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Total Synthesis of Lys^3 Tamandarin M: A Potential Affinity Ligand

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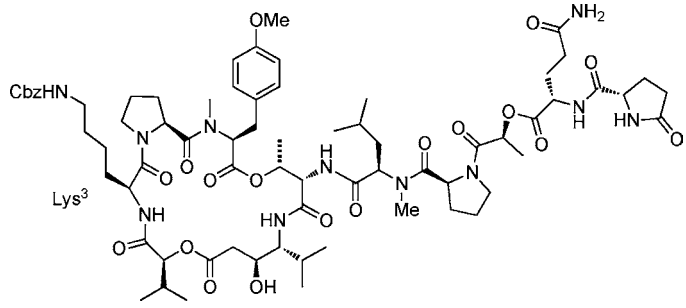
ABSTRACT



The synthesis of Lys^3 tamandarin M is described. This analogue can be used as a protein affinity ligand to probe the mechanism of action of this unique class of molecules.

The didemnins are a family of marine natural products that were isolated by Rinehart in 1984.[1] They consist of a 23-membered depsipeptide macrocycle with a side chain attached to the threonine nitrogen of the macrocycle. The didemnins (Figure 1) have been the subject of many syntheses.[2] Didemnin B (**2**) was the first marine natural product to enter into phase II clinical trials, but the trials were terminated due to toxicity issues associated with dosing levels,[3] and didemnin M (**3**) was later reported by Rinehart in 1994.[4] The difference between didemnins B and M is that the side chain of didemnin M is extended by two additional amino acid residues. It is one of the most potent immunosuppressive reagents known. In 2000, Fenical and Vervoort reported the structure of the structurally related tamandarins A and B[5] (Figure 1) and both molecules were subsequently synthesized.[6] The main difference between didemnins and tamandarins is that tamandarins A and B possess a structurally simpler hydroxyisovaleryl (Hiv) fragment within a 21-membered ring instead of the α-(α-hydroxyisovaleryl)propionyl fragment within a 23-membered ring. This structural distinction results in minor differences in biological activity, but the absence of the highly epimerizable stereocenter present in the didemnins makes the tamandarins a more accessible synthetic target. Although these compounds have been extensively studied, their mechanism of action remains unclear.

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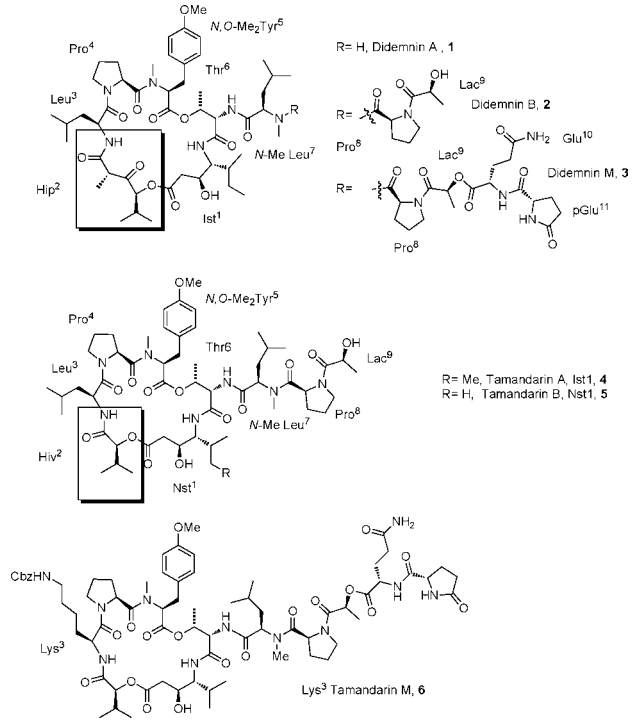
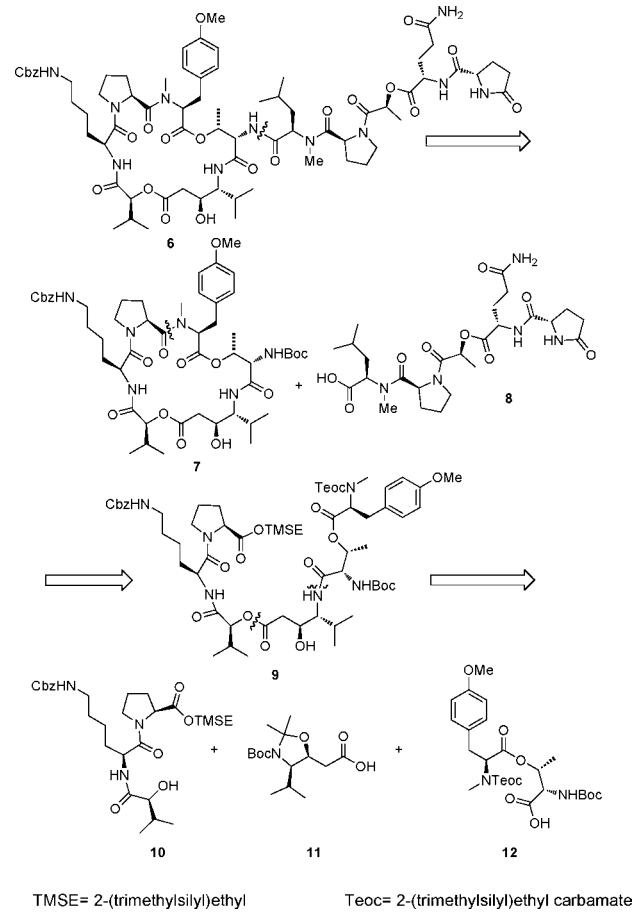


Figure 1. Structures of the didemnins and tamandarins and Lys[3] tamandarin M.

Many of the unresolved questions in the didemnin field are related to its mechanism(s) of action. Previous affinity studies have focused exclusively on the macrocycle by attaching a linker to the *N*-Me-D-Leu residue of didemnin A (**1**).[7] These studies revealed two binding proteins; elongation factor 1α (EF-1α) and palmitoyl protein thioesterase 1 (PPT1). Although the first study showed that an EF-1α-mediated effect upon protein translation explained certain biochemical effects of didemnins, their potent antiproliferative effect remained unexplained.[8] The relevance of PPT1 in mediating antiproliferative activities of didemnins was questioned.[9] Since the residues in both didemnins and tamandarins are the major contributors to the biological activity, it is possible that the molecular targets mediating the activity did not recognize the immobilized side chain. A probe with a fully elaborated side chain would be optimal for affinity studies since the side chain is vital for biological activity.[3] Lys[3] Tamandarin M (**6**) was designed with the replacement of the leucine residue with a lysine residue to allow for attachment of a biological probe far removed from the intact side chain (Figure 1).

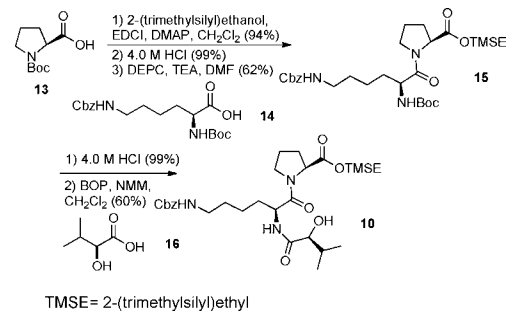
The first step in the retrosynthetic analysis was disconnection of the side chain from the macrocycle to afford **7** and **8** (Scheme 1). The macrocycle (**7**) was then opened at the Pro[4], Me$_2$-Tyr[5] junction to form linear precursor **9**. This site of closure was chosen due to previous reports showing that this position for cyclization has consistently given the highest yields.[2c,10] This linear precursor was then divided into three fragments; the Pro-Lys-Hiv fragment (**10**), the norstatine fragment (**11**) which has been previously synthesized,[11] and the Me$_2$Tyr-Thr fragment (**12**).

Scheme 1. Retrosynthetic Analysis



The synthesis began with the formation of the Pro-Lys-Hiv fragment **10** (Scheme 2). Boc-L-proline (**13**) was esterified with 2-(trimethylsilyl)ethanol to form the TMSE

Scheme 2



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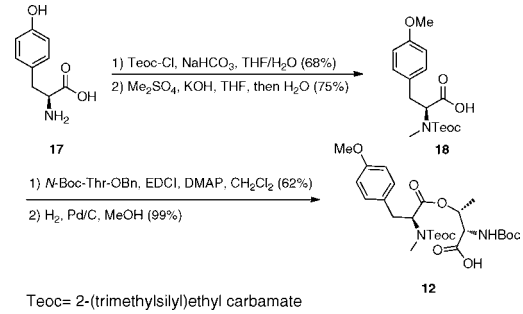
ester. Removal of the Boc group followed by coupling to **14** with diethyl cyanophosphonate (DEPC) gave dipeptide **15** in good yield.[2c] Boc removal followed by a BOP mediated coupling with 2-hydroxy-3-methylbutanoic acid (**16**) yielded the Pro-Lys-Hiv fragment (**10**).

The Tyr-Thr fragment was prepared next (Scheme 3). L-Tyrosine (**17**) was first protected as its 2-(trimethylsilyl)

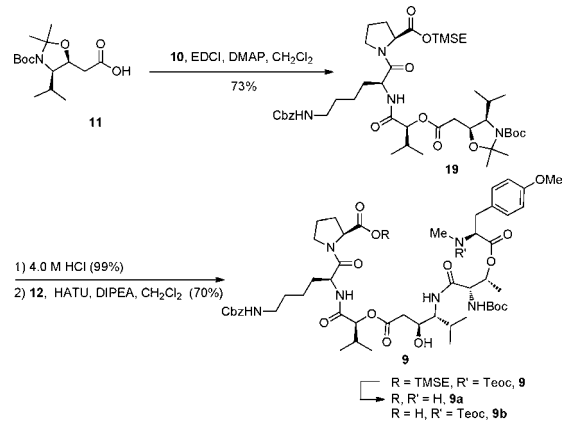
Scheme 3



ethoxycarbonyl (Teoc) carbamate. Dimethylation then subsequent hydrolysis yielded acid **18**. The resulting acid was esterified with *N*-Boc-Thr-OBn using EDCI to furnish the desired benzyl ester. Hydrogenolysis led to acid **12** in quantitative yield.

With all fragments in hand the linear precursor was assembled (Scheme 4). Esterification of acid **11** with alcohol

Scheme 4



10 in the presence of EDCI and DMAP furnished **19**. Treatment of **19** with HCl removed the Boc group and fragmented the oxazolidine to yield a salt which was immediately coupled to acid **12** in the presence of HATU to yield the desired linear precursor **9**.

Unfortunately, all attempts to remove the protecting groups of **9** failed to give the desired product (**9a**). Initial treatment with TBAF resulted in hydrolysis of the Tyr-Thr ester (Table 1, entry 1). Treatment with other fluoride sources also did

Table 1. Screening Different Conditions to Effect Protecting Group Removal

entry	reagent and solvent	temperature (°C)	result
1	TBAF, THF	0 or 25	Hydrolysis of Tyr-Thr ester
2	TBAF, THF AcOH	25	No reaction
3	TBAF on SiO_2	25	**9b**
4	TAS-F, DMF	25 or 40	**9b**
5	TAS-F, CH_3CN	25, 40, or 80	**9b**
6	TAS-F, CH_2Cl_2 KF, CH_3CN	25, 40, or 50	**9b**
7	18-crown-6	70	Decomposition
8	HF·Py	25	No reaction

not yield the free linear precursor. TAS-F[12] and TBAF on silica gel[13] removed the TMSE ester only under a variety of conditions to yield **9b** (Table 1, entry 3−6). Treatment with potassium fluoride at elevated temperatures only resulted in decomposition (Table 1, entry 7). Finally treatment with HF-pyridine resulted in recovered starting material. We were able to remove the TMSE group under fluoride ion conditions, but the Teoc group was unnaffected.

A change in protecting group strategy was necessary. A linear precursor was designed in which the Me_2Tyr^5 nitrogen was protected as a Boc carbamate, and the Thr^6 nitrogen was protected as a Troc carbamate (**20**) (Figure 2). This

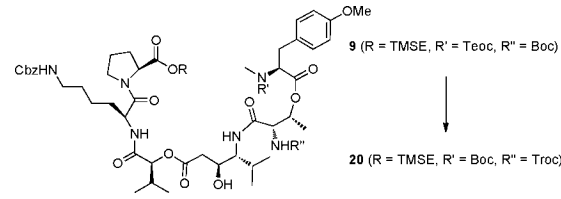


Figure 2. Modifications of protecting groups.

strategy would allow for a one step deprotection of the linear precursor followed by cyclization. The TMSE group was kept the same since it could be removed with fluoride sources and also could be removed along with the Boc group in one step using TFA.[14] The only fragment of the molecule that needed revision was the Me_2Tyr^5-Thr^6 portion. With this change in protecting groups all retrosynthetic disconnections remained unchanged.

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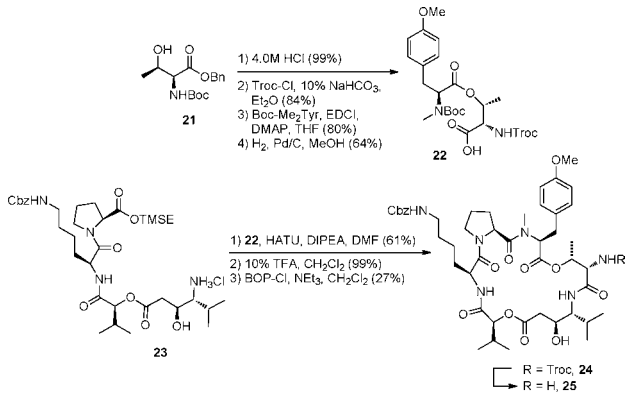
Benzyl ester **21** was treated with acid to remove the Boc group and the free amine was subsequently protected as its Troc carbamate (Scheme 5). Esterification of tyrosine deriva-

Scheme 5



tive[15] with this *N*-Troc-Thr-OBn and hydrogenolysis yielded acid **22**. An HATU-mediated coupling of acid **22** with **23** led to the a linear precursor. Treatment of this linear precursor with 10% TFA led to removal of both terminal protecting groups in quantitative yield. Cyclization with BOPCl led to product **24** in modest yield. Unfortunately the Troc group could not be removed to yield **25**. A variety of reducing conditions including Zn,[16] Cd,[17] Cd/Pb,[18] Zn/Pb,[19] or SmI_2[20] would not promote the removal of this protecting group.

At this point we surmised that a protecting group on the tyrosine nitrogen might not be needed (Scheme 6). Tyrosine derivative **26**[3] was esterified with *N*-Boc-Thr-OBn (**21**). This product was exposed to hydrogenolysis conditions to remove the Cbz carbamate and benzyl ester to form **27**.

Coupling of acid **27** to amine salt **23** resulted in the desired product. Treatment of this linear precursor with TASF removed the desired silyl protecting group. Cyclization with

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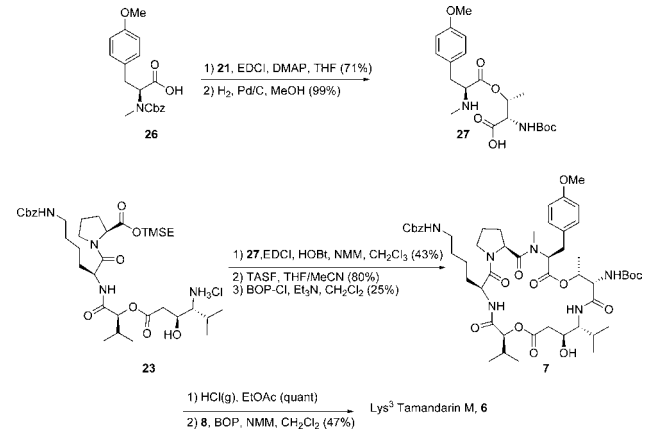
Scheme 6



BOP-Cl provided macrocycle **7** in modest yield. The Boc group of **7** was removed with HCl and the product coupled to the side chain acid **8** to yield Lys[3] Tamandarin M (**6**).[21] At this point the lysine residue will stay protected as Cbz carbamate until the biological probe is ready to be attached.

In conclusion, a tamandarin/didemnin M analogue has been designed and synthesized. This product will be used as a biological probe in affinity studies to clarify the mode of action of this unique class of natural products. This is the first time a tamandarin analogue with an intact side chain that allows for attachment to a tag on a remote location on the macrocycle has been synthesized, leaving an intact side chain for biological studies. Results of this study will be reported in due course.

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Supporting Information Available: Experimental procedures and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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