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# Synthesis and DNA damaging ability of enediyne–polyamine conjugates

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**Abstract**—Polyamine–enediyne conjugates were synthesized and exhibited potent DNA damaging ability under physiological conditions. The extent of their activity was shown to depend upon the polyamine length that regulates the DNA binding affinity of the conjugates, and enhanced DNA damaging activities were observed under slightly acidic conditions.


Polymethylene polyamines, such as spermidine and spermine are natural products and have interesting biological activities. Recently, due to their significant biological activities, a variety of artificially designed polyamines and their conjugates have been developed.[1] Since polyamines interact with the phosphate moiety of nucleic acid purely by charge interactions and by hydrogen bonding in their cationic form, these compounds constitute a class of DNA binding molecules. The increasing interest in hybrid molecules composed of polyamines and known anticancer drugs, intercalates and other biological active agents is due to efforts to enhance the activity and specificity of these drugs.[2] In addition, a polyamine transporter specifically mediates the uptake of extracellular polyamines into cells and this transporter is up-regulated in tumor cells, which require large amounts of polyamines; therefore polyamines themselves continue to attract significant attention as potential anticancer drugs.[3] Recently, we developed artificially designed enediyne drugs possessing characteristic triggering devices.[4] In the course of our studies, we investigated regulation of the reactivity of α,3-didehydrotoluene biradicals which are prone to act as zwitter ionic species having low bioactivities,[5] and reported that α,3-didehydrotoluene biradicals possessing electron withdrawing groups at the benzylic position exhibited enhanced radical characteristics.[6b,c] Previously, we reported the design, synthesis, and DNA cleaving activities of enediynes that we designed possessing a cyanohydrin moiety as a triggering device (Fig. 1).[6a]

Here, we report the synthesis and DNA damaging ability of the enediyne drugs **1**–**4** possessing a monoamine, a diamine, a triamine, and a tetraamine module, respectively, as a DNA binding group (Fig. 2).

Enediynes **1** and **5** were synthesized from a common precursor **8**, as shown in Scheme 1. The THP-protected cyanohydrin **6** was treated with LDA and propargylbromide followed by a Pd-mediated coupling

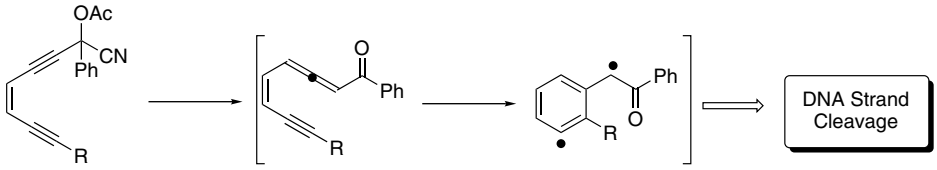


**Figure 1.**

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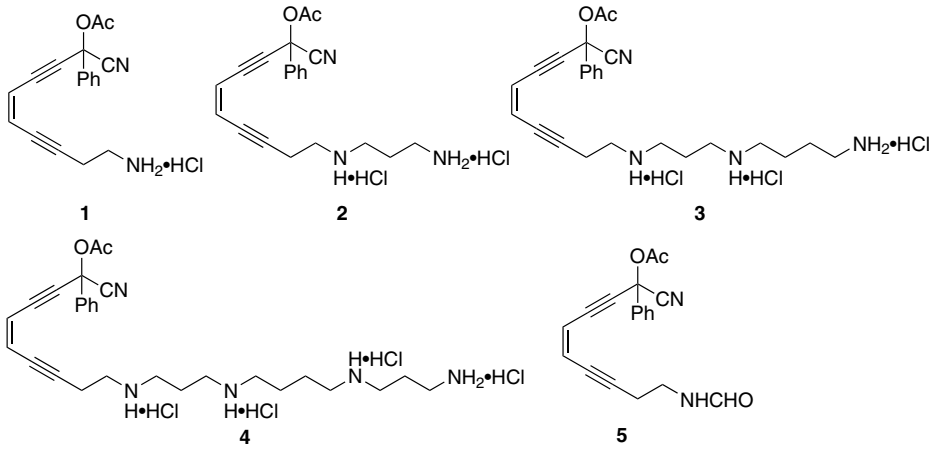
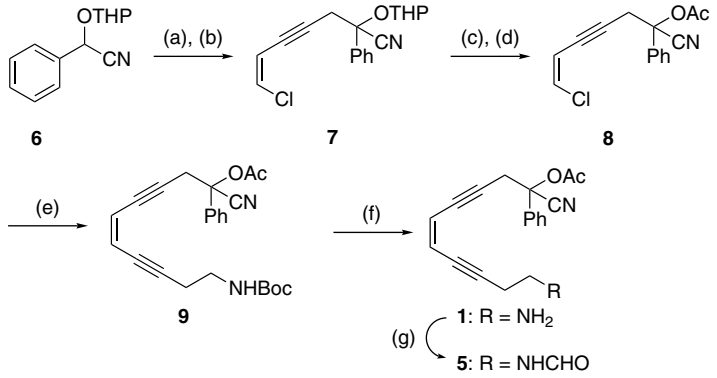



**Figure 2.**

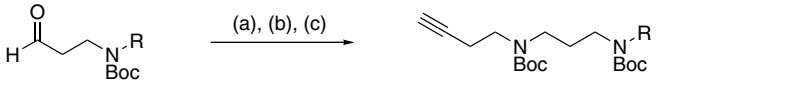



**Scheme 1.** Reagents and conditions: (a) LDA /THF-HMPA, −78 °C, then propargylbromide, −78 °C, 43%; (b) (*Z*)-dichloroethylene, $Pd(PPh_3)_4$, CuI, *n*-$BuNH_2/C_6H_6$, rt, 96%; (c) *p*-TsOH/MeOH, rt; (d) EDC, AcOH, DMAP/$CH_2Cl_2$, 0 °C, 78% (two steps); (e) *N*-Boc-1-amino-3-butyne, $Pd(PPh_3)_4$, CuI, $Et_2NH/C_6H_6$, rt, 95%; (f) HCl/1,4-dioxane, rt, 88%; (g) EDC, $HCO_2H$, DMAP/$CH_2Cl_2$, rt, 89%.

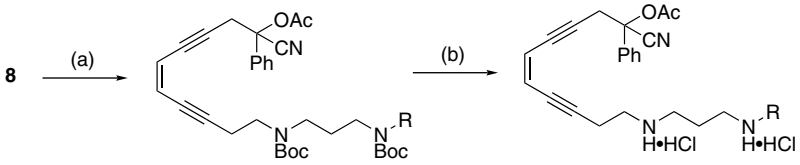
reaction with (*Z*)-dichloroethylene to give enyne **7**. After removal of the THP protection using *p*-TsOH, the resulting alcohol was acetylated to give enyne **8**. The common precursor **8** was coupled with *N*-Boc-1-amino-3-butyne[7] under the Sonogashira condition to afford enediyne **9**, which was treated with HCl to give enediyne **1**. The reference enediyne **5**, the amide group of which is incapable of playing the role of a DNA binding group, was prepared by formylation of enediyne **1**.

Polyamine–enediyne conjugates **2**–**4** were synthesized similarly to enediyne **1** using polyamine–alkynes **11a**–**11c**, which were prepared as described in Scheme 2. The parent aldehydes **10a**,[8] **10b**,[9] and **10c**[10] were treated with 1-amino-3-butyne to give the corresponding aldimines and subsequent reduction using $NaBH_4$ and Boc protection afforded polyamine–alkynes **11a**–**11c**. The common precursor **8** was coupled with polyamine–alkynes **11a**–**11c** in the presence of Pd catalyst to give enediynes **12a**–**12c**, which were treated with HCl in dioxane affording targeting enediynes **2**–**4**, as depicted in Scheme 3.

DNA cleaving assays of synthetic enediynes **1**–**5** were carried out using *Col E1* plasmid DNA in phosphate buffer solutions (pH 6.0 and 9.0) in the presence of the enediyne drugs (10–1000 μM) and the mixture was incubated for 24 h at 37 °C. The resultant DNA fragments were separated by electrophoresis on agarose gel and visualized by ethidium bromide staining. The results are summarized in Figure 3 (pH 6.0) and Figure 4 (pH 9.0).




**Scheme 2.** Reagents and conditions: (a) 1-amino-3-butyne, $MgSO_4/CHCl_3$, rt; (b) $NaBH_4$/MeOH, 0 °C; (c) $(Boc)_2O$, aq NaOH (1 M)/1,4-dioxane, rt.




**12a**: R =H (58%)
**12b**: R = $(CH_2)_4NHBoc$ (73%)
**12c**: R = $(CH_2)_4NBoc(CH_2)_3NHBoc$ (68%)

**2**: R =H (77%)
**3**: R = $[(CH_2)_4NH_2]$•HCl (65%)
**4**: R = $[(CH_2)_4NH(CH_2)_3NH_2]$•2HCl (66%)

**Scheme 3.** Reagents and conditions: (a) **12**, $Pd(PPh_3)_4$, CuI, $Et_2NH/C_6H_6$, rt; (b) HCl/1,4-dioxane, rt.

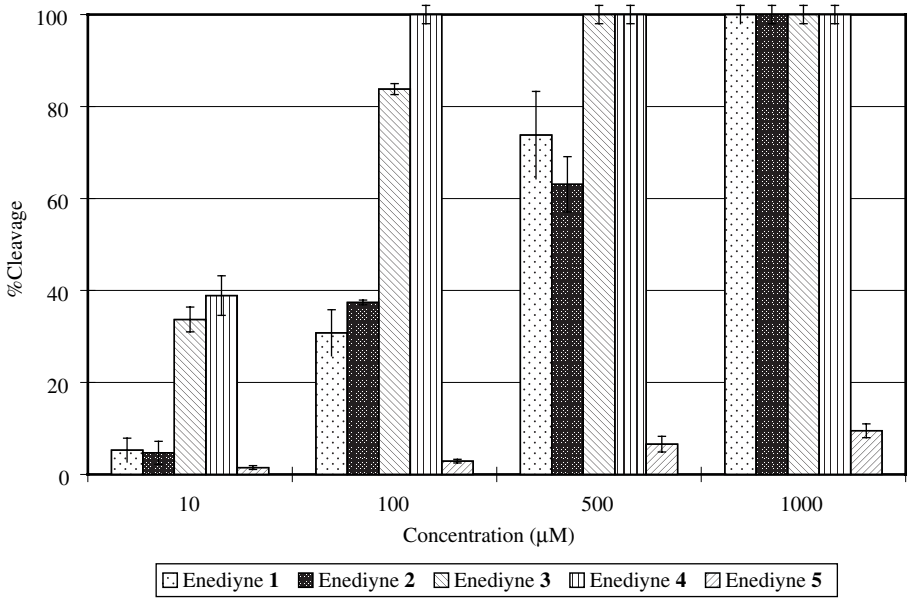



**Figure 3.** DNA cleaving assay using the enediyne drugs **1**–**5** at pH 6.0. Col E1 DNA (12.5 μg/ml) was incubated for 24 h at 37 °C with the enediyne drugs (10, 100, 500 and 1000 μM) in pH 6.0 phosphate buffers, and analyzed by electrophoresis (1% agarose gel, ethidium bromide staining). Results are presented as mean values ± SD of three runs. A control reaction mixture without the addition of any drug was incubated and the mean value of three runs was used as the background to be subtracted from the obtained values.

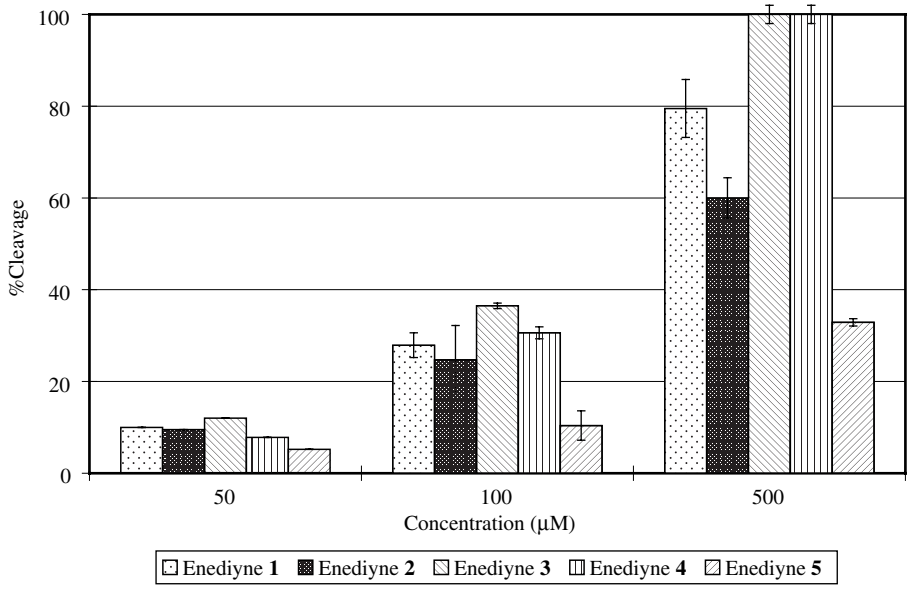



**Figure 4.** DNA cleaving assay using enediyne drugs **1**–**5** at pH 9.0. Col E1 DNA (12.5 μg/ml) was incubated for 24 h at 37 °C with the enediyne drugs (50, 100, and 1000 μM) in pH 9.0 phosphate buffer, and analyzed by electrophoresis (1% agarose gel, ethidium bromide staining). Results are presented as mean values ± SD of three runs. A control reaction mixture without the addition of any drug was incubated and the mean value of three runs was used as the background to be subtracted from the obtained values.

The results in Figure 3 show that the enediynes having a polyamine moiety exhibited potent DNA damaging ability while the reference amide–enediyne **5** showed only low bioactivity. Even enediyne **1**, which has only one amino group, showed considerably enhanced activity compared to the reference **5**, and the extent of bioactivity was similar to that of diamine **2**. Further, triamine **3** and tetraamine **4** showed a similar level of bioactivity, but were more potent than monoamine **1** and diamine **2**.

In basic buffer solution, as shown in Figure 4, the bioactivities of the drugs **1**–**4** were somewhat reduced compared to those in acidic buffers, while higher activity than the reference **5** was still observed. In addition, the order of bioactivity of enediynes **1**–**4** was similar to that observed in acidic buffer solution. Considering the reaction mechanisms in which the hydrolytic removal of the acetyl group of the cyanohydrin moiety initiates the cascade reaction leading to the biradical generation, it is only reasonable that the reference enediyne **5** showed relatively higher DNA damaging ability at pH 9.0 than at pH 6.0. Indeed, when the reactions were monitored by TLC, the enediyne drugs were still detected after 24 h at pH 6.0, while they were consumed completely after 24 h at pH 9.0.[11] It is noteworthy that enediynes **1**–**4** exhibited more potent DNA cleaving activity in acidic buffer solutions than in basic buffers and their activities were higher than the reference **5** at both pH. These results indicated that the polyamine moiety directed the drugs to DNA efficiently by electrostatic interactions and hydrogen bonding in their cationic form at pH 6.0. In contrast, since protonation of the polyamine moiety should occur only partially at pH 9.0,[12] the directivity of the drugs toward DNA should be reduced and, as a result, bioactivity diminished. Further, to confirm the interaction of polyamine–enediyne conjugates **1**–**4** with DNA, we performed a fluorescence quenching assay[13] based upon the displacement of ethidium bromide (EthBr) from DNA caused by the conformational change of DNA induced by binding of the polyamine–enediyne conjugates **1**–**4**. The results are summarized in Table 1.

While this assay method offers only a qualitative comparison of DNA-binding ability of reagents that interact with DNA in a similar fashion, a clear correlation between the release of EthBr and the DNA cleaving activity was observed.

**Table 1.** Fluorescence quenching assay of polyamine–enediyne conjugates **1**–**4**

| Enediyne | % Release of EthBr[a] | % Cleavage of DNA[b] |
|---|---|---|
| **1** | 5.39 ± 0.35 | 5.3 ± 2.6 |
| **2** | 5.86 ± 0.37 | 4.7 ± 2.5 |
| **3** | 12.82 ± 0.20 | 33.7 ± 2.8 |
| **4** | 14.40 ± 0.23 | 38.9 ± 4.3 |

[a] Calf thymus DNA (2 μg/mL), EthBr (1.27 μM) and the polyamine conjugate (10 μM) were mixed in phosphate buffer solution (pH 6.0, 500 μL) and after 1 min, fluorescence intensity ($\lambda_{ex} = 260$ nm, $\lambda_{em} = 600$ nm) was measured ($F1$). Similarly, $F2$ (DNA+EthBr) and $F3$ (only DNA) were measured. % Release of EthBr was calculated as follows and the values indicated are mean values ± SDs of three runs. % Release of EthBr $= [1 - (F3 - F1)/(F3 - F2)] \times 100$.

[b] Selected data from Figure 3.

In conclusion, we developed enediyne model compounds having a polyamine module as DNA binding groups. Our synthesized polyamine–enediyne conjugates showed potent DNA damaging ability at acidic pH and their bioactivity depends upon their binding affinity to DNA.

## Acknowledgements

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