Received: 14 December 2010

Revised: 25 January 2011

(wileyonlinelibrary.com) DOI 10.1002/mrc.2750

Published online in Wiley Online Library: 31 March 2011

The observed and calculated ¹H and ¹³C chemical shifts of tertiary amines and their *N*-oxides

Radek Pohl, Martin Dračínský, Lenka Slavětínská and Miloš Buděšínský*

A series of model tertiary amines were oxidized *in situ* in an NMR tube to amine *N*-oxides and their ¹H and ¹³C NMR spectra were recorded. Next, the chemical shifts induced by oxidation ($\Delta\delta$) were calculated using different GIAO methods investigating the influence of the method [Hartree-Fock (HF), Moeller-Plesset perturbation, density functional theory (DFT)], the functional applied in the DFT (B3LYP, BPW, OPBE, OPW91) and the basis set used [6-31G*, 6-311G**, 6-311++G** and 6-311++G(3df,3pd)]. The best results were obtained with the HF/6-311++G** and OPBE/6-311++G** methods. The computation/experiment comparison approach was used for the configuration prediction of chiral amine *N*-oxides – (*R*) and (*S*)-agroclavine-6-*N*-oxide. Copyright © 2011 John Wiley & Sons, Ltd.

Supporting information may be found in the online version of this article.

Keywords: NMR; ¹H; ¹³C; in situ oxidation of tertiary amines; calculated chemical shifts; HF; MP2; DFT

Introduction

N-oxides of tertiary amines bearing different substituents on the nitrogen atom are chiral compounds with *N*-central chirality. Typical examples of such compounds can be found in various classes of alkaloids isolated from natural sources, e.g. (+)-bulbocapnine- β -*N*-oxide^[1] from *Glaucium fimbrilligerum*, (+)-5,17-dehydromatrine *N*-oxide^[2] from *Euchresta japonica*, pericine *N*-oxide^[3] from *Kopsia arborea* and (4*S*)-corynoxeine *N*-oxide^[4] from *Uncaria rhynchophylla*. Chiral *N*-oxides have also found application in asymmetric catalysis, e.g. in asymmetric cyanosilylation of ketones^[5] in borane-mediated reduction of ketones^[6] or in asymmetric allylation of aldehydes.^[5]

In the early years of NMR when the configuration of chiral *N*-oxides was determined by chemical evidence^[7] or X-ray,^[8] the effect of the N-O group in amine *N*-oxides was observed for the first time in ¹H NMR. The reports dealing with the configuration determination of chiral *N*-oxides by NMR spectroscopy are mainly based on nuclear Overhauser effect (NOE) contacts of alkyl substituents on the nitrogen atom with other parts of the molecule.^[1,9–12] The ¹J_{C,C} coupling constants were also used for the discrimination of *N*-epimeric amine oxides.^[13]

In the literature, there are several examples of the combination of experimental NMR data with calculated NMR parameters (shielding nuclear constants and scalar couplings) addressing various stereochemical problems, e.g. the conformation of sparteine *N*oxides,^[14,15] the conformation and configuration of tertiary amines via GIAO-derived ¹³C chemical shifts and a multiple independent variable regression analysis,^[16] the conformation of *N*-substituted piperidines and pyrrolidines^[17] or the relevance of the calculated ¹H, ¹³C and ¹⁵N shieldings in amines in conformational analysis.^[18] GIAO NMR calculations combined with probability analysis were also used for the assignment of stereochemistry when the NMR data were available only for one diastereoisomer.^[19] Therefore, we decided to test the experiment/calculation comparison approach in the prediction of chiral *N*-oxide configuration. This article deals mainly with the *in situ* oxidation of model achiral tertiary amines 1a-5a to amine *N*-oxides 1b-5b (Fig. 1), a description of the methodology and a screening of appropriate calculation methods. The application of the method is then demonstrated on diastereoisomeric *N*-oxides – (*R*) and (*S*)-agroclavine-6-*N*-oxides.

Experimental

All the amines **1a**–**5a**, the deuterated solvents (CDCl₃ and CD₂Cl₂) and *m*-chloroperbenzoic acid (MCPBA) used in this work were obtained commercially from Sigma-Aldrich Co. The amine *N*-oxides **1b**–**5b** were prepared by an *in situ* oxidation of corresponding amines **1a**–**5a** with MCPBA in an NMR tube with a CDCl₃ solution.

The NMR spectra were measured on a Bruker Avance 600 (with ¹H at 600.13 MHz and ¹³C at 150.9 MHz frequency) using a 5-mm TXI cryo-probe and about 5-10 mg of sample in 0.6 ml of CDCl₃. The chemical shifts are given in δ -scale [with the ¹H shifts referenced to TMS and the ¹³C referenced to CDCl₃ using δ (CDCl₃) at 77.00 ppm].

The typical experimental conditions for the ¹H NMR spectra were 16 scans, a spectral width of 6 kHz and an acquisition time of 5 s, yielding 60 K data points. The free induction decays (FIDs) were zero-filled to 128 K data points.

* Correspondence to: Miloš Buděšínský, Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic. E-mail: budesinsky@uochb.cas.cz

Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czech Republic



Figure 1. The structures of model tertiary amines and their N-oxides.

The typical experimental conditions for the APT ¹³C NMR spectra were 64 scans, a spectral width of 30 kHz and an acquisition time of 1 s, yielding 60 K data points. The FIDs were zero-filled to 128 K data points.

The two-dimensional (2D) homonuclear (H,H-COSY and H,H-rotating frame NOE spectroscopy) and 2D heteronuclear [H,C-heteronuclear single-quantum correlation and H,C-HMBC) experiments were performed when needed for the structural assignments of the signals (with standard 2D NMR pulse sequences of Bruker software being used). For compounds **4a** and **5a**, the low-temperature ¹H and ¹³C NMR spectra (at 0, -25, -50 and -75°C) in CD₂Cl₂ were also measured.

The geometry optimizations and chemical-shift calculations were performed using the Gaussian 03 or Gaussian 09 software package.^[20] The molecular geometries were optimized at the B3LYP/6-311++G^{**} level of theory *in vacuo*. The nuclear mag-

netic shielding constants were calculated at different levels of theory including the Hartree-Fock (HF) approximation, Moeller-Plesset perturbation (MP2) theory and density functional theory (DFT). In the DFT calculations, B3LYP, BPW and the exchange-correlation functionals OPBE and OPW91 were used. In all the methods, four basis sets [$6-31G^*$, $6-311G^{**}$, $6-311++G^{**}$ and 6-311++G(3df,3pd)] were used.

Results

Experimental NMR data

The oxidation reactions were monitored by the ¹H and ¹³C NMR spectra as shown for the in situ oxidation of N-methylpyrrolidine 3a in Fig. 2. Since MCPBA could in general also protonize the starting amine, we measured the NMR spectra of ammonium trifluoroacetates obtained by an insitu protonation of the corresponding amines with trifluoroacetic acid (TFA) to determine the resonances that could originate from protonized forms. Protonation is manifested in the ¹ H NMR spectra by the appearance of an NH proton and its coupling with protons in vicinal positions. However, the protonated form of tertiary amines was not observed after the addition of MCPBA to amines 1a-5a probably due to the low acidity of MCPBA (pKa = 3.82) in comparison with TFA (pKa= 0.23). The protonation of **1a-5a** does not significantly change the ¹³C chemical shifts and the protonation-induced ¹³C chemical shift is -3.0 to 0.6 ppm for C- α , whereas the oxidation-induced ¹³C chemical shift is 9.7–13.4 ppm for C- α . In ¹H NMR, both the protonation and oxidation of **1a-5a** change the ¹H chemical shifts dramatically and the induced ¹H chemical shifts for H- α are 0.45-1.40 and 0.89-1.69 ppm, respectively.



Figure 2. The *in situ* protonation and oxidation of *N*-methylpyrrolidine **3a** monitored by the ¹H (left) and APT ¹³C NMR (right) spectra: (a) *N*-methylpyrrolidine **3a** in CDCl₃; (b) the spectra after an addition of TFA – the formation of protonized **3a**; (c) the spectra after an addition of MCPBA – the formation of **3b**.

Table 1. The 'H and 'SC mean absolute errors (in ppm) of selected computational methods for the calculation of the induced chemical shift in <i>N</i> -oxidation of 1 and 2									
Basis set HF		MP2	B3LYP	BPW	OPBE	OPW91			
MAE for the ¹ H induced chemical shift									
6-31G*	0.39	0.37	0.34	0.31	0.33	0.32			
6-311G**	0.41	0.41	0.34	0.31	0.35	0.34			
6-311++G**	0.44	0.45	0.39	0.34	0.36	0.36			
6-311++G(3df,3pd)	0.42	0.46 ^a	0.37	0.33	0.35	0.35			
MAE for the ¹³ C induced chemical shift									
6-31G*	0.75	2.48	2.12	2.30	1.77	1.77			
6-311G**	0.79	2.47	2.27	2.43	1.69	1.88			
6-311++G**	0.60	2.10	1.70	1.87	1.06	1.06			
6-311++G(3df,3pd)	0.74	3.36 ^a	1.81	1.98	1.12	1.12			
^a Calculated for 1 only.									

Selection of calculation method

Two simple amines 1a and 2a and their N-oxides 1b and 2b were used for the selection of the appropriate calculation method. The molecular geometry of 1a and 2a and their oxidized products 1b and 2b are well defined, and there is no worry about a possible conformation equilibrium. The nuclear magnetic shielding constants were calculated (see Supporting information) for 1a, 1b, 2a and 2b employing the methods listed in Table 1.

Calculation of ¹H and ¹³C chemical shifts induced by oxidation $(\Delta \delta \text{ values})$

The calculated oxidation-induced chemical shifts ($\Delta \delta_{calc}$ = $\sigma_{\rm amine} - \sigma_{\rm aminoxide}$) were compared with the experimentally observed values ($\Delta \delta_{obs} = \delta_{aminoxide} - \delta_{amine}$), and the mean absolute errors (MAEs) were calculated according to Eqn (1):

$$\mathsf{MAE} = \frac{1}{n} \sum_{i=1}^{n} |\Delta \delta_{i(\mathsf{calc})} - \Delta \delta_{i(\mathsf{obs})}| \tag{1}$$

The results obtained by the different calculation methods are compared in Table 1.

We experienced very similar ¹H MAE for all the tested methods (0.31–0.46 ppm), reflecting a serious underestimation of $\Delta\delta$ (see also Supporting information). This is in agreement with our previous observation for the oxidation-induced chemical shift of sulfoxides,^[21] which might be explained by neglecting the solvent effect and vibrational averaging.^[22] Previous calculation^[23,24] showed that using the polarizable continuum model for the inclusion of the solvent effect did not improve the results of the calculation.

A much more diverse situation is seen for ¹³C, where the MAE appeared in the 0.60-3.36 ppm interval depending on the method. Table 1 shows that the HF method provided the best results (lowest MAEs) with only a small effect of the basis set. Rather surprisingly, the highest MAEs were in general obtained by the MP2. For the DFT method, we tested the popular B3LYP hybrid functional, which yielded slightly better results than the BPW functional. The OPBE and OPW91 functionals, which were revealed as promising DFT functionals for the calculation of nuclear shielding constants,^[25,26] were found to be more advantageous than the other DFT functionals. The selection of the basis set appeared to be important and the addition of diffusion functions to heavy atoms and hydrogens significantly improved the MAEs.

Calculation of the ¹H and ¹³C chemical shifts (δ values)

The values of $\Delta \delta$ are not always accessible especially if one wonders about an unknown N-oxide configuration of one particular compound. In this case, the referencing of the calculated shielding constant to a reference compound is necessary. The most used reference compound is TMS, but methane^[27] as the simplest hydrocarbon was also used for the ¹H and ¹³C referencing in the theoretical calculations. We referenced the calculated nuclear shielding constants of 1a, 1b, 2a and 2b to TMS [$\delta_{ref}(H) = 0$ ppm, $\delta_{ref}(C) = 0$ ppm] and methane [$\delta_{ref}(H) =$ 0.22 ppm, $\delta_{ref}(C) = -4.63$ ppm]^[28] calculated at the same level of theory according to Eqn (2):

$$\delta_i = \sigma_{\rm ref} - \sigma_i + \delta_{\rm ref} \tag{2}$$

The results are summarized in Table 2. It is obvious that using a different reference compound will provide different values of δ . Going from $6-31G^*$ to $6-311++G^{**}$, the differences are smaller. For the ¹H data, the chemical shift differences using two reference compounds are 0.32, 0.28, 0.03 and 0.12 ppm for HF/6-31G*, HF/6-311++G**, OPBE/6-31G* and OPBE/6-311++G**, respectively. For the ¹³C data, the differences using two reference compounds are 5.5, 3.3, 3.0 and 1.3 ppm for HF/6-31G*, HF/6-311++G**, OPBE/6-31G* and OPBE/6-311++G**, respectively. HF/6-31G* underestimated both the ¹H and ¹³C chemical shifts, but the other methods listed in Table 2 have performed relatively well. Therefore, even the inexpensive OPBE/6-31G* method could be used for the prediction of amine N-oxide chemical shift, especially ¹³C.

Conformation and NMR chemical shifts of 3a-5a and 3b-5b

The shielding of a nucleus is very sensitive to the neighboring environment, and it is therefore not surprising that chemical shifts reflect the conformation of a molecule. When we consider the calculation of chemical shifts, we have to be careful with the conformational equilibria, and weighted NMR parameters according to the Boltzmann distribution of possible conformers should be used. Therefore, we decided to explore the conformation equilibria for compounds **3a**–**5a** and **3b**–**5b**.

The conformation of **3a** was studied by microwave spectroscopy^[29] or by gas electron diffraction.^[30] We used the known concept of pseudorotation^[31] and calculated the energies

						0005		OPPE	
		6-31G*		6-311++G**		6-31G*		6-311++G**	
¹ H chemical shifts									
1a	2.22	2.06	1.74	2.06	1.78	2.06	2.09	2.16	2.28
1b	3.44	2.77	2.45	2.69	2.40	3.08	3.12	3.06	3.21
2a	2.85	2.49	2.17	2.51	2.22	2.68	2.71	2.85	2.97
	1.53	1.26	0.94	1.28	0.99	1.32	1.35	1.41	1.53
	1.74	1.37	1.05	1.23	0.94	1.64	1.67	1.50	1.62
2b	3.82	2.95	2.63	2.83	2.54	3.01	3.04	2.96	3.08
	2.09	1.62	1.30	1.61	1.32	1.95	1.98	1.99	2.11
	2.15	1.45	1.13	1.37	1.08	1.66	1.69	1.65	1.77
				¹³ C chemical s	hifts				
1a	47.57	41.82	36.32	43.78	40.49	45.06	42.06	46.40	47.76
1b	60.17	55.21	49.71	56.97	53.68	60.26	57.26	60.32	61.68
2a	47.63	42.02	36.52	45.48	42.19	47.90	44.90	51.23	52.59
	26.57	24.48	18.78	27.76	24.37	27.44	24.44	30.47	31.83
	20.58	18.67	13.17	22.31	19.02	23.23	20.23	26.07	27.43
2b	61.07	56.94	51.44	59.90	56.61	63.94	60.94	66.21	67.57
	25.94	23.70	18.20	26.29	23.63	28.49	25.49	30.85	32.21
	19.72	18.39	12.89	22.17	18.88	22.56	19.56	25.56	26.92

^a Referenced to tetramethylsilane, calculated at the same level of theory. ^b Referenced to methane, calculated at the same level of theory.

^b Referenced to methane, calculated at the same level of theory.



Figure 3. The conformation search for *N*-methylpyrrolidine **3a.** (a) The energy map for 201 conformers with different *P* and ϕ_{max} and (b) the energy profile for $\phi_{max} = 45^{\circ}$.

(B3LYP/6-31G^{**}) for 201 conformers with a variation of the pucker amplitude ϕ_{max} (0° \rightarrow 50°, 5° step) and phase angle P (0° \rightarrow 360°, 18° step; for the definition of ϕ_{max} and P, see Supporting information). The preferred conformations were found for $\phi_{max} = 45^{\circ}$, $P = 90^{\circ}$ and $\phi_{max} = 40^{\circ}$, $P = 270^{\circ}$ as shown in Fig. 3. In the subsequent step, the preferred conformers were fully optimized using B3LYP/6-311++G^{**}, and ΔG_{298} was obtained by vibrational analysis (Fig. 4).

In the case of **3b**, we considered the similar conformations with the CH₃ group in the axial and equatorial positions and calculated the corresponding ΔG_{298} (Fig. 4). In six-membered rings **4a**, **5a**, **4b** and **5b**, CH₃ can again occupy the axial or equatorial position. The calculated ΔG_{298} of optimized conformers **4a**_{ax}, **4a**_{eq},**5a**_{ax}, **5a**_{eq}, **4b**_{ax}, **4b**_{eq},**5b**_{ax} and **5b**_{eq} (Fig. 4) showed that the predominant conformation is the one with a CH₃ group in the equatorial position, which is in agreement with the published knowledge.^[32–34]

The experimental ¹H NMR spectra of **4a** and **5a** showed in CDCl₃ at 25 °C a significant line broadening of the α and γ (in the case of **4a**) protons caused by a nitrogen inversion and the chemical exchange of the axial and equatorial protons. This process can be slowed down by cooling the sample to -75 °C. At this temperature, sharp signals of one conformer **4a**_{eq} and **5a**_{eq} are observed.

We calculated the ¹H and ¹³C nuclear shielding constants for two conformers of **3a,b-5a,b** using the OPBE/6-311++ G^{**} method



Figure 4. The conformation preferences for 3a-5a and 3b-5b.

Table 3. A comparison of the experimental and calculated ^{a 13} C chemical shifts of <i>N</i> -oxides $3b_{eq}-5b_{eq}$ and $3b_{ax}-5b_{ax}$									
	3b exp	3b_{eq} calc	3b_{ax} calc	4b exp	4b_{eq} calc	4b_{ax} calc	5b exp	5b _{eq} calc	5b_{ax} calc
C -α	68.6	70.4	73.2	66.4	67.2	72.2	65.0	66.5	71.2
C -β	21.9	26.6	24.9	20.9	24.2	28.4	61.4	64.5	67.1
C -γ	-	-	-	21.3	25.5	25.9	-	-	-
CH₃	54.4	55.7	55.2	57.8	61.1	53.4	59.0	61.2	52.9
MAE		2.6	2.8		2.9	5.6		2.3	6.0
$^{\rm a}$ OPBE/6-311++G**, referenced to TMS, calculated at the same level of theory.									

(see Supporting information). To demonstrate the importance of the correct molecular geometry on the calculated chemical shifts, we compared the experimental and calculated ¹³C chemical shifts of *N*-oxides **3b**_{eq} – **5b**_{eq} and **3b**_{ax} – **5b**_{ax} (Table 3).

The results in Table 3 show that the calculated ¹³C chemical shifts, especially C- α and CH₃ in the vicinity of the N-O group, of major conformers **3b**_{eq}-**5b**_{eq} are closer to the experimental values. The MAEs for **3b**_{eq}-**5b**_{eq} are lower than those for

 $3b_{ax}-5b_{ax}$, which demonstrates not only the necessity of a properly chosen molecular geometry for the calculation of the chemical shifts but also the relevance of the calculated chemical shifts in conformation analysis.

Determination of N-O configuration in agroclavine 6-N-oxides

The main goal of our project is to find the method for an unambiguous assignment of the N-O configuration in chiral amine *N*-oxides containing at least one additional element of chirality. To demonstrate the computation/experiment comparison approach, we calculated the nuclear-shielding constants and referenced the chemical shifts for agroclavine **6a**, 6*S*-agroclavine *N*-oxide *S***-6b** and 6*R*-agroclavine *N*-oxide *R***-6b (Fig. 5) and compared them with the already published NMR data.^[11]**

As arises from the DFT calculations, agroclavine **6a** can exist as a mixture of two conformers differing by the nitrogen configuration. First, the geometry of the two conformers of **6a** was optimized using B3LYP/6-311++G^{**} with a vibrational analysis providing the conformation equilibrium (Fig. 6) and then the geometry of *N*-oxides **S-6b** and *R***-6b** was optimized by the same method. For **S-6b** and *R***-6b**, only one conformer was found and considered for the calculation of NMR parameters.



Figure 5. The structure and numbering of agroclavine 6a, 6S-agroclavine N-oxide S-6b and 6R-agroclavine N-oxide R-6b.



Figure 6. The conformers of agroclavine 6a.

Table 4.	The observed and calculated chemical shifts for 6a, S-6b and R-6b									
	ба		S-6b		<i>R</i> -6b		S-6b		<i>R</i> -6b	
	obs ^a	calc ^b	obs ^a	calc ^b	obs ^a	calc ^b	ind;obs ^c	ind;calc ^d	ind;obs ^c	ind;calc ^d
Proton										
H-2	6.91	6.85	6.95	6.91	6.96	6.98	0.04	0.06	0.05	0.13
H-4ax	2.72	2.94	3.24	3.80	2.83	2.73	0.52	0.86	0.11	-0.21
H-4eq	3.33	3.21	3.40	3.06	3.89	4.65	0.07	-0.15	0.56	1.44
H-5	2.47	2.69	3.37	3.60	3.42	3.32	0.90	0.91	0.95	0.63
H-7a	2.93	2.88	3.67	3.38	3.90	3.60	0.74	0.50	0.97	0.72
H-7b	3.24	3.30	4.07	4.35	3.90	3.77	0.83	1.05	0.66	0.47
H-9	6.22	6.59	6.37	6.65	6.26	6.55	0.15	0.06	0.04	-0.04
H-10	3.68	3.98	4.25	4.97	3.69	3.86	0.57	0.99	0.01	-0.12
H-12	6.91	7.21	7.02	7.37	6.93	7.17	0.11	0.16	0.02	-0.04
H-13	7.06	7.43	7.12	7.43	7.09	7.39	0.06	0.00	0.03	-0.04
H-14	7.14	7.20	7.19	7.26	7.19	7.29	0.05	0.06	0.05	0.09
H-17	1.80	1.82	1.84	1.85	1.85	1.91	0.04	0.03	0.05	0.09
NC H ₃	2.47	2.44	3.28	3.01	3.10	2.87	0.81	0.57	0.63	0.43
Carbon										
C-2	119.7	113.3	120.7	113.8	120.8	115.0	1.0	0.5	1.1	1.7
C-3	112.1	112.2	110.2	110.8	110.6	112.3	-1.9	-1.4	-1.5	0.1
C-4	27.7	30.4	22.9	24.9	23.1	25.5	-4.8	-5.5	-4.7	-4.9
C-5	65.7	64.0	74.6	75.5	77.4	77.5	8.9	11.5	11.7	13.5
C-7	61.6	61.8	73.4	74.6	75.4	78.0	11.8	12.8	13.8	16.1
C-8	135.6	132.2	128.2	126.6	130.8	132.3	-7.4	-5.5	-4.8	0.2
C-9	121.2	121.0	121.7	121.4	121.2	120.7	0.5	0.5	0.0	-0.2
C-10	42.1	44.6	38.7	41.4	41.8	45.7	-3.5	-3.2	-0.3	1.2
C-11	133.0	130.1	131.1	128.7	130.7	128.3	-1.9	-1.4	-2.3	-1.9
C-12	113.3	111.3	114.2	111.9	114.2	111.5	0.9	0.6	0.8	0.2
C-13	123.7	120.6	124.2	120.7	124.1	120.3	0.5	0.1	0.5	-0.3
C-14	110.1	104.6	110.7	105.2	111.1	105.8	0.6	0.5	1.0	1.2
C-15	132.8	129.0	135.6	128.5	135.9	129.2	2.8	-0.5	3.1	0.2
C-16	127.8	123.2	127.1	122.2	127.5	123.2	-0.7	-1.1	-0.3	0.0
C-17	21.2	23.7	21.0	24.0	20.8	23.6	-0.2	0.4	-0.4	-0.1
N C H₃	41.3	40.3	58.5	57.4	49.8	48.9	17.3	17.1	8.6	8.7

^a Measured in CD₃OD; see reference [11].

 $^{\rm b}$ OPBE/6-311++ $\rm G^{**};$ referenced to TMS, calculated at the same level of theory.

^c Observed chemical shift induced by oxidation ($\Delta \delta_{obs} = \delta_{aminoxide} - \delta_{amine}$).

^d Calculated chemical shift induced by oxidation ($\Delta \delta_{calc} = \sigma_{amine} - \sigma_{aminoxide}$).

The nuclear-shielding constants were calculated afterwards on the optimized geometries using the OPBE/6-311++G^{**} method. The data for **6a** were weighted according to the calculated ratio of the conformers at 298 K. We calculated the oxidation-induced chemical shifts ($\Delta \delta_{calc} = \sigma_{amine} - \sigma_{aminoxide}$), experimentally observed values ($\Delta \delta_{obs} = \delta_{aminoxide} - \delta_{amine}$) and also the chemical

shifts referenced to TMS, calculated by the same method. The results are presented in Table 4.

The ¹H NMR data clearly reflect the orientation of the N-O group. For example, in **S-6b**, where the N-O group is axial, the H-4ax and H-10 protons (for the structure, see Supporting information) are significantly deshielded by the known 1,3-diaxial



Figure 7. The optimized geometries of S-6b and R-6b with the specified 1,3-diaxial interaction of the N-O group and the corresponding proton.



Figure 8. The correlation between the calculated (referenced to TMS) and observed ¹³C and ¹H chemical shifts of 6a, S-6b and R-6b.

interaction between a particular proton and the N-O group, but hardly any change in chemical shift for those protons is observed for **R-6b** (Fig. 7), whereas, on the other hand, H-4eq is deshielded because of the parallel orientation of C-H4eq and the N-O bonds. The experimentally observed effect is nicely reproduced by the calculations as follows from Table 4. A dramatic change in the ¹³C chemical shift is observed for the carbon of CH₃-N. Depending on its arrangement, the chemical shift can differ by about 10 ppm for the two diastereoisomers **S-6b** and **R-6b** owing to the γ -gauche interaction of N-CH₃ in **R-6b**. Again, the observed chemical shifts for N-CH₃ correlate very well with those calculated.

To present the performance of the OPBE/6-311++G^{**} calculation method, we correlated the calculated referenced chemical shifts with those observed for **6a**, **S-6b** and **R-6b** as shown in Fig. 8. As arises from the correlations, the calculated ¹³C chemical shifts can be used for the unambiguous assignment of N-O configuration in chiral amine *N*-oxides. The correlation for ¹H is less satisfactory, but some effects, e.g. 1,3-diaxial interaction, could be useful in determining the configuration.

Conclusions

We have tested several computation methods of ¹H and ¹³C chemical-shift calculation on a series of model tertiary amines and their achiral N-O counterparts. The HF/6-311++G^{**} method worked well for the induced chemical shifts. OPBE/6-311++G^{**} provided the best results from the tested DFT methods yielding

satisfactory results for both oxidation-induced and referenced chemical shifts of tertiary amines and their *N*-oxides.

We confirmed that a correct molecular geometry is an essential requirement for obtaining realistic chemical shifts by calculation. In addition, the calculated chemical shifts can help in conformation analysis in some cases.

The computation/experiment comparison approach was successfully applied to the N-O group configuration of 6S-agroclavnine N-oxide **S-6b** and 6R-agroclavnine N-oxide **R-6b**, indicating that the method can be used for determining the configuration of chiral N-oxides. This preliminary finding will be subjected to more extensive study with N-oxides of alkaloids and other chiral tertiary amines in our next publication.

Acknowledgements

This work was supported by the Czech Science Foundation (Grant No. 203/09/1919).

Supporting information

Supporting information may be found in the online version of this article.

References

[1] A. Shafiee, K. Morteza-Semnani, M. Amini, J. Nat. Prod. **1998**, 61, 1564.

- [2] S. Ohmiya, H. Otomasu, J. Haginiwa, I. Murakoshi, *Phytochemistry* 1978, 17, 2021.
- [3] K. Lim, O. Hiraku, K. Komiyama, T. Koyano, M. Hayashi, T. Kam, J. Nat. Prod. 2007, 70, 1302.
- [4] B. Ma, C. Wu, J. Yang, R. Wang, Y. Kano, D. Yuan. Helv. Chim. Acta 2009, 92, 1575.
- [5] A. Malkov, P. Kocovsky, *Eur. J. Org. Chem.* **2007**, 29.
- [6] I. O'Neil, C. Turner, S. Kalindjian, *Synlett* **1997**, 777.
- [7] N. Mandava, G. Fodor, *Can. J. Chem.* **1968**, *46*, 2761.
- [8] C. Huber, G. Fofdor, N. Mandava, *Can. J. Chem.* **1971**, *49*, 3258.
- [9] G. Caldwell, A. Gauthier, J. Mills, *Mag. Reson. Chem.* **1996**, *34*, 505.
 [10] G. Caldwell, A. Gauthier, J. Mills, M. Greco, *Mag. Reson. Chem.* **1993**,
- [10] G. Caldwell, A. Gauthier, J. Mills, M. Greco, *Mag. Reson. Chem.* **1993**, 31, 309.
- [11] V. Kren, J. Nemecek, V. Prikrylova, Collect. Czech. Chem. Commun. 1995, 60, 2165.
- [12] S. Pospisil, P. Sedmera, P. Halada, L. Havlicek, J. Spizek, *Tetrahedron Lett.* 2004, 45, 2943.
- [13] F. Potmischil, H. Herzog, J. Buddrus, *Mag. Reson. Chem.* **1998**, *36*, 240.
- [14] J. Thiel, W. Boczon, P. Fiedorow, B. Jasiewicz, M. Knychala, J. Mol. Struct. 2002, 642, 15.
- [15] B. Jasiewicz, *Molecules* **2008**, *13*, 3.
- [16] A. Sebag, D. Forsyth, M. Plante, J. Org. Chem. 2001, 66, 7967.
- [17] C. Lobato-Garcia, P. Guadarrama, C. Lozada, R. Enriquez, D. Gnecco, W. Reynolds, J. Mol. Struct. 2006, 786, 53.
- [18] I. Alkorta, J. Elguero, *Mag. Reson. Chem.* **2004**, *42*, 955.
- [19] S. Smith, J. Goodman, J. Am. Chem. Soc. 2010, 132, 12946.
- [20] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, X. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada,

M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, V. N. Staroverov, R. Kobayashi, K. N. Kudin, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, V. G. Zakrzewski, R. L. Martin, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, D. J. Fox, Gaussian, Inc., Wallingford, CT, 2009.

- [21] M. Dracinsky, R. Pohl, L. Slavetinska, M. Budesinsky, Mag. Reson. Chem. 2010, 48, 718.
- [22] M. Dracinsky, J. Kaminsky, P. Bour, J. Chem. Phys. 2009, 130, 094106.
- [23] M. Dracinsky, P. Bour, J. Chem. Theory Comput. 2010, 6, 288.
- [24] M. Dracinsky, J. Kaminsky, P. Bour, J. Phys. Chem. B 2009, 113, 14698.
- [25] Y. Zhang, A. Wu, X. Xu, Y. Yan, Chem. Phys. Lett. 2006, 421, 383.
- [26] A. Wu, P. Zhang, X. Xu, W. Yan, J. Comp. Chem. 2007, 28, 2431.
- [27] V. Ananikov, Cent. Eur. J. Chem. 2004, 1, 196.
- [28] G. Fulmer, A. Miller, N. Sherden, H. Gottlieb, A. Nudelman, B. Stoltz, J. Bercaw, K. Goldberg, Organometallics 2010, 29, 2176.
- [29] W. Caminati, F. Scappini, J. Mol. Spect. **1986**, 117, 184.
- [30] G. Pfafferott, H. Oberhammer, J. Boggs, J. Am. Chem. Soc. 1985, 107, 2309.
- [31] C. Altona, M. Sundaralingam, J. Am. Chem. Soc. 1972, 94, 8205.
- [32] E. Eliel, D. Kandasamy, C. Yen, K. Hargrave, J. Am. Chem. Soc. 1980, 102, 3698.
- [33] F. dos Santos, C. Tormena, J. Mol. Struct. (Theochem) 2006, 763, 145.
- [34] T. Rosenau, A. Hofinger, A. Potthast, P. Kosma, *Polymer* **2003**, *44*, 6153.