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SYNTHESIS AND SPECTRAL STUDIES OF TETRAZEPINONES

By

Bertrand J. JEAN-CLAUDE

A thesis submitted to the Faculty of Graduate Studies

and Research of McGill University in partial

fulfilment of the requirements for the Degree of Doctor of Philosophy

Department of Chemistry

McGill University

Montreal, Quebec

Canada

August 1992

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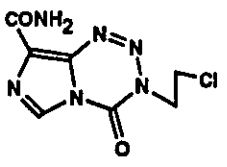
To my Family

To Carrole

ABSTRACT

In order to develop a novel analog of the antitumour agent Mitozolomide **1**, the diazotization of a variety of N-(2-aminophenyl)-N'-alkylureas was studied. The diazotization of N-(2-aminophenyl)-N'-alkylureas of type **2a** gave benzotriazole derivatives **3** (R=CONHalkyl). However, that of N-methyl-N-(2-aminophenyl)-N-alkylureas **2b** gave 1,2,3,5-benzotetrazepinones **4** after the neutralization of the reaction mixture. This finding allowed the synthesis of bi- and tricyclic tetrazepinones. Electronic effects on the stability of the tetrazepinone ring system were studied by varying substituents at the benzene moiety. The stability of the tetrazepinones increased with increasing electronwithdrawing character of the substituent. Variation of substituents at N5 showed that their stability decreases with increasing steric bulk of the substituents. The decomposition of benzotetrazepinones generally gave N-phenyl-N,N'-dimethylureas, 1-alkylbenzotriazoles and 2-hydroxy-benzimidazoles. The synthesis of stable pyrido-1,2,3,5-tetrazepinones is also described. The tetrazepinones were generally characterized by ^{1}H and ^{13}C NMR, X-ray diffraction, mass spectroscopy and microanalysis. The existence of the triazene chain was usually confirmed by ^{15}N NMR spectroscopy after specific labelling of the central nitrogen (N2).



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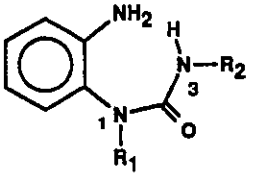
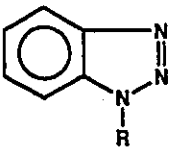


2a R_1=H
b R_1=alkyl



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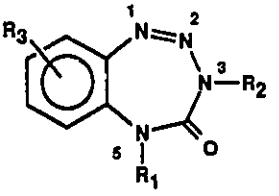

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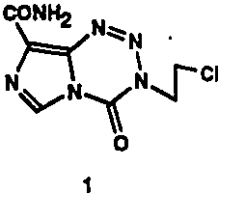
RESUME

Dans le but de développer un nouvel analogue de l'agent antitumoral Mitozolomide **1**, une étude de la diazotation d'une variété de N-(2-aminophényl)-N'-alkylurées du type **2a** a été entreprise. Les conformations préférentielles de ces urées dans le diméthyl sulfoxyde (DMSO) ont été déterminées. La diazotation de ces urées donne lieu à la formation des dérivés benzotriazoliques **3** (R=CONHalkyle) . La diazotation des N-(o-aminophényl)-N,N'-dialkylurées **2b** a donné lieu, par contre, à la formation des 1,2,3,5-benzotétrazépinones **4** après neutralisation du mélange réactionnel. Cette découverte a permis de synthétiser des tétrazépinones bi- et tricycliques. L'effet des substituants du noyau aromatique sur la stabilité du noyau 1,2,3,5-tétrazépin-4-one a été étudié. Les résultats ont démontré que la stabilité du système 1,2,3,5-tétrazépin-4-one augmente avec le caractère électrodonneur du substituant du noyau aromatique. Nous avons également trouvé que la stabilité du noyau 1,2,3,5,-tétrazépinone diminue avec l'encombrement stérique du substituant de la position 5. La décomposition des benzotétrazépinones dans un milieu alcalin génère des produits dépourvus de l'ion diazonium, les N-phényl-N,N'diméthylurées et des produits résultant de l'aromatisation du noyau tétrazépinone, les alkyl-1-benzotriazoles et les hydroxy-2-benzimidazoles. Les tétrazépinones ont généralement été caracterisés par RMN ^{15}N après le marquage isotopique de l'azote N2. Leur structure a également été confirmée par RMN ^{1}H et ^{13}C , par crystallographie aux rayons X , par analyse élémentaire ainsi que par spectroscopie de masse.



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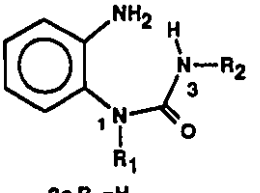

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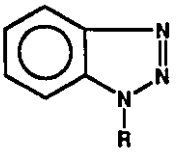
b R_1=alkyl



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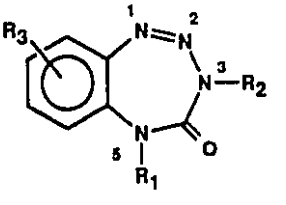

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ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my research director, Professor George Just, for his guidance and encouragement throughout my stay in his laboratory.

I also wish to thank:

The Chemistry Department, McGill University for teaching assistantships,

Professor Orval A. Mamer and M. Nadim Saadé for recording mass spectra,

Dr Rosi Hynes and Dr James F. Britten for determining the X-ray structures described in this thesis,

Dr. Françoise Sauriol for assistance and advice in using the XL-200 and XL-300 NMR Spectrometers,

Dr Fred Morin for running solid state NMR spectra,

M. Sorin Damian and Zoe Damian for performing antitumour tests,

all my coworkers, especially Dr Robert Hambalek, Kristina Martha Katharina Kutterer, Roger Théberge, Irene Idziak, Bin Meng, Dr Hang Tang, Philippe Faury and Denis Labrecque for discussions and friendships,

My parents, Anastasie and Alfred, my sister Marie-Dominique and brothers Frantzy, Joseph and Alfred Jr for support.

I also would like to thank my wife Carrole Jean-François for her love and support.

Tant qu'il y a de la vie, il y a de l'espoir

Where there is a will, there is always a way

GLOSSARY OF ABBREVIATIONS

A427	lung *carcinoma* cell line
A498	renal *carcinoma* cell line
A549	lung *adenocarcinoma* cell line
atm	atmosphere
Ar	Aryl
BOC	*tert*-Butoxycarbonyl
br	broad
t-Bu	tert-butyl
°C	degree Celsius
calcd	calculated
CBz	carbobenzylxoxy
CDD 19 LU	normal human fibroblasts
CIDNP	Chemically Induced Dynamic Nuclear Polarization
δ	chemical shift in parts per million
d	doublet
DMF	*N,N*-dimethyl formamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
Et	ethyl
eq	equivalent
g	grams
HT-29	colon cancer
hrs	hours
HRMS	High Resolution Mass Spectroscopy

HRMS	High Resolution Mass Spectroscopy
Hz	Hertz
HS 578T	breast cancer cell line
IC_{50}	concentration that kills 50% of the cell population
IMR-90	normal human fibroblasts
INEPT	Insensitive Nuclei Enhanced by Polarization Transfer
i-Pr	*iso*-propyl
IR	infrared
J	coupling constant (in NMR)
LOX	malignant *melanoma* cell lines
m	multiplet
m/z	mass charge ratio (in mass spectrometry)
Me	methyl
MHz	megahertz
min	minute
mM	millimoles per liter
m. p.	melting point
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser Effect
OVCAR 3	ovarian cancer lines
PC-3	prostatic *carcinoma*
ppm	part per million
q	quartet
s	singlet (NMR); second (s)
SF126	brain tumour cell line
SW 260	colon-rectal *adenocarcinoma*
t	triplet

THP	tetrahydropyranyl
TLC	Thin Layer Chromatography
UM-SCC-21A	head and neck squamous cancer cell line
UV	ultraviolet

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Chapter 1

The purpose of this chapter is to present a succinct review of the three major classes of antitumour drugs, the mechanism of action of which is based on the generation of alkyldiazonium species. This will give a better understanding of the design of the tetrazepinone ring system. Additional generalities are provided, where appropriate, in each subsequent chapter.

Owing to the wide use of ^{15}N NMR spectroscopy in the characterization of the tetrazepinones described in this thesis, a brief review of the application of this technique to the structure and conformation determination of nitrogen compounds is also given.

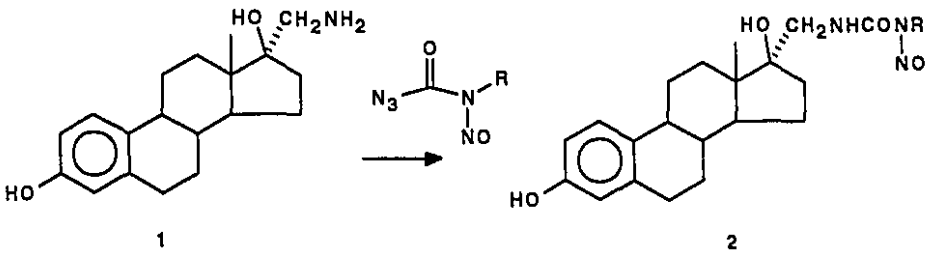
1.1 Nitrosoureas.

The development of the chemistry of nitrosoureas resulted from the discovery of the antitumour activity of N-methyl-N-nitrosourea by the random screening programme of the National Cancer Institute[1]. The identification of N-(2-chloroethyl)-N'-substituted nitrosoureas as potent agents against murine neoplasms has made nitrosoureas a class of antitumour drugs of considerable interest[2-4].

a. Chemistry

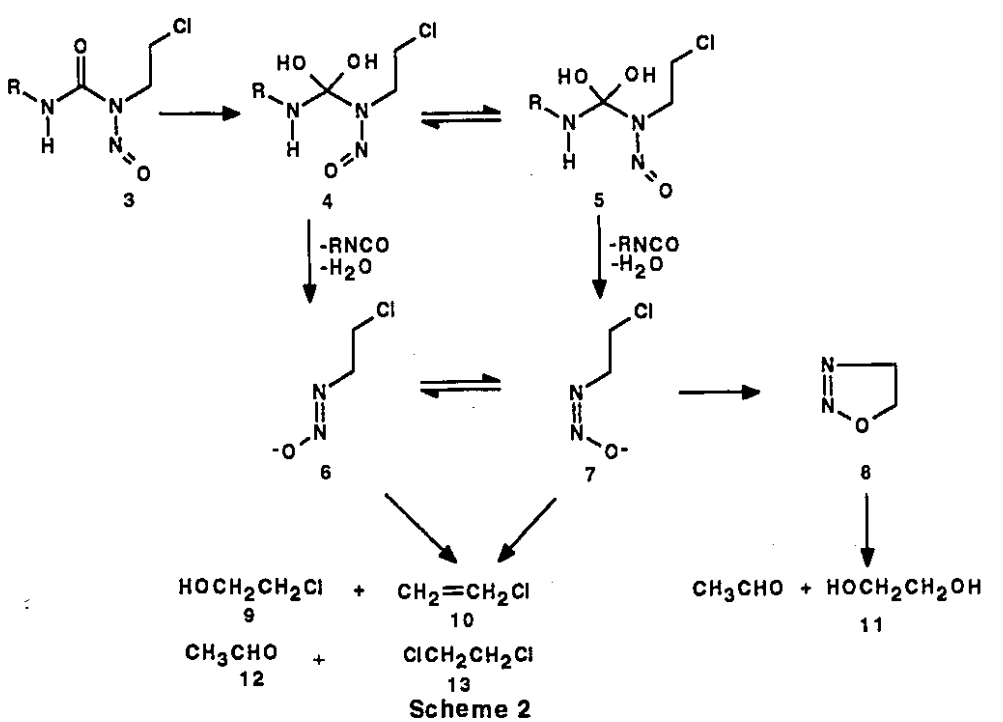
The synthesis of nitrosoureas involves the nitrosylation of 1,3-dialkylureas with sodium nitrite[5] or dinitrogen tetroxide[6]. Nitrosylation, in general, occurs at the electron-richer or the less bulky nitrogen of the ureido function. In cases where selectivity cannot be achieved, the N-alkyl-N-nitroso moiety is introduced by the condensation of an amine with an activated N-alkyl-N-nitroso carbamate[8-10] as shown in Scheme 1 . In this case, an N-

alkyl-nitroso group was attached to the methylamino derivative **1** of oestradiol to give nitrosourea **2**, which was designed to selectively target breast tumour cells .

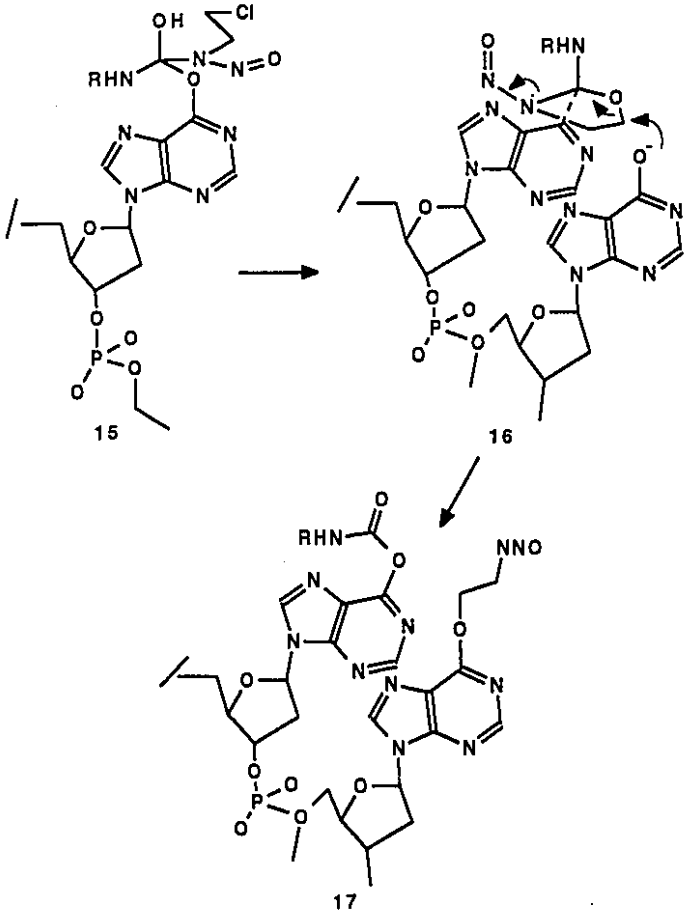


Scheme 1

Nitrosoureas react with nucleophiles to give carbamoylated and alkylated species[11]. The hydrolyses of 1-chloroethyl-nitrososureas have been extensively studied[11-15]. Lown *et al.*[13] suggested that the addition of water to the ureido carbonyl gives tetrahedral intermediates **4** and **5** which collapse to yield *cis* and *trans* alkyl diazotate **6** and **7**. The *trans* rotamer is hydrolyzed to give 2-chloroethanol, acetaldehyde and the chorinated derivatives **10** and **13** . The *cis* isomer cyclizes to give 1,2,3-oxadiazoline **8** that can also decompose to acetaldehyde and ethanediol.



Scheme 2



Scheme 3

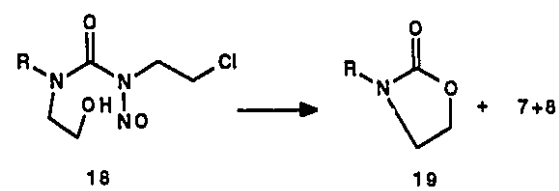
b. Mechanism of antitumour action

Owing to their strong alkylating power, nitrosoureas have long been the subject of several studies on DNA alkylation[16-21]. The most common conclusion from these studies is that chloroethylnitrosoureas cause DNA intra- and interstrand cross-links. They cause alkylation of DNA selectively on runs of 2 or 3 guanines at N-7 or O-6[16a]. To account for this preference, Buckley *et al.*[15] suggested the addition of the O-6 guanine to the carbonyl of nitrosoureas to give a tetrahedral intermediate like **15** similar to the one generated by the addition of water (**4** or **5**). This intermediate can cyclize to give **16** which may be attacked by the O-6 of a neighbouring guanine to give species **17** that is potentially able to alkylate a base of the complementary DNA strand to form a cross-link. (Scheme 3; the 4-amino group

was omitted for clarity). Buckley was the first to suggest a direct interaction of the entire drug with DNA.

c. Structure-activity relationship.

It is now admitted that chloroethyl or methyl groups on the NNO moiety of the nitrosourea is critical for appreciable antitumour activity. 2-Chloroethylating agents are more active than their methylating congeners. This is due, as stated earlier, to their ability to form DNA inter- and intrastrand cross links. In order to selectively target cancer cells or infected tissues, several attempts to link them to biological carriers have been reported[8-10]. In general, nitrosoureas attached to specific biological carriers show antitumour activity lower[8-10] than that of the free drug and fail to show selectivity. Even the attachment of nitrosourea at several positions in nucleosides did not give any satisfying results[22-23]. Only nitrosoureas linked to mono or disaccharides[24-28] show pronounced antitumour activity and reduced toxicity. Chlorozotocin[29] **20** showed reduced bone barrow toxicity in mammals. The reason for this particular activity is still unclear. It was believed that the

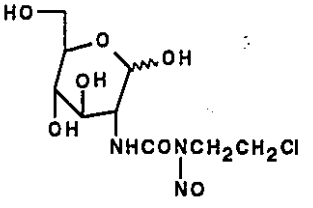


18 19

Scheme 4

low toxicity of nitrosoureas linked to sugar was due to their low carbamoylating power. In fact, it was shown that sugar linked nitrosoureas are self-activated by internal addition of the β or γ hydroxy group to the carbonyl to give a cyclic carbamate of type **19** in the manner summarized in Scheme 4. In phosphate buffer, activation by a γ hydroxyl group was found to be slower than by a β group.This intramolecular reaction eliminates the possible formation of carbamoylating species which are generally believed to be responsible for the

toxicity of nitrosoureas in humans. Unfortunately several compounds bearing β hydroxy groups were found to show bone marrow toxicity comparable with other non-hydroxylated nitrosoureas. The most potent drug and the least toxic to the bone marrow was found to be **21**, which contained a maltosyl group. This drug is presently in clinical trial in Japan.



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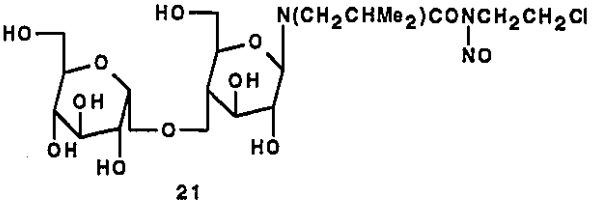

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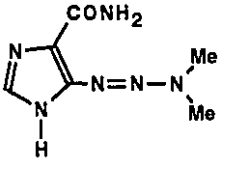
1.2 Open chain triazenes.

Open chain 1-aryl-3-alkyl triazenes have been found to possess interesting antitumour activity. Among them Dacarbazine (Dimethyl-triazenyl-imidazole-4-carboxamide, DTIC) **22** is the single most active drug now used in the treatment of *malignant melanoma*[2-4].



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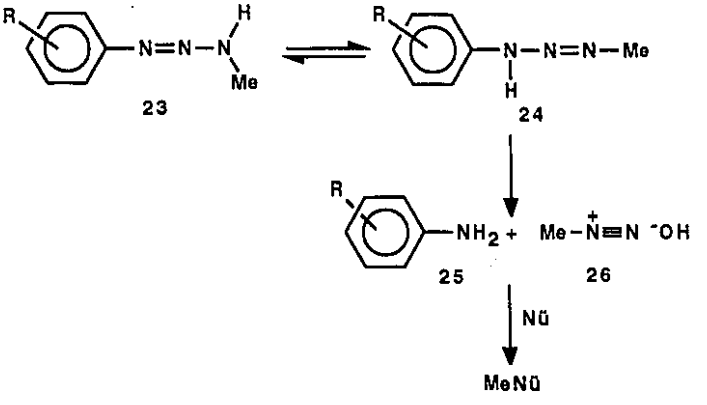
a. Chemistry

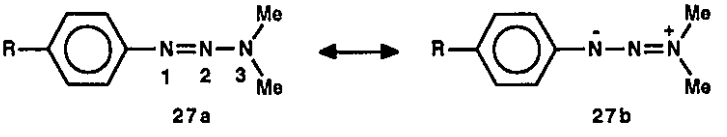
1-Aryl-3-alkyl triazenes are synthesized by the coupling of an alkylamine with an aromatic diazonium salt[30]. This reaction gives an NNN-alkyl linkage which is essential for antitumour activity because it is responsible for the generation of the alkyldiazonium species[3,4,31-33]. The mechanism of the aqueous decomposition of triazenes is believed to be the protolysis of tautomer **24** to give aniline of type **25** and diazotate **26** which is responsible for the alkylation of biological nucleophiles. It has already been shown that an

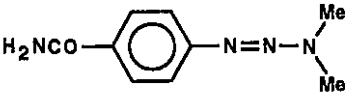
electron withdrawing group at the benzene ring decreases the rate of the protolysis of triazene[34].



Scheme 5

The structure and conformation of arylalkyltriazenes have been extensively studied. Nuclear magnetic resonance studies have shown a remarkable restriction to rotation around the N2N3 bond[35], which indicates that resonance structures of type **27b** contributes to the stability of these compounds. The double bond character of N2N3 was also confirmed by X-ray crystallography which showed bond distance of 1.309 Å* in Dacarbazine **22** and 1-(4-carboxamide)-benzotriazene **27c**[36,37a].



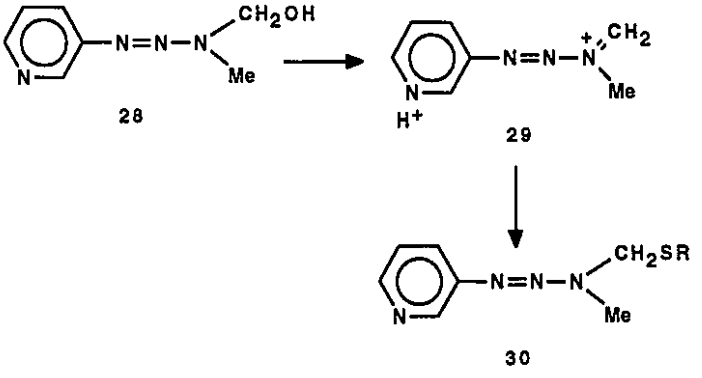


Although monoalkyltriazenes are known to be responsible for the generation of the alkyldiazonium species, interest was mostly in the development of 1-aryl-3-dialkyltriazenes because the latter have a longer life-time in aqueous solution[31,32]. They can be dealkylated to monoalkyltriazene by metabolic oxidation and exhibit interesting antitumour activity.

---

* The bond distance in N, N'-dimethylhydrazine is 1.45 Å[37b]

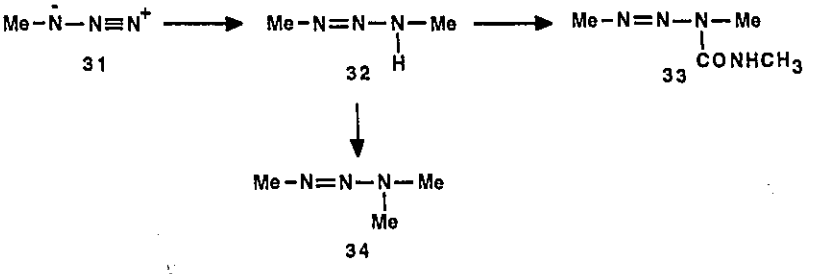
Thus, the assumption that the oxidative demethylation might generate a stable carbinol intermediate has stimulated the interest in preparing N-hydroxymethyltriazene compounds of type **28**[38-40]. When an aryl diazonium salt is mixed with methylamine and formaldehyde, a stable carbinol results, and if the OH group is acetylated, the resulting acetoxy group becomes a leaving group, the high nucleofugacity of which has allowed the preparation of several derivatized aryltriazenes[41-44]. More recently, Iley *et al.*[44] found that pyridyltriazenes resist protolysis in trifluoroacetic acid due to the protonation of the ring nitrogen. This finding has permitted the preparation of thioethers which were designed to be lyase inhibitors[45]. It was assumed that under acidic conditions N-hydroxymethyl triazenes are in equilibrium with their iminium ion **29** which can be trapped by thiol nucleophile to give thioethers **30** (R=glutathinonyl, R/S cysteinyl, N-acetylcysteinyl). Despite the interesting features presented by these molecules (e.g. attachment to biological molecules), they did not show any significant biological activity.



Scheme 6

Another class of antitumour alkyltriazenes, the 1,3-dialkyltriazenes, has been developped by Smith and Michejda[46,47]. The stability of structures **32-34** shows that an aromatic ring is not critical to the stability of the alkyltriazene chain. 1,3-Dialkytriazenes are synthesized by the addition of a Grignard reagent to an azide of type **31**[47]. The other alkyl group can then be introduced by an activation of monoalkyltriazene **32** with potassium hydride followed by treatment with iodomethane. Urea **33** is obtained by treatment of **32**

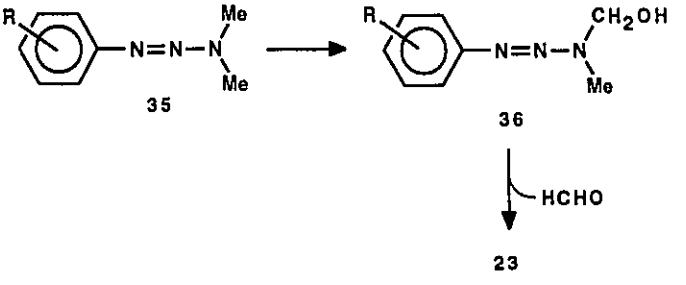
with methyl isocyanate. Like their aryl congeners, the 1,3-dialkyltriazenes, are readily hydrolysed to generate diazonium species[48].



Scheme 7

b. Mechanism of action

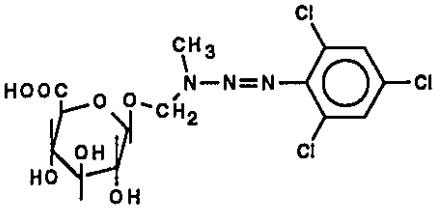
Dialkylaryltriazenes do not generate alkyldiazotate in solution. They have however been found to be antitumour active *in vivo*. Metabolic studies have shown that in fact dimethyltriazenes **35** are demethylated to give the monomethyltriazene. It is believed that the demethylation occurs via a hydroxylation of one of the methyl goups to give carbinol



Scheme 8

36 which can generate the monoalkyltriazene by loss of formaldehyde as outlined above (Scheme 8). The chemical synthesis of the methylols of type **36** has facilitated their identification as a metabolite of dimethylaryltriazenes[32,33,42,44]. The identification of the glucuronoconjugate **37**[38] in the urine of rats fed with trichlorophenyltriazene was an additional confirmation that hydroxymethylaryltriazenes are metabolites of dimethylaryltriazenes.

Hydroxymethylaryltriazenes are readily deformyltated in water, alcoohol or aqueous methylamine. Iley *et al.*[45a] recently suggested a mechanism involving a 6-membered ring transition state **38** for the base catalyzed deformylation of hydroxymethylpyridyltriazenes.



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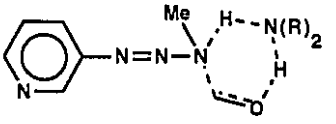


38

The fact that the mechanism of action of aryltriazenes is based on DNA damaging effect is now well established. Most impressive was a study by Meer *et al.*[45b] who observed O-6-[methyl- ^{14}C] guanine and [methyl- ^{14}C] guanine in tissues of rats intraperitoneally infused with [methyl- ^{14}C] DTIC. Dacarbazine (DTIC) has been found to cause DNA strand breaks *in vitro* and, like nitrosoureas to methylate DNA selectively in guanine rich regions of DNA strands[16].

c. Structure-activity relationship

The relative instability of monoalkyltriazene has made them less attractive as suitable drugs. The fact that dimethyltriazenes need metabolic oxidation causes a lowering of their bioavailability. For this reason, several aryltriazenes have not shown antitumour activity high enough to enable them to be introduced into clinic. Even DTIC which is the unique drug of the triazene series to be in clinical use presents the serious disadvantage of being photolabile[49].

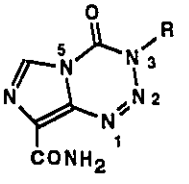
Noteworthy is that mono or diethyl triazenes do not show any antitumour activity[16]. It is believed that this may be due to rapid repair of ethylated DNA. The methyl or the chloroethyl groups seem to be critical structural requirements for an active drug.

The search for potential pro-monoalkyltriazenes remained of considerable interest until it was found that imidazotetrazinones could generate monoalkyl triazene upon hydrolysis without any metabolic oxidation.

1.3 Mitozolomide

The remarkable activity observed for 8-carbamoyl-3-(2-chloroethyl) imidazo-[5,1-d]-1,2,3,5-tetrazine-4-(3H)-one (Mitozolomide) **39b** against murine xenograft tumours[50] has stimulated the development of several substituted imidazo- and pyrazolo tetrazinones[51-53] . Mitozolomide offers the advantage of being administered orally[50,54,55].

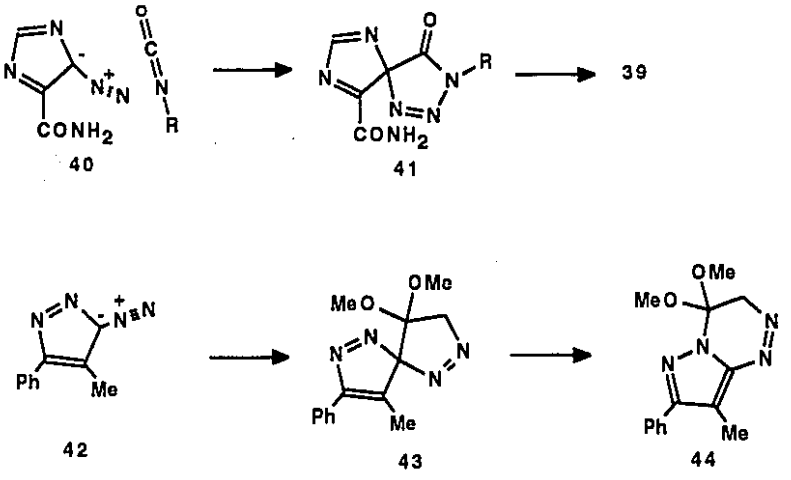
a. Chemistry



39a R=Me
b R=CH_2CH_2Cl

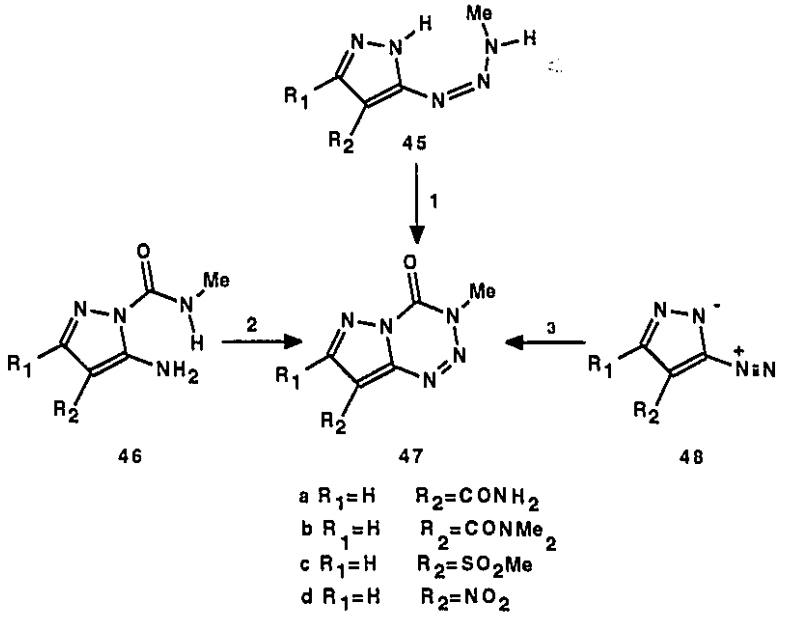
Temozolomide **39a** and Mitozolomide **39b** are synthesized by the condensation of alkyl isocyanates with 5-diazoimidazole zwitterion **40**[51,52]. Although this method for the synthesis of imidazotetrazinone was already known long before the first synthesis of mitozolomide[51,54], the mechanism of this reaction is still unclear. Ege[54] suggested that imidazotetrazinones are the result of a direct concerted 4+2 cycloaddition of alkylisocyanates to the zwitterion **40**. However Padwa[55] proposed a mechanism in which a dipolar (3+2) cycloaddition leads to the formation of an unstable spirobicyle **41** that

rearranges by a 1,5-shift to imidazotetrazinone of type **39** . This mechanism is supported by the fact that stable 3+2 adducts **43** was isolated in the synthesis of pyrazotriazine **44** by the condensation of 2-diazopyrazole **42** with 1,1-dimethoxyethene.



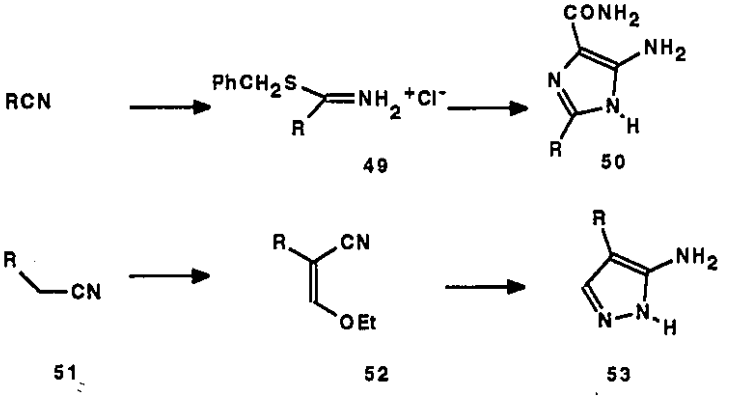
Scheme 9

In addition to the direct condensation of the diazonium zwitterion with alkyl isocyanate in non-hydroxylic solvents (method 3, Scheme 10), two other approaches were developed[56] for the synthesis of pyrazolotetrazinones. One of the strategies (method 1) consists in treating the diazoimidazole zwitterion with the desired alkylamine to form monoalkylimidazotriazenes of type **45**. Treatment of this triazene with phosgene or diimidazocarbonyl give the cyclic pyrazolotetrazinone. Method 2 consists in forming a 2-amino-pyrazol urea that spontaneously cyclizes to the 1,2,3,5-tetrazinone upon diazotization. Method 3 is the most commonly used in the preparation of imidazo-1,2,3,5-tetrazinones.



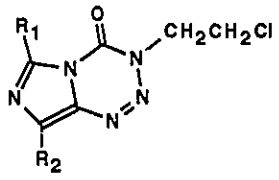
Scheme 10

Although the formation of the 1,2,3,5-tetrazinone ring is the major concern of this section, it is also relevant to discuss some aspects of the preparation of their substituted aminopyrazole and aminoimidazole precursors. Some typical methods for the synthesis of these ring systems are given in Scheme 11. The thioimidate **49**, obtained from the treatment of a substituted nitrile with dry hydrochloric acid in benzylthiol, was condensed with α-amino-α-cyanoacetamide to give imidazoles **50**. The synthesis of the pyrazole proceeded by the reaction of the substituted nitrile with ethylorthoformate to give **52** which is then treated with the substituted hydrazines to give the desired pyrazoles **53**.



Scheme 11

Interest has been recently turned to the synthesis of imidazotetrazinone containing an 8-carboxylic acid group which would allow an easy access to the formation of esters, thioesters and substituted amides[53] at the 8-position. The carboxylic acid was obtained in a pure form by the nitrosylation of the carboxamido group of mitozolomide in sulfuric acid. Refluxing the carboxylic acid in thionyl chloride afforded the acyl chloride **54a** which was converted to the desired carboxy functional groups. No decomposition of the tetrazinone ring system was observed during these reactions. This shows that imidazotetrazinones are very stable molecules. In contrast to their triazene congeners, they are not acid labile .

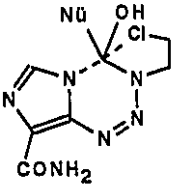


54

a R_1=H R_2=COCl
b R_1=H R_2=COOMe
c R_1=H R_2=NHMe
d R_1=H R_2=$CONMe_2$
e R_1=H R_2=SO_2Me
f R_1=H R_2=SO_2NH_2
g R_1=H R_2=NO_2

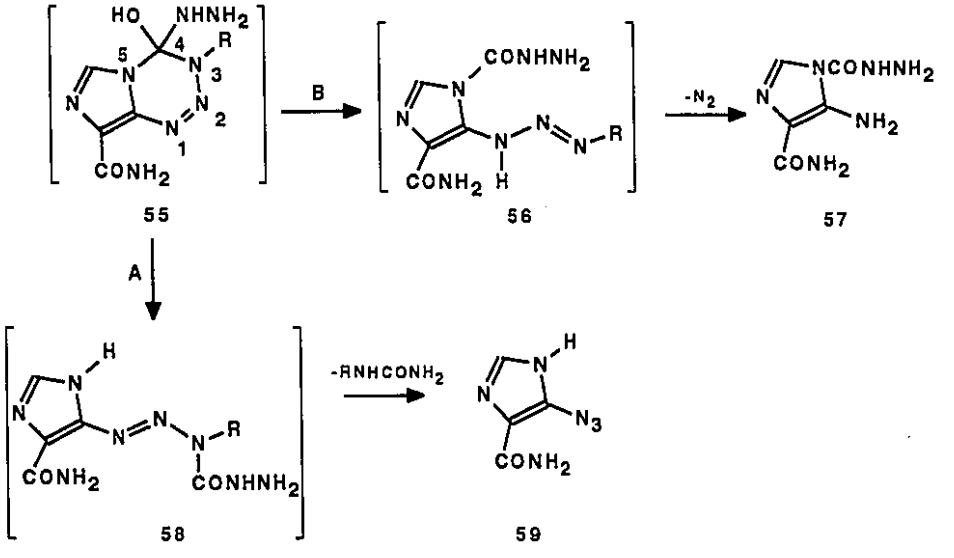
Although in **54a**, nucleophilic additions occurred peferentially on the acyl chloride group, the ureido moiety of the imidazotetrazinone ring is very sensitive to nucleophiles. At pH 8, its hydrolysis leads to the formation of monomethylimidazotriazenes[57]. As previously described for nitrosoureas, the hydrolysis of the ureido moiety of the 1,2,3,5-tetrazinone ring is believed to proceed via a tetrahedral intermediate following the addition of water to the carbonyl[57,58]. Nucleophilic addition may follow the same route. The tetrahedral intermediate can collapse via the breaking of the 3,4 or the 4,5 bond. In pyrazolotetrazinones bond breaking generally occurs at the 4,5 side. However in imidazotetrazinone, the breaking depends on the substituents at N-3. For alkyl substituents

such as methyl, ethyl or isopropyl groups, bond breaking occurs preferentially at the 3,4 side. For the haloalkyl substituent, however, the 4,5 bond is more fragile. It has been suggested that this preference is due to the anchimeric assistance of the chlorine atom that weakens the 4,5 bond in the tetrahedral intermediate **55**.



55

N-alkylimidazotetrazinone are alcoholysed to carbamates, and ammonolysed to ureas . As shown in Scheme 12, the hydrazinolysis of Mitozolomide reported by Baig[57] gave azide **59** (path A) as the major product, whereas that of the 3-alkyl substituted compound gave carbazide **57** (path B). The products are the result of bond breakage at either the 3,4 or the 4,5 bond.



Path A R=CH_2CH_2Cl
Path B R=Me, Et, Pr, CH_2CH_2OMe

Scheme 12

b. Mechanism of action

Mitozolomide was first believed to cause carbamoylation of DNA instead of alkylation. This was based on the fact that it was found to decompose into 2-chloroethyl isocyanate and 5-amino-imidazole[57] when it was heated in water or organic solvent. However extensive biochemical studies by Gibson[59,60] showed that, in fact, mitozolomide causes DNA alkylation to the same extent as alkylsulfates, nitrosoureas and triazenes. Incubation of mitozolomide with DNA strands showed damage resulting from alkylation of DNA in runs of three or four guanines. Very high levels of DNA inter- and intrastand cross-links and DNA-protein links were reported in human colon tumour cells[61] treated with mitozolomide. It should be remembered that the alkylation studies were carried out by incubating the alkylating agent with bacterial plasmids or by analyzing the nuclear DNA of cells previously exposed to alkylating drugs.

c. Structure-activity relationship

The observed enhanced activity of Mitozolomide may mainly be due to its alkylating power. The N-3 methyl substituted tetrazinone (Temozolomide) is antitumour active but less than the chloroethyl substituted mitozolomide. Variation of substituents at the positions 6 and 8 in imidazotetrazinone was undertaken in an attempt to establish the structural requirements for an active drug. The structure-activity relationships at the 8-position shows that 8-N-methyl-carboxamide-imidazotetrazinones **54c**, 8-methyl sufonyl **54e**, 8-sulfamoyl **54f**, are as active as mitozolomide. The 8-phenyl, 8-nitro, 8-methylester, 8-cyano groups were deleterious to antitumour activity against the same tumour model (TLX55 Lymphoma). 8-N-dimethylcarboxamide **54d** was found to be 5 times more active *in vivo* than *in vitro*. Metabolic studies have shown that this was due to the fact that the dimethylcarboxamido compound is dealkylated to the monomethyl amide *in vivo*. In the 6-position, the activity decreases as the bulkiness of the alkyl substituents augments . It is believed that a bulky

substituent at the 6-position may retard the hydrolysis of the ureido moiety . The structure-activity relationship for the imidazotetrazinones is similar to that of their pyrazolo congeners. Most active were pyrazolotetrazinones **47a, 47b.**

CONCLUSION

Nitrosoureas and aryltriazenes are a known class of antitumour drugs for over 20 years. Although their mechanism of action has been extensively studied, several key questions are still awaiting answers. How are they delivered to tumour cells? Is the alkyldiazotate liberated upon nucleophilic attack by DNA bases as suggested by Buckley or does the alkylation occur via a biological carrier that may transfer the alkyl group to DNA ?

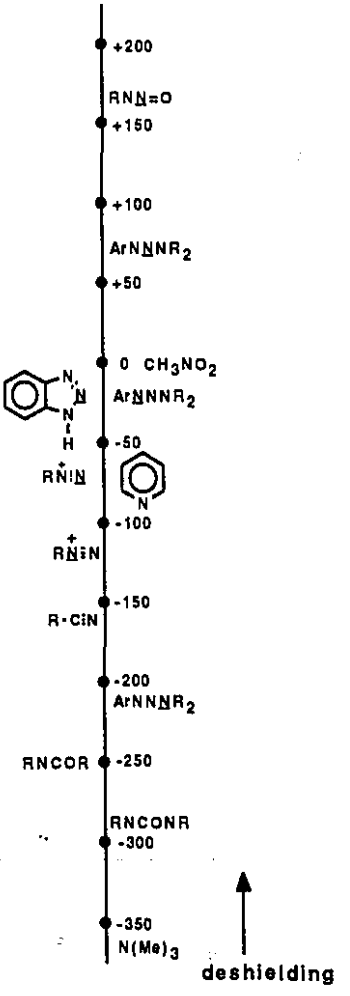
The structure-activity relationship of imidazotetrazinones which has been known for more than 10 years have raised more questions. As discussed earlier, imidazotetrazinone bearing an 8-carboxamide or sulfonamide groups (hydrogen bond donors) are extremely active compared with several other derivatives bearing cyano, nitro or ester groups. This would mean that some hydrogen bonding interaction may be involved somewhere in the activation path. However the 8-methyl sulfone derivative (a hydrogen bond aceptor) is as active as mitozolomide. Despite the contradicting results, it clearly appears that the non-alkylating moiety of these drugs plays an important role in their antitumour action.

Progress in the development of an alkyldiazotate generator has been rather slow. This may presumably be due to conflicting or misleading structure activity relationships studies. This problem stems from the fact that cancer is an extremely complicated cell disorder. The disordered proliferation of cells can be accompanied by the random expression of several silent genes, which may bring resistance to antitumour drugs by an unpredictable mechanism. To date, the best recognized mechanism of resistance to alkylating agents is that

of Mer+, cell phenotypes[84]. In contrast to the Mer+ cell lines, the Mer- are devoid of O-6-alkyl-guanine transferase, a suicide enzyme that dealkylates DNA.

1.4. ^{15}N NMR SPECTROSCOPY

Among all techniques used in the characterization of nitrogen compounds, nitrogen NMR spectroscopy is one of the most useful[62-66]. The low natural abundance of ^{15}N (I=1/2, 0.36%) has always been the major limitation of this technique. This problem can nowadays easily be circumvented by the use of powerful spectrometer (300-500 MHz) or specific labelling[13] . The INEPT[67] and DEPT[68] pulse sequences have also facilitated the detection of the N nucleus at concentration as low as 0.001 M.



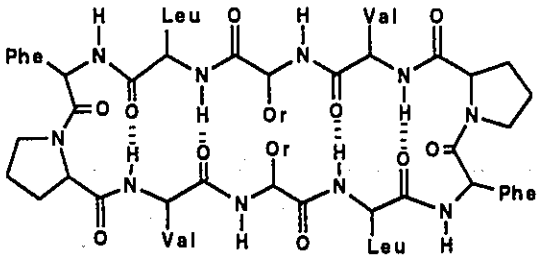
Scheme 13

a. Chemical Shifts

The ^{15}N shifts generally reflect the electron density and bond order at the nitrogen. For instance, the shifts of alkylamine, relative to nitromethane, are in general around -340 ppm whereas those of amides and nitrile are around -250 and -137 ppm respectively[62]. ^{15}N shifts cover a 500-800 ppm range. Within this range all the nitrogen functionalities are observed with very distinct chemical shifts as exemplified by Scheme 13.

The shifts of the nitrogen nucleus in N-alkylamines[67], N-alkylamides[68] and ureas[69], are markedly influenced by substituents effects (α, β, γ). Branching alkyl substituents α to the nitrogen causes a shielding effect whereas additional substitution in the β has a deshielding effect. The γ effect is shielding. Correlation studies have permitted the establishment of the size of these effects . In alkylamines, ureas and amides the β effect deshields by 11 to 20 ppm whereas the α and γ effects shield by around -1 to -6 ppm These topological parameters cause the nitrogen in triethylamine to be more deshielded than in monomethylamine.

The protonation of amines in general can show either a shielding or a deshielding effect[62]. In general, when the lone pair is involved in n-π delocalization as in anilines[70], the effect is shielding whereas when the lone pair is not delocalized as in alkyl amines, it is deshielding presumably due to a decrease in the electron density at nitrogen.

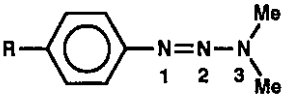


60

The shifts of the ^{15}N nitrogen, are solvent sensitive[71,72]. Chemical shift changes of up to 5 ppm can be observed when going from a protic to an aprotic solvent. This property has been used in several conformational studies on cyclic peptides. An interesting example is that of gramicidin S **60**. All nitrogens in peptide bonds with the carbonyl exposed to the solvent (Pro, Orn, Phe), are more deshielded in trifluoroethanol than in DMSO. Nitrogens in peptide bonds with the hydrogen exposed to the solvent (Leu, Val), are more deshielded in DMSO than in trifluoroethanol . This has permitted the establishment of the orientation of the peptide bonds in Gramicidin S[61,71].

The ^{15}N shifts have been extensively used in the characterization of compound with high nitrogen content. ^{15}N NMR studies were undertaken to establish the contribution of the dipolar resonance structure **27b** to the stability of dimethylaryltriazenes. It was found that N1 was more shielded as the electronwithdrawing character of the substituents on the benzene ring increased[73,74]. The shifts of N2 and N3 were more deshielded as electron-withdrawing character of R increased. Typical ^{15}N shifts values are given in Table 1. It is interesting to notice the 16 ppm shielding of N1 when going from **62** (R=MeO) to **66** (R=NO_2)

Table 1.-^{15}N Shifts of substituted 1-aryl-3,3-dimethyltriazenes

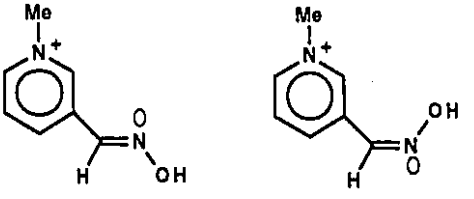


compound	R	N1	N2	N3	σ
61	MeO	-22.80	66.35	-228.16	-0.27
62	4-Me	-23.68	66.12	-226.29	-0.17
63	H	-25.49	69.22	-224.23	0.00
64	4-Cl	-30.99	69.25	-221.83	0.23
65	NO_2	-39.26	73.01	-210.64	0.78

b. Coupling constants

In ^{15}N NMR spectroscopy, the NH coupling constants were found to be the largest[62].Their values parallel the s-character of the hybrid orbital involved in the N-H bond. For instance, for methylamine, $^{1}J_{NH}$ is 62 Hz whereas in amides[75] and nitriles[76], it is around 90 Hz and 120 Hz respectively. The strong dependence of $^{1}J_{NH}$ on the hybridization of the nitrogen has been proven by the Hammett correlation observed for substituted anilines. The more electronwithdrawing the aromatic substituent, the larger were the coupling constants.

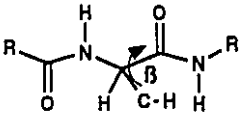
Geometrical dependence has been observed for $^{1}J_{NH}$. In amides it takes a lower value for the *cis* than for the *trans* conformation (*cis* and *trans* refer to the orientation of the proton as to the carbonyl)[77-79].



$^{2}J_{NH}$=2·5Hz $^{2}J_{NH}$=14·5Hz

66 **67**

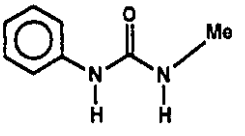
Values for $^{2}J_{NH}$ range from 2 to 20 Hz . These values are characteristic of lone pair orientation in trigonal nitrogen. When the lone pair is oriented *cis* to the proton $^{2}J_{NH}$ can be as large as 20 Hz. Conversely, when the lone pair is oriented *trans* to the proton this value can be as low as 2 Hz. This is well illustrated by oximes **66** and **67** shown above[80].



68

Due to its potential application to the determination of torsion angle in peptides, $^3J_{NH}$ has attracted both theoretical and experimental interest[81,82]. The geometrical dependence of $^3J_{NH}$ values was found to correspond to the the equation $^3J(N\text{-}CO\text{-}CH)=A\cos^2\beta + B\cos\beta + C$. Despite the effort to apply the $^3J_{NH}$ values to geometry assignment, the very narrow range of the latter coupling constants has imposed severe limitation to their use.

One of the most important aspects of NH couplings[82,83] is their use in the determination of exchange rates in nitrogen functionalities. This gives a qualitative idea of the basicity of the nitrogen. Nitrogen coupled signals broaden when they are involved in base or acid catalyzed exchanges with protons. Line shape analysis can allow to estimate the rates of exchange. Using this method, Roberts[83] has shown that in HN-phenyl-N'-methylurea **69**, the N-methyl exchanges 50 times faster than the N-phenyl in acid. In base, the results are the reverse. These results parallel the relative acidity and basicity of the two nitrogens in **69**.



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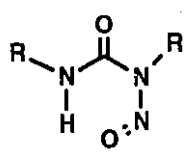
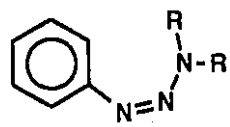
Chapter 2.- Design and Limitations.

a.-Design

In the preceding chapter, we have presented the fundamental characteristics of the three classes of alkyldiazotate generators. In light of this general overview, one should expect that a novel pro-monoalkyltriazene, in order to show antitumour activity, may contain the following features: (a) a potentially hydrolyseable ureido moiety as in **I** and **III** (b) a 1,2,3-triazene moiety as in **II** and **III** (c) an aromatic ring that may facilitate the variation of substituents. All these features are assembled in the benzotetrazepinone structure **IV**.



I



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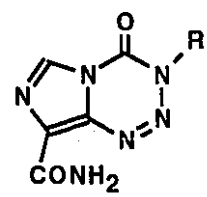
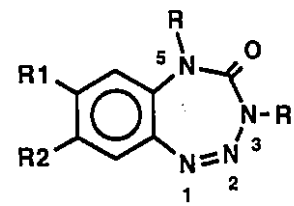


III

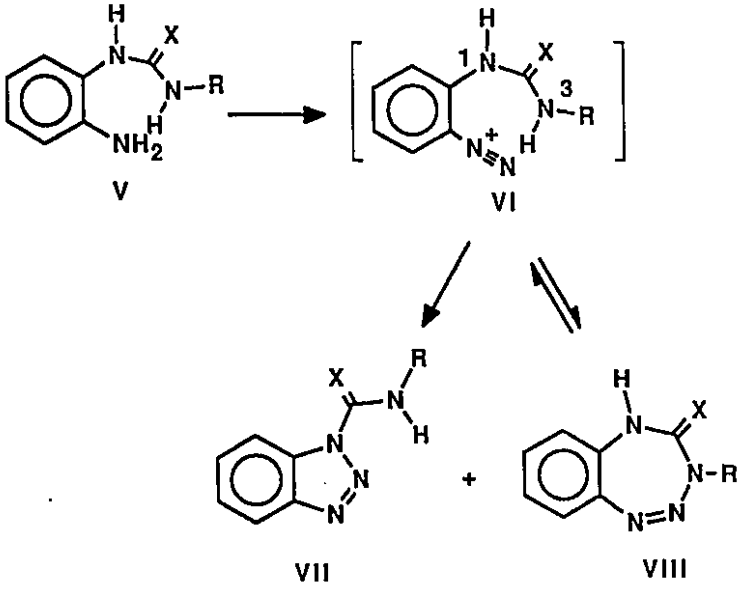


IV

b. Limitations

The synthesis of structure **IV** cannot be achieved by method 3 generally used in the synthesis of mitozolomide (see chapter 1). It is chemically impossible to prepare 2-amino-benzenediazonium derivative which is a short-lived intermediate in the formation of benzotriazole. For the same reason one cannot apply method 1 which would consist in forming the diazonium ion and coupling it with alkylamine to form a monoalkyltriazene which could be cyclized to tetrazepinone by treatment with phosgene. Method 2 was then applied since it involves the formation of a stable 2-aminoarylurea prior to diazotization.

The ureas were obtained by monocarbamoylation of o-phenylenediamine or by treating 2-nitrophenylisocyanate with the desired amines, followed by the catalytic reduction of the resulting 2-nitroaryl urea. Unfortunately, the diazotization of this ureas gave selectively an N-carbamoyl benzotriazole derivative of type **VII** that precipitated from the reaction mixture. No bi-cyclic tetrazepinone of type **VIII** was isolated.



a R=Me	X=O	[85%]
b R=Me	X=S	[76%]
c R=i-Pr	X=O	[72%]
d R=tBu	X=O	[81%]
e R=Phe	X=O	[90%]

Modification of the substituents at N-3 was attempted in an effort to understand these results. Diazotization of **Va-e** gave only the benzotriazoles **VIIa-e**. Neither electron donating nor electronwithdrawing substituents at N-3 influenced the direction of the cyclization. The introduction of an electronwithdrawing group like phenyl that would slow the protolysis of **VIII** did not give any successful result. Interest was lost in further study of the phenyl substituents since only alkyl groups are required for antitumour activity.

In an effort to rationalize these results, a study on the conformation of the 2-aminophenylureas was undertaken. Details are given in the attached paper.

EXPERIMENTAL

Typical method for the synthesis of **VII**, and **e** is given below. The general method for the preparation **VIIIa, c** and **d** is presented in chapter 3.

N-Methyl-1H-benzotriazole-1-thiocarboxamide (VIIb).-

To a solution of o-phenylenediamine (3g, 27 mmol) in chloroform (50 mL) was added methylisothiocyanate (2g, 1eq). The mixture was stirred for 2 hrs and evaporated under vacuum to give **Vb** as a pale yellow powder in quantitative yield, m. p. 120-123 ^{o} C. 200 MHz ^{1}H NMR $CDCl_3$ δ: 7.63 (br s, 1H, ArN*H*CS), 7.19 (t, J=8.8, 1H, Ar), 7.15 (d, 1H, J=8.8, Ar), 6.7 (m, 2H, Ar), 5.85 (br s, N*H*CH_3), 3.5 (br s, 2H, NH_2), 3.05 (d, 3H, J=4.7, NHC*H*$_3$). Thiourea **Vb** (2g, 11 mmol) was dissolved in 2N HCl (20 mL) and diazotized with sodium nitrite (0.8 g in 10 mL of water). The temperature was kept between 0-5 °C and the pale yellow precipitate that formed was filtered and recrystallized from chloroform to give **VIIb** as yellow pellets (1.51g, 72%), m.p. 110° C. IR (KBr disc) ν (cm^{-1}): 3391 (NH), 1245 (C=S); 300 MHz ^{1}H NMR ($CDCl_3$) δ: 8.92-7.5 (m, 4H, Ar), 3.38 (d, 3H, J=5) ; 75.4 MHz ^{13}C NMR ($CDCl_3$) δ: 176 (s), 147 (s), 131 (s), 126 (d), 120 (d), 116 (d), 32 (q); Anal. calcd for $C_8H_8N_4S$: C, 49.99; H, 4.20;, N, 29.16; Found: C, 50.48, H, 4.45; N, 29.25

N-Phenyl-1H-benzotriazole-1-carboxamide (VIIe).- To a solution of o-phenylene diamine (3g, 27 mmol) in chloroform (50 mL) was added dropwise phenyl isocyanate (3g, 1eq) at 0 °C and the precipitate that formed after 30 min was filtered to give Ve as a white powder in quantitative yield, m. p. 175°C. 200 MHz ^{1}H NMR $CDCl_3$ δ: 7.1-6.4 (m, 10H, Ar, NHCO), 6.25 (br, 1H, NHCO), 2.58 (br s, 2H, NH_2). This powder (2g, 8.8 mmol) was diazotized as described above to give **VIIe** (1.87g, 90%) as a white crystalline residue, m. p. 170°C. IR ($CDCl_3$) ν (cm^{-1}): 3200 (NH), 1720 (C=O); 200 MHz ^{1}H NMR ($CDCl_3$) δ: 9.2

(br s, 1H, NHCO), 8.31 (d, 1H, J=8.3, Ar), 8.12 (d, J=8.3, 1H, Ar). 7.95 (m, 1H, Ar), 7.7 (m, 2H, Ar), 7.5 (m, 3H, Ar), 7.22 (m, 1H, Ar) Anal calcd for $C_{13}H_{10}N_4O$: C, 64.54; H, 4.20; N, 23.55. Found : C, 65.11; H, 4.46; N, 23.22.

^{15}N NMR, ^{1}H-NOE DIFFERENCE SPECTROSCOPY AND CONFORMATION OF N-(*O*-AMINOPHENYL)-N'-ALKYL UREAS[a] .

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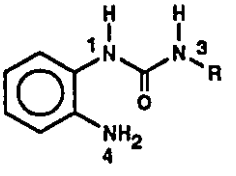
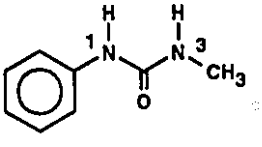
[a] This paper was published in *Magn. Res. in Chem.*, 1992, **30**, 571

ABSTRACT: The ^{15}N NMR spectra of N-(-*o*-aminophenyl)-N'-substituted ureas **1a-1f** and their hydrochloride salts were taken in DMSO. The chemical shifts of N1 were found to be more shielded in ureas **1a-1f** than in N-phenyl-N'-substituted urea **2**. The shifts of N3 varied with substituents. Proton exchanges at the latter nitrogen were observed when the spectra of the hydrochloride salts were taken. The preferential *trans-trans* orientation of the protons with respect to the carbonyl in the ureido group was confirmed by NOE difference spectroscopy. In the solid, the conformation of **1b**, which was determined by X-ray diffraction, was found to be similar to the suggested solution conformation **A**. The ^{15}N shifts of **1b** in the solid were determined by ^{15}N CP-MAS NMR.

INTRODUCTION

As part of our program to synthesize heterocyclic compounds by the diazotization of 1-(*o*-aminophenyl)-3-alkyl ureas,[1] we have undertaken a ^{15}N NMR study of the latter. Our interest was in determining their conformation, and their behaviour under acidic conditions. ^{15}N chemical shifts and NH couplings are informative about the geometry and interactions of nitrogen compounds.[1-5]





1a **R = H**
1b **R = methyl**
1c **R = iso-propyl**
1d **R = tert-butyl**
1e **R = phenyl**
1f **R = para-methoxyphenyl**

2

Although a great deal of NMR results have been reported about aromatic ureas,[6-10] little is known about their preferred conformation in polar solvents. In this report, we discuss an interesting class of aromatic ureas bearing an amino group *ortho* with respect to the ureido moiety. ^{15}N NMR data and ^{1}H NOE difference spectroscopy

results are used to determine their conformations in DMSO. In order to allow comparisons between conformations in solution and the solid state, the X-ray structure of **1b** was determined. Its ^{15}N NMR spectrum was also taken in the solid state.

This study is of more general interest because of the increasing development of *supra*-molecules[11,12] (H-bond donor-acceptor systems), and the use of ureido functions to create β-pleated sheet models for conformational analysis in peptides[13]. The ^{15}N NMR study of aromatic ureido compounds seems then to be highly desirable.

RESULTS AND DISCUSSION

Chemical Shifts.

The chemical shifts and one-bond NH coupling constants of ureas **1** and those of **2** are given in Table 1. The ^{15}N shifts (in DMSO) reported for 1-(phenyl)-3-methyl urea[14a, 23] **2** are quite different from those observed for its *o*-amino derivatives **1a-1f**, as expected. The N1 nitrogens in all ureas (**1a-1f**) are shielded by around 8 ppm when compared to N1 in **2** (δ N1 in **1**: around -285 ppm, δN1 in **2**: -276 ppm). This shielding may be due to the γ effect of the *o*-amino group.

The ^{15}N chemical shifts of N3 in compounds **1** are greatly affected by the nature of the substituent R (Table 1). Although the *iso*-propyl and *tert*-butyl are somewhat stronger electron donating groups than the methyl, they cause a considerable deshielding of N3 (around 25 ppm) in **1c** and **1d** when compared with **1a**. This must be due to the substituent effect β. It has already been shown that the α-effect shields the nitrogen where the β-effect deshields it[3,15]. Values for α and β have already been estimated for

Table 1.- Chemical shifts, in ppm, and one-bond ^{15}N-H coupling constants, in Hz, of 1-(o-aminophenyl)-3-substituted ureas

No	R	δ_{N1}	$^1J_{N1H}$	δ_{N3}	$^1J_{N3H}$	δ_{N4}	$^1J_{N4H}$
1a	H	-284	90	-301	90	-327	-
1b	Me	-286	88	-310	90	-326	83
1c	iPr	-285	89	-278	88	-327	-
1d	tBu	-284	87	-274	90	-327	81
1e	Phe	-282	85	-272	90	-327	83
1f	p-MeOPhe	-282	85	-276	87	-327	-
2[14]	Me	-276	90	-305	90	-	-

alkyl substituted ureas[3,15] (α = -4.9, β= +14.3). Although suggested equations for theoretical predictions of chemical shifts of the latter compounds are not applicable to aromatic ureas, β effects help to understand the remarkable deshielding of N3 in **1c** and **1d**. Despite the presence of an aminoaromatic substituent on N1, the α and β effects on the chemical shifts of N3 are quite similar to those observed on the shifts of amides and aliphatic ureas. The fact that N3 is more deshielded in the bis-aromatic ureas **1e** and **1f** may predominantly be caused by the conjugation of its lone pair with the phenyl substituent (δN3 in **1e**=-272ppm, δN3 in **1f**= -276 ppm as opposed to δN3 in unsubstituted compound **1a**, -301 ppm).

The chemical shifts of N4 for all substituted ureas **1a-1f** are quite similar (around -326 ppm (Table 1)). They are not responsive to substituent changes at N3. Their values are quite similar to that of the amino nitrogen in *o*-phenylenediamine[4,14b] (-328 ppm) . In the hydrochloride salts (Table 2), the shifts of N4 follow a shielding trend whereas those

of N1 and N3 are more deshielded when compared with their corresponding non-protonated ureas.

The chemical shifts of the proton attached to N1 in all alkyl substituted ureas **1a-1f** were around 8 ppm. The N3 proton appeared at around 6 ppm as a broad quartet in **1b**, broad doublet in **1c** and broad singlet in **1d** . The chemical shift of the NH_2 group was around 4.5-5 ppm. The assignment of ^{15}N shifts of the two NH_2 groups in the N3 unsubstituted urea **1a** was ascertained by correlating them to the shifts of their attached proton in a 2D HETCOR experiment (Fig. 1). The more deshielded proton (7.55 ppm)

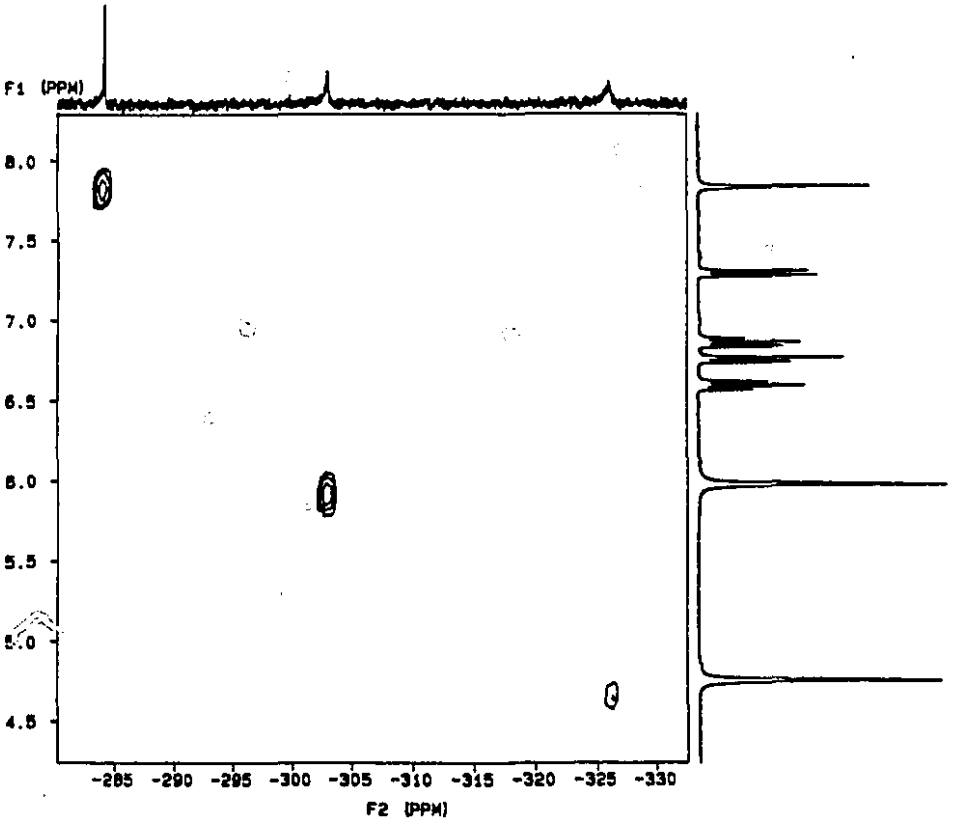


Fig. 1. The 2-D (^{15}N-^{1}H) NMR spectrum of compound **1a** in DMSO-d_6.

which can be assigned to H-N1 is correlated, as expected, to the most deshielded ^{15}N peak at -284 ppm . The ^{15}N peak at -301 ppm is assigned to the ureido NH_2 since it is related to the 5.7 ppm singlet. The peak at -327 ppm can be assigned to the anilino NH_2 since it is correlated to the 4.4 ppm singlet. This experiment also confirms the assignment of the chemical shift of N1 in **1b-1f** since they are all around -284 ppm.

Table 2.- Chemical shifts, in ppm, and one-bond ^{15}N-H coupling constants, in Hz, of 1-(o-aminophenyl)-3-substituted urea hydrochloride salts

No	R	δ_{N1}	$^1J_{N1H}$	δ_{N3}	$^1J_{N3H}$	δ_{N4}
1a	H	-281	90	-301	-	-331
1b	Me	-280	89	-306	-	-328
1d	tBu	-280	91	-272	-	-328
1e	Phe	-277	89	-269	87	-328
1f	p-MeO	-280	87	-275	86	-327

Coupling constants.

It has already been shown that the value of $^1J_{NH}$ is linearly dependent on the percent of s character of the nitrogen orbitals contributing to the NH bond ($\%s=0.34|^1J_{NH}|$)[2,5,16]. The higher the value of $^1J_{NH}$, the higher is the bond order at the nitrogen. Values up to 93.6 Hz have already been reported for amides[15] (Table 1). No such high value was observed for ureas **1a-1f**. The values of $^1J_{NH}$ for N1 in all the compounds vary between 85 and 90 Hz. The lowest values for $^1J_{N1H}$ (85 Hz) were observed for the bis-aromatic compounds **1e** and **1f**. These results show a slight pyramidal geometry of this nitrogen in these two compounds. Values of 87-90 Hz were

observed for $^1J_{N3H}$ for all the analyzed ureas. These results suggest a somewhat more trigonal geometry for N3 in both the alkyl and the aromatic substituted compounds.

Values of $^1J_{NH}$ for N4 vary between 81 and 83 Hz. These values suggest a pyramidal geometry for these nitrogens. Due to fast proton exchanges, no NH couplings were observed for the ammonium salts.

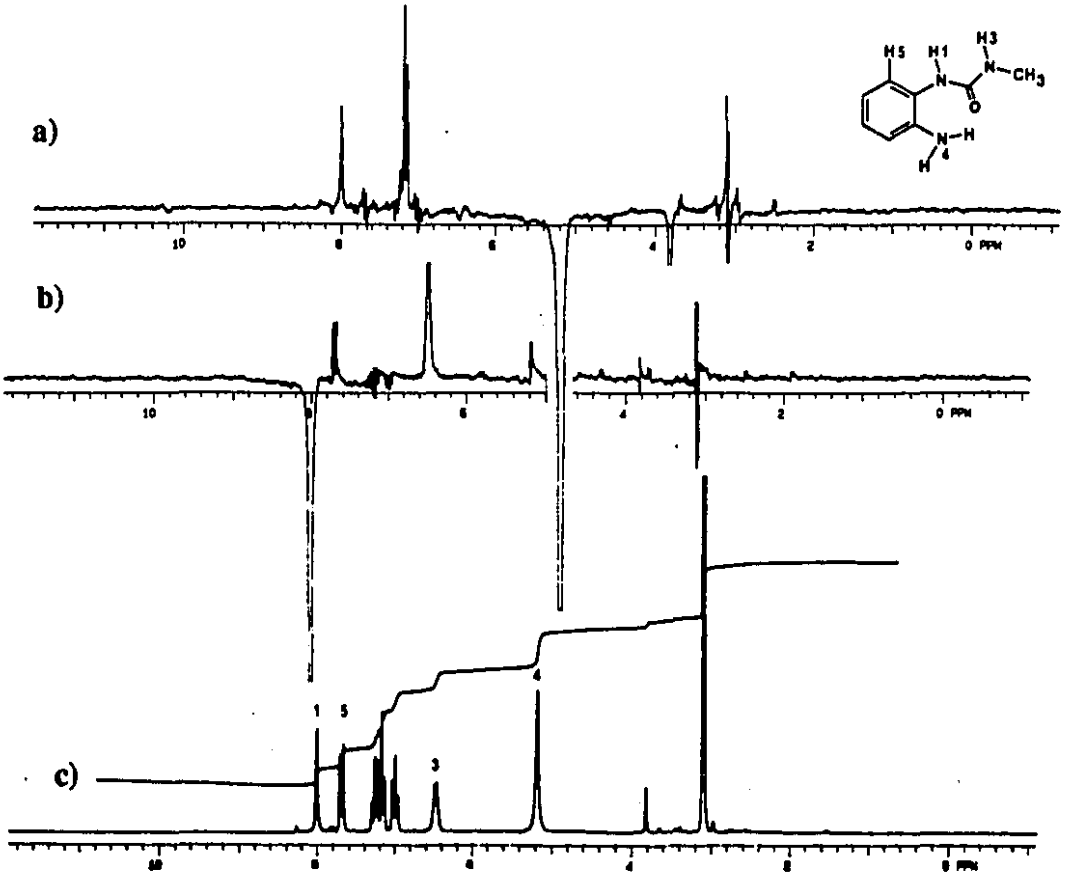


Fig 2. a) NOE difference spectrum of **1b** when the NH_2 signal is irradiated
b) NOE difference spectrum of **1b** when the N1H ptoton signal is irradiated
c) ^1H NMR spectrum of **1b** at 200 MHz in DMSO-d_6

Conformations

In order to determine the orientation of the ureido and amino protons in ureas **1** in solution, NOE experiments were carried out. Irradiation of the proton attached to N1 caused a significant increase in the intensity of the N3 proton signal in **1b-1f** (Fig. 2, 3), showing that they are *trans*-oriented with respect to the carbonyl as in conformer **A** or **B** (Scheme 1). In **1b**, irradiation of the N1 proton showed significant increase in the signal corresponding to the neighboring *ortho* proton. This could not be observed in **1c-1f** because of the overlap of the signals.

These results clearly indicate the presence of conformation **A** in which the *ortho* aromatic protons and the one attached to N1 are in close proximity. Irradiation of the proton attached to N1 has caused the increase of the signal corresponding to the NH_2 protons in all the NOE experiments. This confirmed the presence of conformations in which the N1 proton is pointed toward the amino group as in **B**, **D** and **F** (Scheme 1).

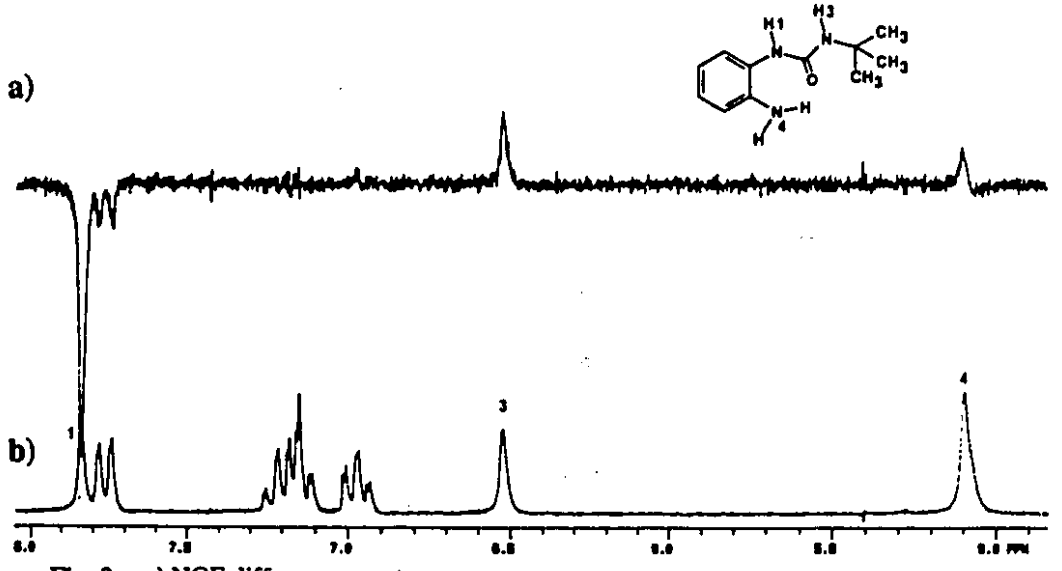
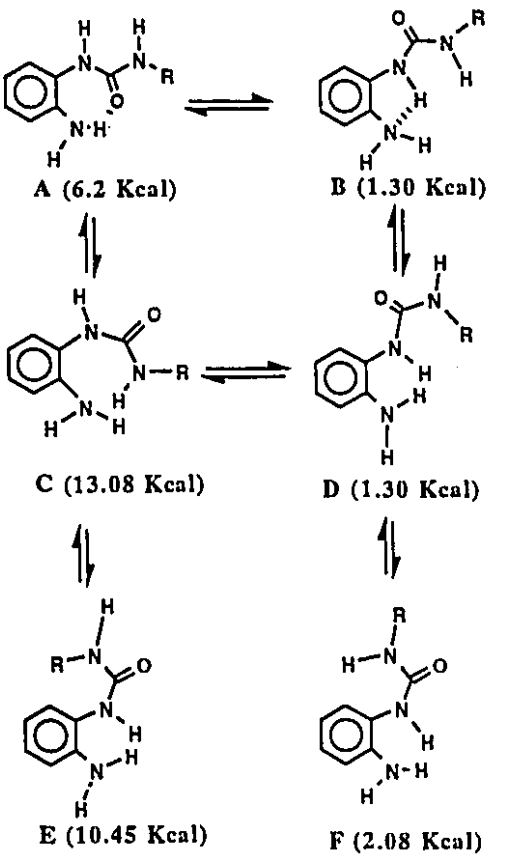


Fig. 3.- a) NOE difference spectrum
b) Spectrum of *tert*-butyl compound **1d** in DMSO-d_6 (only part of the spectrum containing the amine resonances is shown for simplicity).

The equilibrium between **A** and **B** is consistent with the experimental results. The two conformers are stabilized by intramolecular hydrogen bonds . Although it was not possible to confirm the orientation of the two ureido protons in conformers **D** and **F**, their equilibrium with **A** and **B**, should not be rejected since their energies are not very different from those of **A** and **B**. Molecular mechanics calculations[25,26] gave G values of 1.30 Kcal for **B** and **D**, 2.08 for **F** and 6.2 for **A** (Scheme 1). The low energy differences between these structures (0-4 Kcal) suggest rapid equilibria between **A**, **B**, **D**, and **F** at room temperature. Conformers **C** (13.8 Kcal) and **E** (10.45 Kcal), due to their high energies, should contribute only little to the equilibrium mixture. In fact, the existence of conformers like **E**, where the two ureido substituents are oriented *trans-trans* to the carbonyl, has already been contested in several studies on ureas and thioureas[6,8,22], on the basis of steric factors.

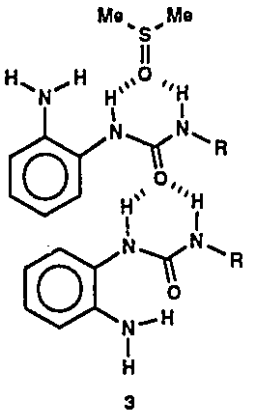
Noticeable differences in $^1J_{NH}$ ranging from 2-4 Hz have already been reported for the conformation in which the proton is *cis* to the carbonyl and the one in which the proton is *trans* to the carbonyl in N-methylformamide[15]. In general, $^1J_{NH}$ value for the *cis* conformer is always smaller than that for the *trans* conformer. For instance values of 93.6 and 90.2 Hz have been assigned to the *trans* and *cis* conformations of N-methylformamide[15]. No such assignments have been reported for ureas despite their similarity to amide. For nitrosoureas, however Lown[17] attributed the 92 Hz value of $^1J_{NH}$ to a *trans*-conformation of the CONHR moiety. Our results show that for ureas **1**, the $^1J_{NH}$ values have little geometrical significance. For values varying from 87 to 90 Hz , NOE experiments confirmed a *transoid* orientation of the proton with respect to the carbonyl in the CONHR moiety.



Scheme 1*

*Calculations were performed for R=Me

On the other hand, the preferential *trans-trans* orientation of the protons as to the carbonyl may be due to the formation of hydrogen bonds with the solvent (DMSO) as shown in **3**. Self-association can also favour this orientation.The equilibria between amide dimers and their monomers have already been suggested in a study on conformation of acetamides[18] in solution. For ureas **1**, the urea-urea type dimer could be as depicted in **3**.



3

Conformation in solid state.

The X-ray structure of **1b**[24] (Fig. 4) shows that only one conformation exists in the solid and it is similar to the solution conformation **A** . The X-ray structure shows a 2.975(4) Å bond distance between the carbonyl oxygen and N4 . These results suggest the presence of an intramolecular hydrogen bond. In the crystal lattice, the intermolecular bond distances between the carbonyl oxygen of one molecule and the two ureido nitrogens of a neighbouring one are 2.855(5) Å and 3.009(5) Å. This indicates the presence of hydrogen bonds between the ureido protons of one molecule and the carbonyl oxygen of a neighbouring one as in **3**. These results also suggest the stability of the self-associated system depicted in **3**. Bond angles around N1 and N3 are 124.2°(4) Å and 122.7°(4) Å . The trigonality of these nitrogens indicates a high sp^2 character. It is also important to notice that, as in solution, the two ureido protons are oriented *trans* to the carbonyl in the solid state.

The ^{15}N NMR of compound **1b** was run in solid state and the spectrum is shown in Fig. 4. The more deshielded peak at -280 ppm was assigned to N1 and the resonances of N3 and N4 were at -304 and -318 ppm respectively. Although ^{15}N NMR chemical shifts have a strong medium dependency,[1,2,3] the values converted to the nitromethane

scale were quite similar to those obtained in solution. The peak for N4 at -318 ppm is broad presumably due to insufficient decoupling power.

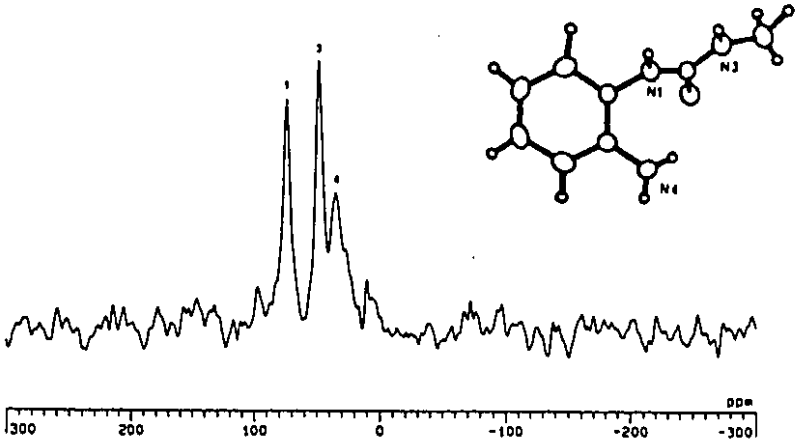


Fig 4.- ORTEP view and solid state (CP-MAS) ^{15}N NMR spectrum of **1c**. Conversion to the nitromethane scale gave: δN1=-280 ppm, δN3=-304 ppm, δN4=-318 ppm)

Acid catalyzed exchanges.

In order to have insight to the behaviour of 1-(*o*-aminophenyl)-3-substituted ureas under acidic conditions, ^{15}N NMR spectra of some of their dry hydrochloride salts were taken in DMSO. Results are given in Table 2.

In a study on exchange rates in ureas, Roberts[15] reported that at acidic pHs, the hydrogens at N3 in **2** exchange 50 times as fast as those on the N1-Aryl moiety . The same trend was observed for ureas **1**. Proton exchanges were observed at N3 in compounds **1a, 1b, 1d,** whereas negligible exchanges occured at N1 (Fig. 5). For the bis-aromatic compounds **1e** and **1f**, no exchanges were observed at N1 and N3 (Fig. 6). These results are in accordance with the relative basicity of the two nitrogens. As expected, rapid exchanges were observed at N4 in all the analyzed compounds.

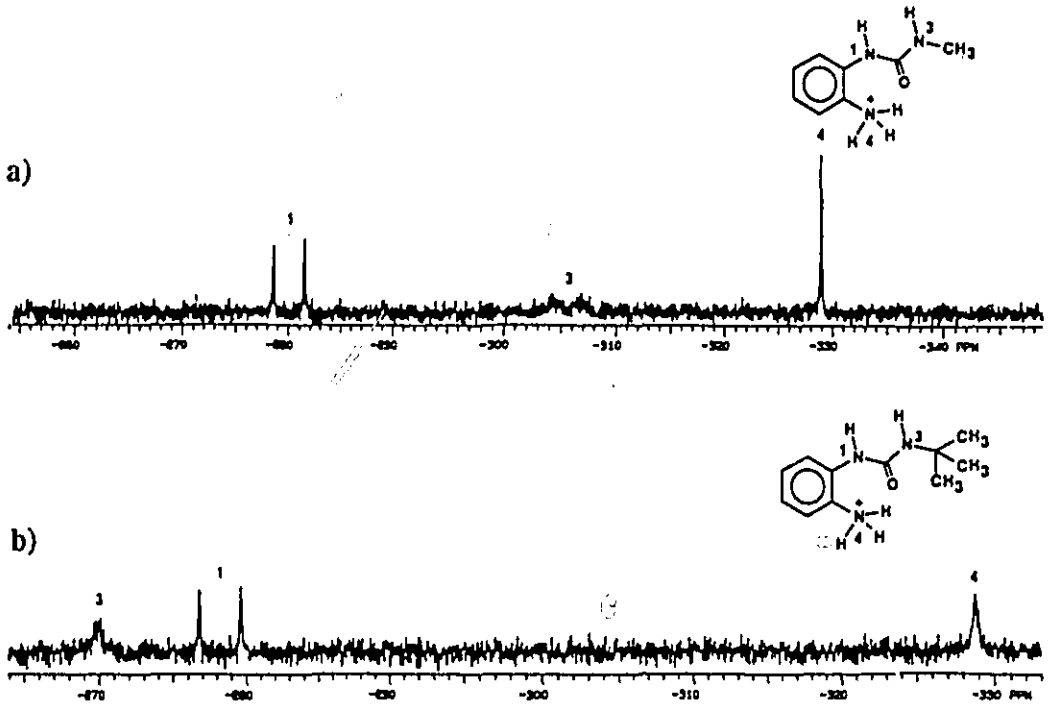


Fig 5.- a) ^{15}N NMR of the ammonium salt of **1b** (N-methyl signal at -306 ppm is coalescent)
b) ^{15}N NMR spectra of the ammoniun salt of **1d** (N-*tert*-butyl appears as a broad singlet at -272 ppm)

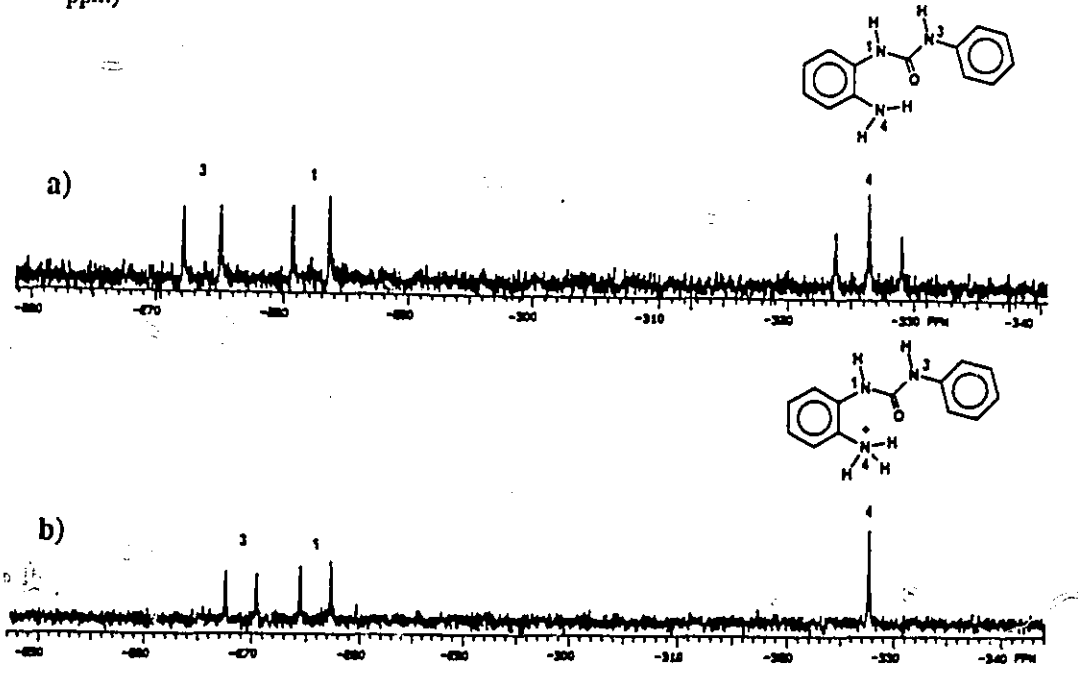


Fig 6.- a) The ^{15}N NMR spectrum of **1e** (recorded with Standard Pulse).
b) ^{15}NMR spectrum of the ammonium salt of compound **1e** (doublets for N1 (-277 ppm) and N3 (-269 ppm) are sharp, whereas the triplet for NH_2 changes into a singlet.)

The rates of exchanges at N3 in compounds **1a**, **1b**, **1d**, were estimated by line-shape analyses[19] . Proton exchanges at N3 in the N-tert-butyl substituted compound **1d** were found to be faster than at N3 in **1a** and **1b**. This may be due to the higher basicity of the N-tert-butyl moiety (Fig 5b).

EXPERIMENTAL

The ^{15}N NMR spectra were recorded on a Varian XL-300 NMR spectrometer and nitromethane was used as a standard set at 0 ppm. All signals appear upfield from nitromethane and hence chemical shifts are negative. All ^{15}NMR spectra were recorded at 30.4 MHz using a multinuclear 10-mm probe and the concentration of each sample was 1M in DMSO-d_6 (99% deuterated). The compounds were sparingly soluble in $CDCl_3$ so that it was not possible to run comparative natural abundance ^{15}NMR experiments in the latter solvent. All spectra were aquired with full NOE effect and the parameters were as follows: relaxation delay, 2s; 90° pulse, 18 μs. The spectral width was 15000 Hz and with 32K data point gave an acquisition time of 1.07 s and a digital resolution 0.93 Hz per point. The FIDs were in general obtained after 1000 transients (line broadening, 0.3 Hz). ^1H NMR spectra and NOE experiments were carried out in DMSO.

The parameters for the HETCOR experiment were as follows: spectral with, 1214 Hz in F1 and 1589 Hz in F2; acquisition time, 0.081s; pulse width, 20 μs; number of transients, 3000; number of increments 84 and $^1J_{NH}$=88 Hz.

The refocussed INEPT pulse sequence[20,21] was used to detect the ^{15}N signals of some of the free base ureido compounds. The values of t_D were set at 3 ms in all cases since the average $^1J_{NH}$ coupling constant was around 87 Hz ($t_D = 1/4^1J_{NH}$). The

refocussing period t_k was also given the same value. The advantage of this pulse sequence is that it allows the recording of spectra in a relatively short time but unfortunately, due to its strong dependency on the NH coupling constants, no signal enhancement was observed for nitrogens involved in even slight exchanges . When the INEPT pulse sequence was used, peaks for N4 were not often observed. Even with extensive drying of the compounds and the recording of the spectrum with standard pulse sequence, some broadening of the triplets were still observed for certain compounds. Some values of $^1J_{N4H}$ are missing in Table 1 due to the fact that the N4H triplets were too broad in certain cases. The spectra of the HCl salts of the ureas were run with standard pulse mode. The line-shape calculations were performed with the computer program described by Harris[19].

The ^{15}N solid state NMR spectrum was obtained on a Chemagnetics CMX-300 at 30.3 MHz. The Cross Polarization/Magic Angle Spinning (CP-MAS) technique was used with a contact time of 1 ms, a recycle time of 2s and a spinning speed of 5 kHz. The spinning speed was high enough to circumvent side bands. The chemical shifts obtained relative to ammonium nitrate were converted to the nitromethane scale by the equation: δN(nitromethane) = δN (ammonium nitrate)-351. The 351 increment was obtained by substracting the solution chemical shift of an ^{15}N labelled N-Methyl-N-carbamoylbenzotriazole[1] obtained in solution relative to nitromethane, from the solid state shift relative to ammonium nitrate.

All compounds were prepared according to methods reported in a separate paper[1].

ACKNOWLEDGEMENT

We thank the Natural Sciences and Engineering Research Council of Canada and Fonds FCAR, Province de Quebec, for financial support. We are also grateful to Dr Francoise Sauriol and Dr Fred Morin for discussion and for their assistance in running NMR spectra. We thank Dr James F. Britten for X-ray analysis.

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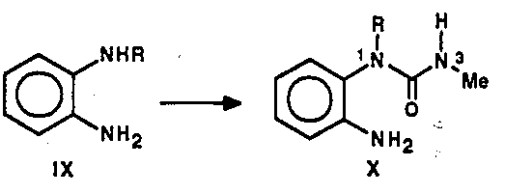
Chapter 3.-

General Overview.-

Since the formation of benzotriazoles resulting from the diazotization of the 2-aminophenyl ureas would be made impossible by attaching a methyl group to N1 of the ureas of type **X**, the synthesis of 5-substituted tetrazepinones was attempted. The fact that one of the main goals of the project was also to synthesize tetrazepinones bearing substituent at the 5-position encouraged this approach.



The synthesis of the ureas generally proceeded according to the following general scheme:

a) The N-alkyl-1,2-phenylenediamine of type **IX** was allowed to react with methylisocyanate to give the urea of type **X**. Since the nucleophilicity of amines parallels their basicity, the carbamoylation occurs selectively at the secondary amine .

b) If selectivity could not be achieved because the N-alkylamine was bulky or because the reactivity of the NH_2 group was increased by an electronic effect, the latter group was protected either with a BOC or a CBz group.

SYNTHESIS OF BI- AND TRICYCLIC TETRAZEPINONES[a]

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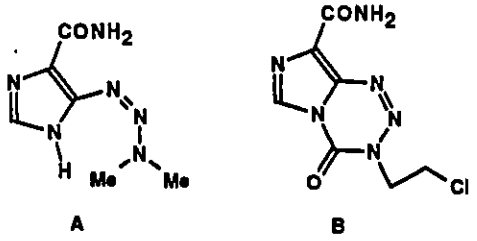
[a] This paper was published in *J. Chem. Soc. Perkin. Trans. 1*, 1991, 2525

ABSTRACT: Diazotization of N,N'-dialkyl-N-(o-aminophenyl) ureas **7**, **13**, and **17** gave 3,5-dimethyl-*3H*-1,2,3,5-benzotetrazepin-4(*5H*)-one **8**, 3-methyl-6,7-dihydro-1,2,3,5-tetrazepino[7,6,5-ij]quinolin-4-(3H)-one **14** and 3-methyl-6,7-dihydro[1,4]oxazino[4,3,2,5-ef]-1,2,3,5-benzotetrazepin-4(*3H*)-one **19**. The structure of these bi- and tricyclic systems were confirmed by ^{1}H, ^{13}C and ^{15}N NMR data. X-ray diffraction of compound **14** shows that the tetrazepinone ring is nonplanar.

INTRODUCTION

Dimethyltriazene **A** (Dacarbazine) shows antineoplastic activity against malignant melanoma[1,2]. Its mechanism of action is based on metabolic demethylation to generate the monoalkyl triazene[2-4], the cytotoxicity of which is due to its ability to methylate DNA[5]. Imidazotetrazinone **B** (Mitozolomide), a cyclic analog of **A**, has recently been shown to release a similar metabolite upon hydrolysis[6,7] and to display potent activity against leukemia, murine xenografts, Lewis lung and colon carcinoma[8]. One of its advantages over dimethyltriazene **A** is that it can generate the cytotoxic monoalkyl triazene without the host metabolic activation[9].

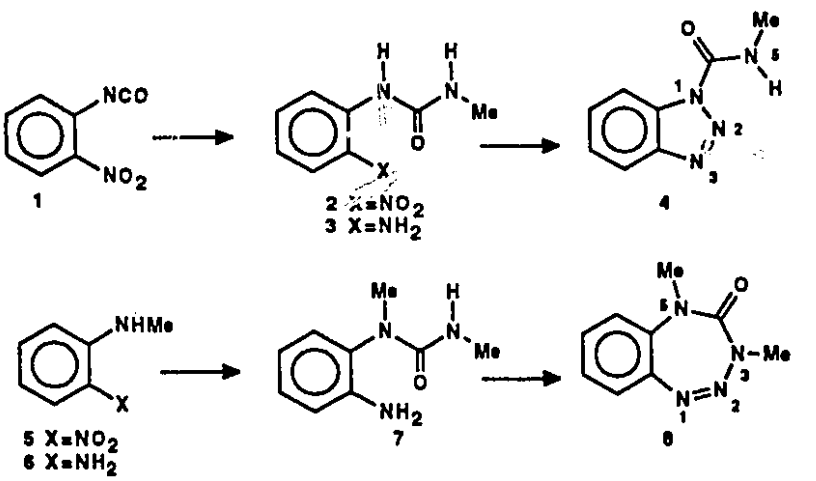


Those results suggest that other cyclic compounds containing the N=N-N-(alkyl)-CO-N moiety may be precursors of monoalkyltriazenes and display similar or more pronounced antitumor activity. In light of these considerations, we have undertaken the synthesis of a novel seven-membered 1,2,3,5-tetrazepine-4-one ring system which contains, like compound **B**, both the ureido function and the alkyltriazene linkage.

We are reporting here the synthesis of bi- and tricyclic tetrazepinone derivatives, based on an adaptation of the strategy developed for the synthesis of B[10]. ^{15}N NMR data were used in confirming their structure. We are also reporting the confirmation of one of the structures by X-ray diffraction.

RESULTS AND DISCUSSION

The approach we chose to study was the diazotization of substituted monoureas of type **3**. The diazonium salt can then cyclize to provide either substituted benzotriazole of type **4** or benzotetrazepinone of type **8**.



Scheme 1

Treatment of the commercially available 2-nitrophenylisocyanate **1** with methylamine provided **2**, which was reduced catalytically to give the amine **3**. Diazotization gave the triazole **4**, m.p. 70°C. The triazole structure was assigned from the ^{1}H NMR which showed a methyl signal at 3.15 ppm as a doublet, coupled with the NH proton at 7.31 ppm, which appeared as a broad singlet. The stability of this compound allowed the recording of a Standard Pulse ^{15}N NMR spectrum at the natural

abundance level (Fig. 3). Four distinct peaks were observed and their assignments are based on literature values[11,12]. Specific labelling by carrying out diazotization with $Na^{15}NO_2$ showed that the signal at -12 ppm corresponded to the central nitrogen N2. Chemical shift values of -58 and -29 ppm were assigned to N3 and N1 and the more shielded nitrogen N5 resonated at -298 ppm with one-bond N-H coupling constant value of 90 Hz.[11]

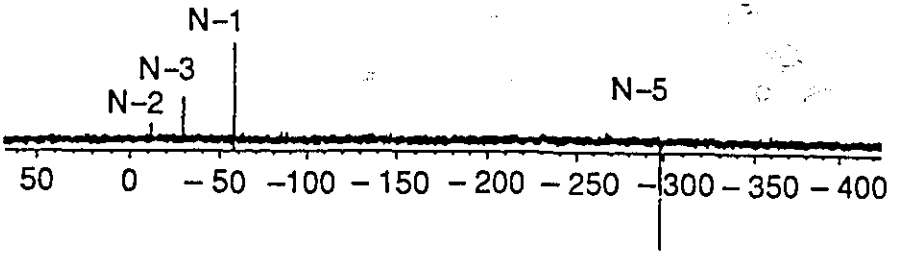


Fig. 1 ^{15}N NMR spectrum of **4** (decoupled with NOE effect)

Since replacement of the methyl group in **3** by other alkyl or aryl groups did not seem to affect the mode of cyclization, it was decided to alkylate the amino group carrying the carbamoyl function. N-methyl-2-nitro-aniline **5** was reduced to the corresponding amine **6**, and the resulting diamine selectively carbamoylated with methyl isocyanate to provide **7**. Diazotization, followed by adjusting the pH of the solution to **8**, gave a yellow powder, mp 40°C, in 52% yield. Its elemental composition was confirmed, by its microanalysis and chemical ionization mass spectrum, which in addition to MH^+, showed a large MH^+-28 peak due to the loss of nitrogen. In the ^1H NMR spectrum, the two methyl signals appeared as singlets and in the ^{15}N NMR spectrum, the central nitrogen of the triazene function showed up as a quartet ($^3J_{NH}$=2.70 Hz) at + 72 ppm, as confirmed by carrying out the diazotization with $Na^{15}NO_2$. The fact that the peak showed up as a quartet ($^3J_{N2H}$) confirmed the presence of the N2-N3-CH_3

moiety. The value of the chemical shift of N2 is similar to those previously reported for the central nitrogens in 1-aryl-3,3-dialkyl triazenes (around 70 ppm)[14,15,20].

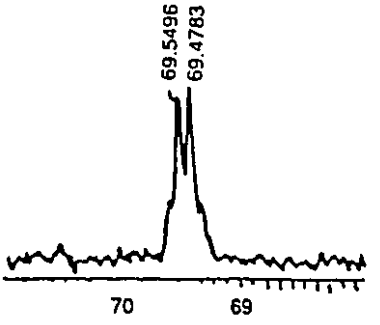
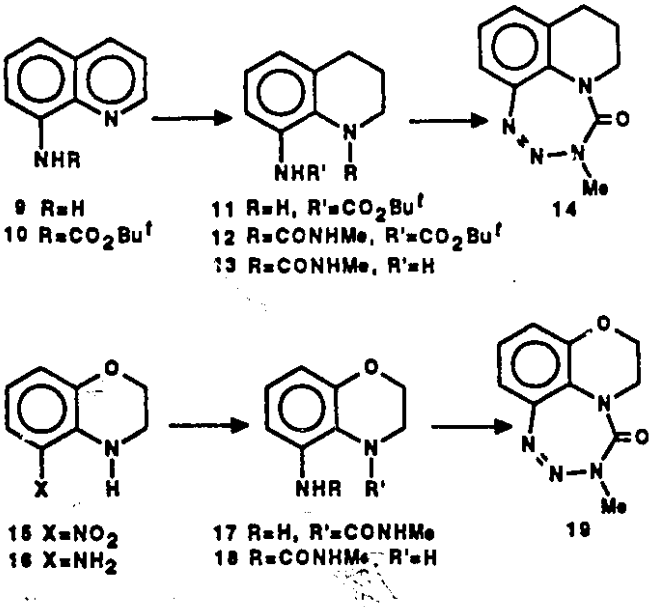


Fig. 2 ^{15}N NMR spectrum of the N-2 specifically labelled compound 14

Tricyclic tetrazepinone **14** was prepared as shown in Scheme 2. 8-aminoquinoline as its *tert*-butoxycarbonyl derivative **10** upon catalytic reduction gave the tetrahydroquinoline **11**. This after conversion into urea **12** by methyl isocyanate, was hydrolyzed to the amino urea **13**.



Scheme 2

Diazotization, followed by adjusting the pH to 8, gave tetrazepinone **14**, the structure of which was assigned on the basis of ^{1}H, ^{13}C, ^{15}N NMR spectra, chemical ionization mass

spectroscopy and elemental analysis. In addition to MH^+, the mass spectrum showed a large MH^+-28 peak due to loss of nitrogen. In the ^{15}N NMR spectrum, the resonance for the central nitrogen atom appeared as a quartet at 69.50 ppm, $^3J_{N2H}$=2.70 Hz (Fig. 1).

The synthesis of the morpholino analog of **19** proceeded in a similar fashion. 2-amino-3-nitrophenol was treated with dibromoethane under basic condition and the resulting nitrobenzoxazine **15** reduced to **16**. Treatment with one equiv. of methyl isocyanate gave **17** as the major product which was separated by chromatography from **18**. Diazotization with $NaNO_2$ or $Na^{15}NO_2$ followed by neutralization, gave **19**. The structure assigned was based on elemental analysis and NMR data. In the ^1H NMR spectrum, the methyl signal appeared as a singlet at 3.43 ppm, and the O-CH_2CH_2-N moiety as a pair of triplet at 4.24 and 3.82 ppm. As in the tetrazepinones **8** and **14**, the central nitrogen of the triazene function showed up as a quartet at 68.50 ppm, $^3J_{N2H}$=2.70 Hz.

In order to further ascertain the structure and geometry of the 1,2,3,5-tetrazepinone ring, the X-ray structure of compound **14** was determined. As shown in Fig. 3a, N5, N3 and O4 were found to be coplanar as expected for a ureido moiety. However, nitrogen N2 of the N1=N2 bond deviated from the plane of the aromatic ring by 0.5384 Å (Fig. 3a, 3b). This deviation makes the 1,2,3,5-tetrazepinone an essentially nonplanar seven-membered ring. Its geometry is strikingly different from that of the 1,2,3,5-triazinone ring in compound **B** for which N2 does not significantly deviate from planarity as conclusively demonstrated by X-ray diffraction results[13]. The geometry of the tetrazepinone ring is similar to that of the diazepinone ring system in the diazepam- related drugs, the X-ray structures of which were recently described by Gilman[21].

It is noteworthy that ^{1}H and ^{13}C chemical shifts of all tetrazepinones were slightly more deshielded than in their ureido precursors. Values around 2.70 ppm for the methyl doublets were observed for ureas **7, 13, 17**, whereas they are at around 3.20 ppm

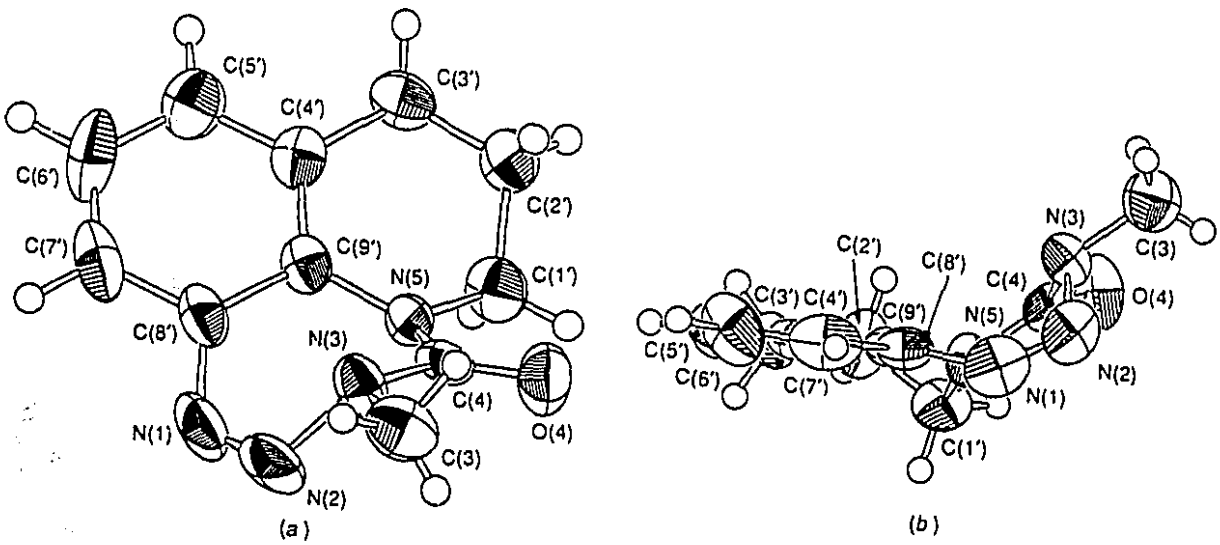


Fig. 3 Ortep wiews[23] of the crystallographically determined molecular structure for compound **14** (50% probability ellipsoid). For clarity arbitrary thermal parameters were assigned to the hydrogen atoms.

for the methyl singlets in the corresponding tetrazepinones. The same trend was observed for the carbonyl ^{13}C chemical shifts which were around 159 ppm in the ureas and around 161 ppm in the corresponding tetrazepinones. This is in agreement with the fact that the electron withdrawing effect of the diazo group decreases the electron density at the nitrogens and carbons in the ureido moiety. It is also interesting to notice the striking difference between the chemical shift of the central nitrogen (N2) in the benzotriazole **4** (-12 ppm) and in the tetrazepinones **8, 14** and **19** (around + 70 ppm). In **4**, the greater shielding of the central nitrogen (N2) is due to the fact that it experiences additional screening from the electronic current in the aromatic triazole ring. In the non-aromatic tetrazepinones, the chemical shift values are analogous to those of the central nitrogen in open-chain 1-aryl-3,3-dialkyl triazenes. Electron delocalization occurs to a

much lesser extent and N3 shows significant sp^3 character, as confirmed by X-ray diffraction results (Fig. 3).

Table 1. Non-hydrogen bond lengths (Å) for 14

O(4)-C(4)	1.21(2)	C(1')-C(2')	1.51(2)
N(5)-C(1')	1.51(2)	C(2')-C(3')	1.48(2)
N(5)-C(9')	1.40(1)	C(3')-C(4')	1.50(2)
N(5)-C(4)	1.33(1)	C(4')-C(5')	1.39(2)
N(3)-N(2)	1.49(2)	C(4')-C(9')	1.42(2)
N(3)-C(4)	1.45(2)	C(5')-C(6')	1.38(2)
N(3)-C(3)	1.45(2)	C(6')-C(7')	1.36(2)
N(2)-N(1)	1.23(1)	C(7')-C(8')	1.39(2)
N(1)-C(8')	1.41(2)	C(8')-C(9')	1.41(2)

Table 2. Non-hydrogen bond angles (°) for 14

C(1')-N(5)-C(9')	115.6(8)	C(5')-C(4')-C(9')	119(1)
C(1')-N(5)-C(4)	117(1)	C(4')-C(5')-C(6')	122(1)
C(9')-N(5)-C(4)	123(1)	C(5')-C(6')-C(7')	119(1)
N(2)-N(3)-C(4)	111.8(9)	C(6')-C(7')-C(8')	122(1)
N(2)-N(3)-C(3)	109(1)	N(1)-C(8')-C(7')	118(1)
C(4)-N(3)-C(3)	115(1)	N(1)-C(8')-C(9')	122(1)
N(3)-N(2)-N(1)	119(1)	C(7')-C(8')-C(9')	119(1)
N(2)-N(1)-C(8')	124(1)	N(5)-C(9')-C(4')	120(1)
N(5)-C(1')-C(2')	107.1(9)	N(5)-C(9')-C(8')	121.2(9)
C(1')-C(2')-C(3')	112(1)	C(4')-C(9')-C(8')	119(1)
C(2')-C(3')-C(4')	115.2(9)	O(4)-C(4)-N(5)	125(1)
C(3')-C(4')-C(5')	120(1)	O(4)-C(4)-N(3)	119.0(9)
C(3')-C(4')-C(9')	120(1)	N(5)-C(4)-N(3)	116(1)

EXPERIMENTAL

Melting points were measured on a Gallenkamp block and are uncorrected. Thin-layer and flash chromatography were performed on silica gel 60 F_{254} aluminum plates and Merck Silica Gel 60 (230-400 mesh) respectively. ^1H NMR spectra were recorded on a Varian XL-200 at 200 MHz. ^{13}C NMR spectra were obtained at 75.40 MHz on a Varian XL-300. All ^1H NMR spectra were run in $CDCl_3$ or in DMSO-d_6 and chemical shifts are reported downfield from TMS. Low and high resolution mass spectra were recorded in a HP 5984A or LKB 9000 and Du Pont 21-492B instruments.(J values are in

reported downfield from TMS. Low and high resolution mass spectra were recorded in HP 5984A and Du Pont 21-492B instruments.(J values are in Hz and ν_{max} in cm^{-1}). All compounds were shown to be homogeneous by TLC and high-field NMR, or to have a purity of > 95% by elemental analysis.

Compounds **1**, **5**, **9** and **15** were obtained from Aldrich Chemical Co.

^{15}N NMR spectra were taken at 30.40 MHz on a Varian XL-300 and chemical shifts are reported upfield from nitromethane, which was used as external standard. The 90° pulse width was 18s and the pulse interval was set at 3s. Spectra were obtained after 100 scans for the ^{15}N enriched compounds when sample concentrations were around 0.10 M in $CDCl_3$ (gated coupled)[11] and after 1024 scans for the natural abundance spectrum at a 0.80 M concentration (decoupled with NOE effect).

All reactions were monitored by thin layer chromatography (TLC).

N-Methyl-(2-aminophenyl-N'-methyl-urea (3).

To a stirred solution of compound **1** (2g, 12.20 mmol) in 10 mL of methylene dichloride was added dropwise 2.50 mL of 40% aqueous methylamine. The mixture was then stirred for 15 min and the pale yellow precipitate was filtered and hydrogenated in 25 mL of methanol containing 300 mg of a 10% Pd-C catalyst, at 3 atm for 20 min. Filtration and evaporation gave 1.8g (89.42%) of a white powder that was recrystallized from methanol; m.p. 170°C; IR (KBr disc) ν_{max}: 3415 (NH, NH_2), 1676 (C=O); 200 MHz ^1H NMR (DMSO) δ: 8.0 (br s, 1H, ArNHCO-), 7.86 (d, J=7.86, 1H, Ar), 7.26-7.17 (m, 2H, Ar), 7.02 (t, J=7.94, 1H, Ar), 6.00 (br q, J=4.36, 1H, -CON*H*CH_3), 5.20 (s, 2H, NH_2), 3.10 (d, J=4.6, 3H, CONHC*H*$_3$); 75.40 MHz ^{13}C NMR ($CDCl_3$) δ: 156.70 (s), 140.70 (s), 125.40 (s), 124.00 (d), 123.60 (d), 116.52 (d), 115.50 (d), 26.30 (q).

N-Methyl-1H-benzotriazole-1-carboxamide (4).

To a solution of **3** (1g) in 20 mL of 5M HCl, 2.5 mL of of 17% aqueous solution of sodium nitrite was added dropwise at 0°C with constant stirring. The resulting white precipitate was extracted with methylene dichloride and purified on silica gel with a 4:3:2 mixture of chloroform/hexane/ethyl acetate to give 0.9g of **4** (85%); m.p. 70°C; IR (KBr disc) ν_{max}: 3380-3210 (NH), 1744 (C=O); 200 MHz ^{1}H NMR ($CDCl_3$) δ: 8.30 (d, J=8.30, 1H, Ar), 8.10 (d, J=8.40, 1H, Ar), 7.70 (t, J=8.24, 1H, Ar), 7.45 (t, J=8.20, 1H, Ar), 7.31 (br s, 1H,-CON*H*CH_3), 3.15 (d, J=5.00, 3H,CONHC*H*$_3$); 75.40 MHz ^{13}C NMR ($CDCl_3$) δ: 150 (s), 146 (s), 132 (d), 125 (d), 120 (d), 114 (d), 25 (q); 30.40 MHz ^{15}N NMR ($CDCl_3$) δ: -12.22 (s), -29.28 (s), -58.00 (s), -298.48 (d, $^1J_{NH}$=90 Hz). Anal. Calcd for $C_8H_8N_4O$: C, 54.54; H, 4.58; N, 31.80. Found C, 54.90; H, 4.62; N, 32.06.

N-(2-aminophenyl)-N,N'-dimethylurea (7).

N-methyl-2-nitroaniline **5** (4g) was catalytically reduced to N-methyl-o-phenylenediamine **6** according to the method described for compound **3**. It was obtained as a brown liquid (2.6, 81%). Amine **6** (2g) was dissolved in a 100 mL of chloroform and stirred at 0°C with methyl isocyanate (1 eq). After 30 min, the brown solution was concentrated under vacuum and purified on silica gel (methanol/CCl_4: 4:1) to give **7** as a brown oil that solidified on standing (2.5g, 86%); m.p. 180°C; IR (KBr disc) ν_{max}: 3400, 3200 (NH_2, NH), 3000 (CH), 1686 (C=O) ^{1}H NMR ($CDCl_3$) δ: 7.2-6.8 (m, 4H, Ar), 4.75 (br s, 2H, NH_2) 4.25 (br s, 1H, -CO-N*H*CH_3), 3.16 (s, 3H, ArN(CH_3)-CO), 2.70 (d, J=4.6, 3H, CONHC*H*$_3$); 75.40 MHz ^{13}C NMR ($CDCl_3$) 159.5 (s), 144.2 (s), 129 (d), 128 (s), 118 (d), 116.2 (d), 34.8 (q), 26.5 (q).

3,5-Dimethyl-3H-1,2,3,5-benzotetrazepin-4(5H)-one (8).

To a solution of **7** (2g) in 25 HCl (10 mL) was added dropwise 5 mL of an 8% aqueous sodium nitrite at 0°C. The mixture was then extracted with methylene dichloride to

remove impurities and the pH of the clear aqueous layer was adjusted to 8 with a 5% solution of sodium bicarbonate. The precipitate that formed was extracted with methylene dichloride and purified on silica gel (ethyl acetate/hexane: 7:3) to give 1.1g (52%) of **8** as a yellow powder ; m.p. 40°C (effervescence at 100°C); IR ($CHCl_3$) ν_{max}: 3000 (C-H), 1680 (C=O); 200 MHz ^1H NMR ($CDCl_3$) δ: 7.45-7.08 (m, 4H Ar), 3.40 (s, 3H $ArN(CH_3)CO$-), 3.23 (s, 3H, $N{=}NNCH_3$); 75.40 ^{13}C NMR ($CDCl_3$) δ: 162 (s), 142.30 (s), 138.5 (s), 132 (d), 128 (d), 124.5 (d), 121 (d), 38 (q), 36 (q) ^{15}N NMR ($CDCl_3$) δ : 72 (q, $^3J_{NH}$=2.70, N_2); MS (CI, NH_3, m/z, I%) 191.09 (MH^+,-N_2, 28.72), 134.00 (MH^+-MeNCO, 14.91); HRMS exact mass calcd. for $C_9H_{11}N_4O$ (M+1): 191.0933. Found: 191.0932. Anal. calcd for $C_9H_{10}N_4O$: C, 56.83; H 5.30; N, 29.46. Found: C, 57.00; H, 5.49; N, 29.32.

8-(N-*tert*-butoxycarbonylamino)-1,2,3,4-tetrahydroquinoline (11).

A solution of 8-aminoquinoline **9** (2g) and di-t-butyl-dicarbonate (3.5g, 1.15 eq) in dioxane (50 mL) was refluxed for three days under nitrogen. Evaporation under vacuum gave **10** as a brown oily compound which was sufficiently pure to be used for further reactions; 200 MHz ^1H NMR ($CDCl_3$) δ : 9.00 (br s, 1H, NHCOOt-Bu), 8.70 (d, 1H, J=4.2, Ar), 8.40 (d, 1H, J=4.2, Ar), 8.40 (d, 1H, J=8.00, Ar), 8.10 (d, 1H, J=8, Ar) 7.5-7.30 (m, 3H, Ar), 1.80 (s, 9H, COOOt-Bu). A solution of **10** and 10% Pd-C (1g) in methanol (30 mL) was hydrogenated at 3 atm overnight. Filtration and evaporation gave a colorless crystalline residue in quantitative yield; m.p. 110°C; IR ($CHCl_3$) ν_{max}: 3400, 3200 (NH_2, NH), 3000 (CH), 1715 (C=O); 200 MHz ^1H NMR ($CDCl_3$) δ: 7.10. (d, J=7.50, 2H Ar), 6.72 (d, J=7.50, 2H, Ar) 6.63, (t, J=7.20, Ar), 6.20 (br s, NHCOOt-Bu), 3.28 (t, J=6.3, 2H, $-CH_2H_2C\mathit{H}_2$-NH-), 2.80 (t, J=6.40, 2H, $C\mathit{H}_2CH_2CH_2NH$-), 1.88 (quintet, J=6.3, 2H, $-CH_2C\mathit{H}_2CH_2$-NH-), 1.50 (s, 9H, COOtBu).

8-N-(*tert*-butoxycarbonylamino)-N-methyl-1,2,3,4-tetrahydroquinoline-1-carboxamide (12).

A solution of **11** (2.8g, 11.3 mmol) and 0.7 mL (1 eq) of methyl isocyanate in chloroform (25 mL) was stirred for 26 hours. The solvent was evaporated and a white crystalline residue was obtained in quantitative yield; m.p. 135°C; IR (KBr disc) ν_{max}: 3300 (NH), 3000 (CH), 1716, 1653 (C=O); 200 MHz ^{1}H NMR ($CDCl_3$) δ: 7.84 (d, J=8, 1H, Ar), 7.16 (t, J=8, 1H, Ar), 6.87 (d, J=8, 1H, Ar), 6.76 (s, 1H, N*H*-COOtBu), 4.60 (br q, 1H, CON*H*CH_3) 3.60 (br, 2H, $CH_2CH_2CH_2$NH(CO-), 2.74 (d, J=4.70, 3H, -COHNCH_3), 2.68 (t, J=7.30, 2H, -C$H_2CH_2CH_2$NCO-), 1.94 (quintet, J=6.70, 2H,-$CH_2CH_2CH_2$NCO-) , 1.64 (s, 9H, tBu).

N-Methyl-8-amino-1,2,3,4-tetrahydroquinoline-1-carboxamide (13).

A suspension of **12** (1.5g) in trifluoroacetic (10 mL) acid-water mixture (85:15) was stirred overnight. Dilution with 20 mL of water and neutralization with a 5% sodium bicarbonate solution gave the deprotected urea as a brown solid (0.7g, 70%); m.p. 130°C; IR (KBr disc) ν_{max}: 3380, 3300 (NH), 3000 (CH), 1638 (C=O); 200 MHz ^{1}H NMR ($CDCl_3$) δ: 6.95 (t, J=8.00, 1H, Ar), 6.60 (d, J=8.00, 2H, Ar), 4.95 (br s, 1H, CON*H*CH_3), 3.83 (br s, 4H, -$CH_2CH_2CH_2$-NCO-, NH_2 overlap), 2.76 (d, J=4.70, 3H, -CONHCH_3), 2.60 (t, J=7.24, 2H, -C$H_2CH_2CH_2$NCO-), 1.90 (quintet, 2H, J=7.00, $CH_2CH_2CH_2$NCO-).

3-Methyl-6,7-dihydro-1,2,3,5-tetrazepino[7,6,5-ij]-4-quinolin-4(3H)-one (14).

To a solution of **13** (0.7g, 0.5 mmol) in 5% HCl (10 mL) was added dropwise at 0° C with stirring 5 mL of a 5% aqueous solution of sodium nitrite. Extraction with two 20 mL portions of methylene dichloride removed impurities. The pH of the clear aqueous layer containing the diazonium salt was adjusted to 8 with a 5% solution of Na_2CO_3 at 0°C. The brown precipitate that formed was extracted with methylene dichloride.

Evaporation of the solvent gave 0.55g (76%) of a pure brown powder; m.p. 85°C (effervescence at 100° C) ; IR ($CHCl_3$) ν_{max}: 3030 (CH), 1690 (C=O); 200 MHz ^1H NMR ($CDCl_3$) δ: 7.25-7.00 (m, 3H, Ar), 4.24 (t, J=5.72, 2H,-$CH_2CH_2CH_2NCO$)-), 3.33 (s, 3H, N=NNCH_3), 2.80 (t, J=6.66, 2H, -$CH_2CH_2CH_2NCO$-), 1.88 (quintet, J=6.1, 2H, -$CH_2CH_2CH_2N$-CO-); 75.40 MHz ^{13}C NMR ($CDCl_3$) δ: 161.00 (s), 142.50 (s), 135.97 (s), 132.39 (d), 131.20 (s), 126.23 (d), 124.98 (d), 44.81 (t), 38.12 (q), 28.53 (t), 23.07 (t); 30.40 MHz ^{15}N NMR ($CDCl_3$): 70 ($^3J_{NH}$=2.7, N2); MS (CI, NH_3, m/z, I%) 217.10 (MH^+, 100), 189.00 (MH^+-N_2) 160.00 (53.00); HRMS exact mass calcd for $C_{11}H_{13}N_4O$ (M+1): 217.1089. Found: 217.1089. Anal. Calc. for $C_{11}H_{12}N_4O$: C, 61.09; H, 5.53; N, 25.91. Found: C, 61.11; H, 5.55; N, 25.90.

5-Nitro-2,3-dihydro-1,4-benzoxazine (15).

2-Amino-3-nitrophenol (3g, 19 mmol) in 15 mL of DMF and 2.7 mL of 1,2-dibromoethane was heated at reflux and 0.75g (2 eq) of KOH was added portionwise. It was kept under reflux for two days. The solution was poured into 30 mL of water at 0°C. Filtration and column chromatography of the red filtrate on silica gel (ethyl acetate/hexane: 2:3) gave 1.5 g (43%) of **15** as yellow crystals; m.p. 100°C; IR ($CHCl_3$) ν_{max}: 3300 (NH), 1531 (NO_2); 200 MHz ^1H NMR ($CDCl_3$) δ: 7.90 (br s, 1H, NH), 7.74 (d, J=8.70, 1H, Ar), 6.90 (d, J=8.70, 1H, Ar), 6.60 (t, J=8.77, 1H, Ar), 4.24 (t, J=4.50, 2H, -O-CH_2CH_2-NH-), 3.64 (q, J=4.7, 2H, -O-CH_2CH_2-NH-); MS (EI, m/z, %) 180 (M^+, 42.00) 134 (M-NO_2, 26).

5-Amino-2,3-dihydro-1,4-benzoxazine (16).

A solution of benzoxazine **15** (0.7g) and 10% Pd-C catalyst (0.3g) in methanol (15 mL) was hydrogenated at 3 atm . Filtration and evaporation gave a brown liquid in quantative yield. IR (KBr disc) ν_{max}: 3300, 3200 (NH_2, NH), 3000 (CH); 200 MHz ^1H

NMR ($CDCl_3$) δ: 6.65 (t, J=8.40, 1H, Ar), 6.4 (t, J=8.20, 2H, Ar), 4.18 (t, J=4.50, 2H, -O-$CH_2$$CH_2$-NH-), 3.42 (t, J=4.30, 2H, -O-$CH_2$$CH_2$-NH-), 3.10 (br s, 3H, NH, NH_2).

5-Amino-N-methyl-2,3-dihydro-1,4-benzoxazine-4-carboxamide (17).

Compound **16** (0.7g) was dissolved in chloroform (25 mL) and methyl isocyanate (0.2 mL, 1 eq) was added dropwise at 0°C. The mixture was stirred for 5 hrs, concentrated under vacuum and chromatographed on silica gel (ethyl acetate/hexane 1:1) to give 0.4g (41%) of a white crystalline residue; 200 MHz ^1H NMR ($CDCl_3$) δ: 6.90 (t, J=8.00, 1H, Ar). 6.30 (m, 2H, Ar), 5.60 (br s, 1H, CONHCH$_3$), 4.2 (t, J=4.53, 2H, -O-$CH_2$$CH_2$-NCO-), 3.80 (br s, 4H, -O-$CH_2$$CH_2$-NCO-, NH_2 overlap), 2.80 (d, J=4.6, 2H,CONHCH_3); 75.40 MHz ^{13}C NMR ($CDCl_3$) δ: 159.13 (s), 150.54 (s), 142.79 (s), 128.69 (d), 116.25 (s), 109.11 (d), 108.82 (d), 67.46 (t), 43.37 (t), 28.91 (q).

3-Methyl-6,7-dihydro[1,4]oxazino[4,3,2-ef]-1,2,3,5-benzotetrazepin-4(5H)-one (19).

To a solution of urea **17** (0.4, 5 mmol) in 15 mL of 5% HCl was added dropwise at 0°C with stirring, 5 mL of a 7% aqueous sodium nitrite. The mixture was then stirred for 10 additional minutes and its pH was adjusted to 9 with a 5% sodium carbonate solution and extracted with three 5 mL portions of methylene dichloride. Evaporation of the solvent gave 0.25g (60%) of **19** as a pure brown powder; m.p. 110°C; IR (KBr disc) ν_{max}: 3000 (C-H), 1691 (C=O); 200 MHz ^1H NMR ($CDCl_3$) δ: 7.20-6.90 (m, 3H, Ar), 4.24 (t, J=4.30, 2H, -O-$CH_2$$CH_2$-NCO-), 3.82 (t, J=4.36, 2H,-O-$CH_2$$CH_2$-NCO-), 3.43 (s, 3H, N=NNCH_3) ; 75.40 MHz ^{13}C NMR ($CDCl_3$) δ: 161.00 (s) 146.53 (s), 142.43 (s), 126.00 (d), 124.17 (s), 120.60 (d), 119.20 (d), 66.38 (t), 41.40 (d), 38.12 (q); 30.40 MHz ^{15}N NMR ($CDCl_3$) δ: 68.5 (q, $^3J_{NH}$=2.7). Anal. calcd for $C_{10}H_{10}O_2N_4$: C, 55.04; H, 4.62; N 25.68. Found C, 55.56; H, 4.77; N, 25.33.

X-ray crystallography.- Crystals of compound **14** were obtained from slow evaporation of 1:1 ethyl acetate/hexane mixture.

Crystal data.- $C_{11}H_{12}N_4O$, M = 216.24. Monoclinic, a = 8.621 (2), b = 14.450(2), c = 8.891 (2) Å, B=108.81 (2)°, V=1048.5 Å^3 (5) (by least-squares refinement on diffractometer angles for 20 automatically centred reflections) space group $P2_1/n$, (alt. $P2_1/c$, No. 14), Z=4, D_x =1.370 g cm^{-3}. Small yellow needles, thermosensitive. Crystal dimensions: 100x0.100x0.100mm, μ(Mo-K_α)=7.21 cm^{-1}.

Data Collection and Processing.- Rigaku AFC6S diffractometer, ω-2θ mode with ω scan width =1.52 + 0.30 tanθ, scan speed 32 deg min^{-1}, graphite-monochromated Mo-K_α radiation, temperature 20 ± 1; 1746 reflections measured 1630 unique after absorption correction (max. min. transmisssion factors= 1.00, 0.95) giving 742 with I>2σ (I). The intensities of three representative reflections remained constant throughout data collection indicating crystal and electronic stability (no decay correction was applied). The data were corrected for Lorentz and polarization effects. Low temperature probe was not available.

Structure Analysis and Refinement.- All non-hydrogen atom positions from direct methods[18] using the TEXSAN crystallographic sotware package of Molecular Structure Corporation[19]. All hydrogen-atom positions from a Fourier difference map. All positional and thermal parameters (non-hydrogen atoms: anisotropic; hydrogens: isotropic) and an extinction parameter were refined by full-matrix least square. Final R and R_w were 0.087, and 0.072 for 742 observed reflections and 152 variable parameters. The weighting scheme $w=4F_o^2/s^2(F_o^2)$ obtained from counting statistics gave satisfactory agreement analyses. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.28 and -0.33 eÅ^{-3}, respectively. Neutral atom

scattering factors were taken from Cromer and Waber[20]. Anomalous dispersion effects were included in F_{calc};[21] the values for $\Delta f'$ and $\Delta f''$ were those of Cromer[22]. Figures were drawn with ORTEPII.[23]*

ACKNOWLEDGEMENT: We thank Natural Sciences and Engineering Research Council of Canada and Fonds FCAR, Province de Quebec, for financial support. We are also grateful to Dr Jim Britten for X-ray structure analysis, Dr Francoise Sauriol for technical assistance in running NMR spectra, Dr John Finkenbine, and Dr Orval Mamer of the Biomedical Mass Spectrometry Unit, McGill University, for running mass spectra.

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* A complete list of bond lengths and angles, hydrogen atom coordinates and thermal parameters has been deposited at the Cambridge Crystallographic Data Centre.

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Chapter 4

General overview

In the preceding chapter we showed that it was possible to synthesize tetrazepinones by diazotizing N-(2-aminophenyl)-N,N'-dialkylureas provided that the the mixture was alkalinified. In this chapter we describe the synthesis and stability of benzotetrazepinones bearing substiuents at the benzene moiety.

We also show that the formation of tetrazepinones results from a relatively stable aryldiazonium intermediate, the concentration of which varies with the pH of the reaction mixture. We also describe the structure of the products resulting from the decomposition of tetrazepinones in chloroform and in aqueous solution.

1,2,3,5-TETRAZEPINONES II.-SYNTHESIS AND STABILITY OF SUBSTITUTED BENZO-1,2,3,5-TETRAZEPIN-4-ONES.

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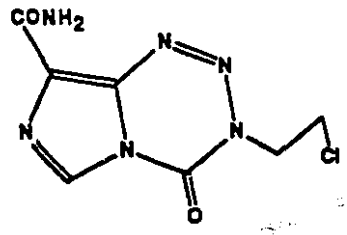
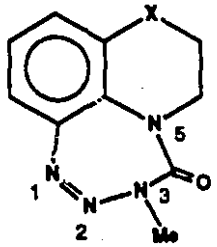
ABSTRACT: The syntheses of 7- and 8-substituted-dimethyl-*3H*-1,2,3,5-benzotetrazepin-4-(*5H*)-ones **6b-e**, and **10** are described. X-ray diffraction of nitrobenzotetrazepinone **6e** showed that its tetrazepinone ring is nonplanar. The stability of the tetrazepinones increased with the electronwithdrawing character of the substituent. The unstable tetrazepinones **6c** and **10** decomposed in chloroform at room temperature to benzotriazole derivatives **13c** and **11**. The decomposition of **6a, c, d** at alkaline pH gave benzotriazoles **13a, c, d** and ureas **14a, c, d**. Tetrazepinone **6e** decomposed to 5-nitro-2-hydroxy-benzimidazole **15e** in quantitative yield. In addition to **13d** and **14d**, the decomposition of **6d** showed a small amount of 2-hydroxybenzimidazole **15d**. At acidic pHs, the benzotetrazepinones were found to be in equilibrium with their corresponding diazonium ureas **5**.

INTRODUCTION.

The discovery of the antitumour activity of imidazo-1,2,3,5-tetrazin-4-one (mitozolomide) **A**[1] has stimulated interest in designing and synthesizing compounds containing the N=N-N(alkyl)CO moiety[2,3,4]. Recently, we reported the synthesis of the tricyclic system **B** and the bi-cyclic compound **6a**[4] featuring the novel 1,2,3,5-tetrazepin-4-one ring. This system contains, like Mitozolomide **A**, the potential pro-monoalkytriazene N=N-N-(alkyl)-CO-N moiety which is essential for antitumour activity[5,6]. Because mitozolomide can be hydrolyzed to the active monoalkyltriazene[6], it does not need metabolic activation to show cytotoxicity.



A



B, X=CH_2, O

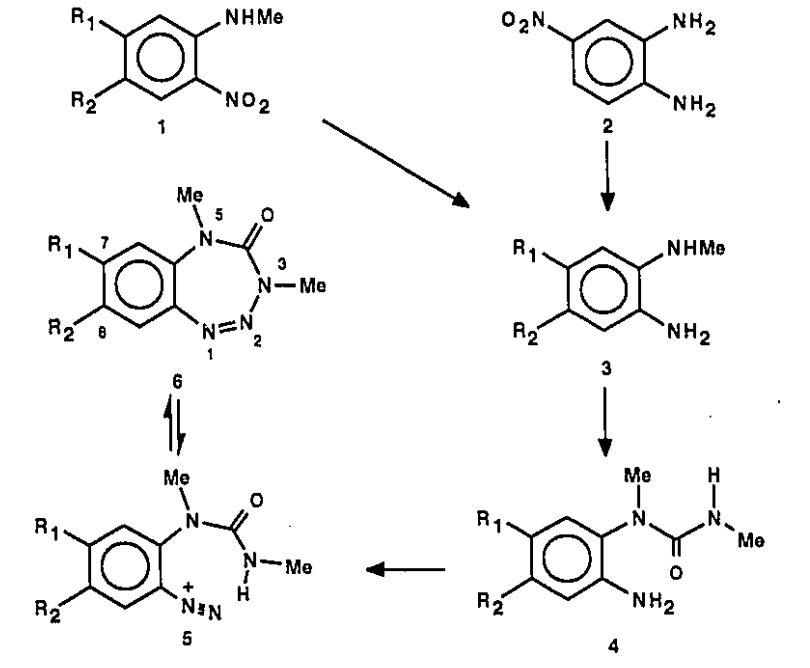
We now synthesized a series of benzotetrazepinones bearing substituents on the benzene ring . Our goal was to determine the effect of these substituents on the stability of the benzo-fused seven-membered ring. The benzotetrazepinones have generally been characterized by ^{1}H, ^{13}C , ^{15}N NMR and elemental analysis.

In this paper, we also show that, in aqueous solution, the benzotetrazepinones are in equilibrium with their diazonium urea precursors. The ratio of these two species varies with the pHs of the solution and the nature of the ring substituents. The products resulting from the decomposition of the tetrazepinones at alkaline pHs are also described.

RESULTS AND DISCUSSION

a. Synthesis

Tetrazepinone **6a** was synthesized according to a published method[4]. The synthesis of **6b, c** and **d**, proceeded according to scheme 1. N-Methyl-2-nitroanilines **1b, 1c**, and **d**, obtained from the treatment of the corresponding 2-nitroanilines with sodium hydride followed by addition of methyl iodide, were reduced catalytically to diamines **3b, 3c**, and **3d**. Selective carbamoylation[1] at the N-methylamino group by reaction with methyl isocyanate gave ureas **4b, c** and **d**. Diazotization of the resulting ureas with a sodium nitrite solution containing $Na^{15}NO_2$ where appropriate, and adjustment of the pH to 8 provided the tetrazepinones **6b, c** and **d** in the yields given in Scheme 1.



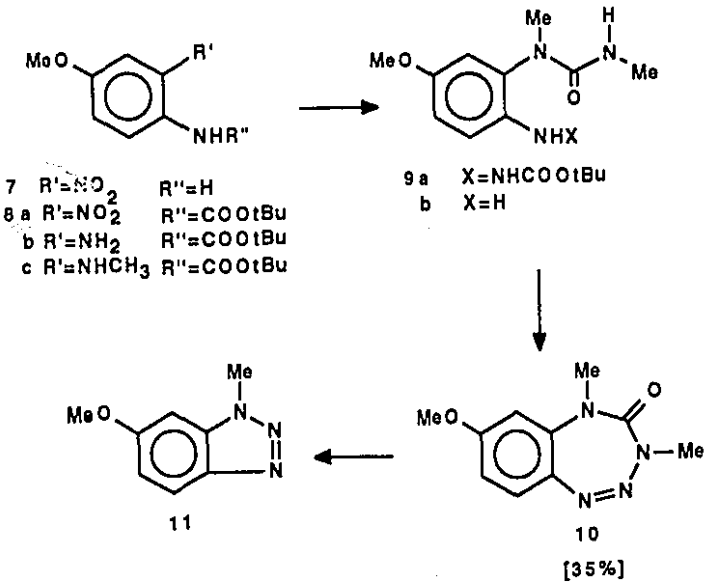
a R_1=H R_2=H [50%]
b R_1=Me R_2=H [32%]
c R_1=H R_2=OMe [61%]
d R_1=H R_2=Cl [71%]
e R_1=NO_2 R_2=H [90%]

Scheme 1

Similarly, 5-nitro-1-(N-methyl)-1,2-phenylenediamine **3e**, obtained from the direct methylation of **2** with methyl iodide under basic condition, was carbamoylated with methyl isocyanate to give urea **4e**. Diazotization of the urea as described, followed by adjustment of the pH to 6 gave 7-nitro-benzotetrazepinone **6e** as yellow needles in almost quantitative yield.

The structures of compounds **6b-e** were assigned on the basis of ^{1}H, ^{13}C, ^{15}N NMR and elemental analysis. In the ^{1}H NMR spectra, the methyl shifts appeared at around 3.40 ppm (N3-Me) and 3.25 ppm (N5-Me). In their urea precursors, the same protons were around 3.10 and 2.70 ppm respectively. The ^{13}C shifts of the methyl groups attached to N3 were more deshielded in the tetrazepinones than in their urea precursors (around 26 ppm in **4b-e** and around 35 ppm in **6b-e**). The ^{15}N resonances for the N2

label were in general observed at around 70 ppm (Table 3) and varied with the electronic character of the substituent at the benzene ring.



Scheme 2

The synthesis of tetrazepinone **10** proceeded according to Scheme 2. 2-Nitro-4-methoxyaniline **7** was treated with NaH and di-*tert*-butyl dicarbonate to give urethane **8a**, which was catalytically reduced to the corresponding amine **8b**. The latter was methylated to yield **8c**, which was treated with methyl isocyanate to provide urea **9a**. Deprotection of **9a** in trifluoroacetic acid , followed by neutralization gave amine **9b** which was diazotized. Adjustment of the pH to 8 gave tetrazepinone **10** in 35 % yield (crude) as a brown powder that effervesced slowly at room temperature.

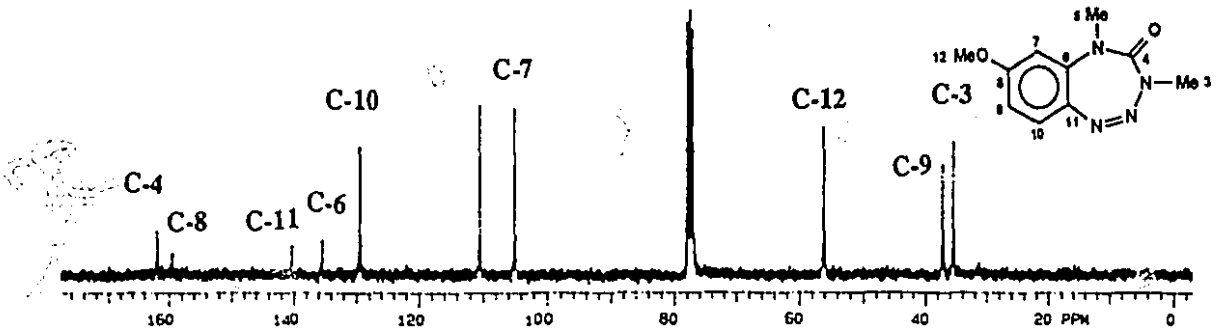


Fig 1.-^{13}C NMR spectrum of fresh **10**

Satisfactory ^{1}H, ^{13}C, ^{15}N NMR spectra could be obtained for **10** if they were taken with freshly prepared samples. In the ^{1}H NMR spectra, the two ureido methyl groups appeared as singlets at 3.34 (N3Me) and 3.26 ppm (N5Me), whereas for their urea precursor the same methyl groups appeared as a singlet at 3.17 and as a doublet at 2.74 ppm. In **10**, the ^{13}C shifts of the methyl groups attached to N5 and N3 were at 36.82 and 35.13 ppm respectively (Fig. 1). In urea **9b**, they were at 35.47 and 27.54 ppm. The ^{15}N label appeared at 63 ppm. We have already shown that the use of ^{15}N NMR spectroscopy to detect ^{15}N labeled N2 in 1,2,3,5-tetrazepinones is an efficient method to assign their structure[4]. Tetrazepinone **10** was stable enough in solution to allow the detection of the shifts of N1, N3 and N5 by ^{15} NMR at the natural abundance level (Table 1). Detailed discussion about the ^{15}N shifts of all the substituted tetrazepinones is given in the ^{15}N NMR section.

The major product isolated from the decomposition mixture, after one week standing at room temperature, was found to be benzotriazole **11**, the structure of which was confirmed by ^{1}H NMR and mass spectroscopy. In the ^{1}H NMR spectrum, the methyl group appeared as a singlet at 4.20 ppm. In addition to M^{+}, the mass spectrum showed a M^{+}-28 peak due to loss of nitrogen.

b. X-ray crystallography.

The X-ray structure of **6e** shows that its fused 1,2,3,5-tetrazepinone ring is essentially non-planar (Fig. 2, 3), as it is in tricyclic system **B** ($X=CH_2$)[4]. Although the nitro group is *para* to the triazene chain, it does not seem to greatly affect the pyramidality of N3 in the solid. Bond angles (Table 2) around this nitrogen are 108° (5) for N2-N3-C1 and 114°(5) for both N2-N3-C2 and C1-N3-C2. The torsion angle between the aromatic ring C5-C6 and the diazo linkage N1-N2 is 141.5°(8), showing a marked deviation of the triazene chain from coplanarity with the benzene ring. The bond

distance between N2-N3 (1.446(7) Å) (Table 1) suggests a single bond character of this linkage, and the pyramidality of bonding about N3 suggests a somewhat sp^3 character of the latter nitrogen. These results constrast with those reported for open-chain benzo- and imidazotriazenes[13,14] and mitozolomide A[15] in which bond lengths of N2-N3 (1.305 Å, 1.309 Å, 1.374 Å), and the planarity of bonding around N3, indicate its

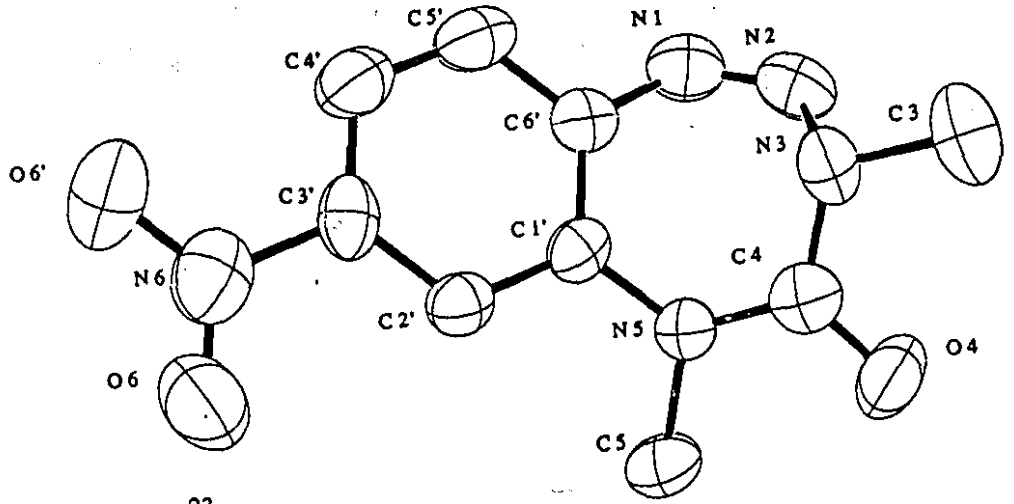


Fig 2.- ORTEP[23] view of the crystallographically determined structure for **6e** (50% probability ellipsoid). For clarity, hydrogen atoms have been omitted .

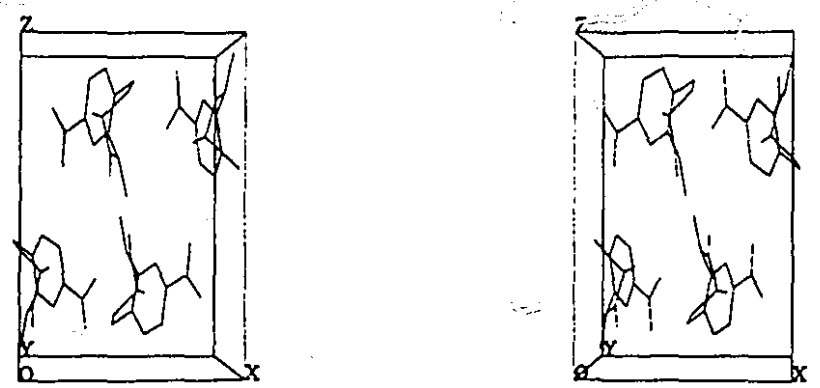


Fig 3.- Stereoviews of the contents of the unit cell of crystals of **6e**.

Table 1. Non-hydrogen bond lengths (Å) for 6e

O(1)-C(2) 1.228(9)	N(4)-C(3) 1.466(6)
O(2)-N(5) 1.205(7)	N(4)-C(4) 1.407(9)
O(3)-N(5) 1.227(8)	N(5)-C(8) 1.473(10)
N(1)-N(2) 1.253(10)	C(4)-C(5) 1.399(8)
N(1)-C(5) 1.409(9)	C(4)-C(9) 1.383(10)
N(2)-N(3) 1.446(7)	C(5)-C(6) 1.384(10)
N(3)-C(1) 1.489(9)	C(6)-C(7) 1.375(11)
N(3)-C(2) 1.419(8)	C(7)-C(8) 1.374(9)
N(4)-C(2) 1.361(10)	C(8)-C(9) 1.389(9)

Table 2. Non-hydrogen bond angles (°) for 6e

N(2)-N(1)-C(5) 122.5(5)	N(3)-C(2)-N(4) 116.2(6)
N(1)-N(2)-N(3) 117.4(5)	N(4)-C(4)-C(5) 120.3(6)
N(2)-N(3)-C(1) 108.0(5)	N(4)-C(4)-C(9) 119.9(5)
N(2)-N(3)-C(2) 114.5(5)	C(5)-C(4)-C(9) 119.6(6)
C(1)-N(3)-C(2) 114.3(6)	N(1)-C(5)-C(4) 124.2(6)
C(2)-N(4)-C(3) 115.3(5)	N(1)-C(5)-C(6) 115.2(5)
C(2)-N(4)-C(4) 121.5(5)	C(4)-C(5)-C(6) 120.1(7)
C(3)-N(4)-C(4) 119.0(6)	C(5)-C(6)-C(7) 121.4(5)
O(2)-N(5)-O(3) 123.6(7)	C(6)-C(7)-C(8) 116.9(6)
O(2)-N(5)-C(8) 119.0(6)	N(5)-C(8)-C(7) 119.0(6)
O(3)-N(5)-C(8) 117.5(5)	N(5)-C(8)-C(9) 117.0(5)
O(1)-C(2)-N(3) 120.5(7)	C(7)-C(8)-C(9) 124.1(6)
O(1)-C(2)-N(4) 123.3(6)	C(4)-C(9)-C(8) 117.7(5)

considerable sp^2 character. These results suggest that the triazene chain in tetrazepinones is only weakly conjugated with the phenyl ring or that the lone pair of N3 is only delocalized to a minor extent.

c. Stability.

The U.V spectra of the tetrazepinones **6a-e** and **10** at pH 8-10 were quite variable. However at acidic pHs, a very distinct peak appeared above 300 nm in the spectra of **6a-e** and **10** presumably due to the presence of diazonium species. The same spectra were observed when freshly diazotized ureas were kept at acidic pHs. It is known

that diazonium salts absorb in the range of 300-450 nm in the UV[18,19]. The presence of the diazonium ion was further confirmed by ^{15}N NMR spectroscopy which showed a peak at around -60 ppm for ^{15}N labelled **6a**, **6c** and **6e** at acidic pHs. We could also observed a doublet at around -300 ppm ($^{1}J_{NH}$=91 Hz) in the spectra of **6a** and **6c** at acidic pHs. The -60 ppm shift values have already been reported for benzene diazonium salts[17a,17b] and doublets around -300 ppm in general correspond to N3H of an aromatic ureido compound[16]. The existence of the diazonium ion was further confirmed by the IR spectrum of the residue resulting from the evaporation of an acidic mixture of **6a** which showed a strong peak at 2300 cm^{-1}. In summary, the UV, IR and ^{15}N NMR data results suggest the presence of a diazonium urea species of type **5** at acidic pH.

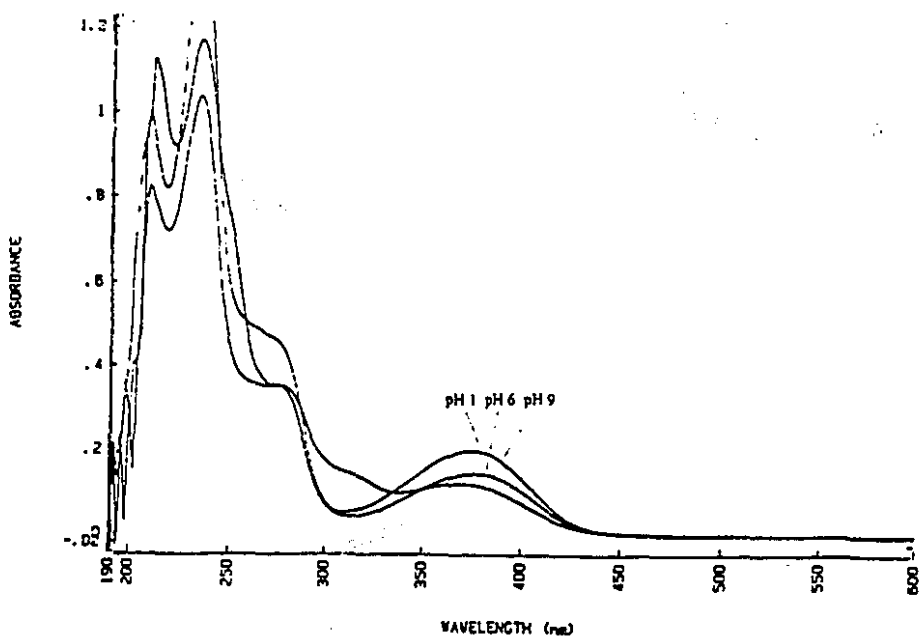


Fig 4.- UV spectra obtained when **6a** was dissolved in water at pH 1, 7 and 9

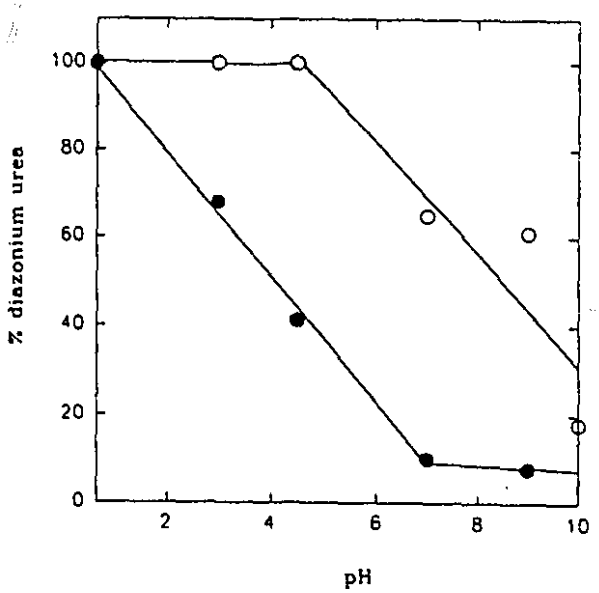
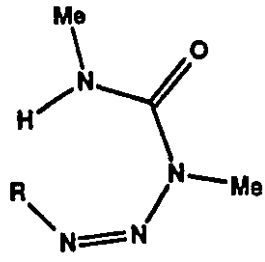


Fig 5. Variation of the diazonium urea/benzotetrazepinone equilibrium ratio for **6a** (O) and **6e** (●) with the pH of the aqueous solution

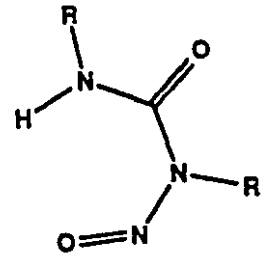
Since the UV gave peaks (300-400 nm) that disappeared or diminished upon alkalinification (Fig. 4), we assumed that this was due to a decrease in the concentration of the diazonium species **5a-e** which are in equilibrium with the cyclic tetrazepinone forms **6a-6e**. We therefore used the UV measurements to establish the pH dependence of diazonium urea/benzotetrazepinone ratio. Typical pH dependent plots are shown in Fig 5. Although there is some scatter, the trend is unequivocal: the concentration of the diazonium urea is higher at acidic pHs. Also, diazonium ureas containing electron withdrawing groups formed tetrazepinones at lower pH than those containing electron donating group. As shown in Fig. 5, while the equilibrium lies toward the formation of tetrazepinone **6e** (tetrazepinone/diazourea: 9:1) at pH 7, it still favours the formation of diazonium urea in the aqueous solution of **6a** (tetrazepinone/diazonium urea: 1:3).

The acid lability of the tetrazepinone ring is in accordance with the known instability of triazenes derivatives in acidic medium[12a-12c, 22]. Recently, Smith[12a, 12b] reported the scission of open-chain ureidotriazene **12a** into a diazonium species and

N,N'-dimethyl-urea at acidic pH. This decomposition pattern is analogous to that of tetrazepinones which in fact are cyclic ureidotriazenes.



12a



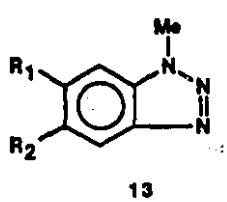
12b

Tetrazepinones **6b**, c, and **10** decompose in chloroform as confirmed by their ^{15}NMR spectra which showed an additional peak at around -5 ppm for the ^{15}N label. Thin layer chromatography showed the formation of a single decomposition product at lower Rf than the corresponding tetrazepinone. The concentration of this compound in the decomposition of **6c** was high enough to allow its isolation by flash chromatography. ^{1}H NMR and elemental analysis confirmed that it was 1-methyl-5-methoxy-benzo-1*H*-triazole **13c**. This is in agreement with the observed -5 ppm shift which is typical of N2 in benzotriazole derivatives[4]. In its proton NMR spectrum, the N-methyl singlet was more deshielded than in its parent benzotetrazepinone spectrum (4.2 ppm for benzotriazole and 3.4 ppm for the corresponding benzotetrazepinone). The mechanism by which such a compound is generated may involve the ring opening of **6c** to give a diazonium urea zwitterion similar to **5c** which may cyclize to benzotriazole **13c** with loss of methyl isocyanate.

The peak at around -5 ppm in the ^{15}N NMR spectra of **6b**, **6c**, and **10** was not observed in those of **6a**, **6d** and **6e** and thin layer chromatography did not show the appearance of any decomposition products. It appeared that tetrazepinones bearing electron withdrawing group were more stable than those bearing electron donating

groups. The methoxy substituted tetrazepinones decomposed in the solid and in solution whereas the chloro- and nitro-substituted compounds show stability at room temperature both in solution and in the solid. The nitrobenzotetrazepinone was stable enough for recrystallization and X-ray diffraction at room temperature.

The aqueous decomposition of nitrosoureas[24] **12b** and imidazotetrazinones[25] of type **A** at alkaline pHs is initiated by the hydrolysis of the ureido moiety. Nitrosoureas are hydrolyzed to an alkyl diazotate and an alkyl isocyanate moiety, whereas imidazotetrazinone **A** is known to decompose to a monoalkyltriazene, which in turn, liberates an alkyldiazonium species[25]. A similar decomposition pattern was expected for benzotetrazepinones. However, the products isolated from the aqueous decomposition of benzotetrazepinones **6a, 6c-e** after stirring overnight at room temperature at pH 9 were completely different from those expected. Tetrazepinones **6a, c, d** gave mainly ureas **14** whereas **6e** afforded benzimidazole **15e** as the major product. The decomposition was studied at pH 9 because the concentration of tetrazepinone was found to be maximal at alkaline pHs.



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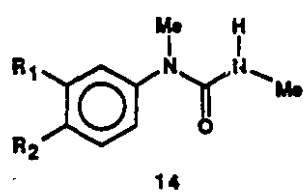

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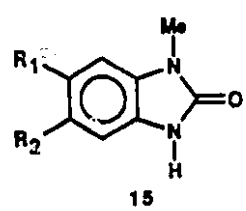
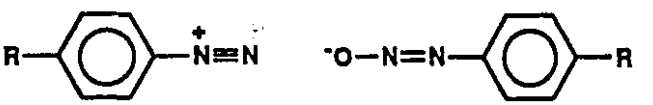


15

a $R_1=R_2=H$
c $R_1=H$ $R_2=OMe$
d $R_1=H$ $R_2=Cl$
e $R_1=NO_2$ $R_2=H$

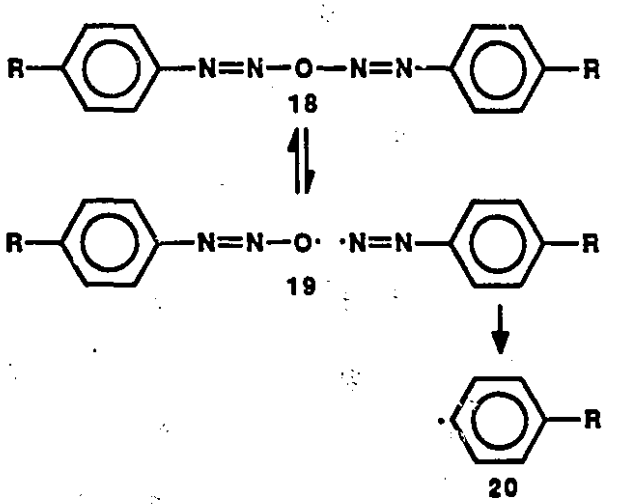
Ureas **14a, c, d** obtained from **6a, c, d** presumably result from the dediazoniation[16] of the diazonium urea species which are always present in aqueous solution at up to pH 9. The structure of **14a** was confirmed by its independent synthesis. Treatment of N-methylaniline with methyl isocyanate gave a colorless solid residue, the

NMR and melting point of which were identical to those of **14a**. The structure of ureas **14c-d** was assigned by ^1H NMR and mass spectroscopy. The presence of a singlet at around 3.1 ppm and a doublet at around 2.7 ppm confirmed the existence of the $N(CH_3)CONHCH_3$ moiety. The mass spectra showed strong M^+-MeNCO peaks.



16 **17**

The mechanism of the dediazoniation of aryl diazonium in alkaline mixture has been extensively studied[17a,25]. Dreher, Niederer, Rieker, Schwartz and Zollinger[17a] showed by [CINDP] ^{15}N NMR spectroscopy that the mechanism of alkaline dediazoniation of p-chloro-benzenediazonium involved homolytic processes. They suggested that the arenediazonium **16** and the diazotate **17** resulting from the addition of hydroxide ion to **17**, may couple to give diazoanhydride **18** . The latter may collapse homolytically to the radical pair **19**, which may in turn generate the aryl radical **20** responsible for the formation of benzene derivatives by hydrogen abstraction. They reported a 148 ppm shift for N2 in the diazotate **17** and -69 ppm for N2 in diazonium ion **16**.



18

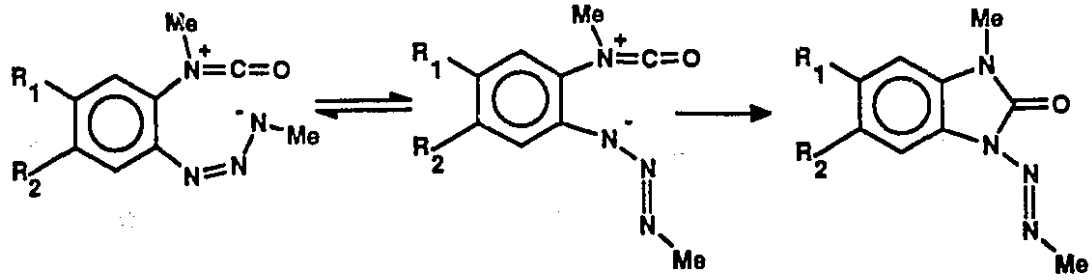
19

20

Scheme 3

Interestingly, when we ran the ^{15}N NMR of the crude decomposition mixture of the N2 labelled compound **6a**, a peak was observed at 170 ppm indicating the possible presence of an aryl diazohydroxy urea species of type **17**. These results indicate that the mechanism of the decomposition of **6a**, **6c-d** could parallel that described for benzene diazonium species as outlined in Scheme 3. We also observed a peak at -3 ppm indicating the presence of benzotriazole **13a** and a peak at 305 ppm indicating the N3 of a ureido function, presumably that of the dediazonation product **14a**. We now know that the ^{15}N shift of the N3-methyl ureido nitrogen in aromatic ureas is around 302 ppm[16].

More interesting was the decomposition of the nitro substituted compounds **6e**, which, when stirred overnight in aqueous sodium carbonate or aqueous methylamine gave a quantitative yield of **15e**. This mode of decomposition may be favoured by the polarity of the solvent since the same result was also obtained when **6e** was kept in ethanol or in water. We know from the X-ray structure of this compound that the N5-C4 bond has a double bond character and the C4-N3 a single bond character. This is in agreement with the possibility that in polar solvent, it may partly exist as dipole **21** which may adopt conformation **22** . The latter may rapidly cyclize to **23** which in turn could be hydrolyzed to benzimidazole **15e**. In an effort to trap intermediate species involved in this mode of decomposition, the ^{15}N NMR of N2 labelled **6e** in the presence of benzylamine was studied. In addition to the N2 labelled tetrazepinone signal at 80 ppm, a strong peak at 69 ppm and two other small signals at 66 and 22 ppm were observed. The strong signal may probably be due to intermediate **23**. Owing to the complexity of the mixture resulting from this experiment, we were unable to identify the products responsible for these peaks.



21 22 23

A similar 2-hydroxybenzimidazole derivative **15d** was also observed in the decomposition of **6d**, although as a minor product. It seems then that this decomposition pathway is favoured by electronwithdrawing group on the phenyl ring.

In summary, the aqueous decomposition of tetrazepinones does not seem to involve the hydrolysis of the ureido moiety like in mitozolomide. This is in accordance with the fact that the carbonyl in imidazotetrazepinone is in a more electron deficient environment (IR ν_{max}: 1725-1790 cm-1)[2] than that of benzotetrazepinone (IR, ν_{max}: 1690-1700 cm^{-1}).

d. ^{15}N NMR

The ^{15}N parameters for all tetrazepinones are summarized in Table 3. The shifts of N5 are around -277 ppm except in **6c** for which δ_{N5} is at -283 ppm. This high shielding may be due to the electron donating character of the methoxy group located *para* to it. This shielding indicates a significant electron density at N5, and may account for the high proclivity of **6c** to decompose to benzotriazole **13c** . Since this decomposition may occur via a diazonium urea of type **5c**, an electron rich N1 may accelerate cyclization to benzotriazole. In the triazene chain, the resonances of N1 are more shielded as the electron donating character augments (e. g. p -CH_3 (**6b**), δN1=45ppm, p-NO_2 (**6e**), δN1=42ppm). A similar trend was observed for the ^{15}N shifts of substituted open-chain aryltriazene and was attributed to the contribution of the dipolar resonance structure of type **24** in which an electronattracting substituent should

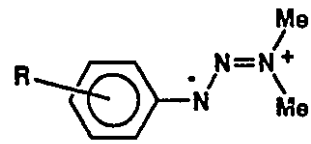
Table 3.-^{15}N NMR chemical shifts, in ppm, of substituted-1,2,3,5-benzotetrazepin-4-ones

No	R_1	R_2	N1	N2	N3	N5
6a	H	H	45.50	71.20	-197.30	-278.00
6b[a]	H	CH_3	45.10	68.50	-197.72	-277.60
6c[a]	H	OCH_3	45.80	74.60	-199.00	-283.00
6d	H	Cl	43.30	75.30	-195.60	-278.00
6e[b]	NO_2	H	42.40	80.40	-193.00	-277.50
9[a, c]	OCH_3	H	44.00	63.00	-197.72	-276.00

[a] A peak at around -5 ppm was also observed due to the formation of a benzotriazole derivative.
[b] A peak at -15 ppm was observed for the nitro group
[c] A peak at -49 ppm was also observed presumably due to the N2 of a diazonium species.

electron-attracting substituent should cause an increase in the electron density at N1[9,11a, 11b]. It should be noted that the contribution of dipolar resonance structures of type **24** to the shielding of N1 in tetrazepinones is weak when compared with the same effect in open-chain triazenes in which a 16 ppm shielding results from the replacement of a p-Me by a p-nitro group (e. g. p* -CH_3, δN1=-23.68, p-NO_2, δN1=-39.26) . The same change in benzotetrazepinones only caused a 3 ppm shielding . This may well be due to the electronwithdrawing effect of the carbonyl that decreases the contribution of the N3 lone pair to n-π delocalization toward the triazene moiety.

* This position is relative to the triazene chain



24

The shifts of N3 showed a deshielding trend as the electronwithdrawing character of substituents increased. This is also in agreement with the resonance contribution of type **24** . As electron withdrawing character of R increases, due to the development of a positive charge at N3, the electron density at the latter nitrogen should normally decrease.

The shifts of N2 are very sensitive to substituent changes (e.g. p-OCH_3, 63 ppm, p-NO_2, 80.40). This effect shows that there is an important electronic interaction between the substituent on the phenyl ring and the diazo linkage in the benzotetrazepinones.

e. Conclusion

This study conclusively demonstrates that 1,2,3,5-tetrazepin-4-one ring is stabilized by electronwithdrawing groups on the phenyl ring, and destabilized by electron donating groups. The unstable tetrazepinones aromatized by loosing methyl isocyanate. Higher yields of tetrazepinones are obtained when ureas containing electron withdrawing groups are diazotized. The formation of the tetrazepinones is more favoured at basic pHs.

f. Biological activity

The antitumour activity of the tetrazepinones was evaluated against 12 human cancer cell lines and their activity compared with that of the clinical drug N'-cyclohexyl-N-(2-chloroethyl)-N-nitrosourea (CCNU)[25]. Tricyclic tetrazepinones **B**, and bi-cyclic tetrazepinones **6d, e** showed interesting antitumour activity. Detailed biological evaluation of tetrazepinones will be reported elsewhere.

EXPERIMENTAL

Melting points were measured on a Gallenkamp block and are uncorrected. Thin-layer and flash chromatography were performed on silica gel 60 F_{254} aluminum plates and Merck Silica Gel 60 (230-400 mesh) respectively. ^{1}H NMR spectra were recorded on a Varian XL-200 at 200 MHz. ^{13}C NMR spectra were obtained at 75.40 MHz on a Varian XL-300. All ^{1}H NMR spectra were run in $CDCl_3$ or in DMSO-d_6 and chemical shifts are reported downfield from TMS and all coupling constants are in Hz. Mass spectra were recorded on a Kratos MS25RFA. All compounds were shown to be homogeneous by TLC and high-field NMR, or to have a purity of > 95% by elemental analysis.

The 2-nitroanilines were purchased from Aldrich Chemical Company.

^{15}N NMR spectra were taken at 30.40 MHz on a Varian XL-300 and chemical shifts are reported up- and downfield from nitromethane, which was used as external standard. The 90° pulse width was 18 μs and the pulse interval was set at 3s. The temperature of the probe was maintained at 0° C for the spectra of diazonium salts and at 20° C for the natural abundance spectra. Spectra were obtained after 100 scans for the ^{15}N enriched compounds when sample concentration were around 0.10 M in $CDCl_3$ (gated coupled) and after about 9000 scans for natural abundance spectra at concentrations around 0.5 M.

All reactions were monitored by thin layer chromatography (TLC)

5-Methyl-1-(N-methyl)-2-phenylenediamine (3b). A solution of 5-methyl-2-nitroaniline (3g, 19.7 mmol) in dimethyl formamide (DMF) (50 mL) was treated with NaH (600 mg). When the gas evolution ceased, methyl iodide (1 mL) was added dropwise at 0° C and the solution was stirred for 1 hr after which it was diluted with cold water (100 mL). N,5-dimethyl-2-nitroaniline **2l** precipitated as yellow crystals which were dried under vacuum (3g, 92%); TLC (20% ethyl acetate in hexane), Rf=0.5; m. p. 90° C; 200 MHz ^{1}H NMR ($CDCl_3$) δ: 8.03 (d, 1H, J=8.82, Ar), 6.58 (s, 1H,Ar), 6.43 (d, 1H, J=8.72, Ar) 6.10 (br s, 1H, N*H*CH_3), 2.98 (d, 3H, J=5.10, NHC*H*$_3$), 2.33 (s, 3H, ArC*H*$_3$); CIMS (*iso*-butane) *m/z* (relative intensity) 167 (MH^+, 100%)

A solution of **2b** and 10% Pd-C (500 mg) was hydrogenated in methanol (25 mL) at 2 atm. Filtration and evaporation gave **3b** as a brown liquid in quantitative yield, TLC (10% methanol in methylene chloride), Rf=0.50; 200 MHz ^{1}H NMR ($CDCl_3$) δ: 6.6 (d, 1H, J=8.2, Ar), 6.45 (overlap of d and s, 2H, Ar), 3.2 (br s, 2H, NH_2), 2.83 (s, 3H, NCH_3), 2.25 (s, 3H, ArC*H*$_3$). (N-alkyl-1,2-phenylene diamines darken when exposed to light; they must be used immediately after their preparation)

4-Methoxy-1-(N-methyl)-1,2-phenylenediamine (3c). As described for **3b**; the N-methyl-2-nitroaniline **2c** was obtained from 2-nitro-4-methoxyaniline (2g, 12 mmol) in DMF (50 mL), NaH (600 mg) and of methyl iodide (1 mL) (Yield: 2g, 92%): reddish needles, m.p 120° C; TLC (80% hexane in ethyl acetate), Rf=0.5; 200 MHz ^{1}H NMR ($CDCl_3$) δ: 8.00 (br s, 1H, N*H*CH_3), 7.60 (d, 1H, J=3.0, Ar), 7.17 (dd, 1H, J=9.26, J=3, Ar), 6.8 (d, 1H, J=9.26, Ar), 6.10 (br s, 1H, N*H*CH_3), 3.80 (d, 1H, J=5.10, OCH_3), 3.01 (d, 3H, J=3.56, NHC*H*$_3$); CIMS (*iso*-butane) *m/z* 183 (MH^+, 100%).

A solution of **2c** (1.9 g) and 10% Pd-C (350 mg) in methanol (20 mL) was hydrogenated at 2 atm. Filtration and evaporation gave **3c** as a brown liquid in

quantitative yield; TLC (10% methanol in methylene dichloride), Rf=0.8; 200 MHz ^{1}H NMR ($CDCl_3$) δ: 6.8 (d, 1H, J=8.6 , Ar), 6.25 (s, 1H, Ar), 6.20 (d, 1H, Ar), 3.7 (s, 2H, NH_2), 2.7 (s, 3H, NHCH_3).

4-Chloro-1-(N-methyl)-1,2-phenylenediamine (3d). As described for 3b; the N-methyl-2-nitroaniline **2d** was obtained from 2-nitro-4-chloro-aniline (2g, 11.6 mmol) and methyl iodide (1 mL) and NaH (600 mg) in DMF (50 mL) (yield: 2g, 90%), TLC 80% hexane in ethyl acetate), Rf=0.5: yellow needles, m. p. 100° C. 200 MHz ^{1}H NMR ($CDCl_3$) δ: 9.14 (d, 1H, J=2.6, Ar), 8.6 (br s, 1H, N*H*CH_3), 8.30 (dd, 1H, J=9.60, J=2.6, Ar), 6.92 (d, 1H, J=9.60, Ar), 3.15 (d, 3H, J=5.2, NHCH_3); CIMS (*iso*-butane) *m/z* 187 (MH^+, 100%).

A solution of **2d** (1.9 g) and 10% Pd-C (350 mg) in methanol (20 mL) was hydrogenated at 3 atm. Filtration and evaporation gave **3d** as a brown liquid in quantitative yield; TLC (10% methanol in methylene dichloride), Rf=0.7; 200 MHz ^{1}H NMR ($CDCl_3$) δ: 6.8 (overlap of d and s, 2H, J=8, Ar), 6.5 (d, 1H, J=8, Ar) 3.4 (br singlet, 3H, NHCH3, NH_2), 2.8 (d, 3H, J=5, NHCH_3).

5-Nitro-1-(N-methyl)-2-phenylenediamine (3e).-To a solution of 4-nitro-1,2-phenylenediamine **1** (6g, 39.2 mmol) in DMF (50 ml) was added dropwise methyl iodide (2 mL, 0.8 eq) and 40 % sodium carbonate (10 mL). The solution was stirred overnight and the solvent was evaporated under vacuo. The dark-red residue that resulted was chromatographed on silica gel to give **3e** (4g, 48 %) as a yellow solid; m.p. 120°C; TLC (40% hexane in ethyl acetate), Rf=0.30; 200 MHz ^{1}H NMR ($CDCl_3$) δ:7.68 (d, 1H, J=7, Ar), 7.5 (s, 1H, Ar), 6.65 (d, J=7, Ar), 3.9 (br s, NH_2), 3.2 (br s, 1H, N*H*CH_3), 2.9 (d, 3H, J=8, NHCH_3); CIMS (*iso*-butane), *m/z* 168 (MH^+, 100).

N-(2-Amino-5-methylphenyl)-N-methyl-N'-methylurea (4b).

A solution of **3b** (2.2 g, 16.6 mmol) in methylene chloride (100 mL) was treated with methyl isocyanate (0.4 ml, 1eq) at room temperature. The mixture was kept overnight and evaporated under reduced pressure. Purification by column chromatography (5% methanol in methylene dichloride) gave **4b** (2g, 64 %) as a pale brown powder, m. p. 118° C; 200 MHz ^{1}H NMR ($CDCl_3$) δ: 6.95 (d, 1H, J=8, Ar) 6.85 (s, 1H, Ar), 6.72 (d, 1H, J=8, Ar), 4.25 (br s, 1H, CON*H*CH_3), 3.7 (s, 2H, NH_2), 3.15 (s, 3H, Ar*C*H_3NCO), 2.71 (d, J=4.6, CONHC*H*$_3$), 2.2 (s, 3H, $ArCH_3$); 75.40 MHz ^{13}C NMR ($CDCl_3$) δ: 158.28 (s), 141.24 (s), 129.87 (d), 128.88 (d), 128.46 (s), 127.42 (s), 116.52, 36.43 (q), 27.43 (q), 20.21 (q).

N-(2-Amino-4-methoxyphenyl),N-(methyl)-N'-(methyl)-urea (4c).

As described for **4b**; From **3b** (1g, 7 mmol) and methyl isocyanate (0.4 mL, 1eq) in methylene dichloride (50 mL) . Purification by column chromatography (5% methanol in methylene dichloride) gave **4c** (1.2g, 86.4 %) as a white powder, m.p 135°C; IR ($CDCl_3$) ν: 3400 (NH, NH_2), 3000 (CH), 1649 (C=O) cm^{-1}; 200 MHz ^{1}H NMR ($CDCl_3$) δ: 6.92 (d, 1H, J=8.3, Ar), 6.31 (s, 1H, Ar), 6.30 (d, 1H, J=8.3, Ar), 4.4 (br s, 1H, CON*H*CH_3) 3.7 (s, 3H, OCH_3), 3.15 (s, 3H, ArNC*H*$_3$CO), 2.71 (d, J=4.5, $CONHCH_3$); 75.4 MHz ($CDCl_3$) δ: 158.09 (s), 152.95 (s), 137.35 (s), 128.37 (s), 117.5862 (d), 115.61 (d), 113.62 (d), 55.80 (q), 35.47 (q), 27.54 (q).

N-(2-Amino-4-chlorophenyl)-N-methyl-N'-methylurea (4d). As described for **4b**. From **3d** (2g, 14 mmol) and methyl isocyanate (1 mL, 1eq) in methylene dichloride (100 mL). Purification by column chromatography (5% methanol in methylene dichloride) gave **4d** (2.1g, 75 %) as a white powder; m. p. 150 °C; 200 MHz ^{1}H NMR ($CDCl_3$) δ: 6.85 (d, J=8.3, Ar), 6.75 (dd, 1H, J=2.0, Ar), 6.67 (dd, 1H, J=2.0, J=8.3), 4.4 (br q, 1H, CON*H*CH_3), 4.20 (s, 2H, NH_2), 3.15 (s, 3H, ArNC*H*$_3$CO), 2.70 (d, 2H,

J=4.0, CONHCH_3; 75.4 MHz ^{13}C NMR ($CDCl_3$) δ: 159.38 (s), 146.33 (s), 136.00 (s), 131.30 (d), 127.24 (s). 120.14 (d), 117.23 (d), 36.80 (q), 28.85 (q).

N-(2-Amino-5-nitrophenyl)-N-methyl-N'-methylurea (4e).

A solution of of **3e** (680 mg, 4.12 mmol) in acetonitrile (15 mL) was treated with methyl isocyanate (0.30 mL) and kept overnight without stirring. The pale yellow precipitate that formed was filtered and dried under vacuum to give **4e** (650 mg, 72%) as a yellow powder; m.p. 180-185° C; 200 MHz ^{1}H NMR (DMSO) δ: 8.39 (dd, 1H, J=8.8, J=2.5, Ar), 8.36 (d, 1H, J=2.5, Ar), 7.23 (d, 1H, J=8.8, Ar), 7.11 (s, 2H, NH_2), 6.30 (q, H, J=5, *H*NCH_3), 3.40 (s, 3H, ArCH_3), 2.99 (d, 3H, J=5, NHCH_3) ; 75.4 MHz ^{13}C NMR ($CDCl_3$) δ: 157.17 (s), 152.60 (s), 135.60, 126.37 (d), 126.14 (s), 125 (d), 114.01 (d), 35.12 (q), 26.70 (q).

3,5,7-Trimethyl-*3H*-1,2,3,5-benzotetrazepin-4(*5H*)-one (6b).

Urea **4b** (1g, 5.55 mmol) was dissolved in 2N HCl (20 mL) and diazotized with 20% aqueous [5%^{15}N] $NaNO_2$ (2 mL); (enrichment was obtained by mixing 20 mg of $Na^{15}NO_2$ with 380 mg of $NaNO_2$). The solution was extracted three times with methylene dichloride. After adjusting its pH to 8, the aqueous layer was reextracted six times with 100 mL portions of hexanes. The solvent was evaporated to give **6b** (360 mg , 36%) as a pale brown powder, m. p. 60° C. IR ($CDCl_3$) ν: 3000 (CH), 1682 (C=O) cm^{-1}; UV (methanol) λ_{max} (ε): 240 (11000) 374 (6000); 200 MHz ^{1}H NMR ($CDCl_3$) δ: 7.3 (d, 1H, J=8, Ar), 7.04 (d, dd, 1H, J=1.2, J=8, , Ar), 6.9 (d, 1H, J=1.2, Ar), 3.37 (s, 3H, N=NNCH_3), 3.26 (s, 3H, ArN(CH_3)CO), 2.40 (s, 3H, ArCH_3); 75.40 MHz ^{13}C NMR ($CDCl_3$) δ: 160.77 (s), 143.30 (s), 140.46 (s), 139.59 (s), 128.30 (s), 126.56 (s), 121.27 (s), 38.05 (q), 36.25 (q), 22.86 (q). Anal. Calcd for $C_{10}H_{12}N_4O$: C, 58.81; H, 5.92; N, 27.44. Found: C, 59.18; H, 5.88; N, 27.23.

3,5-Dimethyl-8-methoxy-*3H*-1,2,3,5-benzotetrazepin-4(*5H*)-one (6c).

Urea **4c** (720 mg, 3.6 mmol) in 2N HCl (10 mL) was diazotized with 20% aqueous [5% ^{15}N] $NaNO_2$ (1.3 mL). The mixture was extracted with three 20 ml portions of methylene dichloride . The pH of the aqueous layer was adjusted to 8 with a saturated sodium carbonate solution, and the precipitate that formed was extracted 5 times with 25 mL portions of methylene dichloride. The solvent was dried over anydrous potassium carbonate and evaporated to give **6c** as a brown powder; m. p. 109° C; IR ($CDCl_3$) ν : 1690 (C=O) cm^{-1}; UV (methanol) $\lambda_{max}(\varepsilon)$: 238 (17640), 302 (2940) ; 200 MHz ^1H NMR ($CDCl_3$) δ: 7.05 (overlap of d, 2H, J=9, Ar), 7.1 (s, 1H, Ar), 3.85 (s, 3H, OCH_3), 3.4 (s, 3H, $N{=}NNCH_3$) 3.25 (s, 3H, $ArNCH_3CO$) ; 75.40 MHz ^{13}C NMR ($CDCl_3$) δ: 159.37 (s), 155.34 (s), 141.97 (s), 131.45(s), 120.49 (d), 118.61 (d), 109.60 (d), 55.73 (q), 36.80 (q), 34.94 (q). Anal. calcd for $C_{10}H_{12}N_4O_2$: C, 54.54; H, 5.49; N, 25.44. Found: C, 54.47; H, 5.53; N, 25.26.

3,5-Dimethyl-8-chloro-*3H*-1,2,3,5-benzotetrazepin-4(*5H*)-one (6d).-

As described for **6c**; from **4d** (1.2g, 5.61 mmol) and $NaNO_2$ (0.387 g) in 2N HCl (15 mL) (0.9g, 71.3%): brown powder, m. p. 85° C. IR ($CDCl_3$) ν: 3000 (CH) 1696 (C=O) cm^{-1}; UV (methanol) λ_{max} (ε): 260 (5825), 291 (1947); 200 MHz ^1H NMR ($CDCl_3$) δ:7.4 (s, 1H, Ar), 7.35 (d, 1H, J=9, Ar, 7.05 (d, 1H, J=9, Ar), 3.4 (s, 3H, $N{=}NNCH_3$), 3.25 (s, 3H, $ArNCOCH_3$); 75.40 MHz ^{13}C NMR ($CDCl_3$) d: 159.06 (s), 141.95(s), 136.97 (s) 131.08 (d), 129.61 (s), 128.63 (d), 120.96 (d), 36.90 (q), 35.04 (q). Anal calcd for $C_9H_9N_4ClO$: C, 48.10; H, 4.00; N, 24.50. Found: C , 47.92; H, 4.04; N, 24.50.

3,5-Dimethyl-7-nitro-*3H*-1,2,3,5-benzotetrazepin-4(*5H*)-one (6e).

From **4e** (1.2 g, 5.35 mmol) in 2N HCl (15 mL) and 20% aqueous [5% ^{15}N] $NaNO_2$ (2 mL). The pH of the aqueous layer was adjusted to 6 and the precipitate that formed was extracted 6 times with 20 mL portions of methylene dichloride; The solvent was dried

over anhydrous sodium carbonate and evaporated to give **5e** (1.13, 90%) as a pale yellow powder which was recrystallized from methylene dichloride: yellow needles, m. p. 119° C. IR ($CDCl_3$) 3000 (CH) 1702 (C=O) cm^{-1}; UV λ_{max} (ε): 248 (10464) 316 (436; 200 MHz ^1H NMR ($CDCl_3$) δ: 8.2 (d, J=8, Ar), 8.00 (s, 1H, Ar), 7.8 (d, J=8, Ar), 3.4 (s, 3H, N=NNCH_3), 3.25 (s, 3H, ArNCOCH_3); 75.4 MHz ^{13}C NMR ($CDCl_3$) δ: 158.8 (s), 146.5 (s), 144.5 (s), 139.5 (s), 128 (d), 118.5 (d), 114.8 (d), 36.7 (q), 34.5 (q). Anal. calcd for $C_9H_9N_5O_3$: C, 45.96; H, 3.86; N, 29.78. Found: C, 46.13; H, 3.70; N, 29.62.

4-methoxy-2-nitro-N-tert-butoxycarbonylaniline (8a).

To a solution of 4-methoxy-2-nitroaniline (4 g , 12.6 mmol) in THF (25 mL) was added NaH (1g) in portions . When the gas evolution ceased, di-*tert*-butyl dicarbonate (7.2g, 1eq) was added in portions. The solution was then diluted with dioxane (100 mL) and heated at reflux overnight. The excess of NaH was quenched with cold methanol . The solvents were then pumped off and the residue suspended in methylene dichloride. After extraction with water, the methylene dichloride layer was dried over $MgSO_4$ and evaporated under reduced pressure. The resulting yellow residue was chromatographed on a silica gel column (30% ethyl acetate in hexane) to give **8a** as a yellow oil that solidified on standing (4g, 50%), m. p. 66° C. IR ($CDCl_3$) ν: 3400 (NH), 3000 (CH), 1727 (C=O) cm^{-1}; 200 MHz ^1H NMR ($CDCl_3$) δ: 9.4 (s, 1H, *H*NCOO-tBu), 8.41 (d, 1H, J=9, Ar), 7.61 (dd, 1H, J=3, Ar), 7.2 (dd, 1H, J=3, J=9, Ar), 3.87 (s, 3H, OCH_3), 1.51 (s, 9H, tBu).

1-N-Methyl-2-N-tert-butoxycarbonyl-4-methoxy-1,2-phenylenediamine (8c)

A solution of **8a** and 10% Pd-C (500 mg) in methanol (20 mL) was hydrogenated at 3 atm. Filtration and evaporation gave **8b** as a brown oil in quantitative yield; IR ($CDCl_3$) ν: 3400 (NH, NH_2), 3000 (CH), 1716 (C=O) cm^{-1}; ^1H 200 MHz ($CDCl_3$) δ: 7.05 (d, 1H, J=9.8, Ar), 6.30 (overlap of s and d, 2H, Ar), 6.00 (br s, 1H, N*H*COOtBu), 4.00 (overlap

of d and s, 5H, OCH_3, NH_2), 1.45 (s, 9H, tBu). To a solution of this oil (3g, 12.6 mmol) in acetonitrile was added all at once methyl iodide (770 mg, 1eq) and 40% aqueous sodium carbonate (3.3 mL) . After 16 hrs, the solvent was evaporated and the resulting yellow residue purified on silica gel (30% ethyl acetate in hexane) to give amine **8c** (1.5g, 47%) as a pale yellow powder, m. p. 83°C. IR ($CDCl_3$) ν: 3025 (CH), 3400 (NH), 1716 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.05 (d, 1H, J=8.75, Ar), 6.2 (overlap of d and s, 2H, Ar), 5.8 (br s, 1H, N*H*COOtBu), 4.2 (br s, 1H, N*H*CH_3) 3.8 (s, 3H, OCH_3), 2.8 (s, 3H, NCH_3), 1.45 (s, 9H, tBu).

N-2-Amino-5-methoxylphenyl-N'-methylurea (9b).

Amine **7c** (1 g, 4.2 mmol) was treated overnight with methyl isocyanate (0.3 mL, 1eq) in methylene dichloride (50 mL). The solvent was evaporated to give **9a** as a clear oil in quantitative yield. 200 MHz ^1H NMR ($CDCl_3$) δ: 8.01 (d, 1H, J=9.1, Ar), 6.85 (dd, 1H, J=9.1, J=3.0, Ar) 6.68 (dd, J=3.0, 1H, Ar), 6.57 (s, 1H, N*H*COOtBu), 3.76 (s, 3H, OCH_3, 2.86 (s, 3H, ArNCH_3), 2.73 (d, 3H, HNC*H*$_3$) 1.50 (s, 9H, tBu). A solution of **9a** (1g) in trifluoacetic acid (10 mL) was heated to 45°C for 10 min after which it was cooled to 0°C and neutralized with a saturated sodium carbonate solution. Extraction and evaporation of the solvent gave **9b** (500 mg, 74%) as a brown powder, m.p 120° C. IR ($CDCl_3$) ν: 3400-3200 (NH, NH_2), 1649 (C=O) cm^{-1}; 200 MHz ^{1}HNMR ($CDCl_3$) δ: 6.60-6.80 (4H, Ar), 6.4 (br s, N*H*CH_3), 3.72 (s, 3H, OCH_3), 3.17 (s, 3H, Ar$N(CH_3)$, 2.71 (d, 3H, J=4.52, NHC*H*$_3$); 75.4 MHz ^{13}C NMR ($CDCl_3$) d:152.94 (s), 137.35 (s), 128.37 (s), 117.59 (d), 115.61 (d), 113.62 (d), 55.70 (q), 35.47 (q), 27.54 (q) ; CIMS (*iso*-butane) *m/z* 210 (MH^+, 100), 178 (MH^+-MeOH, 11), 153 (MH^+-MeNCO, 65)

3,5-Dimethyl-7-methoxy-*3H*-benzotetrazepin-4(*5H*)-one (10).-

As described for **5b**; from **8b** (450 mg) in 5N HCl (7 mL) and 15 % aqueous [5% ^{15}N] $NaNO_2$ (1.2 mL); yield: 151 mg, 36 %); brown powder, m. p. 61°C (effervescence); IR

($CDCl_3$) ν: 3100 (CH) 1691 (C=O) cm^{-1}; 200 MHz ^1H NMR ($CDCl_3$) δ: 7.37 (d, 1H, J=8.9, Ar), 6.75 (dd, J=8.9, J=2.6, Ar), 6.55 (d, J=2.6, 1H, Ar), 3.8 (s, CH_3), 3.3 (s, 3H, CH_3) ; 75.40 ^{13}C NMR ($CDCl_3$) d: 161.7 (s), 140.14 (s), 135.28 (s), 129.20 (d), 110.19 (d), 104.92 (d), 57.80 (q), 36.82 (q), 35.13 (q). The compound decomposed slowly at room temperature, therefore no satisfactory elemental analysis was obtained. Anal. calcd for $C_{10}H_{12}N_4O_2$: C, 54.54; H, 5.49; N, 25.44. Found: C, 53.6; H, 5.30; N, 23.91

1-Methyl-*1H*-6-methoxybenzotriazole (11) The brown oil resulted from the decomposition of solid **10** (250 mg) after two weeks, was purified on silica gel (50% hexane in ethyl acetate) to give **11** (150 mg) as a yellow crystalline residue; m. p. 84° C; 200 MHz ^1H NMR ($CDCl_3$) δ: 7.88 (d,1H, J=8.8, Ar), 7.00 (d, 1H, J=8.8, Ar), 6.76 (s, 1H, Ar), 4.2 (s, 3H, OMe), 3.9 (s, 3H, NMe); EIMS *m/z* 163 (M^+, 51), 135 (M^+-N_2, 26), 120 (M^+-CH_3N_2, 100)

1-Methyl-*1H*-5-methoxybenzotriazole (13c). The NMR solution (3mL, 0.75 M) of **13** was purified on silica gel (50% ethyl acetate in hexane) to give benzotriazole **13c** as a white crystalline residue (300 mg) , m.p 125° C. 200 MHz ^1H NMR ($CDCl_3$) δ: 7.36 (d, overlap of d and s, 2H, J=8.8, Ar), 7.15 (d, 1H, J=8.8, Ar), 4.2 (s, 3H, OMe), 3.8 (s, NMe). Anal. calcd for $C_8H_9N_3O$; C , 58.88; H 5.76; N, 25.75 . Found: C, 58.42; H, 5.47; N, 25.50.

UV Analysis.- A 0.3 ml aliquot of a $2*10^{-5}$M solution of the tetrazepinone was added to cells containing 2.5 ml of water at different pHs. The mixture was stirred and the UV absorbance was read at room temperature at 374 nm for **6a**, 366 nm for **6b**, 395 nm for **6c**, 398 nm for **6d**, 408 nm for **6e**. The percentage of diazourea was calculated by the following equation: [(Absorbance at the desired pH/Absorbance at pH 1)]*100. (We assumed that at pH 1 the concentation of the diazourea is maximal).

General procedure for the alkaline decomposition of 6a, 6c-e.

A solution of the tetrazepinones (0.3 M) in saturated aqueous sodium carbonate was stirred for 25 hrs. The resulting brown solution was extracted with methylene dichloride and the aqueous phase neutralized and evaporated under reduced pressure. The resulting solid residue was reextracted with methanol. The methylene dichloride and methanol extracts were combined and purified on silica gel with the appropriate solvent system (in general 5% hexane in ethyl acetate).

1-Methyl-1H-benzotriazole (13a).

From the decomposition of **6a** (500 mg); purification on a silica gel column (5% hexane in ethyl acetate) gave **13a** as a brown oil (60 mg); 200 MHz ^{1}H NMR ($CDCl_3$) δ:8.2 (d, 1H, J=8, Ar), 7.8-7.4 (m, 3H, Ar), 4.25 (s, 3H, CH_3); EIMS *m/z* 133 (M^+, 58), 105 (M^+-28, 100); HRMS, calcd for $C_7H_7N_3$ (M^+) :133.06399. Found: 133.06590.

N-Phenyl-N,N'-dimethylurea (14a): From the decomposition of **6a** (500 mg); purification on a silica gel column (5% hexane in ethyl acetate) gave **14a** as a crystalline solid (250 mg); m. p. 68 °C; 200 MHz ^{1}H NMR ($CDCl_3$) δ: 7.45-7.1 (overlap of m , 5H, Ar), 4.23 (br s, CON*H*CH_3), 3.2 (s, 3H, ArN(C*H*$_3$)CO), 2.7 (d, 3H, J=4.7, CONHC*H*$_3$); EIMS *m/z* 164 (M^+, 43), 106 (M^+-CH_3NHCO, 100), 77 (M^+-$CH_3NCONHCH_3$); HRMS calcd. for $C_9H_{12}N_2O$ (M^+): 164.09495. Found: 164.09530. Anal. calcd for $C_9H_{12}N_2O$: C, 65.83; H, 7.37 ; N, 17.06 . Found: C,65.59 ; H, 7.75; N, 17.17

5-Methoxybenzotriazole: From the decomposition of **6c** (100 mg); the purification of the mixture on a silica gel column (35% hexane in ethyl acetate) gave a white solid (35 mg). The ^{1}H NMR and the mass spectrum were identical to those of **13c** previously isolated from the decompositon of **6c** in chloroform.

N-p-Methoxyphenyl)-N, N'-dimethylurea (14c) From the decomposition of **6a** (100 mg); purification on a silica gel column (35% hexane in ethyl acetate) gave **14c** as a brown oil (30 mg); 200 MHz ^{1}H NMR ($CDCl_3$) δ: 7.05 (d, 1H, J=9, Ar), 6.9 (d, 1H, J=9, Ar), 3.8 (s, 3H, OCH_3), 3.2 (s, 3H, $ArN(CH_3)CO$), 2.7 (d, 3H, J=5, $CONHCH_3$); EIMS *m/z* 194 (M^+, 78), 137 (M^+-CH_3NCO, 49), 122 (M^+-CH_3NCONH, 100); HRMS calcd. for $C_{10}H_{14}N_2O$ (M^+) :194.10552. Found: 194.10502

N-(4-Chlorophenyl)-N,N'-dimethylurea (14d) From the decomposition of **6d** (100 mg); purification on a silica gel column gave **14d** as a brown oil (50 mg); 200 MHz ^{1}H NMR ($CDCl_3$) δ:7.3 (d, 1H, J=6.6, Ar), 7.1 (d, 1H, J=6.6, Ar), 3.2 (s, 3H, $ArN(CH_3)CO$), 2.7 (d, 3H, J=5, $CONHCH_3$); EIMS *m/z* : 198 (M^+, 70), 141 (M^+-CH_3NCO, 100); HRMS calcd for $C_9H_{11}N_2OCl$ (M+): 198.05599 . Found: 198.05440

2-Hydroxy-6-chlorobenzimidazole (15d) From the decomposition of **6a** (100 mg); purification on a silica gel (5% hexane in ethyl acetate) column gave **15d** as a white powder (9 mg); m. p. 240° C (dec);^{1}H NMR $CDCl_3$ δ: 9.85 (s, 1H, NHCO), 7.15 (overlap of d of s, 2H, Ar), 6.85 (d, 1H, J=5, Ar), 3.4 (s, 3H, CH_3); EIMS *m/z* 182 (M^+, 55) , 153 (M^+-CH_3N, 44) 105 (M^+-77, 100); HRMS calcd for $C_8H_7N_2ClO$ (M^+) :182.02469 . Found: 182.02110

2-Hydroxy-6-nitrobenzimidazole (15e) From the decomposition of **6e** (100 mg); the yellow precipitate that formed was filtered and dried under vacuum at room temperature (yield: 80 mg) . The same result was observed when it was stirred in ethanol, aqueous ethanol, methylamine (40%) or in water at neutral pH; m. p. 260° C (dec.); 200 MHz ($CDCl_3$) δ: 9.2 (s, 1H, NH), 8.1 (d, J=8, Ar), 7.9 (s, 1H, Ar), 7.1 (d, 1H, J=8, Ar), 3.5 (s, 3H, CH_3); EIMS *m/z* 193 (M^+, 100), 147 (M^+-NO_2, 55); HRMS calcd for $C_8H_7N_3O_3$ (M^+): 193.04874 ; Found: 193.04730. (Compound **15e** was also synthesized by treating **3e**

with triphogene (0.33 eq) in the presence of triethylamine (1eq) . The resulting compound was identical to **15e**.)

^{15}N NMR study.- A solution of **4b** or **4c** (500mg) in 3 mL of 2N HCl was maintained at 0 ^{o}C and 1ml of a 30% solution of $NaNO_2/Na^{15}NO_2$ was added dropwise. After 20 min the solution was transferred to a 10 mm NMR tube and analyzed at a probe temperature of $0^{o}C$.

X-ray Crystallography.- Crystals of compound **6e** were obtained from slow evaporation of methylene dichloride.

Crystal data.- $C_9H_9N_4O$, M = 216.24. Orthorombic, a = 8.0120 (8), b = 10.962 (9), c = 11.9290 (10) , V=1048.5 A^3 (16) (by least-squares refinement on diffractometer angles for 26 automatically centred reflections) space group $P2_1/n$, Z=4, Dx =1.491 g cm^{-3}. Small yellow needles. Crystal dimensions: 0.25x0.20x0.20 mm, $\mu(Cu\text{-}K_\alpha)$=0.10 mm^{-1}

Data Collection and Processing.- Enraf-Nonius CAD4 (ω-2θ) mode, scan speed 4 deg min^{-1}, Cu-K_α radiation, temperature: 25 ^{o}C; 2797 reflections measured 560 were unique giving 552 with $I>2\sigma(I)$. The intensities of three representative reflections dropped by 0.45% throughout data collection indicating crystal and electronic stability (decay correction was applied). The data were corrected for Lorentz and polarization effects.

Structure Analysis and Refinement.- All non-hydrogen atom positions from direct methods[27], using the NRC VAX[28] system of crystal solving programs. All hydrogen-atom positions from a Fourier difference map. All positional and thermal parameters (anisotropic) and an extinction parameter were refined by full-matrix least square. Final R and Rw were 0.033, and 0.022 for 552 observed reflections and 154 variable parameters. The weighting scheme $w=4F_o^2/s^2(F_o^2)$ obtained from counting statistics gave satisfactory

agreement analyses. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.11 and -0.14 eA^{-3}, respectively. Neutral atom scattering factors were taken from Cromer and Waber[29,30]. Anomalous dispersion effects were included in F_{calc}; the values for $\Delta f'$ and $\Delta f''$ were those of Cromer[30]. Figures were drawn with ORTEPII[23].

ACKNOWLEDGMENT:

We thank the Natural Sciences and Engineering Research Council of Canada and Fonds FCAR, Province de Quebec, for financial support. Dr Françoise Sauriol for assistance and advices in running NMR spectra, M. Nadim Saadé for running mass spectra. We are also grateful to Sorin Damian and Zoe Damian for performing antitumour tests.

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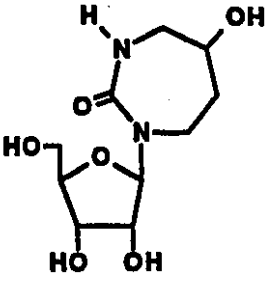
Chapter 5

General overview

The increasing interest in the chemistry of antiviral and antitumour compounds has stimulated the design of several nucleoside analogs. Diazepinone nucleoside **XI** has been found to be a potent inhibitor of cytidine deaminase. In light of this result, we thought it of interest to investigate the stability of benzotetrazepinones containing a cyclopentyl and hydroxypropyl substituent at N-5. The latter are known carbohydrate mimics.



XI

TETRAZEPINONE III.-ON THE STABILITY OF 5-HYDROXYPROPYL- AND 5-CYCLOPENTYL-1,2,3,5-TETRAZEPIN-4-ONES.

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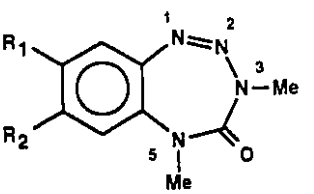
ABSTRACT: The synthesis of 3-methyl-5-(3'-hydroxy-*n*-propyl)-*3H*-1,2,3,5-benzotetrazepin-4-(*5H*)-ones **5a**, **b** and 3-methyl-5-cyclopentyl-*3H*-1,2,3,5-tetrazepin-4-(*5H*)-ones **11a**, **b** are described. Tetrazepinones **5a** and **11a** were found to be very unstable at room temperature whereas their nitrated derivatives **5b-11b** were more stable under the same conditions.The major compounds isolated from their decomposition were benzotriazole derivatives **6** and **12a**, **b**. The benzotetrazepinones as well as the benzotriazoles were characterized by the ^{15}N NMR of their labelled central nitrogen and high resolution mass spectroscopy.

INTRODUCTION

Recently, we described the synthesis of bi- and tricyclic tetrazepinones **1** (R_1=R_2=H) and **2**[1], which were found to be stable at room temperature. In a second report[2], we studied the effect of varying substituents at the benzene ring on the stability of the 1,2,3,5-tetrazepin-4-one moiety in bi-cyclic systems of type **1**. We have shown in the latter study that the stability of benzo-1,2,3,5-tetrazepinones increased with increasing electron withdrawing character of the substituents on the aromatic ring. Tetrazepinones containing electron donating group at the phenyl ring showed marked tendency to decompose to benzotriazole derivatives by loosing a molecule of methyl isocyanate . The desire to synthesize nucleoside analogs bearing tetrazepinone as a base prompted us to verify the stability of systems bearing cyclic or acyclic substituents at N5. Thus, the synthesis of **5** and **11** were attempted.



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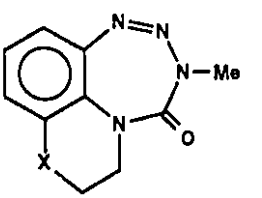
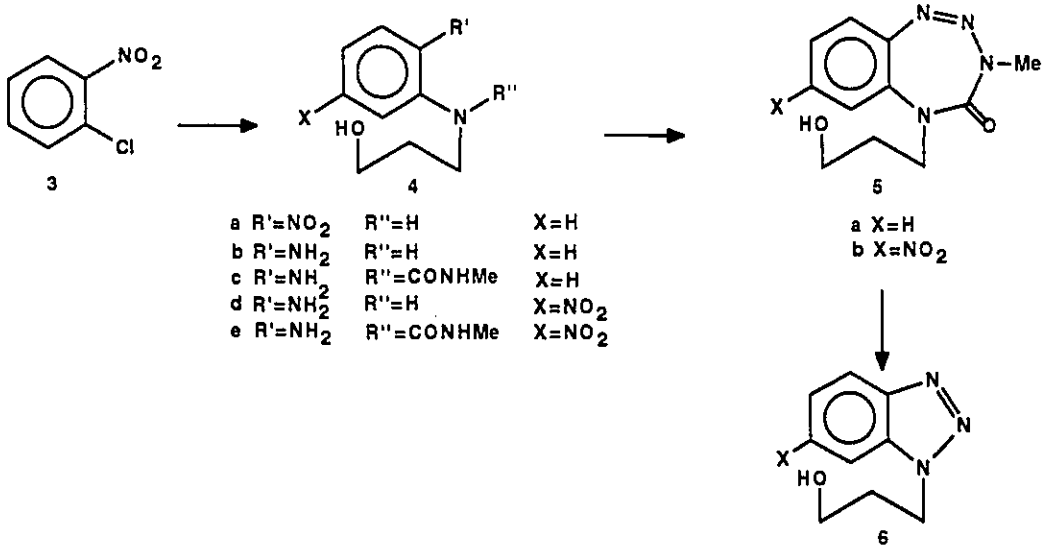


2, X=CH_2, O

This study is of more general interest because of the increasing development of novel nucleoside analogs[8-13]. It is now known that analogs in which the sugar moiety is replaced by aliphatic alcohols, carbocyclic systems[9], 2-hydroxyethoxymethyl group[10], can be potent antiviral drugs. It was also shown that analogs bearing modified bases such as imidazo[4,5-e][1,4]diazepine[11] or 6-amino-5-nitro-pyrimidine[12] exhibit interesting biological activity.

RESULTS AND DISCUSSION

The synthesis of compound **5a** proceeded according to Scheme 1. 2-Chloro-nitrobenzene **3** was reacted with 3-aminopropanol to give **4a**, which was catalytically reduced to **4b**. The latter compound was treated with methyl isocyanate to give urea **4c**. The amine function of **4c** was diazotized with [5% ^{15}N] $NaNO_2$ in order to provide the corresponding diazonium salt specifically labelled at N2. The labelling of N2 in triazene and nitroso derivatives by the use of $Na^{15}NO_2$ is now well documented[3-7].



Scheme 1

Adjustment of the pH to 8, followed by multiple extractions gave **5a** as a clear oil in 28% yield (crude). This oil started effervescing after a short period on drying at room temperature. Satisfactory ^{13}C, ^{1}H and ^{15}N NMR could be obtained with freshly prepared samples.

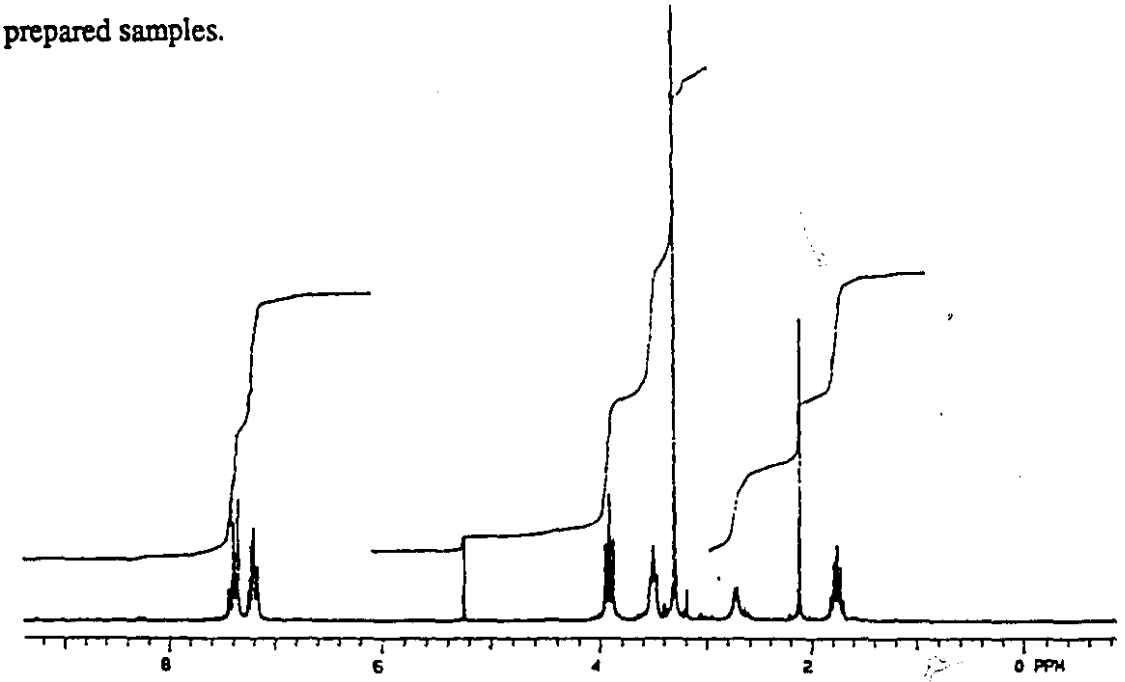


Fig. 1.-^{1}H NMR spectrum of freshly prepared tetrazepinone **5** in $CDCl_3$. (Peaks at 5.2 and 2.2 ppm are due to methylene dichloride and acetone).

In the ^{1}H NMR (Fig. 1), the N3-methyl group appeared as a singlet at 3.3 ppm and the $C1H_2$ appeared as a triplet at 3.9 ppm. The ^{13}C shifts of the tetrazepinone ring were quite similar to those reported for compound **1**[1] (Table 1). In tetrazepinone **5**, the carbonyl appeared at 160.34 ppm and the N3-methyl group at 38.10 ppm whereas the same groups were at 162.00 and 38.00 ppm in **1**. The structure of **5** was further ascertained by ^{15}N NMR spectroscopy that showed the resonance of the N2 label at 75 ppm as a broad quartet. The stability of **5a** was sufficient to allow the observation of the resonances of N1, N3 and N5 at the natural abundance level. Peaks were observed at 45.13 ppm for N1, -197.30 ppm for N3 and at -267 ppm for N5. The assignment was based on literature values[1,2,4].

Table 1* .- Comparison between the ^{13}C NMR parameters of tetrazepinones 5a and 11a with those of 1 (R1=R2=H)[1]

1	5a	11a
162.00	160.34	160.07
142.30	143.90	143.67
138.50	138.26	140.15
132.00	132.50	132.00
128.00	128.30	127.70
124.50	126.14	126.00
121.00	122.51	123.50
38.00	38.10	37.80

The decomposition of **5a** in solution was observed by the appearance of an additional peak at around -5 ppm in its ^{15}N NMR spectrum (overnight) (Fig. 2b) . The same result was observed if the solution was kept dry at room temperature overnight. We now know that this peak corresponds to N2 in benzotriazole derivatives[1,2]. The solution was then purified and the major decomposition product was found to be benzotriazole **6a.** In its ^{1}H NMR spectrum, the $C1H_2$ appeared as a triplet at 4.8 ppm. The absence of a singlet at 3.3 ppm, confirmed the disappearance of the MeNCO moiety. The structure of the benzotriazole **6a** was further confirmed by high resolution mass spectrometry.

These results show that tetrazepinone **5a** is an unstable compound that at room temperature readily decomposes to a benzotriazole derivative.

* The N-5 substituent ^{13}C shifts are not listed

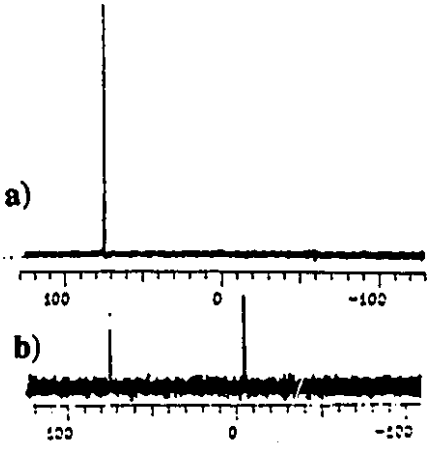
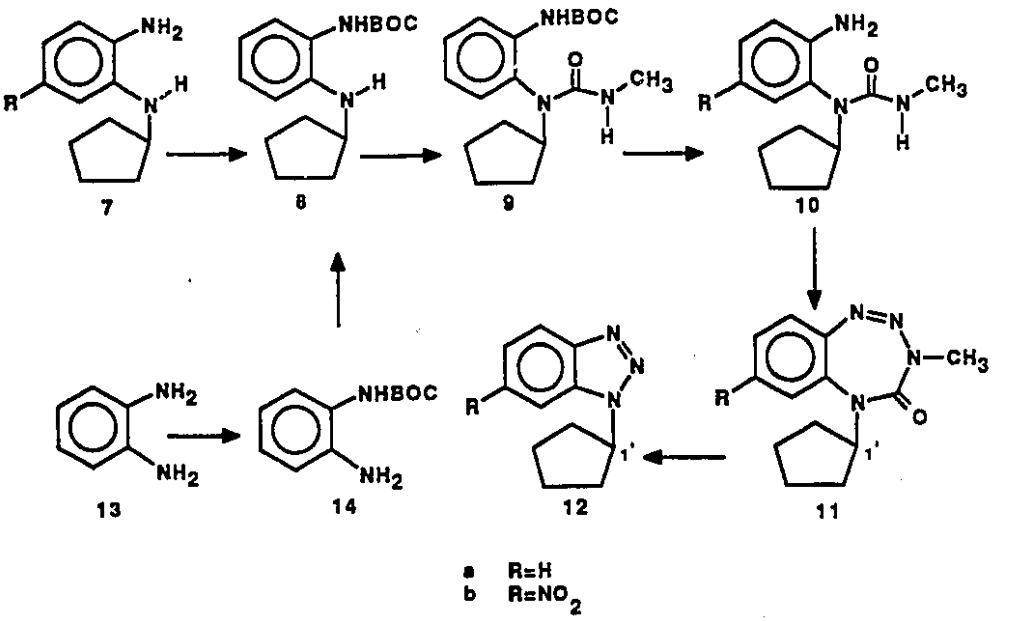


Fig. 2.- a. ^{15}N NMR of freshly prepared tetrazepinone **5a** in $CDCl_3$ (decoupled)
b. ^{15}NMR of **5a** after standing (dry) at room temperature overnight. (Another peak appeared in the -5 ppm region, indicating formation of benzotriazole derivative **6**).

In a previous study, it was found that a nitro group at the benzene ring stabilizes the tetrazepinone ring systems. We therefore synthesized **5b**, expecting enhanced stability of the tetrazepinone ring system. Thus, 4-nitro-1,2-phenylenediamine was treated with 3-bromopropanol to give **4d** which was carbamoylated with methyl isocyanate to provide **4e**. The diazotization of the latter aminourea followed by adjustment of the pH to 6 and extraction with methylene dichloride gave tetrazepinone **5b** as a pale yellow oil, which was stable at room temperature. Its structure was confirmed by ^{15}N NMR which showed a quartet (J=2.8 Hz) at around 78 ppm for the central nitrogen and by high resolution mass spectroscopy. Besides the molecular ion [280 (MH^+, 67%)], fragments due to loss of nitrogen [252 (MH^+-29, 100%)] and methyl isocyanate [223 (MH^+-57, 67%)] were observed. These results show that the incorporation of a nitro group to the benzene ring exerted an important stabilizing effect. In contrast to **5a**, tetrazepinone **5b** could be kept at room temperature for 5 days without decomposing .



Scheme 2

We then attempted the synthesis of system **11a** bearing a more bulky group (Scheme 2). Thus, a large excess of *o*-phenylenediamine was refluxed with cyclopentyl bromide overnight to give **7a.** Since the direct reaction of diamine **7a** with methyl isocyanate afforded a complex mixture of compounds, an adaptation of the previously reported methodology for the selective carbamoylation of aromatic diamine[1,2] was applied. Thus, diamine **7a** was condensed with di-*tert*-butyl-dicarbonate to provide **8,** the structure of which was confirmed by condensing a large excess of o-phenylenediamine **13** with di-*tert*-butyl dicarbonate and treating the resulting urethane **14** with cyclopentylbromide under basic condition. Since the compound synthesized by this method was found to be identical to **8,** we proceeded further by treating it with methyl isocyanate to form **9** which was hydrolyzed in acidic aqueous methanol to give urea **10a** . Diazotization of the latter with $Na^{15}NO_2$ and adjustment of the pH of the mixture to 8 followed by multiple extraction gave **11a** as a pale brown oil.

The structure of **11a** was assigned by ^{1}H, ^{13}C, ^{15}N NMR and mass spectroscopy. In the proton NMR, the methyl group appeared as a singlet at 3.3 ppm, if the tetrazepinone was unlabelled at N2, or as a doublet ($^{3}J_{NH}$=2.8 Hz) if N2 was 99% enriched. H'-1 appeared as a quintet at 4.12 ppm. The ^{13}C parameters of the benzotetrazepinone ring in **11a** are similar to those of **1** and are given in Table 1. The presence of the N2N1Me moiety of the tetrazepinone ring system was also confirmed by the appearance of a quartet ($^{3}J_{NH}$=2.8 Hz) at 74 ppm (Fig. 3a, b) in the ^{15}N NMR spectrum . In the mass spectrum, in addition to the molecular ion [246, ($M^{+}(^{15}N)$, 7.2%)], a fragment due to the loss of nitrogen [217 (M^{+}-28), 34.7] was observed as well as a strong peak due to loss of methyl isocyanate [189, (M^{+}-57), 100%].

After standing at room temperature overnight, the ^{15}N NMR of the N2 labelled **11a** showed the appearance of a peak at around -5 ppm which indicates the formation of a benzotriazole derivative (Fig 4a). Purification of the mixture gave benzotriazole **12a**. In its ^{1}H NMR, the H-1' appeared as a quintet at 5.2 ppm. The absence of the methyl singlet at 3.3 ppm confirmed the disappearance of the methyl isocyanate moiety. The benzotriazole structure as well as the ^{15}N shift of its N2 label were confirmed by the independent preparation of benzotriazole **12a** from the simple diazotization of diamine **7a** with ^{15}N enriched sodium nitrite (Fig. 4 b).

We then attempted to stabilize the ring system **11a** by introducing a nitro group at the benzene moiety as described earlier. Thus, 4-nitro-1,2-phenylenediamine was treated with cyclopentyl bromide to give **7b**, which was carbamoylated with methyl isocyanate to form **10b**. The diazotization of the latter aminourea with $Na^{15}NO_2$ followed by adjustment of the pH to 6 and extraction with methylene dichloride gave **11b** as a pale yellow oil which after 5 days at room temperature was completely converted to nitrobenzotriazole **12b**. The structure of the tetrazepinone **11b** was

confirmed on the basis of its ^{15}N NMR spectrum, which showed a quartet (J=2.8 Hz) at around 83 ppm for the labelled central nitrogen. In the mass spectrum, besides the molecular ion [291, (MH^+ (^{15}N), 78%)], a fragment due to the loss of nitrogen [262 (M^+-29), 75%] was observed as well as a strong peak due to loss of methyl isocyanate [234, (M^+-57), 100%]. The ^1H NMR of benzotriazole **12b** showed the quintet for H'-1 at 5.4 ppm (the shift of H-1' was at 4.2 ppm in the spectrum of the parent tetrazepinone **11b**). The structure of **12b** was further confirmed by high resolution mass spectrometry.

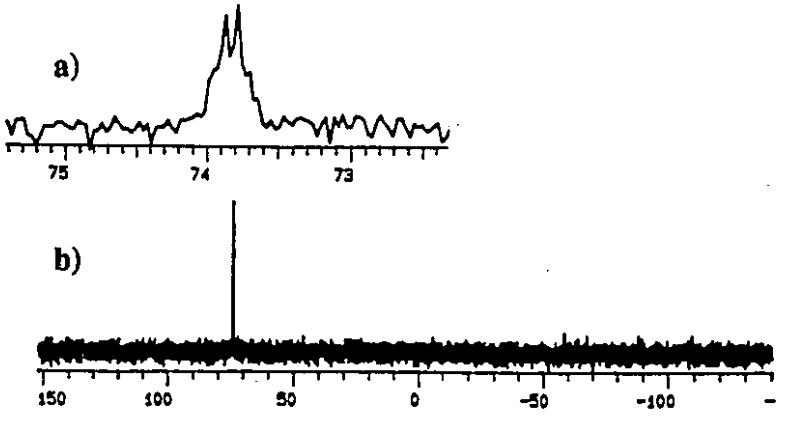


Fig. 3.- a.. ^{15}N NMR of freshly prepared **11a** (coupled)
b. ^{15}N NMR of freshly prepared tetrazepinone **11a** in $CDCl_3$ (decoupled)

The order of stability of the 4 tetrazepinones in this series seems to be the following : **5b>11b>5a>11a**. The preferred decomposition pathway for these tetrazepinones was the loss of the methyl isocyanate moiety to give a ring contraction product. This decomposition pathway has already been reported for benzotetrazepinones bearing electron donating groups at the benzene ring. It is important to notice that while tetrazepinones **5a** **11a** and **b** were relatively unstable at room temperature,

1 (R1=R2=H) and **1** (R1=NO_2, R2=H) which contain a methyl group at N5 were stable under the same condition[1]. Also, tetrazepinones **2** in which N5 is involved in a cyclic system are more stable than **5a**, **11a** and **b**. It appears that large substituents at the 5-position destabilize the tetrazepinone ring system.

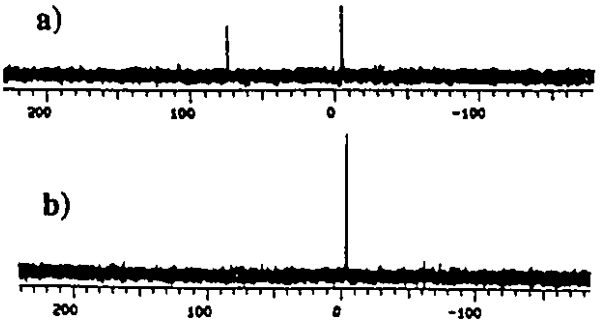
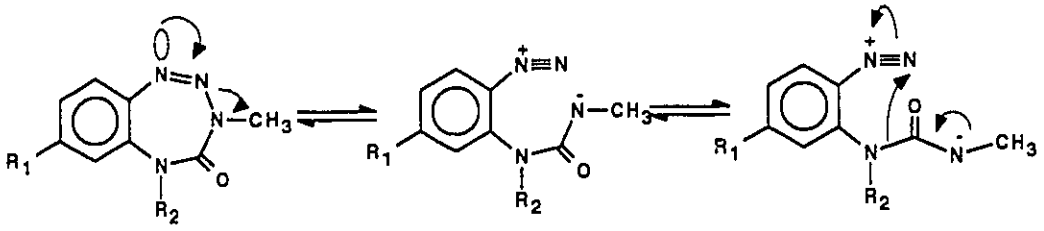


Fig. 4.- a.. ^{15}N NMR spectrum of **11a** after standing (dry) at room temperature overnight. (Another peak appeared in the -5 ppm region, indicating the formation of benzotriazole **12a**)
b.^{15}N NMR of independently synthesized **12a**

Tetrazepinones are rare examples of molecules bearing a triazene chain which do not seem to be stabilized by n-π delocalization. X-ray crystallography results have shown that the N2N3 linkage in tetrazepinones **1** (R_1=H, R_2=NO_2) and **2** (X=CH_2) have a remarkable single bond character (1.45 Å)* . This may account for their possible tendency to exist as an acyclic diazourea of type **16**. In tetrazepinones containing bulky substituents at the 5-position, the cyclic-acyclic equilibrium may lie toward the formation of the acyclic form since the latter would offer a higher degree of freedom to the substituent or a higher torsional angle between the N5-R2 moiety and the aromatic ring . The zwitterion of type **16** could also be in equilibrium with **17** which could collapse to a benzotriazole derivative as indicated in Scheme 3. This would account for the fact that the

* In N, N'-dimethylhydrazine the NN bond distance is 1.45 Å[14]

tetrazepinones bearing large substituents at N5 are prone to decompose to benzotriazoles by loosing a molecule of methyl isocyanate.



15 16 17

Scheme 3

The stabilizing effect exhibited by the nitro group is based on the fact that it may disfavour the ring opening via an N2N3 bond breaking by destabilizing the developing positive charge on N1. It should be remembered that in contrast to the nitro group, a substituent *para* to the triazene chain was found to destabilize the 1,2,3,5-tetrazepinone ring system by promoting rapid conversion to benzotriazole derivative.[2]

EXPERIMENTAL

Melting points were measured on a Gallenkamp block and are uncorrected. Thin-layer and flash chromatography were performed on silica gel 60 F_{254} aluminum plates and Merck Silica Gel 60 (230-400 mesh) respectively. ^1H NMR spectra were recorded on a Varian XL-200 at 200 MHz. ^{13}C NMR spectra were obtained at 75.40 MHz on a Varian XL-300. All ^1H NMR spectra were run in $CDCl_3$ or DMSO and chemical shifts are reported downfield from TMS (J values are in Hz). Mass spectra were recorded on a Kratos MS25RFA or an HP5984A. All compounds were shown to be homogeneous by TLC and high-field NMR, or to have a purity of > 95% by elemental analysis.

All samples were dried *in vacuo* at room temperature before elemental analysis

All samples were dried *in vacuo* at room temperature before elemental analysis

^{15}N NMR spectra were taken at 30.40 MHz on a Varian XL-300 and chemical shifts are reported upfield from nitromethane, which was used as external standard. The 90° pulse width was 18 μs and the pulse interval was set at 3s. The temperature of the probe was around 20° C. Spectra were obtained after 100 scans for the ^{15}N enriched compounds when sample concentrations were around 0.10 M in $CDCl_3$ (gated coupled) and after about 9000 scans for natural abundance spectra at concentrations around 0.5 M.

All reactions were monitored by thin layer chromatography (TLC).

N-(3-Hydroxypropyl)-2-nitroaniline (4a).

A solution of 2-chloronitrobenzene (2g, 12.7 mmol) in 3-aminopropanol (10 mL) was heated to 180° C for 5 min after which it was cooled and concentrated under vacuum. The resulting dark-red residue was purified on silicagel to give 2g (81%) of **4a** as a red oil. 200 MHz, ^1H NMR ($CDCl_3$) δ: 8.14 (overlap of s and d, 2H, J=8.6, Ar, NH), 7.36 (t, 1H, J=8.6, Ar), 6.7 (d, 1H, J=8.6, Ar), 6.62 (t, 1H, J=8.6, Ar) 3.8 (t, 2H, J=6, $NCH_2CH_2CH_2$), 3.42 (t, 2H, J=6, $NCH_2CH_2CH_2$) 1.97 (quintet, 2H, J=6, $NCH_2CH_2CH_2$), 1.68 (br s, 1H, OH); CIMS (*iso*-butane) *m/z* (relative intensity) 197 (MH^+, 100) 151 (MH^+-NO_2, 11)

N-(2-Phenylamino)-N-(3-hydroxypropyl)-N'-methylurea (4c).

Amine **4a** (1.5g) was reduced with 10% Pd-C (500 mg) in methanol (10 mL) to give **4b** as a violet oil in quantitative yield. 200 MHz ^1H NMR δ: 6.8-6.5 (m, 4H, Ar), 3.8 (t, 2H, J=6, $NCH_2CH_2CH_2$), 3.26 (overlap of t and s, 4H, J=6, $NCH_2CH_2CH_2$, NH_2), 1.9 (overlap of quintet and s, 2H, $NCH_2CH_2CH_2$, OH). (N-alkyl phenylenediamines darken when exposed to light. They must be used immediateley after their isolation). To a solution of diamine **4b** (1g, 6mmol) was added 0.4 mL (1eq) of methyl isocyanate. The

solution was kept overnight, concentrated under vacuum and purified on a silica gel column (5% methanol in methylene dichloride) to give urea **4c** (1g , 75%) as a white powder, m. p. 118° C. 200 MHz ^{1}H NMR ($CDCl_3$) δ: 7.2 (t, 1H, J=8.8, Ar), 7.0 (d, 1H, J=8.8, Ar), 6.8 (t, 2H, Ar), 4.4 (br s, 2H, NH_2), 3.9 (br m, 2H, $NCH_2CH_2CH_2$), 3.65 (br m , 2H, $NH_2CH_2CH_2CH_2$), 2.7 (d, 1H J=6, $CONHCH_3$) 1.6 (br m, 3H, $NCH_2CH_2CH_2$, OH); EIMS m/z 223 (M^+, 42). 166 (M^+-57, 15), 121 (M^+-102, 100); Anal calc. for $C_{11}H_{17}N_3O_2.0.5H_2O$: C, 56.80; H 7.76; N 18.01. Found: C 57.18;H 7.60, N, 17.87.

3-Methyl-5-(3-hydroxypropyl)-1,2,3,5-benzotetrazepine-4-one (5a).

Urea **4c** (1g, 5.55mmol) was dissolved in 2N HCl (20 mL) and diazotized with 2mL of aqueous 20% [5% ^{15}N] $NaNO_2$. After 30 min, the solution was extracted three times with methylene dichloride, after which its pH was adjusted to 8. The aqueous layer was extracted six times with three 50 mL portions of methylene chloride. The solvent was evaporated to give 300 mg (28%, crude) of **5a** as a clear oil. The resulting compound started effervescing on standing. It was consequently kept in solution for subsequent analysis. 200 MHz ^{1}H NMR ($CDCl_3$) δ: 7.36 (d, 2H, J=8, Ar), 7.2 (overlap of d and t, 2H, Ar) 3.9 (t, 2H, J=7, $NCH_2CH_2CH_2$), 3.5 (br t, 2H, $NCH_2CH_2CH_2$), 3.3 (s, 3H, $N{=}NNCH_3$), 2.65 (br s, 1H, OH), 1.75 (quintet, 2H, J=7, $NCH_2CH_2CH_2$); 75.4 MHz ^{13}C NMR ($CDCl_3$) 160.34 (s), 143.90 (s), 138.26 (s), 132.50 (d), 128.30 (d), 126.14 (d), 122.51 (d), 60.58 (t), 45.80 (t), 38.01 (t), 32.24 (t); 30.4 MHz ($CDCl_3$) δ: 68.5 (N2 label), 45.1 (N1), -197.7 (N3), -277.6 (N5). (A peak at -5.2 ppm was also observed due to formation of benzotriazole **6**)

1-(3'-Hydroxypropyl)-*1H*-benzotriazole (6).-

The dark chloroform solution of **5a** (250 mg) which resulted from the NMR experiment (overnight at room temperature) was evaporated and the resulting dark oily residue

purified on a silica gel column (5% hexane in ethyl acetate) to give 150 mg of **6**; 200 MHz ^{1}H NMR ($CDCl_3$) δ: 8.00 (d, J=8, 1H, Ar), 7.6 (d, 1H, J=8, Ar), 7.4 (t, 1H, J=8, Ar), 7.3 (t, 1H, Ar), 4.8 (t, 2H, J=7, $HOCH_2CH_2CH_2$-) , 3.6 (br t, 2H, $HOCH_2CH_2CH_2$-), 3.05 (br s, 1H, OH), 2.2 (quintet, 2H, J=7, $HOCH_2CH_2CH_2$-); 30.4 MHz ^{15}N NMR ($CDCl_3$) δ: -5.2 (N2); EIMS *m/z* 177 (M^+, 12.7), 91 (M^+-$HOCH_2CHCH_2$ -N_2), 100); HRMS exact mass calcd for $C_9H_{11}N_3O$ (M^+): 177.09021. Found: 177.09080

N-(3'-Hydroxypropyl)-5-nitro-1,2 phenylenediamine (4d).

A solution of 4-nitro-1,2-phenylenediamine (2g, 12.7 mmol) and 3-bromopropanol in 25 mL of a 1:1 methanol/dimethylformamide mixture was heated at reflux overnight. The solvents were evaporated under vacuo and the resulting dark-red residue chromatographed on silica gel (30% hexane in ethyl acetate) to give **4d** (1 g, 36%) as a red powder, m. p. 89° C; 200 MHz ^{1}H NMR ($CDCl_3$) δ: 7.66 (dd, 1H, J=8.7, J=2.46 Ar), 7.44 (d, 1H, J=2.46, Ar), 6.64 (d, 1H, J=8.7, Ar), 3.85 (t, 2H, J=6.02, $NCH_2CH_2CH_2OH$), 3.6-3.3 (br s, 4H, NH_2, NH, OH), 3.30 (t, 2H, J=6.02, $NCH_2CH_2CH_2OH$), 1.9 (quintet, 2H, J=6.02, $NCH_2CH_2CH_2OH$).

N-(5-Nitro-2-aminophenyl)-N-(3-hydroxypropyl)-N'-methylurea (4e)

A solution of **4d** (400 mg, 1.9 mmol) and methyl isocyanate (0.12, 1 eq) in acetonitrile (20 mL) was kept overnight, concentrated under vacuo and diluted with water (10 mL). The precipitate that formed was filtered to give **4e** (400 mg, 78%) as a yellow powder wich was dried under vacuum at room temperature, m. p. 135-137 °C ; 200 MHz ^{1}H NMR ($CDCl_3$) δ: 8.1 (dd, 1H, J=2.47, J=9, Ar), 7.9 (d, 1H, J=2.47, Ar), 6.9 (d, 1H, J=9, Ar), 4.25 (br s, 1H, $CONHCH_3$), 3.8 (overlap of m, 4H, $CH_2CH_2CH_2OH$), 2.7 (overlap of d and s, 5H, $CONHCH_3$, NH_2), 1.8 (br m, 3H, OH, CH_2); EIMS *m/z* 268 (M^+, 20.6), 211 (M^+-MeNCO, 19.1), 166 (M^+-102, 100) Anal. calc. for $C_{11}H_{16}N_4O_4.H_2O$: C 46.15; H 6.29; N, 19.58. Found C, 46.59; H, 6.08; N, 20.24

3-Methyl-5-(3'-hydroxypropyl)-7-nitro-*3H*-1,2,3,5-benzotetrazepine-4(5H)-

-one (5b).

As described for **5a**; from **4e** (400 mg) in 2N HCl (10 mL): yellow oil, 300mg (72%) ; 200 MHz ^{1}H NMR ($CDCl_3$) δ: 8.10 (overlap of s and d, 1H, J=8.85, Ar), 7.98 (d, 1H, J=8.85, Ar), 4.0 (t, 2H, J=6, $CH_2CH_2CH_2OH$), 3.6 (br m, 2H, $CH_2CH_2CH_2OH$), 3.4 (s, 3H, $N{=}NNCH_3$), 2.19 (br s, OH, D_2O exchangeable), 1.8 (quintet, J=6.00, $CH_2CH_2CH_2OH$); 75.4 MHz ($CDCl_3$) δ: 158.02 (s), 148.57 (s), 145.99 (s), 138 (s) 128 (d), 119.4 (d) 117.01 (d), 59.20 (t), 44.77 (t) 36.90 (q), 30.81 (t); 30.4 MHz ^{15}N NMR ($CDCl_3$) δ: 78.8 (q, J=2.8, q); CIMS (NH_3) *m/z* 280 (MH^+, 67.3) 252 (MH^+-28, 100), 223 (MH^+-57, 66.64); HRMS calcd for $C_{11}H_{14}O_4N_5$ (MH^+) 280.10458. Found: 280.10459

1-N-(Cyclopentyl)-2-phenylenediamine (7a).

o-Phenylene diamine (5 g, 46.2 mmol) cyclopentyl bromide (1 mL, 9.4 mmol) and 10 mL of a saturated sodium carbonate solution as a suspension in methanol (25 mL) was heated at reflux for 20 hrs. Water was added and the brown mixture extracted with methylene dichloride. The solvent was concentrated under vacuum and the resulting oil chromatographed on silica gel (30% hexane in ethyl acetate) to give 7 (1g , 60%) as a brown oil; 200 MHz ^{1}H NMR ($CDCl_3$) δ: 6.85-6.20 (m, 4H, Ar), 3.80 (quintet, 1H, J=4.52, H-1'), 3.40 (br s, 3H, NH, NH_2), 2.1-1.4 (m, 8H, CH_2s); CIMS (*iso*-butane) *m/z* 176 (MH^+,100).

1-(N-Cyclopentyl)-2-(N-tert-butylcarbamoyl)-phenylenediamine (8)

Method A

A solution of 1-N-(cyclopentyl)-2-phenylenediamine (500 mg, 2.8 mmol) and di-*tert*-butyl-dicarbonate (589 mg, 1eq) in methylene dichloride (20 mL) was heated at reflux

for 1 hr under nitrogen. Evaporation under reduced pressure followed by purification on silica gel (50% ethyl acetate in hexane) gave **8** (700 mg . 89%) as a white powder, m.p. 91° C . 200 MHz ^{1}H NMR ($CDCl_3$) d: 7.4-6.6 (m, 4H, Ar), 6.2 (s, 1H, N*H*COOtBu), 3.8 (br m, 2H, H-1'), 2.2-1.4 (m, 21H, CH_2, tBu);

Method B

A solution of *o*-phenylenediamine (4 g, 37 mmol) and di-*tert*-butyl-dicarbonate (2.5 g, 0.33 eq) in tetrahydrofuran (20 mL) was heated at reflux for 1 hr under nitrogen. Evaporation under reduced pressure followed by purification on silica gel (50% ethyl acetate in hexane) gave **8** (1.9 g, 76 %) as a white crystalline residue, m. p. 98° C, 200 MHz ^{1}H NMR $CDCl_3$ d:7.4-6.6 (m, 4H, Ar), 6.2 (s, 1H, N*H*COOtBu), 3.8 (br s, 2H, NH_2), 1.4 (s, 9H, tBu). A mixture of urethane **14** (1g, 4.78 mmol), cyclopentyl bromide (0.5 mL, 1 eq), 40% aqueous sodium carbonate (2 mL) and methanol (20 mL) was heated at reflux for 3 days. The solvents were evaporated, water (10 mL) was added and the resulting mixture extracted with methylene dichloride. The white oil resulting from the evaporation of the methylene dichloride was purified on a silica gel column (50% hexane in ethyl acetate) to give 400 mg (30%) of a white crystalline residue the melting point and ^{1}H NMR of which were identical to those of **8**.

N-Cyclopentyl-N-methylcarboxamide-(N-*tert*-butylcarbamoyl)-1,2-phenylenediamine (9)

A solution of **8** (600 mg, 1eq) and methyl isocyanate (0.14 mL, 1eq) in methylene dichloride (25mL) was kept overnight and the solvent was evaporated to give a white residue in quantitative yield, m. p. 110° C. 200 MHz ^{1}H NMR ($CDCl_3$) δ: 8.23 (d, 1H, J=8.1, Ar), 7.45 (m, 1H, Ar), 7.1 (m, 2H, Ar), 6.82 (s, 1H, N*H*COOtBu) 4.8 (m, 1H, H-1'), 4.00 (br s, 1H, CON*H*CH_3) 2.70 (d, 3H, J=4.7, CONHC*H*$_3$), 2.01-1.40 (overlap of s and m, 21H, cyclopentyl, t-Bu); 75.4 MHz ^{13}C NMR ($CDCl_3$) δ: 158.51, 153.03, 138.40,

131.37, 130.19, 126.48, 123.574, 119.95, 81.54, 58.31, 30.54, 28.85, 28.64, 27.81, 22.95, 22.81. CIMS (NH_3) *m/z* 334 (MH^+, 100), 233 (MH^+-NHCOOtBu, 72.5),

1-(N-Cyclopentyl-N-methylcarboxamide)-1,2-phenylene diamine (10a)

A solution of **10** (400 mg) in a 5:1 mixture of methanol and 2N aqueous HCl was stirred overnight. Dilution with water and neutralization with 5% sodium carbonate gave **10a** as a pale yellow solid in quantitative yield, m.p. 100° C. 200 MHz ^1H NMR $CDCl_3$ δ: 8.2 (t, 1H, J=7.2, Ar), 7.02 (d, 1H, J=7.2 , Ar), 6.70 (overlap of t and d, 2H, Ar) 4.8 (br quintet, 1H, H1'), 4.20 (br q, H, CON*H*Me), 3.8 (br s, 2H, NH_2), 2.70 (d, 3H, J=4.7, CONHCH_3), 2.1-1.10 (overlap of m, 8H, cyclopentyl); EIMS *m/z* 233 (M^+, 100), 175 (M^+-57, 40)

3-Methyl-5-cyclopentyl-*3H*-1,2,3,5-benzotetrazepin-4-(*5H*)-one (11a)

To a solution of urea **6c** (150 mg, 0.85 mmol) in 2N HCl (5 mL) was added dropwise at 0° C 6% aqueous $Na^{15}NO_2$ (1 mL) · The mixture was stirred for an additional 10 min , after which it was extracted twice with methylene chloride. The pH of the aqueous solution was adjusted to 8 with aqueous sodium carbonate after which it was extracted. Evaporation of the mixture gave **11a** (60 mg , 29%) as a pale brown oil; 200 MHz ^1H NMR ($CDCl_3$) δ: 7.40-7.10 (overlap of m, 4H, Ar), 4.13 (quintet, 1H, J=8, H-1') , 3.33 (s, 3H, $NNNCH_3$), 2.4-1.5 (overlap of m, 8H, cyclopentyl CH_2); 75.4 MHz ^{13}C NMR ($CDCl_3$) δ: 160.08 (s), 143.67 (s), 140.15 (s), 131.90 (d), 127.69 (d), 126.00 (d), 123.50 (d), 63.54 (d), 37.70 (q), 32.48 (d), 32.28 (t), 32.20 (d), 26.57 (t); 30.4 MHz ^{15}N NMR ($CDCl_3$) δ: 73.81 (q, J=2.8, N2); CIMS (*iso*-butane) *m/z* 246 (MH^+(^{15}N), 7), 217 (MH^+-^{15}N=N, 35), 189 (MH^+-MeNCO, 100)

1-Cyclopentyl-*1H*-benzotriazole (12a) .

After standing at room temperature overnight, tetrazepinone **11a** was found to be completely converted to **12a**; 200 MHz ^1H NMR ($CDCl_3$) δ: 8.04 (d, J=8, 1H, Ar), 7.6-7.25 (overlap of m, 3H, Ar), 5.2 (quintet, 1H, J=7, H-1'), 2.4-1.6 (overlap of m, 8H, cyclopentyl CH_2) ; 30.4 MHz ^{15}N NMR ($CDCl_3$) δ: -5.0 (N2); EIMS m/z (relative intensity) 188 [$M^+(^{15}N)$, 67] , 91 (M^+- 97, 100); HRMS exact mass calcd for $C_{11}H_{13}N_3$ [M^+ (^{14}N)]: 188.10797. Found: 188.1086

5-Nitro-1-(N-Cyclopentyl)-1,2-phenylenediamine (7b)

As described for **7a**. From 4-nitro-1,2-phenylene diamine (2g, 0.013 mmole) and cyclopentyl bromide (1.3 mL) and saturated sodium carbonate (10 mL) in methanol (50 mL) overnight (red oil, yield: 500 mg, 17 %); 200 MHz ^1H NMR ($CDCl_3$) d: 7.65 (dd, 1H, J=8.4, Ar), 7.52 (s, 1H, Ar), 6.65 (d, 1H, J=8.4, Ar), 4.9 (br, 2H, NH_2, D_2O exchangeable), 3.8 (br quintet, 1H, H-1'), 3.1 (br s, 1H, NH, D_2O exchangeable) 2.2-1.23 (br, 8H , cyclopentyl).

N-Cyclopentyl-N-(5-Nitro-2-aminophenyl-N'-methylurea (10b)

As described for **10a**; From **7b** (500 mg, 2.2 mmol) and methyl isocyanate (1mL) in chloroform (10 mL); The precipitate that formed was filtered to give **10b** (350 mg, 56.2 %) as a yellow powder; m. p. 209° C (dec); Yield: 350 mg, 56.2%; 200 MHz ^1H NMR (DMSO) d: 7.9 (d, 1H, J=9, Ar), 7.6 (s, 1H, Ar), 6.8 (d, 1H, J=9, Ar), 6.6 (s, 2H, NH_2, D_2O exchangeable), 5.6 (br q, 1H, CON*H*CH_3), 4.6 (br quintet, 1H, H-1'), 2.45 (br s, 3H, CONHC*H*$_3$), 1.9-1.1 (m, 8H, cyclopentyl); EIMS *m/z* 278 (M^+, 100), 221 (M^+-MeNCO, 58.2). Anal. calc. for $C_9H_{13}N_3O_3.0.5H_2O$: C, 54.3, H, 6.62, N 19.51. Found: C, 53.83; H 6.58; N 19.52.

3-Methyl-7-nitro-5-cyclopentyl-*3H*-1,2,3,5-benzotetrazepin-4(*5H*)-one (11b)

As described for **5a**; from **10b** (169 mg); the tetrazepinone **11b** was obtained as a yellow oil (100 mg, 56%); 200 MHz ^{1}H NMR ($CDCl_3$) δ : 8.1 (overlap of d and s, 2H, Ar), 7.5 (d, J=8.3, 2H, Ar), 4.25 (quintet, 1H, J=6, H-1'), 3.4 (s, 3H, N=NNCH_3) 2.2-1.5 (m, 8H, cyclopentyl) 75.4 MHz ($CDCl_3$) δ : 158.2 (s), 148.6 (s), 146.5 (s) 139.9 (s) 127.83 (d), 119.8 (d), 118.4 (d), 62.66 (d), (d) 36.95 (q), 31.20 (t), 25.57 (t); 30.4 MHz ^{15}N NMR ($CDCl_3$) δ: 83.3 (q, J=2.8, N2); CIMS (NH_2) *m/z* 291 (MH^+(^{15}N), 78), 262 (MH^+-N=^{15}N), 75), 234 (MH^+-MeNCO, 100);

1-Cyclopentyl-5-nitro-1H-benzotriazole (12b).

From the complete decomposition of **11b** after 5 days at room temperature in the solid: 200 MHz ^{1}H NMR ($CDCl_3$) δ: 8.6 (s, 1H, Ar) 8.2 (overlap of d, 2H, Ar), 5.2 (quintet, J=6.76, H-1) , 2.4 (m, 4H, CH_2s), 2.0 (overlap of m, 4H, CH_2 cyclopentyl); EIMS *m/z* (relative intensity) 233 (M^+(^{15}N), 30), 175 (M^+-cyclopentyl, 28); HRMS exact mass calcd for $C_{11}H_{12}O_2N_4$ (M^+(^{14}N)): 232.09603. Found: 232.09627

ACKNOWLEDGEMENT

We thank the National Research Council of Canada (NSERC) for financial support. We are also grateful to Nadim Saadé and Prof. Orval Mamer of the Medical Mass Spectrometry unit for running mass spectra.

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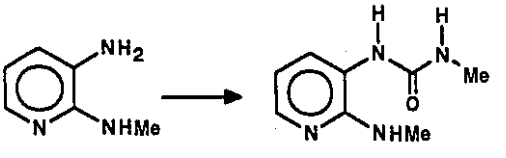
Chapter 6

General overview

The finding that electron-withdrawing groups stabilize the 1,2,3,5-tetrazepinone ring system has encouraged the development of tetrazepinone fused with pyrimidine and pyridine rings, since the latter ring systems are known to be electron-deficient with respect to benzene.

Attempts to synthesize pyridimidine fused tetrazepinone failed because it was not possible to synthesize the urea precursors. However, the formation of N-(3-amino-2-pyridyl)-N'-alkylurea was successfully achieved.



XI XII

In contrast to N-methyl-1,2-phenylene diamine, the reaction of 2,3-diaminopyridine **XI** gave **XII**, selectively carbamoylated at the amino group. We then adapted previously described methodology which consisted of protecting the primary amino group. We chose a carbobenzyloxy group because it was easy to remove by catalytic hydrogenation. This general method had allowed the synthesis of tetrazepinones containing a methyl group, a 4'-hydroxybutyl or a cis-cyclopentyl carbinol group at the 5-position.

TETRAZEPINONE IV.- SYNTHESIS OF PYRIDINE FUSED TETRAZEPINONES.

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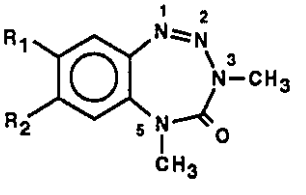
ABSTRACT: The cyclization of N-(2-amino-3-pyridyl)-N, N'-dimethylurea **3c** by diazotization failed to produce 3,5-dimethyl-3*H*-pyrido-[2,3-f]-1,2,3,5-tetrazepin-4-(3*H*)-one **4**, whereas that of N-(3-amino-2-pyridyl)-N,N'-dimethyl urea **9** gave 3,5-dimethyl-3*H*-pyrido-[3,2-f]-1,2,3,5-tetrazepin-4-(3*H*)-one **10**. This finding allowed the synthesis of 4'-hydroxybutyl-pyridotetrazepinone **15** and ± cis-4-cyclopentylcarbinol-pyridotetrazepinone **22**.

INTRODUCTION.

Recently, we reported the synthesis and stability of bicyclic benzo-1,2,3,5-tetrazepinones bearing substituents at the benzene ring[1,2]. We have shown that their stability increases with increasing electron-withdrawing character of these substituents. Since the pyridine ring, due to its ring nitrogen, is electron-withdrawing, it was predicted that its fusion with the 1,2,3,5-tetrazepinone system would give rise to bicyclic compounds the stability of which would be similar to that of the bicyclic systems **1a**, **b**.



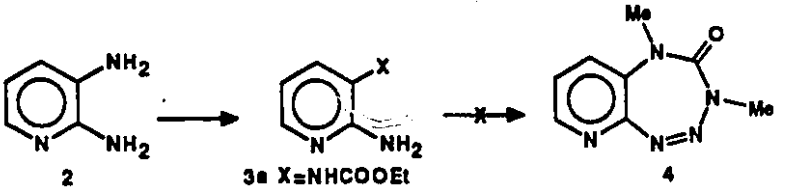
1a R_1=Cl R_2=H
b R_1=H R_2=NO_2

We now report the synthesis and ^{15}N NMR of bi-cyclic pyridotetrazepinones. We also report the synthesis of nucleoside analogs **13** and **18** bearing pyrido-1,2,3,5-tetrazepinones as bases. It is now known that base and sugar modified nucleoside analogs can show interesting antiviral activity[3-8].

RESULTS AND DISCUSSION.

We first attempted the synthesis of pyridotetrazepinone **4** in which the triazene chain is attached to the 2-position of the pyridine ring . Treatment of a large excess of 2,3-diaminopyridine **2** with ethyl chloroformate under basic condition gave the monocarbamate **3a** which was reduced with lithium aluminium hydride to 2-amino-3-methylamino pyridine **3b.** Treatment of this amine with methyl isocyanate gave urea **3c,** the diazotization of which, followed by neutralization, gave a complex mixture. The ^{15}N NMR spectrum of the crude mixture showed two peaks at around 180 and 170 ppm indicating the formation of nitroso species, one of which may be similar to **5** which was isolated from the attempted diazotization of N-(2-amino-3-pyridyl)-N'-methyl urea **3d.** The nitroso group of **5** was at 184 ppm. The chemical shifts of the nitrogen in nitroso compounds is known to be in the 180-190 ppm range (downfield from nitromethane).[9-11]

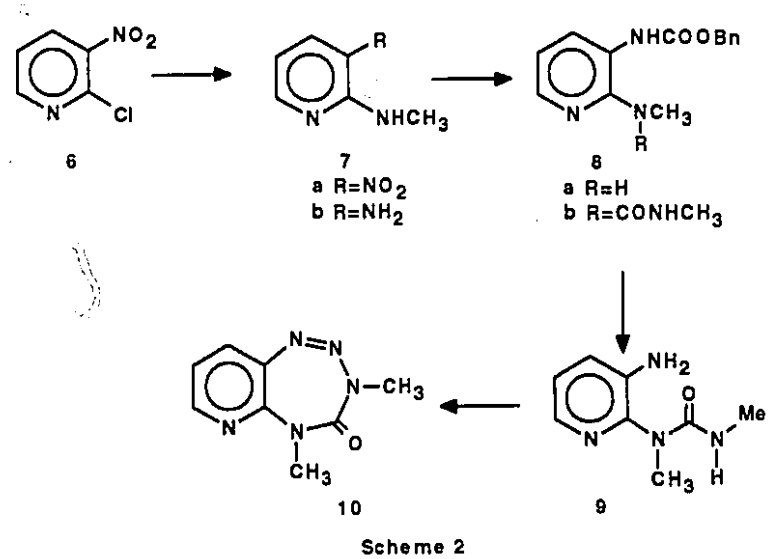
The formation of tetrazepinones results from a relatively stable 2-diazonium-aryl urea intermediate[1,2]. It appeared that under acidic conditions, the ureido moiety of ureas **3c** and **d** was more reactive towards the nitrosylating agent.



2 — 3a X=NHCOOEt; b X=NHMe; c X=NMeCONHMe; d X=NHCONHMe; 5, X=NHCONMeNO.HCl — 4

Scheme 1

We then attempted the synthesis of pyridotetrazepinone **10** in which the triazene moiety of the tetrazepinone would be at the 3-position of the pyridine ring. The preparation of **10** proceeded according to Scheme 2. 2-Chloro-3-nitropyridine **6** was

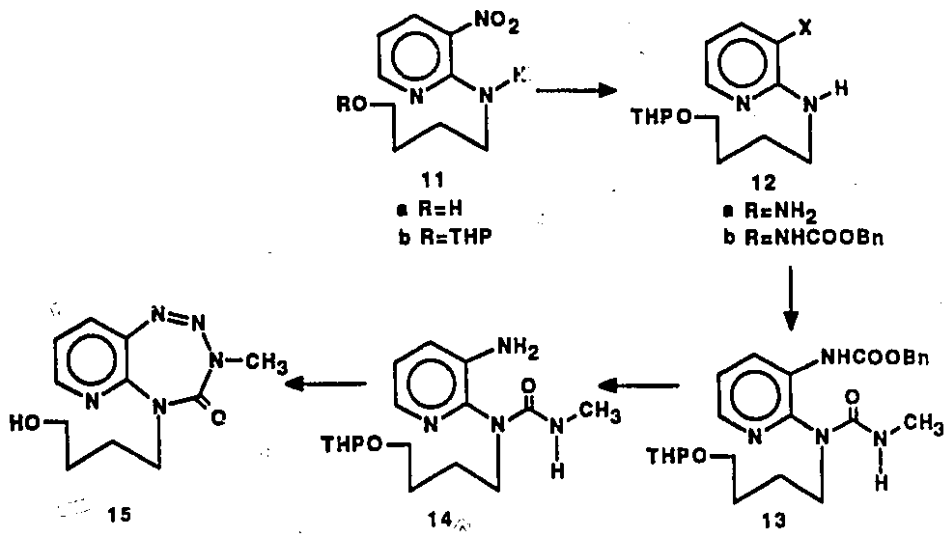


Scheme 2

treated with aqueous methylamine to give N-methylamine **7a** which was reduced to **7b**. Since the reaction of **7b** with methyl isocyanate gave a compound selectively carbamoylated at the primary amino function, it was decided to protect the latter with a carbobenzyloxy group by treating it with benzyl chloroformate. Treatment of the resulting urethane **8a** with methyl isocyanate provided urea **8b**. The amino group was then deprotected by catalytic hydrogenation to give **9**. Diazotization of this compound with [6% ^{15}N] $NaNO_2$ and adjustment of the pH to 6 gave the selectively labelled tetrazepinone **10** as a white powder the elemental composition of which was confirmed by microanalysis and high resolution mass spectometry. Its ^{15}N NMR will be discussed below.

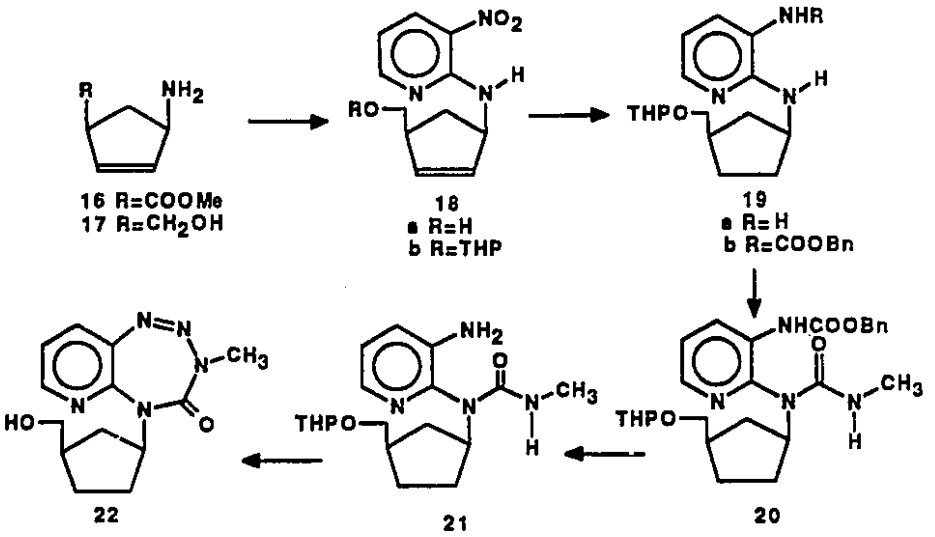
The synthesis of tetrazepinone **15** proceeded according to Scheme 3. 2-Chloro-3-nitropyridine **6** was reacted with 4-aminobutanol to give the aminoalcohol **11a**. The hydroxyl function was protected with a tetrahydropyranyl group to give **11b**. The

nitro group in **11b** was then reduced to amine **12a** and its benzyloxycarbamate derivative **12b** was then treated with methyl isocyanate to give **13**. Catalytic hydrogenation using 10% Pd-C catalyst gave the urea **14** . This urea was diazotized with [6% ^{15}N] $NaNO_2$ in 2N HCl. Neutralization of the reaction mixture gave **15** that could be analyzed by ^{15}N NMR and high resolution mass spectroscopy.



Scheme 3

We then applied the methodology described above to the synthesis of **22**, the structure of which is similar to that of carbocyclic nucleoside analogs[12,13]. Amine **16** was prepared according to a modification of the methodology described by Daluge and Vince[12]. Thus, ± 2-azabicyclo[2.2.1]-hept-5-en-3-one[12] was methanolized under acidic condition to give the amino-ester **16** as an ammonium salt. This compound could also be obtained by hydrolyzing ± 2-azabicyclo[2.2.1]-hept-5-en-3-one to an amino acid which could be esterified by the Chan's method[14]. The amino-ester **16** was reduced with lithium aluminium hydride at -60° C to give ± amino alcohol **17**. This route is an alternative to the rather lengthy synthesis reported by Daluge and Vince[12].



Scheme 4

Amine **17** was refluxed with 2-chloro-3-nitropyridine **6** to provide **18a**, the alcohol function of which was protected as its tetrahydropyranyl ether **18b**. The nitro group and the double bond were simultaneously reduced by catalytic hydrogenation to give **19a**. Urea **21** was obtained as described for **8** and **11**. Thus, the diamino pyridine **19a** was treated with benzyl chloroformate under basic condition to give urethane **19b**, which was converted to urea **20** by reaction with methyl isocyanate. The carbobenzyloxy group of the latter compound was removed by catalytic hydrogenation to give **21** which was diazotized with [99% ^{15}N] $NaNO_2$ to give tetrazepinone **22**.

The structures of the tetrazepinones in this series were further confirmed by ^{1}H NMR, ^{13}C NMR, ^{15}N NMR and high resolution mass spectroscopy. In the ^{1}H NMR spectra, the N3 methyl groups of **10** and **15** appeared as a singlet and that of **22** showed up as a doublet (coupled with ^{15}N) at around 3.4 ppm. In the ^{13}C NMR spectra, the N3-methyl groups appeared at around 38 ppm. In the mass spectra of **10**, **13** and **22**, the molecular ions and strong MH^{+}-N_2 peaks were observed.

We were able to run the natural abundance ^{15}N NMR spectra of tetrazepinones **10** and **15** (Fig. 1) which showed peaks at -85 ppm for the pyridine ring nitrogen, at 38 ppm for N1 and -194 ppm for N-3. The assignment was based on literature values[1,11,15]. N5 was more shielded in **10** (263 ppm) than in **15** (251 ppm). This is due to the β effect which is known to be deshielding for the nitrogen nucleus[13]. The ^{15}N labelled N2 in **10**, **15** and **22** showed up as a quartet (^3J=2.7 Hz) at around 74 ppm (Fig. 2). This coupling between the methyl proton and the central nitrogen confirmed the presence of the CH_3NNN moiety[1,2].

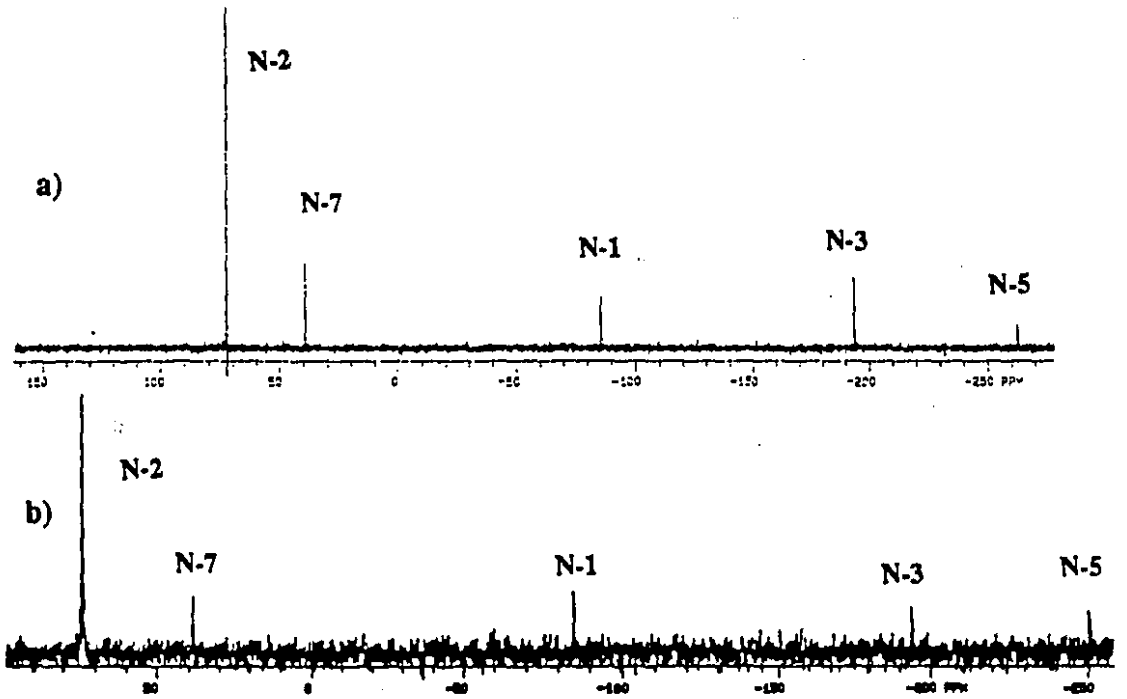


Fig 1. a)^{15} NMR spectrum of **10** in $CDCl_3$
b)^{15}N NMR spectrum of **15** in $CDCl_3$

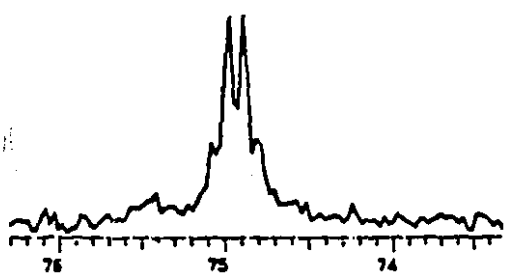


Fig. 2. ^{15}N NMR spectrum of the N2-label of **22**

We were able to run the natural abundance ^{15}N NMR spectra of tetrazepinones **10** and **15** (Fig. 1a, b) which showed peaks at -85 ppm for the pyridine ring nitrogen N-7, at 38 ppm for N-1 and -194 ppm for N-3. The assignment was based on literature values[1,11,15]. N5 was more shielded in **10** (-263 ppm) than in **15** (-251 ppm). This is due to the β effect which is known to be deshielding for the nitrogen nucleus[13]. The ^{15}N labelled N2 in **10**, **15** and **22** showed up as a quartet (^3J=2.7 Hz) at around 74 ppm (Fig. 2). This coupling between the methyl proton and the central nitrogen confirmed the presence of the CH_3NNN moiety[1,2].

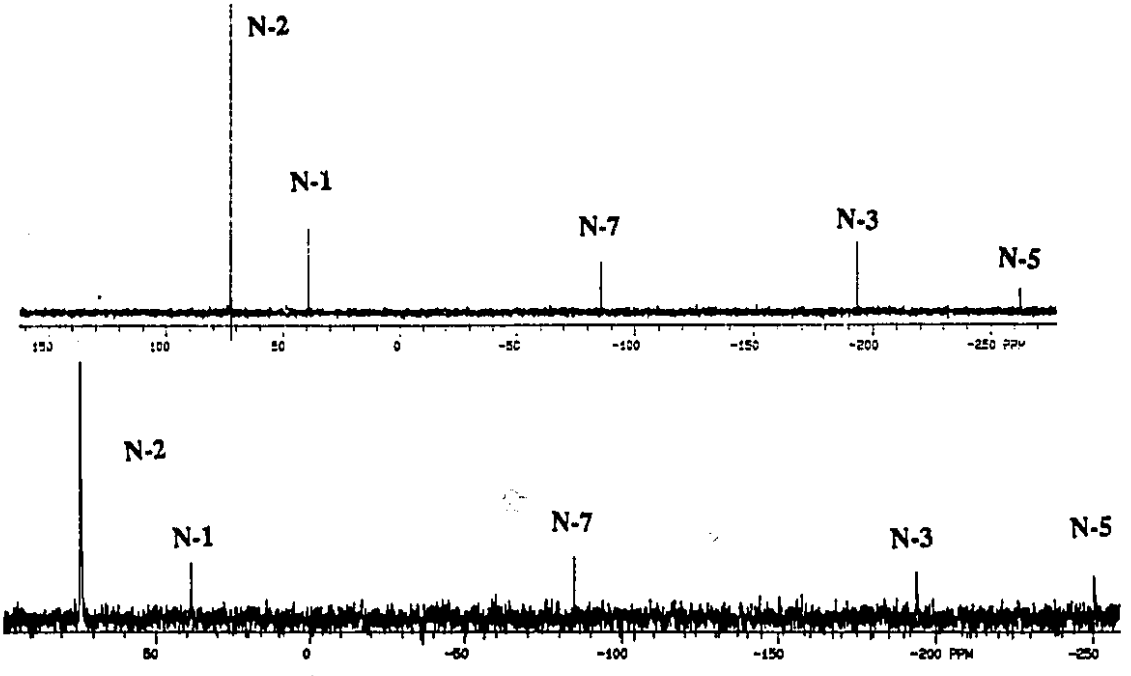


Fig 1. a)[15] NMR spectrum of **10** in $CDCl_3$. (The atom numbering corresponds to that of the ORTEP view, p.130)
b)^{15}N NMR spectrum of **15** in $CDCl_3$

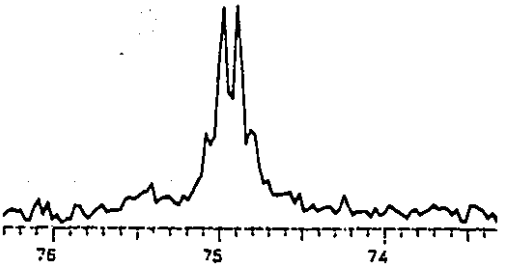


Fig. 2. ^{15}N NMR spectrum of the N2-label of **22**

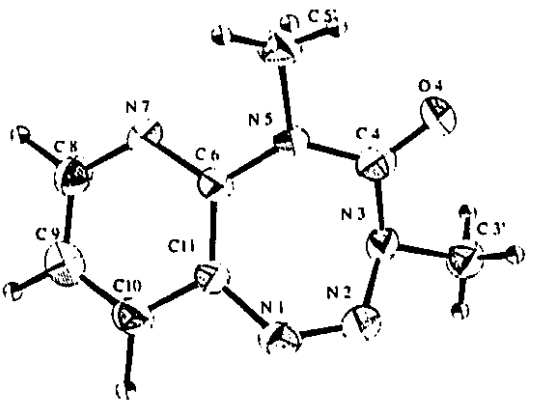


Fig. 3 ORTEP view[23] of the crystallographically determined molecular structure for compound **10** (50% probability ellipsoid). For clarity arbitrary thermal parameters were assigned to the hydrogen atoms.

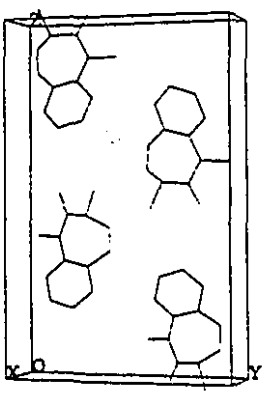


Fig. 4. Stereoview of the contents of the unit cell of crystals of **10**.

The conformation of **10** in the solid was determined by X-ray crystallography (Fig. 3, 4). Bond angles around N3 were 107.7° (5) (Table 2) for N2-N3-C1 and around 116°(5) for both N2-N3-C2 and C1-N3-C2. The torsion angle between the pyridine ring (C10-C11) and the diazo linkage N1=N2 was 145.8° (7), showing a marked deviation of N2 from coplanarity with the benzene ring. The bond distance between N2-N3 (1.450 (7) Å) (Table 1) suggests a single bond character of this linkage, and the pyramidal orientation of bonding about N3 indicates an sp^3 character of the latter nitrogen. These results are similar to those observed for the nitrobenzotetrazepinone **1** ($R=NO_2$)[2] except for the torsion angle between the diazo linkage (N1=N2) and the aromatic ring which

was slightly higher in **10** (141.5° in nitrobenzotetrazepinone **1**; 145.8° in **10**). These results also confirm that the triazene chain in tetrazepinones is only weakly conjugated with the aromatic ring or that the lone pair of N3 is only delocalized to a minor extent.

It is interesting to notice that, in contrast to their benzotetrazepinones congenors, the pyridotetrazepinones containing large groups at the 5-position were not prone to be converted to their corresponding triazoles by loosing their methyl isocyanate moiety.

Table 1. Non-hydrogen bond length (Å) for 10

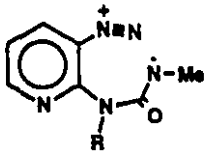
O(4)-C(4)	1.212(6)	C(6)-C(11)	1.386(7)
N(1)-N(2)	1.239(6)	C(8)-C(9)	1.387(8)
N(1)-C(11)	1.426(7)	C(9)-C(10)	1.373(8)
N(2)-N(3)	1.450(7)	C(10)-C(11)	1.391(7)
N(3)-C(3')	1.479(7)	C(3')-H(3'A)	0.99(5)
N(3)-C(4)	1.406(7)	N(7)-C(8)	1.336(7)
N(5)-C(4)	1.376(7)	N(7)-C(6)	1.334(7)
N(5)-C(5')	1.474(6)	N(5)-C(6)	1.413(7)

Table 2. Non-hydrogen bond angles (°) for 10

N(2)-N(3)-C(3')	107.7(4)	N(2)-N(1)-C(11)	122.5(5)
N(2)-N(3)-C(4)	116.6(5)	N(5)-C(6)-N(7)	115.7(4)
C(3')-N(3)-C(4)	113.1(4)	N(5)-C(6)-C(11)	122.1(5)
C(4)-N(5)-C(5')	116.0(4)	N(7)-C(6)-C(11)	121.9(5)
C(4)-N(5)-C(6)	121.9(5)	N(7)-C(8)-C(9)	124.3(5)
C(5')-N(5)-C(6)	119.2(4)	O(4)-C(4)-N(3)	122.2(5)
N(1)-C(11)-C(6)	125.2(5)	N(1)-N(2)-N(3)	118.6(4)
O(4)-C(4)-N(5)	121.8(5)	N(1)-C(11)-C(10)	115.2(5)
N(3)-C(4)-N(5)	116.0(4)	C(6)-C(11)-C(10)	119.1(5)
C(6)-N(7)-C(8)	117.9(4)	C(8)-C(9)-C(10)	117.0(5)
O(4)-C(4)-N(3)	122.2(5)	N(1)-C(11)-C(10)	115.2(5)
N(3)-C(4)-N(5)	116.0(4)	C(6)-C(11)-C(10)	119.1(5)
C(9)-C(10)-C(11)	119.6(5)	N(1)-C(11)-C(6)	125.2(5)

(No pyridotriazole peak was observed in the spectrum of **15** which was run overnight at room temperature). Since this mode of decomposition may occur via a diazonium intermediate of type **23** , the electron deficiency of N5 in pyridotetrazepinones may retard the cyclization to benzotriazole. Also, the electron-withdrawing effect of the

pyridine ring would destabilize a positive charge development on N1 and consequently retard the formation of the diazonium species of type **23**. This study conclusively shows that a pyridine moiety exerts a stabilizing effect on the 1,2,3,5-tetrazepinone ring system.



23

Tetrazepinone **10** was found to show interesting antitumour activity against a variety of human cancer cell lines. Detailed biological evaluation of tetrazepinones will be reported elsewhere.

EXPERIMENTAL

Melting points were measured on a Gallenkamp block and are uncorrected. Thin-layer and flash chromatography were performed on silica gel 60 F_{254} aluminum plates and Merck Silica Gel 60 (230-400 mesh) respectively. ^{1}H NMR spectra were recorded on a Varian XL-200 at 200 MHz. ^{13}C NMR spectra were obtained at 75.40 MHz on a Varian XL-300. All ^{1}H NMR spectra were run in $CDCl_3$ or in DMSO-d_6 and chemical shifts are reported downfield from TMS. J values are in Hz. Low and high resolution mass spectra were recorded on a Kratos MS25RFA, HP5984A, or an LKB9000 spectrometer. All compounds were shown to be homogeneous by TLC and high-field NMR, or to have a purity of > 95% by elemental analysis.

^{15}N NMR spectra were taken at 30.40 MHz on a Varian XL-300 and chemical shifts are reported upfield from nitromethane, which was used as external standard. The 90^{o} pulse width was 18 μs and the pulse interval was set at 3s. The temperature of the

probe was around 20° C for the natural abundance spectra. Spectra were obtained after 100 scans for the ^{15}N enriched compounds when sample concentration were around 0.10 M in $CDCl_3$ (gated coupled) and after about 9000 scans for natural abundance spectra at concentrations around 0.5 M.

All reactions were monitored by thin layer chromatography (TLC).

2-Amino-3-(ethoxycarbonylamino)-pyridine (3a)

To a solution of 2,3-diaminopyridine (3 g, 27 mmol) in tetrahydrofuran (THF) (100 mL) was added dropwise 1 mL (0.33 eq) of ethyl chloroformate. The solution was stirred for 30 min and 40% aqueous sodium carbonate (8 mL) was added. When the gas evolution ceased (after 40 min) the mixture was filtered and the THF evaporated. Water (25 mL) was added and the resulting brown mixture extracted with methylene dichloride. The organic layer was concentrated under vacuo and chromatographed on a silica gel column (5% methanol in ethyl acetate) to give 1.2 g (66%) of a clear oil that solidified on standing, m. p. 78° C . 200 MHz ^1H NMR ($CDCl_3$) δ: 7.85 (d, 1H, J=8 , H-6), 7.60 (d, 1H, J=4 , H-4), 6.80 (s, 1H, N*H*COO), 6.65 (dd, 1H, J= 4, J=8 , H-5), 4.75 (br s, 2H, NH_2), 4.20 (q, 2H, J=8 , C*H*$_2$CH$_3$), 1.25 (t, 3H, J=8, CH$_2$C*H*$_3$).

3-(N-methyl)-2,3-diaminopyridine (3b).

A solution of **3a** (1g, 5.52 mmol) in THF (10 mL) was added dropwise to a suspension of lithium aluminium hydride (0.8 g) in 10 mL of the same solvent. The temperarure was kept at around 45° C during the addition and the mixture was stirred for 6 hrs at room temperature. The excess of lithium aluminium hydride was quenched with the dropwise addition of 10% aqueous sodium hydroxide (10 mL) at 0° C and the solid residue that formed was filtered. The solvents were evaporated and water (20 mL) was added to give a cloudy solution which was extracted with methylene dichloride. The organic layer was

separated, dried over magnesium sulfate and evaporated under vacuo to give **3b** (300 mg, 46%) as a clear oil, TLC (20% methanol in ethyl acetate), Rf=0.4. 200 MHz ^1H NMR ($CDCl_3$) δ: 7.56 (d, 1H, J=4, H-6), 6.73 (m, 2H, H-4, H-5), 4.19 (br, 2H, NH_2), 3.30 (br, 1H, N*H*CH_3), 2.81 (d, 3H, J= 5.2, NHC*H*$_3$)

N-(2-amino-3-pyridyl)-N,N'-dimethylurea (3c).

To a solution of 3-N-methyl-2,3-diaminopyridine **3b** (300 mg, 2.5 mmol) in methylene dichloride (20 mL) was added dropwise methyl isocyanate (0.2 mL, 1eq). The mixture was kept for 16 hrs and the solvent was evaporated to give **3c** as a white solid in quantitative yield, m. p. 100° C. IR ($CDCl_3$) ν (cm-1): 3400 (NH, NH_2), 3000 (CH), 1662 (C=O); 200 MHz ^1H NMR ($CDCl_3$) δ: 7.97 (d, 1H, J=4.5, H-6), 7.28 (d, 1H, J=7.62, H-4), 6.63 (dd, 1H, J=4.5, J=7.6, H-5), 5.08 (br s, 2H, NH_2), 4.01 (br q, 1H, CON*H*CH_3), 3.1 (s, 3H, PyrNC*H*$_3$CO), 2.68 (d, 3H, J=4.7, CO NHC*H*$_3$); 75.3 MHz ^{13}C NMR ($CDCl_3$) δ: 159.11 (s), 157.58 (s), 149.24 (d), 138.42 (d), 123.98 (s), 115.73 (d), 36.68 (q), 28.84 (d); CIMS (*iso*-butane) *m/z* (relative intensity) 181 (MH^+, 100), 149 (MH^+-32, 25), 124 (MH^+-57, 47).

N-(2-amino-3-pyridyl)-N'-methyl-N'-nitrosourea (5). To a solution of 2,3-diaminopyridine **3b** (1 g, 2.5 mmol) in THF (20 mL) was added dropwise methyl isocyanate (0.6 mL, 1eq) . The precipitate that formed after 30 min was filtered to give **3d** as a white powder in quantitative yield, m. p. 120° C. 200 MHz ^1H NMR ($CDCl_3$) δ: 7.67 (overlap of d and s, 3H, H-6, H-4, PyrN*H*CO), 6.63 (dd, 1H, J=4.5, J=7.6, H-6), 6.01 (br q, 1H, CON*H*CH_3), 5.65 (br s, 2H, NH_2), 2.68 (d, 3H, J=4.7, CONHC*H*$_3$); Urea **3d** (500 mg) was dissolved in a concentrated HCl (5 mL) and [6%^{15}N $NaNO_2$ (200 mg in 2 mL of water) was added dropwise. The white precipitate that formed was filtered and dried under vacuum at room temperature (500mg, 81%): white powder, m. p. 80° (dec). The free base could be obtained by redissolving the powder in 5% sodium

carbonate, followed by extraction. The ^1H NMR was run immediately after evaporating the solvent. 200 MHz ^1H NMR ($CDCl_3$) δ: 8.1 (d, J=8, H-6), 7.7 (d, 1H, J=8, H-4), 6.8 (dd, J=5, J=8, H-5), 4.7 (brs, 2H, NH_2), 3.3 (s, 3H, $ONNCH_3$); 30.4 MHz ^{15}N NMR (5.HCl in D_2O) δ: 184 (N2 label). Anal. calcd for $C_7H_8N_5OCl$. $0.5H_2O$: C, 34.93; H, 4.99; N, 29.1. Found: C, 35.01; H, 4.99; N, 28.82

2-(N-methylamino)-3-nitro-pyridine (7a).

A suspension of 2-chloro-3-nitro-pyridine (2.4 g) in 40% aqueous methylamine (20 mL) was heated to 70° C until all solids dissolved. The solution was kept at room temperature for 30 min and the yellow crystals that formed (2g, 88%) were filtered and dried under vacuum, m. p. 50° C. 200 MHz ^1H NMR ($CDCl_3$) δ: 8.4 (overlap of d and s, 2H, H-4, H-6), 8.2 (br s, 1H, N*H*CH_3), 6.63 (dd, J=4.5, J=8.2, H-5), 3.2 (d, 1H, J=5.01, NHC*H*$_3$)

2-(N-methylamino)-3-(N-benzyloxycarbonylamino)-pyridine (8a).

A solution of 2-(N-methylamino)-3-nitro-pyridine **7a** (1.8g) and 10% Pd-C (500 mg) in methanol (25 mL) was hydrogenated at 2 atm . The catalyst was filtered and the solvent evaporated to give **7b** as a brown oil in quantitative yield. 200 MHz ^1H NMR ($CDCl_3$) δ: 7.7 (d, 1H, J=5, H-6), 6.81 (d, 1H, J=7.3, H-4), 6.5 (dd, J=5, J=7.3, H-5), 4.1 (br s, 1H, *H*NCH_3), 3.2 (br s, 2H, NH_2), 3.0 (s, 3H, NHC*H*$_3$). To a stirred solution of 2-N-methyl-2,3-diamino pyridine (1.6 g, 13 mmol), 40 % aqueous sodium carbonate (5 mL) was added dropwise benzyl chloroformate (1 mL) . The mixture was stirred for 30 min and extracted with water (40 mL). The organic layer was separated and dried over magnesium sulfate and evaporated to give **8a** as a brown oil, which was sufficiently pure to be used for further reactions. 200 MHz ^1H NMR ($CDCl_3$) δ: 8.01 (d, 1H, J=5.0 , H-5), 7.4 (overlap of s, 6H, Ar, H-4), 6.57 (dd, 1H, J=5, J=7.6, H-5), 6.2 (s, 1H, N*H*COOBn) , 5.17 (s, 2H, CH_2Ar), 2.95 (d, 3H, J=4.6, NHC*H*$_3$))

2-(N,N'-dimethyl ureido)-3-(N-benzyloxycarbonylamino)-pyridine (8b).

A solution of the carbamate **8a** (1.6 g, 6.2 mmol) in methylene dichloride (25 mL) was treated with methyl isocyanate (0.3 mL) at room temperature. The mixture was kept overnight and evaporated under reduced pressure. The resulting clear oil was purified on a silica gel column (5% methanol in methylene dichloride) to give **8b** (1.6 g, 82.5%) as a white powder, m. p. 145-147° C. 200 MHz ^{1}H NMR ($CDCl_3$) δ: 8.4 (d, 1H, J=8.2, H-5), 8.1 (d, 1H, J=4.7, H-6), 7.4 (br s, 6H, Ar, N*H*COOBn), 7.2 (dd, 1H, J=4.7, J=8, H-5), 5.2 (s, 2H, OCH_2Ar), 4.6 (br s, 1H, CON*H*CH_3), 3.2 (s, 3H, PyrN(CH_3)CO), 2.70 (d, 3H, J=4.8, CONHCH_3). Anal. calcd for $C_{16}H_{18}N_4O_3$: C, 61.13; H, 5.77; N, 17.33. Found: C, 61.53; H, 5.83; N, 18.06

N-(3-amino-2-pyridyl)-N,N'-dimethylurea (9).

Urea **8b** (1.2 g) was dissolved in methanol (20 mL) containing 10% Pd-C (200 mg) . The mixture was kept under hydrogen for 4 hrs at 3 atm. The catalyst was filtered and the solvent evaporated to give **9** as a clear oil in quantitative yield. IR ($CDCl_3$) ν (cm^{-1}): 3400 (NH, NH_2), 1659 (C=O); 200 MHz ^{1}H NMR ($CDCl_3$) δ: 7.93 (d, 1H, J=4, H-6), 7.09 (overlap of d, 1H, H-4, H-5), 4.7 (br s, 1H, CON*H*CH_3), 3.9 (br s, 2H, NH_2), 3.2 (s, 3H, PyrN(CH_3)CO-), 2.7 (d, 3H, J=4.8, 1H, CONHCH_3); 75.3 MHz ^{13}C NMR ($CDCl_3$) δ: 159.19 (s), 143.04 (s), 140.31 (s), 140.02 (d), 125.61 (d), 125.24 (d), 35.41 (q), 28.80 (q); CIMS (*iso*-butane) *m/z* 181 (MH^+, 83), 124 (MH^+-57, 100)

3,5-Dimethyl-pyrido-*3H*-1,2,3,5-tetrazepin-4(*5H*)-one (10).

Urea **9** (1 g, 3.9 mmol) was dissolved in 2N HCl (15 mL) and 27% [6% ^{15}N] $NaNO_2$ (10 mL) was added dropwise at 0° C. After 30 min, the solution was extracted three times with 25 mL portions of methylene dichloride. The aqueous layer was removed and its pH adjusted to 6 with sodium carbonate, after which it was reextracted three times with 25 mL portions of methylene dichloride. The solvent was dried over anhydrous

potassium carbonate and evaporated to give **10** (780 mg, 74.2 %) as a white powder: needles from chloroform, m. p. 90° C. IR ν (cm^{-1}): 3027 (CH), 1701.33 (C=O); 200 MHz ^1H NMR ($CDCl_3$) δ: 8.4 (d, 1H, J=4.73, H-6), 7.8 (d, 1H, J=8, H-4), 7.2 (dd, 1H, J=4.7, J=8, H-5), 3.4 (s, 3H, $N{=}NNCH_3$), 3.3 (s, 3H, $N(CH_3)CO$); 75.4 MHz ($CDCl_3$) δ: 160.15 (s), 151.70 (d), 149.6 (s), 137.3 (s), 137.2 (d), 120.8 (d), 38.3 (q), 34.5 (q); 30.4 MHz ^{15}N NMR ($CDCl_3$) δ: 72.3 (N-2 label), 39.1 (N-1), -85 (N-9), -194 (N-3), -263 (N-5); CIMS (NH_3) *m/z* 192 (MH^+, 88), 164 (MH^+-N_2, 100), 135 (MH^+-MeNCO, 3). HRMS exact mass calcd. for $C_8H_{10}N_5O$ (MH^+): 192.08853; Found: 192.08850; Anal. calcd for $C_8H_9N_5O$: C, 50.26; H, 4.71; N 36.65. Found: C, 50.41, H, 4.62; N, 36.46.

2-N-[1'(4'-hydroxybutyl]-3-nitro-2-aminopyridine (11a).

To a solution of 2-chloro-3-nitropyridine (5 g, 31.6 mmol) in methanol (10 mL) were added 4-aminobutanol (2 mL), sodium bicarbonate (3.3 g) and water (10 mL). The mixture was heated at reflux overnight and the solvents evaporated to give a dark residue. Methylene dichloride (20 mL) was added and the insoluble solids filtered. The filtrate was concentrated under reduced pressure and chromatographed on silica gel to give **11a** (4.1 g , 60.2 %) as a yellow oil. 200 MHz ^1H NMR ($CDCl_3$) δ: 8.6 (d, J=4.76, H-6), 8.18 (overlap of d and br s, 2H, NH), 7.44 (dd, J=4.76, J=8, H-5), 3.6 (overlap of t, 4H, CH_2OH, CH_2NH), 2.4 (br s, 1H, OH) , 1.7 (overlap of m, 4H, CH_2CH_2); Anal. calcd for $C_9H_{13}N_3O_3$: C, 51.17; H, 6.20; N, 19.90. Found: C, 50.85; H, 6.35; N, 19.64.

2-[N-1'-(4'-tetrahydropyranyloxy)butyl]-2,3-diaminopyridine (12a).

To a solution of **11a** (4g, 18.6 mmol) in methylene dichloride (100 mL) was added 3,4-2*H*-dihydropyrane (2mL) and a catalytic amount of p-toluene sulfonic acid (85 mg). The methylene dichloride solution was kept overnight and washed with 5 % aqueous sodium carbonate, dried and evaporated to give **12a** as a yellow oil which was sufficiently pure to be used for further steps; 200 MHz ^1H NMR ($CDCl_3$) δ:7.68 (d, 1H, J=4, H-6), 6.8 (d, 1H,

J=4, H-6), 6.8 (d, 1H, J=6, H-4), 6.49 (dd, J=6, J=4, H-5), 4.56 (s, 1H, OCHO), 3.66-3.3 (m, 6H, CH_2Os, CH_2N), 1.8-1.4 (br m, 10H, CH_2s)

A solution of **11b** (4.5 g) and 10% Pd-C (100 mg) in methanol was hydrogenated at 2 atm for 1 hr. The catalyst was filtered and the solvent evaporated to give **12b** as a brown oil . 200 MHz ($CDCl_3$) δ: 7.69 (d, 1H, J=5.3, H-6), 6.83 (dd, J=5.3, J=7.3, H-4), 6.51 (dd, J=5.3, J=7.3, H-5), 4.5 (1H, OCHO), 3.8-3.2 (overlap of m, 4H, CH_2O, CH_2N), 1.8-1.2 (overlap of m, 4H, CH_2s, NH_2).

N-(3-benzyloxycarbonylamino-2-pyridyl)-N'-[1'-(4'-tetrahydropyranyloxy)butyl]-N'-methylurea (13).

Amine **12a** (2 g, 7.49 mmol) was treated with benzyl chloroformate (0.6 mL, 1eq) in methylene dichloride (15 mL). Saturated aqueous sodium bicarbonate was added and the mixture stirred at room temperature for 1 hr. The methylene dichloride layer was dried over magnesium sulfate and evaporated to give **12b** as a pure brown oil (TLC, 30% hexane in ethyl acetate, Rf=0.2) which was immediately redissolved in pyridine (15 mL) and treated overnight with methyl isocyanate (1.2 mL). The pyridine was azeotroped with toluene and the resulting dark residue chromatographed on silica gel (5% methanol in methylene dichloride) to give **13** (2 g, 62 %) as a brown powder, m. p. 62 °C. 200 MHz ^1H NMR $CDCl_3$ δ: 8.45 (d, 1H, J=8.0, H-6), 8.20 (d, 1H, J=4.74, H-4), 7.37 (s, 5H, Ar), 7.26 (overlap of d and s, 1H, J=8.2, H-5, N*H*COOBn), 5.18 (s, 2H, CH_2, Ar), 4.7 (s, 1H, OCHO) ,4.48 (br q, 1H, CON*H*CH_3), 3.66 (m, 4H, CH_2N, CH_2O), 3.4 (m, 2H, CH_2O), 2.7 (d, 3H, J=5, 3H, CONHC*H*$_3$), 1.8-1.4 (overlap of m, 10H, CH_2s). Anal calcd for $C_{24}H_{32}N_4O_5$: C, 63.14; H, 7.07; N, 12.27. Found: C 62.87, H, 7.04, N 12.44

A solution of urea **13** (1.5 g) and 10% Pd-C (250 mg) in methanol (10 mL) was hydrogenated at 2 atm for 1 hr. The solution was filtered and evaporated to give **14** as a clear oil in quantitative yield. 200 MHz ^{1}H NMR ($CDCl_3$) δ: 7.9 (d, 1H, J=5, H-5), 7.08 (overlap of d, 2H, H-4, H-6), 4.6 (br q, 1H, CH_3NHCO), 4.49 (s, 1H, OCHO), 3.96 (br s, 2H, NH_2), 3.69 (overlap of m, 4H, CH_2), 3.4 (m, 2H, CH_2), 2.71 (d, 3H, J=4.7 Hz, $CONHCH_3$), 1.5 (overlap of m, 10H, CH_2) ; 75.3 MHz ^{13}C NMR ($CDCl_3$) δ: 158.74 , 143 , 140, 140.23, 125.39, 125.23, 100.10, 68.5, 63.7, 54.73, 47.92, 32.19, 28.74, 28.37, 27.11, 26.78, 20.93; CIMS *m/z* 323 (MH^+, 23), 239 (MH^+-dihydropyran, 100), 182 (MH^+-dihyropyran-MeNCO, 22.3).

3-Methyl-5-[1'-(4'-hydroxybutyl)]-*3H*-pyrido-1,2,3,5-tetrazepin-4(*5H*)-one (15).

Urea **14** (700 mg, 2.1 mmol) was dissolved in 2N HCl (15 mL) and [6% ^{15}N] $Na^{15}NO_2$ was added dropwise at 0° C. The solution was extracted three times with 25 mL portions of methylene dichloride. The aqueous layer was separated and its pH adjusted to 8 with sodium carbonate, after which it was reextracted three times with 25 mL portions of methylene dichloride. The solvent was evaporated and the resulting brown oil was purified on a silica gel column (5% hexane in ethyl acetate) to give **15** as a clear oil (300 mg , 74.1 %) ; ν (cm^{-1}): 3400 (OH), 3000 (CH), 1696 (C=O); 200 MHz ^{1}H NMR ($CDCl_3$) δ: 8.4 (d, J=4.73, H-6), 7.8 (d, J=8, H-4), 7.2 (dd, 1H, J=4.7, J=8, H-5) 4.0 (t, 2H, J=6.92, $-CH_2CH_2N$), 3.6 (t, 2H, J=6.92, CH_2CH_2O), 3.4 (s, 3H, $N=NNCH_3$), 1.8 (quintet, 3H, J=6.46, CH_2, OH), 1.6 (m, 2H, CH_2); 75.4 MHz ^{13}C NMR ($CDCl_3$) δ: 159.37 (s), 151.74 (d), 148.89 (s), 138.13 (s), 137.47 (d), 121.1 (d), 63.38 (t), 46.80 (t), 38.39 (q), 29.98 (t), 24.50 (t); 30.4 MHz ^{15}N NMR ($CDCl_3$) δ: 73.5 (q, J=2.8, N-2 label), 38.6 (N-1), -85 (N-9), -194 (N-3), -250.8 (N-5); CIMS (NH_3) *m/z* 250 (MH^+, 35.61), 222 (MH^+-N_2, 100), 193 (MH^+-MeNCO, 8); HRMS calcd for $C_{11}H_{15}N_5O_2$ (MH^+): 250.13034. Found: 250.13040.

(±)Cis-1-amino-4 -hydroxymethyl-cyclopent-2-ene (17).

A solution of ± 2-azabicyclo[2.2.1]hept-5-en-3-one (2g) in dry methanol (20 mL) ssaturated with dry HCl at 0°C was kept overnight at room temperature under nitrogen. Evaporation of the solvent gave the aminoester **16** as an oil in quantitative yield. ^1H NMR data identical to published values.

To a suspension of the aminoester hydrochloride (1g, 5.6 mmol) in dry THF (50 mL) was added lithium aluminium hydride (400 mg in 10 ml of THF) at -80° C. The temperature of the mixture was then brought to room temperature and it was stirred for 5 hrs. The excess of lithium aluminium hydride was quenched with 10 % aqueous sodium hydroxide and the solution was filtered. Evaporation of the solvents gave **17** (0.5g, 83%) as a pale brown oil which was sufficiently pure to be used for further steps. 200 MHz ^1H NMR ($CDCl_3$) δ: 5.78 (s, 2H, HC=CH), 3.96 (dd, 1H, J=2, J=7, H-1), 3.55 (d, J=3.4, CH_2OH), 2.86 (m, 1H, H-4), 2.65 (br s, 2H, NH_2), 2.87 (m, 1H, C*H*H'), 1.37-1.30 (m, 1H, CH*H*').

(±) cis-[4-[(3-nitro-2-pyridyl]-amino]-2-cyclopentenyl]carbinol (18a).

To a solution of amine **17** (1.5g, 4.48 mmol) in tetrahydrofuran (25 mL) was added, 2-chloro-3-nitro-pyridine (0.73g) and pyridine (0.6 mL) . This mixture was heated at reflux overnight. The solvents were evaporated and the residue chromatographed on a silica gel column (30% hexane in ethyl acetate) to give **18a** as a yellow oil (500 mg, 46%) that solidified on standing, m. p. 85° C. 200 MHz ^1H NMR ($CDCl_3$) δ: 8.42 (d, 2H, J=6.56, H-6, H-4), 6.6 (dd, 1H, J=4, J=6.56, , H-5), 5.92 (s, 2H, HC=CH), 5.45 (m, 1H, H-1') 3.69 (t, 2H, J=5, CH_2OH), 2.92 (m, 1H, H'-4) 2.8 (m, 1H, C*H*H'), 1.6 (m, 1H, C*H*H'). CIMS *m/z* 236 (MH^+, 81.2) 204 (MH^+- 32, 81.9), 140 (MH^+-96, 100) . Anal. calcd for $C_{11}H_{15}N_3O_3$: C, 56.11; H, 5.52; N, 17.85. Found C, 56.43; H, 5.60, N, 18.56;

(±)Cis-3-tetrahydropyranyloxymethyl-N-(3-amino-2-pyridyl)-cyclopentylamine (19a).

Compound **18b** was prepared as described for **11b**. From **18a** (400 mg) 3,4-*2H*-dihydropyrane (2 mL), *p*-toluene sulfonic acid (25 mg) in methylene dichloride (10 mL) overnight. The solvent was evaporated to give **18b** as a yellow oil in quantitative yield. 200 MHz ^{1}H NMR ($CDCl_3$) δ: 8.43 (overlap of d, J=6.33, H-6, H-4), 8.22 (br s, 1H, PyrNH), 6.65 (dd, J=4, J=7.5, H-6), 6.1-5.8 (m, 2H, HC=CH), 5.4 (m, 1H, H-1'), 4.6 (s, 1H, OCHO), 3.8-3.3 (m, 4H, CH_2Os), 3.0 (br m, 1H, H'-4), 2.8 (m, C*H*H'), 1.8-1.4 (overlap of m, 6H, CH_2)

Compound **18b** (500 mg) was dissolved in methanol (20 mL) and hydrogenated in the presence of 10 % Pd-C (125 mg) at 3 atm. The catalyst was filtered and the solvent was evaporated to give **19a** (400 mg) as a brown solid, m. p. 98° C. 200 MHz ^{1}H NMR ($CDCl_3$) δ: 7.71 (d, 1H, J=5.08, H-6), 6.77 (dd, J=7.3, H-4), 6.41 (dd, J=5.08, J=7.3, H-5), 4.6 (s, 1H, OCHO), 4.25 (overlap of m and br s, 2H, NH, H-1' D_2O exchangeable), 3.8-3.3 (m, 4H, CH_2Os), 3.15 (br s, 2H, NH_2, D_2O exchangeable), 2.45-1.01 (m, 12H, CH_2). Anal calcd for $C_{16}H_{23}N_3O_2$: C 65.95, H 8.65, N 14.42; Found C 66.10, H 8.63, N 14.44

(±)Cis-4-tetrahydropyranyloxymethyl-N-(3-benzyloxycarbonylamino-2-pyridyl)-cyclopentylamine (20)

As described for **11d.** Amine **19a** (372 mg, 1.2 mmol) was treated with benzyl chloroformate (0.16 mL, 1eq) and 40% aqueous sodium carbonate (5 mL) as a suspension in methylene dichloride. The mixture was stirred for 30 min and the methylene dichloride layer was washed with water (20 mL), dried over magnesium sulfate and evaporated to give **19b** as a pure oil in quantitative yield, TLC (30% hexane in ethyl acetate), Rf=0.7. 200 MHz ^{1}H NMR ($CDCl_3$) δ: 7.82 (overlap of d and s, 2H,

J=5.6, H-6, N*H*COOBn), 7.05-7.41 (overlap of m, 6H, Ar, H-4), 6.6 (dd, 1H, J=5.6, J=7.6, H-5), 5.2 (s, 2H, C*H*$_2$Ar), 4.5 (s, 1H, HCHO), 4.4 (br m, 1H, H-1'), 3.8-3.3 (m, 4H, CH_2Os), 2.45-1.2 (br m, 13H, CH_2)

A solution of **19b** (400 mg, 0.9 mmol) in pyridine (10 mL) and methyl isocyanate (0.1 mL, 2eq) was kept overnight. The pyridine was azeotroped with toluene and the resulting brown residue was purified on a silica gel column (5% methanol in methylene dichloride to give **20** (420 mg) as a white powder, m.p. 90° C. 200 MHz ($CDCl_3$) δ: 8.6 (d, 1H, J=7, H-4), 8.2 (s, 1H, J=4, H-6), 7.2 (overlap of m, 6H, H-5, Ar), 5.2 (s, 2H, C*H*$_2$Ar), 4.7 (m, 1H, H-1'), 4.4 (s, 1H, OCHO), 4.02-3.1 (m, 5H, CH_2Os, CH), 2.7 (d, 3H, CONHC*H*$_3$), 2.2-1.0 (br s, 13H, CH_2s). Anal calcd for $C_{26}H_{34}N_4O_5$: C, 64.71; H, 7.10; N, 11.61. Found: C, 64.85; H, 7.18; N, 11.71.

N-[(±)Cis-4'-tetrahydropyranyloxymethyl-1'-cyclopentyl)]-N-(3-amino-2-pyridyl)-N'-methylurea (21)

Urea **20** (420 mg) in methanol was hydrogenated in the presence of 10% Pd-C (100 mg) and the solution was filtered and evaporated to give **21** as clear oil in quantitative yield. IR ($CDCl_3$) ν (cm^{-1}) :3400 (NH_2, NH), 3000 (CH), 1636 (C=O); 200 MHz ^{1}H NMR ($CDCl_3$) δ: 7.9 (d, 1H, J=4, H-6), 7.05 (overlap of d and t, 2H, H-4, H-5), 4.7 (overlap of s and m, 2H, NH, H-1'), 4.05-3.25 (overlap of m, 6H, CH_2Os, NH_2), 2.7 (d, 3H, J=5, CONHC*H*$_3$), 2.4-1.4 (br m, 13H, CH_2s)

Cis-4'-[3,5-Dimethyl-pyrido-(*3H*)-1,2,3,5-tetrazepin-4-(*5H*)-one-5-yl]-cyclopentylcarbinol (22).

Urea **21** (300 mg, 3.9 mmol) was dissolved in 2N HCl (10 mL) and diazotized with [99%] $Na^{15}NO_2$ at 0° C. The solution was extracted three times with 25 mL portions of methylene dichloride. The aqueous layer was separated and its pH adjusted to 8 with

sodium carbonate, after which it was reextracted three times with 25 mL portions of methylene dichloride. The solvent was evaporated and the resulting brown oil was purified on a silica gel column (5% hexane in ethyl acetate) to give **22** as a clear oil (135 mg, 63 %); IR ($CDCl_3$) ν (cm^{-1}) : 3400 (OH), 3000 (CH), 1696 (CO); 200 MHz ^1H NMR ($CDCl_3$) 8.4 (d, J=4.73, H-6), 7.8 (d, J=8, H-4), 7.2 (dd, 1H, J=4.7, J=8, H-5), 4.45 (quintet, 1H, J=5, H'-1), 3.8 (m, 2H, CH_2OH), 3.4 (d, J=2.8, 3H, $CH_3N^{15}N$), 2.4-1.2 (overlap of m, 8H, CH_2s, OH); 75.4 MHz ^{13}C NMR ($CDCl_3$) δ: 159.56 (s), 151.32 (d), 149.6 (s), 138.46 (s), 136.80 (d) 121.33 (d), 120.8 (d), 68.46 (t), 61.42 (d), 42.22 (d) 38.127 (q), 34.2 (t); 30.47 (t), 28.77 (t); 30.4 MHz ^{15}N NMR $CDCl_3$ δ: 72.3 (q, $^3J_{NH}$=2.8, N-2 label); CIMS (NH_3) *m/z* 277 ($MH^+(^{15}N)$, 9), 248 ($MH^+-^{15}N_2$, 95.0), 220 (MH^+-MeNCO, 79.5), 150 (MH^+-127, 100). This compound could not be obtained with a high degree of purity. Anal. calc. for $C_{13}H_{16}N_5O_2$: C, 56.7; H, 6.18 ; N, 25.45. Found: C, 57.06; H, 6.63; N, 22.63

X-ray Crystallography.- Crystals of compound **5e** were obtained from slow evaporation of methylene chloride.

Crystal data.- $C_8H_9N_5O$, M = 191.19. Orthorombic, *a* = 3.8868 (11), b = 12.2820 (24), c = 18.549 (3) , V=885.5 A^3 (3) (by least-squares refinement on diffractometer angles for 25 reflections with 2θ angle in the range of 20-25° angle) space group $P2_1/n$, Z=4, D_{calc} =1.434 mg $.m^{-3}$. Small colorless needles. Crystal dimensions: 0.45x0.10x0.05 mm.

Data Collection and Processing.- Rigaku AFC6S diffractometer, θ/2θ scan mode graphite monochromator, Mo-K_α radiation, temperature: 25 °C; 871 reflections measured 575 were unique giving 449 reflections with I>2σ(I). No correction was made for absorbtion.

Structure Analysis and Refinement.- All non-hydrogen atom positions from direct methods, using the TEXSAN crystallographic software package of Molecular Corporation . All hydrogen-atom positions from a Fourier difference map. All positional and thermal parameters (anisotropic) and an extinction parameter were refined by full-matrix least square. Final R and Rw were 0.037, and 0.036 for 449 observed reflections and 93 variable parameters. The weighting scheme w=4Fo2/s2(Fo2) obtained from counting statistics gave satisfactory agreement analyses. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.15 and -0.17 eA^{-3}, respectively. Neutral atom scattering factors were taken from Cromer and Waber[20]. Anomalous dispersion effects were included in Fcalc; the values for $\Delta f'$ and $\Delta f''$ were those of Cromer[22]. Figures were drawn with ORTEPII[23].

ACKNOWLEDGMENT:

We thank the Natural Sciences and Engineering Research Council of Canada and Fonds FCAR, Province de Quebec, for financial support. Dr Françoise Sauriol for assistance and advices in running NMR spectra, M. Nadim Saadé of this department, Dr Orval Mamer of the Biomedical Mass Spectrometry Unit, McGill University, for running mass spectra. We are also grateful to Sorin Damian and Zoe Damian for performing antitumour tests.

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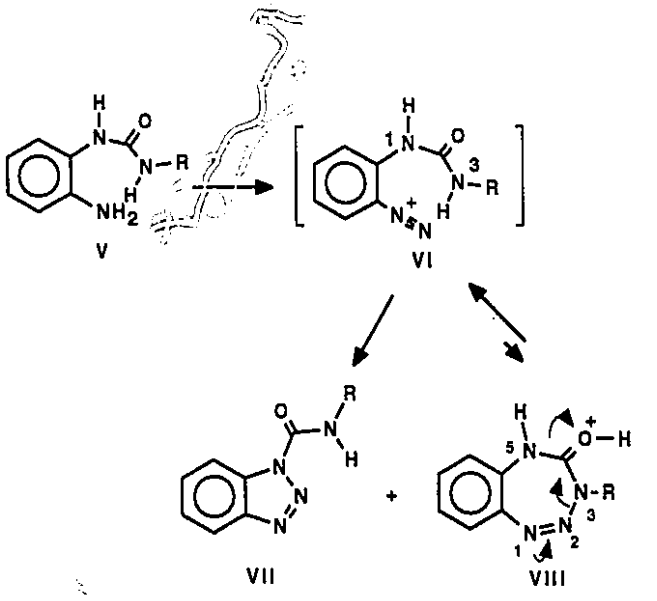
GENERAL CONCLUSION

The finding that tetrazepinones are subject to rapid acid catalyzed hydrolysis accounts for the failure of their attempted synthesis (chapter 2) by the spontaneous cyclization of the diazonium ureas **VI** at acidic pHs. We found that the concentration of the acyclic diazonium ureas alkylated at N-1 increased with increasing acidity of the medium. This means that at acidic pHs, diazonium ureas unsubstituted at N1 do not tend to form tetrazepinones. Consequently, they cyclize to stable benzotriazoles of type **VII** as outlined below. The alkylation of N1 stabilizes the diazonium urea enough to allow the adjustment of the pH of the solution and since at neutral or basic pHs, the equilibrium **VI-VIII** lies toward the formation of the tetrazepinones, the latter could be isolated by extraction with an organic solvent or by simple filtration when they appeared as precipitates.



The conformation of tetrazepinones in the solid was found to present four fundamental structural features as confirmed by their X-ray structures: (a) planarity of N5CO moiety (b) pyramidal geometry of N3 (c) deviation of the N=N linkage from the plane of the aromatic ring (d) single bond character of the N2N3 linkage.

In solution, the pyramidal geometry of N3 was indirectly inferred from ^{15}N NMR which showed that n–π delocalization in the triazene chain of tetrazepinones occurs to a much lesser extent than in open-chain aryltriazenes which possess a planar N1=N2N3 linkage. We explained this observation by the fact that the electronwithdrawing effect of the carbonyl may decrease the availability of the lone pair of N3 for conjugation with the diazo linkage.

We have seen throughout this work that bulky substituents at the 5-position and electron-donating groups at the benzene ring destabilize the 1,2,3,5-tetrazepinone ring system. Conversely, when fused with a phenyl ring containing an electron-withdrawing group or a pyridine ring, tetrazepinones were found to be stable. More importantly, we could confer stability to a 5-hydroxypropylbenzotetrazepinone by introducing a nitro group *para* to the diazo linkage. We also observed that the incorporation of N5 to a third ring (tricyclic systems) gave rise to the formation of stable tetrazepinones.

Although the mechanism of the decomposition of tetrazepinones was not rigorously studied, we believe that their conversion to benzotriazole occurred via the acyclic diazonium urea intermediate. The existence of tetrazepinones as acyclic diazonium ureas may be favoured by electrondonating groups that can stabilize the diazonium ion by resonance conjugation or by bulky substituents since in this form, the ureido moiety is free to adopt the least sterically hindered conformations, e. g. conformations with a reasonable torsion angle between the substituent and the phenyl ring. We found by X-ray crystallography that the torsion angle between the N1-H bond and the plane of the aromatic ring in N-(2-aminophenyl)-N'-methylurea is 58.92°. For a stable N1-cyclopentylurea, for instance, this angle possibly needs to be higher. The preferential existence of tetrazepinones, containing electrondonating or bulky groups, as acyclic

diazonium urea accounts for their instability since the latter form may be prone to decompose to benzotriazole derivatives by losing a molecule of methyl isocyanate. Obviously, this destabilizing effect was not observed for tricyclic systems in which the N5 substituent is locked in a ring.

The cyclization of the diazonium urea zwitterion seems to be controlled by the reactivity of N1. The tetrazepinone bearing a methoxy group *para* to N1 substantially autodecomposes to benzotriazole in solution. Pyridotetrazepinones in which N1 is electrondeficient did not seem to be prone to decompose to their corresponding triazoles.

The decomposition of the benzotetrazepinones in alkaline solution gave benzotriazoles and dediazoniated products which may derive fom the diazonium urea that still exist at a low concentration at basic pHs. In polar solvents, nitrobenzotetrazepinones decompose mostly via the breaking of the C4N3 bond . The fact that this compound loses a CH_3NN moiety is an indirect proof that it is possibly an alkylating agent.

Bi- and tricyclic tetrazepinones were found to be antitumour active and the activity seemed to parallel their stability.

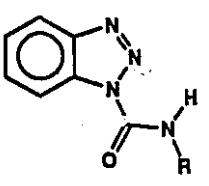
All these results lead to the final conclusion that the future development of tetrazepinones should be directed toward the synthesis of polycyclic or electron- deficient systems such as imidazo- or pyrazolotetrazepinones. *This is based on the fact that tricyclic and electron-deficient tetrazepinones are stable molecules.*

CONTRIBUTION TO KNOWLEDGE

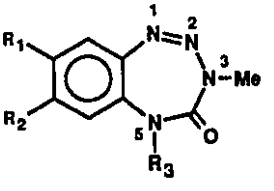
1. We have described the preferred conformations of N-(2-aminophenyl)-N'-methylureas in DMSO and shown that their diazotization gives benzotriazole derivatives of type **I**.

2. We have shown that the diazotization of N-(2-aminophenyl)-N'-dialkylurea gives tetrazepinones **II** following the neutralization of the reaction mixture.

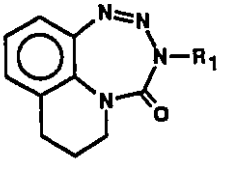
3. We have synthesized tricyclic system **III** and shown its conformation in the solid by X-ray diffraction.



I



II

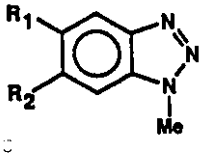


III

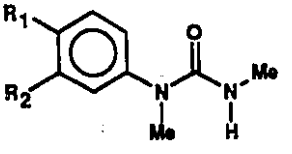
4. The tetrazepinones were shown to be in equilibrium with their diazonium urea precursor. The equilibrium lies toward the formation of tetrazepinones at neutral or basic pHs.

5. We have shown that the stability of bicyclic tetrazepinones **II** increases with increasing electronwithdrawing character of the substituents at the aromatic ring.

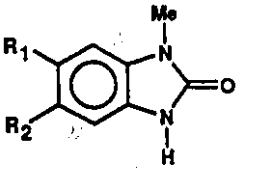
6. We have identified benzotriazoles **IV**, ureas **V** and benzimidazoles **VI** as decomposition products of benzotetrazepinones in base.



IV



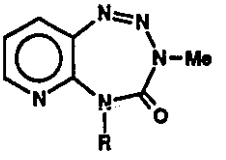
V



VI

7. We have shown that bulky substituents at N5 favour the conversion of benzotetrazepinones to their corresponding benzotriazoles.

8. We have synthesized and determined the X-ray structure of pyrido-tetrazepinones **VII** and developed synthetic methods for the synthesis of nucleoside mimics containing pyridotetrazepinone as a base.



VII

9. Bicyclic benzo- and pyridotetrazepinones, tricyclic tetrazepinones of type **II** were found to show interesting antitumour activity against human cancer cell lines as summarized in the appendix section (pp. 172 and 181).

Je crois que le XXe siècle aura été marqué par des progrès importants dans la connaissance et la compréhension de l'infiniment petit. La contribution du chimiste aura été inestimable. Grâce à son imagination et son instrumentation, il aura ouvert au monde une fenêtre diaphane sur l'infiniment petit. Les êtres les plus invisibles et les plus infimes sont souvent les plus menaçants pour l'existence humaine. C'est en ce sens que le chimiste, par son exploration continue de l'infiniment petit, aura été de ceux qui jouent le rôle de sentinelle pour l'humanité.

I believe that the XXth century will have been marked by important advances in the knowledge and understanding of the infinitesimal. The chemist will have given an invaluable contribution. Thanks to his imagination and his instrumentation, he will have opened to the world a diaphanous window on the infinitesimal. The most invisible and the most miniscule beings are often the most threatening for humankind. In that sense, by continuously exploring the infinitesimal, the chemist will have played the role of a sentinel for the world.

Bertrand J Jean-Claude

APPENDICES

APPENDIX I.-

STRUCTURE OF N-(2-AMINOPHENYL)-N'-METHYLUREA[a,b]

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McGill University,
Montreal, PQ, Canada H3A 2K6

[a] This paper was accepted for publication in *Acta Crystallogr. C*

[b] The X-ray structure was determined by Dr James F. Britten

ABSTRACT:

$C_8H_{11}N_3O$; M_r=165.19 monoclinic; $P2_1/n$; a=10.3997 (14), b=4.6395 (12), c=18.2211 (11) Å, β=100.994 (7)°; V=868.0 (2) $Å^2$; Z=4; D_x=1.2271 Mg/m^3; CuK_α (λ=1.54178 Å); graphite monochromator; μ=6.82 cm^{-1}; F(000)=352: T=293K; 1471 unique reflections; R=0.054; R_w=0.045 for 887 reflections with I>2σ(I). The ureido moiety is approximately planar and makes a dihedral angle of 58.92° (16) with the least square plane of the aromatic ring. The structure showed that the carbonyl is oriented toward the amino group and the distance between the ureido oxygen O(2) and N(4) was 2.975 (4) Å.

INTRODUCTION

An NMR study on the conformation of N-(o-aminophenyl)-N'-alkylureas has stimulated interest in determining the geometry of the title compound in the solid state. NOE difference spectroscopy results (Jean-Claude and Just, 1992) confirmed the existence in dimethyl sulfoxide (DMSO) of conformations in which the two protons of the ureido moiety are oriented *trans-trans* as to the carbonyl. This paper deals with the crystal structure of one of the members of this class of aromatic urea, the N'-(2-phenylamino)-N-methylurea.

EXPERIMENTAL*

The title compound was prepared according to the method described by Jean-Claude and Just (1991). Its recrystallization from slow evaporation of methanol gave coulourless needles, m. p. 170°C, one of which ($0.50x0.10x0.14mm^3$) was used for data collection at 293° K using the ω/2θ scanning mode. Data were collected on a

* This section has been written by Dr James F. Britten

Rigaku AFC6S diffractometer with graphite monochromated CuKα radiation; 1561 reflections (+h, min 0, max 10; +k, min 0, max 5; ±l, min -20, max +20) were collected with $3^{o}<2\theta<120^{o}$. Three standard reflections indicated crystal and electronic stability; 25 centered reflections ($61.6^{o}<2\theta<80^{o}$) were used to determine the unit cell. The data were reduced to 1471 unique reflections ($R_{int}=0.097$) with Lorentz polarization and absorption (azimuthal scan: transmission range 0.93-1.00) corrections applied; 887 reflections were considered observed ($I>2\sigma(I)$). The space group was determined to be $P2_1/n$.

The structure was solved by direct methods. The program SIR88 (Burla, Camalli, Cascarano, Giacovazzo, Polidari, Spagna and Viterbo, 1989) revealed all non-hydrogen atoms, which were refined anisotropically using the Texsan (1985) software package. Hydrogen atoms were found from a difference map synthesis, and were refined isotropically. The final cycle of full matrix, least-squares refinement, minimizing $\Sigma w\ (F_o-F_c)^2$ with a 6:1 reflection to parameter ratio, showed a maximum shift/error of 0.11. Maximum peak on a final difference map was +0.19 e/Å^3 , R=0.054, Rw=0.045, and S=2.40 with weights based on counting statistics. Scattering factors were taken from Cromer and Waber (1974) and anomalous dispersion corrections for the non-hydrogen atoms were from Cromer (1974).

DISCUSSION

The final atomic coordinates and equivalent isotropic temperature factors are given in Table 1*. The bond distances and angles are listed in Table 2 . The structure and labelling of the title compound are shown in Fig. 1, and a stereo packing diagram is given in Fig. 2.

The ureido moiety is planar (χ^2=3.696). The plane of the aromatic ring is defined by atoms C(1), C(2), C(3), C(4) C(5), C(6) and makes a dihedral angle of 58.92° (16) with the plane of the ureido system. This geometry suggests that the lone pair on N1 is presumably delocalized mainly through the N(1)-C(2') bond of the ureido group. It would consequently be less conjugated to the aromatic ring (N(1)-C(1) [1.414 (6) Å], N(1)-C(2') [1.356 (6) Å]).

The distance between N(4) and C(2') is 2.975 (4) Å and the bond angles around N(4) are C(2)-N(4)-H [113.19°], H(1)-N(4)-H [111.90°]. These values indicate a pyramidal geometry for N(4).

In the crystal lattice (Fig. 2), the ureido moiety of one molecule is oriented anti-parallel as to that of another molecule, and the intermolecular distances O(2')-N(3) and O(2') -N(1) are 3.009 (5) Å and 2.855 (5) Å respectively.

The geometry of the title compound in the solid is similar to that of one of its major conformations in solution, as confirmed by NMR spectroscopy results (B. J. Jean-Claude and G. Just, 1992).

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*Complete tables of anisotropic thermal parameters, bond distances and angles involving H-atoms, torsion angles and structure factors have been deposited with the Bristish Library Document Supply Centre as Supplementary Publication No. SUP 00000 (00 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England

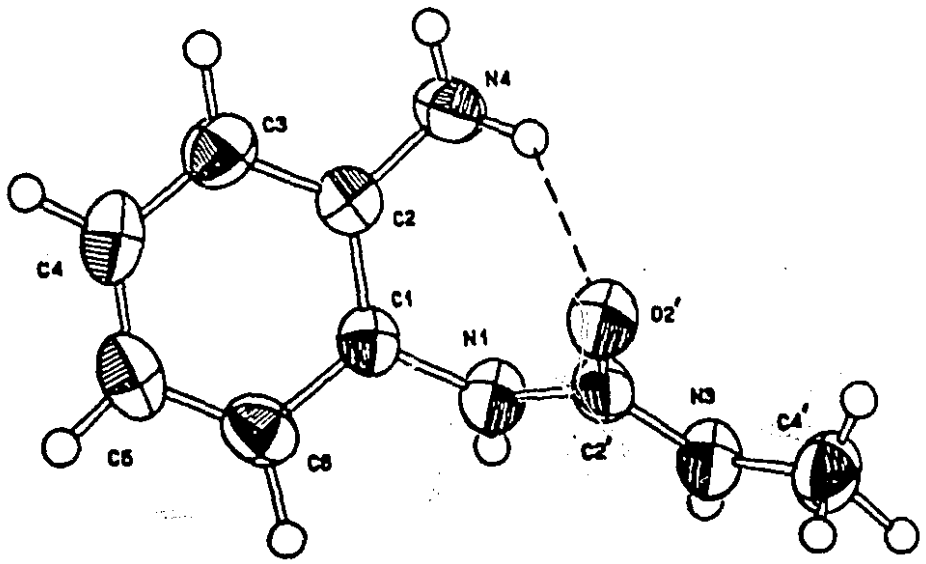

Fig. 1. ORTEP plot (Johnson, 1976) of N-(2-aminophenyl)-N'-methylurea with 50% probability ellipsoid. Hydrogen atoms were given an arbitrary radius for clarity.

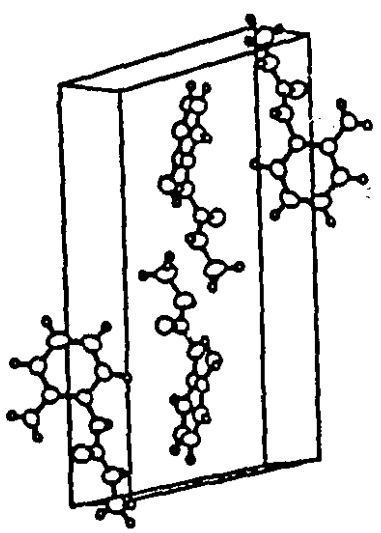

Fig. 2. Stereo ORTEP plot (Johnson, 1976) of the unit cell viewed down the *b* axis with *a* parallel to the bottom of the page. Hydrogens have been omitted for clarity.

ACKNOWLEDGEMENT

We wish to thank the Natural Research Council of Canada for financial support. We are also grateful to Dr Rosi Hynes for discussions.

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CROMER, D. T. & J. T. Waber (1974), International Tables for X-ray Crystallography, vol IV, The Kynoch Press, Birmingham, Table 2.2 A.

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Table S-1.1. Anisotropic Thermal Parameters

ATOM	U11	U22	U33	U12	U13	U23
O(2')	0.077(2)	0.025(2)	0.050(2)	-0.001(2)	0.008(2)	-0.003(2)
N(1)	0.072(3)	0.021(2)	0.046(2)	0.001(2)	0.009(2)	-0.003(2)
N(3)	0.092(3)	0.027(2)	0.046(2)	0.006(2)	0.014(2)	-0.003(2)
N(4)	0.048(2)	0.047(2)	0.057(3)	0.006(2)	0.015(2)	0.001(2)
C(1)	0.048(3)	0.033(2)	0.040(2)	-0.004(2)	0.009(2)	-0.003(2)
C(2)	0.040(2)	0.036(3)	0.046(2)	-0.008(2)	0.008(2)	-0.002(2)
C(3)	0.058(3)	0.046(3)	0.052(3)	-0.000(3)	0.004(2)	0.003(3)
C(4)	0.082(4)	0.067(4)	0.042(3)	-0.011(3)	0.012(3)	0.006(3)
C(5)	0.063(3)	0.072(4)	0.053(3)	-0.007(3)	0.024(3)	-0.010(3)
C(6)	0.047(3)	0.052(3)	0.055(3)	-0.001(3)	0.011(2)	-0.011(3)
C(2')	0.049(3)	0.032(2)	0.042(2)	0.002(2)	0.011(2)	-0.001(2)
C(4')	0.092(4)	0.060(4)	0.044(3)	0.013(3)	0.003(3)	-0.005(3)

Table S-1.2. Bond Angles (°) Involving the Hydrogen atoms

atom	atom	atom	angle	atom	atom	atom	angle
C(1)	N(1)	H(7)	114.97	C(4)	C(5)	H(5)	126.16
C(2')	N(1)	H(7)	118.09	C(6)	C(5)	H(5)	113.69
C(2')	N(3)	H(8)	119.12	C(1)	C(6)	H(6)	115.27
C(4')	N(3)	H(8)	116.92	C(5)	C(6)	H(6)	124.21
C(2)	N(4)	H(1)	113.30	H(3)	C(4')	H(9)	114.31
C(2)	N(4)	H(2)	113.19	H(3)	C(4')	H(10)	116.34
H(1)	N(4)	H(2)	111.90	H(3)	C(4')	H(11)	115.23
C(2)	C(3)	H(3)	120.43	H(9)	C(4')	H(10)	91.75
C(4)	C(3)	H(3)	118.56	H(9)	C(4')	H(11)	107.34
C(3)	C(4)	H(4)	115.20	H(10)	C(4')	H(11)	109.26
C(5)	C(4)	H(4)	124.67				

Table S-1.3. Intermolecular Contacts

atom	atom	distance	ADC(*)	atom	atom	distance	ADC(*)
O(2')	H(8)	2.202	54501	C(4)	H(10)	3.182	4
O(2')	H(7)	2.410	54501	C(4)	H(4)	3.314	75603
O(2')	H(5)	2.696	64502	C(4)	H(9)	3.320	54504
O(2')	H(6)	3.031	64502	C(4)	H(9)	3.366	4
O(2')	H(11)	3.170	75503	C(4)	H(5)	3.584	75603
N(1)	H(1)	3.229	56501	C(5)	H(4)	3.229	75603
N(1)	H(6)	3.336	64502	C(5)	H(8)	3.450	64502
N(1)	H(2)	3.461	56501	C(5)	H(6)	3.502	64502
N(3)	H(5)	3.057	65502	C(6)	H(6)	2.855	64502
N(3)	H(11)	3.217	75503	C(6)	H(3)	3.528	56501
N(4)	H(2)	2.268	75502	C(2')	H(11)	3.220	75503
N(4)	H(3)	3.088	75502	C(2')	H(6)	3.306	64502
N(4)	H(7)	3.117	54501	C(2')	H(8)	3.362	54501
N(4)	H(10)	3.294	75503	C(2')	H(5)	3.365	64502
N(4)	H(2)	3.359	74502	C(2')	H(7)	3.512	54501
N(4)	H(11)	3.550	75503	C(2')	H(5)	3.572	65502
N(4)	H(1)	3.560	75502	C(4')	H(3)	3.009	44404
C(1)	H(6)	3.016	64502	C(4')	H(1)	3.155	75503
C(1)	H(2)	3.497	75502	C(4')	H(11)	3.351	75503
C(1)	H(3)	3.546	56501	H(1)	H(10)	2.648	75503
C(1)	H(7)	3.579	54501	H(1)	H(7)	2.769	54501
C(2)	H(2)	2.833	75502	H(1)	H(11)	2.787	75503
C(2)	H(7)	3.122	54501	H(1)	H(2)	2.851	75502
C(3)	H(10)	2.992	4	H(1)	H(3)	3.037	75502
C(3)	H(9)	3.198	54504	H(1)	H(8)	3.501	54501
C(3)	H(2)	3.494	75502	H(2)	H(2)	2.525	75502
O(2')	N(3)	2.855(5)	54501	N(4)	N(4)	3.130(5)	75502
O(2')	N(1)	3.009(5)	54501	N(4)	N(4)	3.130(5)	74502
O(2')	C(2')	3.406(5)	54501	N(4)	C(2)	3.582(6)	74502
O(2')	C(5)	3.479(6)	64502	C(3)	C(6)	3.496(6)	54501
N(1)	N(4)	3.545(6)	56501				

Table S-1.4. Torsion Angles (°)

(1)	(2)	(3)	(4)	angle
O(2')	C(2')	N(1)	C(1)	-7.2(7)
O(2')	C(2')	N(1)	H(7)	-167
O(2')	C(2')	N(3)	C(4')	7.8(8)
O(2')	C(2')	N(3)	H(8)	175
N(1)	C(1)	C(2)	N(4)	1.6(6)
N(1)	C(1)	C(2)	C(3)	177.7(4)
N(1)	C(1)	C(6)	C(5)	-177.0(4)
N(1)	C(1)	C(6)	H(6)	11
N(1)	C(2')	N(3)	C(4')	-173.5(5)
N(1)	C(2')	N(3)	H(8)	-6
N(3)	C(2')	N(1)	C(1)	174.0(4)
N(3)	C(2')	N(1)	H(7)	14
N(4)	C(2)	C(1)	C(6)	-174.9(4)
N(4)	C(2)	C(3)	C(4)	174.7(4)
N(4)	C(2)	C(3)	H(3)	-6
C(1)	C(2)	N(4)	H(1)	-42
C(1)	C(2)	N(4)	H(2)	-171
C(1)	C(2)	C(3)	C(4)	-1.5(6)
C(1)	C(2)	C(3)	H(3)	178
C(1)	C(6)	C(5)	C(4)	-0.1(7)
C(1)	C(6)	C(5)	H(5)	-178
C(2)	C(1)	N(1)	C(2')	64.9(6)
C(2)	C(1)	N(1)	H(7)	-134
C(2)	C(1)	C(6)	C(5)	-0.4(6)
C(2)	C(1)	C(6)	H(6)	-172
C(2)	C(3)	C(4)	C(5)	1.0(7)
C(2)	C(3)	C(4)	H(4)	179
C(3)	C(2)	N(4)	H(1)	142
C(3)	C(2)	N(4)	H(2)	13
C(3)	C(2)	C(1)	C(6)	1.2(6)
C(3)	C(4)	C(5)	C(6)	-0.1(8)
C(3)	C(4)	C(5)	H(5)	178
C(4)	C(5)	C(6)	H(6)	171
C(5)	C(4)	C(3)	H(3)	-178
C(6)	C(1)	N(1)	C(2')	-118.5(5)
C(6)	C(1)	N(1)	H(7)	42
C(6)	C(5)	C(4)	H(4)	-178
C(2')	N(3)	C(4')	H(9)	69
C(2')	N(3)	C(4')	H(10)	174
C(2')	N(3)	C(4')	H(11)	-56
H(3)	C(3)	C(4)	H(4)	0
H(4)	C(4)	C(5)	H(5)	0
H(5)	C(5)	C(6)	H(6)	-7
H(8)	N(3)	C(4')	H(9)	-98
H(8)	N(3)	C(4')	H(10)	7
H(8)	N(3)	C(4')	H(11)	137

The sign is positive if when looking from atom 2 to atom 3 a clockwise motion of atom 1 would superimpose it on atom 4.

APPENDIX II. SUPPLEMENTARY DATA FOR THE X-RAY STRUCTURE OF 3-METHYL-6,7-DIHYDRO-1,2,3,5-TETRAZEPINO[7,6,5,ij]-QUINOLIN-4(3H)-ONE[d]

[d] The X-ray structure was determined by Dr James F. Britten

Table S-2.1. positional parameters for the structure of 14 (chapter 3)

atom	x	y	z	B(eq)
O(4)	0.118(1)	0.3758(6)	0.6848(9)	5.3(4)
N(5)	0.370(1)	0.3321(6)	0.842(1)	3.2(4)
N(3)	0.337(1)	0.4116(6)	0.602(1)	3.8(4)
N(2)	0.439(1)	0.3437(8)	0.549(1)	5.0(6)
N(1)	0.574(1)	0.3226(8)	0.641(1)	4.9(6)
C(1')	0.300(1)	0.2929(8)	0.963(1)	4.3(6)
C(2')	0.334(1)	0.3619(9)	1.097(1)	4.9(6)
C(3')	0.513(1)	0.3776(8)	1.173(1)	4.3(6)
C(4')	0.611(1)	0.3765(8)	1.062(1)	3.5(5)
C(5')	0.777(2)	0.3971(9)	1.117(1)	4.9(6)
C(6')	0.871(1)	0.395(1)	1.017(2)	6.3(8)
C(7')	0.801(2)	0.370(1)	0.863(2)	5.2(7)
C(8')	0.634(1)	0.3527(8)	0.799(1)	3.6(5)
C(9')	0.536(1)	0.3557(7)	0.899(1)	3.1(5)
C(4)	0.265(2)	0.3706(8)	0.713(1)	3.4(5)
C(3)	0.222(2)	0.455(1)	0.464(2)	5.6(8)
H(4)	0.1851	0.2843	0.9167	5.2
H(5)	0.3505	0.2354	1.0013	5.2
H(6)	0.2831	0.4187	1.0566	5.9
H(7)	0.2906	0.3387	1.1750	5.9
H(8)	0.5262	0.4367	1.2230	5.1
H(9)	0.5539	0.3311	1.2502	5.1
H(10)	0.8275	0.4126	1.2257	5.9
H(11)	0.9843	0.4116	1.0566	7.7
H(12)	0.8681	0.3642	0.7978	6.2
H(1)	0.16(1)	0.505(5)	0.51(1)	5(2)
H(2)	0.28(1)	0.494(6)	0.42(1)	5(2)
H(3)	0.15(1)	0.420(5)	0.40(1)	5(2)

Table S-2.2 Bond distances (Å) and Angles (°) involving the hydrogen atoms

C(1′)	H(4)	0.949	C(5′)	H(10)	0.954
C(1′)	H(5)	0.950	C(6′)	H(11)	0.950
C(2′)	H(6)	0.947	C(7′)	H(12)	0.948
C(2′)	H(7)	0.951	C(3)	H(1)	1.062
C(3′)	H(8)	0.954	C(3)	H(2)	0.949
C(3′)	H(9)	0.946	C(3)	H(3)	0.841

N(5)	C(1′)	H(4)	109.88	H(8)	C(3′)	H(9)	109.45
N(5)	C(1′)	H(5)	109.87	C(4′)	C(5′)	H(10)	119.41
C(2′)	C(1′)	H(4)	110.12	C(6′)	C(5′)	H(10)	118.87
C(2′)	C(1′)	H(5)	110.21	C(5′)	C(6′)	H(11)	120.43
H(4)	C(1′)	H(5)	109.60	C(7′)	C(6′)	H(11)	120.59
C(1′)	C(2′)	H(6)	108.92	C(6′)	C(7′)	H(12)	118.76
C(1′)	C(2′)	H(7)	108.59	C(8′)	C(7′)	H(12)	119.19
C(3′)	C(2′)	H(6)	109.16	N(3)	C(3)	H(1)	103.39
C(3′)	C(2′)	H(7)	108.70	N(3)	C(3)	H(2)	106.71
H(6)	C(2′)	H(7)	109.65	N(3)	C(3)	H(3)	115.01
C(2′)	C(3′)	H(8)	107.64	H(1)	C(3)	H(2)	100.85
C(2′)	C(3′)	H(9)	108.31	H(1)	C(3)	H(3)	108.72
C(4′)	C(3′)	H(8)	107.93	H(2)	C(3)	H(3)	120.02
C(4′)	C(3′)	H(9)	108.23				

Table S-2.3 Anisotropic Thermal Parameters

	U11	U22	U33	U12	U13	U23
O(4)	0.042(5)	0.111(8)	0.049(5)	-0.007(6)	0.017(4)	0.002(5)
N(5)	0.042(6)	0.055(7)	0.028(5)	-0.009(6)	0.017(5)	-0.001(5)
N(3)	0.051(6)	0.060(7)	0.039(6)	0.007(6)	0.023(5)	0.007(5)
N(2)	0.082(9)	0.077(9)	0.044(7)	-0.006(8)	0.042(7)	-0.010(6)
N(1)	0.067(8)	0.073(8)	0.066(9)	-0.001(7)	0.050(7)	-0.004(7)
C(1')	0.07(1)	0.053(8)	0.055(8)	-0.012(7)	0.037(7)	0.008(7)
C(2')	0.063(9)	0.09(1)	0.044(7)	-0.02(1)	0.033(7)	-0.006(8)
C(3')	0.071(9)	0.06(1)	0.030(6)	0.002(8)	0.019(6)	0.004(7)
C(4')	0.043(7)	0.034(7)	0.057(8)	0.001(6)	0.017(7)	0.009(6)
C(5')	0.054(8)	0.07(1)	0.063(9)	0.000(8)	0.014(7)	0.006(8)
C(6')	0.030(7)	0.11(1)	0.10(1)	0.001(8)	0.016(8)	0.02(1)
C(7')	0.06(1)	0.06(1)	0.10(1)	0.009(8)	0.054(9)	0.01(1)
C(8')	0.046(8)	0.036(7)	0.063(9)	0.006(6)	0.031(7)	0.003(6)
C(9')	0.043(7)	0.033(7)	0.047(7)	0.000(6)	0.022(6)	0.008(6)
C(4)	0.062(8)	0.044(8)	0.030(7)	-0.010(8)	0.024(6)	-0.009(6)
C(3)	0.09(1)	0.08(1)	0.05(1)	0.00(1)	0.025(9)	-0.003(9)
H(4)	0.0662					
H(5)	0.0662					
H(6)	0.0744					
H(7)	0.0744					
H(8)	0.0647					
H(9)	0.0647					
H(10)	0.0750					
H(11)	0.0970					
H(12)	0.0787					
H(1)	0.06(2)					
H(2)	0.06(2)					
H(3)	0.06(2)					

Table S-2.4. Torsion Angles (°)

(1)	(2)	(3)	(4)	angle
O(4)	C(4)	N(5)	C(1′)	-1(2)
O(4)	C(4)	N(5)	C(9′)	-156(1)
O(4)	C(4)	N(3)	N(2)	-126(1)
O(4)	C(4)	N(3)	C(3)	-1(2)
N(5)	C(1′)	C(2′)	C(3′)	-60(1)
N(5)	C(9′)	C(4′)	C(3′)	-3(2)
N(5)	C(9′)	C(4′)	C(5′)	178(1)
N(5)	C(9′)	C(8′)	N(1)	-3(2)
N(5)	C(9′)	C(8′)	C(7′)	-175(1)
N(5)	C(4)	N(3)	N(2)	56(1)
N(5)	C(4)	N(3)	C(3)	-178(1)
N(3)	N(2)	N(1)	C(8′)	6(2)
N(3)	C(4)	N(5)	C(1′)	177.2(9)
N(3)	C(4)	N(5)	C(9′)	-22(2)
N(2)	N(1)	C(8′)	C(7′)	-145(1)
N(2)	N(1)	C(8′)	C(9′)	42(2)
N(1)	N(2)	N(3)	C(4)	-75(1)
N(1)	N(2)	N(3)	C(3)	156(1)
N(1)	C(8′)	C(7′)	C(6′)	-177(1)
N(1)	C(8′)	C(9′)	C(4′)	173(1)
C(1′)	N(5)	C(9′)	C(4′)	-24(1)
C(1′)	N(5)	C(9′)	C(8′)	151(1)
C(1′)	C(2′)	C(3′)	C(4′)	36(1)
C(2′)	C(1′)	N(5)	C(9′)	55(1)
C(2′)	C(1′)	N(5)	C(4)	-102(1)
C(2′)	C(3′)	C(4′)	C(5′)	175(1)
C(2′)	C(3′)	C(4′)	C(9′)	-4(2)
C(3′)	C(4′)	C(5′)	C(6′)	179(1)
C(3′)	C(4′)	C(9′)	C(8′)	-178(1)
C(4′)	C(5′)	C(6′)	C(7′)	-2(2)
C(4′)	C(9′)	N(5)	C(4)	131(1)
C(4′)	C(9′)	C(8′)	C(7′)	0(2)
C(5′)	C(4′)	C(9′)	C(8′)	3(2)
C(5′)	C(6′)	C(7′)	C(8′)	5(2)
C(6′)	C(5′)	C(4′)	C(9′)	-2(2)
C(6′)	C(7′)	C(8′)	C(9′)	-4(2)
C(8′)	C(9′)	N(5)	C(4)	-53(2)

Table S-2.5. Least square planes

---------- Plane number 1 ----------

Atoms Defining Plane	Distance	esd
C(4′)	0.0045	0.0109
C(5′)	0.0025	0.0126
C(6′)	-0.0152	0.0152
C(7′)	0.0155	0.0130
C(8′)	-0.0424	0.0110
C(9′)	-0.0491	0.0104
C(3′)	0.0438	0.0115
N(5)	-0.0077	0.0088
N(1)	0.0580	0.0108

Additional Atoms	Distance
C(1′)	0.6417
C(2′)	-0.0689
N(2)	-0.5392
C(4)	-0.8688
N(3)	-1.5243

Mean deviation from plane is 0.0265 angstroms
Chi-squared: 83.9

---------- Plane number 2 ----------

Atoms Defining Plane	Distance	esd
N(5)	-0.0035	0.0087
C(9′)	0.0101	0.0104
C(8′)	-0.0113	0.0110
N(1)	0.0054	0.0108

Additional Atoms	Distance
N(2)	-0.6452
N(3)	-1.6057
C(4)	-0.9247

Mean deviation from plane is 0.0076 angstroms
Chi-squared: 2.4

Dihedral angles between least-squares planes

plane	plane	angle
2	1	3.51

APPENDIX III. ADDITIONAL DATA FOR THE X-RAY STRUCTURE OF 3,5-DIMETHYL-3H-7-NITRO-3H-BENZO-1,2,3,5-TETRAZEPIN-4(5H)-ONE[a] AND SUPPLEMENTARY MATERIAL FOR CHAPTER 4

[a] The X-ray structure was determined by Dr Rosi Hynes

Table S-3.1. positional parameters for the structure of 6e (chapter 4)

	x	y	z	Beq
O 4	0.0660(5)	0.0820 (5)	0.0943(3)	6.16(22)
O 6	0.3092(5)	-0.52154	0.1120(4)	6.88(24)
O 6′	0.3605(6)	-0.5812 (5)	0.2796(4)	8.8 (3)
N 1	-0.0351(6)	-0.1042 (7)	0.3871(4)	4.8 (3)
N 2	-0.0175(6)	0.0060 (6)	0.3618(4)	5.1 (3)
N 3	0.1095(6)	0.0359 (5)	0.2801(4)	4.6 (3)
N 5	0.0368(6)	-0.1161 (6)	0.1489(3)	4.0 (3)
N 6	0.3001(6)	-0.5091 (7)	0.2122(5)	5.6 (3)
C 3	0.1555(8)	0.1664 (6)	0.2956(6)	6.1 (4)
C 4	0.0682(7)	0.0040 (7)	0.1682(5)	4.4 (4)
C 5	0.0039(8)	-0.1483 (7)	0.0316(4)	5.3 (3)
C 1′	0.0925(7)	-0.2073 (7)	0.2229(4)	3.5 (3)
C 2′	0.1661(6)	-0.3120 (7)	0.1812(4)	3.6 (3)
C 3′	0.2139(6)	-0.4012 (6)	0.2576(5)	4.0 (3)
C 4′	0.1909(7)	-0.3921 (7)	0.3714(5)	4.9 (3)
C 5′	0.1093(7)	-0.2900 (7)	0.4099(4)	4.8 (3)
C 6′	0.0615(7)	-0.1973 (7)	0.3379(4)	3.7 (3)

Beq is the mean of the principal axes of the thermal ellipsoid.

Table S-3.2 Anisotropic Thermal Parameters

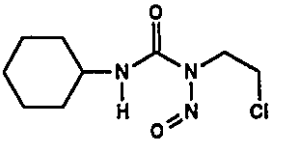
	u11	u22	u33	u12	u13	u23
O 4	10.0(3)	5.9(3)	7.5(3)	0.9 (3)	0.9(3)	2.4(3)
O 6	9.3(3)	8.0(3)	8.9(3)	1.9 (3)	-0.8(3)	-2.3(3)
O 6′	15.3(5)	6.5(3)	11.5(4)	3.1 (3)	-2.1(4)	1.7(3)
N 1	5.9(4)	7.4(4)	5.1(3)	-0.2 (3)	0.4(3)	-0.2(3)
N 2	5.7(4)	8.3(5)	5.5(4)	0.3 (3)	0.3(3)	-1.7(3)
N 3	6.0(4)	4.5(3)	6.9(4)	-0.18(25)	0.8(3)	-0.8(3)
N 5	6.6(4)	4.2(3)	4.5(3)	-0.4 (3)	-0.3(3)	0.1(3)
N 6	6.7(4)	5.5(4)	9.0(5)	-1.1 (3)	-1.8(4)	0.5(4)
C 3	6.6(5)	5.3(4)	11.5(6)	-0.7 (4)	0.1(4)	-1.6(4)
C 4	4.4(4)	6.7(6)	5.8(5)	0.6 (4)	0.4(3)	0.1(4)
C 5	8.9(4)	6.8(5)	4.4(4)	0.2 (3)	-2.0(3)	0.7(3)
C 1′	4.0(3)	4.4(3)	4.7(4)	-1.0 (3)	-0.2(3)	0.8(3)
C 2′	4.9(4)	4.6(3)	4.4(4)	-0.8 (3)	-0.2(3)	0.3(3)
C 3′	4.1(3)	3.2(3)	7.8(4)	-0.7 (4)	-0.6(3)	0.1(4)
C 4′	7.7(5)	5.7(4)	5.3(4)	-1.0 (4)	-1.4(3)	1.2(4)
C 5′	6.6(4)	7.1(4)	4.4(3)	-1.3 (4)	-0.6(3)	1.2(4)
C 6′	4.2(4)	5.2(4)	4.7(4)	-0.6 (3)	0.0(3)	0.1(4)

Table S-3.3 Torsion Angles (°)

C	5	N	1	N	2	N	3	-1.0(| 3)	N	2	N	1	C	5	C	4	-46.2(| 4)
N	2	N	1	C	5	C	6	141.5(| 8)	N	1	N	2	N	3	C	1	-157.9(| 7)
N	1	N	2	N	3	C	2	73.7(| 5)	N	2	N	3	C	2	O	1	122.5(| 7)
N	2	N	3	C	2	N	4	-59.3(| 5)	C	1	N	3	C	2	O	1	-2.7(| 3)
C	1	N	3	C	2	N	4	175.5(| 8)	C	3	N	4	C	2	O	1	1.6(| 3)
C	3	N	4	C	2	N	3	-176.6(| 7)	C	4	N	4	C	2	O	1	158.5(| 8)
C	4	N	4	C	2	N	3	-19.7(| 3)	C	2	N	4	C	4	C	5	50.0(| 5)
C	2	N	4	C	4	C	9	-135.3(| 7)	C	3	N	4	C	4	C	5	-153.9(| 7)
C	3	N	4	C	4	C	9	20.8(| 4)	O	2	N	5	C	8	C	7	174.4(| 8)
O	2	N	5	C	8	C	9	-7.8(| 3)	O	3	N	5	C	8	C	7	-6.3(| 4)
O	3	N	5	C	8	C	9	171.5(| 8)	N	4	C	4	C	5	N	1	4.9(| 3)
N	4	C	4	C	5	C	6	176.8(| 8)	C	9	C	4	C	5	N	1	-169.8(| 8)
C	9	C	4	C	5	C	6	2.1(| 3)	N	4	C	4	C	9	C	8	-177.9(| 7)
C	5	C	4	C	9	C	8	-3.2(| 4)	N	1	C	5	C	6	C	7	174.1(| 8)
C	4	C	5	C	6	C	7	1.5(| 4)	C	5	C	6	C	7	C	8	-3.7(| 4)
C	6	C	7	C	8	N	5	-179.8(| 8)	C	6	C	7	C	8	C	9	2.6(| 4)
N	5	C	8	C	9	C	4	-176.9(| 7)	C	7	C	8	C	9	C	4	0.8(| 4)

Table 3-4.- *In vitro* antitumour activity of bi-and tricyclic tetrazepinones (IC_{50}, μM)

	CCNU	6a	6c	6d	6e	B (X=CH_2)	B (X=O)
A549	54.8	not signif.	1000	114.1	142.8	1000	32.1
A427	340.6	56.6	1000	437.5	8.3	24.9	302.6
A498	127.6	not signif.	63.1	311.8	164.8	45.3	211.1
HS 578T	>1000	>1000	>1000>1	>1000	>1000	-	>1000
LOX	52.3	123.7	275.4	104.1	30.4	23.8	9.6
OVCA3	126.8	not signif.	338	>1000	878	39.8	64.4
PC-3	155.2	316.2	322	102.1	142.1	36.8	23.1
SF126	18.7	125.8	323.4	34	69.9	100	10.6
UMSCC-21A	126.9	>1000	1000	-	1000	61.4	>1000
HT29	>1000	>1000	358.9	119	251	-	-18.3
SW260	21.6	>1000	173.7	>1000	136.1	27.1	>1000
IMR-90	>1000	-	1000	>1000	>1000	1000	-



CCNU

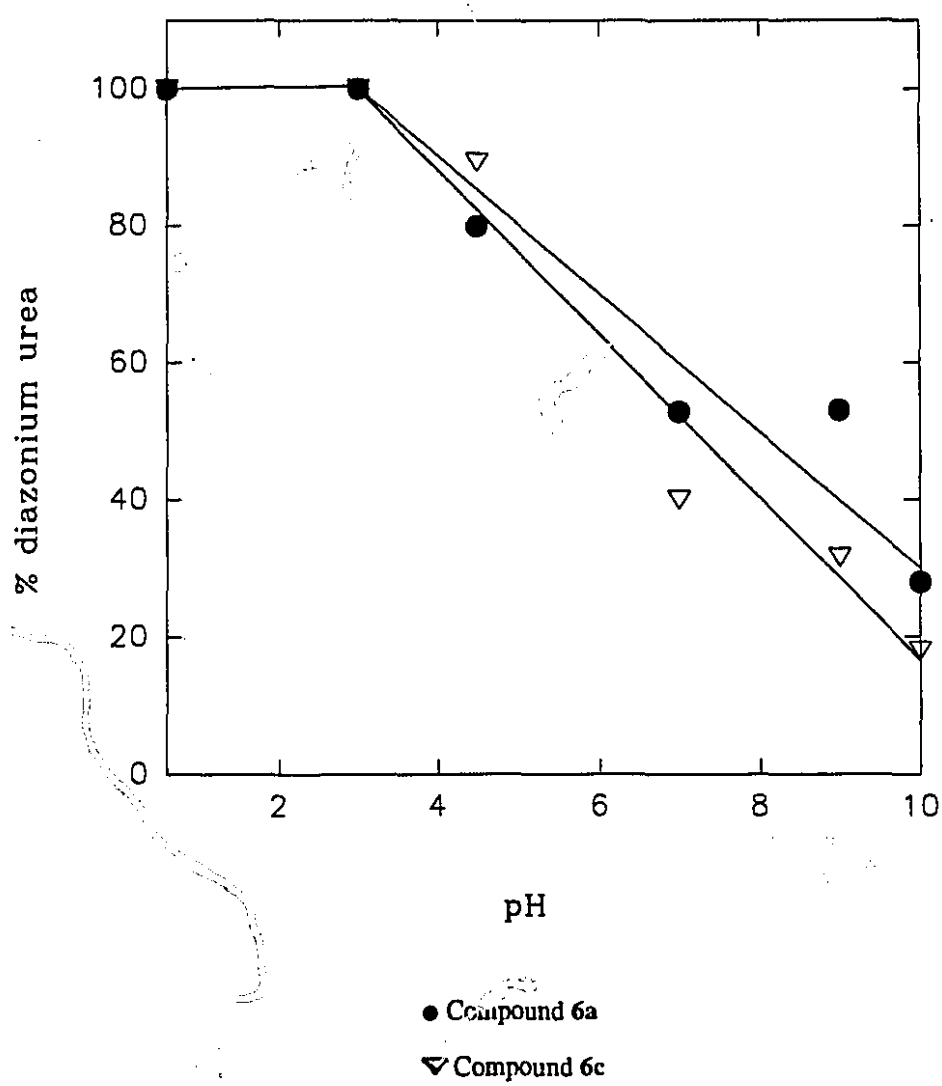


Fig S-3.1 Variation of diazonium urea/tetrazepinone ratio when methoxybenzotetrazepinone 6a and 6c are dissolved in water at different pHs

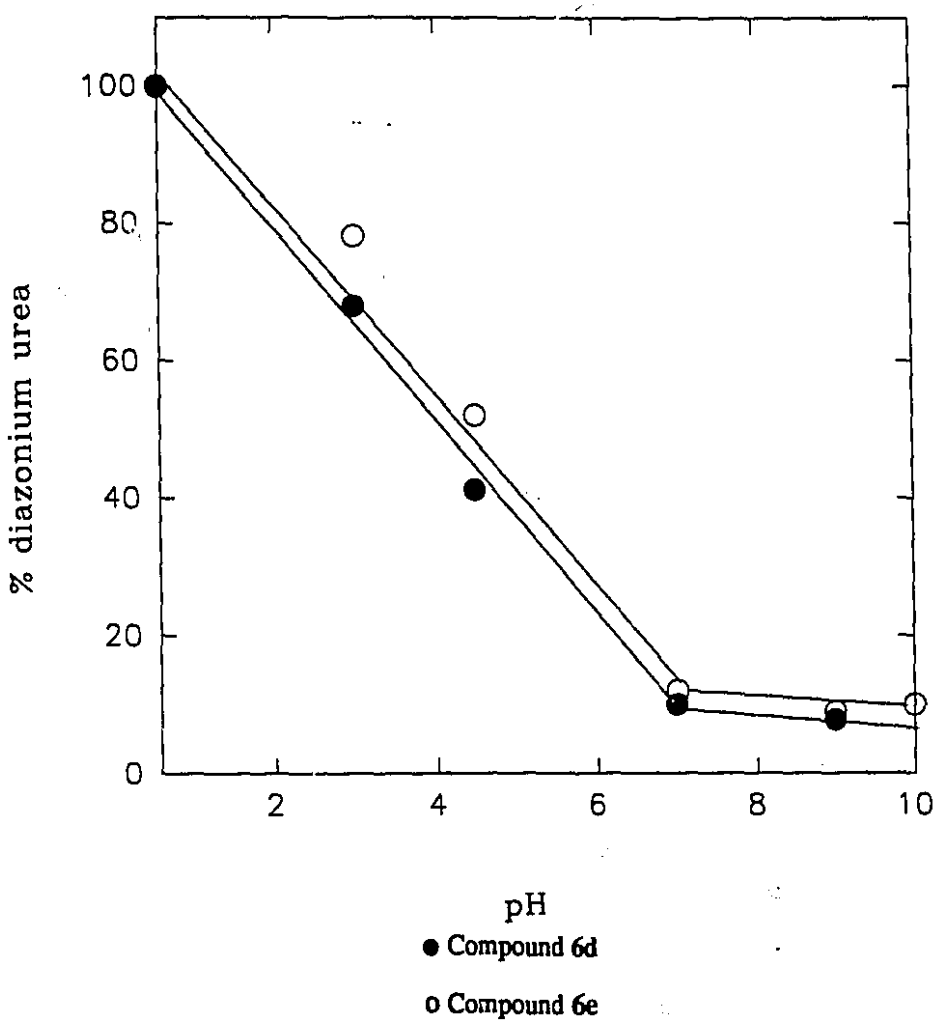


Fig S-3.2 Variation of diazonium urea/tetrazepinone ratio when methoxybenzotetrazepinone **6d** and **6e** are dissolved in water at different pHs

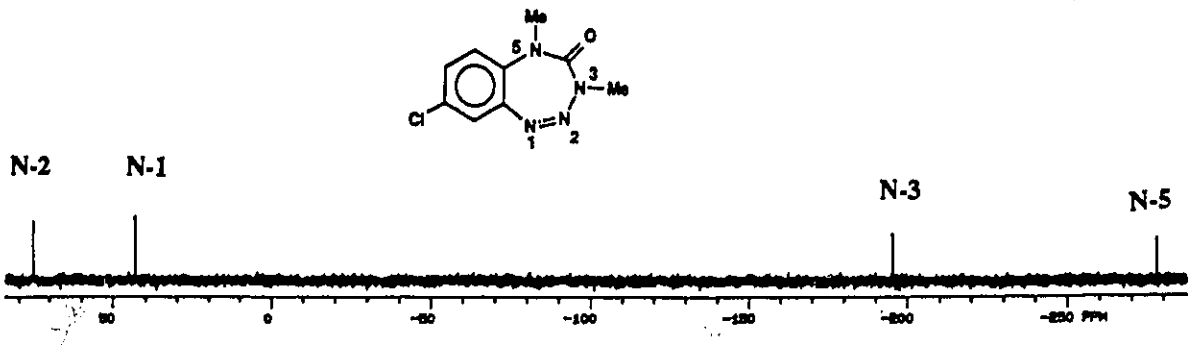


Fig S-3.3 Natural abundance ^{15}N NMR spectrum of 6d

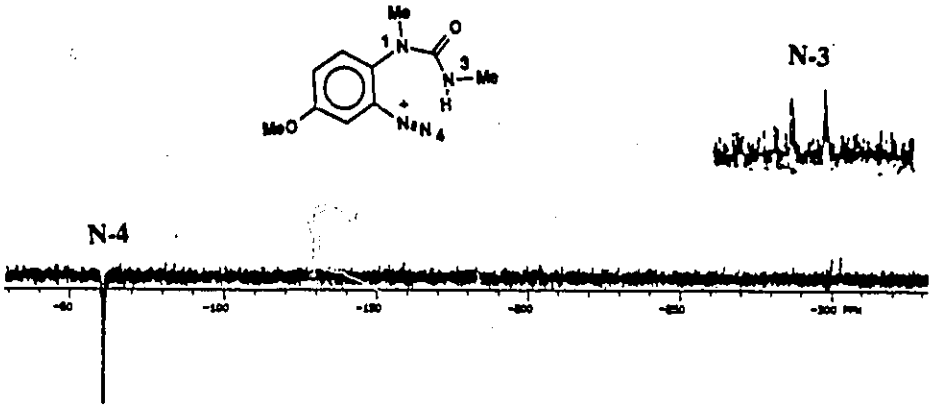


Fig S-3.4 ^{15}N NMR spectrum of 6c specifically labelled at N4 in 2N HCl. Expansion of the N-3 doublet at 305 ppm is shown above the spectrum

APPENDIX IV. SUPPLEMENTARY MATERIAL FOR CHAPTER 6 AND ADDITIONAL DATA FOR THE X-RAY STRUCTURE OF 3,5-DIMETHYL-PYRIDO[2,3]-1,2,3,5-TETRAZEPIN-4(5H)-ONE[a]

[a] The X-ray structure was determined by Dr Rosi Hynes

Table S-4.1. positional parameters for the structure of 10 (chapter 5)

	x	y	z	Beq
O 4	0.16209	0.6566(3)	0.98465(18)	3.96(20)
N 1	0.1288 (16)	0.8867(3)	1.14729(23)	3.8 (3)
N 2	0.1644 (16)	0.8907(3)	1.08097(22)	3.9 (3)
N 3	0.3628 (14)	0.8060(3)	1.04574(22)	2.97(23)
N 5	0.1327 (14)	0.6537(3)	1.10647(21)	2.74(23)
N 7	0.3641 (16)	0.6155(3)	1.21933(22)	3.6 (3)
C 3'	0.4735 (21)	0.8482(5)	0.9747 (3)	4.1 (3)
C 4	0.2097 (19)	0.7025(4)	1.0416 (3)	3.4 (3)
C 5'	-0.0006 (21)	0.5415(4)	1.1024 (3)	3.8 (3)
C 6	0.2700 (17)	0.6921(4)	1.1723 (3)	2.8 (3)
C 8	0.4818 (22)	0.6477(4)	1.2836 (3)	4.1 (3)
C 9	0.5062 (19)	0.7556(5)	1.3051 (3)	4.0 (4)
C10	0.3826 (20)	0.8329(4)	1.2583 (3)	3.6 (3)
C11	0.2730 (17)	0.8017(4)	1.1900 (3)	2.8 (3)
H3'A	0.578 (16)	0.920 (4)	0.985 (3)	4.9 (14)
H3'B	0.249 (14)	0.869 (4)	0.948 (3)	6.2 (17)
H3'C	0.624 (20)	0.783 (5)	0.950 (3)	8.5 (19)
H5'A	-0.156 (17)	0.530 (4)	1.0583 (23)	5.3 (14)
H5'B	0.205 (16)	0.501 (4)	1.0882 (24)	5.1 (14)
H5'C	-0.081 (15)	0.519 (3)	1.1486 (19)	2.5 (10)
H8	0.563 (14)	0.589 (4)	1.3183 (24)	4.4 (14)
H9	0.605 (15)	0.776 (4)	1.3594 (25)	4.6 (13)
H10	0.370 (13)	0.909 (3)	1.2729 (20)	2.3 (10)

Beq is the mean of the principal axes of the thermal elipsoid for atoms refined anisotropically. For hydrogens, Beq = Biso.

Table S-4.2 Anisotropic Thermal Parameters

	u11	u22	u33	u12	u13	u23
O 4	7.4(3)	4.30(23)	3.30(20)	-0.7(3)	-1.1(3)	-0.71(19)
N 1	6.8(4)	3.0 (3)	4.5 (3)	-0.5(3)	-1.6(3)	-0.68(24)
N 2	7.3(4)	3.0 (3)	4.4 (3)	-0.4(3)	-0.9(3)	0.23(23)
N 3	4.8(4)	2.96(25)	3.50(25)	-0.6(3)	-0.2(3)	-0.01(23)
N 5	4.5(4)	2.67(23)	3.23(25)	-1.2(3)	0.0(3)	0.00(21)
N 7	6.9(5)	3.4 (3)	3.21(23)	0.4(3)	-0.4(3)	0.54(24)
C 3'	6.7(6)	4.5 (4)	4.4 (4)	-0.4(4)	-0.2(4)	1.7 (3)
C 4	4.9(5)	3.7 (3)	4.2 (3)	-0.1(4)	-1.2(4)	0.3 (3)
C 5'	6.6(6)	3.4 (3)	4.4 (3)	-2.3(4)	1.0(4)	0.0 (3)
C 6	4.6(5)	2.8 (3)	3.1 (3)	0.2(4)	0.0(3)	-0.1 (3)
C 8	7.9(6)	3.8 (4)	3.8 (3)	0.4(4)	-1.5(4)	0.9 (3)
C 9	6.5(6)	5.2 (4)	3.5 (3)	-0.3(5)	-0.3(4)	0.5 (3)
C10	6.4(5)	3.7 (3)	3.7 (3)	-0.3(4)	1.3(4)	-1.6 (3)
C11	4.6(5)	3.0 (3)	3.1 (3)	0.4(4)	0.5(3)	-0.2 (3)

Table S-4.3. Bond distances (Å) and Angles (°) involving all atoms

O(4)-C(4)	1.212(6)	C(3')-H(3'C)	1.10(6)
N(1)-N(2)	1.239(6)	C(5')-H(5'A)	1.03(5)
N(1)-C(11)	1.426(7)	C(5')-H(5'B)	0.98(6)
N(2)-N(3)	1.450(7)	C(5')-H(5'C)	0.95(4)
N(3)-C(3')	1.479(7)	C(6)-C(11)	1.386(7)
N(3)-C(4)	1.406(7)	C(8)-C(9)	1.387(8)
N(5)-C(4)	1.376(7)	C(8)-H(8)	1.02(5)
N(5)-C(5')	1.474(6)	C(9)-C(10)	1.373(8)
N(5)-C(6)	1.413(7)	C(9)-H(9)	1.11(5)
N(7)-C(6)	1.334(7)	C(10)-C(11)	1.391(7)
N(7)-C(8)	1.336(7)	C(10)-H(10)	0.98(4)
C(3')-H(3'A)	0.99(5)		
C(3')-H(3'B)	1.03(5)		

N(2)-N(1)-C(11)	122.5(5)	H(5'A)-C(5')-H(5'B)	101(4)
N(1)-N(2)-N(3)	118.6(4)	H(5'A)-C(5')-H(5'C)	118(5)
N(2)-N(3)-C(3')	107.7(4)	H(5'B)-C(5')-H(5'C)	110(4)
N(2)-N(3)-C(4)	116.6(5)	N(5)-C(6)-N(7)	115.7(4)
C(3')-N(3)-C(4)	113.1(4)	N(5)-C(6)-C(11)	122.1(5)
C(4)-N(5)-C(5')	116.0(4)	N(7)-C(6)-C(11)	121.9(5)
C(4)-N(5)-C(6)	121.9(5)	N(7)-C(8)-C(9)	124.3(5)
C(5')-N(5)-C(6)	119.2(4)	N(7)-C(8)-H(8)	117(3)
C(6)-N(7)-C(8)	117.9(4)	C(9)-C(8)-H(8)	118(3)
N(3)-C(3')-H(3'A)	105(3)	C(8)-C(9)-C(10)	117.0(5)
N(3)-C(3')-H(3'B)	105(3)	C(8)-C(9)-H(9)	120.2(23)
N(3)-C(3')-H(3'C)	105(3)	C(10)-C(9)-H(9)	122.7(23)
H(3'A)-C(3')-H(3'B)	102(4)	C(9)-C(10)-C(11)	119.6(5)
H(3'A)-C(3')-H(3'C)	120(5)	C(9)-C(10)-H(10)	120.4(24)
H(3'B)-C(3')-H(3'C)	115(4)	C(11)-C(10)-H(10)	120.0(24)
O(4)-C(4)-N(3)	122.2(5)	N(1)-C(11)-C(6)	125.2(5)
O(4)-C(4)-N(5)	121.8(5)	N(1)-C(11)-C(10)	115.2(5)
N(3)-C(4)-N(5)	116.0(4)	C(6)-C(11)-C(10)	119.1(5)
N(5)-C(5')-H(5'A)	112(3)		
N(5)-C(5')-H(5'B)	101(3)		
N(5)-C(5')-H(5'C)	110.1(25)		

Table S-4.4 Torsion Angles (°)

C11	N 1	N 2	N 3	-0.6(3)	N 2	N 1	C11	C 6	-42.8(4)
N 2	N 1	C11	C10	145.8(7)	N 1	N 2	N 3	C 3′	-159.7(7)
N 1	N 2	N 3	C 4	72.0(5)	N 2	N 3	C 4	O 4	122.5(6)
N 2	N 3	C 4	N 5	-60.7(4)	C 3′	N 3	C 4	O 4	-3.2(3)
C 3′	N 3	C 4	N 5	173.6(7)	C 5′	N 5	C 4	O 4	1.8(3)
C 5′	N 5	C 4	N 3	-175.0(7)	C 6	N 5	C 4	O 4	162.3(7)
C 6	N 5	C 4	N 3	-14.5(3)	C 4	N 5	C 6	N 7	-140.4(6)
C 4	N 5	C 6	C11	46.2(4)	C 5′	N 5	C 6	N 7	19.5(3)
C 5′	N 5	C 6	C11	-153.9(6)	C 8	N 7	C 6	N 5	-177.2(7)
C 8	N 7	C 6	C11	-3.8(4)	C 6	N 7	C 8	C 9	1.4(3)
N 5	C 6	C11	N 1	3.0(3)	N 5	C 6	C11	C10	174.1(7)
N 7	C 6	C11	N 1	-170.0(7)	N 7	C 6	C11	C10	1.1(3)
N 7	C 8	C 9	C10	3.7(3)	C 8	C 9	C10	C11	-6.3(4)
C 9	C10	C11	N 1	176.1(7)	C 9	C10	C11	C 6	4.2(3)
C 6′	N 1	N 2	N 3	-1.0(3)	N 2	N 1	C 6′	C 1′	-46.2(4)
N 2	N 1	C 6′	C 5′	141.5(8)	N 1	N 2	N 3	C 3	-157.9(7)
N 1	N 2	N 3	C 4	73.7(5)	N 2	N 3	C 4	O 4	122.5(7)
N 2	N 3	C 4	N 5	-59.3(5)	C 3	N 3	C 4	O 4	-2.7(3)
C 3	N 3	C 4	N 5	175.5(8)	C 5	N 5	C 4	O 4	1.6(3)
C 5	N 5	C 4	N 3	-176.6(7)	C 1′	N 5	C 4	O 4	158.5(8)
C 1′	N 5	C 4	N 3	-19.7(3)	C 4	N 5	C 1′	C 2′	-135.3(7)
C 4	N 5	C 1′	C 6′	50.0(5)	C 5	N 5	C 1′	C 2′	20.8(4)
C 5	N 5	C 1′	C 6′	-153.9(7)	O 6	N 6	C 3′	C 2′	-7.8(3)
O 6	N 6	C 3′	C 4′	174.4(8)	O 6′	N 6	C 3′	C 2′	171.5(8)
O 6′	N 6	C 3′	C 4′	-6.3(4)	N 5	C 1′	C 2′	C 3′	-177.9(7)
C 6′	C 1′	C 2′	C 3′	-3.2(4)	N 5	C 1′	C 6′	N 1	4.9(3)
N 5	C 1′	C 6′	C 5′	176.8(8)	C 2′	C 1′	C 6′	N 1	-169.8(8)
C 2′	C 1′	C 6′	C 5′	2.1(3)	C 1′	C 2′	C 3′	N 6	-176.9(7)
C 1′	C 2′	C 3′	C 4′	0.8(4)	N 6	C 3′	C 4′	C 5′	-179.8(8)
C 2′	C 3′	C 4′	C 5′	2.6(4)	C 3′	C 4′	C 5′	C 6′	-3.7(4)
C 4′	C 5′	C 6′	N 1	174.1(8)	C 4′	C 5′	C 6′	C 1′	1.5(4)

Table S-4.5.-Comparison between the cytotoxicity of CCNU and Tetrazepinone 10 against human cancer cells (IC_{50}, μM)

	CCNU	10
A549	239.6	1000
A427	213.17	31.8
A498	87	77.6
LOX	14.4	24.8
OVCAR3	69.1	31.6
PC-3	48.5	43.8
SF126	13.8	43
UMSCC-21A	100	98.9
SW260	28.8	28.1
IMR90	1000	1000
CDD 19 LU	691	461.6