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SYNTHESIS OF PEPTIDOMIMETIC ANALOGUES OF ECHINOCANDINS

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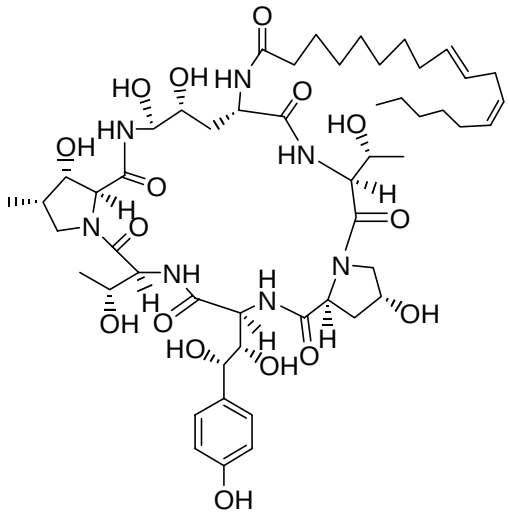
Abstract – Novel peptidomimetic compounds with partial structures being the same as that of echinocandin B, a well known antifungal lipopeptide, have been synthesized. The structures of these compounds were confirmed by NMR and MS. The synthesized compounds were tested for their in vitro antifungal activity. The results suggested that the hydroxyproline-threonine section in the north-western position of the echinocadin is important for the activity.

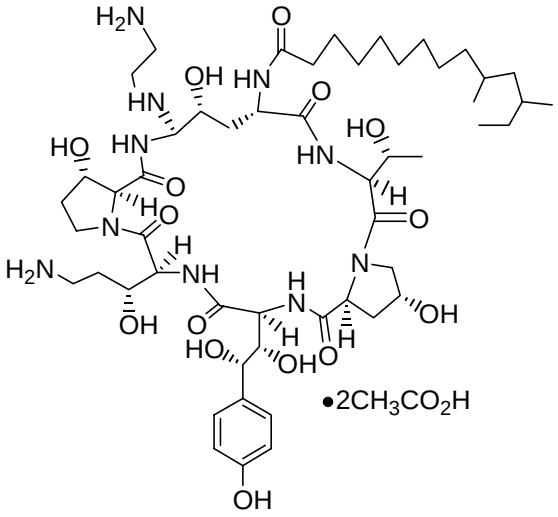
INTRODUCTION

Life-threatening fungal infections have increased dramatically in recent years in immunocompromised patients such as those undergoing cancer chemotherapy, organ transplant, and patients with AIDS. As the conventionally used antifungal drugs, amphotericin B and azoles, have drawbacks with respect to side effects and the development of drug resistance, much attention has been paid to the development of antifungal agents with new mechanism of action in recent years. [1,2] (1, 3)-β-D-glucan synthase is an attractive target for the development of new antifungal agents as it is an essential enzyme for fungal cell wall synthesis. The cell wall is unique to lower eukaryotes, thus inhibitors of this enzyme would be expected to have an excellent therapeutic value. The search for novel inhibitors of glucan synthesis has resulted in the discovery of the lipopepetide series, typified by echinocandin B, and the liposaccharide series, typified by papulacandin. Since the lipopeptide series also demonstrated good in vivo anti-fungal activity, more research has been carried out on the synthesis of echinocandin analogues. Detailed

structure-activity relationships of the echinocandin derivatives have been described in literatures.[3-6] The Merck research group has isolated pneumocandin Bo[7,8] and following several functional group modifications,[6,9] produced L-743872 (MK-0991, caspofungin) (Figure 1) which was the first agent from the echinocandin class approved for the treatment of life-threatening fungal infections.[10,11] However, the price of this drug is still very high.[10] A bioactive analogue with a simple structure and an uncomplicated synthetic route is still needed. For this purpose, we designed and synthesized peptidomimetic compounds, with structural analogy to echinocadins. This article describes the synthesis and anti-fungal evaluation of these peptidomimetic compounds.



Echinocandin B



Caspofungin

Figure 1 Structures of echinocandin B and caspofungin

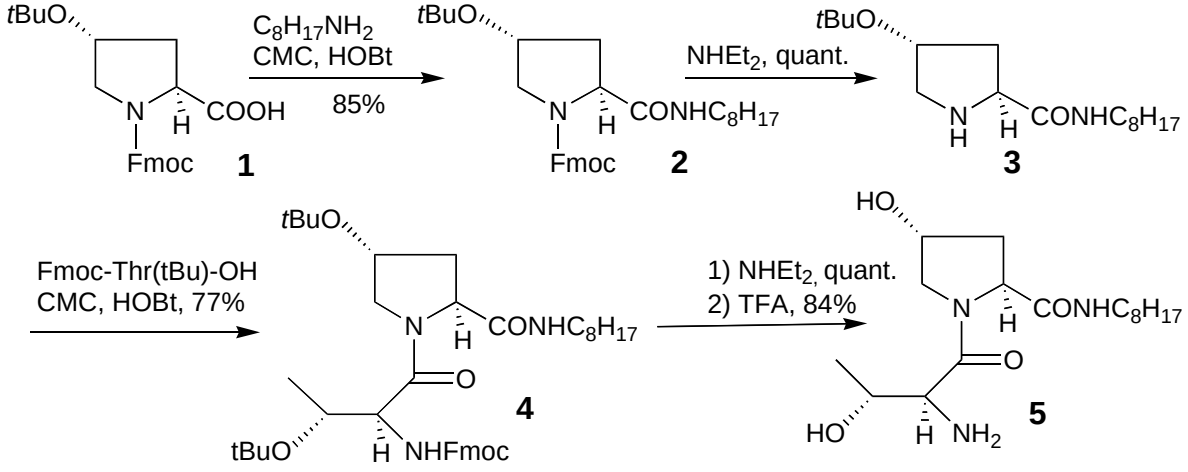
RESULTS AND DISCUSSION

At the beginning, we focused on the hydroxyproline and threonine section, as this motif appears twice, both in the south-eastern and in the north-western sites, in the structures of echinocadins. Thus, we synthesized the dipeptide derivatives (**5** and **11**). Both of them contained a lipophilic side chain in their structures.

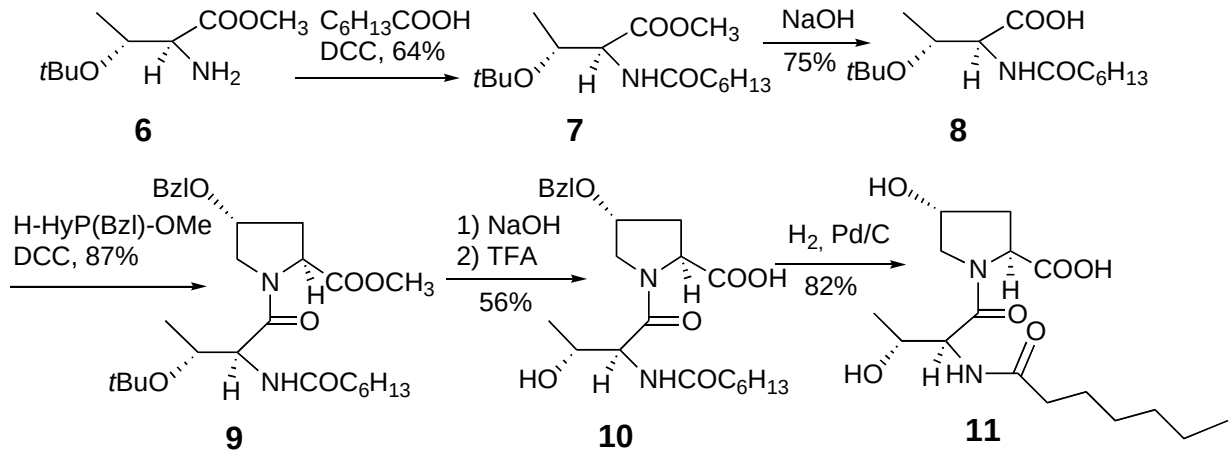
Schemes1 and 2 depict the chemical synthesis routes for compounds (**5** and **11**). Peptide bond formation was achieved by coupling the partially protected amino acids in the presence of either *N, N′*-dicyclohexylcarbodiimide (DCC) or *N*-cyclohexyl-N′-(2-morpholinoethyl)carbodiimide methyl-*p*-toluenesulfonate (CMC) and 1-hydroxybenzotriazole hydrate (HOBt). The side chains were introduced by reaction of the partially protected amino-acid (**1**) with $C_8H_{17}NH_2$ and **6** with $C_6H_{13}COOH$. $NHEt_2$ and TFA were used for the deprotection of Fmoc and *t*-Bu groups, respectively.

Compounds (**5** and **11**) were tested for their antifungal activity. No inhibitory activity was observed at a concentration as high as 64 μM in either of these compounds; suggesting that other structural parts of

echinocadin are also necessary for the activity. Thus, compound (**20**), a tripeptide with a linker to form a ring having the same size as the ring of echinocadin was synthesized.



Scheme 1 Synthesis of compound (**5**)

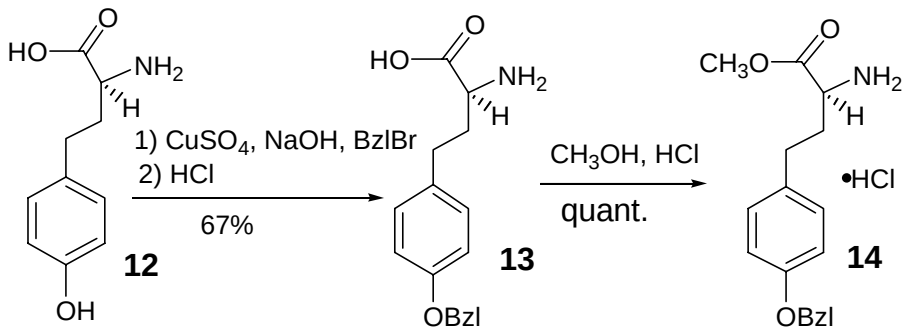


Scheme 2 Synthesis of compound (**11**)

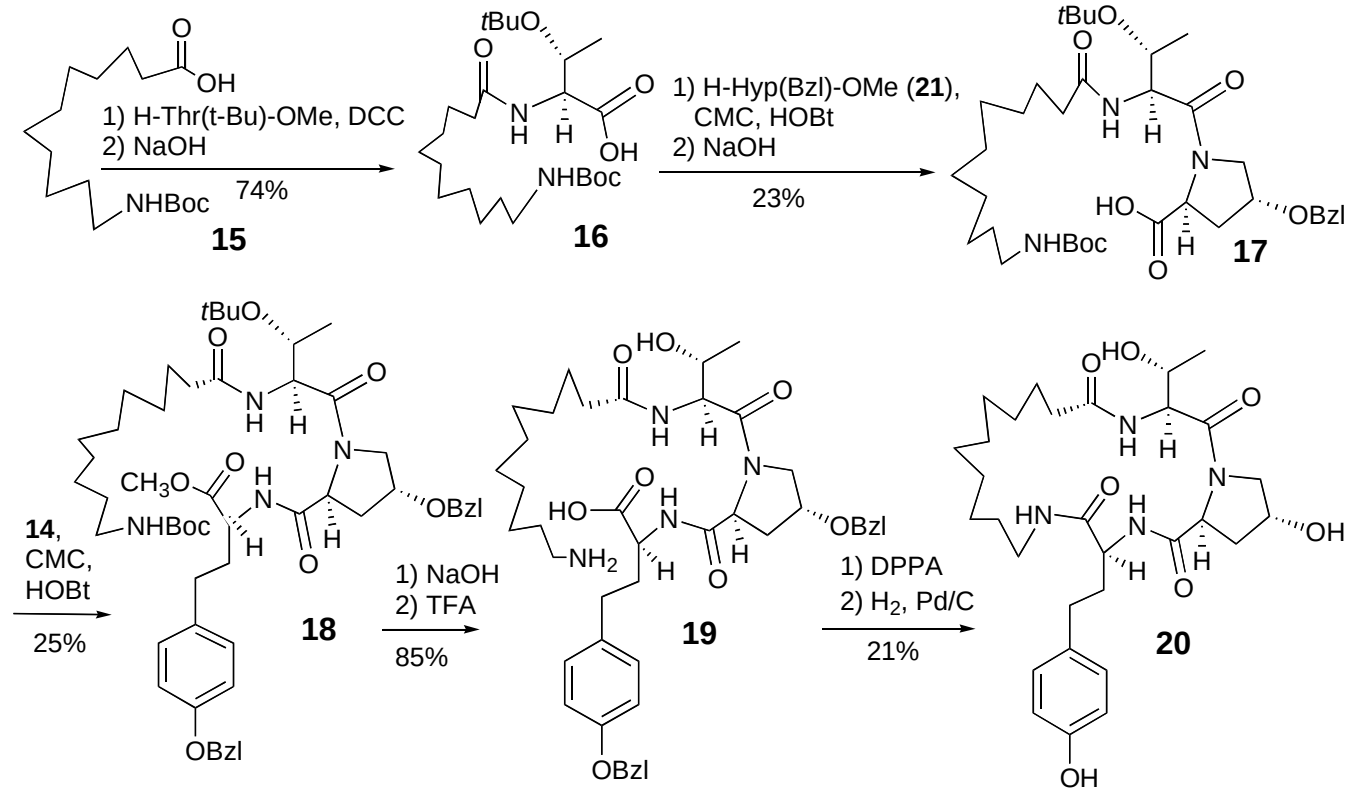
One of the building blocks, H-homotyr(Bzl)-OMe (**14**), was not commercially available and thus prepared from homotyrosine as shown in Scheme 3. The carboxyl and amino groups of homotyrosine (**12**) were protected with a cupric complex. This complex was then reacted with benzyl bromide to form the *O*-benzylated compound which was treated with HCl to give **13**. Methylation of compound (**13**) yielded **14**.

As shown in Scheme 4, compound (**20**) was synthesized by using 11-(Boc-amino)undecanoic acid (**15**) as the starting material. Methyl esters of amino acids (H-Thr(*t*-Bu)-OMe, H-Hyp(Bzl)-OMe and **14**) were then used for the elongation of the peptidomimetic compound in the presence of either DCC or CMC and HOBt. The side chains of these amino acids were protected with either a *t*-Bu or Bzl group, which allowed the saponification of the ester bond without affecting the side chain moieties. The acyclic tripeptide (**18**) was deprotected to give the compound (**19**) with free amino and carboxylic acid groups.

Cyclization was accomplished with diphenylphosphoryl azide (DPPA) and sodium bicarbonate in DMF.



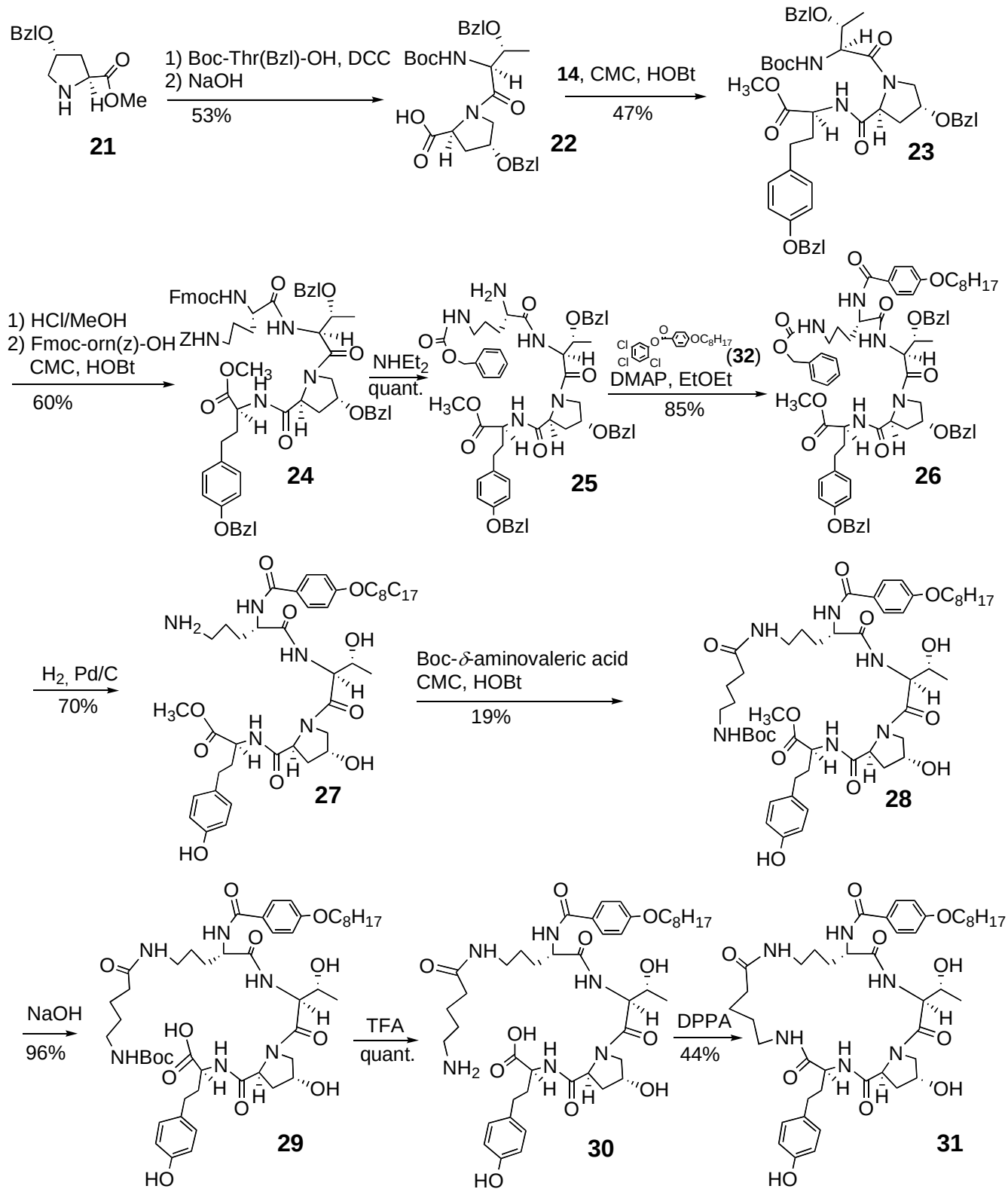
Scheme 3 Synthesis of H-Hty(Bzl)-OMe.HCl



Scheme 4 Synthesis of compound (**20**)

The tripeptide compound (**20**) and the acyclic compound (**19**) did not show antifungal activity in spite of possessing a homotyrosine group in their structures. These results confirmed the reported structure-activity relationships for echinocadins, that both the L-homotyrosine and the fatty acid side chain are essential for the anti-fungal activity.[3,4] Next, we synthesized compound (**31**), whose structure possessed both the above mentioned essential components and had the same ring size as echinocadin.

Scheme 5 illustrates the synthesis of compound (**31**). Peptide elongation was carried out by coupling the partially protected amino acids in the presence of either DCC or CMC and HOBt. Linking of the side chain was difficult. Several attempts by reacting *p*-octyloxybenzoic acid with **25** in the presence of DCC or CMC and HOBt failed. Compound (**26**) was finally synthesized by reacting **25** with an active ester,



Scheme 5 Synthesis of compounds (**26-31**)

p-octyloxybenzoic acid trichlorophenly ester (**32**), which was synthesized from *p*-octyloxybenzoic acid and 2,4,5-trichlorophenol in the presence of DCC.[12] Hydrogenation of **26** resulted in **27**. Compound (**27**) was allowed to react with Boc-δ-aminovaleric acid in the presence of CMC and HOBt to obtain **28**. Complete deprotection of **28** yielded compound (**30**) which was treated with DPPA and

sodium bicarbonate in DMF to yield the cyclic compound (**31**).

The cyclic compound (**31**) as well as the acyclic analogues (**26-30**) did not show antifungal activity. These results suggested that the other two amino acids in the north-western part of echinocadin, hydroxylproline and threonine, are also important for the antifungal activity of these lipopeptides. It has been reported that the homotyrosine and the lipophilic side chain are two essential groups for the activity in echinocandin series.[3,4] Zambias et al. also proposed that the orientation of homotyrosine ring with respect to the lipopholic side chain may be the determining factor for the antifungal activity of echinocandins.[3] The present finding suggests that, through hydrogen bonds and other interactions, the two amino acids in the north-western part are possibly responsible for maintaining a suitable orientation of the two essential groups. The lack of activity of all these compounds may also suggest that the interactions between the hydroxylproline-threonine of the north-western part of echinocadin and the enzyme are essential. It is also possible that these two amino acids confer a suitable physicochemical property to these compounds and, as a result, facilitate their penetration into the fungal cell wall.

Considering the above hypothesis, we designed a new series of compounds based on the relative steric orientations of the phenolic ring and the lipopholic side chain. The synthesis and anti-fungal evaluation of these new compounds are now being undertaken. Promising antifungal activity was observed in some of the initial compounds in this series. These results will be published later.

EXPERIMENTAL[13]

General Method

NMR spectra were obtained on a Bruker AVANCE 500MHz or a JEOL JNM-AL 300 NMR spectrometer with TMS as internal standards. APCI and ESI MS were measured on an Agilent 1100 series LC/MSD system. HRFAB-MS was measured with a JMS-700 apparatus with a resolution of 5000 and with *m*-nitrobenzyl alcohol as the matrix. Silica gel and octadecyl-functionalized silica gel (ODS) used in column chromatography was obtained from Sigma-Aldrich. Organic solvents for chromatography were obtained from Fisher Scientific. All the protected amino-acids used were of L-configuration. H-Hty-OH.HBr was purchased from Watanabe Chemical Industrial Corporation, Japan. Other chemicals were obtained from Sigma-Aldrich or Bachem companies.

Chemical Synthesis of Compound (5)

To a DMF (20 ml) solution of Fmoc-Hyp(*t*-Bu)-OH (**1**) (0.55 g, 1.34 mmol), CMC (0.72 g, 1.70 mmol) and HOBT (0.28 g, 2.07 mmol) was added octylamine (0.145 g, 1.12mmol) dropwise. The mixture was stirred at rt for 15 h. Water was added to the mixture and extracted with EtOAc. The EtOAc extract was choromatographed on a SiO_2 column, eluted with hexane:EtOAc (6:4) to give compound (**2**) (500 mg,

0.96 mmol, 85.7%). Compound (**2**) was treated with 1.6 ml of $NHEt_2$ in 20 ml DMF for 2h at rt. The mixture was partitioned with EtOAc and H_2O. The EtOAc layer was concentrated to dryness to give crude **3**. To a DMF (20 ml) solution of crude **3** was added Fmoc-Thr(*t*-Bu)-OH (0.47 g, 1.18 mmol), HOBT (0.215 g, 1.59 mmol) and CMC (0.69 g, 1.63 mmol). The mixture was stirred at rt overnight, and partitioned with EtOAc and H_2O. The EtOAc layer was chromatographed on a SiO_2 column and eluted with hexane:EtOAc (7:3) to obtain **4** (500 mg, 0.74 mmol, 76.8%). Compound (**4**) was treated with $NHEt_2$ in DMF in the same manner as above, concentrated and further treated with 90 % TFA (10 ml) at rt for 30 min, purified on an ODS column eluted with 40% MeOH to yield compound (**5**) (210 mg, 0.611 mmol, overall yield 54.6% from octylamine). ^{1}H NMR (DMSO-d_6): 0.87 (3H, t, *J*=7.0 Hz, CH_3), 1.24 (13H, br., 5 x CH_2+Thr-CH_3), 1.37 (2H, br., CH_2), 1.80 (1H, m, Hyp-3a), 2.07 (1H, m, Hyp-3b), 3.00 (1H, m, Hyp-5), 3.60 (2 H, br., NCH_2), 3.81 (1 H, m, Thr- Hβ), 3.90 (1 H, br., Thr-Hα), 4.38 (1H, br., Hyp-4), 4.40 (1H, t., J=8.0 Hz, Hyp-2); Positive FAB-MS m/z 344.3 ($[M+H]^+$, 100%); Positive HR-FAB-MS m/z 344.2552 ($[M+H]^+$, $C_{17}H_{34}N_3O_4^+$, Calc. 344.2549).

Synthesis of compound (11)

H-Thr(*t*-Bu)-OMe. HCl (**6**, 3.4 g, 15.1 mmol) and heptanoic acid (2.10 g, 16.1 mmol) were reacted in the presence of DCC (3.4 g, 16.5 mmol) at rt overnight. The filtrate was purified with a SiO_2 column eluted with CH_2Cl_2 to get 2.9 g (9.62 mmol) of **7**. Compound (**7**) (2.9 g) was saponified with 1 N NaOH (20 ml) in 30 ml of MeOH at rt for 2h. The mixture was neutralized with 1N HCl to pH 5-6 then extracted with EtOAc. The EtOAc extract was concentrated to dryness to get **8** (2.06 g, 7.17 mmol, 47.5% from H-Thr(*t*-Bu)-OMe. HCl). Compound (**9**) (290 mg, 0.57 mmol, 86.9% yield from **8**) was synthesized from **8** (190 mg, 0.661 mmol) and H-HyP(Bzl)-OMe.HCl (230 mg, 0.85 mmol) by using the same method as above. Compound (**9**) (180 mg, 0.36 mmol) was saponified with NaOH and worked up as above. The product was treated with 90% TFA at rt for 30 min and concentrated to dryness to obtain **10** (88 mg). Hydrogenation was carried out as following: To 10 ml EtOH solution of **10** (83 mg, 0.191 mmol) was added 15 mg of Pd/C. The mixture was vigorously stirred under a hydrogen balloon for 15 h. The mixture was filtered and the filtrate was concentrated. The residue was chromatographed on a SiO_2 column eluted with $CHCl_3$-MeOH (8:2) to obtain compound (**11**) (overall yield: 18.6 % from H-Thr(*t*-Bu)-OMe. HCl). ^{1}H NMR ($CDCl_3$+CD_3OD 8:1): 0.92 (3H, t, *J*=7.0 Hz, CH_3), 1.20 (3H, d, *J*=6.0 Hz CH_3), 1.36 (6H, br., CH_2), 1.67 (2H, m, CH_2), 2.09 (1H, m, Hyp-3a), 2.30 (2 H, t, J=7.0 Hz, CH_2), 2.42 (1H, m, Hyp-3b), 3.77 (1 H, dd, J=3.0, 12.0 Hz, Hyp-5a), 4.00 (br., overlapped with H_2O of CD_3OD, Hyp-5b), 4.20 (1.4 H, m., Thr-Hα), 4.58 (1H, br., Hyp-H_4), 4.63 (2H, m. Hyp-H_2 + Thr-Hβ); Positive FAB-MS m/z 345.3 ($[M+H]^+$, 40%); Positive HR-FAB-MS m/z 345.2039 ($[M+H]^+$, $C_{16}H_{29}N_2O_6^+$, Calc. 345.2026).

Synthesis of compound (20)

H-Hty-OH.HBr (**12**) (2.04g, 7.38 mmol) was dissolved in 2 N NaOH (15 ml) and a solution of cupric sulfate (pentahydrate, 1.25 g, 5 mmol) in H_2O (5 ml) was added. The mixture was heated to 60 °C then cooled to rt, and diluted with methanol (35 ml). To the mixture, 2N NaOH (1.5 ml) was added followed by the addition of benzyl bromide (1.75 g, 10mmol). The mixture was vigorously stirred at rt for about 1.5 h. The purple-blue precipitate was collected on a filter, washed with a mixture of methanol (5 ml) and H_2O (17.5 ml), then with methanol alone (2.5 ml) and air-dried. The powder was suspended in 2 N HCl (5 ml), sonicated and filtered, the precipitate was washed with 2N HCl (2 x 5 ml) and H_2O (2 x 5ml) to obtain H-Hty(Bzl)-OH (**13**) as a white powder (1.4 g, 4.91 mmol, 66.5 %).[14] Compound (**13**) (1.4 g, 4.91 mmol) was refluxed with 20 ml of dried MeOH and 14 ml of 4.0 M HCl dioxane for 3h and then concentrated to dryness to obtain H-Hty(Bzl)-OMe.HCl (**14**) (Quant.). To a CH_2Cl_2 (10 ml) solution of H-Thr(*t*-Bu)-OMe.Cl (451 mg, 2 mmol) and 11-(Boc-amino)undecanoic acid (**15**) (602 mg, 2mmol) was added DCC (412 mg, 2 mmol). The mixture was stirred at rt for 4h and the filtrate was chromatographed on a SiO_2 column and eluted with CH_2Cl_2-MeOH (96:4) to obtain a residue (0.7 g, 73.8 %). The residue was saponified and worked up as usual to obtain **16** (quant.). H-Hyp(Bzl)-OMe.HCl (**21**) (544 mg, 2.0 mmol) in H_2O (2 ml) was treated with Na_2CO_3 (305 mg, 2.88 mmol) and the mixture was extracted with ether, dried over Na_2SO_4 and concentrated to dryness to get the free amine form. This was dissolved in DMF (10 ml) followed by the addition of **16** (916 mg, 2 mmol), CMC (2 mmol) and HOBT (2 mmol). The reaction mixture was stirred at rt overnight and chromatographed on an ODS column to obtain a residue which was saponified and worked up as usual to obtain **17**. To a solution of H-Hty(Bzl)-OMe (**14**) (153 mg, 0.511 mmol) and **17** (285 mg, 0.43 mmol) in DMF was added CMC(212 mg, 0.5 mmol) and HOBT (68 mg, 0.5 mmol). The reaction mixture was stirred at rt overnight, passed through an ODS column and eluted with 90-100% MeOH to obtain (**18**) (100 mg, 0.106 mmol, 24.7% yield from **17**). Compound (**18**) (100 mg) was deprotected with NaOH then TFA as usual to get an acyclic compound (**19**). Compound (**19**) was dissolved in anhydrous DMF (30 ml) and cooled to 0 °C. To the solution was added diphenylphosphoryl azide (DPPA, 25 μl) dropwise followed by the addition of $NaHCO_3$ (36 mg). The reaction mixture was stirred at 0 °C under nitrogen for 48h, concentrated to dryness and purified on a SiO_2 column. The major fraction eluted with $CHCl_3$-MeOH (95:5) was hydrogenated with a hydrogen balloon and Pd/C followed by purification on a SiO_2 column using $CHCl_3$-MeOH (8:2) to obtain compound (**20**) (11 mg, 18.0 % yield from **18** and 1.00 % yield from H-Hyp(Bzl)-OMe.HCl); ^{1}H NMR ($CDCl_3$+CD_3OD 19:1): 1.26-1.39 (15H, m, undec-6xCH_2+Thr-CH_3), 1.56 (2H, m, undec-CH_2), 1.63, 1.80 (1H each, m, undec-CH_2), 1.68 (2H, m, homo-β-2H), 1.91 (1H, m, Hyp -3-Ha), 2.37 (3H, m, Hyp -3-Hb+ undec-CH_2), 2.54 (1H, m, homo-γ-1H), 2.65(1H, m, homo-γ-1H), 3.15 , 3.26 (1H each, m, undec-CH_2), 3.49 (1H, d, J=10.0 Hz, Hyp -5-Ha), 3.80 (1H, d, J=10.0 Hz, Hyp -5-Hb), 4.15 (2H, m, Thr-α+βH), 4.37

(1H, s, Hyp -4-H), 4.56 (1H, t, J=8.5 Hz, Hyp -2-H), 4.78 (1H, br., homo-α-H), 6.72 (2H, d, J=8.0 Hz, homo-aromatic), 7.00 (2H, d, J=8.0 Hz, homo-aromatic), some small signals between 6.8-7.5 ppm (amide NH). Positive APCI-MS: 575.3 $[M+H]^+$ (100%). Positive FAB-ES m/z 575.3 ($[M+H]^+$, 7%); Positive HR-FAB-MS m/z 575.3442 ($[M+H]^+$, $C_{30}H_{47}N_4O_7^+$, Calc. 575.3445).

Synthesis of compounds (26-31)

The free amine form of **21** was prepared according to the procedure described above, and was dissolved in CH_2Cl_2 (40 ml). To this solution, Boc-Thr(Bzl)-OH (5.56 g, 18 mmol) and DCC (4.12 g, 20 mmol) were added. The reaction mixture was stirred at rt overnight, purified on an ODS column. The 80 % MeOH eluted part was saponified with NaOH and worked up as usual to get **22** in 53.2 % yield. H-Hty(Bzl)-OMe.Cl (**14**) (prepared as described in scheme 3, 2.7 g, 8.01 mmol) and **22** (4.95 g, 9 mmol) were dissolved in 400 ml DMF. To this solution, CMC (3.81 g, 9 mmol) and HOBt (1.22 g, 9 mmol) were added. The reaction mixture was stirred at rt for 18 h, then partitioned with EtOAc/H_2O. The EtOAc layer was chromatographed on an ODS column and eluted with 90% MeOH to obtain **23** (3.0 g, 3.78 mmol, 47.2 % yield from H-Hty(Bzl)-OMe). Compound (**23**) was refluxed in 21 ml of 4 N HCl in dioxane and 30 ml of dry MeOH for 2 h, then concentrated to dryness. Part of the residue (2.5 g, 3.6 mmol) was dissolved in DMF (40 ml). To this solution at 0 °C, was added Fmoc-Orn(Z)-OH (1.76 g, 3.6 mmol), CMC (1.7 g, 4 mmol) and HOBT (0.54 g, 4 mmol), followed immediately by the addition of $NaHCO_3$ (1.5 g, 18 mmol). The mixture was stirred overnight at rt. H_2O (50 ml) was added to the mixture at 0 °C and it was extracted with AcOEt. The AcOEt layer was chromatographed on an ODS column and eluted with MeOH-H_2O to obtain **24** (2.5 g, 2.15 mmol, 59.7 % yield from **23**) from the 95% eluted part. To a solution of 4-(octyloxy)benzoic acid (6.9 g) in Et_2O (220 ml) was added 2,4,5-trichlorophenol (5.5 g) and DCC (5.7 g). The reaction mixture was stirred at rt overnight and filtered. The filtrate was applied on SiO_2 column and eluted with hexane-EtOAc (9:1) to obtain **32** (9.7 g, 81.9 %) as a white powder. Compound (**24**) (1.4 g, 1.20 mmol) was treated with 1.5 ml of $NHEt_2$ in 15 ml of DMF at rt for 2h. The reaction mixture was concentrated to dryness. The residue was dissolved in 40 ml of DMF then **32**(1.4g, 3.99 mmol) and 4-dimethyaminopyridine (0.15 g) were added to this solution. The mixture was stirred at rt for 15 h under nitrogen then at 60 °C for 2h. Additional 4-dimethyaminopyridine (0.1 g) was added and the mixture was stirred at rt for 20h, concentrated to dryness and passed through a SiO_2 column eluted with hexane-EtOAc (1:1) to obtain **26** (1.2 g, 1.02 mmol, 85.0 % yield from **24**). Positive FAB-MS m/z 1174.5 ($[M+H]^+$, 1%); Positive HR-FAB-MS m/z 1174.6155 ($[M+H]^+$, $C_{69}H_{84}N_5O_{12}^+$, Calc. 1174.6116). Compound (**26**) (1.1 g, 0.938 mmol) was hydrogenated under a balloon of hydrogen for 4 days with 10% Pd/C (0.9 g) in MeOH-THF at rt. The mixture was filtered and the filtrate was concentrated to dryness to get **27** as a pale yellow powder (503 mg, 0.654 mmol, 69.7 % yield from **26**). Positive API-MS $[M+H]^+$:

770.3 (100 %). To a DMF (6 ml) solution of **27** (390 mg, 0.507 mmol) and 5-*tert*-butoxycarbonylamino-pentanoic acid (130 mg, 0.60 mmol) was added CMC (297 mg, 0.7 mmol) and HOBT (95 mg, 0.7 mmol). The reaction mixture was stirred at rt overnight. A spoonful of molecular sieves (3Å) was added and the mixture was stirred for an additional overnight. After being filtered and concentrated to dryness, the mixture was chromatographed on an ODS column and eluted with MeOH-H_2O (1:1-1:0) to obtain **28** (95 mg, 0.0980 mmol, 19.3% yield from **27**). Positive APCI-MS: 969.4 ($[M+H]^+$, 15%). Compound (**28**) (90 mg, 0.0929 mmol) was saponified and worked up as usual to obtain a white powder (**29**, 85 mg, 0.0890 mmol, 95.8% yield from **28**). Positive FAB-MS m/z 977 ($[M+Na]^+$, 6%); Negative FAB-MS m/z 953 ($[M-H]^-$, 100%); Negative HR-FAB-MS m/z 953.5226 ($[M-H]^-$, $C_{49}H_{73}N_6O_{13}^-$, Calc. 953.5236). Compound (**29**) (82 mg, 0.0859 mmol) was treated with 90 % TFA at rt for 30 min. concentrated to dryness to obtain **30**. Positive FAB-MS: m/z 855.5 ($[M+Na]^+$, 7%); Negative FAB-MS: m/z 853.4 ($[M-H]^-$, 8%); Negative HR-FAB-MS m/z 853.4696 ($[M-H]^-$, $C_{44}H_{65}N_6O_{11}$, Calc. 853.4711). To a solution of **30** (70 mg, 0.0819 mmol) in DMF (30 ml) at 0 °C under N_2 was added DPPA (25 μl), followed immediately by the addition of $NaHCO_3$ (36.4 mg) in one portion. The mixture was stirred at 0 °C to rt for 48 h. To the mixture, 5 ml of H_2O was added. The mixture was concentrated to dryness and chromatographed on a SiO_2 column eluted with $CHCl_3$-MeOH 100:0-80:20 to obtain compound (**31**) as a white powder (30 mg, 0.0358 mmol, 43.8 % yield from **30** and 0.719% overall yield from H-Hyp(Bzl)-OMe). ^{1}H NMR ($CDCl_3$+CD_3OD 19:1): 0.89 (3H, t, *J*=6.5 Hz, side-chain-CH_3), 1.28 (11H, m, side-chain- 4xCH_2 + Thr-CH_3), 1.45 (4H, m, side-chain-CH_2+ornCH_2), 1.63 (4H, m, orn-β-2H+ penta -γ-2H), 1.80 (6H, m, side-chain-2H + penta-β-2H + Homo-β- 2H), 2.25 (3H, m, penta-α-2H+ Hyp -3-Ha), 2.35 (1H, dd, *J*=8.0, 13.0 Hz, Hyp -3-Hb), 2.54 (1H, m, Homo-γ-1H), 2.71 (1H, overlapped with solvent, homo-γ-1H), 2.90 (1H, m, penta-δ-2H), 3.07 (1H, dd, *J*=5.7, 12.7 Hz, Orn-δ-Ha), 3.37 (1H, m, Orn-δ-Hb), 3.49 (1H, d, *J*=10.0 Hz, Hyp -5-Ha), 3.74 (1H, d, *J*=10.0 Hz, Hyp -5-Hb), 3.99 (2H, t, *J*=6.5 Hz, side-chain-2H), 4.15 (2H, m, Thr-α+β-2H), 4.37 (1H, s, Hyp -4-H), 4.56 (1H, t, *J*=8.5 Hz, Hyp -2-H), 4.78 (1H, s, Homo-α-H), 5.22 (1H, s, Orn-α-H), 6.72 (2H, d, *J*=8.0 Hz, Homo-aromatic), 6.91 (2H, d, *J*=8.0 Hz, side-chain-aromatic), 7.00 (2H, d, *J*=8.0 Hz, Homo-aromatic), 7.80 (2H, d, *J*=8.0 Hz, side-chain-aromatic), some small signals between 6.6-9.6 ppm (amide NH); Positive API MS: 859 ($[M+Na]^+$, 100%). Positive HR-FAB-MS: 837.4778 ($[M+H]^+$, $C_{44}H_{65}N_6O_{10}^+$; Calc. 837.4762).

Biological Activity

The antifungal activity of compounds (**5**, **11**, **19**, **20** and **26**-**31**) was assessed against the following strains: *Candida albicans* ATCC 90028, *Candida krusei* ATCC 6258, *Aspergillus flavus* IFM 41935, *Aspergillus fumigatus* IFM 40808, *Trichophyton mentagrophytes* IFM 40769, *Trichophyton Rubrum* IFO 6204. Cytotoxicity was evaluated in Hela and CEM cells.

None of the compounds showed antifungal activity against any of the strains tested, up to concentration of 64 μg/ml or to the concentrations of their saturated solutions. Furthermore, none of these compounds showed cytotoxicity at the same concentrations.

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