THE SYNTHESIS OF [1',3'-^{3}H]4-(4'-AZIDO-5',6',7',8'-TETRAHYDRO-5',5',8',8'-TETRAMETHYL-2'-ANTHRACENYL)BENZOIC ACID AS A PROBE OF THE RETINOIC ACID RECEPTOR

Marcia I. Dawson,*[1] Peter D. Hobbs,[1] Albert Dorsky,[2] and Hiromi Morimoto[2]
[1]SRI International, Menlo Park, CA 94025
[2]National Tritium Labeling Facility, Berkeley, CA 94720

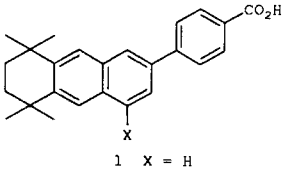
SUMMARY

The synthesis of [1',3'-^{3}H]4-(4'-azido-5',6',7',8'-tetrahydro-5',5',8',8'-tetramethyl-2'-anthracenyl)benzoic acid is described. This retinoid was designed as a photoaffinity probe of the receptor sites of cellular retinoic acid-binding protein and the nuclear retinoic acid receptor protein. The [^{3}H]azidoretinoid was prepared from 1,2,3,4-tetrahydro-1,1,4,4-tetramethyl-7-(4-methylphenyl)-5-nitroanthracene in five steps in 15% yield (89% radiochemical purity by HPLC). ^{1}H and ^{3}H NMR was used to confirm the sites of ^{3}H substitution on the ring.

KEYWORDS: [1',3'-^{3}H]4-(4'-azido-5',6',7',8'-tetrahydro-5',5',8',8'-tetramethyl-2'-anthracenyl)benzoic acid, photoaffinity probe, retinoid, cellular retinoic acid-binding protein.

INTRODUCTION

(*E*)-Retinoic acid (RA) is the most active natural retinoid at controlling cell differentiation and reversing the preneoplastic transformation of cells. If the toxic and teratogenic side effects of RA could be ameliorated, compounds of this class would have great therapeutic potential as preventive agents for cancer. Knowledge of the tertiary structure of the receptor site of the RA-binding proteins--cellular RA-binding protein[1] and nuclear RA receptor proteins[2-4]--would be an invaluable aid in the design of improved analogs. To probe the geometry of the receptor sites, we have undertaken the synthesis of a RA analog having a photoaffinity labeling group that would specifically bind to the receptor site and form a stable covalent bond with residues at this site on irradiation. The polyaromatic retinoid 4-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-anthracenyl)benzoic acid (**1**) appeared to be an ideal candidate for introduction of the photoaffinity



1 X = H
2 X = N_3

0362–4803/90/010089–10$05.00

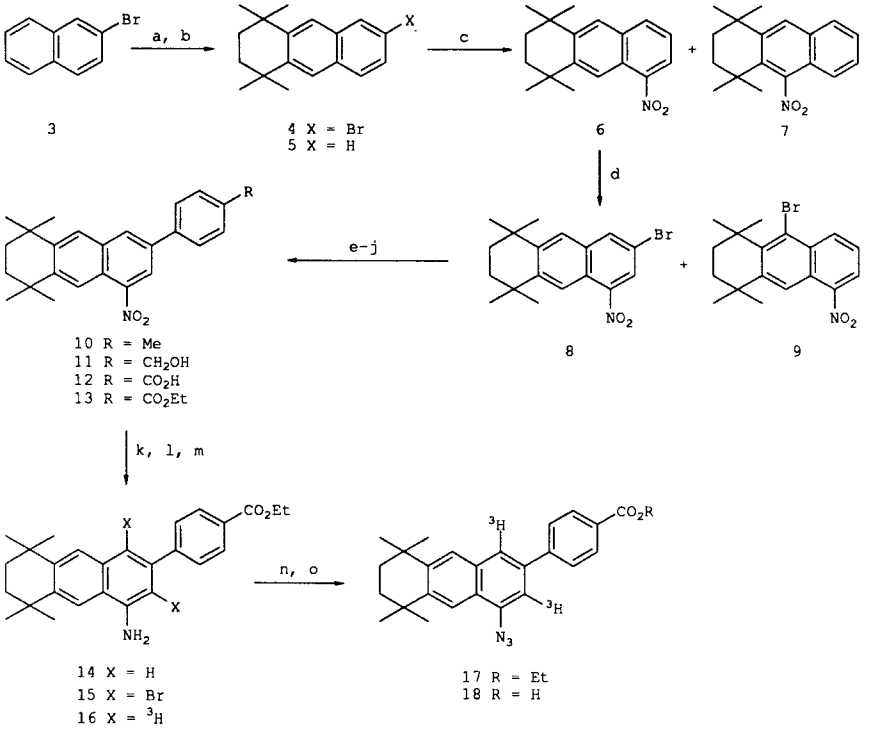
label. This compound had activity comparable to that of RA (E_{50} 0.01 nM) in reversing epithelial keratinization in retinoid-deficient hamster trachea in organ culture, bound to the cellular RA-binding protein receptor site, and did not have the light- and oxygen-sensitive double-bond system of RA.[5] The azido group was selected as the photoaffinity labeling group because of the elegant low-temperature photolysis work of Platz using an aryl azide to label α-chymotrypsin.[6] Introduction of the azido group at the 4-position of the tetrahydroanthracenyl ring of **1** only decreased activity in the tracheal organ culture assay by 50% and, therefore, **18** was chosen as our inital tritiated target for probing receptor structure.

RESULTS AND DISCUSSION

Because of the azido group in **2** , the introduction of the radiolabel by tritium exchange in the final step of the synthesis was not possible. An alternate method of hydrogenolysis of bromo groups with tritium gas was used to introduce the radiolabel on an intermediate before the azido group was introduced. Therefore, the location for tritium depended on the bromination pattern of the aromatic ring system. Our goals were to prepare material with high specific activity and to introduce the radiolabel as late in the synthesis as possible. The azido group could not be introduced by the most obvious route of nitration, reduction, and diazotization of tritiated **1** because nitration under a variety of conditions (e.g., NO_2BF_4[7] and $HNO_3/H_2SO_4/MeNO_2$[8]) produced a mixture of products having nitro groups at the 1, 9, and 10-positions of the tetrahydroanthracenyl ring. Therefore, the more circuitous approach, outlined in Figure 1, was used.

A cycloalkylation reaction was used to produce the saturated ring of the tetrahydroanthracene ring system. In order to inhibit bisalkylation--the standard product of Friedel-Crafts alkylation of naphthalene with 2,5-dichloro-2,5-dimethylhexane[9]--one of the rings of the naphthalene was blocked at the 2-position by a bromo group. 1-Nitronaphthalene could not be used at this step because it failed to react under Friedel-Crafts conditions. With the tetrahydroanthracene ring (**4**) formed, the next step was introduction of the nitro group. Because nitration of **4** would occur at the 1,9, and 10-positions of the ring system, alternate methods were investigated. Attempted nitration of 5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-anthracenylboronic acid--a potential intermediate for the oxidative coupling to form the biaryl ring system--produced only nitrophenols from oxidation of the B-C bond. Therefore, to optimize yields it was necessary to debrominate **4** giving **5**, which was then nitrated under very mild conditions to avoid ring opening. A mixture of the 5- and 9-nitrotetrahydroanthracenes (**6** and **7**) was obtained, separated, and characterized. The bromination of **6** proved to be problematic. The highest yield (40%) of 7-bromo-5-nitro-**8** was obtained using Br_2/Fe in the absence of solvent;[10] Br_2/Ag_2SO_4/90% H_2SO_4, which has been used for *m*-nitration of deactivated aromatic compounds,[11] gave a 30% yield. Both methods produced the 2,9-dibromide and dinitrated products. Separation of the bromination mixture was best accomplished by several chromatographies because crystallization (hexane, CH_2Cl_2/hexane, or EtOH) only gave mixtures.

The tetrahydroanthracene ring was linked to the phenyl ring using a Pd(0)-catalyzed biaryl coupling of the aryl bromide **8** with tolylboronic acid, which is tolerant of the NO_2 function.[12] The methyl group on the product (**10**) was then transformed to the ethyl carboxylate using standard methodology, namely bromination, solvolysis, hydrolysis, oxidation, and esterification. Reaction of the benzyl bromide intermediate with $CaCO_3$ in aq. dioxane at reflux was slow so a two-step procedure (KOAc/DMF at reflux; K_2CO_3) was used to produce the benzyl alcohol **11**. Saponification of the benzyl acetate had to be performed under very mild conditions to avoid destruction of the nitro group. Because the NO_2 group deactivated the tetrahydroanthracene ring to oxidation, the benzyl alcohol group of **11** could be oxidized to the carboxylic acid (**12**) with Jones reagent. In contrast, in the parent structure lacking the nitro group polar, yellow by-products were also obtained.



a: $ClCMe_2CH_2CH_2CMe_2Cl$, $AlCl_3$, CH_2Cl_2, -10° to -5°C; b: Mg, THF, 50°C; aq. NH_4Cl; c: 70% HNO_3/HOAc, $MeNO_2$, H_2SO_4, 0-20°C; d: Br_2, Fe, 20-75°C; e: $Pd[P(C_6H_5)_3]_4$, 4-$(HO)_2B$-C_6H_4Me, 2 M aq. Na_2CO_3, EtOH/$C_6H_5CH_3$; aq. H_2O_2; f: NBS, $(C_6H_5CO_2)_2$, CCl_4, 77°C; g: KOAc, DMF, 100°C; h: K_2CO_3, EtOH; i: CrO_3, aq. H_2SO_4/acetone j: CH_3CHN_2, Et_2O, 0°C; k: H_2, 5% Pd(C), EtOAc; l: Br_2, $CHCl_3$, 0°C; m: $^3H_2$, 10% Pd(C), EtOAc, Et_3N; n: *n*-BuONO, TFA, EtOH, 0°C; aq. NaN_3, 0-20°C; o: KOH, aq. EtOH; aq. H_2SO_4.

Fig. 1. Synthesis of [^{3}H]azidoretinoid **18**.

The nitro group of **13** was reduced giving the oxygen- and light-sensitive amine **14**, which on bromination afforded **15**. Initial studies were performed on unlabeled material. Water-insoluble **14** was diazotized in a mixed solvent system. The azido ester (**[1',3'-H]17** (71%) was chromatographed to remove colored by-products and then saponified giving **2** (90%).

Model reactions for the introduction of tritium employed $^2H_2$. Mass spectral analysis indicated that 80% introduction of deuterium at the two halogen positions of **15** occurred. In contrast, similar conditions [50 wt% 5% Pd(C)] using $^3H_2$ led to incomplete hydrogenolysis. More stringent conditions [300 wt% 10% Pd(C)] were successful. The diazotization reaction on labeled **16** gave the azido ester **17**, which was hydrolyzed to **18** without characterization. HPLC, UV, and NMR were used to confirm the structure and chemical and isotopic purity of **18** by comparison with **2**. The radiochemical purity of **18**, having a specific activity of 41 Ci/mmol, was 89% by HPLC. Comparison of the ^3H and ^1H NMR spectra of **18** with the ^1H NMR spectrum of **2** showed specific ^3H substitution at the 1' and 3'-positions of the tetrahydroanthracene ring.

EXPERIMENTAL

The following instruments were used for characterization: melting points (Thomas Hoover Unimelt capillary melting point apparatus, uncorrected); IR spectra (Perkin-Elmer 710B spectrophotometer); UV spectra (unlabeled compounds: Perkin-Elmer 552 spectrophotometer; labeled compounds: Hewlett- Packard 104A diode array detector coupled to HPLC); NMR spectra (unlabeled ^1H: Varian XL 400 and Jeol FX 90Q spectrometers; labeled ^1H and ^3H: Bruker NR/300 spectrometer); HPLC (labeled: Waters Associates 510 pump, equipped with a Hewlett-Packard 104A diode array UV detector, Berthold ^3H detector, and Tracor display). Liquid scintillation counting was performed on a Packard 1500 Tri-Carb instrument. Merck silica gel 60 was used for chromatography. TLC was performed on Analtech plates.

1,2,3,4-Tetrahydro-1,1,4,4-tetramethylanthracene (5). To a stirred solution of 16.1 g (77.7 mmol) of 2-bromonaphthalene (**3**) and 14.2 g (77.6 mmol) of 2,5-dichloro-2,5-dimethylhexane[8] in 100 mL of CH_2Cl_2, cooled in a -10°C bath was added 1.2 g (9.0 mmol) of $AlCl_3$ in three aliquots over a 15-min period with protection from moisture. The dark-red suspension, which no longer produced HCl gas, was stirred for 15 min more, while the bath temperature was maintained at -10°C to -5°C, and then was poured onto ice (300 g), and extracted with CH_2Cl_2 (100 mL). The extract was washed with water (2 x 50 mL), dried (Na_2SO_4), and concentrated at reduced pressure to give a gum, which was extracted with hexane (150 mL) and filtered to remove the by-product 1,2,3,4,7,8,9,10-octahydro-1,1,4,4,7,7,10,10-octamethylnaphthacene,[8] m.p. 308-309°C. The solution was concentrated to approximately 60 mL, refiltered, and reconcentrated. Crude 6-bromo-1,2,3,4-tetrahydro-1,1,4,4-tetramethylanthracene (**4**) crystallized on standing.

A solution of 24 g of **4** in 120 mL of dry THF was added over a 30-min period, under argon, to 2.4 g (0.10 g-at) of Mg turnings with mechanical stirring and heating in a 50°C oil bath. To initiate the reaction, 0.1 mL of MeI was added after 10 mL of the solution of **4** had been added. The

dark solution was heated at reflux for 1 h and cooled in ice, while water (5 mL) was added with stirring over a 15-min period, giving an orange suspension. The suspension was poured into aq. NH_4Cl (50 mL) and extracted with hexane (2 x 100 mL). The yellow extract was washed with water (2 x 30 mL), dried ($MgSO_4$), and concentrated. The crude material was chromatographed (silica gel, hexane) to give 14.8 g (80% from **3**) of **5** as white needles, m.p. 81.5-82.5°C (toluene); IR ($CHCl_3$) 1590, 1340, 1290, 1275, 1130, 1100, 1010, 940 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.42 (s, 12H, $C(CH_3)_2$), 1.78 (s, 4H, $(CH_2)_2$), 7.30 (m, 2H, 6,7-ArH), 7.66 (m, 2H, 5,8-ArH), 7.72 (s, 2H, 9,10-ArH). Anal. calcd for $C_{18}H_{22}$: C, 90.69; H, 9.31. Found: C, 90.67; H, 9.60.

1,2,3,4-Tetrahydro-1,1,4,4-tetramethyl-5-nitroanthracene (6). To a stirred suspension of 19.76 g (82.9 mmol) of **5** in 150 mL of $MeNO_2$ was added, with cooling in an ice bath, a solution of 8.3 g (92 mmol) of 70% HNO_3 in 30 mL of glac. HOAc, followed by 0.3 g (2.9 mmol) of 95% H_2SO_4. The pale-yellow suspension was stirred at ice-bath temperature for 4 h while 1.50 g (14.5 mmol) of 95% H_2SO_4 was added in three aliquots. The suspension was allowed to warm to room temperature and stirred for 18 h [TLC (2% EtOAc/hexane) R_f 0.36 (5-nitro **6**) and 0.44 (9-nitro **7**)]. The thick, yellow suspension was filtered, and the solid was extracted with $MeNO_2$ (75 mL) and water (200 mL). The filtrate was diluted with CH_2Cl_2 (200 mL) and washed successively with 10% aq. Na_2CO_3 (300 mL) and dil. brine (2 x 100 mL), dried (Na_2SO_4), and concentrated. The resultant yellow solid was combined with that obtained by filtration and chromatographed (silica gel, 1% EtOAc/10% CH_2Cl_2/hexane) three times to remove all traces of **7** to give 11.06 g (47%) of **6** as yellow needles, m.p. 143-144°C (hexane); IR ($CHCl_3$) 1595, 1520, 1340, 1310 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.39 and 1.40 (2s, 12H, $C(CH_3)_2$), 1.78 (s, 4H, $(CH_2)_2$), 7.39 (dd, J = 7.9 Hz, J = 8.2 Hz, 1H, 7-ArH), 7.85 (s, 1H, 9-ArH), 8.00 (d, J = 8.2 Hz, 1H) and 8.13 (dd, J = 7.9 Hz, J = 1.5 Hz, 1H) (6,8-ArH), 8.55 (s, 1H, 10-ArH). Anal. calcd for $C_{18}H_{21}NO_2$: C, 76.28; H, 7.47; N, 4.94. Found: C, 76.57; H, 7.46; N, 4.93.

7-Bromo-1,2,3,4-tetrahydro-1,1,4,4-tetramethyl-5-nitroanthracene (8). An mixture of 10.27 g (36.2 mmol) of **6** and 0.5 g (0.009 g-at) of Fe powder was treated with 11.0 g (68.8 mmol) of Br_2 over a 5-min period at room temperature with mechanical stirring. An exothermic reaction took place and HBr was evolved. The black mixture was stirred with heating in a 75°C oil bath for 2 h, cooled, and extracted with CH_2Cl_2 (200 mL). The suspension was filtered and washed with water, 10% aq. $NaHSO_3$, and water (25-mL portions), dried (Na_2SO_4), and concentrated. The mixture (**6**, 7-bromo-5-nitro-**8**, 9-bromo-5-nitro-**9**, and further bromination products) was chromatographed (silica gel, 1% EtOAc/5% CH_2Cl_2/hexane) to

give crude **8** as a yellow-orange solid. This material was dissolved in 25% CH_2Cl_2/hexane, filtered, and concentrated. The residue was rechromatographed twice (silica gel, 0.5% EtOAc/5% CH_2Cl_2/hexane) to give 5.33 g (40%) of **8** as large yellow crystals, m.p. 115-117°C (hexane); IR ($CHCl_3$) 1585, 1520, 1330 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40 (s, 12H, $C(CH_3)_2$), 1.78 (s, 4H, $(CH_2)_2$), 7.78 (s., 1H, 9-ArH), 8.20 (m, 2H, 6,8-ArH), 8.48 (s, 1H, 10-ArH). Anal. calcd for $C_{18}H_{20}NO_2Br$: C, 59.65; H, 5.56; N, 3.87; Br, 22.06. Found: C, 59.87; H, 5.55; N, 3.83; Br, 22.31.

1,2,3,4-Tetrahydro-1,1,4,4-tetramethyl-7-(4-methylphenyl)-5-nitroanthracene (10). A two-phase mixture of 0.31 g (0.86 mmol) of **6**, 0.21 g (1.54 mmol) of *p*-tolylboronic acid,[13] and 0.075 g (0.065 mmol) of $[(C_6H_5)_3P]_4Pd(0)$ in 1.0 mL of EtOH and 4.5 mL of $CH_3C_6H_5$, containing 1.2 mL (2.4 mmol) of 2 M aq. Na_2CO_3, was degassed (argon, three times), then heated at reflux with magnetic stirring for 23 h and cooled. The orange suspension was treated with 0.5 mL of 30% H_2O_2 and stirred at room temperature for 16 h to give a yellow organic phase and a black aq. suspension. The mixture was diluted with CH_2Cl_2 (20 mL), washed with dil. brine (2 x 10 mL), dried (Na_2SO_4), and concentrated. The orange oil was chromatographed (silica gel, 15% CH_2Cl_2/hexane) to give 0.29 g (91%) of **10** as large yellow crystals, m.p. 128-130°C (hexane); IR ($CHCl_3$) 1600, 1510, 1335 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.42 (s, 12H, $C(CH_3)_2$), 1.81 (s, 4H, $(CH_2)_2$), 2.40 (s, 3H, $ArCH_3$), 7.30 (d, J = 8 Hz, 2H, 3,5-ArH), 7.62 (d, J = 8 Hz, 2H, 2,6-ArH), 7.90 (s, 1H, 9'-ArH), 8.20 (m, 1H) and 8.43 (d, J = 1 Hz, 1H) (6',8'-ArH), 8.56 (s, 1, 10'-ArH). Anal. calcd for $C_{25}H_{27}NO_2$: C, 80.39; H, 7.29; N, 3.75. Found: C, 80.32; H, 7.47; N, 3.69.

7-(4-Hydroxymethylphenyl)-1,2,3,4-tetrahydro-1,1,4,4-tetramethyl-5-nitroanthracene (11). A solution of 367 mg (0.98 mmol) of **10** and 229 mg (1.29 mmol) of NBS in 8 mL of CCl_4 was treated with a crystal of dibenzoyl peroxide, and then heated at reflux with stirring for 4.5 h. Additional portions of the peroxide were introduced at 1.75 and 2.75 h. The orange suspension was cooled and filtered (4-mL CCl_4 rinse). The filtrate was concentrated, and the orange oil was treated with 0.50 g (5.1 mmol) of anhydrous KOAc and 4.0 mL of dry DMF and heated at 100°C for 1.5 h. The cooled, brown solution was partitioned between dil. brine (30 mL) and 33% CH_2Cl_2/hexane (15 mL). The organic extract was washed with water (3 x 10 mL), dried (Na_2SO_4), and concentrated. The orange-brown oil was treated with 0.5 g (3.6 mmol) of anhydrous K_2CO_3 and 5 mL of EtOH and stirred at room temperature for 48 h. The orange solution was decanted from the precipitate and concentrated. The precipitate and brown oil were combined and treated with water (15 mL) and extracted with ether (2 x 10 mL). the yellow extract was washed with brine (5

mL), dried (Na_2SO_4), and concentrated to give a yellow gum, which was chromatographed (silica gel, 5-25% EtOAc/20% CH_2Cl_2/75-55% hexane) to give 265 mg (69%) of **11** as yellow crystals, m.p. 134-136°C (CH_2Cl_2/hexane); IR ($CHCl_3$) 3600, 3300, 1605, 1510, 1335 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.42 (s, 12H, $C(CH_3)_2$), 1.60 (broad s, 1H, OH, exchanged D_2O), 1.81 (s, 4H, $(CH_2)_2$), 4.78 (s, 1H, CH_2O), 7.49 (d, J = 8 Hz, 2H, 3,5-ArH), 7.72 (d, J = 8 Hz, 2H, 2,6-ArH), 7.92 (s, 1H, 9'-ArH), 8.22 (d, J = 2 Hz, 1H) and 8.43 (d, J = 2 Hz, 1H) (6',8'-ArH), 8.56 (s, 1H, 10'-ArH). Anal. calcd for $C_{25}H_{27}NO_3$: C, 77.09; H, 6.99; N, 3.60. Found: C, 76.73; H, 7.09; N, 3.47.

Ethyl 4-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-4-nitro-2-anthracenyl)benzoate (13). A solution of 165 mg (0.42 mmol) of **11** in 1 mL of acetone in a 15-mL centrifuge tube was treated with three 0.2-mL volumes of Jones reagent (prepared from 0.27 g of CrO_3, 0.58 g of water, and 0.42 g of H_2SO_4) at 10-min intervals while stirring at room temperature. TLC (25% EtOAc/hexane) indicated only **12** (R_f 0.0). After being stirred for 10 min more, the yellow suspension was diluted with water (10 mL) and centrifuged. The precipitate was washed with water (4 x 5 mL) by centrifugation. The yellow powder was dried at reduced pressure (0.05 mm) to give 170 mg (99%) of the carboxylic acid **12**, which was treated at 0°C with excess CH_3CHN_2 in Et_2O. The solution was allowed to stand at ice-bath temperature for 10 min, before excess CH_3CHN_2 was destroyed by the dropwise addition of HOAc until gas evolution ceased. The solution was diluted with 5 mL of EtOAc, washed with water (2 x 5 mL), dried (Na_2SO_4), and concentrated. The yellow gum was chromatographed (silica gel, 5% EtOAc/hexane) to give 145 mg (79%) of **13** as yellow crystals, m.p. 108-110°C (hexane); IR ($CHCl_3$) 1710, 1610, 1520, 1340, 1280 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40 (t, J = 7 Hz, 3H, $CH_2C\underline{H}_3$), 1.42 (s, 12H, $C(CH_3)_2$), 1.82 (s, 4H, $(CH_2)_2$), 4.42 (q, J = 7 Hz, 2H, $C\underline{H}_2CH_3$), 7.77 (d, J = 8 Hz, 2H, 3,5-ArH), 7.94 (s, 1H, 9'-ArH), 8.18 (d, J = 8 Hz, 2H, 2,6-ArH), 8.26 (broad s, 1H) and 8.44 (broad s, 1H) (1',3'-ArH), 8.56 (s, 1H, 10'-ArH); UV (EtOH) λ_{max} 222 (ε 3.3 x 10^4), 280 (ε 2.2 x 10^4), 292 nm (ε 2.2 x 10^4). Anal. calcd for $C_{27}H_{29}NO_4$: C, 75.15; H, 6.77; N, 3.25. Found: C, 75.15; H, 6.82; N, 3.17.

Ethyl 4-(4-Amino-5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-anthracenyl)benzoate (14). A solution of 118 mg (0.273 mmol) of **13** in 6 mL of EtOAc, containing 25 mg of 5% Pd(C) was stirred under H_2 until uptake was complete (4.75 h). The suspension was filtered (2 x 5-mL EtOAc rinse). The yellow-green filtrate was concentrated to a yellow gum, which was chromatographed (silica gel, 5% EtOAc/20% CH_2Cl_2/hexane) to give 104 mg (94%) of **14** as an oxygen- and light-sensitive, yellow glass; IR ($CHCl_3$) 3375, 1700, 1605, 1270, 1220 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.41 and 1.42 (2s, 12H, $C(CH_3)_2$), 1.44 (t, J = 7 Hz, 3H, $CH_2C\underline{H}_3$),

1.84 (s, 4H, $(CH_2)_2$), 3.9 (broad s, 2H, NH_2), 4.45 (q, J = 7 Hz, 2H, $C\underline{H}_2CH_3$), 6.98 (d, J = 2 Hz, 1H, 3'-ArH), 7.52 (broad s, 1H, 1'-ArH), 7.72 (d, J = 8 Hz, 2H, 3,5-ArH), 7.80 (s, 1H) and 7.85 (s, 1H) (9',10'-ArH), 8.10 (d, J = 8 Hz, 2H, 2,6-ArH). Anal. calcd for $C_{27}H_{31}NO_2$: C, 80.76; H, 7.78; N, 3.49. Found: C, 80.87; H, 7.55; N, 3.47.

Ethyl 4-(4-Amino-1,3-dibromo-5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-anthracenyl)benzoate (15). To a solution of 50 mg (0.125 mmol) of **14** in 1.0 mL of $CHCl_3$ was added with stirring and cooling in an ice bath a solution of 60 mg (0.375 mmol) of Br_2 in 0.50 mL of $CHCl_3$. The red solution was stirred at ice-bath temperature while protected from light and moisture for 3.25 h at which time TLC (10% EtOAc/hexane) on an aliquot washed with 10% aq. Na_2CO_3 showed **15** (R_f 0.40) and no **14** (R_f 0.16). The solution was diluted with $CHCl_3$ (2 mL) and washed with 10% aq. $NaHSO_3$ (2 mL), water (1.5 mL), 10% aq. Na_2CO_3 (1.5 mL), and water (2 x 1.5 mL), dried (Na_2SO_4), and concentrated. The pink crystalline residue was chromatographed (silica gel, 7.5% EtOAc/hexane) to give 63 mg (90%) of **15** as oxygen- and light-sensitive, white crystals, m.p. 217-219°C (decomp.) (EtOAc/hexane); IR ($CHCl_3$) 3380, 1705, 1605, 1270 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.42 (t, J = 7 Hz, 3H, $CH_2C\underline{H}_3$), 1.43 (s, 12H, $C(CH_3)_2$), 1.80 (s, 4H, $(CH_2)_2$), 4.41 (q, J = 7 Hz, 2H, $C\underline{H}_2CH_3$), 4.76 (broad s, 2H, NH_2), 7.29 (d, J = 8 Hz, 2H, 3,5-ArH), 7.74 (s, 1H, 10'-ArH), 8.15 (d, J = 8 Hz, 2H, 2,6-ArH), 8.24 (s, 1H, 9'-ArH); UV (EtOH) λ_{max} 222 (ε 6.5 x 10^4), 262 (ε 7.5 x 10^4), 344 nm (ε 7.1 x 10^3); EI-MS 557 (M^+, $C_{27}H_{29}{}^{79}Br_2NO_2$). Anal. calcd for $C_{27}H_{29}Br_2NO_2$: C, 57.98; H, 5.23; Br, 28.58; N, 2.50. Found: C, 58.26; H, 5.26; Br, 28.87; N, 2.39.

[1',3'-^{3}H]4-(4'-Azido-5',6',7',8'-tetrahydro-5',5',8',8'-tetramethyl-2'-anthracenyl)benzoic Acid (18). Reaction steps had to be performed in dim light. Intermediates and products were stored at -20°C. A solution of 30 mg (53.6 μmol) of **15** and 48 μL (35 mg, 342 μmol) of Et_3N in 1.5 mL of EtOAc, containing 90.6 mg of dried (100°C) 10% Pd(C), was hydrogenated with $^3H_2$ at atmospheric pressure and room temperature with stirring for 5.75 h. The progress of the reaction was monitored by HPLC [(μ-Bondapak C_{18} column eluted with 25% water/MeOH at 2.0 mL/min with detection at 260 nm) t_R 6.19 (98.5% **[1',3'-^{3}H]16**, UV λ_{max} 287 nm), 8.88 min (1.5%)] comparison with unlabeled **14** [t_R 6.11 min (UV λ_{max} 285 nm)]. The suspension was treated with MeOH (2 x 2 mL) and concentrated at reduced pressure. The resi- due was extracted with EtOAc (6 x 1.5 mL) and filtered (glass-fiber disk), and the filtrate was lyophilized to dryness.

To the crude labeled amine **16** in 0.5 mL of EtOH was added with stirring and cooling in an ice bath 80 μL (1.04 mmol) of TFA, followed by 13 μL (111 μmol) of *n*-butyl nitrite, and the

solution was stirred for 0.5 h. To the red-brown solution was added 80 μL (246 μmol) of precooled (ice-bath) 20% aq. NaN_3. A red semisolid separated out. This mixture was stirred at ice-bath temperature for 0.5 h and at room temperature for 0.5 h. To the now yellow suspension was added 5 mL of 5% aq. $NaHCO_3$ amd 1.5 mL of CH_2Cl_2, and the mixture was stirred for 3 min. The phases were separated by pipet, and the aq. phase was extracted with CH_2Cl_2 (3 x 1 mL). The orange organic phase was washed with water (2 x 1.5 mL) with stirring and then lyophilized. Crude ester **17** [TLC (silica gel, 3% EtOAc/10% CH_2Cl_2/hexane) R_f 0.55] was purified by chromatography (10 g of silica gel, 3% EtOAc/10% CH_2Cl_2/hexane) and lyophilized.

The dark-yellow solid dissolved in 0.6 mL of EtOH was stirred with 0.12 mL of 40% aq. KOH (0.73 mmol) under argon at room temperature for 2.7 h. To the resulting dark-orange suspension was added 6.4 mL of 0.25 N H_2SO_4 in 10% aq. brine and the yellow suspension was stirred for 15 min and filtered (medium-porosity sintered-glass frit, 5 x 1.5-mL water rinse) to give a yellow-brown solid, which was dried under reduced pressure and dissolved in 10 mL of C_6H_6. The yellow solution was filtered and lyophilized to give 6.9 mg (692 mCi at 41 Ci/mmol) (32% radiochemical yield from **15**) of **18**; ^3H NMR ($C_6{}^2H_6$) δ 7.16 (s, 1H, 1'-Ar^{3}H), 7.55 (s, 0.79H relative to 7.16-ppm signal height, 3'-Ar^{3}H); ^1H NMR ($C_6{}^2H_6$) δ 1.36 and 1.38 (2 s, 12H, 5',8'-$C(CH_3)_2$), 1.66 (s, 4H, 6',7'-$(CH_2)_2$), 7.12 (s, 0.21H, 1'-ArH), 7.37 (d, J = 8 Hz, 2H, 3,5-ArH), 7.51 (s, 0.28H, 3'-ArH), 7.76 (s, 1H, 9'-ArH), 8.27 (d, J = 8 Hz, 2H, 2,6-ArH), 8.31 (s, 1H, 10'-ArH), (estimated by comparison of peak heights in ^1H NMR spectrum of **2** and **18**); HPLC (μ-Bondapak C_{18}, 0.1% TFA/5% water/MeOH at 1.0 mL/min, 260 nm) t_R 4.2, 4.8, and 5.4 (11% total ^3H), 6.4 min ([^3H]**18**); UV (0.1% TFA/5% water/MeOH) λ_{max} (relative absorbance) **18**: 236 (0.89), 283 (1.00), 319 nm (0.40), compared to **2**: 236 (0.88), 281(1.00), 319 nm (0.40).

ACKNOWLEDGMENT

Support of this work by USPHS grant CA32428 (M.I.D.) is gratefully acknowledged.

REFERENCES

1. Sani BP, Condon SM, Banerjee C.K. - Biochim. Biophys. Acta 624:226 (1980).
2. Petkovich M., Brand N,J, Krust A, Chambon P. - Nature 330:444 (1987).
3. V. Giguere, Ong E.S., Segui P., Evans R.M.-Nature 330:624 (1987).
4. Benbrook D., Lernhardt E., Pfahl M. - Nature 333:669 (1988).
5. Sani B.P., Wille J.J., Jr., Dawson M.I., Hobbs P.D., Dorsky A., Morimoto H. - (unpublished results).
6. Platz M.S., Kanakarajan K., Goodrich R.P., Young M.J.T., Soundararajan S. - Proc. SPIE Int. Soc. Opt. Eng. (New Dir. Photodyn. Ther.) 847:57 (1988).
7. Kuhn S.J., Olah G.A. - J. Am. Chem. Soc. 83:4564 (1961).
8. Bruson H.A., Kroeger J.W. - J. Am. Chem. Soc. 62:36 (1940).
9. Johnson J.R., Gauerke C.G. - In: Org. Syn., Coll. Vol. 1 (Blatt A.H., ed.), John Wiley and Sons, Inc., New York, 1964, p 123.

10. Derbyshire D.H., Waters W.A. - J. Chem. Soc. 573 (1950).
11. Miyaura N., Yanagi T., Suzuki A. - Syn. Commun. 11:513 (1981).
12. Washburn R.M., Billig F.A., Bloom M., Albright C.F., Levens E. - In: Adv. Chem. Ser., Vol. 32 (Gould R.R., ed.), American Chemical Society, Washington, D.C., 1961, p 208.