MAGNETIC RESONANCE IN CHEMISTRY, VOL. 36, 87–91 (1998)

^{15}N NMR Study of Bi- and Tricyclic 1,2,3,5-Tetrazepin-4-ones

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Received 18 September 1996; revised 5 August 1997; accepted 8 August 1997

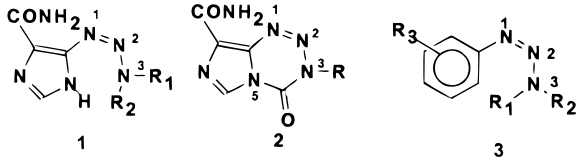
ABSTRACT: The ^{15}N NMR spectra of the novel seven-membered 1,2,3,5-tetrazepinone ring systems were studied. The chemical shift of N-2 was found to be significantly responsive to substituent changes at the phenyl ring. As the electron-withdrawing character of the substituents increased, N-2 became more deshielded. Studies on the diazotization of the 2-aminourea **6c** using specific labeling showed that the tetrazepinones are in equilibrium with their acyclic diazourea form **9**, and that this equilibrium lies towards the formation of the 1,2,3,5-tetrazepinone at neutral or basic pH. Solid-state NMR data indicated that N-3 was significantly more delocalized in the 1,2,3-triazene moiety of the tetrazinone 2 than in the electron-deficient tetrazepinone 5f. © 1998 John Wiley & Sons, Ltd.

KEYWORDS: NMR; ^{15}N NMR; electronic structures; tetrazepinone derivatives

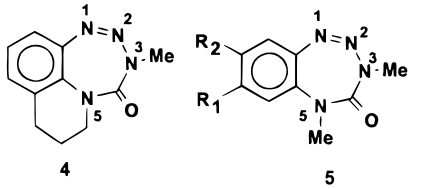
INTRODUCTION

The use of dacarbazine (**1**, $R_1 = R_2 = Me$) as an antineoplastic agent and the discovery of the pronounced antitumour activity of temozolomide (**2**, R = Me) have evoked considerable interest in the determination of structure and conformation of 1,2,3-triazene derivatives.[1–4] To this effect, the x-ray structures of **1**, **2** and **3** have been determined.[1,2,4] ^{15}N NMR studies have been carried out to investigate the effect of substituents on the chemical shifts of the nitrogens in the triazene chain of compounds of type **3**.[5]

We have already reported the synthesis of **4** and **5c** that contain, like **1**, **2** and **3**, the 1,2,3-triazene moiety.[6] In an effort to understand the electronic effects of substituents on the stability of the 1,2,3,5-tetrazepin-4-one ring system, the ^{15}N NMR of tetrazepinones **5** was studied. It is now known that ^{15}N NMR shifts can give insight into electron delocalization in the triazene chain of 1-aryl-3,3-dimethyltriazenes.[7–9]



1 **2** **3**



4 **5**

a R_1=OCH_3 R_2=H
b R_1=CH_3 R_2=H
c R_1=H R_2=H
d R_1=H R_2=OCH_3
e R_1=H R_2=Cl
f R_1=NO_2 R_2=H

In this paper, we discuss (a) the mechanism by which the 1,2,3,5-tetrazepin-4-one ring is formed and (b) the correlation between substituent constants and the ^{15}N shifts of the substituted benzotetrazepinones **5a–f**. We also discuss the solid-state NMR of **2** and **5f**, for which x-ray crystallographic data are already available.[4,10] To our knowledge, this is the first report dealing with the ^{15}N NMR of cyclic ureidotriazenes.

EXPERIMENTAL

All compounds were synthesized according to methods reported elsewhere.[5,10] Compounds **5a–f** were specifically labelled at N-2 to facilitate assignments. Temozolomide (**2**, R = Me) was obtained from Shering Plough Research Institute.

^{15}N NMR spectra were measured on a Varian XL-300 spectrometer and nitromethane was used as an external standard set at 0 ppm. Signals that appeared upfield from nitromethane are negative and those downfield are positive. All ^{15}N NMR spectra were recorded at 30.4 MHz using a multinuclear 10 mm probe and the concentration of each sample was *ca.* 0.5 M in $CDCl_3$. No relaxation agent was added owing to the instability of benzotetrazepinones under acidic conditions. The best results were obtained with a relaxation delay of 25 s (45° pulse = 8 μs). Satisfactory FIDs could be obtained with *ca.* 2000 transients. The spectra were acquired without NOE effect. The spectral window was 27 000 Hz and with 32K data points gave an acquisition time of 1.07 s and a digital resolution of 0.93 Hz.

^{15}N NMR studies on the diazotization of the aminourea **6c** were performed in a 10 mm NMR tube. Briefly, **6c** (0.6 g) was dissolved in 2.5 M HCl (3 ml) and after the

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Contract grant sponsor: National Cancer Institute of Canada (NCIC).
Contract grant sponsor: Luigi Barba Fund.

 CCC 0749-1581/98/020087–05 $17.50

addition of sodium nitrite (6% ^{15}N enriched) the temperature of the probe was kept at 0–5 °C and ^{15}N spectra were measured as described. The pH adjustments were performed at 0–5 °C using saturated sodium hydrogencarbonate solution.

^{15}N solid-state NMR spectra were obtained on a Chemagnetics CMX-300 spectrometer at 30.3 MHz. The cross-polarization magic angle spinning (CP/MAS) technique was used with a contact time of 1 μs, a recycle time of 2 s and a spinning speed of 5 kHz. The chemical shifts obtained relative to ammonium nitrate were converted to the nitromethane scale as described previously[11] [$\delta N_{(nitromethane)} = \delta N_{(ammonium\ nitrate)} - 351$].

All semi-empirical (PM3[12,13]) and *ab initio* (3–21G, 6–31G)[14,15] calculations were performed using the GAMESS package[16,17] on a Sun Sparc10 workstation. Full geometry optimization was allowed in each calculation, and all optimizations were carried out in Cartesian as opposed to internal coordinates. All quantum mechanical calculations used RHF wavefunctions. Equilibrium geometries were verified as such by force constant analysis.

RESULTS AND DISCUSSION

^{15}N NMR and the formation of tetrazepinones

In order to gain insight into the mechanism by which tetrazepinones were formed, we analyzed the diazotization of the unsubstituted urea **6c**. When **6c** was dissolved in 2.5 M HCl, the peak at *ca.* −335 ppm (NH_2) was sharper than that at *ca.* −303 ppm (N-3), indicating that proton exchanges[11] at the amino group were more rapid than those at the ureido nitrogen N-3, as expected [Fig. 1(c)]. These results suggest equilibria **6** ⇌ **7** ⇌ **8**.

When the amine was diazotized in 2.5 M HCl, the diazonium ion was detected by ^{15}N NMR at −60 ppm and at pH 5 negligible exchange was observed at N-3. This suggests the presence of the diazonium species **9**. The ^{15}N shifts of diazonium ions in aqueous solutions are now well documented.[17,18] The presence of the diazonium ion was further confirmed by an IR spectrum taken after evaporation of the solution to dryness. This showed a sharp peak at 2260 cm^{-1}. When the pH was raised to 8 with sodium hydrogencarbonate, a precipitate formed and the conditions were no longer favorable for NMR analysis. The precipitate was extracted from CH_2Cl_2 and the resulting solid was assigned the 3-methyltetrazepinone structure **5c** with N-2 resonating at around −71 ppm.

When the solid was redissolved in 2.5 M HCl, the diazonium ion reappeared. The results *in toto* suggest the equilibria depicted in Scheme 1. We have already described the conformation of 2-aminophenylureas in solution and in the solid.[11,19] Based on the latter study, we suggest that, in aqueous solution, the urea may adopt conformations **6**, **7** or **8**, which resemble a possible transition state of the tetrazepinone ring closure. The 1,2,3,5-tetrazepin-4-one ring appears to be in equilibrium with its acyclic diazonium urea precursor of type **9**. Under acidic conditions, the equilibrium lies toward the formation of **9**, resulting from protonated species **10**. Neutralization drives the equilibrium toward the formation of the 1,2,3,5-tetrazepin-4-one ring. Pro-

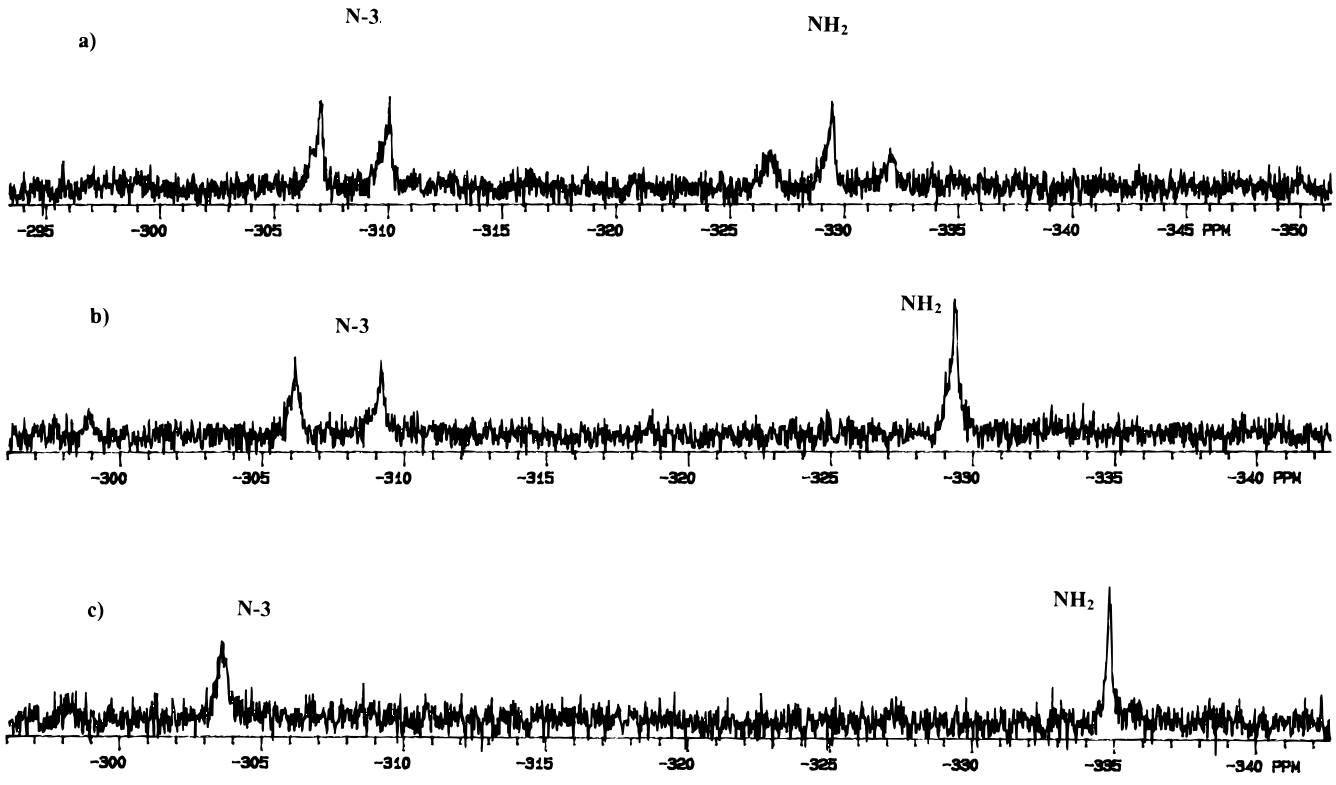
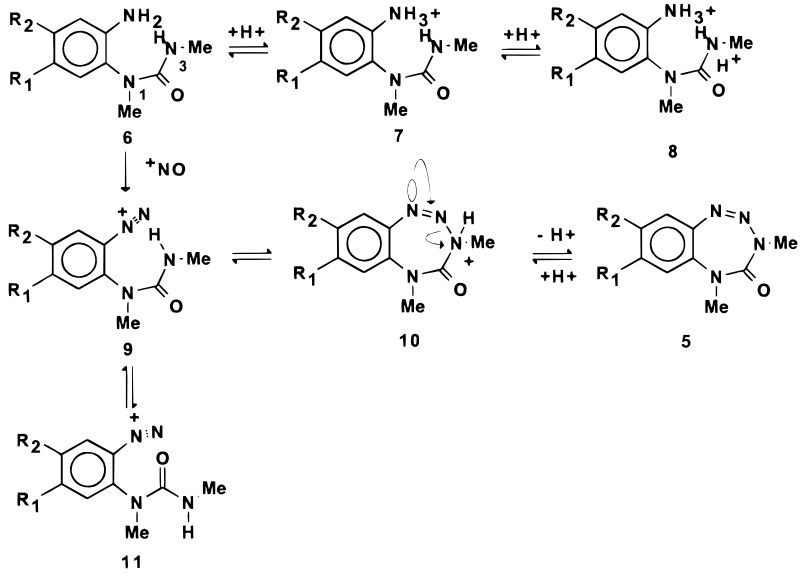


Figure 1. ^{15}N NMR spectra of urea **6a** (a) at pH 9, (b) at pH 4 and (c) in 2.5 M HCl.



Scheme 1

tolysis of the tetrazepinone ring is reminiscent of the scission of acylazourea **12** to give alkyldiazonium species **13** and urea **14** at low pHs, as described by Smith and co-workers[20,21] (Scheme 2).

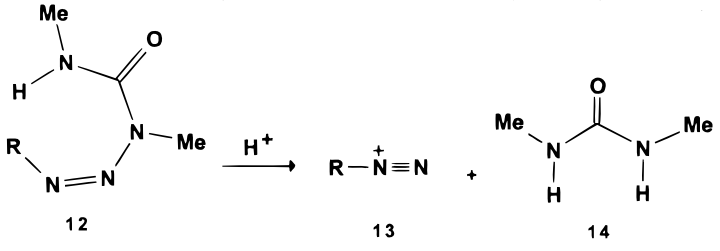
As shown in Fig. 1(c), the ureido moiety of **6** was protonated in 2.5 M HCl (O or N protonation). However, at pH 4, almost no exchange was observed at N-3 [Fig. 1(b)]. Similarly, in the same pH range (pH 5), N-3 in the diazotized urea did not appear to be protonated but the system remained acyclic with a diazonium peak detectable in the -60 ppm range. This suggests that neutralization of the ureido moiety may not be a requirement for cyclization. Also, at basic pH (pH 9), N-3 in the urea precursor did not seem to be prone to deprotonate [Fig. 1(a)], thus excluding the hypothesis that proton abstraction would occur at N-3 prior to cyclization. Hence it is likely that the mechanism by which the tetrazepinone is formed is based on the deprotonation of the tetrazepinium ion **10** as shown in Scheme 1.

^{15}N NMR of tetrazepinones

The chemical shift assignment was based on ^{15}N specific labeling and literature values. In open-chain 1-aryl-3-alkyltriazenes of type **3**, the shifts of N-1, N-2 and N-3 are in the range -23, $+67$ and -227 ppm, respectively.[5,7–9] Wilman[5] reported that N-1 was shielded by electron-withdrawing substituents on the aromatic ring whereas N-3 was deshielded. This is in agreement with the proposed overlap of the N-3 lone pair to form an extended π-system (see resonance structure **15**). In contrast, N-1 was about half as shielded in the tetrazepinone ring system, with a positive shift value ranging from $+45$ to $+43$ ppm. A positive value ($+23$ ppm) has already been reported for N-1 in the open-chain triazenes of type **3** only when N-3 was acetylated.[5] The situation is similar to the tetrazepinone ring structure in which N-3 is carbamoylated. Cross-conjugation with the ureido moiety decreases the involvement of the N-3 lone pair in the adjacent triazene extended π-system.

The most striking difference between the electronic character of tetrazepinones and those of open-chain triazenes is the strong substituent dependence of the N-2 shifts [$\delta_{N\text{-}2}$ in **5a** = 63 ppm, $\delta_{N\text{-}2}$ in **5f** = 80.4 ppm (Table 1)]. The correlation between the shifts of N-2 and the Hammett substituent constants for benzotetrazepinones is shown in Fig. 2. Poor correlations were obtained for N-1, N-3 and N-5. However, it is noteworthy that N-1 as in the triazene series was slightly shielded by electron-withdrawing groups, which suggests resonance structure **16**.

The dependence of the shifts of N-2 on the electronic character of the aromatic ring may be due to the stereoelectronic effect of the N-1 lone pair, which may exert a destabilizing effect on the N-2—N-3 linkage because of its transoid orientation [24, 25] (see structure **10**, Scheme 1). This effect would contribute to an elongation of the N-2—N-3 bond and an increase in the sp character of N-2. Calculations using PM3[12,13] and *ab initio* (3–21G, 6–311G)[14,15] methods consistently showed an elongation of the N-2—N-3 bond as the electron-donating character of the substituents was increased (Table 2). Resonance structure **17** or **18** would



Scheme 2

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

No.	R_1	R_2	σ (*m*, *p*)[a]	N-1	N-2	N-3	N-5
5a	OCH_3	H	−0.27 (*p*)	44.00	63.00	−197.72	−276.00
5b	CH_3	H	−0.17 (*p*)	45.10	68.50	−197.72	−277.60
5c	H	H	0	45.50	71.20	−197.30	−278.00
5d	H	OCH_3	0.12 (*m*)	45.43	74.02	−199.00	−283.00
5e	H	Cl	0.37 (*m*)	43.30	75.30	−195.60	−278.00
5f	NO_2	H	0.78 (*p*)	42.40	80.40	−193.00	−277.50 −15.9 (NO_2)

[a] The substituents positions are relative to the triazene chain.

contribute only little to the tetrazepinones.

It appears that an electron-withdrawing group capable of destabilizing the nascent diazonium ion should deshield N-2 and further stabilize the N-2—N-3 linkage. The observed data are in accordance with this assumption. The tetrazepinone **5a** bearing a methoxy group *para* to the triazene was the most unstable of the series and slowly lost the methylisocyanate moiety to give the corresponding benzotriazole in solution.[10] In contrast, the nitro derivative **5f** was stable enough for x-ray crystallography. A minor peak at around −5 ppm was observed in the spectra of N-2 labelled **5a**,**b** and **d** containing an electron-donating group. This peak corresponded to N-2 in benzotriazole structures as determined by NMR and mass spectrometry and elemental analysis of the isolated compounds.[10] Additional peaks in the −49 to −60 ppm region were observed in the spectra of **5a** and **d**. Peaks in the −49 to −60 ppm region in the spectrum of **5a** may correspond to N-2 in diazonium species or in benzotriazolinium ions of type **19**, which may precede the loss of the alkylisocyanate moiety following cyclization of **11**.

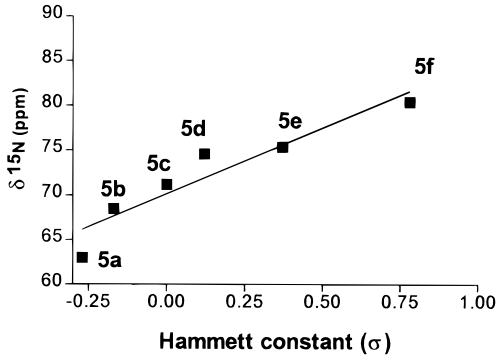


Figure 2. Correlation between Hammett substituent constants (σ) and ^{15}N shifts of benzotetrazepinones **5**.

The ^{15}N shifts of the tricyclic system **4** was almost in the same range as those of tetrazepinones **5b** and **c**. The approximately −7 ppm deshielding of N-5 compared with **5c** is probably due to β-effects exerted by the β-methylene substituent. As previously described, α-effects shield the nitrogen where the β-effect is deshielding[8–10,22,23]. Tricyclic tetrazepinone **4** was stable in the solid and in solution.

Solid-state ^{15}N NMR

Because of the clinically significant antineoplastic activity of **2** and its structural similarity with the tetrazepinones, we thought it of interest to compare its ^{15}N NMR spectrum with that of the most electron-deficient tetrazepinone, **5f**. Moreover, no ^{15}N shifts have yet been reported for **2** in the solid. Because of its limited solubility in chloroform and DMSO, ^{15}N NMR studies in the solid were more appropriate. This results are given in Table 3. Temozolomide showed a negative shift value for N-1 (−21 ppm),[1] suggesting a delocalization of N-3 similar to that observed in open-chain triazenes. More importantly, N-2 was more shielded in the tetrazinone ring of **2** (+31 ppm) than in the nitro-benzotetrazepinone **5f** (89 ppm) in the solid. These shielded shifts are probably due to the high electron density in the six-membered ring tetrazinones. As an example, $\delta_{N\text{-}2}$ in the aromatic benzotriazole is *ca.* −13 ppm.[6] It is also noteworthy that N-3 was more shielded in the tetrazepinone ($\delta_{N\text{-}3} = -172$ ppm) than in the

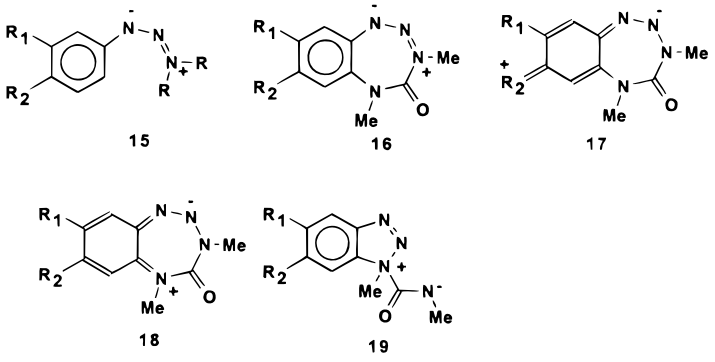
Table 2. N-2 and N-3 PM3 and ab initio bond lengths and calculated N-2, N-3 PM3 partial atomic charges

	N-2/N-3 bond length (Å)			PM3 MOPAC charges		
No.	PM3	3–21G	6–311G	N-2	N-3	N-2 + N-3
5a	1.4523	1.4771	1.4274	−0.0363	−0.0872	−0.1235
5c	1.4522	1.4743	1.4247	−0.0288	−0.0915	−0.1203
5f	1.4459	1.4693	1.4188	−0.0048	−0.0929	−0.0977
4	1.4487	1.4723	1.4227	−0.0287	−0.0899	−0.1186

Table 3. Solid-state ^{15}N NMR of imidazotetrazinone 1 and nitrobenzotetrazepinone 5f

No.	N-1	N-2	N-3	N-5
2	326 (−25)[a]	382 (+31)	251 (−100)	178 (−173)[b]
5f	—[c]	441 (+89)	167 (−184)	88 (−263)[d]

[a] Values in parentheses are converted to the nitromethane scale.
[b] The amide nitrogen resonated at −270 ppm.
[c] Not detected.
[d] The nitro nitrogen was observed at −7 ppm.



tetrazinone ring ($\delta_{N\text{-}3} = -99$ ppm). This is also in accordance with a more significant delocalization of the N-3 lone pair in the tetrazinone ring. Finally, the N-5 shift in the tetrazepinone was in the same range as that of the amido nitrogen in **2** ($\delta_{N\text{-}5}$ in **5f**, −263 ppm; δ_{NHCO} in **2**, −270 ppm). This confirms the high amide character of the N-5—CO linkage in the 1,2,3,5-tetrazepin-4-one ring.

CONCLUSION

The tetrazepinones are among the rare 1,2,3-triazene-containing heterocyclic systems in which little conjugation occurs in the 1,2,3-triazene moiety. This property was evidenced by ^{15}N NMR shifts and also by x-ray crystallographic data which showed an essentially non-planar conformation for the ring system and an almost pyramidal character for N-3.[6,10] Studies on the tetrazepinones are now being directed towards the synthesis of systems containing a strong electron-withdrawing group *para* to the triazene chain and an electron-deficient N-5. We hope that this will promote a shortening of the N-2—N-3 bond and perhaps gives rise to compounds with novel chemical and physical properties.

Acknowledgements

We thank the National Cancer Institute of Canada (NCIC) and the Luigi Barba Fund for financial support. We are also grateful to Dr Francoise Sauriol for technical assistance in running NMR spectra and to Dr. Fred Morin for running solid spectra.

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