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Are nicotinoids protonated on the pyridine or the amino nitrogen in the gas phase?

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ABSTRACT: The gas-phase basicities (GBs) of 12 nicotinoids were calculated for the two potential sites of protonation, the sp^2 pyridine and the sp^3 amino nitrogen atoms, at the B3LYP/6–311 + G(3df,2p)//B3LYP/6–31G(d,p) level and estimated from substituent effects on the GBs of 2-substituted pyrrolidines and *N*-methylpyrrolidines. It was found that, in contrast to the Nsp^3 protonation in water, nicotinoids with a secondary amino nitrogen (substituted nornicotines, anabasine, anatabine) are protonated on the pyridine nitrogen. Nicotinoids with a tertiary amino nitrogen (substituted nicotines, *N*-methylanabasine, *N*-methylanatabine) are protonated on either the pyridine or the amino nitrogen, depending on the electronic effects of the substituents and the strength of an intramolecular $CH\cdots Nsp^3$ hydrogen bond. Copyright © 2005 John Wiley & Sons, Ltd.
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KEYWORDS: protonation site; nicotinoids; DFT calculations; substituent effects; gas-phase basicity

INTRODUCTION

The site of protonation of nicotine, which bears two basic nitrogen atoms, the pyridine and the pyrrolidine nitrogens, depends on their relative intrinsic strengths and possibly on the solvent.[1] In water, the first pK_a (8.0)[2] corresponds to the protonation of the pyrrolidine nitrogen (Nsp^3), whereas the pK_a of the pyridine nitrogen (Nsp^2) can be estimated as ca 5.7 from the pK_a of pyridine (5.20)[3] and the substituent effect of the 3-(*N*-methylpyrrolidine-2-yl) group.[1,3–5] Therefore, in the solvent water, the protonation on the pyrrolidine nitrogen is preferred by ca 13.3 kJ mol^{-1} on the Gibbs energy scale (Scheme 1). In the gas phase, Fourier transform ion cyclotron resonance (FT-ICR) measurements and density functional theory (DFT) calculations of the Gibbs energy of the deprotonation reaction, defining the gas-phase basicity (GB) of each nitrogen site, showed[1] that the basic strengths of each site become very close (Scheme 2).

In the case of nornicotine, the influence of solvation on the preferred protonation site is even more dramatic: the site of protonation is changed from the sp^3 nitrogen in water to the sp^2 nitrogen in the gas phase (Scheme 3).[1]

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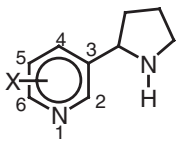
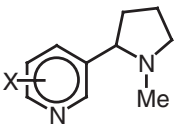
The problem of the site of protonation of nicotine-related molecules (nicotinoids) is important not only in physical organic chemistry but also in pharmaceutical chemistry, since it is known that it is the monoprotonated form of nicotine that is biologically active.[6] The binding of nicotine to nicotinic acetylcholine receptors occurs through a cation–π interaction between the protonated nitrogen of nicotine and aromatic residues of the receptors and/or a hydrogen bond between the pyrrolidine N^+—H and a backbone carbonyl in the region of the binding site.[7–10]

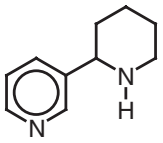
In our laboratories, an earlier study was devoted to the site(s) of protonation of nicotine (**1**) and nornicotine (**2**) in the gas phase, i.e. to the intrinsic base strength of each nitrogen atom.[1] In the present work, we extend our investigations to the 12 new nicotinoids **3**–**14**. Through the theoretical calculation of the intrinsic base strength of each nitrogen atom of these dibasic compounds, we aim to identify the influence of various structural effects on the selectivity of the protonation site. We select compounds **3**–**14** in order to study (i) various electronic effects (field-inductive, resonance, polarisability) of substituents in *ortho* and *meta* positions of the pyridine rings of nornicotine (**3**–**6**) and nicotine (**7**–**10**), (ii) the extension of a five-membered pyrrolidine ring to a six-membered piperidine ring (**11**, **12**) and (iii) the introduction of a double bond in a piperidine ring (**13**, **14**).



X = H (**2**), 6-Me (**3**)
5-C≡CH (**4**),
5-Br (**5**), 5-NO_2 (**6**)



X = H (**1**), 6-t-Bu (**7**)
6-Me (**8**),
5-C≡CH (**9**),
5-Br (**10**)



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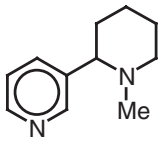

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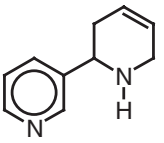

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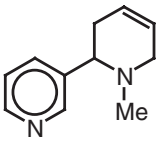

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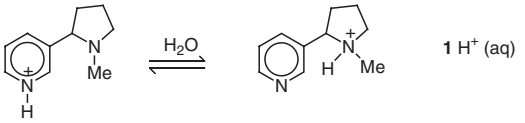
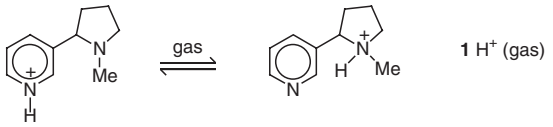


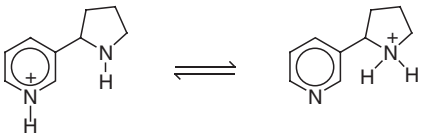
$\Delta G(\text{aq}) = \text{RTln10}\,[pK_a(\text{Nsp}^2) - pK_a(\text{Nsp}^3)] = -13.3\ \text{kJ mol}^{-1}$

Scheme 1



$\Delta G(\text{gas}) = GB(\text{Nsp}^2) - GB(\text{Nsp}^3) = -2\ \text{kJ mol}^{-1}$

Scheme 2



$\Delta G(\text{aq}) = -19.1\ \text{kJ mol}^{-1}$
$\Delta G(\text{gas}) = +20.8\ \text{kJ mol}^{-1}$

Scheme 3

For these nicotinoids, we calculate the *GB* values of each nitrogen atom at the B3LYP/6–311 + G(3df,2p)// B3LYP/6–31G(d,p) level of theory. The difference in *GB* values, $GB(\text{Nsp}^2) - GB(\text{Nsp}^3)$, allows the calculation of the equilibrium constant *K* (at a given temperature) of the proton exchange reaction (Scheme 4) and, consequently, of the percentage of each protonated form. It can be seen in Scheme 4 that a difference of 10 kJ mol^{-1} will give one major form, whereas smaller differences will lead to mixtures of forms protonated on the pyridine nitrogen (written $\text{Nsp}^2\ \text{H}^+$) or on the amino nitrogen ($\text{Nsp}^3\ \text{H}^+$).

GEOMETRIES OF THE UNPROTONATED AND PROTONATED NICOTINOIDS

The determination of theoretical *GB*s required a preliminary investigation of the conformation of unprotonated (neutral), Nsp^2 protonated and Nsp^3 protonated forms of the studied nicotinoids. While several *ab initio* and DFT computational studies of nicotine (**1**) and nornicotine (**2**) conformation have recently been made,[1,11,12] we are not aware of any conformational studies on the nicotinoids **3**–**14**. In this work, 36 new neutral and protonated species were considered at the B3LYP/6–31G(d,p) level of theory, using the Gaussian 98[13] suite of programs.

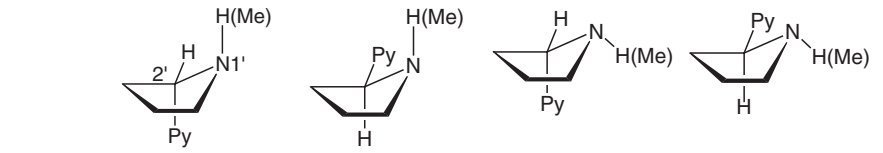
The specification of nicotinoid conformation requires the definition of three stereochemical features:

1. The position of the N1′ substituents (H or Me) relative to the pyridine (Py) ring. Four conformers are possible, as illustrated in Fig. 1 for the example of a pyrrolidine ring with an envelope form.
2. The relative orientation of the two rings, described by the dihedral angle C2C3C2′H2′. The pyridine and the pyrrolidine (piperidine, tetrahydropyridine) rings are always found roughly perpendicular to one another[1,11,12] (see below, Table 3) and two *syn* and *anti* rotamers have been identified[1,11,12] from the

$\text{Nsp}^2\text{H}^+ \overset{K}{\rightleftharpoons} \text{Nsp}^3\text{H}^+$
$\Delta G = GB(\text{Nsp}^2) - GB(\text{Nsp}^3) = -RT\ \ln K$

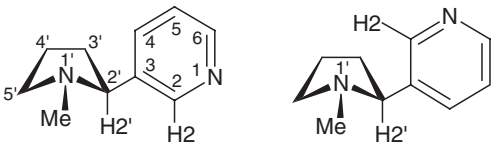
ΔG_{298} (kJ mol^{-1})	K_{298}	% $\text{Nsp}^3\ \text{H}^+$	Protonation site
10	0.02	2	Nsp^2
5	0.13	12	Nsp^2 and Nsp^3
1	0.67	40	Nsp^2 and Nsp^3
0	1	50	$\text{Nsp}^2 = \text{Nsp}^3$

Scheme 4



	axial-axial (ax, ax)	axial-equatorial (ax, eq)	equatorial-axial (eq, ax)	equatorial-equatorial (eq, eq)
This work				
Ref. 11	*trans*	*cis*	*cis*	*trans*

Figure 1. Possible conformers of (nor)nicotine arising from the (pseudo)-axial and equatorial positions of the methyl (hydrogen) group and of the pyridine (Py) ring. Correspondence between the abbreviations of the (nor)nicotine conformers used in this work and in Ref. 11



Syn *Anti*

Figure 2. *Syn* and *anti* rotamers for the (eq, eq) conformer of nicotine

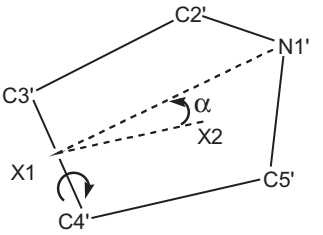


Figure 3. Definition of the ring wagging and twisting coordinates. Points X1 and X2 are the middles of the bonds C3′C4′ and C2′C5′, respectively. The dihedral angle X2C4′X1N1′ (which is equal to X2 C3′X1N1′) defines the wagging coordinate α. The dihedral angle X2X1C4′C5′ (which is equal to X2X1C3′C2′) defines the twisting coordinate β. $\alpha = 0$ and $\beta = 0$ if the ring is planar; $\alpha = 0$ and $\beta \neq 0$ when the ring is twisted; $\alpha \neq 0$ and $\beta = 0$ when the ring adopts the envelope form

respective positions of hydrogen atoms H2 and H2′, as illustrated in Fig. 2 for the example of the (eq, eq) conformer of nicotine.

3. The conformation of the pyrrolidine, piperidine or tetrahydropyridine rings. In the case of pyrrolidine, the ring can adopt (i) an envelope conformation with the N1′ atom out of plane, (ii) a twist conformation or (iii) a conformation between the envelope and twisted forms, which can be specified by two coordinates,[14] the ring wagging angle α and the ring twisting angle β. These angles are defined in Fig. 3.

In the case of neutral nicotine, all computational studies[1,11,12] and a recent gas electron diffraction experiment[12] led to the conclusion that the most stable conformer is (eq, eq, *syn*) (see Fig. 2). The B3LYP/6–31G(d,p) calculations of this work showed that (i) the (eq, eq, *syn*) conformer is more stable than the (eq, eq, *anti*) by 2.0 kJ mol^{-1}, (ii) the C2C3C2′H2′ dihedral angle is 18 ° (pyridine and pyrrolidine rings are roughly perpendicular) and (iii) $\alpha = 16$ ° and $\beta = 1$ °, i.e. the pyrrolidine ring adopts an envelope conformation with the N1′ atom out of plane. The same kind of geometry was found for the other neutral nicotinoids. In all cases, we found that (i) the (eq, eq, *syn*) rotamers have a lower energy than the (eq, eq, *anti*) between 1.3 and 2.4 kJ mol^{-1}, (ii) the two rings are roughly perpendicular, the dihedral angles C2C3C2′H2′ being between 11 and 24 ° and (iii) the dihedral angles α (15–16 °) and β (0–3 °) show that the pyrrolidine rings adopt the envelope form. In the case of neutral anabasine **11** and *N*-methylanabasine (**12**), the six-membered ring exists in a chair conformation. In the case of neutral anatabine (**13**) and *N*-methylanatabine (**14**), the nitrogen atoms are above the plane C3′C4′ C5′C6′ defined by the double bonds and the C2′ atoms are under this plane (Fig. 4). Tables 1–3 present the results of our B3LYP/6–31G(d,p) optimisations of geometries. Table 1 compares the relative energies of the (eq, eq, *syn*) and (eq, eq, *anti*) conformers, Table 2 the dihedral angles C2C3C2′H2′ measuring the approximate perpendicularity between the rings and Table 3 the dihedral angles α and β specifying the conformation of the pyrrolidine ring.

When nicotinoids are protonated on the amino (Nsp^3) nitrogen atom, the results in Table 3 show that the pyrrolidine ring adopts a nearly twisted conformation (except **7** and **10**) while Table 2 shows that the two rings remain approximately perpendicular to one another. Table 1 shows that the (eq, eq, *syn*) conformer remains generally the most stable one but that, in most cases, the energy difference becomes very weak and is even of the opposite sign for *N*-methylanabasine (**13**) and anatabine (**14**).

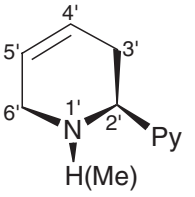


Figure 4. Conformation of the tetrahydropyridine rings of anatabine (**13**) and *N*-methylanatabine (**14**)

Table 1. Conformational energy differences (kJ mol^{-1}) between *syn* and *anti* forms of neutral, Nsp^2 protonated and Nsp^3 protonated nicotinoids [B3LYP/6–31G(d,p) calculations]

No.	Nicotinoid	Neutral *syn*/*anti*[a]	Nsp^2 protonated *anti*/*syn*[a]	Nsp^3 protonated *syn*/*anti*[a]
3	6-Methylnornicotine	2.3	6.2	8.7
2	Nornicotine	2.4	6.0	2.9
4	5-Ethynylnornicotine	1.9	4.7	0.2
5	5-Bromonornicotine	2.4	5.1	0.2
6	5-Nitronornicotine	1.3	6.3	0.1
7	6-*tert*-Butylnicotine	1.8	4.4	2.1
8	6-Methylnicotine	1.9	4.5	8.7
1	Nicotine	2.0	4.6	0.1
9	5-Ethynylnicotine	1.6	4.7	0.7
10	5-Bromonicotine	2.1	4.0	0.4
11	Anabasine	2.2	4.1	0.2
12	*N*-Methylanabasine	2.0	2.8	−0.3
13	Anatabine	2.2	3.4	−0.1
14	*N*-Methylanatabine	2.0	2.0	0.03

[a] Low-energy/high-energy conformer.

Table 2. Dihedral angle C2C3C2′H2′ (°) between the two rings of the most stable conformer of neutral and protonated forms of nicotinoids [B3LYP/6–31G(d,p) calculations]

No.	Nicotinoid	Neutral (eq, eq, *syn*)	Nsp^3 protonated (eq, eq, *syn*)	Nsp^2 protonated (eq, eq, *anti*)
3	6-Methylnornicotine	20.2	−10.4	−130.1
2	Nornicotine	20.9	−9.8	−129.9
4	5-Ethynylnornicotine	22.0	−8.2	−130.8
5	5-Bromonornicotine	22.0	−7.6	−130.7
6	5-Nitronornicotine	23.7	−4.9	−128.7
7	6-*tert*-Butylnicotine	17.8	−8.6	−139.2
8	6-Methylnicotine	17.6	−6.0	−140.6
1	Nicotine	18.3	−8.0	−138.7
9	5-Ethynylnicotine	19.0	−6.1	−141.9
10	5-Bromonicotine	19.3	−5.7	−140.5
11	Anabasine	18.6	−13.9	−152.7
12	*N*-Methylanabasine	15.1	−29.4	−158.8
13	Anatabine	17.2	−8.6	−157.2
14	*N*-Methylanatabine	11.3	−2.7	−163.9

In contrast to the neutral and Nsp^3 protonated nicotinoids, the Nsp^2 (pyridine) protonated forms are more stable in the (eq, eq, *anti*) conformation. The relative energies of the (eq, eq, *anti*) conformers are in the range +2.0 to +6.3 kJ mol^{-1} (Table 1). Brodbelt *et al.*,[15] in the case of the nicotine analogue 3-(*N*,*N*-dimethylaminomethyl)pyridine and Graton *et al.*,[1] in the case of nicotine (**1**) and nornicotine (**2**), have explained this

Table 3. Wagging angle α and twisting angle β (°) of the pyrrolidine ring of the most stable conformer of neutral and protonated nicotinoids [B3LYP/6–31G(d,p) calculations]

No.	Nicotinoid	Neutral (eq, eq, *syn*)		Nsp^3 protonated (eq, eq, *syn*)		Nsp^2 protonated (eq, eq, *anti*)	
		α	β	α	β	α	β
3	6-Methylnornicotine	16.2	1.3	1.9	21.2	6.6	17.6
2	Nornicotine	16.2	2.0	2.3	21.1	6.3	17.8
4	5-Ethynylnornicotine	16.1	1.5	3.1	20.9	6.6	17.7
5	5-Bromonornicotine	16.2	0.2	1.7	21.3	6.5	17.6
6	5-Nitronornicotine	15.4	0.6	1.2	21.4	6.3	17.7
7	6-*tert*-Butylnicotine	15.5	1.1	14.9	0.8	8.4	15.3
8	6-Methylnicotine	15.5	1.1	6.7	18.7	8.7	15.8
1	Nicotine	15.5	1.1	5.2	20.2	8.6	15.8
9	5-Ethynylnicotine	15.3	2.7	5.6	19.4	9.2	15.3
10	5-Bromonicotine	15.4	1.2	15.0	2.1	8.9	15.5

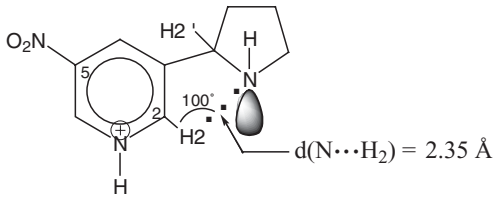


Figure 5. In the (eq, eq, *anti*) conformer of 5-nitronornicotine (**6**) protonated on the pyridine Nsp^2 nitrogen, the positive hydrogen of the C2—H bond points to the lone pair of the pyrrolidine Nsp^3 nitrogen and establishes a C2—H···Nsp^3 intramolecular hydrogen bond inside a five-membered ring

conformational change on Nsp^2 (pyridine) protonation by the formation of a C—H···N(sp^3) intramolecular hydrogen bond (Fig. 5), which is significantly shorter (and hence stronger) in the (eq, eq, *anti*) than in the (eq, eq, *syn*) conformer.

The same explanation can be applied to the (eq, eq, *anti*) preference of the 12 new nicotinoids studied in this work, since we found the same kind of C2—H···N intramolecular hydrogen bond in all these compounds. The length d(N···H) and the angle θ (C2H2N1′) of the hydrogen bond are shown in Table 4. It is interesting that the shortest (strongest) hydrogen bond is found for 5-nitronornicotine (**6**) (2.350 Å; 1 Å = 0.1 nm), which shows the greatest *syn* preference (6.3 kJ mol^{-1}), while the longest (weakest) one is for *N*-methylanatabine (**14**) (2.592 Å) with the least *syn* preference (2.0 kJ mol^{-1}). Indeed, a significant correlation [Eqn (1)] is observed between the relative energy of the (eq, eq, *anti*) conformer (Table 1) and the hydrogen bond length (Table 4):

$$\Delta E(anti/syn) = 43.6 - 16.1d(\mathrm{N}\cdots\mathrm{H}) \qquad (1)$$

$n = 14$, correlation coefficient $r = 0.934$

Table 4. Geometry of the intramolecular C—H···Nsp^3 hydrogen bond in the (eq, eq, *anti*) conformer of the Nsp^2 protonated form of nicotinoids: N···H_2 bond length, *d*, and angle, θ (C2H2N′1) [B3LYP/6–31G(d,p) calculations]

No.	Nicotinoid	d (Å)	θ (°)
3	6-Methylnornicotine	2.375	99.3
2	Nornicotine	2.363	99.6
4	5-Ethynylnornicotine	2.369	99.3
5	5-Bromonornicotine	2.368	99.3
6	5-Nitronornicotine	2.350	99.9
7	6-*tert*-Butylnicotine	2.433	97.6
8	6-Methylnicotine	2.418	98.0
1	Nicotine	2.410	98.0
9	5-Ethynylnicotine	2.421	97.7
10	5-Bromonicotine	2.416	97.9
11	Anabasine	2.453	95.4
12	*N*-Methylanabasine	2.540	94.2
13	Anatabine	2.500	94.2
14	*N*-Methylanatabine	2.592	92.8

This correlation (in units of kJ mol^{-1} and Å) supports the analysis of the nicotinoid conformational change *syn* → *anti* in terms of the formation of an intramolecular hydrogen bond.

Optimized geometries of the 36 structures investigated are available from the authors upon request.

GB CALCULATIONS

The gas-phase basicity (*GB*) of a base B is defined as the Gibbs energy change for the BH^+ (g) → B(g) + H^+(g) deprotonation reaction. The *GB* values were calculated at 298.15 K and 1 bar by the following equations:

$$\Delta G = \Delta H - T\Delta S \qquad (2)$$

$$\Delta H = \Delta E_{\mathrm{elec}} + \Delta ZPVE + \Delta E_{\mathrm{vib}}(T) + 5/2RT \qquad (3)$$

$$\Delta S = S(\mathrm{H}^+) + S(\mathrm{B}) - S(\mathrm{BH}^+) \qquad (4)$$

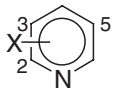
where ΔE_{elec} represents the difference between the electronic energies of the most stable conformation of the products and the reactants at 0 K, $\Delta ZPVE$ is the difference in the zero-point vibrational energies of BH^+ and B, $\Delta E_{\mathrm{vib}}(T)$ accounts for the change in the population of vibrational levels at a temperature T, $5/2RT$ contains the classical term for translation, rotation and the conversion factor of energy to enthalpy and ΔS is the entropy contribution. For H^+, a value of $S = 108.95$ J K^{-1} mol^{-1} at 298 K was employed.[16]

The rotational entropy component is calculated from the B3LYP/6–31G(d,p) geometries. For the vibrational contribution to the entropy and enthalpy, the vibrational frequencies of B and BH^+ were evaluated within the harmonic approximation at the B3LYP/6–31G(d,p) level and scaled by the empirical factor 0.9804.[17] The electronic energies were obtained using a more extended and flexible basis set than for geometries, i.e. at the B3LYP/6–311 + G(3df,2p)//B3LYP/6–31G(d,p) level. This strategy of calculation takes into account the size and number of species studied and has already been justified in our previous work devoted to nicotine and nornicotine.[1] More generally, the good performance of this calculation level has been well documented in the literature.[18–20]

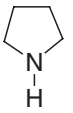
The *GB* values are given in Table 5 for each of the 12 new nicotinoids investigated. Two theoretical values are calculated, one for each protonation site, the pyridine sp^2 and the amino sp^3 nitrogen atoms. The difference between these theoretical values, ΔGB(theor.), should allow identification of the protonation site, as described in Scheme 4, assuming no calculation errors.

These errors arise from the set of approximations used for the calculation of geometries and energies or properties, such as the approximate correlation term introduced by the density functional model, the truncation of the basis set or the harmonic approximation[21] and the

temperature value used in the calculation (298.15 K) being lower than the experimental FTICR value (~338 K). We need to estimate these calculation errors on the *GB* values of nicotinoids. A comparison between the calculated and measured *GB* values for model molecules structurally very similar to the studied nicotinoids should allow the quality of the calculations to be judged. We calculated the *GB* values of 19 models (test molecules) bearing one nitrogen protonation site, for which the experimental *GB* values have been measured. These test molecules are the pyridines **2** and **15**–**22**, pyrrolidines **23**–**26**, piperidine **27**, 1,2,3,6-tetrahydropyridine (**28**), *N*-methylpyrrolidines **29**–**32** and *N*-methylpiperidine (**33**). Table 6 compares the experimental *GB*s[1,22] with the theoretical values calculated at the B3LYP/6–311 + G(3df,2p)//B3LYP/6–31G(d,p) level.



X: 2-*t*-Bu (**15**), 3,5-Me_2 (**16**), 2-Me (**17**), 3-Et (**18**), 3-Me (**19**), H (**20**), 3-Br (**21**), 3-NO_2 (**22**)



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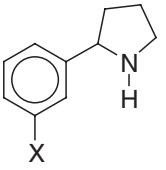
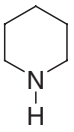


X: CF_3 (**24**), F (**25**), H (**26**)



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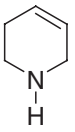

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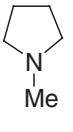

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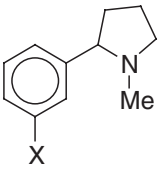
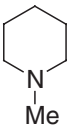


X: CF_3 (**30**), F (**31**), H (**32**)



33

It is striking that the sign of the difference between the computed and experimental values depends on the nature of the protonated nitrogen atom. In the series of tertiary amines **29**–**33**, the calculated *GB*s are always less than the experimental values by 1.2–5.2 kJ mol^{-1} (−3.1 kJ mol^{-1} on average). In contrast, in the series of secondary amines **23**–**28** and pyridines **2** and **15**–**22**, the calculated *GB*s are overestimated in comparison with the experimental values by 2.3–6.5 kJ mol^{-1} (+3.8 kJ mol^{-1} on average) in secondary amines and 2.8–9.9 kJ mol^{-1} (+6.9 kJ mol^{-1} on average) in pyridines.

The differences *GB*(theor.) − *GB*(exp.) result from a combination of calculation and measurement errors. In so far as the experimental *GB*s are believed to be accurate to within 1–2 kJ mol^{-1} on a relative basis within the series of nitrogen bases considered here (see Table 1 in Ref. 1) and since the sign of this difference is systematically and not randomly distributed, we attribute the largest part of the differences to systematic errors of calculation.

A significant cancellation of errors can be obtained by considering isodesmic reactions, such as the proton exchange reaction $B_1H^+ + B_2 \rightarrow B_2H^+ + B_1$ between bases B_1 and B_2. The comparison of very similar bases, B_1 and B_2, enables maximum advantage to be taken of the cancellation of errors on both sides of the reaction. The calculation of the *GB* values of each nitrogen atom of the studied nicotinoids by the method of isodesmic proton exchanges is described in the following section.

ISODESMIC PROTON EXCHANGES

Consider a pair of structurally similar bases, B_1 and B_2. Their *GB* values, *GB*(theor., B_1) and *GB*(theor., B_2), have been calculated at the same level of theory, with some errors, and only the experimental *GB* value of B_2, *GB*(exp., B_2), is known. A corrected theoretical value of the basicity of B_1, *GB*(theor. corr., B_1), can be obtained by using the isodesmic proton exchange reaction (5):

$$B_1H^+ + B_2 \rightarrow B_2H^+ + B_1 \quad (5)$$

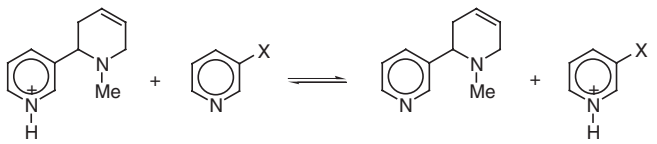
since an important cancellation of calculation errors is expected for the theoretical Gibbs energy of this reaction, ΔG(theor., 5), which is given by Eqn (6). For a given base B_2, the corrected gas phase basicity of B_1 is given by Eqn (7).

$$\Delta G(\text{theor.}, 5) = GB(\text{theor.}, B_1) - GB(\text{theor.}, B_2) \quad (6)$$

$$GB(\text{theor. corr.}, B_1) = GB(\text{exp.}, B_2) + \Delta G(\text{theor.}, 5) \quad (7)$$

The method can be applied to each protonation site, Nsp^2 and Nsp^3, of a nicotinoid B_1, provided that a structurally similar pyridine and amine, respectively, are chosen as bases B_2. Thus, we obtain the corrected basicities of each site, *GB*(theor. corr., B_1, Nsp^2) and *GB*(theor. corr., B_1, Nsp^3). It is recommended to carry out several isodesmic reactions (5) by varying the base B_2 and to take the mean of the results, $\overline{GB}$(theor. corr., B_1, Nsp^2) or $\overline{GB}$(theor.corr., B_1, Nsp^3). The standard deviation of the mean gives a quantitative measure of how well the isodesmic method performs. This method will be illustrated below on the example of

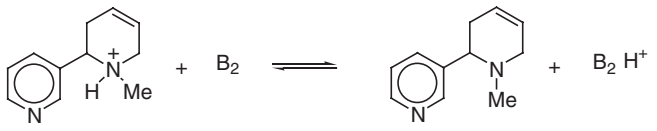
N-methylanatabine (**14**). We calculated the basicity of the Nsp^2 protonation site of *N*-methylanatabine using isodesmic reaction (8):



(8)

As base B_2, we chose from Table 6: nornicotine, pyridine and pyridines *meta*-substituted by alkyl groups. We did not use *ortho*-substituted pyridines and pyridines *meta*-substituted by electron-withdrawing groups Br and NO_2. The details of the calculations (in $kJ\,mol^{-1}$) are shown in Scheme 5. We find $\overline{GB}$(theor.corr., *N*-methylanatabine, Nsp^2) $= 922.9 \pm 1.4\,kJ\,mol^{-1}$ (where the indicated uncertainty is the 95% confidence interval calculated from Student's *t*-test for four degrees of freedom).

In order to calculate the basicity of the Nsp^3 protonation site, we used isodesmic reaction (9), choosing the tertiary amines of Table 6 as bases B_2:



(9)

The details of the calculations (in $kJ\,mol^{-1}$) are shown in Scheme 6. We found $\overline{GB}$(theor.corr.,*N*–methylanatabine, Nsp^3) $= 931.3 \pm 2.3\,kJ\,mol^{-1}$.

Thus, in *N*-methylanatabine, the mean theoretical corrected basicity of the Nsp^3 nitrogen atom remains at $8.4 \pm 3.7\,kJ\,mol^{-1}$ above that of the Nsp^2 nitrogen atom. According to Scheme 4, this corresponds to 87–99% of *N*-methylanatabine protonated on the Nsp^3 atom. In summary, B3LYP/6–311 + G(3df,2p)//B3LYP/6–31G(d,p) calculations, corrected by our empirical isodesmic method, lead to the conclusion that the preferred site of protonation of *N*-methylanatabine is the amino nitrogen atom in the gas phase.

The isodesmic method has been applied to all the nicotinoids studied. For each nicotinoid, we calculated:

- the mean theoretical corrected basicity, $\overline{GB}$(theor. corr.), for each protonation site, Nsp^2 and Nsp^3;
- the difference in basicity between the two sites, $\Delta\overline{GB} = \overline{GB}(Nsp^2) - \overline{GB}(Nsp^3)$;
- the equilibrium constant K of the proton exchange reaction $Nsp^2H^+ \rightleftharpoons Nsp^3H^+$ (see Scheme 4);
- the percentage of nicotinoid protonated on the Nsp^3 site, $\%Nsp^3H^+$.

Table 5. Theoretical gas-phase basicity, *GB* ($kJ\,mol^{-1}$), of the sp^2 pyridine and sp^3 amino nitrogens of nicotinoids and basicity difference $\Delta GB = GB\ (Nsp^2) - GB\ (Nsp^3)$ [B3LYP/6–311 + G(3df,2p)//B3LYP/6–31G(d,p) calculations]

No.	Nicotinoid	Site	*GB* (theor.)	ΔGB (theor.)
3	6-Methylnornicotine	Nsp^2	958.1	
		Nsp^3	924.3	33.8
2	Nornicotine	Nsp^2	939.5	
		Nsp^3	916.0	23.5
4	5-Ethynylnornicotine	Nsp^2	930.7	
		Nsp^3	912.9	17.8
5	5-Bromonornicotine	Nsp^2	917.0	
		Nsp^3	903.1	13.9
6	5-Nitronornicotine	Nsp^2	883.1	
		Nsp^3	882.8	0.3
7	6-*tert*-Butylnicotine	Nsp^2	961.7	
		Nsp^3	940.0	21.7
8	6-Methylnicotine	Nsp^2	952.8	
		Nsp^3	936.0	16.8
1	Nicotine	Nsp^2	936.0	
		Nsp^3	926.9	9.7
9	5-Ethynylnicotine	Nsp^2	928.4	
		Nsp^3	923.5	4.9
10	5-Bromonicotine	Nsp^2	915.3	
		Nsp^3	915.7	−0.4
11	Anabasine	Nsp^2	934.8	
		Nsp^3	920.8	14.0
12	*N*-Methylanabasine	Nsp^2	936.6	
		Nsp^3	935.9	0.7
13	Anatabine	Nsp^2	930.1	
		Nsp^3	912.4	17.7
14	*N*-Methylanatabine	Nsp^2	930.9	
		Nsp^3	928.1	2.8

The results of these calculations, as well as the conclusion about the protonation site(s), are presented in Table 7.

EMPIRICAL ESTIMATION OF *GB* VALUES

It is interesting to compare the *GB*s calculated by quantum chemistry methods with those estimated empirically by using the Taft–Topsom methodology.[23] In this method, the substituent effect on *GB*, δGB, is described by a multilinear structure–energy relationship [Eqn (10)] in terms of substituent constants, σ, and reaction constants, ρ, corresponding to three assumed additive interaction mechanisms between the substituent and the reaction (protonation) site:

$$\delta GB = \rho_F\sigma_F + \rho_R\sigma_R + \rho_\alpha\sigma_\alpha \qquad (10)$$

These are called field/inductive (F), resonance (R) and polarisability (α) effects. For example, the basicity of the Nsp^2 nitrogen of 3-substituted pyridines can be estimated[4] through the equation

$$\delta GB(Nsp^2) = -95.4\sigma_F - 68.2\sigma_R - 17.6\sigma_\alpha \qquad (11)$$

Provided that the substituent constants σ_F, σ_R and σ_α of the *meta*-substituent are known. 5-Substituted nornicotines

Table 6. Comparison of (B3LYP/6–311 + G(3df,2p)//B3LYP/6–31G(d,p)) theoretical and experimental gas-phase basicities, *GB* (kJ mol^{-1}), of test molecules related to nicotinoids

No.	Molecules	*GB* (theor.)	*GB* (exp.)	D^a
Pyridine series (Nsp^2 protonation site)				
15	2-*tert*-Butylpyridine	936.6[b]	929.8[d]	+6.8
16	3,5-Dimethylpyridine	933.4[c]	923.5[d]	+9.9
17	2-Methylpyridine	923.3[c]	917.3[d]	+6.0
18	3-Ethylpyridine	923.6[b]	915.5[d]	+8.1
19	3-Methylpyridine	919.2[c]	911.6[d]	+7.6
20	Pyridine	905.2[c]	898.1[d]	+7.1
21	3-Bromopyridine	884.3[b]	878.1[d]	+6.2
22	3-Nitropyridine	844.4[b]	841.6[e]	+2.8
2	3-(2-Pyrrolidinyl) (nornicotine)	939.5[c]	932.4[c]	+7.3
Secondary amine series (Nsp^3 protonation site)				
23	Pyrrolidine	920.9[c]	915.3[d]	+5.6
24	2-(3-Trifluoromethylphenyl)pyrrolidine	916.1[c]	912.7[c]	+3.4
25	2-(3-Fluorophenylpyrrolidine	923.6[c]	917.1[c]	+6.5
26	2-Phenylpyrrolidine	936.0[c]	933.5[c]	+2.5
27	Piperidine	923.2[b]	920.6[c]	+2.6
28	1,2,3,6-Tetrahydropyridine	914.5[b]	912.2[f]	+2.3
Tertiary amine series (Nsp^3 protonation site)				
29	*N*-Methylpyrrolidine	933.5[c]	934.7[c]	−1.2
30	*N*-Methyl-2-(3-Trifluoromethylphenyl)pyrrolidine	922.5[c]	927.7[c]	−5.2
31	*N*-Methyl-2-(3-fluorophenyl)pyrrolidine	932.5[c]	937.6[c]	−5.1
32	*N*-Methyl-2-phenylpyrrolidine	946.2[c]	948.5[c]	−2.3
33	*N*-Methylpiperidine	937.9[b]	940.1[d]	−2.2

[a] $D = GB$ (theor.) − GB (exp.).
[b] This work.
[c] Ref. 1.
[d] Ref. 22.
[e] From the 3-NO_2 substituent effect reported in Ref. 4.
[f] Measured in this work according to the procedure given in Ref. 1. Uncertainty (as defined in Ref. 1) is ± 0.3 kJ mol^{-1}.

2–6 and nicotines **7–10** are *meta*-substituted pyridines and the *GB*s of the Nsp^2 protonation site might be estimated through Eqn (11). Unfortunately, on Nsp^2 protonation, an intramolecular C—H···Nsp^3 hydrogen bond is created and this fourth interaction mechanism, enhancing the pyridine nitrogen basicity, is not taken into account by the $\rho\sigma$ Eqn (11). Hence there is no simple way to estimate GB(Nsp^2) of substituted nicotines and nornicotines.

$GB_{(theor., B_1, Nsp^2)}$	X	$GB_{(theor., B_2)}$	ΔG (8)	$GB_{(exp., B_2)}$	$GB_{(theor.\ corr., B_1, Nsp^2)}$
930.9	H	905.2	+ 25.7	898.1	923.8
	3-Me	919.2	+ 11.7	911.6	923.3
	3-Et	923.6	+ 7.3	915.5	922.8
	3,5-Me_2	933.4	- 2.5	923.5	921.0
	3-(2-pyrrolidinyl)	939.7	- 8.8	932.4	923.6
					922.9 (Mean)

Scheme 5

$GB_{(theor., B_1, Nsp^3)}$	B_2	$GB_{(theor., B_2, Nsp^3)}$	ΔG(9)	$GB_{(exp., B_2)}$	$GB_{(theor.\ corr., B_1, Nsp^3)}$
928.1	**30**	922.5	+ 5.6	927.7	933.3
	29	933.5	- 5.4	934.7	929.3
	31	932.5	- 4.4	937.6	933.2
	32	946.2	- 18.1	948.5	930.4
	33	937.9	- 9.8	940.1	930.3
					931.3 (Mean)

Scheme 6

Table 7. Results of the proton isodesmic exchange method: corrected theoretical *GB*s for each protonation site, $\overline{GB}(\mathrm{Nsp^2})$ and $\overline{GB}(\mathrm{Nsp^3})$, basicity differences, $\Delta\overline{GB} = \overline{GB}(\mathrm{Nsp^2}) - \overline{GB}(\mathrm{Nsp^3})$, equilibrium constant of the proton exchange, *K*, percentage of form protonated on the amino nitrogen, % $\mathrm{Nsp^3\ H^+}$, and conclusion about the protonation site (*GB*s in kJ mol^{-1})

B_1	B_2	$\overline{GB}(\mathrm{Nsp^2})$	$\overline{GB}(\mathrm{Nsp^3})$	$\Delta\overline{GB}$	*K*	% $\mathrm{Nsp^3H^+}$	Site(s)
6-Methylnornicotine	**2, 15, 17, 20 23–28**	951.4 ± 0.8	920.5 ± 1.9	30.9 ± 2.7	10^{-6}–10^{-5}	0	$\mathrm{Nsp^2}$
Nornicotine	**16, 18, 19, 20 23–28**	931.3 ± 1.9	912.2 ± 1.9	19.2 ± 3.8	10^{-4}–2×10^{-3}	0	$\mathrm{Nsp^2}$
5-Ethynylnornicotine	**2, 18–22 23–28**	924.2 ± 2.0	909.1 ± 1.9	15.1 ± 3.9	5×10^{-4}–10^{-3}	0–1	$\mathrm{Nsp^2}$
5-Bromonornicotine	**2, 20–28 23–28**	911.2 ± 3.3	899.3 ± 1.9	11.9 ± 5.1	10^{-3}–6×10^{-2}	0–6	$\mathrm{Nsp^2}$
5-Nitronornicotine	**2, 20–22 23–28**	877.1 ± 3.3	879.0 ± 1.9	−1.7 ± 5.1	3×10^{-1}–16	20–94	$\mathrm{Nsp^2}$, $\mathrm{Nsp^3}$
6-*tert*-Butylnicotine	**15, 17, 20 29–33**	955.1 ± 1.4	943.2 ± 2.3	11.9 ± 3.7	2×10^{-3}–4×10^{-2}	0–4	$\mathrm{Nsp^2}$
6-Methylnicotine	**15, 17, 20 29–33**	946.2 ± 1.4	939.2 ± 2.3	7.0 ± 3.7	1×10^{-2}–27×10^{-2}	1–21	$\mathrm{Nsp^2}$
Nicotine	**16, 18, 19, 20 29–33**	928.4 ± 1.9	930.1 ± 2.3	−1.7 ± 4.2	4×10^{-1}–11	26–91	$\mathrm{Nsp^2}$, $\mathrm{Nsp^3}$
5-Ethynylnicotine	**18–22 29–33**	922.0 ± 2.6	926.7 ± 2.3	−4.7 ± 4.9	9×10^{-1}–43	48–98	$\mathrm{Nsp^2}$, $\mathrm{Nsp^3}$
5-Bromonicotine	**20–22 29–33**	909.3 ± 5.6	918.9 ± 2.3	−9.0 ± 7.9	15×10^{-1}–9×10^{2}	61–100	$\mathrm{Nsp^3}$
Anabasine	**2, 16, 18–20 23–28**	926.8 ± 1.4	917.0 ± 1.9	9.9 ± 3.3	5×10^{-3}–7×10^{-2}	0–7	$\mathrm{Nsp^2}$
N-Methylanabasine	**2, 16, 18–20 29–33**	928.6 ± 1.4	939.1 ± 2.3	−10.5 ± 3.7	15–3×10^{2}	94–100	$\mathrm{Nsp^3}$
Anatabine	**2, 16, 18–20 23–28**	922.1 ± 1.4	908.6 ± 1.9	13.6 ± 3.3	10^{-3}–2×10^{-2}	0–2	$\mathrm{Nsp^2}$
N-Methylanatabine	**2, 16, 18–20 29–33**	922.9 ± 1.4	931.3 ± 2.3	−8.4 ± 3.7	7–132	87–99	$\mathrm{Nsp^3}$

However, the basicity of the nitrogen atom of the pyrrolidine ring can be obtained by the $\rho\sigma$ methodology. In the series of 2-substituted pyrrolidines, the *GB* variations result only from the field/inductive and polarisability effects, since there is no resonance effect in a saturated ring. By means of known *GB*s, σ_F and σ_α values, we established in a previous study[1] the following equations for the prediction of the basicity (in kJ mol^{-1}) of the pyrrolidine nitrogen in series of 2-substituted pyrrolidines and 2-substituted *N*-methylpyrrolidines, respectively:

$$GB(\mathrm{Nsp^3}, \text{2-substituted pyrrolidines}) = 915 - 206\sigma_F - 33\sigma_\alpha \quad (12)$$

$$GB(\mathrm{Nsp^3}, \text{2-substituted } N\text{-methylpyrrolidines}) = 935 - 197\sigma_F - 29\sigma_\alpha \quad (13)$$

The application of these equations to the prediction of $GB(\mathrm{Nsp^3})$ of substituted nicotines and nornicotines requires the knowledge of the σ_F and σ_α constants for the substituted pyridyl groups that are present in the structure of nicotinoids **1–10**. We calculated these constants using the methods developed by Topsom[24] and Exner *et al.*[25] (σ_F) and Hehre *et al.*[26] and Carsky *et al.*[27] (σ_α). Table 8 gives values of σ_F and σ_α for substituents relevant to the series of substituted nicotines and nornicotines. Their use in Eqns (12) and (13) furnishes the empirical $GB(\mathrm{Nsp^3})$ of nicotinoids **3–10** (Table 9). A comparison between empirical and theoretical basicities is shown in Table 9. The agreement between the two methods is satisfactory since the mean absolute deviation is only 1.3 kJ mol^{-1}.

CONCLUSIONS

For an isolated molecule, the site of protonation of nicotinoids bearing a secondary amine function (NH) is generally the sp^2 nitrogen of the pyridine ring. Such is the case of nornicotine (**2**), substituted nornicotines **3–6**,

Table 8. Values of field/inductive (σ_F) and polarisability (σ_α) substituent constants of substituted pyridyl groups

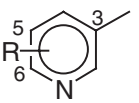
Substituent	R	σ_F	σ_α
	6-*t*-Bu	+0.120	−0.95
	6-Me	+0.119	−0.91
	H	+0.143	−0.88
	5-C≡CH	+0.183	−0.95
	5-Br	+0.225	−0.93
	5-NO_2	+0.318	−0.95

Table 9. Comparison of the basicity of the pyrrolidine nitrogen atom of nicotinoids **3**–**10** calculated empirically by the $\rho\sigma$ method, *GB*(emp.) and theoretically by DFT calculations corrected by the isodesmic method, $\overline{GB}$(theor., corr.)

No.	Substituent	*GB* (emp.)	$\overline{GB}$ (theor., corr.)	Difference[a]
Substituted nornicotines				
3	6-Me	920.3	920.5	−0.2
4	5-C≡CH	908.7	909.1	−0.4
5	5-Br	899.3	899.3	0
6	5-NO_2	880.6	879.0	+1.6
Substituted nicotines				
7	6-*t*-Bu	938.6	943.2	−4.6
8	6-Me	937.7	939.2	−1.5
9	5-C≡CH	926.2	926.7	−0.5
10	5-Br	917.3	918.9	−1.6

[a] Difference = *GB*(emp.) − $\overline{GB}$(theor., corr.).

anabasine (**11**) and anatabine (**13**). In 5-substituted nornicotines, only the 5-NO_2 substituent is able to reverse (partly) the protonation site in favour of the amino nitrogen atom, because its extreme electron-withdrawing effect strongly decreases the basicity of the pyridine nitrogen. Therefore, the conclusion about the site of protonation put forth in a previous study on nornicotine is strengthened for this class of nicotinoids.

The site of protonation of nicotinoids bearing an *N*-Me tertiary amine function may be either the sp^2 nitrogen of the pyridine ring or the sp^3 amino nitrogen. If one considers protonated nicotine $\mathbf{1H}^+$ as a mixture of the Nsp^3 and Nsp^2 monoprotonated forms in equilibrium, this proton exchange equilibrium can be shifted:

- towards the Nsp^2 monoprotonated form by means of a polarisable alkyl substituent in the 6-position of the pyridine ring (nicotinoids **7** and **8**).
- towards the Nsp^3 monoprotonated form by means of electron-withdrawing substituents (C≡CH, Br) in the 5-position of the pyridine ring (**9**, **10**) or by increasing the size (and hence the polarisability) of the ring bearing the amino nitrogen [*N*-methylanabasine (**12**)].

In addition to these electronic substituent or ring-size effects, another effect operates in *N*-methylanatabine (**14**), the intramolecular $CH\cdots Nsp^3$ hydrogen bond. By comparing the experimental *GB*s of pyrrolidine (**23**) (915.3 kJ mol^{-1}) and 2,5,6-tetrahydropyridine (**28**) (912.2 kJ mol^{-1}), it appears that the enhancing basicity effect of ring extension (+5.3 kJ mol^{-1} on going from pyrrolidine to piperidine) is offset by the double bond introduction (−8.4 kJ mol^{-1} on going from piperidine to 2,5,6-tetrahydropyridine) and 2,5,6-tetrahydropyridine is less basic by 3.1 kJ mol^{-1} than pyrrolidine. However, the percentage of the Nsp^3 protonated form increases on going from nicotine **1** to *N*-methylanatabine (**14**). This is the consequence of a weaker hydrogen-bond stabilisation of the Nsp^2 protonated form in **14**, because the intramolecular $CH\cdots Nsp^3$ hydrogen bond is the weakest one in this nicotinoid [d(N···H) = 2.592 Å in **14** instead of 2.410 Å in **1**].

Since nicotinoids bind to their nicotinic acetylcholine receptors (nAChRs) partly through their protonated Nsp^3 nitrogen atom[10] inside a receptor site with a strong aromatic, and hence hydrophobic, character,[9] our findings might be useful in rationalizing the site of protonation of nicotinoids. Pharmaceutical chemists should question whether the site of protonation of nicotinoids (previously considered to be always the Nsp^3 amino nitrogen atom) might not be changed when nicotinoids are transferred from water to the hydrophobic pocket of their receptor. In that case, the structural effects favouring the Nsp^2 protonation should decrease the binding to nAChRs (all other binding factors being kept constant). That the site of protonation of nornicotine is the sp^2 pyridine nitrogen, whereas a significant percentage of nicotine is protonated on the sp^3 amino nitrogen, might be one of the many factors that explain why nicotine binds much better to nAChRs than nornicotine.[28]

Supplementary material

Separate ΔH and ΔS contributions to ΔG are available in Wiley-Interscience.

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