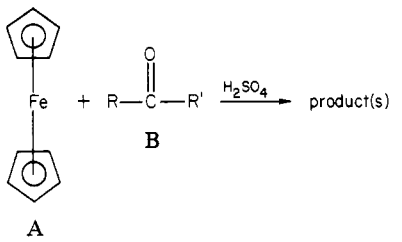
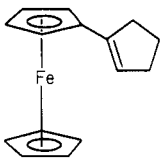
Table I



carbonyl compd	ratio A/B, mmol	ratio H_2SO_4/H_2O, mL	rctn time, min	rctn temp, °C	yield of alcohol, %	yield of olefin(s), %	product mp, mp, °C	lit. mp, °C (ref)
CH_2O (formalin)	2.5/2.6	8/1	1	−15	53		77-78	81-82 (*a*)
CH_2O (formalin)	5.0/5.0	8/0	2	−50	57		77-78	81-82 (*a*)
CH_3CHO	2.5/2.5	8/1	1	0	54		77-78	73-75 (*b*)
cyclohexanone	10/10	8/0	5	0		83	66-66.5	(*c*)
acetone	2.5/10	8/0	5	0	33	36	57-59 62-63	77.5-78.5 (*d*)
benzaldehyde	2.5/2.5	8/1.5	1	−10	42		80-81	81-82 (*e*)
2-pentanone	2.5/2.5	8/1	1	0	25		liquid	
propionaldehyde	2.5/2.5	8/0	1	−15	39	trace	liquid	(*f*)
2-butanone	10/10	8/0	1	0	trace	54[h]		(*f*)
cyclopentanone	10/100	10/0	90	27		83	68-69	64-65 (*g*)

[a] Reference 6, text. [b] Arimoto, F. S.; Haven, A. C., Jr. *J. Am. Chem. Soc.* **1955**, *77*, 6295. [c] Reference 5, text. [d] Rinehart, K. L.; Michejda, C. J.; Kittle, P. A. *J. Am. Chem. Soc.* **1960**, *82*, 2082. [e] Weliky, N.; Gould, E. S. *J. Am. Chem. Soc.* **1957**, *79*, 2742. [f] Schlogl, K.; Mohar, A. *Monatsh. Chem.* **1961**, *92*, 219. [g] Weinmayr, V. *J. Am. Chem. Soc.* **1955**, *77*, 3009. [h] Mixture of 2-ferrocenyl-1-butene and 2-ferrocenyl-2-butene.

with formaldehyde. In most of these syntheses, other products were obtained in small amounts and were not identified. The reaction of ferrocene with benzaldehyde in concentrated sulfuric acid did not provide a substantial amount of a compound, different from phenylferrocenylcarbinol, that has not yet been identified. Ketones, on the other hand, are not as well-behaved. While product yields were generally higher, mostly olefinic products were obtained. With acetone, 2-ferrocenyl-2-propanol (**6**) as well as the corresponding olefin, 2-ferrocenylpropene, was isolated; relative amounts depended on reaction conditions. Cyclopentanone and cyclohexanone gave 1-ferrocenylcyclopentene (**7**) and 1-ferrocenylcyclohexene (**8**), respectively, both in yields of 83%.



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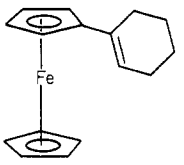

8

Experimental Section

Melting points were obtained on a Fisher-Johns apparatus and are uncorrected. Because of the sensitive nature of some of the syntheses reported here, general descriptions of syntheses will contain parenthetical notes that refer to specific changes for some problem compounds. Table I summarizes our results and provides details of reaction temperature, time, and concentrations of reactants.

Typical Procedure for Reaction of Ferrocene with Aldehydes in Concentrated Sulfuric Acid. To a solution of 8 mL of concentrated sulfuric acid and 1 mL of water cooled to −10 °C (ice–HCl bath) was added 2.5 mmol of the aldehyde. After stirring of the mixture for about 1 min, 2.5 mmol of ferrocene was added. The mixture was vigorously stirred for 1 min, after which 10 mL of ice water was added dropwise over a 2-min period. In some cases, the separation of a solid was observed, but in all successful runs, the solid redissolved to produce a deep red solution. The reaction mixture was poured into about 150 mL of cold water and extracted four times with 20 mL of diethyl ether. The ether solution was washed with aqueous sodium bicarbonate solution and dried with magnesium sulfate and the solvent removed under reduced pressure. The oil or waxy solid thus produced was chromatographed on silica gel, first using hexane as the eluting solvent and gradually increasing the ether content of the eluting solvent to a maximum ether concentration of 30%. Alcohol products were normally recrystallized from hexane.

Typical Procedure for Reaction of Ferrocene with Ketones in Concentrated Sulfuric Acid. To 8 mL of concentrated sulfuric acid cooled to 0 °C was added 10 mmol of the ketone (in the case of cyclopentanone, 100 mmol was added). This mixture was stirred for 1 min, and 10 mmol of ferrocene was added. The mixture was stirred for 5 min, followed by addition of 10 mL of ice water over a 2-min period. The workup procedure followed that described for aldehydes above. The olefinic products were purified by chromatography on silica gel, using hexane as eluting solvent. In the cases of cyclohexanone and cyclopentanone, chromatography was not necessary; the cyclohexenyl- and cyclopentenylferrocene products, respectively, were purified directly by recrystallization from absolute ethanol.

Registry No. **3**, 1273-86-5; **5**, 1277-49-2; **6**, 12093-87-7; **7**, 12260-67-2; **8**, 33183-07-2; CH_3CHO, 75-07-0; phenylferrocenylmethanol, 1277-68-5; 2-ferrocenyl-2-pentanol, 12302-37-3; 1-ferrocenyl-1-propanol, 1294-04-8; 2-ferrocenyl-1-butene, 32614-19-0; 2-ferrocenyl-2-butene, 12289-38-2; formalin, 50-00-0; cyclohexanone, 108-94-1; acetone, 67-64-1; benzaldehyde, 100-52-7; 2-pentanone, 107-87-9; propionaldehyde, 123-38-6; 2-butanone, 78-93-3; cyclopentanone, 120-92-3; ferrocene, 102-54-5; 2-ferrocenylpropene, 31725-14-1.

A Practical Enzymatic Synthesis of (S_P)-Adenosine 5′-*O*-(1-Thiotriphosphate) ((S_P)-ATP-α-S)[1]

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(S_P)-Adenosine 5′-*O*-(1-thiotriphosphate) ((S_P)-ATP-α-S) is an analogue of ATP useful in studying the mecha-

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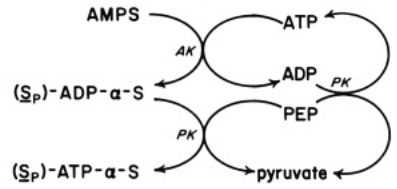
Scheme I. Enzymatic Synthesis of (S_P)-ATP-α-S



nisms of nucleotidyl transfer reactions and for introducing the phosphothioate group into RNA.[3] ATP-α-S was first synthesized by a stereorandom chemical method that produced the S_P and R_P diastereomers in nearly equal amounts.[4] Subsequently, a small-scale (<1 mmol) stereospecific synthesis of (S_P)-ATP-α-S was reported that used a coupled enzyme system comprising adenylate kinase (AK) and pyruvate kinase (PK) (Scheme I).[5,6] We have developed this procedure into a convenient synthesis of (S_P)-ATP-α-S, applicable to preparations on a 20-mmol scale, by using PAN-immobilized enzymes (PAN is a copolymer of acrylamide and *N*-acryloxysuccinimide) and simplifying the synthesis of the starting material and the isolation of the product. The starting material, adenosine 5′-*O*-(monothiophosphate) (AMPS), is prepared by a straightforward thiophosphorylation procedure that provides a solution of AMPS of sufficient purity to be used directly, without purification, in the subsequent enzymatic reaction. The phosphoenol pyruvate[11] required in this synthesis is easily prepared, and the enzymes required are inexpensive, easily immobilized, and readily recycled.

Experimental Section

General Procedures. UV measurements were made at 25 °C with a Perkin-Elmer Model 552 spectrophotometer. ^{31}P NMR spectra were determined at 121.5 MHz on a Bruker Model WM 300 spectrometer. NMR samples were prepared in 1.0 M Tris–HCl, pH 8.5, containing 50 mM EDTA and 20% D_2O. Chemical shifts are reported relative to external 1 M H_3PO_4 in D_2O. HPLC analyses were carried out on a Waters Associates system equipped with a differential ultraviolet detector operating at 254 nm, using a Waters Radial-PAK C-18 column (5 mm × 10 cm, 10-μm particle size). The mobile phase was 5 mM Waters PIC Reagent A containing 10–20% acetonitrile.

Materials. Enzymes, biochemicals, and Dowex-1×8 (200–400 mesh) were obtained from Sigma. Thiophosphoryl chloride was obtained from Aldrich and distilled before use. Triethyl phosphate was obtained from Aldrich and was distilled from BaO before use. PAN was prepared as described previously.[7]

Adenosine 5′-*O*-(Monothiophosphate) (AMPS). Adenosine (13.4 g, 50 mmol) and 120 mL of triethyl phosphate were added to a 250-mL flask equipped with a magnetic stirring bar, condensor, and inlet for argon. The suspension (under argon) was heated rapidly to reflux and maintained at that temperature until a homogenous solution was obtained (ca. 4 min).[8] The mixture was cooled to 5 °C, and 2,6-dimethylpyridine[9] (16.1 g, 150 mmol) and thiophosphoryl chloride (15.3 g, 90 mmol) were then added sequentially. After 45 min the resulting suspension was poured into petroleum ether (bp 35–57 °C, 500 mL), and the solvents were decanted. The residual white solid was washed with petroleum ether (2 × 300 mL) and hydrolyzed by stirring with water (100 mL) at 4 °C for 1 h. The solution was adjusted to pH 7.0 with 5 N KOH and extracted with diethyl ether (2 × 100 mL) and petroleum ether (2 × 100 mL). The resulting aqueous solution (200 mL) contained 40 mmol (80% yield) of AMPS and was used in the following reaction without further purification;[10] ^{31}P NMR δ −58.7 (s).

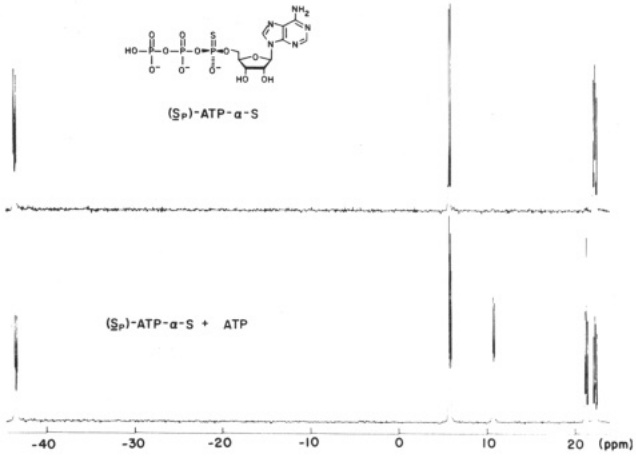


Figure 1. (Upper) 121.5 MHz ^{31}P NMR spectrum of (S_P)-ATP-α-S, 50 mM in 1.0 M Tris–HCl (pH 8.5) (4:1 H_2O:D_2O). (Lower) ^{31}P NMR spectrum of (S_P)-ATP-α-S and ATP under similar conditions.

(S_P)-Adenosine 5′-*O*-(1-Thiotriphosphate) ((S_P)-ATP-α-S). A solution (800 mL) containing potassium phosphoenol pyruvate[11] (PEP, 11.0 g, 100 mmol), AMPS (38 mmol, 190 mL of 0.2 M solution), dithiothreitol (1.43 g, 9.3 mmol), $MgCl_2$·$6H_2O$ (0.08 g, 0.4 mmol), and ATP (0.1 mmol, 2.0 mL of 50 mM solution) was placed in a 2-L round-bottomed flask equipped with a magnetic stirring bar, pH electrode, and argon atmosphere and deoxygenated with argon and adjusted to pH 8.0 with 5 N KOH. Adenylate kinase (EC 2.7.4.3, 3000 U[12]) and pyruvate kinase (EC 2.1.1.40, 5100[12] U) coimmobilized in 250 mL of PAN gel[7] were added, and the resulting suspension was heated to 37 °C. The reaction mixture was maintained at pH 8.0 by the occasional addition of 5 N KOH. After 5 days the enzyme-containing polyacrylamide gel was separated by decantation. The recovered enzymatic activities (calculated as the percentage of starting activities) were AK, 63% and PK, 84%. The turnover numbers (mole of product/mole of enzyme) were AK, 1.0×10^5 and PK, 2.9×10^5.

The phosphate-containing species were adsorbed from the supernatant onto Dowex-1 (250 g, 200–400 mesh, HCO_3^- form) at 4 °C, and the resin was washed with deionized water (1.5 L). Inorganic thiophosphate, PEP, ATP, and other impurities were desorbed by washing with 0.01 M HCl–0.02 M NaCl solution (4 L). (S_P)-ATP-α-S was then desorbed by washing the resin with 0.02 M HCl–0.8 M NaCl solution (3.5 L). The eluent was adjusted to pH 8.0 with 5 N KOH and $BaCl_2$ (34.0 g, 166 mmol) was added. The precipitate was collected by centrifugation and washed se-

(1) Supported by the National Institutes of Health, GM 30367.
(2) Damon Runyon–Walter Winchell Cancer Fund Postdoctoral Fellow, 1983–1985.
(3) Eckstein, F. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 423–439 and references therein.
(4) Eckstein, F.; Goody, R. S. *Biochemistry* **1976**, *15*, 1685–1691.
(5) Frey, P. A.; Sheu, K. F. R. *J. Biol. Chem.* **1977**, *252*, 4445–4448.
(6) Jaffe, E. K.; Cohn, M. *Biochemistry* **1978**, *17*, 652–657.
(7) Pollak, A.; Blumenfeld, H.; Wax, M.; Baugh, R. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1980**, *102*, 6324–6336. (PAN is a copolymer of acrylamide and *N*-acryloxysuccinimide.)
(8) We have found it essential to start with homogeneous solutions of adenosine in order to obtain reproducibly high yields of AMPS. At the concentration used here adenosine becomes soluble in triethyl phosphate at ca. 160–170 °C. Control experiments showed adenosine does not decompose in the short time (<5 min) during which it is heated.
(9) 2,6-Dimethylpyridine was found to promote more rapid and complete thiophosphorylation than pyridine under these reaction conditions.
(10) This solution contained AMPS (40 mmol), inorganic thiophosphate (10 mmol), triethyl phosphate (20 mmol), and other unidentified impurities that did not contain phosphorus. The composition of this solution remained constant over a 24-h period when stored at 4 °C.
(11) Hirschbein, B. L.; Mazenod, F. P.; Whitesides, G. M. *J. Org. Chem.* **1982**, *47*, 3766–6769.
(12) Enzymes were assayed according to Bergmeyer et al. Bergmeyer, H. U. "Methods of Enzymatic Analysis"; Verlag Chemie, New York, 1974. Assays were carried at 25 °C. One unit (U) of enzymatic activity is defined as that amount of enzyme that catalyzes the formation of 1 μmol of product/min at 25 °C. Note that the activities of AK and PK were measured with use of their natural substrates. The activities of the enzymes with the nucleotide phosphothioates are considerably lower. At 1.0 mM MgATP, the apparent K_m for AK-catalyzed phosphorylation of AMPS is 0.31 mM and that for AMP is 0.11 mM at pH 8.0 and 27 °C. The apparent V_{max} for AMP is 39 times that for AMPS.[5]

quentially with water (200 mL), 50% aqueous EtOH (200 mL), and acetone (200 mL). After drying, the isolated solid (17.3 g) contained 90% $Ba_2(S_P)$-ATP-α-S[13] by weight (20 mmol, 53% yield from AMPS), no detectable (R_P)-ATP-α-S, and <3% ATP (determined by HPLC and ^{31}P NMR);[13] ^{31}P NMR δ -43.5 (d, P_α), +5.8 (d, P_γ), +22.3 (dd, P_β); $J_{P_\alpha-P_\beta}$ = 27.2 Hz, $J_{P_\beta-P_\gamma}$ = 20.0 Hz (Figure 1).

Registry No. (S_P)-ATP-α-S, 58976-48-0; (S_P)-ATP-α-S·2Ba, 88157-74-8; AMPS, 19341-57-2; adenosine, 58-61-7; PEP, 138-08-9; ATP, 56-65-5; PK, 9001-59-6; AK, 9013-02-9.

(13) The $Ba_2(S_P)$-ATP-α-S was solubilized for analysis by stirring with 2 equiv of $(NH_4)_2SO_4$ (pH 8.0, 4 °C, 1 h). The $BaSO_4$ was removed by centrifugation.

Synthesis of 4-Alkenoic and 4-Alkynoic Esters via Alkylation of O-Silylated Ketene Acetals

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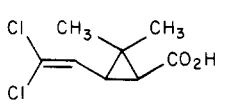
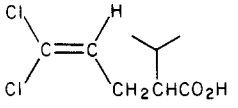
Received June 6, 1983

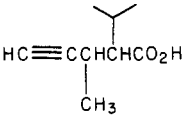
In recent years considerable interest has been shown in insecticidal esters derived from 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (1). Cleavage of the



1



2



3

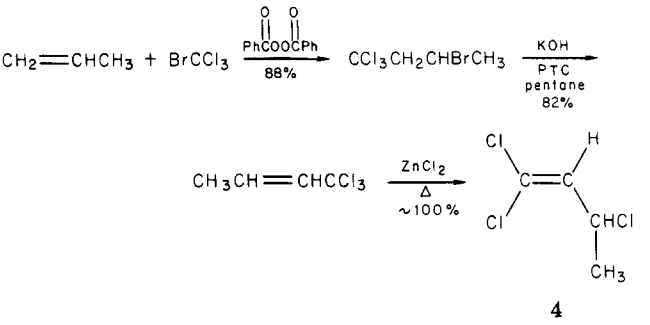
C_2–C_3 bond in 1 yields 5,5-dichloro-2-(1-methylethyl)-4-pentenoic acid (2). In this paper we report the synthesis, by direct allylation of O-silylated ketene acetals, of several esters of acids related to 2 but bearing one or two alkyl groups at C_3. We also report the synthesis of an ester of the 4-alkynoic acid 3 using a similar procedure.[1]

The alkylation of O-silylated ketene acetals has been shown to proceed efficiently with halides that can readily form a stablized carbonium ion in the presence of the Lewis acid catalyst. Thus, *tert*-butyl,[2] secondary benzyl,[3] prenyl,[3] methoxymethyl,[3] and 2-norbornyl halides[4] all give good yields when condensed with O-silylated ketene acetals at room temperature in the presence of a Lewis acid catalyst. However, primary and secondary halides do not undergo reaction with these ketene acetals even under forcing conditions.[5] The success of the alkylation procedure is thus quite dependent upon the halide.

It has been found that 1,1-dichloroallyl halides (with at least one alkyl substituent at C_3) will react smoothly under Lewis acid catalysis with O-silyated ketene acetals to give esters of the desired acid (Table I). The O-silylated ketene acetals were prepared from known or commercially available esters by using published procedures.[6]

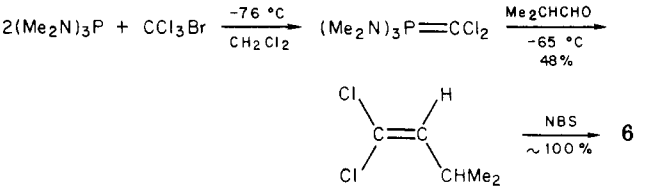
(1) Biological activity of certain esters of these acids will be reported elsewhere.
(2) Reetz, M. T.; Schwellnus, K. *Tetrahedron Lett.* **1978**, 1455.
(3) Paterson, I. *Tetrahedron Lett.* **1979**, 1519.
(4) Reetz, M. T.; Sauerwald, M.; Walz, P. *Tetrahedron Lett.* **1981**, *22*, 1101.
(5) Under the conditions reported in this paper, isopropyl iodide would not react with the ketene acetals.

Scheme I



4

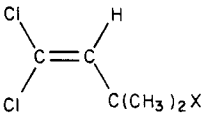
Scheme II



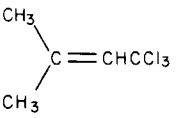
The 1,1,3-trichloro-1-butene (4) required in the synthesis of several of the esters in Table I was prepared as shown in Scheme I.

The use of KOH/pentane with a phase-transfer catalyst for the dehydrobromination of 1,1,1-trichloro-3-bromobutane resulted in twice the yield of 1,1,1-trichloro-2-butene reported in the literature.[7] The latter compound was quantitatively isomerized at room temperature to the desired allylic halide 4 by using a catalytic amount of zinc chloride.

The halide 5 required in the synthesis of several of the desired esters is reported to have been synthesized by refluxing 1,1,1,3-tetrachloro-3-methylbutane with anhydrous ferric chloride in benzene.[8] We found that these conditions did not produce 5 but gave a mixture of 4,4,4-trichloro-2-methyl-1-butene, 1,1,1-trichloro-3-methyl-2-butene, and starting material.



5, X = Cl
6, X = Br



7

A possible precursor to 5 is 7, which has reportedly been synthesized by dehydrohalogenation of 1,1,1-trichloro-3-bromo-3-methylbutane with KOH/ethanol at 0 °C.[7] We found that these conditions gave a mixture of 7 and 4,4,4-trichloro-2-methyl-1-butene. This mixture could not be readily separated by fractional distillation.

The allylic bromide 6 was synthesized as shown in Scheme II. The 1,1,3-trichloro-1-pentene shown in Table I was synthesized by a variation of Scheme I in which 1-butene and carbon tetrachloride were the starting materials.

The synthesis of 1,1-dichloro-3-methyl-1-butene was accomplished via a slight modification of a literature procedure for preparing 1,1-dihaloalkenes.[9]

It has also been found that secondary propargyl halides will alkylate O-silylated ketene acetals. This provides a

(6) Ainsworth, C.; Chen, F.; Kuo, Y. N. *J. Organomet. Chem.* **1972**, *46*, 59.
(7) Nesmeyanov, A. N.; Freidlina, R. K.; Zakharkin, L. I.; Belayvskii, A. B. *Zh. Obschi. Khim.* **1956**, *26*, 1070; *Chem. Abstr.* **1956**, *50*, 16658*i*.
(8) Topchiev, A. V.; Bogomolova, N. T.; Gol'dfarb, Y. Y. *Dokl. Akad. Nauk. SSSR* **1956**, *107*, 420; *Chem. Abstr.* **1956**, *50*, 14503*e*.
(9) Salmond, W. G. *Tetrahedron Lett.* **1977**, 1241.

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