ABSTRACT

Part A

Consideration of the importance of chiral substituted proline derivatives led us to study the radical cyclization of N-arylsulphonyl-N-allyl-3-bromo-L-alanine ester. Our primary objective was to find out the parameters controlling the stereoselectivity.

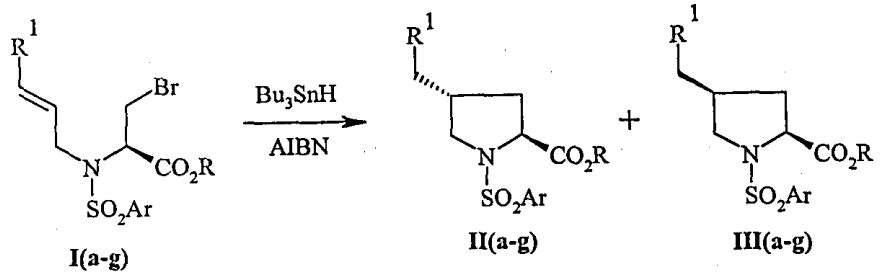


Figure 1

Thus, the various substrates (**Ia-Ig**) were synthesized from L-serine and then subjected these compounds to radical cyclization conditions. Quite surprisingly, the diastereoselectivity, as determined from ^{1}H NMR, was found to be dependent upon the nature of sulphonyl aryl group. Incorporation of more electron withdrawing *p*-nitrosulphonyl or *p*-chloro phenyl sulphonyl, resulted in slight increase of diastereoselectivity, with the *trans* isomer predominating. The most dramatic change was seen when the amine was protected with 2-naphthyl sulphonamide. Thus for the benzyl ester (**Id**) a ratio of 8:1 of the *trans* and *cis* isomers were obtained. Replacement of benzyl with benzhydryl caused an remarkable increase of selectivity from 8:1 to 33:1. The results of cyclization are shown in **Table 1.**

Table 1: Cyclization of Various Bromides (Ia-Ig)

Entry	Substituents	Substrate	Products and their ratio	Combined percent yield
1	R_1=H, R=Bn Ar=p-Tolyl	**Ia**	**II a** and **III a** 3:1	80
2	R_1=H, R=Bn Ar=p-nitrophenyl	**Ib**	**II b** and **III b** 4:1	83
3	R_1=H, R=Bn Ar=p-Chlorophenyl	**Ic**	**II c** and **III c** 4:1	85
4	R_1=H, R=Bn Ar=2-naphthyl	**Id**	**II d** and **III d** 8:1	85
5	R_1=Ph, R=Bn Ar=p-Tolyl	**Ie**	**II e** and **III e** 3:1	82
6	R_1=Ph, R=$CHPh_2$ Ar=p-Tolyl	**If**	**II f** and **III f** 2:1	90
7	R_1=H, R=$CHPh_2$ Ar=2-naphthyl	**Ig**	**II g** and **III g** 33:1	96

Enediynes are another class of compounds that caught our attention. These compounds have the ability to form diradicals *via* Bergman Cyclization. Controlling the Bergman Cyclization in enediynes is an active area of research in recent years. To design a useful enediyne, it is essential to know the parameters that affect the kinetics of BC. Towards that end, we prepared various bisaldimino enediynes (**IVa-IVc**). Differential scanning calorimetric measurements were then recorded for (**IVa**) and (**IVb**). Expectedly, the onset temperature for both the enediynes were quite high. For (**IVa**) the exothermic rise started at ~ 140 ^{0}C while for (**IVb**) it recorded a temperature of ~ 200 ^{0}C to start the onset of BC.

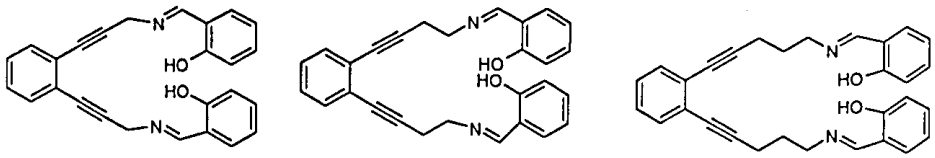
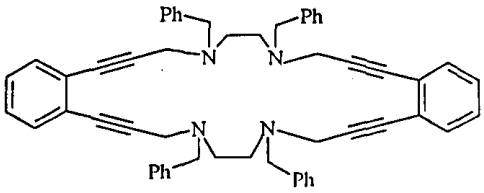


Figure 2

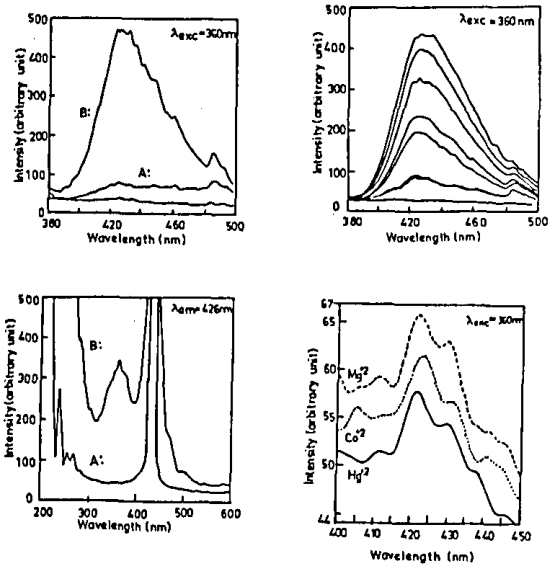
The thermal behaviour of the enediynes (**IVa-IVb**), after complexation, was just opposite. Thus the enediyne (**IVa**) under complexation with metal ions, led to an elevation of onset temperature of BC while for the other one, the onset temperature is reduced by ~ 40 ^{0}C. Thus, we have demonstrated the dependence of thermal behaviour of the two enediynes on the length of spacer, both in neat as well as under metal complexation conditions.

The ability of the enediynes (**IVa-c**) to form complexes with metal ions turned our attention to study their photophysical properties, specially to find the sort of change in the absorption / fluorescence behaviour of the enediynes upon complexation. Fluorescence sensing of ions is of great interest as sensors in biomedical research and in molecular information processing. We initially studied the fluorescence behaviour of the tetraazaenediyne (**V**), which was earlier synthesized in our laboratory by Dr. J. C. Shain. The fluorescence spectrum of this enediyne in DMSO showed extremely weak emission at 426 nm when excitation was done at 360 nm. However, the Ni^{2+}-complex of enediyne (**V**) displayed broad but intense maxima at 426 nm in the emission spectrum with the excitation wavelength fixed at 360 nm (**Figure 4**). The concentration was kept at a level at which neither the starting enediyne nor blank DMSO showed any detectable maxima. The intensity of the emission maximum of the Ni-complex enhanced gradually as the concentration of the complex was increased. Thus, for the first time, we have been able to demonstrate chelation enhanced fluorescence (CHEF) in an enediyne. The enediyne was infact able to show similar behaviour with other metal ions also, ranging from transition metals to alkaline earth metal ions. These metal ions showed an emission maxima at a wavelength ranging from 418-435 nm. However, not much selectivity was observed.



V

Figure 3



Fluorescence Spectra of Tetraaza Enediyne (V)

Figure 4

The observation of CHEF in the macrocyclic enediyne (V) encouraged was to explore the fluorescence behaviour of the bis-aldimino enediynes. In these molecules, the enediyne moiety is the site of photonic transactions for both the excitation and emission. The receptor is the site for guest complexation and decomplexation. The spacer is the methylenes, which act as bridge between fluorophore and receptor. In case of enediyne (**IVa**), the spacer is just a methylene while in case of (**IVb**) and (**IVc**), a two and three-carbon chain separates the fluorophore and the receptor.

The solution containing (**IVa**) failed to show any fluorescence as was evident by the absence of any emission when the molecule was excited at its λ_{max} of 250 nm. However, when Cu (II) ions were added to the same solution, a strong emission was now observed at λ_{max} of 450 nm. Thus the PET mechanism by which the receptor, in this case the salicylaldimine, was quenching the emission from the fluorophore was efficiently blocked by Cu (II) ions. A similar effect was observed for Ni (II), Co (II) as well as nontransition metal ions like Mg (II) and Ca (II). The situation was exactly reversed in the case of other enediynes (**IVb**) and (**IVc**) which itself shows a strong fluorescence at λ_{max} of 450 nm when the excitation was carried out at its UV-absorption maxima of 250 nm. However in the presence of metal ions, the fluorescence intensity decreased to an extent that is dependent upon the type of the metal ion. Cu (II) was found to be the most efficient quencher of fluorescence. Thus PET is more efficient in the metal ion bound state of the receptor than in its free state in case of (**IVb**) and (**IVc**).

In summary, we have shown for the first time the use of an enediyne moiety as a probe for fluorescence studies. The observation of reversing the fluorophoric activity by subtle change of spacer length from one to two or three carbon atoms is also interesting. Since fluorescence devices may be useful for various purposes, our results may open a new type of research in the field of enediynes.

Another structural feature in Z-enediynes that caught our attention in the presence of a reverse-turn associated with the two acetylene atom. With this idea, we prepared several enediynyl amino acid containing peptides and studied their reactivity and conformations.

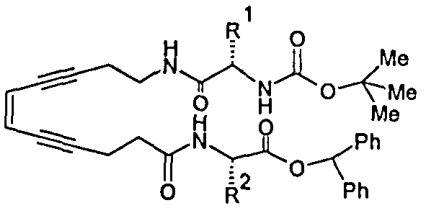


Figure 5

The synthesis of the peptides were carried out according to scheme shown below:

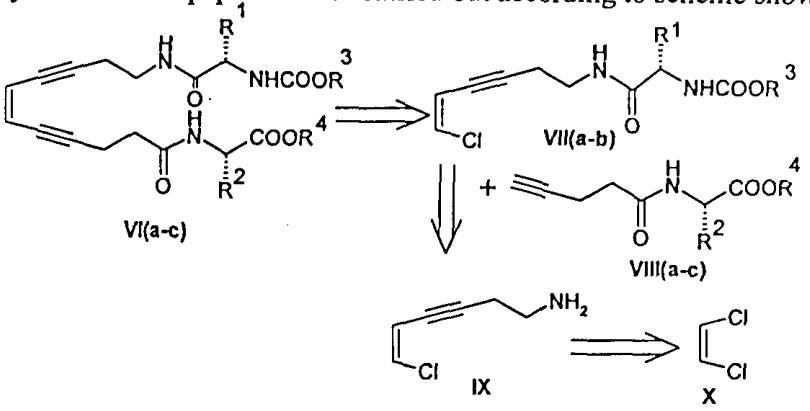


Figure 6

Measurement of thermal reactivity by DSC revealed the onset temperature for phe-ED-phe was found to be higher than the corresponding ala-ED-ala. The third peptide failed to show any clear cut exothermic peak.

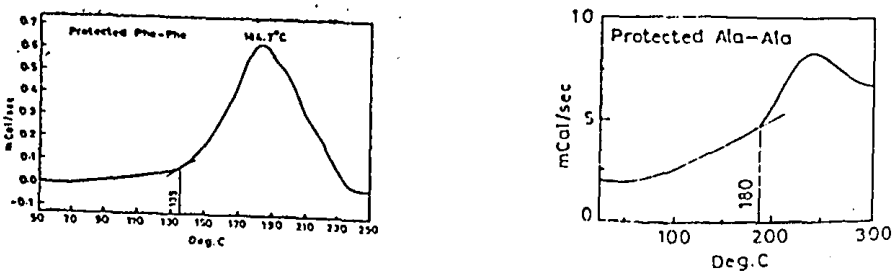


Figure 7

Circular dichroism (CD) spectra of the fully protected peptides were recorded which revealed some noticeable features.

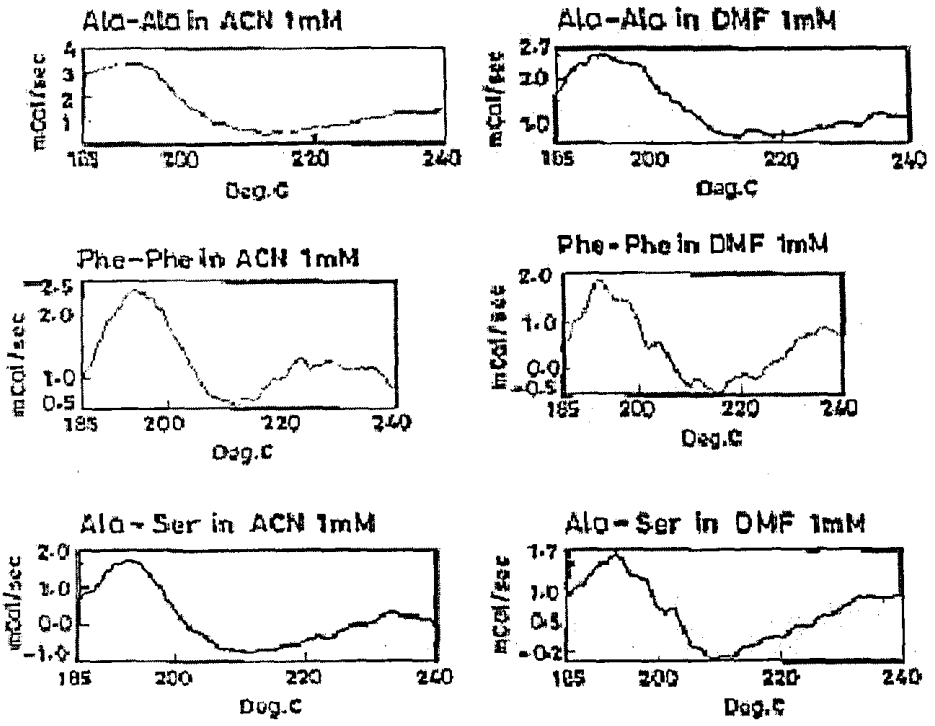
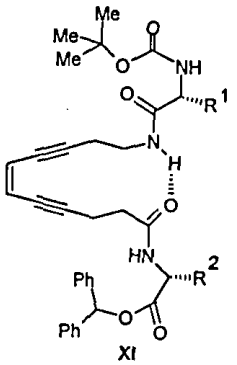


Figure 8

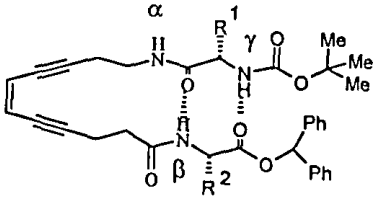
In the figures, there was clear indication of a β-sheet like characteristics in all the peptides. This is apparent from the appearance of broad but definite minima at~ 211 nm. However, along with the minima, there was also a maxima at ~192 nm which is typical α-helix of a peptide. Although our peptides are too small to adopt any fully helical structure, the appearance of this maxima probably indicates the presence of conformation (XI) in solution for all the peptides which involves H-bond formation between the N-H and amide carbonyl, both the functional groups belonging to the enediyne moiety.



XI

Figure 9

The minima at ~211nm would probably indicate presence of conformation (XII) formed by H-bond between the β-NH and α-carbonyl as well as between the γ-NH and the γ-carbonyl in equilibrium with conformation (**XI**).



XII

Figure 10

Variable temperature ^{1}H-NMR was next used to probe intramolecular H-bonding in the peptides. The temperature dependence of the amide protons (including the one protected as carbamate) were recorded in the temperature range of 25-60 ^{0}C in d_6-DMSO. The variation of chemical shift (Δδ/ΔT) for all the three types of N-H's in the three peptides are shown in (**Table 2**).

Table 2: Variation of Chemical Shift with Temperature (Δδ/ΔT)

Peptide	α-NH (Δδ/ΔT) ppb	β-NH (Δδ/ΔT) ppb	γ-NH (Δδ/ΔT) ppb
(VIa) (AA)	-5.5	-6.0	-9.9
(VIb) (PP)	-5.5	-6.1	-10.2
(VIc) (AS)	-6.4	-6.9	-10.0

In view of the above data, we propose that there is significant proportion of β-sheet like conformation as was indicated by CD-measurements as well as the generally higher (Δδ/ΔT) values for the chemical shifts of β and γ N-H's. However, the results also indicated the presence of other conformations as well, specially the α-NH as being intramolecularly H-bonded. In solution, the peptide mainly adopts two particular structural motifs, as indication by two conformations. The variable temperature experiments indicate that the conformation resembling β-sheet type motifs are more predominant. The situation is represented in **Figure 11**

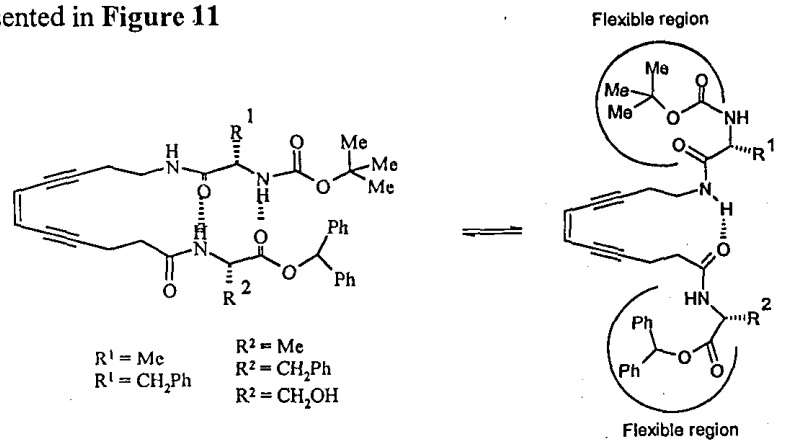
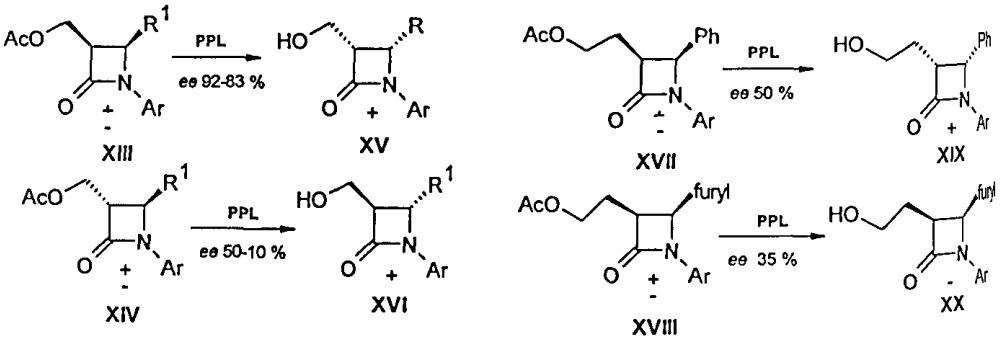


Figure 11

Part B

Pig Pancreatic Lipase (PPL) has been extensively used to catalyze the hydrolysis of wide range of esters and also in transesterification reactions. The enantioselectivity of such transformations depends primarily on the nature and stereochemistry of substituents within the vicinity of the ester functionality and also on the nature of media. Seebach and later on Jones proposed active site models for PPL. Interestingly, although both the models recognize the same four binding pockets, they are locally enantiomeric which has led to a complex situation. In our laboratory, we have been studying the PPL-catalyzed hydrolysis of a number of β-lactam acetates in order to access them in enantiopure / enriched

form. During such a study, a wide range of stereoselectivity was observed. While the hydrolysis of *cis* 3-acetoxymethyl-4-aryl β-lactams (**XIII**) proceeded with a high degree of *ee* thus making the hydrolyzed products useful for possible side chain mimics of Taxol, the corresponding *trans* isomers (**XIV**) showed much less selectivity. The interesting point is, however, the switching of the active site models for the *cis* and the *trans* isomers. While the selectivity of the hydrolysis of *cis* isomers can be explained on the basis of Jone's model, the *trans* isomers prefer to bind according to the Seebach model. Increasing the acetate chain length in the *cis* series has an adverse effect on the *ee*. But the point of interest is that changing the C-4 substituent from phenyl to a furyl in this series caused a shift in binding mode. All these results can be explained if one assumes the dominant roles that the large hydrophobic and the small polar pockets play during binding of the substrates. The catalytic site may be considered flexible enough to accept an α or a β acetate (**Figure 12**).



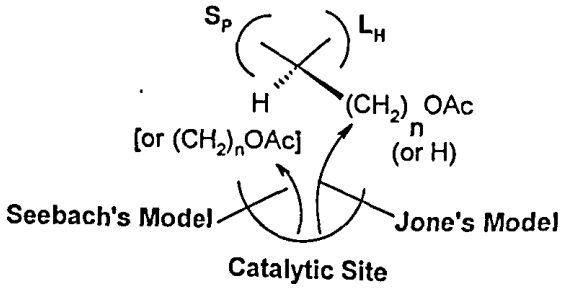


Figure 12