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DFT-GIAO ^{1}H NMR chemical shifts prediction for the spectral assignment and conformational analysis of the anticholinergic drugs (−)-scopolamine and (−)-hyoscyamine

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The relatively large chemical shift differences observed in the ^{1}H NMR spectra of the anticholinergic drugs (−)-scopolamine 1 and (−)-hyoscyamine 2 measured in $CDCl_3$ are explained using a combination of systematic/molecular mechanics force field (MMFF) conformational searches and gas-phase density functional theory (DFT) single point calculations, geometry optimizations and chemical shift calculations within the gauge including/invariant atomic orbital (GIAO) approximation. These calculations show that both molecules prefer a *compact* conformation in which the phenyl ring of the tropic ester is positioned under the tropane bicycle, clearly suggesting that the chemical shift differences are produced by the anisotropic effect of the aromatic ring. As the calculations fairly well predict these experimental differences, diastereotopic NMR signal assignments for the two studied molecules are proposed. In addition, a cursory inspection of the published ^{1}H and ^{13}C NMR spectra of different forms of 1 and 2 in solution reveals that most of them show these diastereotopic chemical shift differences, strongly suggesting a preference for the *compact* conformation quite independent of the organic or aqueous nature of the solvent.

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

Keywords: GIAO; ^{1}H NMR; tropane; diasterotopic assignment

Introduction

The tropane alkaloids[1–3] (−)-scopolamine **1** and (−)-hyoscyamine **2** (Scheme 1) isolated from species of the Solanaceae family are among the first discovered anticholinergic agents.[4] Pharmacological uses of **1** are based on the central neural system (CNS) depressant action and include the treatment of parkinsonism, uveitis, iritis and motion sickness. In contrast, **2** or its racemate atropine is a CNS stimulant used as a preoperative agent to reduce secretions before surgery.[4]

A number of studies have dealt with the NMR properties of these compounds and their conformational preferences in the solid and solution states with great attention to the equatorial/axial ratio of the *N*-Me group either as free bases or as salts.[5–16] The assignment of the ^{1}H and ^{13}C NMR spectra for both compounds have been recorded, discussed and corrected several times, and particular interest has arisen for solution measurements to the unusually large chemical shift differences of diastereotopic protons and carbons of the tropane squeleton (i.e. 0.67 ppm difference between the H6 and H7 signals of **1** as a free base in $CDCl_3$). A very early study[5] of **1** evidenced these observations and proposed the phenyl ring in the tropic ester moiety as responsible for the chemical shift difference of the H-6/H-7 signals, noting this to be the *first observation of magnetic non-equivalence induced in vicinal rather than geminal protons, and where protons are separated from the asymmetric centre by as many as six chemical bonds*. Additionally, it was proposed that the magnitude of the observed difference suggests a conformational preference toward orientation of the phenyl ring in proximity to these protons. Later, Leete *et al*.[8] depicted a conformation of **2** where the asymmetric α-carbon is close to H-6*endo* and H-7*endo*, while Feeney *et al*.[9] proposed a conformer in which the benzene ring is folded under the tropane ring and close to the protons geminal to the oxirane ring, in a later so-called compact conformation. This particular disposition was later corroborated by 2D NOESY experiments as cross peaks between aromatic and H-6/H-7 signals in **1** were detected.[13] In addition, a single crystal X-ray study of the sesquihydrate of **1**[17] afforded this *compact* conformation similar to the one proposed by Feeney *et al*., and an equivalent result was obtained for the hydrobromide of **2** when crystallized from an aqueous solution.[18] Nevertheless, an anhydrous conformational pseudopolymorph of **1** HBr with an '*extended*' conformation, where the phenyl group in the tropic acid moiety is anti to the tropane ring, can also be obtained by recrystallization from ethanol/acetone.[19]

As expected by the highly conformationally dependent nature of the anisochronicity observed in these compounds, all

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above studies proposed full assignment of the tropane ring diastereotopic pairs of nuclei in which tabulated data are in all cases assigned arbitrary.

The gauge including/invariant atomic orbital (GIAO) approximation[20] for the prediction of chemical shifts has been used in various applications including conformational analysis,[21] stereochemistry,[22] spectral assignment[23] and the assessment of aromaticity,[24] as exemplified by very recent work. Additionally, these calculations were used in the configurational assignment of natural 6β-hydroxyhyoscyamine diastereoisomers **3** and **4** (Scheme 1) which are 3α-tropoyloxy tropane alkaloids closely related to **1** and **2**.[25] This study shows that the tropic acid moiety prefers an equivalent *compact* conformation which fairly well explains the chemical shift differences of nuclei that share the same chemical environment between diatereoisomers, and leads to the correct stereochemical assignment, in accordance to that informed earlier using vibrational circular dichroism (VCD).[26]

In continuation to our studies of the NMR properties of α-tropoyloxy tropanes, herein we present the use of ^{1}H NMR chemical shift prediction within the GIAO and DFT methodologies to obtain a full assignment of the anisochronous diastereotopic pairs of nuclei observed for the free bases of **1** and **2** in $CDCl_3$ solutions. Accordingly, some conclusions about the solution-state conformational preferences of these anticholinergic drugs and their salts in different media are also proposed.

Experimental

Samples of (−)-scopolamine hydrobromide and (−)-hyoscyamine hydrobromide were commercially acquired from Sigma–Aldrich and the free bases were obtained by liquid–liquid extraction (10 times with $CHCl_3$) of basic solutions (pH 10 with NH_4OH) of the drugs. ^{1}H NMR measurement was done on a Varian Mercury spectrometer at 300 MHz using $CDCl_3$ solutions containing TMS as the internal standard. The spectra were assigned with the aid of COSY measurements and are in agreement with the literature values.

The conformational distribution for both bases were determined using a systematic conformational search within the molecular mechanics force field (MMFF), considering an energy cutoff of 10 kcal/mol, and starting each search individually from *axial* and *equatorial* *N*-Me orientations. The MMFF94 energies[27] of each conformation were further improved using single point energy calculations at the DFT-B3LYP/6-31G(d) level of theory. A Boltzmann distribution based on the DFT energies allowed the choice of the most important conformations, which were used as input files for geometry optimizations at the B3LYP/6-31G(d) level of theory. Frequency calculations confirmed the optimized geometries to be local minima and delivered values of free energy. Absolute shieldings (AS) were predicted at the DFT-B3LYP/6-311G++(d,p) level of theory, which has shown excellent results with moderated computational expenses.[28] Molecular mechanics calculations were performed using the Spartan'04 modeling software[29] whereas DFT calculations were performed using the Gaussian 03 W software package.[30]

Results and Discussion

Conformational distribution

The preferred conformations of **1** and **2** were obtained by a combination of molecular mechanics systematic searches followed by ab initio single point energy calculations, geometry optimizations and free energy calculations at the B3LYP/6-31G(d) level of theory, and are shown in Figs 1 and 2, respectively. Additionally, relative energies and abundances for the conformations of each compound are summarized in Tables 1 and 2. In each case, eight low energy conformations accounted for 97.8 and 98.0% of the entire MMFF conformational distribution for **1** and **2**, respectively. The *equatorial* *N*-Me orientation is clearly preferred over the more energetic *axial* *N*-methyl disposition for both compounds with *equatorial* to *axial* ratios of 19 : 1 and 41 : 9 for **1** and **2**, respectively, as expected to avoid steric hindrance between the *N*-Me and H-2*endo*/H-4*endo* atoms present in the last orientation. The higher preference toward the *equatorial* disposition of **1** when compared to **2** can be explained by the additional electrostatic repulsion produced between the lone pair electrons in sp^3 orbitals of the oxirane oxygen and the *N*-Me nitrogen, which are oriented toward

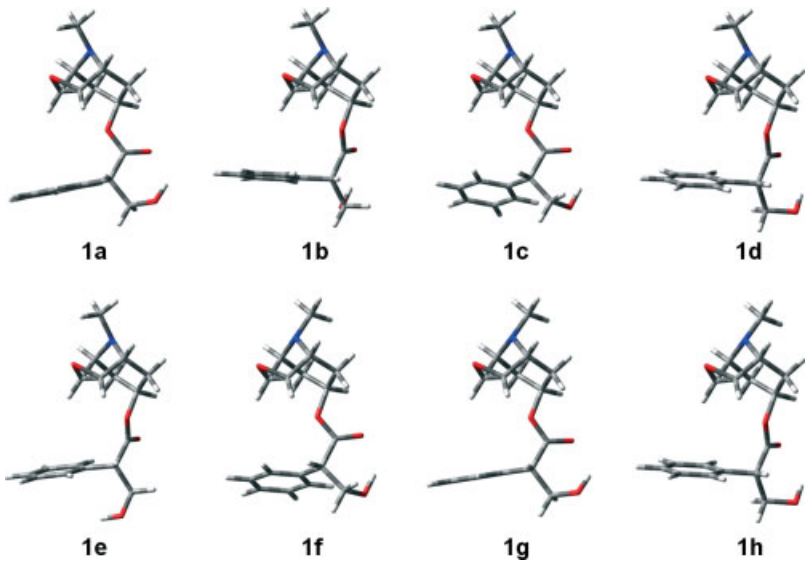


Figure 1. The eight low energy conformations of **1**.

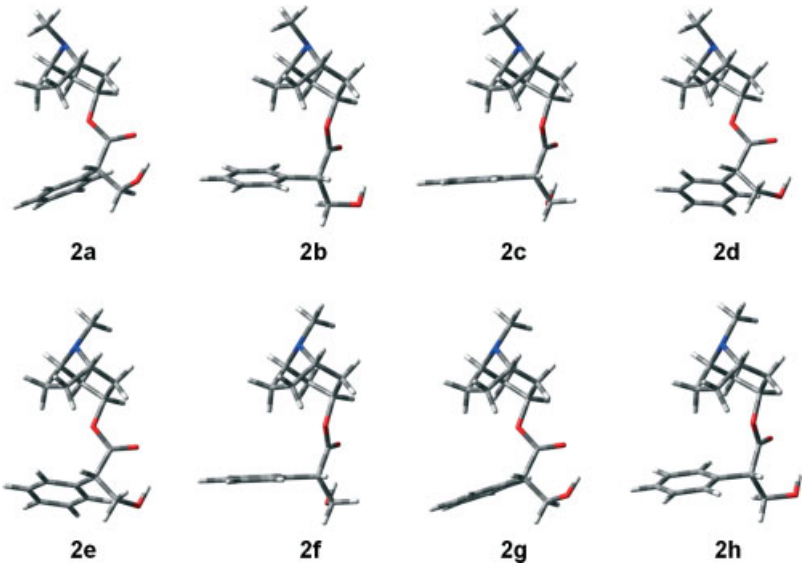


Figure 2. The eight low energy conformations of **2**.

Table 1. Calculated relative energies (kcal/mol), relative free energies and abundances (%) of the eight more stable conformations of (−)-scopolamine **1**

Conformer	ΔE_{MMFF}[a]	ΔE_{OPT}[a]	ΔG_{OPT}[a]	$\%_{OPT}$[b]
1a	1.94	0.62	0.00	33.92
1b	1.75	0.00	0.20	24.30
1c	3.12	0.62	0.30	20.38
1d	1.12	0.24	0.33	19.31
1e	0.00	2.34	1.83	1.54
1f	8.38	3.92	2.97	0.22
1g	7.34	3.94	3.00	0.22
1h	6.58	3.53	3.37	0.11

MMFF conformers were found using a systematic search and their geometries were further optimized at the B3LYP/6-31G(d) level of theory.
[a] Relative to the lowest energy conformer in the MMFF (E_{MMFF} **1e** = 69.26 kcal/mol) and DFT (E_{OPT} **1b** = −637579.98 kcal/mol and ΔG_{OPT} **1a** = −637383.29 kcal/mol) levels of theory.
[b] Calculated using the optimized free energies of the relevant conformers.

Table 2. Calculated relative energies (kcal/mol), relative free energies and abundances (%) of the eight more stable conformations of (−)-hyoscyamine **2**

Conformer	ΔE_{MMFF}[a]	ΔE_{OPT}[a]	ΔG_{OPT}[a]	$\%_{OPT}$[b]
2a	1.76	0.40	0.00	32.03
2b	1.16	0.24	0.20	22.92
2c	1.78	0.00	0.51	13.63
2d	2.81	0.30	0.53	13.08
2e	4.81	1.22	1.07	5.30
2f	4.10	0.99	1.14	4.66
2g	3.93	1.27	1.18	4.36
2h	3.43	1.15	1.23	4.02

MMFF conformers were found using a systematic search and their geometries were further optimized at the B3LYP/6-31G(d) level of theory.
[a] Relative to the lowest energy conformer in the MMFF (E_{MMFF} = 49.94 kcal/mol) and DFT (E_{OPT} **2c** = −591164.13 ΔG_{OPT} **2a** = −590955.07 kcal/mol) levels of theory.
[b] Calculated using the optimized free energies of the relevant conformers.

each other when the *axial* disposition is present. In the same way, a very similar overall conformational preference of the tropic acid moiety present in both bases is observed, with both of them showing *compact* dispositions of the residue in the lowest energy conformations. As pointed out earlier, the same preferences have been informed for the related C-6 hydroxy tropane alkaloids **3** and **4**.[26]

Prediction of chemical shifts and spectral assignment

AS for all hydrogen atoms in each geometrically optimized conformation of **1** and **2** were calculated using the GIAO approximation at the B3LYP/6-311G++(d,p) level of theory, and further used to generate weighted average values for each atom considering the previously obtained conformational distribution. The pertinent data are summarized in Supporting Information Tables S1 and S2 for **1** and **2**, respectively.

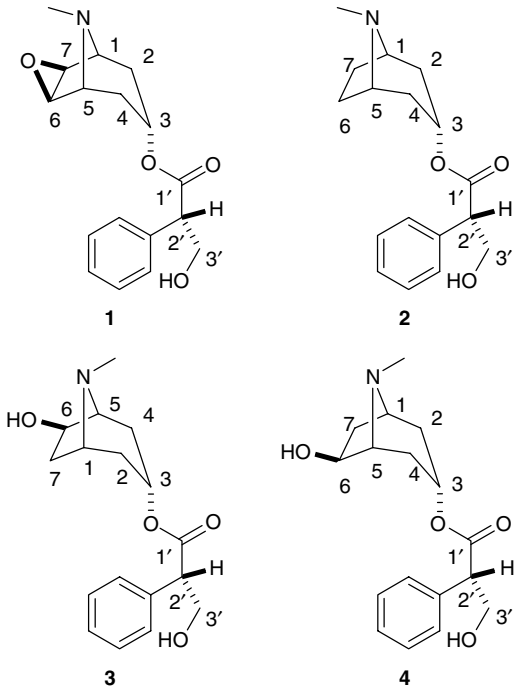
To make these average values suitable for comparison with the corresponding experimental data, AS were converted to theoretical chemical shifts using two described methodologies: the direct subtraction of the calculated absolute shielding of TMS (δ_{TMS}) and the linear scaling method (δ_{LS}),[31] as shown in Supporting Information Tables S1 and S2 for **1** and **2**, respectively. In both cases, the theoretical chemical shifts were compared with two different sets of experimental chemical shifts (δ_{exp}), namely the two possible assignments for the diastereotopic tropane nuclei: the C-2/C-1/C-7 side atoms upfield of the C-4/C-5/C-6 side atoms (assignment A_{1U}), and the C-4/C-5/C-6 side atoms upfield of the C-2/C-1/C-7 side atoms (assignment A_{5U}). From these comparisons, values of average absolute differences (Δ_{aa}) and root mean square errors (rms) were obtained (Table 3), allowing to know which assignment compares best with the calculated chemical shifts and therefore suggests the correct

Table 3. Average absolute differences (Δ_{aa}), root mean square errors (rms) and selectivity percentages ($\%_{sel}$) of correlations between theoretical chemical shifts (trough linear scaling and TMS subtraction), and both possible assignments (A_{1U} and A_{5U}) for the experimental chemical shifts of **1** and **2**

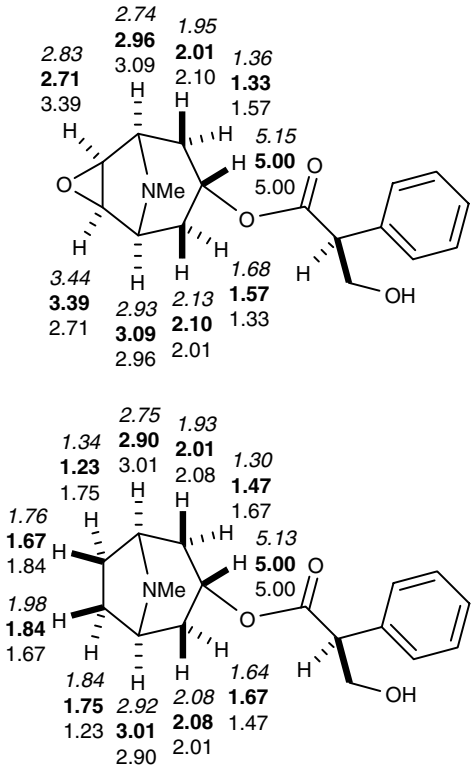
	1						**2**					
	A_{1U}		A_{5U}		$\%_{sel}$		A_{1U}		A_{5U}		$\%_{sel}$	
Error	δ_{LS}	δ_{TMS}	δ_{LS}	δ_{TMS}	δ_{LS}	δ_{TMS}	δ_{LS}	δ_{TMS}	δ_{LS}	δ_{TMS}	δ_{LS}	δ_{TMS}
Δ_{aa}	0.13	0.16	0.26	0.29	92.1	77.0	0.11	0.11	0.21	0.21	84.8	83.8
Rms	0.15	0.19	0.32	0.35	110.0	80.2	0.12	0.13	0.26	0.26	110.2	97.9

choice. The two assignment attempts for **1** and **2** are also given in Supporting Information Tables S1 and S2, respectively, while Scheme 2 compares the linear scaling values with the two possible data assignments.

In the case of the protons in **1** increases of 92.1 and 110.0% in the values of Δ_{aa} and rms error, respectively, on going from assignment A_{1U} to A_{5U}, clearly suggest that the H-2*endo*, H-2*exo*, H-1 and H-7 signals are the ones appearing upfield the H-4*endo*, H-4*exo*, H-5 and H-6 signals, respectively. Accordingly, the 84.8 and 110.2% increases in the same values for the protons in **2** suggest the same behavior, were the H-2*endo*, H-2*exo*, H-1, H-7*endo* and H-7*exo* signals appear upfield the H-4*endo*, H-4*exo*, H-5, H-6*endo* and H-6*exo* signals, respectively. Furthermore, while the later results were obtained using linearly scaled AS, a lower degree of selectivity (percentage of increase in errors and differences) is observed for theoretical chemical shifts obtained using the TMS absolute shielding subtraction. This is an expected result because in the first case the methodology allows elimination of systematic errors inherent to the calculation model concerning nondirectional solvent effects and temperature which remain when the second methodology is used.[28]



Scheme 1. Structures of (−)-scopolamine **1**, (−)-hyoscyamine **2**, (3*R*,6*R*,2′*S*)-6β-hydroxyhyoscyamine **3** and (3*S*,6*S*,2′*S*)-6β-hydroxyhyoscyamine **4**.



Scheme 2. ^{1}H NMR theoretical chemical shifts using linear scaling (δ_{LS}) (italics font) and experimental chemical shifts for the two possible diastereotopic assignments δ_{Exp} (A_{1U}) (boldface) and δ_{Exp} (A_{5U}) (normal) of **1** (top) and **2** (bottom).

It is important to notice that for both assignments (A_{1U} and A_{5U}) the pro-*S* H-3′ and pro-*R* H-3′ diastereotopic hydrogens were assigned arbitrarily to avoid a second variable for the comparison.

Chemical shift differences and solution-state conformational preferences

As discussed earlier, the chemical shift differences observed between diastereotopic pairs of nuclei in the tropane ring of **1** and **2** appear to be closely related to the conformational preferences of these bases, and therefore it is important to evaluate whether these differences are being well predicted by the chemical shift calculations. Table 4 shows the calculated ($\Delta\delta_{LS}$ and $\Delta\delta_{TMS}$) and experimental ($\Delta\delta_{exp}$) differences for these nuclei, and as can be observed the calculations predicted fairly well the experimental values.

Additionally, the conformational dependence of these differences is further supported by the CP-MAS ^{13}C NMR spectra of

Table 4. Chemical shift differences of theoretical [through linear scaling ($\Delta\delta_{LS}$), TMS subtraction ($\Delta\delta_{TMS}$) and the Haigh–Mallion ring current theory ($\Delta\delta_{HM}$)] and experimental values for the A_{1U} assignment ($\Delta\delta_{exp}$) of diatereotopic pairs of nuclei in **1** and **2**

	1				**2**			
Nucleus	$\Delta\delta_{LS}$	$\Delta\delta_{TMS}$	$\Delta\delta_{HM}$	$\Delta\delta_{exp}$	$\Delta\delta_{LS}$	$\Delta\delta_{TMS}$	$\Delta\delta_{HM}$	$\Delta\delta_{exp}$
6endo-7endo	0.63	0.53	0.21	0.68	0.50	0.49	0.13	0.52
6exo-7exo	–	–		–	0.22	0.21	0.04	0.17
5-1	0.19	0.20	0.09	0.13	0.17	0.17	0.07	0.11
4endo-2endo	0.18	0.18	0.04	0.09	0.15	0.16	0.05	0.07
4exo-2exo	0.32	0.33	0.17	0.24	0.34	0.33	0.15	0.20

The $\Delta\delta_{HM}$ values were calculated using the lowest energy conformers of **1** and **2**.

related solids, in which important features arise when contrasted to the anisochronicity observed in the solution spectra of the free bases.[15] While the sesquihydrate hydrobromide of **1** HBr and the hydrobromide of **2**, both packed in the *compact* conformation, show similar diastereotopic chemical shift differences as those found in solution (i.e. C-6/C-7 differences of 0.7 and 0.4 ppm for **1** in the solid and solution states, respectively, and C-6/C-7 differences of 0.9 and 0.5 ppm for **2** in the solid and solution states, respectively), the anhydrate in the *extended* conformation shows only unresolved C-2/C-4, C-1/C-5 and C-6/C-7 signals, clearly pointing toward a close relation between this anisochronicity and the *compact* conformation.[15]

These pieces of evidence are in total agreement with the aromatic anisotropic shielding effect being responsible for the differences in chemical shift observed between diastereotopic pairs of nuclei in bases **1** and **2**. Therefore, it is very unlikely that other conformations, which lack the tropic acid moiety oriented toward and under the tropane ring, are predominant in solutions that experimentally show these chemical shift differences. From there its follows that the presence or absence of these differences can be used to obtain conformational information in solutions of these bases and other structurally related molecules. Consequently, an inspection of the ^{1}H and ^{13}C NMR spectra described in the literature for solutions of **1** and **2**, which clearly show the presence of anisochronous C-6/C-7 or H-6/H-7 diatereotopic pairs, points toward the preference of *compact* conformations of these bases in solution, rather independently of the aqueous or organic nature of the used solvent.

As the chemical shift differences are induced by an aromatic ring, other approaches to calculate ring current effects can be used with similar results, as evidenced for 6-hydroxy-hyocyamine diastereoisomers[25] where the equation developed by Martin *et al*. was tested.[32] Of particular relevance is the use of the methodology developed by Haigh and Mallion[33–35] which in the present case provided the $\Delta\delta_{HM}$ data given in Table 4, which also compare well with the experimental values. The latter approach is an improvement of the classical Bovey-Johnson methodology.[36]

Conclusions

The ^{1}H NMR spectra of **1** and **2** in solution show relatively large chemical shift differences that can be explained using a combination of systematic/MMFF conformational searches and gas-phase DFT single point calculations, geometry optimizations and chemical shift calculations within the GIAO approximation. These calculations showed that both molecules prefer a *compact* conformation in which the phenyl ring of the tropic ester is positioned under the tropane bicycle, clearly suggesting that these differences are produced by the anisotropic effect of the aromatic ring. This is further supported by the CP-MAS ^{13}C NMR spectra of related solids, in which only those packed in a *compact* disposition present similar chemical shift differences.

The calculations predicted fairly well these experimental differences showing that the H-2*endo*, H-2*exo*, H-1 and H-7 signals in **1** are those appearing upfield the H-4*endo*, H-4*exo*, H-5 and H-6 signals, respectively. In the same way the H-2*endo*, H-2*exo*, H-1, H-7*endo* and H-7*exo* signals in **2** appear upfield the H-4*endo*, H-4*exo*, H-5, H-6*endo* and H-6*exo* signals, respectively.

Finally, a cursory inspection of published ^{1}H and ^{13}C NMR spectra of different forms of **1** and **2** measured in solution reveals that most of them show these diastereotopic pair chemical shift differences, strongly suggesting a preference for the *compact* conformation rather independently of the organic or aqueous nature of the used solvent.

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Supporting information

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

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