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Published in:
A C S Combinatorial Science

Link to article, DOI:
10.1021/acscombsci.8b00014

Publication date:
2018

Document Version
Peer reviewed version

Link back to DTU Orbit

Citation (APA):
Oxidative Modification of Tryptophan-Containing Peptides

Jonas Petersen, † Katrine E. Christensen, † Mathias T. Nielsen, ‡ Kim T. Mortensen, † Vitaly V. Komnatnyy, † Thomas E. Nielsen, †‡§ and Katrine Qvortrup †*†

1 Department of Chemistry, Technical University of Denmark, DK-2800 Kongens Lyngby, Denmark
2 Department of Immunology and Microbiology, University of Copenhagen, DK-2200 Copenhagen, Denmark
3 Singapore Centre for Environmental Life Sciences Engineering, Nanyang Technological University, Singapore 637551

ABSTRACT: We herein present a broadly useful method for the chemoselective modification of a wide range of tryptophan-containing peptides. Exposing a tryptophan-containing peptide to 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) resulted in a selective cyclodehydration between the peptide backbone and the indole side chain of tryptophan to form a fully conjugated indolyl-oxazole moiety. The modified peptides show a characteristic and significant emission maximum at 425 nm, thus making the method a useful strategy for fluorescence labeling.

KEYWORDS: solid-phase peptide synthesis, fluorescent labeling, tryptophan, site-selective protein modification

INTRODUCTION

Fluorescence labeling of proteins and peptides is fundamental for the study of biological systems, as it can provide detailed visualization of complex cellular processes.1 The visualization of biological processes has been crucial for our understanding of molecular dynamics and the development of new potent drugs. Nowadays, the most common approach to fluorescence labeling of proteins comprises the introduction of fluorescent small molecules to the nucleophilic side chain of lysine, serine, threonine, or cysteine residues in a peptide or protein of interest.2 However, such strategies often suffer from poor site selectivity, where multiple residues are modified. Though less established, chemoselective functionalization of other residues, such as methionine,3 glucose,4 arginine,5 N-terminal serine/threonine,6 tyrosine,7 and tryptophan,8 has been described. Among these residues, tryptophan is particularly interesting because of its scarce abundance in proteins. With a natural abundance of only 1.09%,9 many proteins of interest will contain only a single or few tryptophan residues accessible for functionalization, thus enabling high control of the position for modification. Furthermore, the relative large size of organic dyes, including undesired physiochemical properties may give rise to several challenges, that compromise the biologically active activity of the labeled target. Therefore, labeling strategies that introduce minimal structural perturbation to the peptide of interest is of high importance. Herein, we describe our efforts toward the oxidative modification of small peptides containing tryptophan. The conjugated nature of the generated indolyl-oxazole moiety emits blue-fluorescence,10 which may advantageously be utilized for spectroscopic studies of biological systems. For instance, the indolyl-oxazole moiety of diazonamide A derivatives has been utilized as intrinsic fluorophores for in vitro cellular uptake studies.11 In addition, the indolyl-oxazole scaffold is present in a variety of naturally occurring biologically active compounds including those shown in Figure 1,12−15 as well as cyclic derivatives such as the diazonamides.11

RESULTS AND DISCUSSION

Using standard reagents for solid-phase peptide synthesis, the 55 HMBA linker was easily immobilized and synthetically elaborated on an amino-functionalized ChemMatrix resin (Table 1). Initially, a range of conditions for the oxidative cyclodehydration of model compound 6 (Scheme 1) was examined.16 Oxidation of the α-carbon of indoles has been performed with the dehydrogenating agent DDQ to form the 61 keto-indole derivative.16 Therefore, it was expected that DDQ,62 could be a suitable reagent for the oxidative cyclodehydration of 63 tryptophan. Furthermore, cyclodehydration of keto-indoles has been carried out with a mixture of triphenylphosphine, metallic iodine, and triethylamine in CH₂Cl₂ to form a conjugated indolyl-oxazole moiety.17 Various solvents were screened (entries A−G), and it was disclosed that exposure of a 68 tryptophan-containing oligopeptide to DDQ (4 equiv) in 69 MeCN led to near-quantitative conversion into the desired 70 indolyl-oxazole product 9 (Scheme 1). Interestingly, the 71 peptide was fully converted to the desired indolyl-oxazole 72 derivative with only two equivalents of DDQ (entry J). The 73 reaction most likely occurs via the αβ-unsaturated imine 74 (Scheme 1). However, in reactions where partial formation of the ketone product 8 was initially observed, it was noted that prolonged reaction times resulted in full conversion to the desired cyclo-dehydrated product 9.

Received: January 30, 2018
Revised: April 13, 2018
Published: May 2, 2018
The sequence tolerance of the site-selective tryptophan oxidation protocol was investigated through the synthesis of a combinatorial library of natural amino acids and common protective groups (entry L-AO). Gratifyingly, the developed protocol produced a diverse set of indolyl-oxazole compounds. The synthesized compounds were purified by preparative HPLC before yield determination and NMR analysis.

**Figure 1.** Biologically active indolyl-oxazole natural products.

**Table 1. Chemical Data for the Indolyl-oxazoles 10A–AQ**

<table>
<thead>
<tr>
<th>Entry</th>
<th>AA</th>
<th>Conditions</th>
<th>Solvent</th>
<th>Purity (^a) (%)</th>
<th>Yield (^b) (%)</th>
<th>Entry</th>
<th>AA</th>
<th>Conditions</th>
<th>Solvent</th>
<th>Purity (^a) (%)</th>
<th>Yield (^b) (%)</th>
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<td>A</td>
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<td>CH(_3CN)</td>
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<td>30</td>
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<td>THF</td>
<td>40</td>
<td>64</td>
<td>Y</td>
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<td>DMF</td>
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<td>AA</td>
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<td>86</td>
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<td>AH</td>
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<td>AK</td>
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<td>CH(_3CN)</td>
<td>77</td>
<td>31</td>
<td>AL</td>
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<td>CH(_3CN)</td>
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<td>13</td>
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<td>AM</td>
<td>Asn</td>
<td>DDQ (2 equiv)</td>
<td>CH(_3CN)</td>
<td>&gt;95</td>
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<td>40</td>
<td>AN</td>
<td>Asn(Trt)</td>
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<td>25</td>
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<td>CH(_3CN)</td>
<td>81</td>
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<td>18</td>
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<td>16</td>
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<td>18</td>
<td>AQ</td>
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<td>T</td>
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<td>15</td>
<td></td>
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</table>

\(^a\)Crude purities. \(^b\)All compounds were purified by preparative HPLC before yield determination and NMR analysis.
The protocol showed compatibility with a wide range of peptides and generally only the desired product was observed by UP-LCMS (see Supporting Information). Unfortunately, non-protected lysine residues were not tolerated (entry AG). Here a range of byproducts was observed by UPLC, including a nucleophilic addition of the lysine side-chain amino group to the conjugated imine (7) as well as a Michael reaction between the amino group and DDQ.

The isolated yields of indolyl oxazole peptides are in the range typically observed for solid-phase synthesis followed by preparative HPLC purification. From our results, we cannot identify a correlation between purity and isolated yields neither is there apparent structure-yield correlation.

Furthermore, the methodology was investigated for peptides containing more than one tryptophan residue (entry AP−AQ). Using two and three equivalents of DDQ, respectively, peptides containing two or three indolyl-oxazole moieties were obtained (see Supporting Information).

In order to investigate the potential of the technique for fluorescent labeling, the fluorescence properties of the indolyl-oxazole containing peptide 10(JL-AO) was measured and compared to the emission spectrum of the corresponding nonoxidized peptides 11(JL-AO), Scheme 2.

As shown in Figure 2, the indolyl-oxazole containing peptides show a remarkably change in fluorescence with a distinct band appearing at 425 nm. Importantly, this absorption is not affected by the presence of aromatic side chain functionalities in naturally occurring amino acids.

Having identified conditions allowing for oxidative cyclo-dehydration of tryptophan in various peptides, we sought to demonstrate the use of this methodology in the labeling of biological relevant peptides. GLP-1 is a 30 amino acid-containing peptide hormone that possesses several pharmacological properties, making it a subject of intensive investigation. Gratifyingly, when exposing GLP-1(12), to the DDQ conditions the desired fluorescence labeled indolyl-oxazole analog 13 was formed (Figure 2) with a satisfactory conversion of 85%, as confirmed by HPLC.

Currently, the methodology has only been demonstrated for immobilized peptides that tolerate acetonitrile. It would be desirable to adapt this chemistry to aqueous conditions, thereby allowing indolyl-oxazole formation in proteins.
require the development of a more stable dehydrogenation reagent, which resists hydrolysis in aqueous solutions.

### CONCLUSIONS

In summary, we report a method that allows for the chemoselective labeling of tryptophan-containing peptide residues. DDQ-mediated oxidative cyclization leads to the installation of an indolyl-oxazole moiety with unique fluorescence properties. We further demonstrate that the indolyl-oxazole moiety selectively may be installed in a pharmaceutically relevant peptide, thereby emphasizing the important potential of the methodology to illuminate biological mechanism of relevance to drug discovery.

### EXPERIMENTAL SECTION

**General Methods.** All reagents and materials used were purchased from ordinary chemical suppliers and were used without purification. The solvents used were of standard HPLC grade. Solid-phase synthesis was carried out using plastic-syringe techniques. Flat-bottomed PE-syringes were fitted with PTFE filters and situated in Teflon valves equipped with Teflon tubing allowing for a moderate vacuum to be applied to the syringes.

Yields of solid-phase synthesis protocols are corrected for salt contents and given as percentage of product mass recovery to the theoretical product loading mass, calculated from the resin loading (4 mmol/g) as specified by the supplier.

Products were analyzed on a Waters Alliance reverse-phase HPLC system consisting of a Waters 2695 Separations Module equipped with a Symmetry C18 column (3.5 μm, 4.6 × 75 mm, column temp 25 °C, flow rate 1 mL/min) and a Waters Photodiode Array Detector (detecting at 215 nm). Preparative RP-HPLC was carried out on a Waters Alliance reverse-phase HPLC system consisting of a Waters 2545 Binary Gradient Module equipped with an xBridge Prep BEH130 C18 column OBD (5 μm, 19 × 100 mm, column temp 25 °C, flow rate 20 mL/min), a Waters Photodiode Array Detector (detecting at 210–600 nm), a Waters UV Fraction Manager and a Waters 2767 Sample Manager. Elution was carried out in a linear reversed phase gradient combining water and acetonitrile (buffered with 0.1% (v/v) TFA).

1D and 2D NMR spectra were recorded using a Varian Unity Inova-500 MHz, a Varian Mercury-300 MHz instrument, a Bruker Ascend-400 MHz instrument equipped with a 5 mm Prodigy cryoprobe or a Bruker Avance-800 MHz instrument, equipped with a 5 mm cryoprobe TCI, in DMSO-d$_6$ or CDCl$_3$ using the residual DMSO or CHCl$_3$ solvent peaks, respectively, as the internal standard. All 13C NMR spectra were proton decoupled. DQF-COSY, HSQC, HMBC, and 2D NOESY spectra were acquired using standard pulse sequences.

LC-DAD-HRMS was performed on an Agilent 1100 LC system equipped with a Agilent Technologies Diode Array Detector and a Luna C18 column (3 μm, 50 mm × 2 mm, column temp 40 °C, flow rate 400 μL/min). Separation was achieved using a linear reversed phase gradient (20% to 100% organic in 8 min, hold for 2 min, 100% to 20% organic in 1 min, hold for 4 min) again combining water and acetonitrile (buffered with 20 mM HCO$_2$H). The LC was coupled to a 189 Micromass LCT orthogonal time-of-flight mass spectrometer,

![Figure 3. Fluorescent labeling of GLP-1.](image-url)
The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

We thank the Carlsberg Foundation, and the Technical University of Denmark for financial support.

**REFERENCES**


**AUTHOR INFORMATION**

**Corresponding Author**

*K-E-mail: kaqvo@kemi.dtu.dk.*

**ORCID**

Katrine Qvortrup: 0000-0003-3828-2069

**Notes**

The authors declare no competing financial interest.