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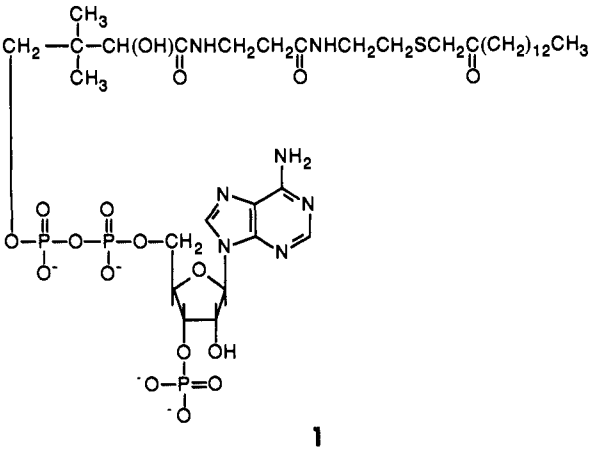
Communications to the Editor

S-(2-Oxopentadecyl)-CoA, a Nonhydrolyzable Analogue of Myristoyl-CoA, Is a Potent Inhibitor of Myristoyl-CoA:Protein *N*-Myristoyltransferase

Sir:

Several viral and cellular proteins have recently been shown to be covalently modified during biosynthesis by N-myristoylation of their amino-terminal glycine residues.[1] Protein N-myristoylation has attracted much attention due to the involvement of myristoylated proteins in the malignant transformation of cells and in the assembly of virus particles. In many cases, the presence of the myristoyl group is essential to the proper functioning of these proteins. For example, pp60$^{v\text{-}src}$, the transforming protein product of the Rous sarcoma virus oncogene is a myristoylated protein.[2] The association of pp60$^{v\text{-}src}$ with the plasma membrane, where it exerts its biological effects, is stabilized by the presence of the *N*-myristoyl group. Nonmyristoylated variants of pp60$^{v\text{-}src}$ retain protein-tyrosine kinase activity but remain soluble and are transformation defective.[3] Myristoylated viral structural proteins include the gag proteins of several retroviruses (including HIV p17) and the VP4 capsid proteins of several picornaviruses.[1,4] When examined, the myristoylation of retroviral gag proteins has been shown to be essential for the assembly[5] or maturation[6] of virus particles.

The N-myristoyltransferase (NMT) that catalyzes protein myristoylation has been proposed as a novel target for the action of antitumor and antiviral agents. Studies on NMT activity in cell-free systems using synthetic peptides as substrates indicate that myristoyl-CoA serves as the myristoyl group donor.[7] Since myristoyl-CoA appears to bind to the transferase prior to the binding of the peptide substrate,[8] it is reasonable to suggest that fatty acyl CoA analogues might prove to be effective inhibitors of NMT. In designing such inhibitors, we initially sought a compound that would structurally resemble myristoyl-CoA, but would not participate in the transferase reaction. Our target compound was *S*-(2-oxopentadecyl)-CoA (1), a nonhydrolyzable, methylene-bridged analogue of myristoyl-CoA.



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The synthetic approach to 1 was based on a final alkylation of coenzyme A using the procedure of Ciardelli and co-workers[9] due to the availability of chloromethyl ketone analogues of fatty acids and knowledge of the selective modification of the active thiol group in coenzyme A.[10] The synthesis of 1 is outlined in Scheme I. Myristoyl

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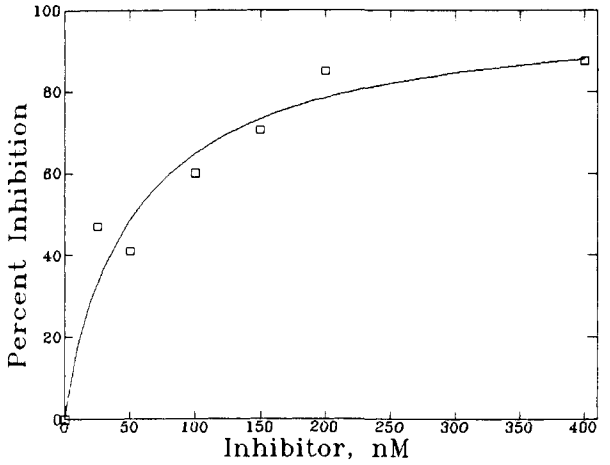

Figure 1. Effect of 1 on the activity of mouse brain *N*-myristoyltransferase. Increasing concentrations of 1 were tested for their ability to inhibit the transfer of [^{3}H]myristate from [^{3}H]-myristoyl-CoA to a synthetic peptide substrate. Reactions contained 0.2 mM peptide and 0.3 μM [^{3}H]myristoyl-CoA (20 Ci/mmol).

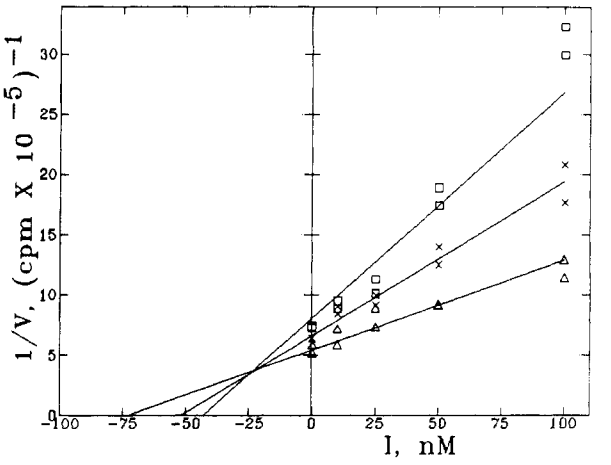

Figure 2. Kinetic constants for the interaction of 1 with NMT. Varying concentrations of 1 were tested for their ability to inhibit the activity of NMT at fixed [^{3}H]myristoyl-CoA concentrations of 0.5 μM (□), 0.75 μM (x) or 1.5 μM (△). All reactions contained 0.2 mM peptide.

chloride was treated with diazomethane to give 1-diazo-2-pentadecanone, mp 55–57 °C, in 79% recrystallized (petroleum ether) yield. Conversion of the resulting diazo group to the corresponding chloride by reaction with dry HCl gas afforded after recrystallization (Et_2O) a 81% yield of the desired chloromethyl ketone intermediate, mp 53–55 °C. Alkylation of coenzyme A dissolved in lithium carbonate buffered solution with a large molar excess of the ketone in the absence of oxygen produced the *S*-(2-oxopentadecyl)-CoA (1), in 87% yield as its lithium salt, mp 269–270 °C dec, after separation from the excess chloromethyl ketone by precipitation in acetone and purification by chromatography.[11]

Compound 1 inhibited NMT activity in vitro in a dose-dependent manner (Figure 1). For these assays NMT was partially purified from mouse brain homogenates by fractionation with ammonium sulfate (25%–55%) followed by chromatography on DEAE-cellulose using the procedure described by Towler et al.[12] for the isolation of NMT from rat liver. NMT activity was assayed by a modification of the procedure of Towler and Glaser,[13] which monitored the transfer of [^{3}H]myristate from [^{3}H]myristoyl-CoA to a synthetic acceptor peptide with the sequence Gly-Asn-Ala-Ala-Ala-Ala-Arg-Arg(NH_2). In our analyses [^{3}H]myristoyl-peptide was separated from radiolabeled reactants by applying a 50-μL aliquot to a 0.5-mL column of CM-Sepharose equilibrated in 20 mM Tris/HCl, pH 7.8. [^{3}H]Myristate and [^{3}H]myristoyl-CoA were eluted from the column with 20 mM Tris·HCl, pH 7.8. The [^{3}H]myristoyl-peptide, which remained bound to the resin, was quantified by liquid scintillation counting. A detailed description of this assay procedure will be published elsewhere.

An analysis of the kinetics of NMT inhibition indicated that 1 was a competitive inhibitor with respect to myristoyl-CoA with $K_i = 2.4 \times 10^{-8}$ M as determined from Dixon plots (Figure 2). This makes 1 one of the most potent inhibitors of NMT activity to be described. The K_i was considerably lower than the apparent K_m of mouse brain NMT for myristoyl-CoA (4×10^{-7} M), the natural substrate for the transferase. The presence of the methylene bridge between the carbonyl group and the sulfur in 1 apparently did not lower but rather increased the affinity of the analogue for the acyl-CoA binding site. These results indicate that the acyl-CoA binding site on NMT can accommodate some structural variation in the substrate. This is consistent with the observation that fatty acyl CoAs with acyl chain lengths of 10, 12, 14, or 16 carbons are bound by NMT with similar affinities (although only those of 12 and 14 carbons are reasonable substrates).[7] NMT will also recognize fatty acyl CoAs bearing several different heteroatom-substituted fatty acid analogues and transfer these analogues, often with an efficiency comparable to that of myristate.[8,14]

The data confirm the hypothesis that analogues based on the structure of myristoyl-CoA have the potential to be potent inhibitors of NMT. Since initial studies suggest that 1 does not readily penetrate living cell membranes, additional analogues of this type will need to be synthesized for use as therapeutic agents. Efforts are currently under way to (1) determine the structural requirements that are necessary for the interaction of myristoyl-CoA analogues with the transferase and (2) determine what modifications can be made to mask the charged residues on these analogues.

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(11) *S*-(2-Oxopentadecyl)-CoA (1), white powder from MeOH, $[\alpha]^{25}_D = -31°$ (c = 0.1, H_2O), showed a single UV (254 nm) quenching and phosphate positive spot on two-dimensional silica gel TLC (R_f = 0.57) and Cellulose TLC (R_f = 0.84) with the solvent *n*-BuOH/HOAc/H_2O) (5:2:3). Its elemental composition was determined by the high-resolution FAB mass spectrum [m/z 1016.3679 $(M + H)^+$, calcd for $C_{36}H_{61}N_7O_{17}P_3SLi_4$ 1016.3698; m/z 1010.3622 $(M + H - Li)^+$, calcd for $C_{36}H_{61}N_7O_{17}P_3SLi_3$ 1010.3616]. The IR, UV, MS, ^{1}H NMR, and ^{13}C NMR (2D COSY and HETCOR) spectra were consistent with the assigned structure.

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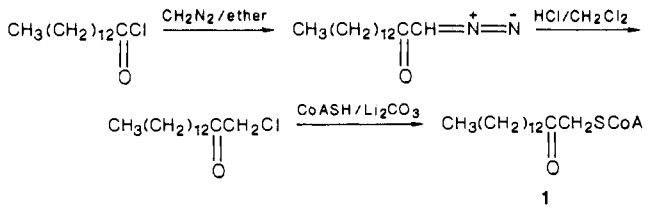
Scheme I



Department of Chemistry, The Ohio State University.

Registry No. 1 (free acid), 121124-66-1; 1·4Li, 121124-67-2; CoA, 85-61-0; NMT, 110071-61-9; $CH_3(CH_2)_{12}COCl$, 112-64-1; $N_2{=}CHCO(CH_2)_{12}CH_3$, 90670-23-8; $ClCH_2CO(CH_2)_{12}CH_3$, 121097-11-8.

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Articles

C2,N^6-Disubstituted Adenosines: Synthesis and Structure–Activity Relationships

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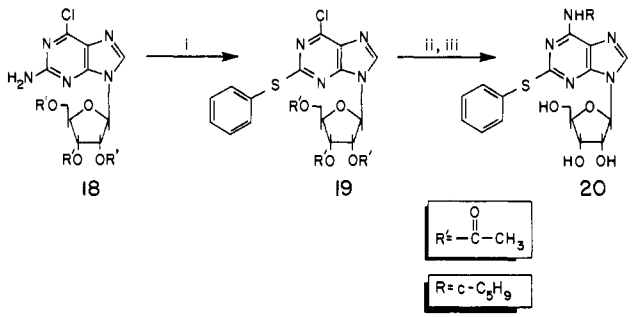
Extracellular adenosine receptors have been divided into two major subtypes, called A_1 and A_2. Substitution of the adenosine molecule with appropriate groups at C2 or N^6 is known to impart selectivity for the A_2 receptor over the A_1 receptor. In the present study, we investigated whether substitution at both C2 and N^6 would have additive effects on the A_2/A_1 affinity ratio, thereby providing compounds with greater A_2 selectivity than presently available agents. Disappointingly, additivity appeared to hold only when an A_1-selective group was present at N^6. For instance, 2-(phenylamino) substitution of the A_1-selective agonist *N*^{6}-cyclopentyladenosine resulted in a 70-fold shift in selectivity in favor of the A_2 receptor, but the same substitution applied to the A_2-selective agonist *N*^{6}-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)ethyl]adenosine resulted in a 100-fold loss of affinity with no change in A_2 selectivity.

Adenosine causes a variety of physiological responses, which are mediated by two subtypes of extracellular receptors, called A_1 and A_2. These two receptor subtypes can be distinguished on the basis of structure–activity relationships,[1-3] and specific receptor binding assays exist for both subtypes.[4,5] Considerable effort has been devoted to the search for adenosine agonists with improved selectivity for A_1 or A_2 receptors. Although agonists with 1000-fold or greater selectivity for the A1 receptor are known,[6] until recently the most A_2-selective agonist was 2-(phenylamino)adenosine (CV-1808, compound **7** in Table II),[7] which shows only 5-fold A_2 selectivity.[5] Very recently, *N*^{6}-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)ethyl]-adenosine (compound **50** in Table IV) was shown to possess about a 30-fold selectivity for the A_2 receptor.[8] Because **7** is substituted at C2, whereas **50** is substituted at N^6 we became interested in the possibility that the functional groups responsible for conferring selectivity on these two compounds might interact with independent sites on the adenosine receptor, thereby allowing additive enhancement of selectivity by combining structural modifications at both positions. Because many other C2 and N^6 groups with widely differing effects on A_1 and A_2 affinity have been reported (see Tables II and IV),[7,9-11] we also tested representative combinations of these groups for their effects on adenosine-receptor selectivity. Our results indicate that the effects of C2 and N^6 substitution are only

Scheme I[a]



[a] (i) PhSSPh, isoamyl nitrite, CH_3CN, Δ; (ii) RNH_2, DME, Et_3N, room temperature; (iii) MeOH–NH_3, room temperature.

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