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Synthesis and cytotoxicity evaluation of diastereoisomers and N-terminal analogues of tubulysin-U

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ABSTRACT

Tubulysins are potent anti-mitotic natural compounds and a scalable and efficient synthetic route for generation of its analogues has been developed and extended to the synthesis of diastereoisomers and N-terminal analogues of tubulysin-U. Structure–activity-relationship studies on these synthetic analogues reaffirmed the significance of native stereochemistry of tubulysins for optimal biological activity and cytotoxicity. However, while modification of Tup stereochemistry has only minor effect on the tubulysins cytotoxicity, Tuv stereochemistry is critically important and modification of either Tuv stereocentre produced a dramatic drop in cytotoxicity.

Introduction

Diagnosis and treatment of cancer has been arguably the fastest developing area of modern day biomedical research. Selectivity of anti-cancer drugs towards tumour cells over normal cells is a key factor for achieving therapeutic efficacy, and highly potent tubulysins may turn out to be extremely effective tools in this regard, particularly if coupled to a monoclonal antibody (MAb)—the essence of targeted drug delivery. Tubulysins are tetrapeptide natural products produced in rather small quantities by two different species of *Myxobacteria*[1] (Fig. 1).

Tubulysins are amongst the most potent known anti-mitotic compounds. Mechanistically, they bind to tubulin and disintegrate microtubules of dividing cells, thereby inducing apoptosis.[2] Structurally, they are composed of three non-proteinogenic amino acids (Tubuvaline/Tuv, Tubuphenylalanine/Tup or Tubutyrosine/Tut and *N*-methyl pipecolic acid/Mep) and proteinogenic Isoleucine/Ile.

Several natural structural variants of tubulysins **1** have been described—tubulysin D being the most cytotoxic. All these tubulysin derivatives possess the same N-terminal amino acid—*N*-methyl pipecolic acid, followed by the natural isoleucine fragment. The functional group differences in the non-proteinogenic Tuv and Tup/Tut fragments give rise to the majority of the analogues—Tubulysins A–I, U, V, Y and Z (Table 1).

Several reports on the total synthesis of tubulysins and their analogues[3] including structurally simplified 'Tubugis' compounds, *N*-methyl tubulysins, pre-tubulysins, etc., have been reported, all due to overwhelming interest in their potential as novel anticancer agents. The development of a library of stereoisomers of tubulysins could shed light on the key binding interactions of tubulysins with tubulin as a function of the overall stereochemistry of the molecule. Tamura and co-workers have demonstrated that stereoisomers of tubulysin D possessing different configurations at the Tuv stereogenic centres are at least three orders less cytotoxic,

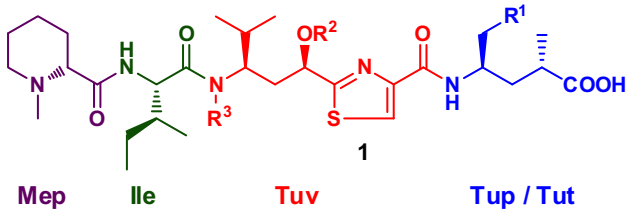

Figure 1. Structure of tubulysins **1**.

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Table 1
Natural tubulysins

Tubulysin	R^1	R^2	R^3
A	*p*-OH-Ph	Ac	$-CH_2OCO$-*i*-Butyl
B	*p*-OH-Ph	Ac	$-CH_2OCO$-*n*-Propyl
C	*p*-OH-Ph	Ac	$-CH_2OCOEt$
D	Ph	Ac	$-CH_2OCO$-*i*-Butyl
E	Ph	Ac	$-CH_2OCO$-*n*-Propyl
F	Ph	Ac	$-CH_2OCOEt$
G	*p*-OH-Ph	Ac	$-CH_2OCOCH{=}C(CH_3)_2$
H	Ph	Ac	$-CH_2OCOMe$
I	*p*-OH-Ph	Ac	$-CH_2OCOMe$
U	Ph	Ac	H
V	Ph	H	H
Y	*p*-OH-Ph	Ac	H
Z	*p*-OH-Ph	H	H

even though the inhibition of tubulin polymerization is comparable to that of the native analogue.[4] The significance of natural (*R*)-configuration of the Tuv *O*-acetate functionality and its influence on the binding with tubulin was reported also by Wipf et al.[5] More recently, while this Letter was in preparation, Yang et al. reported that the epimer of tubulysin U having (*S*)-stereochemistry at the Tup C-4 maintained significant potency against several cell lines.[6] It would be justifiably prominent to obtain a reliable full picture of the structure–activity-relationship (SAR) profile of different stereoisomers of tubulysins, with the potential of developing more active and stable analogues and hence to reaffirm the significance of native stereochemistry in active tubulysins.

Herein, we report the total synthesis of different diastereoisomers and N-terminal analogues of tubulysin U for implementing a SAR study designed to investigate the importance of native stereochemistry and N-terminus of the molecule for its optimal cytotoxic activity.

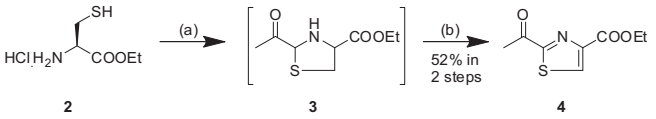
Results and discussion

We have reported a scalable synthesis of Tubulysin U and V in 2007[7] and we very recently demonstrated the effect of variations in functional groups in the central Tuv fragment[3k,8] on the cytotoxicity of various tubulysin U analogues. Even though tubulysins have attracted the interests of the medicinal chemistry community ever since their discovery and quite a few reports have been dedicated to studies on structural variations in Tuv and Tup/Tut fragments, only less than a handful surveyed the significance of the tubulysins' native stereochemistry.[4–6]

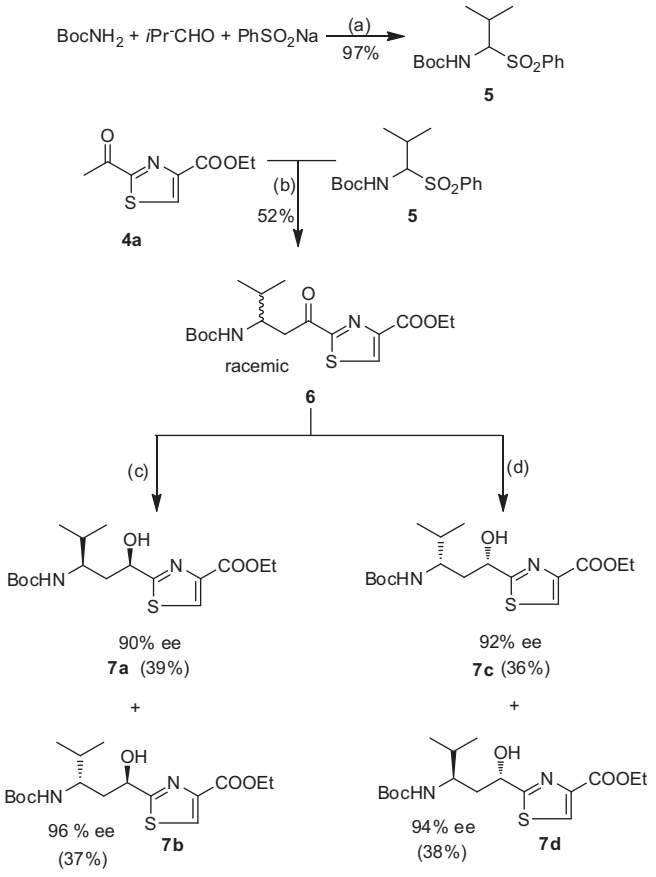
With a reliable and scalable synthetic strategy in hand, we decided to probe the effect on cytotoxicity of systematically altering the stereochemistry of different carbon centres in two of the non-proteinogenic amino acids (Tuv and Tup) in tubulysin U, alongside a couple of N-terminal analogues.

The total synthesis of all the analogues started with the generation of the thiazole ring of the Tubuvaline fragment. Condensation of L-cysteine hydrochloride ethyl ester **2** with methyl glyoxal in the presence of sodium bicarbonate gave a thermally unstable thiazoline **3**, which on MnO_2 mediated oxidation afforded the 2-acyl thiazole ester **4** in 52% overall yield (Scheme 1).

The enol derivative of thiazole **4** was coupled with α-amino sulfone **5** obtained by a one-pot reaction involving Boc-carbamate, isobutyraldehyde and benzene sulfinic acid sodium salt in the presence of formic acid, to give the corresponding β-amino ketone **6** as a racemate. The stereopure precursors of Tuv **7a–d** were obtained by the (*S*)- or (*R*)-CBS-oxazoborolidine/$BH_3{\cdot}Me_2S$ mediated reduction of ketone **6**, followed by chromatographic separation of the diastereoisomers (ratio 1:1). The ee of each stereoisomer was determined by HPLC (Scheme 2).



Scheme 1. Synthesis of 2-acyl thiazole ethyl ester. Reagents and conditions: (a) methyl glyoxal aqueous solution, $NaHCO_3$, $EtOH:H_2O$ (1:1), overnight; (b) MnO_2, MeCN, 65 °C, overnight.



Scheme 2. Synthesis of the four Tuv stereoisomers. Reagents and conditions: (a) formic acid, $THF:H_2O$, 24 h.; (b) NaH, THF, 2–3 h; (c) (*S*)-(−)-2-methyl-CBS-oxazaborolidine, $BH_3{\cdot}Me_2S$, THF, 0 °C, 2–3 h; (d) (*R*)-(+)-2-methyl-CBS-oxazaborolidine, $BH_3{\cdot}Me_2S$, THF, 0 °C, 2–3 h.

The relative configuration of the two stereocentres was confirmed by single crystal X-ray diffraction[8] of **7b** and further correlation with enantiopure *O*-Ac-Cbz-Tuv-OEt as reported by Wipf et al.[9]

The next step was the synthesis of the second non-proteinogenic amino acid fragment Tup. We have earlier reported the synthesis of Tup bearing its natural stereochemistry,[3k,7] but that was not found to be quite efficient in generating the diastereoisomers in good yields and stereopurity, presumably due to the challenging chromatographic separations and protecting group manipulations involved. Hence, we decided to extend the strategy that was reported more recently[8] and, satisfactorily, we were able generate the desired isomers of Tup in their stereopure form (Scheme 3).

Acylation of (−)-menthol using bromoacetyl bromide followed by treatment with triphenylphosphine generated ylide **9** which was methylated using MeI to afford **10** and the stage was set for a Wittig reaction involving the corresponding aldehyde **11** obtained by the oxidation of protected L- or D-phenylalaninol.