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DNA-Cleavage Studies on *N*-substituted Monocyclic Enediynes: Enhancement of Potency by Incorporation of Intercalating or Electron Poor Aromatic Ring and Subsequent Design of a Novel Phototriggerable Acyclic Enediyne

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Abstract—A number of novel *N*-substituted enediynes (azaenediynes) **1**–**4** were synthesized as DNA cleaving agents. Enhancement of DNA cleavage potency was observed with those compounds which could interact with DNA through intercalation of the extended aromatic ring or through electrostatic attraction with electron poor aromatic ring. An acyclic enediyne **5** with a novel phototriggerable device was also synthesized and its DNA-cleaving activity was established. © 2000 Elsevier Science Ltd. All rights reserved.

Naturally occurring enediynes, first reported[1] about twelve years ago, continue to draw the attention of the scientific community[2] and harnessing the powerful DNA-cleaving activity of this class of molecules is an area of high priority in antitumor drug design.[3] For the past few years we have been studying[4] the effect of substituting a saturated carbon in cyclic enediynes with nitrogen on the kinetics of Bergman cyclization.[5] Out of several possible advantages of incorporating nitrogen in the enediyne framework, one particularly important one is the scope of attaching different ligands on to the nitrogen for possible enhancement of the DNA-cleaving activity. In an earlier communication,[4a] we have reported that the azaenediyne (**1**) with a *p*-toluene sulphonamido group had a half-life of ~36 h at 30 °C, undergoing cycloaromatization to the tetrahydroisoquinoline system. This gave rise to the possibility of using **1** as a DNA cleaving agent at the biological temperature. In this communication, we report the result of our study of its DNA cleaving activity along with three other azaenediynes **2**–**4**, all of them differing in the nature of the aryl group attached to the sulphonamide (Fig. 1). We also synthesized a novel acyclic enediyne **5** and its DNA cleavage activity under phototriggerable condition is also reported here.

The target enediynes **1**–**4** were prepared according to our published procedure.[4a] The chloroeneyne amine **6** was first converted into the corresponding sulphonamides **7**–**10**. A palladium (0)-mediated coupling[6] between **7**–**10** and propargyl alcohol proceeded well, resulting in the formation of the acyclic enediyne alcohols **11**–**14**. Mesylation of the alcohols followed by intramolecular *N*-alkylation with K_2CO_3 in DMF produced the desired enediynes in high yields (Scheme 1).

The DNA cleavage activity of the enediynes **1**–**4** was investigated using supercoiled *p*-bluescript DNA isolated[6] in the Form I. Form II band represents the single strand cleaved product. Figure 1 showed the DNA cleavage profile of compound **1** at various concentrations. The electrophoresis result clearly demonstrated the ability of **1** to cause single strand scission of supercoiled DNA thereby generating the Form II. However, judging from the intensity of the bands, the extent of cleavage was around 50% even at a concentration of 112 μmol/mL. Figures 2 and 3 showed the result of incubation of the other enediynes **2**–**4** and DNA. Amongst them, the enediyne **2** with a chlorophenyl sulphonamido group showed much less conversion to Form II compared to either **3** or **4** both of which are superior cleaving agents. The presence of electron withdrawing nitrophenyl sulphonamide in **3** might have resulted in increased affinity[7] for negatively charged DNA. The

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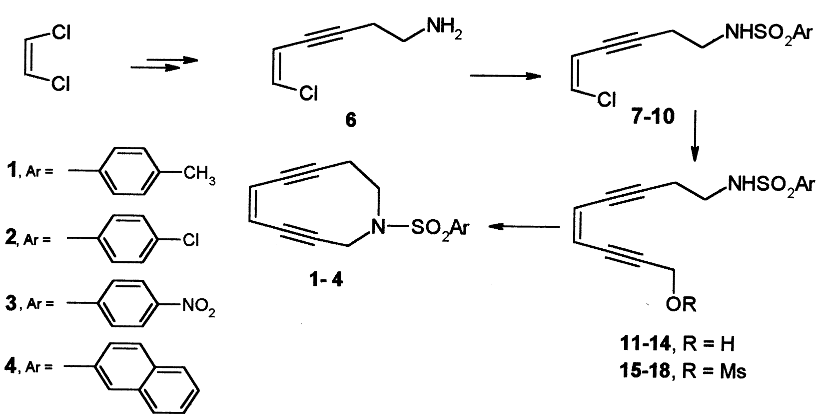


Scheme 1.

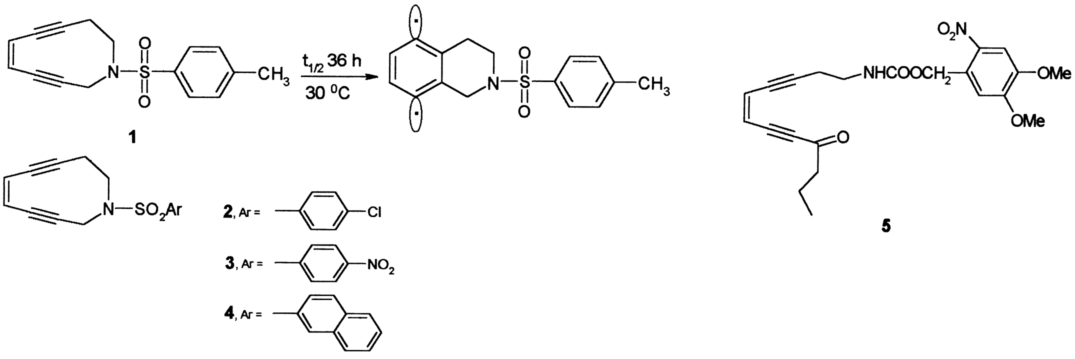


Figure 1.

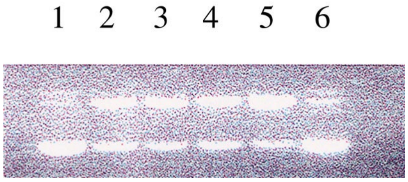


Figure 2. Lanes 1: DNA + TAE buffer (4 °C); 2: DNA + enediyne**1** (14μM/mL) 3: DNA + **1** (28μM/mL); 4: DNA + **1** (56 μM/mL) 5: DNA + **1** (112μM/mL) 6: DNA kept at 37 °C.

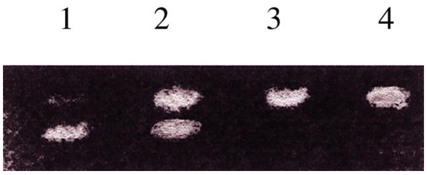


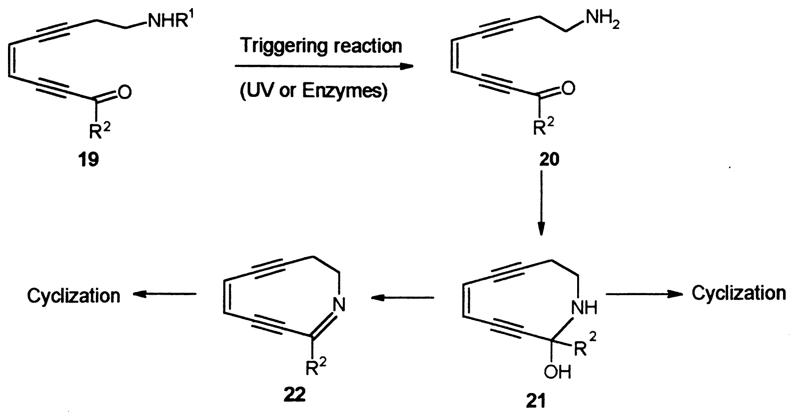
Figure 3. Lanes 1: DNA 2: DNA + chloroenediyne **2** (8μM/mL); 3: DNA + naphthylenediyne (16 μM/mL); 4: DNA + nitroenediyne **3** (8 μM/mL).

enediyne **4** possessing an intercalating naphthalene moiety expectedly exhibited higher efficiency compared to **1** or **2**. Between **3** and **4**, the enediyne **3** showed slightly better cleaving efficiency as indicated in lanes **2** and **3** (Fig. 4). At a concentration of 8 μmol/mL, **3** showed complete conversion to Form II while **4**, at a similar concentration, showed around 80% single strand cleavage.

The above experiments demonstrated that the azaenediynes are indeed capable of cleaving double stranded DNA. However, one problem associated with these compounds is their inherent instability at room temperature. Although these could be stored without any decomposition at 0 °C for months, it would be appropriate if we can design an enediyne molecule which is stable at the biological temperature and is convertible to the cyclic 10-membered azaenediyne only after the occurrence of certain triggering reaction. The in situ generated azaenediyne then shows the DNA-cleavage. After careful consideration of various aspects, we came out with a design of an enediyne **5** which would satisfy the above pre-conditions. The R^1 group was chosen in such a way that would make the nitrogen a poor nucleophile; after removal of R^1, the N regains its nucleophilicity and is expected to carry out a nucleophilic ring closure as detailed in Scheme 2.

One point we like to mention here is the possible formation of an intermolecular product. However, since that will produce a large macrocyclic ring, cycloaromatization from such a product is ruled out. The nitrogen was protected as the 5-nitro veratryl carbamate (N-Voc) which was chosen because of its ease of removal under photolytic conditions[8] (irradiation at $\lambda_{max} = 350$ nm).

The synthesis of enediyne ketone followed a similar sequence of reactions as described earlier for the synthesis of azaenediynes. The chloro eneyne amine **6** was first converted into the N-nitro veratryloxy derivative **23**



Scheme 2.

by acylation with 6-nitro veratryl chloroformate in the presence of DMAP. A second round of Pd (0)-mediated coupling with hex-1-yn-3-ol furnished the enediyne alcohol **24** in 70% yield. Final oxidation to the ketone **5** was carried out with PCC in CH_2Cl_2 (yield 50%). The synthesis is shown in Scheme 3.

The ketone **5** in acetonitrile was mixed with *p*-bluescript supercoiled DNA (mainly Form I) in Tris-acetate buffer (pH 8.0) and was irradiated with UV-light (high wave length band at 350 nm) at the room temperature of 30 °C. As a parallel experiment, a mixture of DNA (in Tris-acetate buffer) and the ketone (in CH_3CN) was kept at 37 °C in an incubator to check the affinity under non-UV conditions. Samples were analyzed by agarose gel electrophoresis. Figure 5 shows the gel pattern after 6 h. It was quite clear that the enediyne ketone **5** had started showing cleavage of the DNA under UV-irradiation. The extent of cleavage became more clear when the gel electrophoresis (shown in Figure 6) was run using individual samples undergoing UV (24 h) and non-UV/thermal (30 h) exposure for 24 h. It was established beyond doubt that the ketone was indeed able to convert the Form I to the cleaved-DNA (Form II).

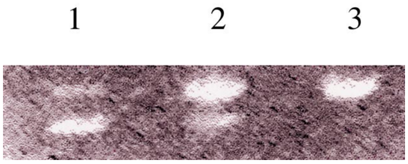


Figure 4. Lanes 1: DNA; 2: DNA + naphthyl enediyne **4** (8μM/mL); 3: DNA + nitroenediyne **3** (8 μM/mL).

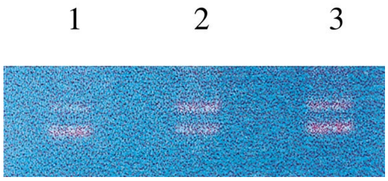
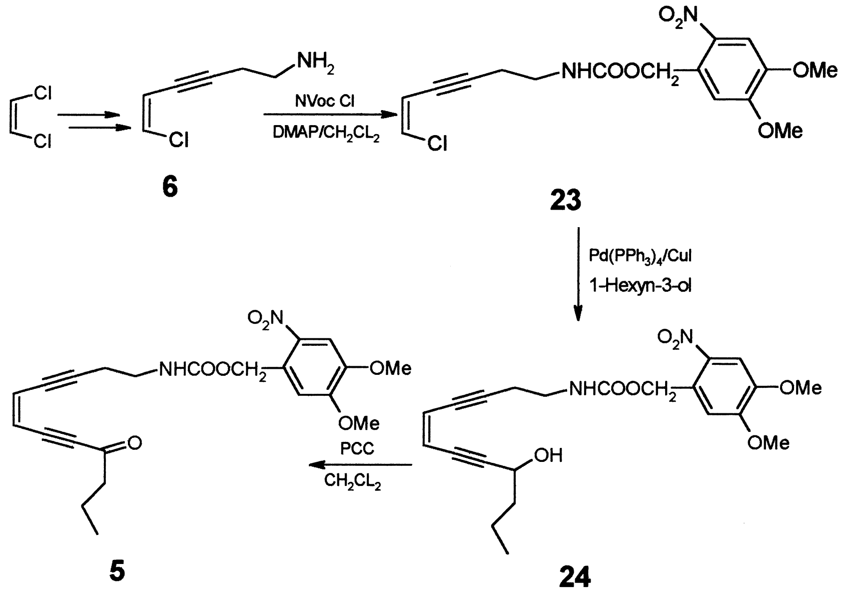


Figure 5. Lanes: 1, DNA; 2: DNA + Enediyne **5** (10 μM/mL) + hν After 6 h; 3: DNA + 5 (10 μM/mL) at 37 °C after 6 h.



Scheme 3.

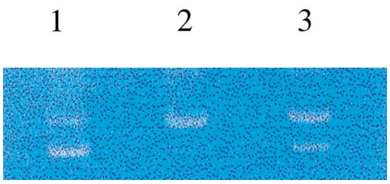


Figure 6. Lanes: 1, DNA; 2: DNA + **5** (10 μM/mL) + hν after 24 h; 3: DNA + 5 (10 μM/mL) at 37 °C after 30 h.

A closer look at the gel electrophoresis pattern in Figure 6 revealed that the ketone **5** was able to cause DNA-cleavage even under the non-UV conditions. However, the extent of cleavage was less compared to when it was irradiated with UV. The cleavage under non-UV conditions could possibly arise from the ability of the ketone **5** to act as Michael acceptor similar to propargylic sulphones.[9] Current studies are aimed towards unraveling the precise mechanism of DNA cleavage.

Selected spectral data

1-(4-Methylphenylsulfonyl)-1-azacyclodec-5-ene-3,7-diyne (1). δ_H 2.45 (3H, s, tosyl-CH_3), 2.77 (2H, t, J = 5.0 Hz, CH_2CH_2N), 3.53 (2H, t, J = 5.0 Hz, CH_2CH_2N), 4.09 (2H, s, $CCCH_2N$) 5 .84 (2H, bs, CH=CH), 7.30 (2H, d, J = 8.1 Hz, Ar-H), 7.75 (2H, d, J = 8.1 Hz, Ar-H); δ_C 21.58, 22.45, 42.16, 51.15, 83.82, 89.53, 94.39, 96.54, 122.18, 124.48, 127.39, 129.75, 136.11, 143.46; Mass (EI) m/z 285 (M^+).

1-(4-Chlorophenylsulphonyl)-1-azacyclodec-5-ene-3,7-diyne (2). δ_H 2.76 (2H, t, J = 4.8 Hz, NCH_2CH_2), 3.58 (2H, t, J = 4.8 Hz, CC-CH_2CH_2), 4.16 (2H, s, CC-CH_2N), 5.77, 5.89 (2×1H, ABq, J = 10.9 Hz CH=CH), 7.16–7.51 (2H, m, Ar-H), 7.79–7.83 (2H, m, Ar-H); Mass (EI) m/z 305, 307 (M^+).

1-(4-Nitrophenylsulphonyl)-1-azacyclodec-5-ene-3,7-diyne (3). δ_H 2.73 (2H, t, J = 5.3 Hz, N-CH_2-CH_2), 3.69 (2H, t, J = 5.3 Hz, N-CH_2-CH_2), 4.28 (2H, s, N-CH_2-CC), 5.82 (2H, s, CH=CH), 8.05–8.12 (2H, m, Ar-H), 8.30–8.35 (2H, m, Ar-H); ν_{max} 3475, 1960, 1636, 1527, 1350, 1163, 1098, 859; Mass (EI) m/z 316 (M^+).

1-(Naphthylsulphonyl)-1-azacyclodec-5-ene-3, 7-diyne (4). δ_H 2.81 (2H, t, J = 5.0 Hz, NCH_2CH_2), 3.61 (2H, t, J = 5.0 Hz, NCH_2CH_2), 4.20 (2H, s, CC-CH_2N), 5.82, 5.87 (2×1H, ABq, J = 10.9 Hz, CH=CH), 7.60–7.65 (2H, m, Ar-H), 7.81–7.98 (4H, m, Ar-H), 8.47 (1H, d, J = 1.47 Hz, Ar-H); Mass (EI) m/z 321 (M^+).

1-[6-Nitro-3,4-dimethoxybenzyloxycarbonylamino]-6-chloro-hex-5-ene-3-yne (23). δ_H 2.62 (2H, dt, J = 1.8, 6.13 Hz, CH_2N), 3.38–3.44 (2H, m, CC-CH_2), 3.93 (3H, s, OCH_3), 3.95 (3H, s, OCH_3), 5.30 (1H, bs, NH), 5.51 (2H, s, $COOCH_2$), 5.81–5.84 (1H, m, ClCH=CH), 6.34 (1H, d, J = 7.38 Hz, ClCH), 6.98 (1H, s, Ar-H), 7.70 (1H, s, Ar-H);Mass (CI) m/z 369, 371 (MH^+).

1-[6-Nitro-3,4-dimethoxybenzyloxycarbonylamino]-9-hydroxy-dodec-5-ene-3,7-diyne (24). δ_H 0.89 (3H, dt, J = 3.5, 11.0 Hz, CH_3), 1.41–1.46 (2H, m, $MeCH_2$), 1.61–1.68 (2H, m, $MeCH_2CH_2$), 2.59 (2H, t, J = 5.9 Hz, CC-CH_2), 3.35–3.39 (2H, m, CH_2N), 3.9 (3H, s, OCH_3), 3.92 (3H, s, OCH_3), 4.49–4.51 (1H, m, CHOH), 5.47 (2H, s, $COOCH_2$), 5.75–5.81 (3H, m, NH, CH=CH), 6.97 (1H, s, Ar-H), 7.65 (1H, s, Ar-H); Mass (CI) m/z 431 (M^+); HRMS calcd for $C_{22}H_{26}N_2O_7$ $+H^+$ 431.1819 found 431.1824.

1-[6-Nitro-3,4-dimethoxybenzyloxycarbonylamino]-9-oxo-dodec-5-ene-3,7-diyne (5). δ_H 0.90 (3H, t, J = 7.2 Hz, CH_3), 1.64–1.71 (2H, m, Me-CH_2), 2.54 (2H, t, J = 7.2 Hz, $MeCH_2CH_2$), 2.67 (2H, t, J = 5.9 Hz, CC-CH_2), 3.38 (2H, t, J = 5.9 Hz, CH_2N), 3.92 (3H, s, OCH_3), 3.94 (3H, s, OCH_3), 5.51 (2H, s, $COOCH_2$), 5.88 (1H, d, J = 10.7 Hz, ClCH=CH), 6.09 (1H, bs, NH), 6.10 (1H, d, J = 10.7 Hz, ClCH), 7.04 (1H, s, Ar-H), 7.68 (1H, s, Ar-H); Mass (CI) m/z 429 (MH^+). HRMS calculated for $C_{22}H_{24}N_2O_7$ $+H^+$ 429.1663 found 429.1668.

DNA cleavage experiments

The DNA cleavage experiments were performed using supercoiled plasmid *p*-bluescript in Tris acetate-EDTA buffer (pH = 8). The plasmid DNA in TAE buffer (40 μL) was incubated for 24 h containing various concentrations of the enediynes **1**–**4** in acetonitrile (20 μL) at 37 °C. The samples were then mixed with sucrose solution containing bromophenol blue and loaded on 0.7% agarose gel having ethidium bromide (0.5 μg/mL). After electropheresis, the bands were visualized under UV-light and photographed. For the studies with the ketone **5** the solution was taken in a quartz cell and irradiated at 350 nm using a medium pressure Hg-lamp.

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