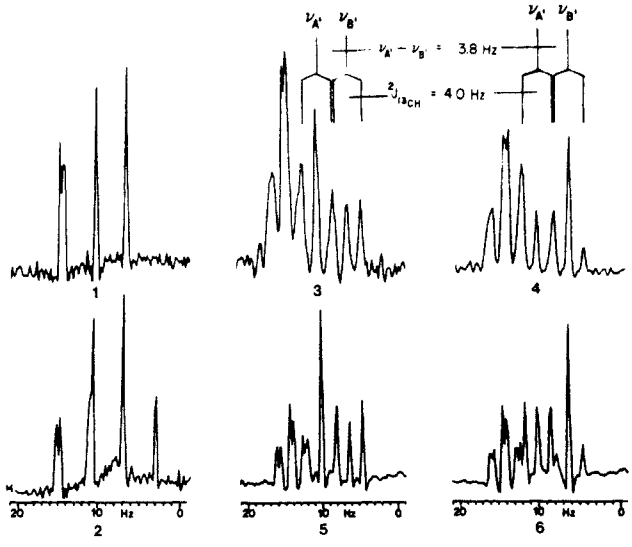

Figure 1. ^1H NMR signal for the C-2 protons of [1,1,2,3,3-$^2H_5$]-propane-1,3-diol mono-*O*-acetyl-D-mandelate observed with broad-band deuterium decoupling. (1) Product from (*R*)- or (*S*)-malonate with broad-band ^{13}C decoupling; (2) product from (*R*)- or (*S*)-malonate without ^{13}C decoupling; (3) product from (*S*)-malonate with selective ^{13}C decoupling of upfield (57.96 ppm) resonance; (4) product from (*S*)-malonate with selective ^{13}C decoupling of downfield (62.05 ppm) resonance; (5) product from (*R*)-malonate with selective ^{13}C decoupling of downfield resonance; (6) product from (*R*)-malonate with selective ^{13}C decoupling of upfield resonance.

CH_3CN/H_2O 3:7) and subjected to proton NMR analysis (solvent benzene-d_6, 7.1 T, Bruker WM 300). The different species present in the sample and the relevant NMR parameters are shown in Chart I. Since the esterification does not discriminate between the two hydroxymethyl groups of 1,3-propanediol, each pure enantiomer of malonate will give two species of the monester; i.e., (*R*)-malonate gives **1** + **2** and (*S*)-malonate **3** + **4**. Exchange of the malonate will also produce some diprotio species, **5** + **6** by C-D bond cleavage, and some monoprotio species of opposite configuration, **3** + **4** from (*R*)-malonate and **1** + **2** from (*S*)-malonate, by C-H bond cleavage. Figure 1 displays the ^1H NMR signal for the C-2 protons of the propanediol moiety. Upon broad-band decoupling of both ^{13}C and deuterium, species **5** + **6** show the center part of an AB system[12] and species **2** + **3** and **1** + **4** give single resonances 3.8 Hz apart which are deuterium-shifted 6.0-Hz upfield from the resonance frequencies of the diprotio species. Upon removal of the ^{13}C decoupling, each of these resonances splits into two due to the 4.0-Hz ^{13}C-^1H two-bond coupling. Sets **1** + **2** and **3** + **4** can be distinguished by ^2H broad-band decoupling, with simultaneous single-frequency ^{13}C decoupling, of either the upfield or the downfield ^{13}C-enriched carbon. Irradiation of C-1 (downfield) leaves H_A' of **1** and H_B' of **3** as doublets but collapses H_B' of **2** and H_A' of **4** into singlets. The intensity ratio of these two singlets gives the ratio of species **1** + **2** to **3** + **4** in the sample and hence the minimum ratio of *R*/*S* isomers in the original malonate. The opposite pattern was observed when the C-3 (upfield) ^{13}C resonance was selectively irradiated.

Analysis of the spectra showed that the sample from (*R*)-malonate contained **1** + **2** and **3** + **4** in a 2:1 ratio, indicating at least 34% ee *R* isomer in the original malonate. The ratio for the (*S*)-malonate-derived material was 1:2 of **1** + **2** and **3** + **4**, corresponding to 34% ee *S* isomer. These are minimum values, because the derivatization procedure involves considerable exchange and racemization, as evidenced by the presence of 24% diprotio species in the propanediol monoester. From the measured exchange in the oxidation of unlabeled malate in D_2O, less than 10%,[8] and the deuterium enrichment of the starting malate, it follows that the chiral purity of the original dipotassium malonate samples is greater than 80% ee.

The work reported here demonstrates the feasibility of generating chiral versions of malonic acid of good enantiomeric purity. It sets the stage for stereochemical studies on the biosynthesis of polyketides, provided conditions can be worked out for the incorporation of these substrates into products without complete obliteration of the stereochemical information by exchange. Work is under way to try to implement such applications.

Acknowledgment. We are indebted to the Purdue University Biochemical Magnetic Resonance Laboratory (supported by NIH Grant RR01077) and The Ohio State University Chemical Instrument Center for NMR and mass spectral measurements, to the Los Alamos Stable Isotope Resource (supported by NIH Grant RR02231) for labeled compounds, and to the National Science Foundation (Grant CHE-8408412) for financial support. We thank the National Institutes of Health for a postdoctoral fellowship (GM 10207 to J.B.). This project was initiated while the senior author was a visitor in the laboratory of Professor H. Simon at the Technical University of Munich under a Humboldt Senior Scientist Award. He thanks Prof. Simon for his hospitality and stimulating discussions and the Humboldt Foundation for financial support.

(12) The ^2H-broad-band decoupled ^1H NMR spectrum of the (*S*)-(+)-*O*-acetyl-D-mandelate monoester of [1,1,3,3-$^2H_4$]propane-1,3-diol displays a 0.6-Hz doublet for the methylene protons. These are the center two lines of an AB quartet; the outer two lines were too weak to be observed. The separation between the center lines is predicted to be $|((\nu_A - \nu_B)^2 + J^2)^{1/2} - J|$ (Becker, E. D. "High Resolution NMR"; Academic Press: New York, 1969; pp 135-138). Since $(\nu_A - \nu_B)$ was measured with the chiral samples, $^2J_{HH}$ could be calculated to be 12 Hz.

Flavin-Catalyzed Reductive Dioxygen Activation with *N*-Methyldihydronicotinamide

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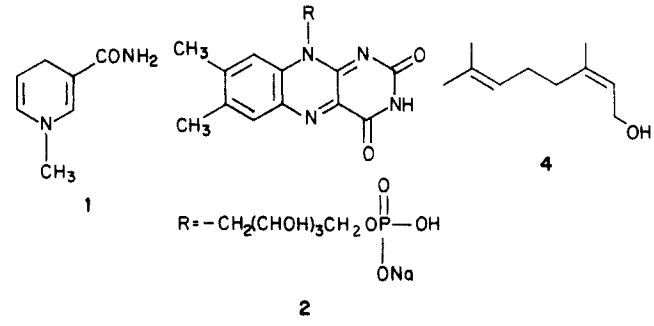
Mechanistic studies[1] and synthetic application of the P-450 type reaction[2] are currently attracting the interest of chemists. Surprisingly, however, components used for artificial O_2 activation systems have little structure similarity to those in the native enzyme systems: native, protoporphyrin-Fe/NADH/FAD·FMN/cys-S$^-$, O_2 (lipoic acid); artificial, TPPS·Mn/H_2/colloidal Pt/N-MeIm, O_2 (benzoic anhydride).

We now wish to report that MeNAH (**1**), a NADH analogue,



efficiently activates O_2 in the presence of FMN (flavin mononucleotide) (**2**) and TPPS·Mn (tetraphenylporphyrintetra-

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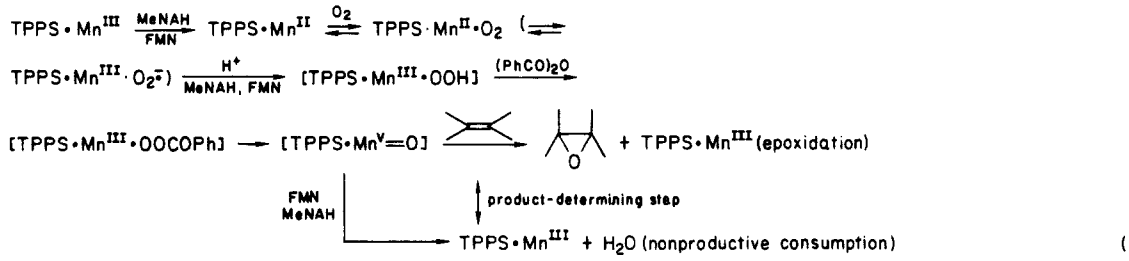

Table I. Rate Constants in the Artificial O_2 Activation System[a]

system	rate constant, M^{-1} s^{-1}
MeNAH + O_2[b]	0.00035 (s^{-1})
Mn^{III}•TPPS + MeNAH[c]	0.1
Mn^{III}•TPPS + MeNAH[d] (catalyzed by FMN)	160
MeNAH + O_2 (catalyzed by FMN)	10.3[e]

[a] In 20 mL of H_2O–EtOH (1:1 v/v) at 25 °C, pH 7.4 (phosphate). [b] MeNAH 60 mg, O_2 1 atm, *N*-MeIm 200 mg, and cyclohexene 150 mg; decrease of MeNAH was followed at 363 nm. Product ratio of hydrated MeNAH to $MeNA^+$ was determined by ^1H NMR to be less than 0.1. [c] Mn^{III}•TPPS 7.8 × 10^{-6} M, MeNAH 0.21 M, *N*-MeIm 0.12 M, decrease of Mn^{III}•TPPS was followed at 469 nm. [d] Pseudo second order with respect to Mn^{III}•TPPS and MeNAH; Mn^{III}•TPPS 7.8 × 10^{-6} M, MeNAH (0.22–2.2) × 10^{-2} M, FMN 1.0 × 10^{-4} M, and *N*-MeIm 0.12 M; increase of Mn^{II}•TPPS was followed at 616 nm. [e] Second order with respect to MeNAH and FMN; 1 atm O_2, 25 °C, pH 7.4.

sulfonate–Mn complex) (**3**), to give epoxides from olefins. Regioselective (>90% at C_6,C_7-terminal) epoxidation takes place for nerol (**4**). The system is one of the most efficient artificial O_2 activation systems and may help shed some light on the mechanism by native P-450 enzyme systems.

Reduction of TPPS•Mn^{III} to TPPS•Mn^{II} with MeNAH proceeds smoothly in H_2O–EtOH (1:1, v/v) at 25 °C, pH 7.4, with a second-order rate constant of (1.0 ± 0.05) × 10^{-1} M^{-1} s^{-1}. On the other hand, the direct reduction of O_2 with MeNAH takes place only much more slowly with second-order rate constant of ca. 3.5 × 10^{-3} M^{-1} s^{-1} (see Table I).

Flavin in P-450 reductase is known to serve as an efficient catalyst for transport of electron from NADH to porphyrin–Fe in the native P-450.[3] Catalytic activity of FMN for the conversion from TPPS•Mn^{III} to TPPS•Mn^{II} was investigated (see Table I). The Mn^{III} → Mn^{II} conversion is the slowest step in the reductive O_2 activation cycles.[1c] Acceleration of the Mn^{III} reduction by factor of 1600 was observed when FMN was present in 1.0 × 10^{-4} M concentration. Addition of FMN to the O_2 activation system consisting of $TPPS_4$•Mn, *N*-MeImd, and MeNAH gave the artificial total O_2 activation system, where structure of each component is very close to that of the native P-450 system: native, P•Fe^{III}•SR, flavoprotein, NADH, acid, O_2; artificial, P•Mn^{II}•NMeImd, FMN, MeNAH, $(PhCO)_2O$ (or acid), O_2.

Satisfactory epoxide formation from olefins was observed with good turnover frequencies and numbers (see Table II) by use of the MeNAH/FMN electron transport system. Thus, as a typical example, 2 mg of Mn^{III}•$TPPS_4$, 200 mg of *N*-methylimidazole, 150 mg of cyclohexene (or 1.00 g of nerol), and 10 mg of FMN were dissolved in 20 mL of aqueous EtOH (1/1 vol/vol) buffered at pH 7.4 with phosphate. Into the solution saturated with O_2 were added 226 mg of benzoic anhydride and 60 mg of MeNAH. The mixture was vigorously stirred at 25 °C under O_2 and the reaction mixture was analyzed by GLC. The results are summarized in Table II. In EtOH–H_2O (1:1 v/v) at 25 °C (Table II) the artificial system showed both large turnover frequencies of 3.6 and 9.0 (mol of product/mol of Mn•TPPS) min^{-1} and turnover number of 60 and 260 (mol of product/mol of TPPS•Mn employed) for the epoxidation of cyclohexene and nerol, respectively. These values are among the best reported for artificial O_2 activation systems. Epoxides were major products (60.1–58.0 mol/mol) from olefins and allylic oxidation took place only ca. 6 mol/mol.

We have found out, interestingly enough, that the similar total artificial systems containing an Fe center instead of a Mn center displayed much lower turnover of 0.80 min^{-1}.

The most significant characteristic of the present MeNAH–FMN–TPPS•Mn system is the favorable ratio of epoxide formation relative to undesirable nonproductive consumption of the oxene intermediate in the product-determining step[1c] (eq 1). The observed rate constant ratio, $r = k$(epoxide formation)/k(oxene nonproductive consumption) was 0.03 or MeNAH consumption ratio, $v_{epoxide}/v_{nonproductive}$, was 0.50 under the present conditions (which gives chemical yield of the epoxide based on MeNAH to be 33%) for the present artificial P-450 system in H_2O–EtOH (1:1, v/v) at 25 °C, where v's are overall rate under the conditions. In contrast in our previous total system consisting TPPS•Mn–*N*-MeImD–col Pt–H_2–O_2, observed r value was considerably smaller: ca. 10^{-7} under the corresponding conditions (or H_2 consumption ratio, v/v, 0.057). Improvement of r and v/v values may be due to very facile transfer of a second electron (to Mn^{II}•O_2) from MeNAH held within the first solvent cage. This occurs after first rate-determining electron transfer (converting Mn^{3+} to Mn^{2+}) had taken place. Further (third etc.) electron transfer (leading to nonproductive oxene consumption) may not be so rapid. It requires further collision with another MeNAH molecule in outer solvent sphere.

Table II. Epoxide Formation by an Artificial O_2 Activation System[b,f]

substrate, 10^3 mol	M•TPPS, 10^6 mol	MeNAH, 10^4 mol	FMN, 10^5 mol	$(PhCO)_2O$, 10^3 mol	reaction time, min	total amount of products, 10^6 mol	turnover number[a]	products (% ratio)	
nerol, 6.5	Mn, 1.8	4.3	2.0	1.0	5	81	45	6,7-epoxide (ca. 90)	2,3-epoxide (ca. 10)
nerol, 6.5	Mn, 1.8	21[c]	2.0	5.0[c]	120	470	260[d]	6,7-epoxide (90)	2,3-epoxide (10)
cyclohexene, 1.8	Mn, 1.8	4.3	2.0	1.0	5	31	17.5[e]	epoxide	cyclohexenone (10)
cyclohexene, 1.8	Mn, 1.8	21[c]	2.0	5.0[c]	120	110	60	epoxide (90)	cyclohexenone (10)
cyclohexene, 1.8	Fe, 1.8	4.3	2.0	1.0	5	7.2	4.0	epoxide (90)	cyclohexenone (10)

[a] Mol of products formed/mol of M•TPPS employed. [b] *N*-MeIm 2.4 × 10^{-3} mol, H_2O 10 mL, EtOH 10 mL, pH 7.4 (phosphate), O_2 1 atm, 25 °C. [c] The reaction started with MeNAH 4.3 × 10^{-4} mol, $(PhCO)_2O$ 1.0 × 10^{-3} mol; after every 15 min., MeNAH 2.1 × 10^{-4} mol and $(PhCO)_2O$ 0.5 × 10^{-3} mol were further added. [d] 86% of Mn•TPPS remained after 260 turnovers. [e] 86% of Mn•TPPS remained after 17.5 turnovers, indicating much lower reactivity of cyclohexene than nerol. [f] Any system lacking one or more component(s) did not show P-450 type activity, except the system where $(PhCO)_2O$ is replaced by acid.

If correct, this mechanism would suggest the chemical significance of a NADH-flavin couple in the native P-450 system.[4]

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Electrophilic Glycinates: New and Versatile Templates for Asymmetric Amino Acid Synthesis

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The number of naturally occurring α-amino acids has grown substantially beyond the roughly 20 amino acids normally found in proteins; over 500 are now known.[1] In addition, there has been a tremendous surge of interest in the asymmetric preparation of relatively inaccessible unnatural amino acids whose potential biological properties and general synthetic utility are just beginning to be realized. Of the methods presently available, there is a general lack of access to optically pure α-monosubstituted α-amino acids and derivatives in both the D and L configuration. Several groups have recently reported the asymmetric alkylation of amino acid derived enolates[2] to furnish α-disubstituted amino acids and, in one approach,[3] the enolates of optically active lactim ethers of diketopiperazines furnishes the α-monosubstituted α-amino acids. The more classical approaches involving the asymmetric hydrogenation of prochiral dehydro amino acid derivatives[4] or hydrogenation of chiral dehydro amino acid derivatives[5] suffer from the range of substitution accessible on the α-"R" group and the variations in the percent asymmetric synthesis (i.e., % ee). In this preliminary account, we wish to report a new and general method for preparing both D- and L-α-monosubstituted α-amino acids via C-C bond-forming reactions on *electrophilic* glycinates[6] that is complementary to the existing enolate-based methodologies.

According to Tischler et al.,[7] D,L-*erythro*-α,β-diphenyl-β-hydroxyethylamine is efficiently resolved on large scale through the agency of the derived glutamate salts to furnish both optically pure antipodes of **1**.[7] Sequential N-alkylation with ethyl bromoacetate (Et_3N, THF, 25 °C), Schotten-Baumann acylation (BnOCOCl, $NaHCO_3$(aq), CH_2Cl_2), and cyclization (catalytic *p*-TsOH, PhH, reflux) furnished the optically pure lactone **2** (mp 200 °C; $[\alpha]^{25}_D$ -66.7°, *c* 0.815, CH_2Cl_2) in 65% overall yield from **1**. Bromination of **2** was realized by treatment with 1 equiv of NBS in warm CCl_4 to afford after filtration of insoluble succinimide the bromide **3** as an amorphous white powder. The bromide **3** is produced in essentially quantitative yield[8] (crude, by ^1H NMR) but decomposes upon exposure to silica gel chromatography. The bromide can be stored indefinitely as a solid in the dark and is directly used for the subsequent C-C coupling reactions as described in Scheme I.

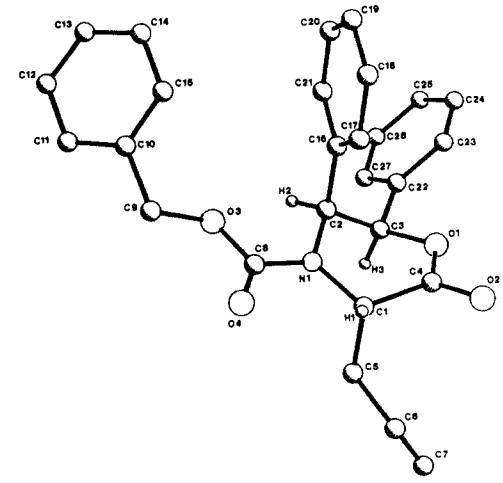
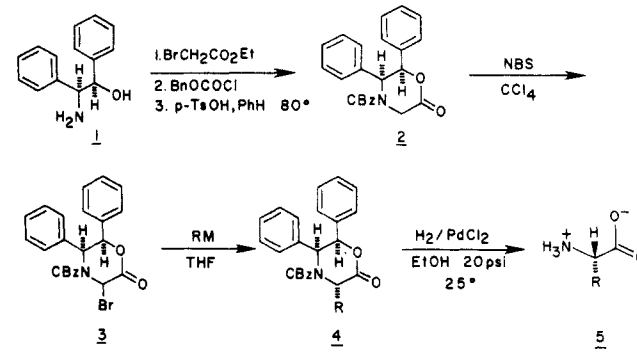


Figure 1. Molecular Structure of **4** (R = $CH_2CH{=}CH_2$). Atoms are shown as spheres of fixed, arbitrary radius.

Scheme I



The bromoglycinate **3** is a very reactive electrophile toward a variety of carbon nucleophiles; those described herein constitute a superficial initial screening and are representative of many possible extensions and variations. A typical procedure is described below for the preparation of β-ethylaspartate.

To a stirred solution of lactone **2** (0.2 g, 0.51 mmol) in refluxing CCl_4 (60 mL) was added NBS (0.11 g, 0.62 mmol). The mixture was allowed to reflux for 35 min, cooled to 0 °C, filtered, and evaporated to afford the bromide **3** as a white powder which was used directly for the next step. The bromide **3** (0.108 g, 0.23 mmol) was dissolved in THF (4 mL) and the (*tert*-butyldimethylsilyl)ketene acetal of ethyl acetate (0.11 mL, 0.58 mmol) was added followed by a solution of anhydrous $ZnCl_2$ (1.5 mL of a 0.17 N solution in THF) at 25 °C. The reaction was allowed to stir for 1 h at 25 °C, poured into H_2O, and extracted thoroughly with CH_2Cl_2. The combined extracts were dried over anhydrous sodium sulfate, filtered, evaporated, and separated on PTLC silica gel (3:1, hexanes/EtOAc) to afford 78.6 mg (71%) of the lactone **4** (R = CH_2CO_2Et). This material was dissolved in absolute EtOH (3 mL) plus THF (1 mL), $PdCl_2$ (13.2 mg) was added, and the system was flushed with H_2 and hydrogenated at 20 psi for 24 h at 25 °C. Filtration of the catalyst through Celite, concentration, and addition of Et_2O precipitates the zwitterionic amino acid (**5**, R = CH_2CO_2Et) (25 mg, quantitative) as a white powder. The percent asymmetric synthesis (% ee) on this and the other amino acids listed in the table was determined by acylating[9] the crude amino acid with (+)-α-methoxy-α-(tri-

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