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Absolute configurations of tubulin inhibitors taltobulin (HTI-286) and HTI-042 characterized by X-ray diffraction analysis and NMR studies

Chuansheng Niu [a,*], Douglas M. Ho [b], Arie Zask [a], Semiramis Ayral-Kaloustian [a]

[a] *Wyeth Research, Pearl River, NY 10965, USA*
[b] *Department of Chemistry, Princeton University, Princeton, NJ 08544, USA*

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ABSTRACT

The stereochemistry of the tubulin inhibitors taltobulin HTI-286 (**2**) and HTI-042 (**3**) was determined by utilizing the DPFGSE 1D NOE experiment. Single crystal X-ray diffraction analysis further confirmed the absolute configuration of these two compounds, which carry the (*S*,*S*,*S*)-configuration necessary for biological activity.

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The natural product hemiasterlin,[1] (**1**, Fig. 1), isolated from marine sponges,[2] is one member of a family of tripeptides consisting of three sterically congested amino acids. Hemiasterlin and its relatives are potent antimitotic agents that effectively inhibit tubulin polymerization by binding to the Vinca-peptide-binding site.[3] The synthetic analog taltobulin (**2**, HTI-286),[4] in which the *N*-methylindole ring was replaced with a phenyl group, was found to be a potent cytotoxin and mitotic blocker like **1**, vincristine and paclitaxel (Taxol™).[5] In particular, compound **2** exhibited more potent activity than **1** against paclitaxel-resistant cell lines in vitro and in vivo.[5,6] Therefore, taltobulin (**2**) was selected for further development for the treatment of advanced malignant solid tumors.[7] The absolute stereochemistry of **2** was proposed as the (*S*,*S*,*S*)-configuration at its three chiral centers based on the synthetic strategy and the stereochemistry of **1**.

Extensive structure–activity relationship (SAR) studies of HTI-286 analogs were conducted in our laboratories. One superior analog, HTI-042 (**3**), was discovered.[8] Compound **3**, which incorporated a methoxy functional group on the phenyl ring, not only retained the potency of **2**, but also showed a better safety window than **2** and complete cures in certain xenograft models. HTI-042 was also selected as a development candidate.

One of the important aspects of our research was to confirm the stereochemistry of taltobulin (**2**) and HTI-042 (**3**). An efficient and straightforward synthetic approach for the total synthesis of HTI-286, HTI-042, and other analogs of **2** for SAR studies is illustrated in Scheme 1.[8,9] Methylation of the pyruvic acid derivative **4** with iodomethane in the presence of sodium hydride gave the *gem*-dimethyl compound **5**. Reductive amination of **5** produced amino acid **6**. Coupling **6** to dipeptide **7** in the presence of PyBOP provided a mixture of two diastereoisomers **8** and **9**, which were easily separated by HPLC. The desired ester **8**, with the right stereochemistry

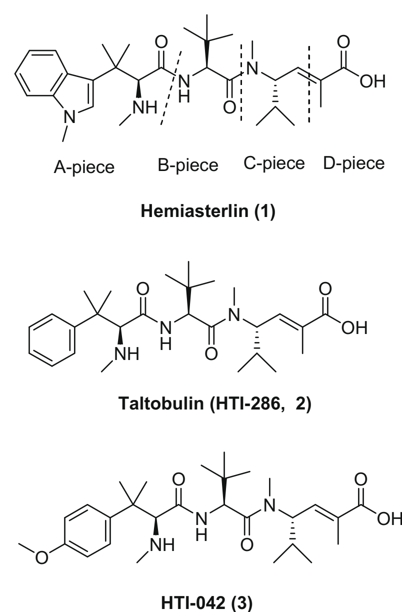

Figure 1.

* Corresponding author. Tel.: +1 845 602 5996; fax: +1 845 602 5561.
E-mail address: niuc@wyeth.com (C. Niu).

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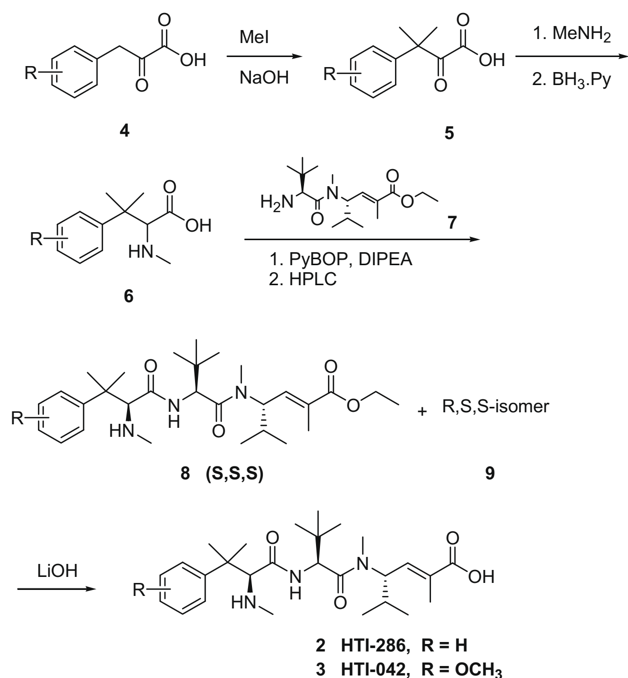

Scheme 1. Synthesis of HTI-286 and analogs.

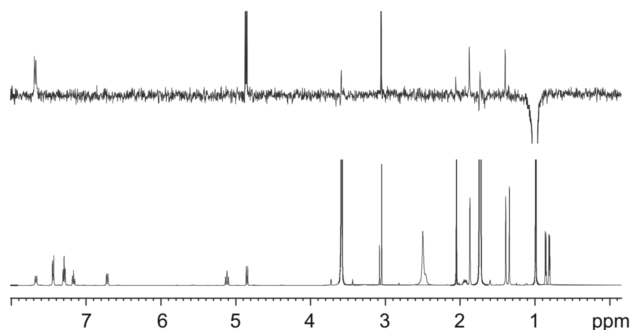

Figure 2. DPFGSE 1D NOE with selective excitation of *tert*-butyl group of HTI-286.

predicted as (*S,S,S*) was then hydrolyzed with lithium hydroxide, providing the target analog **10**. SAR studies revealed that changing the stereochemistry at each chiral center of the tripeptides resulted in reduction of the activity.[8] To facilitate the design and synthesis of novel analogs with demonstrated cytotoxic and tubulin binding abilities, it was necessary to confirm the stereochemistry of HTI-286 and its analogs. To date there have been no reports on determination of the stereochemistry of synthesized analogs such as **2** and **3** by X-ray crystal analysis. However, the structure of the methyl ester of natural product **1** was reported.[10] We hereby report studies on the absolute configuration of the synthetic analogs HTI-286 and HTI-042.

The stereochemistry of compounds **2** and **3** was expected to be *S,S,S* based on the synthetic strategy, HPLC retention time, and bioassay data.[8,11] To confirm the stereochemistry at the α-carbon of the *N*-Me-β-dimethyl phenylalanine residue in these hemiasterlin derivatives and to assign the stereochemistry of **2**, we utilized the double-pulsed field gradient spin echo (DPFGSE) 1D NOE experiment. All the NOE data discussed in this Letter were acquired using THF-d_8. This solvent provided a very well dispersed 1D ^{1}H NMR spectrum and appeared to encourage the occurrence of one significant solution conformation. This observation was evidenced by the extreme magnitudes of the homonuclear coupling constants (both small and large) for **2**. An example of the 1D NOE data acquired from selective excitation of the *proR*-methyl group of the *N*-Me-β-dimethyl phenylalanine residue is shown in Figure 2. As can be seen, the strong NOE correlations between the *proR*-methyl (CH_3-20), the *N*-3 Me, and the *tert*-butyl group (**12a–c**; Fig. 3) require that all of these atoms be on the same face of the molecule. When this information is coupled with the fact that the *proS*-methyl (CH_3-19) shows correlations only to CH_3-20 and the H-14 proton, it becomes clear that this methyl must be on the opposite face of the molecule from CH_3-20 and the stereochemistry at C-14 must be *S*.[8,12]

Some of the most important NOE correlations for the analysis of HTI-286 are shown in Figure 3. According to these studies, the stereochemistry of HTI-286 was in fact the predicted *S,S,S* configuration at the three chiral centers, and this compound appeared to fold back on itself along its length. These observations were confirmed by single-crystal X-ray diffraction.

Crystals of HTI-286 (**2**) and the hydrochloride salt of HTI-042 (**3**) were grown from acetonitrile/water solutions as colorless prisms for a pentahydrate acetonitrile solvate, and colorless needles for a tris(acetonitrile) solvate, respectively. The Flack absolute structure parameter for **3** refined to −0.03(11) for (*S,S,S*) and 1.01(11) for (*R,R,R*). These values are consistent with the correctness of the (*S,S,S*) assignment for **3** based on anomalous dispersion. Using **3** as a heavy-atom derivative, the absolute configuration of **2** is also (*S,S,S*).[13]

Perspective views of **2** and **3** are given in Figure 4. Also included is a view of the methyl ester of **1** to facilitate comparisons. Note that it was drawn as the (*R,R,R*) instead of the (*S,S,S*) isomer in an earlier publication.[10] The tripeptides **1–3** differ only in their A-piece amino acids, so it is perhaps not surprising that the most noticeable conformational differences in their X-ray structures appear near the A-piece ends of these molecules. These differences are largely due to torsional degrees of freedom in the O=C–C–N units in the A-piece amino acid. Referring to Figure 4, from top to bottom, the O=C–C–N torsion angles are 155.7(4)°, −45.5(5)°, and −45.9(3)°, respectively. Note that the A-piece geminal dimethyl group is a key component for potent activity, yet the torsion angles for **2** and **3** forces their dimethyl groups to be completely out of synch with the dimethyl groups in **1**. Presumably these inhibitors assume the same torsion angle upon binding to tubulin,[14] since they have similar biological activity.

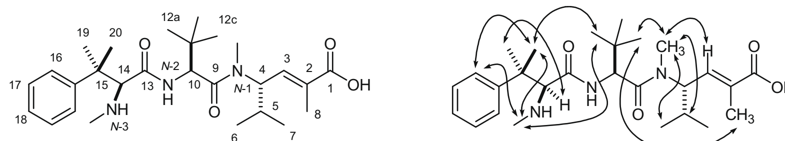

Figure 3. Key NOE enhancements observed for the assignment of C-14 stereochemistry.

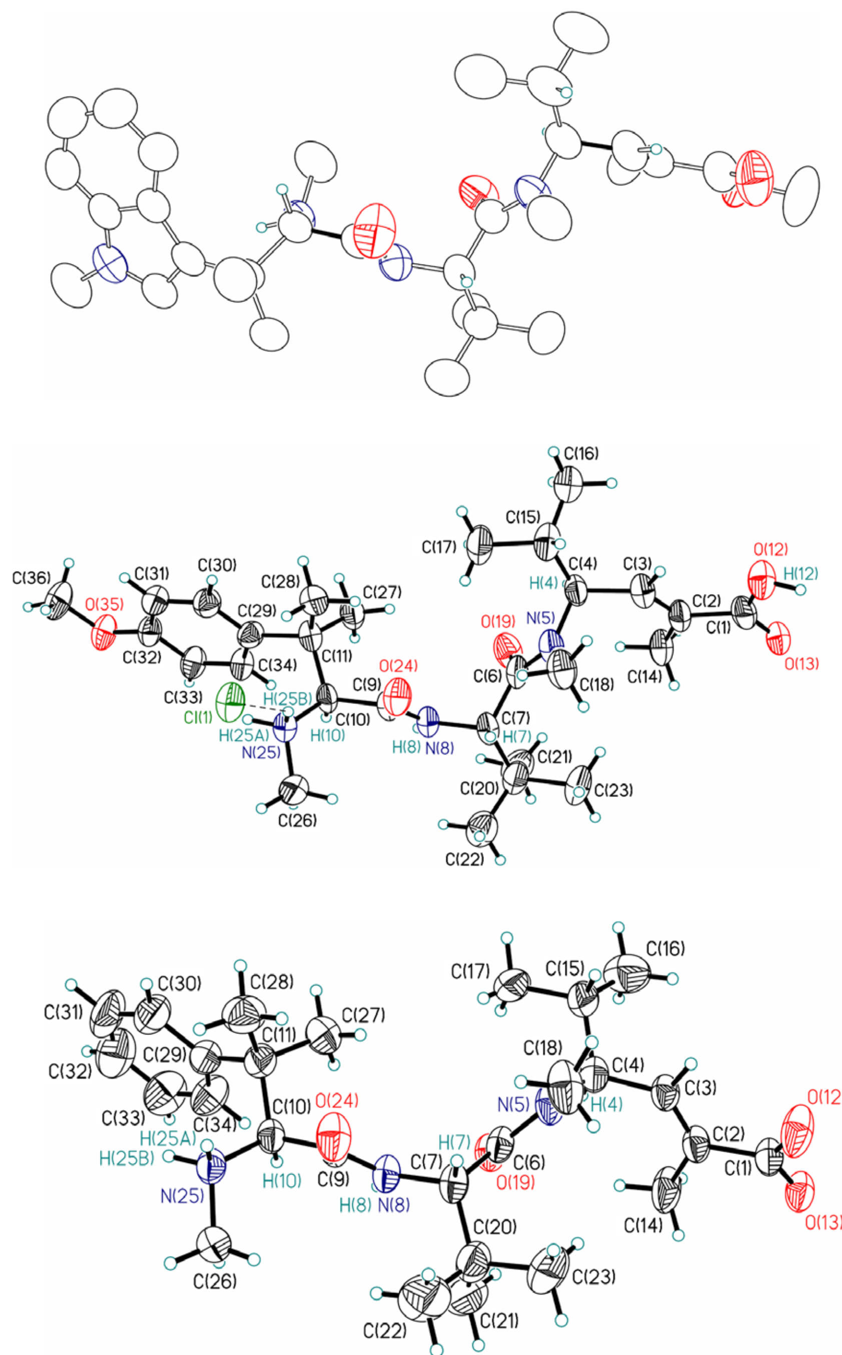

Figure 4. The molecular structures of hemiasterlin methyl ester (top), HTI-042 (middle) and HTI-286 (bottom) obtained by X-ray diffraction analysis.

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