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A novel concept to activate enediynes for DNA cleavage

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A novel concept is presented to activate enediynes *via* a biscumulenic intermediate using photoinduced electron transfer (PET).

The antibacterial and antitumor properties of naturally occurring enediynes are related to their ability to form σ biradicals *via* Bergman cyclisation at physiological conditions thus inducing single and double strand DNA cleavage.[1] Hence, the design of simple enediynes that mimic the natural antitumor antibiotics has been the focus of intense research.[2] However, unless the enediyne is implemented in a strained ring system,[3] it will require substantial heating to undergo the biradical cyclisation. To overcome this obstacle direct photochemical conversion of enediynes to *para*-benzyne diyls has been studied.[4]

Herein, we wish to report about a fundamentally novel concept to activate enediynes for DNA cleavage using electron transfer—quite different from earlier approaches[5]—which may develop into a venue for photoactive prodrugs with long wavelength activation. In this approach enediynes are designed as potential precursors to *s-cis* biscumulenes[6] of the general structure **1**.

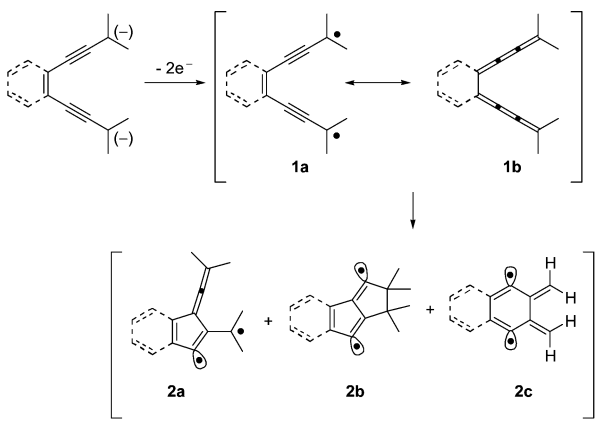
Knowing the high reactivity of cumulenes,[7] one expects **1** to undergo a manifold of unprecedented cyclisations, potentially generating biradicals **2a–c**. As both intermediates, the biscumulene **1** and the biradical **2**, should be highly reactive and difficult to observe, we decided to test our concept directly for its ability to trigger DNA double strand cleavage.

Since a two-electron oxidation (as in Scheme 1) is not really practical to activate enediynes under physiological conditions we envisaged a photoinduced electron transfer (PET) strategy to **4** (compare with **1**) starting from **3**. Upon photoexcitation of the acridinium unit an intramolecular ET is expected to afford an acridine radical and a guaiacol radical cation (Scheme 2). As silyl enol and silyl phenol radical cations are prone to rapid O–Si bond cleavage with concommitant loss of a 'silyl cation'[8] **3** should photochemically afford **4**. Alternatively, **4** should also form *via* desilylation of **3** in presence of a silophile.

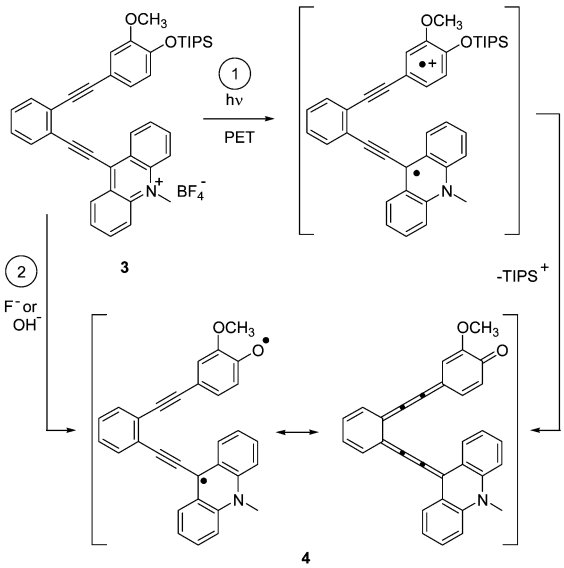
The preparation of **3** was readily accomplished using two consecutive Sonogashira couplings starting from 1,2-diiodobenzene and a final methylation of the acridine with Meerwein's salt. **3** is a thermally very stable enediyne as it cyclises at temperatures only above 200 °C ($k^{220\,°C} = 1.5 \times 10^{-2}$ s^{-1}). Cyclic voltammetry investigations of **3** in acetonitrile at scan rates of 100–1000 mV s^{-1} revealed two irreversible waves, one at $E_{pa} = +1.01$ V_{Fc} for the oxidation of the guaiacol unit and one at $E_{pc} = -0.63$ V_{Fc} for the reduction of the acridinium part.† From UV-Vis and fluorescence data $E_{0,0}$ at 482 nm was derived, which allows us to calculate the potential of the photoexcited acridinium unit in **3** as $E^*_{red} = 1.87$ V_{Fc}, certainly sufficient to oxidise the guaiacol as described in pathway 1 (Scheme 2). Indeed, **3** does not exhibit any relevant fluorescence as other acridinium salts.

At first, various experiments were performed to analyse the binding of **3** to DNA. The affinity of **3** to calf thymus DNA was determined by UV-Vis titration to be $K = 4.5 \times 10^6$ M^{-1}. With increasing DNA concentration a decrease of the absorbance was observed. Moreover, an isosbestic point emerged at 520 nm indicating that exclusively one type of **3**-DNA complex was formed. In LD (linear dichroism,[9] Fig. 1B) spectra, the band at 260 nm decreased upon increasing concentration of **3** indicative of a reduced orientation of the DNA along the flow lines. Moreover, (Fig. 1C) a remarkable reduction of the LD_r band (reduced LD) suggests that the molecules of **3** are characterized by a low degree of orientation.‡ More important is the analysis of the spectra in the chromophore absorption region (300–600 nm). Despite a significant absorption in this region (Fig. 1A) the LD spectra (Fig. 1B) does not show any signal, indicating that the compound is externally bound to the DNA helix. In light of the increased flexibility indicated by the difficulty in orienting DNA, it is possible that the enediyne is bound 'end-on' to the DNA.

The DNA photocleavage activity of enediyne **3** was examined at 300, 350 and 419 nm. Irradiation at 419 nm was more



Scheme 1 Concept to activate enediynes by two-electron oxidation *via* intermediate biscumulenes.



Scheme 2

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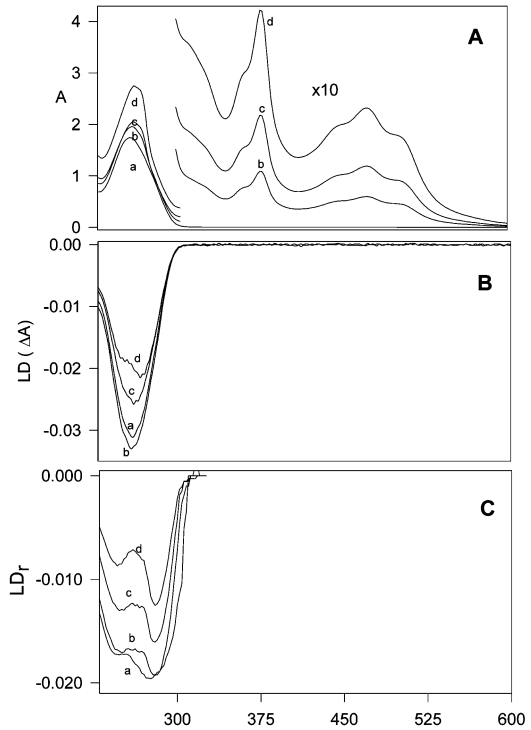


Fig. 1 Absorbance A (A), linear dichroism LD (B), and reduced linear dichroism LD_r (C) spectra of mixtures of **3** and salmon-testes DNA at different dye–DNA ratios (a = 0.00, b = 0.02, c = 0.04, d = 0.08, in 0.01 M ETN buffer/CH_3CN (3 : 2), pH = 7.0; ETN = EDTA/Tris/NaCl).

efficient than at shorter wavelengths leading to almost complete destruction of the supercoiled pBR322 DNA. Importantly, apart from single strand cleavage, double strand cleavage is also observed (Fig. 2).

Since acridinium salts, like other quaternary *N*-heterocycles, may induce DNA cleavage upon irradiation through PET from the DNA (*e.g.* guanine) to the photoexcited acridinium,[10] the mode of action of **3** may not necessarily follow the mechanistic scenario proposed in Scheme 2. As a consequence, salts **5** (E_{pc} = −0.63 V_{Fc}; E^*_{red} = 1.76 V_{Fc}) and **6** (E_{pc} = −0.71 V_{Fc}; E^*_{red} = 1.86 V_{Fc}) were prepared to model the acridinium unit in **3**. Both possess sufficiently high excited state redox potential to oxidise guanine (E_{ox} = 0.86[11a]; 0.95 V_{Fc}[11b]) (Scheme 3).

DNA photocleavage of **5** and **6** was investigated under the same conditions as **3**. Importantly, while both equally showed some single strand DNA cleavage, no double strand cleavage was observed. It appears that the quenching of the fluorescence

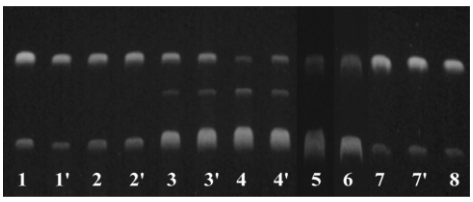
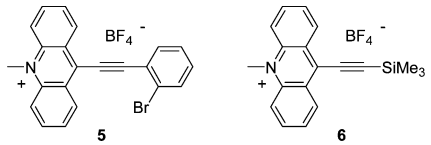


Fig. 2 Photocleavage of supercoiled plasmid DNA pBR322 by **3** (Lanes 1–4), **5** (Lane 5), and **6** (Lane 6) at 419 nm. Reaction mixtures were prepared by addition of appropriate stock solutions to a total volume of 15 μl containing 0.15 μg of plasmid DNA buffered to pH 8 with 10 mM Tris and 1 mM EDTA. Irradiations were performed in a Rayonet photoreactor RPR-100 (16 lamps, λ_{exc} = 419 nm). The mixtures were analyzed on a 1% agarose gel at 80 V for approximately 2 h. Lanes 1,1′: 1 mmol of **3**, ½ h; lanes 2,2′: 1 mmol of **3**, 1 h; lanes 3,3′: 1 mmol of **3**, 2 h; lanes 4,4′: 1 mmol of **3**, 4 h; lane 5: 1 mmol of **5**, 4 h; lane 6: 1 mmol of **6**, 4 h; lanes 7,7′: only DNA pBR322, 4 h, with irradiation; lane 8: only DNA pBR322 as standard.



Scheme 3

of **5** and **6** by DNA finally leads to single strand cleavage only. Notably, as enediyne **3** exhibits almost no relevant fluorescence (**5** and **6** are more fluorescent by a factor of 180 and 260, respectively), it has to cleave DNA by a different mechanism. To support our hypothesis that **3** is activated for DNA cleavage *via* the reactive biscumulene **4**, we treated **3** with NaOH to split off the TIPS group (Scheme 2, pathway 2). Indeed, the reaction of **3** + NaOH in the presence of pBR322, leads to extremely efficient DNA cleavage.

To summarise, we disclose a novel photochemical route at long wavelength and a new thermal variant to activate enediynes for DNA cleavage. There is evidence that the mode of action may involve a biscumulenic intermediate, the fate of which is still speculative. In particular, the finding of DNA double strand cleavage highlights the occurrence of biradical intermediates along a later stage of the reaction pathway so that further studies on this and related enediynes are warranted.

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Notes and references

† Potentials are provided *vs.* ferrocene (Fc).

‡ Alternatively, the orientation angle is about 55°, the angle at which LD_r is 0, see ref. 9*a*).

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