EurJOC
European Journal of Organic Chemistry

A Journal of
ChemPubSoc Europe

Accepted Article

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Authors: Sergej Hermann, Daniel Sack, and Hans-Achim Wagenknecht

To be cited as: *Eur. J. Org. Chem.* 10.1002/ejoc.201800319

Link to VoR: http://dx.doi.org/10.1002/ejoc.201800319

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COMMUNICATION

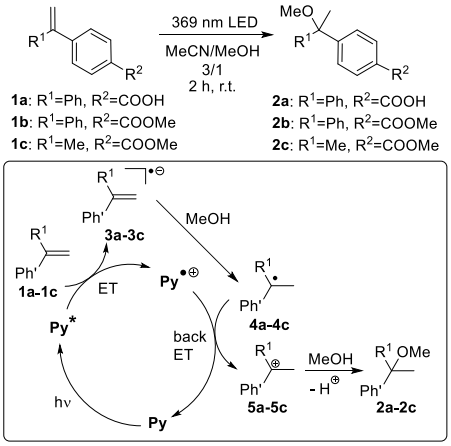
Proline-rich short peptides with photocatalytic activity for the nucleophilic addition of methanol to phenylethylenes

Sergej Hermann,[a] Daniel Sack,[a] and Hans-Achim Wagenknecht*[a]

Abstract: Short proline-rich peptides were synthesized and modified with 1-(N,N-dimethylamino)pyrene by copper(I)-catalyzed cycloaddition. They perform photoredox catalysis of the nucleophilic addition of methanol to 1,1-diphenylethylene derivatives into products with Markovnikov orientation. The common additive triethylamine is avoided because forward and backward electron transfer is controlled by substrate binding. A free carboxylic function in the substrate allows more precise substrate binding and defines the electron transfer path better than the unspecific exciplex formation with the substrate bearing a carboxylic ester. A proline-type turn is an advantage for photoredox catalysis, but a proline-induced helix is not required. This is the first successful example for introducing secondarily structured peptides to photoredox catalysis.

Over the last ten years, peptides were developed as efficient catalysts for a variety of important classes of organic reactions, including acylations, epoxidations and C-C bond formation, in particular aldol-like reactions. Especially the pioneering work by Miller *et al.*[1] and Wennemers *et al.*[2] evidenced the huge potential of small peptides as organocatalysts. On the one hand they pointed out that short peptides consisting of less than 10 amino acids show astonishing catalytic activities, excellent enantioselectivities and broad substrate scopes despite their lower structural complexity compared to larger proteins or real enzymes. On the other hand, short peptides are synthetically well accessible, thus can be simply varied by their structure, and allow chemical transformations in a variety of both organic and aqueous solvents. However, short peptides with photoredox catalytic activity are rarely found in literature. Early examples are peptides as models for DNA photolyase that cleave thymidine-thymidine dimers by photoinduced electron transfer processes.[3] Recently, we published the synthesis of first peptide-based photoredox catalysts for the nucleophilic addition of methanol to styrene derivatives.[4]

Visible light provided by sunlight is an unlimited and thereby "green" natural energy source. In the laboratory, LEDs are cheap and energy-saving light sources for reproducible photocatalysis. However, visible light does not provide enough energy to perform important transformations in organic chemistry regarding carbon bond energies. For instance, blue light (λ=440 nm) has an energy of 270 kJ/mol that is not sufficient to cleave a typical C-C bond of 350 kJ/mol. Photoredox catalysis was developed as an important method to overcome this principal problem.[5-14] It applies photoinduced electron transfer processes instead of sensitization in order to generate reactive radicals and radical ions. After chemical transformation of those reactive intermediates to the desired products, back electron transfer closes the photoredox catalytic cycle. Transition metal complexes, mainly $[Ru(bpy)_3]Cl_2$, are broadly applied photoredox catalysts.[11] Most recently, organic dyes, in particular eosin Y,[12] 9-mesityl-10-methyl-acridiniumperchlorat[13] and rhodamine 6G[14] are used to enhance the sustainability and to broaden the substrate and reaction scopes. We used 1-(*N*,*N*-dimethylamino)pyrene and 1,7-dicyano-perylene-3,4:9,10-tetracarboxylic acid bisimide as photoredox catalysts for the nucleophilic addition of alcohols to 1,1-diphenylethylene (**1**) and other styrene derivatives to products with Markovnikov-[15] and anti-Markovnikov orientation,[16] respectively. These photocatalytic reactions, however, required triethylamine and thiophenol, respectively, as additives to enhance the photocatalytic efficiency by shuttling forward and backward electron and proton transfers. Herein, we report short proline-rich peptides modified with 1-(*N*,*N*-dimethylamino)pyrene (**Py**) that not only allow to avoid triethylamine during photoredox catalysis, but additionally give a closer look on their photocatalytic activity by means of nucleophilic additions of MeOH to 1,1-diphenylethylene derivatives **1a**-**1c** into products **2a**-**2c** with Markovnikov orientation (Scheme 1). Especially the influence of the secondary structure induced by proline residues on the photocatalytic activity of the peptides was investigated.



Scheme 1. Photocatalytic Markovnikov-type nucleophilic addition of MeOH to substrates **1a**-**1c** and proposed photocatalytic mechanism for the conversion to products **2a**-**2c** using **Py**-containing peptides as photoredox catalysts.

By excitation at approximately 400 nm, a singlet energy of E_{00}= 3.1 V adds to the oxidation potential of 1-(*N*,*N*-

[a] Dipl.-Chem. Sergej Hermann, Prof. Hans-Achim Wagenknecht
Institute of Organic Chemistry
Karlsruhe Institute of Technology (KIT)
Fritz-Haber-Weg 6, 76131 Karlsruhe, Germany
E-mail: Wagenknecht@kit.edu

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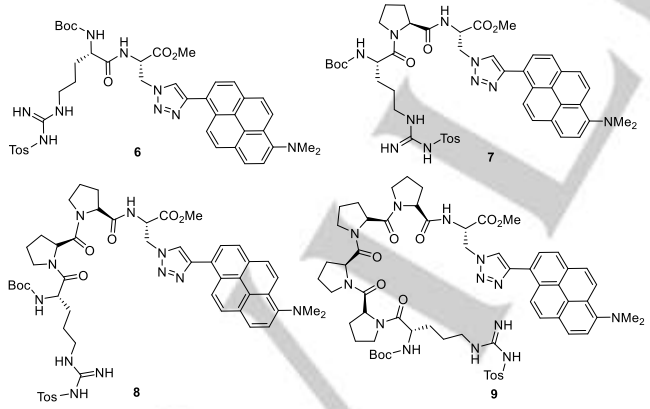
dimethylamino)pyrene (**Py**) E_{ox}=0.91 V yielding a excited state potential of approximately E^*_{ox}=-2.2 V (vs. NHE). This is sufficiently high to photoreduce threefold alkylated olefins and styrenes to their radical anions **3a**-**3c**.[13] These radical anions undergo rapid protonation and the neutral radicals **4a**-**4c** get reoxidized to the corresponding cations **5a**-**5c** that add MeOH as nucleophile into the products **2a**-**2c** with Markovnikov orientation. The chromophore **Py** was attached using the copper(I)-catalyzed cycloaddition to β-azido-L-alanine as anchor point at the C-terminus of the photocatalytic peptides. The synthesis of the ethynyl-substituted chromophore and the subsequent cycloaddition to the Boc-protected **Py**-modified L-alanine (**Py**Ala) followed our recently published procedure.[4] Using the Boc-strategy in solution, the following peptides **6**-**9** with the general sequence Boc-Arg(Tos)-$(Pro)_n$-**Py**Ala-OMe and n = 0, 1, 2 and 4 were prepared (Scheme 2). L-prolines (Pro) were chosen as intervening amino acids in order to vary the distance between proposed substrate binding site (Arg) and the photocatalytic unit (**Py**Ala) and to generate a proline-based helix as secondary structure in the catalysts.[17,18] The tosyl and Boc groups at the N-terminal arginines and the methyl esters at the C-terminal **Py**Ala residues remained on the peptides to improve their solubility in MeCN during photoredox catalysis. All four synthesized peptides **6**-**9** were purified by flash column chromatography and fully characterized by NMR spectroscopy and HR mass spectrometry (see Supporting Information). Compared to the isolated chromophore **Py** with an absorption maximum at 356 nm, all four peptides and the triazolyl pyrene amino acid building block show a pyrene absorption that is bathochromically shifted to approximately 378 nm (see Supporting Information). Accordingly, the triazole linkage to the peptides provide shifts the excitation to longer wavelengths, which is an advantage since not only LEDs at 369 nm but also LEDs at 385 nm can be used for irradiation (see Table 1 footnote).



Scheme 2. Photocatalytic peptides **6**-**9** with the **Py** chromophore as side chain in an Ala derivative. The schematic drawings show only the chemical constitution and configuration of the peptides and should not imply a precise conformation.

The photoredox catalytic experiments were performed by irradiation using a 369 nm LED under strict exclusion of oxygen. We showed that the peptides **6** and **7** are able to efficiently control the forward and backward electron transfer between the substrate **1a** and the chromophore moiety **Py** that Et_3N as additive and electron shuttle is no longer required. This works also in substochiometric amounts of the peptides (25 mol%).[4] We assume that the Arg side chain provides the substrate binding site for the carboxylic function of substrate **1a**-**2c** by hydrogen bonding and/or electrostatic interactions. We examined the photoredox reaction of substrate **1a** with MeOH in the presence of peptides **6**-**9** but with even lower catalyst loadings of 5 mol% and 2.5 mol% (Table 1). For the experiments with 5 mol% photocatalyst, the yield of product **2a** increases from 19% with peptide **6** to 100% with peptide **7** and decreases again down to 30% with peptide **8** and 44% with peptide **9**. The corresponding irradiations with 2.5 mol% photocatalysts show a similar trend but on an overall lower yield level and with less pronounced differences between the proline-bearing peptides.

Table 1. Yields of the photoredox catalytic transformation of substrate **1a**-**1c** to products **2a**-**2c**, respectively, in the presence of 2.5 and 5.0 mol% peptides **6**-**9**: 10 mM substrate in MeCN (1.5 mL) and MeOH (0.5 mL), 25 °C, λ_{exc}=369 nm (LED), 12 h. The yields were determined by GC-MS and NMR spectroscopy.

Peptide	[mol%]	**1a**	**1b**	**1c**
Boc-Arg(Tos)-**Py**Ala-OMe (**6**)	5[b]	19	92	5
	2.5	8	63	n.d.
Boc-Arg(Tos)-Pro-**Py**Ala-OMe (**7**)	5	100	100	44
	2.5	37	71[a]	n.d.
Boc-Arg(Tos)-Pro_2-**Py**Ala-OMe (**8**)	5	30	97	16
	2.5	38	83	n.d.
Boc-Arg(Tos)-Pro_4-**Py**Ala-OMe (**9**)	5	44	100	<5
	2.5	38	83	n.d.

[a] λ_{exc}=385 nm: 80%. [b] Control reaction without irradiation did not yield conversion and product.

In order to gain more insights, the fluorescence quenching of the peptides **6**-**9** by the substrate **1a** was examined by Stern-Volmer plots (see Supporting Information).[19] The Stern-Volmer constants of the peptides **6**-**9** are K_{SV}=0.18-0.19 mM^{-1}, slightly lower than that with **Py** alone (K_{SV}=0.20 mM^{-1}), but clearly evidencing photoinduced electron transfer from the **Py** moiety to the substrate **1a** as the key step of the photoredox catalytic cycle. However, only peptide **7** gives product **2a** in quantitative yield. If substrate binding to the Arg moiety is assumed, the length of the electron transfer path increases from peptide **6** to **9** by each additional Pro residue (from none in **6** to 4 Pro in **9**). Based on the principle that the electron transfer rate shows an exponential dependence on the distance the photoredox catalytic activity differs in the peptides.[20] However, the shortest distance between pyrene and Arg in peptide **6** is obviously not the best with respect to the photoredox catalytic activity. It can be assumed that both forward and backward electron transfer occurs on such a fast timescale in this smallest peptide that the slower chemical

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transformation from radical anion **3a** to cation **5a** and finally to product **2a** works inefficiently. The photoredox catalysis works ~~more~~ efficiently with the larger distance in peptide **7** and gives quantitative yield of product **2a**. The longer distances over two and four Pro residues in peptides **8** and **9**, respectively, are again less productive, however, the yield increases from 30% with peptide **8** to 44% with peptide **9.** These observable differences can be assigned to the Pro-induced secondary structure that yield an advantageous orientation between the substrate and the chromophore in particular in peptide **7**.[21,22] In fact, circular dichroism spectra (Figure 1) reveals a proline-type II helix in peptide **9**. This type of helix typically consists of 3 residues per helix turn. That means that both the Arg side chain as proposed binding site and the chromophore **Py** in peptide **9** may be located on the same side of the helix and the distance between them is smaller compared to peptide **8** that has a slightly different Pro-incuded secondary structure according to its circular dichroism. In conclusion it became clear that one Pro is an obvious advantage for photoredox catalysis, but longer peptides with more prolines are not better.[17]

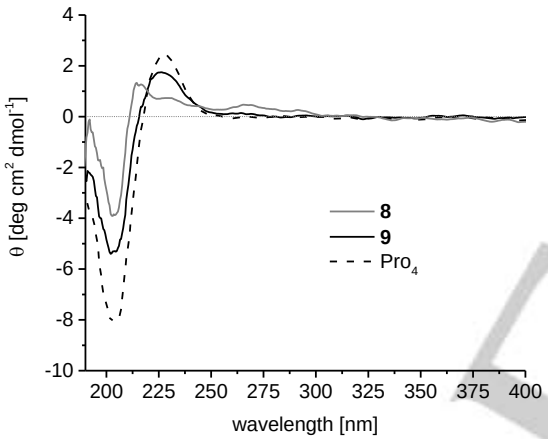

Figure 1. Circular dichroism of **8** (100 μM) and **9** (50 μM) in comparison to Boc-Pro_4-OMe (100 μM) as reference peptide in MeCN at 25 °C.

To elucidate the importance of specific substrate binding via the Arg side chain, photocatalytic irradiations with the methyl ester substrates **1b** and **1c** in the presence of peptides **6**-**9** were performed. The photoredox catalytic results with substrate **1c** are similar to those with substrate **1a**, but show lower yields, including the best conversion in the presence of peptide **7** (5 mol%) with a yield of 44% for product **2c**. Interestingly, all four peptides show quantitative or nearly quantitative conversion to product **1b** if 5 mol% were used in the photoredox catalytic experiments. Only in the presence of peptide **6** a slightly smaller yield of 92% was obtained. Clearly, there is no significant distance dependence of the electron transfer as for substrate **1a** indicating that the electron transfer path is different and shorter for this substrate **1b**. The corresponding Stern-Volmer plots (Figure 2), representatively done for substrates **1a** and **1b**, show only partial fluorescence quenching of the **Py** moiety in peptide **7** by substrate **1b** but an additional emission side band that occurs hypsochromically shifted at higher concentrations. This fluorescence results from excitation of a ground state complex between the electron-poor substrate **1b** and the electron-rich chromophore **Py** that provides not only the preferred binding mode for this substrate but also promotes the photoredox catalytic transformation. It must be noted here, that an exciplex can also be found with substrate **1a** but only to a very small extent and generally supports an electron transfer as initial step of this photoredox catalysis. Inoue *et al.* described similar exciplexes between 1,1-diphenyl-1-alkenes and naphthalene esters.[23] The exciplex formation, in particular between substrate **1b** and the **Py** chromophore in peptides **6**-**9,** competes with specific substrate binding at the Arg side chain and explains why in contrast to substrate **1a**, the peptide structure seems to play only a minor role for photoredox catalysis and the conversion of substrate **1b**. In order to support this, the photoredox catalytic addition to **1b** was performed with MeOH, EtOH, *i*PrOH and *t*BuOH as a row of nucleophiles with increasing steric hindrance. In this row, 100%, 100%, 68% and 12% yields of the corresponding products were obtained after irradiation in the presence of 25 mol% 1-(*N*,*N*-dimethylamino)pyrene as photoredox catalyst. In comparison, 100%, 68%, 10% and 0% yields of the corresponding products were detected the presence of 25 mol% of peptide **7**. These values show that the exciplex formation as preferred binding of substrate **1a** to peptide **7** is influenced by the steric hindrance of the peptide side chains.

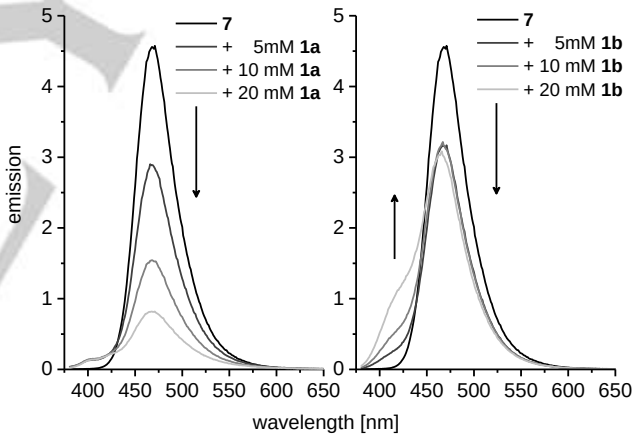

Figure 2. Fluorescence quenching of **7** (2 μM) by substrates **1a** (left) and **1b** (right) in MeCN. Excitation λ_{exc}=365 nm.

In conclusion, short proline-rich peptides modified with 1-(*N*,*N*-dimethylamino)pyrene allow to avoid triethylamine during photoredox catalysis because they control the forward and backward electron transfer by substrate binding. Thereby, closer insights into the mechanism of photoredox catalysis, in particular the nucleophilic addition of methanol to 1,1-diphenylethylene derivatives **1a**-**1c** into the products **2a**-**2b** with Markovnikov orientation were gained. The free carboxylic function of substrate **1a** allows more precise substrate binding. Thereby the electron transfer path is clearly defined and can be controlled by the distance and by the secondary structure of the photoredox catalytic peptide. A proline-type turn is an advantage for photoredox catalysis, but a proline-induced helix is not required.

The ester function of substrate **1b** promotes an unspecific binding by explic formation with the chromophore that significantly limits the control of photoredox catalysis by the peptides. These results provide an important basis for the better understanding of function and mode of action of binding sites in photoredox catalysis. They show the high potential of short peptides and encourage the development of peptides for other reactions to improve the efficiency and selectivity. In particular, short proline peptides provide the possibility to introduce enantioselectivity to this type of photoredox catalysis.

Experimental Section

All experimental details are described in the Supporting Information.

Acknowledgements

We gratefully acknowledge financial support by the Deutsche Forschungsgemeinschaft (grant Wa 1386/16-1 and GRK 1626 „Chemical Photocatalysis“) and the Karlsruhe Institute of Technology (KIT).

Keywords: pyrene • chromophore • photochemistry • addition • nucleophile

[1] a) S. J. Miller, *Acc. Chem. Res.* **2004**, *37*, 601-610; b) E. A. C. Davie, S. M. Mennen, Y. Xu, S. J. Miller, *Chem. Rev.* **2007**, *107*, 5759.

[2] a) J. D. Revell, H. Wennemers, *Curr. Opin. Chem. Biol.* **2007**, *11*, 269-278; b) H. Wennemers, *Chem. Commun.* **2011**, *47*, 12036-21041; c) B. Lewandowski and H. Wennemers, *Curr. Opin. Chem. Biol.* **2014**, *22*, 40-46.

[3] T. Carell, L. T. Burgdorf, L. M. Kundu, M. Cichon, *Curr. Opin. Chem. Biol.* **2001**, *5*, 491-498.

[4] S. Hermann, H.-A. Wagenknecht, *J. Pept. Sci.* **2017**, *23*, 563-566.

[5] I. Ghosh, L. Marzo, A. Das, R. Shaikh, B. König, *Acc. Chem. Res.* **2016**, *49*, 1566-1577.

[6] T. P. Yoon, *Acc. Chem. Res.* **2016**, *49*, 2307-2315.

[7] E. Meggers, *Chem. Commun.* **2015**, *51*, 3290-3301.

[8] S. Staveness, I. Bosque, C. R. Stephenson, *Acc. Chem. Res.* **2016**, *49*, 2295-2306.

[9] D. Ravelli, M. Fagnoni, A. Albini, *Chem. Soc. Rev.* **2013**, *42*, 97-113.

[10] S. Fukuzumi, K. Ohkubo, *Org. Biomol. Chem.* **2014**, *12*, 6059-6071.

[11] M. H. Shaw, J. Twilton, D. W. C. MacMillan, *J. Org. Chem.* **2016**, *81*, 6898-6926.

[12] D. P. Hari, B. König, *Chem. Commun.* **2014**, *50*, 6688-6699.

[13] K. A. Margrey, D. A. Nicewicz, *Acc. Chem. Res.* **2016**, *49*, 1997-2006.

[14] I. Ghosh, B. König, *Angew. Chem. Int. Ed.* **2016**, *55*, 7676-7679.

[15] A. Penner, E. Bätzner, H.-A. Wagenknecht, *Synlett* **2012**, *23*, 2803-2807.

[16] M. Weiser, S. Hermann, H.-A. Wagenknecht, *Beilstein J. Org. Chem.* **2015**, *11*, 568-575.

[17] T. Schnitzer, M. Wiesner, P. Krattiger, J. D. Revell, H. Wennemers, *Org. Biomol. Chem.* **2017**, *15*, 5877-5881.

[18] S. Dobitz, M. R. Aronoff, H. Wennemers, *Acc. Chem. Res.* **2017**, *50*, 2420-2428.

[19] O. Stern, M. Volmer, *Physikal. Z.* **1919**, *20*, 183-188.

[20] R. A. Marcus, N. Sutin, *Biochim. Biophys. Acta* **1985**, *811*, 265-322.

[21] A. L. Rucker, C. T. Pager, M. N. Campbell, J. E. Qualls, T. P. Creamer, *Proteins: Struct., Funct., Bioinf.* **2003**, *53*, 68-75.

[22] R. Schweitzer-Stenner, F. Eker, A. Perez, K. Griebenow, X. Cao, L. A. Nafie, *Pept. Sci.* **2003**, *71*, 558-568.

[23] S. Asaoka, T. Wada, Y. Inoue, *J. Am. Chem. Soc.* **2003**, *125*, 3008-3027.

10.1002/ejoc.201800319

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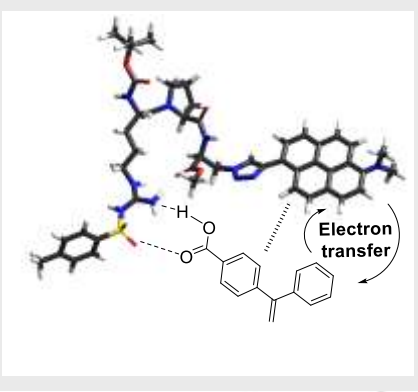
Entry for the Table of Contents

Layout 1: **Topic: Photoredox catalysis**

COMMUNICATION

One proline does the turn: The influence of the secondary structure of pyrene-modified short peptides on photoredox catalysis was studied. One proline is sufficient to yield efficient photoredox catalytic activity.



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Proline-rich short peptides with photocatalytic activity for the nucleophilic addition of methanol to 1,1-diphenylethylenes