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A double branched photosensitive prodrug: synthesis and characterization of light triggered drug release

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ABSTRACT

A novel *o*NB based, double branched photosensitive prodrug **1**, and a biphenyl counterpart **2** were designed and synthesized. Their photo-triggered drug release properties were studied by HPLC and UV–vis spectra. The isobestic points in UV–vis spectra of prodrug **1** indicated that homogeneous photolysis reaction happened upon xenon-based light irradiation. HPLC analysis confirmed that (antitumor) chlorambucil was released quickly accompanied with photobyproducts. Prodrug **1** has a half-time ($t_{1/2}$) of 12.4 min, indicated a good sensitivity towards the light irradiation, making it a promising candidate for applications where UV light is limited.

Introduction

Photoremovable protecting groups (PPGs), either prompted by one-photon excitation or two-photon excitation, have attracted much attention in the area of drug delivery systems (DDS) in recent years.[1] The release of active drug molecules from PPGs irradiated by photons pave the way for effective DDS, as light can be used as extraneous non-physical contact stimulus with precise spatiotemporal control.[2]

Among the photolabile protecting platforms developed, the wavelength used to irradiate PPGs usually fall in the UV range, except for coumarin series (λ_{max} = 400–500 nm),[3] due to their intrinsic mono-aromatic ring system. This is a major drawback for their in vivo biological applications because (1): light scatting and tissue chromophores absorbance cause poor penetration depth and (2): long-term exposure to UV radiation is harmful to biological tissues.[4] Thus, there is an application-driven research for molecules sensitive to visible light or IR light as drug carriers in designing efficient photoresponsive DDS.[1b,5]

o-Nitrobenzyl (*o*NB) derivative, which was first introduced as photosensitive protecting group in 1970,[6] has gained much attention due to its well-studied photolysis mechanism.[7] It is also easy to be synthetically accessed and has efficient photosensitivity. It had become one of the most widely used PPGs. However, as mentioned above, the major bottle neck for its biological/pharmacological application is that the efficient release of caged molecule requires UV lights, which is detrimental to the organism. Molecular engineering has already been started by researchers to address this problem.[8] Two effective strategies employed to facilitate long wavelength absorption are the introduction of electro-donating and/or electro-withdrawing substitutes to promote the internal charge transfer (ICT), and the elongation of D–π–A backbone to increase the molar absorptivity.[9] On the other hand, it has to be emphasized that structural modification towards PPGs is not guaranteed to promote their photolytic performance, and in fact, can sometimes have a negative impact on their photochemical properties.[10] Jullien, Goeldner, Bolze and Zhu groups recently reported the design of donor–acceptor biphenyl *o*NB and styryl-2-nitrobenzyl (SNB) derivatives as PPGs, which showed better photosensitivities by either one-photon or two-photon irradiation compared with classical 4,5-dimethoxy-2-nitrobenzyl (DMNB) protecting group.[1b,8,11]

Therefore, to better understand the structure–property relationship for developing efficient photoremovable DDS, we designed and synthesized an *o*NB based photosensitive prodrug **1** (shown in Fig. 1), a double branched dipolar molecule containing two *o*NB moieties binding two chlorambucil molecules, and **2**, a monomeric counterpart.[11a]

The designed prodrug **1** has two D–π–A backbones constructed by di(ethylene glycol) fragments and opposite nitro groups bound together with the conjugated three aromatic rings. It is expected to

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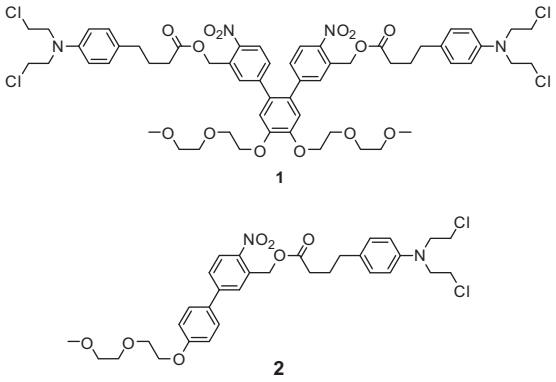
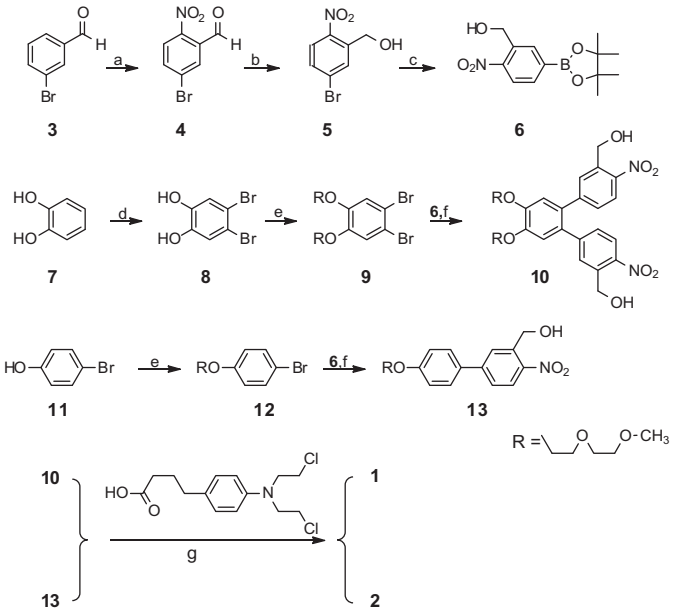


Figure 1. Structures of synthesized prodrugs.



Scheme 1. Synthesis of prodrugs. Reagents and conditions: (a) HNO_3, H_2SO_4, rt; (b) $NaBH_4$, MeOH, rt; (c) B_2pin_2, $PdCl_2(dppf)$, KOAc, toluene, 110 °C; (d) Br_2, chloroform, rt; (e) ROTs, K_2CO_3, DMSO, 80 °C; (f) $PdCl_2(dppf)$, Na_2CO_3, EtOH, toluene, 110 °C; (g) DCC, DMAP, rt.

increase electron delocalization as well as electron density, which are favourable for long wavelength absorbance and molar absorptivity enhancement, thus helping to achieve better photolytic performance. This letter also studied and presented the photochemical property upon xenon-based light irradiation of designed prodrugs.

Design and synthesis of prodrugs

Design

As drug vehicles, drug-loading capacity is an important parameter that has to be considered. Throughout literatures, peer to peer is the most commonly adopted mode for PPGs as drug carriers; usually one carrier molecule only binds one drug molecule due to its specified linker position, that is, the maximal payload capacity is 100%. In order to improve the payload capacity, combining two or more photolabile groups into one molecule seems to be a convenient approach. Bolze had reported a series of symmetrical PPGs for caging of two glutamate molecules, one of which (BNSF) shows 5 GM (1 GM = 10^{-50} cm^4 s $photon^{-1}$) of two-photon uncaging cross section at 800 nm.[12] Thus, to increase the drug-loading capacity as well as to retain good photosensitivity, a double branched prodrug **1** was presented in this Letter. The payload capacity, by virtue of the two *o*NB moieties that conjugated with a mutual benzene core, was consequently doubled.

Synthesis

The synthetic methods are outlined in Scheme 1. Commercially available 3-bromo benzaldehyde was nitrified to get compound **4** in the presence of nitric acid and sulfuric acid with an isomer. The mixture was not separated and was reduced directly by $NaBH_4$ in methanol to get **5** in 62% total yield. Aryl borate **6** was obtained by the palladium catalysed coupling of **5** and Bis(pinacolato)diboron (B_2pin_2) in 87% yield. Compound **8** came from the bromination of pyrocatechol by bromine in chloroform. Double alkylation of **8** afforded **9** in nearly quantitative yield. Suzuki coupling of **6** with **9** in the presence of $PdCl_2(dppf)$ and potassium acetate in toluene produced the key intermediate: double branched carrier **10** in 67% yield. Finally, prodrug **1** was obtained by the esterification of benzylic hydroxyl with chlorambucil using DCC/DMAP in 93% yield. Prodrug **2** was prepared in an analogous way in 95% yield.

Results and discussion

To evaluate the photoresponsive properties of the prodrugs, time-dependent UV–vis absorption spectra and HPLC analysis were employed. The absorption spectra of prodrugs are shown in Figure 2. Both prodrugs showed broad absorbance from ca. 280 nm to 430 nm. Interestingly, although molar extinction coefficient of prodrug **1** was larger than that of prodrug **2** (17.304 M^{-1} cm^{-1} vs 7.328 M^{-1} cm^{-1}) due to the larger conjugation system, its maximal absorption was located at 306 nm. While the maximal absorption of prodrug **2** was recorded at 342 nm, the 36 nm blue shift may be ascribed to the relatively rigid bulky molecular core of prodrug **1**, which was constructed by three closely conjugated aromatic rings.

Photo-induced drug release was carried out by exposing solutions of prodrugs in acetonitrile (10^{-6} M) to xenon-based light with an UV400 filter to cut off wavelengths below 400 nm. Due to the weak absorption of both prodrugs at wavelengths beyond 400 nm, the output power was increased to 190 mW cm^{-2}. As shown in Figure 3a, the initial maximal absorbance of prodrug **1**

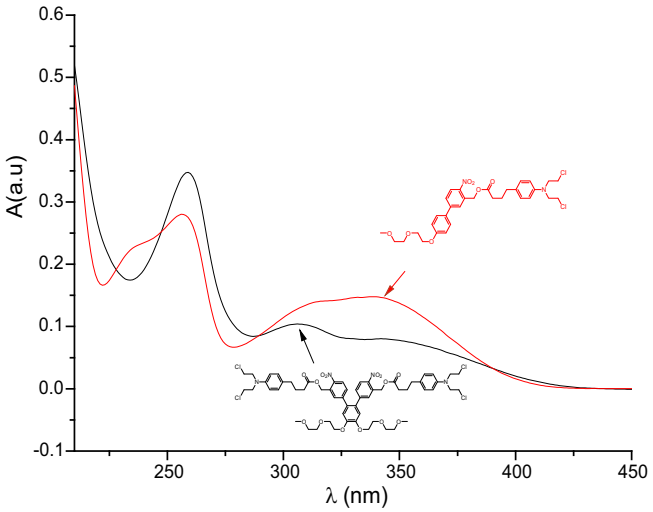


Figure 2. UV–vis spectra of prodrugs.

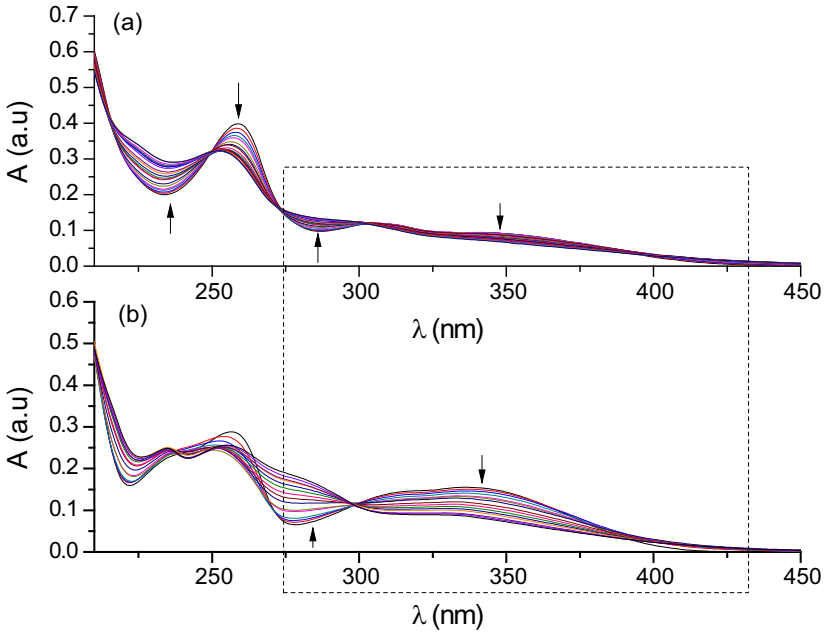


Figure 3. Evolution of UV–vis spectra of prodrug **1** (a), and prodrug **2** (b) upon irradiation ($\lambda \geqslant 400$ nm, 190 mW cm^{-2}) as a function of time from 0 min to 180 min and 0 min to 120 min, respectively. The dashed square shows the similar photolysis behaviours.

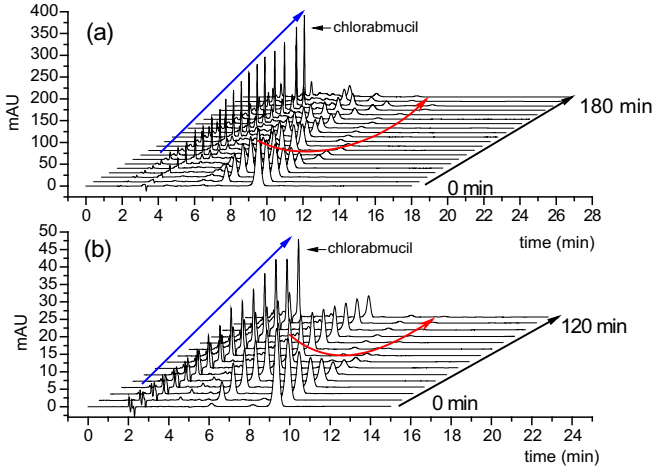


Figure 4. Evolution of HPLC spectra upon irradiation by the light of prodrug **1** (a), and prodrug **2** (b), respectively. The red arrow shows the decay of prodrug and the blue arrow shows the rise of the drug.

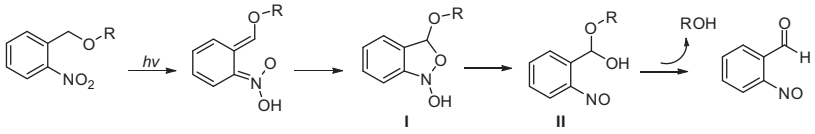
at 306 nm was gradually decreased, suggesting that the drug release was triggered and proceeded smoothly; the isobestic points appeared at 251 nm, 274 nm and 303 nm, which indicated that a homogeneous photolysis reaction occurred upon irradiation.[11e] As illustrated in the dashed square of Figure 3, the changes of UV–vis spectra of prodrug **1** and prodrug **2** were quite similar, indicating that they had similar photolysis behaviours.

The photolytic properties of prodrugs were further investigated by HPLC, which are shown by the results plotted in Figure 4. The photolytic release of prodrugs was confirmed by monitoring the evolution of component peaks. As depicted in Figure 4, the red arrows showed the consumption of prodrugs and the blue arrows showed the increase of drugs (chlorambucil). Both compounds showed good photosensitivity towards the light. As can be seen in spectra, prodrug peaks quickly declined and nearly disappeared within 60 min, while chlorambucil peaks emerged and grew rapidly.

Surprisingly, the decrease of prodrug peaks was not in line with the recorded increase of chlorambucil peaks; in other words, a lag effect was observed. The increase of chlorambucil was found to correlate with the applied irradiation time even though the prodrugs had vanished. It seems that before releasing of chlorambucil, the prodrug molecule was changed into an intermediate, which was subsequently decomposed into parent chlorambucil and a photobyproduct. This phenomenon might be explained by the photolytic mechanism of *o*NB based PPGs proposed by Wirz and coworkers:[13] (Scheme 2).

The formation of a cyclic benzisoxazoline intermediate I upon irradiation, which was irreversibly changed into hemiacetal II, followed by the release of a guest molecule to obtain 2-nitrosobenzaldehyde as byproduct. The existence of both I and II can be evidenced by time-resolved infrared (TRFR) spectra.

The photolytic behaviour of prodrug **1** was a bit more complicated than that of prodrug **2**, more than one of the photobyproducts were recorded in the HPLC spectra. This may be attributed to the unfinished photolysis procedure within the recording time, as there were two *o*NB moieties to be photolysed; moreover, the



Scheme 2. Proposed photolytic mechanism of *o*NB based prodrug.

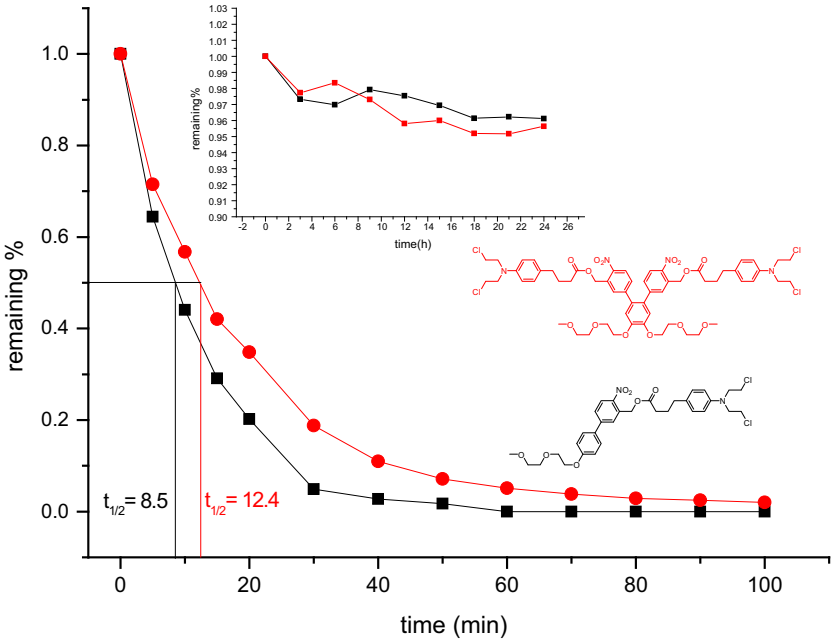


Figure 5. Change of remaining rates of prodrugs; inset shows the stability of prodrugs in acetonitrile solutions kept in the dark within 24 h, remaining rates of both prodrugs were larger than 95%.

internal filtering effect of byproducts can also cause incomplete photolysis.[1b]

Photo-induced drug release of prodrugs was studied by measuring the remaining rates of prodrugs in response to the light. The data plotted in Figure 5 displayed that both prodrugs had good photorelease efficiency. Although prodrug **1** contained two drug molecules, its half-time ($t_{1/2}$) upon irradiation was 12.4 min, compared with that of 8.5 min of prodrug **2**. Prodrug **1** also showed a good photosensitivity. Solution stability of prodrug **1** was investigated by keeping the acetonitrile solution in the dark, followed by measuring the remaining rate using HPLC at sequential time intervals. As shown in Figure 5 (inset), similar to prodrug **2**, the remaining rate of prodrug **1** was still greater than 95% even after being protected from light for 24 h, indicating a good stability as a photon responsive prodrug.

Conclusions

A doubled branched prodrug **1** and a counterpart molecule, prodrug **2** were synthesized and their photochemical properties were studied by UV–vis and HPLC analysis. The preliminary results showed that although the double branched prodrug **1** contained two *o*NB moieties, they did not interfere with each other and gave rise to negative impacts on photochemical properties. Prodrug **1** had good photosensitivity towards xenon-based light irradiation, which made it a promising candidate for applications where UV light is limited. Taking advantage of the two *o*NB moieties, the drug-loading capacity was doubled; moreover, we are also speculating that different drug molecules or one drug molecule plus a signal molecule can be delivered simultaneously to provide synergistic effect or to achieve active targeting ability, which is a challenging yet attractive objective for current therapeutic research.

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Supplementary data

Supplementary data (general procedures for HPLC and UV–vis measurements; experimental section including ^{1}H and ^{13}C NMR for new compounds **1**, **2**, **10** and **13**) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2016.01.064.

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