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SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY OF A CYCLIC ANALOG OF DOLASTATIN 10

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Abstract

A cyclic analog of the natural antiproliferative compound dolastatin 10 was synthesized by introducing an ester link between the N- and C-terminal residues which were modified accordingly. The final macrolactonization was performed by using isopropenyl chloroformate and DMAP as reagents. This analog exhibits submicromolar antiproliferative activity against the L1210 and HT29 cell lines and inhibits *in vitro* tubulin polymerization (IC_{50}, 39 μM).

Keywords: *antineoplastic; antiproliferative agent; depsipeptide; marine metabolites*

Dolastatins are members of a family of antineoplastic pseudopeptides isolated from the sea hare *Dolabella auricularia*.[1,2] Dolastatin 10[3] and a closely related analog[4] show promising antiproliferative properties and were selected as potentially chemotherapeutic agent to undergo clinical assays. Dolastatin 10 typically behaves as an antimitotic agent and inhibits *in vitro* microtubule assembly by binding to the β subunit of tubulin near the vinca domain.[5] However, its precise molecular interactions are unknown.

Recently, we showed by NMR studies that dolastatin 10 exists in solution as two different conformations (a bended and an extended forms) corresponding to a *cis-trans* isomerization of the Dil-Dap amide bond[6]. Similar results were obtained by other groups.[7-9] Locking of one of these forms could provide insight into the biological relevance of such an equilibrium, thus allowing a better understanding of the molecular interactions

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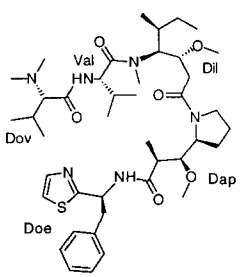
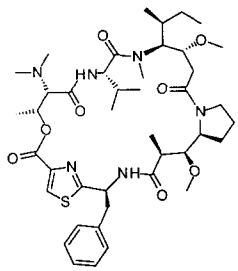
involved. Scrutiny of the bent structure showed that it should be possible to insert an ester linkage between the side chain of the Dov residue and the thiazole ring without affecting the general shape of the molecule. This implies the replacement of the Dov unit by a threonine derived residue and the introduction of a carboxylic function onto the thiazole ring. We describe herein the synthesis and the biological evaluation of compound **1**.



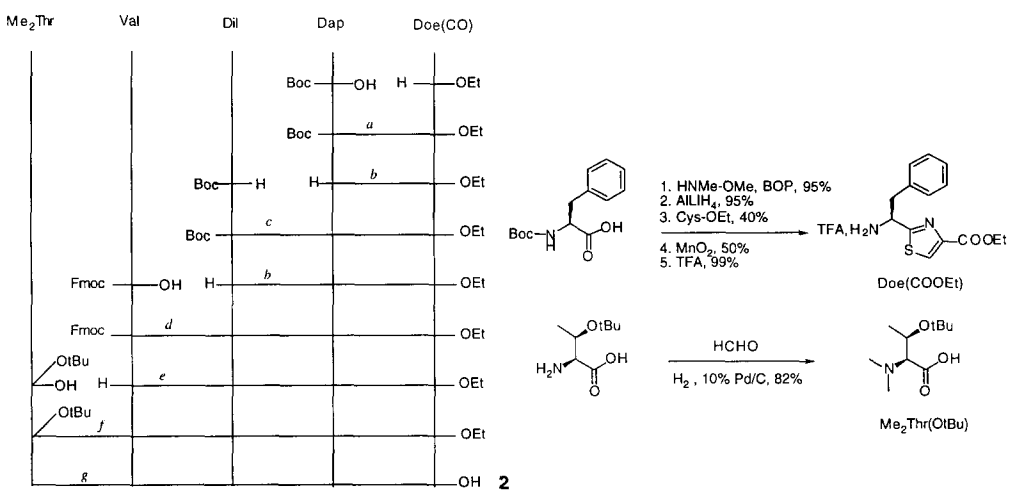
Dolastatin 10



1

Owing to the pseudopeptide nature of the target molecule **1**, five cyclization sites (four amide bonds and one ester bond) could be envisaged. However, we assumed that the modified linear precursor **2** would adopt a conformation very similar to that of dolastatin 10 so that the carboxylic and the hydroxyl groups could be close enough to each other to react easily. Such a situation could not be guaranteed for the other sites. Thus, the synthesis of compound **1** was performed by using the stepwise procedure depicted in scheme 1.

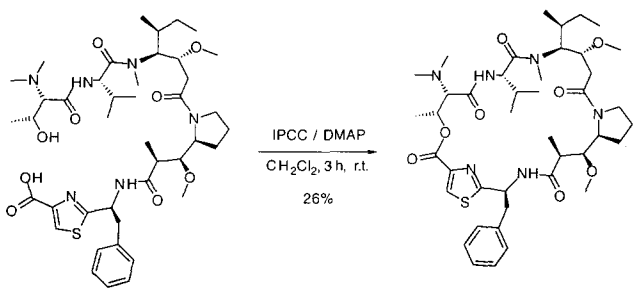
Scheme 1



(a) BOP, Et_3N, CH_2Cl_2, 70%; (b) TFA; (c) BOP, Et_3N, CH_2Cl_2, 73% for two steps; (d) BroP, iPr_2EtN, CH_2Cl_2, 76% for two steps; (e) Et_2NH, DMF; (f) DCC, CH_2Cl_2, 66% for two steps; (g) (*i*) TFA, (*ii*) 2N NaOH, MeOH, 92%.

Carboxylated dolaphenine [Doe(CO) in scheme 1] and Me_2Thr(OtBu) were synthesized according to known procedures (Scheme 1).[10,11] The protected dolaisoleuine (Dil) and dolaproine (Dap) residues were obtained as described earlier.[12] Compound **2** was obtained with an overall yield of 24% relative to Doe(CO).

Scheme 2



2 **1**

Cyclization was performed by a mixed anhydride activation according to our previously described procedure (Scheme 2).[13] After purification by reverse phase HPLC and lyophilization, compound **1** was obtained in moderate yield (26%) as a white solid, homogeneous by HPLC[14] and capillar electrophoresis.[15,16] Its structure was ascertained by its mass spectroscopic properties (FAB+ and HRFAB).[17] NMR spectra recorded at 600 Mhz in various solvents were complicated by the presence of 3 conformers.

Cytotoxic activity of **1** was tested on human colon adenocarcinom (HT-29) and on murine leukemia (L1210) cell lines and compared with that of the parent compound and dolastatin 15. The effect of the compounds on tubulin polymerization was also evaluated. The results are given in the Table.

Table : Inhibition of Cell Growth (IC_{50}, nM) and *in vitro* Tubulin Polymerization (IC_{50}, μM)

compound	cell line		tubulin
	HT-29	L1210	polymerization
Dolastatin 10	0.06	0.03	2.2
Dolastatin 15	0.29	0.13	17
1	58.5	110.8	39

In comparison with the parent compound, **1** exhibited a loss of cell growth inhibitory activity equal to about three orders of magnitude. The difference between the two compounds was less pronounced with respect to the effects on *in vitro* tubulin polymerization, but it should be noted that this assay does not correlate with the inhibition of cell growth.[18-20]

These results are quite encouraging in view of the strong constraint introduced by the cyclization. Although the loss of activity was significant, the IC_{50} value remained at a submicromolar level. However, they

do not allow us to ascertain the contribution of a bent conformation of dolastatin 10 involving a *cis* conformation of the Dil-Dap bond on the biological activity. In particular, the complexity of the NMR spectra was quite unexpected and studies are currently in progress to elucidate the conformations of **1**. The lower biological activity could be explained by the blockage in **1** of the modified Dov residue which has been shown to be crucial for the antiproliferative activity of dolastatin 10.[19,21] Modifications leading to less constrained cyclic compounds are being examined and will be reported in due course.

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