Conformationally Defined Retinoic Acid Analogues. 5. Large-Scale Synthesis and Mammary Cancer Chemopreventive Activity for (2*E*,4*E*,6*Z*,8*E*)-8-(3′,4′-Dihydro-1′(2′*H*)-naphthalen-1′-ylidene)-3,7-dimethyl-2,4,6-octatrienoic Acid (9cUAB30)

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Retinoids that activate the nuclear retinoid X receptors (RXRs) display potential for chemoprevention of breast cancer. We previously reported that 9cUAB30 (**1**) is an RXR-selective retinoid. To explore its in vivo chemopreventive activity, multigram quantities of **1** were needed. Here, we describe a modified synthesis that yields up to 100 g of **1**. We further demonstrate that **1** is very effective in the prevention of *N*-methyl-*N*-nitrosourea induced mammary cancers in rats without signs of toxicity.

In humans, the estrogen receptor antagonist Tamoxifen is used as adjuvant therapy for high-risk patients,[1] and currently it is the only chemopreventive agent for breast cancer approved by the Food and Drug Administration. Even though the benefits of Tamoxifen are substantial, long-term administration is not without risks. Women who undergo Tamoxifen therapy have higher levels of endometrial cancers, since Tamoxifen acts as an agonist rather than an antagonist in the endometrium.[2] Other chemopreventive agents that can be used alone or in combination with Tamoxifen are needed. Since retinoids are capable of inducing apoptosis and cell differentiation, these agents have been explored for their chemopreventive effects. The efficacy of retinoids as chemopreventive agents has been demonstrated for numerous animal models of carcinogenesis including skin, breast, oral cavity, lung, hepatic, gastrointestinal, prostatic, and urinary bladder cancers.[3]

Anzano et al.[4] showed that 9-*cis*-retinoic acid is a potent retinoid for the prevention of *N*-methyl-*N*-nitrosourea (MNU) induced mammary carcinogenesis. However, since the effective dose is near the toxic dose, this agent may not be suitable for long-term administration needed in chemoprevention. The all-*trans*-retinoic acid is much less effective in these assays, suggesting that retinoids that activate the retinoid X receptors (RXRs) may be more efficacious in mammary cancer chemoprevention. Gottardis et al.[5] demonstrated that Targretin, an RXR-selective ligand, prevented the appearance of MNU-induced rat mammary tumors. Furthermore, this retinoid had less toxicity than 9-*cis*-retinoic acid. When either 9-*cis*-retinoic acid or Targretin was used in combination with Tamoxifen, increased chemopreventive efficacy was observed over either agent alone.[6] Recently we reported a new RXR-selective retinoid, **1**,[7] which is a conformationally constrained analogue of 9-*cis*-retinoic acid that locks the tetraene chain in a defined conformation. The retinoid **1** binds to RXRs and efficiently induces transcription mediated by these receptors over retinoic acid receptors (RARs). To evaluate the effectiveness of this retinoid in reducing the MNU-initiated mammary cancers in rats, multigram quantities of **1** were required. Here, we report a new synthesis of **1** suitable for the efficient preparation of multigram quantities and the results of mammary cancer chemopreventive assays and toxicity in rats.

Chemistry

Our previously reported[7] synthesis of **1** is shown in Scheme 1. In this procedure, a Reformatsky reaction between α-tetralone (**2**) and ethyl 4-bromo-3-methyl-2-butenoate (**3**) directly provided the acid **5** (86%). Reduction of the acid to the alcohol **6** (67%) [1:1 (9*Z*) to (all-*E*) mixture], followed by oxidation, provided the aldehyde (9*Z*)-**7** (69%) [1:1 (9*Z*) to (all-*E*) mixture; pure (9Z) isomer obtained by flash chromatography]. A Horner−Emmons condensation between aldehyde (9*Z*)-**7** and triethyl phosphonosenecioate (**8**) provided the ester **9** (78%) as a 2:1 mixture of (9*Z*) and (9*Z*,13*Z*) isomers. These isomers were separated by HPLC, and the desired (9*Z*)-**9** was hydrolyzed under basic conditions (98% yield) to give **1**.

While satisfactory for a small scale, this methodology was not amenable for large-scale synthesis of multigram quantities needed for the chemoprevention studies. The oxidation of the alcohol to the aldehyde required the use of large amounts of MnO_2 and molecular sieves, and the purification became extremely tedious at larger scale. Oxidation of 100 g of alcohol **6** would require 1 kg of MnO_2 and 0.5 kg of powdered molecular sieves. Separation of the product would require washing the MnO_2 with about 15 L of solvent, and during this process, a considerable amount of aldehyde decomposes. At this scale, the yield of the aldehyde is expected to be considerably lower than 69%. Furthermore, the Horner−Emmons reaction resulted in a 2:1 mixture of

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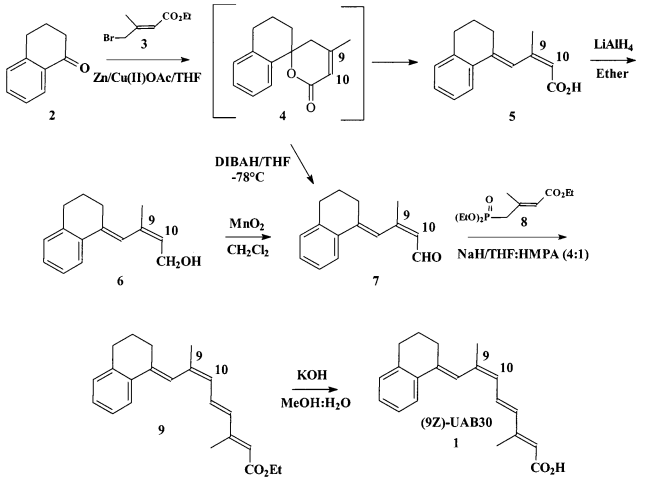
Scheme 1



(9*Z*)-**9** and (9*Z*,13*Z*)-**9**. This is another problematic step because the separation of the two isomers requires HPLC, which is impractical for large scales, and because one-third of the aldehyde **7** is wasted by conversion to the undesired di-*Z* ester **9**. These limitations prompted us to develop an alternative synthetic methodology more amenable for a large-scale synthesis.

The modified synthetic methodology is also depicted in Scheme 1. As shown, the first step also involved a Reformatsky reaction between 1-tetralone (**2**) and ethyl 4-bromo-3-methyl-2-butenoate (**3**) in the presence of Zn and $Cu(OAc)_2$ in THF.[8] However, conditions were used to favor the formation of the intermediate δ-lactone **4**. This reaction was performed on a 100 g scale to yield approximately 100 g (70%) of **4**. Another major change was the controlled reduction of **4** by DIBAH, followed by ring opening and elimination, to provide the aldehyde **7** (75%) [5:1 (9*Z*) to (all-*E*)]. The isomers were readily separated on flash silica, and triethyl phosphonosenecioate **8** was used to olefinate (9Z)-**7** under modified Horner−Emmons conditions to produce the ester **9**. Under these conditions, the use of excess HMPA as the solvent resulted in the desired ester **9** as a 9:1 mixture of (9*Z*,13*E*)-**9** and (9*Z*,13*Z*)-**9**, which were separated by selective crystallization. Pure (9*Z*,13*E*)-**9** was hydrolyzed under basic conditions to give the pure acid **1** in 78% yield.

Biology

Female Sprague−Dawley rats were obtained from Harlan Sprague-Dawley, Inc., at 23 days of age. The animals were housed five per cage and maintained on a Teklad (4%) rodent diet throughout the study. At 50 days of age, the rats received one intravenous injection of MNU via the jugular vein. MNU was purchased from the NCI Chemical Repository. The animals ($n = 20$ per group) were administered with **1** or 9-*cis*-retinoic acid in the diet continuously after MNU injection, beginning at 53 days of age (3 days after the carcinogen). The compounds were incorporated into the diet using a Patterson−Kelly liquid−solid blender. Stability of the retinoids during feeding was verified by HPLC. The groups were as follows: group 1, **1** (200 mg/kg diet); group 2, **1** (100 mg/kg diet); group 3, 9-*cis*-retinoic acid (100 mg/kg diet); group 4, diet only. The rats were weighed once per week, palpated for mammary tumors twice per week, and checked daily for signs of toxicity. The study was terminated 140 days after MNU treatment. All mammary tumors were histologically classified as adenocarcinomas.

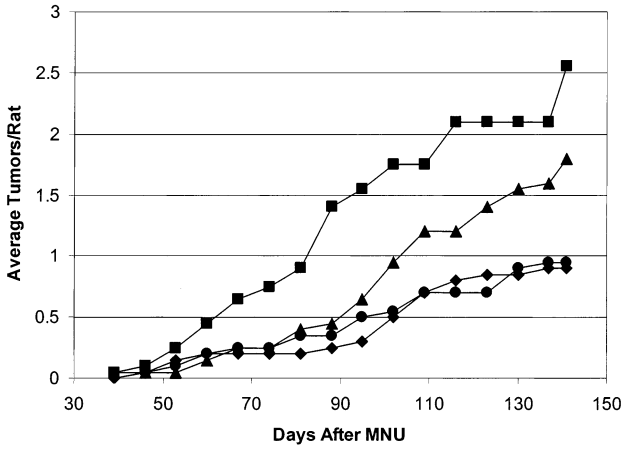


Figure 1. Average tumor formation versus days after MNU administration for female rats fed a 4% Teklad diet with 9cUAB30 (200 mg/kg diet, diamond; 100 mg/kg diet, triangle), with 9-*cis*-retinoic acid (100 mg/kg diet, circle), or without retinoid (MNU control, square).

Results and Discussion

1 and 9-*cis*-retinoic acid were tested in the MNU-induced mammary cancer model currently used by the National Cancer Institute to evaluate the efficacy of chemopreventive agents.[4] At termination of the study, the average number of mammary cancers per rat for the control group was 2.6. **1** at the 200 and 100 mg/kg diet dose levels reduced the number of mammary cancers by 63% and 29%, respectively (Figure 1). The use of 9-*cis*-retinoic acid at the 100 mg/kg diet dose level (a level just below the toxic dose for this compound) decreased the multiplicity of cancers by 65%. Both the high dose of **1** and 9-*cis*-retinoic acid greatly delayed the time of appearance of the mammary cancers (Figure 1). As shown in Table 1, retinoid **1** did not alter body or liver weights of the rats significantly at either dose level. Other signs of retinoid clinical toxicity were not observed. Since mammary cancers are highly sensitive to ovarian hormone changes, we measured the weight of the ovaries and uterus at the end of the study and

Table 1

retinoid	final body weight (g)	organ weight (g) per 100 g of body weight		
		liver	uterus	ovaries
MNU control	269 ± 9[a]	3.22 ± 0.05	0.18 ± 0.03	0.046 ± 0.002
1 (100 mg/kg diet)	271 ± 3	2.96 ± 0.07	0.16 ± 0.01	0.046 ± 0.002
1 (200 mg/mg diet)	272 ± 9	3.11 ± 0.09	0.21 ± 0.02	0.052 ± 0.005
9-*cis*-retinoic acid (100 mg/kg diet)	254 ± 7	3.48 ± 0.09	0.18 ± 0.01	0.040 ± 0.003

[a] Values are the mean ± SEM; $N = 5$. Statistical differences between groups were not observed.

monitored the estrus cycles of the rats in the various groups. Since major differences between groups were not observed, it appeared that **1** modified the carcinogenic process by a mechanism other than through hormonal modifications. Anzano et al. reported similar efficacy for 9-*cis*-retinoic acid in mammary cancer chemoprevention using this MNU assay.[4]

Conclusions

Synthetic methodology suitable for generating **1**, and presumably related retinoids, on a multigram scale was developed. Retinoid **1** was found to be highly active in the prevention of mammary carcinogenesis in rats and was completely nontoxic at the highest tested dose when administered orally. This and related retinoids warrant further evaluation as potential therapeutic agents in mammary chemoprevention.

Experimental Section

Melting points were obtained on an electrothermal melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ARX 300 spectrometer. UV/vis spectra were recorded on Varian Cary 100 Conc spectrophotometer in methanol. IR spectra were recorded using a Bomem MB series FT IR spectrometer. Mass spectra were recorded on a MicroMass platform LCZ spectrometer. Atlantic Microlabs of Atlanta, GA, provided combustion analyses. Solvents and liquid starting materials were distilled prior to use. Reactions and purifications were conducted with deoxygenated solvents under inert gas (N_2) and in subdued lighting. Flash chromatography was performed using Selecto Scientific silica gel (40 μm). Ethyl 4-bromo-3-methylbut-2-enoate (**3**) was prepared by the reaction of ethyl 3,3-dimethylacrylate with *N*-bromosuccinimide.[9−11] Triethyl phosphonosenecioate (**8**) was prepared via the Arbusov reaction.[12] Tetrahydrofuran was distilled from sodium metal/benzophenone ketyl. Diethyl ether, benzene, and dichloromethane were purchased from Fischer as anhydrous solvents. HMPA was distilled from calcium hydride.

7,8-Benzo-4-methyl-1-oxaspiro[5.5]undec-3-en-2-one (4). A mixture of zinc dust (150 g) (<10 μm, Aldrich, catalog no. 20,998-8) and copper(II) acetate monohydrate (15 g, Acros) in 500 mL of glacial acetic acid was stirred under nitrogen for 1 h in a 1000 mL one-neck, round-bottomed flask. The mixture was diluted with anhydrous ether (500 mL) and filtered with suction, and the Zn−Cu complex was washed successively with anhydrous ether (3 × 300 mL) and dry benzene (3 × 300 mL). The mixture was then transferred into a flame-dried 2000 mL three-neck flask fitted with a nitrogen inlet, condenser, and addition funnel. Freshly distilled THF (distilled from Na/benzophenone) (200 mL) was added to the flask, which was heated to about 90 °C in an oil bath. The reaction mixture was then treated dropwise with a solution of tetralone **2** (100.0 g, 684.9 mmol, freshly distilled) and bromoester **3** (220.0 g, 1063 mmol, freshly distilled) in 400 mL of THF (dry). Vigorous bubbling occurred during the addition. The mixture was stirred at reflux for an additional 3.5 h. The reaction mixture was cooled to room temperature, and water (200 mL) and HCl (2 N, 500 mL) were added. The mixture was diluted with 1000 mL of ether and filtered, and the acid layer was separated. The organic layer was washed with water (2 × 200 mL), NaOH (1 N, 2 × 250 mL), and brine (2 × 250 mL). It was then dried (Na_2SO_4) and evaporated to give an oil. This oil was subjected to distillation on a high-vacuum pump (0.1 mm) at 60 °C. The distillate was discarded, and the remaining thick oily residue solidified upon addition of hexanes. This mixture was cooled, filtered, and washed with hexanes to give 108 g (69.2%) of **4** (R_f = 0.3, 50:50 ether/hexane) as a white solid: mp 67−69 °C; MS *m*/*z* 229 (M^+); ^{1}H NMR ($CDCl_3$) δ 7.5−7.54 (m, 1H), 7.2−7.25 (m, 2H), 7.07−7.1 (m, 1H), 5.92 (s, 1H), 2.7−2.9 (m, 3H), 2.5 (d, 1H), 1.98−2.23 (m, 3H), 2.01 (s, 3H), 1.67−1.78 (m, 1H).

(2*Z*,4*E*)-4-(3′,4′-Dihydro-1′(2′*H*)-naphthalen-1′ylidene)-3-methyl-2-butenal (7). To a flame-dried three-neck, round-bottomed flask fitted with a nitrogen inlet, addition funnel, and rubber septum was added lactone **4** (20.0 g, 87.6 mmol). To this was added 400 mL of THF (freshly distilled from Na/benzophenone). The resulting solution was cooled to −78 °C in a dry ice/acetone bath. The reaction mixture was treated with diisobutylaluminum hydride (88.0 mL, 87.6 mmol, 1 N solution in THF, Aldrich) dropwise over a period of 45 min. After 2 h of stirring at −78 °C, an additional amount of DIBAH (8.80 mL, 8.76 mmol) was added dropwise. After an additional 3 h of stirring, more DIBAH (8.80 mL, 8.76 mmol) was added dropwise, and stirring continued at −78 °C for an additional 20 h. The reaction mixture was quenched with 20 mL of water, and the dry ice bath was removed. After reaching room temperature, the mixture was warmed to about 35 °C in a water bath and 60 mL of 18% HCl was added. The mixture was stirred for 10 min at 35−40 °C. The reaction mixture was diluted with ether (200 mL), washed with water (3 × 100 mL) and brine (2 × 100 mL), dried (Na_2SO_4), and evaporated (rotary evaporator, water bath temperature of <35 °C) to give 18 g of an oil, which was purified by column chromatography (silica gel, 40 cm × 7 cm, 1:6 ether/hexanes, all column solvents purged with nitrogen) to give 10 g of (9*Z*)-**7** (R_f = 0.3) and 2.5 g of (all-*E*)-**7** (R_f = 0.25) (75% combined yield). The (9*Z*)-**7** was crystallized from hexanes/ether: mp 65−66 °C; IR 1662 (C=O), 1609 (C=C) cm^{-1}; UV λ_{max} 295 (ϵ 6000); MS *m*/*z* 213 (M^+ + H); ^{1}H NMR ($CDCl_3$) δ 9.64 (d, 1H), 7.64 (m, 1H), 7.13−7.25 (m, 3H), 6.57 (s, 1H), 6.0 (d, 1H), 2.86 (t, 2H), 2.50 (t, 2H), 2.09 (s, 3H), 1.82−1.90 (m, 2H).

(2*E*,4*E*,6*Z*,8*E*)-Ethyl 8-(3′,4′-Dihydro-1′(2′*H*)-naphthalen-1′-ylidene)-3,7-dimethyl-2,4,6-octatrienoate (9). Sodium hydride (60% suspension in mineral oil, 2.95 g, 73.8 mmol) was placed in a flame-dried three-neck, round-bottomed flask fitted with a nitrogen inlet, addition funnel, and rubber septum. Freshly distilled THF (from Na/benzophenone, 400 mL) was added, followed by freshly distilled **8** (19.45 g, 73.67 mmol). The resulting brown mixture was stirred for 15 min, and freshly distilled HMPA (50 mL) was introduced through a syringe. The flask was covered with aluminum foil, and stirring was continued for 15 min. The aldehyde **7** (14.20 g, 66.98 mmol) in 100 mL of dry THF was added dropwise from the addition funnel (covered with aluminum foil). The reaction mixture was stirred for an additional 2.5 h, was quenched with 50 mL of water, and then diluted with 500 mL of ether. The aqueous layer was separated and washed with 100 mL of ether. The combined organic layers were washed with brine (2 × 150 mL), dried (Na_2SO_4), and evaporated to give a crude oil (35 g), which was suspended in methanol (75 mL, degassed with nitrogen). Ether was added until the mixture was homogeneous (about 20 mL), and the solution was cooled overnight at 0 °C to give a crystalline solid. This solid was filtered, washed with methanol, and dried to give 14 g of pure product (9*Z*)-**9** as one isomer: mp 64−65 °C; IR 1706 (C=O), 1602 (C=C) cm^{-1}; UV λ_{max} 328 nm (ϵ 29 300); MS *m*/*z* 323 (M^+ + H); ^{1}H NMR ($CDCl_3$) δ 7.62−7.68 (m, 1H), 7.11−7.22 (m, 3H), 6.65 (dd, 1H), 6.5 (s, 1H), 6.23 (d, 1H), 6.1 (d, 1H), 5.75 (s, 1H), 4.15 (q, 2H), 2.85 (t, 2H), 2.40 (dt, 2H), 2.22 (s, 3H), 1.97 (s, 3H), 1.78−1.87 (m, 2H), 1.27 (t, 3H).

(2*E*,4*E*,6*Z*,8*E*)-8-(3′,4′-Dihydro-1′(2′*H*)-naphthalen-1′-ylidene)-3,7-dimethyl-2,4,6-octatrienoic Acid (9cUAB30, 1). Ester **9** (12.00 g, 37.26 mmol) was suspended in methanol (640 mL, degassed with nitrogen) and warmed to about 60 °C. This mixture was treated with KOH solution (20.90 g, 372.7 mmol, in 220 mL of distilled and degassed water). The resulting mixture was stirred at reflux for 1 h, cooled to 0 °C in an ice bath, and diluted with 300 mL of ice-cold water. The mixture was slowly acidified with ice-cold 2 N HCl to about pH 2. The resulting precipitate was filtered, and the solid was redissolved in 500 mL of ether. The organic solution was washed with brine (3 × 150 mL), dried (Na_2SO_4), and concentrated on a rotary evaporator to about 75 mL of volume. The residual solution was diluted with 100 mL of degassed hexanes and cooled at 0 °C for about 12 h. The resulting yellow crystals

were filtered and dried to give 8.5 g (78%) of pure (9*Z*)-**1** (9*Z*-9cUAB30): mp 175−176 °C; IR 1672 (C═O), 1594 (C═C) cm^{-1}; UV λ_{max} 328 nm (ϵ 30 200); MS *m*/*z* 295 ($M^+ + H$); ^1H NMR ($CDCl_3$) δ 11.00 (br, 1H), 7.6−7.67 (m, 1H), 7.15−7.21 (m, 2H), 7.11−7.14 (m, 1H), 6.68 (dd, 1H), 6.47 (s, 1H), 6.25 (d, 1H), 6.12 (d, 1H), 5.77 (s, 1H), 2.85 (t, 2H), 2.40 (dt, 2H), 2.22 (s, 3H), 1.98 (s, 3H), 1.79−1.87 (m, 2H).

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