An Improved Synthesis of a Hydroxymethyl Tricyclic Ketone from Cyclohexanone, the Key Processes for the Synthesis of a Highly Potent Anti-inflammatory and Cytoprotective Agent

Akira Saito,[a] Suqing Zheng,[a] Motohiro Takahashi,[a] Wei Li,[a] Iwao Ojima,[a,b] Tadashi Honda*[a,b]

[a] Institute of Chemical Biology and Drug Discovery, Stony Brook University, Stony Brook, New York 11794, USA

[b] Department of Chemistry, Stony Brook University, Stony Brook, New York 11794, USA
Fax +1(631)6327942; E-mail: tadashi.honda@stonybrook.edu

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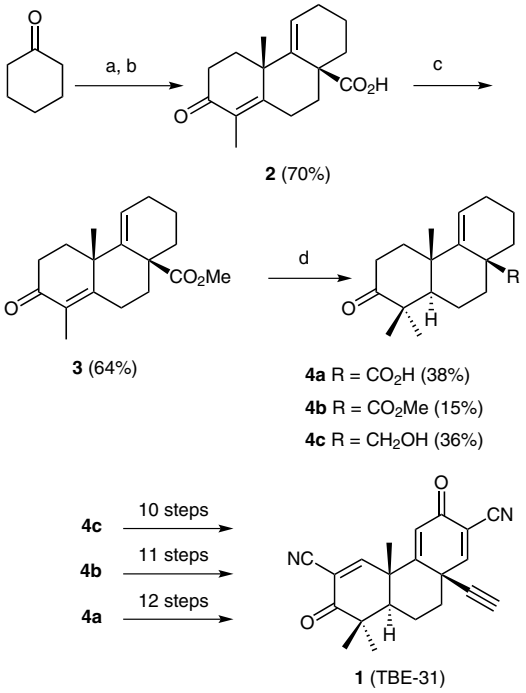
Abstract: An improved synthesis of hydroxymethyl tricyclic ketone, (±)-(4a*S*,8a*S*)-8a-(hydroxymethyl)-1,1,4a-trimethyl-3,4,4a,6,7,8,8a,9,10,10a-decahydrophenanthren-2(1*H*)-one, in five steps (34% yield) from cyclohexanone has been successfully established. Accordingly, 10 grams of a highly potent anti-inflammatory and cytoprotective agent, (±)-(4b*S*,8a*R*,10a*S*)-10a-ethynyl-4b,8,8-trimethyl-3,7-dioxo-3,4b,7,8,8a,9,10,10a-octahydrophenanthrene-2,6-dicarbonitrile (TBE-31), was obtained in 15 steps (9.2% overall yield) via the hydroxymethyl tricyclic ketone from 32 grams of cyclohexanone.

Key words: anti-inflammatory agents, antitumor agents, oxidation, reduction, reductive methylation, regioselectivity

Tricyclic compound **1** (code number in house, TBE-31, Scheme 1) is the most potent activator of the Keap1/Nrf2/ARE (antioxidant response element) pathway so far.[1] The oral administration of **1** indicated excellent oral bioavailability, and resulted in a dose-dependent induction of cytoprotective enzymes, NAD(P)H:quinone oxidoreductase 1 (NQO1), and glutathione *S*-transferase (GST) in the stomach, skin, and liver.[2] Furthermore, long-term (five days per week for four weeks) daily topical applications in small quantities (200 nmol) of **1** caused a robust systemic induction of the Keap1/Nrf2/ARE pathway and decreased 6-thioguanine incorporation into DNA of skin, blood, and liver of azathioprine-treated mice, indicating extraordinary bioavailability and efficacy.[3] Tricyclic compound **1** is orally highly active against aflatoxin-induced liver cancer in rats.[4] Thus, we envision the preclinical and future clinical studies on **1** as a first in class therapeutic agent for the treatment of inflammation and cancer.

For this objective, initially we had to improve the synthesis of hydroxymethyl tricyclic ketone **4c**, (±)-(4a*S*,8a*S*)-8a-(hydroxymethyl)-1,1,4a-trimethyl-3,4,4a,6,7,8,8a,9,10,10a-decahydrophenanthren-2(1*H*)-one, from cyclohexanone in order to obtain 10–100 grams of **1**. Herein, we report the improved synthesis of **4c** from cyclohexanone.

We have previously reported the synthesis of **4c** from cyclohexanone, but under these processes the reductive methylation step of **3** gives three compounds **4a**–**c**, whose separation requires column chromatography. Moreover,



Scheme 1 Previous synthesis of **4c**. *Reagents and conditions*: (a) Me_2CO_3, NaH, KH, THF; (b) 1-chloropentan-3-one, Na, MeOH; (c) Cs_2CO_3, Me_2SO_4, DMF; (d) Li, NH_3, H_2O, MeI, THF.

the consequent conversion of **4a** and **4b** into TBE-31 requires additional steps than that of **4c** (Scheme 1).[1,5] Although these three intermediates **4a**–**c** were very useful for the synthesis of various other tricyclic compounds,[6] the previously reported synthesis[1] of **1** could not give a sufficient amount of **1** for preclinical studies. For the efficient synthesis of **1**, only intermediate **4c** is necessary. Thus we envisioned that, if the new compound **8a** can be obtained in a few steps from the previously reported acid **2**, which is prepared in two steps from cyclohexanone,[6,7] reductive methylation of **8a** would only produce **4c** (Scheme 2).

Initially we attempted to directly prepare **8a** from **2** by selective reduction of the carboxyl group with diborane (strategy A). This method did not give **8a**, instead it gave some undesired compounds without UV absorption at 254 nm on TLC. Secondly, we tried to obtain **8a** through selective reduction of acyl chloride **5** with reducing agents (strategy B in Scheme 2). Acyl chloride **5** was prepared in

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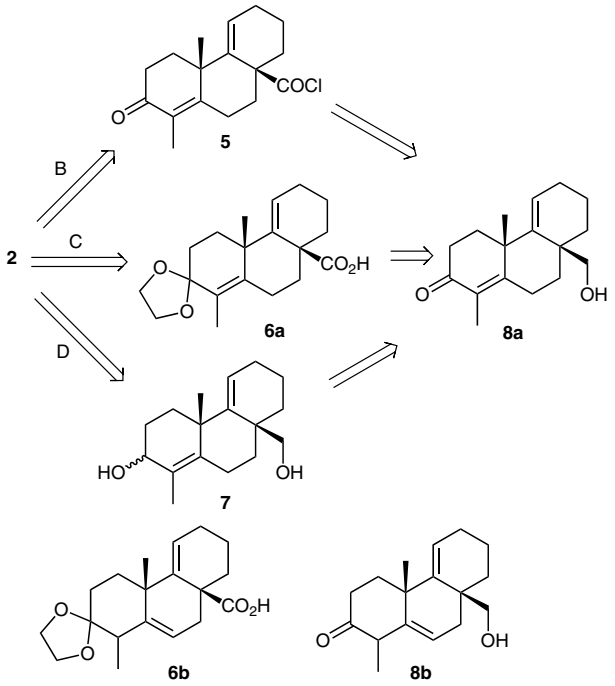
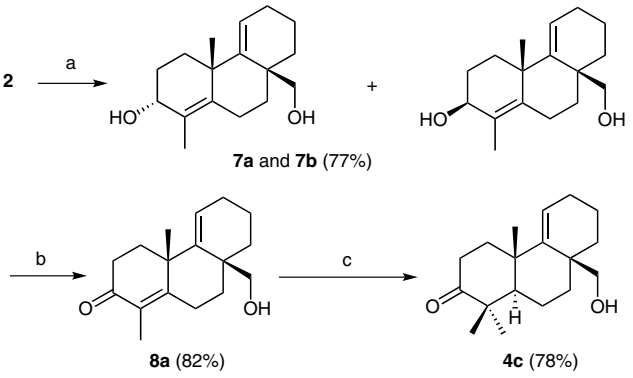
quantitative yield from **2** with thionyl chloride. Attempted reduction of **5** with sodium borohydride was unsuccessful and starting material **5** was recovered unchanged. Reduction of **5** with zinc borohydride also did not give **8a**, but instead gave inseparable compounds whose structures could not be elucidated. Thirdly, we planned to reduce the carboxyl group to the hydroxymethyl group after protection of the carbonyl group of **2** (strategy C in Scheme 2). Ketalization of **2** with ethylene glycol in the presence of pyridinium *p*-toluenesulfonate in toluene was very slow. A mixture of **6a** and **6b** was obtained in only 49% yield and **2** was recovered in 39% yield after reflux for 24 hours. Reduction of the mixture with lithium aluminum hydride, followed by deprotection with pyridinium *p*-toluenesulfonate in acetone, afforded **8a** and **8b**. Reductive methylation of the mixture gave **4c** in very low yield (22%).



Scheme 2 Strategies B, C, and D for the synthesis of **8a** from **2**

Finally, we thought that selective oxidation of the allyl alcohol in **7** would give the desired enone **8a** (strategy D in Scheme 2 and Scheme 3). Reduction of **2** with sodium bis(2-methoxyethoxy)aluminum hydride (Red-Al) in toluene afforded a mixture of new α-alcohol **7a** [NMR (400 MHz, $CDCl_3$): δ = 3.87, s at 2β-H], and new β-alcohol **7b** [NMR (400 MHz, $CDCl_3$): δ = 4.01, dd, *J* = 7.2 and 7.2 Hz at 2α-H] in 77% yield (ratio = 1:3). Selective oxidation of the mixture with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone in 1,4-dioxane[8] produced the new desired enone **8a** in 82% yield. As we expected, only **4c** was obtained by reductive methylation of **8a** in 78% yield. Overall, **4c** was synthesized in five steps (34% yield) from cyclohexanone. Thus, our objective has been successfully achieved by strategy D.



Scheme 3 New synthesis of **4c**. *Reagents and conditions*: (a) Red-Al, toluene; (b) DDQ, 1,4-dioxane; (c) Li, NH_3, H_2O, MeI, THF.

From **4c**, tricyclic compound **1** was synthesized in 10 steps (27% yield) similar to the previously published synthetic sequence.[1]

In summary, according to the improved synthesis of **4c** from cyclohexanone, we have successfully obtained 10 grams of tricyclic compound **1**, a highly potent anti-inflammatory and cytoprotective agent, in 15 steps from cyclohexanone (32 grams, 9.2% overall yield). We are planning to have 100 grams of **1** synthesized using this method by a custom synthesis company for further biological evaluation including a subacute toxicological test.

Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectra were measured on a Bruker Avance 400 NMR spectrometer referenced to δ = 7.27 in $CHCl_3$ (^1H NMR) and δ = 77.23 in $CDCl_3$ (^{13}C NMR) as internal standards. LR-MS and HRMS data were obtained on a Micromass 70-VSE (EI method) and Micromass Q-Tof Ultima (ESI+ method). Elemental analyses were performed by Atlantic Microlab Inc. All samples prepared for elemental analysis were dried at 50–60 °C at reduced pressure (<0.13 mbar) in a National Appliance Company model 5831 vacuum oven. TLC was performed using plates precoated with silica gel 60 F_{254}. Flash column chromatography used silica gel (230–400 mesh). Anhyd THF and CH_2Cl_2 were obtained from a solvent purification system. All other solvents (analytical grade) including anhyd solvents and reagents were used as received. All experiments were performed under a N_2 atmosphere.

2-(Methoxycarbonyl)cyclohexanone[6]

A mixture of NaH (60% oil dispersion, 10 g, 250 mmol, 3.1 equiv) and dimethyl carbonate (18.02 g, 200 mmol, 2.5 equiv) in anhyd THF (50 mL) was heated under reflux. To the mixture was added a solution of cyclohexanone (7.8 g, 80 mmol) in anhyd THF (20 mL) dropwise using a syringe pump over a period of 1 h; 2 min after the addition of the cyclohexanone–THF solution began, KH (30% oil dispersion, 0.9 g) was added to initiate the reaction. When the addition was complete, the mixture was heated under reflux for an additional 30 min. The mixture was cooled in an ice-water bath, and then it was hydrolyzed by slow addition of 3 M aq AcOH (75 mL) and poured into brine (100 mL). The aqueous mixture was extracted with CH_2Cl_2 (4 × 150 mL). The combined extracts were dried ($MgSO_4$), filtered, and concentrated in vacuo to give a thick yellow liquid (17.2 g). The liquid was distilled under reduced pressure to give the product (11.3 g, 91%) as a colorless liquid; bp 38–43 °C/0.067–0.01 mbar, bath temp: 75–78 °C.

(±)-(4a*S*,8a*S*)-1,4a-Dimethyl-2-oxo-2,3,4,4a,6,7,8,8a,9,10-decahydrophenanthrene-8a-carboxylic Acid (2)[7]

To anhyd MeOH (256 mL) cooled in an ice-water bath, Na metal (11.8 g, 0.512 mol, 4.0 equiv) was added. When the Na was completely dissolved in MeOH, 2-(methoxycarbonyl)cyclohexanone (20.0 g, 0.128 mol) was added and the mixture was heated to reflux. To the mixture at reflux 1-chloropentan-3-one (40 mL, 0.30 mol, 2.3 equiv) was added using a syringe pump over 14 h. After the addition was complete, the mixture was heated under reflux for an additional 6 h. The MeOH was removed in vacuo, and CH_2Cl_2 (200 mL) and 5% aq HCl (200 mL) were added to acidify the mixture. The acidic mixture was extracted with CH_2Cl_2 (200 mL). The organic solution was extracted with 1 M aq NaOH (2 × 300 mL). The basic solution was washed with CH_2Cl_2 (500 mL) and acidified with concd aq HCl (100 mL) to give a precipitate. The mixture containing precipitates was extracted with CH_2Cl_2 (2 × 300 mL). The combined extracts were washed with brine (500 mL), dried ($MgSO_4$), filtered, and then concentrated in vacuo to give **2** (27.1 g, 77%) as an amorphous solid that was used in the next reaction without further purification.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.88 (t, *J* = 3.8 Hz, 1 H), 2.66–2.43 (m, 5 H), 2.25 (ddd, *J* = 2.2, 2.2, 18.5 Hz, 1 H), 2.20–2.07 (m, 1 H), 2.07–1.97 (m, 2 H), 1.88–1.76 (m, 1 H), 1.77 (s, 3 H), 1.73–1.62 (m, 2 H), 1.55 (ddd, *J* = 3.4, 13.7, 13.7 Hz, 1 H), 1.46–1.36 (m, 1 H), 1.29 (s, 3 H).

(±)-(2*R*,4a*S*,8a*S*)-8a-(Hydroxymethyl)-1,4a-dimethyl-2,3,4,4a,6,7,8,8a,9,10-decahydrophenanthren-2-ol (7a) and (±)-(2*S*,4a*S*,8a*S*)-8a-(Hydroxymethyl)-1,4a-dimethyl-2,3,4,4a,6,7,8,8a,9,10-decahydrophenanthren-2-ol (7b)

To a solution of **2** (13.6 g, 49.6 mmol) in toluene (550 mL) heated to 110 °C, a 3.5 M solution of Red-Al in toluene (56.6 mL, 198 mmol, 4.0 equiv) was added. The solution was stirred at this temperature for 14 h. The mixture was cooled in an ice-water bath and then 1 M aq NaOH (4 mL) and H_2O (14 mL) were successively added. The organic layer was washed with 1 M aq NaOH (400 mL), H_2O (400 mL), and brine (400 mL), then it was dried ($MgSO_4$), filtered, and concentrated in vacuo to give a residue. The residue was filtered through a pad of silica gel (hexanes–EtOAc, 2:1) to afford **7a** and **7b** [9.97 g, 77% as a mixture of two diastereomers (1:3)] as a colorless solid. Analytical samples of **7a** and **7b** were obtained by flash column chromatography (hexanes–EtOAc, 3:1).

7a

Mp 145–146 °C.

^{1}H NMR ($CDCl_3$): δ = 5.80 (dd, *J* = 3.8, 3.8 Hz, 1 H), 3.87 (s, 1 H), 3.78 and 3.76 (ABq, *J* = 11.0 Hz, each 1 H), 2.45 (ddd, *J* = 1.5, 1.5, 14.6 Hz, 1 H), 2.19–2.08 (m, 3 H), 1.99–1.74 (m, 5 H), 1.78 (d, *J* = 0.7 Hz, 3 H), 1.69 (m, 1 H), 1.67–1.52 (m, 2 H), 1.47 (br s, 1 H), 1.33 (br s, 1 H), 1.23–1.13 (m, 2 H), 1.14 (s, 3 H).

^{13}C NMR ($CDCl_3$): δ = 147.1, 140.6, 125.6, 123.9, 69.5, 66.6, 40.4, 39.5, 36.3, 35.1, 31.6, 28.3, 28.2, 25.9, 22.3, 17.91, 17.85.

MS (ESI+): *m*/*z* = 245.2 $[M - H_2O + H]^+$.

HRMS (ESI+): *m*/*z* $[M - H_2O + H]^+$ calcd for $C_{17}H_{25}O$: 245.1900; found: 245.1899.

Anal. Calcd for $C_{17}H_{26}O_2 \cdot 0.25\ H_2O$: C, 76.50; H, 10.01. Found: C, 76.74; H, 9.94.

7b

Mp 147–150 °C.

^{1}H NMR ($CDCl_3$): δ = 5.71 (dd, *J* = 3.8, 3.8 Hz, 1 H), 4.01 (dd, *J* = 7.2, 7.2 Hz, 1 H), 3.76 (s, 2 H), 2.44 (ddd, *J* = 3.9, 3.9, 14.6 Hz, 1 H), 2.19 (m, 1 H), 2.13 (dd, *J* = 3.9, 4.9 Hz, 1 H), 2.11 (dd, *J* = 1.1, 4.0 Hz, 1 H), 2.04 (m, 1 H), 1.92–1.70 (m, 5 H), 1.73 (s, 3 H), 1.63–1.52 (m, 2 H), 1.39 (br s, 1 H), 1.30 (br s, 1 H), 1.22 (s, 3 H), 1.25–1.05 (m, 2 H).

^{13}C NMR ($CDCl_3$): δ = 146.6, 139.7, 127.1, 123.6, 71.4, 66.9, 40.9, 39.6, 36.6, 35.6, 34.3, 29.8, 29.7, 25.8, 22.8, 17.9, 15.2.

MS (ESI+): *m*/*z* = 285.2 $[M + Na]^+$.

HRMS (ESI+): *m*/*z* $[M + Na]^+$ calcd for $C_{17}H_{26}O_2Na$: 285.1830; found: 285.1825.

Anal. Calcd for $C_{17}H_{26}O_2$: C, 77.82; H, 9.99. Found: C, 77.93; H, 9.91.

(±)-(4a*S*,8a*S*)-8a-(Hydroxymethyl)-1,4a-dimethyl-4,4a,6,7,8,8a,9,10-octahydrophenanthren-2(3*H*)-one (8a)

A mixture of **7a** and **7b** (14.9 g, 57.0 mmol) and DDQ (19.4 g, 85.5 mmol, 1.5 equiv) in 1,4-dioxane (710 mL) was stirred at 40 °C for 12 h. After removal of 1,4-dioxane in vacuo, the resulting residue was dissolved in CH_2Cl_2 (500 mL). The mixture was washed with 1 M aq NaOH (500 mL), H_2O (500 mL), and brine (500 mL), dried ($MgSO_4$), and concentrated in vacuo to give a residue that was filtered through a pad of silica gel (hexanes–EtOAc, 2:1) to afford **8a** as a crystalline solid (12.1 g, 82%); mp 135–136 °C.

^{1}H NMR ($CDCl_3$): δ = 5.74 (dd, *J* = 3.8, 3.8 Hz, 1 H), 3.85 and 3.74 (ABq, *J* = 11.2 Hz, each 1 H), 2.59 (ddd, *J* = 4.2, 5.7, 15.7 Hz, 1 H), 2.58–2.41 (m, 3 H), 2.20–2.13 (m, 2 H), 2.10–2.05 (m, 2 H), 2.01 (ddd, *J* = 4.2, 5.7, 13.6 Hz, 1 H), 1.86–1.80 (m, 2 H), 1.78 (d, *J* = 0.9 Hz, 3 H), 1.67–1.58 (m, 2 H), 1.32 (s, 3 H), 1.30–1.14 (m, 2 H).

^{13}C NMR ($CDCl_3$): δ = 198.6, 163.4, 145.3, 128.4, 124.6, 66.3, 41.1, 39.4, 35.0, 34.2, 34.1, 33.1, 27.6, 25.6, 25.0, 17.5, 11.4.

MS (ESI+): *m*/*z* = 261.2 $[M + H]^+$.

HRMS (ESI+): *m*/*z* $[M + H]^+$ calcd for $C_{17}H_{25}O_2$: 261.1855; found: 261.1849.

Anal. Calcd for $C_{17}H_{24}O_2$: C, 78.42; H, 9.29. Found: C, 78.26; H, 9.26.

(±)-(4a*S*,8a*S*)-8a-(Hydroxymethyl)-1,1,4a-trimethyl-3,4,4a,6,7,8,8a,9,10,10a-decahydrophenanthren-2(1*H*)-one (4c)

Li (sliced ribbon 98%, 2.40 g, 339 mmol, 7.2 equiv) was added to liquid NH_3 (400 mL), and then the solution was stirred at –78 °C for 1 h. A solution of **8a** (12.5 g, 48.0 mmol) and H_2O (864 mg, 48.0 mmol, 1.0 equiv) in THF (192 mL) were added dropwise and the mixture was stirred under reflux at –33 °C (bp of NH_3, with the aid of a CCl_4 bath) for 1 h. The mixture was cooled to –78 °C and isoprene (approx. 10.5 mL) was injected until the blue color disappeared turning the solution cloudy white. To this mixture, THF (69.6 mL) and MeI (69.6 mL, 1.12 mol, 23 equiv) were successively added dropwise. The mixture was stirred under reflux at –33 °C for 1 h. After removal of the NH_3 with the aid of a N_2 stream, sat. aq NH_4Cl soln (100 mL) was added and the aqueous mixture was extracted with EtOAc (3 × 100 mL). The extract was dried ($MgSO_4$), filtered, and then concentrated in vacuo to give a residue that was filtered through a pad of silica gel (hexanes–EtOAc, 3:1) to afford **4c** as a crystalline solid (10.3 g, 78%); mp 109–110 °C.

^{1}H NMR ($CDCl_3$): δ = 5.67 (dd, *J* = 3.8, 3.8 Hz, 1 H), 3.68 (s, 2 H), 2.68 (ddd, *J* = 6.6, 12.5, 15.7 Hz, 1 H), 2.43 (ddd, *J* = 3.3, 5.9, 15.7 Hz, 1 H), 2.20–1.00 (m, 14 H), 1.20 (d, *J* = 0.6 Hz, 3 H), 1.08 (s, 3 H), 1.06 (s, 3 H).

^{13}C NMR ($CDCl_3$): δ = 217.0, 148.0, 123.0, 67.0, 54.1, 47.9, 39.7, 39.0, 38.0, 37.1, 36.7, 34.9, 26.1, 26.0, 22.6, 21.8, 19.7, 18.1.

MS (EI): *m*/*z* = 276 $[M]^+$, 245, 227, 203.

HRMS (EI): *m*/*z* $[M]^+$ calcd for $C_{18}H_{28}O_2$: 276.2089; found: 276.2082.

Anal. Calcd for $C_{18}H_{28}O_2$: C, 78.21; H, 10.21. Found: C, 77.92; H, 10.12.

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Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synthesis.

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