

## DIDEMNIN B : COMPARATIVE STUDY AND CONFORMATIONAL APPROACH IN SOLUTION.

B. BANAIGS<sup>a</sup>, G. JEANTY<sup>a</sup>, C. FRANCISCO<sup>a\*</sup>, P. JOUIN<sup>b</sup>, J. PONCET<sup>b</sup>,  
A. HEITZ<sup>b</sup>, A. CAVE<sup>b</sup>, J. C. PROMÉ<sup>c</sup>, M. WAHL<sup>d</sup>, F. LAFARGUE<sup>d</sup>.

<sup>a</sup> Laboratoire de Chimie Marine (GERAP), Université de Perpignan 66025 Perpignan France .

<sup>b</sup> Centre CNRS-INSERM de Pharmacologie et d'Endocrinologie, BP 5055, 34033 Montpellier CEDEX, France.

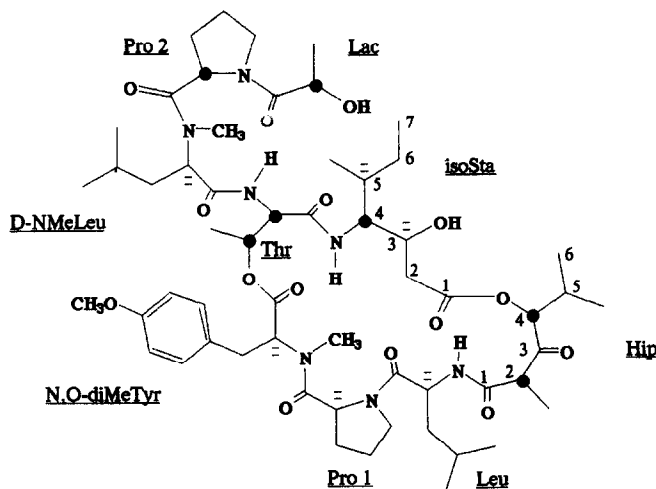
<sup>c</sup> Centre CNRS de Biochimie et de Genetique Cellulaires, 31062 Toulouse , France.

<sup>d</sup> Laboratoire Arago, 66650 Banyuls-sur-mer, France.

(Received in Belgium 12 October 1988)

**Abstract :** A comparative study of isodidemnine-1 and didemnin B is presented using spectroscopic methods, partial degradation and partial synthesis. This leads to the conclusion of the presence of a single depsipeptide, namely didemnin B, with (3S,4R,5S) isostatine instead of the previous statine residue. An attempt to determine the whole conformation in solution of didemnin B by using 2D-NMR is also described .

Over the last decade, the widely-distributed order of Aplousobranchiata (Phylum Chordata, Class Ascidiacea), one of the colonial form of Urochordata, has been the object of intense chemical scrutiny by several research groups [1]. The initial interest for the family of Didemnidae - probably derivated from the family of Polycitoridae in the course of evolution [2] - was prompted by the potent activity found against viruses and tumor cells [3] from a Caribbean tunicate (*Trididemnum* sp, possibly *Trididemnum cyanophorum*) [4]. This led to the isolation of a new class of novel cyclic depsipeptides called the di-



**Figure 1 : Structure of Didemnin B**

demnins [5], and to numerous reports of pharmacological studies of these new agents [6]. More recently, the structure of another didemnin was published [7] and the structure of the former didemnin B revised. [8]

Along with our continued interest in the novel natural products chemistry of marine organisms, we have begun a systematic study of Didemnidae with respect to their chemical defenses. Various metabolites from marine tunicates have been shown in our lab [9], to possess antimicrobial, cytotoxic or antiparasitoy properties, in agreement with the need for soft-bodied organisms to develop the art of chemicals (from lipids to peptides) against their predators. During these studies, we

have worked on *Trididemnum cyanophorum* (Guadaloupe) which was known to be the host of an unicellular cyanophyta (*Synechocystis trididemni*) [10], and found didemnin-like products. Our first results [11] convinced us that the situation was unclear between the various closely related species of Caribbean *Trididemnum* (*cyanophorum*, *solidum* or *palmae*), and between the various described peptides (isodidemnin-1, former didemnin B or the revised structure). Therefore, we decided to present a complete study, including information and results obtained by comparison of isodidemnin-1 and our major product. Considering the high activity of these agents, we have also undertaken efforts to determine the drug conformation in solution, using 2D-NMR means. [12,13]

The most abundant compound from *Trididemnum cyanophorum* (harvested by Scuba techniques near "Ilet Pigeon" - Cousteau's reservation) was readily identified as a peptide by mass spectrometry (see experimental). Its close relationship with didemnin B was based on  $^1\text{H}$  NMR, including 2D-NMR (table 1), and on GC/MS of the heptafluorobutyl propyl ester derivatives of the constituent amino acids. All these results were in agreement with those already described by Rinehart. From a pharmacological point of view, we found lesser activities ( $\text{ID}_{50} = 0.19 \text{ nM}$ ) during in vitro B16 melanoma and P388 screenings, but also lower toxicity during in vivo P388 tests, as some mice were found to survive.

Table 1:  $^1\text{H}$ - $^{13}\text{C}$  NMR DATA (360/90 MHz)

Residue	$^1\text{H}$		$^{13}\text{C}$	Residue	$^1\text{H}$		$^{13}\text{C}$
	$\text{CDCl}_3$	$\text{Pyr-D}_5(-20^\circ\text{C})$			$\text{CDCl}_3$	$\text{CDCl}_3$	
<b>Isohistidine</b>							
NH	7.15 (d,9.5)	7.63 (d,9)		C-H	4.32 (q,7)	4.48 (q,7)	65.4 (d)
4C-H	4.03 (dt,9.5;3)	4.73 (ddd,10;9;1.5)	55.0 (d)	CH <sub>3</sub>	1.23 (d,7)	1.47 (d,7)	20.2 (q)
5C-H	1.77 (m)	2.57 (m)	33.9 (d)	OH	3.34 (bs)		
5C-CH <sub>3</sub>	0.8 (overlap)	1.15 (d,7)	14.7 (q)	<b>Proline 1*</b>			
6C-H <sub>2</sub>	1.15 (overlap)	1.67 (m,7;?;?)	24.9 (t)	$\alpha\text{C-H}$	4.77 (dd,8;4.5)	4.70 (m)	56.7 (d)
	1.35 (overlap)	1.44 (m,7;?;?)		$\beta\text{C-H}_2$	1.91 (m)- 2.16 (m)	1.64 (m) 1.78 (m)	27.9 (t)
7C-H <sub>3</sub>	0.85 (overlap)	1.08 (t,7)	11.4 (q)	$\gamma\text{C-H}_2$	1.91 (m) 2.16 (m)	1.38 (m) 1.41 (m)	24.9 (t)
3C-H	4.01 (overlap)	4.83 (t,10;10)	67.3 (d)	$\delta\text{C-H}_2$	3.51 (m) 3.61 (m)	3.23 (m) 3.46 (m)	46.8 (t)
2C-H <sub>2</sub> b	3.21 (d,16.8)	4.40 (d,18)	29.7 (t)	<b>N-methylLeucine</b>			
	a 2.55(dd,16.8;10)	3.09 (dd,10;18)		N-CH <sub>3</sub>	3.08 (s)	3.18 (s)	31.4 (q)
OH	2.97 (bs)			$\alpha\text{C-H}$	5.32(dd,12;2)	5.79(dd,11.5;3.5)	54.3(d)
<b>Leucine</b>							
NH	7.75 (d,7.5)	8.59 (d,9)		$\beta\text{C-H}_2$	1.64 (m) 1.77 (m)	1.65 (m) 1.95 (m)	36.2 (t)
$\alpha\text{C-H}$	4.74 (bt,9.5;?)	5.17 (ddd,9;2.5;8.5)	49.4 (d)	$\gamma\text{C-H}$	1.34 (m)	1.40 (m,7;6.5)	27.0(d)
$\beta\text{C-H}_2$	1.56 (m) 1.15 (m)	1.79(ddd,12;10.5;2.5) 1.51(ddd,12;8.5;4.5)	41.1 (t)	$\gamma\text{C-CH}_3$	0.80 (d,7) 0.80 (d,7)	0.94 (d,7) 0.80 (d,6.5)	(a) (a)
$\gamma\text{C-H}_2$	1.45 (m)	1.76 (m,10.5;7;4.5)	24.8 (t)	<b>Threonine</b>			
$\gamma\text{C-CH}_3$	0.85 (d,7) 0.85 (d,7)	0.93 (d,7) 0.79 (d,7)	(a) (a)	NH	7.59 (d,5)	8.45 (d,6)	
<b>Hydroxyisovalerylpropionyl unit</b>							
2C-H	4.17 (q,7)	4.76 (q, 6.5)	49.4(d)	$\alpha\text{C-H}$	4.49 (dd,5;2)	5.04 (dd,6;1.5)	56.8(d)
2C-CH <sub>3</sub>	1.27 (d,7)	1.75 (d,6.5)	15.1(q)	$\beta\text{C-H}$	5.34(qd,2;6.5)	5.81(qd,1.5;6.5)	69.9(d)
4C-H	5.11 (d,3.5)	5.67 (d,3.5)	81.1(d)	$\beta\text{C-CH}_3$	1.32 (d,6.5)	1.79 (d,6.5)	16.0(q)
5C-H	2.29 (m)	2.44(m,7;7;3.5)	31.4(d)	<b>Proline 2*</b>			
5C-CH <sub>3</sub>	0.82 (d,7) 0.82 (d,7)	0.83 (d,7) 0.75 (d,7)	16.8(q) 18.5(q)	$\alpha\text{C-H}$	4.58 (dd,7;?)	4.65 (m)	56.1(d)
<b>N,O-dimethylTyrosine</b>							
N-CH <sub>3</sub>	2.50 (s)	2.51 (s)	38.6(q)	$\beta\text{C-H}_2$	1.71 (m) 2.06 (m)	1.90 (m) 2.00 (m)	28.3(t)
$\alpha\text{C-H}$	3.53(overlap)	4.32 (dd,10.5;4)	65.8(d)	$\gamma\text{C-H}_2$	1.96 (m) 2.06 (m)	1.67 (m) 1.67 (m)	26.0 (t)
$\beta\text{C-H}_2$	3.11(dd,4;15.7) 3.30(dd,10;15.7)	3.51(dd,10.5;14) 3.59 (dd,14;4)	33.8 (t)	$\delta\text{C-H}_2$	3.55 (m) 3.63 (m)	3.67 (m) 3.97 (m)	46.8 (t)
AromHo	6.79 (d,7)	7.02 (d,8.5)	129.6 (d)				
Hm	7.01 (d,7)	7.27 (d,8.5)	129.8 (d)				
OCH <sub>3</sub>	3.63 (s)	3.70 (s)	54.7 (q)				

(a) Methyl groups from leucine units: 23.6;23.3;21.3;20.8

Carbonyl groups:204.7;174.0;173;171.7;171.4;170.5;169.6;169.5;165.5

\*The identification for Pro 1 and 2 was based on NOESY results in  $\text{Pyr-D}_5$  and in  $\text{CDCl}_3$ .

**Structure elucidation:** The most interesting structural fact was displayed by 2D-NMR ( $^1\text{H}$ - $^1\text{H}$  COSY) in  $\text{C}_6\text{D}_6$ , where a conspicuous triplet was observed corresponding to a methyl group belonging to an isostatine (isoSta) instead of statine. This result was confirmed by  $^1\text{H}$ - $^{13}\text{C}$  heteronuclear COSY 2D-NMR (table 1), leading to the final macrocyclic structure presented in figure 1.

Next, we decided to study the stereochemistry of the various residues, except for the Hip (hydroxyisovaleryl propionyl unit) already revised by M. M. Joullié [14]. We used Marfey's method [15] in combination with HPLC to determine the absolute configuration of amino acids from our product after hydrolysis with 6N hydrochloric acid, in comparison with available commercial samples or synthetic ones (isoSta for instance). So, we were able to reach our aim [16], eliminating several cases like D allothreonine which had been found to be a good candidate in regard of both scalar and NOE couplings data. All our results were in complete agreement with those recently described: L configuration for MeTyr(Me), Leu, Pro and Thr, D configuration for MeLeu and (3S,4R,5S) for isoSta. Taking into account the revision of the structure for didemnin B by Rinehart [17], it was clear that we had isolated the same product.

On the contrary, isodidemnin-1 looked different with its positive optical rotation (+70°) and its (3S,4R,5R) or (3R,4S,5S) isoSta [7]. As isodidemnin-1 was also a very potent drug, our first idea was to study both depsipeptides in order to compare their conformation in solution. After the first spectroscopic analysis, we suspected that isodidemnin-1 and didemnin B were in fact the same product.

In order to confidently prove it, we focused our attention to a fragment of didemnin containing the isostatyl residue and undertook the synthesis of this fragment. The cyclodepsipeptide didemnin B containing two alkali-labile bonds, the specific cleavage of these bonds should lead to the peptide Lac - Pro - DMeLeu - Thr - isoSta. Parallel alkaline hydrolysis of isodidemnin-1 and of our sample, followed by methylation with diazomethane [7], gave a complex mixture from which only the expected peptide was identified after isolation by silica gel chromatography ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  92/8) followed by reverse phase HPLC. The natural peptide from both origins gave the same HPLC retention time and identical  $^1\text{H}$  NMR spectra. In an attempt to unambiguously assign the stereochemistry of the isoSta residue, this peptide was compared first with synthetic Lac - Pro - D MeLeu - Thr - (3R,4S,5S) isoSta -OMe related to the presumptive evidences previously produced and then with the synthetic analogue in which (3R,4S,5S) isoSta was replaced by the (3S,4R,5S) diastereoisomer in agreement with this study and the recent revised structure for didemnin B. [8]

The peptide sequence was readily produced by coupling isoStaOEt to Lac - Pro - DMeLeu - Thr in order to minimize the number of steps after the introduction of isoSta (the most inaccessible of the five starting residues). Furthermore, we deliberately chose to couple the readily obtained ethyl ester of isoSta, instead of the required methyl ester, in order to submit both synthetic and natural compounds to the same experimental conditions previous to the final purification (i.e. saponification followed by diazomethane methylation). The BOP reagent [18] was successfully employed to prepare the dipeptides Lac-ProOtBu and DMeLeu-ThrOMe. Subsequent coupling of both segments was best achieved, but only in acceptable yield, by using BOP-Cl as previously described [19], assuming that segment coupling on a prolyl residue would produce minimum epimerisation. The (3S,4R,5S) isomer of isoSta was obtained from D allo Ile and the (3R,4S,5S) isomer from Ile following a reported procedure [20]. Since this exotic amino acid was located at the carboxy terminus of the target sequence, we used BOP activation for coupling Lac-Pro-DMeLeu-Thr with isoSta in order to minimize racemisation [18]. Saponification of the carboxyl group in the final peptide, followed by  $\text{CH}_2\text{N}_2$  treatment, produced the expected sequence. Chromatographic purification afforded the pure methyl ester protected peptide in 40% overall yield. Only the synthetic compound built up from the (3S,4R,5S) isoSta had a close spectroscopic (table 2) identity with the natural fragments. As expected from the presence of N-alkyl amino acids in this sequence, several minor rotamers were visible in the NMR spectrum of both synthetic and natural peptides. The diastereomeric purity was ascertain from HPLC analysis. All these results confirmed the (3S,4R,5S) configuration for isoSta in both natural depsipeptides, thus providing a confident proof for the reality of a single depsipeptide: didemnin B.

**Conformational study:** Then, we undertook an evaluation of the conformation of Didemnin B in solution [21] by means of 2D-NMR techniques (NOESY mainly). Due to the size of the peptide, it has been necessary to perform NOESY at 253°K in

Table 2 :  $^1\text{H}$  NMR data for the major rotamer of peptides  
Lac-Pro-DMeLeu-Thr-isoSta-OMe (DMSO  $d_6$ )

Residues	with (3R,4S,5S) isoSta			with (3S,4R,5S) isoSta		
	$\delta$	mult.	J	$\delta$	mult.	J
<b>Lactic acid</b>						
1C-H	4.33	dq	6.4;6.8	4.33	t	6.8
1C-CH <sub>3</sub>	1.16	d	6.4	1.16	d	6.8
OH	4.93	d	6.8	4.92	d	6.8
<b>Proline</b>						
$\alpha$ C-H	4.74	dd	7.3;6.3	4.74	bt	7.3+?
$\beta$ C-H <sub>2</sub>	2.22-1.67	m		2.21-1.65	m	
$\gamma$ C-H <sub>2</sub>	2.01-1.87	m		2.01-1.89	m	
$\delta$ C-H <sub>2</sub>	3.78-3.48	m		3.77-3.48	m	
<b>N-methyl Leucine</b>						
N-CH <sub>3</sub>	3.02	s		3.02	s	
$\alpha$ C-H	5.06	dd	4.4;10.7	5.05	dd	4.4;10.8
$\beta$ C-H <sub>2</sub>	1.67-1.65	m		1.66-1.59	m	
$\gamma$ C-H	1.40	m		1.38	m	
$\gamma$ C-CH <sub>3</sub>	0.89-0.82	d	6.2	0.89-0.82	d	6.2
<b>Threonine</b>						
N-H	7.31	d	9.8	7.31	d	7.8
$\alpha$ C-H	4.16	dd	9.8;4.4	4.14	dd	7.8;4.9
$\beta$ C-H	3.99	m		3.99	m	
$\beta$ C-CH <sub>3</sub>	1.02	d	6.4	1.04	d	6.3
OH	4.86	d	7.3	4.90	d	6.8
<b>isoStatine</b>						
N-H	7.18	d	8.3	7.33	d	9.3
4C-H	3.65	m		3.77	m	
5C-H	1.74	m		1.85	m	
5C-CH <sub>3</sub>	0.79	d	6.3	0.81	d	6.3
6C-H <sub>2</sub>	1.50-1.02	m		1.20-1.09	m	
7C-CH <sub>3</sub>	0.83	t	6.4	0.81	t	6.4
3C-H	3.86	m		3.81	m	
2C-H <sub>2</sub>	2.55-2.12	dAB	15.1;2.4;10.2	2.60-2.12	dAB	15.1;2;9.7
OH	4.92	d	7.3	4.92	d	6.8
OMe	3.58	s		3.58	s	

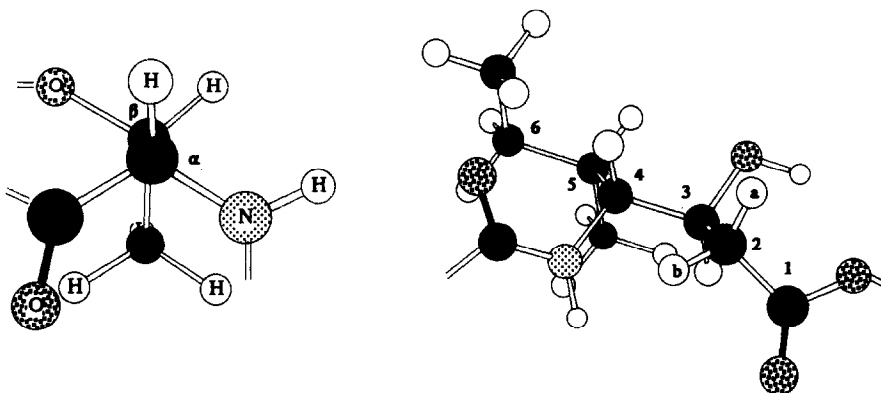
order to see the negative enhancements, as nothing could be observed at room temperature - ROESY techniques were also employed (mainly in  $\text{C}_6\text{D}_6$ ) at room temperature and the observed positive enhancements were found to be consistent with the NOESY data -. The molecule looked rigid under the experimental conditions, as  $^1\text{H}$  NMR data in several solvents had shown didemnin B to present one set of well-resolved signals. For instance, the large differences (in all the solvents) concerning chemical shifts and vicinal coupling constants between 3C-H and the geminal diastereotopic isoSta protons 2C-Ha and 2C-Hb, were indicative of the rigidity of the backbone macrocycle. Thus, its principal conformation was much easier to determine than expected. Having established the unequivocal assignment of the NMR spectra (in  $\text{CDCl}_3$ ,  $\text{C}_6\text{D}_6$  and Pyridine- $\text{D}_5$ ) of our depsipeptide, the NOESY data allowed us to confirm the amino acids sequence, except for the ester bond linkages (Scheme 1).

Also, some internal enhancements were displayed during these experiments, mainly in Thr (between NH and C  $\beta\text{H}/\text{C}\beta\text{CH}_3$ ) and isoSta (between NH and C3H/C2Hb/C5CH<sub>3</sub>, between C4H and C6CH<sub>3</sub>). Some conformational information was obtained with the help of scalar coupling constants (scheme 2). A trans conformation for the Pro 1-MeTyr(Me), Pro 2-DMeLeu and Thr-isoSta peptidic bonds was confidently assigned as NOE enhancements occurred between NH or NCH<sub>3</sub> and the  $\alpha$  proton of the preceding amino acid. A similar conclusion was reached for Pro 1-Leu and Pro 2-Lac amidic bonds due to the NOEs between both protons  $\delta\text{CH}_2$  of Pro 1 and Pro 2 respectively with  $\alpha$ -CH Leu and  $\alpha$ -CH Lac. A trans conformation was confidently given to the bond Hip-Leu, based on a valuable NOE enhancement between C2H Hip and NH Leu. Though the NOE data did not directly yield information about the DMeLeu-Thr peptidic bond, it was necessary to have a trans conformation to explain the important intensity of NOE enhancement between NCH<sub>3</sub> DLeu and NHThr. This NOE result, indicating

Scheme 1 : NOE results (from NOESY and ROESY experiments).

	Lac	Pro 2	DMeLeu	Thr	isoSta	Hip	Leu	Pro 1	MeTyr(Me)
d NN			N-----N		N-----N				N-----N
d αN		α-----N		α-----N		2-----N		α-----N	
d αδ	2-----δ							α-----δ	
Medium and long range	3-----δ			α-----N α-----β		2-----N	β-----δ		γ-----Ho

----- Strong NOEs      ----- Medium NOEs      ----- Weak NOEs



Scheme 2 : Threonine and ( 3S, 4R, 5S ) isoStatine conformations

Table 3: NH/αCH variations according to solvents and to temperature

Residue	CDCl <sub>3</sub>	Pyr-D <sub>5</sub>	Δδ	CDCl <sub>3</sub> + drop MeOH	Δδ	345°K Pyr-D <sub>5</sub>	253°K Pyr-D <sub>5</sub>	Δδ	DMSO
NH iSta	7.15	7.56	0.41	7.28	0.13	7.56	7.67	0.11	6.92
NH Leu	7.75	8.47	0.72	7.81	0.06	8.47	8.63	0.16	7.85
NH Thr	7.59	8.25	0.66	7.64	0.05	8.25	8.49	0.24	7.77
NCH <sub>3</sub> Leu	3.08	3.24	0.16	3.19	0.11	3.24	3.18	0.06	3.05
NCH <sub>3</sub> Tyr	2.50	2.64	0.14	2.55	0.05	2.64	2.51	0.13	2.50
αCH Tyr	3.53	4.20	0.67	?	?	4.20	4.32	0.12	4.06
αCH Pro 1	4.77	4.75	0.02	4.87	0.10	4.75	4.70	0.05	4.63
αCH Leu	4.74	5.19	0.45	4.80	0.06	5.19	5.17	0.02	4.73
H2 Hip	4.17	4.72	0.55	4.03	0.14	4.72	4.76	0.04	3.96
H4 Hip	5.11	5.65	0.54	4.99	0.12	5.65	5.67	0.02	4.94
αCH isoSta	4.03	4.70	0.67	3.90	0.13	4.70	4.73	0.03	3.82
αCH Thr	4.49	5.07	0.58	4.54	0.05	5.07	5.04	0.03	4.35
αCHMeLeu	5.32	5.80	0.48	5.39	0.07	5.80	5.79	0.01	5.05
αCH Pro 2	4.58	4.72	0.14	4.70	0.12	4.72	4.65	0.07	4.35
CH Lac	4.32	4.53	0.21	4.47	0.15	4.53	4.48	0.05	4.04

a close spatial proximity, could be only arranged for such a trans conformation, with a special spatial position for the NCH<sub>3</sub>, leading to a β or γ turn in the dangling tripeptide fragment that placed the DMeLeu, Pro 2 and Lac residues outside the cycle. The trans peptide bond conformation, already mentioned involving Pro 2 and DMeLeu, increased this arrangement. A β turn [22], with DMeLeu and Pro 2 occupying the corner positions, was chosen to explain the low-field chemical shift of αCH-DMeLeu (δ = 5.80 ppm) due to coplanarity with CO Pro 2, with a likely hydrogen bond between the CO Lac (i) and the NH Thr (i+3) as a conformational consequence. These results suggested a general shape for the molecule.

During these studies, we noted some spatial effects across the ring - NH Leu- αCH Thr, NH Leu-2CHb isoSta, αCH Thr-βCH Leu, 2CHb isoSta-αCH Thr -, indicating an internal NH Leu and the possibility of an unexpected arrangement for the isoSta residue. In order to confirm those points, some parameters (temperature, solvents) of COSY experiments were changed (table 3). Interestingly, the effects involving the NH groups of Leu and isoSta are almost temperature independent (<0.002 ppm/°K in the range from 253 to 345°K), in agreement with an expected internal orientation [23] that could be related to strong hydrogen-bridges inside the cycle. The low solvation for both NH isoSta and NH Leu was also pointed out, in accord with two possible hydrogen-bonds from NH isoSta to CO Leu and CO MeTyr(Me). This rationalized the NOE enhancements βCH Leu-αCH Thr and NH Leu-2CHb isoSta. With the support of an internal NH Leu and of isoSta (already organised, scheme 2), we returned to the Hip in order to reach conformational conclusions as NOE results were lacking for this residue. To rationalise this lack of long distance spin interactions, one of its carbonyl groups (3C) had to be oriented perpendicular to the medium plane of the cycle to look after the whole conformation for the rest of the cycle (accordingly to the whole NOE results). That conformation for the molecule allowed us to explain the turning up of the peptidic propagation in the cycle, as a bend was found out from isoSta to Leu with Hip occupying the corner position. As the Hip residue is not an amino acid, 9 atoms are involved in this bend, leading to an intermediate turn between β (10 atoms) and γ (7 atoms). Finally, that bend (CO isoSta-Hip-NH Leu) and the extended row \* Leu-Pro 1-MeTyr(Me) confirmed the conclusion that the other ester bond (CO MeTyr(Me)- O Thr) was involved in a second turn to build the ring, the CO Pro 1 being in a perpendicular orientation to the medium plane (figure 2).

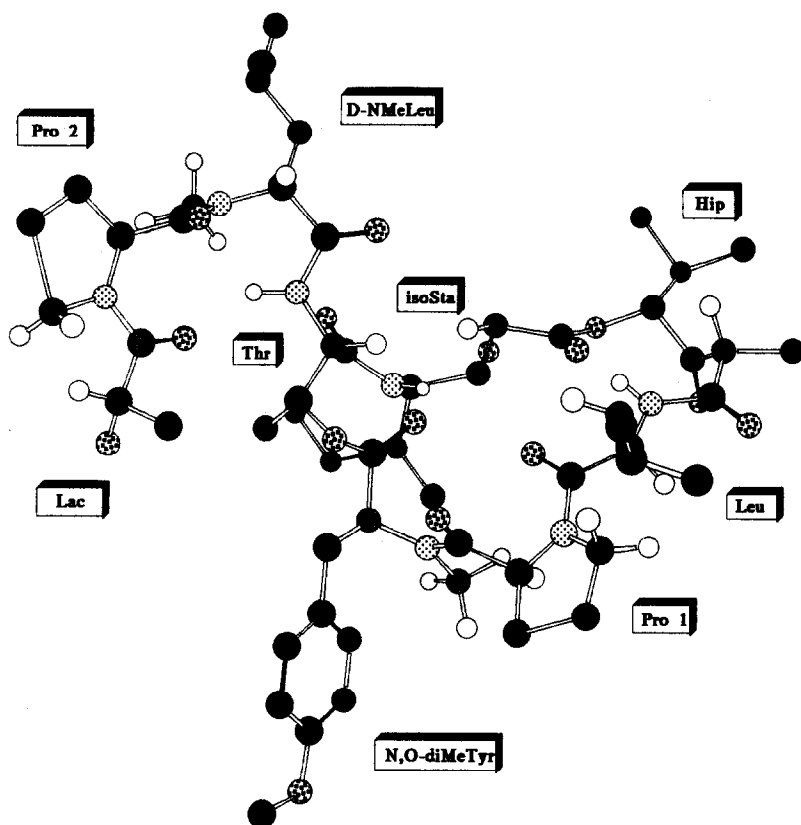
In conclusion, we should like to point out the following points: (a) it is obvious that all didemnin B-like depsipeptides described until now from Caribbean tunicates, are in fact the same one, namely didemnin B - with (3S,4R,5S) isoSta instead of Sta - (b) the NMR conformational analysis showed NH Leu and NH isoSta to be oriented inward (c) the β turn conformation for the side chain that could play a part in the drug-receptor interactions in regard to the different averages of activity from didemnin B to didemnin A (which is without such an organised side chain). However, didemnin A is also a very potent drug indicating that the conformational picture of the cycle is, by far, the most valuable knowledge (d) the overall organisation of isoSta with a free hydroxyl group that could be related with the activity of such compounds according to the protease inhibitory effect of pepstatin (which is dependent on the free OH group of Sta [24], with the same absolute configuration for the carbon bearing this OH functionality in pepstatin and didemnin B) and the antineoplastic activity of dolastatin 10. [25]

The results of our study led to the conclusion that didemnin B in solution looked like an isolated rather than a solvated molecule, from a conformational point of view. The resulting overall molecular shape (or at least, the main one that dominated the equilibrium) was found to assume a conformational constancy from CDCl<sub>3</sub> to DMSO. The most surprising fact concerned the tail Lac-Pro 2-DMeLeu-Thr where a similar conformation was found in DMSO and in apolar solvents \*\*. A likely explanation of such an effect could be related to the presence of a proline rigidifier and a collection of sterically demanding side-chains all around this tail (aromatic ring, Leu, isoSta). We are currently undertaking NMR additional experiments in protic solvents for a better understanding of this stable conformation that could be related to the lipophilic nature of didemnin B.

\* This random arrangement was based on the great intensity of NOEs for MeTyr(Me), Leu, Pro 1 and on the vicinal NH / α CH scalar coupling (<sup>3</sup>J = 9.5Hz) for Leu.

\*\* See the overall integrity of chemical shifts in table 3. More, actual 1D NOE experiments in DMSO indicate the presence of effects between NCH<sub>3</sub> Leu/NH Thr, CH<sub>3</sub> Lac/βCH<sub>2</sub> Pro 2 and αCH Pro2/NCH<sub>3</sub>Leu, in agreement with an overall similar conformation for this fragment in any solvents

**Figure 2 : Computer-generated drawing of Didemnin B in solution.  
Most of hydrogens are omitted for clarity.**



## EXPERIMENTAL

**General:** IR and UV spectra were recorded respectively on a Perkin Elmer model 621 and a Pye-Unicam model SP8/300 spectrophotometers. Optical rotations were recorded on a Roussel Jouan polarimeter, model T71, using a 0.5 cm microcell.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra (1D or 2D) were recorded on a 360/90 MHz Bruker WM-360 spectrometer. Melting points were determined using a Buchi melting-point apparatus. Specific optical rotations were measured on a Schmidt and Haensch Polartron D apparatus. GC/MS experiments and FAB mass spectra were obtained on a ZAB HS (VG - analytical, Manchester, UK) operating at 8 keV accelerating voltage. For FAB/MS, a Xenon beam of 8 keV was focused on the target covered with Ca, 1 mg of sample dissolved into 2 ml of a 1:1 mixture of glycerol and thioglycerol. For GC/MS experiments, a combined EI/CI source was used. The electron energy was 70 eV (EI) and 50 eV (CI). Scan speed was 1 sec/decade. A 0.32 mm (i.d.) x 30 m fused silica column, coated with OV1 was used. Helium was the carrier gas. BOP reagent (Benzotriazolyl-oxo-tris(dimethylamino)phosphonium hexafluoro-phosphate) was obtained from Sempa-Chimie. Amino acids derivatives were of the L form and were purchased from Bachem or Novabiochem. HPLC analysis were performed using Ultrasphere ODS Beckman 10x250 mm (column A) or Ultrasphere ODS Beckman 4.6x150 (column B).

Abbreviations and symbols follow the recommendations of IUPAC-IUB Joint Commission on Biochemical Nomenclature (*Eur. J. Biochem.* 1984, 9, 138). In addition the following abbreviations are used: **BOP**: (1H-1,2,3-benzotriazol-1-yloxy)-tris(dimethylamino)-phosphonium hexafluorophosphate; **BOP-Cl**: N,N'-bis-(2-oxo-3-oxazolindinyl)-phosphonic chloride; **COSY**: two dimensional correlated spectroscopy; **DIEA**: diisopropylethylamine; **isoSta**: 4-amino-3-hydroxy-5-methyl heptanoic acid; **Lac**: L - lactic acid; **NOESY**: two dimensional nuclear Overhauser enhancement spectroscopy; **Sta**: 4-amino-3-hydroxy-6-methylheptanoic acid; **TFA**: trifluoroacetic acid.

**Collection/extraction**: *Trididemnum cyanophorum* was collected at "Ilet Pigeon" (Guadeloupe) in March 1985. After collection, samples were stored in ethyl alcohol. The alcohol was decanted and the whole animal was repeatedly extracted with 50%  $\text{CH}_2\text{Cl}_2$  in MeOH. The resulting dark green solutions were evaporated to dryness under vacuum and the residue was diluted with water, transferred to separatory funnels and extracted with ether (3 x 1 liter). The organic layer was evaporated to yield a 2.5 g extract. The aqueous phase was then extracted with n-butanol (3 x 1 liter). The solvent was removed to give a green solid which was extracted with  $\text{CH}_2\text{Cl}_2$  (3 x 1 liter). Evaporation of the organic solutions yielded 1 g of a dark brown solid.

**Chromatographic procedures**: The 1g  $\text{CH}_2\text{Cl}_2$  extract was loaded onto a LH-20 column which was packed in MeOH and elution was allowed to proceed. Four fractions were collected and the content of each fraction was evaluated by TLC (reverse phase C18, MeOH/ $\text{H}_2\text{O}$  9/1 or 7/3), using chlorine vapor as visualization reagent. The main fraction (634 mg) was loaded on a Lowbar silica gel column which was eluted in step gradient fashion with a MeOH/ $\text{CH}_2\text{Cl}_2$  fraction. The main cyclopeptide was contained in the 97/3  $\text{CH}_2\text{Cl}_2$ /MeOH fraction. For data accumulation and testing, it was further purified by reverse phase HPLC, monitoring by differential refractometers. Essentially pure compound was obtained as a white amorphous solid (50 mg) on a C18 reverse phase column (50 x 1 cm) with MeOH/Water 7/3 as solvent for this purification.

**Physical data**: UV(MeOH):209(20000),223(14800),277(7050),284(6090); $[\alpha]_{\text{D}}^{20} = -70^\circ$  (c0.06,  $\text{CHCl}_3$ )

IR( $\text{CHCl}_3$ ):3680,3600,2970,2940,2880,1725,1650,1605,1540,1510

HR FAB/MS gave  $\text{MH}^+$  at 1112.6511 (calculated 1112.6416 for  $\text{C}_{57}\text{H}_{89}\text{N}_7\text{O}_5$ ). The fragment ions at m/z 1040, 943 and 816 corresponded to the sequential cleavage of the side chain, m/z 861, 771, 743, 686, 555, 307 and 210 to the cleavage of the ring. In the MIKE spectrum of m/z 1112, the large loss of CO indicated a cyclic peptidic structure. The amino acid analysis was performed by GC/MS of the N-heptafluorobutyl-O-propyl ester derivatives after hydrolysis by 6N HCl for 14 h at 110 °C in a sealed tube. The unusual amino acids were characterized as follows: N-MeLeu (EI: m/z 296, 100%, M - 87; m/z 254, 30%, loss of propene from m/z 296; m/z 228, 10%,  $\text{C}_3\text{F}_7\text{-CONH}(\text{CH}_3)\text{H}^+$ ; CI ( $\text{CH}_4$ ): m/z 384, 40%,  $\text{MH}^+$ ; m/z 324, 100%, loss of propanol; m/z 296, 80%, loss of CO from m/z 324). MeTyr(Me) (EI: m/z 360, 7%, M-87; m/z 220, 60%, M -  $\text{C}_3\text{F}_7\text{CON}(\text{CH}_3)\text{H}$ ; m/z 121, 100%,  $\text{CH}_3\text{OC}_6\text{H}_4\text{CH}_2^+$ ; CI ( $\text{CH}_4$ ): m/z 448, 40%,  $\text{MH}^+$ ; m/z 388, 100%, loss of propanol; m/z 360, 80%, loss of CO from m/z 388, m/z 221, 50%,  $\text{MH} - \text{C}_3\text{F}_7\text{CON}(\text{CH}_3)\text{H}$ ; isoSta (EI: 552, 3%, M -  $\text{C}_4\text{H}_9^+$ ; m/z 510, 10%, loss of propene from m/z 552; m/z 464, 15%, loss of propylformate from m/z 552; m/z 282, 100%, C-C cleavage between amino and hydroxylic functions; CI ( $\text{CH}_4$ ): m/z 610, 40%,  $\text{MH}^+$ ; m/z 396, 100%, loss of  $\text{C}_3\text{F}_7\text{COOH}$ ; m/z 336, 80%, loss of propanol from m/z 396, m/z 308, 40%, loss of CO from m/z 336; m/z 282, 20%, same origin as in the EI spectrum; m/z 214, 70%,  $\text{C}_3\text{F}_7\text{-CONH}_3^+$ ).

$^1\text{H}$  NMR(in  $\text{C}_6\text{D}_6$ ): isoSta: 7.54(NH),4.67(C4H),2.33(C5H),1.09(C5- $\text{CH}_3$ ),1.46-1.71 (C6- $\text{H}_2$ ),1.16(C7- $\text{H}_3$ ),4.53 (C3H),4.12(C2- $\text{H}_2$ )

Leu:8.26(NH),5.07( $\alpha\text{CH}$ ),1.05-1.07( $\gamma\text{C-CH}_3$ );

Hip:4.54(C2H),1.68(C2- $\text{CH}_3$ ),5.56(C4H),2.48(C5H),0.88-0.89(C5- $\text{CH}_3$ );

MeTyr(Me):2.20(N $\text{CH}_3$ ),3.40( $\alpha\text{CH}$ ),3.23-3.36( $\beta\text{CH}_2$ ),6.72-6.82(Aromatics), 3.36 (O $\text{CH}_3$ );

Thr:8.02(NH),4.89( $\alpha\text{CH}$ ),5.92( $\beta\text{CH}$ ),1.90( $\beta\text{C-CH}_3$ );

DMeLeu:2.97(N $\text{CH}_3$ ),5.57( $\alpha\text{CH}$ ),1.98-1.70( $\beta\text{CH}_2$ ),1.05-1.07( $\gamma\text{C-CH}_3$ );

Lactic-acid:4.27(CH),1.38( $\text{CH}_3$ );

Pro1:4.25( $\alpha\text{CH}$ ),1.35( $\beta\text{CH}_2$ ),1.27( $\gamma\text{CH}_2$ ),3.01-3.22( $\delta\text{CH}_2$ );

Pro2:4.43( $\alpha\text{CH}$ ),1.35( $\beta\text{CH}_2$ ),1.27( $\gamma\text{CH}_2$ ),2.55( $\delta\text{CH}_2$ ).

**Alcaline hydrolysis of didemnin**: 5 mg of didemnin (our sample or isodidemnin-1) were solubilized in cooled (5°C) methanol



(100 $\mu$ l) and 1N NaOH (20 $\mu$ l) was added. After 30 min., 1N HCl (20 $\mu$ l) was added followed by CH<sub>2</sub>N<sub>2</sub> until persistent yellow coloration. The solvent was removed under a stream of argon and the crude residue was applied on a short silica gel column (5g) and eluted with MeOH/CH<sub>2</sub>Cl<sub>2</sub> (8/92). After evaporation by bubbling argon of the major fraction (Rf 0.33 CH<sub>2</sub>Cl<sub>2</sub>/MeOH 95/5), the peptide was further purified by HPLC (MeOH/Water 75/25) column A; analysis HPLC column B (MeOH, 1% TFA/H<sub>2</sub>O, 1% TFA 50/50)  $t_r$  = 6.15 min. Methanol was removed under reduced pressure and the remaining water solution was freeze-dried and lyophilized.

**Synthesis:** Acetyl - lactyl - proline tert-butyl ester (1): BOP (17.68 g, 40 mmol) and DIEA (diisopropylethyl amine, 6.9 ml, 40 mmol) were stirred with a solution of acetyl-lactic acid (5.28 g, 40 mmol) and of proline tert-butyl ester (3.42 g, 20 mmol) in methylene chloride (50 ml). After 2 h at room temperature, the reaction mixture was diluted with EtOAc (150 ml) and washed with KHSO<sub>4</sub> 5% (2x30 ml), then with Na<sub>2</sub>CO<sub>3</sub> 5% (2x30 ml) and saturated NaCl (30 ml). The mixture was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure. Chromatographic purification (open column of silica gel with ethyl acetate/hexane 50/50 as solvent mixture) yielded 5.30 g (93%) of (1) as a colorless oil.  $[\alpha]_D^{25}$  = -120° (c 1, MeOH); <sup>1</sup>H NMR (DMSO d<sub>6</sub>) 5.13 (q, J = 6.8 Hz, 1H), 4.17 (dd, J = 5.2 and 8.6 Hz, 1H), 3.66-3.57 (m, 1H), 3.53-3.43 (m, 1H), 2.21-2.08 (m, 1H), 2.02 (s, 3H), 1.97-1.87 (m, 1H), 1.37 (s, 9H), 1.32 (d, J = 6.8 Hz, 3H). Anal. Calcd. for C<sub>14</sub>H<sub>23</sub>NO<sub>5</sub>: C, 58.93; H, 8.12; N, 4.91. Found: C, 58.80; H, 8.10; N, 5.05.

Acetyl - lactyl proline(2) : (11.98 g, 42 mmol) was stirred in trifluoroacetic acid (20 ml) at room temperature for 2 h. After removing the solvent under reduced pressure, (2) was crystallised by triturating in diethyl ether (7.02 g, 73%). mp 146-148 °C;  $[\alpha]_D^{25}$  = -114° (c 1, MeOH); <sup>1</sup>H NMR (DMSO d<sub>6</sub>) 5.14 (q, J = 6.9 Hz, 1H), 4.25 (dd, J = 5.2 and 9.5 Hz, 1H), 3.66-3.56 (m, 1H), 3.56-3.45 (m, 1H), 2.24-2.06 (m, 1H), 2.02 (s, 3H), 1.98-1.88 (m, 1H), 1.88-1.78 (m, 1H), 1.31 (d, J = 6.9 Hz, 3H). Anal. Calcd. for C<sub>10</sub>H<sub>15</sub>NO<sub>5</sub>: C, 52.40; H, 6.60; N, 6.11. Found: C, 52.60; H, 6.75; N, 6.00.

[N,N-(benzyloxycarbonyl)-methyl-D-leucyl] - threonine methyl ester (3): BOP (6.63 g, 15 mmol) and DIEA (5.7 ml, 15 mmol) were added to a CH<sub>2</sub>Cl<sub>2</sub> solution (20 ml) of threonine methyl ester (2.54 g, 15 mmol) and of Z-(NMe)-D-leucine (4.19 g, 15 mmol) - prepared from Z-D-leucine according to McDermott and Benoiton [26] -. The work-up already described for (1) afforded crude (3) as a colorless oil (5.73 g, 97%).  $[\alpha]_D^{25}$  = +40° (c 1, MeOH); <sup>1</sup>H NMR (DMSO d<sub>6</sub>) 7.74 (m, 1H), 7.40-7.26 (m, 5H), 5.13 (s, 2H), 4.95 (m, 1H), 4.89-4.68 (m, 1H), 4.30 (dd, J = 2.0 and 9.5 Hz, 1H), 3.62 (s, 3H), 2.80 (s, 3H), 1.64-1.56 (m, 2H), 1.48-1.34 (m, 1H), 1.05 (d, J = 7.7 Hz, 3H), 0.94-0.78 (m, 6H). Anal. Calcd. for C<sub>20</sub>H<sub>30</sub>N<sub>2</sub>O<sub>6</sub>: C, 60.90; H, 7.67; N, 7.10. Found: C, 60.75; H, 7.82; N, 7.25.

Acetyl - lactyl - prolyl - [(N-methyl)-D-leucyl] - threonine methyl ester (4): To a solution of (3) (3.94 g, 10 mmol) in MeOH (50 ml) were added 1N HCl (12 ml) and palladium 10% on charcoal (0.9 g). The mixture was stirring for 1 h at room temperature with bubbling hydrogen. The catalyst was then filtered off and the solvent evaporated in vacuo. The resulting amino hydrochloride was crystallised in MeOH/diethyl ether - 2.58 g, 87%; mp 211-212°C;  $[\alpha]_D^{25}$  = -52° (c 1, MeOH). Freshly prepared and finely ground [27] BOP-Cl (2.20 g, 8.6 mmol) was added with triethylamine (1.21 ml, 8.6 mmol) to a solution of (2) - 1.98 g, 8.6 mmol in CH<sub>2</sub>Cl<sub>2</sub> (10 ml) -. The mixture was stirred at room temperature until BOP-Cl was solubilized (2 h). D-MeLeu-Thr-OMe, HCl (2.56 g, 8.6 mmol) was then added with triethylamine (1.21 ml, 8.6 mmol) and the resulting mixture stirred overnight. The aforementioned work-up [see (1)] furnished crude (4) which was purified by crystallisation in diethyl ether (2.92 g, 72%); mp 169-170°C;  $[\alpha]_D^{25}$  = +12° (c 1, MeOH); <sup>1</sup>H NMR (DMSO d<sub>6</sub>) 7.38 (d, J = 8.3 Hz, 1H), 5.21 (q, J = 6.8 Hz, 1H), 5.16 (dd, J = 4.4 and 9.8 Hz, 1H), 4.87 (d, J = 9.3 Hz, 1H), 4.80 (d, J = 7.8 Hz, 1H), 4.29 (dd, J = 3.4 and 8.3 Hz, 1H), 4.18-4.07 (m, 1H), 3.81-3.73 (m, 1H), 3.63 (s, 3H), 3.55-3.42 (m, 1H), 3.00 (s, 3H), 2.29-2.17 (m, 1H), 2.12-1.85 (m, 2H), 2.01 (s, 1H), 1.75-1.47 (m, 3H), 1.43-1.33 (m, 1H), 1.30 (d, J = 6.8 Hz, 3H), 1.07 (d, J = 6.7 Hz, 3H), 0.89 (d, J = 6.2 Hz, 3H), 0.81 (d, J = 6.2 Hz, 3H). Anal. Calcd. for C<sub>22</sub>H<sub>37</sub>NO<sub>8</sub>: C, 56.04; H, 7.91; N, 8.91. Found: C, 55.79; H, 7.82; N, 8.96.

Lactyl - prolyl - [(N-methyl)-D-leucyl] - threonine (5): 1N NaOH (15 ml) was added dropwise to a cold solution of (4) - 2.36 g, 5 mmol - in MeOH (10 ml) and stirred for 20 min. Then 1N HCl (15 ml) was added and the solvent evaporated under reduced pressure. The clear solution was saturated with NaCl and extracted four times with CH<sub>2</sub>Cl<sub>2</sub> (20 ml). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated. (5) was crystallized by triturating in diethyl ether (2.00 g, 96%); mp 158-159°C;  $[\alpha]_D^{25}$  = +56° (c 1, MeOH). Anal. Calcd for C<sub>19</sub>H<sub>33</sub>N<sub>3</sub>O<sub>7</sub>: C, 54.93; H, 8.01; N, 10.11. Found: C, 55.09; H, 7.92; N, 9.96.

Lactyl - propyl - [(N-methyl)-D-leucyl] - threonyl - (3R,4S,5S)- isostatatin methyl ester (6a): Coupling between (5) - 0.91 g, 2.2 mmol - and the trifluoroacetate salt of the (3R,4S,5S)-isostatatin ethyl ester - 0.63 g, 2 mmol - was accomplished in the same conditions already described for (3) and afforded the corresponding peptide as a foam (1.10 g). This crude residue was saponified to furnish the acid (0.9 g) which was promptly solubilized in MeOH and treated with CH<sub>2</sub>N<sub>2</sub> until persistent yellow colour. After the solvent was removed, the residue was purified by low pressure chromatography on a Lowbar column (Merck; size B; elution with MeOH/ CH<sub>2</sub>Cl<sub>2</sub> 5/95) to afford (6a) as a foam (0.5 g, 43%).  $[\alpha]_D^{25}$  = +41° (c 1, MeOH); <sup>1</sup>H NMR (see table 2). Anal. Calcd. for C<sub>28</sub>H<sub>50</sub>N<sub>4</sub>O<sub>9</sub>: C, 57.32; H, 8.59; N, 9.55. Found: C, 57.05; H, 8.65; N, 9.70. HPLC column B (MeOH, 1%TFA/Water, 1%TFA 50/50)  $t_r$  = 6.14 min. TLC Rf 0.33, CH<sub>2</sub>Cl<sub>2</sub>/MeOH 95/5.

Lactyl - prolyl - [(N-methyl)-D-leucyl] - threonyl - (3S,4R,5S) - isostatatin methyl ester (6b): By a similar procedure, (6b) was obtained as a foam (0.47 g, 40%).  $[\alpha]_D^{25}$  = +36° (c 1, MeOH); <sup>1</sup>H NMR (see table 2). Found for C<sub>28</sub>H<sub>50</sub>N<sub>4</sub>O<sub>9</sub>: C, 57.06; H, 8.69; N, 9.48. HPLC column B (MeOH, 1%TFA/Water, 1%TFA 50/50)  $t_r$  = 8.49 min. TLC Rf 0.27, CH<sub>2</sub>Cl<sub>2</sub>/MeOH 95/5.

## Acknowledgements

This research was supported by INSERM Grants (n° 841005 and 841014). We have to thank M. Guyot for a generous gift of isodidemnin-1. We would like to thank the Service de microanalyses du C.N.R.S. (Ecole Nationale Supérieure de Chimie de Montpellier) for elemental analysis results and SANOFI-RECHERCHES (Toulouse) for pharmacological screenings.

## REFERENCES

- 1 (a) C. Ireland and P.J. Scheuer, *J. Am. Chem. Soc.*, **1980**, *102*, 5688; (b) K.L. Rinehart, J. B. Gloer, R.G. Hugues, H.E. Denis, P.J. McGovern, E.G. Swynenberg, D.A. Stringfellow, S.L. Kuentzel and H.L. Li, *Science*, **1981**, *212*, 933; (c) C. Ireland, A.R. Durso, R.A. Newman and M.P. Hacker, *J. Org. Chem.*, **1982**, *47*, 1807; (d) M. Guyot, C. Belaud and E. Morel, *J. Chem. Res. (S)*, **1983**, 188 and (*M*), 1823; (e) D. Gouffès, S. Moreau, N. Helbecque, J.L. Bernier, J.P. Hénichart, Y. Barbin, D. Laurent, J.F. Verbiest, *Tetrahedron* **1988**, *44*, 451; (f) K.L. Rinehart, V. Kishnou, K.C. Bibli, R. Sakai, D.W. Sullins, K.M. Li, *J. Nat. Prod.*, **1988**, *51*, 1.
- 2 F. Lafargue and M. Wahl, *Ann. Inst. Oceanogr.*, **1987**, *63* (1), 1.
- 3 K.L. Rinehart, P.D. Shaw, L.S. Shield, J.B. Gloer, G.C. Harbour, M.E.S. Koker, D. Samain, R.E. Schwartz, A.A. Tymiak, D.L. Weller, M.H.G. Munro, G.T. Carter, R.G. Hugues, H.E. Renis, E.B. Swynenberg, H.L. Li, D.A. Stringfellow, J.J. Vavra, J.H. Coats, G.E. Zurenko, S.L. Kuentzel, G.J. Bakus, R.C. Brusca, L.L. Craft, D.N. Young and J.L. Connor, *Pure Appl. Chem.*, **1981**, *53*, 795.
- 4 J.B. Gloer, Ph.D. thesis (1983), University of Illinois.
- 5 K.L. Rinehart, J.B. Gloer, J.C. Cook, S.A. Mizaak, T.A. Scahill, *J. Am. Chem. Soc.*, **1981**, *103*, 1857.
- 6 (a) T.J. Jiang, R.H. Liu, S.E. Salmon, *Cancer Chemother. Pharm.*, **1983**, *11*, 1; (b) L.H. Li, L.G. Timmins, T.L. Wallace, W.C. Krueger, M.D. Prairie, W.B. Im, *Cancer Letters*, **1984**, *23*, 279; (c) S.L. Crampton, E.G. Adams, S.L. Kuentzel, L.H. Li, G. Badiner, B.J. Bhuyan, *Cancer Res.*, **1984**, *44*, 1796; (d) D.W. Montgomery and C.F. Zukosky, *Transplantation* **1985**, *40*, 49; (e) H.G. Chun, B. Davies, D. Hoth, M. Suffness, J. Plowman, K. Flora, C. Grieshaber, B. Leyland-Jones, *Investigat. New Drugs*, **1986**, *4*, 279.
- 7 M. Guyot, D. Davoust, E. Morel, *C.R. Acad. Sci.*, **1987**, *305*, 681.
- 8 K.L. Rinehart, V. Kishore, S. Nagarayan, R.J. Lake, J.B. Gloer, K. Li, F.A. Bozich, R.E. Maleczka, W.L. Todsén, M.H.G. Munro, D.W. Sullins, R. Sakai, *J. Am. Chem. Soc.*, **1987**, *109*, 6846.
- 9 M. Wahl, Ph.D. thesis (1987), University of Kiel.
- 10 F. Lafargue and G. Duclaux, *Ann. Inst. Oceanogr.*, **1979**, *55*(2), 163.
- 11 B. Castro, P. Jouin, A. Cave, M. Dufour, B. Banaigs, C. Francisco in "Peptides Chemistry and Biologie", Proceedings of the 10th American Peptide Symposium, G.R. Marshall Ed., **1988**, Leiden, 656.
- 12 (a) W.P. Aue, E. Bartholdi, R.R. Ernst, *J. Chem. Phys.*, **1976**, *64*, 2229; (b) G. Bodenhausen, R. Freeman, *J. Magn. Reson.*, **1977**, *28*, 471; (c) A. Bax, F. Mehlkoph, J. Smidt, *J. Magn. Reson.*, **1980**, *40*, 213; (d) A. Bax, R. Freeman, *J. Magn. Reson.*, **1981**, *44*, 542.
- 13 (a) J. Jeener, B.H. Meier, P. Bachmann, R.R. Ernst, *J. Chem. Phys.*, **1979**, *69*, 4546; (b) S. Macura, Y. Huang, D. Suter, R.R. Ernst, *J. Magn. Reson.*, **1981**, *43*, 259.
- 14 W.R. Ewing, K.L. Bhat, M.M. Joullie, *Tetrahedron*, **1986**, *42*, 5863.
- 15 P. Marfey, *Carlsberg Res. Commun.*, **1984**, *49*, 591.
- 16 B. Banaigs, G. Jeanty, J.M. Aracil, C. Francisco, *J. of Chromatography* (submitted to publication).
- 17 K.L. Rinehart, 10th American Peptide Symposium, St. Louis, May 23-28, **1987**.
- 18 (a) B. Castro, J.R. Dormoy, J.G. Evin, C. Selve, *Tetrahedron Lett.*, **1975**, 1219; (b) P. Rivaille, J.P. Gaudron, B. Castro, G. Milhaud, *Tetrahedron* **1980**, *36*, 3413; (c) R.M. Wenger, *Helv. Chim. Acta*, **1983**, *66*, 2672; (d) J.A. Ferhents, R. Seyer, A. Heitz, P. Fulcrand, B. Castro, P. Corvol, *Int. J. Pept. Protein Res.*, **1986**, *28*, 620.
- 19 C. Van Der Auwera, M.J.O. Anteunis, *Int. J. Peptide Protein Res.*, **1987**, *29*, 574.
- 20 P. Jouin, J. Poncet, M.N. Dufour, I. Maugras, A. Pantaloni, B. Castro, *Tetrahedron Lett.*, **1988**, 2661.
- 21 H.R. Looali, H. Kesler, H. Oschkinat, H.P. Weber, T.J. Petcher, A. Widmer, *Helv. Chim. Acta*, **1985**, *68*, 682.
- 22 R. Chandrasekaran, A. Lakshminarayan, U. Pandya, G. Ramachandran, *Biochim. Biophys. Acta*, **1973**, *303*, 14.
- 23 (a) L.G. Pease, C.H. Nin, G.J. Zimmermann, *J. Am. Chem. Soc.*, **1979**, *101*, 184; (b) E.S. Stevens, N. Sugawara, G.M. Bonara, C. Toniolo, *J. Am. Chem. Soc.*, **1980**, *102*, 7048.
- 24 W.S. Liu, S.C. Smith, G.I. Glover, *J. Med. Chem.*, **1979**, *22*, 577.
- 25 G.R. Pettit, Y. Kamano, C.L. Herald, A.A. Tuinman, F.E. Boettner, H. Kizu, J.M. Schmidt, L. Baczynskyj, K.B. Tomer, R.J. Bontems, *J. Am. Chem. Soc.*, **1987**, *109*, 6883.
- 26 J.R. McDermott, N.L. Benoiton, *Can. J. Chem.*, **1973**, *51*, 1915.
- 27 C. Van der Auwera, M.J.O. Anteunis, *Bull. Soc. Chim. Belg.*, **1986**, *95*, 203.