Electroreductive Cleavage of 2-Methylene-9,10-anthraquinone (Maq) Esters of Carboxylic Acids and N-Substituted Carbamic Acids: Protecting Groups for Carboxylic Acids and Primary Amines

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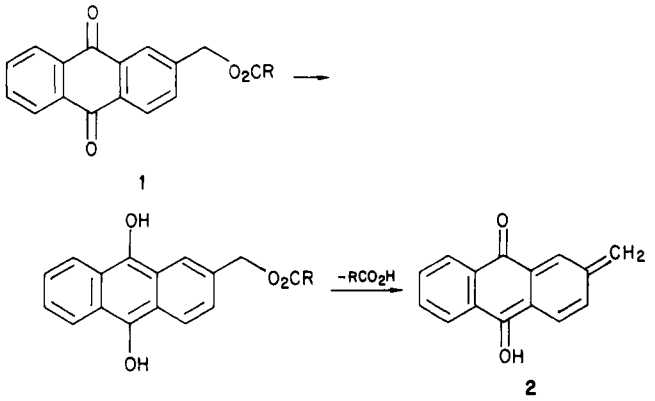
The 2-methylene-9,10-anthraquinone (Maq) ester of γ-(benzyloxycarbonylamino)butyric acid (**3a**) and the Maq ester of *N*-(3-benzyloxycarbonylpropyl)carbamic acid (**4**) were synthesized as possible γ-aminobutyric acid (GABA) delivery systems. Electrochemical reduction of these Maq esters at −0.5 V (SCE) in 50% aqeuous THF buffer (pH 7.0) produced their corresponding anthrahydroquinones, which failed to give detectable cleavage products after several hours. In contrast, reduction of **3a** and **4** in DMF at −0.9 V (−1.2 V) produced their radical anions (dianions), which formed the Maq cleavage products 2-methyl-9,10-anthraquinone and 2-(hydroxymethyl)-9,10-anthraquinone in combined yields of >80%. Comparable yields of the other cleavage products, the GABA esters, were also found. This work demonstrates that electrochemical reduction can be used as an alternative method for deprotection of Maq esters of carboxylic acids used in the synthesis of peptides. In addition, the synthesis of **4** and its reductive cleavage shows that Maq esters of carbamates can serve as protecting groups of primary amines.

Previous work in this laboratory has demonstrated that the neurotransmitters dopamine, glutamate (Glu), and γ-aminobutyric acid (GABA) can be attached to a polystyrene backbone via a cathodically cleavable, cationic isonicotinamide unit.[1] When these polymers were coated onto glassy carbon electrodes, cathodic current resulted in the release of the neurotransmitters. Since very small amounts of neurotransmitter can, in principle, be delivered quantitatively to a specific location by using microelectrodes,[1b] this method has the potential for becoming an alternative to existing modes of drug delivery to single neurons.[2] A limitation intrinsic to the polystyrene/isonicotinamide system is that only a fraction of the attached neurotransmitter is released upon reduction.[1] This results in part because the cathodic cleavage reaction is not clean. In addition, the amount released does not increase proportionately with the thickness of the polymer film, suggesting that charge propagation through the polymer[3] is slow and only those units near the carbon surface are active.

Our goal at the outset of this work was to prepare a better delivery system for neurotransmitters; one that would release a high percentage of its bound neurotransmitter upon electroreduction. Our choice of a cathodically cleavable functional group was the 2-methylene-9,10-anthraquinone (Maq) ester (**1**). Kemp and Reczek have shown that the Maq ester can be used as a carboxyl protective group in peptide synthesis.[4] Mild reducing agents converted the Maq ester to its corresponding hydroquinone, which reportedly underwent cleavage to the peptide and quinone methide intermediate **2**. Tautomerization of **2** gave 2-methyl-9,10-anthraquinone, which was formed quantitatively. Utilizing the Maq ester in the present study broadens the scope of this work. Successful cathodic cleavage of Maq esters not only makes this functional group a candidate for incorporation into a drug delivery system but also suggests an alternative method for deprotection in the synthesis of peptides. Furthermore, there is considerable interest in the cleavage of reduced Maq esters since a similar cleavage is believed to occur in the structurally related antitumor antibiotics, daunomycin and 11-deoxydaunomycin.[5]



In this work we attached GABA to the Maq group in two different ways as shown in structures **3** and **4**. In **3** the amino end group of GABA is protected as a benzyl carbamate, whereas in **4** the carboxylic acid end group of GABA is protected as a benzyl ester. The phenyl group was incorporated into compound **3b** to further activate the hydroquinone cleavage. The electroreduction of **3** and **4** in aqueous buffer and DMF was studied. In aqueous

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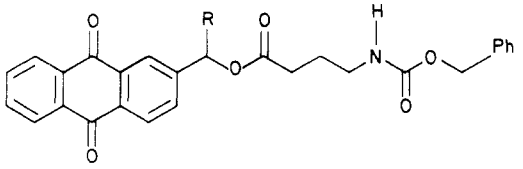
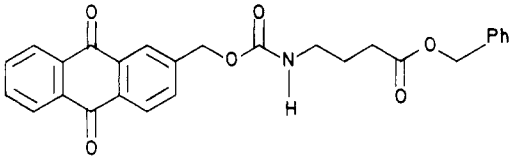


3a, R=H
b, R=Ph



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buffer the anthrahydroquinones of **3** and **4** were formed, which failed to give detectable cleavage products after several hours. In contrast, reduction in DMF produced their corresponding anion radicals or dianions, which cleaved in high yields.

Experimental Section

Electrochemical Measurements. Electrochemical experiments were performed with a Princeton Applied Research (PAR) potentiostat, Model 173, in conjunction with a PAR 175 universal programmer. Voltammograms were recorded on a Houston Omnigraphic 2000 x–y recorder. Although potential measurements were referred to Ag/AgCl in 50% aqueous THF buffer (0.10 M KCl, 0.050 M phosphate) and a saturated calomel electrode (SCE) in DMF, all potentials in the text are referred to SCE (−67 mV vs. Ag/AgCl in the pH 7.0, 50% aqueous THF buffer).

Cyclic Voltammetry. A one-compartment cell was used. The working electrode was a glassy carbon disk (A = 0.090 cm^2) set in a Teflon tube. Prior to measurements on each solution this electrode was cleaned and polished with 0.30 and 0.050 μm α alumina (Buehler), wiped with a tissue, and sonicated in water for 3–5 min. Pretreatment of the electrode consisted of cycling the potential between +1.5 and −1.5 V (SCE) in aqueous phosphate buffer (pH 7.0) 5 times at a scan rate of 100 mV s^{-1}. A graphite rod served as a counterelectrode. In aqueous test solutions (0.10 M Cl^-) an Ag/AgCl reference electrode was used directly. In DMF it was necessary to separate the SCE reference electrode from the test solution to prevent leakage of aqueous solution into the nonaqueous medium. This was accomplished by using in sequence a course glass frit, a 10-cm tube (0.5 cm diameter) containing a DMF (0.50 M $LiClO_4$)/methyl cellulose gel and then directly an aqueous agar (1.0 M NaCl), and a saturated KCl solution in which the SCE was suspended.

General Procedure for Constant Potential Electrolyses in 50% Aqueous THF Buffer. A three-compartment cell was used for the electrolyses. The center compartment, containing a carbon sponge (pretreated by soaking in concentrated HNO_3 for 1–2 min, washing with distilled water, and drying in an oven at 150–200 °C), was separated from both the reference electrode and counterelectrode compartments by a glass frit (medium) and aqueous agar. The counterelectrode was a carbon sponge and the reference compartment contained saturated KCl and the SCE. Approximately 10 mL of 50% aqueous THF buffer (0.10 M KCl, 0.050 M phosphate) was introduced into the center compartment. After deoxygenation with N_2, the background current was measured. The compound to be reduced was added (10–40 mg) and the resulting solution was again deoxygenated. After the electrolysis was completed (1–3 h), as evidenced by constant current with time, the contents of the center compartment were transferred to an Erlenmeyer flask where a stream of air was passed through the solution for 1 h to ensure complete oxidation of anthrahydroquinone to anthraquinone. The resulting mixture was extracted with 3 × 15 mL of CH_2Cl_2. The CH_2Cl_2 extracts were combined, dried over $MgSO_4$, and stripped of solvent in a rotatory evaporator under reduced pressure. The mass of the residue was determined and its NMR spectrum recorded. In the case of **3b**, the only compound that underwent reductive cleavage in this medium, NMR spectra of different ratios of **3b** and its cleavage product, 2-benzyl-9,10-anthraquinone, were recorded so that yields of the latter compound could be determined from the NMR spectra of the **3b** electrolysis mixtures.

General Procedure for Constant Potential Electrolysis in DMF. The three-compartment cell described above was also used in the electrolyses in DMF (0.1 M $LiClO_4$). The compartments, however, were separated by glass frits and DMF/methyl cellulose gel. The reference compartment contained the DMF electrolyte, which was separated from SCE by the series of salt bridges described earlier. Pretreated carbon sponge was used as the working electrode and the counterelectrode. Approximately 10 mL of the DMF electrolyte was introduced into the center compartment and the solution was deoxygenated with N_2. After the background current of this solution was measured, the compound to be reduced was added and the resulting solution was once again deoxygenated. After the electrolysis was complete, the contents of the center compartment were transferred to a separatory funnel, with CH_2Cl_2 for rinsing. Approximately 20 mL of water was added and the resulting mixture was extracted with 3 × 15 mL of CH_2Cl_2. The aqueous layer was set aside and the CH_2Cl_2 extracts were combined and washed with 3 × 20 mL of water. The water washings were combined with the original aqueous layer and set aside for chromatographic analysis. The CH_2Cl_2 solution was dried over $MgSO_4$, filtered, and stripped of most of its solvent in a rotatory evaporator under reduced pressure. A small amount of solvent, presumably DMF, was removed by passing a stream of N_2 over the liquid, leaving a faintly yellow solid. This solid was weighed and chromatographed on silica gel. 2-Methyl-9,10-anthraquinone was rapidly eluted from the column with CH_2Cl_2 followed by a trace of an unidentified yellow solid. After ca. 50 mL of CH_2Cl_2 has passed through the column, the column was eluted with CH_2Cl_2–CH_3OH (ca. 50:1) to give 2-(hydroxymethyl)-9,10-anthraquinone.

Chromatographic Analysis of the Aqueous Extracts of DMF Electrolyses. The aqueous extracts from the electrolysis of **4** in DMF were analyzed directly for $PhCH_2O_2C(CH_2)_3NH_3^+$ by using a Beckman Amino Acid Analyzer, Model 118 BL. The column was 640 × 9 mm Beckman PA 35; the column temperature was 75 °C; the eluant was citrate buffer (pH 4.12); and the flow rate was 104 mL/h. The retention time was identical with that of a sample of $PhCH_2O_2C(CH_2)_3NH_3^+$ $PhSO_3^-$ under the same conditions. The aqueous extracts from the electrolysis of **3b** containing $PhCH_2O_2CNH(CH_2)_3CO_2^-$ were made basic with NaOH (ca. 10.0 g/100 mL of extract) and heated at 95 °C for 45–60 min to hydrolyze the carbamate. After cooling, the pH of the solution was decreased to 2–3 with the addition of concentrated HCl. Analysis with the Beckman Amino Acid Analyzer gave a peak with a retention time identical with that obtained from a solution of GABA. Analysis prior to the NaOH hydrolysis showed that less than 5% GABA was present in the catholyte.

Chemicals. DMF was dried by heating reagent DMF at 60 °C over CaH_2 for 6–10 h before distillation at 45–50 °C under reduced pressure. Further drying was accomplished by stirring the distillate over neutral Al_2O_3 (dried under vacuum at 160–170 °C for several hours) prior to redistillation at 45–50 °C and repeating the procedure. The dry DMF was stored over molecular sieves (4 Å) and under Ar. Reagent grade THF was distilled at atmospheric pressure just prior to use. The 50% aqueous THF buffer (0.10 M KCl, 0.050 M phosphate) was prepared by dissolving KCl and KH_2PO_4 in 450 mL water, adding 500 mL of THF, adjusting the pH to the desired level with the addition of KOH, and adding sufficient water to make 1 L. All other chemicals were obtained from Aldrich Chemical Co. or Fisher Scientific Co. and were used without further purification.

γ-(Benzyloxycarbonylamino)butanoic Acid (5). To 13.0 mL of 2.00 M NaOH (26.0 mmol) was added 2.58 g (25.0 mmol) of γ-aminobutyric acid. To the resulting solution were added simultaneously an additional 13.0 mL of the base and 3.80 mL (26.6 mmol) of benzyl chloroformate with vigorous stirring. The mixture was stirred in an ice bath for 1 h and then at room temperature for an additional 1 h. The reaction mixture was extracted with 3 × 50 mL of ether to remove unreacted benzyl chloroformate. The aqueous layer was acidified with concentrated HCl (pH 3) and cooled in an ice bath. The resulting crystals were suction filtered, washed with 0.10 M HCl, and air-dried to give 5.10 g (86%) of **5**; mp 64–65 °C (lit.[6] mp 66–67 °C). Recrystal-

lization from ethyl acetate/hexane afforded an analytically pure sample: mp 65–67 °C; ^{1}H NMR ($CDCl_3$, 80 MHz) δ 7.32 (s, 5 H, Ar), 5.09 (s, 2 H, benzylic), 3.22 (q, 2 H, J = 6.4 Hz), 2.36 (t, 2 H, J = 6.4 Hz), 1.80 (m, 2 H, J = 6.4 Hz); IR (neat film) 3340, 3070, 3040, 2980, 2930, 1690, 1550, 1450, 1275, 1260, 1210, 1140, 1020, 745, 720, 690 cm^{-1}. Anal. Calcd for $C_{12}H_{15}NO_4$: C, 60.75; H, 6.37; N, 5.90. Found: C, 60.87; H, 6.40; N, 6.00.

Anthraquinone 3a. To a solution of 0.460 g (1.93 mmol) of 2-(hydroxymethyl)-9,10-anthraquinone (**11**), 0.462 g (1.95 mmol) of **5**, and 0.238 g (1.95 mmol) of 4-(dimethylamino)pyridine in 120 mL of CH_2Cl_2 was added a solution of 0.453 g (2.19 mmol) of dicyclohexylcarbodiimide in 30 mL of CH_2Cl_2. The mixture was stirred at room temperature overnight. After filtration, the solvent was removed under reduced pressure in a rotatory evaporator using a 35 °C water bath. Ethyl acetate (50 mL) was added to the residue with stirring. A small amount of undissolved solid was removed by filtration. The filtrate was washed with 100 mL of 1 M $NaHSO_4$, 50 mL of saturated $NaHCO_3$, and 3 × 50 mL of water and dried over $MgSO_4$. After filtration, the solvent was removed under reduced pressure in a rotatory evaporator and the residue was recrystallized from boiling ethyl acetate, giving 0.378 g (43%) of **3a**: mp 134–136 °C; ^{1}H NMR ($CDCl_3$, 80 MHz) δ 8.37–8.26 (m, 4 H, Ar), 7.86–7.74 (m, 3 H, Ar), 7.33 (s, 5 H, Ar), 5.26 (s, 2 H, benzylic), 5.09 (s, 2 H, benzylic), 4.8 (b, 1 H, NH), 3.27 (q, 2 H, J = 6.4 Hz), 2.49 (t, 2 H, J = 7.2 Hz), 1.89 (m, 2 H, J = 7.2); IR (KBr) 3340, 1735, 1685, 1670, 1592, 1535, 1335, 1280, 1185, 710. Anal. Calcd for $C_{27}H_{23}NO_6$: C, 70.89; H, 5.07; N, 3.06. Found: C, 70.93; H, 5.15; N, 3.26.

Benzyl γ-aminobutyrate, benzenesulfonic acid salt (6) was prepared as previously described.[1d]

Benzyl *N*-[(2,3,5-Trichlorophenoxy)carbonyl]-γ-aminobutyrate (7). To a solution of 7.70 g (21.9 mmol) of **6** in 100 mL of CH_2Cl_2 was added 10 mL of Et_3N dropwise while the temperature of the mixture was maintained at 1–2 °C with an ice bath. To this mixture was added a solution of 7.46 g (28.7 mmol) of 2,3,5-trichlorophenyl chloroformate in 50 mL of CH_2Cl_2 dropwise while the temperature was maintained at −5 °C. After the addition, the reaction mixture was stirred at −5 °C for 2 h. The CH_2Cl_2 solution was washed with 3 × 100 mL of 0.5 M HCl, 3 × 100 mL of 2.0 M $NaHCO_3$, and 3 × 50 mL of water, dried over $MgSO_4$, and decolorized with activated charcoal. After filtration, the solvent was removed under reduced pressure in a rotatory evaporator and the residue was recrystallized from ethyl acetate/hexane to give 6.50 g (71%) of **7**: mp 71–72 °C; ^{1}H NMR ($CDCl_3$, 80 MHz) δ 7.50–7.24 (m, 7 H, Ar), 5.3 (b, 1 H, NH), 5.13 (s, 2 H, benzylic), 3.33 (q, 2 H), 2.47 (t, 2 H), 1.93 (m, 2 H); ^{13}C NMR (Me_2SO-d_6) δ 173.089, 153.563, 147.538, 137.207, 131.341, 131.308, 129.731, 129.069, 128.645, 128.612, 127.433, 126.680, 66.397, 41.199, 31.604, 25.556; IR (KBr) 3370, 3090, 3070, 3030, 2950, 2920, 1730, 1525, 1460, 1355, 1260, 1240, 1230, 1210, 1180, 1175, 1125, 1095, 1080, 750, 700 cm^{-1}. Anal. Calcd for $C_{18}H_{16}Cl_3NO_4$: C, 51.89; H, 3.87; N, 3.63; Cl, 25.52. Found: C, 52.01; H, 4.04; N, 3.42; Cl, 25.68.

Benzyl γ-Isocyanatobutyrate (8). To a solution of 14.1 g (33.8 mmol) of **7** and 10.0 mL (78.8 mmol) of chlorotrimethylsilane in 250 mL of dry toluene was added 15 mL (108 mmol) of Et_3N. The mixture was heated at a gentle reflux for a period of 3 h. After cooling in an ice bath, a precipitate was removed by filtration and the solvent distilled off under N_2 at atmospheric pressure. The residue was pyrolized at 170 °C for a period of 1.5 h under N_2 at atmospheric pressure. Vacuum fractional distillation of the pyrolysis products gave two fractions. The first fraction (86–90 °C, 0.05 Torr) consisted of 2,3,5-trichlorophenyl trimethylsilane ether: ^{1}H NMR ($CDCl_3$, 80 MHz) δ 7.43–6.97 (m, 2 H, Ar), 0.30 (s, 9 H, Me). The second fraction gave 3.79 g (51%) of **8**: bp 118–122 °C (0.08 torr); ^{1}H NMR ($CDCl_3$, 80 MHz) δ 7.35 (s, 5 H, Ar), 5.13 (s, 2 H, benzylic), 3.38 (t, 2 H), 2.51 (t, 2 H), 1.93 (m, 2 H); IR (neat film) 3100, 3080, 3050, 2970, 2900, 2300, 1740, 1460, 1360, 1250, 1165, 1080, 850, 750, 735, 695 cm^{-1}. Anal. Calcd for $C_{12}H_{13}NO_3$: C, 65.74; H, 5.98; N, 6.39. Found: C, 65.59; H, 6.07; N, 6.31.

Anthraquinone 4. A solution of 1.78 g (4.94 mmol) of 2-(hydroxymethyl)-9,10-anthraquinone and 1.10 g (5.85 mmol) of **8** in 80 mL of dry toluene–dioxane (2:1) was stirred at 50–55 °C over a period of 3 h. The reaction mixture was cooled to room temperature and stripped of its solvent in a rotatory evaporator under reduced pressure. Dry toluene (50 mL) was added to the residue and some insoluble material was removed by filtration. Removal of solvent under reduced pressure in a rotatory evaporator gave a residue which was recrystallized from acetone, giving 0.637 g (27%) of **4**: mp 123–125 °C; ^{1}H NMR ($CDCl_3$, 80 MHz) δ 8.37–8.24 (m, 4 H, Ar), 7.85–7.60 (m, 3 H, Ar), 7.33 (s, 5 H, Ar), 5.24 (s, 2 H, benzylic), 5.11 (s, 2 H, benzylic), 4.9 (b, 1 H, NH), 3.28 (q, 2 H), 2.44 (t, 2 H), 1.89 (m, 2 H); IR (KBr) 3400, 1745, 1720, 1675, 1595, 1520, 1450, 1335, 1295, 1275, 1260, 1165, 935, 715, 695 cm^{-1}; NH_3 CIMS, m/e 475 ([M + 18]$^+$); HRMS calcd for $C_{20}H_{16}NO_5$ (M − OCH_2Ph) 350.1029, found 350.0999. Anal. Calcd for $C_{27}H_{23}NO_6$: C, 70.89; H, 5.07; N, 3.06. Found: C, 70.70; H, 4.99; N, 3.02.

2-(Bromomethyl)-9,10-anthraquinone. A suspension of 12.5 g (56.2 mmol) of 2-methyl-9,10-anthraquinone, 9.47 g (53.2 mmol) of *N*-bromosuccinimide, and 0.85 g of benzoyl peroxide in 300 mL of dry CCl_4 was heated at a gentle reflux for a period of 12 h. The reaction mixture was cooled and suction filtered. The solid was triturated in 200 mL of methanol, filtered, and rinsed with methanol. Recrystallization from ethyl acetate gave 7.40 g (46%) of 2-(bromomethyl)-9,10-anthraquinone: mp 199–201 °C (lit.[4] mp 198–201 °C); ^{1}H NMR ($CDCl_3$, 80 MHz) δ 8.30–8.19 (m, 4 H, Ar), 7.85–7.74 (m, 3 H, Ar), 4.58 (s, 2 H, benzylic); IR (KBr) 3060, 3030, 1675, 1590, 1385, 1320, 1295, 1230, 980, 935, 710, 675 cm^{-1}.

2-Benzyl-9,10-anthraquinone. To a suspension of 10.8 g (36.0 mmol) of 2-(bromomethyl)-9,10-anthraquinone in 300 mL of dry benzene, cooled in an ice bath, was added 15.2 g (114 mmol) of $AlCl_3$. The mixture was warmed to room temperature and stirred at 60–65 °C for a period of 48 h. The reaction mixture (dark brown) was cooled in an ice bath, and 200 g of crushed ice was added with vigorous stirring, transforming the reaction mixture into a gray suspension. To this suspension was added 20 mL of concentrated HCl and the resulting mixture was extracted with 200 mL of ether. The ether layer was separated, washed with 3 × 100 mL of water, dried over $MgSO_4$, and decolorized with activated charcoal. Removal of solvent under reduced pressure gave a solid that was recrystallized from methanol to give 8.10 g (75%) of 2-benzyl-9,10-anthraquinone: mp 140–142 °C; ^{1}H NMR ($CDCl_3$, 80 MHz) δ 8.34–8.13 (m, 4 H, Ar), 7.87–7.35 (m, 3 H, Ar), 7.31–7.25 (m, 5 H, Ar), 4.13 (s, 2 H, benzylic); IR (KBr) 3080, 3060, 3020, 1675, 1595, 1495, 1455, 1320, 1290, 985, 930, 730, 710, 695 cm^{-1}. Anal. Calcd for $C_{21}H_{14}O_2$: C, 84.54; H, 4.73. Found: C, 84.63; H, 4.71.

2-(α-Bromobenzyl)-9,10-anthraquinone (9). A suspension of 2.17 g (7.29 mmol) of 2-benzyl-9,10-anthraquinone, 1.31 g (7.34 mmol) of *N*-bromosuccinimide, and 0.3 g of benzoyl peroxide in 60 mL of CCl_4 was heated at reflux for 3 h. The reaction mixture was cooled to room temperature and filtered to remove insoluble materials. The solvent in the filtrate was removed under reduced pressure in a rotatory evaporator. The residue was dissolved in 15 mL of hot acetone, 10 mL of dry methanol was added, and the resulting solution was slowly cooled, giving 1.76 g (64%) of **9**: mp 110–112 °C; ^{1}H NMR ($CDCl_3$, 80 MHz) δ 8.38–8.24 (m, 4 H, Ar), 7.96–7.73 (m, 3 H, Ar), 7.45–7.25 (m, 5 H, Ar), 6.36 (s, 1 H); IR (KBr) 3060, 1675, 1590, 1455, 1335, 1295, 980, 935, 710, 700 cm^{-1}. Anal. Calcd for $C_{21}H_{13}O_2Br$: C, 66.86; H, 3.47; Br, 21.18. Found: C, 67.04; H, 3.56; Br, 21.37.

Anthraquinone 3b. A solution of 1.286 g (5.42 mmol) of **5** in 13.7 mL of 0.4 M tetra-*n*-butylammonium hydroxide was stirred at room temperature for 0.5 h. Most of the water was removed under reduced pressure. The remaining water was removed by adding 70 mL of benzene to the liquid residue and heating the mixture at reflux in a Dean–Stark apparatus. The benzene was then removed from the tetra-*n*-butylammonium salt of **5** under reduced pressure. The residue was dissolved in 50 mL of dry DMF. To this solution was added 2.00 g (5.31 mmol) of **9**, and the mixture was heated at 60–65 °C for 8 h with stirring. After cooling, the solvent was removed under reduced pressure and the residue was dissolved in 100 mL of CH_2Cl_2. This solution was washed with 3 × 50 mL of water, dried over $MgSO_4$, and reduced in volume to ca. 3 mL. Chromatography of this solution on a silica gel (60–200 mesh) column (1.5 × 24 in.) followed by elution with

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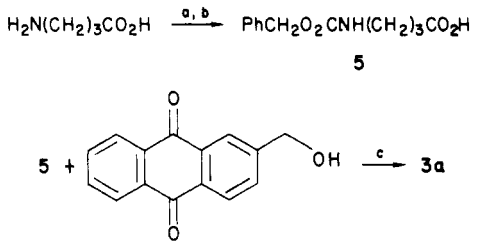
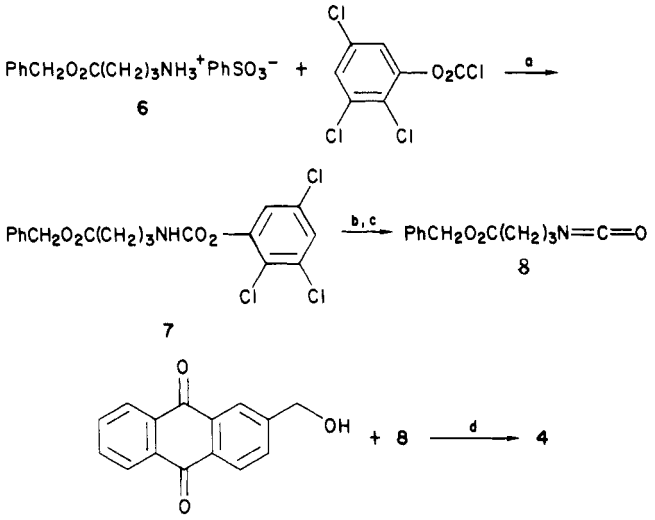
Scheme I[a]



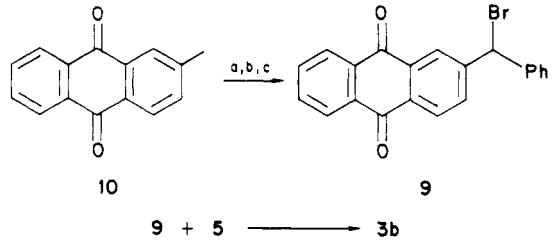
[a] (a) NaOH. (b) $PhCH_2O_2CCl$. (c) 4-(Dimethylamino)-pyridine, dicyclohexylcarbodiimide.

Scheme II[a]



[a] (a) Et_3N, −5 °C. (b) $ClSiMe_3$, Et_3N. (c) 170 °C, 1.5 h. (d) 50–55 °C, 3 h.

Scheme III[a]



[a] (a) NBS, CCl_4, $(PhCO_2)_2$. (b) PhH, $AlCl_3$, 60–65 °C. (c) NBS, CCl_4, $(PhCO_2)_2$.

600 mL of CH_2Cl_2 gave starting materials and an unidentified side product. Elution with 500 mL of methanol gave a solution that was dried over anhydrous $MgSO_4$ and decolorized with activated charcoal. Removal of solvent under reduced pressure gave a residual oil that was dissolved in 5 mL of boiling ether. Slow cooling of this solution overnight in a refrigerator gave 0.349 g (12%) of light yellow crystals of **3b**: mp 131–133 °C; ^1H NMR ($CDCl_3$, 80 MHz) δ 8.34–8.18 (m, 4 H, Ar), 7.82–7.67 (m, 3 H, Ar), 7.34 (s, 5 H, Ar), 7.31 (s, 5 H, Ar), 6.96 (s, 1 H), 5.07 (s, 2 H, benzylic), 4.9 (b, 1 H, NH), 3.24 (q, 2 H), 2.54 (t, 2 H), 1.90 (m, 2 H); IR (KBr) 3360, 3080, 3040, 2970, 2950, 1730, 1695, 1675, 1590, 1540, 1450, 1330, 1300, 1280, 1260, 1165, 1140, 1000, 930, 740, 710, 700 cm^{-1}. Anal. Calcd for $C_{33}H_{27}NO_6$: C, 74.28; H, 5.10; N, 2.63. Found: C, 74.17; H, 5.20; N, 2.26.

Results

Synthesis of Anthraquinones 3 and 4. The synthetic pathways to the GABA-bound anthraquinones **3** and **4** are outlined in Schemes I–III. All of the new compounds had satisfactory elemental analyses and their NMR and IR spectra were consistent with their structures. Anthraquinone **3b** was prepared with R = phenyl to activate the anthrahydroquinone cleavage and to serve as a model to demonstrate the feasibility of attaching **3a** to a polystyrene backbone. Thus, substitution of polystyrene for benzene in the Friedel–Crafts alkylation step in the synthesis of **3b** should provide an entry into the polymer delivery system.

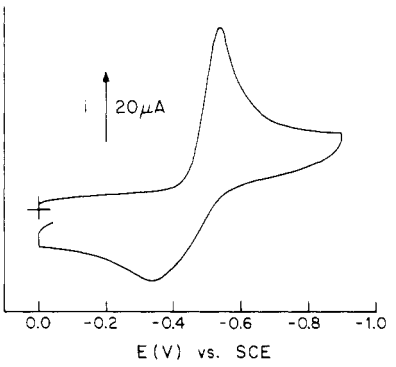


Figure 1. Cyclic voltammogram of 1.5 mM **3a** in 50% aqueous THF buffer (pH 7.0) at a sweep rate of 100 mV s^{-1}.

Table I. Cathodic Peak Potentials Measured by Cyclic Voltammetry

	50% aq THF[a]	DMF[b]	
compd	Ep[c]	Ep(1)[c]	Ep(2)[c]
10	−0.523	−0.93	−1.25
3a	−0.553	−0.91	−1.23
4	−0.535	−0.87	−1.21

[a] pH 7.0 buffer (0.10 M KCl, 0.050 M phosphate). [b] 0.10 M $LiClO_4$. [c] Reference is SCE, sweep rate = 100 mV s^{-1}.

Although the focus of this work is on the cleavage of the Maq group, our choice of the benzyloxycarbonyl protecting group in **3** deserves comment. This group can be cleaved by electroreduction, albeit at rather high negative potentials (ca. −2.6 V vs. SCE).[7] Deprotection by chemical treatment would be very difficult without cleavage of the Maq group as well. A better protecting group for chemical cleavage would be *tert*-butoxycarbonyl, which can be cleaved by reagents such as CF_3CO_2H under mild conditions.[8]

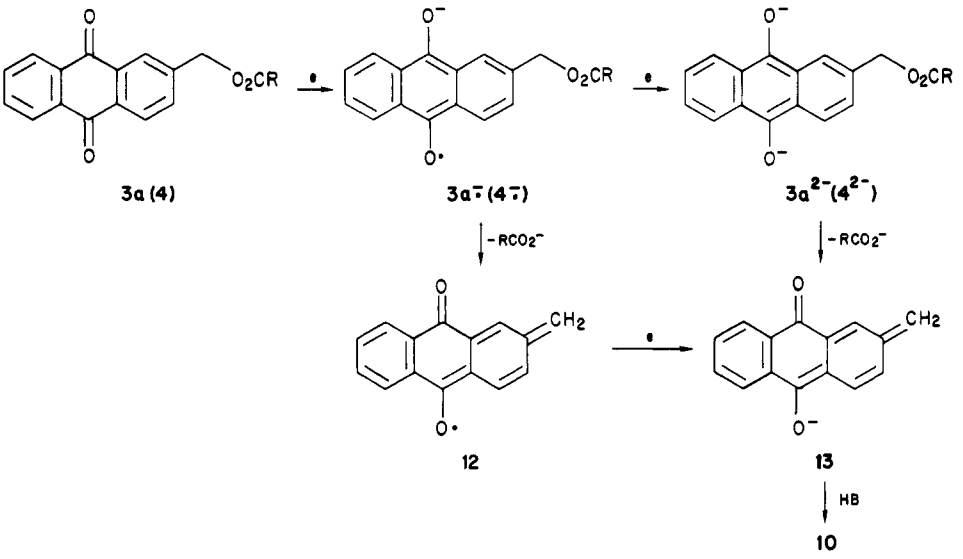
Electroreduction of 3, 4, and 10 in 50% Aqueous THF. The electrochemistry of quinones has been extensively studied in both aqueous and nonaqueous media.[9] In aqueous media, one reversible wave is generally observed for a quinone/hydroquinone couple corresponding to a $2e/2H^+$ process. Since anthraquinones **3** and **4** are not appreciably soluble in water, it was necessary to employ a solvent mixture to study their electrochemistry. The 50% aqueous THF buffer (0.10 M KCl, 0.050 M phosphate) proved to be a suitable solvent. Concentrations of at least 3.0 mM for **3** and **4** could be realized. Furthermore, adsorption was not a problem in this solvent mixture as evidenced by the constancy of $i_p/\nu^{1/2}$ (i_p is the peak potential and ν is the scan rate) in the cyclic voltammograms of these compounds. In aqueous THF buffer containing higher percentages of water (e.g., 60%) $i_p/\nu^{1/2}$ increased with increasing ν, probably resulting from adsorption.

(7) Mairanovsky, V. G. *Angew. Chem., Int. Ed. Engl.* **1976**, *15*, 281 and references therein.

(8) (a) Carpino, L. A. *J. Am. Chem. Soc.* **1957**, *79*, 4427. (b) McKay, F. C.; Albertson, N. F. *J. Am. Chem. Soc.* **1957**, *79*, 4686. (c Anderson, G. W.; McGregor, A. C. *J. Am. Chem. Soc.* **1957**, *79*, 6180.

(9) Chambers, J. Q. In "The Chemistry of the Quinonoid Compounds"; Patai, S., Ed.; Wiley: London, 1974; Chapter 14.

Scheme IV



Cyclic voltammograms of 2-methyl-9,10-anthraquinone (**10**), **3a**, and **4** were recorded in 50% aqueous THF buffer (pH 7.0) at a glassy carbon electrode. Each of these anthraquinones gave a single cathodic wave and a very broad anodic wave on the reverse scan as is shown in Figure 1 for **3a**. The cathodic peak potentials (Ep) for these compounds are quite similar (Table I). The difference in cathodic and anodic peak potential (ΔEp) was found to be 241, 323, and 289 mV, respectively, considerably larger than the 30-mV separation expected for a reversible 2e/2H$^+$ couple.

Constant potential reduction of 1–3 mM solutions of **3a**, **4**, and **10** were conducted in 50% aqueous THF buffer (pH 7.0) at a carbon sponge at potentials 20–40 mV more negative than their respective Ep values. The electrolyses were terminated when the current no longer decreased appreciably with time (60–80 min). The catholyte was exposed to O_2 (air) for at least 1 h prior to workup to ensure complete oxidation of anthrahydroquinone to anthraquinone.

The results from the exhaustive electrolyses of **3a**, **4**, and **10** were essentially identical. Each compound consumed 1.9–2.1 F/mol (corrected for background current) of charge, and 85–95% of starting anthraquinone was isolated following air oxidation and workup. No cleavage product **10** was detected (based upon TLC and ^{1}H NMR) from the electrolysis of either **3a** or **4**. Similar results were obtained at pH 5. It is clear that cleavage of the anthrahydroquinones derived from **3a** and **4** occurs at very slow rates under the above conditions.

The electrolysis results from **3b** in 50% aqueous THF buffer were slightly more encouraging as expected. Reduction of **3b** at –0.57 V for 1 h (pH 7.0) resulted in a 15% yield of the cleavage product, 2-benzyl-9,10-anthraquinone. The yield was increased to 50% by conducting the electrolysis for longer periods of time (ca. 3 h). Slightly lower yields of 2-benzyl-9,10-anthraquinone were obtained at pH 5.0 (38% with t = 3 h). Control experiments demonstrated that hydrolysis of **3b** did not occur under the above conditions.

Electroreduction of 3a, 4, and 10 in DMF. In aprotic media such as DMF a quinone is typically reduced in two separate one-electron steps to its radical anion and dianion.[9] Such is the case for 2-methyl-9,10-anthraquinone (**10**). A cyclic voltammogram (CV) of a 1.0 mM solution of **10** in DMF (0.10 M $LiClO_4$) is shown in Figure 2a. Two cathodic waves are observed at –0.92 and –1.24 V (SCE) with ν = 20 mV s^{-1}. Both reductive processes are quasi-reversible at this scan rate with ΔEp values of 65 and 80 mV, respectively. Coulometry experiments confirmed that both waves represent one-electron processes.

CV's of **3a** and **4** are quite similar under identical conditions and display two cathodic waves with peak potentials that nearly coincide with those of **10** (Table I). In Figure 2b is shown a CV of 1.0 mM **3a** with ν = 20 mV s^{-1}. The second wave is quite broad and has a peak current that is higher than that expected for a one-electron process. As the scan rate is increased the difference in peak currents decreases and at $\nu \gtrsim 100$ mV s^{-1} cyclic voltammograms of **3a** and **10** closely resemble each other.

Constant potential electrolysis of **3a** at a carbon sponge in the DMF electrolyte at the first reduction wave (–0.88 V) for 2–3 h gave the cleavage products **10** and 2-(hydroxymethyl)-9,10-anthraquinone (**11**) in yields of 50–65% and 35–30%, respectively. Electrolysis of **3a** at the second wave (–1.2 V) gave similar ratios of **10** and **11** in comparable yields, but shorter times were required (ca. 1 h). The yield of the other cleavage product from **3a** was also determined. The carbamate $PhCH_2O_2CNH(CH_2)_3CO_2^-$ in the aqueous fraction from the workup procedure was hydrolyzed and the resulting GABA analyzed on an amino acid analyzer. Yields of over 80% were found which is in agreement with the combined yields of **10** and **11**.

The results from the constant potential reduction of **4** at –0.88 V were similar to those from **3a** except that the ratio of **10**:**11** was somewhat higher (65–80% for **10** and 20–15% for **11**). Since N-substituted carbamates rapidly decarboxylate,[10] the aqueous fraction contained the benzyl ester of GABA, $PhCH_2O_2C(CH_2)_3NH_3^+$, which was analyzed directly. Yields of up to 83% were found for the GABA ester.

Discussion

The constant potential electrolysis results for **3a** and **4** show that these Maq esters undergo very slow cleavage in aqueous media via their corresponding anthrahydroquinones but cleave at appreciable rates in DMF via the radical anions or dianions. Good yields of the anthraquinone cleavage products, **10** and **11**, and the GABA esters were obtained in the DMF electrolyte. The dianions of these Maq esters appear to undergo efficient cleavage

(10) Johnson, S. L.; Morrison, D. L. *J. Am. Chem. Soc.* **1972**, *94*, 1323.

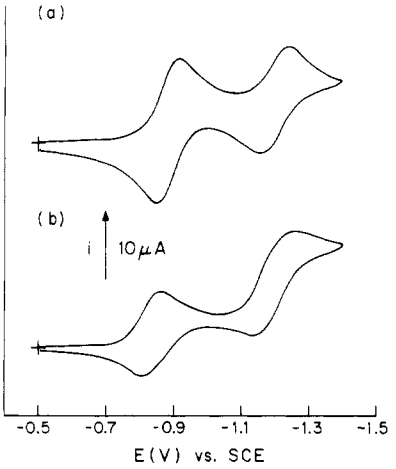


Figure 2. (a) Cyclic voltammogram of 1.0 mM 2-methyl-9,10-anthraquinone (**10**) in DMF (0.10 M $LiClO_4$) at a sweep rate of 20 mV s^{-1}. (b) Cyclic voltammogram of **3a** in DMF (0.10 M $LiClO_4$) at a sweep rate of 20 mV s^{-1}.

and this allows shorter times for electrolysis.

In Scheme IV are outlined several likely pathways leading to the major anthraquinone cleavage product **10**. Reduction of **3a** (**4**) at -0.88 V gives the radical anion $\mathbf{3a}^{-\cdot}$ ($\mathbf{4}^{-\cdot}$) which undergoes cleavage to the neutral radical **12**. Since $\mathbf{3a}^{-\cdot}$ ($\mathbf{4}^{-\cdot}$) is reduced at -1.2 V, it is reasonable to conclude that **12** is rapidly reduced to **13** at -0.88 V. One-electron reduction of **12** produces the anion **13** which is presumably protonated by trace amounts of water in the DMF medium or by the electrolyte to give **10**. Alternatively, reduction of **12** could occur by electron transfer from $\mathbf{3a}^{-\cdot}$ ($\mathbf{4}^{-\cdot}$) and $\mathbf{10}^{-\cdot}$. Reduction of **3a** (**4**) at -1.2 V gives its dianion $\mathbf{3a}^{2-}$ ($\mathbf{4}^{2-}$) which can cleave to give **13** directly. Indeed, the cyclic voltammetric peak currents at 20 and 100 mV s^{-1} suggest that this cleavage occurs on the time scale of seconds.

One possible route to the minor anthraquinone cleavage product, the alcohol **11**, is nucleophilic attack of water or hydroxide ion on **12** or **13**. However, this pathway does not appear to be operative. Reduction of **3a** in DMF containing 1% water gave **10** and **11** in approximately the same relative yields as in dry DMF. Furthermore, reduction of **3a** at -0.88 V in the presence of 0.10 M potassium ethyl xanthate, a nucleophile that has been used to successfully trap the quinone methide derived from 11-deoxydaunomycin,[5] gave only **10** and **11** as anthraquinone products in yields that were similar to those obtained in the absence of the nucleophile. A second possible source of **11** is the base catalyzed hydrolysis (saponification) of **3a** (**4**). Reductive cleavage of **3a** (**4**) to **10** in DMF increases the basicity of the medium (see Scheme IV). It follows then that electrolysis of increasing concentrations of **3a** (**4**) should lead to increasing basicity and presumably higher yields of **11**. This was not found to be the case. Electrolysis at **3a** at -0.88 V in the concentration range of 2-8 mM produced **11** in yields of 27-34%. A more serious problem with the saponification mechanism, though, is that very little free GABA is formed in the electrolysis of **3a** or **4**. It is unlikely that only the ester adjacent to the Maq group in **3a** and **4** would be hydrolyzed without hydrolysis of the ester at the opposite end of the chain also occurring in at least one case. As a final probe into the question of how **11** is formed, **3a** (2.0 mM) was reduced at -0.88 V in the presence of varying concentrations of **10** (2.0-13.0 mM). The yield of **11** nearly doubled. Thus, **10** or $\mathbf{10}^{-\cdot}$ somehow catalyzes the "hydrolytic" conversion of **3a** to **11**. We have not explored this further.

As mentioned in the introduction one of our goals in this work was to use the GABA-bound Maq esters as drug delivery systems. This work shows that these systems will have limited use in this application. Very slow cleavage rates in aqueous media will make GABA delivery under physiological conditions impractical. A second goal in this work was to provide an alternative method for deprotecting Maq esters of carboxylic acids used in the synthesis of peptides. The high yield of cleavage products from the electroreduction of **3a** in DMF at only moderately negative potentials clearly shows that this goal was accomplished. Furthermore, synthesis of **4** and its reductive cleavage to the benzyl ester of GABA in high yields demonstrates that Maq esters of carbamates can serve as excellent protecting groups of primary amines.

Acknowledgment. This work was supported by the National Institutes of Health.

Registry No. **3a**, 92013-50-8; **3b**, 92013-56-4; **4**, 92013-54-2; **5**, 5105-78-2; **5** Bu_4N^+ salt, 92013-58-6; **6**, 86272-16-4; **7**, 92013-51-9; **8**, 92013-52-0; **9**, 92013-55-3; **11**, 17241-59-7; $NH_2(CH_2)_3CO_2H$, 56-12-2; $PhCH_2OC(O)Cl$, 501-53-1; $2,4,5\text{-}Cl_3C_6H_2OC(O)Cl$, 16947-69-6; $ClSiMe_3$, 75-77-4; $2,4,5\text{-}Cl_3C_6H_2OSiMe_3$, 1014-29-5; $PhCH_2OC(O)(CH_2)_3N{=}C(OSiMe_3)O\text{-}2,4,5\text{-}Cl_3C_6H_2$, 92013-53-1; $PhCH_2OC(O)(CH_2)_3NH_3^+$, 92013-59-7; 2-(bromomethyl)-9,10-anthraquinone, 7598-10-9; 2-methyl-9,10-anthraquinone, 84-54-8; 2-benzyl-9,10-anthraquinone, 49658-23-3.

Synthesis of the Non-K-Region Dihydrodiols of 7-Methylbenz[*c*]acridine

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The syntheses of the four non-K-region *trans*-dihydrodiols of 7-methylbenz[*c*]acridine (**2**) are described. The *trans*-8,9- and -10,11-dihydrodiols **13** and **16** were prepared from 7-methyl-8,9,10,11-tetrahydrobenz[*c*]acridine (**3**) via the *trans*-8,9-diacetoxy-7-methyl-8,9,10,11-tetrahydrobenz[*c*]acridine (**10**) and its 10,11-isomer (**14**) by selective benzylic bromination followed by dehydrobromination. The *trans*-tetrahydro diacetates were obtained through the alkenes **6** and **7** and their epoxide derivatives. *trans*-1,2- and -3,4-dihydrodiols **22** and **24** were similarly prepared from the *trans*-1,2- and -3,4-diacetates of 7-methyl-1,2,3,4-tetrahydrobenz[*c*]acridine (**19** and **20**). The latter were products of the Prevost reaction on mixed 3,4- and 1,2-dihydro-7-methylbenz[*c*]acridines (**17** and **18**).

7-Methylbenz[*c*]acridine (**2**) is a polycyclic azaaromatic compound with significant carcinogenic potency[1] and is structurally analogous to 7-methylbenz[*a*]anthracene, a potent carcinogenic polycyclic hydrocarbon.[2] For poly-

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