Notes

An Efficient and Stereoselective Synthesis of 9-*cis*-Retinoic Acid

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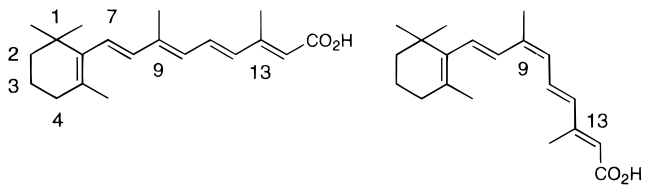
Introduction

Retinoids are metabolites, derivatives, and synthetic analogues of vitamin A which exert numerous biological effects *in vivo* by binding to and activating nuclear retinoid receptors, which results in the alteration of gene expression.[1] *all-trans*-Retinoic acid (ATRA, **1**) (Figure 1)[2] is known to modulate proliferation and differentiation of a variety of cell types and has proved useful for the treatment of dermatological diseases and certain cancers.[3] Recently, 9-*cis*-retinoic acid (9-*cis*-RA, **2**) was identified as a novel endogenous hormone in mammalian tissues.[4] It has been shown to play an important role as a modulator of nuclear transcription of cells through the retinoic acid receptors ($RAR_{\alpha,\beta,\gamma}$) as well as retinoid X receptors ($RXR_{\alpha,\beta,\gamma}$).[5] 9-*cis*-RA (ALRT1057) is in phase II clinical trials for the treatment of several cancers including renal cell carcinoma, non-Hodgkin's lymphoma, and acute promyelocytic leukemia, among others.[6] As a result of its biological profile and importance, we needed to prepare this compound in large quantities, which prompted us to devise several synthetic schemes for its preparation.[7]

The synthesis of 9-*cis*-RA (**2**) presented some challenges, among which were the following (1) introducing the acid-sensitive trisubstituted 9,10-*cis* double bond in an efficient manner and preserving its geometric integrity, (2) securing the *trans*-geometry of the 13,14-double bond, and (3) utilizing readily available, inexpensive starting materials and reagents, as well as avoiding the use of chromatography in order to simplify the process for large scale production. Compound **2** has previously been prepared on a kilogram scale in six steps starting from β-cyclocitral with an overall yield of ~11%.[7a] It has also been prepared on milligram scale by photoisomerization of ATRA in a low 5% yield.[8]



all-trans-Retinoic Acid (ATRA) **1**

9-cis-Retinoic Acid (*9-cis*-RA) **2**

Figure 1.

We wish to describe herein an efficient approach for the preparation of 9-*cis*-RA (**2**) starting from commercially available and inexpensive β-ionone (Scheme 1). The key step in this synthesis relies on the geometrically highly selective introduction of a *cis* trisubstituted double bond via a 1,4-conjugate addition of dimethyl cuprate to acetylenic nitrile **5**, to give almost exclusively the crucial and desired 9,10-*cis* double bond. Thus, readily available β-ionone **3** was converted to dieneyne **4** in 80% yield after distillation according to the reliable Negishi procedure.[9] Treatment of **4** with *n*-BuLi in THF at −78 °C followed by the addition of phenyl cyanate[10] gave, after basic workup, nitrile **5** in 95% yield. 1,4-Conjugate addition of dimethyl cuprate to **5**, in THF at −78 °C, afforded in nearly quantitative yield trienenitrile **6**, with a high level of stereocontrol (>98:2 *cis:trans*).[11,12] Reduction of nitrile **6** to the trienealdehyde **7** using Dibal, in hexanes at −78 °C, proceeded in 95% yield, accompanied by about 10% isomerization to the undesired *trans*-aldehyde (Scheme 1). Homologation of aldehyde **7** (as a 9:1 2-*cis*:2-*trans*

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(2) The traditional retinoid numbering system is being used in the text when referring to 9-*cis*-RA (**2**) and *all-trans*-RA (**1**). The IUPAC nomenclature was used in the Experimental Section.

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(11) Ratio determined by 400 MHz ^{1}H-NMR.

(12) For 1,4-addition to acetylenic esters: (a) Corey, E. J.; Katzenellenbogen, J. A. *J. Am. Chem. Soc.* **1969**, *91*, 1851. (b) Ernst, L.; Hopf, H.; Krause, N. *J. Org. Chem.* **1987**, *52*, 398. For 1,4-addition to acetylenic nitriles: (c) Westmijze, H.; Vermeer, P. Westmijze, H., Kleijn, H., Vermeer, P. *Tetrahedron Lett.* **1979**, 3327; *Synthesis* **1978**, 454 and references cited therein. For the preparation of a mixture of nitriles: (d) Cainelli, G.; Cardillo, G.; Contento, M.; Grasselli, P. Umani Ronchi A. *Gazz. Chim. Ital.* **1973**, *103*, 117. (e) Andriamialisoa, Z.; Valla, A.; Zennache, S.; Giraud, M.; Potier, P. *Tetrahedron Lett.* **1993**, *34*, 8091.

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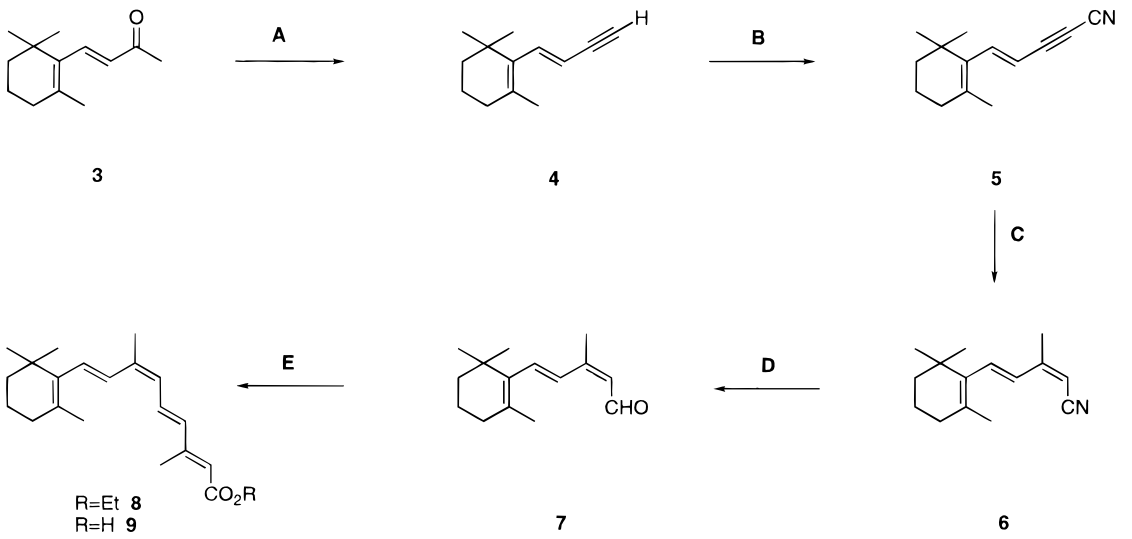
Scheme 1[a]



[a] A: (i) LDA, THF, −78 °C; (ii) $ClP(O)(OEt)_2$; (iii) LDA; H_2O. B: (i) *n*-BuLi, THF; (ii) PhOCN, −78 to 25 °C. C: MeLi, CuI, THF, −78 °C. D: Dibal-H, −78 °C, hexanes. E: (i) *n*-BuLi, DMPU, THF, $(EtO)_2P(O)CH_2C(CH_3)CHCO_2Et$, −40 °C; (ii) KOH, EtOH, 70 °C.

mixture) under Wittig−Horner−Emmons[13] conditions with diethyl 3-(ethoxycarbonyl)-2-methylprop-2-enyl phosphonate (*n*-BuLi, DMPU, −40 to +25 °C) afforded ester **8** in 89% yield with a 15:1 ratio of 13-*trans*:13-*cis* isomers as determined by ^{1}H NMR. Saponification of ester **8** using KOH in ethanol at 70°C for 3 h gave the desired 9-*cis*-retinoic acid in 90% yield. Recrystallization from a mixture of ethanol/water (10:1) afforded geometrically pure 9-*cis*-RA (**2**) (98.7% as determined by HPLC) in 83% yield after recrystallization.

We have described the synthesis of 9-*cis*-RA (**2**) in six steps (five steps *vide infra*) and ∼50% overall yield starting from readily available and inexpensive β-ionone. All steps proceeded in high yields, affording the desired products without the use of chromatography. Process developments aimed at conducting the reactions at higher temperatures and scale-up feasibility are underway.

Experimental Section

All the reactions were carried out under a nitrogen atmosphere except where stated. The organic solvents were purchased from Fisher Scientific, THF was distilled from Na (metal) in the presence of benzophenone, and hexanes were distilled from LAH, and stored over 4 Å sieves. Thin layer chromatography was performed on Merck Kieselgel 60 F-254 plates. ^{1}H and ^{13}C NMR spectra were recorded on Bruker 400 MHz spectrometer. Elemental analysis was carried out at Huffman Laboratories, Co.

***(E)*-5-(2,6,6-Trimethyl-1-cyclohexen-1-yl)pent-4-en-2-ynenitrile (5).** A 500 mL flame-dried flask was charged with (*E*)-4-[1-(2,6,6-trimethylcyclohexen-1-yl)]but-3-en-1-yne[9] (**4**) (10.45 g, 60.0 mmol) in THF (125 mL) and cooled to −78 °C. A solution of *n*-BuLi in hexanes (2.25 M; 27.5 mL, 61.5 mmol) was slowly added over 20 min, and the slightly dark mixture was stirred for an additional 10 min. Phenyl cyanate (prepared from phenol and cyanogen bromide according to a reported method)[10] (7.50 mL, 66 mmol) in THF (25 mL) was introduced via cannula, and the mixture was allowed to warm to room temperature. Sodium hydroxide (20 mL of a 6 N solution) was added, and the mixture was extracted with ethyl acetate (250 mL). The organic layer was further washed with 1 N NaOH (2 × 50 mL), water (3 × 30 mL), and brine (2 × 30 mL) and dried over $MgSO_4$, and the solvents were evaporated. The resulting residue, which was virtually pure (by TLC), was nevertheless filtered through a short pad of silica gel using hexanes as eluent in order to remove the base-line materials. The solvent was evaporated to give 10.88 g (91% yield) of **5** as a slightly yellow oil: R_f 0.75 (hexanes); $bp_{0.05}$ 77−80 °C; n^{20}_D 1.5612; IR (neat) 2933, 2320, 2251 (CN), 1590 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.11 (d, J = 16.4 Hz, 1 H), 5.54 (d, J = 16.4 Hz, 1 H), 2.07 (t, J = 4.0 Hz, 2 H), 1.76 (s, 3 H), 1.60 (m, 2 H), 1.46 (m, 2 H), 1.04 (s, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 151.3, 137.8, 137, 129.9, 115.8, 106.4, 106.3, 84.1, 40.2, 34.5, 34.1, 29.1, 22.1, 19.2; HRMS for $C_{14}H_{17}N$ calcd 199.1362, found 199.1385.

***(2Z, 4E)*-3-Methyl-5-(2,6,6-trimethyl-1-cyclohexen-1-yl])-penta-2,4-dienenitrile (6).** A 500 mL flame-dried, round bottom flask was charged with anhydrous copper iodide (10.9 g, 57.0 mmol) and THF (150 mL) and then cooled to 0 °C. A solution of methyllithium (42.8 mL of a 1.4 M solution in Et_2O) was slowly added over a 10 min period until the solution became clear and colorless. The cuprate solution was cooled to −78 °C, and a solution of nitrile **5** (5.44 g, 27.3 mmol) in THF (75 mL) was added dropwise. The mixture was stirred at this temperature for 45 min followed by addition of a saturated ammonium chloride solution (100 mL). The reaction mixture was allowed to warm to room temperature, EtOAc (200 mL) was added, and the mixture was washed with 2% NaOH (2 × 30 mL) followed by saturated NH_4Cl (2 × 30 mL), water (2 × 30 mL), and brine (2 × 30 mL). The organic layer was dried over $MgSO_4$, and the solvent was evaporated to provide a residue which was virtually pure by TLC. The residue was filtered through a short pad (1 in.) of silica gel using hexanes:EtOAc (9:1) as eluent in order to remove base-line materials. The solvent was evaporated to give 5.38 g (92% yield) of **6** as a slightly yellow-orange oil: R_f 0.75 (hexanes:EtOAc (9:1)); $bp_{0.1}$ 78−84 °C; n^{20}_D 1.5528; IR (neat) 2933, 2250, 1590 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.70 (d, J = 16 Hz, 1 H), 6.59 (d, J = 16.4 Hz, 1 H), 5.09 (s (1 H), 2.06 (s 3 H), 2.07 (t, J = 4.0 Hz, 2 H), 1.75 (s, 3 H), 1.62 (m, 2 H), 1.47 (m, 2 H), 1.04 (s, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.0, 136.8, 136.3, 133.28, 130.3, 117.4, 95.0, 39.7, 34.3, 29.1, 21.9, 19.4, 19.2; HRMS for $C_{15}H_{21}N$ calcd 215.1675, found 215.1699.

***(2Z,4E)*-3-Methyl-5-(2,6,6-trimethyl-1-cyclohexen-1-yl)-penta-2,4-dienal (7).** A 250 mL flame-dried, round bottomed flask was charged with nitrile **6** (1.88 g, 8.76 mmol) and hexanes (100 mL) and cooled to −78 °C. Dibal (9.0 mL of a 1.0 M solution in hexanes, 9.0 mmol) was added dropwise at such a rate that the internal reaction temperature did not exceed −70 °C. The mixture was stirred for 5 min until TLC analysis (hexanes: EtOAc (9:1)) indicated that the reaction was complete (starting material R_f 0.75; product R_f 0.70). A saturated solution of Rochelle salt (20 mL) was added, and the mixture was warmed to room temperature. EtOAc (200 mL) was added and the mixture washed using water (2 × 50 mL) and brine (2 × 50 mL) and then dried over $MgSO_4$. Removal of the solvents provided a virtually pure residue (by TLC): 1.81 g, 95% yield; n^{20}_D 1.5660;

(13) Ratios vary between 10:1 to 15:1 on different runs and concentrations.

IR (neat) 2958, 2928, 2866, 1668, 1614 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 10.16 (d, J = 8.0 Hz), 7.16 (d, J = 16.0 Hz), 6.63 (d, J = 16.0 Hz), 5.88 (d, J = 8.0 Hz, 1H, C*H*CHO), 2.69 (s, 2 H, ring), 2.14 (s, 3 H), 1.80 (s, 3 H), 1.11 (s, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.3, 155.4, 137.4, 136.7, 132.8, 127.9, 128, 39.6, 34.4, 33.3, 29.1, 21.9, 21.3, 19.2; HRMS for $C_{15}H_{22}O$ calcd 218.1671, found 219.1709 (M + H).

***(2E,4E,6Z,8E)*-Ethyl 3,7-Dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)nona-2,4,6,8-tetraenoate (8).** A solution of diethyl 3-(ethoxycarbonyl)-2-methylprop-2-enylphosphonate (1.4 g, 5.36 mmol) in anhydrous THF (25.0 mL) was cooled to 0 °C and treated with anhydrous DMPU (2.0 mL) and *n*-BuLi in hexanes (2.25 mL of 2.35 M solution, 5.32 mmol). The mixture was stirred at this temperature for 20 min and then cooled to −78 °C. A solution of aldehyde **7** (964 mg, 4.46 mmol) in THF (20.0 mL) was slowly added, and the reaction mixture was stirred at −78 °C for an additional 60 min. The mixture was allowed to warm to 0 °C as the reaction went to completion (monitored by TLC). A saturated solution of ammonium chloride (5 mL) was added, and the mixture was extracted using EtOAc (3 × 30 mL). The combined organic layers were washed with water (2 × 25 mL) and brine (30 mL), dried over $MgSO_4$, and concentrated. The residue was purified on a short silica gel column (1.5 in.) to give 1.18 g (89% yield) of the desired ester **8** (∼15:1 ratio of 13-*trans*:13-*cis* isomers) as a yellow-orange oil: IR (neat) 2928, 1709 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ (ppm) 7.08 (dd, J = 15, 11.3 Hz, 1 H), 6.65 (d, J = 16 Hz, 1 H), 6.29 (d, J = 15 Hz, 1 H), 6.23 (d, J = 15 Hz, 1 H), 6.06 (d, J = 11.3 Hz, 1 H), 4.17 (m, J = 7 Hz, 2 H), 2.7 (s, 2 H), 2.33 (s, 3 H), 2.03 (s 3 H), 1.82 (s, 3 H) 1.29 (t, J = 7 Hz, 3 H), 1.1 (s, 6 H); MS 328 (M), 255, 219, 175, 161, 119.

***(2E,4E,6Z,8E)*-3,7-Dimethyl-9-(2,6,6-trimethyl-1-cyclohexen-1-yl)nona-2,4,6,8-tetraenoic Acid (9) (9-*cis*-Retinoic Acid).** A solution of ethyl tetraenoate **8** (1.1 g, 33 mmol) in ethanol (40 mL) was treated with 1 N KOH (20 mL) at 70 °C for 3 h, cooled to room temperature, acidified with 10% (v:v) HCl, and then extracted with EtOAc (2 × 40 mL). The organic layer was washed with water (2 × 20 mL) and brine (2 × 20 mL) and dried over $MgSO_4$, and the solvent was evaporated. The residue was recrystallized (two times) by dissolving it in a minimum volume of a 9:1 mixture of ethanol:water (∼20 mL per gram of material). The desired 9-*cis*-retinoic acid (**2**) was thus obtained in a highly pure form (by ^1H NMR) as light yellow solid: yield 83%; mp 188−190 °C; IR (KBr, cm^{-1}) 2914, 1670, 1583; ^1H NMR ($CDCl_3$ 400 MHz) δ 7.20 (dd, J = 15.0 Hz, 1 H), 6.65 (d, J = 16.0 Hz, 1 H), 6.28 (d, J = 16.0 Hz, 1 H), 6.25 (d, J = 15.0 Hz, 1 H), 6.06 (d, J = 11.0 Hz, 1 H), 5.80 (s, 1 H), 2.35 (s, 3 H), 2.05 (t, J = 6.6 Hz, 2 H), 2.01 (s, 3 H), 1.75 (s, 3 H), 1.64 (m, 2 H), 1.49 (m, 2 H), 1.04 (s, 6 H). Anal. Calcd for $C_{20}H_{28}O_2$: C, 79.95; H, 9.39; O, 10.65. Found: C, 79.97; H, 9.38; O, 10.66.

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Supporting Information Available: Copies of ^1H-NMR and/or ^{13}C-NMR of compounds **5**−**7** and **9**. Elemental analysis, HPLC analysis, and IR for **9** (9 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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