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Synthesis of bicyclic enediynes that possess a photosensitive triggering device and exhibit strong DNA cleaving activity†

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The synthesis of a bicyclic enediyne capable of photosensitive triggering and conjugated to a pyrrole-imidazole polyamide is described. Using UV irradiation, this hybrid molecule is shown to exhibit 100-fold stronger potency for DNA cleavage, in an *in vitro* assay, as compared to the enediyne without the DNA-localizing pyrrole-imidazole.

DNA-cleaving molecules not only serve as anticancer drug candidates; they also are useful as chemical probes for the modulation of cell functions.[1] Enediyne antitumor antibiotics are a family of natural products exhibiting DNA cleavage activity.[2] These products are stable in their natural form and undergo Masamune–Bergman cyclization of the enediyne moiety in a 9- or 10-membered ring to provide a benzene biradical after chemical activation.[3] The benzene biradical abstracts the hydrogen in a DNA sugar backbone and results in DNA cleavage. There are many reports on the synthesis of artificial enediyne derivatives, which can generate a biradical by chemical activation.[4] We have reported on the synthesis of 9- and 10-membered enediyne precursors, which undergo β-elimination under ambient conditions to generate highly reactive enediynes.[5] However, the spatial and temporal control of enediyne reactivity towards cycloaromatization is difficult to achieve.

Photopromoted cycloaromatization is an effective way to control the biradical formation of enediynes.[6] This involves the use of stable precursors in the dark, which generate a biradical by irradiation at an appropriate wavelength. The direct UV irradiation of the enediyne moiety enables the acceleration of its cycloaromatization.[7] However, the efficiency of the activation method is low. On the other hand, biradical formation by irradiation of a photosensitive triggering device of stable enediynes or enediyne precursors is an alternative, and effective, method for the control of biradical formation. Nicolaou and co-workers first demonstrated the photo-induced cycloaromatization of a dynemycin analogue possessing an *o*-nitrobenzyl ether as a photosensitive triggering device.[8] Popik and co-workers recently reported on a cyclopropenone-containing enediyne precursor.[9] The cyclopropenone acts as a precursor of acetylene. Herein, we report on the synthesis of bicyclic enediynes that possess photosensitive triggering devices and exhibit strong DNA cleavage activity.

We designed the *trans*-fused bicyclic 10-membered enediyne **1** possessing an *o*-nitrobenzyl ether as a photosensitive triggering device with an azido group as a connecting device (Scheme 1). The alkyloxy substituents of the *o*-nitrobenzyl group improved the sensitivity toward UV irradiation.[10] UV irradiation of the enediyne **1** promoted cleavage of the *o*-nitrobenzyl ether to provide the *trans*-fused hemiketal **3**. After epimerization of the *trans*-fused hemiketal **3** to the *cis*-fused hemiketal **4**, the *cis*-fused hemiketal **4** could undergo cycloaromatization to generate a *p*-benzene biradical. Transition state analysis of Masamune–Bergman cyclization of the *trans*- and the *cis*-fused hemiketals **3** and **4** to **5** and **6** based on DFT calculation at the B3LYP/6-31G(d) level by Gaussian 03[11] indicated that transition states **TS-B** of Bergman cyclization of the *cis*-fused enediyne **4** would be more favorable than **TS-A** of the *trans*-fused enediyne **3** ($\Delta\Delta H$ = 24.9 kJ mol^{-1}). These results suggested that the photopromoted cleavage of the ketal can initiate the generation of a biradical from **1**. In addition, copper-catalyzed Huisgen [3 + 2] cycloaddition can use the azido group for coupling with DNA binding molecules **2** that possess a terminal alkyne.[12,13]

The synthesis of a bicyclic enediyne bearing an azido group is shown in Scheme 2. Treatment of the terminal alkene **7** with butyllithium at −78 °C for 30 min, followed by addition of 1.5 equivalents of butenoride **8** afforded hemiketal **9** in 78% yield. The ethoxyethyl ether at the C2 position of **8** is important for yielding the mono adduct **9**. Treatment of the hemiketal **9** with 1,1,1-trimethoxyethane and a catalytic amount of PPTS in MeOH at room temperature resulted in removal of the ethoxyethyl ethers and ketalization with methanol to provide the methyl ketal **10** in 85% yield. The methyl ether **10** was converted to the *o*-nitrobenzyl ether **12** by treatment with benzyl alcohol **11** under acidic conditions in the presence of MS5A (47%). The primary alcohol **12** was converted to bromide **13** in 82% yield. Cyclization of bromoalcohol **13** was examined. A solution of bromoalcohol **12** was slowly

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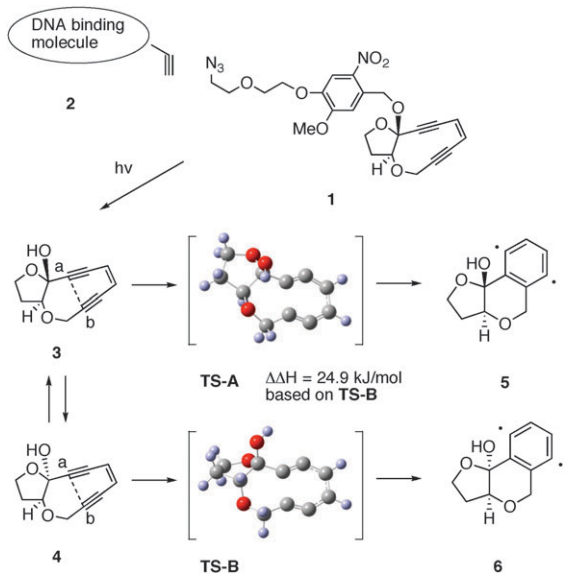
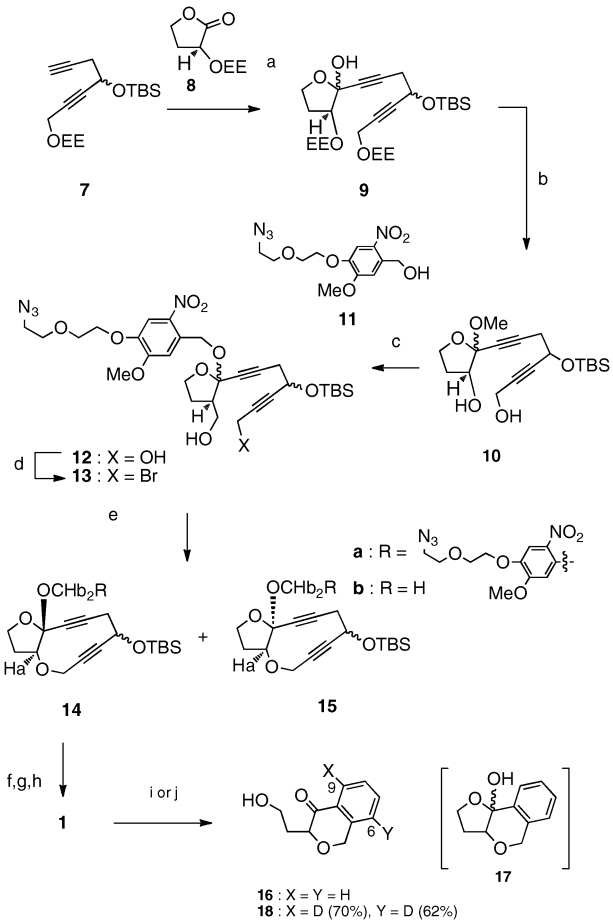


Scheme 1 Design of a bicyclic enediyne **1** with a photosensitive triggering device.

added to a suspension of NaH in THF in the presence of a small amount of H_2O at room temperature to provide the *trans*- and *cis*-fused 10-membered cyclic ethers **14a** and **15a** in 30% and 12% yields, respectively. The relative stereochemistry of the bicyclic ethers **14a** and **15a** was determined by comparison with the ^{1}H NMR spectra of the corresponding methyl derivatives **14b** and **15b**, the relative stereochemistries of which were determined by the NOE experiments.[14] Removal of the TBS ether from **14a** and mesylation of the secondary alcohol, followed by β-elimination of the resulting mesyl ester treated with DBU at room temperature, provided the *trans*-fused 10-membered enediyne **1** in 32% yield. The enediyne **1** was stable enough to stock as an acetonitrile solution at −20 °C for more than a month. In addition, it should be noted that the *cis*-fused cyclic ether **15a** was not converted to the corresponding *cis*-fused enediyne by the same procedure.

The photochemical activation of enediyne **1** was examined. UV light irradiation (360 nm) to a solution of enediyne **1** in THF : H_2O (3 : 1) for 30 min at 0 °C, followed by stirring at room temperature for 30 min without UV irradiation provided the aromatic product **16** in 25% yield instead of the tricyclic product **17**. Use of THF-d_8 : H_2O (3 : 1) as a solvent for the reaction resulted in the aromatic product **18** in 25% yield with 62% and 70% deuteration at the 6 and 9 positions, respectively. However, comparison with the reactivity of the enediynes **3** and **4** towards the cycloaromatization failed because the difficulty of isolation of **3** and **4**.

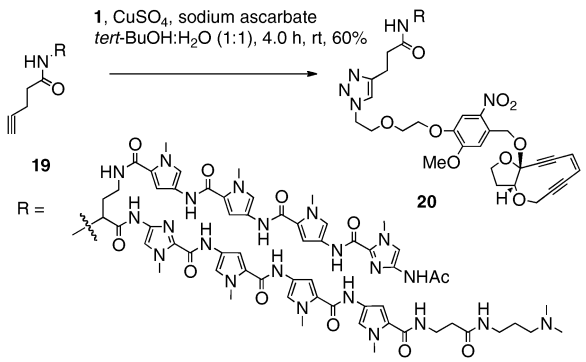
To demonstrate the utility of the enediyne **1**, the synthesis of the 10-membered enediyne **20** conjugated with a hairpin pyrrole-imidazole polyamide through an *o*-nitrobenzyl ether was examined (Scheme 3). The pyrrole-imidazole polyamide unit acted as an effective miner groove binder.[15,16] The enediyne **1** was treated with 1.5 equivalents of the pyrrole-imidazole polyamide **19** bearing a terminal alkyne in the presence of $CuSO_4$ (1.0 equiv.) and sodium ascorbate (2.0 equiv.) in *tert*-BuOH : H_2O (1 : 1) for 4 h at room temperature. Purification



Scheme 2 *Reagents and conditions*: (a) butyllithium, THF, −78 °C, 30 min then **8**, THF, 78%; (b) 1,1,1-trimethoxyethane, cat. PPTS, MeOH, 85%; (c) **11**, PPTS, MS5A, CH_2Cl_2, 47%; (d) CBr_4, PPh_3, 2,6-lutidine, rt, 82%; (e) NaH, THF, rt, 30% for **14**, 12% for **15**; (f) TBAF, THF; (g) MsCl, NEt_3, CH_2Cl_2, 0 °C; and, (h) DBU, THF, rt. 33% for **1**. (i) UV irradiation at 360 nm, THF : H_2O (3 : 1), 5 °C, 30 min, then rt, 30 min; (j) UV irradiation at 360 nm, THF-d_8 : H_2O (3 : 1), 5 °C, 30 min, then rt, 30 min

of the reaction mixture by a reverse phase HPLC provided the conjugated enediyne **20** in 60% yield.

DNA cleavage experiments using enediyne **1** were examined (Fig. 1a). A Form I DNA was incubated with enediyne **1** (1.0 mM) under UV irradiation (365 and 302 nm) for 5, 10 and



Scheme 3 Synthesis of the hybrid enediyne **20** by the coupling of bicyclic enediyne **1** with a pyrrole-imidazole polyamide **19**.

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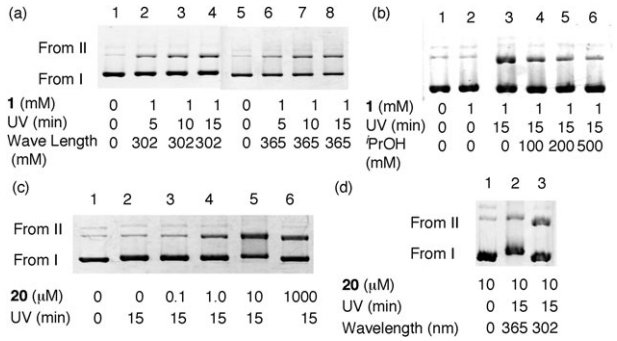

Fig. 1 Cleavage of pGEX-4T-1 plasmid DNA by enediynes **1** and **20**. (a) Lanes 1 and 5: DNA alone incubated in the dark for 15 min at 23 °C; lanes 2–4: DNA incubated with 1 mM of **1** and UV irradiation at 302 nm for 5, 10, and 15 min; and, lanes 6–8: DNA incubated with 1 mM of **1** and UV irradiation at 365 nm for 5, 10 and 15 min. (b) Lane 1: DNA alone incubated for 15 min at 23 °C; lane 2: DNA incubated in the dark with 1 mM of **1**; lanes 3–6: DNA incubated with 0, 100, 200, and 500 mM of ^{i}PrOH and 1 mM of **1** and UV irradiation at 302 nm. (c) Lane 1: DNA alone incubated in the dark for 15 min at 23 °C; lanes 2–5: DNA incubated with 0, 0.1, 1.0, and 10 μM of **20** and UV irradiation at 302 nm; lane 6: DNA incubated with 1 mM of compound **1** and UV irradiation at 302 nm. (d) Lane 1: DNA alone incubated in the dark for 15 min at 23 °C; lanes 2 and 3: DNA incubated with 10 μM of **20** and UV irradiation at 365 nm and 302 for 15 min, respectively.

15 min. The mixtures were directly analyzed by agarose gel electrophoresis. The enediyne **1** did not cause DNA cleavage without UV irradiation (lanes 1 and 5). UV irradiation promoted Form I DNA cleavage to Form II (lanes 2–4 and 6–8). In addition, no significant differences were observed in the cleavage of DNA by enediyne **1** regardless of whether UV irradiation was at 302 or 365 nm. The effect of isopropyl alcohol as a radical scavenger on the DNA cleavage of **1** is shown in Fig. 1b. Isopropyl alcohol reduced the generation of Form II DNA from Form I DNA. These results suggest that radical species cause DNA cleavage.

DNA cleavage experiments using enediyne **20** were examined. (Fig. 1c). Incubation of Form I DNA with enediyne **20** at varying concentrations (0, 0.1, 1.0, and 10 μM) under UV irradiation (302 nm) resulted in cleavage to Form II (lanes 3–5). Lane 6 showed DNA cleavage of non-hybrid molecule **1** (1000 μM). These results indicate that the conjugated enediyne **20** exhibited more than 100-fold stronger DNA cleavage activity compared with non-conjugated enediyne **1**. We additionally found an unexpected effect of UV wavelength on the DNA cleavage of **20**. UV irradiation at 302 nm was more effective for the cleavage of DNA by the hybrid enediyne **20** (Fig. 1d). However, the mechanism responsible for the difference is not yet clear.

In conclusion, we described the synthesis of a hybrid bicyclic enediyne **20** possessing a photosensitive triggering device and its pyrrole-imidazole polyamide. The *trans*-fused bicyclic enediyne **1** is stable in the dark and undergoes Masamune–Bergman cyclization after photochemical activation initiated by cleaving the *o*-nitrobenzyl ether. The azido moiety of cyclic enediyne **1** can be used for conjugation with DNA binding molecules, such as pyrrole-imidazole polyamide, by copper-catalyzed Huisgen [3 + 2] cycloaddition. The pyrrole-imidazole polyamide hybrid **20** exhibited more than 100-fold stronger DNA cleaving activity than **1**.

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