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SYNTHESIS OF AN IODINE-125-LABELED RETINOID

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SUMMARY

The carrier-free synthesis of 4-(5',6',7',8'-tetrahydro-4'-[^{125}I]iodo-5',5',8',8'-tetramethyl-2'-anthracenyl)benzoic acid (4'-[^{125}I]TTAB) is described. Site specific radioiodination was accomplished by electrophilic destannylation of the tri(*n*-butyl)tin analog of the retinoid with Na^{125}I and chloramine-T. 4'-[^{125}I]TTAB (33 μCi, 1.65% radiochemical yield based on Na^{125}I consumed) was purified by reversed-phase HPLC.

KEYWORDS: 4-(5',6',7',8'-tetrahydro-4'-[^{125}I]iodo-5',5',8',8'-tetramethyl-2'-anthracenyl)-benzoic acid; 4'-[^{125}I]TTAB; electrophilic destannylation.

INTRODUCTION

The retinoid 4-(5',6',7',8'-tetrahydro-5',5',8',8'-tetramethylanthracen-2'-yl)-benzoic acid (**1**) is an ideal candidate for radiolabeling because of its high biological activity[1] and stability, and the finding that functionalization of its tetrahydroanthracenyl ring system does not appreciably decrease activity in bioassays used to assess cancer chemoprevention activity. For example, incorporation of an iodo group at the 4-position of this ring (**4**) only reduced by one-third the ED_{50} value for inhibiting the induction of ornithine decarboxylase by 12-*O*-tetradecanoylphorbol-13-acetate in mouse epidermis (Dawson and Chao, unpublished).

Therefore, we undertook the carrier-free synthesis of the radioiodinated analog to provide material of high specific activity (theoretical specific activity 2170 Ci/mmole based on Na^{125}I used) for biological labeling studies of proteins having low intracellular concentrations.

RESULTS AND DISCUSSION

Mais *et al.*[2] reported the facile conversion of an iodinated prostanoid to its radioiodinated analog through the tri(*n*-butyl)stannane intermediate. Clanton *et al.*,[3] Blaszczak *et al.*,[4] and McBride *et al.*[5] have also successfully used the radioiododestannylation method for the preparation of ^{125}I-labeled compounds. This method appeared to be ideal

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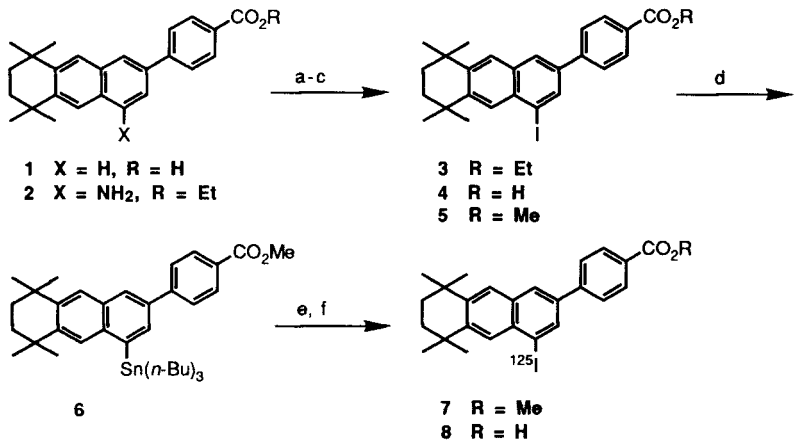
for the transformation of **4** to its radioiodinated analog **8** (Scheme 1). Labeling could be conducted in the penultimate step with a very high degree of site selectivity, thereby eliminating the need for extensive purification steps. The starting material for this synthesis was the 4'-amino analog **2** of the ethyl ester of **4**.[6] Diazotization under nonaqueous conditions and treatment with HI introduced the iodo group at the 4-position of the tetrahydroanthracene ring, giving ethyl ester **3**, which was hydrolyzed to **4**. Iodoretinoid **4** was then converted to the corresponding methyl ester **5** to facilitate ester hydrolysis. The milder conditions for methyl ester hydrolysis would minimize deiodination that occurred during this step. Treatment of **5** with bis[tri(*n*-butyl)tin] in the presence of Pd(0) afforded stannylated retinoid **6** and the methyl ester of **1**. Retinoid **6** was isolated (70%) by preparative TLC (silica gel, 5:1 hexane/EtOAc), and was shown by HPLC and TLC analyses to be free from iodoretinoid **5**, which if present could reduce the specific activity of ^{125}I-labeled retinoid **8** that was subsequently prepared. Stannylated retinoid **6** was then converted to iodoretinoid **5** using non-radioactive NaI and chloramine-T to determine reaction conditions and to establish the purity of the final product. Iodination of **6** for 1 h at room temperature gave an 82:18 mixture of iodoretinoid **5** and the methyl ester of **1**, which resulted from destannylation of **6**, and no starting material. The mixture of iodoretinoid **5** and the methyl ester of **1** was hydrolyzed to give a 78:22 mixture of iodoretinoid **4** and retinoid **1**. The products from the cold run were analyzed and identified by comparison of authentic samples using HPLC and TLC [HPLC (Novapak C_{18}, 2% H_2O/MeCN, 2 mL/min, 260 nm) t_R 2.0 (**8**), 4.6 (methyl ester of **1**), 9.6 (**5**), 54 min (**6**); (10% aq. HOAc/10% THF/80% MeCN, 1 mL/min) t_R 6.0 (**1**), 11.8 (**8**), 24 min (**5**); TLC (5 × 20-cm silica gel, 20% EtOAc/80% hexane), R_f 0.65 (**5**), 0.67 (methyl ester of **1**), 0.76 (**6**); (5 × 20-cm C_{18}, 2% H_2O/98% MeCN) R_f 0.05 (**6**), 0.26 (**5**), 0.4 (methyl ester of **1**), 0.4 (**8**)].



Scheme 1. a) *n*-BuONO, TFA, EtOH; aq. HI; b) KOH, aq. EtOH; aq. H_2SO_4; c) CH_2N_2, Et_2O; d) $[(n\text{-}Bu)_3Sn]_2$, $[(C_6H_5)_3P]_4Pd$, $Pd(OAc)_2$, THF; e) $Na^{125}I$, chloramine-T, MeOH, Na phosphate, pH 7.5; f) 40% aq. KOH; aq. HCl.

In this nonradioactive run, we found that the electrophilic displacement of the tri(*n*-butyl)tin group by iodine, followed by hydrolysis of the ester group, afforded iodoretinoid

8, which was readily purified from noniodinated retinoid **1** by HPLC. Therefore, stannylated retinoid **6** was treated with $Na^{125}I$ and chloramine-T under the same conditions. Radioiododestannylation and subsequent *in situ* hydrolysis produced 33 μCi of 4'-[^{125}I]TTAB (**8**), which was isolated by reversed-phase HPLC in 1.65% yield.

EXPERIMENTAL

4-(5',6',7',8'-Tetrahydro-4'-iodo-5',5',8',8'-tetramethylanthracen-2'-yl)-benzoic Acid (4). To a solution of 103 mg (0.26 mmol) of **2** in 2.5 mL of EtOH, cooled in an ice-bath, was added 1.0 mL of TFA. To this violet solution was added with stirring at ice-bath temperature 0.12 mL (1.0 mmol) of *n*-butyl nitrite. After 30 min, the now dark-red solution was treated with 1.0 mL of 50% aq. HI, and the ice-bath was removed. The brown suspension was stirred at ambient temperature for 16 h. Slight gas evolution was observed. The now black suspension was diluted with 40 mL of H_2O and extracted with 20 mL of $CHCl_3$. The purple extract was washed with 10% $NaHSO_3$ (10 mL) and H_2O (2 × 10 mL). The orange solution was dried (Na_2SO_4) and concentrated to give after chromatography on silica gel (1.5 × 20-cm, 5% EtOAc/95% hexane) 87 mg (66%) of the ethyl ester **3** as a colorless glass: IR ($CHCl_3$) λ_{max} 1715, 1610, 1280, 1110, 1020, 905, 845 cm^{-1}; 400 MHz ^{1}H NMR ($CDCl_3$) δ 1.40 and 1.44 (2 s, 12 H, $C(CH_3)_2$), 1.41 (t, J = 7.2 Hz, 3 H, $CH_2\underline{CH}_3$), 1.78 (m, 4 H, $(CH_2)_2$), 4.40 (q, J = 7.2 Hz, 2 H, $\underline{CH}_2CH_3$), 7.72 (dm, J = 8.6 Hz, 2 H, 3,5-ArH), 7.76 (s, 1 H, 9'-ArH), 7.97 (d, J = 1.4 Hz, 1 H, 3'-ArH), 8.00 (s, 1 H, 10'-ArH), 8.12 (dm, J = 8.6 Hz, 2 H, 2,6-ArH), 8.23 (d, J = 1.7 Hz, 1 H, 1'-ArH); EI-MS 512 (M^+); EI-HRMS calcd. for $C_{27}H_{29}IO_2$ 512.1211, found 512.1201 (M^+).

A suspension of 75 mg (0.15 mmol) of ethyl ester **3** in 3 mL of EtOH and 0.5 mL of 40% aq. KOH was stirred at 20°C under argon for 19 h. The resultant clear solution was concentrated to 1 mL under an argon stream. The suspension was acidified with 10 mL of 1 N H_2SO_4, stirred at 20°C for 1.25 h, and filtered. The precipitate was washed with H_2O (5 × 5 mL). The white solid was dried at reduced pressure and extracted into 30 mL of hot MeOH. The extract was concentrated to give 72 mg of crude **4**. A 66-mg sample was crystallized (CH_2Cl_2/hexane) to give 46 mg (70%) of **4** as white crystals: HPLC (Waters Radialpak Novapak C_{18}, 8-mm × 10-cm, 10% 1% aq. HOAc/90% MeOH, 2.0 mL/min, 260 nm) t_R 7.9 (3.5%) (**1**), 13.6 min (96.5 %) (**4**); HPLC (10% 1% aq. HOAc/10% THF/80% MeOH) t_R 3.5 min (3.5%), 6.2 min (96.5%). Samples were purified as required using the latter HPLC system to give pure **4** as white crystals, mp 250-251°C: HPLC (10% 1% aq. HOAc/10% THF/80% MeCN) t_R 11.8 min (100%); IR ($CHCl_3$) λ_{max} 2350-3200, 1685, 1610, 1300, 1020, 840 cm^{-1}; 400 MHz ^{1}H NMR ($CDCl_3$) δ 1.43 and 1.47 (2 s, 12 H, $C(CH_3)_2$), 1.82 (m, 4 H, $(CH_2)_2$), 7.80 (dm, J = 8.6 Hz, 2 H, 3,5-ArH), 7.81 (s, 1 H, 9'-ArH), 8.03 (d, J = 1.1 Hz, 1 H, 3'-ArH), 8.04 (s, 1 H, 10'-ArH), 8.22 (dm, J = 8.6 Hz, 2 H, 2,6-ArH), 8.27 (d, J = 1.7 Hz, 1 H, 1'-ArH); UV (EtOH) λ_{max} 311 nm (ε 1.56 x 10^4); EI-HRMS calcd. for $C_{25}H_{25}IO_2$ 484.0898, found 484.0867 (M^+).

Methyl 4-{4'-[Tri(*n*-butyl)stannyl]-5',6',7',8'-tetrahydro-5',5',8',8'-tetramethylanthracen-2'-yl}benzoate (6). Methyl ester **5** was prepared as required from

4 by esterification (CH_2N_2) and purified by HPLC (Novapak C_{18}, 1% H_2O/99% MeCN) t_R 7.8 min (100%). Satisfactory purification of **5** was not obtained using silica-based HPLC.

To a stirred mixture of 3.9 mg (7.8 μmol) of **5** and 0.55 mL of Et_3N in 3 mL of THF (distilled from benzophenone ketyl) in a 5-mL round-bottomed flask equipped with a stirring bar and fitted with an argon bubbler was added 7.50 μL (19.5 μmol) of $[(n\text{-}Bu)_3Sn]_2$ (Aldrich), followed by 3.23 mg (2.8 μmol) of $[(C_6H_5)_3P]_4Pd$ (Aldrich) and 0.77 mg of $Pd(OAc)_2$. The heterogeneous mixture was heated at reflux for 1 h, at which time it was transformed to a clear, brown solution, which subsequently deposited a black precipitate. After 3 h, the reaction mixture was cooled to room temperature and filtered through Celite. The filtrate was concentrated under a stream of argon, and the residue was purified by chromatography on analytical TLC plates (EM silica gel 60 Å, F-254, 250-μm thickness, 5 × 20-cm, 17% EtOAc/83% hexane) to give 3.6 mg (70%, estimated by UV absorbance at 282 nm) of **6** as an oil, R_f 0.76; 400 MHz ^1H NMR ($CDCl_3$) δ 0.885 (t, J = 7.3 Hz, 9 H, CH_2CH_3), 1.41 (s, 12 H, $C(CH_3)_2$), 1.65 (m, 18 H, $Sn(CH_2)_3CH_3$), 1.80, (s, 4 H, $(CH_2)_2$), 3.90 (s, 3 H, OCH_3), 7.8-8.2 (m, 8 H, ArH); UV (EtOH) λ_{max} 232 (ε 3.76 × 10^4), 282 (ε 4.16 × 10^4), 316 nm (ε 1.97 × 10^4); DCI-MS (NH_3) 663 (M+H); DCI-MS (CH_4) calcd. for $C_{38}H_{55}O_2Sn$ 663.3223, found 663.3244 (M+H).

4-(5',6',7',8'-Tetrahydro-4'-[^{125}I]iodo-5',5',8',8'-tetramethyl-2'-anthracenyl)benzoic Acid (8). A 40-μL aliquot of a solution prepared from 3.6 mg (5.4 μmol) of **6** in 100 μL of MeOH was diluted with 50 μL of 0.2 M Na phosphate buffer, pH 7.5, and then added to 19.17 μL of a cold (0°C) aqueous solution of $Na^{125}I$ [0.77 nmole; 104.35 μCi/μL, specific activity 17.40 mCi/μg (2176 Ci/mmol) of iodide, NEN, Lot No. 1111391A]. A 20-μL aliquot of freshly prepared chloramine-T solution [4.22 mg (18.5 μmol) of chloramine-T in 1.0 mL of buffer) was added. After 30 min at room temperature, 40 μL of 40% aq. KOH was added to the crude methyl ester **7** to hydrolyze the ester group. This reaction mixture was stirred for 2 h at ambient temperature, then was acidified with 500 μL of 3 N HCl, and extracted with $CHCl_3$ (3 × 3 mL). The extract was concentrated to 100 μL under a stream of argon. The residue was purified by reversed-phase HPLC (10% 1% aq. HOAc/10% THF/80% MeCN, 1.0 mL/min) R_f 3.8 and 11.8 min (**8**). The 11.8-min peak was collected and concentrated to give 33 μCi of **8** (1.65% yield based on $Na^{125}I$, assuming a specific activity of 2176 Ci/mmol). A theoretical maximum specific activity of 2176 Ci/mmole can be assigned since [^{125}I]-TTAB (**8**) can be separated from the starting material (**6**).

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