

## 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<sub>50</sub>, 39  $\mu$ M). © 1998 Elsevier Science Ltd. All rights reserved.

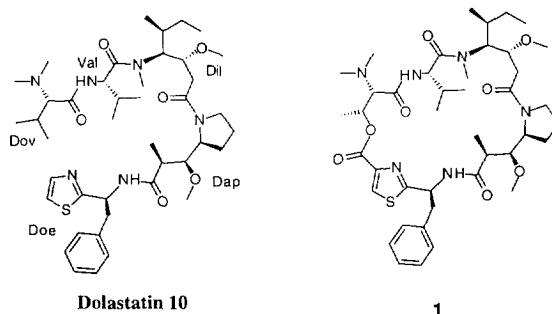
**Keywords:** antineoplastic; antiproliferative agent; depsipeptide; marine metabolites

Dolastatins are members of a family of antineoplastic pseudopeptides isolated from the sea hare *Dolabella auricularia*.<sup>1,2</sup> Dolastatin 10<sup>3</sup> and a closely related analog<sup>4</sup> show promising antiproliferative properties and were selected as potentially chemotherapeutic agent to undergo clinical assays. Dolastatin 10 typically behaves as an antimetabolic agent and inhibits *in vitro* microtubule assembly by binding to the  $\beta$  subunit of tubulin near the vinca domain.<sup>5</sup> 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<sup>6</sup>. Similar results were obtained by other groups.<sup>7-9</sup> 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

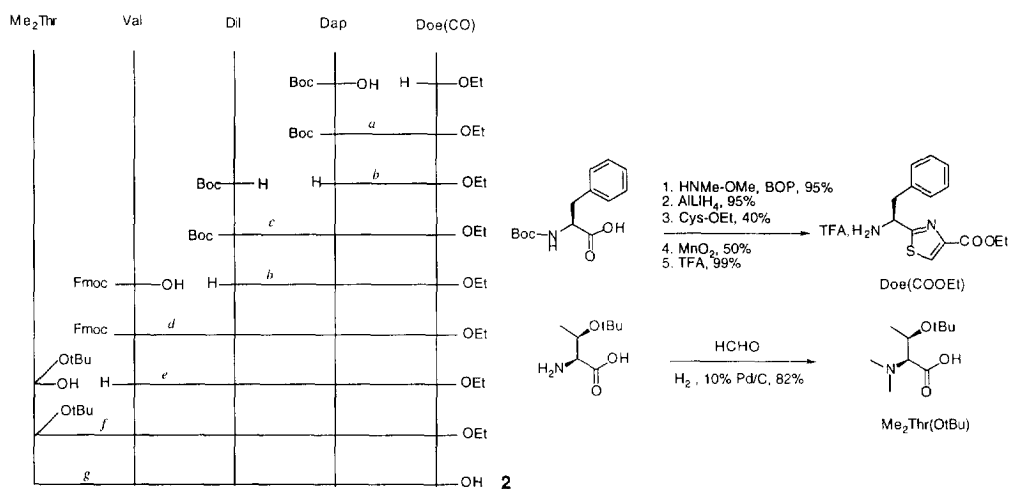
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involved. Scrutiny of the bent structure showed that it should be possible to insert an ester linkage between the side chain of the Dov residue and the thiazole ring without affecting the general shape of the molecule. This implies the replacement 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**.



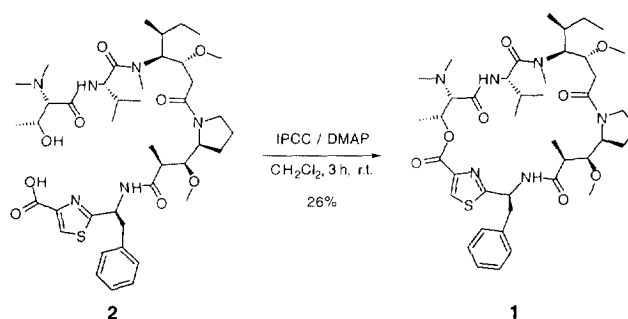
Owing to the pseudopeptide nature of the target molecule **1**, five cyclization sites (four amide bonds and one ester bond) could be envisaged. However, we assumed that the modified linear precursor **2** would adopt a conformation very similar to that of dolastatin **10** so that the carboxylic and 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,  $\text{Et}_3\text{N}$ ,  $\text{CH}_2\text{Cl}_2$ , 70%; (b) TFA; (c) BOP,  $\text{Et}_3\text{N}$ ,  $\text{CH}_2\text{Cl}_2$ , 73% for two steps; (d) BrOP,  $\text{iPr}_2\text{EtN}$ ,  $\text{CH}_2\text{Cl}_2$ , 76% for two steps; (e)  $\text{Et}_3\text{N}$ , DMF; (f) DCC,  $\text{CH}_2\text{Cl}_2$ , 66% for two steps; (g) (i) TFA, (ii) 2N NaOH, MeOH, 92%.

### Scheme 2



Cytotoxic activity of **1** was tested on human colon adenocarcinoma (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<sub>50</sub>, nM) and *in vitro* Tubulin Polymerization (IC<sub>50</sub>, 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

These results are quite encouraging in view of the strong constraint introduced by the cyclization. Although the loss of activity was significant, the IC<sub>50</sub> 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.<sup>19,21</sup> Modifications leading to less constrained cyclic compounds are being examined and will be reported in due course.

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14. Column: Kromasil C8 5mm 4.6 x 150 mm. Mobile phase : acetonitrile 0.1% TFA / water 0.1% TFA (gradient from 50% to 100% in 10 min at a flowrate of 1.5 mL/min).  $t_R$  = 3.04 min
15.  $t_m$  = 18.0 min in the conditions described in ref 16.  $t_m$  = 18.9 min for dolastatin 10.
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17. MS (FAB)  $m/z$  (%) 813 (M+H<sup>+</sup>, 85), 781 (M-MeOH+H<sup>+</sup>, 10); HRMS (FAB) calcd for C<sub>42</sub>H<sub>65</sub>N<sub>6</sub>O<sub>8</sub>S 813.4585, found 813.4583.
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