Design, synthesis and DNA-cleaving efficiency of photoswitchable dimeric azobenzene-based C_2-symmetric enediynes†

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Designed azobenzene-based enediyne-amino acid C_2-symmetric hybrids have been synthesized and the role of amino acid linker in stabilizing the *Z* form has been demonstrated; DNA-binding and cleavage studies have established higher reactivity of the *Z*-isomers.

Symmetry plays an important role in many chemical and biological processes.[1,2] Many key proteins and enzymes exist as homo dimers with two-fold axis of symmetry namely the C_2-symmetry. These are biologically active only in this homo dimeric form.[3–5] Various research groups have designed C_2-symmetric DNA-cleaving agents[6] and studied their cleavage efficiency.[7] Enediynes have now been established as a fertile ground for designing novel DNA-cleaving agents.[8] Although many reports of ingenious design of enediynes are there in the literature,[9] including from our own laboratory,[10] as such there is no report of how the biological activity of enediynes depends upon their symmetry property. In order to study such effects, we have designed novel hybrid molecules consisting of azo benzene,[11] enediyne and amino acid. These molecules have different symmetry groups in the *E* and *Z* forms. Their synthesis and chemical as well as biological reactivities are reported herein which nicely demonstrated the importance of geometric considerations while designing new enediynes. Since C_2-symmetry is our prime consideration, two types of molecules **Type I** and **Type II** were targeted (Fig. 1). Both the types consisted of azobenzene, enediyne and amino acid which are joined by two carbon linkers. Use of same amino acid in **Type I** systems retains the C_2 while incorporation of dissimilar amino acids or same amino acid with different configuration in **Type II** molecules breaks the C_2-symmetry. The latter molecules were synthesized for comparing the activity of the C_2 and non-C_2 symmetric molecules. The actual target molecules belonging to different classes are shown in Fig. 1. We wanted to address the following question: can perturbation of symmetry element affect the DNA-cleaving potential of enediyne?

We first carried out the MM2 calculation to find out the energy-minimized conformation of **Type I** molecules **1a** and **2a**. The exercise revealed a crescent shape conformation for the *Z*-isomer, while the *E*-isomer has a zigzag conformation. The complementarity of conformation between the *Z*-isomer and the helical shape of DNA may ensure higher binding with *ds*-DNA and hence higher cleaving efficiency. The stability imparted by the π-stacking interaction[12] between the benzene rings of the phenylalanine-based system is expected to slow down the kinetics of thermal *Z* to *E* re-isomerisation. It is interesting to note that the energy minimization of the *Z*-form of the non C_2-symmetric molecule **2e** containing D- and L-phenylalanine showed very little π-stacking interactions in the *Z*-isomer. For **2d**, the isopropyl group of valine lies away from the phenyl group.

The synthetic endeavour towards **1a**/**1b** started with the preparation of the azobenzene-amino acid diesters **3a**/**3b**. These were hydrolysed to the free acids **4a**/**4b**. The corresponding dipotassium salts **5a**/**5c** were esterified with 2-bromoacetylamine **6** in DMF.[13] The target molecules **1a**/**1b** were isolated pure in 85% yield after column purification (Si-gel, DCM : MeOH = 30 : 1). The corresponding aliphatic system **1c** was also prepared *via* a similar route (Scheme 1). The synthesis of **Type II** molecules was similarly carried out. The structures of all the compounds were in agreement with ^{1}H, ^{13}C NMR as well as mass spectral data (HRMS).

The configuration around the N–N double bond was first determined by recording the UV spectrum in CH_3CN. For the *trans* compounds a strong λ_{max} at 363 nm appeared typical of the *trans* azobenzenes.[14] For the *cis* isomers a characteristic λ_{max} at higher wavelength of 440 nm was observed. The

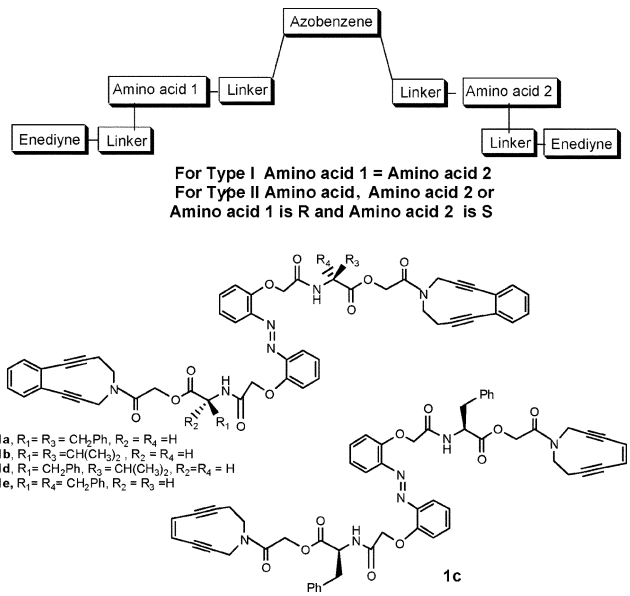

Fig. 1 Structures of target enediynes.

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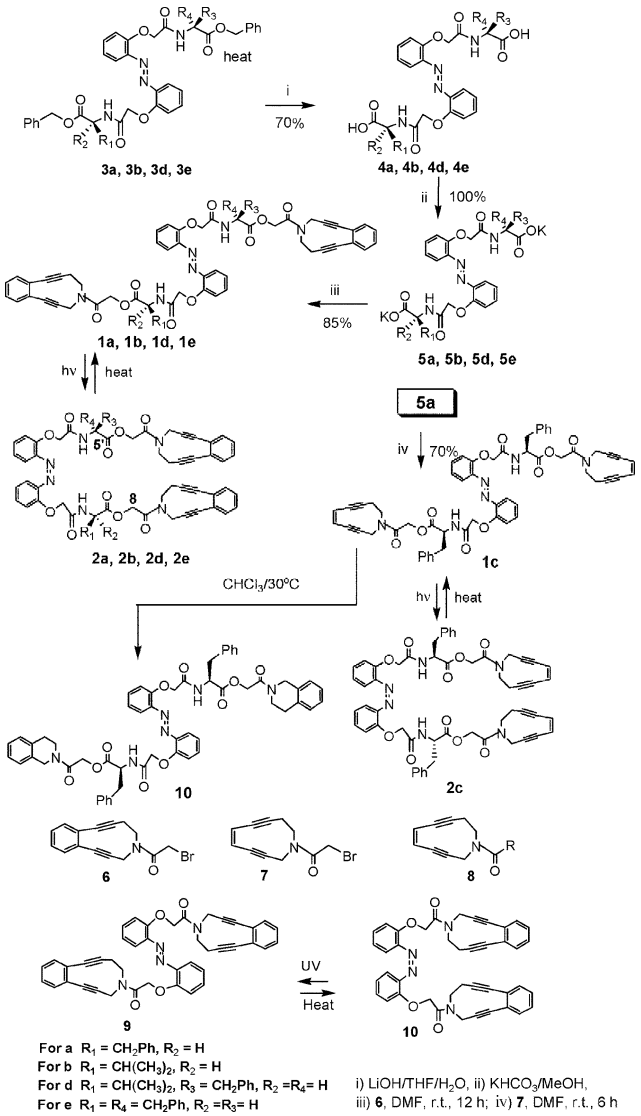

Scheme 1 Synthesis of azobenzene enediyne amino acid hybrids.

NOESY spectrum taken on a mixture of *E* and *Z* isomers showed cross peaks between the proton at C-8 and the benzylic methylene at C-5′ for only the *Z*-isomer. It is also to be noted that the aromatic protons in all the *cis* isomers appeared upfield suggesting less conjugation due to non-planarity of the azobenzene ring which is the case for the *cis* form. Final confirmation about the geometry came from the single-crystal X-ray structure of the valine based ester **3b** which clearly showed the *trans* disposition of the two arms (Fig. 2). The structures of other azo systems were deduced from a comparison of characteristic NMR signals with those of **3b**.

The reactivity of the enediynes towards Bergman cyclization (BC)[15] as studied by differential scanning calorimetry (DSC)[16] indicated similar onset temperatures (80–90 °C) for the aromatic enediynes which is very similar to what was observed for isolated enediynyl amides.[13] The solution phase kinetics performed on **1a** did show slow BC kinetics at 45 °C with a half life of 7 days. For the non-aromatic enediyne, the *E* isomer **1c** showed an onset temperature of 50 °C for BC while in solution

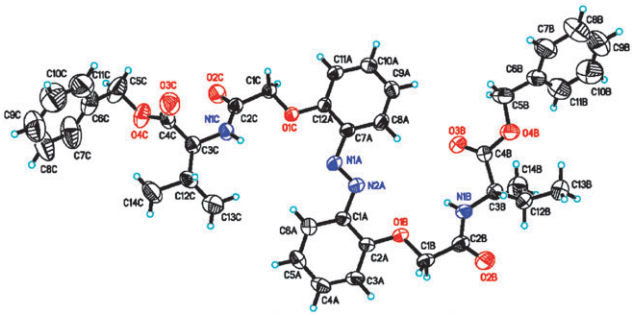

Fig. 2 X-Ray crystal structure of **3b** (CCDC 676458, ESI†).

($CDCl_3$) it has a half life of 22 h at 30 °C as compared to 36 h for the simple sulfonamide of same ring size.[17] The non-aromatic enediynes **1c**/**2c** are the most reactive ones while the aromatic ones, although quite stable at room temperature did show slow cyclization and hence are candidates for studying the DNA-cleavage activity under biological conditions. The formation of cycloaromatized products was confirmed by mass spectrometric data which showed peaks at $MH^+ + 4$ corresponding to the addition of four hydrogens to the two sets of diradicals.

The photoisomerization was done by irradiation (354 nm) of CH_2Cl_2 solution of the *E*-enediynes for 4–5 h. The half lives for the thermal re-isomerization of the *Z*-enediynes to the corresponding *E*-forms are shown in the Table 1. Incorporation of amino acids as spacers has significantly slowed down the *Z* to *E* conversion as compared to systems without amino acid **9** and **10**.

For the assessment of DNA-cleaving potential, the various enediynes (both *E* and *Z* isomers at μmol concentrations) were separately incubated with pBR322 plasmid DNA. The incubations were carried out at 20 °C (to suppress the re-isomerization) and the other at 37 °C. For enediynes **1a**/**2a**, the gel pattern clearly indicated better cleaving efficiency (~2×) for *Z* isomer **2a** as compared to the *E* isomer **1a** (Fig. 3). The valine-based enediynes **1b**/**2b** were, however, found to be poor DNA-cleavers after 2 h incubation both at 20 and 37 °C thus making it difficult to compare the relative cleaving efficiencies of the *E* and *Z* isomers.

For the non-benzenoid enediynes **1c**/**2c**, the gel pattern after 2 h clearly indicated higher cleaving potency for the *Z*-isomer (~30%) as compared to the *E*-isomer. However, with time, as more and more *Z* form is converted to the *E*-isomer, the extent of cleavage became almost the same. The other interesting and highly important observation is the generation of linear DNA (form III) for the aliphatic enediynes; the *Z*-form showing generation of form III after 2 h of incubation at 20 °C while at 37 °C both *E* and *Z* forms showed linear cuts. It is possible that the linear cut observed for the *E*-isomer is due to the presence of the *Z*-form (~15%) under equilibrating

Table 1 Kinetics of *Z* to *E* thermal isomerization

Compound	$t_{1/2}$(20 °C)/h	Compound	$t_{1/2}$(20 °C)/h
2a	30.0	**2d**	12.0
2b	19.0	**2e**	19.0
2c	7.2	**10**	2.3

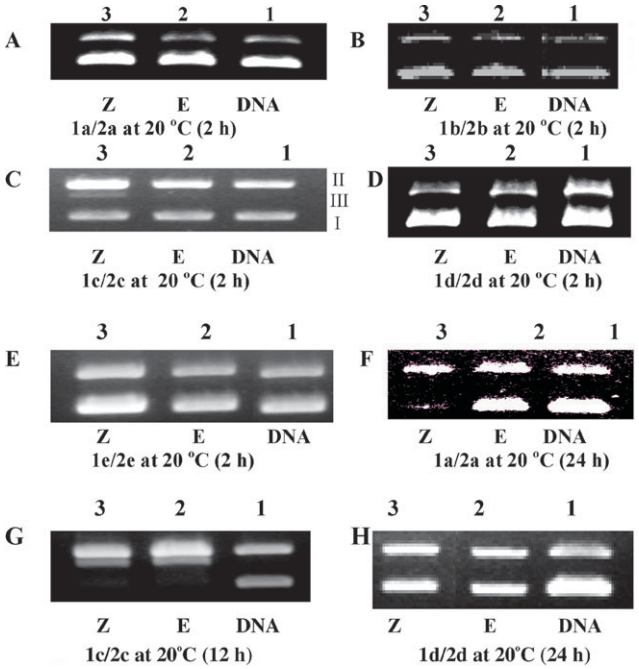

Fig. 3 pBR322 DNA cleavage experiment of compounds **1a–1e**/**2a–2e**; For **A–H**: lane 1: control DNA in TAE buffer (pH 8, 7 μL) + CH_3CN (5 μL); lane 2: DNA in TAE buffer (pH 8, 7 μL) + *E* isomer (0.02 mM) in CH_3CN (5 μL); lane 3: DNA in TAE buffer (pH 8, 7 μL) + *Z*-isomer (0.02 mM) in CH_3CN (5 μL).

conditions. It may be noted that the isolated non-benzenoid enediyne **8** is a much inferior cleaving agent as compared to **1c**/**2c**; the extent of cleavage even after 12 h incubation was much less (only 50% as compared to 85%). It also failed to show formation of any linear form under similar conditions.[17] The non-C_2-symmetric molecules **1d**/**2d** and **1e**/**2e** have been found to be very poor DNA-cleavers. All these point to the importance of having an azobenzene endowed with C_2-symmetry in the design.

The DNA-binding studies by absorption titrations involving addition of a solution of calf thymus DNA to a fixed concentration of the probe[18] indicated higher degree of hypochromism[19] for the *Z*-isomer **2a** as compared to **1a**. The binding constant (using the Benesi–Hildebrand equation) revealed greater binding affinity for enediyne **2a** (2.5× as compared to **1a**). Thus the higher DNA-cleavage efficiency for the *Z*-isomer is correlated to its binding efficiency.

Thus, we have successfully designed and synthesized azobenzene-based enediyne–amino acid C_2-symmetric hybrids. A new way of modulating the biological activity of this class of molecules by introducing a symmetry element like C_2 has been firmly established. Future research will concentrate on exploiting this idea to generate water-soluble cytotoxic agents. Since *E* to *Z* isomerization is done photochemically, our work opens up the possibility of enhancing the DNA-cleaving efficiency of the C_2-symmetric enediynes *via* photoirradiation.

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