

SYNTHESIS OF A NEW CLASS OF RETINOID,  
<sup>3</sup>H-LABELLED TTNPB,<sup>1</sup> WITH A HIGH SPECIFIC ACTIVITY

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SUMMARY

Synthetic procedures for tritiation of a retinoidal benzoic acid derivative, (E)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl-6,7-<sup>3</sup>H<sub>2</sub>)-1-propenyl]-benzoic acid, (TTNPB), are described. Tritium was introduced by catalytic hydrogenation. The final product, TTNPB-<sup>3</sup>H<sub>2</sub> (9), had a specific activity of 24.1 Ci/mole and a radiochemical purity of 95.6%.

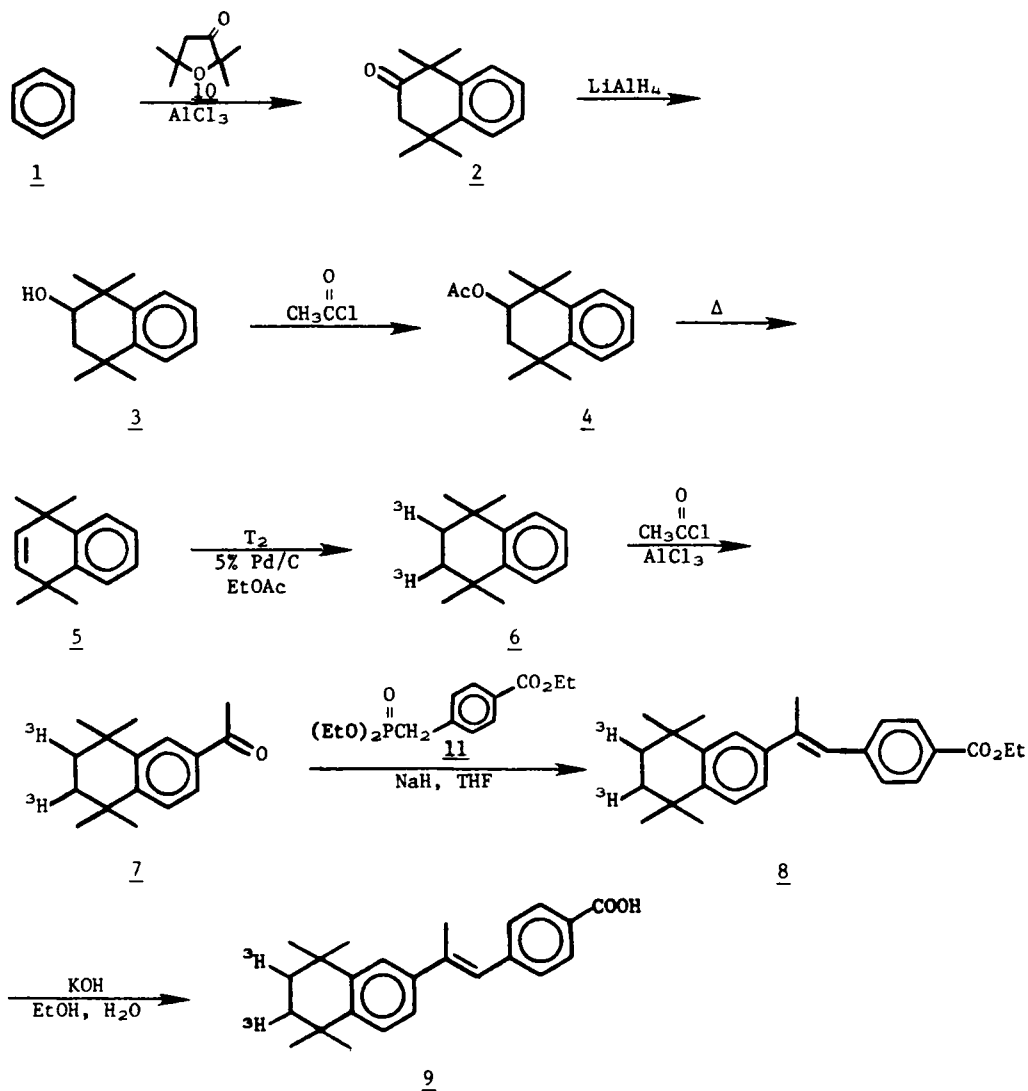
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INTRODUCTION

The development of new retinoids for chemoprevention of cancer and as adjuvants for chemotherapy requires radiolabelling for assaying biological activity. The compound TTNPB (9), a new aromatic analogue of vitamin A acid, has been reported previously<sup>1</sup> to be more active than retinoic acid in preventing progression of skin papilloma (induced by 7,12-dimethylbenzanthracene and promoted by croton oil) in the mouse<sup>2</sup> and in reversing keratinization of retinoid-deficient hamster tracheal organ cultures.<sup>3</sup>

To study the stability and pharmacokinetics of TTNPB in vivo and in vitro and therefore evaluate its therapeutic potential, the incorporation of radiolabels with a high specific activity into the molecule was clearly necessary.

The method proposed for this tritium labelling is shown in Scheme 1.



Scheme 1

#### SYNTHESIS

Benzene (**1**) was reacted with 2,2,5,5-tetramethyltetrahydro-3-keto-furan in the presence of aluminum chloride to yield crystalline ketone (**2**). In our hands, the published procedures<sup>4,5</sup> yielded a mixture of compounds, usually with very

little ketone (2) plus mostly rearrangement products, which were almost impossible to separate. Changing the reaction conditions by drastically lowering the reaction temperature did not impact the yield of (2), but reduced the amount of rearrangement products and allowed the ketone (2) to be isolated and purified. Reduction with lithium aluminum hydride gave quantitative yields of alcohol (3), which was then acetylated to give the acetate (4). Pyrolysis of 4 in a closed Carius tube at 340-350°C for 1.5 hr finally led to the needed unsaturated compound (5).

The catalytic hydrogenation of (5) with tritium gas (25 Ci, at 1 atm and at room temperature), using a Toepler pump, afforded 1,1,4,4-tetramethyl-1,2,3,4-tetrahydronaphthalene-2,4-<sup>3</sup>H<sub>2</sub> (6) in a quantitative yield. The Friedel-Crafts reaction of di-tritiated compound (6) with acetyl chloride proceeded satisfactorily to give the labeled ketone (7). The Emmons-Horner condensation of the phosphonate (11) and the labeled ketone (7) yielded crystalline ester (8). The cis:trans ratio in the crude ester (8) turned out to be 1:5. Without purification the cis/trans mixture (8) was saponified in ethanolic KOH solution and the desired trans acid (9) was isolated by fractional recrystallization.

The final analysis of the acid (9) by reverse-phase HPLC [eluants: (1) CH<sub>3</sub>CN:1% HOAc = 7:3, (2) EtOH:1% HOAc = 8:2] equipped with a Berthold-HPLC radioactivity monitor showed 99% chemical purity and ≥95% radiochemical purity, respectively, in the two solvent systems.

#### EXPERIMENTAL

Unless otherwise noted, all the reactions described here were performed under yellow light and under argon atmosphere. Melting points are uncorrected. Solvents were dried or distilled before use. Radioassays were carried out in 10 mL of ScintiVerse II cocktail (Fischer Scientific Co.) with internal standards and radioactivity was determined with a Beckman LS-250 liquid scintillation system. Analyses by HPLC were performed using a Waters 6000A solvent delivery system,

Model 450, with a variable wavelength detector, and an RCM-100 radial compression module, Radial Pak ( $C_{18}$  or silica, 8 mm ID  $\times$  10 cm). Radiochemical purities were measured by Berthold-HPLC Radioactivity Monitor LB 503.

1,1,4,4-Tetramethyl-2-tetralone (2). To a stirred mixture of dry 2,2,5,5-tetramethyltetrahydro-3-keto-furan (10) (23.68 g, 0.16 mole; Alfa Products) in 80 mL of dry benzene (70.24 g, 0.9 mole) was added anhydrous, powdered  $AlCl_3$  (45.5 g, 0.34 mole) gradually over a period of 25 min while the mixture was being maintained below  $40^\circ C$  by external cooling (an ice bath). Then the mixture was slowly warmed to room temperature and stirring was continued for an additional 1.5 hr while the inside temperature was kept between  $38^\circ$  and  $39^\circ C$ . The evolution of HCl gas was observed during this period. The reaction mixture was poured into 250 mL of ice and water containing 25 mL of concentrated HCl. The mixture was extracted with ether ( $3 \times 100$  mL) and the combined ether extract was washed with water (100 mL), saturated  $NaHCO_3$  solution (100 mL), and then again with water (100 mL). The organic layers were dried over anhydrous  $Na_2SO_4$  and concentrated to give 30.56 g of dark syrup. This material was taken up in twice its volume of petroleum ether ( $30-60^\circ C$ ) and kept at  $-15^\circ C$  to afford 7.76 g of a light yellow crystalline product. The remaining mother liquor was evaporated to a syrup and distilled through a  $3/4" \times 18"$  Vigreux column at 0.25 torr. Fraction 1, a light yellow liquid (bp  $55-104^\circ C$ ) was discarded. Fraction 2, a yellow syrup (3.48 g, bp  $110-115^\circ C$ ), fraction 3, an orange-yellow syrup (8.98 g, bp  $115-118^\circ C$ ), fraction 4 (1.44 g, bp  $123^\circ C$ ), and fraction 5 (4.58 g, bp  $123-126^\circ C$ ) were collected. Fractions 2 and 3 were combined and crystallized to give 3.81 g of solid. Purification of the combined crude product by successive recrystallizations from petroleum ether with the aid of decolorizing charcoal gave 5.44 g (17% yield) of 2 as colorless stout prisms, mp  $75-77^\circ C$  (lit.<sup>4</sup>  $78-79^\circ C$ ).

1,1,4,4-Tetramethyl-2-tetralol (3). To a solution of 3 g (79.05 mmoles) of  $LiAlH_4$  in 50 mL of anhydrous ether, a solution of the ketone (2) (16.4 g, 81.1 mmoles) in 100 mL of ether was added slowly, with continuous stirring at room

temperature. The mixture was refluxed for 4 hr and then quenched with saturated NH<sub>4</sub>Cl solution. The ether layer was decanted and the aqueous layer was extracted with ether (3 × 50 mL). The combined ether extract was dried over anhydrous MgSO<sub>4</sub> and evaporated, affording a syrup. Recrystallization from EtOH gave 15.8 g (90% yield) of 3 as colorless crystals, mp 85°C (lit.<sup>6</sup> 86–87°C).

1,1,4,4-Tetramethyl-2-acetoxytetralin (4). To a solution of 3 (20.4 g, 0.1 mole) and freshly distilled N,N-dimethylaniline (24.2 g, 0.2 mole) in 150 mL of anhydrous ether was slowly added freshly distilled AcCl (14.1 g, 0.18 mole) in 50 mL of ether. The mixture was heated under reflux for 20 hr. Ice water was added and the ether layer was washed with 1N HCl, saturated NaHCO<sub>3</sub> solution, and, finally, with water to neutrality. The ether layer was dried over anhydrous MgSO<sub>4</sub> and was concentrated to give 26.4 g of light yellow oil. The crude acetate (4) was chromatographed on alumina column (100 g, activity I, neutral). Combined fractions 1 and 2 (each 100 mL of benzene eluant) were recrystallized from petroleum ether to give 22.9 g (93% yield) of 4 as colorless crystals, mp 65–66.5°C (lit.<sup>6</sup> 66–67°C).

1,1,4,4-Tetramethyl-1,4-dihydronaphthalene (5). The acetate (4) (2.69 g, 10.8 μmoles) was decomposed by heating in a thick-walled Carius tube at 300–315°C for 1.5 hr. The reaction product was dissolved in ether, washed with saturated NaHCO<sub>3</sub> solution and then with water, and finally dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The concentrate (~1.8 g) was chromatographed on a column of silica gel (25 g, packed with hexane). The fractions containing product (fractions 1–5, 50 mL of hexane each) were combined and concentrated, affording 1.48 g of colorless liquid. The crude product was distilled through a 3/4" × 18" Vigreux column at ~150°C/0.12 torr to give 1.35 g (0.7 μmole, 66% yield) of a colorless liquid. The liquid product (5) showed one component by vapor-phase chromatography (VPC); IR lacked carbonyl; NMR bands at 0.94 (singlet), 5.03 (singlet), and 6.83 δ (A<sub>2</sub>B<sub>2</sub> symmetrical multiplet) in the ratio 12:2:4 assigned to the four CH<sub>3</sub>s, the -CH=CH group, and four aromatic hydrogens, respectively. Mass spectrum was (M<sup>+</sup>) 186.

1,2,3,4-Tetrahydro-1,1,4,4-tetramethylnaphthalene-2,3-<sup>3</sup>H<sub>2</sub> (6). A mixture of 77.0 mg (0.41 μmole) of the dihydronaphthalene (5) (98.5% chemical purity

according to VPC) and 82.8 mg of 5% Pd/C (Aldrich) in 5 mL of ethylacetate was stirred at room temperature for 30 min. The catalyst was removed by filtration through Celite and the filtrate was recharged with 81 mg of fresh 5% Pd/C. The flask containing the dihydronaphthalene (5), fresh 5% Pd/C, 8 mL of ethylacetate, and a magnetic stirring bar was attached to the Toepler system. The whole system was degassed three times and 25 Ci (0.86 m Atom of  $T_2$ , 0.43 mmole of  $T_2$  of carrier-free tritium gas) (New England Nuclear) was introduced into the reaction flask. After completion of the  $T_2$  transfer, the mixture was stirred for 15 hr at room temperature. The catalysts were removed by filtration through Celite and the ethylacetate was removed by careful vacuum transfer, leaving ~85 mg of oily residue. The crude product (6) (total activity of ~10 Ci, 100% chemical purity by VPC) was taken up in 10 mL of  $ClCH_2CH_2Cl$  and used without further purification in the acetylation step.

6-Acetyl-1,2,3,4-tetrahydro-1,1,4,4-tetramethylnaphthalene-2,3- $^3H_2$  (7). To a cooled, stirred mixture of 85 mg (0.44 mmole, ~10 Ci) of tetrahydronaphthalene (6) in 10 mL of  $ClCH_2CH_2Cl$  and 124 mg (0.93 mmole) of anhydrous, powdered  $AlCl_3$  was added 0.75 mL (827 mg, 10 mmoles) of  $AcCl$  in 4 mL of  $ClCH_2CH_2Cl$  dropwise over a period of 20 min. The dark solution was slowly warmed to room temperature. After stirring for an additional 1 hr, the mixture was quenched with 20 mL of ice water and extracted with ether (2 × 20 mL). The combined ether extract was washed with water (3 × 20 mL) and dried over anhydrous  $MgSO_4$ . The dried, crude residue consisted of 85% of acetylated product (7) and 15% of unreacted starting material (6) according to VPC. Purification of the crude residue by chromatography (silica gel, hexane/ether gradient) gave 60 mg (0.25 mmole, total activity of 4 Ci) of the methyl ketone (7).

(E,Z)-4-[2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl-6,7- $^3H_2$ )-1-propenyl]benzoic acid ethyl ester (8). To a cooled, stirred suspension of 93.6 mg of NaH (60% in oil dispersion, 56 mg, 2.34 mmoles equivalent) in 4 mL of dry THF was added a mixture of 60 mg (0.25 mmole, 4 Ci) of the tritiated methyl ketone (7), 160 mg (0.73 mmole) of 15-Crown-5-ether, and 310 mg of diethyl 4-carboethoxy benzyl phosphonate (11) in 2 mL of THF in a syringe. The solution became dark

red. After being stirred for 20 hr at room temperature, the reaction mixture was poured into 15 mL of ice water and 15 mL of brine solution. The mixture was extracted with ether (3 × 30 mL) and the combined ether extract was washed repeatedly with water (3 × 30 mL) until it became neutral. The ether solution was dried over anhydrous MgSO<sub>4</sub> and concentrated, affording 72.8 mg of yellow oil. The oily residue was purified on a silica gel column (10 g, ether/hexane gradient) to give 23.3 mg of crude esters. Analytical HPLC (CH<sub>3</sub>CN:H<sub>2</sub>O = 8:2, 2 mL/min, 304 nm) indicated two isomers: t<sub>R</sub>: 27 min (15% Z isomer); t<sub>R</sub>: 30 min (80% E isomer). The mixture had a total activity of 1.3 Ci (32.5% radiochemical yield) and was saponified in the next step without further purification.

(E)-4-[2-(5,6,7,8-Tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl-6,7-<sup>3</sup>H<sub>2</sub>)-1-propenyl]benzoic acid (TTNPB-<sup>3</sup>H<sub>2</sub>) (9). To a stirred solution of 10 mg (0.026 mole, 55.7 mCi) of the tritiated ester (8) in 5 mL of EtOH was added 5 mL of 0.1N KOH. The mixture was heated in a 70-75°C oil bath for 1.5 hr. After cooling, the solution was acidified with 1 mL of 1N H<sub>2</sub>SO<sub>4</sub> (to pH 2) and extracted with ether (3 × 10 mL). The combined ether extract was washed with water (2 × 10 mL), saturated NaCl solution (10 mL), and again with water (10 mL). The ether extract was dried over anhydrous MgSO<sub>4</sub> and concentrated, affording 18.3 mg of yellow solid. The crude products were passed through a silica gel column with hexane. Analytical HPLC (EtOH:1% HOAc = 8:2, 1 mL/min, 304 nm) indicated two isomers of the acid: t<sub>R</sub>: 9 min (15% Z isomer); t<sub>R</sub>: 12 min (80% E isomer). The E acid (9) was obtained by successive fractional recrystallization from CH<sub>2</sub>Cl<sub>2</sub>/hexane. Finally, the E-acid was purified by minipreparative HPLC, (EtOH:1% HOAc = 8:2). The HPLC retention time and the UV spectrum (λ<sub>max</sub> 297 nm, ε = 22600, EtOH) of the purified E-acid (9) were identical to those of authentic unlabeled E-acid. Compound 9 had total activity of 148.93 mCi (25.6% radiochemical yield), specific activity of 24.1 Ci/mole, and radiochemical purity of 95.6%.

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