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[2 + 2] Photocycloadditions of thiomaleimides†

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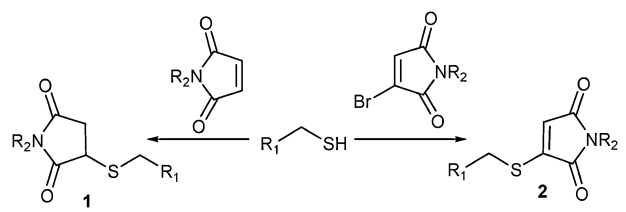
Thiomaleimides, generated by the addition of bromomaleimides to thiols including cysteine, undergo highly efficient [2 + 2] photocycloadditions.

The maleimide motif has found widespread use in a range of fields due its diverse reactivity profile. It is a highly reactive conjugate acceptor and as such can be employed effectively in selective reactions with thiols, such as cysteine residues in proteins. As a result it is one of the most widely used functional groups in bioconjugation.[1] Maleimides are also photoactive, undergoing reactions including [2 + 2] photocycloadditions,[2] which have been exploited in applications ranging from total synthesis[3] to polymer cross-linking.[4]

We envisaged a situation in which these two modes of reactivity could be combined. The product of the addition of a thiol to a maleimide is the saturated thiosuccinimide **1** (Scheme 1), which can no longer undergo the [2 + 2] photocycloadditions. In contrast when a thiol is added to a bromomaleimide an addition-elimination reaction takes place and the product is the unsaturated thiomaleimide **2**. We have shown this reaction to have broad applications in bioconjugation *via* the highly efficient, selective and reversible modification of single cysteine residues and disulfide bonds in proteins.[5] We postulated that such thiomaleimides may also retain the photoactivity of the maleimides, and we report here on our results.

Irradiation of hexylthiomaleimide **3** with a 125 W medium pressure mercury lamp in pyrex glassware afforded after just five minutes a single dimeric product **4** in quantitative yield (Table 1). This is an extremely rapid reaction in comparison to maleimide itself which undergoes dimerization in one hour under the same conditions (see ESI). The reaction proceeds in acetonitrile or water/acetonitrile (95 : 5) down to at least 72 μM (see ESI),



Scheme 1 Addition of a thiol to a maleimide *vs.* a bromomaleimide.

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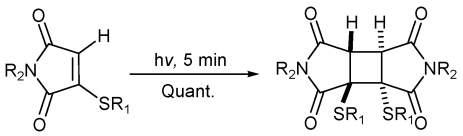
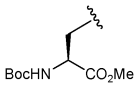
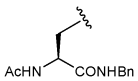
Table 1 Photochemical dimerisation of thiomaleimides



Entry	R_1	R_2	Starting Material	Dimer Product
1	n-Hex	H	**3**	**4**
2	n-Hex	Me	**5**	**6**
3		H	**7**	**8**[a]
4		H	**9**	**10**[a]

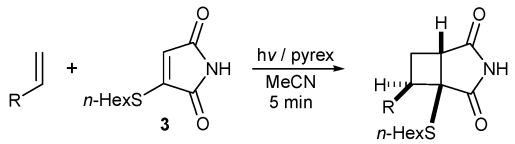
[a] Obtained as a mixture of diastereomers.

indicating that it could be applied in conditions suitable for bioconjugation. The dimerisation also proceeds quantitatively when the nitrogen is methylated (entry 2). By analysing NMR J-couplings and nuclear Overhauser effects (NOEs) from the irradiation of a 1 : 1 mixture of the N–H and N–Me substrates **3** and **5** we deduced that the dimers formed are the *exo* head-to-head products (see ESI). Head-to-head dimers were expected according to FMO theory and in line with literature precedent, whilst the *exo*- orientation can be explained by avoidance of the worst of the steric clashes.

To test the hypothesis that this strategy could be used on substrates containing cysteine, we irradiated a solution of *N*-Boc-Cys(Mal)-OMe **7**[5a] resulting in the formation of dimeric products **8** in just 5 min. The dimerisation of *N*-AcCys(Mal)NHBn **9** was also carried out, as analysis of the conveniently visible ^{13}C satellite peaks provided further evidence for the *exo* head-to-head outcome in these reactions (See ESI). These dimers can be considered as photochemically formed disulfide mimics, in which the two cysteine residues are bridged by a two carbon linker, and which are stable to reductive conditions. Indeed treatment of **4** with excess reducing agent, β-mercaptoethanol, showed no reaction (see ESI).

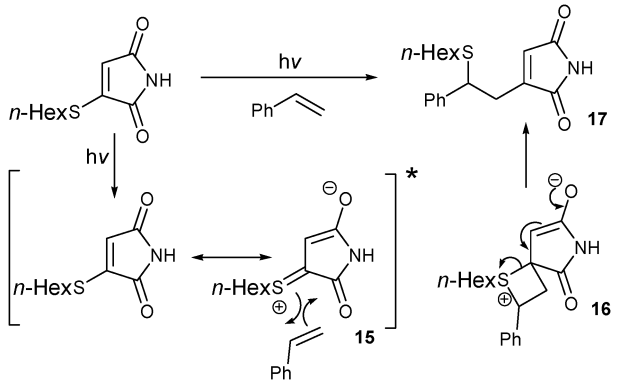
We investigated suitable olefinic partners for the [2 + 2] photocycloaddition as alternatives to this dimerisation. We were pleased to find that with a variety of olefins the expected [2 + 2] photocycloaddition takes place, again in just 5 min (Table 2). Excess olefin is always necessary to stop the competing

Table 2 Thiomaleimide-alkene photocycloadditions



Entry	*R*	Equiv	Product	Yield
1	n-Bu	300	**11**	41%
2		300	**12**	49%
3		10	**13**	48%
4	Ph	10	**14**	70%[a]

[a] 30% insertion product **17** was also isolated.

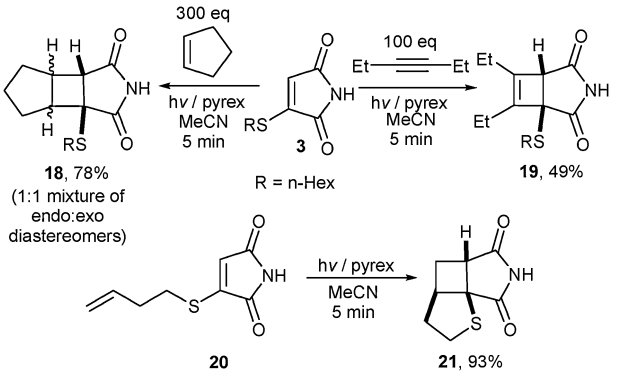


Scheme 2 Insertion of styrene in to the C–S bond.

thiomaleimide dimerisation reaction. The products isolated were all *exo* 'head-to-head'.

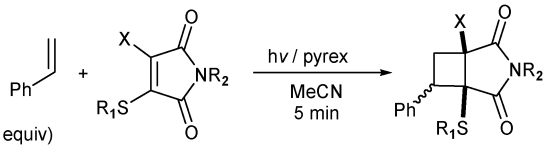
By far the best partner (entry 4) was styrene; only requiring 10 equivalents and forming the cyclobutane **14** in 70% yield. An intriguing isomeric product **17** was also isolated in 30% yield, derived from an insertion of the styrene in to the C–S bond (Scheme 2). The mechanism for this unprecedented side-reaction is not known, but one possibility is best visualized by drawing a resonance form of the excited state, **15**, which represents the double bond character present in the C–S bond. A [2 + 2] photocycloaddition would thus afford zwitterionic intermediate **16** which then undergoes a fragmentation to afford the product **17**. Alternative mechanisms such as *via* electron transfer or *via* homolysis of the C–S bond are also possible. It is intriguing to note that maleimides are also known to undergo alkene insertions into the imide, as widely demonstrated by Booker-Milburn *et al.*,[6] and thus demonstrate an incredibly varied photochemical reactivity profile. Importantly for potential applications in bioconjugation the two products **14** and **17** represent an overall 100% attachment of the styrene to the maleimide.

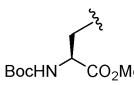
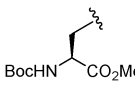
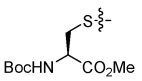
The broad potential scope for this [2 + 2] photocycloaddition is further exemplified by the reactions of thiomaleimide **3** with cyclopentene to form tricyclic product **18**, with hex-3-yne to form cyclobutene **19**, and the intramolecular cycloaddition of thiomaleimide **20** to afford **21** (Scheme 3).



Scheme 3 Further photocycloadditions of thiomaleimides.

Table 3 Styrene photocycloadditions with thiomaleimides



Entry	R_1	R_2	X	Yield
1	n-Hex	Me	H	70%[a]
2	n-Hex	CH_2Cy	H	64%[b]
3	n-Hex	Ph	H	80%[b,c]
4		H	H	63%[d]
5	n-Hex	H	n-HexS	70%[b,e]
6		H		60%[b,e]

[a] Combined yield of two separable diastereomers. [b] Combined yield of two inseparable diastereomers. [c] 1% of the C–S insertion product also isolated. [d] Combined yield of four inseparable diastereomers. [e] Irradiation time 20 min.

With styrene demonstrated as a suitable olefenic partner for the intermolecular [2 + 2] photocycloaddition, we also explored the effect of varying the thiomaleimide (Table 3). Initially the effect of maleimide N-substitution on the outcome of the reaction was considered (entries 1–3). Alkyl and aryl substituents were tolerated. Notably alkyl substituents led to complete elimination of the C–S insertion by-product, whilst in the aryl case it was present in just 1% yield. The cysteine thiomaleimide **7** also underwent effective [2 + 2] photocycloaddition with styrene (entry 4) to afford four diastereomeric cyclobutanes as a mixture. Again no C–S bond insertion product was isolated in this case. We then switched our attention to dithiomaleimides, which upon irradiation with styrene afforded dithio-cyclobutanes (entries 5 and 6), following a slightly extended irradiation time of 20 min. Interestingly irradiation of these dithiomaleimides independently led to no observable dimerisation even after 8 h, possibly due to increased steric effects of the tetrasubstituted double bonds.

Low wavelength, high energy UV exposure can cause undesirable side-reactions in proteins and other biomolecules.[7] To minimize such photochemical degradation UV wavelengths

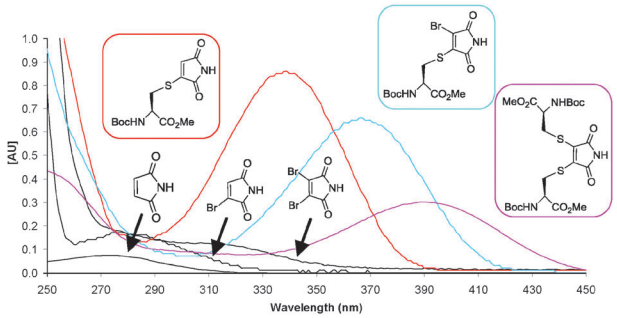


Fig. 1 Overlayed UV absorbance spectra of maleimides at 1mM in acetonitrile.

above 350 nm are sought.[8] Thiomaleimides may prove more useful than simple maleimides for photochemical applications in such areas as successive substitutions of the maleimide cause a bathochromic shift, as seen in Fig. 1. Maleimide itself absorbs at a λ_{max} of around 273 nm, whereas the thiomaleimide absorbs at a λ_{max} of 339 nm and the dithiomaleimide at λ_{max} of 393 nm. An increase in extinction coefficients of the thiomaleimides compared to maleimide is also apparent from this figure, which in part explains the increased efficiency of their photocycloadditions.

In conclusion, thiomaleimides readily generated by the addition of thiols to bromomaleimides undergo highly efficient [2 + 2] photocycloadditions. This can be utilized to form dimers, or in the presence of suitable olefins such as styrene to afford the corresponding cyclobutanes. The bathocromic shift observed for thiomaleimides, along with the efficiency of the photochemical reactions, suggests these compounds will be more effectively employed in [2 + 2] photocycloadditions than simple maleimides. We envisage broad potential applications including photochemical bioconjugation, cross-linking studies, and surface modification.

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