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Synthesis and DNA cleavage reaction characteristics of enediyne prodrugs activated via an allylic rearrangement by base or UV irradiation☆

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Abstract—A number of enediyne prodrugs **1**–**5** possessing an (*E*)-3-hydroxy-4-(2′-hydroxy-1′-phenylethylidene)cyclodeca-1,5-diyne scaffold have been synthesized via the Sonogashira coupling and an intramolecular Nozaki–Hiyama–Kishi reaction as the key steps. Upon incubation with enediyne prodrugs **4** and **5** possessing a free hydroxymethyl group on the exocyclic double bond, circular supercoiled DNA (Form I) underwent single strand cleavage into circular relaxed DNA (Form II) in buffer solution at pH 8.5, while the silylated analogs **1**–**3** showed very weak DNA cleavage activity. Alternatively, the silylated analogs **1**–**3** could be activated by UV irradiation via a photochemical alkene isomerization followed by an allylic rearrangement to form the putative epoxy enediyne, resulting in efficient DNA cleavage similar to the level observed with the prodrugs **4** and **5**.

1. Introduction

Naturally occurring enediyne antitumor antibiotics[1,2] possessing either a 9- or 10-membered ring enediyne core structure have shown potent antitumor activity that was believed to result from DNA cleavage.[3,4] The cyclic enediyne core could be activated toward cycloaromatization under physiological conditions, furnishing a 1,4-benzenoid diradical species that abstracts hydrogen atoms from DNA backbone to induce DNA strand scission responsible for lethal damages of cells. More recently, antitumor activity of enediyne antibiotics has been demonstrated to be attributable to not only DNA cleavage but also RNA cleavage, protein agglomeration, and apoptosis.[5]

After disclosure of the mechanism of DNA cleavage by a family of naturally occurring enediynes, a great number of efforts were devoted to design and synthesize various types of enediyne-mimic compounds.[6] The most importance in designing enediyne model compounds is how to regulate the reactivity using a simple triggering device, by which a stable precursor can be transformed in situ to a highly reactive enediyne ready for cycloaromatization under physiological conditions. A large number of activation strategies have been hitherto proposed, involving the formation of enyne–allenes or enyne–cumulenes via nucleophilic attack, photolysis or elimination of a substituent.[7]

In our previous studies,[8] we have established methodologies of generating enediynes in situ via rearrangement of an allylic double bond under neutral,[9] acidic,[10] and lanthanide (III) catalysis[11,12] conditions, respectively. We also have developed three synthetic routes to the 10-membered ring enediyne precursors: (a) intramolecular acetylide addition toward aldehyde;[10c] (b) intramolecular Sonogashira cross-coupling reaction;[13] and (c) intramolecular Nozaki–Hiyama–Kishi reaction.[12b,c] The precursors prepared by these synthetic routes were confirmed to be converted into 10-membered ring

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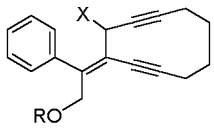
enediynes via an allylic cation intermediate in buffer solution.[14] A more remarkable result was that introduction into enediyne precursors with a neighboring nucleophilic group could assist in the rearrangement of allylic esters to cyclic enediynes. These enediyne precursors would represent a synthetic model system that mimics the intramolecular allylic rearrangement involved in the activation of maduropeptin chromophore artifacts.[8]

As an extension of our work on the development of antitumor prodrugs through activation into enediyne formation, we synthesized the enediyne prodrugs **1**–**5** (Fig. 1) possessing an exocyclic 2′-hydroxy-1′-phenylethylidene unit and evaluated their cytotoxicity and DNA cleavage potency. Some results on the synthesis and cytotoxicity were communicated in a short letter before[15] and the details on synthesis and DNA cleavage reactions are reported here. Among the enediyne prodrugs synthesized, compounds **4** and **5** possessing a free hydroxy group showed potent DNA cleavage activity, which was induced by spontaneous allylic rearrangement in basic buffer solutions, while the silyl ether-protected analogs **1**–**3** could be activated by photoirradiation to undergo an alkene isomerization–allylic rearrangement process, resulting in efficient DNA cleavage reaction as observed for the enediyne prodrugs **4** and **5**.

2. Results and discussion

2.1. Chemistry

Synthesis of the enediyne prodrugs **1**–**5** from the commercially available starting material, 2-hydroxyacetophenone (**6**), is outlined in Scheme 1. Following protection of the hydroxyl group of **6**, the resultant silyl ether **7** was subjected to a Horner–Wadsworth–Emmons reaction to give the unsaturated ester **8** in a quantitative yield as a 40:60 mixture of *E* and *Z* isomers. After column chromatographic separation, the pure (*E*)-**8** was treated with bromine to form a dibromo adduct, which, without further purification, was subjected to a base-induced elimination of HBr to give the vinyl bromide **9** as a 70:30 mixture of *E* and *Z* isomers. Unfortunately, (*Z*)-**8** could not be transformed into **9** with recovery of the starting materials quantitatively. Our initial attempts were made to carry out the cross-coupling of **9** with 1,7-octadiyne under the catalysis of Pd(0) and Cu(I). However, a mixture of the desired cross-coupling product and the debromination by-product **8** (as *E* and *Z* mixtures) was obtained from the pure (*E*)-**9** in 14–38% combined yields in THF, MeCN or DMF in the presence of Et_3N or K_2CO_3 at 22–80 °C.[17] On the other hand, the cross-coupling of the minor isomer (*Z*)-**9** with 1,7-octadiyne under similar conditions afforded the desired product in 54–75% yields, which was free of the debromination by-product **8.** Reduction of (*E*)-**9** with Dibal-H afforded the alcohol (*E*)-**10** in 96% yield. We tried to reduce (*Z*)-**9** by Dibal-H in a similar manner, but successful result was not obtained due to decomposition of the materials. From the bromo alcohol **10** the acyclic enediyne **11** was obtained in 75% yield via the Sonogashira coupling of **10** with 1,7-octadiyne. Unexpectedly, isomerization of the double bond occurred during the cross-coupling reaction and **11** was obtained as a 46:54 mixture of *E* and *Z* isomers. Conversion of **11** into the iodoalkyne **12** was achieved in 90% yield by treatment of the terminal alkyne with iodine and morpholine in PhMe at 60 °C.[18] The *E* and *Z* isomers of the iodoalkyne **12** were separated and the pure (*E*)-**12** was oxidized to the aldehyde (*E*)-**13** in a quantitative yield by treating with PDC. In contrast, (*Z*)-**12** decomposed into unidentified materials in a similar PDC oxidation. The double bond configurations of compounds **8**–**13** described above were determined according to the NOE experiments (see Figures **S1**–**S6** in Supplementary data). The intramolecular Nozaki–Hiyama–Kishi reaction of the aldehyde with the iodoalkyne moiety within (*E*)-**13** was carried out with 3 equiv of $CrCl_2$ and 1 equiv of $NiCl_2$[12b,c,16,18a] in THF at room temperature under high dilution conditions to give the exocyclic enediyne alcohol **1** in 24% yield. The rest of the materials was decomposed and could not be identified. Acetylation of the alcohol **1** was performed with Ac_2O–DMAP to give the acetate **2** in 84% yield. Alternatively, treatment of **1** with $MeOCH_2CO_2H$ under the DCC–DMAP conditions furnished the methoxyacetate **3** in 52% yield. Finally, desilylation with PPTS in MeOH at room temperature transformed both silyl ethers **2** and **3** into the corresponding alcohols **4** and **5**, respectively, in excellent yields.

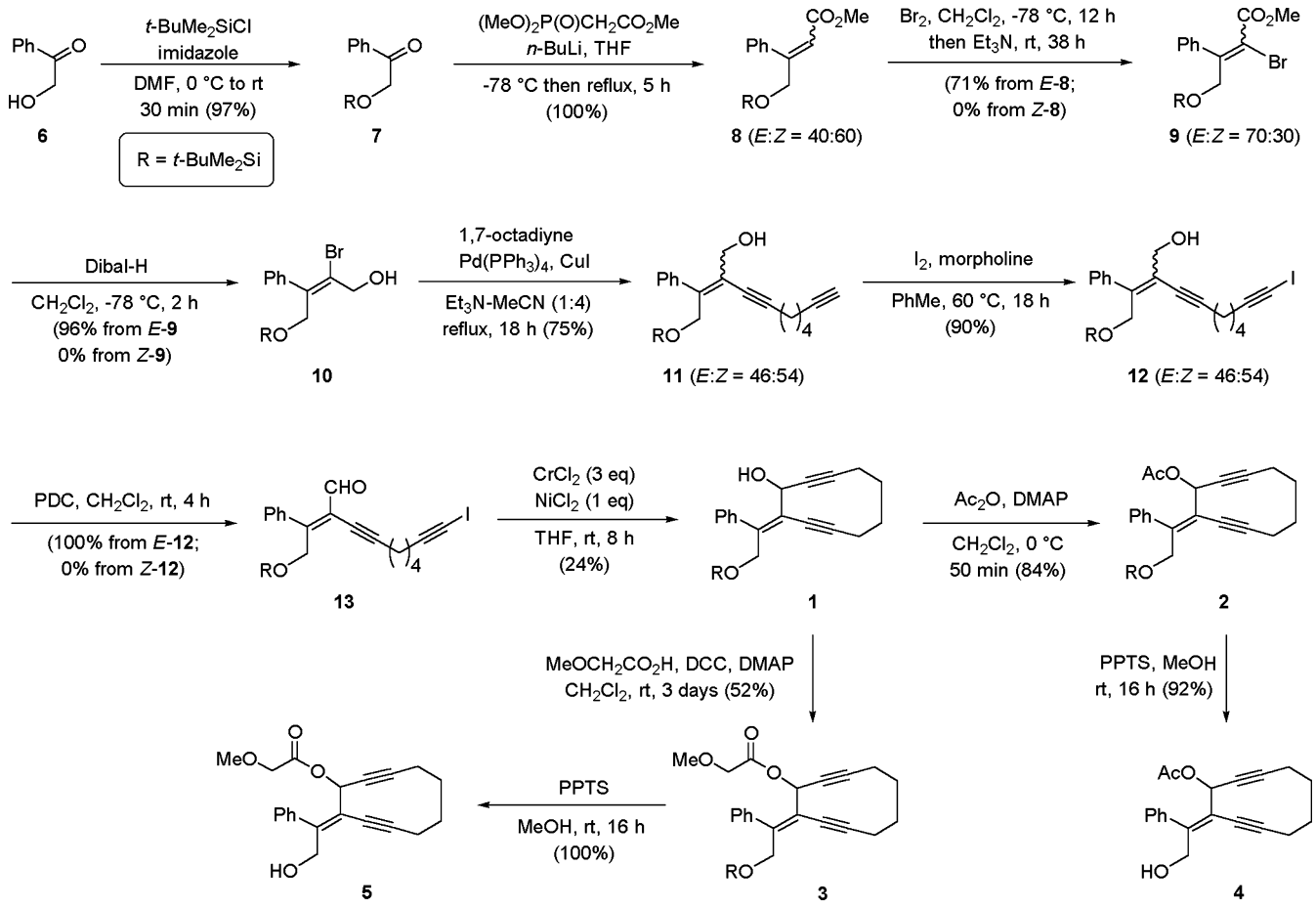


1: R = *t*-BuMe_2Si, X = OH
2: R = *t*-BuMe_2Si, X = OAc
3: R = *t*-BuMe_2Si, X = OC(O)CH_2OMe
4: R = H, X = OAc
5: R = H, X = OC(O)CH_2OMe

Figure 1. Molecular structures of enediyne prodrugs **1**–**5**.

2.2. DNA cleavage

The DNA cleavage activity of enediyne prodrugs **1**–**5** was evaluated by monitoring the conversion of circular supercoiled DNA (Form I) to circular relaxed (Form II) or linear (Form III) DNA. ΦX174 RFI supercoiled DNA (Form I) was incubated with various enediyne prodrugs in TAE buffer solution (pH 8.5) containing 20% DMSO at 37 °C for 72 h, and then the reaction mixtures were analyzed by gel electrophoresis over 1% agarose gel (ethidium bromide stain). Figure 2 shows the representative gel picture (A) observed at various drug concentrations and the corresponding scanning densitometry results (B). The enediyne prodrugs **1**–**3** with a silyl-protected hydroxy group were generally very weak in causing DNA damage. In contrast, the enediyne prodrugs **4** and **5** exhibited DNA cleaving activity in a concentration-dependent manner. About 49% and 41% net DNA strand scissions were observed for the acetate



Scheme 1. Synthesis of enediyne prodrugs **1**–**5**.

4 and the methoxyacetate **5**, respectively, at 1.0 mM drug concentration. However, formation of Form III DNA was not observed for the action of the enediyne prodrugs **1**–**5**.

Figure 3 illustrates the time dependency of DNA cleavage by the enediyne prodrugs **1**–**5** evaluated with 1.0 mM drug concentration under the identical conditions as described in Figure 2. The extents of DNA cleavage by **4** and **5** increased with incubation time (12%, 30%, and 37% for **4**; 18%, 27%, and 31% for **5** after 24, 48, and 72 h incubation, respectively) and significant cleavages of about 30% were observed after incubation for 48 h. Although the cleavage values are slightly lower than those given in Figure 2, the relative DNA cut potency is consistent with the fact that the acetate **4** is more active than the methoxyacetate **5** in pH 8.5 buffer at 37 °C for 72 h. Moreover, DNA strand breakage by the silyl-protected analogs **1**–**3** was negligible at different incubation durations. On the basis of the results shown in Figures 2 and 3, we can conclude that the enediyne prodrugs **1**–**3** are not activated in basic buffer at pH 8.5 while the enediyne prodrugs **4** and **5** may undergo a self-assisted allylic rearrangement in pH 8.5 buffer to migrate the exocyclic double bond into the endocyclic position, resulting in formation of a reactive 10-membered enediyne (vide infra).

It was reported that irradiation of 2,5-dihydrofuran induced a photochemical [1,3]-shift of an oxygen substituent to form 3,4-epoxybut-1-ene.[19a] A similar [1,3]-shift of allylic hydroxyl or methoxy group occurred under photochemical conditions in 8-hydroxy- and 8-methoxy-germacrene B.[19b,19c] The medium-sized ring of the germacrene B derivatives was crucial for the photochemical [1,3]-shifts. Photochemistry of the 4-ethyl-4-methyl disubstituted 3-alkylidene-2-naphthalenol derivatives was reported[20a] but the results of the photochemical [1,3]-OH shift were not reproduced.[20b] As compared to [1,3]-shifts of allylic alcohol photochemical isomerization of *E* and *Z* configurations of alkenes was known to be much faster.[19d,20] We anticipated that if a similar photochemical [1,3]-shift of the oxygenated group X in **1**–**5** (Fig. 1) or a photochemical isomerization of the exocyclic double bond takes place (vide infra), enhanced DNA cleavage potency would be observed under UV irradiation. For examination of the photoinduced DNA cleavages by the enediyne prodrugs **1**–**5**, we carried out the comparative experiments without and with photoirradiation at 365 nm. The mixtures of ΦX174 RFI DNA with 1.0 mM prodrugs **1**–**5** in TAE buffer solution containing 20% DMSO were irradiated for 4 and 8 h, respectively, followed by incubation along with the non-irradiated controls for 72 h. The representative gel picture and the corresponding scanning densitometry results are shown in Figure 4. It was confirmed that the

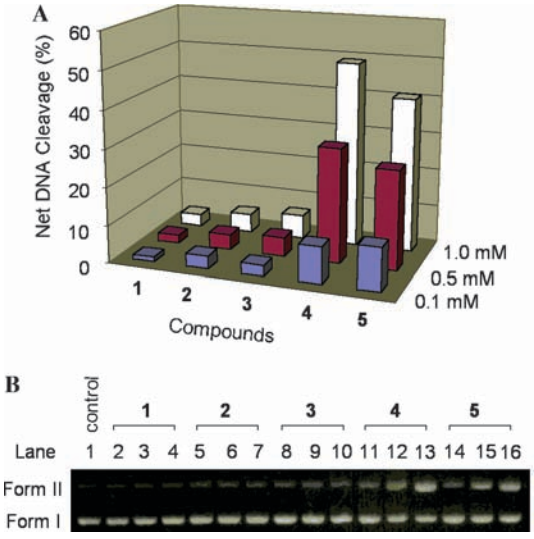


Figure 2. (A) Gel picture of concentration-dependent DNA cleavage by enediyne prodrugs **1**–**5**. (B) Scanning densitometry results of the gel picture shown in (A). ΦX174 RFI DNA (50 μM/bp) was incubated for 72 h at 37 °C without drug (control) or with 0.1 mM (lanes 2, 5, 8, 11, and 14), 0.5 mM (lanes 3, 6, 9, 12, and 15) and 1.0 mM (lanes 4, 7, 10, 13, and 16) of **1**–**5** in TAE buffer (pH 8.5) containing 20% DMSO and analyzed by gel electrophoresis (1% agarose gel, ethidium bromide stain). Form I is the supercoiled DNA. Form II and Form III bands represent the single- and double-strand cleaved DNA products, respectively (see Ref. 24). The percentage of net DNA cleavage was calculated by the following equation: $\{[(\text{Form II})_s/[(\text{Form I})_s + (\text{Form II})_s] \times 100\} - \{(\text{Form II})_c/[(\text{Form I})_c + (\text{Form II})_c] \times 100\}$. The subscripts 's' and 'c' refer to as the samples and control(s), respectively. The net DNA cleavage values are 9.7%, 30.3%, and 49.0% for **4** and 11.4%, 26.2%, and 40.8% for **5**, respectively, at 0.1, 0.5, and 1.0 mM drug concentrations. TAE buffer = Tris + acetic acid + EDTA.

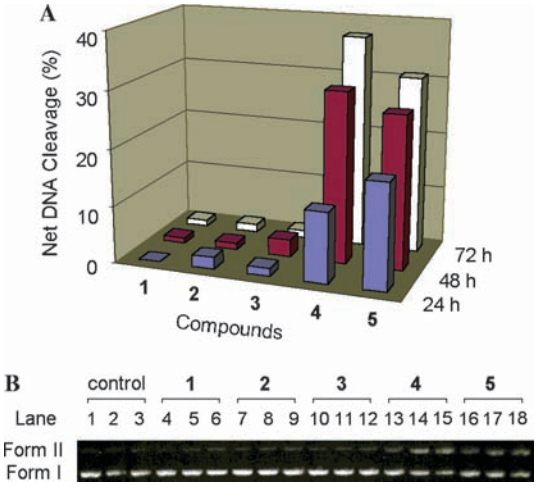


Figure 3. (A) Gel picture of time-dependent DNA cleavage by enediyne prodrugs **1**–**5**. (B) Scanning densitometry results of the gel picture shown in (A). ΦX174 RFI DNA (50 μM/bp) was incubated without drug (controls) or with 1.0 mM of **1**–**5** at 37 °C for 1 day (lanes 1, 4, 7, 10, 13, and 16), 2 days (lanes 2, 5, 8, 11, 14, and 17) and 3 days (lanes 3, 6, 9, 12, 15, and 18) in TAE buffer (pH 8.5) containing 20% DMSO and analyzed by gel electrophoresis (1% agarose gel, ethidium bromide stain). The percentage of net DNA cleavage was calculated in the same manner as given in Figure 2. The net DNA cleavage values are 12.3%, 30.0%, and 37.2% for **4** and 18.4%, 26.8%, and 30.8% for **5**, respectively, at 24, 48, and 72 h incubation time.

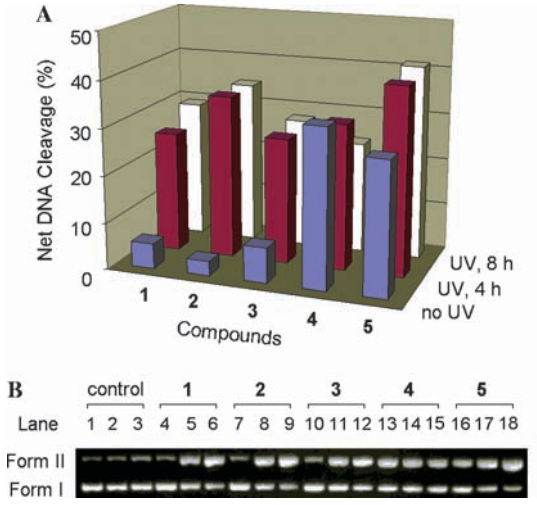
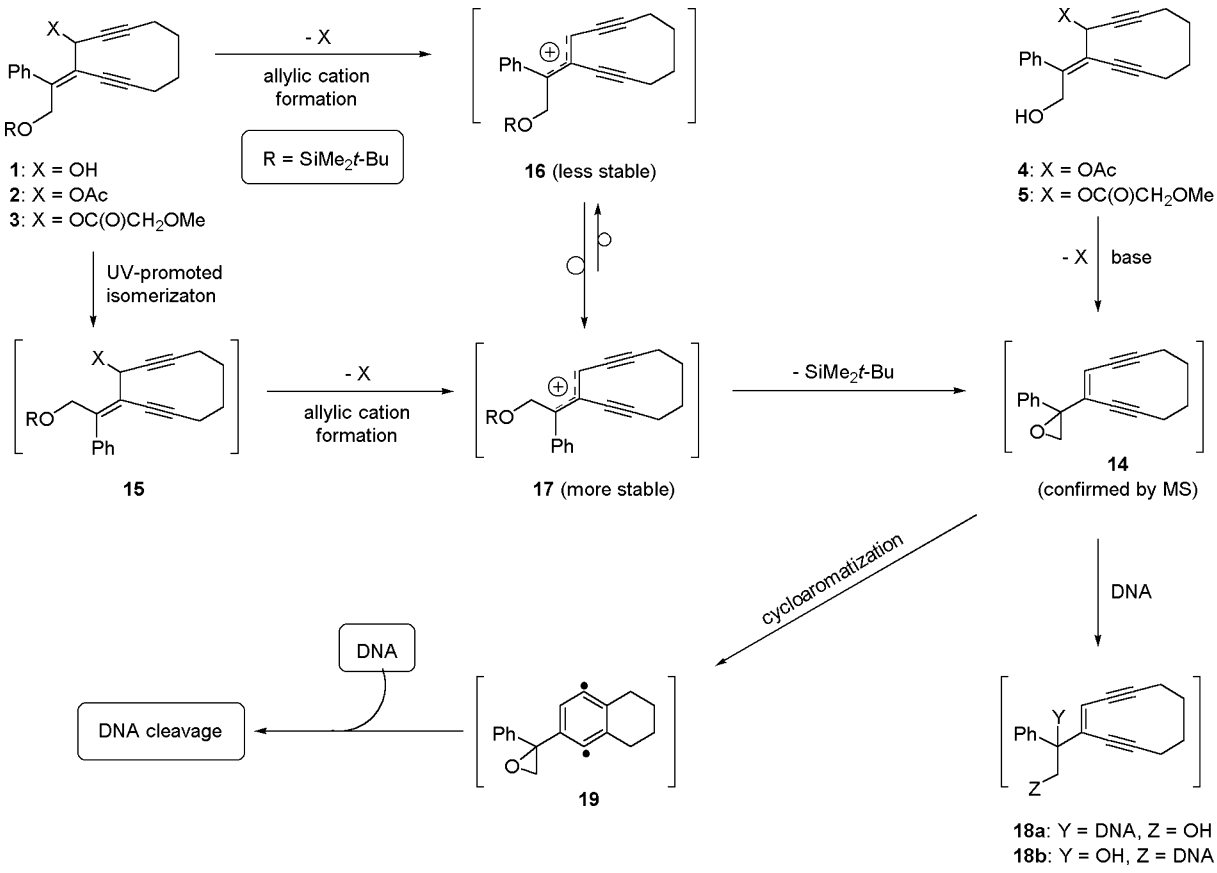


Figure 4. (A) Gel picture of UV-promoted DNA cleavage by enediyne prodrugs **1**–**5**. (B) Scanning densitometry results of the gel picture shown in (A). ΦX174 RFI DNA (50 μM/bp) was incubated without drug (controls) or with 1.0 mM of **1**–**5** for 72 h at 37 °C without UV irradiation (lanes 1, 4, 7, 10, 13, and 16) or with UV irradiation for 4 h (lanes 2, 5, 8, 11, 14, and 17) and 8 h (lanes 3, 6, 9, 12, 15, and 18), respectively, in TAE buffer (pH 8.5) containing 20% DMSO and analyzed by gel electrophoresis (1% agarose gel, ethidium bromide stain). The percentage of net DNA cleavage was calculated in the same manner as given in Figure 2. The net DNA cleavage values are 5.4%, 25.6%, and 29.4% for **1**, 3.1, 34.7 and 34.7% for **2**, 7.5%, 26.8%, and 27.7% for **3**, 33.6%, 31.0%, and 23.3% for **4** and 28.3%, 39.9%, and 41.3% for **5**, respectively, without UV irradiation and with UV irradiation for 4 and 8 h.

enediyne prodrugs **1**–**3** with a silyl-protected hydroxy group could be activated by UV irradiation and the DNA cleavage values are 26%, 35%, and 27% (4 h UV irradiation) and 29%, 35%, and 28% (8 h UV irradiation) for **1**–**3**, respectively. The results indicate that 4 h UV irradiation prior to incubation in pH 8.5 buffer is sufficient for activating **1**–**3**, resulting in about 30% net DNA strand scission comparable to those of the enediyne prodrugs **4** and **5**. We also observed some interesting behaviors of the enediyne prodrugs **4** and **5** under photoirradiation. Without UV irradiation, the acetate **4** caused 34% net DNA cleavage which is higher than 28% damaged by the methoxyacetate **5**. However, the potency of **4** decreased to 31% and 24% of net DNA cut after 4 and 8 h UV exposure, respectively. In contrast, the methoxyacetate **5** produced enhanced DNA cleavage potency to 40% and 41% after UV irradiation for 4 and 8 h. Therefore, the DNA scission potency with 4 h UV irradiation follows the order of **5** (40%) > **2** (35%) > **4** (31%) > **3** (27%) ≈ **1** (26%).

2.3. Mechanisms of action

We considered that the enediyne prodrugs **4** and **5** were converted into the epoxy enediyne **14** in basic buffer via an intramolecular S_N2' substitution reaction with the departure of acetate or methoxyacetate anion (Scheme 2). Conversion of **4** and **5** into **14** is also possible to involve an allylic cation intermediate, which is trapped intramolecularly by the free hydroxy group.[14] According to the pK_a values of acetic acid (4.76) and methoxy-



Scheme 2. Proposed mechanisms of base- and UV-promoted DNA cleavage by enediyne prodrugs **1**–**5**.

acetic acid (3.53), the methoxyacetate **5** should be easily converted into **14** than the acetate **4**. It seems to be not consistent with a higher potency of **4** than that of **5** in DNA strand scission and cytotoxicity.[15] One possibility is that the acetate **4** slowly releases the reactive species, while the methoxyacetate **5** serves as a fast-acting DNA cleaver. Non-DNA cutting decomposition of the epoxy enediyne **14** might occur in the action of **5**.

According to our previous studies on allylic rearrangement of acyclic 1,2-dialkynylallyl alcohols,[10d] cleavage of X in **1**–**3** to form the less stable sickle allylic cation **16** is difficult as compared to formation of the more stable sickle allylic cation **17** from the isomeric precursors **15**. It accounts for the inactivity of the silyl-protected analogs **1**–**3** in pH 8.5 buffer. Upon UV irradiation, a fast isomerization of the exocyclic double bond should occur to convert **1**–**3** into the alkene isomers **15**.[19d,20] The latter decomposed in buffer to the sickle allylic cation **17** followed by attack of the neighboring silyloxy group with migration of the double bond into the endocyclic position. After loss of the silyl group from the intermediate, the epoxy enediyne **14** is formed. It was found that the acetate **2** was slightly more potent than the methoxyacetate **3** under photochemical conditions. The results are parallel to those of non-UV-activated DNA damage by **4** and **5** shown in Figure 3. Involvement of a common reactive species **14** for DNA cleavage of **1**–**5** under UV irradiation is reasonably supported. We cannot definitely rule out the possibility of direct conversion of **1**–**5** into a 10-membered ring enediyne of the structure **18** [*Y* = OH, OAc, and OC(O)CH_2OMe; *Z* = *t*-BuMe_2SiO] under photochemical conditions but we are confident that it is not a main reaction pathway due to a fast alkene isomerization under photoirradiation.[19,20] The diminished activity of **4** with UV activation is unusual according to our discussion given above. We suspect that a non-DNA cutting pathway might be competing with formation of **14** and the details are not known.

We made a lot of effort in the isolation of the epoxy enediyne **14** but without success due to its instability during purification over silica gel. Therefore, we tried to detect its presence in solution by mass spectrometry. The compound **4** (1.0 mM) in TAE buffer solution (pH 8.5) containing 20% DMSO was incubated at 37 °C for 72 h in the absence of a DNA substrate and the mixture was subjected to mass analysis under the +FAB conditions. Figures 5 and 6 show the recorded mass spectrum of the crude reaction mixture and the possible fragment ions, respectively. The peak *m*/*z* 249 ($M+H^+$) corresponds to the ion **III** (the protonated epoxy enediyne **14**) or **II** (formed from the protonated **4** by loss of HOAc) as shown in Figure 6. We carefully examined the mass spectrum of **4** obtained under +FAB conditions (spectrum not shown) and found, in addition to the base peak *m*/*z* 249 ($M+H^+-$HOAc), the molecular ion *m*/*z* 309 ($M+H^+$, 10% of relative intensity). However, in the mass spectrum shown in Figure 5, the ion *m*/*z*

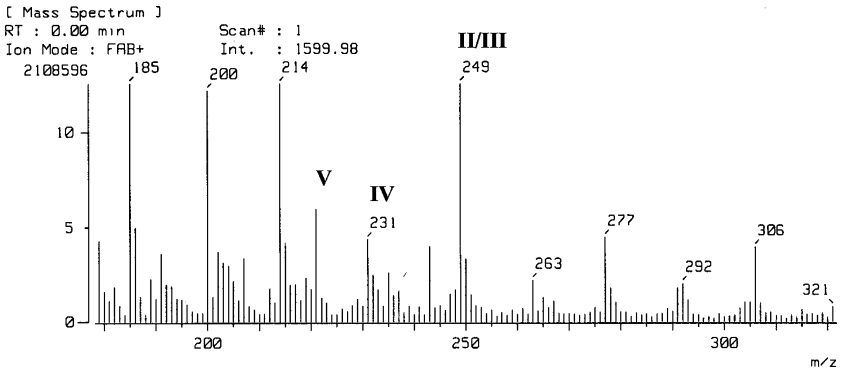


Figure 5. Mass spectrum of the reaction mixture of the enediyne prodrug **4** (1.0 mM) in TAE buffer (pH 8.5) containing 20% DMSO after incubated at 37 °C for 72 h. The reaction mixture was analyzed with a JEOL JMX-SX 102 A spectrometer under +FAB conditions. The structures of the ions **II**–**V** are found in Figure 6.

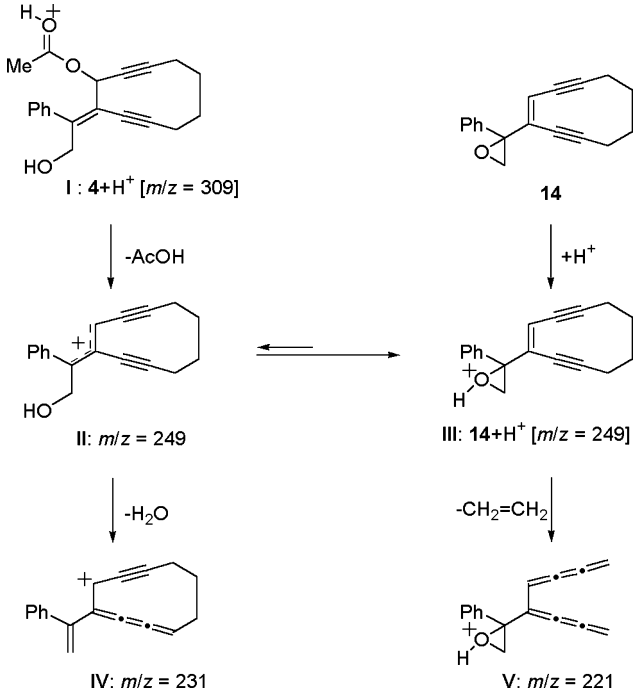


Figure 6. Proposed structures for the molecular and fragment ions seen in the mass spectra of **4** (not shown) and **14** (given in Fig. 5).

309 was not seen. The results imply that the ion of *m*/*z* 249 in Figure 5 is for the structure **III**. The mass peaks *m*/*z* 231 and *m*/*z* 221 are the fragments of the ions **II** and **III**, and they were observed in all mass spectra of **1**–**5** measured under +CI conditions by using CH_4 and NH_3 as the ion source. In combination with the DNA cleavage data presented in Figures 2 and 3, we suggest that compounds **4** and **5** incubated at pH 8.5 underwent a base-promoted allylic rearrangement to form the epoxy enediyne **14**. The latter thus generated in situ can undergo thermal cycloaromatization to form 1,4-benzenoid diradical species **19**, thereby resulting in DNA strand cleavage through abstraction of hydrogen atoms from the sugar-phosphate backbone (Scheme 2).[1a,3] On the other hand, there is a possibility for the epoxy enediyne **14** damaging DNA via base alkylation through the epoxy moiety[21] to form the DNA alkylation products **18a**,**b**, which then undergo a similar thermal cycloaromatization described for **14** in Scheme 2. Although reactions of epoxy compounds with DNA have been extensively reported in the literature,[22] we have not experimentally confirmed for **14** and the related precursors in this study. Nevertheless, the epoxy enediyne **14** represents a synthetic model system for mimicking the intramolecular allylic rearrangement for the activation of the maduropeptin chromophore artifacts.[23]

Cytotoxicity of compounds **1**–**5** was evaluated against P388 cancer cell line.[15] Compounds **1**–**3** with a silyl-protected hydroxy group exhibited similar IC_{50} values of 8.6–17.2 μM, while compounds **4** and **5** with a free hydroxyl group gave IC_{50} values of 39.0–54.2 μM. Obviously, the cytotoxicity data are opposite to the observed DNA cleavage activity of these compounds in the absence of UV activation (Figs. 2 and 3). One possibility is that the protected compounds **1**–**3** converted into the active enediyne species slowly than compounds **4** and **5**, resulting in a long-lasting action on the cancer cells. However, the nature of the difference in two assays for compounds **1**–**5** is not clear.

3. Conclusion

We have designed and synthesized the enediyne prodrugs **1**–**5** possessing an (*E*)-3-hydroxy-4-(2′-hydroxy-1′-phenylethylidene)cyclodeca-1,5-diyne scaffold and evaluated their DNA cleavage reactions in pH 8.5 buffer without and with UV irradiation. The enediyne prodrugs **4** and **5** exhibited concentration- and time-dependent DNA strand scission in basic buffer (pH 8.5) at 37 °C, while the silyl-protected analogs **1**–**3** are almost inactive. The results suggest that a base-promoted intramolecular allylic rearrangement takes place to convert **4** and **5** into a reactive epoxy enediyne **14**, which can cause DNA strand cleavage most likely via hydrogen atom abstraction from the sugar-phosphate backbone. Under photochemical conditions, the enediyne prodrugs **1**–**3** could be activated to enter the alkene isomerization–allylic rearrangement cascade of reactions, leading to formation of the same epoxy enediyne **14**. An enhanced

potency for the methoxyacetate **5** with UV irradiation was observed although the acetate **4** gave the opposite result for some unknown reasons. With the help of mass spectrometry, the putative epoxy enediyne **14** was detected in the incubation mixture of the acetate **4** under the conditions used for DNA cleavage experiments. Based on the results, the enediyne prodrugs **1**–**3** are identified to be potentially useful for photoactivated prodrugs. Thus, enediyne precursors **1**–**5** are potential prototype compounds for a new class of antitumor prodrugs.

4. Experimental

4.1. General methods

All reactions were carried out under a dry nitrogen atmosphere using freshly distilled solvents unless otherwise noted. Reagents were purchased from suppliers and used without further purification. Tetrahydrofuran (THF) and toluene were distilled over sodium and benzophenone. Dichloromethane, acetonitrile, and DMF were distilled over calcium hydride. Reactions were monitored by thin-layer chromatography (TLC) on E. Merck silica gel plates (Silica Gel 60 F_{254}) using UV light and 7% ethanolic phosphomolybdic acid and heat as the visualizing methods. Flash column chromatography was carried out on E. Merck silica gel (230–400 mesh). NMR Spectra were recorded on a 300 MHz spectrometer at ambient temperature. Chemical shifts are reported in ppm related to residual chloroform (δ = 7.26 in ^{1}H NMR and δ = 77.0 in ^{13}C NMR). Multiplicity is designated as singlet (s), doublet (d), doublet of doublet (dd), triplet (t), triplet of doublet (td), or multiplet (m). IR spectra were measured on a FT-IR spectrophotometer. Mass spectra were recorded under +CI or +FAB conditions.

4.2. Synthesis of enediyne prodrugs

4.2.1. 2-(*tert*-Butyldimethylsilyloxy)acetophenone (7). To a solution of 2-hydroxyacetophenone (2.000 g, 14.7 mmol) and imidazole (1.500 g, 22.0 mmol) in dry DMF (30 mL) cooled in an ice-water bath (0 °C) was added *tert*-butyldimethylsilyl chloride (2.657 g, 17.6 mmol) followed by stirring the resultant mixture at room temperature for 30 min. The reaction was quenched with cold water (250 mL) and extracted with EtOAc (3× 80 mL). The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, 5% EtOAc–hexane) to give **7** (3.563 g, 97%) as a colorless oil; R_f = 0.51 (10% EtOAc–hexane); IR (neat) 2930, 2857, 1707, 1255, 1155 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (d, J = 6.9 Hz, 2H), 7.57 (t, J = 7.4 Hz, 1H), 7.45 (t, J = 7.5 Hz, 2H), 4.93 (s, 2H), 0.95 (s, 9H), 0.15 (s, 6H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 197.2, 134.8, 133.1, 128.5 (2×), 127.8 (2×), 67.5, 25.9 (3×), 18.6, −5.2 (2×); MS (+CI) *m*/*z* (relative intensity) 251 (M+H$^+$, 31), 235 (100); HRMS (+FAB) calcd for $C_{14}H_{23}O_2Si$ (M+H$^+$) 251.1468, found 251.1483.

4.2.2. (*E*)- and (*Z*)-4-(*tert*-Butyldimethylsilyloxy)-3-phenylbut-2-enoic acid methyl ester [(*E*)- and (*Z*)-8]. To a solution of trimethyl phosphonoacetate (2.8 mL, 17.1 mmol) in dry THF (30 mL) cooled in a dry ice–acetone bath (−78 °C) was added *n*-BuLi (1.6 M in hexane, 13.3 mL, 21.3 mmol) followed by stirring at the same temperature for 30 min. To the resultant mixture was added a solution of **7** (3.563 g, 14.2 mmol) in dry THF (20 mL). The resultant mixture was then allowed to warm up to room temperature and stirred for 5 h at the same temperature. The reaction was quenched with saturated aqueous NH_4Cl (10 mL) and extracted with EtOAc (3× 10 mL). The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, 1% EtOAc–hexane) to give (*Z*)-**8** (1.729 g, 60%) and (*E*)-**8** (2.613 g, 40%).

Compound (*E*)-**8**. A colorless oil; R_f = 0.46 (10% EtOAc–hexane); IR (neat) 2953, 2857, 1716, 1633, 1257, 1171, 1098 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50–7.46 (m, 2H), 7.37–7.33 (m, 3H), 6.05 (t, J = 1.0 Hz, 1H), 5.19 (d, J = 1.2 Hz, 2H), 3.77 (s, 3H), 0.77 (s, 9H), 0.02 (s, 6H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 166.3, 158.8, 139.0, 128.6, 127.9 (2×), 127.6 (2×), 117.1, 59.7, 51.4, 25.8 (3×), 18.2, −5.2 (2×); MS (+CI) *m*/*z* (relative intensity) 307 (M+H$^+$, 100); HRMS (+FAB) calcd for $C_{17}H_{27}O_3Si$ (M+H$^+$) 307.1729, found 307.1734.

Compound (*Z*)-**8**. A colorless oil; R_f = 0.60 (10% EtOAc–hexane); IR (neat) 2954, 2858, 1732, 1659, 1223, 1158 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37–7.33 (m, 3H), 7.19–7.16 (m, 2H), 6.23 (t, J = 2.3 Hz, 1H), 4.35 (d, J = 1.8 Hz, 2H), 3.58 (s, 3H), 0.97 (s, 9H), 0.13 (s, 6H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 166.4, 158.2, 137.2, 127.9, 127.9 (2×), 127.2 (2×), 114.5, 66.9, 51.1, 26.0 (3×), 18.5, −5.3 (2×); MS (+CI) *m*/*z* (relative intensity) 307 (M+H$^+$, 100); HRMS (+FAB) calcd for $C_{17}H_{27}O_3Si$ (M+H$^+$) 307.1729, found 307.1735.

4.2.3. (*E*)- and (*Z*)-2-Bromo-4-(*tert*-butyldimethylsilyloxy)-3-phenylbut-2-enoic acid methyl ester [(*E*)- and (*Z*)-9]. To a solution of (*E*)-**8** (2.622 g, 8.6 mmol) in dry CH_2Cl_2 (43 mL) cooled in a dry ice–acetone bath (−78 °C) was added bromine (0.88 mL, 17.1 mmol) in dry CH_2Cl_2 (10 mL) through a dropping funnel, followed by stirring at the same temperature for 12 h. To the resultant mixture was added triethylamine (6.0 mL, 42.8 mmol), followed by stirring at the same temperature for 38 h. The reaction was then quenched with saturated aqueous NH_4Cl (20 mL) and extracted with CH_2Cl_2 (3× 20 mL). The combined organic layer was washed with saturated aqueous $Na_2S_2O_3$ (50 mL) and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, 1% EtOAc–hexane) to give a 70:30 mixture of (*E*)-**9** and (*Z*)-**9** (2.326 g, 71%).

Compound (*E*)-**9**. A yellow oil; R_f = 0.54 (10% EtOAc–hexane); IR (neat) 2953, 2930, 2857, 1734, 1232, 1101 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33–7.28

(m, 3H), 7.20–7.15 (m, 2H), 4.67 (s, 2H), 3.51 (s, 3H), 0.76 (s, 9H), −0.03 (s, 6H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 164.9, 149.0, 137.6, 127.9, 127.9 (2×), 127.7 (2×), 109.6, 66.2, 52.7, 25.7 (3×), 18.2, −5.4 (2×); MS (+CI) *m*/*z* (relative intensity) 387 ($M+2+H^+$, ^{81}Br, 30), 385 ($M+H^+$, ^{79}Br, 29), 255 (M^++2−OSiMe$_2$*t*-Bu, ^{81}Br, 100), 253 (M^+−OSiMe$_2$*t*-Bu, ^{79}Br, 100); HRMS (+FAB) calcd for $C_{17}H_{26}BrO_3Si$ ($M+H^+$, ^{79}Br) 385.0835, found 385.0830.

Compound (*Z*)-**9**. A yellow oil; R_f = 0.50 (10% EtOAc–hexane); IR (neat) 2953, 2930, 2857, 1732, 1252, 1106 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.42–7.30 (m, 3H), 7.23–7.19 (m, 2H), 4.65 (s, 2H), 3.88 (s, 3H), 0.79 (s, 9H), −0.05 (s, 6H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 164.7, 150.8, 139.1, 127.9, 127.8 (2×), 127.8 (2×), 110.3, 64.6, 53.1, 25.8 (3×), 18.3, −5.5 (2×); MS (+CI) *m*/*z* (relative intensity) 387 ($M+2+H^+$, ^{81}Br, 87), 385 ($M+H^+$, ^{79}Br, 91), 255 (M^++2−OSiMe$_2$*t*-Bu, ^{81}Br, 100), 253 (M^+−OSiMe$_2$*t*-Bu, ^{79}Br, 100); HRMS (+FAB) calcd for $C_{17}H_{26}BrO_3Si$ ($M+H^+$, ^{79}Br) 385.0835, found 385.0819.

4.2.4. (*E*)-2-Bromo-4-(*tert*-butyldimethylsilyloxy)-3-phenylbut-2-en-1-ol [(*E*)-10]. To a solution of (*E*)-**9** (1.782 g, 4.6 mmol) in dry CH_2Cl_2 (23 mL) cooled in a dry ice–acetone bath (−78 °C) was added Dibal-H (1.0 M in THF, 23.1 mL, 23.1 mmol), followed by stirring at the same temperature for 1 h. The reaction was then quenched with methanol (20 mL) at −78 °C, followed by warming to room temperature. Saturated aqueous NH_4Cl (20 mL) was added and the resultant mixture was stirred at room temperature for 1 h and extracted with CH_2Cl_2 (3× 30 mL). The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, 5% EtOAc–hexane) to give (*E*)-**10** (1.589 g, 96%) as a pale yellow oil; R_f = 0.49 (20% EtOAc–hexane); IR (neat) 3391, 2930, 2858, 1945, 1875, 1803, 1728, 1644, 1471, 1256, 1086, 1004 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34–7.30 (m, 3H), 7.22–7.20 (m, 2H), 4.62 (s, 2H), 4.47 (s, 2H), 0.87 (s, 9H), 0.00 (s, 6H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 142.2, 141.3, 128.0, 128.0 (2×), 127.4 (2×), 127.2, 65.5, 64.2, 25.8 (3×), 18.3, −5.4 (2×); MS (+CI) *m*/*z* (relative intensity) 359 ($M+2+H^+$, ^{81}Br, 11), 357 ($M+H^+$, ^{79}Br, 16), 341 (M^++2−OH, ^{81}Br, 100), 339 (M^+−OH, ^{79}Br, 100); HRMS (+FAB) calcd for $C_{16}H_{26}BrO_2Si$ ($M+H^+$, ^{79}Br) 357.0886, found 357.0898.

4.2.5. (*E*)- and (*Z*)-2-[2′-(*tert*-Butyldimethylsilyloxy)-1′-phenylethylidene]deca-3,9-diyn-1-ol [(*E*)- and (*Z*)-11]. To a suspension of $Pd(PPh_3)_4$ (0.144 g, 0.12 mmol) and CuI (0.047 g, 0.25 mmol) in degassed CH_3CN (0.8 mL) were added (*E*)-**10** (0.445 g, 1.20 mmol), triethylamine (1.2 mL), and 1,7-octadiyne (0.5 mL, 3.70 mmol) in degassed CH_3CN (4.0 mL). The reaction mixture was refluxed with stirring in dark for 18 h. The reaction was quenched with saturated aqueous NH_4Cl (5 mL) and extracted with EtOAc (3× 10 mL). The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, 5% EtOAc–hexane) to give a 46:54 mixture of (*E*)-**11** and (*Z*)-**11** (0.357 g, 75%).

Compound (*E*)-**11**. A yellow oil; R_f = 0.34 (20% EtOAc–hexane); IR (neat) 3406, 2930, 2858, 2216, 2118, 1463, 1256, 1096 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35–7.28 (m, 3H), 7.18–7.15 (m, 2H), 4.69 (s, 2 H), 4.01 (s, 2H), 2.52–2.48 (m, 2H), 2.30–2.25 (m, 2H), 1.98 (t, J = 2.7 Hz, 1H), 1.79–1.72 (m, 4H), 0.79 (s, 9H), −0.04 (s, 6H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 147.3, 137.8, 128.6 (2×), 127.8 (2×), 127.3, 121.0, 96.8, 83.9, 77.9, 68.7, 65.7, 62.6, 27.7, 27.7, 26.0 (3×), 19.3, 18.3, 18.1, −5.3 (2×); MS (+CI) *m*/*z* (relative intensity) 383 ($M+H^+$, 18), 381 ($M-H^+$, 26), 365 (M^+−OH, 85), 251 (M^+−OSiMe$_2$*t*-Bu, 58), 233 (100); HRMS (+FAB) calcd for $C_{24}H_{33}O_2Si$ [$(M-H)^+$] 381.2250, found 381.2249.

Compound (*Z*)-**11**. A yellow oil; R_f = 0.40 (20% EtOAc–hexane); IR (neat) 3430, 2930, 2858, 2216, 2118, 1463, 1256, 1109, 1074 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41–7.27 (m, 5H), 4.52 (s, 2 H), 4.36 (s, 2H), 2.27–2.23 (m, 2H), 2.13–2.07 (m, 2H), 1.95 (t, J = 2.7 Hz, 1H), 1.53–1.38 (m, 4H), 0.88 (s, 9H), −0.04 (s, 6H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 145.8, 140.9, 128.3 (2×), 127.7 (2×), 127.2, 123.8, 94.6, 84.1, 80.3, 68.4, 63.7, 62.7, 27.3, 27.2, 25.9 (3×), 19.1, 18.3, 18.0, −5.3 (2×); MS (+CI) *m*/*z* (relative intensity) 383 ($M+H^+$, 18), 381 ($M-H^+$, 44), 365 (M^+−OH, 43), 251 (M^+−OSiMe$_2$*t*-Bu, 87), 233 (94); HRMS (+FAB) calcd for $C_{24}H_{33}O_2Si$ [$(M-H)^+$] 381.2250, found 381.2255.

4.2.6. (*E*)- and (*Z*)-2-[2′-(*tert*-Butyldimethylsilyloxy)-1′-phenylethylidene]-10-iododeca-3,9-diyn-1-ol [(*E*)- and (*Z*)-12]. To a solution of iodine (5.002 g, 19.7 mmol) in toluene (100 mL) was added morpholine (4.6 mL, 52.6 mmol). The resultant mixture was heated at 60 °C with stirring for 40 min. To the resultant mixture was added a 46:54 mixture of (*E*)-**11** and (*Z*)-**11** (2.422 g, 6.6 mmol) in toluene (100 mL), followed by stirring at 60 °C for 18 h. The reaction mixture was allowed to cool down and purified directly by flash column chromatography (silica gel, hexane then 20% EtOAc–hexane) to give a 46:54 mixture of (*E*)-**12** and (*Z*)-**12** (2.925 g, 90%).

Compound (*E*)-**12**. A yellow oil; R_f = 0.34 (20% EtOAc–hexane); IR (neat) 3401, 2929, 2857, 2215, 1255, 1095, 1005 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34–7.27 (m, 3H), 7.19–7.13 (m, 2H), 4.69 (s, 2H), 4.01 (s, 2H), 2.52–2.42 (m, 4H), 1.80–1.68 (m, 4H), 0.80 (s, 9H), −0.03 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 147.3, 137.8, 128.6 (2×), 127.8 (2×), 127.3, 121.0, 96.7, 94.0, 78.0, 68.7, 65.7, 62.6, 27.8, 27.7, 25.9 (3×), 20.5, 19.3, 18.3, −5.2 (2×); MS (+CI) *m*/*z* (relative intensity) 509 ($M+H^+$, 7), 491 (M^+−OH, 68), 345 (100); HRMS (+FAB) calcd for $C_{24}H_{32}IO_2Si$ [$(M-H)^+$] 507.1216, found 507.1220.

Compound (*Z*)-**12**. A yellow oil; R_f = 0.40 (20% EtOAc–hexane); IR (neat) 3419, 2929, 2857, 2216, 2117, 1256, 1109, 1073, 1005 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41–7.26 (m, 5H), 4.52 (s, 2H), 4.36 (d, J = 5.1 Hz, 2H), 2.28–2.22 (m, 4H), 1.50–1.35 (m, 4H), 0.88 (s,

9H), 0.04 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 145.9, 140.9, 128.2 (2×), 127.7 (2×), 127.2, 123.8, 94.5, 94.2, 80.4, 68.4, 63.7, 62.7, 27.3, 27.2, 25.9 (3×), 20.4, 19.1, 18.3, −5.3 (2×); MS (+CI) *m*/*z* (relative intensity) 509 ($M+H^+$, 9), 491 (M^+-OH, 65), 250 (100); HRMS (+FAB) calcd for $C_{24}H_{32}IO_2Si$ [$(M-H)^+$] 507.1216, found 507.1194.

4.2.7. (*E*)-2-[2′-(*tert*-Butyldimethylsilyloxy)-1′-phenylethylidene]-10-iododeca-3,9-diynal [(*E*)-13]. To a suspension of (*E*)-**12** (0.782 g, 1.5 mmol) and powdered 4Å molecular sieves in dry CH_2Cl_2 (7.7 mL) was added PDC (0.578 g, 3.1 mmol), followed by stirring at room temperature for 4 h. The reaction mixture was filtered through a short plug of silica gel with rinsing by CH_2Cl_2. The filtrate was concentrated under reduced pressure and the residue was purified by flash column chromatography (silica gel, 3% EtOAc–hexane) to give (*E*)-**13** (0.776 g, 100%) as a yellow oil; R_f = 0.60 (20% EtOAc–hexane); IR (neat) 2930, 2858, 2225, 2118, 1683, 1586, 1257, 1111 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.36 (s, 1H), 7.38–7.36 (m, 3H), 7.26–7.23 (m, 2H), 4.90 (s, 2H), 2.56–2.51 (m, 2H), 2.46–2.42 (m, 2H), 1.76–1.70 (m, 4H), 0.76 (s, 9 H), −0.02 (s, 6H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 190.5, 165.6, 134.6, 129.6 (2×), 128.9, 127.8 (2×), 122.6, 100.7, 93.9, 73.5, 68.6, 65.3, 27.7, 27.6, 25.7 (3×), 20.5, 19.4, 18.2, −5.3 (2×); MS (+CI) *m*/*z* (relative intensity) 507 ($M+H^+$, 100); HRMS (+FAB) calcd for $C_{24}H_{30}IO_2Si$ [$(M-H)^+$] 505.1060, found 505.1073.

4.2.8. (*E*)-4-[2′-(*tert*-Butyldimethylsilyloxy)-1′-phenylethylidene]cyclodeca-1,5-diyn-3-ol (1). To a suspension of anhydrous $CrCl_2$ (0.134 g, 1.10 mmol) and $NiCl_2$ (0.048 g, 0.37 mmol) in dry THF (74 mL) was added (*E*)-**13** (0.187 g, 0.37 mmol) in dry THF (10 mL), followed by stirring at room temperature for 8 h. The reaction was quenched with cold water (30 mL) and brine, and extracted with EtOAc (3× 50 mL). The combined organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, 10% EtOAc–hexane) to give **1** (34.2 mg, 24%) as a yellow oil; R_f = 0.46 (20% EtOAc–hexane); IR (neat) 3436, 2928, 2856, 2213, 1462, 1361, 1254, 1095 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38–7.27 (m, 5H), 5.42 (s, 1H), 4.71 and 4.64 (ABq, J = 12.3 Hz, 2H), 2.58–2.18 (m, 4H), 1.85–1.64 (m, 4H), 0.78 (s, 9H), −0.04 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 146.1, 137.2, 128.8 (2×), 127.8 (2×), 127.4, 124.1, 101.7, 91.8, 82.7, 79.5, 66.6, 64.0, 27.7 (2×), 25.8 (3×), 21.7, 20.7, 18.3, −5.2 (2×); MS (+CI) *m*/*z* (relative intensity) 381 ($M+H^+$, 10), 363 (M^+-OH, 95), 249 ($M^+-OSiMe_2t$-Bu, 100), 233 (15), 231 (18), 221 (37); HRMS (+FAB) calcd for $C_{24}H_{33}O_2Si$ ($M+H^+$) 381.2250, found 381.2248.

4.2.9. (*E*)-3-Acetoxy-4-[2′-(*tert*-butyldimethylsilyloxy)-1′-phenylethylidene]cyclodeca-1,5-diyne (2). To a solution of **1** (47.7 mg, 0.13 mmol) and DMAP (15.3 mg, 0.13 mmol) in dry CH_2Cl_2 (1.3 mL) was added acetic anhydride (24 μL, 0.25 mmol), followed by stirring at room temperature for 50 min. The reaction mixture was filtered through a short plug of silica gel with rinsing by CH_2Cl_2. The filtrate was concentrated under reduced pressure and the residue was purified by flash column chromatography (silica gel, 5% EtOAc–hexane) to give **2** (44.4 mg, 84%) as a pale brown oil; R_f = 0.60 (20% EtOAc–hexane); IR (neat) 2930, 1220, 1092 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35–7.23 (m, 3H), 7.12–7.06 (m, 2H), 5.53 (s, 1H), 4.71 and 4.64 (ABq, J = 12.3 Hz, 2H), 2.58–2.15 (m, 4H), 1.95 (s, 3H), 1.86–1.64 (m, 4H), 0.78 (s, 9H), −0.07 (s, 6H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 169.3, 149.0, 137.0, 128.6, 128.2 (2×), 127.8 (2×), 127.6, 119.8, 102.4, 92.3, 79.3, 66.6, 65.4, 27.7 (2×), 25.8 (3×), 21.8, 21.0, 20.8, 18.3, −5.3 (2×); MS (+CI) *m*/*z* (relative intensity) 423 ($M+H^+$, 5), 363 (M^+-OAc, 100), 249 (10), 233 (14), 231 (10); HRMS (+FAB) calcd for $C_{26}H_{35}O_3Si$ ($M+H^+$) 423.2355, found 423.2352.

4.2.10. (*E*)-4-[2′-(*tert*-Butyldimethylsilyloxy)-1′-phenylethylidene]-3-(methoxyacetoxy)-cyclodeca-1,5-diyne (3). To a solution of **1** (50.2 mg, 0.13 mmol), DCC (0.272 g, 1.3 mmol), and DMAP (0.161 g, 1.3 mmol) in dry CH_2Cl_2 (1.3 mL) was added methoxyacetic acid (0.10 mL, 1.3 mmol), followed by stirring at room temperature for 3 days. The reaction mixture was filtered through a short plug of silica gel with rinsing by CH_2Cl_2. The filtrate was concentrated under reduced pressure and the residue was purified by flash column chromatography (silica gel, 5% EtOAc–hexane) to give **3** (30.9 mg, 52%) as a colorless oil; R_f = 0.46 (20% EtOAc–hexane); IR (neat) 2929, 2856, 2236, 2212, 1761, 1463, 1254, 1178, 1126 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31–7.24 (m, 3H), 7.10–7.07 (m, 2H), 5.65 (d, J = 2.1 Hz, 1H), 4.70 and 4.63 (ABq, J = 12.3 Hz, 2H), 3.96 and 3.84 (ABq, J = 16.5 Hz, 2H), 3.38 (s, 3H), 2.55–2.14 (m, 4H), 1.86–1.50 (m, 4H), 0.78 (s, 9H), −0.07 (s, 6H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 168.5, 149.3, 136.9, 128.1 (2×), 127.9 (2×), 127.7, 119.5, 102.5, 92.8, 79.3, 78.9, 69.5, 66.6, 65.6, 59.4, 27.7 (2×), 25.8 (3×), 21.7, 20.8, 18.3, −5.3 (2×); MS (+CI) *m*/*z* (relative intensity) 470 ($M+NH_4^+$, 21), 380 [$M-C(O)CH_2OCH_3+H^+$, 100], 363 [$M^+-OC(O)CH_2OCH_3$, 81], 250 (27), 233 (24); HRMS (+FAB) calcd for $C_{27}H_{37}O_4Si$ ($M+H^+$) 453.2462, found 453.2448.

4.2.11. (*E*)-3-Acetoxy-4-(2′-hydroxy-1′-phenylethylidene)cyclodeca-1,5-diyne (4). To a solution of **2** (32.9 mg, 7.8×10^{-2} mmol) in MeOH−H_2O (10:1, 1.1 mL) was added PPTS (2.0 mg, 7.8×10^{-3} mmol), followed by stirring at room temperature for 16 h. The reaction mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel. 30% EtOAc–hexane) to give **4** (22.0 mg, 92%) as a colorless oil; R_f = 0.57 (50% EtOAc–hexane); IR (neat) 3445, 2929, 2859, 2237, 2215, 1739, 1371, 1221 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35–7.29 (m, 3H), 7.18–7.12 (m, 2H), 5.57 (s, 1H), 4.69 and 4.63 (ABq, J = 12.9 Hz, 2H), 2.60–2.16 (m, 4H), 1.96 (s, 3H), 1.90–1.66 (m, 4H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 169.2, 148.4, 136.6, 128.4 (2×), 128.1, 127.8 (2×), 120.7, 103.2, 92.5, 79.1, 66.5, 65.3, 27.6 (2×), 21.7, 20.9, 20.8; MS (+CI) *m*/*z* (relative

intensity) 291 (M^+-OH, 30), 249 (M^+–OAc, 100), 233 (17), 231 (47), 221 (86); MS (+FAB) *m/z* (relative intensity) 309 ($M+H^+$, 10), 249 (M^+-OAc, 100), 231 (85), 221 (100); HRMS (+FAB) calcd for $C_{20}H_{21}O_3$ ($M+H^+$) 309.1491, found 309.1486.

4.2.12. (*E*)-4-(2′-Hydroxy-1′-phenylethylidene)-3-(methoxyacetoxy)cyclodeca-1,5-diyne (5). To a solution of **3** (20.5 mg, 4.5×10^{-2} mmol) in MeOH−H_2O (10:1, 1.7 mL) was added PPTS (1.1 mg, 4.5×10^{-3} mmol), followed by stirring at room temperature for 16 h. The reaction mixture was concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, 30% EtOAc–hexane) to give **5** (15.3 mg, 100%) as a colorless oil; $R_f = 0.49$ (50% EtOAc–hexane); IR (neat) 3436, 2930, 2860, 2236, 2212, 1757, 1183, 1124 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$). δ 7.40–7.30 (m, 3H), 7.15–7.13 (m, 2H), 5.69 (s, 1H), 4.67 and 4.61 (ABq, $J = 12.6$ Hz, 2H), 3.96 and 3.84 (ABq, $J = 16.5$ Hz, 2H), 3.38 (s, 3H), 2.55–2.13 (m, 4H), 1.83–1.64 (m, 4H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 168.5, 148.6, 136.5, 128.5 (2×), 128.3, 127.7 (2×), 120.4, 103.3, 93.1, 79.0, 78.7, 69.4, 66.5, 65.5, 59.4, 27.6 (2×), 21.7, 20.8; MS (+CI) *m/z* (relative intensity) 356 ($M+NH_4^+$, 18), 266 [$M-C(O)CH_2OCH_3+H^+$, 100], 249 [$M^+-OC(O)CH_2OCH_3$, 43], 233 (28), 231 (8). 221 (6); HRMS (+FAB) calcd for $C_{21}H_{23}O_4$ ($M+H^+$) 339.1596, found 339.1604.

4.3. DNA cleaving assay

Photoirradiation at 365 nm was carried out using a ULTRA-VIOLET PRODUCTS NTFL-40 transilluminator. An ATTO sequencing gel electrophoresis apparatus was used for agarose gel electrophoresis. The gels were analyzed by scanning densitometry with an ATTO Lane Analyzer (version 3). DNA cleavage studies on the enediyne prodrugs **1**–**5** were performed by the use of supercoiled, covalently closed, circular ΦX174 RFI double-stranded DNA (Form I). Solution of 50.0 μM/bp (micromolar per base pair) of ΦX174 RFI DNA and the enediyne prodrugs at various concentrations in TAE buffer (pH 8.5) containing 20% DMSO (total volume 10 μL) was incubated at 37 °C for the indicated times without or with UV-irradiation using a transilluminator ($\lambda_{ex} = 365$ nm). The resultant mixtures were then analyzed by gel electrophoresis (1% agarose gel, ethidium bromide stain). DNA cleavage was determined by the formation of relaxed circular DNA (Form II) and linearized DNA (Form III). The gels were placed on a UV transilluminator ($\lambda_{ex} = 365$ nm) and photographed with Polaroid 667 film. The relative densities of various DNA bands on the gel pictures were quantified by scanning densitometry with an ATTO Lane Analyzer. Since Form III DNA was not observed in this study, the percentage of net DNA cleavage was calculated by the following equation: $\{[(\text{Form II})_s/[(\text{Form I})_s + (\text{Form II})_s]\times100\} - \{(\text{Form II})_c/[(\text{Form I})_c + (\text{Form II})_c]\times100\}$. The subscripts 's' and 'c' refer to as the samples and control(s), respectively. The results are given in Figures 2–4.

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Supplementary data

Figures S1–S6 for the NOE data of the compounds **8**–**13**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2005.12.040.

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