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Communications to the Editor

Design and Syntheses of Potent and Selective Dipeptide Inhibitors of *Candida albicans* Myristoyl-CoA:Protein *N*-Myristoyltransferase

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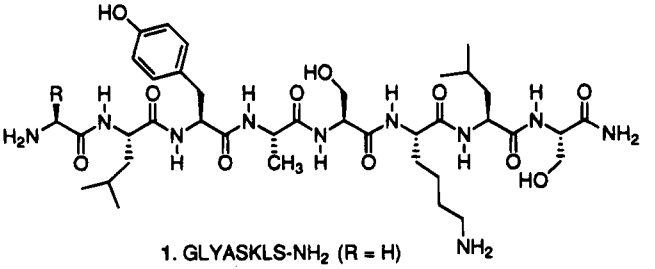
Myristoyl-CoA:protein *N*-myristoyltransferase (NMT; EC 2.1.3.97), is a monomeric enzyme[1,2] which transfers the rare cellular fatty acid myristate from myristoylCoA to the *N*-terminal glycine of eukaryotic cellular proteins with diverse functions.[3,4] A number of proteins encoded by enveloped and nonenveloped viruses are *N*-myristoylated and also are required for viral replication.[5] Different proteins utilize their covalently bound myristoyl moiety for different purposes, including regulation of protein–protein and protein–lipid interactions.[4]

NMT is encoded by a single copy gene in humans, yeast, and pathogenic fungi.[6–8] Introduction of temperature-sensitive NMT alleles into *Cryptococcus neoformans* and *Candida albicans* by homologous recombination has provided proof that these pathogenic fungi require NMT for their viability.[9,10] These observations suggest that selective inhibitors of fungal NMTs could represent a new class of fungicidal agents for treating systemic fungal infections in the rapidly increasing population of immunocompromised patients.[11]

The design of NMT inhibitors was guided by the observation that although the acylCoA binding sites of NMTs are highly conserved,[9,12] their peptide substrate specificities have diverged.[2,7,9,13,14] This difference in substrate specificity was exploited in order to develop the first potent and selective peptidomimetic inhibitors of NMT. The strategy emerged from the identification of a high-affinity octapeptide substrate GLYASKLS-NH_2, **1** (Figure 1, K_m = 0.07, 0.6 and 0.7 μM for *Saccharomyces cerevisiae*,[15] *C. albicans*,[14] and human NMT,[2] respectively), which is derived from the N-terminal fragment of Arf2p (ADP ribosylation factor 2), a protein that must be myristoylated by NMT for expression of its essential biological function.[16] Peptide structure–activity relationship (SAR) studies using octapeptides in which each amino acid residue in **1** was individually replaced with alanine 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 key enzyme recognition functionalities that are imperative for tight binding. The substitution of alanine for glycine provided a competitive inhibitor ALYASKLS-NH_2, **2** (Figure 1, K_i = 5, 8, and 35 μM for *S. cerevisiae*,[15] *C. albicans*,[14] and human NMT,[14] respectively).[17] Analog **2** was the first example of a peptide with a residue other than glycine at the N-terminus that was capable of binding to NMT.



1. GLYASKLS-NH_2 (R = H)
2. ALYASKLS-NH_2 (R = CH_3)

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

Structural modifications of **1**, which were obtained by replacing the amino acid residues at positions 1–4 with an 11-aminoundecanoyl moiety and removing the C-terminal Ser-carboxamide, led to the identification of a tripeptide lead **3** (Chart 1) with micromolar potency against *Candida* NMT (IC_{50} = 1.2 μM, Table 1). Previous SAR studies[14] indicated that the leucine residue in **3** contributed a modest factor of approximately 10 to the binding of the inhibitor. Subsequent removal of the leucine carboxamide group afforded the isoamylamide

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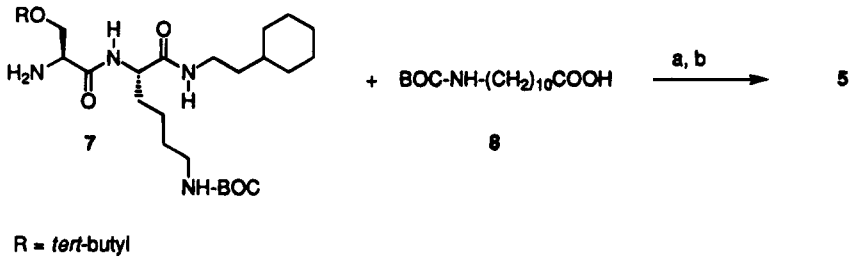
Table 1. Potency and Selectivity of Peptidomimetic Inhibitors of NMT[a]

	C. albicans NMT		human NMT		
compd	IC_{50}, μM	$K_{i(app)}$, μM	IC_{50}, μM	$K_{i(app)}$, μM	selectivity[b]
3	1.20 ± 0.14	ND	0.23 ± 0.01	ND	0.2
4	0.78 ± 0.11	ND	0.42 ± 0.06	ND	0.5
5	0.11 ± 0.03	0.070 ± 0.01	0.50 ± 0.37	0.09 ± 0.02	4.5
6	0.30 ± 0.03	0.13 ± 0.004	1.14 ± 0.61	ND	3.8
10	1.60 ± 0.07	0.76 ± 0.01	11.4 ± 0.49	4.2 ± 0.7	7.1
16	0.42 ± 0.15	0.59 ± 0.07	16.0 ± 3.0	ND	38
17	0.056 ± 0.01	0.031 ± 0.003	14.1 ± 4.3	ND	250

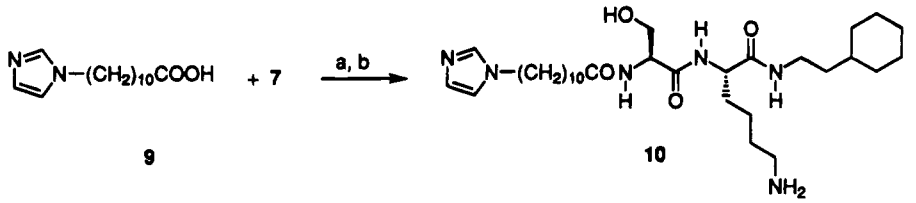
[a] Potency against the indicated NMT as assessed by IC_{50} using the peptide GNAASARR-NH_2 at its apparent K_m (see text) and myristoyl-CoA at 1 μM, and by the apparent K_i versus the peptide substrate GNAASARR-NH_2 determined with the myristoyl-CoA at 1 μM. [b] Selectivity is the ratio of the IC_{50}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

Scheme 1[a]



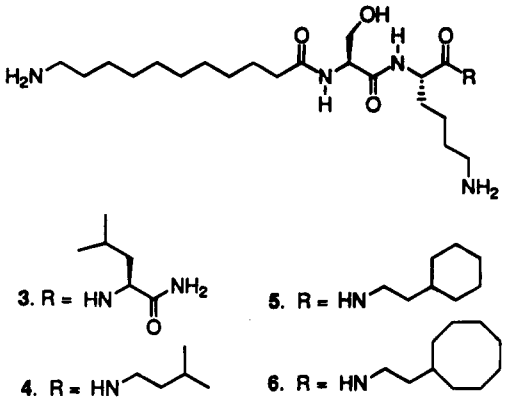
[a] Reagents: (a) DCC, HOBt, CH_2Cl_2–DMF, 0 °C to room temperature; (b) trifluoroacetic acid, room temperature, 3 h.

Scheme 2[a]



[a] Reagents: (a) DCC, HOBt, CH_2Cl_2–DMF, 0 °C to room temperature; (b) trifluoroacetic acid, room temperature, 3 h.

Chart 1. 11-Aminoundecanoyl-Substituted Peptide Amide Inhibitors of *Candida albicans* NMT

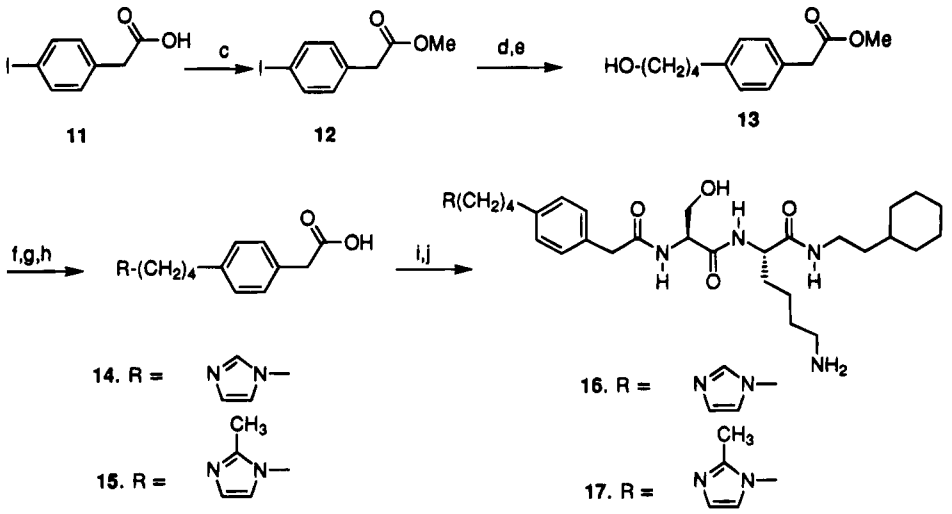


4 (IC_{50} = 0.78 μM) with no loss in binding affinity. Further replacement of the isoamyl group in **4** with several other aliphatic and aromatic residues led to the identification of the (cyclohexylethyl)amide **5** (IC_{50} = 0.11 μM) as the most potent inhibitor in this series. However, the introduction of the larger 2-cyclooctylethyl group (**6**, IC_{50} = 0.3 μM) decreased the inhibitory activity.

The synthesis of the cyclohexylethyl analog **5**[18] was accomplished (Scheme 1) by the condensation of the *tert*-butyl-protected dipeptide amine **7**[18] with BOC-protected aminoundecanoic acid **8**[19] using DCC/HOBt. After deprotection, the desired product was isolated by reverse-phase liquid chromatography to give **5** as its trifluoroacetate salt. The dipeptide **5** not only exhibited significantly improved potency (K_i = 0.07 μM, Table 1) but also displayed a modest 5-fold selectivity versus human NMT. In order to further augment this potency and selectivity, efforts were focused on 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. A series of N-linked imidazole compounds with varying chain lengths between the imidazole and the serine residue was synthesized as illustrated for the analog **10** (Scheme 2). The acid precursor **9**[20] was obtained by the alkylation of sodium imidazolide with the ω-iodo acid in the presence of 18-crown-6 in dimethylformamide. Subsequent reaction of **9** with dipeptide amine **7** in the presence of DCC/HOBt, followed by deprotection, provided the imidazole-substituted analog **10**. The inhibitory activity was found to be a function of the connecting chain length, with a minimum IC_{50} of 1.6 μM (K_i = 0.76 μM, Table 1) for **10**.

At this stage, further enhancements to attain potencies below 1 μM with these imidazole-containing compounds presented a synthetic challenge. We reasoned that the potency of these inhibitors might be improved by introducing conformational constraints in the flexible linear linker chain using a 1,4-disubstituted phenyl ring. A facile synthetic methodology was developed for the construction of the key intermediates, N-linked imidazole-substituted phenylacetic acids **14** and **15**,[18] as outlined in Scheme 3. The commercially available

Scheme 3[a]



[a] Reagents: (c) 4 N HCl/dioxane, MeOH, room temperature, 16 h; (d) butyn-1-ol, $PdCl_2(PPh_3)_2$, CuI, Et_3N, CH_3CN, room temperature, 3 h; (e) H_2, Pd/C (5%), 40 psi, room temperature, 4 h; (f) $(PhO)_3PMeI$, CH_3CN, room temperature, 4 h; (g) 1 M LiOH, MeOH–H_2O, room temperature, 2.5 h; (h) RNa, DMF, 18-crown-6, 60 °C, 2.5 h; (i) DCC/HOBt, DMF, + **7**; (j) trifluoroacetic acid, 3 h.

4-iodophenylacetic acid **11** was converted to the methyl ester **12** in the presence of methanolic HCl and coupled with butyn-1-ol in the presence of $PdCl_2(PPh_3)_2$, CuI, and Et_3N in acetonitrile to afford **13** after reduction of the triple bond. The iodination of **13** followed by methanolysis and reaction of the resulting iodo acid with sodium imidazolide in DMF in the presence of 18-crown-6 in catalytic amounts led to the formation of the N-1 imidazole-substituted phenylacetic acid **14**. The condensation of **14** with the amine **7**, using DCC/HOBt, followed by deprotection, furnished the desired product **16** as its trifluoroacetate salt. Remarkably, the incorporation of a 1,4-disubstituted phenyl ring in **16** imparted increased potency (IC_{50} = 0.42 μM) versus **10** and significantly improved selectivity (40-fold versus human NMT, Table 1). Compound **16** is the first example of a competitive peptidomimetic NMT inhibitor (K_i = 0.59 μM) having an amphoteric group at the N-terminus which displayed a potency below 1 μM against the *Candidal* enzyme. More strikingly, we discovered that a seemingly minor structural modification involving substitution of imidazole by 2-methylimidazole, as exemplified by **17**, further improved the potency (IC_{50} = 0.056 μM) and selectivity (250-fold versus human NMT, Table 1). The kinetic analysis of inhibition (Figure 2) indicated that **17** was a competitive inhibitor with respect to the peptide substrate GNAASARR-NH_2 (K_m = 20 μM) with a K_i = 0.031 μM, a significant potency improvement over the substrate **1**. To the best of our knowledge, compound **17** represents the most potent and selective peptidomimetic *Candida* NMT inhibitor reported to date. Another pertinent observation is that the imidazole-substituted phenylacetyl moiety in **17** effectively mimics the tetrapeptide GLYA and imparts high affinity to the inhibitor.

In conclusion, the first potent and selective peptidomimetic inhibitors of the NMT enzyme from *C. albicans* have been identified. With a weak octapeptide inhibitor, ALYASKLS-NH_2 (MW = 850), as a starting point, a dipeptide inhibitor, **17** (IC_{50} = 56 nM, 250-fold selective, MW 596), was identified. An unprecedented phenomenon is that 2-methylimidazole functions as a substitute for the N-terminal primary amino group of

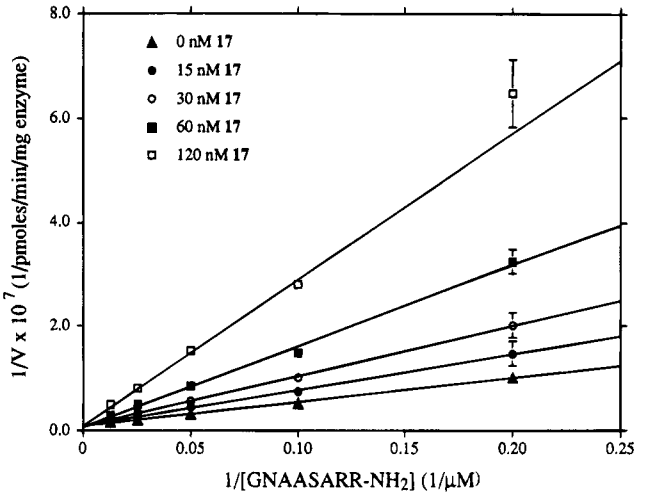


Figure 2. Lineweaver–Burk plot of 1/*V* vs 1/[GNAASARR-NH_2] for inhibitor **17** with *Candida* NMT. The concentration of peptide substrate was varied from 5 to 80 μM (ca. $^1/_8K_m$ to $2K_m$) with a fixed myristoyl-CoA concentration of 1 μM and 12 ng of NMT. A replot of the slopes vs 1/[**17**] yielded a $K_{i(app)}$ for inhibitor **17** vs GNAASARR-NH_2 of 31 ± 3 nM. Error bars were plotted as data points ± standard deviation of the triplicate measurements. Error bars that would obscure the data points were omitted for clarity.

NMT peptide ligands and imparts unexpectedly high affinity and selectivity. The synthetic methodology described herein allows the preparation of a variety of heteroatom or heterocyclic substituted 4-phenylacetic acid analogs. The knowledge gathered from this SAR data has been further utilized to develop totally non-peptidic NMT inhibitors which will be published elsewhere in the near future. Further synthetic details and the biological data on these novel NMT inhibitors will be reported in forthcoming publications.

Acknowledgment. The authors thank Mr. James F. Zobel for providing amino acid analyses and Mr. James P. Doom and Charles A. Gloeckner of Monsanto Co. for obtaining FAB mass spectral data.

Supplementary Material Available: Experimental procedures, including ^{1}H-NMR data, amino acid analyses, HPLC

data, and high-resolution FAB mass spectral data for the intermediates and final products (5 pages). Ordering information is given on any current masthead page.

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(17) All the K_i's are reported as apparent inhibition constants, competitive versus the peptide substrate GNAASARR-NH_2. *C. albicans* and human NMTs were expressed in *E. coli* and were purified to apparent homogeneity using protocols described in earlier publications (refs 1 and 13). IC_{50} and K_i determinations of inhibitors with recombinant *Candida* (ref 13) and human (ref 7) NMT were made using a radiochemical HPLC end-point assay as described previously (refs 1 and 2). The reported IC_{50}s represent an average of two or more determinations. Inhibitor stock solutions were prepared at 22 mM in DMSO; the final concentration of DMSO in each assay was 0.5% (v/v). IC_{50} assays with *Candida* NMT employed variable amounts of inhibitor or buffer with 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 of 0.2 M *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid), pH 7.4, 2 mM DL-dithiothreitol, 0.2 mM ethylene glycol bis(β-aminoethyl ether) *N,N,N',N'*-tetraacetic acid, and the reaction was initiated by the addition of 50 μL of a *Candida* NMT solution (7–12 ng/50 μL). Assays with human NMT were identical except that they used 60–75 ng/50 μL of enzyme solution and 0.22 nmol of GNAASARR-NH_2. The reaction was quenched after 10 min at 24 °C by the addition of ice-cold MeOH, and the reaction products were separated and measured using HPLC and in-line scintillation counting (ref 2). K_i determinations versus the peptide substrate were carried out in a similar manner, except that the GNAASARR-NH_2 concentration was varied between 5 and 80 μM (*Candida* NMT) or between 0.5 and 8 μM (human NMT), and that the reaction times were lengthened or shortened as needed to achieve the maximum signal possible without incurring substrate depletion. The data were analyzed by nonlinear regression analysis of double reciprocal (Lineweaver–Burk) plots with the program *k*-cat (BioMetallics, Princeton, NJ). Both competitive and noncompetitive models were tested in each case.

(18) All new compounds exhibited spectral and analytical data consistent with the structure; experimental details (^{1}H-NMR, ^{13}C-NMR, HPLC data, and amino acid analyses) are provided in the supplementary material.

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