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Light-Triggered Ruthenium-Catalyzed Allylcarbamate Cleavage in Biological Environments

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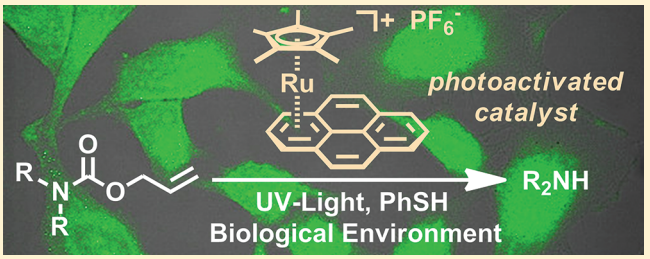
ABSTRACT: The sandwich complex [Cp*Ru(η^6-pyrene)]-PF_6 (Cp* = η^5-$C_5(CH_3)_5$) serves as a photoactivatable catalyst for the conversion of *N*-allylcarbamates into their amines in the presence of thiophenol under biorelevant conditions (water, air, plus aliphatic thiols) and even in mammalian cells. This new phototriggered substrate/catalyst pair points towards applications in chemical biology and medicinal chemistry where signal amplification is combined with spacial and temporal control.



The modulation, sensing, and imaging of biological processes in biomedical research typically relies on stoichiometric events, whereas the catalytic initiation of biological processes provides signal amplification, for example, by turning over caged substrates multiple times, by catalytically labeling or deactivating target biomolecules, and by catalytically activating prodrugs.[1] This advantage of catalytic turnover has been successfully exploited in the area of enzyme-triggered bioimaging.[2] However, the design of bioorthogonal synthetic catalyst/substrate pairs which can passively diffuse into cells, such as a typical tool compound used in chemical biology studies, is a highly formidable challenge and no useful systems exist to date.[3−5] We recently reported that the organometallic half-sandwich complex [Cp*Ru(COD)Cl] (**1**; COD = 1,5-cyclooctadiene) can catalyze the conversion of *N*-allylcarbamates into their amines in the presence of thiols, water, and air.[3] We here wish to disclose a photoactivated version of this catalytic system, namely [Cp*Ru(η^6-pyrene)]PF_6 (**2**) (Scheme 1), which allows light-triggered catalysis with temporal and in the future also spatial control.[6,7]

RESULTS AND DISCUSSION

The sandwich complex [Cp*Ru(η^6-pyrene)]PF_6 was first reported by Mann et al. and was demonstrated to be quite stable toward thermal replacement of the pyrene moiety.[8,9] On the basis of the known photochemical lability of Ru(η^6-arene) sandwich coordination modes,[10] we envisioned that the released solvent-coordinated fragment [Cp*Ru(solvent)$_3$]$^+$ should be capable of serving as a catalyst for a variety of transformations. Indeed, we found that the complex **2** (10 mol %), upon irradiation with $\lambda \geq 330$ nm for 5 min, catalyzes the cleavage of a representative selection of allylcarbamates **3a−d** to their respective amines **4a−d** in good yields of 70−83% in the presence of 5 equiv of thiophenol at room temperature and under air (Scheme 1).[11] It is noteworthy that no products were formed in the absence of UV light.

In order to test the photoinduced catalytic cleavage of allylcarbamates under biologically more relevant conditions, we used the previously developed bis-allyloxycarbonyl-modified rhodamine 110 (**5**) (Scheme 2).[3] This "caged" fluorophore is nonfluorescent but can be converted to the highly fluorescent rhodamine 110 (**6**) upon carbamate cleavage, thus allowing us to monitor the photocatalysis by simple fluorescence readout. Accordingly, upon UV photolysis, the caged rhodamine **5** in the presence of β-mercaptoethanol, PhSH, and catalyst **2** (20 mol %) resulted in the rapid formation of rhodamine 110 (**6**) with maximum yields already reached after approximately 3 min (Figure 1). The formation of rhodamine 110 correlated with the formation of the fluorescent pyrene in the course of the photoactivation of **2**, as demonstrated in Figure 1. Further data shown in Table 1 reveal that no significant amounts of rhodamine 110 are formed without catalyst (Table 1, entry 1) or in the absence of thiols (Table 1, entry 2). Obviously, thiols are required for the Ru-catalyzed cleavage of the allylcarbamates (Table 1, entries 3−6). In fact, the more nucleophilic thiophenol (Table 1, entry 5) or combinations of aliphatic and aromatic thiols (Table 1, entries 5 and 6) work significantly better than aliphatic thiols alone (Table 1, entries 3 and 4).

Finally, we investigated the photoactivated allylcarbamate cleavage in mammalian cells. For this, cultured HeLa cells were first incubated with the caged fluorophore **5** (100 μM), followed by a washing step, so that the caged fluorophore **5** was only located within the HeLa cells but not in the culture

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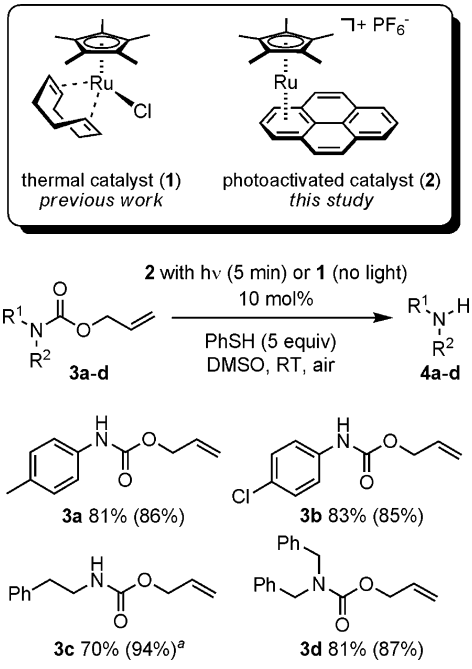
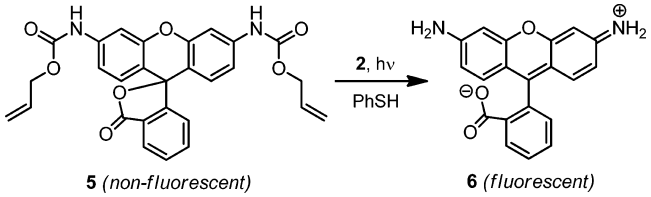
Scheme 1. Previous Thermal and New Photoactivated Ruthenium Catalysts ($\lambda \geq 330$ nm) for the Cleavage of Allylcarbamates under Biorelevant Conditions[b]



[a]Isolated as BOC-protected amine. [b]The yields for the thermal reaction are shown in parentheses for comparison and are taken from ref 3.

Scheme 2. *N,N*-Bisallyloxycarbonylrhodamine 110 (5) as a "Caged" Fluorophore



medium. At this stage, no rhodamine 110 fluorescence could be detected (Figure 2A), after the following addition of either complex **2** alone or complex **2** together with thiophenol (Figures 2B,C). However, the addition of complex **2** followed by a 10 min irradiation led to a small increase in fluorescence (6-fold) (Figure 2D), whereas a much stronger fluorescence increase was observed upon photolysis in the presence of complex **2** together with thiophenol: an irradiation time of 5 min resulted in an increase in the overall fluorescence intensity by 35-fold (Figure 2E), whereas an irradiation time of 10 min increased the fluorescence intensity further to overall 70-fold (Figure 2F).[12] This benefit of thiophenol is consistent with our model experiments shown in Table 1. Furthermore, the distribution of fluorescence within the cellular cytosol implies that the ruthenium sandwich complex is membrane-permeable.[13,14]

CONCLUSION

We here demonstrated that, upon photoactivation, [Cp*Ru(η^6-pyrene)]PF_6 is capable of catalyzing the conversion of *N*-allylcarbamates to their respective amines in the presence of thiophenol under biologically relevant conditions and even in

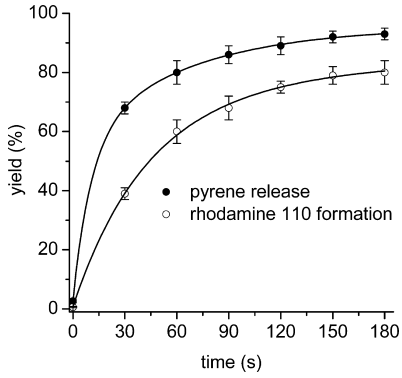


Figure 1. Fluorescence-monitored formation of rhodamine 110 and pyrene in the photoinduced catalytic conversion **5** → **6** in the presence of 20 mol % [Cp*Ru(η^6-pyrene)]PF_6. Reaction conditions: caged rhodamine **5** (0.5 mM) in DMSO/H_2O (1/1) with β-mercaptoethanol (5 mM), PhSH (5 mM), and catalyst **2** (0.1 mM) under photolysis with $\lambda \geq 330$ nm for the monitored reaction time. Yields were determined by fluorescence measurements (pyrene, λ_{ex} 319 nm, λ_{em} 390 nm; rhodamine 110, λ_{ex} 488 nm, λ_{em} 520 nm), the data were fitted to an exponential equation, and standard deviations from three independent experiments are given.

Table 1. Light-Induced Catalytic Cleavage of Bisallyloxycarbonylrhodamine 5 with [Cp*Ru(η^6-pyrene)]PF_6 under Biologically Relevant Conditions[a]

entry	amt of cat. (mol %)	thiol	yield (%)[b]
1	none	ME	0.5 ± 0.1
2	20	none	1.2 ± 0.3
3	20	ME	13 ± 2
4	20	Cys	14 ± 2
5	20	PhSH	93 ± 4
6	20	ME + PhSH	90 ± 3

[a]General reaction conditions: caged rhodamine **5** (0.5 mM) and catalyst **2** (0.1 mM) in 1/1 DMSO/sodium phosphate buffer (1 mM, pH 7.25) at 37 °C for 10 min under irradiation at $\lambda \geq 330$ nm. The concentrations of β-mercaptoethanol (ME), PhSH, and cysteine (Cys) were all 5 mM. [b]Yields were determined by fluorescence measurements, and the standard deviations from three independent experiments are provided.

living mammalian cells. This phototriggered substrate/catalyst pair might become an attractive tool for future applications that require spatial and temporal control.

EXPERIMENTAL SECTION

Compound Synthesis. Allylcarbamates **3a−d**,[3,15] [Cp*Ru(pyrene)]PF_6 (**2**),[8] and *N,N*-bisallyloxycarbonylrhodamine 110 (**5**)[3] were synthesized according to published procedures.

Photoinduced Cleavage of *N*-Allyloxycarbonylanilines. A representative example is given: 4-chloro-*N*-allyloxycarbonylaniline (15 mg, 0.071 mmol), [Cp*Ru(pyrene)]PF_6 (4 mg, 0.007 mmol, 10 mol %), and thiophenol (36 μL, 0.35 mmol) were dissolved in DMSO (105 μL), and the solution was irradiated for 5 min with a 200 W Hg(Xe) arc lamp (Newport) in combination with a $\lambda \geq 330$ nm filter. Afterward, the reaction mixture was stirred overnight at room temperature in air. The resulting solution was diluted 10-fold with water, extracted with Et_2O (5 × 5 mL), dried over Na_2SO_4, concentrated in vacuo, and purified by silica gel flash column chromatography with hexane/ethyl acetate (5/1 to 3/1) to afford 7.8 mg (83%) of 4-chloroaniline. Analogous reactions have been performed under thermal conditions with [Cp*Ru(COD)Cl].[3]

Photoinduced Cleavage of Caged Rhodamine 5. Biscarbamate **5** (0.5 mM) and ruthenium complex **2** (0.1 mM) in 1/1 DMSO/

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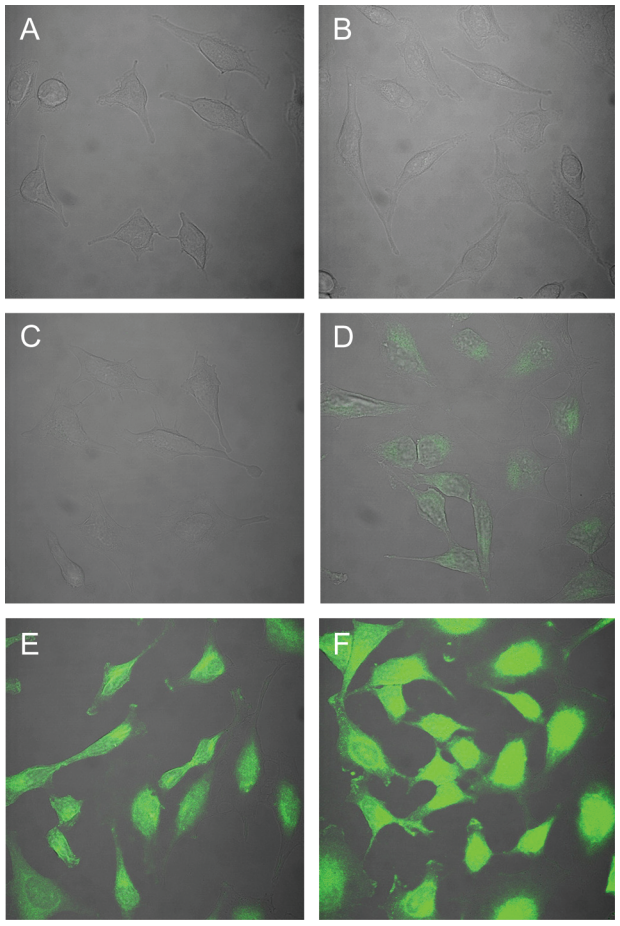

Figure 2. Confocal fluorescence imaging of $[Cp^*Ru(pyrene)]PF_6$-induced uncaging of the bisallylcarbamate-protected rhodamine 110 (**5**) inside HeLa cells. HeLa cells were incubated with caged rhodamine **5** (100 μM) for 25 min and then washed with PBS buffer. (A) after the washing step; (B) after the addition of ruthenium complex **2** (20 μM) and a 10 min incubation time; (C) after the addition of ruthenium complex **2** (20 μM) and thiophenol (1 mM) and a 10 min incubation time without photolysis; (D) ruthenium complex **2** (20 μM) added and photolyzed for 10 min with $\lambda \geq 330$ nm; (E) ruthenium complex **2** (20 μM) and thiophenol (1 mM) added and photolyzed for 5 min with $\lambda \geq 330$ nm; (F) ruthenium complex **2** (20 μM) and thiophenol (1 mM) added and photolyzed for 10 min with $\lambda \geq 330$ nm.

sodium phosphate buffer (1 mM, pH 7.25) were irradiated with $\lambda \geq 330$ nm at 37 °C for 10 min in the absence or presence of additional thiols (5 mM each). Yields were determined by measurement of the generated fluorescence of rhodamine 110 with a SpectraMax M5 fluorescence plate reader from Molecular Devices (λ_{ex} 488 nm, cutoff 495 nm, λ_{em} 520 nm) and comparison with a calibration curve prepared with rhodamine 110 (Table 1).[3]

Fluorescence Confocal Imaging of Mammalian Cells. HeLa cells were cultured in Dulbecco's modified Eagle medium (DMEM, Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS, Biochrom), glutamine (2 mM), and penicillin/streptomycin (100 μg/mL). One day in advance of the experiments, approximately 1×10^5 cells were plated on standard-bottom dishes (35/12/1.5 mm) in media (2 mL). Rhodamine biscarbamate **5** in DMSO (10 μL, 20 mM stock) was added to the standard-bottom dishes containing HeLa cells and briefly agitated to reach a homogeneous concentration of 100 μM. Following incubation for 25 min at 37 °C, the medium was removed and cells were washed successively with PBS buffer (2×1 mL) and replaced by 1 mL of fresh medium. Then, $[Cp^*Ru(\eta^6\text{-pyrene})]PF_6$ (4 μL of a 5 mM stock solution in DMSO) was added, resulting in a catalyst concentration of 20 μM. After moderate agitation for a few seconds, the standard-bottom dishes were exposed to light of $\lambda \geq 330$ nm for 5 min. Afterward, the medium was removed and washed one time with PBS buffer. Then, the cells were fixed and permeabilized with cold methanol for 5 min at −20 °C. Subsequently, methanol was removed and dishes containing cells were stored at −20 °C until fluorescence images were recorded with a LSM 510 META confocal microscope (Zeiss). Images comprised phase contrast and green fluorescence channels. The image analysis was performed with the software Image J as reported recently.[16]

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Notes

The authors declare no competing financial interest.

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