EurJOC
European Journal of Organic Chemistry

Research Article
doi.org/10.1002/ejoc.202201372

Chemistry Europe
European Chemical Societies Publishing

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A Light-Induced Decarboxylative-Elimination of Substituted Maleimides as a Strategy Towards Triggered Photorelease

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Herein we report on photodecarboxylations of various substituted maleimides, resulting in an elimination reaction. Furthermore, we establish facile wavelength tunability through modulation of the maleimide double bond substituents. We envisage that these versatile reagents, which are readily constructed and diversified by nucleophilic substitution reactions on bromomaleimides, will offer new opportunities for triggered photorelease.

Introduction

Photochemical reactions that lead to controlled bond cleavages of linkers are of widespread application as they offer the possibility of light-stimulated molecular release with spatiotemporal control.[1] For example, photocleavable protecting groups (PPGs) provide an exquisite approach to reveal functional groups upon irradiation, or to act as phototriggers for activation of biological processes.[2] Within the field of bioconjugation, there is significant interest in the development of photocleavable uncaging groups;[3–5] for example, allowing the controlled activation of biomolecules,[6,7] or the release of attached cargo.[8–10] PPGs can also be extended for non-biological applications, for example, as protecting groups in multi-step organic syntheses,[11] solid-state syntheses[12] and photolithography,[13] making PPGs widely sought molecules.

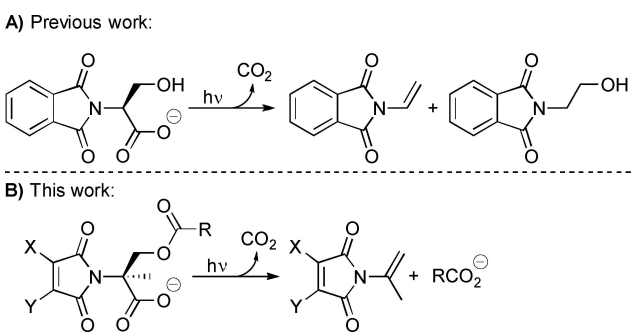
There are currently numerous PPGs in existence with the most prevalent being the *o*-nitrobenzyl group.[14] The ketoprofen molecule was the first PPG discovered to undergo cleavage via a photodecarboxylation mechanism.[15] As well improved kinetics, this cleavage strategy has advantages in biological applications over the traditional *o*-nitrobenzyl PPG which releases a cytotoxic side-product.

We, and others, have reported widely on the use of bromomaleimides, and related substituted maleimides, for the construction of peptide and protein conjugates via selective cysteine modification.[16–22] We have also shown that thiomaleimides generated in this way are efficient chromophores, undergoing [2+2] photocycloaddition reactions[23] with the ability to control stereoselectivity,[24] and with applications including photochemical disulfide rebridging[7] and polymer cross-linking.[25] Intriguingly, we have also observed that *C*-terminal cysteinyl maleimides undergo a photochemical decarboxylation side-reaction.[7]

Griesbeck et al. have shown that phthaloyl amino acids, both in their original and acetylated forms, undergo decarboxylative-eliminations,[26,27] albeit with the competing formation of a decarboxylation-protonation product (Scheme 1A).[26] Inspired by this, we aimed to explore the possibility that such a photochemical release reaction could be transferred to substituted maleimides (Scheme 1B).

Results and Discussion

We chose to focus our initial attention on dithiomaleimides, as they do not undergo [2+2] photodimerisations,[23] and have the added benefit of being readily accessed in disulfide rebridged conjugates.[16,18,20,28] Thus, our first targeted compound was dithiomaleimide-acetylated serine construct, compound **3**, to offer a convenient comparison to the work by Griesbeck and co-workers.[26,27] A four-step synthetic route starting from the activated dibromomaleimide carbamate **1**[29] was required for the synthesis (Scheme 2). Carbamate **1** was



Scheme 1. A) Previous work on the photodecarboxylation reaction of phthaloyl amino acids.[26] **B)** The work described herein on the photodecarboxylation reaction of substituted maleimides.

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Supporting information for this article is available on the WWW under https://doi.org/10.1002/ejoc.202201372

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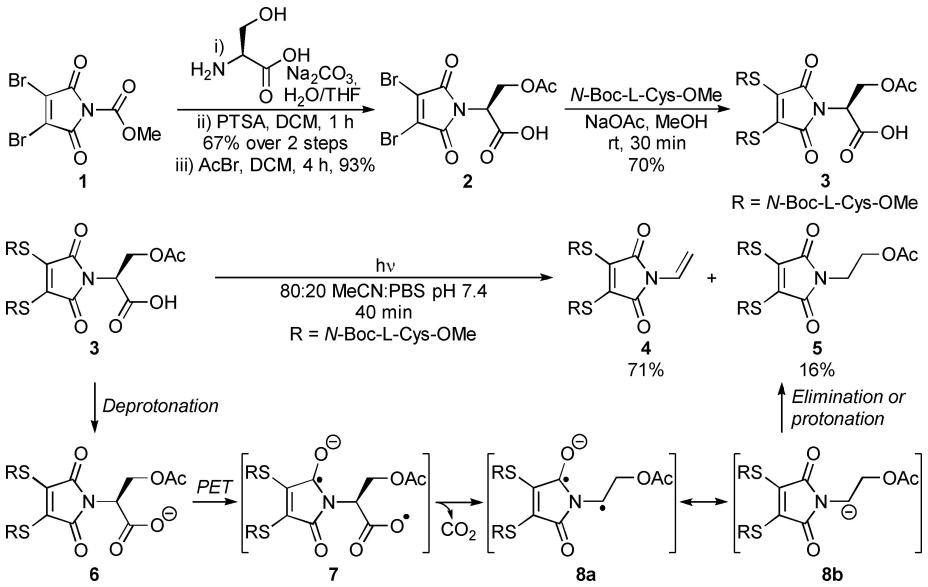
doi.org/10.1002/ejoc.202201372



Scheme 2. Synthesis and irradiation of model dithiomaleimide **3**.

reacted with L-serine to produce a dibromomaleimide-serine. Acetylation was achieved in the presence of acetyl bromide in base free conditions to give dibromomaleimide **2**, and finally substitution with *N*-Boc-L-Cys-OMe resulted in the generation of dithiomaleimide **3**. Of significance, the absorption maximum of dithiomaleimide **3** was 402 nm, which attractively would allow selective excitation using a violet light LED. However, for convenience whilst exploring substrate scope, the irradiations were initially conducted using a broad emission medium pressure mercury lamp (125 W, most significant emission at 365 nm) in an 80:20 MeCN:phosphate buffered saline (PBS) pH 7.4 solvent system. Full consumption of starting material **3** was achieved within 40 min.

As with the phthalimide variants,[26,27] the photodecarboxylation was followed by two competing pathways: elimination to produce enamide **4** (71%) or protonation to produce dithiomaleimide **5** (16%). The mechanism for this reaction is proposed to be a photoinduced electron transfer (PET) from the carboxylate **6** to the electronically excited maleimide chromophore, to form the diradical anion **7**. This then undergoes a decarboxylation to form **8a**, which is a resonance form of carbanion **8b**. Elimination of the acetate leaving group afforded enamide **4**, or protonation afforded maleimide **5**. This mechanism would match that proposed by Griesbeck et al. for the phthalimide system.[26] It is also possible that the decarboxylation-elimination pathway is concerted,[27] however this appears less likely based on the competitive formation of the protonated product.

We then set out to incorporate a methyl group in the serine α-position, with the hope that it would favor the elimination pathway. We postulated that the methyl may perhaps discourage the protonation, relative to the elimination, due to the additional steric bulk. The synthetic route (Figure 1A) allowed direct attainment of dibromomaleimide-α-Me-serine **10** from dibromomaleic acid **9** and α-Me-L-serine. From here, we chose to attach a phenylacetic acid cargo to allow more convenient detection of the cargo upon irradiation. This attachment was achieved via a reaction with phenylacetyl chloride, which produced a mixture of halomaleimides due to halogen exchange and was directly converted to dicysteinylmaleimide **11**.

Dithiomaleimide **11** was irradiated in an increased buffer content solvent system of 50:50 MeCN:phosphate buffer (PB) pH 7.4. The photoreaction of dithiomaleimide **11** was found to reach completion in just 10 min (Figure 1B). Analysis of the reaction by LCMS showed that it was remarkably efficient, cleanly generating enamide **12** (Figure 1C) and phenylacetic acid (see Supporting Information Figure 3, observed using an LCMS selected ion recording (SIR) method). ^{1}H NMR analysis was also carried out on the reaction mixture (Figure 1D), with the loss of the diastereotopic protons in dithiomaleimide **11**, growth of the alkene protons in enamide **12**, and formation of phenylacetic acid, establishing complete conversion of starting material was attained. Further analysis of the NMR integrations confirmed that a $\geq$90% yield of the enamide **12** and phenylacetic acid was achieved in this reaction (see Supporting Information Figure 2); and prolonged irradiation for 40 min led to no further change (see Supporting Information Figure 1) confirming that the phenylacetic acid released did not undergo secondary photoreactions. Notably no protonation product was observed proving the successful impact of the α-methyl substituent. Attempts at chromatography purification resulted in the isolation of enamide **12** in 70% yield and dithiomaleimide **13** in 19% yield. This was assumed to be as a result of enamide hydrolysis upon exposure to the mildly acidic silica; it should be noted that a trace peak of dithiomaleimide **13** (*m/z* 564) was also observed in the LCMS chromatogram post-irradiation (Figure 1C and Supporting Information Figure 4).

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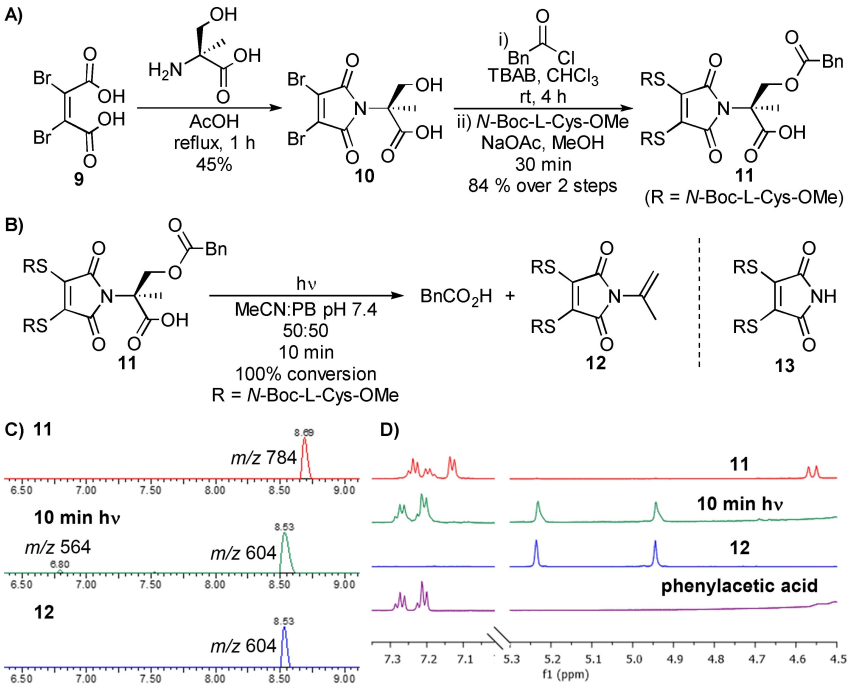

Figure 1. A) Synthesis of optimized dithiomaleimide **11** with incorporation of a methyl substituent in the serine α-position. **B)** Irradiation of dithiomaleimide **11** to give phenylacetic acid and enamide **12**. Compound **13** was then observed following column chromatography, assumed to be due to acid catalyzed hydrolysis of enamide **12**. **C)** Monitoring the irradiation by LCMS to show enamide **12** formation. **D)** Monitoring the irradiation by ^{1}H NMR shows enamide **12** and phenylacetic acid formation.

We envisaged that tunability of this effective photodecarboxylation-elimination strategy could potentially be achieved through modulating the maleimide double bond substituents to give a selection of prospective PPGs, triggered by differing wavelengths of light. We chose an alkyl aminobromomaleimide, an aryl aminobromomaleimide, an aminothiomaleimide and an aminomaleimide, as they have somewhat different UV–Vis absorption spectra (Figure 2B).[30–32] In addition, we imagined amino-substituted maleimides to be particularly useful in biological applications, due to their hydrolytic and thiol stability.[32] We chose to use a benzoic acid cargo for ease of synthesis with benzoyl bromide, to prevent the previously observed halogen exchange, and the successful syntheses are shown in Figure 2A. Dithiomaleimide **15** and alkyl aminobromomaleimide **16** were obtained in facile one-step addition-elimination reactions of dibromomaleimide **14** with *N*-Boc-L-Cys-OMe and propylamine, respectively. A two-step synthesis was planned for aminothiomaleimide **18**. Excess dibromomaleimide **14** was reacted with *N*-Boc-L-Cys-OMe with aims to give a thiobromomaleimide. Some disubstitution was still observed, which was removed upon flash chromatographic purification. However, separation of the excess starting reagent **14** and the thiobromomaleimide proved difficult. Thus, the mixture was taken forwards and reacted with aniline to give both aminobromomaleimide **17** and aminothiomaleimide **18**. Finally, the synthesis of an aminomaleimide was started with the reaction of bromomaleic anhydride **19** with α-Me-L-serine. Surprisingly, only the acetylated bromomaleimide-serine **20** was isolated, likely due to acetylation in the acetic acid solvent. As this could still be used for proof of principle photodecarboxylation, bromomaleimide-serine **20** was reacted with propylamine to generate aminomaleimide **21**.

We then attempted irradiation of this library of substituted maleimide chromophores using the aforementioned ^{1}H NMR and LCMS monitoring methods developed. In each case, these showed that the photodecarboxylation-elimination pathway was successfully followed to produce the respective enamides and release the carboxylic acid, albeit to different extents. The irradiation times, % conversion of starting material and UV–Vis absorption data is summarized in Figure 2C. Interestingly, both maleimides with aromatic amines, compounds **17** and **18**, did not appear to reach completion after 2 h irradiation (see Supporting Information Figure 14 and Supporting Information Figure 18 for ^{1}H NMR data and Supporting Information Figure 16 and Supporting Information Figure 20 for LCMS data). A direct evaluation of alkyl aminobromomaleimide **16** and aryl aminobromomaleimide **17** highlights that the reduced conversion, even with longer irradiation, is possibly associated with the presence of the aromatic substituent. Previously, O'Reilly and co-workers observed the fluorescence quenching of substituted maleimides when aromatic rings were directly conjugated.[33,34] Likely, a similar mechanism is quenching the excited state of aryl aminobromomaleimide **17** here.

With dithiomaleimide **15** undergoing the fastest photoreaction, an NMR method using the *o*-nitrobenzaldehyde chemical actinometer was used to approximate the quantum yield of benzoic acid photorelease to be 0.4 ± 0.1 upon irradiation at 365 nm (see Supporting Information Section 4 for details).[24,35,36] This confirms the highly efficient nature of the maleimide photodecarboxylative-elimination. The quantum yield for a phthalimide

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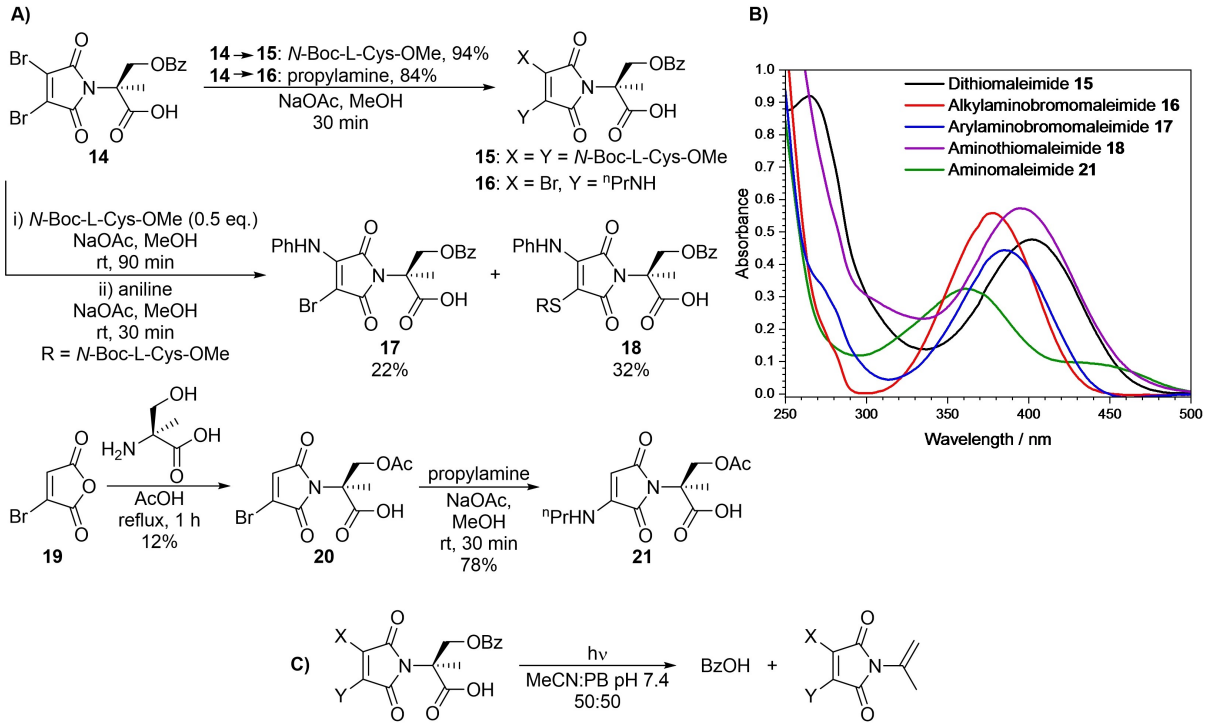

Molecule	Irradiation Time (min)	% Conversion	λ_{max} (nm)	ε (cm^{-1} M^{-1})
15	10	100	402	3200
16	50	100	377	3700
17	120	66	385	3000
18	120	18	395	3900
21*	30	100	362	2100

Figure 2. A) Synthesis of a library of substituted maleimides **15**–**18** and **21**. **B)** UV–Vis absorption spectra for the library of maleimides **15**–**18** and **21** at 0.15 mM in MeCN. **C)** % photoconversion upon irradiation of the maleimides **15**–**18** and **21** as 1.5 mM solutions after the stated irradiation time, along with their UV–Vis absorption data. *Note, for aminomaleimide **21**, acetic acid is released instead of benzoic acid.

photodecarboxylation is reported as 0.40±0.05, upon irradiation at 300 nm.[27] Thus the dithiomaleimide chromophore **15** not only has a comparable quantum yield to phthalimides but at a significantly higher wavelength of light.

As the various substituted maleimides display different UV–Vis absorption profiles (Figure 2B) and undergo photorelease, we envisioned these may allow wavelength-selective cleavages; albeit aware that greater differences in λ_{max} would ultimately be required to optimize such selectivity.[37] We chose to show this potential through an irradiation of a mixture of dithiomaleimide **15** and aminobromomaleimide **16**. An initial 40 min irradiation using a 470 nm LED torch resulted in photorelease from both dithiomaleimide **15** and aminobromomaleimide **16** to produce their corresponding enamides in a ratio of 6:1 (determined by ^{1}H NMR, see Supporting Information Figure 30), illustrating some selectivity of this photoreaction. A subsequent 55 min irradiation using a 365 nm LED torch then resulted in complete photorelease from aminobromomaleimide **16** (see Supporting Information Figure 29).

This serves as initial proof of concept that maleimide chromophores can facilitate wavelength-selective photorelease, which could provide a new platform for orthogonal PPGs.

Conclusions

In conclusion, we have reported the discovery of a novel photoreactive pathway of substituted maleimides; namely a photodecarboxylation-elimination reaction. A competing protonation pathway was inhibited successfully through incorporation of an α-methyl substituent. Dithiomaleimides were found to undergo an efficient photodecarboxylation, and the maleimide core was then shown to be readily diversified to afford a range of tunable wavelength chromophores, which all underwent photorelease. The prospective wavelength-selectivity of these chromophores was also exemplified using dithiomaleimides and aminobromomaleimides and we imagine improved selectivity will be possible through exploring a further range of substituted maleimides. We envisage that discovery of these novel maleimide-based PPGs for efficient photorelease, with their facile construction by nucleophilic substitution reactions, will have applications in the construction of diverse photocleavable conjugates.

Acknowledgements

We gratefully acknowledge the EPSRC for funding.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: photodecarboxylation · photocleavable linker · phototrigger · maleimide · thiomaleimide

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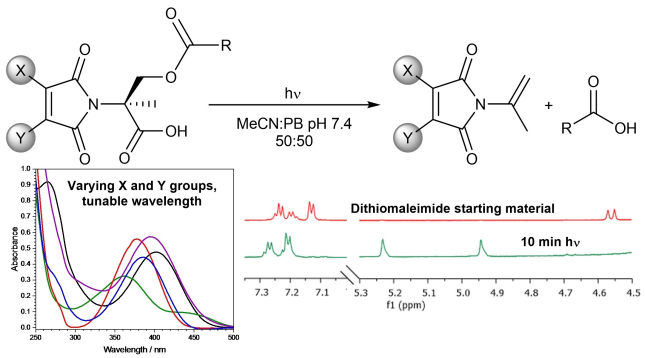
Manuscript received: November 22, 2022
Revised manuscript received: March 24, 2023



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A Light-Induced Decarboxylative-Elimination of Substituted Maleimides as a Strategy Towards Triggered Photorelease

A photodecarboxylation-elimination reaction of various substituted maleimides is described, with convenient wavelength tunability through modulation of the maleimide substituents. These versatile reagents, which are readily constructed and diversified by nucleophilic substitution reactions on bromomaleimides, offer enticing new opportunities for triggered photorelease.