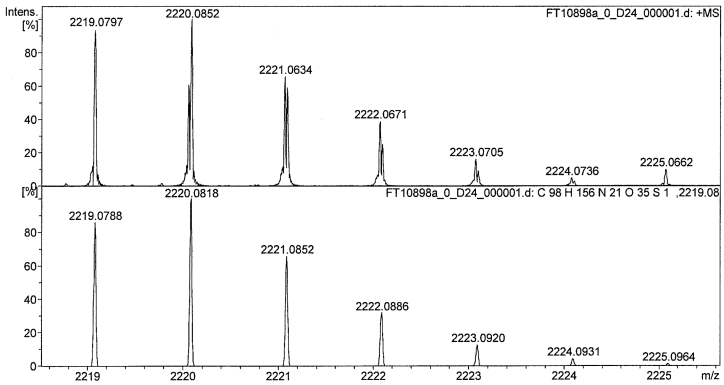


Fig. S34 High resolution MALDI spectrum of purified probe **B.**

(MALDI-TOF *m/z* calcd. 2219.0788 for $C_{98}H_{156}N_{21}O_{35}S$, found 2219. 0797 ($[M+H]^+$)

5. Enzyme test using MMP2

Probe A
$MPEG_{12}$-Fmoc-Lys(MC)-GABA-Gly-Pro-Leu-Gly-Val-Arg-Gly-Cys(DNP)-Ala-NH_2

Probe B
$MPEG_{12}$-Fmoc-Lys(MC)-GABA-Gly-Val-Arg-Leu-Gly-Pro-Gly-Cys(DNP)-Ala-NH_2

Table S1. Reaction set up for Enzyme cleavage experiment

run	Substrate	MMP2	Inhibitor	Reaction time [min] (MS spectra)	Reaction time [min] (fluorescent spectra)
R1	probe A	–	–	0, 120, 200 min (Fig. S35a-c)	0, 240 min (Fig. S42)
R2	probe A	+	–	0, 120, 200 min (Fig. S36a-c)	240 min (Fig. S43)
R3	probe A	+	+	0, 120, 200 min (Fig. S37a-c)	240 min (Fig. S44)
R4	probe B	–	–	0, 120, 200 min (Fig. S38a-c)	0, 240 min (Fig. S45)
R5	probe B	+	–	0, 120, 200 min (Fig. S39a-c)	240 min (Fig. S46)
R6	probe B	+	+	0, 120, 200 min (Fig. S40a-c)	240 min (Fig. S47)

(a)

(b)

(c)

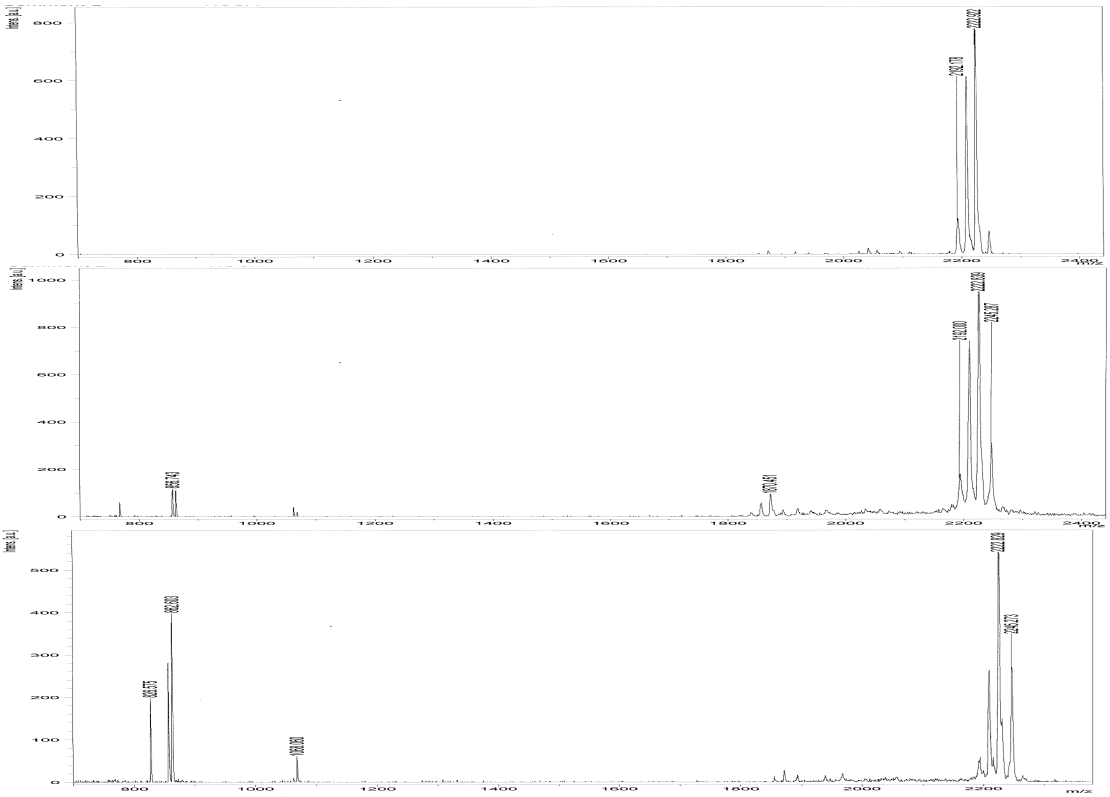


Fig. S35 MALDI-TOF-MS spectra of R1 (a) 0 min, (b) 120 min, (c) 200 min.

(a)

(b)

(c)

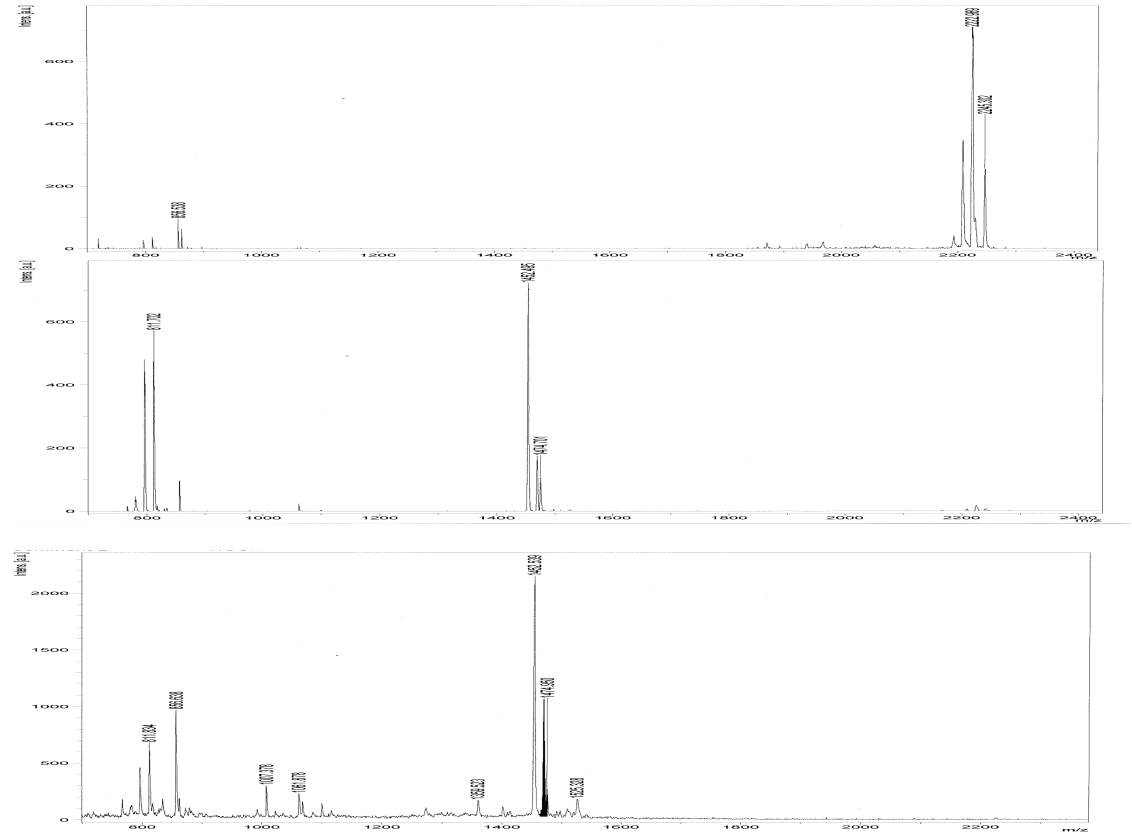


Fig. S36 MALDI-TOF-MS spectra of R2 (a) 0 min, (b) 120 min, (c) 200 min.

(a)

(b)

(c)

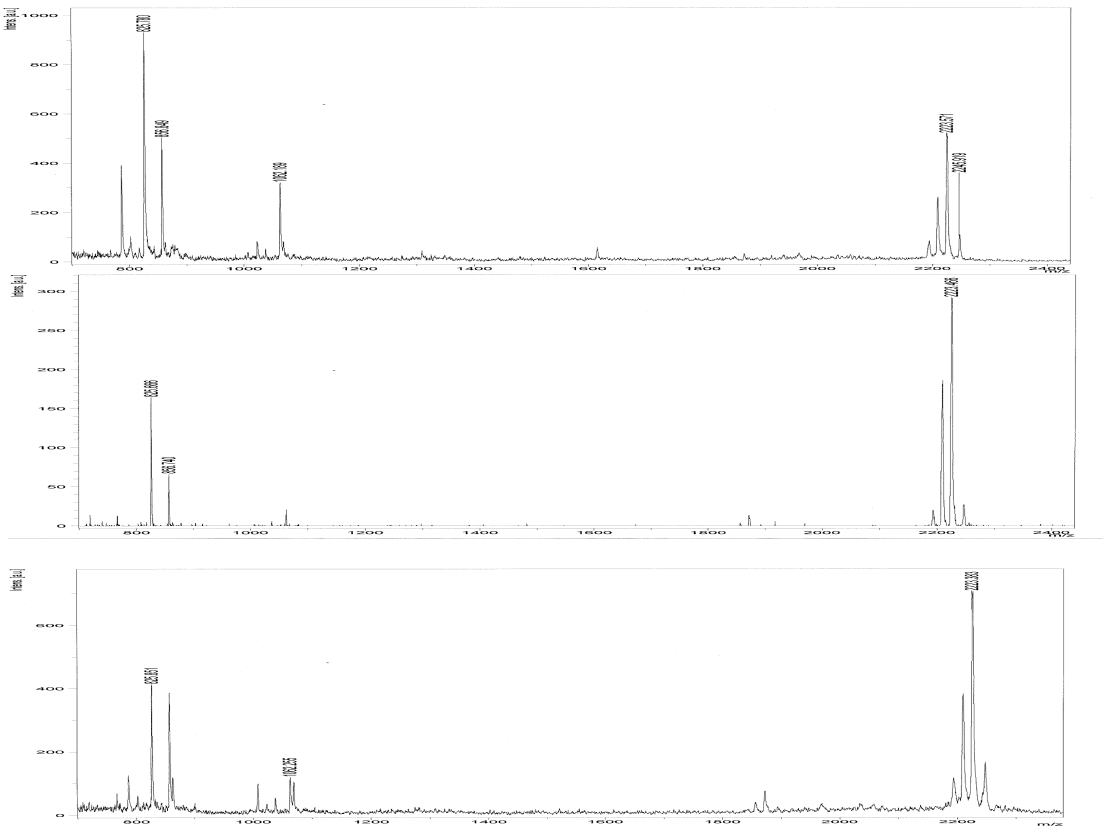


Fig. S37 MALDI-TOF-MS spectra of R3 (a) 0 min, (b) 120 min, (c) 200 min.

(a)

(b)

(c)

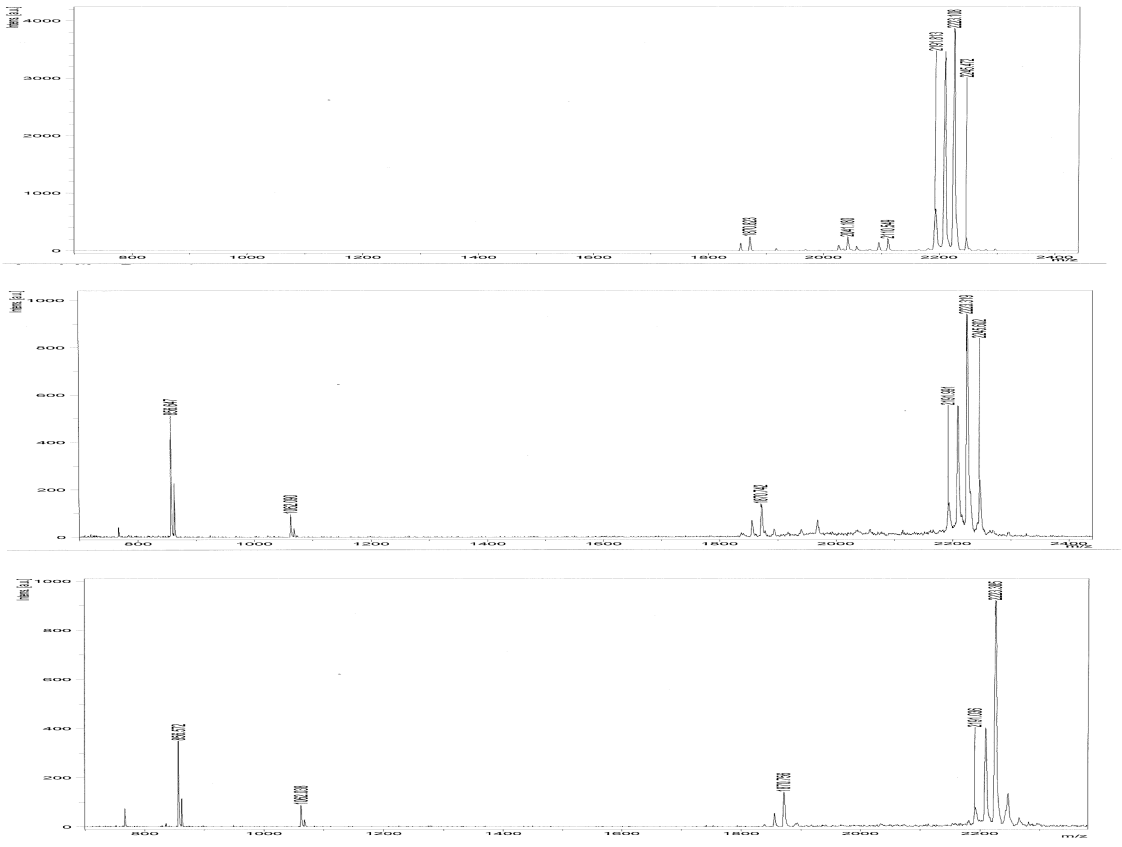


Fig. S38 MALDI-TOF-MS spectra of R4 (a) 0 min, (b) 120 min, (c) 200 min.

(a)

(b)

(c)

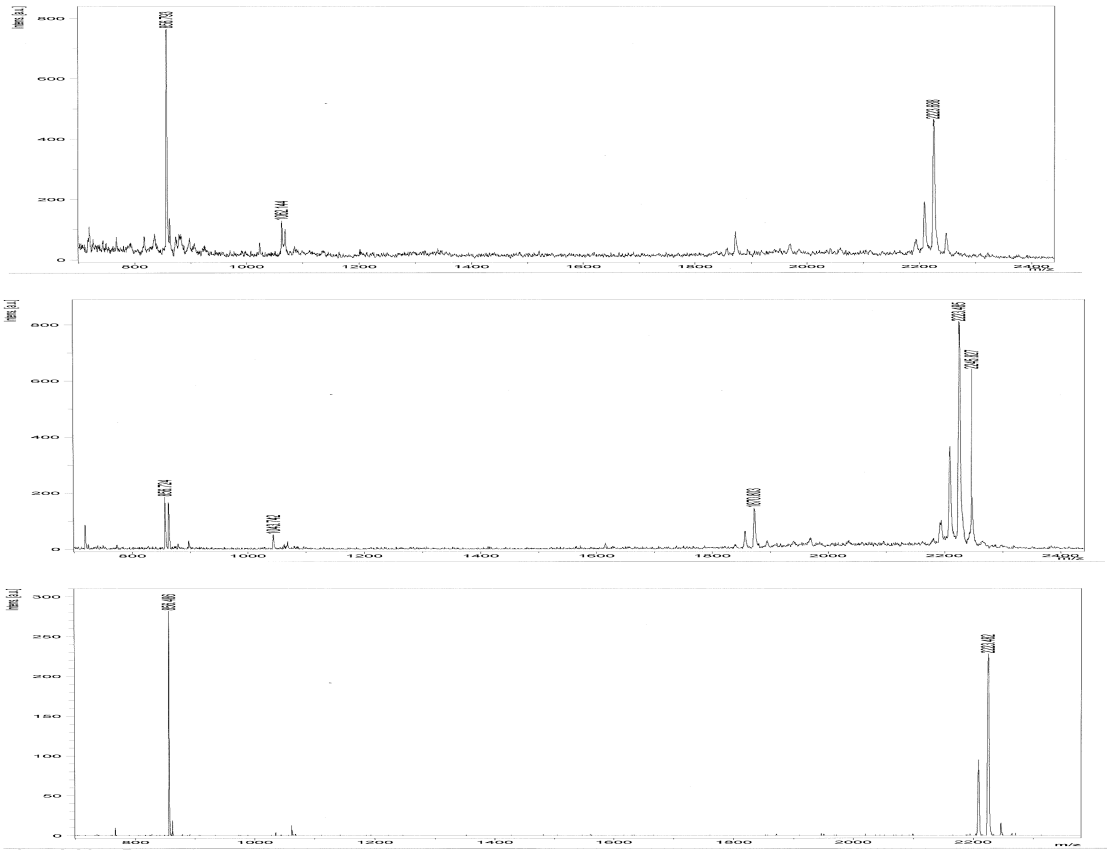


Fig. S39 MALDI-TOF-MS spectra of R5 (a) 0 min, (b) 120 min, (c) 200 min.

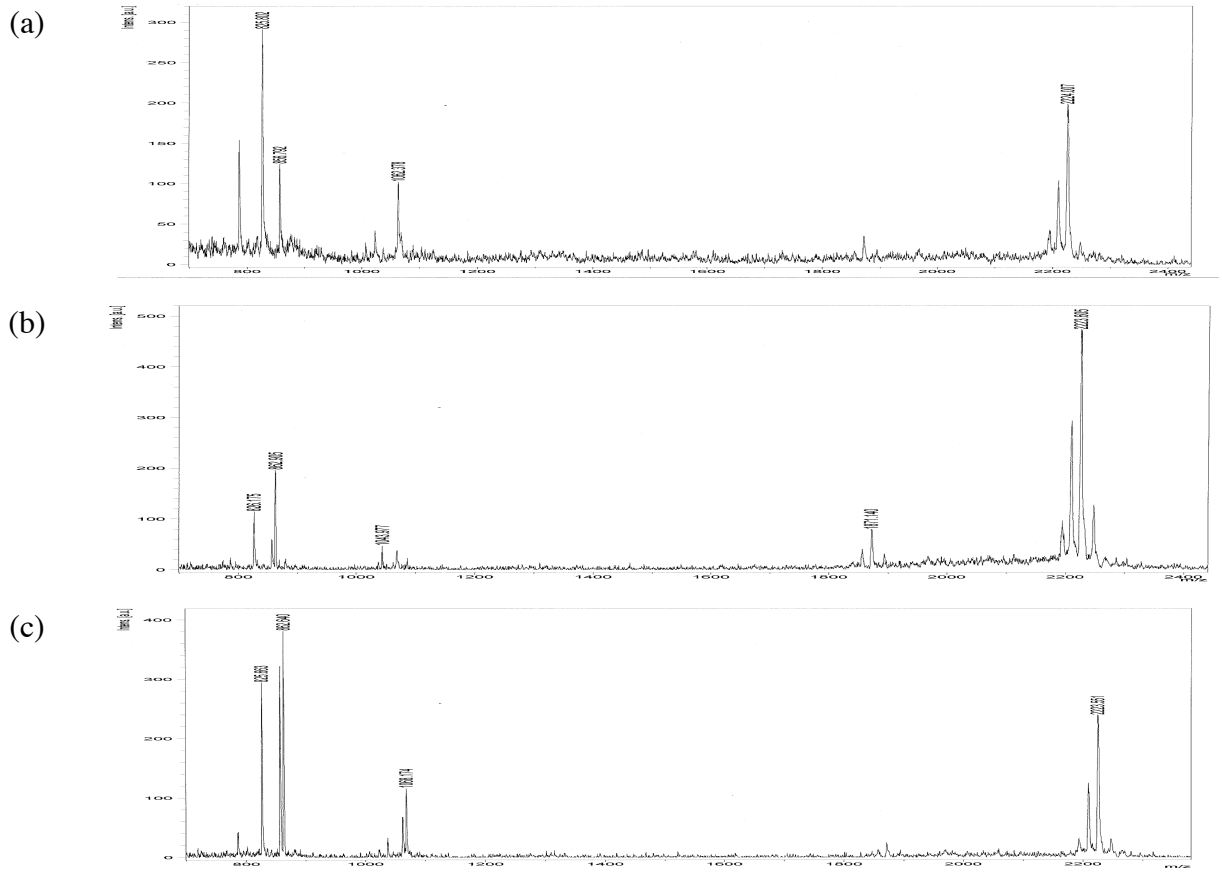


Fig. S40 MALDI-TOF-MS spectra of R6 (a) 0 min, (b) 120 min, (c) 200 min.

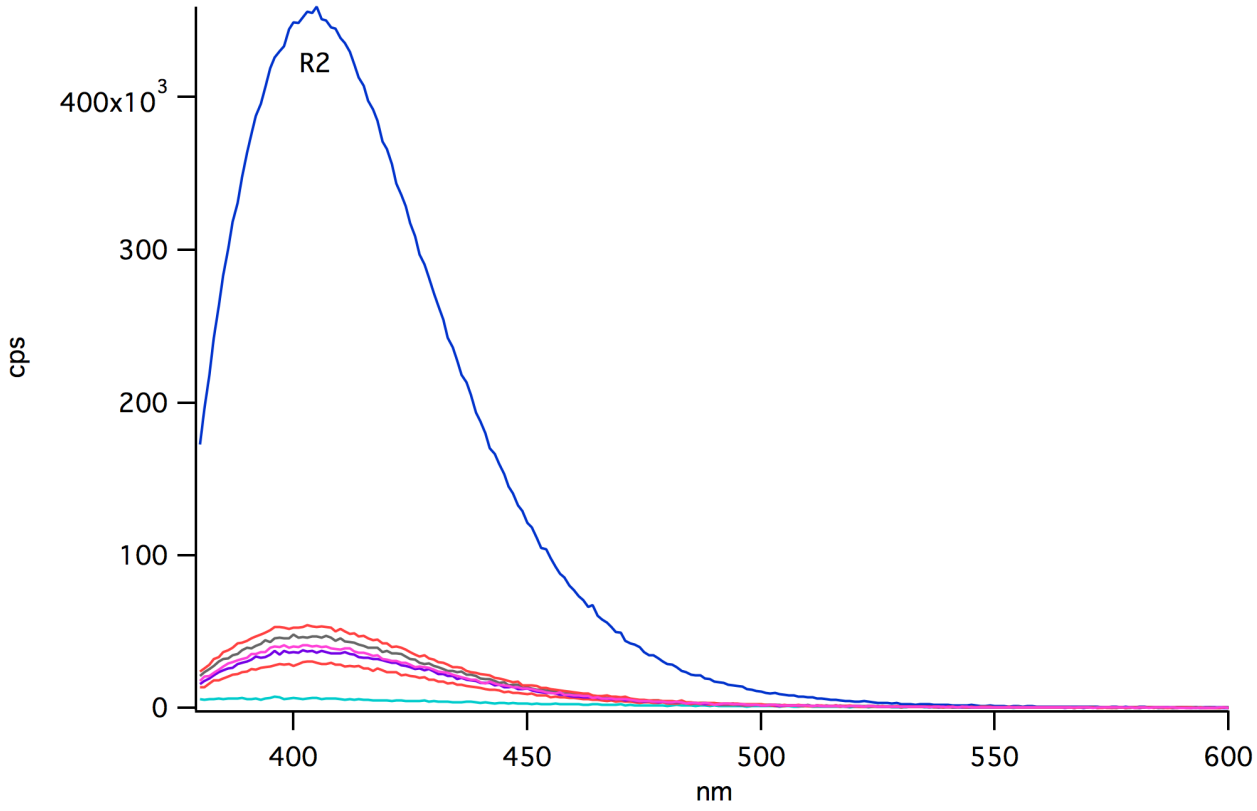


Fig. S41 Overlaid Fluorescence Spectra of R1-R6 (4 hr)

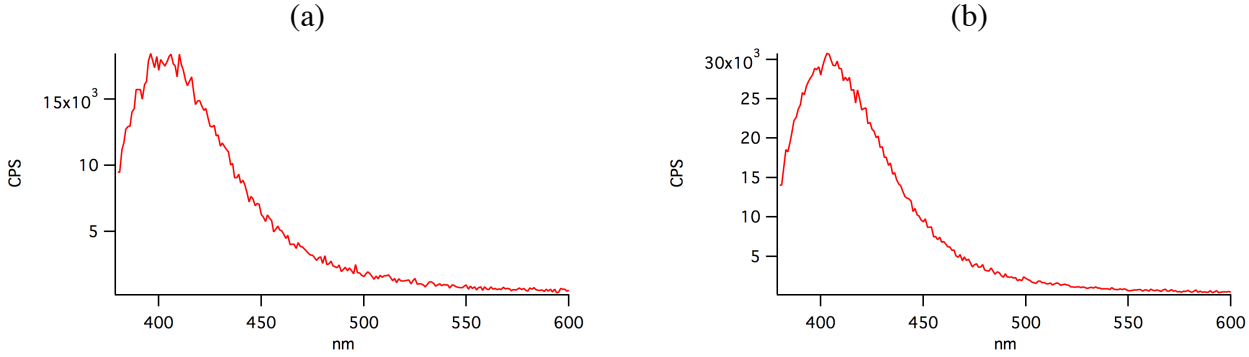


Fig. S42 Fluorescence Spectra of R1 (a) 0 hr (b) 4 hrs.

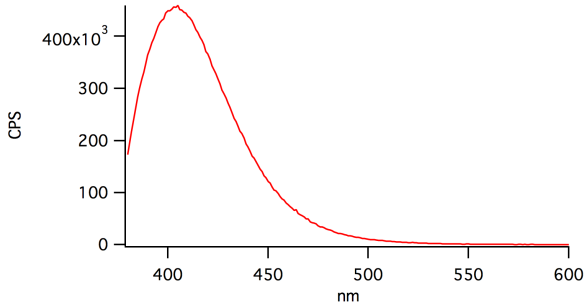


Fig. S43 Fluorescence Spectra of R2 (4 hr).

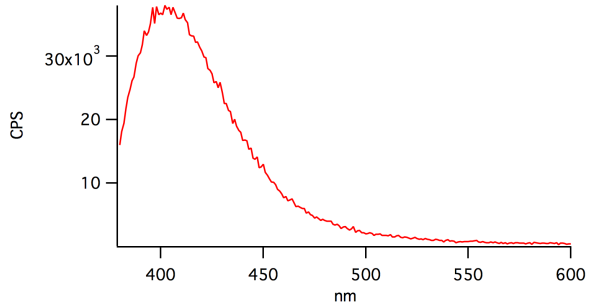


Fig. S44 Fluorescence Spectra of R3 (4 hr).

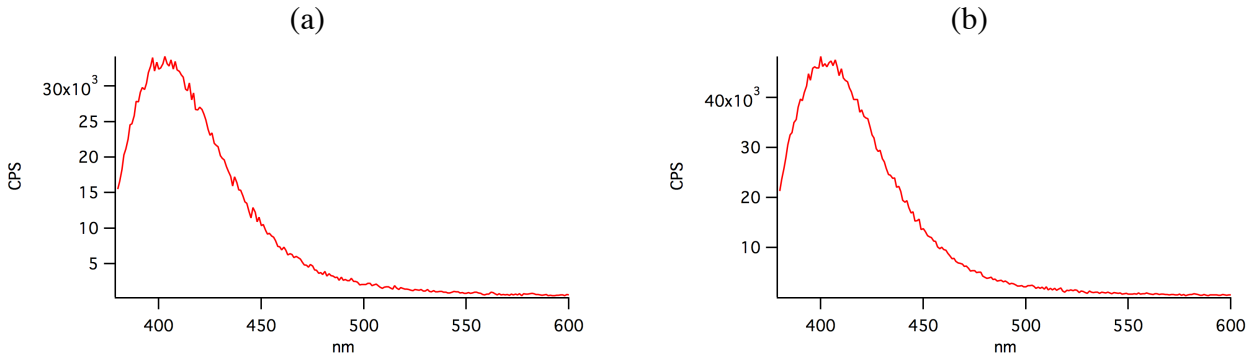


Fig. S45 Fluorescence Spectra of R4 (a) 0 hr (b) 4 hrs.

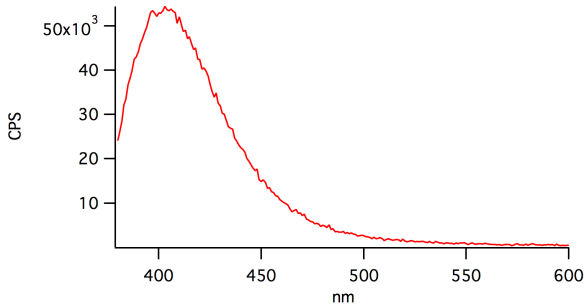


Fig. S46 Fluorescence Spectra of R5 (4 hr).

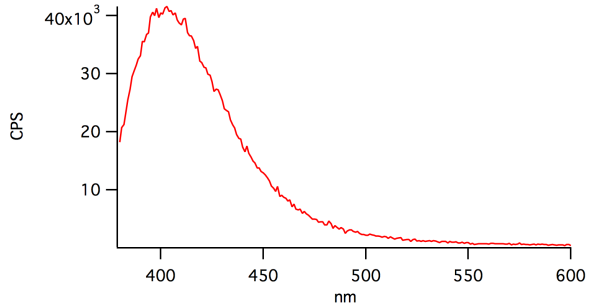


Fig. S47 Fluorescence Spectra of R6 (4 hr).