Supporting Information
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An Efficient Hemisynthesis of 20- and 21-[13C]-Labeled 21-Cortexolone: A Model for the Study of Skin Sensitization to Corticosteroids

Emilie Claudel, Cécile Arbez-Gindre, Valérie Berl and Jean-Pierre Lepoittevin*

Institut de Chimie, Laboratoire de Dermatochimie, UMR 7177, CNRS et Université de Strasbourg, 4, rue Blaise Pascal, F-67070 Strasbourg, France. Fax: +33 368 851 527
E-mail: jplepoit@unistra.fr

This Supporting Information contains additional experimental procedures and characterization details for the intermediates 3, 3a, 8, 9, 10, as well as NMR spectra for compounds 1a and 1b.

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**General information**

All air- or moisture-sensitive reactions were conducted in flame-dried glassware under an atmosphere of dry argon. All solvents used were of reagent grade. Anhyd solvents were freshly distilled before use. THF and Et$_2$O were distilled from sodium/benzophenone. CH$_2$Cl$_2$ was dried over P$_2$O$_5$ before distillation. Petroleum ether (PE) used refers to the fraction boiling in the range 35–60 °C. Unless otherwise noted, reactions were magnetically stirred and monitored by TLC with 0.25 mm Merck precoated silica gel plates. Flash chromatography was performed with silica gel 60 (particle size 0.040-0.063 mm) supplied by Merck, Geduran. Yields refer to chromatographically and spectroscopically pure compounds, unless otherwise stated. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker AC300 spectrometer in CDCl$_3$ unless otherwise specified. Chemical shifts are reported in ppm ($\delta$) and CHCl$_3$ was used as internal standard ($\delta = 7.26$ ppm). IR spectra were obtained on a Perkin-Elmer FT-IR 1600 spectrometer; peaks are reported in cm$^{-1}$. Melting points were determined on a Büchi Tottoli 510 apparatus and are uncorrected. Elemental analyses were collected at the Service de microanalyse of the University of Strasbourg (France).

**Experimental Section:**

**Caution:** Skin contact with cortexolone derivatives must be avoided. As potential sensitizing substances, these compounds must be handled with care.

**17β-Cyano-17α-hydroxy-androst-4-en-3-one (3).**

To a suspension of the commercially available diketone (10.00 g, 34.91 mmol) in MeOH (78 mL) were added KCN (9.6 g, 146.64 mmol, 4.2 equiv) and slowly AcOH (3.5 mL, 61.09 mmol, 1.75 equiv). The reaction mixture was stirred at r.t. for 3 h and then allowed to stand for one night. AcOH (7.0 mL, 122.18 mmol, 3.5 equiv) was added once more. The solution was stirred for 2 h and allowed to stand for 4 h. H$_2$O (50 mL) was then added and the white precipitate filtered off, washed with H$_2$O (100 mL) and dried under reduced pressure to give 3 (10.94 g, 34.91 mmol, quant) as a white solid; mp 211-212°C (Lit.$^1$ 169-172°C). [$\alpha$]$_{D}^{20} +149$ (c 2.0, CHCl$_3$). {Lit.$^1$ [$\alpha$]$_{D}^{20} +146$ (c 1.0, CHCl$_3$)}.

IR (CHCl$_3$): 3587 (OH), 2360 (CN), 1662 (C=O), 1615 cm$^{-1}$ (C=C).
$^1$H NMR (300 MHz, CDCl$_3$): $\delta = 0.97$ (s, 3 H, H-18), 1.19 (s, 3 H, H-19), 0.91-1.88 (m, 12 H), 1.96-2.07 (m, 2 H), 2.24-2.49 (m, 5 H), 3.27 (s, 1 H, OH-17), 5.74 (s, 1 H, H-4).

$^{13}$C NMR (75 MHz, CDCl$_3$): $\delta = 16.2$ (C-18), 17.4 (C-18 or C-19), 20.4 (C-11), 23.9 (C-15), 29.4, 31.9, 32.7, 33.8, 35.7, 36.0 (C-8), 38.1, 38.6 (C-10), 47.6 (C-14), 49.1 (C-13), 53.1 (C-9), 77.7 (C-17), 120.9 (C-20), 124.0 (C-4), 171.1 (C-5), 199.9 (C-3).

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$[20-^{13}$C$]$-17$\beta$-Cyano-17$\alpha$-hydroxy-androst-4-en-3-one (3a).

Starting from the commercially available diketone (5.04 g, 17.60 mmol) and K$_{13}$CN (4.9 g, 73.91 mmol, 4.2 equiv) and using the same procedure as for the synthesis of 3 gave 3a (4.70 g, 14.96 mmol, 85%) as a white solid; mp 211-212°C.

$^1$H NMR (300 MHz, CDCl$_3$/MeOD): $\delta = 0.81$ (s, 3 H, H-18), 1.06 (s, 3 H, H-19), 0.76-1.93 (m, 14 H), 2.13-2.36 (m, 5 H), 3.99 (s, 1 H, OH-17), 5.58 (s, 1 H, H-4).

$^{13}$C NMR (75 MHz, CDCl$_3$/MeOD): $\delta = 15.9$ (C-18), 17.0 (C-19), 20.1 (C-11), 23.5 [d, $^3J$($^{13}$C,C) = 3.1 Hz, C-15], 29.1, 31.6, 32.5, 33.4, 35.2, 35.7 (C-8), 37.6, 38.4 (C-10), 43.3 [d, $^3J$($^{13}$C,C) = 3.1 Hz, C-14], 48.7 (C-13), 52.9 (C-9), 76.9 [d, $^1J$($^{13}$C,C) = 62.3 Hz, C-17], 121.1 ($^{13}$C-20), 123.3 (C-4), 172.6 (C-5), 200.7 (C-3).

SCI Finder registry number [135441-75-7].

17$\alpha$-Hydroxy-pregn-4-en-3,20-dione (8).

To a solution of 7 (1.27 g, 3.13 mmol) in EtOH and H$_2$O (90:10, 100 mL) were added CaCO$_3$ (313 mg, 3.13 mmol, 1 equiv) and MeI (9.7 mL, 156.50 mmol, 50 equiv). The reaction mixture was stirred for 20 h under reflux, cooled down, diluted with CH$_2$Cl$_2$ (200 mL). The aqueous phase was extracted with CH$_2$Cl$_2$ (2 x 100 mL). The combined organic layers were dried (MgSO$_4$), concentrated under reduced pressure and the residue purified by column chromatography over silica (PE, EtOAc 25%) to give 8 (1.03 g, 3.13 mmol, quant) as a white solid; mp 212-213°C [Lit.$^2$ 213-215°C (acetone)]. $[\alpha]_D^{20}$ +89 (c 1.5, CHCl$_3$). [lit.$^2$ $[\alpha]_D^{20}$ +97 (c 0.5, CHCl$_3$)].

IR (CHCl$_3$): 3492 (OH), 1698 (C=O), 1662 (C=O), 1615 cm$^{-1}$ (C=C).

$^1$H NMR (300 MHz, CDCl$_3$): $\delta = 0.72$ (s, 3 H, H-18), 0.90-1.13 (m, 2 H), 1.16 (s, 3 H, H-19), 1.28-1.45 (m, 3 H), 1.51-1.88 (m, 8 H), 1.97-2.04 (m, 1 H), 2.20-2.45 (m, 4 H), 2.24 (s, 3 H, H-
21), 2.66 (ddd, $^3 J = 2.9$ Hz, $^2 J = 11.5$ Hz, $^2 J = 14.5$ Hz, 1 H, H-16$, 2.88 (br s, 1 H, OH-17), 5.70 (d, $J = 1.1$ Hz, 1 H, H-4$).

$^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ = 15.3 (C-18), 17.3 (C-19), 20.5 (C-11), 23.8 (C-15), 27.8 (C-21), 30.0, 32.0, 32.8, 33.4, 33.9, 35.4 (C-8), 35.6, 38.5 (C-10), 48.0 (C-13), 49.9 (C-14), 53.3 (C-9), 89.8 (C-17), 123.8 (C-4), 171.1 (C-5), 199.6 (C-3), 211.6 (C-20).

SCI Finder registry number [68-96-2].

21-bromo-17$\alpha$-hydroxy-3-(1-pyrrolidinium-1-yliden)-pregn-5-en-20-one chloride (9).

To a suspension of 8 (5.00 g, 15.13 mmol) in MeOH (20 mL) heated in reflux was added pyrrolidine (1.7 mL, 21.18 mmol, 1.4 equiv). The reaction mixture was stirred for 2 h under reflux and then cooled to 0°C. The white precipitate was filtered off, washed with MeOH (60 mL) and dried under reduced pressure. To the residue (1.00 g, 2.61 mmol) solubilized in EtOH (50 mL) was added concd HCl (1.1 mL, 13.04 mmol, 5 equiv). The solution was treated with Br$_2$ (2 mL, 3.91 mmol, 1.5 equiv) in EtOH (10 mL) which was added over 30 minutes. The reaction mixture was stirred for 4 h, filtered off and washed with cold EtOH (50 mL). The solid was isolated and the filtrate was concentrated and crystallized in EtOH to give 9 (1.275 g, 2.56 mmol, 98%) as a yellow solid; mp 244-247°C [Lit.$^3$ 269°C (EtOH)]. $[\alpha]_{20}^D +128$ (c 2.0, CHCl$_3$).

$^1$H NMR (300 MHz, CDCl$_3$ / %DMSO): $\delta$ = 0.49 (s, 3 H, H-18), 0.91-2.14 (m, 18 H), 1.06 (s, 3 H, H-19), 2.41-2.44 (m, 2 H), 2.51-2.60 (m, 1 H, H-16$\beta$), 2.67-2.87 (m, 2 H), 3.71-3.95 (m, 5 H, 2 x H-1’, 2 x H-4’ and OH-17), 4.07 (A part of an AB system, $J_{AB} = 15.1$ Hz, 1 H, H-21), 4.36 (B part of an AB system, $J_{AB} = 15.1$ Hz, 1 H, H-21), 6.19 (s, 1 H, H-4$\beta$).

$^{13}$C NMR (75 MHz, CDCl$_3$ / %DMSO): $\delta$ = 14.7 (C-18), 17.1 (C-19), 20.3 (C-11), 23.1 (C-15), 24.1 (C-2’ or C-3’), 24.2 (C-2’ or C-3’), 26.9, 30.0, 31.6, 33.0, 33.7, 34.3, 35.0 (C-8), 35.7, 39.2 (C-10), 47.1 (C-13), 49.5 (C-9 or C-14), 52.3 (C-9 or C-14), 52.4 (C-1’ or C-4’), 52.8 (C-1’ or C-4’), 89.6 (C-17), 115.6 (C-4), 172.1 (C-3 or C-5), 182.6 (C-3 or C-5), 203.3 (C-20).

SCI Finder registry number [23712-55-2].

21-bromo-17$\alpha$-hydroxypregn-4-en-3,20-dione (10).

To a solution of 9 (200 mg, 0.37 mmol) in EtOH and H$_2$O (83:17, 17 mL) were added K$_2$CO$_3$ (142 mg, 1.04 mmol, 2.8 equiv). The reaction mixture was stirred at r.t. for 2h and then concentrated under reduced pressure. Water (20 mL) was added and the precipitate filtered off,
washed with H₂O (50 mL) and dried under reduced pressure to give 10 (113 mg, 0.28 mmol, 76%) as a white solid; mp 217-219°C (Lit.⁴ 223-224°C). [α]D²⁰ +130 (c 1.0, CHCl₃). {Lit.⁵ [α]D²⁰ +129 (c 1.013, dioxane}).

IR (KBr): 3422 (OH), 1710 (C=O), 1651 cm⁻¹ (C=O).

¹H NMR (300 MHz, CDCl₃): δ = 0.72 (s, 3 H, H-18), 1.18 (s, 3 H, H-19), 0.74-2.18 (m, 14 H), 2.23-2.49 (m, 4 H), 2.64-2.84 (m, 2 H, H-16β and OH-17), 4.16 (A part of an AB system, Jₐₙ = 15.0 Hz, 1 H, H-21), 4.39 (B part of an AB system, Jₐₙ = 15.0 Hz, 1 H, H-21), 5.73 (s, 1 H, H-4).

¹³C NMR (75 MHz, CDCl₃): δ = 14.8 (C-18), 17.1 (C-19), 20.4 (C-11), 23.3 (C-15), 30.2, 31.7, 32.5, 33.6, 33.9, 35.3, 35.4 (C-8), 35.6, 38.3 (C-10), 47.3 (C-13), 49.8 (C-14), 52.9 (C-9), 89.8 (C-17), 123.5 (C-4), 171.1 (C-5), 199.3 (C-3), 203.6 (C-20).

SCI Finder registry number [20380-17-0].

References
