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Synthesis and Biological Evaluation of Tamandarin B Analogues

Javier Adrio,[†] Carmen Cuevas,[§] Ignacio Manzanares,[§] and Madeleine M. Joullié*,[‡]

Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104-6323, Departamento de Quimica Organica, Faculdad de Ciencias, Universidad Autonoma de Madrid, 28049 Madrid, Spain, and Pharma Mar S.A., Sociedad Unipersonal, Avd. de los Reyes, 1.P.I. La Mina Norte 28770 Colmenar Viejo, Spain

mjoullie@sas.upenn.edu

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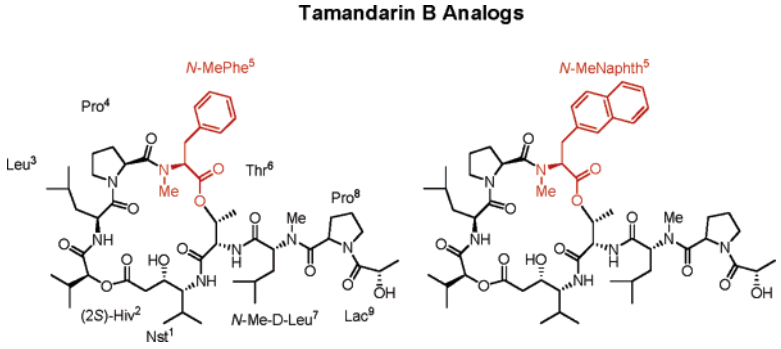
ABSTRACT



The synthesis of two tamandarin B analogues in which the *N*,*O*-Me$_2$Tyr^5 unit was replaced by *N*-Me-phenylalanine (*N*-MePhe^5) and (*S*)-2-(methylamino)-3-(naphthalen-2-yl)propanoic acid (*N*-MeNaphth^5) is described. The choice of the macrocyclization site was crucial to achieve satisfactory macrolactamization. Coupling between norstatine (Nst^1) and threonine (Thr^6) afforded only a 15% yield, while lactamization between proline (Pro^4) and the aromatic moiety could be achieved in 65% yield.

Tamandarins A and B (**1** and **2**) are two cyclic depsipeptides isolated from a Brazilian ascidian by Fenical and co-workers.[1] Their structures are similar to that of didemnin B (**3**), an ascidian metabolite isolated by Rinehart et al. in 1981 (Figure 1).[2] The two species that produce **1** and **3** originate from remote geographic locations but use a common method to defend themselves and their vulnerable larvae by producing these structurally similar but not identical depsipeptides.[3] Some members of the family exhibited outstanding biological activity, especially a manifest capacity to inhibit cell proliferation, which has stimulated much effort into their development for therapeutic use.[4] Tamandarins A (**1**) and B (**2**), recently synthesized in our laboratory,[5] were reported to exhibit much of the same biological activity as didemnin B (**3**) and aplidine (**4**), maintaining similar levels of in vitro antitumor activity as well as protein biosynthesis inhibition properties,[1,4] suggesting that the propionic acid unit results only in a minor conformational modification and may not

[‡] University of Pennsylvania.
[†] Universidad Autónoma de Madrid.
[§] Pharma Mar S.A.

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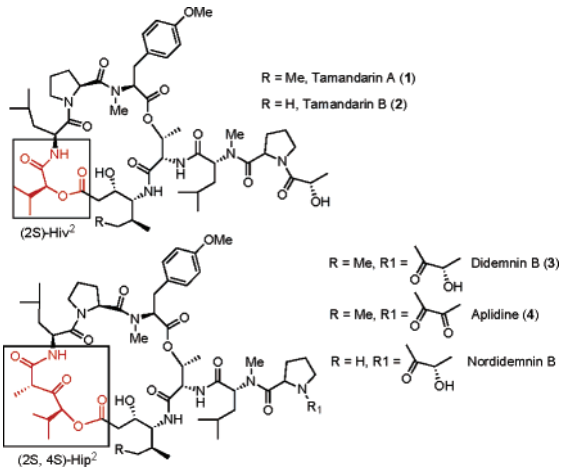


Figure 1. Structures of didemnins and tamandarins.

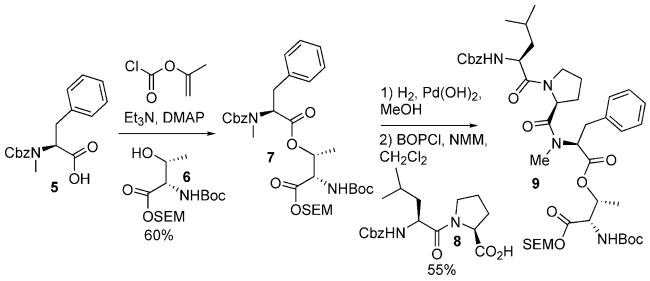
be required for bioactivity. Furthermore, the synthesis of several side chain analogues of tamandarin A have shown that structural modifications can afford compounds with biological activity similar to that of didemnin B analogues.[5] These observations support the belief that tamandarins are simplified didemnin mimics. This concept was corroborated by fluorescence studies of the predator−prey interactions of **1** and **3**.[3]

There is a large amount of information on the structure−activity relationships (SAR) of the side chains of didemnins.[6] However, the length and complexity of the synthetic routes for accessing the didemnins have hindered the development of large numbers of macrocycle-modified compounds. However, a few such analogues are known. The role of the *N*,*O*-Me_2Tyr[5] residue of didemnin B was reported to be important for biological activity. To determine the importance of aromaticity at this position, two analogues, *N*-MeLeu[5] and *N*-MePhe[5], were synthesized and shown to retain antitumor activity and the ability to inhibit protein synthesis.[9c]

The easier synthetic access to tamandarin derivatives could accelerate the preparation of new analogues and the development of structure−activity relationships for these natural products. To the best our knowledge, no tamandarins with modified macrocycles have been reported.

The *N*,*O*-Me_2Tyr[5] residue at position 5 of the didemnins was shown to be a potentially important pharmacophoric element, which could interact with a hydrophobic pocket in the receptor because it extends out of the main body of the

Scheme 1



molecule in the solid state[7] or in solution.[8] Since comparable cytotoxic activity was observed in didemnins when residue 5 was replaced with either aliphatic hydrophobic amino acids,[9] such as *N*-MeLeu,[5] or aromatic residues, such as *N*-MePhe,[5,4e] we decided to prepare two tamandarin B analogues containing hydrophobic residues by replacing *N*,*O*-Me_2Tyr[5] with *N*-Me-phenylalanine (*N*-MePhe[5]) and (*S*)-2-(methylamino)-3-(naphthalen-2-yl)propanoic acid (*N*-MeNaphth[5]). The synthesis previously described for tamandarin B[5b] was used as the basis for the synthesis of our *N*-MePhe[5] analogue, differing only in the preparation of the tetrapeptide moiety, which consists of Leu[3], Pro[4], Phe[5], and Thr[6] residues linked in sequence. We began our synthesis with commercially available Cbz-L-Phe, which was methylated using dimethyl sulfate to afford Cbz-*N*-MePhe **5**. The coupling of the resulting acid with Boc-Thr-OSEM **6** formed ester **7**. Subsequent hydrogenolysis and coupling with acid **8** yielded the desired tetrapeptide **9** (Scheme 1).

Hydrogenolysis of tetrapeptide **9** provided the free amine **10**, which was coupled with the activated pentafluorophenyl PFP ester **11** to afford the linear precursor **12** in 57% yield (Scheme 2). Selective cleavage of the SEM ester in the presence of the Boc and TIPS protective groups was achieved with $MgBr_2 \cdot Et_2O$.[10] The Cbz group of the resulting acid was removed to obtain the corresponding amino acid by hydrogenolysis using $Pd(OH)_2$ as the catalyst. Macrocyclization with HATU gave the Boc derivative **13**. The macrocycle (**13**) was treated with HCl (gas) in dioxane, which successfully removed both TIPS and Boc protective groups. The macrocycle salt **14** was coupled with the corresponding side chain **15** (prepared as previously described).[4a]

Unfortunately, the macrolactamization reaction took place in only 15% yield. Several attempts to improve this yield by changing the solvent, the base, and the coupling reagent failed. Because we required a robust protocol for the synthesis of different analogues, the difficulties found in the macrocyclization step led us to modify the synthetic strategy. Since the first total synthesis of didemnins reported by Rinehart et al.[11] in 1987, several different macrocyclization strategies have been described. Among them, a protocol first reported by Shioiri[12] and then by Lloyd-Williams and Giralt[13]

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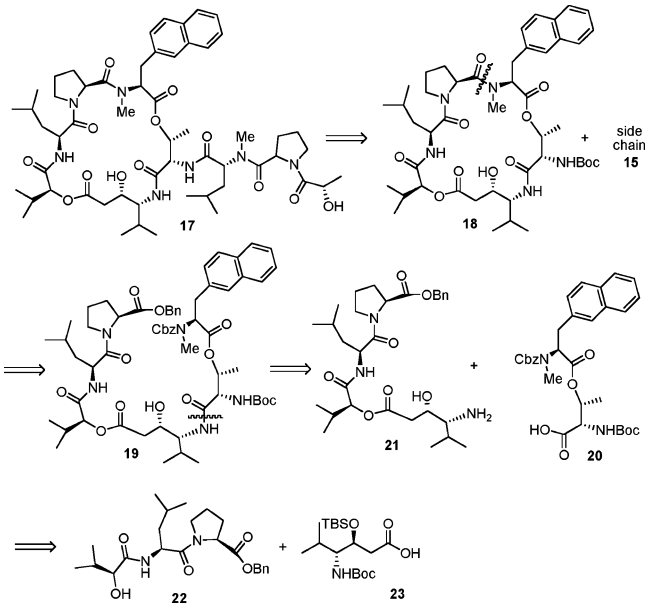
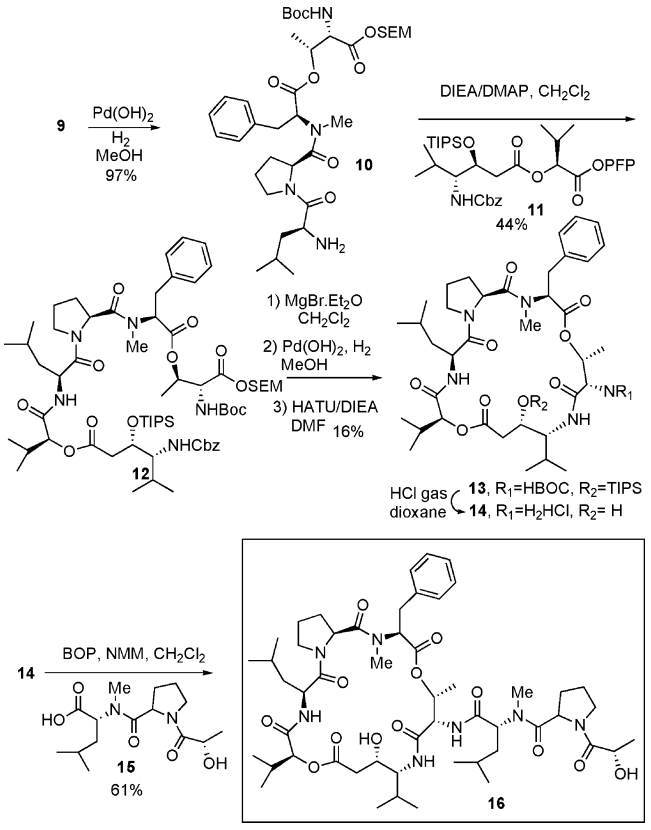


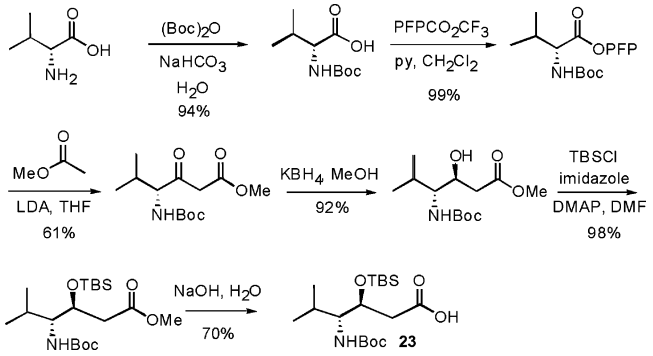
Figure 2. Retrosynthetic analysis of compound **17**.

selected the site between Pro[4] and *N*,*O*-Me$_2$Tyr[5] to afford the macrolactam. We assumed that the same approach could be applied to tamandarin analogues and changed the macrolactamization site for the synthesis of other tamandarin B analogues.

Scheme 2



Scheme 3

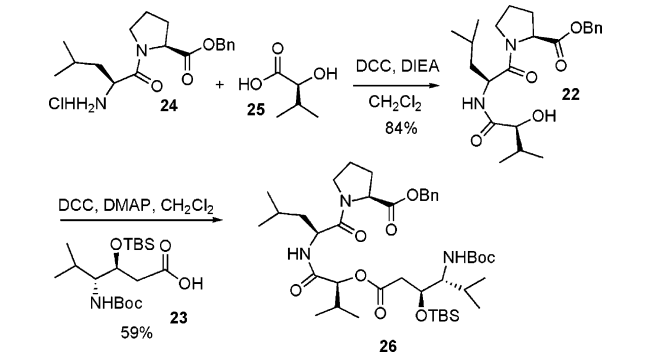


A retrosynthetic analysis for the synthesis of *N*-MeNaphth[5] (**17**) using this approach is shown in Figure 2. The formation of the macrocyclic core is the key step, and the site between Pro[4] and *N*,*O*-Me$_2$Tyr[5] was selected as the point of macrocyclization. Linear precursor **19** was disconnected into units **20** and **21**. The depsipeptide **21** was prepared by coupling alcohol **22** and norstatine **23** (Figure 2).

The synthesis of the norstatine moiety was performed as described in our previous work,[5b] but using Boc instead of Cbz as the nitrogen protecting group and TBDMS instead of TIPS as the oxygen protecting group (Scheme 3).

The coupling between Leu-Pro-OBn **24** and the hydroxyvaleric acid **25** using DCC and diisopropyl ethylamine in CH_2Cl_2 afforded alcohol **22** in good yield. Subsequently, reaction with norstatine **23** employing DCC and DMAP gave depsipeptide **26** (Scheme 4).

Scheme 4



The coupling between threonine phenacyl ester **27** and *N*-L-3-(2-naphthyl)alanine **28** using DCC/DMAP afforded the desired intermediate **20** in 68% yield. The phenacyl ester was removed using Zn in AcOH/H_2O in 60% yield (Scheme 5).

Depsipeptide **21**, obtained by acidic deprotection of compound **26**, was coupled with acid **20** using HBTU, HOBt,

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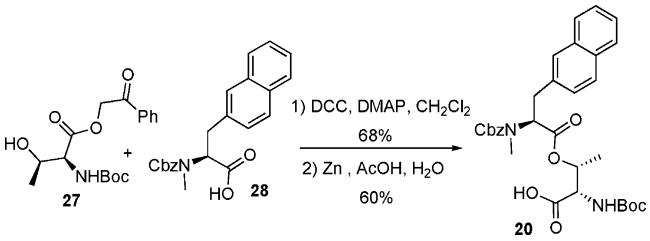
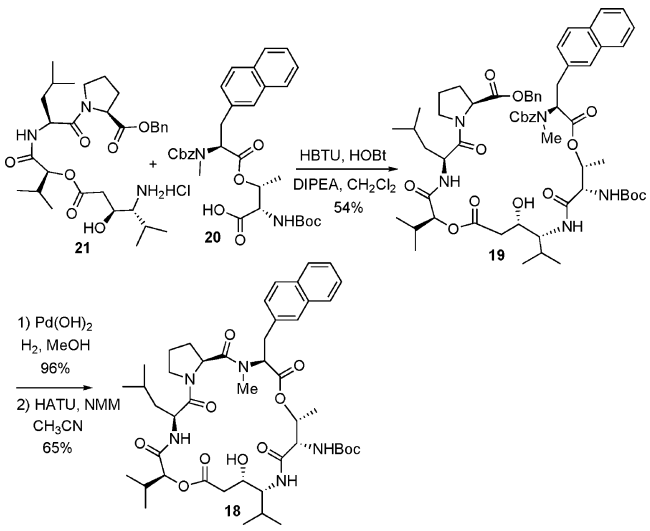
Scheme 5



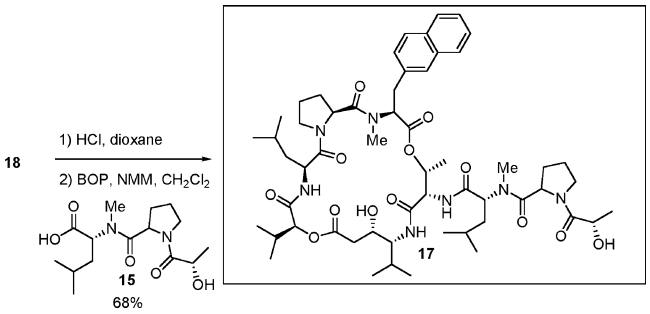
and DIPEA to afford the linear precursor **19** in 54% yield. The benzyl protecting groups were removed by hydrogenation in 96% yield. Finally, the macrocyclization (HATU/NMM/CH_3CN) afforded the desired macrocycle **18** in 65% yield (Scheme 6).

Scheme 6



The coupling of the hydrochloride salt of macrocycle **18** with the lactyl side chain **15** gave the corresponding tamandarin B analogue **17** (Scheme 7).

Scheme 7



A panel of 14 human tumor cell lines was used to evaluate the cytotoxic potential of the tamandarin B analogues **16** and **17**: prostate carcinoma tumor cells (DU-145 and LN-CaP), ovarian cells sensitive (IGROV) or resistant (IGROV-ET) to ET-743, SK-BR-3 breast adenocarcinoma, MEL-28 malignant melanoma, A-549 lung carcinoma NSCL, K-562 chronic myelogenous leukemia, PANC-1 pancreatic epitheloid carcinoma, HT-29 colon carcinoma cells, LoVo lymph node metathesis cells and the corresponding LoVo-Dox cells resistant to Doxorubicin, and cervix epitheloid carcinoma (HeLa) or resistant (HeLa-Apl) to aplidine.

Table 1. Data of in vitro Cytotoxicity (GI_{50}, nM) of Compounds **2**, **16**, and **17**

	tamandarin B **2**	*N*-MePhe **16**	*N*-MeNaphth **17**
DU-145	7.08	2.37	2.17
LN-CaP	5.84	1.52	1.98
IGROV	7.31	2.94	2.09
IGROV-ET	173	215	159
SK-BR-3	5.44	0.66	1.83
MEL-28	3.03	2.74	4.14
A-549	7.62	4.56	6.96
K-562	8.47	23.2	20.0
PANC-1	14.0	3.81	6.24
HT-29	6.32	4.77	4.60
LOVO	30.5	26.1	6.84
LOVO-DOX	1250	358	429
HELA	3.90	1.17	2.81
HELA-APL	59.7	44.5	36.4

A conventional colorimetric assay[14] was set up to estimate GI_{50} values, that is, the drug concentration that causes 50% cell growth inhibition after 72 h continuous exposure to the test molecules. Tamandarin B (**2**) is included in the test for comparison. The results obtained are shown in Table 1.

The results revealed that the cytotoxic activity of analogues **16** and **17** is generally better than that of **2**. This remarkable activity suggests that analogues **16** and **17** might be used as scaffolds for further development of new types of antitumor compounds.

In conclusion, two tamandarin B analogues involving structural changes in the macrocycle have been synthesized and shown to have enhanced cytotoxic activity against many human tumor cell lines.

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Supporting Information Available: Experimental procedures and characterization data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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