J. Med. Chem. **1998,** *41,* 996−1000

Notes

Novel Biologically Active Nonpeptidic Inhibitors of MyristoylCoA:Protein *N*-Myristoyltransferase

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Received December 29, 1997

A new class of biologically active nonpeptidic inhibitors of *Candida albicans* NMT has been synthesized starting from the octapeptide ALYASKLS-NH_2 (**2**). The synthetic strategy entailed the preparation of novel protected Ser-Lys mimics **9** and **12** from (*S*)- or (*R*)-3-iodotyrosine and then grafting key enzyme recognition elements in a stepwise manner. Like **2**, compounds **16**, **17**, and **18** are competitive *Candida* NMT inhibitors that bind to the peptide recognition site of the enzyme. Moreover, **16**−**18** have an affinity comparable to that of **2** even though they are devoid of peptide bonds. In contrast to **2**, these nonpeptidic inhibitors exhibit antifungal activity.

Introduction

The design and synthesis of biologically active nonpeptidic inhibitors of disease targets have been a significant challenge in the discovery of novel therapeutic agents. Nonetheless, rapid progress has been made over the last decade as potent nonpeptidic agents have been identified for increasingly complex systems including antagonists of the RGD-based fibrinogen receptor,[1] as well as HIV-I protease inhibitors.[2] For the past few years we have been interested in finding novel antifungal agents with a unique mode of action. The enzyme myristoylCoA:protein *N*-myristoyltransferase (NMT; EC 2.1.3.97) is an attractive target for the generation of such agents.[3] NMT catalyzes the cotranslational transfer of myristate (C14:0) from myristoylCoA to the *N*-terminal glycine residue of several eukaryotic proteins, and is present in a variety of pathogenic fungi.[3] Genetic studies have demonstrated that *C. albicans* and *C. neoformans* require NMT for their viability.[4,5] Although the atomic structure of NMT is not yet known, several potent and selective peptidomimetic inhibitors have been developed by exploiting the differences in peptide substrate specificities between fungal and human NMTs.[6,7] This report describes the design and synthesis of the first biologically active inhibitors of fungal NMTs where the key enzyme recognition elements are tethered to novel scaffolds devoid of peptide bonds.

The synthetic design emanated from the previously described high-affinity octapeptide substrate GLYASKLS-NH_2 **1** (Figure 1). Earlier studies have revealed that the primary amino group of glycine, the hydroxyl group of serine-5, and ϵ-amino group of lysine are the critical

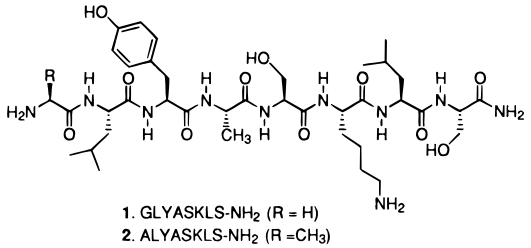

Figure 1. Octapeptide substrate (**1**) and inhibitor (**2**) of *Candida albicans* NMT.

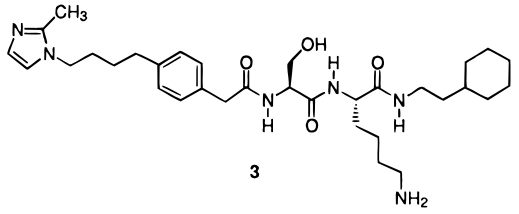

Figure 2. Dipeptide inhibitor of *Candida albicans* NMT.

enzyme recognition elements responsible for imparting high affinity to **1**.[8] Substitution of glycine in **1** with alanine provided the ALYASKLS-NH_2 inhibitor **2** ($K_{i(app)}$ = 15.3 ± 6.4 μM), which did not exhibit antifungal activity even at concentrations of >100 μM. Subsequent replacement of the first four amino acids in **2** with a *p*-[(2-methyl-1-imidazol-1-yl)butyl]phenylacetyl group and substituting the C-terminal Leu-Ser dipeptide fragment with a 2-cyclohexylethyl amide moiety provided the potent dipeptide NMT inhibitor **3** ($K_{i(app)}$ = 0.031 ± 0.003 μM, Figure 2). Despite its excellent potency, no antifungal activity was detected against logarithmically growing cultures of *C. albicans* (EC_{50} > 100 μM).[6]

The lack of antifungal activity for **3** may be partially attributed to the remaining Ser-Lys dipeptide bond

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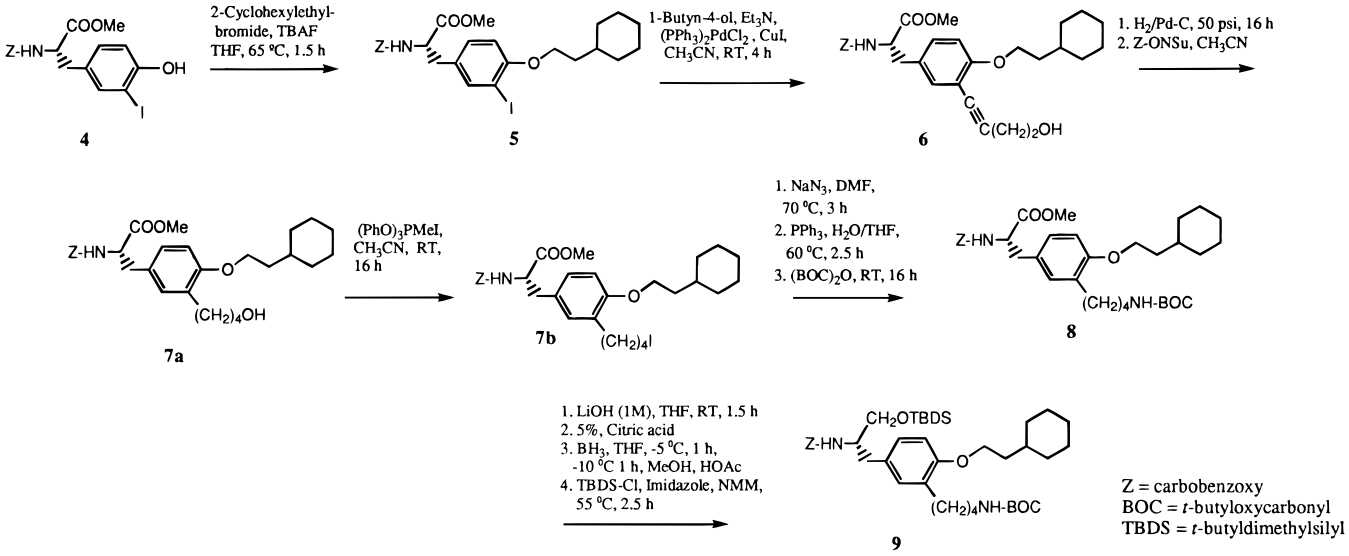
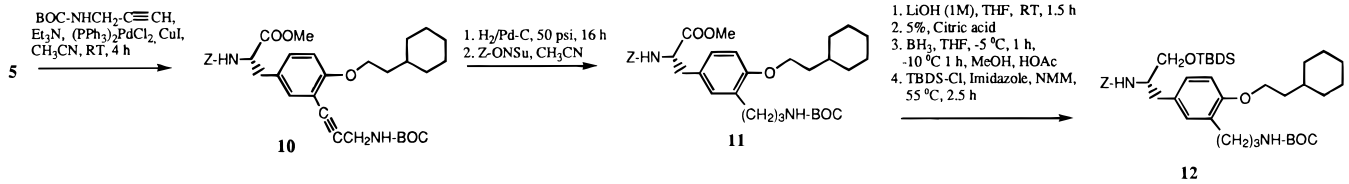
S0022-2623(98)00001-6 CCC: $15.00
Published on Web 02/18/1998

Scheme 1. Synthesis of Ser-Lys Dipeptide Scaffold **9**



Scheme 2. Synthesis of Ser-Lys Dipeptide Scaffold **12**



which could be cleaved in vitro. Consequently, we sought to attenuate the peptide character of **3** in order to improve its metabolic stability as well as its absorption and intracellular distribution characteristics. This rationale led to the design and synthesis of the novel Ser-Lys dipeptide scaffolds **9** and **12**, embodying the key enzyme recognition elements in a core structure derived from tyrosine.

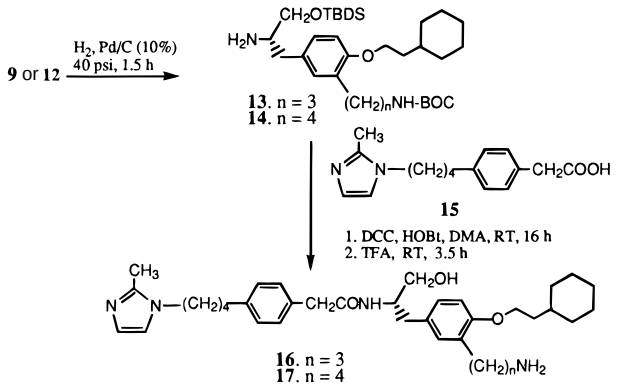
Chemistry

The synthesis of **9** and **12** began from commercially available L-3-iodotyrosine as shown in Scheme 1. First, alkylation of the phenol in the Z-protected L-tyrosine derivative **4** with 2-cyclohexylethyl bromide afforded the cyclohexylethyl ether **5**. Palladium-catalyzed coupling of **5** with 1-butyn-4-ol, in the presence of triethylamine and CuI in acetonitrile, gave the corresponding alkyne product **6**. After the reduction of the triple bond by catalytic hydrogenation, the Z-group was reintroduced to give **7a**, which was then subjected to iodination using methyltriphenoxyphosphonium iodide to afford **7b**. Reaction of **7b** with sodium azide followed by reduction with triphenylphosphine in THF/H_2O, and subsequent protection of the resulting primary amine with $(BOC)_2O$ yielded **8**. The ester group in **8** was subjected to methanolysis using 1 M LiOH. After acidification, the resulting acid was reduced to the primary alcohol and converted to the silylated derivative **9**.

Analogue **12**, having the aminopropyl chain, was also synthesized starting from **5** according to Scheme 2. The iodotyrosine derivative **5** underwent smooth palladium catalyzed insertion of BOC-protected propargylamine in the presence of CuI and triethylamine to provide the propargyl derivative **10**. Catalytic hydrogenation of **10** followed by carbobenzoxylation gave **11**. Subsequent saponification of the methyl ester group in **11** followed by diborane reduction of the corresponding carboxylic acid, and silylation of the resulting primary alcohol yielded **12**.

Amines **13** and **14** generated by the hydrogenation of **9** and **12** (Scheme 3), were individually condensed with the imidazole-substituted phenylacetic acid **15**[9] using DCC/HOBt. The crude reaction products were treated with trifluoroacetic acid, and the final compounds **16** and **17** were isolated as trifluoroacetate salts by reverse-phase HPLC.[9]

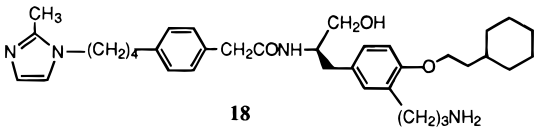
Scheme 3. Syntheses of Nonpeptidic NMT Inhibitors



The synthesis of the *R* enantiomer **18** (Figure 3) was also achieved following the same methodologies as outlined in Schemes 2 and 3, commencing from the corresponding *R* isomer of **5**.

Results and Discussion

All seven peptide bonds and seven of the eight chiral center have been removed from the starting octapeptide



18

Figure 3. Nonpeptidic inhibitor of *Candida albicans* NMT.

inhibitor, **2**. The first four residues (ALYA) were replaced by a *p*-[(2-methylimidazol-1-yl)butylphenyl]-acetyl group in which the imidazole moiety represented the key N-terminal amino recognition element at alanine. The C-terminal tetrapeptide SKLS was replaced by a new chiral tyrosinol scaffold that retains the serine alcohol recognition element and presents the lysine amine moiety in the form of an attached 3-aminobutyl side chain.[8] The hydrophobic interaction[3] fulfilled by leucine-7 in **2** was represented by the 4-cyclohexylethyl ether. This design assumed that these nonpeptidic analogues would have a similar binding mode and orientation to that of **2**.

The nonpeptidic analogues **16**, **17**, and **18** were evaluated for inhibition of purified *C. albicans* and human NMTs (Table 1). Like **2**, the *S* and *R* isomers **16** and **18**, are competitive *Candida* NMT inhibitors with respect to the peptide substrate GNAASARR-NH_2. Compounds **16** and **18** have K_is of 20 and 9 μM, respectively. Remarkably, these values are comparable to that observed for **2** (15 μM). The modest 2−4-fold selectivity[3] of **2** for the fungal versus the human enzyme was maintained by **16**. These results indicate that none of the amide bonds in **2** are necessary to generate an inhibitor directed against the NMT peptide recognition site.

The homologue **17** with an aminobutyl side chain is also an inhibitor with a K_i of 9 ± 1 μM. It is evident from these results that there is no clear chirality preference within this nonpeptidic series. This differs from previous results with either dipeptide[6] or tripeptide[3,6] inhibitors. The findings reported here suggest that it may be possible to develop potent inhibitors of NMT that are *achiral* as long as they maintain the three critical enzyme recognition elements.

While the octapeptide **2** was devoid of biological activity, compounds **17** and **18** were fungicidal against *C. albicans* (Table 1), with MFCs of 150 and 250 μM, respectively. In contrast, **16** is fungistatic only against *C. albicans*. Compounds **17** and **18** are also fungicidal against *C. neoformans* (e.g., MFC = 200 μM for **18**).

In order to probe the origin of the fungicidal activity for **17**, human NMT was expressed in *C. neoformans* in place of the functional wild-type fungal enzyme. There was a reproducible 2-fold reduction in the fungicidal activity of **17** when tested against a strain containing human NMT.[10] The fungicidal effect was not due to cell lysis but was accompanied by a rapid inhibition of protein synthesis.[10] The 2-fold difference in antifungal activity of **17** against strains containing fungal versus human NMT mirrors the observed differences in its K_i against the human and fungal enzymes. Together, these results indicate that the fungicidal effects of **17** involve inhibition of protein N-myristoylation in *C. neoformans*.

Conclusion

A new class of nonpeptidic NMT inhibitors which embody only *one* chiral center and exhibit *fungicidal* activity has been discovered. Further optimization of the spatial orientation of the critical recognition elements should lead to even more potent and selective NMT inhibitors with improved antifungal activity.

Table 1. Enzyme Potency and Antifungal Activity of Nonpeptidic NMT Inhibitors

compd	IC_{50} (μM)[a] *C. albicans* NMT	IC_{50} (μM)[a] human NMT	EC_{50} (μM)[b] 24 h, *C. albicans*
16	29.3 ± 0.7	62.3 ± 5.7	56 ± 17
17	20.0 ± 0.03	64	49 (MFC = 150 μM)
18	17.5	6.5	77 (MFC = 250 μM)

[a] Potency against the indicated NMT as assessed by IC_{50} using the peptide substrate GNAASARR-NH_2 at its apparent K_m and myristoylCoA at 1 μM. [b] EC_{50}s were determined as described in ref 6.

Experimental Section

Unless otherwise stated, starting materials were obtained from commercial suppliers and were used without further purification. All reactions were performed under anhydrous conditions in an atmosphere of argon. Nuclear magnetic proton were recorded on a Varian XL-300 spectrometer, and chemical shifts (δ) are reported in ppm relative to tetramethylsilane. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad peak. Low-resolution mass spectra were recorded on a VG40-250T instrument, and high-resolution mass spectra were recorded on a Finnigan MAT 90 mass spectrometer operating in the FAB mode. The purity of all the compounds was shown to be >95% by ^{1}H NMR and chromatographic techniques. Analytical reverse-phase high-performance liquid chromatography (HPLC) was carried out using a Waters Delta-Pak cartridge (C-18, 8 × 100 mm), using a linear gradient of (A) water containing 0.05% trifluoroacetic acid and (B) acetonitrile containing 0.05% TFA, at a flow rate of 1 mL/min. The elution was carried out with a linear gradient from 10 to 90% of B in 30 min, and the separation was monitored by UV absorbance at 215 nm.

Final compounds were purified by reverse-phase HPLC using a Waters Delta-Pak cartridge (C-18, 40 × 100 mm, 15 μm) and eluting with a linear gradient consisting of 5−70% of B in 30 min. The flow rate was adjusted to 70 mL/min, and the separtion was monitored by UV absorbance at 215 nm. The appropriate fractions were pooled and freeze-dried, and the products were isolated as TFA salts.

3-Iodo-*N*-(phenylmethoxycarbonyl)-L-tyrosine Methyl Ester (4). To a suspension of 3-iodotyrosine methyl ester hydrochloride (5.7 g, 0.156 mol) in dichloromethane (50 mL) was added *N*-methylmorpholine (1.6 g, 0.016 mol). After 15 min, *N*-(benzyloxycarbonyl)oxysuccinimide (4.0 g, 0.016 mol) was added, and the resulting mixture was stirred at 10 °C for 1 h and at room temperature for 2 h. The reaction mixture was diluted with dichloromethane (25 mL), washed successively with 5% citric acid (2 × 20 mL) and water (3 × 25 mL), and dried (Na_2SO_4). The solution was concentrated, and the resulting substance was crystallized from 25% EtOAc in hexane to afford 5.4 g (74%) of **4** as a white powder: R_f = 0.58 (EtOAc:hexane, 1:1 v/v); ^{1}H NMR ($CDCl_3$) δ 7.35 (m, 5H), 7.28 (m, 1H), 6.96 (m, 1H), 6.87 (d, 1H, J = 8.1 Hz), 5.1 (q, 2H), 4.61 (m, 1H), 3.73 (s, 3H), 3.0 (m, 2H); FAB-MS m/z 462 (M + Li); HRMS calcd for $C_{18}H_{18}NIO_5Li$ (M + Li) 462.0389, found 462.0392.

***O*-(Cyclohexylethyl)-3-iodo-*N*-(phenylmethoxycarbonyl)-L-tyrosine Methyl Ester (5).** A mixture of **4** (0.8 g, 0.00176 mol), 2-cyclohexylethyl bromide (0.5 g, 0.0026 mol), and tetrabutylammonium fluoride (1.1 g) in dry THF (10.0 mL) was heated to reflux under nitrogen for 1 h. Thereafter, the solvent was distilled under reduced pressure, and the residue was partitioned between cold 5% citric acid (25 mL) and dichloromethane (30 mL). The organic phase was washed with water (2 × 20 mL), dried (Na_2SO_4), and concentrated under

reduced pressure. The resulting syrup was purified by silica gel flash chromatography using 20% EtOAc in hexane to give 0.6 g (60%) of **5** as a colorless syrup: $R_f = 0.42$ (EtOAc:hexane, 3:7 v/v); ^{1}H-NMR ($CDCl_3$) δ 7.51 (s, 1H), 7.35 (m, 5H), 6.98 (dd, 1H, J = 2.1, 8.4 Hz), 6.67 (d, 1H, J = 8.4 Hz), 5.2 (br, 1H), 5.1 (d, 2H, J = 3.0 Hz), 4.6 (m, 1H), 4.0 (t, 2H, J = 6.6 Hz), 3.73 (t, 3H), 3.00 (m, 2H), 1.73 (m, 9H), 1.26 (m, 2H), 0.96 (m, 2H); FAB-MS m/z 572 (M + Li); HRMS calcd for $C_{26}H_{32}NIO_5Li$ (M + Li) 572.1484, found 572.1475.

***O*-(Cyclohexylethyl)-3-(4-hydroxy-1-butynyl)-*N*-(phenylmethoxycarbonyl)-L-tyrosine Methyl Ester (6).** To a solution of **5** (1.4 g, 0.025 mol) and 1-butyn-4-ol (0.26 g, 0.0037 mol) in acetonitrile (8 mL) were added triethylamine (0.36 g, 0.0036 mol), $(Ph_3)_2PPdCl_2$ (0.1 g, 0.00014 mol), and CuI, and the mixture was stirred at room temperature for 4 h under argon atmosphere. The reaction mixture was then concentrated under reduced pressure, and the residue was partitioned between 5% citric acid (20 mL) and EtOAc (30 mL). The organic phase was washed with water (2 × 20 mL), dried (Na_2SO_4), and concentrated to dryness. The resulting dark brown substance was purified by silica gel flash chromatography using 25% EtOAc in hexane as the eluent to afford 1.2 g (96%) of **6** as a pale yellow syrup: $R_f = 0.16$ (EtOAc:hexane, 3:7 v/v); ^{1}H NMR ($CDCl_3$) δ 7.34 (s, 5H), 7.1 (d, 1H, J = 2.1 Hz), 6.95 (m, 1H), 6.74 (d, 1H, J = 8.4 Hz), 5.2 (br, 1H), 5.1 (s, 2H), 4.6 (m, 1H), 4.01 (t, 2H, J = 6.3 Hz), 3.8 (q, 2H, J = 6.3 Hz), 3.72 (t, 3H), 3.0 (m, 2H), 2.71 (t, 2H, J = 6.3 Hz), 2.08 (t, 1H, OH), 1.5−1.85 (m, 8H), 1.2−1.4 (m, 4H); FAB-MS m/z 508 (M + H); HRMS calcd for $C_{30}H_{38}NO_6$ (M + H) 508.2699, found 508.2709.

***O*-(Cyclohexylethyl)-3-(4-hydroxybutyl)-*N*-(phenylmethoxycarbonyl)-L-tyrosine Methyl Ester (7a).** A solution of **6** (1.2 g, 0.0024 mol) in MeOH (25 mL) and acetic acid (0.2 mL) was hydrogenated at 50 psi in the presence of 5% Pd/C (1.00 g) for 16 h. The catalyst was removed by filtration, and the filtrate was concentrated to dryness under reduced pressure. The resulting residue was dissolved in dichloromethane (10 mL), *N*-methylmorpholine (0.32 g, 0.0032 mol), and (benzyloxycarbonyl)succinimide (0.50 g, 0.002 mol) were added, and the mixture was stirred at room temperature for 16 h. The reaction mixture was then diluted with dichloromethane (25 mL), washed successively with 5% citric acid (2 × 10 mL), saturated sodium bicarbonate (2 × 15 mL), and water, and dried (Na_2SO_4). After removal of the solvent under reduced pressure, the residue was purified by silica gel flash chromatography using 50% EtOAc in hexane to afford 0.81 g (86%) of **7a** as a colorless syrup: ^{1}H NMR ($CDCl_3$) δ 7.34 (s, 5H), 6.84 (s, over m, 2H), 6.71 (d, 1H, J = 8.3 Hz), 5.22 (br, 1H), 5.1 (m, 1H), 3.95 (t, 2H, J = 8.0 Hz), 3.73 (s, 3H), 3.62 (m, 2H), 3.0 (m, 2H), 2.55 (t, 2H), 1.8−1.5 (m, 7H), 1.4−1.2 (m, 6H), 1.1−0.8 (m, 4H); FAB-MS m/z 512 (M + H); HRMS calcd for $C_{30}H_{42}NO_6$ (M + H) 512.3012, found 512.3049.

***O*-(Cyclohexylethyl)-3-(4-iodobutyl)-*N*-(phenylmethoxycarbonyl)-L-tyrosine Methyl Ester (7b).** To a solution of **7a** (0.8 g, 0.0016 mol) in acetonitrile (5 mL) was added triphenoxymethylphosphonium iodide (1.00 g, 0.0022 mol), and the mixture was stirred at room temperature for 3 h. The reaction mixture was concentrated, and the residue was partitioned between EtOAc (25 mL) and cold 0.25 N NaOH (15 mL). The organic phase was washed successively with 0.25 N NaOH (2 × 15 mL) and water (3 × 15 mL), dried (Na_2SO_4), and concentrated to dryness under reduced pressure. The material thus obtained was purified by silica gel flash chromatography to furnish 0.75 g of **7b** as a colorless liquid: ^{1}H-NMR ($CDC1_3$) δ 7.4 (s, 5H), 6.85 (m, 3H), 6.72 (d, 1H, J = 8.5 Hz), 5.15 (br, 1H), 5.1 (d, 2H, J = 2.4 Hz), 4.62 (m, 1H), 3.95 (t, 2H, J = 6.6 Hz), 3.73 (s, 3H), 3.17 (t, 2H, J = 7.7 Hz), 3.0 (m, 2H), 2.55 (t, 2H, J = 7.7 Hz), 1.8−1.6 (m, 11H), 1.25 (m, 4H), 1.04 (m, 2H); FAB-MS m/z 622 (M + H); HRMS calcd for $C_{30}H_{41}NIO_5$ (M + H) 622.2028, found 622.2000.

***O*-(Cyclohexylethyl)-3-[4-[[(1,1-dimethylethoxy)carbonyl]amino]butyl]-*N*-(phenylmethoxycarbonyl)-L-tyrosine Methyl Ester (8).** A mixture of **7b** (0.75 g, 0.0011 mol) and **so**dium azide (0.23 g, 0.0035 mol) in DMF (2.5 mL) was heated at 70 °C for 3 h. DMF was distilled in vacuo, and the residue was partitioned between water (10 mL) and EtOAc (25 mL). The organic phase was washed with brine, dried (Na_2SO_4), and concentrated to dryness under reduced pressure to afford 0.58 g of the corresponding azide as a pale yellow syrup which was used without further purification in the following step.

A solution of the azide (0.4 g, 0.00075 mol) in THF (6 mL) and water (0.2 mL) was treated with a solution of triphenylphosphine (0.3 g, 0.0015 mol) in THF (6 mL), and the mixture was stirred at room temperature for 30 min. Thereafter, the reaction mixture was heated at 60 °C for 2.5 h under an atmosphere of nitrogen and concentrated in vacuo. The residue was dissolved in dichloromethane (5 mL) and cooled in an ice bath, $(BOC)_2O$ (0.16 g, 0.00075 mol) was added, and the mixture was stirred at room temperature for 1.5 h. The reaction mixture was then diluted with dichloromethane (20 mL), washed with saturated sodium bicarbonate (2 × 15 mL), and brine (2 × 15 mL), dried (Na_2SO_4), and concentrated to dryness under reduced pressure. The resulting material was purified by silica gel flash chromatography using 25% EtOAc in hexane containing 0.1% triethylamine to furnish 0.4 g (88%) of **8** as a colorless syrup: $R_f = 0.41$ (EtOAc:hexane, 3:7 v/v); ^{1}H-NMR ($CDCl_3$) δ 7.33 (s, 5H), 6.82 (m, 2H), 6.72 (d, 1H, J = 8.5 Hz), 5.21 (br, 1H), 5.09 (d, 2H, J = 3.3 Hz), 4.6 (m, 1H), 3.94 (t, 2H, J = 6.6 Hz), 3.72 (s, 3H), 3.12 (m, 2H), 3.05 (m, 2H), 2.56 (m, 2H), 1.8−1.6 (m, 8H), 1.43 (s over m, 10H), 1.27 (m, 4H), 0.95 (m, 4H); FAB-MS m/z 617 (M + H); HRMS calcd for $C_{35}H_{50}N_2O_7Li$ (M + Li) 617.3778, found 617.3741.

4-(Cyclohexylethoxy)-3-[4-[[(1,1-dimethylethoxy)carbonyl]amino]butyl]-β-[[(1,1-dimethylethyl)dimethylsilyl]methoxy]-*N*-(phenylmethoxycarbonyl)-(*S*)-benzeneethanamine (9). A mixture of **8** (0.44 g, 0.0007 mol) and 1 M LiOH (1.75 mL) in THF (1 mL) was stirred at room temperature for 1.5 h. It was then diluted with 5% citric acid (10 mL) and extracted with EtOAc (2 × 10 mL). The combined organic extracts were washed successively with brine (2 × 10 mL), dried (Na_2SO_4), and concentrated to dryness. The resulting residue was dried in a desiccator overnight over NaOH pellets to afford 0.4 g of the corresponding acid, which was subjected to reduction in the following step without purification.

To a solution of the acid in THF (5 mL) was added dropwise BH_3−THF complex (1 M, 2 mL), and the mixture was stirred at −5 °C for 1 h and at 10 C for 1 h. Thereafter, the reaction was quenched by the addition MeOH (3 mL) containing acetic acid (0.3 mL) and concentrated to dryness under reduced pressure. The resulting residue was partitioned between saturated sodium bicarbonate (10 mL) and dichloromethane (25 mL). The organic phase was washed with brine (2 × 15 mL), dried (Na_2SO_4), and concentrated to dryness. The residue was dried in a desiccator overnight to afford 0.3 g of the corresponding primary alcohol, which was dissolved in acetonitrile (3.0 mL). *tert*-Butyldimethylchlorosilane (0.11 g, 0.00073 mol), imidazole (0.03 g), and *N*-methylmorpholine (0.08 g, 0.0007 mol) were added. The resulting mixture was stirred at 60 °C under a nitrogen atmosphere for 2.5 h and concentrated under a reduced pressure. The residue was partitioned between 5% citric acid (10 mL) and dichloromethane (20 mL). The organic phase was washed with brine, dried (Na_2SO_4), and concentrated to dryness. The residue thus obtained was purified by silica gel flash chromatography using 12% EtOAc in hexane to furnish 0.22 g (47%) of **9** as a colorless syrup: ^{1}H NMR ($CDCl_3$) δ 7.32 (s, 5H), 6.95 (m, 2H), 6.8 (d, 1H, J = 8.2 Hz), 5.1 (s, 2H), 4.95 (br, 1H), 4.5 (m, 1H), 3.94 (t, 2H, J = 6.6 Hz), 3.85 (br, 1H), 3.51 (d, 2H, J = 3.3 Hz), 3.1 (m, 2H), 2.72 (d, 2H, J = 6.9 Hz), 2.55 (m, 2H), 1.8−1.4 (m, 10H), 1.42 (s, 9H), 1.22 (m, 4H), 0.9 (s over m, 12H), 0.027 and 0.022 (2s, 6H); FAB-MS m/z 697 (M + H); HRMS calcd for $C_{40}H_{65}N_2O_6Si$ (M + H) 697.4612, found 697.4642.

***N*-[1-[[3-(Aminobutyl)-4-(cyclohexylethoxy)phenyl]methyl]-2-hydroxyethyl]-4-[(2-methyl-1*H*-imodazol-l-yl]butyl]-(*S*)-benzeneethanamine (17).** A solution of **9** (0.2 g, 0.0003 mol) in EtOH (5.0 mL) was hydrogenated at 30 psi

in the presence of 10% Pd/C (0.05 g) for 1.5 h. The catalyst was removed by filtration, the filtrate was concentrated under reduced pressure, and the residue was dried in vacuo for 4 h to afford the corresponding amine, which was used without purification in the following step.

To a solution of the acid **15** (0.14 g, 0.00045 mol) in dimethylacetamide (2 mL) and dichloromethane (2.0 mL) were added DCC (0.093 g, 0.00045 mol) and HOBt (0.075 g), and the mixture was stirred at 0 °C for 1.5 h. The reaction mixture was then added to a solution of the amine as prepared above, in dimethylacetamide (0.5 mL), DMAP (0.018 g) and *N*-methylmorpholine (0.046 g, 0.00045 mol) were added, and the mixture was stirred at room temperature for 16 h. After the removal of the solvents in vacuo, the residue was partitioned between cold 0.25 N NaOH (5 mL) and EtOAc (20.0 mL). The organic phase was washed with saturated sodium bicarbonate (2 × 5 mL) and brine, dried (Na_2SO_4), and concentrated under reduced pressure. The resulting substance (0.2 g) was stirred with trifluoroacetic acid (2 mL) at room temperature for 3.5 h and concentrated under reduced pressure. The residue was then purified by reverse-phase HPLC using 5−70% acetonitrile in water (30 min gradient) at a flow rate of 70 mL/min. Appropriate fractions were pooled and freeze-dried to yield **17** as a hygroscopic white powder: t_R = 24.2 min; ^{1}H NMR (CD_3OD) δ 7.41 (d, 1H, J = 2.1 Hz), 7.35 (d, 1H, J = 2.1 Hz), 7.01 (d, 2H, J = 7.8 Hz), 6.94 (m, 4H), 6.77 (d, 1H, J = 8.1 Hz), 4.08 (t, 3H, J = 7.2 Hz), 3.96 (m, 2H), 3.48 (d, 2H, J = 1.2 Hz), 3.34 (q, 2H, J = 5.1 Hz), 2.8−2.45 (s over m, 11H), 1.8−1.3 (m, 15H), 1.3−1.15 (m, 2H), 0.95 (m, 2H); FAB-MS m/z 603 (M + H); HRMS calcd for $C_{37}H_{55}N_4O_3$ (M + H) 603.4274, found 603.4320.

***N*-[1-[[3-(Aminopropyl)-4-(cyclohexylethoxy)phenyl]-methyl]-2-hydroxyethyl]-4-[(2-methyl-1*H*-imodazol-1-yl)-butyl]-(*S*)-benzeneethanamine (16):** t_R = 23.6 min; ^{1}H NMR (CD_3OD) δ 7.46 (d, 1H, J = 2.1 Hz), 7.41 (d, 1H, J = 2.1 Hz), 7.02 (m, 6H), 6.79 (d, 1H, J = 8.4 Hz), 4.1 (t, 3H, J = 7.5 Hz), 4.01 (t, 2H, J = 6.6 Hz), 3.52 (d, 2H, J = 4.8 Hz), 3.41 (s, 2H), 2.81 (m, 4H), 2.66 (m, 7H), 2.0−1.4 (m, 14H), 1.27 (m, 3H), 1.05 (m, 2H); FAB-MS m/z 589 (M + H); HRMS calcd for $C_{36}H_{53}N_4O_3$ (M + H) 589.4118, found 589.4121.

***N*-[1-[[3-(Aminopropyl)-4-(cyclohexylethoxy)phenyl]-methyl]-2-hydroxyethyl]-4-[(2-methyl-1*H*-imodazol-l-yl]-butyl]-(*R*)-benzeneethanamine (18):** t_R = 23.63 min; ^{1}H NMR (CD_3OD) δ 7.44 (d, 1H, J = 1.8 Hz), 7.39 (d, 1H, J = 1.8 Hz), 7.02 (m, 6H), 6.78 (d, 1H, J = 8.1 Hz), 4.12 (t, 3H, J = 7.4 Hz), 4.00 (t, 2H, J = 6.6 Hz), 3.52 (d, 2H, J = 5.1 Hz), 3.41 (s, 2H), 2.81 (m, 4H), 2.64 (s over m, 5H), 2.02−1.2 (m, 15H), 1.2−1.08 (m, 4H), 1.04 (m, 2H); FAB-MS m/z 589 (M + H); HRMS calcd for $C_{36}H_{53}N_4O_3$ (M + H) 589.4118, found 589.4130.

***O*-(Cyclohexylethyl)-3-(4-hydroxy-1-propynyl)-*N*-(phenylmethoxycarbonyl)-L-tyrosine Methyl Ester (10):** yield 72%; pale yellow syrup; ^{1}H NMR ($CDCl_3$) δ 7.34 (s, 5H), 7.12 (d, 1H, J = 2.1 Hz), 6.9 (m, 1H), 6.82 (d, 1H, J = 8.4 Hz), 5.15 (br, 1H), 5.10 (s, 2H), 4.65 (br, 1H), 4.58 (m, 1H), 4.18 (d, 2H, J = 7.1 Hz), 4.01 (t, 2H, J = 6.6 Hz), 3.72 (s, 3H), 3.0 (m, 2H), 1.82−1.6 (m, 8H), 1.46 (s, 9H), 1.4−1.1 (m, 3H), 0.95 (m, 2H); FAB-MS m/z 597 (M + H); HRMS calcd for $C_{34}H_{49}N_2O_7$ (M + H) 597.3534, found 597.3494.

***O*-(Cyclohexylethyl)-3-[[4-[(1,1-dimethylethoxy)-carbonyl]amino]propyl]-*N*-(phenylmethoxycarbonyl)-L-tyrosine methylester (11):** yield 67%; R_f = 0.25 (EtOAc:hexane, 3:7 v/v); ^{1}H NMR ($CDCl_3$) δ 7.33(s, 5H), 6.85 (m, 2H), 6.71 (d, 1H, J = 8.3 Hz), 5.4 (br, 1H), 5.10 (m, 2H), 4.7 (br, 1H), 4.6 (m, 1H), 3.95 (t, 2H, J = 6.6 Hz), 3.72 (s, 3H), 3.05 (m, 4H), 2.58 (m, 2H), 1.8−1.6 (m, 10H), 1.44 (s, 9H), 1.25 (m, 3H), 1.05−0.8 (m, 2H); FAB-MS m/z 603 (M + Li); HRMS calcd for $C_{34}H_{48}N_2O_7Li$ (M + Li) 603.3622, found 603.3651.

4-(Cyclohexylethoxy)-3-[[4-[(1,1-dimethylethoxy)-carbonyl]amino]propyl]-β-[[(1,1-dimethylethyl)dimethylsilyl]methoxy]-*N*-(phenylmethoxycarbonyl)-(*S*)-benzeneethanamine (12): ^{1}H NMR ($CDCl_3$) δ 7.33 (s, 5H), 6.98 (m, 2H), 6.70 (d, 1H, J = 8.2 Hz), 5.1 (s, 2H), 4.98 (br, 1H), 4.7 (br, 1H), 3.96 (t, 2H, J = 5.1 Hz), 3.85 (br, 1H), 3.50 (d, 2H, J = 8.5 Hz), 3.18 (m, 2H), 2.75 (d, 2H, J = 6.9 Hz), 2.58 (m, 2H), 1.74 (m, 9H), 1.4 (s over m, 9H), 1.25 (m, 4H), 0.91 (s over m,11H), 0.037 and 0.03 (2s, 6H); FAB-MS m/z 689 (M + Li); HRMS calcd for $C_{39}H_{62}N_2O_6SiLi$ (M + Li) 689.4537, found 689.4555.

Acknowledgment. The authors thank Mr. James P. Doom and Charles A. Gloeckner of Monsanto Co. for providing FAB mass spectral data.

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JM980001Q