Acyl Pyrophosphates: Activated Analogues of Pyrophosphate Monoesters Permitting New Designs for Inactivation of Targeted Enzymes[1]

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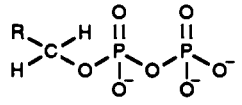
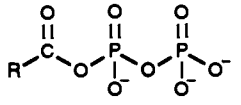
We report a new class of reactive biological analogues, acyl pyrophosphates, and a demonstration of their use as enzyme inactivators. Pyrophosphate monoesters are intermediates in many biological processes, including terpene and steroid biosynthesis and protein regulation.[2,3] The enzymes involved in promoting these processes are significant targets for inactivation in the rational design of bioactive agents.[4] The design of inactivators can be approached systematically if the pyrophosphate ester can be replaced by an analogue that will react irreversibly after binding to a protein.[5] Analogues of pyrophosphate esters have been prepared in which oxygen atoms of the pyrophosphate group are replaced by sulfur atoms, nitrogen atoms, or methylene groups. These materials bind to enzymes, and many function as alternative substrates.[6] However, their properties do not permit them to be inactivators. We reasoned that an alternative general approach would be to modify the primary alcohol group which forms the ester linkage to the pyrophosphate group. Conversion of the methylene group to a carbonyl group will enhance electrophilic reactivity and provide for acylation of adjacent nucleophiles in the enzyme binding site, in analogy to reactions of acyl phosphate monoesters with enzymes[7] and other proteins.[8] The resulting material is an acyl pyrophosphate, a class of molecule that has not previously been isolated.[9]



pyrophosphate ester



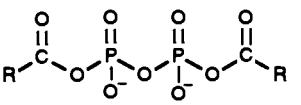
acyl pyrophosphate

While anhydrides of carboxylic and phosphoric acids can be prepared efficiently by reaction of an acyl halide and the salt of a phosphate ester[10] or by reaction of an anhydride with an aqueous phosphate solution,[11] we find that these methods do not extend to the production of acyl pyrophosphates. Reaction of tetra-*n*-butylammonium salts of pyrophosphoric acid with acid chlorides produces mixtures of the acyl pyrophosphate and a bis(acyl) pyrophosphate (observed by ^{31}P NMR) in methylene chloride. Reaction of an acid chloride with sodium pyrophosphate gives neither product. Reaction of a carboxylic anhydride with sodium pyrophosphate gives only a very low yield of acyl pyrophosphate.[9]

Table I. Acyl Pyrophosphates Synthesized from Carboxylic Acids

acyl moiety	reactn time, h	% yield (±5%)[a]
acetyl	2	95
isopentenoyl	2	95
benzoyl	5	95
p-methylbenzoyl	5	95
p-nitrobenzoyl	2	85
phenylacetyl	2	95
dimethylacryloyl	1.5	25-50
geranoyl	1.5	35
farnesoyl	1.5	35
N-acetylvalyl	2	95
N-acetylleucyl	2	95

[a] Determined from ^{31}P NMR spectra of reaction mixtures.



bis(acyl) pyrophosphate

Acyl pyrophosphates can be produced without contaminating bis(acyl) pyrophosphates (analyzed by ^{31}P NMR) by addition of 1 equiv of tetra-*n*-butylammonium disodium hydrogen pyrophosphate[12] to a solution of the carboxylic acid and dicyclohexylcarbodiimide[13] in dichloromethane. The solution is kept at room temperature for 2-3 h. Dicyclohexylurea is removed by precipitation from the reaction solution at -20 °C for 5-10 h, followed by filtration, evaporation, dissolution of the product in acetonitrile, and extraction with hexane to remove residual dicyclohexylurea.[14]

A diverse group of carboxylic acids were efficiently converted to the corresponding acyl pyrophosphates by this method (Table I). All materials had appropriate NMR spectra and high-resolution mass spectra for the parent peak (FAB (-) with glycerol or NBA matrix).

Acyl pyrophosphates disproportionate at room temprature in nonhydroxylic solvents (dichloromethane, tetrahydrofuran) to give bis(acyl) pyrophosphates and inorganic pyrophosphate. The stability of acyl pyrophosphates depends on the associated cation. When $(n\text{-}Bu_4N)_3HP_2O_7\cdot 3H_2O$ was used, the resulting acyl pyrophosphates disproportionated much faster than those from $(n\text{-}Bu_4N)Na_2HP_2O_7\cdot 3H_2O$.[15] Addition of anhydrous hydrogen chloride or acetic acid to the dichloromethane solution did not affect the rate of disproportionation. Solutions stored at -20 °C also slowly decompose, with bis(acyl) pyrophosphates as the major products.

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(12) Woodside, A. B.; Huang, Z.; Poulter, C. D. *Org. Synth.* **1988**, *66*, 211. Disodium dihydrogen pyrophosphate was dissolved in water, and 1 equiv of tetra-*n*-butylammonium hydroxide was added. Water was removed by lyophilization to yield a hygroscopic powder, $n\text{-}Bu_4N\cdot Na_2\cdot H\cdot P_2O_7\cdot 3H_2O$.
(13) For acyl phosphate formation with this reagent, see: Berg, P. *J. Biol. Chem.* **1958**, *233*, 608.
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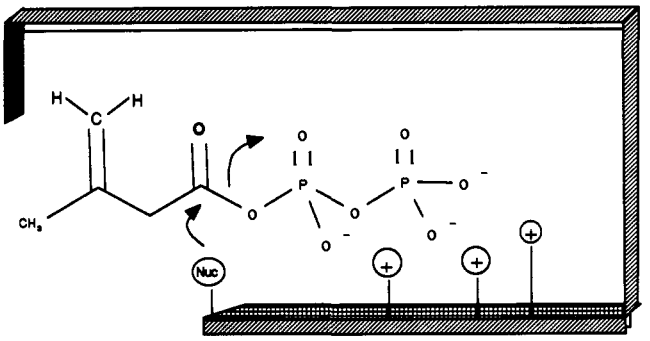
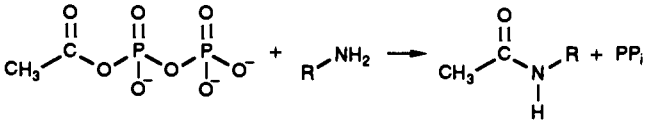


Figure 1. Schematic model for the binding of an acyl pyrophosphate as a substrate analogue.

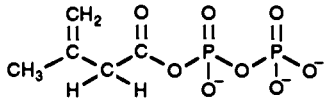
Amines and thiols are acylated by acyl pyrophosphates in organic solvents and in aqueous solution.



Reaction of acyl pyrophosphates (50 mM) with carboxyl-terminal-protected amino acid esters (250 mM) in phosphate buffer (200 mM) at pH 7.0 produces N-acylated amino acid esters (63% yield after workup and purification of reaction of valine ethyl ester and phenylacetyl pyrophosphate). Lysine ethyl ester is preferentially acylated at the ϵ-amino group under these conditions.

Acyl pyrophosphates hydrolyze in wet organic solvents or in water to form inorganic pyrophosphate and the corresponding acids. Inorganic pyrophosphate does not incorporate ^{18}O from the hydrolysis of 0.25 M isopentenoyl diphosphate in 0.75 M pH 7.0 HEPES buffer at 37 °C containing 25% $H_2{}^{18}O$ (^{31}P NMR ^{18}O analysis[16-18]). Thus, the hydrolysis occurs by acylation of water with pyrophosphate as the intact leaving group. Between pH 3.0 and 7.0, the hydrolysis of phenylacetyl pyrophosphate follows first-order kinetics (monitored by ^{31}P NMR), $k_{obsd} = 2.7 \pm 0.5 \times 10^{-5}$ s^{-1}, pH 6.2, 37 °C, 0.5 M potassium maleate buffer ($t_{1/2}$ = 7.2 h).

As a test of the ability of an acyl pyrophosphate to inactivate an enzyme that utilizes a related substrate, farnesyl synthetase from yeast (EC 2.5.1.1) was incubated with 0.25 mM isopentenoyl pyrophosphate.



isopentenoyl pyrophosphate

This enzyme is likely to contain nucleophilic groups in its substrate binding site.[15] Activity was monitored by using an acid lability assay with geranyl pyrophosphate and [1-^{14}C]isopentenyl pyrophosphate.[19] The enzyme lost all activity during a 2-min preincubation at 37 °C (the kinetics of the process were not determined due to the complexity of the assay). Activity could not be recovered by addition of excess substrate. Preincubation with large amounts of substrate protects the enzyme from inactivation. A schematic model for the inactivation reaction is shown in Figure 1.

Addition of the hydrolysis products of isopentenoyl pyrophosphate, isopentenoic acid, and inorganic pyrophosphate under the same conditions gave no inactivation, although competitive inhibition (due to the pyrophosphate[19]) was observed. The specificity of inhibition is further demonstrated by our observation that acetyl pyrophosphate does not inactivate the enzyme (concentrations up to 5 mM).

These results indicate that acyl pyrophosphates can be conveniently prepared and possess reaction patterns that permit them to be used as enzyme inactivators. Detailed evaluations of these materials are necessary to determine their potential for specific applications.[20]

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New Molecular Complements to Imides. Complexation of Thymine Derivatives

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Molecular recognition of imides (Scheme I) is of interest in

Scheme I

chromatographic resolutions,[1] thymine receptors,[2] liquid crystals,[3] and molecular tapes and sheets,[4] as well as theoretical[5] and experimental[6] evaluations of secondary effects in hydrogen bonding. We describe here new systems for base-pairing to thymines which offer unusual affinities and promise for the catalysis of reactions involving thymines.

The receptors are prepared from the readily available xanthene-1,8-dicarboxylic acid **1**, and they resemble those derived from Kemp's triacid[7] but have more spacious interiors.[8] Esterification with phenol (DCC, CH_2Cl_2, 0 °C) or naphthylethanol gave the mono esters[9] **2** (Scheme II). Activation ($SOCl_2$) and then coupling with suitable amines gave the amide esters **3a-c** (not shown), which were heated with biguanide (2 equiv) in ethanol to give the receptors **4a-c** (30-40% overall from **1**).[9] The ester **4d** was prepared from **2a** by sequential treatment with $SOCl_2$ and

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