# COUPLING OF STEROID *O*-(CARBOXYMETHYL)OXIME DERIVATIVES WITH AMINO ALCOHOLS*

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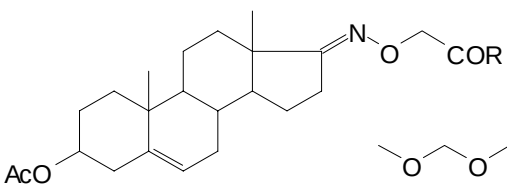
New approach to the preparation of steroids with connecting bridge, based on an *O*-carboxymethyloxime (CMO) structure, and with terminal hydroxy group, is presented. 17-CMO derivatives of 3β-acetoxy- and 3β-methoxymethoxyandrost-5-en-17-one were condensed with α,ω-amino alcohols to give derivatives with a chain of seven to nine atoms. After THP-protection, these compounds were converted to 3-keto-4-ene derivatives. An alternative synthesis consisted in transformation of 17-CMO derivatives with bonded amino acids by reduction of the terminal carboxyl. The resulting compounds were designed as building blocks for the preparation of bis-haptens for sandwich immunoassays.

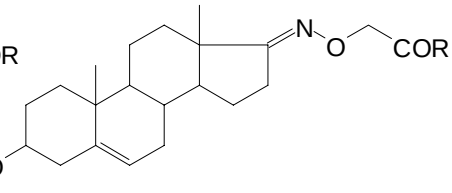
**Key words:** Steroid synthesis; Haptens; Connecting bridge.

Mikola et al.[2] studied reactions of steroid ketones with *O*-(ω-hydroxyalkyl)hydroxylamines which gave ω-hydroxy substituted *O*-alkyloximes. The preparation of starting hydroxylamines[2] was rather complicated and we decided to synthesize derivatives with similar connecting bridge from the available steroid (*O*-carboxymethyl)oximes (CMO). In the oxime derivatives reported by Mikola a chain of six or eight atoms bearing a terminal hydroxyl group was introduced instead of the original carbonyl group. The use of a CMO derivative requires condensation of the carboxyl group with a suitable α,ω-substituted linear synthon containing at one end a hydroxyl group or its precursor. We used synthons with two to four carbon atoms that corresponded to a resulting chain of seven to nine atoms, not counting the terminal hydroxyl group. For attaching to the carboxyl group we chose the amide bond and consequently worked with amino alcohols as derivatives of choice. We already made use of a similar approach[3] in the condensation of CMO derivatives with amino acids or short peptides (Gly, β-Ala, GlyGly).

We started with the known[3] (17*E*)-CMO derivatives **1** and **7** and used mixed anhydrides for the activation of the CMO carboxyl[3]. Protection of the hydroxyl in ω-hydroxy-

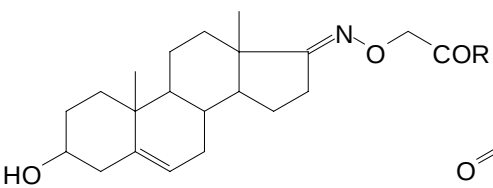
* Part CCCXXXI in the series On Steroids; Part CCCXXX: see ref.[1].

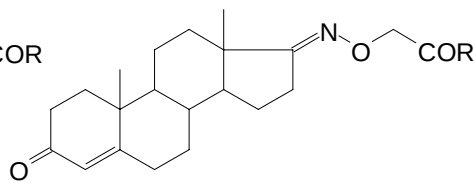



| | R |
|---|---|
| **1** | OH |
| **2** | $NH(CH_2)_2OH$ |
| **3** | $NH(CH_2)_3OH$ |
| **4** | $NHCH_2CH(OH)CH_3$ |
| **5** | $NH(CH_2)_4OH$ |
| **6** | $NHC(CH_3)_2CH_2OH$ |

| | R |
|---|---|
| **7** | OH |
| **8** | $NH(CH_2)_2OH$ |
| **9** | $NH(CH_2)_3OH$ |
| **10** | $NHCH_2COOCH_2CH_3$ |
| **11** | $NHCH_2COOH$ |
| **12** | $NH(CH_2)_2COOCH_3$ |
| **13** | $NH(CH_2)_2COOH$ |




| | R |
|---|---|
| **14** | $NH(CH_2)_2OH$ |
| **15** | $NH(CH_2)_3OH$ |
| **16** | $NHCH_2CH(OH)CH_3$ |
| **17** | $NH(CH_2)_4OH$ |
| **18** | $NHC(CH_3)_2CH_2OH$ |
| **19** | $NH(CH_2)_2OTHP$ |
| **20** | $NH(CH_2)_3OTHP$ |
| **21** | $NHCH_2CH(OTHP)CH_3$ |
| **22** | $NH(CH_2)_4OTHP$ |
| **23** | $NHC(CH_3)_2CH_2OTHP$ |

| | R |
|---|---|
| **24** | $NH(CH_2)_2OH$ |
| **25** | $NH(CH_2)_3OH$ |
| **26** | $NHCH_2CH(OH)CH_3$ |
| **27** | $NH(CH_2)_4OH$ |
| **28** | $NHC(CH_3)_2CH_2OH$ |

alkylamines was not necessary because the amino group was more reactive. The reaction of **1** with 2-aminoethanol, 3-amino-1-propanol, 1-amino-2-propanol, 4-amino-1-butanol, and 2-amino-2-methyl-1-propanol gave the corresponding substituted amides **2–6**. Their IR spectra displayed hydroxyl (about 3 600 $cm^{-1}$), amide N–H (about 3 400 $cm^{-1}$), and amide I and II (about 1 660 and 1 535 $cm^{-1}$) bands. The $^1H$ NMR spectra exhibited characteristic signals of protons on carbon atoms bearing hydroxyl and amide groups at δ 3.1–3.9, and an N–H signal at δ 6.3–6.7, together with signals of the parent CMO derivative. In the spectrum of **6**, the N–H signal formed a broad singlet (δ 6.28) and the $CH_2OH$ protons gave an AB system with $J$ = 12 Hz.

The second approach we employed consisted in the transformation of derivatives with a chain, obtained by elongation with amino acids. CMO derivatives with bonded amino acids can be reduced at the terminal carboxyl to give the corresponding ω-hydroxy derivatives.

The 17-CMO derivatives with bonded amino acids were prepared by hydrolysis of the corresponding ethyl and methyl esters **10** and **12**. The former ester is known[3] and the latter was prepared by the same procedure[3]. In this case, the 3-hydroxyl group was protected with the methoxymethyl group because an acetyl protective group could be hydrolyzed with the slightly alkaline aqueous suspension of sodium borohydride. Reduction[4] of the mixed anhydride, prepared from glycine derivative **11**, gave 30% of the desired hydroxy derivative **8**; the β-alanine derivative **13** afforded 41% of the hydroxy derivative **9**. In both reactions a considerable amount of the starting acid was recovered (56% and 42%, respectively). Compounds **8** and **9** were independently prepared from derivative **7** by coupling with 2-aminoethanol and 3-amino-1-propanol, respectively.

The protecting group in position 3 of the steroid skeleton was removed by hydrolysis with sodium hydroxide (acetates) or hydrochloric acid (methoxymethyl derivatives). Compounds **2–6**, **8**, and **9** were thus converted into the corresponding hydroxy derivatives **14–18**.

For the preparation of 3-keto-4-ene derivatives, the hydroxyl group in acetates **2–6** was first blocked by reaction with 3,4-dihydro-2*H*-pyran and then hydrolyzed to give the 3-hydroxy derivatives **19–23**. These compounds were subjected to modified[5] Oppenauer oxidation and after acid-catalyzed cleavage of the THP group converted into the corresponding derivatives **24–28**. Their IR spectra displayed characteristic bands at 1 666 $cm^{-1}$ (overlapping conjugated ketone and amide I bands) and 1 616 $cm^{-1}$ (conjugated double bond). The $^1H$ NMR spectra corresponded to those of the starting acetates (except signals of the acetyl group, H-3α, and H-6). The H-19 signal was shifted downfield by about 0.15 ppm and the H-4 signal was observed at about δ 5.75 as a broad singlet or a narrow doublet ($J$ = 1–1.5 Hz).

Steroid derivatives containing in position 17 a spacer with terminal hydroxyl group may be used in preparation of bis-haptens by reaction with a carboxyl group of another

steroid CMO-derivative. In sandwich immunoassays, unsymmetrical steroid bis-haptens were found to be more specific[6] (low cross-reactivity) than symmetrical bis-haptens.

## EXPERIMENTAL

Melting points were determined on a micro melting point apparatus Boetius (Germany). Optical rotations were measured at 25 °C on a Perkin–Elmer 141 MC polarimeter. Infrared spectra (wavenumbers in $cm^{-1}$) were recorded on Bruker IFS 88 spectrometer. $^1$H NMR spectra were taken on a Varian UNITY-200 spectrometer (200 MHz, FT mode) at 23 °C in deuteriochloroform with tetramethylsilane as internal standard. Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) and multiplet widths (*W*) in Hz. Thin-layer chromatography was performed on silica gel G (ICN Biochemicals), detection by spraying with concentrated sulfuric acid followed by heating. Preparative TLC was carried out on plates 200 × 200 × 0.4 mm, column chromatography on silica gel 60–120 μm. Prior to evaporation in vacuo on a rotary evaporator (bath temperature 50 °C), solutions in organic solvents were dried over anhydrous sodium sulfate.

General Procedure for Coupling 17-CMO Derivatives with Amino Alcohols

A solution of ethyl chloroformate in THF (*c* 1 mol $l^{-1}$, 2.2 ml) was added dropwise at –5 °C to a solution of the 17-CMO derivative (2.0 mmol) and *N*,*N*-diisopropylethylamine (383 μl, 2.2 mmol) in THF (12 ml). The reaction mixture was stirred at –5 °C for 40 min and then a solution of the amino alcohol (4.4 mmol) in THF (2 ml) was added. After stirring at 0 °C for 4 h, the reaction mixture was poured into brine (100 ml). The product was twice extracted with ethyl acetate, the combined extracts were washed with water (2 times), dried and the solvent was evaporated. The residue was chromatographed on a column of silica gel (30 g) in benzene–acetone (9 : 1).

*(17E)-3*β*-Acetoxyandrost-5-en-17-one O-{[N-(2-hydroxyethyl)carbamoyl]methyl}oxime* (**2**). Reaction of compound **1** (807 mg, 2.0 mmol) with 2-aminoethanol (266 μl, 4.4 mmol) afforded 801 mg (90%) of product **2**, m.p. 125–127 °C (dichloromethane–hexane), $[\alpha]_D$ –37° (*c* 1.7, chloroform). IR (chloroform): 3 624 (OH); 3 431 (NH); 1 725 (C=O); 1 668, 1 536 (CONH); 1 254 (C–O). $^1$H NMR: 0.93 s, 3 H (3 × H-18); 1.04 s, 3 H (3 × H-19); 2.04 s, 3 H ($CH_3COO$); 3.48 q, 2 H, $J$ = 5.1 ($NHCH_2$); 3.75 q, 2 H, $J$ = 5.1 ($CH_2OH$); 4.50 s, 2 H ($OCH_2CO$); 4.60 m, 1 H, $W$ = 32 (H-3α); 5.39 bd, 1 H, $J \approx 4.5$ (H-6); 6.67 bt, 1 H, $J \approx 5$ (NH). For $C_{25}H_{38}N_2O_5$ (446.6) calculated: 67.24% C, 8.58% H, 6.27% N; found: 67.42% C, 8.74% H, 6.03% N.

*(17E)-3*β*-Acetoxyandrost-5-en-17-one O-{[N-(3-hydroxypropyl)carbamoyl]methyl}oxime* (**3**). Reaction of compound **1** (807 mg, 2.0 mmol) with 3-amino-1-propanol (337 μl, 4.4 mmol) afforded 800 mg (87%) of product **3**, m.p. 139–140 °C (dichloromethane–hexane), $[\alpha]_D$ –35° (*c* 2.2, chloroform). IR (chloroform): 3 624 (OH); 3 431 (NH); 1 725 (C=O); 1 662, 1 536 (CONH); 1 254 (C–O). $^1$H NMR: 0.93 s, 3 H (3 × H-18); 1.04 s, 3 H (3 × H-19); 2.03 s, 3 H ($CH_3COO$); 3.48 q, 2 H, $J$ = 6.2 ($NHC\mathbf{H}_2$); 3.75 t, 2 H, $J$ = 5.3 ($CH_2OH$); 4.49 s, 2 H ($OCH_2CO$); 4.60 m, 1 H, $W$ = 32 (H-3α); 5.39 bd, 1 H, $J \approx 4.5$ (H-6); 6.53 bt, 1 H, $J \approx 6$ (NH). For $C_{26}H_{40}N_2O_5$ (460.6) calculated: 67.80% C, 8.75% H, 6.08% N; found: 67.61% C, 8.28% H, 5.95% N.

*(17E)-3*β*-Acetoxyandrost-5-en-17-one O-{[N-(2-hydroxypropyl)carbamoyl]methyl}oxime* (**4**). Reaction of compound **1** (807 mg, 2.0 mmol) with 1-amino-2-propanol (350 μl, 4.4 mmol) afforded 890 mg (97%) of product **4**, m.p. 167–169 °C (acetone–hexane), $[\alpha]_D$ –36° (*c* 1.7, chloroform). IR (chloroform): 3 620 (OH); 3 433 (NH); 1 725 (C=O); 1 668, 1 535 (CONH); 1 254 (C–O, acetate); 1 032 (C–O). $^1$H NMR: 0.92 s, 3 H (3 × H-18); 1.04 s, 3 H (3 × H-19); 1.20 d, 3 H, $J$ = 6.4 ($CH_3CH$–O); 2.03 s, 3 H ($CH_3COO$); 3.15 m and 3.90 m, 2 × 1 H ($NHC\mathbf{H}_2$); 3.50 m, 1 H (CHOH); 4.48 s, 2 H ($OCH_2CO$); 4.60 m, 1 H, $W$ = 32 (H-3α); 5.39 bd, 1 H, $J \approx 4.5$ (H-6); 6.67 bt, 1 H, $J \approx 5.5$ (NH).

For $C_{26}H_{40}N_2O_5$ (460.6) calculated: 67.80% C, 8.75% H, 6.08% N; found: 68.05% C, 8.52% H, 5.91% N.

*(17E)-3*β*-Acetoxyandrost-5-en-17-one O-{[N-(4-hydroxybutyl)carbamoyl]methyl}oxime* (**5**). Reaction of compound **1** (807 mg, 2.0 mmol) with 4-amino-1-butanol (406 μl, 4.4 mmol) afforded 793 mg (84%) of product **5**, m.p. 102–104 °C (dichloromethane–hexane), $[\alpha]_D$ –34° (*c* 1.6, chloroform). IR (chloroform): 3 607 (OH); 3 432 (NH); 1 725 (C=O); 1 668, 1 536 (CONH); 1 254 (C–O). $^1$H NMR: 0.93 s, 3 H (3 × H-18); 1.05 s, 3 H (3 × H-19); 2.04 s, 3 H ($CH_3COO$); 3.36 q, 2 H, *J* = 6.1 (NHC**H**$_2$); 3.68 bt, 2 H, *J* ≈ 5 (C**H**$_2$OH); 4.46 s, 2 H ($OCH_2CO$); 4.60 m, 1 H, *W* = 32 (H-3α); 5.39 bd, 1 H, *J* ≈ 4.5 (H-6); 6.37 bt, 1 H, *J* ≈ 6 (NH). For $C_{27}H_{42}N_2O_5$ (474.6) calculated: 68.32% C, 8.92% H, 5.90% N; found: 68.27% C, 8.95% H, 5.99% N.

*(17E)-3*β*-Acetoxyandrost-5-en-17-one O-{[N-(1-hydroxy-2-methyl-2-propyl)carbamoyl]methyl}oxime* (**6**). Reaction of compound **1** (807 mg, 2.0 mmol) with 2-amino-2-methyl-1-propanol (392 mg, 4.4 mmol) afforded 832 mg (88%) of product **6**, m.p. 122–124 °C (ether), $[\alpha]_D$ –34° (*c* 1.5, chloroform). IR (chloroform): 3 632, 3 624 (OH); 3 405 (NH); 1 725 (C=O); 1 658, 1 536 (CONH); 1 380, 1 350 ($C(CH_3)_2$); 1 254 (C–O). $^1$H NMR: 0.94 s, 3 H (3 × H-18); 1.05 s, 3 H (3 × H-19); 1.30 s, 6 H ($C(CH_3)_2$); 2.04 s, 3 H ($CH_3COO$); 3.58 and 3.64 AB system, 2 H, *J*(A,B) = 12 (C**H**$_2$OH); 4.41 s, 2 H ($OCH_2CO$); 4.63 m, 1 H, *W* = 32 (H-3α); 5.39 bd, 1 H, *J* ≈ 4.5 (H-6); 6.28 bs, 1 H (NH). For $C_{27}H_{42}N_2O_5$ (474.6) calculated: 68.32% C, 8.92% H, 5.90% N; found: 68.12% C, 8.63% H, 5.65% N.

*(17E)-3*β*-Methoxymethoxyandrost-5-en-17-one O-{[N-(2-hydroxyethyl)carbamoyl]methyl}oxime* (**8**). Reaction of compound **7** (811 mg, 2.0 mmol) with 2-aminoethanol (266 μl, 4.4 mmol) afforded 717 mg (80%) of product **8**, m.p. 122–125/138–140 °C (methanol), $[\alpha]_D$ –33° (*c* 1.0, chloroform). IR (chloroform): 3 626, 3 400 (OH); 3 432 (NH); 1 668, 1 535 (CONH); 1 148, 1 102, 1 037 ($CH_3OCH_2O$); 1 066 (C–O). $^1$H NMR: 0.94 s, 3 H (3 × H-18); 1.04 s, 3 H (3 × H-19); 3.38 s, 3 H ($CH_3O$); 3.48 q, 2 H, *J* = 5.3 ($NHCH_2$); 3.75 m, 2 H ($CH_2OH$); 4.50 s, 2 H ($OCH_2CO$); 4.70 s, 2 H ($OCH_2O$); 5.37 bd, 1 H, *J* ≈ 4.5 (H-6); 6.70 bt, 1 H, *J* ≈ 5 (NH). $^1$H NMR (after addition of trichloroacetyl isocyanate): 0.93 s, 3 H (3 × H-18); 1.03 s, 3 H (3 × H-19); 3.37 s, 3 H ($CH_3O$); 3.42 m, 1 H, *W* = 32 (H-3α); 3.69 q, 2 H, *J* = 5.3 ($NHCH_2$); 4.39 t, 2 H, *J* = 5.3 ($CH_2OOC$); 4.50 s, 2 H ($OCH_2CO$); 4.69 s, 2 H ($OCH_2O$); 5.36 bd, 1 H, *J* ≈ 4.5 (H-6); 6.71 bt, 1 H, *J* ≈ 5 (NH); 8.56 s, 1 H ($CCl_3CONHCOO$). For $C_{25}H_{40}N_2O_5$ (448.6) calculated: 66.94% C, 8.99% H, 6.24% N; found: 66.85% C, 8.73% H, 6.21% N.

*(17E)-3*β*-Methoxymethoxyandrost-5-en-17-one O-{[N-(3-hydroxypropyl)carbamoyl]methyl}oxime* (**9**). Reaction of compound **7** (811 mg, 2.0 mmol) with 3-amino-1-propanol (337 μl, 4.4 mmol) afforded 681 mg (74%) of product **9**, m.p. 117–118 °C (acetone–hexane), $[\alpha]_D$ –32° (*c* 1.7, chloroform). IR (chloroform): 3 628 (OH); 3 400 (NH); 1 662, 1 536 (CONH); 1 148, 1 103, 1 036 ($CH_3OCH_2O$). $^1$H NMR: 0.93 s, 3 H (3 × H-18); 1.03 s, 3 H (3 × H-19); 3.37 s, 3 H ($CH_3O$); 3.48 q, 2 H, *J* ≈ 6 ($NHCH_2$); 3.65 t, 2 H, *J* = 5.5 ($CH_2OH$); 4.49 s, 2 H ($OCH_2CO$); 4.69 s, 2 H ($OCH_2O$); 5.36 bd, 1 H, *J* ≈ 4.5 (H-6); 6.54 bt, 1 H, *J* ≈ 5.5 (NH). For $C_{26}H_{42}N_2O_5$ (462.6) calculated: 67.50% C, 9.15% H, 6.06% N; found: 67.45% C, 9.12% H, 5.97% N.

(17*E*)-3β-Methoxymethoxyandrost-5-en-17-one *O*-{[*N*-(Carboxymethyl)carbamoyl]methyl}oxime (**11**)

Aqueous 0.4 M potassium hydroxide solution (20 ml) was added to a solution of ester[3] **10** (981 mg, 2.0 mmol) in methanol (50 ml). After stirring at room temperature for 5 h the reaction mixture was acidified with dilute hydrochloric acid (1 : 4) and the solvents were evaporated in vacuo. The residue was partitioned between water and ethyl acetate and the aqueous phase was extracted with ethyl acetate. The combined extracts were washed with water (3 times) and dried. Evaporation of the solvent in vacuo afforded 890 mg (96%) of acid **11**, m.p. 154–156 °C (acetone–water), $[\alpha]_D$ –32° (*c* 1.8,

chloroform). IR (chloroform): 3 500–2 500 (COOH); 3 420 (NH); 1 734 (C=O); 1 672, 1 539 (CONH); 1 148, 1 103, 1 036 (C–O). $^1$H NMR: 0.93 s, 3 H (3 × H-18); 1.03 s, 3 H (3 × H-19); 3.38 s, 3 H ($OCH_3$); 3.44 m, 1 H, *W* = 32 (H-3α); 4.12 d, 2 H, *J* = 4.9 ($NHCH_2CO$); 4.55 s, 2 H ($OCH_2CO$); 4.70 s, 2 H ($OCH_2O$); 5.37 bd, 1 H, *J* ≈ 4.5 (H-6); 6.92 t, 1 H, *J* = 4.9 (NH). For $C_{25}H_{38}N_2O_6$ (462.6) calculated: 64.91% C, 8.28% H, 6.06% N; found: 65.05% C, 7.98% H, 5.84% N.

(17*E*)-3β-Methoxymethoxyandrost-5-en-17-one *O*-{{*N*-[2-(Methoxycarbonyl)ethyl]carbamoyl}-methyl}oxime (**12**)

To a solution of 17-CMO derivative **7** (811 mg, 2.0 mmol) and *N*,*N*-diisopropylethylamine (766 μl, 4.4 mmol) in THF (12 ml) was added dropwise at –5 °C 1 M solution of ethyl chloroformate in THF (2.2 ml). The reaction mixture was stirred at –5 °C for 40 min and then β-alanine methyl ester hydrochloride (307 mg, 2.2 mmol) was added. After stirring at 0 °C for 1 h, and at room temperature for 2 h, the reaction mixture was poured into brine (100 ml). The product was extracted with ethyl acetate, the extract was washed with water (2 times), dried and the solvent was evaporated. The residue was chromatographed on a column of silica gel (50 g) in benzene–ether (80 : 20) to give 692 mg (71%) of compound **12**, m.p. 92–94 °C (ether), $[\alpha]_D$ –23° (*c* 2.1, chloroform). IR (chloroform): 3 435 (NH); 1 731 (C=O); 1 670, 1 532 (CONH); 1 148, 1 103, 1 037 (C-O). $^1$H NMR: 0.93 s, 3 H (3 × H-18); 1.04 s, 3 H (3 × H-19); 2.55 t, 2 H, *J* = 6.1 ($NHCH_2C\mathbf{H}_2CO$); 3.37 s, 3 H ($OCH_3$); 3.57 q, 2 H, *J* = 6.1 ($NHC\mathbf{H}_2CH_2CO$); 3.69 s, 3 H ($COOCH_3$); 4.47 s, 2 H ($OCH_2CO$); 4.69 s, 2 H ($OCH_2O$); 5.36 bd, 1 H, *J* ≈ 4.5 (H-6); 6.87 bt, 1 H, *J* ≈ 5.5 (NH). For $C_{27}H_{42}N_2O_6$ (490.5) calculated: 66.10% C, 8.63% H, 5.71% N; found: 66.35% C, 8.75% H, 5.52% N.

(17*E*)-3β-Methoxymethoxyandrost-5-en-17-one *O*-{[*N*-(2-Carboxyethyl)carbamoyl]methyl}oxime (**13**)

Ester **12** (981 mg, 2.0 mmol) was hydrolyzed by the same procedure as ester **10** in the preparation of **11**. Crystallization of the residue from ether–hexane afforded 850 mg (89%) of acid **13**, m.p. 128–131 °C, $[\alpha]_D$ –30° (*c* 1.5, chloroform). IR (chloroform): 3 500–2 500 (COOH); 3 452 (NH); 1 712 (C=O, acid dimer); 1 670, 1 533 (CONH); 1 147, 1 103 (C–O). $^1$H NMR: 0.92 s, 3 H (3 × H-18); 1.03 s, 3 H (3 × H-19); 2.60 t, 2 H, *J* = 5.8 ($NHCH_2C\mathbf{H}_2CO$); 3.38 s, 3 H ($OCH_3$); 3.42 m, 1 H (H-3α); 3.58 q, 2 H, *J* = 5.8 ($NHC\mathbf{H}_2CH_2CO$); 4.49 s, 2 H ($OCH_2CO$); 4.70 s, 2 H ($OCH_2O$); 5.37 bd, 1 H, *J* ≈ 4.5 (H-6); 6.94 bt, 1 H, *J* ≈ 6 (NH). For $C_{26}H_{40}N_2O_6$ (476.6) calculated: 65.52% C, 8.46% H, 5.88% N; found: 65.63% C, 8.45% H, 6.01% N.

(17*E*)-3β-Methoxymethoxyandrost-5-en-17-one *O*-{[*N*-(2-Hydroxyethyl)carbamoyl]methyl}oxime (**8**)

A solution of ethyl chloroformate in THF (*c* 1 mol $l^{-1}$, 0.6 ml) was added dropwise to a solution of acid **11** (231 mg, 0.5 mmol) and *N*,*N*-diisopropylethylamine (105 μl, 0.6 mmol) in THF (3 ml) cooled at –5 °C. The reaction mixture was stirred for 40 min at –5 °C and then transferred via cannula during 10 min to a cooled (ice bath) suspension of sodium borohydride (47 mg, 1.25 mmol) in water (1 ml). The reaction mixture was stirred at room temperature for 2 h and then poured into dilute hydrochloric acid (1 : 4, 10 ml). The product was extracted with ethyl acetate (2 times), the combined extracts were washed with dilute hydrochloric acid and water, dried and the solvent was evaporated. The residue was chromatographed on a column of silica gel (20 g). The starting acid **11** (130 mg, 56%) was eluted with benzene–acetone (9 : 1), further elution with the same solvent mixture afforded 70 mg (30%) of compound **8**, m.p. 121–124/136–138 °C (methanol), $[\alpha]_D$ –31° (*c* 0.7, chloroform), identical with the product prepared from the 17-CMO derivative **7** and 2-aminoethanol.

(17*E*)-3β-Methoxymethoxyandrost-5-en-17-one *O*-{[*N*-(3-Hydroxypropyl)carbamoyl]methyl}oxime (**9**)

The title compound was prepared from acid **13** (285 mg, 0.5 mmol) by the same procedure as the hydroxy derivative **8** from acid **11**. Chromatography on a column of silica gel (20 g) in benzene–acetone (9 : 1) afforded 120 mg (42%) of the starting acid **13** and 95 mg (41%) of compound **9**, m.p. 117–118 °C (methanol), $[\alpha]_D$ –32° (*c* 1.8, chloroform), identical with the product prepared from the 17-CMO derivative **7** and 3-amino-1-propanol.

General Procedure for Deprotection of 3-Acetoxy and 3-Methoxymethoxy Derivatives

*A*) To a solution of the acetate (0.2 mmol) in THF (2 ml) and methanol (1 ml) was added 0.4 M aqueous sodium hydroxide (0.5 ml). After stirring at room temperature for 4 h the reaction was neutralized with dilute hydrochloric acid (1 : 4) and the solvents were evaporated in vacuo. The residue was dissolved in ethyl acetate and water and the aqueous phase was extracted with ethyl acetate. The combined organic phases were washed with water, dried and the solvent was evaporated. The residue was crystallized from acetone–ether, unless stated otherwise.

*B*) Concentrated hydrochloric acid (50 μl) was added to a solution of methoxymethoxy derivative (0.2 mmol) in benzene (3 ml) and methanol (3 ml). After stirring at 42 °C for 9 h the solvents were evaporated in vacuo and the residue was dissolved in dichloromethane. The solution was washed with water, dried and the solvent was evaporated. The residue was chromatographed on a preparative silica gel plate in benzene–acetone (1 : 1).

*(17E)-3β-Hydroxyandrost-5-en-17-one O-{[N-(2-hydroxyethyl)carbamoyl]methyl}oxime* (**14**). Deprotection of acetate **2** (90 mg) by method *A* afforded 70 mg (86%) of hydroxy derivative **14**, m.p. 176–179 °C, $[\alpha]_D$ –40° (*c* 1.3, chloroform). IR (chloroform): 3 610 (OH); 3 433 (NH); 1 669, 1 534 (CONH); 1 048 (C–O). $^1$H NMR: 0.92 s, 3 H (3 × H-18); 1.02 s, 3 H (3 × H-19); 3.45 q, 2 H, $J$ = 5 (NHC**H**$_2$); 3.50 m, 1 H, $W$ = 32 (H-3α); 3.71 t, 2 H, $J$ = 5 (C**H**$_2$OH); 4.48 s, 2 H (OCH$_2$CO); 5.35 bd, 1 H, $J \approx 4.5$ (H-6); 6.69 bt, 1 H, $J \approx 5$ (NH). For $C_{23}H_{36}N_2O_4$ (404.6) calculated: 68.29% C, 8.97% H, 6.92% N; found: 68.45% C, 9.03% H, 6.73% N.

Deprotection of methoxymethoxy derivative **8** (90 mg) by method *B* gave 60 mg (74%) of hydroxy derivative **14**, m.p. 175–178 °C (acetone–ether), $[\alpha]_D$ –36° (*c* 1.2, chloroform), identical with the product prepared by method *A*.

*(17E)-3β-Hydroxyandrost-5-en-17-one O-{[N-(3-hydroxypropyl)carbamoyl]methyl}oxime* (**15**). Deprotection of acetate **3** (92 mg) by method *A* afforded 56 mg (70%) of hydroxy derivative **15**, m.p. 166–168 °C, $[\alpha]_D$ –39° (*c* 1.5, chloroform). IR (chloroform): 3 610 (OH); 3 430 (NH); 1 662, 1 536 (CONH); 1 086, 1 061, 1 014 (C–O). $^1$H NMR: 0.93 s, 3 H (3 × H-18); 1.03 s, 3 H (3 × H-19); 3.48 q, 2 H, $J$ = 6 (NHCH$_2$); 3.65 t, 2 H, $J$ = 5.3 (CH$_2$OH); 4.49 s, 2 H (OCH$_2$CO); 5.36 bd, 1 H, $J \approx 4.5$ (H-6); 6.54 bt, 1 H, $J \approx 6$ (NH). For $C_{24}H_{38}N_2O_4$ (418.6) calculated: 68.87% C, 9.15% H, 6.69% N; found: 68.95% C, 9.36% H, 6.83% N.

Deprotection of methoxymethoxy derivative **9** (92 mg) by method *B* gave 69 mg (83%) of hydroxy derivative **15**, m.p. 164–166 °C (acetone–ether), $[\alpha]_D$ –34° (*c* 1.2, chloroform), identical with the product prepared by procedure *A*.

*(17E)-3β-Hydroxyandrost-5-en-17-one O-{[N-(2-hydroxypropyl)carbamoyl]methyl}oxime* (**16**). Deprotection of acetate **4** (92 mg) by method *A* afforded 65 mg (77%) of hydroxy derivative **16**, m.p. 150–155 °C, $[\alpha]_D$ –36° (*c* 1.8, chloroform). IR (chloroform): 3 612 (OH); 3 432 (NH); 1 668, 1 536 (CONH); 1 060, 1 050 (C–O). $^1$H NMR: 0.93 s, 3 H (3 × H-18); 1.03 s, 3 H (3 × H-19); 1.19 d, 3 H, $J$ = 6.1 (C**H**$_3$CH–O); 3.15 m and 3.94 m, 2 × 1 H (NHC**H**$_2$); 3.50 m, 2 H (C**H**OH of propyl moiety and H-3α); 4.49 s, 2 H (OCH$_2$CO); 5.35 bd, 1 H, $J \approx 4.5$ (H-6); 6.66 bt, 1 H, $J \approx 5.5$ (NH). For $C_{24}H_{38}N_2O_4$ (418.6) calculated: 68.87% C, 9.15% H, 6.69% N; found: 68.97% C, 9.27% H, 6.95% N.

*(17E)-3β-Hydroxyandrost-5-en-17-one O-{[N-(4-hydroxybutyl)carbamoyl]methyl}oxime* (**17**). Deprotection of acetate **5** (95 mg) by method *A* afforded 71 mg (82%) of hydroxy derivative **17**, m.p. 154–156 °C, $[\alpha]_D$ –36° (*c* 1.5, chloroform). IR (chloroform): 3 614, 3 500 (OH); 3 431 (NH); 1 668, 1 537 (CONH); 1 058 (C–O). $^1$H NMR: 0.93 s, 3 H (3 × H-18); 1.03 s, 3 H (3 × H-19); 3.35 q, 2 H, *J* = 6.4 (NHC**H**$_2$); 3.52 m, 1 H, *W* = 32 (H-3α); 3.67 t, 2 H, *J* = 6 (C**H**$_2$OH); 4.46 s, 2 H ($OCH_2CO$); 5.35 bd, 1 H, *J* ≈ 4.5 (H-6); 6.67 bt, 1 H, *J* ≈ 6 (NH). For $C_{25}H_{40}N_2O_4$ (432.6) calculated: 69.41% C, 9.32% H, 6.48% N; found: 69.25% C, 9.15% H, 6.76% N.

*(17E)-3β-Hydroxyandrost-5-en-17-one O-{[N-(1-hydroxy-2-methyl-2-propyl)carbamoyl]methyl}oxime* (**18**). Deprotection of acetate **6** (95 mg) by method *A* afforded 66 mg (76%) of hydroxy derivative **18**, m.p. 174–176 °C, $[\alpha]_D$ –39° (*c* 1.7, chloroform). IR (chloroform): 3 600, 3 500 (OH); 3 405 (NH); 1 658, 1 536 (CONH); 1 063, 1 049 (C–O). $^1$H NMR: 0.94 s, 3 H (3 × H-18); 1.03 s, 3 H (3 × H-19); 1.29 s, 6 H (2 × $CH_3$); 3.53 m, 1 H, *W* = 32 (H-3α); 3.61 s, 2 H (C**H**$_2$OH); 4.41 s, 2 H ($OCH_2CO$); 5.34 bd, 1 H, *J* ≈ 4.5 (H-6); 6.28 bs, 1 H (NH). For $C_{25}H_{40}N_2O_4$ (432.6) calculated: 69.41% C, 9.32% H, 6.48% N; found: 69.53% C, 9.42% H, 6.26% N.

General Procedure for Preparation of THP-Protected Derivatives

4-Toluenesulfonic acid monohydrate (2 mg) and 3,4-dihydro-2*H*-pyran (114 μl, 1.25 mmol) were added to a solution of the hydroxy acetate (1 mmol) in dioxane (2 ml). After stirring at room temperature for 3 h, a solution of potassium hydroxide (70 mg, 1.25 mmol) in 75% aqueous methanol (400 μl) was added and the mixture was refluxed for 20 min. After cooling, the mixture was poured into ether (200 ml), the solution was washed with brine, dried and the solvent was evaporated. The residue was chromatographed on a column of silica gel (40 g, pretreated with ammonia vapor for 24 h) in benzene–ether (1 : 1).

*(17E)-3β-Hydroxyandrost-5-en-17-one O-{{N-[2-(2-tetrahydropyranyloxy)ethyl]carbamoyl}methyl}oxime* (**19**). Compound **2** (446 mg) afforded 300 mg (61%) of amorphous product **19**, $[\alpha]_D$ –32° (*c* 1.4, chloroform). IR (chloroform): 3 608 (OH); 3 435 (NH); 1 669, 1 533 (CONH); 1 138, 1 124, 1 060, 1 035 (C–O). $^1$H NMR: 0.92 s, 3 H (3 × H-18); 1.03 s, 3 H (3 × H-19); 3.52 m, 5 H (H-3α, NHC**H**$_2$, 2 × H-6 of THP group); 3.81 m, 2 H ($CH_2$OTHP); 4.50 s, 2 H ($OCH_2CO$); 4.58 bs, 1 H (H-2 of THP group); 5.35 bd, 1 H, *J* ≈ 4.5 (H-6); 6.72 bt, 1 H, *J* ≈ 5 (NH). For $C_{28}H_{44}N_2O_5$ (488.7) calculated: 68.82% C, 9.08% H, 5.73% N; found: 69.05% C, 8.78% H, 5.52% N.

*(17E)-3β-Hydroxyandrost-5-en-17-one O-{{N-[3-(2-tetrahydropyranyloxy)propyl]carbamoyl}methyl}oxime* (**20**). Compound **3** (460 mg) gave 351 mg (70%) of product **20**, m.p. 78–88 °C (ether), $[\alpha]_D$ –30° (*c* 1.5, chloroform). IR (chloroform): 3 608, 3 400 (OH); 3 431 (NH); 1 669, 1 534 (CONH); 1 061 (C–O). $^1$H NMR: 0.93 s, 3 H (3 × H-18); 1.03 s, 3 H (3 × H-19); 3.46 m, 5 H (H-3α, NHC**H**$_2$, 2 × H-6 of THP group); 3.82 m, 2 H ($CH_2$OTHP); 4.46 s, 2 H ($OCH_2CO$); 4.55 bs, 1 H (H-2 of THP group); 5.36 bd, 1 H, *J* ≈ 4.5 (H-6); 6.46 bt, 1 H, *J* ≈ 5 (NH). For $C_{29}H_{46}N_2O_5$ (502.7) calculated: 69.29% C, 9.22% H, 5.57% N; found: 69.44% C, 9.31% H, 5.68% N.

*(17E)-3β-Hydroxyandrost-5-en-17-one O-{{N-[2-(2-tetrahydropyranyloxy)propyl]carbamoyl}methyl}oxime* (**21**). Compound **4** (460 mg) gave 325 mg (64%) of amorphous product **21**, $[\alpha]_D$ –32° (*c* 1.1, chloroform). IR (chloroform): 3 608 (OH); 3 433 (NH); 1 668, 1 534 (CONH); 1 075, 1 048, 1 032 (C–O). $^1$H NMR: 0.93 s, 3 H (3 × H-18); 1.03 s, 3 H (3 × H-19); 1.15 d, 1.5 H, *J* = 6.4 (CH(OTHP)C**H**$_3$); 1.23 d, 1.5 H, *J* = 6.4 (CH(OTHP)C**H**$_3$); 3.17–3.32 m, 1 H (one H of NHC**H**$_2$); 3.42–3.62 m, 3 H (CH-OTHP, H-3α, 1 × H-6 of THP group); 3.83–3.96 m, 2 H (one H of $NCH_2$ and 1 × H-6 of THP group); 4.50 s, 1 H and 4.51 s, 1 H ($OCH_2CO$); 4.61 m, 0.5 H and 4.67 m, 0.5 H (H-2 of THP group); 5.36 bd, 1 H, *J* ≈ 4.5 (H-6); 6.57 bt, 0.5 H, *J* ≈ 6 and 6.91 bt, 0.5 H, *J* ≈ 6 (NH). For $C_{29}H_{46}N_2O_5$ (502.7) calculated: 69.29% C, 9.22% H, 5.57% N; found: 69.54% C, 9.34% H, 5.39% N.

*(17E)-3β-Hydroxyandrost-5-en-17-one O-{{N-[4-(2-tetrahydropyranyloxy)butyl]carbamoyl}methyl}oxime* (**22**). Compound **5** (474 mg) gave 420 mg (81%) of amorphous product **22**, $[\alpha]_D$ –31° (*c* 1.4, chloroform). IR (chloroform): 3 609, 3 500 (OH); 3 433 (NH); 1 669, 1 534 (CONH); 1 079, 1 034 (C–O). $^1$H NMR: 0.93 s, 3 H (3 × H-18); 1.03 s, 3 H (3 × H-19); 3.30–3.60 m, 5 H (H-3α, NHC**H**$_2$, 2 × H-6 of THP group); 3.71–3.91 m, 2 H ($CH_2$OTHP); 4.46 s, 2 H ($OCH_2CO$); 4.56 bs, 1 H (H-2 of THP group); 5.36 bd, 1 H, $J \approx 4.5$ (H-6); 6.30 bt, 1 H, $J \approx 5.5$ (NH). For $C_{30}H_{48}N_2O_5$ (516.7) calculated: 69.73% C, 9.36% H, 5.42% N; found: 69.65% C, 9.13%H, 5.26% N.

*(17E)-3β-Hydroxyandrost-5-en-17-one O-{{N-[1-(2-tetrahydropyranyloxy)-2-methyl-2-propyl]carbamoyl}-methyl}oxime* (**23**). Compound **6** (474 mg) gave 330 mg (64%) of amorphous product **23**, $[\alpha]_D$ –30° (*c* 1.3, chloroform). IR (chloroform): 3 608, 3 500 (OH); 3 411 (NH); 1 679, 1 529 (CONH); 1 076, 1 065, 1 035 (C–O). $^1$H NMR: 0.93 s, 3 H (3 × H-18); 1.03 s, 3 H (3 × H-19); 1.41 s, 6 H (NHC(C**H**$_3$)$_2$); 3.40 and 3.65 AB system, 2 H, $J$(A,B) = 9.4 (C**H**$_2$OH); 3.52 m, 2 H (H-3α, 1 × H-6 of THP group); 3.82 m, 1 H (1 × H-6 of THP group); 4.39 s, 2 H ($OCH_2CO$); 4.59 bs, 1 H (H-2 of THP group); 5.37 bd, 1 H, $J \approx 4.5$ (H-6); 6.46 bs, 1 H (NH). For $C_{30}H_{48}N_2O_5$ (516.7) calculated: 69.73% C, 9.36% H, 5.42% N; found: 69.84% C, 9.05% H, 5.57% N.

General Procedure for Oppenauer Oxidation of 3-Hydroxy Derivatives

1-Methyl-4-piperidone (246 μl, 2.0 mmol) was added under argon to a solution of the hydroxy derivative (0.5 mmol) in toluene (15 ml). A part (3 ml) of toluene was distilled off and 1 M solution of aluminum isopropoxide in toluene (1.0 ml) was added. After refluxing under argon for 4 h, the mixture was cooled, diluted with ether (150 ml) and washed successively with 5% aqueous citric acid, water, aqueous potassium hydrogen carbonate solution, and water. After drying, the solvents were evaporated. The residue was dissolved in benzene (6 ml) and methanol (6 ml), 4-toluenesulfonic acid monohydrate (95 mg, 0.5 mmol) was added and the mixture was heated at 42 °C for 3 h. The solvents were evaporated, the residue was dissolved in ethyl acetate and water, and the aqueous layer was extracted with ethyl acetate. The combined extracts were washed with water, aqueous potassium hydrogen carbonate solution and water, dried, and the solvent was evaporated. The residue was chromatographed on three preparative silica gel plates in benzene–acetone (6 : 4, twice developed).

*(17E)-Androst-4-ene-3,17-dione 17-{O-{[N-(2-hydroxyethyl)carbamoyl]methyl}oxime}* (**24**). Compound **19** (244 mg) afforded 112 mg (56%) of ketone **24**, m.p. 136–139 °C (ether), $[\alpha]_D$ +99° (*c* 1.3, chloroform). IR (chloroform): 3 629 (OH); 3 403 (NH); 1 666 (C=O ketone and amide I); 1 615 (C=C); 1 535 (amide II); 1 089 (C–O). $^1$H NMR: 0.96 s, 3 H (3 × H-18); 1.20 s, 3 H (3 × H-19); 3.47 q, 2 H, $J$ = 5 (NHC**H**$_2$); 3.74 q, 2 H, $J$ = 5 (C**H**$_2$OH); 4.49 s, 2 H ($OCH_2CO$); 5.74 bs, 1 H (H-4); 6.66 bt, 1 H, $J \approx 5$ (NH). For $C_{23}H_{34}N_2O_4$ (402.5) calculated: 68.63% C, 8.51% H, 6.96% N; found: 66.45% C, 8.32% H, 7.01% N.

*(17E)-Androst-4-ene-3,17-dione 17-{O-{[N-(3-hydroxypropyl)carbamoyl]methyl}oxime}* (**25**). Compound **20** (251 mg) afforded 134 mg (64%) of amorphous ketone **25**, $[\alpha]_D$ +95° (*c* 1.6, chloroform). IR (chloroform): 3 628, 3 400 (OH); 3 431 (NH); 1 664 (C=O ketone and amide I); 1616 (C=C); 1 536 (amide II); 1 059 (C–O). $^1$H NMR: 0.96 s, 3 H (3 × H-18); 1.21 s, 3 H (3 × H-19); 3.48 q, 2 H, $J$ = 6.2 (NHC**H**$_2$); 3.66 bt, 2 H, $J \approx 5$ (C**H**$_2$OH); 4.49 s, 2 H ($OCH_2CO$); 5.75 bd, 1 H, $J$ = 1.2 (H-4); 6.54 bt, 1 H, $J \approx 6$ (NH). For $C_{24}H_{36}N_2O_4$ (416.6) calculated: 69.20% C, 8.71% H, 6.72% N; found: 69.35% C, 8.97% H, 6.53% N.

*(17E)-Androst-4-ene-3,17-dione 17-{O-{[N-(2-hydroxypropyl)carbamoyl]methyl}oxime}* (**26**). Compound **21** (251 mg) afforded 114 mg (55%) of amorphous ketone **26**, $[\alpha]_D$ +85° (*c* 1.6, chloroform). IR (chloroform): 3 620, 3 500 (OH); 3 433 (NH); 1 666 (C=O ketone and amide I); 1 616 (C=C); 1 535 (amide II); 1 088 (C–O). $^1$H NMR: 0.95 s, 3 H (3 × H-18); 1.18 d, 3 H, $J$ = 6.1 (C**H**$_3$CH(OH)); 1.19 s, 3 H (3 × H-19); 3.14 m and 3.94 m, 2 × 1 H (NHC**H**$_2$); 3.50 m, 1 H (C**H**OH); 4.48 s, 2 H ($OCH_2CO$); 5.73 bd, 1 H,

$J$ = 1.4 (H-4); 6.64 bt, 1 H, $J \approx 5$ (NH). For $C_{24}H_{36}N_2O_4$ (416.6) calculated: 69.20% C, 8.71% H, 6.72% N; found: 69.28% C, 8.84% H, 6.81% N.

*(17E)-Androst-4-ene-3,17-dione 17-{O-{[N-(4-hydroxybutyl)carbamoyl]methyl}oxime}* (**27**). Compound **22** (258 mg) afforded 127 mg (59%) of amorphous ketone **27**, $[\alpha]_D$ +85° (*c* 1.9, chloroform). IR (chloroform): 3 628, 3 500 (OH); 3 433 (NH); 1 666 (C=O ketone and amide I); 1 616 (C=C); 1 536 (amide II); 1 088 (C–O). $^1$H NMR: 0.97 s, 3 H (3 × H-18); 1.22 s, 3 H (3 × H-19); 3.37 bq, 2 H, $J \approx 6.5$ (NHC**H**$_2$); 3.68 bt, 2 H, $J \approx 6$ (C**H**$_2$OH); 4.47 s, 2 H (OCH$_2$CO); 5.75 bd, 1 H, $J$ = 1.4 (H-4); 6.35 bt, 1 H, $J \approx 5$ (NH). For $C_{25}H_{38}N_2O_4$ (430.6) calculated: 69.74% C, 8.90% H, 6.51% N; found: 70.03% C, 9.07% H, 6.63% N.

*(17E)-Androst-4-ene-3,17-dione 17-{O-{[N-(1-hydroxy-2-methyl-2-propyl)carbamoyl]methyl}oxime}* (**28**). Compound **23** (258 mg) afforded 109 mg (51%) of ketone **28**, m.p. 202–205 °C (ether), $[\alpha]_D$ +81° (*c* 1.6, chloroform). IR (chloroform): 3 500 (OH); 3 406 (NH); 1 662 (C=O ketone and amide I); 1 617 (C=C); 1 535 (amide II); 1 061 (C–O). $^1$H NMR: 0.97 s, 3 H (3 × H-18); 1.21 s, 3 H (3 × H-19); 1.30 s, 6 H (NC(CH$_3$)$_2$); 3.61 s, 2 H (C**H**$_2$OH); 4.41 s, 2 H (OCH$_2$CO); 5.75 bd, 1 H, $J$ = 1.5 (H-4); 6.26 bs, 1 H (NH). For $C_{25}H_{38}N_2O_4$ (430.6) calculated: 69.74% C, 8.90% H, 6.51% N; found: 69.53% C, 8.60% H, 6.33% N.

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