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Visible-light-induced cleavage of 4-α-amino acid substituted naphthalimides and its application in DNA photocleavage†

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A new kind of visible-light photocleavable molecule, 4-α-amino acid substituted naphthalimide, is reported. The cleavage occurred at the C–N bond between the 4-amino and the amino acid residue and released a 4-aminonaphthalimide. A lysine substituted naphthalimide exhibited a strong DNA photocleavage activity when irradiated with a blue light LED.

Organic molecules capable of releasing active moieties after light irradiation are termed photocages or photocleavable (photolabile, photoreleasable, photoremovable or photoactivatable) molecules. These molecules are extensively explored in biological applications, organic synthesis, and photolithographic techniques as tools for spatial and temporal control.[1] However, most of the photocleavable molecules require irradiation with short wavelength light (<400 nm),[1b] which limits their applications in living biosystems. Therefore, great efforts have been made to develop photocleavable chromophores that absorb visible light or near-IR light,[2] especially the photocleavable fluorophores that have the ability to act both as a "phototrigger" for active molecule release and a "fluorophore" for active molecule visualization. So far, only a few fluorophores have been modified as photocleavable groups under visible light, such as coumarin,[3] xanthene,[4] perylene[5] and organic–inorganic hybrid ruthenium compounds.[6]

Naphthalimide derivatives have been used in a wide variety of fields. As a class of DNA-intercalating agents, they have been extensively explored as antitumor agents.[7] Due to the electronic push–pull structure, 3- and 4-aminonaphthalimides show strong absorption and emission with a large Stokes shift in the visible region, and have been widely developed as fluorescent and colorimetric probes for detection of ions and bioactive molecules,[8] as well as for cellular imaging.[9] These probes are considered to have high photostability when irradiated at their absorption maxima.[9a,10] To the best of our knowledge, no photocleavable naphthalimide has been reported. Here, we report the photocleavage property of 4-α-amino acid substituted naphthalimides under visible light irradiation. A designed lysine substituted naphthalimide (DNLys) was demonstrated to have a strong DNA photocleavage activity when irradiated with a blue light LED (465–470 nm).

In our research, we observed that a lysine substituted naphthalimide, **1**, could be gradually cleaved under room light. Because 4-aminonaphthalimides are very stable under room light, we suspected that the photocleavage of **1** might relate to the α-carboxyl group of lysine. Therefore we synthesized seven 4-α-amino acid substituted naphthalimides (compounds **1–7**) and eight 4-amino substituted naphthalimides (without an α-carboxyl group) (compounds **8–15**) (Fig. 1). These 4-aminonaphthalimides were dissolved in ethanol, ethanol containing 1% trifluoroacetic acid (TFA) or ethanol containing 1% ammonia, put in glass bottles and then exposed to daylight for 2 h. The thin layer chromatography (TLC) assay showed that all the seven α-amino acid substituted naphthalimides were photocleaved (compared with lane a) in all the three solutions, however other 4-amino substituted naphthalimides were not changed except for compound **13** in 1% TFA (Fig. 1). It is worth noting that **13** (which contains a γ-carboxyl group) was photocleaved in a 1% TFA solution, but not under neutral and basic conditions (1% ammonia). This set of results suggests that 4-α-amino acid substituted naphthalimides are highly photolabile.

To understand the mechanism of the photocleavage of 4-α-amino acid substituted naphthalimides, we compared the photocleaved fluorescent products of **1** and **2** after irradiation in an ethanol solution by HPLC with detection at 430 nm (Fig. S1†). The HPLC results showed that the photocleaved fluorescent products of both compounds were identical. The ^{1}H NMR and ^{13}C-NMR spectra of the photocleaved fluorescent

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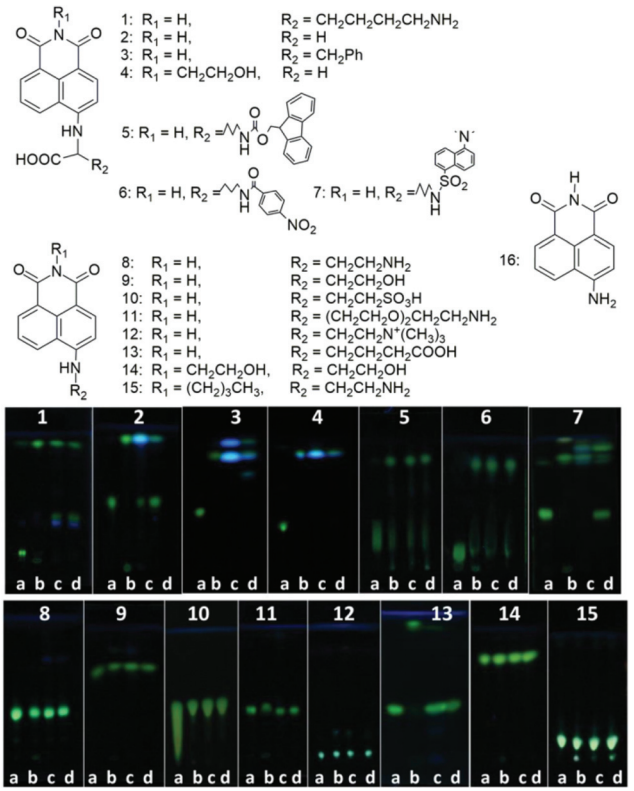

Fig. 1 Photocleavage of 4-amino substituted naphthalimides. Top: structures of 4-amino substituted naphthalimides; **1–7**: substituted by α-amino acids; **8–15**: substituted by other amino compounds. Bottom: TLC analysis of 4-amino substituted naphthalimides (**1–15**) after exposure to daylight for 2 h; lane a: without irradiation; lane b: irradiated in 1% TFA; lane c: irradiated in ethanol; lane d: irradiated in 1% ammonia.

product (Fig. S2†) only showed ^{1}H signals higher than 6.5 ppm and ^{13}C signals higher than 105 ppm, suggesting that no saturated alkyl was linked on the product. The EI-MS showed the molecular ion peak at *m*/*z* 212, which corresponded to compound **16** (Fig. 1).

The other part of the photocleaved products of 4-α-amino acid substituted naphthalimides was investigated with compound **6**, because the nitrobenzoyl moiety of **6** can be monitored using a UV detector (270 nm). HPLC analysis showed that a peak (15 min) of the photocleaved product only with the UV absorption at 270 nm (without absorption at 430 nm) was observed (Fig. S3†). However, this product was unstable, and changed to many other compounds during purification (Fig. S4 and S5†). In the ESI-MS analysis of the reaction mixture of photocleaved **6**, we found one group of molecules with molecular weights of 296, 280, 264, 250, and 232, and the MS/MS analysis of these molecules (296, 280, 264 and 250) showed fragment ions of 233 and 150, which suggests a series of molecules containing the nitrobenzoyl moiety (Fig. S6†).

Based on the structure of 4-α-amino acid substituted naphthalimides, the formation of a five-membered-ring intramolecular hydrogen-bond between the α-carboxyl group and the amino group may be critical for the photocleavage of these 4-α-amino acid substituted naphthalimides, which can explain

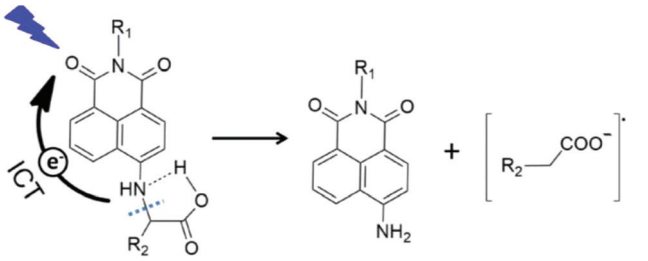

Fig. 2 The photocleavage of 4-α-amino acid substituted naphthalimides.

why other 4-amino-1,8-naphthalimides (without an α-carboxyl group) cannot be photocleaved (Fig. 2). 4-Amino-1,8-naphthalimides are known to be intramolecular charge transfer (ICT) fluorophores with "push–pull" substituent pairs (electron donor/acceptor).[8] The photochemical excitation leads to the ICT from the 4-amino group to the excited imide, resulting in the cleavage of the C–N bond of the α-amino acid and the intramolecular hydrogen transfer from the α-carboxyl group to the amino group to produce compound **16** and a radical of the amino acid residue. Subsequently the radial may react with the surrounding molecules and/or further decompose to form various products.

Naphthalimides represent an important class of DNA binders that have been extensively explored as antitumor agents, and a few of them have been shown to exhibit the activity of photoinduced DNA cleavage under UV light irradiation.[11,12] It is well known that UV-light can cause DNA damage. The above results have shown the photocleavage property of 4-α-amino acid substituted naphthalimides, this property may endow them with the ability to photocleave DNA. To prove this hypothesis, we synthesized a lysine substituted naphthalimide, DNLys (*N*-dimethylaminopropyl-4-(1-carboxyl-5-amino-amylamino)-1,8-naphthalimide), and its photocleaved product, DNNH (*N*-dimethylaminopropyl-4-amino-1,8-naphthalimide) (Fig. 3 and S7†). The introduction of the dimethylaminopropyl group and lysine is to increase the affinity of naphthalimide to DNA, because the amino groups bear positive charges under physiological conditions[9d] and can enhance the DNA cleavage efficiency of the light-activated DNA cleaver.[13] The absorption and emission spectra of DNLys and DNNH showed that the maximum absorption was in the range of 435–445 nm (Fig. S8†), and the maximum emission was in the range of 545–550 nm (Fig. S9†). The addition of DNA slightly decreased their absorption and slightly increased their emission.

The photocleavage experiment of DNLys was performed under a LED array (3 W; 465–470 nm). 82% of DNLys was observed to be cleaved to DNNH after exposure to blue light in methanol for 1 h (Fig. S10†). However, the photocleavage of DNLys in phosphate buffered saline (PBS, pH 7.4) was much slower than that in methanol (Fig. 3), only 23% of DNLys was cleaved in 4 h. It is interesting that the addition of DNA could accelerate the photocleavage of DNLys in PBS, and 19%, 32% and 63% of it were cleaved in 1, 2 and 4 h (Fig. 3). These

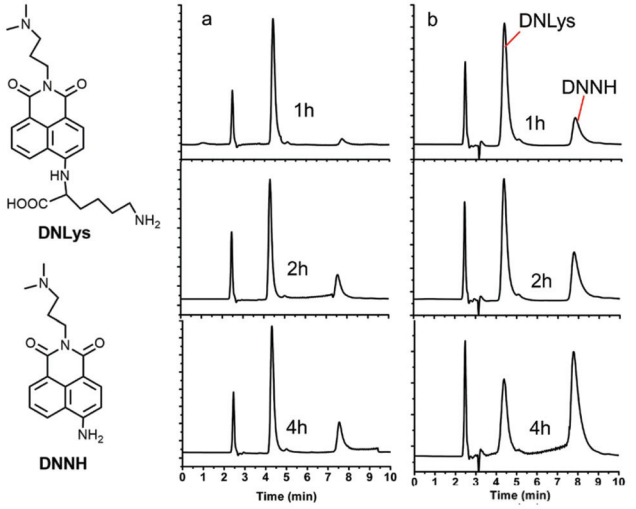

Fig. 3 The structure of DNLys/DNNH and HPLC analysis of the photocleaved DNLys (200 μM). (a) Photocleaved in pH 7.4 PBS; (b) photocleaved in pH 7.4 PBS in the presence of CT-DNA (100 μM in base pairs). Samples were irradiated under 465–470 nm LED light for 1, 2, and 4 h at room temperature, and then applied for the RP-HPLC assay, mobile phase: methanol–H_2O (0.1% TFA), 4 : 6; detected at 245 nm.

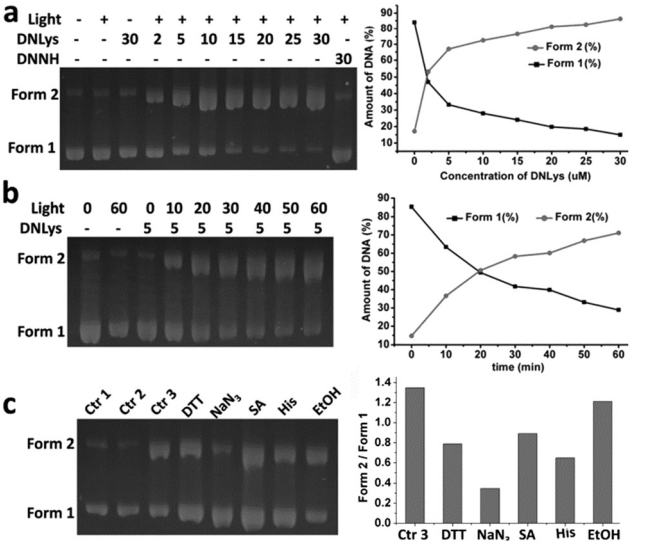

Fig. 4 Cleavage of closed supercoiled pBR322 DNA (0.5 μg/20 μL) irradiated under blue LED light at room temperature. a. (left) Agarose gel assay of DNA cleaved at different concentrations (μM) of DNLys or DNNH after 0.5 h irradiation; (right) quantified plots of DNA as a function of DNLys concentration. b. (left) Gel assay of DNA cleaved by DNLys (5 μM) after irradiation for different times (min); (right) quantified plots of DNA as a function of irradiation time. c. Gel (left) and quantified (right) assay of effects of additives on DNA cleavage by DNLys (5 μM) after 0.5 h irradiation, Ctr 1: DNA only without irradiation; Ctr 2: DNA only; Ctr 3: DNA + DNLys; additives: DTT (50 mM); NaN_3 (100 mM); SA (salicylic acid, 20 mM); His (histidine, 5 mM); EtOH (ethanol, 2 M).

results suggest that the interaction of DNA and DNLys could promote the photocleavage of DNLys and implied the feasibility for DNA cleavage. These results further confirm that 4-α-amino acid substituted naphthalimides can be photocleaved by visible light and produce stable fluorescent 4-amino naphthalimides.

The DNA photocleavage experiments were performed using a closed supercoiled pBR322 DNA under irradiation with a blue LED at room temperature. The cleaved DNA was analyzed on 1% agarose gel. After cleavage, supercoiled pBR322 DNA (form I) would convert to relaxed circular DNA (form II). As shown in Fig. 4a, DNLys did not cleave DNA without irradiation. After irradiation for 0.5 h, DNA cleavage was observed in the presence of different concentrations of DNLys, the cleavage efficiency increased with the increase of DNLys concentration. 2–5 μM DNLys could cause most of the DNA to be cleaved under irradiation. As expected, 30 μM DNNH (photocleavage product of DNLys) did not cause notable DNA cleavage under the same conditions. The kinetics of DNA photocleavage showed that the intact DNA greatly decreased with the irradiation time, and most of the plasmid DNA was consumed within 30 min of irradiation (Fig. 4b). The control experiments showed that very little DNA damage was observed in the absence of DNLys even after irradiation for 60 min. Since DNNH also undergo the photo-induced ICT process, these results suggest that the DNA cleavage was related to the photocleavage of DNLys, and not to the ICT excited state of 4-aminonaphthalimides.

The photoinduced DNA damage by synthetic DNA cleavage agents usually involves the reactive oxygen species, such as hydroxyl radicals, singlet oxygen and superoxide anions.[14] In order to determine whether the reactive oxygen species are responsible for DNA cleavage by light-activated DNLys, standard scavengers and inhibitors of reactive oxygen species were added respectively to the reaction system, *i.e.* singlet oxygen scavengers (sodium azide (NaN_3) and histidine (His)), superoxide radical scavenger (dithiothreitol (DTT)), and hydroxyl radical scavengers (salicylic acid (SA) and ethanol (EtOH)). As shown in Fig. 4c, a certain inhibition effect of all the test scavengers was observed. Among them, the singlet oxygen scavenger, NaN_3 and histidine showed the strongest and the second strongest inhibition effect on the DNA cleavage. DNA photocleavage is usually caused by reactive oxygen species or the direct reaction with the light-activated DNA cleaver.[13b–d] This set of results suggests that the DNA photocleavage in the presence of DNLys may involve the reactive oxygen species, especially the singlet oxygen.[15]

The preceding results have shown that the DNA cleavage was only caused by the photocleavage of DNLys, and not caused by the irradiated DNNH, suggesting that the singlet oxygen was not generated from the photosensitized 4-aminonaphthalimide. The photocleavage of DNLys produced a radical of amino acid residues, thus the singlet oxygen may be generated by the reaction of dissolved oxygen with the excited radical states. Even so, we could not exclude the direct mechanism of DNA damage by the excited radical states because of the high activity of the radical. Further research will focus on the mechanistic details of photocleavage of 4-α-amino acid

substituted naphthalimides, as well as of the DNA cleavage by the irradiated DNLys.

The above results showed that 4-α-amino acid substituted naphthalimides could be photoactivated with blue light and release a fluorescent product, 4-amino naphthalimide. The 4-α-amino acid substituted naphthalimides can be easily synthesized by substitution reaction of 4-bromine-1,8-naphthalimides with different α-amino acids. Furthermore, through the reaction with the amino acid residues, the 4-α-amino acid substituted naphthalimides (*e.g.* lysine and glutamic acid substituted) could be further linked to peptides, proteins and other biologically interesting molecules. In addition, the substituent at the N-atom at the 9-position of naphthalimides (imide N-atom) can be easily changed to different molecules by reaction of 4-bromine-1,8-naphthalanhydride with the corresponding compounds containing the NH_2 group. Therefore the 4-α-amino acid substituted naphthalimide fluorophore could act as a multifunctional platform for the construction of light-control systems by linking different functional molecules. The visible light activation makes the constructed systems hold the potential in biological applications, *e.g.* spatial and temporal control of drug delivery. The fluorescence emission of 4-α-amino acid substituted naphthalimides and their photocleaved products makes the constructed systems work in visualization. The photoactivation of DNLys showed the photocleavage ability towards DNA, suggesting its potential application in phototherapy after further modifying the 4-α-amino acid substituted naphthalimides to enhance the DNA binding ability.

Conclusions

In summary, we describe the photocleavage property of 4-α-amino acid substituted naphthalimides upon blue light irradiation. The photocleavage of these molecules occurred at the C–N bond between 4-amino and the amino acid residue and released a fluorescent product, 4-aminonaphthalimide. DNA was found to enhance the photocleavage of a lysine-substituted naphthalimide (DNLys), which caused the cleavage of DNA. Because of the ease of the modification on the 4-position and 9-position of the naphthalimide ring, this finding provided a multifunctional platform for the construction of light-control systems.

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