Batch and flow synthesis of disulfides by visible-light-induced TiO2 photocatalysis

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Batch and flow synthesis of disulfides by visible light induced TiO$_2$ photocatalysis

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Abstract: A mild and practical method for the preparation of disulfides via visible light induced photocatalytic aerobic oxidation of thiols has been developed. The method involves the use of TiO$_2$ as a heterogeneous photocatalyst. The catalyst’s high stability and recyclability makes this method highly practical. The reaction can be substantially accelerated in a continuous-flow packed-bed reactor, which enables a safe and reliable scale-up of the reaction conditions. The batch and flow protocol described herein can be applied to a diverse set of thiol substrates for the preparation of homo- and hetero-dimerized disulfides. Furthermore, biocompatible reaction conditions (room temperature, visible light, neutral buffer solution, no additional base) have been developed, which permits the rapid and chemoselective modification of densely functionalized peptide substrates without recourse to complex purification steps.

Several synthetic approaches reported in the literature suggest viable strategies for the formation of symmetrical and unsymmetrical disulfides.\(^1\) In Nature, disulfides play a pivotal role in protein folding and oligomerization.\(^2\) Disulfides are also fundamental to the production of many fine chemicals and pharmaceuticals, as well as vulcanizing reagents for industrial-scale rubber production.\(^3\) Nevertheless, most synthetic methods require the use of harsh reaction conditions, such as strong oxidants and high reaction temperatures. More recently, milder and greener approaches have been developed, including aerobic oxidation of thiols in presence of molecular oxygen or air, catalyzed by transition metal complexes.\(^4\)

Owing to the prevalence of disulfides in proteins, a number of biocompatible and bioorthogonal methodologies have also been developed.\(^5\) In such cases, S–S bond formation serves as a controlled trigger for oxidative folding or as a method to enable bioconjugation.\(^6\) The redox-sensitive nature of disulfide bonds also renders them viable candidates for drug delivery systems.\(^7\) Nevertheless, rapid, straightforward and biocompatible strategies are still in demand to obviate the need for long reaction times and activating agents (e.g. Ellman’s reagent or methanethiosulfonate reagents).\(^8\) \(^9\) We recently reported a photocatalytic approach to symmetrical disulfides catalyzed by the organic dye Eosin Y.\(^10\) Despite the mild reaction conditions and fast reaction times of our approach, the use of Eosin Y as a homogeneous photocatalyst still necessitates an additional purification step to recover catalyst and isolate the target disulfide. In peptide and protein modification, purification steps are undesired and often problematic, especially for delicate proteins that are prone to denature. Furthermore, the use of a homogeneous catalyst in general might lead to bioincompatibility issues, since these catalysts tend to give undesired binding interactions.\(^11\)

In our efforts to develop efficient synthetic tools for chemical biology purposes, our attention was drawn to the use of TiO$_2$ as a cheap and recyclable photocatalyst of aerobic S–S bond formation.\(^11\) To date, the main application of TiO$_2$ photocatalysis has been in the water treatment industry,\(^11\) while its use for organic synthesis remains scarcer.\(^11\)\(^12\) In light of the high energy band-gap of nanoscale TiO$_2$ (3.2 eV for the anatase form), its photocatalytic activity is typically associated with UV-light irradiation.\(^12\)\(^13\) However, given the incompatibility of UV light to peptides and proteins,\(^15\) we reasoned that visible-light photoredox catalysis would be better suited to chemical biology applications owing to the milder reaction conditions (e.g. room temperature and visible light). In this regard, several recent reports have shown that surface interactions between nanoscale TiO$_2$ and some organic substrates, such as amines, can overcome the innate lack of visible-light absorption.\(^16\) In particular, Chen and co-workers showed that the use of triethylamine (TEA) allows for visible light TiO$_2$ excitation, which in turn enables the selective aerobic oxidation of sulfides to sulfoxides.\(^17\) Here, we report the use of TiO$_2$ as catalyst for the visible light-mediated formation of symmetrical and unsymmetrical disulfide bonds. The chemistry is amenable to intramolecular S–S bond formation in complex peptides under biocompatible reaction conditions (buffer solution, no additional base required, visible light, simple purification by filtration or centrifugation). Furthermore, we demonstrate that TiO$_2$ can be repeatedly recycled without noticeable catalyst deactivation, which enabled the development of a highly effective continuous-flow protocol with much reduced reaction times.

We began our study with benzyl mercaptan 1, a relatively unreactive substrate which proved challenging for our Eosin Y based-protocol (Table 1). In the absence of any light or base, almost no reaction was observed (Table 1, Entry 1 and 2). Upon light irradiation, 33% of the target disulfide product 2 was observed (Table 1, Entries 3 and 4). It should be noted that photooxidative disulfide formation is possible in the presence of a

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Supporting information for this article is available on the WWW under http://
base under visible light irradiation in the absence of any photocatalyst as shown by Yoon et al.\(^\text{16}\) However, the transformation has a very narrow scope and is limited to those thiols of which the corresponding thiolate absorbs in the visible light region, e.g. \(\rho\)-NO\(_2\)-thiophenol. Improved results were obtained when the light source was switched from CFL (compact fluorescent light) to white LEDs (Table 1, Entries 5, 6-7). Interestingly, a 28% yield of 2 was attained when a carbonate buffer was used as the base (Table 1, Entry 7). This result is significant as it provides opportunities to perform this chemistry under biocompatible conditions. The lower yield can be explained by the triple phase-transfer reaction conditions, i.e. oxygen to liquid, reactants to TiO\(_2\) and base from the water to the organic phase. In all cases, no overoxidation to the corresponding sulfoxide and sulfones was observed due to a careful management of the reaction time (Table 1).

Table 1: Optimization table of solvents, light sources and base\(^a\)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Light source</th>
<th>Solvent</th>
<th>Base</th>
<th>Yield (%)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No light</td>
<td>CH(_3)CN</td>
<td>TMEDA</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>CFL</td>
<td>CH(_3)CN</td>
<td>No base</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>CFL</td>
<td>CH(_3)CN</td>
<td>TMEDA</td>
<td>32</td>
</tr>
<tr>
<td>4</td>
<td>CFL</td>
<td>EtOH</td>
<td>TMEDA</td>
<td>33(^c)</td>
</tr>
<tr>
<td>5</td>
<td>White LED</td>
<td>EtOH</td>
<td>TMEDA</td>
<td>43(^c)</td>
</tr>
<tr>
<td>6</td>
<td>White LED</td>
<td>EtOH</td>
<td>TMEDA</td>
<td>75(^c)</td>
</tr>
<tr>
<td>7</td>
<td>White LED</td>
<td>H(_2)O/CH(_3)CN (1:3)</td>
<td>Carbonate buffer</td>
<td>28(^c)</td>
</tr>
</tbody>
</table>

\(^a\)Standard reaction conditions: 1 (1 mmol), TMEDA (1 mmol), TiO\(_2\) (Aeroxide® P25, 12.5 mol %), EtOH (2 mL), O\(_2\), visible light irradiation, 1.5 hours. \(^b\)Yield determined with GC-MS and internal standard. \(^c\)Reaction time 5 hours. \(^d\)Reaction time 8 hours.

To reduce the risks associated with the handling of molecular oxygen and to overcome potential gas-liquid mass transfer limitations, we further concentrated our efforts on transferring this transformation to a continuous-flow protocol (see Supporting Information).\(^{19}\) Initial attempts to introduce a slurry of TiO\(_2\) in the liquid phase into a capillary microreactor immediately proved impractical. We found that the TiO\(_2\) nanoparticles tended to aggregate in the presence of TMEDA leading to rapid clogging of the microreactor. Next, a packed-bed reactor strategy was used in which the TiO\(_2\) nanoparticles were loaded in a glass packed-bed reactor. A bed consisting purely of TiO\(_2\) resulted in an excessive drop in pressure, which could be remedied by adding small glass beads to the bed (60 mg TiO\(_2\), 310 mg glass beads, 200 µL void for the combined gas-liquid phase, ID 3 mm, 4 cm length) (see Supporting Information). To circumvent potential leaching of TiO\(_2\) from the reactor, the packed-bed was flushed with a solution of TMEDA (1 M in EtOH) prior to the reaction. This caused the TiO\(_2\) particles to aggregate and effectively avoided any leaching of these small particles, which can cause clogging at the reactor outlet. Substantial rate accelerations (from 8 h in batch to 5 min in flow) were observed when performing this reaction in flow. This can be attributed to the enhanced gas-liquid mass transfer characteristics and the improved irradiation of the reaction mixture (Scheme 1).\(^{19a, 22}\)

To verify whether the TMEDA-induced aggregation of TiO\(_2\) nanoparticles might be influencing their catalytic properties, we analysed the size and the morphology of the TiO\(_2\) nanoparticles by scanning electron microscopy (SEM) and X-ray diffraction (XRD).\(^{16, 23}\) Samples of the catalyst were collected at the inlet and outlet of the microreactor after reaction, and were dried and compared to a sample of fresh TiO\(_2\). By both SEM and XRD, we were unable to detect any substantial difference between the different samples, suggesting that the composition of TiO\(_2\) remained unchanged, and that the catalyst was stable under our reaction conditions (see Supporting Information). However, further dynamic light scattering (DLS) experiments proved that large TiO\(_2\) aggregates were formed in the presence of TMEDA (see Supporting Information).

Next, we evaluated the influence of the increased particle size of TiO\(_2\) in presence of TMEDA with respect to potential deactivation and recyclability of the catalyst. The conversion of thiophenol to disulfide 3 was continuously monitored in a continuous-flow packed-bed reactor for >28 hours. No decrease in reaction yield was observed, thus proving the stability of the catalyst and the reliability of the continuous-flow set-up in terms of catalyst leaching (see Supporting Information). Furthermore, in batch, the TiO\(_2\) nanoparticles were separated by centrifugation and reused up to ten consecutive times with no impact on the reaction yield (see Supporting Information).

With the optimized batch and flow conditions in hand, we examined the scope of the aimed transformation with an array of (hetero)aromatic thiols (Scheme 1). Disulfides 2 to 7 were obtained in good to excellent yields (72-96% in batch vs 60-99% in flow).

Scheme 1. Comparison of flow and batch yields for symmetrical disulfide formation. Reaction conditions in batch: thiol (1 mmol), TMEDA (1 mmol), TiO\(_2\) (Aeroxide® P25, 12.5 mol %), EtOH (2 mL), O\(_2\), visible light irradiation. The yields reported are isolated. For the detailed reaction conditions in flow see Supporting Information.
The formation of unsymmetrical disulfides via oxidative coupling is rarely described in literature, most likely due to the selectivity issues (i.e., homo- or hetero-dimerization) arising under the reaction conditions.\textsuperscript{11a}\textsuperscript{24} Previous reports of unsymmetrical disulfide formation often rely on the use of reactive reagents.\textsuperscript{25} Under our reaction conditions, the use of an excess of the less reactive thiol (i.e., 5 equiv. of the aliphatic thiol) overcame statistical formation of homo- and hetero disulfides and allowed us to obtain a diverse range of unsymmetrical aryl-alkyl disulfides 8-13 in good yields (23-86%) with good selectivity to the unsymmetrical disulfide (< 5%) of the symmetrical disulfide of the aromatic thiol; more symmetrical disulfide is formed from the less reactive thiol due to the large excess) (Scheme 2).

![Scheme 2](image)

These results demonstrate the potential of our methodology for the formation of symmetrical and unsymmetrical disulfide derivatives as handles for bioconjugation. However, larger peptide sequences are typically only stable at neutral or slightly basic pH. A further evaluation of the reaction conditions revealed that the chemistry could be carried out in aqueous phosphate buffer (pH=7.4) in the absence of TMEDA. These reaction conditions are practical in a chemical biology laboratory and suitable for working with sensitive and complex biomolecules, such as peptides, proteins, and in principle, antibodies. Practically, only buffer solution, TiO\textsubscript{2}, visible light irradiation and atmospheric conditions are required to carry out the transformation.

To probe these new reaction conditions, an intramolecular disulfide bond formation reaction was carried out to yield the cyclic nonapeptide Oxytocin starting from its reduced form 16. Oxytocin is a hormone peptide produced in mammals and associated with the brain modulation of several social and non-social behaviours.\textsuperscript{23} The active form of Oxytocin 17 was obtained within only 30 minutes (Scheme 4, see also Supporting Information). Notably, the methodology also worked on a crude sample of the Oxytocin precursor isolated by precipitation after cleavage from the solid-phase resin, emphasizing the feasibility of the transformation on impure peptide mixtures. After the reaction, the TiO\textsubscript{2} nanoparticles were easily separated by centrifugation and Oxytocin was purified by standard preparative scale LC-MS.

![Scheme 4](image)

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In conclusion, we have developed a convenient protocol for the preparation of disulfides via visible-light photocatalytic aerobic oxidation of thiols with TiO$_2$ as a recyclable photocatalyst. Unsymmetrical disulfides were also prepared in good yield using an excess of the less reactive coupling partner. The use of a continuous-flow TiO$_2$ packed-bed reactor substantially reduced the reaction times (from hours in batch to minutes in flow). The stability of the TiO$_2$ photocatalyst was investigated and no noticeable degradation was observed in both batch and flow. We note as well that the mild reaction conditions (room temperature, visible light, neutral buffer solution) and the facile catalyst recuperation by filtration makes this protocol particularly attractive for routine application in biomolecule modification, as exemplified in the synthesis of Oxytocin and peptide 19.

**Experimental Section**

**General batch procedure:** In an oven-dried vial equipped with a magnetic stirrer and a PTFE septum, 10 mg (12.5 mol %) of TiO$_2$ was added to a mixture of thiol (1.0 mmol) and N,N,N'-tetramethylethylenediamine (TMEDA) (1.0 mmol) in EtOH (2.0 mL). The vial was subjected to visible light irradiation and oxygen was added to the reaction mixture through bubbling. The reaction was stirred at 1000 rpm and monitored by TLC. When full conversion was reached, the reaction mixture was adsorbed on silica and purified by flash chromatography to give the desired disulfide.

**General continuous-flow procedure in a packed-bed reactor:** A 5 mL syringe containing 0.5 M substrate and a 5 mL syringe containing 0.5 M TMEDA were mounted on a single syringe pump. The liquid flowrate (per syringe) was fixed at 8.3 μL/min. The two liquid streams were merged in a T-Mixer and to this stream O$_2$ was added at a flowrate of 50 μL/min by means of a mass flow controller. After reaching steady state, a reaction sample was collected until 1 mmol of product was collected. This sample was purified by flash chromatography and analyzed by NMR and IR.

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**Keywords:** disulfide formation • continuous flow • heterogeneous photocatalysis • TiO$_2$ • visible light

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COMMUNICATION


A mild and practical method for the preparation of disulfides via visible light induced photocatalytic aerobic oxidation of thiols has been developed. The method involves the use of TiO₂ as a heterogeneous photocatalyst, which provides high stability and recyclability. The batch and flow protocol described herein can be applied to a diverse set of thiol substrates for the preparation of homo- and hetero-dimerized disulfides and can be used for peptide modification.

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