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Complete ^{1}H, ^{13}C and ^{15}N NMR assignment of tirapazamine and related 1,2,4-benzotriazine *N*-oxides

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^{1}H, ^{13}C and ^{15}N NMR measurements (1D and 2D including $^{1}H-^{15}N$ gs-HMBC) have been carried out on 3-amino-1, 2,4-benzotriazine and a series of *N*-oxides and complete assignments established. *N*-Oxidation at any position resulted in large upfield shifts of the corresponding N-1 and N-2 resonances and downfield shifts for N-4 with the exception of the 3-amino-1,2,4-benzotriazine 1-oxide in which a small upfield shift of N-4 was observed. Density functional GIAO calculations of the ^{15}N and ^{13}C chemical shifts [B3LYP/6-31G(d)//B3LYP/6-311+G(2d,p)] gave good agreement with experimental values confirming the assignments. The combination of ^{13}C and ^{15}N NMR provides an unambiguous method for assigning the ^{1}H and ^{13}C resonances of *N*-oxides of 1,2,4-benzotriazines. Copyright © 2006 John Wiley & Sons, Ltd.

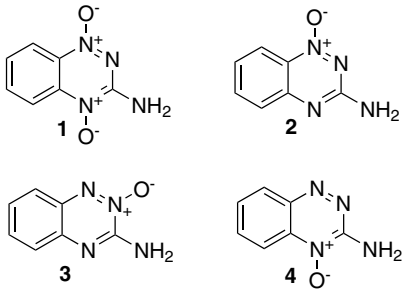
KEYWORDS: NMR; ^{1}H NMR; ^{13}C NMR; ^{15}N NMR; HMBC; benzotriazine; *N*-oxide; tirapazamine; density functional; GIAO

INTRODUCTION

Tirapazamine (**1**) (TPZ, 3-amino-1,2,4-benzotriazine 1,4-dioxide) is a bioreductive antitumor agent[1] that is selectively toxic to hypoxic cells[2] and is currently undergoing Phase III clinical trials in combination with chemo-radiotherapy.[3] TPZ is activated by one-electron reductases[4] to form a radical anion. Under hypoxic conditions, found in an increasing range of solid human tumors,[5,6] the radical anion may be protonated[7] and fragmented to produce a cytotoxic species. This species has been proposed to be either a hydroxyl radical[8] or, via elimination of water, a benzotriazinyl radical.[9] Either reactive species may then react with DNA to produce DNA sugar radicals[10] and the 1-oxide **2**.

As part of a program to develop new analogs[11] of **1,** we wished to unequivocally assign *N*-oxide isomers, isolated from the oxidation of 1,2,4-benzotriazine analogs, and also from bioreductive metabolism studies (Scheme 1). Some ambiguity between the assignments of 2-oxide **3** and 4-oxide **4** existed in the literature[12,13] and the 4-oxide **4** had not been characterized. During the course of this work, Gates *et al*. assigned the 2-oxide **3** and 4-oxide **4** by X-ray crystallography,[14] but did not assign the ^{1}H and ^{13}C spectral data.

The availability of inverse-detection methods has made it possible to acquire ^{15}N data for compounds without isotope enrichment, despite its low natural abundance and gyromagnetic ratio.[15] *N*-Oxidation of alkylamines leads to a considerable downfield shift of the oxidized nitrogen[15,16] while the perturbations at non-oxidized nitrogens in the same molecule are considerably smaller and can shift either upfield or downfield. When the nitrogen atom is part of a conjugated aromatic system, the situation is far less straightforward.[17] In general, the chemical shift of the atom being oxidized does move upfield, but the shifts of other nitrogen atoms in the ring concerned can also experience significant changes, at times greater than those of the oxidized nitrogen atom. Oxidation at N-1 of 1,2,4-benzotriazine has been shown to change the chemical shift of N-1 by 114 ppm upfield.[18] In an effort to see if ^{15}N chemical shift differences on oxidation were large enough to allow us to determine the N–O position in the 3-amino-1,2,4-benzotriazines, we studied the nor-oxide **5**, the 3 mono-*N*-oxides **2**–**4** and the dioxide TPZ **1** by ^{1}H, ^{13}C and ^{15}N NMR spectroscopy.



Scheme 1. *N*-oxides of 3-amino-1,2,4-benzotriazine.

EXPERIMENTAL

Preparation of compounds

Preparation of 1,2,4-benzotriazin-3-amine 1-oxide (**2**). A mixture of 2-nitroaniline (10.0 g, 72.4 mmol) and cyanamide

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(15.2 g, 0.36 mmol) was melted at 100 °C, cooled to *ca* 40 °C and conc. HCl (10 ml) was added dropwise. The resulting exotherm was allowed to subside and the mixture was heated at 100 °C for 1 h. The mixture was cooled to *ca* 40 °C and 30% sodium hydroxide solution (50 ml) added carefully. The mixture was stirred at 100 °C for 2 h, cooled to 25 °C, diluted with water (50 ml) and stirred for 30 min. The suspension was filtered, washed with water (2 × 10 ml), washed with diethyl ether (2 × 5 ml) and dried under vacuum to give 1-oxide **2** (10.3 g, 88%) as a yellow powder: mp (methanol/ethyl acetate) 267–269 °C [lit.[19] mp (ethanol) 269 °C]; ^{1}H NMR δ 8.14 (d, J = 8.7 Hz, 1 H, H-8), 7.79 (dd, J = 8.6, 7.0 Hz, 1 H, H-6), 7.54 (d, J = 8.6 Hz, 1 H, H-5), 7.32–7.38 (m, 3 H, H-7, NH_2).

Preparation of 1,2,4-benzotriazin-3-amine 1,4-dioxide (**1**). Compound **1** was prepared by the method of Seng and Ley.[20] Oxidation of 1-oxide **2** using peracetic acid gave 1,4-dioxide **1** as a red powder: mp (acetic acid) 198–199 °C (lit.[20] 220 °C); 1 H NMR δ 8.21 (d, J = 8.8 Hz, 1 H, H-8), 8.14 (d, J = 8.7 Hz, 1 H, H-5), 8.04 (br s, 2 H, NH_2), 7.95 (ddd, J = 8.7, 7.1, 1.4 Hz, 1 H, H-6), 7.58 (ddd, J = 8.8, 7.1, 1.4 Hz, 1 H, H-7).

Preparation of 1,2,4-benzotriazin-3-amine (**5**). A solution of 1-oxide **2** (1.98 g, 14.3 mmol) and sodium dithionite (5.0 g, 28.7 mmol) in 70% aqueous ethanol (100 ml) was heated at reflux temperature for 3 h. The hot suspension was filtered and the filtrate extracted with chloroform (3 × 50 ml). The combined organic fraction was dried and the solvent evaporated. The combined solid and extracts were purified by chromatography, eluting with a gradient (0–2%) of methanol/chloroform, to give benzotriazine **5** (1.58 g, 67%) as a yellow solid: mp (methanol/chloroform) 200–203 °C (lit.[13] mp 207 °C); ^{1}H NMR δ 8.20 (dd, J = 8.3, 0.9 Hz, 1 H, H-8), 7.78–7.83 (m, 1 H, H-6), 7.62 (br s, 2 H, NH_2), 7.54 (d, J = 8.4 Hz, 1 H, H-5), 7.43–7.48 (m, 1 H, H-7).

Preparation of 1,2,4-benzotriazine 1-oxide (**6**). *t*-Butyl nitrite (90%, 1.05 ml, 8.0 mmol) was added to a stirred solution of 1-oxide **2** (254 mg, 1.6 mmol) in dimethylformamide (10 ml) and the solution stirred at 60 °C for 2 h. The solvent was evaporated and the residue partitioned between ethyl acetate (50 ml) and water (50 ml). The organic fraction was washed with water (2 × 25 ml), washed with brine (20 ml), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–10%) of ethyl acetate/dichloromethane, to give **6** (120 mg, 52%) as a pale yellow solid: mp (ethyl acetate/dichloromethane) 138–139 °C [lit.[12] mp (methanol) 138–140 °C]; ^{1}H NMR δ 9.19 (s, 1 H, H-3), 8.41 (d, J = 8.6 Hz, 1 H, H-8), 8.10–8.13 (m, 2 H, H-5, H-6), 7.90–7.94 (m, 1 H, H-7).

Preparation of 1,2,4-benzotriazin-3-ol 1-oxide (**7**). Sodium nitrite (10.0 g, 145 mmol) was added in small portions to a stirred solution of 1-oxide **2** (11.7 g, 72 mmol) in trifluoroacetic acid (80 ml) at 0 °C and the mixture stirred at 20 °C for 2 h. The mixture was poured into ice/water (800 ml) and stirred for 30 min. The precipitate was filtered, washed with water, and dried to give 3-hydroxy-1-oxide **7** (9.40 g, 80%) as a yellow powder: mp 209–212 °C; [lit.[19] mp (H_2O) 219 °C]; ^{1}H NMR δ 8.14 (d, J = 8.4 Hz, 1 H, H-8), 7.77–7.81 (m, 1 H, H-6), 7.54 (d, J = 8.4 Hz, 1 H, H-5), 7.88–7.92 (m, 3 H, H-7, NH_2); ^{13}C NMR δ 160.2, 148.7, 135.6, 129.8, 125.8, 124.6, 119.8.

Preparation of 3-chloro-1,2,4-benzotriazine 1-oxide (**8**). A mixture of alcohol **7** (9.30 g, 57.0 mmol), dimethylformamide (0.5 ml), and phosphoryl oxychloride (40 ml) was stirred at reflux temperature for 1 h and then poured onto ice and stirred for 30 min. The resulting solid was filtered and washed with water (3 × 10 ml). The filtrate was extracted with ethyl acetate (3 × 50 ml), dried, and the solvent evaporated. The combined solids were purified by chromatography, eluting with 5% ethyl acetate/petroleum ether, to give the 3-chloride **8** (9.52 g, 92%) as a pale yellow powder: mp (dichloromethane) 119–119.5 °C [lit.[12] (methanol) 117–118 °C]; ^{1}H NMR δ 8.38 (dd, J = 8.7, 1.0 Hz, 1 H, H-8), 8.16 (ddd, J = 8.3, 7.0, 1.3 Hz, 1 H, H-6), 8.06 (dd, J = 8.2, 1.0 Hz, 1 H, H-5), 7.90 (ddd, J = 8.7, 6.9, 1.3 Hz, 1 H, H-7); ^{13}C NMR δ 155.3, 146.9, 137.2, 133.9, 131.5, 128.0, 119.9.

Preparation of 1,2,4-benzotriazine (**9**). A solution of 3-chloride **8** (300 mg, 1.65 mmol) in ethanol/ethyl acetate (1 : 1, 50 ml) was stirred vigorously with palladium on carbon (100 mg) under H_2 (60 psi) for 2 h. The mixture was filtered through celite, washed with ethanol (2 × 10 ml), and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–5%) of methanol/dichloromethane, to give **9** (90 mg, 33%) as a white solid: mp (ethyl acetate/petroleum ether) 75–76 °C [lit.[21] mp (light petrol) 76–77 °C]; ^{1}H NMR δ 10.11 (s, 1 H, H-3), 8.59 (d, J = 8.6 Hz, 1 H, H-8), 8.16–8.23 (m, 2 H, H-5, H-6), 8.09 (ddd, J = 8.6, 7.0, 1.0 Hz, 1 H, H-7).

Oxidation of 1,2,4-benzotriazin-3-amine (**5**). A solution of *m*-chloroperbenzoic acid (0.89 g, 3.6 mmol) in dichloromethane (5 ml) was added dropwise to a stirred solution of **5** (0.50 g, 3.4 mmol) in 10% methanol/dichloromethane (50 ml) at 20 °C and the solution stirred at 20 °C for 3 h. The solution was washed with dilute aqueous NH_3 solution (50 ml), dried, and the solvent evaporated. The residue was purified by chromatography, eluting with a gradient (0–5%) of methanol/chloroform, to give (i) 1,2,4-benzotriazin-3-amine 2-oxide (**3**) (405 mg, 70%) as a yellow powder: mp (methanol/dichloromethane) 175–180 °C [lit.[13] mp (acetic acid) 200 °C]; ^{1}H NMR δ 8.20 (br s, 2 H, NH_2), 7.68 (d, J = 8.2 Hz, 1 H, H-8), 7.60–7.65 (m, 1 H, H-6), 7.53 (d, J = 7.6 Hz, 1 H, H-5), 7.45–7.49 (m, 1 H, H-7); followed by (ii) 1,2,4-benzotriazin-3-amine 1-oxide (**2**) (65 mg, 11%) as a yellow powder: mp 266–268 °C, spectroscopically identical with the sample prepared above; and (iii) 1,2,4-benzotriazin-3-amine 4-oxide (**4**) (51 mg, 9%) as pale yellow solid: ^{1}H NMR δ 8.29 (d, J = 8.6 Hz, 1 H, H-8), 8.20 (br s, 2 H, NH_2), 8.16 (d, J = 8.6 Hz, 1 H, H-5), 7.91 (ddd, J = 8.6, 7.0, 1.2 Hz, 1 H, H-6), 7.65 (ddd, J = 8.6, 7.0, 1.1 Hz, 1 H, H-7); MS m/z 162 (M^+, 100%), 146 (10); HRMS calcd for $C_7H_6N_4O$ (M^+) m/z 162.0542, found 162.0540.

NMR

NMR data were collected on a Bruker Avance 400 spectrometer operating at frequencies 400.13 MHz (^{1}H), 100.62 MHz (^{13}C) and 40.55 MHz (^{15}N) and equipped with a 5-mm, triple-resonance (^{1}H/^{13}C/^{15}N), inverse-detection probe with

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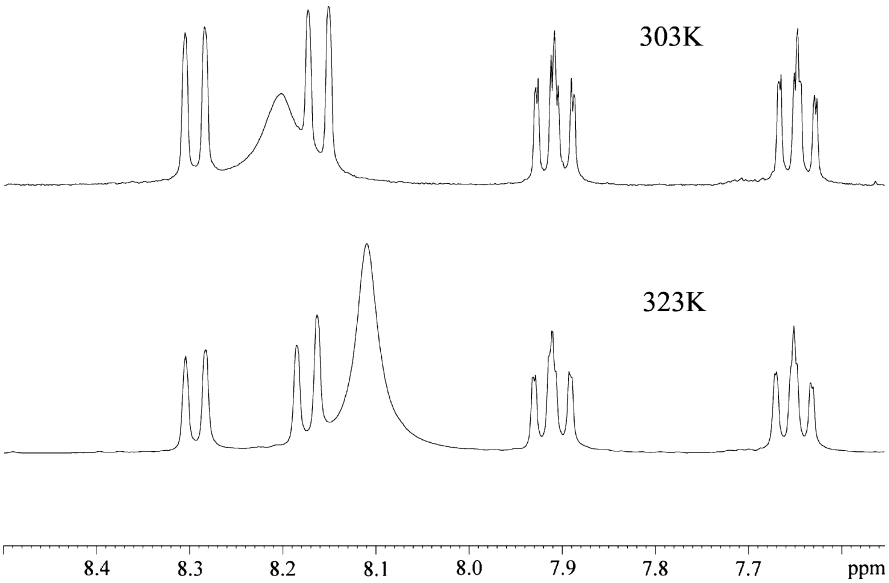

Figure 1. ^{1}H NMR spectrum of 1,2,4-benzotriazin-3-amine 4-oxide (**4**) at 303 K and 323 K.

a self-shielded z-gradient coil for 2D experiments (90° pulse width 7.6/15.8/40 μs). A 5-mm ^{1}H/^{13}C dual probe (90° pulse width 12.0/7.56 μs) was used to observe standard 1D spectra, which were obtained in $(CD_3)_2SO$ at 303 K, and referenced to Me_4Si. Broadening of the amine NH_2 peak made it necessary to heat the samples to 323 K for observation of all expected cross peaks in the 2D spectra (Fig. 1). Typical experimental parameters for one-dimensional ^{1}H/^{13}C spectra were as follows: spectral width 17/240 ppm, data points 32 K/64 K, spectral resolution 0.21/0.40 Hz, number of scans 32/2000, acquisition time 2.42/1.25 s, relaxation delay 0.1/0.75 s, flip angle 30/50°.

Assignments were determined using ^{1}H–^{1}H gs-COSY, ^{1}H–^{13}C gs-HSQC and gs-HMBC, and ^{1}H–^{15}N gs-HMBC two-dimensional experiments using standard pulse sequences from the Bruker pulse library. The pulse conditions are as follows:

^{1}H–^{1}H gs-COSY: Spectral width of 8 ppm was used in both dimensions and the acquisition data size was 2048 points. One transient was acquired per increment, with a 1.5 s relaxation delay, for a total of 256 experiments.

^{1}H–^{13}C gs-HSQC and gs-HMBC: A spectral width of 8 ppm in F_2 and 50 ppm (120–170 ppm) in F_1 was used and the HSQC/HMBC experiments were optimized for C–H coupling of 140/8.3 Hz. Typically, acquisition data size was 2048 points and the number of increments for time evolution was 256. The number of scans per increment was 2/4 with 0.5/1 s delays between transients. Gradient ratios were 40 : 10 and 50 : 30 : 40% for the ^{1}H–^{13}C gs-HSQC and gs-HMBC experiments, respectively.

^{1}H–^{15}N gs-HMBC: A spectral width of 8 ppm in F_2 and 400 ppm in F_1 was used and the experiments were optimized for N–H coupling of 10 Hz. Acquisition data size was 2048 points and the number of increments for time evolution was 128. The number of scans per increment varied between 212 and 360 depending on the time available to optimize signal to noise, and the delay between transients was set to 1.5 s. Data sets were zero-filled and Fourier transformed to give a final matrix of 2048 × 1024 points using sine-bell weighting functions. The gradient ratio was 70 : 30 : 50%. Data were referenced to liquid NH_3 using urea as an external standard (CH_3NO_2 = 380 ppm).

COMPUTATIONAL STUDIES

All geometries were optimized with either the B3LYP/6-31G(d) or BPW91/6-31G(d) density functional models using the program Gaussian 03.[22] NMR chemical shieldings were then calculated using the GIAO method with either the B3LYP/6-311+G(2d,p) or BPW91/6-311(d,p)[23] models. ^{15}N and ^{13}C chemical shifts were then calculated with reference to chemical shieldings, using the same models, for urea and tetramethylsilane, respectively. The chemical shifts for nitrogen in the benzotriazine molecules were then calculated relative to urea (at 75 ppm). Both models gave similar trends, and the B3LYP/6-31G(d)//B3LYP/6-311+G(2d,p) results are used in the discussion. Natural bond orbital analyses were carried out using the NBO program.[24]

RESULTS AND DISCUSSION

We used a modified procedure of Mason and Tennant[13] to unambiguously synthesize 1-oxide **2** (Scheme 2). Oxidation of **2** with trifluoroperacetic acid gave the 1,4-dioxide **1**. Reaction of 1-oxide **2** with sodium dithionate in refluxing EtOH gave the nor-oxide **5**. Reaction of 1-oxide **2** with *t*-butyl nitrite in DMF gave the 3-H 1-oxide **6**, while diazotization of **2** to the 3-hydroxy-1-oxide **7** and chlorination gave the 3-chloro-1-oxide **8**. Catalytic hydrogenation of **8** gave the parent 1,2,4-benzotriazine **9**. Treatment of 3-amino-1,2,4-benzotriazine (**5**) with MCPBA[14] gave four products. The major product was the 2-oxide **3** (79%), with small amounts of 1-oxide **2** (11%) and 4-oxide **4** (9%), and trace amounts of the 3-hydroxy-1-oxide **6**.

Unambiguous assignment of the nitrogen resonances was dependent on cross peaks between the amine NH_2 peak and

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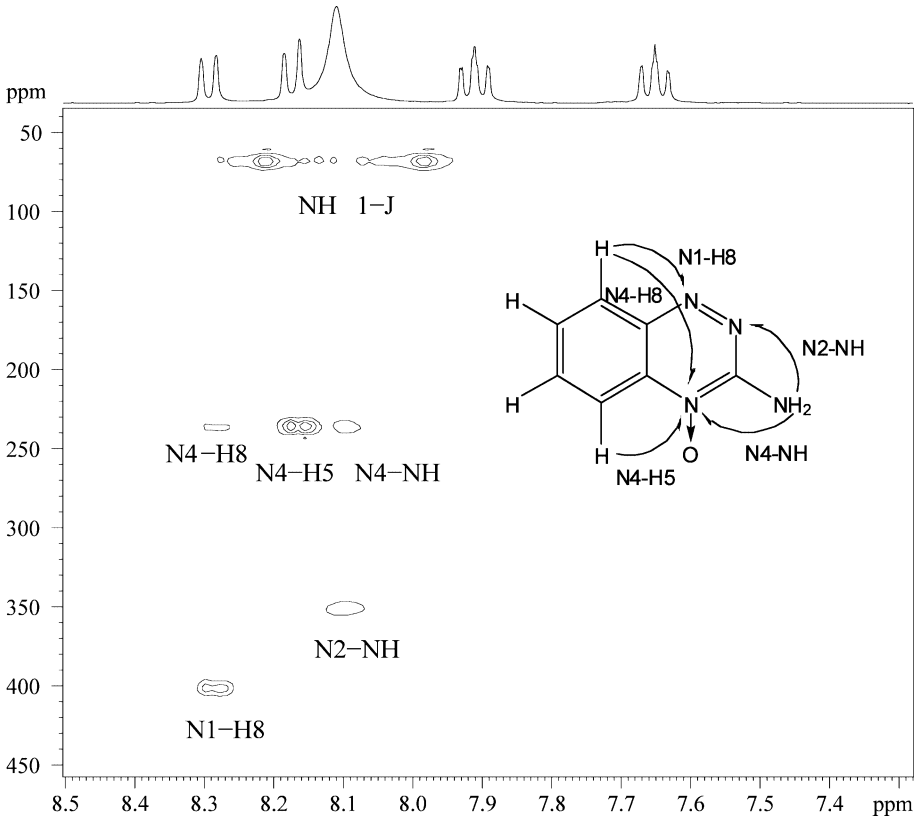
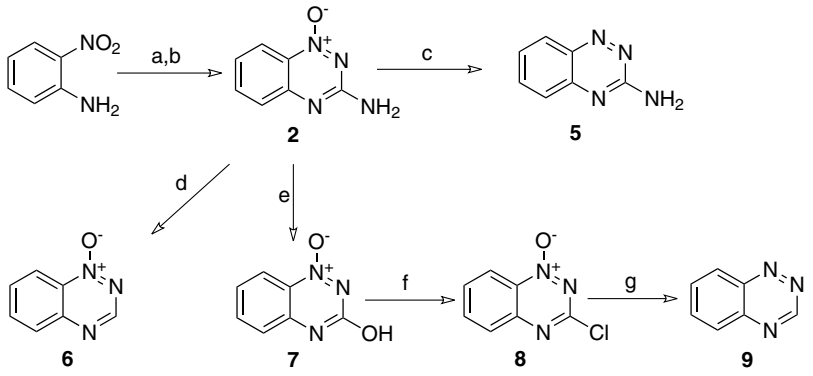


Figure 2. ^{1}H–^{15}N gs-HMBC spectrum of 1,2,4-benzotriazin-3-amine 4-oxide (**4**).



Scheme 2. Synthesis of 1,2,4-benzotriazines. Reagents: (a) NH_2CN, cHCl; (b) NaOH; (c) $Na_2S_2O_4$, 70% aq. EtOH; (d) *t*-butyl nitrite, DMF; (e) $NaNO_2$, TFA; (f) $POCl_3$, DMF; (g) H_2, Pd/C, EtOH/EtOAc.

N-2 and N-4. The amine NH_2 peak was frequently broad; hence it was necessary to heat the samples to 323 K for observation of all expected cross peaks in the ^{1}H–^{15}N HMBC spectra (Fig. 1). Once the assignment of nitrogen resonances was complete, cross peaks between H-8 and N-1 and H-5 and N-4 allowed unambiguous assignment of those protons. Subsequent COSY, HSQC, and ^{1}H–^{13}C HMBC led to the full assignment of the proton and carbon resonances.

The natural-abundance, 10-Hz optimized ^{1}H–^{15}N HMBC spectrum of the 4-oxide **4** is shown in Fig. 2. The doublet arising from 1–*J* coupling of the 3-NH_2 group is obvious at 69.6 ppm. In addition to this direct correlation, long-range couplings from the amine protons to N-2 and N-4 (350.4 and 236.9 ppm) could be observed. Observation of coupling from the aromatic proton at 8.16 ppm to the nitrogen at 236.9 ppm allowed assignment of the aromatic proton as H-5 and the nitrogen as N-4. Coupling was observed from the aromatic proton H-8 at 8.29 ppm to the remaining nitrogen at 400.5 ppm, as was a weak 4–*J* coupling from H-8 to N-4. Standard two-dimensional spectroscopy was then used to assign all the proton and carbon resonances in the molecule. A similar procedure was used for all the other molecules studied and the unambiguous assignments are shown in Table 1. 1,2,4-Benzotriazine (**9**) and the corresponding 1-oxide **6** were studied as well, and their nitrogen NMR agrees with that published by Witanowski[18] (Table 2).

Oxidation of the parent benzotriazine **5** caused the N-1 resonance to shift upfield by 55–154 ppm with the largest shift for the 1,4 dioxide **1** and the smallest for the 4-oxide **4**. The chemical shift of N-2 was similarly affected in all cases

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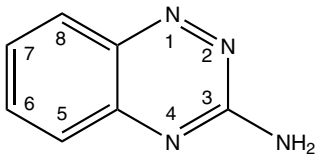
Table 1. ^{1}H, ^{13}C and ^{15}N chemical shift data for compounds **1–5**



	Nor-oxide **5**			1-Oxide **2**			2-Oxide **3**			4-Oxide **4**			1,4-Dioxide **1**		
Position	^{1}H	^{13}C	$^{15}N^{a}$	^{1}H	^{13}C	$^{15}N^{a}$	^{1}H	^{13}C	$^{15}N^{a}$	^{1}H	^{13}C	$^{15}N^{a}$	^{1}H	^{13}C	$^{15}N^{a}$
1	–	–	455.5	–	–	332.7	–	–	335.4	–	–	400.5	–	–	301.1
2	–	–	383.7	–	–	293.6	–	–	297.4	–	–	350.4	–	–	283.9
3	–	160.4	–	–	160.2	–	–	149.1	–	–	153.6	–	–	151.3	–
4	–	–	209.9	–	–	204.8	–	–	216.0	–	–	236.9	–	–	220.6
4a	–	141.8	–	–	148.7	–	–	133.7	–	–	133.4	–	–	138.3	–
5	7.54	125.8	–	7.54	125.8	–	7.53	123.9	–	8.16	115.8	–	8.14	117.0	–
6	7.80	135.6	–	7.79	135.6	–	7.62	130.8	–	7.91	134.9	–	7.95	135.3	–
7	7.47	124.7	–	7.35	124.6	–	7.47	125.8	–	7.65	127.0	–	7.58	126.8	–
8	8.20	129.2	–	8.14	119.8	–	7.68	124.1	–	8.29	129.5	–	8.21	121.0	–
8a	–	142.0	–	–	129.8	–	–	135.5	–	–	142.7	–	–	130.5	–
NH_2	7.61	–	79.0	7.34	–	79.2	8.20	–	73.4	8.20	–	69.6	8.04	–	71.2

[a] ^{15}N chemical shifts are referenced relative to liquid NH_3 at 0 ppm.

Table 2. ^{1}H, ^{13}C and ^{15}N chemical shift data for compounds **6** and **9**

	Nor-oxide **9**			1-Oxide **6**		
Position	^{1}H	^{13}C	$^{15}N^{a}$	^{1}H	^{13}C	$^{15}N^{a}$
1	–	–	445.0 (448.2)[b]	–	–	331.4 (334)[b]
2	–	–	401.0 (403.4)[b]	–	–	317.1
3	10.11	153.6	–	9.19	154.0	–
4	–	–	279.0 (281.9)[b]	–	–	265.5
4a	–	140.1	–	–	146.8	–
5	8.19	128.4	–	8.12	128.8	–
6	8.20	136.3	–	8.12	136.1	–
7	8.09	131.7	–	7.92	131.5	–
8	8.59	129.0	–	8.41	119.6	–
8a	–	147.6	–	–	134.9	–

[a] ^{15}N chemical shifts are referenced relative to liquid NH_3 at 0 ppm.
[b] Ref. 18.

but with a slightly smaller range (33–100 ppm). The N-4 resonance showed significantly different results. In only one case, 1-oxide **2**, was an upfield shift of 5 ppm observed. In all other *N*-oxides, the shift was downfield, with the largest shift of 27 ppm for the 4-oxide **4**, while the 2-oxide **3** and the 1,4-dioxide **1** showed similar, small downfield shifts of 6 ppm and 11 ppm, respectively.

At the time the original ^{1}H NMR assignments for tirapazamine were reported, many of the peaks were unresolved.[12] Long-range correlations between ^{1}H and ^{15}N can also be used to assign the now-resolved ^{1}H resonances. During the course of our work, Fuchs *et al.*[14] unambiguously differentiated the 2-oxide **3** and 4-oxide **4** using X-ray crystallography. Their unassigned ^{1}H and ^{13}C NMR data are in close agreement with the data presented here and provide independent confirmation of our method of assignment.

The calculated ^{15}N and ^{13}C GIAO [B3LYP/6-31G(d)//B3LYP/6-311+G(2d,p)] chemical shifts for compounds **1–5** were obtained by reference to urea and tetramethylsilane, respectively (Table 3). The calculated ^{13}C and ^{15}N NMR chemical shifts plotted *versus* the corresponding experimentally assigned chemical shifts (Figs 3 and 4, respectively) showed reasonable agreement when compared to the experimental data, with linear relationships being observed. However, the slopes of these lines deviated from unity as is commonly observed. This has been ascribed to an overestimation of the negative paramagnetic contribution to the chemical shieldings.[25] The diamagnetic and paramagnetic contributions to the chemical shieldings in the benzotriazines were then calculated. As expected, the diamagnetic contributions (σ_{dia}) varied only slightly for the different

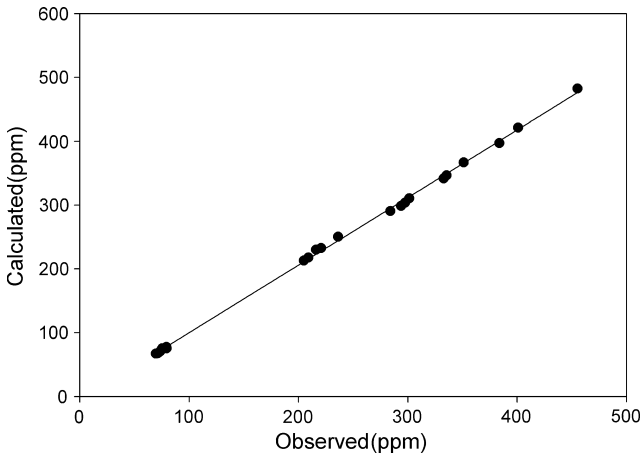


Figure 3. Plot of calculated (Chestnut-scaled) ^{15}N chemical shifts for compounds **1–5** *versus* experimental shifts. (Slope = 1.0594, R = 0.9995).

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Table 3. Calculated chemical shifts B3LYP/6-31G(d)//B3LYP/6-311+G(2d,p)[a]

	Nor-oxide **5**		1-Oxide **2**		2-Oxide **3**			4-Oxide **4**	1,4-Dioxide **1**	
Position	^{13}C	^{15}N[b]	^{13}C	^{15}N[b]	^{13}C	^{15}N[b]	^{13}C	^{15}N[b]	^{13}C	^{15}N[b]
1	–	515.2	–	363.0	–	369.7	–	449.4	–	328.3
	–	*482.2*	–	*341.9*	–	*346.6*	–	*421.5*	–	*310.5*
2	–	422.2	–	315.7	–	321.6	–	388.8	–	307.0
	–	*397.3*	–	*298.7*	–	*303.9*	–	*366.9*	–	*290.6*
3	166.3	–	167.5	–	154.7	–	162.9	–	159.0	–
	160.9	–	*162.0*	–	*149.5*	–	*157.6*	–	*153.8*	–
4	–	230.2	–	224.9	–	242.1	–	263.7	–	245.1
		217.7	–	*213.0*	–	*230.0*	–	*250.4*	–	*232.5*
4a	147.7	–	156.5	–	139.7	–	142.0	–	146.0	–
	142.1	–	*151.3*	–	*134.7*	–	*136.9*	–	*141.0*	–
5	132.6	–	132.7	–	130.1	–	123.6	–	123.7	–
	127.7	–	*127.7*	–	*125.3*	–	*118.9*	–	*119.1*	–
6	140.5	–	140.5	–	135.8	–	139.9	–	139.6	–
	135.6	–	*135.6*	–	*131.0*	–	*135.0*	–	*134.6*	–
7	128.9	–	128.7	–	131.7	–	131.2	–	130.2	–
	124.3	–	*124.1*	–	*127.0*	–	*126.5*	–	*125.5*	–
8	137.9	–	126.9	–	131.0	–	137.7	–	128.4	–
	132.9	–	*122.2*	–	*126.2*	–	*132.7*	–	*123.7*	–
8a	151.2	–	138.7	–	144.4	–	154.7	–	138.9	–
	146.4	–	*134.1*	–	*139.6*	–	*149.9*	–	*134.3*	–
NH_2	–	77.8	–	76.1	–	70.5	–	66.9	–	67.7
	–	*77.4*	–	*75.8*	–	*70.6*	–	*67.4*	–	*67.9*

[a] Values in italics are scaled using the equation $\sigma_{dia} + k\sigma_{para}$: ^{13}C ($k = 0.952$) Ref. 20, ^{15}N ($k = 0.916$) this work.
[b] ^{15}N chemical shifts are referenced relative to liquid NH_3 at 0 ppm.

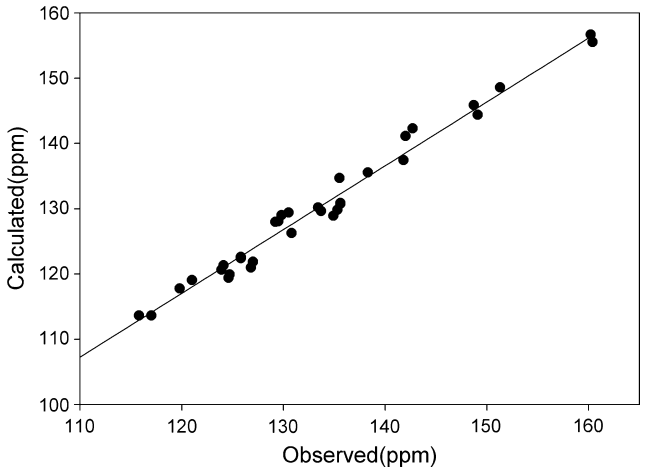

Figure 4. Plot of calculated (Chestnut-scaled) ^{13}C chemical shifts for compounds **1–5** *versus* experimental shifts. (Slope = 1.0338, $R = 0.9881$).

carbon and nitrogen atoms while the paramagnetic contributions (σ_{para}) covered the whole range of chemical shifts. Following the recent work of Chesnut,[26] a scaling parameter, k, for the overestimation of the paramagnetic contribution was obtained from least-squares fits to plots of σ_{para} *versus* the difference between the observed nitrogen chemical shifts δ and σ_{dia} (calculated). The value of k (0.916) was in good agreement with that reported by Chesnut of 0.926. In the case of the ^{13}C chemical shifts the scaling constant from Chesnut of 0.952 was used.[26] Scaled chemical shieldings were then calculated from $\sigma_{dia} + k\sigma_{para}$ and chemical shifts recalculated (Table 3). Figures 3 and 4 show plots of the calculated chemical shifts *versus* the experimental values for the nitrogen and carbon chemical shifts, respectively. The calculated ^{15}N chemical shifts are of the same order as found experimentally and the insensitivity of the N-4 shift relative to N-1 and N-2 for each analog is also reproduced.

Large variations in chemical shifts in aminopyridines and aminobenzenes have been associated with changes

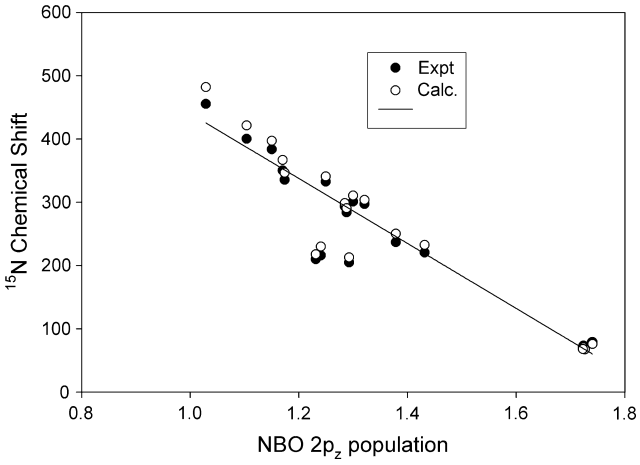

Figure 5. Plot of calculated (Chestnut-scaled) ^{15}N chemical shifts for compounds **1–5** *versus* N 2p$_z$ population.

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in the N(π) $2p_z$ electron population and its effect on the paramagnetic term.[23,27,28] Fig. 5 shows a plot of the calculated and experimental ^{15}N chemical shifts as a function of the N(π) $2p_z$ NBO population.[24] A linear relationship similar to that reported by Barfield[23] was found; however the N-4 chemical shifts for compounds **1–3** deviated markedly. The origin of this deviation can be traced to the insensitivity of the paramagnetic term to variation of the benzotriazines **1–5**.

CONCLUSIONS

^1H, ^{13}C and ^{15}N NMR measurements were carried out on 3-amino-1,2,4-benzotriazine **5** and a series of *N*-oxides **1–4**, as well as the parent 1,2,4-benzotriazine **9** and its 1-oxide **6**. Unambiguous assignment of the ^{15}N peaks and correlation to ^1H resonances using $^1H-^{15}N$ gs-HMBC spectra allowed assignment of H-5 and H-8. This information was then used to fully assign the proton and carbon spectra of all the compounds studied. *N*-Oxidation at any position resulted in large upfield shifts of the corresponding of N-1 and N-2 resonances; however *N*-oxidation resulted in downfield shifts for N-4 with the exception of the 1-oxide **2** in which a small (5 ppm) upfield shift was observed. Calculation of ^{15}N and ^{13}C GIAO [B3LYP/6-31G(d)//B3LYP/6-311+G(2d,p)] gave good agreement with experimental values, especially when the paramagnetic contribution to the chemical shielding was scaled. This variation was attributed to changes in the N(π) $2p_z$ electron population and was most evident for the N-4 chemical shifts of compounds **1–3**. The combination of ^{13}C and ^{15}N NMR provides an unambiguous method for assigning the ^1H and ^{13}C resonances of *N*-oxides of 1,2,4-benzotriazines, which is in close agreement with calculated values from GIAO calculations. Furthermore, the data is in agreement with unassigned ^1H and ^{13}C data from previous X-ray crystallographic determinations.[14]

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