Research Article

Synthesis of the Nakanishi Ring-Locked Retinoid

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An optimized synthetic route to prepare ring-locked retinoid 1a has been developed. We fully describe a purification protocol that provides isomerically pure 1a in support of on-going proof of concept studies for the development of therapeutic agents to treat human ADRP. Additionally, we have found that isomerically pure 1a can be stored in amber vials under argon at −20°C for use over time (up to six months) without degradation. Thus, enabling 1a to be an accessible and valuable biological tool.

Previous studies using P23H mutant opsin as an in vitro model of human Autosomal Dominant Retinitis Pigmentosa (ADRP) found that ring-locked retinoid 1a (Figure 1, carbon numbering shown based on 11-cis-retinal) was able to act as a pharmacological chaperone [1–3]. Compound 1a induced the mutant protein to fold properly and undergo normal cellular transport and degradation suggesting 1a could be a potential therapeutic agent for the prevention of ADRP [1–3]. This ring-locked analog of 11-cis-retinal has also been used extensively to elucidate the mechanism of photoactivation of rhodopsin [4–6].

We required a reliable synthesis of isomerically pure compound 1a. Its synthesis, along with the spectral data, has been reported twice in the literature [1, 7], in addition to the syntheses of a number of related ring-locked retinoids [4–6, 8]. The original synthetic route reported by Nakanishi resulted in the preparation of mixtures of multiple geometric isomers of 1 (E/Z-isomers among C7-C8, C9-C10, and C13-C14) and did not describe specific HPLC conditions for resolution of the final isomerically pure products. Our synthetic strategy differs from the two reported syntheses in two fundamental ways (discussed below) enabling ready access to 1a. Two key bond-forming events distinguish this synthesis: the C7-C8 double bond was originally established via a Julia olefination, while the connection between C13 and C14 was accomplished via a Petersen olefination. In each case, both E- and Z-isomers of the desired products were reported to have formed [7]. In contrast, our use of a Wittig reagent in the formation of the C7-C8 double bond afforded the E-isomer as the sole product in the former olefination; and in the case of the latter, the requisite Petersen reagent, a silylated acetaldehyde tert-butylamine, was not readily accessible in our hands, and an alternate methodology was instead utilized [7, 9]. A Horner-Emmons-Wadsworth strategy (utilizing diethyl cyanomethylphosphonate) was found to be an advantageous replacement for the penultimate formation of the C13-C14 double bond in two steps (as opposed to three as previously reported) [1]. In particular, using the nitrile Horner-Emmons-Wadsworth (Scheme 2) reagent obviated the need for a final manganese dioxide oxidation which resulted in a more efficient and reliable synthesis endgame for the preparation of 1a. The key changes we have introduced to the synthetic route as described by Noorwez et al. [1] allowed us to increase the yields and geometric purity of key intermediates (7, 11). Herein, we report the full details of the synthesis, purification, characterization, and stability of isomerically pure 1a.

Starting from commercially available cycloheptenone 2, the allylic acetate 4 was prepared via a two-step sequence of radical bromination with NBS and 1,1′-azobis(cyclohexanecarbonitrile) (ACHN) followed by displacement of the allylic bromide 3 with potassium acetate under phase transfer catalyst conditions with tetrabutylammonium bromide (TBAB, Scheme 1). The ketone underwent
Horner-Emmons-Wadsworth olefination with diethyl(1-cyanoethyl)phosphonate to provide a 50% yield of 5 and 6 with an E/Z ratio of 2:1. The isomers were separated by column chromatography, and the geometry of the double bond was established by NOE (Figure 2). The allylic acetate 5 was hydrolyzed with potassium carbonate in methanol to furnish the allylic alcohol 7 in excellent yield and purity. The alcohol was protected as the TBS ether and 9 remained fixed during this three-step reaction sequence. The trans geometry was confirmed by NOE experiments (key interactions are shown in Figure 2).

The Wittig olefination reagent, β-cyclogeranyl triphenylphosphonium bromide, was prepared following established methodology [10, 11]. Reaction conditions using n-BuLi as the base resulted only in decomposition. However, the desired tetraene 11 was obtained when potassium tert-butoxide was employed as the base in the presence of imidazole and DMAP, and then the nitrile was reduced with DIBAL-H to provide aldehyde 9 in 77% yield over two steps. The aldehyde 9 was carried forward without purification.

Importantly, the double-bond geometry (between C9 and C10) remained fixed during this three-step reaction sequence. The trans geometry was confirmed by NOE experiments (key interactions are shown in Figure 2).

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Scheme 1: Reagents and conditions: (a) (i) NBS, ACHN, benzene, reflux, 2 h, and 34%; (ii) KOAc, TBAB, THF/H2O, r.t., 4 days, and 17%; (b) NaH, (EtO)2P(O)(CH(CH3)CN, THF, 0°C to r.t., and 20 h; (c) isomer separation by SiO2 column chromatography, E 33%, and Z 17%; (d) K2CO3, MeOH, r.t., 1.5 h, and 99%; (e) TBSCI, imidazole, DMAP, CH2Cl2, r.t., 1 h, and 80%; (f) DIBAL-H, Et2O, –78°C to 0°C, 2 h, and 96%.

Companion automated chromatographer using generic silica gel cartridges from Silicycle.

4-Oxo-cyclohept-2-en-1-yl Acetate (4) (See [12]). A solution of cycloheptenone (2, 80% tech. grade, 8.00 g, 73.0 mmol), NBS (17.5 g, 98.0 mmol), and ACHN (180 mg, 0.730 mmol) in benzene (150 mL) was combined at r.t. under argon then heated at reflux for 3 h. The reaction mixture was cooled to room temperature, then cooled to 0°C in an ice bath, and diluted with hexanes (200 mL). The succinimide
Figure 2: Key NOEs used to establish the geometry of the tetra-substituted double bond corresponding to the double bond between C9 and C10 of 1.

Table 1: Summary of the literature [1, 7] and experimental (exp) 1H NMR chemical shifts of key resonances (δ reported in ppm) for C13-C14 E and Z-isomers of 1.

<table>
<thead>
<tr>
<th>Proton</th>
<th>C13-C14 Z-isomer</th>
<th>C13-C14 E-isomer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent</td>
<td>CDCl3</td>
<td>CDCl3</td>
</tr>
<tr>
<td>H-7</td>
<td>6.32 ( — )</td>
<td>6.40</td>
</tr>
<tr>
<td>H-8</td>
<td>6.52 ( — )</td>
<td>6.61</td>
</tr>
<tr>
<td>H-11</td>
<td>7.02 ( 7.00 )</td>
<td>7.07</td>
</tr>
<tr>
<td>H-12</td>
<td>5.79 ( 5.78 )</td>
<td>5.80</td>
</tr>
<tr>
<td>H-14</td>
<td>10.12 ( 10.11 )</td>
<td>10.15</td>
</tr>
</tbody>
</table>

precipitate was removed by vacuum filtration, and the filtrate was concentrated in vacuo to provide 3 as a dark brown oil. The crude bromide 3 was dissolved in THF (75 mL) at room temperature. To this solution was added water (50 mL), potassium acetate (28.0 g, 285 mmol), and tetrabutylammonium bromide (230 mg, 0.730 mmol). The biphasic mixture was stirred vigorously at r.t. for 4 days under argon. The reaction mixture was then transferred to a separatory funnel and extracted with ether (3 × 100 mL). The combined organic phases were washed with saturated NaHCO3 (2 × 150 mL), brine (150 mL), dried over MgSO4, filtered, and concentrated in vacuo. The product was purified by column chromatography (330 g silica gel cartridge, gradient elution 0–20% EtOAc-hexanes), yield of 4, 20 g (12 mmol, 17%), and recovered 3, 4.6 g (24 mmol, 34%).

\[ R_f = 0.21 \text{ (EtOAc-hexanes, 1:8).} \]

1H NMR (600 MHz, CDCl3): δ = 6.42 (dd, 1H, J = 12.5, 3.5 Hz, H-2), 6.00 (dd, 1H, J = 12.5, 1.5 Hz, H-3), 5.56 (m, 1H, H-1), 2.66–2.56 (m, 2H, H-5), 2.22–2.15 (m, 1H, H-7), 2.10–2.06 (s, 3H, O2CCH3), 1.90–1.81 (m, 3H, H-6, H-7).

13C NMR (150 MHz, CDCl3): δ = 18.0 (C6), 31.6 (C7), 42.8 (C5), 71.9 (C1), 131.3 (C3), 144.3 (C2), 169.9 (O2CCH3), 202.3 (C4).

MS (APCI): m/z [M + H]+ = 169.1.

(E)-4-(1-Cyanoethylidene)cyclohept-2-en-1-yl Acetate (5) (See [12]).

Into a dried flask fitted with an addition funnel was placed NaH (60% dispersion in oil, 200 mg, 84.0 mmol) and THF (140 mL). The slurry was cooled in an ice bath, and the addition funnel was charged with a solution of diethyl(1-cyanoethyl)phosphonate (1.06 mL, 6.00 mmol) in THF (45 mL). The phosphonate solution was added slowly over 5 minutes to the NaH slurry at 0°C. The reaction mixture was then warmed to r.t. over 30 minutes. A solution of 4 (900 mg, 5.40 mmol) in THF (30 mL) was added via addition funnel to the phosphonate solution over 5 minutes. The reaction mixture was then stirred at r.t. for 18 h. The reaction was quenched by the addition of water (200 mL) and then extracted with ether (3 × 150 mL). The combined organic phases were dried over MgSO4, filtered, and concentrated in vacuo. The E- and Z-isomers were separated by column chromatography (120 g silica gel cartridge, isocratic elution 10% EtOAc-hexanes); yield of 5, 0.36 g (1.8 mmol, 33%) and 6, 0.23 g (1.1 mmol, 17%).

\[ R_f = 0.43 \text{ (EtOAc-hexanes, 1:8).} \]

1H NMR (600 MHz, CDCl3): δ = 6.36 (d, 1H, J = 12.0 Hz, H-5), 5.96 (dd, 1H, J = 12.0, 2.0 Hz, H-4), 5.50 (m, 1H, H-6), 2.85 (td, 1H, J = 14.0, 5.0 Hz, H-9), 2.60–2.53 (m, 1H, H-9), 2.09 (s, 3H, O2CCH3), 2.03–1.97 (m, 1H, H-7), 1.95 (s, 3H, C2-CH3), 1.91–1.75 (m, 3H, H-7, H-8).

13C NMR (150 MHz, CDCl3): δ = 16.3 (C2-CH3), 21.1 (O2CCH3), 21.9 (C8), 32.0 (C7), 34.2 (C9), 72.0 (C6), 106.4 (C3), 119.6 (C2), 127.1 (C5), 138.3 (C4), 151.9 (C1), 170.1 (O2CCH3).

MS (APCI): m/z (%): 205.1 (40), 163.1 (100).
(Z)-4-(1-Cyanoethylidene)cyclohept-2-en-1-yl Acetate (6).

\[ R_f = 0.33 \text{ (hexanes-EtOAc, 8 : 1).} \]

$^1$H NMR (600 MHz, CDCl$_3$): $\delta = 6.64$ (d, 1H, $J = 12.0$ Hz, H-5), 5.90 (dd, 1H, $J = 12.0$, 2.0 Hz, H-4), 5.51 (m, 1H, H-6), 2.62 (td, 1H, $J = 15.0$, 5.0 Hz, H-9), 2.40–2.31 (m, 1H, H-9), 2.07 (s, 3H, O$_2$CH$_3$), 2.04–1.98 (m, 1H, H-7), 1.96 (s, 3H, C$_2$-CH$_3$), 1.86–1.67 (m, 3H, H-7, H-8).

$^{13}$C NMR (150 MHz, CDCl$_3$): $\delta = 16.1$ (C$_2$CH$_3$), 20.7 (C8), 21.1 (O$_2$CCH$_3$), 29.8 (C9), 31.9 (C7), 71.7 (C6), 106.7 (C3), 119.0 (C2), 130.0 (C5), 136.7 (C4), 152.7 (C1), 170.1 (O$_2$CCH$_3$).

LRMS (EI): $m/z$ (%) = 205.1 (15), 163.1 (100).

(E)-2-(4-Hydroxycyclohept-2-en-1-ylidene)propanenitrile (7) (See [12]). To a solution of 5 (320 mg, 1.60 mmol) in methanol (10 mL) at 0°C was added K$_2$CO$_3$ (110 mg, 0.780 mmol). The reaction mixture was stirred for 15 minutes at 0°C, then warmed to r.t., and stirred for 1 h. The reaction mixture was poured into water (25 mL) and extracted with EtOAc (3 × 20 mL). The combined organic phases were then washed with brine (15 mL), dried over MgSO$_4$, filtered, and concentrated in vacuo. The product was purified by column chromatography (40 g silica gel cartridge, gradient elution 5–40% EtOAc-hexanes); yield of 7, 0.25 g (1.6 mmol, 99%).

\[ R_f = 0.11 \text{ (EtOAc-hexanes, 1 : 6).} \]

$^1$H NMR (300 MHz, CDCl$_3$): $\delta = 6.26$ (d, 1H, $J = 12.0$ Hz, H-5), 6.07 (d, 1H, $J = 12.0$ Hz, H-4), 4.48 (m, 1H, H-6), 2.79 (td, 1H, $J = 10.0$, 5.0 Hz, H-9), 2.47 (m, 2H, H-9, OH), 2.00 (m, 1H, H-7), 1.92 (s, 3H, C$_2$-CH$_3$), 1.87–1.61 (m, 3H, H-7, H-8).

$^{13}$C NMR (75 MHz, CDCl$_3$): $\delta = 16.2$ (C$_2$CH$_3$), 22.1 (C8), 34.2 (C9), 35.6 (C7), 69.9 (C6), 105.4 (C3), 119.8 (C2), 125.4 (C5), 143.0 (C4), 152.7 (C1).

MS (APCI): $m/z$ [M + H]$^+$ = 164.1, 146.1 (–H$_2$O).

(E)-2-(4-(tert-Butyldimethylsilyl)oxy)cyclohept-2-en-1-ylidene)propanenitrile (8) (See [12]). To a solution of 7 (220 mg, 1.30 mmol), imidazole (410 mg, 6.00 mmol), and DMAP (3.00 mg, 0.030 mmol) in CH$_2$Cl$_2$ at 0°C was added tert-butylidimethylsilyl chloride (TBSCI, 240 mg, 1.60 mmol). The reaction mixture was stirred at 0°C for 15 minutes and then warmed to r.t. for another 1.5 h. The reaction was quenched by the addition of water (25 mL).
The organic phase was removed, and the aqueous phase was extracted with CH$_2$Cl$_2$ (3 × 15 mL). The combined organic phases were washed with brine (30 mL), dried over MgSO$_4$, filtered, and concentrated in vacuo. The product was purified by column chromatography (40 g silica gel cartridge, gradient elution 0–5% EtOAc-hexanes); yield of 8, 0.30 g (1.1 mmol, 80%).

$$R_f = 0.36 \text{ (EtOAc-hexanes, 1 : 20).}$$

$^1$H NMR (600 MHz, CDCl$_3$): $\delta = 6.23$ (dd, 1H, $J = 12.0$, 2.0 Hz, H-5), 6.03 (dd, 1H, $J = 12.0$, 2.0 Hz, H-4), 4.49–4.43 (m, 1H, H-6), 2.82 (td, 1H, $J = 14.0$, 5.0 Hz, H-9), 2.52 (ddd, 1H, $J = 14.0$, 9.5, 5.0 Hz, H-9), 1.96–1.89 (m, 4H, H-7, C2–C3), 1.84–1.70 (m, 3H, H-7, H-8), 0.90 (s, 9H, Si(CH$_3$)$_3$), 0.10 (s, 3H, Si(CH$_3$)$_2$), 0.09 (s, 3H, Si(CH$_3$)$_2$).

$^{13}$C NMR (150 MHz, CDCl$_3$): $\delta = 133.0$ (C2), 143.6 (C5), 153.9 (C4), 191.0 (C1).

$^1$H NMR (600 MHz, CDCl$_3$): $\delta = 6.52$ (d, 1H, $J = 16.0$ Hz), 6.49 (dd, 1H, $J = 12.0$, 2.0 Hz), 6.20 (d, 1H, $J = 16.0$ Hz), 5.70 (dd, 1H, $J = 12.0$, 2.0 Hz), 4.53–4.46 (m, 1H), 2.71 (td, 1H, $J = 13.5$, 5.0 Hz), 2.31–2.20 (m, 1H), 2.10–1.94 (m, 2H), 1.90 (s, 9H), 1.88–1.76 (m, 2H), 1.74 (s, 3H), 1.72–1.54 (m, 4H), 1.47 (m, 2H), 1.06 (s, 3H), 1.05 (s, 3H) 0.92 (s, 9H), 0.12 (s, 3H), 0.11 (s, 3H).

MS (ES): m/z [M + H]$^+$ = 400.1.

To a solution of 11 (390 mg, 0.960 mmol) in THF (32 mL) in the dark, at 0°C, was added TBAF (1 M in THF, 1.90 mL, 1.92 mmol). The reaction mixture was stirred in the dark at 0°C for 5 h. The reaction was quenched by the addition of water (35 mL) and extracted with EtOAc (3 × 35 mL). The combined organic layers were washed with brine (50 mL), dried over Na$_2$SO$_4$, filtered, and concentrated in vacuo. The allylic alcohol (540 mg, yellow solid, $R_f = 0.24$ (hexanes-EtOAc 10 : 1)) was dissolved in CH$_2$Cl$_2$ (15 mL) and cooled to −20°C in the dark. MnO$_2$ (85% activated, 1.70 g, 20.0 mmol) was added, and the mixture was stirred for 10 minutes in the dark at −20°C. The reaction mixture was then warmed to r.t. and stirred for 2 h in the dark. The reaction mixture was then vacuum filtered through a pad of Celite, and the cake was washed with CH$_2$Cl$_2$ (5 × 10 mL). The bright yellow filtrate was concentrated in vacuo. The product was purified by column chromatography (40 g silica gel cartridge, gradient elution 0–20% EtOAc-hexanes); yield of 12, 0.13 g (0.45 mmol, 47%).

$$R_f = 0.18 \text{ (EtOAc-hexanes, 1 : 20).}$$

$^1$H NMR (600 MHz, CDCl$_3$): $\delta$ ppm 7.39 (d, 1H, $J = 12.0$ Hz), 6.61 (d, 1H, $J = 16.0$ Hz), 6.45 (d, 1H, $J = 16.0$ Hz), 5.96 (d, 1H, $J = 12.0$ Hz), 2.65 (t, 2H, $J = 6.5$ Hz), 2.62 (t, 2H, $J = 6.5$ Hz), 2.06 (m, 5H), 1.96–1.87 (m, 2H), 1.76 (s, 3H), 1.70–1.62 (m, 2H), 1.57 (s, 1H), 1.51 (m, 2H), 1.07 (s, 6H).

minutes at r.t., then the flask was wrapped in aluminum foil. A solution of the 12 (130 mg, 0.450 mmol) in THF (6 mL) was added to the reaction mixture, and the reaction was stirred for 3 h at r.t. in the dark. The reaction was poured into 30 mL of ice water and then extracted with ether (4 × 20 mL). The combined organic phases were washed with brine (20 mL), dried over MgSO₄, filtered, and concentrated in vacuo. The intermediate nitrile was purified by column chromatography (40 g silica gel cartridge, isocratic 5% EtOAc-hexane); yield of pentaene nitrile 0.14 g (0.45 mmol, 99%).

\[ R_f = 0.36 \text{ (EtOAc-hexanes, 1:50).} \]

To a solution of the nitrile (140 mg, 0.450 mmol) in Et₂O (60 mL) at −78 °C in the dark was added DIBAL-H (1 M in CH₂Cl₂, 3.00 mL, 3.00 mmol). The reaction mixture was stirred at −78 °C for 10 minutes, then warmed to r.t., and stirred for 30 minutes in the dark. It was then poured into a vigorously stirred slurry of silica gel (5.50 g, ~40 times the mass of the nitrile) and ether (20 mL) under argon at r.t. The slurry was stirred for 30 minutes at r.t. The silica gel was removed by filtration under argon, and the silica gel was washed with ether (100 mL). The bright yellow filtrate was concentrated in vacuo, yield of E/Z 1:1 (0.450 mmol, 96%, E:Z ratio ~1:1). The product was purified by HPLC using a normal phase semiprep column (Phenomenex Luna 5 μ silica 100 Å, 250 mm × 10 mm × 5 mm), isocratic elution 5% ether-hexanes, and flow rate of 10 mL min⁻¹ and monitored at 350 and 210 nm. For optimal separation of isomers 1 mg aldehyde in 0.2 mL hexanes (HPLC grade) was injected per run. This provided 1a with an isomeric purity of 96% by HPLC (AUC at 350 nm), and 1b with an isomeric purity of >98% by HPLC (AUC at 350 nm) (see Figure 3, panels 1a and 1b at t₀).

\[ R_I = 8.13 \text{ min; } R_f = 0.35 \text{ (EtOAc-hexanes, 1:20).} \]

1H NMR (400 MHz, CDCl₃): δ ppm 10.07 (d, 1H, J = 8.0 Hz, H-15), 6.95 (d, 1H, J = 11.5 Hz, H-11), 6.57 (d, 1H, J = 16.0 Hz, H-8), 6.38 (d, 1H, J = 16.0 Hz, H-7), 6.26 (d, 1H, J = 11.5 Hz, H-12), 5.97 (d, 1H, J = 8.0 Hz, H-14), 2.89 (t, 2H, J = 6.5 Hz, H-21), 2.60 (t, 2H, J = 6.5 Hz, H-20), 2.12–2.00 (m, 3H, C9-CH₃), 1.94 (m, 2H, H-4), 1.76 (s, 3H, C5-CH₃), 1.71–1.60 (m, 2H, H-3), 1.51 (m, 2H, H-2), 1.13–1.03 (s, 6H, 2xCH₂-CH₃).

MS (ES): m/z [M + H]⁺ = 311.0.

(Z)-2-((E)-4-((E)-4-(2,6,6-trimethylcyclohex-1-en-1-y1)but-3-en-2-ylidene)cyclohept-2-en-1-ylidene) Acetaldehyde (1b).

\[ R_I = 7.69 \text{ min; } R_f = 0.35 \text{ (EtOAc-hexanes, 1:20).} \]

1H NMR (400 MHz, CD₂Cl₂): δ ppm 10.15 (d, 1H, J = 8.0 Hz, H-15), 7.07 (m, 2H, H-11, H-12), 6.61 (d, 1H, J = 15.5 Hz, H-8), 6.40 (d, 1H, J = 15.5 Hz, H-7), 5.80 (d, 1H, J = 8.0 Hz, H-14), 2.57 (t, 2H, J = 7.0 Hz, H-21), 2.47 (t, 2H, J = 7.0 Hz, H-20), 2.03 (m, 5H, H-4, C9-CH₃), 1.95–1.85 (m, 2H, H-21), 1.76 (s, 3H, C5-CH₃), 1.72–1.62 (m, 2H, H-3), 1.30 (m, 2H, H-2), 1.07 (s, 6H, 2xCH₂-CH₃).

MS (ES): m/z [M + H]⁺ = 311.0.

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References