ABSTRACT

Consideration of the fact that aryl fused enediynes are much less reactive than the corresponding aliphatic enediynes led us to synthesize a new class of salicylaldimine-based nonaromatic enediynes **1(A-C)** and study their thermal reactivity (**Figure 1**). Our aim was to activate these enediynes by metal ion complexation to such an extent that the cyclization takes place under ambient conditions. Although the activation barrier for cycloaromatization was demonstrated to be considerably changed upon complexation, the compounds failed to be activated enough for the cyclization to take place at physiological temperature. Nevertheless, interesting dependence of reactivity on length of spacer separating the imine and the enediyne and on the nature of metal ion was observed. The results are shown in **Table 1.**

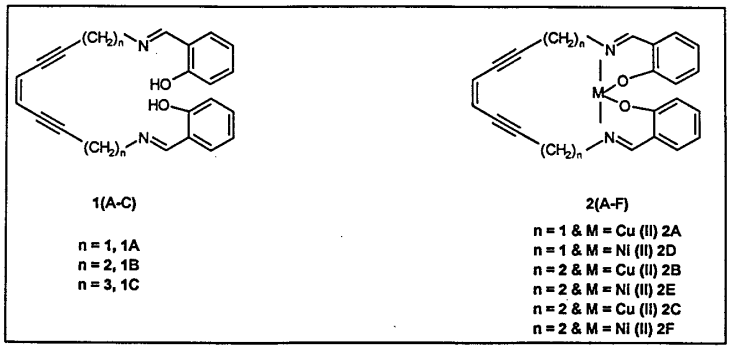
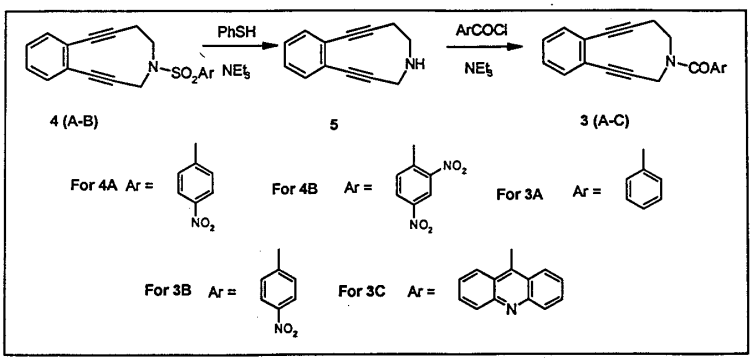


Figure 1: Our Target Non-benzene-fused Bis-salicylaldimino Enediynes

Table 1: Onset Temperature of BC of Enediynes and their Complexes

Ligand	Onset Temperature of BC (°C)	Complex	Onset Temperature of BC (°C)
1A	115	Ni (II)	121.6
		Cu (II)	140.6
1B	155	Ni (II)	146.9
		Cu (II)	139.0
1C	167	Ni (II)	204
		Cu (II)	156

A novel class of benzene-fused amido enediynes **3(A-C)** were synthesized (**Scheme 1**) from the amine **5**. The reactivity of these amido enediynes were determined and compared with corresponding sulphonamido enediynes **4(A-B)**. NMR studies in organic solvents showed higher reactivity of sulfonamides (**Table 2**). However, in the solid state, the amides were more reactive towards cyclization as indicated by DSC studies (**Figure 2**). These amido enediynes, inspite of having a benzene-fused ring, were also shown to possess DNA-cleavage activity (**Figure 3** and **4**). The activity was dependent upon the nature of the aryl group in the amide. Attachment of electron poor nitro or intercalating acridine moiety enhanced the cleavage efficiency.



Scheme 1: Synthesis of Amido Enediynes

Table 2: Kinetic Parameters for the Cyclization of the Enediynes in $CDCl_3$

Compound number	Rate constant (per h) at 70 °C	Activation energy $E^{\#}$ (Kcal/mole)	Half life at 70 °C in h
3A	$3.7x10^{-3}$	24	188
3B	$5.1x10^{-3}$	------	136
4A	$1.43x10^{-2}$	20	49
4B	$2.43x10^{-2}$	------	28

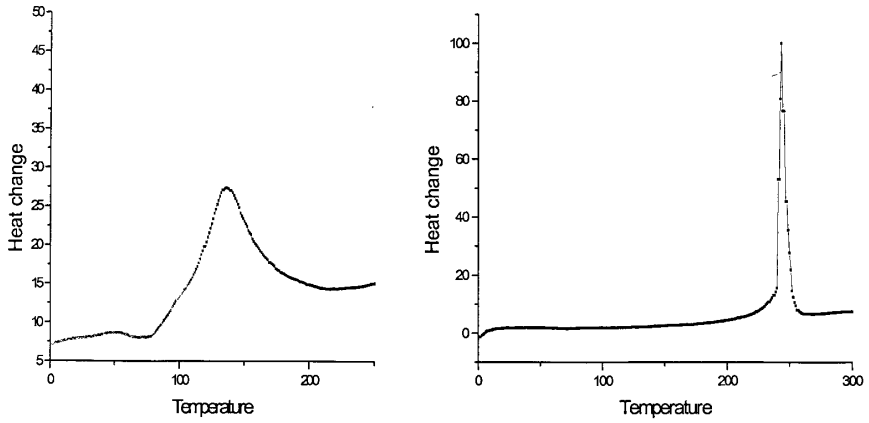


Figure 2: DSC Curves of **3B** and **4A**

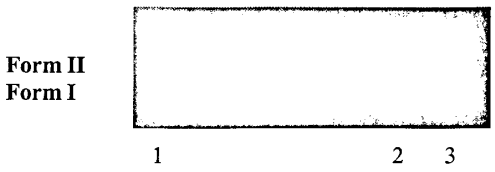


Figure 3: Interaction of supercoiled DNA (in Tris-acetate buffer, pH 8.0) and various enediynes in acetonitrile; incubation was continued upto 48 h and analyzed by agarose (0.7%) gel electrophoresis using ethidium bromide stain: Lanes 1: DNA; Lanes 2: DNA + enediyne **3B** (40 μmol); Lanes 3: DNA + enediyne **3A** (40 μmol).

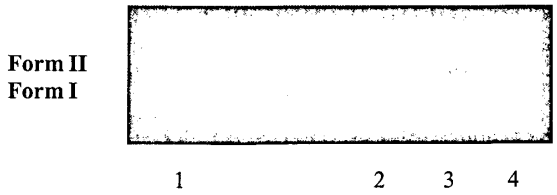
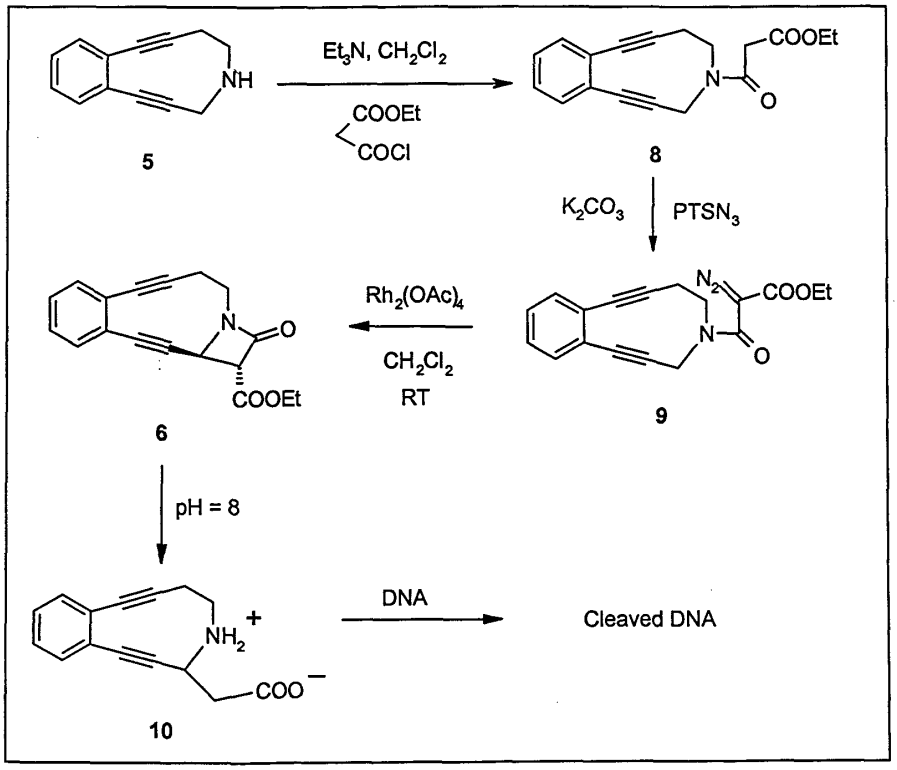
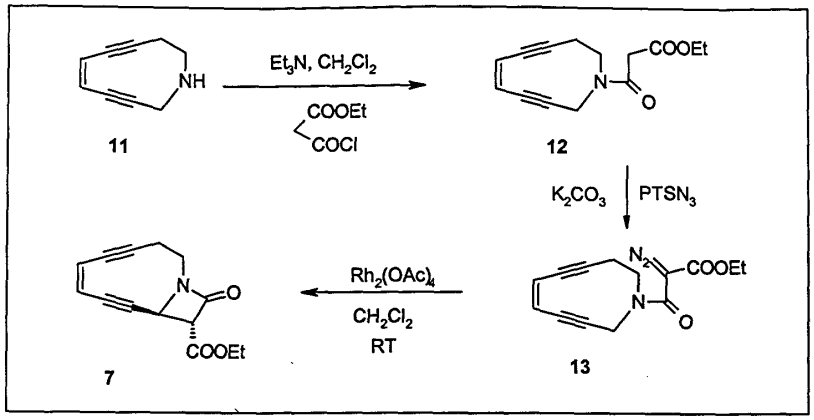


Figure 4: Interaction of supercoiled DNA (in Tris-acetate buffer, pH 8.0) and various enediynes in acetonitrile; incubation was continued upto 48 h and analyzed by agarose (0.7%) gel electrophoresis using ethidium bromide stain: Lanes 1: DNA; Lanes 2: DNA + enediyne **4A** (1 mmol); Lanes 3: DNA + enediyne **3C** (40 μmol); Lanes 4: DNA + enediyne **3B** (40 μmol).

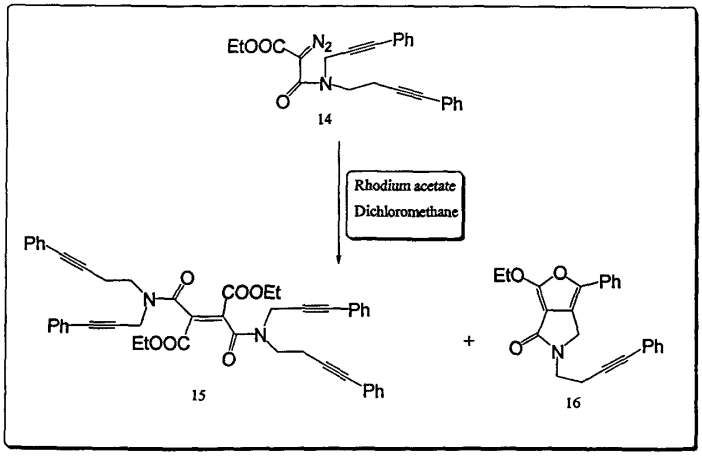
A new stereoselective route to β-lactam fused aromatic as well as non-aromatic enediynes **6** and **7** was developed (**Scheme 2** and **Scheme 3**). The key step was the insertion of carbenes onto preformed enediynes. The carbenes were generated from the corresponding diazo amides **9** and **13**. The reaction produced only one product, namely the *trans* β-lactam-fused enediynes demonstrating complete control of regio and stereo selectivity in the insertion reaction. Interestingly, during a model study of carbene insertion of an acyclic diyne **14**, only a dimer **15** and a γ-lactam **16** (generated via addition of the carbene on to a triple bond) could be isolated (**Scheme 4**). This demonstrated the importance of using a cyclic template for generation of a β-lactam.



Scheme 2: Carbene Mediated Synthesis of β- Lactam Fused Enediyne



Scheme 3: Carbene Mediated Synthesis of β- Lactam Fused Aliphatic Enediyne



Scheme 4: Carbene Insertion of Acyclic diynes

The conjugated acid of the ring-opened amino enediyne **10** was proposed to be the cleaving agent. This was supported by the DNA-cleavage activity of intermediate amino enediyne **5**. The latter, in fact, can even cause double strand cleavage of pBR 322 as evident from agarose gel electrophoresis experiments (**Figure 5**).

Figure 4: Interaction of supercoiled DNA (in Tris-acetate buffer, pH 8.0) and enediyne **6** in acetonitrile; incubation was continued upto 48 h and analyzed by agarose (0.7%) gel electrophoresis using ethidium bromide stain: Lanes 1: DNA; Lanes 2: DNA + enediyne **6** (50 μmol); Lanes 3: DNA.

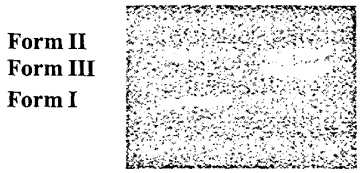


Figure 5: Interaction of supercoiled plasmid DNA (pBR 322) (in Tris-acetate buffer, pH 8.0) and enediyne **5** in acetonitrile; incubation was continued up to 24 h and analyzed by agarose (0.7%) gel electrophoresis using ethidium bromide stain: Lanes 1: DNA; Lanes 2: DNA: + enediyne **5** (50 μmol).

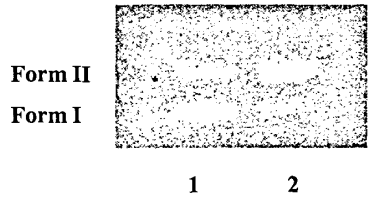


Figure 6: Interaction of supercoiled plasmid DNA pBlueScript SK + (in Tris-acetate buffer, pH 8.0) and enediynes **5** in acetonitrile; incubation was continued up to 24 h and analyzed by agarose (0.7%) gel electrophoresis using ethidium bromide stain: Lanes 1: DNA; Lanes 2: DNA + enediyne **5** (50 μmol).