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# SYNTHESIS OF MYRISTIC ACID ANALOGS WITH ANTI-HIV ACTIVITY THAT MAY BE RESISTANT TO METABOLIC PROCESSING BY ß-OXIDATION

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**Abstract:** Novel classes of oxatetradecanoic acid analogs **10,11,13,16**, and **21** were synthesized as alternative substrates for myristoylCoA:protein N-myristoyltransferase. These compounds inhibit replication of human immunodeficiency virus I (HIV-I) in acutely infected CD4-positive human T lymphocyte cell lines. The antiviral activity and potential metabolic stability of these compounds may make attractive therapeutic agents.

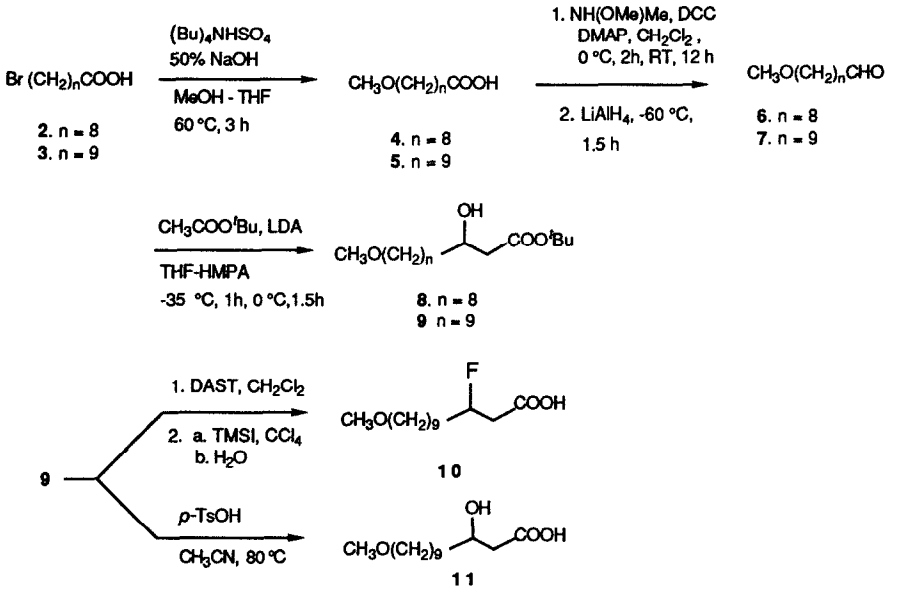
Human myristoylCoA:protein N-myristoyltransferase (NMT, E.C. 2.1.3.97) is a 416 residue,[2] monomeric enzyme that catalyzes the co-translational attachment of a tetradecanoyl group, via an amide bond, to the amino-terminal glycine residue of a number of cellular proteins as well as proteins encoded by the genomes of enveloped and nonenveloped viruses.[3] N-myristoylation of several of these viral proteins is necessary for viral replication and/or the production of infectious virions. The gag polyprotein precursor ($Pr55^{gag}$) of human immunodeficiency virus I (HIV-1) represents such an example.[4,5] We have synthesized a variety of myristic acid analogs which, when converted to their acylCoA derivatives by acylCoA synthetases, serve as alternative substrates for NMT.[6-8] One such compound, 13-oxatetradecanoic acid $CH_3O(CH_2)_{12}COOH$ (**1**, O-13) is efficiently incorporated into HIV-1 $Pr55^{gag}$, causes redistribution of the protein from cytosolic fractions, reduces the efficiency of its proteolytic processing by viral protease, and produces marked decreases in viral replication in both acutely and chronically infected human T-lymphocyte cell lines at concentrations that are not toxic to these cells.[9,10]

13-Oxatetradecanoic acid is a promising antiviral agent that is readily absorbed from the gastrointestinal tract. However, it is metabolized rapidly *in vivo via* ß-oxidation (half life in the plasma of fed mice after intravenous administration = 12-15 min.). In order to circumvent this problem, we embarked on the synthesis of α, ß and γ-substituted surrogates of **1**. In this report we wish to disclose the efficient syntheses of α-fluoro (**21**), β-fluoro (**10**), β-hydroxy (**11**), γ -hydroxy (**16**), and α,β-unsaturated (**13**) derivatives, some of which inhibit HIV-I replication in acutely infected T-lymphocytes at doses which do not cause cellular toxicity.

The synthesis of compounds **10** and **11** (Scheme I) began with the novel conversion of bromo acids **2** and **3** under phase transfer conditions to methoxy substituted acids **4** and **5** in 80% and 70% yield, respectively.[11] It is interesting to note that reaction of bromo acids with sodium methoxide gives poor yields of the methoxyacids.[12] Compounds **4** and **5** were converted to their respective aldehydes **6** and **7** *via* the formation of *N*-methoxy methyl amides and subsequent reduction by $LiAlH_4$.[13] The aldol condensations of **6** and **7** with the anion generated from

*t*-butyl acetate by the action of LDA in THF-HMPA at -70 °C furnished the corresponding hydroxy esters **8** and **9** in 60% and 65% yield respectively.

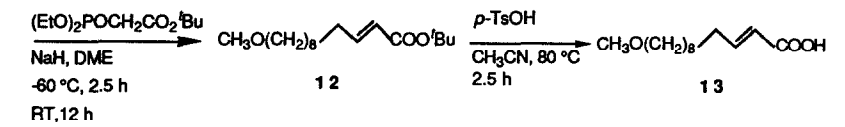
**SCHEME I**




The transformation of **9** to **10** was effected without observable elimination using DAST in $CH_2Cl_2$ at room temperature in 65% yield, followed by removal of the *t*-butyl ester group using TMSI[14] to afford **10**[15] as a crystalline solid. The ß-hydroxy acid **11** was obtained by the reaction of **9** in refluxing acetonitrile with catalytic *p*-toluenesulfonic acid.[16]

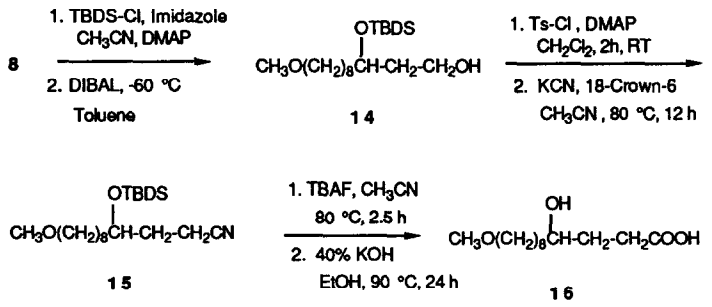
The Wadsworth-Emmons reaction of **7** (Scheme II) with the ylid generated from *t*-butyl diethylphosphonoacetate using NaH in DME at -60 °C gave the *trans* ($J$ = 15.6 Hz) α,ß unsaturated ester **12** in 85% yield. Subsequent reaction of **12** in refluxing acetonitrile with catalytic *p*-tolenesulfonic acid provided the free acid **13**.[17]

**SCHEME II**



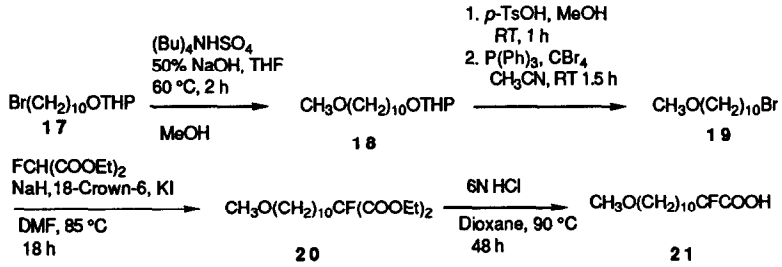
The γ-hydroxy acid **16** was synthesized in six steps starting from the ß-hydroxy ester **8** (Scheme III). The ester **8** was first converted to the silyl derivative and subjected to reduction with DIBAL at -60 °C to afford the alcohol **14** in 68% yield. Tosylation of **14** followed by reaction with KCN/18-crown-6 furnished the cyano derivative **15**. Subsequent reactions involved desilylation of **15** using TBAF followed by heating in 40% KOH and acidfication to obtain the acid **16**.[17]

**SCHEME III**




The α-fluoro analog **21** was obtained in five steps (Scheme IV) starting from the THP-protected bromo derivative **17**. Thus, the reaction of **17** under phase transfer conditions in the presence of MeOH afforded **18** in 80% yield. After the removal of the THP group in **18** in the presence of catalytic *p*-toluenesulfonic acid, the resulting alcohol was converted to the corresponding bromo compound **19**, by reaction with $P(Ph)_3/CBr_4$ in acetonitrile. The conversion of **19** to **20** was effected in 70% yield by reacting with diethylfluoromalonate in the presence of NaH and catalytic KI in DMF at 85 °C. Further reaction of the ester **20** in 6N HCl followed by decarboxylation, furnished the α-fluoro-O-13 analog **21**[17] in 52% yield.

**SCHEME IV**




Analogs **10**,**11**,**13** and **16** produced a dose-dependent reduction in HIV-1 production in acutely infected CD4+ CEM cells (Table I). The $ID_{50}$ value of 13-oxatetradecanoic acid (**1**) was 1-5 μM (n = 4 experiments, each

**Table I**

| Compound | AcylCoA Produced[a] (% myristoylCoA) *Pseudomonas* | Human | Acylpeptide Produced[a] (% myristoylpeptide) Yeast NMT | Human NMT | Antiviral Effect $ID_{50}$ (μM) | $TD_{50}$ (μM) |
|---|---|---|---|---|---|---|
| $CH_3O(CH_2)_{12}COOH$ (1) | 175 ± 4 | 75 ± 8 | 109 ± 3 | 84 ± 2 | 1-5 | 80-190 |
| $CH_3O(CH_2)_9CHCH_2COOH$ (11) <br> OH | 114 ± 5 | <5 | <5 | <5 | 9-20 | 280 |
| $CH_3O(CH_2)_8CH(CH_2)_2COOH$ (16) <br> OH | [b] | [b] | 107 ± 5 | <5 | 77 | >400 |
| $CH_3O(CH_2)_{10}CHCOOH$ (21) <br> F | 14 ± 1 | 26 | 14 ± 1 | <5 | not active | >100 |
| $CH_3O(CH_2)_9CHCH_2COOH$ (10) <br> F | 94 ± 5 | <5 | <5 | 5 | 1-5 | 300 |
| $CH_3O(CH_2)_9CH{=}CHCOOH$ (13) | 78 ± 3 | 12 ± 1 | 7 ± 1 | 5 | 5 | >80 |

[a] The *in vitro* acylCoA synthetase and NMT assays are described in detail in refs 5 and 18. [b] Not determined.

done in duplicate).[18] The $ID_{50}$ values for **10**, **11**, and **13** were comparable: 3-fluoro-13-oxatetradecanoic acid (**10**) = 1-5 μM, 3-hydroxy-13-oxatetradecanoic acid (**11**) = 9-20 μM, and 2*E*-13-oxatetradecenoic acid (**13**) = 5 μM (n = 2-3 experiments, each in duplicate). Movement of the hydroxyl group from C3 to C4 was associated with at least a four fold reduction in antiviral potency while movement of the fluoro group from C3 to C2 yielded an inactive analog (Table I). For each of the biologically active compounds, the dose that produced >50% killing of uninfected cells ($TD_{50}$) was at least ten fold greater than the corresponding $ID_{50}$ values (Table I).

*In vitro* studies using partially purified human myristoylCoA synthetase,[19] tritiated CoA[5,19] and the myristate analogs show that the efficiency of conversion of 13-oxatetradecanoic acid (**1**) to its acylCoA derivative is84% that of tetradecanoic acid while the efficiency of conversion of 3-hydroxy- and 3-fluoro-13-oxatetradecanoic acid were ≤5% of tetradecanoic acid. In contrast, the efficiency of conversion of 2-fluoro-13-oxatetradecanoic acid was 14% that of C14:0(Table I). These compounds can be converted to their CoA derivatives by the less specific *Pseudomonas* acylCoA synthetase (see Table I) and tested as substrates for human NMT purified from an erythroleukemia cell line.[19] Incubation of the analog CoAs with human NMT plus a radiolabelled octapeptide representing residues 1-8 of the HIV-1 $Pr55^{gag}$ (Gly-Ala-Arg-[$^3$H]Ala-Ser-Val-Leu-Ser-$NH_2$)[5] revealed that the amount of analog peptide produced was ≤5% that of myristoylpeptide (the amount of 13-oxatetradecanoylpeptide

produced in the single point *in vitro* assay[5] was 84 ± 2% of the amount of tetradecanoyl peptide. Similar results were obtained with purified[19] *S. cervisiae* NMT (Table I).

NMT has an ordered sequential reaction mecahanism with myristoylCoA binding to enzyme prior to peptide.[20] More detailed kinetic studies of the most potent antiviral compound 3-fluoro-13-oxatetradecanoic acid, indicated that binding of analog CoA to the enzyme produces alterations in both peptide $K_m$ (200 μM versus 70 μM with myristoylCoA) and $V_m$ (10% that observed with myristoylCoA). Paige et al[21] have noted that 2-fluoro- and 2-hydroxytetradecanoic acids inhibit incorporation of exogenous labeled myristate into cellular N-myristoylproteins produced by a cultured murine T-cell lymphoma cell line (LSTRA). Synthesis of radiolabeled 4-hydroxy-, 3-hydroxy- and 3-fluoro-13-oxatetradecanoic acids will be required to determine if their antiviral effects reflect any alterations in cellular NMT activity *in vivo* and/or their incorporation into HIV-I $Pr55^{gag}$. Furthermore, enantioselective synthesis of the hydroxy and fluoro substituted analogs of **1** may yield more potent anti-HIV agents.

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11. A mixture of 10-bromodecanoic acid (5.0 g, 0.02 mol), 50% sodium hydroxide (20 mL) and tetrabutyl ammoniun hydrogen sulphate (1.5 g, 4.4 mmol) in methanol (100 mL) and THF (50 mL) was heated at 60 °C for 2.5 h under nitrogen. The reaction mixture was cooled, concentrated under reduced pressure, diluted with water (200 mL), acidified with cold conc. HCl (30 mL) and extracted with $CH_2Cl_2$ (200 mL). The organic phase was washed with water ( 2 x 50 mL), dried ($Na_2SO_4$) , concentrated and the residue was purified by

flash chromatography over silica gel using 15% EtOAc in hexane as eluent to give 3.2 g of **5** (80%). $^1$H-NMR($CDCl_3$, 300 MHz): 1.3 (m, 10H), 1.6 (m, 4H), 2.35 (t, 2H, $J$ = 7.5 Hz), 3.33 (s, 3H), 3.37 (t, 2H, $J$ = 6.6Hz). Mass spectrum(FAB): *m/z* 203 (M++H); exact mass *m/z* calculated: $C_{11}H_{22}O_3Li$(M+Li) 209.1729, found 209.1776.

Compound **4** was prepared in a similar manner using 9-bromononanoic acid, yield 70%.

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15. DAST (0.15 g, 0.9 mmol) was added to a solution of **9** (0.2 g, 0.66 mmol) in $CH_2Cl_2$ and stirred at -20 °C for 0.5 hr and room temperature for 2 hr. The reaction mixture was then cooled to -30 °C and added saturated $NaHCO_3$ (5 mL) and $CH_2Cl_2$ (15 mL). The organic phase was washed with water, dried ($Na_2SO_4$), concentrated and the residue was purified by flash chromatography over silicagel using 5% EtOAc in hexane to give 0.15 g, of syrupy substance which was dissolved in $CCl_4$ (5 mL) and treated with TMSI (0.6 mL) and stirred at room temperature for 45 mins. The resulting solution was concentrated, added $CH_2Cl_2$ (25 mL) and cold water (5 mL). The organic phase was washed with water, dried ($Na_2SO_4$) concentrated and residue was crystallized from hexane to give 65 mg of **10** as shiny white flakes. $^1$H-NMR ($CDCl_3$, 300 MHz): 1.29-1.65 (m, 16H), 2.5-2.8 (m, 2H), 3.34 (s, 3H), 3.38 (t, 2H, $J$ = 6.6 Hz), 4.85 & 5.01 (m, 1H, $J_{H,F}$ = 48.3 Hz). $^{19}$F-NMR ($CDCl_3$, 300 MHz): 180.3 (m). Mass spectrum (FAB): *m/z* 255 (M+Li); exact mass *m/z* calculated: 255.1948, found: 255.1938(M+Li).

16. $^1$H-NMR ($CDCl_3$, 300 MHz): 1.29-1.6 (m, 16H), 2.54 (m, 2H), 3.35 (s, 3H), 3.37 (t, 2H, $J$ = 6.6 Hz), and 4.1(m, 1H). Mass spectrum (FAB): *m/z* 253 (M+Li); exact mass *m/z* calculated: 253.1991, found: 53.1959 (M+Li).

17. All new compounds gave analytical and spectral data consistent with their structures. Detail experimental procedures will be published elsewhere.

18. Target compounds were tested for HIV-1 growth inhibition in acutely infected CEM cells by an automated tetrazolium based colorimetric assay as described by Pauwles *et al*; *J. Virol. Methods* **1988**, *20*, 309-321 and refs 5 and 6. Note that $ID_{50}$ is the concentration of the compound that produces a 50% inhibition of virus-induced cytopathic effect.

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