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8 Advanced NMR techniques for structural characterization of heterocyclic structures

Diana C. G. A. Pinto, Clementina M. M. Santos and Artur M. S. Silva
Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

Abstract

^{1}H and ^{13}C NMR spectra remain the first tool used by chemists to perform the structure elucidation of their products on a routine basis. It is common to provide NMR data on both proton and carbon spectra based on one-dimensional experiments. The increasing complexicity of natural compounds and their synthetic related derivatives implies the use of some more recent 1D and 2D NMR techniques. The purpose of this publication is to describe the main ^{1}H, ^{13}C and ^{15}N NMR features of three to six-membered heterocyclic compounds and also to discuss the application of several 1D and mainly 2D NMR techniques in the structure elucidation of these compounds. A brief discussion of these NMR techniques from the point of view of structural elucidation of organic compounds will be also considered.

Correspondence/Reprint request: Dr. Artur M. S. Silva, Department of Chemistry, University of Aveiro, 3810-193 Aveiro Portugal. E-mail: arturs@dq.ua.pt

1. Introduction

Nitrogen heterocyclic compounds are broadly distributed in Nature, including amino acids, purines, pyrimidines and many other natural products, whereas others displayed important biological activities. Therefore, it is important to know their important NMR features, such as ^{1}H, ^{13}C and ^{15}N NMR chemical shifts, and how one can use advance NMR techniques for the structural characterisation of these heterocyclic compounds.

^{1}H and ^{13}C NMR spectra remain the first tool used by chemists to perform the structure elucidation of their heterocyclic compounds on a routine basis. It is common to provide NMR data on both proton and carbon spectra based on one-dimensional experiments, but often only proton resonances are assigned. The increasing complexicity of natural compounds and their synthetic related derivatives implies the use of some more recent 1D and 2D NMR techniques. NMR spectra of these complex heterocycles contain several resonance lines which cannot be resolved in a conventional 1D NMR experiment. The interpretation of these NMR data requires correlations between different nuclei which are implicitly contained in 1D spectra but often difficult to extract. Multidimensional NMR spectra provide both increased resolution and correlations which are easy to analyze. The price to pay is more complex NMR experiments which consist of a series of *rf* pulses separated by short time periods (delays) during which no external *rf* is applied. This pulse sequence is then followed by the recording of the resulting magnetization.

Nitrogen NMR spectroscopy has great importance for structural analysis, since *N*-containing functional groups and N atoms in molecular skeletons are frequently encountered. Nitrogen has two NMR active nuclei, ^{15}N which gives sharp lines but is very insensitive and ^{14}N which is a medium sensitive nucleus but its signals are usually significantly broadened by quadrupolar interactions sometimes to the extent that they are unobservable on a high resolution NMR spectrometer. The 1D ^{15}N NMR experiment is much less sensitive than ^{1}H and ^{13}C NMR experiments, it yields narrow lines and has a large chemical shift range. Its low natural abundance (0.37%) makes its low sensitivity even worse so it is often enriched for NMR [1-3]. A typical analysis of a ^{15}N NMR spectrum consists of matching expected chemical shifts to the expected moieties. Heteronuclear coupling with protons is observed, one bond couplings are of the order of 90 Hz and smaller 2, 3 and 4-bond coupling are also observed. In some cases, very weak satellite signals can be observed in the ^{1}H spectrum of NH signals and can be used to confirm the assignment of NH signals. The 1D ^{14}N NMR experiment is much less sensitive than ^{1}H and has a much larger chemical shift range. Its signals are broadened by quadrupolar interactions and the larger the molecule and the more asymmetric the nitrogen's environment, the broader the signal. Molecules that are significantly larger than urea yield signals too broad to be observed with a high-resolution NMR spectrometer. However, the nitrogen chemical shift range is wide and so may be readily used for distinguishing nitrogen species for very small molecules. Heteronuclear coupling is rarely observed with ^{14}N because of its quadrupolar broadening. Carbon signals of nitriles and NH protons are often broadened by residual coupling to ^{14}N.

The purpose of this report is to describe the main NMR features of the most common and important classes of nitrogen heterocyclic compounds and also to discuss the application of several 1D and 2D NMR techniques in the structure elucidation of

these compounds. A brief discussion of these NMR techniques from the point of view of structural elucidation of organic compounds will be also considered.

2. NMR techniques for structural characterization of heterocyclic compounds

In ^{1}H-^{1}H COSY, the 1D spectrum is displayed along each axis with a contour projection of this spectrum along the diagonal axis. Off-diagonal peaks represent proton shift correlations (or proton couplings). In ^{1}H-^{13}C or ^{1}H-^{15}N COSY, the ^{13}C or ^{15}N NMR spectrum appears along one axis and the ^{1}H NMR spectrum along the other, H-C or H-N shift correlation is evidenced by spots in the 2D display. ^{1}H-^{1}H COSY simply identifies which protons are coupled with each other (typically geminal and vicinal couplings) and ^{1}H-^{13}C or ^{1}H-^{15}N COSY identifies all proton and carbon or nitrogen atoms which are connected by a ^{13}C-^{1}H or ^{15}N-^{1}H coupling over one bond. The latter are also of great use in assigning proton resonances when the carbon or nitrogen resonances for the same sites are known and *vice-versa*. TOCSY (TOtal Correlated SpectroscopY) is a 2D NMR method that produces a similar spectrum to ^{1}H-^{1}H COSY experiment, except that cross peaks will be observed among all protons of the same spin-system. For example, if A is coupled to B and B is coupled to C, but A is not coupled to C, there will still be a cross peak connecting A and C. This 2D is particularly advantageous in cases of severe resonance overlapping, for which ^{1}H-^{1}H COSY spectra can often leave ambiguities (*e.g.* carbohydrates and proteins). An essentially identical 2D NMR spectra of TOCSY is HOHAHA (HOmonuclear HArtmann-HAhn spectroscopy) which differ only in some technical details in the originally published sequences.

The first techniques of heteronuclear shift correlations employed detection of ^{13}C, the lower-γ nuclide, being the high-resolution in the ^{13}C dimension their great advantage. The basic pulse sequence of the heteronuclear 2D shift correlation HETCOR provides correlations of ^{13}C-^{1}H that are connected by one-bond coupling constant $^{1}J_{C/H}$. However, it is often desirable to be able to observe cross-signals for C, H spin pairs connected by two- or three-bond coupling ($^{2}J_{C\text{-}H}$ or $^{3}J_{C\text{-}H}$). The COLOC experiment can offer this information, upon adjustment of delays for long-range C-H coupling constants (generally they are optimized for a $J_{C\text{-}H} \approx 10$ Hz). During the last fifteen years, the approach to data collection has fundamentally changed to one in which the proton, high-γ nucleus, is observed, being the carbon or nitrogen (heteronucleus) detected indirectly; this changing has give rise to some experiments, frequently referred as "inverse" shift correlations. These are two techniques in widespread use that provide single bond heteronuclear shift correlation, known as HMQC and HSQC. The correlation data given by these two methods are essentially equivalent, the finer details differences have, for routine spectroscopy, little consequence or no consequence at all. The experiment used to establish correlations between carbons or nitrogen and neighbouring protons, over more than one bond, is designated as HMBC. To observe connectivity between carbon and proton two and three bonds away, a long-range coupling constant of $J_{C\text{-}H}$ = 7-10 Hz should be used. In some cases these long-range correlations have been established by using 1D selective INEPT experiment, which give the connectivity of a selected proton, by irradiation of its corresponding resonance, to the carbon atoms to which it is coupled, and can be optimized for different long-range $J_{C\text{-}H}$ coupling.

The HSQC/HMQC and HMBC experiments or some of their sensitivity improving variants have also been used to assign the ^{15}N resonances of some heterocyclic compounds. Generally the used one-bond coupling constant $^{1}J_{N\text{-}H}$ is around 90 Hz while those of the long-range coupling constant is normally optimised to $J_{C\text{-}H}$ = 6-10 Hz [3-7].

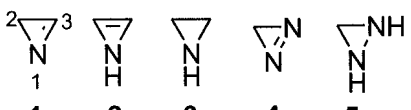
Nuclear Overhauser enhancement (NOE) is the enhancement produced in the signal of a nearby proton when a selected proton is irradiated. The magnitude of the enhancement is related to the distance between the protons. 1D NOE studies are generally presented in literature as NOE difference spectra, in which a series of spectra shows the effect on the original spectrum, while 2D NOESY experiment encapsulates all of this information in one spectrum. However, these techniques has a great problem for mid-sized molecules with masses around 1000-2000 daltons where the NOE effect becomes vanishingly small. With the increasing interest in larger molecules of organic chemistry together with the wider availability of high-field instruments, the measurement of NOE effects in the rotating frame constituted an alternative solution. For small molecules the magnitude of the ROE effect is similar to that of NOE effect, whilst for larger molecules it reaches a maximum for homonuclear spins, but under no circumstances does it became zero. ROE experiment is more frequently performed as 2D technique where it is called ROESY (Rotating frame Overhause Effect SpectroscopY which has also been known as CAMELPSIN – Cross-relaxation Appropriate for Minimolecules Emulated by Locked SPINs).

3. Three-membered heterocyclic compounds - azirines and aziridines

Azirine **1** and its dihydro-derivatives, aziridines **3**, can be seen as the most simple of all heterocyclic systems. They are a three-membered ring with two carbons and one nitrogen atom. Although numerous derivatives of 1-azirine **1** and aziridine **3** ring systems are known and have been fully characterized, 2-azirine **2** ring systems are known only as transient intermediates [8]. The two isomeric forms of azirine **1** and **2** have been designated by *Chemical Abstracts* and IUPAC as 2*H*-azirine and 1*H*-azirine, respectively [9]. This nomenclature and the corresponding numbering will be used in this chapter.

1-Diazirine **4** and saturated diaziridine **5** are also three-membered heterocyclic compounds containing two nitrogens and one carbon atom in their structure [10].

The properties of three-membered hetereocycles are mostly a result of the great bond angle strain (Bayer strain), making these compounds highly chemical reactive. Although 2*H*-azirine **1** is thermally instable and has to be stored at very low temperature, substituted aziridines are more stable. Aziridine **3** was known as ethylene imine and they may present considerable toxicity. 1-Diazirines **4** are structural isomers of diazoalkanes and in liquid state can decompose explosively. *N*-atoms of diaziridines **5** are configurationally stable so that stereoisomerism is possible [8].



1 **2** **3** **4** **5**

^{1}H NMR spectrum of the parent compound 2*H*-azirine **1** shows chemicals shifts of H-2 and H-3 at δ 1.26 and 9.93 ppm, respectively, and in the ^{13}C NMR spectrum C-2 resonates at δ 14.4 ppm and C-3 at d 164.2 ppm. H-2 resonances of substituted 2*H*-azirines appears at δ 0.20-4.00 ppm and the ^{13}C NMR spectra of these azirines shows absorptions at δ 19.0-45.0 ppm for the C-2 and δ 160.0-170.0 ppm for C-3 [11-13]. Therefore, ^{13}C NMR data of small heterocycles are scarce and ^{13}C-^{1}H coupling constants are often obtained from ^{13}C satellites in the ^{1}H NMR spectra of these compounds [14].

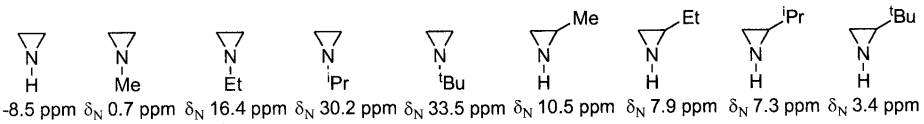
In the three-membered ring systems, the geminal coupling constant (J_{gem}) is usually smaller than J_{cis} and J_{trans}, being J_{cis} usually greater than J_{trans} (Table 1). A study envolving 64 aziridines showed average values for the coupling constants of J_{gem} = 1.4 Hz, J_{trans} = 3.3 Hz and J_{cis} = 6.4 Hz and they seem to be more dependent on the number of nonbonding electron pairs at the heteroatom than on its electronegativity [8, 15]. These vicinal coupling constants of *cis* and *trans* related protons generally allows to determine the stereochemistry at C-2 and C-3 of many aziridines.

Protons attached to aziridine ring carbons resonate at *ca*. δ 1.5 ppm and those that are attached to nitrogen tend to be at low δ values (*ca*. 1.0 ppm). The carbon resonance of the parent aziridine **3** is observed at δ 18.2 ppm and when bearing alkyl and / or aryl substituents they appear at δ 30-50 ppm [8].

Table 1. NMR data (δ, ppm; range of coupling constants) of three-membered heterocyclic systems [8].

Skeleton	δ (^{1}H)	δ (^{13}C)	J_{gem}	J_{cis}	J_{trans}
cyclopropane	0.22	-2.2	-3 to -1	6-12	-4 to 8
2*H*-azirine C-2	1.3	14.4	---	---	---
C-3	9.9	164.2	---	---	---
aziridine	1.48	18.2	0.9-4	5-9	2-7
1-diazirine	0.4	---	---	---	---
diaziridine	1.2	56.0	---	---	---

^{15}N NMR has been of great interest to establish the effect of the ring substitution on the chemical shifts of the aziridinyl nitrogen (relative to ammonia). Substitution on one or both β-carbons shifts the ^{15}N resonance downfield relative to unsubstituted aziridine **3**. Aziridine **3** nitrogen appears in the ^{15}N NMR spectrum, relative to anhydrous ammonia, at δ – 8.5 ppm. *N*-alkylation shifts this chemical shift downfield: *N*-Me **6** at δ 0.7 ppm, *N*-Et **7** at δ 16.4 ppm; *N*-*i*Pr **8** at δ 30.2 ppm and *N*-^{t}Bu **9** at δ 33.5 ppm; *C*-alkylation has the reverse effect **10-13** [14].

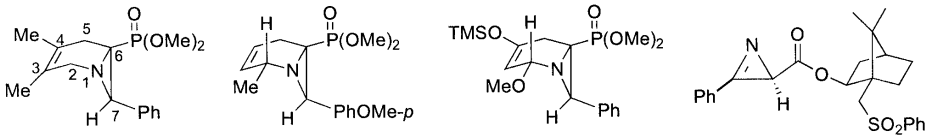


The ^{13}C NMR spectroscopy is very useful for the characterization of azirine derivatives, namely haloazirines containing chloro, bromo and even iodo atoms in the position 2 of the heterocyclic ring. The chemical shift of the sp^{3} carbon (C-2) of the azirine ring appear at δ 13-63 ppm, depending on the substitution pattern, and typically

presents low intensity, whereas the chemical shift of the sp^2 carbon (C-3) appears at δ 155-167 ppm [16]. The ^{13}C NMR spectra of azirines containing other substituents such as phenyl, methyl and ester groups fit exactly the characteristics described above for the ^{13}C NMR spectra of 2-haloazirines [17].

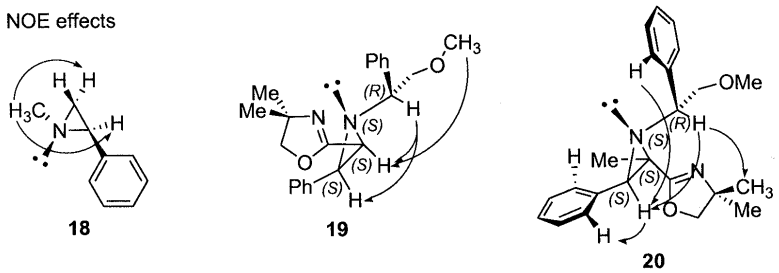
The stereochemistry and/or regiochemistry of azirine-fused compounds containing 3-phosphorated substituents were accomplished by NMR data, namely by COSY and NOESY/NOE experiments. H-7 of **14-16** appears as a doublet in the narrow range of δ 3.12-3.27 ppm, indicating an anisotropic shielding effect by the C=C double bond and coupling with the phosphorus atom (J_{PC} = 6 Hz). Irradiation of H-7 of **14** resulted in enhancement of the protons signals of C-3 and C-4 methyl groups. The regiochemistry assignment in the synthesis of **15** and **16** is supported by the C-5 resonance appearing at δ 22.1 and 27.4 ppm, respectively, and by the coupling constants with the phosphorus atom (J_{PC} = 11.0 Hz). A COSY spectrum of **15** have revealed cross-ring coupling between H-2 and H-5 [18].

The NOESY spectrum of **17** presented correlation between H-2 of the azirine ring with the *ortho* protons of the C-3 phenyl group, which pointed out for the *R*-configuration of C-2 of this compound [19].



14 **15** **16** **17**

In order to understand some reaction mechanism and the possible nitrogen inversion of some *N*-methyl-2-phenylaziridines, their configuration has been assigned by NOESY spectra. For example, in derivative **18** the methyl group correlates with the H-2 and one of H-3 protons supporting its presence in the same side of the molecule and putting the nitrogen lone-pair on the same side of the phenyl group which confirms the existence of only one invertomer [20]. Similar studies related with the nitrogen invertomer assignment have been carried out to establish the absolute configuration of the aziridine ring of compounds **19** and **20** [21].

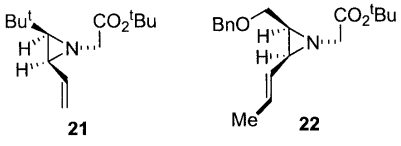


18 **19** **20**

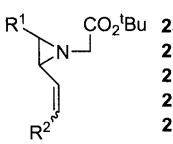
The stereochemistry of *N*-substituted vinylaziridines **21** and **22** was also performed by NOESY spectra. The vinylaziridine **21** showed interaction between the α-carbonyl protons and the *t*-BuCH and C*H*CH_2 while in the case of vinylaziridine **22** interactions

between the α-carbonyl protons and both the aziridine methine protons have been observed. These data support the presence of a single nitrogen invertomer. However, the ^{1}H NMR spectra of compounds **23a-e** at room temperature consists in two sets of lines indicating an equilibrium of two invertomers [22].

Variable temperature studies on aziridines and diaziridines show a remarkable range of nitrogen inversion rates [23]. Electron delocalizing substituents on small-ring nitrogen lower the inversion barrier; substituents bearing unshared electron pairs raise the inversion barrier to levels at which enantiomers can be isolated [24]. Pure invertomers have also been obtained of *N*-haloaziridines and *N*-alkoxyaziridines [8,25,26].

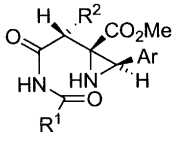


21 **22**



23a $R^1 = CH_2OBn$, $R^2 = H$
23b $R^1 = CH_2OBn$, $R^2 = (Z)$-Me
23c $R^1 = CH_2OBn$, $R^2 = (E)$-Me
23d $R^1 = C_6H_{13}$, $R^2 = H$
23e $R^1 = C_5H_{11}$, $R^2 = (Z)$-Me

^{1}H NMR spectrum of poly-substituted aziridines **24** and **25** shows two doublets ($J \sim$ 9 Hz) corresponding to CH and NH proton resonances of the aziridine ring, at δ 2.40-3.39 and 2.83-3.09 ppm, respectively. The addition of D_2O exchanges the label proton and the CH tends to show up as a sharp singlet [27,28]. In fat-derived aziridines **26** the protons of the aziridine ring appears as a multiplet at δ 1.94 ppm, while the carbon resonances of C-12 and C-13 (carbons atoms of the aziridine ring) appeared at δ 34.67 and 34.82 ppm, respectively [29].



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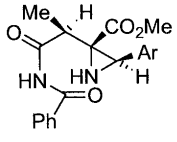

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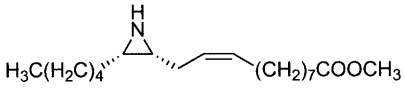

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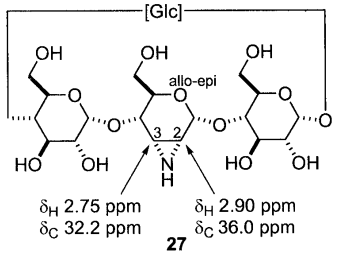
Structural characterization of aziridine-fused to sugar units **27** needs the homonuclear COSY and heteronuclear HMQC spectra to assign the proton and carbon resonances of the sugar unities. These spectra were also important to assign the proton and carbon resonances of their aziridine ring [30]. The presence of the aziridine ring in compounds **28,29** was supported by the characteristic upfield shifted signals for the C*H*NNs protons (δ 3.33-3.95 ppm) and upfield shift of their carbon atoms in positions 3 and 4 (δ 38-47 ppm) [31].



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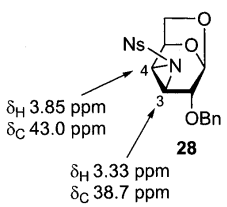

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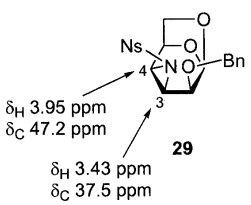

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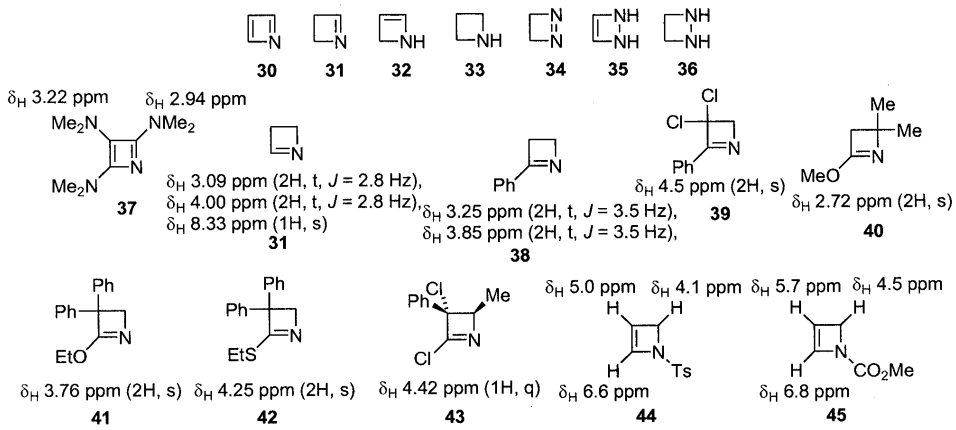
4. Four-membered heterocycles - azetes, azetines and azetidines

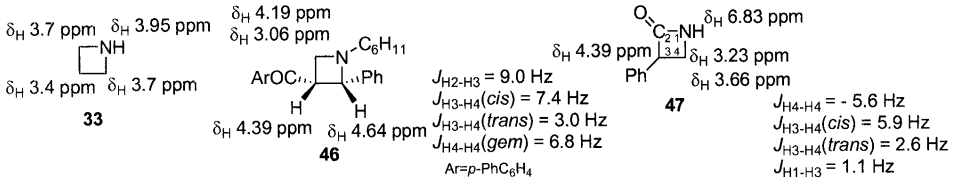
Azete **30**, 1-azetine **31**, 2-azetine **32** and the saturated azetidine **33** are four-membered heterocyclic compounds containing one nitrogen atom in their structure, while 1-diazetine **34**, 3-diazetine **35** and diazetidine **36** have two nitrogen atoms. In four-membered heterocycles, the ring strain is less than in the corresponding three-membered compounds, similar to cyclobutane.

Azete **30** itself hasn't been synthesized yet but it would be expected to be thermally unstable and extremely reactive [10]. The spectral data indicated in derivative **37** were obtained at low temperature and cited as evidence for the azete structure. Unsubstituted 1-azetine **31** is a colourless liquid which undergoes polymerization within a few seconds at 20ºC but their derivatives possess greater stability [32]. Structures **31,38-43** present ^{1}H NMR data of some derivatives of 1-azetines.

Little is known about the structure of 2-azetines; the parent system **32** has not been reported [33]. NMR studies of 1-substituted 2-azetines shows rapid nitrogen inversion and the proton chemical shifts of this type of compounds are presented in structures **45** and **46** [33-35].

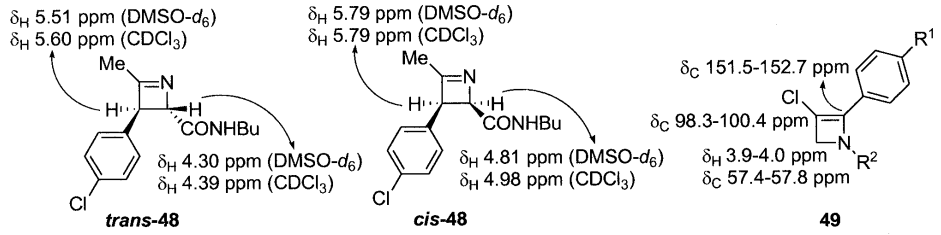
Azetidine **33** also called trimethyleneimine is thermally stable and less reactive than aziridine. 3-Diazetine **35** is also known as 1,2-dihydro-1,2-diazete. The preparation of the unsubstituted diazetidine **36** hasn't been achieved yet; however numerous diazetidines are known [10]. In azetidine derivatives the geminal proton-proton coupling constants (J_{gem}) on the carbons adjacent to nitrogen are 5-7.5 Hz, and J_{cis} is larger than J_{trans}. The substituent on the nitrogen atom of azetidine can be either axial or equatorial and rapid inversion at nitrogen normally occurs. The magnitude of the vicinal coupling constants (J^{cis}_{H3-H4} *ca.* 6-7.5 Hz, J^{trans}_{H3-H4} *ca.* 3.0 Hz) is widely used in the assignment of stereochemistry. Long-range coupling constants between ring protons are common and in structure **47** it is presented one example [8].



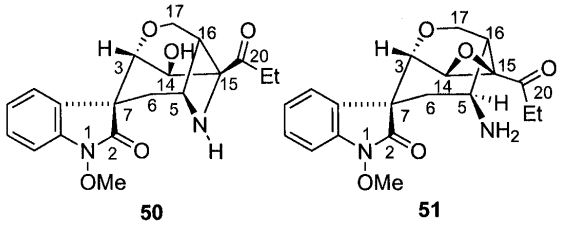


The group of Sakurai studied the synthesis of 1-azetine derivatives (*e.g*. **48**) through a photochemical process of α-dehydroamino acids [36,37]. NMR analysis revealed the formation of *cis* and *trans* isomers being their stereochemistry based on the values of the vicinal coupling constants of the azetine ring ($J_{H3\text{-}H4}$). The *cis*-coupling constant values are higher (*ca.* 10 Hz) than that of the the *trans*-isomer (*ca*. 7 Hz). ^{1}H-^{1}H and ^{1}H-^{13}C COSY spectra helped to confirm the structures **48**.

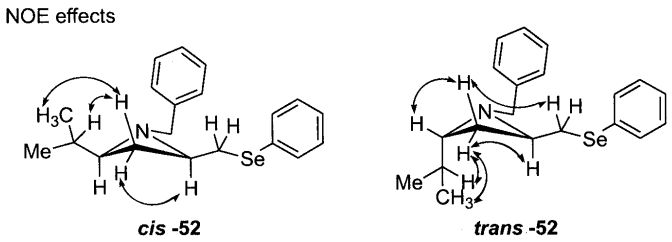
1-Aryl-3-chloro-2-azetines **49** can be prepared from 2-aryl-3,3-dichloroazetidines and their ^{1}H and ^{13}C NMR spectra are enough to characterize this type of structures [38].



^{1}H-^{13}C HMBC spectra help in the structure reinvestigation of gelsemoxonine **50**, an azetidine containing an oxindole alkaloid moiety isolated from *Gelsemium elegans* Benth [39]. The structure of this compound was described by Lin *et al*. as an unusual *N*4/C20 seco-indole alkaloid **51** [40]. The acetylation of **50** gave an unexpected diacetylated derivative (δ_H 2.02 and 1.91 ppm) which raised doubts regarding the structure of **51**. The ^{1}H-^{13}C HMBC spectrum of **50** in pyridine-d_5 at – 30ºC, taking advantage that the signal of the azetidine NH becomes sharp and well defined at lower temperatures, showed connectivities between this proton and carbons C-14 and C-20 (δ 210.9 ppm). These data allowed the construction of an azetidine ring consisting of the NH, C-15, C-16 and C-5 positions. This structure was confirmed by X-ray crystallographic analysis.

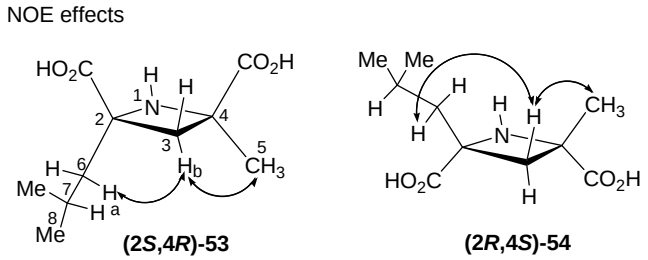


The configurational assignment of several azetidines derivatives was made from ^{1}H NMR data based on the coupling constants values of the heterocyclic protons; J_{trans} presents lower values (5.8-7.9 Hz) than J_{cis} (8.4-8.9 Hz) as expected for the azetidine ring. The relative configuration was further confirmed by NOE experiments [41-44]. The NOE correlation observed in the NOESY spectra of *cis*-**52** and *trans*-**52** are depicted in their structures and allowed the assignment of their configuration.



***cis* -52** ***trans* -52**

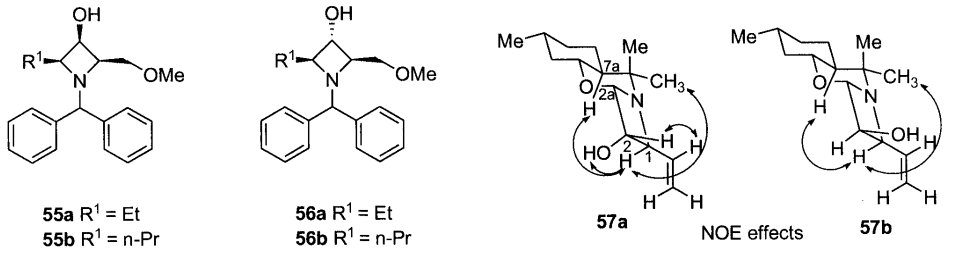
The structures of two natural enantiomeric azetidine-type aminoacids **53** and **54** were also established by NMR spectroscopy and their configuration also established by using NOESY experiments. In compound **53** there is a strong NOE correlation between H-b and H-a and H-5, suggesting that the 2-isobutyl group and the 4-methyl group were oriented on the same face of the azetidine ring [45]. The main NOE correlations observed in the NOESY of **54** are presented in the corresponding structure. The NOE effects were also very important to the stereochemistry assignment of tricyclic azetidines [46].



(2*S*,4*R*)-53 **(2*R*,4*S*)-54**

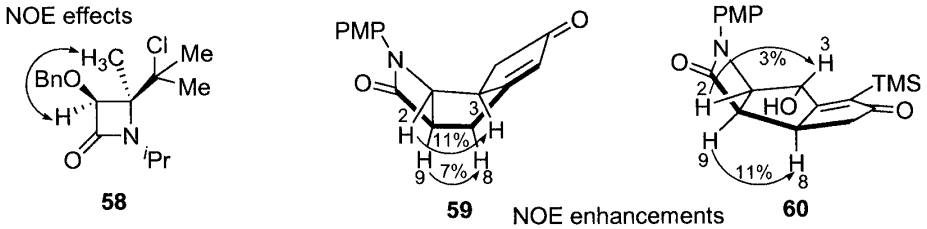
Azetidin-3-ols can be found in nature as 2,4-disubstituted derivatives in a sphingosine-type alkaloids. They can act as active moieties of many compounds having important pharmacological properties. The configuration of azetidinols **55** and **56** couldn't be determined by 1D or 2D NOE experiments due to the proximity of the H-2 and H-4 proton resonances, it has been assigned by their coupling constants. The *cis* isomers **55a** and **55b** showed higher coupling constants values $J_{H2\text{-}H3}$ and $J_{H3\text{-}H4}$ of 6.3 Hz, than the corresponding *trans* isomers **56a** and **56b** (J = 5.6 Hz) [47].

The stereochemistry of azetidin-3-ols **57a** and **57b** was confirmed by NOESY experiments. The difference observed in the NOESY spectrum was the correlation between H-2 and H-α of the vinyl substituent in the *trans* isomer **57a** and the absence of this correlation in the *cis* isomer **57b**, which allowed to assign the relative disposition of H-1 and H-2 [48].

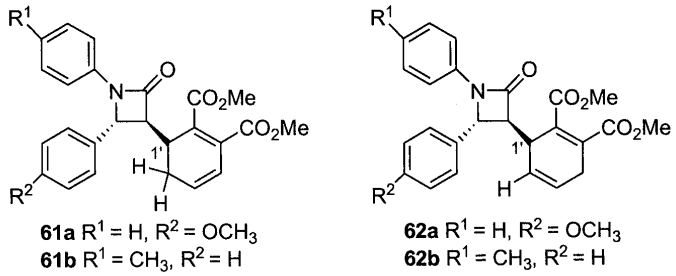


The coupling constants between H-3 and H-4 of the azetidin-2-one ring allow the determination their stereochemistry. Such coupling constants of vicinal protons are reported to be J = 5-6 Hz for the *cis* derivatives and J = 0-2 Hz for the *trans* derivatives [45,49,50]. Therefore NOE experiments are also very important for the establishment of the stereochemistry of this type of compounds. The *cis* stereochemistry of **58** was confirmed by NOE experiments, which revealed correlation between the H-3 and the 4-methyl group [50].

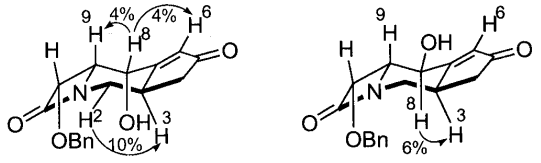
For the compound **59** NOE irradiation of H-2 resulted on 11% enhancement of the signal corresponding to H-3 in the *syn*-stereochemistry for the hydrogens of the central five-membered ring, and for similar derivatives with a *anti*-configuration **60**, irradiation of H-9 resulted in an enhancement of the signal due to H-8 [46].



COSY correlations allowed the assignment of two possible structures of azetidin-2-one **61a,b** and **62a,b**, obtained in the Diels-Alder reaction of α-dienylazetidinones with dimethylacetylene dicarboxylate. The correlation between both methylene protons with H-1', which in turn was coupled with only one of the olefinic protons, assigned the formation of the adducts **61a,b** instead of **62a,b** [51].



In complex systems of fused tricyclic 2-azetidinones, NOE effects were of great use to the configuration assignment. In the case of azetidinones **63a** and **63b**, having a six membered ring fused to the β-lactam, NOE irradiation on H-8 resulted in a 4% enhancements in the signals of H-9 and H-6 for compound **63a** while in the case of compound **63b** a 6% enhancement on H-3 signal was observed. On this basis, compound **63a** has a *syn* H8-H9 / *anti* H8-H3 stereochemistry, while in compound **63b** has an *anti* H8-H9 / *syn* H8-H3 stereochemistry. This type of approach was also applied to the stereochemistry determination of azetidinones having a six-membered ring fused to the C3-C4 bond of the β-lactam ring [46].



63a NOE enhancements **63b**

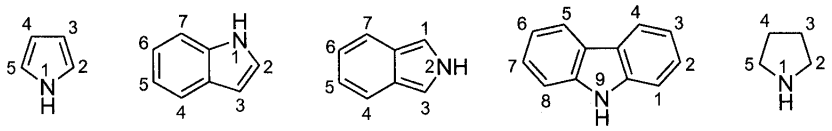
The ^{15}N NMR spectrum of azetidine **33** is similar to that of the aziridine **3**. Unsubstituted azetidine has its ^{15}N resonance at δ 25.3 ppm (relative to anhydrous ammonia) and *N*-*t*-butylazetidine presents their resonances at δ 52 ppm [14].

5. Five-membered heterocyclic compounds

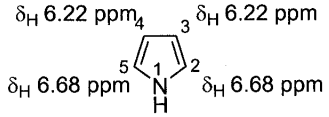
5.1. Pyrroles, indoles and carbazoles

Pyrrole **64** is an aromatic five-membered ring which contains one nitrogen atom in its structure. When a pyrrole ring is fused to a benzene ring in the 2,3-position originates the benzo[*b*]pyrrole known as indole **65**; when is fused to a benzene in the 3,4-position gives the benzo[c]pyrrole, also known as isoindole **66**. Pyrrole can also be fused with two benzene rings, in 2,3- and 4,5-positons to gives carbazole **67**, a benzo[*b*]indole. If the pyrrole ring is fully saturated it is denominated as pyrrolidine **68** [10].

The chemical shifts in the ^{1}H NMR spectrum of pyrrole are in the typical region of the aromatic compounds, being the α-hydrogen assigned at lower fields. The ring protons of pyrrole are also coupled to the NH (see structure **64**) [52]. The chemical shifts of all the protons in the pyrrole ring depend on the solvent used (Table 2).



64 **65** **66** **67** **68**



64

J_{H2-H3} = 2.70 Hz
J_{H2-H4} = 1.44 Hz
J_{H2-H5} = 1.87 Hz
J_{H3-H4} = 3.35 Hz

$J_{H1-H2} = J_{H1-H5}$ = 2.58 Hz
$J_{H1-H3} = J_{H1-H4}$ = 2.46 Hz

Table 2. Variation in the ^{1}H NMR chemical shifts of pyrrole with solvent.[a]

Solvent	H-2,5	H-3,4	Ref.
neat	6.32	6.14	[52]
hexane	6.32	5.94	[52,53]
cyclohexane	6.51	6.11	[52,54]
CCl_4	6.62	6.05	[52,55]
benzene	5.85	5.80	[52,53]
$CDCl_3$	6.68	6.22	[52]
acetone	6.58	5.89	[52,53]
THF	6.52	5.92	[52,56]

[a]Chemical shift of the N*H* resonance is profoundly solvent-dependent.

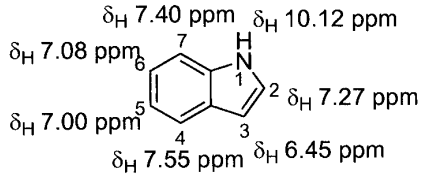
The presence of a *N*-methyl group in the pyrrole ring leads to upfield shifts in the remaining ring proton resonances while electron withdrawing substituents (*e.g.* formyl, acyl, alkoxycarbonyl) on that position causes substantial downfields shifts of the α-proton resonances and much smaller downfields shifts for those of the β-protons (Table 3).

Table 3. Variation in the ^{1}H NMR chemical shifts of *N*-substituted pyrroles.

N-Substituent (Solvent)	Δδ(H-2,5)[a]	Δδ(H-3,4)[a]	Ref.
Me (CCl_4)	-0.25	-0.13	[52,55]
CHO ($CDCl_3$)	+0.59	+0.18	[52,57]
COMe (CCl_4)	+0.53	+0.08	[52,58]
COBu^t ($CDCl_3$)	+0.77	+0.03	[52]
CO_2Me (CCl_4)	+0.51	+0.05	[52,57]
Ph (CCl_4)	+0.30	+0.10	[52,57]

[a] $\Delta\delta = \delta_{NR} - \delta_{NH}$ in ppm.

The presence of a benzene ring on the [*b*] face of the pyrrole ring has a small effect on the chemical shifts of the heterocyclic protons. H-5 and H-6 of indole ring (*e.g.* **65**) have similar chemical shifts and protons H-4 and H-7 appears further downfield. The chemical shift of the indolic N*H* proton is also very depend in the used solvent and as in the pyrrole it is also coupled to the ring protons with coupling constants of $J_{H1\text{-}H2}$ = 2.5 Hz, $J_{H1\text{-}H3}$ = 2.0 Hz and $J_{H1\text{-}H4}$ = 0.8 Hz [in $(CD_3)_2CO$] [59]. In the ^{1}H NMR spectra of the benzo[*c*]fused derivatives (*e.g.* **66**), the resonances of H-1 and H-3, adjacent to the heteroatom, are at lower field than the corresponding ones in the benzo[*b*]isomers [60,61].



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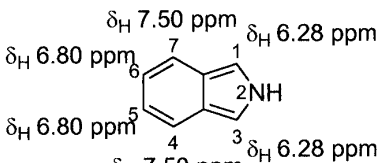


66

Similar to ^{1}H resonances, the ^{13}C NMR chemical shifts of pyrrole and *N*-methylpyrrole are also solvent dependent (Table 4).

^{1}H NMR data for carbazole **67** are given in Table 5. A study involving carbazole and 13 *N*-substituted derivatives reveals little changes in proton chemical shifts on changing solvent from acetone to carbon tetrachloride [52]. Therefore the chemical shifts of carbazole are not significantly altered by *N*-substitution and by changing solvent from chloroform-d_1 to DMSO-d_6 (Table 5).

^{13}C NMR data of some indoles are given in the table 7.

Table 4. Variation of ^{13}C NMR chemical shifts (δ, ppm) of pyrrole and *N*-methylpyrrole with solvent [62].

	Pyrrole		*N*-Methylpyrrole		
Solvent	C-2,5	C-3,4	C-2,5	C-3,4	NMe
neat (+ 10% C_6H_{12})	118.9	108.6	122.4	109.4	36.0
cyclohexane	118.2	108.6	121.5	109.0	35.7
CH_2Cl_2	118.8	109.2	122.6	109.4	36.9
dioxane	117.7	108.0	121.6	108.0	35.4
acetone	118.0	107.8	121.7	108.1	35.4
benzene	118.1	108.7	122.1	109.2	35.6
DMSO	116.3	106.1	120.2	106.1	34.3

Table 5. ^{1}H NMR data (δ, ppm) for carbazole (in acetone-d_6) [59].

	H-1	H-2	H-3	H-4
δ	7.49	7.36	7.16	8.08
J_1	---	8.21	0.89	0.67
J_2	---	---	7.17	1.18
J_3	---	---	---	7.80

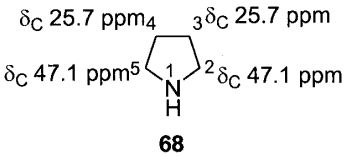
Table 6. ^{13}C NMR data (δ, ppm) for carbazole and *N*-substituted derivatives.

Substituent	C-1,8	C-2,7	C-3,6	C-4,5	C-4a,4b	C-8a,9a	Solvent	Ref.
none	110.8	125.4	118.4	120.0	122.6	139.6	DMSO-d_6	[63]
none	110.6	125.9	119.5	120.4	123.5	139.6	$CDCl_3$	[64]
1-Me	108.2	125.5	118.7	120.1	122.7	140.9	$CDCl_3$	[65]
1-COMe	115.8	126.9	123.2	119.4	126.0	138.3	$CDCl_3$	[63]
1-Ph	109.5	126.6	119.9	120.4	122.7	140.1	$CDCl_3$	[63]

Table 7. ^{13}C NMR chemical shifts (δ, ppm) for substituted indoles.

Substituent	C-2	C-3	C-3a	C-4	C-5	C-6	C-7	C-7a	Solvent	Ref.
None	123.7	101.8	127.0	119.9	121.1	119.0	110.4	134.8	$CDCl_3$	[66]
None	124.7	102.1	128.3	120.8	121.8	119.8	111.4	135.6	Dioxane-d_8	[67,68]
1-Me	128.6	100.8	128.4	119.1	120.7	121.3	109.0	136.6	$CDCl_3$	[65]
1-COMe	125.0	108.7	130.2	120.6	123.4	124.8	116.0	135.2	$CDCl_3$	[65]
2-CO_2H	126.1	106.8	127.7	123.5	121.2	119.3	111.9	136.3	DMSO-d_6	[66]
3-COMe	133.4	116.2	124.4	122.0	120.9	120.9	111.4	135.9	DMSO-d_6	[66]
3-CHO	138.1	118.2	124.2	123.3	122.0	120.8	112.3	137.1	DMSO-d_6	[66]
3-OCOMe	121.6	119.8	129.2	114.4	116.9	118.8	111.8	133.3	DMSO-d_6	[66]
5-CN	126.0	102.5	126.9	125.5	101.6	123.8	111.5	136.8	$CDCl_3$	[66]
5-OMe	124.3	101.6	127.7	111.6	153.1	101.8	111.9	130.3	$CDCl_3$	[66]
6-OMe	123.2	102.4	122.3	121.2	110.0	156.5	94.8	136.6	$CDCl_3$	[66]
7-OMe	123.6	102.8	126.9	120.2	113.5	102.1	146.7	129.6	$CDCl_3$	[66]

The NMR spectrum of isoindole **66** and pyrrolidine **68** systems are very simple due to the symmetry of the molecules. For instance, the ^{13}C NMR spectrum of *N*-methyl-isoindole **69** presents only five carbon resonances and that of pyrrolidine **68** only two [52].



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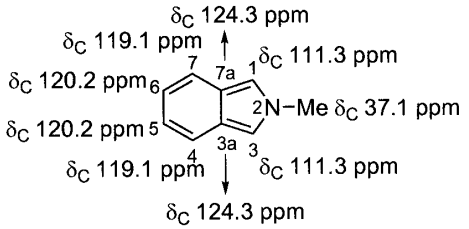

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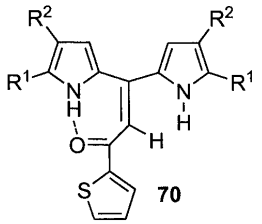
NMR spectroscopy is an important method for the investigation of hydrogen bonds in 1- and 2-substituted pyrroles [69,70]. The strong N-H···O intramolecular hydrogen bond in the Z-isomers of 2-(2-acylethenyl)pyrroles **70** causes the deshielding of the proton and nitrogen atoms by respectively Δδ 5-6 and 15 ppm and a decrease in the absolute value of the $^{1}J_{NH}$ coupling constant of 2 Hz in $CDCl_3$ and 4.5 Hz in DMSO-d_6, relatively to the other NH group not involved in an hydrogen bond. However, the N-H···N intramolecular hydrogen bond in 2-(2'-pyridyl)pyrrole **71** originates a high-frequency shift of the H-1 signal (Δδ ~ 1 ppm) and a low frequency shift of the pyridine nitrogen resonance (Δδ 8-19 ppm) relatively to those of 2-(3'- and 4'-pyridyl)pyrroles. These data are indicative of the different nature of the hydrogen bonding, predominantly covalent or electrostatic. Obviously the N-H···O hydrogen bond of **70** is much stronger than the N-H···N of **71** (the shift in the H-1 resonance of **70** is 6 times bigger) and depends in turn on geometry of the hydrogen bridge; the former is mainly covalent and the latter is predominantly electrostatic [69]. The N-H···N intramolecular hydrogen bonding in 2-(2'-pyridyl)pyrrole **71** is similar to the C-H···N intramolecular hydrogen bonding between the α-hydrogen of the vinyl group and the pyridine nitrogen in 1-vinyl-2-(2'-pyridyl)pyrrole **72** and similar compounds. The C-H···N intramolecular hydrogen bond also leads to an increase of the $^{1}J_{C\alpha\text{-}Hx}$ coupling constant of *ca* 5 Hz [69,70].



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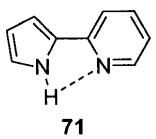

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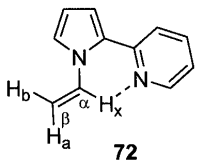
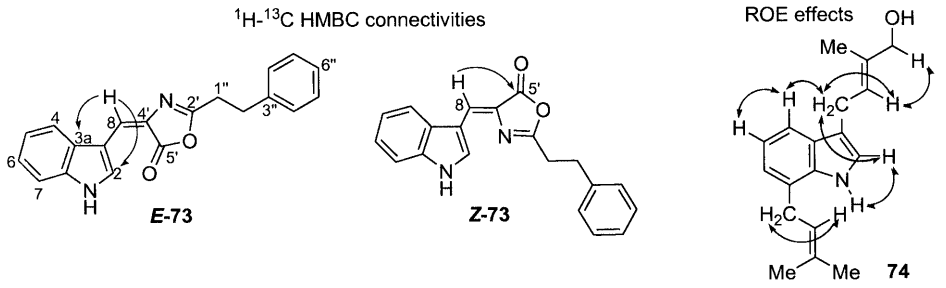


72

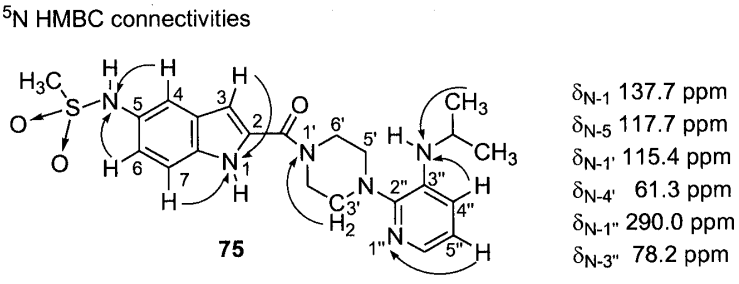
^{1}H-^{13}C HMBC correlations were very useful to establish the substituents position of indole **73**, being assign to position 3 due to the connectivities of H-8 (vinylic singlet) with one quaternary carbon (C-3a) and C-2. The stereochemistry of the double bound was also established by this technique, where H-8 shows coupling with C-5'; the

coupling constants of J = 4.9 and 11.1 Hz indicates the presence of the *Z*-**73** and *E*-**73** configuration, respectively [71].

The NH proton of the indole skeleton can be identified by exchange with D_2O, and also by the correlation between NH and H-2 observed in the ROESY spectrum. All the correlations observed in the ROESY spectrum of 3,7-disubstituted indole **74** were essential for the structural determination of this natural alkaloid [72].



Delavirdine **75** is a complex nitrogenous pharmaceutical agent which contains an indole ring in its structure and has six unique nitrogens in a variety of different electronic environments. The long-range couplings in the GHNMQC (gradient-enhanced hydrogen-nitrogen multiple quantum coherence) spectrum of delavirdine **75** allowed the assignments of the nitrogen atoms contained in the structure (see **75**). The molecule contains three protonated nitrogen resonances (N-1, N-5 and N-3''), but only two of them appear as doublets; N-1 J = 99 Hz and N-3'' J = 85 Hz. The protonated nitrogen of the acidic 5-sulfonamidomethyl moiety is shown to be sufficiently acidic in DMSO-d_6 solution at room temperature to preclude the observation of a direct response for this ^{1}H-^{15}N heteronuclear pair. Variable temperature studies in pyridine-d_5 solution demonstrated that it is possible to slow "exchange" almost, if not completely, between – 20°C and – 30°C and to block the process by converting delavirdine **75** to its mesylate, which allowed the direct response doublet, with full intensity, to be readily observed in DMSO-d_6 solution at room temperature. The study of long-range coupling and the chemical shifts of nitrogen allowed the orientation and structural elucidation of a variety of alkaloids [73].



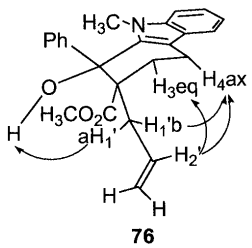
The indole ring can be found in a variety of biologically active natural compounds and their association to give bis-indoles structures has also been reported in literature. Several *bis*-indoles alkaloids have been isolated and they often exhibit more potent biological activities than the monomeric units. The structural elucidation of natural and synthetic derivatives have been accomplished using the two dimensional NMR techniques, mainly COSY and HMBC experiments [74 -77].

Specific NOE correlation between protons of the indolyl and the linkage molecule allowed the assignment of the positions of the indolyl moieties and the relative configuration of each compound [76,78,79]. NOESY analysis of **76** revealed a *trans*-diaxial orientation of the phenyl and allyl groups in the indole-fused system, namely by the NOE effects observed between H-2' of the allyl group and both H-3_{eq} and H-4_{ax}, H-1'b with H-4_{ax} and of H-1'a with the tertiary alcohol proton [80].

^{1}H-^{13}C HMBC spectra were very helpful to identify the position of sugar derivatives in alkaloids indole-type, specially the correlation of anomeric proton with the carbon in which the sugar moiety is connected, together with the coupling constant of this proton (J = 5.5 Hz), which indicates the position and the β-configuration of the sugar residue [77,81]. One of these examples can be seen in structure **77** [82,83].^{1}H-^{13}C and ^{1}H-^{15}N HMBC spectra were very important to establish the structure of natural bis-indole alkaloids (*e.g.* 77) [81-85].

Carbazoles appear as natural and synthetic alkaloids in a free-form, fused to other heterocyclic molecules or even as constituents in organic polymers [86-88]. Proton resonances of H-1 and H-8 of a carbazole nucleus appears in the aromatic zone at a quite downfield δ ~ 7-8 ppm and appears each one as a doublet (J = 8 Hz) when there isn't substituents in the vicinal position [89,90]. In the case of *N*-formylcarbazole it is interesting to note the appearance of two broad signals for the formyl group in both ^{1}H and ^{13}C NMR, which is due to the existence of two rotamers arising out of restricted rotation around the N-CHO bond [89].

NOE effects



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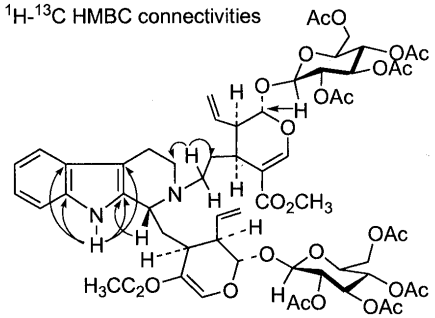
^{1}H-^{13}C HMBC connectivities



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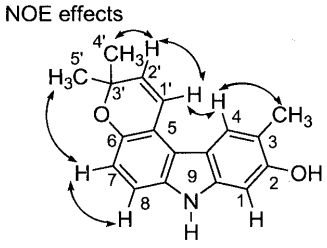
Glycoborinine **78** is a carbazole alkaloid isolated from the roots of *Glycosmis arborea* and the ^{1}H-^{13}C HMBC spectrum of this compound give valuable information for the establishment of its structure (H-1 --> C-2, C-3, C-4a and C-9a; H-7 --> C-5, C-6 and C-8a; H-1' --> C-4b, C-5 and C-6; H-2' --> C-5) and in the assignment of their carbon resonances (Table 8). The structure assignment was also confirmed by NOE interactions (see structure **78**) [89].

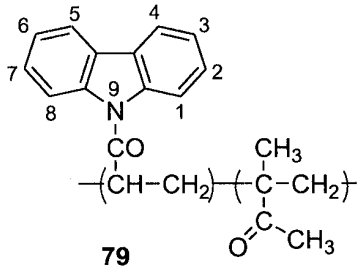


78

Table 8. ^{13}C NMR data (δ, ppm) of glycoborinine **78.**

Carbon	δ (ppm)	Carbon	δ (ppm)
C-1	96.5	C-8	110.4
C-2	154.5	C-8a	135.8
C-3	117.4	C-9a	141.6
C-4	123.8	C-10	16.7
C-4a	116.6	C-1'	120.9
C-4b	119.3	C-2'	131.2
C-5	115.0	C-3'	75.5
C-6	146.1	C-4'	27.2
C-7	113.5	C-5'	27.2

NMR spectroscopy is probably the most effective technique to determine intramolecular (sequence determination and configuration) and intermolecular (chemical composition) chain structure of polymers. Complete spectra assignments of the ^{1}H and ^{13}C NMR spectra of dimers, trimers and more complex polymers containing carbazole as a monomer were done by using 2D NMR experiments, such as HSQC, HMBC and TOCSY spectra [91-93]. The synthesis of copolymers of *N*-acryloylcarbazole (A) and methyl methacrylate (M) **79** can be produced in different ratios of A *versus* M. The composition A/M of the copolymer can be determined by ^{1}H NMR spectrum, which gives relative intensities of the aromatic and aliphatic proton resonances [93].



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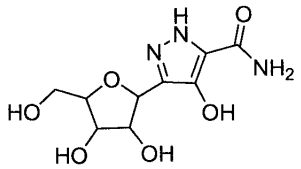
NMR can also be very useful in the structural elucidation of new pyrrolidine derivatives or pyrrolidine fragments involved in organic synthesis, as reactants or intermediates [94,95].

5.2. Pyrazoles and indazoles

Pyrazoles are five-membered ring systems with three carbons and two nitrogen atoms in the 1- and 2-positions and are among the azole family one of the most studied group of compounds. These studies are mainly due to their biological applications; it seems that the presence of the pyrazole ring enhance the activity of compounds as was detected for some cyclooxygenase inhibitory activity and anti-inflammatory agents [96]. Motivated by the discovery of the natural pyrazole *C*-glycoside pyrazofurim **80**, which is an antibiotic with a broad spectrum of antimicrobial and antiviral activities [97], much attention has been focused towards pyrazoles activities. Nevertheless, their characterization has never been neglected.

The assignment of pyrazole structures is usually carried out by NMR spectroscopy, detailed proton and carbon NMR data can be found in the chapter of Elguero in *Comprehensive Heterocyclic Chemistry* which summarize the information about pyrazole derivatives, but also contains references to the original literature [98]. Ten years later Elguero *et al*. published a review on the ^{13}C NMR spectra of pyrazoles were they report carbon-13 chemical shifts and coupling constants of 1168 pyrazoles [99]. More recently it can be found some pyrazole derivatives characterisations where authors use 2D NMR spectroscopy [100 - 102].

Pyrazoles (1*H*-pyrazole **81**) and their benzoderivatives (1*H*-indazole **82** and 2*H*-indazole **83**) have specific behaviour that they usually are treated together but separated from other azoles. It is important notice that these azole systems have characteristic chemical shifts but their tautomerism must be taken into account in their characterisation. However, in *N*-substituted pyrazoles the H-3 and H-5 became inequivalents (Table 9). *N*-substitution of 1*H*-indazole does not affect significantly the chemical shifts of the heterocyclic ring but in the case of *N*-substitution of 2*H*-indazole one can detect higher shifts (Table 11). The same effects can be detected in the ^{13}C NMR chemical shifts for these types of compounds (Tables 10 and 12).



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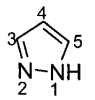

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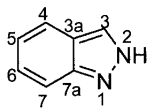

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Table 9. ^{1}H NMR spectral data (δ, ppm) of pyrazole derivatives.

Compound	Solvent	H-3	H-4	H-5
pyrazole[a]	CCl_4	7.61	6.31	7.61
4-methylpyrazole	$CDCl_3$	7.36	-	7.36
4-phenylpyrazole	DMSO	8.00	-	8.00
1-methylpyrazole	$CDCl_3$	7.49	6.22	7.35
1-phenylpyrazole	$CDCl_3$	7.72	6.46	7.87
1-acetylpyrazole	$CDCl_3$	7.71	6.44	8.25
1-tosylpyrazole	DMSO	7.88	6.58	8.45

[a]δ_{NH} 12.64 ppm

Table 10. ^{13}C NMR spectral data (δ, ppm) of pyrazole derivatives.

Compound	Solvent	C-3	C-4	C-5
pyrazole	acetone	133.4	104.6	133.4
1-methylpyrazole	$CDCl_3$	138.7	105.1	129.3
1-phenylpyrazole	$CDCl_3$	140.7	107.3	126.2
1-acetylpyrazole	$CDCl_3$	143.6	109.3	127.8
1-tosylpyrazole	DMSO	145.8	109.6	132.4

Table 11. ^{1}H NMR spectral data (δ, ppm) of indazole derivatives.

Compound	Solvent	H-3	H-4	H-5	H-6	H-7	NH
indazole	DMSO	8.08	7.77	7.11	7.35	7.55	13.04
1-methylindazole	$CDCl_3$	7.94	7.71	7.10	7.34	7.59	-
2-methylindazole	$CDCl_3$	7.67	7.56	7.02	7.26	7.68	-

Table 12. ^{13}C NMR spectral data (δ, ppm) of indazole derivatives.

Compound	Solvent	C-3	C-3a	C-4	C-5	C-6	C-7	C-7a
indazole	DMSO	133.4	122.8	120.4	120.1	125.8	110.0	139.9
1-methylindazole	$CDCl_3$	132.4	123.8	120.8	120.2	125.9	108.6	139.7
2-methylindazole	$CDCl_3$	123.1	121.8	119.6	121.2	125.4	116.8	148.7

Claramunt *et al.* collected ^{15}N NMR data from the literature for pyrazole and indazole derivatives and other heterocyclic compounds, and completed the review with the study of new compounds in different solvents [103]. One of the most important aspects of these NMR analyses is the fact that nitrogen atoms are directly involved in the proton exchange process, so the best way to study tautomerism must be the observation of nitrogen resonances (Table 13). In fact there can be found several tautomeric studies for pyrazoles using, among other techniques, NMR spectroscopy mostly ^{1}H, ^{13}C and ^{15}N NMR [104 - 106]. For example, in the solid state the ^{13}C and ^{15}N CP/MAS NMR spectra of 1*H*-pyrazole-3-(*N*-butyl)carboxamide showed that it exist as this tautomer, while in solution both tautomers are present in a ratio that depends on the temperature (at 293K, 90% 3-substituted/10% 5-substituted) (Table 14) [105].

Table 13. ^{15}N NMR spectral data of 1*H*-pyrazole derivatives (δ, ppm).

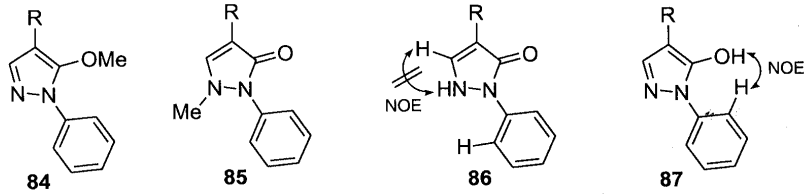
Compound	Solvent	N-1	N-2
pyrazole	DMSO	-173.1	-79.8
pyrazole	$CDCl_3$	-132.2	-132.2
1-methylpyrazole	DMSO	-178.4	-71.6
1-methylpyrazole	$CDCl_3$	-180.8	-76.5
indazole	DMSO	-196.3	-66.1
1-methylindazole	DMSO	-203.8	-57.6
2-methylindazole	DMSO	-162.1	-92.3

Table 14. ^{13}C and ^{15}N data (δ, ppm; $^{1}J_{NH}$, Hz) of 1*H*-pyrazol-3-(*N*-butyl)carboxamide.

Temp.	Tautomer	C-3	C-4	C-5
213 K	3-subst	148.80	105.61	130.58
	5-subst	140.11	104.21	138.55
298 K[a]	3-subst	147.0	105.6	131.4
		N-1(H)	N-2	NH (amide)
213 K	3-subst	- 176.2	- 82.1	- 256.4
		$^{1}J_{NH}$ 106		$^{1}J_{NH}$ 89
	5-subst	- 172.0	- 72.5	- 252.3
				$^{1}J_{NH}$ 92
298 K[a]	3-subst	- 169.4	- 75.8	- 247.0

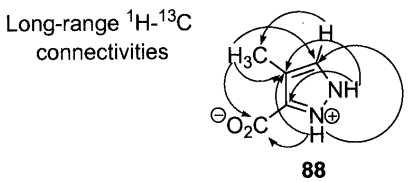
[a]CP/MAS

The tautomerism of 3(5)-unsubstituted-pyrazolones *versus* the corresponding hydroxypyrazoles was study by NMR. The magnitude of the $^{2}J_{C4\text{-}H3(5)}$ spin coupling constant allowed the unambiguous differentiation between 1*H*-pyrazol-5-ol and 1,2-dihydro-3*H*-pyrazol-3-one. 1*H*-pyrazol-5-ol and 2,4-dihydro-3*H*-pyrazol-3-ones exhibit ^{2}J = 9-11 Hz while this coupling constant in 1,2-dihydro-3*H*-pyrazol-3-one in considerable reduced to ^{2}J = 5-4 Hz. This can be mainly attributed to the removal of the lone-pair at N-1 pyrazole in the latter due to protonation or alkylation [107]. These conclusions were taken by using fixed tautomers such as *O*-methyl **84** and *N*-methyl derivatives **85** as pyrazoles and pyrazolones, respectively. Considering that pyrazolones **86** showed $^{2}J_{C4\text{-}H3(5)}$ values in the range of 9.5 to 11.2 Hz, it seems strong evidence that they are present in the hydroxyl form **87**. These data was also confirmed by ^{13}C NMR data and by NOE effects.

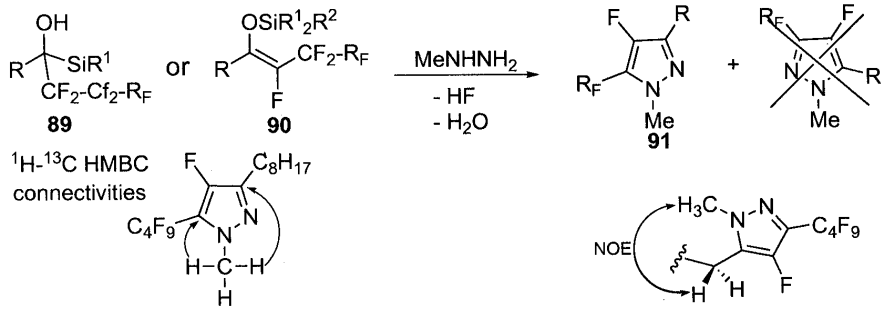


R = H, COMe, COPh, CO(2-thienyl), COCH=CHPh(*E*)

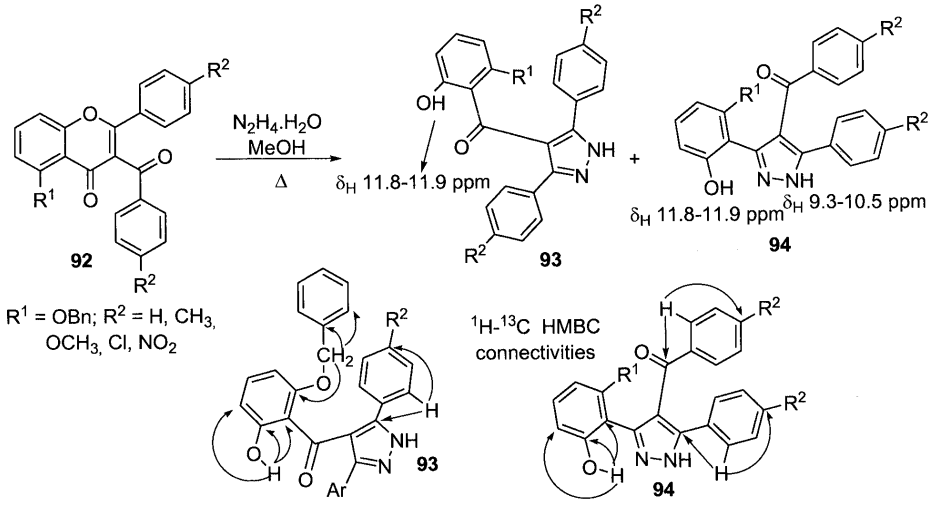
NMR experiments are also used in simple structural characterisations. For example the structure of some natural pyrazoles have been confirmed by the connectivities found in SINEPT (long-range ^{1}H-^{13}C couplings) experiments (*e.g.* 4-methylpyrazol-3(5)-carboxylic acid **88** isolated from the methanol extract of the sponge *Tedania anhelans* [108]).



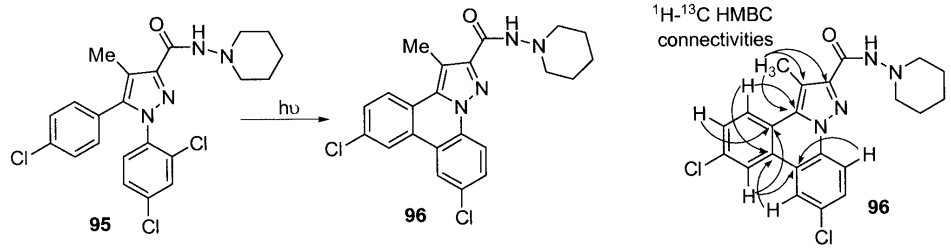
NMR experiments are also essential in the characterization of synthetic pyrazoles, for instance the regiochemistry of the synthesis of 4-fluoro-5-(perfluoroalkyl)pyrazoles **91**, obtained from the reaction of 1-(trialkylsilyl)perfluoroalkanols **89** or 1-alkyl-1-(trialkylsilyloxy)perfluoroalk-1-enes **90** with methylhydrazine, was confirmed by ^{1}H-^{13}C HMBC and NOE experiments [109].



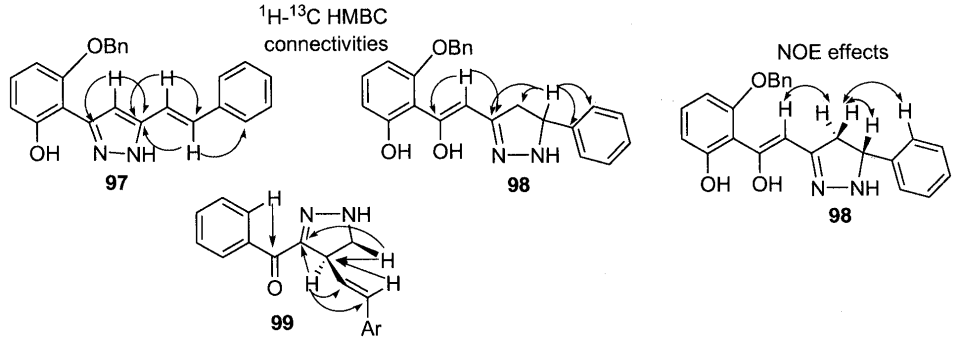
The reaction of 3-aroylflavones **92** with hydrazine hydrate gave an isomeric mixture of aroylpyrazoles **93** and **94**. The structure of aroylpyrazoles **93** can be assigned based on the high frequency value of the resonance of the hydroxyl proton (δ 11.8-11.9 ppm) due to the hydrogen bond with the carbonyl group. In the case of aroylpyrazoles **94** one can detect two broad singlets (δ 11.8-11.9 and 9.3-10.5 ppm) due to the proton resonances of the hydroxyl and NH groups, indicating the absence of prototropy in this case because of the hydrogen bond between the hydroxyl proton and the nitrogen N-2 of the pyrazole ring. Nevertheless, the isomeric aroylpyrazoles structures were fully confirmed by connectivities found in HMBC spectra [101].



The CB_1 cannabinoid antagonist *N*-(piperidinyl)-5-(4-chlorophenyl)-4-methyl-1*H*-pyrazol-3-carboxamide **95** undergoes a photocyclisation reaction giving a single product which structure is based on a pyrazolo[1,5-*f*]phenanthridine **96**. The structure of this compound was established by 2D NMR techniques, namely COSY, HSQC, HMBC and ROESY. The ROESY spectrum showed that the pyrazole methyl group and a trisubstituted aryl ring were adjacent and the connectivities deduced from ^{1}H-^{13}C HMBC spectrum allowed to establish the full structure (see structure **96**) [110].



The caracterisation of several styrylpyrazole derivatives **97-99** recently synthesised was accomplished using several 2D NMR experiments, mainly ^{1}H-^{13}C HMBC spectrum allowing the establishment of several important connectivities and NOE effects to assign the stereochemistry [101,102].



5.3. Imidazoles and benzimidazoles

1,3-Diazole, commonly known as imidazole **100**, is a five-membered ring system with three carbons and two nitrogen atoms in the 1- and 3-positions. The nitrogen bears a hydrogen atom (N-1) seems as a pyrrole-like N-atom; the other (N-3) resembles a pyridine-like N-atom [10]. Ring fused derivatives with or without a bridgehead nitrogen atom can be obtained with this system. Benzimidazole **101** is one of that derivatives and has a benzene ring fused in the 4,5-positions of the imidazole ring. The partially and fully reduced derivatives of imidazole are no longer aromatic and are described as 2-imidazoline **102**, 3-imidazoline **103**, 4-imidazoline **104** and imidazolidine **105**. The NMR spectra of imidazole **100** and benzimidazole **101** show the presence of two tautomeric forms, which are rapidly equilibrating on the NMR timescale.

Imidazole is a full aromatic neutral compound, so the chemical shifts of their protons are around δ 7-8 ppm (Table 15) and the vicinal proton-proton coupling constant are very small ($J \sim 1$ Hz).

NMR spectra of imidazoles unsubstituted on nitrogen are very difficult to obtain due to their poor solubility in non-polar solvents. *N*-Acetylation of these derivatives allows them to become soluble in standard NMR solvents [$CDCl_3$ and DMSO-d_6] (Table 16). Table 17 presents the ^{1}H NMR chemical shifts of some imidazole derivatives.

While in neutral solutions of imidazole **100** and benzimidazole **101** rapid exchangeable *N*-hydrogens are verified, in the *N*-methylazoles the chemical shifts are "fixed" and shifted downfield by adjacent nitrogen atoms.

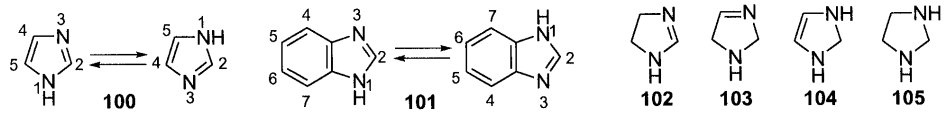


Table 15. ^{1}H NMR data (δ, ppm) of imidazole.

Solvent	H-2	H-4	H-5	ref
$CDCl_3$	7.86	7.25	7.25	[111]
	7.73	7.14	7.14	[10]
D_2O	7.73	7.14	7.14	[10]

Table 16. ^{1}H NMR data (δ, ppm) *N*-methylimidazole.

Solvent	H-2	H-4	H-5	ref
$CDCl_3$	7.47	7.08	6.88	[112]
	7.43	7.05	6.90	[113]
	7.41	7.03	6.87	[114]
C_6D_{12}	7.41	7.05	6.88	[115]
D_2O	7.63	7.13	7.03	[116]
	7.57	6.99	7.07	[114]
	7.57	7.00	7.07	[114]
$(CD_3)_2SO$	7.55	6.88	7.08	[114]

Table 17. ^{1}H NMR chemical shifts (δ, ppm) of some imidazole derivatives [111].

Compound	H-2	H-4	H-5	1-Me	2-Me	4-Me	5-Me
2-Me	---	6.96	6.96	---	2.36	---	---
4-Me	7.47	---	6.81	---	---	2.23	---
1,2-Me	---	6.79	6.73	3.52	2.30	---	---
1,4-Me_2	7.20	---	6.53	3.49	---	2.15	---
1,5-Me_2	7.27	6.68	---	3.42	---	---	2.10
2,4-Me_2	---	---	6.54	---	2.21	2.04	---
4,5-Me_2	7.45	---	---	---	---	2.19	2.19
2,4,5-Me_3	---	---	---	---	2.23	1.98	1.98
2-Br-1-Me	---	7.04	7.04	3.64	---	---	---
5-Br-1-Me	7.59	7.07	---	3.63	---	---	---
1-Me-4-NO_2	7.54	---	7.87	3.90	---	---	---
1-Me-5-NO_2	7.64	8.09	---	4.05	---	---	---

The neutral aromatic system can be converted into a monocationic **106** as well as a monoanionic **107** system and the ^{1}H NMR chemical shifts are undoubtedly affected. Anion formation result in shifts to higher field; conversely, in the cations the downfield is especially great to the H-2 resonance [10].

Similar to what is observed in the proton spectra, the ^{13}C NMR spectra of imidazoles due to the fast tautomerism, render two of the chemical shifts equivalent for the NH derivatives (Table 18).

In table 19 are presented ^{13}C NMR chemical shifts of some benzimidazole derivatives.

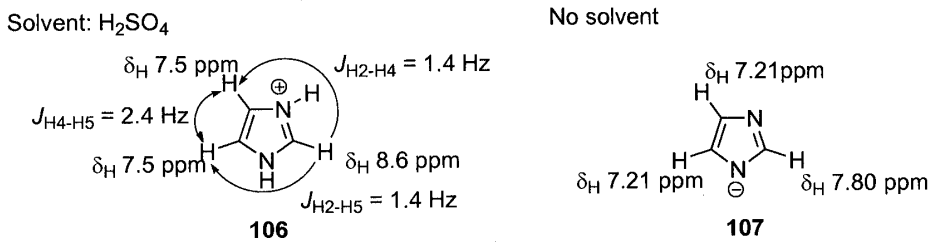


Table 18. ^{13}C NMR chemical shift (δ, ppm) of imidazole derivatives.

Compound	C-2	C-4	C-5	C=O	Me	C-1'	C-2'	C-3'	C-4'	Solvent	ref
imidazole	136.2	122.3	122.3	---	---	---	---	---	---	$CDCl_3$	[117]
	136.3	122.8	122.8	---	---	---	---	---	---	CD_2Cl_2	[62]
	135.9	122.0	122.0	---	---	---	---	---	---	---	[111]
	135.4	121.9	121.9	---	---	---	---	---	---	$CDCl_3$	[10]
1-Ac	136.2	130.5	115.9	166.3	22.3	---	---	---	---	$CDCl_3$	[65]
1-Me	138.7	130.2	121.0	---	34.2	---	---	---	---	$CDCl_3$	[62]
	138.8	130.1	121.0	---	34.0	---	---	---	---	CD_2Cl_2	[62]
	138.3	129.6	120.3	---	32.6	---	---	---	---	Dioxane-d_8	[62]
1-Ph	135.0	129.9	117.8	---	---	136.7	121.0	129.4	128.0	---	[111]
2-Ph	146.7	122.7	122.7	---	---	130.0	125.2	128.4	128.3	---	[111]
1-Me-2-Ph	147.2	127.8	121.9	---	34.4	130.1	128.0	128.0	128.0	---	[111]
1-Me-4-Ph	141.7	137.4	122.2	---	---	133.6	124.3	128.1	126.2	---	[111]

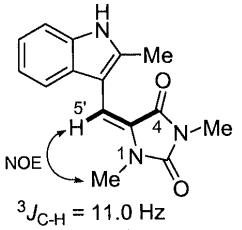
Table 19. ^{13}C NMR chemical shift (δ, ppm) of benzimidazole derivatives.

Compound	C-2	C-4	C-5	C=O	Me	C-6	C-7	Solvent	ref
benzimidazole	144.4	110.1	122.7	---	---	121.9	119.4	$CDCl_3$	[118]
	141.5	115.4	122.9	---	---	122.9	115.4	CD_3OD	[10]
1-Me	143.1	119.7	121.5	---	30.4	122.4	108.9	$CDCl_3$	[65]
2-Me	152.9	115.1	123.1	---	14.3	123.1	115.1	CD_3OD	[119]
1-Ac	141.2	120.1	124.7	166.9	23.3	125.5	115.1	$CDCl_3$	[65]

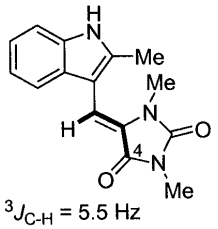
(*Z*)- And (*E*)-orientation of several aplysinopsins analogues was differentiated on the basis of carbon-proton coupling constants $^{3}J_{C4\text{-}H5'}$ being smaller (2-6 Hz) for *cis*-configuration and higher (8-12 Hz) for *trans*-configuration around the C=C double bond (see structures *E*-**108** and *Z*-**108**). In the case of **108**, the (*E*)-stereochemistry was also confirmed by NOE effect between the vinylic proton H-5' and the methyl group in N-1 [120].

Intensive NOE experiments allowed the identification of (*E*)-configuration at the C=N double bond of 2-aminoimidazoles **109** and a preferential occurrence of *s-trans* conformation. The absence of NOE between N=CH proton and the amino protons in additional to the NOE correlation between imidazole H-5 and N=CH proton was essential regarding the stereochemistry of compound **109** [121].

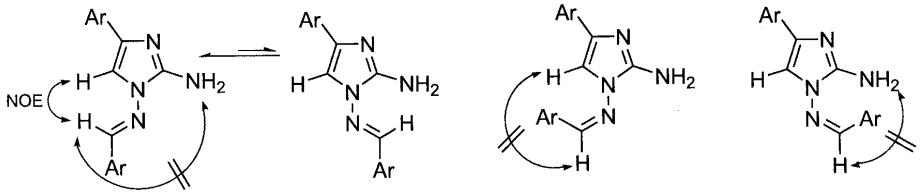
NOE difference measurements were also used to obtain substantial informations for the conformation and configuration of complex structures as benzimidazole derivatives [122,123].



(*E*)-108



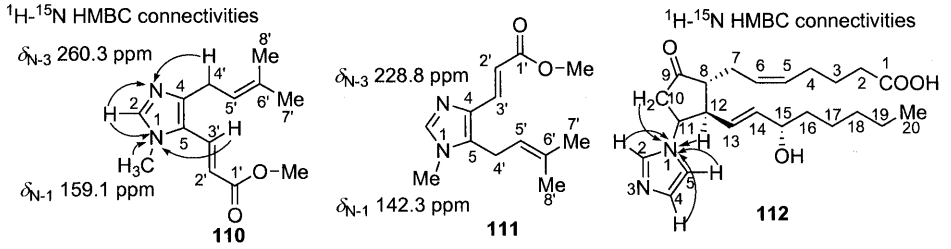
(*Z*)-108



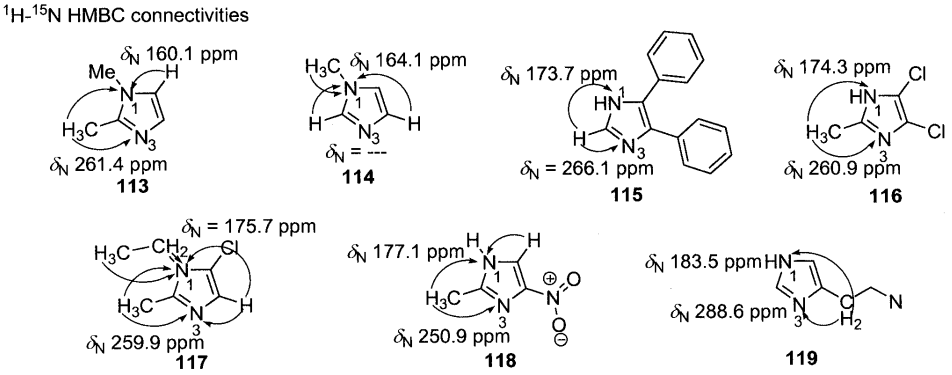
s-trans-(*E*)-109 *s-cis*-(*E*)-109 *s-trans*-(*Z*)-109 *s-cis*-(*Z*)-109

Long-range heteronuclear ^{1}H-^{13}C coupling constants, such as TOCSY-based methods and HMBC-based correlation techniques have also been used in the unequivocal assignments of imidazoles derivatives, but ^{1}H-^{15}N HMBC technique is also very important in the structural characterization of imidazole derivatives [124,125]. ^{1}H-^{15}N HMBC spectra was essential to assign the correct position of the two side chains in visoltricin **110** which is the inverse of their isomer fungerin **111**, two compounds presenting important biological activities. For **110** H-2 of imidazole ring correlated with nitrogen resonances of N-1 and N-3. H-3' and *N*-methyl protons showed long-range correlation with N-1, while H-4' correlates with the nitrogen signal of N-3 [124].

^{1}H-^{15}N HMBC spectrum of ^{15}N-labeled imidazole-prostaglandin E_2 adduct **112** allowed the confirmation of covalent attachment site of the imidazole ring to the prostaglandin framework [125]. N-1 of imidazole ring correlated with imidazole protons H-2, H-4 and H-5 and also the two prostaglandin protons H-10 and H-12, identifying this latter correlation the nitrogen N-1 as site bond to the carbon C-11 of prostaglandin nucleus.

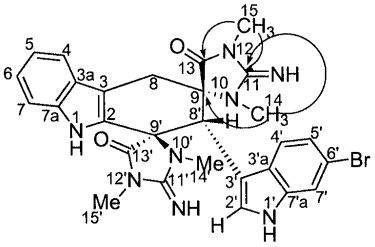


Long-range ^{1}H-^{15}N correlations of heterocyclic compounds optimized with CIGAR-HMBC experiments revealed strong correlations of protons with "pyrrolic-like" nitrogen than to the "pyridine-like" nitrogen atoms of imidazoles and pyrazoles [126] and that very long-range (four and five-bond) correlations of methyl groups and the nitrogens of aromatic heterocycles are observed. Some examples of these conclusions are depicted in structures **113-119.**



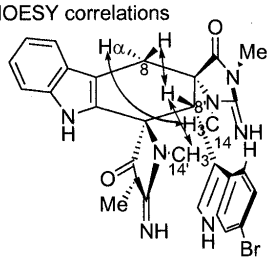
The imidazolidinones presents a C=O in positions 2-, 4- or 5- of the imidazole moiety. Structural elucidation of both natural and synthetic derivatives was accomplished by NMR spectroscopy. Tubastrindole A **120** is a natural alkaloid containing in his structure two indoles and two imidazolidinones. The latter nucleus was confirmed by ^{1}H-^{13}C HMBC correlations and the stereochemical orientation was elucidated by NOESY experiments. The 14-methyl protons (δ 3.30 ppm), attached to a nitrogen atom, were correlated with the quaternary carbon C-9 and guanidine carbon C-11. The 15-methyl protons (δ 2.71 ppm) correlate with C-13 and C-11. The other imidazolidinone moiety chemical shifts were established by similar ^{1}H-^{13}C HMBC correlations. The stereochemistry of the chiral centers was shown by the NOESY correlations where the NOE between H-8 and H-8' indicates that these hydrogens are in same face of the ring and NOEs between H-8α and H-14 and between H-8' and H-14' indicates the stereochemical orientation of two spiro-junctions [127].

^{1}H-^{13}C HMBC connectivities



120

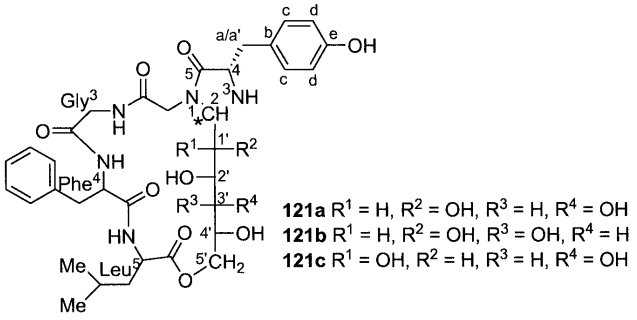
NOESY correlations



120

Structural studies involving 2D NMR spectroscopy of carbohydrate-containing bicyclic diasteriomeric imidazolidinone derivatives have been made in order to provide the relative geometry of the imidazolidinone ring moiety [128,129]. For the *trans/cis* assignments of the two diasteriomeric compounds (*2S*)- and (*2R*)- of **121a-c** COSY and TOCSY experiments were carried out in order to obtain the relative configuration of the imidazolidinone ring. In the COSY spectra of the *trans* isomers four-bond correlation between H-2 and H-4 was observed while in the spectra of *cis* isomers this correlation were not present, just the three-bond correlation of H-2 to H-1' of the pentitolyl residue. TOCSY maps of both isomers showed strong correlations of H-2 with the protons from the pentitolyl spin-system but in the *trans* isomers a progressively weaker correlations of H-2 with H-4 and H-a/H-a' of the imidazolidinone ring were observed. These experiences allowed concluding that the major isomer had a *trans* arrangement of the bulky groups placed at positions 2 and 4 of the imidazolidinone ring.

Histidine, is an essential amino acid having an imidazole ring in their structure. The tautomeric state of the neutral imidazole side chain, the protonation state and the existence of hydrogen bonds are some of the properties that can be monitorised by 2D NMR techniques, concerning the imidazole ring chemical shifts [130,131]. Sequence-specific assignments of histidine in labelled proteins and in smaller histidine-containing compounds was achieved using modern techniques as TOCSY and TROSY-based pulse sequences [131,132].



121a R^1 = H, R^2 = OH, R^3 = H, R^4 = OH
121b R^1 = H, R^2 = OH, R^3 = OH, R^4 = H
121c R^1 = OH, R^2 = H, R^3 = H, R^4 = OH

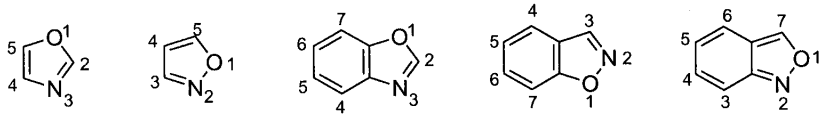
The imidazole core can appear as a substituent in a complex molecule, can be derivatised in several positions of the imidazole ring and often appears as fused to other systems. NMR spectroscopy can provide specific information about the position where

the imidazole binds to a complex molecule, the kind and positions of the substituents and, what are the positions of the imidazole moiety fused to the remain structure.

5.4. Oxazoles, isoxazoles and its benzoderivatives

Azoles are a class of five-membered heteroaromatic compounds derived from cyclopentadiene. If one carbon is replaced by a nitrogen atom and another by a oxygen atom, we have two possible structures: 1,3-oxazole, also known as oxazole **122**, and 1,2-oxazole, also known as isoxazole **123.** Normally in literature concerning the chemistry of oxazole **122** and isoxazole **123** also are referred their parent benzoderivatives, benzoxazole **124** and the two possible isomers of benzisoxazole, 1,2-benzisoxazole **125** and 2,1-benzisoxazole **126** which are sometimes designated as indoxazene and anthranil, respectively [133].

Brown and Ghosh have published ^{1}H NMR data for a variety of substituted oxazoles and for the parent oxazole **122** (Table 20) [134]. This study of substituent effect on the proton shifts of oxazole allowed concluding that electron-releasing substituents cause upfield shift and electron-withdrawing substituents cause downfield shift. Wasylishen *et al*. have published identical data for isoxazole **123** and some of their alkyl derivatives (Table 20) [135]. ^{13}C NMR chemical shifts of these unsubstitued azoles are present in Table 22 [10,133].



122 **123** **124** **125** **126**

Table 20. ^{1}H NMR spectra data (δ, ppm) of oxazole and isoxazole derivatives.

Compound	H-2	H-3	H-4	H-5	Solvent
oxazole	7.95	-	7.09	7.65	CCl_4
oxazole	8.25	-	7.25	7.97	D_2O
2-CH_3-oxazole	-	-	7.17	7.78	D_2O
4-CH_3-oxazole	8.10	-	-	7.59	D_2O
2-CH_3,4-CH_3-oxazole	-	-	-	7.49	D_2O
2-CH_3,5-CH_3-oxazole	-	-	6.64	-	D_2O
4-CO_2H-oxazole	8.54	-	-	8.27	D_2O
4-$CO_2C_2H_5$-oxazole	8.66	-	-	8.40	D_2O
4-CO_2NH_2-oxazole	8.47	-	-	8.26	D_2O
2-CH_3,4-$CO_2C_2H_5$-oxazole	-	-	-	8.42	D_2O
4-$CO_2C_2H_5$-oxazole	8.20	-	-	7.91	$CDCl_3$
2-CH_3,4-$CO_2C_2H_5$-oxazole	-	-	-	8.17	$CDCl_3$
2-C_6H_5-oxazole	-	-	7.26	7.74	$CDCl_3$
4-C_6H_5-oxazole	7.91	-	-	7.91	$CDCl_3$
5-C_6H_5-oxazole	7.90	-	7.34	-	$CDCl_3$
5-*p*-Cl-C_6H_5-oxazole	7.94	-	7.36	-	$CDCl_3$
5-*p*-OCH_3-C_6H_5-oxazole	7.87	-	7.25	-	$CDCl_3$
2-$CO_2C_2H_5$,5-C_6H_5-oxazole	-	-	7.50	-	$CDCl_3$
2-$CO_2C_2H_5$,5-*p*-Cl-C_6H_5-oxazole	-	-	7.54	-	$CDCl_3$
2-$CO_2C_2H_5$,5-*p*-OCH_3-C_6H_5-oxazole	-	-	7.41	-	$CDCl_3$
2-$CO_2C_2H_5$,5-*p*-NO_2-C_6H_5-oxazole	-	-	8.01	-	$CDCl_3$
isoxazole	-	8.15	6.28	8.39	CS_2
5-CH_3-isoxazole	-	8.02	5.93	-	CS_2
3-CH_3,5-CH_3-isoxazole	-	-	5.73	-	CS_2
4-CH_3,5-CH_3-isoxazole	-	7.95	-	-	CS_2

The ^{1}H NMR data of benzoxazole **124** [133], 1,2-benzisoxazole **125** [136] and 2,1-benzisoxazole **126** [136] are collected in Table 21 and a complete description of the chemical shifts of ^{13}C NMR spectra was found only for benzoxazole **124** (Table 22).

Table 21. ^{1}H NMR chemical shifts (δ, ppm) of the benzoxazole and benzisoxazoles.

Compound	H-2	H-3	H-4	H-5	H-6	H-7	Solvent
benzoxazole	7.46	-	7.67	7.80	7.79	7.73	CD_3OD
1,2-benzisoxazole	-	8.85	7.69	7.25	7.47	7.58	neat
2,1-benzisoxazole	-	7.70	7.28	6.96	7.57	8.83	neat

Table 22. ^{13}C NMR chemical shifts (δ, ppm) of oxazole, isoxazole and benzoxazole.

Compound	C-2	C-3	C-4	C-5	C-6	C-7	C-3a	C-7a	Solvent
oxazole	150.6	-	125.4	138.1	-	-	-	-	$CDCl_3$
isoxazole	-	149.1	103.7	157.9	-	-	-	-	$CDCl_3$
benzoxazole	152.6		120.5	125.4	124.4	110.8	140.1	150.5	$CDCl_3$

In these earlier studies the azoles derivatives were simple ones, usually alkylated and the authors were concerned in comparing different azoles [137,138] or in studying solvent-induced shifts [139]. The first publication, to our knowledge, concerning the use of NMR technique to establish structures a little bit more complicated oxazoles is the work of Buchan and Turner [140] where they use ^{13}C NMR to determine the substitution pattern of isoxazole derivatives. With their simple methodology they were able to distinguish isomeric structures.

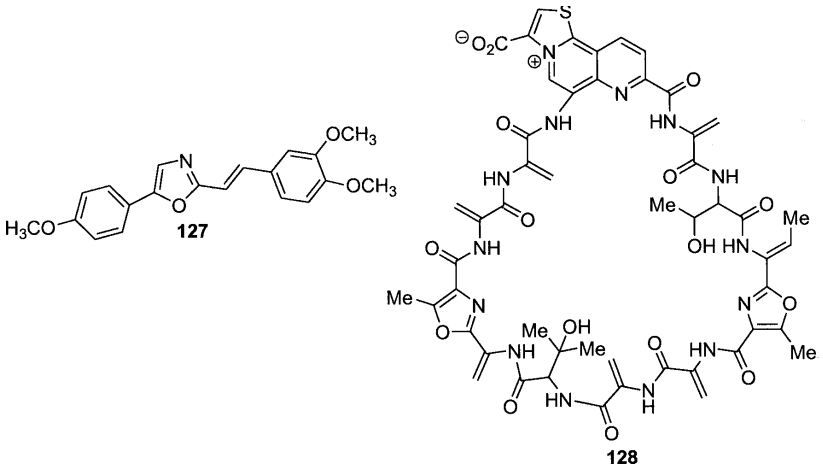
The interest in oxazoles is connected with the Second World War, when was made an effort to synthesise penicillin thinking at that time that penicillin has an oxazole nucleus. Obviously several strategies were developed to synthesise new oxazole derivatives [141]. Naturally occurring oxazoles were considered rare until the 1980s when a number of unprecedented natural compounds containing oxazole nucleus were isolated. Since 1986 there has been a dramatic increase in the number of examples and structural complexity of bioactive natural products containing an oxazole ring. Marine organisms are rich sources of these novel metabolites [142-146].

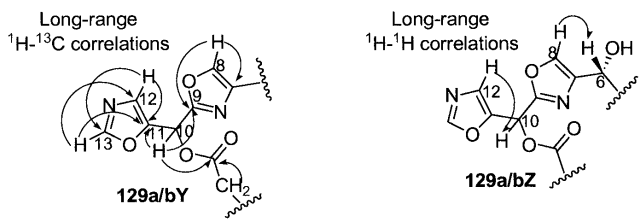
The prevalence of oxazoles and isoxazoles cores in biologically active molecules [96,147,148] also stimulated the need for methodologies towards their syntheses. These two aspects stimulate also efficient ways to perform their unequivocal characterization. The ^{1}H and ^{13}C NMR spectra remain the primary tools but some complex structures require 2D NMR experiments. These 2D NMR techniques start to be used in the characterisation of natural derivatives, mainly to establish the structure of the substituents and the substitution pattern of the oxazole nucleus [149-152] but also to determine if there are more than one nucleus and how they are linked [153-157].

The oxazole is not found in nature in the free state, being the oxazole ring of all oxazole-containing natural products substituted, some are relatively simple 2,5-disubstituted oxazoles as is the case of annuloline **127**, the first oxazole natural product isolated, characterisated and synthesised [158]. Probably the most complex of the naturally occurring substances containing an oxazole ring is the antibiotic bernimamycin

A **128**, which present a 2,4-disubstituted pattern [133]. Obviously the advanced NMR techniques are a powerful tool in structural elucidation of the more complex ones.

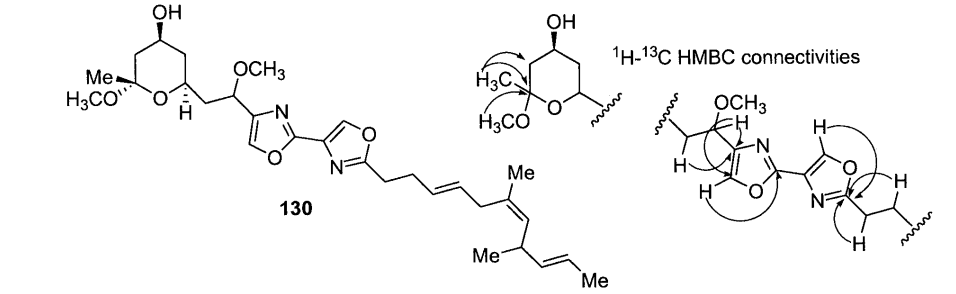
To our knowledge the first example of structural characterisation of oxazoles using 2D NMR spectroscopy is the work of Adamczeski *et al.* describing the spectroscopic data to support the structures of bengazoles A **129a** and B **129b**, a bis(oxazole)-type compound isolated from a marine sponge which exhibit anthelminthic activity [159]. As can be seen by simple structure analysis the difference of the two heterocyclic compounds is the skeleton of the R-oxycarbonyl group, which can be distinguished by the multiplicity in ^{1}H NMR spectra. The authors pointed out that they have evidences for the presence of the two oxazole rings, one monosubstituted and other disubstituted, from the characteristic NMR shifts and coupling constants $^{1}J_{CH}$ values (see structure **129a/b-X**). The establishment of the linkage between the oxazole rings and the position of the substituents was established from both of the long-range ^{1}H-^{13}C and ^{1}H-^{1}H COSY spectra (see structures 1**29a/b-Y** and **129a/b-Z**), which support the structure of these compounds.

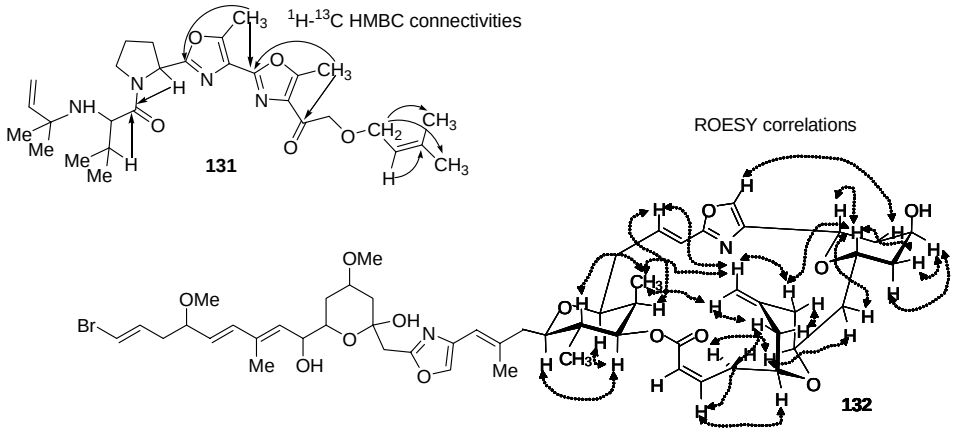




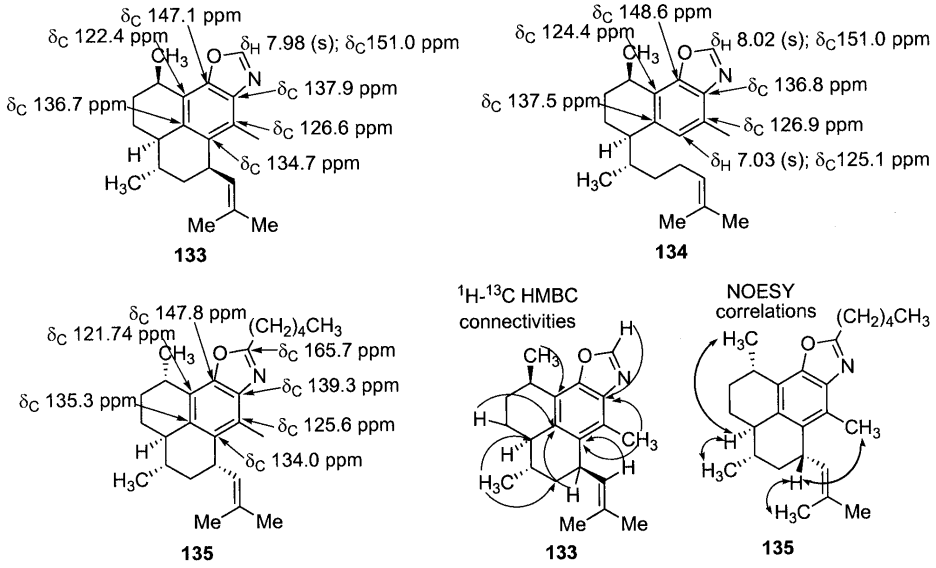
The structure of hennoxazole A **130**, a natural compound isolated from the sponge *Polyfibrospongia* sp. and that is active against herpes simplex virus type 1, was established by comparing their NMR spectra with those of known compounds (to prove the presence of two oxazole rings attached to each other) but also with some important connectivities found in its HMBC spectrum [160].

The structure of complex natural compounds having oxazole rings were established by a combination of several 2D NMR techniques [COSY, TOCSY (HOHAHA), HMQC, HMBC] [161-167]. As examples of the NMR experiments utility to assign the structure of these compounds it is shown the connectivities found in the ^{1}H-^{13}C HMBC spectrum of muscoride A **131**, a new oxazole peptide alkaloid isolated from *Nostoc muscorum* [161], and the ROESY correlations of a new cytostatic macrolide, isolated from the marine sponge *Phorbas* sp., which establish the relative stereochemistry about the macrolide ring and the solution conformation of the molecule **132** [162].

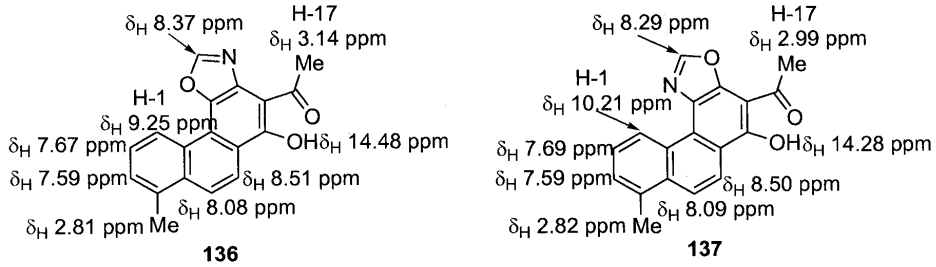




Three natural benzoxazole alkaloids pseudopteroxazole **133**, *seco*-pseudopteroxazole **134** and homopseudopteroxazole **135** have been isolated from *Pseudopterogorgia elisabethae*. Their structures have been established by using 2D NMR experiments; where the NOESY spectra were used to assign the stereochemistry of the cyclohexane rings and the ^{1}H-^{13}C HMBC spectra were used to confirm the substitution pattern of the benzoxazole ring [168,169].

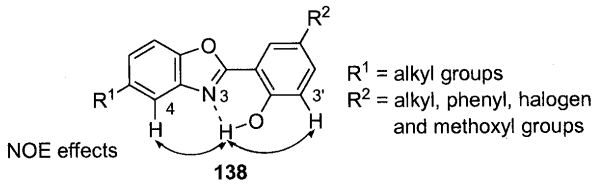


Several benzoxazole derivatives have been isolated from the genus Salvia and fully characterise by using 2D NMR experiments [170,171]. The most recent natural benzoxazole derivatives salviamine B **136** and isosalviamine E **137** were isolated from *Salvia yunnanensis*, and their structures were established by using COSY, HMBC, HMQC and NOESY spectra [171]. The detailed analysis of the ^{1}H NMR data of salviamines and isosalviamines allowed the development a method to distinguish these two isomers. Thus the H-1 resonance shifts downfield to δ 10.21 ppm and H-17 shifts up field to δ 2.99 ppm in the *iso* series, in comparison to the salviamine series, where the H-1 and H-17 signals occur at δ 9.25 and 3.14 ppm, respectively.



The important biological activities found for some natural benzoxazole derivatives stimulated their synthesis and the development or application of several NMR experiments to assign their structures [172-174]. For instance NOESY experiments were used to determine the orientation of the hydroxyl group in compound **138** and to suggest a strong intramolecular hydrogen bond of the hydroxyl proton with the nitrogen atom of the oxazole ring [174].

Katritsky *et al*. [175] published detailed NMR information of several synthetic benzisoxazoles and oxazoles derivatives based on gradient selected *g*HMQC, *g*HMBC and *g*HMQC-TOCSY trying to eliminate the inconsistent data previously published for those compounds and also to demonstrate the effectiveness of the *g*HMQC-TOCSY spectra to identify spin systems due to highly overlapping proton resonances. They showed that one can conclude on the presence of an oxazole vs isoxazole rings on the basis of the magnitude of the heteronuclear one-bond coupling constants ($^1J_{C8\text{-}H8}$ = 231 Hz for benzoxazoles and 210 Hz for benzisoxazoles).



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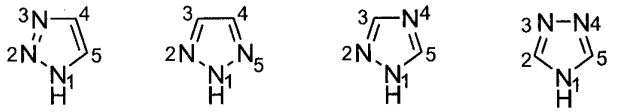
5.5. Triazoles and benzotriazoles

Triazoles are a class of aromatic five-membered heterocyclic compounds having three nitrogen atoms in the ring. As a consequence there are four possible systems to represent this azoles, 1,2,3-triazole (1*H*-1,2,3-triazole) **139**, 1,2,5-triazole (2*H*-1,2,3-triazole) **140**, 1,2,4-triazole (1*H*-1,2,4-triazole) **141** and 1,3,4-triazole (4*H*-1,2,4-triazole) **142**. As can be noticed 1,2,3-triazole **139** and 1,2,5-triazole **140** are tautomers as well as 1,2,4-triazole **141** and 1,3,4-triazole **142**. In order to distinguish the 1,2,3-triazoles from 1,2,4-triazoles they are named in literature respectively as υ-triazoles and s-triazoles. Probably one of the first systematic studies of ^{1}H NMR spectra of these azoles and their simple derivatives is the work of Barlin and Baterham [112]; their chemical shift data has small differences with those indicated by Wamhoff [176] and Polya [177]. In 1968 Creagh and Truitt studied the tautomerism of 1,2,4-triazole and they conclude that NMR proton chemical shift depend not only on the solvent used but also on its purity and on the temperature used during the acquisition of the spectra [178]. Some of those chemical shifts are recorded in Table 23, as well as the chemical shifts of the *N*-methyl derivatives.



139 **140** **141** **142**

Table 23. ^{1}H NMR spectral data (δ, ppm) of triazole derivatives.

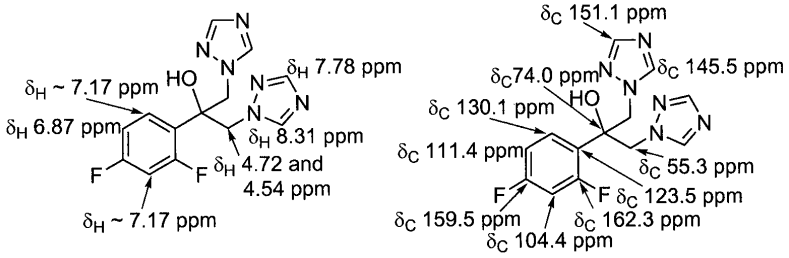
Compound	Solvent	H-3	H-4	H-5	NH
1,2,3-triazole	DMSO-d_6	-	7.91	7.91	13.50
1,2,4-triazole	DMSO-d_6	8.25	-	8.25	13.90
1-methyl-1,2,3-triazole	$CDCl_3$	-	7.74	7.49	-
2-methyl-1,2,3-triazole	$CDCl_3$	-	7.57	7.57	-
1-methyl-1,2,4-triazole	$CDCl_3$	7.94	-	8.09	-
4-methyl-1,2,4-triazole	$CDCl_3$	8.23	-	8.23	

Weigert and Roberts in their study on the ^{13}C NMR of five-membered aromatic heterocyclic compounds referred, for the first time, the chemical shifts of 1,2,3- and 1,2,4-triazoles [179]. Later Elguero *et al*. presented a systematic study of the ^{13}C NMR of five-membered nitrogen heterocycles and naturally occurring 1,2,3- and 1,2,4-triazoles were included [178]. Table 24 summarize some of those ^{13}C NMR chemical shifts found for simple triazoles.

Table 24. ^{13}C NMR spectral data (δ, ppm) of triazole derivatives.

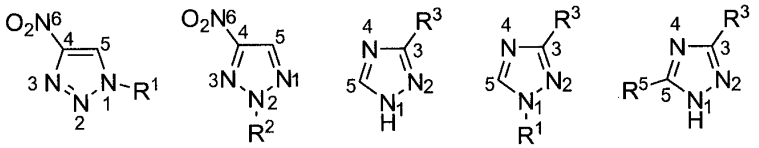
Compound	C-3	C-4	C-5	Solvent
1,2,3-triazole	-	130.3	130.3	DMSO-d_6
1,2,4-triazole	146.2	-	146.2	DMSO-d_6
1-methyl-1,2,3-triazole	-	132.6	124.8	DMSO-d_6
2-methyl-1,2,3-triazole	-	133.2	133.2	DMSO-d_6
1-methyl-1,2,4-triazole	150.4	-	143.5	DMSO-d_6
4-methyl-1,2,4-triazole	143.1	-	143.1	DMSO-d_6

Triazole derivatives have been reported to possess interesting biological activities such as anti-inflammatory [181] and antimicrobial [182] activities, which are an incentive to fully characterise these type of azoles. For example the authenticity of the fluconazole **143** structure, a well-known antifungal agent since 1982, was assigned by NMR only in 1996 [183].



143

The mobile protons at the ring nitrogen atoms of 1,2,3- and 1,2,4-triazoles are responsible for the tautomerism and consequently isomerism after substitution and constitutes a problem in the structure analysis of these compounds. Licht *et al*. investigated the tautomer equilibrium of several nitrated triazoles and bistriazoles **144-148** and they showed that the ^{1}H and ^{13}C NMR data are controlled by the type of substituents in the triazole ring, and the ^{15}N NMR signals are controlled by the pyridine or pyrrole character of the nitrogen atoms (Tables 25 and 26) [184]. Essential information is obtained from the coupling behaviour of triazoles: heteronuclear coupling constants $J_{C\text{-}H}$ of the 1,2,3-triazole structures ($^{1}J_{C5\text{-}H5}$ 206-210 Hz and $^{2}J_{C4\text{-}H5}$ 8-9 Hz) are smaller than those found for 1,2,4-triazole structures ($^{1}J_{C3\text{-}H3}$ 215-234 Hz and $^{3}J_{C5\text{-}H3}$ 13-16 Hz). For protons attached to carbons it was found $^{2}J_{N\text{-}H}$ coupling constants of 7-8 Hz for pyrrole-like nitrogen atoms and of 11-13 Hz for pyridine-like nitrogen atoms. $^{3}J_{N\text{-}H}$ is only observed for 1,2,3-triazoles ($^{3}J_{N2\text{-}H5}$ 11-12 Hz) although the same atom sequence N-N-C-H exists in 1,2,4-triazoles.



144 **145** **146** **147** **148**

Table 25. NMR data (δ, ppm) of 1,2,3-triazole structures **144** and **145**.

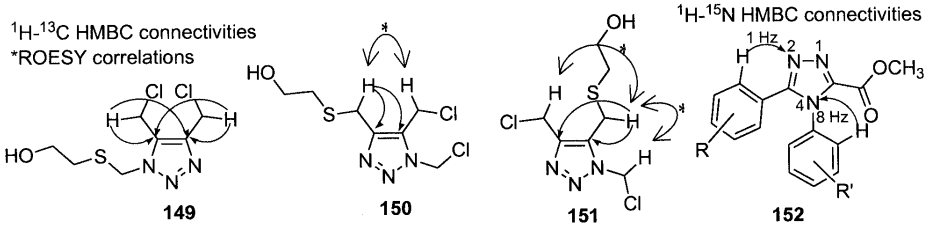
Structure	H-5	C-4	N-1	N-2	N-3	N-6	Substituent
144	9.1	154	- 106	- 52	- 44	- 25	R^1 = H
144	10.2	157	- 135	- 33	- 26	- 19	R^1 = dinitrophenyl
145	9.2	156	- 44	- 139	- 59	- 30	R^2 = dinitrophenyl
145	9.0	155	- 44	- 134	- 59	- 27	R^2 = triazolyl
145	9.0	156	- 45	- 138	- 60	- 28	R^2 = *C*-nitrotriazolyl
145	9.2	156	- 44	- 139	- 59	- 28	R^2 = *N*-nitrotriazolyl
145	9.2	158	- 43	- 140	- 58	- 27	R^2 = dinitrophenyltriazolyl

Table 26. NMR data (δ, ppm) of 1,2,4-triazole structures **146**, **147** and **148**.

Structure	H-1	H-5	C-3	C-5	N-1	N-2	N-4	N-3	Substituent
146	14.1	8.9	163	146	- 161	- 93	- 139	- 26	$R^3 = NO_2$
146	14.8	8.8	157	146	- 166	- 109	- 145		R^3 = nitrotriazolyl
147	-	10.1	153	144	- 106	- 119	- 140		$R^1 = NO_2$, R^3 = nitrotriazolyl
147	-	9.6	160	151	- 170	- 105	- 137		R^1 = dinitrophenyl, R^3 = nitrotriazolyl
148	13.2	6.9	161	158	- 200	- 108	- 179	- 24	$R^3 = NO_2$, $R^5 = NH_2$
148	15.2	-	151	160	- 101	- 138	- 156		R^3 = nitrotriazolyl, $R^5 = NO_2$

NOE effects have been used to prove regiospecific substitution of several triazoles or to establish the conformation of triazole oligomers, but in some cases is combined with other 2D NMR experiments [185-187]. Al-Masoudi *et al.* have been interested in study several triazole-type compounds because they exhibit anticancer activity [188], and recently they published the synthesis of new derivatives where they used ROESY spectra to confirm the position of the chloromethyl groups in the triazole moiety of **149**, **150** and **151** [189]. Meanwhile the *C*- or *N*-site attachment of the thioether group was concluded mostly from HMQC and HMBC experiments. In the case of triazole **149** the chemical shift of the S-(CH_2)-N group appeared at higher field (δ 5.37 ppm) in comparison to S-(CH_2)-C groups of triazoles **150** and **151** (δ 3.38 and 3.34 ppm, respectively).

The application ^{1}H-^{15}N HMBC experiment have been used in the characterization of 1,2,4-triazole derivatives [6]. An experiment with a long-range coupling constant optimised for 8 Hz allowed to assign N-4 and a coupling constant of 1 Hz was necessary to observe a four bond correlation between H-*ortho* of the 3-aryl ring to N-2 of 1,2,4-triazole **152**.



In the family of triazoles it is worthy mention their benzo derivatives 1,2,3-benzotriazole (1*H*-benzo[*d*][1,2,3]triazole) **153** and 2,1,3-benzotriazole (2*H*-benzo[*d*][1,2,3]triazole) **154**. In fact several benzotriazoles have shown potential biological activities such as antimycobacterial [190], antitumor [191] and anti-inflammatory [192] activities, just to mention a few. As can be seen by structures **153** and **154** these triazoles are also tautomeric forms. The study of the annular tautomerism of benzotriazole and its acetone adducts in the +30/-90ºC temperature range allowed to determine the corresponding prototropy barrier (10.8 kcal/mol at 294K) [193].

Katritzky *et al.* have used 2D NMR experiments to characterize dimers of benzotriazoles and for studies on the conformation of 2-(benzotriazol-1-yl)-substituted tetrahydrofurans [194,195].

5.6. Tetrazoles

Tetrazoles are a class of aromatic five-membered heterocyclic compounds having four nitrogen atoms in the ring. Tetrazoles have been studied by NMR spectroscopy, being probably one of the first studies the analyses of the substitution effect in the NMR spectra of tetrazoles [196]. One of the most important aspects of the proton and carbon NMR spectra of the tetrazole ring deals with the fact that this azole system is represented by two tautomeric forms, 1,2,3,4-tetrazole (1*H*-1,2,3,4-tetrazole) **155** and 1,2,3,5-tetrazole (2*H*-1,2,3,4-tetrazole) **156** which present a singlet, at δ 9.5 ppm corresponding to proton H-5 in ^{1}H NMR spectra [196] and a signal at δ 144.2 ppm corresponding to carbon C-5 in ^{13}C NMR [180], when the spectra were obtained in D_2O.

A systematic study of this ring proton NMR spectra involving the effects of small substituent groups in the chemical shifts and how they can be used to distinguish between the two isomeric systems 1-methyl-1,2,3,4-tetrazole **157** and 2-methyl-1,2,3,4-tetrazole **158** (1-methyl-1,2,3,5-tetrazole) (Table 27) have been published [112]. A long-range coupling was observed over four bonds between the H-5 and 1-NCH^3 protons (*J* 0.45 Hz) for structure **157** but not for the structure **158**, where there is a five bond separation between the H-4 and 1-NCH_3 protons. These two methyltetrazole isomers were also object of study by ^{13}C NMR spectroscopy [179] but the most systematic study on this subject was the work of Elguero *et al.* (Table 28) [180].

Table 27. $^{1}H^{a}$ and $^{13}C^{b}$ NMR spectral data (δ, ppm) of methyltetrazoles.

Compound	H-5	1-NCH_3	2-NCH_3	C-5	1-NCH_3	2-NCH_3
1-methyl-1,2,3,4-tetrazole	8.98	4.27	-	144.2	33.7	-
2-methyl-1,2,3,4-tetrazole	8.60	-	4.46	153.4	-	38.8

[a]Spectra measured in $CDCl_3$. [b]Spectra measured in dioxane

These first studies indicate that one of the most important aspects of the structural characterisation of tetrazoles is the differentiation between structural isomers. For that reason *N*-alkylated tetrazoles and its 5-substituted derivatives have been the focus of many investigations [112,180].

Sveshnikov and Nelson published the unequivocal assignment of ^{13}C and ^{15}N NMR chemical shifts for isomeric 1,5- **159a,b** and 2,5-disubstituted **160a,b** tetrazoles using long-range ^{1}H-^{13}C, ^{1}H-^{15}N coupling constants together with $^{1}J_{N\text{-}C}$ [197]. $^{3}J_{C5\text{-}NMe}$ = 2.1-2.8 Hz for 2,5-isomers are bigger than those of 1,5-isomers $^{3}J_{C5\text{-}NMe}$ = 0.75 Hz (only detected for one derivative). The resonance of C-5 of 2,5-isomers resonates at Δδ 9.2-12.2 ppm to higher frequency than those of the 1,5-isomers. $^{1}J_{C5\text{-}N1}$ are smaller in absolute values than the corresponding $^{1}J_{C5\text{-}N2}$ by 1.9-2.1 Hz. The ^{15}N NMR chemical shifts and the absolute values of the long-range $J_{N\text{-}H}$ coupling constants, which can be used in the structural characterisation of these compounds, are presented in Table 28.

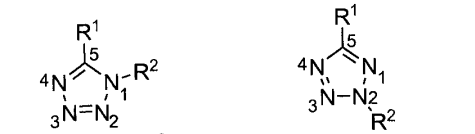
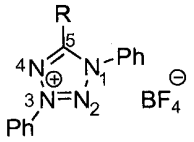


Table 28. ^{15}N NMR data (δ, ppm; *J*, Hz) of tetrazole derivatives.

	N-1	N-2	N-3	N-4
159a	- 151.35 d, ^{2}J = 9.3 q, ^{2}J = 2.0	- 8.29 m	14.29 d, ^{3}J = 3.0	- 48.64 d, ^{2}J = 12.1
159b	- 153.67 m	- 7.49 q, ^{3}J = 1.9	10.19 s	- 52.06 q, ^{3}J = 1.9
160a	- 75.81 m	- 103.62 q, ^{2}J = 2.3	2.03 q, ^{3}J = 1.5	- 48.00 q, ^{3}J = 1.1
160b	- 76.57 d, ^{2}J = 15.3	- 71.52 m	- 2.18 s	- 48.91 d, ^{2}J = 12.6

Jaźwiński *et al*. investigate mesoionic compounds containing a tetrazolium ring (*e.g.* **161a-c**) [198] and they used essentially ^{15}N NMR data to characterize them (Table 29). ^{1}H-^{15}N HMBC cross-polarization technique were used to identify N-1 and N-3 resonances, due to the connectivities found with the *ortho*-protons of the adjacent phenyl rings. The half-width of the ^{14}N NMR signals can be used to assign the presence of positive charges on nitrogen atoms. These types of signals are rather broad due to rapid ^{14}N quadrupolar relaxation and tend to be relatively narrow when the nitrogen atom in questions bears a formal charge. N-3 signal of the studied compounds **161a-c** is relatively sharp suggesting that N-3 has a formal positive charge. In the case of **161a** N-1 is also a sharp signal suggesting that some positive charge is located in N-1 and N-3.



161a R = H
161b R = Cl
161c R = N_3

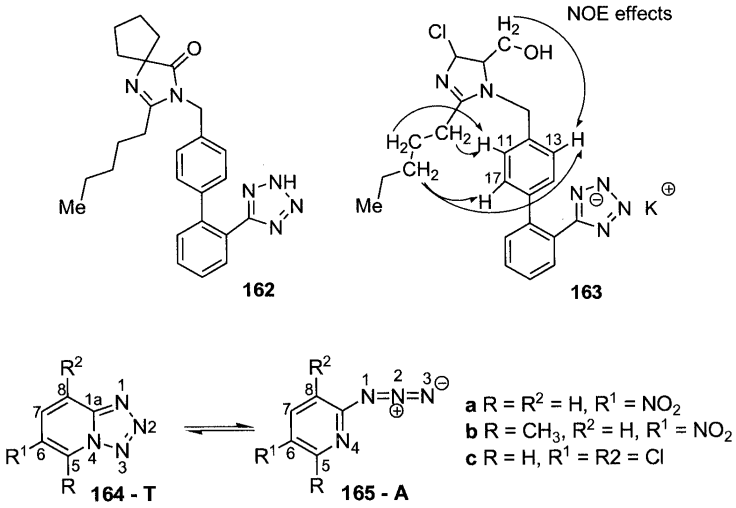
Table 29. ^{15}N NMR chemical shifts (δ, ppm with respect to CH_3NO_2 signal at 0 ppm) of some 1,3-diphenyl-1,2,3,4-tetrazolium derivatives.

Compound	N-1	N-2	N-3	N-4
161a	- 129.8 (550)[a]	- 33.5	- 92.4 (550)[a]	- 73.3
161b	- 132.7	- 22.4	- 90.7 (260)[a]	- 73.3
161c	- 153.1	- 31.8	- 97.5[b] (560)[a]	- 96.6[b]

[a] ^{14}N signal half-with (Hz) in parenthesis. All ^{14}N measurements were taken on acetone-d_6 solutions. [b] The assignment can be opposite

The 5(4)-biphenyltetrazole sub-unit is common to a number of angiotensin II inhibitors [199] and for that reason several authors are interested in the study this kind of compounds. Bauer *et al.* studied the characterisation of irbesartan **162**, a novel anti-hypertensive agent, by using a series of 1D and 2D NMR experiments to assign their structure. They studied the prototropic tautomerism of irbesartan **162** by solid-state NMR and conclude that this molecule is a rare example of desmotropic behaviour, whereby the isolated crystal forms (two forms designated as A and B) are stable in solid state yet related through a tautomeric equilibrium in solution [200]. Mavromoustakos *et al.* described the structure elucidation of losartan **163** [201], a drug used against hypertension [199]. This work was essentially based on NOESY spectroscopy coupled with theoretical calculations to assign the losartan **163** structure but also to understand the mode of action of this new drug.

It is worth mention the characterisation of tetrazolo[1,5-*a*]pyridine derivatives **164** because they present a tetrazole ring fused with another aromatic heterocyclic ring but also because they are being used as precursors of 1,3-diazepines, potential anti-AIDS drugs [202]. Cmoch *et al.* have studied this type of tetrazoles in order to characterize them



but also to understand their tautomeric equilibrium with the azide form **165** [7, 203-205]. Small differences have been found in the ^{1}H NMR of both forms, a relative large differences in the ^{13}C NMR data but especially big differences have been observed in the ^{15}N NMR chemical shifts (Tables 30 and 31).

Table 30. ^{13}C chemical shifts (δ, ppm) for the azide (A) and tetrazole (T) forms of some compounds **164-165**.

Compound	C-1a		C-5		C-6		C-7		C-8	
	A	T	A	T	A	T	A	T	A	T
a	160.0	149.1	145.5	125.1	141.0	139.8	134.0	126.5	113.8	116.3
b	157.3	148.3	155.1	138.3	141.2	139.3	135.8	128.1	111.8	113.1
c	150.0	146.8	145.3	122.2	123.2	121.1	138.1	132.3	127.5	124.9

Table 31. ^{15}N chemical shifts (δ, ppm) for the azide (A) and tetrazole (T) forms of some compounds **164-165**.

Compound	N-1		N-2		N-3		N-4		NO_2	
	A	T	A	T	A	T	A	T	A	T
a	- 275.1	- 66.2	- 144.6	28.6	- 140.7	- 31.8	- 96.3	- 133.8	- 23.4[a]	- 17.1[a]
b	- 270.0	- 65.9	- 144.0	25.8	- 141.9	- 28.5	- 91.5	- 128.7	- 12.3	- 18.3
c	- 276.1	- 66.3	- 143.3	20.8	- 141.4	- 28.0	- 90.8	- 129.8		

[a]Assignments may be reversed

6. Six-membered heterocyclic compounds

6.1. Pyridines and piperidines

Pyridine **166** is the simplest heterocycle of the azine type. Is similar to benzene ring where a CH group is replaced by a nitrogen atom. The nomenclature of substituted pyridines is presented as α-, β-, γ- or as numbers 2-, 3-, 4-. Methylpyridines are known as picolines, dimethylpyridines as lutidines and 2,4,6-trimethylpyridines as collidines.

Pyridone type compounds, namely 2-pyridone [pyridin-2(1*H*)-one] **167** and 4-pyridone [pyridin-4(1*H*)-one] **168**, are in equilibrium with their corresponding tautomeric forms, 2- and 4-hydroxypyridines, respectively. Structural investigation of the pyridones indicates that the keto form is favoured in solid state and also in most solvents by solvation, except in light petroleum at higher dilution [10].

The saturated cycle piperidine **169** has the same nomenclature than pyridine and the molecule exists in a chair from. The N-H and N-CH_3 in piperidine **169** and *N*-methylpiperidine **170**, respectively, prefers the equatorial position in chair conformation [10].

Table 32 presents the ^{1}H NMR chemical shifts for pyridine derivatives in $CDCl_3$. These data confirm pyridine as a delocalized 6π-heteroarene with a diamagnetic ring current; the individual ring positions have different π-electron densities due to anisotropic effect of the nitrogen. The proton chemical shifts of pyridine show that the α-position is the most deshielded one. Pyridine can be described mesomerically by canonical structure in which the π-electron density is lowest on the 2-, 4- and 6-atoms, and highest on the *N*-atom. ^{1}H NMR data of pyridones **167** and **168** confirm that they also must be regarded as π-delocalized systems with aromatic character [206]. Similar to what is observed in ^{1}H NMR spectra, the ^{13}C NMR data of pyridine **166** and pyridones **167** and **168** confirm that they must be regarded as π-delocalized systems with aromatic character (Table 32) [10].

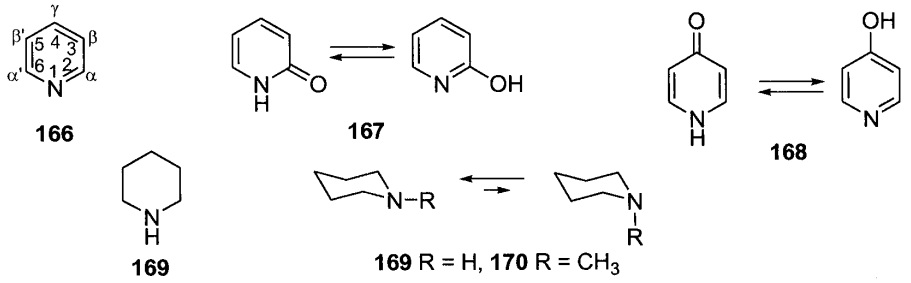


Table 32. ^{1}H and ^{13}C NMR data (δ, ppm) of pyridine derivatives.

	pyridine	2-pyridone	4-pyridone	piperidine
H-2	8.59	---	7.98	2.77
H-3	7.38	6.60	6.63	1.52
H-4	7.35	7.30	---	1.52
H-5	7.38	6.60	6.63	1.52
H-6	8.59	7.23	7.98	2.77
C-2	149.8	162.3	139.8	47.5
C-3	123.6	119.8	115.9	27.2
C-4	135.7	140.8	175.7	25.5
C-5	123.6	104.8	115.9	27.2
C-6	149.8	135.2	139.8	47.5

^{1}H and ^{13}C NMR data of pyridine derivatives are very susceptible to structure variation and sensitive to solvent and dilution effects (Table 33) [207,208]. The effects can arise from the aromatic ring current, the perturbation of the π- and σ-electrons by the nitrogen atom, the magnetic anisotropy of the nitrogen atom, and the electrostatic influence of the lone pair dipole. Protonation of pyridine results in shielding of the α carbon atoms and deshielding of the β and γ carbon atoms, and these effects can be accounted in terms of additivity parameters (Table 33). The upfield protonation parameter of γ carbon has been assigned to changes in the C-N bond order, while the β and γ parameters have been assigned to change polarization effects. The parameters are highly reproducible for monoprotonation but deviate significantly from additivity for diprotonated heterocycles [209,210].

^{13}C NMR chemical shifts data for a number of monosubstituted pyridines are given in table 34 [211,212]. As well in ring protons, the ring carbon atoms in α-positions suffers the most heavily deshielding effect, those at γ-position to the nitrogen are also

Table 33. ^{13}C NMR chemical shifts (δ, ppm) of pyridine in various solvents [208].

Solvent	C-2	C-3	C-4
CCl_4	149.7	123.2	135.1
$CDCl_3$	149.9	123.8	136.0
EtOH (95 %)	150.1	125.1	137.7
$(CF_3)_2CHOH$	148.9	126.7	140.7
CF_3CO_2H	141.9	128.5	148.6
H_2SO_4	142.6	129.4	149.7

Table 34. ^{13}C NMR chemical shifts (δ, ppm) of monosubstituted pyridines[a].

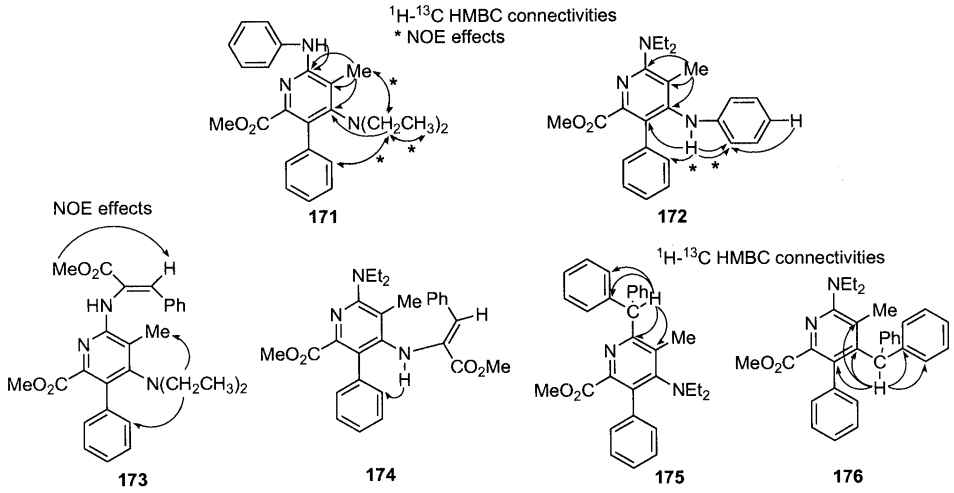
Substituent position	Substituent	C-2	C-3	C-4	C-5	C-6
2	Br	142.9	129.0	139.5	123.7	151.0
	CHO	153.1	121.6	137.5	128.3	150.3
	CN	133.8	129.2	137.9	127.8	151.5
	COMe	153.9	121.4	136.9	127.5	149.3
	Me	158.7	123.5	136.1	120.8	149.5
	NH_2[b]	160.9	109.5	138.5	113.6	148.7
	OH[b,c]	162.3	119.8	140.8	104.8	135.2
	OMe[b]	163.1	110.5	138.7	116.7	146.6
3	Br	151.7	121.6	139.1	125.4	148.7
	CHO	152.0	132.1	136.2	124.8	155.0
	CN	153.2	110.5	140.6	124.8	153.8
	COMe	150.1	123.9	132.5	121.5	153.8
	Me	150.9	133.1	136.4	123.4	147.3
	NH_2[b]	137.7	145.7	122.0	125.1	138.8
	OH[b]	137.8	153.5	121.4	123.8	140.0
	OMe[b]	137.3	155.2	120.0	123.8	141.4
4	Br	152.6	127.6	133.2	127.6	152.6
	CHO	151.3	123.6	141.7	123.6	151.3
	CN	151.7	126.4	120.5	126.4	151.7
	COMe	151.2	121.6	143.0	121.6	151.2
	Me	150.1	125.0	147.0	125.0	150.1
	NH_2[b]	148.5	110.4	155.8	110.4	148.5
	OH[b,c]	139.8	115.9	175.7	115.9	139.8
	OMe[b]	150.7	109.8	164.9	109.8	150.7

[a] Neat liquids, unless otherwise specified; [b] In DMSO-d_6; [c] Compound in N-H keto form

deshielded relative to benzene (δ 128.5 ppm) while those in a β-position are more benzene-like. Substituent effects follow the same general trend as substituted benzenes. The chemical shifts of the ring carbon atoms in a *para* position to the substituent are heavily affect relatively to the unsubstituted heterocycle and the ring carbon atoms in *meta* position are little affected by the presence of the substituent.

The α carbon signal of piperidine **169** is shifted downfield by about 20 ppm relative to cyclohexane resonance (δ 27.7 ppm), while in *N*-methylpiperidine it appears at δ 56.7 ppm. The β and γ carbon atoms of piperidine are upfield of the cyclohexane resonance by 0-7 ppm [213].

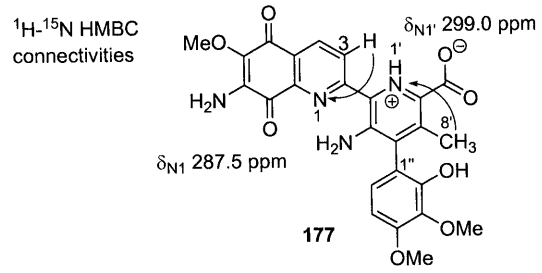
1D and 2D NMR spectroscopy have also been applied to the structural elucidation of pyridine derivatives. ^{1}H-^{13}C HMBC in combination with NOE difference spectra allowed the structural elucidation of pyridine regioisomers formed by the addition of ynamines to vinylcarbodiimides and ketenimines [214]. The differentiation of regioisomers **171** and **172** was based on the ^{1}H-^{13}C HMBC connectivities depicted in the corresponding structures and supported by the NOE effects produced on the *ortho* protons of both phenyl rings of the molecule when the NH signal is saturated for pyridine **172** and of those produced in both methyl groups and in the *ortho* protons of C-phenyl ring when the methylenic protons signal was saturated for pyridine **171**. The differentiation of compounds **173** from **174** and of **175** from **176** was achieved in the same way as described for **171** and **172**. The differentiation of the former pair is mainly based on the NOE effects while the latter one was mainly based on the ^{1}H-^{13}C HMBC connectivities.



The combination of these two techniques with ^{15}N NMR data was used for the full assignment of molecules containing one or more pyridine rings [215]. The ^{15}N NMR chemical shifts of streptonigrin **177,** an antitumour antibiotic which exhibits a broad spectrum activity against a range of human cancers, were assigned by ^{1}H-^{15}N HSQC and HMBC experiments (see structure **177**).

In phenazopyridine derivatives 1**78a-c** the ^{1}H-^{15}N HMBC (long-range coupling constants were optimised for 2 Hz) are of great useful for the assignment of their structures. For compound **178a** the pyridine ring nitrogen was assigned by the correlation with H-5 and 6-NH_2 while N-a and N-b nitrogen atoms were correlated with H-4 and H-*ortho* of the phenyl ring, respectively. In the case of the acetylated product **178c** the correlations between one amido signal and the H-5 signal allowed to assign it as the 6-amido group [216].

One-bond and long-range ^{1}H-^{13}C correlations are required to the unequivocal assignment of ^{13}C chemical shifts in many pyridine-containing compounds, such as metallocomplexes [217] or simple molecules as hydropyridones [218].



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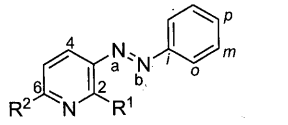
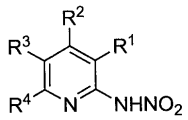
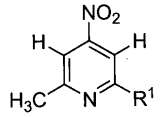


178a $R^1 = NH_2$, $R^2 = NH_2$
178b $R^1 = NH_2$, $R^2 = NHAc$
178c $R^1 = NHAc$, $R^2 = NHAc$

^{15}N NMR chemical shifts provided direct information for the amino-imino tautomerism for pyridine derivatives. For example, ^{15}N NMR resonance of pyridine nitrogen in 2-nitraminopyridine is $\Delta\delta \sim 20$-30 ppm shielded compared with 3-nitraminopyridine and 2-methylnitraminopyridine, where the imino tautomers could not exist [219]. These studies were extended to other 3-, 4-, 5- and 6-methyl-2-nitraminopyridines **179a-d** and $^{15}N_{ring}$ NMR resonances are strongly shielded (varying from δ -170.9 to – 197.0 ppm) when compared with pyridine (δ - 63.2 ppm). This effect is explained by the protonation of the pyridine nitrogen. The dominant imino tautomer is supported by the absence of the $^{15}N_{nitro}$ resonance in the ^{1}H-^{15}N HMBC spectra, since it is always situated four bonds from the nearest proton and also on the weak correlation of $^{15}N_{amino}$ signal with the pyridine ring protons. In the 4-nitro-substituted derivatives **180a-d** which have the amino forms, the $^{15}N_{ring}$ NMR resonance is about 40 ppm more deshielded than the 4-nitro-substituted with imino forms [220].



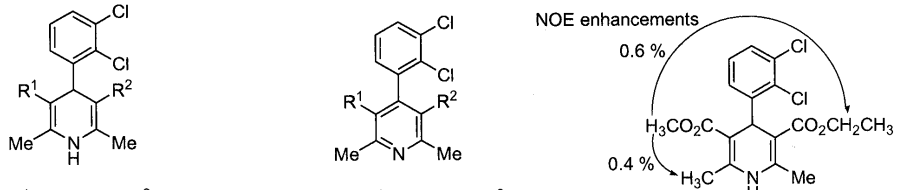
179a $R^1 = CH_3$, $R^2 = R^3 = R^4 = H$
179b $R^1 = H$, $R^2 = CH_3$, $R^3 = R^4 = H$
179c $R^1 = R^2 = H$, $R^3 = CH_3$, $R^4 = H$
179d $R^1 = R^2 = R^3 = H$, $R^4 = CH_3$



180a $R^1 = NHCH_3$
180b $R^1 = NHCH_2CH_3$
180c $R^1 = NCH_3NO_2$
180d $R^1 = NCH_2CH_3NO_2$

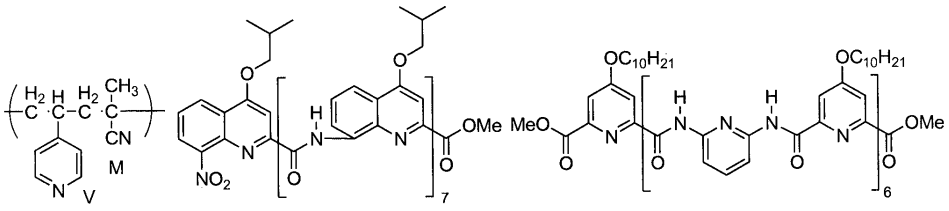
The study over symmetric pyridines is quite complex and the total ^{1}H and ^{13}C NMR assignments require the use of 2D NMR techniques. First studies in felodipine **181a**, a calcium channel blocker used for hypertension management, and its derivatives **181b-f**

could not distinguish the 2,6-dimethyl groups [221]. However, with the help of NOESY (NOE enhancements in the structure of **181a**) and also computer aided molecular modelling (CAMM), for spatial distances calculations between protons, these assignments have been accomplished [222].



181a $R^1 = CO_2CH_3$, $R^2 = CO_2CH_2CH_3$
181b $R^1 = CO_2CH_2CH_3$, $R^2 = CO_2CH_2CH_3$
181c $R^1 = CO_2CH_3$, $R^2 = CO_2CH_3$
181d $R^1 = CO_2CH_3$, $R^2 = CO_2CH_2CH_3$
181e $R^1 = CO_2CH_2CH_3$, $R^2 = CO_2CH_2CH_3$
181f $R^1 = CO_2CH_3$, $R^2 = CO_2CH_3$

NMR spectroscopy has become an important technique for the investigation of polymers microstructure [223,224]. Brar *et al.* have been studied the microstructure of 4-vinylpyridine-methacrylonitrile copolymers **182** (V/M) [225-227]. The copolymer composition was determined by quantitative $^{13}C\{^1H\}$-NMR spectrum. The highly complex and overlapped ^1H and $^{13}C\{^1H\}$ NMR spectra of these copolymers have been assigned completely with the help of ^{13}C NMR DEPT-135, HSQC and TOCSY experiments. The α-methyl region overlapped with methine and methylene region in the ^1H NMR spectra could be resolved with the help of 2D HSQC spectra. To understand the connectivity and confirm the various couplings in the copolymer chain, TOCSY experiments with small short mixing time (4 ms) were recorded [227]. This experiment allowed observing the three bond coupling between protons of different directly coupled groups in the V/M copolymer. NMR protocols developed for solving the solution structures of α-peptides have also been developed for solving the structure of complex pyridine- and quinoline-derived oligoamide foldamers **183** and **184** [228].

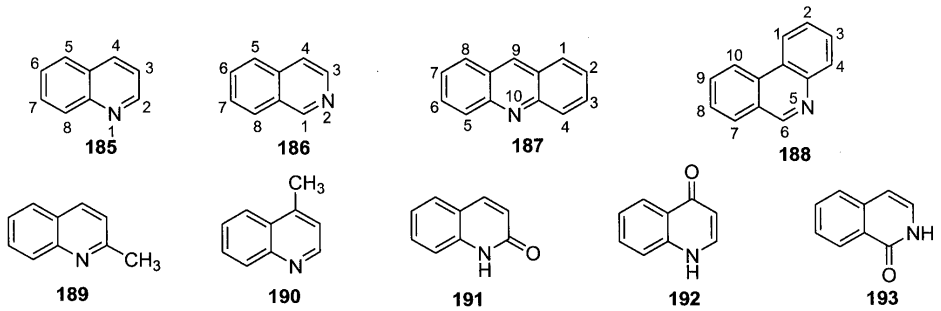


182 **183** **184**

6.2. Quinolines and isoquinolines

The topology of pyridine allows two neutral benzene-annulated products, quinoline (benzo[*b*]pyridine) **185** and isoquinoline (benzo[*c*]pyridine) **186**. They can be seen as naphthalene derivatives in which one of its α-CH groups is replaced by nitrogen in quinoline **185** and in case of isoquinoline **186** the β-CH group is replaced by nitrogen. Two neutral dibenzannulated products can also be derived from pyridine, the linear annulated acridine **187** and the angular annulated phenanthridine (dibenzo[*b*,*d*]pyridine) **188**.

2- And 4-methylquinoline (quinaldine **189** and lepidine **190**, respectively), 2-quinolone (carbostiril) **191** and 4-quinolone **192** are important derivatives of quinoline. 1-Isoquinolone [isoquinolin-1(2*H*)-one, isocarbostiril] **193** is the most important derivative of isoquinoline [10].



Quinoline **185** has structural and spectroscopy analogies to naphthalene while isoquinoline **186** is closely related with both naphthalene and pyridine (Table 35 presents the ^{1}H NMR chemical shifts of these compounds). In these compounds, the fusion of one aromatic ring to the pyridine unit, changes the electronic distribution in the pyridine portion of the resultant molecules **185** and **186**. The chemical shifts of remaining ring carbon atoms in the pyridine portion of the molecule are consequently different from those in the parent pyridine, although the difference is usually less than 10 ppm [206]. ^{13}C NMR chemical shift of the condensed azine systems **185-188** are given in Table 36 [10,206].

Table 35. ^{1}H NMR chemical shifts δ, ppm) for quinoline and isoquinoline (in $CDCl_3$).

	H-1	H-2	H-3	H-4	H-5	H-6	H-7	H-8
quinoline	---	8.81	7.26	8.00	7.68	7.43	7.61	8.05
isoquinoline	9.15	---	8.45	7.50	7.71	7.57	7.50	7.87

Table 36. ^{13}C NMR chemical shifts (δ, ppm) for quinoline derivatives (in $CDCl_3$).

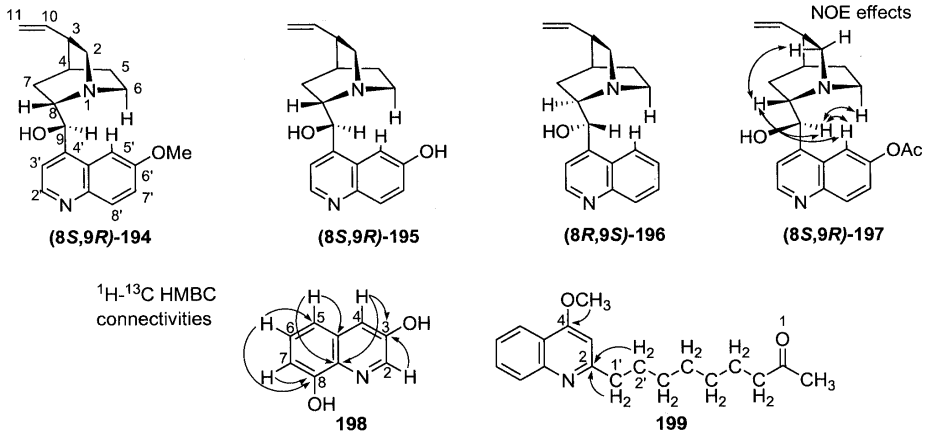
Carbon	quinoline	isoquinoline	acridine	phenanthridine
C-1	---	152.5	129.5	121.0
C-1a	---	---	126.6	126.6
C-2	150.3	---	128.3	127.0
C-3	120.8	143.1	125.5	128.3
C-4	135.7	120.4	130.3	130.4
C-4a	128.0	135.7	149.1	144.1
C-5	127.6	126.5	130.3	---
C-6	126.3	130.6	125.5	153.1
C-7	129.2	127.2	128.3	129.8
C-8	129.3	127.5	129.5	128.2
C-8a	148.1	128.8	126.6	---
C-9	---	---	135.9	126.6
C-10	---	---	---	121.3
C-10a	---	---	149.1	123.7

In the ^{15}N NMR spectroscopy, pyridine-type nitrogen absorbs at relatively low field (δ 63 ppm for pyridine **166**, δ 72 ppm for quinoline **185** and δ 68 ppm for isoquinoline **186**), therefore they are very sensitive to substituent effects being particularly important when strong electron-donating substituents (to the aza-nitrogen) are present (upfield shifts up to 60 ppm may be observed – 2-NH_2). ^{15}N NMR chemical shifts suffer the influence of hydrogen bonding solvents, being shifted to upfield; the extent of which depends on the proton-donor ability of the solvent and the acceptor ability of the base (shifts of some 20 ppm are commonly found). When the lone pair of the pyridine-type nitrogen is protonated, the nitrogen chemical shifts move ~ 100 ppm to higher field [206,229,230].

Several natural quinoline alkaloids have been isolated and fully characterized by extensive NMR techniques [231,232]. Compounds **194-197** are composed by two relatively rigid entities, an aromatic quinoline ring and an aliphatic quinuclidine ring connected by a methane bridge differing only in their configuration at the C-8 and C-9 positions, as reported before for similar synthetic compounds [233,234]. The stereochemistry (8*R*,9*S* configuration for **196** and 8*S*,9*R* configuration for **194, 195** and **197**) was determined by COSY and NOESY experiments (NOESY data showed only for **197**). This stereochemistry and / or the substituents at C-6' determined the insect antifeedant activity of these alkaloids.

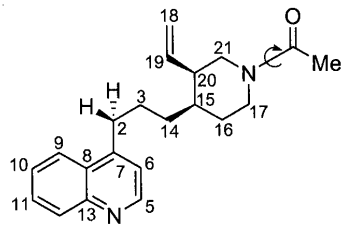
Quinoline alkaloids bearing oxygen at the 3-position are rare in nature [235]; only a few examples having a 3-hydroxyquinoline moiety have been reported. Jineol **198** is one of that natural alkaloid possessing citotoxic activity which structure was elucidated by ^{1}H-^{13}C HMBC (see structure **198**) and NOE experiments (significant NOE effects were observed between the H-4 and H-5 protons when either H-4 or H-5 was irradiated). Methylation and acetylation of Jineol **198** confirmed the position of the two hydroxyl groups [236].

^{1}H-^{13}C HMBC is also important to assign the substituent position on the quinoline structures (*e.g.* **199**). For example, the 4-methoxy protons displayed a correlation with the quaternary carbon C-4 and both H-1' and H-2' correlates with the downfield quaternary carbon C-2, allowing the assignments of a 2-(nonan-8-one)-4-methoxyquinoline structure **199** [237].

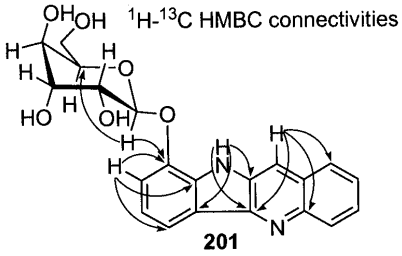


The existence of an equilibrium of two rotational or geometric isomers of the *N*-acetyl derivative **200** was deduced from their ^{1}H NMR spectra. The basis for the assignment of the configuration of the two isomers is the difference in the chemical shifts of the H-17β in the *E* and in *Z* isomers, due to the deshielding effect by the carbonyl amide group, *cis* to H-17β and H-21β in the *E* and *Z* forms, respectively. The ^{13}C NMR spectra showed signals of a 4-substituted quinoline moiety and also exhibits significant chemical shifts differences for C-17 (δ 41.8/46.4 ppm) and C-21 (δ 52.3/46.0 ppm) in the two isomers. ROESY spectrum helped in the assignments of the configuration of both isomers; the interaction between the methyl group of the major isomer and the protons H-21α,β assigned the configuration *E* and the cross-peaks between the methyl group of the minor isomer and the methylene proton H-17β assigned the *Z* configuration [238].

The presence of a glycoside unit in natural quinoline fused system **201** was established on the basis of 1D and 2D NMR data (mainly COSY and ^{1}H-^{13}C HMBC spectra). The presence of one anomeric proton and their coupling constant of J = 7.7 Hz in the ^{1}H NMR spectrum indicate that **201** are a monoglycoside with a β configuration [239].

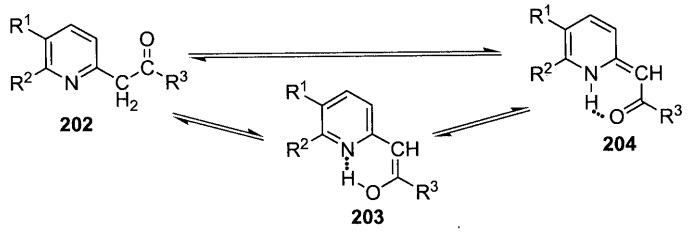


200



201

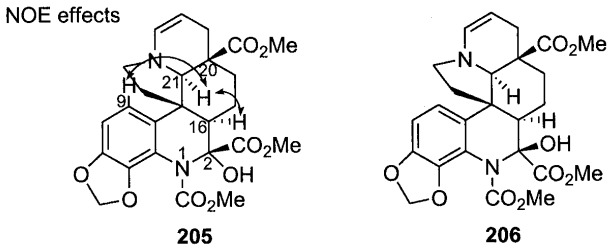
^{15}N NMR methodology was applied to the study of prototropic tautomerism in quinoline derivatives. For example, these tautomeric forms are expected to be present in solutions of 2-phenylacylpyridine: ketimine **202**, enaminone **204** and enolimine **203**. ^{15}N NMR chemical shifts of the ketimine tautomers vary in the range δ -65 to -75 ppm whereas those of the enolimine and enaminone forma are shielded due to the intermolecular hydrogen bonding, significantly varying in the limits δ -120 to -126 and -226 to -228.5 ppm, respectively. The tautomer ratio is affected by solvent, temperature and substituent. In general enolimine **203** form is favoured by non-polar solvents, which conserve the strong intramolecular hydrogen bond. In an aqueous solution of 2-phenylacylpyridine the ratio **202:203** is equal to 12:1 but the **203** form predominates in cyclohexane solution [240-242].



202 **203** **204**

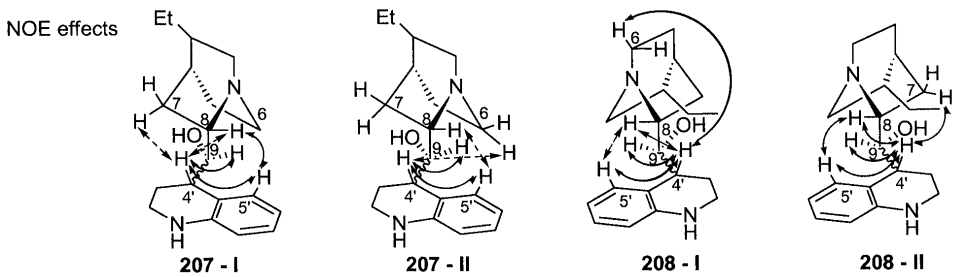
Substitution effects and the establishment of their position in the hydroquinolines skeleton has been under study and several derivatives have been fully characterized by the combination of 1D and 2D NMR techniques [243-246].

The structure of natural and synthetic hydroquinolines has been established by spectroscopic analysis. Mersinine A **205** and mersinine B **206**, two natural epimers obtained from a Malayan Kopsic species showed very similar NMR spectral data except for the resonance of H-6, 2-O*H* and C-16, leading to the assignment of the epimeric center at C-2. The stereochemistry of both epimers was assigned by NOE experiments: irradiation of H-9 result in the enhancement of H-21 signal; while irradiation of H-21 causes enhancement of H-9 as well as H-16, allowing the assignment of the stereochemistry of H-16 as α. Epimer **205** is converted into the more stable **206** upon treatment wih a dilute alcoholic NaOH solution [247].

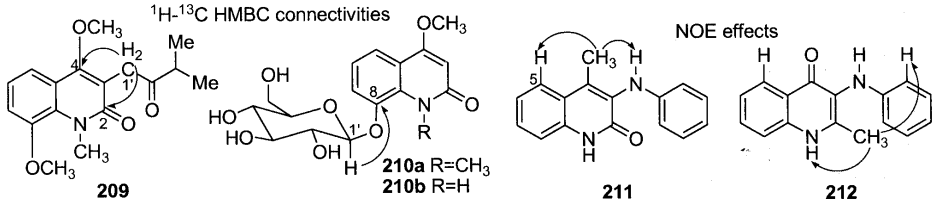


Different hydroquinolines obtained by the hydrogenation of two isomeric quinoline alkaloids were investigated by NMR spectroscopy, being NOESY experiments of great use to confirm the existence of two conformers in the hydroderivatives. The proton H-4' (new formed quiral center) gave NOESY signals as represented in structures **207-I,II** and **208-I,II** [248].

2- And 4-quinolones can be found in nature as simple structures or bearing different substituents, such as long alkyl chains, hydroxyl, methoxyl or sugar moieties [232, 249-253]. The C-H long range correlation between H-1' and C-2 and C-4 in the ^{1}H-^{13}C HMBC spectrum of **209** suggests the location of the side chain at C-3 on the 2-quinolone nucleus. Compounds **210a,b** are rare examples of quinoline glycosides from natural sources isolated from *Echinops gmelinii* (compositae) [254]. The correlations found in the ^{1}H-^{13}C HMBC spectrum of **210a** between the anomeric proton at δ 5.62 (H-1') and C-8 signal (δ 147.2 ppm) indicates that the glucosidic position is at C-8.

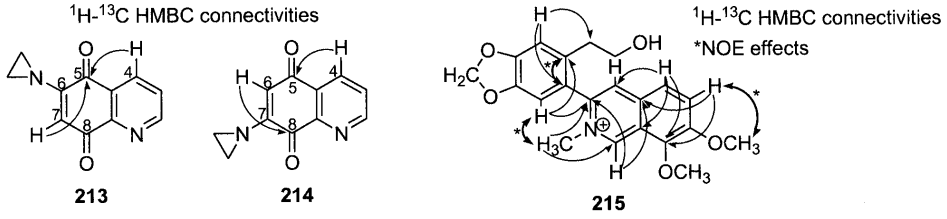


NOE experiments allowed the structure establishment of two synthetic isomers of 2- and 4-quinolone type compounds **211** and **212**, respectively. The irradiation of the methyl groups promotes an enhancement at the aromatic H-5 and N-H of the substituent moiety of compound **211** and at NH of the quinoline nucleus and the two *ortho* protons of the phenyl ring of compound **212** [255].



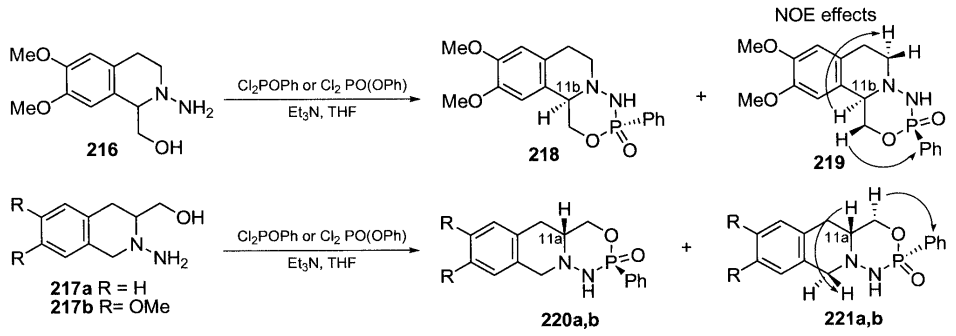
The treatment of quinolone-5,8-diones with aziridine leads to the formation of two isomers that have to be distinguished by ^{1}H-^{13}C HMBC experiments. The connectivities of H-4 and H-7 with carbon C-5 identified the 6-aziridinyl isomers **213** whereas the 7-aziridinyl isomers **214** showed a 3-bond correlation between H-4 and C-5 and also between H-6 and C-8 [256].

Both natural and synthetic derivatives of isoquinoline type compounds were examined and identified by measuring various 2D NMR spectra, including COSY, HMBC and NOESY spectra. HMBC and NOE interactions observed for Usambanoline **215**, a natural isoquinoline isolated from the stems of *Zanthoxylum usambarense*, were used to elucidate its structure [257].



Regiomeric tetrahydroisoquinoline hydrazine alcohols **216** and **217** were cyclised with phenylphosphonic dichloride. Two *P*-2 epimeric diasteriomers, differing in the *cis* or *trans* positions of the *P*-substituent and the hydrogen at annelation (H-*an*) were formed. NOESY cross peaks allowed the determination of stereochemistry and conformations of the hydroisoquinoline products. The orientation of H-*an* (*e.g.*, H-11b for **218** and **219** and H-11a for **220** and **221**) and the protons connected to the carbons adjacent to the annelation allowed the assignment of two different type of annelation products. The stereochemistry was established by the NOE observed from the *P*-phenyl group to the annelation products [258].

Complete ^{1}H and ^{13}C NMR chemical shifts assignments of other synthetic hydroisoquinoline derivatives were made based on 2D NMR spectroscopy. The stereochemistry of these compounds was elucidated based on the value of the ^{1}H-^{1}H vicinal coupling constants, which were measured in the phase-sensitive DQCOSY spectrum [259].



The structure elucidation of quinoline moiety in metallocomplex structures [260,261], of novel natural alkaloids pyridoacridine-type compounds [262-263], of some synthetic hydroacridines [264] and the identification, configuration and conformational analysis of some synthetic quinoline products belonging to the antimalarian drugs [265-267] are a few examples where 1D and 2D NMR experiments have also been extensively used.

6.3. Pyridazines, pyrimidines, pyrazines and its benzoderivatives

Substitution of two carbon atoms of a benzene ring by nitrogen atoms may occur in three ways, giving rise to pyridazine (1,2-diazabenzene) **222**, pyrimidine (1,3-diazabenzene) **223** and pyrazine (1,4-diazabenzene) **224**. Among all these diazines, pyrazine ring **224** present the simplest ^{1}H NMR spectrum due to the symmetry of the ring, one singlet corresponding to the four equivalent protons. Pyridazine **222**, also present ring symmetry, but in the ^{1}H NMR spectrum appeared two signals of an A_2X_2 type. Finally, pyrimidine **223** is the diazine nucleus which presents more signals, the ^{1}H NMR spectrum present three different types of protons, H-2 the most deshielding one, the equivalent ones H-4 and H-6 and the most shielded proton H-5 (Table 37). This molecule characteristic symmetry also explains the number of signals which appear in ^{13}C NMR spectra of these compounds (Table 38) [10].

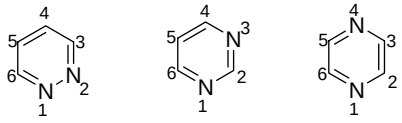


Table 37. ^{1}H NMR data (δ, ppm; $CDCl_3$ as solvent) of diazines.

Compound	H-2	H-3	H-4	H-5	H-6
pyridazine	-	9.17	7.52	7.52	9.17
pyrimidine	9.26	-	8.78	7.46	8.78
pyrazine	8.59	8.59	-	8.59	8.59

Table 38. ^{13}C NMR data (δ, ppm; $CDCl_3$ as solvent) of diazines.

Compound	C-2	C-3	C-4	C-5	C-6
pyridazine	-	153.0	130.3	130.3	153.0
pyrimidine	158.4	-	156.9	121.9	156.9
pyrazine	145.9	145.9	-	145.9	145.9

It seems that pyrazine nucleus occur in well-known aroma substances [268,269] and are important in non-enzymatic Maillard processes observed in foods [270]. These aspects stimulated their study but as Sommer *et al*. [271] emphasised the characteristic nucleus of pyrazines implies that they can appeared as pure substances or as mixtures of isomers. For that reason they developed a method, based on several 1D and 2D NMR experiments, which allowed the determination of the substitution pattern of pyrazines. In their detailed work they present data for mono- di- and trisubstituted pyrazines, being the first ones used as an additional comparison for shift, vicinal and long-range coupling constants values. The most important aspect of their work is the use of ^{1}H-^{15}N HMBC experiments at the natural abundance to distinguish di- and trisubstituted isomers. The reliability of the method was the data resulting from ^{1}H-^{13}C HSQC, ^{1}H-^{13}C HMBC, INADEQUATE and FLOCK experiments. For instance they were able to distinguish and fully characterise the isomers 2-ethoxy-3-methylpyrazine **225** and 2-ethoxy-6-methylpyrazine **226**, and also the isomers 2-acetyl-3,5-dimethylpyrazine **227** and 2-acetyl-3,6-dimethylpyrazine **228**, based on the ^{15}N NMR chemical shifts and on the ^{1}H-^{15}N HMBC correlations (Table 39).

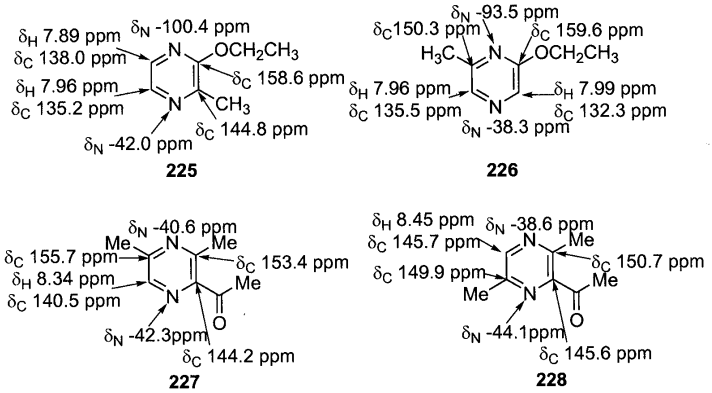
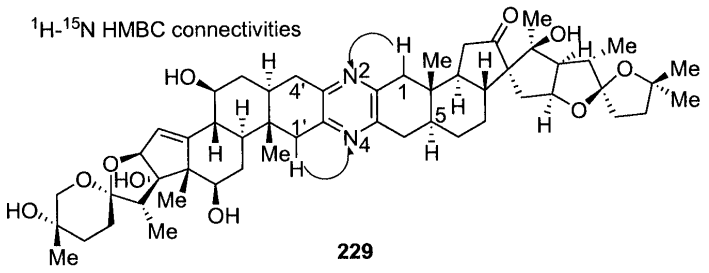


Table 39. ^{1}H-^{15}N correlations in ^{1}H-$^{1}5N$ HMBC spectra of di- and trisubstituted pyrazines.

Compound	δ (^{15}N) (ppm)		δ (^{15}H) (ppm)			Coupling pathway
			subs./H	at position in	molecule	
	N-1	N-4	3	5	6	$^{15}N \leftrightarrow {}^{1}H$
225	- 100.4				7.89 (H)	N-1 ↔ H-6
		- 42.0	2.47 (CH_3)	7.96 (H)		N-4 ↔ C-3-CH_3; N-4 ↔ H-5
227	- 42.3				8.34 (H)	N-1 ↔ H-6
		- 40.6	2.80 (CH_3)	2.60 (CH_3)		N-4 ↔ C-3-CH_3; N-4 ↔ C-5-CH_3
228	- 44.1				2.58 (CH_3)	N-1 ↔ C-6-CH_3
		- 38.6	2.77 (CH_3)	8.45 (H)		N-4 ↔ C-3-CH_3; N-4 ↔ H-5

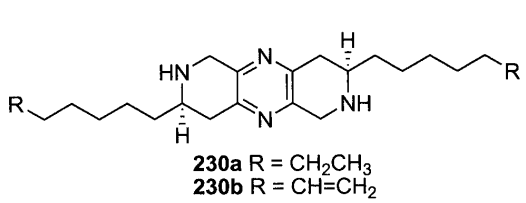
As was demonstrated by Rizzi, pyrazines appear in foods and are responsible for some Maillard reactions [270]. Jun *et al.* in their study of the stability of glucosamine [272], an important amino sugar found in a various plant tissues [273], found, among other compounds, a mixture of pyrazine derivatives which were characterised by NMR spectroscopy, mainly by COSY experiments.

Fukuzawa *et al.* used NOESY, COSY, HMQC and specially ^{1}H-^{15}N-HMBC (see structure **229**) experiments to determine the orientation of the two steroidal units attached to the pyrazine ring in ritterazine A **229**, a cytotoxic metabolite isolated from the tunicate *Ritterella tokioka* [274].

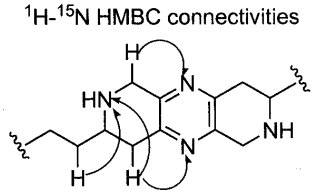


229

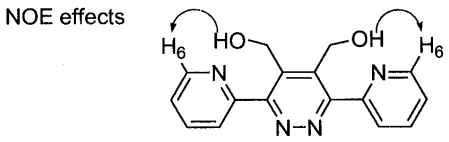
Chill *et al*. also isolated two cytotoxic alkaloids, barrenazine A **230a** and B **230b**, from an unidentified tunicate [275]. These compounds present an unusual heterocyclic skeleton that include a pyrazine ring and their structures were elucidated mainly by 2D NMR experiments, ^{1}H-^{15}N HMBC correlations were consistent with proposed structures and used as final prove.



230a R = CH_2CH_3
230b R = $CH{=}CH_2$

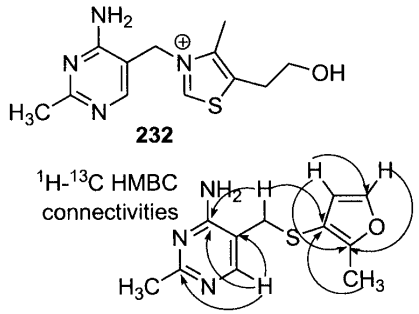


Pyridazine nucleus seems also to present important biological properties, such as inhibition of acetylcholinesterase which is important to enhance memory in Alzheimer's disease patients [276], and as inhibition of phosphodiesterase III which could be considered as new antiplatelet agents [277]. The synthesis and derivatisation of several simple pyridazine/pyridazinone derivatives with potential applications have been characterised by 1D and 2D NMR experiments, nevertheless their structure simplicity the NMR data was important to assign their structure [278-280]. For instance, NOESY spectrum was necessary to demonstrate the through-space coupling of hydroxyl protons with the H-6 protons of 4,5-bis(hydroxymethyl)-3,6-di(2-pyridyl)pyridazine **231** and prove that exists an intramolecular hydrogen bond between the hydroxyl proton and the pyridine nitrogen [278].



231

Thiamin, vitamin B1 **232**, is an example of an important pyrimidine ring containing compound since it is a precursor for the roasted or cooked meat aroma [281]. Jhoo *et al.* have studied the thermal degradation of thiamin and used 1D and 2D NMR experiments to fully characterise the major decomposition product 2-methyl-4-amino-5-(2-methyl-3-furylthiomethyl)pyrimidine **233** [282]. The ^{1}H-^{1}H COSY spectrum proved the presence of two olefinic proton signals correlated to each other (protons at δ 6.24 and 7.29 ppm). The HMBC spectrum decisively showed important connectivities that ultimately confirmed the structure (see structure **233**).



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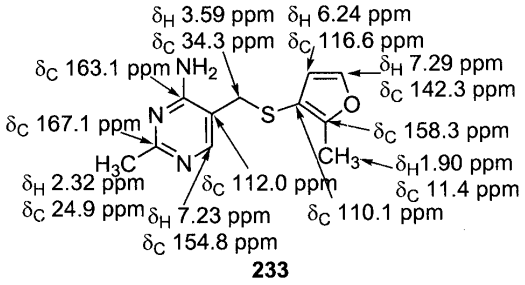
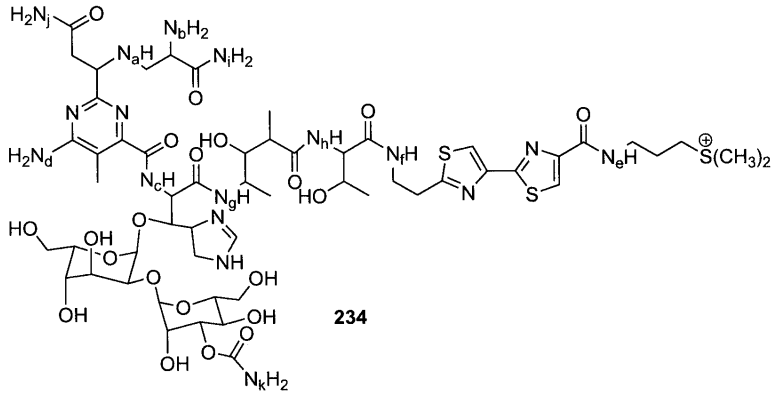


233

Xia *et al.* characterise a much more complicated pyrimidine ring containing the natural compound bleomycin **234**, a clinical useful antineoplastic drug [283]. A combination of ^{1}H, ^{1}H-^{15}N HSQC and HMBC and ^{1}H-^{1}H COSY and NOESY NMR spectroscopy was used to completely assign the proton and nitrogen atoms chemical shifts of bleomycin and derivatives. Another important aspect of their work was the use of NMR spectroscopy to define the internal axial ligand to Co(III), used as model to understand the natural complex of Fe(III). The apparent large upfield shifts of N-a, N-b and N-c in Co(III) complex are consistent with their direct complexation to a positively charged metal ion. In addition, the missing Nc-H correlation in the HSQC spectrum confirmed that N-c nitrogen was deprotonated when Co(III) complex was formed. The relatively large ^{15}N NMR chemical shifts of N-d in metal-free bleomycin **234** (δ 84.1 ppm) and the Co(III) complex (δ 96.2 ppm) are in agreement with the direct bonding of the aromatic pyrimidine ring nitrogen with the metal center (Table 40).

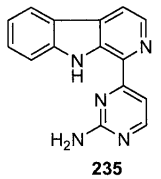


234

Table 40. ^{1}H-^{15}N HSQC cross-peaks of bleomycin **234** and their Co(III) complex (δ, ppm).

	bleomycin		Co(III) complex			bleomycin		Co(III) complex	
	^{15}N	^{1}H	^{15}N	^{1}H		^{15}N	^{1}H	^{15}N	^{1}H
N-a	35.1	-	11.2	5.92	N-g	118.4	8.18	114.4	8.87
N-b	36.5	-	-9.7	4.18/3.14	N-h	133.9	8.44	133.6	-
N-c	118.8	9.13	94.8	-	N-i	108.9	8.08/7.54	108.5	7.78/6.99
N-d	84.1	6.49	96.2	7.90/7.68	N-j	113.6	7.76/7.12	112.5	7.99/7.28
N-e	116.8	8.94	115.9	8.56	N-k	75.9	6.57/6.22	76.1	6.72/6.11
N-f	119.4	8.42	117.6	8.52					

In a recent work Costa *et al*. isolated from *Annona foetida* a new alkaloid possessing antileishmanial activity [284], which structure were assigned by 1D and 2D NMR experiments. The author's results indicate that this new alkaloid is a derivative of annomontine **235**, the *N*-hydroxyannomontine **236**. The ^{1}H NMR spectrum obtained for the new compound was similar to the known annomontine except the absence of the N-H indole hydrogen signal (characteristic chemical shift at δ 11.81 ppm) and the presence of a N-OH signal at δ 15.08 ppm indicating a hydrogen bond with a nitrogen atom of the pyrimidine ring. Nevertheless, the authors obtained detailed ^{1}H-^{13}C HMBC correlations to confirm the proposed structure (see structure **236**).



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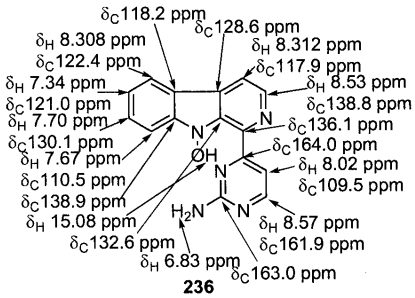

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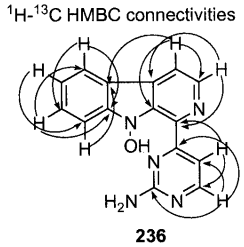
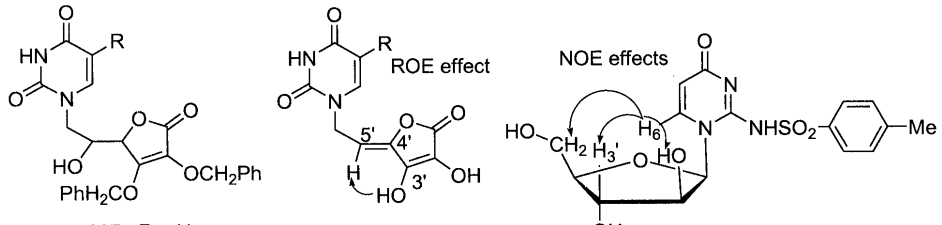


236

Cytosine, uracil and thymine, the well known bases of DNA and RNA, are derivatives of pyrimidine. Due to their importance in life process they were used as models to obtained similar compounds hoping to obtain new derivatives with important biological activities. Raić-Malić *et al.* synthesised and characterised a few derivatives **237a-c** and **238a-c** and detected that some exhibit appreciable antitumour cell activity [285]. The characterisation of this compounds was done by NMR experiments including ROESY and ^{1}H-^{13}C HMBC; one important aspect of the characterisation was the establishment of the Z-configuration of the C4'=C5' double bond of **238a-c** and that was achieved by the interactions found in ROESY spectrum between 3'-hydroxyl proton the methine proton H-5'.

NOESY spectra are commonly used to establish the configuration of pyrimidine derivatives [286,2987]. For instance Krizmanić *et al.* confirm the configuration of **239**.

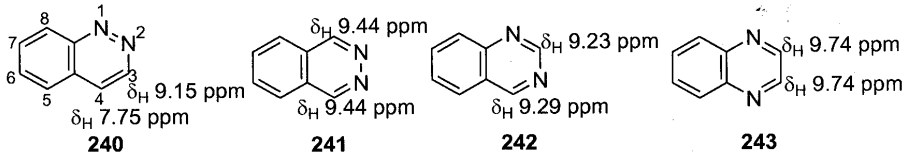


237a R = H
237b R = F
237c R = CF_3

238a R = H
238b R = F
238c R = CF_3

239

The benzo derivatives of the above referred diazines, generally refered as benzodiazines, are cinnoline **240**, phthalazine **241**, quinoxaline **242** and quinazoline **243**. The aromatic protons in the benzenoid ring appear as an AA'BB' spin system and/or resemble those of the parent heterocycles quinoline and isoquinoline [10]. The most important ones and easily identified are the protons of the heterocyclic ring which present characteristic chemical shifts (indicate in the compound structures). The carbon assignments were made for all carbons in all benzodiazines and again the resonances of the carbons of the heterocyclic ring present larger differences in their chemical shifts (Table 41) [10].

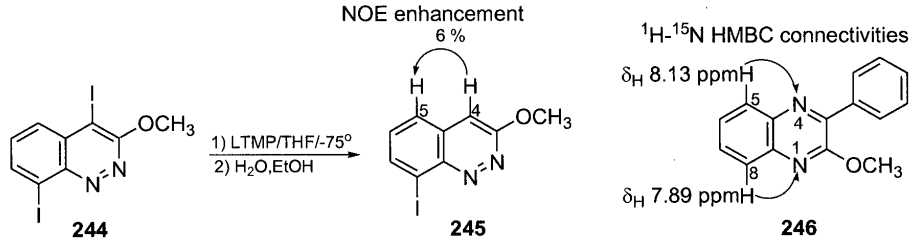


240 **241** **242** **243**

Table 41. ^{13}C NMR spectral data (δ, ppm; $CDCl_3$ as solvent) of some benzodiazines.

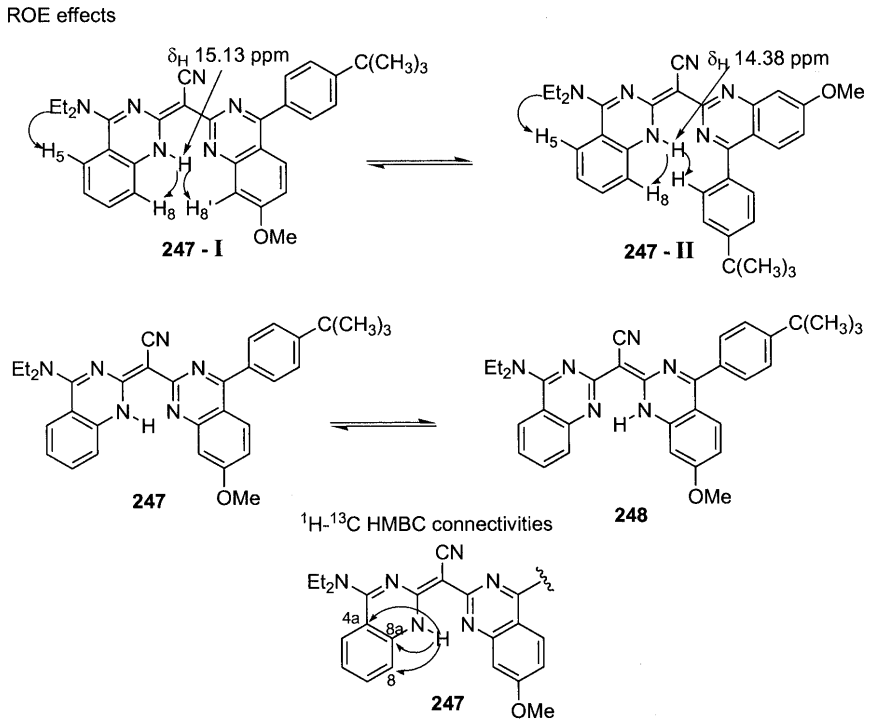
Compound	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-4a	C-8a
cinnoline	-	-	146.1	124.7	127.9	132.3	132.1	129.5	126.8	151.0
phthalazine	152.0	-	-	152.0	127.1	133.2	133.3	127.1	126.3	126.3
quinazoline	-	160.5	-	155.7	127.4	127.9	134.1	128.5	125.2	150.1
quinoxaline	-	145.5	145.5	-	129.8	129.9	129.8	129.8	143.2	143.2

Quéguiner *et al*. studied the metallation of benzodiazines and in doing that they synthesised and characterised several derivatives. One of their reports is on metallation of cinnolines [288] and in all the obtained derivatives used NOE experiments to confirm some chemical transformations. For instance they confirm the transformation of cinnnoline **244** into cinnoline **245** by NOE difference spectrum, upon irradiation of H-4 signal in cinnoline **245** a 6 % enhancement of the signal at δ 7.62 ppm, assigned to H-5, was observed. Later, they reported the metallation of quinazoline, quinoxaline and phthalazine derivatives [289,290] and in some of these cases unambiguous structure determinations were carried out using ^{1}H-^{15}N HMBC experiments. For example the ^{1}H NMR spectra of quinoxaline **246** showed two double of doublet at δ 8.13 and 7.89 ppm which could be assigned equally to proton H-5 and proton H-8. In the ^{1}H-^{15}N HMBC spectrum clearly is observed a correlation between nitrogen N-1 signal (at δ 265 ppm) and the proton signal at δ 7.89 ppm and between nitrogen N-4 (at δ 334 ppm) and the proton signal at δ 8.13 ppm. These data allowed unambiguous determination of the chemical shifts of protons H-5 and H-8 in quinoxaline **246**.



Gehring and Daltrozzo applied several 2D NMR experiments, namely COSY, NOESY, ROESY, EXSY, HMQC and HMBC to determine both the rotamer and tautomer equilibrium of new H-chelates of several quinazoline-2-acetonitrile derivatives [291]. For instance, in the case of quinazoline-2-acetonitrile dye **247** there can be an equilibrium between rotamers **247-I** ⇆ **247-II** and a tautomerization equilibrium between **247** ⇆ **248.** The structure determination of the rotamers **247-I** and **247-II** was mainly based on NOE effects between the proton of the H-bridge and other protons of the system found in the ROESY spectra. The ROESY spectra present ROEs between the Et_2N group and proton H-5 at δ 7.78 and 7.72 ppm, respectively. With the DQF-COSY it is possible to assign the signals of protons H-6, H-7 and H-8: starting with the signal of H-5 at δ 7.78 ppm, is found a coupling with H-6 at δ 7.25 ppm, which in turn couples with H-7 at δ 7.66 ppm and this one couples with H-8 at δ 7.45 ppm. The signals of the other rotamer can be assigned in the same way. The ROESY spectra show ROE effects between the chelate proton at δ 15.13 ppm and H-8 at δ 7.45 ppm and H-8' at δ 7.10 ppm. Therefore, these protons must belong to structure **247-I**. The chelate proton at δ 14.38 ppm exhibits ROE effects to proton H-8 at δ 6.92 ppm and δ 7.85 ppm, the *ortho* protons of the phenyl group. Therefore, this rotamer has the structure **247-II**. The relative amounts of the two rotamers were determined by the signal integrals and was detected that the rotamer **247-I** is the major one.

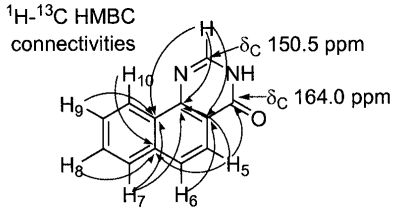
The ^{1}H-^{13}C HMBC spectra show couplings between the chelate proton and carbons C-4a, C-8a and C-8 for both rotamers **247-I** and **247-II**. There are no couplings to carbon atoms of the second quinazoline system. Therefore, it can be inferred that both rotamers mainly or only exist as tautomer **247**.



Benzodiazines can also be found in natural compounds. As an example it can be referred the work of Morita *et al*. [292] on the isolation and structural characterisation of the alkaloid samoquasine A **249** from the seeds of *Annona squamosa*. In the characterisation they used 1D and 2D NMR experiments, in particularly the interpretation of the ^{1}H-^{1}H COSY and HOHAHA spectra revealed proton connectivities from H-5 to H-6 and from H-7 to H-10. In addition, connectivities indicated by ^{1}H-^{13}C HMBC confirmed a 1,2-disubstituted naphthalene skeleton. An amide carbonyl carbon and a signal at δ 150.5 ppm assignable to the carbon between two nitrogen atoms were included as part of a pyrimidine ring.

Dal Piaz *et al*. studied the synthesis and complete characterisation of diazine fused compound isoxazolo[3,4-*d*]pyridazin-7(6*H*)-ones and doing so they used several 1D an 2D NMR experiments [293]. For instance in the case of 3-methyl-4-(2-thienyl)isoxazolo[3,4-*d*]pyridazin-7(6*H*)-one **250** the assignment of ^{15}N NMR signals of N-5 and N-6 was based on INEPT (refocused and decoupled) or ^{1}H-$^{1}5N$ HMBC experiments. The signal due to carbon C-7 could be identified independently by heteronuclear ^{13}C-^{1}H NOE difference experiments. Finally, the signal due to N-1 had to

be determined in one-pulse ^{15}N NMR experiments because this nitrogen atom lacks coupling suitable for polarization transfer.



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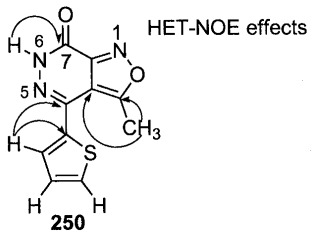
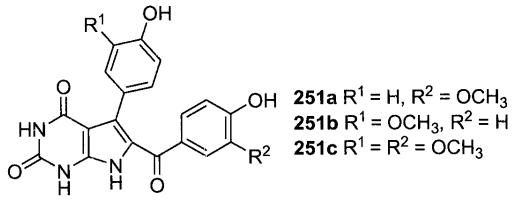
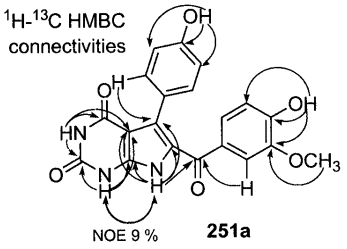


250

The structure of the pyrrolopyrimidine alkaloids **251a-c**, isolated from a tunicate *Cystodytes* sp., were elucidated on the basis of spectroscopic data, mainly NOE experiments and ^{1}H-^{13}C HMBC correlations. The ^{1}H-^{15}N HSQC spectrum allowed the unequivocal assignment of the three nitrogen atoms [294].

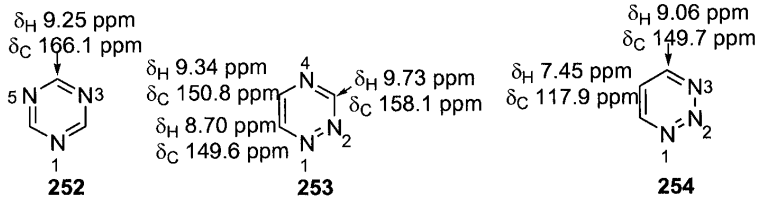




251a

6.4. Triazines

Among the triazine systems, the 1,3,5-triazine **252** derivatives are the oldest known, 1,2,4-triazine **253** derivatives are probably the most studied ones and 1,2,3-triazine **254** derivatives are the poor relation amongst the triazine family. The ^{1}H and ^{13}C NMR spectra of 1,3,5-triazine **252** are quite simple, due to the symmetry of the molecule there is only one single signal in each spectrum. The least studied of the class 1,2,3-triazine **254** present also a symmetry and in consequence protons H-4 and H-6 are equivalent so the ^{1}H and ^{13}C NMR spectra present only two signals. Protons H-4 and H-6 appeared as doublet (J = 6.0 Hz) and proton H-5 as a triplet. Finally, 1,2,4-triazine **253** present, as expected, three signals in both ^{1}H and ^{13}C NMR spectra [10]; their proton chemical shifts are affected by the used solvent (Table 42) [295].



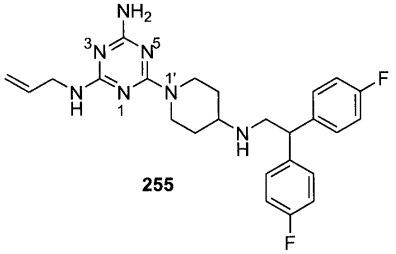
252 **253** **254**

Table 42. ^{1}H NMR spectral data (δ, ppm) of 1,2,4-triazine **253** in various solvents.

Solvent	H-3	H-5	H-6
$CDCl_3$	9.73	9.34	8.70
CCl_4	9.63	9.24	8.53
CD_3OD	9.86	9.52	8.93
DMSO-d_6	9.75	9.42	8.88
C_6D_6	9.45	8.68	7.92

The 1,3,5-triazine ring is important in drug research since it is present on compounds having biological activities (*e.g*. study on structure-activity relationships of P2-receptor antagonists [296]). Sometimes the main problem in structural characterisation of these compounds are not the 1,3,5-triazine and/or 1,2,4-triazine nucleus but the substituent groups [297-299].

Multidrug resistance (MDR) is now recognised as a major cause of failure of cancer chemotherapy. It seems that some 1,3,5-triazine derivatives do contain chemical features known to be important for MDR reversing activity. Amm *et al*. study a few examples of this type of compounds, for example derivatives **255** and **256**, in order to gain information on the structure-activity relationship. In their first study [299] they used NMR spectroscopy, particularly ^{15}N NMR spectroscopy to identify the sterically less hindered nitrogen atom of the 1,3,5-triazine and consequently the one that will be easily protonated. Due to conformational problems, the ^{15}N NMR spectra of **255** and **256** at room temperature exhibit very complex pattern except in the aliphatic amino nitrogen, where a single signal occur. Spectra recorded at 350 K, contain well resolved signals: the triazine nitrogens give signals around δ– 205 ppm (they are scarcely dependent of the proximate amino groups) and the resonances of the aniline nuclei are located in a narrow (less than 10 ppm) range, around δ– 285 ppm. As expected, the protonation of **255** occur first at the secondary aliphatic amine. The second protonation results in several changes in the spectrum. Two signals were strongly displaced. A resolved triplet at δ ~ -270 ppm was assigned to the N-3 atom of triazine (^{3}J = 4 Hz with the NH of each side-chain; the other two triazine nitrogens N-1 and N-5 gave a doublet with the same coupling constant). The other signal at δ ~- 260 ppm was then assigned to the piperidine nitrogen N-1'. The very strong deshielding of N-3 clearly demonstrates that the second protonation occur at this site even if does not result in a detectable $^{1}J_{N\text{-}H}$ coupling interaction. The significant deshielding observed in the N-1' in the piperidine results from the changes in electron delocalisation through the conjugated system. The referred conformational problems of compounds **255** and **256** at room temperature led to study the quantitative analysis of the conformers in solution at low temperature [300].



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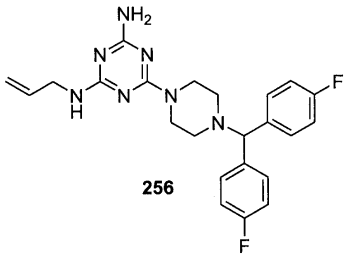
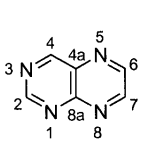


256

Birkett *et al.* studied by NMR experiments the rotational isomerism involving the side-chains of several 1,3,5-triazine derivatives [301,302]. In their study they used 1D and 2D NMR experiments establish the structure of the possible tautomers at different temperatures.

6.5. Pteridines

Pteridine **257** has been used as trivial name for the condensed pyrazino[2,3-*d*]pyrimidine ring system, which is numbering according to the IUPAC rules (see structure **257**). The ^{1}H NMR spectrum of pteridine **257** contains two singlets which can be assigned to the protons of the pyrimidine ring and a pair of doublets from the adjacent H-6 and H-7 protons. The spectroscopic data, especially the strong downfield shifts of the proton and carbon resonances are characteristic of pteridine as a strong π-deficient heteroaromatic system [10].



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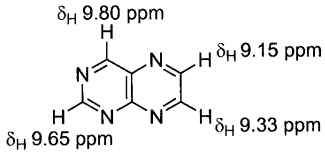

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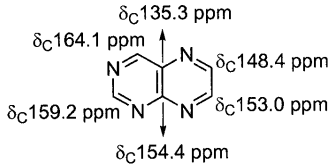
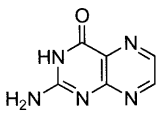


257

Most naturally occurring pteridines have an amino group in the 2-position and a hydroxyl group in the 4-position, thus limiting structural variations of different substituents in positions 6- and 7-. The term "pterin" is used for the most favoured tautomeric formula of this compound, 2-aminopteridin-4-one **258**. Other naturally occurring pteridines are lumazine **259** (pteridine-2,4-dione), and xanthopterin **260**, isoxanthopterin **261**, leucopterin **262**, which can be described as 6-oxo-, 7-oxo- and 6,7-dioxo-pterins, respectively [303].



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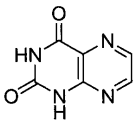

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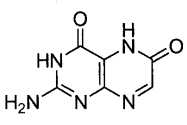

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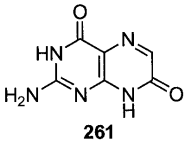

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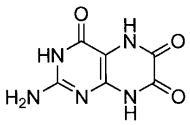


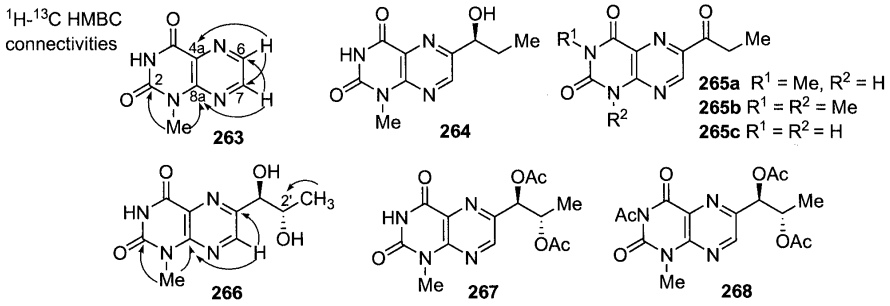
262

^{1}H NMR studies of pteridine **257** and its 2-, 4- and 7-methyl derivatives in $CDCl_3$ have resulted in the assignment of the ^{1}H signals. The low solubility of pterin **258** and its naturally occurring derivatives in aprotic solvents does not allow easy investigation of the neutral forms, but mono- and di-cations taken in trifluoroacetic acid or fluorosulfonic acid are well characterized [303].

2-Phenylpteridine and its 4- and 7-monomethyl, 4,7- and 6,7-dimethyl and 4,6,7-trimethyl derivatives [304] as well as the corresponding 4-phenylpteridines series and its 2- and 7-methyl, 2,7- and 6,7-dimethyl and 2,6,7-trimethyl derivatives, exists as neutral molecules in aqueous solution, essentially as unhydrated species [305].

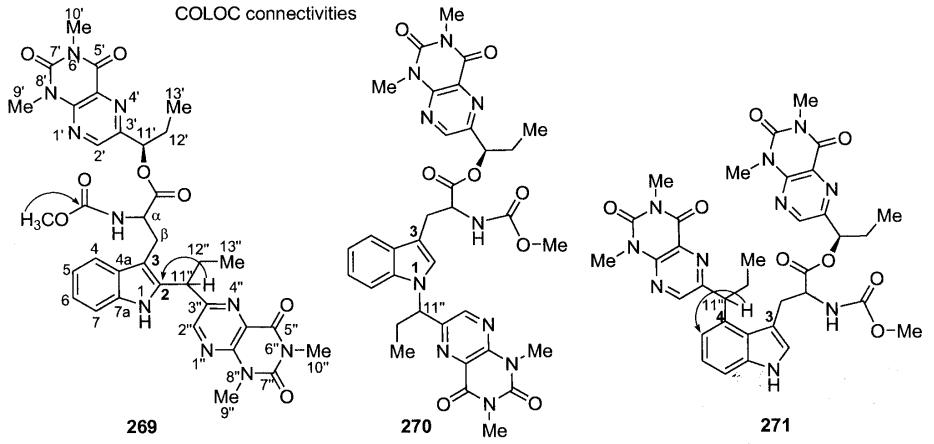
Among simple halogenopteridines only the 2-, 4-, 6- and 7-chloro as well as the 6,7-dichloro derivatives have been synthesized and structurally investigated [306-310].

Pteridines are widely distributed in animal kingdom, being for example 1-methylpteridine-2,4-dione **263** isolated from lithistid sponge *Corallistes fulvodesmus* [311], leucettidine **264** from the Bermaudian calcareous sponge *Leucetta microraphis* and other leucettidine analogues **265a-c** from the free polychaete *Odontosyllis undecimonta* of Toyama Bay in Japan [312]. Structural elucidation of leucettidine **264** was deduced by analysis of spectral data of other pteridines of known configuration [313]. The assignments of chemical shifts and stereochemistry of the side chain of the natural pteridine **266** was accomplished using the NMR data of the known pteridine **263** and by the results of it reaction with Ac_2O in pyridine that gave both products **267** of diol acetylation and product **268** of the further N-3 acetylation [312]. The structure of some of the referred pteridine derivatives was made by several spectroscopic analyses, with special emphasis to ^{1}H-^{13}C HMBC correlations (see structures **263**, **266**).

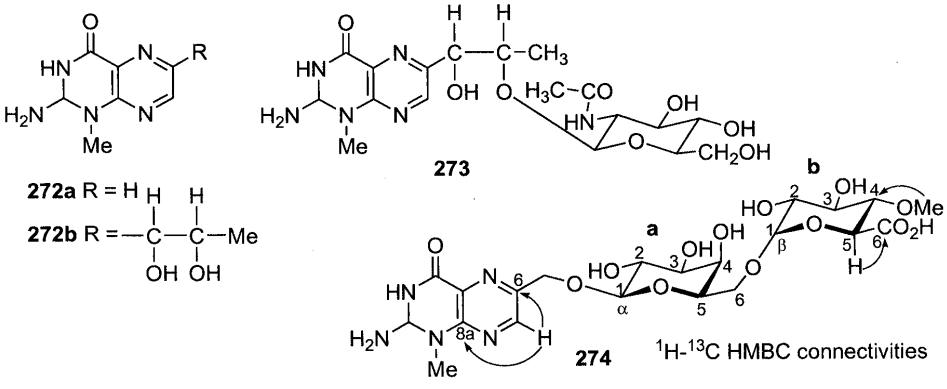


More complex pteridines have been isolated from marine sources. Compounds **269-271** share some common structural features: two pteridine units, each of which bears a three carbon side chain, a tryptophan core with a methyl carbamate substituent and different pteridine substitution patterns. Pseudoanchynazine A **269** has pteridine substitution at C-2 of indole unit, pseudoanchynazine B **270** at N-1 and pseudoanchynazine C **271** at C-4, in all cases the tryptophan carboxyl group forms an ester with the second pteridine moiety [314].

The structure of these three compounds **269-271** is very similar and 2D NMR experiments were used in the full assignment of ^{1}H and ^{13}C NMR chemical shits. The main structural features are: in the ^{1}H NMR spectrum of compound **270** indole H-2 was evident while NH-1 was absent. This, together with the downfield chemical shift for C-11'' and H-11'' (δ 60.5 and 5.62 ppm, respectively) indicated nitrogen substitution at C-11''; therefore, the identity of **270** as the N-1 substituted isomer of **269** was established. In the case of compound **271**, ^{1}H NMR signals indicated three connected protons in the indole nucleus, which placed the pteridine substituent either at C-4 or C-7. Analysis of the 2D NMR spectra, especially COLOC (correlation between H-11'' and C-5) and NOESY (correlation between H-11'' and H-5) indicated substitution at C-4. The COLOC connectivitites of H-11'' to C-2 of compound **269** allowed the identification of linkage point of pteridine to indole [314].

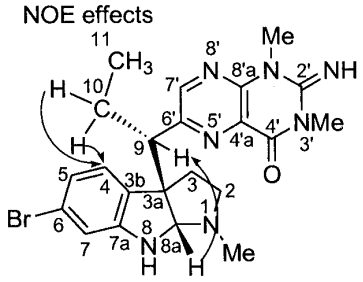


Pterins are the most common pteridines found in nature. The parent pterin **272a** and several glycoside derivatives have been isolated and characterised (β-glycosidic linkage was confirmed by the coupling constant of the anomeric proton J ~ 6-8 Hz) [312, 315-317]. The structure elucidation of pterin glycoside lumipterin **273** was based on 1D and 2D NMR experiments but also based on the cleavage of the glycosidic bond and in the characterisation of the resulting aglycone biopterin **272b** [318]. Cyanopterin **274** is an example of a pterin bearing two sugar unities at C-6. The large anomeric coupling of J = 7.6 Hz confirm the β-configuration of galactose (ring a). The ROE effects and the HMBC connectivities allowed to assign the structure of **274**: the low field H-6 (δ 3.74 and 3.88 ppm) shows a transglycosidic ROE contact to the anomeric proton of the second carbohydrate moiety (δ 4.96 ppm). The ROE contact of H-4 and the methyl group at 3.47 ppm and the low-field shift of C-4 (δ 83.1 ppm) of the sugar b identifying the α-configuration of 4-*O*-methylated glucoronic acid (ring b). The carboxylic acid was identified by the connectivity found in the HMBC spectrum between the carbonyl carbon at δ 174.6 ppm to the proton H-5 of the glucoronic acid (sugar b) [317].



Pterin without sugar residues are also found in nature. Urochordamine A **275** and urochordamine B **276** are two examples and their structures were determined through the use of COSY and ^{1}H-^{13}C HMBC measurements and the relative stereochemistry was deduced by NOESY experiments. The cross peaks between H-8a and H-9 for both **275** and **276** secured *cis* relationship of the fused pyrroles. Both H-10 methylene protons showed NOE effects with proton H-4 of the benzene ring in **275**, whereas one of the H-10 methylene protons in **276** shows NOE effects with H-3 methylene protons, thereby suggesting that the stereochemistry at C-9 was opposite in **275** and **276** [319].

^{1}H-^{13}C HMBC correlations between the H-9 with C-3, C-3a, C-3b, C-8a, C-10, C-11, C-6' and C-7' and of the most deshielded proton H-7' (δ 8.02 ppm, s) with C-6' and C-8'a allowed the assignment of pterin substituent at position C-6'.



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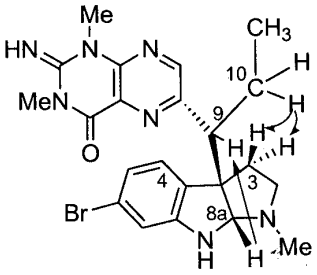

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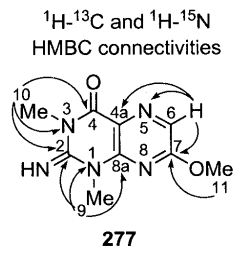
^{1}H-^{15}N and ^{1}H-^{13}C HMBC measurements were used for the characterization of a simple natural pterin **277** [320] substituted at C-7 (see structure **277**). Extensive studies in full assignment and stereochemistry of methotrexato conjugates with short-chain alkylamino acids **278a-c** were performed using 2D NMR techniques such as COSY and ROESY experiments [321]. NOE measurements are also essential for the configuration determination of tetrahydrobiopterin analogues [322].



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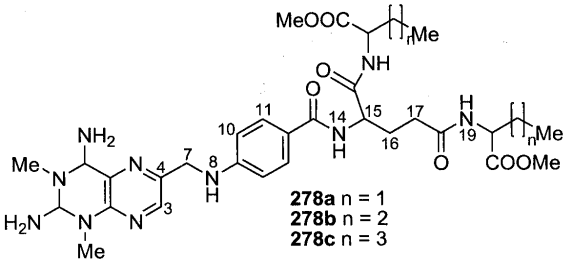


278a n = 1
278b n = 2
278c n = 3

Table 43. ROE effects of pterins **278a-c.**

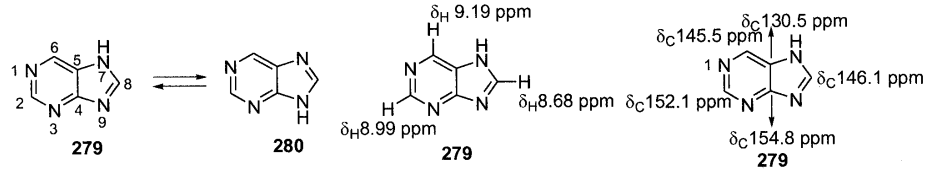
	ROE effects		ROE effects		ROE effects
H-3	H-7, H-8, H-10	H-11	H-10, H-14, H-15	H-16	H-14, H-15
H-8	H-3, H-10	H-14	H-11, H-15, H-6, H-17	H-17	H-14, H-15
H-10	H-3, H-7, H-8	H-15	H-16, H-17, H-19	H-19	H-15

NMR methods for the study of motion in protein continue to improve and a number of studies of protein-ligand complexes relevant to drug design have been reported. NMR studies have been proved to be particularly successful for obtaining detailed information about structure and binding site flexibility and identification of the location of the binding site and the conformation of the ligand within it. Understanding binding site will be important in designing novel ligands as well as the affinity for new candidate ligands [323]. Structural studies of the complex protein-cofactor in phenylalanine hydrolase and protein-ligand in dihydrofolate reductase are two examples of complex molecules having a pterin residue and which have been analysed by NMR spectroscopy [324-326].

6.6. Purines

For historical reasons, the numbering of purine (imidazo[4,5-*d*]pyrimidine) does not comply with IUPAC rules. Purine exists in two tautomeric forms, namely 7*H*-purine **279** and 9*H*-purine **280**, which are in equal concentration in solution (annular tautomerism). In solid state, the 7*H*-form is dominant.

The NMR spectroscopic data of the pyrimidine ring are comparable with those of the parent system. However, the protons are strongly shifted downfield in the imidazole ring when compared with the parent system [10].



Purine nucleosides are known as adenosine (A) **281**, guanosine (G) **282**, inosine (I) **283** and xanthosine (X) **284**. The structures shown for these nucleosides **281-284** are the commonly accepted predominant tautomers, and it will be shown bellow that the ^{15}N NMR chemical shifts are consistent with these structures (Table 44) [327].

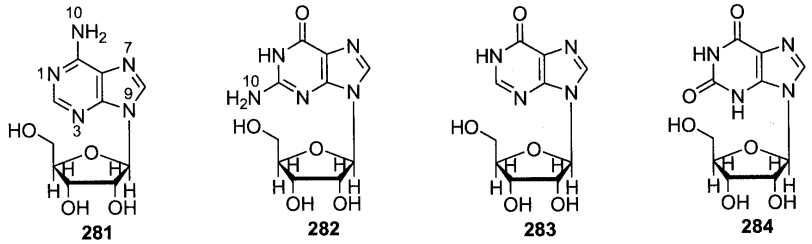


Table 44. ^{15}N chemical shifts (δ, ppm) for the purine and their nucleosides (in DMSO-d_6).

Compound	N-1	N-3	N-7	N-9	N-10
purine[b]	230.4	245.6	169.4	173.2	---
aenosine	203.1	215.8	220.5	149.3	60.7
ganosine	127.1	146.1	226.8	149.8	52.8
iosine	154.1	193.8	228.6	154.3	---
xanthosine	133.5	93.5	227.8	145.7	---

^{15}N NMR spectroscopy was especially effective for investigate the protonation and tautomeric behaviour of the nitrogens of purine derivatives. The small separation of 4 ppm between N-7 and N-9 resonances for purine **279** in neutral water indicates that the NH-7 and NH-9 tautomers are present in nearly equal amounts. This is not the case when DMSO is used as solvent, wherein the chemical shifts for N-7 and N-9 are δ 166.8 and 187.3 ppm, respectively, being the tautomer **280** more favoured. The ^{15}N NMR chemical shift changes on protonation are strongly indicative of essentially exclusive protonation of purine **279** on the nitrogen N-1 in both solvents DMSO and water. N-1 resonances move upfield with increasing trifluoroacetic acid concentration, while the chemical shifts of nitrogens N-3, N-7 and N-9 remain almost unchanged.

A complete protonation of 9-methyl derivative originate an upfield shift of N-1 resonance by 83.9 ppm, while the resonances of N-3, N-7 and N-9 are shifted downfield by 6.4, 1.6 and 9.8 ppm, respectively, indicating once again, essentially exclusive protonation of N-1 for this derivative. Protonation of 7-methylpurine in water also appears to occur essentially at N-1, which present an upfield shift of 77.0 ppm. The resonances of nitrogens N-3 and N-7 here move downfield by 4.0 amd 7.0 ppm, respectively, while resonance of nitrogen N-9 changes very little [328].

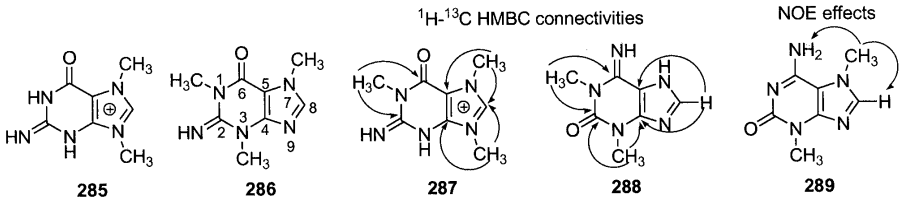
Table 45. ^{15}N chemical shifts (δ, ppm) for the purine derivatives[a].

Compound	N-1	N-3	N-7	N-9
purine	109.7	124.8	181.6	185.7
purine (DMSO-d_6)	98.4	116.0	166.8	187.3
9-methylpurine	109.3	132.7	145.0	244.1
7-methylpurine	108.5	116.7	229.4	143.0

[a]In ppm upfield from external D$^{15}NO_3$. The spectra were taken in aqueous solution unless otherwise indicated.

The changes in ^{15}N shift on protonation can be dramatic, especially for azine nitrogen. On addition of acid to an adenosine solution the resonance assigned to N-1 is shifted 71.7 ppm to higher field, whereas all the other nitrogen resonances only shift slightly, showing that protonation in solution takes place mainly at N-1 but do not exclude lesser amounts of protonation at the other nitrogens. In guanosine, N-7 is the most basic nitrogen atom and their resonance is moved upfield by 66.3 ppm on addition of acid [329].

In the past decades, several groups have reported the isolation of methylated guanine base analogues from sponges, including 7,9-dimethylguanine (herbipoline) **285** [330], 1,3,7-trimethylguanine **286** [331] and 1,7,9-trimethylguanine **287** [332]. The isolation of some isoguanines such as 1,3-dimethyl **288** [333] and 3,7-dimethyl **289** [334] and their characterization as also been reported using NMR techniques (mainly HMBC and NOE measurements).

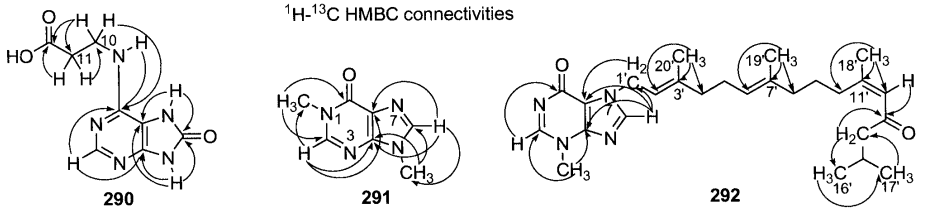


285 **286** **287** **288** **289**

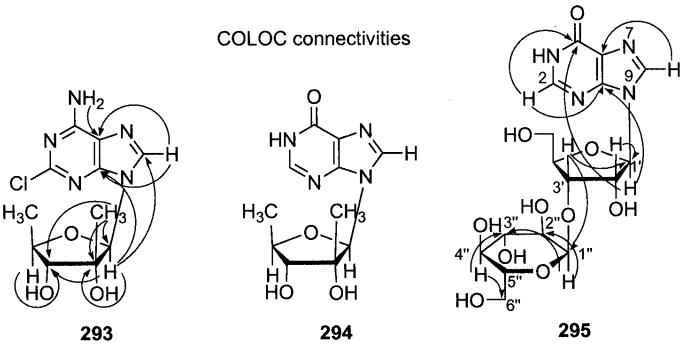
^{1}H-^{13}C HMBC experiments was also of great importance in the establishment of structure and substitution pattern of novel natural purines derivatives **290-292** [335-337]. NOE in combination with ^{1}H-^{15}N HMBC data allowed distinguishing between a number of possible substitution patterns in **291**. Irradiation of H-2 (δ 8.48 ppm) promotes an enhancement of N1-CH_3 and N9-CH_3 by 12 and 10%, respectively. Confirmation of 1,9-methylation pattern was achieved by interpretation of ^{1}H-^{15}N HMBC data for **291**: a) H-2 correlates with nitrogen N-1 and N-3; b) N1-methyl protons to nitrogen N-1; c) H-8 to nitrogen N-7 and N-9; and N9-CH_3 methyl protons to nitrogen N-9.

COSY experiments supported the proton-proton coupling relationship in **290** and **292**. COSY spectrum of **290** presents correlation between the signal a δ 2.54 ppm (H-11, triplet) and that at δ 3.60 ppm (H-10 quartet), and this signal in turn showed further coupling to an exchangeable proton at δ 6.54 ppm, resulting in an isolated spin system $XCH_2CH_2X'H$.

The proton resonances of the terminal isopropyl moiety of the tetraprenyl side-chain of **292** were assigned from a COSY spectrum while ^{1}H-^{13}C HMBC correlations confirm the structure of this side-chain and their linkage to N-7 of the bicyclic moiety of **292**.

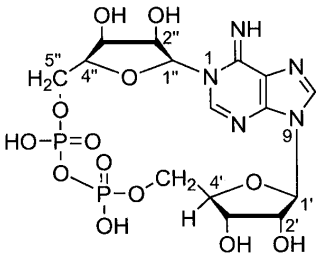


Numerous bioactive purine derivatives having a glycosyl residue have been isolated from marine sources and many NMR techniques have been used for complete structural elucidation [338,339]. COSY, COLOC and NOE experiments allowed assignments of ^{1}H and ^{13}C NMR chemical shifts as well as stereochemistry of trachycladines A **293** and B **294** (COLOC connectivities are show in the structures) [338]. The structure of a nucleoside isolated from the Crustacean *Ligia exotica* was elucidated as 3'-*O*-(α-D-glucosyl)inosine **295** by extensive analysis of 2D NMR spectra. The ^{13}C NMR chemical shift for the anomeric carbons C-1'' (δ_C 100.0 ppm) and the coupling constant for the anomeric proton of the glucose unit ($J_{H1''-H2''}$ = 3.4 Hz) indicate that the stereochemistry of glucoside is via an α-linkage [340].



Purine nucleotides can be cyclized by an enzymatic way. Aplysic cyclase catalyses the conversion of NAD (nicotinamide adenine dinucleotide), NGD (nicotinamide guanine dinucleotide) and NHD (nicotinamide hypoxanthine dinucleotide) into cyclic ADP-ribose (cADPR), cyclic GDP-ribose (cGDPR) and cyclic HDP-ribose (cHDPR), respectively. In these cyclic nucleotides, the new formed glycosyl bonds are attached on the nitrogen N-1 of the adenine nucleus on cADPR **296** and on nitrogen N-7 of the purine rings in the cases of cGDPR **297a** and cHDPR **297b**. ^{1}H-^{13}C HMBC spectrum of cGDPR **297b** showed that protons H-1' and H-1'' showed intense cross-peaks with carbon C-8 and H-8 is the only aromatic proton exhibited cross-peaks with carbon C-1' and C-1''. These data confirm the new glycosyl bond attached onto the N-7 position of the guanine ring [341].

^{1}H-^{13}C HMBC spectrum of cHDPR **297a** allowed distinguishes between C-2 and C-8. Proton H-8 showed correlation with C-5 and H-2 is coupled with C-6 and both showed one bond coupling with the protons attached to them. Once again, correlations of H-8 with C-1' and C-1'' and of C-8 and H-1' and H-1'' clearly show the glycosyl linkage on the N-7 position of the hypoxanthine ring [341].



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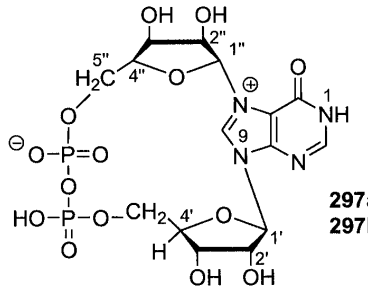
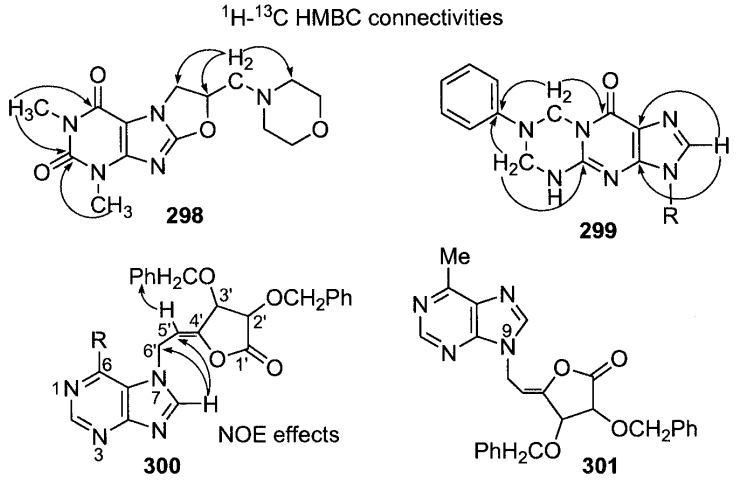


297a R = NH_2
297b R = H

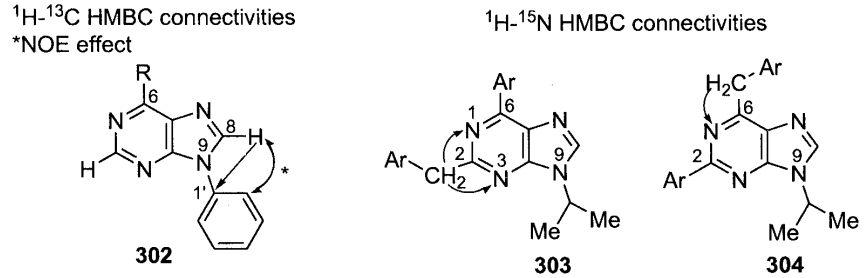
Compounds **298** and **299** are two examples of purine base fused to other cyclic structures. The results derived from ^{1}H-^{13}C HMBC (see structures) and NOESY spectra were especially informative about the substitution pattern in the fused analogs [342,343].

The structure of synthetic purine derivatives was determined on the basis of the ^{1}H and ^{13}C NMR chemical shifts and on the proton-proton coupling constants [344]. The regioisomers **300** and **301** were distinguished by the chemical shifts pattern of the purine moiety. Thus, H-8 resonance of the N-7 isomer **300** was shifted downfield (δ 8.88 ppm) relative to the corresponding one (δ 8.15 ppm) in the N-9-substituted molecule **301**. On the contrary, the signal of H-2 was shifted upfield (δ 8.30 ppm) in the N-7 derivative **300** relative to the H-2 signal (δ 8.75 ppm) of the N-9 derivative **301**. Furthermore, the equivalent *N*-methylene protons were more deshielded (δ 5.31 ppm) in the N-7 isomer **300** than the corresponding ones (δ 5.12 ppm) of the N-9 isomer 301. Vicinal coupling constant of protons in the side chain was greater in the N-9 than in the N-7 regioisomer. Later, the same group used NOESY experiments to establish the *E*-configuration across the C-4' and C-5' double bond (see structure **300**) [345].



Regioselectivity in the preparation of novel purine derivatives was proved by means of 2D NMR techniques. For compounds **302** the correlation in the ^{1}H-^{13}C HMBC spectra, between C-1 of the aryl substituent and H-8 in the purine indicate that the aryl group was located on the nitrogen of the imidazole moiety. Furthermore, NOESY spectra of these compounds displayed correlations between the *N*-aryl protons and H-8, but neither between the aryl protons and H-2 or of the protons of the 6-substituent [346].

^{1}H-^{15}N HMBC spectra was also used to distinguish between regioisomers **303** and **304**. The assignment of compound **303** was based on the presence of cross-peaks between methylene protons and nitrogens N-1 and N-3, while in the case of compounds **304** only the connectivity between methylene protons and nitrogen N-1 was observed [347].



1D and 2D NMR spectroscopy are important tools for the elucidation of synthetic purine nucleosides [348,349], novel complexes between purine derivatives and transition metals ions [350-352], the three-dimensional structures of nucleic acids [353,354] and the conformation of coenzymes and analogues in order to establish the coenzymatically active conformation at the enzyme active site [355].

7. References

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