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Click and photo-unclick chemistry of aminoacrylate for visible light-triggered drug release†

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"Click and Photo-unclick Chemistry" of aminoacrylates is proposed for a new photo-labile linker. Adducts are built in 2 steps with good yields and cleaved rapidly by tissue penetrable visible light (690 nm) with a photosensitizer. Facile synthesis, release of mother drug, and stability and cleavage in medium are demonstrated.

The use of light as an external signal is a very appealing tool for the spatio-temporal release of bio-active molecules. However, applications have been limited mostly to the cellular level due to the use of high energy UV light causing cellular damage and limited tissue penetration (<1 mm).[1] To apply this exciting tool in living animals, new strategies should be invented where active compounds can be released by the tissue penetrable low energy light (preferably, >650 nm).[2] Unfortunately, the energy of longer wavelength light is too low to directly initiate cleavage of most covalent bonds.

One clever approach has been proposed by taking advantage of the unique reaction of singlet oxygen that can be generated by the combination of photosensitizer and low energy light. Spontaneous cleavage of dioxetanes following 2+2 cycloaddition reactions of singlet oxygen with olefins has been explored for the site-specific release of bioactive molecules (Fig. 1). Electron rich heteroatom-substituted olefins, such as vinyl dithioethers, vinyl diethers and vinyl monoethers,

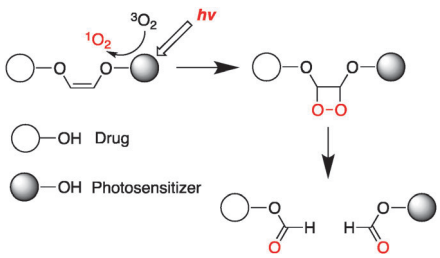

Fig. 1 [2+2] oxidation of vinyl diether by singlet oxygen, and subsequent cleavage of dioxetane for the release of drug.

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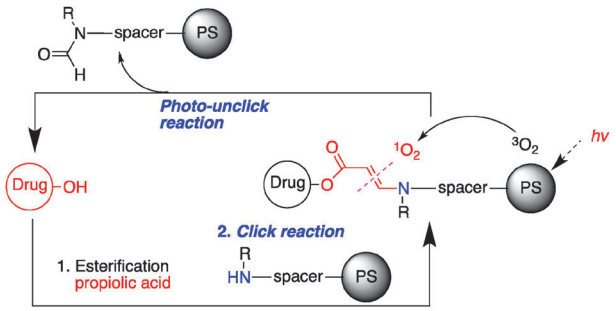

Fig. 2 Facile synthesis and cleavage of aminoacrylate; and release of a parent drug after its oxidative cleavage.

were incorporated in liposomes,[3] cyclodextrin dimers,[4] and prodrugs.[5] However, vinyl mono-ethers might not be the optimum choice for this application due to competition between the 2+2 cycloaddition reaction and the ene reaction.[6] On the other hand, the synthetic methods for vinyl diethers and dithioethers are very scarce and the available reaction conditions are not practical due to the low yield and non-stereospecificity.[7] Another concern with vinyl diether linkers is regeneration of the parent drug. Formyl groups at the cleaved products might attenuate the activity of the drug (*e.g.*, Drug–O–CHO). Here, we propose aminoacrylates (β-enamino esters) as a new linker for the "click and photo-unclick chemistry" for low energy light-controlled release of active compounds. Adducts can be easily synthesized, cleaved rapidly by visible light, and are stable in aqueous media (Fig. 2).

Due to the limitations of the previously investigated linkers, we started searching for new linkers and turned our attention to β-enamino ketones. In our previous screening, these linkers showed relatively fast photo-oxidation by singlet oxygen (64%, in 60 min).[6] Inspired by the oxidation rate of β-enamino ketone, we designed analogues of β-enamino esters (compounds **2** and **3**, Chart 1) as new linker candidates that could readily be prepared through high yield reactions (esterification and thiol–yne type reaction).[8] The esterification of 4-phenylphenol with propynoic acid was performed by the Steglich esterification with DCC and DMAP at 0 °C (to RT) to give biphenyl propiolate (**1**).[9] The thiol–yne type reaction of **1** with diethylamine or piperidine gave **2** and **3** in 89% and 80% yields, respectively, at RT in 10–15 min.

To examine the scope of the preparation and photo-oxidation, we then prepared analogues by replacing the nitrogen with

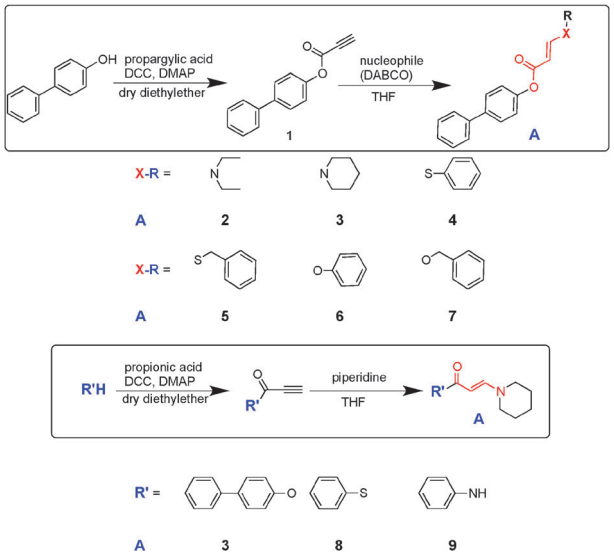

Chart 1 Reaction sequence and prepared substrates for heteroatom-acrylate, aminoacylthioate, and aminoacrylamide (**R′H**: 4-phenylphenol, thiophenol, or aniline).

sulfur or oxygen [thio-acrylate (**4**,**5**) and oxy-acrylate (**6**,**7**)], or the oxygen with nitrogen or sulfur [amino-acrylthioate (**8**) and aminoacrylamide (**9**)] (Chart 1). These compounds were rapidly prepared under mild reaction conditions (RT, air, 15–20 min), giving excellent yields for all substrates (80–95%). All the products from the click reaction step gave *E* isomers based on the coupling constant of the two olefinic protons, J = 12–15 Hz.

To evaluate the rate of oxidation by singlet oxygen, the model compounds **2–7** were irradiated by a diode laser (690 nm, 200 mW cm^{-2}, 25 min) in the presence of the photosensitizer 5-(4-methoxyphenyl)-10,15,20-tetraphenyl-21,23-dithiaporphyrin (Fig. S1 and Table S1, ESI†). The reaction of olefins with singlet oxygen was monitored by the decrease of olefinic proton peaks in the ^{1}H-NMR spectra. (*Z*)-1,2-Bis(phenylthio)ethylene was used as positive control. While **4–7** did not show any reactivity (0%), compounds **2** and **3** showed significant decrease of the olefinic proton peaks (62 and 72%) in 25 min of irradiation. Oxidation of only the nitrogen analogues is probably due to electron donation from the nitrogen to the carbonyl group making the olefinic bond electron rich for attack by singlet oxygen.

The aminoarylthioate (**8**) and aminoacrylamide (**9**) were evaluated under the same oxidation conditions. Interestingly, both **8** and **9** also showed fast reaction with singlet oxygen, 60 and 100%, respectively. Among aminoacrylate (**3**), aminoarylthioate (**8**), and aminoacrylamide (**9**), compound **9** showed the fastest reaction rate, 100% disappearance of olefinic protons in 25 min.

From the GC-MS analysis of the cleaved mixture of compound **8**, apart from the expected product, thiophenol, diphenyl disulfide was also detected with a number of minor side products. Diphenyl disulfide seemed to be formed during the GC-MS experimental procedure since it was also observed in GC-MS data of the thiophenol standard sample (ESI†). This conclusion was also supported by the fact that the doublet peak at 7.5 ppm from diphenyl disulfide was not observed in the ^{1}H-NMR of the cleaved mixture of **8**. Even though **9** showed the fastest reaction with singlet oxygen, it gave even more side products in GC-MS than **8** (ESI†). However, the aminoacrylate linker system (**3**) was selected for further investigation because it gave the clean product, 4-phenylphenol.

To examine if the cleavage was mediated by singlet oxygen, compound **3** was tested with a singlet oxygen quencher (1,4-diazabicyclo[2.2.2]octane, DABCO) (**3** and DABCO, Fig. S1, ESI†). It was observed that oxidation of the vinylic bond was greatly reduced (72% → 31%) suggesting the role of singlet oxygen.

One key requirement for delivery systems is re-generation of the active form of parent molecules after release. However, in the oxidative cleavage of vinyl diether linkers, two formyl products were produced which do not spontaneously decompose to give alcohol products (Fig. 1).[10] Interestingly, from the model compound **3**, we could recover 4-phenylphenol after the irradiation in addition to one amide product, 1-formyl piperidine. The two products were confirmed by GC-MS analysis (ESI†).

Since the aminoacrylates **2** and **3** showed a fast reaction with singlet oxygen (62 and 72%) at a comparable rate with the control (vinyl dithioether), we further designed model systems **10–13** with a spacer to accommodate two alcoholic model compounds (*e.g.*, 4-phenylphenol and phenol). All were prepared using high yield click reactions (84–90%). Compounds **11**, **12**, and **13** showed faster reaction than **10**, presumably due to the weaker electron withdrawing effect of the ester bond to the enamino group in **11** and **12** (Table S1, ESI†). Using the spacers of **12** and **13**, we tried to prepare prototypes (compounds **14** and **15**) having both the linker and a photosensitizer (**PS**) in one molecule. Prototypes **14** and **15** were successfully prepared but **15** gave a lower isolated yield (65%) due to the loss in the purification step. Indeed, both showed much faster oxidation reaction (89% in 10 min for **14**; and 79% in 15 min for **15**) than **12** and **13** even faster than the control (87% in 25 min).

As an example of aminoacrylate linker with a biologically relevant molecule, the model prodrug (compound **16**) was prepared from Estrone. It showed a photo-oxidation of 90% in 10 min similar to compound **14.** Compound **16** successfully released Estrone after irradiation (ESI†).

To examine photo-cleavage and stability of the linker in an aqueous solution (Dulbecco's Modified Eagle Medium with

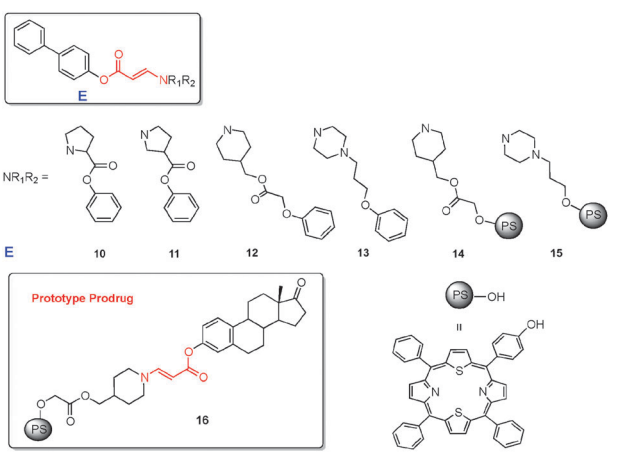

Chart 2 Prepared model substrates and the prototype prodrug.

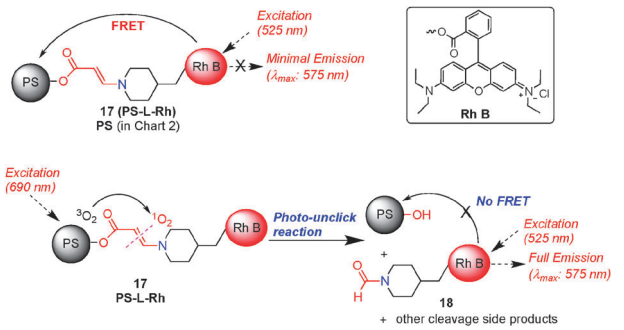

Fig. 3 Model compound, PS-L-Rh, for monitoring the cleavage of the linker using FRET.

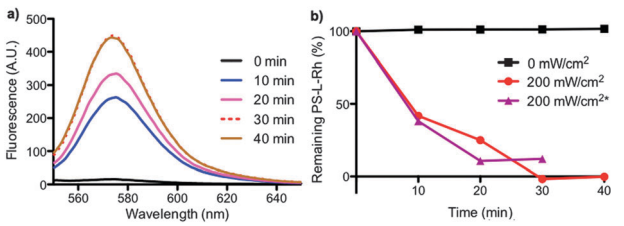

Fig. 4 Photocleavage of **17** in media: (a) fluorescence intensity (excitation at 525 nm) after the irradiation, (b) photocleavage of **17** with or without irradiation in media: ***17** kept for 7 days in the media in the dark before the experiment.

5% fetal bovine serum) using FRET (fluorescence resonance energy transfer), compound **17** (**PS-L-Rh**) was designed and prepared by conjugating two dyes [hydroxyl-dithiaporphyrin (PS, Chart 2) and rhodamine B (Rh B)] with the aminoacrylate linker (Fig. 3).[11] In **PS-L-Rh**, Rh B is a donor and PS (dithiaporphyrin) is an acceptor of the FRET. Fluorescence (λ_{em}: 575 nm, excitation at 525 nm) of the Rh group is quenched by the PS group when they are close *via* the linker. However, once the two dyes are apart after the cleavage of the linker, the fluorescence intensity of the Rh group increases dramatically since the FRET is not possible. Time-dependent increase of Rh emission upon irradiation (690 nm diode laser at 200 mW cm^{-2}) was first confirmed with **PS-L-Rh** in $CHCl_3$. Complete ($\sim$100%) cleavage was achieved in 10 min, giving about 8-fold increase in Rh fluorescence intensity. This rate is consistent with the cleavage data of **16** (in $CDCl_3$, 90% cleavage in 10 min) monitored by NMR (Fig. S1 and Table S1, ESI†). Compound **17** successfully released hydroxyl dithiaporphyrin, after irradiation (ESI†). The conversion yield from **17** to hydroxyl-dithiaporphyrin by the photo-unclick reaction seemed to be high (estimated by TLC, $>$80%). Then, **PS-L-Rh** in media was irradiated using the same irradiation conditions. It showed $\sim$100% cleavage in 30 min (Fig. 4). The slower cleavage in medium may be, in part, due to the lower concentration of oxygen (0.27 mM in media *vs.* 2.4 mM in $CHCl_3$ at atmospheric pressure)[12] and the shorter lifetime of singlet oxygen (2 μs in media *vs.* 60 μs in $CHCl_3$).[13]

The stability of the aminoacrylate linker of **PS-L-Rh** in media and $CHCl_3$ was investigated by monitoring the fluorescence emission (575 nm) of the Rh group excitation at 525 nm. Up to 7 days, no increase of the Rh emission was observed ($<\pm$ 12%). To ensure the intactness of the linker, the **PS-L-Rh** kept in the dark for 7 days was irradiated (690 nm diode laser at 200 mW cm^{-2}) and the fluorescence was determined. It showed similar kinetic data with those of the fresh sample (Fig. 4b), indicating that the aminoacrylate was stable in medium at least up to 7 days in the dark.

In conclusion, we proposed and proved the concept of "click and photo-unclick chemistry" using nucleophile-yne type reaction and photo-oxidative cleavage of electron-rich olefins using singlet oxygen. Among aminoacrylate, aminoacrylamide, and amioacrylthiolate, aminoacrylate seemed to be best suited for applications for the release of active compounds due to its fast photo-oxidation without unnecessary oxidation products. In addition, we proved that the aminoacrylate linker was cleaved rapidly by the irradiation of long wavelength visible light (690 nm) and was stable under dark conditions in the biological medium. This combination of click and photo-unclick chemistry would find important applications in the spatio-temporal release of not only drugs but also other bioactive molecules. Since the release can be triggered by tissue penetrable low energy light, this simple but unique chemistry will be applicable in the visible light-controlled release of biologically important molecules at the tissue level.

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