ChemComm

COMMUNICATION

View Article Online
View Journal

Cite this: DOI: 10.1039/d5cc03361f

Received 13th June 2025,
Accepted 3rd July 2025

DOI: 10.1039/d5cc03361f

rsc.li/chemcomm

Lighting up peptides: direct dehydroalanine formation from diselenides *via* Rose Bengal photocatalysis†

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A visible-light-driven method for the site-selective conversion of diselenide-containing peptides to dehydroalanine (Dha) is presented. Using Rose Bengal as a photosensitizer in aqueous conditions, this photocatalytic process proceeds *via* oxidative cleavage of the Se–Se bond followed by β-elimination to afford Dha with high yield and chemoselectivity. This mild and bioorthogonal strategy enables downstream peptide functionalization and offers a practical route for post-synthetic peptide editing.

Dehydroalanine (Dha) is a naturally occurring, non-proteinogenic amino acid found in numerous peptides, particularly those exhibiting antibiotic activity, such as lantibiotics.[1,2] Peptides containing Dha are of significant interest due to the unique chemical properties imparted by its α,β-unsaturated carbonyl structure. This functional motif enables selective chemical modifications of peptides under mild reaction conditions.[3,4]

Methods reported to date for the generation of dehydroalanine (Dha) from natural amino acids typically require prior activation of amino acid side chains—for example, activation of the hydroxyl group of serine or modification of the thiol group of cysteine.[5–10] An alternative approach involves oxidative elimination of phenylselenocysteine using sodium periodate ($NaIO_4$), although this transformation generally requires extended reaction times of up to 12 hours.[11,12] More recently, Shirakawa *et al.*[13] described a direct conversion of cysteine to Dha in peptides using cesium carbonate, achieving moderate yields. However, this method lacks chemoselectivity, as serine residues are also susceptible to the same transformation. Given the high frequency of serine in peptide sequences, this lack of specificity can present significant challenges when applying the reaction to longer or more complex peptides.

In recent years, the development of light-driven chemical transformations has seen rapid progress, particularly those utilizing visible light.[14–19] Such photochemical reactions often employ photosensitizers, including Rose Bengal (RB) and eosin Y. Notably, a recent study by Waliczek *et al.*[20] reported a visible-light-induced transformation of peptides bearing cysteine residues into alanine, mediated by a phosphine reagent, RB, and LED-based visible light irradiation. There is a growing interest in the development of chemoselective reactions that proceed with high efficiency, utilize environmentally benign solvents—especially water—and require only catalytic or sub-stoichiometric amounts of reagents.[21,22] Such transformations align with the principles of green chemistry and offer significant advantages in terms of sustainability and process safety. Herein, we report visible-light-induced dehydroalanine formation from selenocystine-containing peptides mediated by Rose Bengal.

We began by synthesising model peptide **1** (see Fig. S4–S6, ESI†), which was obtained as a diselenide homodimer. The peptide was assembled manually on a solid support according to the commonly used Fmoc strategy (details in ESI†). We additionally applied sonication during coupling steps and piperidine-based Fmoc deprotection, which significantly reduced reaction times.[23] As previously reported, we developed a highly efficient method for the desulfurization of peptides to alanine using the photosensitizer Rose Bengal (RB). In the course of applying this methodology to selenocystine-containing peptides, we observed that, in the absence of the phosphine reductant TCEP, the oxidized selenocystine residue was converted to dehydroalanine. The reaction was performed in aqueous buffer at pH 6, under open-air conditions, for 3 hours, using a sub-stoichiometric amount of RB. Illumination was provided by an office lamp equipped with an LED bulb. Although the desired transformation occurred, the reaction proceeded with only moderate efficiency.

LC-MS analysis of the reaction mixture confirmed the formation of the target peptide, but also revealed a major by-product corresponding to an oxidized form of selenium. The observed exact mass and characteristic isotopic distribution were consistent with the presence of a seleninic acid moiety, indicating partial overoxidation of the selenocysteine residue

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† Electronic supplementary information (ESI) available. See DOI: https://doi.org/10.1039/d5cc03361f

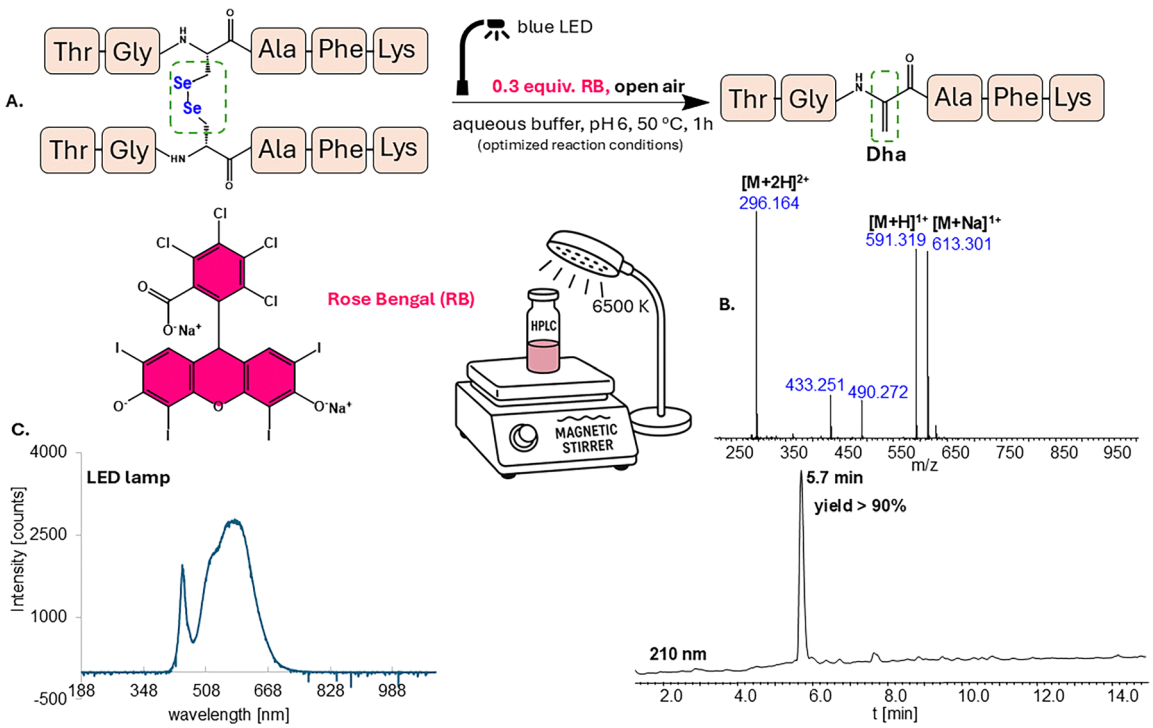
under the applied conditions. In subsequent experiments, we systematically optimized the reaction conditions. Reaction mixtures were analyzed by RP-HPLC-ESI-MS. Chromatographic analysis of irradiated samples across the pH range 5–8 (see Fig. S22–S28, ESI†) indicated that pH 6 gave the highest yield of the desired peptide, approximately 60%. Noteworthy, irradiating a sample previously deoxygenated with nitrogen and closed for the reaction led to a significant decrease in reaction efficiency (see Fig. S29, ESI†). This experiment clearly demonstrates the necessary contribution of oxygen to the reaction conditions. We also evaluated the effect of the amount of RB used (0.1–0.5 equiv.). While 0.1 equiv. RB proved insufficient, no significant improvement in yield was observed above 0.2 equiv. (see Fig. S30–S34, ESI†). Based on these trials, 0.3 equiv. RB was identified as the optimal loading, notably remaining sub-stoichiometric relative to the peptide substrate.

Further experimental studies revealed that heating the sample after irradiation for 1 hour at approximately 50 °C led to a shift in the LC-MS chromatogram signal ratio in favor of the desired product. This observation suggested that the compound initially presumed to be a by-product (seleninic acid moiety) is, in fact, an intermediate in the pathway of obtaining dehydroalanine. Based on these findings, a photochemical reaction was performed at pH 6 using 0.3 equivalents of RB, with simultaneous stirring and heating at 50 °C on a magnetic stirrer (Scheme 1). The reaction progress was monitored by LC-MS (see Fig. S35–S39, ESI†). Analysis of the resulting data indicated that under these conditions, the target product was obtained after 1 hour with a yield exceeding 90%. We also

Table 1 Optimization of photochemical conversion

	RB (eq.)	pH	Solvent	Time (h)	Temp.	Yield
1	0.3	5	H_2O	3	Ambient	19%
2	0.3	6	H_2O	3	Ambient	67%
3	0.3	7	H_2O	3	Ambient	55%
4	0.3	8	H_2O	3	Ambient	35%
5	0.1	6	H_2O	3	Ambient	62%
6	0.2	6	H_2O	3	Ambient	80%
7	0.3	6	H_2O	3	Ambient	85%
8	0.4	6	H_2O	3	Ambient	86%
9	0.5	6	H_2O	3	Ambient	88%
10	0.5	6	DMF	3	Ambient	Trace
11	0.3	6	H_2O	1	50 °C	> 90%
12	0.3	6	25% ACN	1	50 °C	> 90%

evaluated the reaction under aqueous–organic conditions. The results indicate that a moderate proportion (20–30% v/v) of organic co-solvents such as acetonitrile is compatible with the reaction conditions (see Fig. S40, ESI†). In contrast, solvents such as DMF or NMP lead to decomposition of the peptide substrate, resulting in complex mixtures of poorly defined products (Table 1). To demonstrate the feasibility and chemoselectivity of the developed method, additional peptide **2–6** models (Scheme 2) were synthesized on solid support (see Fig. S7–S21, ESI†). The resulting peptides, isolated as diselenide homodimers, were purified by RP-HPLC and characterized by HR-MS. These compounds were then subjected to



Scheme 1 (A) Schematic representation of RB-mediated conversion of peptide **1** to Dha-containing analog. (B) MS spectrum and HPLC chromatogram obtained for desired product. (C) Spectral characteristic of LED lamp.

Peptide **1** (H–TGUAFK–NH_2)$_2$

Peptide **2** (H–SIGUGFKA–NH_2)$_2$

Peptide **3** (Ac–UFAAK–NH_2)$_2$

Peptide **4** (H–GDFQAPGIGU–NH_2)$_2$

Peptide **5** (Ac–KUAAAUQFKA– NH_2)$_2$

Peptide **6** (H–USGFKGC–NH_2)$_2$

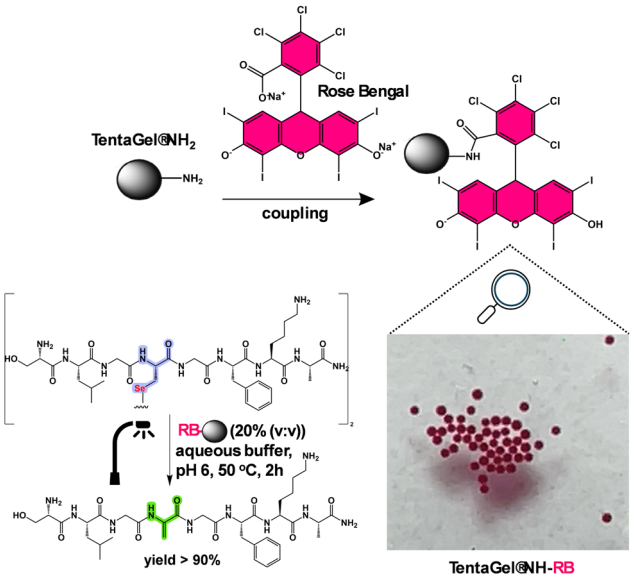
Scheme 2 Sequences of model peptides **1–6**.

photochemical transformation under previously optimized reaction conditions. As anticipated, peptides **2–5** underwent efficient photoconversion, yielding the corresponding dehydroalanine derivatives in 90–95% yield after one hour of irradiation at elevated temperature (see Fig. S41–S55, ESI†). Notably, no significant by-products were detected.

Of particular interest is the peptide **5**, which contains two selenocysteine residues within its sequence. When exposed to the same photochemical conditions, peptide **5** was converted into a product containing two Dha moieties. Based on the obtained results and available literature, we propose that this method represents the most efficient direct approach for the synthesis of Dha to date, with additional advantages in terms of chemoselectivity and reaction kinetics.

Peptide **6**, containing both an oxidized selenocysteine (Sec) and a cysteine (Cys) residue within its sequence, requires separate discussion. In this case, the outcome of the irradiation was somewhat unexpected (see Fig. S56 and S57, ESI†). The preliminary experiments in our research group demonstrated that irradiation of peptides containing a cysteine residue in the presence of RB leads exclusively to its oxidation, resulting in the formation of disulfide bonds. However, studies on peptide **6** revealed that, although the desired product was formed in low yield, the major component of the post-reaction mixture was a cyclic compound featuring a mixed Se–S bridge. This product was resistant to further transformation and did not convert to Dha. These findings indicate that, although the reaction described in this manuscript shows high selectivity toward Sec residues, the presence of an additional Cys residue in the sequence can interfere with the desired transformation. Therefore, Cys may need to be selectively protected—*e.g.*, using an Acm group—to avoid side reactions. Notably, cysteine is a relatively low-abundance amino acid in proteins (1–2%), unlike serine, which is significantly more common (6–8%). Importantly, the recently developed Cs_2CO_3-mediated method is not selective for serine and leads to its conversion to Dha.[13]

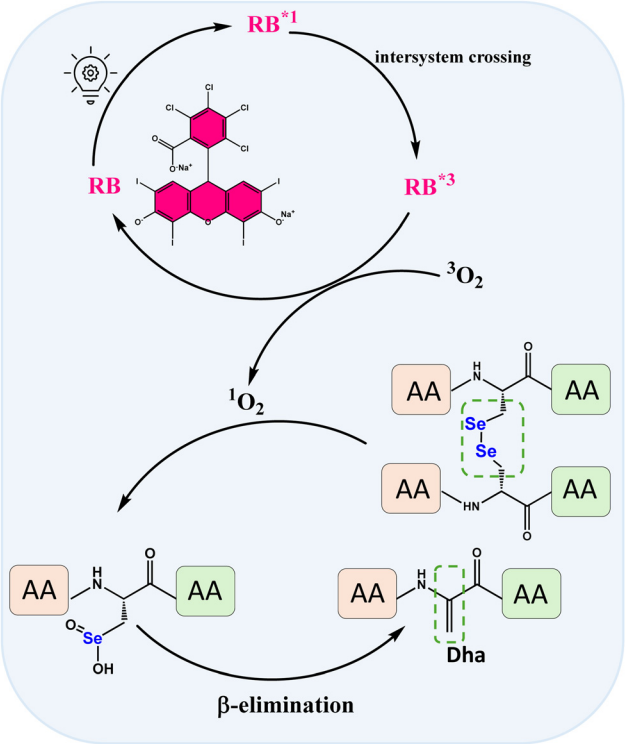
As part of our study, we investigated the use of a commonly employed iridium-based photocatalyst for the photochemical transformation of peptide diselenides.[23] To this end, peptide **2** was subjected to irradiation under various conditions: in pH 6 buffer, in water, in water–acetonitrile mixtures (50 : 50 and 90 : 10, v/v), and under deoxygenated conditions. In all cases, only trace amounts of the desired product were detected (see Fig. S57–S59, ESI†). These results indicate that, under the tested conditions, the iridium photocatalyst is not effective for promoting this transformation, in contrast to the presented RB-based protocol.



Scheme 3 Rose Bengal immobilization on TentaGel NH_2 resin. Schematic representation for the conversion of peptide **2** using immobilized RB.

To render the photochemical transformation method more practical and elegant, Rose Bengal was immobilized on a solid support.[24] Exploiting the carboxylic acid functionality of RB, we anchored the dye to an amine-terminated TentaGel resin *via* amide bond formation. For this purpose, a PEG-linked TentaGel NH_2 resin was reacted with RB in the presence of diisopropylcarbodiimide as the coupling agent in DMF for 3 h. Successful immobilization was readily confirmed by the characteristic intense pink color of the resin. Representative enlarged resin particles are shown in Scheme 3. The photochemical transformation was then evaluated using peptide **2** under the previously optimized conditions. The RB-functionalized resin was added to the reaction mixture, which was irradiated with an LED lamp while stirring magnetically. The target product was again obtained in high yield; however, the reaction time required extension to at least 2 h (see Fig. S60 and S61, ESI†). This slower kinetics is consistent with the heterogeneous nature of the system, as the immobilized RB is not fully exposed to the aqueous phase. Nevertheless, the overall conversion remained efficient, and the photocatalyst could be conveniently removed by simple filtration after completion of the reaction.

Based on our experimental observations—specifically, the requirement for molecular oxygen and the detection of selenic acid as an intermediate in the early stages of the reaction—along with literature data on the photochemical behavior of Rose Bengal, we propose the following reaction mechanism (Scheme 4). Upon visible light irradiation, Rose Bengal is excited to its singlet state, which rapidly undergoes intersystem crossing to the triplet state. Triplet RB then reacts with molecular oxygen to generate singlet oxygen ($^1O_2$), establishing oxidative conditions in the reaction medium. Under these



Scheme 4 Proposed mechanism for RB-mediated Dha formation in peptides containing an oxidized selenocysteine residue.

conditions, diselenide bond within peptide is oxidized and can undergo cleavage of the Se–Se bond, leading to the formation of seleninic acid species (see Scheme 4). In the final step, the selenium – containing group undergoes oxidative β-elimination (promoted by increased temperature), resulting in the formation of a Dha residue *via* C–Se bond cleavage and the formation of a double bond at the β-position.[25]

In conclusion, we have developed an efficient method for the photochemical conversion of selenocystine to dehydroalanine in peptides using Rose Bengal as a photosensitizer in aqueous conditions. The protocol affords yields exceeding 90%, is time-efficient (1 hour), and requires only a sub-stoichiometric amount of RB in combination with a custom-built blue LED light source. Compared to the existing methods, this approach offers significant advantages in terms of simplicity, efficiency and chemoselectivity. Selenocysteine serves as a valuable tool for protein and peptide editing, as it can be incorporated into proteins through genetic engineering techniques and into peptides using a commercially available Fmoc-protected amino acid derivative.

M. W and P. S conceptualized the project. M. W and M. S. conducted the experiments. All authors contributed to the experimental design and data analysis. M. W. drafted the initial manuscript, and all authors discussed and commented on the manuscript.

Conflicts of interest

There are no conflicts to declare.

Data availability

The data supporting this article have been included as part of the ESI.†

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