with 5% then 10% MeOH in $CHCl_3$ as eluents. The intermediate diethyl ester was hydrolyzed (see 3). The crude product could not be precipitated or crystallized, but it was separated in 12 portions on a 21.1 × 250 mm^2 Regis C_{18} column. The column was eluted with a 5% to 15% MeCN gradient in 0.1% aqueous trifluoroacetic acid at 13.5 mL/min. The combined product was reeluted from the same column with 18% MeCN–0.1% TFA. The product-containing fractions were combined and lyophylized to a white powder: yield 0.53 g (12%); mp 150 °C dec; NMR (D_2O) δ 2.1 (m, 1 H, $CHCH_2CH_2$), 2.2 (m, β-H on Glu), 2.3 (m, 1 H, $CHCH_2CH_2$), 2.34 (t, J = 7.5 Hz, γ-CH_2 on Glu), 2.55 (m, β-H), 4.1 (m, CHC=O), 4.16 (t, J = 8.8 Hz, NCH_2), 4.35 (m, α-H), 7.71 (d, J = 8.6 Hz, 3′- and 5′-H), 7.91 (d, J = 8.6 Hz, 2′- and 6′-H); FAB-MS (thioglycerol) m/e 445 (M + 1), 429 (M + 1-NH_2); UV (0.1 N NaOH) λ_{max} (ϵ) 278 (16 500); IR (KBr) cm^{-1} 1689, 1655, 1638, 1608, 1570, 1502, 1426, 1400, 1294, 1201, 1140, 1109, 1025, 854, 805, 773, 725. Anal. ($C_{19}H_{20}N_6O_7 \cdot {}^3/_5TFA \cdot {}^{12}/_5H_2O$) C, H, N, F.

Biological Tests. The details of the GAR-Tfase, AICAR-Tfase, and FPGS assays were reported previously.[28]

MTX Uptake Assay. The ability of compounds to block the uptake of radiolabeled MTX into Molt-4 cells was used as a measure of the affinity of the test compounds for the reduced folate transport system as discussed in an earlier publication.[46]

Cell Culture Method for Evaluation of Compounds as Antitumor Agents. Cells and Medium. Molt-4 T-cell leukemia and MCF-7 human breast adenocarcinoma cells, obtained from the American Type Culture Collection (ATCC), were grown in RPMI 1640 medium supplemented with 10 nM calcium leucovorin as the folate source, 10% dialyzed fetal calf serum, penicillin, streptomycin, and, for MCF-7, sodium pyruvate (110 μg/mL).

Cytotoxicity Assay. Cells were seeded into 96-well plates using a Perkin-Elmer Pro/pette. MCF-7 cells were seeded at 15 000 cells per well in 150 μL of medium. Prior to the addition of drugs, cultures were incubated for 24 h at 37 °C. Compounds were added at 2× concentration in 150 μL of medium and each concentration was assayed in triplicate. Cultures were incubated for 72 h in a 37 °C humidified incubator at 5% CO_2. Inhibition of cell growth was measured using the MTT dye reduction assay.

MTT Dye Reduction Assay. Cell dilutions for a standard curve were prepared from a 72-h log-phase culture. Serial dilutions were seeded in triplicate in 96-well plates and incubated at 37 °C for 1 h. MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was dissolved in phosphate buffered saline at 5 mg/mL and sonicated for 30 s. Using the Perkin-Elmer Pro/pette, 200 μL of medium was removed and 100 μL of MTT was added to the wells of the standard curve and test plates. Suspension cultures were spun for 5 min at 1000 rpm before removing medium from the wells. Plates were incubated for 1 h at 37 °C on a platform shaker. Following this incubation, 100 μL of medium was removed from the wells and 100 μL of dimethyl sulfoxide was added to each well. The plates were sonicated for approximately 10 s to solubilize the precipitated formazan dye. The absorbance of each well was measured using a Titertek Multiskan MC microtiter plate reader at 570 nm with a reference wavelength of 750 nm.

Acknowledgment. The authors wish to acknowledge the expert technical assistance of Dr. Gary Martin, Aris Ragouzeos, and Ron Crouch with NMR studies; Dr. Lester Taylor and Robert Johnson with mass spectroscopic studies; and Emma Rose and Patricia Parker for biological studies. The authors also wish to thank Prof. John Hynes for helpful discussions.

Registry No. 2, 126632-31-3; 2·2TFA, 139348-96-2; 3, 135439-31-5; 4, 129166-73-0; 5, 132497-50-8; 6, 124656-55-9; 7, 126632-27-7; 8, 126632-26-6; 9, 126632-28-8; 10, 126632-29-9; 11, 126632-30-2; 12·$^3/_5$TFA, 139348-92-8; 13, 118252-48-5; 14b, 139348-93-9; 15b, 139348-94-0; 16a, 118252-53-2; 16b, 139348-95-1; 17a (Z = 2′-F, di-*tert*-butyl ester), 126632-33-5; 17a (Z = 3′-F), 126632-36-8; 17a (Z = 3′-Me), 126632-38-0; 17a (Z = 3′-MeO), 126632-41-5; 17a (Z = 2′-Cl), 126632-42-6; 17b, 139348-97-3; 18a, 403-21-4; 18d, 5081-36-7; 19a, 126632-34-6; 19c, 126632-47-1; 19d, 126632-39-1; 20 (R = Et, Z = H), 13726-52-8; 20a, 126632-35-7; 20b, 85803-27-6; 20c, 126632-37-9; 20d, 126632-40-4; 20e, 80014-92-2; 21a, 739-33-3; 22a, 126632-43-7; 22b, 129166-68-3; 22c, 74226-00-9; 23a, 126632-44-8; 23b, 129166-69-4; 23c, 132497-46-2; 24a, 126632-45-9; 24b, 129166-71-8; 24b (ethyl ester), 129166-70-7; 24c, 132497-48-4; 24c (ethyl ester), 132497-47-3; 25a, 126632-46-0; 25b, 129166-72-9; 25c, 132497-49-5; 26, 139348-98-4; 27, 86364-65-0; 28, 139348-99-5; 29, 139349-00-1; 30, 139349-01-2; MTX, 59-05-2; FPGS, 63363-84-8; GAR-Tfase, 9032-02-4; AICAR-Tfase, 9032-03-5; 4-$H_2NC_6H_4CO_2Et$, 94-09-7; $(4\text{-}EtO_2CC_6H_4S)_2$, 20057-83-4; $Br(CH_2)_4Br$, 110-52-1; $Br(CH_2)_3Br$, 109-64-8; $NCCH_2CO_2Et$, 105-56-6; $HN{=}C(NH_2)_2 \cdot HCl$, 50-01-1; $HN{=}CHNH_2 \cdot HOAc$, 40730-94-7; $HN{=}C(NH_2)NHNH_2 \cdot H_2CO_3$, 2582-30-1; H-Glu-(OEt)-OEt·HCl, 1118-89-4; $Cl(CH_2)_3COCl$, 4635-59-0; $(CO_2Et)_2$, 95-92-1.

(46) Patil, S. D.; Jones, C.; Nair, M. G.; Galivan, J.; Maley, F.; Kisliuk, R. L.; Gaumont, Y.; Duch, D.; Ferone, R. Folate Analogues. 32. Synthesis and Biological Evaluation of 2-Desamino-2-methyl-N^{10}-propargyl-5,8-dideazafolic Acid and Related Compounds. *J. Med. Chem.* 1989, 32, 1284–89.

Inhibition of Pig Kidney L-Aromatic Amino Acid Decarboxylase by 2,3-Methano-*m*-tyrosines

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Departments of Chemistry and Biochemistry, School of Chemical Sciences, University of Georgia, Athens, Georgia 30602.
Received April 8, 1991

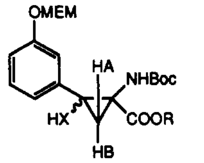
Both racemic (*E*)- and (*Z*)-2,3-methano-*m*-tyrosines (9*E* and 9*Z*) have been synthesized from a common intermediate, monoester (*Z*)-1-(ethoxycarbonyl)-2-[3-[(2-methoxyethoxy)methoxy]phenyl]cyclopropanecarboxylic acid (5). Quinine and ephedrine, respectively, were used to resolve their *N*-*tert*-butoxycarbonyl (Boc) derivatives. Among the compounds prepared, the (+)-(*E*)-diastereomer of 9 is the most potent inhibitor of L-aromatic amino acid decarboxylase (Dopa decarboxylase), having a K_i of 22 μM, with the (−)-*Z*-diastereomer (9*Z*) second at K_i = 49 μM. (+)-9*E* is a 45-fold more potent inhibitor of DDC than its acyclic analogue, D-*m*-tyrosine.

Introduction

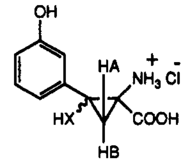
L-Aromatic amino acid decarboxylase (Dopa decarboxylase, DDC) is a pyridoxal 5′-phosphate (PLP) dependent enzyme which catalyzes the decarboxylation of L-dopa and 5-hydroxy-L-tryptophan, thus playing a critical role in the biosynthesis of the important neurotransmitters, epinephrine, norepinephrine, and serotonin.[1] The possible involvement of catecholamines in a number of clinical

(1) Pletscher, A.; Gey, K. F.; Burkard, W. P. Inhibitors of Monoamine Oxidase and Decarboxylase of Aromatic Amino Acids. *Handb. Exp. Pharmak.* 1966, 19, 593–735.

Table I. ^{1}H NMR Spectral Data of (*E*)- and-(*Z*)-2,3-Methano-*m*-tyrosine



6E : R=Et[a], 6Z: R=Me[a]
7Z and 7E : R=H[a]



9E[b] and 9Z[b]

	chemical shift, δ						coupling constants, Hz		
compd	Ar	H_N	H_X	H_B	H_A	R	J_{AB}	J_{AX}	J_{BX}
9*E*	7.37; 6.67 (2d)	-	3.13 (t)	2.3 (dd)	1.83 (dd)		-7.1	10.9	9.1
9*Z*	7.2; 6.8 (2d)	-	2.81 (t)	1.85 (dd)	1.51 (dd)		-5.7	10.2	8.6
6*E*	7.2; 6.83 (2d)	5.53	2.7 (t)	2.0 (dd)	1.47 (dd)	3.72; 0.72			
6*Z*	7.55; 7.05 (2d)	4.92	3.8 (t)	2.36 (m)	2.0 (m)	3.64 (s)			
7*E*	7.16; 6.8 (2d)	5.54	2.7 (t)	2.1 (dd)	1.52 (dd)		-5.7	10.3	8.7
7*Z*	7.26; -6.85 (2d)	4.71	2.99 (t)	2.17 (m)	1.75 (m)				

[a] Solvent, $CDCl_3$; internal reference, tetramethylsilane group. [b] Solvent D_2O; internal reference, *p*-dioxane.

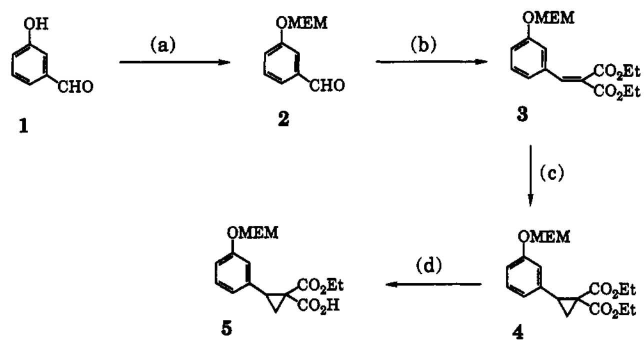
disorders, including hypertension, has generated a great deal of interest in the preparation and evaluation of potential inhibitors of DDC. Dopa hydrazide (Carbidopa) is used as a peripheral DDC inhibitor in the treatment of Parkinson's syndrome.[2] α-Methyldopa (Aldomet), an antihypertensive agent, inhibits DDC in vitro by a slow transamination reaction which inactivates the PLP cofactor,[3] but the antihypertensive effects may not be related to DDC inhibition.[4] In some early work, Bernabe and co-workers synthesized a number of racemic (*Z*)-1-amino-2-arylcyclopropanecarboxylic acids and reported that many of them inhibited DDC.[5] Among the most potent of these was the 3-hydroxyphenyl-substituted compound.

Synthesis of 2,3-methano-*m*-tyrosine, a rigid analogue of D-*m*-tyrosine, a known DDC inhibitor,[6] and determination of the inhibitory potencies of its stereoisomers should afford insight into the steric requirements of the enzyme active site, since it might be expected that the *configuration* of the most active rigid stereoisomer might approximate the *conformation* of the bound amino acid during enzymatic decarboxylation. As expected, significant differences in the inhibitory potencies of the stereoisomers of 2,3-methano-*m*-tyrosine were found, and the results are presented herein.

Results and Discussion

Synthesis. The syntheses of both racemic (*E*)- and (*Z*)-2,3-methano-*m*-tyrosines were accomplished from a common intermediate, monoester 5 (Scheme I). The MEM ether[7] 2, prepared in 82% overall yield from 3-hydroxybenzaldehyde by standard methods,[8] provided the necessary protection of the phenolic hydroxyl group throughout the subsequent synthetic steps and was readily removed, along with the *N*-*tert*-butoxycarbonyl (Boc) group, by nonaqueous acid. Knoevenagel condensation of 2 with diethyl malonate gave the benzalmalonate 3, which was cyclopropanated with dimethylsulfoxonium methylide,[9] affording the desired cyclopropane derivative 4. Surprisingly, when dimethyl malonate was used in the Knoevenagel condensation, decarbomethoxylation occurred, as evidenced by the disappearance of one methyl ester singlet in the ^{1}H NMR spectrum.

Scheme I[a]



[a] Reagents: (a) MEM-Cl, PTS; (b) $CH_2(CO_2Et)_2$, piperidine; (c) $(CH_3)_3SOI$, NaH; (d) NaOH–EtOH.

Treatment of diethyl ester 4 with 1 equiv of sodium hydroxide gave the monoester 5, resulting from hydrolysis of the less-hindered ester function, as determined by NMR spectroscopy. It remained to convert either the carboxylic acid or ester function of 5 into the *tert*-butylcarbamate group to obtain the desired amino acid derivatives, *N*-Boc-(*E*)- and -(*Z*)-2,3-methano-*m*-tyrosines (7*E* and 7*Z*). Thus, the azide afforded by treatment of 5 (Scheme II) with diphenyl phosphorylazide[10] was smoothly converted

(2) Marsden, C. D.; Parker, J. D.; Rees, J. E. A Year's Comparison of Treatment of Patients with Parkinson's Disease with Levodopa Combined with Carbidopa Versus Treatment with Levodopa Alone. *Lancet* **1973**, No. 2, 1459–1462.
(3) O'Leary, M. H.; Baughn, R. L. Decarboxylation Dependent Transamination Catalyzed by Mammalian 3,4-Dihydroxyphenylalanine Decarboxylase. *J. Biol. Chem.* **1977**, *252*, 7168–7173.
(4) Sjoerdsma, A. Methyldopa. *Brit. J. Clin. Pharmacol.* **1982**, *13*, 45–49.
(5) Bernabe, M.; Cuevas, O.; Fernandez-Alvarez, E. D'eriv'es du Cyclopropane. V. Preparation of Amino-1-aryl-2-cyclopropanecarboxylic Acids and Preliminary Results of Their Interaction with Dopadecarboxylase. *Eur. J. Med. Chem.* **1979**, *14*, 33–45.
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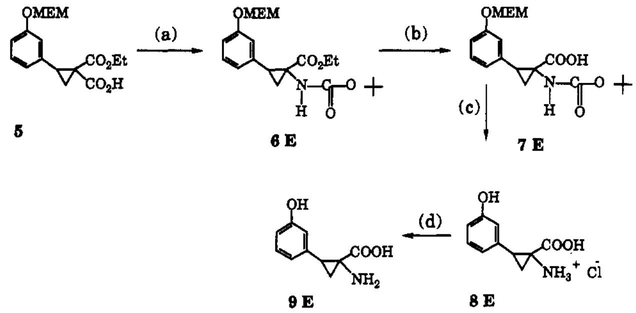
(7) Corey, E. J.; Grass, J. L.; Ulrich, P. A New General Method for Protection of the Hydroxy Function. *Tetrahedron Lett.* **1976**, 809–812.
(8) Van Heerden, F. R.; Van Zyl, J. J.; Rall, G. J. H.; Brandt, E. V.; Roux, D. G. Phase-Transfer Catalyst: A General Method of Methoxymethylation of the Hydroxy Function. *Tetrahedron Lett.* **1978**, 661–662.
(9) Corey, E. J.; Chaykovsky, M. Dimethyloxosulfonium Methylide ($(CH_3)_2SOCH_2$) and Dimethylsulfonium Methylide ($(CH_3)_2SCH_2$). Formation and application to Organic Synthesis. *J. Am. Chem. Soc.* **1965**, *87*, 1353–1364.

Scheme II[a]



[a]Reagents: (a) DPPA, TEA, *t*-BuOH, Δ; (b) NaOH-EtOH; (c) 4 N HCl-dioxane; (d) Amberlite, IRA-400, 3% HOAc.

Table II. ^{13}C NMR Spectral Data of (*E*)- and (*Z*)-2,3-Methano-*m*-tyrosine

6E : R=Et[a], **6Z** : R=Me[a]
7Z and **7E** : R=H[a]

9E[b] and **9Z**[b]

compd	chemical shifts, δ				
	C_4	C_3	C_2	C_5	C_1
9*E*	39.71	38.93	16.93		169.7
9*Z*	39.64	29.64	16.99		172.04
6*E*	41.0	35.39	20.98	156.5	173.7
6*Z*	39.32	32.0	21.2	156.7	172.1
7*E*	40.6	35.5	20.8	156.5	173.6
7*Z*	39.54	33.17	21.33	156.9	177.25

[a]Solvent, $CDCl_3$; internal reference, $CDCl_3$, 77.0 ppm. [b]Solvent, D_2O; internal reference, *p*-dioxane, 66.7 ppm.

into the carbamate 6*E* after Curtius rearrangement and alcoholysis of the resulting isocyanate with *tert*-butyl alcohol. Vigorous alkaline hydrolysis of the hindered ester function of 6*E* gave the crystalline racemic (*E*)-2,3-methano-*m*-tyrosine derivative 7*E*.

(*Z*)-2,3-Methano-*m*-tyrosine (7*Z*) was synthesized from **5** by the somewhat longer route shown in Scheme III. The monoester **5** failed to give a well-characterized hydrazide, but we found that treatment of its potassium salt with refluxing ethanolic hydrazine gave a hydrazide (**10a**) which upon subsequent nitrosation yielded an oily azide (**10b**). This intermediate, necessarily esterified with diazomethane before the Curtius rearrangement, gave the crystalline (*Z*)-2,3-methano-*m*-tyrosine derivative (6*Z*) in 75% overall yield from **5**. Saponification of 6*Z* furnished the desired racemic carboxylic acid 7*Z*. Hydrogen chloride cleavage of the blocking groups converted both 7*Z* and 7*E* into the free amino acids, 9*E* and 9*Z*, after ion exchange neutralization of their hydrochlorides.

All of the intermediates and final products were characterized by high-field ^{1}H and ^{13}C NMR (Tables I and II). Chemical shifts, splitting patterns, and coupling constants were consistent with the expected configurational assignments. The chemical and physical properties of 9*E* and 9*Z* were quite similar to those of the corresponding stereoisomers of 2,3-methanophenylalanine.[11a] The isolated

(10) Shioiri, T.; Ninomiya, K.; Yamada, S. Diphenylphosphoryl Azide. A New Convenient Reagent for a Modified Curtius Reaction and for the Peptide Synthesis. *J. Am. Chem. Soc.* **1972**, *94*, 6203–6205.

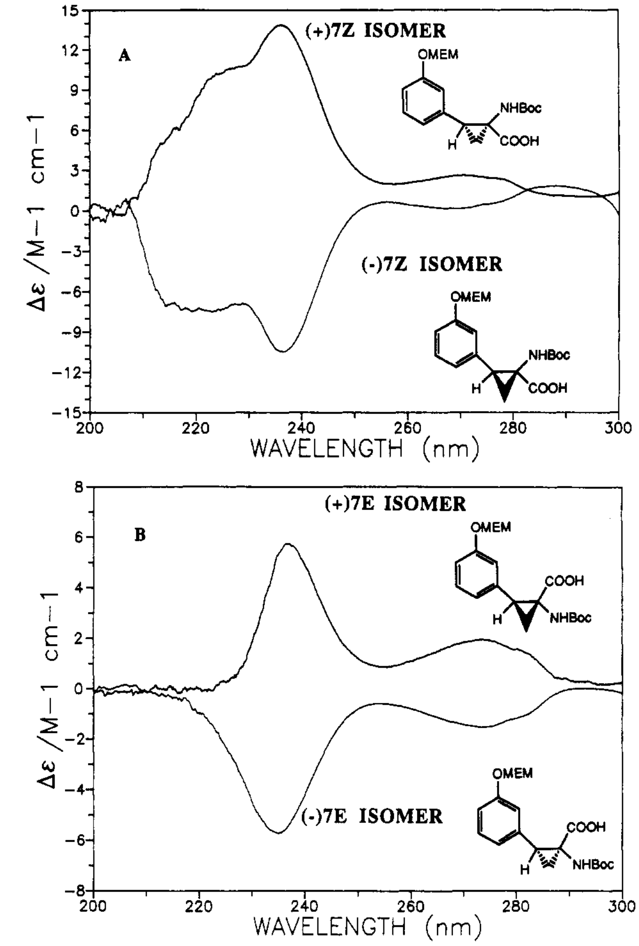


Figure 1. CD spectra of *N*-Boc-*O*-MEM-(*E*)- and (*Z*)-2,3-methano-*m*-tyrosines in CH_3CN: (A) (+)-7*E* and (−)-7*E*, and (B) (−)-7*Z* and (−)-7*Z*.

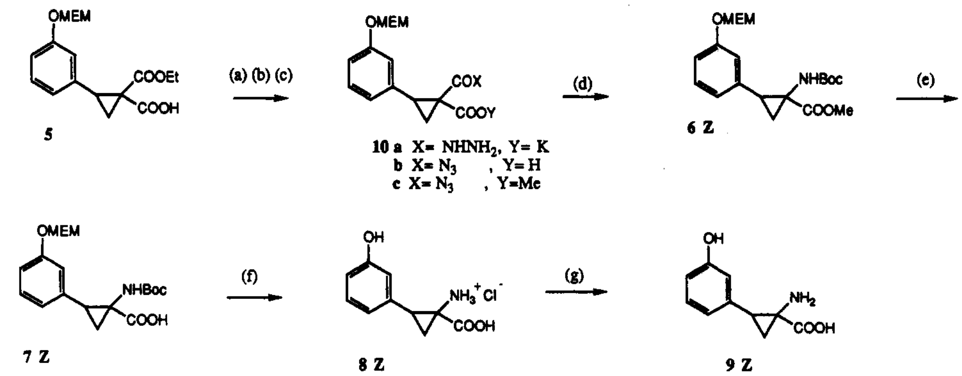
cyclopropane proton, H_X, appeared as a well-resolved triplet in each case and the geminal protons, H_A and H_B, appeared as doublets of doublets in all the derivatives except 6*Z* and 7*Z*, appearing in the latter as broad multiplets. This may be due to a slow equilibrium between s-cis- and s-trans-isomers of the carbamate moiety in 6*Z* and 7*Z*. Unambiguous assignment of the cyclopropane ring protons in 6*E*, 9*E*, and 9*Z* was allowed by their chemical shifts and coupling constants, i.e., H_X is always downfield of H_A and H_B, due to deshielding by the aromatic ring, and $J_{cis} > J_{trans}$ for vicinal protons on cyclopropane rings.[12] Interestingly, the cyclopropane proton coupling constants obtained for 9*E* and 9*Z* are virtually identical with those of the methanophenylalanines.[11a]

Some useful trends are shown in the ^{13}C NMR spectra (Table II). While both C-3 and C-4 of all the (*E*)-isomers appear downfield of C-3 and C-4 in the (*Z*)-isomers, the reverse is true of C-2 (except in the amino acids, 9*E* and 9*Z*) and of the carbonyl carbon atom, C-1. Notably, derivatives of (*E*)- and (*Z*)-2,3-methanophenylalanine[11a] ex-

(11) (a) King, S. W.; Holt, E. M.; Stammer, C. H. Synthesis of Racemic (E)- and (Z)-1-Amino-2-phenylcyclopropanecarboxylic Acid: (E)- and (Z)-"Cyclopropylphenylalanine." *J. Org. Chem.* **1982**, *47*, 3270–3273. (b) Kimura, H.; Stammer, C. H. Resolution and Deblocking of Racemic N-(Bezyloxycarbonyl)cyclopropylphenylalanine. *J. Org. Chem.* **1983**, *48*, 2440–2441.

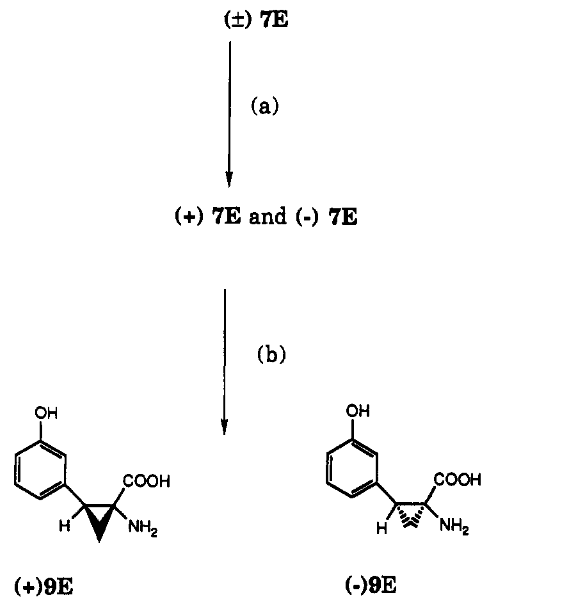
(12) (a) Jackman, L. M.; Sternhell, S. *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*; Pergamon Press: Oxford, 1972; pp 286–288. (b) Goudemer, A. In *Stereochemistry: Fundamentals and Methods*; Kagan, H. B., Ed.; Thieme: Stuttgart, 1977; Vol. 1, p 77.

Scheme III[a]



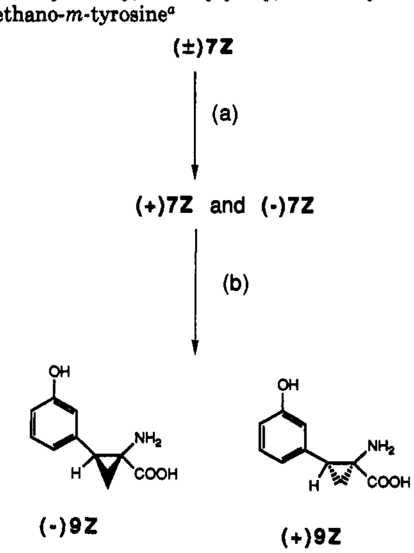
[a] Reagents: (a) [i] KOH, EtOH, [ii] H_2NNH_2; (b) HONO; (c) CH_2N_2, Et_2O; (d) $PhCH_3$, Δ, *t*-BuOH; (e) NaOH–EtOH; (f) 4 N HCl–dioxane; (g) Amberlite, IRA-400, 3% HOAc.

Scheme IV. Resolution of *N*-[(11-Dimethylethoxy)carbonyl]-*O*-[(2-methoxyethoxy)methyl]-(*E*)-2,3-methan-*m*-tyrosine[a]



[a] Reagents: (a) (i) quinine–acetone, (ii) 1 M NaOH; (b) (i) 4 N HCl–dioxane, (ii) Amberlite IRA-400, 3% HOAc.

Scheme V. Resolution of *N*-[(1,1-Dimethylethoxy)carbonyl]-*O*-[(2-methoxyethoxy)methyl]-(*Z*)-2,3-methano-*m*-tyrosine[a]



[a] Reagents: (a) (i) (−)-ephedrine–3-pentanone/hexane, (ii) 1 M NaOH; (b) (i) 4 N HCl–dioxane; (ii) Amberlite IRA-400, 3% HOAc.

hibited the same trends, suggesting that ^{13}C chemical shifts may be useful for diastereochemical assignment of aromatic 2,3-methano amino acid isomers.

Resolution of *N*-Boc-*O*-MEM-(*E*)-2,3-methano-*m*-tyrosine (7*E*) was achieved by crystallization of its quinine salt from acetone (Scheme IV) while the (*Z*)-isomer (7*Z*) was resolved by crystallization of its ephedrine salt from 3-pentanone (Scheme V). In the absence of crystals acceptable for X-ray crystallography, the absolute configurations of the resolved materials were inferred by comparison of their CD spectra with those of 2,3-methanophenylalanines of known configuration (Figure 1, parts A and B) and by their elution order from a chiral column (Resolvosil, containing covalently linked bovine serum albumin) (Figure 2, parts A and B). Earlier work from these laboratories showed that the (2*S*)-configuration required a negative 214-nm CD peak, shown in the (*E*)-(2*S*,3*R*)-2,3-methanophenylalanine.[11b] In the case of the 2,3-methano-*m*-tyrosines, this peak is red-shifted to about 235 nm, due to the electron-donating oxygen substituent. Consequently, the levorotatory isomers of the 2,3-methano-*m*-tyrosines were assigned pro tem the (2*S*)-configuration, which is spatially related to the L-configuration of biogenic amino acids. Also the levorotatory forms

Table III. Comparison of Inhibition of (*E*)- and (*Z*)-2,3-Methano-*m*-tyrosine with (+)-D-*m*-Tyrosine

amino acids	K_i, M	relative inhibition
(+)-D-*m*-tyrosine	1.0×10^{-3} [a]	1
(+)-9*E*	2.2×10^{-5}	45.4
(−)-9*E*	6.3×10^{-4}	1.6
(+)-9*Z*	3.3×10^{-4}	3.0
(−)-9*Z*	4.9×10^{-5}	20.4

[a] From Voltattorni, C. B., et al. (See ref 6.)

of both (*E*)- and (*Z*)-isomers were more strongly retained on the Resolvosil column, which is known to bind L-amino acids selectively. Although this is to our knowledge the first example of the separation of a cyclopropyl amino acid on Resolvosil, a large number of natural and unnatural amino acids and derivatives are known to be separable, and in all cases the L-isomer is retained longer.[13] (Differences

(13) (a) Allenmark, S.; Bomgren, B.; Boren, H. Direct Liquid Chromatographic Separation of Enantiomers on Immobilized Protein Stationary Phases. III. Optical Resolution of a Series of N-Aroyl D, L-Amino Acids by High Performance Liquid Chromatography on Bovine Serum Albumin Covalently Bound to Silica. *J. Chromatogr.* **1983**, *264*, 63–68. (b) Allenmark, S.; Anderson, S. Direct Liquid Chromatographic Separation of Enantiomers on Immobilized Protein Stationary Phases. V. Optical Resolution of N-(2,4-Dinitrophenyl) and Dansyl-D,L-Amino Acids. *J. Chromatogr.* **1986**, *351*, 231–328.

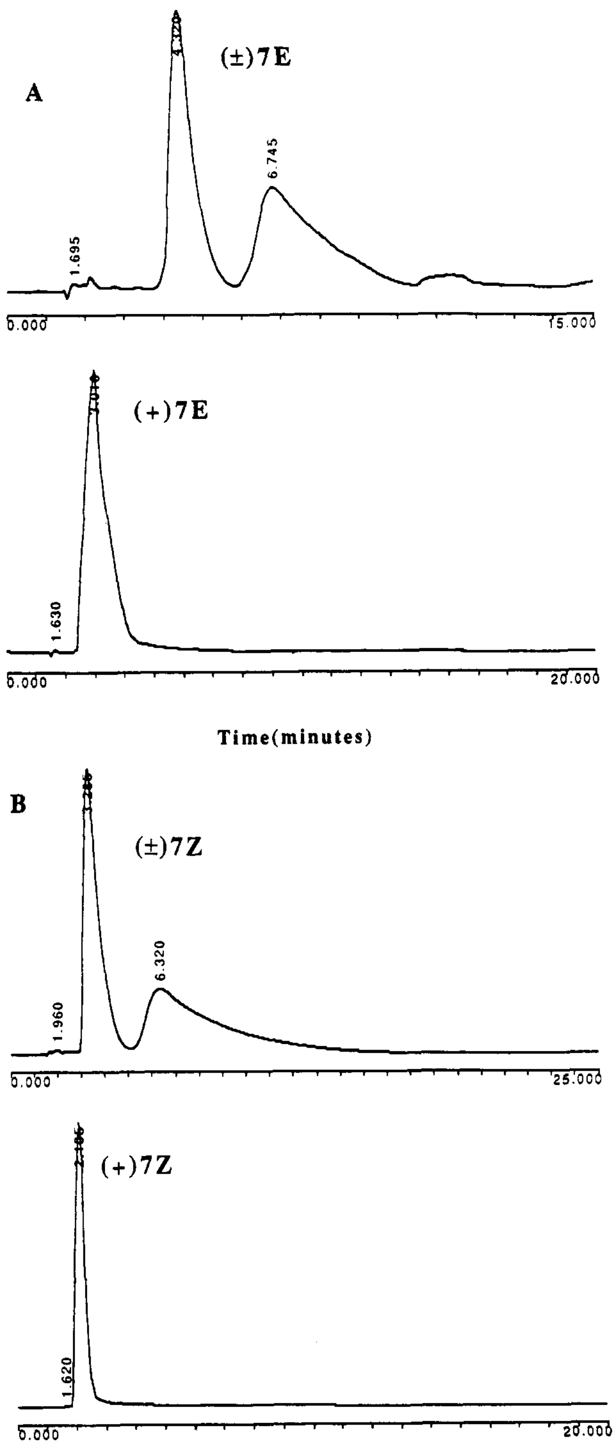


Figure 2. High-performance liquid chromatography of *N*-Boc-*O*-MEM-(*E*)- and (*Z*)-2,3-methano-*m*-tyrosines: (A) (±)-7*E* and (+)-7*E*, and (B) (±)-7*Z* and (+)-7*Z*.

in the retention times for the dextrorotatory isomers in Figure 2 are due to differences in buffer composition.)

Enzyme Inhibition. We have utilized a continuous spectrophotometric assay to measure DDC activity.[14] All of the stereoisomers of 2,3-methano-*m*-tyrosines were found to be linear competitive inhibitors of DDC (Figure 3, parts A and B); however they differed significantly in

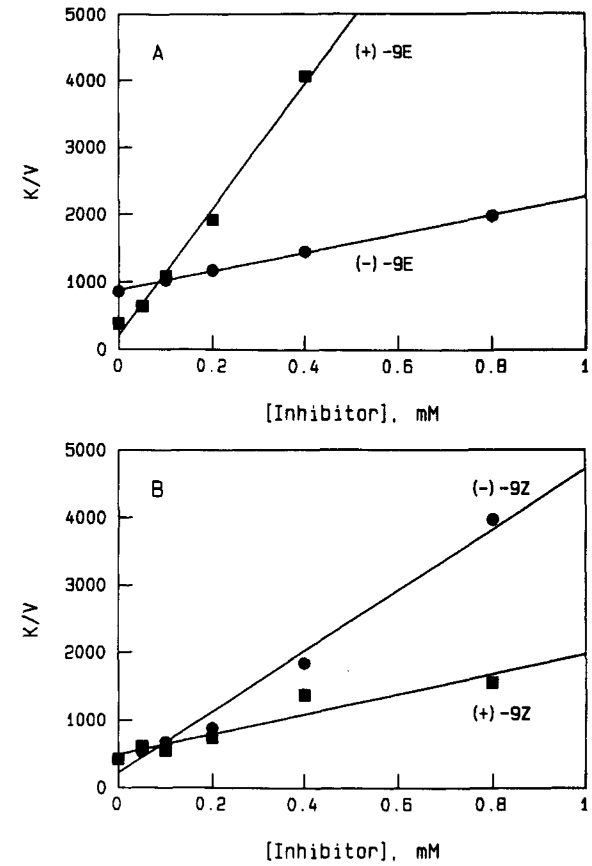


Figure 3. Inhibition of Dopa decarboxylase by (*E*)- and (*Z*)-2,3-methano-*m*-tyrosine: (A) (+)-9*E* and (−)-9*E*, and (B) (+)-9*Z* and (−)-9*Z*.

their K_i values (Table III). The (+)-(*E*)-enantiomer [(+)-9*E*], assigned the (2*R*,3*S*)-configuration, corresponding to that of a D-amino acid, was the most potent inhibitor, showing a K_i of 22 μM (Table III), 45-fold greater than that of D-*m*-tyrosine. Among the rigid compounds prepared, this isomer must therefore approximate the bound conformation of the natural substrate amino acid most closely. It should be noted that L-*m*-tyrosine is an excellent substrate for DDC, with a V_{max} twice that of L-Dopa.[6] However, the potency of the (−)-(2*S*,3*S*)-(*Z*)-enantiomer [(−)-9*Z*], with K_i = 49 μM, and having not only the L- but also the (*Z*)-configuration, was at first difficult to rationalize. It does appear, however, that the (3*S*)-configuration binds optimally to DDC, the configuration at C-2 appearing to be less important. These results are consistent with the known stereochemical specificity of DDC, which binds both D- and L-amino acids with equal affinity,[6] but decarboxylates only L-amino acids. The potent inhibition of DDC by (+)-(*E*)-2,3-methano-*m*-tyrosine suggests that cyclopropane analogues of inhibitory amino acids may be more potent inhibitors of other PLP-dependent enzymes than the known inhibitors. It may also be possible to increase the inhibitory potency of these cyclopropanes toward DDC by further structural modification.

Experimental Section

Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analysis were carried out by Atlantic Microlab, Atlanta, GA. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded with a JEOL FX 90Q (operating at 90.0 and 22.5 MHz), Bruker AC 250 (operating at 250.1 and 62.4 MHz), or Bruker AC 300 (operating at 300.1 and 75.4 MHz) spectrometer, using tetramethylsilane (TMS) or *p*-dioxane (3.53 ppm downfield from TMS) as internal standards for ^{1}H NMR

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and p-dioxane (66.7 ppm downfield from TMS) or $CDCl_3$ (77.0 ppm downfield from TMS) as internal standards for ^{13}C NMR. Ultraviolet spectra were taken on a Gilford Response UV-vis spectrophotometer. Circular dichroism spectra were taken on a JASCO J-500C spectropolarimeter at ambient temperature in acetonitrile.

TLC was performed on Whatman precoated silica gel plates with the following solvent systems (v/v): (I) EtOAc–hexane (1:1), (II) EtOAc–hexanes (2:3); (III) $CHCl_3$–EtOH–acetic acid (90:10:1), (IV) EtOAc–acetic acid (100:1); (V) n-BuOH–acetic acid–water (4:1:5), upper layer; (VI) $CHCl_3$–MeOH–acetic acid (5:2:1).

Anhydrous $MgSO_4$, Na_2SO_4, and K_2CO_3 (Baker) was used to dry the organic solutions during workups: 60–230 mesh (Baker) and 32–63 M (Woelm) silica gel were used for gravity and flash chromatography, respectively. Solvents were evaporated during workup of reaction mixtures on a rotating evaporator.

Enzyme Purification and Assay. Dopa decarboxylase was isolated and purified from pig kidney (Pel-Freeze) according to the procedure described by Borri-Voltatorni,[15] except that Bioacryl-BP-1000 (supplied by Supelco) was added to the crude extract and centrifuged to obtain a clear solution. After the DEAE–cellulose column, we used phenyl Sepharose CL-4B,[16] followed by Sephacryl S-300 chromatography, which gave a highly purified enzyme. The purity was checked by polyacrylamide gel electrophoresis which showed two subunits and is in accordance to previous results reported by Voltatorni.[17] The specific activity of the purified enzyme was 3.87 μmol min^{-1} mg^{-1}. The assay of Dopa decarboxylase was performed using a continuous spectrophotometric procedure described by Scrivens et al.[14] The CO_2 kits used during these experiments were purchased from Sigma Chemical Co. Phosphoric acid solution (1 M) was used to adjust the pH of the reagent solution to 6.8. Typical solutions contained the following: CO_2 solution 500 μL, PLP 6 μL (2 mmol), Dopa 2–32 μL (5 mmol), and inhibitor 0–60 μL (10 mmol), which was adjusted to a total volume 600 μL by H_2O. The method involved the trapping of liberated CO_2 with phosphoenolpyruvate, catalyzed by phosphoenolpyruvate carboxylase. The resultant oxaloacetate was in turn reduced by malate dehydrogenase to L-malate, with concomitant oxidation of NADH to NAD. Thus, the decarboxylation was followed continuously by observing the decrease in absorbance at 340 nm. K_i values were determined from the plot of (K_m/V_{max})apparent vs [inhibitor], as shown in Figure 3. The slope of this plot is $K_m/(V_{max}K_I)$, and the intercept is the value of K_m/V_{max} in the absence of inhibitor. The K_i value is provided by the ratio of intercept to slope.

HPLC Methods. The high-pressure liquid chromatography was performed on Rainin HPLC system. The chiral column (Resolvosil, from Macherey-Nagel) was used to check the purity of the resolved compounds. The racemic compound showed two resolved peaks of the enantiomers; the resolved compound showed one peak. The (+)-enantiomers of the (E)- and (Z)-isomers elute early. This corresponds to D-amino acids, which are not retained strongly on the column. The (–)-enantiomers interact more strongly with the column, so they are eluted later (Figure 2, parts A and B). The column was eluted with 0.02 M potassium phosphate buffer, pH 6.8, at flow rate of 1 mL/min, and the absorbance was detected at 265 nm. In the case of the resolved compounds the elution was performed with 0.025 M potassium phosphate buffer, which resulted in shorter retention time than the racemic compounds.

3-[(2-Methoxyethoxy)methoxy]benzaldehyde (2). A solution of m-hydroxybenzaldehyde (27.5 g, 225 mmol) and NaOH (9.9 g, 247.5 mmol) in water (125 mL), containing 6 mL of Aliquat 336, was stirred at room temperature for 20 min. MEM chloride[9] (12.9 mL, 113 mmol) in CH_2Cl_2 (160 mL) was added dropwise at 0 °C over a period of about 20–30 min. The reaction mixture was stirred overnight. The aqueous phase was separated and extracted with CH_2Cl_2 (2 × 100 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated to dryness in vacuo to give a dark brown oil, which was chromatographed on a silica gel column (10 × 50 cm) with hexane–ether (2:1) to give 16.8 g (70%) of 2 as a yellow oil: ^1H NMR ($CDCl_3$) δ 0.0 $(CH_3)_4Si$), 3.3 (s, 3 H, OCH_3), 3.43–3.57 (m, 2 H, OCH_2), 3.7–3.84 (m, 2 H, OCH_2), 5.23 (s, 2 H, $PhOCH_2$), 7.2–7.6 (m, 4 H, ArH), 9.83 (s, 1 H, CHO).

Diethyl [3-[(2-Methoxyethoxy)methoxy]benzal]malonate (3). A solution of 2 (5.4 g, 25.71 mmol), diethyl malonate (32.91 mmol, 4.99 mL), piperidine (2.0 mL), and acetic acid (14 mL) in toluene (50 mL) was heated to reflux overnight with azeotropic removal of water (oil bath temperature 140 °C). The reaction mixture was cooled, ether (50 mL) was added, and after separation, the organic phase washed with 1 N $KHSO_4$ (3 × 30 mL) and 5% $NaHCO_3$, (3 × 30 mL). The organic phase was dried over $MgSO_4$ and evaporated to dryness in vacuo to give a yellow oil which was chromatographed on a silica gel column (4.0 × 20 cm) using hexane–ether (2:1) to give 8.12 g (89%) of 3: R_f (I) 0.40; ^1H NMR ($CDCl_3$) δ 0.0 $(CH_3)_4Si$), 1.07–1.33 (t, J = 8 Hz, 6 H, $CO_2CH_2CH_3$), 3.27 (s, 3 H, OCH_3), 3.33–3.47 (m, 2 H, OCH_2), 3.60–3.73 (m, 2 H, OCH_2), 4.0–4.33 (dq, J = 10 Hz, 4 H, $CO_2CH_2CH_3$), 5.00 (s, 2 H, $PhOCH_2$), 6.8–7.2 (m, 4 H, ArH), 7.5 (s, 1 H, CH=C); ^{13}C NMR δ 14.02, 14.2, 58.9, 61.9, 67.6, 71.4, 75.6, 93.3, 116.95, 118.4, 122.9, 125.8, 129.7, 134, 141.6, 148.6, 157.3, 164.6.

Diethyl 2-[3-[(2-Methoxyethoxy)methoxy]phenyl]cyclopropane-1,1-dicarboxylate (4). In a three-neck flask flame dried under nitrogen and fitted with septum, stopper, and condenser was placed NaH (97%) (0.50 g, 20.53 mmol) in 15 mL of DMSO. Next was added solid $(CH_3)_3SOI$ (4.517 g, 20.53 mmol) slowly in three portions at ca. 10 °C. The reaction mixture was stirred for 30 min at room temperature, and a solution of 3 (4.059 g, 13.60 mmol) in THF (25 mL) was added dropwise over 20 min. After stirring 1 h at room temperature and 1 h at 50–60 °C, the reaction mixture was poured into 10–15 mL of ice-cold water and extracted with ether (3 × 50 mL), and the organic extracts were washed with brine (2 × 20 mL), dried over K_2CO_3, filtered, and evaporated in vacuo. The crude product was chromatographed on a silica gel column (4.0 × 20 cm), eluant hexane–ether (2:1), to obtain 3.45 g (68.5%) of pure product 4 as an oil: R_f (II) (0.61); ^1H NMR ($CDCl_3$) δ 0.0 $(CH_3)_4Si$), 0.77–0.97 (t, J = 10 Hz, 3 H, (Z)-$CO_2CH_2CH_3$), 1.2–1.4 (t, J = 10 Hz, 3 H, (E)-$CO_2CH_2CH_3$), 1.67 (dd, 1 H, ∇H),[19] 2.13 (dd, 1 H, ∇H), 3.13 (1 H, ∇H), 3.3 (s, 3 H, OCH_3), 3.43–3.6 (m, 2 H, OCH_2), 3.67–4.0 (m, 2 H, OCH_2, 2 H, q, J = 20 Hz, (Z)-$CO_2CH_2CH_3$), 4.23 (q, J = 20 Hz, 2 H, (E)-$CO_2CH_2CH_3$), 5.17 (s, 2 H, $PhOCH_2$), 6.67–7.23 (m, 4 H, ArH); ^{13}C NMR δ 13.7, 14.0, 18.7 (∇CH_2), 31.8 (∇CH), 37.2 (∇C), 58.6, 60.9, 61.3, 67.4, 71.4, 93.2, 115, 116.6, 121.9, 128.9, 136.21, 156.9, 166.4, 169.6.

(Z)-1-(Ethoxycarbonyl)-2-[3-[(2-Methoxyethoxy)methoxy]phenyl]cyclopropanecarboxylic Acid (5). To a solution of 4 (5.5 g, 14.905 mmol) in EtOH (10 mL) was added dropwise a solution of NaOH (0.596 g, 14.905 mmol) in 10 mL of water. The cloudy solution was stirred for 40 h at room temperature when TLC showed completion. After evaporation of the ethanol the residue was diluted with (10 mL) water and was extracted with ether (2 × 30 mL). The aqueous phase was acidified to pH 2 with $KHSO_4$ solution and extracted with ethyl acetate (3 × 30 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$, and evaporated to give a colorless oil, which was chromatographed on a silica gel column (4.0 × 20 cm) by eluting with 1% acetic acid in EtOAc to obtain 4.6 g (95.6%) of pure 5 as an oil: R_f (III) 0.82; ^1H NMR ($CDCl_3$) δ 0.0 $(CH_3)_4Si$), 0.72 (t, J = 8 Hz, 3 H, $CO_2CH_2CH_3$), 2.1–2.4 (m, 2 H, 2 ∇H), 3.2 (t, 1 H, ∇H), 3.32 (s, 3 H, OCH_3), 3.4–3.57 (m, 2 H, OCH_2), 3.6–3.83 (m, 4 H, OCH_2, $CO_2CH_2CH_3$), 5.13 (s, 2 H, OCH_2O), 6.67–7.23 (m, 4 H, ArH); ^{13}C NMR 13, 21.3 (∇CH_2), 33.2 (∇CH), 39.8 (∇C), 58.9, 62.2,

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(19) We use the ∇ symbol to mean "cyclopropyl".

67.5, 71.37, 93.2, 115.5, 117.28, 122.7, 129.2, 135.7, 157, 170.9, 172.5.

***N*-[(1,1-Dimethylethoxy)carbonyl]-*O*-[(2-methoxyethoxy)methyl]-(*E*)-2,3-methano-*m*-tyrosine Ethyl Ester (6*E*).** In a three-neck flask (flame dried) under nitrogen fitted with a rubber septum stopper, and condenser was added a solution of **5** (3.2 g, 9.49 mmol), triethylamine (2.1 mL, 15.15 mmol), and diphenyl phosphorylazide (2.3 mL, 10.4 mmol) in *t*-BuOH (dry). The reaction mixture was refluxed for 24 h, and the solvent was evaporated under vacuo. The residue was taken into ethyl acetate (100 mL) and washed with 5% citric acid (2 × 20 mL), followed by 5% $NaHCO_3$ (2 × 20 mL). The organic phase was dried over anhydrous K_2CO_3, filtered, and concentrated in vacuo. The crude product was chromatographed on a silica gel column (2.5 × 23 cm, 60–230 mesh; Baker) with ether–hexane (1:4) which yielded 2.95 g (77%) of 6*E* as an oil: R_f (diethyl ether) 0.8.

***N*-[(1,1-Dimethylethoxy)carbonyl]-*O*-[(2-methoxyethoxy)methyl]-(*E*)-2,3-methano-*m*-tyrosine (7*E*).** To a solution of 6*E* (2.95 g, 7.217 mmol) in EtOH (10 mL) was added a solution of NaOH (432 mg, 10.81 mmol) in water (10 mL) and refluxed overnight; the ethanol was evaporated, and 15 mL of water was added, which was acidified to pH 2 with $KHSO_4$, and extracted with ethyl acetate (2 × 50 mL). The organic extracts were washed with brine (2 × 15 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The residue was chromatographed on a silica gel column (2.5 × 23 cm, 60–230 mesh; Baker) with EtOAc–hexane (1:1) to obtain 2.47 g (90%) of 7*E*: R_f (diethyl ether) 0.2; mp 130 °C; ^1H NMR ($CDCl_3$) δ 0.0 ($(CH_3)_4Si$), 1.45 (s, 9 H), 1.52 (dd, 2 H, ∇HA), 2.1 (dd, 1 H, ∇HB), 2.7 (dd, 1 H, ∇HX), 3.3 (s, 3 H, OCH_3), 3.5 (t, 2 H, OCH_2), 2.8 (t, 2 H, OCH_2), 5.2 (s, 2 H, $-OCH_2O-$), 5.54 (1 H, NH), 6.8–7.16 (m, ArH), 8.8 (s, 1 H COOH); ^{13}C NMR ($CDCl_3$) δ 20.98 (∇CH_2), 28.25, 35.39 (∇CH), 41 (∇C), 58.7, 67.2, 71.5, 80.2, 93, 115, 116.9, 123, 128.9, 136.6, 156.5, 173.7 (COOH). Anal. ($C_{19}H_{27}O_7N$) C, H, N.

(*E*)-2,3-Methano-*m*-tyrosine Hydrochloride (8*E*). In a three-necked flask, flame dried under nitrogen, was placed a solution of 7*E* (0.63 g, 1.5 mmol) in dioxane (9 mL), *m*-cresol (1 mL), and 4 N HCl in dioxane (10 mL) was added dropwise at 0 °C. After 4 h at room temperature the reaction was complete by TLC, the solvent was evaporated in vacuo, and the residue was triturated with ether to give a solid. This was reprecipitated from 2-propanol–ether to give 400 mg (87%) of (±)-8*E*: R_f (IV) 0.5; ^1H NMR (D_2O) δ 1.83–2.3 (dd, 2 H, ∇H), 3.13 (t, 1 H, ∇H), 6.67–7.3 (m, 4 H, ArH); ^{13}C NMR (D_2O) δ 16.9 (∇CH_2), 38.9 (∇CH), 39.7 (∇C), 114.6, 115.8, 121.2, 129.8, 135.3, 153.3, 169.7.

***N*-[(1,1-Dimethylethoxy)carbonyl]-*O*-[(2-methoxyethoxy)methyl]-(*Z*)-2,3-methano-*m*-tyrosine Methyl Ester (6*Z*).** To a stirred solution of **5** (7 g, 20.52 mmol) in absolute ethanol (20 mL) was added a filtered solution of 1.31 g (20.5 mmol) of KOH (87.5% assay) in absolute ethanol (20 mL) at room temperature. After 1 h the solution was evaporated, and the residual syrup was crystallized from THF–*n*-hexane to yield 7.36 g (100%) of solid potassium salt of **5**. The salt was dissolved in H_2NNH_2–H_2O (45 mL, 85% solution in water) containing EtOH (7 mL). After refluxing for 36 h (oil bath temp 110–115 °C), the solution was evaporated in vacuo and reevaporated three times after addition of EtOAc to give 7.0 g (100%) of hydrazide **10a**. To a cold solution of **10a** (7.0 g, 20.28 mmol) and $NaNO_2$ (1.54 g, 22.3 mmol) in water (50 mL) was added 1 M sulfuric acid (40 mL) dropwise. The two-phase mixture was stirred for 2.5 h at 0–4 °C, and the aqueous phase was separated and extracted with ether (3 × 50 mL). The combined ethereal extracts were washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give 2.5 g (39%) of the azide (**10b**): R_f (III) 0.78. Treatment of **10b** with ethereal diazomethane, generated from diazald (2.12 g, 9.85 mmol), KOH (591 mg, 10.56 mmol), water (2 mL), and carbitol (6 mL) according to the optimized procedure of Hudlicky[18] gave 2.18 g (89%) of methyl ester **10c**. This was heated to reflux in *t*-BuOH for 2 days, at which time TLC showed no starting material left. The solution was washed with 10% citric acid (2 × 15 mL) and 10% $NaHCO_3$ (2 × 15 mL), dried over K_2CO_3, filtered, and evaporated in vacuo. The crude product chromatographed on a silica gel column (2.5 × 23 cm) using hexane and ether (1:1) to obtain 2.15 g (87%) of 6*Z* as an oil: ^1H NMR ($CDCl_3$) δ 0.0 ($(CH_3)_4Si$), 1.6 (s, 9 H), 2.0 (dd, 1 m, ∇HA), 2.36 (dd, 1 H, ∇HB), 3.8 (dd, 1 H, ∇HB), 3.64 (s, 3 H, OCH_3), 3.82 (q, 2 H, OCH_2), 4.02 (s, 3 H), 4.09 (q, 2 H, OCH_2), 4.92 (s, 1 H, NH), 5.52 (s, 2 H, $-OCH_2O-$), 7.05–7.55 (m, 4 H, ArH).

***N*-[(1,1-Dimethylethoxy)carbonyl]-*O*-[(2-methoxyethoxy)methyl]-(*Z*)-2,3-methano-*m*-tyrosine (7*Z*).** To a solution of 6*Z* (2.1 g, 5.31 mmol) in EtOH (6 mL) was added a solution of NaOH (425 mg, 10.63 mmol) in water (6 mL). The reaction mixture was stirred at room temperature overnight. The ethanol was evaporated, the residue was diluted with 10 mL of water, and the solution was acidified to pH 2 with $KHSO_4$ and extracted with EtOAc (3 × 30 mL). The combined organic extracts were washed with brine (2 × 20 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The crude product was chromatographed on a silica gel column (2.5 × 23 cm, 60–230 mesh; Baker) with ether–hexane (1:1) to obtain 1.7 g (85%) of 7*Z* as a solid which was recrystallized with EtOAc–hexane: R_f (diethyl ether) 0.2; mp 124 °C; ^1H NMR ($CDCl_3$) δ 0.0 ($(CH_3)_4Si$), 1.28 (s, 9 H), 1.75 (s, 1 H, ∇HA), 2.17 (s, 1 H, ∇HB), 2.99 (t, 1 H, ∇HX), 3.39 (s, 3 H, OCH_3), 3.56 (q, 2 H, OCH_2), 3.82 (s, 2 H, OCH_2), 4.71 (s, 1 H, NH), 5.26 (s, 2 H, $-OCH_2O-$), 6.85–7.26 (m, 4 H, ArH); ^{13}C NMR (*p*-dioxane) δ 21.33 (∇ CH_2), 27.98, 33.17 (∇CH), 39.54 (∇C), 58.9, 67.38, 715, 80.13, 93.32, 114.99, 116.77, 122.2, 129.25, 136.12, 156.9, 177.25 (COOH). Anal. ($C_{19}H_{27}NO_7$) C, H, N.

(*Z*)-2,3-Methano-*m*-tyrosine Hydrochloride (8*Z*). To a solution of 7*Z* (285 mg, 0.75 mmol) in dioxane (4 mL) and *m*-cresol (0.1 mL) was added 4 N HCl in dioxane (7 mL) dropwise at 0 °C. The reaction mixture was stirred at 0 °C for 4 h, the solvent was evaporated in vacuo, and the residue was saturated with ether to give a solid. This was recrystallized from 2-propanol–ether to give 150 mg (87.3%) of 8*Z*: R_f (BAW) 0.76; ^1H NMR (D_2O) δ 2.02 (acetone), 1.51 (q, 1 H, ∇HA), 1.85 (q, 1 H, ∇HB), 2.81 (q, 1 H, ∇HX), 6.8–7.2 (m, 4 H, ArH).

(*Z*)-2,3-Methano-*m*-tyrosine (9*Z*). The HCl salt 8*Z* was passed through acetate resin using 3% aqueous acetic acid as an eluent. The fraction containing free amino acid was collected which was lyophylized to give 110 mg (92%) of (±)-9*Z* as a solid: R_f (BAW) 0.6; mp 190–203 °C dec; ^1H NMR (DCl in D20) δ 2.02 (acetone), 1.49 (q, 1 H, ∇HA), 1 (q, 1 H, ∇HB), 2.75 (q, 1 H, ∇HX), 6.5–7.01 (m, 4 H, ArH); ^{13}C NMR (*p*-dioxane) δ 16.99 (∇CH_2), 29.64 (∇CH), 38.61 (∇C), 115.68, 116.6, 121.6, 130.54, 133.35, 158.89, 172.04 (COOH). Anal. ($C_{10}H_{13}NO_4$) C, H, N.

***N*-[(1,1-Dimethylethoxy)carbonyl]-*O*-[(2-methoxyethoxy)methyl]-(*E*)-2,3-methano-*m*-tyrosine (7*E*) Quinine Salt.** A mixture of (±)-7*E* (550 mg, 1.36 mmol) and quinine (441 mg, 1.36 mmol) was dissolved in acetone (12 mL). It was kept at 0 °C for 6 days. A white solid was collected by centrifugation and dried in vacuo to provide a crude resolved compound **7a** (450 mg), which was recrystallized three times with acetone: mp 135–143 °C; $[\alpha]^{20}_D$ −71.5° (*c* 2, EtOH). The mother liquor collected from the above experiment (**7b**) was evaporated to dryness to provide a crude resolved compound of **7b** (550 mg): mp 60–65 °C; $[\alpha]^{20}_D$ −60° (*c* 2, EtOH). A suspension of **7a** (450 mg) was taken into 7 mL of 1 M NaOH and extracted with $CHCl_3$ (3 × 20 mL) and the extract discarded. The aqueous layer was acidified with 4 N HCl and extracted with $CHCl_3$ (3 × 50 mL). This organic layer was washed with water (1 × 20 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo which was recrystallized from EtOAc–hexane to yield 227 mg (82.5%) of (−)-7*E* as a solid: mp 130 °C; $[\alpha]^{20}_D$ −2.6° (*c* 4, EtOH); ^1H NMR ($CDCl_3$) δ 0.0 ($Si(CH_3)_4$), 1.45 (s, 9 H), 1.55 (dd, 1 H, ∇HA), 2.12 (dd, 1 H, ∇HB), 2.8 (t, 1 H, ∇HX), 3.35 (s, 3 H, OCH_3), 3.55 (t, 2 H, OCH_2), 3.75 (t, 2 H, OCH_2), 5.22 (s, 2 H, $-OCH_2O-$), 5.48 (s, 1 N, NH), 6.85–7.15 (m, 4 H, ArH). Anal. ($C_{19}H_{27}NO_7$) C, H, N.

A suspension of **7b** (550 mg) was taken into 7 mL of 1 N NaOH and extracted with $CHCl_3$ (3 × 20 mL), and the extract was discarded. The aqueous layer was acidified with 4 N HCl and extracted with $CHCl_3$ (3 × 50 mL). This organic layer was washed with water (1 × 20 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The product was recrystallized from EtOAc–hexane to yield 255 mg (93%) of (+)-7*E* as a solid: mp 129 °C; $[\alpha]^{20}_D$ +2.6° (*c* 4, EtOH); ^1H NMR ($CDCl_3$) δ 0.0 ($Si(CH_3)_4$), 1.45 (s, 9 H), 1.55 (dd, 1 H, ∇HA), 2.1 (dd, 1 H, ∇HB), 2.74 (dd, 1 H, ∇HX), 3.32 (s, 3 H, OCH_3), 3.55 (t, 2 H, OCH_2), 3.75 (t, 2 H, OCH_2), 5.2 (s, 2 H), 5.52 (s, 1 H, NH), 6.8–7.15 (m, 4 H, ArH). Anal. ($C_{19}H_{27}NO_7$) C, H, N.

(−)-(*E*)-2,3-Methano-*m*-tyrosine [(−)-9*E*]. To a solution of (−)-7*E* (100 mg, 2.47 mmol) and *m*-cresol (0.15 mL) in dioxane (3 mL) was added dropwise 4 N HCl in dioxane (2.5 mL) at 0 °C.

The reaction mixture had stirred for 4 h at room temperature when TLC showed completion of reaction. It was evaporated to an oil, which was triturated with ether to give 46 mg (81%) of (-)-*8E* as a solid: mp 200–210 °C dec; R_f (BAW) 0.7. Anal. ($C_{10}H_{12}O_3Cl$) C, H, N.

The HCl salt (-)-*8E* (40 mg, 0.174 mmol) was passed through acetate resin (Amberlite). The acetate resin was generated by passing 3% aqueous acetic acid through Amberlite IRA-400 OH. The fraction containing free amino acids was lyophylized to obtained 32 mg (95%) of (-)-*9E* as a white solid: mp 195–207 °C dec; R_f (BAW) 0.3 $[\alpha]^{20}_D$ -4.8° (*c* 1, 1 N HCl). ^1H NMR (DCl in D_2O 2%) δ 3.53 (*p*-dioxane), 1.73 (dd, 1 H, ∇HA), 1.98 (dd, 1 H, ∇HB), 2.95 (dd, 1 H, ∇HX), 6.6–7.06 (m, ArH); ^{13}C NMR (*p*-dioxane) δ 16.8 (∇CH_2), 30.8 (∇CH), 39.64 (∇C), 114.6, 115.84, 121.2, 129.8, 135.3, 155.3 (aromatic carbon), 169.83 (COOH).

(+)-(*E*)-2,3-Methano-*m*-tyrosine [(+)-9*E*]. To a solution of (+)-*7E* (100 mg, 2.47 mmol) and *m*-cresol (0.15 mL) in dioxane (3 mL) was added dropwise 4 N HCl in dioxane (2.5 mL) at 0 °C. The reaction mixture was completed in 4 h at room temperature. The solvent was evaporated to an oil which was triturated with ether to obtain 48 mg (84.5%) of (+)-*8E* as a solid: R_f (BAW) 0.7; mp 200–210 °C dec. Anal. ($C_{10}H_{12}O_3NCl$) C, H, N.

The HCl salt (+)-*8E* (60 mg, 0.26 mmol) was passed through acetate resin. The fraction containing free amino acids was lyophilized to obtain 45 mg (81.5%) of (+)-*9E* as a white solid: mp 195–205 °C dec; R_f (BAW) 0.3; $[\alpha]^{20}_D$ +4.7° (*c* 1, 1 N HCl); ^1H NMR (DCl in D_2O) δ 3.53 (*p*-dioxane), 1.73 (dd, 1 H, ∇HA), 1.96 (dd, 1 H, ∇HB), 2.95 (dd, 1 H, ∇HX), 6.6–7.06 (m, 4 H, ArH); ^{13}C NMR (*p*-dioxane), 16.8 (∇CH_2), 30.8 (∇CH), 39.64 (∇C), 114.6, 115.8, 121.2, 129.8, 135.35, 155.3 (aromatic carbon), 169.8 (COOH).

***N*-[(1,1-Dimethylethoxy)carbonyl]-*O*-[(2-methoxyethoxy)methyl]-(*Z*)-2,3-methano-*m*-tyrosine (7*Z*) (-)-Ephedrine Salt.** A mixture of (±)-*7Z* (500 mg, 1.31 mmol) and (-)-ephedrine (216.8 mg, 1.31 mmol) was dissolved in 3-pentanone (6 mL) and 1 mL of hexane. It was kept at room temperature for 6 days; a white solid was collected by centrifugation and dried in vacuo to provide a crude resolved compound **7a** (340 mg), which was recrystallized three times with 3-pentanone and hexane to obtain 302 mg of **7a** as a solid: mp 114–120 °C; $[\alpha]^{20}_D$ -18.3° (*c* 1, $CHCl_3$). The mother liquor collected from the above experiment **7b** was evaporated to dryness to obtain 397 mg of **7b** as an oil, $[\alpha]^{20}_D$ -5.7° (*c* 1, $CHCl_3$).

A suspension of **7a** (302 mg) was taken into 10 mL of 1 M NaOH and extracted with $CHCl_3$ (3 × 30 mL), and the extract was discarded. The aqueous layer was acidified with 4 N HCl and extracted with $CHCl_3$ (3 × 40 mL). This organic layer was washed with brine (1 × 15 mL) and water (1 × 15 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo and was recrystallized from EtOAc–hexane to obtain 180 mg (86%) of (-)-*7Z* as a solid: mp 128–129 °C; $[\alpha]^{20}_D$ -28.7° (*c* 2, EtOH); ^1H NMR ($CDCl_3$) δ 0.0 (Si(CH_3)$_4$), 1.20 (s, 9 H), 1.72 (dd, 1 H, ∇HA), 2.04 (dd, 1 H, ∇HB), 2.91 (dd, 1 H, ∇HB), 3.32 (s, 3 H, OCH_3), 3.50 (q, 2 H, OCH_2), 3.75 (s, 2 H, OCH_2), 4.67 (s, 1 H, NH), 5.19 (s, 2 H, -OCH_2O-), 6.82–7.23 (w, 4 H, ArH); ^{13}C NMR (*p*-dioxane) δ 21.22 (∇CH_2), 27.89 (-c-), 33.07 (∇CH), 39.49 (∇C), 58.8, 67.30, 71.45, 80.0, 93.28, 114.9, 116.7, 122.0, 129.19, 136.05, 156.85, 177.19 (COOH). Anal. ($C_{19}H_{27}NO_7$) C, H, N.

A suspension of **7b** (397 mg) was taken into 10 mL of 1 M NaOH and extracted with $CHCl_3$ (3 × 30 mL), and the extract was discarded. The aqueous layer was acidified with 4 N HCl and extracted with $CHCl_3$ (3 × 40 mL). The organic layer was washed with brine (1 × 15 mL) and water (1 × 15 mL), dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The product was recrystallized from EtOAc–hexane to obtain 230 mg (92%) of (-)-*7Z* as a solid: mp 128–129 °C; $[\alpha]^{20}_D$ +29.1° (*c* 2, EtOH); ^1H NMR ($CDCl_3$) δ 0.0 (Si(CH_3)$_4$), 1.2 (s, 9 H), 1.7 (dd, 1 H, ∇HA), 2.0 (dd, 1 H, ∇HB), 2.9 (dd, 1 H, ∇HB), 3.3 (s, 3 H, OCH_3), 3.48 (q, 2 H, OCH_2), 3.74 (s, 2 H, OCH_2), 4.65 (s, 1 H, NH), 5.15 (s, 2 H, -OCH_2O-), 6.8–7.2 (w, 4 H, ArH); ^{13}C NMR (*p*-dioxane) δ 21.2 (∇CH_2), 27.88 (-c-), 33.0 (∇CH), 39.47 (∇CH), 58.7, 67.29, 71.43, 79.9, 93.27, 114.87, 116.7, 121.9, 129.15, 136.0, 156.83, 177.14 (COOH). Anal. ($C_{19}H_{27}NO_7$) C, H, N.

(-)-(*Z*)-2,3-Methano-*m*-tyrosine [(-)-9*Z*]. To a solution of (-)-*7Z* (85 mg 0.26 mmol) and *m*-cresol (0.1 mL) in dioxane (3 mL) was added dropwise 4 N HCl in dioxane (2.5 mL) at 0 °C. When TLC showed completion of reaction, the solution was evaporated to an oil, which was triturated with diethyl ether to give a solid. This was recrystallized from 2-propanol–diethyl ether to give 38 mg (74%) of (-)-*8Z*: R_f (BAW) 0.72; mp 200–210 °C dec; $[\alpha]^{20}_D$ -30.2° (*c* 2, H_2O); ^1H NMR (D_2O) δ 2.02 (acetone), 1.5 (q, 1 H, ∇HA), 1.84 (q, 1 H, ∇HB), 2.8 (q, 1 H, ∇HX), 6.78–7.17 (m, 4 H, ArH).

The HCl salt (-)-*8Z* (30 mg, 0.13 mmol) was passed through acetate resin (Amberlite). The fresh acetate resin was generated by passing 3% aqueous acetic acid through Amberlite IRA-400 OH resin. The compound was loaded on the column and eluted slowly with 3% acetic acid–water, small fractions were collected which was checked by TLC, and those fractions showing a ninhydrin-positive spot were collected and lyophilized to obtain 22 mg (88%) of (-)-*9Z* as a white solid: mp 195–205 °C dec; R_f (BAW) 0.3. $[\alpha]^{20}_D$ -32.2° (*c* 1.5, IN HCl); ^1H NMR (DCl in D_2O_2%) δ 2.02 (acetone), 1.48 (q, 1 H, ∇HA), 1.79 (q, 1 H, ∇HB), 2.74 (q, 1 H, ∇HX), 6.4–7.0 (m, 4 H, ArH); ^{13}C NMR (*p*-dioxane) δ 16.9 (∇CH_2), 29.59 (∇CH), 38.57 (∇C), 115.6, 116.52, 121.5, 130.49, 133.27, 158.8, 171.95 (COOH). Anal. ($C_{10}H_{13}NO_4$) C, H, N.

(+)-(*Z*)-2,3-Methano-*m*-tyrosine [(+)-9*Z*]. To a solution of (+)-*7Z* (100 mg, 0.26 mmol) and *m*-cresol (0.15 mL) in dioxane (3 mL) was added dropwise 4 N HCl in dioxane (4 mL) at 0 °C. The reaction mixture was stirred for 4 h at 0 °C. When TLC showed completion of reaction, it was evaporated to an oil, which was triturated with diethyl ether to give a solid. This was recrystallized from 2-propanol–diethyl ether to give 45 mg (75%) of (+)-*8Z*: R_f (BAW) 0.72; mp 200–210 °C dec; $[\alpha]^{20}_D$ +30.1° (*c* 2, H_2O); ^1H NMR (D_2O) δ 2.02 (acetone), 1.5 (q, 1 H, ∇HA), 1.85 (q, 1 H, ∇HB), 2.8 (q, 1 H, ∇HX), 6.8–7.2 (m, 4 H, ArH).

The HCl salt of (+)-*8Z* (40 mL, 0.17 mmol) was passed through acetate resin (Amberlite). The fresh acetate resin was generated by passing 3% aqueous solution of acetic acid through Amberlite IRA-400 OH resin. After regeneration, the compound was loaded on the column and eluted slowly with 3% acetic acid–water, fractions showing a ninhydrin-positive spot were collected and lyophilized to obtain 30 mg (89%) of (+)-*9Z* as a white solid: mp 195–205 °C dec; R_f (BAW) 0.3; $[\alpha]^{20}_D$ +32.4° (*c* 2, 1N HCl); ^1H NMR ($CDCl_3$, in D_2O 2%) δ 2.02 (acetone), 1.47 (q, 1 H, ∇HA), 1.78 (q, 1 H, ∇HB), 2.72 (q, 1 H, ∇HX), 6.35–6.9 (m, 4 H, ArH); ^{13}C NMR (*p*-dioxane) δ 16.9 (∇CH_2), 29.58 (∇CH), 38.55 (∇C), 115.55, 116.50, 121.5, 130.48, 133.28, 158.8, 171.9 (COOH). Anal. ($C_{10}H_{13}NO_4$) C, H, N.

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