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Supporting Information

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Ruthenium-Induced Allylcarbamate Cleavage in Living Cells

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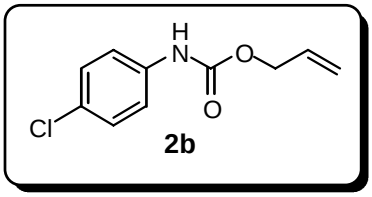
Synthesis of Allyl Carbamates

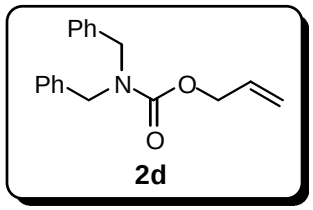
NMR spectra were recorded on a Bruker AM-500 (500 MHz), Bruker DMX-360 (360 MHz), or Bruker DMX-300 (300 MHz) spectrometer. Low-resolution mass spectra were obtained on an LC platform from Micromass using ESI technique. High-resolution mass spectra were obtained with a Micromass AutoSpec instrument using either CI or ES ionization. Infrared spectra were recorded on a Perkin Elmer 1600 series FTIR spectrometer. Solvents and reagents were used as supplied from Aldrich, ACROS, or STREM.

The allyl carbamates were synthesized by the reaction of the respective amine with allyl chloroformate in presence of pyridine. Compounds **2a**[1] and **2c**[2] have been reported before.



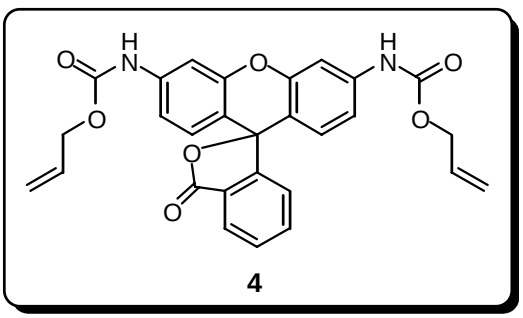
2b

Compound 2b. Pyridine (1.27 mL, 15.7 mmol) was added to a stirred solution of *p*-chloroaniline (2.00 g, 15.7 mmol) in CH_2Cl_2 (15 ml) at room temperature. The reaction was subsequently cooled to 0 °C and allyl chloroformate (1.83 mL, 17.3 mmol) was added dropwise. The resulting light pink solution was stirred for two hours while warming to room temperature. Thereafter, the reaction mixture was dried onto silica gel and subjected to column chromatography with hexane/ethyl acetate (10:1) as the eluting solvent. The resulting product was isolated in quantitative yield (3.32 g) as clear needle-like crystals. ^{1}H-NMR (360 MHz, $CDCl_3$) δ (ppm) 7.33 (m, 2H), 7.26 (m, 2H), 6.71 (br s, 1H), 5.96 (m, 1H), 5.36 (dd, J = 17.1, 1.4 Hz, 1H), 5.26 (dd, J = 10.2, 1.1 Hz, 1H), 4.66 (dt, J = 5.7, 1.2 Hz, 2H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ (ppm) 153.4, 136.6, 132.4, 129.1, 128.6, 120.1, 118.5, 66.1. IR (thin film) ν (cm^{-1}) = 3331, 3117, 2941, 2881, 2760, 1886, 1818, 1700, 1597, 1532, 1493, 1401, 1307, 1227, 1061, 1004, 930, 824, 763, 673. HRMS calcd for $C_{10}H_{10}ClNO_2$ (M^+) 211.0400, found (M^+) 211.0408.



2d

Compound 2d. Pyridine (0.420 mL, 5.21 mmol) was added to a stirred solution of dibenzylamine (1.03 g, 5.21 mmol) in CH_2Cl_2 (8 ml) at room temperature. The reaction was subsequently cooled to 0 °C and allyl chloroformate (1.83 mL, 17.3 mmol) was added dropwise. The resulting bright yellow solution was allowed to warm to room temperature while stirring overnight. The reaction mixture was then dried onto silica gel and subjected to column chromatography with hexane/ethyl acetate (10:1) as the eluting solvent. The resulting product was isolated as a clear oil (1.15 g, 78 %). ^{1}H-NMR (300 MHz, DMSO-d^6) δ (ppm) 7.28 (m, 10H), 5.94 (m, 1H), 5.23 (ddt, J = 17.3, 1.7, 1.7 Hz, 1H), 5.16 (ddt, J = 10.5, 1.5, 1.4 Hz, 1H), 4.63 (dt, J = 5.2, 1.5 Hz, 2H), 4.41 (s, 4H). ^{13}C-NMR (75 MHz, DMSO-d^6) δ (ppm) 156.6, 138.3, 134.0, 129.2, 128.3, 128.1, 127.9, 117.5, 65.9, 49.5, 49.0. IR (thin film) ν (cm^{-1}) = 3086, 3063, 3029, 2936, 1952, 1875, 1807, 1700, 1604, 1495, 1453, 1415, 1366, 1230, 1115, 992, 954, 755, 700. HRMS calcd for $C_{18}H_{19}NO_2$ $(M+H)^+$ 282.1494, found $(M+H)^+$ 282.1486.



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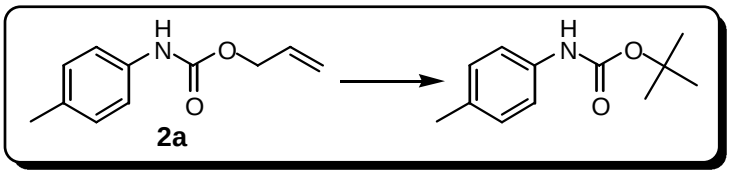
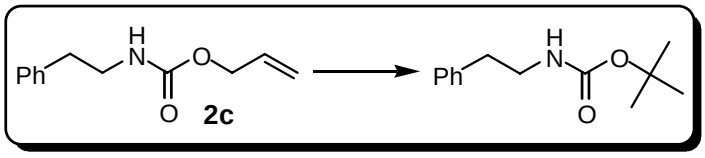
Compound 4. Rhodamine 110 (250 mg, 0.717 mmol) was dissolved in dry DMF (1.5 mL) under argon and cooled to 0

°C. In a separate flask, pyridine (174 μL, 2.15 mmol) was dissolved in DMF (0.6 mL). To the solution containing rhodamine 110 were added simultaneously the solutions containing pyridine and allylchloroformate (152 μL, 1.43 mmol) dropwise. The resulting red reaction mixture was allowed to warm to room temperature while stirring overnight. Thereafter, 10 mL of EtOAc was added to the metallic red solution which was transferred to a separatory funnel and washed twice with 5% HCl (10 mL each). Small amounts of saturated $NaHCO_3$ were used to eliminate emulsion formation. The ethyl acetate layer was collected and combined with two further ethyl acetate washings (10 mL each), washed with saturated $NaHCO_3$ (10 mL), dried with anhydrous $MgSO_4$, and concentrated *in vacuo*. Trace amounts of DMF were removed under low pressure overnight. The resulting oil was subjected to column chromatography on a short column with hexane/ethyl acetate (2:1) as the eluting solvent. The resulting product was isolated as a white solid (93.0 mg, 26%). ^{1}H-NMR (360 MHz, DMSO-d^6) δ (ppm) 10.07 (s, 2H), 8.00 (d, J = 7.4 Hz, 1H), 7.78 (td, J = 7.4, 1.2 Hz, 1H), 7.71 (td, J = 7.4, 1.0 Hz, 1H), 7.56 (d, J = 2.0 Hz, 2H), 7.27 (d, J = 7.5 Hz, 1H), 7.14 (dd, J = 8.7, 2.1 Hz, 2H), 6.70 (d, J = 8.7 Hz, 2H), 5.98 (m, 2H), 5.36 (ddt, J = 17.2, 1.7, 1.6 Hz, 2H), 5.24 (ddt, J = 10.4, 1.6, 1.4 Hz, 2H), 4.62 (dt, J = 5.5, 1.4 Hz, 4H). ^{13}C-NMR (125 MHz, DMSO-d^6) δ (ppm) 168.5, 152.9, 152.3, 150.8, 141.3, 135.5, 132.9, 130.0, 128.3, 125.6, 124.6, 123.8, 117.6, 114.3, 112.3, 105.0, 81.8, 64.8. IR (thin film) ν (cm^{-1}) = 3316, 2940, 1737, 1612, 1546, 1528, 1409, 1322, 1286, 1222, 1106, 1060, 990, 870, 760. HRMS calcd for $C_{28}H_{22}N_2O_7$ $(M+H)^+$ 499.1505, found $(M+H)^+$ 499.1487.

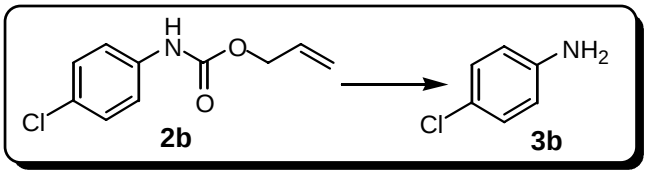
Determination of Isolated Yields of the Allylcarbamate Deprotection



Procedure for the cleavage of 2a and the isolation of the BOC-protected amine. After stirring carbamate **2a** (250 mg, 1.31 mmol) overnight with Cp*Ru(COD)Cl (50.0 mg, 0.131 mmol) and thiophenol (671 μL, 6.55 mmol), BOC anhydride (1.71 g, 7.86 mmol) was added to the reaction. The reaction was then cooled to 0 °C and DMAP (320 mg, 2.62 mol) was added portionwise. The reaction was stirred for 1 hour while warming to room temperature, before dry loading onto silica gel column and eluting with 15:1 Hexane/EtOAc. Yield over two steps was 86% (233 mg).

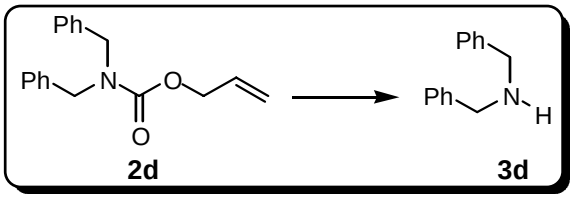


Procedure for the cleavage of 2c and the isolation of the BOC-protected amine. After stirring carbamate **2c** (272 mg, 1.33 mmol) overnight with Cp*Ru(COD)Cl (50.4 mg, 0.132 mmol) and thiophenol (680 μL, 6.63 mmol), BOC anhydride (1.74 g, 7.96 mmol) was added to the reaction. The reaction was then cooled to 0 °C and DMAP (259 mg, 2.12 mol) was added portionwise. The reaction was stirred for 1 hour while warming to room temperature, before dry loading onto silica gel column and eluting with 15:1 Hexane/EtOAc. Yield over two steps was 96% (276 mg).



Procedure for the cleavage of 2b and the isolation of the free amine 3b. Carbamate **2b** (277 mg, 1.31 mmol) was stirred overnight at room temperature with Cp*Ru(COD)Cl (49.8 mg, 0.131 mmol) and thiophenol (671 μL, 6.55 mmol). Amine **3b** was then purified by column chromatography after dry loading the entire reaction and eluting with 10:1 Hexane/EtOAc (142 mg, 85%).

Procedure for the cleavage of 2d and the isolation of the free amine 3d. Carbamate **2d** (216 mg, 0.769 mmol) was stirred overnight at room temperature with Cp*Ru(COD)Cl (29.2 mg, 0.0769 mmol) and thiophenol (394 μL, 3.84 mmol). Amine **3d** was then isolated by column chromatography after dry loading the entire reaction and eluting with 10:1 Hexane/EtOAc (132 mg, 87%).



Studies by Gas Chromatography

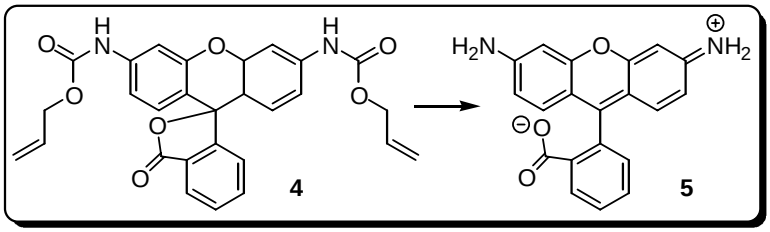
General reaction procedure. To a 5 mL round bottom flask was added carbamate **2a** (50.0 mg, 0.262 mmol), which was dissolved in the given solvent (1.3 mL). After the carbamate was completely dissolved, Cp*Ru(COD)Cl (10.0 mg, 0.0262 mmol) was added and stirred until the solution became clear golden orange. Finally, thiophenol (134 μL, 1.31 mmol) was added to the reaction causing the solution to darken. The reaction was stirred at the appropriate temperature overnight before addition of the n-decane standard (14.6 μL, 0.0751 mmol). The reaction was then diluted 50-fold before GC analysis.

Reaction procedure for reactions containing both aromatic and aliphatic thiols. To a 5 mL round bottom flask was added carbamate **2a** (50.0 mg, 0.0262 mmol), which was dissolved in the given solvent (1.3 mL). After the carbamate was completely dissolved, Cp*Ru(COD)Cl was added and stirred until the solution became clear golden orange. At this point, benzeneethanethiol (176 μL, 1.31 mmol) and then thiophenol (134 μL, 1.31 mmol) were added to the reaction causing the solution to darken. The reaction was stirred at the appropriate temperature overnight before the addition of the n-decane standard (14.6 μL, 0.0751 mmol). The reaction was then diluted 50 times before GC analysis.

Determination of GC-yields. All GC-yields for the conversion of allylcarbamate **2a** to *p*-methylaniline **3a** were determined from a standard curve using an n-decane internal standard on an Agilent HP5890 instrument with a 25 meter HP Ultra 2, crosslinked 5% phenylmethylsilicone column. The standard curve was acquired by recording the ratios of *p*-toluidine/decane with 1.5 mM decane and 1, 2, 3, 4, and 5 mM *p*-toluidine. The ratios at each concentration were determined in triplicate from independently prepared samples and averaged to make the final standard curve.

GC method. The separation method used an initial hold time of 2 min at 80 °C, a ramp of 10 °C/min to a final oven temperature of 250 °C, and a final hold time of 10 min. The identity of peaks was verified by GS/MS and by comparing with the retention times of known samples (retention times: thiophenol = 3.4 min, n-decane = 3.7 min, *p*-toluidine = 4.9 min, benzeneethanethiol = 6.7 min, allylphenyl thioether = 7.3 min, phenyl disulfide = 15.4 min).

Uncaging of Allyloxycarbonyl Protected Rhodamine 110 (4):



Reaction conditions. Reaction 1 (Entry 1 in Figure 1b): To 95 μL of DMSO was added 5 μL of a stock solution of **4** (20 mM in DMSO), followed by 100 μL of water.

Reaction 2 (Entry 2 in Figure 1b) : To 95 μL of DMSO was added 5 μL of a stock solution of **4** (20 mM in DMSO), followed by 93 μL of cell extract and 7 μL of glutathione solution (100 mM in water).

Reaction 3 (Entry 3 in Figure 1b): To 91 μL of DMSO was added 5 μL of a stock solution of **4** (20 mM in DMSO), followed by 93 μL of cell extract, 7 μL of a glutathione solution (100 mM in water), and 4 μL of a stock solution of Cp*Ru(COD)Cl (5 mM in DMSO).

Reaction 4 (Entry 4 in Figure 1b): To 84 μL of DMSO was added 5 μL of a stock solution of **4** (20 mM in DMSO), followed by 7 μL of a thiophenol solution (100 mM in DMSO), 93 μL of cell extract, 10 μL of a glutathione solution (100 mM in water), and 4 μL of a stock solution of Cp*Ru(COD)Cl (5 mM in DMSO).

All reactions were agitated thoroughly with a micropipette and then shaken at 120 rpm at 37 °C for 2.5 hours before determining the yield. All yields of **5** were determined with the help of a standard curve using a Spectramax Gemini XS fluorescence plate reader (Molecular Devices, Sunnyvale, CA) using 96 well-plate format, an excitation wavelength of 488 nm, a 495 nm cutoff, and an emission wavelength of 520 nm. The standard curve was prepared from two independent measurements containing all components of reaction 4 (Figure 1), substituting protected fluorophore **4** for rhodamine 110 (**5**) with 500, 450, 400, 350, 300, and 250 μM concentrations. Each concentration was diluted 500-fold into 1:1 MeOH:H_2O (100 μL) before measuring fluorescence. The obtained values from the two independent experiments were then averaged to give the final linear plot.

Preparation of the cell extract. The cell lysate was prepared by growing BL21DE3pLysS strain *E. coli* in LB broth, Miller media (Fisher) containing ampicillin (100 μg/mL) and chloramphenicol (34 μg/mL). One liter of LB media was inoculated with 5 mL of starting culture and grown overnight at 37 °C and 250 rpm. The culture was spun at 6000 rpm for 15 min and the obtained pellets (totally 8.3 g) were resuspended in 30 mL of phosphate buffered saline (PBS) and sonicated (3x 45 sec at 6 min intervals). Lysed cells were then spun down at 17,000 rpm for 55 min at 4 °C and the supernatant was decanted. The solution was stored at –80 °C and used as the whole cell extract.

Live Cell Imaging

Preparation of cell cultures. HeLa cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Invitrogen) supplemented with 10% Fetal Bovine Serum (FBS, Biowhittaker Molecular Applications), glutamine (2 mM), and penicillin/streptomycin (100 μg/mL). One day before imaging, approximately 350,000 cells were plated on glass-bottom dishes (35/10/1.7 mm, Warner Instruments, Amsterdam, ND) in media (ca. 2 mL).

Fluorescence microscopy. All images were taken using an Olympus FV10-ASW confocal laser scanning biological microscope for availability and a 40× water-immersion objective lens (NA = 1.15). Excitation of HeLa cells containing deprotected rhodamine 110 at 488 nm and/or $DiIC_{18}(5)$ at 633 nm was carried out with a HeNe laser. Emission was collected at 500-555 nm for rhodamine 110 and at 645-745 nm for $DiIC_{18}(5)$.

Membrane staining procedure. Immediately before experiments, 10 μL of fluorophore 1,1'-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine, 4-chlorobenzenesulfonate salt ($DiIC_{18}(5)$ solid, Molecular Probes) in DMSO (1.5 mg/mL) was added to the glass-bottom dishes containing the HeLa cells and agitated carefully until homogenous. The cells were allowed to incubate with the fluorophore for 25-45 min.

Protocol for the uncaging of allyloxycarbonyl protected rhodamine 110 (4). To the glass-bottom dishes containing HeLa cells and 2 ml of media was added protected rhodamine **4** in DMSO (10 μL, 20 mM) that was agitated until homogenous. The cells were then incubated at 37 °C for 25 minutes. Following incubation, media was removed and cells were washed successively with PBS (2×1 mL) before replacing 1 mL of the media and beginning live imaging. Then, catalyst **1** (20 or 40 μM) with or without thiophenol (0.5 or 1.0 mM) was added in 1 mL of media to the dish containing cells and agitated moderately for a few seconds.

Cell Viability MTT Assay

Cell viability following exposure to the conditions used for cellular imaging was assessed using (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) with an assay from Molecular Probes (Vyrant MTT assay kit).

HeLa cells were plated in 200 μL DMEM in 96-well microplates with a density of 13,000 cells/well and cells were grown for 24 hours. Thereafter, 2 μL of a 10 mM DMSO solution of protected fluorophore (**4**) was added to each of 6 wells (100 μM final concentration) and incubated at 37 ^{o}C for 25 min. After 25 minutes, the media was removed from the wells containing protected fluorophore and an additional row of 6 wells containing HeLa cells. At this time, 150 μL of PBS was added to each of these wells simulating a washing. The PBS from the wells incubated with protected fluorophore was then removed and replaced with 180 μL media containing 40 μM Cp*Ru(COD)Cl (**1**) and 500 μM thiophenol. The PBS in the other wells was removed and replaced with 180 μL fresh media. The cells were then incubated for 10 minutes at 37 ^{o}C. After 10 minutes, the media was removed from all 12 wells and replaced with 150 μL of PBS, which was subsequently removed and replaced with 200 μL of fresh media. The cells were then incubated for 24 hours before removing the media from the 12 treated wells from above, an additional row of 6 wells containing untreated cells, and a row of 6 wells containing no cells. All media was then replaced with 182 μL of fresh media along with 18 μL of MTT (5 mg/mL) before being incubated for 3 hours at 37 ^{o}C. After 3 hours, 155 μL of media containing MTT was removed from each of the wells (4 rows x 6 wells = 24 wells) and replaced with 90 μL of DMSO. After incubating an additional 10 minutes at 37 °C, the absorbance of each well was measured at 550 nm.

A background correction was carried out by subtracting the average background absorption of the wells containing no cells from the average absorbance of wells containing cells. The percent viability was calculated by dividing the average corrected absorbance of the treated wells with the average corrected absorbance of the untreated wells.

Cells exposed to the control washing sequence containing no additives showed a 42 % survival rate as compared to cells exposed to no washings. Cells exposed to the imaging conditions including Ru-catalyst (40 μM), thiophenol (500 μM), and protected fluorophore (100 μM) also showed a 42 % survival rate. These results indicate that cell viability is not adversely affected by these reagents under the used conditions.

References

1.) Greck, C.; Bischoff, L.; Ferreira, F.; Genêt, J. P. *J. Org. Chem.* **1995**, *60*, 7010-7012.
2.) Stafford, J. A.; Gonzales, S. S.; Barrett, D. G.; Suh, E. M.; Feldman, P. L. *J. Org. Chem.* **1998**, *63*, 10040-10044.