Synthetic Peptides

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Peptide Fragment Coupling Using a Continuous-Flow Photochemical Rearrangement of Nitrones**

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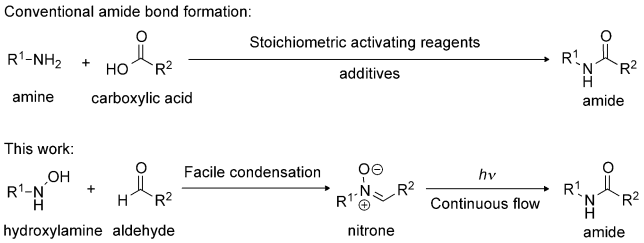
Amide bonds are prevalent in nature as the key chemical linkage of peptides and proteins and are also found in pharmaceutically relevant compounds and important synthetic polymers.[1] Conventional amide bond formation relies on the condensation of carboxylic acids and amines and generally uses stoichiometric amounts of activating agents and other additives.[2] However, the use of activating agents in peptide synthesis can compromise their utility because of drawbacks such as epimerization, high cost, and waste generation.[2b,3] In addition, despite the power of native chemical ligation (NCL) strategies for fragment assembly of polypeptides,[4] their reliance on the presence of *N*-terminal cysteine residues limits their broader application and suggests a need for alternative amide bond formation processes.[2a] Toward this goal, we herein describe a continuous flow approach of amide bond construction that proceeds by way of photochemical rearrangement of a nitrone with stereochemical fidelity and, notably, is thus well suited for peptide fragment coupling (Scheme 1).



Scheme 1. Photochemical amide synthesis.

Photochemical rearrangements of nitrone to oxaziridine,[5] and oxaziridine to amide[6] have been individually documented starting in the 1950s. However, there are only sporadic reports of a one-pot photochemical rearrangement of nitrones to amides,[7] and to the best of our knowledge, no peptide bond formation using this approach has been described.[8] This photochemical amide bond formation process is attractive for several reasons, including: 1) a lack of stoichiometric amounts of activating agents or additives for nitrone formation from hydroxylamines and aldehydes,[9] 2) the ability to effect peptide ligation at a range of amino acid residues, and 3) bypassing isolation of the often unstable oxaziridine intermediates.[6] In addition, by using a straightforward, easily assembled, continuous-flow reactor system,[10] the efficiency of this photochemical process is greatly enhanced.

Continuous-flow photochemical processes have well established advantages over conventional batch transformations in reaction efficiency, yield, reproducibility, and material throughput.[11,12] In batch reactions, light penetration is limited to a narrow layer within the reaction mixture. Whereas in continuous-flow setups, the narrower reaction channels and increased surface-to-volume ratio greatly enhance light absorption, even at high substrate concentrations. High material throughput can be realized simply by flowing the reaction mixture for a longer period of time. Furthermore, because the irradiation period can be precisely controlled by the flow rate, over-irradiation-related side reactions and decomposition pathways are often minimized or avoided altogether.

The photochemical continuous-flow reactor system that we constructed is depicted in Figure 1. Quartz tubing was placed around the water-cooled quartz immersion well of a 450 W medium-pressure mercury lamp. The reaction solution was introduced into the tubing using a syringe pump and collected into a flask after passing through a 20 psi back-pressure regulator. The complete UV setup was operated safely within an aluminium foil-lined photobox that was easily accommodated by a standard laboratory fume hood.[13]

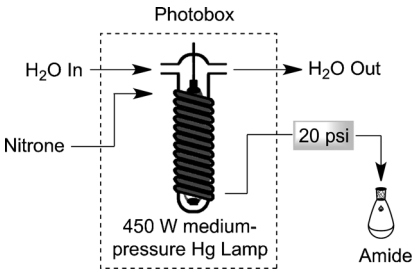


Figure 1. Continuous flow photochemical reactor.

We first tested the photochemical rearrangement of simple alkyl-aryl nitrone **1a** in the continuous-flow system.

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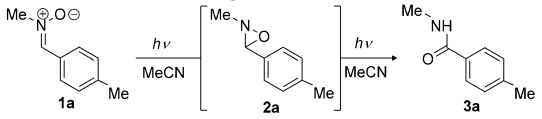
Nitrone **1a** was readily prepared from methyl hydroxylamine hydrochloride and *p*-tolualdehyde.[14] MeCN was found to be optimal among the solvents evaluated (tetrahydrofuran (THF), MeCN, dimethylformamide (DMF), isopropanol (*i*PrOH), benzene) and was used as the reaction solvent for further study.[15]

Optimization of the reaction conditions is summarized in Table 1. We initially chose 0.02 M as the substrate concentration and used a cooling water bath to maintain the reaction temperature at 40 °C. With a residence time (t_R) of 30 minutes,

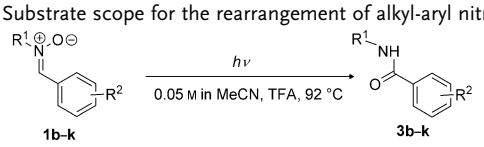
Table 1: Photochemical rearrangement of nitrone **1a** to amide **3a**.



Entry[a]	Concentration [M]	t_R [min]	TFA [equiv]	T [°C]	Ratio (**1a**:**2a**:**3a**)[b]
1	0.02	30	–	40	nd:2:3
2	0.02	30	0.1	40	nd:nd:1
3	0.05	30	0.1	40	nd:1:26
4	0.05	10	0.1	40	1:11:21
5	0.05	10	0.1	92	nd:1:19
6[c]	0.05	10	0.25	92	nd:nd:1
7	0.1	30	0.25	92	1:4:21

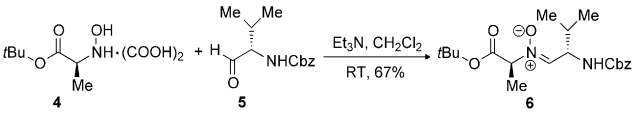
[a] All experiments were conducted using 0.625 mL quartz tubing with an inner diameter (i.d.) of 0.762 mm. [b] All ratios were determined by ^{1}H NMR studies. nd = not detected. [c] the yield of isolated **3a** was 93%.

Table 2: Substrate scope for the rearrangement of alkyl-aryl nitrones.



Entry[a]	Nitrone	R^1	R^2	t_R [min]	TFA [equiv]	Yield [%]
1	**1b**	Me	*p*-OMe	10	–	83
2	**1c**	Me	*m*-OMe	15	–	70
3	**1d**	Me	3,4-(OCH_2O)	5	0.1	74
4	**1e**	Me	*o*-Me	15	0.25	60
5	**1f**	Me	2,4,6-Me	10	0.25	65
6[b]	**1g**	Me	*p*-Br	20	0.25	90
7	**1h**	Me	*p*-F	15	0.25	84
8	**1i**	Me	*p*-CF_3	10	0.1	81
9	**1j**	Bn	*p*-OMe	10	–	87
10	**1k**	Bn	*p*-Me	10	0.25	89

[a] All experiments were conducted using quartz tubing (0.460 mL or 0.625 mL, i.d. = 0.762 mm). See the Supporting Information for details. [b] A Pyrex sleeve (280 nm cutoff) was placed around the UV lamp to prevent debromination.



Scheme 2. Synthesis of nitrone **6**. Cbz = benzyloxycarbonyl.

all the starting nitrone **1a** was consumed, but the oxaziridine intermediate **2a** was not fully converted into the amide product **3a** (Table 1, entry 1). The addition of a catalytic amount of trifluoroacetic acid (TFA, 0.1 equiv) facilitated conversion of oxaziridine into amide,[16] and afforded nearly exclusive amide formation with a 30 minute t_R, even at 0.05 M substrate concentration (Table 1, entry 3). Removal of the cooling water bath resulted in a rise of the reaction temperature to 92 °C and further expedited amide formation (entry 5). Finally, increasing the amount of TFA to 0.25 equivalents drove the reaction to completion, with a 10 minute t_R, and gave **3a** in 93% yield of the isolated product (entry 6). Further increasing the substrate concentration led to diminished conversion (entry 7). Thus, 0.05 M was used as the reaction concentration for further studies.

With these conditions in hand, a series of alkyl–aryl nitrone substrates were investigated (Table 2). In general, formation of amides from nitrones with either electron-rich or electron-poor aryl group proceeded smoothly with good to excellent yields of the isolated product. Substrates with electron-rich aryl groups tended to require a shorter t_R and in some cases did not require TFA to accelerate the subsequent rearrangement (Table 2, entries 1–3, 9).

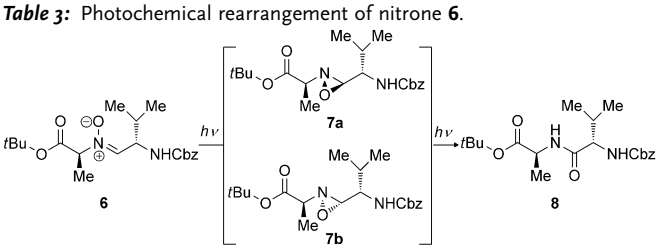
Encouraged by the feasibility of the photochemical rearrangement of alkyl-aryl nitrones to form amides, we next investigated peptide bond formation employing this transformation. Nitrone **6** was readily synthesized from Ala-derived hydroxylamine **4** and Val-derived aldehyde **5** (Scheme 2).[14,17]

When a 0.05 M solution of nitrone **6** in MeCN was subjected to the continuous flow photochemical conditions, the rearrangement was facile and neither TFA nor heating was required to promote the reaction (Table 3). Entries 1–4 demonstrate the reaction outcome with different residence times. The oxaziridine intermediate from nitrone **6** existed as a 3:2 mixture of two diastereomers **7a** and **7b**. The stereochemistry of the oxaziridine rings in **7a** and **7b** was tentatively assigned as shown. A ten minute t_R was sufficient for full conversion of both the starting nitrone and the oxaziridine intermediates, and dipeptide **8** was isolated in 59% yield (Table 3, entry 4). In comparison, the corresponding batch reaction with a ten minute reaction time only gave a mixture of oxaziridines and amide in 7:1 ratio (entry 5).

Concentration is paramount in photochemical transformations, according to the Beer–Lambert law, and a clean reaction is rarely obtained at a preparatively useful concentration such as 0.1 M in batch processes.[18] We were thus pleased to find that increasing the initial substrate concentration to 0.1 M required only a marginally longer residence time (12.5 minutes) for complete conversion, and more importantly, no detrimental effect on the yield of isolated **8** was observed (entry 7). It is important to note that no epimerization on either stereogenic center in dipeptide **8** was observed by ^{1}H NMR spectroscopy or HPLC analysis.[13]

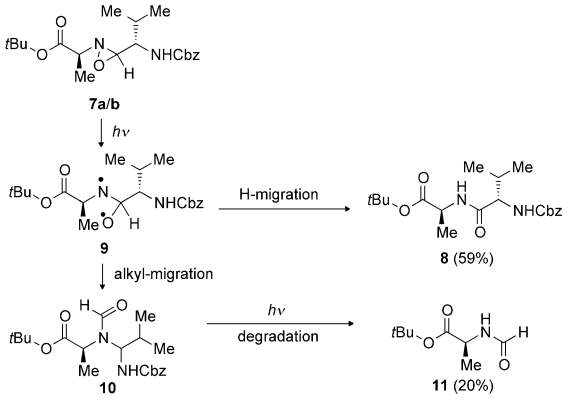
Interestingly, in the rearrangement of nitrone **6**, another amide (**11**) was isolated in 20% yield along with the desired dipeptide **8**. The mechanism of formation of **11** is proposed in Scheme 3.[8b,19] Upon irradiation, homolytic cleavage of the

Table 3: Photochemical rearrangement of nitrone **6**.



Entry[a]	t_R [min]	Concentration [M]	Ratio (**6**:**7**:**8**)[b]
1	1	0.05	1:9[c]:1
2	2.5	0.05	nd:4:3
3	5	0.05	nd:1:5
4	10	0.05	nd:nd:1[d]
5[e]	–	0.05	nd:7:1
6	10	0.1	nd:1:6
7	12.5	0.1	nd:nd:1[d]

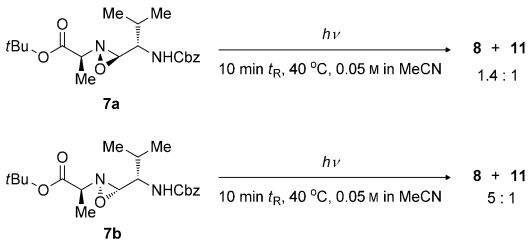
[a] All entries were conducted in MeCN at 40 °C using a quartz tubing (0.625 mL, i.d. = 0.762 mm). [b] All ratios were determined by ^{1}H NMR studies. nd: not detected. [c] **7a**:**7b** = 3:2. [d] Yield of isolated **8** is 59%. [e] Batch reaction was conducted in a quartz NMR tube (i.d. = 5 mm) placed next to the quartz tubing for ten minutes.



Scheme 3. Proposed mechanism for the formation of **11**.

weak N–O bond in the oxaziridine intermediate generates *N*,*O*-diradical **9**. H-migration leads to the desired amide (**8**), while migration of the alkyl group gives aminal **10**, which further degrades to amide **11**. The ratio of **8** and **11** remains constant throughout the course of the reaction. It is also worth noting that similar by-products were not observed in the photochemical rearrangement of alkyl-aryl nitrones **1a**–**k**.

The formation of **11** is somewhat surprising, because both previously reported theoretical studies[20] and experimental data[21] suggest that only the substituent *anti* to the nitrogen lone pair migrates during this process. Because nitrone **6** exists exclusively as its *Z*-stereoisomer,[22] we expect both oxaziridines **7a** and **7b** to be *trans*-substituted. In both cases, therefore, an H atom should be at the *anti* position and migrate selectively. To understand the stereochemical course of the rearrangement better, **7a** and **7b** were prepared from epoxidation of the corresponding imine and separated chromatographically.[8b,23] When **7a** and **7b** were subjected individually to the photochemical conditions, each provided

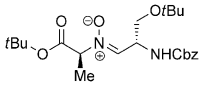
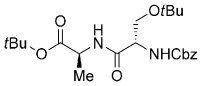
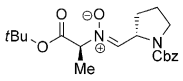
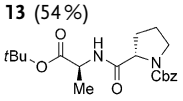
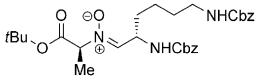
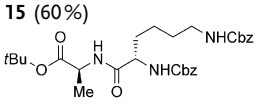
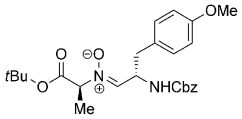
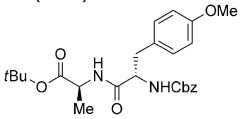
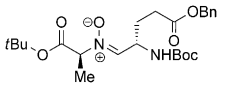
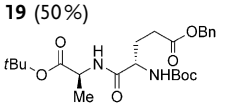
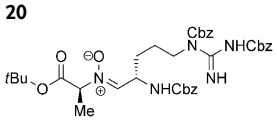
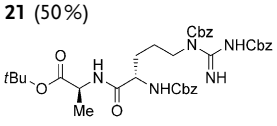


Scheme 4. Individual photochemical rearrangement of **7a** and **7b**.

a mixture of **8** and **11**, however in different ratios (Scheme 4). This information, together with a previous theoretical study that showed the substituent *anti* to the nitrogen lone pair migrates with no significant activation barrier,[20] prompts us to propose that nitrogen inversion in the oxaziridine ring competes with N–O bond cleavage in this process. The difference between the selectivity of **7a** and **7b** could be explained by disparate rates of nitrogen inversion owing to different spatial environments. Further investigation of the rearrangement process and efforts to increase the selectivity are currently ongoing.

Next, a broader range of dipeptide formation was investigated (Table 4). All nitrone starting materials were readily synthesized from the corresponding hydroxylamines and aldehydes.[14,17] Although we had demonstrated that the initial substrate concentration could be as high as 0.1 M (Table 3,

Table 4: Photochemical formation of dipeptides from nitrones.

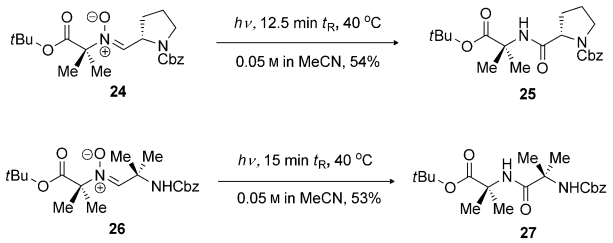
Entry[a]	Substrate	Product[b]
1	 **12**	 **13** (54%)
2	 **14**	 **15** (60%)
3	 **16**	 **17** (56%)
4	 **18**	 **19** (50%)
5[c]	 **20**	 **21** (50%)
6[d]	 **22**	 **23** (37%)

[a] All experiments were conducted at 0.05 M in MeCN at 40 °C with t_R = 10 min using quartz tubing (0.460 mL or 0.625 mL, i.d. = 0.762 mm) unless otherwise noted. See the Supporting Information for details. [b] Yields of the isolated product are shown in parenthesis. [c] t_R = 5 min. [d] Experiment was conducted in benzene with t_R = 20 min.

entry 7), the solubility of some of the substrates in MeCN was limited to 0.05 M. All reactions proceeded smoothly under continuous flow photochemical conditions to give the corresponding dipeptide in reasonable to good yields. The rearrangement reaction tolerated a variety of functional groups. Nitrones derived from protected amino acids with hydrophobic side chains (Val, **6**), polar uncharged side chains (Ser, **12**), aromatic rings (Tyr, **18**), charged side chains (Lys, **16**; Glu, **20**; and Arg, **22**), and proline (**14**) were all amenable substrates for this transformation. Amides of the type shown for **11** were also observed in all entries, with yields of the isolated product ranging from 10–19 %.[13]

S-protected cysteine-derived nitrones (*S*-Bn, *S*-*t*Bu, *S*-trityl) are not suitable substrates for this transformation because of the decomposition of the oxaziridine intermediates.[24] Despite this limitation, the substrate scope of this photochemical process represents a high degree of complementarity to NCL and allows peptide fragment coupling of a much broader range of amino acid residues.

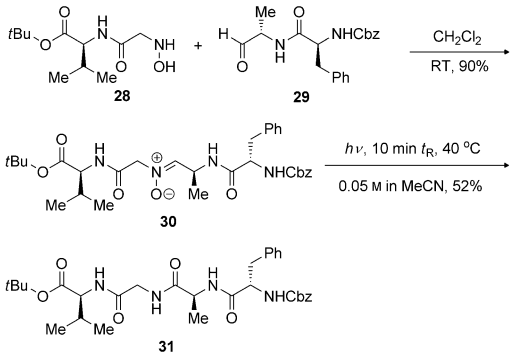
To expand the scope of this photochemical peptide bond formation process further, sterically hindered nitrones **24** and **26** were prepared.[14,25] Upon irradiation, dipeptides **25** and **27** were obtained without compromised yields, albeit longer residence times were required (Scheme 5).



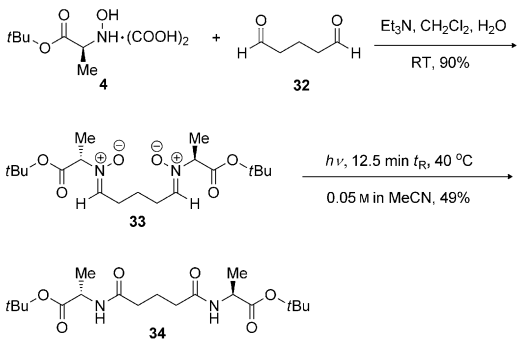
Scheme 5. Sterically hindered peptide bond formation.

We next investigated the formation of a longer peptide (Scheme 6). Nitrone **30** was prepared from Val/Gly-derived hydroxylamine **28** and Phe/Ala-derived aldehyde **29**.[14,17] When a solution of **30** (0.05 M in MeCN) was subjected to the described photochemical conditions ($t_R = 10$ min), tetrapeptide **31** was isolated in 52 % yield.

The double photochemical rearrangement of bis-nitrone **33** also proceeded smoothly, giving the symmetrical diamide



Scheme 6. Formation of the tetrapeptide **31** from nitrone **30**.



Scheme 7. Photochemical bi-directional amide bond formation.

34 in 49 % yield (Scheme 7).[26] This result suggests that this transformation might find applications in protein engineering to create multifunctional chimeric proteins.[27]

In conclusion, we have demonstrated a general and versatile approach for amide bond formation using a continuous-flow photochemical rearrangement of nitrones. Not only can this method be used to synthesize simple amides, but also to generate more complex peptide bonds. Importantly, this transformation represents a novel approach for peptide fragment coupling and expands the current scope of protein synthesis. In addition, by adopting a continuous-flow setup, the efficiency of the photochemical process can be significantly enhanced. Further investigations of its applications in cyclic peptide synthesis and protein ligation are currently ongoing.

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Keywords: amides · continuous flow · nitrones · peptides · photochemistry

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