A New Method for the Synthesis of Optically Active α-Amino Acids and Their N^{α} Derivatives *via* Acylamino Malonates

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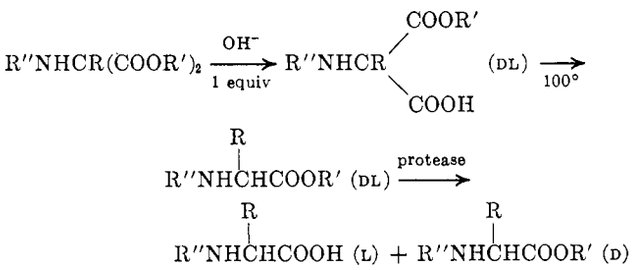
A diethyl 2-acyl(or 2-benzyloxycarbonyl)amino-2-alkyl(or 2-aralkyl)malonate is half-saponified in good yield to the DL monoester. The monoester is smoothly and quantitatively decarboxylated at 100° (*e.g.*, by refluxing in dioxane) to yield the DL-acylamino acid ethyl ester. This derivative is resolved directly by enzymic hydrolysis of the ester group (*e.g.*, chymotrypsin, subtilisin, etc.) to yield the L-acyl(or benzyloxycarbonyl)amino acid, which can be used directly for further peptide synthesis. In addition, the unchanged D derivative is obtained. Alternatively, the optically active amino acids can be recovered by total hydrolysis or by anhydrous cleavage, *e.g.*, by HBr, of their derivatives. The following compounds were synthesized by this method: *N*-acetyl-β-(*o*-methylphenyl)-L-alanine (**4**), *N*-acetyl-β-(*o*-methylphenyl)-D-alanine ethyl ester (**5**), *N*-acetyl-β-(2-naphthyl)-L-alanine (**7**), *N*-acetyl-β-(2-naphthyl)-D-alanine (**9**), *N*-acetyl-β-(2-naphthyl)-D-alanine ethyl ester (**8**), β-(6-quinolyl)-L-alanine (**13**), β-(6-quinolyl)-D-alanine dihydrochloride (**15**), *N*-acetyl-β-(6-quinolyl)-D-alanine ethyl ester (**14**), and *N*-benzyloxycarbonyl-L-phenylalanine (**20**). The preparation of dimethyl benzyloxycarbonylaminomalonate (**16**) is also described.

The malonic ester synthesis is frequently used for the preparation of α-amino acids. One of its advantages is that the intermediate substituted acylamino dialkyl malonates often crystallize well and are easily purified. Yields are generally good. Also, the necessary halogeno derivatives are in many cases easily accessible. To obtain optically active products, the racemic amino acid is usually first generated by simultaneous total hydrolysis and decarboxylation by heating in strong acid. After reacylation, the racemate is resolved enzymically by means of an acylase preparation. Resolution by means of chymotrypsin was reported after reacylation and reesterification.[2] Stereospecific enzymic synthesis (*e.g.*, of phenylhydrazides by means of papain) has also been employed.[3]

In the present paper we describe a much simpler method which converts the substituted malonic ester directly to an acylamino acid ester racemate under very mild conditions. The latter compound is then directly resolved taking advantage of the stereospecific esterase activity of proteolytic enzymes. The method is also applicable to the direct synthesis of benzyloxycarbonyl-L-amino acids (from benzyloxycarbonylaminomalonate) for use in peptide synthesis.

Results and Discussion

Diethyl acetamidomalonate and dimethyl benzyloxycarbonylaminomalonate (**16**) (prepared from commercial dimethyl aminomalonate) were used as starting materials and alkylated in the usual way with the appropriate halogen compounds. The intermediates were checked by tlc before proceeding. The N-substituted L-amino acid and D-amino acid ester were obtained by the reactions indicated in the following scheme.



Partial Hydrolysis of the Diester.—As can be seen from Figure 1, there is a very pronounced difference in the rate of saponification of the first ester group and the second. The reason for this is the electrostatic repulsion between the ionized monoester produced in the first stage of saponification and the hydroxyl ion catalyzing further saponification. Thus by controlling the reaction conditions the DL monoester can be obtained in satisfactory yield. Separation and purification of the monoester–monoacid from any residual diester is simple when there are no additional ionizable groups present; however, one may easily proceed to the decarboxylation step without purification at this stage as exemplified in the straightforward synthesis of the racemic amino acid esters **12** and **18**.

Decarboxylation of the Monoester.—Figure 2 shows the course of decarboxylation of monoethyl malonate derivative **2**, as followed by monitoring the evolution of carbon dioxide.[4] It is seen that at 100° (*e.g.*, in refluxing dioxane), without the addition of strong acid, the reaction was of first order with a half-time of about 10 min. That means that within 1 hr the reaction was practically quantitative. For complete decarboxylation of the benzyloxycarbonylaminomalonate **18** refluxing in dioxane for 24 hr was necessary. It is unknown whether this slower rate is a general behavior of benzyloxycarbonylaminomalonates. Purification is easily possible at this stage, since the product is either nonionizable altogether or at least considerably less acidic than the starting material.

Enzymic Resolution.—The decarboxylation product, being an ester of a DL-acylamino acid, serves directly as the substrate for a stereospecific esterase. A number of proteolytic enzymes available in a highly pure and stable form exhibit this type of activity. The amount of enzyme necessary for resolution at a reasonable rate is often very small. In the present study we used chymotrypsin and subtilisin.

The reaction can be followed by alkali uptake at constant pH (*e.g.*, using an automatic titration device) as shown in Figure 3. In the case of esters of low

(1) (a) Deceased November 1, 1972. (b) Recipient of a postdoctoral fellowship from the Swiss National Fund for the Advancement of Science.

(2) T. N. Pattabiraman and W. B. Lawson, *Biochem. J.*, **126**, 659 (1972).

(3) For a review see, *e.g.*, J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 1, Wiley, New York, N. Y., 1961, p 707.

(4) A. Patchornik and Y. Shalitin, *Anal. Chem.*, **33**, 1887 (1961).

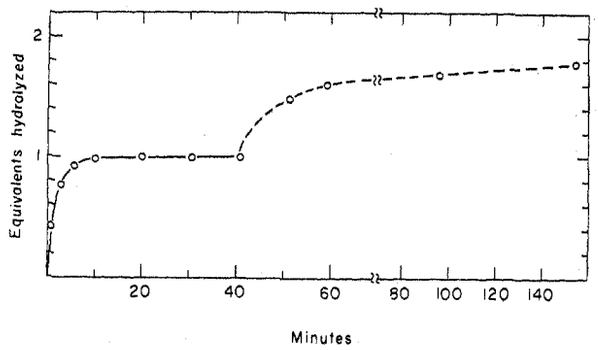


Figure 1.—The time course of partial hydrolysis of diethyl (*o*-methylbenzyl)acetamidomalonate (1) (128 mg) at room temperature in 0.75 ml of ethanol containing 0.3 ml of 3.9 *N* NaOH. Ordinate: equivalents hydrolyzed as determined in aliquots by titration with 0.01 *N* HCl of the excess base present. On heating to 80° (dotted line) the second ester group is seen to be hydrolyzed.

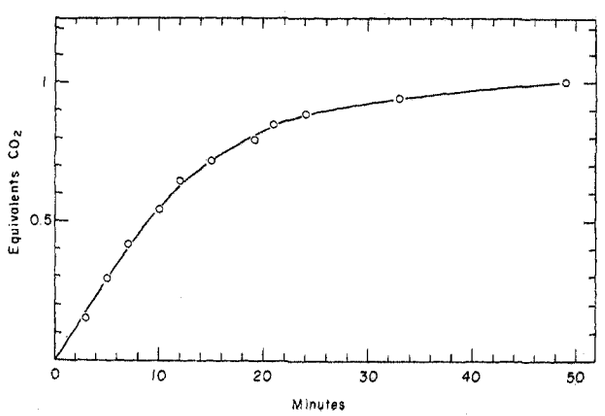


Figure 2.—The time course of decarboxylation of monoethyl (*o*-methylbenzyl)acetamidomalonate (2) (20 mg) in 2 ml of dioxane at 100°. CO_2 was trapped into benzylamine and titrated continuously with 0.11 *M* sodium methoxide.[4]

solubility one can work in suspension, in order to avoid the need for large volumes; thus the amount of enzyme required to achieve the optimal concentration is kept low. However, the suspension must be thin enough to allow proper stirring, mainly to prevent local accumulation of the alkali solution added to keep the pH constant. Alternatively, instead of using the pH-Stat, it may be possible to add enough buffer at the outset in order to prevent the pH from dropping too much during the hydrolysis.

The hydrolysis product (the L form of the acylamino acid) goes into solution at the pH values necessary for most enzymes, and the unhydrolyzed D form is removed at the end of the reaction by filtration, followed by extraction, or by extraction alone. If there is no protonatable group in the side chain, the L-acylamino acid is recovered from the aqueous solution after acidification. However, for instance, in the case of the quinoline derivatives, one either hydrolyzes directly to the free L-amino acid or alternatively isolates the L-acylamino acid as a salt, according to the need in further use of the products.

The advantages of the method described here are (a) hydrolysis and decarboxylation conditions are very mild; (b) one of the reaction intermediates is itself the substrate for enzymic resolution; (c) a variety of proteolytic enzymes can be employed for resolution

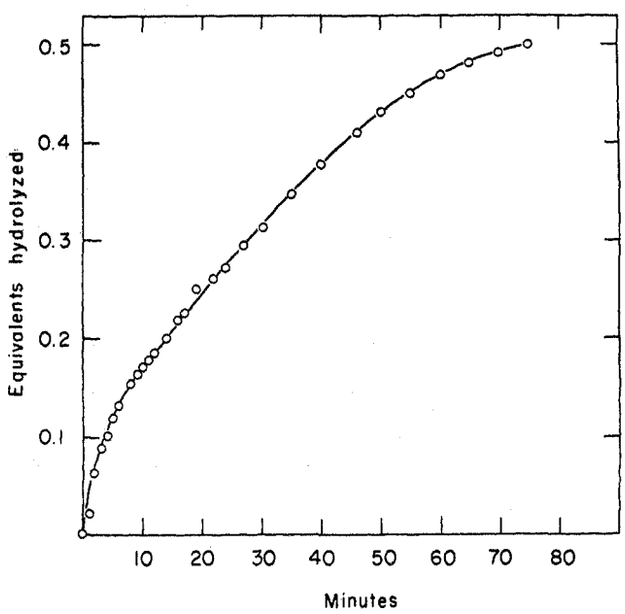


Figure 3.—Enzymic resolution of *N*-acetyl-β-(*o*-methylphenyl)-DL-alanine ethyl ester (3) with chymotrypsin. The course of the reaction was followed by measuring the alkali uptake in a pH-Stat assembly. For details see Experimental Section.

and these are available in a high state of purity and only minute amounts are necessary; (d) amino malonate can be N blocked by a variety of reagents before introducing the R group, a feature which should be of advantage in further peptide synthesis using the new N-blocked L-amino acid. Furthermore, it should be possible to introduce R groups carrying functions blocked by different moieties, thus facilitating differential deblocking in later synthetic steps.

Experimental Section

Melting points were determined in capillaries and are uncorrected.

Materials.—Diethyl acetamidomalonate and dimethyl aminomalonate hydrochloride were from Fluka AG (Buchs, Switzerland) and were used without further purification. α-Monobromoxylene, 2-methylnapthalene, and 6-methylquinoline were products of Eastman Kodak Company (Rochester, N. Y.); the latter two were distilled or recrystallized prior to use. α-Chymotrypsin was from Worthington Biochemical Corporation (Freehold, N. J.) and subtilisin Carlsberg from Novo Industri A/S (Copenhagen, Denmark). 2-Bromomethylnaphthalene was prepared according to Chapman, *et al.*[5]

Diethyl (*o*-Methylbenzyl)acetamidomalonate (1).—Diethyl acetamidomalonate (6.5 g, 30 mmol) was dissolved in a solution of sodium (0.72 g, 31.2 mmol) in 45 ml of dry ethanol. α-Bromoxylene (4.2 ml, 30.8 mmol) was added at room temperature and the reaction mixture was refluxed for 1 hr. The neutral, still boiling mixture was gradually mixed with 90 ml of hot water, slowly cooled to room temperature, and seeded. If still basic, the reaction mixture was neutralized with acetic acid prior to dilution with water. After 2 hr in an ice bath the crystalline precipitate was filtered off, washed with water, and dried, yield 8.05 g (84%), mp 83–84°. *Anal.* Calcd for $C_{17}H_{23}O_5N$ (321.4): C, 63.53; H, 7.21; N, 4.36. Found: C, 63.59; H, 7.13; N, 4.21.

Monoethyl (*o*-Methylbenzyl)acetamidomalonate (2).—To a clear solution of diester 1 (6.4 g, 20 mmol) in 37.5 ml of ethanol, 15 ml of 3.9 *N* NaOH was added. The reaction at room temperature was followed by titrating the excess base in aliquots withdrawn at different time intervals. After 40 min the reaction mixture was acidified with 6 *N* HCl to about pH 2. The ethanol

(5) N. B. Chapman and S. F. A. Williams, *J. Chem. Soc.*, 5044 (1952).

was evaporated and the remaining aqueous mixture was extracted with five 100-ml portions of ethyl acetate (to the first extraction some ethanol had to be added to obtain clear phases). The combined extracts were washed twice with saturated NaCl solution, dried (Na_2SO_4), and evaporated to dryness. The solid residue was triturated with a small amount of ethyl acetate (to remove some yellow impurities), suspended in petroleum ether (bp 30–60°), filtered after 12 hr at 4°, and dried, yield 5 g (86%), mp 126–128° dec, neut equiv calcd 293.3, found 284 (nonaqueous titration with sodium methoxide in dioxane). *Anal.* Calcd for $C_{15}H_{19}NO_5$ (293.3): C, 61.42; H, 6.53; N, 4.78. Found: C, 61.56; H, 6.42; N, 4.60.

N-Acetyl-β-(o-methylphenyl)-DL-alanine Ethyl Ester (3). Decarboxylation of Monoester.—Monoester **2** (4 g) was dissolved in 50 ml of absolute dioxane and refluxed for 45 min. By this time titratable acid had practically disappeared. The dioxane was evaporated and the oil obtained was dissolved in 30 ml of ethyl acetate, washed with $NaHCO_3$ solution and saturated NaCl solution, and dried (Na_2SO_4), and the solvent was evaporated. The oily residue crystallized on seeding and trituration with petroleum ether. The crystals were collected, washed with petroleum ether, and dried, yield 2.6 g (78%), mp 63–64°. *Anal.* Calcd for $C_{14}H_{19}O_3N$ (249.3): C, 67.44; H, 7.68; N, 5.62. Found: C, 67.65; H, 7.55; N, 5.63.

Enzymic Resolution of N-Acetyl-β-(o-methylphenyl)-DL-alanine Ethyl Ester (3). N-Acetyl-β-(o-methylphenyl)-L-alanine (4).—Ester **3** (18 g, 73 mmol) was suspended in 600 ml of 0.1 *N* KCl containing 400 mg of $NaHCO_3$ (as a buffer, to prevent pH jumping on addition of alkali, which can inactivate the enzyme). Solid α-chymotrypsin (40 mg) was added and the hydrolysis was run at 37° at pH 7–7.5. The reaction mixture was stirred efficiently by a magnetic stirrer and the pH was kept constant by means of a pH-Stat (2 *N* NaOH delivered from a buret equipped with a magnetic valve). After 120 min the unchanged ester of the D isomer **5** was filtered off and the clear filtrate was extracted with several portions of ethyl acetate. The aqueous solution was acidified with 6 *N* HCl (10 ml) and the free acid **4** was extracted into four portions of ethyl acetate which were combined, washed with saturated NaCl solution, dried (Na_2SO_4), and concentrated. The crystals formed were recrystallized from 60 ml of ethyl acetate, filtered, washed with petroleum ether, and dried, yield 6.2 g (78%), mp 156–157°, $[\alpha]^{25}D$ +40.6° (*c* 10, CH_3OH). *Anal.* Calcd for $C_{12}H_{15}O_3N$ (221.3): C, 65.14; H, 6.83; N, 6.33. Found: C, 65.01; H, 6.87; N, 6.20. (An additional 1.3 g, mp 150°, was precipitated from the mother liquor by petroleum ether, total yield 94%.)

N-Acetyl-β-(o-methylphenyl)-D-alanine Ethyl Ester (5).—The ester of the D isomer, collected by filtration and ethyl acetate extraction of the aqueous hydrolysis mixture as described above, was dissolved in ethyl acetate, washed twice with $NaHCO_3$ and once with saturated NaCl solution, and dried (Na_2SO_4), and the solution was evaporated to dryness. Reprecipitation from ethyl acetate (70 ml)–petroleum ether (300 ml) yielded 5.5 g (64%) of colorless needles, mp 77–78°, $[\alpha]^{25}D$ 4.84° (*c* 10, CH_3OH). *Anal.* Calcd for $C_{14}H_{19}O_3N$ (249.3): C, 67.44; H, 7.68; N, 5.62. Found: C, 67.58; H, 7.61; N, 5.74. The optical purity of this material was checked by gas-liquid chromatography.[6] No L isomer could be detected under conditions that would have revealed 1% contamination.

Diethyl (2-naphthylmethyl)acetamidomalonate (6) was prepared by treating 2-bromomethylnaphthalene with diethyl acetamidomalonate as described for **1**, yield *ca.* 75%, mp 109°. *Anal.* Calcd for $C_{20}H_{23}NO_5$ (357.4): C, 67.3; H, 6.45; N, 3.92. Found: C, 68.6; H, 6.42; N, 4.51.

The following compounds were prepared by partial alkaline hydrolysis, decarboxylation, and enzymic resolution with α-chymotrypsin of the malonate **6** as outlined above for derivatives **2** and **3**.

N-Acetyl-β-(2-naphthyl)-L-alanine (7) had mp 178° (lit.[2] mp 181–182°), $[\alpha]^{24}D$ +41.4° (*c* 1.81, CH_3OH:DMF 1:2). *Anal.* Calcd for $C_{15}H_{15}NO_3$ (257.3): N, 5.44. Found: N, 5.20. Titration equiv: calcd 257.3; found 278 (nonaqueous titration with sodium methoxide in DMF).

N-Acetyl-β-(2-naphthyl)-D-alanine ethyl ester (8) had mp 133–136°, $[\alpha]^{24}D$ −21.5° (*c* 2.11, CH_3OH:DMF 1:2). *Anal.* Calcd for $C_{17}H_{19}NO_3$ (285.3): C, 71.56; H, 6.71; N, 4.91. Found: C, 71.27; H, 6.57; N, 5.02.

N-Acetyl-β-(2-naphthyl)-D-alanine (9).—Ester **8** (285 mg) was hydrolyzed at room temperature in 2 ml of ethanol containing 1.5 equiv of aqueous NaOH (6 *N*) for 2 hr. Following dilution with water the mixture was acidified with 6 *N* HCl to about pH 2 and the ethanol was evaporated. The crystalline product was collected and recrystallized from ethyl acetate, yield 60%, mp 170–171°, $[\alpha]^{24}D$ −40.8° (*c* 2.07, CH_3OH:DMF 1:2). *Anal.* Calcd for $C_{15}H_{15}NO_3$ (257.3): C, 70.07; H, 5.88; N, 5.44. Found: C, 69.80; H, 5.65; N, 5.42. Titration equiv: calcd 257; found 266 (nonaqueous titration with sodium methoxide in DMF).

6-Chloromethylquinoline[7] (10) was synthesized from 6-hydroxymethylquinoline[8] obtained from 6-methylquinoline *via* the 6-aldehyde.[9]

Diethyl (6-Quinolylmethyl)acetamidomalonate (11).—Chloride[10] **10** (7.65 g, 36 mmol) was added to diethyl acetamidomalonate (15.6 g, 72 mmol) dissolved in 60 ml (72 mmol) of 1.2 *M* ethanolic sodium ethoxide. The mixture was refluxed for 6 hr. After cooling the salt was filtered off, the filtrate was evaporated to dryness, the residue was taken up in 500 ml of ethyl acetate, and the organic layer was extracted with 3 × 150 ml of ethyl acetate, and the organic layer was extracted with 3 × 150 ml of ice-cold 4 *N* HCl. The combined extracts were carefully neutralized by the addition of 10 *N* NaOH with stirring and cooling to give a voluminous precipitate which was reextracted with 3 × 200 ml of ethyl acetate. After the organic extract was washed (H_2O), dried ($MgSO_4$), and evaporated, the residual solid was recrystallized from ethyl acetate–hexane, 8.7 g (67.5%), mp 150–151° (lit.[11] mp 156°). An additional 1.2 g (9.2%), mp 149–150°, was obtained from the concentrated mother liquor. *Anal.* Calcd for $C_{19}H_{22}N_2O_5$ (358.4): C, 63.67; H, 6.19; N, 7.82. Found: C, 63.82; H, 6.28; N, 7.81.

N-Acetyl-β-(6-quinolyl)-DL-alanine Ethyl Ester (12).—Partial hydrolysis of diethyl malonate **11** (9.0 g, 25.1 mmol) in 200 ml of ethanol was accomplished by adding 6.5 ml (40 mmol) of 6.1 *N* NaOH and stirring for 1 hr at ambient temperature, after which period no more of the diester could be detected by tlc. The partly precipitated sodium salt of the monoacid–monoester was dissolved by adding 2 volumes of water, and the solution was neutralized with 3.6 ml (40 mmol) of 11.1 *N* HCl and evaporated to dryness. The solid residue, after drying over KOH *in vacuo*, was suspended in 200 ml of dioxane and refluxed for 90 min. Finely powdered NaCl (left from the partial hydrolysis) was removed by filtering the cold solution. Evaporation of the dioxane left an oily residue which solidified on scratching and was recrystallized from ethyl acetate–ether to yield 6.65 g (92.6%) of colorless racemic ester **12**, mp 130–131°. *Anal.* Calcd for $C_{16}H_{18}N_2O_3$ (286.4): C, 67.11; H, 6.34; N, 9.78. Found: C, 67.23; H, 6.25; N, 9.78.

Enzymic Resolution of N-Acetyl-β-(6-quinolyl)-DL-alanine Ethyl Ester. β-(6-Quinolyl)-L-alanine (13).—Powdered racemic ester **12** (4.0 g, 14 mmol) was suspended in 100 ml of 0.1 *M* KCl (10^{-4} *M* in KH_2PO_4 to prevent pH jumping on addition of alkali). After the pH was adjusted to 7.6 hydrolysis was initiated by adding 3.5 mg of subtilisin Carlsberg to the vigorously stirred mixture. The pH was kept constant by means of a pH stat (0.5 *N* NaOH as titrant). Alkali uptake (6.95 mmol) ceased after 130 min. The crystalline ester of the D isomer **14** was collected by filtration. The filtrate was concentrated to about 30 ml, extracted with 3 × 20 ml of $CHCl_3$, and evaporated to dryness. The residue (the acetyl L acid) was hydrolyzed by refluxing overnight in 40 ml of 6 *N* HCl.[12] The hydrochloric

(6) We are greatly indebted to Dr. B. Feibush, Department of Chemistry, Weizmann Institute of Science, for performing this analysis. The chromatographic system was a modification(U. Beitler and B. Feibush, unpublished work) of the method of E. Gil-Av, B. Feibush, and R. Charles-Sigler, *Tetrahedron Lett.*, 1009 (1966).

(7) C. E. Kaslow and J. M. Schlatter, *J. Amer. Chem. Soc.*, **77**, 1054 (1955).

(8) V. M. Rodionov and M. A. Berkenheim, *J. Gen. Chem. USSR*, **14**, 501 (1944); *Chem. Abstr.*, **39**, 4606 (1945).

(9) V. M. Rodionov and M. A. Berkenheim, *J. Gen. Chem. USSR*, **14**, 330 (1944); *Chem. Abstr.*, **39**, 4076 (1945).

(10) 6-Bromomethylquinoline[7] might also be an adequate starting material for this reaction. However, an attempt to obtain this compound by brominating 6-methylquinoline with *N*-bromosuccinimide failed.

(11) V. N. Konyukhov, L. N. P'yankova, and K. Yu. Bobarykina, *Khim. Geterotsikl. Soedin., Akad. Nauk Latv. SSR*, 140 (1965); *Chem. Abstr.*, **63**, 5733e (1965).

(12) Alternatively N^{α}-acetyl-β-(6-quinolyl)-L-alanine could be isolated as its potassium salt (KOH as titrant in the enzymic hydrolysis) as follows: the aqueous filtrate was evaporated to complete dryness, the residual salt was extracted with ethanol, the extract was filtered and concentrated, and the potassium salt was precipitated with ether.

acid was evaporated, the residue was redissolved in 20 ml water, and the free L acid was isolated by isoelectric precipitation at pH 7. Recrystallization from water gave 1.25 g (83%) of β-(6-quinolyl)-L-alanine (**13**), mp 250–255° dec (lit.[11] mp 246–247° for the racemic compound), $[\alpha]^{24}D$ +14.8 ± 1° (*c* 1.69, 5 *N* HCl). *Anal.* Calcd for $C_{12}H_{12}N_2O_2$ (216.24): C, 66.65; H, 5.59; N, 12.96. Found: C, 66.72; H, 5.53; N, 12.91.

***N*-Acetyl-β-(6-quinolyl)-D-alanine ethyl ester (14)**, collected by filtration and chloroform extraction of the hydrolysis mixture as described above, was dried over NaOH *in vacuo*. Recrystallization from ethyl acetate–hexane yielded 1.84 g (92%) of colorless needles, mp 145–146°, $[\alpha]^{24}D$ +29.2 + 1° (*c* 4.47, CH_3OH). *Anal.* Calcd for $C_{16}H_{18}N_2O_3$ (286.4): C, 67.11; H, 6.34; N, 9.79. Found: C, 67.16; H, 6.34; N, 9.66.

β-(6-Quinolyl)-D-alanine Dihydrochloride (15).—Ethyl ester **14** (1.25 g) was hydrolyzed in 30 ml of 6 *N* HCl at reflux temperature overnight. Evaporation of the HCl and recrystallization of the product from methanol–2-propanol gave 1.18 g (93%) of the free D acid in the form of its dihydrochloride, mp 270–271° dec, $[\alpha]^{24}D$ −15.1 ± 1° (*c* 1.25, 5 *N* HCl). Titration equiv: calcd 96.4; found 98.4 (nonaqueous titration with sodium methoxide in DMF).

Dimethyl *N*-Benzyloxycarbonylaminomalonate (16).—To dimethyl aminomalonate hydrochloride (5.5 g, 30 mmol) in 60 ml of ice-cold water–dioxane (9:1, v/v) benzyl chloroformate (4.5 ml, 32 mmol) was added in small portions over a period of about 20 min. The reaction mixture was vigorously stirred and cooled in an ice bath while the pH was kept between 9 and 10 by dropwise addition of 4 *N* NaOH. When the uptake of alkali had ceased the mixture was adjusted to pH 7 with 1 *N* HCl and the precipitated product was collected by filtration and dried *in vacuo* over KOH. Recrystallization from ether–petroleum ether yielded 7.4 g (88%) of colorless prisms, mp 57–58°. *Anal.* Calcd for $C_{13}H_{15}NO_6$ (281.26): C, 55.51; H, 5.38; N, 4.98. Found: C, 55.70; H, 5.38; N, 4.74.

***N*-Benzyloxycarbonyl-DL-phenylalanine Methyl Ester (18).**—Benzyl chloride (1.09 ml, 9.45 mmol) was added with stirring into a solution of malonate **16** (2.65 g, 9.45 mmol) in 10 ml of 0.945 *M* ethanolic sodium ethoxide. The mixture was kept at reflux temperature for 3 hr, cooled, and filtered, the filtrate was evaporated, and the residue was taken up in 200 ml of ethyl acetate. The organic layer was washed with 5% $NaHCO_3$, 1 *N* HCl, and water, dried ($MgSO_4$), and evaporated to give a yellowish oil which was homogeneous in tlc; the oil was used without further purification for the subsequent steps.

For partial hydrolysis 2.6 g of the oil in 15 ml of ethanol containing 1.64 ml of 6.1 *N* NaOH was allowed to stand at room temperature for 45 min, 50 ml of water was added, the pH was adjusted to 6 with 1 *N* HCl, most of the ethanol was evaporated, and the aqueous solution was acidified with 1 *N* HCl (pH 2–3) and extracted with 2 × 100 ml of ethyl acetate. Washing (H_2O), drying ($MgSO_4$), and evaporating the ethyl acetate extract gave the monoacid–monoester **17** as a colorless oil, which was characterized as its dicyclohexylamine salt, mp 152° (from ethanol–ether). Titration equiv: calcd 538; found 535 (nonaqueous titration with $HClO_4$ in acetic acid). *Anal.* Calcd for $C_{31}H_{42}N_2O_6$ (538.69): C, 69.12; H, 7.86; N, 5.20. Found: C, 69.32; H, 7.66; N, 5.40.

Decarboxylation was accomplished by refluxing a solution of 2.1 g of the oily monoacid–monoester in 50 ml of dioxane for 20 hr. Evaporation of the solvent and crystallization from ether–petroleum ether gave 1.67 g (71% based on initial benzyloxycarbonylaminomalonate **16**) of **18**, mp 78–79°. *Anal.* Calcd for $C_{18}H_{19}NO_4$ (313.34): C, 68.99; H, 6.11; N, 4.47. Found: C, 69.24; H, 5.90; N, 4.36.

Enzymic Resolution of *N*-Benzyloxycarbonyl-DL-phenylanine Methyl Ester.—Ester **18** (450 mg, 1.56 mmol) suspended in 50 ml of 0.1 *M* KCl (10^{-5} *M* in KH_2PO_4) was hydrolyzed in the presence of 5 mg of chymotrypsin at 37° and pH 7.6 (pH stat, 0.1 *N* NaOH as titrant). The uptake of alkali (0.78 mmol) virtually stopped after 24 hr. The unchanged ester of the D isomer **19** was filtered off and the aqueous filtrate was extracted with 2 × 50 ml of ethyl acetate to gain some additional D isomer **19** by evaporation of the organic extract. Total yield of Z-D-Phe-OMe (**19**) after one recrystallization from ether–petroleum ether was 222 mg (90%), mp 80°, $[\alpha]^{24}D$ +1.6 ± 0.3° (*c* 2.5, CH_3OH). *Anal.* Calcd for $C_{18}H_{19}NO_4$ (313.34): C, 68.99; H, 6.11; N, 4.47. Found: C, 69.28; H, 6.32; N, 4.67.

The aqueous layer obtained above was acidified with dilute HCl and extracted with 2 × 50 ml of ethyl acetate. Washing (H_2O), drying ($MgSO_4$), and evaporating the organic extract gave Z-L-Phe (**20**) in a 72% yield (51% based on benzyloxycarbonylaminomalonate **16**) after one recrystallization from ethyl acetate–petroleum ether, mp 82–84° (lit.[13] mp 88–89°), $[\alpha]^{24}D$ +6 ± 1° (*c* 2.62, acetic acid) (lit.[13] $[\alpha]D$ +5.1°). Titration equiv: calcd 299; found 294 (nonaqueous titration with sodium methoxide in DMF). Removal of the carbobenzoxy group with HBr in acetic acid yielded L-Phe, $[\alpha^{24}D$ −36.2 ± 1.5° (*c* 2.09, H_2O) (lit.[13] $[\alpha]D$ −34.4°).

Registry No.—**1**, 5440-53-9; **2**, 37447-32-8; **3**, 37439-96-6; **4**, 37439-97-7; **5**, 37439-98-8; **6**, 37447-33-9; **7**, 37439-99-9; **8**, 37440-00-9; **9**, 37440-01-0; **10**, 2644-82-8; **11**, 2644-83-9; **12**, 37440-02-1; **13**, 37440-03-2; **14**, 37440-04-3; **15**, 37440-05-4; **16**, 37447-35-1; **17** dicyclohexylamine salt, 37447-36-2; **18**, 32563-40-9; **19**, 37440-07-6; **20**, 1161-13-3; diethyl acetamidomalonate, 1068-90-2; 2-bromo-*o*-xylene, 89-92-9; 2-bromomethylnaphthalene, 939-26-4; dimethylaminomalonate hydrochloride, 16115-80-3; benzyl chloroformate, 501-53-1.

(13) E. Grassmann and E. Wünsch, *Chem. Ber.*, **91**, 462 (1958).