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First Total Synthesis of Tubulysin B

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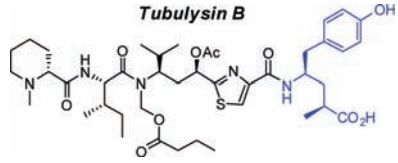
ABSTRACT



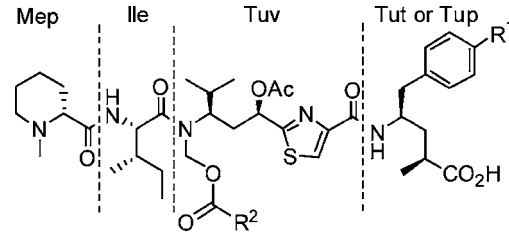
The first total synthesis of tubulysin B is described. The aziridine route to tubuphenylalanine (Tup) of the tubulysin D/U-series could not be transferred to the synthesis of tubutyrosine (blue moiety). Therefore, tubutyrosine (Tut) was synthesized by a Wittig olefination/diastereoselective catalytic reduction sequence. Interestingly, the C-2 epimer of tubulysin B has a cytotoxic activity almost identical to the natural diastereomer.

Tubulysins comprise a natural product family of highly active antimitotic tetrapeptides isolated by Höfle's group from myxobacteria culture broths.[1] At the cell level, they inhibit tubulin polymerization and induce apoptosis.[1,2]

Tubulysins are composed of four amino acids (Figure 1): *D*-*N*-methyl pipecolic acid (Mep), *L*-isoleucine (Ile), tubuvaline (Tuv), which is itself based on two condensed amino acids, and tubutyrosine (Tut) or tubuphenylalanine (Tup). In addition, an unusual tertiary amide *N*,*O*-acetal ester is present in the most active tubulysins.

Tubulysins A and D are representative for the basic structures of the most potent members. Their cell growth inhibitory activity exceeds that of well-known chemotherapeutic agents like taxol or vinblastine by 10-fold to more than 100-fold. Therefore, tubulysins are excellent candidates for the development of novel anticancer drugs. A significant drawback for further applications is the limited availability by fermentation due to a very low production yield.

Although some tubulysins have recently been synthesized by us and others, either all are simplified analogues or the synthetic routes contain steps that proved difficult to repeat, especially under scale up conditions. Most important, all belong to the *D*-series with a C-terminal tubuphenylalanine.[3,4] In contrast to that, so far only tubulysins A and B from the Tut-series advanced to early preclinical use. Thus, only tubulysins A and B have been used in various cancer cell targeting bioconjugate approaches with very encouraging results. They are also the only tubulysins carried on to animal experiments.[5]



Tubulysin A (**1**), R^1= OH, R^2= $CH_2CH(CH_3)_2$
Tubulysin B (**2**), R^1= OH, R^2= $CH_2CH_2CH_3$
Tubulysin D (**3**), R^1= H, R^2= $CH_2CH(CH_3)_2$

Figure 1. Natural tubulysins A, B (with Tut, R^1 = OH), and D (with Tup, R^1 = H).

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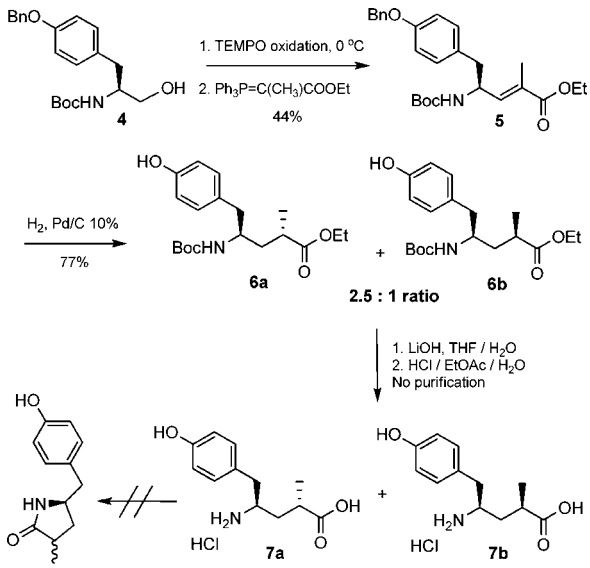
The synthesis of Tut-containing tubulysins like tubulysin A and B has not been accomplished so far. Herein, the first total synthesis of tubulysin B and its C2-epimer is described.

The Tut subunit turned out to be more challenging than anticipated. Despite the structural analogy with Tup, the Tut synthesis could not be accomplished in sufficient yield following the aziridine route as described previously for the synthesis of Tup.[3] This is quite surprising since the *p*-hydroxy group is electronically decoupled; i.e., the phenol is benzylic, not phenylic. Neither the free hydroxy nor protected forms were efficient in the aziridine opening reaction. In addition, the Tut intermediates and the product form the corresponding γ-lactams more easily than the Tup-analogues. This unexpectedly different reactivity of phenylethyl (homobenzylic) substituents vs *p*-hydroxyphenylethyl was also observed by others.[6]

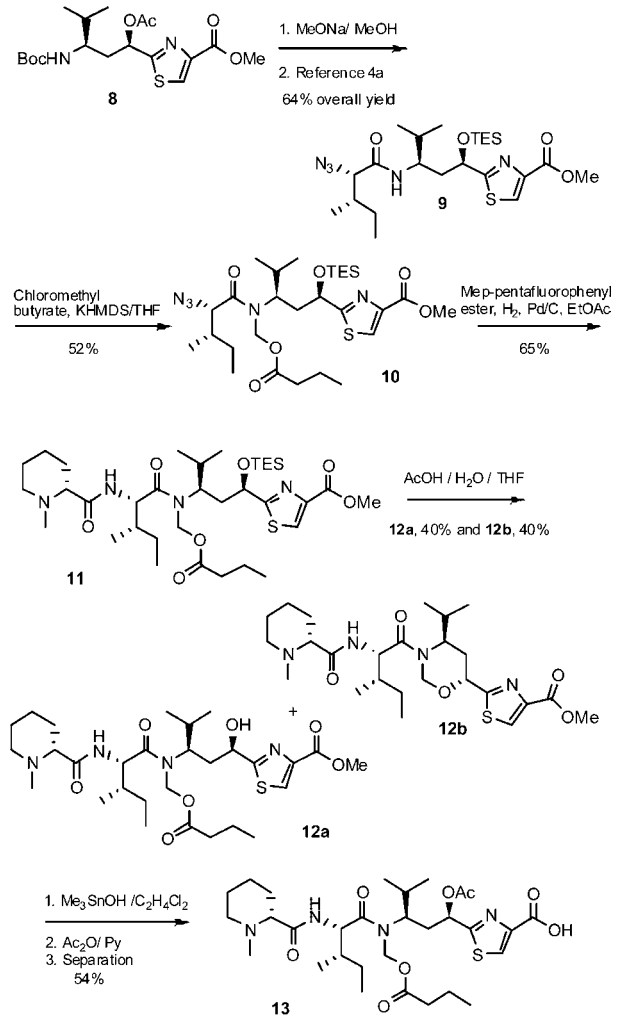
The synthesis of the key intermediate, the α,β-unsaturated ester **5**, was achieved in 44% overall yield by oxidation of the commercially available *N,O*-protected tyrosinol **4** to the aldehyde followed by a Wittig olefination. Complete epimerization was observed when a Swern oxidation was carried out instead of the TEMPO protocol. Ethyl ester cleavage, subsequent menthyl ester formation, and catalytic hydrogenation (e.g., with Pd/C, or Noyori catalyst) rendered a mixture of diastereoisomers that was difficult to separate by flash column chromatography. Instead, a direct catalytic reduction of the Wittig product over Pd on charcoal[7] affords a mixture of C2-diastereomers (2.5:1 ratio) that is easily resolved by preparative HPLC. Despite several attemps, crystals suitable for X-ray analysis could not be obtained, and the unambiguous stereochemical assigment of the diastereoisomers could not be accomplished at this stage. Thus, both compounds were submitted to basic hydrolysis directly followed by removal of the Boc protecting group to afford the corresponding amino acid salts **7a** and **7b** (Scheme 1). This protocol avoids the formation of undesirable γ-lactams.[8] The diastereomeric building blocks were separately carried on to tubulysin B and its C2-epimer. Gratifyingly, the diastereomer with the natural stereochemistry turned out to be the major one (in 53% yield), which is in agreement with previous results in similar systems.[6]

The convergent synthesis of the tripeptide **13** (Scheme 2) was accomplished starting from the uncommon amino acid tubuvaline, which was synthesized featuring a three-component approach.[3] The introduction of the rare *N,O*-acetal proved to be a difficult task even following the route described by Ellman and co-workers.[4a] In our hands, an undesired cyclization to the six-membered acetal took place

Scheme 1. Synthesis of the Tubutyrosine (Tut) Hydrochloride Salts **7a** and **7b**



Scheme 2. Synthesis of Tripeptide **13**



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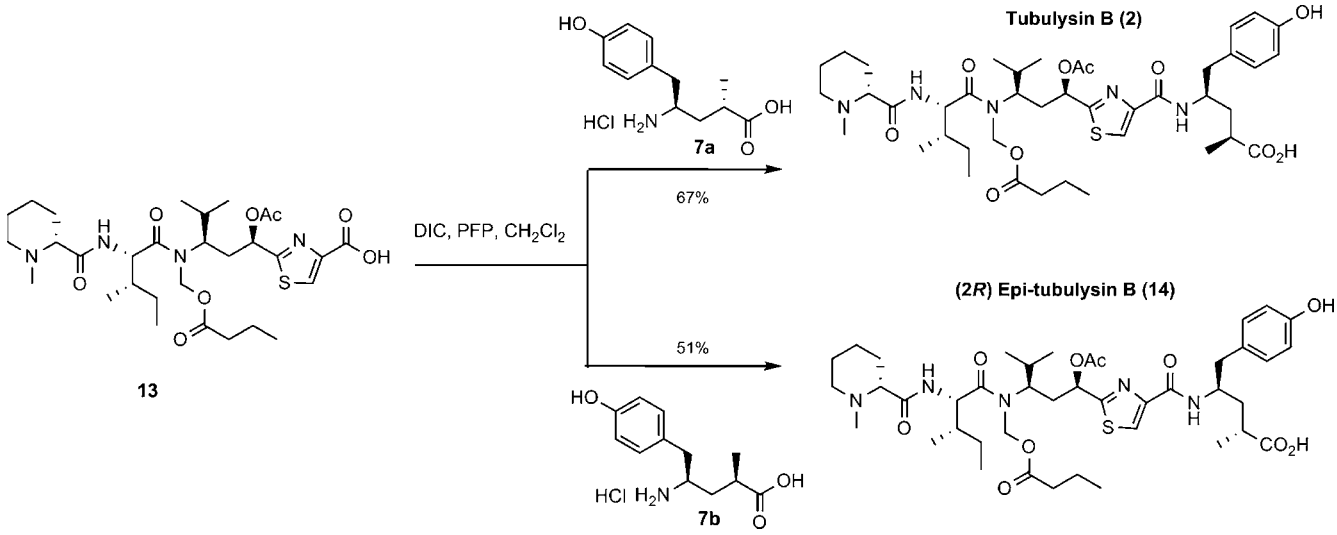
Scheme 3. Synthesis of Tubulysin B (**2**) and Its C-2 Epimer



after removal of the TES protecting group under acidic conditions. A mixture of compounds **12a** and **12b** was obtained. Several attempts to avoid or separate this byproduct were not successful. Preparative HPLC allowed some separation but with a low α-value. Eventually, the crude mixture of compounds **12** was treated with Me_3SnOH to cleave the methyl ester. Acetylation of the secondary alcohol was carried out following a standard procedure,[4b] to afford the separable tripeptide **13** in 54% overall yield from **12a** after HPLC purification.

Finally, the hydrochloride salts of tubutyrosine and its epimer (**7a** and **7b**) were coupled to the free acid **13** through pentafluorophenyl ester formation, to render synthetic tubulysin B (**2**) and the tubulysin B (2*R*)-epimer (**14**) in 67% and 51% yield, respectively (Scheme 3).

Interestingly, the differences of tubulysin B and its C2-epimer are minimal in almost all aspects. R_f values in TLC and biological activity (v.i.) are identical within error limits, and even analytical HPLC retention times and NMR spectra are very similar. Only a comparative look at the NMR spectra reveals the differences (see Supporting Information for details), and a comparison of lists of shift values is not conclusive—similar to earlier observations we made in the tubulysin U/V-series.[3]

The biological activity of natural tubulysin B (provided by R&D Biopharmaceuticals) along with that of the synthetic compounds **2** and **14** was evaluated against human cancer cell lines, using taxol as a reference compound. Interestingly, there are no significant differences between the GI_{50} values of natural, synthetic, and epimeric tubulysin B (Table 1). A relative importance of the stereocenter at C-2 might be expected considering earlier reports in another series.[9] Thus, the measurements were repeated in an independent lab which verified that the activity of both compounds is comparable to that of the isolated natural metabolite. This fact reveals that the stereochemistry of the methyl group at C-2 in tubulysin B is of minor importance for the cytotoxic activity.

Table 1. Cytotoxic Activity (GI_{50} Values [nM])

compound	PC-3[a]	HT-29[b]
nat. tub B (**2**)	0.3	0.5
synt. tub B (**2**)	1.1	1.0
(2*R*)-tub B (**14**)	0.8	1.4
taxol	7.2	-

[a] Human prostate cancer cell line. [b] Human colon cancer cell line.

In summary, a short, convergent, and stereoselective synthesis of tubulysin B and its equally active non-natural C-2 epimer has been performed.

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Supporting Information Available: Experimental procedures and spectral data of all new compounds, including selected copies of ^1H and ^{13}C NMR spectra, HPLC chromatograms, and HRMS (ESI-FT-ICR) spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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