Table I. Lipase-Catalyzed Hydrolyses of (±)-**1**

lipase	pH	time, h	ster pref	c[7]	ee[6] acid	ee[6] ester	E[7]
Mucor meihei[a]	8	22	*R*	0.489	94	90	101
Pseudomonas sp.[b]	8	40	*R*	0.508	90	93	65
Candida cylindracea[c]	8	44	*R*	0.560	74	94	23
Porcine pancrease[d]	8	87	*S*	0.111	32	4	2

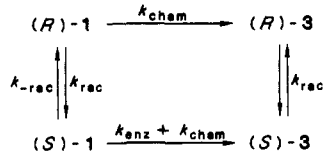
[a] Amino, MAP. [b] Boehringer, Mannheim. [c] Meito-Sangyo Ltd., OF-360. [d] Sigma Type II.

Table II. Protease-Catalyzed Hydrolyses of (±)-**1**

protease	pH	time, h	ster pref	c[7]	ee[6] acid	ee[6] ester	E[7]
Streptomyces griseus[a]	8	74	*S*	0.500	>96	>96	>100
Aspergillus saitoi[b]	6	312	*R*	0.455	96	80	>100
Bacillus subtilis[c]	8	74	*S*	0.392	96	62	97
Aspergillus oryzae[d]	8	74	*S*	0.578	70	>96	≥21

[a] Sigma Type XXI. [b] Sigma Type XIII. [c] Amano, Protease N. [d] Amano, Protease 2A.

Scheme I. Proposed Competing Reaction Pathways



pecific hydrolysis of (±)-**1**. Among some 15 lipases examined, several of these were found to be highly enantiospecific (Table I) but, unfortunately, they all preferentially cleaved the (+)-*R* ester (**1**). The only exception was the porcine pancreatic lipase (PPL), which exhibited low enantiospecificity (E = 2). This rather unexpected stereochemical outcome prompted us to continue our search for a suitable enzyme among the proteases. It is gratifying to note that in contrast to the lipases, most of the proteases have the desired *S* stereochemical preference. Some of these are listed in Table II. Particularly noteworthy is the protease of *Streptomyces griseus*, which exhibited very high enantiospecificity (E = >100). The availability of an enzyme with the required stereochemical features allowed us to proceed with the design of the in situ racemization experiment.

Our initial experiments were conducted in organic–aqueous biphasic media with trioctylamine as the base. Although complete conversion was achieved, the ee of the product was low and variable (ranging from 20% to 70%). This observation led us to induce the racemization in a purely aqueous alkaline medium, for the *S. griseus* protease is known to be stable at alkaline pH.

To achieve the desired objective, the following competing reaction kinetics (Scheme I) must be considered for the delineation of the precise experimental coonditions: (a) the rate of racemization (k_{rac}) should proceed faster than the rate of chemical hydrolysis (k_{chem}); (b) enzymatic hydrolysis (k_{enz}) of (*S*)-**1** should be much faster than racemization (k_{-rac}); (c) racemization of the acid ($k_{rac'}$) should not occur.

When (±)-**1** was exposed to *S. griseus* protease at pH 9.7, it was completely transformed to the acid (*S*)-**3** (ee = 0.76).[6] The 12% of the (*R*)-**3** formed is the result of both racemization ($k_{rac'}$) of (*S*)-**3** and competing nonenzymic hydrolysis (k_{chem}) of (*R*)-**1**, which indicates a low k_{rac}/k_{chem} ratio. While we made no attempts to suppress $k_{rac'}$ (e.g., by the precipitation of (*S*)-**3** as a salt), we were able to decrease k_{chem} or increase the ratio k_{rac}/k_{chem} by the use of the ethyl ester (±)-**2** as the substrate. The following procedure, developed after much experimentation, is presented to illustrate the feasibility of this catalytic process and its suitability for large-scale use: to 145 mg (0.51 mmol) of (±)-**2**, suspended in 35 mL of 0.2 M carbonate (HCO_3/NaOH) buffer (pH 9.7), was added 22 mg of *S. griseus* protease. The resulting suspension was stirred for 24 h at 22 °C. The reaction mixture was then extracted with CH_2Cl_2 to remove traces of remaining ester. Acidification of the aqueous layer to pH 2.0 resulted in the precipitation of (*S*)-**3**. The white precipitate was extracted into CH_2Cl_2. after the solution was dried over Na_2SO_4, the solvent was evaporated in vacuo to give 120 mg (92%) of (*S*)-**3** (ee = 0.85).[6] One crystallization (88 mg) from ethyl acetate–hexane (1:5) afforded 68 mg of (*S*)-(−)-**3** (ee = 0.94); $[\alpha]^{23}_D$ = −165° (*c* 0.85, MeOH).

It is noteworthy that the final step in the synthesis of Ketorolac[4] is the nonenzymic hydrolysis of its ester to yield the racemate (±)-**3**. In contrast, enzymatic second-order asymmetric hydrolysis of (±)-**2** allows one to obtain the eutomer[8] (*S*)-(−)-**3** of high optical purity at comparable chemical yields.

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(6) The enantiomeric excess (ee) of optically active **3** was determined by ^{1}H NMR measurement of its methyl ester in the presence of Eu(hfc)$_3$.

(7) The enantiomeric ratio (E value) is calculated from $E = [\ln [(1 - c)(1 - ee_s)]]/[\ln [(1 - c)(1 + ee_s)]]$, where $c = ee_s/(ee_s + ee_p)$. See ref 1 for a detailed discussion of these calculations.

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Nuclease Activity of 1,10-Phenanthroline–Copper Ion. Chemistry of Deoxyribose Oxidation

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The 1,10-phenanthroline–cuprous complex with hydrogen peroxide as a coreactant makes single-stranded breaks in B DNA in an oxidative reaction which is sequence dependent but proceeds at deoxyribose residues linked to any of the four bases, adenine, guanine, cytidine, and thymine.[1] The reaction efficiency at any sequence position depends on the stability of the $(OP)_2Cu^+$–DNA complex (step a of eq 1) adjacent to the deoxyribose attacked by the reactive copper–oxo species (I).[2,3]

$$(OP)_2Cu^+ + DNA \xrightarrow{\text{step a}} (OP)_2Cu^+\text{–DNA} \xrightarrow{H_2O_2} \underset{\text{I}}{(OP)_2Cu^{2+}\text{–oxo–DNA}} + OH^- \rightarrow \text{oligonucleotide products} \quad (1)$$

The proposed reaction scheme presented in Scheme I has been based on the analysis of products of the nucleolytic activity using uniformly labeled poly(dA-T),[4] 5′- and 3′-labeled restriction fragments,[5] and the self-complementary dodecamer 5-^{32}P-CGCGAATTCGCG as substrates.[6] These studies demonstrated the generation of free bases and 3′- and 5′-monophosphate ester termini. With the 5′ ^{32}P-labeled dodecamer, 3′-phosphoglycolates could be detected in minor amounts which varied with sequence position. In addition, an intermediate was trapped which did not comigrate with any known standards and converted to the 3′-phosphate upon treatment with piperidine or storage at −20 °C. In this paper, we report the isolation of 5-methylene-2-5*H*-furanone

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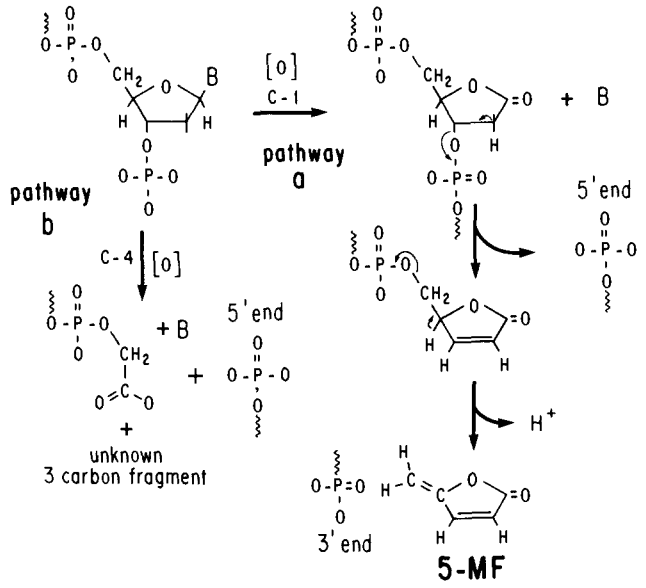
Scheme I



(5-MF),[7-9] the deoxyribose derivative predicted in the formation of 3′-phosphomonoester termini, the major pathway of the reaction.

For the isolation of 5-MF, the essential coreactants, $(OP)_2Cu^+$ and H_2O_2, were generated by superoxide produced by cobalt-60 γ radiolysis[10] in Tris buffer containing $(OP)_2Cu^{2+}$, O_2, and the target DNA. Superoxide accomplishes the formation of $(OP)_2Cu^+$ by the one-electron reduction of the cupric complex and the production of H_2O_2 by its spontaneous dismutation.[11] Under these conditions, hydroxyl radicals are scavenged by Tris, and DNA scission in the absence of $(OP)_2Cu^{2+}$ or O_2 is not significant. An authentic sample of 5-MF is stable to these reaction conditions. In contrast, 5-MF does not survive our usual method of potentiating the nucleolytic activity which involves adding thiol to an aerobic solution of $(OP)_2Cu^{2+}$.

The use of ^{32}P poly(dA-T)[4,12] as the substrate provides an approach to assess the yield of 5-MF per single stranded break. Subsequent to digestion of the synthetic alternating copolymer by 1,10-phenanthroline–copper in the presence of γ radiation as described in the legend to Figure 1, the reaction mixture was heated at 90 °C for 1 h to ensure the quantitative conversion of the intermediate to 3′-phosphomonoester and 5-MF and then divided into two aliquots. To assess the number of single-stranded breaks, one aliquot was digested with alkaline phosphatase and the ^{32}P-inorganic phosphate was extracted by 1:1 isobutanyl alcohol/benzene as the molybdate complex.[13] The other aliquot was then analyzed for 5-MF by using HPLC (see Figure 1 for further details). The chromatogram revealed a product which migrated identically with an authentic sample of 5-MF. The yield of 5-MF measured by this method was approximately 0.5 mol per mol of phosphate released or 1 mol of 5-MF per single-stranded break. This value is the upper limit of the yield due to the possibility of incomplete phosphatase hydrolysis. Since pathway B produces 1 mol of hydrolyzable phosphate and phosphoglycolate termini but no 5-MF, a reduced yield of 5-MF would be consistent with the proposed chemistry.

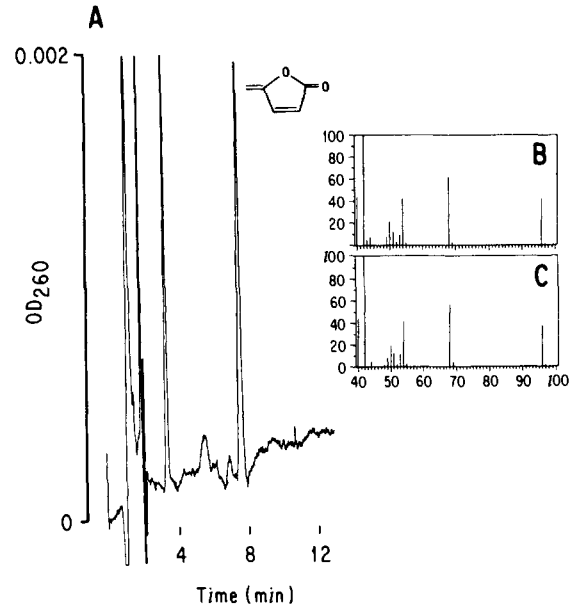


Figure 1. Product analysis by HPLC and mass spectroscopy. (A) Reaction mixtures (1200 μL) containing ^{32}P poly(dA-T), 25 μg/mL; 1,10-phenanthroline, 170 μM; $CuSO_4$, 38 μM; NaCl, 25 mM; and Tris-HCl, 50 mM pH 7.0 were exposed to 30 000 rad of ^{60}Co radiation (approximately 1000 rad/min). 80% the radioactive label was rendered acid soluble.[17] The sample was heated at 90 °C for 1 h and then stored at −20 °C until HPLC analysis. Up to 600 μL was injected per run. Separation was achieved on an Alltech Econosphere C-18 column (5 μm, 4.6 × 0.250 mm) eluted with 4% CH_3CN/96% H_2O at 2 mL/min. The column is protected with an Upchurch precolumn filled with E. Merck Perisorb RP-18. Detection is at 260 nm. A product with an identical retention time as an authentic sample of 5-MF was observed. (B) Mass spectrum of an authentic sample of 5-MF following gas chromatography. (C) A reaction mixture with concentrations identical with (A) but using calf thymus DNA was analyzed by GC/MS.[13] A product with an identical gas chromatographic retention time as authentic 5-MF yielded the 70-eV mass spectrum presented.

The isolation of 5-MF was further confirmed by digesting calf thymus DNA with 1,10-phenanthroline–copper ion and subjecting a CH_2Cl_2 extract of the product mixture to gas chromatography/mass spectral analysis. The mass spectrum of the major component of the extract is identical with that of authentic 5-MF.[14] (Parent ion, m/e 96; see inset of Figure 1 for complete spectrum.)

The isolation of 5-MF as the major deoxyribose oxidation product of the nucleolytic activity of 1,10-phenanthroline–copper strongly supports the reaction pathway outlined in Scheme I

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(13) The yield of 5′- and 3′-phosphomonoesters for cleaved [^{32}P]poly(dA-T) was determined by using alkaline phosphatase in the following manner. Digests (1200–1300 μL) were prepared as described in the legend of Figure 1 by using [^{32}P]poly(dA-T) (3.31×10^5 cpm/μg); 80% of the radioactive label was rendered acid soluble. Following irradiation, the samples were heated in order to decompose any metastable intermediates. Samples which were to be analyzed for phosphate were divided pairwise into 135-μL aliquots. Bacterial alkaline phosphatase (0.2 unit, Worthington LS0005129) was added to one aliquot and an equal volume of water to the other. The aliquots were then heated at 65 °C for 15 min. Another 0.2 unit of bacterial alkaline phosphatase was added and incubation continued for an additional 15 min. One hundred microliters of each sample was then diluted and acidified by the addition of 80 μL of 5 M HCl and 220 μL of water. The inorganic phosphate was then analyzed by using the molybdic acid method of: Hackney, D.; Stempel, K. E.; Boyer, P. D. *Methods Enzymol.* **1980**, *64* 60–83.
(14) Duplicate 500-mL samples were prepared with the same composition as described in the legend for Figure 1 with the exception that calf thymus DNA replaced poly(dA-T). One sample was exposed to 30 000 rads of ^{60}Co radiation approximately 1000 rad/min). The other sample was allowed to stand at 25 °C for the same amount of time. Both samples were heated at 90 °C for 2 h, treated with 0.425 mmol of $ZnCl_2$ (to complex the excess 1,10-phenanthroline) and 25 g of NaCl, and then extracted with three 30-mL portions of dichloromethane. The solvent was stripped from the combined extracts and the residue taken up in 75 μL of acetonitrile and stored at −70 °C. GC/MS analysis was carried out with a Perkin-Elmer Sigma 3 gas chromatograph interfaced to a Kratos MS-25 mass spectrometer. The GC was fitted with a capillary column (30 mm × 0.25 mm i.d.) coated with DB-5 (0.25-μm film thickness). Isothermal separation was carried out at 80 °C; the injector and interface were maintained at 150 °C. Mass spectra were taken at 70 ev. See insert of Figure 1 for spectra. There was no peak in the gas chromatography for the control sample (nonirradiated).

leading to 3'-phosphomonoester termini. Since the hydrogen atom of C-1 is in the interior of the minor groove of B DNA, the coordination complex must react with B DNA from a binding site within the minor groove. The sequence-dependent reactivity observed with biologically functional DNA therefore reflects a conformational variability of this structural domain.[15] Possibly the 3'-phosphomonoester formed by hydroxyl radicals generated chemically[16,17] or cobalt-60 γ radiation is produced via a parallel mechanism.[18]

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Superconductivity above Liquid Nitrogen Temperature: Preparation and Properties of a Family of Perovskite-Based Superconductors

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Over the last decade, the search for high-temperature superconducting materials remained virtually stagnant. This situation changed radically with the discovery of Bednorz and Müller[1] of superconductivity above 30 K in a layered perovskite oxide composed of La, Ba, and Cu. Improvements in the superconducting transition temperature (T_c) to ~45 K by Sr substitution and identification of the phase responsible for superconductivity $(La_{2-x}Ba(or\ Sr)_xCuO_y$ where x is typically between 0.1–0.3)[2-6] followed rapidly. The next major advance was immediate and dramatic. Wu, Chu, and co-workers reported[7] a new material based on the starting composition $Y_{1.2}Ba_{0.8}CuO_y$ with T_c well above 90 K. Nearly simultaneous reports by other groups[8,9] confirmed these results. The Y–Ba–Cu material was a mixture of several unidentified phases and only a small fraction of the sample actually was superconducting. Recently, the structure of this new superconductor was identified as an oxygen-defect perovskite corresponding to the composition $Y_1Ba_2Cu_3O_y$.[10-12]

In this paper, we report on the synthesis of single-phase $Y_1Ba_2Cu_3O_y$ and show how the preparation conditions play a dramatic role in determining the superconducting properties. Also,

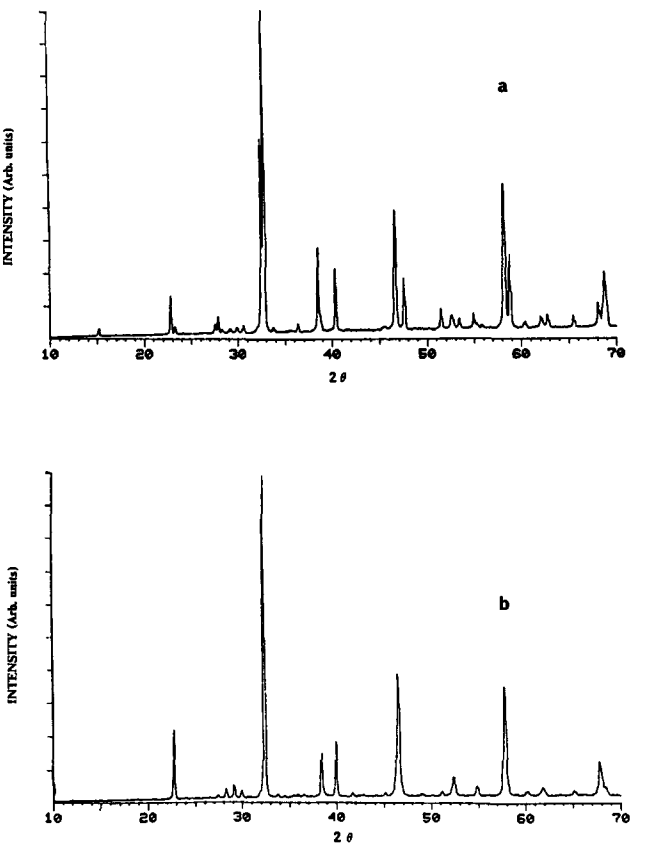

Figure 1. X-ray powder diffraction patterns for (a) $Y_1Ba_2Cu_3O_y$ and (b) $Pr_1Ba_2Cu_3O_y$.

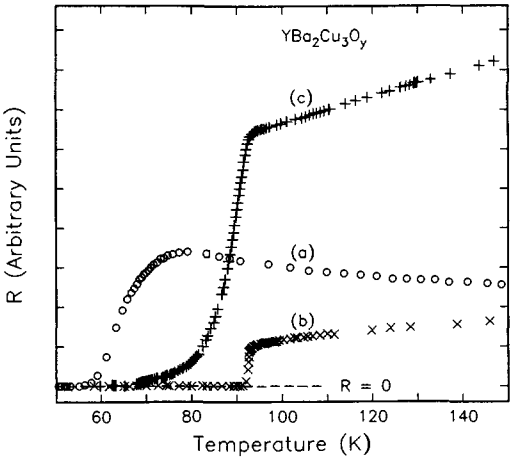

Figure 2. Plot of four-probe electrical resistivity vs. temperature for $Y_1Ba_2Cu_3O_y$ under various preparative conditions: (a) fast removal of pellets from oxygen anneal at 900 °C; (b) slow cooling of oxygen annealed sample from 900 to 200 °C over 5 h; (c) same as (b) except air anneal.

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