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SYNTHESIS AND IN VITRO CYTOTOXICITY OF DIASTEREOISOMERICALLY MODIFIED DOLASTATIN 15 ANALOGUES

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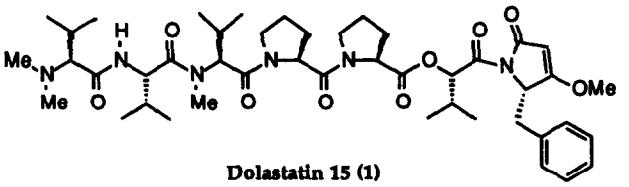
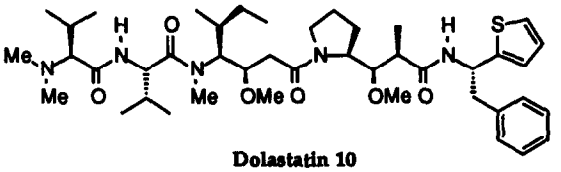
Key words: Dolastatin; Depsipeptide; Cytostatic activity; Antiproliferative effect

Abstract : Dolastatin 15 **1**(4S,7S) is a potent cytostatic depsipeptide of marine origin. Analysis of the effects of the stereochemistry in the non-peptide moiety on activity revealed that chirality inversion in the aromatic terminal region preserves the antiproliferative activity.

Dolastatin 15 **1**(4S,7S) is a cytostatic depsipeptide isolated from the marine molusc *Dolabella auricularia*.[1] Its linear structure is related to that of dolastatin 10, another potent antineoplastic metabolite of the same origin.[2] Detailed biochemical analysis has revealed a qualitatively similar activity profile for both agents, although dolastatin 10 presents higher potency in most studies.[3-5] These agents are antimitotic and specifically interact with tubulin.[6,7] Their cell cycle phase selectivity was confirmed by typical G2/M metaphase arrest analysis.[5,8]



Dolastatin 15 (1)

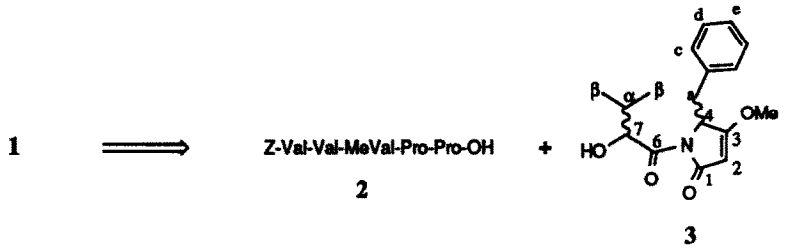


Dolastatin 10

The recent development of synthetic procedures for the preparation of dolastatin 10[9] was accompanied by a preliminary structure-activity analysis.[10] Surprisingly, no such analysis has been performed on dolastatin 15. Having developed an efficient synthesis of dolastatin 15[11] we evaluate here the stereoisomerical requirements of the two chiral centers C-4 and C-7[12] present in the non-peptide moiety of the molecule on its cytotoxic activity.

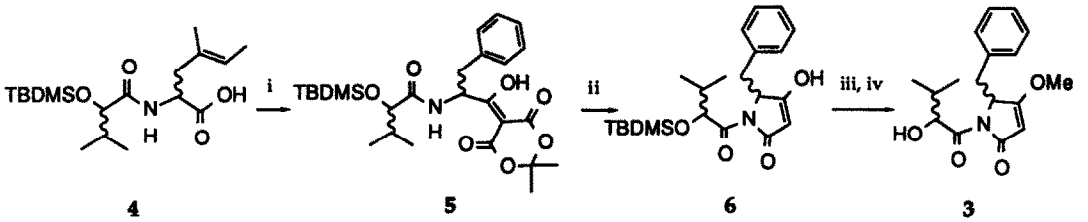
Chemistry

The general convergent strategy used for the preparation of dolastatin 15 (Scheme 1) [11,13] allows



Scheme 1. Retroynthesis of dolastatin 15 analogues

modifications of acyl-pyrrolidinone **3** and subsequent coupling with the same pentapeptide **2**. We obtained the four diastereoisomers of the N-(hydroxy-isovaleryl)-pyrrolidinone **3** by condensation of protected N-(hydroxyisovaleryl)-phenylalanine **4** with Meldrum's acid, cyclisation of the adduct **5** followed by methylation of the corresponding tetramic acid intermediate **6** under Mitsunobu conditions, and finally deprotection of the hydroxyl (Scheme 2).[11] Since activation of an N-acylamino acid as in **4** proceeds with epimerisation, we did not attempt to selectively obtain each diastereoisomer of **3** independently. We prepared both **3**(4S7S)/**3**(4R7S) and **3**(4S7R)/**3**(4R7R) pairs of diastereoisomers starting from N-[(2*S*)-2-hydroxy-isovaleryl]-D,L-phenylalanine **4**(7S) and the 2*R* countrepart **4**(7R), respectively. Compound **3**(4S7S) was separated from **3**(4R7S) by silica gel column chromatography. The **3**(4S7R)/**3**(4R7R) pair was separated in the same way..



Scheme 2. i: Meldrum's acid (1.1 equiv), dimethylaminopyridine (2.5 equiv), isopropenyl chlorocarbonate (1.1 equiv), CH_2Cl_2, -10 °C, 15 min; ii: Δ , CH_3CN, 30 min, 85% for two steps; iii: Ph_3P (1.2 equiv), MeOH (1.2 equiv), DEAD (1.2 equiv), THF, rt, 16 h, 60%; iv: CF_3CO_2H, rt, 5 min, 85%.

The absolute configuration of compounds **3**(4S7S) and **3**(4R7S) was determined in a previous study.[11] The absolute configuration of their respective enantiomers **3**(4R7R) and **3**(4S7R) were assessed by comparison of their physical properties.[14] By using described procedures,[11] each alcohol **3** was coupled with pentapeptide **2** and the resulting depsipeptide deprotected and methylated to give the corresponding compound **1**.[15]

Antiproliferative activity

The *in vitro* antiproliferative potency of both dolastatin 10 and dolastatin 15 has been extensively studied on bone marrow and lymphoma cell lines. Dolastatin 15 has a marked antiproliferative effect on normal human and murine bone marrow progenitor cells and against different human lymphoma cells (IC_{50}, 0.012 to 0.12

nM),[4,8] as well as on continuous human leukemia cell lines and peripheral blood cells from patients with acute myeloid leukemia (IC50, 0.1 to 1 nM)[3]. Therefore, we tested the four diastereoisomers of dolastatin 15 for their effect on the proliferation of different human and murine cell lines and primary cells (Table). The assays included growth factor-dependent proliferation of primary murine bone marrow cell, interleukin-2- dependent proliferation of the murine cytotoxic T cell line CTLL, growth factor-independent proliferation of the human leukemia T cell line Jurkat[16] as well as murine leukemia L1210 proliferation[17]. As shown in the Table, the compounds inhibit the proliferation of all cell types with IC50 values in the nanomolar range. Dolastatin 15 **1**(4S7S) was the most potent; the diastereomers **1**(4S7R) and **1**(4R7S) were approximately 2-fold less potent and **1**(4R7R) isomer showed a 10- to 100-fold decreased activity. None of the compounds showed selectivity for any cell type.

Table. Effects of dolastatin 15 (D15) analogues **1** on growth inhibition (IC_{50}, nM)

	Bone marrow	Jurkat	CTLL	L1210
1(4S,7S)	17	5	3	0.15
1(4S,7R)	40	10	8	0.30
1(4R,7S)	50	10	10	0.31
1(4R,7R)	120	700	400	1.78

In the course of an ongoing structure-activity relationship analysis of dolastatin 10 analogues, it was recently demonstrated that inversion of the configuration at the aromatic terminal residue only moderately decreases its cytotoxic activity and its inhibitory effect on tubulin polymerisation.[18] Having prepared dolastatins 10[19] and 15,[11] we compared their conformation in solution. We observed a high degree of flexibility in the aromatic region of both compounds. In that context, it is not surprising to observe only a small decrease in activity by inverting the configuration of C-4 in dolastatin 15. Moreover, dolastatin 10 exists in two different conformations corresponding to *cis-trans* isomerisation of the Dil-Dap amide bond (manuscript submitted), whereas all the amide bonds are *trans* in dolastatin 15 (unpublished results). A conformational study of all the analogues presented here is currently under investigation to determine whereas the decrease in growth inhibiting activity could be correlated with a modification of the configuration of one or more of the amide bonds.

Acknowledgement

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14. Physical and spectral data of **3**.

Compound	$[\alpha]_D^{20}$ (c 1, MeOH)	H^1 NMR, 360 MHz (DMSO *d6*) δ(ppm) Hβ	Hα	Ha	OMe	H6	H4	H2	Hc,d,e
3(4S7S)[3(4R7R)]	+313(-300)	0.82, 0.88	1.85-1.96	3.00, 3.50	3.82	4.82-4.84	4.90	5.08	6.92-7.25
3(4R7S)[3(4S7R)]	-232(+244)	0.85, 0.95	1.82-1.90	3.10, 3.30	3.84	4.76-4.77	4.90	5.14	6.92-7.24

15. Physical and spectral data of **1** (H^1 NMR data are consistent with the assigned structures).

Compound	$[\alpha]_D$ [a]	mp (°C)	HPLC, rt (mn)[b]	MS(FAB+) m/e[c]
1(4R7S)	-285	159 - 161	5.50	838 (9)
1(4R7R)	-290	151 - 153	6.66	838 (19)
1(4S7R)	-25	140 - 142	6.75	838 (9)

a: *c* 0.2, MeOH; b: C8, Ultrabase 5 μm, B(CH_3CN, 0.1% TFA)/A(H_2O, 0.1% TFA), 1.5 ml/mn, 40 to 70% B in 10 mn; c: MH^+(relative intensity).

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