Efficient synthesis of novel oxime analogues of the hormone 1α, 25-dihydroxyvitamin D_3

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Calcitriol analogues which contained an oxime in the side chain were synthesised in five steps from an intermediate 22-nor Ketone. The key intermediate enones were synthesised by the Wittig–Horner olefination and Wittig olefination respectively. Reduction, oximation, photoisomerisation and deprotection gave the target compounds with the oxime at C-24.

Keywords: calcitriol, oximation, Wittig–Horner olefination, Wittig olefination

The metabolite of vitamin D, 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$, calcitriol, **1**] (Fig. 1) is well known to regulate calcium, phosphorus homeostasis in vertebrate organisms[1] and plays a very important role on cellular differentiation, proliferation, and the functioning of the central nervous system.[2] Furthermore, there is evidence that calcitriol may generate biological responses through regulation of gene expression by signaling through the nuclear vitamin D receptor (VDR) transcription factor.[3,4] Although calcitriol can play a very important biological role, the side effects including hypercalciuria, hypercalcinaemia, nephrocalcinosis, nephrolithiasis and soft tissue calcification, limit its application.[5] Consequently, vitamin D analogues are being evaluated currently as potential drug candidates to overcome the side effects of calcitriol.[6] Moreover,[7,8]as the basic site of the active metabolite, the side chain of vitamin D is regarded as an important region for modification.[1] For example, analogues with catabolism-blocking side chain modifications including olefinic,[9] acetylenic[10] ketonic[11] and oxime[12] groups have been developed and their high antiproliferative activity and low calcium activity has been reported.

Side chain oxime analogue **2** and oxime O-methyl ether **3** have been prepared by using Horner-Wadsworth-Emmons (HWE) coupling of the A-ring with the C,D-ring and possess better biological activity than calcitriol (**1**).[12] Some 24-oxime derivatives have been used as antagonists of vitamin D. For example, the antagonistic bioactivity of **4** is very effective. This was prepared from the known 24-oxocholesterol acetate.[13] Recently, several steroidal natural products with an oxime group isolated from marine sponges have been shown to have unique biological activities,[14] such as anticancer activity,[15] antibacterial activity, [16]antihyperglycemic agents, [17] and potential for the treatment of rheumatoid arthritis.[18] However, there are few reports of their chemical synthesis.

Based on our prior work, [19-21] we now report an effective method for the synthesis of three types of novel oxime analogues **5–7**. We suggest that these compounds may have desirable pharmacological properties (*e.g.*, high metabolic stability) and biological activity (*e.g.*, lower hypercalcaemia).[12]

Result and discussion

Synthesis of **5** *by Wittig–Horner reaction and photo-isomerisation*

For the synthesis of **5,** we started from aldehyde **8** (Scheme 1), which is readily obtained in large quantities from vitamin D_2 using the procedures originally described by Calverley[22] modified by our group.[21] Wittig–Horner coupling of **8** with phosphonate **9**[23] was best carried out in THF at room temperature with NaH as base. Under these conditions, the enone **11a** was obtained in 88% yield. Reaction of **11a** with $Na_2S_2O_4$ and $NaHCO_3$ under phase transfer conditions gave ketone **12a** in 67% yield. The reaction was also carried out with Mg and MeOH as the reducing agent, but the yield was less than 30%. Subsequently, the ketone **12a** was transformed to give the oxime **13a** with the yield of 97% by direct reaction with hydroxylamine hydrochloride. In the final two steps, removal of the silyl protecting groups of **13a** with TBAF in THF afforded a 82% yield of triol **14a**, and photoisomerisation of **14a** using anthracene(An) as sensitiser finally gave the target oxime analogue **5** in 63% yield. At the beginning of our research work, the target oxime analogue **5** was obtained by photoisomerisation and subsequent deprotection. The photoisomerisation reaction was incomplete and it was hard to separate the isomers. The production could not be used directly in next step. Consequently we carried out the deprotection first, and then the photoisomerisation. After photoisomerisation, the products were purified directly by semi-preparative HPLC (10% H_2O/CH_3OH).

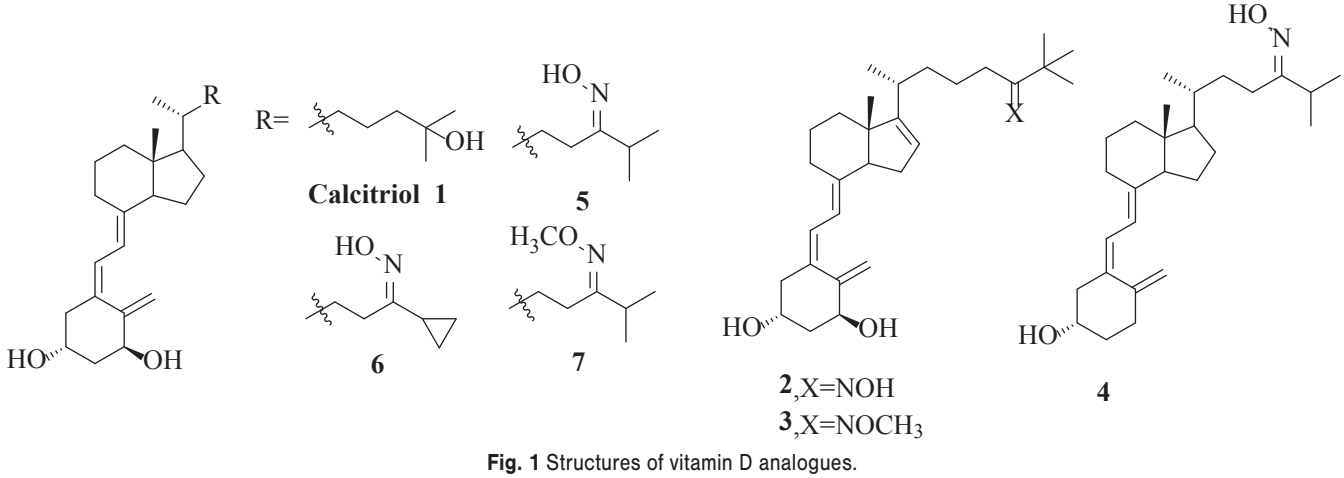

Fig. 1 Structures of vitamin D analogues.

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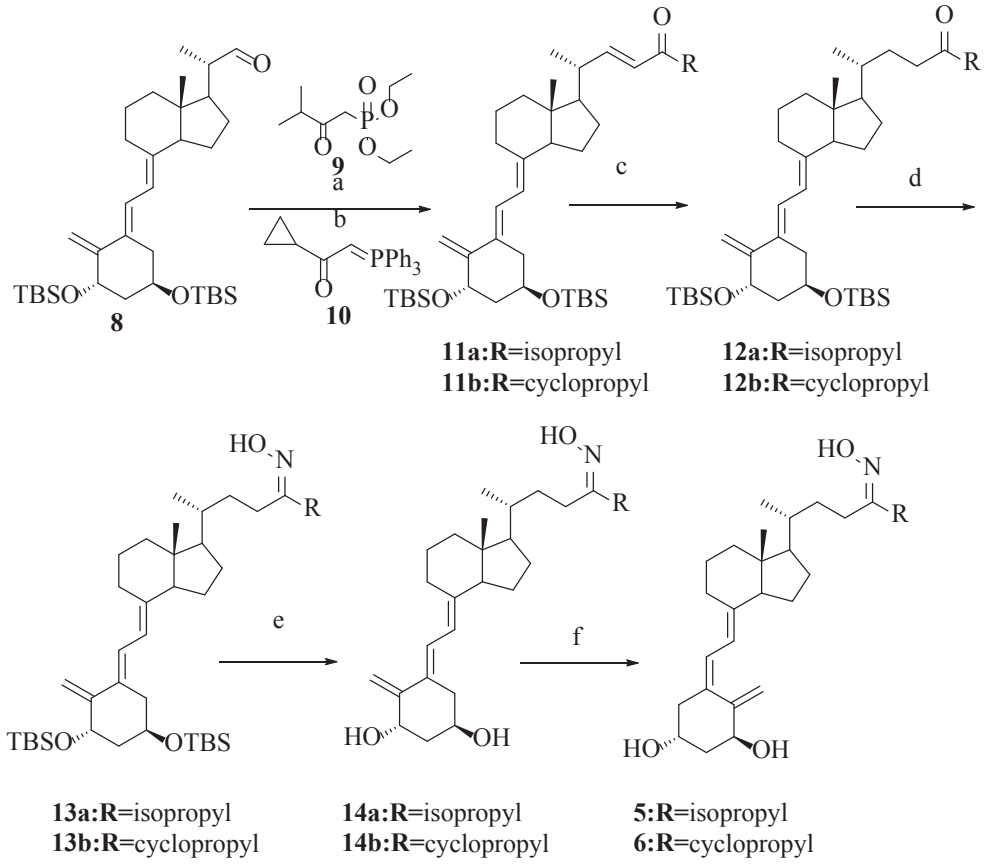
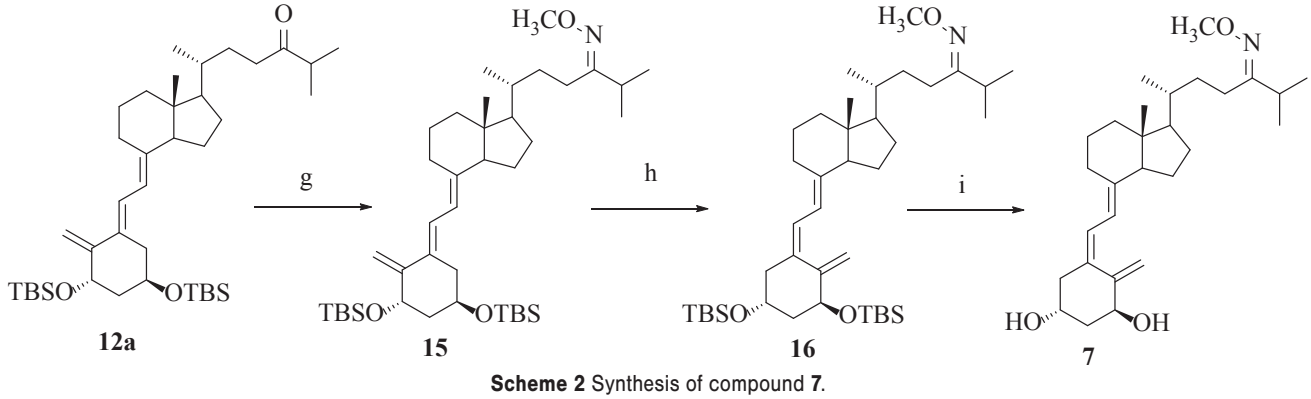


Scheme 1 Synthesis of compounds **5** and **6**.

(a):NaH, THF; (b):DMSO 105°C; (c): $Na_2S_2O_4$, $NaHCO_3$, $(C_{10}H_{21})_3NMeCl$, $PhCH_3$, H_2O, 80°C; (d):$NH_2OH{\cdot}HCl$ Et_3N; (e): n-Bu_4NF ,THF,60°C; (f):An, *hv*, CH_3OH, 20°C.



Scheme 2 Synthesis of compound **7**.

(g):$NH_2OCH_3{\cdot}HCl$, Et_3N; (h):An, *hv*, toluene, 20°C; (i): n-Bu_4NF ,THF,60°C

Synthesis of cyclopropyl oxime analogue **6**

The synthesis of the target compound **6** was similar to that of the oxime analogue **5** (Scheme 1). Treatment of aldehyde **8** with a molar excess of cyclopropylcarbonylmethylenetriphenylphosphorane(**10**, readily obtained from cyclopropyl methyl ketone) in dimethyl sulfoxide at 105°C for 4h led to the isolation of the pure enone **11b** in 54% yield.[23] Reaction of **11b** with $Na_2S_2O_4$, followed by direct reaction with hydroxylamine hydrochloride gave the oxime **13b**. Removal of the silyl protecting group with TBAF gave triol **14b,** which upon reaction with anthracene as sensitiser in CH_3OH at room temperature yielded cyclopropyl oxime analogue **6**. In the last step, we explored the different catalytic effect between anthracene or 9-acetylanthracene. We found that the yield of **6** was much higher when anthracene was used as sensitiser. The crude producti **6** was purified by semi-preparative HPLC (20% H_2O/CH_3OH).

Synthesis of oxime O-methyl ether **7**

As a starting compound for the synthesis of the oxime O-methyl ether **7** we chose the key intermediate **12a** (Scheme 2). The introduction of the oxime O-methyl ether **15** using hydroxylamine O-methyl ether in anhydrous ethanol proceeded without destroying the acid-sensitive conjugated triene unit of this seco-steroid. Then, using anthrancene as triplet-sensitiser in toluene **15** was converted to the (5Z)-vitamin derivative **16**. Removal of the TBS protective groups with TBAF in THF completed the synthesis of oxime O-methyl ether **7**. In contrast to the last two steps of the route to compounds **5** and **6**, the target oxime O-methyl ether **7** was obtained by photoisomerisation and subsequent deprotection. The reason was that the photoisomerisation reaction mixture was easily purified by using silica gel column chromatography easily, to give the (5Z)-vitamin derivatives **16**.

Conclusion

In conclusion, we have synthesised three kinds of novel oxime analogues of the hormone 1α,25-dihydroxyvitamin D_3, oxime analogue **5**, cyclepropyl oxime analogue **6** and oxime O-methyl ether **7** by oximation reaction without destroying the acid-sensitive conjugated triene unit of this seco-steroid. The total yield of three target compounds **5**, **6** and **7** were 29%, 19% and 27%,respectively. The successful synthesis of these products can provide an experimental foundation for modifying the side chain of vitamin D to develop bioactive molecules, and hence the discovery of new drugs. The study of the biological activity of compounds **5**, **6** and **7** has been carried out in our group.

Experimental

All the reactions were performed in oven-dried glassware under an atmosphere of ultra high purity argon. Anhydrous THF was distilled from sodium metal immediately prior to use. All chemicals were purchased from commercial suppliers and used without further purification unless otherwise noted. Column chromatography was performed using silica gel (300–400 mesh).^{1}H and ^{13}C NMR spectra were recorded at 500 MHz and 126 MHz with a Bruker Avance III 500 NMR spectrometer in $CDCl_3$. Chemical shifts (δ) are reported downfield from internal Me_4Si (δ 0.00). HRMS spectra analyses were performed on an Agilent 6540Q-TOF MS. HPLC was carried out using a LC-6AD equipped with one 6 mL/min preparative pump head using Shim-pack PERP-ODS(H) KIT 10 mm×250mm (semipreparative) columns packed with C-18-bonded silica and a SPD-20A UV-C variable-wavelength detector set at 254 nm. TLC plates were visualised by observation under UV lamp. Melting points were determined in capillaries and without correction.

The starting aldehyde **8** was obtained from vitamin D_2 as described previously. Aldehyde **8**[21]: m.p. 111–113°C,(lit.[22]113–115°C); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.59 (d, *J* = 3.2 Hz, 1H), 6.45 (d, *J* = 11.4 Hz, 1H), 5.84 (d, *J* = 11.4 Hz, 1H), 4.97 (d, 2H), 4.63–4.47 (m, 1H), 4.22 (s, 1H), 2.94–2.84 (m, 1H), 2.56 (dd, *J* = 14.1, 5.1 Hz, 1H), 1.12 (t, 4H), 0.59 (s, 3H), 0.10–0.04 (m, 12H). ppm;^{13}C NMR(75 MHz, $CDCl_3$): δ 204.9, 153.6, 142.2, 135.9, 121.5, 116.8, 106.6, 70.1, 67.1, 55.6, 51.3, 49.7, 46.2, 43.8, 40.2, 36.5, 28.8, 26.4, 25.8, 25.7, 23.3, 22.6, 18.2, 18.0, 13.5, 12.4,–4.8, –5.0 ppm; MS (ESI, *m/z*) $[M+H]^+$:573.56.

1(S),*3*(R)-*Bis(tert-butyldimethylsilyloxy)-20*(R)-*(3'-isopropyl-3'-oxypropyl-1'*(E)-*enyl)-9,10-secopregna-5*(E),*7*(E),*10(19)–triene*(**11a**): Sodium hydride (60%, 0.241 g, 6.02 mmol) was added to anhydrous THF (20 mL) under argon atmosphere, diethylphosphono-3-methyl-2-butanone (**9**, 1.782 g, 8.02 mmol) was added dropwise to the reaction mixture. The reaction mixture was stirred for 1 h at room temperature under Ar. Then aldehyde **8** (2.200 g, 3.839 mmol) in dry THF (10 mL) was added. The reaction mixture was heated to reflux for another 2h with an argon flow, cooled to room temperature, and treated with water (100 mL). The aqueous layer was extracted with ethyl acetate (3×50 mL), which was in turn washed with saturated brine (3×50 mL) and water (2×50 mL) and dried over sodium sulfate. After removal of solvent, the residue was purified by chromatography (SiO_2, PE: Et_2O = 100:3) to give **11a** [21] (2.186 g, 87.7%) as white solid. m.p. 105–106°C, (lit.[21] 105–106°C) ; ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.73 (dd, *J* = 15.7, 9.0 Hz, 1H), 6.46 (d, *J* = 11.4 Hz, 1H), 6.09 (d, *J* = 15.7 Hz, 1H), 5.83 (d, *J* = 11.4 Hz, 1H), 4.97 (d, 2H), 4.66–4.45 (m, 1H), 4.23 (s, 1H), 2.98–2.71 (m, 2H), 2.57 (dd, *J* = 14.1, 4.9 Hz, 1H), 2.29 (t, 2H), 2.10–1.84 (m, 3H), 1.82–1.64 (m, 4H), 1.11 (m, 10H), 0.86 (m, 20H), 0.59 (s, 3H), 0.07 (s, 12H) ppm;^{13}C NMR (126 MHz, $CDCl_3$): δ 204.6, 153.7, 152.4, 142.8, 135.8, 126.4, 121.7, 116.8, 106.8, 70.3, 67.3, 56.3, 55.5, 46.2, 44.0, 40.5, 40.4, 38.4, 36.7, 29.0, 27.6, 26.0, 25.9, 23.6, 22.4, 19.6, 18.7, 18.6, 18.4, 18.2, 12.4, –4.7, –4.8 ppm.

1(S),*3*(R)-*Bis(tert-butyldimethylsilyloxy)-20*(R)-*(3'-isopropyl-3'-oxypropyl)-9,10-secopregna-5*(E),*7*(E), *10(19)–triene*(**12a**): Enone **11a**(0.304 g, 0.474 mmol)was dissolved in toluene (15 mL) and distilled water (15 mL) and sodium bicarbonate (0.790 g, 9.405 mmol), sodium dithionite (1.630 g, 9.405 mmol) and methyltridecylammonium chloride (0.150 g,0.310 mmol) were added. The reaction mixture was stirred vigorously for 3h at reflux under Ar. After being cooled to room temperature, the reaction mixture was extracted with ethyl acetate (3×20 mL). The organic layer was washed with water (2×30 mL), brine (2×30 mL) and dried over sodium sulfate. The solvent was removed by rotary evaporation. The resulting white solid was chromatographed on silica gel, eluting with a gradient of Et_2O in PE (PE: Et_2O = 100:3), to give the desired product **12a** [21] (0.204 g, 66.9%) as white solid. m.p. 76–78°C (lit.[21] 76–78°C); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.46 (d, *J* = 11.4 Hz, 1H), 5.82 (d, *J* = 11.5 Hz, 1H), 4.96 (d, 2H), 4.54 (dd, *J* = 9.2, 4.2 Hz, 1H), 4.22 (d, *J* = 2.4 Hz, 1H), 2.87 (d, *J* = 13.1 Hz, 1H), 1.09 (m, 7H), 0.54 (s, 3H), 0.08–0.04 (m, 12H). ppm; ^{13}C NMR (126 MHz, $CDCl_3$): δ 215.5, 153.8, 143.4, 135.6, 121.8, 116.6, 106.7, 70.3, 67.3, 56.5, 46.0, 44.0, 41.0, 40.7, 37.4, 36.6, 35.8, 29.9, 29.1, 27.7, 26.0, 25.9, 23.6, 22.4, 18.7, 18.5, 18.4, 18.2, 12.1, –4.7, –4.8 ppm.

1(S),*3*(R)-*Bis(tert-butyldimethylsilyloxy)-20*(R)-*(3'-isopropyl-3'-hydr-oxyiminopropyl)-9,10-secopregna-5*(E),*7*(E), *10(19)–triene N-oxide* (**13a**): A 50 mL round-bottom flask was charged with **12a** (0.500 g, 0.777 mmol) dissolved in 25 mL of anhydrous alcohol. Hydroxylamine hydrochloride (1.100 g, 15.540 mmol, 20 equiv.) and triethylamine (5 mL) were added, and the reaction was brought to reflux for 3h at which point TLC analysis showed complete consumption of starting material and the appearance of a new, more polar product **13a**(0.496 g, 97%). This material was purified directly using silica gel column chromatography (10% ethyl acetate/PE) to give a white solid. m.p.109–112°C;^{1}H NMR (500 MHz, $CDCl_3$): δ 6.46 (d, *J* = 9.8 Hz, 1H), 5.84 (d, *J* = 11.4 Hz, 1H), 4.99 (s, 1H), 4.94 (s, 1H),4.52 (m, 1H), 4.31–3.93 (m, 1H), 3.04 -2.74 (m, 1H), 0.55 (s, 3H), 0.17–0.03 (m, 11H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.02, 153.58, 143.23, 135.30, 121.68, 116.38, 106.53, 77.21, 76.96, 76.70, 70.16, 67.18, 56.37, 56.26, 55.97, 45.87, 43.88, 40.47, 36.70, 36.49, 33.58, 31.70, 28.89, 27.57, 25.81, 25.75, 23.48, 23.35, 22.21, 20.06, 19.97, 18.52, 18.19, 18.02, 11.96, –4.82, –4.96 ppm; HRMS (ESI Positive) calcd for $C_{39}H_{71}NO_3Si_2Na$, $[M+Na]^+$680.4870, found 680.4870.

20(R)-*(3'-isopropyl-3'-hydroxyiminopropyl)-1*(S),*3*(R)-*dihydroxy-9,10-secopregna-5*(E),*7*(E),*10(19)- triene N-oxide* (**14a**)*:* A solution of **13a** (0.080 g,0.122 mmol) was dissolved in tetrahydrofuran (25 mL) and then 1M tetrabutylammonium fluoride solution in tetrahydrofuran (1.5 mL) was added. The reaction mixture was heated to 60°C for 1.5h. After being cooled to room temperature, the reaction mixture was poured into water (100 mL). The aqueous layer was extracted with ethyl acetate (3×50 mL). The organic layer was washed with water (2×50 mL), brine (2×50 mL) and dried over sodium sulfate. The residue was purified by chromatography (silica gel, PE:EA=1:1)to give **14a** (0.043 g,82.2%)as a colourless solid. m.p.77-80°C;^{1}H NMR (500 MHz, $CDCl_3$): δ 6.55 (d, *J* = 11.3 Hz, 1H), 5.87 (d, *J* = 11.4 Hz, 1H), 5.24-4.98 (m, 1H), 4.95 (s, 1H), 4.58-4.39 (m, 1H), 4.32-4.02 (m, 1H), 3.05-2.62 (m, 2H), 0.71-0.37 (m, 3H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.28, 145.05, 132.83, 123.14, 115.91, 109.65, 77.22, 76.97, 76.72, 71.01, 65.70, 56.40, 55.92, 45.88, 41.82, 40.34, 36.60, 33.58, 31.70, 28.99, 27.49, 23.47, 23.17, 22.23, 20.08, 20.00, 18.51, 12.06 ppm; HRMS (ESI Positive) calcd for $C_{27}H_{43}NO_3Na$, $[M+Na]^+$ 452.3141, found 452.3140.

20(R)-*(3'-isopropyl-3'-hydroxyiminopropyl)-1*(S),*3*(R)-*dihydroxy-9,10-secopregna-5*(Z),*7*(E),*10(19)- triene N-oxide* (**5**): Oxime **14a** (0.024 g, 0.100 mmol), anthracene (0.020 g) and triethylamine (2 mL) in methanol (35 mL) was stirred at room temperature under Ar. The reaction mixture was then irradiated using a 500W high pressure UV lamp through a brown uranium quartz filter for 10h. The solution was concentrated under reduced pressure. The residue mixture was purified directly using reverse phase HPLC chromatography (10% H_2O/methanol) to give 0.015 g of **5** as colourless foam (62.5%).^{1}H NMR (500 MHz, $CDCl_3$): δ 6.40 (d, *J* = 11.2 Hz, 1H),6.04 (d, *J* =11.2Hz, 1H), 5.42 (s, 1H), 5.01 (s, 1H), 4.54-4.36 (m, 1H), 4.36–4.13 (m, 1H), 2.93–2.71 (m, 1H), 0.65 (s, 3H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 166.68, 147.64, 143.04, 132.85, 124.91, 117.00, 111.64, 77.18, 76.92, 76.67, 70.75, 66.80, 56.27, 55.98, 45.87, 45.22, 42.83, 40.40, 36.61, 33.54, 31.81, 29.61, 29.00, 27.46, 23.51, 23.16,

22.21, 20.06, 19.97, 18.97, 18.52, 11.92, 0.92, –0.10 ppm; HRMS (ESI Positive) calcd for $C_{27}H_{43}NO_3Na$, $[M+Na]^+$ 452.3141, found 452.3141.

1(S),*3*(R)-*Bis(tert-butyldimethylsilyloxy)-20*(R)-*(3'-isopropyl-3'-methyliminopropyl)-9,10-secopregna-5*(E),*7*(E),*10(19)–triene N-oxide* (**15**): Ketone **12a** (0.300 g, 0.466 mmol) was slowly added with stirring to a solution of methoxylamine hydrochloride (1.558 g, 18.64 mmol) and triethylamine(5 mL) in anhydrous alcohol (50 mL) at r.t., The mixture was stirred at 80°C for 8h. Ethyl acetate and water were added, and the organic phase was washed with brine, dried with Na_2SO_4, and evaporated. The residue was dissolved in CH_2Cl_2, applied on a silica column, and washed with PE/diethyl ether (200:1) to produce **15**(0.297 g, 94.6%) as a white solid. m.p.85–87°C;^{1}H NMR (500 MHz, $CDCl_3$): δ 6.48 (d, *J* = 11.4 Hz, 1H), 5.85 (d, *J* = 11.4 Hz, 1H), 4.98 (d, 2H), 4.56 (m, 1H), 4.24 (s, 1H), 3.81 (s, 4H), 2.85 (m, 1H), 0.57 (s, 4H), 0.18–0.03 (m, 16H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.73, 153.57, 143.19, 135.30, 121.66, 116.38, 106.55, 77.21, 76.96, 76.71, 70.18, 67.17, 60.94, 60.85, 56.37, 55.93, 45.85, 43.89, 40.45, 36.68, 36.51, 36.26, 33.63, 32.15, 28.88, 27.61, 27.50, 27.38, 27.18, 25.80, 25.74, 23.47, 23.40, 22.20, 20.19, 20.10, 19.18, 18.62, 18.53, 18.18, 18.01, 11.94, –4.83, –4.97 ppm; HRMS (ESI Positive) calcd for $C_{40}H_{73}NO_3Si_2$, $[M+H]^+$ 672.5207, found 672.5204.

1(S),*3*(R)-*Bis(tert-butyldimethylsilyloxy)-20*(R)-*(3'-isopropyl-3'-methyliminopropyl)-9,10-secopregna-5*(Z), *7*(E), *10(19)–triene N-oxide* (**16**): A solution of oxime ether **15** (0.160 g, 2.38 mmol), anthracene (0.200 g) and triethylamine (5 mL) in toluene was irradiated for 8h with a 500W high pressure mercury arc lamp in an analogous manner as was described above for **5**. The solution was concentrated under vacuum, and the residue was applied to a silica column, and washed with PE/diethyl ether (200:1) to produce **16** (0.124 g,77.5%) as colourless foam. ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.24 (d, *J* = 11.2 Hz, 1H), 6.02 (d, *J* = 11.2 Hz, 1H), 5.18 (s, 1H), 4.87 (d, *J* = 1.5 Hz, 1H), 4.34 (m, 1H), 4.30–4.06 (m, 1H), 3.79 (d, *J* = 1.9 Hz, 4H), 2.80 (m, 1H), 0.54 (s, 4H), 0.26–0.05 (m, 17H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.77, 148.27, 140.86, 134.94, 123.09, 117.87, 111.11, 77.20, 76.95, 76.69, 71.99, 67.47, 60.95, 60.85, 56.27, 55.93, 45.98, 45.71, 44.77, 40.52, 36.71, 36.28, 33.63, 33.19, 32.17, 28.80, 27.63, 27.53, 27.43, 27.19, 25.79, 23.43, 22.11, 20.19, 20.10, 19.18, 18.64, 18.54, 18.17, 18.07, 11.90, -4.74, -4.84, -5.14 ppm; HRMS (ESI Positive) calcd for $C_{40}H_{73}NO_3Si_2$, $[M+H]^+$ 672.5207, found 672.5195.

20(R)-*(3'-isopropyl-3'-methyliminopropyl)-1*(S),*3*(R)-*dihydroxy-9,10-secopregna-5*(Z),*7*(E),*10(19)- triene N-oxide* (**7**)*:* The protected analogue **16**(0.100 g, 0.15 mmol) was dissolved in THF (30 mL), and TBAF (1M in THF, 2 mL) was added. The reaction mixture was refluxed to 60°C for 2h. The reaction was quenched with H_2O and extracted with ethyl acetate (3×50 mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated in vacuum to give the crude product which was purified by column chromatography (50% ethyl acetate in PE) to afford **7**(0.042 g,63.5%) as colourless foam. ^{1}H NMR (500 MHz, $CDCl_3$): δ6.36 (d, *J* = 11.2 Hz, 1H), 6.01 (d, *J* = 11.1 Hz, 1H), 5.31 (s, 1H), 4.98 (s, 1H), 4.38 (s, 1H), 4.21 (d, *J* = 3.0 Hz, 1H), 3.78 (d, *J* = 2.7 Hz, 4H), 2.81 (d, *J* = 14.2 Hz, 1H), 0.54 (s, 3H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.92, 147.57, 142.96, 132.96, 124.82, 117.02, 111.70, 77.23, 76.97, 76.72, 70.69, 66.72, 60.96, 60.85, 56.27, 55.89, 45.84, 45.16, 42.78, 40.38, 36.64, 33.60, 32.13, 28.99, 27.42, 27.21, 23.51, 23.34, 22.22, 20.19, 20.09, 19.17, 18.51, 11.92 ppm; HRMS (ESI Positive) calcd for $C_{28}H_{45}NO_3Na$, $[M+Na]^+$ 466.3297, found 466.3293.

1(S),*3*(R)-*Bis(tert-butyldimethylsilyloxy)-20*(R)-*(3'-cyclopropyl-3'-oxypropyl-1'*(E)-*enyl)-9,10-secopregna-5*(E),*7*(E),*10(19)–triene*(**11b**): Obtained by Wittig coupling of aldehyde **8**(8 g, 0.014mol) with phosphorane **10** (12 g, 0.035 mol) in dimethyl sulfoxide (50 mL). The reaction mixture was refluxed to 105°C for 4h.The enone was purified on a silica column, using a PE/diethyl ether (50:1) solvent system, to give enone **11b** (4.8 g) [22] in 53.7% yield as a white solid. m.p. 123–124°C, (lit.[22] 123–124°C); ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.79 (dd, *J* = 15.7, 8.9 Hz, 1H), 6.48 (d, *J* = 11.4 Hz, 1H), 6.19 (d, *J* = 15.7 Hz, 1H), 5.85 (d, *J* = 11.4 Hz, 1H), 5.01 (s, 1H), 4.97 (s, 1H),4.56 (dd, *J* = 9.1, 4.0 Hz, 1H), 4.24 (s, 1H), 3.00–2.73 (m, 1H), 2.58 (dd, *J* = 14.1, 5.1 Hz, 1H), 2.38–2.26 (m, 2H), 0.61 (s, 3H), 0.15–0.03 (m, 13H) ppm.

1(S),*3*(R)-*Bis(tert-butyldimethylsilyloxy)-20*(R)-*(3'-cyclopropyl-3'-oxypropyl)-9,10-secopregna-5*(E),*7*(E),*10(19)–triene* (**12b**)*:* Starting with **11b**(1.200 g, 1.88 mmol), a hydrogenation procedure similar to that used to make **12a** was used to obtain **12b**[22] (0.824 g, 68.4%) as a white solid. m.p. 93–94°C, (lit.[22] 93–94°C); ^{1}H NMR (500 MHz, $CDCl_3$): δ6.46 (d, *J* = 11.4 Hz, 1H), 5.83 (d, *J* = 11.4 Hz, 1H), 4.98 (s, 1H), 4.94 (s, 1H),4.59–4.38 (m, 1H), 4.16 (s, 1H), 2.83 (m, 1H), 0.65–0.38 (m, 3H), 0.09–0.04 (m, 9H) ppm.

1(S),*3*(R)-*Bis(tert-butyldimethylsilyloxy)-20*(R)-*(3'-cyclopropyl-3'-hydroxyiminopropyl)-9, 10-secopregna-5*(E),*7*(E),*10(19)–triene N- oxide* (**13b**): Using the procedure for the synthesis of **13a** from **12a**, **13b** (0.051 g, 0.078 mmol) was obtained from **12b** (0.050 g, 0.078 mmol) in 100% yield as a white solid. m.p. 118–120°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ6.46 (d, *J* = 11.3 Hz, 1H), 5.83 (d, *J* = 11.3 Hz, 1H), 4.98 (s, 1H), 4.94 (s, 1H),4.54 (d, *J* = 5.1 Hz, 1H), 4.22 (s, 1H), 2.85 (m, 1H), 2.59–2.47 (m, 1H), 0.79–0.61 (m, 5H), 0.54 (d, *J* = 8.1 Hz, 3H), 0.16–0.01 (m, 14H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.00, 161.92, 153.59, 143.22, 135.33, 132.28, 121.66, 116.39, 114.20, 106.54, 99.94, 77.20, 76.94, 76.69, 75.17, 70.17, 67.17, 60.01, 56.36, 56.17, 56.02, 45.87, 43.88, 40.46, 36.49, 36.44, 36.12, 33.08, 31.83, 28.88, 27.56, 25.80, 25.74, 23.72, 23.47, 22.19, 18.53, 18.19, 18.02, 14.06, 11.96, 8.28, 5.09, 5.03, –4.83, –4.97 ppm; HRMS (ESI Positive) calcd for $C_{39}H_{69}NO_3Si_2Na$, $[M+Na]^+$ 678.4714, found 678.4713.

20(R)-*(3'-cyclopropyl-3'-hydroxyiminopropyl)-1*(S),*3*(R)-*dihydroxy-9,10-secopregna-5*(E),*7*(E),*10(19)- triene N-oxide* (**14b**): Using the procedure for the synthesis of **14a** from **13a**, compound **14b** (0.047 g, 0.110 mmol) was obtained from **13b**(0.050 g, 0.156 mmol) in 70.5% yield as a white solid. m.p. 97–110°C; ^{1}H NMR (500 MHz, $CDCl_3$): δ6.54 (d, *J* = 11.0 Hz, 1H), 5.87 (d, *J* = 11.1 Hz, 1H), 5.09 (s, 1H), 4.95 (s, 1H), 4.47 (s, 1H), 4.15 (m, 1H), 2.79 (m, 3H), 0.77–0.61 (m, 4H), 0.59–0.42 (m, 4H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.96, 151.60, 144.92, 132.93, 123.08, 115.96, 109.64, 77.23, 76.98, 76.73, 70.98, 65.66, 56.40, 56.15, 55.98, 45.88, 41.84, 40.35, 36.61, 36.35, 36.07, 31.79, 29.62, 28.99, 27.49, 23.47, 22.23, 18.54, 14.05, 12.07, 5.01 ppm; HRMS (ESI Positive) calcd for $C_{27}H_{41}NO_3Na$, $[M+Na]^+$ 450.2984, found 450.2977.

20(R)-*(3'-cyclopropyl-3'-hydroxyiminopropyl)-1*(S),*3*(R)-*dihydroxy-9,10-secopregna-5*(Z),*7*(E),*10(19)- triene N-oxide*(**6**): Prepared from oxime **14b** by following the same procedure described for **7**. Compound **6** (0.010 g, 0.024 mmol) from **14b** (0.047 g, 0.011 mmol) as colourless foam in 72.3% yield. ^{1}H NMR (500 MHz, $CDCl_3$): δ6.38 (d, *J* = 11.3 Hz, 1H), 5.98 (d, *J* = 11.3 Hz, 1H), 5.33 (s, 1H), 5.00 (s, 1H), 4.44 (s, 1H), 4.23 (s, 1H), 2.94–2.73 (m, 1H), 2.60 (d, *J* = 10.3 Hz, 1H), 1.08–0.94 (m, 3H), 0.55 (s, 3H) ppm; ^{13}C NMR (126 MHz, $CDCl_3$) δ 163.41, 147.62, 143.04, 132.84, 124.93, 117.00, 111.67, 77.18, 76.93, 76.67, 70.77, 66.81, 56.27, 56.02, 45.87, 45.22, 42.83, 40.40, 36.34, 31.89, 29.62, 29.01, 27.46, 23.67, 23.51, 22.21, 18.53, 13.93, 11.94, 5.11, 5.03, -0.09 ppm; HRMS (ESI Positive) calcd for $C_{27}H_{41}NO_3Na$, $[M+Na]^+$ 450.2984, found 450.2961.

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Electronic Supplementary Information

^{1}H NMR, ^{13}C NMR, HRMS data can be found in the ESI available through stl.publisher.ingentaconnect.com/content/stl/jcr/supp-data.

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References

1 F. Baeke, T. Takiishi, K. Hannelie, G. Conny and M.Chantal, *Curr. Opin. Pharmacol.,* 2010, **10**, 482.
2 K. Z.Guyton, T. W. Kensler and G. H. Posner, *Annu. Rev. Pharmacol. Toxicol.,* 2001, **41**, 421.

3 E. T. Luz, D. Q. Tan, D. Basel, H. Jonathan, X.H. Liao, P. Ana, L. G. James and H. W. John, *PNAS,* 2008, **105**, 8250.
4 J. W. Pike, *Annu. Rev. Nutr.,* 1991, **11**,189.
5 M. Sonako and J. Glenville, *Mol Cancer Ther.* 2006, **5**, 797.
6 K.W. Colston, G. Pirianov, E. Bramm, K.J. Hamberg and L. Binderup, *Breast Cancer Res Treat.,* 2003, **80**, 303.
7 T. Hidetoshi, I. Masaki, K. Motoshi, I. Y. Akemi, H.Yoshio and I. Hajime, *J. Dermatol. Sci.,* 2003, **31**, 21.
8 N. Yusuke, K. Yuko, I. Keisuke, O. Eiji, N. Jun-ichi, I. Seiichi, T. Kazuya, T. Aya, H. Yuichi and N. Kazuo, *J. Med. Chem.,* 2006, **49**, 2398.
9 P. Anita, M. Maura and C. Michal, *Steroids*, 2013, **78**, 1003.
10 M. Hubert , J. L. Hong, P. Bradford, S. Nanjoo and R. U. Milan, *J. Med. Chem.,* 2009, **52**, 5505.
11 L. Gilles, P. Giuseppe, U. Milan, M. Stanislaw, M. Hubert, J. Pawel, C. Caroline, V. Paul, S. Brenden, R. Matthew, R. G. Satyanarayana and A. Luciano, *J. Med. Chem.,* 2009, **52**, 2204.
12 H. P. Gary, A. H. Bethany,P. Sara, D.Patrick and W. K. Thomas, *J. Med. Chem.,* 2002, **45**, 1723.
13 K. Bruce, K. S. Heinrich and F. D. Hector, *Arch. Biochem. Biophys.,*1983**, 221**,38.
14 X.J. Zhang and J. G. Cui *Chin. J. Org. Chem.,* 2010, **30**, 655 (in Chinese).
15 S.-J. Choi, J.-E. Lee, S.-Y. Jeong, I. Isak, S.-D. Lee, E.-J. Lee and S. K. Lee, *J. Med. Chem.,* 2010, **53**, 3696.
16 Y. H. Chang, K. K. Young, H. L Yong and H. K. Jin, *Bioorg. Med. Chem. Lett.,* 1998, **8**, 221.
17 Y. Hiroaki, T. Makoto, Y. Eriko, F. Sachie, F. Toshihiko, Y. Makoto, I. Aya and H. Yuka, *Bioorg. Med. Chem. Lett.,* 2000, **10**, 373.
18 T. B. Jeffrey, S. D. Michael, N. Amr, C. Marianne, C. T. Theodore, T. James, A. Saadat and D. Li, *J. Med. Chem.,* 2010, **53**, 8650.
19 W. Guo, Z.-J. Fang, T.-C. Huang and L.-J. Han, *Chin. J. Magn. Reson.,* 2013, **30**, 417 (in Chinese).
20 W. Guo, Z.-J. Fang, T.-C. Huang and L.-J. Han, *Chem. Reag.*, 2013, **35**, 851 (in Chinese).
21 W. Guo, Z.-J. Fang, H.-L. Li and Y.-N. Liu, *J.Chem.Res.,* 2014, **38**, 231.
22 M.J. Calverley, *Tetrahedron*, 1987, **43**, 4609.
23 S.R.Jones and B.S. Selinsky, *J. Org. Chem*., 1998, **63**, 3786.