A Direct Stereoselective Preparation of a Fish Pheromone and Application of the Zinc Porphyrin Tweezer Chiroptical Protocol in Its Stereochemical Assignment

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ABSTRACT A two-step stereoselective preparation of a goldfish pheromone, 17α,20β-dihydroxy-4-pregnen-3-one, is reported from the readily available cortexolone in 64% overall yield. The (20*S*)-epimer was also synthesized in three steps from cortexolone with an overall yield of 47%. A microscale chiroptical technique based on a host/guest complexation mechanism between the substrate and a dimeric metalloporphyrin host (tweezer) was used to confirm the stereochemical assignment, while Density Functional Theory (DFT) calculations were employed to explain the high stereoselectivity induced by the 17α-hydroxyl and C18-methyl groups. *Chirality 25:575–581, 2013.*

KEY WORDS: goldfish pheromone; stereoselective reduction; cortexolone; circular dichroism; DFT; computational modeling; zinc porphyrin tweezer

INTRODUCTION

We herein report a direct enantioselective preparation of 17α,20β-dihydroxy-4-pregnen-3-one, (20*R*)-**1**, a goldfish (*Carassius auratus*) pheromone, from cortexolone (Scheme 1). The pheromone is at least a thousand times more expensive than the readily available corticosteroid, cortexolone. The female goldfish releases the pheromone causing an increased sperm production in males.[1–4]

An extensive body of research[5–9] conducted on the pheromone's biological activity emphasizes its importance and its potential to be a key element for the aquaculture of other organisms.[10–15] Outstanding work by Sorensen and coworkers on sea lampreys (*Petromyzon marinus*) strongly suggests that other fish pheromones have a related steroidal skeleton.[7,8,16,17] Previous syntheses include the classical[18] and enzymatic reduction of 17-hydroprogesterone precursor.[19] The synthetic approach employed in this article owes its strength to both its stereoselective nature and the ease of its extension to a wide variety of other potentially biologically active pheromones.

The initial synthetic approach was based on a one-pot hydride reduction of the readily generated chlorocortexolone **2** (Scheme 2). Reduction of **2** generates chloroalcohol **3**, which, under reductive conditions in the presence of the alkoxide, may close up to form epoxide **4**. The epoxide, in the presence of an additional equivalent of hydride may open up from the less hindered side to afford target pheromone **1** after workup. Epoxide **4** itself can be used as a key intermediate in the generation of multiple variants of the pheromone in an attempt to target other aquatic species. For instance, nucleophilic opening of the epoxide using alkyl aluminates may afford the general target structure **5**.

EXPERIMENTAL

General

Anhydrous solvents were dried and distilled (THF from potassium, CH_2Cl_2 from CaH_2). Solvents used were either high-performance liquid chromatography (HPLC) grade or reagent grade. Column and flash chromatography were performed on silica gel 60 F254. ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on 400 or 360 MHz spectrometers. $CDCl_3$ and DMSO-d_6 were used as NMR solvents and their signals were used as internal standards. Chemical shifts were expressed in ppm, followed by multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; quin, quintet; m, multiplet; br, broad), and the number of corresponding protons. High-resolution mass spectrometry (HRMS) of positive ions was obtained on an AccuTOF mass spectrometer with a DART ion source. The circular dichroism (CD) spectra were measured in millidegrees and normalized into ε_{max} [$Lmol^{-1}\,cm^{-1}$]/λ [nm] units.

21-Chloro-17α-hydroxy-4-pregnen-3,20-dione (2). Cortexolone (500 mg, 1.44 mmol) and mesyl chloride (330 mg, 2.88 mmol) were dissolved in CH_2Cl_2 (20 ml), and a catalytic amount (2 mg) of DMAP was slowly added and the mixture was refluxed for 4 h. The crude was diluted with 10 ml of CH_2Cl_2 and washed with brine (10 ml) three times. The organic layer was then dried with Na_2SO_4 and the solvent was evaporated. Purification by column chromatography and recrystallization in EtOAc afforded **2** (443 mg, 85%), mp = 227-230 °C; IR ν/cm^{-1} = 3495 (br, 17-OH), 1724 (C = O, at C20), 1637 (C = C-C = O); ^1H NMR (360 MHz, DMSO-d_6) δ = 5.63 (s, 1H, *H*-C = C), 5.54 (s, 1H, 17-O*H*), 4.77 (d, *J* = 16.8 Hz, 1H of CH_2Cl), 4.47 (d, *J* = 16.9 Hz, 1H of CH_2Cl), 2.55 (t, *J* = 13.2 Hz, 1H, 16β-*H*), 2.40 (dt, *J* =14.9, 3.4 Hz, 2H: overlap of 6β-H_{ax} and 2β-H_{ax}), 2.24 (m, 1H, 6α-H_{eq}), 2.15 (m, 1H, 2α-H_{eq}), 1.96 (dq, *J* = 13.3, 2.3 Hz, 1H, 1β-H_{eq}), 1.79 (m, 1H, 7β-H_{eq}), 1.66-1.75 (m, 2H, overlap of 12β-H_{eq} and 14-*H*), 1.51-1.66 (m, 4H, overlap of 15α-*H*, 1α-H_{ax}, 8-*H*, and 11α-H_{eq}), 1.30-1.50 (m, 3H, overlap of 16α-*H*, 11β-H_{ax}, and 12α-H_{ax}), 1.23 (m, 1H, 15β-*H*), 1.14 (s, 3H, 19-CH_3), 1.01 (dq, *J* = 11.8, 2.9 Hz, 1H, 7α-H_{ax}), 0.91

Additional Supporting Information may be found in the online version of this article.

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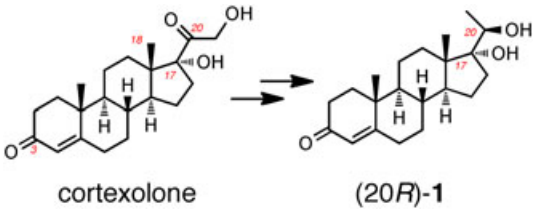
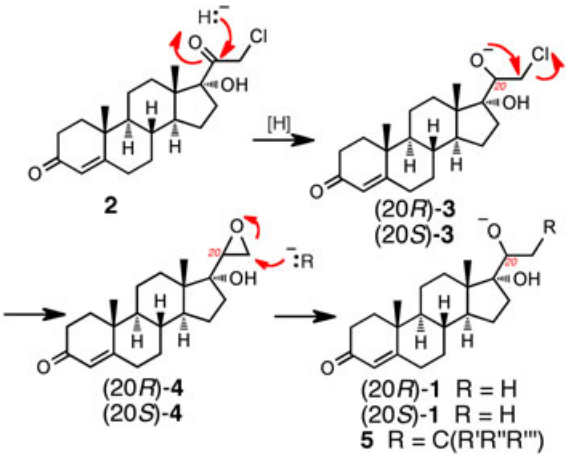


Scheme 1. Fish pheromone in two steps from cortexolone.



Scheme 2. General concept of a one-pot basic reduction. The stereochemistry of C20 was intentionally not shown here since it is dependent on the reductive method employed as explained below.

(dt, J = 11.9, 3.1 Hz, 9-*H*), 0.56 (s, 3H, 18-CH_3); HRMS *m/z* calcd. for $C_{21}H_{30}ClO_3$ $[M+H]^+$ 365.1883, found 365.1813 (displaying typical isotopic chlorine pattern shown in the Supporting Information).

17α-Hydroxyprogesterone (6). Intermediate **2** (182 mg, 0.5 mmol) was dissolved in 30% NH_3 in MeOH (20 ml). A solution of 45% $Ti_2(SO_4)_3$ in dilute sulfuric acid (4 mmol, 2.35 ml) was added at once. The reaction mixture was stirred for 10 h, resulting in the formation of a suspension of white powder (TiO_2) in a dark solution. The mixture was concentrated, extracted with EtOAc (20 ml) three times; the organic layers were combined, dried with Na_2SO_4, and concentrated under reduced pressure. Silica gel column chromatography afforded a major fraction of methylketone **6** (137 mg, 0.414 mmol) in 83% yield, R_f = 0.55 (50% EtOAc in hexanes), mp = 280-285 °C; ^{1}H NMR (360 MHz, DMSO-d_6): δ = 5.63 (s, 1H, *H*-C = C), 5.25 (s, 1H, 17-O*H*), 2.53 (dt, J = 13.2, 3.1 Hz, 1H, 16β-*H*), 2.39 (dt, J =14.9, 3.4 Hz, 2H: overlap of 6β-H_{ax} and 2β-H_{ax}), 2.24 (dt, J = 13.3, 2.2 Hz, 1H, 6α-H_{eq}), 2.15 (dt, J = 13.3, 2.2 Hz, 1H, 2α-H_{eq}), 2.08 (s, 3H, 21-CH_3), 1.96 (dq, J = 13.3, 2.3 Hz, 1H, 1β-H_{eq}), 1.79 (m, 1H, 7β-H_{eq}), 1.66-1.75 (m, 2H, overlap of 12β-H_{eq} and 14-*H*), 1.51-1.66 (m, 4H, overlap of 15α-*H*, 1α-H_{ax}, 8-*H*, and 11α-H_{eq}), 1.30-1.50 (m, 3H, overlap of 16α-*H*, 11β-H_{ax}, and 12α-H_{ax}), 1.23 (m, 1H, 15β-*H*), 1.12 (s, 3H, 19-CH_3), 0.99 (dq, J = 11.8, 2.9 Hz, 1H, 7α-H_{ax}), 0.88 (dt, J = 11.9, 3.1 Hz, 9-*H*), 0.53 (s, 3H, 18-CH_3).

17α, 20β-Dihyroxy-4-pregnen-3-one [(20*R*)-1]. Pheromone (20*R*)-**1** was obtained in a one-pot reductive procedure after the dechlorination of **2** with Ti^{3+} to generate **6** in situ. To the latter formed after 10 h of reaction time, $NaBH_4$ (23 mg, 0.61 mmol) was added. The reduction was complete in 30 min, after which the mixture was extracted with EtOAc. The organic layers were combined, washed with brine, dried over Na_2SO_4, rotavaped, and then purified on a silica gel column using 25% EtOAc/hexanes. Product (20*R*)-**1** (125 mg, 0.376 mmol) was obtained with an overall yield of 64% from cortexolone, and showed identical NMR spectra to the authentic pheromone. R_f = 0.43 (50% EtOAc/hexanes), mp = 199-203 °C; IR ν/cm^{-1} = 3444 (br, OH), 2938 (aliphatic C-H), 1659 (C = C-C = O); ^{1}H NMR (400 MHz, DMSO-d_6): δ = 5.62 (s, 1H, *H*-C = C), 4.04 (d, J =7.1 Hz, 1H, 20-O*H*), 3.75 (app quin, J = 6.6 Hz, 1H, 20-*H*), 3.43 (s, 1H, 17-O*H*), 2.39 (m, 2H: overlap of 6β-H_{ax} and 2β-H_{ax}), 2.15-2.24 (m, 2H, 6α-H_{eq} overlap with 2α-H_{eq}), 1.95 (m, 1H, 1β-H_{eq}), 1.80 (m, 1H, 7β-H_{eq}), 1.64-1.75 (m, 2H, overlap of 12β-H_{eq} and 14-*H*), 1.42-1.63 (m, 5H, overlap of 16β, 15α-*H*, 1α-H_{ax}, 8-*H*, and 11α-H_{eq}), 1.20-1.41 (m, 3H, overlap of 16α-*H*, 11β-H_{ax}, and 12α-H_{ax}), 1.19 (m, 1H, 15β-*H*), 1.18 (s, 3H, 19-CH_3), 1.00 (d, J = 6.3 Hz, 3H, 21-CH_3 overlap with m, 1H, 7α-H_{ax}), 0.82 (m, 1H, 9-*H*), 0.76 (s, 3H, 18-CH_3); HRMS *m/z* calcd. for $C_{21}H_{33}O_3$ $[M+H]^+$ 333.2430, found 333.2392.

21-Chloro-17α,20α-dihydroxy-4-pregnene-3-one [(20*S*)-3]. Intermediate **2** (200 mg, 0.55 mmol) was dissolved in 20 ml of anhydrous THF, and 2.5 ml of 0.5 M HAlO in anhydrous THF were added while stirring. The mixture was stirred at room temperature for 3 h, then extracted with EtOAc (20 ml) three times. The combined organic layers were washed with brine (15 ml) three times, dried over Na_2SO_4, and concentrated in vacuo and purified on silica gel to afford 198 mg (98% yield) of chloroalcohol (20*S*)-**3**. R_f = 0.49 (50% EtOAc/hexanes), mp = 194-197 °C; IR ν/cm^{-1} = 3430 (17-OH, 20-OH), 2940 (CH), 1656 (C = C-C = O); ^{1}H NMR (360 MHz, DMSO-d_6): δ = 5.62 (s, 1H, *H*-C = C), 4.93 (d, J = 7.7 Hz, 1H, 20-O*H*), 3.86 (s, 1H, 17-O*H*), 3.69 (m, 2H, 21-CH_2Cl), 3.51 (m, 1H, 20-*H*), 2.37 (dt, J =14.9, 3.4 Hz, 2H: overlap of 6β-H_{ax} and 2β-H_{ax}), 2.24 (dt, J = 13.3, 2.2 Hz, 1H, 6α-H_{eq}), 2.15 (dt, J = 13.3, 2.2 Hz, 1H, 2α-H_{eq}), 1.95 (dq, J = 13.3, 2.3 Hz, 1H, 1β-H_{eq}), 1.66-1.82 (m, 3H, 7β-H_{eq}, 12β-H_{eq} and 14-*H*), 1.42-1.62 (m, 6H, overlap of 16β-*H*, 16α-*H*, 15α-*H*, 1α-H_{ax}, 8-*H*, and 11α-H_{eq}), 1.20-1.40 (m, 2H, overlap of 11β-H_{ax}, and 12α-H_{ax}), 1.15 (m, 1H, 15β-*H* overlap with s, 3H, 19-CH_3), 0.99 (m, 1H, 7α-H_{ax}), 0.88 (m, 1H, 9-*H*), 0.78 (s, 3H, 18-CH_3); HRMS *m/z* calcd. for $C_{21}H_{32}ClO_3$ $[M+H]^+$ 367.2040, found 367.2085 (displaying typical isotopic chlorine pattern shown in the Supporting Information).

17-Hydroxy-20,21-epoxy-4-pregnene-3-one [(20*S*)-4]. Intermediate (20*S*)-**3** (125 mg, 0.345 mmol) was dissolved in MeOH (15 ml). A solution of NaOH (40 mg, 0.68 mmol) dissolved in 5 ml of water was added to the solution, and the mixture was stirred at room temperature for 2 h. The crude was then extracted with EtOAc and the organic layers were combined and washed with brine and dried over Na_2SO_4. It was then concentrated under reduced pressure to afford a nearly quantitative yield of epoxide (20*S*)-**4** (110 mg, 96%). R_f = 0.47 (50% EtOAc/hexanes), mp = 235-239 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ = 5.63 (s, 1H, *H*-C = C), 4.01 (s, 1H, 17-O*H*), 3.01 (dd, J = 4.0, 2.8 Hz, 1H, 20-*H*), 2.67 (dd, J = 5.2, 2.8 Hz,

1H, 21-H_S), 2.58 (dd, J = 5.2, 4.0 Hz, 1H, 21-H_R), 2.40 (dt, J =14.9, 3.4 Hz, 2H: overlap of 6β-H_{ax} and 2β-H_{ax}), 2.24 (dt, J = 13.3, 2.2 Hz, 1H, 6α-H_{eq}), 2.15 (dt, J = 13.3, 2.2 Hz, 1H, 2α-H_{eq}), 1.96 (dq, J = 13.3, 2.3 Hz, 1H, 1β-H_{eq}), 1.75-1.87 (m, 2H, overlap of 7β-H_{eq} and 14-*H*), 1.49-1.68 (m, 6H, overlap of 16α-*H*, 16β-*H*, 1α-H_{ax}, 12β-H_{eq}, 8-*H*, and 11α-H_{eq}), 1.30-1.46 (m, 3H, overlap of 11β-$H_{ax,}$ 12α-H_{ax}, and 15α-*H*), 1.22 (m, 1H, 15β-*H*), 1.16 (s, 3H, 19-CH_3), 0.99 (m, 1H, 7α-H_{ax}), 0.85 (m, 1H, 9-*H*), 0.81 (s, 3H, 18-CH_3); HRMS *m/z* calcd. for $C_{21}H_{31}O_3$ $[M+H]^+$ 331.2273, found 331.2292.

17α,20α-Dihyroxy-4-pregnen-3-one [(20*S*)-1]. Intermediate (20*S*)-**3** (323 mg, 0.88 mmol) was dissolved in 30% NH_3 in MeOH (20 ml). A solution of 45% $Ti_2(SO_4)_3$ (1.76 mmol, 1.0 ml) was added at once. The reaction mixture was stirred for 10 h, resulting in the formation of a suspension of white powder (TiO_2) in a dark solution. The mixture was concentrated, extracted with EtOAc (20 ml) three times; the organic layers were combined, dried with Na_2SO_4, and concentrated under reduced pressure. Silica gel column chromatography afforded (163 mg, 0.49 mmol, 56%) in 47% overall yield from cortexolone. Alternatively, intermediate (20*S*)-**4** (100 mg, 0.303 mmol) was dissolved in 10 ml of anhydrous THF. An amount of 2.0 ml of 0.5 M HAlO in anhydrous THF was added into the solution while stirring. The reaction mixture was heated in an oil bath at 65 °C overnight, then extracted with EtOAc. The organic layers were combined, washed with brine twice, dried over Na_2SO_4, then concentrated under reduced pressure. Silica gel column chromatography using 25% EtOAc/hexanes afforded (20*S*)-**1** (70 mg, 0.21 mmol, 69%) in 55% overall yield from cortexolone. R_f = 0.40 (50% EtOAc/hexanes), mp = 203-206 °C. Mixed melting points of 1:1 (wt%) of (20*R*)-**1** and (20*S*)-**1** was measured as 188–195 °C; and the melting point of a 1:1 (wt%) mixture of a sample of the commercially available pheromone (20*R*)-**1** with (20*S*)-**1** was measured as 190–195 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 5.73 (s, 1H, *H*-C = C), 3.86 (quin, J = 6.3 Hz, 1H, 20-*H*), 3.49 (d, J = 5.53 Hz, 1H, 20-O*H*), 2.31-2.48 (m, 3H, overlap of 2β-H_{ax}, 2α-H_{eq}, 6β-H_{ax}), 2.24-2.31 (m, 1H, 6α-H_{eq}), 1.98-2.10 (m, 2H, 16β-*H* and 1β-H_{eq}), 1.82-1.90 (m, 1H, 7β-H_{eq}), 1.81 (s, 1H, 17-O*H*), 1.66-1.81 (m, 5H, overlap of 16α-*H*, 15α-*H*, 1α-H_{ax}, 14-*H*, and 12β-H_{eq}), 1.51-1.66 (m, 3H, overlap of, 11α-H_{eq}, 8-*H*, and 12α-H_{ax}), 1.35-1.48 (m, 1H, 11β-H_{ax}), 1.23 (m, 1H, 15β-*H*), 1.21 (d, J = 6.5 Hz, 3H, 21-CH_3), 1.19 (s, 3H, 19-CH_3), 1.10 (m, 1H, 7α-H_{ax}), 0.98 (m, 1H, 9-*H*), 0.78 (s, 3H, 18-CH_3); HRMS *m/z* calcd. for $C_{21}H_{33}O_3$ $[M+H]^+$ 333.2430, found 333.2916.

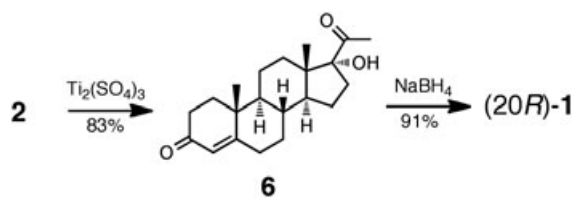
General procedure for conjugate ester 8. Conjugation of *bis*-Boc carrier acid[20,21] **7** with the 2° chiral alcohol of the pheromones (commercially available pheromone, (20*R*)-**1**, and (20*S*)-**1** respectively): a solution of (20*R*)-**1** (5.3 mg, 0.16 mmol, 1 eq), *bis*-Boc carrier **7** (20.0 mg, 0.60 mmol, 4 eq), EDC (32.1 mg, 0.167 mmol, 10 eq) and DMAP (2.4 mg, 0.019 mmol, 1.2 eq) in anhydrous CH_2Cl_2 (8 ml) was stirred in a 50-ml round-bottom flask overnight under nitrogen. The mixture was diluted with CH_2Cl_2 (10 ml), washed with a saturated $NaHCO_3$ solution, and then with brine. The solution was dried with Na_2SO_4 and then the solvent was rotavaped. The residue was quickly purified by pipette column chromatography (CH_2Cl_2/MeOH, 40:2, R_f = 0.42) to yield the *bis*-Boc derivative of conjugate ester (20*R*)-**8** as an oily film (4.0 mg, 39% yield); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 6.75 (br s, 1H, N-*H*), δ = 5.62 (s, 1H, *H*-C = C), 4.92 (m, 1H, 20-*H*), 4.12 (br s, 1H, 17-O*H*) overlap with 4.11 (s, 2H, (C = O)CH_2NBoc), 3.88 (m, 2H, -NBocCH_2CH_2CH_2NHBoc), 2.90 (m, 2H, CH_2CH_2NHBoc), 2.39 (m, 2H: overlap of 6β-H_{ax} and 2β-H_{ax}), 2.24 (m, 1H, 6α-H_{eq}), 2.15 (m, 1H, 2α-H_{eq}), 1.97 (m, 2H, $CH_2CH_2CH_2$) overlap with 1.96 (m, 1H, 1β-H_{eq}), 1.45-1.81 (m, 8H, 7β-H_{eq},12β-$H_{eq,}$14-*H*, 16β-*H*, 15α-*H*, 1α-H_{ax}, 8-*H*, and 11α-H_{eq}), 1.37 (s, 9H, NH*B*oc), 1.34 (s, 9H, N*B*oc), 1.30-1.45 (m, 3H, overlap of 16α-*H*, 11β-$H_{ax,}$ and 12α-H_{ax}), 1.22 (m, 1H, 15β-*H*) overlap with 1.21 (br s, 3H, 21-CH_3), 1.13 (s, 3H, 19-CH_3), 0.96 (m, 1H, 7α-H_{ax}), 0.84 (m, 1H, 9-*H*), 0.67 (s, 3H, 18-CH_3); HRMS *m/z* calcd. for $C_{31}H_{51}N_2O_6$ $[M\text{-Boc}+H]^+$ 547.3747, found 547.3779.

Host-guest complex between 9 and the Zn tetraphenylporphyrin tweezer[20,21]. The purified **8** (1.0 mg) was dissolved in 0.5 ml CH_2Cl_2, and 0.1 ml TFA was added to the solution to form the TFA salt of **9**. The mixture was stirred under argon for 2 h. The solvent was evaporated and the residue was further dried on the vacuum line. The residue was suspended in MeOH and washed with Na_2CO_3 solution to yield **9**. The MeOH was then evaporated and the compound was redissolved in anhydrous CH_2Cl_2 (0.3 ml). A 10-μL aliquot of the solution was added to the solution of Zn porphyrin tweezer (commercially available) in CH_2Cl_2 (1 μM). The CD spectra were then recorded according to the published procedure.[20,21]

RESULTS AND DISCUSSION

Chlorination of cortexolone was performed using mesyl chloride and DMAP to afford chlorocortexolone **2** in 85% yield (not shown).[22] Reduction of **2** using sodium borohydride ($NaBH_4$) generated chloroalcohol (20*R*)-**3**. Although (20*R*)-**3** was expected to be close to the epoxide under basic conditions, the isolation and characterization of that intermediate preceded further basic treatment. Chloroalcohol (20*R*)-**3** was isolated in ca. 98% yield. Only the (20*R*)-**3** (i.e., 20β)[23] was obtained and there was no evidence for a mixture of diastereomers by either thin-layer chromatography (TLC) or NMR. The reduction is stereocontrolled by the presence of the 17-α-hydroxy and the C18-methyl groups according to the discussion below in conjunction with computational molecular modeling.

Treatment of (20*R*)-**3** with KOH resulted in the formation of epoxide (20*R*)-**4** in about 96% yield. Reduction of the epoxide with $NaBH_4$ was unsuccessful under the mild conditions needed to avoid excessive reduction of the ketone or alkene function of ring A. An alternative approach using Ti^{3+} proved successful as a more direct pathway to obtaining target pheromone (20*R*)-**1**. Reductive dechlorination[24] of chloroketone **2** with Ti^{3+} generated hydroxyprogesterone **6**, which was followed by $NaBH_4$ reduction[18] leading to (20*R*)-**1** in an overall 76% yield from **2** (Scheme 3). The latter reduction is both time-sensitive and dependent on the equivalency of $NaBH_4$. As

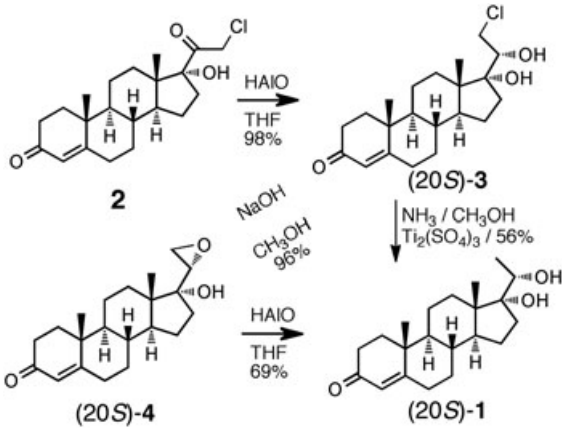


Scheme 3. Synthesis of the (20*R*)-epimer (the pheromone). These two steps were combined in a one-pot transformation.

previously reported,[18] we also observed the reduction of both the C3 enone and the C20 ketone with increased reaction times (>30 min) and $NaBH_4$ equivalencies (>1.1 eq). Careful control of reaction conditions favored the C20 over the C3 reduction as discussed below with the aid of computational calculations. We were also able to combine the dechlorination and reduction steps in one pot with an overall yield of 64% for the conversion of cortexolone to (20*R*)-**1** in just two steps. The target structure showed identical spectra to those of the commercially available sample of the pheromone.

We aimed to accomplish an equaly stereoselective preparation of the pheromone C20 epimer, and provide a rationale for the stereoselectivity. Although it was evident that aluminumoxyhydride (HAlO) was capable of exhibiting selectivity towards reducing ketones, the presence of the 17-OH guided such reduction to the C20 over the C3 ketone which was reduced preferentially in the case of progesterone.[25] The HAlO reduction of chlorocortexolone, shown in Scheme 4, generated the diastereomer of the chloroalcohol that was obtained by $NaBH_4$ reduction. Further reductive dechlorination was achieved using Ti^{3+} on (20*S*)-**3** to afford the C20 epimer (i.e., 20α) of the pheromone, (20*S*)-**1** (three steps from cortexolone, overall 47% yield). Alternatively, basic treatment of (20*S*)-**3** led to epoxide (20*S*)-**4** which upon selective reduction with HAlO afforded (20*S*)-**1** in an overall 55% yield (over four steps) from cortexolone.

We proceeded to characterize the stereochemistry of the secondary alcohol of both C20 epimers using a previously established microscale chiroptical method developed by Nakanishi, Berova, and colleagues.[20,21] The advantages of this chiroptical method, over traditional derivatization methods combined with 2D NMR analysis, is the unambiguous determination of configuration with microscale quantities. Li et al.[26,27] elegantly described how the tweezer methodology could be applied to *erythro* and *threo* diols; however, none of the substrates studied contained a stereogenic tertiary alcohol. Pheromone (20*R*)-**1** was coupled to a bidentate carrier **7** (Scheme 5) to afford conjugate (20*R*)-**8**. The derivatization was carried out on the secondary alcohol with no interference from the vicinal tertiary alcohol, as previously observed for the ginkgolide derivative reported by Kurtan et al.[20] Deprotection of (20*R*)-**8** with trifluoroacetic acid (TFA) resulted in (20*R*)-**9** which was complexed with a *bis*-chromophoric zinc porphyrin tweezer (Fig. 1).[20,21] The well-established chiroptical method takes advantage of the presence of a large moiety (L) and a medium moiety (M) on the secondary alcohol carbon, in which the small hydrogen (H) will align syn to the ester carbonyl.



Scheme 4. Synthesis of the (20*S*)-epimer of the pheromone, (20*S*)-**1**.

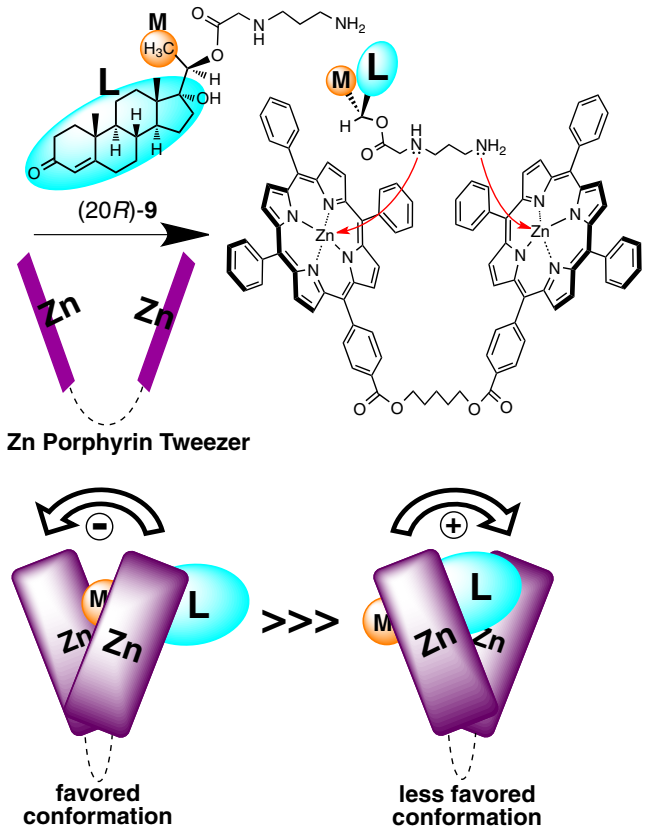
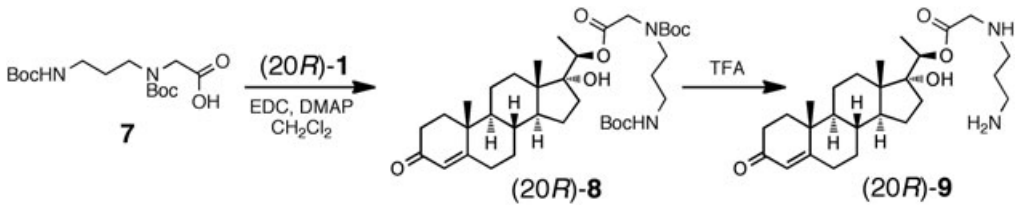


Fig. 1. The diamino conjugate of (20*R*)-**9** was complexed with Zn porphyrin tweezer leading to two populations of conformers, a favored one and a less favored one. The predominantly favored conformer(s) lead to a negative exciton couplet, as expected. The purple rectangular shapes, highlighting the twist of the complex, represent the transition moments of the two porphyrins in the tweezer; L and M represent the large and medium groups, respectively. The (20S)-9 was also complexed leading to the opposite twist for its predominant conformer, resulting in an opposite couplet (not shown in this figure).



Scheme 5. Coupling the secondary alcohol to a bidentate carrier **7**. This was carried out for both authentic and synthesized pheromone (20*R*)-**1** as well as the (20*S*)-**1**, forming (20*R*)-**9** and (20*S*)-**9** (not shown), respectively.

In this case, M is the methyl group and the L group is an unambiguously much larger group; namely, the steroid moiety. Therefore, it is expected that there will be a clear overpopulation of the favored conformation of the diamino conjugate with the Zn porphyrin tweezer, in which the L group will preferentially reside away from the crevice of the complex. This induces a negative twist for the transition moments of the Zn porphyrin twist, which results in a negative exciton couplet. The resultant complex was subjected to CD studies in methylcyclohexane (Fig. 2). Similarily, the C20 epimer, (20*S*)-**1**, was subjected to conjugation with **7** (Scheme 5), followed by deprotection and complexation with the tweezer prior to CD studies. As predicted on the basis of the steric size of the substituents flanking the stereogenic center,[20,21,28–30] as described above, the exciton coupled CD spectrum of the tweezer complex formed from (20*R*)-**1** (compound (20*R*)-**9**) had a negative exciton couplet, reflective of the negative twist of the transition moments of the porphyrin tweezer and indicative of an (*R*) absolute configuration, whereas the complex formed from (20*S*)-**1** (compound (20*S*)-**9**) had a positive couplet, predicting an (*S*) absolute configuration.

Molecular modeling Density Functional Theory (DFT) calculations at the (R)B3LYP/6-31G(d) level were computed to elucidate the pathway of the reduction of the carbonyl at C20 to yield either the pheromone, (20*R*)-**1**, or its epimer (20*S*)-**1**. The ground state equilibrium geometries, i.e., lowest energy conformers, of all synthesized molecules and suggested stable intermediates (**[2-HAlO]**, **[6]**$^-$ and **[6-BH$_3$]**) were optimized in the gas phase at 298 K. All ground states were true minima with no imaginary vibrational frequencies (see Electronic Supporting Information for energy differences between conformers of the optimized structures).[31–35]

The equilibrium geometry of methylketone **6** has an H-bond between the OH at C17 and the oxygen of the carbonyl at C20. This exposes the *re* face of the carbonyl toward nucleophilic attack, as the opposite *si* face is hindered by the C18-methyl group (Fig. 3).[36]

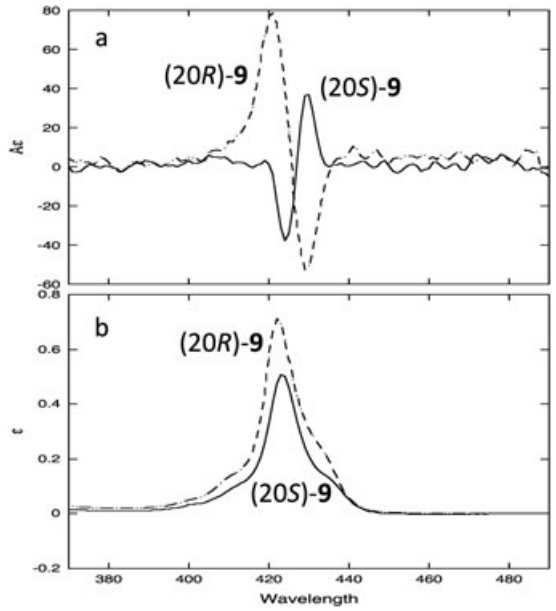


Fig. 2. (**a**) CD spectra of the complexes of (20*R*)-***9*** (dashed) and of (20*S*)-**9** (solid) with Zn porphyrin tweezer. (**b**) Respective UV spectra of these complexes: (20*R*)-***9*** (dashed) and of (20*S*)-**9** (solid).

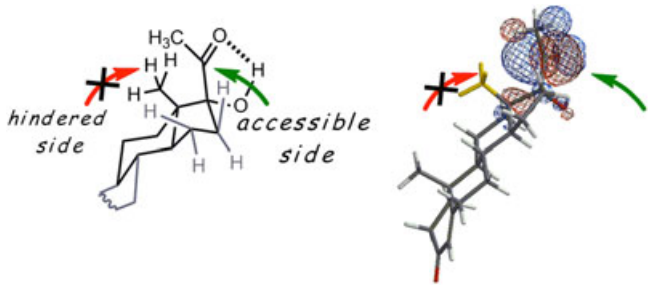


Fig. 3. Hydride attack on LUMO + 1 is hindered from the *si* face due to the C18 methyl (highlighted in yellow for clarity).

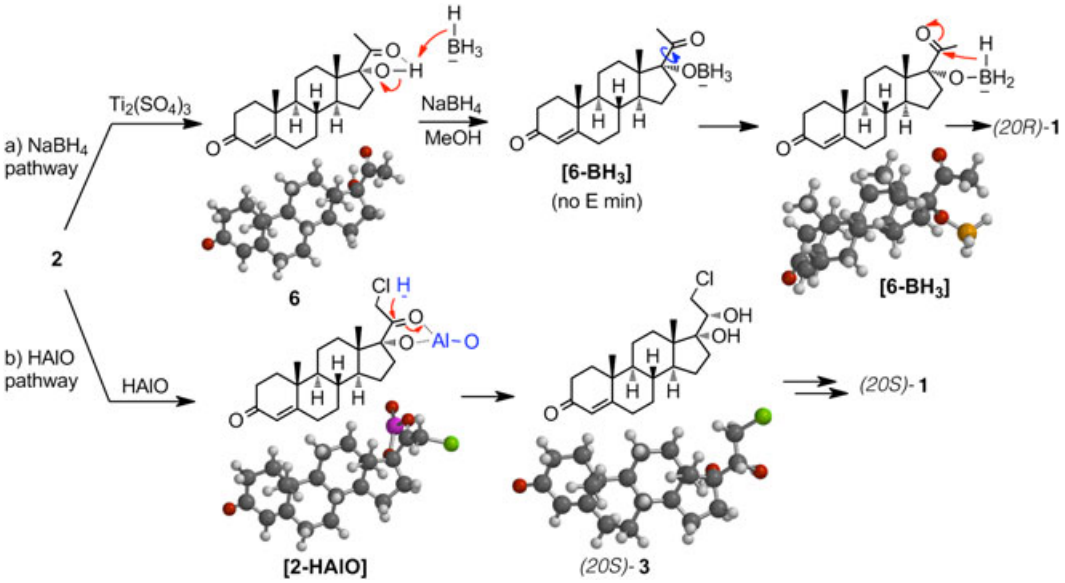


Fig. 4. (**a**) $NaBH_4$ and (**b**) HAlO proposed mechanisms with optimized structures. Structures **[6]**$^-$, **[2-HAlO]** and **[6-BH$_3$]** are the only suggested stable intermediates that were not isolated and characterized.

In the case of the reduction of **6** with $NaBH_4$ in MeOH the (20*R*)-epimer is formed exclusively; i.e., the (20*S*) is not formed. The sodium cation is not as oxophilic as aluminum in HAlO and will therefore not chelate the two oxygens at C17 and C20 (as shown in HAlO reduction, below). Thus, the methoxide ion of the basic methanolic solution abstracts the proton from the 17-OH, resulting in a near 180-degree rotation of the C17-C20 bond to minimize repulsion between the two oxygen atoms (**6** → **[6]**$^-$). This exposes the *si* face of the carbonyl, contrary to the case presented in Fig. 3. Intermediate **[6]**$^-$ is then reduced from the accessible *si* face of the carbonyl, leading to the formation of the (20*R*)-epimer, i.e., pheromone (20*R*)-**1** as shown in Fig. 4a. A similar stereochemical outcome would result in a site delivery of the hydride from a **[6-BH$_3$]** complex at the C17 alkoxy. Previous work by Evans and co-workers also report similar high stereoselectivities in related β-hydroxyketone reductions using borohydrides.[37,38] In the case of HAlO, the more oxophilic aluminum coordinates the two oxygen atoms on C17 and C20 while the hydride attacks the carbonyl from the nonhindered *re* face leading to an exclusive stereoselection for the (20*S*)-epimer (Fig. 4b).[39]

CONCLUSION

We developed a two-step procedure for the construction of a goldfish pheromone in an overall 64% yield with exclusive enantioselectivity from a rather inexpensive starting material. A second procedure, using a fairly newly developed aluminum reducing agent (HAlO), was designed for a three-step synthesis of the C20 epimer of the pheromone in an overall 47% yield. Future studies with collaborators will be focused on elucidating the difference in the biological activities of the two C20 epimers, as well as novel constructs, based on the general structure **5**, resulting from the opening of the intermediate epoxide **4**. Moreover, transition state computations should provide more insight to the proposed reaction mechanisms.

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23. Although using (20R) and (20S) is less ambiguous to denote the stereochemistry at acyclic stereocenters such as C20, we used the traditional notations of 20β and 20α, respectively, to refer to the names of the pheromone and its epimer, as in previous reports. The use of 20α/β maintains consistency with the α-hydroxy substituent already present in the name, as in 17α-hydroxyprogesterone. The ambiguous nature of the 20β and 20α notation is inherent in their dependence on the orientation of the drawn side chain at C17. Since progesterone is conventionally drawn with the methyl group oriented to the right-hand side of ring D (e.g., 17-hydroxyprogesterone in Scheme 3), confusion arises when the product of the reduction at C20 is drawn with the same orientation of the methyl group, which would depict the opposite stereochemical information if the α/β notation is used. It is worth clarifying that the α/β assignment on the side chain is based on the parent structure of cholesterol, in which the methyl group is drawn to the left and in the back. For added clarity, we used

20R and 20S in the discussion of the reduction at C20 throughout the manuscript.

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