SYNTHESIS OF LINEAR STEROID OLIGOESTERS BASED ON ETIENIC ACID[+,++]

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Linear oligoesters based on etienic acid (3β-hydroxyandrost-5-ene-17β-carboxylic acid) containing 2–4 steroid units were prepared employing mixed anhydride esterification. NMR spectra of the oligomeric steroids have been fully assigned.

Keywords: Mixed anhydrides; Esters; NMR spectroscopy; Oligoesters; Oligomers; Steroids.

The steroid skeleton has a great potential for the synthesis and self-assembly of larger molecular structures because of its functionalization capabilities and rigidity. Constructs consisting of several steroid units are thus very interesting due to their potential biological activity and self-assembly properties as well as for physico-chemical and biological studies. Dimeric steroids were established as synthetic byproducts[3] and also found in natural environment[4]. The literature shows several examples, where steroid acids (in most cases cholic acids) were used in various synthetic procedures[5].

Recently, a study was published on the linear chaining of steroid units through amide linkages[6] and heterocyclic moieties[7,8]. The approach we selected is ester chaining of 3β-hydroxyandrost-5-ene-17β-carboxylic acid (**1**; etienic acid).

The model important feature for the development of synthetic pathway, is in absence of numerous functional groups on rings A, B and C; however, with the possibility of inserting it later for the sake of control of intra- and

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++Presented in part: ref.[2]

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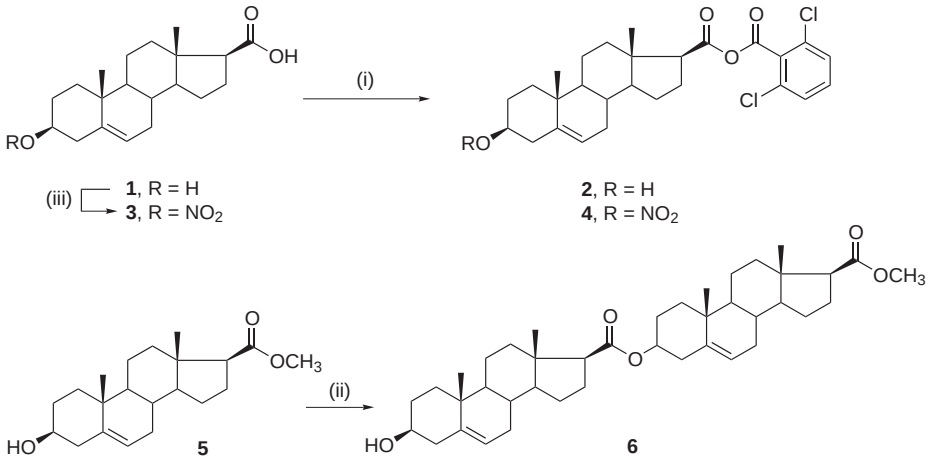
intermolecular interactions. The planned presence of modifying functionalities will influence the interaction capabilities of the oligoesters with phase boundaries and membranes. Compared with frequently used cholic acids, there is, a difference in the length of side chain terminated with carboxyl group on ring D. This may cause more difficult access of functional groups to chemical transformations. The single bonds in ester groups offer flexibility of the linkage compared with the previously synthesized amide or pyrazine-linked steroids. Examples of use of the ester bond for connecting steroid units are limited to cholic acid derivatives area[9–11] (for reviews on cholic acid oligomers, see refs[5,12]).

Target issues of the project developed in this study involve the incorporation of one or several steroid units into systems, where they can play crucial role as chirality-inducing and self-assembling principles in supramolecular processes[13–16].

We tried several ways of ester synthesis aiming at desired etienyl etienates. Direct self-esterification of etienic acid using acid catalysis and different dehydration agents did not lead to reasonable results. The same condensation with *N*,*N*′-dicyclohexylcarbodiimide showed some conversion; however, the yields were very poor as the main product was very difficult to separate from nitrogen containing by-products. Similar was the experience with isolated intermediates containing benzimidazol-1-yl, *N*-succinimidyl, or benztriazol-1-yl groups with or without a hypernucleophilic catalyst 4-(dimethylamino)pyridine (DMAP). Finally, we were able to develop a pathway, which successfully employs the principles taken from macrolactone condensations. We employed a modified[17] condensation method using 2,6-dichlorobenzoyl chloride[18] (DCBC). A question arose, whether or not to isolate the mixed anhydride.

In the search for suitable condensation, we compared two methods (Scheme 1) of steroid esterification, one, where we prepared mixed anhydride **2** as a key intermediate and reacted it further with etienic acid (**1**) or its methyl ester **5** and the other, which employed the condensation of etienic acid (**1**) with 2,6-dichlorobenzoyl chloride mediated by the 4-(dimethylamino)pyridine, without isolation of mixed anhydride **2**. Dimeric steroid **6** was then prepared by its condensation with methyl ester **5**. However, even here we did not achieve satisfactory results. So, we attempted the preparation of dimeric steroid ester **6** in step-by-step reaction with intermediates isolated. After having rather negative experience with *O*-acetyl protecting group, which was not orthogonal to the etienyl etienate ester, we used etienic acid **2** with 3-hydroxyl protected by *O*-nitro group, orthogonally to the etienyl etienate ester. Compound **3** was prepared by ester-



(i) DCBC, DMAP, toluene; (ii) **2**, toluene; (iii) HNO_3, Ac_2O, CH_2Cl_2

SCHEME 1

ification of etienic acid **1** with acetyl nitrate. *O*-Nitro protecting group is easily removable using zinc in acidic medium[19]. Hence, compound **3** was prepared and transformed to mixed anhydride **4**. The compound **4** was used without isolation in the next step for condensation with **5** to prepare dimeric steroid **7** (Scheme 2). *O*-Nitro group was removed by zinc in acidic medium to yield **6**. To distinguish the ester groups in oligoesters of etienic acid we used simple notation[6], the hydroxy group in position 3 of one steroid unit was denoted as "west" (primed) and the methyl carboxylate in position 17 of the second steroid unit was denoted as "east" (non-primed), both units being linked with the 17′→3 ester bridge. ^{13}C NMR spectra of **5** and **6** are presented in Table II. As it follows from Table II, the ^{13}C-chemical shifts of rings A and B of "west" steroid unit in compound **6** and those of rings C and D of "east" steroid units correspond well to the same in compound **5**.

Hydroxy derivative **6** was subjected to condensation with the mixed anhydride **4** to yield *O*-nitro derivative **8**, which was deprotected giving tristeroid analogue **9**. Finally, mixed anhydride **4** was condensed with tristeroid **9** to yield tetrasteroid **10**. Then, by deprotecting of *O*-nitro derivative **10**, we finally prepared hydroxy derivative **11** with four steroid skeletons linked by simple ester bonding.

Condensation and deprotection leading to tetrasteroids proceeded rather slowly, but in a very good yield. The higher the oligomers the less soluble

5
(i)
(ii)
7, R = NO2
6, R = H
6
(i)
(ii)
8, R = NO2
9, R = H
9
(i)
(ii)
10, R = NO2
11, R = H
OCH3
RO
7, 6
8, 9
10, 11
(i) 3, DCBC, DMAP, toluene; (ii) Zn, THF, AcOH

SCHEME 2

they were, but soluble enough for usual manipulations and characterization. All the products were purified by column chromatography on silica gel. The structures of steroid oligomers were determined mainly by NMR spectra. 1D-^{1}H and ^{13}C APT spectra together with 2D-^{1}H, ^{1}H-COSY and 2D-^{1}H, ^{13}C-HMQC spectra were used for structure assignment of ^{1}H and ^{13}C signals. NMR results were summarized in Tables I and II. As discussed above with derivatives **5** and **6**, there is a very good consistency in data sets for the particular units also in tri-, and tetrasteroids (**9** and **11**).

In conclusion, reasonable synthetic way together with purification and characterization scheme were developed for the preparation of etienic acid oligomers linked with ester bonds. The mechanistic, physico-chemical and sol-gel properties of the oligosteroids prepared are the subject of a forthcoming study. Compounds **6**, **9** and **11** are designed for a broad application in the attachment of one or more steroid units to any suitable backbone. The compounds could be used as steroid macrolactone synthetic blocks for the preparation of derivatives similar to those utilized in the studies of transmembrane transport, their incorporation into membrane, or their self-assembly potential.

EXPERIMENTAL

Melting points were determined on a Boetius micro-melting point apparatus (Germany). Optical rotations were measured on an Autopol IV (Rudolph Research Analytical, Flanders, U.S.A.) polarimeter at room temperature, which was corrected to 20 °C; $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1} with concentration in 10 g l^{-1}. IR spectra (wavenumbers in cm^{-1}) were recorded on a Perkin–Elmer PE 580 spectrometer in chloroform solutions (temperature 23 °C), unless stated otherwise. ^{1}H NMR spectra of compounds **3**, **7**, **8** and **10** were taken on

TABLE I
Characteristic ^{1}H NMR chemical shifts of etienic acid methyl esters **5**, its dimer **6**, trimer **9** and tetramer **11** in $CDCl_3$

Proton	**5**	Disteroid **6**		Tristeroid **9**			Tetrasteroid **11**			
		west	east	west	middle	east	west	middle	middle	east
H-3	3.524 m	3.524 m	4.634 m	3.524 m	4.634 m	4.634 m	3.524 m	4.634 m	4.634 m	4.634 m
H-6	5.350 dt	5.350 m	5.374 m	5.350 m	5.374 m	5.374 m	5.350 m	5.374 m	5.374 m	5.374 m
H-18	0.674 s	0.687 s	0.674 s	0.687 s	0.687 s	0.673 s	0.686 s	0.686 s	0.686 s	0.673 s
H-19	1.012 s	1.011 s	1.029 s	1.010 s	1.029 s	1.029 s	1.010 s	1.028 s	1.028 s	1.028 s
OCH_3	3.673 s	–	3.672 s	–	–	3.672 s	–	–	–	3.672 s

a Varian Unity-200 (at 200 MHz) while ^{1}H and ^{13}C NMR spectra of compounds **5**, **6**, **9** and **11** were recorded on a Varian Unity-500 (^{1}H at 499.87 MHz and ^{13}C at 125.70 MHz). All NMR spectra were measured 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 recorded and used for structure assignment of signals in compounds **5**, **6**, **9** and **11**. Mass spectra were recorded on an VG Analytical ZAB-EQ spectrometer (FAB method was used). Microanalysis were taken on elemental analyzer Perkin–Elmer 2400 Series II CHNS/O. Thin-layer chromatography (TLC) was performed on silica gel G (ICN Biomedicals, detection by spraying with concentrated sulfuric acid followed by heating); in S1 (toluene–ether 9:1), S2 (chloroform–methanol 90:1),

TABLE II
^{13}C NMR chemical shifts of etienic acid methyl esters **5**, its dimer **6**, trimer **9** and tetramer **11** in $CDCl_3$

Carbon	**5**	Disteroid **6**		Tristeroid **9**			Tetrasteroid **11**			
		west	east	west	middle	east	west	middle	middle	east
C-1	37.26	37.26	37.01	37.25	37.00	37.00	37.26	37.01	37.01	37.01
C-2	31.61	31.61	27.90	31.60	27.89[a]	27.91[a]	31.62	27.93	27.93	27.90
C-3	71.70	71.70	73.37	71.69	73.37	73.37	71.70	73.38	73.38	73.38
C-4	42.25	42.24	38.50	42.23	38.50	38.50	42.25	38.51	38.51	38.51
C-5	140.83	140.81	139.82	140.80	139.79	139.83	140.81	139.84	139.83	139.81
C-6	121.36	121.39	122.20	121.38	122.24	122.19	121.39	122.23	122.23	122.19
C-7	31.81	31.80	31.80	31.79	31.79	31.79	31.80	31.80	31.80	31.80
C-8	32.00	31.99	31.94	31.98	31.94	31.94	31.99	31.95	31.95	31.95
C-9	50.06	50.09	49.94	50.08	49.97	49.94	50.09	49.98	49.98	49.95
C-10	36.55	36.54	36.65	36.53	36.64	36.64	36.54	36.65	36.65	36.65
C-11	20.91	20.84	20.98	20.84	20.92	20.97	20.85	20.93	20.93	20.98
C-12	38.19	38.37	38.13	38.36	38.32	38.12	38.37	38.33	38.33	38.13
C-13	43.95	43.93	43.94	43.93	43.92	43.92	43.93	43.93	43.93	43.94
C-14	56.14	56.29	56.05	56.28	56.21	56.05	56.30	56.22	56.22	56.06
C-15	24.56	24.54	24.54	24.54	24.54	24.54	24.55	24.55	24.55	24.55
C-16	23.64	23.45	23.63	23.45	23.45	23.62	23.46	23.46	23.46	23.64
C-17	55.19	55.27	55.16	55.26	55.24	55.15	55.28	55.25	55.25	55.16
C-18	13.31	13.27	13.31	13.27	13.27	13.30	13.28	13.28	13.28	13.31
C-19	19.38	19.35	19.38	19.35	19.35	19.38	19.35	19.35	19.35	19.38
C-20	174.56	173.43	174.57	173.44	173.44	174.56	173.44	173.44	173.44	174.57
OCH_3	51.21	–	51.21	–	–	51.21	–	–	–	51.22

[a] Interchangeable.

S3 (toluene–acetone 30:1) and S4 (chloroform–methanol 45:1) systems. Preparative TLC was carried out on 200 × 200 mm plates (layer thickness 0.4 mm). For column chromatography, neutral silica gel SiliTech 32-63, 60 Å (ICN Biomedicals) or Silpearl (Kavalier) was used. Prior to evaporation on a rotary evaporator in vacuum (bath temperature 40 °C, pressure 1.5 kPa), solutions in organic solvents were dried over anhydrous $MgSO_4$. All products were dried in vacuum oven at 50 °C. For reactions, toluene dried over sodium was used. Celite was, prior to filtrations, washed with chloroform.

3β-Nitratoandrost-5-ene-17β-carboxylic Acid (**3**)

Nitric acid (65%; 2.7 ml, 39 mmol) was added dropwise with stirring and cooling (–25 °C) to acetic anhydride (13.6 ml). After stirring and cooling for 10 min, a solution of **1** (2.09 g, 6.6 mmol) in dichloromethane (30 ml) was added dropwise at –25 °C during 30 min. The mixture was stirred at –25 °C for 4 h, poured into a mixture of ice (150 g) and aqueous ammonia (25%, 30 ml), kept at +5 °C overnight and filtered. The product was washed with cool water and dried in an oven, affording 2.15 g (90%) of **3**. TLC: R_F 0.25 (S1), 0.5 (S3) and 0.35 (S4). M.p. 210–212 °C; $[\alpha]_D^{20}$ –12.3 (*c* 0.13, $CHCl_3$). IR: 3510 (OH monomer); 2749, 2719, 2664, 2627, 2583, 2556 (OH dimer); 1734 (C=O monomer); 1700 (C=O dimer); 1670 (C=C); 1626 (NO_2); 1419 (dimer COOH); 1276 (NO_2); 973 (NOC); 868 (N–O); 598 (NO_2). ^{1}H NMR: 5.46 bd, 1 H (*J* = 4.9, H-6); 4.81 m, 1 H (H-3); 1.03 s, 3 H (3 × H-19); 0.76 s, 3 H (3 × H-18). MS, *m/z*: 363 (M^+, $C_{20}H_{29}NO_5$), 348, 320, 317, 300, 285, 271, 253, 243, 233, 216, 207, 192, 183, 171, 159, 147, 129, 119, 107, 81, 74, 43, 41, 28. For $C_{20}H_{29}NO_5$ (363.5) calculated: 66.09% C, 8.04% H, 3.85% N; found: 65.77% C, 8.06% H, 3.62% N.

Methyl 3β-[(3β-Nitratoandrost-5-ene-17β-carbonyl)oxy]androst-5-ene-17β-carboxylate (**7**)

To nitrate **3** (500 mg, 1.4 mmol, dried in vacuum for 2 h), dry toluene (12 ml), DCBC (0.2 ml, 1.4 mmol) and DMAP (40 mg, 0.33 mmol) were added. The mixture was refluxed in oil bath for 2 h. After cooling to room temperature (overnight), methyl ester **5** (458 mg, 1.4 mmol, dried in vacuum for 2 h) in dry toluene (10 ml), DCBC (0.2 ml, 1.5 mmol) and DMAP (2.62 g, 21.5 mmol) were added. The mixture was heated at 90 °C in oil bath for 2 h. The mixture was cooled to the room temperature and water (50 ml) was added. Compound **7** was extracted with ether and the extract was washed with water, saturated aqueous $NaHCO_3$ and water, affording 900 mg of crude dimer **7**. After column chromatography on silica gel (120 g) in toluene and crystallization (toluene–ether), 780 mg (84%) of dimer **7** were obtained. TLC: R_F 0.75 (S1) and 0.85 (S2). M.p. 228–230 °C; $[\alpha]_D^{20}$ +41.5 (*c* 0.135, $CHCl_3$). IR: 1720 (C=O); 1670 (C=C); 1626 (NO_2); 1292, 1288, 1276, 598 (NO_2); 888, 868, 852 (NO); 975 (NOC); 1436 ($COOCH_3$); 1382, 1372 (CH_3); 1198 (C–O middle ester); 1171 ($COOCH_3$). ^{1}H NMR: 5.45 bd, 1 H (*J* = 4.9, H-6′); 5.38 bd, 1 H (*J* = 4.3, H-6); 4.80 m, 1 H (H-3′); 4.63 m, 1 H (H-3); 3.68 s, 3 H ($COOCH_3$); 1.03 s, 6 H (3 × H-19 and 3 × H-19′); 0.69 s, 3 H (3 × H-18′) and 0.67 s, 3 H (3 × H-18). MS, *m/z*: 678 (M^+, $C_{41}H_{59}NO_7$), 662, 645, 631, 615, 577, 391, 328, 315, 300, 283, 279, 262, 255, 239, 211. For $C_{41}H_{59}NO_7$ (677.9) calculated: 72.64% C, 8.77% H, 2.07% N; found: 72.50% C, 8.93% H, 2.03% N.

Methyl 3β-[(3β-Hydroxyandrost-5-ene-17β-carbonyl)oxy]androst-5-ene-17β-carboxylate (**6**)

Nitrate **7** (569 mg, 0.8 mmol) was mixed with THF (24 ml), acetic acid (6 ml) and water (1.2 ml). Zinc powder (900 mg, 13.7 mmol) was added stepwise with stirring in 25 portions

during 3 h. The mixture was filtered through a celite layer, which was then washed with chloroform. The filtrate was washed with water, saturated aqueous $NaHCO_3$ and water, affording 499 mg of compound **6**. After column chromatography on silica gel (115 g) in chloroform and crystallization (toluene–ether), 477 mg (90%) of compound **6** were obtained. TLC: R_F 0.4 (S2). M.p. 230–232 °C; $[\alpha]_D^{20}$ +10.0 (*c* 0.10, $CHCl_3$). IR: 3608 (OH); 1720 (C=O); 1669 (C=C); 1436 ($COOCH_3$); 1383 (CH_3); 1198, 1173 (C–O); 1054, 1044 (C–O, C–OH). For ^1H and ^{13}C NMR spectra, see Tables I and II. MS, *m/z*: 633 (M^+, $C_{41}H_{60}O_5$), 631, 613, 423, 399, 391, 385, 375, 361, 345, 330, 329, 315, 283, 255, 215, 181, 161, 149, 121, 91, 81. For $C_{41}H_{60}O_5$ (632.9) calculated: 77.81% C, 9.56% H; found: 77.63% C, 9.60% H.

Methyl 3β-({3β-[(3β-Nitratoandrost-5-ene-17β-carbonyl)oxy]androst-5-ene-17β-carbonyl}oxy)androst-5-ene-17β-carboxylate (**8**)

To nitrate **3** (150 mg, 0.41 mmol, dried in vacuum for 2 h), dry toluene (15 ml), DCBC (0.06 ml, 0.41 mmol) and DMAP (40 mg, 0.33 mmol) were added. The mixture was refluxed in oil bath for 2 h. After cooling to room temperature (overnight), hydroxy derivative **6** (261.6 mg, 0.41 mmol, dried in vacuum for 2 h) in dry toluene (10 ml), DCBC (0.1 ml, 0.7 mmol) and DMAP (808 mg, 6.62 mmol) were added. The mixture was heated at 90 °C in oil bath for 2 h. The mixture was cooled to room temperature and water (50 ml) was added. Compound **8** was extracted with ether and the extract was washed successively with water, saturated aqueous $NaHCO_3$ and water, affording 398 mg of crude trimer **8**. After column chromatography on silica gel (100 g) in toluene and crystallization (toluene–ether), 240 mg (60%) of trimer **8** were obtained. TLC: R_F 0.85 (S1 and S4) and 0.80 (S2). M.p. 238–241 °C; $[\alpha]_D^{20}$ +16.7 (*c* 0.12, $CHCl_3$). IR: 1720 (C=O); 1671 (C=C); 1626, 1293, 1276 (NO_2); 868, 858 (N–O); 599 (NO_2); 977 (NO–C); 1436 ($COOCH_3$); 1383, 1373 (CH_3); 1196, 1171 (C–O). ^1H NMR: 5.45 bd, 1 H (*J* = 4.9, H-6″); 5.37 bd, 2 H (*J* = 4.6, H-6 and 1 × H-6′); 4.78 m, 1 H (H-3″); 4.62 m, 2 H (H-3 and 1 × H-3′); 3.67 s, 3 H ($COOCH_3$); 1.03 s, 9 H (3 × H-19, 3 × H-19′ and 3 × H-19″); 0.69 s, 6 H (3 × H-18′ and 3 × H-18″) and 0.67 s, 3 H (3 × H-18). MS, *m/z*: ($C_{61}H_{87}NO_9$), 593, 545, 531, 513, 471, 438, 429, 405, 389, 363, 349, 329, 316, 303, 274, 232, 225, 197, 181, 163, 149, 115, 91, 89, 73. For $C_{61}H_{87}NO_9$ (978.3) calculated: 74.89% C, 8.96% H, 1.43% N; found: 74.72% C, 9.10% H, 1.76% N.

Methyl 3β-({3β-[(3β-Hydroxyandrost-5-ene-17β-carbonyl)oxy]androst-5-ene-17β-carbonyl}oxy)androst-5-ene-17β-carboxylate (**9**)

Nitrate **8** (240 mg, 0.25 mmol) was mixed with THF (15 ml), acetic acid (1.5 ml) and water (0.3 ml). Zinc powder (928 mg, 14.2 mmol) was added with stirring in 23 portions during 3.5 h. The mixture was filtered through a celite layer, which was then washed with chloroform. The filtrate was washed with water, saturated aqueous $NaHCO_3$ and water, affording 214 mg of compound **9**. After column chromatography on silica gel (80 g) in chloroform and crystallization (toluene–ether), 190 mg (83 %) of pure compound **9** were obtained. TLC: R_F 0.15 (S1), 0.20 (S2) and 0.25 (S4). M.p. 269–273 °C; $[\alpha]_D^{20}$ +14.6 (*c* 0.11, $CHCl_3$). IR: 3608 (OH); 1719 (C=O); 1669 (C=C); 1436 ($COOCH_3$); 1383, 1374 (CH_3); 1197, 1172 (C–O); 1043 (C–OH). For ^1H and ^{13}C NMR spectra, see Tables I and II. MS, *m/z*: ($C_{61}H_{88}O_7$), 912, 862, 819, 803, 787, 771, 743, 727, 712, 695, 681, 667, 653, 635, 623, 607, 587, 575, 561, 543, 529, 515, 495, 483, 467, 453, 437, 423, 393, 303, 287, 225, 198, 181, 171, 154, 137, 91. For $C_{61}H_{88}O_7$ (933.6) calculated: 78.50% C, 9.50% H; found: 77.92% C, 9.62% H.

Methyl 3β-{[3β-({3β-[(3β-Nitratoandrost-5-ene-17β-carbonyl)oxy]androst-5-ene-17β-carbonyl}oxy)androst-5-ene-17β-carbonyl]oxy}androst-5-ene-17β-carboxylate (**10**)

To nitrate **3** (78 mg, 0.22 mmol, dried in vacuum for 2 h), dry toluene (10 ml), DCBC (0.05 ml, 0.36 mmol) and DMAP (40 mg, 0.33 mmol) were added. The mixture was refluxed in oil bath for 1 h. After cooling to room temperature (overnight), hydroxy derivative **9** (190 mg, 0.2 mmol, dried in vacuum for 2 h) in dry toluene (10 ml), DCBC (0.05 ml, 0.36 mmol) and DMAP (400 mg, 3.28 mmol) were added. The mixture was heated at 90 °C in oil bath for 2 h. The mixture was cooled to room temperature and water (50 ml) was added. Compound **10** was extracted with ether and the extract was washed successively with water, saturated aqueous $NaHCO_3$ and water, affording 250 mg of crude tetramer **10**. After column chromatography on silica gel (70 g) in toluene and crystallization (toluene–ether), 232 mg (89%) of tetramer **10** were obtained. TLC: R_F 0.80 (S4). M.p. 221–223 °C; $[\alpha]_D^{20}$ +13.3 (*c* 0.13, $CHCl_3$). IR: 1722 (C=O); 1670 (C=C); 1624, 1291, 1278 (NO_2); 866, 859 (N–O); 600 (NO_2); 973 (NO–C); 1435 ($COOCH_3$); 1383, 1373 (CH_3); 1197, 1171 (C–O). ^{1}H NMR: 5.44 bd, 1 H (*J* = 5.2, H-6‴); 5.38 bd, 3 H (*J* = 4, H-6, 1 × H-6′ and 1 × H-6″); 4.80 m, 1 H (H-3‴); 4.63 m, 3 H (H-3, 1 × H-3′ and 1 × H-3″); 3.67 s, 3 H ($COOCH_3$); 1.03 s, 12 H (3 × H-19, 3 × H-19′, 3 × H-19″ and 3 × H-19‴); 0.69 s, 9 H (3 × H-18′, 3 × H-18″ and 3 × H-18‴) and 0.67 s, 3 H (3 × H-18). MS, *m/z*: ($C_{81}H_{115}NO_{11}$), 979, 767, 653, 561, 546, 485, 393, 389, 301, 279, 225, 207, 197, 181, 165, 149, 115, 91. For $C_{81}H_{115}NO_{11}$ (1278.8) calculated: 76.08% C, 9.06% H, 1.10% N; found: 76.87% C, 8.92% H, 1.31% N.

Methyl 3β-{[3β-({3β-[(3β-Hydroxyandrost-5-ene-17β-carbonyl)oxy]androst-5-ene-17β-carbonyl}oxy)androst-5-ene-17β-carbonyl]oxy}androst-5-ene-17β-carboxylate (**11**)

Nitrate **10** (232 mg, 0.18 mmol) was mixed with THF (10 ml), acetic acid (1.5 ml) and water (0.3 ml). Zinc powder (1.5 g, 23 mmol) was added with stirring in 35 portions during 8 h. The mixture was filtered through a celite layer, which was then washed with chloroform. The filtrate was successively washed with water, saturated aqueous $NaHCO_3$ and water, affording 218 mg of crude compound **11**. After column chromatography on silica gel (100 g) in chloroform and crystallization (toluene–ether), 188.5 mg (84%) of pure compound **11** were obtained. TLC: R_F 0.40 (S4). M.p. 274–277 °C; $[\alpha]_D^{20}$ +25.9 (*c* 0.10, $CHCl_3$). IR: 3610 (OH); 1719 (C=O); 1671 (C=C); 1436 ($COOCH_3$); 1383, 1375 (CH_3); 1196, 1172 (C–O); 1043 (C–OH). For ^{1}H and ^{13}C NMR spectra, see Tables I and II. MS, *m/z*: ($C_{81}H_{116}O_9$), 534, 513, 485, 409, 393, 358, 347, 335, 317, 303, 273, 225, 197, 181, 137, 91. For $C_{81}H_{116}O_9$ (1233.8) calculated: 78.85% C, 9.48% H; found: 77.89% C, 9.54% H.

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REFERENCES

1. Slavíková B., Kasal A., Chodounská H., Krištofíková Z.: *Collect. Czech. Chem. Commun.* **2002**, *67*, 30.
2. Reschel M., Černý I., Pouzar V., Drašar P.: *Chem. Listy* **2001**, *95*, 765.
3. Crabbe P., Zderic J.: *Bull. Soc. Chim. Belg.* **1961**, *70*, 403.
4. Pettit G. R., Inoue M., Camano Y., Herald D. L., Arm C., Dufresne C., Christie N. D., Schmidt J. M., Doubek D. L., Krupa T. S.: *J. Am. Chem. Soc.* **1988**, *110*, 2006.
5. Davis P. A., Bonar-Law R. P., Sanders J. K. M.: *Comp. Supramol. Chem.* **1996**, 264.
6. Černý I., Buděšínský M., Pouzar V., Drašar P.: *Collect. Czech. Chem. Commun.* **2001**, *66*, 933.
7. Černý I., Pouzar V., Buděšínský M., Drašar P.: *Collect. Czech. Chem. Commun.* **2000**, *65*, 1597.
8. Lotowski Z., Morzycki J. W., Niewczas I. S., Zdanowicz M.: *Collect. Czech. Chem. Commun.* **2002**, *67*, 47.
9. Li Y., Dias J. R.: *Org. Prep. Proced. Int.* **1996**, *28*, 203.
10. Li Y., Dias J. R.: *Synthesis* **1997**, 425.
11. Zhu X.-X., Nichifor M.: *Acc. Chem. Res.* **2002**, *35*, 539.
12. Li Y., Dias J. R.: *Chem. Rev. (Washington, D. C.)* **1997**, *97*, 283.
13. Dukh M., Černý I., Urbanský M., Pouzar V., Král V., Drašar P.: Czech 290491 (June 5, 2002); Czech Pat. Appl. PV3098-99 (August 31, 1999).
14. Dukh M., Černý I., Pouzar V., Král V., Drašar P.: *Chem. Listy* **2001**, *95*, 749.
15. Dukh M., Drašar P., Černý I., Pouzar V., Shriver J. A., Král V., Sessler J. L.: *Supramol. Chem.* **2002**, *14*, 237.
16. Dukh M., Kalvoda L., Šaman D., Černý I., Pouzar V., Král V., Drašar P.: *Chem. Listy* **2002**, *96*, 400.
17. Inaga J., Hirata K., Saeki H., Katsuki T., Yamaguchi M.: *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989.
18. Gao H., Dias J. R.: *Eur. J. Org. Chem.* **1998**, 720.
19. Černý I., Pouzar V., Drašar P., Havel M.: *Collect. Czech Chem. Commun.* **1992**, *57*, 362.