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A CHIRAL RECOGNITION ELEMENT WITH AN UNUSUAL SIZE CONSTRAINT AFFECTS THE POTENCY AND SELECTIVITY FOR PEPTIDOMIMETIC INHIBITORS OF *CANDIDA ALBICANS* MYRISTOYL-COA:PROTEIN *N*-MYRISTOYLTRANSFERASE[¥]

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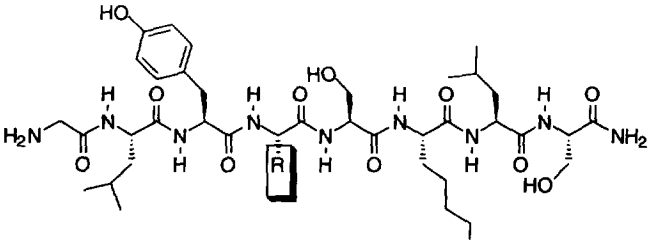
Abstract: Beginning with a high affinity octapeptide substrate GLYASKLS-NH_2 (**2**, K_m = 0.6 μM) a potent dipeptide *Candida* NMT inhibitor **18a** (IC_{50} = 20 nM) was identified. The structure-activity relationship studies suggest that the α-methyl group with an (*R*) configuration at the benzylic position, imparts maximum selectivity and potency against *Candida* NMT. The synthetic design, chiral separation of the diastereomers **18a** and **18b**, and in vitro potency of this novel class of NMT inhibitors are described.

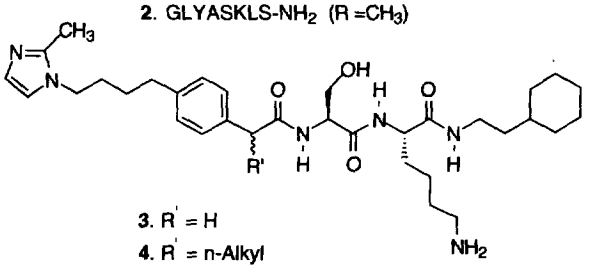
Myristoyl-CoA:protein *N*-myristoyltransferase (NMT; EC 2.1.3.97), is a cytosolic monomeric enzyme that catalyzes the cotranslational transfer of myristate (C14:0) from myristoyl-CoA to the *N*-terminal glycine residue of eukaryotic proteins.[1,2] Recent genetic studies have demonstrated that pathogenic fungi such as *C. albicans* and *Cryptococcus neoformans* require NMT for their viability,[3] suggesting that potent and selective inhibitors of fungal NMTs might lead to new fungicidal agents with a novel mode of action.[4] While potent analog inhibitors of myristoyl-CoA have have been identified, they exhibit little selectivity for human NMT.[5] In contrast, each of these enzymes exhibit distinctly different specificities[6,7] toward peptide substrates. These differences in substrate specificity were exploited to identify potent and selective peptidomimetic NMT inhibitors. Here, we report the design, synthesis, and kinetic properties of **18a**, the most potent (IC_{50} = 20 nM, K_i = 27 nM) peptidomimetic inhibitor of *Candida* NMT reported to date, which also exhibits significant selectivity (400-fold) versus human NMT. This remarkable selectivity and specificity for the fungal enzyme is unprecedented, and is quite surprising since human NMT recognizes a much wider variety of protein substrates than its fungal counterpart.

Our synthetic endeavor originated from the tight-binding octapeptide substrate **2**, 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.[8] The identification of the *N*-terminal amino group of

[¥]Dedicated with great respect to Professor Nelson J. Leonard on the occasion of his 80th birthday



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



3. R' = H
4. R' = n-Alkyl

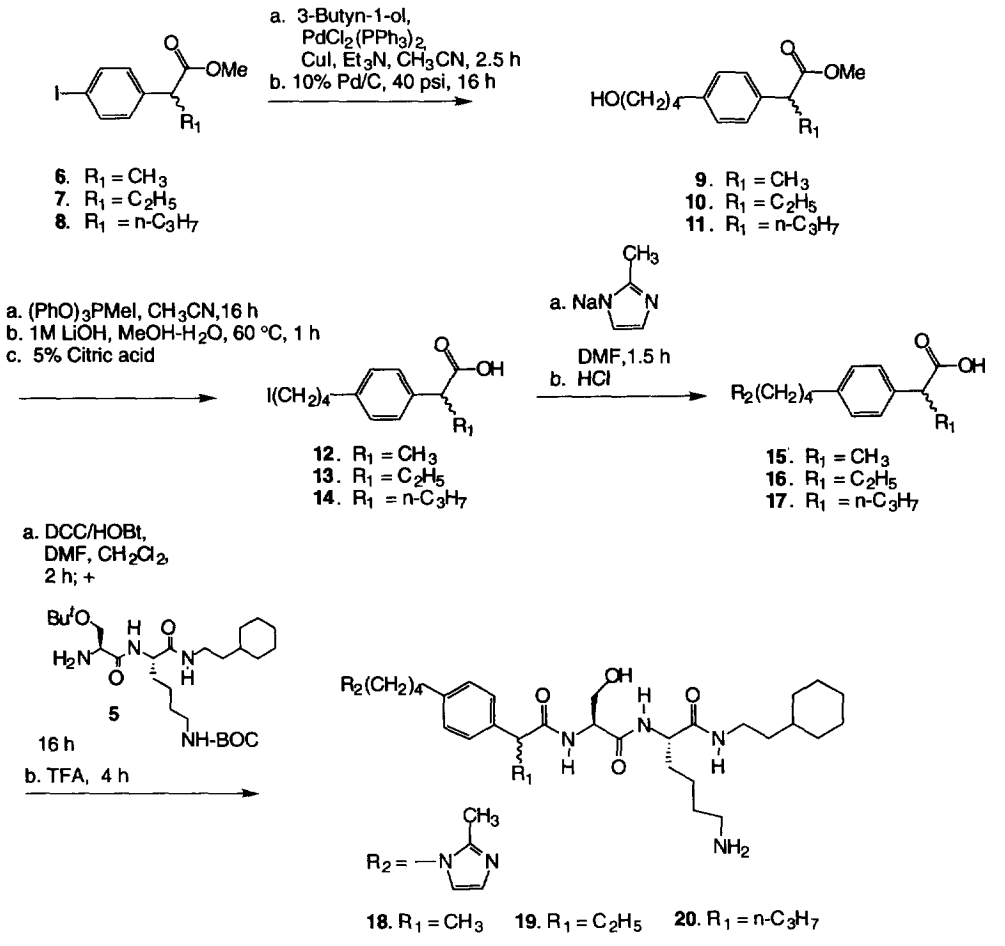
glycine-1, the hydroxyl group of serine-5, and the ε-amino group of lysine-6 as the three key enzyme recognition elements needed for tight-binding led to the synthesis of **3** (IC_{50} = 60 nM, 250-fold selective versus human NMT).[9] In this communication we disclose a fourth recognition element in **2** that culminated in the design and synthesis of **18a** with improved potency and selectivity.

The rationale for the synthesis of **18a** evolved from the observation that the substitution of glycine for alanine-4 in **2** to provide **1** (K_m = 4.6 μM for *C. albicans*) resulted in a tenfold decrease in the apparent K_m versus **2**,[10] indicating that the side chain methyl group of alanine-4 is also an important recognition element in enhancing binding affinity of the inhibitor. This result suggests that analogs of **3** with an α-alkyl substituent (**4**, Figure 1) might exhibit higher potencies. This set the stage for a general synthetic strategy that would allow the introduction of an alkyl substituent R′ at the benzylic position α to the carboxamide group in **3** in order to probe the steric effects on potency and selectivity at this position.

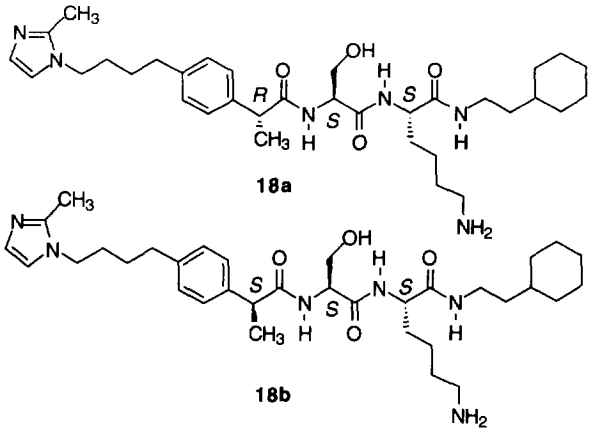
The synthesis (Scheme 1) began with the alkylation of methyl 4-iodophenylacetate with methyl iodide in the presence of NaH in THF to afford *(R,S)* methyl 2-(4-iodophenyl)propionate **6**. The ester **6** was coupled with 3-butyn-1-ol using catalytic amounts of $PdCl_2(PPh_3)_2$ and CuI in the presence of triethylamine, and the resulting product was subjected to catalytic reduction to afford the hydroxy ester **9**. The reaction of **9** with methyl triphenoxyphosphonium iodide, followed by hydrolysis and acidification provided the iodo acid **12**.

The condensation of the acid **12** with 2-methylimidazole in the presence of NaH yielded the corresponding imidazole substituted carboxylic acid **15**. Subsequent activation of **15** using DCC/HOBt, and coupling with the amine **5**,[9] followed by deprotection and purification by preparative reverse-phase HPLC, provided **18** as a diastereomeric mixture. The *(R,S)* ethyl (**19**), and *(R,S)* n-propyl (**20**) analogs were synthesized by a similar reaction sequence as outlined in Scheme 1.[11]

Scheme 1



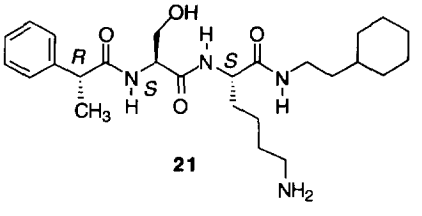
The chiral separation of **18** was achieved by HPLC using a chirobiotic-V column packed with the chiral selector vancomycin[12] linked to silica gel as the stationary phase, and eluting with 23% acetonitrile in ammonium phosphate buffer at pH 4.2 to afford the corresponding diastereomers **18a** and **18b**.[13] The proton NMR spectra of **18a** and **18b** revealed that the purity of these diastereomers was >95%. The assignment of absolute configuration at the benzylic chiral centers was established by comparing the ^{1}H NMR spectra of **18a** and **18b** with those of the reference compounds **21** and **22**.[14] The chemical shift of the methyl



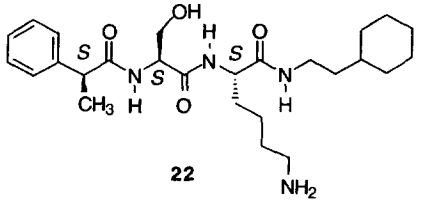
18a

18b

group in (*S,S,S*) **22** exhibits a characteristic downfield shift when compared with the corresponding (*R,S,S*) diastereomer **21**.



21



22

A similar downfield shift of the methyl signal was also observed in **18b** when compared with its diastereomer **18a**. Furthermore, the chemical shifts of the α-methine protons for the serine and lysine residues in **18a** and **18b**, as confirmed by proton-COSY experiments, were identical with the corresponding chemical shift values observed for **21** and **22**.

These analogs were evaluated for their inhibition of recombinant human and *Candida* NMTs as described[9] previously. The data (Table 1) suggest that the observed potency and selectivity are a function of the size and orientation of the substituent at the benzylic position. While the α-methyl group in **18** imparts maximum selectivity and potency versus **3**, the introduction of a bulkier group such as n-propyl (**20**) results in dramatically reduced potency against *Candida* NMT, and enhanced activity against human NMT. The incorporation of an α-ethyl group (**19**) retains potency comparable to the α-methyl group against *Candida* NMT, but its selectivity is reduced significantly.

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

Compd	IC_{50}, nM (*C. albicans* NMT)	IC_{50}, nM (human NMT)	Selectivity[b]
18	40 ± 2.83	8000 ± 420	200
18a	19.98 ± 0.99	8200	410
18b	307	49000	160
19	42 ± 12.73	1540 ± 10	37
20	130 ± 10	490 ± 10	4.0

[a]Potency against the indicated NMT as assessed by IC_{50} using the peptide GNAASARR-NH_2 at its apparent K_i and myristoyl-CoA at 1 μM, and apparent K_i versus the peptide substrate GNAASARR-NH_2 determined with myristoyl-CoA at 1 μM. [b]Selectivity is the ratio of the IC_{50} against human NMT to the IC_{50} against *Candida albicans* NMT.

An evaluation of the individual isomers of **18** revealed that the (*R,S,S*) diastereomer **18a** is a highly potent inhibitor with an IC_{50} of 20 ± 0.99 nM for *Candida* NMT, and exhibits 400-fold selectivity versus human NMT (Table 1). The corresponding (*S,S,S*) isomer **18b** was about 15-fold less potent with an IC_{50} of 307 nM against the *Candidal* enzyme and displayed only 150-fold selectivity versus human NMT. A more detailed kinetic analysis of **18a** revealed it to be a competitive inhibitor with respect to the peptide substrate GNAASARR-NH_2 (K_m = 20 μM) with a K_i = 27 nM. Notably, the (*R*) methyl group in **18a** that corresponds to the (*S*) alanine methyl configuration in **2**, confers a dual-effect on the biological activity of **3** by augmenting potency and enhancing selectivity.

Thus, we have identified a new chiral recognition element whose size affects the potency and selectivity of peptidomimetic NMT inhibitors. Furthermore, we have demonstrated by synthesis and chiral separation, that the concept of incorporating a methyl group with (*R*) stereochemistry results in a high-affinity ligand for *Candida* NMT. The competitive kinetic behavior of these peptidomimetic inhibitors indicates that these compounds bind to the NMT peptide binding site. Moreover, the structure-activity relationship (SAR) trends parallel that observed in the original peptide substrates and provide additional evidence that **18a** and its analogs function as true peptidomimetics of **1** and **2**.

Acknowledgment

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13. **18a**: R_t = 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); amino acid analysis: Ser 1.00 (1.00) Lys 1.00 (1.00); HRMS calcd for $C_{34}H_{57}N_6O_4$ (MH^+) 611.4285, found 611.4273.

    18b: R_t = 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); amino acid analysis: Ser 1.00 (1.00) Lys 1.00 (1.00); Ser 1.00 (0.99) Lys 1.00 (1.01); HRMS calcd for $C_{34}H_{57}N_6O_4$ (MH^+) 611.4285, found 611.4254.
14. These were synthesized by reacting either (*R*) or (*S*)-2-phenylpropionic acids with the amine **5** using DCC/HOBt, treating the resulting compounds with TFA, followed by reverse-phase HPLC purification.

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