A PREPARATION OF HIGH SPECIFIC ACTIVITY [11,12-^{3}H]-9-*cis*-RETINOIC ACID

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SUMMARY

A selective total synthesis of [11,12-^{3}H]-9-*cis*-retinoic acid **1** at a specific activity of 46 Ci/mmole is described. The alkyne **4**, efficiently prepared in two steps from readily available starting materials, was partially reduced to 9,11-di-*cis*-retinoic acid **3** with both deuterium and tritium gas. Selective isomerization of **3** to **1** is discussed, as well as non-selective direct conversions of **4** to **1**.

Key words: 9-*cis*-retinoic acid, deuteruim, tritium, high specific activity

INTRODUCTION

Interest in the biological role of nuclear retinoid receptors has intensified with the recent discovery that 9-*cis*-retinoic acid (9-*cis*-RA), **1**, an endogenous retinoid, is a ligand for the retinoid X (RXR) and retinoic acid (RAR) receptors .[1,2,3] In contrast, all *trans*-RA binds only to the RARs, although it has been demonstrated to regulate gene function *via* the RXRs in cell based cotransfection assays.[3,4] Radiolabelled ligands are essential tools for studies of retinoid receptor biology and while [11,12-^{3}H]-all-*trans*-RA is commercially available, the 9-*cis*-isomer is not. Two synthetic approaches to radiolabelled 9-*cis*-RA have recently appeared involving photo isomerization of [11,12-^{3}H]-all-*trans*-RA[5] and a total synthesis of [11-^{3}H]-9-*cis*-RA.[6] In connection with our research efforts, we recently required millicurie quantities of high specific activity 9-*cis*-RA. Herein we describe the discovery of a selective and practical route to [11,12-^{3}H]-9-*cis*-retinoic acid.

Several factors influenced our synthetic design. The pioneering work of Robeson *et al.* demonstrated the facile isomerization of the 13-*cis*-olefin of 9,13-di-*cis*-RA **2** to generate **1** (Scheme I).[7] In their total synthesis of 9-*cis*-RA **1**, the final step involved a selective iodine catalyzed *cis/trans* isomerization of the 13,14-*cis*-olefin of **2**. The relative stability of the 9,10-*cis*-olefin in this isomerization encouraged us to undertake the synthesis of 9,11-di-*cis*-RA **3** which, with a selective isomerization of the 11-*cis* olefin, would provide access to **1**.[8] Preparation of the di-*cis* isomer **3** by partial reduction of the acetylene **4** would allow introduction of the tritium radiolabel late in the synthesis. Ample precedent exists for partial acetylene reduction as a method of tritium incorporation in retinoid syntheses.[9] As a result, acetylene **4** became our primary target.

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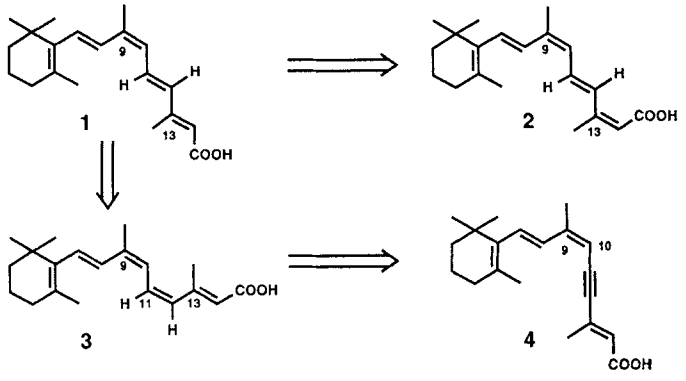
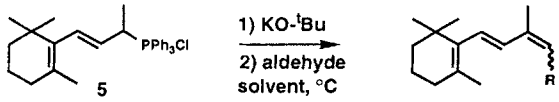
Scheme I

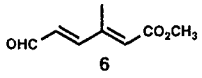
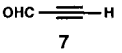
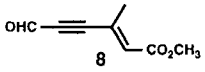


DISCUSSION

For the construction of the critical 9,10-*cis*-olefin in **4**, we chose to explore the Wittig reaction of the ylid derived from phosphonium salt **5**, originally described by Pommer and Sarnecki in their convergent approach to retinoic acids.[10] As is frequently observed in Wittig approaches to trisubstituted olefins, the ylid of **5** reacted with **6** to give a mixture of geometric isomers.[11] In an attempt to optimize conditions favoring *cis*-olefin formation, we undertook a study of reactions of **5** as summarized in Table I.

Table I



Entry	Aldehyde	T (°C)	Solvent	Selectivity cis/trans
1	 6	-10	DMF/NaOMe	(45/55)[9]
2	 7	0 - 20	THF/MeOH	57/43
3	 8	0 - 20	THF	70/30
4	8	-78 - 20	THF	82/18

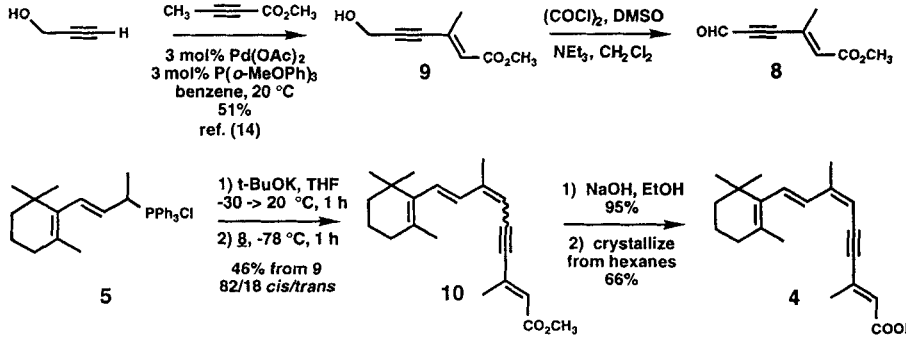
Phosphonium salt **5** (1.25 eq., 0.5 M) was stirred at in the indicated solvent at -30 °C under a nitrogen atmosphere and treated with potassium t-butoxide (1.2 eq.). After warming to 20 °C, the phosphorane was cooled to the indicated temperature and treated with aldehyde (1.0 eq., 0.5 M). After stirring 1 h and warming to ambient temperature, the reactions were worked up (NH_4Cl, Et_2O) and product isolated by column chromatography. Isomeric ratios determined by GCMS and ^1H NMR.

We were able to confirm the Pommer and Sarnecki result: reaction of purified **5** with sodium methoxide and dienal **6**[12] in dimethylformamide at -10 °C provided a 45/55 *cis/trans* isomeric ratio of isolated products (entry 1). Modest *cis* selectivity was observed with propargyl

aldehyde **7**[13] at 0 °C (entry 2). The more reactive enynal **8**, prepared in two steps from propargyl alcohol,[14] exhibited 70/30 *cis/trans* selectivity under similar conditions (entry 3). The ratio was improved to 82/18 at -78 °C (entry 4).

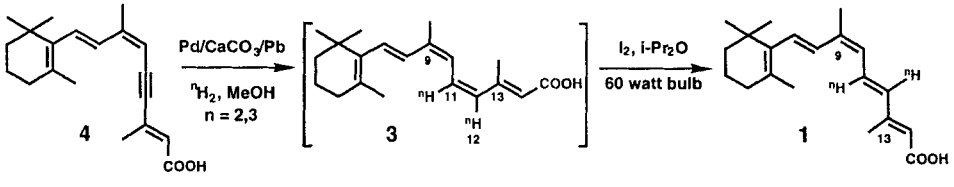
Having achieved modest selectivity for the desired *cis*-isomer **10** in the Wittig condensation, we were gratified to find that after saponification (NaOH/EtOH) of the ester **10** mixture, the 9-*cis*-isomer **4** selectively crystallized from hexanes in 66% yield. The total synthesis is shown in Scheme II.

Scheme II



The partial reduction of **4** with deuterium gas using Lindlar catalyst at room temperature was investigated (Scheme III). Reduction was observed to be faster in CH_3OH than in EtOAc (<2 vs. 24 h) as monitored by TLC or ^1H NMR. The crude reduction product was a mixture of deuterated **3** and **1** (~9/1 by ^1H NMR). Isomerization of the mixture to deuterated 9-*cis*-RA **1** under the conditions of Robeson *et al.*[7] was complete in 30-90 min as determined by ^1H NMR spectroscopy. On larger scales (~50 mg), deuterated **1** was readily purified by recrystallization in 43% yield (see experimental).[16]

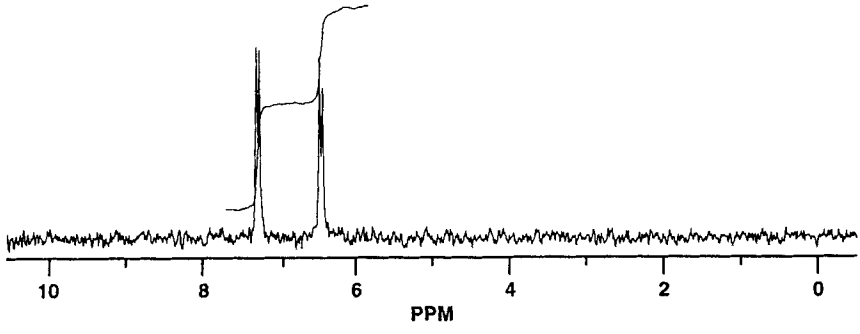
Scheme III



HPLC analysis of the crude reduction mixture containing 9,11-di-*cis*-RA **3** and 9-*cis*-RA **1** along of purified **1**[15] revealed identical retention times for both **3** and **1** by all conventional methods.[16] For tritium incorporation experiments, an analytical method was required to determine the extent of isomerization and product purity of high specific activity material. Resolution of these isomers was achieved after considerable effort utilizing a Hypercarb S graphite column.[17]

With this separation technique available, analyses of the tritium incorporation and *cis/trans* isomerization were possible. The partial reduction of **4** with tritium gas using Lindlar catalyst did not proceed in the same manner as the deuterium incorporation experiments. Tritiations carried out using Lindlar catalyst (in the presence or absence of quinoline) and tris(triphenylphosphine)ruthenium(II) chloride failed to give any significant reduction of **4** to **3**. Reduction was successful when catalyzed by 10% palladium on carbon but yielded material with a specific activity lower than expected (28 mCi/mmol). Satisfactory reduction of **4** with tritium gas was ultimately achieved with the soluble tris(triphenylphosphine)rhodium(I) chloride (Wilkinson's catalyst) in benzene/ethanol at room temperature. Unexpectedly, HPLC analysis of the crude tritiation product on a Hypercarb S column[17] revealed a mixture containing both [11,12-^{3}H]-9,11-di-*cis*-retinoic acid **3** and [11,12-^{3}H]-9-*cis*-retinoic acid **1**. Partial purification of the crude tritiation product was achieved by reverse phase HPLC[16] and Hypercarb S analysis of the partially purified material showed a 1:1 mixture of **1** to **3**. Separation of these isomers was achieved on a Hypercarb S column[17] and providing [11,12-^{3}H]-9-*cis*-retinoic acid **1** and [11,12-^{3}H]-9,11-di-*cis*-retinoic acid **3** (46 Ci/mmol). The proton decoupled ^{3}H NMR spectrum of the methyl ester of **1** (generated by $TMSCHN_2$ treatment of **1**) clearly shows the trituim label at the 11 and 12 positions (7.33 and 6.46 ppm respectively) as shown in Figure I.

Figure I



Proton decoupled ^{3}H NMR spectrum of [11,12-^{3}H]-9-*cis*-retinoic acid methyl ester at 360 MHz.

Isomerization of tritiated **3** under the conditions of Robeson *et al.*[7] was monitored by Hypercarb S HPLC. Complete conversion of **3** to **1** occurs in 10 min. Isomerization over 60 min produces an unidentified peak at the expense of **1**. While this isomerization method of preparation of tritiated **1** from tritiated **3** remains an option, sufficient quantities of tritiated **1** are produced by the non-selective Wilkinson's catalyst reduction thus enhancing the brevity of the overall process.

In summary, the preparation of isomerically pure 11,12-dehydro-9-*cis*-retinoic acid, **4**, has been achieved in 30% yield from the known precursors, phosphonium salt **5** and enyne **9**. Partial reduction of **4** with deuterium and subsequent isomerization proceeds in 43% yield to generate [11,12-^{2}H]-9-*cis*-retinoic acid, **1**. Partial reduction of **4** with tritium gas generates mCi quantities high specific activity [11,12-^{3}H]-9-*cis*-retinoic acid, **1** (46 Ci/mmol).

EXPERIMENTAL

General: All solvents and reagents were used as supplied by the manufacturer. Radiochemical purity and mass spectroscopy determinations were carried out by the Quality Control Department of Amersham International plc using a Spectra Physics SP8780 HPLC pumping system and a Jeol JMS DX 300 mass spectrometer with the appropriate data capture systems. HPLC purifications were carried out using a Gilson 305/306 pumping system. Radioactivity was measured on a Wallac 1209 liquid scintillation counter. The Hypercarb S column was purchased from Shandon and the Zorbax ODS column was packed in-house.

Preparation of phosphonium salt, **5**: 5-(2,6,6-trimethylcyclohexen-1-yl)-3-methyl-3-hydroxy-1,4-pentadiene (prepared as described in ref. 9) (6.0 g, 34.1 mmol) and triphenylphosphine (8.93 g, 34.1 mmol) were dissolved in CH_2Cl_2 (100 mL) and treated with 1*N* HCl/Et_2O (70 mL). After 48 h at 20 °C, the reaction mixture was concentrated *in vacuo* to give an oily residue which was chromatographed on silica gel eluting with 8% MeOH/CH_2Cl_2 (R_f 0.3) Concentration from THF provides a yellow foam 14.2 g, (88%) used as such in subsequent reactions.

Preparation of methyl-6-hydroxy-3-methyl-hex-4-yn-2-enoate, **9**: This material was prepared as described in ref. (12) using tris(2-methoxyphenyl)phosphine in the catalytic system. Propargyl alcohol (2.86 g, 51 mmol), methylbutynoate (5.0 g, 51 mmol) and palladium acetate (286 mg, 1.3 mmol) were stirred in degassed benzene (100 mL) and tris(2-methoxyphenyl)phosphine (448 mg, 1.3 mmol) was introduced. An exothermic reaction occurred and the solution darkens. Stirring was continued for 2 h. Concentration and chromatography on silica gel eluting with 35% acetone in hexanes (R_f 0.4) provided **9** as an oil, 4.0 g (51%). ^{1}H NMR (300 MHz, $CDCl_3$) d 6.03 (d, J = 1.5 Hz, 1H), 4.40 (d, J = 5.5 Hz, 2H), 3.69 (s, 3H, CH_3), 2.26 (d, J = 1.5 Hz, 3H).

Preparation of methyl-3-methyl-hex-6-al-4-yn-2-enoate, **8**: To a solution of $(COCl)_2$ (3.62 mL, 40.9 mmol) in anhydrous CH_2Cl_2 (100 mL) cooled in a dry ice/acetone bath under nitrogen was carefully added DMSO (4.65 g, 65.5 mmol). After 5 min., a solution of propargyl alcohol **9** (2.52 g, 16.4 mmol) in CH_2Cl_2 (10 mL) was added. After stirring for 10 min, the resulting white slurry was treated with Et_3N (11.4 mL, 81.8 mmol) over 5 min.. The resulting thick slurry was allowed to warm to 0 °C then poured into H_2O (200 mL) and CH_2Cl_2 (100 mL). The layers were separated and the organic layer was washed with 1 N HCL solution (50 mL) and saturated aqueous $NaHCO_3$ (2 x 100 mL). After filtration through cotton, concentration afforded 2.43 g of **8** as an oil which was used immediately in the next step.

Preparation of methyl-11,12-dehydro-9-*cis*-retinoic acid, **10**: Phosphonium salt **5** (8.0 g, 16.8 mmol) was cooled to -30 °C in anhydrous THF (50 mL) under nitrogen and treated with t-BuOK (1.85 g, 16.6 mmol). This mixture was stirred and allowed to warm to room temperature over 1 h. The resulting deep red-brown solution was re-cooled to -78 °C and treated with a solution of freshly prepared aldehyde **8**, (2.43 g, 16.0 mmol) in THF (10 mL). After 10 min at -78 °C, the reaction mixture was allowed to warm to 0 °C over 1 h and poured into H_20 (100 mL). The pH was adjusted to 5 with 1 N HCl solution and the mixture was extracted with Et_2O (2 x 100 mL). After washing with H_20 (2 x 50 mL) and saturated NaCl solution (2 x 50 mL), the organic layer

was dried ($MgSO_4$), filtered and concentrated to give an oily solid. Triphenylphosphine oxide residues were removed by filtration of a 30% CH_2Cl_2/hexane solution of the crude product through a silica gel pad (8 x 8 mm). The filtrate was concentrated and chromatographed on silica gel eluting with 30% CH_2Cl_2/hexanes to yield crude product **10**, 2.33 g (46% from **9**, R_f 0.28). This product was a mixture of *cis*-**10** (82%) and *trans*-**10** (18%) as determined by GCMS *m/e* 312 (M^+), and ^1H NMR analysis of the H_{14} resonances (s, 1H): *cis* **4**, δ 5.49, *trans* **4**, δ 5.56.

Preparation of 11,12-dehydro-9-*cis*-retinoic acid, **4**: Crude methyl-11,12-dehydro-9-*cis*-retinoic acid, **10** (2.33 g, 7.53 mmol) was stirred in ethanol (30 mL) under nitrogen and treated with 1N NaOH solution (15 mL). The solution was stirred at 50 °C for 2 h, then cooled and poured into H_2O (100 mL). 1N HCl solution (15 mL) was added slowly with stirring and the pH was adjusted to 2. The product was extracted with Et_2O (2 x 50 mL), washed with H_2O (50 mL), saturated NaCl solution (50 mL), then dried ($MgSO_4$), filtered and concentrated to an oil which solidified upon standing, 2.1 g (94%). Recrystallization from hexanes provided homogeneous 11,12-dehydro-9-cis-retinoic acid, **4**, in three crops (1.47 g, 66%). m.p. 105 - 106 °C; R_f 0.24 (20% acetone/hexanes); ^1H NMR (300 MHz, $CDCl_3$) δ 6.80 (d, J = 16.0 Hz, 1H), 6.41 (d, J = 16.0 Hz, 1H), 6.02 (br s, 1H), 5.49 (s, 1H), 2.34 (s, 3H, CH_3), 2.06 (m, 2H, CH_2), 1.99 (s, 3H, CH_3), 1.77 (s, 3H, CH_3), 1.62 (m, 2H, CH_2), 1.47 (m, 2H, CH_2), 1.07 (s, 6H, CH_3); GCMS *m/e* 298 (M^+).

Preparation of [11,12-^{2}H]-9,11-di-*cis*-retinoic acid, **3**: 11,12-Dehydro-9-*cis*-RA, **4**, (54 mg, 0.18 mmol) and Lindlar catalyst Pd/ $CaCO_3$/Pb (22 mg) were stirred in degassed MeOH (5 mL) under a slight positive pressure of deuterium gas for 2 h at room temperature. TLC indicated complete reaction (35% acetone/hexanes, **4**, R_f 0.55; **3**, R_f 0.57). The reaction was filtered through Celite and concentrated *in vacuo* to provide **3** as a solid, 56 mg. Crude ^1H NMR (300 MHz, $CDCl_3$) δ 6.60 (d, J = 16.0 Hz, 1H), 6.38 (br s, 1H), 6.24 (d, J = 16.0 Hz, 1H), 5.85 (s, 1H), 2.32 (s, 3H, CH_3), 1.98 (m, 5H, CH_2,CH_3), 1.71 (s, 3H, CH_3), 1.60 (m, 2H, CH_2), 1.46 (m, 2H, CH_2), 1.02 (s, 6H, CH_3). Approximately 10% of deuterated **1** was present; see ^1H NMR below. HPLC on Hypercarb S : t_r 7.2 min.[17]

Preparation of [11,12-^{2}H]-9-*cis*-retinoic acid, **1**: The crude reaction product containing [11,12-^{2}H]-9,11-di-*cis*-retinoic acid, **3**, (56 mg, 0.18 mmol) was dissolved in anhydrous Et_2O (10 mL) in a Pyrex flask and treated with iodine in Et_2O (50 μL of a 0.5 mg/mL solution). This was exposed to a 60 Watt incandescent bulb for 1.5 h at room temperature. $Na_2S_2O_3$ (s, 100 mg) was added and stirred 5 min then the reaction was filtered through a silica gel plug in a Pasteur pipette. The filtrate was concentrated and triturated with hexanes. The product was filtered and dried to afford **1** as a yellow solid, 23 mg (43%). TLC R_f 0.57 (35% acetone/hexanes); ^1H NMR (300 MHz, $CDCl_3$) δ 6.62 (d, J = 16.0 Hz, 1H), 6.26 (d, J = 16.0 Hz, 1H), 6.04 (s, 1H), 5.77 (s, 1H), 2.33 (s, 3H, CH_3), 2.03 (t, 2H, J = 6.0 Hz, CH_2), 1.98 (s, 3H, CH_3), 1.73 (s, 3H, CH_3), 1.61 (m, 2H, CH_2), 1.46 (m, 2H, CH_2), 1.02 (s, 6H, CH_3). HRMS for $C_{20}H_{26}O_2D_2$: calcd. 302.2215; found 302.2219. HPLC on Hypercarb S: t_r 18.4 min.[17]

Preparation of [11,12-^{3}H]-9-*cis*-retinoic acid, **1**: 11,12-Dehydro-9-*cis*-RA, **4** (3 mg, 0.01 mmol) and Wilkinson's Catalyst (5 mg, 0.005 mmol) were stirred in a solution of benzene:ethanol (1:1,

2 mL) under 3 Ci (0.051 mmol) of tritium gas for 16 h at ambient temperature. Labile tritium was removed by rotary evaporations from methanol and catalyst was removed by passing the crude tritiated material through a short bed of silica gel eluting in hexane:acetone (7:3). Partial purification of the crude [11,12-^{3}H]-9-*cis*-retinoic acid was carried out by HPLC on a Zorbax ODS column eluting in CH_3OH:H_2O:CH_3CN:*i*-PrOH:AcOH (125:150:150:75:1) to yield approximately 100 mCi of **1**.

Hypercarb S purification of [11,12-^{3}H]-9-*cis*-retinoic acid, **1**: [11,12-^{3}H]-9-*cis*-retinoic acid, **1**, was separated from [11,12-^{3}H]-9-di-*cis*-retinoic acid, **3**, and other impurities by HPLC on a Hypercarb S column with gradient (hexane:toluene:AcOH (90:10:0.1) to hexane:toluene:AcOH (40:60:0.1)) elution over 45 min at 1 mL/min. Retention time for **1** is approximately 35 min. HPLC analysis showed a radiochemical purity of >95%[a] for **1** with <1% [11,12-^{3}H]-9-di-*cis*-retinoic acid, **3**, present[b] to afford 15 mCi of **1**. Mass spectrometry (on the methyl ester, generated from $TMSCHN_2$) gave a specific activity of 46 Ci/mmol.

a.) Reverse phase HPLC on a Nucleosil ODS eluting in 0.05M ammonium acetate pH 7.6:acetonitrile (35:65).
b) Hypercarb S column with gradient elution from hexane:toluene AcOH (75:25:0.5) to toluene AcOH (100:0.5).

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16. See ref. (1, 2, 5) and experimental section for conditions. HPLC Conditions: Zorbax ODS (250 x 4.6mm). Eluent [CH_3CN:MeOH:i-PrOH:H_2O:AcOH/300:250:150:300:2] Flow rate 1 mL/min UV 340 nm.

17. HPLC Hypercarb S. (5000E) (100 x 4.6 mm) Solvent A hexanes:AcOH/500:0.5: Solvent B toluene:AcOH/500:0.5; Gradient 0 to 80%B over 20 min. flow rate 1 mL/min.