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Synthesis and Inhibitory Activity of Difluoroketone Substrate Analogs of *N*-Myristoyltransferase.[1]

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Abstract: Two fluorinated nonhydrolyzable analogs of myristoyl-coenzyme A were synthesized and tested for inhibitory activity against *N*-myristoyltransferase (NMT). *S*-(2,2-Difluoro-3-oxohexadecyl)-coenzyme A (**3**) and *S*-(3,3-difluoro-2-oxopentadecyl)-coenzyme A (**2**) were prepared by alkylation of coenzyme A and were purified by reverse phase chromatography. Inhibition of NMT was observed with **3** and **2**, with IC_{50}'s of 110 nM and 80 nM, respectively, in an *in vitro* assay developed in our laboratory. The known unfluorinated analog *S*-(2-oxopentadecyl)-coenzyme A (**1**) was found to have an IC_{50} of 7 nM. At 100 μM in D_2O, **3** was 59% hydrated and **2** was 88% hydrated.

INTRODUCTION

N-Myristoyltransferase (NMT, EC 2.3.1.97) catalyzes the transfer of myristic acid (n-tetradecanoic acid) from myristoyl-coenzyme A to the N-terminus of a variety of cellular and viral proteins.[2] NMT exhibits an absolute requirement for an N-terminal glycine. The amino acid sequence of the next 5-10 residues has been shown to modulate the transferase activity both *in vitro* and *in vivo*.[3] Of the natural fatty acyl-CoA esters, NMT displays high selectivity for myristoyl-CoA.[3a,4] Cellular proteins that are myristoylated include the *src* family of tyrosine kinases and the GTP-binding subunits of heterotrimeric G proteins. Among mammalian retroviruses, myristoylation of the *gag* polyprotein has been found in HIV, HTLV-1 and HTLV-2, and simian immunodeficiency virus.[5]

The presence of the myristoyl group is important for proper functioning of the myristoylated proteins. Blocking myristoylation of the HIV *gag* protein by site-directed mutagenesis of the requisite N-terminal glycine has been shown to block assembly and release of infectious virus.[6] Similar experiments with Moloney murine leukemia virus,[7] Mason-Pfizer monkey virus[8] and spleen necrosis virus[9] have shown that N-terminal myristoylation of the *gag* protein is required for viral assembly and/or release in these viruses

as well. Myristoylation is also important in viruses outside of the retroviridae.[5] For example, myristoylation of a capsid protein is required for infectivity in poliovirus.[10] Finally, it has been found that blocking myristoylation of the v-*src* oncogene eliminates its ability to transform cells,[11] perhaps by preventing recognition by a specific binding protein.[12]

Two methods for altering the function of cellular myristoylation have been explored. In one approach, analogs of myristic acid that function as substrates for NMT are employed, causing alterations in the hydrophobicity and biological activity of myristoylated proteins. Many such alternate substrates that contain heteroatom substituents or sites of unsaturation have been studied.[13] Several of these substrate analogs have been shown to inhibit HIV replication in tissue culture.[14] Substrate analogs with antifungal[15] and antitrypanosomal[16] activities have also been identified. In the second approach, inhibitors of NMT have been sought in order to directly alter the level of protein myristoylation.[17] The most active inhibitors are nonhydrolyzable analogs of myristoyl-CoA,[2d,18] of which 2-oxopentadecyl-CoA 1 is the most potent inhibitor of NMT reported to date.[18a,19]

We have sought to test whether more potent inhibitors could be designed based upon the postulated mechanism of action of NMT.[20] The catalytic mechanism must proceed through one or more tetrahedral intermediates in which the carbonyl group of myristic acid has become sp^3 hybridized. Electron-deficient ketones have been incorporated into inhibitors of many hydrolytic enzymes[21] because of their ability to mimic such tetrahedral intermediates by reversible formation of hydrates and hemiketals (Figure 1).

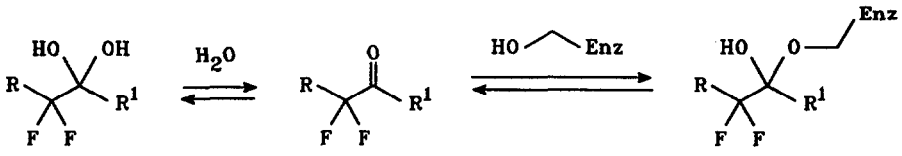
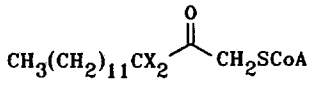


Figure 1.

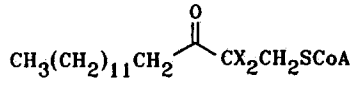
Replacement of the scissile amide or ester bond in substrate analogs with an α,α-difluoroketone or α,α,α-trifluoroketone group has produced many potent inhibitors of serine proteases,[22] esterases[23] and acyltransferases.[24] It has been noted, however, that cysteine proteases are not particularly sensitive to inhibition by polyfluoroketones.[22a]

We have synthesized and tested two fluorinated analogs of myristoyl-CoA, 2 and 3, that contain electron-deficient ketones which should enhance their ability to form hemiketals or hydrates within the active site of NMT. We have also synthesized the unfluorinated analog 1, in order to directly compare

fluorinated and unfluorinated compounds. To aid in the interpretation of the inhibition data, the extents of hydration of the fluorinated compounds, **2** and **3**, were investigated in aqueous solution using ^{19}F NMR.



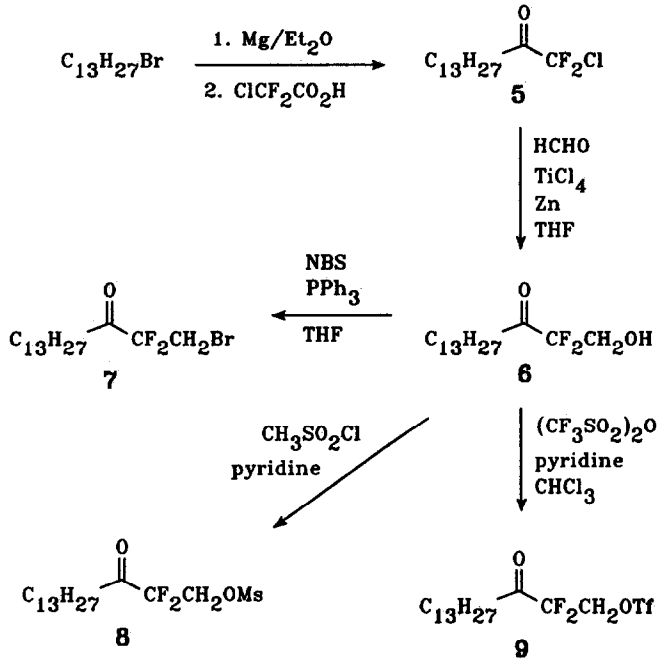
2 X = F
1 X = H



3 X = F
4 X = H

RESULTS

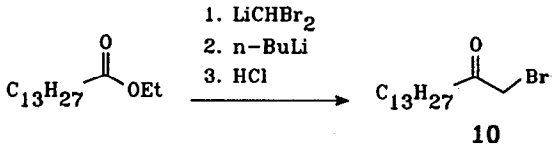
In order to synthesize alkylated coenzyme A target structures **2** and **3**, we required electrophilic fluoroketone derivatives that possess a good leaving group. For the synthesis of these intermediates, two separate syntheses were required. Compound **1** was also prepared. Its synthesis required bromomethylketone **10**.



Scheme 1.

The β-bromoketone **7** and the corresponding mesylate **8** and triflate **9**, precursors to **3**, were synthesized as shown in Scheme 1. The reaction between fluorinated acids and organometallics can be controlled to give good yields of ketones,[25] and chlorodifluoroacetic acid was found to react with the Grignard reagent $H_{27}C_{13}MgBr$ to give chloroketone **5** in 70% yield. Directed aldol reaction of chloroketone **5** with paraformaldehyde, mediated by zinc-titanium tetrachloride reagent,[26] gave β-hydroxyketone **6** in 30% yield after six hours. No increase in yield was seen after longer reaction time (15 hours). Hydroxyketone **6** was converted to bromide **7** (59%) by treatment with two equivalents each of NBS and triphenylphosphine.[27] Use of less than two equivalents of the reagents resulted in lower yields. Both THF and DMF were suitable solvents, although higher yields were obtained in THF. Mesylate **8** was prepared in 95-100% yield from β-hydroxyketone **6** by reaction with methanesulfonyl chloride in pyridine. Also, triflate **9** was prepared in 73% from **6** by reaction with triflic anhydride in chloroform.

The synthesis of **1** required the preparation of bromomethylketone **10** (Scheme 2). The bromoketone **10** was synthesized from ethyl myristate by the method of Kowalski.[28] This alternative to the classical synthesis of bromomethylketones via diazoketones worked well for the unfluorinated target **10**.



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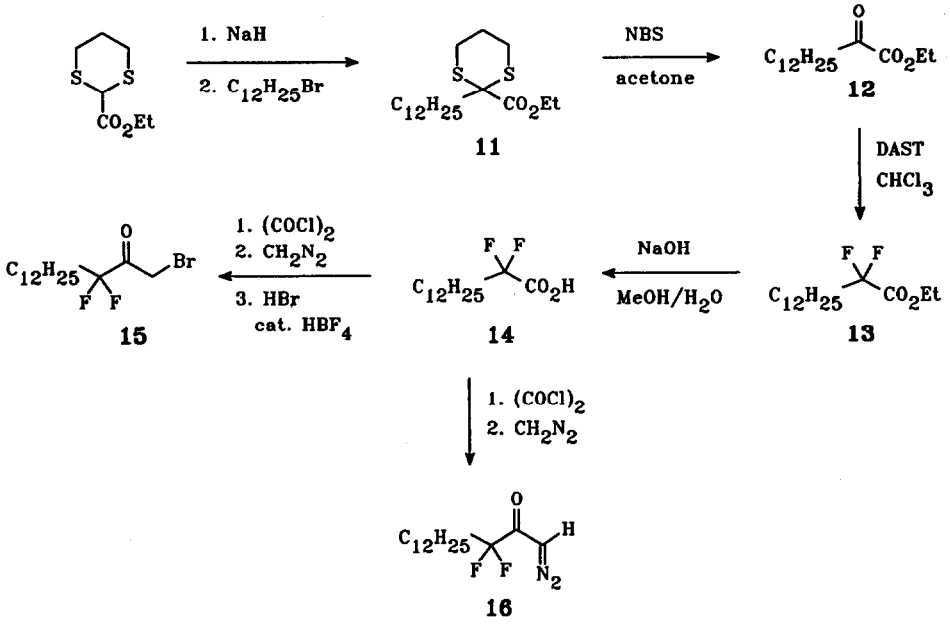
Scheme 2.

The fluorinated bromomethylketone **15**, intermediate for target compound **2**, was also prepared (Scheme 3). Alkylation of the sodium salt of 2-carboethoxy-1,3-dithiane with 1-bromododecane afforded dithiane **11** (> 90%). Oxidative hydrolysis of **11** to the corresponding α-ketoester **12** was accomplished in high yield with NBS in wet acetone.[29] Excess NBS (nine equivalents) was necessary for complete hydrolysis. The α-ketoester **12** was fluorinated with diethylaminosulfur trifluoride (DAST) in $CHCl_3$ to give ethyl 2,2-difluoromyristate **13**, in good yields. Higher yields were obtained if the DAST was added in several portions over the course of the reaction rather than in one portion at the beginning. Conversion of difluoroester **13** to bromomethylketone **15** utilizing the method used to prepare **10** was unsuccessful, producing a low yield of **15** and unidentified byproducts. A three step sequence, via diazoketone **16**, produced good yields of **15**. Alkaline hydrolysis of α,α-difluoroester **13** provided α,α-difluoro acid **14**, which was reacted with a ten molar excess of oxalyl chloride in toluene. The crude acyl chloride was isolated and reacted with excess CH_2N_2 in Et_2O to give diazoketone **16**. Small samples of **16** were purified

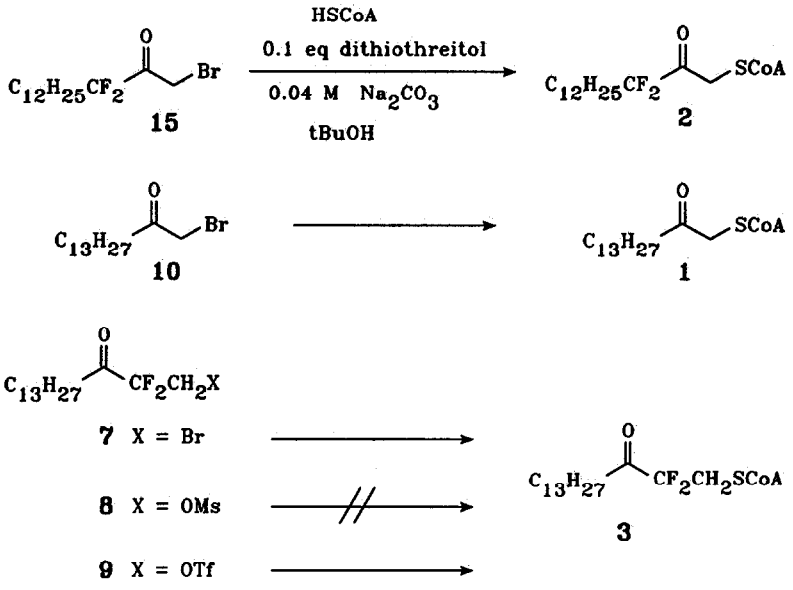
by chromatography on silica gel in order to test this diazoketone for inhibition against NMT. However, **16** was usually not isolated, but instead carried directly on to bromomethylketone **15** by treatment with aqueous HBr in the presence of a catalytic amount of HBF_4.[30] In the reaction of **16** with HBr, it is important to keep the reaction time short (≤ 30 min). Longer reaction times resulted in a decrease in yield of **15**.



Scheme 3.

The final step in the preparation of compounds **1**, **2** and **3** is alkylation of coenzyme A. Coenzyme A was alkylated following the known procedure for the selective modification of the thiol group in coenzyme A (Scheme 4). Coenzyme A was reacted with excess alkylating agent (5-10 equivalents) under nitrogen in a solvent system in which both reactants were soluble (1:2 0.04M Na_2CO_3 / *t*-BuOH). Bromomethylketones **15** and **10** reacted readily to give **2** and **1**, respectively. In both cases, the reaction was complete after six hours, and only small amounts of oxidized coenzyme A (CoAS-SCoA) were formed. In the reaction of **15** with coenzyme A, excess **15** could not be recovered. Instead, a mixture of higher molecular weight compounds was obtained. Mesylate **8** did not react with coenzyme A under these reaction conditions, and coenzyme A was instead oxidized to the disulfide CoAS-SCoA. Because of the failure of the mesylate to alkylate coenzyme A, the β-bromoketone **7** and the triflate **9** were synthesized. β-Bromoketone **7** reacted with coenzyme A, though not as readily as did α-bromoketones **15** and **10**. The reaction required 24 hours for completion, and produced a very low yield of **3**. The unreacted β-bromoketone **7** could be isolated by

solvent extraction from the reaction products and recycled. The reaction of triflate 9 with coenzyme A afforded a considerably higher yield of 3, despite the fact that 9 was prone to hydrolysis (to 6) under the reaction conditions. After removal of excess alkylating agent, the alkyl-CoA's were purified by reverse phase chromatography on C18 silica gel, eluting with 50% 10 mM phosphate buffer (pH = 7.5) / 50% CH_3CN. Phosphate salts were removed by $HClO_4$ precipitation of the alkyl-CoA from aqueous solution.[31] All three compounds showed a single spot on TLC in $BuOH/H_2O/AcOH$ = 5:3:2 (visualized with UV light, I_2 vapor, or 2,4-dinitrophenylhydrazine), and gave ^1H and ^{13}C NMR spectra consistent with the assigned structures.



Scheme 4.

The ^{19}F NMR spectra of compounds 2 and 3 in DMSO-d_6 and D_2O are shown in Figure 2. Both ketone and hydrate signals are upfield of trifluoroacetic acid. The ketone forms appear between -28 and -31 ppm and the hydrates appear between -38 and -40 ppm. In DMSO-d_6, both compounds are present mainly as ketone, with the small amount of hydrate present likely due to trace water in the DMSO-d_6 (Fig. 2b and 2d). The signals are triplets due to coupling with adjacent methylene protons.

At 14 mM in D_2O, the ketone and hydrate signals for 2 and 3 are broadened considerably (Fig. 2a and 2c), which is attributed to aggregation. Similar broadening of signals has been observed with 3-octylthio-1,1,1-trifluoro-propan-2-one in D_2O, where the signal linewidth of the resonance for the aggregated hydrate

was twice that of the resonance for the non-aggregated hydrate.[32] Integration of the signals in Figures 2a and 2c showed that **2** is 54% hydrated and **3** is 9% hydrated at 14 mM in D_2O. Lowering the concentration to 2.0 mM produced no change in the linewidth of ketone and hydrate signals, and little change in their chemical shifts (≤ 0.1 ppm). However, there was a slight increase in the relative amount of hydrate for both **2** (56% hydrated) and **3** (12% hydrated). At 0.1 mM in D_2O, the hydrate and ketone signals are triplets, indicating the compounds are not significantly aggregated at this concentration. Again, there was a small change in ketone and hydrate chemical shifts (0.4 - 0.6 ppm) on lowering the concentration. However, there was a substantial increase in the relative amount of hydrate for both **2** (88% hydrated) and **3** (59% hydrated). At equal concentrations, fluoroketone **2** is hydrated to a greater extent than is fluoroketone **3**, perhaps due to the thioether being an α-substituent in **2** and a β-substituent in **3**.

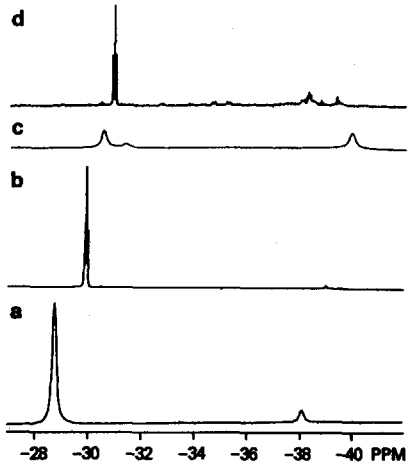


Figure 2. ^{19}F NMR of **3** and **2**: (a) **3**, 14 mM in D_2O; (b) **3**, 8.6 mM in DMSO-d_6; (c) **2**, 14 mM in D_2O; (d) **2**, 14.5 mM in DMSO-d_6. Ketone signals are between -28 and -31 ppm. Hydrate signals are between -38 and -40 ppm. Chemical shifts are relative to CF_3CO_2H at 0.0 ppm.

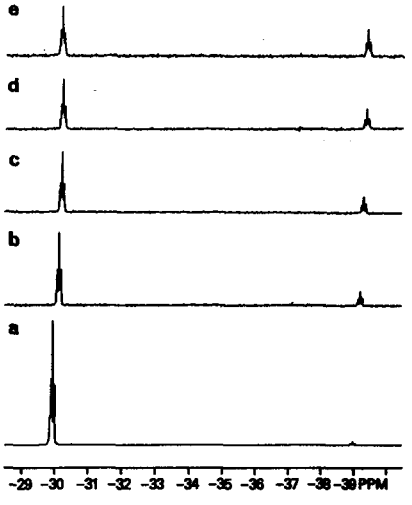


Figure 3. ^{19}F NMR of **3** in DMSO-d_6 + D_2O: (a) DMSO-d_6, (b) DMSO-d_6 + 5% D_2O, (c) DMSO-d_6 + 10% D_2O, (d) DMSO-d_6 + 15% D_2O, (e) DMSO-d_6 + 20%. Spectra in (b)-(e) are on solutions obtained by incremental addition of D_2O to the solution of **3** in DMSO-d_6 in (a). The initial concentration of **3** in DMSO-d_6 was 8.6 mM. Chemical shifts are relative to CF_3CO_2H at 0.0 ppm.

^{19}F NMR of the compounds in DMSO-d_6/D_2O was also investigated. Shown in Figure 3 are spectra of **3** in DMSO-d_6/D_2O. The DMSO-d_6/D_2O solutions were obtained by addition of D_2O to a solution of

3 in DMSO-d_6. An increase in linewidth of ketone and hydrate signals was observed on addition of the D_2O (Fig. 3b-3e). The percent hydration of 3 was determined in DMSO-d_6/D_2O (Table 1). As expected, the extent of hydration increased with increasing amounts of D_2O. Similar results were obtained when the same experiment was conducted with 2 (spectra not shown). Again, there was an increase in signal linewidth on addition of D_2O. Percent hydration of 2 in DMSO-d_6/D_2O is listed in Table 1.

TABLE 1. Percent Hydration[a] for 3 and 2 in DMSO-d_6/D_2O

solvent DMSO-d_6/D_2O	3	2
100:0	3.4	14
95:5	17	50
90:10	24	63
85:15	31	70
80:20	35	75
0:100	59	88

[a] Determined by integration of hydrate and ketone signals in ^{19}F NMR.

TABLE 2. Inhibition of NMT Activity at 10 μM.[a]

Compound	NMT activity (%)[b]	N[c]
1	1.5 ± 0.2	3
2	4.7 ± 0.7	6
3	11. ± 1	6
16	105 ± 8	6

[a] Compounds were tested for their ability to inhibit the transfer of [^{3}H]-myristate from [^{3}H]myristoyl-coenzyme A to the peptide substrate Gly-Asn-$(Ala)_4$-$(Arg)_2$-NH_2. Compounds were tested at a 10 μM concentration.
[b] NMT activity = activity with inhibitor/activity of positive control x 100%.
[c] Number of assays.

Compounds 1, 2 and 3 were tested for their ability to inhibit the transfer of [^{3}H]-myristate from [^{3}H]-myristoyl-coenzyme A to the peptide substrate Gly-Asn-$(Ala)_4$-$(Arg)_2$-NH_2.[33] The effect of a 10 μM concentration of the compounds on the activity of yeast NMT is summarized in Table 2. As expected, 1 inhibited NMT activity nearly completely (to less than 2% of uninhibited controls). Strong inhibition was also observed with the fluoroketone analogs 2 and 3. Diazoketone 16 was also tested, since diazoketones have been found to be inhibitory for cysteine peptidases[34] and some serine peptidases,[35] but it did not inhibit NMT (Table 2). The IC_{50} values were determined to be 80 nM for 2, 110 nM for 3, and 7 nM for 1 (see discussion below) (Figure 4). No evidence of time dependence of the inhibition was observed in the inhibition of NMT by these three compounds.

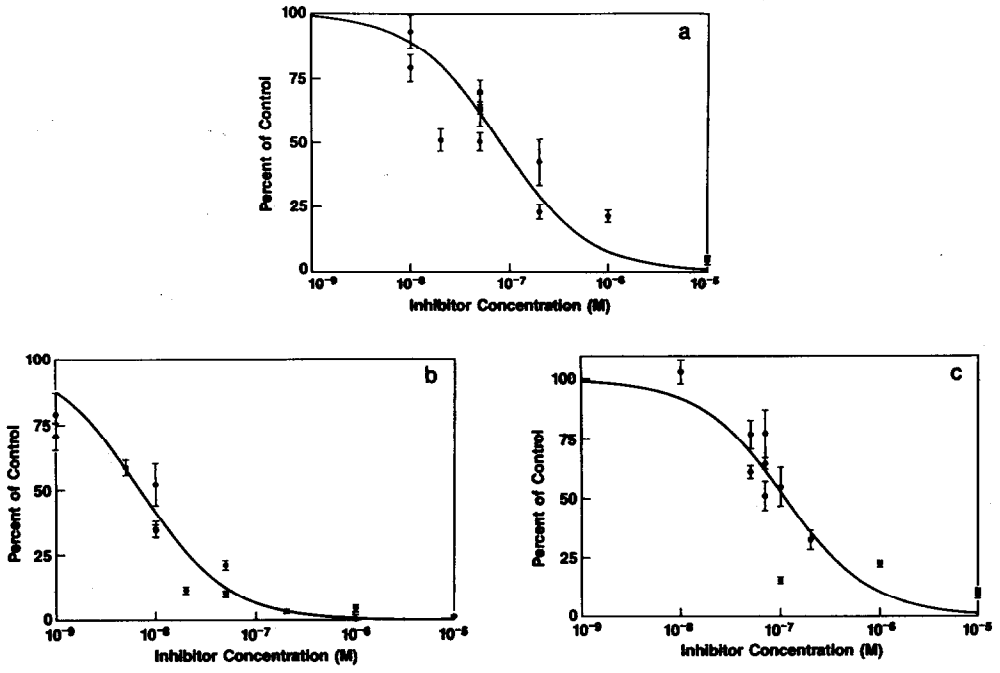


Figure 4. Inhibition of NMT by compounds (a) **2**, (b) **1**, and (c) **3**. The NMT assay was carried out as described in the experimental section. In the positive control, 100% corresponds to an NMT activity of 0.9 μM/min/mg protein. The plotted data are the averages of duplicate or triplicate determinations. The curves were generated using the equation for competitive inhibition with: (a) IC_{50} = 80 nM, K_m = 0.7 μM, [S] = 5 μM; (b) IC_{50} = 7 nM, K_m = 0.7 μM, [S] = 5 μM; (c) IC_{50} = 110 nM, K_m = 0.7 μM, [S] = 5 μM.

DISCUSSION

The catalytic mechanism of NMT is at present unknown, although it has been established that the yeast[20a] and human[20b] enzymes display ordered Bi-Bi kinetics. Several lines of evidence suggest that there is a nucleophilic amino acid in the active site of NMT that participates in the normal catalytic cycle, possibly by formation of an acyl-enzyme intermediate with an ester or thioester linkage.[36] However, an enzyme mechanism that does not involve a covalent myristoyl-enzyme intermediate is also compatible with the observed data.[2d] Inhibitor designs based on electron-deficient fluoroketones have been successful both when the enzyme mechanism involves a covalent intermediate,[37] as well as with enzymes where non-covalent catalysis occurs.[38] In the former cases, the ketone form of the fluoroketone inhibitor reacts with an active site hydroxyl in the enzyme to form a hemiketal. In the latter cases, the hydrated form of the inhibitor has been identified within the active site of the enzyme. In view of the fact that the most potent

inhibitor of NMT described to date is a ketone analog of myristoyl-CoA (*S*-(2-oxopentadecyl)-CoA),[18a,19] we undertook the synthesis of an α,α-difluoroketone derivative of this compound. We also prepared an α,α-difluoroketone derivative of the homologous ketone inhibitor of NMT, **4**.[18b]

All three compounds synthesized (**1**, **2**, **3**) are powerful inhibitors of NMT with sub-micromolar IC_{50} values. Of the three, the unfluorinated **1** is the most potent. In our system, **1** showed an IC_{50} of 7 nM, which agrees well with the previously reported K_i value of 10-14 nM for yeast NMT[20a] (other K_i values reported are 24 nM for mouse brain NMT[19] and 56 nM for human NMT[20b]). The fluorinated analog **2**, with an IC_{50} of 80 nM, is less potent. The least potent inhibitor is fluoroketone **3**, with an IC_{50} of 110 nM. However, **3** is more potent than its unfluorinated analog **4** for which a K_i 250 nM was reported by Wagner and Rétey.[18b] Although a quantitative comparison of **3** and **4** cannot be made at present, a qualitative comparison is justified by: a) the similarity of the methodologies used by Wagner and Rétey[18b] and in this paper (both use yeast NMT and the same peptide substrate) and b) the analyses in this work were performed with a [^{3}H]-myristoyl-CoA concentration equal to its K_m, under which conditions a competitive inhibitor will display an IC_{50} of approximately twice its K_i value.

As with many polyfluoroketones in aqueous solution, the effective concentration of the ketone forms of **2** and **3** may be reduced due to an unfavorable hydrate/ketone equilibrium.[39] Based upon literature precedent,[40] it is likely to be the ketone forms of **1**, **2**, **3** and **4** which bind to NMT. In at least one case where the hydrate was identified in the enzyme-inhibitor complex, it was determined that the ketone bound initially, followed by enzyme catalyzed hydration of the ketone.[40c] It is therefore important to estimate the concentrations of the ketone forms of **2** and **3** under the enzyme assay conditions.

Measurements of the extent of hydration of fluoroketones **2** and **3** by ^{19}F NMR in D_2O were complicated by the self-association of the inhibitors. As noted earlier, at 14 mM and 2.0 mM in D_2O, the signals for **2** and **3** were broadened considerably, compared to the signals in DMSO-d_6 at the same concentrations (Figure 2). The line broadening is a result of the microenvironment around the inhibitor molecules when they are in an aggregate. At lower concentrations, the compounds would be aggregated to a lesser extent, especially below the critical micelle concentration (CMC). Because of the similarity in the structures of **2**, **3** and myristoyl-coenzyme A, the CMC's for **2** and **3** are expected to be similar to the CMC for myristoyl-coenzyme A, which is 210 μM.[41] The difference in signal linewidth in the spectra of the inhibitors at 2.0 mM (wide linewidth) and 100 μM (narrow linewidth) suggests that the CMC's of **2** and **3** are between those concentrations. At a 100 μM concentration in D_2O, both **2** and **3** exist predominantly in the hydrated form (88% and 59%, respectively). In DMSO-d_6, the signals for **2** and **3** have narrow linewidths, indicating no self-association. On addition of D_2O (up to 20%), there was a small increase in signal linewidth, indicating minimal self-association. For both **2** and **3**, as the amount D_2O was increased (from 0% to 20%) the extent of hydration increased. The extent of hydration in DMSO-d_6 with increasing

amounts of D_2O approached that determined in D_2O at 100 μM.

The inhibitor concentrations in the assays were between 1.0 nM and 10μM, below the estimated CMC. For fluoroketones **2** and **3**, the extents of hydration determined at 100 μM in D_2O were used to correct for the concentration of ketone in the assays. The extent of hydration of the unfluorinated ketone **1** is expected to be on the order of one percent.[42] Then, if the inhibition measured on the bulk compounds is due to interaction of the enzyme with the ketone form, the corresponding IC_{50} values are 10 nM for **2**, 45 nM for **3** and 7 nM for **1**.

If the mechanism of inhibition of NMT by ketone analogs of myristoyl-CoA involves a covalent interaction with an active site hydroxyl group, one would expect improvement in inhibitory activity on incorporation of fluorines α to the ketone carbonyl in the inhibitors. However, for the ketone analogs of myristoyl-CoA **1** and **4**, introduction of fluorines α to the ketone carbonyls had no major effect on inhibitor potency. On the basis of these results, we infer that the mechanism of inhibition by nonhydrolyzable ketone analogs of myristoyl-CoA is unlikely to involve a covalent hemiketal intermediate. The availability of fluoroketones **2** and **3** should now allow the nature of the enzyme-inhibitor complex to be directly investigated by ^{19}F NMR spectroscopy.

EXPERIMENTAL

Materials and Methods. A plasmid expressing yeast NMT cDNA (pBB125) was provided by Jeffrey Gordon (Washington Univ., St. Louis, MO). JM101 *E. coli* was obtained from American Type Culture Collection (Rockville, MD). The peptide substrate, Gly-Asn-$(Ala)_4$-$(Arg)_2$-NH_2, was synthesized and HPLC purified by Peptide Technologies, Gaithersburg MD. The 9,10-[^{3}H]-myristic acid (specific activity 40 Ci/mmol) was from DuPont-NEN, Boston MA, and acyl-CoA synthetase (*Pseudomonas*) was obtained from Sigma Co. St. Louis, MO. Semipreparative purification of alkyl-CoA thioethers was performed by reverse phase chromatography on a Delta-Pak (15μm) 0.8 x 10 cm column (Waters, Milford MA) using a solvent system based upon that used by Causey and Bartlett[43] for analysis of acyl-CoA esters. Purification at larger scale was carried out by reverse phase flash chromatography on Bakerbond C-18 silica gel from North Strong Scientific, Rockville MD. Analytical TLC plates (MN silica gel) were from Brinkmann, Westbury NY, and were visualized with iodine (I), 2,4-dinitrophenylhydrazine (H), or UV light (U). The AG-1 ion-exchange resin (X-8, 200-400 mesh) was supplied by Bio-Rad Labs, Hercules CA. Starting materials and reagents were obtained from Aldrich Chemical Co, Milwaukee WI, Sigma Co., St. Louis MO, Fluka USA, Lake Ronkonkoma NY or Lancaster Synthesis, Windham, NH.

Melting points were determined using a Thomas Hoover capillary melting point apparatus and are uncorrected. ^1H NMR were determined on a Varian XL-200 at 200 MHz or on a Varian Gemini 300 at 300 MHz, with TMS (0.0 ppm) as an internal standard. ^{13}C NMR were determined at 75.5 MHz on a Varian Gemini 300. Chemical shifts are reported relative to TMS, using the solvent as internal standard; $CDCl_3$ at 77.0 ppm or DMSO-d_6 at 39.5 ppm. ^{19}F NMR spectra were determined on a Varian 300XL at 282.2 MHz and were not proton decoupled. Chemical shifts are reported relative to CF_3CO_2H (0.00 ppm), with upfield shifts designated as negative. Fast atom bombardment (FAB) mass spectra were run on a JEOL SX102 mass spectrometer, using xenon atoms. Chemical ionization (CI) mass spectra were acquired on a Finnigan 4600 mass spectrometer. Elemental analyses were performed by Galbraith Laboratories, Knoxville TN.

CAUTION. Diazomethane (CH_2N_2) is hazardous. Directions for the safe handling of CH_2N_2 can be found in Organic Syntheses.[44] All operations involving CH_2N_2 were conducted in a fume hood. Diazomethane was prepared from 1-methyl-3-nitro-1-nitrosoguanidine using a modification[45] of the procedure described by Arndt[46] and was used immediately after preparation.

1-Chloro-1,1-difluoro-2-pentadecanone (5). In a 3-neck round bottom flask equipped with a condenser and an addition funnel was placed 2.28 g (94 mmol) of magnesium turnings and enough Et_2O to cover the turnings. A solution of 20 mL (78 mmol) of 1-bromotridecane in 80 mL of Et_2O was placed in the addition funnel. Approximately 10 mL of the bromotridecane solution was added to the flask. After the reaction initiated, the bromotridecane solution was added dropwise at a rate to maintain reflux. When the addition was complete, the mixture was refluxed for 20 min. The reaction mixture was cooled to between 10° and 15°C, and a solution of 2.2 mL (26.1 mmol) of chlorodifluoroacetic acid in 16 mL of Et_2O was added dropwise. After the addition was complete, the mixture was stirred for two hours at 10-15°C. To the reaction mixture was added 90 mL of 10% HCl and 50 mL of Et_2O. After stirring for 10 min, the phases were separated. The aqueous phase was extracted with Et_2O (4 x 25 mL). The combined Et_2O extracts were washed with saturated aqueous $NaHCO_3$ (25 mL), washed with saturated aqueous NaCl (2 x 20 mL), dried with Na_2SO_4, and stripped of solvent. Column chromatography on silica gel with hexane yielded 5.43 g (70%) of ketone **5** as a colorless oil: TLC hexane, R_f = 0.23 (H); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.75 (t, J = 7 Hz, 2H), 1.68 (m, 2H), 1.26 (m, 20H), 0.88 (t, J = 7 Hz, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 191.7 (t, J = 29 Hz), 119.8 (t, J = 305 Hz), 35.2, 32.0, 29.7, 29.6, 29.4, 29.3, 28.9, 23.0, 22.8, 14.2; ^{19}F NMR (282.2 MHz, $CDCl_3$) δ 7.2 (s); MS (FAB) 297 (MH^+), 211. Anal. Calcd for $C_{15}H_{27}ClF_2O$: C, 60.70; H, 9.17; Cl, 11.94; F, 12.80. Found: C, 60.81; H, 9.22; Cl, 11.82; F, 12.42.

2,2-Difluoro-1-hydroxy-3-hexadecanone (6). To a flask containing 0.988 g (15.1 mmol) of zinc dust was added 10 mL of THF. The suspension was cooled to 0°C, and approximately 0.1 mL (0.9 mmol) of titanium tetrachloride was added. The suspension was stirred at room temperature for 15 min. Then, a solution of 1.48 g (5.0 mmol) of **5** and 0.451 g (15.0 mmol) of paraformaldehyde in 15 mL of THF was added dropwise. The reaction mixture was stirred at room temperature for 6.5 h. The reaction was quenched with aqueous NH_4Cl. Approximately 50 mL of Et_2O were added, and the mixture was filtered through glass wool (to remove the zinc). The aqueous and organic phases were separated. The aqueous phase was extracted with Et_2O (3 x 25 mL). The combined Et_2O extracts were washed once with saturated aqueous NaCl, dried with Na_2SO_4, and the solvent was removed. Column chromatography on silica gel with $CHCl_3$ afforded 0.437 g (30%) of β-hydroxyketone **6** as a white waxy solid: mp 44-50°C; TLC $CHCl_3$, R_f = 0.20 (H); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.98 (t, J = 13 Hz, 2H), 2.72 (t, J = 7 Hz, 2H), 1.63 (m, 2H), 1.26 (m, 20H), 0.88 (t, J = 7 Hz, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 201.5 (t, J = 30 Hz), 114.6 (t, J = 254 Hz), 61.9 (t, J = 29 Hz), 37.0, 32.0, 29.7, 29.6, 29.5, 29.4, 29.3, 29.0, 22.8, 22.5, 14.2; ^{19}F NMR (282.2 MHz, $CDCl_3$) δ -40.8 (t, J = 13 Hz); MS (CI, NH_3) 310 ($M + NH_4^+$), 292. Anal. Calcd for $C_{16}H_{30}F_2O_2$: C, 65.72; H, 10.34; F, 12.99. Found: C, 66.02; H, 10.45; F, 12.82.

1-Bromo-2,2-difluoro-3-hexadecanone (7). To a solution of 929 mg (3.18 mmol) of **6** and 1.131 g (6.35 mmol) of NBS in 40 mL of THF were added 1.667 g (6.36 mmol) of triphenylphosphine in portions over 15 min. The reaction mixture was stirred at 50°C for 7 h. Then, 0.5 mL of MeOH were added, and the mixture was stirred for 15 min at 50°C. The reaction mixture was added to water and Et_2O, and the aqueous and organic layers were separated. The aqueous phase was extracted with Et_2O (4 x 40 mL). The combined Et_2O extracts were washed three times with 50 mL of saturated aqueous $NaHCO_3$, washed twice with 25 mL of saturated aqueous NaCl, dried with Na_2SO_4, and the solvent was removed. The crude material was triturated in 3:1 hexane/Et_2O. The insoluble material was filtered, and the filtrate was stripped of solvent. The material recovered from the filtrate was purified by column chromatography on silica gel: first, with 3:1 hexane/Et_2O; second, with 10% CH_2Cl_2 in hexane, to yield 665.4 mg (59%) of β-bromoketone **7** as an oil: TLC 3:1 hexane/Et_2O, R_f = 0.65 (H); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.69 (t, J = 13 Hz, 2H), 2.74 (t, J = 7 Hz, 2H), 1.64 (m, 2H), 1.26 (m, 20H), 0.88 (t, J = 7 Hz, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 199.3 (t, J = 30 Hz), 113.7 (t, J = 254 Hz), 37.1, 32.0, 29.7, 29.6, 29.4, 29.3, 28.9, 27.6 (t, J = 29 Hz), 22.8, 22.5, 14.2; ^{19}F NMR (282.2 MHz, $CDCl_3$) δ -31.0 (t, J = 13 Hz); MS (FAB) 355 (M(Br81) - H), 353 (M(Br79) - H), 211. Anal. Calcd for $C_{16}H_{29}BrF_2O$: C, 54.09; H, 8.23; F, 10.69; Br,

22.49. Found: C, 54.38; H, 8.23; F, 10.58; Br, 22.75.

2,2-Difluoro-1-methanesulfonyl-3-hexadecanone (8). A solution of 202 mg (0.69 mmol) of **6** in 0.5 mL of pyridine was cooled to between -5° and 0°C. To this solution was added dropwise a solution of 150 μL (1.9 mmol) of methanesulfonyl chloride in 0.5 mL of pyridine. The reaction mixture was stirred for three hours at 0°C. Approximately 2 mL of 10% HCl were slowly added. The white precipitate that formed was filtered, washed with 0.5 mL of 10% HCl, washed with 3 mL of water, and air dried to afford 258 mg (100%) of mesylate **8**, which was used without further purification: mp 50-53°C; TLC $CHCl_3$, R_f = 0.47 (H); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.56 (t, J = 13 Hz, 2H), 3.09 (s, 3H), 2.73 (t, J = 7 Hz, 2H), 1.64 (m, 2H), 1.26 (m, 20H), 0.88 (t, J = 7 Hz, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 198.9 (t, J = 29 Hz), 112.6 (t, J = 257 Hz), 65.3 (t, J = 30 Hz), 38.0, 36.6, 32.0, 29.7, 29.6, 29.4, 29.3, 28.9, 22.7, 22.4, 14.2; ^{19}F NMR (282.2 MHz, $CDCl_3$) δ -38.5 (t, J = 13 Hz); MS (FAB) 371 (MH^+), 211.

2,2-Difluoro-1-trifluoromethanesulfonyl-3-hexadecanone (9). A solution of 250 mg (0.85 mmol) of **6** and 69 μL (0.85 mmol) of pyridine in 1.5 mL of $CHCl_3$ was cooled to between -5° and 0°C. To the cooled solution was added 0.2 mL (1.19 mmol) of triflic anhydride. The reaction mixture was stirred for 25 min at -5° to 0°C. The reaction mixture was washed with three 1.0 mL portions of cold 10% HCl and with 1.0 mL of saturated aqueous NaCl. The $CHCl_3$ was evaporated. Column chromatography on silica gel with $CHCl_3$ afforded 262.2 mg (73%) of triflate **9** as an oil: TLC $CHCl_3$, R_f = 0.68 (H); ^{1} H NMR (300 MHz, $CDCl_3$) δ 4.78 (t, J = 12 Hz, 2H), 2.75 (t, J = 7 Hz, 2H), 1.65 (m, 2H), 1.26 (m, 2H), 0.88 (t, J = 6 Hz, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 198.8 (t, J = 29 Hz), 118.5 (q, J = 320 Hz), 111.7 (t, J = 259 Hz), 70.0 (t, J = 30 Hz), 36.3, 31.8, 29.5, 29.4, 29.3, 29.1, 28.7, 22.6, 22.2, 14.0; ^{19}F NMR (282.2 MHz, $CDCl_3$) δ 1.6 (s, 3F), -38.7 (t, J = 12 Hz, 2F); MS (CI, NH_3) 442 ($M + NH_4^+$).

1-Bromo-2-pentadecanone (10). A solution of LDA was prepared by adding *n*-BuLi (5.5 mL of a 1.6 M solution in hexane, 8.8 mmol) to a solution of diisopropylamine (1.34 ml, 9.6 mmol) in anhydrous THF at 0°C. After stirring under nitrogen at 0°C for 30 min, the LDA was added over 10 min via cannula to a stirring solution of dibromomethane (0.62 mL, 8.8 mmol) in 12 mL of THF held at -100°C. After an additional 10 min at -100°C, a solution of ethyl myristate (1.02 g, 4 mmol) in 8 mL of THF was added through a steel cannula which was cooled in dry ice. The resulting suspension was stirred at -100°C for 20 min, after which *n*-BuLi (3.8 mL, 6 mmol) was added. After stirring for 10 min at -100°C, the resulting orange solution was added through a cannula cooled with dry ice to a -78°C solution of HCl in ethanol (2M, prepared by cautiously adding 6 ml of acetyl chloride to 40 mL of anhydrous ethanol). After several hours at -78°C, the solution was poured into 100 mL of ethyl acetate, and washed successively with 2 x 50 mL of water, 2 x 50 mL of 10% H_2SO_4, 2 x 50 mL of sat. $NaHCO_3$, and 50 mL of sat. NaCl. Drying over Na_2SO_4 and removal of solvent afforded 1.28 g of crude product as an orange semisolid, mp 51-57°C. Recrystallization from 5 mL of hexane gave 0.686 g (56%) of bromoketone **10** as a tan powder, mp 59.5-61.5°C; TLC hexane/Et_2O = 3:1, R_f = 0.58 (H); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.88 (s, 2H), 2.64 (t, J = 6 Hz, 2H), 1.62 (m, 2H), 1.26 (m, 20H), 0.88 (t, J = 6 Hz, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 202.0, 39.9, 34.3, 32.0, 29.7, 29.6, 29.5, 29.4, 29.1, 24.0, 22.8, 14.2.

2-Carboethoxy-2-dodecyl-1,3-dithiane (11). In a 100 mL flask were placed 1.14 g of NaH, 80% dispersion in mineral oil (38.0 mmol NaH). The mineral oil was removed by washing with hexane. To the flask were added 35 mL of benzene and then 5.0 mL (31.7 mmol) of 2-carboethoxy-1,3-dithiane. The suspension was cooled in an ice-bath. Then a solution of 8.4 mL (35.0 mmol) of 1-bromododecane in 12.9 mL of DMF was added dropwise. The reaction mixture was stirred, and allowed to warm to room temperature, overnight (14 h). To the reaction mixture was added 15 mL of water to react with excess NaH. The aqueous and organic phases were separated. The organic layer was washed three times with 25 mL of water. The combined aqueous phases were neutralized with HCl and washed once with 15 mL of benzene. The benzene phases were combined, dried with Na_2SO_4, and stripped of solvent. The product showed two spots on TLC in hexane, R_f = 0.75 and 0.10 (I). The low R_f material proved to be dithiane **11**, and was purified by column chromatography on silica gel. The column was eluted with hexane until the high R_f material was removed, then with 25% acetone in hexane to yield 10.65 g (93%) of dithiane **11** as a light brown oil: TLC 25% acetone in hexane, R_f = 0.59 (I); ^{1}H NMR (200 MHz, d_6-acetone) δ 4.22 (q, J = 7 Hz, 2H), 3.29 (ddd, J = 14, 12, 2.5 Hz, 2H), 2.66 (ddd, J = 14, 4.5, 3.2 Hz, 2H), 2.16 (dtt, J = 13.8, 4.5,

2.5 Hz, 1H), 1.78 (dtt, J = 13.8, 12, 3.2 Hz, 1H), 1.47 (m, 2H), 1.30 (m, 23H), 0.89 (t, J = 6 Hz, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 170.8, 61.7, 52.8, 39.0, 31.9, 29.8, 29.7, 29.5, 29.4, 29.3, 28.0, 24.9, 24.3, 22.7, 14.3, 14.2; MS (FAB) 360 (M^+), 287. Anal. Calcd for $C_{19}H_{36}O_2S_2$: C, 63.28; H, 10.06. Found: C, 63.43; H, 10.19.

Ethyl 2-oxotetradecanoate (12). A solution of 42.79 g (240.4 mmol) of NBS in 425 mL of 97:3 acetone/water was cooled in an ice-bath. A solution of 9.61 g (26.65 mmol) of **11** in 25 mL of 97:3 acetone/water was added dropwise to the NBS solution. The reaction mixture was stirred for 10 min at ice-bath temperature. The mixture was added to 100 mL of 1:1 CH_2Cl_2/hexane and 150 mL of aqueous Na_2SO_3, and stirred until the color faded from the organic layer. The aqueous and organic phases were separated. The organic phase was washed with 3 x 50 mL of aqueous Na_2SO_3, 50 mL of water, 4 x 50 mL of saturated aqueous $NaHCO_3$, 50 mL of water, and 2 x 50 mL of saturated aqueous NaCl. Then the organic layer was dried with Na_2SO_4, and the solvent was removed. The crude material was purified by column chromatography on silica gel with CH_2Cl_2 to yield 7.00 g (90%) of ketoester **12** as an oil: TLC CH_2Cl_2, R_f = 0.49 (H); ^1H NMR (300 MHz, $CDCl_3$) δ 4.32 (q, J = 7 Hz, 2H), 2.82 (t, J = 7 Hz, 2H), 1.68 (m, 2H), 1.37 (t, J = 7 Hz, 3H), 1.26 (m, 18H), 0.88 (t, J = 7 Hz, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 194.6, 161.1, 62.3, 39.3, 32.0, 29.7, 29.4, 29.0, 23.1, 22.7, 14.2; MS (CI, NH_3) 288 ($M + NH_4^+$). Anal. Calcd for $C_{16}H_{30}O_3$: C, 71.07; H, 11.18. Found: C, 71.29; H, 11.25.

Ethyl 2,2-difluorotetradecanoate (13). A solution of 6.46 g (23.9 mmol) of **12** in 50 mL of ethanol-free $CHCl_3$ was cooled in an ice-bath. Then, 6.40 mL (48.4 mmol) of diethylaminosulfur trifluoride (DAST) were added dropwise. After the addition was complete, the reaction mixture was stirred at room temperature for four hours. Another 3.20 mL (24.2 mmol) of DAST were added, and the mixture was stirred for an additional two hours. The reaction mixture was poured into ice-water and stirred vigorously for five minutes. The organic and aqueous phases were separated. The aqueous phase was extracted with $CHCl_3$ (4 x 50 mL). The combined $CHCl_3$ extracts were washed with saturated aqueous $NaHCO_3$ (4 x 25 mL), with water (2 x 20 mL), and with saturated aqueous NaCl (20 mL). The chloroform was dried with Na_2SO_4, and the solvent was removed. The crude material was purified by column chromatography on silica gel with 10% ethyl acetate in hexane to yield 5.032 g (72%) of difluoroester **13** as a light orange oil: TLC 10% ethyl acetate in hexane, R_f = 0.58 (I); ^1H NMR (300 MHz, $CDCl_3$) δ 4.32 (q, J = 7 Hz, 2H), 2.05 (m, 2H), 1.45 (m, 2H), 1.35 (t, J = 7 Hz, 3H), 1.26 (m, 18H), 0.88 (t, J = 6 Hz, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 164.3 (t, J = 33 Hz), 116.3 (t, J = 250 Hz), 62.7, 34.6 (t, J = 23 Hz), 32.0, 29.7, 29.6, 29.4, 29.3, 29.1, 22.8, 21.5, 14.2, 14.1; ^{19}F NMR (282.2 MHz, $CDCl_3$) δ -30.2 (t, J = 17 Hz); MS (FAB) 293 (MH^+), 273. Anal. Calcd for $C_{16}H_{30}F_2O_2$: C, 65.72; H, 10.34; F, 12.99. Found: C, 66.15; H, 10.47; F, 12.54.

2,2-Difluorotetradecanoic acid (14). To a solution of 4.41 g (15.1 mmol) of **13** in 90 mL of MeOH was added 90 mL of 1.25 N NaOH. The reaction mixture was refluxed for three hours. The MeOH was removed, and the mixture was acidified with 10 mL of concentrated HCl. Dichloromethane (75 mL) was added to dissolve the organic material. The aqueous and organic phases were separated. The aqueous phase was extracted with CH_2Cl_2 (4 x 30 mL). The combined CH_2Cl_2 extracts were washed with saturated aqueous NaCl (2 x 25 mL) and dried with Na_2SO_4. Removal of solvent yielded 3.16 g (79%) of acid **14**: mp 39.5-41.5°C; TLC 90:10:1 hexane/Et_2O/AcOH, R_f = 0.15 (I); ^1H NMR (300 MHz, $CDCl_3$) δ 5.16 (s, br), 2.08 (m, 2H), 1.49 (m, 2H), 1.26 (m, 18H), 0.88 (t, J = 7 Hz, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 167.8 (t, J = 33 Hz), 116.2 (t, J = 249 Hz), 34.4 (t, J = 23 Hz), 32.0, 29.7, 29.6, 29.5, 29.4, 29.3, 29.1, 22.8, 21.4, 14.2; ^{19}F NMR (282.2 MHz, $CDCl_3$) δ -30.7 (t, J = 17 Hz). Anal. Calcd for $C_{14}H_{26}F_2O_2$: C, 63.61; H, 9.91; F, 14.37. Found: C, 62.38; H, 10.17; F, 14.33.

1-Bromo-3,3-difluoro-2-pentadecanone (15). To a solution of 200 mg (0.76 mmol) of **14** and 1.0 mL (11.6 mmol) of oxalyl chloride in 15 mL of toluene were added two drops of DMF. The reaction mixture was stirred at room temperature for two hours. The excess oxalyl chloride and the toluene were removed under vacuum at room temperature. The crude acid chloride was dissolved in 4 mL of Et_2O, and added to a solution of diazomethane in Et_2O (100 mL). After 15.5 h, the solution volume was reduced to approximately 40 mL. Then, 5 mL of 48% HBr and 50 μL of 48% HBF_4 were added. The two phase reaction mixture was stirred vigorously for 30 minutes at room temperature. The mixture was diluted by

the addition of water and Et_2O. The organic and aqueous phases were separated. The aqueous phase was extracted with Et_2O (3 x 10 mL). The combined Et_2O extracts were washed with 5% NH_4OH (2 x 5 mL), washed with saturated aqueous NaCl (5 mL), and dried with Na_2SO_4. Removal of solvent afforded the product as an oil, which was a mixture of bromoketone **15** and a second compound. The product showed two close running spots on TLC in various solvent systems. The best separation with the least amount of tailing on the higher R_f spot was achieved in CH_2Cl_2 (R_f = 0.80, **15**; 0.71, second compound (H)). Column chromatography on silica gel with CH_2Cl_2 afforded some purification of bromoketone **15**. Bromoketone **15** was used without further purification. Data for bromoketone **15**: TLC CH_2Cl_2, R_f = 0.80 (H); ^1H NMR (300 MHz, $CDCl_3$) δ 4.28 (s, 2H), 2.05 (m, 2H), 1.46 (m, 2H), 1.26 (m, 18H), 0.88 (t, J = 7 Hz, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 192.2 (t, J = 33 Hz), 118.2 (t, J = 252 Hz), 44.5, 33.1 (t, J = 22 Hz), 31.9, 29.6, 29.5, 29.4, 29.2, 29.0, 22.7, 21.2, 14.1; ^{19}F NMR (282.2 MHz, $CDCl_3$) δ -29.3 (t, J = 17.7 Hz); MS (CI, NH_3) 360 (M(Br81) + NH_4^+), 358 (M(Br79) + NH_4^+), 340, 338.

1-Diazo-3,3-difluoro-2-pentadecanone (16). To a solution of 500 mg (1.9 mmol) of **14** and 2.4 mL (27.9 mmol) of oxalyl chloride in 35 mL of toluene were added three drops of DMF. The reaction mixture was stirred at room temperature for two hours. The excess oxalyl chloride and the toluene were removed under vacuum at room temperature. The crude acid chloride was dissolved in 6 mL of Et_2O and added to a solution of diazomethane in Et_2O. After 15 h, the Et_2O was evaporated. The crude material was purified by column chromatography on silica gel with CH_2Cl_2, affording 349.8 mg (64%) of diazoketone **16** as a yellow oil: TLC CH_2Cl_2, R_f = 0.51 (H, U); ^1H NMR (300 MHz, $CDCl_3$) δ 5.75 (s, 1H), 2.03 (m, 2H), 1.45 (m, 2H), 1.26 (m, 18H), 0.88 (t, J = 7 Hz, 3H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 185.6 (t, J = 32 Hz), 118.7 (t, J = 252 Hz), 54.3, 33.4 (t, J = 23 Hz), 31.9, 29.6, 29.4, 29.3, 29.2, 22.7, 21.4, 14.1; ^{19}F NMR (282.2 MHz, $CDCl_3$) δ -30.7 (t, J = 17 Hz); MS (CI, NH_3) 306 (M + NH_4^+), 278.

General procedure for the alkylation of coenzyme A. *S*-(2-oxopentadecyl)-coenzyme A (1). A solution of 215 mg (253 μmol) of coenzyme A and 3.91 mg (25.4 μmol) of dithiothreitol in 21.5 mL of 0.04 M Na_2CO_3 was stirred under nitrogen for 30 min. A solution of 387.1 mg (1.27 mmol) of **10** in 43 mL of *t*-BuOH was then added. The reaction mixture was stirred at room temperature under nitrogen until all coenzyme A was reacted as determined by TLC (*n*-butanol/water/acetic acid = 5:3:2). Reactions typically took six hours, after which the *t*-BuOH was removed on a rotary evaporator. The precipitated bromoketone **10** was removed by filtration on a celite pad. The filtrate was lyophilized to yield 415.5 mg of crude product. The crude product was purified by reverse phase chromatography on C18 silica gel. The crude product was loaded on the column (5 x 2 cm) in acidic H_2O (pH = 3). The column was eluted successively with acidic H_2O (pH = 3), 90% 10 mM phosphate buffer (pH = 7.5) / 10% CH_3CN, 50% 10 mM phosphate buffer (pH = 7.5) / 50% CH_3CN. Alkylated coenzyme A **1** eluted with 50% 10 mM phosphate buffer (pH = 7.5) / 50% CH_3CN, and fractions containing **1** were combined and lyophilized. The material, containing **1** and phosphate salts, was dissolved in 6 mL of water. Upon addition of 10 mL of 5% $HClO_4$, **1** precipitated. The precipitate was collected by filtration, washed with 10 mL of 0.8% $HClO_4$, washed with three 3 mL portions of acetone, and dried. The yield of **1**, as a white powder, was 178 mg (71%): mp dec 134°C; TLC BuOH/H_2O/AcOH = 5:3:2, R_f = 0.37 (H, U); ^1H NMR (300 MHz, DMSO-d_6) δ 8.67 (s, 1H), 8.39 (s, 1H), 8.03 (t, J = 6 Hz, 1H), 7.74 (t, J = 6 Hz, 1H), 5.98 (d, J = 5 Hz, 1H), 4.79 (m, 1H), 4.71 (m, 1H), 4.40 (br, 1H), 4.18 (br, 2H), 3.89 (m, 1H), 3.73 (s, 1H), 3.55 (m, 1H), 3.39 (s, 2H), 3.31 (m, 2H), 3.17 (m, 2H), 2.52 (t, J = 7 Hz, 2H), 2.25 (t, J = 7 Hz, 2H), 1.44 (m, 2H), 1.22 (m, 20H), 0.92 (s, 3H), 0.84 (t, J = 7 Hz, 3H), 0.74 (s, 3H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 205.9, 172.2, 170.6, 150.6, 148.4, 145.9, 141.5, 118.6, 87.4, 82.2, 74.2, 73.5, 73.1, 72.3, 65.0, 37.9, 35.2, 34.9, 31.4, 30.9, 29.1, 29.0, 28.9, 28.8, 28.6, 23.3, 22.2, 21.1, 19.1, 14.0; MS (FAB) 990 (M - H), 766, 563, 506, 426; HRMS (FAB, neg. ion) calcd for $C_{36}H_{63}N_7O_{17}P_3S$ (M - H) 990.3214, obsd 990.3150.

***S*-(3,3-Difluoro-2-oxopentadecyl)-coenzyme A (2).** Following the procedure described for **1**, 200 mg (236 μmol) of coenzyme A were reacted with 417.0 mg (1.22 mmol) of **15**, in the presence of 3.62 mg (23.5 μmol) of dithiothreitol. The reaction took six hours, yielding 444.3 mg of crude product. After purification, 142.4 mg (59%) of **2** were obtained as a light orange powder: mp dec 133.5°C; TLC BuOH/H_2O/AcOH = 5:3:2, R_f = 0.47 (H, U); ^1H NMR (300 MHz, DMSO-d_6) δ 8.65 (s, 1H), 8.39 (s, 1H), 8.07 (t, J = 5.7 Hz, 1H), 7.75 (t, J = 5.7 Hz, 1H), 5.99 (d, J = 5.2 Hz, 1H), 4.80 (m, 1H), 4.71 (m, 1H),

4.42 (br, 1H), 4.20 (br, 2H), 3.91 (dd, J = 9, 5 Hz, 1H), 3.81 (s, 2H), 3.75 (s, 1H), 3.54 (dd, J = 9, 4 Hz, 1H), 3.30 (m, 2H), 3.22 (m, 2H), 2.55 (t, J = 7 Hz, 2H), 2.27 (t, J = 7 Hz, 2H), 2.04 (m, 2H), 1.23 (m, 20H), 0.93 (s, 3H), 0.85 (t, J = 6 Hz, 3H), 0.75 (s, 3H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 195.8 (t, J = 30 Hz), 171.9, 170.4, 150.5, 148.1, 145.9, 141.1, 118.4 (t, J = 252 Hz), 118.3, 87.0, 81.7, 73.6, 73.0, 72.5, 71.8, 64.4, 37.2, 34.7, 34.3, 32.2 (t, J = 21 Hz), 30.8, 30.3, 28.5, 28.3, 28.2, 28.1, 28.0, 21.6, 20.5, 20.2, 18.5, 13.4; ^{19}F NMR (282.2 Hz, DMSO-d_6) δ -30.5 (t, J = 18 Hz); MS (FAB) 1026 (M - H), 766, 599, 506, 426; HRMS (FAB, neg. ion) calcd for $C_{36}H_{61}F_2N_7O_{17}P_3S$ (M - H) 1026.3026, obsd 1026.3018.

***S*-(2,2-Difluoro-3-oxohexadecyl)-coenzyme A (3).** Following the procedure described for **1**, 300 mg (354 μmol) of coenzyme A were reacted with 750 mg (1.77 mmol) of **9**, in the presence of 5.42 mg (35.1 μmol) of dithiothreitol. The reaction was allowed to proceed for 20 hours, yielding 706.8 mg of crude product, which was a mixture of **3** and coenzyme A disulfide. The products were separated by reverse phase chromatography. The coenzyme A disulfide eluted with 90% mM phosphate buffer (pH = 7.5) / 10% CH_3CN and **3** eluted with 50% 10 mM phosphate buffer (pH = 7.5) / 50% CH_3CN. After $HClO_4$ precipitation, 81.6 mg (22%) of **3** were obtained as a white powder: mp dec 133°C; TLC BuOH/H_2O/AcOH = 5:3:2, R_f = 0.44 (H, U); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.61 (s, 1H), 8.44 (s, 1H), 8.04 (t, J = 5 Hz, 1H), 7.75 (t, J = 6 Hz, 1H), 6.00 (d, J = 5 Hz, 1H), 4.80 (br, 1H), 4.73 (br, 1H), 4.42 (br, 1H), 4.18 (br, 2H), 3.91 (dd, J = 9, 5 Hz, 1H), 3.75 (s, 1H), 3.53 (dd, J = 9, 4 Hz, 1H), 3.35-3.2 (m, 4H), 3.27 (t, J = 16 Hz, 2H), 2.75 (t, J = 7 Hz, 2H), 2.63 (t, J = 7 Hz, 2H), 2.28 (t, J = 6 Hz, 2H), 1.51 (m, 2H), 1.24 (m, 20H), 0.94 (s, 3H), 0.85 (t, J = 7 Hz, 3H), 0.74 (s, 3H); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 200.8 (t, J = 30 Hz), 172.3, 170.9, 151.9, 148.7, 147.5, 141.2, 118.9, 117.5 (t, J = 254 Hz), 87.5, 82.0, 73.9, 73.4, 72.9, 72.3, 63.1, 38.0, 36.5, 35.1, 34.8, 32.6 (t, J = 26 Hz), 31.8, 31.3, 29.0, 28.9, 28.8, 28.7, 28.1, 22.1, 19.6, 18.9, 13.9; ^{19}F NMR (282.2 MHz, DMSO-d_6) δ -29.6 (t, J = 16 Hz); MS (FAB) 1040 (M - H), 613, 506, 426; HRMS (FAB, neg. ion) calcd for $C_{37}H_{63}F_2N_7O_{17}P_3S$ (M - H) 1040.3182, obsd 1040.3160.

NMT Enzyme Assay.[47] *Saccharomyces cerevisiae* NMT was expressed in *E. coli* strain JM101 which had been transformed with the plasmid pBB125.[3c] Large scale cultures were grown as previously described,[3c] and packed cells (10 min at 4000g) were disrupted by sonication in lysis buffer containing phenylmethyl-sulfonylfluoride and EDTA.[48] After removal of cell debris by centrifugation (15 min at 10,000g), the resulting cell lysate was stored at -78°C (500 μL aliquots in polypropylene tubes). Once thawed, this enzyme solution and dilutions thereof were held at 4°C, and used directly in NMT assays. High specific activity [^{3}H]-myristoyl-CoA was prepared by enzymatic synthesis using acyl-CoA synthetase,[20b,49] and 0.3 μCi was used per assay (total myristoyl-CoA concentration 0.5 μM). The peptide substrate was an octamer (Gly-Asn-$(Ala)_4$-$(Arg)_2$-NH_2) modeled after the N-terminal sequence of the catalytic subunit of cAMP-dependent protein kinase, and was present in the assay mixture at 200μM concentration. The enzymatic reaction was carried out for 10 min at 30°C in an assay buffer containing 7.5 mM Tris, 0.75 mM dithiothreitol, 0.075 mM ethylene glycol bis(2-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid and 0.25% DMSO. After stopping the reaction by immersion in a boiling water bath for 2 min, the unreacted [^{3}H]-myristoyl-CoA was removed by absorption onto Bio-Rad AG-1 X-8 ion-exchange resin and the tritiated myristoyl-peptide product was quantitated in the supernatant by scintillation counting. Assays were performed in triplicate, and assays containing inhibitors were compared to identical uninhibited controls after subtraction of appropriate blanks lacking the peptide substrate.

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