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Synthesis of *N,N*-dimethylaminopyrene-modified short peptides for chemical photocatalysis

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The synthesis of peptide-based photocatalysts that use 1-*N,N*-dimethylaminopyrene as chromophore and their application in photocatalysis is reported. The copper(I)-catalyzed alkyne-azide cycloaddition was applied as key step to prepare the peptide–pyrene conjugates in quantitative yields for different short peptide sequences. The photocatalysts were evaluated for the nucleophilic addition of methanol to 1,1-diphenylethylenes to products with Markovnikov-type orientation The short peptides contain arginine as substrate binding site during photocatalysis, and thus, the reaction was performed without the additive triethylamine that was previously applied as electron shuttle. Full conversion of the substrate and good yields for the addition product were achieved. Copyright © 2017 European Peptide Society and John Wiley & Sons, Ltd.

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Chemical photocatalysts are organic dyes or transition metal complexes that efficiently couple the physical process of light absorption to a chemical reaction by means of time, space, and energetics, especially by photoinduced transfer of energy or electrons [1–8]. The principal problem for this type of photochemistry was the use of visible light, provided by sunlight as an essentially unlimited and thereby 'green' natural light source or LEDs as cheap and energy-saving artificial light sources. This problem has been solved by photoinduced electron transfer instead of energy transfer processes, commonly named as photoredox catalysis, a research field that has been established over the past decade [1]. The 'working horse' for photoredox catalysis is mainly $[Ru(bpy)_3]^{2+}$ [9,10], but nowadays, also organic compounds like eosin Y [11] and 9-mesityl-10-methyl-acridiniumperchlorat [8,12] are applied to strengthen the sustainability by avoiding transition metal complexes. We recently published the photocatalysis of the nucleophilic addition of methanol and other alcohols to 1,1-diphenylethylene (**1**) and other styrene derivatives to products with Markovnikov-orientation and anti-Markovnikov-orientation by 1-(*N,N*-dimethylamino)pyrene (**Py**) and 1,7-dicyano-perylene-3,4:9,10-tetracarboxylic acid bisimide, respectively [13,14]. Especially with this perylene bisimide derivative as photocatalyst, the yields of nucleophilic methanol addition to styrene derivatives were higher than those obtained with 9-mesityl-10-methyl-acridiniumperchlorat as photocatalyst [12]. One of the major drawbacks of these photocatalytic conversions was the use of additives, especially triethylamine, as electron shuttle to promote the efficiency of forward and backward electron transfers. In order to avoid these additives, it looked reasonable to design short peptides with binding sites that fix the substrates for the electron transfer process during photocatalysis. Short peptides have been successfully applied for enantioselective catalysis, mainly by Miller *et al.* [15] and by Wennemers *et al.* [16] but not yet for chemical photocatalysis. Moreover, short peptides have the advantage that they are soluble both in polar organic solvents and in aqueous solutions. Herein, we report the synthesis of short peptides modified with **Py** and the first elucidation of their photocatalytic activity with respect to nucleophilic additions to styrenes in the Markovnikov orientation.

Py as photocatalyst has an oxidation potential of E = 0.91 V (vs. NHE), together with the singlet state energy of E_{00} = 3.1 V; the excited state has a potential of approximately E* = −2.2 V, which is sufficiently high to reduce styrene derivatives [17]. Moreover, **Py** has the highest extinction in the range around 350–370 nm and hence allows to apply 369 nm LEDs as cheap and reliable light source. In order to attach this pyrene chromophore to short peptides, it looked reasonable to apply the copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) because this gives an easy and flexible access to a variety of peptide conjugates. A commonly applied amino acid for this type of bioconjugation is β-azido-L-alanine (**1**, in the Boc-protected form) that can be synthesized from L-serine in two steps according to literature [18]. The chromophore building block **2** for the CuAAC was designed as the pyrene derivative with *N,N*-dimethylamino and ethynyl substituents in the 1 and 6 positions, respectively. The synthesis of this building block **2** (Scheme 1) started with methylation of 1-aminopyrene (**3**) by methyl iodide in 98% yield. Treatment with *N*-bromosuccinimide gave bromination of **4** at the 6-position in 96% yield. Pyrene **5** was coupled to trimethylsilyl-acetylene by a Pd-catalyzed, Sonogashira-type reaction. After immediate cleavage of the trimethylsilyl group by tetrabutylammonium fluoride, the building block **2** was achieved in 96% yield over two steps.

Initially, we synthesized the Boc-Arg(Tos)-Arg(Tos)-N_3Ala-OMe and Boc-Arg(Tos)-Phe-N_3Ala-OMe as precursors for the potential photocatalytic peptides using the β-azido-L-alanine building block **2** (='N_3Ala') and the Boc strategy for solution peptide synthesis. It was tried to subsequently functionalize these peptides by the pyrene building block **2** in the presence of Cu(I). We found MS evidence for Boc-Arg(Tos)-Arg(Tos)-**Py**Ala-OMe (**8**) and Boc-Arg(Tos)-Phe-**Py**Ala-OMe (**9**) (Supporting Information); however, the

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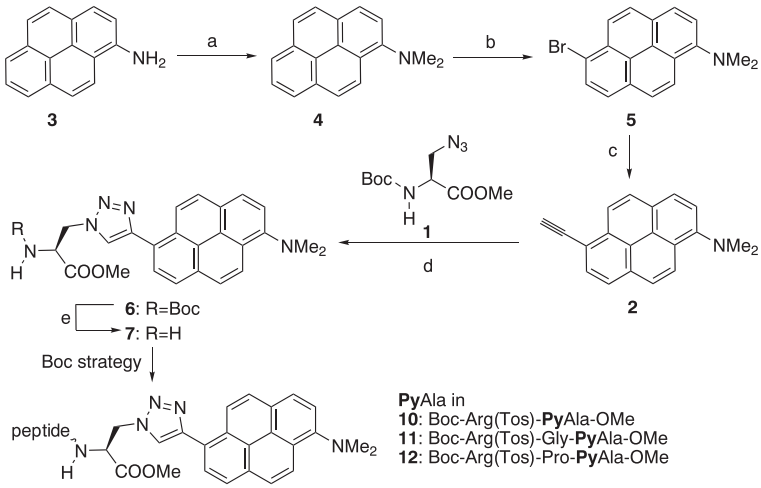
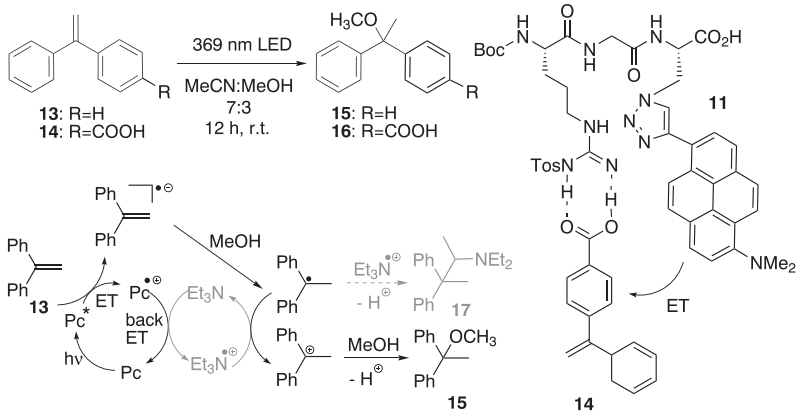


Scheme 1. Synthesis of pyrene building block **2** and CuAAC with Boc-protected β-azido-L-alanine (**1**) to the Boc-protected β-pyrenyl-L-alanine building block **6** for synthesis of the functionalized peptides **10**–**12**: (A) MeI, K_2CO_3, DMF, 2 h 120 °C; 98%; (B) NBS, $CHCl_3$, 16 h, r.t.; 96%; (C) trimethylsilyl-acetylene, Pd $(PPh_3)_2Cl_2$, Pd(dppf)Cl_2, CuI, NEt_3, tetrahydrofuran, 16 h, 80 °C; then, tetrabutylammonium fluoride (1 M in tetrahydrofuran), CH_2Cl_2, 1 h, r.t.; 96%; (D) sodium ascorbate, Cu$(MeCN)_4PF_6$, TBTA, CH_2Cl_2:DMF:MeOH = 1:1:3, 1 h, r.t.; quant.; (E) HCl (4 M in dioxane), 1 h, r.t.; quant.

yields were very low and the purity not satisfying. Hence, the synthetic strategy was changed, and the CuAAC was performed on the level of the Boc-protected peptide building block **1**. Accordingly, the reaction of **1** with **2** in CH_2Cl_2/dimethylformamide/MeOH solvent mixture in the presence Cu$(MeCN)_4PF_6$ and sodium ascorbate for 1 h at room temperature gave the corresponding pyrene-modified alanine building block **6**. The yield of the β-pyrenyl-L-alanine (**7**, gives later '**Py**Ala' in peptides) after removal of the t-butyloxycarbonyl (BOC) group by treatment with 4 M HCl in dioxane was quantitative. This building block was successfully applied to synthesize the dipeptides Boc-Arg(Tos)-**Py**Ala-OMe (**10**), Boc-Arg(Tos)-Gly-**Py**Ala-OMe (**11**), and Boc-Arg(Tos)-Pro-**Py**Ala-OMe (**12**) in 65–70% yield. The tosyl protecting group was left on the peptides to enhance their solubility in organic solvents during photocatalysis. All peptides were purified by column chromatography and fully characterized by nuclear magnetic resonance spectroscopy and high-resolution mass spectrometry.

The photocatalytic activity of the three peptides **10**–**12** in comparison with **Py** was determined using a high-power LED for irradiation at 369 nm, a Peltier temperature control element and a stirrer. In accordance with our previous publications, 1,1-diphenylethylene (**13**) and 4-(phenyl-1-vinyl)-benzoic acid (**14**) were applied as substrates for photocatalytic and nucleophilic addition of MeOH (Scheme 2 and Table 1). The conversion of **13** and formation of **15** could be identified and quantified by gas chromatography mass spectrometry, the conversion of **14** and formation of **16** by nuclear magnetic resonance spectroscopy. As previously reported for substrate **13** [13], the photocatalytic conversion of both **13** and similarly of **14** can only be completed with 39% yield of product **15** and 23% of product **16**, respectively, if Et_3N is added to the solution. Side products are the adduct of Et_3N (**17**) and **13**, which we both identified recently [14]. This is the starting point for photocatalytic improvement.

A more detailed look on the mechanism (Scheme 2) and the problem of inefficient back electron transfer indicates that loss of polar attraction after rapid protonation of the substrate radical anion may lead to diffusion and separation of the photocatalyst from the intermediate product-forming radical cation. As it can be assumed that back electron transfer is a strongly distance dependant process, the photocatalyst may not be regenerated and removed from the catalytic cycle. This scenario could potentially be improved if a substrate binding site is available on the photocatalyst that keeps the substrate in the vicinity of the pyrene chromophore as long as it is required for forward and back



Scheme 2. Photocatalytic Markovnikov-type nucleophilic addition of MeOH to substrates **13** and **14**, proposed photocatalytic mechanism for the conversion of **13** using Et_3N as electron shuttle (Pc = photocatalyst **Py**, or peptides **10**, **11,** or **12**) and proposed binding of substrate **14** to peptide **11**.

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Table 1. Photocatalytic nucleophilic addition of MeOH to **13** and **14** by photocatalysts (Pc) **Py**, or peptides **10**–**12**[a]

Line	Substrate	Pc (mol%)	Time (h)	Additive	Conversion %	Yield %
1	**13**	**Py** (100)	3	—	17[b]	**15**: 17[b]
2	**13**	**Py** (100)	3	Et_3N[c]	100[b]	**15**: 39[b]
3	**14**	**Py** (100)	3	—	9[d]	**16**: 9[d]
4	**14**	**Py** (100)	3	Et_3N[c]	79[d]	**16**: 23[d]
5	**13**	**10**, **11,** or **12** (100)[e]	12	—	—[b]	**15**: —[b,g]
6	**13**	**10**, **11,** or **12** (100)	12	Et_3N[c]	58–100[b]	**15**: —[b,g,h]
7	**14**	**10**, **11,** or **12** (100)	12	—	100[f]	**16**: 100[f]
8	**14**	**10**, **11,** or **12** (100)[e]	12	Et_3N[c]	—[f]	**16**: —[e,h]
9	**14**	**10**, **11,** or **12** (25)	12	—	99–100[f]	**16:** 99–100[f]

[a]Reaction conditions: **13** or **14** (2 mM) in MeCN:MeOH = 7:3 (4 ml), argon atmosphere, 12 h, 25 °C, 369-nm high-power LED.
[b]**13** and **15** identified and quantified by gas chromatography mass spectrometry.
[c]5 vol%.
[d]**14** and **16** identified by high-performance liquid chromatography mass spectrometry and quantified by high-performance liquid chromatography ultraviolet.
[e]Destruction of Pc.
[f]**14** and **16** identified and quantified by nuclear magnetic resonance spectroscopy.
[g]Only traces of product **15**.
[h]**17** was obtained as product.

electron transfer process in the time course of the complete photocatalytic cycle. Accordingly, the peptides **10**–**12** as photocatalyst bear an arginine side chain that served as binding site especially for the substrate **14** carrying the complementary carboxylic acid, presumably by hydrogen bonding. The photocatalytic experiments (Table 1) revealed that especially the MeOH addition to substrate **14** photocatalyzed by all three peptides **10**–**12** run with quantitative conversions and also quantitative yields of **16** (after 12 h irradiation). The comparable irradiations with **14** in the presence of Et_3N as additive lead to photodestruction of the photocatalysts **10**–**12** and thus only little conversions. The question if the binding of the carboxylic acid of substrate **14** to the peptidic photocatalysts **10**–**12** was required can be answered by the corresponding irradiations with substrate **13**. Without Et_3N, only traces of **15** were detected, whereas in the presence of the Et_3N additive, the Et_3N-adduct **17** was observed as the main but undesired product. That showed clearly that the binding site interactions between the peptidic photocatalysts and the substrate **14** played a central role for successful photocatalysis and that the short peptides **10**–**12** represent significantly improved photocatalyst for this type of reaction as they allow to avoid Et_3N as additive. Concerning the stoichiometry, we reported that **Py** can be applied in catalytic amounts (10 mol%) for this type of nucleophilic addition to styrene derivatives [14]. We performed similar experiments with 25 mol% of the peptides **10**, **11,** and **12**; the conversions and yields were similarly good as with stochiometric amounts.

In conclusion, we showed that the electron-rich **Py** chromophore could be covalently conjugated to short peptides using the CuAAC. However, this copper(I)-catalyzed 'click' functionalization had to be performed on the level of the amino acid building block, in our case using β-azido-L-alanine (**1**) because this gave an easy and flexible access to a variety of peptide conjugates. Corresponding conjugation attempts with short peptides bearing the β-azido group as side chain failed presumably because of the instability of the azido functionality under the acidic conditions of Boc deprotection. The photocatalytic activities of the three peptides **10**–**12** were determined in comparison with **Py** for the nucleophilic addition of MeOH to the diphenylethylene substrates (**13** and **14**) yielding the corresponding products in Markovnikov orientation (**15** and **16**). The peptides **10**–**12** bear an *N*-terminal arginine side chain that served as potential binding site by hydrogen bonding especially for the substrate **14** carrying the complementary carboxylic acid. As a result, the functionalized and synthesized peptides represent significantly improved photocatalysts in comparison with **Py** for this type of reaction as they allow avoiding Et_3N as additive. These results underscore the significant potentials of short peptides for chemical photocatalysis.

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