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Advances in the Universal NMR Database Approach. 2′-Substituted Taxanes as Probes for an Improved Protocol of Diastereomeric Differentiation

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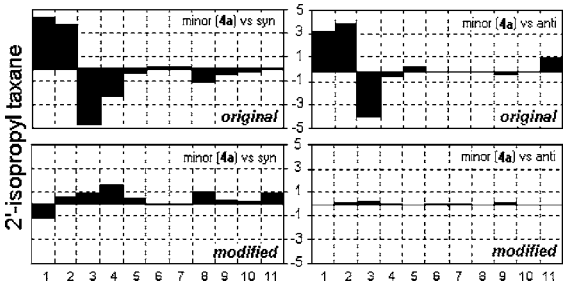
ABSTRACT



The configuration of the α-substituted α-hydroxy-β-aminoester moiety in a series of 2′-substituted taxanes was analyzed according to the recently proposed Universal NMR Database (*UDB*) approach. A critical analysis of the results showed that modifications regarding chemical shift adjustment (so as to render the shifts virtually connectivity independent) were necessary to get consistent stereoassignments in this set of compounds. On this basis, a modified *UDB*-based strategy, especially tailored to the configurational assignment of densely substituted diastereomeric fragments, is proposed.

We have recently accomplished a nondiastereoselective entry into the 2′-methyltaxanes **2a**,**b** based on the addition of Grignard reagents to the corresponding 2′-dehydrotaxane **1**.[1] The configurational assignment of the diastereomeric addition products was approached using two spectroscopic methods, the *UDB* (Universal NMR DataBase) and the *J*-based analyses. We have now extended this synthetic and structural elucidation efforts to various homologues and analogues of 2′-methyltaxanes, with the two-fold aim of investigating their biological profile and use them as a spectroscopic probes to assess the applicability of the *UDB* method to highly functionalized fragments of complex natural products. While the biological profile of these compounds will be discussed in an independent publication, we present here a modification of the *UDB* method developed and validated for the

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(1) Dambruoso, P.; Bassarello, C.; Bifulco, G.; Appendino, G.; Battaglia, a.; Guerrini, a.; Fontana, G.; Gomez-Paloma, L. *Tetrahedron. Lett.* **2005**, accepted for publication.

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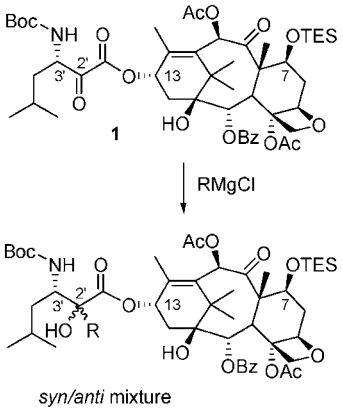
configurational assignment of 2′-alkyltaxanes side chain and tailored for the study of densely substituted diastereomeric fragments.

Scheme 1 summarizes yield and diastereoselection observed for the addition of the archetypal alkyl, alkenyl, and aryl Grignard reagents to the 2′-dehydrotaxane **1**. For sake of comparison, the results observed with MeMgCl are also reported.

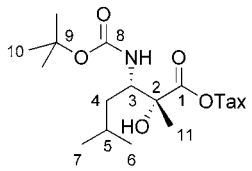
Scheme 1. Addition of Various Grignard Reagents to the 2′-Dehydrotaxane **1**



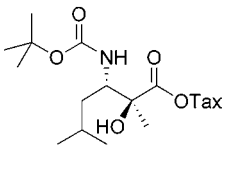
taxane	R	solvent	T (°C)	yield	min/maj
2a/2b	Me	THF	-78	56 %	1:2.6
3a/3b	Et	THF	-78	56 %	1:3.2
4a/4b	^{i}Pr	THF	-78	57 %	1:9.0
5a/5b	^{t}Bu	THF	-78	37 %	1:1.1
6a/6b	Ph	DCM/THF 4:1	-78	80 %	1:1
7a/7b	Vinyl	DCM/THF 4:1	-78	82 %	1:1

As reported in detail in another account,[1] the original *UDB* approach[2] failed to provide a clear-cut configurational assignment of the newly formed stereocenter of the 2′-methyltaxanes **2a,b**. Given the propensity of the baccatin core to hydrophobically cluster with the C-13 side chain,[3] it seems reasonable to assume that this interaction underlies the inability of a c.s. (chemical shift) database (DB), built on diastereomeric isoserine methyl esters, to correctly represent the (empirically adjusted) c.s. of their corresponding taxane esters. We therefore anticipated that, if a suitable correcting factor could be found, any ambiguity related to structural differences between the simplified DB elements and the real systems could be overcome. Alternatively, recently reported methods to assign the configuration in flexible compounds based on the *ab initio* calculation of ^{13}C c.s. and their comparison with experimental counterparts[4] could be employed. Nevertheless, an unambiguous configurational assignment of taxane side chains **3a**,**b**−**7a**,**b** could eventually be obtained without resorting to sophisticated calculations, simply by introducing a different type of correcting factor in the *UDB* approach.

A database was built starting from the epimeric 2′-methyltaxanes **2a**,**b**.[1] Then, the correcting factor was computed for each carbon *i* by calculating the difference between the mean c.s. value of the two C-2′ taxane epimers with unknown configuration ($m_i^{epimers}$) and the mean c.s. value of the two DB elements (m_i^{DB}). This may be expressed in mathematical terms as: $CF_i = m_i^{epimers} - m_i^{DB}$. Then, for each position *i*, an adjusted c.s. value was computed as follows: $c.s._i = c.s._i - CF_i$, where $c.s._i$ is the adjusted value while $c.s._i$ is the plain experimental value.[5] A given pair may be examined by inspection of the differences $\Delta\delta_i$'s between adjusted and reference (database) c.s. at each position *i*. For instance: $\Delta\delta_i^{(major\ vs\ syn)} = c.s._i^{major} - c.s._i^{DB-syn}$ and plotted as a histogram. Only small variations are expected for stereochemically homogeneous pairs, as highlighted in Figure 1.



2a
(2',3')-*syn*



2b
(2',3')-*anti*

It is noteworthy that the correcting factor proposed herein represents, on average, the contribution to the chemical shift of the skeletal diversity between a DB element and the real compound under study. For this reason, such a factor can be used to account for the difference in chemical space between the DB elements and the real compounds under study.

By simple comparison of the two histograms of each epimeric compound, the relative configuration (C2′−C3′ *syn* or *anti*) could be easily and unambiguously deduced. A validation of this *UDB* analysis was obtained by submitting each pair of epimeric 2′-substituted taxanes to the *J*-based analysis (Supporting Information, Table S6). To our delight, no discrepancy was found in the results of these two configurational approaches.

In general, two numeric parameters can be introduced to evaluate the reliability of an *UDB* analysis, namely, the total absolute deviation (TAD) and reliability index (RI). The first is calculated as follows: $TAD = \Sigma_i|\Delta\delta_i|$, where $\Delta\delta_i = c.s_i^{epimer} - c.s._i^{DB}$. The second is a normalized value expressing how larger is the higher TAD with respect to the lower one. For a given pair, $RI = [(TAD^{higher} - TAD^{lower})/TAD^{lower}]*1000$. Other interesting parameters that may also

(2) (a) Kobayashi, Y.; Lee, J.; Tezuka, K.; Kishi, Y. *Org. Lett.* **1999**, *1*, 2177−2180. (b) Lee, J.; Kobayashi, Y.; Tezuka, K.; Kishi, Y. *Org. Lett.* **1999**, *1*, 2181−2184. (c) Kobayashi, Y.; Tan, C.-H.; Kishi, Y., *Helv. Chim. Acta* **2000**, *83*, 2562−2571. (d) Kobayashi, Y.; Tan, C.-H.; Kishi, Y. *Angew. Chem., Int. Ed.* **2000**, *39*, 4279−4281. (e) Tan, C.-H.; Kobayashi, Y.; Kishi, Y. *Angew. Chem., Int. Ed.* **2000**, *39*, 4282−4284. (f) Kobayashi, Y.; Tan, C.-H.; Kishi, Y. *J. Am. Chem. Soc*, **2001**, *123*, 2076−2078.

(3) Snyder, J. P.; Nettles, J. H.; Cornett, B.; Downing, K. H.; Nogales, E. *PNAS*, **2001**, *98*, 5312−5316.

(4) (a) Barone, G.; Gomez-Paloma, L.; Duca, D.; Silvestri, A.; Riccio, R.; Bifulco, G. *Chem. Eur. J.* **2002**, *8*, 3233−3239. (b) Barone, G.; Gomez-Paloma, L.; Duca, D.; Silvestri, A.; Riccio, R.; Bifulco, G. *Chem. Eur. J.* **2002**, *8*, 3240−3245.

(5) Calculated in this way, the adjusted chemical shifts tend to be virtually connectivity independent.

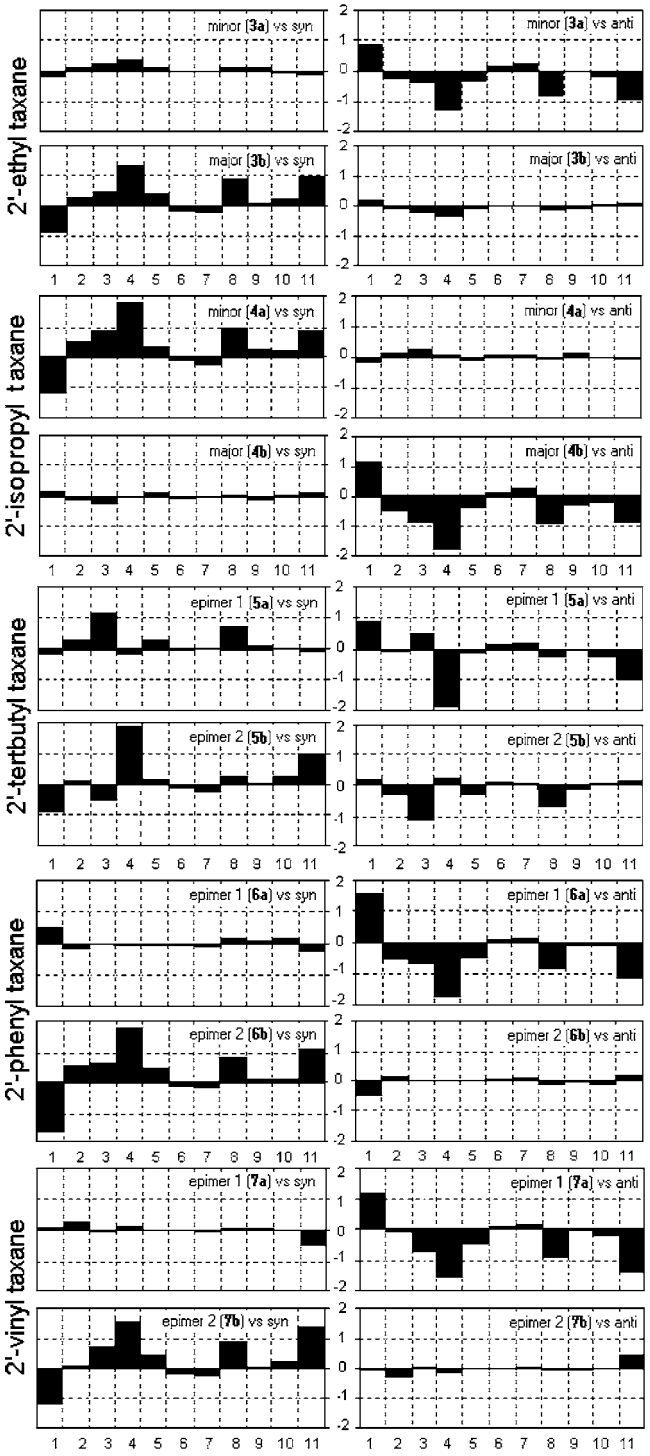
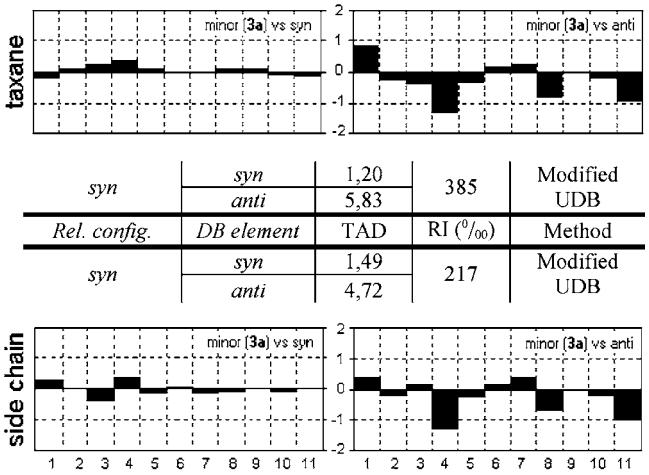


Figure 1. Modified *UDB* analysis of 2′-substituted taxanes.[6] Horizontal and vertical axes show carbon number and $\Delta\delta$ values.

be computed in this context are the deviations of the adjusted c.s. from mean DB c.s., that is, $d(\text{major})_i = c.s._i^{\text{major}} - m_i^{\text{DB}}$ and $d(\text{minor})_i = c.s._i^{\text{minor}} - m_i^{\text{DB}}$. In a way, such deviations should represent the contribution of a given configuration to the chemical shift. In other words, the study of these deviations can be informative on the effects that a given configurational pattern exerts on a particular carbon framework.

Interestingly, our modified *UDB* analysis does not seem crucially dependent upon the choice of the DB elements, provided that the molecular skeleton of the DB correctly reproduces that of the compounds under investigation. Thus, Figure 2 shows a comparison between the modified UDB



syn	*syn*	1,20	385	Modified UDB
	anti	5,83		
Rel. config.	*DB element*	TAD	RI (‰)	Method
syn	*syn*	1,49	217	Modified UDB
	anti	4,72		

Figure 2. Effect caused by the nature of DB elements on a modified UDB analysis in the case of 2′-ethyltaxane **3a**.

analysis performed on the minor epimer of the 2′-ethyltaxane **3a** with the DB elements set as 2′-methyltaxanes **2a**,**b** or their corresponding side chain methyl esters.[7] In both cases, the RI values were large enough to allow a confident analysis. As expected, the use of the two diastereomeric 2′-methyltaxanes as DB elements made the analysis more reliable, as in the latter case the DB elements are particularly close in chemical space to the real compounds.

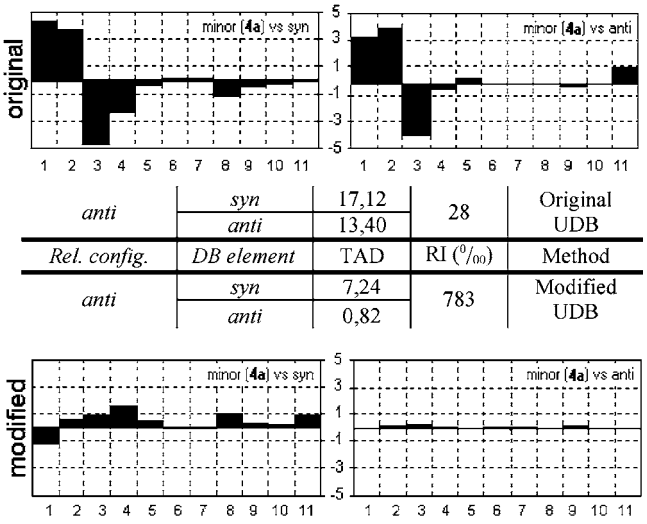
Finally, Figure 3 shows a comparison between the plots of *UDB* analyses performed by using the original approach versus the new one described here. Both analyses were carried out on the minor epimer of 2′-*iso*propyltaxane **4a**, setting the two epimeric 2′-methyltaxanes **2a**,**b** as DB elements.

While TAD's values point to the same configurational assignment with both strategies, more conclusive results, as expressed by RI values (28‰ for the original and 783‰ for the modified), can be obtained with the modified method.

The *UDB* analysis oultined here is particularly suited for large series of diastereomeric compounds, like those generated by combichem. In this case, a *J*-based analysis would be very time-consuming and probably inadequate for the task. It should be pointed out that the completion of the full

(6) The NMR spectra of the 2′-*tert*butyl taxanes **5a**,**b** revealed a mixture of two conformers for each 2′-epimer, one being largely predominant to the other. This *UDB* analysis was performed on the major rotamers (see also the Supporting Information).

(7) Roers, R.; Verdine, G. L. *Tetrahedron Lett.* **2001**, *42*, 3563−3565.



anti	*syn*	17,12	28	Original UDB
	anti	13,40		
Rel. config.	*DB element*	TAD	RI (‰)	Method
anti	*syn*	7,24	783	Modified UDB
	anti	0,82		

Figure 3. Comparison of original and modified UDB analysis plots for the epimeric 2′-isoproproyltaxanes **4a**, using the epimeric 2′-methyltaxanes **2a**,**b** as DB elements.

J-based analysis of the diastereomeric pairs **2a**,**b**−**7a**,**b** required almost 30 days for the acquisition, elaboration, and interpretation of the NMR data, whereas the modified *UDB* approach could be carried out in just a few hours, once the full NMR assignments were available. On the other hand, when a single compound like a novel natural product has to be elucidated from a configurational/conformational standpoint, a detailed NMR investigation using the *J*-based method is certainly reliable and therefore preferable in many cases. As a final remark, we would like to stress the key relevance of the conformational similarity of the DB elements and those under investigation. In our set of 2′-substituted taxanes, homogeneous configurational isomers displayed a homogeneous conformational behavior too (Figure 4). Thus, all *syn* compounds could be represented by the same dominant conformer, and the same holds true for the *anti* products. This homogeneity in the conformational features is a crucial requisite for a successful application of any *UDB*-based strategy,[8] notwithstanding what correcting factors are chosen or data elaboration is implemented.

Figure 4. Predominant conformers for syn and anti 2′-substituted taxanes. See the Supporting Information for details.

In conclusion, an investigation on the configurational assignment of a series of semi-synthetic 2′-substituted taxanes has led to the development of a modified version of the Universal NMR Database (*UDB*) approach that was validated by *J*-based analysis. The modification proposed herein for the *UDB* method is entirely based on the choice of a suitable correcting factor and is especially suited for the configurational analysis of diastereomeric mixtures obtained in various strategies aimed at the generation of chemical diversity (combichem, diversity-oriented synthesis,[9] diverted total synthesis[10]), and represents a significant advancement in the field of configurational analysis.

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Supporting Information Available: NMR data, *J*-based, and *UDB* analyses for the collection of 2′-substituted taxanes. This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) When our modified analysis was applied to different conformers of a pair of diastereomers (minor conf. *syn* vs major conf. *anti* and vice versa), no confident configurational assignment could be obtained. See Supporting Information Figures S1−S3.

(9) Burke, M. D.; Berger, E. M.; Schreiber, S. L. *Science* **2003**, *302* (5645), 613−618.

(10) Njardarson, J. T.; Gaul, C.; Shah, B.; Hjang, X.-Y.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2004**, *126*, 1038−1040.