PREPARATION OF (17*E*)-3β-HYDROXYANDROST-5-EN-17-ONE (*O*-CARBOXYMETHYL)OXIME DERIVATIVES WITH SHORT PEPTIDE CHAIN*

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17-(*O*-Carboxymethyl)oxime (CMO) derivatives of 3β-acetoxy-, 3β-methoxymethoxy-, 3β-hydroxyandrost-5-en-17-one, and of -androst-4-ene-3,17-dione (*IV – VI, XXIX*) were prepared. Methods for the attachment of amino acids to the CMO group and for the peptide chain elongation were tested. The mixed anhydride method was used for linking the first amino acid unit (Gly, βAla, or Gly-Gly dipeptide), further units were added by the *N*-hydroxysuccinimide method. Compounds with one to four amino acids were prepared. This concept is suitable for the preparation of haptens with variable bridge length for the binding studies.

The sensitivity of steroid immunoassays depends in substantial part on the diminution of undesired binding to steroid – label attachment in the labelled antigen (bridge effect[1]). This effect can be reduced or completely avoided by using the different bridge in immunogen (steroid – protein attachment) and in labeled antigen (steroid – label attachment). The application of amino acids as building blocks of the bridge enables its easy elongation and furthermore discrete variation of the bridge length. Single glycine unit was used for bridge elongation in haptens, derived from estradiol by introducing 12-carboxyl group[2], and from 11-deoxycortisol modified by 4-carboxymethylmercapto group[1].

The aim of our work is in corroborating of chemical synthesis of (*O*-carboxymethyl)oximino (CMO) steroids elongated with one or more simple amino acids and consequently in giving tools for the preparation of haptens with different length of connecting bridge. As parent skeletons the 17-CMO derivatives of 3β-hydroxyandrost-5-en-17-one and androst-4-en-3,17-dione were chosen and one to four units of glycine and/or β-alanine were attached.

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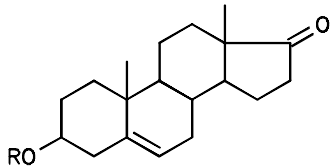
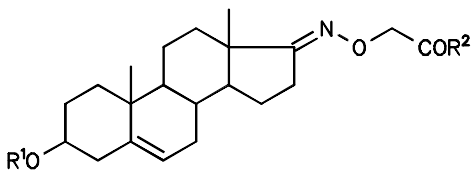


	R
I	Ac
II	MOM
III	H



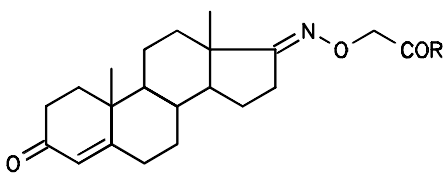
	R^1	R^2
IV	Ac	OH
V	MOM	OH
VI	H	OH
VII	H	OMe
VIII	H	$OCH(CH_3)_2$
IX	Ac	Gly–OEt
X	MOM	Gly–OEt
XI	H	Gly–OEt
XII	H	Gly–OH
XIII	Ac	βAla–OMe
XIV	H	βAla–OH
XV	Ac	Gly–Gly–OEt
XVI	MOM	Gly–Gly–OEt

	R^1	R^2
XVII	H	Gly–Gly–OEt
XVIII	H	Gly–Gly–OH
XIX	H	Gly–βAla–OMe
XX	H	Gly–βAla–OH
XXI	H	βAla–Gly–OEt
XXII	H	βAla–Gly–OH
XXIII	H	βAla–βAla–OMe
XXIV	H	βAla–βAla–OH
XXV	H	Gly–Gly–Gly–OEt
XXVI	H	Gly–Gly–Gly–OH
XXVII	H	Gly–Gly–Gly–Gly–OEt
XXVIII	H	Gly–Gly–Gly–Gly–OH

MOM = CH_2OCH_3

Starting 17-CMO derivatives of 3β-hydroxyandrost-5-en-17-one with acetyl or methoxymethyl (MOM) protecting group in position 3 (compounds *IV* and *V*) were prepared by reaction of (*O*-carboxymethyl)hydroxylamine with corresponding 17-ketones *I* and *II*. For the 17-CMO derivative of androst-4-ene-3,17-dione (compound *XXIX*) multistep transformation (VI → VII → VIII → XXX → XXIX) of the 3-hydroxy derivative *VI* was needed, because the selective oximation of androst-4-ene-3,17-dione gave only 3-CMO derivative beside a minor amount of the 3,17-bis(CMO) derivative. In the first step the 17-CMO group of 3-hydroxy derivative *VI* was protected as a methyl ester *VII* by reaction with diazomethane. Attempted Oppenauer oxidation[3] of this product was accompanied by partial transesterification of methyl ester and resulted in a mixture of only 10% of desired product, 20% of corresponding isopropyl ester, and further unidentified polar products. Original methyl ester *VII* was therefore transesterified into more stable isopropyl ester *VIII* by reaction with 2-propanol. The Oppenauer oxidation proceeded with isopropyl ester *VIII* more smoothly, and whole sequence including subsequent alkaline hydrolysis gave a satisfactory yield of the 17-CMO derivative *XXIX* (48% from oxime *VI*).

For addition of the first amino acid unit (Gly, βAla, or Gly-Gly dipeptide) the mixed anhydride method was used. The ester protection from terminal amino acid was removed by the alkaline hydrolysis. In the case of simultaneous use of acetyl protection of the hydroxy group in position 3, this group was also deblocked. Derivatives with the MOM protecting group can be selectively deprotected by hydrochloric acid in ethanol



	R
XXIX	OH
XXX	$OCH(CH_3)_2$
XXXI	Gly–OEt
XXXII	βAla–OMe
XXXIII	Gly–Gly–OEt
XXXIV	Gly–OH
XXXV	βAla–OH
XXXVI	Gly–Gly–OH

and in this way the 3-hydroxy derivatives with protected terminal amino acid are available. With methods mentioned above the 17-CMO derivatives with one (Gly: *IX* – *XII*, *XXXI*, *XXXIV*; βAla: *XIII*, *XIV*, *XXXII*, *XXXV*) and two (Gly-Gly: *XV*–*XVIII*, *XXXIII*, *XXXVI*) amino acid linked were prepared.

Further amino acid units were added by the activated ester method[4] with *N*-hydroxysuccinimide, which can be used even in presence of the unprotected 3-hydroxy group. With this method the compounds with two (Gly-Gly: *XVII*; Gly-βAla: *XIX*; βAla-Gly: *XXI*; βAla-βAla: *XXIII*), three (Gly-Gly-Gly: *XXV*), and four (Gly-Gly-Gly-Gly: *XXVII*) amino acids were synthesized.

The configuration on the C=N double bond of 17-CMO derivatives *IV*, *V*, *IX*, and *XXXI* was studied in detail. Comparing their ^{13}C NMR spectra (Table I) with spectra of unsubstituted 17-oximine derivatives with (17*Z*)- and (17*E*)-configuration (*XXXVII* and *XXXVIII*, respectively) reveals that the shifts of the affected carbon atoms (C-13, C-16, and C-18) correspond to the (17*E*)-isomer. Also ^{1}H NMR shift of H-18 in 5-ene derivatives *IV* and *V* (0.94 ppm) is very close to value found for (17*E*)-isomer *XXXVII* (0.93 ppm, ref.[5]). All derivatives in whole series gave one distinct signal for H-18 in ^{1}H NMR spectrum supporting the (17*E*)-configuration (Tables II and III). Other characteristic signals are the singlet of OCH_2CO group in region 4.29 – 4.59 ppm, the doublet of $NHCH_2CO$ grouping in glycine unit (3.74 – 4.05 ppm), or triplet and quadruplet of $NHCH_2CH_2CO$ of β-alanine (2.22 – 2.61 and 3.26 – 3.59 ppm).

The results achieved on model derivatives are stimulating, all syntheses in this series proceeded with high yields without isomerization, and used the inexpensive reagents. The use of ethyl group for glycine synthon protection was satisfactory for our purposes, application of the 2,2,2-trichloroethyl group[1] led in our case to partial degradation of the product during its zinc/acetic acid cleavage.

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 (Table II and III) were taken on a Varian UNITY-200 (200 MHz, FT mode) and ^{13}C NMR spectra (Table I) on a Varian UNITY-500 (125.7 MHz, FT mode) at 23 °C in deuteriochloroform with tetramethylsilane as internal standard unless stated otherwise. Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) and width of multiplets (*W*) in Hz. For ^{13}C NMR spectra the number of directly bonded hydrogen atoms was determined from the proton decoupled "attached proton test" (refs[6,7]). FAB mass spectra were recorded on a VG Analytical ZAB-EQ spectrometer. Thin-layer chromatography was performed on silica gel G (ICN Biochemicals), with detection by spraying with concentrated sulfuric acid followed by heating. For column chromatography silica gel 60 – 120 μm was used. Prior to evaporation on rotary evaporator in vacuo (bath temperature 50 °C), solutions in organic solvents were dried over anhydrous sodium sulfate.

General Procedure for Preparation of 17-CMO Derivatives *IV – VI*

The mixture of ketone (20 mmol), (*O*-carboxymethyl)hydroxylamine hemihydrochloride (4.37 g, 20 mmol), and pyridine (70 ml) was heated under stirring at 60 °C for 6 h. Toluene (70 ml) was added and the solvents were evaporated in vacuo, the rest of pyridine was removed by coevaporation with another portion (70 ml) of toluene. The residue was dissolved in chloroform (300 ml), washed with water (3 times), and dried. Removal of the solvent afforded the product which could be directly used in the next step. Analytical sample was obtained by crystallization from methanol–ethyl acetate.

TABLE I

Parameters of ^{13}C NMR spectra of 17-CMO derivatives *IV*, *V*, *IX*, *XXXI*, *XXXVII*, and *XXXVIII* in deuteriochloroform. For other conditions see Experimental

Carbon	*IV*[a]	*V*[b]	*IX*[c]	*XXXI*[d]	*XXXVII*[e]	*XXXVIII*[f]
1	36.89	37.15	36.89	35.66	36.9	37.0
2	27.65	28.80	27.65	33.67[g]	27.2	27.8
3	73.79	76.82	73.69	199.29	73.6	73.9
4	38.03	39.48	38.03	124.05	38.0	38.2
5	139.81	140.89	139.86	172.35	139.2	140.1
6	121.96	121.07	121.90	32.60[h]	121.7	122.1
7	31.27	31.25	31.20	31.40[h]	31.2	31.4
8	31.20	31.32	31.27	34.96	31.2	31.4
9	50.04	50.20	50.07	53.80[i]	50.2	50.4
10	36.65	36.80	36.64	38.60	36.6	35.2
11	20.43	20.47	20.43	20.51	20.4	20.6
12	33.74	33.80	33.78	33.89[g]	33.9	34.1
13	44.19	44.22	44.17	44.19	43.6	45.8
14	53.92	54.01	53.96	53.18[i]	54.0	54.2
15	23.23	23.24	23.28	23.17	23.3	23.3
16	26.09	26.10	26.08	26.02	25.0	29.1
17	173.41[j]	173.47[j]	170.45[k]	170.41[j]	170.2	171.2
18	16.89	16.91	16.91	17.32[l]	16.9	13.7
19	19.29	19.35	19.26	17.08[l]	19.3	19.4
O**C**H_2CO	69.79	69.81	72.42	72.48	–	–
OCH_2**C**O	174.48[j]	174.21[j]	170.45[k]	170.36[j]	–	–

[a] Other signals: 21.39 (**C**H_3COO); 170.63 (CH_3**C**OO). [b] Other signals: 55.16 (CH_3O); 94.62 (OCH_2O). [c] Other signals: 14.10 (**C**H_3CH_2O); 21.34 (**C**H_3COO); 40.88 (NH**C**H_2CO); 61.47 (CH_3**C**H_2O); 169.61 (NHCH_2**C**O); 172.83 (CH_3**C**OO). [d] Other signals: 14.11 (CH_3CH_2O); 40.89 (NHCH_2CO); 61.52 (CH_3CH_2O); 169.67 (NHCH_2CO). [e] (17*E*)-Oximinoandrost-5-en-3β-yl acetate, values taken from literature[5]. [f] (17*Z*)-Oximinoandrost-5-en-3β-yl acetate, values taken from literature[5]. [g–j, l] Signals can be mutually interchanged. [k] Overlapped signals.

TABLE II

^{1}H NMR spectra of 17-CMO derivatives *IV* – *XXVIII*. Measured in $CDCl_3$, unless stated otherwise. For other conditions see Experimental

Compound	18-H_3 s	19-H_3 s	6-H bd[a]	3-H m[b]	OCH_2CO s	NCH_2CO d (*J*)	NCH_2CH_2CO q (*J*)	NCH_2CH_2CO t (*J*)	NH t (*J*)
IV[c]	0.94	1.04	5.39	4.60	4.59	–	–	–	–
V[d]	0.94	1.03	5.36	3.43	4.57	–	–	–	–
VI[e]	0.85	0.96	5.30	[f]	4.43	–	–	–	–
VII[e,g]	0.85	0.96	5.28	[f]	4.54	–	–	–	–
VIII[h]	0.93	1.03	5.36	[f]	4.53	–	–	–	–
IX[c,i]	0.94	1.04	5.39	4.61	4.53	4.08 (5.1)	–	–	6.82 (≈5)
X[d,i]	0.94	1.04	5.37	3.43	4.53	4.08 (5.2)	–	–	6.83 (≈5)
XI[i]	0.94	1.03	5.37	3.53	4.53	4.08 (4.9)	–	–	6.84 (≈5)
XI[e,j]	0.87	0.96	5.28	[f]	4.53	4.08 (4.9)	–	–	7.84 (≈5)
XII[e]	0.86	0.96	5.28	[f]	4.37	3.79 (6.2)	–	–	7.70 (6.2)
XIII[c,k]	0.93	1.04	5.39	4.61	4.47	–	3.57 (6.0)	2.35 (6.0)	6.88 (6.0)
XIV	0.92	1.03	5.37	3.54	4.48	–	3.58 (5.9)	2.61 (5.9)	6.90 (5.9)
XIV[e]	0.86	0.96	5.29	[f]	4.30	–	[f]	2.38 (≈7)	7.44 (5.9)
XV[c,i]	0.93	1.04	5.39	4.61	4.53	4.03 (5.4) 4.06 (5.4)	–	–	6.73 (≈5) 7.00 (≈5)
XVI[d,i]	0.93	1.03	5.35	3.43	4.53	4.04 (5.5) 4.05 (5.5)	–	–	6.62 (5.5) 6.97 (5.5)
XVII[i]	0.93	1.03	5.36	3.53	4.53	4.03 (5.4) 4.05 (5.4)	–	–	6.99 (5.4) 6.99 (5.4)
XVII[e,j]	0.86	0.96	5.29	[f]	4.37	3.78 (5.8) 3.84 (5.8)	–	–	7.69 (5.8) 8.25 (5.8)

TABLE II
(*Continued*)

Compound	18-H_3 s	19-H_3 s	6-H bd[a]	3-H m[b]	OCH_2CO s	NCH_2CO d (*J*)	NCH_2CH_2CO q (*J*)	NCH_2CH_2CO t (*J*)	NH t (*J*)
XVIII	0.86	0.96	5.28	[f]	4.37	3.76[l] (5.8)	–	–	7.69 (5.8) 8.14 (5.8)
XIX[k]	0.94	1.03	5.37	3.54[m]	4.52	3.96 (5.3)	3.54[m]	2.56 (6.2)	6.63 (≈6) 6.98 (≈5)
XX[e]	0.89	0.96	5.28	[f]	4.37	3.69 (5.6)	3.26 (5.8)	2.37 (5.8)	7.60 (5.5) 7.91 (5.5)
XXI[i]	0.92	1.03	5.36	3.53	4.47	4.00 (5.5)	3.59 (≈6)	2.49 (≈6)	6.28 (5.5) 7.01 (5.5)
XXII[e]	0.85	0.96	5.29	3.27[m]	4.30	3.74 (5.8)	3.27[m]	2.30 (6.5)	7.46 (5.6) 8.26 (5.8)
XXIII[k]	0.92	1.03	5.36	3.53[m]	4.46	–	3.53[m,n]	2.39 (6.1) 2.52 (6.1)	6.28 (5.5) 7.01 (5.5)
XXIV[e]	0.85	0.96	5.28	3.26[m]	4.29	–	3.26[m,n]	2.23 (6.7) 2.36 (6.9)	7.48 (5.6) 7.99 (5.5)
XXV[i]	0.93	1.03	5.36	3.52	4.52	4.02[o] (5.4)	–	–	6.92 (5.4) 7.06 (5.4) 7.22 (5.4)
XXVI[e]	0.86	0.96	5.28	[f]	4.38	3.76 m[o]	–	–	7.67 (5.5) 8.16[p] (5.6)

TABLE II
(*Continued*)

Compound	18-H_3 s	19-H_3 s	6-H bd[a]	3-H m[b]	OCH_2CO s	NCH_2CO d (*J*)	NCH_2CH_2CO q (*J*)	NCH_2CH_2CO t (*J*)	NH t (*J*)
XXVII[e,j]	0.86	0.96	5.29	[f]	4.38	3.74 (5.5) 3.76 (5.8) 3.79 (≈7) 3.82 (5.8)	– – – –	– – – –	7.69 (5.5) 8.14 (5.8) 8.17 (≈7) 8.24 (5.8)
XXVIII[e]	0.86	0.96	5.28	[f]	4.38	3.76 m[r]	–	–	7.69 (5.3) 8.14 m[s]

[a] $J \approx 4.5$. [b] $W \approx 32$. [c] Other signal: 2.04 s, 3 H (CH_3COO). [d] Other signal: 4.70 s, 2 H and 3.38 s, 3 H (CH_3OCH_2O). [e] Measured in CD_3SOCD_3. [f] Undeterminable value. [g] Other signal: 3.64 s, 3 H (CH_3OOC). [h] Other signals: 5.08 heptet, 1 H and 1.25 d, 6 H, J = 6.2 ($(CH_3)_2CHOOC$). [i] Other signals: 4.22 q, 2 H and 1.29 t, 3 H, J = 7.2 (CH_3CH_3OOC). [j] Other signals: 4.09 q, 2 H and 1.19 t, 3 H, J = 7.2 (CH_3CH_2OOC). [k] Other signal: 3.69 s, 3 H (CH_3OOC). [l] 2 × NCH_2CO. [m] Overlapped signals. [n] 2 × NCH_2CH_2CO. [o] 3 × NCH_2CO. [p] 2 × NH. [r] 4 × NCH_2CO. [s] 3 × NH.

(17E)-3β-Acetoxyandrost-5-en-17-one (O-carboxymethyl)oxime (IV). Treatment of ketone *I* (6.61 g, 20 mmol) afforded 7.86 g (97%) of the product *IV*, m.p. 225 – 229 °C (decomposition), $[\alpha]_D$ −42° (*c* 1.8, chloroform). IR spectrum (chloroform): 3 500 – 2 700 (COOH); 1 768, 1 727 (C=O); 1 255, 1 032 (C–O). For $C_{23}H_{33}NO_5$ (403.5) calculated: 68.46% C, 8.24% H, 3.47% N; found: 68.67% C, 8.03% H, 3.56% N.

(17E)-3β-Methoxymethoxyandrost-5-en-17-one (O-carboxymethyl)oxime (V). From ketone[8] *II* (6.61 g, 20 mmol) were obtained 7.43 g (92%) of the product *V*, m.p. 157 – 159 °C, $[\alpha]_D$ −37° (*c* 1.7, chloroform). IR spectrum (chloroform): 3 500 – 2 700 (COOH); 1 768, 1 734 (C=O); 1 147, 1 106, 1 037 (C–O). For $C_{23}H_{35}NO_5$ (405.5) calculated: 68.12% C, 8.70% H, 3.45% N; found: 67.77% C, 9.00% H, 3.26% N.

(17E)-3β-Hydroxyandrost-5-en-17-one (O-carboxymethyl)oxime (VI). This compound was prepared from ketone *III* (5.76 g, 20 mmol) by the above mentioned general procedure with the different work-up. The residue after evaporation of toluene was triturated with water (250 ml), crystalline product was collected on the filter, washed with water and dried in vacuo over phosphorus pentoxide. Yield 6.73 g (93%) of the product *VI*, m.p. 230 – 235 °C (decomposition), $[\alpha]_D$ −31° (*c* 1.4, chloroform–methanol 1 : 1). Literature[9] gives m.p. 264 – 266 °C (decomposition), $[\alpha]_D^{24}$ −37.9° (ethanol).

General Procedure for Coupling of 17-CMO Derivatives with Esters of Amino Acids via Mixed Anhydride Method

To a solution of 17-CMO derivative (2.0 mmol) and *N*,*N*-diisopropylethylamine (766 μl, 4.4 mmol) in tetrahydrofuran (12 ml) cooled at −5 °C was added dropwise 1 M solution of ethyl chloroformate in tetrahydrofuran (2.2 ml). Reaction mixture was stirred at −5 °C for 40 min and then amino acid ester hydrochloride (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). Product was extracted with ethyl acetate, the extract was washed with water (2 times), dried and solvent was evaporated. The residue was chromatographed on a column of silica gel (50 g) in benzene–ether (80 : 20).

*N-[[[(17E)-3*β*-Acetoxyandrost-5-en-17-ylidene]amino]oxy]acetylglycine ethyl ester* (IX). Coupling of compound *IV* (807 mg, 2.0 mmol) with glycine ethyl ester hydrochloride (307 mg, 2.2 mmol) afforded 880 mg (90%) of product *IX*, m.p. 127 – 130 °C (dichloromethane–ether), $[\alpha]_D$ −37° (*c* 1.6, chloroform). IR spectrum (chloroform): 3 423 (NH); 1 742 shoulder, 1 727 (C=O); 1 675, 1 531 (CONH); 1 255, 1 032 (C–O). For $C_{27}H_{40}N_2O_6$ (488.5) calculated: 66.37% C, 8.25% H, 5.73% N; found: 66.63% C, 8.03% H, 5.66% N.

*N-[[[(17E)-3*β*-Methoxymethoxyandrost-5-en-17-ylidene]amino]oxy]acetylglycine ethyl ester* (X). Coupling of compound *V* (811 mg, 2.0 mmol) with glycine ethyl ester hydrochloride (307 mg, 2.2 mmol) afforded 903 mg (92%) of product *X*, m.p. 103 – 105 °C (light petroleum–ether), $[\alpha]_D$ −33° (*c* 1.9,

TABLE III

^{1}H NMR spectra of 17-CMO derivatives *XXIX* – *XXXVI*. Measured in deuteriochloroform. For other conditions see Experimental

Compound	18-H_3 s	19-H_3 s	4-H bd (*J*)	OCH_2CO s	NCH_2CO d (*J*)	NCH_2CH_2CO q (*J*)	NCH_2CH_2CO t (*J*)	NH bt (*J*)
XXIX	0.95	1.20	5.76 (1.1)	4.59	–	–	–	–
XXX[a]	0.96	1.20	5.74 (1.2)	4.57	–	–	–	–
XXXI[b]	0.97	1.20	5.74 (1.2)	4.52	4.07 (4.9)	–	–	6.80 (≈5)
XXXII[c]	0.97	1.21	5.75 (1.2)	4.47	–	3.57 (5.9)	2.55 (5.9)	6.86 (≈5)
XXXIII[b]	0.97	1.21	5.75 (1.2)	4.53	4.04 4.05 (5.1) (5.1)	–	–	6.67 6.99 (≈5) (≈5)
XXXIV	0.97	1.21	5.77 (1.2)	4.55	4.11 (4.8)	–	–	6.91 (≈5)
XXXV	0.95	1.20	5.75 (1.0)	4.48	–	3.57 (≈6)	2.59 (≈6)	6.94 (≈6)
XXXVI	0.96	1.20	5.75 (1.3)	4.53	4.05 m[d]	–	–	7.21 7.26 (4.5) (4.5)

[a] Other signals: 5.08 heptet, 1 H and 1.25 d, 6 H, *J* = 6.2 ($(CH_3)_2CHOOC$). [b] Other signals: 4.22 q, 2 H and 1.29 t, 3 H, *J* = 7.2 (CH_3CH_2OOC). [c] Other signals: 3.69 s, 3 H (CH_3OOC). [d] 2 × NCH_2CO.

chloroform). IR spectrum (chloroform): 3 422 (NH); 1 743 (C=O); 1 676, 1 529 (CONH); 1 255 (C–O, ester); 1 148, 1 103, 1 065, 1 043 (C–O). For $C_{27}H_{42}N_2O_6$ (490.5) calculated: 66.10% C, 8.63% H, 5.71% N; found: 66.34% C, 8.44% H, 5.56% N.

*N-[[[(17E)-3*β*-Acetoxyandrost-5-en-17-ylidene]amino]oxy]acetyl*-β-*alanine methyl ester* (XIII). Coupling of compound *IV* (807 mg, 2.0 mmol) with β-alanine methyl ester hydrochloride (307 mg, 2.2 mmol) afforded 915 mg (94%) of product *XIII*, m.p. 111 – 113 °C (ether–hexane), $[\alpha]_D$ –29° (*c* 2.1, chloroform). IR spectrum (chloroform): 3 435 (NH); 1 728 (C=O); 1 670, 1 531 (CONH); 1 255, 1 032 (C–O). For $C_{27}H_{40}N_2O_6$ (488.5) calculated: 66.37% C, 8.25% H, 5.73% N; found: 66.46% C, 8.43% H, 6.01% N.

N-[[[(17E)-3-Oxoandrost-4-en-17-ylidene]amino]oxy]acetylglycine ethyl ester (XXXI). Coupling of compound *XXIX* (720 mg, 2.0 mmol) with glycine ethyl ester hydrochloride (307 mg, 2.2 mmol) afforded 670 mg (75%) of product *XXXI*, m.p. 124 – 126 °C (ether), $[\alpha]_D$ +94° (*c* 2.1, chloroform). IR spectrum (chloroform): 3 423 (NH); 1 742 (C=O, ester); 1 669 (C=O, conjugated ketone and amide I); 1 529 (NH, amide II); 1 089, 1 061, 1 022 (C–O). Mass spectrum, *m/z*: 445 (M + 1). For $C_{25}H_{36}N_2O_5$ (444.6) calculated: 67.54% C, 8.16% H, 6.30% N; found: 67.68% C, 8.33% H, 6.38% N.

N-[[[(17E)-3-Oxoandrost-4-en-17-ylidene]amino]oxy]acetyl-β-*alanine methyl ester* (XXXII). Coupling of compound *XXIX* (720 mg, 2.0 mmol) with β-alanine methyl ester hydrochloride (307 mg, 2.2 mmol) afforded 712 mg (80%) of product *XXXII*, m.p. 120 – 123 °C (ether), $[\alpha]_D$ +93° (*c* 2.3, chloroform). IR spectrum (chloroform): 3 435 (NH); 1 731 (C=O, ester); 1 668 (C=O, conjugated ketone and amide I); 1 531 (NH, amide II); 1 088, 1 057, 1 013 (C–O). Mass spectrum, *m/z*: 445 (M + 1). For $C_{25}H_{36}N_2O_5$ (444.6) calculated: 67.54% C, 8.16% H, 6.30% N; found: 67.46% C, 8.43% H, 6.03% N.

General Procedure for Coupling of 17-CMO Derivatives with Glycylglycine Ethyl Ester via Mixed Anhydride Method

To a solution of 17-CMO derivative (2.0 mmol) and *N*,*N*-diisopropylethylamine (383 μl, 2.2 mmol) in tetrahydrofuran (12 ml) cooled at –5 °C 1 M solution of ethyl chloroformate in tetrahydrofuran (2.2 ml) was added dropwise. After stirring at –5 °C for 40 min glycylglycine ethyl ester hydrochloride (432 mg, 2.2 mmol) was added. Then 1 M solution of *N*,*N*-diisopropylethylamine in tetrahydrofuran (3.3 ml) was added dropwise during 10 min. Stirring was continued at 0 °C for 1 h and at room temperature for 2 h and then the reaction mixture was poured into brine (100 ml). Product was extracted with ethyl acetate, the extract was washed with water (2 times), dried and solvent was evaporated. The residue was chromatographed on a column of silica gel (50 g) in benzene–ethyl acetate (80 : 20).

*N-[[[(17E)-3*β*-Acetoxyandrost-5-en-17-ylidene]amino]oxy]acetylglycylglycine ethyl ester* (XV). Compound *IV* (807 mg, 2.0 mmol) afforded 883 mg (81%) of amorphous product *XV*, $[\alpha]_D$ –31° (*c* 1.8, chloroform). IR spectrum (chloroform): 3 419, 3 330 (NH); 1 741 (C=O); 1 727 (C=O, acetate); 1 669, 1 521 (CONH); 1 253, 1 032 (C–O). For $C_{29}H_{43}N_3O_7$ (545.7) calculated: 63.83% C, 7.94% H, 7.70% N; found: 63.54% C, 7.93% H, 7.66% N.

*N-[[[(17E)-3*β*-Methoxymethoxyandrost-5-en-17-ylidene]amino]oxy]acetylglycylglycine ethyl ester* (XVI). Compound *V* (811 mg, 2.0 mmol) afforded 989 mg (90%) of amorphous product *XVI*, $[\alpha]_D$ –28° (*c* 2.1, chloroform). IR spectrum (chloroform): 3 419 (NH); 1 744 (C=O, ester); 1 669, 1 522 (CONH); 1 148, 1 103, 1 037 (CH_3OCH_2O). For $C_{29}H_{45}N_3O_7$ (547.7) calculated: 63.60% C, 8.28% H, 7.67% N; found: 66.42% C, 8.47% H, 7.56% N.

N-[[[(17E)-3-Oxoandrost-4-en-17-ylidene]amino]oxy]acetylglycylglycine ethyl ester (XXXIII). Compound *XXIX* (720 mg, 2.0 mmol) afforded 696 mg (69%) of amorphous product *XXXIII*, $[\alpha]_D$

+77° (*c* 1.6, chloroform). IR spectrum (chloroform): 3 419 (NH); 1 745 (C=O); 1 666 (C=O, conjugated ketone and amide I); 1 520 (NH, amide II); 1 090, 1 035 (C–O). Mass spectrum, *m/z*: 502 (M + 1). For $C_{27}H_{39}N_3O_6$ (501.6) calculated: 64.65% C, 7.84% H, 8.38% N; found: 64.54% C, 7.93% H, 8.66% N.

General Procedure for Coupling via *N*-Hydroxysuccinimide Active Esters

To a mixture of carboxylic component (0.5 mmol), *N*-hydroxysuccinimide (92 mg, 0.8 mmol), 4-dimethylaminopyridine (2.5 mg, 20 μmol), and tetrahydrofuran (5 ml) 1 M solution of *N,N′*-dicyclohexyl carbodiimide in benzene (1 ml) was added. Reaction mixture was stirred for 6 h at room temperature, diluted with ethyl acetate (8 ml), separated *N,N′*-dicyclohexylurea was filtered off and the solvents were evaporated in vacuo. To the residue tetrahydrofuran (7 ml) and amino acid ester hydrochloride or glycylglycine ethyl ester (1.0 mmol) were added, the mixture was cooled at –5 °C and 1 M solution of *N,N*-diisopropylethylamine in tetrahydrofuran (1 ml) was added dropwise. After stirring at 0 °C for 2 h the reaction mixture was poured to ethyl acetate (300 ml). The organic phase was washed with water (2 times), dried and solvents were evaporated in vacuo. The residue was chromatographed on a column of silica gel (20 g), benzene–acetone (90 : 10) washed out non polar impurities and the same solvent mixture in ratio 70 : 30 eluted the product.

*N-[[[(17E)-3*β*-Hydroxyandrost-5-en-17-ylidene]amino]oxy]acetylglycylglycine ethyl ester* (XVII). Coupling of acid *XII* (210 mg, 0.5 mmol) with glycine ethyl ester hydrochloride (140 mg, 1.0 mmol) afforded 216 mg (85%) of the amorphous product *XVII*, $[\alpha]_D$ –36° (*c* 1.7, chloroform). IR spectrum (chloroform): 3 607 (O–H); 3 421, 3 330 shoulder (NH); 1 745 (C=O); 1 669, 1 522 (CONH). Mass spectrum, *m/z*: 504 (M + 1). For $C_{27}H_{41}N_3O_6$ (503.6) calculated: 64.39% C, 8.21% H, 8.34% N; found: 64.14% C, 8.34% H, 8.56% N.

*N-[[[(17E)-3*β*-Hydroxyandrost-5-en-17-ylidene]amino]oxy]acetylglycyl-*β*-alanine methyl ester* (XIX). Coupling of acid *XII* (210 mg, 0.5 mmol) with β-alanine methyl ester hydrochloride (140 mg, 1.0 mmol) afforded 223 mg (88%) of the amorphous product *XIX*, $[\alpha]_D$ –29° (*c* 2.3, chloroform). IR spectrum (chloroform): 3 609 (O–H); 3 431, 3 338 (NH); 1 734 (C=O); 1 668, 1 522 (CONH); 1 200, 1 063, 1 050 (C–O). Mass spectrum, *m/z*: 504 (M + 1). For $C_{27}H_{41}N_3O_6$ (503.6) calculated: 64.39% C, 8.21% H, 8.34% N; found: 64.12% C, 7.99% H, 8.55% N.

*N-[[[(17E)-3*β*-Hydroxyandrost-5-en-17-ylidene]amino]oxy]acetyl-*β*-alanylglycine ethyl ester* (XXI). Coupling of acid *XIV* (216 mg, 0.5 mmol) with glycine ethyl ester hydrochloride (140 mg, 1.0 mmol) afforded 240 mg (92%) of the amorphous product *XXI*, $[\alpha]_D$ –31° (*c* 2.6, chloroform). IR spectrum (chloroform): 3 609 (O–H); 3 430 (NH); 1 742 (C=O); 1 669, 1 518 (CONH); 1 200, 1 063, 1 048 (C–O). Mass spectrum, *m/z*: 518 (M + 1). For $C_{28}H_{43}N_3O_6$ (517.7) calculated: 64.97% C, 8.37% H, 8.12% N; found: 65.23% C, 8.08% H, 7.89% N.

*N-[[[(17E)-3*β*-Hydroxyandrost-5-en-17-ylidene]amino]oxy]acetyl-*β*-alanyl-*β*-alanine methyl ester* (XXIII). Coupling of acid *XIV* (216 mg, 0.5 mmol) with β-alanine methyl ester hydrochloride (140 mg, 1.0 mmol) afforded 230 mg (89%) the amorphous product *XXIII*, $[\alpha]_D$ –28° (*c* 2.2, chloroform). IR spectrum (chloroform): 3 606 (O–H); 3 438 (NH); 1 734 (C=O, ester); 1 668, 1 517 (CONH). Mass spectrum, *m/z*: 518 (M + 1). For $C_{28}H_{43}N_3O_6$ (517.7) calculated: 64.97% C, 8.37% H, 8.12% N; found: 64.75% C, 8.55% H, 7.98% N.

*N-[[[(17E)-3*β*-Hydroxyandrost-5-en-17-ylidene]amino]oxy]acetylglycylglycylglycylglycine ethyl ester* (XXV). Coupling of acid *XII* (210 mg, 0.5 mmol) with glycylglycine ethyl ester hydrochloride (196 mg, 1.0 mmol) afforded after chromatography in benzene–acetone (50 : 50) 217 mg (88%) of the amorphous product *XXV*, $[\alpha]_D$ –30° (*c* 1.8, chloroform). IR spectrum (chloroform): 3 605 (O–H); 3 424, 3 350 (NH); 1 741 (C=O); 1 669, 1 527 (CONH); 1 088, 1 063, 1 050, 1 040, 1 020. Mass

spectrum, *m*/*z*: 561 (M + 1). For $C_{29}H_{44}N_4O_7$ (560.7) calculated: 62.12% C, 7.91% H, 9.99% N; found: 62.35% C, 8.14% H, 9.76% N.

*N-[[[(17E)-3*β*-Hydroxyandrost-5-en-17-ylidene]amino]oxy]acetylglycylglycylglycylglycine ethyl ester* (XXVII). Coupling of acid *XVIII* (238 mg, 0.5 mmol) with glycylglycine ethyl ester hydrochloride (196 mg, 1.0 mmol) afforded after chromatography in dichloromethane–ethanol (80 : 20) 205 mg (66%) of the amorphous product *XXVII*, $[\alpha]_D$ −22° (*c* 1.6, chloroform–methanol 1 : 1). IR spectrum (KBr pellet): 3 370 (OH); 3 302 (NH); 1 742 (C=O, ester); 1 651, 1 550 (CONH); 1 218, 1 061, 1 028 (C–O, ester). For $C_{31}H_{47}N_5O_8$ (617.7) calculated: 60.27% C, 7.67% H, 11.34% N; found: 60.35% C, 7.47% H, 11.56% N.

General Procedure for Alkaline Hydrolysis of 17-CMO Derivatives with Attached Amino Acid or Peptide Residue

To a solution of ester (1.0 mmol) in methanol (25 ml) 0.4 M aqueous potassium hydroxide solution (10 ml) was added. After stirring for 5 h at room temperature the reaction mixture was acidified with diluted hydrochloric acid (1 : 4) and the solvents were evaporated in vacuo. The residue was triturated with water, the product was collected on the filter, washed with water and dried over phosphorus pentoxide in vacuo.

For preparation of compounds *XXXIV* – *XXXVI* an alternative method of isolation of product was used: the residue was distributed between ethyl acetate and water, 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 the product.

*N-[[[(17E)-3*β*-Hydroxyandrost-5-en-17-ylidene]amino]oxy]acetylglycine* (XII). *Method A.* Hydrolysis of ester *IX* (490 mg, 1.0 mmol) afforded 364 mg (89%) of product *XII*, m.p. 225 – 232 °C (decomposition), $[\alpha]_D$ −27° (*c* 1.7, methanol). IR spectrum (KBr pellet): 3 500 – 2 500 (COOH); 3 412, 3 320 shoulder (NH); 1 738 (C=O); 1 665, 1 547 (CONH). Mass spectrum, *m*/*z*: 419 (M + 1). For $C_{23}H_{34}N_2O_5$ (418.5) calculated: 66.01% C, 8.19% H, 6.69% N; found: 66.24% C, 8.44% H, 6.56% N. *Method B.* Hydrolysis of ester *XI* (447 mg, 1.0 mmol) afforded 335 mg (80%) of compound *XII*, m.p. 223 – 230 °C (decomposition), identical with product prepared under *A*.

*N-[[[(17E)-3*β*-Hydroxyandrost-5-en-17-ylidene]amino]oxy]acetyl-*β*-alanine* (XIV). Hydrolysis of ester *XIII* (490 mg, 1.0 mmol) afforded 386 mg (89%) of product *XIV*, m.p. 175 – 178 °C, $[\alpha]_D$ −46° (*c* 1.8, dimethyl sulfoxide). IR spectrum (KBr pellet): 3 500 – 2 500 (COOH); 3 413 (NH); 1 743, 1 722 shoulder (C=O); 1 677, 1 529 (CONH); 1 191, 1 061, 1 050 (C–O). Mass spectrum, *m*/*z*: 433 (M + 1). For $C_{24}H_{36}N_2O_5$ (432.6) calculated: 66.64% C, 8.39% H, 6.48% N; found: 66.87% C, 8.22% H, 6.48% N.

*N-[[[(17E)-3*β*-Hydroxyandrost-5-en-17-ylidene]amino]oxy]acetylglycylglycine* (XVIII). *Method A.* Hydrolysis of ester *XV* (546 mg, 1.0 mmol) afforded 366 mg (77%) of product *XVIII*, m.p. 119 – 122 °C, $[\alpha]_D$ −22° (*c* 1.6, methanol). IR spectrum (KBr pellet): 3 500 – 2 500 (COOH); 3 392 (NH); 1 734 (C=O); 1 668, 1 536 (CONH); 1 220, 1 060 (C–O). Mass spectrum, *m*/*z*: 476 (M + 1). For $C_{25}H_{37}N_3O_6$ (475.6) calculated: 63.16% C, 7.84% H, 8.84% N; found: 63.24% C, 7.56% H, 8.56% N. *Method B.* Hydrolysis of ester *XVII* (504 mg, 1.0 mmol) afforded 376 mg (79%) of compound *XVIII*, m.p. 122 – 125 °C, identical with product prepared under *A*.

*N-[[[(17E)-3*β*-Hydroxyandrost-5-en-17-ylidene]amino]oxy]acetylglycyl-*β*-alanine* (XX). Hydrolysis of ester *XIX* (504 mg, 1.0 mmol) afforded 290 mg (59%) of amorphous product *XX*, $[\alpha]_D$ −42° (*c* 1.8, dimethyl sulfoxide). IR spectrum (KBr pellet): 3 500 – 2 500 (COOH); 3 383, 3 340 shoulder, 3 084 (NH and OH); 1 731 (C=O, acid); 1 686, 1 659, 1 536 (CONH); 1 088, 1 062, 1 013 (C–O). Mass spectrum, *m*/*z*: 490 (M + 1). For $C_{26}H_{39}N_3O_6$ (489.6) calculated: 63.78% C, 8.03% H, 8.58% N; found: 64.05% C, 7.86% H, 8.29% N.

*N-[[[(17E)-3*β*-Hydroxyandrost-5-en-17-ylidene]amino]oxy]acetyl*-β-*alanylglycine* (XXII). Hydrolysis of ester *XXI* (518 mg, 1.0 mmol) afforded 314 mg (64%) of amorphous product *XXII*, $[\alpha]_D$ −39° (*c* 2.2, dimethyl sulfoxide). IR spectrum (KBr pellet): 3 500 – 2 500 (COOH); 3 390 (NH); 3 237, 3 083 (NH and OH); 1 745 (C=O, acid); 1 647, 1 628, 1 559, 1 536 (CONH); 1 088, 1 068, 1 016 (C–O). Mass spectrum, *m*/*z*: 490 (M + 1). For $C_{26}H_{39}N_3O_6$ (489.6) calculated: 63.78% C, 8.03% H, 8.58% N; found: 64.01% C, 7.89% H, 8.77% N.

*N-[[[(17E)-3*β*-Hydroxyandrost-5-en-17-ylidene]amino]oxy]acetyl*-β-*alanyl*-β-*alanine* (XXIV). Hydrolysis of ester *XXIII* (518 mg, 1.0 mmol) afforded 372 mg (74%) of amorphous product *XXIV*, $[\alpha]_D$ −42° (*c* 1.5, dimethyl sulfoxide). IR spectrum (KBr pellet): 3 500 – 2 500 (COOH); 3 380, 3 320, 3 089 (NH and OH); 1 723 (C=O, acid); 1 663, 1 632, 1 564, 1 539 (CONH); 1 188, 1 089, 1 061, 1 018 (C–O). Mass spectrum, *m*/*z*: 504 (M + 1). For $C_{27}H_{41}N_3O_6$ (503.6) calculated: 64.39% C, 8.21% H, 8.34% N; found: 64.12% C, 7.95% H, 8.65% N.

*N-[[[(17E)-3*β*-Hydroxyandrost-5-en-17-ylidene]amino]oxy]acetylglycylglycylglycine* (XXVI). Hydrolysis of ester *XXV* (560 mg, 1.0 mmol) in methanol (60 ml) afforded 446 mg (83%) of amorphous product *XXVI*, $[\alpha]_D$ −34° (*c* 2.5, dimethyl sulfoxide). IR spectrum (KBr pellet): 3 500 – 2 500 (COOH); 3 350 shoulder (OH); 3 291 (NH); 1 745 (C=O, acid); 1 651, 1 549 (CONH); 1 062 (C–O). Mass spectrum, *m*/*z*: 533 (M + 1). For $C_{27}H_{40}N_4O_7$ (532.6) calculated: 60.89% C, 7.57% H, 10.52% N; found: 61.04% C, 7.36% H, 10.66% N.

*N-[[[(17E)-3*β*-Hydroxyandrost-5-en-17-ylidene]amino]oxy]acetylglycylglycylglycylglycylglycine* (XXVIII). Hydrolysis of ester *XXVII* (617 mg, 1.0 mmol) in methanol (60 ml) afforded 450 mg (76%) of amorphous product *XXVIII*, $[\alpha]_D$ −35° (*c* 1.6, dimethyl sulfoxide). IR spectrum (KBr pellet): 3 500 – 2 500 (COOH); 3 300 (OH); 3 270 (NH); 1 744 (C=O, acid); 1 647, 1 552 (CONH); 1 065, 1 030 (C–O). For $C_{29}H_{43}N_5O_8$ (589.7) calculated: 59.07% C, 7.35% H, 11.88% N; found: 59.24% C, 7.56% H, 11.66% N.

N-[[[(17E)-3-Oxoandrost-4-en-17-ylidene]amino]oxy]acetylglycine (XXXIV). Hydrolysis of ester *XXXI* (444 mg, 1.0 mmol) afforded 404 mg (97%) of amorphous product *XXXIV*, $[\alpha]_D$ +91° (*c* 2.4, chloroform). IR spectrum (chloroform): 3 500 – 2 500 (COOH); 3 420 (NH); 1 732 (C=O, acid); 1 666 (C=O, conjugated ketone and amide I); 1 532 (NH, amide II); 1 104, 1 062, 1 012 (C–O). Mass spectrum, *m*/*z*: 417 (M + 1). For $C_{23}H_{32}N_2O_5$ (416.5) calculated: 66.32% C, 7.74% H, 6.73% N; found: 66.57% C, 7.54% H, 6.48% N.

N-[[[(17E)-3-Oxoandrost-4-en-17-ylidene]amino]oxy]acetyl-β-*alanine* (XXXV). Hydrolysis of ester *XXXII* (444 mg, 1.0 mmol) afforded 406 mg (94%) of amorphous product *XXXV*, $[\alpha]_D$ +71° (*c* 2.2, chloroform). IR spectrum (chloroform): 3 500 – 2 500 (COOH); 3 431 (NH); 1 714 (C=O, acid); 1 666 (C=O, conjugated ketone and amide I); 1 533 (NH, amide II); 1 104, 1 065, 1 013 (C–O). Mass spectrum, *m*/*z*: 431 (M + 1). For $C_{24}H_{34}N_2O_5$ (430.5) calculated: 66.95% C, 7.96% H, 6.51% N; found: 66.77% C, 8.17% H, 6.39% N.

N-[[[(17E)-3-Oxoandrost-4-en-17-ylidene]amino]oxy]acetylglycylglycine (XXXVI). Hydrolysis of ester *XXXIII* (500 mg, 1.0 mmol) afforded 434 mg (92%) of amorphous product *XXXVI*, $[\alpha]_D$ +73° (*c* 1.9, chloroform). IR spectrum (chloroform): 3 500 – 2 500 (COOH); 3 408 (NH); 1 732 (C=O, acid); 1 665 (C=O, conjugated ketone and amide I); 1 530 (NH, amide II); 1 105, 1 063, 1 013 (C–O). Mass spectrum, *m*/*z*: 474 (M + 1). For $C_{25}H_{35}N_3O_6$ (473.5) calculated: 63.41% C, 7.45% H, 8.87% N; found: 63.27% C, 7.54% H, 8.57% N.

N-[[[(17*E*)-3β-Hydroxyandrost-5-en-17-ylidene]amino]oxy]acetylglycine Ethyl Ester (*XI*)

Concentrated hydrochloric acid (1.0 ml, 12.0 mmol) was added to a solution of methoxymethoxy derivative *X* (980 mg, 2.0 mmol) in a mixture of benzene (50 ml) and ethanol (50 ml). The mixture was warmed to 50 °C for 3 h, the solvents were evaporated in vacuo, the residue was dissolved in

ethyl acetate. The extract was washed successively with water, saturated aqueous potassium hydrogen carbonate solution, water and dried. After evaporation of the solvent, the residue was chromatographed on a column of silica gel (70 g). Benzene–acetone (95 : 5) washed out non polar impurities, and the same solvent mixture (90 : 10) washed out 678 mg (76%) of amorphous hydroxy derivative *XI*, $[\alpha]_D$ −35° (*c* 2.1, chloroform). IR spectrum (chloroform): 3 606 (O–H); 3 422 (NH); 1 741 (C=O); 1 674, 1 531 (CONH); 1 048, 1 021 (C–O). Mass spectrum, *m/z*: 447 (M + 1). For $C_{25}H_{38}N_2O_5$ (446.6) calculated: 67.24% C, 8.58% H, 6.27% N; found: 67.34% C, 8.34% H, 6.56% N.

N-[[[(17*E*)-3β-Hydroxyandrost-5-en-17-ylidene]amino]oxy]acetylglycylglycine Ethyl Ester (*XVII*)

Concentrated hydrochloric acid (0.55 ml, 6.6 mmol) was added to a solution of methoxymethoxy derivative *XVI* (550 mg, 1.0 mmol) in a mixture of benzene (28 ml) and ethanol (28 ml). The mixture was warmed to 50 °C for 3 h, the solvents were evaporated in vacuo, the residue was dissolved in ethyl acetate. The extract was washed successively with water, saturated aqueous potassium hydrogen carbonate solution, water and dried. After evaporation of the solvent, the residue was chromatographed on a column of silica gel (50 g). Benzene–acetone (85 : 15) washed out non polar impurities, and the same solvent mixture (70 : 30) washed out 405 mg (80%) of amorphous hydroxy derivative *XVII*, $[\alpha]_D$ −34° (*c* 2.1, chloroform) identical with product prepared by coupling of compound *XII* with glycine ethyl ester.

(17*E*)-Androst-4-ene-3,17-dione 17-(*O*-Carboxymethyl)oxime (*XXIX*)

To a solution of ester *XXX* (1.8 g, 4.5 mmol) in methanol (100 ml) 0.4 M aqueous potassium hydroxide (45 ml) was added. After stirring for 8 h at 45 °C the reaction mixture was neutralized with dilute hydrochloric acid (1 : 4) and the solvents were evaporated in vacuo. The residue was dissolved in 5% aqueous sodium hydrogen carbonate and this solution of sodium salt was washed thoroughly with ethyl acetate. After acidification with dilute hydrochloric acid the product was extracted with ether, the extract was washed with water (2 times) and dried over anhydrous sodium sulfate. Evaporation of the solvent in vacuo afforded 1.24 g (77%) of amorphous product *XXIX*, $[\alpha]_D$ +104° (*c* 2.1, chloroform). IR spectrum: 3 500 – 2 500 (COOH); 1 767, 1 736 (C=O, acid); 1 664, 1 618 (C=C–C=O); 1 108, 1 097, 1 070 (C–O). ^{1}H NMR spectrum: 5.76 bs 1 H (H-4); 4.59 s, 2 H (OCH_2COO); 1.20 s, 3 H (3 × H-19); 0.95 s, 3 H (3 × H-18). For $C_{21}H_{29}NO_4$ (359.5) calculated 70.17% C, 8.13% H, 3.90% N; found: 70.05% C, 8.35% H, 3.75% N.

(17*E*)-Androst-4-ene-3,17-dione 17-(*O*-Carboxymethyl)oxime Isopropyl Ester (*XXX*)

Compound *VI* (3.55 g, 9.8 mmol) was dissolved in methanol–ether mixture (220 ml, 1 : 1), cooled with ice and excess of ethereal diazomethane solution was added dropwise. After 15 min stirring with cooling, the solvents and excess of diazomethane were evaporated in vacuo. The residue (3.6 g) contained mainly methyl ester *VII* (TLC, benzene–acetone 1 : 1). Methyl ester *VII* (3.6 g) was dissolved in benzene (50 ml) and 2-propanol (250 ml) and concentrated hydrochloric acid (2 ml) was added. After stirring at 60 °C for 40 h, the solvents were evaporated in vacuo, the residue was dissolved in benzene, washed with water (2 times) and dried over anhydrous sodium sulfate. Evaporation of the solvents afforded 3.67 g of crude isopropyl ester *VIII* (TLC, benzene–acetone 1 : 1). 1-Methyl-4-piperidone (6.15 ml, 50 mmol) was added under argon to a solution of *VIII* (3.67 g) in toluene (120 ml). A part (10 ml) of toluene was distilled off and 1 M aluminum isopropoxide (9.1 ml) was added. After refluxing under argon for 2 h, the mixture was cooled, diluted with ether (250 ml) and washed successively with dilute hydrochloric acid (1 : 4), water, saturated aqueous potassium carbonate solution and water. After drying over anhydrous sodium sulfate and evaporation of sol-

vents in vacuo, the residue was chromatographed on a column of silica gel (150 g) in benzene–ether (97 : 3). Yield 2.45 g (62% calculated on compound *VI*) of title compound *XXX*, m.p. 62 – 65 °C (pentane), $[\alpha]_D$ +114° (*c* 1.7, chloroform). IR spectrum (tetrachloromethane): 1 756, 1 734 (C=O, ester); 1 679, 1 619 (C=C–C=O); 1 204, 1 109 (C–O). For $C_{24}H_{35}NO_4$ (401.5) calculated 71.79% C, 8.79% H, 3.49% N; found: 72.05% C, 8.55% H, 3.25% N.

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