CHAPTER-2

An effective strategy towards synthesis of steroidal isoxazolidine, isoxazoline and isoxazole via stereoselective intramolecular 1,3-Dipolar cycloaddition.
Introduction

The monumental work of Huisgen and coworkers in the early 1960’s has led to the general concept of 1,3-dipolar cycloaddition reaction. Over the years this reaction has been developed into a useful method for synthesis of five membered heterocyclic ring. Many 1,3-dipolar species are readily available and react with a wide variety of dipolarophile. The reaction proceeds through the formation of two carbon-carbon sigma bond with a considerable amount of steric and electronic rearrangement.

Intemolecular 1,3-Dipolar cycloadditions are usually bimolecular and involve the addition of a 1,3-dipole to a multiple bond system leading to a five membered heterocycles.

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    &\quad \\
    &\quad \\
\end{align*}
\]

1,3-Dipole is basically a three atom system over which four \( \pi \)-electrons are distributed as in the case of allyl anion system. The three atoms can be a wide variety of combinations of carbon, oxygen and nitrogen. The dipolarophile can virtually be any double bond or triple bond.

Huisgen and co-workers have systematically studied the mechanism of 1,3-dipolar cycloaddition. The principal question that arose when considering the regiospecificity of 1,3-dipolar cycloaddition, is whether the two new \( \sigma \)-bonds formed on addition of the 1,3-dipole to the dipolarophile are formed simultaneously or one after another. The mechanism that has emerged from Huisgen’s group is that of a single step four centre cycloaddition in which the two new bonds are both partially formed in the transition state although not necessarily to the same extent. A symmetry energy correlation diagram reveals that such a thermal cycloaddition reaction is an allowed process. A proposed alternative mechanism is a two step process involving spin paired diradical intermediate.

When the 1,3-dipole and the dipolarophilic part are in the same molecule cycloaddition is intramolecular and leads to a new bicyclic system. Thus intramolecular cycloadditions are amenable to the construction of inherently more complex product than intermolecular cycloadditions. Markedly different regioselectivity controlled by the
geometric constraints of bringing the 1,3-dipole into correct internal alignment for reaction with the dipolarophile is often observed in an intramolecular cycloaddition. Sometimes these geometric constraints overwhelm the normal regiochemical preferences dictated by electronic factors. The greater steric constraints inherent to intramolecular cycloaddition often affords higher diastereofacial discrimination; accordingly, these reactions can exhibit very high stereoselectivity. With all these advantages intramolecular cycloaddition is certainly a powerful synthetic tool for heterocyclic synthesis.8

The example of such process was first reported by Le Bel and Whang in 1959.9 Since this initial report many papers dealing with intramolecular 1,3-dipolar cycloadditions have been published and considerable use has been made of this reaction for organic synthesis.10,11 The intramolecular cycloadducts have also been used to generate synthons for the solution of other synthetic tasks.

In the present discussion it is aimed to review the common characteristics of the intramolecular 1,3-dipolar cycloaddition with special emphasis on the nitrone and nitrile oxide cycloaddition.

Nitrones

Intramolecular cycloaddition of nitrone is possible only when alkene and the nitrone moieties are suitably arranged in the same molecule.12 This useful synthetic idea was first investigated by Le Bel and Whang,9,12 using molecules in which the nitrone was separated by a propylene or a butylene chain. The unsaturated nitrone was not isolated but was generated either by oxidation of an N-alkenyl hydroxylamine or by condensation of an unsaturated aldehyde with N-methyl hydroxylamine.

\[
\text{CH}_3\text{N(OH)CH}_2\text{(CH}_2\text{)}_3\text{CH=CH}_2 \xrightarrow{\text{H}_2\text{O}} \quad \begin{array}{c}
\text{H}_2\text{C} \\
\text{C=CH}
\end{array} \\
\text{CH}_2=\text{CH(CH}_2\text{)}_3\text{CHO} \xrightarrow{\text{CH}_3\text{NHOOH}} \quad \begin{array}{c}
\text{O} \\
\text{N} \\
\text{CH}_3
\end{array} \\
\quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad
hydroxylamine was shown to have the trans ring fusion. In this case the stereochemistry was found to be temperature dependent with increasing proportions of cis isomer at higher reaction temperature.\textsuperscript{14} At 300°C the trans fused isoxazolidine undergoes partial isomerisation to the cis isomer. This process was rationalised by thermal cycloreversion of the isoxazolidine into nitrone followed by ring closure to give product of opposite stereochemistry. Retro 1,3-dipolar cycloaddition have been noted with intermolecular example of nitrone olefin adducts\textsuperscript{15} and provide reasonable analogy for the foregoing interconversion.

In order to rationalise the stereochemical result, Le Bel suggested that the transition state leading to cis ring fusion requires the potential six membered carbocycles to adopt twist conformation. A slightly deformed chair arrangement will produce the trans isomer. Examination of models indicate that orbital overlap for 1,3-addition is favourable for a cis-ring juncture for both syn and anti configuration of the intermediate nitrone. In the transition leading to trans isomer, a significant interaction develops between the 1-H and 5-methylene group which allows the seemingly less favourable twist arrangement of the tetramethylene chain leading to cis isomer to compete successfully. This would account for the observed isomer ratio of 2:1(cis-trans) in the condensation of 6-heptenal with methyl hydroxyl amine. With citronellal the trans product is more stable than the cis one.

A most noteworthy feature of the intramolecular cycloaddition of nitrone derivative is the fact that a cyclopentane ring with three contiguous asymmetric centre is constructed stereospecifically in a single step from an acyclic precursor. An example of usefulness of this methodology is reported by Vinick and co-workers.\textsuperscript{16}
Nowadays, 1,3-dipolar cycloaddition is used in almost all branches of organic chemistry to synthesise molecules of higher stereopreferences.

Oppolzer and co-workers have extended the intramolecular 1,3-dipolar cycloaddition reaction to include nitrone alkenes separated by chains that generally incorporate a benzene ring and a hetero atom to produce isoxazolidines. In most of the cases examined, the isoxazolidines were found to contain a cis ring junction between the heteroring.

The ether oxygen of the above example can be replaced by a formamide group, which results in the formation of the related tetrahydro quinolene product.

The intramolecular cyclization of allenic nitrones affords several novel bicyclic products. Treatment of 5,6-heptadien-2-one with N-methyl hydroxyl amine gives a transient nitrone which produces the unsaturated bicyclic isoxazolidine by intramolecular cycloaddition at the terminal double bond of the allenic function.
The reaction of hydroxylamine with silver carbonate on celite, a reagent known to oxidise hydroxylamine to nitrones,\textsuperscript{20} produces cycloadducts in 19\%(trans isomer) and 23\% (cis isomer) yields respectively.\textsuperscript{21} Two orientations of addition of the N-alkenyl nitrene to the un-symmetrical dipolarophile are possible. But the cis ring fusion prefers, probably due to steric effects. Oppolzer\textsuperscript{11} has noted a high degree of selectivity in the intramolecular addition of methylene nitrene to 1,2-disubstituted olefinic group, where bonding occurs with the same orientation.

The reaction of C-benzoyl nitrones with allyl amine has been reported to afford tetrahydro-5H-pyrrolo[3,4-c]-isoxazoles.\textsuperscript{22} These cycloadducts result from the initial formation of an imine which then undergoes an intramolecular 1,3-dipolar cycloaddition.

An interesting sequence consisting of intramolecular nitrene cycloaddition followed by isoxazolidiene hydrogenolysis that leads to a triply functionalized perhydroquinolizine has been described.\textsuperscript{23}

A series of sulphur substituted dienyl nitrones are conveniently prepared from 3-sulfolene precursors. Intramolecular 1,3-dipolar cycloaddition reactions gave the fused byclic isoxazolidines in a stereoselective manner.\textsuperscript{24}
Since the discovery of the general concept of 1,3-dipolar cycloadditions, numerous cycloadditions involving nitrile oxides have already been described.

The most widely employed method of preparation of nitrile oxide is via chlorination of oximes followed by a base treatment. This method has the disadvantage because of the use of hazardous chlorine gas and undesired chlorination at other activated sites leading to serious side reactions.\(^{25}\)

Nitrile oxide can be conveniently generated by the oxidation of aldoximes with NO\(_2\). This method of nitrile oxide preparation has been reported for oxidation of 2-allyloxybenzaldehyde oxime to give the fused ring compound through an intramolecular 1,3-dipolar cycloaddition of nitrile oxide.\(^{26}\)

Treatment of nitro compound with phenylisocyanate in presence of triethyl amine leads to the formation of nitrile oxide.\(^{27}\) This strategy is used for the following examples.

Macrocyclization of the olefinic nitrile oxide to the ansa macrolide skeleton has been carried using clorax as an oxidising agent in a phase transfer catalytic reaction condition.\(^{28}\)
A large number of aldehyde oximes were transformed into the corresponding nitrile oxides in good yield by using 1 equivalent of NCS (N-chloro succinimide) in DMF followed by a base treatment. 

\[
\text{ArCH=OH} \xrightarrow{\text{NCS}} \text{Ar-CH(Cl)=N-OH} \xrightarrow{\text{base}} \text{Ar-C=N}^+\text{O}^- 
\]

Recently chloramin-T has been reported to use effectively for conversion of oximes into the nitrile oxide in one step for in situ 1,3-dipolar cycloaddition reaction. This method was used to synthesis the isoxazolidine from ortho-hydroxy-O-allyl ether benzaldehyde oxime in 84% yield.

1,3-Dipolar cycloaddition in steroids

There are few examples for use of 1,3-dipolar cycloaddition reactions in steroids. Some of them are recorded in the introductory part of chapter-1a.

Oximation of the C-20-steroidal aldehyde followed by treatment of N-chlorosuccinimide/Et3N affords a nitrile oxide, which underwent intermolecular 1,3-dipolar cycloaddition with alkenes (e.g. 3-methyl-1-butene) to form isoxazoline (1:1 mixture of C-24 isomer)
The insitu 1,3-dipolar cycloaddition reaction of nitrile oxide with 3,3-dimethylpropargyl alcohol affords steroidal isoxazole in 90% yield.\(^{32}\)

Reaction of 3,20-dioxo-pregna-4,16-diene with nitronic ester (MeO\(_2\)CCH=N(O)OMe in dry CH\(_2\)Cl\(_2\) at 14000 atmospheric pressure for 15-17 h is reported to give N-methoxy(16,17-d)1',2'-oxazolo derivative of pregnane in 65-80% yield.\(^{33}\)

Pregnadiene (R=H,Ac) undergoes 1,3-dipolar cycloaddition with MeON(O)=CH-CO\(_2\)Me in the presence of BF\(_3\).Et\(_2\)O in CH\(_2\)Cl\(_2\) at 20\(^{\circ}\)C to give (16,17-d)-4'H-1',2'-oxazole derivative of steroid.\(^{34}\)

High pressure induced cycloaddition of nitronic ester of 16-Dehydro-20-oxo-
steroids is reported to give steroidal[16α,17α-d]-tetrahydro-1',2'-isoxazoles regiospecifically. Generally both modes (exo-endo) of dipolarophile approach to the dipole are occurred, all four possible isomers have been isolated and their preferred conformations were established. The conversion of unstable stereomers to stable forms proceeds as a simultaneous N-inversion and isoxazolidine cyclic conformational change.

This transformation was confirmed from PMR data, the 3'H signal at δ 3.60 ppm (J=7.5 Hz) is the characteristic of isoxazolidine.

There is no report for intramolecular 1,3-dipolar cycloaddition in steroidal D-ring however one example at A\B ring is recorded below.

Reaction of the 1(10)-unsaturated trans-5-oxo 5,10-secosteroid with N-methyl hydroxylamine has been reported to give a non-isolable nitrone that undergoes a spontaneous transannular cyclisation with formation of the N-methyl isoxazolidine derivative in over 60 % yield.

The geometry of the trans cyclo-decenone ring in the modified steroid compound is favourable for trans annular 1,3-dipolar cycloaddition. In contrast the diastereomeric 1(10) unsaturated cis nitrone system does not undergo intramolecular 1,3-dipolar cycloaddition since the double bonds between C-1 and C-10 and the trigonal C-5 atom of the cis-cyclodecenone ring are too far apart to permit the internal cyclization reaction to occur.

**Results and discussion**

In continuation to the search for synthesising novel heterosteroid of probable biological importance, it was planned in this chapter to synthesise novel D-ring fused heterosteroid utilising intramolecular 1,3-dipolar cycloaddition.
The strategy for preparation of a novel steroidal substrate for intramolecular cycloaddition is shown as below.

17-Acetamidoandrost-5,16-diene-3β-acetate(1) was prepared from 16-Dehydro pregnenolone acetate oxime via Beckmann rearrangement using POCI₃ pyridine system at 0°C (described in chapter-1).

The enamide 1 thus obtained was formylated at C-16 position with Vilsmeier reagent (10 molar equivalent) at 10-15°C for 4 h to prepare 16-formyl-17-acetamidoandrost-5,16-diene-3β-acetate (2).

The product 2 melted at 236-37°C and showed an Rf value 0.3 (in chloroform). The product was characterised as below.

The IR(KBr) spectrum of the compound showed an absorption peak at $\nu_{max}$ 3250 cm⁻¹ for N-H-stretching. This shifting of absorption position for NH group from normal
value of 3300 cm\(^{-1}\) was probably due to the hydrogen bonding of N-H proton with the neighbouring formyl group, and the position of this absorption peak was not concentration dependent indicating that the hydrogen bonding is intramolecular. This assumption was also supported by the \(^1\)HNMR(CDCl\(_3\)) spectrum of the product in which the broad singlet at \(\delta\) 10.75 integrated for one proton was assigned to the hydrogen bonded N-H proton because of its characteristic down field shift. Deuterium exchange experiment in \(^1\)H NMR showed absence of the peak at \(\delta\) 10.75. The sharp singlet peak at \(\delta\) 9.08 integrated for one proton was accounted for the formyl proton at C-16 position. The mass spectrum(El) of the compound showed fragmentation peak at m/z 339 due to loss of CH\(_3\)COOH fragment from the molecular ion along with other fragmentation ion peak at m/z 311, 297,282 and 269. The compound gave satisfactory elemental analysis and showed a specific rotation of -37.50° at a temperature of 23°C(D-line of sodium light) which indicated that the product was optically pure and the reaction proceeds stereoselectively with the maintenance of stereochemistry of the different stereogenic centres. The same product was also prepared by treating 16-DPA oxime with 10 molar equivalents Vilsmeier reagent at 10-15°C for 3-4 h as was mentioned in the chapter 1a. The two products were proved to be identical by comparing the spectral data, elemental analysis, mixture melting points.

\(3\beta\)-acetoxy-16-formyl-17(allylacetamido)androst-5,16-diene(3a)

N-Allylation of 16-formyl-17-acetamidoandrost-5,16-diene-3\(\beta\)-acetate(2) with allyl bromide was carried under phase transfer catalytic reaction condition to give the corresponding \(3\beta\)-acetoxy-16-formyl-17(allyl acetamido)androst-5,16-diene(3a).

A solution of 2 (1 molar equivalent) in CH\(_2\)Cl\(_2\) was treated with 1.5 molar equivalents of allyl bromide, tetrabutylammonium bromide (0.5 molar equivalent) and 30%KOH(aqueous) in a round bottomed flask for 16 h at room temperature. The product was extracted with dichloromethane washed with water, dried over sodium
sulphate. Removal of dichloromethane afforded N-allylated product 3a in 67% yield. The product 3a gave a melting point 170°C (recrystallised from ethanol) and Rf value (0.3) in ethyl acetate:hexane (20:80).

The IR (KBr) spectrum of the compound showed no absorption peak between 3100-3400 cm\(^{-1}\) which was a clear indication of the absence of NH proton in the molecule. In \(^1\)HNMR spectrum of the product the peak at \(\delta\) 10.75 for the amide proton of the compound (2) was absent however two additional peaks comprising a broad singlet at \(\delta\) 5.06 and a multiplet at \(\delta\) 4.44 appeared which were accounted for the olefinic protons of allyl group. The mass spectrum (El) gave the molecular ion peak at m/z 439 and the remaining fragmentation peaks were at m/z 411, 397, 396, 379, 369, 351, 337, and 309. The product 3a gave satisfactorily elemental analysis for the molecular formula C\(_{27}\)H\(_{37}\)NO\(_4\) and showed a specific rotation of -58.46° at 23°C.

**Steroidal isoxazolidine**

When compound (3a) was stirred at room temperature with two molar equivalent of N-methyl hydroxyl amine hydrochloride for 2 h in ethanol on usual work up it afforded a mixture of two products (vide TLC). The \(^1\)HNMR spectrum of the crude compound showed two sharp singlet peaks at \(\delta\) 3.5 and 3.45 for N-methyl group of two isomeric nitrones and a sharp singlet at \(\delta\) 7.20 integrated for one proton.

The nitrone thus formed was dried with the help of a high performance vacuum pump and stirred at 80°C in toluene for 12 h. From the TLC it was evident that out of the two nitrones formed only one underwent cycloaddition to give the isoxazolidine (4) in 48% yield.
The product was characterised with the help of spectral data, optical rotation and molecular modelling.

The IR (KBr) spectrum of the product showed broad absorption band at $\nu_{\text{max}}$ 1720 cm$^{-1}$ and 1660 cm$^{-1}$, for C=O stretching vibrations of the 3-acetyl and 17-N-acetyl groups. The $^1$HNMR spectrum of the product showed absence of a signal at $\delta$ 7.2 for nitrone. Absence of this signal at $\delta$ 7.2 as well as allylic group signal supported the cycloaddition reaction of nitrone with the allylic group. The mass spectrum (EI) of the product showed a molecular ion peak at m/z 468 and a peak appeared at m/z 422 was assigned to the loss of diagnostic CH$_3$NHO group from the molecule. The peak at m/z 396 was assigned to the successive loss of C$_2$H$_2$[(M$^+$-CH$_3$COOH)-C$_2$H$_2$]. The product gave satisfactory elemental analysis for the molecular formula C$_{28}$H$_{40}$N$_2$O$_4$ and showed a specific rotation value of $[\alpha]_D^{23} = -176.21^\circ$ [c=1, CHCl$_3$].

3β-acetoxy-17-(allylacetamido)-androst-5,16-diene-16-formyl oxime (5a)

Oximation of ethanol solution of aldehyde (3a) with hydroxyl amine hydrochloride for 0.5 h at room temperature rendered a white solid product in 78 % yield which was characterised as 3β-acetoxy-17-(allylacetamido)-androst-5,16-diene-16-formyl oxime (5a). The product was recrystallised from ethanol. It showed an $R_f$ value 0.15 in Toluene:acetone = 95:5 and melted at 182°C.

The IR(KBr) spectrum of the compound showed a medium absorption band at 3320 cm$^{-1}$ which was assigned to the O-H stretching of the oxime group. The $^1$HNMR (CDCl$_3$) spectrum of the product showed a sharp singlet at $\delta$ 7.5 integrated for one proton, which was assigned to the imine proton of oxime. The mass spectrum (EI) of the compound showed distinct peaks at m/z 454 and 455 which were assigned to the M$^+$ and (M$^+$+I) ion peaks respectively.
Steroidal isoxazoline

The general procedure of preparation of isoxazoline from oxime consist of cycloaddition of nitrile oxide with olefins. The various methods of nitrile oxide preparation is described in the introductory part of this chapter. However this procedure has certain disadvantages like poor yields, low temperature reaction conditions and multistep reaction sequences.

A newly developed procedure has employed chloramin-T in ethanol as efficient reagent for preparation of nitrile oxide directly from aldoximes for cycloaddition in one step which is comprised of excellent yield and stereoselectivity. Therefore this strategy was chosen for carrying 1,3-dipolar cycloaddition reaction intramolecularly in a suitably designed steroidal molecule comprising an aldoxime and N-allyl group.

When steroidal oxime derivative (5a) was reacted in refluxing ethanol, the formation of a new product and absence of starting material was observed after 3 h vide TLC. The reaction was worked up by reducing the volume of ethanol in a rotavapour and pouring the content into ice cold water. It was extracted with dichloromethane, washed with water and solvent removed to obtain a solid product of (6) in 78% yield. It was purified by recrystallisation from methanol. Melting point of the solid was determined as 210-11°C and in TLC, it showed an Rf value 0.19 (solvent CHCl₃:MeOH=99:1). The product was characterised from the spectral and elemental analysis as below.

The IR(KBr) spectrum of the compound showed absence of a band at 3250 cm⁻¹ due to oxime OH. It showed absorption band at 1715 and 1650 cm⁻¹ for acetyl carbonyl groups. The ¹H NMR (CDCl₃) spectrum of the product showed absence of sharp singlet near δ 7.5 for imine proton and also allyl olefinic protons near δ 5.44 and 5.04 were absent which rendered proof for cyclo addition. The multiplet at δ 3.71 integrated for one proton was due to the characteristic tertiary alkane proton of the ring juncture and the two double doublet at δ 4.21 and 3.38 integrated for one proton each was
accounted for the alkane protons $\alpha$ and $\alpha'$ of the ring juncture. Other two $\alpha$ and $\alpha'$ proton appeared in coincidence with the multiplet at $\delta$ 4.57 and 3.71. In the $^{13}$C NMR spectrum the two carbonyl carbon peaks appeared at $\delta$ 170.8 and 168.3. The signals at $\delta$ 161.1, 155.03 and 140.4 were accounted for the three olefinic carbons at D and E ring of the molecule. The signals at $\delta$ 122.3 and 114.4 were accounted for the olefinic carbon at C-5 and C-6. The signals at $\delta$ 74.19 and 60.12 were assigned to the $\alpha$ and $\alpha'$ carbon of the ring juncture and the signal at $\delta$ 71.47 was due to the tertiary carbon at the ring juncture. The signal at $\delta$ 51.05, 50.48, 48.45 and 48.77 were due to the two acetyl methyl and two angular methyl carbons of the molecule. The remaining thirteen skeletal alkyl carbons appeared between $\delta$ 38.48 and 15.93. The mass spectrum (EI) of the compound showed peak at m/z 452 which was assigned to the molecular ion peak and the peaks at m/z 410 and 350 were due to the successive loss of ketene (CH$_2$=C=O) and acetic acid (CH$_3$COOH) fragments from the molecular ion. The product showed satisfactory elemental analysis and gave a specific rotation value $[\alpha]_D^{23} = -184.3^0$ (c=1, CHCl$_3$).

**Steroidal isoxazole (7)**

The following strategy was followed towards synthesis of ring fused isoxazole.

![N-propargylation](image)

The N-propargylation of substrate 2 to 3b was achieved with propargyl bromide under PTC in a reaction condition similar to that followed for allylation. The product 3b melted at 180°C and in TLC showed an Rf value 0.3 (in ethyl acetate: hexane;
20:80). The IR (NaCl) spectrum of the product showed a medium absorption band at $v_{\text{max}}$ 3250 cm$^{-1}$ and a weak band at 2145 cm$^{-1}$ due to the alkyne moiety. The strong absorption bands at 1718 and 1652 cm$^{-1}$ were assigned to the C=O stretching of acetyl groups. The $^1$H NMR spectrum of the product showed a sharp singlet at $\delta$ 9.35 for C-16 formyl proton, however absence of an amide proton signal near $\delta$ 10.00 rendered proofs for alkylation. Finally the Mass spectrum (EI) of the product showed peak at m/z 437 assigned to the molecular ion and the peak at m/z 409 and 394 due to successive loss of CO(28) and CH$_3$ (15) fragment from the molecular ion. The compound 3b gave satisfactory elemental analysis and had a specific rotation $\left[\alpha\right]_D^{23} = -61.23^0$ (c=1, CHCl$_3$).

Oximation of product 3b to 5b was accomplished with hydroxylamine hydrochloride in ethanol. The product 5b melted at 125°C and in TLC showed an $R_f$ value 0.3 (CHCl$_3$/MeOH:98/2). The IR (NaCl) spectrum of the product showed a medium absorption band at $v_{\text{max}}$ 3300 cm$^{-1}$ for the oxime OH stretching. The medium absorptions at $v_{\text{max}}$ 3250 and 2145 cm$^{-1}$ were due to the alkyne moiety. The strong absorption bands at $v_{\text{max}}$ 1720 and 1650 cm$^{-1}$ were assigned to the C=O stretching of acetyl groups. The $^1$H NMR spectrum showed absence of formyl peak, however a sharp singlet appeared at $\delta$ 7.68 which was assigned to the imine proton. The Mass spectrum (EI) of the product showed molecular ion peak at m/z 452. The peak at m/z 392 and 350 were due to (M$^-$-CH$_3$COOH) and [(M$^-$-60)-CH$_2$=C=O] fragmentation ions respectively. The compound gave satisfactory elemental analysis for molecular formula C$_{27}$H$_{36}$N$_2$O$_4$ and showed specific rotation value $\left[\alpha\right]_D^{23} = -78.3^0$ (c=1.11, CHCl$_3$).

The oxidation of oxime derivative (5b) was accomplished by reacting chloramin-T in refluxing ethanol. The reaction was worked up by reducing the volume of ethanol in a rotavapour, pouring into ice cold water and extracted with dichloromethane, washed with water dried over Na$_2$SO$_4$ and solvent removed to give (7) in 58% yield. It was purified by column chromatography and recrystallisation from methanol. Melting point of the solid was determined as 156°C and in TLC, it showed an Rf value 0.29 (solvent CHCl$_3$:MeOH=99:1). The product was characterised from the spectral and elemental analysis as below.

The IR(KBr) spectrum of the compound showed absence of peak at 3250 cm$^{-1}$ for
oxime OH. It showed absorption bands at 1715 and 1650 cm$^{-1}$ for acetyl carbonyl groups. The $^1$H NMR (CDCl$_3$) spectrum of the product showed absence of sharp singlet near $\delta$ 7.5, supporting proof for cyclo addition. The additional peak at $\delta$ 8.92 was assigned to the isoxazolo ring proton. The mass spectrum (EI) of the compound showed molecular ion peak at m/z 450 and the peaks at m/z 408 and 348 were due to the successive loss of ketene (CH$_2$=C=O) and acetic acid (CH$_3$COOH) fragments from the molecular ion. The product showed satisfactory elemental analysis and gave a specific rotation value $[\alpha]_D ^{23} = -145.3^0$ (c=1, CHCl$_3$).

The stereochemistry of the product (4) and (6) were determined based on a molecular model study and $^1$HNMR spectral anlysis. The molecular model study of the transition state of (A) and (B) showed higher strain associated with (B), because of the close proximity of the N-methyl group of the nitrone intermediate with steroidal C-18 methyl and thus disfavoured an exo but favoured an endo isomer. This cis/endo geometry was further supported by $^1$HNMR spectrum which showed a characteristic coupling constant of J=4.4 Hz and 5.1 Hz for the two methylene protons adjacent to oxygen atom of isoxazoline. This low value of coupling constant is characteristic proof of cis ring juncture and hence bridged protons of [4,3,0] pyridoisoxazolidine are assigned cis/endo geometry.

**Conclusion**

The stereoselective 1,3-dipolar cycloaddition reaction is the first example of its kind in steroids. The study showed the influence and role of steroidal unit in dipole cycloaddition which favours a cis / endo isomer only. Further the prepared steroidal isoxazoline are considered to be important because i) many of D-ring annelated
heterosteroids have recently shown anti tumour activity and ii) isoxazolidines are key intermediates to a broad range of biologically active substance via 1,3-amino alcohol.

**Experimental section**

**16-Dehydroprogrenoloneacetate oxime:** Prepared by the procedure discussed in Chapter-1.

**17-Acetamidoandrost-5,16-diene-3β-acetate(1):** Prepared by the procedure discussed in Chapter-1.

**Preparation of 3β-acetox-y-16-formyl-17-acetamido-androst-5,16-diene(2).**

To a prepared Vilsmeier reagent(0.026 mol) in a 250mL round bottomed flask (from 2.41 mL POCI₃ and 2.01 mL of DMF at 0°C) was added a solution of 3β-acetox-y 17-acetamido-androst-5-16-diene(1g,0.0026 mol) in 100mL dry CH₂Cl₂ under N₂ atmosphere and stirred at 10-15°C for 4 h, during which the colour of the reaction turned red. The reaction mixture was poured slowly into ice cold water(250 mL), stirred for 15 minutes and the organic layer was distilled out in a rotavapour to obtain an organic solvent free aqueous layer. The aqueous layer was basified with powdered KOH and stirred at 40°C for 15 minutes during which a solid separated out. It was extracted with CHCl₃ (3x50mL), washed with water (3 x 20mL) and the combined extract was dried over Na₂SO₄. Removal of the solvent rendered the product 2 sufficiently pure to proceed for the next reaction step. Yield, 845mg (81.5%); mp. 236-37°C (ethyl acetate); TLC in CHCl₃ (Rf =0.3); IR (KBr)v max 3250, 2940, 1725, 1625, 1500, 1240, 1060, cm⁻¹; ¹H NMR, (CDCl₃ ) δ 10.75 (bs, 1H), 9.08 (s, 1H), 5.10 (m, 1H), 4.33 (m, 1H), 2.05 (s, 3H), 1.80 (s, 3H), 0.98 (s, 3H), 0.85 (s, 3H), 2.55-1.15 (m, 17H); Mass spectrum (El) m/z 339, 311, 297, 282, 269; Anal. Calcd. for C₂₄H₃₃N0₄: C, 72.15; H, 8.33; N, 3.51. Found : C, 72.25; H,8.30; N, 3.40; [α]₀²³ = -37.6°(c=1.2, CHCl₃).

Reaction of 16-DPA oxime also gave the product (2) under Vilsmeier reaction condition, the procedure was discussed in chapter-1

**3β-Acetox-y-17-(N,N-allylacetamido)-16-formyl-androst 5,16-diene(3a):**

To a stirred solution of 3β-acetox-y-16-formyl-17-acetamido-androst-5,16-diene(1.59g,0.004 mol) and allyl bromide (0.51mL, 0.006 mol) in dichloromethane...
(200mL) was added a solution of aqueous KOH (30%, 50mL) and tetrabutyl ammonium bromide (644mg, 0.002 mol) at room temperature and stirred vigorously for 16h. The progress of the reaction was monitored with the help of TLC (using ethyl acetate:hexane: 20:80 as eluant). After completion of the reaction, the organic layer was separated out and the aqueous layer was extracted with dichloromethane (2 x 50mL). The combined organic extract was washed with water (3 x 30mL), dried over Na₂SO₄, solvent was removed in rotavapour and the residue was kept in ethanol at room temperature when a solid product crystallised out. Yield 1.2g (68.5%); mp 170°C (ethanol); TLC in ethylacetate:hexane=20:80 (Rf =0.3); IR (NaCl) ν max 2955, 2925, 1720, 1655, 1250, 1050 cm⁻¹; ¹H NMR (CDCl₃) δ 9.39(s,1H), 5.67(bs,1H), 5.27(bs,1H), 5.06(bs,2H), 4.44(m,2H), 3.48 (m,1H), 1.89(s,3H), 1.87(s,3H), 0.94(s,6H), 2.2-1.42 (m, 17H); Mass spectrum (El) m/z 439 (M⁺), 440 (M⁺+1), 411 (M⁺-28), 396 [(M⁺ -28)-15], 380, 379, 370, 351, 337; Anal.Calcd. for C₂₇H₃₇NO₄ : C, 73.80; H, 8.42; N, 3.18. Found : C.73.75; H.8.40; N, 3.19; [α]D²³ = -58.46° (c=1, CHCl₃).

3β-Acetoxy-17(N,N-propargylacetamido)-16-formyl-androst-5,16-diene (3b):

To a solution of 1.2g (0.003 mol) of 3β-acetoxy-16-formyl-17-acetamido-androst-5,16-diene (2) in CH₂Cl₂(150mL) was added 644 mg (0.002 mol) of TBAB, 0.53 mL(0.006 mol) of propargyl bromide and 30mL of 40% KOH solution. The reaction mixture was stirred vigorously for a period of 12 h and after completion of the reaction, monitored with the help of TLC (ethyl acetate:hexane=20:80) the organic layer was separated and the aqueous layer was extracted with CH₂Cl₂ (2x20mL). The combined extract was washed with water (3 x 30mL), dried over Na₂SO₄ and CH₂Cl₂ was removed by ordinary distillation to give a gummy product, which was crystallised from ethanol to obtain a white solid product 3b. Yield 819mg (62.5%); mp 180°C(ethanol); TLC in hexane:ethyl acetate = 80:20 (Rf = 0.3); IR (KBr) ν max 3250, 2950, 2915, 2145, 1718, 1652, 1250, 1050, 775 cm⁻¹; ¹H NMR δ 9.35 (s, 1H), 5.18 (bs, 1H), 4.5 (m, 1H), 4.2 (m, 2H), 3.5 (m, 1H), 1.89 (s, 3H), 1.85 (s, 3H), 0.90 (s, 6H), 2.2-1.5 (m, 17H); Mass spectrum (El) m/z 437(M⁺), 438 (M⁺+1), 409(M⁺-28), 394[(M⁺ -28)-15], 377(M⁺-60); Anal. Calcd. for C₂₇H₃₈NO₄: C, 74.14; H,8.00; N,3.20; Found: C, 74.09; H, 8.05; N, 3.12. [α]D²³ = -61.23° (c=1, CHCl₃).
Steroidal isoxazolidine (4)

To a solution of 439mg (0.001 mol) of 3β-acetoxy-17-(allylacetamido)-16-formyl-androst-5,16-diene (3a) in 50mL ethanol in a 150mL round bottomed flask was added solid N-methyl hydroxyl amine hydrochloride (85mg, 0.001 mol) and stirred at room temperature for 2 h. During this period of stirring the whole amount of compound got dissolved and TLC showed completion of reaction. It was worked up by evaporating ethanol in a rotavapour, pouring the content into 100mL of water and basified with NaHCO₃. Extraction with CH₂Cl₂, washing with water, drying over Na₂SO₄, removal of solvent and chromatographic separation afforded a crude nitro compound which was dried in a high performance vacuum pump. Dry toluene (100 mL) was added into the round bottom flask and under nitrogen atmosphere, the content was heated at 80°C and stirred for a period of 12 h. Removal of toluene in a rotavapour at 60°C gave a product which was purified by preparative thin layer chromatography using solvent system MeOH:CHCl₃=99.5:0.5. Yield 224 mg (48%); mp 138-40°C; TLC in MeOH:CHCl₃ /98:2(Rf = 0.24); IR (KBr)ν max 2950, 1720, 1665, 1250, 1050 cm⁻¹; ¹H NMR (CDCl₃) δ 5.30 (bs, 1H), 5.07-5.02 (dd, 1H, J=4.4 Hz, J' = 5.1 Hz), 4.47 (m, 1H), 4.09 (m, 1H), 3.80 (m, 1H), 3.41 (d, 1H), 3.33-3.29 (dd, 1H, J=4.5 Hz, J' = 5.1 Hz), 2.75 (m, 1H), 2.61 (s, 3H), 2.02 (s, 3H), 1.90 (s, 3H), 0.95 (s, 3H), 0.86 (s, 3H), 2.19-1.42 (m, 17H); Mass spectrum (EI) m/z 468 (M⁺), 437 [M⁺-31(CH₃NH₂)], 422 [M⁺-46(CH₃NHO)], 396 [(M⁺-46)-26(C₂H₂)], 381; Anal. Calcd. for C₂₈H₄₈N₂O₄: C, 71.79; H, 8.54; N, 5.98. Found: C, 71.31; H, 8.22; N, 5.24; [α]D²⁵ = -176° (c=1, CHCl₃).

3β-Acetoxy-17(allylacetamido)-androst-5,16-diene-16-formyl oxime (5a):-

To a stirred mixture of 3β-acetoxy-17-(allylacetamido)-16-formyl-androst-5,16-diene (439mg, 0.001 mol) in ethanol (100mL) in a 250mL round bottomed flask, was added 105mg (0.0015 mol) of hydroxylamine hydrochloride and the reaction was stirred at room temperature for 0.5 h. During this period of stirring the initially insoluble starting material got completely dissolved. The reaction mixture was poured into 250mL ice cold water, neutralised with NaHCO₃ and stirred for 15 minutes when a white solid product precipitated out. It was extracted with CHCl₃ (2 x 50mL), washed with water (20mL), dried over Na₂SO₄ and solvent removed in a rotavapour. Recrystallisation from ethanol gave a white solid of 5a, Yield 355mg (78.3%); mp 182°C (ethanol); TLC in...
toluene/acetone : 95/5 (Rf. = 0.15). IR (KBr) ν_{max} 3320, 2950, 1720, 1650, 1250, 1050 cm\(^{-1}\); \(^1\)H NMR(CDC\(_3\)) δ 7.5 (s, 1H), 5.2 (bs, 1H), 5.1 (s, 1H), 4.8 (m, 2H), 4.4 (m, 3H), 1.9 (s, 3H), 1.8 (s, 3H), 0.98 (s, 6H), 2.3-1.4 (m, 17H); Mass spectrum(EI) m/z 454 (M\(^+\)), 455 (M\(^+\)+1); Anal. Calcd. for C\(_{27}\)H\(_{38}\)N\(_2\)O\(_4\) : C, 71.36; H, 8.37; N, 6.16. Found: C, 70.91; H, 8.33; N, 5.88; [α]\(^D\)\(_{23}\) = -57.5° (c=1, CHCl\(_3\)).

3β-Acetoxy-17-(propargylacetamido)-androst-5,16-diene-16-formyloxime (5b):

Yield 339mg(75%); mp 125\(^0\)C(ethanol); TLC in CHCl\(_3\)/MeOH : 98/2 (Rf = 0.26); IR (NaCl) ν_{max} 3300, 3225, 2950, 2915, 2145, 1720, 1650, 1250, 1050 cm\(^{-1}\); \(^1\)H NMR δ 7.68 (s, 1H), 5.25 (bs, 1H), 4.71 (m, 1H), 4.48 (m 2H), 3.9 (m, 1H), 2.00 (s, 6H), 1.1 (s, 6H), 2.3-1.5 (m, 17H); Mass spectrum (EI) m/z 452 (M\(^+\)), 392 (M\(^+\)-60), 350[(M\(^+\)-60)-42]; Anal. Calcd. for C\(_{27}\)H\(_{38}\)N\(_2\)O\(_4\) : C, 71.68; H, 7.96; N,6.19. Found : C, 71.03; H, 7.92; N, 5.98; [α]\(^D\)\(_{23}\) = -78.3° (c=1.11, CHCl\(_3\)).

Steroidal isoxazoline(6):

454 mg (0.001 mol) of 3β-acetoxy-17-(allylacetamido)-androst-5,16-diene-16-formyl oxime (5a) was dissolved in dry ethanol(100mL) in a 250mL round bottomed flask. To it was added 400mg (0.002 mol) chloramin-T and the flask was fitted with a reflux condenser and heated at 78\(^0\)C under stirring for 3 h. Progress of the reaction was monitored with the help of TLC(CHCl\(_3\):MeOH=98:2). After completion, of the reaction the volume of ethanol was reduced to one fourth and it was poured into a beaker containing 200mL water, extracted with CH\(_2\)Cl\(_2\) (3 x 50 mL), washed with water (2 x 20 mL), dried over Na\(_2\)SO\(_4\). Removal of solvent gave a white solid product which was recrystallised from methanol to obtain a pure white crystal of (6). Yield 352mg(78%); mp 210\(^0\)C; TLC in CHCl\(_3\):MeOH/99:1(Rf = 0.18); IR (KBr) ν_{max} 2915,1715, 1650, 1250,1175,1050, 775. \(^1\)H NMR (CDCl\(_3\)) δ 5.40 (s, 1H), 4.57 (bs, 2H), 4.21 (dd, 1H), 3.71 (m, 2H), 3.38 (dd, 1H), 2.24 (s, 3H), 2.02 (s, 3H), 1.33 (s, 3H), 1.06 (s, 3H), 2.48-1.57 (17H,m); \(^13\)C NMR (CDCl\(_3\)) δ 170.8, 168.325, 161.103, 155.037, 140.41, 122.388, 114.482, 74.196, 71.478, 60.120, 51.05, 50.48, 48.95, 48.77, 38.45, 37.13, 37.03, 33.60, 31.34, 30.92, 28.08, 27.84, 24.30, 21.76, 20.75, 19.54, 15.93. Mass spectrum (EI) m/z 452 (M\(^+\)), 410 (M\(^+\)-42), 392 (M\(^+\)-42-18), 350 (410-60), 335 (-15). Anal.Calcd. for C\(_{27}\)H\(_{38}\)N\(_2\)O\(_4\) : C, 71.68; H,7.96; N,6.19, Found: C, 71.53; H, 7.82; N, 5.98; [α]\(^D\)\(_{23}\) = -184.3° (c=1.2, CHCl\(_3\)).
Steroidal isoxazole(7):

452 mg (0.001 mol) of 3β-acetoxy-17-(propargylacetamido)-androst-5,16-diene-16-formyl oxime (5b) was taken in dry ethanol (100 mL) in a 250 mL round bottomed flask. To it was added 400 mg (0.002 mol) chloramin-T and the flask was fitted with a reflux condenser and heated at 78°C under stirring for 2 h. Progress of the reaction was monitored with the help of TLC, using a solvent system of CHCl₃:MeOH=98:2. After completion of the reaction the volume of ethanol was reduced and it was poured into 200 mL water, extracted with CH₂Cl₂ (3 x 50 mL), washed with water (2 x 20 mL), dried over Na₂SO₄. Removal of solvent gave a white solid product which was crystallised from methanol to obtain a pure white crystal of (7). Yield 261 mg (58%); mp 156°C (methanol); TLC in CHCl₃:MeOH /99:1 (Rf = 0.29); IR (KBr) νₚₓₚₚ 2915, 1715, 1650, 1250, 1175, 1050 cm⁻¹; ¹H NMR (CDCl₃) δ 8.92 (s, 1H), 5.40 (s, 1H), 4.57 (bs, 1H), 3.72 (m, 2H), 2.24 (s, 3H), 2.02 (s, 3H), 1.33 (s, 3H), 1.06 (s, 3H), 2.48-1.57 (17H, m); Mass spectrum (EI) m/z 450 (M⁺), 408 (M⁺-42), 348 (M⁺-60); Anal. Calcd. for C₂₇H₃₄N₂O₄: C, 72.00; H, 7.55; N, 6.22. Found: C, 72.13; H, 7.42; N, 6.10; [α]D²³ = -145.3 (c=1, CHCl₃).

References
