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Total Synthesis and Biological Evaluation of Tamandarin B Analogues

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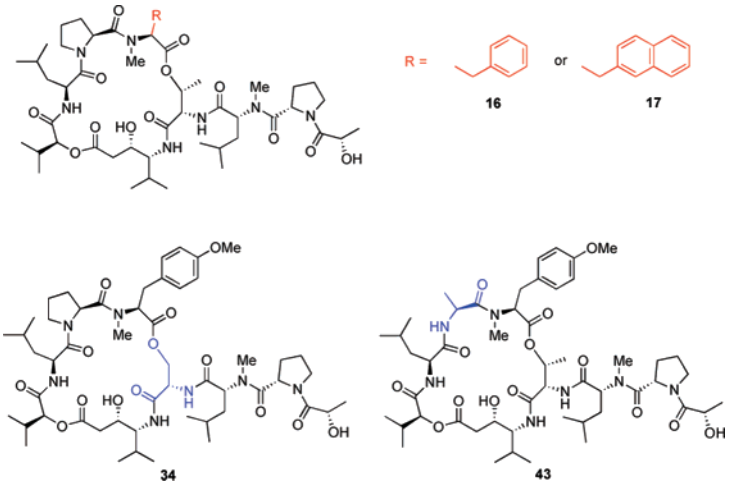

Tamandarins A and B are a class of marine natural cyclodepsipeptides with structures and biological activities closely related to those of the didemnins. The easier synthetic access to tamandarins accelerates the preparation of new macrocyclic derivatives of this family of antitumor, antiviral, and immunosuppressive compounds. The optimization of the previously reported synthetic route to tamandarins by changing the macrolactamization site from Nst[1] and Thr[6] to Pro[4] and *N*,*O*-Me$_2$Tyr[5] residues led to a significant improvement in the reaction yield. Using this new synthetic approach, four new macrocyclic analogues of tamandarin B were prepared and evaluated for anticancer activity. These results provide further insight into the structure−activity relationship of the tamandarins and didemnins.

Introduction

Nature is an inventive chemist and has been a vast source of biologically active compounds, which have been successfully employed in the treatment of different diseases. In spite of the tremendous biodiversity present in the ocean, marine organisms do not have a history of use in traditional medicine as strong as that of terrestrial organisms. However, in recent years the number of marine-derived compounds undergoing preclinical and early clinical development has increased exponentially.[1,2]

Due to its impressive in vitro and in vivo biological activities, the didemnin family is a clear example of marine compounds with potential therapeutic value.[3] The didemnins were isolated from *Tridemnum solidum*, a Caribbean tunicate, by Rinehart and co-workers in 1981.[4] Didemnin B (**1**), the initial lead congener, has shown potent antitumor, antiviral, and immuno-

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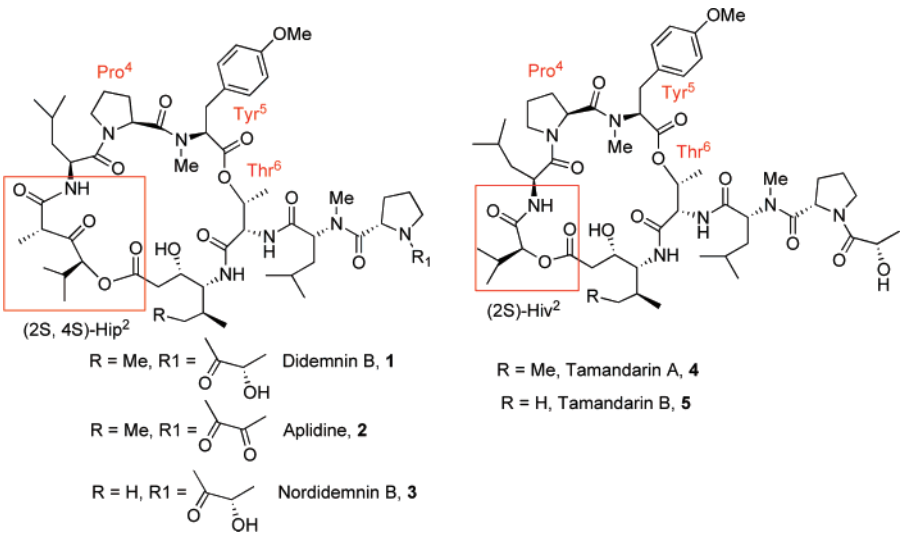

FIGURE 1. Structures of didemnins and tamandarins.

suppressive activities, as well as the ability to inhibit in vitro protein biosynthesis and to induce rapid apoptosis.[5−8] The antitumor action of these compounds resulted in the first human clinical trials in the U.S. of a marine natural product against cancer, and additional clinical trials of a second-generation didemnin, dehydrodidemnin B (aplidine; **2**), are underway.[1,2,9] However, the mechanisms by which didemnins produce these biological activities are still under investigation.

In 2000, Fenical and co-workers reported the structures of tamandarins A and B (**4** and **5**), two cyclic depsipeptides isolated from a Brazilian ascidian.[10] Their structures are related to those of didemnins, differing only in that the macrocyclic core of tamandarins A and B contains the Hiv (α-hydroxyvaleryl) instead of the more complex Hip (α-(α-hydroxyvaleryl)-propionyl) moiety (Figure 1). The two species that generate products **1** and **4** originate from remote geographic locations but use a common method to defend their vulnerable larvae by producing these structurally similar depsipeptides.[11]

Tamandarins A and B were reported to exhibit much of the same biological activity as didemnin B and aplidine, maintaining similar levels of antitumor activity and protein biosynthesis inhibition properties.[10] In addition, the synthesis of several analogues of tamandarins A and B has shown that equivalent structural modifications result in similar biological activities for both didemnins and tamandarins.[12−15] Furthermore, fluorescence studies of the predator−prey interactions revealed similar behaviors for both depsipeptide families.[11] These observations support the hypothesis that tamandarins are simplified didemnin mimics, indicating that the absence of the propionic acid unit results only in minor conformational modification and may not greatly affect bioactivity.

The first studies of structure−activity relationships of didemnins showed that the biological activities were highly dependent on the structure of the side chain.[3] This observation caused the majority of structure−activity studies to focus on this region.[3] The macrocyclic core itself presents levels of biological activity considerably lower than those reported for the corresponding didemnins.[16,17] However, besides the importance of the side chain, it is difficult to identify a concrete structure−activity model for the didemnin family. The length and complexity of the existing synthetic routes for accessing didemnins[6,18−22] have hindered the development of a large number of macrocycle-modified analogues in which the importance of each residue was individually tested. Only a few examples of didemnin analogues modified at the Hip-isostatine[17,23−25] (residues 1 and 2) or at the *N*,*O*-Me$_2$Tyr^5

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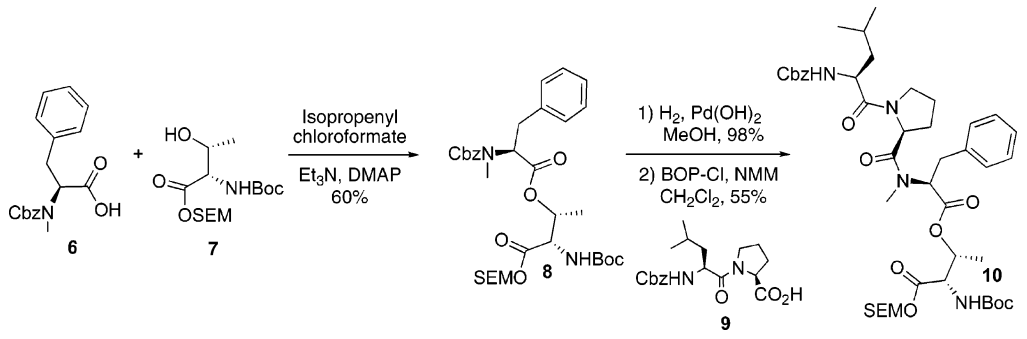
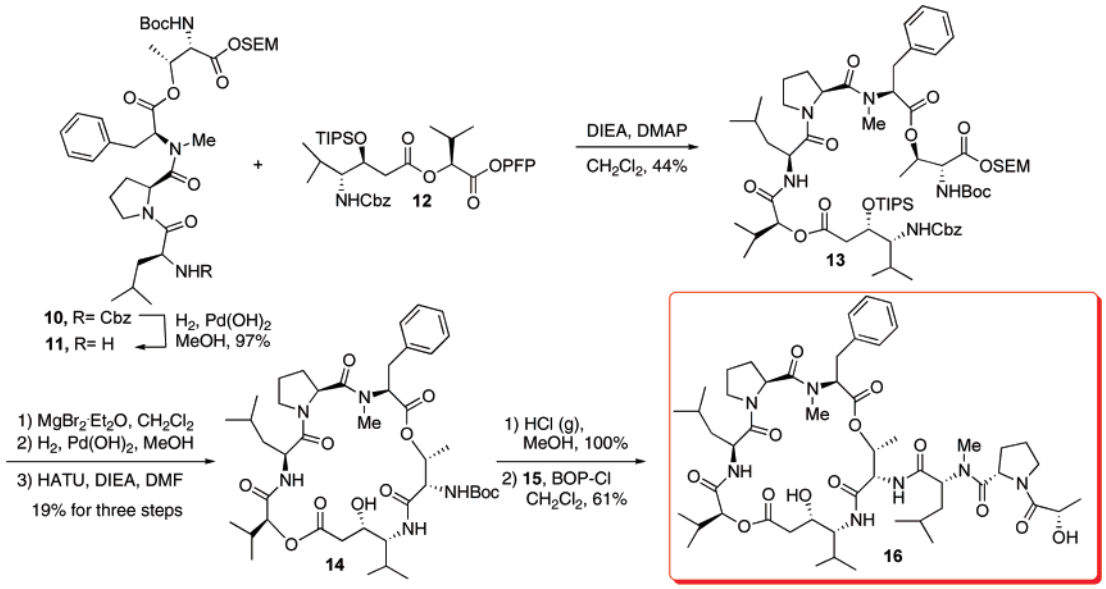
SCHEME 1



SCHEME 2



position have been reported to date.[7,26−28] Also, no drastic conformational change occurred when L-Pro[4] was substituted with a D-Pro residue.[29]

In this context, we envisage that the easier synthetic access to tamandarin derivatives could accelerate the preparation of new macrocyclic analogues to facilitate the elucidation of the didemnin pharmacophore. Tolerance for modification in the tetrapeptide region of didemnin B (**1**) resulted in several syntheses of analogues modified in this region. The [*N*,*O*-Me_2-Tyr^5] at position 5 of didemnin is thought to be an important pharmacophoric element, because it projects outward from the main body of the molecule in the solid state and in solution.[30,31] We prepared two didemnin B analogues in which the tyrosine moiety was replaced with *N*-Me-leucine and *N*-Me-phenylalanine.[26−28] These analogues exhibited biological activity similar to that of didemnin B, indicating that changes in this position are tolerated.

In order to evaluate whether tamandarins are fully competent mimics of didemnins, we began our study by preparing a tamandarin B analogue in which the *N*,*O*-Me_2Tyr^5 residue was replaced with *N*-Me-phenylalanine (*N*-$MePhe^5$) and (*S*)-2-(methylamino)-3-(naphthalen-1-yl)propanoic acid (*N*-$MeNaphth^5$).[32] Next, we prepared a macrocycle that was modified at the Pro^4 and Thr^6 positions, where the tolerance for variation was not well investigated except for changing L-proline to D-proline.[29] We now report the synthesis and cytotoxic evaluation of four new tamandarin B analogues, providing further insight into the structure−activity relationships of the didemnin family.

Results and Discussion

Synthesis of *N*-$MePhe^5$-Tamandarin B. The synthesis of *N*-Me-Phe^5-tamandarin B was achieved on the basis of methodology previously reported in our research groups for the preparation of tamandarins A and B,[12−15] differing only in the preparation of the tetrapeptide moiety. We began the synthesis

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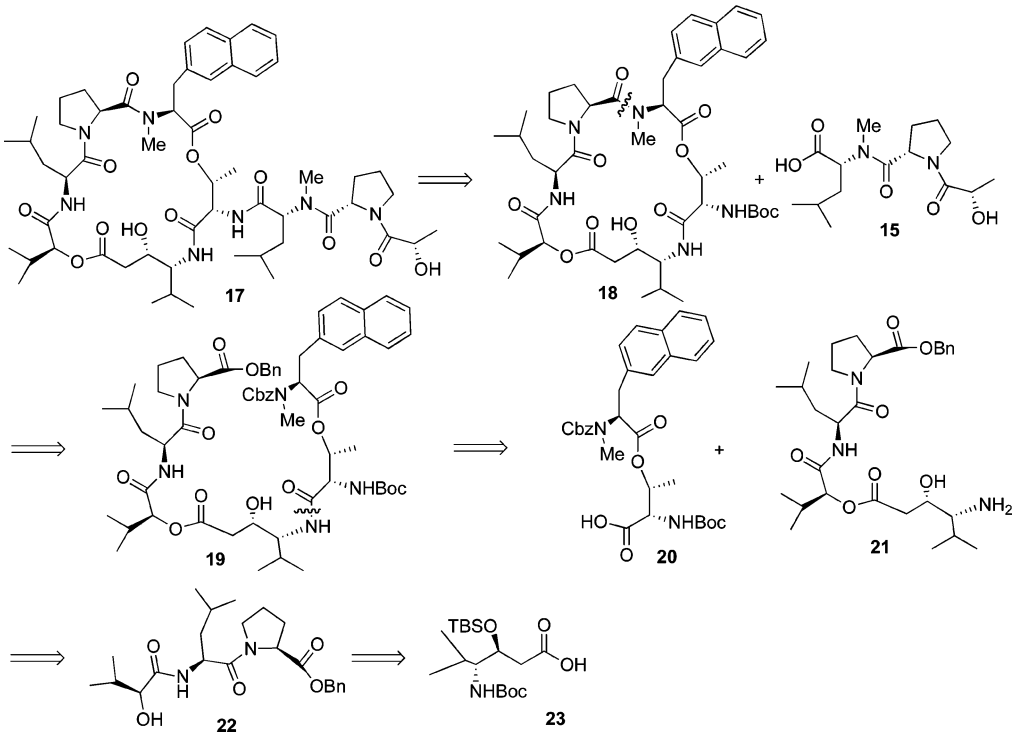

FIGURE 2. Retrosynthetic analysis of *N*-MeNaphth[5]-tamandarin B (**17**).

with the coupling of Cbz-*N*-MePhe (**6**) with Boc-Thr-OSEM (**7**) to afford the corresponding dipeptide ester **8** in good yield. Subsequent hydrogenolysis with $Pd(OH)_2$ and coupling with acid **9** yielded the desired tetrapeptide **10** (Scheme 1).

Hydrogenolysis of the Cbz group of tetrapeptide **10** [H_2, $Pd(OH)_2$] provided the free amine **11**, which was coupled with the previously described PFP ester **12**[15] to afford the linear precursor **13** in 43% overall yield. The cleavage of the SEM group was achieved using $MgBr_2 \cdot Et_2O$ without affecting the Boc and TIPS protecting groups[33] to furnish the corresponding acid, which was converted to the free amine under usual hydrogenolysis conditions. Subsequent macrolactamization with HATU in CH_2Cl_2 give the *N*-Boc-protected macrocycle **14** (19%, three steps overall). Reaction with HCl (gas) in dioxane, to remove both the TIPS and Boc protective groups, afforded the hydrochloride salt, which was coupled with the corresponding side chain **15** (prepared as previously described)[12−15] to afford the desired Phe[5]-tamandarin B (**16**; Scheme 2).

Several attempts to improve the low yield obtained in the macrocyclization (only 19%) by changing the solvent, the base, and the coupling agent failed. Taking into account the requirement of an efficient protocol that would allow the preparation of several macrocycle derivatives, we decided to modify the synthetic strategy by changing the macrocyclization site.[18] Since the first total synthesis of didemnins reported by Rinehart et al. in 1987,[21] several different macrocyclization approaches have been reported. Among them, the protocol reported by Shioiri[18] and then by Lloyd-Williams and Giralt[12] effected the cyclization between Pro[4] and *N*,*O*-Me_2Tyr[5] to obtain the corresponding macrolactam in 76% yield, the highest yield reported to date. On the basis of this precedent, we decided to apply this strategy in the synthesis of the next tamandarin B analogues.

Synthesis of *N*-MeNaphth[5]-Tamandarin B. The hydrophobicity of the residue at position 5 in didemnins was believed to be an important factor in the biological activity.[34] For example, the replacement of this moiety with more polar *N*-Me-L-tyrosine resulted in loss of the cytotoxic and immunosuppressive activity.[17] The incorporation of other aromatic hydrophobic amino acids at this position appeared worthwhile; therefore, we decided to prepare a tamandarin B analogue by replacing *N*,*O*-Me_2Tyr[5] with the more hydrophobic *N*-Me-L-3-(2-naphthyl)-alanine.

A retrosynthetic analysis for the synthesis of *N*-MeNaphth[5]-tamandarin B (**17**) is shown in Figure 2. The final step involves the addition of the side chain **15** to the macrocycle **18**. The bond between Pro[4] and *N*-MeNaphth[5] was selected as the macrocyclization site. The linear precursor **19** was disconnected into the dipeptide Boc-Thr-Cbz-*N*-MeNaphth (**20**) and the Norst-Hiv-Leu-Pro unit (**21**). Depsipeptide **21** was prepared by coupling alcohol **22** and norstatine **23** (Figure 2).

The synthesis began with the preparation of the norstatine moiety **23**, which was carried out by following the procedure previously described in our research group, but changing the protecting groups (Boc instead of Cbz at nitrogen and TBS instead of TIPS at the oxygen; Scheme 3).

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

The synthesis of acid **20** (Figure 2) began with the protection of the acid and amino groups of threonine as a SEM ether and Boc carbamate. Unfortunately the coupling with Cbz-*N*-Me-

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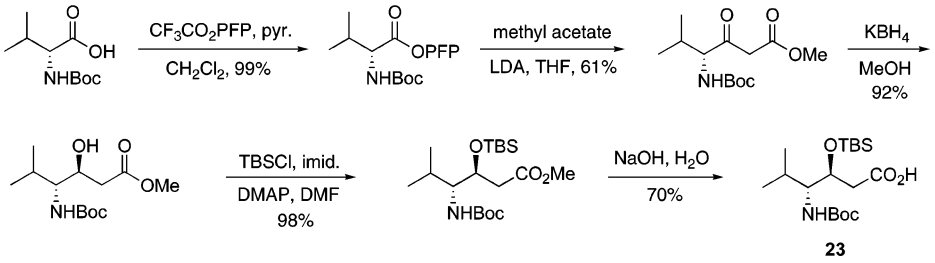
SCHEME 3



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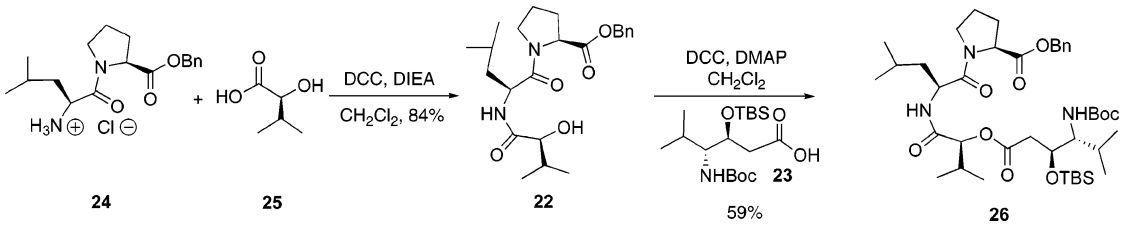
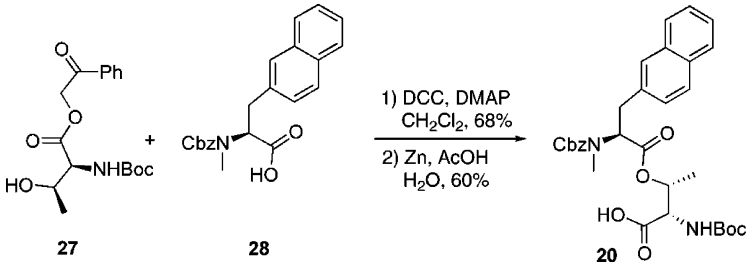


SCHEME 5



(2-naphthyl)alanine using DCC/DMAP occurred in very low yield (5%), affording a compound without the SEM moiety as the major product of the reaction. This problem could be solved by changing the protecting group on the ester moiety. Therefore, *N*-Boc-threonine was protected as a phenacyl ester by reaction with bromoacetophenone to afford **27**. The subsequent coupling with Cbz-*N*-Me-3-(2-naphthyl)alanine **28** using DCC/DMAP afforded the desired dipeptide in 68% yield. The phenacyl group was then removed using Zn in AcOH/H_2O to give acid **20** in 60% yield (Scheme 5).

Depsipeptide **21**, obtained by acidic deprotection of compound **26**, was coupled with acid **20** using HBTU, HOBt, and DIPEA to afford the linear precursor **19**. The benzyl protecting groups were removed simultaneously by hydrogenation in 96% yield. The macrocyclization (HATU/NMM/CH_3CN) afforded the desired macrocycle **18** in 65% yield. The coupling of the hydrochloride salt of macrocycle **18** with the lactyl side chain **15** gave the corresponding tamandarin B analogue **17** (Scheme 6).

Synthesis of Ser^6-Tamandarin B. The conformation of the threonine residue in several macrocycles is believed to play a critical role in shaping the overall molecular conformation, as this residue forms the junction of the cyclic and linear peptide chains. In several antibiotic peptides, the threonine residue forms an ester bond between the threonine hydroxyl and the C-terminal carboxyl in the cyclic part of the molecule, and the threonine amino group is acylated to form a linear peptide chain.[35] Replacing this residue with serine should assess the importance of this moiety.

The synthesis of the Ser^6-tamandarin B was performed in a way similar to that described for the preparation of *N*-MeNaphth^5-tamandarin B but using *N*,*O*-Me_2Tyr instead *N*-MeNaphth^5 and serine instead of threonine. The synthesis of Ser-*N*,*O*-Me_2Tyr dipeptide is described in Scheme 7. The acid group of *N*-Boc-serine was protected as a phenacyl ester to afford **29** in 98% yield. The subsequent coupling with *N*-Cbz-Me_2Tyr **30** using DCC/DMAP furnished the desired dipeptide in 80% yield. The phenacyl group was then removed using Zn in AcOH/H_2O to give the acid **31** in 55% yield (Scheme 7).

Next, depsipeptide **21** was coupled with acid **31** using HBTU, HOBt, and DIPEA to afford the protected linear precursor **32** in 65% yield. The benzyl protecting groups were removed by hydrogenation in 99% yield to give rise to the corresponding linear precursor. Finally, macrocyclization (HATU/NMM/CH_3CN) afforded the desired cyclic peptide **33** in 57% yield. With the macrocycle in hand, the Ser^6-tamandarin B analogue **34** was prepared by attaching the side chain to the macrocycle hydrochloride salt in the presence of BOP and NMM (Scheme 8).

Synthesis of Ala^4-Tamandarin B. To the best of our knowledge, no successful synthetic changes in the Leu^3-Pro^4 region of the didemnins have been reported to date, except for the replacement of Leu^3 with Lys^3.[36] The fact that the Leu^3-Pro^4 region exists in a β-turn conformation in the solid state has led to speculation that this conformational feature may be a determining element in the biological activity of this family of compounds. However, the substitution of L-proline by D-proline in naturally occurring D-Pro^4-didemnin B apparently

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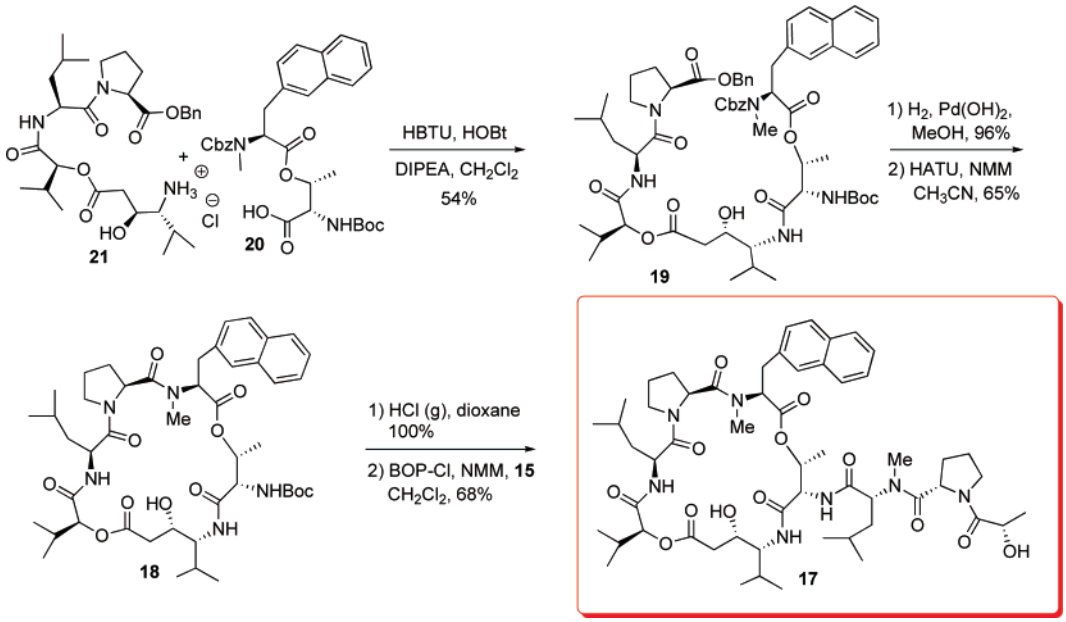
SCHEME 6



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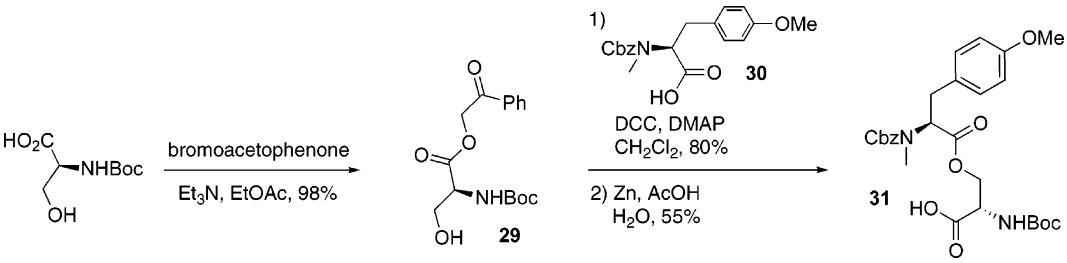
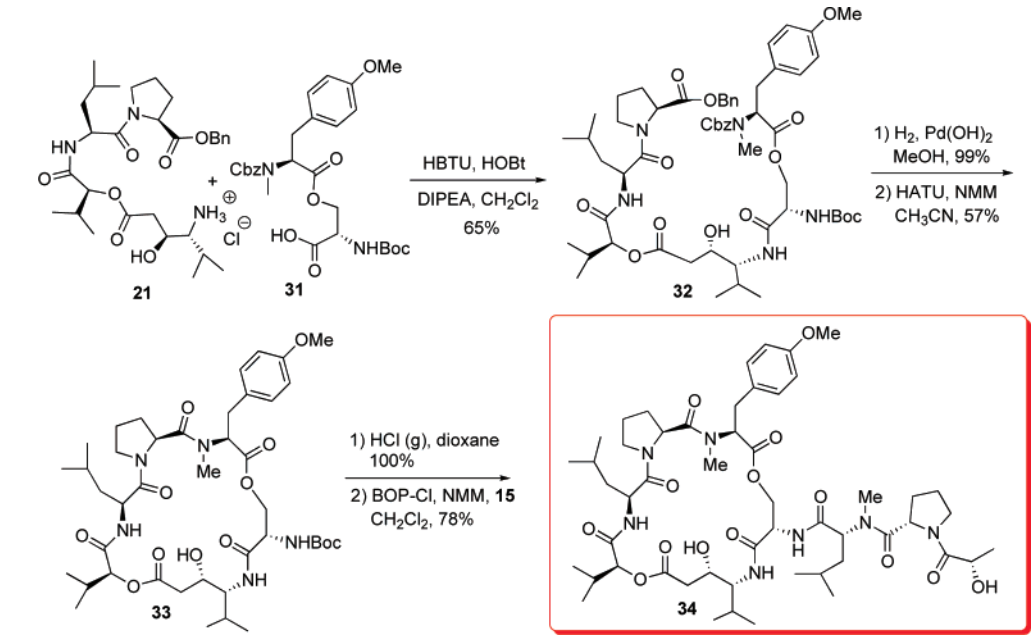


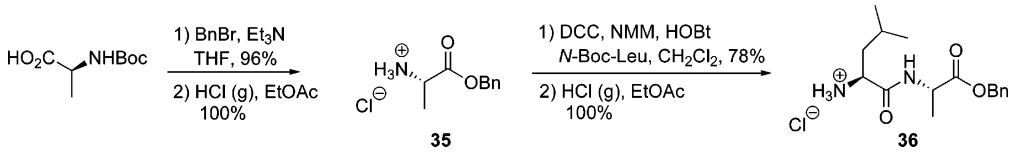
SCHEME 8



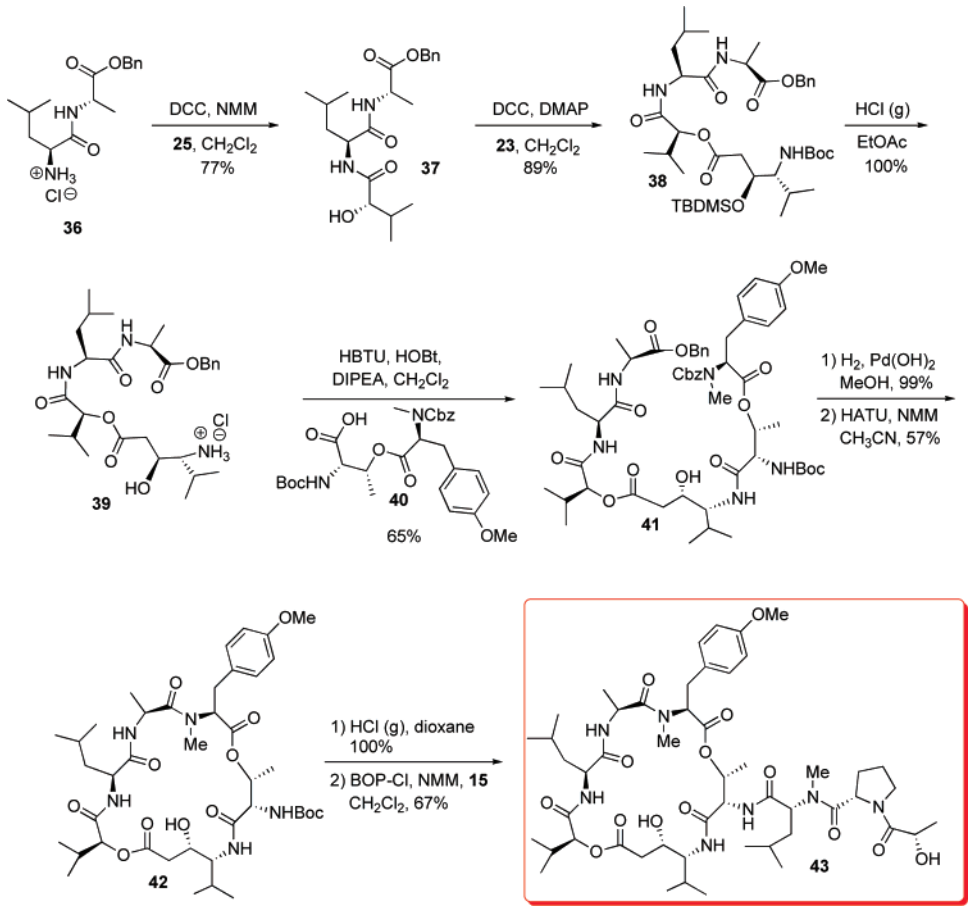
did not cause much change in the conformation of the didemnin macrocycle, preserving a similar cytotoxic activity.[29] In order to understand the role of the L-Pro[4] residue in the biological activity of tamandarins, we decided to replace the proline residue with L-alanine.

The synthesis of the Ala[4]-tamandarin B derivative was performed by following the same synthetic sequence used for the synthesis of the *N*-MeNaphth[5] and Ser[6] analogues. First, *N*-Boc-L-alanine was protected as its benzyl ester in excellent yield (96%). Removal of the Boc group with a solution of HCl

SCHEME 9



SCHEME 10



in EtOAc afforded the hydrochloride salt **35**, which was successfully coupled with *N*-Boc-L-leucine using DCC/NMM/HOBt to give the corresponding dipeptide in 78% yield. Finally, the hydrochloride salt **36** was obtained by cleavage of the Boc group using a solution of HCl in EtOAc (Scheme 9).

The reaction of dipeptide **36** with hydroxyvaleric acid **25** in the presence of DCC/NMM afforded **37** in 77% yield. Furthermore, esterification of acid **23** with alcohol **37** gave fragment **38** in 89% yield. Next, the Boc and TBS groups were removed in quantitative yield using a solution of HCl in EtOAc to give **39**. The coupling between **39** and dipeptide **40** afforded the linear precursor **41**. Hydrolysis of benzyl protecting groups and macrocyclization (HATU, NMM, CH_3CN) afforded the macrocyclic core **42** of a tamandarin B analogue, with alanine in place of Pro.[4] The coupling of the hydrochloride salt of macrocycle **42** with the lactyl side chain gave the desired Ala[4]-tamandarin B analogue **43** (Scheme 10).

Biological Evaluation of the Synthetic Analogues. A panel of 14 human tumor cell lines was used to evaluate the cytotoxic potential of the tamandarin B analogues **16**, **17**, **34**, and **43**: 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.

A conventional colorimetric assay[37] was set up to estimate GI_{50} values: i.e., the drug concentration that causes 50% cell growth inhibition after 72 h of continuous exposure to the test molecules. Tamandarin B (**5**) is included in the test for comparison. The results obtained are shown in Table 1.

These results show that the modification accomplished in *N*,*O*-Me_2Tyr and Thr[6] are well tolerated, since the cytotoxic activities of analogues **16**, **17**, and **34** are similar or in some cases superior to that of **5**. However, the replacement of Pro[4] with alanine effected a considerable decrease in activity, suggesting that Pro[4] is a critical structural element required for cytotoxic activity. This outstanding activity suggests that

(37) Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107−1112.

TABLE 1. Data of in Vitro Cytotoxicity (GI_{50}, nM) of the Compounds 5, 16, 17, 34, and 43

	Tamandarin B (**5**)	N-MePhe[5] (**16**)	N-MeNaphth[5] (**17**)	Ser[6] (**34**)	Ala[4] (**43**)
DU-145	7.08	2.37	2.17	6.79	1810
LN-CaP	5.84	1.52	1.98	2.71	548
IGROV	7.31	2.94	2.09	11.1	1990
IGROV-ET	173	215	159	456	
SK−BR-3	5.44	0.66	1.83	3.74	6860
MEL-28	3.03	2.74	4.14	16.6	1500
A-549	7.62	4.56	6.96	51.5	5850
K-562	8.47	23.2	20.0	78.0	5870
PANC-1	14.0	3.81	6.24	34.9	3750
HT-29	6.32	4.77	4.60	23.7	4220
LOVO	30.5	26.1	6.84	38.9	3550
LOVO-DOX	1250	358	429	2390	9840
HELA	3.90	1.17	2.81	3.76	1180
HELA-APL	59.7	44.5	36.4	411	9840

analogues **16**, **17**, and **34** might be used as scaffolds for further development of new types of antitumor compounds.

Conclusions

We have completed four total syntheses of tamandarin B analogues using a new synthetic strategy based on the change of the macrocyclization site. 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. The changes achieved in *N*,*O*-Me_2-Tyr and Thr[6] residues were well tolerated and even enhanced the cytotoxic activity against several human tumor cell lines. However, the substitution of Pro[4] by alanine resulted in dramatically reduced activity, indicating that conformational and spatial effects are critical for antitumor activity.

Experimental Section

[*N*-MePhe[5]]-Tamandarin B Protected Macrocycle (14). To a solution of the fully protected linear precursor **13** (140 mg, 0.11 mmol) dissolved in CH_2Cl_2 (5 mL), at 0 °C was added MgBr·Et_2O (85 mg, 0.33 mmol). The reaction mixture was stirred at 0 °C for 2 h and overnight at room temperature. The mixture was diluted with CH_2Cl_2, and the organic layer was washed with 10% HCl (100 mL) and NaCl (100 mL, saturated), dried (Na_2SO_4), filtered, and concentrated. The resulting acid (130 mg) was obtained as a white foam and used directly in the next step. To a solution of crude acid (130 mg, 0.30 mmol) in MeOH (5 mL) under argon was added $Pd(OH)_2$ (28 mg). The reaction mixture was purged with H_2 and stirred overnight under a H_2 atmosphere (1 atm). The mixture was filtered through Celite. The filtrate was concentrated to yield the linear precursor (87 mg, 78%) as a white foam, which was used directly in the next step. The crude amino acid linear precursor (87 mg, 0.08 mmol) was dissolved in DMF (9 mL) and cooled to 0 °C. HATU (40 mg, 0.10 mmol) was added, followed by the dropwise addition of DIEA (46 μL, 0.26 mmol). The reaction mixture was stirred at 0 °C for 1 h and then overnight. The reaction mixture was concentrated in vacuo, diluted with EtOAc (10 mL), washed with 10% HCl (10 mL), 5% $NaHCO_3$ (10 mL), and NaCl (10 mL, saturated), dried (Na_2SO_4), filtered, and concentrated. The crude oil was purified by column chromatography (silica gel, EtOAc/Hex 5:1) to yield the protected macrocycle **14** (10 mg, 19% three steps overall) as a white foam. $R_f = 0.40$ (AcOEt/Hex 3/7). $[\alpha]_{20}^{D} = -36.1$ ($c = 1$, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 0.79−1.09 (m, 18H), 1.09−1.10 (s, 21H), 1.27 (d, $J = 6.4$ Hz, 3H), 1.44 (s, 9H), 1.56−1.70 (m, 3H), 1.73−1.81 (m, 1H), 1.99−2.23 (m, 4H), 2.27 (dd, $J = 17.0$ and 2.9 Hz, 1H), 2.42 (dd, $J = 17.0$ and 3.0 Hz, 1H), 2.50 (s, 3H), 2.61 (dd, $J = 17.1$ and 6.5 Hz, 1H), 2.98−3.00 (m, 1H), 3.19−3.23 (m, 1H), 3.30−3.32 (m, 1H), 3.42 (dd, $J = 9.8$ and 4.9 Hz, 1H), 3.57 (dd, $J = 10$ and 4.4 Hz, 1H), 3.60−3.62 (m, 1H), 3.63−3.64 (m, 1H), 4.03−4.21 (m, 1H), 4.32−4.36 (m, 1H), 4.37−4.45 (m, 1H), 4.57−4.60 (m, 1H), 4.60−4.69 (m, 1H), 4.88−4.92 (m, 1H), 4.94 (d, $J = 6.0$ Hz, 1H), 5.80 (m, 1H), 7.12−7.17 (m, 2H), 7.28−7.31 (m, 3H), 7.48−7.51 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 12.2, 12.6, 15.1, 16.5, 17.5, 18.0, 18.2, 18.8, 19.6, 20.7, 21.0, 23.5, 24.9, 27.9, 28.1, 30.1, 31.0, 35.1, 38.5, 39.7, 40.9, 47.7, 48.1, 55.5, 56.9, 59.3, 65.9, 68.8, 69.9, 71.8, 80.1, 81.2, 126.9, 128.7, 129.5, 138.1, 155.9, 168.7, 170.9, 171.0, 172.2. IR (neat): 3331, 2926, 1743, 1636, 1513, 1456 cm^{-1}.

[*N*-MePhe[5]]-Tamandarin B (16). To a solution of the Boc-protected macrocycle **14** (10 mg, 0.01 mmol) in HPLC-grade EtOAc was added a solution of HCl in EtOAc. The resulting solution was stirred at room temperature for 2 h. The solution was concentrated and the residue diluted with CH_2Cl_2 and concentrated again to yield the hydrochloride salt (quantitative yield) as a white solid, which was used directly in the next step. To a mixture of the macrocycle amine salt (9 mg, 0.01 mmol) and side chain (6.1 mg, 0.015 mmol) in CH_2Cl_2 (0.50 mL) at 0 °C was added BOP (8.4 mg, 0.015 mmol) and NMM (6 μL, 0.05 mmol). After 30 min at 0 °C, the reaction was stirred at room temperature overnight. The reaction mixture was treated with NaCl solution (2 mL, saturated) and extracted with EtOAc (2 × 10 mL). The organic layers were washed with 10% HCl (5 mL), 5% $NaHCO_3$ (5 mL), and NaCl (5 mL, saturated), dried (Na_2SO_4), filtered, and concentrated. The crude oil (8 mg, 61%) was purified by HPLC (HyperPrep PEP 100 C18, isocratic, CH_3CN/H_2O 85/15; flow 7 mL/min, 270 nm) to afford **16** as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 0.83−1.02 (m, 24H), 1.25 (d, $J = 6.2$ Hz, 3H), 1.28 (m, 3H), 1.39 (d, $J = 6.2$ Hz, 3H), 1.43 (d, $J = 6.3$ Hz, 3H), 1.31−1.57 (m, 4H), 1.63−1.67 (m, 1H), 1.75−1.82 (m, 1H), 1.95−2.05 (m, 3H), 2.08−2.29 (m, 3H), 2.40 (dd, $J = 7.4$ and 17.2 Hz, 1H), 2.50 (s, 3H), 3.10 (s, 3H), 3.10−3.17 (m, 1H), 3.20−3.22 (m, 1H), 3.30−3.29 (m, 1H), 3.39−3.47 (m, 1H), 3.50−3.61 (m, 2H), 3.80−3.84 (m, 1H), 3.93−4.00 (m, 1H), 4.28−4.30 (m, 1H), 4.30−4,33 (m, 1H), 4.62−4.65 (m, 1H), 4.66 (t, $J = 4.5$ Hz, 1H), 4.83 (t, $J = 5.5$ Hz, 1H), 5.05 (d, $J = 4.4$ Hz, 1H), 5.23 (m, 3H), 5.40 (q, $J = 3.8$ Hz, 1H), 7.05−7.11 (m, 2H), 7.19−7.25 (m, 3H), 7.45−7.59 (m. 2H), 7.75−7.80 (m, 1H).

[2-Naphthylala[5]]-Tamandarin B Macrocycle (18). To a solution of protected linear precursor **19** (60 mg, 0.054 mmol) in MeOH under argon was added $Pd(OH)_2$ (21 mg). The reaction mixture was purged with H_2 and stirred overnight under a H_2 atmosphere (1 atm). The mixture was filtered through Celite. The filtrate was concentrated to yield the free linear precursor (45 mg, 96%) as a yellow oil, which was used in the next step without purification. The crude amino acid linear precursor (45 mg, 0.050 mmol) was dissolved in CH_3CN (10 mL) and cooled to 0 °C. HATU (46 mg, 0.12 mmol) was added, followed by the dropwise addition of NMM (11 μL, 0.10 mmol). The reaction mixture was stirred at 0 °C for 1 h and then overnight. The reaction solvent was evaporated in vacuo, diluted with EtOAc (10 mL), washed with 10% $KHSO_4$ (10 mL), 5% $NaHCO_3$ (10 mL), and NaCl (10 mL, saturated), dried (Na_2SO_4), filtered, and concentrated. The crude oil was purified by column chromatography (silica gel, EtOAc/Hex 2/1) to yield the protected macrocycle **18** (28 mg, 65%) as a white foam. $R_f = 0.26$ (AcOEt/Hex 1/2). $[\alpha]_D^{20} = -55.2$ ($c = 1$, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 0.90−1.02 (m, 24H), 1.29 (d, $J = 6.6$ Hz, 3H), 1.48 (s, 9H), 1.71−1.85 (m, 2H), 2.05−2.09 (m, 2H), 2.19−2.23 (m, 2H), 2.52 (s, 3H), 2.96 (dd, $J = 4.7$ and 16.9 Hz, 1H), 3.46 (dd, $J = 10.7$ and 14.0 Hz, 1H), 3.67 (dd, $J = 4.3$ and 13.9 Hz, 1H), 3.73−3.77 (m, 2H), 3.78−3.83 (m, 1H), 3.99−4.05 (m, 1H), 4.39 (dd, $J = 3.1$ and 10.3 Hz, 1H), 4.56−4.59 (m, 1H), 4.92 (t, $J = 12.0$ Hz, 1H), 4.97−5.00 (m, 2H), 5.00−5.02 (m, 1H), 7.36−7.37 (m, 1H), 7.49−7.52 (m, 2H), 7.56−7.58 (m, 2H), 7.72−7.74 (m, 1H), 7.76−7.79 (m, 2H), 7.98−8.00 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 15.6, 18.4, 189, 18.9, 20.6, 21.2, 23.9, 25.3, 25.4, 28.0, 28.4, 28.5, 30.8, 35.6, 39.0, 39.3, 39.5, 47.2, 48.8, 56.3,

57.5, 60.7, 66.0, 69.3, 71.9, 79.6, 80.7, 126.3, 126.9 127.5, 127.8, 128.2, 128.5, 128.9, 132.8, 133.9, 135.9, 156.2, 169.0, 170.1, 170.9, 171.7, 172.0 173.2. IR (neat): 3339, 2962, 2872, 2248, 1742, 1665, 1635, 1529, 1451, 1167, 851 cm^{-1}.

[2-Naphthylala[5]]-Tamandarin B (17). To a solution of the Boc-protected macrocycle **18** (28 mg, 0.032 mmol) in HPLC-grade dioxane (3 mL) was added a solution of HCl in dioxane (3 mL). The resulting solution was stirred at room temperature for 2 h. The solution was concentrated and the residue diluted with CH_2Cl_2 and concentrated again to yield the hydrochloride salt (quantitative yield) as a white solid, which was used directly in the next step. To a mixture of the macrocycle amine salt (25 mg, 0.031 mmol) and side chain **15** (14.6 mg, 0.046 mmol) in CH_2Cl_2 (4 mL) at 0 °C was added BOP (20.3 mg, 0.046 mmol) and NMM (14 μL, 0.12 mmol). After 30 min at 0 °C, the reaction was stirred at room temperature overnight. The reaction mixture was treated with NaCl solution (2 mL, saturated) and extracted with EtOAc (2 × 10 mL). The organic layers were washed with 10% HCl (5 mL), 5% $NaHCO_3$ (5 mL), and NaCl (5 mL, saturated), dried (Na_2SO_4), filtered, and concentrated. The crude oil (8 mg, 68%) was purified by HPLC (HyperPrep PEP 100 C18, isocratic, CH_3CN/H_2O 85/15; flow 7 mL/min, 270 nm) to afford **17** as a white foam. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 0.82−0.96 (m, 24H), 1.09−1. 28 (m, 14H), 1.31 (d, J = 6.8 Hz, 3H), 1.39 (d, J = 6.8 Hz, 3H), 1.64 (m, 3H), 2.10 (m, 4H), 2.48 (s, 3H), 3.10 (s, 3H), 3.09−3.15 (m, 2H), 3.35−3.41 (m, 2H), 3.43−3.82 (m, 5H), 4.29−4.33 (m, 1H), 4.42−4.47 (m, 1H), 4.65−4.70 (m, 1H), 4.72 (t, J = 6.8 Hz, 1H), 4.87 (t, J = 8.3 Hz, 1H), 5.02 (d, J = 5.3 Hz, 1H), 5.29−5.32 (m, 2H), 7.24−7.27 (m, 1H), 7.45−7. 63 (m, 4H), 7.67−7.83 (m, 4H).

[Ser[6]]-Tamandarin B Macrocycle (33). To a solution of the protected linear precursor **32** (206 mg, 0.19 mmol) in MeOH (15 mL) under argon was added $Pd(OH)_2$ (81 mg). The reaction mixture was purged with H_2 and stirred overnight under a H_2 atmosphere (1 atm). The mixture was filtered through Celite. The filtrate was concentrated to yield the free linear precursor (150 mg, 91%) as a yellow oil, which was used in the next step without purification. The crude amino acid linear precursor (150 mg, 0.17 mmol) was dissolved in CH_3CN (30 mL) and cooled to 0 °C. HATU (160 mg, 0.42 mmol) was added, followed by the dropwise addition of NMM (38 μL, 0.34 mmol). The reaction mixture was stirred at 0 °C for 1 h and then overnight. The reaction mixture was concentrated in vacuo, diluted with EtOAc (15 mL), washed with 10% $KHSO_4$ (10 mL), 5% $NaHCO_3$ (10 mL), and NaCl (10 mL, saturated), dried (Na_2SO_4), filtered, and concentrated. The crude oil was purified by column chromatography (silica gel, EtOAc/Hex 2/1) to yield the protected macrocycle **33** (130 mg, 89%) as a white foam. R_f = 0.23 (AcOEt/Hex 1/2). $[\alpha]_D^{20}$ = −56.1 (c = 1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 0.89−1.05 (m, 18H), 1.45 (m, 9H), 1.62−1.74 (m, 1H), 2.00−2.20 (m, 4H), 2.55 (m, 3H), 2.81 (dd, 1H), 2.99(dd, 1H), 3.15 (dd, 1H**),** 3.36 **(**dd, 1H), 3.53 (m, 1H), 3.67 (m, 1H), 3.73 (m, 1H), 3.80 (s, 3H), 3,91 (m, 1H), 4.10 (m, 1H), 4.19 (m, 1H), 4.42 (td, 1H), 4.48 (m, 1H), 4.51 (dd, 1H), 4.59 (m, 1H), 4,91 (m, 1H), 5.06 (m, 2H), 6.84 (d, 2H), 7.01 (d, 2H), 7.77 (d, 2H), 8.55 (d, 2H). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 17.4, 17.8, 18.7, 20.2, 20.6, 21.4, 23.5, 24.9, 25.1, 26.0, 28.0, 28.1, 28.3, 28.6, 28.8, 30.2, 30.7, 34.1, 38.6, 46.1, 47.0, 48.7, 48.9, 52.4, 55.3, 57.2, 62.9, 63.7, 65.6, 68.7, 69.3, 78.7, 80.5, 114.1, 129.8, 130.3, 155.6, 158.6, 169.1, 170.8, 171.1, 171.2. IR (neat): 3314, 2924, 2359, 1742, 1630 cm^{-1}. HRMS: m/z calcd for $C_{57}H_{79}N_5O_{15}Na$ $(M + Na)^+$ 1096.5470, found 1096.5498.

[*N*-Ser[6]]-Tamandarin B (34). To a solution of the Boc-protected macrocycle **33** (20 mg, 0.024 mmol) in HPLC-grade dioxane (10 mL) was added a solution of HCl in dioxane (10 mL). The resulting solution was stirred at room temperature for 2 h. The solution was concentrated and the residue diluted with CH_2Cl_2 and concentrated again to yield the hydrochloride salt (18 mg, quantitative yield) as a white solid, which was used directly in the next step. To a mixture of the macrocycle amine salt (18 mg, 0.023 mmol) and side chain (11 mg, 0.035 mmol) in CH_2Cl_2 (3 mL) at 0 °C was added BOP (15 mg, 0.035 mmol) and NMM (10 μL, 0.092 mmol). After 30 min at 0 °C, the mixture was stirred at room temperature overnight. The reaction mixture was treated with saturated NaCl solution (2 mL) and extracted with EtOAc (2 × 10 mL). The organic layers were washed with 10% HCl (5 mL), 5% $NaHCO_3$ (5 mL), and NaCl (5 mL, saturated), dried (Na_2SO_4), filtered, and concentrated. The crude oil (24 mg) was purified by HPLC (C18, isocratic, CH_3CN/H_2O 85/15; flow 7 mL/min, 270 nm) to obtain **34** as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.82−0.96 (m, 24H), 1.09−1.28 (m, 14H), 1.39−1.43 (m, 3H), 1.64−1.67 (m, 2H), 1.88−1.98 (m, 2H), 2.10−2.15 (m, 2H), 2.58 (s, 3H), 2.90−2.65 (m, 1H), 3.05 (s, 3H), 3.09−3.12 (m, 1H), 3.35−3.43 (m, 1H), 3.43−3.82 (m, 5H), 3.79 (s, 3H), 3.98−4.05 (m, 1H), 4.12−4.17 (m, 1H), 4.45−4.50 (m, 1H), 4.62−4.66 (m, 1H), 4.67−4.70 (m, 2H), 5.05 (d, J = 5.3 Hz, 1H), 5.29−5.32 (m, 2H), 6.80 (d, J = 8.7 Hz, 2H), 7.02 (d, J = 8.6 Hz, 2H), 7.65 (d, J = 6.7 Hz, 1H), 8.09 (d, J = 9.5 Hz, 1H), 8.45 (d, J = 8.9 Hz, 1H). HRMS: m/z calcd for $C_{52}H_{81}N_7O_{14}Na$ $(M + Na)^+$ 1050.5739, found 1050.5731.

[Ala[4]]-Tamandarin B Macrocycle (42). To a solution of the protected linear precursor (102 mg, 0.09 mmol) in MeOH (88 mL), under argon, was added $Pd(OH)_2$ (44 mg). The reaction mixture was purged with H_2 and stirred overnight under a H_2 atmosphere (1 atm). The mixture was filtered through Celite, and the filtrate was concentrated to yield the free linear precursor (77 mg, 96%) as a yellow oil. The crude amino acid linear precursor (77 mg, 0.91 mmol) was dissolved in CH_3CN (20 mL) and cooled to 0 °C. HATU (815 mg, 0.22 mmol) was added, followed by the dropwise addition of NMM (20 μL, 1.82 mmol). The reaction mixture was stirred at 0 °C for 1 h and then overnight at room temperature. The reaction mixture was concentrated in vacuo, diluted with EtOAc (15 mL), washed with 10% $KHSO_4$ (15 mL), 5% $NaHCO_3$ (15 mL), and NaCl (15 mL, saturated), dried (Na_2SO_4), filtered, and concentrated. The crude oil was purified by column chromatography (silica gel, EtOAc/Hex 2/1) to yield the protected macrocycle **42** (45 mg, 60%) as a white foam. R_f = 0.24 (AcOEt/Hex 1/2). $[\alpha]_D^{20}$ = −33.09 (c = 1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 0.92−1.04 (m, 18H), 1.16 (d, J = 6.8 Hz, 3H), 1.17−1.30 (m, 3H), 1.49 (s, 9H), 1.51−1.61 (m, 1H), 1.73−1.75 (m, 1H), 1.83−1.86 (m, 1H), 1.93−1.97 (m, 1H), 2.30−2.40 (m, 1H), 2.57−2.39 (m, 1H), 3.00−3.05 (m, 1H), 3.08 (s, 3H), 3.19−3.21 (m, 1H), 3.49−3.51 (m, 1H), 3.82 (s, 3H), 3.98−4.00 (m, 1H), 5.01−5.06 (m, 2H), 5.27−5.30 (m, 1H), 5.56−5.66 (m, 1H), 6.67−6.69 (m, 1H), 6.88 (d, J = 7.9 Hz, 2H), 7.00−7.09 (m, 1H), 7.12 (d, 2H). ^{13}C NMR (125 MHz, $CDCl_3$, δ): 15.1, 18.4, 18.9, 20.9, 21.1, 23.2, 24.0, 24.8, 24.9, 25.5, 28.0, 28.1, 30.1, 33.7, 34.4, 39.01, 39.3, 45.4, 47.1, 51.2, 55.3, 69.3, 71.9, 79.5, 80.6, 114.1, 114.2, 129.9, 132.3, 156.1, 169.01, 170.9, 171.5, 172.3, 173.2. HRMS: m/z calcd for $C_{41}H_{65}N_5O_{12}Na$ $(M + Na)^+$ 842.4527, found 842.4545.

[*N*-Ala[4]]-Tamandarin B (43). To a solution of the Boc-protected macrocycle **42** (11 mg, 0.013 mmol) in HPLC-grade dioxane (5 mL) was added a solution of HCl in dioxane (5 mL). The resulting solution was stirred at room temperature for 4 h. The solution was concentrated and the residue diluted with CH_2Cl_2 and concentrated again to yield the hydrochloride salt (quantitative yield) as a white solid, which was used directly in the next step. To a mixture of the macrocycle amine salt (10 mg, 0.013 mmol) and side chain (6.2 mg, 0.019 mmol) in CH_2Cl_2 (2 mL) at 0 °C was added BOP (8.4 mg, 0.019 mmol) and NMM (6 μL, 0.052 mmol). After 30 min at 0 °C, the reaction was stirred at room temperature overnight. The reaction mixture was treated with NaCl solution (5 mL, saturated) and extracted with EtOAc (2 × 10 mL). The organic layers were washed with 10% HCl (5 mL), 5% $NaHCO_3$ (5 mL), and NaCl (5 mL, saturated), dried (Na_2SO_4), filtered, and concentrated. The crude oil (10 mg, 77%) was purified by semipreparative HPLC (C18, isocratic, CH_3CN/H_2O 85/15; flow 7 mL/min, 270 nm) to give **43** as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 0.82−1.46 (m, 32H), 1.46−2.10 (m, 12H), 2.16 (m, 4H), 2.65 (m,

1H), 2.82−3.01 (m, 1H), 3.05 (s, 3H), 3.20 (s, 3H), 3.49−3.55 (m, 2H), 3.63−3.92 (m, 5H), 3.85 (s, 3H), 4.09 (m, 1H), 4.32−4.43 (m, 2H), 4.60−4.65 (m, 1H), 4.75 (t, J = 8.0 Hz, 1H), 5.01 (d, J = 5.3 Hz, 1H), 5.24 (s, 2H), 6.64−6.66 (m, 1H), 6.85 (d, J = 8.7 Hz, 2H), 7.14 (d, J = 8.8 Hz, 2H), 7.22−7.23 (m, 1H). LRMS: *m*/*z* calcd for $C_{51}H_{81}N_7O_{14}$ $(M + H)^+$ 1016.2, found 1016.4.

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Supporting Information Available: Text and figures giving experimental procedures, ^{1}H and ^{13}C NMR spectra for new compounds, and HPLC data for compounds **16**, **17**, and **43**. This material is available free of charge via the Internet at http://pubs.acs.org.

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