Lysine–enediyne conjugates as photochemically triggered DNA double-strand cleavage agents†

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Received (in Cambridge, UK) 10th November 2004, Accepted 23rd December 2004
First published as an Advance Article on the web 25th January 2005
DOI: 10.1039/b417012a

Statistical analysis of DNA-photocleavage by two types of lysine–enediyne conjugates confirms that more double-strand breaks are produced than can be accounted for by coincident single-strand breaks.

In recent years, considerable effort has been invested into development of synthetic reagents that selectively cleave DNA under irradiation with visible or UV light without the use of metals or reducing agents.[1] The ability of these chemical systems to generate reactive organic intermediates on demand represents a promising approach to new antitumor therapeutic strategies.

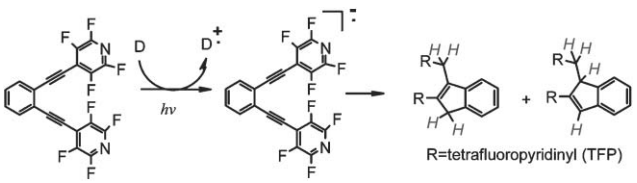
Enediyne antibiotics are among the most potent natural anticancer agents. Their biological activity stems from cyclization of the enediyne moiety to a reactive *p*-benzyne diradical followed by simultaneous cleavage of both strands of duplex DNA (double-stranded cleavage, or ds). Since ds lesions are believed to be more biologically important than single-strand (ss) breaks,[3] numerous studies have focused on developing new DNA ds photocleavage agents utilizing the photochemical version of the Bergman cyclization.[2] Despite these efforts, only one of the photoactivated enediynes reported in the literature shows ds DNA cleavage at μM concentrations (10^{-6}–10^{-5} M)[2a] and it still remains unclear whether the photochemical version of the Bergman cyclization provides true ds DNA cleavage instead of accumulation of random ss breaks.

Our interest in the design of new molecular systems for DNA cleavage was caused by the discovery of photochemical transformation of tetrafluoropyridinyl (TFP) substituted enediynes into indenes.[4] Since this reaction is accompained by four formal H-atom abstractions, we suggested that this chemistry can also be used for development of potent DNA photocleaving agents.

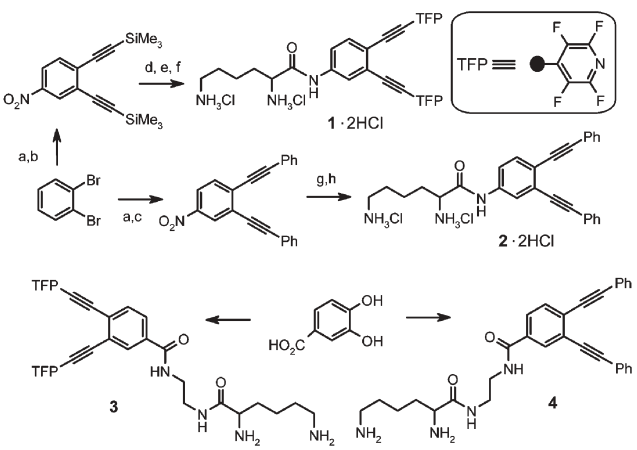
By taking advantage of the ability of positively charged amino acids to bind to DNA,[5] we designed hybrid molecular systems **1**, **3**, which combine bis-TFP enediyne and lysine moieties *via* two linkers of different length. For comparison, we also synthesized bis-Ph enediynes **2** and **4** expected to undergo the photoBergman cyclization.[2f,h] Synthesis of enediynes **1** and **2** is outlined in Scheme 2. Nitration of dibromobenzene yielded 3,4-dibromonitrobenzene which was coupled with acetylenes under Sonogashira conditions. The products were reduced with $SnCl_2$ to afford anilines which were acylated with $Boc_2LysOH/POCl_3$ in pyridine. Deprotection with HCl provided the requisite lysine–enediyne conjugates **1** and **2** as diammonium salts. Lysine–enediyne conjugates **3** and **4** were synthesized in a similar fashion from 3,4-dihydroxybenzoic acid as described in the ESI†.

The ability of enediynes to cleave DNA under irradiation was investigated using conversion of supercoiled plasmid DNA into the respective relaxed circular and linear forms (Forms II and III). The relative amounts of the three DNA forms were determined by densitometric analysis of the gel electrophoresis bands at different irradiation times.

As shown in Fig. 1, irradiation of plasmid pB322 in the presence of conjugate **1** generates linear DNA before all of the supercoiled DNA is converted to the relaxed circular form (lanes 3–9). Under conditions where all of the three DNA forms are present at the same time, one can gain a more thorough insight into the nature of the cleavage using the statistical test of Povirk.[6,7] This test assumes a Poisson distribution of strand cuts and calculates average number of ss-(n_1) and ds-breaks (n_2) per DNA molecule (Table 1).[8] In the case of enediyne **1**, the n_1/n_2 ratio decreases before reaching



Scheme 1 C1–C5 cyclization of bis-TFP enediynes.



Scheme 2 (a) HNO_3, rt, 75%; (b) Me_3SiCCH, $PdCl_2(PPh_3)_2$, CuI, $(i\text{-}Pr)_2NH$, 78%; (c) PhCCH, $PdCl_2(PPh_3)_2$, CuI, $(i\text{-}Pr)_2NH$, 72 h, rt, 76%; (d) C_5F_5N, CsF, DMF, 24 h, rt, 65%; (e) $SnCl_2$, THF, 3 h, rt, 79%; (f) 1. $Boc_2Lys(OH)$, $POCl_3$, Py, −20 °C, 1 h, rt, 2. HCl, CH_2Cl_2, 41%; (g) $SnCl_2$, THF, 3 h, rt, 75%; (h) 1. $Boc_2Lys(OH)$, $POCl_3$, Py, −20 °C → rt, 1 h; 2. HCl, CH_2Cl_2, 39%.

† Electronic supplementary information (ESI) available: Full experimental details. See http://www.rsc.org/suppdata/cc/b4/b417012a/

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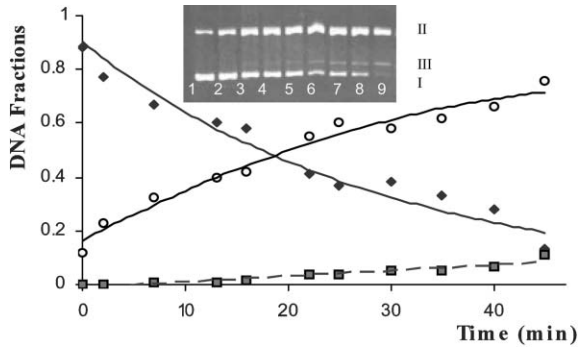

Fig. 1 Photochemical cleavage of pBR322 supercoiled DNA (30 μM) by **1** (20 μM) in phosphate buffer (20 mM, pH 8.0). Lanes 1–9, DNA + **1** + *hν* for 2, 7, 13, 16, 22, 25, 30, 35 and 45 min of irradiation ($\lambda > 305$ nm) respectively. The relative amounts of the three DNA forms are given by diamonds (Form I), hollow circles (Form II) and squares (Form III). The lines are used only to organize the data.

Table 1 Statistical efficiency of single-strand and double-strand break formation by **1** as a function of irradiation time

	Relative amounts (%)			Number of ss-breaks (n_1) and ds-breaks (n_2) per molecule		
Time/min	Form I	Form II	Form III	n_1	n_2	n_1/n_2
0	88.1	11.9	0	0.13	0	
2	75.2	24.8	0	0.29	0	
7	64.0	35.2	0.8	0.44	0.008	53
13	54.0	44.5	1.5	0.60	0.014	44
16	52.9	45.5	1.6	0.62	0.016	40
22	50.0	48.3	1.7	0.68	0.017	40
25	32.0	63.6	4.4	1.09	0.046	24
30	38.9	56.2	4.9	0.90	0.052	17
35	29.5	65.0	5.5	1.16	0.059	20
40	22.1	68.9	9.0	1.41	0.100	14

a plateau suggesting that ss and ds events are kinetically independent and that Form II → Form III conversion occurs at a slower rate than the initial scission. However, at all times the range of n_1/n_2 values (14–53) is significantly smaller than expected from a completely random process.[9]

The other lysine–enediyne conjugates **2–4** are also capable of causing DNA cleavage efficiently: less than 10% of supercoiled DNA remains after 45 min of radiation. Importantly, in every case the n_1/n_2 values cannot be accounted by coincident random ss-breaks (Tables 2).[9,10] Comparable n_1/n_2 values, in the range of 6–20, have been observed for iron bleomycin.[11] The dynamics of cleavage are interesting—in contrast to **1**, the n_1/n_2 values for enediynes **2–4** remain relatively constant with time. The n_1/n_2 ratios are noticeably smaller for the enediynes **3** and **4** where the DNA-cleaving moiety is attached to the lysine residue through a longer linker. These observations suggest that DNA-photocleaver interaction plays an important role in the DNA cleavage and that the longer tether may allow for better alignment of the enediyne for interaction with opposing DNA strands.

The interaction of lysine–enediyne conjugates with DNA was investigated using fluorescence quenching binding assay[12] based on displacement of ethidium bromide by enediynes. The C_{50} values (the concentration of conjugate leading to a 50% reduction in fluorescence intensity of bound ethidium bromide) of compounds **1–4** are, on average, an order of magnitude smaller than C_{50} of spermidine (respectively 0.9 (**1**), 5.0 (**2**), 1.3 (**3**), 1.5 (**4**) vs. 29 μM [36 μM in ref. 12*a*]). This result suggests that non-electrostatic components contribute significantly to binding of lysine–enediyne conjugates to DNA. Interestingly, the similarity in binding of enediynes **3** and **4** correlates well with the respective n_1/n_2 ratios.

Table 2 Statistical efficiency of single-strand and double-strand break formation by enediynes **2–4** as a function of irradiation time

	n_1/n_2 (n_2)		
Time/min	**2**	**3**	**4**
17	35 (0.03)	15 (0.05)	13 (0.06)
22	35 (0.04)	17 (0.05)	10 (0.10)
27	35 (0.05)	12 (0.09)	13 (0.09)
32	26 (0.09)	10 (0.11)	13 (0.12)
37	28 (0.09)	14 (0.13)	11 (0.14)
42	29 (0.10)	14 (0.17)	10 (0.19)

In conclusion, we have unambiguously shown that photoactivated enediynes can cause true non-random ds DNA cleavage. Further research will concentrate on understanding chemistry responsible for the cleavage, optimizing DNA binding and studying its correlation with the cleavage efficiency.

The authors are grateful to the National Science Foundation (CHE-0316598) and to the Material Research and Technology (MARTECH) Center at Florida State University for partial support of this research, to the 3M Company for an Untenured Faculty Award, to Olga Barykina and Charlene Townsend for their help with the experimental work, to Professor Tim Cross and Dr Alla Korepanova for the use of the experimental facilities and Professor Debra Mohler (Emory) for helpful discussions.

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7 Experimental conditions: **1** (20 μM, 0–45 min), **2** (20 μM, 0–45 min), **3** (25 μM, 0–45 min), **4** (25 μM, 0–45 min). The solution (100 μL) containing enediyne, DNA (0.02 μg μL^{-1}, 30 μM per bp) in 20 mM potassium phosphate buffer was incubated for 1 h at 25 °C. After incubation, 10 samples (10 μL of each) in a Pyrex tubes (OD 0.11 in.) were sealed tightly with Parafilm. The sample was placed at a distance of 20 cm from 200 W Hg–Xe lamp (Spectra-Physics, Laser & Photonics Oriel Instruments with long pass filter with 324 nm cut-on wavelength) and cooled with the stream of air. The relative quantities of the supercoiled, nicked, and linear DNA were calculated by the image analyzer software Total/Lab (Nonlinear Dynamics Ltd., UK). The amount of supercoiled DNA was multiplied by factor of 1.4 to account for reduced ethidium bromide intercalation into supercoiled DNA.
8 For random Poisson distribution the average number of dsb per molecule can be calculated from the equation: $n_2 = 1/[(f_I + f_{II} + f_{III})/f_{III} - 1]$. The average number of ss breaks can be determined from the fraction of supercoiled DNA remaining after irradiation $f_I = \exp[-(n_1 + n_2)]$.
9 Using typical n_1 values obtained from the experiment, one can calculate the expected n_2 from Freifelder–Trubo[10] relation $n_2 = n_1^2(2h + 1)/4L$, where h is the maximum number of unbroken base pairs between single-strand breaks in opposite strands that produces a linear form ($h = 16$), L = the number of phosphodiester bonds per DNA strand ($L = 4361$ for pB322). Under these assumptions, $n_1/n_2 = 1057$ ($n_1 = 0.5$), 529 ($n_1 = 1$), 352 ($n_1 = 1.5$).
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