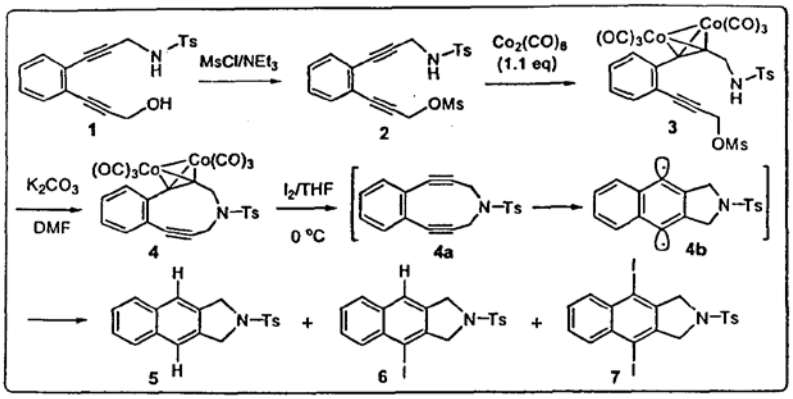
ABSTRACT

Natural enediyne antibiotics are a small family of compounds endowed with potent cytotoxic and antitumour activities. These properties are due to single- and double strand cleavage of DNA, provoked by a benzenoid diradicals formed through Bergman Cyclization (BC) of the enediyne moiety. Through extensive research inputs over the past years, it became clear that cyclic enediynes are more reactive than their acyclic counterparts. The cyclic 9-membered enediynes are very reactive than the 10-membered analogue. Other macrocyclic enediynes of ring size 11 or more are stable at physiological temperature.

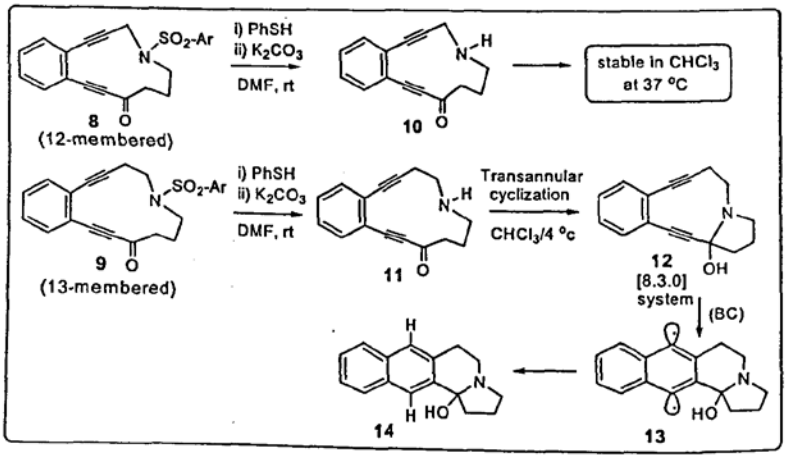
Over the past few years, we have given a lot of efforts to synthesize a 9-membered enediyne containing a nitrogen atom as a replacement for the saturated carbon. The key step in the synthesis of such a system is the intramolecular N-alkylation of an acyclic substrate. Direct intramolecular N-alkylation, however, failed for an intended 9-membered system. We envisioned that complexation of the alkynyl functionality with cobalt carbonyl might facilitate the intramolecular attack by the nitrogen on to the activated propargylic centre. Proximity of the reacting centres due to bending of the otherwise linear C(sp)-C(sp)-C(sp^3) axis and extra flexibility offered by the slight elongation of the triple bond after complexation might also aid the cyclization.

Based on the above logic, we successfully synthesized for the first time a 9-membered N-containing enediyne in complexed form. The synthesis is shown in **Scheme 1**. The results clearly demonstrated that proximity of the reacting centers is more important than the activation of the leaving group by cobalt complexation for the completion of the cyclic network. Decomplexation led to several products formed by abstraction of I/H by the diradical generated *via* BC even at 0 °C thus demonstrating the high reactivity of the 9-membered system.



Scheme 1: Synthesis of 9-membered azaenediyne **4** in complexed form and its reactivity

Keeping in view of the fact that macrocyclic enediynes of ring size 11 or more are stable at physiological temperature, it is possible to broaden the scope of design of novel enediyne, if an intramolecular transannular reaction is conceived to reduce the ring size of a large macrocyclic enediyne, which in turn should enhance the rate of BC. Based on this, we went ahead to synthesize two novel macrocyclic N-substituted enediynyl ketones; one with a 12-membered ring (**8**) and another having a13-membered ring (**9**) **(Scheme 2).**



Scheme 2: Fate of N-substituted enediynyl ketones **8** and **9** after deprotection

Deprotection of the sulphonamido ketone **8** using thiophenol and K_2CO_3 in DMF generated the 12-membered free amino ketone **10 (Scheme 2)**. Compound **10** proved to be stable at room temperature. On the contrary, the 13-membered analog **11** in $CHCl_3$ decomposes slowly at room temperature and consequently involves the transannular addition of amine to carbonyl producing [8.3.0] system **12** and then undergoes BC protocol to generate **14** even at 4 °C.

The mechanism involving generation of diradical **13** is also supported by the interaction of the enediyne with supercoiled DNA. Thus **12** showed single-strand cuts to generate form II when incubated with double stranded plasmid DNA (pBR 322) in the supercoiled form **(Figure 1)**. The corresponding sulphonamido ketone **9**, under similar condition, did not show any DNA damage thus ruling out a possible cleavage mechanism by Michael addition of nucleophilic bases of DNA to the unsaturated carbonyl system. But the protected

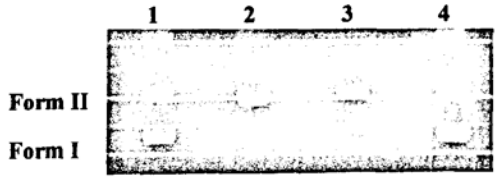


Figure 1: DNA cleavage studies by agarose gel electrophoresis

Lanes: 1. DNA in TAE buffer (pH 8) at 37 °C.
2. DNA in TAE buffer (pH 8) + aminol **(12)** (48 h) at 37 °C.
3. DNA in TAE buffer (pH 8) + aminol **(12)** (24 h) at 37 °C.
4. DNA in TAE buffer (pH 8) + sulphonamide **(9)** (48 h) at 37 °C.

amino ketone **9** in presence of glutathione, a biological thiol, at a pH of 8.0 was able to cleave supercoiled DNA. This observation proved our design based on activation through a biological thiol-mediated triggering of enediyne towards BC.

We had also designed enediyne scaffolds containing azide and olefinic functionality in the two arms of the enediyne. It was expected that intramolecular 1,3-dipolar cycloaddition would lead to the formation of bicyclic enediyne which

would then become reactive (for enediynes with ring size of 10 or less). With this intention, we had synthesized the following two classes of enediyne scaffolds: in one class (represented by **15** and **16**), the terminal alkene was in conjugation with enediyne system. In the other class (represented by **17** and **18**), the alkene was homologated in order to break this conjugation (**Figure 2**).

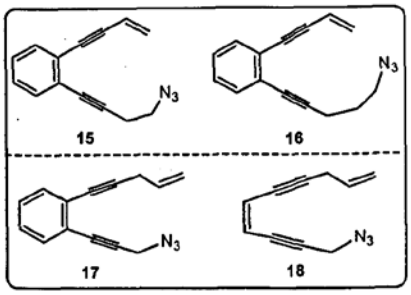
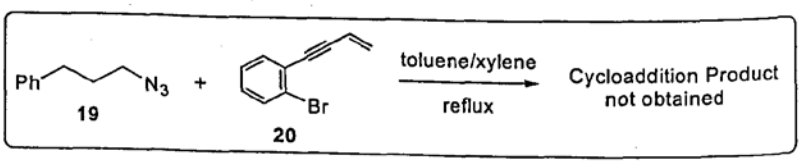


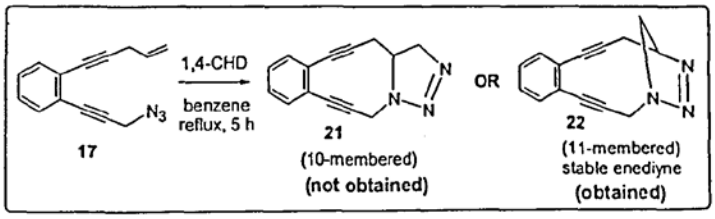
Figure 2: Target enediynes **15-18**

The compound **15** was found to be stable and reluctant to undergo intramolecular cycloaddition. We first thought that the acetylene arm containing the azide was too short to come within a reacting distance for the cycloaddition with alkene to take place. Thus the homologous (compound **16**) was prepared in order to bring the reacting functionalities in closer proximity. But the same result was obtained with **16** as was observed for compound **15**. We then decided to attempt the intermolecular version. Thus, intermolecular cycloaddition reaction between the azide **19** and conjugated eneyne **20** (**Scheme 3**) was attempted. In this case also, no cycloaddition product was isolated indicating poor dienophile character of the alkene due to conjugation with enediyne system. To break this conjugation, we synthesized the compounds **17** and **18**.



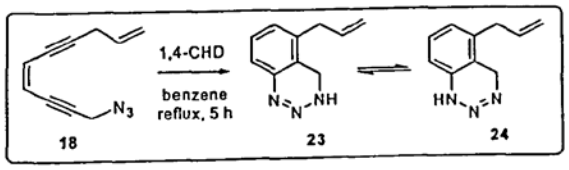
Scheme 3: Approach towards intermolecular 1,3-dipolar cycloaddition

Both the aromatic and non-aromatic azido enediynes (**17** and **18** respectively) were found to be unstable. They slowly decomposed at room temperature. Upon refluxing in benzene for 5 hr in presence of 1,4-CHD, the compound **17** underwent cycloaddition to give one regioisomeric triazoline **22** (**Scheme 4**).



Scheme 4: The fate of compound **17** on refluxing in benzene

The non-armatic enediynyl azide **18**, however, gave the aromatic compound **23/24** (**Scheme 5**) probably *via* azide-alkyne cycloaddition followed by Myers-Saito rearrangement. The compound was also able to cleave the double stranded plasmid DNA in super coiled form.



Scheme 5: The fate of compound **18** on refluxing in benzene