NOVEL HETEROCYCLIC ENEDIYNES. MOLECULAR DESIGN, CHEMICAL SYNTHESIS AND DNA CLEAVAGE

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Abstract-The novel 10-membered oxaenediyne (**4a**) and azaenediynes (**5a~d**) were designed and synthesized in a short procedure, and the azaenediynes (**5a~d**) were found to effectively cleave DNA under both weakly acidic and basic conditions with no additive.

DNA cleaving molecules, particularly those with a simple structure and high efficiency, have considerable potential in chemistry, molecular biology, and medicine.[1] Therefore, much attention has been directed towards the design and synthesis of novel DNA cleaving molecules in relation to the powerful anticancer and DNA cleaving enediyne antibiotics such as neocarzinostatin, calicheamicins, esperamicins, dynemicins, kedarcidin, and C-1027.[2] These molecules undergo Masamune-Bergman or Saito-Myers cyclization under suitable conditions to generate benzenoid diradicals which can then damage DNA.[2] We recently reported that the novel 10-membered thiaenediynes (**1**) were synthesized with high stability and produced the diradical (**3**) *via* the cyclization of the ene-yne-allene (**2**) under basic conditions as shown in Figure 1.[3] Furthermore, we found that some of those possessing a DNA intercalatable moiety effectively cleave DNA under weakly basic conditions without any additive presumably by an alkylation mechanism rather than by a radical mechanism.[3c]

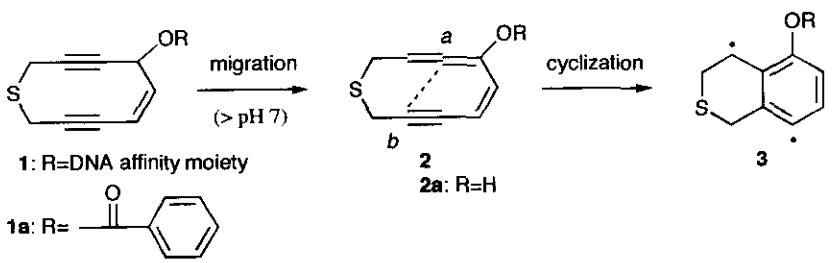


Figure 1

In this communication, we report the molecular design, chemical synthesis and DNA cleaving activity of the novel heterocyclic enediynes, oxaenediyne[4] (**4**) and azaenediyne[5] (**5**) (Figure 2).[6]

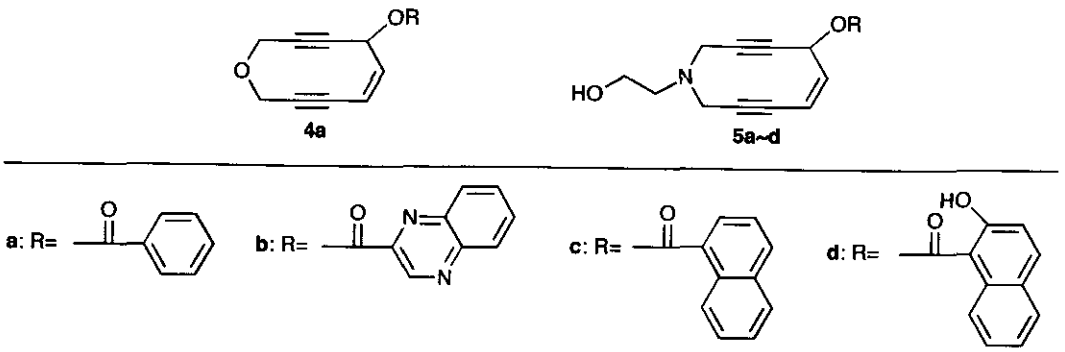
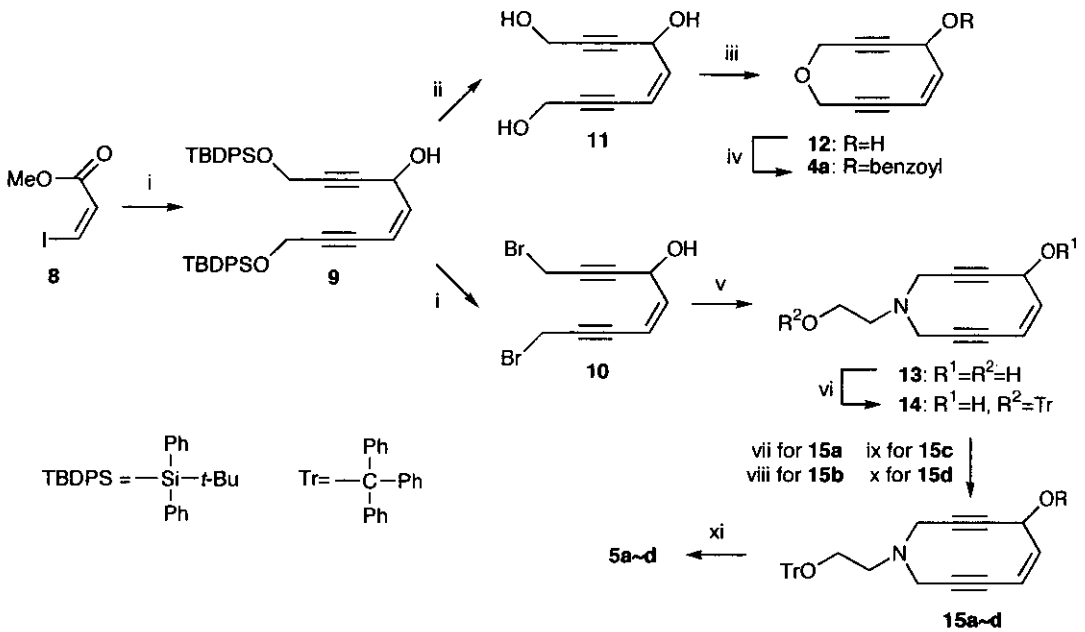


Figure 2

According to Nicolaou's report,[7] the *ab* distances of the ene-yne-allenes (**6**) and (**7**) (Figure 3), which are the key precursors for the cyclization, are important factors for the spontaneous cyclizations that generate the benzenoid diradicals at ambient temperature and must be within *ca.* 3.3 Å. Molecular calculations[8] indicated that the *ab* distances of the oxa-ene-yne-allene (**6**) and the aza-ene-yne-allene (**7**) were 3.08 Å (by AM1) or 3.10 Å (by PM3) and 3.10 Å (by AM1) or 3.13 Å (by PM3), respectively, and these distances were shorter than that of the thia-ene-yne-allene (**2a**).[3c] Considering these points, the novel heterocyclic enediynes (**4**) and (**5**) were expected to have better chemical structures for effective DNA cleavage compared to **1**.

Figure 3

Our synthetic approach for these novel enediynes began with the conversion of the *cis*-vinyl iodide (**8**) into the acyclic enediynes (**9**) and (**10**) in 4 and 7 steps, respectively, using procedures[3] recently developed in our laboratories (Scheme 1). After several abortive attempts, the 10-membered oxaenediyne skeleton was finally constructed by the intramolecular cyclization of the enediyne triol (**11**). Thus, the intramolecular cyclization of **11** prepared from **9** was best effected by using CCl_4 and Ph_3P[9] in DMF at 25 °C for 5 h to afford the 10-membered oxaenediyne (**12**) in 68% yield. Acylation of **12** with a benzoyl group gave the oxaenediyne (**4a**) in 35% yield. On the other hand, the intermolecular cyclyzation of **10** and ethanolamine in the presence of Na_2CO_3 in EtOH at 50 °C under high dilution conditions proceeded smoothly to give the 10-membered azaenediyne (**13**) in 62% yield. After selective protection of the primary alcohol of **13** with a trityl group, several DNA intercalatable aromatic compounds were introduced into the secondary alcohol of **14** to afford the acylated azaenediyne (**15a~d**). Finally, deprotection of the trityl group in **15a~d** under acidic conditions furnished the desired azaenediyne (**5a~d**) in good yields.



Scheme 1 *Reagents and conditions*: i, ref. 3; ii, *n*-Bu_4NF (2.5 equiv), THF, 0 °C, 20 min, 100%; iii, CCl_4 (2.0 equiv), Ph_3P (2.0 equiv), DMF (0.12 M for **11**), 25 °C, 5 h, 68 %; iv, BzCl (2.0 equiv), Et_3N (2.3 equiv), CH_2Cl_2, 25 °C, 0.5 h, 35%; v, $HOCH_2CH_2NH_2$ (1.3 equiv), Na_2CO_3 (1.5 equiv), EtOH (0.004 M for **10**), 50 °C, 48 h, 62%; vi, TrCl (1.5 equiv), Et_3N (2.5 equiv), CH_2Cl_2, 25 °C, 1.5 h, 94%; vii, BzCl (1.5 equiv), Et_3N (2.5 equiv), CH_2Cl_2, 25 °C, 0.5 h, 100%; viii, 2-quinoxaloyl chloride (1.5 equiv), Et_3N (2.5 equiv), CH_2Cl_2, 25 °C, 0.5 h, 100%; ix, 1-naphthoyl chloride (2.0 equiv), Et_3N (2.5 equiv), CH_2Cl_2, 25 °C, 0.5 h, 79%; x, 2-hydroxynaphthalene-1-carboxylic acid (1.1 equiv), WSC·HCl (4.0 equiv), 25 °C, 1 h, 100%; xi, 10% HCl-MeOH, THF-MeOH, 25 °C, 8 h, 95% for **5a**, 65% for **5b**, 72% for **5c**, 66% for **5d**.

The DNA cleaving activities of the novel enediynes (**4a**), (**5a~d**) and (**13**) were assayed with supercoiled ΦX174 DNA (form I) in 20% acetonitrile-Tris-HCl buffer. We first examined the DNA cleaving activities of **4a** and **5a**, both of which have a benzoyl group, under weakly basic and acidic conditions. These results are shown in Figure 4. As expected from the results of the corresponding thiaenediyne (**1a**),[3] the azaenediyne (**5a**) effectively cleaved DNA under weakly basic conditions with no additive and the activity was similar to that of **1a**. Remarkably, **5a** was further found to cleave DNA under weakly acidic conditions and the activity was stronger than that under basic conditions while the thiaenediyne (**1a**) did not show DNA cleaving activity under acidic conditions.[3] On the other hand, unexpectedly, the oxaenediyne (**4a**) only slightly cleaved DNA even at high concentration (10 mM) under both basic and acidic conditions, but these activities were much lower than those of **5a**. The low activity of **4a** may be due to its instability under both basic and acidic conditions, and the high activity of **5a** under acidic conditions may result from the protonated nature that brings about a strong affinity for DNA. The DNA cleaving activities of several azaenediynes (**13**) and (**5a~d**) under weakly basic and acidic conditions are shown in Figures 5 and 6, respectively. It was found that all the azaenediynes (**5a~d**) possessing an aromatic moiety cleaved DNA much more effectively than **13**, and form III DNA (linear DNA) appeared along with form II DNA (open circular DNA) at high concentrations. Interestingly, under acidic conditions, **5a** and **5c** caused strong

cleavage of DNA leading to its small fragments which could not be detected by the electrophoresis technique (lane 2 in Figure 4-(*b*) and lanes 3 and 5 in Figure 6-(*a*)). Furthermore, it was found that the DNA cleaving activity was dependent on the introduced aromatic moiety.

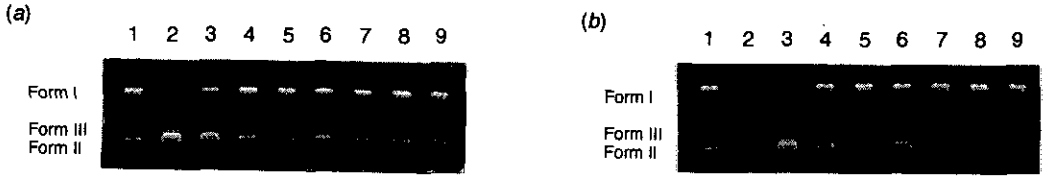


Figure 4 ΦX174 form I DNA (50 μmol dm^{-3} per base pair) was incubated at 37 °C and at (*a*) pH 8.5 and (*b*) pH 6.5 for 24 h with **4a** and **5a** in 20% acetonitrile-Tris-HCl buffer (50 mmol dm^{-3}) and analysed by electrophoresis (0.9% agarose gel, ethidium bromide stain). Lane 1, DNA alone; lanes 2-9: **5a** (10000), **5a** (1000), **5a** (100), **5a** (10), **4a** (10000), **4a** (1000), **4a** (100) and **4a** (10 μmol dm^{-3}), respectively.

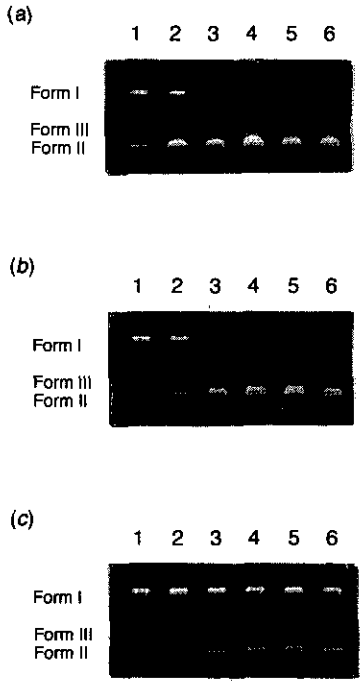


Figure 5 ΦX174 form I DNA (50 μmol dm^{-3} per base pair) was incubated at (*a*) 10 mmol dm^{-3}, (*b*) 1 mmol dm^{-3} and (*c*) 100 μmol dm^{-3} and at 37 °C for 24 h with **13** and **5a-d** in 20% acetonitrile-Tris-HCl buffer (pH 8.5, 50 mmol dm^{-3}) and analysed by electrophoresis (0.9% agarose gel, ethidium bromide stain). Lane 1, DNA alone; lanes 2-6: **13**, **5a**, **5b**, **5c** and **5d**, respectively.

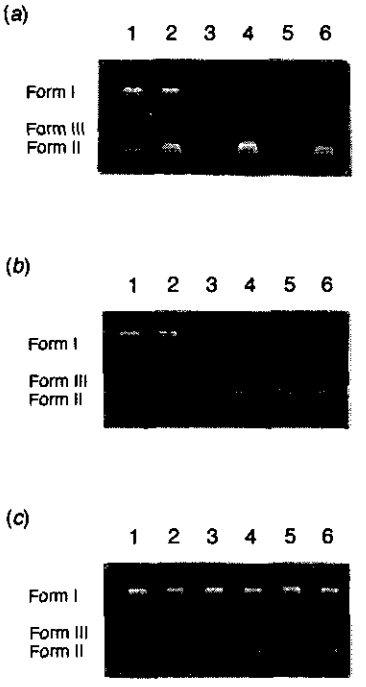


Figure 6 ΦX174 form I DNA (50 μmol dm^{-3} per base pair) was incubated at (*a*) 10 mmol dm^{-3}, (*b*) 1 mmol dm^{-3} and (*c*) 100 μmol dm^{-3} and at 37 °C for 24 h with **13** and **5a-d** in 20% acetonitrile-Tris-HCl buffer (pH 6.5, 50 mmol dm^{-3}) and analysed by electrophoresis (0.9% agarose gel, ethidium bromide stain). Lane 1, DNA alone; lanes 2-6: **13**, **5a**, **5b**, **5c** and **5d**, respectively.

In summary, we have succeeded in the design and synthesis of the highly strained novel 10-membered oxaenediyne (**4**) and azaenediyne (**5**), and found that azaenediyne (**5**) showed effective DNA cleaving activity under both weakly basic and acidic conditions without the addition of any activator. At this stage, the mechanism of DNA cleavage by **5** is not clear, and further study to elucidate the precise mechanism of DNA cleavage and introduction of the novel DNA cleaving moiety into the sequence-specific delivery system are now under investigation.

ACKNOWLEDGEMENTS

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