Visible-Light-Mediated Selective Arylation of Cysteine in Batch and Flow

Cecilia Bottecchia, Maarten Rubens, Smita B. Gunnoo, Volker Hessel, Annemieke Madder, and Timothy Noël*

Abstract: A mild visible-light-mediated strategy for cysteine arylation is presented. The method relies on the use of eosin Y as a metal-free photocatalyst and aryl diazonium salts as arylation agents. The reaction can be significantly accelerated in a microflow reactor, whilst allowing the in situ formation of the required diazonium salts. The batch and flow protocol described herein can be applied to obtain a broad series of arylated cysteine derivatives and arylated cysteine-containing dipeptides. Moreover, the method was applied to the chemoselective arylation of a model peptide in biocompatible reaction conditions (room temperature, phosphate-buffered saline (PBS) buffer) within a short reaction time.

The formation of C–S bonds is of high interest in the fields of organic synthesis and drug discovery.[1] However, due to the undesired coordination between metal catalysts and sulfur atoms, traditional cross-coupling methods are often inadequate strategies for C–S bond formation.[2] Despite the undesired coordination, some transition-metal-catalyzed cross-coupling methods for C–S bond formation have been reported.[3] However, these methods often rely on high reaction temperatures and/or require stoichiometric amounts of a strong base. A well-known strategy largely applied in industry for C–S bond formation is the so-called Stadler–Ziegler reaction, in which a diazonium salt reacts with an aryl thiolate to afford the desired thioether derivative.[4] Starting from the original conditions reported by Stadler and Ziegler, a plethora of methodologies have emerged, allowing milder reaction conditions.[5] Among them, our group reported a mild one-pot procedure for the synthesis of arylsulfides facilitated by photoredox catalysis.[6]

In the interest of developing mild methodologies for chemical biology purposes,[7] we envisaged modifying our procedure to achieve a visible-light-induced protocol for cysteine arylation. Specifically, we directed our attention towards the development of a biocompatible metal-free strategy involving inexpensive organic dyes as photoredox catalysts. In addition, due to the incompatibility of UV light to peptides and proteins, we reasoned that visible light photoredox catalysis would be perfectly suited to chemical biology applications owing to the milder reaction conditions (e.g. room temperature and visible light).

Novel selective chemical modifications of peptides and proteins are of pivotal importance for the study of protein–protein interactions and for the development of novel bioconjugates and drug candidates.[8] Compared to other amino acids commonly targeted for post-translational modifications, cysteine exhibits low natural abundance and a relatively high nucleophilicity.[9] Together, these characteristics account for the generally higher selectivity and the broad reactivity profile typical for post-translational chemical modifications involving cysteine residues. Some of the most widespread strategies for cysteine bioconjugation include disulfide formation,[10] thiol–maleimide reactions,[11] and alkylation with haloalkyl reagents.[12] Other strategies use cysteine as precursor for the formation of dehydroalanine[13] (Dha), or as a handle for nucleophilic aromatic substitution allowing access to perfluorinated staples in peptides and proteins.[14] Moreover, several methodologies relying on thiol–ene[15] or thiol–yne[16] reactions have been reported, often requiring UV irradiation to generate the thyl radical. Fewer records in the literature describe the use of transition metals for cysteine modification. Among them, recent developments illustrate methodologies for cysteine arylation[17] as well as viable protocols for cysteine arylation in proteins.[18] Inspired by these reports, we envisioned that photoredox catalysis could serve our purpose to obtain a mild and straightforward methodology for cysteine arylation. Moreover, we hypothesized that highly electrophilic benzenediazonium salts would be suitable as arylation agents, able to easily generate aryl radicals (E_{red} as high as 0.5 V vs. saturated calomel electrode, SCE) via a single electron transfer (SET) pathway.[19] The generated aryl radicals could then be trapped by the nucleophilic thiol moiety of cysteine.

Thus, we commenced our investigation with the arylation of N-Ac-L-cysteine-OMe 1a using 4-fluorobenzenediazonium tetrafluoroborate in acetonitrile (MeCN) under batch conditions. In the absence of light and photocatalyst, a modest 26% of the desired arylated product 3g was obtained within 2 hours reaction time (Table 1, entry 1). When exposed to
In order to circumvent these issues, we translated in situ formation, PTSA (59\%), 56\%). Moreover, a series of further increasing the amount of 3g (81\% flow) was obtained (Table 1, ortho to 2017). In general, we observed that electron-deficient counter-ion to afford shelf-stable 70% batch), 89\% flow and para and 2017. The Authors. Published by Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

The significant acceleration of reaction kinetics did not significantly increase aryl radical formation (Table 1, entry 3). In order to minimize the risks associated with the handling of potentially explosive diazo intermediates and desiring to simplify our protocol into a one-pot procedure, we investigated the in situ formation of the diazonium salt starting from readily available 4-fluoroaniline, tert-butyl nitrite (t-BuONO, 2.0 equiv) and catalytic amounts of tetrafluoroboric acid (HBF\(_4\), 1.5 mol %) as a benchmark photoredox catalyst, a higher yield of 40\% was obtained (Table 1, entry 3). In order to minimize the risks associated with the handling of potentially explosive diazo intermediates and desiring to simplify our protocol into a one-pot procedure, we investigated the in situ formation of the diazonium salt starting from readily available 4-fluoroaniline, tert-butyl nitrite (t-BuONO, 2.0 equiv) and catalytic amounts of tetrafluoroboric acid (HBF\(_4\), 1.5 mol %). Within 2 hours, product 3g could be isolated in an improved 56\% yield (Table 1, entry 4). Tetrafluoroborate benzenediazonium salts are easily isolated and exhibit higher stabilities as compared to diazonium salts bearing other counterions. However, by implementing the in situ formation of diazonium salts, the counterion choice appeared less restrictive (i.e. no need to use the BF\(_4\) counter-ion to afford shelf-stable diazonium salts). Instead, we chose to catalyze amounts of easy-to-handle para-toluenesulfonic acid (TsOH\(\cdot\)H\(_2\)O), which gave similar results (59\%, Table 1 entry 5). To develop a biocompatible strategy, we further tested the possibility to employ an organic dye, eosin Y, as photocatalyst for our transformation. Gratifyingly, in the presence of 1 mol % of eosin Y, the desired product was obtained in 59\% (Table 1, entry 6). This is in line with recent reports on the ability of eosin Y to be oxidatively quenched by diazonium salts, thus generating aryl radicals.[20] Further increasing the amount of t-BuONO to 3 equiv did not lead to any improvement in yield (Table 1, entry 7). Solvent screening revealed that the reaction afforded lower yields in DMSO (15\%, Table 1, entry 8) but proceeded well in PBS buffer (pH 8, 46\% Table 1, entry 9), a commonly used solvent for peptide and protein modifications.

One of the major limitations of photocatalytic reactions conducted in batch is the inefficient irradiation of the reaction mixture, often resulting in sub-optimal yields and difficulty of scale-up.[28] In order to circumvent these issues, we translated our arylation protocol into a micro-flow procedure. We developed a photomicroreactor assembly consisting of a 3D-printed holder equipped with 0.45 mL PFA micro-capillary tubing (500 \(\mu\)M ID) and 3.12 W white light-emitting diodes (LEDs; see the Supporting Information for micro-reactor details).[21] Remarkably, within only a 30 second residence time, 79\% of compound 3g was obtained (Table 1, entry 10). Due to the evolution of nitrogen gas (consistent with the reduction of diazonium salts), the formation of a slug flow was observed, which ensured optimal mixing efficiency.[22] The significant acceleration of reaction kinetics and increase in product yields can be attributed to the optimal irradiation of the reaction mixture.[20a]

With optimized conditions in hand, we evaluated the scope of our protocol both in batch and in continuous flow (Scheme 1). The arylation reaction tolerated a wide variety of substituents on the aniline coupling partner. Aniline molecules bearing alkyl substituents reacted in modest yields in batch to give the corresponding arylated cysteine derivatives (3a to 3e) and improved yields were obtained in flow for compounds 3a and 3h. Notably, compound 3d (49\% batch vs. 61\% flow) bearing an alkyne moiety could be of use for further functionalization of biomolecules through copper-catalyzed alkyn-azole cycloaddition methods (CuAAC).[23] Anilines bearing both ortho and para substitutents also reacted in modest to good yields to give the desired arylated derivatives 3e and 3f (28\% and 66\% batch vs. 73\% in flow for 3f). In general, we observed that electron-deficient anilines gave higher yields as compared to the electron-rich anilines. This can be attributed to the difference in reactivity of their corresponding diazonium salts. In fact, electron-deficient aryl diazonium salts are less stable and therefore more prone to reduction via SET.[18b] Moreover, a series of fluorinated derivatives was obtained in good to excellent yields (3g to 3m). Specifically, para- and ortho-fluoro (3g 59\% batch, 82\% flow, 3h 70\% batch), para- and meta-trifluoromethyl (3k 60\% batch, 89\% flow and 3l 81\% flow) and trifluoromethoxy (3m 62\% flow) arylated cysteine derivatives were all prepared in good yields. Additionally, perfluoroarylated derivatives 3i (flow 40\%) and 3j (batch 42\%, flow 45\%) were synthesized in satisfactory yields. Similar perfluoroarylated cysteine derivatives have been reported by Pentelute and co-workers as convenient intermediates for peptide stapling.[14a,3]

Next, we explored the potential of our methodology for Cl, Br and I-containing anilines, as all halogenated derivatives.

**Table 1:** Optimization of reaction conditions in batch for cysteine arylation.[a]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Light source</th>
<th>Catalyst</th>
<th>Changes from optimized conditions</th>
<th>Isolated yield [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>no light</td>
<td>none</td>
<td>pre-made diazonium</td>
<td>26</td>
</tr>
<tr>
<td>2</td>
<td>CFL</td>
<td>none</td>
<td>pre-made diazonium</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>CFL</td>
<td>Ru(bpy)_2Cl_2</td>
<td>pre-made diazonium</td>
<td>40</td>
</tr>
<tr>
<td>4</td>
<td>CFL</td>
<td>Ru(bpy)_2Cl_2</td>
<td>in situ formation, HBF_4</td>
<td>56</td>
</tr>
<tr>
<td>5</td>
<td>CFL</td>
<td>Ru(bpy)_2Cl_2</td>
<td>in situ formation, PTSA</td>
<td>59</td>
</tr>
<tr>
<td>6</td>
<td>CFL</td>
<td>eosin Y</td>
<td>none</td>
<td>59</td>
</tr>
<tr>
<td>7</td>
<td>CFL</td>
<td>eosin Y</td>
<td>in situ formation, 3 equiv. BuONO</td>
<td>72%</td>
</tr>
<tr>
<td>8</td>
<td>CFL</td>
<td>eosin Y</td>
<td>DMSO</td>
<td>15</td>
</tr>
<tr>
<td>9</td>
<td>CFL</td>
<td>eosin Y</td>
<td>PBS</td>
<td>46</td>
</tr>
<tr>
<td>10</td>
<td>LEDS</td>
<td>eosin Y</td>
<td>continuous flow</td>
<td>79 (92%)</td>
</tr>
</tbody>
</table>

[a] Standard reaction conditions: 0.5 mmol N-Ac-cysteine-OMe (1a), 4-fluoroaniline (1.3 equiv), t-BuONO (2.0 equiv), 1.5 mol % TsOH\(\cdot\)H\(_2\)O and 1 mol % eosin Y in 5 mL MeCN (0.1 M), white CFL 2 hours reaction time. For pre-made diazonium salts: 4-fluorobenzenediazonium tetrafluoroborate was used in absence of t-BuONO and TsOH\(\cdot\)H\(_2\)O.[b] Yield determined by GC-MS with n-decane as internal standard. [c] For detailed flow conditions, see Scheme 1 and the Supporting Information.
could represent useful synthetic handles for further peptide functionalization. Both ortho- and para-Cl derivatives were obtained in satisfactory yields ($3q$ 78%, $3p$ 62%) as well as the ortho-Br derivative $3n$ (79%). Moreover, para- and meta-I derivatives were synthesized ($3q$ 35%, $3r$ 41%) albeit in slightly diminished yields. The lower yields observed in the presence of an iodine atom could be explained by considering that iodoarene moieties are prone to iodine transfer to aryl radicals, thus affording 1,4-diiodobenzene, which we did observe as a significant side product in our reaction (detected in GC-MS).

Additionally, we explored the possibility of employing keto- and ester-containing anilines, thus obtaining para-methyl ketone and ortho-methoxy ester derivatives $3s$ (78%) and $3t$ (75%) in good yields. Finally, we probed the reactivity of the heterocycle 3-amino-5-Cl pyridine towards our transformation. Gratifyingly, the pyridine-containing cysteine derivative $3u$ was obtained in 69% yield in flow.

Owing to the ease of scalability, our flow protocol could be easily employed to obtain arylated cysteine derivatives on gram scales. Consequently, this notable feature allows one to prepare sufficient quantities for use in automated solid phase peptide synthesis (SPPS). As an example, we performed a continuous-flow scale-up experiment with N-Ac-l-cysteine-OMe $1a$ (5 mmol) and 3-trifluoromethylaniline. Within approximately two hours of operation time, 1.16 g (72%) of derivative $3l$ was obtained.

Encouraged by the results obtained for the arylation of N-Ac-l-Cys-OMe, we prepared a small array of cysteine-containing dipeptides to test the compatibility of our methodology with simple model peptides. Therefore, four dipeptides (4 N-Boc-l-Ala-l-Cys-OMe, 5 N-Boc-l-Leu-l-Cys-OMe, 6 N-Boc-l-Trp-l-Cys-OMe and 7 N-Boc-l-Phe-l-Cys-OMe) were prepared in solution via native chemical ligation, and were subjected to our arylation protocol (Scheme 2). Satisfyingly, N-Boc-l-Ala-l-Cys-OMe afforded the corresponding arylated dipeptides $8a$ (60%), $8b$ (56%) and $8c$ (42%) in good yields. Similarly, good yields were obtained with N-Boc-l-Leu-l-Cys-OMe for derivatives $9a$ to $9d$. A remarkable acceleration and increase in yield was observed when the arylation of N-Boc-l-Leu-l-Cys-OMe was conducted in flow. When attempting the arylation of N-Boc-l-Trp-l-Cys-OMe, we found the presence of indole to be...
incompatible with the in situ diazonium formation. However, when pre-formed diazonium salt was added to N-Boc-L-Trp-L-Cys-OMe, the corresponding arylated derivative 10a was obtained in 39% yield. Finally, N-Boc-L-Phe-L-Cys-OMe afforded the corresponding arylated derivative 11a in 55% yield.

In order to further demonstrate the utility of our methodology, we focused our attention on performing our arylation strategy on more complex peptide substrates. However, we anticipated that the in situ formation of diazonium salts might be incompatible with the delicate nature of peptides and proteins. Keen to adapt our protocol to biologically relevant reaction conditions, we tested the possibility of employing pre-made diazonium salts and aqueous phosphate buffer (pH 8) for our cysteine arylation. Thus, we applied our arylation protocol under these mild reaction conditions on peptide 12, which was used upon resin cleavage without further purification. In the presence of para-F benzenediazonium salt tetrafluoroborate or para-OCF₃ benzenediazonium tetrafluoroborate, full conversion to the desired products 13 and 14 was achieved within 30 minutes as detected by LC/MS (Scheme 3). Notably, no selectivity issues were observed in the presence of lysine and serine residues, and no organic solvent was required, thus demonstrating the excellent compatibility of our protocol with other common post translational modification methods involving these residues.

In conclusion, we reported a one-pot protocol for cysteine arylation via visible light photoredox generation of aryl radicals from their corresponding diazonium salts. In situ formation of diazonium salts starting from readily available anilines reduces the risks associated with the handling of potentially explosive intermediates. An array of arylated cysteine derivatives decorated with a broad range of substituents was obtained in moderate to good yields (17 examples, 28–79%). The implementation of a microflow reactor afforded faster reaction times and increased yields (30 to 150 seconds residence time, 11 examples, 45–89% yield). Moreover, a diverse set of cysteine containing dipeptides was

Scheme 2. Arylation of cysteine-containing dipeptides in batch and flow. a) Reaction conditions for dipeptide arylation in batch are the same as for the arylation of N-Ac-L-cysteine-OMe but on 0.25 mmol scale. b) Reaction conditions for dipeptide arylation in flow are the same as for the arylation of N-Ac-L-cysteine-OMe but on a 1 mmol scale. c) For Trp-Cys pre-made 4-tBu benzenediazonium tetrafluoroborate was used. d) 150 seconds residence time.

Scheme 3. Arylation of a cysteine-containing peptide. 1 equiv of crude peptide 12 (0.47 μmol), 10 equiv diazonium salt, 1 mol% eosin Y in 1 mL PBS buffer (pH 8), white CFL, 30 minutes reaction time.
arylated successfully in batch and in flow (12 examples, 32–86% yield). The reaction was easily scaled-up, affording more than one gram of the arylated cysteine derivative within 2 hours of total operation time. Finally, in biologically relevant conditions, a model peptide containing additional nucleophilic side chains was selectively converted to its Cys-arylated derivative within 30 minutes. Taking into account the simplicity of our reaction conditions (atmospheric conditions, visible light irradiation, short reaction time), we believe that our procedure will be appealing to chemical biologists for post translational chemical modification of cysteine.

Acknowledgements

The authors would like to thank Mark van den Bosch for the preparation of diazonium salts. C.B. and T.N. acknowledge the European Union for a Marie Curie ITN Grant (Photo4-Entrepreneurship for funding). Financial support from Flanders Innovation & Entrepreneurship for funding. F. van den Bossche thanks Flanders Innovation & Entrepreneurship for funding. Financial support from BOF-UGent and IOF-UGent is further gratefully acknowledged.

Conflict of interest

The authors declare no conflict of interest.

Keywords: cysteine arylation · diazonium salts · microreactors · photochemistry · photoredox catalysis

How to cite: Angew. Chem. Int. Ed. 2017, 56, 12702–12707

Angew. Chem. 2017, 129, 12876–12881


Manuscript received: July 1, 2017
Accepted manuscript online: August 14, 2017
Version of record online: September 1, 2017