SYNTHESIS OF 9*E*- AND 9*Z*-LOCKED RETINOIC ACID ANALOGS AS LIGANDS FOR RAR AND RXR

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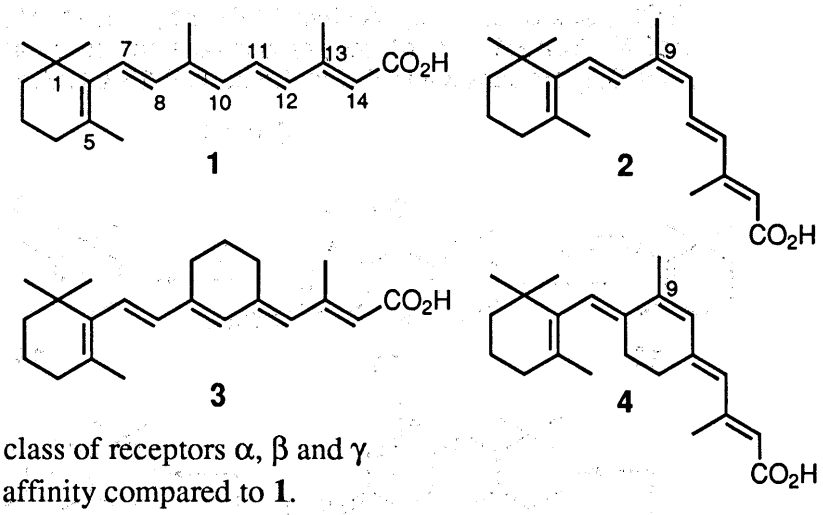
New retinoic acid (RA) analogs 9*E*-locked-RA **3** and 9*Z*-locked-RA **4** were synthesized from dithiane **6** and β-cyclocitral **13**, respectively. Both analogs behaved as agonistic ligands for a mixture of retinoic acid receptor (RAR) and retinoid X receptor (RXR).

KEYWORDS retinoic acid analog; retinoic acid receptor; retinoid X receptor; transcriptional activity; CAT assay

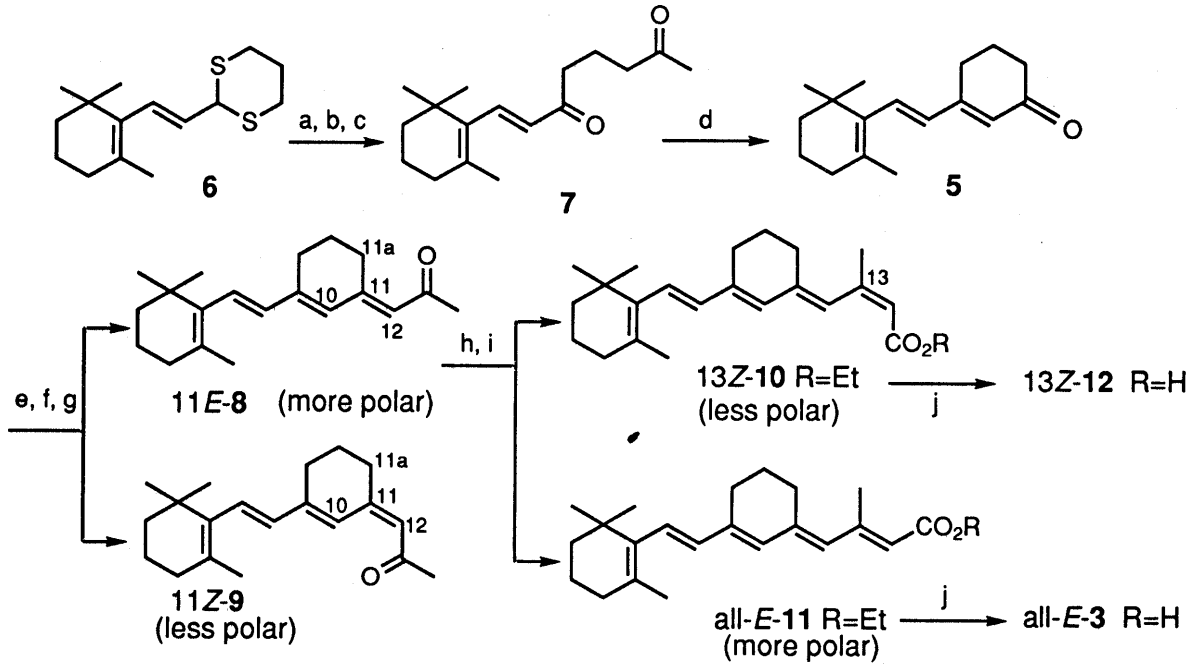
RA is a signaling molecule which plays a role in several types of vitamin A action including growth, differentiation, and development, though not in vision.[1] In the past several years, two distinct classes of nuclear hormone receptors mediating RA-dependent transcription[2] have been identified. The first class is composed of the α, β and γ RARs which bind all-*E*-RA **1**, and the second class of receptors α, β and γ RXRs bind 9*Z*-RA **2** with much higher affinity compared to **1**.



Here we describe syntheses of new RA analogs **3** and **4** having the structure such that 9*E* to 9*Z* and 9*Z* to 9*E* isomerizations were respectively prohibited, in order to investigate the behaviors of these analogs as ligands for RAR and RXR.

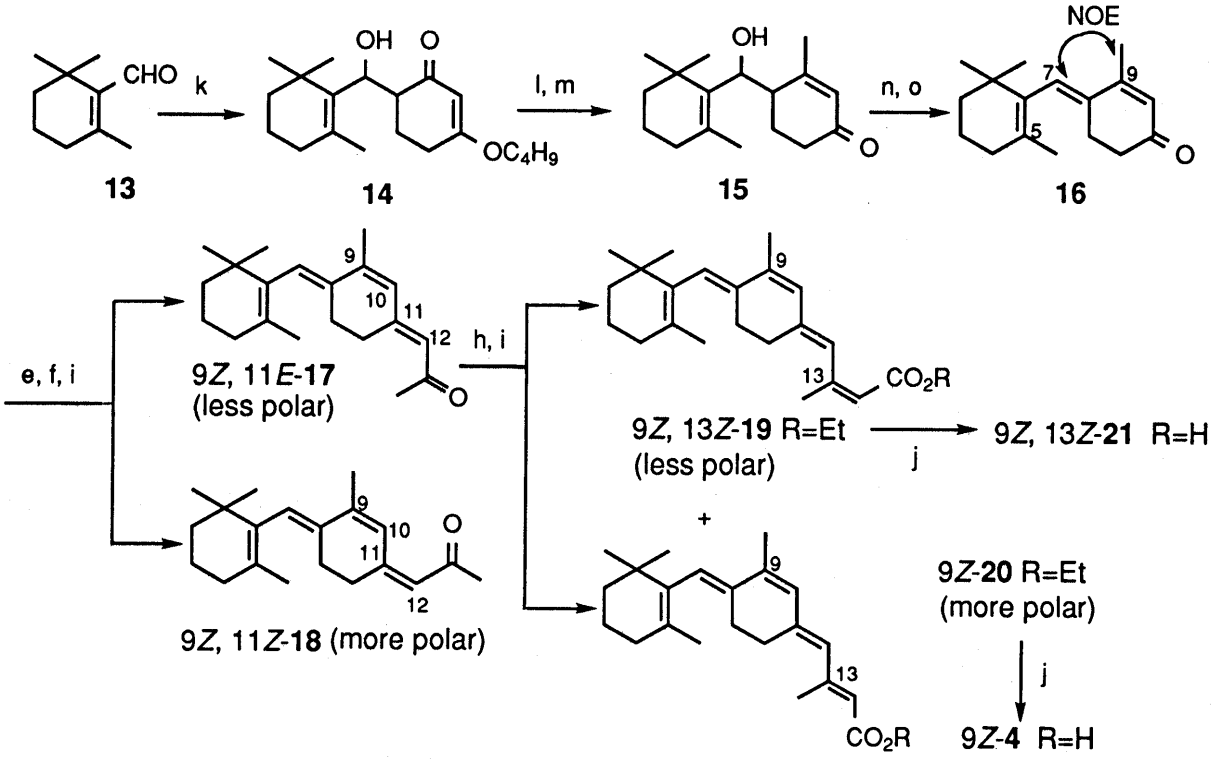
The 9*E*-locked trienone **5** was synthesized by Albeck et al.[3] *via* a Wittig reaction between β-cyclocitrylphosphonium bromide and 3-formyl-2-cyclohexenone. Although we first followed this method, the yields of starting materials were low and the Wittig reaction gave a trace amount of **5**. Therefore we prepared **5** by our original procedure as shown in Chart 1.

Diketone **7**, derived from the reaction of lithium salt of dithiane **6**[4] with 5-chloro-2-pentanone ethylene ketal (Aldrich) and subsequent deprotections of the thioketal and ketal groups (57% in 3 steps), was easily cyclized in the presence of MeONa[5] to afford trienone **5** (quantitative yield), which was unable to directly elongate to the retinoate analog by an Emmons-Horner reaction. Thus, trienone **5** was converted to tetraenones **8** and **9** in a 1:1 ratio in 72% yield [recovered 24% of **5**] by an aldol



a) *n*-BuLi, 5-chloro-2-pentanone ethylene ketal / THF, -78°C, b) HgO, $HgCl_2$ /97% MeOH, r.t., c) *p*-TsOH / acetone, r.t., d) MeONa / THF, r.t., e) LDA, $Me_2NN{=}CMe_2$ / THF, r.t., f) AcOH:THF:H_2O:AcONa (5:2:2:1), r.t., g) low pressure column chromatography, h) LDA, $TMSCH_2CO_2Et$ / THF, -78°C, i) preparative HPLC in the dark, j) 25% NaOH / EtOH, 50°C

Chart 1



k) 3-butoxy-2-cyclohexenone, LDA / THF, -78°C, l) MeLi / THF, -78-0°C, m) 15% H_2SO_4, r.t., n) Ac_2O, Et_3N, DMAP / CH_2Cl_2, r.t., o) DBU / toluene, reflux

Chart 2

type condensation followed by mild deprotection.[6] Peterson olefination (92%) of **8** followed by preparative HPLC gave 13*Z*-isomer **10**[7] and all-*E*-isomer **11**[7] of the retinoate analogs in a 1:1 ratio, which were hydrolyzed to RA analogs **12**[8] and **3**[8], respectively, without double-bond isomerization.

9*Z*-Locked trienone **16** was prepared by a modified procedure of the synthetic method of 11*Z*-locked retinal[9] (Chart 2). Aldol condensation between β-cyclocitral **13**[10] and 3-butoxy-2-cyclohexenone[11] afforded hydroxy ketone **14** (61%), which was converted into 7*E*-trienone **16** as a sole product [observed NOE (nuclear Overhauser effect) between 7-H and 9-Me] by the sequence of the addition of methyl lithium, hydrolysis (60%), acetylation (83%) and elimination (83%). Final transformation of **16** to 9*Z*-locked RAs **21** and **4** was achieved by means of the route used for 9*E*-locked RAs **12**[8] and **3**[8].

Transcriptional activities of synthesized RA analogs **3**, **12**, **4** and **21** were investigated by CAT (chloramphenicol acetyl transferase) assay. All analogs indicated weak activities compared with those of **1** and **2** [**3**; 1/10 of **1** or **2**: **12**, **4** and **21**; 1/100 of **1** or **2**]. This suggests that synthesized analogs **3**, **12**, **4** and **21** exhibited agonistic actions for a mixture of RAR and RXR. Relationship concerning the structure and transcriptional activities is now under study.

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7) Satisfactry ^{1}H- and ^{13}C-NMR, FT-IR, UV-VIS, and MS spectral data were obtained.
8) ^{1}H-NMR data (200 MHz, $CDCl_3$) for compounds **3**, **12**, **4** and **21** are as follows:
For **3** : δ: 1.01 (6H, s, gem-Me), 1.70 (3H, s, 5-Me), 2.31 (3H, s, 13-Me), 2.34 and 2.61 (each 2H, each t-like, J =5 Hz, 9a- and 11a-H_2), 5.75 (1H, s, 14-H), 5.82 (1H, s, 12-H), 6.05 (1H, s, 10-H), 6.09 (1H, d, J =16 Hz, 8-H) and 6.26 (1H, d, J =16 Hz, 7-H). For **12** : δ: 1.01 (6H, s, gem-Me), 1.70 (3H, s, 5-Me), 2.13 (3H, s, 13-Me), 2.32 and 2.54 (each 2H, each t-like, J =6 Hz and 5 Hz, 9a-H_2 and 11a-H_2), 5.64 (1H, s, 14-H), 6.10 (1H, d, J =16.5 Hz, 8-H), 6.18 (1H, s, 10-H) and 6.23 (1H, d, J =16.5 Hz, 7-H) and 6.90 (1H, s, 12-H). For **4** :δ: 0.96 (6 H, br s, gem-Me), 1.47 (3 H, s, 5-Me), 1.98 (3 H, s, 9-Me), 2.30 (3 H, s, 13-Me), 5.72 (1 H, s, 14-H), 5.79 (1 H, s, 12-H), 5.98 (1 H, s, 10-H) and 6.06 (1 H, s, 7-H). For **21** : δ: 0.95 (6 H, br s, gem-Me), 1.46 (3 H, s, 5-Me), 1.97 (3 H, s, 9-Me), 2.11 (3 H, s, 13-Me), 5.63 (1 H, s, 14-H), 6.05 (1 H, s, 10-H), 6.11 (1 H, s, 7-H) and 6.87 (1 H, s, 12-H).
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(Received October 14, 1994; accepted October 25, 1994)