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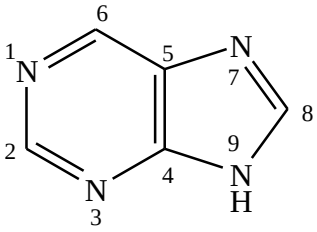
Complexing Properties and Applications of Some Biologically Active Nucleic acid Constituents

M. S. Masoud [a*], M. Sh. Ramadan[a], A. M. Ramadan[a] and M. H. Al-Saify[b]

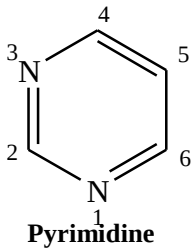
[a] *Chemistry Department, Faculty of Science, Alexandria University, Alexandria, Egypt.*

[b] *Sidi Kerir Petrochemicals Company, Alexandria, Egypt.*

In today's age of molecular biology purines and pyrimidines are probably best known as the basic constituents of the nucleic acids which are biomolecules that store genetic information in cells or that transfer this information from old cells to new cells. There are two groups of nucleic acids: deoxyribonucleic acid and ribonucleic acid, DNA and RNA, respectively(1). DNA codes for the functioning of the cell and RNA is the "worker" that helps get the DNA message out to the rest of the cell. DNA is located mainly in the nucleus of the cell (with a small amount in the mitochondrion of eukaryotic cells); RNA is primarily in the cytosol of the cell. DNA is double stranded and RNA is single stranded. DNA and RNA are long, threadlike polymers of deoxyribonucleotides and ribonucleotides, respectively. The monomeric nucleotides consist of a nitrogenous base, a sugar unit, and one or more phosphate groups. The nitrogenous bases fall into two classes: purines and pyrimidines (1).

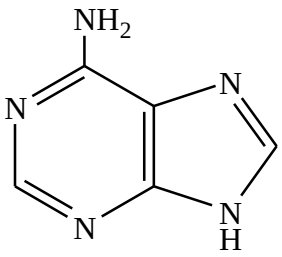


Purine

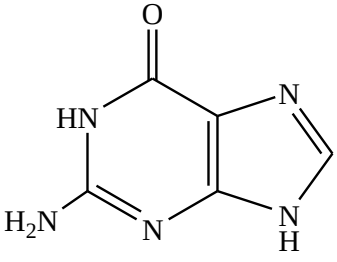


Pyrimidine

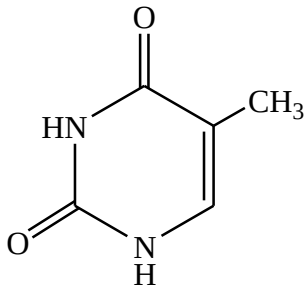
The four bases of DNA are adenine, guanine, thymine, and cytosine. Adenine and guanine are purines; thymine and cytosine are pyrimidines:



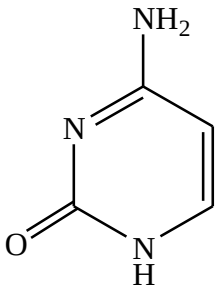
Adenine (A)



Guanine (G)

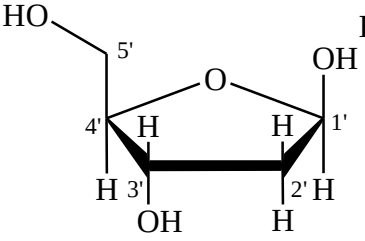


Thymine (T)

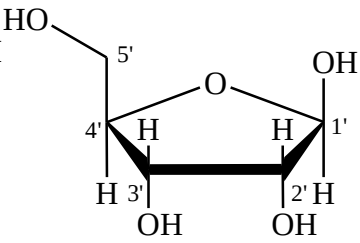


Cytosine (C)

A nucleoside consists of a sugar residue attached to a base unit. The sugar is deoxyribose in DNA and ribose in RNA:

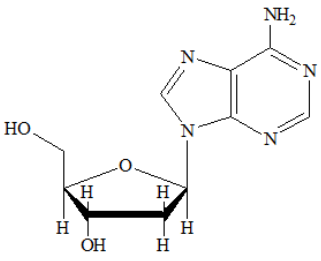


β-D-2-Deoxyribose

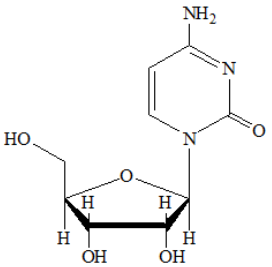


Ribose

The carbon atoms of the sugar units are distinguished from the atoms of the bases by the use of a 'primed' number. The bases are attached to C(1) of the sugar unit, through N(9) of a purine or N(1) of a pyrimidine:



Deoxyadenosine



Cytidine

The names of the four nucleosides in DNA are: deoxyadenosine, deoxyguanosine, deoxythymidine, and deoxycytidine. The four nucleosides of RNA are: adenosine, guanosine, cytidine, and uridine. This last nucleoside contains uracil, a pyrimidine which is used instead of thymine in RNA(1).

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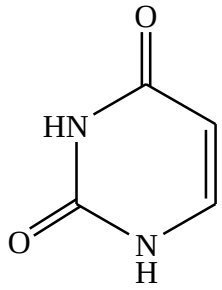
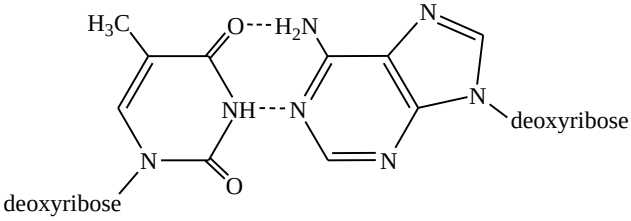
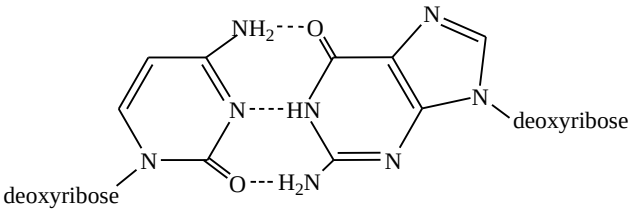


Uracil (U)

Genetic information is maintained in all organisms (with the exception of a few viruses) in the form of DNA. This fact wasn't established until the 1940s and 1950s. Prior to this discovery, it was believed that genetic information was passed on by proteins due to their more complex and differentiated nature. DNA is structured as a double helix and is often referred to as being double stranded. This discovery led to the explanation for the mechanism by which organisms can duplicate and pass on their genetic information. It was known that equal amounts of adenine and thymine and of cytosine and guanine were present in DNA, but the double helix model explained why: the two chains of the helix are held together by hydrogen bonding between (adenine-thymine) and (cytosine-guanine) residues[1].



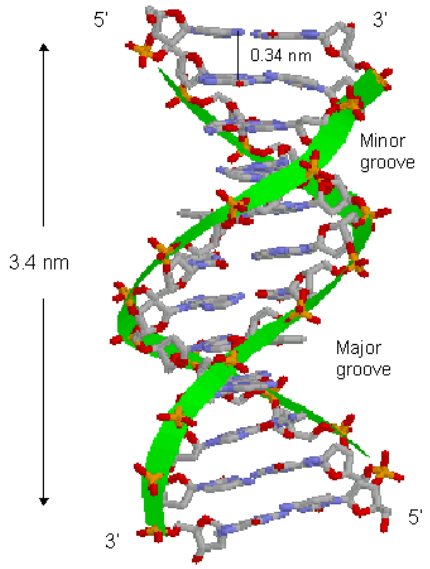
Thymine **Adenine**



Cytosine **Guanine**

Two hydrogen bonds are made between adenine and thymine, while three are formed between cytosine and guanine. Specificity of this base pairing is maintained by the hydrogen bonding in addition to steric factors. The helix is the right size for a purine to pair with a pyrimidine, two purines would be too big to fit, and two pyrimidines are too small and would leave a gap.

The sugar residues form the backbone of the molecule, and are located on the outside of the helix, forming grooves in the surface of the molecule. The interior contains the paired bases[1].



A- Biological activities

Barbituric acid derivatives are a well known class of compounds, many of which are widely used as drugs having such disparate pharmacological activities as depressants, hypnotics and stimulants. Hence, they have been the subject of numerous reports and investigations which have attempted to interpret their mode of action[2]. Additional incentives for studying these compounds stem from observation that they closely resemble several nitrogenous bases found in nucleic acids[3]. Taken in small doses, barbiturates produce depression of sensory function, and in larger doses cause depression of motor functions. Increasing the dose produces sedation, sleep or anaesthesia, and over produces coma and respiratory cessation[3]. Serum lipid peroxidation was measured by thiobarbituric acid reactive material method during physical exercise of different duration[4].

Many pyrimidine nucleobase analogs were evaluated as ligands of Toxoplasma gondii orotate phosphoribosyltransferase (OPRTase) by measuring their ability to inhibit this enzyme in vitro. A structure-activity relationship of ligand binding to OPRTase was formulated using uracil, barbituric acid, and orotic acid as reference compounds.

A necessary feature of the natural base triads for triplex formation is the requirement of a purine (A or G) in the central position[5], since only these provide sets of two hydrogen bond donors / acceptors in the major groove of the double helix. Pyrimidine bases devoid of this feature have incompatible complementarity and lead to triplexes with lower stability.

5-Aminouracil (AUH), a pyrimidine nucleobase analog of T in which 5-Me is replaced by 5-amino group, with hydrogen bonding sites on both sides, is compatible in the central position of triplex triad[6].

A growth-stimulating effect of 5-aminouracil (AUH) at 50, 100, and 150 μM on long-term callus tissue was found during investigations of callus tissue growth. Three years old callus tissues were cut and subcultured in culture medium containing different AUH concentrations. The callus tissues were then collected at 15-day intervals up to 45 days to determine individual callus weight. At 45 days, the callus tissues were collected and submitted to starch gel electrophoresis. The in vivo effect of the various AUH concentrations on onion root tip cell growth was also investigated, and a similar growth response pattern was observed. The interference of AUH with DNA replication for cell proliferation and tissue seems to affect important genes that modulate enzyme-specific activity or degradation of isoenzymes[7].

5-Halogenated-uracils act as chemical mutagens and as inhibitors of nucleic acid synthesis, and are consequently cancerostatic and antiviral agents. Metal ions sometimes have the stabilizing effects on the structure of nucleic acids[8]. 5-Fluorouracil was incorporated into the transfer RNA of *Escherichia coli* under conditions of growth and starvation. The tRNA was isolated and, in the latter case, further methylated in vitro. It was shown that tRNA isolated from growing cells as well as the tRNA methylated in vitro contained reduced levels of 5-methyluridylic acid. An analysis of the relation between the amount of 5-fluorouracil incorporated into the tRNA and the percentage decrease in the methylation of uracil to 5-methyluracil under conditions in vivo and in vitro indicated that the incorporation of 5-fluorouracil into the uracil position yielded non-random 5-methyluracil[9,10].

A number of uracil analogs were tested for their ability to inhibit nuclear and mitochondrial uracil-DNA glycosylase activities: 5-azauracil, uracil, 6-aminouracil, 6-azauracil, 5-aminouracil, and 5-fluorouracil all inhibited activities to variable degrees[11]. Also, 2-thiouracil, a RNA synthesis inhibitor, reduces the fertility of photoperiod sensitive genic male-sterile rice. The inhibition can be released by the application of uracil[12].

Some nucleobase analogous were screened as inhibitors of dihydrouracil dehydrogenase (DHU dehydrogenase) from mouse liver. 5-Nitrobarbituric acid was identified as potent inhibitors of this activity[13].

The inhibitory effects of 5-fluorouracil degradation in rat liver extracts catalyzed by dehydrouracil dehydrogenase (DHU dehydrogenase) were investigated[14]. The inhibitory activities of 5-substituted uracil derivatives decreased time-dependently during preincubation with rat liver extracts, indicating that these compounds are substrates of DHU dehydrogenase. During preincubation, 4,6-dihydroxypyrimidine derivatives were converted to barbituric acid derivatives, which have stronger activities. The inhibitory activity of 2,4-dihydroxypyridine and 3-deazauracil, which was stronger than that of uracil, did not change during preincubation, indicating that this compound is an inhibitor of DHU dehydrogenase[14]. These findings along with an extensive review of literature allowed the formulation of a structure-activity relationship[13].

Several compounds were evaluated as ligands of *Toxoplasma gondii* uracil phosphoribosyltransferase (UPRTase) by examining their ability to inhibit this enzyme in vivo[15]. 2-Thiopyrimidine, 1-deazauracil and 2,4-dithiouracil compounds bound to the enzyme better than two known substrates for *T.gondii* UPRTase, 5-fluorouracil and emimycin, which have antitoxoplasmal activity.

Several selected compounds were evaluated as substrates for *T.gondii* UPRTase, and 2,4-dithiouracil is also a substrate for this enzyme[15]. Also, a number of nucleobase analogs were evaluated as potential inhibitors of *Toxoplasma gondii* uridine phosphorylase (UrdPase)[16]. Based on the inhibition data, a structure-activity relationship for the binding of nucleobase analogs to the enzyme was formulated using uracil as a reference compound. *T.gondii* results are similar to those of mammalian UrdPase and thymidine phosphorylase (dThdPase). 6-benzyl-2-thiouracil, was identified as a potent, specific inhibitor of *T.gondii* UrdPase, relative to mammalian UrdPase and dThdPase[16].

By using an electron-topological approach, the structural and electronic features of many uracil derivatives, inhibitors of thymidine phosphorylase (dThdPase), were evaluated[17]. The approach contained quantum chemistry calculations of the electronic structure. The results allowed for a prognosis of inhibitory activities of the compounds and their rational synthesis[17]. Such features as tautomerization, intramolecular H-bonding, and σ- and π-electron-accepting and donating abilities of substituents were considered[18].

A comparative study of the growth-regulating activity of derivatives of uracil and its 6-aza analog showed that 5-aminouracil (AUH) inhibited lettuce and oat growth more than did uracil, whereas uridine did not inhibit growth. The inhibitory activity of 5-amino-6-azauracil was weak and depended on the time of treatment[19]. However, exposure of Crepis capillaries seeds to X-irradiation induced chromosome aberrations in 25 and 36%, respectively. AUH had no significant effect on chromosomes alone but potentiated the effect of X-irradiation when added at the phase of the cell cycle following irradiation[20]. However, 5-arylazopyrimidines exhibit inhibitory effects against transplantable mouse tumors. At non-toxic dosages, several of these agents showed effective tumor growth inhibition, as evidenced by reduction in tumor size[21,22].

Since most living systems contain metal ions which are essential for proper functioning, question

arises as to study the effect of such metal ions on nucleic acids. Any elucidation of metal ions effects on the pyrimidine nucleus could possibly lead to a better understanding of complex biological processes occurring in living system. Copper, together with magnesium, calcium, iron, zinc, chromium, vanadium and manganese, is one of the essential metallic elements. It possesses great biological activity when associated with certain metal-protein complexes which participate in the transport of oxygen and electronic transfer reactions[23,24].

Platinum group metal complexes of nucleic acid bases and their derivatives attracted considerable attention because of their antitumor and antibacterial activity[25,26]. Platinum(II) and palladium(II) form cis and trans square-planar complexes. The cis isomer is very active. The discovery of cis-dichlorodiamineplatinum(II) (cis-DDP, cisplatin) as a cancer chemotherapeutic agent aroused much interest in the synthesis of more potent and less toxic drugs[27]. The biological activity of cisplatin is due to its ability to bind the guanine-cytosine of the DNA strand and stop the replication process[28]. Cisplatin has been used in treating several human tumors of the genito-urinary type[29].

Platinum pyrimidine blues, which are oligomer compounds derived from cisplatin and pyrimidine bases, are reported to show antitumor activities[30]. The advantage of these platinum blue complexes over the well known cisplatin is their low nephrotoxicities[31]. A series of other platinum greens[32-34] were also synthesized effectively and selectively from aquated cis-$Pt(NH_3)_2I_2$ and pyrimidine derivatives with a carefully controlled amount of H_2O_2 under Ar. This method can be used with different counter anions in the starting aqua-compound like SO_4^{2-}, NO_3^- and ClO_4^-[33,34].

The relation between HPLC behaviour of Pt pyrimidine greens and their biological activity against leukemia cells in vitro was studied[35]. The results demonstrated that Pt-pyrimidine greens consists of three fractions with three retention times. Peak 3 may be responsible for the growth inhibitory activity against the cells[35]. The antitumor activity was affected by synthetic conditions, mainly temperature and nature of ligands. Pt-uracil green synthesized at 40°C showed the highest activity among all prepared Pt-greens[32]. It is evident that high selective Pt-pyrimidine green complexes exhibit remarkable anticancer activities[36-38].

Palladium(II) complexes with potential bidentate ligands containing ON and NS donor atoms have aroused considerable interest due to their use in cancer chemotherapy[39]. PdL_2Cl_2 (L=5-aminouracil (AUH)) and PdL'Cl (L' = dye derived from β-naphthol and AUH) were prepared and studied. These complexes undergo complete decomposition above 215°C to form palladium oxide. The complexes have been screened for their possible antitumor activity in vitro using Dalton's Lymphoma Ascites tumor cells[40]. It is evident that antitumor activity is associated with the cis-configuration of the complexes[41] and for monodentate ligand complexes, the cis-complex may isomerize into the thermodynamically more stable trans-configuration. Bidentate ligands exclude this possibility. In addition, several ternary complexes of platinum(II) and palladium(II) with amino acids and purines, substituted pyrimidines and nucleosides have been reported[42,43]. Some of these complexes are biologically active against human pathogens[44].

6-Amino-2-thiouracil (ATU) exhibits antiviral and chemotherapeutic activities[45]. The X-ray study of this compound showed that the thione form is the predominant in the solid state[46].

Studies on the coordination selectivity of multidentate ligands toward various metal ions are of fundamental importance in various areas, e.g. the design of ligands as therapeutic reagents for the treatment of metal intoxication[47], design of complexes as imaging agents in the body[48], and selective complexation of one isomer can be useful for the separation of diastereomeric ligand pairs[49,50].

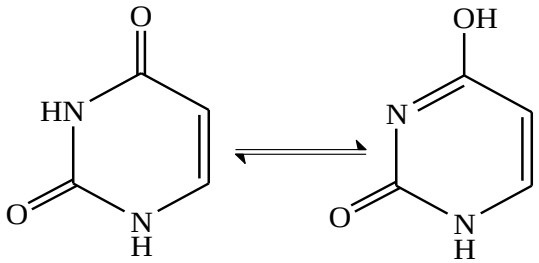
B- Structural chemistry

The binding of cations to biomolecules such as nucleic acid constituents has been studied intensively by X-ray crystallography[51-53]. Crystal structure of the polymeric complex of zinc(II) with cystosine 5'-phosphate, $(C_9H_{12}N_3O_8P)Zn(H_2O)$, showed that the complex is 2-dimensionally polymeric and the Zn atom is tetrahedrally coordinated to N(3) of the pyrimidine, to 2 phosphate O atoms, and to 1 H_2O molecule. The Zn atom also formed weak intramolecular interaction with O(2) of the pyrimidine[54].

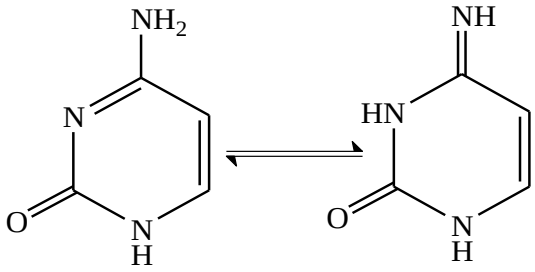
A knowledge of relative stabilities of tautomeric forms of N-heterocyclic molecules as well as of the tautomeric conversion from one tautomeric form to another is important from the point of view of structural chemistry. A large amount of experimental and theoretical work has been performed in order to understand the phenomenon of tautomerism, and to estimate the relative stabilities of tautomeric forms of nucleic acid bases and related model systems[55,56]. An ab initio study is presented for calculations of the relative stability of tautomers of uracil, cytosine, isocytosine and some of other thio derivatives[57]. Substitution of atoms not directly involved in the tautomerism has no appreciable effect on the gas phase enthalpy values. However, thio substitution of the O involved in the tautomerism produces a general and small shift favouring the thiol isomer[57].

The contribution of electron correlation to the relative stabilities of tautomeric forms of nucleic acid bases is assessed[58]. Different levels of many-body perturbation theory (MBPT)[59] up to full fourth-order

(MBPT(4)) and the coupled-cluster (CC)[60] single and double excitation model including the effects of triple excitation contributions are used to study the relative stabilities of models of tautomers of uracil and cytosine, with MBPT(2) being used for the complete molecule. Such "many-body" methods are most appropriate for large molecules because of their size-extensive nature[59]. The general phenomena of lactim-lactam and amino-imino tautomerism is analyzed with reference to formamide, formamidine, formamidic acid, and the lactim-lactam tautomers of 2-oxopyridine.



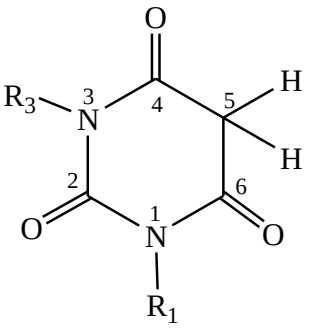
Ura ⇌ Ura*



Cyt ⇌ Cyt*

The electron correlation contributions favoured the lactim tautomer, and are a significant correlation for the relative energies of the lactim-lactam pair of cytosine, although for uracil it is not of importance. In the case of amino-imino tautomers of the bases, the electron correlation contributions are greater for the amino forms[58]. MNDO recalculations with full geometry optimization for the amino-type tautomer of cytosine predicted non-coplanarity of the amino nitrogen atom with heteroaromatic rings. However, bond lengths, valence, bond angles, dipole moments and ionization potentials of the coplanar and non-coplanar forms do not differ significantly[61].

Substituent and solvent effects on tautomeric equilibria of barbituric acid derivatives have been studied intensively[62]. The ^{13}C NMR of N-alkyl and N-aryl substituted barbituric acids showed that they all exhibit C(5) methylene carbon signal of the keto form in DMSO, N-methylformamide, deuteriochloroform, hexadeuterioacetone and monodeuteriomethanol. However, the characteristic absorption spectra of the C(5) "vinyl" group of the enol form could not be detected. Thus, the N-alkyl and N-aryl compounds adopt the triketo form (I) in polar and non-polar solvents were used for studies[62].



(I)

$R_1 = CH_3$, $R_3 = H$

; $R_1 = R_3 = CH_3$

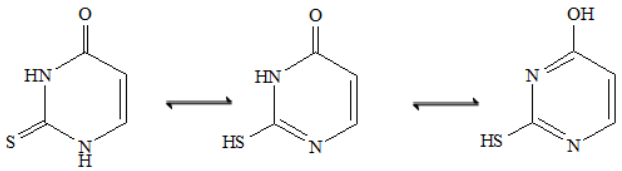
; $R_1 = C_6H_5$, $R_3 = H$

; $R_1 = R_3 = C_6H_5$

2-Thiobarbituric acid derivatives, showed extensive tautomerization[63]. Their ^{13}C chemical shift assignments were achieved by utilizing models from which relative tautomer distribution ratio were determined. These ratios were correlated with the dielectric constant of the various solvents[62].

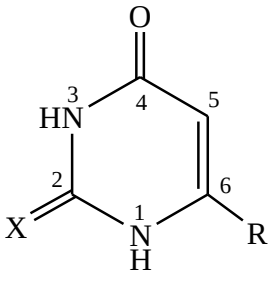
The relative stabilities of a series of tautomers of 2-thiouracil (TU), 6-amino-2-thiouracil (ATU) and 6-aminouracil (6-AU) were investigated using the all-valence CNDO/2 method. For TU and ATU, the thione-enol form is the most stable whereas in 6-AU the keto-enol form is the most stable, indicating that substitution of S does not alter the equilibrium of the tautomers drastically[64].

In our laboratory, Masoud *et al.*[65-71], published a series of papers to give spotlights on the structural chemistry of many pyrimidine compounds and their arylazo derivatives, mainly from tautomerism viewpoint[71]. The ^1H NMR spectrum of 2-thiouracil exhibits only two doublets assigned to C(5) and C(6) protons. The amide protons were not detected in the spectrum and the data suggest that the following species are in rapid equilibrium.



However, the ^1H NMR spectra of arylazothiouracils showed doublet peaks at 5.6-5.9 ppm assigned to H(6) as a result of coupling with N(1) proton. Signals in the 6.9-7.9 ppm range were assigned to the phenyl group. All spectra exhibit a broad peak in the 11.3-12.4 ppm range assigned to NH groups. The absence of peaks for the OH and SH functions suggested that arylazothiouracils exist mainly in the keto-thione form[71].

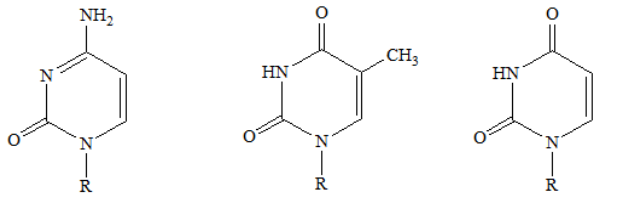
The dissociation constants of substituted uracils (I) and thiouracils (II) were determined spectrophotometrically[72]. The pK_1 values of thiouracils were lower than those of the corresponding uracils. The effect of substituents at the 6-position of these compounds was examined. Amino and Ph groups, compared with alkyl group, decreased pK_1 values. Further, the optimal experimental conditions for the reaction of thiouracils with H_2O_2 were established where uracils were the end product. This reaction was fairly applied to the quantitative determination of thiouracils[72].



I , **X** = O

II , **X** = S

Many pyrimidine nucleosides are important in lipid and carbohydrate metabolism. The thermodynamic parameters (ΔG°, ΔH°, and ΔS° values) were reported potentiometrically for proton ionization of cytosine, cytidine, thymine, thymidine, uracil and uridine[73].



I
Cytosine, R = H
Cytidine, R = ribose

II
Thymine, R = H
Thymidine, R = deoxyribose

III
Uracil, R = H
Uridine, R = ribose

The thermodynamic data indicated that the sites of ionization in thymidine and uridine are sufficiently remote from each other.

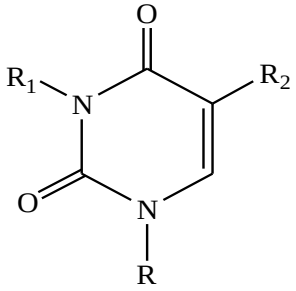
The second proton ionization in each case is largely unaffected by the increased negative charge resulting from the ionization of the first proton. Thymidine has the highest second pK value, probably because of the presence of the deoxyribose moiety[73].

The acid dissociation constants of some 2- and 6-mercaptopyrimidine derivatives with amino or hydroxy substituents were determined spectrophotometrically. A number of significant known regularities were readily apparent in observing the pK_a values. For example, the sulphur-containing compounds are invariably less basic and more acidic than their oxygen analogous. Dithiouracil has pK_a values (6.34 and 11.09) about 1.5 units lower than 2-thiouracil (7.71 and 12.79). A similar behaviour was observed in the case of 4,5-diamino-2,6-dimercaptopyrimidine (2.00 and 5.07) and 2-mercapto-4,5-diamino-6-hydroxypyrimidine (3.35 and 6.61)[74]. Also, the dissociation constants in isopropyl alcohol medium were determined by potentiometric titration with tetrabutylammonium hydroxide. Because of ion-pair formation, the incomplete dissociation of the tetrabutylammonium salt was taken into account in the calculations of pK_a[75].

Electronic-absorption and fluorescene-emission spectra of several pyrimidines were measured at room temperature (298 K) in different solvents[76]. In combination with the ground-state dipole moments of these compounds, the spectral data were used to determine their lowest excited singlet-state dipole moments by solvatochromic method. The effects of the solvent upon the spectral properties and of the structure upon the ground and excited singlet-state dipole moments were discussed[76]. For most of the compounds, the excited singlet-state dipole moments are higher than their ground-state counterparts[76].

Free radical formation by reaction of water radiolysis intermediates produced by X-irradiation with pyrimidine and purine constituents of DNA was studied by ESR spectroscopy[77]. Reactions of electrons and $^{\bullet}OH$ radicals were observed; $H^{\bullet}$ formed a minority and its contribution was difficult to establish. For the $^{\bullet}OH$ radical, the reaction with uracil and cytosine was found to be addition to 5,6-double bond. For all the methylated pyrimidines, H-abstraction from the methyl group was dominant[77].

Carbon-13 NMR spectroscopy, in contrast to ^1H NMR spectroscopy, provided a clear distinction in a variety of N heterocyclic systems between O–Me and nuclear N–Me groups. MeO groups occur in the range δ 53.20-61.87 ppm, nuclear N–Me groups at 34.29-49.62 ppm, and MeS groups at 12.35-14.55 ppm in $CDCl_3$. Data for N– and O–Me derivatives of pyridine-2 and -4-ol, the corresponding pyrimidines, and some S analogs were compared with those for the unmethylated parent compounds[78]. Further, the characterization of mono- and dimethylated 5-substituted uracils I (R, R_1 = H, Me; R_2 = H, Me, Br, NO_2 and NH_2) was examined. Analysis of their physicochemical properties (pK_a, UV, ^1H NMR) afforded structural characteristics of 5-substituted uracils alkylated at the ring nitrogens[79].



I

^{1}H NMR spectroscopy and allied analytical techniques have provided important insights as to the conformation of pyrimidine nucleosides.

A support for the requisite anti conformation of pyrimidine nucleosides in solution was provided from CD spectral data[80]. Additionally, theoretical and experimental ^{1}H, ^{13}C, ^{15}N, and ^{17}O NMR spectral parameters of urcail and 5-nitro-, 5-amino- and 5-carboxyuracils were determined and analyzed[81]. The effect of substiuents on the chemical shifts was studied and the spin coupling constants were measured[81].

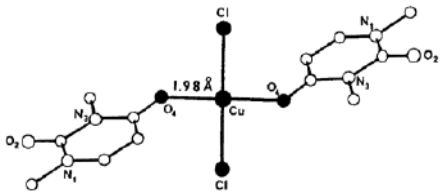
Vibrational assignments[82-89] of six-membered heterocyclic compounds have been reported using IR and Raman spectroscopies. 6-Aminouracil and 6-amino-2-thiouracil (ATU) and their N-deuterated derivatives were determined at 4000-200 cm^{-1} [82]. However, 5-chloro and 5-aminouracils Raman spectra were measured at 2000-50 cm^{-1} using an Ar laser excitation source[83]. Also, IR and far IR spectra of ATU, 2-amino-4-hydroxy-6-methylpyrimidine have been studied[84]. Tautomeric behaviour, effects of solvents and pH on n-π^* and π-π^* transitions of these molecules have been investigated. Bathochromic and hypsochromic shifts observed in electronic transitions on changing from neutral to cationic or anionic forms have been explained[84]. A similar study was carried on the IR spectra of 4-amino-2,6-dihydroxypyrimidine. The thermodynamic functions were evaluated and discussed[85]. The structure of uracil in aqueous solutions of different pH values was achieved by Raman scattering[86,87]. The results obtained reveal that Raman lines associated with the stretching and bending vibration modes of uracil ring around 1000 and 1240 cm^{-1} are sensitive to the pH of the solutions[86]. The data showed considerable promise as a tool for characterizing mixtures of nucleic acid compounds[87].

IR spectroscopic study of uracil derivatives and their hydrogen-bonded complexes with hydrogen chloride in Ar matrixes was reported[88]. The spectral characteristics demonstrate that uracils form a C(4)=O---H–Cl hydrogen bond of intermediate strength. For some of the bases a small amount of the C(2)=O---H–Cl species is identified in Ar matrixes containing an excess of HCl. H-bonding of the C=S group played only a minor role in thiouracils. For all the bases studied, N–H---Cl–H structures were also identified from the shifts of the uracil N–H modes[88]. However, the carbonyl stretching region in somewhat peculiar and ν(C=O) modes were considered the most important modes of nucleic acid base derivatives, which take part in hydrogen bonding[89].

DNA strands can curl up to produce some amazing structures, and they can bind to metal ions. DNA has served as an ingenious storage device for genetic data for more than three billion years. But only few years ago, it has also emerged as a powerful material for building complex structures at the nanometer scale. It has also been known for many years that DNA strands can get involved in coordination chemistry with a variety of metallic species: magnesium ions and the antitumor agent cisplatin (cis-dichlorodiamineplatinum(II)).

There has been a growing interest in the interaction of metal ions with nucleic acids. Such interactions result in physiological effects which can be either detrimental or beneficial[90,91]. However, study of such large biological systems with respect to metal ion binding is very complex due to the presence of a plethora of potential metal binding sites on the nucleobases, the ribose or deoxyribose sugar, and also on the phosphate backbone. Therefore, to aid in the understanding of these important systems, much work has concentrated upon the study of metal ion interaction with isolated nucleobases such as analogous substituted pyrimidines. For instance, 1-methyluracils are considered since they are generally agreed to be good models for the uracils when present in nucleic acids since, the uracils bound through N(1) to the sugar[92].

An ab inito study of tautomerism in uracil, considering the relative stabilities of all tautomers in both gas phase and solution has been performed[93]. It has shown that uracils exist predominantly in the diketo form in both the solid state[94] and in neutral aqueous solution and consequently, coordination of metal ions would be expected to occur through the exocyclic oxygen atoms. 1,3-Dimethyluracil was considered as a reasonable model for neutral uracil (UH) and the crystal structure of its Cu^{II} complex has been reported[95].



Cu(1,3-dimethyluracil)Cl_2

The short (1.98 Å) metal-O(4) bond indicated fairly strong metal-O(4) binding in metal-uracil complexes in accordance with the stability of these complexes to heat[96].

Transition metal salts react with uracil (L) in methanol giving $[ML_4(H_2O)_2]X_2$ where: M = Mn, Fe, Co, Ni and Cu; X = NO_3, Cl or ½ (SO_4) which were characterized by spectral measurements[97]. Uracil is coordinated through the O(4) site. $[ML_4(H_2O)_2]Cl_2$ decomposed at 120-300°C with the loss of 2 H_2O and 2L molecules to give ML_2Cl_2[97]. The Cu(5X-uracil)$_2$.nH_2O complexes (X = NO_2 and I; n = 2 and 1, respectively) were seen to exhibit unique magnetic properties which seemingly depend on the nature of the substituent at the 5-position of the uracil ring[98]. Under high pH, uracil may bind to metal

ion in the form of their monoanion (U) via either N(1) or N(3), with the adjacent carbonyl oxygen atoms offering the additional possibility of chelation and bridging. The data of non-electrolytic $[MU_2(H_2O)_2]$ complexes (M = Mn, Co, Ni and Cu) indicated the hexa-coordination of the metal ion with chelation of the uracil through C(2)=O and N(3). The small change observed for ν(C(4)=O) is probably due to hydrogen bonding between C(4)=O and the coordinated water molecules[99]. Also, $ML_2(H_2O)_4$ (M = Mn, Fe, Co, Ni and Cu; HL = uracil) were prepared and characterized by TGA and electronic and IR spectra[100]. The complexes are non-electrolytes and the M-L binding is through N(3).

In the same manner, some complexes of Cu^{II}, Ni^{II}, Co^{II} and Zn^{II} with uracil, thymine or 2-thiouracil have been synthesized and characterized[101]. Thiouracil acts as a tridentate ligand coordinating through the one carbonyl oxygen, thiocarbonyl S and one N. The Zn^{II} complexes have binuclear structures with tetrahedral arrangement around each zinc atom[101].

Complex formation between Be^{II} or Hg^{II} and 5-substituted uracils was studied at 31°C by a potentiometric method. Stability constants of the uracil complexes were increased in the order 5-nitro < 5-bromo < 5-iodo < 5-Me < 5-amino[102]. Further information on the formation of metal-complex and calculation of equilibrium constants values for Hg^{II} and Be^{II} ions were deduced by applying electrochromatographic method[103]. The method is based on the migration of a metal ion spot on the chromatographic paper strip soaked background electrolytes containing the ligand under electric influence. Ionophoretic mobilities were recorded at different pH's and a plot of mobility versus pH was given[103].

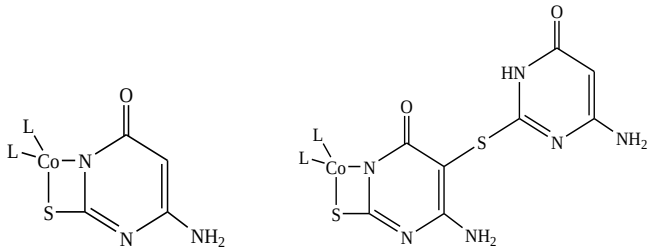
Interaction of Fe^{III}, UO_2^{II}, Mn^{II}, Ni^{II} and Cu^{II} with 4-amino-2,6-dihydroxypyrimidine was studied potentiometrically at room temperature in aqueous medium. The pH titration data were employed for the calculation of proton-ligand and metal-ligand stability constants[104].

A room temperature reaction of $Co(OAc)_2.4H_2O$ with pyrimidine-2-thiones (Hypymt), in MeOH, was carried out in air. The substituent group at a 4-position in the pymt skeleton governs the product. The pymt and its derivatives, gave tris-type Co^{III} complexes mer–$[Co(N–S)_3]$ in good yields. However, the 2-thiouracil derivatives having an –O or a –S atom as the 4-substituent, produced only bis-type Co^{II} complexes $[Co(N–S)_2]$. Such behaviour concerned the different electronic structures of the pyrimidine rings[105].

The study of thiopyrimidines is an area of great interest owing to their multiple potential sites for binding metal ions[106-109]. Thermal studies on metal complexes of pyrimidines and, in particular, of thiopyrimidine derivatives permit coordinated and uncoordinated solvate molecules, such as water or ammonia. Also, dehalogenation and decarboxylation processes may be detected and their enthalpies were calculated[110-112]. The thermal decomposition processes of 6-amino-2-thiouracil (H_2ATU) and its Co^{II}, Ni^{II}, Cu^{I}, Ag^{I}, Zn^{II}, Cd^{II} and Hg^{II} complexes were studied by TG and DSC technique[112]. Spectral studies on the isolated complexes, $[Co(HATU)_2(H_2O)].2H_2O$; $[Ni(HATU)_2(H_2O)_2]$; $Cu(HATU).H_2O$; $Ag(HATU).\ 0.5H_2O$; $Zn(ATU).\ 1.5H_2O$; $Cd\ (ATU).H_2O$; and $Hg(HATU)_2$, have suggested that the coordination mode of H_2ATU to these metal ions can occur in (i) a monoanionic form ($HATU^-$; chelating bidentate form through the N(1) and S(2) atoms), (ii) a dianionic form (ATU^{2-}; bridging ligand where N(1), N(3), O(4) and S(2) atoms being involved)[112]. Relatively stable intermediates in the pyrolytic process were isolated, their chemical nature was inferred by means of other techniques, such as IR and UV-visible-near-IR spectroscopies and magnetic measurements[112,113].

HL (L = thiouracil, 6-amino-2-thiouracil) reacted with $[NEt_4]_2[NiCl_4]$ in MeCN to give $Et_4N\ [NiL_3]$, where Ni^{II} is an octahedrally coordinated[114].

Thermal reaction between trans-$[CoCl_2(en)_2]Cl$, ATU (6-amino-2-thiouracil) and NaOH in the presence of an activated charcoal produced two red complexes I, II.



I **II**

L=en

Complex II has an unusual dimerization of 6-amino-2-thiouracilate ligand where a new bond is formed between the C(5) atom of the coordinated ATU and the S(2) atom of an uncoordinated ATU. This unique reactivity of ATU and the characterization of the resultant complexes were described by elemental analysis, UV-visible, ^1H and ^{13}C NMR spectroscopy and crystal-structure analysis[115].

The behaviour of 6-aminouracil and its N–Me derivatives (L) in various media and their reactions with cis-$[Pt(NH_3)_2Cl_2]$ in H_2O were studied spectroscopically. $[Pt(NH_3)_2L_2](NO_3)_2$ complexes were isolated and characterized[116]. In neutral media, 6-aminouracils act as bidentate ligand, formed a covalent bond with the Pt^{II} through the uracil ring C(5). The

exocyclic amino group acts as an additional donor atom[116].

The chemistry of Au^{III} is much less developed than that of the isoelectronic and often isostructural Pt^{II} atom. The first crystallographically characterized Au^{III} complex with a pyrimidine derivative is the N(3)-bonded compound with 1-methylcytosine[117]. In the same manner, a gold complex was isolated[118] by reacting $HAuCl_4$ with 6-amino-1,3-dimethyl-5- (2-chlorophenylazo) uracil (DZCH) to give $[Au(DZC)Cl_2]$ complex.

X-ray diffraction showed that the crystal of $[Au(DZC)Cl_2]$ contains individuals complex molecules in which the metal has a slightly distorted square-planar coordination. Two cis corners are occupied by Cl ligands. The uracil derivative formed a six-membered chelate ring via the deprotonated amino group and the phenyl-substituted nitrogen atom of the azo group[118].

Au^{III} and Pt^{II} react with 6-amino-2-thiouracil to provide coloured crystalline complexes whose structures were determined through elemental analysis, IR and ^1H NMR spectra. The existence of the divalent oxidation state of Au is inferred from a study of the ESR absorption spectra[119].

The crystal structure of the complex bis (6-amino-1,3-dimethyl-5-phenylazouracilato) palladium(II) was determined by X-ray crystallography[120]. The M-L binding is through the nitrogen atom of the deprotonated amino group and the nitrogen atom of the azo group bonded to the phenyl ring, to give a square-planar PdN_4 geometry with Pd^{II} at the inversion center. The entire molecule is planar with the exception of the phenyl rings which are twisted about the C-N bond away from the coordination plane to overcome inter-ligand steric crowding[120].

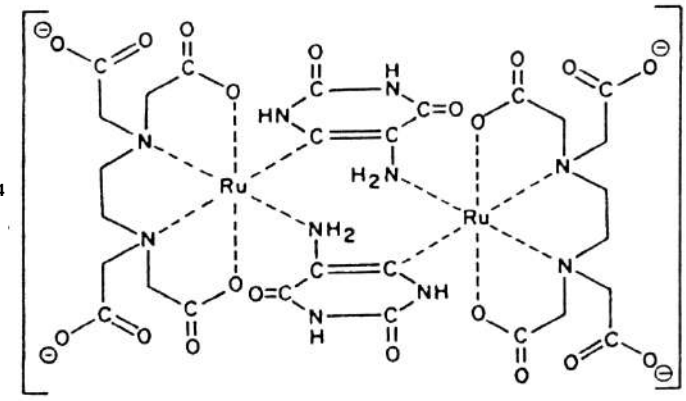
Platinum(II) and palladium(IV) complexes with uracil, 5-aminouracil and its methyl derivatives were prepared and characterized[121-124]. For instance, trans-ML_2X_2 (M = Pd, Pt; X = halo; L = 5-aminouracil, 5-amino-6-methyluracil and 5-amino-1,3,6-trimethyluracil) were synthesized, where L coordinated through the exocyclic amino N atom[123]. However, cis-$[(NH_3)_2Pt(1\text{-}MeUH)_2](NO_3)_2.3H_2O$ and cis-$[(NH_3)_2Pt(1\text{-}MeUH)_2][PtCl_6].2H_2O$ complexes were isolated containing neutral 1-MeUH (1-methyluracil) ligand in the 2-oxo-4-hydroxo tautomeric form which was estimated on the basis of the X-ray results[124].

The crystal structure of the complex bis(μ-6-amino-3-methyl-5-nitrosouracilato-N(5), O(4), N(1), O(2))cadmium(II) ($Cd(AMNU)_2$) is described to determine the coordination properties of a nitrosouracil derivative with the N(3) position blocked[125]. The structure consists of a sequence of complex polymeric sheets, linked by hydrogen bonds. Each ligand is coordinated to two different cadmium atoms, and each cadmium atom is coordinated by four pyrimidine ligands. These ligands are equivalent with one cadmium atom binding through N(5) and O(4) atoms, while the other cadmium atom binds through the N(1) and O(2) sites[125].

Mixed ligand complexes played an important role in biological systems. Binary and ternary complexes of UO_2^{II}, Ni^{II} and Zn^{II} with uracil and nitrilotriacetic acid were studied at 35°C and ionic strength 0.1 M ($HClO_4$). The stability constants of the 1:1:1 mixed-ligand complexes were calculated[126]. Besides, the interaction of UO_2^{II} with 4-amino-6-hydroxy-2-thiopyrimidine (AHTP) in presence of oxalic acid, malonic acid, o-aminophenol and 1,10-phenanthroline has been investigated by potentiometric method. The ternary complexes are formed in stepwise manner in which AHTP behaves as a secondary ligand[127].

Mixed ligand complexes of Cu^{II}, Ni^{II}, Co^{II} and Zn^{II} formed with glycine (gly) and uracil (U) or 2-thiouracil (TU) have been synthesized and characterized[128]. Results showed that glycine is bidentate in all cases; uracil behaves as a bidentate ligand in Cu^{II} complex, $Na_2[Cu(gly)(U)(OH)_2].H_2O$, coordinating through its one carbonyl oxygen and nitrogen, whereas in other cases it is only monodentate, coordinating only through nitrogen. However, the Cu^{II} complex, $[Cu_2(OH)_2(gly)(TU)].2H_2O$, has a binuclear structure, with square-planar arrangement around each copper atom[128].

Mixed ligand complexes of ruthenium (III) edta with uracil, 2-aminopyrimidine, 2-thiocytosine and 5-aminouracil (5-amura) were synthesized and characterized[129]. 5-Aminouracil formed an interesting organometallic complex ($K_4[Ru(edta)(5\text{-}amura)]_2.5H_2O$. In this complex, the carbon C(6) of 5-aminouracil forms a covalent bond with Ru^{III}, with simultaneous coordination of the nitrogen of the exocyclic amino group to a second ruthenium atom forming a 2:2 diligand bridged bimetallic complex[129].



Structure of $K_4[Ru(edta)(5\text{-}amura)]_2.5H_2O$

Mixed ligand complexes of cis-dichloro (ethionine) platinum(II) and cis-dichloro

(ethionine)palladium(II) with substituted pyrimidines, 2-hydroxy-pyrimidinehydrochloride, isocytosine, 2-mercaptopyrimidine, 5-aminouracil, 2-thiocytosine, and 2-thiouracil were synthesized and characterized[130]. Based on the spectral data, ethionine coordinates to the metal ion through S and amino N, leaving a free carboxylic acid group. The 2-thiocytosine and 2-thiouracil ligands are of bidentate behaviour, coordinating to the metal ion through ring N (N(3)) and S while 5-aminouracil coordinates through the amino (NH_2) group[130].

In absolute ethanol medium, copper(II) nitrate reacted with 6-amino-1,3-dimethyl-5-nitrosouracil (HDANU) and 2,2′-bipyridine gave a mixed complex [Cu(DANU) (bipy) (EtOH)](NO_3)[131], consisting of monomeric CuN_4O units in which the copper ion displayed a square-pyramidal coordination[131,132]. The antiferromagnetic (singlet) ground state structure of $[CuLpy]_2.0.5H_2O$ are described (L = di deprotonated form of 6-amino-1,3-dimethyl-5-((2-carboxyphenyl)azo) uracil and py = pyridine)[133], this complex has discrete dinuclear entities from pairwise association of mononuclear fragments via two extended bridging networks Cu–O–C–O---H–N–Cu, which comprised long hydrogen bonds.

To design and synthesize polynuclear metal complexes, one of the best and common strategies is the use of mononuclear complexes as ligands. These complexes must contain donor groups for another metal ion or metal complex with empty coordination sites[134]. The reaction of H_2L [H_2L= N,N′-bis (1,3-dimethyl-5-nitrosopyrimidine-2,4 (1H,3H)-dione-6-yl) propylenediamine] with an excess of Cu $(ClO_4)_2.6H_2O$ in ethanol gave the dinuclear complex [Cu(μ-HL)Cu$(H_2O)_2$(EtOH)][ClO_4]$_3$. This compound reacted with NaCl in ethanol-water (5:1) to afford the mixed-bridged trinuclear copper(II) complex [(Cu(μ-HL)Cu(H_2O)Cu(μ-L))(μ-Cl)][ClO_4]$_2$.4.88H_2O, the crystal structure of which was solved by single-crystal X-ray diffraction[135].

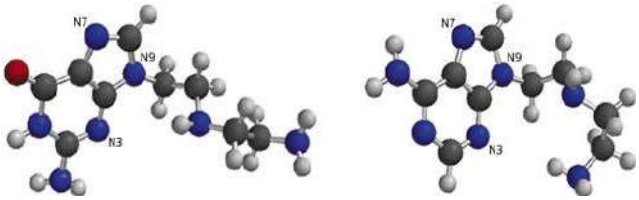
Masoud *et al.*[136-159] published a series of papers to give deeper insight on the structural chemistry of some pyrimidine compounds, azo derivatives beside their complexes. The mode of studies was based on the use of different spectroscopic methods (UV-VIS, IR, NMR, ESR), magnetic susceptibility, thermal and electrical conductivity data.

In a development mainly pioneered by Nadrian Seeman's group at New York University, strand complementarity and the editing procedures provided by modern molecular biology allowed researchers to build two- and three-dimensional objects including cubes, tiles etc. from specifically designed strands of DNA[160]. Two laboratories, those of Bernhard Lippert at the University of Dortmund, Germany, and of Andrew Houlton at the University of Newcastle upon Tyne have used the metal coordination of the DNA building blocks as a tool to construct interesting new molecular structures. Lippert's group systematically exploited the geometrical properties of metal-nucleobase complexes to create pre-designed supramolecular assemblies[161].

Houlton's group extended the approach to other metal centres and more complex geometries. They attached an alkyldiamine 'tether' to the nitrogen N(9) of the purine bases adenine and guanine and studied their interactions with an equimolar concentration of copper nitrate [162]. Evaporation resulted in crystals where both purine bases formed polymeric assemblies, the adenine compound used nitrogens on both sides of its ring system to bind to neighbouring copper ions. The N(3) position, facing the minor groove in DNA, would bind to the same metal ion as the diamine nitrogens, while N(7), which faced the major groove in DNA, would coordinate another metal centre. The guanine variant, in contrast, only used the major groove site, making two bonds (via the tether) to one side and one to the other.

A series of Zn and Cd complexes were prepared. Adenine can act as a tridentate chelator using the N(3) position in combination with the two diamine nitrogens and zinc perchlorate formed a 1:1 complex[162]. The crystal structure of $ZnCl_2$ complex indicated a symmetrical complex of three metal ions bridged by two ligand molecules.

The guanine ligand have its highest metal affinity in the N(7) position, located opposite the alkyldiamine appendix, and favoured oligo- and polymeric assemblies. Reaction of the guanine hydrochloride with cadmium nitrate formed a crystal structure with trimeric layers, each of which links to its neighbour through the N(7). With cadmium sulfate, Houlton's group obtained an interestingly square-shaped tetramer, which in the crystal stacks up to a highly symmetrical tube [162]. The guanine tetramers occurred naturally in the G-rich telomeres capping the chromosome ends, and the new feature of these metal assisted tetramers is that they retain the ability to form Watson-Crick base pairs. Thus, a stack of the Cd_4G_4 complex could serve as a flytrap to catch up to four strands of C-rich DNA.



Alkylamine-tethered guanine (left) and adenine (right) used as building blocks: (grey = carbon, white = hydrogen, blue = nitrogen, red = oxygen)

C- Determination of some drugs

A spectrophotometric method for the determination of drugs containing aromatic amino

group (derivatives of p-aminobenzoic acid and sulfanilic acids) was based on the drug reaction with 5-nitrobarbituric acid in DMF[163]. Additionally, liquid membrane electrode systems responsive to the nicotinium cation were described based on the use of the ion-association complexes of this cation with 5-nitrobarbiturate counter anions in nitrobenzene solvent as ion-exchange sites[164]. The performance characteristics of these electrodes, evaluated according to IUPAC recommendations, revealed fast, stable and near-Nernstian responses for 10^{-2} – 10^{-5} M nicotine over the pH range 3.5-7. Comparison of the nicotine-5-nitrobarbiturate liquid membrane electrode with the reported liquid membrane electrodes for nicotine[165] showed that this electrode, however, had the advantage of higher selectivity for nicotine in the presence of many inorganic and organic cations. This was used satisfactorily for direct determination of nicotine in tobacco smoke[164].

The limits of barbiturate compounds in blood and urine can be detected by thin-layer chromatography with the use of adsorbent resin. The mobile phases for the blood and urine systems were $CHCl_3$-Me_2CO (9:1) and C_6H_6-Me_2CO (85:15), respectively. Detection was done by spraying with diphenylcarbazone-$HgCl_2$ reagent. In most cases the sensitivity could be doubled by subsequent spraying with 1% KOH in MeOH and warming the chromatograms in a stream of air[166].

Methods of argentometric potentiometric titration of uracils were developed with the use of ion-selective electrodes, where an ethanolic sample solution was titrated versus aqueous $AgNO_3$ in a medium of borate buffer (pH 9.3-9.8) using a silver-selective Ag_2S electrode versus SCE. Uracils were also determined by the addition of $AgNO_3$ solution in isopropanol and titration of the liberated acid by piperidine in isopropanol using a glass electrode versus SCE[167]. The use of Pt electrode instead of glass indicator electrode improved the accuracy and extended the applicability of potentiometric titration with alcoholic and aqueous solutions of NaOH, KOH, and Et_4NOH[168]. The potential jumps in sample solutions of pyrimidines in DMSO were substantially better than in DMF or $MeOCH_2CH_2OH$[168].

Thermometric titrimetry is also a valuable procedure for the titration of the mercapto groups which can be titrated as acids in aqueous or in DMF media[169]. The use of acetic acid as a solvent allowed titration of the basic pyrimidine nucleus. Also, a potentiometric titrimetric method[170] for the determination of 2-mercaptopyrimidine and thiouracil derivatives was based on the reaction with $K_2Cr_2O_7$ and back-titration with Fe^{II}.

The polarographic reduction at the dropping mercury electrode of dimercaptopyrimidines has been investigated at pH 1-12[171]. In an alkaline medium, the nucleus of 2,4-dimercaptopyrimidine was reduced in 1 e^-, 1 H^+ process, forming a free radical which was immediately dimerized. The C(4) atom was probably the electroactive centre. Trisubstituted derivatives showed no reduction of the pyrimidine nucleus[171].

1,3-dimethyl-4-amino-5-nitroso-2,6-pyrimidinedione, an intermediate during the synthesis of theophylline, was determined by a spectrophotometric method based on the reaction with a Cu^{II} ion and measurement of the absorbance of the chelate formed at 400 nm. Beer's law was obeyed in the concentration range 40-120 µg/ml[172].

Iodometric determination of 2-thiouracil and its derivatives in alkaline medium was presented[173]. In the volumetric titration with the potentiometric end-point detection, the determinability range was 100-1000 µmol for 2-thiouracil, 30-700 µmol for 6-amino-2-thiouracil and 100-500 µmol for 5-methyl-2-thiouracil. The elaborated method was applied to the determination of 6-propyl-2-thiouracil in drugs with relative standard deviation < 1%[173].

In a sequel of continuation, Masoud *et al.*[174,175], evaluated the acid dissociation constants of uracil, thiouracil, 5-(substituted arylazo) uracil, 5-(substituted arylazo) thiouracil[174], 5-(substituted arylazo) barbituric and thiobarbituric acids[175]. The data obtained potentiometrically in an aqueous medium and in different percentages of ethanol-water media at variable temperatures (25-45°C) and in dioxane-water mixture at 25°C, the compounds exhibit two pK's values corresponding to deprotonation of both the (4)-OH and (2)-OH groups. On the other hand, 5-(carboxy phenylazo) derivatives gave three pK's values. The thermodynamic parameters of ionization were evaluated, and discussed from the electronic character of the substituent and the nature of the hydrogen bond views[174,175].

Dependence between the structure and chromatographic behaviour of nucleic acid components was developed. The distribution constants (K) of uracils and thymines on Sephadex G-10 in column chromatography technique depend on the adsorption properties and the ionization constant of the compounds[176]. In addition, the separation behaviour of various nitrogen-containing compounds on branched-polyfluoroalkylsilane coated silica gel columns was studied[177]. Fluorinated compounds are separated using fluorinated columns. The elution order depends on the number of fluorine atoms in the solutes. The addition of a masking agent of adsorption pointed to the mobile phase improved the separation of pyrimidine compounds, giving rise to symmetrical peaks. Better recognition of geometrical isomers and epimers was achieved on fluorinated packings, which was attributed to the less solvophobic interaction than on an octadecyl siloxane column and the rigid molecular structure of the fluorocarbon chain[177].

To detect a trace amount of nucleic acid bases, a liquid ionization (LI) mass spectrometer was improved in terms of ionization efficiency and ion focusing. This ion source provided better sensitivity for the analyte (uracil) and lower background of water clusters(178).

Violuric acids are the 5-isonitroso derivatives of barbituric acid. These compounds are used in immense analytical applications for the identification and determination of several metal ions(179). Some complexes of these ligands with transition metal ions have been isolated and characterized by spectroscopic and X-ray crystallographic techniques(180-185). The violurato derivatives showed π-acceptor properties acting as strong-field ligands. Complexes of the anions of monomethyl- and dimethyl-violuric acids with Ru^{II}, Rh^{III}, Pd^{II} and Pt^{II} have been isolated and characterized. IR spectra suggest bidentate coordination through the nitroso group and the carbonyl oxygen in position six(182). Diamagnetic salts of the type $M^{+}[FeV_3]^{-}.XH_2O$ have been prepared (where M^{+} = alkali-metal cation, V^{-} = violurate)(183). Extensive studies were reported in our laboratory(184-224) from different angles in solution and solid states for structural chemistry . The studies were focused on different techniques .

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