Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supplementary Information

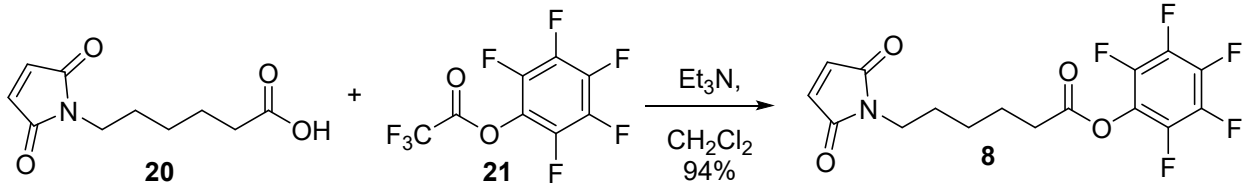
Albumin-ruthenium catalyst conjugate for bio-orthogonal uncaging of alloc group

Kimberly S. Taylor, Madison M. McMonagle, Schaelee C. Guy, Ariana M. Human-McKinnon, Shumpei Asamizu, Heidi J. Fletcher, Bradley W. Davis, and Takashi L. Suyama*

Table of Contents

General Experimental Procedures

Nuclear magnetic resonance (NMR) spectra were acquired on a JEOL ECZS 400 MHz spectrometer. ^{1}H NMR reference values were 7.26 ppm for $CDCl_3$, 2.50 ppm for DMSO-d_6, and 3.31 ppm for MeOH-d_4. ^{13}C NMR reference values were 77.16 ppm for $CDCl_3$, 39.52 ppm for DMSO-d_6, and 49.00 ppm for MeOH-d_4. ^{19}F NMR signals are reported without correction. Thermo VarioScan 3020 plate reader was used to acquire all fluorescence data. ESI-LCMS data were obtained on Shimadzu LCMS-2020 equipped with D2-W PDA. High resolution mass spectrometry (HRMS) data were acquired on Thermo Scientific Q Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometer at West Virginia University Shared Facilities. All TLC analyses were conducted with glass-backed silica gel 60 plates with a 254 nm fluorescent dye from EMD Millipore. Thermo Scientific Pierce™ 0.5 mL 10K MWCO PES protein concentrators were used. All reagents and solvents were purchased from Fisher Scientific. Triethylamine and CH_2Cl_2 were distilled from CaH_2 prior to use. TFA was distilled immediately before use. Dimethylformamide (DMF) and 1,4-dioxane stored over molecular sieves were purchased and used as-is. All other reagents and solvents were used as purchased. Unless otherwise specified, flash column chromatography was performed on a Büchi Flasy Advance system equipped with PDA and ELSD detectors.



The pentafluoro ester **8** was prepared loosely following the reported procedures.[1] Pentafluorophenyl trifluoroacetate (1.45 mL. 8.52 mmol) was added to a solution of 6-maleimidohexanoic acid (1.510 g, 7.102 mmol) in CH_2Cl_2 (30 mL). The reaction mixture was cooled to 0 ˚C and triethylamine (3.5 ml, 25 mmol) at which point a color change to orange was observed. The reaction was allowed to stir under nitrogen overnight. It was then diluted with dichloromethane and washed with 1 N $NaHSO_4$ followed by saturated $NaHCO_3$. The combined organic layers were dried over Na_2SO_4, then decanted and concentrated. Purification by column chromatography (100% hexanes to 50% EtOAc/hexanes) afforded the product as an off white solid (2.528 g, 6.701 mmol, 94%). Characterization of this product **8** was consistent with literature.[2]

Physical State: off-white solid

TLC R_f Value: 0.53 (1:2 EtOAc/hexanes, stained by $KMnO_4$)

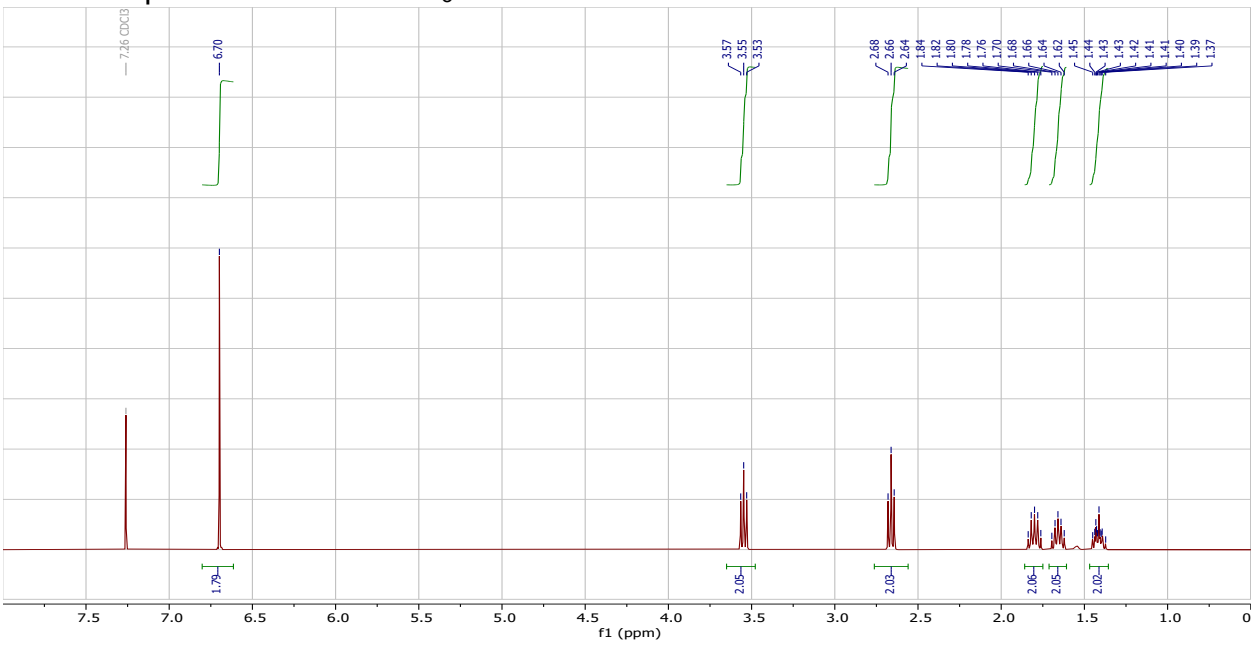
^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.70 (s, 2H), 3.55 (t, *J* = 7.2 Hz, 2H), 2.66 (t, *J* = 7.4 Hz, 2H), 1.80 (dt, *J* = 15.2, 7.5 Hz, 2H), 1.66 (tt, *J* = 7.8, 6.5 Hz, 2H), 1.47 – 1.35 (m, 2H).

^{13}C-NMR (101 MHz, $CDCl_3$): δ 171.0, 169.4, 134.2, 37.6, 33.2, 28.3, 26.1, 24.3.

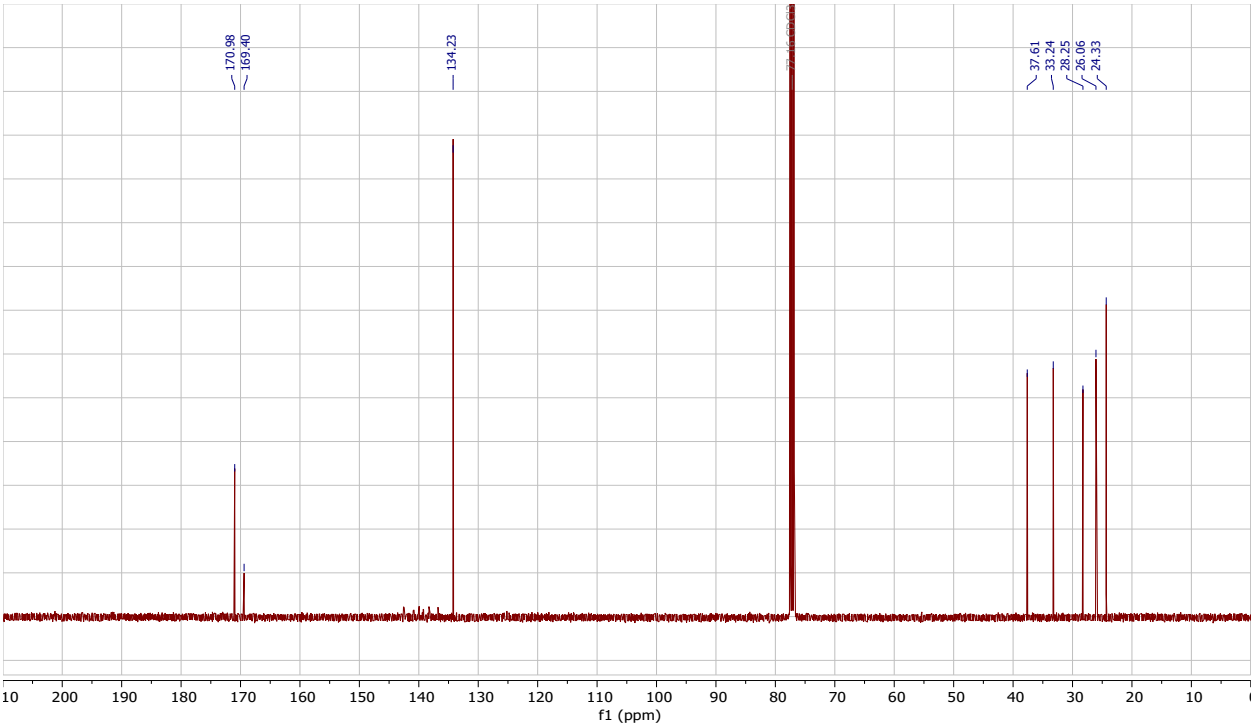
^{19}F NMR (376 MHz, $CDCl_3$): δ -152.6 (m, 2F), -157.9 (t, 1F), -162.2 (m, 2F).

HRMS (ESI pos): $C_{16}H_{12}F_5NO_4Na$ $[M+Na]^+$ calcd 400.05787, found 400.0570.

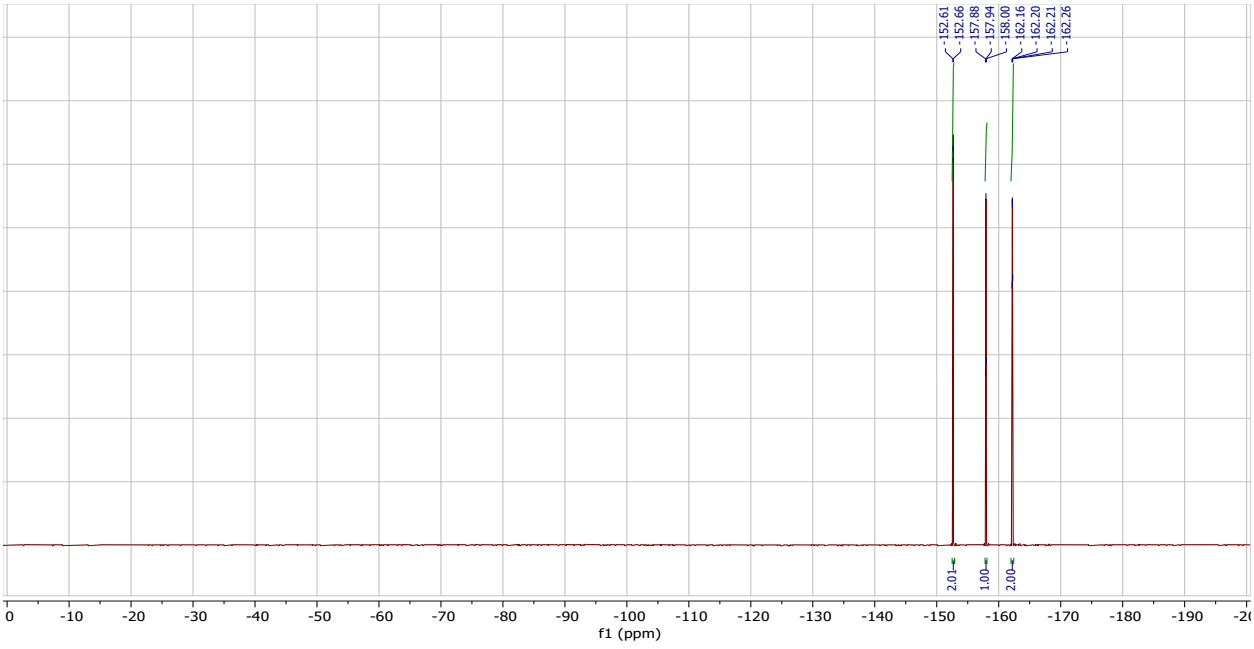
^{1}H NMR spectrum of **8** in $CDCl_3$

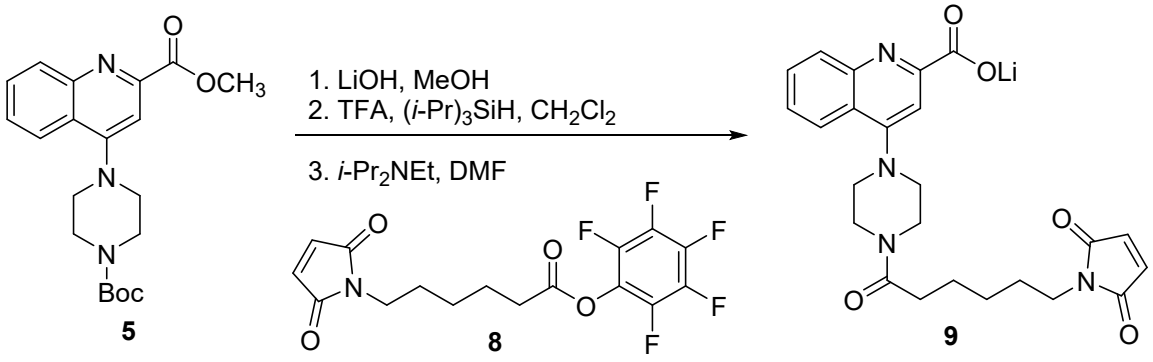


^{13}C NMR spectrum of **8** in $CDCl_3$



^{19}F NMR spectrum of **8** in $CDCl_3$





The methyl ester **5** was prepared according to the literature.[3] To a solution of the methyl ester **5** (177 mg, 0.476 mmol) in H_2O/MeOH (1:49 mixture, 10 mL) under a N_2 atmosphere at rt was added LiOH (13.6 mg, 0.568 mmol, 1.2 eq). The mixture was stirred overnight. Upon confirming complete consumption of the starting material by TLC, the volatiles were removed under vacuum. Then the residues were suspended in benzene (5 mL) and again the volatiles were removed under vacuum to yield 147.5 mg of crude oil. This material was dissolved in CH_2Cl_2 (12 mL) and under a N_2 atmosphere and under stirring, TFA (6 mL) was added at rt. The mixture turned red-orange immediately. After 30 min, the volatiles were removed on a rotary evaporator. Then benzene (10 mL) was added and the volatiles were removed again on a rotary evaporator to yield a red-orange oil. This crude material was dissolved in anhydrous 1,4-dioxane (10 mL) and under a N_2 atmosphere and stirring, the pentafluorophenyl ester **8** (197 mg, 0.522 mmol) and *i*Pr_2NEt (820 µL, 4.82 mmol) were added at 0 °C. The mixture was let warm to rt overnight. Then the mixture was filtered through a pad of Celite®, which was washed with 1,4-dioxane. The volatiles were removed to yield a crude orange oil (700 mg). A portion of this crude (77 mg) was purified on a HPLC equipped with a biphenyl reverse phase column with a gradient mobile phase ranging from 3:7 MeOH/H_2O to 8:2 to obtain a 36 mg of impure zwitterionic product. Any further purification proved to be difficult. This crude material was used for the uncaging experiment without further purification.

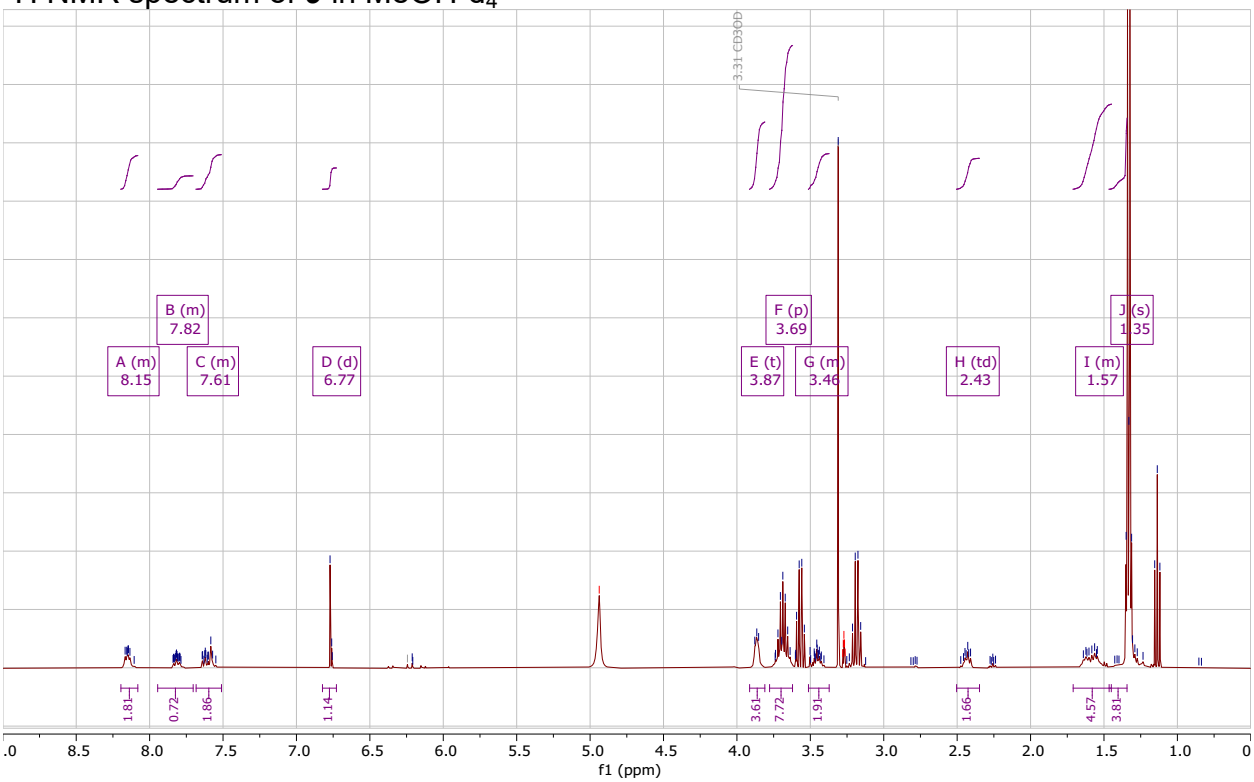
Physical State: orange oil

^{1}H-NMR (400 MHz, MeOH-d_4): δ 8.20 – 8.08 (m, 2H), 7.95 – 7.70 (m, 1H), 7.68 – 7.51 (m, 2H), 6.77 (d, *J* = 4.5 Hz, 2H), 3.87 (t, *J* = 5.2 Hz, 4H), 3.69 (m, 4H), 3.51 – 3.37 (m, 2H), 2.43 (td, *J* = 7.4, 4.6 Hz, 2H), 1.71 – 1.45 (m, 4H), 1.35 (m, 2H).

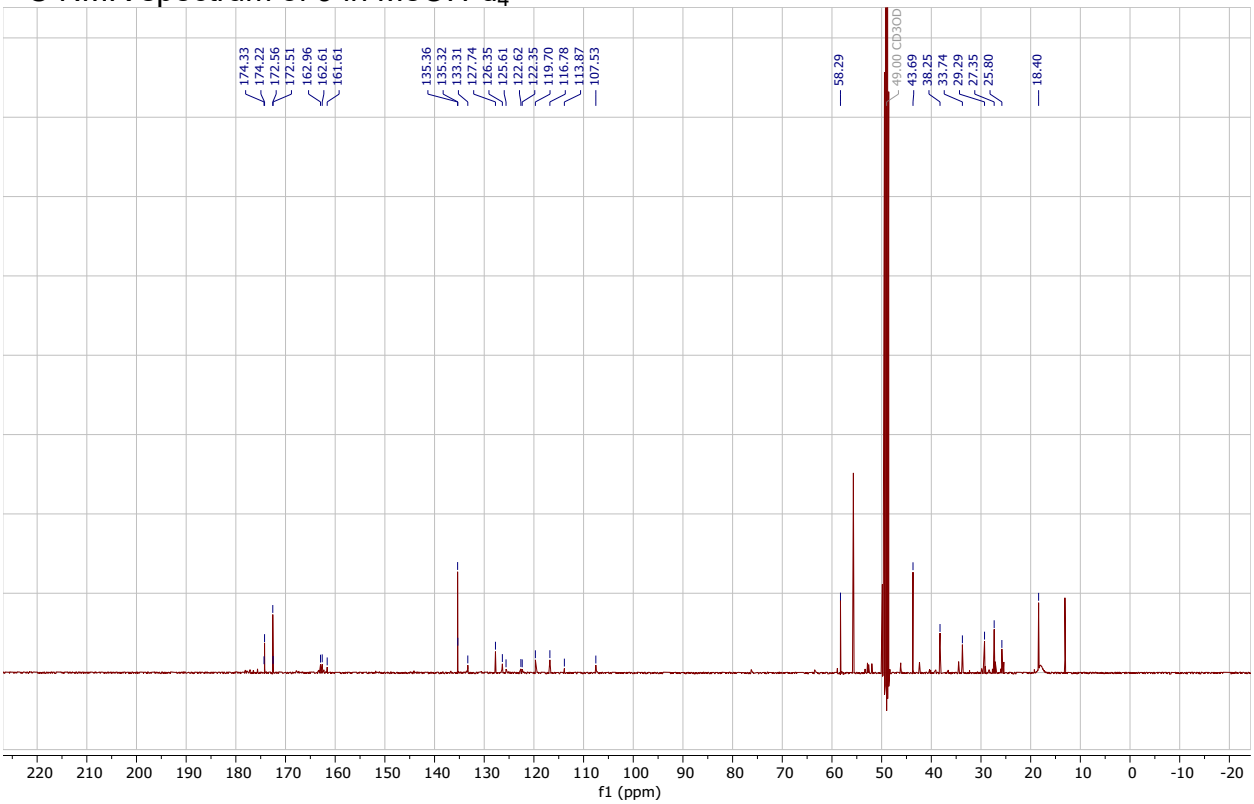
^{13}C-NMR (101 MHz, $CDCl_3$): δ (rotamers present) 174.3, 174.2, 172.6, 172.5, 163.0, 162.6, 161.6, 135.4, 135.3, 133.3, 127.7, 126.3, 125.6, 122.6, 122.4, 119.7, 116.8, 113.9, 107.5, 58.3, 43.7, 38.2, 33.7, 29.3, 27.3, 25.8, 18.4.

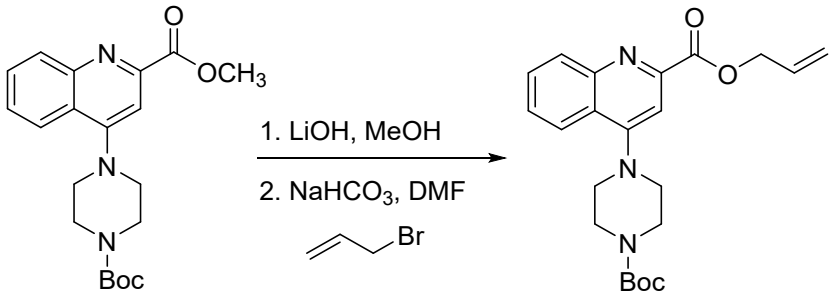
HRMS (ESI pos): $C_{24}H_{26}LiN_4O_5$ $[M+H]^+$ calcd 457.20578, found 457.2042.

^{1}H NMR spectrum of **9** in MeOH-d_4



^{13}C NMR spectrum of **9** in MeOH-d_4





5 **7**

To a solution of the methyl ester **5** (360 mg, 0.969 mmol, prepared according to literature[3]) in MeOH (10 mL) at 0 °C was added LiOH (24 mg, 1.0 mmol, 1.03 eq) under a N_2 atmosphere with vigorous stirring. This reaction mixture was allowed to warm to rt over night. Then the volatiles were removed under vacuum. EtOH (10 mL) was added and the volatiles were removed under vacuum to yield 321 mg of crude red-brown solid. This material was suspended in anhydrous DMF (10 mL) under a N_2 atmosphere. At rt, allyl bromide (140 µL, 1.65 mmol, 1.7 eq) and K_2CO_3 (241 mg, 1.74 mmol, 1.8 eq) were added under stirring. The mixture was allowed to react overnight. Upon diluting with EtOAc (50 mL) and water (20 mL) and mixing well, the phases were separated. The aqueous phase was extracted with EtOAc (20 mL) twice. The combined organic layers were dried over Na_2SO_4. Upon removing the volatiles under vacuum, a crude orange oil was obtained. This material was subjected to flash column chromatography with a gradient mobile phase (1:9 to 1:1 EtOAc/hexanes) to obtain 238 mg of thick pale orange gum **7** (0.599 mmol, 62% yield over 2 steps).

Physical State: pale orange thick gum

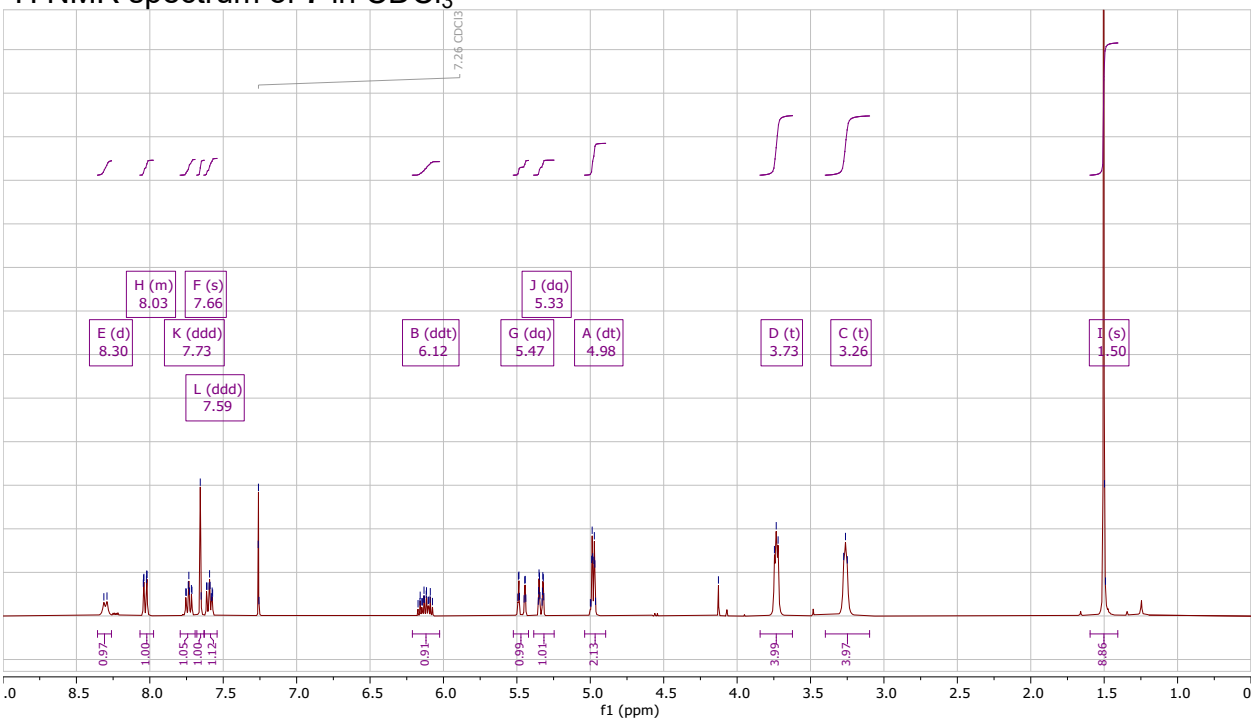
TLC R_f Value: 0.56 (1:1 EtOAc/hexanes)

^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.30 (d, *J* = 8.5 Hz, 1H), 8.07 – 7.97 (m, 1H), 7.73 (ddd, *J* = 8.4, 6.8, 1.4 Hz, 1H), 7.66 (s, 1H), 7.59 (ddd, *J* = 8.3, 6.8, 1.3 Hz, 1H), 6.12 (ddt, *J* = 17.0, 10.3, 5.8 Hz, 1H), 5.47 (dq, *J* = 17.2, 1.5 Hz, 1H), 5.33 (dq, *J* = 10.4, 1.2 Hz, 1H), 4.98 (dt, *J* = 6.0, 1.3 Hz, 2H), 3.73 (t, *J* = 5.0 Hz, 4H), 3.26 (t, *J* = 5.0 Hz, 4H), 1.50 (s, 9H).

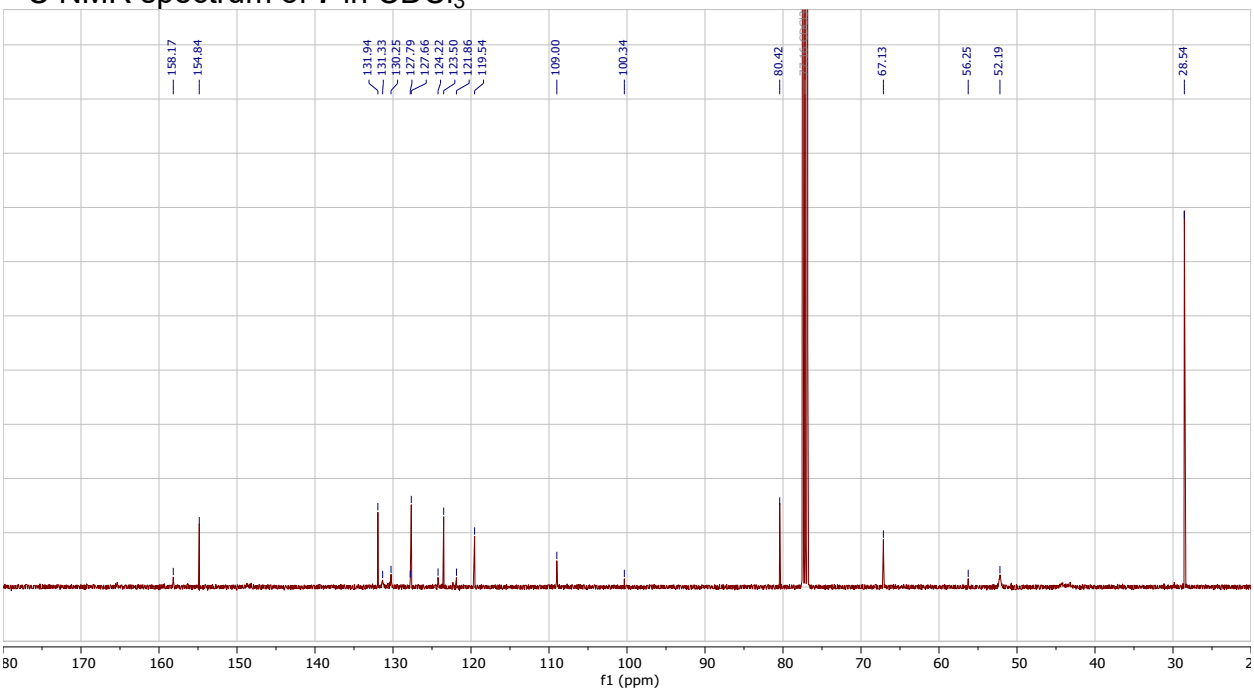
^{13}C-NMR (101 MHz, $CDCl_3$): δ 158.1, 154.8, 131.9, 131.3, 130.2, 127.8, 127.6, 124.2, 123.5, 121.8, 119.5, 109.0, 100.3, 80.4, 67.1, 56.2, 52.2, 28.5.

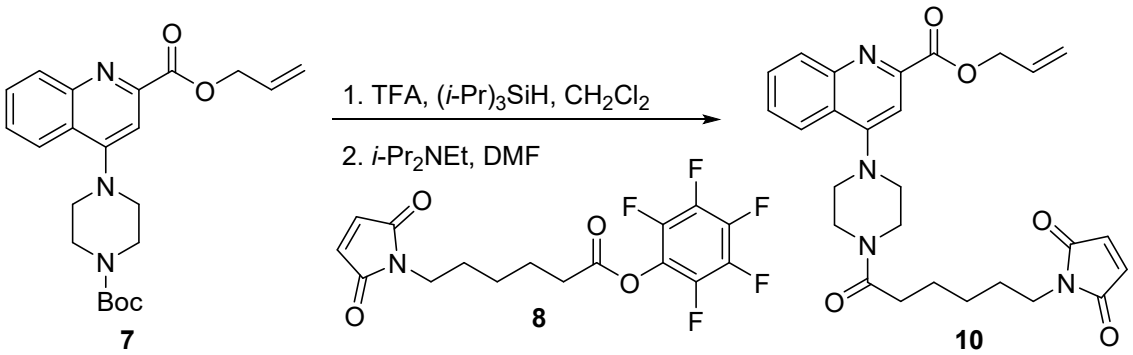
HRMS (ESI pos): $C_{22}H_{28}N_3O_4$ $[M+H]^+$ calcd 398.20743, found 398.2062.

^{1}H NMR spectrum of **7** in $CDCl_3$



^{13}C NMR spectrum of **7** in $CDCl_3$





To a solution of the Boc-carbamate **7** (225 mg, 0.566 mmol) in CH_2Cl_2 (3 mL) at rt were added triisopropylsilane (233 µL, 1.13 mmol, 2.0 eq) and TFA (3 mL) under a N_2 atmosphere while stirring. After 1.5 hrs, the volatiles were removed under vacuum. The red-orange gummy residue was dissolved in CH_2Cl_2 (1.0 mL) and a few mL of Et_2O was added to yield an off-white precipitate. Upon diluting with a few mL of hexanes, the solid was filtered (292 mg). This crude amophous orange solid was dissolved in anhydrous DMF (3 mL) under a N_2 atmosphere. While stirring, diisopropylethylamine (495 µL, 2.83 mmol, 5.0 eq) and the pentafluorophenyl ester **8** (211 mg, 0.559 mmol, 1.0 eq) were added. The mixture was allowed to react for 36 hours at rt. Upon removal of the volatiles under vacuum, the mixture was diluted with EtOAc (50 mL) and washed with saturated $NaHCO_3$ (30 mL) twice, water (30 mL), and brine (30 mL) successively. The organic layer was dried over Na_2SO_4. After removal of the solvent under vacuum, the residue was subjected to flash column chromatography with a gradient mobile phase (1:3 EtOAc/hexanes to 100% EtOAc then to 1:24 MeOH/EtOAc). Upon pooling fractions containing the product identified by TLC and removing the solvents under vacuum, a pale brown oil **10** (254 mg, 0.518 mmol, 91% over 2 steps) was obtained.

Physical State: pale brown oil

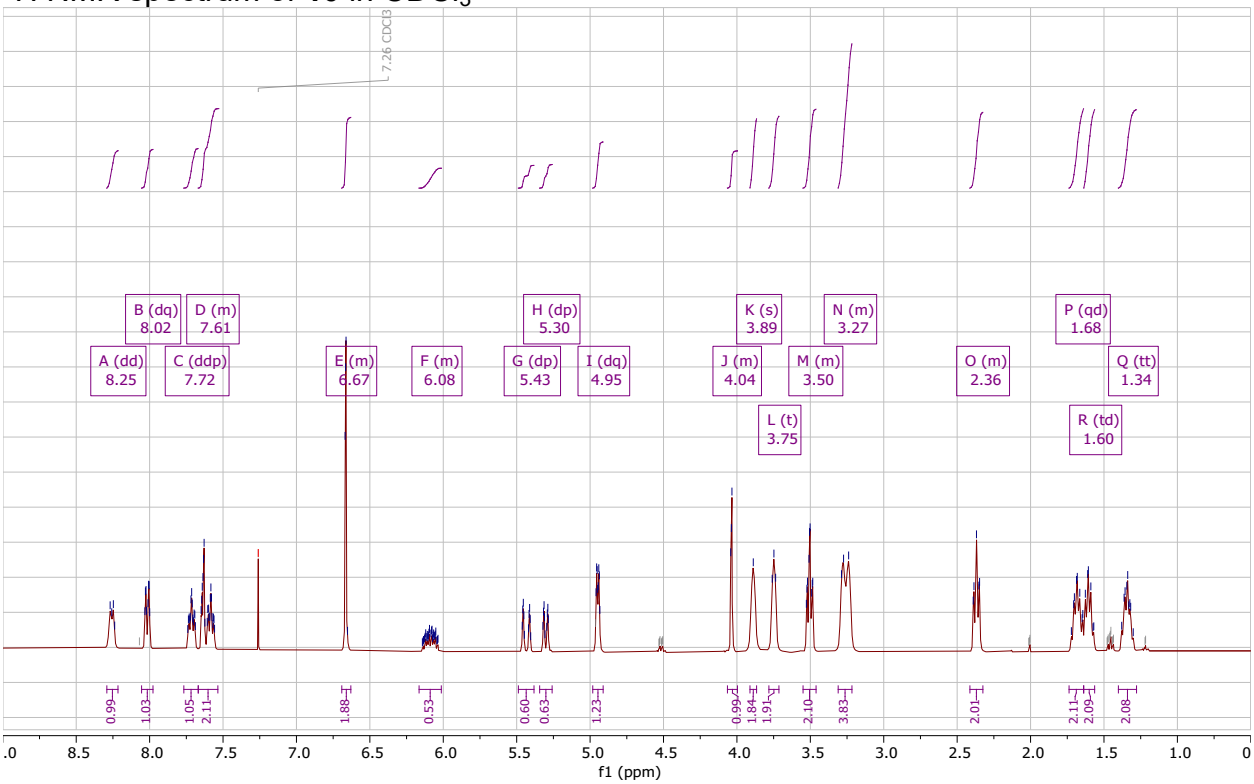
TLC R_f Value: 0.57 (100% EtOAc)

^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.39 (m, 1H), 8.04 (dt, *J* = 8.4, 1.8 Hz, 1H), 7.77 (ddt, *J* = 8.6, 6.9, 1.7 Hz, 1H), 7.69 – 7.58 (m, 2H), 6.69 (s, 2H), 6.13 (ddt, *J* = 16.5, 10.2, 5.9 Hz, 1H), 5.47 (dq, *J* = 17.2, 1.5 Hz, 1H), 5.34 (dq, *J* = 10.4, 1.2 Hz, 1H), 4.99 (dt, *J* = 6.0, 1.3 Hz, 2H), 3.93 (dd, *J* = 10.2, 5.4 Hz, 2H), 3.78 (dd, *J* = 6.1, 3.6 Hz, 2H), 3.54 (t, *J* = 7.2 Hz, 2H), 3.38 – 3.28 (m, 4H), 2.40 (t, *J* = 7.6 Hz, 2H), 1.72 (dt, *J* = 15.6, 7.6 Hz, 2H), 1.63 (q, *J* = 7.5 Hz, 2H), 1.43 – 1.31 (m, 2H).

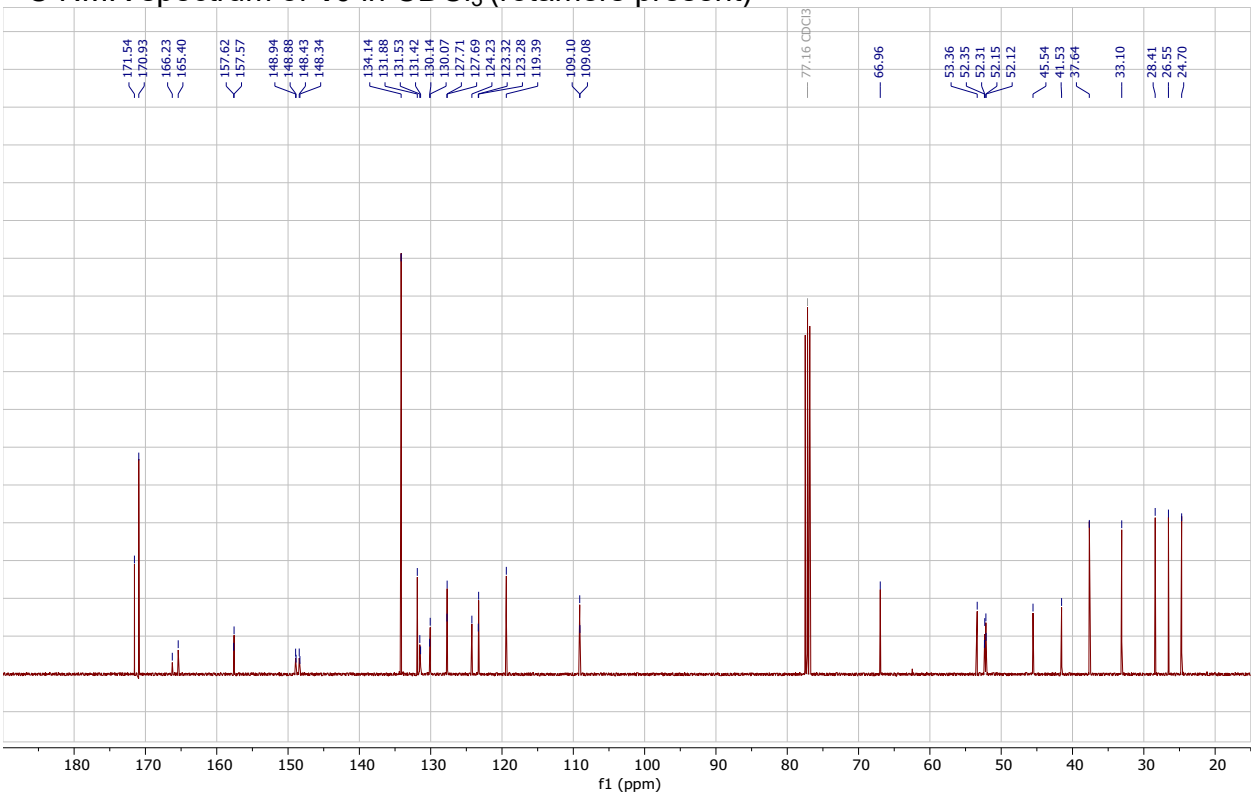
^{13}C-NMR (101 MHz, $CDCl_3$): δ (rotamers present) 171.5, 170.9, 166.2, 165.4, 157.6, 157.6, 148.9, 148.9, 148.4, 148.3, 134.1, 131.9, 131.5, 131.4, 130.1, 130.1, 127.7, 127.7, 124.2, 123.3, 123.3, 119.4, 109.1, 109.1, 67.0, 53.4, 52.3, 52.3, 52.1, 52.1, 45.5, 41.5, 37.6, 33.1, 28.4, 26.5, 24.7.

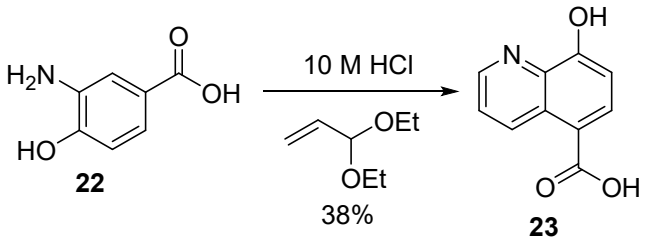
HRMS (ESI pos): $C_{27}H_{31}N_4O_5$ $[M+H]^+$ calcd 491.22890, found 491.2273.

^{1}H NMR spectrum of **10** in $CDCl_3$



^{13}C NMR spectrum of **10** in $CDCl_3$ (rotamers present)





To the aminobenzoic acid **22** (4.081 g, 26.65 mmol) in ice bath was added conc. HCl (10 M aq, 50 mL) slowly under a N_2 atmosphere. A gray suspension resulted. At 0 °C, acrolein diethyl acetal (2.78 mL, 40.0 mmol, 1.5 eq) was added in 3 portions dropwise over 30 minutes. Immediately the reaction mixture was warmed to reflux. The reflux was maintained with stirring overnight. A beige solid suspension in a pale brown solution resulted. Upon cooling to rt, enough conc. aq. ammonia was slowly added under stirring until pH of 9 was reached, which resulted in all of the solids being dissolved. The pH of the solution was then adjusted to ~3 with conc. HCl. A brown solid precipitated, which was filtered and dried under vacuum overnight (1.914 g, 10.12 mmol, 38%). This precipitate was shown to be sufficiently pure by NMR analysis even though it contained some NH_4Cl, and it was used in the subsequent step without further purification. The NMR signals coincided with the literature.[4]

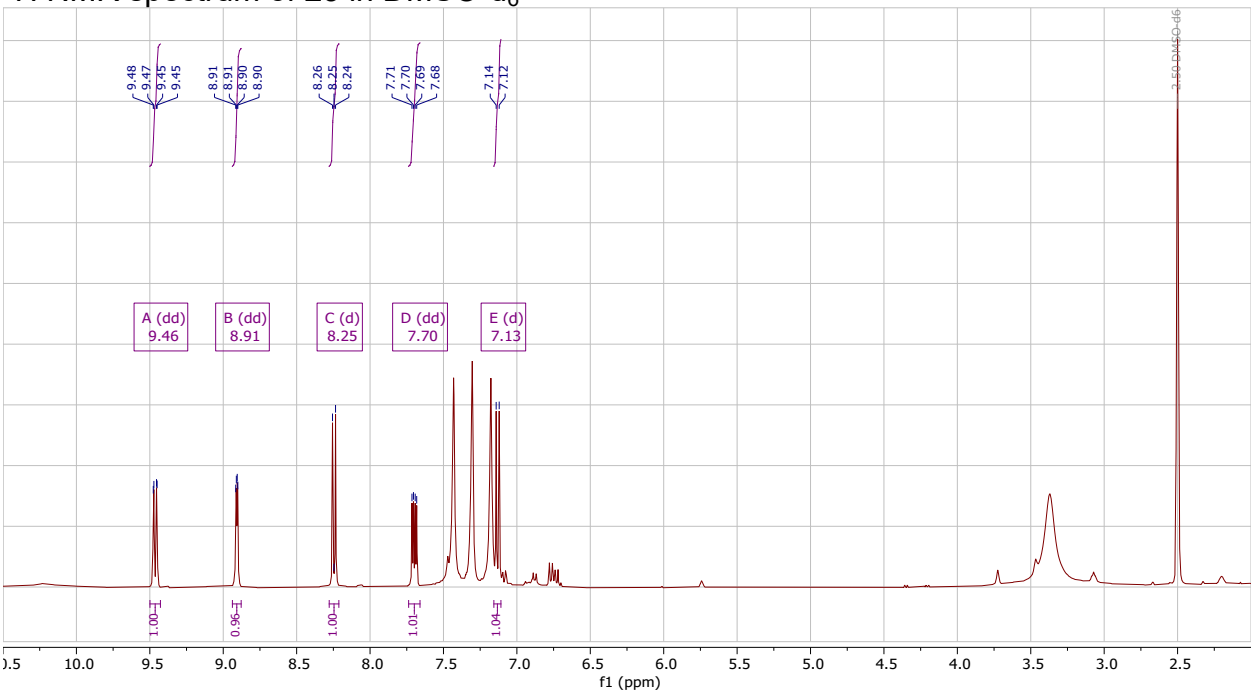
Physical State: brown solid

^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.46 (dd, *J* = 8.8, 1.6 Hz, 1H), 8.91 (dd, *J* = 4.1, 1.6 Hz, 1H), 8.25 (d, *J* = 8.3 Hz, 1H), 7.70 (dd, *J* = 8.8, 4.1 Hz, 1H), 7.13 (d, *J* = 8.3 Hz, 1H).

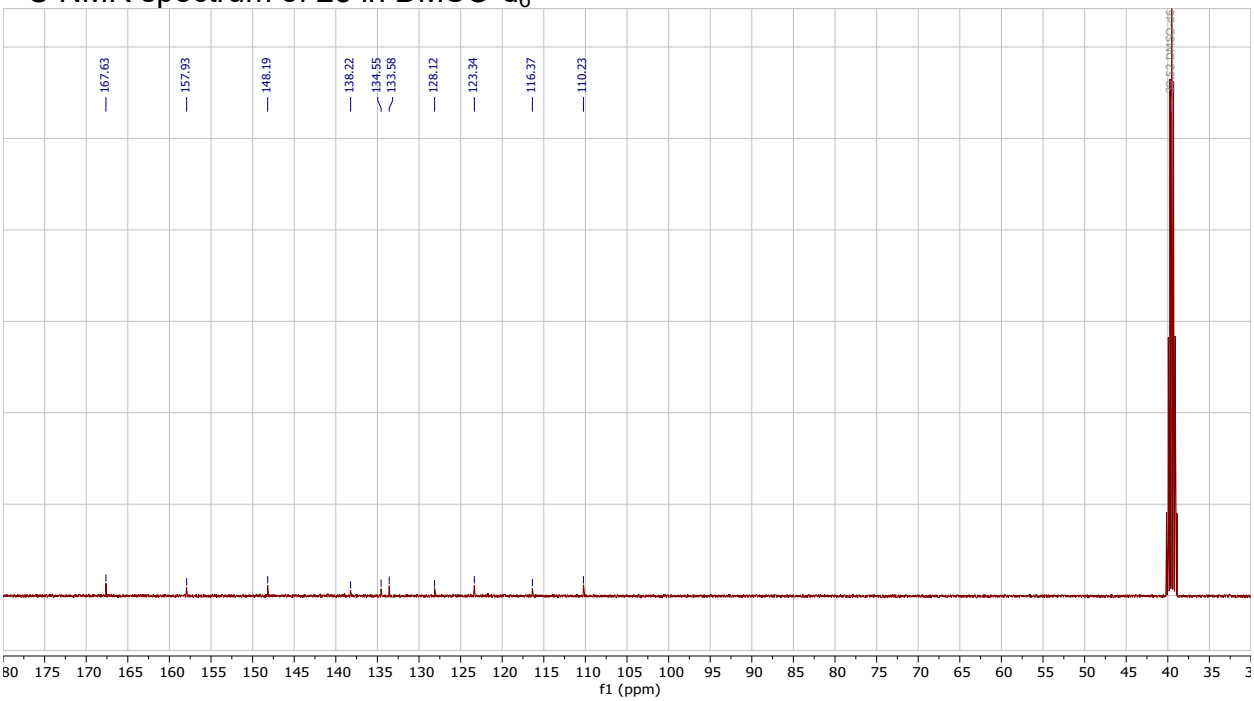
^{13}C-NMR (101 MHz, DMSO-d_6): δ 167.6, 157.9, 148.2, 138.2, 134.6, 133.6, 128.1, 123.3, 116.4, 110.2.

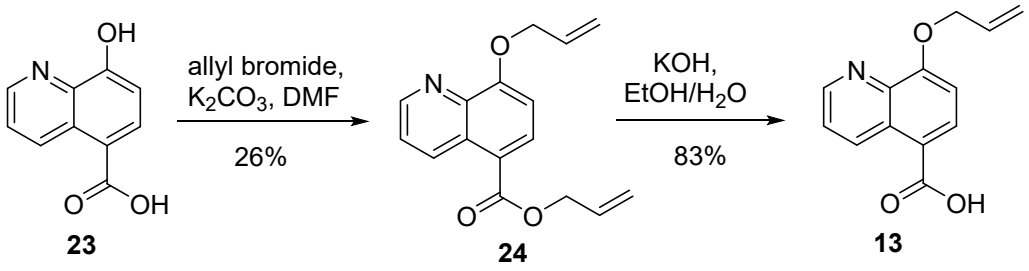
HRMS (ESI pos): $C_{10}H_8NO_3$ $[M+H]^+$ calcd 190.04987, found 190.0495.

^{1}H NMR spectrum of **23** in DMSO-d_6



^{13}C NMR spectrum of **23** in DMSO-d_6





23 **24** **13**

Zimmerman et al's procedures were followed with a slight modification.[4] To a solution of the quinoline carboxylic acid **23** (1.8702 g, 9.8863 mmol) in DMF (25 mL) at rt under a N_2 atmosphere was added K_2CO_3 (8.2787 g, 59.899 mmol, 6.1 eq). Under stirring, allyl bromide (9.8 mL, 113 mmol, 11 eq) was added slowly at rt. The mixture was then heated at 45-50 °C with vigorous stirring overnight. Upon cooling to rt, EtOAc (40 mL) and H_2O (40 mL) were added under stirring. The mixture was stirred for 5 minutes, and the phases were separated. The aqueous phase was extracted with EtOAc (40 mL). The combined organic phase was washed with H_2O (40 mL) four times and with brine (40 mL). The resulting solution was dried over anhydrous Na_2SO_4. Upon decanting and removal of the volatiles under vacuum, the bis-allyl product was purified by flash column chromatography using the Buchi Flash system on an EcoFlex Silica Gel 12 g column with a gradient mobile phase (1:24 EtOAc/hexanes to 100% EtOAc). The fractions containing the desired product were pooled and the solvents were removed under vacuum to yield a brown solid **24**, which was sufficiently pure by NMR (681 mg, 2.529 mmol, 26%).

To a solution of the allyl ester **24** (598 mg, 2.221 mmol) under a N_2 atmosphere in THF/MeOH/H_2O (3 mL, 3 mL, 1.5 mL) at rt was added LiOH (532 mg, 22.2 mmol, 10 eq). This mixture was stirred overnight. All of the volatiles were removed under vacuum. After adding H_2O (7 mL) to the residues, 3 M HCl aqueous solution (~8 mL) was added to neutralize to pH ~3. A white precipitate was formed from the brown solution. Upon filtration, a sticky pale beige solid (**12**) was collected and was dried under vacuum (424 mg, 1.85 mmol, 83%). The NMR data matched the literature.[4]

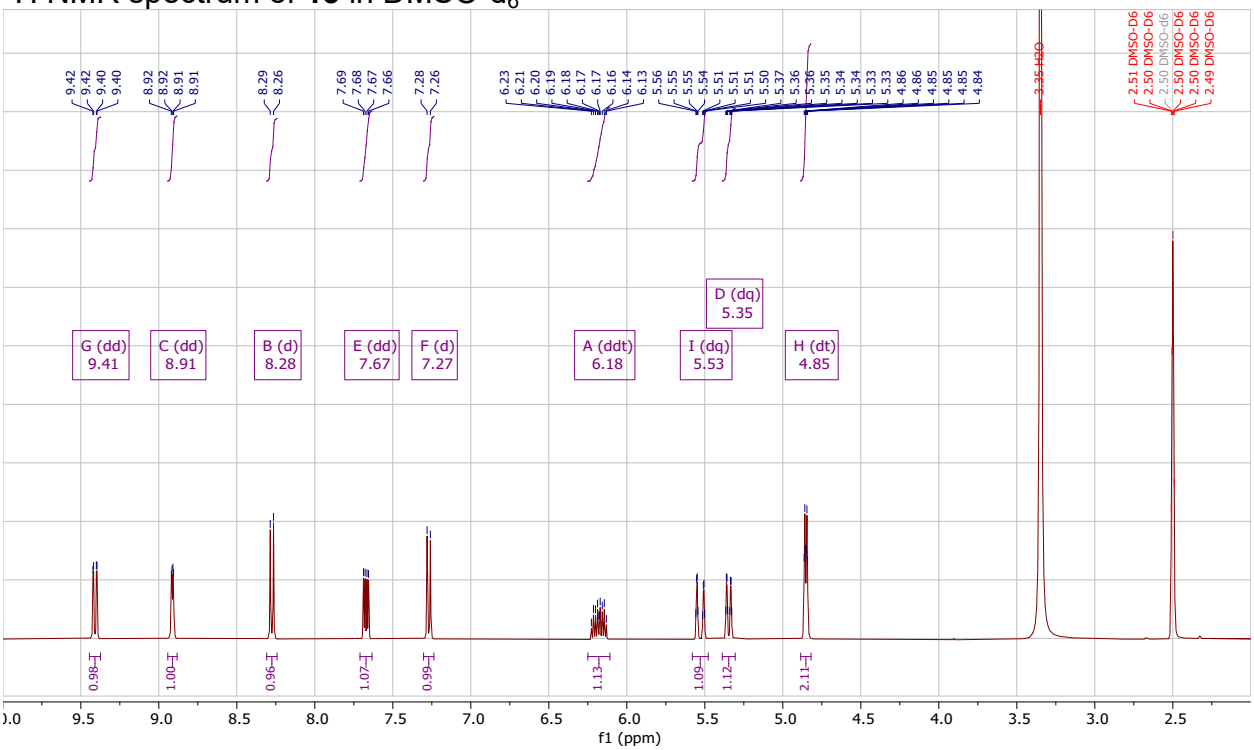
Physical State: beige solid

^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.41 (dd, *J* = 8.8, 1.7 Hz, 1H), 8.91 (dd, *J* = 4.1, 1.7 Hz, 1H), 8.28 (d, *J* = 8.4 Hz, 1H), 7.67 (dd, *J* = 8.8, 4.1 Hz, 1H), 7.27 (d, *J* = 8.4 Hz, 1H), 6.18 (ddt, *J* = 17.3, 10.5, 5.3 Hz, 1H), 5.53 (dq, *J* = 17.2, 1.7 Hz, 1H), 5.35 (dq, *J* = 10.5, 1.5 Hz, 1H), 4.85 (dt, *J* = 5.4, 1.6 Hz, 2H).

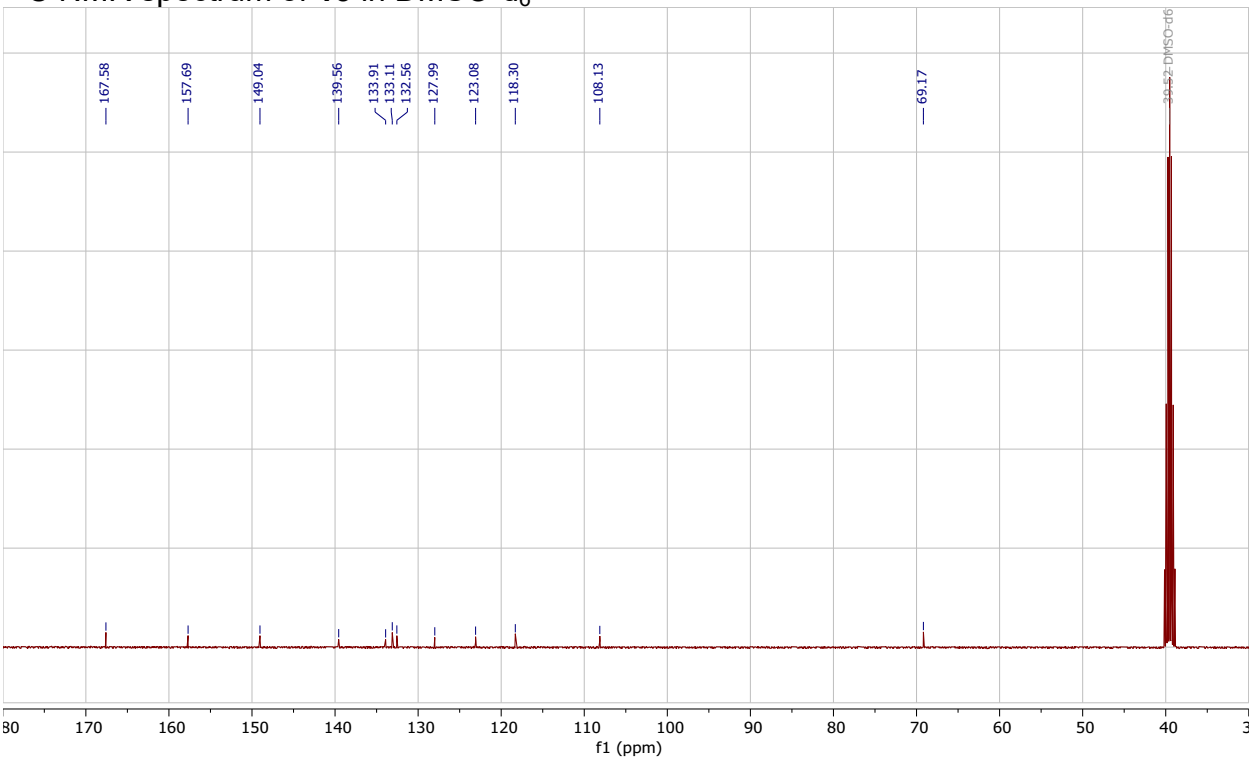
^{13}C-NMR (101 MHz, DMSO-d_6): δ 167.6, 157.7, 149.0, 139.6, 133.9, 133.1, 132.6, 128.0, 123.1, 118.3, 118.3, 108.1, 69.2.

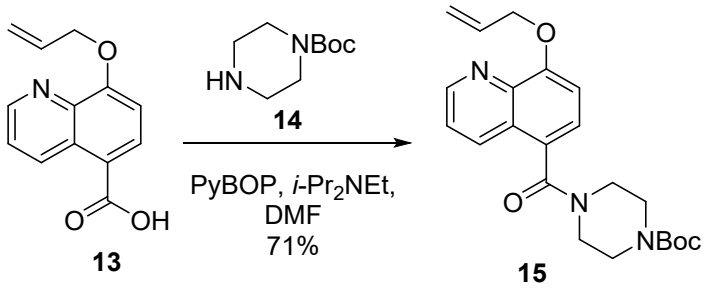
HRMS (ESI pos): $C_{13}H_{12}NO_3$ $[M+H]^+$ calcd 230.08117, found 230.0810.

^{1}H NMR spectrum of **13** in DMSO-d_6



^{13}C NMR spectrum of **13** in DMSO-d_6





To a suspension of the quinoline carboxylic acid **13** (413 mg, 1.802 mmol) and the *N*-Boc-piperazine **14** (391 mg, 2.099 mmol, 1.2 eq) in DMF (10 mL) under a N_2 atmosphere was added PyBOP (1.054 g, 1.9504 mmol, 1.1 eq) and diisopropylethylamine (630 μL, 3.62 mmol, 2 eq) at rt. This mixture was stirred overnight at rt. Then the reaction mixture was diluted with EtOAc (40 mL) and H_2O (20 mL) under stirring. Following phase separation, the aqueous phase was extracted with EtOAc (20 mL). The combined organic phases were washed with H_2O (20 mL) four times and with brine (20 mL) twice. The resulting solution was dried over anhydrous Na_2SO_4 and the volatiles were removed under vacuum to yield a crude orange-brown oil (778 mg), which solidified upon standing. The crude material was purified by flash chromatography using a Buchi Flash system on a EcoFlex Silica Gel 12 g column with a gradient mobile phase (1:4 EtOAc/hexanes to 100% EtOAc). Upon pooling the fractions containing the product and removing the solvents under vacuum, a pale brown solid **15** was obtained (505 mg, 1.271 mmol, 71%).

Physical State: pale brown solid

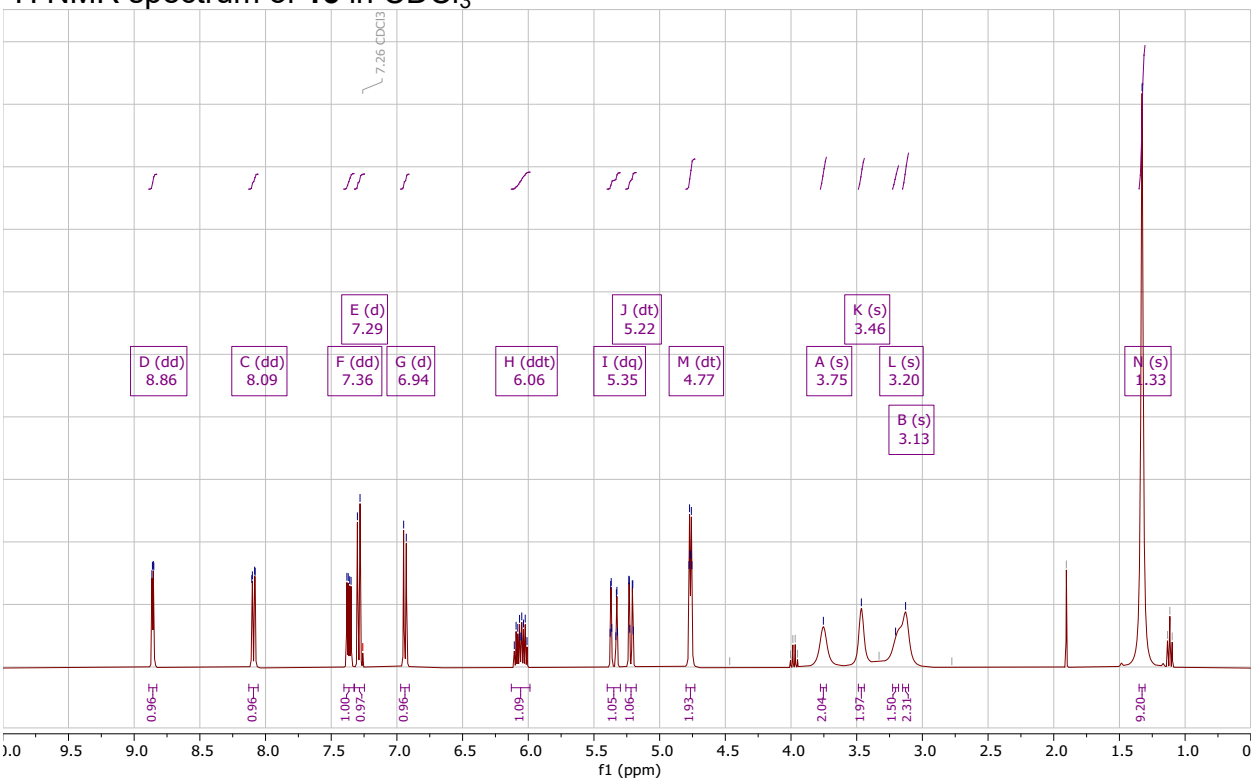
TLC R_f Value: 0.28 (100% EtOAc)

^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.86 (dd, *J* = 4.2, 1.7 Hz, 1H), 8.09 (dd, *J* = 8.5, 1.7 Hz, 1H), 7.36 (dd, *J* = 8.5, 4.2 Hz, 1H), 7.29 (d, *J* = 8.0 Hz, 1H), 6.94 (d, *J* = 8.0 Hz, 1H), 6.06 (ddt, *J* = 17.4, 10.6, 5.4 Hz, 1H), 5.35 (dq, *J* = 17.3, 1.6 Hz, 1H), 5.22 (dt, *J* = 10.5, 1.4 Hz, 1H), 4.77 (dt, *J* = 5.5, 1.6 Hz, 2H), 3.75 (m, 2H), 3.46 (m, 2H), 3.20 (m, 2H), 3.13 (m, 2H), 1.33 (s, 9H).

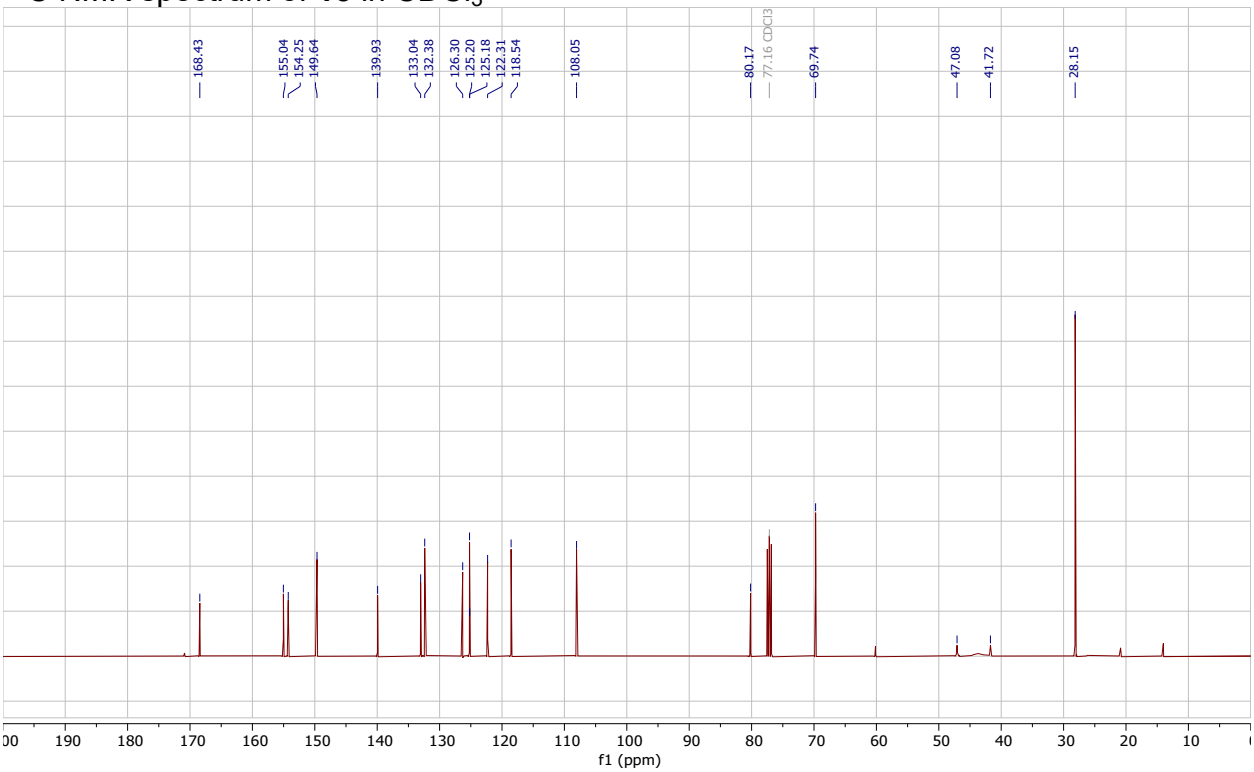
^{13}C-NMR (101 MHz, $CDCl_3$): δ 168.4, 155.0, 154.3, 149.6, 139.9, 133.0, 132.4, 126.3, 125.2, 125.2, 122.3, 118.5, 108.1, 80.2, 69.7, 47.1, 41.7, 28.1.

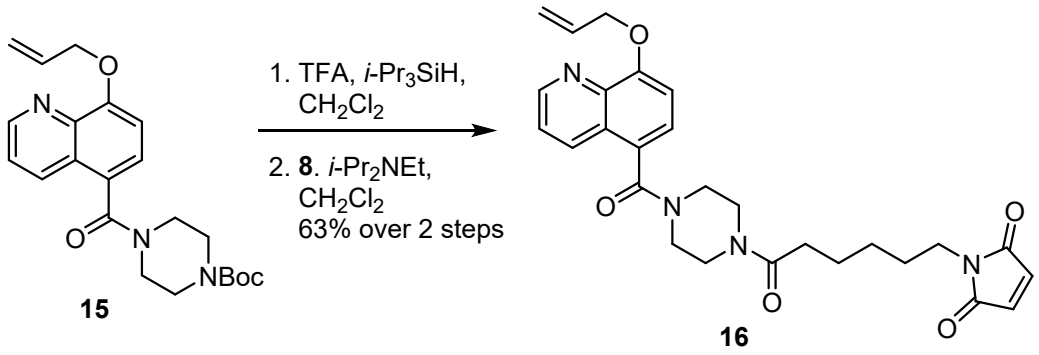
HRMS (ESI pos): $C_{22}H_{28}N_3O_4$ $[M+H]^+$ calcd 398.20743, found 398.2061.

^{1}H NMR spectrum of **15** in $CDCl_3$



^{13}C NMR spectrum of **15** in $CDCl_3$





To a solution of the Boc carbamate **15** (329 mg, 0.828 mmol) in CH_2Cl_2 (5 mL) at rt was added triisopropylsilane (340 μL, 1.65 mmol, 2.0 eq) under a N_2 atmosphere. Upon cooling to 0 °C, TFA (2 mL) was added slowly under stirring. After 50 minutes, the volatiles were removed under vacuum. The resulting sticky gummy was washed with 1:1 Et_2O/hexanes. This crude material was dissolved in DMF (9 mL) and the pentafluorophenyl ester **8** (348 mg, 0.922 mmol 1.1 eq) and diisopropylethylamine (720 μL, 4.13 mmol, 5.0 eq) were added. The reaction mixture was stirred at rt overnight. Upon diluting with EtOAc (100 mL) and H_2O (25 mL), the mixture was partitioned. The aqueous phase was extracted with EtOAc (25 mL). The combined organic phases were washed with H_2O (30 mL) three times and with brine (30 mL), and were dried over anhydrous Na_2SO_4. Upon removal of most of the volatiles under vacuum, this material was purified by flash chromatography using a Buchi Flash system on a EcoFlex Silica Gel 12 g column with a gradient mobile phase (2:3 EtOAc/hexanes to 100% EtOAc then to 1:9 MeOH/EtOAc) to yield 254 mg of thick pale brown oil **16** (0.518 mmol, 63%).

Physical State: pale brown oil

TLC R_f Value: 0.26 (1:9 MeOH/EtOAc)

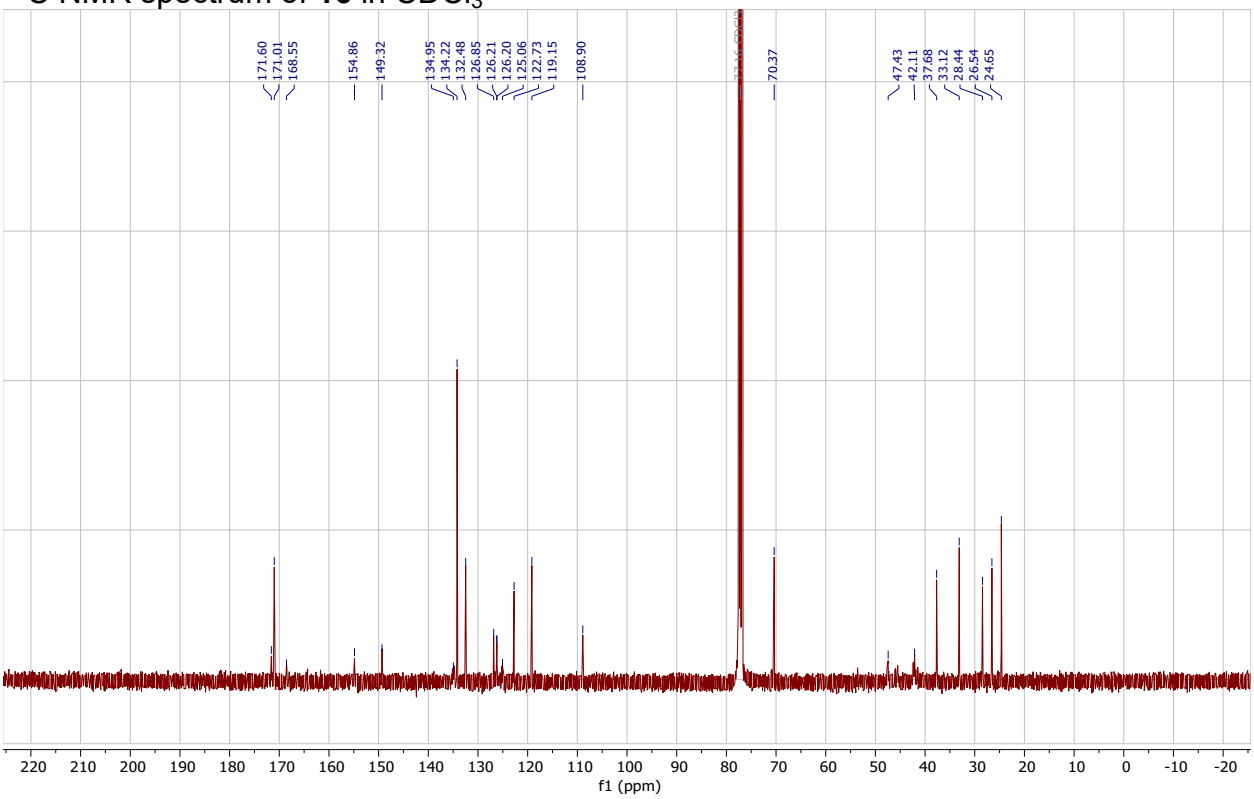
^{1}H-NMR (400 MHz, $CDCl_3$): δ 9.08 – 9.02 (m, 1H), 8.29 (d, *J* = 8.5 Hz, 1H), 7.54 (dd, *J* = 8.7, 4.3 Hz, 1H), 7.45 (d, *J* = 8.0 Hz, 1H), 7.10 (d, *J* = 8.0 Hz, 1H), 6.68 (s, 2H), 6.21 (ddt, *J* = 17.3, 10.7, 5.5 Hz, 1H), 5.50 (dq, *J* = 17.3, 1.5 Hz, 1H), 5.38 (dq, *J* = 10.5, 1.3 Hz, 1H), 5.30 (s, 1H), 4.92 (dt, *J* = 5.6, 1.5 Hz, 2H), 3.91 (s, 2H), 3.77 (s, 1H), 3.63 (s, 1H), 3.51 (s, 3H), 3.29 (s, 2H), 2.36 (s, 1H), 2.26 (s, 1H), 1.63 (s, 4H), 1.33 (s, 3H).

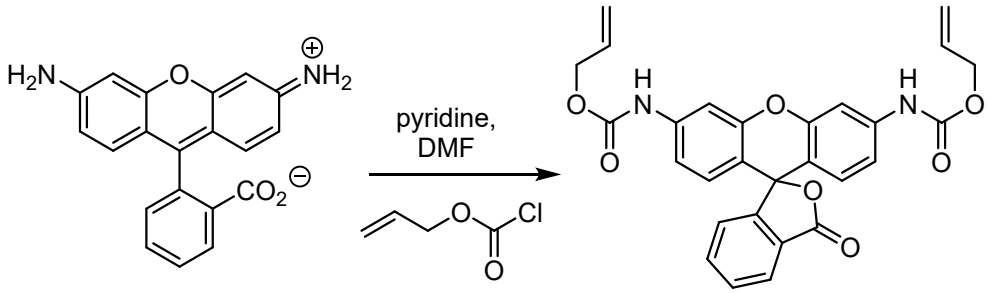
^{13}C-NMR (101 MHz, $CDCl_3$): δ 171.6, 171.0, 168.6, 154.9, 149.3, 135.0, 134.2, 132.5, 126.8, 126.2, 126.2, 125.1, 122.7, 119.2, 108.9, 70.4, 47.4, 42.1, 37.7, 33.1, 28.4, 26.5, 24.7.

HRMS (ESI pos): $C_{27}H_{31}N_4O_5$ $[M+H]^+$ calcd 491.22890, found 491.2274.

^{1}H NMR spectrum of **16** in $CDCl_3$

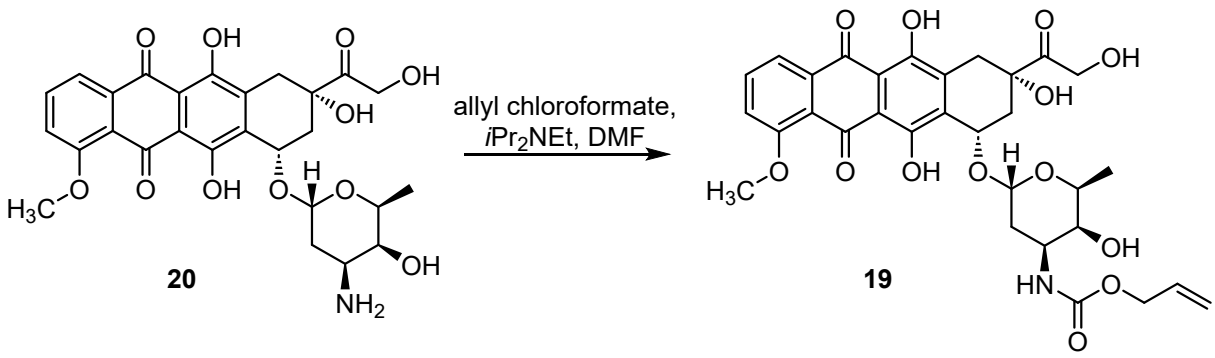
^{13}C NMR spectrum of **16** in $CDCl_3$





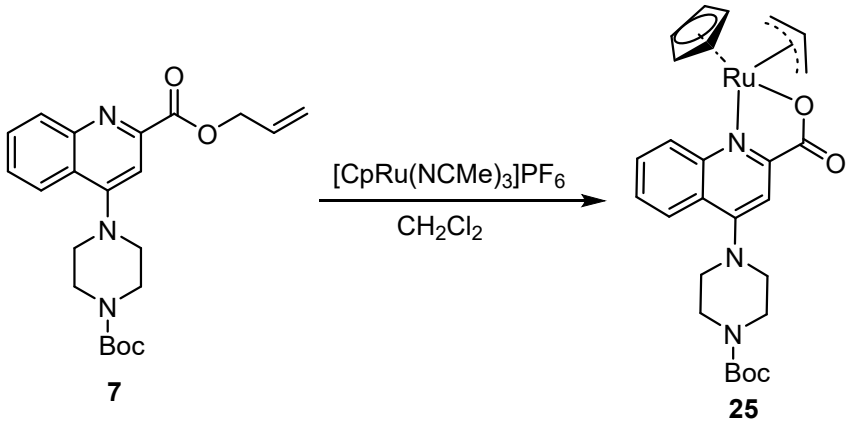
4 **3**

The literature procedures were followed and the analytical data of the product were consistent with Meggers et al's.[5]



20 **19**

The literature procedures were followed and the analytical data of the product were consistent with Rotello et al's and the structure.[6]



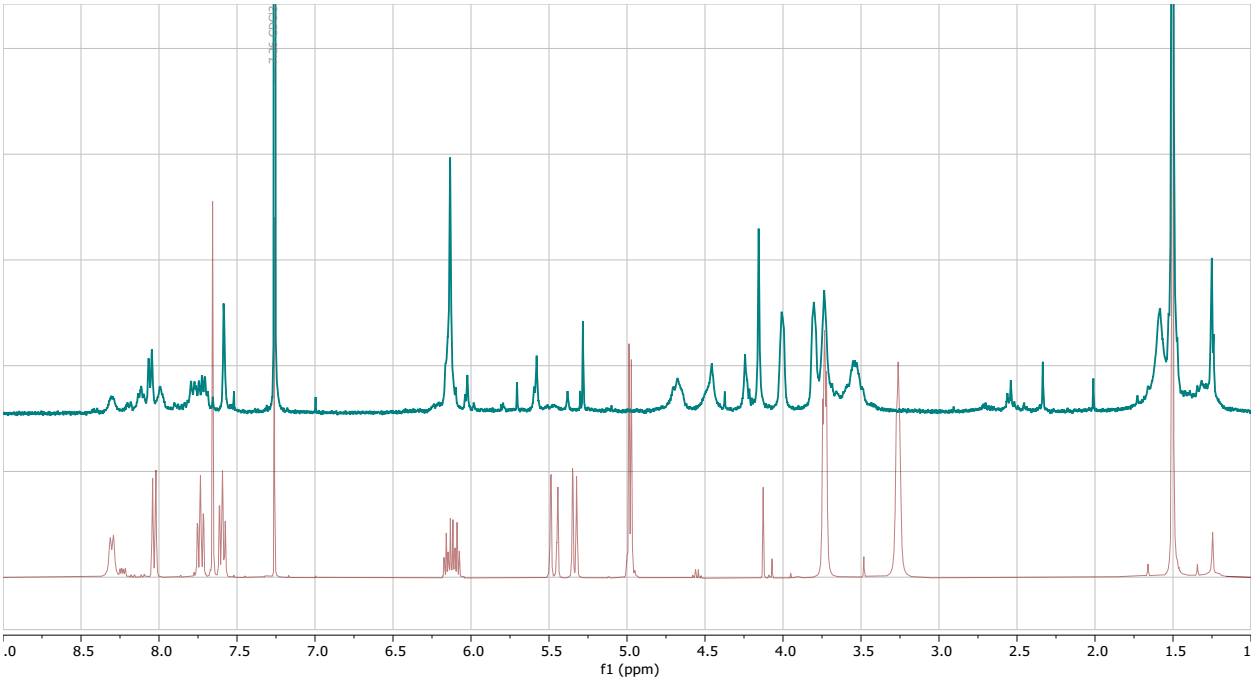
7 **25**

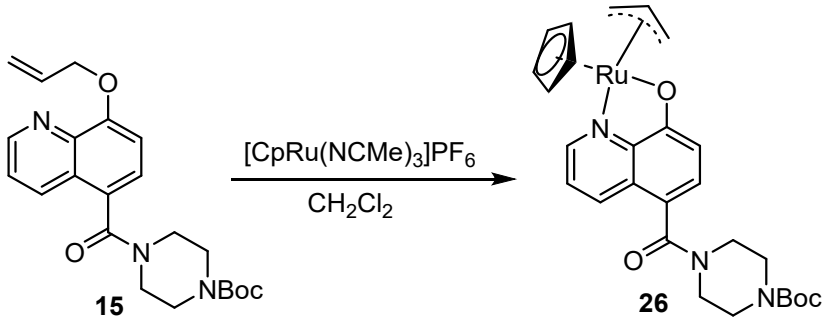
Meggers et al's procedures were followed with a slight modification.[7] Under a N_2 atmosphere, the quinoline allyl ester **7** (1.8 mg, 0.0045 mmol, 1.0 eq) was dissolved in 0.1 mL CH_2Cl_2. To the this solution was added $[CpRu(NCMe)_3]PF_6$ (tris(acetonitrile)cyclopentadienylruthenium hexafluorophosphate, 2.0 mg, 0.0046 mmol, 1.0 eq). After the mixture was gently mixed at rt for 30 min, the volatiles were removed under vacuum. The residue was washed three times with Et_2O. Upon removal of residual solvents in vacuum, a yellow-brown solid **25** was obtained (3.2 mg). This crude material was used in the uncaging reaction without further purification. The expected changes in the ^1H NMR spectrum was observed as shown below. This material was used for the experiment described in entry 7 of Table 1.

Physical State: yellow-brown solid

^{1}H-NMR (400 MHz, $CDCl_3$) : δ 8.20 – 7.91 (m, 2H), 7.91 – 7.67 (m, 2H), 7.58 (s, 1H), 6.08 (d, *J* = 44.4 Hz, 5H), 4.68 (m, 2H), 4.46 (m, 1H), 4.20 (d, *J* = 34.3 Hz, 2H), 4.01 (s, 2H), 3.77 (d, *J* = 26.9 Hz, 6H), 1.50 (d, *J* = 1.8 Hz, 9H).

Stacked ^{1}H NMR spectra of **7** (maroon) and **25** (cyano green) in $CDCl_3$



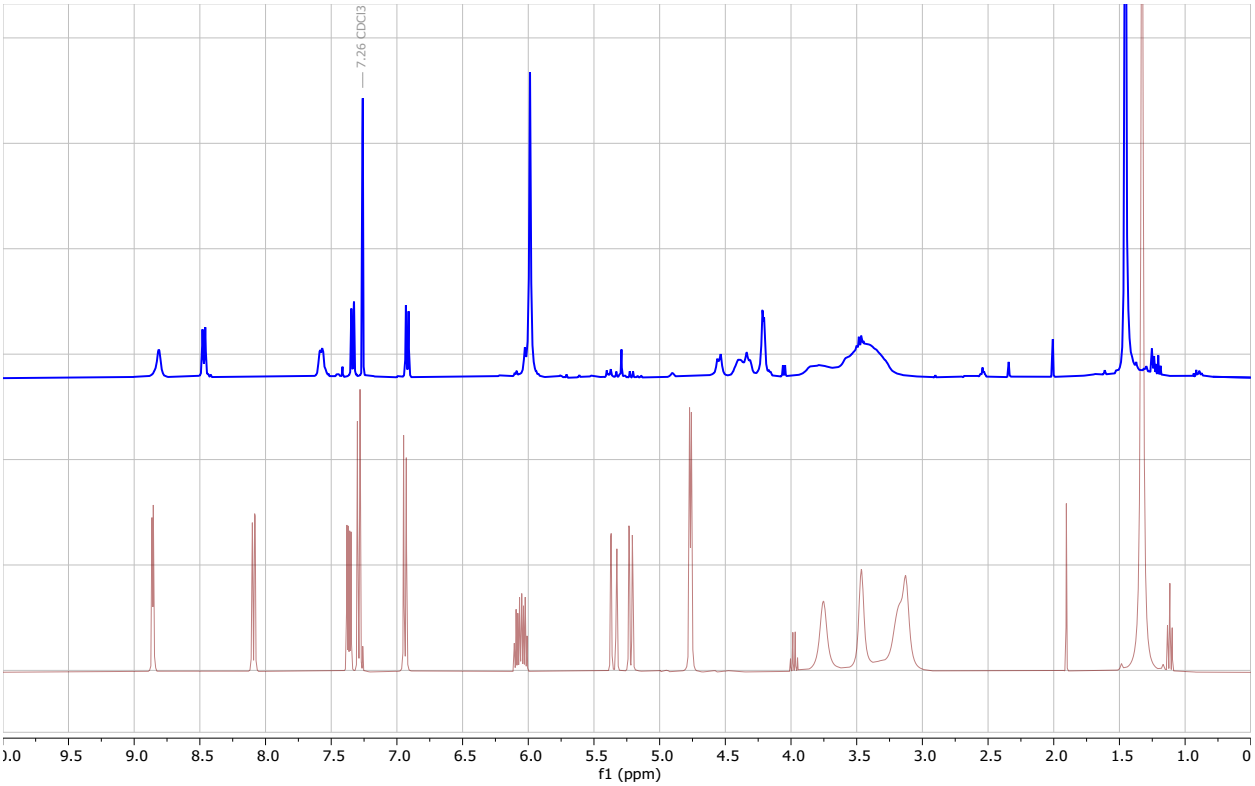


Meggers et al's procedures were followed with a slight modification.[7] Under a N_2 atmosphere, the quinoline allyl ether **15** (6.2 mg, 0.0156 mmol, 1.0 eq) was dissolved in 0.4 mL CH_2Cl_2. To this solution was added $[CpRu(NCMe)_3]PF_6$ (tris(acetonitrile)cyclopentadienylruthenium hexafluorophosphate, 6.8 mg, 0.0157 mmol, 1.0 eq). After the mixture was gently mixed at rt for 30 min, the volatiles were removed under vacuum. The residue was washed three times with Et_2O. Upon removal of residual solvents in vacuum, an orange-brown solid **26** was obtained (12 mg). This crude material was used in the uncaging reaction without further purification. The expected changes in the ^1H NMR spectrum was observed as shown below. This material was used for the experiment described in entry 8 of Table 1.

Physical State: orange-brown solid

^{1}H-NMR (400 MHz, Aetone-d_6): δ 8.81 (s, 1H), 8.47 (d, *J* = 8.3 Hz, 1H), 7.64 – 7.52 (m, 1H), 7.34 (d, *J* = 8.6 Hz, 1H), 7.26 (d, *J* = 1.1 Hz, 1H), 6.92 (d, *J* = 8.2 Hz, 1H), 5.99 (s, 5H), 4.55 (d, *J* = 10.0 Hz, 1H), 4.49 – 4.30 (m, 2H), 4.21 (d, *J* = 5.7 Hz, 2H), 3.79 (s, 3H), 3.45 (m, 5H), 1.46 (s, 9H).

Stacked ^{1}H NMR spectra of **15** (maroon) and **26** (blue) in $CDCl_3$



BSA Conjugation and Uncaging Experiments General Procedures

Materials were added to achieve the final concentrations shown in Table S1 below. Unless otherwise specified, all centrifugation was performed at 15,000 rpm. BSA was treated with 1 mM dithiothreitol (DTT) at least overnight at 4 °C. The treated BSA was placed in a 10K MWCO filter tube and was diluted with 400 µL PBS (pH=6.5). Upon centrifuging for 4 min, the BSA solution was washed again twice by adding an additional 400 µL PBS (pH=6.5) and centrifuging for 4 min. To this BSA solution was added the ligand stock solution (3 mM in DMSO) and the mixture was thoroughly agitated. The mixture was incubated at 37 °C for 2 hours with agitation.

The conjugated BSA was washed twice by adding 400 µL PBS (pH=7.4) and centrifuging for 4 min and again by adding 400 µL PBS (pH=7.4) and centrifuging for 2 min. To this solution was added $[CpRu(NCMe)_3]PF_6$ (tris(acetonitrile)cyclopentadienylruthenium hexafluorophosphate) stock solution (6 mM in MeCN) and the mixture was incubated at rt with agitation for 30 min. This Ru-coordinated material was washed three times by adding 400 µL PBS (pH=7.4) and centrifuging for 4 min. The mixture was transferred to a regular 1.5 mL microcentrifuge tube and diluted with PBS (pH=7.4). To this solution were added glutathione (GSH), nucleophilic additives (if any), and $alloc_2$-rhodamine stock solutions (70 mM GSH in PBS, 4 mM $alloc_2$-rhodamine in DMSO). The total volume was 200 µL in each case. This mixture was incubated at 37 °C overnight with agitation.

The next day, 10 µL of each uncaging reaction mixture was diluted 20 fold in PBS (pH=7.4) in triplicate in a black fluorescence 96-well plate with a transparent bottom. Along side the samples were prepared rhodamine 110 standard solutions for the standard curve, ranging from 100 µM to 1.7 nM in PBS (pH=7.4) in triplicates. A PBS (pH=7.4) solution was used as the triplicate blank. The fluorescence measurements were taken from the top face on a plate reader with 490 nm excitation and 520 nm emmission. The uncaged rhodamine 110 (**4**) concentrations were calculated using the triplicate averages according to the standard curve constructed as shown in Figure S1.

Entry	Ligand	BSA	CpRu	GSH
1	**9**, 150 μM	30 μM	300 μM	3.5 mM
2	**10**, 150 μM	30 μM	450 μM	3.5 mM
3	**10**, 150 μM	30 μM	450 μM	0 mM
4	**16**, 150 μM	30 μM	450 μM	3.5 mM
5	**16**, 150 μM	30 μM	150 μM	0 mM
6	**16**, 150 μM	30 μM	750 μM	3.5 mM
7	**7**, 30 μM	0 μM	30 μM	3.5 mM
8	**15**, 30 μM	0 μM	30 μM	3.5 mM
9	**18**, 30 μM	0 μM	30 μM	3.5 mM
10	none	30 μM	150 μM	3.5 mM
11	none	0 μM	30 μM	3.5 mM
12	none	30 μM	0 μM	3.5 mM

Table-S1. Final concentrations of reagents added to uncaging reactions of alloc$_2$-rhodamine 110.

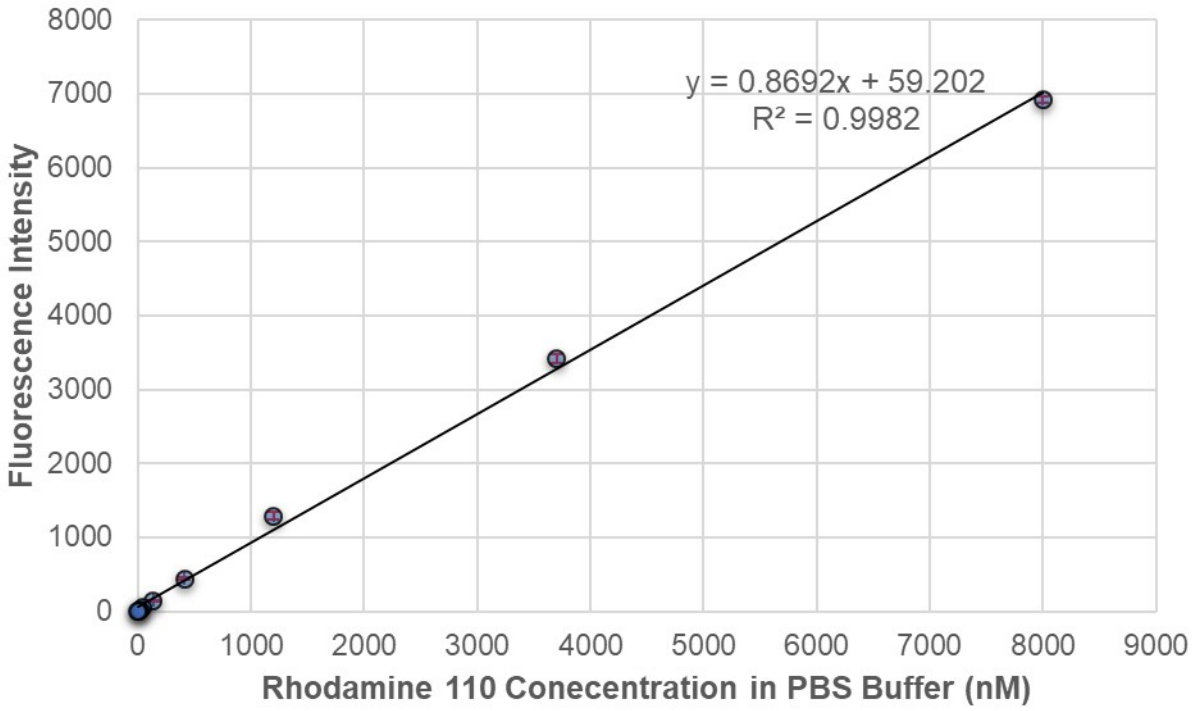


Figure S1. Linear correlation between fluorescence intensity (λ_{ex} 490 nm, λ_{em} 520 nm) and concentration of rhodamine 110 in PBS buffer (pH 7.4). Average of triplicates shown.

Incubation	1:3	1:10	GSH control	Ru control	Ligand control	BSA control	BSA blank	PBS blank
15 min	4%	14%	9%	0%	0%	0%	0%	0%
2hrs	12%	20%	12%	0%	0%	1%	0%	0%
16hrs	1%	4%	0%	0%	0%	0%	0%	0%

Table-S2. Determination of optimal incubation time for ligand conjugation and optimal ligand to BSA ratio. Incubation time: BSA was incubated with the ligand at 37 °C for 15 min, 2 hrs, or 16 hrs. BSA to ligand ratio: BSA was incubated as described above with either 3 eq of ligand or 10 eq of ligand. GSH control: the experiment lacked GSH (3.5 mM), but otherwise identical conditions to the 1:3 ratio experiment. Ru control: $CpRu(NCMe)_3PF_6$ was omitted. Ligand control: the ligand **9** was omitted. BSA control: BSA was omitted. BSA blank: **3** incubated with BSA only. PBS blank: pH 6.5 PBS buffer.

Albumin-Conjugated Uncaging of Alloc-Doxorubicin Procedures

Materials were added to achieve the final concentrations shown in table S3 below. All centrifugation steps were done at 15,000 rpm unless stated otherwise. In MWCO filter tube, reduced BSA 178 μM in PBS with 1 mM DTT was added to 400 μl PBS (pH=6.5) and centrifuged for 5 minutes. The DTT was washed from the sample by adding 400 μl PBS (pH=6.5) and centrifuging for 4 minutes. This step was repeated once. The ligands were then added to respective mixture and incubated at 37°C for 2 hours with agitation. After incubation, CpRu (6 mM in MeCN) was added and incubated at room temp for 30 minutes with agitation. The samples were centrifuged for 4 min and resuspended with 400 μl PBS (pH=7.4). This step was repeated once. The samples were centrifuged for 2 min and resuspended to 180 μl with PBS (pH=7.4) and mixed well. 177 μl of each mixture transferred to respective centrifuge tube. In each tube, GSH (70 mM in PBS), and alloc-doxorubicin (4 mM in MeOH) were added, and the mixtures were incubated overnight at 37°C with agitation. The following day, each sample was diluted 4-fold to 80 μl with H_2O and placed in LCMS vials. The yield of each sample was then determined using a standard curve and LCMS analysis.

	Ru-**12**	Ru-**17**	Ligand Control
BSA	30 μM	30 μM	30 μM
Ligand	**10**, 150 μM	**16**, 150 μM	0 μM
CpRu	450 μM	450 μM	450 μM
19	100 μM	100 μM	100 μM
GSH	3.5 mM	3.5 mM	3.5 mM

Table-S3. Final concentrations of materials for uncaging alloc-doxorubicin (**19**). CpRu refers to $[CpRu(CNMe)_3]PF_6$.

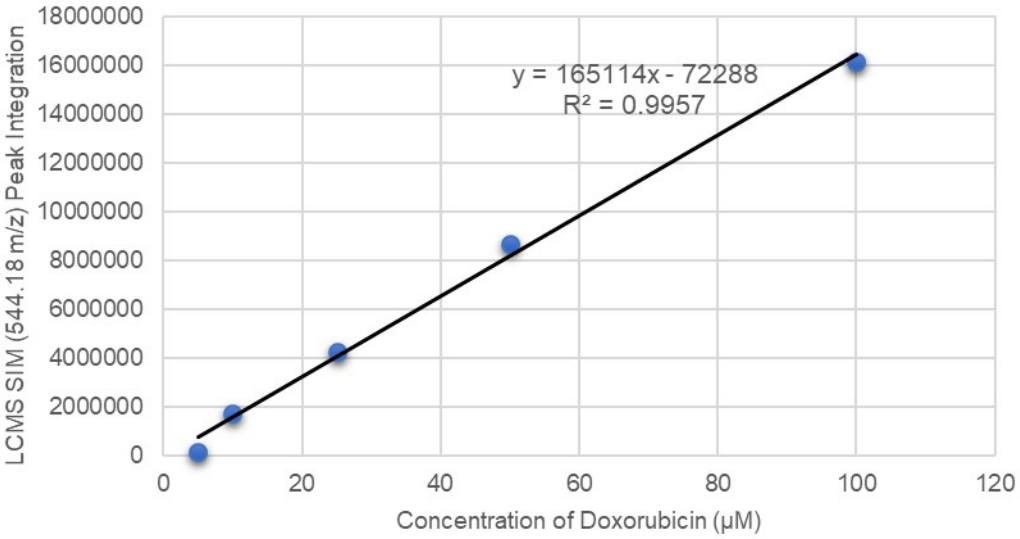
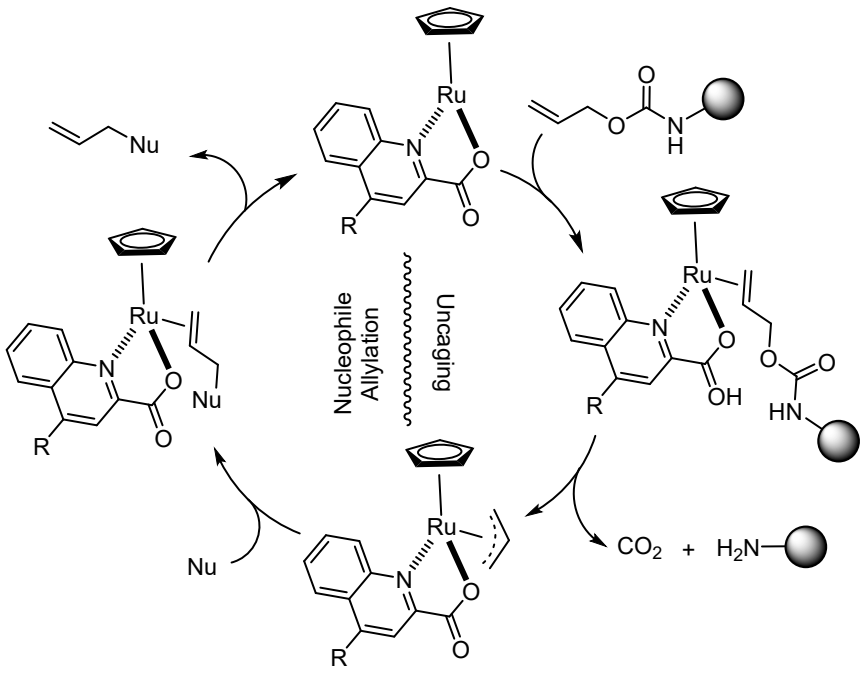


Figure-S2.

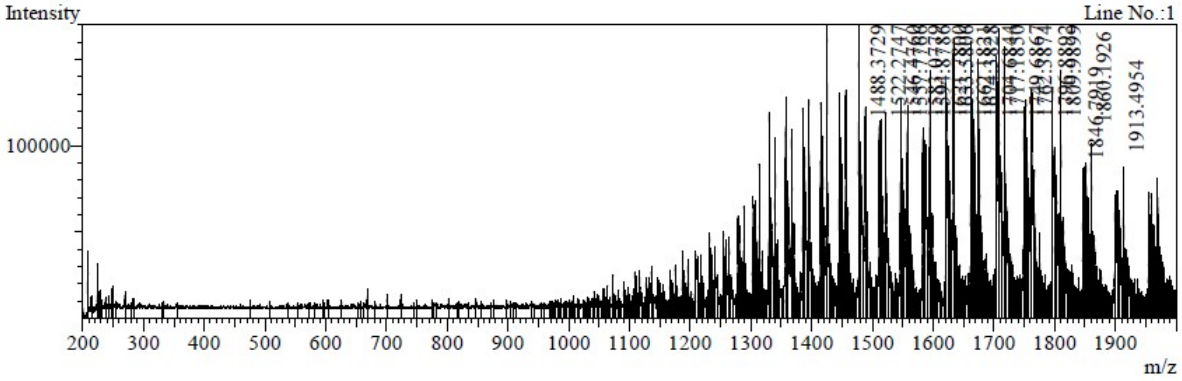
Linear correlation between ESI LCMS single-ion-monitoring (SIM) peak integration obtained for 544.18 m/z (M+H) and concentration of doxorubicin (μM).



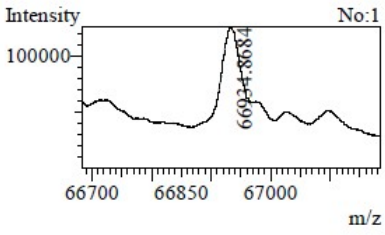
Scheme-S1. Proposed mechanism of alloc uncaging reaction. Adapted from Meggers et al[5]

Evaluation of the conjugation reaction by deconvoluted ESI-LCMS mass spectra

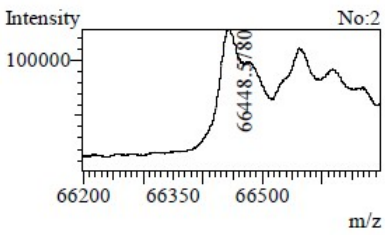
Virtually the same trends were observed for conjugation with both ligands **10** and **16**, which are constitutional isomers of one another.



No.	Name	# Peaks	A.Mass	SD	R.Mass
1	66934.8684	12	1.00728	3.672	66934.8684
2	66448.5780	8	1.00728	3.303	66448.5780

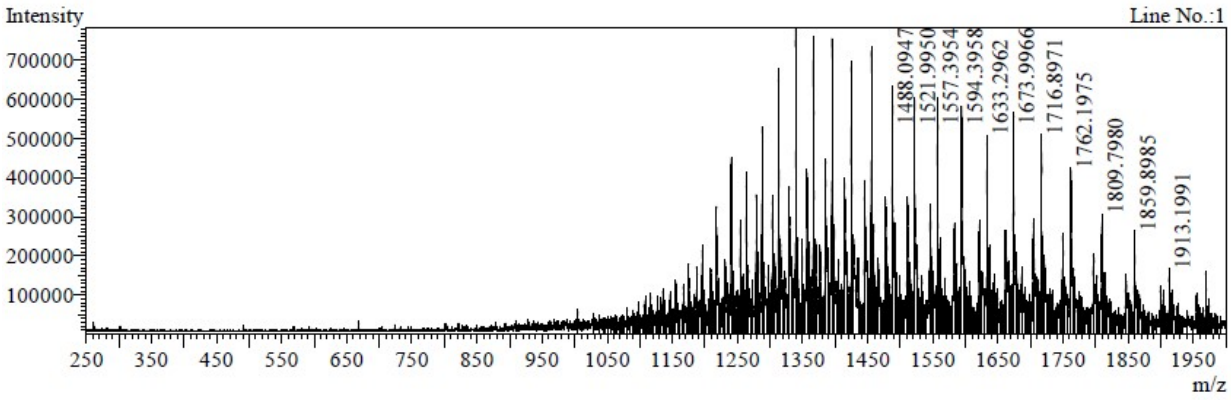


No.	Int.	Charge	Real Charge	m/z	Weight	Mass
1	91675	34	34.000	1969.6983	0.11	66935.4947
2	96350	35	34.999	1913.4954	0.10	66937.0842
3	112850	36	36.002	1860.1926	0.10	66930.6715
4	160000	37	37.001	1809.9899	0.09	66932.3569
5	143350	38	38.001	1762.3874	0.09	66932.4446
6	175625	39	39.002	1717.1850	0.08	66930.9311
7	168975	40	40.000	1674.3828	0.08	66935.0208
8	180950	41	41.000	1633.5806	0.08	66935.5061
9	159100	42	41.995	1594.8786	0.07	66942.5954
10	136150	43	42.996	1557.7766	0.07	66941.0808
11	128375	44	43.999	1522.2747	0.07	66935.7665
12	135075	45	45.002	1488.3729	0.06	66931.4529

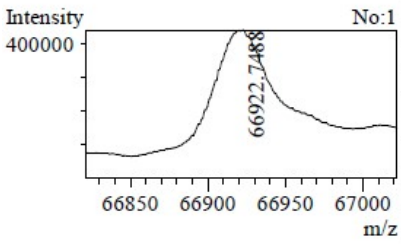


No.	Int.	Charge	Real Charge	m/z	Weight	Mass
1	92325	36	36.000	1846.7919	0.15	66448.2463
2	145975	37	37.001	1796.8892	0.14	66447.6310
3	127975	38	37.999	1749.6867	0.13	66449.8180
4	170750	39	39.003	1704.6844	0.13	66443.4077
5	173175	40	40.001	1662.1821	0.12	66446.9928
6	148250	41	40.998	1621.7800	0.12	66451.6815
7	120550	42	42.001	1583.0779	0.11	66446.9660
8	136950	43	42.996	1546.4760	0.10	66455.1550

Figure-S3. The deconvolution calculations output showing the conjugated mass of 66934.9 (expected 66920.5) and the unconjugated BSA 66448.6 (expected 66430.3) after BSA was treated with the allyl ester ligand **10** and incubated at 37 °C for 30 minutes.



No.	Name	# Peaks	A.Mass	SD	R.Mass
1	66922.7488	12	1.00728	2.508	66922.7488



No.	Int.	Charge	Real Charge	m/z	Weight	Mass
1	159376	34	34.000	1969.2997	0.11	66921.9423
2	167048	35	34.998	1913.1991	0.10	66926.7137
3	264516	36	36.001	1859.8985	0.10	66920.0839
4	307244	37	36.999	1809.7980	0.09	66925.2566
5	423584	38	37.999	1762.1975	0.09	66925.2284
6	511028	39	39.002	1716.8971	0.08	66919.7030
7	565684	40	40.002	1673.9966	0.08	66919.5728
8	509348	41	40.999	1633.2962	0.08	66923.8457
9	582036	42	42.000	1594.3958	0.07	66922.3178
10	606116	43	42.999	1557.3954	0.07	66924.6892
11	603596	44	44.000	1521.9950	0.07	66923.4597
12	634480	45	45.003	1488.0947	0.06	66918.9339

Figure-S4. The deconvolution calculations output showing the conjugated mass of 66922.7 (expected 66920.5) after BSA was treated first with 1 mM DTT followed by removal of DTT by a 10K MWCO filter, and then treated with the allyl ester ligand **10** and incubated at 37 °C for 30 minutes. The unconjugated peaks are shown in the mass spectrum without the algorithm detecting them.

Kinetic Experiments

The BSA-Ru conjugate was prepared as before by treating BSA with 5 eq of the ligand (**10** or **16**) and 25 eq of $[CpRu(NCMe)_3]PF_6$. A solution containing 2.0 μM BSA-Ru catalyst concentration (based on the BSA concentration) with 3.5 mM GSH was challenged with various concentrations of $alloc_2$-rhodamine **3**, ranging from 0.5 to 64 μM. The fluorescence intensity as described earlier was measured every 60 seconds with 160 rpm shaking in between measurements, which was converted to the corresponding rhodamine 110 concentration as shown in Figure-S5 and Figure-S7 below.

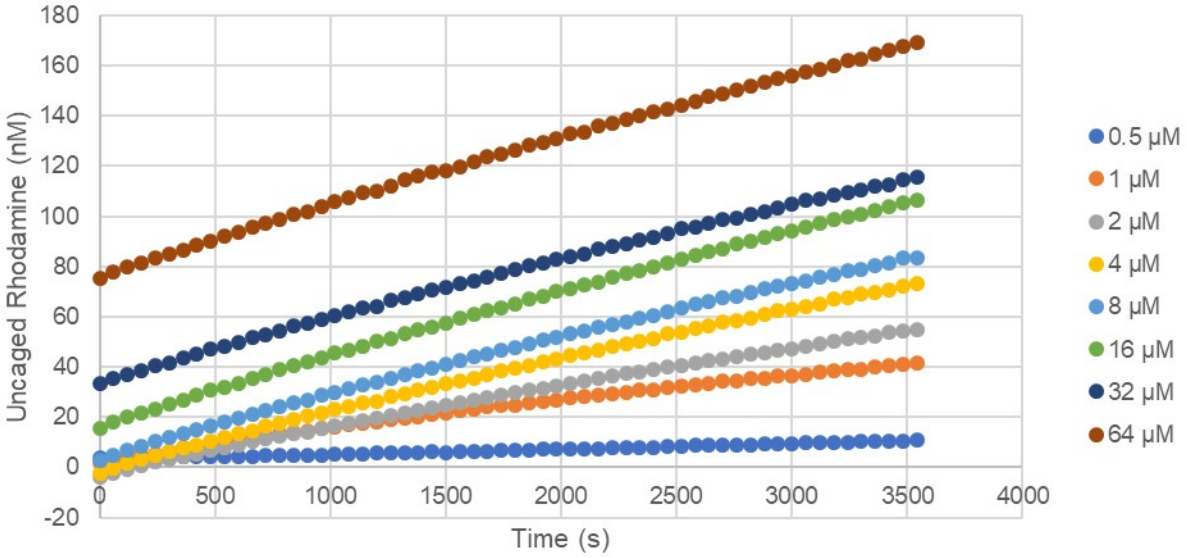


Figure-S5. Uncaging reaction rates by 2.0 μM albumin-Ru-**10** catalyst with various $alloc_2$-rhodamine **3** concentrations.

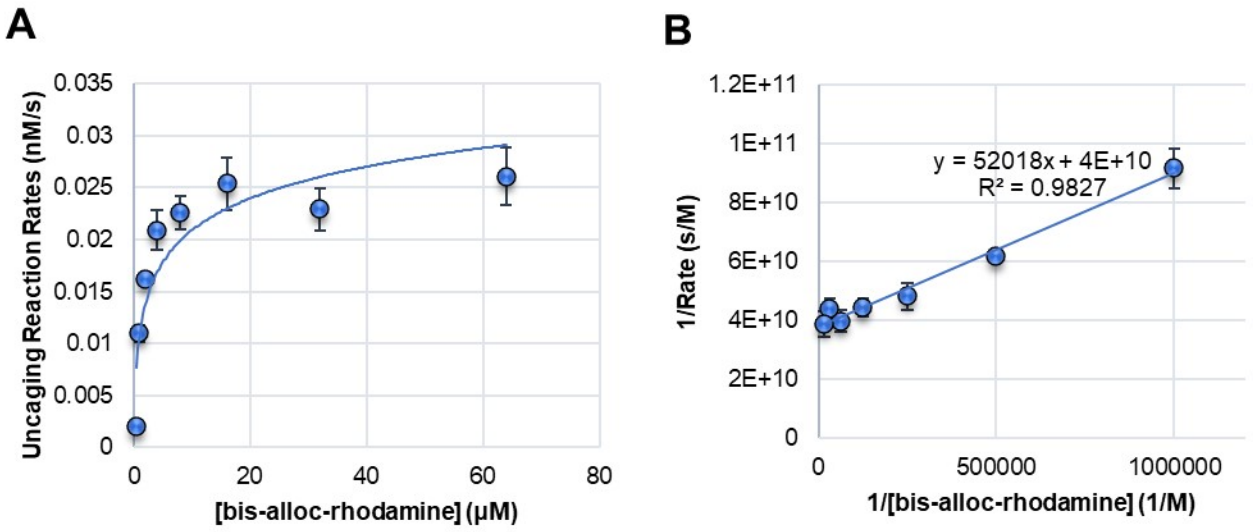


Figure-S6. (**A**) Uncaging reaction rates by 2.0 μM Ru-**12** catalyst plotted against various substrate concentrations show a logarithmic correlation with an estimate of V_{max} at 0.03 nM/sec. (**B**) Lineweaver-Burke plot of the same experiment as **A**. Accordingly, $V_{max} = 2.7*10^{-11}$ (M/s), $K_m = 1.4*10^{-6}$ M, $k_{cat} = 1.3*10^{-5}$ s^{-1}, and the catalytic efficiency (k_{cat}/K_m) = 9.6 $M^{-1}s^{-1}$.

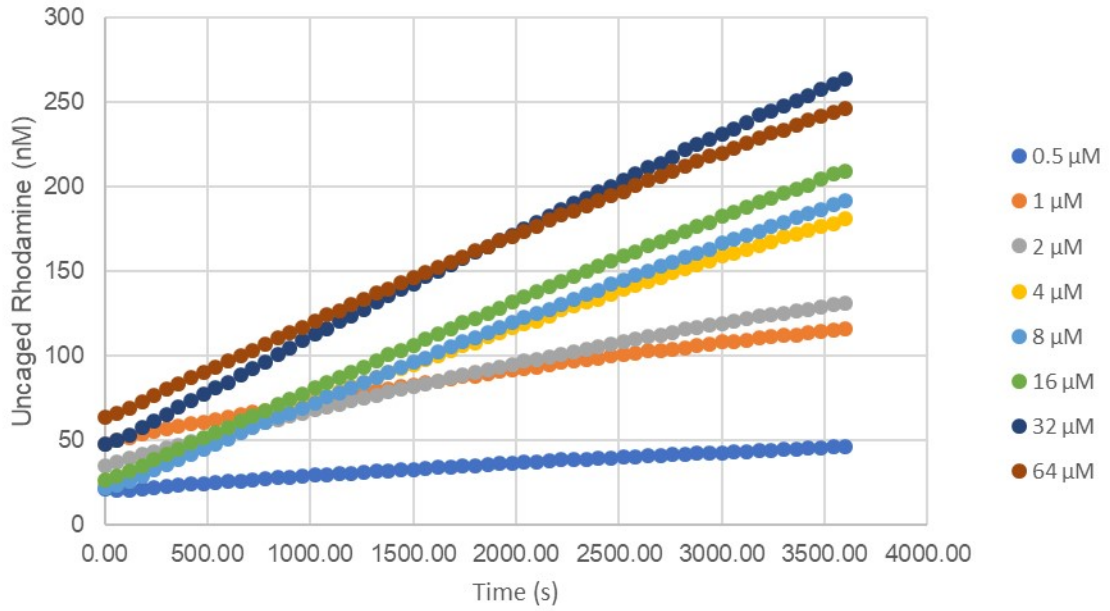


Figure-S7. Uncaging reaction rates by 2.0 μM Ru-**17** catalyst with various $alloc_2$-rhodamine **3** concentrations.

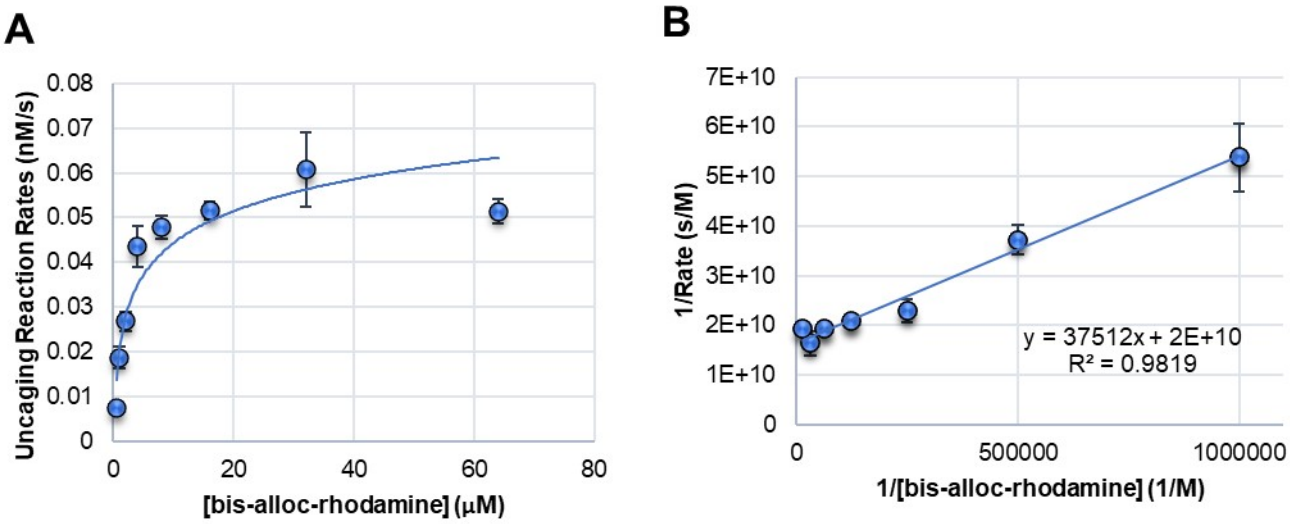


Figure-S8. (**A**) Uncaging reaction rates by 2.0 μM Ru-**17** catalyst plotted against various substrate concentrations show a logarithmic correlation with an estimate of V_{max} at 0.07 nM/sec. (**B**) Lineweaver-Burke plot of the same experiment as **A**. Accordingly, $V_{max} = 6.0*10^{-11}$ (M/s), $K_m = 2.3*10^{-6}$ M, $k_{cat} = 3.0*10^{-5}$ s^{-1}, and the catalytic efficiency (k_{cat}/K_m) = 13 $M^{-1}s^{-1}$.

Catalytic Efficiency Calculation for Ru-PD-L1[8]

In Figure-S3 c of the SI-reference 8 (ref 51 in the main text), the apparent k_2 for uncaging alloc-doxorubicin is given as 25.6 $M^{-1}s^{-1}$ in the supporting information and as 22.1 $M^{-1}s^{-1}$ in the main text (the discrepancy between the SI and the main text may be due to a copy-and-paste error from another paper by the same authors[9]). However, in Figure-S3 b of the same reference,[8] the slope appears to be 0.00153 min^{-1} (rise of 0.23 μM/min and run of 150 μM), which equates to 2.56×10^{-5} sec^{-1}. Using the approximation given in the main article $k_1[S] = k_2[Cat.][S]$, the slope (2.56×10^{-5} sec^{-1}) is k_1 and therefore,

$$k_2 = \frac{k_1}{[Cat.]} = \frac{2.56 \times 10^{-5}\, s^{-1}}{10\ \mu M} = 2.56 \times 10^{-6}\ \mu M^{-1} s^{-1} = 2.56\ M^{-1} s^{-1}$$

Therefore, our catalytic systems appear to have performed slightly better against $alloc_2$-rhodamine (**3**) with the catalytic efficiency of 9.6 $M^{-1}s^{-1}$ and 13 $M^{-1}s^{-1}$ for Ru-**12** and Ru-**17**, respectively, than the Ru-PD-L1 catalyst did against alloc-doxorubicin (cat. eff. 2.56 $M^{-1}s^{-1}$).

SI References:

(1) Prakash, T. P.; Mullick, A. E.; Lee, R. G.; Yu, J.; Yeh, S. T.; Low, A.; Chappell, A. E.; Østergaard, M. E.; Murray, S.; Gaus, H. J.; Swayze, E. E.; Seth, P. P. Fatty Acid Conjugation Enhances Potency of Antisense Oligonucleotides in Muscle. *Nucleic Acids Res.* **2019**, *47* (12), 6029–6044. https://doi.org/10.1093/nar/gkz354.

(2) Park, S.; Lee, M.; Pyo, S.-J.; Shin, I. Carbohydrate Chips for Studying High-Throughput Carbohydrate−Protein Interactions. *J. Am. Chem. Soc.* **2004**, *126* (15), 4812–4819. https://doi.org/10.1021/ja0391661.

(3) Liang, A. D.; Serrano-Plana, J.; Peterson, R. L.; Ward, T. R. Artificial Metalloenzymes Based on the Biotin–Streptavidin Technology: Enzymatic Cascades and Directed Evolution. *Acc. Chem. Res.* **2019**, *52* (3), 585–595. https://doi.org/10.1021/acs.accounts.8b00618.

(4) Garcia, E. S.; Xiong, T. M.; Lifschitz, A.; Zimmerman, S. C. Tandem Catalysis Using an Enzyme and a Polymeric Ruthenium-Based Artificial Metalloenzyme. *Polym. Chem.* **2021**, *12* (46), 6755–6760. https://doi.org/10.1039/D1PY01255J.

(5) Völker, T.; Dempwolff, F.; Graumann, P. L.; Meggers, E. Progress towards Bioorthogonal Catalysis with Organometallic Compounds. *Angew. Chem. Int. Ed.* **2014**, *53* (39), 10536–10540. https://doi.org/10.1002/anie.201404547.

(6) Das, R.; Landis, R. F.; Tonga, G. Y.; Cao-Milán, R.; Luther, D. C.; Rotello, V. M. Control of Intra- versus Extracellular Bioorthogonal Catalysis Using Surface-Engineered Nanozymes. *ACS Nano* **2019**, *13* (1), 229–235. https://doi.org/10.1021/acsnano.8b05370.

(7) Völker, T.; Meggers, E. Chemical Activation in Blood Serum and Human Cell Culture: Improved Ruthenium Complex for Catalytic Uncaging of Alloc-Protected Amines. *ChemBioChem* **2017**, *18* (12), 1083–1086. https://doi.org/10.1002/cbic.201700168.

(8) Zhao, Z.; Wang, X.; Wang, J.; Li, Y.; Lin, W.; Lu, K.; Chen, J.; Xia, W.; Mao, Z.-W. A Nanobody–Bioorthogonal Catalyst Conjugate Triggers Spatially Confined Prodrug Activation for Combinational Chemo-Immunotherapy. *J. Med. Chem.* **2023**, *66* (17), 11951–11964. https://doi.org/10.1021/acs.jmedchem.3c00557.

(9) Zhao, Z.; Tao, X.; Xie, Y.; Lai, Q.; Lin, W.; Lu, K.; Wang, J.; Xia, W.; Mao, Z.-W. In Situ Prodrug Activation by an Affibody-Ruthenium Catalyst Hybrid for HER2-Targeted Chemotherapy. *Angew. Chem. Int. Ed.* **2022**, *61* (26), e202202855. https://doi.org/10.1002/anie.202202855.