Design and Synthesis of Novel Imidazole-Substituted Dipeptide Amides as Potent and Selective Inhibitors of *Candida albicans* MyristoylCoA:Protein *N*-Myristoyltransferase and Identification of Related Tripeptide Inhibitors with Mechanism-Based Antifungal Activity†

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A new class of antifungal agents has been discovered which exert their activity by blockade of myristoylCoA:protein *N*-myristoyltransferase (NMT; EC 2.1.3.97). Genetic experiments have established that NMT is needed to maintain the viability of *Candida albicans* and *Cryptococcus neoformans*, the two principal causes of systemic fungal infections in immunocompromised humans. Beginning with a weak octapeptide inhibitor ALYASKLS-NH_2 (**2**, K_i = 15.3 ± 6.4 μM), a series of imidazole-substituted Ser-Lys dipeptide amides have been designed and synthesized as potent and selective inhibitors of *Candida albicans* NMT. The strategy that led to these inhibitors evolved from the identification of those functional groups in the high-affinity octapeptide substrate GLYASKLS-NH_2 **1a** necessary for tight binding, truncation of the *C*-terminus, replacement of the four amino acids at the N-terminus by a spacer group, and substitution of the glycine amino group with an N-linked 2-methylimidazole moiety. Initial structure−activity studies led to the identification of **31** as a potent and selective peptidomimetic inhibitor with an IC_{50} of 56 nM and 250-fold selectivity versus human NMT. 2-Methylimidazole as the N-terminal amine replacement in combination with a 4-substituted phenacetyl moiety imparts remarkable potency and selectivity to this novel class of inhibitors. The (*S*,*S*) stereochemistry of serine and lysine residues is critical for the inhibitory activity, since the (*R*,*R*) enantiomer **40** is 10^3-fold less active than the (*S*,*S*) isomer **31**. The inhibitory profile exhibited by this new class of NMT ligands is a function of the pK_a of the imidazole substituent as illustrated by the benzimidazole analog **35** which is about 10-fold less potent than **31**. The measured pK_a (7.1 ± 0.5) of 2-methylimidazole in **31** is comparable with the estimated pK_a (∼8.0) of the glycyl residue in the high-affinity substrate **1a**. Groups bulkier than methyl, such as ethyl, isopropyl, or iodo, at the imidazole 2-position have a detrimental effect on potency. Further refinement of **31** by grafting an α-methyl group at the benzylic position adjacent to the serine residue led to **61** with an IC_{50} of 40 nM. Subsequent chiral chromatography of **61** culminated in the discovery of the most potent *Candida* NMT inhibitor **61a** reported to date with an IC_{50} of 20 nM and 400-fold selectivity versus the human enzyme. Both **31** and **61a** are competitive inhibitors of *Candida* NMT with respect to the octapeptide substrate GNAASARR-NH_2 with $K_{i(app)}$ = 30 and 27 nM, respectively. The potency and selectivity displayed by these inhibitors are dependent upon the size and orientation of the α-substituent. An α-methyl group with the *R* configuration corresponding to the (*S*)-methyl-4-alanine in **2** confers maximum potency and selectivity. Structural modification of **31** and **61** by appending an (*S*)-carboxyl group β to the cyclohexyl moiety provided the less potent tripeptide inhibitors **73a** and **73b** with an IC_{50} of 1.45 ± 0.08 and 0.38 ± 0.03 μM, respectively. However, these tripeptides (**73a** and **73b**) exhibited a pronounced selectivity of 560- and 2200-fold versus the human NMT. More importantly **73a** displayed fungistatic activity against *C. albicans* with an EC_{50} of 51 ± 17 μM in cell culture. Compound **73b** also exhibited a similar antifungal activity. An Arf protein gel mobility shift assay for monitoring intracellular myristoylation revealed that a single dose of 200 μM of **73a** or **73b** produced <50% reduction in Arf N-myristoylation, after 24 and 48 h, consistent with their fungistatic rather than fungicidal activity. In contrast, the enantiomer **73d** which had an IC_{50} >1000 μM against *C. albicans* NMT did *not* exhibit antifungal activity and produced no detectable reduction in Arf N-myristoylation in cultures of *C. albicans*. These studies confirm that the observed antifungal activity of **73a** and **73b** is due to the attenuation of NMT activity and that NMT represents an attractive target for the development of novel antifungal agents.

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Introduction

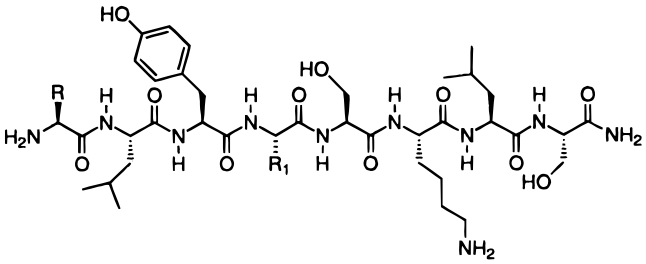
MyristoylCoA:protein *N*-myristoyltransferase (NMT; EC 2.1.3.97) catalyzes the cotranslational transfer of the rare cellular fatty acid myristate (C14:0) from myristoylCoA to the N-terminal glycine residue of a number of diverse eukaryotic cellular protein substrates. These include serine/threonine and tyrosine kinases, protein phosphatases such as calcineurin, the α subunits of heterotrimeric G proteins, retroviral gag polyprotein precursors such as $Pr55^{gag}$ of human immunodeficiency virus I, and the capsid proteins of a number of picornaviruses and papovaviruses. Different proteins utilize their covalently bound myristoyl moiety for different purposes including regulation of protein−protein and protein−lipid interactions.[1−3]

NMT is a cytosolic enzyme. It is encoded by a single copy gene that appears to be represented in a broad range of eukaryotes, e.g. *Saccharomyces cerevisiae*, a variety of pathogenic fungi (*Candida albicans*, *Cryptococcus neoformans*, *Histoplasma capsulatum*), and *Homo sapiens*.[4−7] *C. albicans* NMT has been purified to homogeneity. This monomeric protein of 451 amino acid residues has no known co-factor requirements and exhibits 46% primary sequence identity with the human enzyme.[7]

NMT has an ordered Bi-Bi mechanism.[8] The apoenzyme first binds myristoylCoA, forming a binary complex which results in conformational changes giving rise to a functional peptide binding site and formation of a myristoylCoA:NMT:peptide ternary complex. Catalysis then occurs with the transfer of the myristoyl moiety to the peptide substrate. This is then followed by the sequential release of CoA and the acylpeptide product.

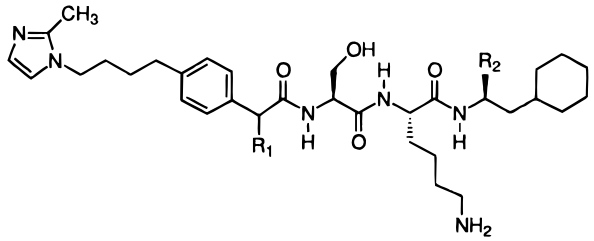
Genetic studies have established that the *NMT* gene is essential for *C. neoformans*,[9] and *C. albicans*.[10] To do so, conditional lethal *nmt* alleles were incorporated into their genomes by homologous recombination. These mutant alleles encode NMTs with reduced affinity for myristoylCoA due to substitution of a conserved glycine near their C-terminus with aspartic acid. The mutant *nmt* alleles produce temperature-sensitive growth arrest and myristic acid auxotrophy. Removal of myristate from the medium results in cell death, thus demonstrating that NMT is required for their viability. Moreover, in the case of *C. albicans*, the myristic acid auxotrophy was quite stable: the reversion rate was only 2.5×10^{-8} (ref 10).

These observations suggested that fungal NMTs may serve as a target for the design and development of a new class of fungicidal agents for treating systemic fungal infections in the rapidly expanding population of immunocompromised patients.[11−13] Subsequent design of inhibitors was guided by the observation that, although the acylCoA binding sites of orthologous NMTs are highly conserved throughout the course of eukaryotic evolution, their peptide substrate specificities have diverged.[5,9,12,14,15] This difference in substrate specificity among NMTs was exploited to develop the first potent and species-selective peptidomimetic inhibitors of the acyltransferase. A high-affinity octapeptide substrate



1a. GLYASKLS-NH_2 (R = H, R_1 = CH_3)
1b. GLYGSKLS-NH_2 (R = R_1 = H)
2. ALYASKLS-NH_2 (R = R_1 = CH_3)

Figure 1. Octapeptide substrates (**1a**,**b**) and inhibitor (**2**).



73a. R_1 = H, R_2 = COOH
73b. R_1 = CH_3, R_2 = COOH

Figure 2. NMT inhibitors with anti-*Candida* activity.

GLYASKLS-NH_2, **1a**, was initially identified (Figure 1, K_m = 0.07, 0.3, and 0.7 μM for *S. cerevisiae*,[16] *C. albicans*,[17] and human[15] NMT, respectively). The octapeptide sequence was derived from the N-terminal fragment of Arf2p (ADP ribosylation factor 2), a yeast protein that must be myristoylated by NMT for expression of its essential biological function.[18] Next, a simple substitution of alanine for glycine in **1a** provided an inhibitor **2** which was competitive for peptide (K_i = 15.3 ± 6.4 μM) and uncompetitive for myristoylCoA (K_i = 31.2 ± 0.7 μM).[16,17] Further *in vitro* kinetic studies using purified *C. albicans* NMT and a panel of peptides having single alanine substitutions at each position of **1a** revealed that the primary amino group of glycine, the hydroxyl group of serine at position 5, and the ε-amino group of lysine at position 6 are the important components involved in recognition by the NMT:myristoylCoA binary complex.[17] Incorporation of these key structural motifs into new scaffolds has generated a series of potent and selective NMT inhibitors as exemplified by **31** and **61**.[12,13] This paper describes their complete synthesis, structure−activity relationship (SAR) studies, and further structural modifications which led to the discovery of related tripeptide NMT inhibitors **73a** and **73b** (Figure 2) with a mechanism-based antifungal activity.

Chemistry

N-Linked imidazole-substituted Ser-Lys dipeptide amides with varying alkyl chain length between the N-1 of imidazole and Ser were synthesized according to the routes shown in Scheme 2. The dipeptide intermediate **4** was conveniently synthesized as outlined in Scheme 1 from the commercially available lysine derivative **3b**. The ω-imidazol-1-ylalkanoic acids **5**−**7**[19a] were activated as hydroxy benzotriazole esters and coupled with the protected amine **4** in dimethylformamide. The resulting products were deprotected by catalytic hydrogenation to afford **8**−**10**. The synthesis of analog **13** was achieved

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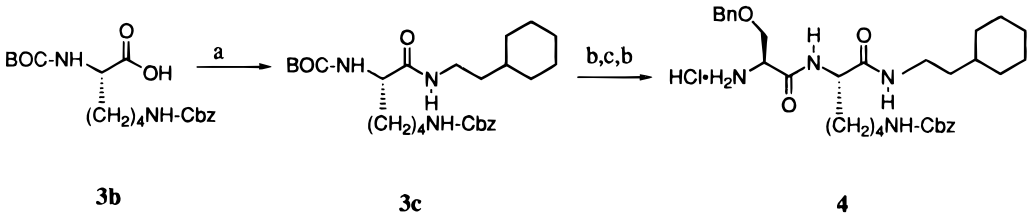
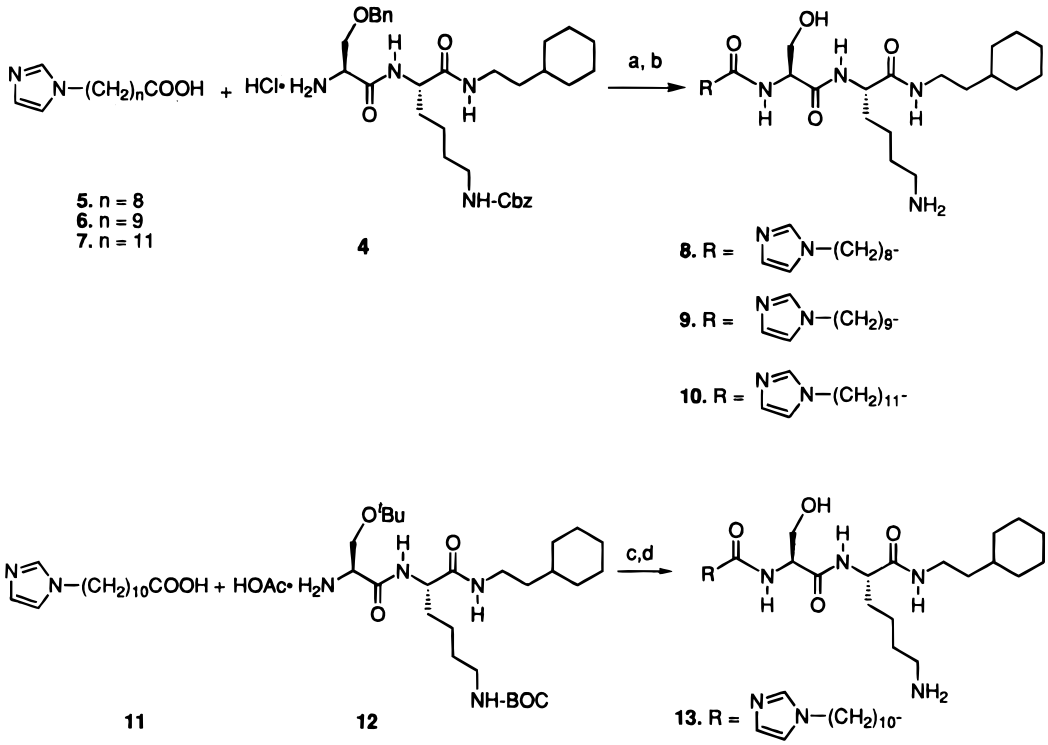
Scheme 1. Synthesis of Ser-Lys Dipeptide Amine Precursor[a]



3b **3c** **4**

[a] Reagents: (a) EDC, HOBt, 2-cyclohexylethylamine, DMF, room temperature, 16 h; (b) 4 N HCl/dioxane, room temperature, 4 h; (c) BOC-Ser(OBn)OSu, DMF, Et_3N, room temperature, 16 h.

Scheme 2. Synthesis of Imidazole-Linked NMT Inhibitors with Varying Chain Lengths[a]



[a] Reagents: (a) HOBt, EDC, Et_3N, DMF, 30 min, room temperature; (b) H_2, Pd/C (10%), MeOH, HCl, room temperature, 18 h, 40 psi; (c) DCC, HOBt, CH_2Cl_2–DMF, 0 °C to room temperature; *N*-methylmorpholine; (d) trifluoroacetic acid, room temperature, 3 h.

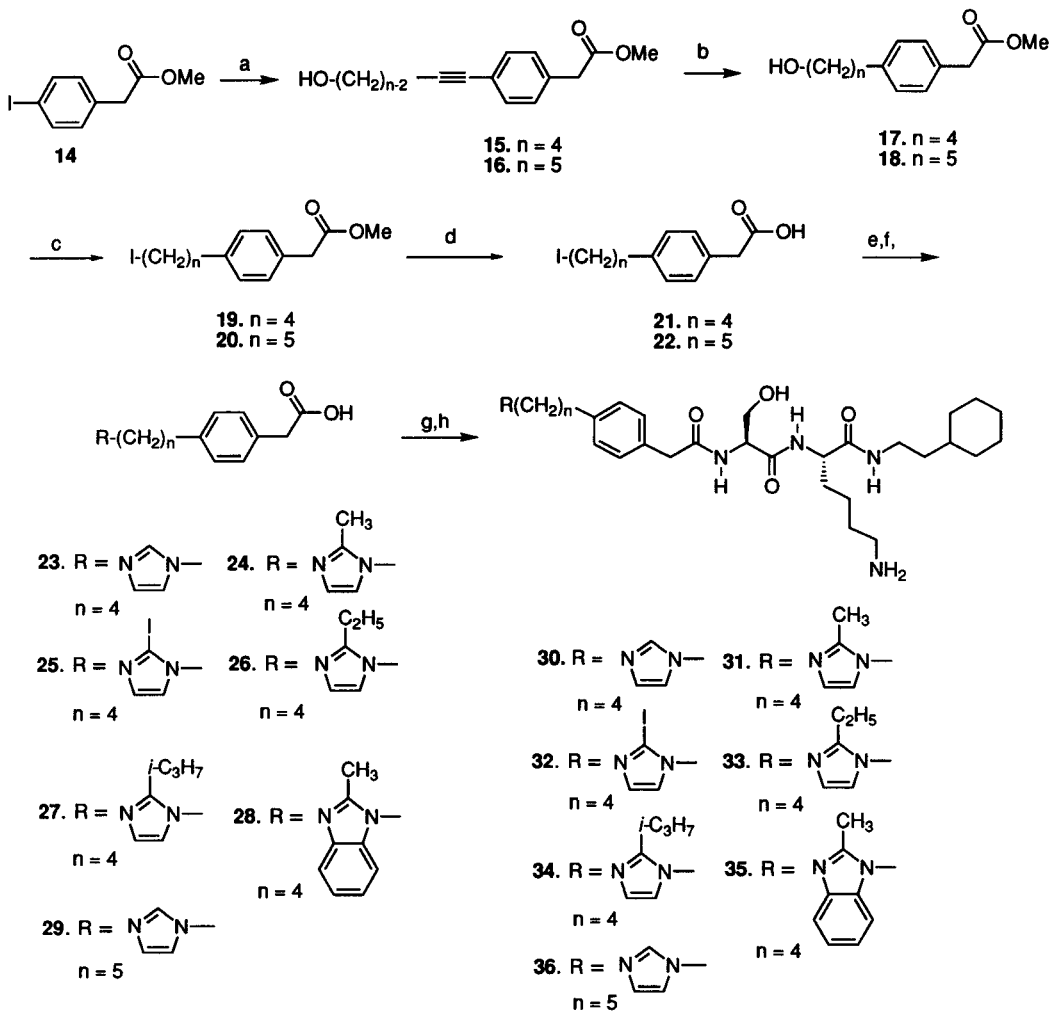
according to Scheme 2 by condensing the acid **11**[19b] with the protected amine **12**[12] via formation of the benzotriazole active ester using DCC and hydroxybenzotriazole, followed by deprotection of the crude product with trifluoroacetic acid, and purification by reverse-phase (C_{18}) HPLC.

Scheme 3 outlines a general methodology for preparing Ser-Lys dipeptides with imidazole, 2-substituted imidazole, and 2-methylbenzimidazole bearing a 4-substituted phenacetyl ring. The commercially available 4-iodophenylacetic acid was esterified in the presence of methanolic HCl to obtain the corresponding methyl ester **14**. Reaction of the ester **14** with butyn-1-ol in the presence of triethylamine, $PdCl_2(PPh_3)_2$, and CuI[20] yielded the acetylenic product **15** which, after catalytic reduction of the triple bond, provided the 4-hydroxybutyl-substituted phenylacetate ester **17**. The hydroxy ester **17** was then converted to the iodo derivative **19** using $(PhO)_3PMeI$ and subjected to alkaline hydrolysis to obtain the iodo acid **21** after acidification. Condensation of the acid **21** with imidazole, 2-methylimidazole, 2-iodoimidazole,[21] 2-ethylimidazole, 2-isopropylimidazole, or 2-methylbenzimidazole in the presence of NaH in DMF or THF yielded the corresponding imidazole-linked phenylacetic acids (**23**–**28**), respectively. These acids were individually coupled with the amine generated from **12**, using DCC/HOBt. The crude products after reaction workup were treated with trifluoroacetic acid and purified by reverse-phase HPLC to afford the final compounds **30**–**35** as trifluoroacetate salts.

Synthesis of **36** was accomplished using a procedure similar to that described above, by condensing **29** with the amine **12** employing DCC/HOBt. The crude product was treated with trifluoroacetic acid and purified by reverse-phase HPLC to obtain **36**.

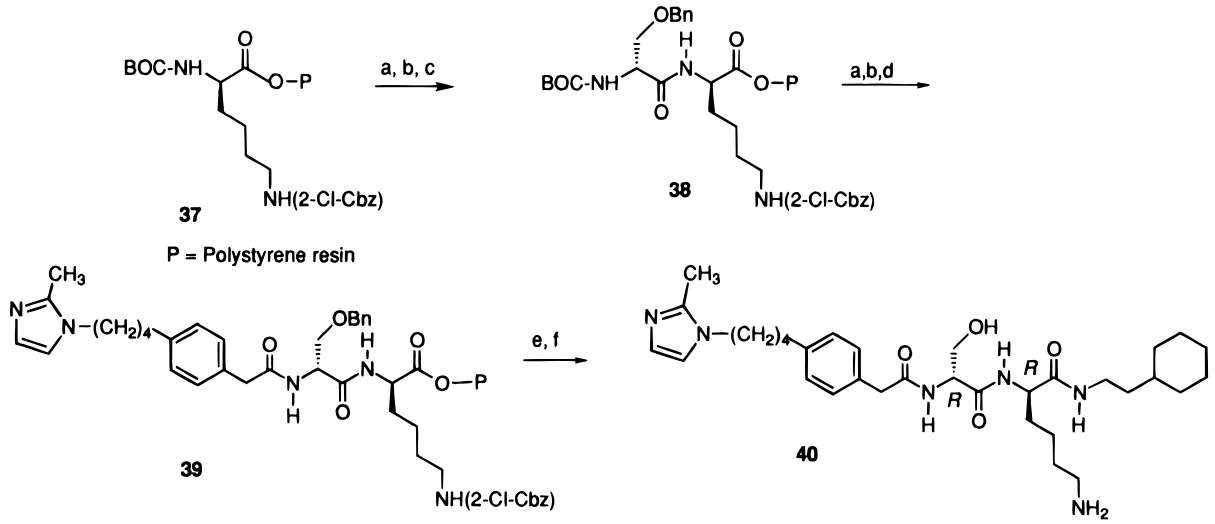
The (*R*,*R*) enantiomer **40**, which is the optical isomer of **31**, was synthesized by solid-phase methodology[22] according to Scheme 4, starting from the commercially available protected *R*-lysine derivative **37** linked to Merrifield's resin. The resin-bound lysine derivative **37** was treated with 50% trifluoroacetic acid in dichloromethane. It was then washed with diisopropylethylamine (DIEA) to generate the free amine and coupled to BOC-D-Ser(OBn)-OH with the aid of DCC/HOBt in dimethylformamide at room temperature to obtain **38**. Exposure of **38** to 50% trifluoroacetic acid in dichloromethane for 30 min, followed by formation of the free amine using DIEA, and coupling with the activated 2-methylimidazole-substituted phenylacetic acid **24** using DCC/HOBt, gave the resin-bound 2-methylimidazole derivative **39**. The final compound **40** was released from the resin by heating **39** with 2-cyclohexylethylamine at

Scheme 3[a]



[a] Reagents: (a) Alkyn-1-ol, $PdCl_2P(Ph_3)_2$, CuI, Et_3N, CH_3CN, room temperature, 3 h; (b) H_2, Pd/C (5%), 40 psi, room temperature, 4 h; (c) $(PhO)_3PMeI$, CH_3CN, room temperature, 4 h; (d) 1 M LiOH, MeOH-H_2O, room temperature, 2.5 h; (e) RNa, DMF, 18-crown-6, 60 °C, 2.5 h; (f) HCl; (g) DCC/HOBt, DMF + **12**, *N*-methylmorpholine; (h) trifluoroacetic acid, room temperature, 3 h.

Scheme 4. Solid-Phase Synthesis of the (*R*,*R*) Isomer[a]

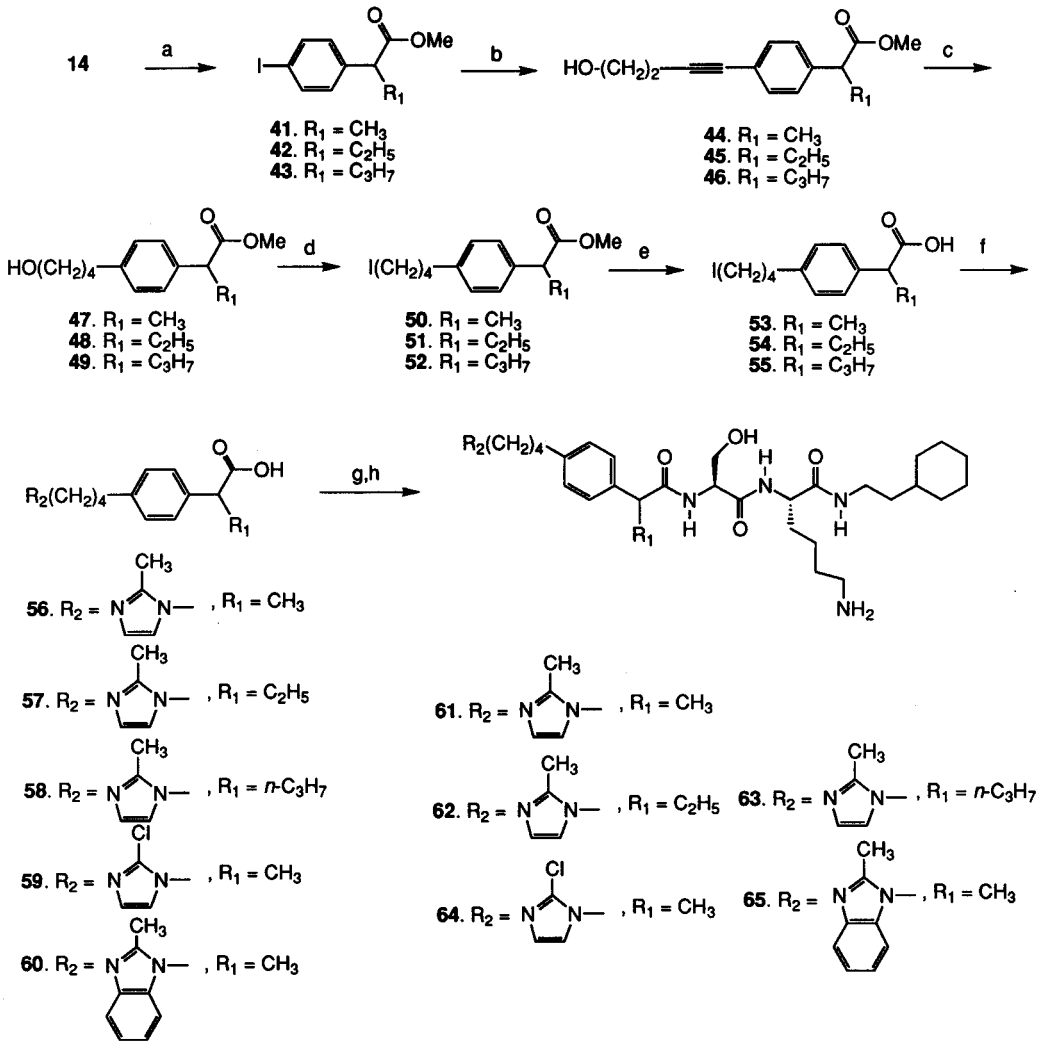


[a] Reagents: (a) 50% TFA/CH_2Cl_2, 30 min, room temperature; (b) DIEA; (c) BOC-D-Ser(*O*Bn)-OH, DCC, 1 h; (d) **24**, DCC, HOBt, DMF, 18 h; (e) 2-cyclohexylethylamine, 50 °C, 5 h; (f) 90% HF/anisole, 0 °C 1 h.

50 °C for 5 h, treating the resin-free material with 90% HF−anisole at 0 °C for 1 h, and purification by reverse-phase HPLC.

The benzylic α-substituted imidazole compounds **61**−**65** were prepared as illustrated in Scheme 5 starting from methyl 4-iodophenylacetate **14**. Synthesis of **61** began with alkylation of **14** with methyl iodide in the presence of NaH in tetrahydrofuran to afford the racemic propionate ester **41**. The ester was then coupled with 3-butyn-1-ol using $PdCl_2(PPh_3)_2$, CuI, and triethylamine. The resulting acetylenic product **44** was purified and subjected to catalytic reduction to obtain

Scheme 5[a]



[a] Reagents: (a) NaH, THF, room temperature, 16 h; (b) 3-butyn-1-ol, $PdCl_2(Ph_3)_2$, CuI, Et_3N, CH_3CN, room temperature, 3 h; (c) H_2, Pd/C (5%), 40 psi, room temperature, 4 h; (d) $(PhO)_3PMeI$, CH_3CN, room temperature, 4 h; (e) 1 M LiOH, $MeOH-H_2O$, room temperature, 2.5 h; (f) RNa, DMF, 18-crown-6, 60 °C, 2.5 h; (g) DCC/HOBt, DMF + **12**, *N*-methylmorpholine; (h) trifluoroacetic acid, room temperature, 3 h.

the hydroxy ester **47**. Treatment of **47** with $(PhO)_3PMeI$, followed by hydrolysis of the ester **50** and acidification, yielded the acid **53**. In the following step, **53** was condensed with sodium 2-methylimidazolide in dimethylformamide to obtain the corresponding imidazole-substituted carboxylic acid **56**. Subsequent activation of **56** using DCC/HOBt and coupling with the free amine generated from **12**, followed by deprotection and purification by reverse-phase HPLC, provided **61** as a diastereomeric mixture. The (*R*,*S*)-ethyl-**62**, (*R*,*S*)-propyl-**63**, and (*R*,*S*)-2-chloroimidazole **64** and (*R*,*S*)-2-methylbenzimidazole **65** analogs were synthesized using a similar reaction sequence as that outlined in Scheme 5.

Chiral separation of **61** was achieved by HPLC (Figure 3) using a chirobiotic-V column packed with the chiral selector vancomycin linked to silica gel[23] as the stationary phase and eluting with 23% acetonitrile in ammonium phosphate buffer at pH 4.2 to afford the corresponding diastereomers **61a** and **61b** having >95% purity as indicated by their ^{1}H-NMR spectra.

Reference dipeptides **68** and **69**, which enabled assignment of absolute configuration at the new chiral center in diastereomers **61a** and **61b**, were prepared according to Scheme 6. The commercially available (*R*)- and (*S*)-phenylpropionic acids **66** and **67** were individually coupled with the free amine generated from **12**, using DCC/HOBt. The resulting products were treated with trifluoroacetic acid, and the dipeptides **68** and **69** were isolated by reverse-phase chromatography.

Scheme 7 illustrates the synthesis of tripeptides **73a**–**73c** starting from the differentially protected lysine derivative **70**. Coupling of **70** with β-cyclohexylalanine methyl ester using DCC/HOBt provided the dipeptide ester **71**. The carbobenzoxy group in **71** was removed by catalytic hydrogenation, and the resulting amine was condensed with the 2-methylimidazole derivative **24** using DCC/HOBt to afford the tripeptide **72**. The ester group in **72** was converted to the corresponding acid with 1 M LiOH followed by acidification, and the resulting crude product was treated with trifluoroacetic acid. The final compound **73a** was isolated by reverse-phase HPLC purification. The racemic α-methyl analog **73b** was prepared and purified in a similar manner as described for **73a**, using the imidazole acid **56** instead of **24**. The tripeptide ester **73c** was prepared by coupling the free amine generated from **72** with the acid **24** via activation using DCC/HOBt, followed by deprotection of the crude product with trifluoroacetic acid and HPLC purification.

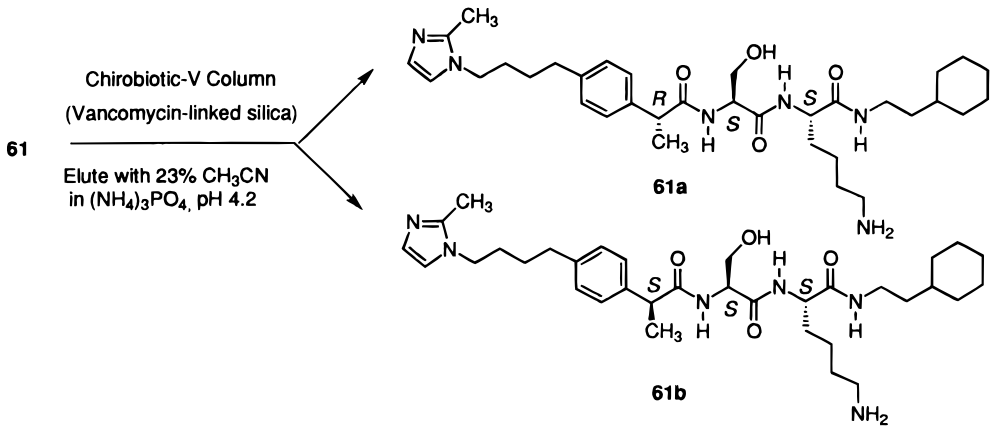
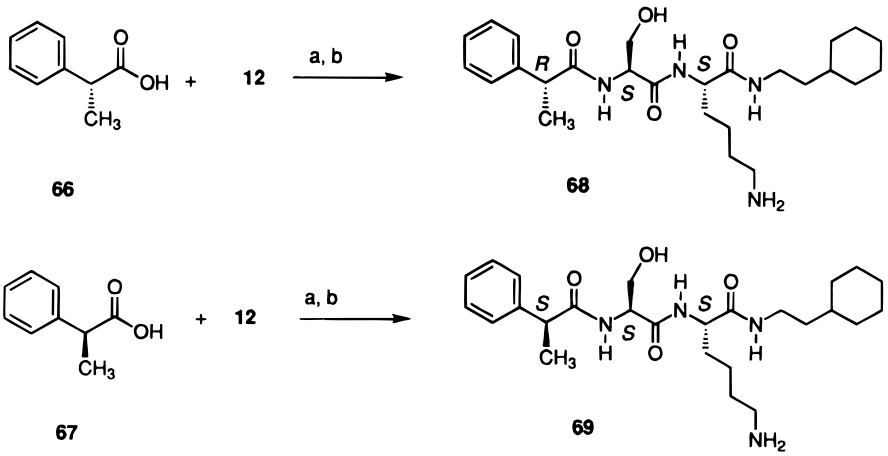


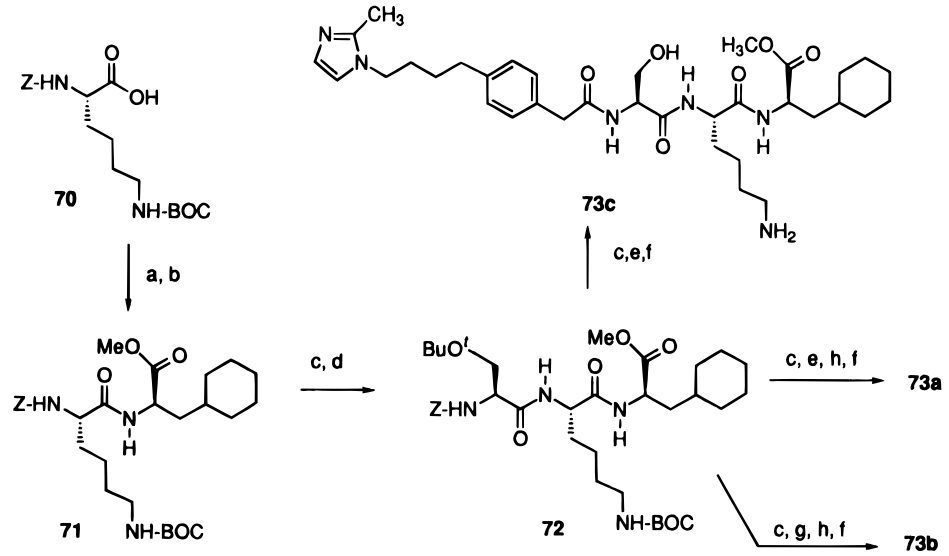
Figure 3. Chiral separation of **61**.

Scheme 6. Synthesis of Reference Peptides[a]



[a] Reagents: (a) DCC, HOBt, 0 °C 1 h, *N*-methylmorpholine, room temperature, 16 h; (b) trifluoroacetic acid, room temperature, 3 h.

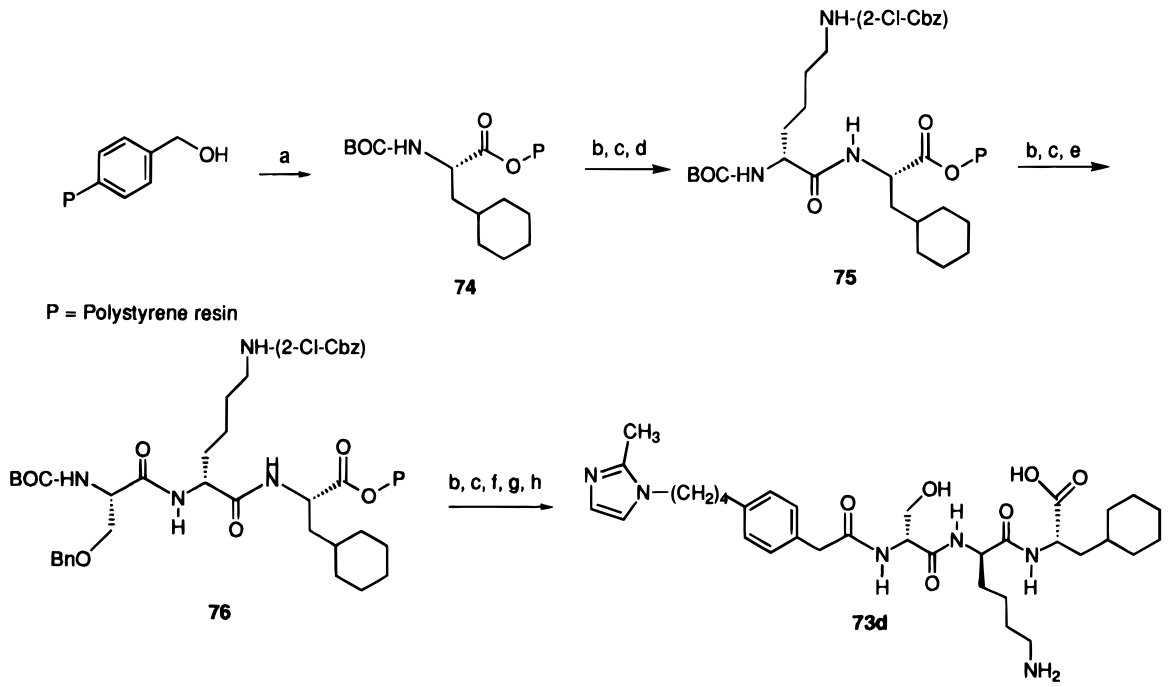
Scheme 7. Synthesis of Imidazole-Substituted Tripeptide NMT Inhibitors[a]



[a] Reagents: (a) DCC, HOBt, dimethylacetamide, CH_2Cl_2, 0 °C, 1 h; (b) L-cyclohexylalanine methyl ester hydrochloride, *N*-methylmorpholine, room temperature, 16 h; (c) H_2, 5% Pd/C, room temperature, 1 h; (d) Z-Ser(*O*^{t}Bu)-OH, DCC, HOBt, CH_2Cl_2, dimethylacetamide, 0 °C, 1.5 h, room temperature, 48 h; (e) **24**, DCC, HOBt, 0 °C, 1.5 h, room temperature, 24 h, (f) trifluoroacetic acid, room temperature, 3 h; (g) **56**, DCC, HOBt, 0 °C, 1.5 h, room temperature, 24 h; (h) 2 M LiOH, dioxane, H_2O, room temperature, 2 h, HOAc.

The enantiomer **73d** was synthesized using solid-phase methods, according to Scheme 8. The first step involved the attachment of BOC-protected D-cyclohexylalanine to the polystyrene resin using DCC to afford **74**. Cleavage of the BOC group in **74** with 50% trifluoroacetic acid, followed by generation of the free amine and condensation with the BOC-protected D-lysine derivative via activation using DCC, gave the dipeptide **75** linked to the resin. This reaction sequence was repeated on **75** to attach the BOC-protected D-serine to the lysine residue on the resin to obtain **76**. In a similar fashion using **76**, the 2-methylimidazole-

Scheme 8. Solid-Phase Synthesis of the (*R,R,R*) Isomer[a]



[a] Reagents: (a) Boc-β-cyclohexyl-D-Ala, DCC, 1 h; (b) 50% TFA/CH_2Cl_2, 30 min, room temperature; (c) DIEA, CH_2Cl_2; (d) Boc-D-Lys(2-Cl-Cbz), DCC, 1 h; (e) BOC-D-Ser(OBn)-OH, DCC, 1 h; (f) **24**, DCC, HOBt, DMF, 18 h; (g) 90% HF/anisole, 0 °C, 1 h; (h) reverse-phase (C_{18}) HPLC, 5−55%.

carboxylic acid **24** was coupled to the serine residue on the resin. The peptide product was simultaneously deprotected and cleaved from the resin and purified by reverse-phase HPLC to yield the tripeptide **73d**.

Enzyme Assay

The final compounds were evaluated for the inhibition of recombinant human and *Candida* NMTs as described previously.[12] Both enzymes were expressed in *E. coli* and were purified to apparent homogeneity according to protocols described in ref 24. Standard solutions of inhibitors were prepared at 22 mM in 0.5% DMSO. The IC_{50} determinations (concentration required for 50% inhibition) involved incubation of the compound at a series of known concentrations with *C. albicans* NMT in the presence of 0.11 nmol of [^{3}H]myristoyl CoA (1 μCi, 9.09 Ci/mmol) and 2.2 nmol of GNAASARR-NH_2 in a total volume of 60 μL. After a 10 min incubation at 24 °C, the reaction was quenched by addition of ice-cold methanol. The products were separated by HPLC on a Vydac-C_4 column and quantitated by in-line scintillation counting.

The reported IC_{50}s represent an average of two independent determinations each done in duplicate. K_i's are reported as apparent inhibition constants and were determined as described in ref 12.

Antifungal Assay

NMT inhibitors were tested for anti-*Candida* activity according to the following protocol.[25] Briefly, 20 mM stock solutions of the inhibitors were prepared in DMSO (1% v/v) and diluted into sterile deionized water to obtain twice the final desired concentration for the cultured-based assay. Portions (100 μL) of the stock solutions were dispensed into triplicate wells of a 96-well microtiter plate (Costar). *C. albicans* strain B311 [a 100 μL portion containing 10^5 cells in 2 × yeast nitrogen broth (YNB)] was distributed into each of the wells. Controls included DMSO alone and amphotericin B (final concentration = 0.05, 1, and 5 μg mL^{-1}; positive control). Plates were incubated at 30 °C, and growth was scored by monitoring the OD_{490} using an ELISA plate reader (Dynatech). EC_{50}, the concentration of the compound inhibiting the growth by 50% compared to untreated controls, was determined at 24 h. All experiments were done in triplicate on at least three occasions. Within a given experiment, the triplicate determinations varied by <10% per sample.

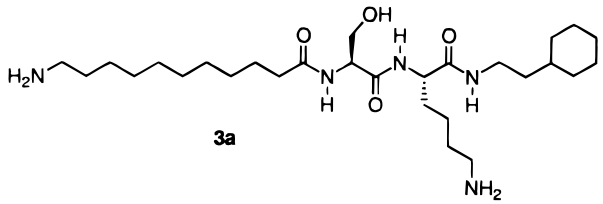


Figure 4. Dipeptide inhibitor of *Candida albicans* NMT.

Results and Discussion

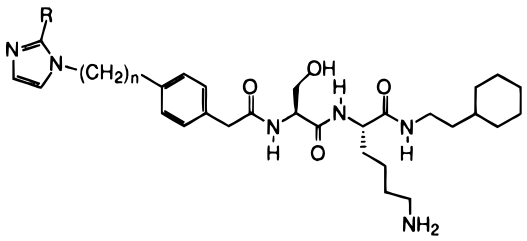
Our initial structural modification of **1a** by replacing the amino acid residues at positions 1−4 with an 11-aminoundecanoyl moiety, and substituting the C-terminal Leu-Ser-carboxamide with a cyclohexylethyl group, led to the identification of the dipeptide amide lead **3a** (Figure 4) with good activity against *C. albicans* NMT (IC_{50} = 0.11 ± 0.03 μM) and little selectivity (7-fold) versus the human enzyme.[12,26] Unfortunately no antifungal activity was detected for **3a** in cell cultures even at concentrations of 100 μM.[26] This may be a consequence of inadequate inhibitor potency or ineffective cellular penetration due to the presence of two basic (pK_a ~10) primary amine groups in **3a**. To overcome this problem, efforts were directed toward finding a less basic replacement for the N-terminal amino group. An N-linked imidazole in place of the N-terminal amino group was one of the choices investigated since the pK_a

Table 1. Potency of Imidazole-Substituted NMT Inhibitors with Varying Chain Lengths[a]

		IC_{50}, μM	
compd	*n*	*C. albicans* NMT	human NMT
8	8	5.5 ± 1.3	ND
9	9	3.0	ND
10	11	3.0	ND
13	10	1.55 ± 0.07	11.35 ± 0.49

[a] Potency against the indicated NMT as assessed by IC_{50} using the peptide GNAASARR-NH_2 at its apparent K_m (see text) and myristoylCoA at 1 μM. ND = not determined.

Table 2. Potency and Selectivity of Imidazole-Substituted NMT Inhibitors[a]



			IC_{50}, μM		
compd	*n*	R	*C. albicans* NMT	human NMT	selectivity[b]
36	5	H	1.2	ND	ND
30	4	H	0.31 ± 0.06	16 ± 2.97	51
31	4	CH_3	0.056 ± 0.0	14.1 ± 4.25	252
33	4	C_2H_5	0.147 ± 0.01	28.2	192
34	4	*i*-C_3H_7	0.2 ± 0.04	>100	ND
32	4	I	1.88 ± 0.03	ND	ND
40	4	CH_3	62.4 ± 13	ND	ND

[a] Potency against the indicated NMT as assessed by IC_{50} using the peptide GNAASARR-NH_2 at its apparent K_m (see text) and myristoylCoA at 1 μM. [b] Selectivity is the ratio of the IC_{50} for human NMT divided by the IC_{50} for *C. albicans* NMT. ND = not determined.

of imidazole (~7.5) is comparable with that of alanine or glycine.[27] A brief structure−activity (SAR) study that varied the connecting chain length between the N-1 nitrogen of imidazole and the serine residue (Table 1) revealed that an 11-carbon atom linker, as exemplified by **13**, conferred maximum potency (IC_{50} = 1.55 ± 0.07 μM) for *C. albicans* NMT. It is pertinent to note that analogs of **3a** containing a much less basic nitrogen heterocycle such as 1,2,4-triazole, or more basic functional groups such as guanidines or piperazines in place of imidazole, were significantly less effective NMT inhibitors.[28]

At this stage, further enhancements to this series to attain potencies below 1 μM presented a synthetic challenge. We reasoned that the activity of these inhibitors might be improved by introducing conformational constraints in the flexible linear linker chain using a 1,4-disubstituted phenyl ring. A recent study had demonstrated that incorporation of a rigidifying element such as 1,4-phenylene moiety β to the undecanoyl carbonyl group in **3a** was well tolerated by the enzyme.[26] As the distance between the ω-amino group and undecanoyl carbonyl functionality is critical for molecular recognition, imidazole compounds **30**−**36** were synthesized with four or five methylene groups connecting the imidazole nitrogen with the aromatic ring. Comparision of the IC_{50}s (Table 2) of **30** (IC_{50} = 0.31 ± 0.06 μM) and its homolog **36** (IC_{50} = 1.2 μM) suggested that placing the imidazole moiety four methylene units from the phenyl ring resulted in a 4-fold enhancement in potency. Furthermore, examination of the potencies of **13** (IC_{50} = 1.55 ± 0.07 μM) and **30** indicated that introduction of conformational restrictions in the flexible alkyl chain linker confers about 5-fold improvement in potency and a 10-fold increase in selectivity versus the human NMT (IC_{50} = 16 ± 2.97 μM). The analog **30** is the first example of a competitive peptidomimetic NMT inhibitor (K_i = 0.59 ± 0.07 μM) having an amphoteric group at the N-terminus and a potency below 1 μM against the *C. albicans* enzyme.

A further improvement in potency was realized by replacing the imidazole with a more basic 2-methylimidazole as exemplified by **31**. It is remarkable that a seemingly minor structural modification of **30**, *i.e.* introduction of a methyl group into an imidazole ring, provided both enhanced potency (IC_{50} = 0.056 ± 0.01 μM, *C. albicans* NMT; and 0.035 ± 0.008 μM, *S. cerevisiae* NMT) and an additional 10-fold improvement in selectivity (250-fold versus human NMT, Table 2). This may be attributable to the measured pK_a of the 2-methylimidazole (7.1 ± 0.5), which is about 3 pH units lower than the measured pK_a (~10.0) of the N-terminal amine group in **3a** and comparable to the estimated pK_a (7.7) of alanine in **2**. Kinetic analysis indicated that **31** is a competitive inhibitor (K_i = 0.031 ± 0.003 μM) with respect to the peptide substrate GNAASARR-NH_2.

Replacement of the 2-methyl group in **31** by electronegative groups which would lower the pK_a by 2−3 units,[29] such as iodo, to obtain **32** (IC_{50} = 1.9 ± 0.03 μM) markedly reduced potency by 36-fold. A similar trend was observed when the 2-methyl group was replaced by bulkier groups such as ethyl or isopropyl, as illustrated by analogs **33** and **34**. Substitution of 2-methylimidazole with less basic heterocycles such as 2-methylbenzimidazole to obtain **35** (IC_{50} = 0.6 ± 0.16 μM) also led to diminished potency.

To further augment the inhibitory activity of NMT inhibitors, we sought additional structural features that might impart higher binding affinity. A comparision of $K_{m(apparent)}$ values of the octapeptide substrates **1a** and **1b** revealed that substitution of alanine-4 in **1a** by glycine to give **1b** resulted in a 10-fold increase in K_m.[17] This observation suggested that the side chain methyl of alanine-4 in **1a** also contributes to binding. Thus, analogs of **31** with an α-alkyl substiutent R_1 at the benzylic position adjacent to serine might provide the conformational bias present in **1a** and consequently exhibit higher potencies. This hypothesis set the stage for synthesis of a series of benzylic α-substituted imidazole compounds **61**−**65**.

The diastereomers **61a** and **61b** were isolated by chiral chromatography as depicted in Figure 3. The assignment of absolute configuration at the benzylic chiral centers was established by comparing the ^{1}H-NMR spectra of **61a** and **61b** with those of reference compounds **68** and **69**. The methyl group in (*S*,*S*,*S*) **69** displayed a characteristic downfield shift when compared with the corresponding (*R*,*S*,*S*) diastereomer **68**. A similar downfield shift of the methyl signal was also observed in **61b** when compared with its diastereomer **61a**. Furthermore, the proton chemical shifts of the α-methine protons for the serine and lysine residues in **61a** and **61b**, as confirmed by proton-COSY experiments, were identical with the corresponding proton chemical shift values observed for **68** and **69**.

Analogs **61**−**65**, **61a**, and **61b** were evaluated for inhibition of *C. albicans* and human NMT (Table 3). The

Table 3. Potency and Selectivity of Benzylic-Substituted NMT Inhibitors[a]

compd	R_1	IC_{50}, μM: *C. albicans* NMT	IC_{50}, μM: human NMT	selectivity[b]
61	CH_3 (racemic)	0.04 ± 0.003	8 ± 0.42	200
61a	CH_3 (*R*)	0.02 ± 0.001	8.2	410
61b	CH_3 (*S*)	0.31	49	160
62	C_2H_5 (racemic)	0.042 ± 0.013	1.54 ± 0.01	37
63	*n*-C_3H_7 (racemic)	0.130 ± 0.01	0.49 ± 0.01	4
64	CH_3 (racemic)	2.05 ± 0.49	142.0	69
65	CH_3 (racemic)	0.86 ± 0.48	119.5 ± 31.8	139

[a] Potency against the indicated NMT as assessed by IC_{50} using the peptide GNAASARR-NH_2 at its apparent K_m and myristoylCoA at 1 μM. [b] Selectivity is the ratio of the IC_{50} for human NMT divided by the IC_{50} for *C. albicans* NMT.

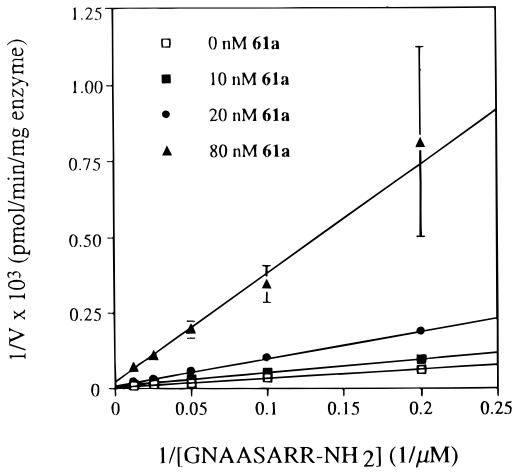


Figure 5. Double-reciprocal plot of 1/*V* vs 1/[GNAASARR-NH_2] for inhibitor **61a** with *C. albicans* NMT. GNAASARR-NH_2 (K_m = 20 μM) concentrations of 5, 10, 20, 40, and 80 μM were used with a fixed [^{3}H]myristoylCoA concentration of 1 μM (9.09 Ci/mmol) and 15 ng of NMT. The secondary plot of slopes vs 1/[**61a**] yielded a $K_{i(app)}$ of 27 ± 7 nM. The data are plotted as the mean of the reciprocal velocity ± the standard deviation of the triplicate measurements. Error bars that would obscure data points are omitted for clarity.

SAR data suggest that the observed potency and selectivity are a function of the size and orientation of the α-substituent at the benzylic position. While the α-methyl group in **61** delivers maximum potency (IC_{50} = 0.04 ± 0.003 μM, *C. albicans* NMT; and 0.017 μM, *S. cerevisiae* NMT) and selectivity versus **31**, the incorporation of a bulkier group such as *n*-propyl (**63**) results in dramatically reduced potency against *C. albicans* NMT and enhanced activity against human enzyme. Inclusion of an α-ethyl group (**62**) retains potency comparable to that of the α-methyl group against the fungal NMT, but its selectivity is reduced significantly.

A detailed analysis of the inhibitory profile of individual isomers revealed that the (*R*,*S*,*S*) diastereomer **61a** is a highly potent inhibitor with an IC_{50} of 20 ± 0.99 nM for *C. albicans* NMT and exhibits 400-fold selectivity versus the human enzyme (Table 3). The corresponding (*S*,*S*,*S*) isomer **61b** is 15-fold less potent (IC_{50} of 310 nM against the fungal enzyme) and "only" 150-fold selective versus human NMT. A more detailed enzyme kinetic analysis of **61a** revealed it to be a competitive inhibitor (K_i = 27 ± 7 nM, Figure 5) with respect to the peptide substrate GNAASARR-NH_2. Importantly, the (*R*)-methyl group in **61a**, which corresponds to the (*S*)-4-alanine methyl configuration in **1a**, confers decreased potency against human NMT while increasing it against the fungal enzyme.

Substitution of the 2-methylimidazole in **61** with less basic groups such as 2-chloroimidazole (**64**) or 2-methylbenzimidazole (**65**, $pK_{a(est)}$ = 6.3) resulted in significantly reduced potency (Table 3). In the case of the 2-chloro analog, although the size of the halo group is comparable to that of the 2-methyl group, the inductive effect is likely to attenuate the pK_a of the imidazole ring which appears detrimental to inhibitory activity.[29] These results suggest that the protonation of the imidazole ring is essential for tight binding of these peptidomimetic ligands to the peptide binding site provided by the myristoylCoA:NMT binary complex.

All of the NMT inhibitors described above were evaluated for their antifungal activity in a cell culture assay. Surprisingly none of the final compounds displayed any growth inhibitory activity (EC_{50} > 100 μM) against *C. albicans* (or *C. neoformans*). Consequently, our synthetic efforts were focused on improving the cell-penetration properties of these NMT inhibitors by installing a carboxyl group β to the cyclohexyl moiety. This approach resulted in the synthesis of tripeptide analogs **73a** and **73b**. Both these analogs were moderate inhibitors of *C. albicans* NMT [IC_{50} = 1.45 ± 0.08 and 0.38 ± 0.03 μM, respectively (Table 4)]. However, both displayed a dramatically enhanced selectivity of 560- and 2200-fold, respectively, toward the *C. albicans* NMT, an unexpected "beneficial" effect of introducing the carboxyl group (Table 4). More importantly, compound **73a** exhibited growth inhibitory activity (Table 4) with an EC_{50} of 51 ± 17 μM, 24 h, after a single dose administration to cultures of a well-characterized amphotericin B-sensitive and fluconazole-sensitive clinical isolate of *C. albicans* (strain B311, ref 25). The compound had no growth inhibitory activity against *C. neoformans* (EC_{50} >100 μM).

An assay has been developed recently to test the effects of *in vitro* inhibitors of purified NMT on cellular NMT activity in exponentially growing cultures of *C. albicans*.[25] The assay takes advantage of the fact that one of the most prominent *C. albicans N*-myristoylproteins is an Arf whose mobility during SDS−polyacrylamide gel electrophoresis is greater when it is N-myristoylated than when it is not. The ratio of N-myristoylated to nonmyristoylated Arf can be taken as a measure of cellular NMT activity. This ratio is defined by Western blot analysis of total cellular proteins isolated prior to and after exposure to an enzyme inhibitor. Studies of *C. albicans* strains with conditional lethal *NMT* gene mutations indicate that a reduction in the level of N-myristoylated Arf to ≤50% of total Arf is associated with growth arrest and death.[25] An Arf protein gel mobility shift assay indicated that a single 200 μM dose of **73a** or **73b** produces a <50% reduction in Arf N-myristoylation after 4 h, which is consistent with their observed fungistatic but not fungicidal activity.[25]

The racemic α-methyl analog **73b** is 4-fold more potent and exhibits similar anti-*Candida* activity as **73a** (Table 4). Like **73a**, **73b** has no growth inhibitory effects on *C. neoformans* (EC_{50} > 100 μM). It is pertinent to note that the tripeptide ester **73c** which is about 18-fold more potent than **73a** did not display any antifungal activity against either organism. This finding suggests that installing a carboxyl group β to the cyclohexyl moiety promotes cellular uptake of NMT inhibitors. The (*R*,*R*,*R*) enantiomer (**73d**) which had an IC_{50} of >1000 μM against *C. albicans* NMT had no

Table 4. Biological Activity of Imidazole-Substituted Tripeptide NMT Inhibitors[a]

	IC_{50}, μM			EC_{50}, μM[c]	
compd	*C. albicans* NMT	human NMT	selectivity[b]	24 h, *C. albicans*	24 h, *C. neoformans*
73a	1.45 ± 0.08	809.5 ± 33.2	560	51 ± 17	>100
73b	0.38 ± 0.03	840 ± 28.3	2200	33	>100
73c	0.081 ± 0.01	22.7 ± 2.7	276	>100	>100
73d	>1000	>1000		>100	>100

[a] Potency against the indicated NMT as assessed by IC_{50} using the peptide GNAASARR-NH_2 at its apparent K_m and myristoylCoA at 1 μM. [b] Selectivity is the ratio of the IC_{50} for human NMT divided by the IC_{50} for *C. albicans* NMT. [c] See text.

growth inhibitory effect against *C. albicans* (or *C. neoformans*) and did not produce any detectable reduction in *C. albicans* Arf N-myristoylation.

Together, these data confirm that the antifungal activity exhibited by peptidomimetic inhibitors **73a** and **73b** is a consequence of attenuation of intracellular *C. albicans* NMT activity. The fungistatic instead of fungicidal activity of **73a** may be attributed to its observed susceptibility to degradation by cellular carboxypeptidases: repeated addition of **73a** to cultures produces a more prolonged growth suppressive effect. The antifungal effects of **73a** and **73b** are species-specific. The lack of broad spectrum activity in these compounds may reflect a number of mechanisms including, for example, differences in their uptake by *C. albicans* and *C. neoformans*, differences in their intracellular stabilities or compartmentalization within these organisms, and/or differences in their recognition by the peptide binding sites of the two fungal NMTs.

Conclusions

We have discovered a new class of potent and selective imidazole-substituted peptidomimetic inhibitors of *C. albicans* NMT, starting from a weak octapeptide inhibitor ALYASKLS-NH_2 **2**. Remarkably, *2-methylimidazole* effectively mimics the pK_a of the alanine residue in **2** and confers high affinity and selectivity in combination with a phenacetyl moiety. We have also identified a chiral recognition element which led to the discovery of the most potent *C. albicans* NMT inhibitor **61a** reported to date. The high affinity and selectivity manifested by **31** and **61a** toward fungal NMTs are notable since the human enzyme recognizes a much wider variety of protein substrates than its fungal counterpart. Furthermore, we have demonstrated by synthesis and chiral separation that incorporation of a methyl group with (*R*) stereochemistry provides a favorable conformational bias and results in a high-affinity ligand for the fungal enzyme. The synthetic protocols described above allow preparation of a variety of heteroatom or heterocyclic substituted 4-phenylacetic acid analogs. The solid-phase methodology adds synthetic diversity and makes a combinatorial approach feasible as a future alternative. The conceptual framework and the synthetic efforts have led to the identification of selective *Candida* NMT inhibitors **73a** and **73b** with a mechanism-based antifungal activity. Incorporation of a carboxyl moiety β to the cyclohexyl group results in a dual affect of enhancing selectivity and imparting antifungal activity. The SAR studies reported in this paper should serve as a basis for future efforts to develop nonpeptidal and more potent inhibitors of NMT as novel antifungal agents.

Experimental Section

Unless otherwise stated, starting materials were obtained from commercial suppliers and were used without further purification. L-Amino acid derivatives were purchased from Sigma. Melting points were determined with a Melt-Temp apparatus and are uncorrected. All reactions were performed under anhydrous conditions in an atmosphere of argon. Nuclear magnetic proton and carbon-13 spectra 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, unless otherwise noted. Amino acid analyses were performed following hydrolysis in 6 N HCl at 150 °C *in vacuo* for 1.5 h using a Beckman 6300 high-performance analyzer. The purity of all the reported compounds was found to be >96% by ^{1}H-NMR and HPLC techniques.

Analytical reverse-phase high-performance liquid chromatography (HPLC) was carried out on 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 5 to 70% 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 separation was monitored by UV absorbance at 215 nm. The appropriate fractions were pooled and freeze-dried, and the products were isolated as TFA salts.

Solid-phase peptide synthesis was carried out on polystyrene resin using BOC-protected amino acid derivatives purchased from Nova-Biochem.

Ser(OBn)-Lys(Cbz)-2-cyclohexylethylamide Hydrochloride (4). A mixture of the lysine derivative **3b** (6.0 g, 0.0158 mol), HOBt (2.07 g, 0.0158 mol), and EDC (3.03 g, 0.0158 mol) in dry DMF (40 mL) was stirred at room temperature. After 1 h, a solution of 2-cyclohexylethylamine (2.01 g, 0.0158 mol) in dry DMF (10 mL) was added, and the mixture was stirred at room temperature for 18 h. The reaction mixture was diluted with dichloromethane (400 mL),washed sequentially with saturated $NaHCO_3$ (2 × 200 mL) and brine (2 × 200 mL), and dried ($MgSO_4$). Removal of the solvent afforded 6.0 g (78%) of **3c** as a white solid: ^{1}H-NMR ($CDCl_3$) δ 7.31−7.34 (m, 5H), 6.15 (s, 1H), 5.28 (s, 2H), 4.90 (s, 1H), 3.17−3.26 (m, 4H), 0.86 −1.84 (m, 28H); HRMS (EI) *m/z* calcd for $C_{27}H_{43}N_3O_5$ 489.3203(M^+), found 489.3208.

This lysine derivative **3c** (6.0 g, 0.012 mol) was stirred with 4 N HCl in dioxane (30 mL) for 1 h at room temperature. The reaction mixture was concentrated under reduced pressure, and the residue was dried *in vacuo* to afford the hydrochloride salt (5.75 g) as a pale yellow solid: ^{1}H-NMR (CD_3OD) δ 7.33−7.34 (m, 5H), 5.06 (s, 2H), 3.74 (m, 2H), 3.31 (m, 4H), 0.90−1.89 (m, 19H); HRMS (EI) *m/z* calcd for $C_{22}H_{35}N_3O_3$ 389.2678 (M^+), found 389.2658. This material was used without purification in the following step.

A mixture of Boc-Ser(OBn)-OH (3.81 g, 0.013 mol), HOBt (1.70 g, 0.013 mol), and EDC (2.47 g, 0.013 mol) in dry DMF (40 mL) was stirred at room temperature. After 1 h, a solution of the hydrochloride salt (5.5 g, 0.013 mol) obtained as above from **3c** in dry DMF (20 mL), and triethylamine (1.30 g, 0.013 mol) were added, and the mixture was stirred at room temperature for 18 h. The reaction mixture was diluted with

dichloromethane (400 mL) and washed successively with saturated $NaHCO_3$ (2 × 200 mL) and brine (2 × 200 mL) and dried ($MgSO_4$). After removal of the solvents under reduced pressure, the resulting pale yellow solid (6.63 g) was stirred with 4 N HCl in dioxane (30 mL) for 1 h at room temperature and concentrated under reduced pressure. The resulting hydrochloride salt was dried *in vacuo* to give 6.27 g of **4** as a pale yellow solid which was used without purification: ^{1}H-NMR (CD_3OD) δ 7.29−7.36 (m, 10H), 5.06 (s, 2H), 4.60 (s, 2H), 4.29−4.34 (m, 1H), 4.09−4.13 (m, 1H), 3.65−3.88 (m, 2H), 3.18−3.07 (m, 4H), 0.86−1.71 (m, 19H); HRMS (EI) *m/z* calcd for $C_{32}H_{46}N_4O_5$ 566.3468 (M^+), found 566.3502. Amino acid analysis: Ser 1.00 (1.00), Lys 1.00 (1.00).

9-(1*H*-Imidazol-1-yl)nonanoyl-Ser-Lys-*N*-(2-cyclohexylethyl)amide (8). A mixture of the acid **5** (0.08 g, 0.34 mmol), HOBt (0.046 g, 0.34 mmol), and EDC (0.065 g, 0.34 mmol) in dry DMF (5 mL) was stirred at room temperature for 40 min. It was then treated with a solution of **4** (0.2 g, 0.34 mmol) and triethylamine (0.034 g, 0.34 mmlo) in dry DMF (2 mL), and the resulting mixture was stirred at room temperature for 18 h. The reaction mixture was diluted with dichloromethane (100 mL) and washed successively with saturated $NaHCO_3$ (2 × 100 mL) and brine (2 × 100 mL) and dried ($MgSO_4$). After removal of the solvent, the residue was purified by silica gel flash chromatography using 1−4.5% methanol in dichloromethane as the eluent to furnish 0.14 g (52%) of the protected dipeptide as a white solid. This was dissolved in methanol (10 mL), 1 N HCl (0.15 mL) and 5% Pd−C (0.10 g) were added, and the resulting suspension was stirred under H_2 (45 psi) for 32 h at room temperature. The catalyst was removed by filtration through Celite, the filtrate was concentrated under reduced pressure, and the residue was purified by reverse-phase HPLC. The appropriate fractions were pooled and concentrated under reduced pressure to give 0.07 g (46.0%) of **8** as a glassy substance: ^{1}H-NMR (CD_3OD) δ 8.95 (s, 1 H), 7.65 (d, 1 H, J = 1.61 Hz), 7.56 (d, 1 H, J = 1.61 Hz), 4.37−4.30 (m, 2H), 4.24 (t, 2 H, J = 7.35 Hz), 3.86−3.81 (m, 1H), 3.74−3.64 (m, 1H), 3.18 (t, 2H, J = 7.25 Hz), 2.92 (t, 2H, J = 7.37 Hz), 2.26 (t, 2H, J = 7.55 Hz), 1.97−0.85 (m, 31H); HRMS *m/z* calcd for $C_{29}H_{52}N_6O_4$ 549.4128 (M + H), found 549.4106. Amino acid analysis: Ser 1.00 (0.97), Lys 1.00 (1.03).

10-(1*H*-Imidazol-1-yl)decanoyl-Ser-Lys-*N*-(2-cyclohexylethyl)amide (9) was prepared in a manner similar to **8** by coupling **6** with **4** in 42% yield as a white glassy substance: ^{1}H-NMR (CD_3OD) δ 8.95 (s, 1 H), 7.65 (s, 1 H), 7.55 (s, 1 H), 4.36−4.31 (m, 2H), 4.24 (t, 2 H, J = 7.34 Hz), 3.86−3.82 (m, 1H), 3.74−3.69 (m, 1H), 3.18 (t, 2H, J = 7.25 Hz), 2.92 (t, 2H, J = 7.39 Hz), 2.26 (t, 2H, J = 7.52 Hz); 1.98−0.85 (m, 33H); HRMS *m/z* calcd for $C_{30}H_{54}N_6O_4$ 563.4285 (M + H), found 563.4290. Amino acid analysis: Ser 1.00 (0.92), Lys 1.00 (1.08).

12-(1*H*-Imidazol-1-yl)dodecanoyl-Ser-Lys-*N*-(2-cyclohexylethyl)amide (10) was prepared in a manner similar to **8** by coupling **7** with **4**: ^{1}H-NMR (CD_3OD) δ 8.95 (s, 1 H), 7.65 (d, 1 H, J = 1.61 Hz), 7.56 (d, 1 H, J = 1.61 Hz), 4.36−4.31 (m, 2H), 3.86−3.81 (m, 1H), 4.24 (t, 2 H, J = 7.25 Hz), 3.74−3.68 (m, 1H), 3.18 (t, 2H, J = 7.25 Hz), 2.92 (t, 2H, J = 7.35 Hz), 2.56 (t, 2H, J = 7.55 Hz), 1.98−0.85 (m, 37H); HRMS *m/z* calcd for $C_{32}H_{58}N_6O_4$ 591.4598 (M + H), found 591.4649.

Methyl 4-(4-Hydroxy-1-butynyl)phenylacetate (15). To a solution of butyn-1-ol (0.77 g, 0.011 mol) and methyl 4-iodophenylacetate (1.5 g, 0.0054 mol) in acetonitrile (10 mL) at 0 °C was added triethylamine (1.5 mL, 0.01 mol), followed by the addition of bis(triphenylphosphine)palladium chloride (0.25 g, 0.36 mmol) and CuI (0.025 g). The reaction mixture was stirred at 0 °C under an argon atmosphere for 30 min and at room temperature for 3 h. The dark-colored reaction mixture was concentrated under reduced pressure, and the residue was partitioned between 5% citric acid (50 mL) and EtOAc (50 mL). The organic phase was washed with 5% citric acid (3 × 15 mL) and water, dried (Na_2SO_4), and concentrated. The resulting material was purified by silica gel flash chromatography using 35% EtOAc in hexane as the eluent to afford 1.0 g (85%) of **15** as a dark-colored liquid: ^{1}H-NMR ($CDCl_3$) δ 7.36 (d, 2H, J = 8.1 Hz), 7.21 (d, 2H, J = 8.1 Hz), 3.82 (q, 2H, J = 6.3 Hz), 3.69 (s, 3H), 3.61 (s, 2H), 2.69 (t, 2H, J = 6.3 Hz), 1.78 (t, 1H, J = 6.3 Hz, hydroxyl); ^{13}C-NMR ($CDCl_3$) δ 172.59, 133.57, 131.68, 129.06, 122.17, 86.59, 81.83, 60.97, 51.96, 40.81, 23.64; FAB-MS *m/z* 219 (M + H); HRMS calcd for $C_{13}H_{15}O_3$ (M + H) 219.1021, found 219.1008.

Methyl 4-(5-hydroxy-1-pentynyl)phenylacetate (16) was prepared in a manner analogous to **15** by coupling 4-pentyn-1-ol with **14** in 83% yield: ^{1}H-NMR ($CDCl_3$) δ 7.34 (d, 2H, J = 8.4 Hz), 7.19 (d, 2H, J = 8.4 Hz), 3.83 (q, 2H, J = 6.0 Hz), 3.69 (s, 3H), 3.6 (s, 2H), 2.54 (t, 2H, J = 6.9 Hz), 1.86 (m, 2H); FABMS *m/z* 233 (M + H); HRMS calcd for $C_{14}H_{17}O_3$ 233.1178, found 233.1190.

Methyl 4-(4-Hydroxybutyl)phenylacetate (17). The ester **15** (10.6 g, 0.0485 mol) was dissolved in methanol (200 mL), 10% Pd−C (1.1g) was added, and the mixture was stirred under a hydrogen (50 psi) atmosphere. After 6 h, additonal 10% palladium on carbon (1 g) was added, and the hydrogenation continued overnight. The catalyst was filtered through Celite, and the filtrate was concentrated and dried *in vacuo* to give 10.20 g (94.5%) of **17** as a pale yellow liquid: ^{1}H-NMR ($CDCl_3$) δ 7.15 (q, 4H, J = 8.1 Hz), 3.69 (s, 3H), 3.65 (t, 2H, J = 8.1 Hz), 3.6 (s, 2H), 2.62 (t, 2H, J = 7.2 Hz), 1.65 (m, 4H), 1.2 (br, 1H); ^{13}C-NMR ($CDCl_3$) δ 172.16, 141.05, 131.08, 128.96, 128.44, 62.32, 51.84, 40.57, 35.06, 32.06, 27.33; FAB-MS *m/z* 223 (M + H); HRMS calcd for $C_{13}H_{19}O_3$ (M + H) 223.1334, found 223.1328.

Methyl 4-(5-hydroxypentyl)phenylacetate (18) was prepared in a manner similar to **17** starting from **16** in 90% yield: ^{1}H-NMR ($CDCl_3$) δ 7.14 (d, 4H, J = 8.1 Hz), 3.69 (s, 3H), 3.65 (m, 2H), 3.59 (s, 2H), 2.6 (t, 2H, J = 7.5 Hz), 1.62 (m, 4H), 1.42 (m, 2H), 1.18 (t, 1H, J = 5.1 Hz); FAB-MS *m/z* 237 (M + H); HRMS calcd for $C_{14}H_{21}O_3$ (M + H) 237.1491, found 237.1486.

Methyl 4-(4-Iodobutyl)phenylacetate (19). To a solution of the hydroxymethyl ester **17** (3.88 g, 17 mmol) in dry acetonitrile (10 mL) was added a solution of the methyl triphenoxyphosphonium iodide (10.26 g, 23 mmol) in dry acetonitrile (100 mL), and the mixture was stirred at 0 °C. The reaction mixture was warmed to room temperature in 12 h and then quenched with excess methanol at 0 °C. Solvents were removed under reduced pressure, and the residue was dissolved in ethyl acetate (500 mL), washed successively with cold 0.2 N NaOH (2 × 500 mL), water (2 × 500 mL), and saturated NaCl (2 × 500 mL), dried over $MgSO_4$, and concentrated. The crude material thus obtained was purified by flash chromatography (10% EtOAc in hexane) to give 4.12 g (71%) of **19** as a clear liquid: ^{1}H-NMR ($CDCl_3$) δ 7.17 (q, 4H, J = 7.92 Hz), 3.69 (s, 3H), 3.60 (s, 2H), 3.20 (t, 2H, J = 6.86 Hz), 2.62 (t, 2H, J = 7.52 Hz), 1.70−1.89 (m, 4H); ^{13}C-NMR ($CDCl_3$) δ 172.02, 140.50, 131.40, 129.15, 128.50, 51.93, 40.67, 34.27, 32.81, 32.03, 6.71; FABMS *m/z* 333 (M + H); HRMS calcd for $C_{13}H_{18}O_2I$ (M + H) 333.0351, found 333.0347.

Methyl 4-(5-iodopentyl)phenylacetate (20) was prepared in a manner similar to **19** starting from **18** in 70% yield: ^{1}H-NMR ($CDCl_3$) δ 7.16 (dd, 4H, J = 8.4 Hz), 3.69 (s, 3H), 3.59 (s, 2H), 3.18 (t, 2H, J = 6.9 Hz), 2.6 (t, 2H, J = 6.9 Hz), 1.85 (m, 2H), 1.52 (m, 2H), 1.45 (m, 2H); FABMS *m/z* 347 (M + H); HRMS calcd for $C_{14}H_{20}O_2I$ (M + H) 347.0508, found 347.0511.

4-(4-Iodobutyl)phenylacetic Acid (21). Ester **19** (21.28 g, 0.064 mol) was dissolved in methanol (160 mL). Lithium hydroxide (6.72 g, 0.16 mol) and water (10 mL) were added, and the reaction mixture was stirred for 18 h at room temperature. The solvents were removed under reduced pressure, the residue was treated with ethyl acetate (600 mL), and the organic solution was washed with 1 N HCl (3 × 300 mL) and brine (3 × 300 mL), dried over $MgSO_4$, and filtered. The filtrate was concentrated, and the residue was dried *in vacuo* to give 17.5 g (85.9%) of **21** as a pale yellow solid: ^{1}H-NMR (CD_3OD) δ 7.16 (q, 4H, J = 8.26 Hz), 3.55 (s, 2H), 3.24 (t, 2H, J = 6.75 Hz), 2.61 (t, 2H, J = 7.35 Hz), 1.68−1.84 (m, 4H); ^{13}C-NMR ($CDCl_3$) δ 178.24, 140.84, 130.70, 129.32, 128.59, 40.63, 34.31, 32.85, 32.02, 6.63; FABMS *m/z* 319 (M + H); HRMS calcd for $C_{12}H_{16}IO_2$ (M + H) 319.0194, found 319.0208.

4-(5-Iodopentyl)phenylacetic acid (22) was prepared in a manner similar to **21** starting from **20** in 89% yield: ^{1}H-NMR ($CDCl_3$) δ 7.16 (dd, 4H, J = 8.1 Hz), 3.6 (s, 2H), 3.18 (t, 2H, J = 7.2 Hz), 2.6 (t, 2H, J = 7.5 Hz), 1.85 (m, 2H), 1.63 (m,

2H), 1.46 (m, 2H), ^{13}C-NMR δ 178.20, 141.28, 130.48, 129.19, 128.50, 40.57, 35.19, 33.29, 30.02, 6.76; FABMS *m/z* 333 (M + H); HRMS calcd for $C_{13}H_{18}IO_2$ (M + H) 333.0350, found 333.0368.

4-[4-(2-Methyl-1*H*-imidazol-1-yl)butyl]phenylacetic Acid (24). To a suspension of sodium hydride (3.17 g, 0.132 mol) in dry DMF (30 mL) at 5 °C was added a solution of 2-methylimidazole (10.11 g, 0.123 mol) in dry DMF (50 mL). The reaction mixture was stirred for 30 min at 5 °C, a solution of the iodo acid **21** (14 g, 0.044 mol) in dry DMF (15 mL) was added, and the mixture was stirred for 1.5 h. The reaction mixture was allowed to warm to room temperature and stirred for 5 h, cooled to 0 °C, and quenched with 1 N HCl. The solution was concentrated, and the residue was dissolved in water and washed several times with ethyl acetate. Water was removed under reduced pressure, and the residue was dried *in vacuo* and treated with ethyl acetate/acetonitrile (1:1 v/v). The solid was filtered and washed with ethyl acetate several times. The solid thus obtained was then washed with absolute ethanol. The ethanol washings were concentrated and dried to obtain 7.63 g (64%) of **24**: ^{1}H-NMR (CD_3OD) δ 7.47 (d, 1H, J = 2.01 Hz), 7.40 (d, 1H, J = 2.01 Hz), 7.17 (q, 4H, J = 8.16), 4.12 (t, 2H, J = 7.25 Hz), 3.56 (s, 2H), 2.67 (t, 2H, J = 7.35 Hz), 2.58 (s, 3H), 1.65−1.88 (m, 4H); FABMS *m/z* 273 (M + H); HRMS calcd for $C_{16}H_{21}N_2O_2$ (M + H) 273.1603, found 273.1635.

4-[4-(1*H*-Imidazol-1-yl)butyl]phenylacetic acid (23) was prepared in a manner similar to **24** using imidazole and **21**: t_R = 14.3 min; ^{1}H-NMR (CD_3OD) δ 8.7 (s, 1H), 7.54 (d, 1H, J = 1.5 Hz), 7.46 (d, 1H, J = 1.5 Hz), 7.16 (dd, 4H, J = 8.4 Hz), 4.22 (t, 2H, J = 7.2 Hz), 3.56 (s, 2H), 2.66 (t, 2H, J = 7.2 Hz), 1.91 (m, 2H), 1.66 (m, 2H); FAB-MS *m/z* 259 (M + H); HRMS calcd for $C_{15}H_{19}N_2O_2$ (M + H) 259.1447, found 259.1429.

4-[4-(1*H*-Imidazol-1-yl)pentyl]phenylacetic acid (29) was prepared in a manner similar to **24** using imidazole and **22**: t_R = 15.46 min; ^{1}H-NMR (CD_3OD) δ 8.6 (s, 1H), 7.49 (d, 1H, J = 1.5 Hz), 7.4 (d, 1H, J = 1.5 Hz), 7.14 (dd, 4H, J = 8.1 Hz), 4.18 (t, 2H, J = 7.2 Hz), 3.55 (s, 2H), 2.6 (t, 2H, J = 7.2 Hz), 1.88 (m, 2H), 1.68 (m, 2H), 1.3 (m, 2H); FABMS *m/z* 273 (M + H); HRMS calcd for $C_{16}H_{21}N_2O_2$ (M + H) 273.1684, found 273.1727.

4-[4-(2-Iodo-1*H*-Imidazol-1-yl)butyl]phenylacetic acid (25) was prepared in a manner similar to **24** starting from 2-iodoimidazole[21] and **21**: t_R = 15.4 min; ^{1}H-NMR (CD_3OD) δ 7.64 (d, 1H, J = 2.1 Hz), 7.53 (d, 1H, J = 2.1 Hz), 7.16 (dd, 4H, J = 8.1 Hz), 4.16 (t, 2H, J = 7.5 Hz), 3.56 (s, 2H), 2.67 (t, 2H, J = 7.5 Hz), 1.87 (m, 2H); FABMS *m/z* 259 (M + H); HRMS calcd for $C_{15}H_{19}N_2O_2$ (M + H) 259.1447, found 259.1429.

4-[4-(2-Ethyl-1*H*-imidazol-1-yl)butyl]phenylacetic acid (26) was prepared in a manner similar to **24** using 2-ethylimidazole and **21** in 45% yield: ^{1}H-NMR (DMSO-d_6) δ 7.65 (d, 1H, J = 2.1 Hz), 7.57 (d, 1H, J = 2.1 Hz), 7.16 (dd, 4H, J = 8.1 Hz), 4.13 (t, 2H, J = 7.5 Hz), 3.53 (s, 2H), 2.94 (q, 2H, J = 7.5 Hz), 2.52 (t, 2H, J = 7.5 Hz),1.75 (m, 2H), 1.58 (m, 2H), 1.26 (t, 3H, J = 7.5 Hz); FAB-MS *m/z* 293 (M + Li); HRMS calcd for $C_{17}H_{22}N_2O_2Li$ (M+Li) 293.1841, found 293.1859.

4-[4-(2-Isopropyl-1*H*-imidazol-1-yl)butyl]phenylacetic acid (27) was prepared in a manner similar to **24** using 2-isopropylimidazole and **21**: ^{1}H-NMR (CD_3OD) δ 7.46 (d, 1H, J = 1.88 Hz), 7.43 (d, 1H, J = 2.15 Hz), 7.17 (q, 4H, J = 8.06 Hz), 4.16 (t, 2H, J = 7.52 Hz), 3.55 (s, 2H), 3.38 (q, 1H, J = 6.98 Hz), 2.68 (t, 2H, J = 7.25 Hz), 1.67−1.86 (m, 4H), 1.34 (d, 6 H); HRMS *m/z* calcd for $C_{18}H_{24}N_2O_2$ 301.1916 (M + H), found 301.1859.

4-[4-(2-Methyl-1*H*-benzimidazol-1-yl)butyl]phenylacetic Acid (28). A mixture of 2-methylbenzimidazole (0.19 g, 1.44 mmol) and **21** (0.2 g, 0.63 mmol) in dry THF (10 mL) containing NaH (0.038 g, 1.58 mmol) was heated to reflux for 1 h under a nitrogen atmosphere. The reaction mixture was acidified with acetic acid (0.2 mL) and concentrated to dryness, and the residue was purified by reverse-phase (C_{18}) HPLC to obtain 0.18 g (62%) of the title compound **28** as a hygroscopic substance: t_R = 18.0 min; ^{1}H-NMR (CD_3OD) δ 7.75 (m, 2H), 7.57 (2d, 2H, J = 3.0 Hz), 7.16 (q, 4H, J = 8.4 Hz), 4.42 (t, 2H, J = 7.5 Hz), 3.55 (s, 2H), 2.81 (s, 3H), 2.68 (t, 2H, J = 7.5 Hz), 1.92 (m, 2H), 1.78 (m, 2H); HRMS calcd for $C_{20}H_{21}N_2O_2$ (M − H) 321.1603, found 321.1582.

(*R,S*)-Methyl 2-(4-Iodophenyl)propionate (41). Sodium hydride (0.095 g, 3.96 mmol, 80% suspension) was added to a solution of the methyl 4-iodophenylacetate **14** (1.0 g, 3.62 mmol) in dry THF (10 mL), and the mixture was stirred at 5 °C. After 30 min, iodomethane (0.68 g, 4.8 mmol) was added, and the mixture was stirred at room temperature. Additional iodomethane (0.46 g, 3.2 mmol) was added after 2 h, and the mixture was stirred at room temperature overnight for 16 h. Acetic acid (0.2 mL) was added, and the reaction mixture was concentrated under reduced pressure. The residue was partitioned between EtOAc (30 mL) and 5% citric acid (25 mL). The organic phase was washed with water (2 × 20 mL), dried (Na_2SO_4), and concentrated, and the resulting substance was purified by silica gel flash chromatography using 20% EtOAc in hexane to give 0.7 g of **41** (70%) as a colorless liquid: ^{1}H NMR ($CDCl_3$) δ 7.64 (d, 2H, J = 8.4 Hz), 7.03 (2H, J = 8.4 Hz), 3.66 (m over s, 4H), 1.46 (d, 3H, J = 7.2 Hz); ^{13}C-NMR ($CDCl_3$) δ 174.31, 140.09, 137.61, 129.46, 92.52, 52.05, 44.89, 18.35; FABMS *m/z* 291 (M + H); HRMS calcd for $C_{10}H_{12}IO_2$ (M + H) 290.9882, found 290.9861.

(*R,S*)-Methyl 2-(4-iodophenyl)butyrate (42) was prepared in a manner similar to **41** using ethyl iodide and **14** in 43% yield as a colorless liquid: ^{1}H-NMR ($CDCl_3$) δ 7.64 (d, 2H, J = 8.1 Hz), 7.05 (d, 2H, J = 8.1 Hz), 3.66 (s, 3H), 3.4 (t, 1H, J = 6.7 Hz), 2.1 (m, 1H), 1.78 (m, 1H), 0.88 (t, 3H, J = 7.5 Hz); FABMS *m/z* 305 (M + H); HRMS calcd for $C_{11}H_{14}IO_2$ (M + H) 305.0038, found 305.0030.

(*R,S*)-Methyl 2-(4-Iodophenyl)pentanoate (43). To a solution of **14** (2.5 g, 9.05 mmol) in THF (35 mL) at 0 °C was added NaH (0.23 g, 9.6 mmol). After for 30 min of stirring, a solution of iodopropane (1.1 mL) in 10 mL of THF was added dropwise. The reaction mixture was stirred at room temperature for 1 h, heated at 65 °C for 2 h, and concentrated under reduced pressure. The residue was partitioned between cold 5% citric acid (30 mL) and ethyl acetate (50 mL). The organic phase was washed with water (2 × 25 mL), dried (Na_2SO_4), and concentrated. The residue was purified by silica gel flash chromatography using 20% EtOAc in hexane to to afford 2.1 g (73%) of the title compound as a pale yellow liquid: ^{1}H-NMR ($CDCl_3$) δ 7.65 (dd, 2H, J = 6.6, 1.8 Hz), 7.04 (dd, 2H, J = 6.6, 1.8 Hz), 3.65 (s, 3H), 3.50 (t, 1H, J = 7.2 Hz), 2.0 (m, 1H), 1.65 (m, 1H), 1.24 (m, 2H), 0.9 (t, 3H, J = 7.2 Hz); FABMS *m/z* 318 (M^+); HRMS calcd for $C_{12}H_{15}IO_2$ (M^+) 318.0116, found 318.0065.

(*R,S*)-Methyl 2-[4-(4-hydroxy-1-butynyl)phenyl]propionate (44) was prepared in a manner similar to **15** starting from **41** and 3-butyn-1-ol to afford 96% yield of the title compound as an orange liquid: ^{1}H-NMR ($CDCl_3$) δ 7.37 (d, 2H, J = 8.1 Hz), 7.22 (d, 2H, J = 8.1 Hz), 3.8 (q, 2H, J = 6.9 Hz), 3.69 (m, 1H), 3.66 (s, 3H), 2.69 (t, 2H, J = 6.0 Hz), 1.78 (t, 1H), 1.48 (d, 3H, J = 6.9 Hz); ^{13}C-NMR ($CDCl_3$) δ 174.81, 140.39, 132.01, 127.55, 122.39, 86.68, 82.16, 61.25, 52.20, 45.35, 23.91, 18.49; FABMS *m/z* 233 (M + H); HRMS calcd for $C_{14}H_{17}O_3$ (M + H) 233.1178, found 233.1149.

(*R,S*)-Methyl 2-[4-(4-hydroxy-1-butynyl)phenyl]butyrate (45) was prepared in a manner similar to **15** starting from **42** and 3-butyn-1-ol in 81% yield as an orange liquid: ^{1}H-NMR ($CDCl_3$) δ 7.36 (d, 2H, J = 7.2 Hz), 7.36 (d, 2H, J = 7.2 Hz), 3.81 (q, 2H, J = 6.0 Hz), 3.65 (s, 3H), 3.44 (t, 1H, J = 6.8 Hz), 2.69 (t, 2H, J = 6.0 Hz), 2.15 (m, 1H), 1.78 (m, 2H), 0.87 (t, 3H, J = 7.2 Hz); ^{13}C-NMR ($CDCl_3$) δ 174.36, 138.97, 131.97, 128.03, 122.41, 86.67, 81.92, 61.26, 53.28, 52.10, 26.71, 23.93, 12.19; FABMS *m/z* 247 (M + H); HRMS calcd for $C_{15}H_{19}O_3$ (M + H) 247.1334, found 247.1331.

(*R,S*)-Methyl 2-[4-(4-hydroxy-1-butynyl)phenyl]pentanoate (46) was prepared in a manner similar to **15** starting from **43** and 3-butyn-1-ol in 77% yield as an orange viscous liquid: ^{1}H-NMR ($CDCl_3$) δ 7.35 (d, 2H, J = 8.4 Hz), 7.23 (d, 2H, J = 8.4 Hz), 3.79 (q, 2H, J = 6.6 Hz), 3.65 (s, 3H), 3.54 (t, 1H, J = 6.7 Hz), 2.69 (t, 2H, J = 6.3 Hz), 2.1 (m, 1H), 1.78 (m, 2H), 1.22 (m, 1H), 0.89 (t, 3H, J = 7.2 Hz); FABMS *m/z* 261 (M + H); HRMS calcd for $C_{16}H_{21}O_3$ (M + H) 261.1491, found 261.1523.

(*R,S*)-Methyl 2-[4-(4-hydroxybutyl)phenyl]propionate (47) was prepared in a manner similar to **17** starting from **44** in 98% yield as a pale yellow liquid: ^{1}H-NMR ($CDCl_3$) δ 7.21 (d, 2H, J = 7.8 Hz), 7.13 (d, 2H, J = 7.8 Hz), 3.68 (m, 3H),

3.66 (s, 3H), 2.62 (t, 2H, J = 7.5 Hz), 1.63 (m, 4H), 1.48 (d, 2H, J = 7.2 Hz); ^{13}C-NMR ($CDCl_3$) δ 175.14, 141.14, 137.82, 128.57, 127.28, 62.76, 51.9, 44.94, 35.15, 32.23, 27.4, 18.52; FABMS m/z 237 (M + H); HRMS calcd for $C_{14}H_{21}O_3$ (M + H) 237.1491, found 237.1491.

(*R,S*)-Methyl 2[4-(4-hydroxybutyl)phenyl]butyrate (48) was prepared in a manner similar to **17** starting from **45** in 96% yield as a colorless liquid: ^{1}H-NMR ($CDCl_3$) δ 7.22 (d, 2H, J = 7.8 Hz), 7.12 (d, 2H, J = 7.8 Hz), 3.56 (s over m, 5H), 3.42 (t, 1H, J = 7.5 Hz), 2.62 (t, 2H, J = 7.5 Hz), 2.11 (m, 1H), 1.72 (m, 5H), 1.29 (t, 1H, J = 5.3 Hz), 0.88 (t, 3H, J = 7.5 Hz); FABMS m/z (M + H) 251; HRMS calcd for $C_{14}H_{26}O_3$ (M + H) 251.1647, found 251.1646.

(*R,S*)-Methyl α-propyl-4-(4-hydroxy-1-butyl)benzeneacetate (49) was prepared in a manner similar to **17** starting from **46** to afford 97% yield of the title compound as a colorless liquid: ^{1}H-NMR ($CDCl_3$) δ 7.21 (d, 2H, J = 8.1 Hz), 7.13 (d, 2H, J = 8.1 Hz), 3.67 (t, 2H, J = 8.5 Hz), 3.52 (t, 1H, J = 7.5 Hz), 2.62 (t, 2H, J = 7.5 Hz), 2.05 (m, 1H), 1.64 (m, 5H), 1.20 (m, 3H), 0.90 (t, 2H, J = 7.5 Hz); FABMS m/z 264 (M^+); HRMS calcd for $C_{16}H_{24}O_3$ (M + H) 264.1725, found 264.1693.

(*R,S*)-Methyl 2-[4-(4-iodobutyl)phenyl]propionate (50) was prepared in a manner similar to **19** starting from **47** in 85% yield as a pale yellow viscous liquid: ^{1}H-NMR ($CDCl_3$) δ 7.2 (d, 2H, J = 8.1 Hz), 7.14 (d, 2H, J = 8.1 Hz), 3.69 (q, 1H, J = 6.9 Hz), 3.66 (s, 3H), 3.2 (t, 2H, J = 6.9 Hz), 2.6 (t, 2H, J = 7.5 Hz), 1.85 (m, 2H), 1.7 (m, 2H), 1.48 (d, 3H, J = 7.2 Hz); ^{13}C-NMR ($CDCl_3$) δ 175.02, 140.57, 138.02, 128.55, 127.37, 51.94, 44.93, 34.28, 32.87, 32.05, 18.57, 6.71; FABMS m/z 353 (M + Li); HRMS calcd for $C_{14}H_{20}IO_2$ (M + H) 347.0508, found 347.0509.

(*R,S*)-Methyl 2-[4-(4-iodobutyl)phenyl]butyrate (51) was prepared in a manner similar to **19** starting from **48** in 32% yield as a colorless liquid: ^{1}H-NMR ($CDCl_3$) δ 7.21 (d, 2H, J = 8.1 Hz), 7.12, (d, 2H, J = 8.1 Hz), 3.62 (s, 3H), 3.43 (t, 1H, J = 7.8 Hz), 3.2 (t, 2H, J = 6.6 Hz), 2.61 (t, 2H, J = 7.5 Hz), 2.06 (m, 1H), 1.83 (m, 2H), 1,78 (m, 3H), 0.89 (t, 3H, J = 7.5 Hz); ^{13}C-NMR ($CDCl_3$) δ 174.70, 140.75, 136.73, 128.64, 127.98, 53.04, 51.96, 34.45, 33.03, 32.19, 26.86, 12.30, 6.87; FABMS m/z 361 (M + H); HRMS calcd for $C_{15}H_{22}IO_2$ (M + H) 361.0664, found 361.0647.

Methyl (*R,S*)-2-[4-(4-iodobutyl)phenyl]pentanoate (52) was prepared in a manner similar to **19** starting from **49** in 82% yield as a colorless liquid: ^{1}H-NMR ($CDCl_3$) δ 7.22 (d, 2H, J = 8.1 Hz), 7.12 (d, 2H, J = 8.1 Hz), 3.65 (s, 3H), 3.53 (t, 1H, J = 7.8 Hz), 3.12 (t, 2H, J = 6.6 Hz), 2.61 (t, 2H, J = 7.5 Hz), 2.02 (m, 1H), 1.82 (m, 2H), 1.74 (m, 3H), 1.25 (m, 2H), 0.9 (t, 2H, J = 7.5 Hz); FABMS m/z 374 (M^+); HRMS calcd for $C_{16}H_{23}IO_2$ (M^+) 374.0742, found 374.0762.

(*R,S*)-2-[4-(4-Iodobutyl)phenyl]propionic Acid (53). The ester **50** (1.0 g) was stirred with 1 M LiOH (5.00 mL) containing MeOH (3.75 mL) at 60 °C for 1 h, under a nitrogen atmosphere. The reaction mixture was concentrated under reduced pressure, water (25 mL) was added, and the mixture was extracted with ether (3 × 15 mL). The aqueous extract was acidified with 5% citric acid and extracted with EtOAc (3 × 15 mL). The combined organic extracts were washed with water (3 × 15 mL), dried (Na_2SO_4) and concentrated to give **53** (0.7 g, 73%) as a colorless liquid: ^{1}H-NMR ($CDCl_3$) δ 7.22 (d, 2H, J = 8.1 Hz), 7.15 (d, 2H, J = 8.1 Hz), 3.72 (q, 1H, J = 7.2 Hz), 3.2 (t, 2H, J = 7.2 Hz), 2.6 (t, 2H, J = 7.5 Hz), 1.85 (m, 2H), 1.7 (m, 2H), 1.5 (d, 3H, J = 7.2 Hz); ^{13}C-NMR ($CDCl_3$) δ 181.19, 140.84, 137.14, 128.57, 127.50, 44.89, 34.25, 32.81, 32.00, 17.98, 6.73; FABMS m/z 339 (M + Li); HRMS calcd for $C_{14}H_{20}IO_2$ (M + Li) 339.0432, found 339.0424.

(*R,S*)-2-[4-(4-iodobutyl)phenyl]butyric Acid (54). The ester **51** (0.35 g, 0.97 mmol) was stirred with 1 M LiOH (2.00 mL) containing THF (1.00 mL) at room temperature for 16 h under a nitrogen atmosphere. The reaction mixture was concentrated under reduced pressure, acidified with 5% citric acid, and extracted with EtOAc (2 × 10 mL). The combined EtOAc extracts were washed with water (3 × 15 mL), dried (Na_2SO_4), and concentrated to give 0.26 g (73%) of **54** as a colorless liquid: ^{1}H-NMR ($CDCl_3$) δ 7.21 (d, 2H, J = 8.1 Hz), 3.38 (2t, 2H, J = 6.8 Hz), 3.19 (t, 1H, J = 6.8 Hz), 2.61 (t, 2H, J = 7.5 Hz), 2.1 (m, 1H), 1.85−1.5 (m, 5H), 0.91 (t, 3H, J = 7.5 Hz), FABMS m/z 346 (M^+); HRMS calcd for $C_{14}H_{19}IO_2$ (M^+) 346.0430, found 346.0410.

(*R,S*)-2-[4-(4-Iodobutyl)phenyl]pentanoic Acid (55). The ester **52** (0.7 g, 1.9 mmol) was stirred with 1 M LiOH (3.00 mL) containing THF (5.00 mL) at room temperature for 4 h under a nitrogen atmosphere. The reaction mixture was diluted with water (20 mL) and washed with EtOAc (2 × 15 mL). The aqueous portion was acidified with 5% citric acid and extracted with EtOAc (2 × 15 mL). The combined EtOAc extracts were washed with water (3 × 15 mL), dried (Na_2SO_4), and concentrated to give 0.23 g (50%) of **55** as a colorless liquid: ^{1}H-NMR ($CDCl_3$) δ 7.23 (d, 2H, J = 8.1 Hz), 7.12 (d, 2H, J = 8.1 Hz), 3.62 and 3.54 (t, 1H, J = 7.5 Hz), 3.38 and 3.19 (t, 2H, J = 7.2 Hz), 2.61 (t, 2H, J = 7.5 Hz), 2.15 (m, 1H), 1.83−1.6 (m, 5H), 1.25 (m, 2H), 0.91 (t, 3H, J = 7.2 Hz); FABMS m/z 367 (M + Li); HRMS calcd for $C_{15}H_{21}IO_2Li$ (M + Li) 367.0745, found 367.0816.

(*R,S*)-α-Methyl-4-[4-(2-methyl-1*H*-imidazol-1-yl)butyl]-benzeneacetic Acid (56). Sodium hydride (0.05 g, 80% suspension, 2.1 mmol) was added to a solution of 2-methylimidazole (0.092 g, 1.12 mmol) at 0 °C. After 30 min, a solution of **12** (0.25 g, 0.75 mmol) in DMF (1.5 mL) was added, and the mixture was stirred at 0 °C for 30 min and at room temperature for 1.5 h under an argon atmosphere. After the DMF was distilled *in vacuo*, the residue was treated with 2 N HCl (3 mL) and water (10 mL) and washed with EtOAc (3 × 5 mL). The aqueous phase was freeze-dried, and the residue was washed with 10% CH_3CN in EtOAc (20 mL) and dried to afford 0.14 g (58%) of **56**: t_R = 14.9 min; ^{1}H-NMR (CD_3OD) δ 7.46 (d, 1H, J = 2.1 Hz), 7.39 (d, 1H, J = 2.1 Hz), 7.22 (d, 2H, J = 7.8 Hz), 7.13 (d, 2H, J = 7.8 Hz), 4.12 (t, 2H, J = 7.2 Hz), 3.66 (q, 1H, J = 6.8 Hz), 2.66 (t, 2H, J = 7.5 Hz), 2.57 (s, 3H), 1.85 (m, 2H), 1.67 (m, 2H), 1.41 (d, 3H, J = 7.5 Hz); FABMS m/z 287 (M + H); HRMS calcd for $C_{17}H_{23}N_2O_2$ (M + H) 287.1760, found 287.1754.

(*R,S*)-α-Methyl-4-[4-(2-chloro-1*H*-imidazol-1-yl)butyl]-benzeneacetic Acid (59). A mixture of 2-chloroimidazole[21] (0.056 g, 0.55 mmol), **53** (0.12 g, 0.36 mmol), and NaH (0.025 g, 1.04 mmol) in THF (5.00 mL) was heated at 70 °C for 1 h, under a nitrogen atmosphere with vigorous stirring. The reaction mixture was acidified with acetic acid and concentrated under reduced pressure, and the resulting material was purified by reverse-phase HPLC. The appropriate fractions were combined and freeze-dried to give 0.1 g (66%) of the title compound as a glassy solid: t_R = 22.0 min; ^{1}H-NMR (CD_3OD) δ 7.29 (s, 1H), 7.22 (d, 2H, J = 7.8 Hz), 7.06 (s, 1H), 4.05 (t, 2H, J = 7.2 Hz), 3.66 (q, 1H, J = 7.2 Hz), 2.64 (t, 2H, J = 7.5 Hz), 1.79 (m, 2H), 1.66 (m, 2H), 1.42 (d, 3H, J = 7.2 Hz); FABMS m/z 307 (M + H); HRMS calcd for $C_{16}H_{19}ClN_2O_2$ (M + H) 307.1042, found 307.1021.

(*R,S*)-α-Ethyl-4-[4-(2-methyl-1*H*-imidazol-1-yl)butyl]-benzeneacetic Acid (57). A mixture of **54** (0.12 g, 0.35 mmol) and 2-methylimidazole (0.15 g, 1.85 mmol) in dry THF (5 mL) was heated at 60 °C for 4 h under a nitrogen atmosphere. The reaction mixture was acidified with acetic acid and concentrated under reduced pressure. The resulting product was purified by reverse-phase HPLC. The appropriate fractions were pooled and freeze-dried to give 0.07 g (50%) of **57** as a glassy solid: t_R = 16.6 min; ^{1}H-NMR (CD_3OD) δ 7.47 (d, 1H, J = 2.4 Hz), 7.39 (d, 1H, J = 2.4 Hz), 7.22 (d, 2H, J = 8.1 Hz), 7.15 (d, 1H, J = 8.1 Hz), 4.12 (t, 2H, J = 7.2 Hz), 3.39 (t, 2H, J = 7.5 Hz), 2.67 (t, 2H, J = 7.5 Hz), 2.56 (s, 3H), 2.1 (m, 1H), 1.85 (m, 2H), 0.88 (t, 2H, J = 7.5 Hz); FABMS m/z 301 (M + H); HRMS calcd for $C_{18}H_{25}N_2O_2$ (M + H) 301.1916, found 301.1927.

(*R,S*)-α-Propyl-4-[4-(2-methyl-1*H*-imidazol-1-yl)butyl]-benzeneacetic acid (58) was prepared in a manner similar to **56** using 2-methylimidazole and **55** in 80% yield as a yellow syrup: t_R = 18.25 min; ^{1}H-NMR (CD_3OD) δ 7.47 (d, 1H, J = 2.1 Hz), 7.41 (d, 1H, J = 2.1 Hz), 7.23 (d, 2H, J = 8.4 Hz), 7.15 (d, 1H, J = 8.4 Hz), 4.13 (t, 2H, J = 7.5 Hz), 3.51 (t, 2H, J = 8.1 Hz), 2.68 (t, 2H, J = 7.5 Hz), 2.59 (s, 3H), 2.1 (m, 1H), 1.85 (m, 2H), 1.69 (m, 3H),1.25 (m, 2H), 0.93 (t, 2H, J = 7.5 Hz); FABMS m/z 315 (M + H); HRMS calcd for $C_{19}H_{27}N_2O_2$ (M + H) 315.2072, found 315.2072.

(*R,S*)-α-Methyl-4-[4-(2-methyl-1*H*-benzimidazol-1-yl)-butyl]benzeneacetic acid (60) was prepared in a manner

similar to **56** using 2-methylbezimidazole and **53** in 87% yield: t_R = 18.6 min; ^{1}H-NMR (CD_3OD) δ 7.79 (m, 2H), 7.73 (m, 2H), 7.21 (d, 2H, J = 8.1 Hz), 7.14 (d, 2H, J = 8.1 Hz), 4.44 (t, 2H, J = 7.2 Hz), 3.67 (q, 1H, J = 7.2 Hz), 2.71 (s, 3H), 2.68 (t, 2H, J = 7.2 Hz), 1.93 (m, 2H), 1.76 (m, 2H), 1.42 (d, 3H, J = 7.2 Hz); FABMS m/z 337 (M + H); HRMS calcd for $C_{21}H_{25}N_2O_2$ (M + H) 337.1917, found 337.1903.

(*R,S*)-*N*-[2-[4-[4-(2-Methyl-1*H*-imidazol-1-yl)butyl]phenyl]-1-oxopropyl]-L-seryl-*N*-(2-cyclohexylethyl)-L-lysinamide (61). A mixture of **56** (0.092 g, 0.29 mmol) and hydroxybenzotriazole (0.085 g, 0.57 mmol) in dimethylacetamide (1.5 mL) and dichloromethane (3.0 mL) was treated with DCC (0.62 g, 0.29 mmol) and stirred at 0 °C. After 2 h, a solution of the amine acetate **12** (0.13 g, 0.24 mmol)[25] in dimethylacetamide (0.5 mL) and *N*-methylmorpholine (0.08 mL) were added, and the mixture was stirred at 0 °C for 1 h and then at room temperature for 16 h. The reaction mixture was concentrated *in vacuo*, and the residue was partitioned between cold 0.25 N NaOH (15 mL) and EtOAc (25 mL). The organic phase was washed successively with water (2 × 10 mL), 5% citric acid (2 × 10 mL), and water, dried (Na_2SO_4), and concentrated. The resulting material was washed with a solvent mixture of ether−hexane (1:1 v/v), filtered, and dried to give a pale yellow powder (0.10 g) which was stirred with trifluoroacetic acid (1.5 mL) for 4 h at room temperature. After the removal of TFA under reduced pressure, the residue was purified by reverse-phase HPLC. The appropriate fractions were pooled and freeze-dried to give 0.025 g of **61** as a diastereomeric mixture: t_R = 17.4 min (40%), 17.7 min (60%); ^{1}H-NMR (CD_3OD) δ 7.47 (d, 1H, J = 2.1 Hz), 7.41 (d, 1H, J = 2.1 Hz), 7.25 (d, 2H, J = 8.4 Hz), 7.14 (d, 2H, J = 8.4 Hz), 4.4−4.2 (m, 2H), 4.13 (t, 2H, J = 7.5 Hz), 3.9−3.6 (m, 3H), 3.18 (m, 2H), 2.98−2.84 (2t, 2H, J = 7.2 Hz), 2.66 (t, 2H, J = 7.5 Hz), 2.59 and 2.58 (2s, 3H), 1.85 (m, 3H), 1.8−1.55 (m, 21H), 0.92 (m, 2H); HRMS calcd for $C_{34}H_{54}N_6O_4$ (MH^+) 611.4285, found 611.4265. Amino acid analysis: Ser 1.00 (1.02), Lys 1.00(0.98).

Chiral Separation of 61a and 61b. The racemic material **61** (0.5 mg) was dissolved in acetonitrile (25.0 μL), loaded on an ASTEC vancomycin column (5−μM silica gel, 4.6 × 250 mm), and eluted with 23% acetonitrile containing 0.8% of ammonium phosphate at pH = 4.2, at a flow rate of 1.0 mL/min for 15 min. The separation was monitored by UV absorbance at 220 nm. The separation was repeated by making 19 more injections of 25.0 μL each, containing 0.5 mg of the recemic material. The appropriate fractions were collected, pooled, and desalted by passing through a short C_{18} column (4.6 × 150 mm) and eluting with acetonitrile containing 0.1% trifluoroacetic acid. The fractions were collected and freeze-dried to obtain 5.0 mg of **61a** and 3.6 mg of **61b** as white amorphous substances.

(*R*)-*N*-[2-[4-[4-(2-Methyl-1*H*-imidazol-1-yl)butyl]phenyl]-1-oxopropyl]-L-seryl-*N*-(2-cyclohexylethyl)-L-lysinamide (61a): t_R = 17.56 min; ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.44 (br, 1H), 7.37 (br, 1H), 7.25 (d, 2H, J = 8.0 Hz), 7.14 (d, 2H, J = 8.0 Hz), 4.41 (dd, 1H, Lys α-CH, J = 4.5 Hz, 9.5 Hz), 4.36 (t, 1H, Ser α-CH, J = 7.0 Hz), 4.19 (t, 2H, J = 7.5 Hz), 3.84 (dd, 1H, J = 6.0, 10.5 Hz), 3.78 (q, 1H, J = 7.0 Hz), 3.71 (dd, 1H, J = 7.5, 10.5 Hz), 3.10 (m, 2H), 2.94 (t, 2H, J = 7.0 Hz), 2.65 (t, 2H, J = 8.0 Hz), 2.57 (s, 3H), 1.94 (m, 1H), 1.85 (m, 2H), 1.75−1.62 (m, 10H), 1.48 (m, 1H), 1.41 (d, 3H, J = 7.0 Hz), 1.37 (q, 2H, J = 7.0 Hz), 1.32−1.12 (m, 4H), 0.92 (m, 2H); HRMS calcd for $C_{34}H_{57}N_6O_4$ (M + H^+) 611.4285, found 611.4273. Amino acid analysis: Ser 1.00 (1.00), Lys 1.00 (1.00).

(*S*)-*N*-[2-[4-[4-(2-Methyl-1*H*-imidazol-1-yl)butyl]phenyl]-1-oxopropyl]-L-seryl-*N*-2-cyclohexylethyl)-L-lysinamide (61b): t_R = 17.35 min; ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.45 (br, 2H), 7.25 (d, 2H, J = 8.0 Hz), 7.13 (d, 2H, J = 8.0 Hz), 4.35 (t, 1H, Ser α-CH, J = 6.25 Hz), 4.25 (dd, 1H, Lys α-CH, J = 4.0, 9.75 Hz), 4.14 (br, 2H), 3.84 (dd, 1H, J = 5.5, 10.5 Hz), 3.72 (q, 2H), 3.16 (m, 2H), 2.87 (t, 2H, J = 7.5 Hz), 2.64 (t, 2H, J = 6.5 Hz), 2.56 (br, 3H), 1.87 (m, 3H), 1.74−1.54 (m, 10H), 1.43 (d, 3H, J = 7.0 Hz), 1.42−1.14 (m, 8H), 0.91 (m, 2H); HRMS calcd for $C_{34}H_{57}N_6O_4$ (M + H^+) 611.4285, found 611.4254. Amino acid analysis: Ser 1.00 (0.99), Lys 1.00 (1.01).

(*R*)-*N*-(1-Oxo-2-phenylpropyl)-L-seryl-*N*-(2-cyclohexylethyl)-L-lysinamide (68) was prepared in a manner similar to **13** by coupling (*R*)-phenylacetic acid with **12**. After the workup, the crude product was treated with trifluoroacetic acid and the desired product was isolated by reverse-phase HPLC to afford 27% yield of the title compound as a white powder: t_R = 15.67 min; ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.33 (m, 4H), 7.23 (m, 1H), 4.36 (dd, 1H, Lys α-CH, J = 4.0, 10.0 Hz), 4.28 (dd, 1H, Ser α-CH, J = 5.5, 7.5 Hz), 3.78 (dd, 1H, J = 5.5, 10.5 Hz), 3.75 (q, 1H, J = 7.0 Hz), 3.66 (dd, 1H, J = 7.5, 10.5 Hz), 3.19 (m, 2H), 2.92 (t, 2H, J = 7.5 Hz), 1.95 (m, 1H), 1.71 (m, 8H), 1.5 (m, 1H), 1.44 (d, 3H, J = 7.0 Hz), 1.38 (q, 2H, J = 7.5 Hz), 1.31−1.12 (m, 5H), 0.92 (m, 2H); HRMS calcd for $C_{26}H_{43}N_4O_4$ (MH^+) 475.3284, found 475.3296. Amino acid analysis: Ser 1.00 (1.00), Lys 1.00 (1.00).

(*S*)-*N*-(1-Oxo-2-phenylpropyl)-L-seryl-*N*-(2-cyclohexylethyl)-L-lysinamide (69) was prepared in a manner similar to **13** by coupling (*S*)-phenylacetic acid with **12**. After the workup, the crude product was treated with trifluoroacetic acid and the desired product was isolated by reverse-phase HPLC to afford a 43% yield of the title compound as a white powder: t_R = 15.35 min; ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.33 (m, 2H), 7.30 (m, 2H), 7.22 (m, 1H), 4.36 (t, 1H, Ser α-CH, J = 7.0 Hz), 4.25 (dd, 1H, Lys α-CH, J = 4.5, 7.5 Hz), 3.84 (dd, 1H, Ser β-CH, J = 5.5, 10.5 Hz), 3.75 (q, 1H, J = 7.0 Hz), 3.73 (dd, 1H, Ser β-CH, J = 7.0, 10.5 Hz), 3.15 (m, 2H), 2.82 (t, 2H, J = 7.5 Hz), 1.82 (m, 1H), 1.85 (m, 1H), 1.74−1.5 (m, 8H), 1.45 (d, 3H, J = 7.0 Hz), 1.4−1.12 (m, 8H), 0.92 (m, 2H); HRMS calcd for $C_{26}H_{43}N_4O_4$ (M + H^+) 475.3284, found 475.3296. Amino acid analysis: Ser 1.00 (0.99), Lys 1.00 (1.01).

(*R,S*)-*N*-[2-[4-[4-(2-Methyl-1*H*-imidazol-1-yl)butyl]phenyl]-1-oxobutyl]-L-seryl-*N*-(2-cyclohexylethyl)-L-lysinamide (62) was prepared in a manner similar to **61** by reacting **57** with **12**: t_R = 17.3 min; ^{1}H-NMR (CD_3OD) δ 8.18 (br, 1H, exchangeable), 7.9 (br, 1H, exchangeable), 7.82 (br, 1H, exchangeable), 7.49 (d, 1H, J = 2.1 Hz), 7.42 (d, 1H, J = 2.1 Hz), 7.27 (d, 2H, J = 7.8 Hz), 7.15 (d, 2H, J = 7.8 Hz), 4.41−4.21 (m, 2H), 4.14 (t, 2H, J = 7.2 Hz), 3.9−3.6 (m, 2H), 3.45 (m, 1H), 3.18 (m, 2H), 2.85 (m, 2H), 2.64 (m, 2H), 2.61 and 2.60 (s, 3H), 2.08−1.1 (m, 23H), 0.91 (m, 5H); HRMS calcd for $C_{35}H_{57}N_6O_4$ (M + H^+) 625.4441, found 625.4429. Amino acid analysis: Ser 1.00 (1.01), Lys 1.00 (0.99).

(*R,S*)-*N*-[2-[4-[4-(2-Methyl-1*H*-imidazol-1-yl)butyl]phenyl]-1-oxopentyl]-L-seryl-*N*-2-cyclohexylethyl)-L-lysinamide (63) was prepared in a manner similar to **61** by reacting **58** with **12**: t_R = 19.5 min; ^{1}H-NMR (CD_3OD) δ 7.25 (m, 3H), 7.12 (m, 3H), 4.4−4.21 (m, 2H), 4.03 (t, 2H), 3.8−3.5 (m, 3H), 3.2 (m, 2H), 2.85 (2t, 2H), 2.64 (m, 2H), 2.45 and 2.44 (s, 3H), 2.1−1.6 (m, 16H), 1.6−1.15 (m, 9H), 0.91 (m, 5H); HRMS calcd for $C_{36}H_{58}N_6O_4$ (M + H^+) 639.4598, found 639.4592. Amino acid analysis: Ser 1.00 (1.00), Lys 1.00 (1.00).

(*R,S*)-*N*-[2-[4-[4-(2-Chloro-1*H*-imidazol-1-yl)butyl]phenyl]-1-oxopropyl]-L-seryl-*N*-(2-cyclohexylethyl)-L-lysinamide (64) was prepared in a manner similar to **61** by reacting **59** with **12**: t_R = 22.5 min; ^{1}H-NMR (CD_3OD) δ 7.25 (d, 1H, J = 3.3 Hz), 7.23 (d, 1H, J = 3.3 Hz), 7.2 (d, 1H, J = 1.5 Hz), 7.12 (d, 2H, J = 8.1 Hz), 6.95 (d, 1H, J = 1.5 Hz), 4.34 (m, 1H), 4.26 (m, 1H), 4.01 (t, 2H, J = 7.2 Hz), 3.71 (m, 2H), 3.18 (m, 2H), 2.92, 2.85 (2t, 2H, J = 7.2 Hz), 2.62 (t, 2H, J = 7.2 Hz), 2.0−1.1 (m, 24H), 0.94 (m, 2H); HRMS calcd for $C_{36}H_{58}N_6O_4$ (M + H^+) 631.3739, found 631.3729. Amino acid analysis: Ser 1.00 (1.02), Lys 1.00 (0.98).

(*R,S*)-*N*-[2-[4-[4-(2-Methyl-1*H*-benzimidazol-1-yl)butyl]-phenyl]-1-oxopropyl]-L-seryl-*N*-(2-cyclohexylethyl)-L-lysinamide (65) was prepared in a manner similar to **61** by reacting **60** with **12**: t_R = 22.8 min; ^{1}H-NMR (CD_3OD) δ 7.8 (m, 1H), 7.75 (m, 1H), 7.58 (m, 2H), 7.25 (d, 2H, J = 8.1 Hz), 7.14 (m, 2H), 4.44 (2H, t, J = 6.6 Hz), 4.35 (m, 2H), 3.85−3.6 (m, 2H), 3.16 (m, 2H), 2.93 (t, 2H, J = 6.9 Hz), 2.83 (2s, 3H), 2.67 (t, 2H, J = 7.2 Hz), 1.93 (m, 3H), 1.72 (m, 10H), 1.55−1.1 (m, 11H), 0.92 (m, 2H); HRMS calcd for $C_{38}H_{57}N_6O_4$ (M + H^+) 661.4441, found 661.4465. Amino acid analysis: Ser 1.00 (1.02), Lys 1.00 (0.98).

4-[4-(2-Methyl-1*H*-imidazol-1-yl)butyl]phenylacetyl-Ser-Lys-*N*-(2-cyclohexylethyl)amide (31). To a mixture of **24** (0.095 g, 0.3 mmol) and HOBt (0.066 g, 0.32 mmol) in dimethylacetamide (0.5 mL) and dichloromethane (3.0 mL)

was added DCC (0.66 g, 0.32 mmol), and the mixture was stirred at 0 °C. After 1.5 h, a solution of the amine acetate **12** (0.13 g, 0.24 mmol)[25] in dimethylacetamide (0.5 mL) and *N*-methylmorpholine (0.04 mL) were added and stirred at 0 °C for 1 h and then at room temperature for 16 h. The reaction mixture was concentrated *in vacuo*, and the residue was partitioned between cold 0.25 N NaOH (10 mL) and EtOAc (25 mL). The organic phase was washed successively with water (2 × 10 mL), 5% citric acid (2 × 10 mL), and water, dried (Na_2SO_4), and concentrated. The resulting material was washed with a solvent mixture of ether−hexane (1:1 v/v), filtered, and dried to give a pale yellow powder (0.125 g) which was stirred with trifluoroacetic acid (1.5 mL) for 3.5 h at room temperature. After the removal of TFA under reduced pressure, the residue was purified by reverse-phase HPLC to give **31** (0.08 g) as a white hygroscopic substance: t_R = 18.7 min; ^{1}H-NMR (CD_3OD) δ 7.3−7.15 (m, 6H), 4.34 (m, 2H), 4.08 (t, 2H, J = 7.25 Hz), 3.85 (m, 1H), 3.75 (m, 1H), 3.56 (s, 2H), 3.2 (m, 2H), 2.89 (t, 2H, J = 9.25 Hz), 2−0.8 (m, 23H); ^{13}C-NMR (CD_3OD) 173.59, 173.51, 172.89, 141.39, 134.14, 130.19, 129.50, 122.89, 118.99, 62.70, 56.95, 54.32, 49.67, 42.77, 40.34, 39.28, 37.47, 36.24, 35.51, 34.06, 31.69, 29.91, 28.87, 27.62, 27.46, 27.17, 23.66; HRMS calcd for $C_{33}H_{53}N_6O_4$ (M + H$^+$) 597.4128, found 597.4122. Amino acid analysis: Ser 1.00 (1.00), Lys 1.00 (1.00).

4-[4-(1*H*-Imidazol-1-yl)butyl]phenylacetyl-Ser-Lys-*N*-(2-cyclohexylethyl)amide (30) was prepared in a manner similar to **31** by coupling **23** with **12** as white hygroscopic substance: t_R = 17.2 min; ^{1}H-NMR (CD_3OD) δ 8.42 (s, 1H), 7.8 (br s, 1H, exchangeable), 7.43 (s, 1H), 7.33 (s, 1H), 7.2 (m, 4H), 4.33 (m, 2H), 4.17 (t, 2H, J = 7.5 Hz), 3.85 (m, 1H), 3.75 (m, 1H), 3.56 (s, 2H), 3.19 (m, 2H), 2.88 (t, 2H, J = 7.5 Hz), 2.65 (t, 2H, J = 7.2 Hz), 2−0.8 (m, 23H); HRMS calcd for $C_{32}H_{51}N_6O_4$ 583.3972 (M + H$^+$), found 583.3961. Amino acid analysis: Ser 1.00 (0.99), Lys 1.00 (1.00).

4-[4-(1*H*-Imidazol-1-yl)pentyl]phenylacetyl-Ser-Lys-*N*-(2-cyclohexylethyl)amide (36) was prepared in a manner similar to **31** by coupling **29** with **12** as white hygroscopic substance: ^{1}H-NMR (CD_3OD) δ 8.82 (s, 1H), 7.61 (s, 1H), 7.53 (s, 1H), 7.19 (d, 2H, J = 8.1 Hz), 7.12 (d, 2H, J = 8.1 Hz), 4.34 (m, 2H), 4.22 (t, 2H, J = 7.2 Hz), 3.83 (m, 1H), 3.75 (m, 1H), 3.57 (s, 2H), 3.19 (m, 2H), 2.89 (t, 2H, J = 7.2 Hz), 2.62 (t, 2H, J = 7.2 Hz), 1.92 (m, 3H), 1.72 (m, 10H), 1.8−1.1 (m, 10H), 0.92 (m 2H); HRMS calcd for $C_{33}H_{53}N_6O_4$ 597.4128 (M + H$^+$), found 597.4079. Amino acid analysis: Ser 1.00 (0.99), Lys 1.00 (1.01).

4-[4-(2-Methyl-1*H*-imidazol-1-yl)butyl]phenylacetyl-D-Ser-D-Lys-(2-cyclohexylethyl)amide (40). BOC(2-Cl-Cbz)-D-Lys-Merrifield resin (0.4 g of 0.63 mmol/g substitution) was treated with 50% TFA in CH_2Cl_2 for 30 min in a glass shaker vessel to remove the BOC protecting group. The amino acid-resin was then washed with CH_2Cl_2 (3 × 20 mL), 10% DIEA in CH_2Cl_2 (3 × 20 mL), 2-propanol, and CH_2Cl_2 (3 × 20 mL). A solution of BOC(*O*-Bn)-D-Ser (0.3 g, 1.0 mmol) in CH_2Cl_2 (3 × 20 mL) was treated with DCC (0.1 g, 0.5 mmol) and stirred for 20 min at ambient temperature, and the resulting mixture was then added to the amino acid-resin. After the resin was shaken for 60 min, the resin was washed thoroughly with CH_2Cl_2 (3 × 20 mL), treated with 50% TFA in CH_2Cl_2 for 30 min, and washed again with CH_2Cl_2 (3 × 20 mL), 10% DIEA in CH_2Cl_2 (3 × 20 mL), 2-propanol, and CH_2Cl_2 (3 × 20 mL). The peptide-resin thus obtained was suspended in a solvent mixture of DMF-DMA (5 mL), **24** (0.086 g, 0.25 mmol), DCC (0.057 g, 0.28 mmol), HOBt (0.034 g, 0.25 mmol), and diisopropylethylamine (0.04 mL, 0.5 mmol) were added, and the mixture was shaken for 18 h at room temperature. The product was divided in half, and one portion reacted with a mixture of cyclohexylethylamine (1 mL) in trifluroethanol (0.5 mL) at 50 °C for 5 h under N_2. The free peptide was concentrated under vacuum, and the amino acid side chain protecting groups were removed with 90% HF/anisole for 60 min at 0 °C. The crude peptide was concentrated under vacuum and purified to 99% purity on RP C-18, using a linear gradient of 5−55% acetonitrile (0.05% TFA) in water (0.05% TFA) over 25 min. The product was lyophilized to give 19 mg of **40** as a white powder: t_R = 14.6 min; ES/MS m/z 597 (M + H). Amino acid analysis: Ser 1.00 (1.04), Lys 1.00 (0.96).

4-[4-(2-Ethyl-1-imidazol-1-yl)butyl]phenylacetyl-Ser-Lys-*N*-(2-cyclohexylethyl)amide (33) was prepared in a manner similar to **31** by coupling **26** with **12** as white glassy substance: t_R = 20.8 min; ^{1}H-NMR (CD_3OD) δ 7.49 (d, 1H, J = 1.8 Hz), 7.44 (d, 1H, J = 1.8 Hz), 7.22 (d, 1H, J = 8.1 Hz), 7.15 (d, 1H, J = 8.1 Hz), 4.35 (m, 2H), 4.15 (t, 2H, J = 7.2 Hz), 3.85 (m, 1H), 3.75 (m, 1H), 3.57 (s, 2H), 3.18 (m, 2H), 2.97 (q, 2H, J = 7.5 Hz), 2.9 (t, 2H, J = 7.5 Hz) 2−1.8 (m, 3H), 1.78−1.6 (m, 10 H), 1.55−1.15 (t over m, 11H), 0.92 (m, 2H); HRMS calcd for $C_{34}H_{55}N_6O_4$ (M + H$^+$) 611.4285, found 611.4282. Amino acid analysis: Ser 1.00 (1.00), Lys 1.00 (1.00).

4-[4-(2-Isopropyl-1-imidazol-1-yl)butyl]phenylacetyl-Ser-Lys-*N*-(2-cyclohexylethyl)amide (34). A mixture of the acid **27** (0.047 g, 0.156 mmol), HOBt (0.021 g, 0.156 mmol), and EDC (0.030 g, 0.156 mmol) was stirred in dry DMF (2 mL) at room temperature for 40 min. It was then treated with a solution of **4** (0.085 g, 0.156 mmol) in dry DMF (2 mL) containing *N*-methylmorpholine (0.016 g, 0.312 mmol). After the reaction mixture was stirred at room temperature for 18 h, it was diluted with dichloromethane (100 mL) and washed successively with saturated $NaHCO_3$ (2 × 100 mL) and brine (2 × 100 mL) and dried ($MgSO_4$). Removal of the solvent under reduced pressure provided 90 mg of a yellow oil which was then treated with 4 N HCl in dioxane (5 mL) at room temperature for 2.5 h. The resulting mixture was concentrated under reduced pressure, and the residue was purified by reverse-phase HPLC to give 9 mg (12.5%) of **34** as a glassy substance: ^{1}H-NMR (CD_3OD) δ 7.48 (d, 1H, J = 2.01 Hz), 7.45 (d, 1H, J = 2.01 Hz), 7.18 (ab q, 4H, J = 8.06 Hz), 4.38−4.28 (m, 2H), 4.17 (t, 2H, J = 7.45 Hz), 3.91−3.81 (m, 2 H), 3.75−3.69 (m, 1H), 3.55 (s, 2 H), 3.42 (q, 1 H, J = 7.00 Hz), 3.23−3.14 (m, 2H), 2.94−2.86 (m, 3H), 1.96−0.84 (m, 29H); HRMS m/z calcd for $C_{35}H_{56}N_6O_4$ 625.4441 (M + H), found 625.4410. Amino acid analysis: Ser 1.00 (0.95), Lys 1.00 (1.05).

4-[4-(2-Methylbenzimidazol-1-yl)butyl]phenylacetyl-Ser-Lys-*N*-(2-cyclohexylethyl)amide (35) was prepared in a manner similar to **31** by coupling **28** with **12** as a white substance: t_R = 19.6 min; ^{1}H-NMR (CD_3OD) δ 7.75 (m, 2H), 7.60 (m, 2H), 7.19 (d, 1H, J = 8.1 Hz), 7.14 (d, 1H, J = 8.1 Hz), 4.44 (m, 2H), 4.35 (m, 2H), 3.84 (m, 1H), 3.72 (m, 1H), 3.56 (s, 2H), 3.18 (m, 2H), 2.88 (t, 2H, J = 7.2 Hz), 2.83 (s, 3H), 2.65 (t, 2H, J = 7.2 Hz), 1.95 (m, 3H), 1.82−1.55 (m, 10H), 1.55−1.11 (m, 8H), 0.95 (m, 2H); HRMS calcd for $C_{37}H_{55}N_6O_4$ (M + H$^+$) 647.4285, found 647.4329. Amino acid analysis: Ser 1.00 (0.96), Lys 1.00 (1.04).

4-[4-(2-Iodoimidazol-1-yl)butyl]phenylacetyl-Ser-Lys-*N*-(2-cyclohexylethyl)amide (32) was prepared in a manner similar to **31** by coupling **25** with **12** as a white hygroscopic powder: t_R = 19.5 min; ^{1}H-NMR (CD_3OD) δ 7.60 (d, 1H, J = 1.8 Hz), 7.48 (d, 1H, J = 1.8 Hz), 7.21 (d, 2H, J = 8.1 Hz), 7.15 (d, 2H, J = 8.1 Hz), 4.35 (m, 2H,), 4.16 (t, 2H, J = 7.2 Hz), 3.80 (m, 2H), 3.56 (s, 2H), 3.18 (m, 2H), 2.89 (t, 2H, J = 7.2 Hz), 2.66 (t, 2H, J = 7.5 Hz), 1.85 (m, 2H), 1.66 (m, 10H), 1.55−1.1 (m, 9H), 0.85 (m, 2H); HRMS calcd for $C_{34}H_{50}IN_6O_4$ (M + H$^+$)709.2937, found 709.2884. Amino acid analysis: Ser 1.00 (1.00), Lys 1.00 (1.00).

11-(1*H*-Imidazol-1-yl)undecanoyl-Ser-Lys-(2-cyclohexylethyl)amide (13) was prepared in a manner similar to **31** by coupling **11** with **12** as a white glassy substance: t_R = 19.8 min; ^{1}H-NMR (CD_3OD) δ 8.55 (s, 1H), 7.5 (s, 1H), 7.39 (s, 1H), 4.35 (m, 2H), 4.19 (t, 2H, J = 7.2 Hz), 3.8 (m, 2H), 3.2(m, 2H), 2.94 (m, 2H), 2.27 (t, J = 7.2 Hz, 2H), 2−0.8 (m, 35H); HRMS calcd for $C_{31}H_{57}N_6O_4$ (M + H$^+$) 577.4442, found 577.4452. Amino acid analysis: Ser 1.00 (0.98), Lys 1.00 (1.02).

Cbz-Lys(BOC)-Cha-OMe (71). A solution of BOC-Cha-OMe (1.0 g, 3.5 mmol) in dichloromethane (4.00 mL) was treated with trifluoroacetic acid (1.00 mL), and the mixture was stirred at room temperature for 1 h. After removal of the solvents under reduced pressure, the residue was triturated with ether and filtered. The solid material thus obtained was washed thoroughly with ether and dried in a desiccator under vacuum over NaOH pallets to give the trifluoroacetate salt (0.87 g), which was used without further purification in the following step.

To a solution of Cbz-Lys(BOC)-OH (1.3 g, 3.4 mmol) (**70**) and HOBt (0.6 g, 4.0 mmol) in dichloromethane (8.00 mL) and

dimethylacetamide (2.0 mL) was added dropwise a solution of DCC (0.74 g, 3.6 mmol) in dichloromethane (10 mL), and the mixture was stirred at 0 °C for 1 h at 0 °C. The reaction mixture was filtered, and the filtrate was added to a solution of the trifluoroacetate salt as prepared above in dichloromethane (3.0 mL) and added *N*-methylmorpholine (0.37 g, 3.68 mmol). After being stirred at room temperature for 16 h, the reaction mixture was concentrated under vacuum. The residue was partitioned between EtOAc (40 mL) and 5% citric acid (20 mL). The organic phase was washed successively with 5% citric acid (2 × 20 mL), water (2 × 20 mL), 0.25 N NaOH (2 × 20 mL) and brine and dried (Na_2SO_4). After removal of the solvent, the product was crystallized from EtOAc/hexane to give 1.4 g (73%) of the dipeptide ester **71** as a white powder: mp 98−100 °C; ^{1}H-NMR ($CDCl_3$) δ 7.36 (m, 5H), 6.31 (br, 1H), 4.41 (br, 1H), 5.11 (s, 2H), 4.63 (m, 2H), 4.16 (m, 1H), 3.73 (s, 3H), 3.08 (m, 2H), 2−1.45 (m, 12H), 1.42 (s, 9H), 1.4−0.8 (m, 7H); FAB-MS *m*/*z* 554 (M + Li); HRMS calcd for $C_{29}H_{45}N_3O_7Li$ (M + Li) 554.3418, found 554.3447.

Cbz-Ser(O^{t}Bu)-Lys(Boc)-Cha-OMe (72). A solution of **71** (0.6 g, 1.1 mmol) in MeOH (15 mL) and acetic acid (0.07mL) was hydrogenated at atmospheric pressure, in the presence of 5% Pd/C (0.2 g) for 1 h. The catalyst was removed by filtration, the filtrate was concentrated under reduced pressure, and the resulting amine acetate (FAB-MS 420 (M + Li)) was used without further purification in the next step.

To a solution of Cbz-Ser(O^{t}Bu)-OH (0.4 g, 1.35 mmol) in dimethylacetamide (1 mL) and dichloromethane (5 mL) at 0 °C were added HOBt (0.23 g, 1.53 mmol) and DCC (0.3 g, 1.45 mmol), and the mixture was stirred for 1 h. The reaction mixture was filtered, and the filtrate was added to a solution of the amine acetate in dimethylacetamide (1 mL) containing *N*-methylmorpholine (0.14 g, 1.36 mmol). After the resulting mixture was stirred at room temperature for 16 h, the solvents were removed *in vacuo*, and the residue was partitioned between cold 0.25 N NaOH (25 mL) and ethyl acetate (25 mL). The organic phase was washed successively with cold 0.25 N NaOH (2 × 25 mL), water, 5% citric acid (2 × 15 mL), and water, dried (Na_2SO_4), and concentrated under reduced pressure. The resulting material was crystallized from EtOAc/hexane to give **72** (0.48 g, 63%) as a white powder: m p 108−109 °C; ^{1}H-NMR ($CDCl_3$) δ 7.37 (m, 4H), 7.18 (d, 1H, *J* = 9.6 Hz), 6.41 (br, 1H), 5.68 (br, 1H), 5,12 (s, 2H), 4.69 (m, 1H), 4.59 (m, 1H), 4.43 (q, 1H, J = 7.2 Hz), 4.24 (m, 1H), 3.83 (m, 1H), 3.73 (s, 3H), 3.4 (t, 1H, *J* = 7.5 Hz), 3.07 (m, 2H), 2−1.45 (m, 11H), 1.43−1.2 (m, 3H), 1.19 (s, 9H), 1.12−0.8 (m, 5H); FAB-MS *m*/*z* 697 (M + Li), 641, and 597; HRMS calcd for $C_{36}H_{59}N_4O_9$ (M + H) 691.4282, found 691.4273.

4-[4-(2-Methyl-1-imidazol-1-yl)butyl]phenylacetyl-L-Ser-L-Lys-L-Cha-OH (73a). A solution of **72** (0.2 g, 0.29 mmol) in MeOH (10.0 mL) was hydrogenated at room temperature for 1 h in the presence of 5% Pd/C (0.05 g). The catalyst was removed by filtration, the filtrate was concentrated under reduced pressure, and the residue was dried in a desiccator for 2 h *in vacuo* to afford the amine (FAB-MS *m*/*z* 563 M + Li) which was used as such in the following step.

To a solution of **24** (0.12 g, 0.39 mmol) and HOBt (0.065 g, 0.43 mmol) in dimethylacetamide (2.5 mL) was added DCC (0.085 g, 0.41 mmol), and the mixture was stirred at 0 °C for 2 h. It was then treated with a solution of the amine as prepared above in dimethylacetamide (0.5 mL) containing *N*-methylmorpholine (0.041g, 0.41 mmol), and DMAP (0.005 g) was added. The reaction mixture was stirred at room temperature for 24 h and concentrated *in vacuo*. The residue was partitioned between EtOAc (25 mL) and cold 0.25 N NaOH (10 mL). The organic phase was washed with water (3 × 15 mL), dried (Na_2SO_4), and concentrated under reduced pressure to give an amorphous substance which was crystallized from ether/hexane and stirred with 1 M LiOH, (0.3 mL), containing MeOH (0.2 mL), for 2 h at room temperature. The mixture was acidified with 5% citric acid and extracted with EtOAc (3 × 15 mL). The combined organic extracts were washed with water (3 × 10 mL), dried (Na_2SO_4), and concentrated. The residue was dried *in vacuo* for 16 h and treated with trifluoroacetic acid (1.5 mL). After being stirred for 4 h at room temperature, the solution was concentrated under reduced pressure and residue was purified by reverse-phase HPLC. The appropriate fractions were combined and freeze-dried to afford **73a** (0.053 g, 34%) as a white powder: t_R = 19.8 min; ^{1}H-NMR (CD_3OD) δ 7.45 (d, 1H, *J* = 2.1 Hz), 7.38 (d, 1H, *J* = 2.1 Hz), 7.22 (m, 4H), 4.41 (m, 3H), 4.13 (m, 2H), 3.81 (m, 2H), 3.58 (d, 2H, *J* = 2.4 Hz), 2.92 (m, 2H), 2.67 (m, 2H), 2.57 (s, 3H), 1.95−0.85 (m, 23H); FAB-MS *m*/*z* 641 (M + H); HRMS calcd for $C_{34}H_{53}N_6O_6$ (M + H), 641.4027, found 641.4041. Amino acid analysis: Ser 1.00 (1.02), Lys 1.00 (1.00).

(*R,S*)-*N*-[2-[4-[4-(2-Methyl-1*H*-imidazol-1-yl)butyl]phenyl]-1-oxopropyl]-L-Ser-L-Lys-L-Cha-OH (73b) was prepared in a manner similar to **73a**, starting from **72** and **56**: yield 23% (a white powder); t_R = 21.6 min; ^{1}H-NMR (CD_3OD) δ 7.37 (d, 1H, *J* = 2.1 Hz), 7.30 (d, 1H, *J* = 2.1 Hz), 7.17 (d, 1H, *J* = 7.8 Hz), 7.05 (d, 1H, *J* = 7.8 Hz), 4.35 (m, 2H), 4.23 (m, 1H), 4.03 (t, 2H, *J* = 7.5 Hz), 3.64 (m, 3H), 2.84 (t, 2H, *J* = 7.8 Hz), 2.57 (t, 2H, *J* = 7.5 Hz), 2.49 (s, 3H), 2.0−1.05 (m, 24H), 0.85 (m, 2H); FAB-MS *m*/*z* 655 (M + H); HRMS calcd for $C_{35}H_{55}N_6O_8$ (M + H) 655.4183, found 655.4210. Amino acid analysis: Ser 1.00 (1.00), Lys 1.00 (1.00).

4-[4-(2-Methyl-1*H*-imidazol-1-yl)butyl]phenylacetyl-D-Ser-D-Lys-D-Cha-OH (73d). The peptide was assembled by the solid-phase method of synthesis.[22] To a suspension of hydroxymethylpolystyrene resin (3.0 g, 0.67 mmol of OH/g of resin) in dichloromethane (40 mL) at 0 °C were added Boc-β-cyclohexyl-D-Ala (1.08 g, 4.0 mmol), DCC (0.82 g, 4.0 mmol), and DMAP (0.05 g, 0.4 mmol). The mixture was allowed to warm to room temperature and shaken in a glass vessel for 18 h. The amino acid-resin was washed successively with dichloromethane (25 mL), methanol (25 mL), and dichloromethane (25 mL). The resulting resin **74** was treated with 50% TFA (trifluoroacetic acid) in CH_2Cl_2 (25 mL) for 30 min and washed successively with CH_2Cl_2 (25 mL), 2-propanol (25 mL), 10% DIEA, and dichloromethane (25 mL). This resin was then treated with a stirred solution of BOC-(2-Cl-Cbz)-D-Lys (3.32 g, 8.0 mmol) and DCC (0.82 g, 4.0 mmol) in dichloromethane (40 mL) and shaken for 60 min. The peptide resin was then washed with dichloromethane, methanol, and dichloromethane to obtain dipeptide-resin product **75**. This was treated with 50% trifluoroacetic acid in dichloromethane for 30 min, followed by washing with dichloromethane (25 mL), 2-propanol (25 mL), 10% DIEA, and dichloromethane (25 mL). The resulting resin was treated with a stirred solution of BOC-(*O*-Bn)-D-Ser (2.36 g, 8.0 mmol) and DCC (0.82 g, 4.0 mmol) in dichloromethane (40 mL) and shaken for 60 min. After the resin was washed successively with dichloromethane (25 mL), methanol (25 mL), and dichloromethane (25 mL), the resulting tripeptide-resin product **76** was treated with 50% TFA in dichloromethane (25 mL) and washed with dichloromethane (25 mL), 2-propanol (25 mL), 10% DIEA, and dichloromethane (25 mL). This resin was suspended in DMF/DME (9/1, v/v, 40 mL); **24** (0.2 g, 0.65 mmol), HOBt (0.09 g, 0.65 mmol), DCC (0.15 g, 0.72 mmol), and DIEA (0.11 mL, 0.65 mmol) were added; and the mixture was allowed to shake for 18 h. The crude product was liberated from the resin, and the protecting groups were removed, by treatment with 90% HF/anisole for 60 min at 0 °C. The resulting crude peptide was purified to 98% purity on RP C-18 HPLC, using a linear gradient of 5−55% acetonitrile (0.05% TFA) in water (0.05% TFA) over 25 min. The appropriate fractions were pooled and lyophilized to give 280 mg of **73d** as a white powder: t_R = 19.98 min; ^{1}H-NMR (CD_3OD) δ 7.46 (d, 1H, *J* = 2.1 Hz), 7.39 (d, 1H, *J* = 2.1 Hz), 7.22 (d, 2H, *J* = 8.1 Hz), 7.14 (d, 2H, *J* = 8.1 Hz), 4.42 (m, 3H), 4.12 (t, 2H, *J* = 7.5 Hz), 3.79 (m, 2H), 3.57 (s, 2H), 2.89 (t, 2H, *J* = 7.5 Hz), 2.66 (t, 2H, *J* = 7.5 Hz), 2.58 (s, 3H), 2.0−1.1 (m, 21 H), 0.95 (m, 2H); HRMS calcd for $C_{34}H_{53}N_6O_6$ (M + H$^+$) 641.4027, found 641.4043. Amino acid analysis: Ser 1.00 (0.98), Lys 1.00 (1.00).

4-[4-(2-Methyl-1*H*-imidazol-1-yl)butyl]phenylacetyl-L-Ser-L-Lys-L-Cha-OMe (73c) was prepared in a manner similar to **31** starting from **72** and **24**: t_R = 21.3 min; ^{1}H-NMR (CD_3OD) δ 7.36 (d, 1H, *J* = 2.1 Hz), 7.28 (d, 1H, *J* = 2.1 Hz), 7.21 (d, 2H, *J* = 8.1 Hz), 7.14 (d, 2H, *J* = 8.1 Hz), 4.41 (m,3 H), 4.09 (t, 2H, *J* = 7.5 Hz), 3.81 (m, 2H), 3.69 (s, 3H), 3.57 (s, 2H), 2.89 (t, 2H, *J* = 7.5 Hz), 2.68 (t, 2H, *J* = 7.5 Hz), 2.53 (s, 3H), 2.0−1.1 (m, 21H), 0.85 (m, 2H); FAB-MS *m*/*z* 655 (M +

H); HRMS calcd for $C_{35}H_{55}N_6O_8$ (M + H) 655.4183, found 655.4225. Amino acid analysis: Ser 1.00 (0.95), Lys 1.00 (1.05).

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