LINEAR CHAINING OF ETIENIC ACID DERIVATIVES WITH THE AMIDE BOND. SYNTHESIS OF OLIGOMERIC STEROIDS+

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Synthesis of linear molecules containing two to four steroid units connected with the amide bonds was developed. As a repeating unit, 3β-hydroxyandrost-5-ene-17β-carboxylic acid (etienic acid) was used. An active ester of this acid with *N*-hydroxysuccinimide and its 3-azido analogue were prepared and methods for the preparation of higher oligomers were studied.

Keywords: Steroids; Active esters; Amides; Oligoamides; NMR spectroscopy; Oligomers.

Continuing our efforts in the synthesis of larger linear molecular structures using the steroid skeleton as a rigid building block, we were searching for a suitable method of connecting steroid units less tightly than in the case of steroid pyrazines[2], where the low solubility of even bis-steroid compounds has complicated the condensation to higher fused compounds. The present work utilises the amide bond for connecting steroid units. The amide bond is sufficiently stable during standard chemical transformations, which is an advantage over the pyrazine-condensed steroids. The one-point connection allows more free molecular motion compared with the pyrazine-fused compounds, and thus we expected the formation of more soluble compounds. On the other hand, to achieve a more fixed orientation of steroid units, we needed to keep the linking chain as short as possible. For this reason, we chose derivatives of 3β-hydroxyandrost-5-ene-17β-carboxylic acid (**1**) (etienic acid) and its 3β-azido analogue as an amine source. From a broad palette of amide syntheses, known from peptide chemistry, we chose the active ester method with *N*-hydroxysuccinimide[3] which has the advantage

+ Part CDXI in the series On Steroids; Part CDX see ref.[1]

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of producing a water-soluble side product. Examples of the use of the amide bond for connecting steroid units are limited to the cholic acid area[4,5] (for reviews on cholic acid oligomers see *cf.* refs[6,7]). Cholic acids, however, contain a carboxylic group at the end of a four-carbon side chain, so their reactivity is very probably different from etienic acid derivatives used in this study.

In our initial experiments, we tested a two-steroid condensation. As a key intermediate, we prepared active ester **2** by the *N,N′*-dicyclohexylcarbodiimide-mediated condensation of etienic acid (**1**) and *N*-hydroxysuccinimide. As a precursor for the amine component, we used 3β-azidoandrost-5-en-17β-ol (**3**). This compound has been reported in the literature[8], but in the original paper the configuration in position 17 was left unresolved. In our opinion, borohydride reduction of 17-oxoandrost-5-enes gives exclusively a 17β-hydroxy derivative; additional support is given in Table I, where ^{1}H NMR acetylation shifts for the corresponding 3β-hydroxy derivatives of 17α and 17β configurations are compared (data for diol acetates are taken from ref.[9]).

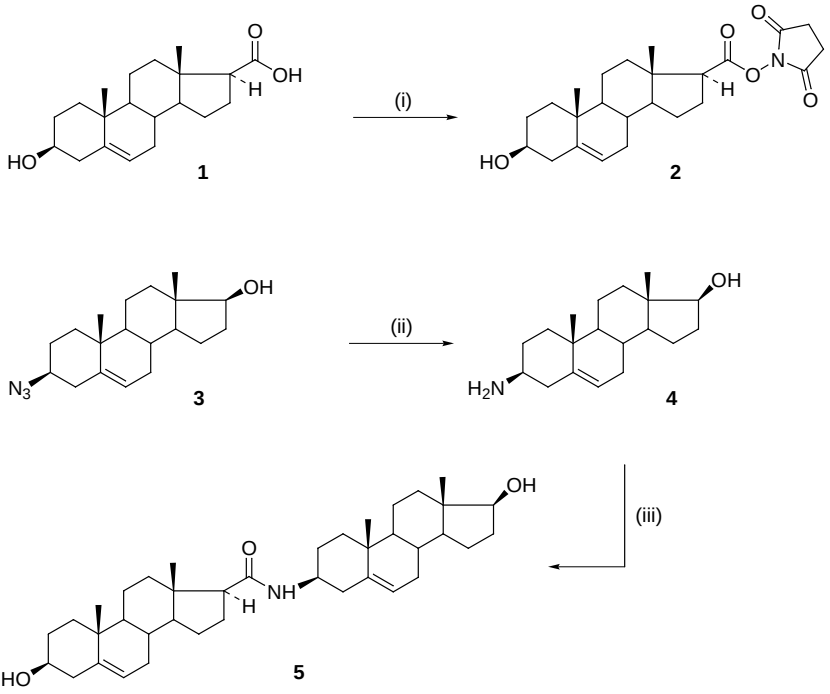
To prepare amine **4**, we employed a sodium borohydride reduction in the presence of nickel(II) chloride, which worked well with other steroid derivatives[10]. The crude amine reacted with active ester **2** in THF rather slowly, but after 24 h most of the reactants were converted into amide **5**

TABLE I
Selected ^{1}H NMR data for epimeric androst-5-en-17-ol derivatives in $CDCl_3$ (coupling constants in parentheses)

Compound	Proton		
	H-17	H-18	H-19
3	3.65 t (8.4)	0.76 s	1.02 s
3β,17β-Diol 3-acetate	3.65 t (8.2)	0.76 s	1.03 s
3β,17α-Diol 3-acetate	3.75 t (5.0)	0.68 s	1.04 s
Acetate[a] of **3**	4.60 dd (7.8, 9.0)	0.80 s	1.01 s
3β,17β-Diol diacetate	4.60 dd (7.5, 9.0)	0.80 s	1.03 s
3β,17α-Diol diacetate	4.84 t (5.0)	0.76 s	1.04 s

[a] The acetate of **3** was prepared by the treatment of **3** with acetic anhydride in pyridine.

(Scheme 1). The presence of the amide bond was confirmed by ^{1}H NMR by an NH doublet at δ 5.13 with the coupling constant J = 8.5 Hz, and by amide bands at 1 657 (amide I) and 1 505 (amide II) cm^{-1} present in the IR spectrum.



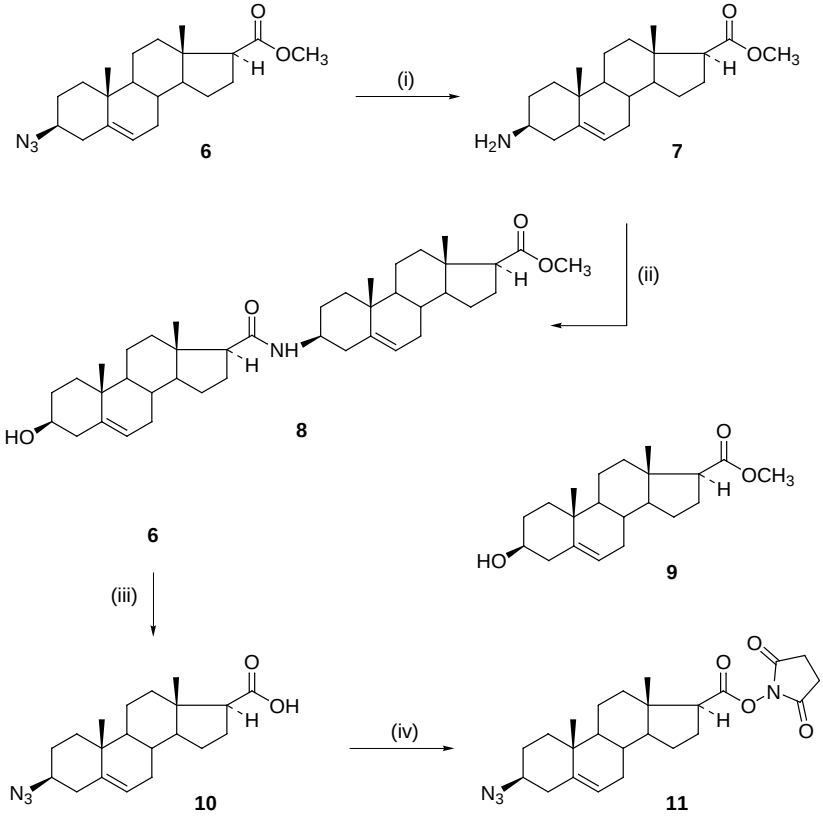
(i) *N*-hydroxysuccinimide, DCC, dioxane; (ii) $NaBH_4$, $NiCl_2$, dioxane, EtOH; (iii) **2**, THF

SCHEME 1

In the second stage, we attempted the preparation of dimeric steroid amides capable of further lengthening. The required intermediate, bearing both azide as the amine precursor and protected carboxylic acid moiety, was methyl 3β-azidoandrost-5-ene-17β-carboxylate (**6**). This compound has been described in the patent literature[11]; it was prepared in connection with the synthesis of growth-regulating activity agents. Its straightforward synthesis was briefly reproduced according to a summary in Chemical Abstracts.

Amine **7** was prepared from azide **6** and a subsequent condensation with the active ester **2** gave disteroid **8** (Scheme 2). This derivative mimics the arrangement of the characteristic groups of methyl ester of etienic acid **9**: the

hydroxy group in position 3 of one steroid unit (denoted as "west") and the methyl carboxylate in position 17 of the second steroid unit (denoted as "east"), both units being linked with the 17′→3 carboxamide bridge. ^{13}C NMR spectra of **8** and **9** are presented in Table II (for both compounds the HMQC method was used in the assignment). When compared, carbon shifts of parts of **9**, including rings A and B on one side and C and D on the other, match well the shifts from the corresponding parts of both steroid units of **8**.



(i) $NaBH_4$, $NiCl_2$, dioxane, EtOH; (ii) **2**, benzene, DMF; (iii) NaOH, THF, MeOH; (iv) *N*-hydroxysuccinimide, DCC, THF

SCHEME 2

When analysing a possible approach to higher-condensed steroids, two ways seem feasible at this point. The first consists in the propagation from the C-terminus including hydrolysis of the methyl ester group in **8**, activation with *N*-hydroxysuccinimide and repeating the above described condensation with amine **7**. The second method uses the N-terminus propagation, for which the analogue of **8** with the azide group in position 3 is required. After its reduction to amine, the condensation with **2** can give a tristeroid derivative.

Preliminary experiments indicated that the former method was applicable, but the active ester on the disteroid reacted with amine **7** only slowly and the reaction did not proceed to completion. To apply the latter method, we needed the 3-azido analogue of **2**, available from acid **10** by the *N*,*N*′-dicyclohexylcarbodiimide mediated condensation with *N*-hydroxysuccinimide. The resulting active ester **11** can be used for further condensation without purification.

In the synthesis of the tristeroid derivative, we initially prepared disteroid **12** with the azido group in position 3′ from amine **7** and active ester **11**. The tristeroid analogue of etienic acid was then obtained by the reduction of **12** to amine **13** and a subsequent condensation with the active ester **2**. The sequence was repeated: from amine **13**, the intermediate tristeroid **15** was obtained, and *via* amine **16**, tetrasteroids **17** and **18** were prepared. Condensation to tetrasteroids proceeded more slowly and the target compounds were obtained in only small amounts. Their properties, however, are promising: taking into account their molecular weight (>1 200), their solubility in organic solvents is satisfactory for usual manipulations and the products can be purified by classic column chromatography on silica gel.

The structural evidence for steroid oligomers is based mainly on ^{13}C NMR spectra. Tables II and III summarize the experimental data and, as discussed before with derivatives **8** and **9**, very good consistency in data sets for the particular units in di-, tri- and tetrasteroids both in 3β-hydroxy (**8**, **9**, **14** and **17**) and 3β-azido (**6**, **12**, **15** and **18**) series was found (Scheme 3).

In conclusion, we developed a method for linear chaining of steroid units capable of linking up to four steroid units. In principle, four steroid units, when assuming a linear arrangement, can reach a 40 Å limit corresponding to the lipid bilayer thickness. Hence, with the appropriate substitution, similar oligomers can be utilised in the studies of transmembrane transport. The question of space arrangement of the individual units in the prepared oligosteroids has not been satisfactorily answered and will be the subject of a separate study. The use of intermediates **2** and **11**, however, is not necessarily limited to the field of steroid oligomers, as they are designed for a

7 + 11
(i)
(ii)
12, R = N3
13, R = NH2
12, 13
13 + 2
13 + 11
(iii)
(iii)
(ii)
14, R = OH
15, R = N3
16, R = NH2
14-16
16 + 2
16 + 11
(iii)
(iii)
17, R = OH
18, R = N3
17, 18
(i) benzene, THF, DMF; (ii) NaBH4, NiCl2, THF, EtOH; (iii) THF, DMF

SCHEME 3

broader application in the attachment of one or more steroid units to any suitable backbone, *e.g.* to peptide or nucleotide chains.

TABLE II
^{13}C NMR chemical shifts of 3-hydroxysteroids **8**, **9**, **14** and **17** in $CDCl_3$

Carbon	**9**	Disteroid **8**		Tristeroid **14**			Tetrasteroid **17**			
		west	east	west	middle	east	west	middle		east
C-1	37.26	37.28	37.90	37.28	37.90	37.90	37.28	37.91	37.91	37.91
C-2	31.61	31.61	29.27	31.61	29.29	29.25	31.62	29.28	29.28	29.26
C-3	71.70	71.70	49.43	71.70	49.41	49.43	71.70	49.41	49.41	49.43
C-4	42.25	42.25	39.85	42.26	39.86	39.86	42.25	39.87	39.87	39.87
C-5	140.83	140.75	140.34	140.76	140.26	140.39	140.75	140.27	140.30	140.38
C-6	121.36	121.42	121.57	121.42	121.63	121.54	121.42	121.62	121.60	121.54
C-7	31.81	31.81	31.75	31.81	31.75	31.75	31.81	31.76	31.76	31.76
C-8	32.00	31.95	31.91	31.96	31.86	31.91	31.96	31.86	31.86	31.92
C-9	50.06	50.14	49.99	50.13	50.06	49.99	50.13	50.06	50.06	49.99
C-10	36.55	36.55	36.60	36.55	36.60	36.60	36.55	36.61	36.61	36.61
C-11	20.91	20.79	21.03	20.79	20.91	21.03	20.79	20.91	20.91	21.03
C-12	38.19	38.68	38.14	38.68	38.63	38.14	38.68	38.63	38.63	38.14
C-13	43.95	43.62	43.93	43.62	43.60	43.93	43.62	43.61	43.61	43.93
C-14	56.14	56.45	56.06	56.45	56.37	56.06	56.45	56.38	56.38	56.06
C-15	24.56	24.51	24.55	24.51	24.51	24.55	24.51	24.51	24.51	24.55
C-16	23.64	23.41	23.65	23.41	23.41	23.65	23.41	23.41	23.41	23.65
C-17	55.19	57.15	55.17	57.14	57.14	55.17	57.14	57.14	57.14	55.17
C-18	13.31	13.06	13.31	13.06	13.06	13.31	13.08	13.08	13.08	13.32
C-19	19.38	19.39	19.36	19.39	19.37	19.37	19.39	19.37	19.37	19.37
C-20	174.56	171.68	174.60	171.70	171.68	174.60	171.71	171.69	171.68	174.60
OCH_3	51.21	–	51.21	–	–	51.21	–	–	–	51.22

TABLE III
^{13}C NMR chemical shifts of 3-azidosteroids **6**, **12**, **15** and **18** in $CDCl_3$

Carbon	**6**	Disteroid **12**		Tristeroid **15**			Tetrasteroid **18**			
		west	east	west	middle	east	west	middle		east
C-1	38.11	38.10	37.90	38.09	37.90	37.90	38.10	37.90	37.90	37.90
C-2	27.91	27.91	29.28	27.90	29.25	29.28	27.90	29.25	29.27	29.29
C-3	61.08	61.08	49.44	61.06	49.41	49.43	61.07	49.42	49.42	49.44
C-4	37.58	37.59	39.86	37.58	39.86	39.86	37.58	39.86	39.86	39.86
C-5	139.92	139.84	140.34	139.82	140.24	140.38	139.83	140.25	140.30	140.38
C-6	122.22	122.27	121.59	122.26	121.64	121.54	122.26	121.64	121.61	121.54
C-7	31.78	31.77	31.77	31.80	31.75	31.75	31.75	31.75	31.75	31.75
C-8	31.92	31.82	31.96	31.81	31.86	31.95	31.82	31.86	31.86	31.95
C-9	50.04	50.10	49.99	50.08	50.05	49.98	50.08	50.05	50.05	49.98
C-10	36.66	36.66	36.61	36.64	36.60	36.60	36.65	36.60	36.60	36.60
C-11	20.83	20.80	20.94	20.78	20.90	20.93	20.80	20.92	20.92	20.95
C-12	38.13	38.62	38.14	38.60	38.60	38.13	38.62	38.61	38.61	38.14
C-13	43.92	43.60	43.93	43.60	43.60	43.92	43.61	43.61	43.61	43.93
C-14	56.09	56.40	56.06	56.36	56.38	56.05	56.37	56.37	56.37	56.06
C-15	24.54	24.50	24.56	24.49	24.50	24.55	24.49	24.51	24.51	24.55
C-16	23.64	23.42	23.65	23.41	23.41	23.64	23.42	23.42	23.42	23.64
C-17	55.18	57.13	55.18	57.11	57.13	55.16	57.12	57.14	57.14	55.18
C-18	13.31	13.06	13.32	13.06	13.07	13.31	13.06	13.07	13.07	13.32
C-19	19.28	19.28	19.37	19.28	19.36	19.36	19.28	19.37	19.37	19.37
C-20	174.52	171.62	174.60	171.64	171.70	174.60	171.66	171.72	171.73	174.61
OCH_3	51.23	–	51.21	–	–	51.21	–	–	–	51.22

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 in chloroform solution, unless stated otherwise. NMR spectra were taken on a Varian UNITY-200 (^{1}H at 200 MHz) or on Varian UNITY-500 (^{1}H at 500 MHz, ^{13}C at 125.7 MHz) instruments at 23 °C, in deuteriochloroform, with tetramethylsilane as internal standard. Chemical shifts are given in ppm (δ-scale) and coupling constants (*J*) in Hz. Homonuclear 2D-COSY and heteronuclear HMQC spectra were used for structural assignment of carbon signals in compounds **8** and **9**. Carbon signals in the other compounds were assigned using APT spectra and the comparison with the data on structurally similar compounds. Mass spectra were recorded on a VG Analytical ZAB-EQ spectrometer. Thin-layer chromatography (TLC) was performed on silica gel G (ICN Biomedicals, detection by spraying with concentrated sulfuric acid followed by heating); preparative TLC was carried out 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 $MgSO_4$.

Succinimidyl 3β-Hydroxyandrost-5-ene-17β-carboxylate (**2**)

To a stirred suspension of etienic acid[12] (**1**) (1.0 g, 3.14 mmol) and *N*-hydroxysuccinimide (506 mg, 4.39 mmol) in dioxane (20 ml) under argon, *N,N′*-dicyclohexylcarbodiimide (1 M solution in benzene, 4.5 ml, 4.5 mmol) was added and the mixture was stirred at room temperature for 5 h. Water (5 drops) was added and, after 5 min stirring, the mixture was transferred to a column with aluminum oxide (50 ml, prewashed with benzene) and the product was eluted with chloroform (100 ml). The chloroform solution was washed with water, dried, and the solvent was evaporated. The residue was dissolved in dry benzene (50 ml), filtered, and the solvent was evaporated. The crystalline product was pure enough for further syntheses. The yield of **2** was 1.2 g (92%), analytical sample was obtained by crystallisation from acetone–hexane mixture, m.p. 235–245 °C (dec.), $[\alpha]_D^{25}$ +45 (*c* 0.98, chloroform). IR: 3 568, 3 478 (O–H); 1 806, 1 782, 1 736 (C=O); 1 669 (C=C); 1 064 (C–OH). ^{1}H NMR (200 MHz): 5.36 m, 1 H (H-6); 3.53 m, 1 H (H-3α); 2.83 d, 4 H, *J* = 1.5 (succinimide H); 2.65 t, 1 H, *J* = 9.2 (H-17α); 1.02 s, 3 H (3 × H-19); 0.84 s, 3 H (3 × H-18). EI MS: 415 (68, M^+), 397 (61), 382 (32), 55 (100). For $C_{24}H_{33}NO_5$ (415.5) calculated: 69.37% C, 8.00% H, 3.37% N; found: 69.42% C, 8.01% H, 3.43% N.

3β-Azidoandrost-5-en-17β-ol (**3**)

This compound was prepared exactly as in ref.[8] The yield after column chromatography on silica gel (100 g) in a mixture of benzene–acetone (100 : 1) was 63%. M.p. 139–141 °C (methanol), $[\alpha]_D^{25}$ –35 (*c* 0.95, chloroform). IR: 3 613, 3 469 (O–H); 2 099 (N_3); 1 669 (C=C); 1 050, 1 041 (C–OH). ^{1}H NMR (200 MHz): 5.38 m, 1 H (H-6); 3.65 t, 1 H, *J* = 8.4 (H-17α); 3.21 m, 1 H (H-3α); 1.02 s, 3 H (3 × H-19); 0.76 s, 3 H (3 × H-18). For $C_{19}H_{29}N_3O$ (315.5) calculated: 72.34% C, 9.27% H, 13.32% N; found: 72.21% C, 9.43% H, 13.40% N. Literature[8] gives for the 17ξ-isomer (see the text) m.p. 138–139 °C, $[\alpha]_D$ –36 (*c* 0.8, chloroform).

N-(17β-Hydroxyandrost-5-en-3β-yl)-3β-hydroxyandrost-5-ene-17β-carboxamide (**5**)

Azide **3** (120 mg, 0.38 mmol) was stirred in a mixture of dioxane (1 ml) and ethanol (5 ml). Sodium borohydride (50 mg, 1.3 mmol) was added and then nickel(II) chloride hexahydrate (0.1 M solution in ethanol, 0.3 ml, 30 μmol) was dripped in. After 30 min stirring, the mixture was chilled in an ice bath, 10% sulfuric acid (1.5 ml) was added and, after a short stirring, the whole mixture was poured into cold dilute aqueous ammonia (*ca* 10%, 20 ml). The solids were filtered off on a column of celite, and the product was eluted with a mixture of benzene–methanol (1 : 1, *ca* 40 ml). The solvents were evaporated, the residue was co-evaporated twice with benzene and dried *in vacuo* over P_2O_5 overnight. This procedure yielded 100 mg (91%) of crude amine **4**, which was used without further purification. To amine **4** (40 mg, *ca* 138 μmol), ester **2** (35 mg, 84 μmol) and THF (0.5 ml) were added and the mixture was stirred at room temperature for 24 h. Water (5 ml) was then added and the solids were filtered off on a column of celite and thoroughly washed with water. The product was then eluted with a mixture of benzene–methanol (1 : 1). The solvents were evaporated, the residue was co-evaporated twice with benzene and dried. After column chromatography on silica gel (15 g) in a mixture of chloroform–methanol (100 : 1), the yield of **5** was 35 mg (70%). M.p. above 250 °C (dec., ethanol), $[\alpha]_D^{25}$ –24 (*c* 0.59, chloroform–methanol 10 : 1). IR: 3 611 (O–H); 3 437 (N–H); 1 667 sh (C=C); 1 657 (amide I); 1 505 (amide II); 1 044 (C–OH). ^{1}H NMR (200 MHz): 5.36 m, 2 H (H-6, H-6′); 5.13 bd, 1 H, *J* = 8.5 (NH); 3.73 m, 1 H (H-3′α); 3.65 t, 1 H, *J* = 8.3 (H-17′α); 3.52 m, 1 H (H-3α); 1.01 s, 6 H (3 × H-19, 3 × H-19′); 0.76 s, 3 H (3 × H-18′); 0.70 s, 3 H (3 × H-18). FAB MS: 590 (M + 1). For $C_{39}H_{59}NO_3$ (589.9) calculated: 79.41% C, 10.08% H, 2.37% N; found: 79.18% C, 10.21% H, 2.50% N.

Methyl 3β-Azidoandrost-5-ene-17β-carboxylate (**6**)

Prepared according to ref.[11], m.p. 104–105 °C (methanol), $[\alpha]_D^{25}$ +3 (*c* 1.25, chloroform). IR: 2 098, 1 277 (N_3); 1 727 (C=O); 1 669 (C=C); 1 436 (COO**CH$_3$**); 1 380 (CH_3); 1 170 (C–O). ^{1}H NMR (500 MHz): 5.39 bd, 1 H, *J* = 5.2 (H-6); 3.67 s, 3 H ($COOCH_3$); 3.21 ddt, 1 H, *J* = 6.3, 4.1 and 11.3 (H-3α); 2.35 t, 1 H, *J* = 9.4 (H-17α); 1.005 s, 3 H (3 × H-19); 0.673 s, 3 H (3 × H-18). For ^{13}C NMR, see Table III. FAB MS: 358 (M + 1). For $C_{21}H_{31}N_3O_2$ (357.5) calculated: 70.55% C, 8.74% H, 11.75% N; found: 70.44% C, 8.88% H, 11.67% N. Literature[11] gives m.p. 106–118 °C (MeOH–H_2O).

Methyl 3β-(3β-Hydroxyandrost-5-ene-17β-carboxamido)androst-5-ene-17β-carboxylate (**8**)

Azide **6** (250 mg, 0.70 mmol) was stirred in a mixture of dioxane (3 ml) and ethanol (10 ml). Sodium borohydride (105 mg, 2.88 mmol) was added and then nickel(II) chloride hexahydrate (0.1 M solution in ethanol, 0.6 ml, 60 μmol) was dripped in. After 30 min stirring, the mixture was chilled in an ice bath, 10% sulfuric acid (2 ml) was added and, after a short stirring, the whole mixture was poured into cold dilute aqueous ammonia (*ca* 10%, 50 ml). The product was extracted with ethyl acetate (50 ml), the extract was washed with dilute ammonia, water and dried. The solvents were evaporated, the residue was co-evaporated twice with benzene and dried *in vacuo* over P_2O_5 overnight. This procedure yielded 220 mg (95%) of crude amine **7**, which was used without further purification. Amine **7** (100 mg, *ca* 0.30 mmol) in benzene (1 ml) was added to a stirred solution of ester **2** (80 mg, 0.19 mmol) in DMF (3 ml) and the mixture was stirred at room temperature for 24 h.

Water (5 ml) and 10% aqueous HCl was then added, the solids were filtered off on a column of celite, which was thoroughly washed with water. The product was then eluted with a mixture of chloroform–methanol (10 : 1). The solvents were evaporated, the residue was co-evaporated twice with benzene and dried. After column chromatography on silica gel (20 g) in a mixture of chloroform–methanol (100 : 1), 103 mg (85%) of **8** was obtained. M.p. above 275 °C (dec., ethanol), $[\alpha]_D^{25}$ –3 (*c* 0.87, chloroform). IR: 3 607 (O–H); 3 436 (N–H); 1 726 (C=O ester); 1 656 (amide I); 1 506 (amide II); 1 436 (COO**CH$_3$**); 1 043 (C–OH). ^{1}H NMR (200 MHz): 5.36 m, 2 H (H-6, H-6′); 5.12 bd, 1 H, *J* = 7.7 (NH); 3.71 m, 1 H (H-3α); 3.67 s, 3 H ($COOCH_3$); 3.52 m, 1 H (H-3′α); 1.01 s, 3 H and 1.00 s, 3 H (3 × H-19, 3 × H-19′); 0.70 s, 3 H (3 × H-18′); 0.67 s, 3 H (3 × H-18). FAB MS: 632 (M + 1). For ^{13}C NMR, see Table II. For $C_{41}H_{61}NO_4$ (631.9) calculated: 77.93% C, 9.73% H, 2.22% N; found: 77.84% C, 9.79% H, 2.10% N.

3β-Azidoandrost-5-ene-17β-carboxylic Acid (**10**)

Ester **6** (800 mg, 2.24 mmol) in THF (5 ml) was stirred with methanolic sodium hydroxide (900 mg NaOH in 11 ml of methanol) under argon at 50 °C for 20 h. The reaction mixture was then poured into ice-cold sulfuric acid (1 M solution, 25 ml) and the product was extracted with ethyl acetate (*ca* 70 ml). The organic solution was washed with water (3×), dried and the solvents were evaporated. The product was co-evaporated twice with benzene, dried, and crystallised from hot methanol giving 520 mg (68%) of acid **10**, m.p. 203–205 °C, $[\alpha]_D^{25}$ –4 (*c* 0.62, chloroform). IR: 3 514 (O–H); 3 092, 2 747, 2 665, 2 561 (O–H dimer); 2 098, 1 279 (N_3); 1 734 (C=O); 1 700 (C=O dimer); 1 670 sh (C=C); 1 420, 1 242 (C–O, C–OH dimer). ^{1}H NMR (200 MHz): 5.39 m, 1 H (H-6); 3.21 m, 1 H (H-3α); 2.40 t, 1 H, *J* = 9.2 (H-17α); 1.01 s, 3 H (3 × H-19); 0.75 s, 3 H (3 × H-18). EI MS: 343 (M^+, 31), 315 (63), 300 (100). For $C_{20}H_{29}N_3O_2$ (343.5) calculated: 69.94% C, 8.51% H, 12.23% N; found: 69.92% C, 8.76% H, 12.21% N.

Succinimidyl 3β-Azidoandrost-5-ene-17β-carboxylate (**11**)

N-Hydroxysuccinimide (225 mg, 1.96 mmol) was dissolved in THF (3 ml) and acid **10** (500 mg, 1.46 mmol) and additional THF (1 ml) were added under stirring at room temperature. Then *N*,*N*′-dicyclohexylcarbodiimide (1 M solution in benzene, 2 ml, 2 mmol) was added and the mixture was stirred for 6 h. After dilution with benzene (3 ml), the mixture was filtered through a column of alumina layered with anhydrous magnesium sulfate. The product was eluted with benzene (20 ml), the organic solution was washed with water, dried, and the solvents were evaporated leaving crystalline ester **11** (620 mg, 97%), pure enough for further syntheses. Analytical sample was crystallised from acetone, m.p. 224–227 °C, $[\alpha]_D^{25}$ +61 (*c* 1.04, chloroform). IR: 2 098, 1 276 (N_3); 1 813, 1 784, 1 740 (C=O); 1 670 (C=C); 1 250 (C–O). ^{1}H NMR (200 MHz): 5.39 m, 1 H (H-6); 3.22 m, 1 H (H-3α); 2.84 d, 4 H, *J* = 1.5 (succinimide H); 2.65 t, 1 H, *J* = 9.2 (H-17α); 1.02 s, 3 H (3 × H-19); 0.84 s, 3 H (3 × H-18). For $C_{24}H_{32}N_4O_4$ (440.5) calculated: 65.43% C, 7.32% H, 17.72% N; found: 65.33% C, 7.51% H, 17.65% N.

Methyl 3β-(3β-Azidoandrost-5-ene-17β-carboxamido)androst-5-ene-17β-carboxylate (**12**)

The crude amine **7** (360 mg, *ca* 1.09 mmol, prepared as in the case of **8**) in benzene (2 ml) and THF (1 ml) was added to a stirred solution of ester **11** (440 mg, 1.00 mmol) in THF

(2 ml) and DMF (1 ml) and the mixture was stirred at room temperature for 24 h. Water and saturated aqueous NaCl were then added and the product was extracted with chloroform. The organic layer was washed with 10% aqueous HCl, saturated aqueous $KHCO_3$ and aqueous NaCl, dried, and the solvents were evaporated. After column chromatography on silica gel (30 g) in a mixture of benzene–acetone (20 : 1), 480 mg (73%) of amide **12** was obtained. M.p. above 225 °C (dec., acetone), $[\alpha]_D^{25}$ +14 (*c* 0.73, chloroform). IR: 3 437 (N–H); 2 098, 1 277 (N_3); 1 738, 1 727 (C=O ester); 1 667 sh (C=C); 1 657 (amide I); 1 505 (amide II); 1 436 (COO**CH$_3$**); 1 238 (C–O). ^{1}H NMR (500 MHz): 5.39 m, 1 H (H-6′); 5.36 m, 1 H (H-6); 5.11 d, 1 H, *J* = 8.3 (NH); 3.74 m, 1 H (H-3α); 3.67 s, 3 H ($COOCH_3$); 3.20 m, 1 H (H-3′α); 1.007 s, 3 H and 0.999 s, 3 H (3 × H-19, 3 × H-19′); 0.700 s, 3 H (3 × H-18); 0.670 s, 3 H (3 × H-18′). For ^{13}C NMR, see Table III. FAB MS: 657 (M + 1). For $C_{41}H_{60}N_4O_3$ (657.0) calculated: 74.96% C, 9.21% H, 8.53% N; found: 74.89% C, 9.30% H, 8.27% N.

Methyl 3β-[3β-(3β-Hydroxyandrost-5-ene-17β-carboxamido)androst-5-ene-17β-carboxamido]-androst-5-ene-17β-carboxylate (**14**)

Azide **12** (167 mg, 0.25 mmol) was stirred in a mixture of THF (2 ml) and ethanol (5 ml). Sodium borohydride (55 mg, 1.45 mmol) was added and then nickel(II) chloride hexahydrate (0.1 M solution in ethanol, 0.33 ml, 0.03 mmol) was dripped in. After 30 min stirring, the mixture was chilled in an ice bath, 10% sulfuric acid (1.5 ml) was added and, after a short stirring, the whole mixture was poured into cold dilute aqueous ammonia (*ca* 10%, 20 ml). The solids were filtered off on a column of celite, and the product was eluted with a mixture of benzene–methanol (1 : 1, *ca* 40 ml). The solvents were evaporated, the residue was co-evaporated twice with benzene and dried *in vacuo* over P_2O_5 overnight. This procedure yielded 139 mg (87%) of crude amine **13**, which was used without further purification. To a crude amine **13** (139 mg, *ca* 0.22 mmol) in THF (1 ml) and DMF (1 ml), ester **2** (115 mg, 0.28 mmol) in THF (1 ml) was added and the mixture was stirred at room temperature for 3 days. After the same work-up as for **12** and column chromatography on silica gel (20 g) in a mixture of benzene–chloroform–acetone (100 : 100 : 1), 150 mg (73%) of amide **14** was obtained. M.p. above 290 °C (dec., chloroform–ether), $[\alpha]_D^{25}$ +3 (*c* 0.71, chloroform). IR: 3 608 (O–H); 3 436 (N–H); 1 725 (C=O ester); 1 656 (amide I); 1 505 (amide II); 1 436 (COO**CH$_3$**); 1 043 (C–OH). ^{1}H NMR (500 MHz): 5.36 m, 3 H (H-6, H-6′, H-6″); 5.13 bd, 1 H and 5.11 bd, 1 H, both *J* = 6.0 (2 × NH); 3.74 m, 2 H (H-3α, H-3′α); 3.67 s, 3 H ($COOCH_3$); 3.52 m, 1 H (H-3″α); 1.013 s, 3 H and 0.999 s, 6 H (3 × H-19, 3 × H-19′, 3 × H-19″); 0.699 s, 3 H and 0.696 s, 3 H (3 × H-18′, 3 × H-18″); 0.669 s, 3 H (3 × H-18). For ^{13}C NMR, see Table II. FAB MS: 932 (M + 1). For $C_{61}H_{90}N_2O_5$ (931.4) calculated: 78.66% C, 9.74% H, 3.01% N; found: 78.56% C, 9.77% H, 2.78% N.

Methyl 3β-[3β-(3β-Azidoandrost-5-ene-17β-carboxamido)androst-5-ene-17β-carboxamido]-androst-5-ene-17β-carboxylate (**15**)

To a crude amine **13** (280 mg, *ca* 0.44 mmol, see the preceding experiment) in THF (2 ml) and DMF (2 ml), ester **11** (195 mg, 0.44 mmol) in THF (1 ml) was added. The mixture was stirred at room temperature for 3 days. After the same work-up as for **12** and column chromatography on silica gel (25 g) in a mixture of benzene–chloroform–acetone (100 : 100 : 1), 265 mg (63%) of amide **15** was obtained. M.p. above 240 °C (dec., acetone), $[\alpha]_D^{25}$ +12 (*c* 0.79, chloroform). IR: 3 436 (N–H); 2 098, 1 278 (N_3); 1 726 (C=O ester); 1 668 sh

(C=C); 1 657 (amide I); 1 505 (amide II); 1 436 (COO**CH$_3$**); 1 238 (C–O). ^{1}H NMR (500 MHz): 5.39 m, 1 H and 5.36 m, 2 H (H-6, H-6′, H-6″); 5.13 bd, 1 H and 5.11 bd, 1 H, both J = 8.2 (2 × NH); 3.74 m, 2 H (H-3α, H-3′α); 3.67 s, 3 H (COOCH$_3$); 3.21 m, 1 H (H-3″α); 1.007 s, 3 H and 0.999 s, 6 H (3 × H-19, 3 × H-19′, 3 × H-19″); 0.699 s, 3 H and 0.696 s, 3 H (3 × H-18′, 3 × H-18″); 0.669 s, 3 H (3 × H-18). For ^{13}C NMR, see Table III. FAB MS: 957 (M + 1). For $C_{61}H_{89}N_5O_4$ (956.4) calculated: 76.61% C, 9.38% H, 7.32% N; found: 76.53% C, 9.45% H, 7.30% N.

Methyl 3β-{3β-[3β-(3β-Hydroxyandrost-5-ene-17β-carboxamido)androst-5-ene-17β-carboxamido]androst-5-ene-17β-carboxamido}androst-5-ene-17β-carboxylate (**17**)

Amine **16** was prepared from ester **15** (90 mg, 94 μmol) according to the procedure for compound **14** (see amine **13**) in 78% yield. To the crude amine **16** (68 mg, *ca* 73 μmol) in THF (1 ml) and DMF (1 ml), ester **2** (35 mg, 84 μmol) in THF (1 ml) was added. The mixture was stirred at room temperature for 3 days. After the same work-up as for **12** and preparative TLC in a mixture of chloroform–acetone (8 : 2), 30 mg (33%) of amide **17** was obtained. M.p. above 300 °C (dec., chloroform–acetone), $[\alpha]_D^{25}$ +6 (*c* 0.72, chloroform). IR (KBr): 3 437, 3403 (N–H); 1 734 (C=O ester); 1 653 (amide I); 1 506 (amide II); 1 435 (COO**CH$_3$**); 1 198 (C–O); 1 057 (C–OH). ^{1}H NMR (500 MHz): 5.36 m, 4 H (H-6, H-6′, H-6″, H-6‴); 5.12 bd, 3 H, J = 8.0 (3 × NH); 3.74 m, 3 H (H-3α, H-3′α, H-3″); 3.67 s, 3 H (COOCH$_3$); 3.52 m, 1 H (H-3‴α); 1.013 s, 3 H (3 × H-19‴); 1.000 s, 9 H (3 × H-19, 3 × H-19′, 3 × H-19″); 0.700 s, 3 H and 0.696 s, 6 H (3 × H-18′, 3 × H-18″, 3 × H-18‴); 0.669 s, 3 H (3 × H-18). For ^{13}C NMR, see Table II. FAB MS: 1 231 (M + 1). For $C_{81}H_{119}N_3O_6$ (1 230.9) calculated: 79.04% C, 9.75% H, 3.41% N; found: 78.82% C, 9.81% H, 3.33% N.

Methyl 3β-{3β-[3β-(3β-Azidoandrost-5-ene-17β-carboxamido)androst-5-ene-17β-carboxamido]androst-5-ene-17β-carboxamido}androst-5-ene-17β-carboxylate (**18**)

To a crude amine **16** (50 mg, *ca* 54 μmol, see the previous experiment) in THF (0.5 ml) and DMF (0.5 ml), ester **11** (24 mg, 54 μmol) in THF (0.5 ml) was added. The mixture was stirred at room temperature for 3 days. After the same work-up as for **12** and preparative TLC in a mixture of chloroform–acetone (100 : 1), 18 mg (26%) of waxy amide **17** was obtained. IR (KBr): 3 440, 3 327 (N–H); 2 092, 1 272 (N_3); 1 736 (C=O ester); 1 665 (C=C); 1 665, 1 626 (amide I); 1 534, 1 502 (amide II); 1 436 (COO**CH$_3$**); 1 244 (C–O). ^{1}H NMR (500 MHz): 5.39 m, 1 H (H-6‴); 5.36 m, 3 H (H-6, H-6′, H-6″); 5.12 bd, 1 H, J = 8.0 (3 × NH); 3.74 m, 3 H (H-3α, H-3′α, H-3″α); 3.67 s, 3 H (COOCH$_3$); 3.21 m, 1 H (H-3‴α); 1.007 s, 3 H (3 × H-19″); 1.000 s, 9 H (3 × H-19, 3 × H-19′, 3 × H-19″); 0.699 s, 3 H and 0.695 s, 6 H (3 × H-18′, 3 × H-18″, 3 × H-18‴); 0.669 s, 3 H (3 × H-18). For ^{13}C NMR, see Table III. FAB MS: 1 256 (M + 1). For $C_{81}H_{118}N_6O_5$ (1 255.9) calculated: 77.47% C, 9.47% H, 6.69% N; found: 77.18% C, 9.57% H, 6.42% N.

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