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2′-Methyl taxanes: synthesis and NMR configurational assignment

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Abstract—Capitalizing on an oxidation–alkylation approach, a non-diastereoselective entry into 2′-methyl taxanes was developed. The issue of configurational assignment at the newly formed side-chain quaternary stereocenter was solved and put into a more general context by integrating information from an alternative diastereoselective synthesis of model compounds and from spectroscopic measurements, critically comparing the *J*-Based and the Universal NMR Database approaches.

Over the past 15 years, the structure–activity relationships of the anticancer drug Taxol™ (paclitaxel) have been intensely investigated both in academia and in industry.[1] Whereas changes on the diterpenoid core were either detrimental or non-influential for bioactivity, an increase of potency was observed for certain modifications of the acyl moieties at C-2 and C-13.[1] Thus, the introduction of a methyl group on the α-carbon of the amino acidic ester group increased cytotoxicity and/or tubulin binding with several types of C-13 side chains.[2] Surprisingly, this general effect has not yet been investigated in a systematic way. Hence, neither the introduction of groups larger than the methyl nor the configurational inversion at C-2′ have been reported (Fig. 1), preventing the translation of these interesting bioactivity data into any of the various binding modes that have been developed for the interaction of tubulin and taxoids.[3] The strategies developed so far for the synthesis of 2′-methyl taxoids are based on the acylation of a protected baccatin III with an α-alkylated side-chain

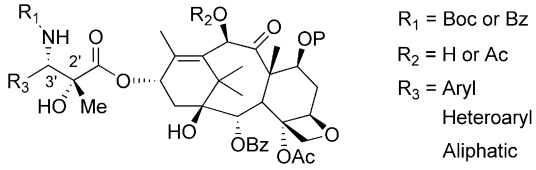

Figure 1. Anticancer 2′-alkyl taxanes (see Ref. 2).

synthon (β-lactam,[2a,b,4] *N*-acyloxazolidine[2c]). The increased steric bulk of the α-carbon is a major impediment to extend this strategy beyond the introduction of a methyl group, since the acylation of the 13-hydroxy group of baccatin III derivatives is a critical reaction, encumbered by the steric bulk.[1] To solve this issue and to pave the way to the introduction of larger alkyl groups, an alternative strategy was pursued, capitalizing on the attack of a carbanion species to a norstatin-type 2′-dehydrotaxoid precursor (Scheme 1).[5] The rationale for this choice was that the baccatin core is relatively inert toward organometallic compounds,[1] while the high electrophilicity of the α-ketoesters carbonyl group should allow the attack of bulky alkyl groups. In the event, this strategy could be successfully implemented, but a low level of diastereoselection was observed in the addition to the 2′-carbonyl. While the obtaining of a pair of C-2′ diastereomers could in principle be perceived as an asset to extend the structure–activity

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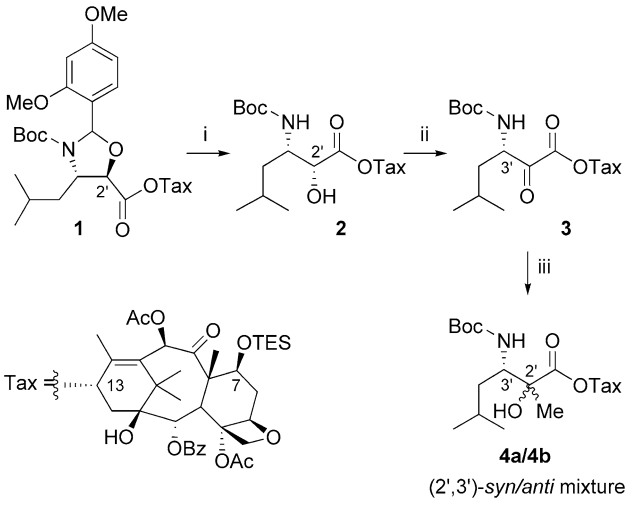

Scheme 1. Semisynthesis of 2′-methyl taxanes: (i) Dowex 50W/8, refluxing MeCN/MeOH 9:1, 83%; (ii) DCC/$CHCl_2COOH$, DMSO, 20 °C, 94%; (iii) MeMgCl, THF, −78 °C, 56% (1:2.6 *syn*/*anti* ratio).

database, this nevertheless raised the issue of the configuration assignment of the newly generated stereocenter.

Our entry into 2′-alkyl taxanes is exemplified by the synthesis of the diastereomeric 2′-methyl taxoids **4a** and **4b** (Scheme 1). The sequence commences with the condensation of a baccatin III derivative and a norstatin side-chain synthon bearing orthogonal protection at their secondary hydroxyls (triethylsilyl: (TES) for the 7-hydroxyl of baccatin III, oxazolidine for the 2′-hydroxyl). After considerable experimentation,[6] selective unmasking of the side-chain hydroxyl could be achieved in excellent yield (83%) with a Dowex 50W/8 resin in refluxing 9:1 MeCN/MeOH, affording the 2′-deprotected taxane **2**. The following oxidation of the 2′-hydroxyl turned out to be a critical step. Thus, within the many oxidants investigated (Cr^{VI}, TPAP, DMSO, TEMPO), only those based on activated DMSO could cleanly oxidize the 2′-hydroxyl. While the Swern protocol was plagued by epimerization at C-3′, with the DMSO–DCC couple (Moffatt oxidation[7]) the configuration at this center was maintained. The α-ketoester obtained in this way (**3**) was stable enough to be purified by gravity column chromatography and stored,[8] as reported for the 2′-dehydroanalogue of paclitaxel, that, however, was configurationally stable at C-3′.[9]

The addition of MeMgCl to **3** occurred uneventfully, affording in 56% isolated yield a 2.6:1 pair of C-2′-epimers.[10] These compounds could be successfully separated by flash chromatography, but their structure elucidation turned out to be a non-trivial problem. Thus, while a reliable assignment was mandatory for structure–activity studies and their translation in terms of active conformation, the stereochemical assignment of adjacent stereocenters in densely functionalized acyclic fragments is still a challenging issue.

Recently, two complementary approaches have shown a great potential to address the issue of acyclic configurational assignment. One relies on the comparison of

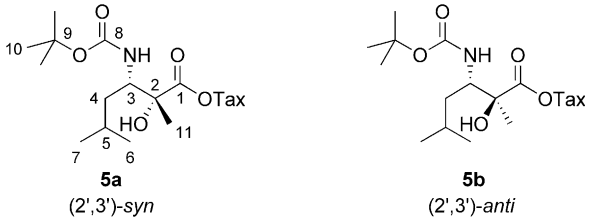

Figure 2. Database references for the *UDB*-based configurational assignment of 2′-methyl taxanes.

^{13}C chemical shift (c.s.) values with a set of suitable reference compounds (*Universal NMR Database* or *UDB*).[11] The other is based on the integration, in terms of spatial relationships, of the angular dependence of $^{3}J_{H,H}$ and $^{2,3}J_{C,H}$ values with key dipolar effects (*J-Based analysis*).[12] Our diastereomeric 2′-methyl taxoids were perceived as an interesting system to evaluate the applicability and relative merits of these methods. *UDB* seemed of more direct and easier application, since NMR data on the epimeric α-methyl norstatin methyl esters **5a** and **5b** have been published.[13] These compounds correspond to the side chains of taxoids **4a** and **4b**, and, capitalizing on their ^{13}C NMR data, a reference database for the configurational assignment was built (Fig. 2).

The reported c.s. of the database elements were then compared to the empirically corrected c.s.[14] for the 2′-methyl taxanes of Scheme 1. The correcting factor accounts for the structural differences due to the presence of the polycyclic taxoid core.

As shown in Figure 3, this comparative analysis did not allow an unambiguous assignment of the relative configuration of the epimeric taxoids **4a** and **4b**. Thus, we turned our attention to the *J-Based analysis*. It was clear from the outset that also the application of this methodology was not free from difficulties and pitfalls, owing to the simultaneous presence of a quaternary stereocenter and a nitrogen functionality. Indeed, the quaternary C-2′ implies the loss of a critical $^{3}J_{HH}$ coupling value, while the nitrogen-bearing C-3′ determines a different range of values for J_{CH} couplings.[15] Hence, an independent validation of the *J*-Based analysis on model compounds of known configuration seemed necessary. To this purpose, a pair of C-3 epimeric 2-methyl-3-thienylisoserines (**6a** and **6b**) and the taxane **7** were employed. Compounds **6a** and **6b** were available from an independent investigation,[16] and had been prepared in a diastereoselective way using the Seebach's strategy of "Self-Regeneration of Stereocenters", a reliable and widely employed synthetic protocol.[17] The taxane **7**, bearing a *syn*-heteroaryl isoserine chain, was instead prepared by condensation of the sodium salt of 7-TES-baccatin III-1,14-carbonate and the protected (3*R*,4*R*)-3-methyl-4-thienyl-azetidin-2-one **8** (Fig. 4).

Application of the *J*-Based analysis on compound **7**, pointed to the existence of a predominant staggered rotamer with *syn*-configuration (Fig. 5). Extension of the *J*-Based analysis on the diastereomeric **6a**–**b** pair

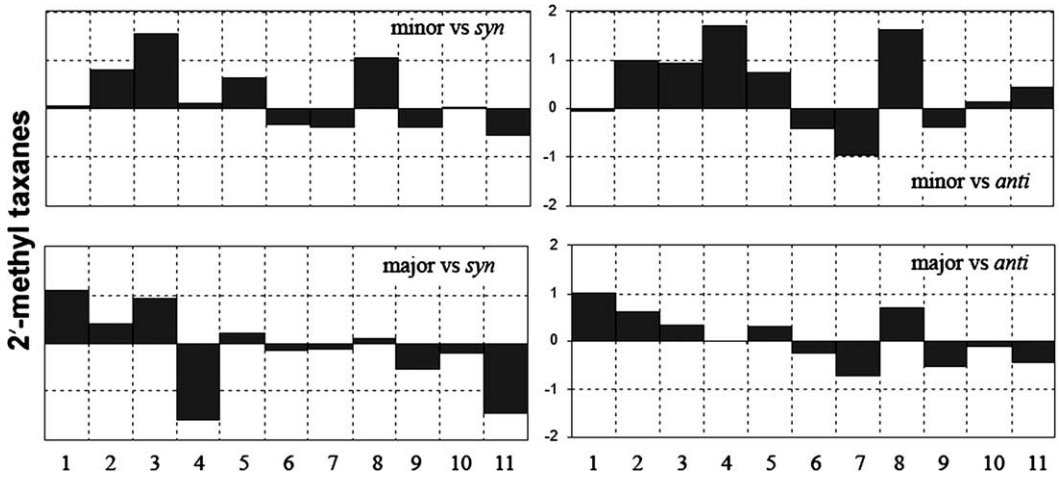

Figure 3. *UDB* analysis of 2′-methyl taxanes. The histogram plots were obtained as follows: (1) ^{13}C c.s. for both the database elements and 2′-methyl taxane structures were predicted using the Schaller program.[14] (2) For each position, the difference between the predicted c.s. of 2′-methyl taxanes and those of database elements was calculated. These differences, that empirically accounts for the structural differences between the database and real system, represent the correcting factor. (3) The measured c.s. (100 MHz, $CDCl_3$) of each 2′-methyl taxane epimers was adjusted using this correcting factor. (4) Finally, within each pair, the Δ between the adjusted c.s. of the examined compound and those of the chosen database element were computed and plotted as histograms. Only small variations are expected for stereochemically homogeneous pairs. Horizontal and vertical axes report carbon number and $\Delta\delta$ in ppm, respectively, as in similar plots throughout the paper.

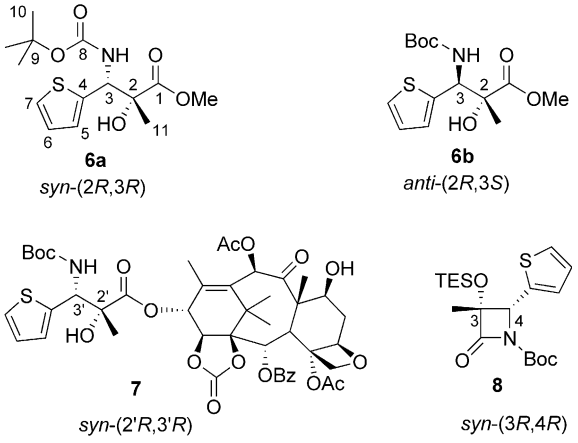

Figure 4. Model compounds for the validation of the *UDB* and the *J-Based* approaches to the configuration of 2′-methyl taxanes.

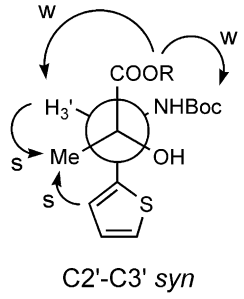

Figure 5. The dominant side-chain conformation of the *syn*-diastereomer as determined by applying the *J*-Based analysis to the 2′-methyl-3′-thienyl-taxane **7**. The intensity of dipolar effects (ROESY) is expressed in terms of three categories: s = strong, m = medium, w = weak throughout the paper. See Table S12 in Supplementary data for measured $^{2,3}J_{CH}$ values.

(Fig. 4; Tables S10 and S11 in Supplementary data) validated the *J*-Based approach on this kind of complex β-amino acid derivatives.

By comparison, a *UDB* analysis of the relative configuration of **7**, using **6a** and **6b** as database constitutive elements, failed (Fig. 6). It is known that, in solution, the baccatin core is engaged in hydrophobic interactions with the apolar elements of the C-13 isoserine side chain,[1] and the conformational variations associated with these phenomena presumably invalidate the *UDB*-based strategy.

Armed with the information gathered with the model compounds **6a**/**6b**–**7**, the *J*-Based approach was performed on the C-2′ epimeric 2′-methyl taxanes **4a** and **4b**. Figure 7 shows the predominant conformer for each diastereomer. The observed pattern of *J*-values and ROESY effects, suggests, with a high level of confidence, that the configuration of the side chain is of the *anti*-type for the major diastereomer, and of the *syn*-type for the minor one. It should be pointed out that the complexity of the system and the presence of several possible chelation sites makes it difficult to predict the stereochemical course of carbonyl addition by any of the known models.

In conclusion, we have developed an original and versatile synthesis of C-2′-alkyl taxoids of the norstatin type, exemplified by the preparation of the diastereomeric 2′-methyl taxanes **4a** and **4b**. In order to assign their configuration, an extensive NMR study was performed, critically evaluating the *UDB* and the *J*-Based approaches to stereochemical assignment. In the light of these results, a significant revision of the *UDB* strategy seems necessary. Indeed, such revision appears

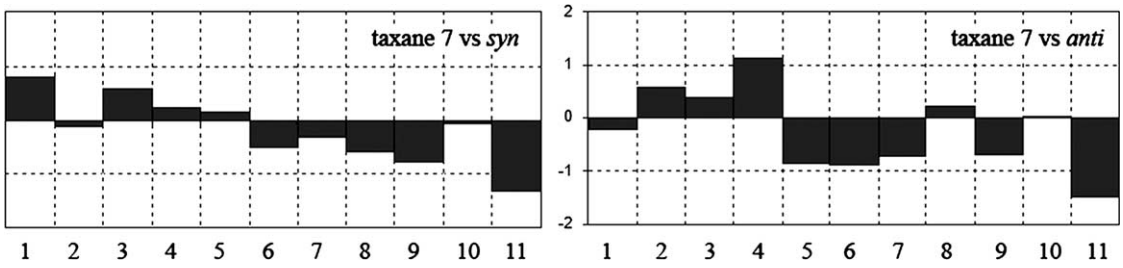

Figure 6. *UDB* analysis performed on the taxane **7** using **6a** and **6b** as database elements.

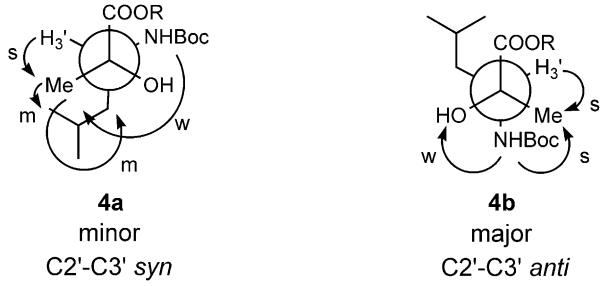

Figure 7. *J*-Based conformational analysis of 2′-methyl taxanes. See Table S13 in Supplementary data for the measured $^{2,3}J_{CH}$ values.

compelling to account for those cases where database elements and the compound under investigation may display a different conformational behavior.

A critical study in which a suitable correcting factor has been devised to extend the application of *UDB* to heavily functionalized acyclic chains was being published elsewhere.[18]

Acknowledgements

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Supplementary data

Experimental synthetic procedures and NMR data for compounds **1**–**7**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2005.03.095.

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