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## SYNTHESIS OF NOVEL 20-OXO-PREGNACALCIFEROL ANALOGS WITH BINDING AFFINITY TO THE PROGESTERONE RECEPTOR

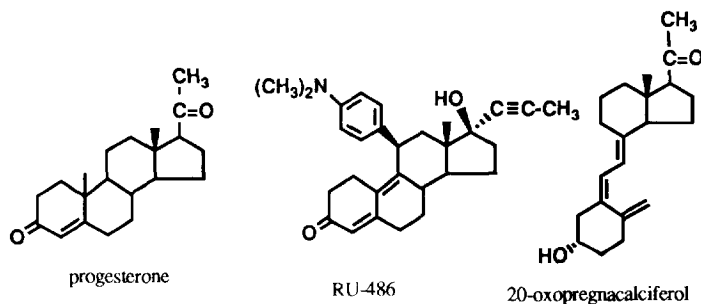
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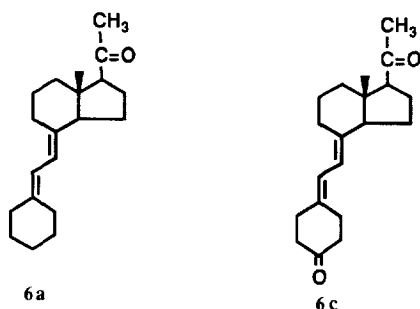
**Abstract:** 3-Desoxy-20-oxo-19-nor-pregnacaliferol (**6a**) and its 2-oxo analog **6c** were prepared from 22-acetoxo-Grundmann's ketone **1** and the corresponding (cyclohexylidene)ethyl phosphine oxides **2a, b** and their binding to the progesterone receptor examined.

RU 486,<sup>1</sup> a 19-nor-steroid developed in the 1980's by Roussel-UCLAF researchers, has strong anti-progesterone and anti-glucocorticosteroid activities. When used in conjunction with synthetic prostaglandins, it terminates pregnancy, which accounts for its wide interest.<sup>2,3</sup>

Many analogs have been prepared,<sup>4</sup> all of which share with RU 486 the intact steroidal AB ring system of progesterone with the conjugated 3-oxo-4-ene moiety. This feature is assumed to be responsible for binding to the progesterone receptor (PR).

Contrary to the general belief that the intact AB rings of progesterone are necessary for binding to the PR while the CD rings are suitably modified, we found that a pregnacaliferol analog with an intact progesterone CD ring system, but with modified AB rings, also bound to the PR with birth control effect in vivo.<sup>5</sup> In our ongoing effort to examine the structural features of the molecule responsible for its binding properties to the PR we became interested in the synthesis of 20-oxopregnacaliferol analogs with 19-nor-3-desoxy-A rings, in which the exocyclic methylene group (carbon 19) and the 3-hydroxy group have been replaced by hydrogen atoms. The compounds prepared were 3-desoxy-20-oxo-19-nor-pregnacaliferol (**6a**) and 3-desoxy-2,20-dioxo-19-nor-pregnacaliferol (**6c**) and their binding to the PR was examined.<sup>6</sup>





For the synthesis of 3-desoxy-20-oxo-19-nor-pregnacalciferol (**6a**) we used our convergent approach, based on Lythgoe's concept and successfully applied by us to other 19-nor-vitamin D compounds.<sup>7</sup> Thus, 22-acetoxy Grundmann's ketone analog **18** was reacted with the conjugate (cyclohexylidene)ethyl diphenylphosphinoxy carbanion derived from **2a** to give the expected diene **3a** (*n*-BuLi, THF, -78 °C, 1 h then 0 °C, 18 h; 57%). Reduction of 22-ester **3a** gave 22-alcohol **4a** (LiAlH<sub>4</sub>, THF, 0 °C, 30 min then 20 °C, 30 min) followed by Swern oxidation to give 22-aldehyde **5a** ((COCl)<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>, DMSO, -60 °C, 30 min then TEA; overall 44%). The next step of the synthesis was Van Rhee's<sup>9</sup> unusual oxygenation procedure which we previously applied to the air sensitive pregnacalciferol system.<sup>5</sup> Aldehyde intermediate **5a** was converted to the 20-keto derivative **6a** in the presence of cupric acetate, complexed with 2,2'-bipyridyl or 1,10-phenanthroline as catalyst, the base 1,4-diazabicyclo[2.2.2]octane (Dabco) and DMF as solvent and air bubbled in the solution for 18 h (60%). The final 3-desoxy-20-oxo-19-nor-pregnacalciferol (**6a**) did bind to the PR (Table 1) but to a significantly smaller degree (40%) than the previously synthesized 20-oxopregnacalciferol.<sup>5</sup> Interestingly, the intermediate 22-alcohol **4a** and the 22-aldehyde **5a** also did bind to the PR, the latter compound binding 1.5 times more than the 20-ketone **6a**. The biological results indicate that an oxygen function in the A ring could be essential for improved binding to the PR. We therefore turned our attention to the 19-norpregnacalciferol analog with an oxo group in the A ring. Looking for a stable derivative with a prolonged half life *in vivo* we excluded positions 4 (and 10) for the introduction of the keto group, because such dienones in the vitamin D<sub>3</sub> series<sup>10</sup> are known to be unstable, light sensitive compounds.<sup>11</sup> Similarly, synthesis of compounds possessing keto groups at C-1 or C-3 would result in the base- and acid-labile beta, gamma-unsaturated ketones. It is well known that the 3-hydroxy polyene system of vitamin D does not survive even indirect oxidations to the 3-keto group. Therefore, our choice fell on a 2-oxo-substituted A ring.

For the synthesis of the 3-desoxy-2,20-dioxo-19-nor-pregnacalciferol (**6c**) we used the synthetic methodology described for **6a**. We chose as our starting material commercially available 1,4-cyclohexanedione mono-ethylene ketal (**7**) as a ring-A building block. Peterson reaction with methyl (trimethylsilyl)acetate in the presence of LDA in THF gave the 4'-ethylenedioxcyclohexylidene ester **8** (-78 °C, 2 h; 97%), which was subsequently reduced to the allylic alcohol **9** (DIBAL-H in toluene at -78 °C, 72%). The alcohol **9** was transformed to the desired ring-A phosphine oxide **2b** by *in situ* tosylation, conversion into the corresponding

phosphine, followed by oxidation with hydrogen peroxide (TsCl, *n*-BuLi, 0 °C then Ph<sub>2</sub>PH, *n*-BuLi, 0 °C, 30 min then H<sub>2</sub>O<sub>2</sub>, CHCl<sub>3</sub>, 0 °C, 1 h; 77% overall yield). Wittig-Horner coupling of the CD ring ketone **1** with the anion of **2b** gave pregnacalciferol analog **3b** (*n*-BuLi, THF, -78 °C, 1 h then 0 °C, 18 h; 28%). Reduction of **3b** gave quantitatively the alcohol **4b** (LiAlH<sub>4</sub>, THF, 0 °C, 30 min then 20 °C, 30 min) which underwent Swern oxidation to the aldehyde **5b** ((COCl)<sub>2</sub>, CH<sub>2</sub>Cl<sub>2</sub>, DMSO, -60 °C, 30 min then TEA; 68%). Van Rhee's<sup>9</sup> air oxygenation of **5b** resulted in the formation of 20-keto compound **6b** (60%). Careful removal of the ethylenedioxy group in the latter was done under controlled conditions with acetic acid-water (4:1) at 70 °C for 15 min, followed by immediate removal of the acetic acid by purging with nitrogen or azeotropic distillation with toluene. HPLC purification gave 2,20-dioxopregnacalciferol analog **6c** (70%).

As shown in the Table 1 3-desoxy-2,20-dioxo-19-nor-pregnacalciferol (**6c**) has almost the same binding activity to the PR, as the previously examined 20-oxopregnacalciferol, whereas the intermediate 22-alcohol **4b**, and the 22-aldehyde **5b** and the mono-protected 2,20-dione **6b** did also bind to the PR but to a lesser degree.

From the PR binding data we expected that the 20-oxopregnacalciferols might inhibit the growth of breast cancer cell lines. This was indeed the case. 20-Oxopregnacalciferol and the intermediates with binding to the PR significantly inhibited the growth of the breast cancer cell line T 47 D. These experiments are in progress.

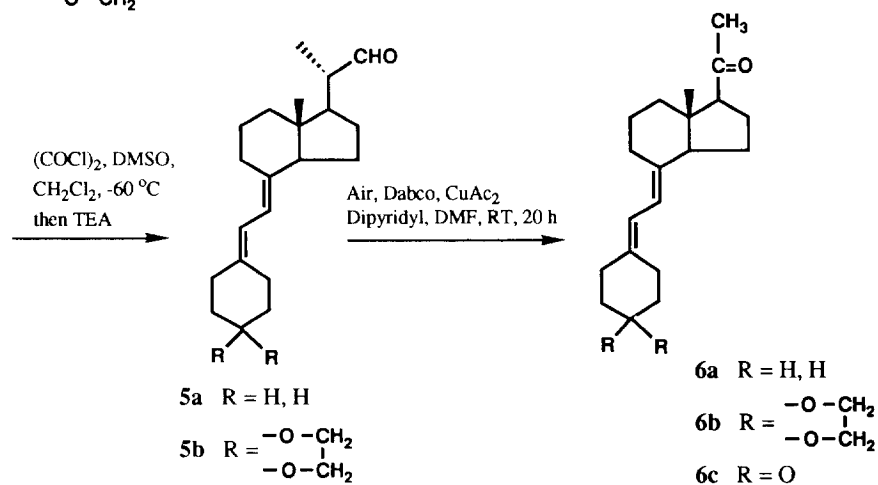
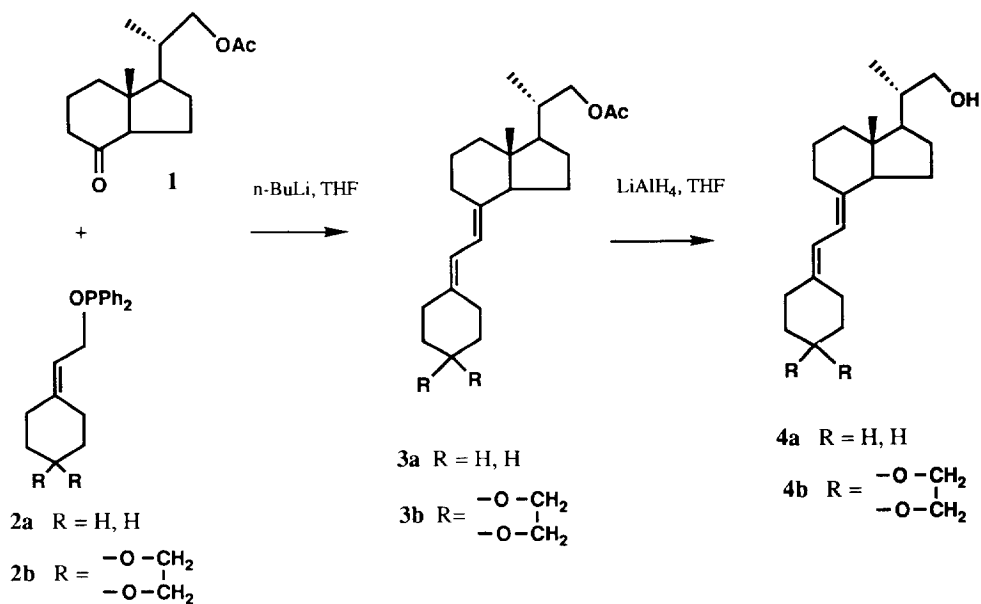
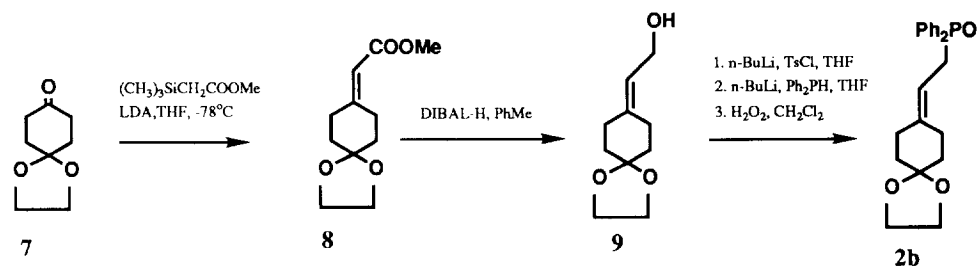
**Table 1. Competitive Binding of the 3-Desoxy-20-oxo-19-nor-pregnacalciferol, its 2-oxo Analog, and Intermediate Compounds to the Progesterone Receptor.**

Test Compound	Total Binding (DPM) ±S.D.	% Inhibition
<sup>3</sup> H-R5020	4758±145	---
+ R5020	750 ±231	84
+ 20-oxopregnacalciferol	1950±170	59
+ <b>4a</b>	4139± 62	13
+ <b>5a</b>	3045± 90	36
+ <b>6a</b>	3616±171	24
+ <b>4b</b>	2881±152	40
+ <b>5b</b>	2546±120	47
+ <b>6b</b>	2531±165	47
+ <b>6c</b>	2036±133	58

#### Experimental Notes:

The source of the progesterone receptor is the cytosolic fraction of MCF-7 cells (human breast cancer cell line) which were dosed with Estradiol 24 hours prior to harvesting and processing.

The cytosolic fractions used in all the binding measurements were freshly prepared; no frozen extracts were used. All competitive compounds were added at 400x-fold excess of the labelled R5020. The final ethanol concentration did not exceed 5% of the total volume of the incubation mixture.



**Analytical Data:** All  $^1\text{H}$  NMR spectra in  $\text{CDCl}_3$  at 400 or 500 MHz, all mass spectra (MS) at 70 eV (EI).

**2b:**  $^1\text{H}$  NMR  $\delta$  1.33 (2H, t,  $J = 6.4$  Hz), 1.56 (2H, t,  $J = 6.3$  Hz), 2.07 (2H, m), 2.20 (2H, m), 3.11 (2H, m), 3.91 (4H, s), 5.30 (1H, q,  $J = 7.0$  Hz), 7.46 (4H, m), 7.52 (2H, m), 7.73 (4H, m); MS  $m/z$  (rel. intensity) 368 ( $\text{M}^+$ , 80), 323 (40), 230 (51), 202 (100), 166 (89).

**3a:**  $^1\text{H}$  NMR  $\delta$  0.58 (3H, s, 18- $\text{CH}_3$ ), 1.03 (3H, d,  $J = 6.8$  Hz, 21- $\text{CH}_3$ ), 2.06 (3H, s,  $\text{OCH}_3$ ), 2.83 (1H, m, 9 $\beta$ -H), 3.80 (1H, dd,  $J = 10.7, 7.7$  Hz, one of 22-H), 4.09 (1H, dd,  $J = 10.7, 3.0$  Hz, one of 22-H), 5.85 (1H, d,  $J = 11.2$  Hz, 7-H), 6.06 (1H, d,  $J = 11.2$  Hz, 6-H); MS  $m/z$  (rel. intensity) 344 ( $\text{M}^+$ , 100), 284 (62), 243 (38), 148 (48), 91 (60), 43 (55); UV (in EtOH)  $\lambda_{\text{max}}$  243, 251.5, 261 nm.

**3b:**  $^1\text{H}$  NMR  $\delta$  0.583 (3H, s, 18- $\text{CH}_3$ ), 1.025 (3H, d,  $J = 7.0$  Hz, 21- $\text{CH}_3$ ), 2.06 (3H, s,  $\text{OCOCH}_3$ ), 2.82 (1H, m, 9 $\beta$ -H), 3.80 (1H, dd,  $J = 10.9, 7.7$  Hz, one of 22-H), 3.97 (4H, s, - $\text{O-CH}_2$ -), 4.09 (1H, dd,  $J = 10.9, 3.0$  Hz, one of 22-H), 5.82 (1H, d,  $J = 11.1$  Hz, 7-H), 6.12 (1H, d,  $J = 11.1$  Hz, 6-H); MS  $m/z$  (rel. intensity) 402 ( $\text{M}^+$ , 23), 342 (6), 206 (12), 175 (11), 133 (11), 99 (22); exact mass calcd for  $\text{C}_{25}\text{H}_{38}\text{O}_4$  402.2770, found 402.2776; UV (in EtOH)  $\lambda_{\text{max}}$  243, 251.5, 261 nm.

**4a:**  $^1\text{H}$  NMR  $\delta$  0.582 (3H, s, 18- $\text{CH}_3$ ), 1.062 (3H, d,  $J = 6.7$  Hz, 21- $\text{CH}_3$ ), 2.83 (1H, m, 9 $\beta$ -H), 3.39 (1H, m, one of 22-H), 3.65 (1H, m, one of 22-H), 5.85 (1H, d,  $J = 11.1$  Hz, 7-H), 6.06 (1H, d,  $J = 11.1$  Hz, 6-H); UV (in EtOH)  $\lambda_{\text{max}}$  243, 251.5, 261 nm.

**4b:**  $^1\text{H}$  NMR  $\delta$  0.584 (3H, s, 18- $\text{CH}_3$ ), 1.062 (3H, d,  $J = 6.6$  Hz, 21- $\text{CH}_3$ ), 2.82 (1H, m, 9 $\beta$ -H), 3.39 (1H, m, one of 22-H), 3.65 (1H, m, one of 22-H), 3.97 (4H, s, - $\text{O-CH}_2$ -), 5.82 (1H, d,  $J = 11.1$  Hz, 7-H), 6.12 (1H, d,  $J = 11.1$  Hz, 6-H); MS  $m/z$  (rel. intensity) 360 ( $\text{M}^+$ , 82), 301 (22), 267 (12), 206 (58); exact mass calcd for  $\text{C}_{23}\text{H}_{36}\text{O}_3$  360.2664, found 360.2663; UV (in EtOH)  $\lambda_{\text{max}}$  243, 251.5, 261 nm.

**5a:**  $^1\text{H}$  NMR  $\delta$  0.606 (3H, s, 18- $\text{CH}_3$ ), 1.138 (3H, d,  $J = 6.6$  Hz, 21- $\text{CH}_3$ ), 2.84 (1H, m, 9 $\beta$ -H), 5.86 (1H, d,  $J = 11.2$  Hz, 7-H), 6.06 (1H, d,  $J = 11.2$  Hz, 6-H), 9.59 (1H, d,  $J = 3.0$  Hz, 22-H); MS  $m/z$  (rel. intensity) 300 ( $\text{M}^+$ , 25), 285 (13), 149 (43), 129 (28), 105 (42), 91 (75), 55 (100); UV (in EtOH)  $\lambda_{\text{max}}$  243, 251.5, 261 nm.

**5b:**  $^1\text{H}$  NMR  $\delta$  0.609 (3H, s, 18- $\text{CH}_3$ ), 1.14 (3H, d,  $J = 6.7$  Hz, 21- $\text{CH}_3$ ), 2.84 (1H, m, 9 $\beta$ -H), 3.97 (4H, s, - $\text{O-CH}_2$ -), 5.83 (1H, d,  $J = 11.1$  Hz, 7-H), 6.12 (1H, d,  $J = 11.1$  Hz, 6-H), 9.59 (1H, d,  $J = 3.0$  Hz, 22-H); MS  $m/z$  (rel. intensity) 358 ( $\text{M}^+$ , 23), 330 (3), 206 (8), 119 (100), 100 (38); exact mass calcd for  $\text{C}_{23}\text{H}_{34}\text{O}_3$  358.2508, found 358.2508; UV (in EtOH)  $\lambda_{\text{max}}$  243, 251.5, 261 nm.

**6a:**  $^1\text{H}$  NMR  $\delta$  0.514 (3H, s, 18- $\text{CH}_3$ ), 2.13 (3H, s, 21- $\text{CH}_3$ ), 2.85 (1H, m, 9 $\beta$ -H), 5.87 (1H, d,  $J = 11.3$  Hz, 7-H), 6.04 (1H, d,  $J = 11.3$  Hz, 6-H); MS  $m/z$  (rel. intensity) 286 ( $\text{M}^+$ , 65), 243 (55), 147 (75), 77 (55), 55 (45), 43 (100); UV (in EtOH)  $\lambda_{\text{max}}$  243, 251.5, 261 nm.

**6b:**  $^1\text{H}$  NMR  $\delta$  0.518 (3H, s, 18- $\text{CH}_3$ ), 2.13 (3H, s, 21- $\text{CH}_3$ ), 2.85 (1H, m, 9 $\beta$ -H), 3.97 (4H, s, - $\text{O-CH}_2$ -), 5.84 (1H, d,  $J = 11.2$  Hz, 7-H), 6.11 (1H, d,  $J = 11.2$  Hz, 6-H); MS  $m/z$  (rel. intensity) 344 ( $\text{M}^+$ , 60), 301 (12), 239 (25), 206 (14), 147 (35), 105 (45), 91 (55), 55 (22); exact mass calcd for  $\text{C}_{22}\text{H}_{32}\text{O}_3$  344.2351, found 344.2366; UV (in EtOH)  $\lambda_{\text{max}}$  243, 251.5, 261 nm.

**6c:**  $^1\text{H}$  NMR  $\delta$  0.526 (3H, s, 18-CH<sub>3</sub>), 2.14 (3H, s, CO-CH<sub>3</sub>), 2.86 (1H, m, 9 $\beta$ -H), 5.83 (1H, d,  $J$  = 11.2 Hz, 7-H), 6.28 (1H, d,  $J$  = 11.2 Hz, 6-H); MS  $m/z$  (rel. intensity) 300 ( $M^+$ , 100), 257 (56), 239 (9), 135 (44); exact mass calcd for C<sub>22</sub>H<sub>28</sub>O<sub>2</sub> 300.2859, found 300.2074; UV (in EtOH)  $\lambda_{\text{max}}$  252.5, shoulders at 245 and 260 nm.

**8:**  $^1\text{H}$  NMR  $\delta$  1.771 (4H, m), 2.38 (2H, t,  $J$  = 6.4 Hz), 3.00 (2H, t,  $J$  = 6.6 Hz), 3.69 (3H, s), 3.98 (4H, s), 5.67 (1H, s); MS  $m/z$  (rel. intensity) 212 ( $M^+$ , 100), 197 (25), 180 (44), 153 (73).

**9:**  $^1\text{H}$  NMR  $\delta$  1.698 (4H, m), 2.28 (2H, t,  $J$  = 6.4 Hz), 2.34 (2H, t,  $J$  = 6.4 Hz), 3.97 (4H, s), 4.16 (2H, d,  $J$  = 6.8 Hz), 5.44 (1H, t,  $J$  = 6.8 Hz); MS  $m/z$  (rel. intensity) 184 ( $M^+$ , 14), 166 (7), 153 (11), 87 (100).

### References and Notes

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