IVAX Drug Research Institute Ltd. Budapest, Hungary

Etiprednol dicloacetate, a new soft glucocorticoid drug candidate. Development of chemistry

Á. Csanádi, Gy. Horváth, T. Szekeres, T. Haskó, L. Ila, J. Ivanics, M. Patthy, J. Salát, G. Seres, I. Pallagi, G. Tóth, F. Szederkényi, A. Kónya, A. Tegdes, N. Bodor, Z. Zubovics

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Zoltán Zubovics, 1045 Budapest, Berlini u. 47–49. Hungary; Zoltan.Zubovics@idri.hu

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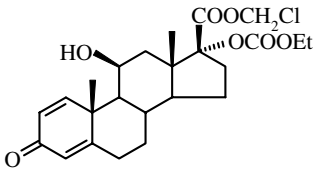
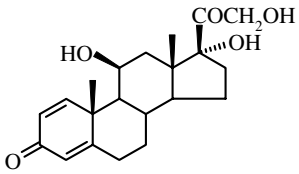
During development of chemistry of the soft drug candidate etiprednol dicloacetate (BNP-166) 1) optimization studies on the three-step chemical synthesis resulted in a process that could be scaled-up to the kg level, 2) the impurity profile was determined, 3) synthetic routes were developed for the preparation of the radiolabeled target compound, and 4) a series of hydroxylated metabolites was prepared.

1. Introduction

Soft analogues of drugs are known to possess chemical structures designed specifically to allow predictable metabolism. Among these analogs those providing inactive metabolites proved to be successful in various therapeutic fields, e.g. as antimicrobials, anticholinergics, antiarrhythmics, corticosteroids, β-blockers, analgetics, etc. (Bodor and Buchwald 2000). Thus, the soft corticosteroid loteprednol etabonate (**1**), which is structurally related to prednisolone (**2a**), shows excellent glucocorticoid activity without having serious side effects (Szelenyi et al. 2000).
This favorable pharmacological profile is attributed to the specific substitutions at positions 17α and 17β, which are isoelectronic/isosteric with the substitution pattern of prednisolone or hydrocortisone and which allow an enzymatic hydrolysis to Δ^1-cortienic acid, a 17β-carboxy metabolite of prednisolone lacking any glucocorticoid activity. After launching the opthalmic drugs Lotemax® and Aldrex® containing **1** as active agent, further studies are in progress to provide other formulations of **1** for use in other therapeutic fields.



LOTEPREDNOL ETABONATE (**1**)



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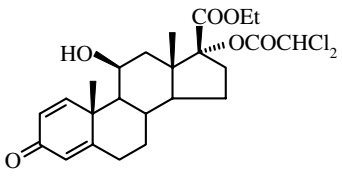


ETIPREDNOL DICLOACETATE, BNP-166 (**6a**)

Simultaneously, development of a second generation of soft corticosteroids led to the discovery of a new family, i.e. to the 17α-dichloroacetoxy analogues and, within this, to the promising drug candidate etiprednol dicloacetate (**6a**, ethyl 17α-dichloroacetoxy-11β-hydroxyandrosta-1,4-diene-3-one-17β-carboxylate, BNP-166).
In the present paper the chemical synthesis, the impurity profile, the synthetic methods affording the radiolabeled compound and the preparation of some potential metabolites of **6a** are described.

2. Investigations, results and discussion

2.1. Synthesis of 6a

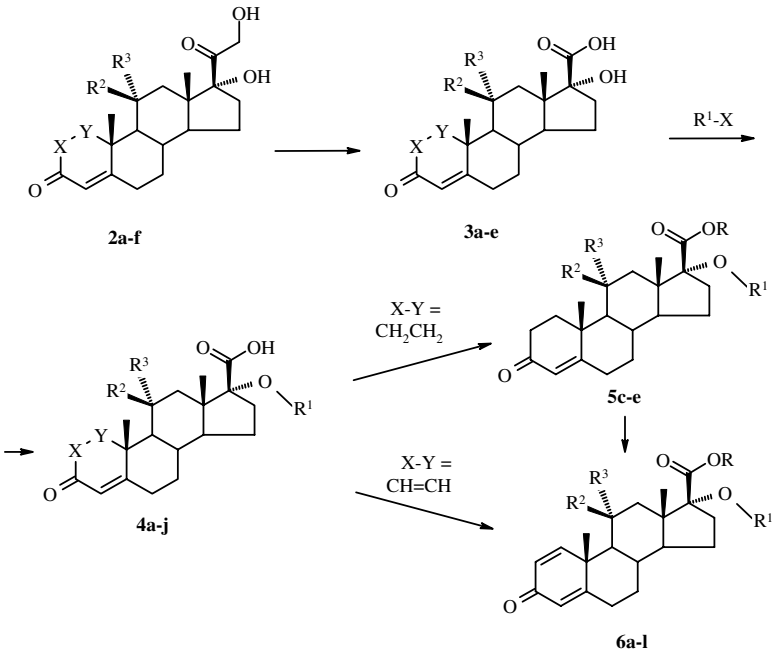
The known chemical synthesis (Bodor 1997) of **6a** (see Scheme 1) consists of three steps including pregnane side chain oxidation of the starting prednisolone (**2a**) to Δ^1-cortienic acid (**3a**) using sodium metaperiodate followed by acylation of 17α-hydroxy with dichloroacetyl chloride to yield **4a** and finally esterification of 17β-carboxy with ethyl iodide/K_2CO_3 in DMF leading to BNP-166 (**6a**), total yield via three steps: 65%.
Although lab scale optimization followed by pilot plant scaling up to the 3.5 kg batch size yielded the product in the desired yield and quality, some analytical problems (see below, under "impurity profile") coming from the use of ethyl iodide in the final step prompted us to modify this latter step, and diethyl sulfate was found to be a suitable reagent to replace ethyl iodide.

2.2. Impurity profile

2.2.1. Isolation and structure elucidation of the impurities

An analysis of the HPLC impurity profile of a number of experimental lab scale and pilot plant batches revealed that **6a** prepared by any variant of the above synthetic procedure usually contained 1–4 minor components in amounts between 0.1–0.5% wherein one or two impurities (e.g. **5c** and/or **6l**) occured regularly while others were detected only in just one or in a few batches. In order to identify any impurity above 0.1%, all these compounds (a

Scheme 1



Compd.	R	R^1	R^2	R^3	X–Y
2a, 3a			OH	H	CH=CH
2b, 3b			$R^2 + R^3$ = oxo		CH=CH
2c, 3c			OH	H	CH_2CH_2
2d, 3d			H	H	CH_2CH_2
2e, 3e			H	OH	CH_2CH_2
2f			H	H	CH=CH
2g			H	OH	CH=CH
2h			$R^2 + R^3$ = oxo		CH_2CH_2
4a, 6a	Ethyl	Dichloroacetyl	OH	H	CH=CH
4b, 6b	Ethyl	Dichloroacetyl	$R^2 + R^3$ = oxo		CH=CH
4c, 5c	Ethyl	Dichloroacetyl	OH	H	CH_2CH_2
4d, 5d	Ethyl	Dichloroacetyl	H	H	CH_2CH_2
4e, 5e	Ethyl	Dichloroacetyl	H	OH	CH_2CH_2
6d	Ethyl	Dichloroacetyl	H	H	CH=CH
6e	Ethyl	Dichloroacetyl	H	OH	CH=CH
4f, 6f	Ethyl	Chloroiodoacetyl	OH	H	CH=CH
4g, 6g	Ethyl	Trichloroacetyl	OH	H	CH=CH
4h, 6h	Ethyl	Formyl	OH	H	CH=CH
4i, 6i	Ethyl	$-CO-CO-NMe_2$	OH	H	CH=CH
4j, 6j	Ethyl	Monochloroacetyl	OH	H	CH=CH
6k	Methyl	Dichloroacetyl	OH	H	CH=CH
6l	Ethyl	H	OH	H	CH=CH

total of 11 entities) were isolated either by classical silica gel column chromatography or by semi-preparative reversed-phase HPLC, and their structures were elucidated by NMR and mass spectroscopy. All the compounds were found to be analogues of **6a**. Thus, the 11-oxo (**6b**), 1,2-dihydro (**5c**), 11-deoxy (**6d**), 11-epi (**6e**), 17α-chloroiodoacetoxy (**6f**), 17α-trichloroacetoxy (**6g**), 17α-formyloxy (**6h**), 17α-(*N,N*-dimethyl)oxamoyloxy (**6i**) and the 17β-methyl ester (**6k**) analogues of BNP-166, Δ^1-cortienic acid ethyl ester (**6l**), as well as a rearranged ring A containing compound **7** were identified.

2.2.2. Origin of the impurities

The presence of these impurities can be attributed to impurities in the starting prednisolone and/or reagents used, and to interactions with the solvent DMF. Thus, by comparison with authentic samples, a HPLC analysis of prednisolone revealed that the commercial product usually contains several contaminants, among others prednisone (**2b**), the 1,2-dihydro analog, i.e. hydrocortisone (**2c**), 11-deoxyprednisolone (**2f**) and 11-epi prednisolone (**2g**), each within the range of 0.1–0.4%. A similar impurity pattern of prednisolone was reported earlier (Görög et al. 1998). During the 3-step synthesis shown in Scheme 1 these impurities underwent the same transformation as prednisolone itself and afforded the impurities **5c**, **6b**, **6d** and **6e**.

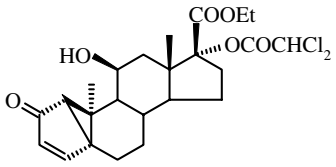
6f was the product of a partial $Cl \rightarrow I$ exchange in the presence of KI formed as a by-product during esterification. By virtue of the new asymmetry center at the side chain α-carbon, the chloroiodoacetoxy compound **6f** exists in the form of two diastereoisomers. Although the two forms were well distingushed both by NMR and HPLC (their ratio was 6 : 4), the minor isomer could not be detected in the chromatograms of impure **6a**, as it was overlapping with the main peak. The amount of this impurity in **6a** could be substantially reduced by a halogen reconversion, i.e. by treatment of impure **6a** with benzyltributylammonium chloride in DMF solution whereupon the amount of the major diastereomer changed from 0.5% to $< 0.1\%$ (HPLC) and in the ^{1}H NMR spectrum the signals of both diastereomers were below the limit of detection.

Furthermore, commercial dichloroacetyl chloride used in the second reaction step as acylating agent contained some trichloro homologue (detected by GC in the form of the corresponding acid after hydrolysis of commercial dichloroacetyl chloride with 1.1 eq. of water in acetone at room temperature as described by Pritchard and Skinner 1950) and thus, upon acylation with dichloroacetyl chloride the trichloroacetoxy compound **6g** was also formed. The formate analogue **6h** is thought to be a product of formylation by DMF (Paquette 1995), while the oxamate **6i** may arise from a chlorine to dimethylamino substitution in **6g** (upon the influence of dimethylamine formed from DMF under the alkaline conditions of esterification) followed by hydrolysis of the residual two geminal chlorine atoms to an oxo group, see also below under Synthesis.

The presence of the methyl ester **6k** was attributed to methyl iodide present as an impurity (0.08–0.24 area% by GC which, due to the large difference in the response factors, corresponds to 0.25–0.76 mol%) in commercial ethyl iodide used in the final esterification step.

The formation of **6l** can be explained by partial hydrolysis of the 17α-dichloroacetoxy group in **6a** to 17α-hydroxy.

Finally, lumiprednisolone (**8**) prepared by the known photorearrangement of prednisolone (**2a**) (Williams et al. 1980) could also be detected in **2a** by HPLC and this compound may be the source of compound **7**. In addition, it was shown that formation of **8** from prednisolone as well as the formation of **7** from **6a** could be provoked not only by monochromatic UV light as described but also by irradiation with a xenone lamp emitting a wide spectrum visible light. This finding suggested that **7** may have been formed not only from **8** present in prednisolone (**2a**) but even directly from **6a**, which is routinely handled without protection from light.



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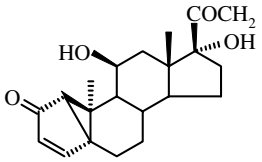

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2.2.3. Synthesis of the impurities

2.2.3.1. Synthesis of the isolated impurities

The majority of the above impurities were synthesized by following the route leading to the parent compound **6a** wherever it was applicable, see Scheme 1. These syntheses served as a further proof of the structures and provided, at the same time, reference samples for later analytical studies.

Thus, the 11-oxo (**6b**) and the 1,2-dihydro (**5c**) analogues were prepared by starting with commercial prednisone (**2b**) or hydrocortisone (**2c**), respectively, and by following the usual 3-step procedure: **2b** → **3b** → **4b** → **6b** and **2c** → **3c** → **4c** → **5c**, respectively.

11-Deoxy BNP (**6d**) and 11-epi BNP (**6e**) were prepared by synthesizing first the corresponding 1,2-dihydro derivatives **5d** and **5e** via the usual route: **2d** → **3d** → **4d** → **5d** and **2e** → **3e** → **4e** → **5e**, respectively, followed by selective 1,2-dehydrogenation of **5d** and **5e**, respectively, with DDQ to afford **6d** and **6e**, respectively. It should be noted that the pregnane side chain oxidation **2e** → **3e** with the usual reagent, $NaIO_4$ was unsuccessful, but could be performed by base catalysed air oxidation in methanol/K_2CO_3 (Kertesz and Marx 1986).

While the starting cortexolone (**2d**) is commercially available, 11-epi hydrocortisone (**2e**) is not. This latter compound was prepared as shown in Scheme 2. Cortisone (**2h**) was ketalized with ethylene glycol/pTsOH and the obtained 3,20-diketal **9** was selectively reduced to the 11α-hydroxy-3,20-diketal **10** with sodium metal in boiling n-propanol, by analogy with a literature method (Heusser et al. 1952). Finally, acidic deketalization (Bernstein et al. 1953) gave the desired **2e**.

Acylation of 17α-hydroxy in Δ^1-cortienic acid (**3a**) with chloroiodoacetyl chloride, trichloroacetyl chloride, acetyl formate or (*N,N*-dimethyl)oxamoyl chloride, respectively, afforded the corresponding 17α-acyloxy compounds **4f–i**, respectively, which upon esterification with ethyl iodide under the usual conditions gave the desired compounds **6f–i**, respectively. Partial transformation of **6a** to the chloroiodoacetyl compound **6f** was observed upon treatment of the parent compound **6a** with NaI in aprotic solvents (acetone, DMF), thus confirming the suggested route of formation. In addition, **6i** was prepared also by reaction of **6g** with dimethylamine in DMF, confirming the above suggestion on the formation of this impurity.

The methyl ester **6k** was obtained by esterification of **4a** with methyl iodide, while direct esterification of Δ^1-cortienic acid (**3a**) with ethyl iodide, without prior acylation of 17α-hydroxy, led to Δ^1-cortienic acid ethyl ester (**6l**).

Finally, compound **7** could be prepared, in full analogy with the known prednisolone → lumiprednisolone rearrangement, by UV irradiation (254 nm) of **6a** in dioxane. Similarity of stereochemistry in these two rearranged compounds was confirmed by NMR and CD spectra.

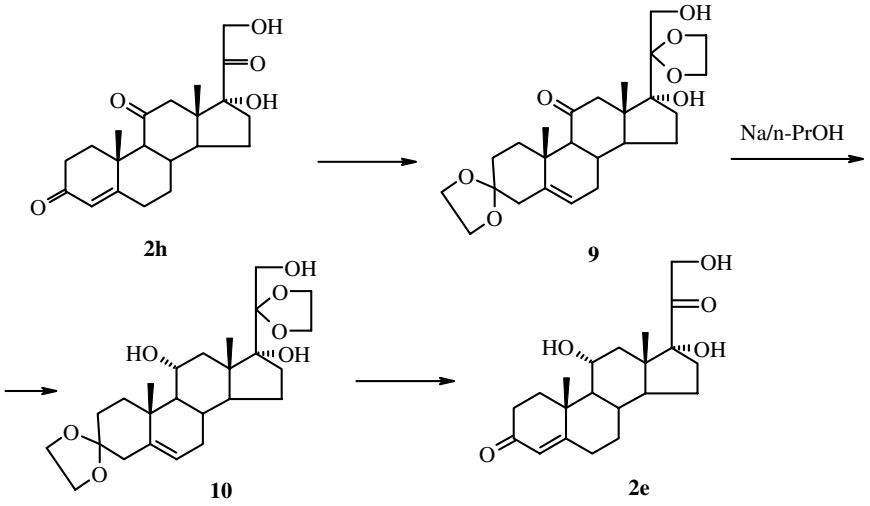
2.2.3.2. Synthesis of additional potential impurities

Since trichloroacetyl chloride was found to be a minor contaminant of the reagent dichloroacetyl chloride, it was assumed that monochloroacetyl chloride, if present as a contaminant in dichloroacetyl chloride, may analogously result in the formation of a 17α-monochloroacetoxy analogue (**6j**). Therefore, this compound was also prepared via the usual route: **3a** → **4j** → **6j**.

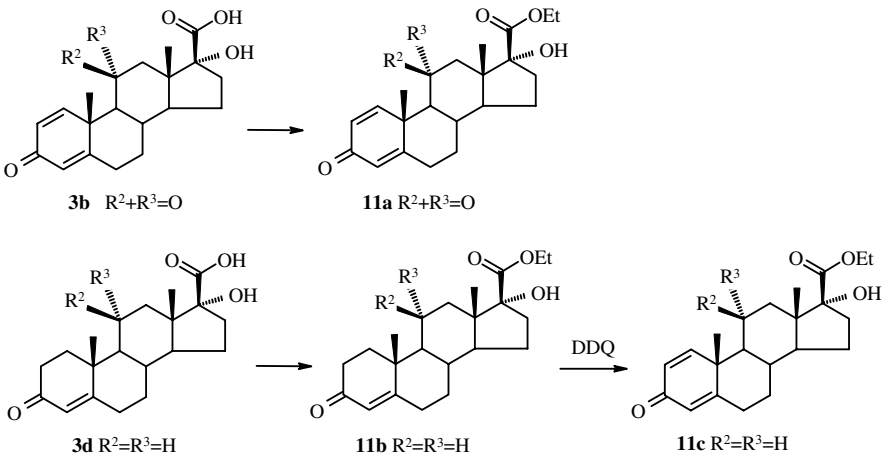
Furthermore, in the light of the presence of **6l** originating in a partial hydrolysis of **6a** the question emerged whether or not other 17α-hydroxy compounds were also formed from other 17α-acyloxy compounds with different 11-substitutions. With the cortienic acid derivatives **3b** and **3d** at hand, the usual esterification (see Scheme 3) afforded the corresponding ethyl esters **11a** (as a potential hydrolysis product derived from **6b**) and **11b**, the latter giving **11c** (a potential hydrolysis product derived from **6d**) after dehydrogenation with DDQ.

HPLC studies revealed that none of the above potential impurities (**6j**, **11a** and **11c**) were present in the parent compound **6a**.

Scheme 2



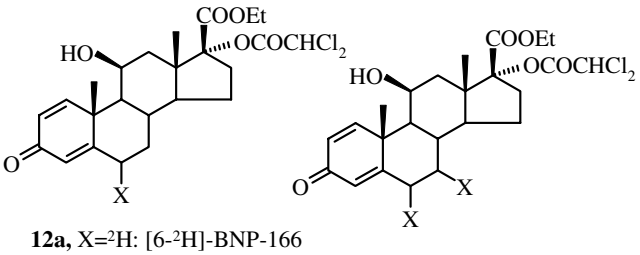
Scheme 3



2.3. Synthesis of radiolabeled BNP-166

Pharmacokinetic studies required radiolabeled BNP-166 with high specific activity. For this purpose the use of the 6-monotritiated (**12b**) and 6,7-ditritiated (**13**) variants of BNP-166 were taken into account.

The possible routes of preparation of these two compounds were studied by performing model reactions using the non-radioactive isotopes ^{1}H and ^{2}H as detailed below.



12a, X=^{2}H: [6-^{2}H]-BNP-166
12b, X=^{3}H: [6-^{3}H]-BNP-166 **13,** X=^{3}H: [6,7-di-^{3}H]-BNP-166

2.3.1. 6-Monotritiated BNP-166 (**12b**)

[6-^{3}H]-BNP-166 (**12b**) was prepared by acid-catalysed hydrogen exchange in the parent compound **6a** at the allylic position C-6.

Preliminary model reactions with $^{2}H_2O$ in dioxane under acid ($^{2}HCl/^{2}H_3PO_4$) catalysis, by analogy with a literature method (Seibl and Gaumann 1963) but at a slightly elevated temperature (60 °C), were successful. NMR and MS data of **12a** confirmed incorporation of deuterium only at the desired position 6 after a post-treatment with $^{1}H_2O$ to back exchange deuterated 11-O^{2}H to 11-O^{1}H.

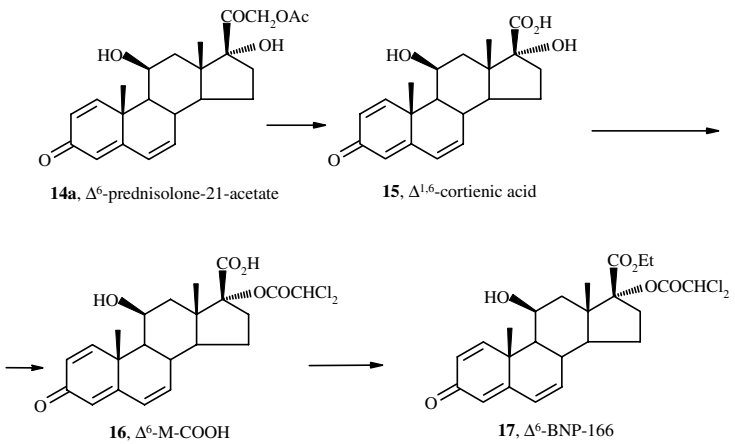
Subsequently, tritium could be incorporated in the same manner, using tritiated water under the same conditions. NMR showed the presence of ~20% ^{3}H at position 6 of **12b** with an α/β ratio of ~2 : 1.

2.3.2. Studies on the synthesis of 6,7-ditritiated-BNP-166 (**13**)

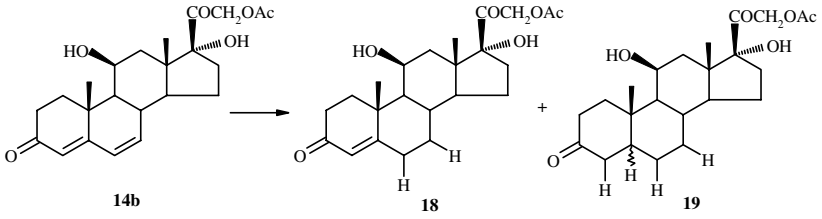
In the light of literature data on the selective catalytic hydrogenation (Pd/$BaCO_3$/ethyl acetate, Angelo and Laibach 1957) of the Δ^6 double bond in the 1,4,6-triene type compound Δ^6-prednisolone-21-acetate (**14a**), model experiments were undertaken in which the hydrogenation of Δ^6-analogues of BNP-166 and of its synthetic intermediates with $^{1}H_2$ was studied.

To this end Δ^6-BNP-166 (**17**) was prepared from the known Δ^6-prednisolone-21-acetate (Angelo and Laubach 1960) by removing first the 21-O-acetyl group and then following the synthesis of the parent compound **6a**, as shown in Scheme 4.

Scheme 4



Scheme 5

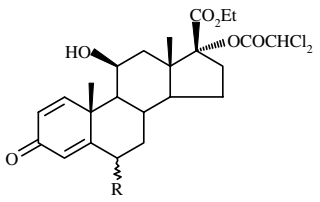


Catalytic hydrogenation of Δ^6-BNP-166 under various reaction conditions led, however, to chlorine loss in the side chain, while the same reaction starting with the Δ^6-intermediates **14a** or **15** gave mixtures of variously hydrogenated products that were the corresponding pregna-1,4-diene-3-one, pregn-4-ene-3-one and pregnane-3-one derivatives. Next, in order to reduce the number of possible hydrogenated products, the corresponding 1,2-dihydro analogue of Δ^6-prednisolone, i.e. Δ^6-hydrocortisone-21-acetate (**14b**) was prepared (Angelo and Laubach 1960) and hydrogenated in the presence of various catalysts in various solvents. Although saturation of the Δ^6-double bond was not selective even in this case (see Scheme 5), the desired, partially saturated product (**18**) and both diastereomers of the fully saturated analogue (**19**) could be identified by NMR and separated by HPLC, thus providing a method for the preparation of [6,7-di-^{3}H]-**18** using $^3H_2$.

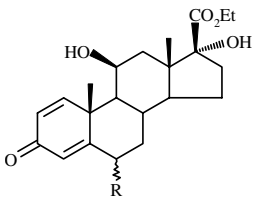
In this case, synthesis of [6,7-di-^{3}H]-BNP-166 (**13**) can be started with ditritiated [6,7-di-^{3}H]-**18** and after saponification of the 21-O-acetate [6,7-di-^{3}H]-1,2-dihydro-BNP, i.e. ditritiated **5c** can be prepared as described for the non-labeled **5c** above. Finally, **13** can be obtained from ditritiated **5c** by 1,2-dehydrogenation with DDQ as was shown by the successful unlabeled model experiment **5c** → **6a**.

2.4. Preparation of hydroxylated metabolites of BNP-166

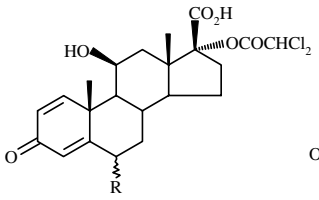
Partial loss of radioactivity during pharmacokinetic studies with [6-^{3}H]-BNP-166 (**12b**) suggested *in vivo* 6-hydroxylation of the substrate. Considering this possibility, and also the known metabolic transformations of BNP-166 (**6a**) into the hydrolysis products **6l** (M−OH), **3a** (M−CA) and **4a** (M−COOH) (Bodor 1997) hydroxylation of the parent molecule and of the above three metabolites in positions 6α and 6β was undertaken using chemical and also microbiological tools with the aim to provide reference samples for identification by HPLC.



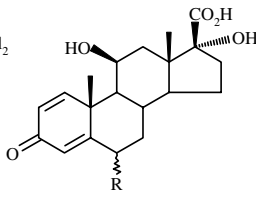
6a R = H
20a R = α-OH
20b R = β-OH



6l R = H
21a R = α-OH
21b R = β-OH



4a R = H
22a R = α-OH
22b R = β-OH



3a R = H
23a R = α-OH
23b R = β-OH

Thus, microbiological hydroxylation of **6a** and of the 17α-hydroxy metabolite **6l**, by means of the strain *Absidia coerulea* IDR 620 afforded the corresponding 6β-hydroxy derivatives **20b** and **21b**, respectively. 6β-Hydroxy-Δ^1-cortienic acid (**23b**) was obtained by alkaline hydrolysis of the corresponding ethyl ester **21b**.

On the other hand, upon chemical hydroxylation of **6a**, **6l** and **3a**, with SeO_2 in boiling dioxane the corresponding 6α-hydroxy derivatives **20a**, **21a** and **23a**, respectively, were isolated, while a similar transformation of **4a** led to an 86 : 14 mixture of the 6α- (**22a**) and 6β-hydroxy (**22b**) derivatives.

3. Experimental

Melting points were determined on a Boetius microscope and are uncorrected. Purity of the compounds was tested on TLC plates (silica gel, Merck) using the solvent systems given below. The spots were visualized under UV light and/or by exposure to iodine vapours. NMR spectra were recorded in $CDCl_3$, DMSO-d_6 or CD_3OD solutions using a Bruker Avance 500 or an AC-250 spectrometer, operating at 500/250 MHz (^{1}H) and 125/62.5 MHz (^{13}C), respectively. Chemical shifts are given on the δ-scale and were referenced to TMS. Pulse programs for the 1D and 2D NMR experiments were taken from the Bruker software library. For structure elucidation and NMR signal assignment ^{1}H, ^{13}C, DEPT-135, ^{1}H,^{1}H-COSY, HMQC, HMBC and ^{1}H,^{1}H-NOESY spectra were recorded. Mass spectra were recorded on a Finnigan MAT 8430 instrument, ion accelerating voltage: 3kV; temperature of the ion source: 250 °C; EI: electron energy: 70 eV; electron current: 500 μA; CI: reagent gas: isobutane. Analytical HPLC was performed, in most cases, using a Waters Breeze HPLC System with a reversed-phase silica column, Hypersil ODS, 3 μm, 100 × 4 mm ID and isocratic elution. The eluent was methanol − aqueous buffer = 60/40 (v/v). The aqueous buffer: 0.1% (v/v) aqueous solution of acetic acid adjusted to pH 4.5 with 2 N NaOH; flow rate: 0.8 ml/min; column temperature: 40 °C, detection at 243 nm. For compounds **18** and **19** HPLC was done on normal phase silica using a Nucleosil SI, 5 μm, 250 × 4 mm ID column and isocratic elution. The eluent was a mixture of n-hexane, methylene chloride, methanol and glacial acetic acid (88 : 8:3.9 : 0.1, v/v); flow rate: 1.5 ml/min. Detection: Waters 486 detector at 255 nm.

Semi-preparative HPLC separations were carried out using the following system: Waters PrepLC 4000 System with a Waters 486 UV-VIS detector; column: Waters Delta Pak C-18, 15 μm, 100 Å, 19 × 300 mm; gradient elution using Milli-Q water (A) and 85% acetonitril in water (B), according to the gradient profile shown in Table 1; detection at 243 nm; flow rate: 16 ml/min.

Gas chromatography was carried out on a CP-9000 instrument. The methyl iodide content of ethyl iodide was measured by following the comparative standard addition method and using a CPSil5CB column (25 m × 0.32 mm),

Table 1: Gradient profile used in HPLC separation

Time point (min)	Component A	Component B
0	60	40
45	20	80
46	60	40
55	60	40

Table 2: Rf-values of compounds 6 in different solvent systems

Solvent system*	Rf value						
	6d	**6b**	**6a**	**6h**	**6e**	**6l**	**6i**
No. 1	0.56	0.50	0.30	0.20	0.10	0.07	0.07
No. 2	0.75	0.74	0.70	0.58	0.49	0.37	0.18

* System No. 1: methylene chloride-acetone 95 : 5;
System No. 2: ethyl acetate – n-hexane 2 : 1

injector and detector temperature: 250 °C, heating rate: 15 °C/min from 45 to 225 °C. On the other hand the trichloroacetyl chloride impurity in dichloroacetyl chloride was determined after derivatization of the hydrolysed crude samples with bistrimethylsilyl trifluoroacetamide on an RH5ms$^+$ column (30 m × 0.25 mm), injector and detector temperature: 250 °C, heating rate: 15 °C/min from 50 to 225 °C.
CD spectra were run on a JASCO 600 instrument. All the compounds gave satisfactory elemental analysis values.

3.1. Isolation of the impurities

Six impurities, i.e. compounds **6b**, **6d**, **6e**, **6h**, **6i** and **6l** with the Rf-values shown in Table 2 were separated from **6a** by TLC and isolated by column chromatography on silica gel from **6a** mother liquors containing the impurities at relatively high concentrations.
Impurities **5c**, **6f**, **6g**, **6k** and **7** were isolated by semi-preparative reversed-phase HPLC under the conditions given above. A more detailed description of the HPLC separation studies will be published elsewhere (Patthy et al.).

3.2. Synthesis

3.2.1. 11-Epi-hydrocortisone (**2e**)

To a solution of **10** (1.64 g, 3.64 mmol) in methanol (180 ml) was added 8% (v/v) aqueous sulfuric acid (24 ml) and the mixture was refluxed for 40 min. Then the solvent was partially evaporated *in vacuo* and the residue was diluted with water (260 ml). The solution was neutralized with saturated aqueous sodium bicarbonate solution (55 ml) and the product was extracted into ethyl acetate. The organic layer was dried over magnesium sulfate and concentrated *in vacuo*. The crude product (1.3 g) was purified by column chromatography on silica gel eluting with methylene chloride – methanol 9 : 1. Yield: 0.56 g (42%), pale yellow crystalline powder. M.p. 206–208 °C.
^{1}H NMR (250 MHz, DMSO-d$_6$): δ 0.55 (3H, s, CH_3-18), 1.24 (3 H, s, CH_3-19), 3.76 (1 H, m, H_β-11), 4.10/4.48 (1 H + 1 H, dd, H-21), 5.60 (1 H, s, H-4), 4.32 (1 H, d, 11-OH), 4.67 (1 H, t, 21-OH), 5.28 (1 H, s, 17-OH).

3.2.2. 11β,17α-Dihydroxyandrosta-1,4-diene-3-one-17β-carboxylic acid (**3a**)

To **2a** (70 g, 0.194 mol) in a mixture of tetrahydrofuran (600 ml) and methanol (220 ml) at 30 °C was added a solution of sodium metaperiodate (120 g, 0.561 mol) in warm (50 °C) water (550 ml) over 25 min. The reaction mixture was stirred at room temperature for 2 h. The organic solvents were removed *in vacuo*. The precipitate was collected by filtration, washed twice with water and without drying dissolved in 0.25 N aqueous sodium hydroxide solution (880 ml). Insoluble impurities were removed by filtration, the clear solution was washed twice with methylene chloride and acidified with 0.5 N hydrochloric acid (550 ml) to pH = 1. The precipitate was collected by filtration, washed three times with water and dried at 40 °C until constant weight. Yield: 63.7 g (95%), white crystalline powder. M.p. 230 °C (decomp.).
^{1}H NMR (250 MHz, DMSO-d$_6$): δ 0.92 (3 H, s, CH_3-18), 1.40 (3 H, s, CH_3-19), 4.36 (1 H, ms, H_α-11), 5.91 (1 H, s, br, H-4), 4.61 (1 H, d, 11-OH), 6.16 (1 H, d, J = 10.0 Hz, CH-1), 7.32 (1 H, d, J = 10 Hz, H-2).

3.2.3. 17α-Hydroxy-11-oxoandrosta-1,4-diene-3-one-17β-carboxylic acid (**3b**)

Prepared as described for **3a**, starting with **2b**. Yield: 84%, white crystalline powder. M.p. 277–282 °C (decomp.).
^{1}H NMR (250 MHz, DMSO-d$_6$): δ 0.60 (3 H, s, CH_3-18), 1.38 (3 H, s, CH_3-19), 6.02 (1 H, d, H-4), 6.08 (1 H, dd, H-2), 7.60 (1 H, d, H-1); ^{13}C NMR (62.7 MHz, DMSO-d$_6$): 15.5 (C-18), 18.5 (C-19), 93.5 (C-17), 123.6 (C-4), 126.8 (C-2), 154.9 (C-1), 167.0 (C-5), 174.0 (CO_2H), 184.8 (C-3), 210.5 (C-11).

3.2.4. 11β,17α-Dihydroxyandrost-4-ene-3-one-17β-carboxylic acid (**3c**)

Prepared as described for **3a**, starting with **2c**. Yield: 84%, white crystalline powder. M.p. 231–234 °C.
^{1}H NMR (250 MHz, DMSO-d$_6$): δ 0.90 (3 H, s, CH_3-18), 1.35 (3 H, s, CH_3-19), 4.25 (1 H, m, H_α-11), 5.55 (1 H, d, H-4); ^{13}C NMR (62.7 MHz, DMSO-d$_6$): 17.0 (C-18), 20.2 (C-19), 31.1 (C-8), 66.3 (C-11), 84.5 (C-17), 121.3 (C-4), 172.2 (C-5), 174.9 (CO_2H), 197.9 (C-3).

3.2.5. 17α-Hydroxyandrost-4-ene-3-one-17β-carboxylic acid (**3d**)

Prepared as described for **3a**, starting with **2d**. Yield: 82%, white crystalline powder. M.p. 223–228 °C (decomp.).
^{1}H NMR (250 MHz, DMSO-d$_6$): δ 0.62 (3 H, s, CH_3-18), 1.18 (3 H, s, CH_3-19), 5.62 (1 H, s, H-4); ^{13}C NMR (62.7 MHz, DMSO-d$_6$): 14.7 (C-18), 16.7 (C-19), 84.4 (C-17), 122.9 (C-4), 170.8 (C-5), 174.9 ($\underline{CO_2}$H), 197.8 (C-3).
MS: EI: $[M]^{+\cdot}$ 332 (100%), m/z 244 (66%), m/z 229 (48%), m/z 124 (30%). CI: $[M + H]^+$ 333 (100%).

3.2.6. 11α,17α-Dihydroxyandrost-4-ene-3-one-17β-carboxylic acid (**3e**)

To a solution of **2e** (0.35 g, 0.96 mmol) in methanol (17.5 ml) was added anhydrous potassium carbonate (0.35 g, 2.5 mmol). Air was bubbled into the stirred suspension at room temperature over 25 min and the reaction mixture was stirred for one day to give a clear solution. After removal of the solvent *in vacuo* the residue was dissolved in water (22 ml) and ethyl acetate (13 ml). The layers were separated, the aqueous layer was washed with ethyl acetate (2 × 20 ml) and then acidified with 2 N hydrochloric acid (3.5 ml). The product was extracted into ethyl acetate (3 × 20 ml). The organic layer was dried over magnesium sulfate and concentrated *in vacuo*. Yield: 0.22 g (65%), pale yellow crystalline powder. M.p. 178–180 °C.
^{1}H NMR (500 MHz, $CDCl_3$ + 1 drop of CD_3OD): δ 0.77 (3 H, s, CH_3-18), 1.28 (3 H, s, CH_3-19), 3.93 (1 H, td, H_β-11), 5.69 (1 H, s, H-4); ^{13}C NMR (125 MHz, $CDCl_3$ + 1 drop of CD_3OD): 16.0 (C-18), 18.2 (C-19), 35.0 (C-8), 43.1 (C-10), 48.1 (C-13), 68.7 (C-11), 85.0 (C-17), 124.1 (C-4), 172.5 (C-5), 175.9 ($\underline{CO_2}$H), 201.2 (C-3).

*3.2.7. 17α-Dichloroacetoxy-11β-hydroxyandrosta-1,4-diene-3-one-17β-carboxylic acid (***4a***)*

Dichloroacetyl chloride (62.5 ml, 95.8 g; 0.65 mol) in methylene chloride (1500 ml) was slowly added to a stirred solution of potassium bicarbonate (139.5 g, 1.39 mol) and **3a** (45.0 g, 0.13 mol) in water (2000 ml) over 2 h. The obtained reaction mixture was acidified with 5 N hydrochloric acid (135 ml) to pH = 1–2. The layers were separated and the aqueous layer was extracted with methylene chloride (2 × 210 ml). The combined organic layers were washed with saturated ammonium chloride solution (2 × 360 ml). The organic layer was stirred with the solution of potassium bicarbonate (17.1 g, 172 mmol) in water (1000 ml) for 30 min. The process was repeated with the solution of potassium bicarbonate (8.6 g, 86.0 mmol) in water (600 ml). The combined aqueous solutions were washed with methylene chloride (135 ml) and then slowly acidified with 2 N hydrochloric acid to pH = 1–2 under stirring. The solution was warmed to 45–50 °C then the precipitated white solids were collected by filtration and washed with water. The obtained white powder was dried at 45 °C *in vacuo* until constant weight. Yield: 55.4 g (93%), white crystalline powder. M.p. 210–214 °C.
^{1}H NMR (250 MHz, DMSO-d$_6$): δ 1.01 (3 H, s, CH_3-18), 1.41 (3 H, s, CH_3-19), 5,94 (1 H, s, H-4), 6.18 (1 H, d, J = 10 Hz, H-2), 6.85 (1 H, s, $CO_2-\underline{CH}Cl_2$), 7.33 (1 H, d, H-1).

3.2.8. 17α-Dichloroacetoxy-11-oxoandrosta-1,4-diene-3-one-17β-carboxylic acid (**4b**)

Prepared as described for **4a**, starting with **3b**. Yield: 79%, white crystalline powder. M.p. 230–234 °C.
^{1}H NMR (250 MHz, DMSO-d$_6$): δ 0.67 (3 H, s, CH_3-18), 1.37 (3 H, s, CH_3-19), 6.02 (1 H, d, H-4), 6.12 (1 H, dd, H-2), 6.83 (1 H, s, $\underline{CO_2}-CHCl_2$), 7.58 (1 H, d, H-1); ^{13}C NMR (62.7 MHz, DMSO-d$_6$): 15.1 (C-18), 18.4 (C-19), 91.8 (C-17), 123.6 (C-4), 126.9 (C-2), 154.9 (C-1), 162.6 ($\underline{CO_2}-CHCl_2$), 166.8 (C-5), 168.6 (COOH), 184.8 (C-3), 208.7 (C-11).

3.2.9. 17α-Dichloroacetoxy-11β-hydroxyandrost-4-ene-3-one-17β-carboxylic acid (**4c**)

Prepared as described for **4a**, starting with **3c**. Yield: 86%, white crystalline powder. M.p. 197–200 °C.
^{1}H NMR (250 MHz, (CD_3OD): δ 1.09 (3 H, s, CH_3-18), 1.49 (3 H, s, CH_3-19), 4.42 (1 H, ddd, $J_1 = J_2 = 4.7$ Hz, $J_3 = 2.1$ Hz H-11), 5.67 (1 H, s, H-4), 6.12 (1 H, dd, H-2), 6.44 (1 H, s, $CO_2-\underline{CH}Cl_2$).

3.2.10. 17α-Dichloroacetoxyandrost-4-ene-3-one-17β-carboxylic acid (**4d**)

Prepared as described for **4a**, starting with **3d**. Yield: 73%, white crystalline powder. M.p. 199–201 °C.
^{1}H NMR (250 MHz, DMSO-d$_6$): δ 0.75 (3 H, s, CH_3-18), 1.15 (3 H, s, CH_3-19), 5.63 (1 H, s, H-4), 6.94 (1 H, s, $CHCl_2$); ^{13}C NMR (62.7 MHz, DMSO-d$_6$): 14.1 (C-18), 16.7 (C-19), 93.2 (C-17), 123.0 (C-4), 163.0 ($\underline{CO_2}-CHCl_2$), 169.3 (C-5), 170.3 ($\underline{CO_2}$H), 197.7 (C-3).
MS: EI: $[M]^{+\cdot}$ 442/444 (22/14%), m/z 314 (31%), m/z 269 (56%), m/z 124 (83%). CI: $[M + H]^+$ 443/445 (6/4%), m/z 315 (100%).

3.2.11. 17α-Dichloroacetoxy-11α-hydroxyandrost-4-ene-3-one-17β-carboxylic acid (**4e**)

Prepared as described for **4a**, starting with **3e**. Yield: 87%, brownish oil. This compound was used in the next step without further purification.

3.2.12. 17α-Chloroiodoacetoxy-11β-hydroxyandrosta-1,4-diene-3-one-17β-carboxylic acid (**4f**)

To a stirred suspension of **3a** (1.57 g, 4.5 mmol) and potassium carbonate (0.94 g, 6.8 mmol) in dimethyl formamide (20 ml) below 5 °C chloroiodoacetyl chloride (3.25 g, 13.6 mmol) was added. The reaction mixture was stirred at room temperature for 2.5 h, then diluted with saturated sodium chloride solution (100 ml) and extracted twice with diethyl ether. The organic layer was dried over magnesium sulfate and concentrated *in vacuo*. The obtained dark residue was purified by column chromatography on silica gel eluting with a 90 : 10 : 0.6 mixture of methylene chloride, methanol and acetic acid. This partially purified product was used without further purification. Yield: 1.22 g (49%), dark oil.

3.2.13. 17α-Trichloroacetoxy-11β-hydroxyandrosta-1,4-diene-3-one-17β-carboxylic acid (**4g**)

Prepared as described for **4a** but using trichloroacetyl chloride instead of dichloroacetyl chloride. Yield: 48%, white crystalline powder. M.p. 249–250 °C (decomp.).

3.2.14. 17α-Formyloxy-11β-hydroxyandrosta-1,4-diene-3-one-17β-carboxylic acid (**4h**)

To **3a** (1.0 g, 2.9 mmol) in anhydrous pyridine (4 ml) a formic acid-acetic anhydride mixture (0.46 ml) (Stevens and VanEs 1964) was added and the mixture was refluxed for 7 h. The obtained precipitate was collected by filtration. Yield: 0.39 g (39%), white crystalline powder. M.p. 233 °C.
^{1}H NMR (250 MHz, DMSO-d_6): δ 0.99 (3 H, s, CH_3-18), 1.40 (3 H, s, CH_3-19), 5.90 (1 H, d, H-4), 6.15 (1 H, dd, H-2), 7.32 (1 H, d, H-1), 8.08 (1 H, s, HC = O); ^{13}C NMR (62.7 MHz, DMSO-d_6): 16.3 (C-18), 20.6 (C-19), 68.0 (C-11), 90.0 (C-17), 121.4 (C-4), 126.9 (C-2), 156.4 (C-1), 160.8 (HCO_2), 170.0 (C-5), 170.0 (CO_2H), 184.9 (C-3).

3.2.15. 17α-(N,N-Dimethyl)oxamoyloxy-11β-hydroxyandrosta-1,4-diene-3-one-17β-carboxylic acid (**4i**)

To a suspension of **3a** (0.5 g, 1.44 mmol) and anhydrous potassium carbonate (0.3 g, 2.16 mmol) in anhydrous dimethyl formamide (10 ml) (*N,N*-dimethyl)oxamoyl chloride (Kirsanov and Molosnova 1959) (1.12 g, 8.2 mmol) was added at 0–5 °C. The reaction mixture was stirred at room temperature for 5.5 h and then diluted with saturated sodium chloride solution (60 ml). The product was extracted into ethyl acetate. The organic layer was dried over magnesium sulfate and concentrated in vacuo. This crude product was used in the next step without further purification. Yield: 1.04 g, colorless oil.

3.2.16. 17α-Chloroacetoxy-11β-hydroxyandrosta-1,4-diene-3-one-17β-carboxylic acid (**4j**)

Prepared as described for **4a** but using monochloroacetyl chloride instead of dichloroacetyl choride. The crude product was purified by column chromatography on silica gel eluting with n-hexane – ethyl acetate 2 : 1. Yield: 26%, yellow crystalline powder. M.p. 200 °C (decomp.).
^{1}H NMR (250 MHz, DMSO-d_6): δ 0.98 (3 H, s, CH_3-18), 1.38 (3 H, s, CH_3-19), 4.32 (1 H, m, H_α-11), 4.36 (2 H, s, $COCH_2Cl$), 5.95 (1 H, d, H-4), 6.18 (1 H, dd, H-2), 7.33 (1 H, d, H-1); ^{13}C NMR (62.7 MHz, DMSO-d_6): 16.3 (C-18), 20.5 (C-19), 40.9 ($CO\underline{CH_2}Cl$), 68.0 (C-11), 91.4 (C-17), 121.4 (C-4), 126.9 (C-2), 156.4 (C-1), 166.0 ($\underline{CO}CH_2Cl$), 169.9 (C-5), 170.1 (COOH), 184.9 (C-3).

3.2.17. Ethyl 17α-dichloroacetoxy-11β-hydroxyandrost-4-ene-3-one-17β-carboxylate (**5c**)

Prepared by method A as described for **6a** below, starting with **4c**. Yield: 63%, white crystalline powder. M.p. 182–184 °C.
^{1}H NMR (500 MHz, $CDCl_3$): δ 1.02 (3 H, s, CH_3-18), 1.45 (3 H, s, CH_3-19), 1.26 (3 H, t, $\underline{CH_3}CH_2O$), 4.20 (2 H, q, $CH_3\underline{CH_2}O$), 2.97 (1 H, m, H_β-16), 4.49 (1 H, m, H_α-11), 5.68 (1 H, d, H-4), 5.92 (1 H, s, $CHCl_2$); ^{13}C NMR (125 MHz, $CDCl_3$): 14.3 ($\underline{CH_3}CH_2O$), 17.2 (C-18), 21.1 (C-19), 24.0 (C-15), 31.4 (C-16), 31.7 (C-8), 32.1 (C-6), 32.8 (C-1), 34.0 (C-2), 35.1 (C-7), 39.4 (C-10), 40.0 (C-12), 47.3 (C-13), 52.3 (C-14), 56.1 (C-9), 61.8 ($CH_3\underline{CH_2}O$), 64.5 ($CHCl_2$), 68.3 (C-11), 93.6 (C-17), 122.6 (C-4), 163.0 ($\underline{CO_2}-CHCl_2$), 168.5 ($\underline{CO_2}-CH_2CH_3$), 171.9 (C-5), 199.5 (C-3).
MS: EI: $[M]^{+\bullet}$ 486/488 (41/28%), m/z 358 (8%), m/z 285 (48%), m/z 267 (100%), m/z 124 (47%). CI: $[M + H]^+$ 487/489 (84/57%), m/z 359 (100%).

3.2.18. Ethyl 17α-dichloroacetoxyandrost-4-ene-3-one-17β-carboxylate (**5d**)

Prepared by method A as described for **6a** below, starting with **4d**. Yield: 79%, pale yellow crystalline powder. M.p. 150–153 °C.
^{1}H NMR (500 MHz, DMSO-d_6): δ 0.72 (3 H, s, CH_3-18), 1.16 (3 H, s, CH_3-19), 1.16 (3 H, t, $\underline{CH_3}CH_2O$), 4.11 (2 H, q, $CH_3\underline{CH_2}O$), 2.80 (1 H, m, H_β-16), 5.64 (1 H, d, H-4), 6.92 (1 H, s, $CHCl_2$); ^{13}C NMR (125 MHz, DMSO-d_6): 13.7 ($\underline{CH_3}CH_2O$), 14.0 (C-18), 16.7 (C-19), 19.7 (C-11), 23.1 (C-15), 29.8 (C-12), 30.8 (C-16), 31.5 (C-1), 31.6 (C-6), 33.3 (C-2), 34.7 (C-8), 34.9 (C-7), 37.9 (C-10), 47.2 (C-13), 49.9 (C-14), 52.6 (C-9), 60.7 ($CH_3\underline{CH_2}O$), 64.7 ($CHCl_2$), 92.9 (C-17), 123.0 (C-4), 163.0 ($\underline{CO_2}-CHCl_2$), 167.7 ($\underline{CO_2}-CH_2CH_3$), 170.2 (C-5), 197.7 (C-3).
MS: $[M]^{+\bullet}$ 470/472 (65/45%), m/z 342 (31%), m/z 269 (100%), m/z 244 (53%), m/z 229 (32%), m/z 124 (64%). CI: $[M + H]^+$ 471/473 (90/61%), m/z 343 (100%).

3.2.19. Ethyl 17α-dichloroacetoxy-11α-hydroxyandrost-4-ene-3-one-17β-carboxylate (**5e**)

Prepared by method A as described for **6a** below, starting with **4e**. The product was isolated by extraction with diethyl ether and concentration of the extract. Yield: 50%, pale yellow crystalline powder. M.p. 134–136 °C.
^{1}H NMR (500 MHz, $CDCl_3$): δ 0.83 (3 H, s, CH_3-18), 1.28 (3H, t, $\underline{CH_3}CH_2O$), 1.34 (3 H, s, CH_3-19), 4.05 (1 H, td, H_β-11), 4.22 (2 H, m, $CH_3\underline{CH_2}O$), 5.76 (1 H, s, H-4), 5.96 (1 H, s, $CHCl_2$).

3.2.20. Ethyl 17α-dichloroacetoxy-11β-hydroxyandrosta-1,4-diene-3-one-17β-carboxylate (**6a**)

3.2.20.1. Method A: By esterification of **4a** with ethyl iodide

To a stirred suspension of **4a** (50 g, 0.109 mol) and anhydrous potassium carbonate (16.58 g, 0.120 mol) in anhydrous dimethyl formamide (500 ml) was added ethyl iodide (13.1 ml, 25.5 g; 0.164 mol) at room temperature. After stirring for 1.5 h the reaction mixture was diluted with saturated aqueous sodium chloride solution (1000 ml) and stirred for 1 h. The obtained precipitate was collected by filtration and washed with water (3 × 180 ml). The crude product (53.2 g) was recrystallized from ethyl acetate (400 ml). Yield: 31.9 g (61%), white crystalline powder. M.p. 201–202.5 °C.
^{1}H NMR (500 MHz, $CDCl_3$): δ 1.06 (3 H, s, CH_3-18), 1.47 (3 H, s, CH_3-19), 1.27 (3 H, t, $\underline{CH_3}CH_2O$), 4.21 (2 H, q, $CH_3\underline{CH_2}O$), 2.97 (1 H, m, H_β-16), 4.53 (1 H, m, H_α-11), 2.36 (1 H, m, H_α-6), 2.59 (1 H, m, H_β-6), 5.91 (1 H, s, $CHCl_2$), 6.03 (1 H, d, H-4), 6.28 (1 H, dd, H-2), 7.29 (1 H, d, H-1); ^{13}C NMR (125 MHz, $CDCl_3$): 14.1 ($\underline{CH_3}CH_2O$), 16.9 (C-18), 21.0 (C-19), 61.6 ($CH_3\underline{CH_2}O$), 64.2 ($CHCl_2$), 31.9 (C-6), 69.9 (C-11), 93.4 (C-17), 122.5 (C-4), 127.9 (C-2), 156.0 (C-1), 162.8 ($\underline{CO_2}$-$CHCl_2$), 169.7 (C-5), 168.3 ($\underline{CO_2}-CH_2CH_3$), 186.5 (C-3).
MS: EI: $[M]^{+\bullet}$ 484/486 (3.9/2.6%), m/z 363/365 (8.5/5.7%), m/z 356 (3%), m/z 283 (21%), m/z 265 (34%), m/z 122 (100%), m/z 121 (41%). CI: $[M + H]^+$ 485/487 (93/64%), m/z 122 (100%).

3.2.20.2. Method B: By esterification of **4a** with diethyl sulfate

The procedure described above (method A) was followed with the difference that diethyl sulfate (21.5 ml, 25.3 g, 0.164 mol) was used instead of ethyl iodide. Yield: 70%.

3.2.20.3. Method C: By dehydrogenation of **5c**:

1,2-Dehydrogenation was carried out as described below for the preparation of **6d**, yield: 96%.

3.2.21. Ethyl 17α-dichloroacetoxy-11-oxoandrosta-1,4-diene-3-one-17β-carboxylate (**6b**)

Prepared by method A as described for **6a**, starting with **4b**. Yield: 60%, white crystalline powder. M.p. 191–197 °C.
^{1}H NMR (250 MHz, $CDCl_3$): δ 0.77 (3 H, s, CH_3-18), 1.49 (3 H, s, CH_3-19), 1.28 (3 H, t, $\underline{CH_3}CH_2O$), 4.21 (2 H, q, $CH_3\underline{CH_2}O$), 3.00 (1 H, m, H_β-16), 5.97 (1 H, s, $CHCl_2$), 6.11 (1 H, d, H-4), 6.25 (1 H, dd, H-2), 7.68 (1 H, d, H-1); ^{13}C NMR (62.7 MHz, $CDCl_3$): 14.0 ($\underline{CH_3}CH_2O$), 15.6 (C-18), 18.8 (C-19), 62.1 ($CH_3\underline{CH_2}O$), 64.1 ($CHCl_2$), 92.3 (C-17), 124.9 (C-4), 127.8 (C-2), 154.6 (C-1), 162.6 ($\underline{CO_2}-CHCl_2$), 165.7 ($\underline{CO_2}-CH_2CH_3$), 167.4 (C-5), 186.1 (C-3), 208.1 (C-11).
MS: EI: $[M]^{+\bullet}$ 482/484 (24/17%), m/z 354 (32%), m/z 281 (100%), m/z 122 (50%), m/z 121 (88%). CI: $[M+ H]^+$ 483/485 (100/62%).

3.2.22. Ethyl 17α-dichloroacetoxyandrosta-1,4-diene-3-one-17β-carboxylate (**6d**)

To **5d** (0.4 g, 0.85 mmol) in anhydrous toluene (40 ml) 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (0.27 g, 1.2 mmol) and benzoic acid (0.21 g, 1.7 mmol) were added. The reaction mixture was heated at 85–90 °C for 51 h. After removal of the precipitate the filtrate was diluted

with methylene chloride (40 ml) and extracted twice with a solution of sodium bicarbonate (0.6 g) in water (40 ml). The combined aqueous layers were back-extracted with methylene chloride (40 ml). The combined organic layers were washed with water (60 ml), dried over magnesium sulfate and concentrated *in vacuo*. The crude product (0.43 g) was purified by column chromatography on silica gel eluting with methylene chloride – acetone 97.5 : 2.5. Yield: 0.24 g (60%), white crystalline powder. M.p. 125–126 °C.
^{1}H NMR (250 MHz, $CDCl_3$): δ 0.82 (3 H, s, CH_3-18), 1.23 (3 H, s, CH_3-19), 1.23 (3 H, t, $\underline{CH_3}CH_2O$), 4.21 (2 H, q, $CH_3\underline{CH_2}O$), 2.95 (1 H, m, H_β-16), 5.89 (1 H, s, $\overline{CHCl_2}$), 6.08 (1 H, d, H-4), 6.25 (1 H, dd, H-2), 7.05 (1 H, d, H-1); ^{13}C NMR (62.7 MHz, $CDCl_3$): 13.7 ($\underline{CH_3}CH_2O$), 14.3 (C-18), 18.3 (C-19), 61.6 ($CH_3\underline{CH_2}O$), 63.9 ($CHCl_2$), 93.6 (C-17), 124.1 (C-4), 127.7 (C-2), 155.3 (C-1), 163.0 ($\underline{CO_2}-CHCl_2$), 168.4 (C-5, and $\underline{CO_2}-CH_2CH_3$), 186.2 (C-3).
MS: EI: $[M]^{+\bullet}$ 468/470 (4.3/2.8%), m/z 340 (22%), m/z 267 (55%), m/z 122 (100%), m/z 121 (25%). CI: $[M + H]^+$ 469/471 (100/73%), m/z 122 (68%).

3.2.23. Ethyl 17α-dichloroacetoxy-11α-hydroxyandrosta-1,4-diene-3-one-17β-carboxylate (**6e**)

Prepared as described for **6d**, starting with **5e**. The crude product was purified by column chromatography on silica gel eluting with methylene chloride – methanol 95 : 5. Yield: 37%, yellow oil.
^{1}H NMR (500 MHz, $CDCl_3$): δ 0.84 (3 H, s, CH_3-18), 1.34 (3H, s, CH_3-19), 1.28 (3 H, t, $\underline{CH_3}CH_2O$), 4.23 (2 H, q, $CH_3\underline{CH_2}O$), 2.99 (1 H, m, H_β-16), 4.10 (1 H, m, H_β-11), 5.93 (1 H, s, $CHCl_2$), 6.12 (1 H, d, H-4), 6.19 (1 H, dd, H-2), 7.77 (1 H, d, H-1); ^{13}C NMR (125 MHz, $CDCl_3$): 14.2 ($\underline{CH_3}CH_2O$), 15.6 (C-18), 18.7 (C-19), 61.8 ($CH_3\underline{CH_2}O$), 64.2 ($CHCl_2$), 92.8 (C-17), 124.8 (C-4), 125.3 (C-2), 158.3 (C-1), 162.8 ($\underline{CO_2}-CHCl_2$), 167.2 (C-5), 168.1 ($\underline{CO_2}-CH_2CH_3$), 186.5 (C-3).
MS: EI: $[M]^{+\bullet}$ 484/486 (10/7%), m/z 363/365 (9/6%), m/z 356 (8%), m/z 283 (35%), m/z 265 (56%), m/z 122 (100%), m/z 121 (65%). CI: $[M + H]^+$ 485/487 (50/33%), m/z 122 (100%).

3.2.24. Ethyl 17α-chloroiodoacetoxy-11β-hydroxyandrosta-1,4-diene-3-one-17β-carboxylate (**6f**)

Prepared by method A as described for **6a**, starting with **4f**. The crude product was isolated by extraction with diethyl ether and was purified by column chromatography on silica gel eluting with ethyl acetate – *n*-hexane 2 : 1. The product was then crystallized by dissolving it in ethyl acetate and precipitating with *n*-hexane. Yield: 10%, white crystalline powder. M.p. 186–187 °C.
^{1}H NMR (500 MHz, DMSO-d_6): δ 0.93/0.94 (3 H, s, CH_3-18), 1.39 (3 H, s, CH_3-19), 1.18/1.22 (3 H, t, $\underline{CH_3}CH_2O$), 2.73 (1 H, m, H_β-16), 4.10 (2 H, q, $CH_3\underline{CH_2}O$), 4.30 (1 H, m, H_α-11), 5.91 (1 H, d, H-4), 6.17 (1 H, dd, H-2), 6.56/6.65 (1 H, s, CHICl), 7.31 (1 H, d, H-1); ^{13}C NMR (125 MHz, DMSO-d_6): 14.5/14.6 ($\underline{CH_3}CH_2O$), 16.6/16.9 (C-18), 17.0/17.4 (CHClI), 21.2 (C-19), 61.3 ($CH_3\underline{CH_2}O$), 92.4/92.5 (C-17), 122.1 (C-4), 127.6 (C-2), 156.9/157.0 (C-1), 166.0 ($\underline{CO_2}-CHICl$), 168.8 ($\underline{CO_2}-CH_2CH_3$), 170.7 (C-5), 185.5 (C-3).
MS: EI: $[M]^{+\bullet}$ 576/578 (6/2%), m/z 455/457 (16/5%), m/z 356 (15%), m/z 283 (26%), m/z 265 (35%), m/z 122 (100%), m/z 121 (44%).

3.2.25. Ethyl 17α-trichloroacetoxy-11β-hydroxyandrosta-1,4-diene-3-one-17β-carboxylate (**6g**)

Prepared by method A as described for **6a**, starting with **4g**. Yield: 49%, white crystalline powder. M.p. 219–222 °C.
^{1}H NMR (250 MHz, $CDCl_3$): δ 1.08 (3 H, s, CH_3-18), 1.49 (3 H, s, CH_3-19), 1.26 (3 H, t, $\underline{CH_3}CH_2O$), 4.24 (2 H, q, $CH_3\underline{CH_2}O$), 3.00 (1 H, m, H_β-16), 4.50 (1 H, m, H_α-11), 6.07 (1 H, d, H-4), 6.31 (1 H, dd, H-2), 7.37 (1 H, d, H-1); ^{13}C NMR (62.7 MHz, $CDCl_3$): 14.0 ($\underline{CH_3}CH_2O$), 16.8 (C-18), 20.9 (C-19), 23.9 (C-15), 30.8 (C-16), 31.3 (C-8), 31.8 (C-6), 33.8 (C-7), 39.4 (C-12), 44.0 (C-10), 47.6 (C-13), 51.6 (C-14), 55.1 (C-9), 61.6 ($CH_3\underline{CH_2}O$), 69.6 (C-11), 89.7 (CCl_3), 95.0 (C-17), 122.3 (C-4), 127.7 (C-2), 156.4 (C-1), 160.0 ($\underline{CO_2}-CCl_3$), 167.8 ($\underline{CO_2}-CH_2CH_3$), 170.1 (C-5), 186.5 (C-3).
MS: EI: $[M]^{+\bullet}$ 518/520/522 (2.9/3.1/1%), m/z 397/399/401 (5.6/5.5/1.7%), m/z 356 (3.5%), m/z 283 (17%), m/z 265 (29%), m/z 122 (100%), m/z 121 (42%). CI: $[M+ H]^+$ 519/521/523 (47/48/17%), m/z 122 (100%).

3.2.26. Ethyl 17α-formyloxy-11β-hydroxyandrosta-1,4-diene-3-one-17β-carboxylate (**6h**)

Prepared by method A as described for **6a**, starting with **4h**. Yield: 86%, off-white crystalline powder. M.p. 255–257 °C.
^{1}H NMR (250 MHz, $CDCl_3$): δ 1.03 (3 H, s, CH_3-18), 1.46 (3 H, s, CH_3-19), 1.28 (3 H, t, $\underline{CH_3}CH_2O$), 4.19 (2 H, q, $CH_3\underline{CH_2}O$), 2.97 (1 H, m, H_β-16), 4.50 (1 H, m, H_α-11), 6.02 (1 H, d, H-4), 6.28 (1 H, dd, H-2), 7.27 (1 H, d, H-1), 7.90 (1 H, s, HCO_2); ^{13}C NMR (62.7 MHz, $CDCl_3$): 14.2 ($\underline{CH_3}CH_2O$), 16.8 (C-18), 21.0 (C-19), 61.3 ($CH_3\underline{CH_2}O$), 90.8 (C-17), 122.5 (C-4), 127.9 (C-2), 156.0 (C-1), 159.8 (HCO_2), 169.1 (C-5), 169.8 ($\underline{CO_2}-CH_2CH_3$), 186.5 (C-3).
MS: EI: $[M]^{+\bullet}$ 402 (2.8%), m/z 356 (12%), m/z 283 (23%), m/z 281 (10%), m/z 265 (43%), m/z 122 (100%), m/z 121 (61%). CI: $[M + H]^+$ 403 (100%), m/z 122 (66%).

3.2.27. Ethyl 17α-(N,N-dimethyl)oxamoyloxy-11β-hydroxyandrosta-1,4-diene-3-one-17β-carboxylate (**6i**)

Prepared by method A as described for **6a**, starting with **4i**. The crude product was isolated by extraction with ethyl acetate and then purified by column chromatography on silica gel eluting with ethyl acetate – *n*-hexane 4 : 1. Yield in two steps: 32% (calculated upon the starting material **3a**), yellow crystalline powder. M.p. 198–201 °C.
^{1}H NMR (500 MHz, $CDCl_3$): δ 1.05 (3 H, s, CH_3-18), 1.46 (3 H, s, CH_3-19), 1.27 (3 H, t, $\underline{CH_3}CH_2O$), 4.24 (2 H, q, $CH_3\underline{CH_2}O$), 2.97 (1 H, m, H_β-16), 2.96 and 3.03 (3 H, 3 H, s, s, NMe_2), 4.49 (1 H, m, H_α-11), 6.02 (1 H, d, H-4), 6.26 (1 H, dd, H-2), 7.24 (1 H, d, H-1); ^{13}C NMR (125 MHz, $CDCl_3$): 14.2 ($\underline{CH_3}CH_2O$), 16.9 (C-18), 21.1 (C-19), 34.0 and 36.6 (NMe_2), 61.6 ($\underline{CH_3}CH_2O$), 93.4 (C-17), 122.5 (C-4), 127.9 (C-2), 155.8 (C-1), 160.9 and 161.9 (OCO/NCO), 169.0 ($\underline{CO_2}-CH_2\ CH_3$), 169.6 (C-5), 186.4 (C-3).
MS: EI: $[M]^{+\bullet}$ 473 (6%), m/z 356 (8%), m/z 352 (2.1%), m/z 283 (77%), m/z 265 (100%), m/z 122 (25%), m/z 121 (39%). CI: $[M + H]^+$ 474 (100%).

3.2.28. Ethyl 17α-chloroacetoxy-11β-hydroxyandrosta-1,4-diene-3-one-17β-carboxylate (**6j**)

Prepared by method A as described for **6a**, starting with **4j**. Yield: 62%, yellow crystalline powder. M.p. 129–135 °C. NMR showed the presence of ~5% 17α-iodoacetoxy analog.
^{1}H NMR (250 MHz, $CDCl_3$): δ 1.03 (3 H, s, CH_3-18), 1.47 (3 H, s, CH_3-19), 1.27/1.28 (3 H, t, $\underline{CH_3}CH_2O$), 4.20 (2 H, q, $CH_3\underline{CH_2}O$), 2.95 (1 H, m, H_β-16), 3.40/4.02 (2 H, s, CH_2I/CH_2Cl), 4.50 (1 H, m, H_α-11), 6.03 (1 H, d, H-4), 6.28 (1 H, dd, H-2), 7.27 (1 H, d, H-1); ^{13}C NMR (125 MHz, $CDCl_3$): −5.4 (CH_2I), 14.3 ($\underline{CH_3}CH_2O$), 16.9 (C-18), 21.0 (C-19), 40.8 (CH_2Cl), 61.8 ($CH_3\underline{CH_2}O$), 61.4 (CH_2Cl), 91.8/92.2 (C-17), 122.6 (C-4), 128.0 (C-2), 155.9 (C-1), 166.1/167.2 ($\underline{CO_2}-CH_2Cl(I)$), 168.9/169.1 ($\underline{CO_2}-CH_2CH_3$), 169.6 (C-5), 186.5 (C-3).
MS: $[M]^{+\bullet}$ 450/452 (2.2/0.9%), m/z 356 (16%), m/z 329/331 (7.4/2.5%), m/z 283 (24%), m/z 265 (40%), m/z 122 (100%), m/z 121 (53%). CI: $[M + H]^+$ 451/453 (15/6%), m/z 122 (100%).

3.2.29. Methyl 17α-dichloroacetoxy-11β-hydroxyandrosta-1,4-diene-3-one-17β-carboxylate (**6k**)

Prepared by method A as described for **6a** but using methyl iodide instead of ethyl iodide. Yield: 76%, white crystalline powder. M.p. 116–119 °C.
^{1}H NMR (250 MHz, DMSO-d_6): δ 0.97 (3 H, s, CH_3-18), 1.42 (3 H, s, CH_3-19), 2.79 (1 H, m, H_β-16), 3.65 (3 H, s, $\underline{CH_3}O$), 4.23 (1 H, m, H_α-11), 6.89 (1 H, s, $CHCl_2$), 5.95 (1 H, d, H-4), 6.19 (1 H, dd, H-2), 7.33 (1 H, d, H-1).
MS: EI: $[M]^{+\bullet}$ 470/472 (2.9/2.1%), m/z 349/351 (7.9/5.1%), m/z 342 (3%), m/z 283 (12%), m/z 265 (28%), m/z 122 (100%), m/z 121 (44%). CI: $[M + H]^+$ 471/473 (19/13%), m/z 122 (100%).

3.2.30. Ethyl 11β,17α-dihydroxyandrosta-1,4-diene-3-one-17β-carboxylate (**6l**)

Prepared by the esterification procedure (method A) described for **6a**, starting with **3a**. Yield: 45%, white crystalline powder. M.p. 233–238 °C.
^{1}H NMR (250 MHz, DMSO-d_6): δ 0.85 (3 H, s, CH_3-18), 1.40 (3 H, s, CH_3-19), 1.26 (3 H, t, $\underline{CH_3}CH_2O$), 4.10 (2 H, q, $CH_3\underline{CH_2}O$), 4.27 (1 H, m, H_α-11), 5.90 (1 H, d, H-4), 6.15 (1 H, dd, H-2), 7.30 (1 H, d, H-1); ^{13}C NMR (62.7 MHz, DMSO-d_6): 16.7 (C-18), 20.6 (C-19), 14.0 ($\underline{CH_3}CH_2O$), 59.6 ($CH_3\underline{CH_2}O$), 30.8 (C-8), 68.3 (C-11), 84.6 (C-17), 121.3 (C-4), 126.3 (C-2), 156.5 (C-1), 170.3 (C-5), 173.0 ($\underline{CO_2}-CH_2CH_3$), 184.9 (C-3).
MS: EI: $[M]^{+\bullet}$ 374 (22%), m/z 253 (41%), m/z 122 (100%), m/z 121 (90%). CI: $[M + H]^+$ 375 (100%).

3.2.31. Ethyl 17α-dichloroacetoxy-11β-hydroxy-1α,5β-cyclo-10α-androst-3-ene-2-one-17β-carboxylate (**7**)

6a (1.5 g, 3.09 mmol) in dry 1,4-dioxane (280 ml) placed in a quartz vessel was irradiated with a 15 W low-pressure mercury lamp (Lighttech, Dunakeszi, Hungary) at 254 nm for 2 h under a nitrogen atmosphere. Then the solvent was evaporated *in vacuo* and the crude product (1.75 g) was purified by repeated column chromatography on silica gel eluting with ethyl acetate – *n*-hexane 2 : 1. The obtained oil was dissolved in ethyl acetate and precipitated with *n*-hexane to give the solid product. Isolated yield: 0.09 g (6%), white crystalline powder. M.p. 162–164 °C.
^{1}H NMR (500 MHz, $CDCl_3$): δ 0.98 (3 H, s, CH_3-18), 1.29 (3 H, s, CH_3-19), 1.27 (3 H, t, $\underline{CH_3}CH_2O$), 4.21 (2 H, q, $CH_3\underline{CH_2}O$), 2.06 (1 H, m, H_α-6), 2.34 (1 H, s, H-1), 2.35 (1 H, m, H_β-6), 2.96 (1 H, m, H_β-16), 4.46

(1 H, m, H_α-11), 5.90 (1 H, d, H-3), 5.94 (1 H, s, $CHCl_2$), 7.28 (1 H, d, H-4); ^{13}C NMR (125 MHz, $CDCl_3$): 14.0 (C-19), 14.1 ($\underline{CH_3}CH_2O$), 16.9 (C-18), 61.6 ($CH_3\underline{CH_2}O$), 64.4 ($CHCl_2$), 24.5 (C-6), 40.1 (C-1), 53.2 (C-5), 67.9 (C-11), 93.7 (C-17), 131.9 (C-3), 162.8 ($\underline{CO_2}-CHCl_2$), 165.2 (C-4), 168.3 ($\underline{CO_2}-CH_2CH_3$), 206.4 (C-2).
MS: $[M]^{+\bullet}$ 484/486 (27/20%), m/z 456/458 (15/10%, $[M\text{-}CO]^{+\bullet}$), m/z 356 (5%), m/z 283 (41%), m/z 265 (83%), m/z 161 (100% [rings A + B + H]$^+$), m/z 121 (50%). CI: $[M + H]^+$ 485/487 (63/54%), m/z 357 (100%).
CD spectrum (0.4 mg/ml, methanol), λ (Δε): 236 (−6.68631), 282 (5.56626), 339 (−2.90836).

3.2.32. Lumiprednisolone (**8**)

The photorearrangement of **2a** was performed by irradiation with the same low-pressure mercury lamp as in the case of **7** at 254 nm as described (Williams et al. 1980). The NMR spectrum corresponds to that described.
CD spectrum (0.4 mg/ml, methanol), λ (Δε): 236 (−5.90805), 283 (6.66909), 338 (−2.67945).

3.2.33. 3,20-Bis(ethylenedioxy)-17α,21-dihydroxypregn-5-ene-11-one (**9**)

A mixture of **2h** (3.0 g, 8.3 mmol) and ethylene glycol (67.5 ml) was concentrated to a volume of 55 ml *in vacuo* (200 Pa). *p*-Toluenesulfonic acid monohydrate (120 mg) was added and the slow distillation was continued with vigorous stirring for about 2 h. The solid residue was slurried with water (80 ml), filtered off and washed twice with water. This product was used in the next step without further purification. Yield: 3.44 g (92%), beige crystalline powder. M.p. 196–199 °C.

3.2.34. 3,20-Bis(ethylenedioxy)-11α,17α,21-trihydroxypregn-5-ene (**10**)

To a solution of **9** (1 g, 2.23 mmol) in boiling n-propanol (267 ml) metallic sodium (9.86 g, 42.9 mmol) was added in small pieces over 3 h. After dilution with water the solution was extracted with methylene chloride (3 × 350 ml). The combined organic layers were washed with water (2 × 400 ml), dried over magnesium sulfate and concentrated in vacuo. The product was used in the next step without further purification. Yield: 1.0 g (99%), off-white crystalline powder. M.p. 258–260 °C.

3.2.35. Ethyl 17α-hydroxy-11-oxoandrosta-1,4-diene-3-one-17β-carboxylate (**11a**)

Prepared as described for **6l**, starting with **3b**. Yield: 92%, white crystalline powder. M.p. 161–163 °C.
^{1}H NMR (250 MHz, $CDCl_3$): δ 0.75 (3 H, s, CH_3-18), 1.42 (3 H, s, CH_3-19), 1.32 (3 H, t, $\underline{CH_3}CH_2O$), 4.25 (2 H, q, $CH_3\underline{CH_2}O$), 6.10 (1 H, d, H-4), 6.22 (1 H, dd, H-2), 7.63 (1 H, d, H-1); ^{13}C NMR (62.7 MHz, $CDCl_3$): 16.1 (C-18), 18.8 (C-19), 14.2 ($\underline{CH_3}CH_2O$), 62.0 ($CH_3\underline{CH_2}O$), 36.4 (C-8), 84.5 (C-17), 124.7 (C-4), 127.7 (C-2), 154.9 (C-1), 166.2 (C-5), 173.2 ($\underline{CO_2}-CH_2CH_3$), 186.2 (C-3).

3.2.36. Ethyl 17α-hydroxyandrost-4-ene-3-one-17β-carboxylate (**11b**)

Prepared as described for **6l**, starting with **3d**. Yield: 95%, white crystalline powder. M.p. 176–177 °C.
^{1}H NMR (500 MHz, DMSO-d_6): δ 0.62 (3 H, s, CH_3-18), 1.14 (3 H, s, CH_3-19), 1.19 (3 H, t, $\underline{CH_3}CH_2O$), 4.04/4.14 (1H + 1H, m, $CH_3\underline{CH_2}O$), 5.62 (1 H, d, H-4).
MS: EI: $[M]^{+\bullet}$ 360 (100%), m/z 244 (47%), m/z 229 (43%), m/z 124 (21%). CI: $[M + H]^+$ 361 (100%).

3.2.37. Ethyl 17α-hydroxyandrosta-1,4-diene-3-one-17β-carboxylate (**11c**)

Prepared as described for **6d**, starting with **11b**. The crude product was purified by column chromatography on silica gel eluting with methylene chloride – acetone 92.5 : 7.5. Yield: 66%, off-white crystalline powder. M.p. 192–194 °C.
^{1}H NMR (250 MHz, $CDCl_3$): δ 0.78 (3 H, s, CH_3-18), 1.25 (3 H, s, CH_3-19), 1.30 (3 H, t, $\underline{CH_3}CH_2O$), 4.22 (2 H, q, $CH_3\underline{CH_2}O$), 6.09 (1 H, d, H-4), 6.25 (1 H, dd, H-2), 7.05 (1 H, d, H-1); ^{13}C NMR (62.7 MHz, $CDCl_3$): 15.2 (C-18), 18.7 (C-19), 14.3 ($\underline{CH_3}CH_2O$), 61.4 ($CH_3\underline{CH_2}O$), 35.6 (C-8), 85.6 (C-17), 124.0 (C-4), 127.6 (C-2), 155.5 (C-1), 168.8 (C-5), 174.0 ($\underline{CO_2}-CH_2CH_3$), 186.3 (C-3).
MS: EI: $[M]^{+\bullet}$ 358 (27%), m/z 237 (7%), m/z 122 (100%), m/z 121 (60%). CI: $[M + H]^+$ 359 (100%).

3.2.38. Ethyl 17α-dichloroacetoxy-11β-hydroxy-[6-^{2}H]androsta-1,4-diene-3-one-17β-carboxylate (**12a**)

Acidic reagent: ^2H_2O (18 mg) and PCl_5 (5 mg) were dissolved in anhydrous 1,4-dioxane (1 ml).
A solution of **6a** (100 mg) in the acidic reagent (0.5 ml) was stirred at 60 °C for 2 h. After cooling to room temperature the stirred mixture was diluted with H_2O (5 ml). The white precipitate was collected by filtration and washed with water (2 × 1 ml). The obtained white product was dried *in vacuo* until constant weight. The treatment with the above acidic reagent followed by the aqueous work-up were repeated once more. Yield: 82.7 mg, white powder.
The ^{1}H NMR spectrum (500 MHz, $CDCl_3$) was identical with the spectrum of **6a** but due to the ^{2}H labeling of the $H_2C(6)$ methylene group the corresponding signals [2.36 (0.86 H, m, H_α-6) and 2.59 (0.72 H, m, H_β-6)] were of reduced intensity.
MS: EI: $[M]^{+\bullet}$ 484/486 (2.6/1.7%), $[M_d]^{+\bullet}$ 485/487 (2.5/1.6%), m/z 363/365 (11.8/7.5%), m/z 356 (3.3%), m/z 357 (3.2%), m/z 283 (18%), m/z 284 (15%), m/z 265 (30%), m/z 266 (25%), m/z 122 (100%, ions [122] and $[121]_d$), m/z 123 (71%, ion $[122]_d$), m/z 121 (38%).
CI: $[M + H]^+$ 485/487 (97/63%), $[M_d + H]^+$ 486/488 (90/58%), m/z 122 (96%, ions [122] and $[121]_d$), m/z 123 (61%, ion $[122]_d$).

3.2.39. Ethyl 17α-dichloroacetoxy-11β-hydroxy-[6-^{3}H]androsta-1,4-diene-3-one-17β-carboxylate (**12b**)

Prepared as described for **12a**, starting with 100 mg of **6a** and 18 mg of tritiated water (ARC, American Radiolabeled Chemicals, Inc., 11624 Bowling Green Drive, St. Louis, MO 63146 U.S.A., Art. 194A, specific activity: 2146 GBq/ml) in 1 ml of 1,4-dioxane. Yield: 81.5 mg, white powder, molar activity: 184.89 GBq/mmol, radiochemical purity: > 95% by HPLC.
The ^{1}H NMR spectrum (500 MHz, $CDCl_3$) was identical with the spectrum of **6a** but due to the ^{3}H labeling of the $H_2C(6)$ methylene group the corresponding signals [2.36 (0.90 H, m, H_α-6) and 2.59 (0.86 H, m, H_β-6)] were of reduced intensity (6β: 13%, 6α: 7%).

3.2.40. 11β,17α-Dihydroxyandrosta-1,4,6-triene-3-one-17β-carboxylic acid (**15**)

Prepared as described for **3a**, starting with 375 mg of Δ^6-prednisolone (Seibl and Gaumann 1963). Yield: 332 mg (96%), white powder. M.p. 261–265 °C (decomp.).

3.2.41. 17α-Dichloroacetoxy-11β-hydroxyandrosta-1,4,6-triene-3-one-17β-carboxylic acid (**16**)

Prepared as described for **4a,** starting with 323 mg of **15**. Yield: 414 mg (97%), white powder. M.p. 165–170 °C.

3.2.42. Ethyl 17α-dichloroacetoxy-11β-hydroxyandrosta-1,4,6-triene-3-one-17β-carboxylate (**17**)

Prepared as described for **6a,** starting with 414 mg of crude **16**. Yield: 321 mg (74%), white powder.
^{1}H NMR (250 MHz, $CDCl_3$): δ 1.10 (3 H, s, CH_3-18), 1.44 (3 H, s, CH_3-19), 1.27 (3 H, t, $\underline{CH_3}CH_2O$), 4.21 (2 H, q, $CH_3\underline{CH_2}O$), 2.97 (1 H, m, H_β-16), 4.59 (1 H, m, H_α-11), 5.91 (1 H, s, $CHCl_2$), 6.00 (1 H, d, H-4), 6.11 (1 H, dd, H-7), 6.30 (1 H, dd, H-6), 6.31 (1 H, dd, H-2), 7.30 (1 H, d, H-1); ^{13}C NMR (62.5 MHz, $CDCl_3$): 14.1 ($\underline{CH_3}CH_2O$), 16.7 (C-18), 22.9 (C-19), 61.8 ($CH_3\underline{CH_2}O$), 64.2 ($CHCl_2$), 68.8 (C-11), 93.0 (C-17), 122.8 (C-4), 128.0 (C-6), 128.6 (C-2), 152.9 (C-1), 162.8 ($\underline{CO_2}-CHCl_2$), 163.0 (C-5), 168.2 ($\underline{CO_2}-CH_2CH_3$), 186.4 (C-3).

3.2.43. 11β,17α-Dihydroxy-21-acetoxypregn-4-ene-3-one (**18**) *and 11β,17α-dihydroxy-21-acetoxy-(5α and 5β)pregnane-3-one* (**19**)

A stirred mixture of **14b** (40 mg, obtained as described by Angelo and Laubach 1960) and 2% Pd/$SrCO_3$ (8.2 mg) in ethyl acetate (5 ml) was hydrogenated at atmospheric pressure for 20 min. The catalyst was filtered off and the filtrate was evaporated in vacuo. Yield: 32 mg, white powder. Reverse phase HPLC of the crude product showed the presence of unchanged starting material (39%, RRT = 1.00), the expected **18** (42%, RRT = 1.07) and the fully saturated products **19** (total of the 5α and 5β isomers: 19%, $RRT_{5\alpha} = 1.58$, $RRT_{5\beta} = 1.66$). On normal phase silica RRT of the product **18** was 1.12 (with RRT of the starting **14b**: 1.00).

3.2.44. 11β,17α-Dihydroxy-21-acetoxy-(5α and 5β)pregnane-3-one (**19**)

A stirred mixture of **14b** (40 mg) and 2% Pd/$SrCO_3$ (8.2 mg) in ethyl acetate (5 ml) was hydrogenated at atmospheric pressure for 2 h. The catalyst was filtered off and the filtrate was evaporated *in vacuo*. Yield: 34 mg, white powder. The product was a 1 : 1 mixture of the 5α and 5β isomers.
^{1}H NMR (500 MHz, $CDCl_3$): δ 0.95/0.96 (3 H, s, CH_3-18), 1.27 (3 H, s, CH_3-19), 2.18 (3 H, s, $\underline{CH_3}CO$), 4.41/4.46 (1 H, m, H_α-11); ^{13}C NMR (125 MHz, $CDCl_3$): 14.1/26.0 (C-19), 17.2 (C-18), 20.5 ($\underline{CH_3}CO$), 57.2/47.8 (C-9), 68.3 (C-11), 89.9 (C-17), 170.7 ($CH_3\underline{CO}$), 204.8 (C-20), 212.0/212.6 (C-3).
MS: EI: $[M]^{+\bullet}$ 406 (7.5%), m/z 346 (6%), m/z 328 (7%), m/z 287 (17%), m/z 269 (13%), m/z 244 (17%), m/z 229 (16%), m/z 43 (100%).
CI: $[M + H]^+$ 407 (100%), m/z 389 (61%).

3.2.45. Ethyl 17α-dichloroacetoxy-6α,11β-dihydroxyandrosta-1,4-diene-3-one-17β-carboxylate (**20a**)

Selenium dioxide (4.0 g, 36.05 mmol) was added to a solution of **6a** (5.0 g, 11.82 mmol) in 1,4-dioxane (500 ml). The obtained clear solution was stirred under reflux for 12 h. The precipitated black solids were filtered off and the filtrate was evaporated *in vacuo*. The obtained brownish residue was purified by column chromatography on silica gel eluting with methylene chloride-methanol 20 : 1. Yield: 19.4%, m.p. 129–134 °C.
^{1}H NMR (500 MHz, $CDCl_3$): δ 1.06 (3 H, s, CH_3-18), 1.45 (3 H, s, CH_3-19), 1.28 (3 H, t, $\underline{CH_3}CH_2O$), 4.22 (2 H, q, $CH_3\underline{CH_2}O$), 2.98 (1 H, m, H_β-16), 4.53 (1 H, m, H_α-11), 4.61 (1 H, m, H_β-6), 5.90 (1 H, s, $CHCl_2$), 6.31 (1 H, dd, H-2), 6.42 (1 H, d, H-4), 7.23 (1 H, d, H-1); ^{13}C NMR (125 MHz, $CDCl_3$): 14.1 ($\underline{CH_3}CH_2O$), 16.9 (C-18), 21.4 (C-19), 61.7 ($CH_3\underline{CH_2}O$), 64.3 ($CHCl_2$), 67.6 (C-6), 69.8 (C-11), 93.3 (C-17), 118.5 (C-4), 128.2 (C-2), 155.3 (C-1), 162.8 ($\underline{CO_2}-CHCl_2$), 170.2 (C-5), 168.2 ($\underline{CO_2}-CH_2CH_3$), 186.1 (C-3).
MS: EI: $[M]^{+\bullet}$ 500/502 (22/14%), m/z 482/484 (19/13%), m/z 299 (43%), m/z 281 (81%), m/z 147 (62%), m/z 138 (44%), m/z 109 (100%). CI: $[M + H]^+$ 501/503 (96/66%), m/z 373 (100%).

3.2.46. Ethyl 17α-dichloroacetoxy-6β,11β-dihydroxyandrosta-1,4-diene-3-one-17β-carboxylate (**20b**)

Microbial hydroxylation was carried out using the strain No. IDR 620 of the *Absidia coerulea* species. The strain was maintained on slants of malt extract-yeast extract medium (malt extract 1%, yeast extract 0.4%, glucose 0.4% and agar 2% in distilled water). After 7 days of cultivation at 28 °C the spores were washed from the slant culture with 5 ml of sterile distilled water. The obtained spore suspension was used for the inoculation of 100 ml of fermentation medium B1 (peptone 0.8%, glucose 1.8%, malt extract 0.8%, corn steep liquor 0.2% and NaCl 0.2%) in an Erlenmeyer flask. The culture was incubated at 25 °C for 3 days on a rotary shaker operating at 250 rpm. Five milliliters of the seed culture were used for the inoculation of 100 ml of the fermentation medium B1 in an Erlenmeyer flask. The culture was incubated at 25 °C for 3 days on a rotary shaker and then the substrate (**6a**, 100 mg/l) was added to the culture in the form of 10 mg/ml solutions in ethanol. After further 3 days of cultivation the hydroxylation was finished and the product was isolated from the fermentation broth.
To this end the fermentation broth was extracted twice with ethyl acetate. The extract was concentrated and *n*-hexane was added to the residue. After stirring at room temperature the solid material, which contained the 6β-hydroxy product, was filtered off and dried. This crude product was purified by column chromatography on silica gel with gradient elution. Step gradient of acetone in *n*-hexane or methanol in methylene chloride were used as eluents. Pure product was obtained by crystallization from ethyl acetate – *n*-hexane. In this manner 114 mg of **20b** were obtained from 10 liters of fermentation broth.
^{1}H NMR (500 MHz, $CDCl_3$): δ 1.09 (3 H, s, CH_3-18), 1.69 (3 H, s, CH_3-19), 1.28 (3 H, t, $\underline{CH_3}CH_2O$), 4.23 (2 H, q, $CH_3\underline{CH_2}O$), 2.99 (1 H, m, H_β-16), 4.56 (1 H, m, H_α-6), 4.56 (1 H, m, H_α-11), 5.90 (1 H, s, $CHCl_2$), 6.14 (1 H, d, H-4), 6.28 (1 H, dd, H-2), 7.26 (1 H, d, H-1).
MS: EI: $[M]^{+\bullet}$ 500/502 (12/8%), m/z 482/484 (21/14%), m/z 299 (40%), m/z 281 (100%), m/z 147 (82%), m/z 138 (98%), m/z 121 (83%). CI: $[M + H]^+$ 501/503 (95/64%), m/z 373 (100%).

3.2.47. Ethyl 6α,11β,17α-trihydroxyandrosta-1,4-diene-3-one-17β-carboxylate (**21a**)

Prepared as described for **20a**, starting with **6l**. Yield: 16.5%, m.p. 132–137 °C.
^{1}H NMR (500 MHz, $CDCl_3$): δ 1.02 (3 H, s, CH_3-18), 1.44 (3 H, s, CH_3-19), 1.33 (3 H, t, $\underline{CH_3}CH_2O$), 4.25 (2 H, q, $CH_3\underline{CH_2}O$), 2.70 (1 H, m, H_β-16), 4.51 (1 H, m, H_α-11), 4.59 (1 H, m, H_β-6), 6.29 (1 H, dd, H-2), 6.40 (1 H, d, H-4), 7.22 (1 H, d, H-1); ^{13}C NMR (125 MHz, $CDCl_3$): 14.3 ($\underline{CH_3}CH_2O$), 17.6 (C-18), 21.4 (C-19), 61.6 ($CH_3\underline{CH_2}O$), 67.8 (C-6), 70.1 (C-11), 85.5 (C-17), 118.4 (C-4), 128.0 (C-2), 155.5 (C-1), 170.5 (C-5), 174.1 ($\underline{CO_2}-CH_2CH_3$), 186.2 (C-3).
MS: EI: $[M]^{+\bullet}$ 390 (24%), m/z 372 (17%), m/z 147 (56%), m/z 138 (40%), m/z 121 (100%). CI: $[M + H]^+$ 391 (100%).

3.2.48. Ethyl 6β,11β,17α-trihydroxyandrosta-1,4-diene-3-one-17β-carboxylate (**21b**)

Prepared as described for **20b**, starting with **6l**. 416 mg of **21b** were obtained from 10 liters of fermentation broth.
^{1}H NMR (500 MHz, $CDCl_3$): δ 1.05 (3 H, s, CH_3-18), 1.67 (3 H, s, CH_3-19), 1.33 (3 H, t, $\underline{CH_3}CH_2O$), 4.24 (2 H, q, $CH_3\underline{CH_2}O$), 2.70 (1 H, m, H_β-16), 4.54 (1 H, m, H_α-11), 4.54 (1 H, m, H_α-6), 6.12 (1 H, d, H-4), 6.26 (1 H, dd, H-2), 7.26 (1 H, d, H-1); ^{13}C NMR (125 MHz, $CDCl_3$): 14.3 ($\underline{CH_3}CH_2O$), 17.6 (C-18), 22.7 (C-19), 61.1 ($CH_3\underline{CH_2}O$), 70.1 (C-11), 73.5 (C-6), 85.5 (C-17), 124.9 (C-4), 127.0 (C-2), 157.3 (C-1), 166.4 (C-5), 174.3 ($\underline{CO_2}-CH_2CH_3$), 186.7 (C-3).
MS: EI: $[M]^{+\bullet}$ 390 (46%), m/z 372 (22%), m/z 147 (54%), m/z 138 (29%), m/z 121 (100%). CI: $[M + H]^+$ 391 (100%).

3.2.49. 17α-Dichloroacetoxy-6α,11β-dihydroxyandrosta-1,4-diene-3-one-17β-carboxylic acid (**22a**) *and 17α-Dichloroacetoxy-6β,11β-dihydroxyandrosta-1,4-diene-3-one-17β-carboxylic acid* (**22b**)

Prepared as described for **20a**, starting with **4a** with the difference that the crude product was taken up in water (150 ml) and saturated sodium bicarbonate solution was added until the semisolid residue was dissolved. The solution was acidified under stirring with 20% aqueous hydrochloric acid to pH = 1–2. The precipitated solids were collected, washed with water and dried in a vacuum oven at 50 °C. Yield: 86.7%, yellowish powder. An analytical sample was prepared by repeated column chromatography on silica gel eluting with a 100 : 15 : 0.2 mixture of methylene chloride, methanol and acetic acid. In this manner an 86 : 14 mixture of the title compounds was obtained. M.p. 205–208 °C.
^{1}H NMR (500 MHz, CD_3OD): δ 1.12 (3 H, s, CH_3-18), 1.48 (3 H, s, CH_3-19), 2.90 (1 H, m, H_β-16), 4.43 (1 H, m, H_α-11), 4.57 (1 H, m, H_β-6), 6.27 (1 H, dd, H-2), 6.36 (1 H, d, H-4), 6.42 (1 H, s, $CHCl_2$), 7.43 (1 H, d, H-1); ^{13}C NMR (125 MHz, CD_3OD): 17.4 (C-18), 22.0 (C-19), 66.0 ($CHCl_2$), 68.4 (C-6), 70.1 (C-11), 95.0 (C-17), 118.8 (C-4), 128.1 (C-2), 159.5 (C-1), 164.8 ($\underline{CO_2}-CHCl_2$), 169.5 (C-5), 175.4 (CO_2H), 188.9 (C-3).

3.2.50. 6α,11β,17α-Trihydroxyandrosta-1,4-diene-3-one-17β-carboxylic acid (**23a**)

Prepared as described for **22a**, starting with **3a**. Yield: 53.9%, m.p. 178–181 °C.
^{1}H NMR (500 MHz, DMSO-d_6): δ 0.91 (3 H, s, CH_3-18), 1.36 (3 H, s, CH_3-19), 2.50 (1 H, m, H_β-16), 4.27 (1 H, m, H_α-11), 4.40 (1 H, m, H_β-6), 6.16 (1 H, dd, H-2), 6.16 (1H, d, H-4), 7.26 (1 H, d, H-1), 12.25 (1 H, broad, COOH); ^{13}C NMR (125 MHz, DMSO-d_6): 17.0 (C-18), 21.2 (C-19), 66.1 (C-6), 68.4 (C-11), 84.7 (C-17), 117.5 (C-4), 127.0 (C-2), 156.4 (C-1), 172.5 (C-5), 174.9 (CO_2H), 185.0 (C-3).
MS: EI: $[M]^{+\bullet}$ 362 (10%), m/z 344 (8%), m/z 300 (52%), m/z 137 (36%), m/z 109 (100%). CI: $[M + H]^+$ 363 (50%), m/z 301 (100%).

3.2.51. 6β,11β,17α-Trihydroxyandrosta-1,4-diene-3-one-17β-carboxylic acid (**23b**)

To **21b** (50 mg, 0.133 mmol) in water (1 ml) at room temperature was added a 1 M solution of sodium hydroxyde (1 ml) and the reaction mixture was stirred overnight. The obtained clear solution was acidified with 20% hydrochloric acid to pH = 1 and extracted with ethyl acetate. The combined organic layers were dried over magnesium sulfate and evaporated in vacuo. Yield: 62%, m.p. 170–190 °C (decomp.).
^{1}H NMR (500 MHz, CD_3OD): δ 1.08 (3H, s, CH_3-18), 1.69 (3 H, s, CH_3-19), 2.69 (1 H, m, H_β-16), 4.45 (1 H, m, H_α-11), 4.45 (1 H, m, H_α-6), 6.10 (1 H, d, H-4), 6.24 (1 H, dd, H-4), 7.46 (1 H, d, H-1); ^{13}C NMR (125 MHz, CD_3OD): 18.0 (C-18), 23.1 (C-19), 70.8 (C-6), 74.3 (C-11), 87.1 (C-17), 124.9 (C-4), 127.1 (C-2), 161.5 (C-1), 171.4 (C-5), 177.1 (CO_2H), 189.5 (C-3).

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