# Prediction of the Structure of Nobilisitine A Using Computed NMR Chemical Shifts

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Ⓢ *Supporting Information*

ABSTRACT: ^{1}H and ^{13}C NMR computed chemical shifts are determined for eight diastereomers of the originally proposed structure of nobilisitine A, which has recently been shown to be incorrect. On the basis of comparison of the computed chemical shifts with those reported experimentally, we predict that the true structure of nobilisitine A is likely the diastereomer shown here or its enantiomer.

Nobilisitine A
Originally-Proposed

Nobilisitine A
Newly-Proposed
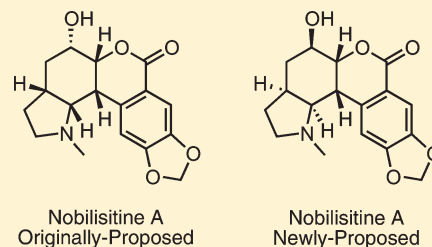
Nobilisitine A is an alkaloid natural product from *Clivia nobilis* that was first isolated and characterized by Evidente and co-workers in 1999.[1] Extensive analysis, primarily focused on ^{1}H and ^{13}C NMR data and comparison to related natural products, led to the proposed structure (**1**) shown in Figure 1. However, very recently Banwell and co-workers accomplished the total synthesis of the enantiomer of **1** (structure and relative configuration confirmed by X-ray crystallography) and reported that the NMR spectra of the synthesized product displayed significant deviations from those of the actual natural product (Table 1).[2] In particular, these authors noted a large difference in the chemical shift for the methyl protons and suggested that the more downfield shift observed for the natural product may be consistent with a structure in which the B and D rings are *anti* to each other (preventing the methyl group from approaching the face of the aromatic ring) rather than *syn* as initially proposed.

We have been interested in utilizing computed ^{1}H and ^{13}C NMR chemical shifts to assist in assigning and correcting misassigned natural product structures and felt that computed chemical shifts could be of value in deducing the correct structure of nobilisitine A. Our approach involves a quantum mechanical treatment of candidate structures in which the geometries and NMR shielding parameters are calculated with affordable density functional theory methods, and the shielding constants are empirically scaled[3,4] using parameters obtained through linear regression analysis of large sets of experimental and computational data. Effort is also made to take into account the effects of solvent and conformational freedom. These techniques have been successfully applied in the past for other natural products.[5] In the present case, we chose to utilize the B3LYP/6-31+G(d,p) level of theory to optimize the geometries of candidate structures in the gas phase and to use the mPW1PW91/6-311+G(2d,p) level of theory to compute the NMR shielding constants in a solvent continuum model.[6] Final predicted chemical shifts relative to TMS in CHCl$_3$ were obtained by applying linear regression parameters

that we have previously determined.[4] Various conformers of each structure were examined systematically (see Supporting Information for details), and those lying within a 2.5 kcal/mol energy window relative to the minimum of lowest energy were utilized in our predictions (weighted averages based on relative free energies at 298.15 K were used).

Because most of the ^{1}H and ^{13}C NMR chemical shifts reported for nobilisitine A are consistent with those found by Banwell and co-workers (Table 1), we decided to focus initially on possible diastereomeric structures. While the five stereocenters allow for 16 possible diastereomers (two enantiomers of each lead to 32 total possible stereoisomers), we narrowed the initial scope to eight by assuming a *cis* ring-fusion for the C/D rings. Although this pentacyclic natural product is quite rigid, several modes of conformational mobility do exist and need to be treated appropriately: the ethylene group in ring D can adopt two conformations, ring C can adopt two different chair forms, the amine can invert, and the hydroxy group can rotate.[7] This flexibility could in principle amount to 24 conformers for each diastereomer, although, not surprisingly, we found that many combinations were either mutually incompatible and/or incompatible with certain stereochemical arrays (i.e., some potential conformers could not be located as minima). In other cases, minima were located but were significantly higher in energy than the lowest energy minimum for a given diastereomer and thus would not be expected to contribute to the observed experimental spectrum. In fact, for several diastereomers, only a single contributing conformer was located, and the largest number of contributing conformers located for any one diastereomer was five (see Supporting Information).

Two sets of firmly established experimental data are available for use in validating our approach. The first is the ^{1}H and [13]C
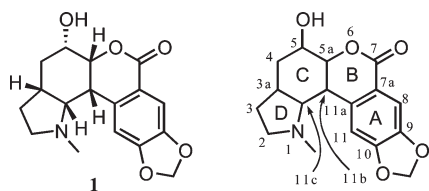
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Figure 1. Proposed structure of nobilisitine A (**1**) and corresponding numbering/lettering system used for the polycyclic framework.[1]

Table 1. Comparison of Experimental NMR Chemical Shifts for Nobilisitine A (Isolated[1]) and for Structure 1 (Synthesized[2])

	^{13}C NMR chemical shifts (ppm)^a			^{1}H NMR chemical shifts (ppm)^a	
nucleus #^{b}	nob. A	(**1**) synthetic	nucleus #^{b}	nob. A	(**1**) synthetic
2	54.9	55.0	2	3.24	3.31
3	30.1	29.8	2	2.30	2.24
3a	**34.7**	**39.4**	3	2.00	1.93
4	33.7	30.9	3	1.62	1.45
5	68.6	69.7	3a	2.27	2.34
5a	81.4	78.0	4	2.02	1.88
7	164.0	164.7	4	1.62	1.78
7a	118.6	121.0	5	3.96	3.74
8	109.7	109.8	5a	4.65	4.61
9	147.2	147.6	8	7.53	7.54
10	152.6	151.9	11	7.05	6.70
11	106.5	106.6	11b	3.34	2.87
11a	137.3	137.8	11c	2.67	2.60
11b	36.6	41.0	N CH$_3$	**2.24**	**1.45**
11c	66.6	67.7	acetal CH$_2$	6.05	6.05
N CH$_3$	41.8	45.2	acetal CH$_2$	6.05	6.05
acetal CH$_2$	102.0	101.9			
	MAD^c	1.5		MAD^c	0.17
	largest outlier^d	$\Delta\delta$ = 4.7		largest outlier^d	$\Delta\delta$ = 0.79

^{a} Both sets of data taken from ref 2. For ^{1}H multiplets, the median of the range is listed here. ^{b} See Figure 1. ^{c} MAD = mean absolute deviation, computed as $(1/n)\Sigma_i^n|\delta_{isol} - \delta_{synth}|$, where δ_{isol} and δ_{synth} refer to the two sets of experimental chemical shifts. ^{d} Largest outliers for each set of data are highlighted in bold text.

NMR data reported for **1** (this structure was confirmed by X-ray crystallography) by Banwell and co-workers[2] We located five conformers of this diastereomer, but only one appears to be significant (the next lowest energy structure lies more than 3.5 kcal/mol higher in energy, based on computed free energies at 298.15 K).[8] Although the X-ray data were not used in our initial search for conformers, this computed conformer is quite similar to that found in the crystallographic structure. The experimental and computed chemical shifts for **1** are shown in Table 2. There is an overall excellent agreement between theory and experiment with an average error (CMAD; corrected mean absolute deviation; see footnote d in Table 2 for definition) of 1.2 ppm for ^{13}C and 0.13 ppm for ^{1}H. Furthermore, the largest deviations are only 3.4 ppm for ^{13}C and 0.31 ppm for ^{1}H (bold in Table 2).[9,10]

The second point of reference is data reported recently for clivonine (**2**, Figure 2), a diastereomer of **1** (again confirmed by X-ray).[11] For this structure, we also located five conformers. In this case, two were found to lie very close in energy to each other (<0.5 kcal/mol apart, based on computed free energies at 298.15 K), so we used the Boltzmann-weighted average (at the same temperature) of their computed shifts for comparison to experimental data. As shown in Table 3, excellent agreement between theory and experiment is again observed.

Table 2. Comparison of Experimental and Computed NMR Chemical Shifts for Structure 1

^{13}C NMR chemical shifts (ppm)		^{1}H NMR chemical shifts (ppm)		
nucleus #^{a}	expt^b	computed^c	nucleus #^{a}	expt^b	computed^c
2	55.0	53.0	2	3.31	3.17
3	29.8	31.3	2	2.24	2.24
3a	39.4	42.2	3	1.93	1.99
4	30.9	31.3	3	1.45	1.36
5	69.7	68.9	3a	2.34	2.37
5a	78.0	77.3	4	1.88	1.67
7	164.7	162.8	4	1.78	1.65
7a	121.0	121.2	5	3.74	3.55
8	109.8	109.7	5a	4.61	4.46
9	147.6	146.2	8	7.54	7.35
10	151.9	151.5	11	6.70	6.58
11	106.6	106.4	11b	2.87	2.81
11a	137.8	139.1	11c	2.60	2.85
11b	41.0	42.3	N CH$_3$	1.45	1.41
11c	67.7	66.5	acetal CH$_2$	6.05	5.97
N CH$_3$	**45.2**	**41.8**	acetal CH$_2$	**6.05**	**5.74**
acetal CH$_2$	101.9	101.1			
	CMAD^d	1.2		CMAD^d	0.13
	largest outlier^e	$\Delta\delta$ = 3.4		largest outlier^e	$\Delta\delta$ = 0.31

^{a} See Figure 1. ^{b} Data taken from ref 2. For ^{1}H multiplets, the median of the range is listed here. ^{c} See Supporting Information for details. ^{d} CMAD = corrected mean absolute deviation, computed as $(1/n)\Sigma_i^n|\delta_{comp} - \delta_{exp}|$ where δ_{comp} refers to the scaled computed chemical shifts. ^{e} Largest outliers for each set of data are highlighted in bold text.
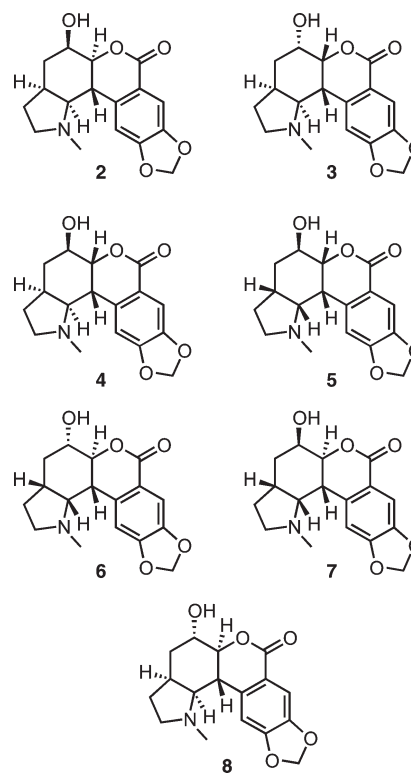

Figure 2. The seven diastereomers of structure **1** examined herein. See Table 4 for information on computed NMR data for each structure.

The remaining diastereomers (Figure 2) were computed in the same way, and Table 4 shows a summary of how the computed data compare to those reported for nobilisitine A (see Figures 1 and 2 for structures). It is clear that all eight diastereomers are similar, with the largest average deviations between their computed shifts and the experimental shifts reported for nobilisitine A being only 2.0 ppm for ^{13}C and 0.37 ppm for ^{1}H. On the basis of these CMADs and the deviations for the outliers in each case, structure 4 displays the best overall agreement with the experimental data.[12,13] Furthermore, in our opinion, structure 4 is also more consistent with the additional experimental data reported previously for nobilisitine A, such as ^{1}H coupling

constants and 2D NMR spectra.[1] Even so, it is clearly a close call to choose a single diastereomer in this situation, highlighting the need for high accuracy if computed chemical shifts are to be used to distinguish between closely related diastereomeric structures. Table 5 shows the computed data for structure 4 compared to the experimental data for nobilisitine A.[1] The match between the experimental and computed values for nobilisitine A and structure 4 is comparable to that seen for structures 1 and 2 (Tables 2 and 3), and the computed values for structure 4 are more consistent with those observed for nobilisitine A than are the experimentally obtained values for 1, the originally proposed structure (especially in terms of largest outliers; see Table 1). Figure 3 shows a ball-and-stick image of the lowest energy conformer of structure 4.

The chemical shift of the N-methyl protons in nobilisitine A warrants particular attention, as Banwell and co-workers identified deviation here as the most obvious indication that the originally proposed structure is incorrect.[2] As noted above, the originally proposed structure places the methyl group *syn* to the aromatic system across the B and C rings and leads to an observed chemical shift (in synthesized structure 1) of 1.45 ppm, which is significantly more upfield than one might expect for N-methyl protons unaffected by a nearby aromatic system.[14] The observed chemical shift of these protons in isolated nobilisitine A of 2.24 ppm prompted the proposal from Banwell and co-workers of a "*cis* B/C *anti*, *cis* C/D" configuration of the natural product, which would place the methyl group well away from the aromatic ring. Of the eight diastereomers examined, only structures 3 and 4 are of this configuration, and structure 3 exhibits some of the largest computed chemical shift deviations (compared to isolated nobilisitine A) of the set. In addition, while the computed chemical shifts for the N-methyl protons in all eight diastereomers examined range from 1.41 to 2.44 ppm (see Supporting Information), the computed value of 2.10 ppm in structure 4 is the closest to the observed value of 2.24 ppm in nobilisitine A and is consistent with a chemical shift observed for "normal" N-methyl protons.[14]

In order to gain greater confidence in our tentative conclusion that the structure of nobilisitine A is 4, we turned to the DP4 probability analysis developed by Smith and Goodman.[15,16] The DP4 analysis was designed specifically for the situation where one set of experimental data is available to which one possible diastereomeric structure out of many must be assigned. The analysis is based on calculated error probabilities for scaled

Table 3. Comparison of Experimental and Computed NMR Chemical Shifts for Structure 2

^{13}C NMR chemical shifts (ppm)			^{1}H NMR chemical shifts (ppm)		
nucleus #[a]	expt[b]	computed[c]	nucleus #[a]	expt[b]	computed[c]
2	52.95	52.04	2	3.29	3.20
3	30.82	32.64	2	2.27	2.44
3a	**33.43**	**36.27**	3	2.53	2.46
4	28.73	28.09	3	2.27	1.88
5	67.41	68.44	3a	2.53	2.32
5a	81.81	80.73	4	2.10	2.18
7	164.68	163.10	4	1.80	1.77
7a	118.69	118.94	5	4.24	4.03
8	109.34	109.20	5a	4.09	4.09
9	146.68	145.71	8	7.46	7.31
10	152.67	152.52	11	7.74	7.90
11	107.15	107.46	11b	3.22	3.22
11a	140.77	142.77	11c	2.89	2.93
11b	33.12	34.15	N CH$_3$	2.53	2.44
11c	69.50	69.83	acetal CH$_2$	6.02	6.01
N CH$_3$	45.22	43.08	acetal CH$_2$	**6.02**	**5.76**
acetal CH$_2$	101.82	100.51			
	CMAD[d]	1.09		CMAD[d]	0.12
	largest outlier[e]	$\Delta\delta = 2.8$		largest outlier[e]	$\Delta\delta = 0.26$

[a] See Figure 1. [b] Data taken from ref 11. For ^{1}H multiplets, the median of the range is listed here. [c] See Supporting Information for details. [d] CMAD = corrected mean absolute deviation, computed as $(1/n)\Sigma_i^n|\delta_{comp} - \delta_{exp}|$ where δ_{comp} refers to the scaled computed chemical shifts. [e] Largest outliers for each set of data are highlighted in bold text.

Table 4. Comparison of Computed NMR Chemical Shifts for Each Diastereomer to Those Reported for Nobilisitine A

|		computed data relative to nobilisitine A experimental data[b]			
		^{13}C chemical shifts (ppm)		^{1}H chemical shifts (ppm)	
structure[a]	relative configuration (positions 3a, 5, 5a, 11b, 11c)	CMAD	largest deviation	CMAD	largest deviation
1	R S R R R	1.9	7.5	0.25	0.83
2	S R S R S	1.9	5.6	0.34	0.90
3	S S R R S	2.0	9.0	0.29	0.88
4	S R R R S	1.4	3.1	0.21	0.65
5	R R R R R	1.4	5.2	0.23	0.79
6	R S S R R	2.0	6.6	0.19	0.67
7	R R S R R	1.7	4.0	0.20	0.71
8	S S S R S	1.6	4.7	0.37	0.88

[a] Structures shown in Figures 1 and 2. [b] Experimental data taken from ref 2.

Table 5. Comparison of Experimental NMR Data for Nobilisitine A and Computed Chemical Shifts for Structure 4

^{13}C NMR chemical shifts (ppm)			^1H NMR chemical shifts (ppm)		
nucleus #[a]	expt[b]	computed[c]	nucleus #[a]	expt[b]	computed[c]
2	54.9	54.2	2	**3.24**	**2.59**
3	30.1	31.3	2	2.30	2.78
3a	34.7	36.9	3	2.00	2.22
4	33.7	30.8	3	1.62	1.64
5	68.6	67.9	3a	2.27	2.14
5a	81.4	79.1	4	2.02	1.84
7	164.0	162.1	4	1.62	1.88
7a	118.6	118.1	5	3.96	3.88
8	109.7	109.6	5a	4.65	4.37
9	147.2	146.2	8	7.53	7.35
10	152.6	151.9	11	7.05	6.82
11	106.5	105.9	11b	3.34	3.15
11a	**137.3**	**140.4**	11c	2.67	2.72
11b	36.6	38.7	N CH$_3$	2.24	2.10
11c	66.6	65.8	acetal CH$_2$	6.05	6.01
N CH$_3$	41.8	39.7	acetal CH$_2$	6.05	5.77
acetal CH$_2$	102.0	101.3			
	CMAD[d]	1.4		CMAD[d]	0.21
	largest outlier[e]	$\Delta\delta = 3.1$		largest outlier[e]	$\Delta\delta = 0.65$

[a] See Figure 1. [b] Data taken from ref 2. For ^1H multiplets, the median of the range is listed here. [c] See Supporting Information for details. [d] CMAD = corrected mean absolute deviation, computed as $(1/n)\Sigma_i^n|\delta_{comp} - \delta_{exp}|$ where δ_{comp} refers to the scaled computed chemical shifts. [e] Largest outliers for each set of data are highlighted in bold text.
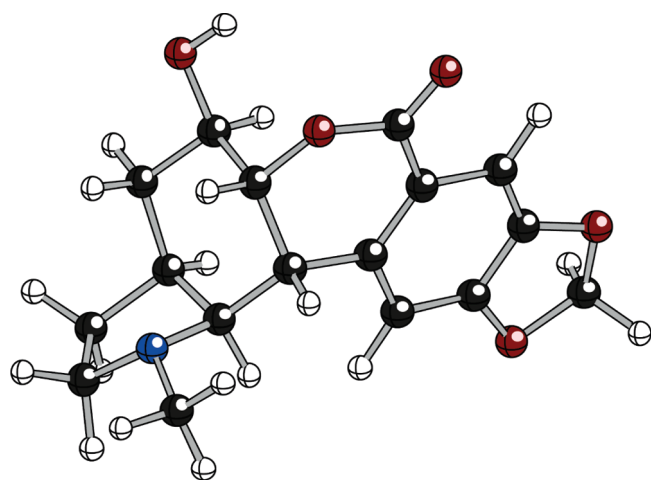
Figure 3. Ball-and-stick representation of the lowest energy conformer of structure **4**.

computed chemical shifts for each hydrogen and carbon atom, assuming a statistical t distribution for these errors. Bayes's theorem is then used to transform the product of the individual error probabilities for each atom in the structure into an overall probability that the structure is correct. For nobilisitine A, when the reported ^{13}C and ^1H NMR chemical shifts were compared to those calculated for all eight diastereomers, DP4 analysis identified structure **4** as most likely, with a probability of 99.8% (the remaining 0.2% probability was assigned to structure **6**).[17] In

applying the DP4 analysis, the researcher has some control in indicating which computed chemical shifts can be assigned to each experimental shift. We chose not to restrict these assignments beyond the types of CH$_n$ groups; for example, the computed and experimental chemical shift values for all methylene protons were allowed to be paired in the most favorable way possible, giving each structure the best chance of a high ranking and avoiding any unintended predisposition for any one structure. We note that carrying out identical analyses using computed data for **1**−**8** and experimental data for **1** led to a 99.7% probability for structure **1**, and using computed data for **1**−**8** and experimental data for structure **2** led to a 99.5% probability for structure **2**, indicating that the analysis is likely to be reliable for **1**−**8**.

Taken collectively, our computational evidence strongly suggests that structure **4** (or its enantiomer) is the true structure of nobilisitine A. This conclusion is in line with the hypothesis of Banwell and co-workers that nobilisitine A likely exhibits an *anti* relationship of the B and D rings (both *cis*-fused) on ring C. We cannot definitively rule out the possibility of a structure with alternate connectivity (although this seems unlikely), but if the true structure of nobilisitine A is indeed a diastereomer of that originally proposed, the most likely candidate is structure **4**. We hope that this study will provide useful direction for future synthetic efforts that will ultimately confirm the true structure of this natural product.

■ ASSOCIATED CONTENT

S **Supporting Information.** Full details and references for computational methods; full details on linear regression parameters; coordinates, NMR data, and complete assignments for all computed NMR spectra; additional references for linear regression and natural product revisions based on computed NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

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(2) Schwartz, B. D.; Jones, M. T.; Banwell, M. G.; Cade, I. A. *Org. Lett.* **2010**, *12*, 5210–5213.

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(4) For additional details on linear regression parameters, see our website: http://cheshirenmr.info.

(5) A recent high-profile example involves hexacyclinol. See: (a) Saielli, G.; Bagno, A. *Org. Lett.* **2009**, *11*, 1409–1412. (b) Williams,

A. J.; Elyashberg, M. E.; Blinov, K. A.; Lankin, D. C.; Martin, G. E.; Reynolds, W. F.; Porco, J. A., Jr.; Singleton, C. A.; Su, S. *J. Nat. Prod.* **2008**, *71*, 581–588. (c) Porco, J. A., Jr.; Su, S.; Lei, X.; Bardhan, S.; Rychnovsky, S. D. *Angew. Chem., Int. Ed.* **2006**, *45*, 5790–5792. (d) Rychnovsky, S. D. *Org. Lett.* **2006**, *8*, 2895–2898. See Supporting Information for additional references.

(6) See Supporting Information for details and references to theoretical methods.

(7) There is also an additional mode of conformational freedom present in the acetal methylene moiety. Here the methylene group can adopt two slightly noncoplanar conformations with respect to the ring. These two conformations are essentially inconsequential since they are nearly degenerate energetically, have very little effect on the computed chemical shifts, and are in an area of the molecule that is not in question. Therefore, they were not explicitly considered in our conformational analyses.

(8) Additional conformers involving hydroxy rotation likely exist but are similarly expected to lie significantly higher in energy than the lowest energy conformer.

(9) (a) The largest deviation in ^{13}C NMR data is associated with the amine methyl group, which is near the face of the aromatic D ring. These two groups likely interact, at least in part, via dispersion interactions that may not be fully accounted for in the B3LYP computed gas phase geometries. Even so, we note that the deviation here is not exceptionally large, and therefore additional effort to account for this error seems unnecessary. (b) The largest deviation in ^{1}H NMR data is associated with an acetal methylene proton and is likely due to the small amount of conformational mobility possible here (see ref 7). Note also that the experimental chemical shifts of these two protons are collectively reported as either a broad singlet or a multiplet (see ref 2).

(10) Complete assignment details are available in the Supporting Information.

(11) Mañas, C. G.; Paddock, V. L.; Bochet, C. G.; Spivey, A. C.; White, A. J. P.; Mann, I.; Oppolzer, W. *J. Am. Chem. Soc.* **2010**, *132*, 5176–5178.

(12) Note that this particular diastereomer was found to have five contributing conformers, the most of any diastereomer examined, which may be expected to lead to slightly increased errors.

(13) Note also that structure **4** actually shows a slightly lower CMAD than does **5** for the ^{13}C NMR data if another significant figure is considered (1.36 ppm compared to 1.44 ppm).

(14) The observed proton chemical shift for trimethylamine is 2.12 ppm. This value is obtained from the online Spectral Database for Organic Compounds (SDBS) at http://riodb01.ibase.aist.go.jp/sdbs/cgi-bin/cre_index.cgi?lang=eng.

(15) Smith, S. G.; Goodman, J. M. *J. Am. Chem. Soc.* **2010**, *132*, 12946–12959.

(16) Use of the DP4 analysis is quite practical, owing to a versatile Java applet that the Goodman group has made available online. The current URL is http://www-jmg.ch.cam.ac.uk/tools/nmr/nmrParameters.html.

(17) The creators of the DP4 probability analysis have noted that in cases of very closely related diastereomers the analysis may somewhat overestimate the probability of the most likely structure.[15]