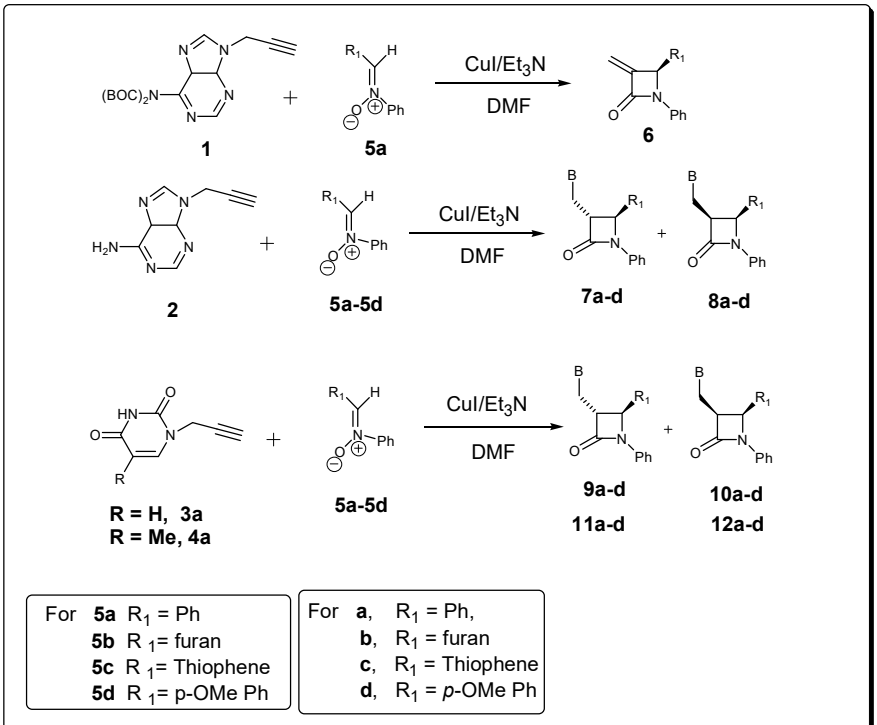
ABSTRACT

This dissertation entitled **"*SYNTHESIS OF ENEDIYNE AND β-LACTAM BASED CHIMERIC SYSTEMS USING 1, 3-DIPOLAR CYCLOADDITION*"** is an embodiment of research towards: (a) synthesis of dual pharmacophores consisting of β-lactams and nucleosides *via* Kinugasa reaction, (b) design, synthesis and reactivity studies of β-lactam fused enediynes *via* intramolecular Kinugasa reaction and (c) synthesis of isoxazoline fused enediynes by a highly regioselective one-step nitrile oxide-alkene [3+2] cycloaddition approach. Towards achieving our goals, several novel β-lactams and bicyclic enediynes were synthesized and their molecular conformation and chemical reactivity were evaluated.

The first work was aspired towards synthesizing the β-lactam nucleobase chimeras *via* Kinugasa reaction and checking the antibacterial activities of the synthesized chimeras against penicillin sensitive *E. coli* strain. The combination of the β-lactam and nucleobase, a chimeric molecule, is a viable bioconjugate as it has the acylating activity of β-lactams as well as the recognition ability of nucleobases. For the synthesis of the target β-lactams, various N-propargyl bases, namely 3-propargyl adenine (**2**) and its di-t-Boc analogue (**1**), 1-propargyl uracil (**3a**)**,** 1-propargyl thymine (**4a**) and 1-propargyl cytosine were prepared. With the propargyl nucleobases in hand, we proceeded to carry out the Kinugasa reaction with the diphenyl nitrone (**5a**). N, N-di-t-Boc propargyl adenine (**1**) was reacted in the first place for better handling due to solubility. However, the reaction with the phenyl nitrone (**5a**) in presence of CuI and Et_3N in DMF produced only the exomethylene β-lactam **6** thus showing the lability of the imidazoline ring as a nucleofugal. We have established the mechanistic possibility for the formation of exomethylene β-lactam.

The reaction with propargyl adenine itself for which the imidazole ring is not activated further by electron withdrawal was attempted first. The lone pair on the N in amino group can now participate in delocalization thus lowering the electron withdrawal further. Gratifyingly, the reaction was successful and we obtained moderate yields of *cis* and *trans* β-lactams **8a-d** and **7a-d** respectively. The reaction of propargyl uracil (**3a**), thymine (**4a**) and adenine (**2**) with the phenyl (**5a**), furan (**5b**), thiophene (**5c**) and *p*-OMe phenyl (**5d**) nitrones were also successful. A mixture of *cis* (**8, 10, 12a-d**) and *trans* β-lactam (**7, 9, 11a-d**) were obtained in respectable yields (60-66%) in all the cases.



Scheme 1: Formation of β-lactams with different propargyl nucleobases

The yield of various nucleoside- β-lactams were improved (70-76%) by mimicking the click chemistry condition using sodium-ascorbate, $CuSO_4$, $5H_2O$, tri-ethyl amine as base and DMF–H_2O (1:2) as solvent. This is mainly because of greater solubility of nucleobases in this solvent system which makes the reaction

more feasible and devoid of formation of any exo-methylene β-lactams.

With the chimeras of β-lactam and nucleobases in hand, their antibacterial activities, if any, were checked using the "holed-plate" assay against penicillin sensitive *E. coli* strain. Among all the chimeras the uracil-β-lactam (initially given as a mixture of *cis* and *trans*) was found to be active against the strain with the activity potency of about 20% of that of ampicillin. Similar activity was found when individual *cis* and *trans* isomers were used for antibacterial screening.

β-lactams, because of its inherent strain and bio-recognizable character, are attractive for use as locking device. Moreover, the advantage lies in the fact that in addition to the strain that it imparts into an enediyne fused on to it, the ring can also be easily opened by nucleophile (thiol), or enzymes like transpeptidase or β-lactamase or under basic conditions. Moreover, being itself a highly strained system, the β-lactam can impart strain in rings fused onto it and can have substantial effect on the reactivity of enediynes. To increase the selectivity and effectiveness, these mimics should also preferably possess suitable handles for appending DNA-binding agents. From this idea, a new class of synthetic enediynes 'lactenediynes' has been developed, which can be regarded as a prodrug.

From synthetic point of view we framed our objectives to devise a new route to synthesize β-lactam fused enediynes in a concerted pathway *via* intramolecular Kinugasa reaction in order to bypass the problem associated with the instability of alkyne or nitrone precursor. Kinugasa reaction is basically a one step synthesis of β-lactams *via* [3+2] cycloaddition between a nitrone and an *in situ* generated Cu (I)-acetylide. The Kinugasa reaction offers several advantages, which include the mild reaction conditions and the availability of a large repertoire of alkynes and nitrones. Crafting of these functionalities on two arms of the same molecule to facilitate an intramolecular reaction is comparatively easier

than the widely used Staudinger reaction which requires the use of an acyl halide, a more reactive functionality.

By this synthetic strategy we have successfully developed a general synthetic route to β-lactam-fused enediynes by intramolecular Kinugasa reaction. The method has widened the scope of Kinugasa reaction in the synthesis of sensitive systems like the oxacyclo and also 11-membered aromatic and corresponding aliphatic carbocyclic analogue (shown in **Figure 1**). The thermal reactivity of enediyne **19** indicated very little effect of endocyclic double bonds. The aliphatic enediyne expectedly showed higher reactivity as compared to the aromatic counterparts.

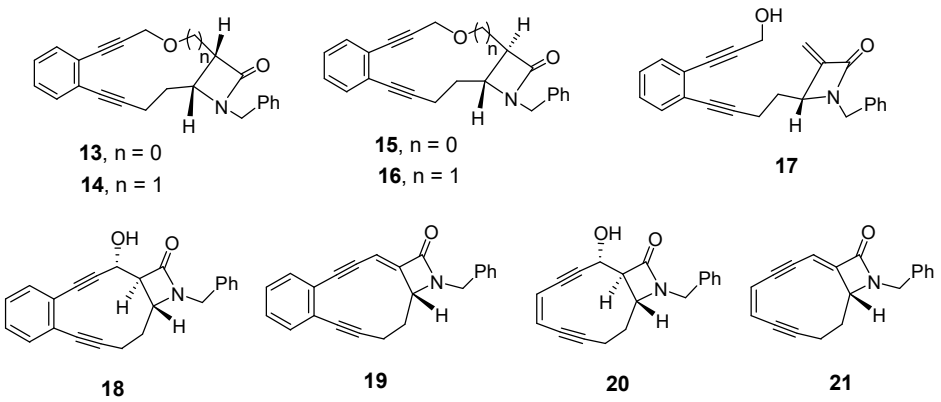


Figure 1: Our Target β-Lactam-fused Enediynes

Synthesis of two stereoisomeric isoxazoline fused enediynes by a highly regioselective one step nitrile oxide-alkene [3+2] cycloaddition approach have been developed. Their thermal reactivity towards Bergman cyclization was also studied. Towards the proposed intramolecular cycloaddition, we needed to incorporate the proper functionality in the two arms of the enediyne, namely the 2-electron dienophile and the 4-electron dipolarophile. The dienophile and the dipolarophile in our case were an alkene and a nitrile oxide respectively between which an intramolecular [3+2] cycloaddition reaction may take place.

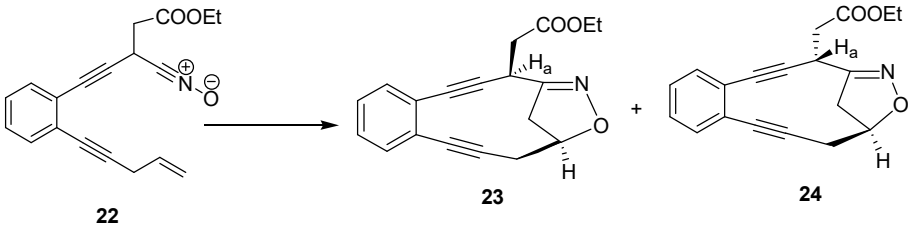


Figure 2: Isoxazoline fused bicyclic enediynes *via* nitrile oxide-alkene cycloaddition

The final cycloaddition was carried out in refluxing benzene at a sufficiently dilute condition (0.003 M) to minimize the intermolecular reaction followed by the *in-situ* formation of nitrile oxide compound (**22**). The final two products **23** and **24** which had same R_f value (R_f = 0.50 in 15:1 hexane/ethyl acetate) were isolated together which could not be separated by conventional chromatographic method. The compounds could only be separated by HPLC using ODS-Diacel column and 85% MeOH/ water as the mobile phase. The overall yield was 75% and the two isomers **23** and **24** were produced in a ration of 1:1 (**Figure 2**). The NOESY spectra of both the compounds were recorded. The cross peaks allowed us to assign the chemical shifts of various protons and the distinguishing feature between the structures is the difference in chemical shifts for the H_a. From the energy minimized structures we have concluded that the front running fraction in HPLC corresponded to structure **23** while the slower fraction corresponded to structure **24**. The thermal reactivity of both the isomers was studied and compared with that of isooxazolidine fused enediynes.