Synthesis of Photolabile "Precursors" of Amino Acid Neurotransmitters

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The synthesis of photolabile precursors of the neurotransmitters for the aspartic acid, glutamic acid, glycine, and γ-aminobutyric acid receptors are reported. These compounds are designed for the rapid, photochemically initiated release of the neurotransmitter. The importance of rapidly photolyzed, inert (toward a neuronal receptor) precursors of neurotransmitters is in chemical kinetic investigations of the reactions involving the neuronal receptors. In the past, such studies have been hampered because of the insufficient time resolution of the available techniques. A photolyzable precursor of carbamoylcholine, an amino-group-containing analogue of acetylcholine, has been used previously to make measurements of currents flowing through the acetylcholine receptor channel within 100 μs.

Membrane proteins form the basis of cellular communication in the nervous system and play an important role in brain function.[2] Central to the transmission processes are chemical reactions in which membrane proteins form transmembrane channels upon interaction with specific chemical signals (neurotransmitters). The membrane proteins include the receptors activated by acetylcholine, γ-aminobutyric acid (GABA), β-alanine, glycine, and glutamate. The application of chemical kinetic investigations, using fast-reaction techniques, to studies of these receptor-mediated reactions is recent;[3,4] the use of such techniques has been restricted to studies of receptor proteins in membrane vesicles that have a time resolution of 5 ms[3] or to measurements with receptor-containing cells[5] that can be resolved only to 20 ms. Kinetic measurements of biological reactions, faster than those currently available, can be obtained by the photolysis of inert precursors of compounds that participate in the reactions. Earlier studies[6-11] demonstrated the utility of photolabile precursors of biologically important phosphates in such investigations. Photolabile precursors of carbamoylcholine, a specific ligand for the acetylcholine receptor, have been synthesized[12,14] and allow chemical kinetic investigations of the receptor-mediated reaction with a 100-μs time resolution. Here we describe the synthesis of photolabile precursors of neurotransmitters that activate neuronal receptors specific for aspartic acid, glutamic acid, glycine, or γ-aminobutyric acid and some initial investigations of the photolysis of a photolabile glycine derivative.

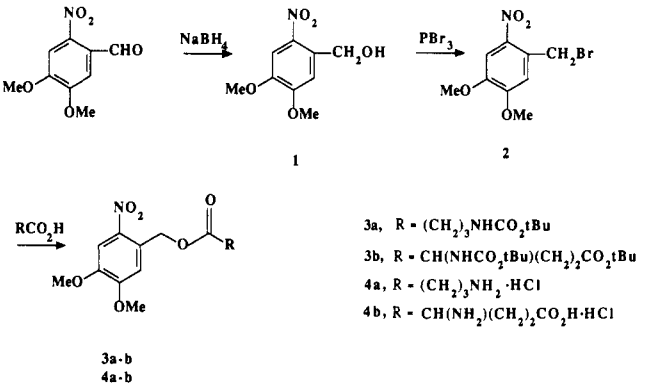
Results and Discussion

Three different pathways were used to synthesize seven photolyzable compounds (Scheme I, compounds **4a** and **4b**; Scheme II, compounds **6c**, **9a–d**) that feature the photolabile moiety (4,5-dimethoxy-2-nitrophenyl)methyl or 1-(4′,5′-dimethoxy-2′-nitrophenyl)ethyl bonded to an amino acid by either an ester or an amine linkage.

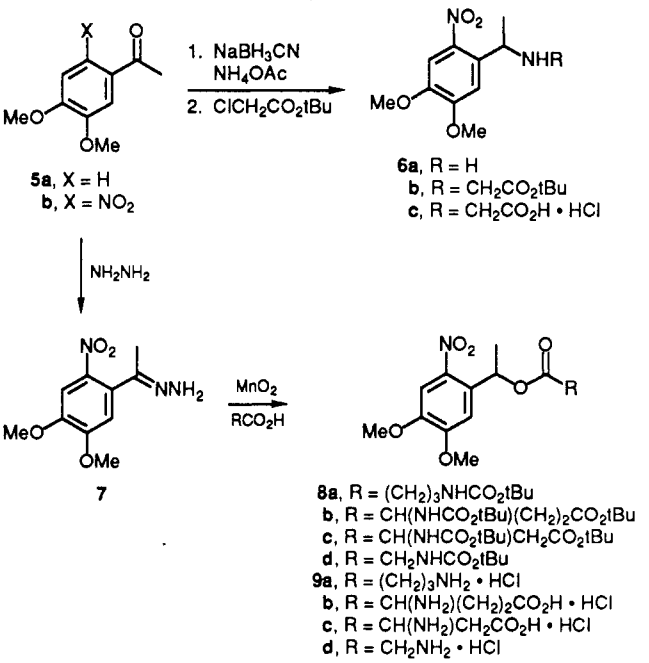
The first approach to the formation of an ester linkage between the amino acid and the photolabile alkyl group involved the synthesis of 1-(bromomethyl)-4,5-dimethoxy-2-nitrobenzene, **2**. The requisite bromide, **2**, was obtained by reduction of 4,5-dimethoxy-2-nitrobenzaldehyde with sodium borohydride to the corresponding alcohol, **1**, followed by reaction of **1** with phosphorus tribromide.

Treatment of N-protected 4-aminobutanoic acid with **2** in the presence of potassium fluoride and acetone gave the corresponding ester, **3a**. Removal of the protecting group by treatment with anhydrous hydrogen chloride in ethyl acetate gave the photolabile derivative of GABA, **4a**.

Scheme I



Scheme II



Similar treatment of *N*-(*tert*-butoxycarbonyl)-L-glutamic acid β-*tert*-butyl ester with **2** gave **3b**, which was then

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(2) Kandel, E. R.; Schwartz, J. H. *Science* **1985**, *218*, 433.

0022-3263/90/1955-1585$02.50/0

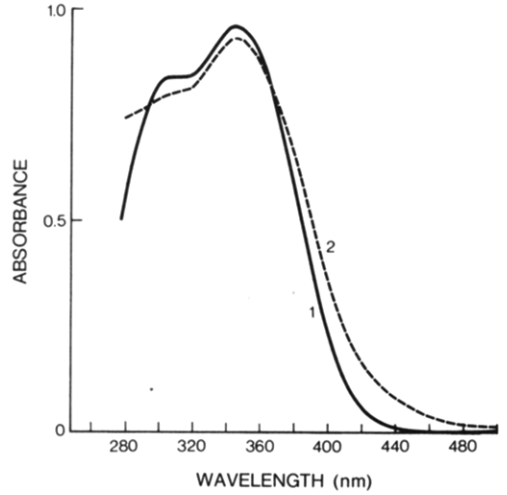

Figure 1. Effect of laser photolysis upon the visible and the ultraviolet spectrum of compound **9d** (photolabile derivative of glycine). An aqueous solution of 0.166 mM compound **9d** (0.6 cm^3) in 0.2 M borate buffer, pH 9.5, was exposed to an excimer laser beam at 308 nm for 130 pulses at about 20 mJ/pulse. A spectrum was recorded before (1) and after (2) photolysis; measurements were made with a Cary 219 spectrophotometer.

deprotected to give the photolabile glutamate, **4b**. The overall yield of compounds **4a** and **4b** was 19 and 10%, respectively.

The second method of esterification required the precursor 4,5-dimethoxy-2-nitroacetophenone, **5b**. Treatment of 3,4-dimethoxyacetophenone, **5a**, with concentrated nitric acid at 18–22 °C for 2 h gave **5b** in 45% yield. Ketone **5b** readily formed 4,5-dimethoxy-2-nitroacetophenone hydrazone, **7**, when mixed with hydrazine hydrate in ethanol and acetic acid. In a modification of the procedure of Walker et al.[12] for the formation of phosphate esters, the hydrazone, **7**, in chloroform was mixed with manganese dioxide. The filtrate from this mixture was stirred in the dark with the appropriately protected amino acids. Esters **8a–d** were prepared by this method and were deprotected to give **9a–d** in overall yields of 16, 11, 5, and 4%, respectively.

The third synthetic route involved the reaction of a substituted ethylamine, 1-(4′,5′-dimethoxy-2′-nitrophenyl)ethyl amine, **6a**, with *tert*-butyl chloroacetate in acetonitrile and a proton scavenger (Proton Sponge), *N,N,N′,N′*-tetramethyl-1,8-napthalenediamine, to give compound **6b**. Removal of the ester-blocking group with anhydrous hydrogen chloride in ethyl acetate gave the photolyzable glycine derivative, **6c**, in an overall yield of 6%. The substituted amine was prepared by reductive amination of 4,5-dimethoxy-2-nitroacetophenone, following the procedure of Borch et al.[13].

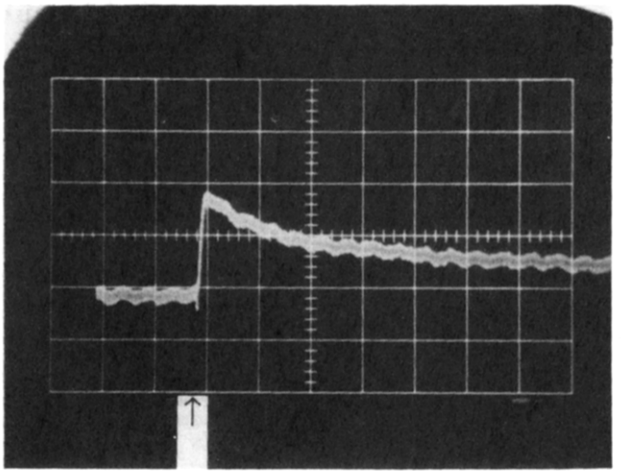

Figure 2. Flash photolysis of 2.5 mM compound **9d**, 0.2 M borate buffer, pH 9.5. The absorbance at 406 nm was recorded before and after a pulse (indicated by an arrow) of about 10 mJ from an excimer laser at 308 nm (X axis = 1 s $division^{-1}$; Y axis = 0.5 mV $division^{-1}$; the increase in absorbance reflects the response time of the recording instrument).

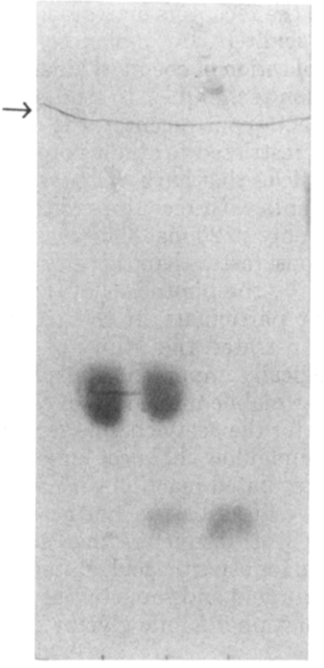

Figure 3. Thin-layer chromatogram of the photolyzable glycine derivative (compound **9d**) before and after photolysis. The material on the chromatogram was detected after treatment with ninhydrin. The solvent used was 1-butanol:glacial acetic acid:H_2O, 4:1:1. An arrow indicates the solvent front: (a) 20 μL of a 3 mM solution of compound **9d** was applied; (b) 3 mM compound **9d** was photolyzed at 308 nm (252 pulses, 4 mJ/pulse, total volume of solution 100 μL and irradiated volume 8 μL), and then 20 μL was applied to the chromatogram; (c) 20 μL of 1.3 mM glycine.

The photolysis characteristics of one of these compounds, the glycine derivative, have been studied. The UV spectra of [1-(4′,5′-dimethoxy-2′-nitrophenyl)ethyl]glycine hydrochloride (**9d** Scheme II) before and after photolysis (130 laser pulses at approximately 20 mJ/pulse) are shown in Figure 1. It can be seen that photolysis results in a decrease in the absorbance of the starting material in the spectra region 290–360 nm and an increased absorbance in the 360–500-nm region. A single 10–20-ns, 10-mJ pulse at 308 nm from an excimer laser delivered to a sample of compound **9d** induced an increase in absorbance followed

(3) Hess, G. P.; Cash, D. J.; Aoshima, H. *Nature* **1979**, *282*, 329.
(4) Hess, G. P.; Udgaonkar, J. B.; Olbricht, W. L. *Ann. Rev. Biophys. Biophys. Chem.* **1987**, *16*, 507.
(5) Udgaonkar, J. B.; Hess, G. P. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 8758.
(6) Doebel, K.; Pfenninger, H. U.S. Patent 3,300,482, **1967**.
(7) Kaplan, J. N.; Forbush, B.; Hoffman, J. F. *Biochemistry* **1978**, *17*, 1929.
(8) Karpen, J. W.; Zimmerman, A. L.; Stryer, L.; Baylor, D. A. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 1287.
(9) McCray, J. A.; Herbette, L.; Kihare, T.; Trentham, D. R. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 7237.
(10) Gurney, A. M.; Lester, H. A. Physiol. *Rev.* **1987**, *67*(2), 583.
(11) Walker, J. W.; Reid, G. P.; McCray, J. A.; Trentham, D. R. *J. Am. Chem. Soc.* **1988**, *110*, 7010.
(12) Walker, J. W.; McCray, J. A.; Hess, G. P. *Biochemistry* **1986**, *25*, 1799.
(13) Borch, R. F.; Bernstein, H. D.; Durst, H. D. *J. Am. Chem. Soc.* **1970**, *93*, 12.

by a slower decrease (Figure 2). The major component of this decrease has a time constant of ~1 s. The absorption maximum of the transient intermediate was found to be near 410 nm. The absorption properties of the transient species are similar to those observed in experiments with nitrobenzyl derivatives of ATP[7] and carbamoylcholine.[12,14] Thin-layer chromatography (Figure 3) shows that photolysis of compound **9d** results in the liberation of glycine. The left lane in the Figure is the chromatograph of **9d**, the middle lane that of photolyzed **9d**, and the right lane that of glycine.

The photolysis results obtained with **9d** at pH 7.0 are similar to those observed by Karpen and colleagues[8] with a 4,5-dimethoxy-2-nitrobenzyl ester of a phosphate, guanosine 3′5′-(cyclic)monophosphate (cGMP). This photolyzable cGMP shows spectral changes at 410 nm, which occur within 100 μs after exposure to an excimer laser pulse at 351 nm. Measurements of the release of cGMP in the photolysis reaction, using the cGMP-sensitive cation channels of retinal rods as an assay, are consistent with the rapid photolysis of this derivative of cGMP.[8]

In the case of a photolabile derivative of carbamoylcholine it has been shown that the whole-cell current-recording technique[15] in combination with laser pulse photolysis allows chemical kinetic measurements to be made of the acetylcholine receptor-mediated reaction directly on the surface of a single cell in the submillisecond time region.[14] The results presented here indicate that photolabile derivatives of amino acid neurotransmitters can be synthesized and that photolysis leads to liberation of the neurotransmitters. The use of these compounds in combination with the whole-cell current-recording technique and laser pulse photolysis may allow chemical kinetic investigations to be made of the diverse receptors in neuronal cells that are activated by a variety of amino-group-containing neurotransmitters.

Experimental Section

Proton NMR spectra were recorded at 200 MHz on a Varian XL200 spectrometer, and spectral data are reported for all compounds. Melting points are uncorrected. Satisfactory combustion analyses were obtained on title compounds **4a,b**, **6c**, and **9a–d**. The purity of all intermediate compounds was judged to be >95% by ^{1}H NMR spectral determinations.

High-Performance Liquid Chromatography. A Dynamax modular macro C18 column equipped with a guard column and UV detector (212 nm) was used for the purification of 1-[1-(4′,5′-dimethoxy-2′-nitrophenyl)ethyl] hydrogen aspartate mono(trifluoroacetate).

Thin-Layer Chromatography (TLC). Thin-layer chromatography was performed using silica gel. The TLC plate was developed for approximately 130 min in 1-butanol:acetic acid:H_2O (4:1:1) and dried. It was sprayed with ninhydrin (0.3% in 95% ethanol) and left overnight in the dark. From a ninhydrin color comparison with standard samples of glycine we estimate that 5–10% of the photolabile precursor of glycine was photolyzed.

Photolysis. An excimer laser (Lumonics 860, 10–20 mJ/pulse at 308 nm) was used. A rectangular quartz cuvette with a 2-mm light-path length was used to measure absorption. The wavelength of the detecting light was selected by a monochromator (McPherson Model 275) and measured by using a photomultiplier (EMI 9635QB).

(4,5-Dimethoxy-2-nitrophenyl)methanol (1). To a stirred mixture of 4,5-dimethoxy-2-nitrobenzaldehyde (2.0 g, 9.5 mmol, Aldrich Chemical Co., 80%) and 40 mL of methanol was added sodium borohydride (0.18 g, 4.8 mmol). After 1 h, 10 mL of water was added, and the mixture was extracted three times with chloroform. The combined chloroform layers were dried and evaporated to an oil, which was purified by chromatography on silica gel using methylene chloride as the eluent; yield 1.4 g (69%); mp 145–147 °C (lit.[6] mp 145–146 °C); ^{1}H NMR ($CDCl_3$) δ 7.70 (s, 1 H), 7.15 (s, 1 H), 4.94 (s, 2 H), 3.98 (s, 3 H), 3.92 (s, 3 H).

1-(Bromomethyl)-4,5-dimethoxy-2-nitrobenzene (2). To a chilled solution of compound 1 (0.372 g, 1.74 mmol) in 50 mL of benzene and 3 drops of dry pyridine was added, dropwise, phosphorus tribromide (0.27 g, 0.093 mL, 1.0 mmol) in 2 mL of benzene. After 24 h, 5 mL of water was added, and the solution extracted with diethyl ether. The solvent was removed by evaporation, and the crude product was recrystallized from toluene and pentane to give 0.313 g (64.6%); mp 131–132 °C; ^{1}H NMR ($CDCl_3$) δ 7.68 (s, 1 H), 6.95 (s, 1 H), 4.87 (s, 2 H), 4.0 (s, 3 H), 3.94 (s, 3 H).

(4,5-Dimethoxy-2-nitrophenyl)methyl 4-[*N*-(*tert*-Butoxycarbonyl)amino]butanoate (3a). Compound **2** (56 mg, 0.20 mmol), 4-[*N*-(*tert*-butoxycarbonyl)amino]butanoic acid (41 mg, 0.20 mmol), and anhydrous potassium fluoride (23 mg, 0.4 mmol) were dissolved in 5 mL of acetone. The solution was heated to 50 °C for 16 h, diluted with 20 mL of water, and then extracted four times with ethyl acetate. The combined ethyl acetate layers were dried, and the solvent was removed by evaporation to give a solid. Purification by chromatography on silica gel using pentane and diethyl ether (1:1) gave 69 mg (85%); mp 96–97 °C; ^{1}H NMR ($CDCl_3$) δ 7.7 (s, 1 H), 7.0 (s, 1 H), 5.5 (s, 2 H), 3.97 (s, 3 H), 3.94 (s, 3 H), 3.15 (q, 2 H, J = 6 Hz), 2.45 (t, 2 H, J = 6 Hz), 1.85 (m, 2 H), 1.35 (s, 9 H).

1-[(4′,5′-Dimethoxy-2′-nitrophenyl)methyl] 5-*tert*-Butyl *N*-(*tert*-Butoxycarbonyl)glutamate (3b). To 1-(bromomethyl)-4,5-dimethoxy-2-nitrobenzene (355 mg, 1.27 mmol), γ-*tert*-butyl *N*-(*tert*-butoxycarbonyl)-L-glutamic acid (385 mg, 1.27 mmol), and anhydrous potassium fluoride (149 mg, 2.6 mmol) was added 5 mL of acetone. The solution was heated to 50 °C for 28 h, diluted with 60 mL of water, and then extracted four times with ethyl acetate. The combined ethyl acetate layers were dried, and the solvent was removed by evaporation to give a yellow oil. Purification by chromatography on silica gel using methylene chloride and diethyl ether (1:1) as the eluent gave 429 mg (69%) of a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.7 (s, 1 H), 7.1 (s, 1 H), 5.5 (d, 2 H, J = 5 Hz), 5.1 (s, 1 H), 3.99 (s, 3 H), 3.92 (s, 3 H), 2.33 (m, 2 H), 2.1 (m, 2 H), 1.39 (s, 9 H), 1.36 (s, 9 H).

(4,5-Dimethoxy-2-nitrophenyl)methyl 4-Aminobutanoate Monohydrochloride (4a). Compound **3a** (70 mg, 0.18 mmol) was stirred with 1 mL of 3 M anhydrous hydrogen chloride in ethyl acetate at room temperature for 1 h. Diethyl ether was added, and the product collected. Recrystallization from ethyl acetate, methanol, and diethyl ether gave 37 mg (61%): mp 168–169 °C; ^{1}H NMR (D_2O) δ 7.55 (s, 1 H), 6.9 (s, 1 H), 5.2 (s, 2 H), 3.82 (s, 3 H), 3.78 (s, 3 H), 2.95 (t, 2 H, J = 7.8 Hz), 2.5 (t, 2 H, J = 7.8 Hz), 1.9 (m, 2 H). Anal. Calcd for $C_{13}H_{19}O_6N_2Cl$: C, 46.64; H, 5.72; N, 8.37. Found: C, 46.88; H, 5.93; N, 8.16.

1-[(4′,5′-Dimethoxy-2′-nitrophenyl)methyl] Hydrogen Glutamate Hydrochloride (4b). Compound **3b** (429 mg, 0.87 mmol) was stirred with 15 mL of anhydrous 3.8 M hydrogen chloride in ethyl acetate at room temperature for 1 h. Diethyl ether was added until the product precipitated. Recrystallization from ethyl acetate gave 122 mg (37%) of a yellow solid: mp 165–167 °C; ^{1}H NMR (D_2O) δ 7.75 (s, 1 H), 7.1 (s, 1 H), 5.5 (m, 2 H), 4.2 (t, 1 H, J = 7 Hz), 3.9 (s, 3 H), 3.85 (s, 3 H), 2.45 (m, 2 H), 2.15 (m, 2 H). Anal. Calcd for $C_{14}H_{19}O_8N_2Cl$: C, 44.39; H, 5.04; N, 7.40. Found: C, 44.59; H, 5.19; N, 7.21.

4,5-Dimethoxy-2-nitroacetophenone (5b). 4,5-Dimethoxyacetophenone (**5a**, 15 g, 0.083 mol, Aldrich) was added over 1 h to 90 mL of concentrated nitric acid with stirring while the internal reaction temperature was kept between 18–22 °C. After the addition was complete, the solution was stirred for an additional hour and then poured into 1200 mL of water. After chilling, the product was collected and recrystallized from ethanol to furnish 8.4 g (45%) of compound **5b**: mp 130–132 °C (lit.[16] mp 133–136

(14) Milburn, T.; Matsubara, N.; Billington, A. P.; Udgaonkar, J. B.; Walker, J. W.; Carpenter, B. K.; Webb, W. W.; Marque, J.; Denk, W.; McCray, J. A.; Hess, G. P. *Biochemistry*, **1989**, *28*, 49.

(15) Hamill, O. P.; Marty, A.; Neher, E.; Sakmann, B.; Sigworth, F. J. *Pflügers Arch. Eur. J. Physiol.* **1981**, *391*, 85.

(16) Minielli, J.; Scarborough, H. Fr. M 3207; *Chem. Abstr.* **1967**, *63*, 13207.

°C); ^{1}H NMR ($CDCl_3$) δ 7.6 (s, 1 H), 6.7 (s, 1 H), 4.0 (s, 6 H), 2.5 (s, 3 H).

[1-(4′,5′-Dimethoxy-2′-nitrophenyl)ethyl]amine (6a). To 4,5-dimethoxy-2-nitroacetophenone (**5b**, 8.25 g, 0.367 mol) was added anhydrous methanol (100 mL), ammonium acetate (38.5 g, 0.5 mol), and sodium cyanoborohydride (2.2 g, 0.035 mol). A drying tube was attached and the mixture stirred at room temperature. After 4 days, 200 mL of methanol was added, and the mixture heated to 60 °C for 24 h. The suspension was filtered, and the filtrate evaporated to a yellow oil. The oil was dissolved in 200 mL of 1 M hydrochloric acid and then extracted with 4 × 50 mL of ether. The ether phase was discarded. The pH of the aqueous phase was raised to 11 by addition of potassium hydroxide. A second ether extraction (4 × 50 mL) removed the amine product. The ether solution was dried ($MgSO_4$) and evaporated to give 8.96 g (23.6%) of a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.5 (s, 1 H), 7.3 (s, 1 H), 4.8 (q, 1 H, J = 6 Hz), 3.98 (s, 3 H), 3.91 (s, 3 H), 1.6 (b, 2 H), 1.43 (d, 3 H, J = 6.8 Hz).

***tert*-Butyl [*N*-[1-(4′,5′-Dimethoxy-2′-nitrophenyl)ethyl]amino]ethanoate (6b).** The substituted amine **6a** (1.17 g, 0.00518 mol) was mixed with 5 mL of acetonitrile and added to a solution of *tert*-butyl chloroacetate (0.780 g, 0.005 18 mol, Aldrich) and Proton Sponge (1.11 g, 0.005 18 mol, Aldrich) in 30 mL of acetonitrile. The mixture was stirred and heated at 60 °C for 15 h. The dark solution was cooled and evaporated to remove acetonitrile. The residue was triturated three times with diethyl ether, and the combined ether layers were reduced to an oil. Purification of the oil by chromatography on silica gel using diethyl ether and pentane, 3:1, as the eluent gave 0.67 g (39%) of a yellow oil: ^{1}H NMR ($CDCl_3$) δ 7.45 (s, 1 H), 7.36 (s, 1 H), 4.47 (q, 1 H, J = 6.4 Hz), 3.96 (s, 3 H), 3.91 (s, 3 H), 3.08 (d, 2 H, J = 2.8 Hz), 2.78 (impurity in starting material), 1.9 (br, 1 H), 1.4 (m, 12 H).

[*N*-[1-(4′,5′-Dimethoxy-2′-nitrophenyl)ethyl]amino]ethanoic Acid Hydrochloride (6c). To compound **6b** (0.67 g, 0.0020 mol) was added 15 mL of anhydrous 4 M hydrogen chloride in ethyl acetate. The solution was stirred at 40 °C for 45 min to give 400 mg (63%) of compound **6c** after recrystallization from a mixture of ethyl acetate, methanol, and diethyl ether; ^{1}H NMR (D_2O) δ 7.68 (s, 1 H), 7.19 (s, 1 H), 5.12 (q, 1 H, J = 7 Hz), 3.93 and 3.80 (s, 6 H), 3.76 (d, 2 H, J = 3.0 Hz), 1.67 (d, 3 H, J = 6.8 Hz). Anal. Calcd for $C_{12}H_{17}O_6N_2Cl$: C, 44.93; H, 5.34; N, 8.74. Found C, 45.12; H, 5.16; N, 8.54.

4,5-Dimethoxy-2-nitroacetophenone Hydrazone (7). To compound **5b** (8.38 g, 0.0372 mol) in 144 mL of ethanol (95%) was added 5.71 g (0.097 mol) of 85% hydrazine hydrate and 7.2 mL of glacial acetic acid. The mixture was heated under reflux for 3 h and then poured into 1 L of water and chilled for 24 h. The yellow precipitate was collected and recrystallized from toluene to give 4.7 g (53%); mp 112–114 °C; ^{1}H NMR ($CDCl_3$) δ 7.6 (s, 1 H), 6.9 (s, 1 H), 4.7 (s, 2 H), 3.9 (s, 6 H), 2.0 (s, 3 H).

1-(4′,5′-Dimethoxy-2′-nitrophenyl)ethyl 4-[(*tert*-Butoxycarbonyl)amino]butanoate (8a). To a solution of compound **7** (1.13 g, 0.00473 mol) in 15 mL of chloroform was added 5 g of manganese dioxide (Aldrich). The mixture was stirred for 7 min in the dark and then filtered by gravity into a solution of 4-[(*tert*-butoxycarbonyl)amino]butyric acid (Sigma) in 20 mL of chloroform. The reaction mixture was stirred in the dark for 2 days and then passed through 1 g of silica gel to decompose excess reactant. The yellow eluent was evaporated to an oil and chromatographed on 40 g of silica gel by using diethyl ether and pentane, 2:1. Fractions collected containing a component with an R_f of 0.3 were pooled to give **8a**: yield 0.516 g (76.3%); mp 83.5–85 °C; ^{1}H NMR ($CDCl_3$) δ 7.5 (s, 1 H), 7.0 (s, 1 H), 6.5 (q, 1 H, J = 7 Hz), 4.0 (s, 3 H), 3.9 (s, 3 H), 3.1 (t, 2 H, J = 7 Hz), 2.3 (t, 2 H, J = 7 Hz), 1.8 (m, 2 H), 1.6 (d, 3 H, J = 6.0 Hz), 1.4 (s, 9 H).

1-[1-(4′,5′-Dimethoxy-2′-nitrophenyl)ethyl] 5-*tert*-Butyl *N*-(*tert*-Butoxycarbonyl)glutamate (8b). To a solution of compound **7** (654 mg, 2.7 mmol) in 10 mL of chloroform was added 5.0 g of manganese dioxide (Aldrich) in the dark. The mixture was stirred for 7 min before being filtered via gravity into a solution of *N*-(*tert*-butoxycarbonyl)-α-L-glutamic acid γ-*tert*-butyl ester (976 mg, 1.9 mmol, Sigma) in 20 mL of chloroform in the dark. The red solution was stirred in the dark for 2 days and then evaporated to a yellow oil, which was chromatographed on 55 g of silica gel by using diethyl ether and pentane, 2:1. Fractions collected containing a component with an R_f of 0.25 were pooled to give a yellow oil, 732 mg (76%); ^{1}H NMR ($CDCl_3$) δ 7.6 (s, 1 H), 7.05 (s, 1 H), 6.5 (br, 1 H), 5.0 (br, 1 H), 4.3 (br, 1 H), 4.0 (s, 3 H), 3.9 (s, 3 H), 2.4 (m, 2 H), 2.2 (m, 2 H), 1.6 (d, 3 H, J = 6 Hz), 1.4 (m, 18 H).

1-[1-(4′,5′-Dimethoxy-2′-nitrophenyl)ethyl] 4-*tert*-Butyl *N*-(*tert*-Butoxycarbonyl)aspartate (8c). To a solution of compound **7** (280 mg, 1.3 mmol) in 10 mL of chloroform was added 2.0 g of manganese dioxide (Aldrich). The mixture was stirred in the dark for 7 min and then filtered by gravity into a solution of the dicyclohexylammonium salt of β-*tert*-butyl-*N*-(tert-butoxycarbonyl)-L-aspartic acid (356 mg, 0.76 mmol, Sigma) in 10 mL of chloroform. The solution was stirred in the dark for 2 days, diluted with 20 mL of methylene chloride and passed through 3 g of silica gel. The yellow eluent was evaporated to an oil and chromatographed on 30 g of silica gel by using diethyl ether and pentane, 2:1. Fractions collected containing a component with an R_f of 0.25 were pooled to give a yellow oil: yield 0.405 g (87%); ^{1}H NMR ($CDCl_3$) δ 7.6 (s, 1 H), 7.1 (s, 1 H), 6.5 (s, 1 H), 5.5 (m, 1 H), 4.5 (m, 1 H), 4.0 (s, 3 H), 3.9 (s, 3 H), 2.8 (m, 2 H), 1.6 (m, 3 H), 1.4 (m, 18 H).

1-[1-(4′,5′-Dimethoxy-2′-nitrophenyl)ethyl] *N*-(*tert*-Butoxycarbonyl)glycinate (8d). To a solution of compound **7** (820 mg, 3.4 mmol) in 20 mL of chloroform was added 3.6 g of manganese dioxide (Aldrich) in the dark. The mixture was stirred for 7 min and then filtered by gravity into a solution of *N*-(*tert*-butoxycarbonyl)glycine (301 mg, 1.7 mmol, Sigma) in 15 mL of chloroform. The solution was stirred for 3 days in the dark and then evaporated to an oil which was chromatographed on 50 g of silica gel by using diethyl ether and pentane, 1.25:1. Fractions containing a component with an R_f of 0.43 were pooled to give a yellow oil, 548 mg (84%); ^{1}H NMR ($CDCl_3$) δ 7.6 (s, 1 H), 7.02 (s, 1 H), 6.5 (q, 1 H, J = 6 Hz), 5.0 (br, 1 H), 4.0 (s, 3 H), 3.9 (s, 3 H), 3.9 (s, 2 H), 1.6 (d, 3 H, J = 6 Hz), 1.4 (s, 9 H).

1-(4′,5′-Dimethoxy-2′-nitrophenyl)ethyl 4-Aminobutanoate Hydrochloride (9a). Compound **8a** (100 mg, 0.24 mmol) was stirred with anhydrous 3 M hydrogen chloride in ethyl acetate for 1 h at room temperature. Diethyl ether was added until the solution turned cloudy. Chilling at 0 °C furnished a crystalline compound which, after recrystallizing from ethyl acetate and diethyl ether, yielded 73 mg (87%); mp 136–137 °C; ^{1}H NMR (D_2O) δ 7.5 (s, 1 H), 7.1 (s, 1 H), 6.2 (q, 1 H, J = 6 Hz), 3.9 (s, 3 H), 3.8 (s, 3 H), 2.9 (t, 2 H, J = 7 Hz), 2.5 (t, 2 H, J = 7 Hz), 1.9 (m, 2 H), 1.5 (d, 3 H, J = 6.6 Hz). Anal. Calcd for $C_{14}H_{21}O_6N_2Cl$: C, 48.21; H, 6.07; N 8.03. Found: C, 48.49; H, 6.18; N, 7.91.

1-[1-(4′,5′-Dimethoxy-2′-nitrophenyl)ethyl] Hydrogen Glutamate Hydrochloride (9b). To 10 mL of anhydrous 3.5 M hydrochloric acid in ethyl acetate was added compound **8b** (704 mg, 0.82 mmol). The solution was stirred at room temperature for 1 h. Diethyl ether was added until the solution turned cloudy. Chilling in an ice bath gave a crystalline compound, which after recrystallization from ethyl acetate and diethyl ether gave a yellow solid, yield 382 mg (58%); mp 88–90 °C; ^{1}H NMR (D_2O) δ 7.6 (s, 1 H), 7.1 (s, 1 H), 6.4 (m, 1 H), 4.2 (m, 1 H), 3.9 (s, 3 H), 3.80 (s, 3 H), 2.5 (m, 2 H), 2.1 (m, 2 H), 1.6 (d, 3 H, J = 7 Hz). Anal. Calcd for $C_{15}H_{21}O_8N_2Cl$: C, 45.90; H, 5.39; N, 7.13. Found: C, 46.07; H, 5.34; N, 6.93.

1-[1-(4′,5′-Dimethoxy-2′-nitrophenyl)ethyl] Hydrogen Aspartate Mono(trifluoroacetate) (9c). To 10 mL of anhydrous 3.5 M hydrogen chloride in ethyl acetate was added compound **8c** (404 mg, 0.82 mmol). The solution was stirred at room temperature for 1 h. Diethyl ether was added until the solution became cloudy. Chilling at 0 °C gave a yellow solid after recrystallization from ethyl acetate and diethyl ether. Purification by reverse-phase HPLC using a gradient of 0.09% trifluoroacetic acid/acetonitrile in 0.09% trifluoroacetic acid/water gave a yellow oil. Crystallization from ethyl acetate and diethyl ether gave 95 mg (25%); mp 83.5–87.5 °C; ^{1}H NMR (D_2O) δ 7.7 (s, 1 H), 7.2 (s, 1 H), 6.5 (m, 1 H), 4.5 (s, 1 H), 4.0 (s, 3 H), 3.95 (s, 3 H), 3.1 (m, 2 H), 1.7 (m, 3 H). Anal. Calcd for $C_{16}H_{19}O_{10}N_2F_3$: C, 42.21; H, 3.98; N, 6.15. Found: C, 42.58; H, 4.57; N, 6.14.

1-[1-(4′,5′-Dimethoxy-2′-nitrophenyl)ethyl]glycine Hydrochloride (9d). Compound **8d** (500 mg, 1.3 mmol) was stirred with anhydrous 3.3 M hydrogen chloride in ethyl acetate for 100 min at room temperature. Diethyl ether was added until the

solution turned cloudy. Chilling at 0 °C furnished a yellow oil which was recrystallized three times from diethyl ether, methanol, and ethyl acetate to give 81 mg (19%); mp 168–170 °C; ^{1}H NMR (D_2O) δ 7.5 (s, 1 H), 7.1 (s, 1 H), 6.4 (q, 1 H, J = 6 Hz), 3.9 (s, 2 H), 3.9 (s, 3 H), 3.8 (s, 3 H), 1.5 (d, 3 H, J = 6 Hz). Anal. Calcd for $C_{12}H_{17}O_6N_2Cl$: C, 44.94; H, 5.31; N, 8.74. Found: C, 44.51; H, 5.41; N, 8.40.

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Registry No. 1, 1016-58-6; 2, 53413-67-5; **3a**, 123642-54-6; **3b**, 123642-55-7; **4a**, 123642-56-8; **4b**, 123642-57-9; **5a**, 1131-62-0; **5b**, 4101-32-0; **6a**, 123642-58-0; **6b**, 123642-59-1; **6c**, 123642-60-4; **7**, 123642-61-5; **8a**, 123642-62-6; **8b**, 123642-63-7; **8c**, 123642-64-8; **8d**, 123642-65-9; **9a**, 123642-66-0; **9b**, 123642-67-1; **9c**, 123674-19-1; **9d**, 123642-68-2; 4,5-$(MeO)_2$-2-$O_2NC_6H_2CHO$, 20357-25-9; (BOC)$NH(CH_2)_3COOH$, 57294-38-9; BOC-L-Glu(OBu-*t*)-OH, 13726-84-6; $ClCH_2COOBu$-*t*, 107-59-5; BOC-L-Asp(OBu-*t*)-OH·DCHA, 1913-12-8; BOC-Gly-OH, 4530-20-5.

Regioselective Routes to Nucleophilic Optically Active 2- and 3-Carene Systems

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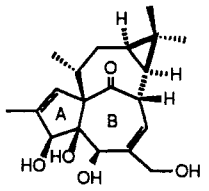
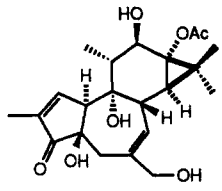
Commercially available (+)-3-carene (**4**) is shown to be capable of efficient conversion to vinyl bromides **28**, **46**, and **49** and to vinyl stannane **44**. All four compounds stem from (+)-3-norcaranone (**23**), an optically pure ketone best prepared by epoxidation of **4**, followed by oxirane ring opening, acetylation, ozonolysis, and $CrCl_2$-promoted reduction. The strong proclivity exhibited by **23** to enolize in the cyclopropyl carbinyl sense is used to advantage to gain entry to **28** and **44**. Remarkably, the tosylhydrazone of (+)-3-norcaranone (**45**) is distinguished from its ketone progenitor **23** by its capacity for highly regioselective deprotonation from the alternative α-position. The crossover has made possible synthetic access to **46** and **49**. Other chemistry of this class of compounds is also presented, including a route to **51**, a vinyl bromide epimeric to **49**. Especially relevant to future work in the ingenol area is the ability of these molecules to serve as nucleophiles. Several reactions involving **28** are provided as exemplary of this property.

Projected syntheses of the tumor-promoting diterpenes ingenol (**1**)[3,4] and phorbol (**2**)[3,5] share in common with the structurally simpler aristolone (**3**)[6] the necessity of fusing a *gem*-dimethylcyclopropane ring to a six-membered carbocycle. In the Ourisson[6a] and Chan[6c] syntheses of **3**, this structural component was introduced by 1,3-dipolar addition of 2-diazopropane to a suitable enone acceptor and subsequent photoextrusion of nitrogen. The key step in Piers' approach[6b] to **3** was a cupric sulfate catalyzed intramolecular diazo ketone variant of the above.



1



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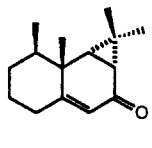

3

To date, the three-membered rings in **1** and **2** have not been introduced in this manner. Instead, tandem dibromocarbene insertion–$Me_2Cu(CN)Li_2$ substitution has been employed,[5a] and a protocol based on Diels–Alder cycloadditions of carbonyl-activated dimethylcyclopropenes has been developed.[4j,44]

Our program goals in the ingenol area[7] necessitated the utilization of preformed C/D ring subunits, *with strong preference given to optically active intermediates readily available from the chiral pool.* This concept is not new, it having been deployed earlier by Yamakawa[4b] and by Funk[4h] as a tool for arriving at right-hand segments of **1**. However, both of these groups proceeded to rupture the

(1) National Science Foundation Graduate Fellow, 1982–1985.
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(3) (a) Hecker, E. *Pure Appl. Chem.* **1977**, *49*, 1423. (b) Hecker, E. In *Carcinogenesie*; Slaga, T. S., Sivak, A., Boutwell, R. K., Eds.; Raven: New York, 1978; Vol. 2 (Mechanism of Tumor Promotion and Carcinogenesis), p 11. (c) Evans, F. J.; Coper, C. J. *Lloydia* **1978**, *41*, 193. (d) Adolf, W.; Hecker, E. *Tetrahedron Lett.* **1980**, *21*, 2887. (e) Falsone, G.; Crea, A. E. G.; Noack, E. A. *Arch. Pharm.* **1982**, *315*, 1026. (f) Sorg, B.; Hecker, E. *Z. Naturforsch., B: Anorg. Chem., Org. Chem.* **1982**, *37B*, 1640. (g) Gotta, H.; Adolf, W.; Opferkuch, H. J.; Hecker, E. *Ibid.* **1984**, *39B*, 683. (h) Rizk, A. M.; Hammouda, F.-M.; El-Missiry, M. M.; Radwan, H. M.; Evans, F. J. *Phytochemistry* **1985**, *24*, 1605. (i) *Naturally Occurring Phorbol Esters*: Evans, F. J., Ed.; CRC: Boca Raton, FL, 1986. (j) *Mechanism of Tumor Promotion*; Slaga, T. J., Ed.; CRC: Boca Raton, FL, 1984; Vols. I–IV.
(4) (a) Paquette, L. A.; Nitz, T. J.; Ross, R. J.; Springer, J. P. *J. Am. Chem. Soc.* **1984**, *106*, 1446. (b) Satoh, T.; Okuda, T.; Kaneko, Y.; Yamakawa, K. *Chem. Pharm. Bull.* **1984**, *32*, 1401. (c) Rigby, J. H.; Moore, T. L.; Rege, S. *J. Org. Chem.* **1986**, *51*, 2398. (d) Funk, R. L.; Bolton, G. L. *J. Am. Chem. Soc.* **1986**, *108*, 4655. (e) Winkler, J. D.; Henegar, K. E.; Williard, P. G. *Ibid.* **1987**, *109*, 2850. (f) Mehta, G.; Pathak, V. P. *J. Chem. Soc., Chem. Commun.* **1987**, 876. (g) Ross, R. J.; Paquette, L. A. *J. Org. Chem.* **1987**, *52*, 5497. (h) Funk, R. L.; Olmstead, T. A.; Parvez, M. *J. Am. Chem. Soc.* **1988**, *110*, 3298. (i) Paquette, L. A.; Ross, R. J.; Springer, J. P. *Ibid.* **1988**, *110*, 6192. (j) Rigby, J. H.; Kierkus, P. Ch. *Ibid.* **1989**, *111*, 4125.
(5) (a) Wender, P. A.; Keenan, R. M.; Lee, H. Y. *J. Am. Chem. Soc.* **1987**, *109*, 4390. (b) Wender, P. A. *Pure Appl. Chem.* **1989**, *61*, 469 and earlier references cited therein.
(6) (a) Berger, C.; Franck-Neumann, M.; Ourisson, G. *Tetrahedron Lett.* **1968**, 3451. (b) Piers, E.; Britton, R. W.; de Waal, W. *Can. J. Chem.* **1969**, *47*, 831. (c) Prasad, C. V. C.; Chan, T. H. *J. Org. Chem.* **1987**, *53*, 120.
(7) (a) For the earlier synthetic thrusts from this laboratory, consult ref. 4a,g,i. (b) See also Paquette, L. A.; Shi, Y.-J. *J. Org. Chem.* **1989**, *54*, 5205. (c) Any intended use of carene synthons, requires, of course, that the carene ring be expanded to approach 1 or be functionalized with a bridgehead hydroxyl to arrive at **2**. These tactics will be detailed elsewhere.

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