COUPLING OF STEROID *O*-(CARBOXYMETHYL)OXIME DERIVATIVES WITH SINGLE-PROTECTED α,ω-DIAMINOALKANES+

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New approach to the synthesis of steroid oximes bearing *O*-substituents with terminal amino groups was described. The easily accessible steroid *O*-(carboxymethyl)oximes were reacted with single-protected Boc-α,ω-diaminoalkanes to give corresponding amide intermediates. From them the Boc protecting groups were cleaved with trifluoroacetic acid to afford the desired steroid derivatives with terminal amino groups. The procedure was succesfully tested on steroids with *O*-(carboxymethyl)oxime group in positions 7 and 17. The decomposition of target products was observed during deprotection of substituted 19-oximes.
Key words: Steroids; *O*-Alkyloximes; Amides; Fluoroimmunoassays; Haptens.

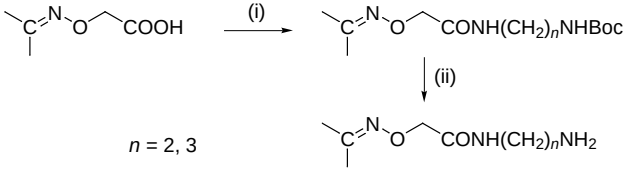
O-Alkyloximes with terminal amino groups were designed as components for steroid fluoroimmunoassay[2]. Known compounds of this class were prepared by the reaction of steroid ketones with corresponding substituted hydroxylamines, *i.e.* with *O*-(4-aminobutyl)- or *O*-(6-aminohexyl)hydroxylamine. The disadvantage of the original method was the rather complicated preparation of the substituted hydroxylamine components.

Our alternative synthetic approach leading to analogous compounds consists in preparation of *O*-(carboxymethyl)oxime derivatives (CMO) and subsequent attachment of a synthon containing amino group. The bonding is accomplished with the amidic bond, which is sufficiently stable for further synthetic use. Unlike the hydroxylamine method, this method is applicable to configurationally pure oximes. This is important for lesser hindered ketones in connection with higher *O*-alkyloxime homologues, *i.e.* for *O*-(2-carboxyethyl)oximes[3] or *O*-(3-carboxypropyl)oximes[4] and the position

+ Part CDVI in the series On Steroids; Part CDV see ref.[1]

3 of steroid skeleton. The strategy is similar to that used in preparation of derivatives with terminal hydroxy group[5]: we used easily available CMOs and suitable simple diamine synthon for the lengthening.

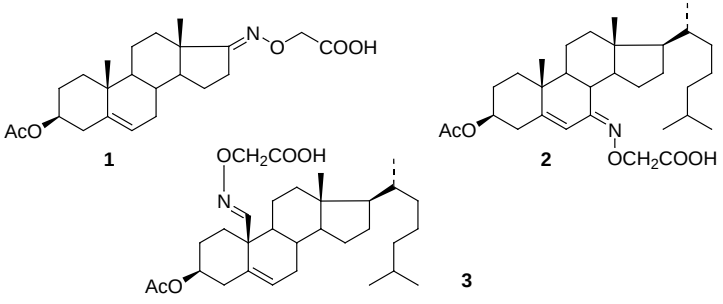
In preliminary experiments *O*-(carboxymethyl)oxime derivatives were reacted with a large excess of α,ω-diaminoalkanes, however only complex inseparable mixtures were obtained (*cf.* literature data[6]). For this reason, another synthesis consisting of two reaction steps was elaborated (Scheme 1). Steroid *O*-(carboxymethyl)oxime derivatives were first transformed to mixed anhydrides[5] and then reacted with single-protected Boc-α,ω-diaminoalkanes[7].



(i) 1. $ClCOOC_2H_5$, iPr_2NEt/THF; 2. $H_2N(CH_2)_nNHBoc$ (ii) TFA/CH_2Cl_2

SCHEME 1

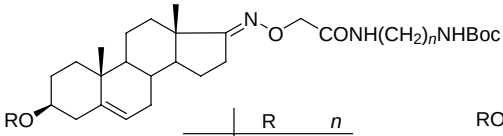
The procedure was applied to steroid *O*-(carboxymethyl)oximes **1**, **2** and **3** with oxime group in positions 17, 7 and 19, respectively. These compounds have typical structural features of biologically active steroids: a hydroxy



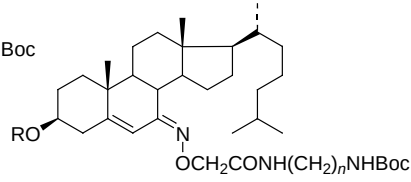
group in position 3 and a double bond in position 5 (the hydroxy group was protected as an acetate). By the reaction with single-protected ethane-1,2-diamine and propane-1,3-diamine, compounds **4**–**9** were prepared in 50–78% yields. In their IR spectra, characteristic N–H bonds and amide I and II bands were present belonging to secondary amides and carbamates. ^{1}H NMR spectra showed characteristic NH signals of secondary amides and carbamates, signals of methylene groups adjacent to nitrogen and intense singlets of the Boc group. The FAB mass spectra displayed a peak of *m/z* [M + 1],

which independently proved the attachment of the $NH(CH_2)_nNH$-$COOC(CH_3)_3$ moiety to the original oxime molecule (*m/z* increment of 142 for $n = 2$ or 156 for $n = 3$).

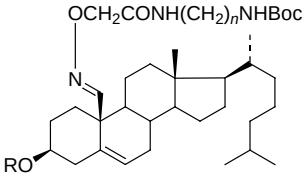
The acetate group in position 3 was selectively cleaved by alkaline hydrolysis: for characterization purposes, hydroxy derivatives **10**–**15** were prepared. In their IR spectra, characteristic O–H bonds were found. The ^1H NMR spectra displayed analogous signals as parent acetates, only H-3 protons were shifted upfield and the methyl singlet of acetate was missing.



	R	*n*
4	Ac	2
5	Ac	3
10	H	2
11	H	3



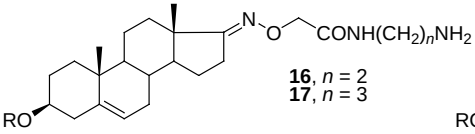
	R	*n*
6	Ac	2
7	Ac	3
12	H	2
13	H	3



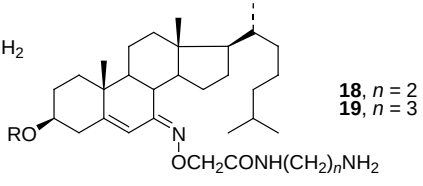
	R	*n*
8	Ac	2
9	Ac	3
14	H	2
15	H	3

To obtain amines, the removal of Boc protecting group was accomplished using trifluoroacetic acid in dichloromethane[8]. The amine formed was then without purification subjected to alkaline hydrolysis to remove the acetate protection in position 3 of the steroid. The prepared amines were purified by chromatography[9] on silica gel in a mixture of chloroform–methanol–isopropylamine. In ^1H NMR spectra, the presence of characteristic NH signals from secondary amide and that of methylene groups adjacent to nitrogen was in accord with their structures.

This procedure was successful for steroid *O*-(alkyl)oximes in positions 7 and 17 (**16**–**19**). For compounds **8** and **9** with oxime group in position 19,



16, $n = 2$
17, $n = 3$



18, $n = 2$
19, $n = 3$

the simultaneous decomposition of oxime moiety was observed during the removal of the Boc protecting group: in the ^{1}H NMR spectra of the resulting complex mixture, the signals belonging to OC**H**$_2$CONH or H-19 were not found.

In conclusion, we developed a simple method for the modifying of *O*-alkyl oxime chain in steroid CMOs. The method is designed for the synthesis of steroid haptens with potential use for fluoroimmunoassays[2] or sandwich immunoassays[10].

EXPERIMENTAL

Melting points were determined on a Boetius micro melting point apparatus (Germany). Optical rotations were measured on a Perkin–Elmer 141 MC polarimeter; $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. IR spectra (wavenumbers in cm^{-1}) were recorded on a Bruker IFS 88 spectrometer. ^{1}H NMR spectra were taken on a Varian UNITY-200 (200 MHz, FT mode) at 23 °C with tetramethylsilane as internal standard. Chemical shifts are given in ppm (δ-scale), coupling constants (*J*) and width of multiplets (*W*) in Hz. Mass spectra (FAB) were recorded on a VG Analytical ZAB-EQ spectrometer. Thin-layer chromatography (TLC) was performed on silica gel G (ICN Biochemicals), with detection by spraying with concentrated sulfuric acid followed by heating. Preparative TLC was done on 200 × 200 mm plates (layer thickness 0.4 mm). For column chromatography, neutral silica gel 60–120 μm was used. Prior to evaporation on a rotary evaporator *in vacuo* (bath temperature 50 °C), solutions in organic solvents were dried over anhydrous Na_2SO_4.

Coupling of Steroid *O*-(Carboxymethyl)oxime with Single-Protected α,ω-Diaminoalkanes – General Procedure

A 1 M solution of ethyl chloroformate in THF (2.2 ml, 2.2 mmol) was added dropwise at –5 °C to a solution of the oxime 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 single-protected α,ω-diaminoalkane[7] (4 mmol) in THF (3 ml) was added. After stirring at 0 °C for 3 h, the reaction mixture was diluted with ethyl acetate (150 ml), washed with 5% aqueous citric acid (2 × 75 ml) and saturated NaCl solution (2 × 75 ml), dried and the solvent was evaporated. The residue was chromatographed on a column of silica gel (50 g) in a mixture of benzene–acetone (98 : 2 to 90 : 10).

*(17E)-3*β*-Acetoxyandrost-5-en-17-one O-[(N-{2-[(tert-butoxycarbonyl)amino]ethyl}carbamoyl)-methyl]oxime* (**4**). Reaction of **1** (ref.[11]; 807 mg, 2.0 mmol) with *tert*-butyl *N*-(2-aminoethyl)-carbamate (641 mg, 4.0 mmol) gave 669 mg (61%) of product **4**, m.p. 144–146 °C (hexane), $[\alpha]_D$ –30 (*c* 1.7, $CHCl_3$). IR ($CHCl_3$): 3 458 (NH, carbamate); 3 436 (NH, sec. amide); 1 725 (C=O, acetate); 1 712 (amide I, carbamate); 1 671 (amide I, sec. amide); 1 531 (amide II, sec. amide); 1 508 (amide II, carbamate); 1 254 (C–O, acetate and carbamate). ^{1}H NMR ($CDCl_3$): 6.74 bt, 1 H, $J \approx 6$ (NH, sec. amide); 5.39 bd, 1 H, $J \approx 4.5$ (H-6); 4.89 bs, 1 H (NHBoc); 4.61 m, 1 H, $W = 32$ (H-3α); 4.48 s, 2 H (OCH_2CO); 3.43 m, 2 H and 3.28 m, 2 H (2 × CH_2NH); 2.04 s, 3 H (AcO); 1.44 s, 9 H (*t*-Bu); 1.04 s, 3 H (3 × H-19); 0.94 s, 3 H (3 × H-18). FAB MS, *m/z*: 546 (M + 1). For $C_{30}H_{47}N_3O_6$ (545.7) calculated: 66.03% C, 8.68% H, 7.70% N; found: 66.32% C, 8.72% H, 7.65% N.

*(17E)-3*β*-Acetoxyandrost-5-en-17-one O-[(N-{3-[(tert-butoxycarbonyl)amino]propyl}carbamoyl)-methyl]oxime* (**5**). Reaction of **1** (ref.[11]; 807 mg, 2.0 mmol) with *tert*-butyl *N*-(3-aminopropyl)-carbamate (697 mg, 4.0 mmol) gave 872 mg (78%) of product amorphous **5**, $[\alpha]_D$ –31 (*c* 1.6, $CHCl_3$). IR ($CHCl_3$): 3 457 (NH, carbamate); 3 430 (NH, sec. amide); 1 725 (C=O, acetate); 1 707 (amide I, carbamate); 1 664 (amide I, sec. amide); 1 533 (amide II, sec. amide); 1 509 (amide II, carbamate); 1 253 (C–O, acetate and carbamate). ^{1}H NMR ($CDCl_3$): 6.98 bt, 1 H, $J \approx 6$ (NH, sec. amide); 5.39 bd, 1 H, $J \approx 4.5$ (H-6); 4.89 bt, 1 H, $J \approx 6$ (NHBoc); 4.61 m, 1 H, $W = 32$ (H-3α); 4.49 s, 2 H (OCH_2CO); 3.43 q, 2 H, $J \approx 6$ and 3.28 q, 2 H, $J \approx 6$ (2 × CH_2NH); 2.04 s, 3 H (AcO); 1.43 s, 9 H (*t*-Bu); 1.04 s, 3 H (3 × H-19); 0.93 s, 3 H (3 × H-18). FAB MS, *m/z*: 560 (M + 1). For $C_{31}H_{49}N_3O_6$ (559.8) calculated: 66.52% C, 8.82% H, 7.51% N; found: 66.43% C, 9.00% H, 7.32% N.

*(7Z)-3*β*-Acetoxycholest-5-en-7-one O-[(N-{2-[(tert-butoxycarbonyl)amino]ethyl}carbamoyl)-methyl]oxime* (**6**). Reaction of **2** (ref.[12]; 1 031 mg, 2.0 mmol) with *tert*-butyl *N*-(2-amino-ethyl)carbamate (641 mg, 4.0 mmol) gave 657 mg (50%) of amorphous product **6**, $[\alpha]_D$ –117 (*c* 1.6, $CHCl_3$). IR ($CHCl_3$): 3 461 shoulder (NH, carbamate); 3 435 (NH, sec. amide); 1 727 (C=O, acetate); 1 710 (amide I, carbamate); 1 661 (amide I, sec. amide); 1 641 (C=N); 1 581 (amide II, sec. amide); 1 507 (amide II, carbamate); 1 251 (C–O, acetate and carbamate). ^{1}H NMR ($CDCl_3$): 6.63 bt, 1 H, $J \approx 5$ (NH, sec. amide); 6.51 d, 1 H, $J = 1.2$ (H-6); 4.88 bt, 1 H, $J \approx 5$ (NHBoc); 4.70 m, 1 H, $W = 32$ (H-3α); 4.50 s, 2 H (OCH_2CO); 3.43 q, 2 H, $J = 5.6$ and 3.27 q, 2 H, $J = 5.6$ (2 × CH_2NH); 2.05 s, 3 H (AcO); 1.43 s, 9 H (*t*-Bu); 1.14 s, 3 H (3 × H-19); 0.92 d, 3 H, $J = 6.4$ (3 × H-21); 0.86 d, 6 H, $J = 6.1$ (3 × H-26 and 3 × H-27); 0.69 s, 3 H (3 × H-18). FAB MS, *m/z*: 658 (M + 1). For $C_{38}H_{63}N_3O_6$ (657.9) calculated: 69.37% C, 9.65% H, 6.39% N; found: 69.25% C, 9.42% H, 6.41% N.

*(7Z)-3*β*-Acetoxycholest-5-en-7-one O-[(N-{3-[(tert-butoxycarbonyl)amino]propyl}carbamoyl)-methyl]oxime* (**7**). Reaction of **2** (ref.[12]; 1 031 mg, 2.0 mmol) with *tert*-butyl *N*-(3-amino-propyl)carbamate (697 mg, 4.0 mmol) gave 896 mg (67%) of amorphous product **7**, $[\alpha]_D$ –112 (*c* 1.8, $CHCl_3$). IR ($CHCl_3$): 3 455 (NH, carbamate); 3 431 (NH, sec. amide); 1 728 (C=O, acetate); 1 708 (amide I, carbamate); 1 666 (amide I, sec. amide); 1 641 (C=N); 1 534 (amide II, sec. amide); 1 508 (amide II, carbamate); 1 252 (C–O, acetate and carbamate). ^{1}H NMR ($CDCl_3$): 6.65 bt, 1 H, $J \approx 6$ (NH, sec. amide); 6.52 d, 1 H, $J = 1.2$ (H-6); 5.00 bs, 1 H (NHBoc); 4.69 m, 1 H, $W = 32$ (H-3α); 4.50 s, 2 H (OCH_2CO); 3.37 q, 2 H, $J = 6.3$ and 3.15 q, 2 H, $J = 5.5$ (2 × CH_2NH); 2.05 s, 3 H (AcO); 1.43 s, 9 H (*t*-Bu); 1.13 s, 3 H (3 × H-19); 0.93 d, 3 H, $J = 6.5$ (3 × H-21); 0.86 d, 6 H, $J = 6.5$ (3 × H-26 and 3 × H-27); 0.68 s, 3 H (3 × H-18). FAB MS, *m/z*: 672 (M + 1). For $C_{39}H_{65}N_3O_6$ (672.0) calculated: 69.71% C, 9.75% H, 6.25% N; found: 69.89% C, 9.58% H, 6.03% N.

*(19E)-3*β*-Acetoxycholest-5-en-19-al O-[(N-{2-[(tert-butoxycarbonyl)amino]ethyl}carbamoyl)-methyl]oxime* (**8**). Reaction of **3** (ref.[12]; 1 031 mg, 2.0 mmol) with *tert*-butyl *N*-(2-amino-ethyl)carbamate (641 mg, 4.0 mmol) gave 916 mg (70%) of amorphous product **8**, $[\alpha]_D$ –79 (*c* 1.4, $CHCl_3$). IR ($CHCl_3$): 3 457 (NH, carbamate); 3 440 shoulder (NH, sec. amide); 1 728 shoulder (C=O, acetate); 1 710 (amide I, carbamate); 1 675 (amide I, sec. amide); 1 534 shoulder (amide II, sec. amide); 1 508 (amide II, carbamate); 1 253 (C–O, acetate and carbamate). ^{1}H NMR ($CDCl_3$): 7.43 s, 1 H (H-19); 6.72 bt, 1 H, $J \approx 5$ (NH, sec. amide); 5.65 bd, 1 H, $J = 4.5$ (H-6); 4.93 bs, 1 H (NHBoc); 4.64 m, 1 H, $W = 32$ (H-3α); 4.55 s, 2 H (OCH_2CO); 3.44 q, 2 H, $J = 5.4$ and 3.26 bs, 2 H (2 × CH_2NH); 2.02 s, 3 H (AcO); 1.44 s, 9 H (*t*-Bu); 0.90 d, 3 H, $J = 6.5$ (3 × H-21); 0.86 d, 6 H, $J = 6.4$ (3 × H-26 and 3 × H-27); 0.64 s, 3 H (3 × H-18). FAB MS, *m/z*: 658 (M + 1). For $C_{38}H_{63}N_3O_6$ (657.9) calculated: 69.37% C, 9.65% H, 6.39% N; found: 69.66% C, 9.39% H, 6.15% N.

(19E)-3β-Acetoxycholest-5-en-19-al O-[(N-{3-[(tert-butoxycarbonyl)amino]propyl}carbamoyl)-methyl]oxime (**9**). Reaction of **3** (ref.[12]; 1 031 mg, 2.0 mmol) with *tert*-butyl *N*-(3-aminopropyl)carbamate (697 mg, 4.0 mmol) gave 1 001 mg (74%) of amorphous product **9**, $[\alpha]_D$ –88 (*c* 1.8, $CHCl_3$). IR ($CHCl_3$): 3 454 (NH, carbamate); 3 437 (NH, sec. amide); 1 728 shoulder (C=O, acetate); 1 708 (amide I, carbamate); 1 671 (amide I, sec. amide); 1 529 (amide II, sec. amide); 1 509 (amide II, carbamate); 1 253 (C–O, acetate and carbamate). ^{1}H NMR ($CDCl_3$): 7.43 s, 1 H (H-19); 6.65 bt, 1 H, $J \approx 5$ (NH, sec. amide); 5.64 bd, 1 H, J = 4.5 (H-6); 5.01 bs, 1 H (NHBoc); 4.63 m, 1 H, W = 32 (H-3α); 4.55 s, 2 H (OCH_2CO); 3.38 q, 2 H, J = 6.4 and 3.15 bs, 2 H (2 × CH_2NH); 2.02 s, 3 H (AcO); 1.43 s, 9 H (*t*-Bu); 0.90 d, 3 H, J = 6.4 (3 × H-21); 0.86 d, 6 H, J = 6.4 (3 × H-26 and 3 × H-27); 0.63 s, 3 H (3 × H-18). FAB MS, *m/z*: 672 (M + 1). For $C_{39}H_{65}N_3O_6$ (672.0) calculated: 69.71% C, 9.75% H, 6.25% N; found: 69.95% C, 9.56% H, 5.98% N.

Hydrolysis of Acetates – General Procedure

An 0.4 M aqueous NaOH (0.5 ml, 0.2 mmol) was added to a solution of acetate (0.2 mmol) in THF (2 ml) and methanol (1 ml). After stirring at room temperature for 5 h, the solvents were evaporated *in vacuo*. The residue was dissolved in ethyl acetate (50 ml) and 5% aqueous citric acid (50 ml) and the aqueous phase was extracted with ethyl acetate (50 ml). Combined extracts were washed with water (2 × 50 ml), dried and evaporated *in vacuo*. The residue was chromatographed on two silica gel plates in a mixture of benzene–acetone (60 : 40).

(17E)-3β-Hydroxyandrost-5-en-17-one O-[(N-{2-[(tert-butoxycarbonyl)amino]ethyl}carbamoyl)-methyl]oxime (**10**). Acetate **4** (109 mg, 0.2 mmol) gave 63 mg (63%) of hydroxy derivative **10**, m.p. 191–192 °C (acetone–ether), $[\alpha]_D$ –33 (*c* 1.6, $CHCl_3$). IR ($CHCl_3$): 3 610 (OH); 3 458 (NH, carbamate); 3 436 (NH, sec. amide); 1 707 (amide I, carbamate); 1 671 (amide I, sec. amide); 1 531 (amide II, sec. amide); 1 509 (amide II, carbamate); 1 251 (C–O, carbamate). ^{1}H NMR ($CDCl_3$): 6.74 bs, 1 H (NH, sec. amide); 5.36 bd, 1 H, $J \approx 4.5$ (H-6); 4.91 bs, 1 H (NHBoc); 4.48 s, 2 H (OCH_2CO); 3.53 m, 1 H, W = 32 (H-3α); 3.43 q, 2 H, J = 6.1 and 3.27 q, 2 H, J = 6.1 (2 × CH_2NH); 1.44 s, 9 H (*t*-Bu); 1.03 s, 3 H (3 × H-19); 0.94 s, 3 H (3 × H-18). For $C_{28}H_{45}N_3O_5$ (503.7) calculated: 66.77% C, 9.01% H, 8.34% N; found: 66.83% C, 9.12% H, 8.35% N.

(17E)-3β-Hydroxyandrost-5-en-17-one O-[(N-{3-[(tert-butoxycarbonyl)amino]propyl}carbamoyl)-methyl]oxime (**11**). Acetate **5** (112 mg, 0.2 mmol) gave 82 mg (79%) of amorphous hydroxy derivative **11**, $[\alpha]_D$ –31 (*c* 1.7, $CHCl_3$). IR ($CHCl_3$): 3 609 (OH); 3 457 (NH, carbamate); 3 431 (NH, sec. amide); 1 705 (amide I, carbamate); 1 664 (amide I, sec. amide); 1 533 (amide II, sec. amide); 1 509 (amide II, carbamate); 1 252 (C–O, carbamate). ^{1}H NMR ($CDCl_3$): 6.98 bt, 1 H, $J \approx 6$ (NH, sec. amide); 5.36 bd, 1 H, $J \approx 4.5$ (H-6); 4.89 bt, 1 H, $J \approx 6$ (NHBoc); 4.49 s, 2 H (OCH_2CO); 3.53 m, 1 H, W = 32 (H-3α); 3.34 q, 2 H, J = 6.4 and 3.16 q, 2 H, J = 5.8 (2 × CH_2NH); 1.43 s, 9 H (*t*-Bu); 1.03 s, 3 H (3 × H-19); 0.93 s, 3 H (3 × H-18). For $C_{29}H_{47}N_3O_5$ (517.7) calculated: 67.28% C, 9.15% H, 8.12% N; found: 67.46% C, 9.17% H, 7.89% N.

(7Z)-3β-Hydroxycholest-5-en-7-one O-[(N-{2-[(tert-butoxycarbonyl)amino]ethyl}carbamoyl)-methyl]oxime (**12**). Acetate **6** (132 mg, 0.2 mmol) gave 104 mg (84%) of amorphous hydroxy derivative **12**, $[\alpha]_D$ –112 (*c* 1.6, $CHCl_3$). IR ($CHCl_3$): 3 610 (OH); 3 461 shoulder (NH, carbamate); 3 435 (NH, sec. amide); 1 707 (amide I, carbamate); 1 670 (amide I, sec. amide); 1 638 (C=N); 1 532 (amide II, sec. amide); 1 507 (amide II, carbamate); 1 252 (C–O, carbamate). ^{1}H NMR ($CDCl_3$): 6.65 bt, 1 H, $J \approx 5.5$ (NH, sec. amide); 6.47 d, 1 H, J = 1.2 (H-6); 4.86 bs, 1 H (NHBoc); 4.50 s, 2 H (OCH_2CO); 3.65 m, 1 H, W = 32 (H-3α); 3.42 q,

2 H, J = 5.6 and 3.25 bt, 2 H, $J \approx 6$ (2 × CH_2NH); 1.43 s, 9 H (*t*-Bu); 1.13 s, 3 H (3 × H-19); 0.92 d, 3 H, J = 6.4 (3 × H-21); 0.86 d, 6 H, J = 6.4 (3 × H-26 and 3 × H-27); 0.68 s, 3 H (3 × H-18). For $C_{36}H_{61}N_3O_5$ (615.9) calculated: 70.21% C, 9.98% H, 6.82% N; found: 70.45% C, 10.02% H, 6.56% N.

*(7Z)-3*β*-Hydroxycholest-5-en-7-one O-[(N-{3-[(tert-butoxycarbonyl)amino]propyl}carbamoyl)-methyl]oxime* (**13**). Acetate **7** (134 mg, 0.2 mmol) gave 112 mg (89%) of amorphous hydroxy derivative **13**, $[\alpha]_D$ –118 (*c* 1.6, $CHCl_3$). IR ($CHCl_3$): 3 610 (OH); 3 455 (NH, carbamate); 3 431 (NH, sec. amide); 1 706 (amide I, carbamate); 1 655 (amide I, sec. amide); 1 639 (C=N); 1 534 (amide II, sec. amide); 1 508 (amide II, carbamate); 1 252 (C–O, carbamate). ^{1}H NMR ($CDCl_3$): 6.72 bt, 1 H, $J \approx 6$ (NH, sec. amide); 6.50 d, 1 H, J = 1.2 (H-6); 4.98 bs, 1 H (NHBoc); 4.51 s, 2 H (OCH_2CO); 3.64 m, 1 H, W = 32 (H-3α); 3.36 q, 2 H, $J \approx 6$ and 3.15 bt, 2 H, $J \approx 5.6$ (2 × CH_2NH); 1.43 s, 9 H (*t*-Bu); 1.12 s, 3 H (3 × H-19); 0.92 d, 3 H, J = 6.4 (3 × H-21); 0.87 d, 6 H, J = 6.4 (3 × H-26 and 3 × H-27); 0.68 s, 3 H (3 × H-18). For $C_{37}H_{63}N_3O_5$ (629.9) calculated: 70.55% C, 10.08% H, 6.67% N; found: 70.67% C, 10.28% H, 6.45% N.

*(19E)-3*β*-Hydroxycholest-5-en-19-al O-[(N-{2-[(tert-butoxycarbonyl)amino]ethyl}carbamoyl)-methyl]oxime* (**14**). Acetate **8** (132 mg, 0.2 mmol) gave 111 mg (90%) of amorphous hydroxy derivative **14**, $[\alpha]_D$ –80 (*c* 1.6, $CHCl_3$). IR ($CHCl_3$): 3 607 (OH); 3 461 shoulder (NH, carbamate); 3 440 (NH, sec. amide); 1 704 (amide I, carbamate); 1 673 (amide I, sec. amide); 1 527 (amide II, sec. amide); 1 511 (amide II, carbamate); 1 253 (C–O, carbamate). ^{1}H NMR ($CDCl_3$): 7.42 s, 1 H (H-19); 6.64 bt, 1 H, $J \approx 5.5$ (NH, sec. amide); 5.62 bd, 1 H, $J \approx 4.5$ (H-6); 5.20 bs, 1 H (NHBoc); 4.52 s, 2 H (OCH_2CO); 3.57 m, 1 H, W = 32 (H-3α); 3.44 q, 2 H, $J \approx 5.5$ and 3.28 bt, 2 H, $J \approx 5.5$ (2 × CH_2NH); 1.44 s, 9 H (*t*-Bu); 0.90 d, 3 H, J = 6.4 (3 × H-21); 0.86 d, 6 H, J = 6.4 (3 × H-26 and 3 × H-27); 0.63 s, 3 H (3 × H-18). For $C_{36}H_{61}N_3O_5$ (615.9) calculated: 70.21% C, 9.98% H, 6.82% N; found: 70.56% C, 10.06% H, 6.53% N.

*(19E)-3*β*-Hydroxycholest-5-en-19-al O-[(N-{3-[(tert-butoxycarbonyl)amino]propyl}carbamoyl)-methyl]oxime* (**15**). Acetate **9** (134 mg, 0.2 mmol) gave 119 mg (95%) of hydroxy derivative **15**, m.p. 105–108 °C (ether), $[\alpha]_D$ –87 (*c* 1.6, $CHCl_3$). IR ($CHCl_3$): 3 609 (OH); 3 454 shoulder (NH, carbamate); 3 437 (NH, sec. amide); 1 704 (amide I, carbamate); 1 671 (amide I, sec. amide); 1 530 (amide II, sec. amide); 1 510 (amide II, carbamate); 1 252 (C–O, carbamate). ^{1}H NMR ($CDCl_3$): 7.42 s, 1 H (H-19); 6.56 bt, 1 H, $J \approx 5.5$ (NH, sec. amide); 5.61 bd, 1 H, $J \approx 4.5$ (H-6); 5.00 bs, 1 H (NHBoc); 4.53 s, 2 H (OCH_2CO); 3.55 m, 1 H, W = 32 (H-3α); 3.37 q, 2 H, J = 6.4 and 3.14 t, 2 H, J = 6.4 (2 × CH_2NH); 1.44 s, 9 H (*t*-Bu); 0.90 d, 3 H, J = 6.4 (3 × H-21); 0.86 d, 6 H, J = 6.4 (3 × H-26 and 3 × H-27); 0.63 s, 3 H (3 × H-18). For $C_{37}H_{63}N_3O_5$ (629.9) calculated: 70.55% C, 10.08% H, 6.67% N; found: 70.45% C, 10.05% H, 6.72% N.

Cleavage of Boc Groups – General Procedure

Trifluoroacetic acid (200 μl, 2.6 mmol) was added dropwise to a solution of a Boc protected derivative (0.2 mmol) in dichloromethane (1 ml) at 0 °C. The mixture was stirred for 40 min at room temperature, cooled to 0 °C, and 4 M solution of KOH in methanol (2 ml) was added. After stirring at room temperature for 12 h, the solvents were evaporated and the residue was chromatographed. For compounds **16** and **17**, the residue was chromatographed on a column of silica gel (10 g) in a mixture of $CHCl_3$–MeOH–isopropylamine (98 : 1 : 1). For compounds **18** and **19**, the residue was chromatographed on two preparative silica gel plates in a mixture of $CHCl_3$–MeOH–isopropylamine (94 : 4 : 2).

*(17E)-3*β*-Hydroxyandrost-5-en-17-one O-{[N-(2-aminoethyl)carbamoyl]methyl}oxime* (**16**). Deprotection of **4** (109 mg, 0.2 mmol) gave 56 mg (69%) of amorphous product **16**, $[\alpha]_D$ –22

(*c* 1.3, MeOH). IR (KBr pellet): 3 415 broad (NH, sec. amide); 3 292 shoulder (NH_2); 1 659 (amide I); 1 536 (amide II); 1 088 (C–NH_2); 1 062, 1 042 (C–O). ^{1}H NMR (CD_3SOCD_3): 7.42 bt, 1 H, $J \approx 5.8$ (NH, sec. amide); 5.29 bd, 1 H, $J \approx 4.5$ (H-6); 4.30 s, 2 H (OCH_2CO); 3.08 q, 2 H, $J = 6.1$ (C**H**$_2$NHCO); 2.55 t, 2 H, $J = 6.4$ (C**H**$_2$$NH_2$); 0.96 s, 3 H (3 × H-19); 0.86 s, 3 H (3 × H-18). For $C_{23}H_{37}N_3O_3$ (403.6) calculated: 68.45% C, 9.24% H, 10.41% N; found: 68.67% C, 9.35% H, 10.13% N.

*(17E)-3*β*-Hydroxyandrost-5-en-17-one O-{[N-(3-aminopropyl)carbamoyl]methyl}oxime* (**17**). Deprotection of **5** (112 mg, 0.2 mmol) gave 59 mg (71%) of amorphous product **17**, $[\alpha]_D$ –19 (*c* 1.0, MeOH). IR (KBr pellet): 3 350 broad (NH); 1 669 (amide I); 1 534 (amide II); 1 087 (C–NH_2); 1 061, 1 041 (C–O). ^{1}H NMR (CD_3SOCD_3): 7.42 bt, 1 H, $J \approx 5.8$ (NH, sec. amide); 5.28 bd, 1 H, $J \approx 4.5$ (H-6); 4.30 s, 2 H (OCH_2CO); 3.09 q, 2 H, $J = 6.1$ (C**H**$_2$NHCO); 2.56 t, 2 H, $J = 6.4$ (C**H**$_2$$NH_2$); 0.96 s, 3 H (3 × H-19); 0.86 s, 3 H (3 × H-18). For $C_{24}H_{39}N_3O_3$ (417.6) calculated: 69.03% C, 9.41% H, 10.06% N; found: 68.97% C, 9.23% H, 9.84% N.

*(7Z)-3*β*-Hydroxycholest-5-en-7-one O-{[N-(2-aminoethyl)carbamoyl]methyl}oxime* (**18**). Deprotection of **6** (132 mg, 0.2 mmol) gave 66 mg (64%) of amorphous product **18**, $[\alpha]_D$ –121 (*c* 1.4, MeOH). IR (KBr pellet): 3 300 broad (NH); 1 683, 1 671 (amide I); 1 640 shoulder (C=N); 1 538 (amide II); 1 078 (C–NH_2); 1 060 (C–O). ^{1}H NMR (CD_3SOCD_3): 7.59 bt, 1 H, $J \approx 6$ (NH, sec. amide); 6.43 bs, 1 H (H-6); 4.31 s, 2 H (OCH_2CO); 3.10 q, 2 H, $J = 6.3$ (C**H**$_2$NHCO); 2.57 t, 2 H, $J = 6.3$ (C**H**$_2$$NH_2$); 1.05 s, 3 H (3 × H-19); 0.90 d, 3 H, $J = 6.4$ (3 × H-21); 0.84 d, 6 H, $J = 6.4$ (3 × H-26 and 3 × H-27); 0.65 s, 3 H (3 × H-18). For $C_{31}H_{53}N_3O_3$ (515.8) calculated: 72.19% C, 10.36% H, 8.15% N; found: 72.34% C, 10.62% H, 7.86% N.

*(7Z)-3*β*-Hydroxycholest-5-en-7-one O-{[N-(3-aminopropyl)carbamoyl]methyl}oxime* (**19**). Deprotection of **7** (134 mg, 0.2 mmol) gave 83 mg (79%) of amorphous product **19**, $[\alpha]_D$ –119 (*c* 1.6, MeOH). IR (KBr pellet): 3 300 broad (NH); 1 668, 1 662 (amide I); 1 646 shoulder (C=N); 1 540 (amide II); 1 079 (C–NH_2); 1 067 (C–O). ^{1}H NMR (CD_3SOCD_3): 7.70 bt, 1 H, $J \approx 6$ (NH, sec. amide); 6.43 bs, 1 H (H-6); 4.31 s, 2 H (OCH_2CO); 3.16 q, 2 H, $J = 6.3$ (C**H**$_2$NHCO); 2.59 t, 2 H, $J = 6.7$ (C**H**$_2$$NH_2$); 1.05 s, 3 H (3 × H-19); 0.91 d, 3 H, $J = 6.4$ (3 × H-21); 0.84 d, 6 H, $J = 6.4$ (3 × H-26 and 3 × H-27); 0.65 s, 3 H (3 × H-18). For $C_{32}H_{55}N_3O_3$ (529.8) calculated: 72.55% C, 10.46% H, 7.93% N; found: 72.64% C, 10.50% H, 7.71% N.

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