SYNTHETIC APPROACH TO 5α-PREGNANOLONE 19-[*O*-(CARBOXYMETHYL)OXIME] DERIVATIVES+

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Received May 7, 2004
Accepted August 13, 2004

Dedicated to Professor Miloslav Černý on the occasion of his 75th birthday.

Syntheses of 5α-pregnanolone derivatives (3-hydroxy-5α-pregnan-20-ones) with 3α- and 3β-configuration and 19-[*O*-(carboxymethyl)oxime] group were developed, starting from 19-hydroxy-20-oxopregn-5-en-3β-yl acetate. After catalytic hydrogenation, acetylation and ketone protection, the acetyl in position 3 was removed. To obtain the 3α-derivative, nitrite epimerization of the intermediate tosylate was performed before the introduction of methoxymethyl protecting group, while the 3β-derivative was protected directly. In both series, deacetylation, oxidation to an aldehyde and *O*-(carboxymethyl)hydroxylamine condenzation followed. Conversion to the methyl ester, simultaneous deprotection of positions 3 and 20, and alkaline hydrolysis gave the corresponding 19-[*O*-(carboxymethyl)oximes]. The nine- and ten-step syntheses described herein (yields 19.5 and 7.3%, respectively) gave the target compounds, designed as haptens for immunological use.

Keywords: Synthesis; Steroids; *O*-(Carboxymethyl)oxime; Haptens; Oximes; Neurosteroids; Hormones.

Neuroactive steroids belong to a group of frequently studied compounds with respect to their physiological role in nervous systems. Recently, an interest has arisen in the reduced metabolites of progesterone, with a focus on the less abundant derivatives of the tetrahydroprogesterone series[2–5], of which allopregnanolone has been the mostly reported compound[3,6–8].

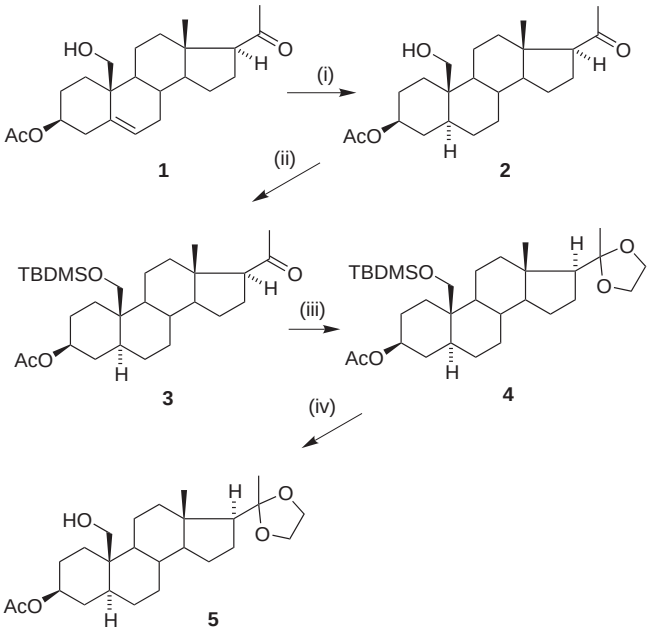
+ Part CDXVIII in the series On Steroids; Part CDXVII see lit.[1]

When analyzed in a biological matrix, pretreatment of samples before the assay is very important, because of the presence of a number of similar steroid derivatives. For this purpose, HPLC separation in combination with mass spectrometry techniques is employed most often[3,4,9,10]. Regardless of these methods, immunoassays are still being used for routine analyses and initial screening[5,8]. So far, several haptens suitable for the generation of antibodies against this type of compounds have been reported. For the conjugation of allopregnanolone (3α-hydroxy-5α-pregnan-20-one), position 11 has been employed most frequently. Thus, the 11α-carboxymethyloxy derivative gave sufficiently selective and frequently used antibodies[6,11,12], the 11-[*O*-(carboxymethyl)oxime] (11-CMO) was also tested[13], and the 11α-hydroxy hemisuccinate has been described[14], but its application in an assay has not been published as yet. Furthemore, 20-CMO was used in an assay[15], and the 21-hydroxy hemisuccinate was prepared for different purposes[16]. As regards others, the use of the 21-hydroxy hemisuccinate of pregnanolone (3α-hydroxy-5β-pregnan-20-one) has been reported[17] and 5β-pregnane-3,20-dione 3-CMO was employed for a nonselective assay connected with chromatographic preselection[5].

In connection with our studies on the 19-substituted derivatives in the androstane and pregnane series[18], we present the syntheses of two 19-CMO haptens from tetrahydroprogesterone isomers, i.e. from isopregnanolone (3β,5α-THP, 3β-hydroxy-5α-pregnan-20-one) and allopregnanolone (3α,5α-THP, 3α-hydroxy-5α-pregnan-20-one).

As a starting compound, we employed 19-hydroxy-20-oxopregn-5-en-3β-yl acetate[19] (**1**), which was hydrogenated over palladium on activated carbon in ethanol in the first step (Scheme 1). This reaction gave the saturated derivative **2** with 5α-configuration, similar to the parent pregnenolone acetate (20-oxopregn-5-en-3β-yl acetate)[20,21]. The next step, i.e. the protection of the 20-oxo group as a dioxolane, was not a simple task. We attempted several methods of ketalization: beside the method using treatment with ethylene glycol, 4-toluenesulfonic acid hydrate, and triethyl formate as a water scavenger, which proved useful in similar cases[18], we employed other standard ways of water removal, including azeotropic distillation and molecular sieves, but the conversions were not complete and the yields were low. Finally, we succeeded in a three-step procedure, in which the 19-hydroxy group was protected as the *tert*-butyldimethylsilyl ether, ketone **3** was subsequently protected using the first of the above methods, and the silyl protecting group from the resultant product **4** was removed with tetrabutylammonium fluoride. This way, ketal **5** was prepared in 78% overall yield.

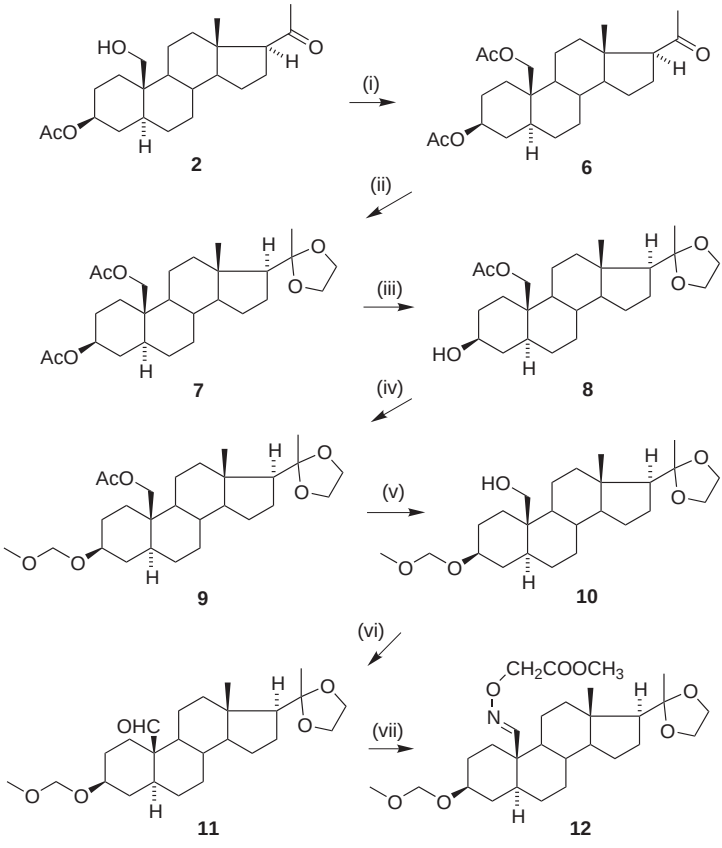
This sequence, however, is not suitable for a larger scale and therefore we explored another way.



(i) H_2, Pd/C, EtOH; (ii) TBDMSCl, imidazole, DMF; (iii) TsOH, ethylene glycol, triethyl orthoformate, benzene; (iv) TBAF, THF

SCHEME 1

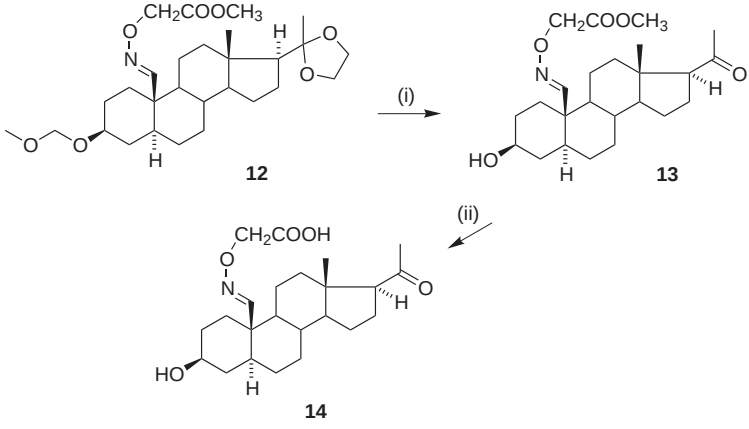
To bypass the use of silyl protection during acetalization, diacetate **6** was prepared (Scheme 2), and converted into ketal **7** by the triethyl orthoformate method. Different reactivity of acetyl groups in positions 3 and 19 allowed selective deprotection of that in position 3 by potassium carbonate in boiling methanol. The resulting derivative **8** was used as a starting point for the preparation of the 3α-epimer (see below). In the 3β-series, position 3 in **8** was protected to give the methoxymethyl derivative **9** and position 19 was then liberated by alkaline hydrolysis. The remaining part of the synthesis of the 3β-epimer was completed in a standard way. First, the 19-hydroxy derivative **10** was oxidized into aldehyde **11** with chromium(VI) oxide–pyridine complex, and subsequently treated with *O*-(carboxymethyl)-hydroxylamine, giving, after diazomethane treatment, CMO methyl ester **12**. The deprotection of positions 3 and 20 leading to ketone **13** was accom-



(i) Ac_2O, Py; (ii) TsOH, ethylene glycol, triethyl orthoformate, benzene; (iii) K_2CO_3, H_2O, MeOH; (iv) $BrCH_2OCH_3$, *N,N*-diisopropylethylamine, benzene; (v), NaOH, MeOH; (vi) CrO_3 - Py, $MgSO_4$, CH_2Cl_2; (vii) 1. *O*-(carboxymethyl)hydroxylamine, Py; 2. CH_2N_2, ether, MeOH

SCHEME 2

plished simultaneously under acidic conditions (Scheme 3). The use of acid-labile protecting groups minimized exposure to bases in the final steps, which is often accompanied by partial isomerization in position 17. The only exception, where alkaline treatment had to be used, was the methyl ester hydrolysis in **13**, which afforded the final CMO derivative **14**, suitable as a hapten for isopregnanolone.



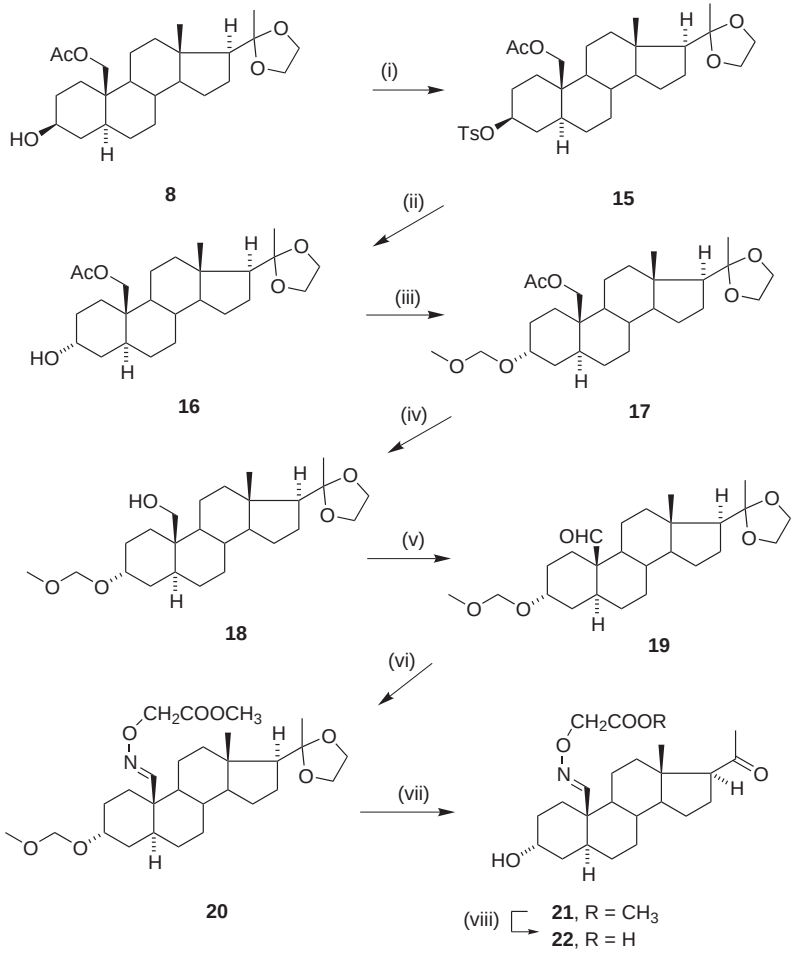
(i) aq. $HClO_4$, benzene, MeOH; (ii) aq. NaOH, THF, MeOH

SCHEME 3

In the synthesis of the allopregnanolone CMO derivative, derivative **8** was epimerized in position 3 (Scheme 4). From available methods, the solvolysis of tosylate with potassium acetate[22] or the Mitsunobu reaction with trifluoroacetic acid have been applied to 3β-hydroxy-5α-pregnan-20-one[23]. We decided to evaluate another method[16,24], which is the modification of a classical nucleophilic substitution of tosylate with nitrite[25]. Tosylate **15**, prepared from **8** by the reaction with 4-toluenesulfonyl chloride in pyridine, was treated with sodium nitrite in hexamethylphosphoramide, and, after chromatography, the 3α-hydroxy derivative **16** was obtained (58% yield from **8**). As in the case of the 3β-hydroxy derivative, subsequent protection of the 3-hydroxy group gave the methoxymethoxy derivative **17**, which was deacetylated to afford the 19-hydroxy derivative **18**, oxidized into aldehyde **19**, and transformed to the CMO methyl ester **20**. Removal of protecting groups in positions 3 and 19, which gave compound **21**, and the final ester hydrolysis into **22** used the same protocol as described for the 3β-epimer.

In general, the protection of the 20-oxo function as a cyclic ketal resulted in problems, caused by its instability. Despite a thorough treatment, excluding contact with an acid during work-up and manipulation, the yields were occasionally lowered by the loss of the protecting group. Nevertheless, the protocol designed yielded sufficient amounts of 19-[*O*-(carboxymethyl)-

oxime]haptens of isopregnanolone and allopregnanolone to be tested for immunoassay use. The overall yields of the syntheses of compounds **14** and **22** from **1** were 19.5% (9 steps) and 7.3% (10 steps), respectively.



(i) TsCl, Py; (ii) $NaNO_2$, HMPA; (iii) $BrCH_2OCH_3$, *N,N*-diisopropylethylamine, benzene; (iv) NaOH, MeOH; (v) CrO_3 - Py, $MgSO_4$, CH_2Cl_2; (vi) 1. *O*-(carboxymethyl)hydroxylamine, Py; 2. CH_2N_2, ether, MeOH; (vii) aq. $HClO_4$, benzene, MeOH; (viii) aq. NaOH, THF, MeOH

SCHEME 4

EXPERIMENTAL

Melting points were determined on a Boetius micro melting point apparatus (Germany). Optical rotations were measured at 25 °C on a Perkin–Elmer 141 MC polarimeter, and $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. Infrared spectra (wavenumbers in cm^{-1}) were recorded on a Bruker IFS 88 spectrometer. ^{1}H NMR spectra were taken on a Bruker Avance-400 instrument (400 MHz, FT mode) at 23 °C in $CDCl_3$ and referenced to TMS as the internal standard. Chemical shifts are given in ppm (δ-scale); coupling constants (*J*) and width of multiplets (*W*) are given in Hz. NMR spectra for the target CMO derivatives (**14** and **22**) were measured on Varian Unity-500 and Bruker Avance-500 instruments (^{1}H at 500 MHz, ^{13}C at 125.7 MHz) under the above conditions. For ^{13}C, secondary referencing was performed using the solvent signal at position δ($CDCl_3$) 77.0, and heteronuclear 2D spectra (HSQC) were used for structural assignment of signals.

Thin-layer chromatography (TLC) was performed on silica gel G (ICN Biochemicals, detection by spraying with concentrated sulfuric acid followed by heating). For column chromatography, Silica gel 60 (Merck, 63–100 μm) or Aluminum oxide 90 neutral (Merck, 63–200 μm, activity III) were used. Prior to evaporation on a rotary evaporator in vacuo (0.25 kPa, bath temperature 40 °C), solutions in organic solvents were dried over anhydrous $MgSO_4$.

19-Hydroxy-20-oxopregn-5-en-3β-yl acetate (**1**) was prepared according to lit.[19] from pregnenolone acetate (Steraloids). For physical constants and spectral data, see lit.[18].

19-Hydroxy-20-oxo-5α-pregnan-3β-yl Acetate (**2**)

The unsaturated derivative **1** (1.84 g, 4.91 mmol) was hydrogenated in ethanol (100 ml) over 10% palladium on activated carbon (200 mg) at room temperature and atmospheric pressure. After 4 h, the consumption of hydrogen stopped, the catalyst was filtered off on celite, and the solvent was removed in vacuo. The crude product (about 1.9 g) was recrystallized from acetone to give 1.17 g (63%) of **2**, m.p. 145–147 °C, $[\alpha]_D$ +82 (*c* 0.30, $CHCl_3$). IR ($CHCl_3$): 3628, 3511 (O–H); 1722 (C=O, acetate); 1700 (C=O, ketone); 1384 (CH_3); 1362 (CH_3, acetate); 1257, 1029 (C–O, acetate). ^{1}H NMR (400 MHz): 4.75 (1 H, tt, *J* = 11.3, *J* = 5.1, H-3α); 3.93 and 3.82 (2 H, AB system, *J* = 11.6, H-19, H-19′); 2.53 (1 H, t, *J* = 8.9, H-17α); 2.11 (3 H, s, 3 × H-21); 2.03 (3 H, s, 3-OAc); 0.65 (3 H, s, 3 × H-18). For $C_{23}H_{36}O_4$ (376.5) calculated: 73.37% C, 9.64% H; found: 73.21% C, 9.50% H.

20,20-(Ethylenedioxy)-19-hydroxy-5α-pregnan-3β-yl Acetate (**5**)

Imidazole (900 mg, 13.22 mmol) and *tert*-butyldimethylsilyl chloride (480 mg, 3.19 mmol) were added to a solution of ketone **2** (300 mg, 0.80 mmol) in DMF (3 ml). The mixture was stirred at room temperature for 2 days, then poured into an ice-cold saturated aqueous $KHCO_3$, and extracted with ethyl acetate (30 ml). The extract was washed with a saturated aqueous $KHCO_3$ and water, dried, and the solvents were evaporated. To a solution of the crude silyl derivative **3** (350 mg, 0.71 mmol) in benzene (4 ml), ethylene glycol (0.5 ml, 8.97 mmol), triethyl orthoformate (1.5 ml, 9.02 mmol), and 4-toluenesulfonic acid monohydrate (5 mg) were added. After stirring at room temperature for 2 h, the same work-up as in the preceding step was used. The crude ketal **4** (350 mg, 0.65 mmol) was deprotected in THF (2 ml) by a 1.0 M solution of tetrabutylammonium fluoride in THF (4 ml, 4 mmol). After standing at room temperature for 3 days, the mixture was diluted with ethyl acetate

(50 ml) and washed successively with a 5% aqueous citric acid, saturated aqueous $KHCO_3$, and water. After drying, the solvent was evaporated, leaving 260 mg (78%) of ketal **5**. An analytical sample was crystallized from methanol, m.p. 179–181 °C, $[\alpha]_D$ +15 (*c* 0.6, $CHCl_3$). IR ($CHCl_3$): 3629, 3507 (O–H); 1724 (C=O, acetate); 1381 (CH_3); 1255, 1026 (C–O, acetate); 1154, 1069, 1045, 950 (ring, dioxolane). ^{1}H NMR (400 MHz): 4.74 (1 H, tt, *J* = 11.3, *J* = 5.0, H-3α); 3.78–4.02 (6 H, OCH_2CH_2O, H-19, H-19′); 2.02 (3 H, s, 3-OAc); 1.29 (3 H, s, 3 × H-21); 0.80 (3 H, s, 3 × H-18). For $C_{25}H_{40}O_5$ (420.6) calculated: 71.39% C, 9.59% H; found: 71.47% C, 9.83% H.

20-Oxo-5α-pregnane-3β,19-diyl Diacetate (**6**)

Monoacetate **2** (6.0 g, 15.9 mmol) was dissolved in pyridine (40 ml), the solution was cooled in an ice bath, and acetic anhydride (8.0 ml, 84.7 mmol) was added. The reaction mixture was left at room temperature overnight. The reaction mixture was then poured into an ice and water mixture (400 ml), the crystals were filtered off, washed with water, and dried. The yield was 6.5 g (97%) of diacetate **6**, sufficiently pure for further processing. An analytical sample was crystallized from methanol, m.p. 116–118 °C, $[\alpha]_D$ +68 (*c* 0.40, $CHCl_3$). IR ($CHCl_3$): 1728 (C=O, acetate); 1701 (C=O, ketone); 1383, 1366 (CH_3); 1247, 1031 (C–O, acetate). ^{1}H NMR (400 MHz): 4.73 (1 H, tt, *J* = 11.3, *J* = 5.0, H-3α); 4.32 and 4.26 (2 H, AB system, *J* = 12.2, H-19, H-19′); 2.50 (1 H, t, *J* = 9.0, H-17α); 2.10 (3 H, s, 3 × H-21); 2.06 (3 H, s, 19-OAc); 2.02 (3 H, s, 3-OAc); 0.61 (3 H, s, 3 × H-18). For $C_{25}H_{38}O_5$ (418.6) calculated: 71.74% C, 9.15% H; found: 71.66% C, 9.31% H.

20,20-(Ethylenedioxy)-3β-hydroxy-5α-pregnan-19-yl Acetate (**8**)

Ethylene glycol (5.0 ml, 89.7 mmol), triethyl orthoformate (15.0 ml, 90.2 mmol), and 4-toluenesulfonic acid monohydrate (100 mg) were added to a stirred solution of diacetate **6** (6.4 g, 15.3 mmol) in benzene (50 ml). After 5 h at room temperature, the reaction mixture was poured into an ice-cold saturated aqueous $KHCO_3$ and extracted with ethyl acetate (350 ml). The extract was washed successively with $KHCO_3$ (2×), water, dried, and the solvent was evaporated. The crude ketal **7** (8.0 g) was dissolved in warm methanol (450 ml) and heated at reflux with a solution of K_2CO_3 (1.0 g, 7.2 mmol) in water (50 ml) for 1 h. Most of the solvent was removed in vacuo, the residue was diluted with water and extracted with a mixture of ether (350 ml) and benzene (50 ml). The organic layer was washed with a saturated aqueous NaCl (2×) and water, dried, and the solvents were evaporated. The foamy residue was crystallized from methanol with several drops of pyridine, giving acetate **8** (5.0 g, 78%), m.p. 144–146 °C, $[\alpha]_D$ +10 (*c* 0.5, $CHCl_3$). IR ($CHCl_3$): 3609, 3456 (O–H); 1730 (C=O, acetate); 1374 (CH_3); 1247, 1039 (C–O, acetate); 1153, 1069, 1058, 950 (ring, dioxolane). ^{1}H NMR (400 MHz): 4.33 and 4.22 (2 H, AB system, *J* = 12.1, H-19, H-19′); 3.83–4.02 (4 H, OCH_2CH_2O); 4.74 (1 H, tt, *J* = 10.9, *J* = 4.9, H-3α); 2.06 (3 H, s, OAc); 1.28 (3 H, s, 3 × H-21); 0.75 (3 H, s, 3 × H-18). For $C_{25}H_{40}O_5$ (420.6) calculated: 71.39% C, 9.59% H; found: 71.07% C, 9.33% H.

20,20-(Ethylenedioxy)-3β-(methoxymethoxy)-5α-pregnan-19-ol (**10**)

Acetate **8** (1.0 g, 2.38 mmol) was dissolved in benzene (20 ml), the resulting solution was cooled in an ice bath, and *N*,*N*-diisopropylethylamine (5 ml, 28.7 mmol) and bromomethyl methyl ether (0.6 ml, 7.4 mmol) were added. The mixture was stirred at room temperature

for 2 h. The mixture was then partitioned between ether (100 ml) and cold 5% aqueous citric acid (100 ml). The organic layer was washed successively with a 5% aqueous citric acid, water, saturated aqueous $KHCO_3$ (2×), and water, dried, and the solvent was evaporated. The crude product (1.2 g) in benzene was applied onto a short column of alumina (30 ml) in benzene and eluted with a mixture of benzene–acetone (10:1). The obtained methoxymethoxy derivative **9** (1.0 g, 90%) was sufficiently pure to be used in the next step without further purification. A solution of sodium hydroxide (1.2 g, 30 mmol) in methanol (100 ml) was added to compound **9** (950 mg, 2.0 mmol), and the mixture was heated at reflux and stirred for 2.5 h. After cooling, a lump of solid carbon dioxide was added, and the mixture was concentrated to a small volume. An ice-cold, saturated aqueous $KHCO_3$ (200 ml) was then added, and the product was extracted with ether (100 ml). The ethereal layer was washed with $KHCO_3$, water, dried, and the solvent was evaporated to give 710 mg (82%) of **10**. An analytical sample was crystallized from methanol with several drops of pyridine, m.p. 161–162 °C, $[\alpha]_D$ +18 (*c* 0.26, $CHCl_3$). IR ($CHCl_3$): 3628, 3492 (O–H); 1374 (CH_3); 1145, 1102, 1038 (C–O–C–O–C); 1069, 1049, 949 (ring, dioxolane). ^{1}H NMR (400 MHz): 4.69 (2 H, s, OCH_2O); 3.83–4.02 (5 H, OCH_2CH_2O, H-19); 3.80 (1 H, bd, J = 11.6, H-19′); 3.55 (1 H, tt, J = 11.1, J = 5.1, H-3α); 3.37 (3 H, s, OCH_3); 1.29 (3 H, s, 3 × H-21); 0.80 (3 H, s, 3 × H-18). For $C_{25}H_{42}O_5$ (422.6) calculated: 71.05% C, 10.02% H; found: 71.11% C, 9.98% H.

(19*E*)-20,20-(Ethylenedioxy)-3β-(methoxymethoxy)-5α-pregnan-19-al *O*-[(Methoxycarbonyl)methyl]oxime (**12**)

Pyridine (2.5 ml, 30.9 mmol) was added dropwise at 0 °C under an argon atmosphere to a stirred suspension of chromium(VI) oxide (1.0 g, 10.0 mmol) and anhydrous magnesium sulfate (1.3 g) in dichloromethane (25 ml), and the stirring continued at 0 °C for 20 min. Subsequently, a solution of compound **10** (670 mg, 1.59 mmol) in dichloromethane (5 ml) was added, and the reaction mixture was stirred under argon at 0 °C for 2 h. After dilution with ether (30 ml), the mixture was filtered through a column of alumina (25 g), which was washed with an ether–dichloromethane mixture (1:1). The filtrate was concentrated, and pyridine was removed from the residue by coevaporation with toluene. The crude aldehyde **11** (650 mg, 1.54 mmol) was dissolved in pyridine (7 ml), *O*-(carboxymethyl)hydroxylamine hemihydrochloride (550 mg, 2.50 mmol) was added, and the mixture was stirred at room temperature for 24 h. Toluene (20 ml) was added, and the solvents were evaporated. The residue was partitioned between ethyl acetate (150 ml) and 5% citric acid (150 ml), and the separated aqueous phase was extracted with ethyl acetate (50 ml). The combined organic phases were washed with 5% citric acid (2×) and water (2×). After drying, the solvent was evaporated. The crude CMO derivative was suspended in ether (20 ml) and methanol (10 ml), and treated with an ethereal solution of diazomethane at 0 °C for 5 min. Excess diazomethane and the solvents were evaporated, and the residue was chromatographed on a column of silica gel (75 g) in a mixture of benzene–ether (50:1 to 20:1). The yield of foamy ester **12** was 710 mg (88%). IR ($CHCl_3$): 1756, 1739 (C=O); 1441, 1375 (CH_3); 1145, 1102, 1038 (C–O–C–O–C); 1070, 1049, 1009, 949 (ring, dioxolane). ^{1}H NMR (400 MHz): 7.62 (1 H, bs, H-19); 4.66 (2 H, s, OCH_2O); 4.63 (2 H, s, OCH_2COO); 3.84–4.00 (4 H, OCH_2CH_2O); 3.76 (3 H, s, $COOCH_3$); 3.53 (1 H, tt, J = 10.9, J = 4.5, H-3α); 3.35 (3 H, s, OCH_3); 1.28 (3 H, s, 3 × H-21); 0.72 (3 H, s, 3 × H-18). For $C_{28}H_{45}NO_7$ (507.7) calculated: 66.25% C, 8.93% H, 2.76% N; found: 66.32% C, 8.98% H, 2.69% N.

(19*E*)-3β-Hydroxy-20-oxo-5α-pregnan-19-al 19-{*O*-[(Methoxycarbonyl)methyl]oxime} (**13**)

The protected derivative **12** (690 mg, 1.36 mmol) was dissolved in a mixture of benzene (6 ml) and methanol (6 ml), and stirred with a 35% aqueous $HClO_4$ (0.6 ml) at 50 °C for 4 h. The reaction mixture was cooled, poured into a cold saturated aqueous $NaHCO_3$, and extracted with ethyl acetate (80 ml). The extract was washed successively with a saturated aqueous $NaHCO_3$ and water, dried, and the solvents were evaporated. The residue was chromatographed on silica gel (20 ml) in a mixture of benzene–acetone (20:1 to 6:1) to give ester **13** (450 mg, 79%) as a solid foam, $[\alpha]_D$ +75 (*c* 0.15, $CHCl_3$). IR ($CHCl_3$): 3608, 3507, 3388 (O–H); 1754 (C=O, OCH_2COO); 1699 (C=O, ketone); 1440, 1386, 1357 (CH_3); 1036 (C–OH). ^{1}H NMR (400 MHz): 7.61 (1 H, bs, H-19); 4.63 (2 H, s, OCH_2COO); 3.75 (3 H, s, $COOCH_3$); 3.63 (1 H, tt, *J* = 11.0, *J* = 4.8, H-3α); 2.51 (1 H, t, *J* = 9.0, H-17α); 2.11 (3 H, s, 3 × H-21); 0.58 (3 H, s, 3 × H-18). For $C_{24}H_{37}NO_5$ (419.6) calculated: 68.71% C, 8.89% H, 3.34% N; found: 68.42% C, 9.06% H, 3.14% N.

(19*E*)-3β-Hydroxy-20-oxo-5α-pregnan-19-al 19-[*O*-(Carboxymethyl)oxime] (**14**)

To a solution of ester **13** (300 mg, 0.72 mmol) in a mixture of THF (5 ml) and methanol (1 ml), 0.4 M sodium hydroxide (3.6 ml, 1.44 mmol) was added dropwise, and the mixture was stirred at room temperature for 5 h. The solution was concentrated in vacuo, diluted with ice-cold water (50 ml) and extracted with ethyl acetate (100 ml). The aqueous phase was acidified with a 5% hydrochloric acid, and crystallization was allowed to proceed at 0 °C for 1 h. The crystals were filtered off, washed with ice-cold water, and air-dried leaving 210 mg (72%) of the CMO derivative **14**. After recrystallization from a methanol–water mixture, the yield was 158 mg (54%), m.p. 190–192 °C, $[\alpha]_D$ +80 (*c* 0.37, $CHCl_3$). IR ($CHCl_3$): 3606, 3480, 3384 (O–H); 1769, 1733 (C=O, OCH_2COO); 1699 (C=O, ketone); 1387, 1357 (CH_3); 1034 (C–OH). ^{1}H NMR (500 MHz): 7.61 (1 H, d, *J* = 1.3, H-19); 4.63 and 4.58 (2 H, AB system, *J* = 16.6, CH_2COO); 3.65 (1 H, tt, *J* = 11.0, *J* = 4.8, H-3α); 2.51 (1 H, t, *J* = 9.0, H-17α); 2.11 (1 H, s, 3 × H-21); 0.55 (3 H, s, 3 × H-18). ^{13}C NMR: 209.72 (C-20), 173.60 (COOH), 153.87 (C-19), 71.15 (C-3), 70.02 (CH_2COO), 63.71 (C-17), 56.59 (C-14), 52.92 (C-9), 44.50 (C-5), 44.04 (C-13), 43.14 (C-10), 38.71 (C-4), 38.60 (C-12), 36.25 (C-8), 32.59 (C-1), 31.73 (C-7), 31.44 (C-21), 31.38 (C-2), 28.00 (C-6), 24.28 (C-15), 22.74 (C-16), 21.59 (C-11), 13.28 (C-18). For $C_{24}H_{37}NO_5$ (405.5) calculated: 68.12% C, 8.70% H, 3.45% N; found: 68.24% C, 8.79% H, 3.28% N.

20,20-(Ethylenedioxy)-3α-hydroxy-5α-pregnan-19-yl Acetate (**16**)

To a solution of compound **8** (3.0 g, 7.1 mmol) in pyridine (30 ml) cooled with ice, 4-toluenesulfonyl chloride (4.0 g, 21.0 mmol) was added, and the mixture was left at room temperature overnight. The mixture was then poured into water with ice (400 ml), the crystalline solids were filtered off and dissolved in a mixture of ether (350 ml) and benzene (50 ml). The solution was washed successively with a 5% aqueous citric acid, saturated aqueous $KHCO_3$ (2×), and water. After drying, the solvent was evaporated to give 3.6 g (88%) of the crude tosylate **15**, sufficiently pure for further processing. Tosylate **15** (3.5 g, 6.1 mmol) was dissolved in hexamethylphosphoramide (65 ml), and sodium nitrite (15 g, 217.4 mmol) was added. The mixture was stirred under argon atmosphere and heated to 90 °C for 2 h. After cooling, the mixture was poured into a saturated aqueous NaCl with ice (250 ml) and extracted with ether (200 ml). The ethereal layer was washed successively with a 5% aque-

ous citric acid (2×), saturated aqueous $KHCO_3$ (2×), and water. After drying, the solvent was evaporated. The residue (2.6 g) was chromatographed on a column of silica gel (150 g) in a mixture of benzene–acetone (50:1 to 20:1). The main fraction (1.5 g, 58%) contained the hydroxy derivative **16**, which was crystallized from an ether–hexanes mixture, m.p. 98–103 °C, $[\alpha]_D$ +18 (*c* 0.38, $CHCl_3$). IR ($CHCl_3$): 3616, 3482 (O–H); 1729 (C=O, acetate); 1375 (CH_3); 1246, 1036 (C–O, acetate); 1175, 1068, 1046, 950 (ring, dioxolane). ^{1}H NMR (400 MHz): 4.29 and 4.23 (2 H, AB system, J = 12.1, H-19, H-19′); 4.09 (1 H, bs, H-3β); 3.83–4.02 (4 H, OCH_2CH_2O); 2.06 (3 H, s, 19-OAc); 1.29 (3 H, s, 3 × H-21); 0.75 (3 H, s, 3 × H-18). For $C_{25}H_{40}O_5$ (420.6) calculated: 71.39% C, 9.59% H; found: 71.11% C, 9.62% H.

20,20-(Ethylenedioxy)-3α-(methoxymethoxy)-5α-pregnan-19-ol (**18**)

The hydroxy derivative **16** (1.2 g, 2.85 mmol) was treated as described for the preparation of **10**. First, intermediate **17** was prepared using benzene (20 ml), *N*,*N*-diisopropylethylamine (6 ml, 28.7 mmol), and bromomethyl methyl ether (0.6 ml, 7.4 mmol); the yield was 1.1 g (83%). Then, deacetylation of **17** (1.07 g, 2.30 mmol) with a solution of sodium hydroxide (1.2 g, 30 mmol) in methanol (100 ml) gave 825 mg (85%) of the hydroxy derivative **18**. An analytical sample was crystallized from methanol with a drop of pyridine, m.p. 122–124 °C, $[\alpha]_D$ +12 (*c* 0.23, $CHCl_3$). IR ($CHCl_3$): 3630, 3492 (O–H); 1374 (CH_3); 1142, 1094, 1038 (C–O–C–O–C); 1067, 1048, 950 (ring, dioxolane). ^{1}H NMR (400 MHz): 4.67 (2 H, m, OCH_2O); 3.83–4.02 (6 H, OCH_2CH_2O, H-19, H-3β); 3.77 (1 H, dd, J = 11.6, J = 5.3, H-19′); 3.38 (3 H, s, CH_3O); 1.29 (3 H, s, 3 × H-21); 0.80 (3 H, s, 3 × H-18). For $C_{25}H_{42}O_5$ (422.6) calculated: 71.05% C, 10.02% H; found: 71.01% C, 10.23% H.

(19*E*)-20,20-(Ethylenedioxy)-3α-(methoxymethoxy)-5α-pregnan-19-al *O*-[(Methoxycarbonyl)methyl]oxime (**20**)

Pyridine (2.9 ml, 35.8 mmol), chromium(VI) oxide (1.2 g, 12.0 mmol), anhydrous magnesium sulfate (1.5 g), and dichloromethane (30 ml) were treated as described for the preparation of **12**, and compound **18** (780 mg, 1.85 mmol) in dichloromethane (7.5 ml) was added to the resulting mixture. After oximation of **19** with *O*-(carboxymethyl)hydroxylamine hemihydrochloride (620 mg, 2.84 mmol) in pyridine (8 ml) and methylation as above, 650 mg (69%) of oxime **20** was obtained. ^{1}H NMR (400 MHz): 7.65 (1 H, bs, H-19); 4.65 (2 H, s, OCH_2O); 4.63 (2 H, s, OCH_2COO); 3.82–4.01 (5 H, OCH_2CH_2O, H-3β); 3.75 (3 H, s, $COOCH_3$); 3.37 (3 H, s, CH_3O); 1.28 (3 H, s, 3 × H-21); 0.72 (3 H, s, 3 × H-18). The product after chromatography contained the corresponding deprotected ketone (2.51 t, J = 8.8, H-17α; 2.11 s, 3 × H-21; 0.58 s, 3 × H-18) and was used in the next step without further purification.

(19*E*)-3α-Hydroxy-20-oxo-5α-pregnan-19-al 19-{*O*-[(Methoxycarbonyl)methyl]oxime} (**21**)

The protected derivative **20** (540 mg, 1.06 mmol) was dissolved in a mixture of benzene (5 ml) and methanol (5 ml), and stirred with a 35% aqueous $HClO_4$ (0.5 ml) at 50 °C for 4 h. The reaction mixture was cooled, poured into a cold saturated aqueous $NaHCO_3$, and extracted with ethyl acetate (70 ml). The extract was washed successively with a saturated aqueous $NaHCO_3$ and water, dried, and the solvents were evaporated. The residue was chromatographed on silica gel (20 ml) in a mixture of benzene–acetone (20:1 to 10:1) to give ester **21** (290 mg, 65%). An analytical sample was crystallized from methanol, m.p.

155–156 °C, $[\alpha]_D$ +101 (*c* 0.26, $CHCl_3$). IR ($CHCl_3$): 3616, 3491 (O–H); 1755 (C=O, OCH_2COO); 1699 (C=O, ketone); 1439, 1385, 1357 (CH_3); 1101 (C–O). ^{1}H NMR (400 MHz): 7.64 (1 H, bs, H-19); 4.62 (2 H, s, OCH_2COO); 4.08 (1 H, bs, H-3β); 3.74 (3 H, s, $COOCH_3$); 2.52 (1 H, t, *J* = 9.0, H-17α); 2.11 (3 H, s, 3 × H-21); 0.58 (3 H, s, 3 × H-18). For $C_{24}H_{37}NO_5$ (419.6) calculated: 68.71% C, 8.89% H, 3.34% N; found: 68.55% C, 8.84% H, 3.45% N.

(19*E*)-3α-Hydroxy-20-oxo-5α-pregnan-19-al 19-[*O*-(Carboxymethyl)oxime] (**22**)

To a solution of ester **21** (240 mg, 0.57 mmol) in a mixture of THF (3.5 ml) and methanol (0.7 ml), 0.4 M aqueous sodium hydroxide (2.8 ml, 1.1 mmol) was added dropwise, and the mixture was stirred at room temperature for 3 h. The solution was concentrated in vacuo, diluted with ice-cold water (40 ml), and extracted with ethyl acetate (70 ml). The aqueous solution was acidified with a 5% hydrochloric acid, and crystallization was allowed to proceed at 0 °C for 1 h. The crystals were filtered off, washed with ice-cold water, and air-dried, leaving 205 mg (88%) of the CMO derivative **22**. After recrystallization from a methanol–water mixture, the yield was 160 mg (69%), m.p. 157–158 °C, $[\alpha]_D$ +97 (*c* 0.40, $CHCl_3$). IR ($CHCl_3$): 3616, 3505, 3109 (O–H); 1769, 1734 (C=O, OCH_2COO); 1699 (C=O, ketone); 1386, 1358 (CH_3); 1003 (C–OH). ^{1}H NMR (500 MHz): 7.65 (1 H, d, *J* = 1.3, H-19); 4.64 (2 H, s, CH_2COO); 4.07 (1 H, p, *J* = 2.8, H-3β); 2.52 (1 H, t, *J* = 9.0, H-17α); 2.11 (1 H, s, 3 × H-21); 0.56 (3 H, s, 3 × H-18). ^{13}C NMR: 209.86 (C-20), 173.86 (COOH), 153.80 (C-19), 69.85 (CH_2COO), 66.37 (C-3), 63.72 (C-17), 56.69 (C-14), 53.03 (C-9), 44.06 (C-13), 43.86 (C-10), 38.70 (C-5), 38.63 (C-12), 36.67 (C-4), 36.25 (C-8), 31.72 (C-7), 31.45 (C-21), 29.31 (C-2), 27.97 (C-1), 27.94 (C-6), 24.26 (C-15), 22.71 (C-16), 21.06 (C-11), 13.24 (C-18). For $C_{23}H_{35}NO_5$ (405.5) calculated: 68.12% C, 8.70% H, 3.45% N; found: 68.35% C, 9.03% H, 3.29% N.

*We thank Ms L. Kloučková for excellent technical assistance. We are indebted to Dr P. Fiedler for recording and interpretation of the IR spectra. Our thanks are due to Ms M. Snopková for the measurements of 400 MHz ^{1}H NMR spectra and Dr M. Buděšínský for taking and interpretation of the NMR spectra of haptens **14** and **22**. Elemental analyses were carried out in the Analytical Laboratory (Dr L. Holasová, Head). This work was carried out as a part of the Academy of Sciences of the Czech Republic research project (Z4 055 905) and supported by the Grant Agency of the Czech Republic (grant No. 203/04/0123).*

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