

# Reactions of cytosine, thymine and uracil toward 2,4,6-trinitro-chlorobenzene

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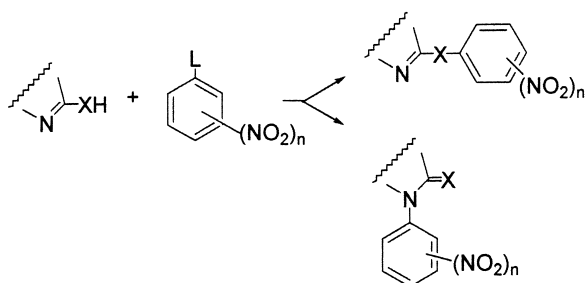
The title reactions are an example of direct introduction of an aryl group into DNA bases.

**Keywords:** cytosine, thymine, uracil, 2,4,6-trinitro-chlorobenzene

The title reactions are an example of direct introduction of an aryl group into DNA bases. The reactions of tautomeric substituted pyrimidines<sup>1</sup> are of interest since they are components of nucleic acids.<sup>2</sup> Moreover, derivatives of cytosine and uracil are potential therapeutic agents.<sup>3</sup>

In previous papers,<sup>4–6</sup> we reported the nucleophilic ability of different centres in derivatives of "aza" heterocycles towards aromatic (or aliphatic) electrophiles, *via* nucleophilic substitution reactions, as illustrated by Scheme 1, where L is the leaving group.

The heterocyclic reagent was either a neutral or charged derivative<sup>7</sup> of thiazole or pyridine systems. 2-Hydroxypyridine, 2-aminopyridine (or related thiazoles) may react as bidentate nucleophiles at both the endocyclic or exocyclic hetero atoms. The C-5 carbon atom of 2-aminothiazole is a third nucleophilic centre in the reaction with 2,4,6-trinitrochlorobenzene.<sup>6</sup>



X = O, S, NH; n = 2, 3

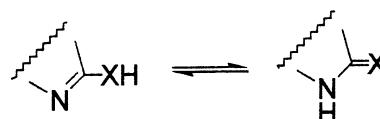
**Scheme 1**

Derivatives of uracil were reported to react with electrophilic reagents not only at both nitrogen atoms (or at the oxygen atoms),<sup>8–11</sup> but also at the C-5 or C-6 carbon atoms.<sup>12,13</sup> Regioselectivity and poly-alkylation reactions complicate the alkylation reaction<sup>10,11,14</sup> of derivatives of nucleic acid bases. Although many examples of alkylation reactions have been reported in the literature, very few examples of arylation reactions of DNA bases have been described.<sup>15</sup>

These heterocyclic derivatives are of interest also as potential biocides and they showed interesting properties in mechanistic<sup>16,17</sup> and tautomerism<sup>18,19</sup> studies involving the heterocyclic ring.

When "aza" heterocycles bear hydroxy, mercapto or amino groups, a possible explanation of their reactivity and regio-

selectivity is the position of the tautomeric equilibrium (see Scheme 2 for 2-substituted heterocycles). Tautomerism produces at least four centres that are reactive toward electrophilic reagents, and it may be responsible for the regiochemistry and reactivity of the considered heterocycles.



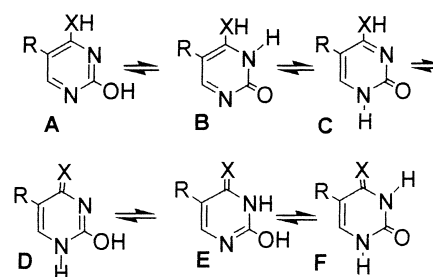
X = O, S, NR

**Scheme 2**

The use of the sodium salt of the heterocyclic derivatives<sup>7</sup> in the reactions with electrophilic reagents, reduces the complication arising from the presence of several tautomeric forms.

With the aim of extending our studies to the reactivity of compounds of greater interest than that of the simple models previously reported,<sup>4</sup> we now report some data concerning the products of the reactions between 2,4,6-trinitrochlorobenzene (1) and heterocycles bearing two potential hydroxy and amino groups: cytosine (2), uracil (3) and thymine (4).

The main tautomeric forms<sup>1,20</sup> of 2, 3, 4 are shown in Scheme 4. Usually, form F is the more populated form for X = O: uracil and thymine are pyrimidine-2,4-diones. In cytosine, the imino forms D, E, F are scarcely populated; tautomeric form C (X = NH) mainly describes cytosine.<sup>21</sup>



X = NH, R = H, cytosine (2)

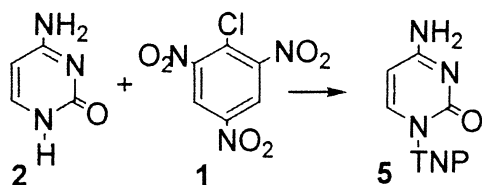
X = O, R = H, uracil (3); R = CH<sub>3</sub>, thymine (4)

**Scheme 4**

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## Results and discussion

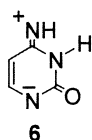
In neutral conditions, *i.e.* without addition of bases, 2,4,6-trinitrochlorobenzene reacts slowly with cytosine at the N-1 of the heterocyclic ring, affording compound **5** in Scheme 5.



*X-ray diffraction analysis of 5:* As far as we are aware, this work represents the first example of an X-ray structural analysis of a compound in which the 2(1H)-pyrimidinone ring is substituted at N-1 with a phenyl ring. The intramolecular bond lengths and angles are in good agreement with the hybridization expected for the atoms involved and also with those found in other cytosine derivatives reported in the literature.<sup>22</sup>

In principle, cytosine may react at four centres, but only **5** was recovered from the reaction mixtures. Our previous studies<sup>19</sup> indicated that the exocyclic  $sp^2$  nitrogen of the amino group atom is a more powerful nucleophile than  $sp^3$  of the amino group. Consequently, and in agreement with literature reports,<sup>1,21</sup> the absence of products reaction arising from the attack of the exocyclic nitrogen may be explained by a balance between its high reactivity and the fact that tautomeric forms **D E F** of **2** in Scheme 4, showing the exocyclic nitrogen in the imino ( $sp^2$ ) form, are scarcely populated.

The availability of the lone pair of the N-3 of **2** is depressed for several reasons.<sup>26</sup> Firstly, the N-3 nitrogen is sterically hindered. Furthermore the electronic effect of groups bonded in  $\alpha$  position to the "aza" N-3 is mainly inductive in character,<sup>27</sup> and the amino group may exert an electron-withdrawing effect on the N-3. This electronic effect overlaps with the electron-withdrawing effect of the adjacent C=O group. On the other hand, the amino group in position 4 may enhance the availability of the lone pair of N-1 as a result of the mesomeric effect as illustrated by **6**.

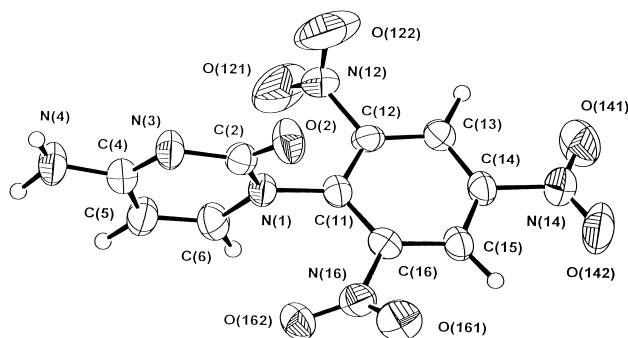


Previously,<sup>4</sup> we observed that the reaction of 4-hydroxypyridine nitrogen toward **1** is 75 times faster than the same reaction of 2-hydroxypyridine. This difference in reaction rate was explained by the fact that the reactive species is the neutral aromatic hydroxypyridine in which the hydroxy group is a more powerful electron-releasing group in position 4 than in position 2 of the pyridine ring. This is because in position 2 the mesomeric (electron-releasing) effect is strongly opposed by the electron withdrawing inductive effect.<sup>27</sup>

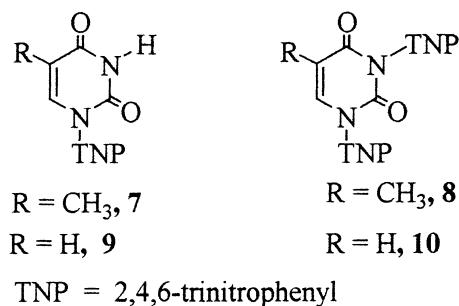
The reaction of **1** and thymine or uracil in THF or in  $CH_3CN$  under neutral conditions is very slow. After 10 days at 40°C no reaction products were detected in the reaction mixtures using TLC analysis. This fact emphasises the activating power of the cytosine amino group, which behaves as a genuine amino group. On the other hand, the hydroxy group in position 4 of **3** and **4** cannot exert its electron donating power because it is mainly in an oxo form.

2- And 4-hydroxypyridines, in neutral medium are more reactive than uracil and thymine toward **1**. Uracil and

thymine nitrogen atoms are mainly amidic in character. This explains their lack of reactivity in comparison with the reactivity of hydroxypyridines. This conclusion agrees with the usual statement that the di-oxo tautomeric form **F** (of both **3** and **4**) is a more populated form than the related oxo forms in mono-hydroxypyridines. Obviously, the sodium salts of **3** and **4** are more reactive than the neutral molecules. The monoanion of uracil and thymine exists in two main tautomeric forms.



**Fig.1** ORTEP plot of compound **5** showing 50% probability displacement ellipsoids. H atoms are drawn as small circles of arbitrary radii.



After one week two main products were separated from the reaction mixtures of mono sodium salt of uracil (or thymine) and **1**. Structures **7** and **8** were assigned on the basis of  $^1H$ ,  $^{13}C$  NMR and MS spectral data, in comparison with spectral data of N alkylated derivatives.<sup>28</sup>

In the case of the reactions between **1** and uracil, we separated compound **9** from the reaction mixture as the main product (see experimental). The  $^1H$  NMR spectrum of the crude reaction product and a chromatographic fraction showed the presence of a small amount (less than 4%) of **10**. From the same reaction mixture, a very small amount (less than 2%) of a compound was also isolated to which structure **11** may be assigned. Structure **11** shows both  $^1H$  NMR signals of H-5 and H-6 of the heterocyclic moiety strongly shifted toward a low field, in a region unusual for non aromatic pyrimidinones, but usual for heteroaromatic structures such as **11**.



## Conclusion

Even if a reaction pathway involving the attack on the oxygen atoms followed by the migration from O to N, observed in similar systems,<sup>4,29</sup> cannot be completely ruled out, we have no evidence for the presence of a pathway involving the attack on the oxygen atoms. It is probable that the reaction products arise by direct attack on the nitrogen atoms, as in the normal two step mechanism of S<sub>N</sub>Ar reactions. This conclusion agrees with the fact that alkylation reactions of uracil and thymine with trimethylphosphate in basic medium,<sup>11</sup> did not reveal any reactions at the oxygen atom. The behaviour reported here parallels that usually observed in alkylation reaction of **2**, **3**, **4**.

In agreement with the reactivity of cytosine, the thymine and uracil salts show that N-1 is a better nucleophile than N-3. In fact, no isomers of **7** and **9**, such as **12** were recovered from the reaction mixtures. The nucleophilicity of nitrogen N-3 is probably depressed by steric hindrance and by the presence of two adjacent carbonyl groups which are strong electron-withdrawing groups. The formation of **8** and **10** probably arises from the attack of **7** and **9** on a second molecule of **1**.

By assuming that the oxygen and nitrogen atoms in salts of thymine and uracil have similar reactivities, the formation of **7** and **9** as main products and the formation of a very small amount of ether **11** may be an indication that the negative charge is mainly located on the aza nitrogen N-1 rather than on the oxygen atoms which have an amide character.

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