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Steroids 54. Amino acylamidosteroids

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Aminosteroids were prepared and acylated with protected amino acids by means of the mixed anhydride or the active ester method. The tert*-butyloxycarbonyl- (BOC) protecting group was eliminated by acidolysis, and the benzyloxycarbonyl- (Z) group by catalytic hydrogenation. 3β- and 6β-Glycylamidosteroids were prepared by indirect amination of chloroacetamido derivatives, formed by the Ritter reaction on the corresponding 3α,5α-cyclo and 5α,6α-epoxy steroids. Water-soluble double salts were produced from the compounds for pharmacological investigations.* © 1996 by Elsevier Science Inc. (*Steroids* **61**:697–702, 1996)

Keywords: aminoalkyl carboxamidosteroid; Ritter reaction; cyclosteroid; epoxysteroid

Introduction

Immunomodulation is an important feature in medicine, involving in certain cases activation of the immune system, and in other cases the immunosuppressant activity of natural and synthetic materials. Immunostimulants are also used as adjuvants in certain therapeutic methods. There are many natural compounds that influence the immune system; one group of them contains peptide structural moieties. A tripeptide segment of the lactoyl oligopeptide FK-156, isolated as a metabolite of a microorganism, was shown to be the minimum structure necessary to elicit immunostimulatory activity.[1]

The immunoregulatory activity of corticosteroids is used in practice, when suppression of the immune system is needed.[2] The advantageous properties of peptides and steroids are thought to be coupled in aminosteroids acylated by amino acids. Researchers at Roussel-Uclaf prepared such pregnane derivatives and reported their immunostimulatory activities.[3,4]

Other workers report the antiarrythmic activity of amino acylamidosteroids.[5]

These results prompted us to prepare amidosteroids with pregnane, androstane, and estratriene skeletons containing the amino acid part in positions 3, 6, 17, and 21.

Experimental

The starting steroids were kindly provided by Gedeon Richter Chemical Works (Budapest, Hungary). All solvents used were of reagent grade, unless otherwise stated. Melting points (m.p.) were determined on a Kofler block and are uncorrected. Optical rotations were measured with a Polamat-A polarimeter (Zeiss, Jena) (c = 1). The reactions were monitored by thin-layer chromatography (TLC) (Merck precoated silica gel F_{254} plates, 0.25 mm thick) in the following solvent systems (ss): (A) acetone/benzene/petroleum ether (3.5:3.5:3 v/v), (B) benzene/methanol (8:2 v/v), and (C) benzene/methanol (7:3 v/v). The spots were detected in ultraviolet light (254 and 366 nm) and visualized by spraying with concentrated H_2SO_4 or with vanillin reagent [vanillin (0.3 g) dissolved in ethanol (10 mL) and conc H_2SO_4 (5 drops) added], followed by heating (120 °C) for 10 min. Elemental analyses were carried out in a KOVO (Czechoslovakia) CHN automatic analyzer after exhaustive drying of the samples in vacuum (8–14 h, 100°C). ^1H NMR spectra were recorded on a Bruker AM 400 instrument.

Aminosteroids

Aminosteroids were prepared from the corresponding ketoximes by $LiAlH_4$ reduction or by Ipaktschi's method ($NaBH_4/NiCl_2$).[6,7]

Acylation of aminosteroids by mixed anhydride method

To a cooled (−15°C) and stirred solution of BOC- or (*Z*)-amino acid (2 mmol) and *N*-ethylmorpholine (2 mmol) in dry dimethylformamide (DMFA, 25 mL), isobutyl chloroformate (2 mmol) was added. The crystalline hydrochloride salt of the amine separated and the temperature rose to −10°C. After 5 min the suspension was cooled again to −15°C, and a cooled (−15°C) dimethylformamide (20–40 mL) solution of the aminosteroid (2 mmol) was added over 5–10 min. The reaction mixture was left to warm to 0°C, and it was maintained at this temperature for 3 h, then at 5°C for 15–20 h. In the case of sterically hindered amines an additional reaction time at room temperature was needed. When TLC showed a complete transformation (a less polar new component), the solution was poured into ice (150 g). The voluminous precipitate was filtered, washed on the filter with water until neutral, and dried (yield: 85–97%). BOC-protected amino acylamidosteroids **3aa, 3ca, 3da, 3fa, 4aa, 4ca, 4da, 4fa, 5ca, 9aa, 9ca,** and **9fa** (Table 1) and the

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Received March 20, 1995; accepted August 12, 1996.

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0039-128X/96/$15.00
PII S0039-128X(96)00202-6

Table 1 Physical constants of N-BOC-amino acylamidosteroids

Compounds	Yield (%)	M.p. (°C)	R_f (B)
3aa	87	102–110	0.68 + 0.72
3ca	95	119–122	0.69 + 0.74
3da	73	114–120	0.72
3fa	93	117–123	0.68
4aa	85	114–119	0.73
4ca	94	117–127	0.70
4da	92	116–121	0.74
4fa	97	112–117 sint. from 107	0.71
5ca	96	100–102	0.69 + 0.73
9aa	88	103–112	0.7
9ca	88	98–105	0.7 + 0.73
9fa	93	100–107	0.76 + 0.8

Table 2 Physical constants of *N-Z* protected amino acylamidosteroids

Compounds	Yield (%)	M.p. (°C)	R_f (A)	$[\alpha]_D^\circ$ ($c = 1$)
1ab	82	Foam	0.50	+19 (EtOH)
1cb	86	95–105	0.75	+34 ($CHCl_3$)
1db	65	Foam	0.70	+24 ($CHCl_3$)
1eb	72	Oily	0.75	+13 (EtOH)
6ab	78	209–211	0.85	+81 (EtOH)
6bb	71	202–207	0.80	+69 ($CHCl_3$)
6cb	73	167–172	0.80	+71 ($CHCl_3$)
6db	75	98–103	0.90	+65 ($CHCl_3$)
6eb	55	169–173	0.85	+72 ($CHCl_3$)
7cb	81	Oily	0.75	+ 6 ($CHCl_3$)

analogous *Z*-protected compounds **1ab, 1cb, 1db, 1eb, 6ab, 6bb, 6cb, 6db,** and **6eb** (Table 2) were prepared in this manner.

Acylation with N-(t-butoxycarbonyl)-L-alanylsuccinimide

20(*R,S*)-Aminopregna-5,16-dien-3β-ol (**9,** 3.15 g, 1 mmol) was dissolved in dry DMFA (30 mL), and *t*-BOC-alanylsuccinimide (2.83 g, 1.05 mmol) was added. After 15 min of stirring, the solution was made neutral by adding triethylamine (5 drops) and was left to stand at room temperature for 20 h, when TLC no longer revealed free amine. 2-(Dimethylamino)ethylamine (0.5 mL) was added, and the solution was saturated with water. The precipitate formed was filtered, washed with water until neutral, and dried. Compound **9ca** was obtained as a white powder (4.22 g, 87%), R_f = 0.76 and 0.80 (twin spots, ss B), m.p. 100–107°C.

Elimination of the BOC protecting group

N-BOC-aminoalkyl carboxamidosteroid (2 mmol) was dissolved in dry cold (5°C) dioxane containing dry hydrogen chloride (4.5 mol). The solution was left to stand at this temperature for 4–20 h.

Table 3 Physical constants and elemental analysis data of compounds **1a–10a**.HCl

Compound	M.p. (°C)	$[\alpha]_D^\circ$	R_f (ss)	Molecular formula	Calculated C	Calculated H	Calculated N	Found C	Found H	Found N
1a	196–200	+28 ($c = 1$, EtOH)	0.38 (B)	$C_{21}H_{36}N_2O_2$ (348.5)	72.36	10.41	8.03	72.05	10.61	8.40
1c	194–196	+30 ($c = 1$, EtOH)	0.49 (B)	$C_{22}H_{38}N_2O_2$ (362.6)	72.88	10.56	7.72	72.79	10.35	7.20
1d	174–177	+34 ($c = 1$, EtOH)	0.65 (B)	$C_{28}H_{42}N_2O_2$ (438.7)	76.66	9.65	6.38	76.42	9.45	6.90
1e	185–187	+17 ($c = 1$, EtOH)	0.60 (B)	$C_{25}H_{44}N_2O_2$ (404.6)	74.20	10.96	6.92	74.48	10.75	6.72
2a.HCl	240 (dec.)	−44 ($c = 1$, EtOH)	0.6 (C)	$C_{23}H_{37}ClN_2O_3$ (425.0)	65.06	8.78	6.60	64.75	8.74	6.30
3a.HCl	219–220		0.32 (C)	$C_{21}H_{37}ClN_2O_2$ (385.0)	65.51	9.69	7.28	65.25	9.85	7.48
3c.HCl	295–304	−27 ($c = 0.5$, MeOH)	0.18 (C)	$C_{22}H_{39}ClN_2O_2 \cdot H_3COH$ (431.1)	64.08	10.05	6.47	63.87	9.61	6.24
3d.HCl	189–193	+27 ($c = 0.5$, DMSO)	0.42 (C)	$C_{28}H_{43}ClN_2O_2 \cdot H_3COH$ (507.2)	68.68	9.34	5.52	69.42	9.08	5.67
3f.HCl	263–267[a] (lit.[8] 256, sint. from 235)	−52 ($c = 1$, EtOH)	0.32 (C)	$C_{24}H_{41}ClN_2O_2 \cdot H_3COH$ (425.1)	65.69	9.92	6.13	65.81	10.18	6.55
4a.HCl[b]	211–215	−97 ($c = 1$, EtOH)	0.30 (C)	$C_{21}H_{35}ClN_2O_2 \cdot H_3COH$ (415.0)	63.66	9.47	6.75	63.78	9.41	7.07
4f.HCl	286–302 (lit.[10] 263)		0.08 (C)	$C_{24}H_{39}ClN_2O_2 \cdot H_3COH$ (455.1)	65.98	9.52	6.16	65.63	9.44	6.39
5c.HCl	255–258	−20 ($c = 0.5$, MeOH)	0.25 (C)	$C_{22}H_{33}ClN_2O_2 \cdot H_3COH$ (230.0)	64.99	8.77	6.59	64.94	8.59	6.86
6a.HCl	265–268 (dec.)	+54 ($c = 1$, EtOH)	0.80 (C)	$C_{23}H_{39}ClN_2O_2$ (411.0)	67.21	9.56	6.82	66.89	9.20	6.85
6b.HCl	160–163	+43 ($c = 1$, EtOH)	0.60 (C)	$C_{25}H_{42}ClN_3O_3$ (468.1)	64.15	9.04	8.98	64.07	8.75	8.77
6c.HCl	185–188	+72 ($c = 1$, EtOH)	0.80 (C)	$C_{24}H_{41}ClN_2O_2$ (425.0)	67.82	9.72	6.59	67.56	9.65	6.34
6d.HCl	170–175	+85 ($c = 1$, EtOH)	0.85 (C)	$C_{30}H_{45}ClN_2O_2$ (501.1)	71.90	9.05	5.59	71.74	9.18	5.27
6e.HCl	168–172	+55 ($c = 1$, EtOH)	0.90 (C)	$C_{27}H_{47}ClN_2O_2$ (467.1)	69.42	10.14	5.99	69.12	9.80	6.06
7c.HCl	152–155 (lit.[7] m.p. 220)[c]	+18 ($c = 1$, EtOH)	0.60 (C)	$C_{24}H_{43}ClN_2O_2$ (427.1)	67.49	10.15	6.52	67.35	10.37	6.30
7d.HCl	172–176C (lit.[7] m.p. 240)[c]	+35 ($c = 1$, EtOH)	0.85 (C)	$C_{30}H_{47}ClN_2O_2$ (503.1)	71.76	9.42	5.57	71.53	9.56	5.34
8a.HCl	200–210 (dec.)	+5 ($c = 1$, EtOH)	0.51 (C)	$C_{23}H_{37}ClN_2O_2$ (409.0)	67.54	9.12	6.85	67.38	9.34	6.72
9c.HCl	216–218		0.2 (B)	$C_{24}H_{38}ClN_2O_2$ (422.0)	68.30	9.08	6.64	67.92	9.17	6.40
10a.HCl	275–280 (dec.)	−9 ($c = 1$, EtOH)	0.80 (C)	$C_{25}H_{41}ClN_2O_5$ (485.0)	61.90	8.52	5.77	61.66	8.33	5.79

[a]Containing crystal methanol.
[b]The base is described in Ref. 9.
[c]20*S* derivatives.

Table 4 Selected ^{1}H NMR data for steroid 17β-isomers **3a–5d**

Compound	Signals ($CDCl_3$/DMSO/TMS) δ, ppm							
	18-H_3	19-H_3	CH_2 or CH[a]	3-H (m)	17-H (q)	6-H	-CO-NH	NH_3^+
3a.HCl	0.71	0.80	3.56	3.50	3.82	–	7.92	8.39
3f.HCl	0.68	0.79[b]	4.34	3.38	3.82	–	8.22	8.50
4a.HCl	0.81	0.99	3.58	3.48	3.84	5.28	7.90	8.40
4f.HCl	0.79	1.03	3.96	3.46	3.78	5.28	8.10	8.40
5d.HCl	0.78	–	3.52	–	3.72	–	8.75	8.90[c]

[a]In the amino acid part.
[b]Literary values: 0.63, 0.77 (on the free base in $CDCl_3$).[8]
[c]Further signals: 3.78 ($ArOCH_3$) and 7.35, 7.75, 7.90 (aromatic hydrogens) ppm.

When TLC showed a complete transformation, the solution was saturated with diethyl ether, and the solid material that separated was filtered and washed with diethyl ether or an ether-acetone mixture until neutral. The filter cake was dried in a desiccator over sodium hydroxide pellets (yield: 70–90%). The material can be recrystallized from methanol or from methanol-ether. Compounds **3a, 3c, 3d, 3f, 4a, 4c, 4d, 4f, 5c, 9c,** and **9f** were prepared by this method (Tables 3 and 4).

Reduction of 20-carbonyl group in 3-Z-aminoalkyl acylamidopregnane derivatives

To a cooled (0°C) suspension of 3α-(*N*-Z-aminoalkyl acylamido)-5α-pregnan-20-ones (**6cb** and **6db,** 1 mmol) in ethanol (25 mL), sodium borohydride (0.75 g, 20 mmol) was added in portions. After 2 h, the reaction mixture was saturated with water, and the resulting precipitate was filtered, washed with water until neutral, and dried over P_2O_5 in a vacuum desiccator. Compounds **7cb** and **7db** were prepared by this process (data on **7cb** are given in Table 2).

Elimination of the Z protecting group

The *N*-Z-aminoalkyl carboxamidosteroids (1 mmol) were dissolved in ethyl acetate and 10% Pd/C was added. Hydrogen gas was bubbled through the stirred suspension for 4 h at room temperature. The catalyst was then removed by filtration and the solvent was evaporated off under reduced pressure. The residue was chromatographed on silica gel, using a mixture of acetone/benzene (1:9 v/v) as eluent. Evaporation of the pure fractions yielded the following bases: **1a, 1c, 1d, 1e, 6a, 6b, 6c, 6d, 6e, 7c,** and **7d** (data on the compounds are given in Table 3).

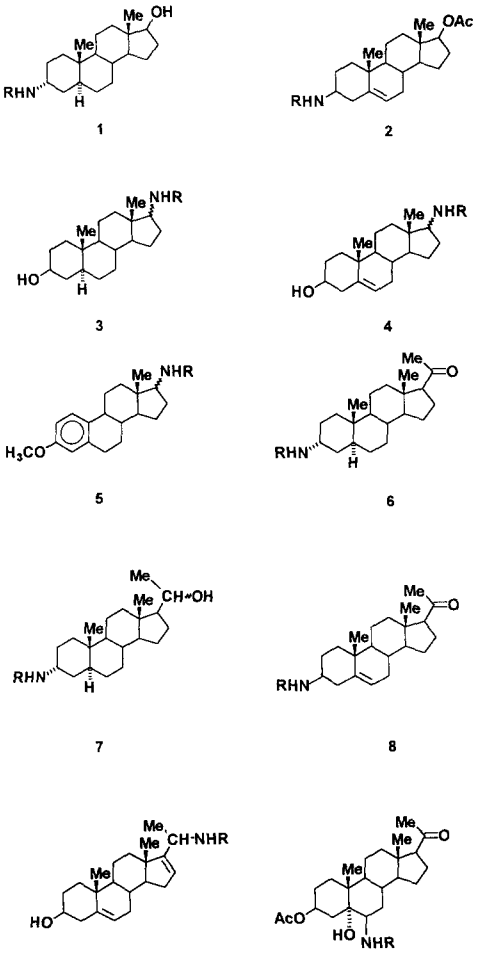
Preparation of 3β-glycylamidoandrost-5-en-17β-ol (2a) and 3β-glycylamidopregn-5-en-20-one (8a) from 3α,5α-cyclosteroids (11, 12) and 3β-acetoxy-6β-glycylamido-5-hydroxy-5α-pregnan-20-one (10a) from 5α,6α-epoxy-pregnane (17)

Ritter reaction. *From 3α,5α-cyclosteroids.* To a cooled (0°C) dichloromethane (10 mL) solution of 3α,5α-cyclo-5α-androstane-6β,17β-diol-6,17-diacetate[11] (**11,** 0.01 mol) chloroacetonitrile (7.5 g, 0.1 mol) and borontrifluoride ethyl etherate (1 mL) were added. After standing for 12 h, the solution, which turned lilac, was washed in turn with water and with saturated $NaHCO_3$ solution, then dried (Na_2SO_4) and evaporated. The residual oil was chromatographed on neutral alumina with benzene. The pure fractions were combined and evaporated, and the resulting product was recrystallized from acetone, affording 3β-chloroacetamidoandrost-5-en-17β-ol 17-acetate (**13**) as white powder. M.p. 212–213°C, $[\alpha]_D = -58°$ (chloroform).

Table 5 Physical properties of hydrophylic salts (**h.t.**[a] and **HCl.t.**[b]) of compounds **2–7**

Compound	M.p. (range °C)	$[\alpha]_D^{20}$ ($c = 1$)	R_f (C)	Molecular formula	Calculated			Found			Solubility in water (%)
					C	H	N	C	H	N	
2a.h.t.	162–167	−25[c]	0.45	$C_{25}H_{40}N_2O_8$ (496.6)	60.46	8.11	5.64	60.64	8.34	5.30	0.3[d]
3c.HCl.t.	270–278	−19[c]	0.15	$C_{25}H_{50}ClN_3O_6S$ (556.2)	53.98	9.06	7.55	53.89	8.94	7.29	0.2
3f.HCl.t.	273–290	−3[c]	0.20	$C_{26}H_{48}ClN_3O_5S$ (550.2)	56.75	8.79	7.63	56.64	8.55	7.48	0.2
4a.HCl.t.	203–208	−78[e]	0.22	$C_{23}H_{42}ClN_3O_5S$ (508.1)	54.37	8.33	8.27	54.14	8.46	8.36	0.5
4c.HCl.t.	266–275	−60[e]	0.12	$C_{24}H_{44}ClN_3O_5S$ (522.2)	55.20	8.49	8.05	55.08	8.23	7.91	0.5[d]
4d.HCl.t.	191–197	+19[e]	0.73 + 0.6	$C_{30}H_{48}ClN_3O_5S.H_2O$ (616.3)	58.47	8.18	6.82	58.58	8.17	6.82	0.4[d]
5c.HCl.t.	255–265	−12[c]	0.12	$C_{24}H_{40}ClN_3O_5S$ (518.1)	55.63	7.78	8.11	55.49	7.81	7.97	0.15
6a.h.t.	122–126	+53[c]	0.60	$C_{27}H_{44}N_2O_8$ (524.7)	61.81	8.40	5.30	61.72	8.34	5.22	0.5
6c.h.t.	115–120	+51[c]	0.60	$C_{28}H_{46}N_2O_8$ (538.7)	62.43	8.60	5.20	62.65	8.45	5.40	0.5
6e.h.t.	125–130	+50[c]	0.80	$C_{31}H_{52}N_2O_8$ (580.8)	64.11	9.02	4.80	64.34	9.18	4.58	0.5
7c.h.t.	125–128	+20[c]	0.70	$C_{29}H_{48}N_2O_8$ (540.7)	62.19	8.94	5.18	62.34	8.75	5.25	0.5
7d.h.t.	132–137	+41[c]	0.80	$C_{34}H_{52}N_2O_8$ (616.8)	66.20	8.49	4.54	66.45	8.36	4.32	0.4

[a]Hemitartrate.
[b]Hydrochloride-taurine double salt.
[c]In MeOH.
[d]In EtOH/H_2O (3:7).
[e]In EtOH/H_2O (7:3).



	R		R		R
1-10	H				
a	Gly	aa	BOC-Gly	ab	Z-Gly
b	GlyGly	ba	BOC-GlyGly	bb	Z-GlyGly
c	L-Ala	ca	BOC-L-Ala	cb	Z-L-Ala
d	L-Phe	da	BOC-L-Phe	db	Z-L-Phe
e	DL-Leu			eb	Z-DL-Leu
f	L-Pro	fa	BOC-L-Pro		

Scheme 1

With 6β-acetoxy-3α,5-cyclo-5α-pregnan-20-one[12] (**12,** 3.5 g, 0.01 mol) as starting compound and working as above, 2.1 g (53%) 3β-chloroacetamidopregn-5-en-20-one (**15**) was obtained. M.p. 170–171°C, $[\alpha]_D$ = +25.9° (chloroform).

From 5α,6α-epoxide. 3β-Acetoxy-5α,6α-oxidopregnan-20-one[13] (**17,** 3.7 g, 0.01 mol) was reacted in the above manner and afforded 2.9 g (62%) 3β-acetoxy-6β-chloroacetamino-5α-hydroxypregnan-20-one (**18**). M.p. 164–165°C, $[\alpha]_D$ = −19.1° (EtOH).

Chloride-azide exchange. Sodium azide (3.25 g, 0.05 mol) was suspended in a dimethylformamide (30 mL) solution of chloroacetamidoandrost-5-ene (**13**) or -pregnane derivatives (**15** or **18,** 0.01 mol), and the stirred suspension was heated at 100°C for 6 h. Water (150 mL) was then added to the resulting brown mixture, which was extracted with benzene (3 × 30 mL). The combined benzene fractions were washed with water, dried on Na_2SO_4, and evaporated to dryness. The residual oil was chromatographed on neutral alumina, with benzene as eluent. The combined pure fractions gave after evaporation a yellowish oil, which crystallized from chloroform/petroleum ether.

3β-Azidoacetamidoandrost-5-en-17β-ol 17-acetate (**14,** 3.1 g, 78%): m.p. 169–173°C, $[\alpha]_D$ = −63° (EtOH).

3β-Azidoacetamidopregn-5-en-20-one (**16,** 2.5 g, 62%): m.p. 153–157°C, $[\alpha]_D$ = +26.3° (chloroform).

3β-Acetoxy-6β-azidoacetamido-5α-hydroxypregnan-20-one (**19,** 2.8 g, 68%): m.p. 124–127°C, $[\alpha]_D$ = −5.1° (EtOH).

Reduction of the azido groups. The above 3β-azidoacetamido derivatives (**14, 16,** or **19,** 0.01 mol) were dissolved in ethanol (200 mL) and shaken in hydrogen in the presence of 10% Pd/C for 6 h. The catalyst was then filtered off, 2 N methanolic hydrogen chloride (10 mL) was added, and the solution was evaporated to dryness in vacuum. The solid residue was suspended in diethyl ether and filtered. These hydrochloride salts were used without recrystallization.

3β-Glycylamidoandrost-5-en-17β-ol 17-acetate (**2a**) was obtained with a yield of 89% (3.6 g, data on the compound are in Table 3).

The yield of 3β-glycylamidopregn-5-en-20-one (**8a**) was 78% (3.1 g, data in Table 3).

3β-Acetoxy-6β-glycylamido-5α-hydroxypregnan-20-one (**10a**) was formed in 78% yield (3.8 g, data in Table 3).

Formation of hydrochloride salts

Amino acylamidosteroids (5 mmol) were dissolved in methanol (50–300 mL) at room temperature, and concentrated hydrochloric acid (5.1 mmol HCl) was added. The solution was stirred for 5 min and evaporated to dryness at a reduced pressure. The residue was recrystallized from methanol or from methanol/diethyl ether.

Formation of hemitartrates

To a stirred methanolic solution of the amino acylamidosteroid base (5 mmol), a concentrated aqueous solution of tartaric acid (0.75 g, 5 mmol) was added. The solution was evaporated to dryness and the residue was recrystallized from ethanol.

Formation of hydrochloride-taurine double salts

To a stirred methanolic (400 mL) solution of the amino acylamidosteroid hydrochloride salt (5 mmol), taurine (0.63 g, 5 mmol) dissolved in water (10 mL) was added. After stirring for a further 5 min, the solution was evaporated to dryness and the crystalline residue was recrystallized from methanol (see Table 5).

Results and discussion

Aminosteroids were prepared by various methods. 3α-Amino-5α-androstane (**1**) and 3α-amino-5α-pregnane (**6**) derivatives were prepared via the known route of β-tosylate → α-azide exchange, followed by reduction.[14] 20ξ-Aminopregnadiene derivative (**9**) was prepared by $LiAlH_4$ reduction, and the 17ξ-amines (**3, 4,** and **5**) by $NaBH_4/NiCl_2$ reduction[6] from the corresponding ketoximes. The acylated aminosteroids **2, 7, 8,** and **10** were not prepared from amino derivatives; their syntheses started from another acylamidosteroid (**7** from **6**) or from 3α,5α-cyclo compounds (**11, 12**)[11,12] and from the 5α,6α-epoxide (**17**), respectively.[13]

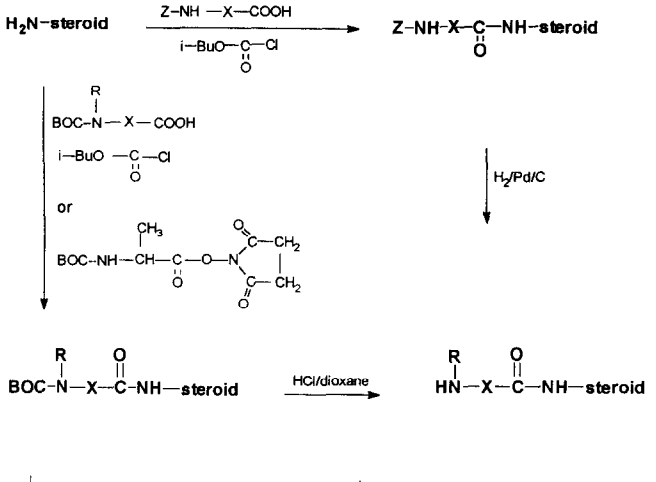
The amines **3, 4, 5,** and **9** were acylated with BOC-amino

acids by the mixed anhydride method,[15] whereas in the case of amines **1** and **6** Z-amino acids were applied in the same mixed anhydride method. Alanyl derivative **9ca** was also prepared by acylation of amine **9** with BOC-alanylsuccinimide.[16]

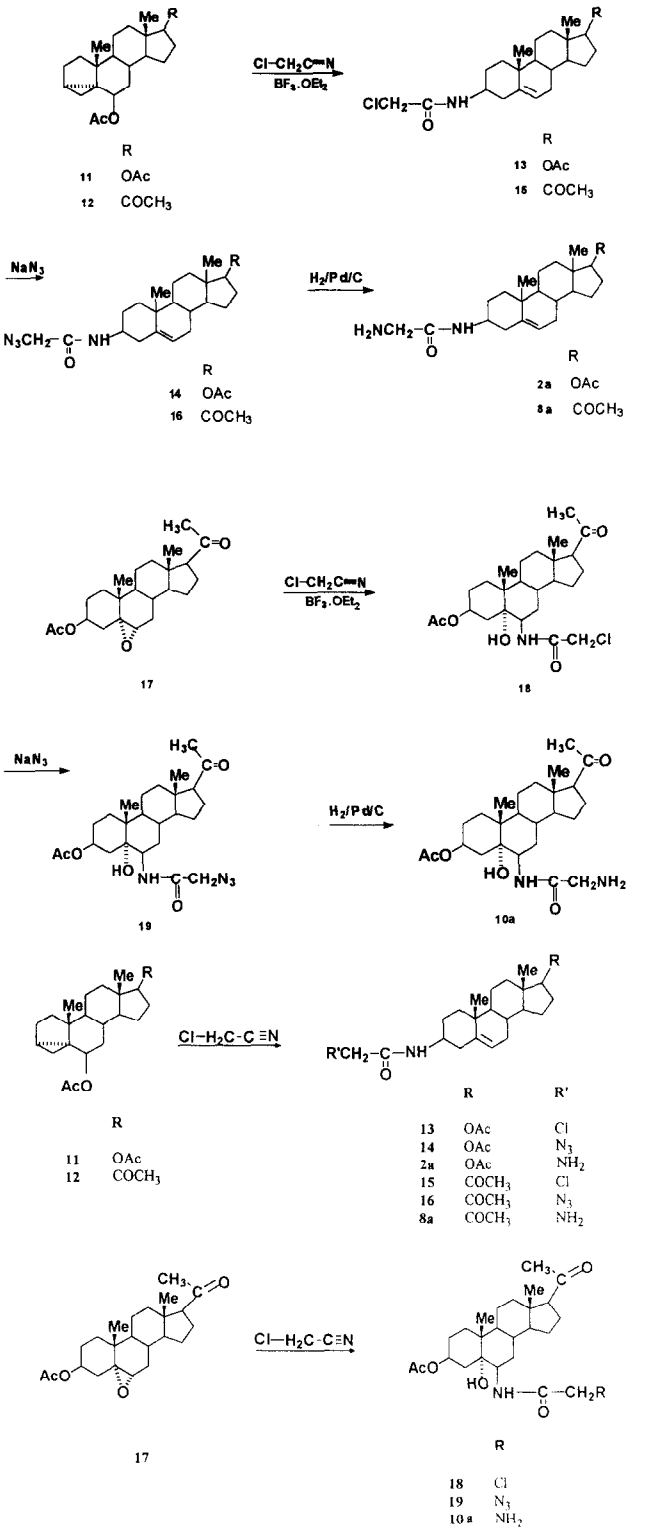
For deprotection of the aminosteroids acylated with BOC-amino acids, dry acidic fission of the BOC group proved to be the best method. Hydrogen chloride in dry dioxane cleaved the urethane bond in a few hours without the formation of by-products. The hydrochloride salt of the aminoalkyl acylamidosteroid was precipitated from the reaction mixture on dilution with water (see Schemes 1 and 2).

As starting aminosteroids, we applied the mixture of epimeric amines in several cases. The isomeric derivatives acylated with amino acids displayed a significant difference in polarity, even if achiral glycine was used. These epimeric modifications can be separated from each other by recrystallization. In this manner we prepared the pure stereoisomers **17β–3a, 17β–3f, 17β–4a, 17β–4f,** and **17β–5d.** The configuration of the C-17 substituent is established on the basis of ^{1}H NMR spectra. The 17α and 17β protons not only exhibit different chemical shifts, but the signals for the 17α-H and 17β-H protons show different coupling patterns with the amide N-H and the C-16 protons characteristic of the C-17 isomers.[17] The C-17 acetamide isomers show the 17 α-H (quasi-axial) as a quartet (d,d,d), whereas the C-17β-H (quasi-equatorial) is a triplet (d,d).[17] In our isomers the 17-H appears as a four-line pattern, indicating its quasi-axial position; therefore, the substituent is assigned the 17β stereochemistry (see Table 4).

Besides the acylation of aminosteroids, other routes are sometimes needed, especially when the aminosteroid is not easily available. Another possibility for the formation of



Scheme 2



Scheme 3

acylamido derivatives is the opening of strained rings with organic nitriles. Cyclic compounds that can afford a carbenium ion under acidic conditions react with nitriles to produce amido-substituted derivatives. This transformation, named the Ritter reaction,[18] was applied to 3α,5α-cyclosteroids[19] and yielded acetamido- and benzamidocholestanes and pregnanes. Application of this reaction for the

opening of 4β,5β-, 5β,6β-, and 5α,6α-epoxysteroids gave 5α-acetamido-4β-hydroxy, 5α-acetamido-6β-hydroxy, and 6β-acetamido-5α-hydroxy derivatives, respectively, in regioselective reactions.[20,21]

We earlier extended this reaction to the opening of steroid oxethanes, when steroidal dihydrooxazines were formed.[22] Instead of the substrate, we have now modified the reagent. Application of chloroacetonitrile to both 3α,5α-cyclo- **(11, 12)** and 5α,6α-epoxysteroid **(17)** resulted in the formation of the corresponding chloroacetamido derivatives **(13, 15, 18).** The presence of the chloro substituent involves the possibility of its transformation to an amino group. This was solved in two steps: exchange to azides **(14, 16, 19)** followed by reduction to the wanted amino derivatives **(2a, 8a, 10a).** Both reactions are regio- and stereoselective. The transformation of 3α,5α-cycloandrostane-6β,17β-diol **(11)** in the above process gave **2a,** whereas on starting from the analogous pregnan-20-one **(12)** we obtained glycylamido derivative **8a.** Both compounds contain the 3-glycylamido group in the β position.

The opening of the 5α,6α-epoxide **(17)** in the Ritter reaction is also regioselective, resulting in 5α-hydroxy-6β-amido substitution **(18).** It follows a modified S_N2 mechanism and causes a vicinal *trans*-diaxial steric arrangement, as demonstrated by Bourgery et al.[21] On this basis, in **10a** a 5α,6β disubstitution can be found (Scheme 3).

The pharmacological tests demanded aqueous solutions, but our compounds are only sparingly soluble in water, even in the form of hydrochloride salts. To increase their hydrophilicity, we formed salts with organic acids. The hemitartrates exhibit better but still not high enough solubility. From the double salts formed from the hydrochlorides with taurine, an aqueous solution with a concentration of 0.5–1.5% can be prepared, which is suitable for *in vitro* pharmacological studies.

The results of immunological screening and antiarrythmic activity investigations will be published later.

Acknowledgments

This work was supported by grant OTKA T 020938 of the Hungarian Academy of Sciences. We thank Mrs. M. Rózsa-Tarjáni for the NMR spectra and Dr. G. Bozóki-Bartók for the elemental analyses.

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