Bioorganometallic Chemistry

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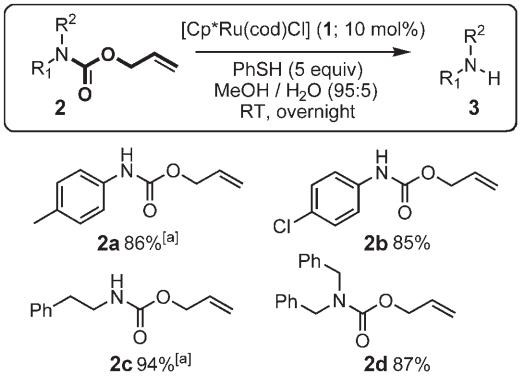
Ruthenium-Induced Allylcarbamate Cleavage in Living Cells**

*Craig Streu and Eric Meggers**

Organometallics are increasingly gaining attention as tools in chemical biology owing to their distinguished physicochemical properties, reactivities, and three-dimensional structures.[1] Along these lines, the exceptional ability of organometallic compounds to catalyze a wide variety of chemical transformations has not yet been sufficiently exploited for chemical biology, but could yield bioactive molecules with novel properties.[2–4] For example, such catalysts could eventually be used to amplify signals by turning over a substrate multiple times, catalytically label or deactivate target biomolecules, or release prodrugs and all this in a cellular environment. However, designing catalysts which work under physiological conditions is a significant challenge owing to the combined presence of air, water, and a plethora of cellular components such as millimolar concentrations of thiols, which are prone to poison organometallic catalysts, especially under protic and aerobic conditions.[5]

With respect to this new aspect of bioorganometallic chemistry, we herein disclose a ruthenium-catalyzed release of amines from their allylcarbamates that tolerates the combination of water, air, and thiols, and we demonstrate the utility of this cleavage reaction in living mammalian cells.[6–10]

In the course of screening several organometallic catalysts for a variety of reactions, we found that the complex [Cp*Ru(cod)Cl] (Cp* = pentamethylcyclopentadienyl, cod = 1,5-cyclooctadiene; **1**) catalyzes the cleavage of allylcarbamates **2** to their respective amines **3** in the presence of an excess of thiophenol in an open flask experiment, tolerating water and air (Scheme 1).[11,12] For example, the reaction of *p*-methylaniline allylcarbamate **2a** (200 mM) with 5 equivalents of thiophenol in the presence of 10 mol% ruthenium catalyst **1**, carried out in MeOH/H_2O (95:5) under air and overnight at room temperature, provides *p*-methylaniline in 86% yield if isolated as the *tert*-butoxycarbonyl (Boc)-protected amine, and 89% yield according to GC analysis (Table 1, entry 1). This deprotection is quite general as it works under the same conditions with high yields also for allylcarbamates of an electron-acceptor-substituted aniline (**2b**, 85% yield) and a primary (**2c**, 94%, isolated as the Boc-protected amine), as well as a secondary amine (**2d**, 87% yield; Scheme 1).[13]



Scheme 1. Reaction of compounds **2** to form the respective amines **3**. [a] Isolated as the Boc-protected amine.

The influence of air, water, and aliphatic thiols on the GC-determined yields of this ruthenium-catalyzed cleavage is shown for substrate **2a** in Table 1. Accordingly, yields are not significantly affected by air and water (Table 1, entries 1–3). In contrast, omitting thiophenol prevents the carbamate cleavage completely in the presence of water and air (Table 1, entry 4). Most importantly for cellular applications, the cleavage reaction can be performed in the presence of

[*] C. Streu, Prof. E. Meggers
Department of Chemistry
University of Pennsylvania
231 South 34th Street, Philadelphia, PA 19104 (USA)
Fax: (+1) 215-746-0348
E-mail: meggers@sas.upenn.edu
Homepage: http://www.sas.upenn.edu/~meggers/

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Table 1: Catalytic cleavage of allylcarbamate **2a** to *p*-methylaniline with [Cp*Ru(cod)Cl].[a]

Entry	Thiol	Solvent	Atm.	*T*	Yield
1	PhSH	MeOH/H_2O (95:5)	air	RT	89%
2	PhSH	MeOH	air	RT	93%
3	PhSH	MeOH/H_2O (95:5)	argon	RT	96%
4	no thiols	MeOH/H_2O (95:5)	air	RT	0%
5	PhSH and $PhCH_2CH_2SH$	MeOH/H_2O (95:5)	air	RT	93%
6	$PhCH_2CH_2SH$	MeOH/H_2O (95:5)	air	RT	34%
7	$PhCH_2CH_2SH$	MeOH/H_2O (95:5)	air	37°C	67%

[a] General reaction conditions: **2a** (200 mM), [Cp*Ru(cod)Cl] (20 mM), thiol (1 M; each), at room temperature (20–23°C) overnight. Yields were determined by GC analysis.

aliphatic thiols, such as benzeneethanethiol, with virtually no influence on the yield of the reaction (93%; Table 1, entry 5). However, if performed at room temperature, the aromatic thiol is necessary because substitution for an aliphatic thiol leads to a significant reduction in yield (34%; Table 1, entry 6). Interestingly, increasing the temperature to 37°C provides the released amine with an improved yield of 67% by using the aliphatic thiol benzeneethanethiol as the only nucleophile.

To evaluate the carbamate cleavage under conditions that more closely resemble a physiological environment, the bis-allyloxycarbonyl-protected rhodamine 110 (**4**; Figure 1a) was synthesized.[14] This caged fluorophore is virtually nonfluorescent (Figure 1b, entry 1) and stable in the presence of *E. coli* cell extract (Figure 1b, entry 2).[15] However, upon allylcarbamate deprotection, strongly green-fluorescent rhodamine 110 (**5**) is released (Figure 1a). This allows for monitoring of the ruthenium-catalyzed reaction by fluorescence analysis. For example, the reaction of **4** with **1** (20 mol%) in the presence of cell extract and an additional glutathione (GSH; 3.5 mM)[16] leads to the release of the fluorophore with a yield for **5** of 6% at 37°C (Figure 1b, entry 3). Under the same conditions, but with additional thiophenol, the yield increases to 80% as measured after 2.5 h (Figure 1b, entry 4), resulting in a turnover number of 8. These results demonstrate that [Cp*Ru(cod)Cl] is able to catalyze the cleavage of allylcarbamates under biological conditions consisting of millimolar concentrations of GSH as well as a plethora of cellular components from the whole-cell extract.

Finally, the caged fluorophore **4** was used as a tool to investigate the ruthenium-induced allylcarbamate cleavage in living mammalian cells. For this, cultured HeLa cells were loaded with caged fluorophore **4** by incubating the HeLa cells with **4** (100 μM) for half an hour, followed by washing the cells with phosphate-buffered saline (PBS) buffer solution, and the subsequent addition of fresh media. Thereafter, catalyst **1** was added to the medium in final concentrations as low as 20 μM. Fluorescence should now only develop inside the cell where the caged fluorophore **4** is located. Indeed, observing the

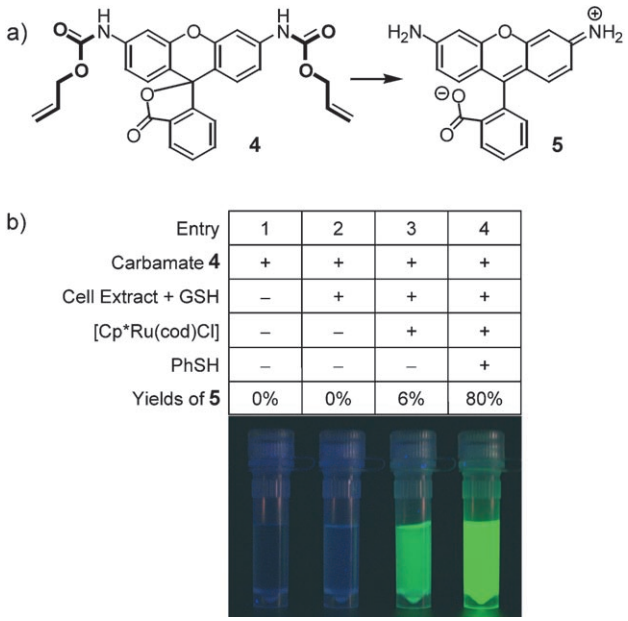

Figure 1. Influence of cell extract on the ruthenium-catalyzed allylcarbamate cleavage. a) Ruthenium-catalyzed deprotection of the caged fluorophore **4** to rhodamine 110 (**5**). b) Fluorescence development with **4**. Reaction conditions: Entry 1: **4** (0.5 mM) in DMSO/H_2O (1:1). Entry 2: **4** (0.5 mM), in DMSO/cell extract (1:1), and GSH (3.5 mM), yielding an overall pH of 7.0. Entry 3: Same as entry 2, but with additional [Cp*Ru(cod)Cl] at 100 μM. Entry 4: Same as entry 2, but with additional [Cp*Ru(cod)Cl] at 100 μM and 3.5 mM PhSH. Reaction mixtures were shaken at 120 rpm for 2.5 h at 37°C. Yields were then determined by comparing the fluorescence of diluted samples with a standard curve of different concentrations of rhodamine 110. For the shown vials, the solutions were diluted 20-fold with H_2O/MeOH (1:1) and excited with a long-wavelength UV lamp.

changes in fluorescence by live-cell imaging with a confocal fluorescence microscope demonstrates that green fluorescence increases in intensity by around 10-fold over a time period of 15 minutes within the cytoplasm of the cells (Figure 2a and b). For comparison, in the absence of thiophenol, the fluorescence increases more modestly by around 3.5-fold, indicating that thiophenol is beneficial, although not absolutely required, for the induction of fluorescence. This is consistent with our model experiments in cell extract (see Figure 1).

Additional cell-staining experiments with the red fluorescent membrane carbocyanine dye $DiIC_{18}$(5) illustrate that the fluorescence only develops in the interior of the cell (Figure 2c–f). In control experiments, we verified that no fluorescence evolves in the absence of ruthenium complex **1** or the absence of the caged fluorophore **4** (data not shown). Together, these experiments demonstrate that ruthenium complex **1** is capable of passing through the cell membrane and inducing the cleavage of allylcarbamates within living cells. Notably, cytotoxicity experiments with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method verify that treatment with the organoruthenium compound **1**, thiophenol, and the caged fluorophore **4** does not influence the viability of the cells (see the Supporting Information).

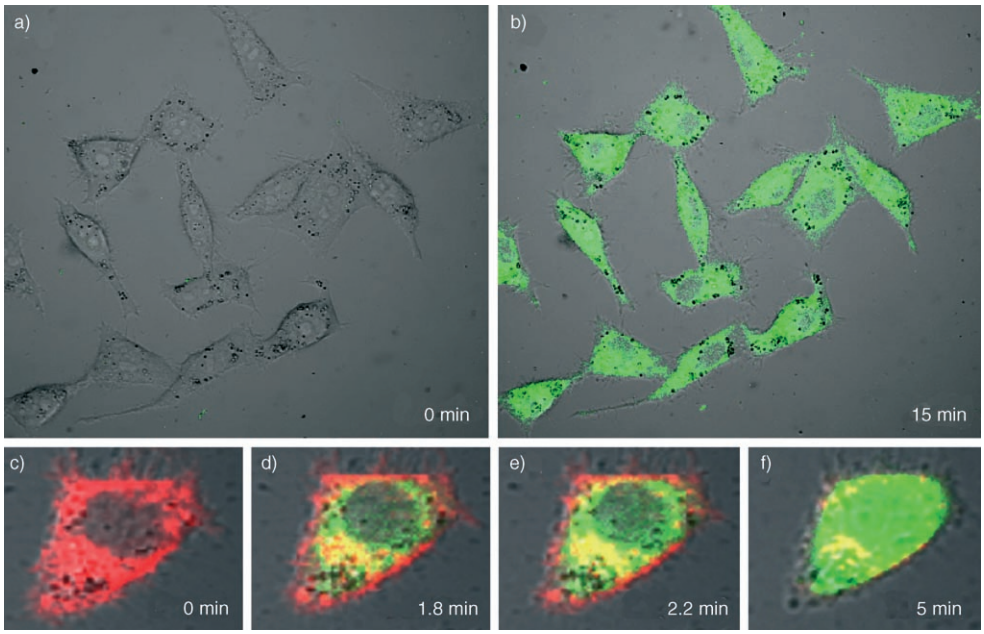

Figure 2. Fluorescence microscopy imaging of ruthenium-induced uncaging of allylcarbamate-protected rhodamine 110 (**4**) inside HeLa cells. Shown are superimposed phase-contrast and fluorescence images. Images a) and b): Cells were preincubated with **4** (100 μM) for 30 min, washed with PBS buffer solution, and then treated with [Cp*Ru(cod)Cl] (20 μM) and thiophenol (500 μM). The shown images are a) right after this addition and b) after 15 min. Images c)–f): Cells were preincubated with **4** (100 μM) for 30 min and at the same time with the membrane carbocyanine dye $DiIC_{18}(5)$ (Molecular Probes). After washing with PBS buffer solution, cells were treated with [Cp*Ru(cod)Cl] (40 μM) and thiophenol (100 μM). The shown images are c) right after this addition and d)–f) after the indicated times.

In conclusion, [Cp*Ru(cod)Cl] has been shown to be capable of inducing the uncaging of amines from their respective allylcarbamates under physiological conditions such as in living mammalian cells. The described reaction is an encouraging starting point towards the goal of designing catalytic organometallics as tools in chemical biology.

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