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Synthesis and DNA damaging ability of enediyne model compounds possessing photo-triggering devices

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Abstract—Enediyne model compounds possessing photo-triggering devices were developed. These enediynes afforded biradicals by UV irradiation and showed DNA cleaving activity. The DNA damage was confirmed to be mainly caused by the biradical, not singlet oxygen.

For many decades, DNA damage induced by photo-irradiation has attracted considerable attention and several types of DNA photocleavers have been exploited. Since the photochemical processes of these agents are generally compatible with other biological processes, they have significant potential as biological probes of DNA, and drugs for photochemotherapy in which ultraviolet, visible, and infrared lights are used together with administered photoactive agents.[1] Now with the recent rapid progress of laser and fiber technology it is becoming possible to introduce an intense beam of these radiations into the depths of the human body and the importance of photochemotherapy has been correspondingly increasing.[2] Since Shiraki and Sugiura reported the photocleavage of DNA by *Dynemicin A* in 1990, this new class of photocleaver has attracted a great deal of attention from researchers, and several enediyne model compounds bearing photo-triggering devices have been developed.[3] In the course of our studies for developing enediyne model compounds generating bioactive carbon biradicals through a characteristic triggering action,[4] we have also investigated the design and synthesis of enediynes activated photochemically. Here, we report the synthesis and DNA cleaving abilities of the enediyne model compounds **1**, **2a**, and **2b** that we designed, activated by photo-irradiation and, by comparison with reference compounds **3** and **4**, we also confirm that the DNA damage was mainly caused by the biradical. The molecular design of targeting arenediynes is depicted in Figure 1. Photochemical removal of the protecting group on the cyanohydrin moiety initiates the reaction cascade including regeneration of the carbonyl group and isomerization to an arenyne–allene, which cycloaromatizes to generate the α,3-didehydrotoluene biradical.[5]

Initially, the syntheses of the alkynes **9a** and **9b** were undertaken as shown in Scheme 1. Hydroxy ester **5**[6] was reacted with 2-nitrobenzyl chloromethyl ether[7] giving acetal **6**, which was treated with aq NH_3 in EtOH affording amide and the following dehydration reaction using Burgess reagent[8] gave nitrile **9a**. Carbonate **9b** was also synthesized from ester **5**. THP protection of **5** afforded acetal **7**, which was converted to nitrile **8** in two steps. The removal of the THP protection followed by the introduction of the carbonate moiety using *p*-nitrophenyl 2-(2-nitrophenyl)propyl carbonate[9] afforded **9b**. Diiodobenzene was coupled with methyl propargyl ether in the presence of Pd catalyst affording arenyne **10**, and the sequential coupling reaction of **10** with **9a** or **9b** under the Sonogashira conditions gave enediynes **1** and **2a**. Enediyne **2b** was also synthesized from **11** in a similar fashion to the synthesis of **2a**.

First, we examined the cycloaromatization reactions of enediynes **1**, **2a**, and **2b** in the presence of 1.1 equiv of Et_3N and excess amounts (50 equiv) of 1,4-cyclohexadiene under UV irradiation ($\lambda > 365$ nm) in degassed MeOH. The results are summarized in Table 1. While carbonate **2a** and **2b** were photolyzed completely within 30 min, a considerably longer reaction time (0.5 h vs 17 h) was needed for the deprotection of acetal **1**.[9b] In

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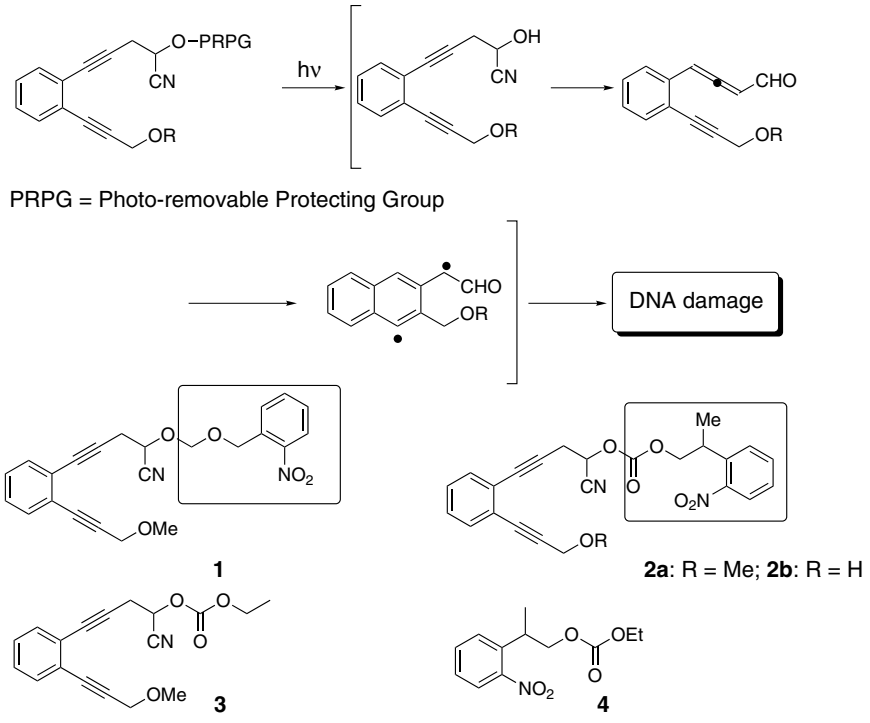
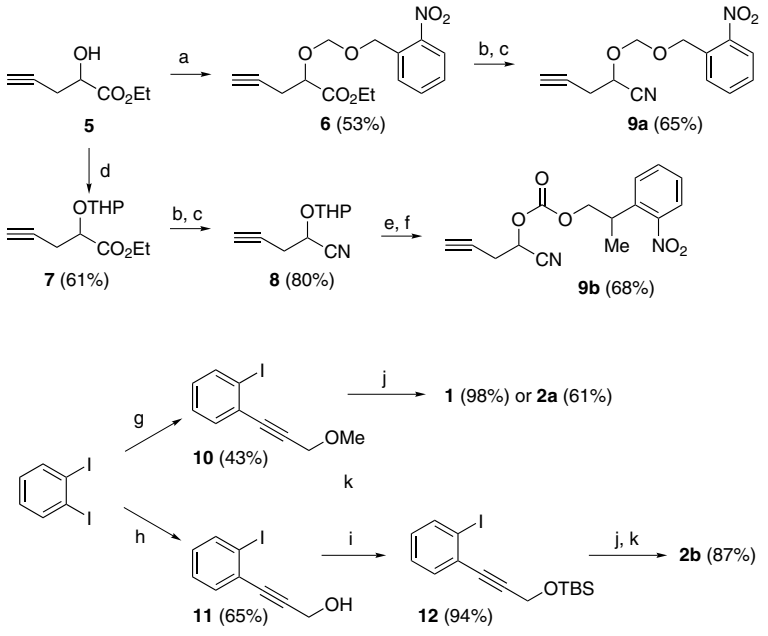


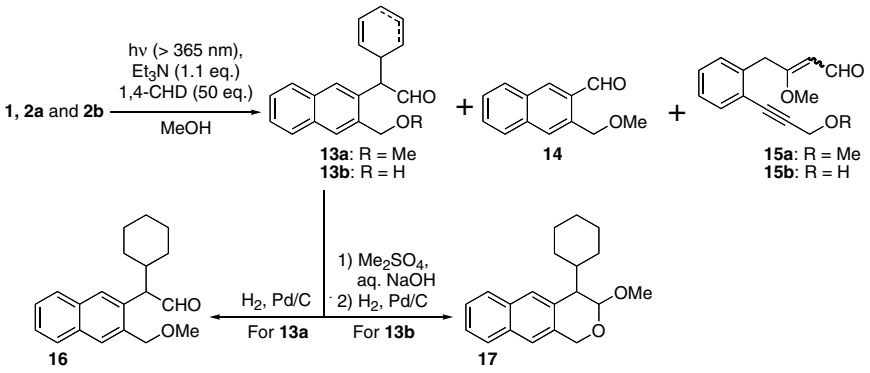
Figure 1.



Scheme 1. Reagents and conditions: (a) 2-Nitrobenzyl chloromethyl ether, NaH/THF, rt. (b) aq NH_3/EtOH, rt. (c) $Et_3N^+SO_2N^-CO_2Me$/THF, rt. (d) DHP, *p*-TsOH/C_6H_6, rt. (e) Amberlyst 15/MeOH, rt. (f) *p*-Nitrophenyl 2-(2-nitrophenyl)propyl carbonate, Py/CH_2Cl_2, rt. (g) Methyl propargyl ether, $Pd(PPh_3)_4$, CuI, Et_3N/toulene, rt. (h) 2-Propyn-1-ol, $Pd(PPh_3)_4$, CuI, Et_3N/toulene, rt. (i) TBSCl, imidazole/CH_2Cl_2, rt. (j) **9a** (or **9b**), $Pd(PPh_3)_4$, CuI, Et_3N/toulene, rt. (k) aq HF/MeCN, 0 °C.

both enediynes **1** and **2a**, the reactions proceeded, giving the products **13a**, **14**, and **15a** accompanied by considerable amounts of unknown polar materials (entries 1 and 2). Adduct **13a** was obtained as an isomeric mixture and then was converted to **16** by catalytic hydrogenation to confirm the structure. In the cycloaromatization reactions of **2b**, **13b** was obtained along with the trace amounts of **15b** (entry 3). **13b** and **15b** were converted to **17** and **15a**, respectively, to confirm the structure. To exclude any possibility that the cascade reaction was initiated by the base promoted deprotection of **2**, we prepared reference enediyne **3**, bearing a photostable protecting group. When cycloaromatization of the reference enediyne **3** was carried out under the same

Table 1. The cycloaromatization reactions of enediynes **1** and **2**



Entry	Enediyne	Time (h)	Products
1	**1**	17	**13a**[a] (8%), **14** (9%), **15a** (16%)
2	**2a**	0.5	**13a**[a] (2%), **14** (3%), **15a** (9%)
3	**2b**	0.5	**13b**[b] (6%), **15b**[c] (trace)
4	**3**	0.5	No reactions

Enediynes, Et_3N (1.1 equiv), and 1,4-cyclohexadiene (50 equiv) were dissolved in MeOH and photo-irradiated using a MUV-202U (MORITEX, 2000 mW cm^{-2}, $\lambda > 365$ nm) for the indicated times.

[a] Compound **13a** was obtained as an isomeric mixture and then was converted to **16** by catalytic hydrogenation to confirm the structure.

[b] Compound **13b** was converted to **17** in two steps and the structure was confirmed.

[c] The structure of **15b** was confirmed by converting to **15a** using Me_2SO_4 and aq NaOH.

conditions, enediyne **3** was recovered almost completely (entry 4). These results indicate that the cascade reaction was initiated photochemically to form the α,3-didehydrotoluene biradical. In addition, the enediyne skeleton itself might undergo a photo-Bergman cyclization reaction,[3d,g] however, in our enediyne systems, no product arising from Bergman cycloaromatization was obtained.

A plausible mechanism of the formation of **13**–**15** is depicted in Figure 2. Photo-irradiation initiates the reaction cascade, consisting of the deprotection, isomerization, and cycloaromatization processes, giving the biradical. The resulting biradical abstracts hydrogen from 1,4-CHD, and finally should afford **13**. The biradical also reacts with adventitious oxygen to give a peroxide, which would decompose to aldehyde **14**.[10] In addition, the intermediary arenyne–allene degrades with the conjugate addition of solvent MeOH affording **15**.

Next, we carried out DNA cleaving assays of synthetic enediynes. Enediynes **2a** and **2b** were incubated with ΦX174R-1 plasmid DNA in a phosphate buffer solution (pH 8.0, containing 20% of acetonitrile) and the mixture was irradiated ($\lambda > 365$ nm). The resultant DNA fragments were separated by electrophoresis on agarose gel and visualized by ethidium bromide staining: the results are summarized in Table 2. Prolonged irradiation time (30 min vs 60 min) and elevated temperature (25 °C vs 37 °C) were effective in improving the DNA damaging abilities of **2b** (entries 4–6), however, in the case of **2a**, these modifications were not so effective (entries 1–3). Although **2a** and **2b** showed a similar degree of facility in the deprotection step (Table 1, entries 2 and 3) and disappeared almost completely after the irradiation, **2a** showed only low activity compared with **2b**. This difference would be attributed to the low solubility of **2a** in the buffer solution. Indeed, enediyne **2b** could dissolve

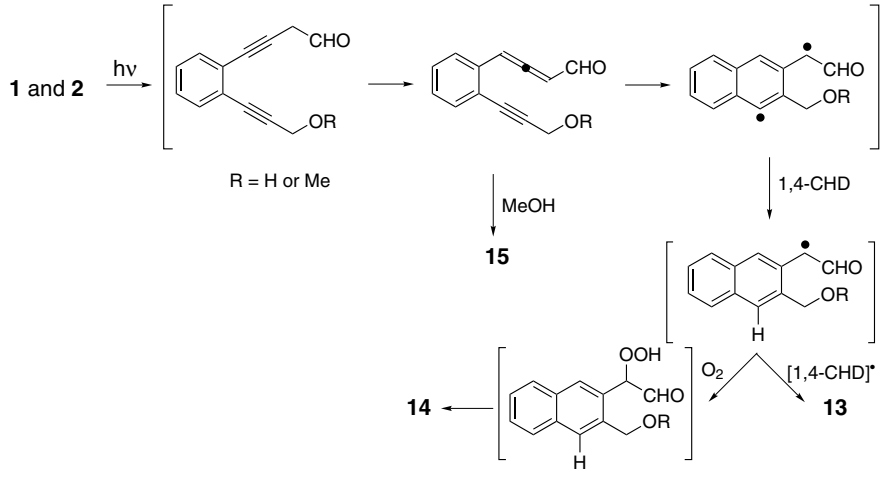


Figure 2.

Table 2. DNA cleaving abilities of synthetic enediynes **1**–**3** and the reference **4**

Entry	Enediyne	Time (min)	Temperature (°C)	% Cleavage[b,c]
1	**2a**[a]	30	25	11 ± 3
2	**2a**[a]	60	25	12 ± 4
3	**2a**[a]	30	37	15 ± 4
4	**2b**	30	25	27 ± 4
5	**2b**	60	25	40 ± 3
6	**2b**	30	37	47 ± 7
7	**3**	30	25	<5
8	**4**	30	25	11 ± 2

ΦX174R-1 DNA (0.46 μg, WAKO Pure Chemical Industries, Ltd) in 20 μL phosphate buffer solution (pH 8.0, containing 20% acetonitrile) with drugs (500 μM) was incubated and photo-irradiated using a VL-30L (VILBER LOURMAT, 1820 μW cm^{-2}, $\lambda > 365$ nm) for indicated times at 25 or 37 °C. Immediately, 15 μL samples were loaded into 1% agarose gel. The running buffer was 20 mM TAE (pH 7.8). Electrophoresis was at 50 V for 8 h. After electrophoresis, gel was stained for 1 h in ethidium bromide (1 μg/mL) and de-stained for 5 min in water. Relative amounts of DNA in Form I, Form II, and Form III were determined by densitometry.

[a] Enediyne **2a** was suspended in phosphate buffer solution.

[b] Values presented are mean value ± SD of three runs. A control reaction mixture without the addition of any drug was incubated and photo-irradiated. The mean value of three runs was used as the background to be subtracted from the obtained values.

[c] No isolable product could be detected on TLC from the reaction mixtures.

into a buffer solution up to a concentration of 500 μM, while **2a** did not dissolve completely even below 100 μM, becoming a suspension. The observed DNA cleavage could be caused by the biradical through the photo-triggered cycloaromatization reaction, however, the possibility of cleavage being due to the singlet oxygen that was yielded by the photosensitization of a nitrophenyl moiety could not be excluded. To clarify this point, we performed a DNA cleaving assay of the reference compounds **4** under the same conditions. Although the reference carbonate **4** damaged DNA to some extent, the degree of cleavage was around 10% (entry 8) and therefore the potent DNA cleavage exhibited by enediyne **2b** should be mainly attributed to the oxidative damage caused by the photoreleased biradicals.[11]

In conclusion, we developed enediyne model compounds bearing photo-triggering devices and showed that these synthetic enediynes exhibited potent DNA damaging activity under UV irradiation conditions. We also clarified that the biradical formation played the important role in the cleavage of DNA in our systems.[12] Further improvement of the model compounds is now underway.

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11. Mechanistically, the σ-radical part of the biradical instead of the resonance-stabilized π-radical part would be responsible for this DNA scission, although the alkylation pathway cannot be ruled out.
12. Since the DNA damage caused by **2a** was of the similar degree to the case of **4**, we cannot attribute the DNA damage to only the biradical.