Russian Chemical Reviews **67** (11) 975 – 998 (1998)
© 1998 Russian Academy of Sciences and Turpion Ltd

UDC 547.577.17.591.147

Substituted androstanes as aromatase inhibitors

I S Levina

Contents

I.	Introduction	975
II.	Classification of steroid aromatase inhibitors and methods for assessment of their activities *in vitro* and *in vivo*	976
III.	Methods for the synthesis of aromatase inhibitors of the androstane series and their activities	976
IV.	Structure – activity relationships in substituted androstanes as aromatase inhibitors	995
V.	*In vivo* activities and applications of major steroid aromatase inhibitors	995
VI.	Conclusion	996

Abstract. The synthesis and structure – activity relationships of inhibitors of steroid aromatase which catalyses the last stage of a multistep biotransformation of cholesterol into estrogens, *viz*., aromatisation of C_{19}-steroids into C_{18}-phenolic steroids, are discussed. Compounds of the androstane series which are structurally related to the natural substrate, *viz*., androst-4-ene-3,17-dione, are the subjects of consideration. The review encompasses problems of synthesis of various substituted androstanes and their aromatase-inhibiting activities and structural requirements for selective specific aromatase inhibitors based on *in vitro* and *in vivo* structure – activity studies of compounds synthesised, their biological properties and the results of clinical trials. Special attention is paid to practical applications of aromatase inhibitors in the treatment of hormone-dependent mammary and ovarian tumours as well as benign prostatic tumours. In writing this report, the author has used all the information currently available in the chemical, biochemical, endocrinological and medicinal literature as well as in patents. The bibliography includes 173 references.

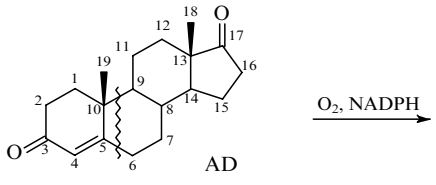
I. Introduction

The search for selective and specific therapeutic steroid agents in the past few decades has given the main impetus to the development of synthesis of modified steroids. It is commonly known that many physiological processes occurring in the organism are controlled by a relatively small set of steroid hormones produced upon cholesterol biosynthesis. These compounds represent small hydrophobic molecules which penetrate easily through membranes and interact with their specific receptors thus regulating gene transcription. One most important factor that stimulated studies of steroids was the discovery that analogues of some endogenous hormones can be used for controlling the synthesis and modifications of steroids involved in proliferation of malignant tumours.

In many clinical situations, estrogens produced in normal or excess quantities play a prominent role in the pathogenesis of various diseases. Therefore, suppression of estrogen biosynthesis is one of the ways to achieve progress in the treatment of such diseases as mammary and ovarian tumours, endometriosis, *etc*.[1–4] The main efforts in the design of therapeutic preparations of the androstane series which are aromatase (estrogen synthase) inhibitors in their pharmacological effect have been directed at the synthesis of drugs against hormone-dependent mammarian tumours. This approach has indisputable advantages over cytotoxic therapy which often causes serious complications.[3,5]

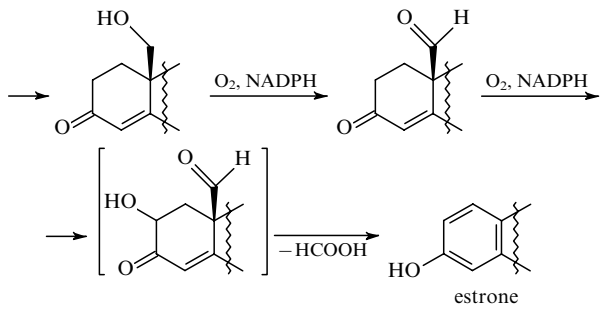
At present, mammary tumours represent the most widespread type of malignant neoplasms and are the second major cause of mortality in women. In the USA alone, casuality of this disease amounts to about 130 000 annually.[6] About a half of these malignant tumours require a source of estrogens for their growth and development. The present-day endocrine therapy relies upon the use of either antiestrogens which act on tumour cells directly, *via* their specific receptors, or high doses of progestins the mechanism of action of which has not been finally established. An alternative strategy consists in the application of aromatase inhibitors (AI) which suppress production of estrogens in the organism.

Aromatase represents an enzyme complex made up of two major components: a flavoprotein (NADPH-cytochrome P-450 reductase) which transfers electrons from NADPH to the terminal enzyme and a specific form of cytochrome P-450 commonly known as aromatase cytochrome P-450. This protein is involved in the binding of the C(19) atom of the steroid substrate and catalyses the multistep reaction resulting in the aromatisation of the ring A of the steroid.[7–9] The conversion of Δ^4-3-ketoandrogens into phenolic estrogens is the ultimate stage in the multienzyme transformation of cholesterol into female sex hormones. Although the details of this mechanism are still questionable, it is believed[10,11] that aromatase performs three successive steps of the androgen oxidation. The first two steps yield 19-hydroxy- and 19-oxo-androgens. However, the site of incorporation of the third hydroxy group is not established. Presumably, hydroxylation occurs at C(2),[12] which results in aromatisation of the ring A of androst-4-ene-3,17-dione (AD). The loss of the angular C(19)-methyl group and *cis*-elimination of the hydrogen atoms from positions 1 and 2 give estrone and formic acid.[3,12–19]



I S Levina N D Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninskii prosp. 47, 117913 Moscow, Russian Federation. Fax (7-095) 135 53 28. Tel. (7-095) 938 36 14

Received 3 October 1997
Uspekhi Khimii **67** (11) 1068 – 1093 (1998); translated by R L Birnova



The enzymic oxidation described above yields an enzyme-bound derivative, which in turn gives the aromatic steroid by virtue of rapid elimination and simultaneous recovery of the unchanged, active enzyme. It is obvious that various structural modifications of the natural substrate (AD) can alter this elimination in such a way that the steroid intermediate will remain covalently bound with the enzyme rather than undergo conversion into the aromatic steroid. Elucidation of the mechanism of androgen aromatisation and demonstration of its possible inhibition has led to successful design and development of a broad range of AI.

Since aromatisation is a unique reaction of steroid biosynthesis and estrogens are its final products, *in vivo* inhibition of aromatase does not affect other essential enzymes involved in this process. This is an important advantage of AI over other inhibitors recommended for therapeutic use (see Refs 4, 20).

Studies of the mechanism of aromatisation of the natural substrate (AD) have specified the type of its structural modification required for the design of AI. It consists in the introduction of substituents into the ring A or at adjacent positions with simultaneous preservation, in the majority of cases, of polar functional groups at C(3) and C(17). The sites for substitution have been identified as C(1), C(4), C(6), C(7), and C(19). The nature of the substituents varies widely depending on the substitution site.

Syntheses of AI often pose serious problems and are as such a considerable progress in both the design of synthetic approaches and their practical implementation. In each particular case, the synthesis of steroid AI employs original approaches which are based on modern methods of synthetic organic chemistry or a combination of several methods. Syntheses of structures with 2,19-bridges or a series of androsta-4,9(11)-diene-3,17-diones carrying a sulfur-containing group at C(10) (Section III.1) provide illustrative examples. Stereochemical aspects of the reactions are very important; modern physicochemical methods are employed for establishing the structure and stereochemistry of the reaction products.

From the standpoint of their chemical structure, AI can be classified according to the site of substitution in the AD molecule. It is this principle that is used in the description of AI syntheses in Section III of the present review. However, it should be remembered that classification of AI according to the mechanism of their action may be different. In this review, this classification was used to analyse the relationships between the structure of AI and their inhibitory activity.

The reviews on steroid AI that cover simultaneously the problems of their synthesis, relationships between their structures and biological properties and pharmacological data are virtually absent. One can mention the book by Zeelen 'Medicinal Chemistry of Steroids',[20] which includes the analysis of steroid and nonsteroid AI, as well as the surveys[9, 15, 21] devoted predominantly to the mechanisms of action of AI and their inhibitory activity *in vitro*.

The present review describes the methods for the synthesis of substituted androstanes active as aromatase inhibitors, the relationships between the structure of AI and the extent of their binding with the enzyme as a criterion for directed search for selective AI, as well as biological properties and practical applications of the most potent steroid preparations currently available.

II. Classification of steroid aromatase inhibitors and methods for assessment of their activities *in vitro* and *in vivo*

Steroid AI have come under the scrutiny of science with the publication of the pioneering work by Brodie *et al.*[22] in 1973. In the subsequent two decades, a vast variety of steroid compounds have been synthesised and assayed for AI activity.

In vitro inhibition of human placenta aromatase is the most common procedure used in the study of modified androgens.[23] This method is used primarily for testing steroids as candidates for AI. The inhibition is evaluated by several parameters. The most important of them are the inhibition constant, K_i, which is determined in experiments where both AI and a steroid substrate (AD) are introduced, and the concentration of AI that results in 50% inhibition (IC_{50}). The analysis of inhibition kinetics, which allows determination of the type of inhibitory activity for the given steroid compound, is also important.

The following methods are used for *in vivo* studies of AI: (1) inhibition of ovarian estrogen secretion; (2) inhibition of peripheral (extraovarian) aromatisation which is the main source of estrogens in post-menopausal women; (3) evaluation of the antitumour effect of AI in an experimental model of a 7,12-dimethylbenzo[*a*]anthracene (DMBA)-induced mammary tumour.

According to the mechanism of their action, AI are classified into reversible and irreversible ones. Compounds which, like usual androgenic substrates, reversibly bind with the active centre of aromatase to form a complex that does not undergo rapid dissociation can serve as rather efficient AI. Competitive aromatase inhibitors that are capable of binding with iron of the cytochrome P-450 heme (*e.g.*, due to the presence of a hetero atom with a specific stereochemical environment in their molecule) can sometimes manifest inhibitory activities both *in vitro* and *in vivo*. A great number of steroid AI are irreversible. Irreversible inhibition of the enzyme implies the conversion of a steroid substrate into an active complex which can irreversibly bind with the enzyme. These compounds are also referred to as 'suicide inhibitors'.[9, 24–26] The latter have a number of salient advantages over competitive reversible AI owing to their high specificity and the ability for irreversible inhibition. Thus, irreversible AI produce long-term inactivation of aromatase *in vivo*.

III. Methods for the synthesis of aromatase inhibitors of the androstane series and their activities

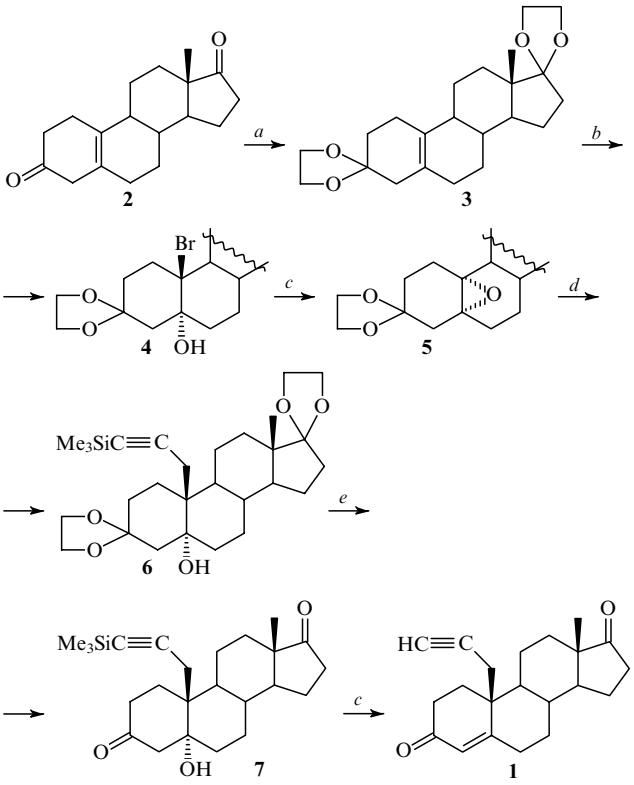
1. 19-Substituted androstanes

19-Substituted androstanes are the most numerous and diverse aromatase inhibitors. It is quite natural because many investigators consider the angular 19-methyl group to be the site of the primary enzyme attack leading to aromatisation.

Formally, the functionalisation of the 19-methyl group of the steroid molecule is a very difficult task. This class of steroids are synthesised mostly from compounds having a 5(10) double bond or from 19-hydroxy(oxo)androstanes using diverse methods of synthetic organic chemistry for their further transformations, such as epoxidation followed by oxirane ring opening with appropriate reagents; substitution of 19-mesyl(tosyl)oxy- and 19-halogeno-derivatives; fluorination; the Wittig, Corey, and Mitsunobu ethynylations, and other sophisticated approaches. All the known methods for the synthesis of 19-substituted androstanes are discussed below in detail.

10β-Propynyl-steroids (19-ethynylandrostanes) synthesised in the 80's simultaneously and independently in several laboratories[14, 27–31] pertain to irreversible suicide inhibitors. Some 19-ethynyl-steroids of the androstane series have been described in patents.[32–35]

19-Ethynylandrost-4-ene-3,17-dione **1** was obtained from commercially available estr-5(10)-ene-3,17-dione **2**. Diketal **3** formed upon protection of the carbonyl groups reacted with *N*-bromosuccinimide to give the bromohydrin **4** which was easily converted into the 5α,10α-epoxide **5**. Epoxide opening with an alkynylcuprate reagent gave the 5α,10β-disubstituted derivative **6** in 60% yield. Deprotection and elimination of the hydroxy group from position 5 of the 3,17-diketone **7** yielded the target androstane **1** in 23% yield starting from the oxirane **5**.[14]

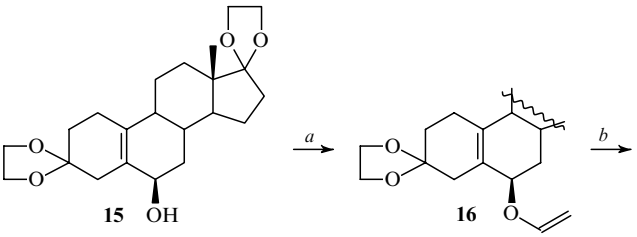


(*a*) $HO(CH_2)_2OH$, H^+, PhH; (*b*) *N*-bromosuccinimide (NBS), DMF; (*c*) NaOMe, MeOH; (*d*) $[Me_3SiC{\equiv}CCH_2]_2CuLi$, Et_2O; (*e*) H^+, Me_2CO.

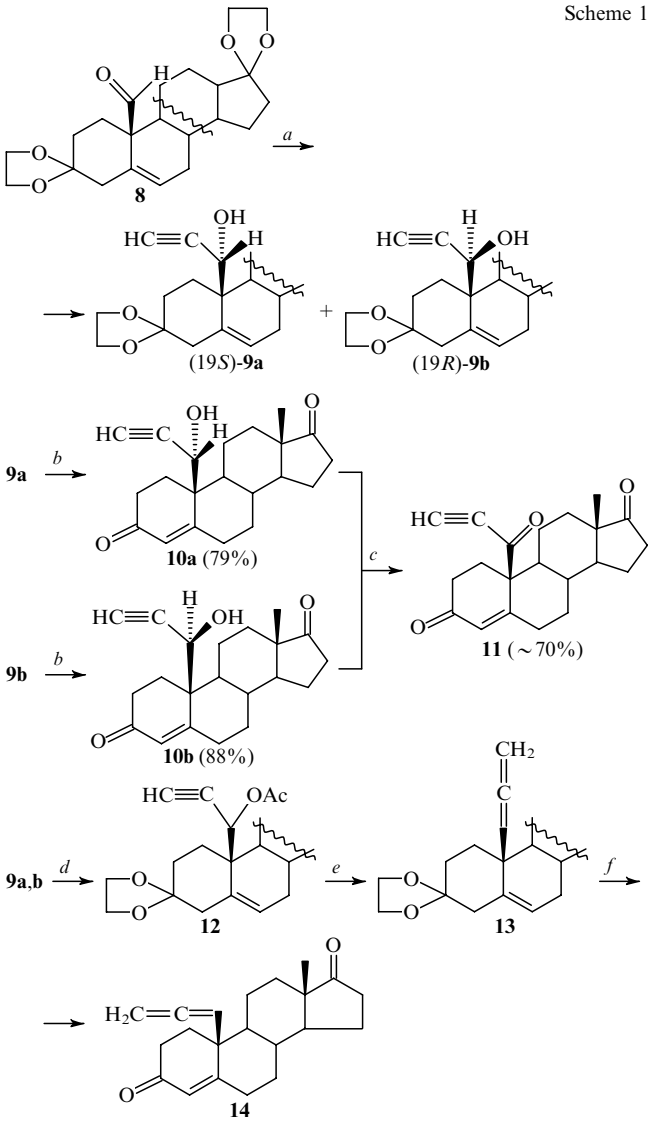
Structurally related diastereomeric 19-ethynyl-19-hydroxy derivatives **10a**,**b** were obtained from the bisketal-aldehyde **8**. The latter reacted with ethynylmagnesium bromide to give a chromatographically separable mixture of (19*S*)-**9a** and (19*R*)-**9b** derivatives in a 3 : 2 ratio (> 90% yield). The authors [27] ascribed the (19*S*)-configuration to the main product by analogy with the stereoselectivity of the reaction of the diketal-aldehyde **8** with methyllithium. After removal of the ketal protection, the diketones **10a**,**b** were obtained in the individual state and further oxidised into the triketone **11** [27] (Scheme 1).

The acetate **12** prepared from 19-ethynyl-19-hydroxy-steroids **9a**,**b** served as the starting compound in the synthesis of an analogous allenic AI (**14**) [29] based on cuprate-initiated reductive elimination of acetic acid. The allene **13** was converted into the final product **14** after deprotection (see Scheme 1).

Yoshida *et al.*[29] have also developed an original procedure for the synthesis of 19-ethynyl-AD **1** using 6β-hydroxyestrenedione bisketal **15** as the starting compound. The vinyl ether **16** obtained

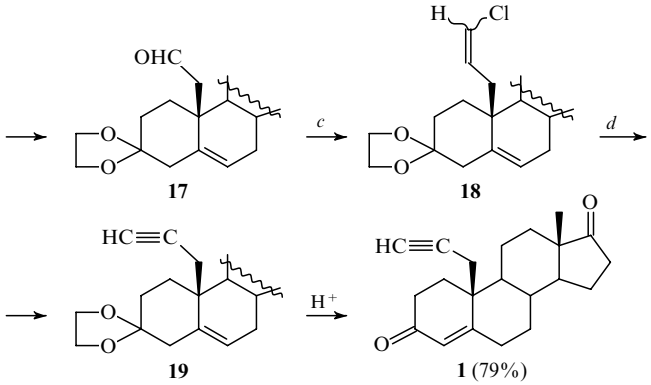


Scheme 1



(*a*) $HC{\equiv}CMgBr$, Δ, THF, 2 h, 94%; (*b*) 10% H_2SO_4, Me_2CO; (*c*) CrO_3, H_2SO_4; (*d*) Ac_2O, Py; (*e*) MeLi, CuI, Et_2O, −78 °C, 50%; (*f*) *p*-TsOH, Me_2CO, 70%.

from it was subjected to the Claisen rearrangement (66% yield). The Wittig reaction of the aldehyde **17** with chloromethylenephosphorane gave a mixture of *cis*- and *trans*-vinyl chlorides **18**, which were dehydrochlorinated with lithium diisopropylamide at

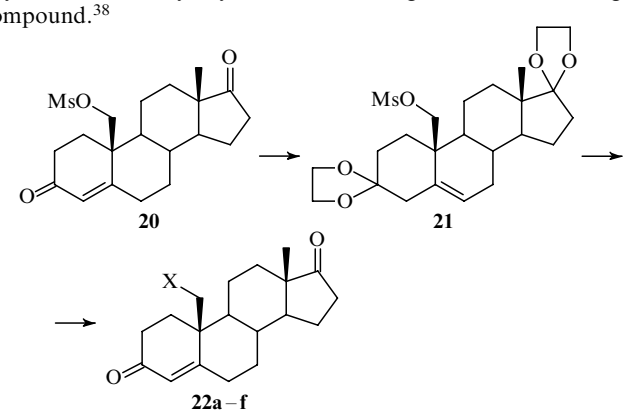


(*a*) $H_2C{=}CHOEt$, $Hg(OAc)_2$, 78%; (*b*) collidine, 165 °C, 4 h, 66%; (*c*) $ClCH{=}PPh_3$, 84%; (*d*) lithium diisopropylamide (LDA), −70 °C, 1 h, 95%.

−70 °C to yield the ethynyl derivative **19** in 95% yield. Removal of the ketal protection gave the final product, *viz*., 19-ethynyl-AD **1**, in high yield.

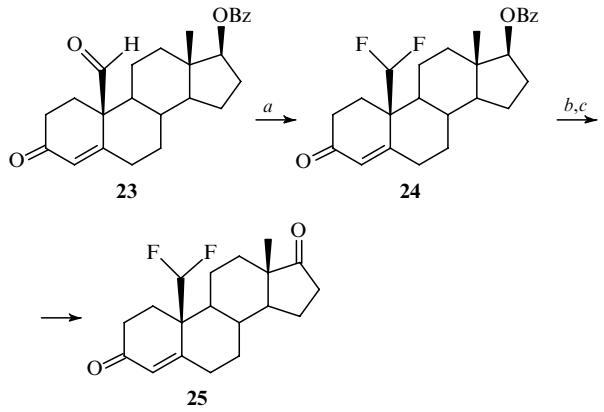
The steroid **1** and its derivatives have been extensively assayed as AI. It was found that 19-ethynyl-AD **1** and its 19-hydroxy- (**10a**,**b**) and 19-oxo-derivatives (**11**) are irreversible suicide inhibitors of aromatase and manifest high specificity [14, 15, 26, 28, 29, 31, 36] (the data on their inhibitory activities are summarised in Table 1). The steroid **1** possessed high activity on oral administration with respect to the inhibition of ovarian aromatase. It is noteworthy that 19-ethynyl-17-propionyloxyandrost-4-ene-3-one manifests only *in vivo* inhibitory activity.[37] The allenic derivative AD **14** also pertains to irreversible AI and has rather high K_i value.[29]

Several 19-halogeno-substituted androstanes **22** were obtained from the bisketal **21** by substitution of 19-methanesulfonates, which is well known in the series of Δ^5-3-acetoxysteroids; 19-mesyloxy diketone **20** being used as the starting compound.[38]



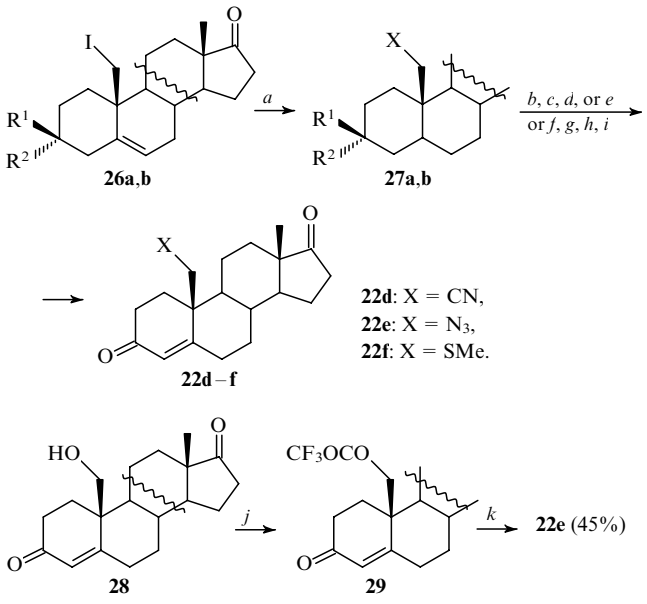
X = Cl (**a**), Br (**b**), I (**c**), CN (**d**), N_3 (**e**), SMe (**f**).

19-Difluoroandrostane **25** was obtained by selective fluorination of 17-benzoyloxy-3-oxoestr-4-en-19-al (**23**) with an excess of diethylaminosulfur trifluoride in a mixture of dichloromethane and trichlorofluoromethane. Removal of the benzoyl protection and oxidation of the resulting alcohol gave 19-difluoro-AD (**25**) which manifested moderate inhibitory activity [39, 40] (see Table 1).



(*a*) excess Et_2NSF_3 (DAST), CH_2Cl_2–$FCCl_3$ (1 : 1), 20 °C, 48 h; (*b*) OH^-; (*c*) CrO_3, H_2SO_4.

The synthesis of androstanes with functional groups at position 19 containing sulfur or nitrogen exploited the ability of 19-iodo derivatives **26a**,**b** for nucleophilic substitution without homoallylic rearrangement.[41] This approach was used to obtain a series of 19-substituted androstanes **27a**,**b** and **22d**–**f**. It is appropriate to mention in this connection the synthesis of 19-azido-AD **22e** described in a patent.[42] Compound **22e** was obtained by esterification of 19-hydroxy-AD **28** with trifluoroacetic anhydride with subsequent treatment of the ester **29** with sodium azide in DMF.



R^1 = OAc, R^2 = H (**a**); R^1, R^2 = $O(CH_2)_2O$ (**b**); X = CN, N_3, SSO_2Me; (*a*) NaCN (X = CN); NaN_3, HMPA (X = N_3); NH_4SSO_2Me (X = SSO_2Me); (*b*) KOH, MeOH; (*c*) CrO_3, H_2SO_4; (*d*) $Al(OPr^i)_3$, cyclohexanone (CHex) (**27a**, X = CN, N_3); (*e*) $(COOH)_2$, EtOH (**27b**, X = CN, N_3); (*f*) LAH, THF; (*g*) MeI; (*h*) K_2CO_3; (*i*) $Al(OPr^i)_3$, CHex (**27a**, X = SSO_2Me); (*j*) $(CF_3CO)_2O$, Py; (*k*) NaN_3, DMF.

19-Substituted AD **22d**–**f** competitively inhibit aromatase.[38, 42] Of special interest is the 19-methylthio derivative **22f** which is the first highly efficient AI (K_i = 1 nM); its inhibitory effect is determined not only by its interaction with the steroid-binding site of aromatase but also by coordination of the steroid sulfur atom with the heme iron of cytochrome P-450.[41]

The attention of several research groups was focussed on 10β- and 19-sulfur-containing AD derivatives as aromatase inhibitors. Compounds with the sulfur atom linked directly with C(10) or separated from the C(10) atom by one or several methylene groups have been synthesised and assayed. Thus the introduction of the SH group at position 10 of estr-4-ene-3,17-dione resulted in an efficient suicide AI **32**. The opening of the 5α,10α-epoxide ring of estrane **5** with potassium sulfide in ethylene glycol in the presence of 18-crown-6 gave 5α,10β-disubstituted estrane **30** in 70% yield. The latter was converted in two steps into 10β-mercaptoestr-4-ene-3,17-dione (**32**) *via* 5α-hydroxy diketone **31**. The yield of the final product was 10% with respect to estr-5(10)-ene-3,17-dione **2**.[14] The corresponding 17β-hydroxy derivative possessing aromatase-inhibiting activity was synthesised by Bednarski and Nelson [14] following an analogous scheme.[43] *In vitro* studies of the biological activity of the thiol **32** revealed that the free thiol group is required for the binding to the enzyme, because the corresponding acetylthio derivative was inactive.[19, 43]

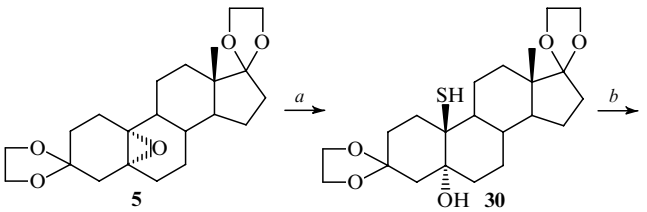
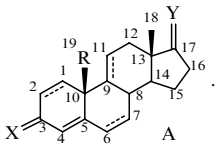


Table 1. Data on *in vitro* inhibition of aromatase from human placenta by 19-substituted androstanes .

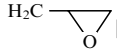
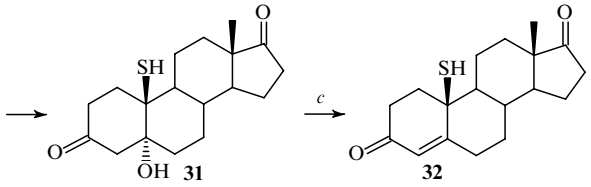
R	X	Y	The position of a double bond [a]			K_i /nM	IC_{50} /nM	Ref.
			Δ^1	Δ^6	$\Delta^{9(11)}$			
Me (ADD)	O	O	+	–	–	320		24
Me (**114**)	O	O	+	+	–	180		171
Me (**183**)	O	O	–	+	–	42		24
Me	H_2	O	–	–	–	37		152, 145
Me	O	H_2	+	–	–	1100		172
Et	O	O	–	–	–	9		15
$(CH_2)_2Me$	O	O	–	–	–	3000		15
$CH=CH_2$	O	O	–	–	–	3		15, 30
$CH_2-C\equiv CH$ (**1**)	O	O	–	–	–	4.3		31
						23		28
						68		15
							31.3	9
$CH_2-CH=CH_2$	O	O	–	–	–	13		31
						56		30
$C\equiv CMe$	O	O	–	–	–	–		31
$CH_2C\equiv CMe$	O	O	–	–	–	26		31
$CH(OH)C\equiv CH$ (**10**)	O	O	–	–	–	49		31
$COC\equiv CH$ (**11**)	O	O	–	–	–	350		31
$CH=C=CH_2$ (**14**)	O	O	–	–	–	14		31
Me	CH_2	O	–	–	–	13		148
CH_2OH	CH_2	O	–	–	–	4.7		148
CHO	CH_2	O	–	–	–	24		94, 148
CHF_2 (**25**)	O	O	–	–	–	1000	1300	15, 40
CH_2N_3 (**22e**)	O	O	–	–	–	22.5		31, 41
SH	O	OH	–	–	–	106		43
CH_2SH (**35**)	O	O	–	–	–	34		43
CH_2SMe (**22f**)	O	O	–	–	–	1	42	41, 46
$(CH_2)_2SMe$ (**55**)	O	O	–	–	–	0.5	22	49, 46
$(CH_2)_2SMe$ (**40a**)	O	O	–	–	+		12	46
$(CH_2)_2SEt$ (**40b**)	O	O	–	–	+		870	46
$(CH_2)_3SMe$ (**51**)	O	O	–	–	+		10000	46
$(CH_2)_2SAc$ (**40c**)	O	O	–	–	+		10000	46
$(CH_2)_2SPh$ (**40d**)	O	O	–	–	+		400	46
$(CH_2)_2S(CH_2)_2OH$ (**40e**)	O	O	–	–	+		1000	46
$(CH_2)_2SCHF_2$ (**40f**)	O	O	–	–	+		1000	46
$(CH_2)_2SSC_6H_3(NO_2)_2$ (**40g**)	O	O	–	–	+		1000	46
$(CH_2)_2SCH_2C\equiv CH$ (**40h**)	O	O	–	–	+		1000	46
$(CH_2)_2SCH_2Me$ (**40i**)	O	O	–	–	+		570	46
$(CH_2)_2SCH_2CH=CH_2$ (**40j**)	O	O	–	–	+		270	46
$(CH_2)_2SCH=CH_2$ (**40l**)	O	O	–	–	+		50	46
$(CH_2)_2SSMe$ (**40m**)	O	O	–	–	+		1000	46
$(CH_2)_2SSPh$ (**40n**)	O	O	–	–	+		1000	46
$(CH_2)_2SC_3H_5$-cyclo (**40o**)	O	O	–	–	+		1000	46
$(CH_2)_2SC\equiv CH$ (**40p**)	O	O	–	–	+		330	46
 [(19*R*)-**59**]	O	O	–	–	–	7		51
[(19*S*)-**59**]	O	O	–	–	–	75		51
 [(19*S*)-**60**]	O	O	–	–	–	1		51
[(19*R*)-**60**]	O	O	–	–	–	75		51
 [(2′*S*)-**64**]	O	O	–	–	–	22		55
 [(19*R*)-**76**]	O	O	–	–	–	3.4		56
[(19*S*)-**76**]	O	O	–	–	–	56		56
[(19*R*)-**78**]	O	β-OH	–	–	–	30		56
[(19*S*)-**78**]	O	β-OH	–	–	–	693		56

Table 1 (continued).

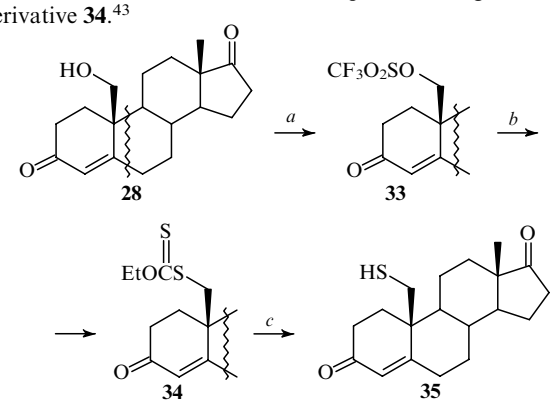
R	X	Y	The position of a double bond [a]			K_i /nM	IC_{50} /nM	Ref.
			Δ^1	Δ^6	$\Delta^{9(11)}$			
$(CH_2)_3-C(2)$-β (**86**)	O	O	–	–	–	24		59
2β,10β-$(CH_2)_2$	O	O	–	–	–	70		11
2β,10β-$CH_2CH(OH)$ [(*R*)-**82a**]	O	O	–	–	–	870		11
2β,10β-$CH_2CH(OH)$ [(*S*)-**82b**]	O	O	–	–	–	926		11
$CH(OH)CH_2Br$ [(19*R*)-**80**]	O	O	–	–	–	27		11
$CH(OH)CH_2Br$ [(19*S*)-**80**]	O	O	–	–	–	1130		11
$CH(OH)CH_2Cl$ [(19*R*) : (19*S*) = 9 : 1]	O	O	–	–	–	63		11
$CH(OH)CH_2I$ [(19*R*) : (19*S*) = 9 : 1]	O	O	–	–	–	11		11

[a] Symbols + and − denote the presence and absence of the double bond in this position, respectively.



(*a*) KSH, $HO(CH_2)_2OH$, 18-crown-6, 70%; (*b*) H^+, Me_2CO, 78%; (*c*) NaOH, MeOH, 32%.

The homologous 19-mercapto-AD **35** was obtained by the reaction of the 19-triflate **33** with potassium ethyl xanthate in the presence of 18-crown-6 with subsequent cleavage of the thio derivative **34**.[43]

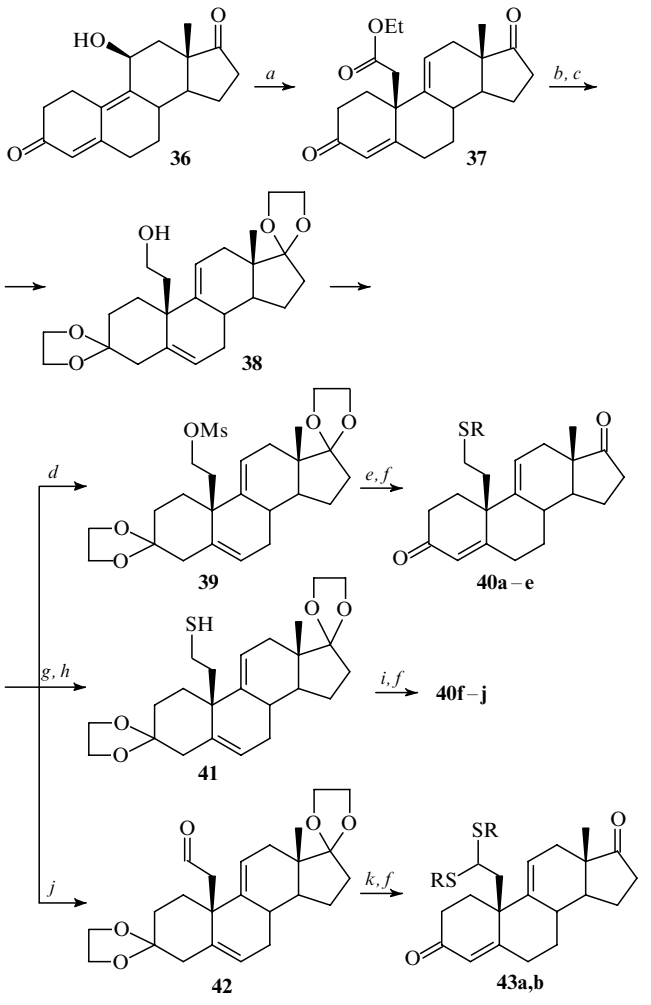


(*a*) $(CF_3SO_2)_2O$, Py; (*b*) EtOCSSK, 18-crown-6, THF, 20 °C, 20 h; (*c*) $H_2N(CH_2)_2NH_2$, THF.

The inhibition constant of the steroid **35** is 34 nM.[43] It represents an irreversible AI; its activity is 16 times as high as that of formestane both *in vitro* and *in vivo* (see Section II). However, the thiol **35** has a limited use because of its insufficient pharmacological stability due to the presence of a free thiol group. To overcome this limitation, its ethylthio derivative, *viz*., 19-ethylthio-AD, has been synthesised [44] (for the relevant patent, see Ref. 45).

One of recent papers devoted to 19-functionalised androstanes presents the results of extensive studies on the synthesis and structure – aromatase affinity relationships of several series of androstane-4,9(11)-diene-3,17-diones carrying a sulfur-containing group, $[(CH_2)_nSR]$, at the C(10) atom, where $n = 0-3$ and R stands for various alkyl, acyl and aryl substituents.[46] 19-Ethoxycarbonylandrosta-4,9(11)-diene-3,17-dione **37** obtained from the allylic alcohol **36** by the [3.3]-Claisen rearrangement was used as the starting compound in these syntheses (Scheme 2).

Scheme 2

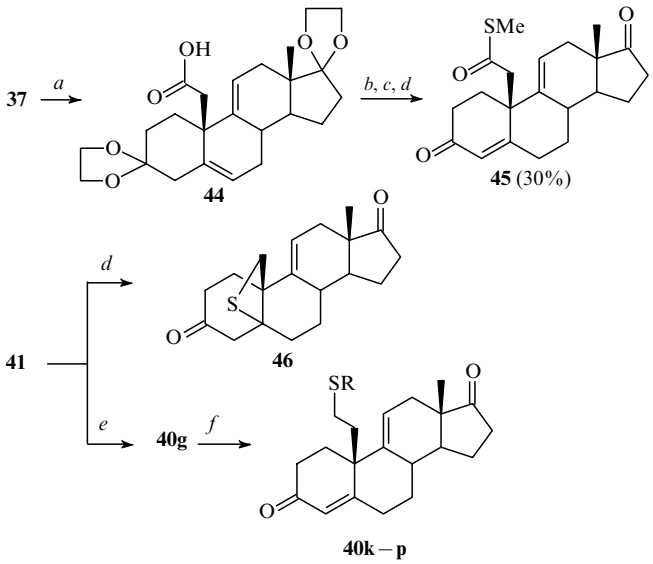


R = Me (**a**, 51%), Et (**b**, 55%)

40: R = Me (**a**), Et (**b**), Ac (**c**), Ph (**d**), $(CH_2)_2OH$ (**e**), CHF_2 (**f**), 2,4-$(NO_2)_2C_6H_3S$ (**g**), $CH_2C\equiv CH$ (**h**), CH_2SMe (**i**), $CH_2CH=CH_2$ (**j**); (*a*) $(EtO)_3CMe$, $EtCO_2H$, 137 °C, 4 h, 81%; (*b*) $HO(CH_2)_2OH$, CH_2Cl_2, Δ, 8 h, 71%; (*c*) LAH, THF, −50 to 20 °C, 2 h, 85%; (*d*) MsCl, Et_3N, CH_2Cl_2, 0 °C, 100%; (*e*) RSNa, DMF; (*f*) HCl; (*g*) AcSH, PPh_3, diethylazodicarboxylate (DEAD), THF, 20 °C, 2.5 h, 60%; (*h*) NH_2NH_2, THF, −30 °C, 72 h, 97%; (*i*) Bu^tOK, THF, RHal; (*j*) $(COCl)_2$, DMSO, CH_2Cl_2, −50 °C; (*k*) RSH, $BF_3 \cdot Et_2O$, −78 to 20 °C.

Thus the reaction of steroid **36** with triethyl orthoacetate in the presence of propionic acid gave the ethoxycarbonyl derivative **37** in > 80% yield.[47] The latter was reduced to alcohol **38** *via* the bisketal. Several methods presented in Scheme 2 have been elaborated for its conversion into various 19-thiomethyl androstane derivatives. One of them consists in conversion of the alcohol **38** into the corresponding mesylate **39**, subsequent substitution of the mesyloxy group by various thiolates and deprotection to give compounds **40a**–**e**. The first member of this series, *viz*., 3,17-diketo analogue of compound **41**, could not be obtained using this approach; various attempts at its deprotection gave compound **46**, which is the product of intramolecular Michael addition (Scheme 3).

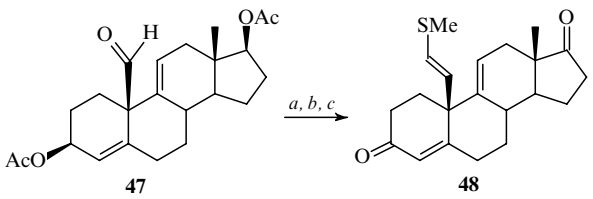
Scheme 3



40 : R = C≡CSiMe$_3$ (**k**), CH=CH$_2$ (**l**), SMe (**m**), SPh (**n**), cyclo-C$_3$H$_5$ (**o**), C≡CH (**p**);
(*a*) KOH, MeOH, 18 h, 95%; (*b*) ClCO$_2$Et, Et$_3$N, 20 °C, 1 h; (*c*) MeSNa, DMF, 2 h; (*d*) HCl; (*e*) 2,4-(NO$_2$)$_2$C$_6$H$_3$SCl, Py, CH$_2$Cl$_2$, 20 °C, 0.5 h; (*f*) RM, THF.

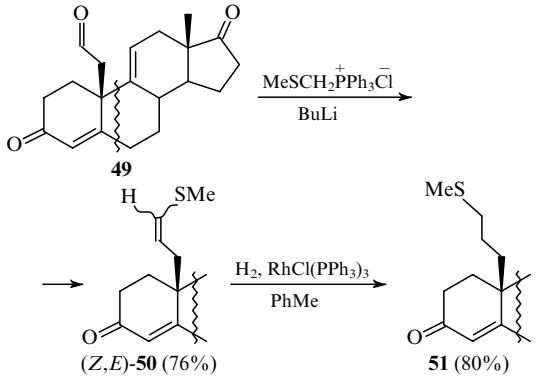
The thiol **41** was used as the starting compound in the synthesis of thio derivatives **40f**–**j**. This has been obtained by the Mitsunobu reaction from the alcohol **38** and thioacetic acid in the presence of PPh$_3$ and diethyl azodicarboxylate followed by hydrazinolysis. Alkylation of the thiol group of compound **41** with alkyl halides or 2,4-dinitrophenylsulfenyl chloride gave compounds **40f**–**j** in good yields (see Scheme 2). Thioacetals **43a**,**b** were prepared by the reaction of the aldehyde **42** obtained by the Swern oxidation of the alcohol **38** with methane- or ethanethiol, respectively, in the presence of boron trifluoriole etherate. Compounds **40k**–**p** were synthesised by the reaction of the disulfide **40g** with various organometallic reagents. The methyl thiocarboxylate **45** was obtained from the key compound **37** in three steps which included hydrolysis of the ester function, activation of the resulting acid **44** with ethyl chloroformate and reaction with sodium methanethiolate (Scheme 3).

Methylthiovinyl steroids **48** and **50** have also been described.[46] The acetoxy derivative **47** was deacetylated into the corresponding 3,17-diol which was introduced into the reaction with diethyl methylthiomethylphosphonate and butyllithium. The thus prepared (10*E*)-methylthiovinyl derivative was further oxidised into steroid **48**.

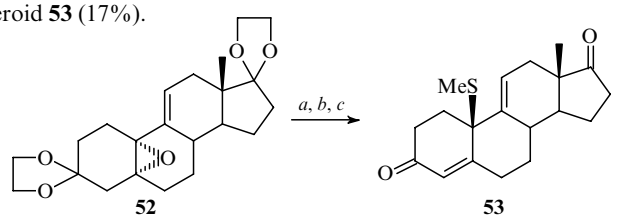


(*a*) KOH, MeOH (78%); (*b*) MeSCH$_2$PO(OEt)$_2$, BuLi (76%); (*c*) Al(OPr^i)$_3$, CHex (74%).

An analogous reaction of the homologous aldehyde **49** with (methylthiomethyl)triphenylphosphorane gave a 1 : 1 mixture of chromatographically separable *E*- and *Z*-isomers **50** in total yield 76%. Hydrogenation of this mixture in the presence of the Wilkinson catalyst gave compound **51** in 80% yield which contained a 3-methylthiopropyl residue at C(10).

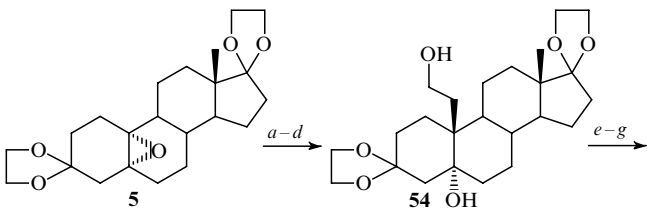


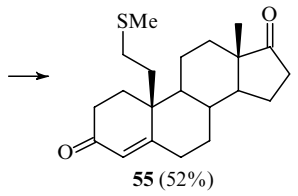
And, finally, the opening of the 5α,10α-epoxide ring of estrane **52** with lithium methanethiolate which is analogous to that described above for compound **5**,[14,43] and subsequent deketalisation and elimination of the 5α-hydroxy group resulted in $\Delta^{4,9(11)}$-steroid **53** (17%).



(*a*) MeSLi, THF, 1 h, 20 °C; (*b*) 6 N HCl; (*c*) 0.1 N NaOH (17%).

The opening of the 5α,10α-epoxide ring of the estrane **5** with monolithium acetylide with subsequent partial hydrogenation of the hydroxy alkyne and hydroboration of the resulting alkene gave the diol **54**. Its mesylation and subsequent reaction of the mesylate with sodium methanethiolate followed by acid hydrolysis gave the ring *C*-saturated analogue of the steroid **40a**, *viz*., 19-methylthiomethyl-AD **55**.





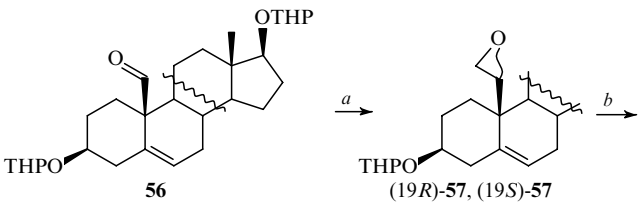
55 (52%)

(*a*) LiC≡CH, ethylenediamine (EDA), 50 °C, 96 h, 81%; (*b*) H_2 – 10% $Pd(OH)_2/BaSO_4$, EtOAc – Py; (*c*) $BH_3 \cdot Me_2S$, THF, 35 °C, 4 h; (*d*) 30% H_2O_2, NaOH, 0 to 20 °C, 12 h, 67%; (*e*) MsCl, Et_3N, CH_2Cl_2, 0 °C, 45 min; (*f*) MeSNa, DMF, 20 °C, 1 h; (*g*) HCl, EtOH, 20 °C, 3 h.

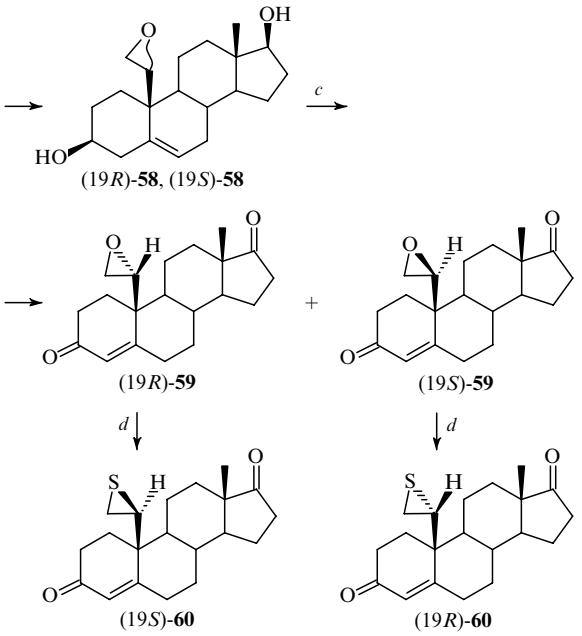
The results of *in vitro* and *in vivo* studies of activities of the majority of compounds synthesised by Lesuisse *et al.*[46] suggest that the steroids with one- or two-carbon chains having a terminal thioether function at position 10 (*e.g.*, **22f**, **40a**,**b**, **55**) are the most efficient AI. The presence or the absence of the 9(11)-double bond does not practically affect their inhibitory activity.[48] Compound **40a** was the most reactive among other compounds assayed and was considered [46] to be one of the best AI available to date. On the contrary, the steroids with the sulfur atom attached directly to the steroid skeleton (**53**) or separated from it by three carbon atoms (**51**) have practically no affinity for the enzyme. The key property of the efficient AI of this series is the ability of the sulfur atom to react with the heme iron of the porphyrin moiety of the enzyme (compounds **22f**, **40a** and **55**), whereas compound **53** reacts only with the lipophilic steroid-binding centre of the enzyme. This behaviour is consistent with the hypothesis [14, 41] that the incorporation of one methylene group between the sulfur atom and position 10 of the steroid skeleton alters the character of the interaction of the steroid substrate with the enzyme. The fact that AI with a two-carbon chain at C(10) react most efficiently with the heme Fe(III) allowed Lesuisse *et al.*[46] to suppose that the iron atom is separated from the 19-methyl group of the substrate by a distance equivalent to two methylene groups at the oxidation steps of AD. This hypothesis is consistent with the generally accepted mechanims of aromatisation.[15, 16] The effect of the substituent at the sulfur atom in the molecule of AD analogues on the inhibition of aromatase has also been studied. Sulfur-containing steroids with methyl, vinyl or ethynyl substituents at the sulfur atom (compounds **40a**, **l** and **p**, respectively) possessed the highest affinity for the enzyme. Substitution of the ethyl group (**40b**) for the methyl group (**40a**) decreases the activity of the steroid substrate 72-fold. Compounds with hydroxyethyl (**40e**) and cyclopropyl groups (**40o**) are completely devoid of activity, while compounds with allyl (**40j**), phenyl (**40d**), prop-2-ynyl (**40h**) and methylthiomethyl (**40i**) groups possess certain activity. Disulfides **40g**,**m**,**n**, thioacetals **43a**,**b** and analogues **48** and **50** with a double bond in the side chain at C(10) are also inactive as AI.

Compound **40a**, which is the most active in this series, decreases the estradiol level and inhibits the growth of endocrine tumours *in vivo* when used at a dose of 0.4 mg kg^{-1}.[49]

Yet another type of 19-substituted androstanes, *viz.*, 10β-(oxiran-2-yl)- and -(thiiran-2-yl)-steroids, represent competitive reversible AI. The synthesis of diastereomeric 10β-(epoxyethyl)estr-4-ene-3,17-diones (19*S*)-**59** and (19*R*)-**59** was carried out in four steps.[50–52]



56 (19*R*)-**57**, (19*S*)-**57**

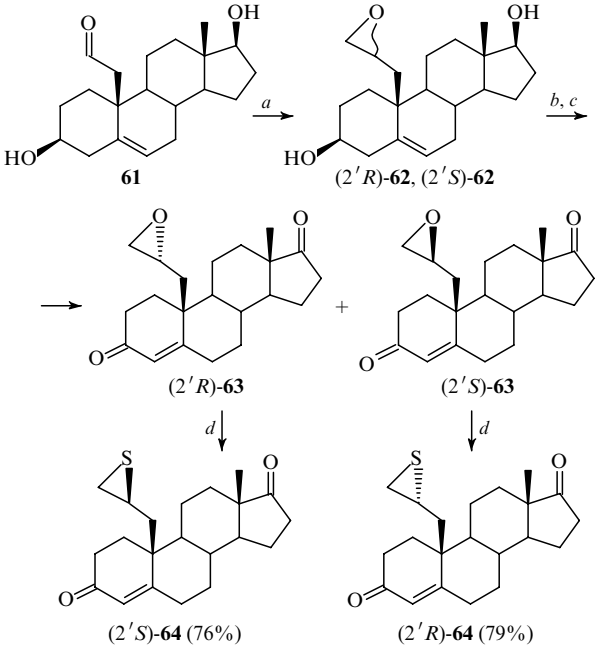


(*a*) $Me_2\overset{+}{S}\overset{-}{C}H_2$ (or $Me_2\overset{+}{S}O\overset{-}{C}H_2$), THF or DMSO; (*b*) $Py \cdot HSO_3C_6H_4Me$, 20 °C, 4 h; (*c*) $Al(OPr^i)_3$, CHex; (*d*) Ph_3PS is picric acid, PhH, 80 °C, 16 h.

Reaction of the tetrahydropyranyl derivative of 19-aldehyde **56** with dimethylsulfonium methylide (or dimethylsulfoxonium methylide) in THF gives a mixture of oxiranes (19*R*)-**57** and (19*S*)-**57** in 26% and 74% yields, respectively. Under other reaction conditions, *i.e.*, in DMSO in the presence of dimethylsulfonium methylide at 0 °C, the ratio of diastereomeric products is changed: the (19*R*)-**57** isomer becomes predominant.[51] Deprotection and chromatographic separation of diols (19*R*)-**58** and (19*S*)-**58** with subsequent Oppenhauer oxidation of each of the diastereomeric alcohols gave individual isomers (19*R*)-**59** and (19*S*)-**59**, respectively. Steroid thio analogues (19*R*)-**60** and (19*S*)-**60** were obtained from oxiranes **59** by reaction of the latter with excess of triphenylphosphine sulfide and picric acid in benzene.[53]

The configuration of isomeric 10β-oxiranes **59** (*R* or *S*) was established from X-ray analysis. It was shown that the oxirane ring in the stereoisomer (19*R*)-**59** is positioned over the ring *A* in the direction towards C(1) and C(2) in a manner similar to that of the 19-hydroxy group in 19-hydroxy-AD **28** . On the contrary, the three-membered ring in the molecule of (19*S*)-**59** is positioned over the ring *B* and directed toward C(6).[31, 51, 54] Presumably, it is these stereochemical peculiarities that are responsible for the difference in the binding and inhibitory activities of these compounds. Both of them are potent competitive AI with K_i 7 and 75 nM, respectively. Thiiranes (19*R*)-**60** and (19*S*)-**60** manifest an even greater stereoselectivity of binding: K_i for (19*R*)-**60** is 1–2 nM and that for (19*S*)-**60** is 75 nM.[51] In another study [54] it was shown that (19*R*)-isomers of oxiranes and thiiranes are more active (36-fold and 80-fold, respectively) than the corresponding (19*S*)-diastereomers. Spectroscopic studies with purified aromatase showed that the inhibition involved reversible binding of the oxygen (or sulfur) atom of the oxirane (or thiirane) with the heme iron of the enzyme.[15, 51, 54] The knowledge of the spatial structure of these compounds made it possible to assume that the modified steroid inhibitor is bound in exactly the same way as does the natural substrate (AD) and that the heme iron is localised in close proximity to the 19-methyl group of the androgen molecule.[54] It is of note that with neither 19-hydroxy, nor 19-methoxy derivatives of AD is such coordination possible.[15] Thus, coordination with the heme iron of the enzyme, together with the binding selectivity inherent in 10β-(oxiran-2-yl)- and -(thiiran-2-yl)- steroids make these AD analogues highly specific aromatase inhibitors.

The synthesis of homologous 19-(oxiran-2-yl)- (**63**) and 19-(thiiran-2-yl)-AD (**64**) continued the series of investigations aimed at establishing the localisation of the aromatase heme iron during its binding with the steroid substrate.[55]

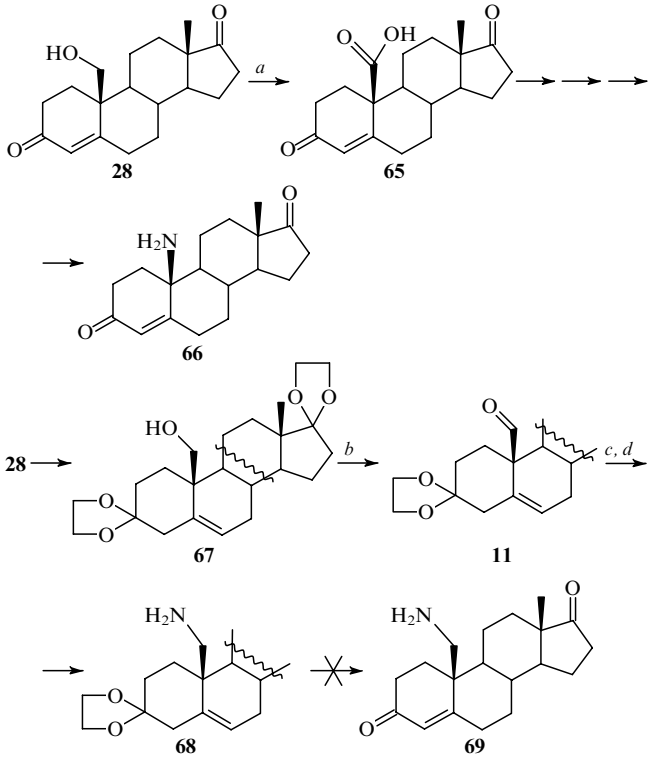


(*a*) $Me_2\overset{+}{S}\overset{-}{C}H_2$ (or $Me_2\overset{+}{S}O\overset{-}{C}H_2$), THF, 4 °C (75%); (*b*) $CrO_3 \cdot Py$, CH_2Cl_2, 25 °C, 20 min; (*c*) diazabicyclononene (DBN), CH_2Cl_2, 25 °C, 18 h; (*d*) Ph_3PS, PhH, Δ, 18 h.

The methodology of the synthesis of these compounds is analogous to those used in the synthesis of 10β-(oxiran-2-yl) and -(thiiran-2-yl) steroids[52] with 3β,17β-dihydroxyandrost-5-ene 19-carbaldehyde **61** as the starting compound.[30] Unlike the 10-formyl analogue **56**, this reacted with both dimethylsulfonium and dimethylsulfoxonium methylide to give a chromatographically separable mixture of diastereomeric oxiranes (2′*R*)-**62** and (2′*S*)-**62** of the same composition (3 : 1) in 75% yield. This result is rationalised by higher spatial accessibility of the aldehyde group in the molecule of compound **61**. After separation into individual diastereomers, epoxy diols **62** were converted into diketones (2′*R*)-**63** and (2′*S*)-**63** by the Collins oxidation with subsequent isomerisation of resulting Δ^5-3,17-diketones with diazabicyclonene. The configuration of epoxide (2′*R*)-**63** was established by X-ray analysis. Reaction of this oxirane with triphenylphosphine sulfide and picric acid gave thiirane (2′*S*)-**64** in 76% yield. Analogously, thiirane (2′*R*)-**64** was obtained from (2′*S*)-**63** in 79% yield. The *R*- and *S*-configuration of thiiranes **64** was ascribed on the basis of the established stereochemistry of the oxirane precursors and the known stereochemical regulation of thiirane formation.[55] Oxiranes **63** and thiiranes **64** are competitive AI; the latter possess higher activity than the corresponding oxiranes. (2′*S*)-Isomers of both compounds are more efficient inhibitors than 2′*R*-diastereomers. The inhibition constant of the strongest inhibitor, *viz*., the thiirane (2′*S*)-**64**, is 22 nM. The oxygen and sulfur atoms in the oxirane (2′*S*)-**63** and the thiirane (2′*S*)-**64** molecules coordinate with the aromatase heme iron during their interaction with the enzyme similarly to the corresponding (19*S*)-diastereomers **59** and **60**. Here, the weaker inhibitor possesses weaker affinity for the heme iron of cytochrome P-450. The configuration of the 2′-centre of the three-carbon chain at C(10) is crucial for the stereoselectivity of inhibition.[55] Thus, strong competitive inhibition of aromatase and coordination of the heteroatom in the steroid substrate are observed even in those cases where the oxygen or the sulfur atom in the three-membered ring are separated from C(10) by a methylene group (*cf*. Ref. 46).

Having considered the AD analogues containing an oxygen or a sulfur atom at positions 10 or 19, one may conclude that sulfur-containing steroids are the most potent inhibitors and that compounds with a methylene group between the steroid skeleton and the heteroatom manifest higher inhibitory activity. A characteristic feature of compounds of this series is the stereoselectivity of their interaction with the enzyme.

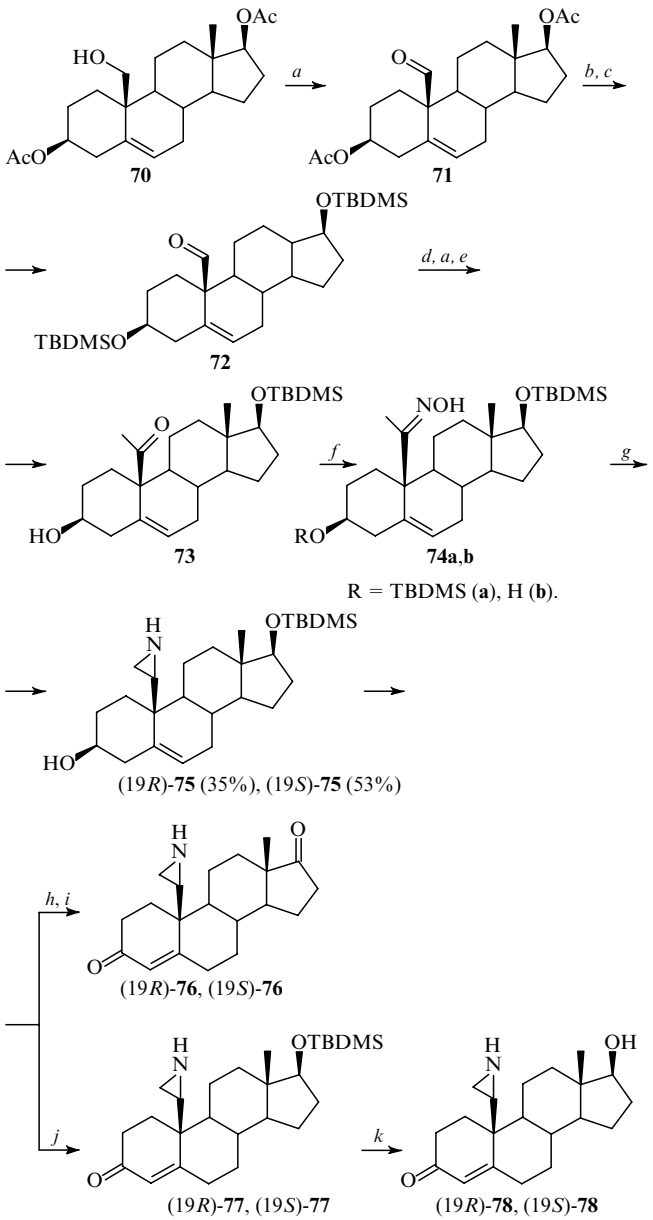
Synthesis of AD derivatives containing nitrogen at position 19, which bind with iron of the aromatase heme by virtue of coordination similarly to sulfur-containing steroids was described by Robinson in 1993,[56] although attempts to synthesise such derivatives were undertaken earlier.[57] Thus 10β-aminoestr-4-ene-3,17-dione **66** was obtained by the Curtius rearrangement of the corresponding derivative of 19-acid **65**. However, all the attempts to obtain its homologue, *viz*., 19-aminomethyl-AD **69**, failed, although its diketal derivative **68** was obtained in high yield from 19-hydroxy-AD **28**. Compounds **66** and acetamido and trifluoroacetamido derivatives of diketone **69** displayed low AI activity.[57]



(*a*) $CrO_3 - H_2SO_4$; (*b*) $Py \cdot CrO_3$ (93%); (*c*) $NH_2OH \cdot HCl$ (88%); (*d*) Ni/Ra, EtOH, 2 M NaOH (89%).

Oxime **74** obtained from the accessible 3,17-diacetate of the triol **70** in seven steps (Scheme 4) was the key compound in the synthesis of 10β-(aziridin-2-yl)-steroids.[56] Oxidation of compound **70** with tetrapropylammonium perruthenate in the presence of *N*-methylmorpholine *N*-oxide as a co-oxidant gave 19-aldehyde diacetate **71** in high yield; the latter was further converted into diol and then into bis-*tert*-butyldimethylsilyl ether **72**. Methylation of the aldehyde **72** with methyllithium occurred in quantitative yield. Oxidation of the 19-hydroxy-19-methyl derivative and selective desilylation of the thus obtained 10β-acetyl derivative gave 17-silyl ether **73**. The latter was refluxed with hydroxylamine hydrochloride in pyridine to give a mixture of key oximes **74a**,**b** in 70% yield. On treatment with lithium aluminium hydride in THF, the oxime **74b** was converted into a mixture of (aziridin-2-yl)-steroids (19*R*)-**75** and (19*S*)-**75** isolated in the individual states with 35% and 53% yields, respectively. Each of them was further converted into (19*R*)- and (19*S*)-10β-(aziridin-2-yl) diketones **76** (in 39% and 41% yields, respectively) *via* the corresponding diols. 17β-Hydroxy analogues (19*R*)- and

Scheme 4



(*a*) Tetrapropylammonium perruthenate (TPAP), *N*-methylmorpholine *N*-oxide (NMO), CH_2Cl_2, 20 °C; (*b*) 10% KOH, MeOH, 90%; (*c*) Bu^tMe_2SiCl ((TBDMS)Cl), DMF; (*d*) MeLi, Et_2O; (*e*) TBAF, THF; (*f*) $NH_2OH \cdot HCl$, Py, Δ; (*g*) LAH, THF, Δ, 18 h; (*h*) HF, H_2O, EtOH, 20 °C, 2.5 h; (*i*) $Al(OPr^i)_3$, CHex; (*j*) $Al(OPr^i)_3$, 4-methylpiperidone; (*k*) HF, H_2O, MeCN, 20 °C, 2 h.

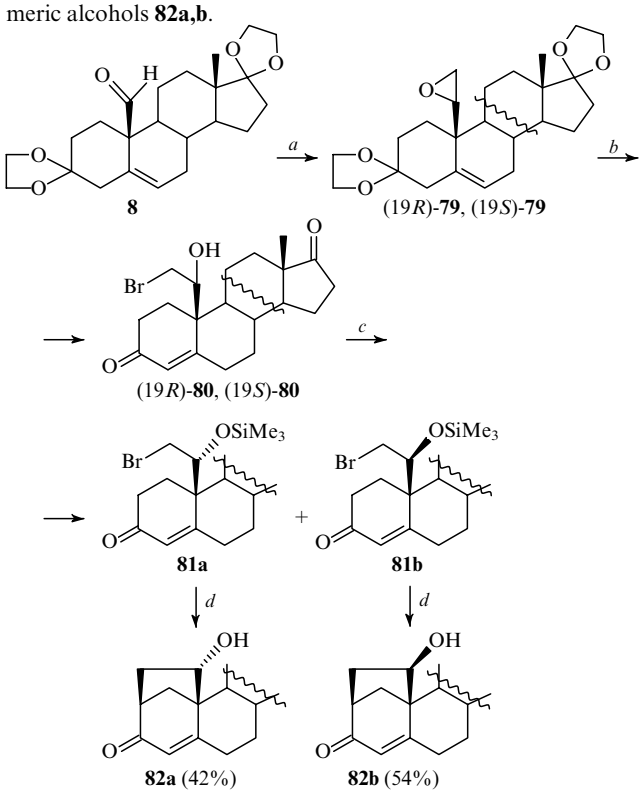
(19*S*)-**78** were synthesised from the aziridines (19*R*)- and (19*S*)-**75** *via* intermediate Δ^4-3-ketones **77**. The stereochemistry of these compounds was established by deamination of diastereomeric aziridines **76** and **78** with sodium nitrite in aqueous acetic acid into the known 10β-vinylestr-4-ene-3,17-diones and the corresponding 17β-alcohols. The configuration of the (aziridin-2-yl)-steroid (19*R*)-**78** was also confirmed by X-ray analysis.

Compounds (19*R*)- and (19*S*)-**76** as well as (19*R*)- and (19*S*)-**78** are potent stereoselective AI with K_i = 3.4, 56.0, 30.0 and 693 nM, respectively. 10β-(Aziridin-2-yl)-steroid (19*R*)-**76** manifested the highest efficiency. Spectroscopic analysis of its interaction with preparations of microsomal aromatase have shown the coordination of the nitrogen atom of the aziridine ring with the iron atom of the cytochrome P-450 heme. Earlier, such a coordination was established for 19-azido-AD **22e**.[17] These results suggest that the orientation and accessibility of the nitrogen atom are crucial for efficient coordination.[56] It should be noted that *N*-acetylation of compounds **76** strongly decreases their inhibitory activity.[56]

The recently described 10β-aziridinoestr-4-ene-3,17 dione was less active in comparison with the 10β-aziridine (19*R*)-**76**.[58]

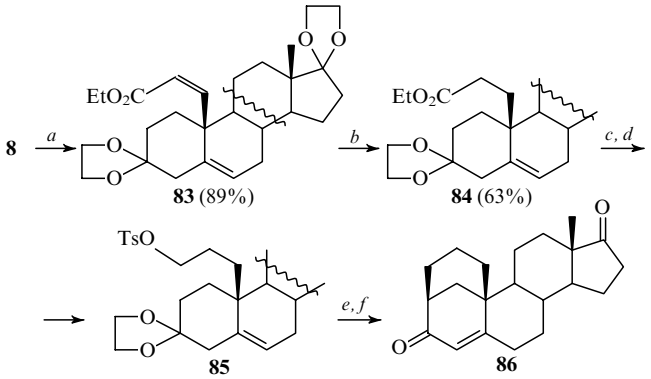
The synthesis of hydroxylated 2,19-bridged structures related to AD was described in 1991.[11] The key compound in this synthesis was 10β-epoxide **79** [a mixture of (19*R*)- and (19*S*)-isomers] obtained by reaction of the aldehyde **8** with dimethylsulfonium methylide.

The opening of the epoxide ring and removal of the ketal protection with 48% HBr in acetone gave a mixture of bromohydrins **80** [the (19*R*)-**80**/(19*S*)-**80** ratio was 7 : 1]. Chromatographically separable trimethylsilyl derivatives **81a** and **81b** were obtained by the action of bis(trimethylsilyl)-acetamide in DMF. Intramolecular alkylation at C(2) under the action of lithium bis(trimethylsilyl)amide in THF and subsequent treatment of the reaction mixture with an acid complete the synthesis of diastereomeric alcohols **82a,b**.



(*a*) $Me_3\overset{+}{S}I^-$, $NaCH_2SOCH_3$, DMSO, THF, 98%; (*b*) HBr, H_2O, Me_2CO; (*c*) $CH_3C(=NSiMe_3)OSiMe_3$ (BSA), DMF; (*d*) $LiN(SiMe_3)_2$, HCl, THF.

The synthesis of diketone **86**, which is homologous to these bridge structures, was carried out in five steps.[59, 60]

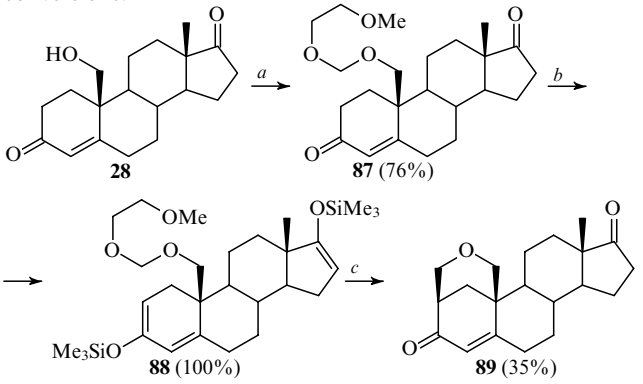


(*a*) $(EtO)_2P(O)CH_2CO_2Et$; (*b*) Mg, MeOH; (*c*) LAH (93%); (*d*) TsCl, Py, 60%; (*e*) H^+ (60%); (*f*) $LiN(SiMe_3)_2$, THF, −78 °C (62%).

In this case, too, the same bisketal **8** was used as the starting compound. Its reaction with triethyl phosphonoacetate gave the unsaturated derivative **83** in high yield, and the double bond was reduced with magnesium in methanol. The ester function in the thus prepared compound **84** was reduced into the alcohol function which was then tosylated. Removal of 3,17-diketal protection in the tosylate **85** and cyclisation of 19-(2-tosyloxyethyl)-AD with lithium bis(trimethylsilyl)amide in THF gave the final product, 2β,19-ethylene-AD **86**.

The section devoted to bridge structures can be completed by the description of a synthesis of 2β,19-methyleneoxy-AD **89**.

The methoxyethoxymethyl ether (MEM) **87** prepared from 19-hydroxymethyl-AD **28** was converted into the homoannular dienol **88** silyl ether. The latter underwent Lewis acid-catalysed intramolecular alkoxyalkylation at C(2) involving the acetal carbon atom of the MEM-protective group to give the bridge steroid **89**. It is noteworthy that the MEM ether group played the role of both the protective group and the reagent in these conversions.



(*a*) $ClCH_2OCH_2CH_2OMe$, $(Me_2CH)_2NEt$, CH_2Cl_2, 20 °C, 20 h; (*b*) LDA, Me_3SiCl, THF, −20 °C, 0.5 h; (*c*) $TiCl_4$, CH_2Cl_2, −20 °C, 0.5 h.

Some sulfur-containing analogues of the steroid **89** have been recently patented.[59]

2β,19-Bridged steroids **82a**,**b**, **86** and **89** appeared to be sufficiently active AI.[11, 59–61] Bromohydrins **80** also manifested a certain AI activity (see Table 1).[11]

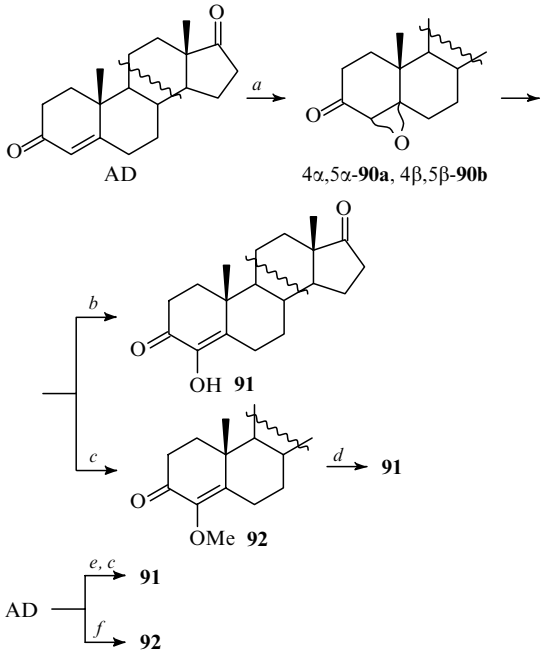
2. 4-Substituted androstanes

Of all substituted androstanes, particularly of those substituted at position 4, 4-hydroxyandrost-4-ene-3,17-dione (4-OHA) **91** is the most popular and well-studied. It was first described in 1977 [62] as a competitive AI. Later, it was shown to represent a suicide irreversible AI; according to different estimates, its K_i ranges from 1 to 50 nM.[19, 55, 62–64] This steroid is the basis of one of a few (if not the only) steroid inhibitors of aromatase that is already used in clinical practice.[65] This compound is known under the trade name 'formestane' and is used for the treatment of hormone-dependent mammary tumours in women in the post-menopausal period.[8, 15, 48, 66–71]

All the syntheses of 4-OHA **91** described in the literature involved acid- or base-catalysed opening of 4,5-epoxides **90a**,**b**. The latter are obtained by treatment of AD with hydrogen peroxide in an alkaline medium.

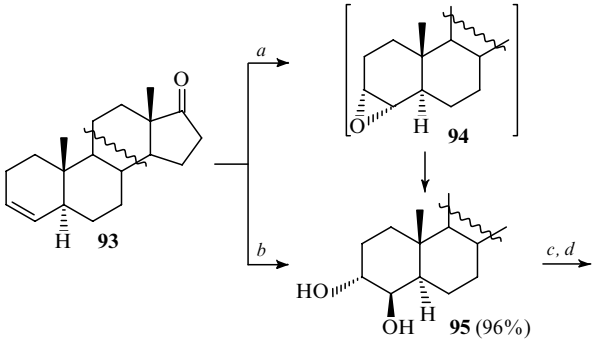
Epoxidation of conjugated steroid ketones is a well-known reaction. In the case of Δ^4-3-ketones, it affords a mixture of 4α,5α- and 4β,5β-oxides in which the latter predominates. The conditions for this reaction have not practically changed since the publication by Bible *et al.*[72] in 1957. However, the overall yields described therein were rather low and poorly reproducible. Various modifications of reaction conditions failed to increase the yields considerably,[73–75] although a successful synthesis of epoxides **90a** and **90b** (1 : 5, total yield 90%) is documented.[48]

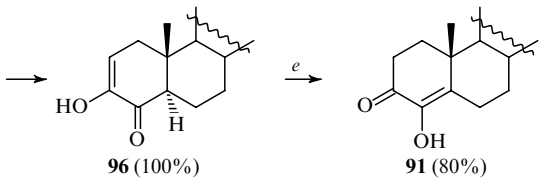
The opening of the epoxide ring in compounds **90a**,**b** results in 4-hydroxy-substituted AD **91** in up to 50% yields.[14, 48, 74–78] The steroid **91** was also obtained in a satisfactory yield by opening of the isomeric epoxides **90a**,**b** and hydrolysis of the ether **92**.[13] Rearrangement of the epoxides **90a**,**b** in the presence of boron trifluoride etherate in benzene and chromatographic separation of side products (*e.g.*, *A*-nor-3-oxo-5α-carbaldehyde) gave the target steroid **91** in 34% yield.[14] The ether **92** was synthesised in 25% yield by treatment of AD with *o*-iodosylbenzoic acid and alkali in methanol.[79] Special mention should also be made of a recently developed procedure for the synthesis of 4-OHA **91** (47% yield) which involves hydroxylation of AD with OsO_4/H_2O_2 with subsequent dehydration of the resulting mixture of 4,5-diols in an alkaline medium.[70]



(*a*) H_2O_2, OH^-; (*b*) AcOH, H_2SO_4, or Me_2CO, H_2O, H_2SO_4, or HF, Py, or $BF_3 \cdot Et_2O$, PhH; (*c*) MeOH, NaOH (62%); (*d*) HCl, dioxane, Δ (53%); (*e*) OsO_4, H_2O_2, Bu^tOH, 20 °C; (*f*) $OIC_6H_4CO_2H$, MeOH, KOH (25%).

Introduction of 4-OHA **91** into the clinical practice and the steadily increasing number of publications devoted to the analysis of the topography of the active site of aromatase using various analogues of 4-OHA **91** (see, *e.g.*, Refs 80 and 81) have stimulated the search for alternative efficient methods of its synthesis. A recent publication,[82] which describes a new approach to the synthesis of 4-OHA **91** by oxidation of easily accessible 5α-androst-3-en-17-one (**93**), is an example. The latter was obtained by reduction of AD with zinc in acetic acid.[83] Treatment of steroid **93** with performic acid generated *in situ* gives *trans*-diaxial diol **95** in 96% yield. Oxidation of androstenone **93** in dichloromethane resulted in 3α,4α-epoxide **94** as the only identified and isolated reaction product. Further oxidation of the diol **95** with dimethyl sulfoxide activated with trifluoroacetic anhydride gives hydroxy ketone **96** in quantitative yield. The latter is isomerised into the final steroid **91** on treatment with a base.

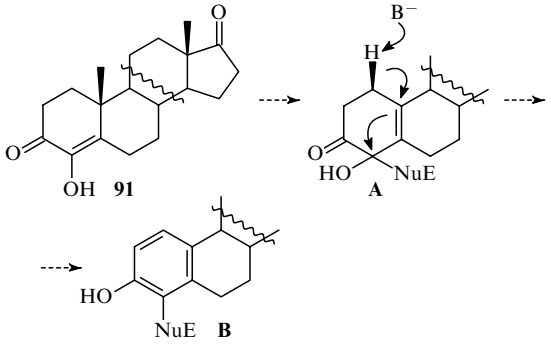




(*a*) H_2O_2, HCO_2H, CH_2Cl_2, 20 °C, 6 h, (*b*) H_2O_2, HCO_2H, 20 °C, 1 h; (*c*) DMSO, $(CF_3CO)_2O$, −60 °C, 3 h; (*d*) Et_3N, −60 °C, 15 min; (*e*) NaOMe, 20 °C, 1 h.

High yields in all the stages of this synthesis and the accessibility of the starting steroid **93** (the authors propose to use a mixture of 5α- and 5β-isomers) illustrate the high outlook of this approach.

The mechanism of the inhibitory effect of 4-OHA **91** on aromatase has not been completely elucidated. An inactivation mechanism, which involves the formation of a covalent bond between the enzyme and position 4 of the steroid substrate (which, in turn, changes the direction of the final stage of elimination), has been proposed by Covey.[24] Protonation of the hydroxy group at position 4 of the substrate and its elimination as a water molecule occurs in the enzyme-bound intermediate **A** (instead of removal of the enzyme as the nucleophile, which normally takes place).



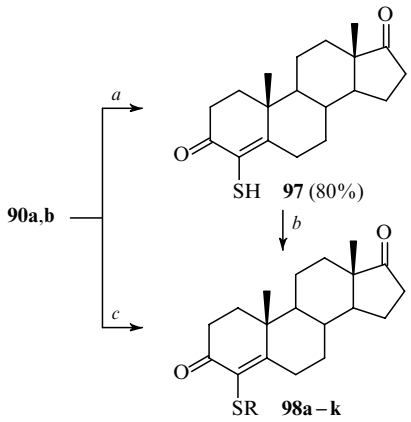
Nu is the nucleophile, E is the enzyme.

Inactivation of the enzyme, which cannot 'kick off' the steroid substrate, follows aromatisation of the steroid ring *A* with the formation of structure **B**. A great number of 4-OHA **91** analogues have been synthesised aimed at elucidating the mechanism of its action and obtaining more efficient aromatase inhibitors. Thus its 6α-methyl analogue obtained by hydrolysis of the corresponding 4,5-epoxide in a mixture of acetic and sulfuric acids has been described.[84] 4-Acetoxy-AD[63,64,85] possessed inhibitory properties, whereas 4-methoxy-AD **92** was inactive.[74] However, recent *in vitro* studies have shown that 4-methoxy-AD **92** suppressed the proliferative activity of cancer cells.[86]

Although 4-OHA **91** is an active AI, it is characterised by low bioavailability and short-term action and manifests *in vivo* activity only on subcutaneous administration, which is due to the ease of its glucuronidation.[67,87,88] The quest for the synthesis and analysis of its various analogues thus becomes understandable. Besides, these studies were aimed at establishing the structural demands for the design of novel AI.

A prominent role among formestane **91** analogues belongs to 4-alkylthio- and 4-arylthio-AD. Several compounds of this series (**98a**–**e**) were obtained in the 80's[89,90] by the opening of epoxides **90a**,**b** with the corresponding thiol reagents. All of them appeared to be efficient competitive AI with K_i ranging from 36 to 73 nM.[90] It was found that the chain length at C(4) of alkylthio analogues of such steroids should not exceed three carbon atoms in order to preserve a sufficiently high inhibitory activity.[90] 4-Phenylthio-AD **98d** (25 and 50 mg per kg per day) inhibited the growth of mammary tumours in rats.[91]

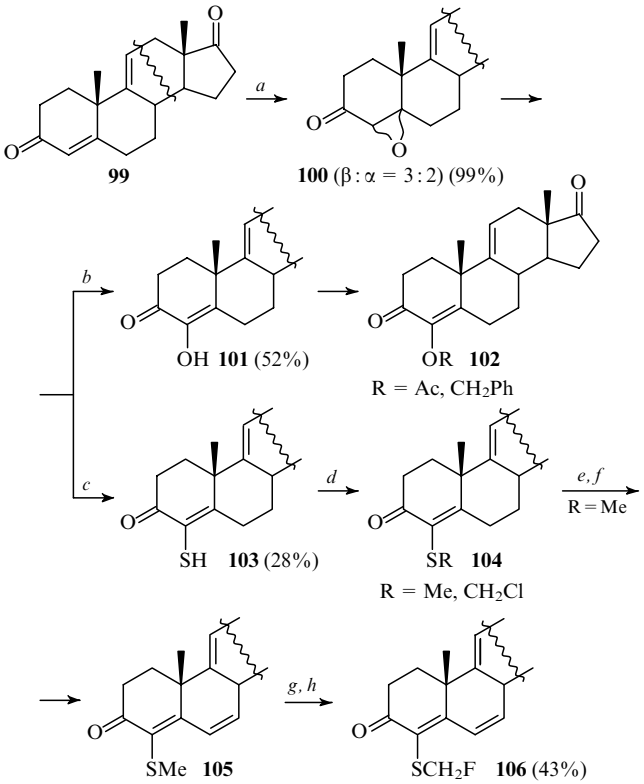
The thiol **97** was synthesised in high yield by the opening of epoxides **90a**,**b** with thioacetic acid in dioxane. Its subsequent alkylation with various functionalised alkyl halides gave a series of



(*a*) AcSH, dioxane, MeOH, HCl; (*b*) RX, Bu^{t}OK, THF; (*c*) RSH; R = Me (**a**), Et (**b**), Pr (**c**), Ph (**d**), CH_2Ph (**e**), CH_2F (**f**), CH_2Cl (**g**), CHF_2 (**h**), CH_2CN (**i**), CH_2OMe (**j**), CH_2SMe (**k**).

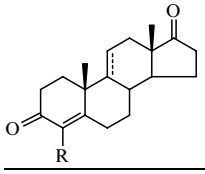
4-alkylthio-AD **98f**–**k** containing hetero atoms in the substituent at C(4).[48,92]

4-Thio-substituted **98f**–**h** were comparable in activity with 4-OHA **91** (see Table 2). The difluoromethyl analogue **98h** was found to be a reversible AI, whereas the fluoromethyl and chloromethyl analogues **98f** and **98g** are suicide AI with K_i = 30 nM (*cf.* K_i for 4-OHA **91** is 27 nM[48]). *In vivo* activity of compound **98f** is comparable with that of 4-OHA **91** and, like the latter, it is inactive on *per os* administration.[48] It is noteworthy that the presence of an additional 9(11)-double bond in these analogues, like in 19-sulfur-containing steroids **40a**–**o**, does not influence their affinity and inhibitory activity with respect to aromatase. A similar conclusion was made from the biochemical analysis[48,88] of some 4-alkyl(aryl)thio-$\Delta^{9(11)}$-AD **102**–**106** (their synthesis is shown below). The most active compound of this series is the steroid **104** (R = CH_2Cl) (K_i = 27 nM).[48]



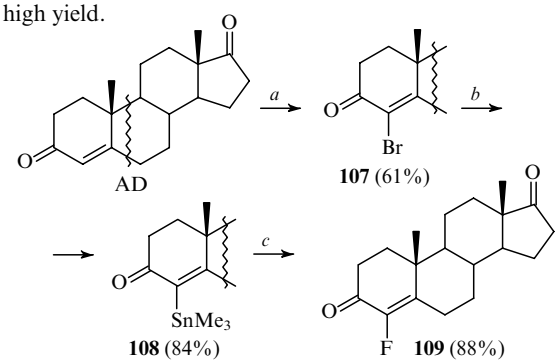
(*a*) H_2O_2, NaOH, CH_2Cl_2, MeOH, 4 °C, 1 h; (*b*) H_2SO_4, AcOH, 4 h, 20 °C; (*c*) AcSH–dioxane, MeOH, HCl; (*d*) RX, Bu^{t}OK, THF; (*e*) $HC(OEt)_3$, EtOH, *p*-TsOH; (*f*) chloranil; (*g*) MCPBA; (*h*) DAST, $CHCl_3$, Δ.

Table 2. Data on *in vitro* inhibition of aromatase from human placenta by 4-substituted androstanes.



R	The presence of a $\Delta^{9(11)}$ double bond	K_i /nM	IC_{50} /nM	Ref.
OH (**91**)	–	10–37		31
		27		48, 123
		50		55
		67		124
			43.7	9
OPh (**176**)	–	412		80
NH_2 (**112**)	–	37		15
SH (**103**)	+	72		48
SCH_2Cl (**98g**)	–	30		48
SCH_2Cl (**104**)	+	27		48
SCH_2F (**98f**)	–	30		48
SCH_2CN (**98i**)	–	169		48

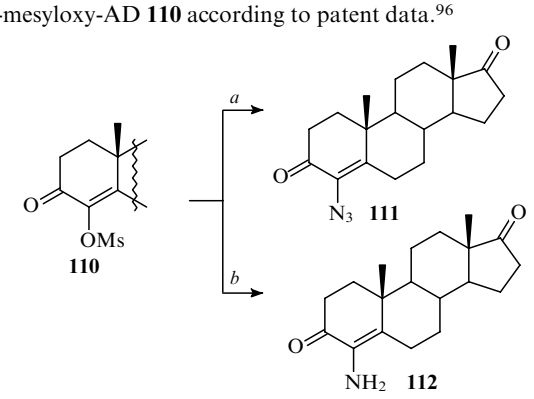
Other 4-substituted AD have been also obtained and assayed as AI. Thus 4-fluoro-AD **109** has been recently prepared from 4-bromo-AD **107**.[93] Conversion of bromide **107** into the 4-trimethylstannylated steroid **108** by hexamethyldistannane catalysed by tetrakis(triphenylphosphine)palladium and its subsequent treatment with cesium fluorosulfate gave 4-fluoro-AD **109** in high yield.



(*a*) Br_2, AcOH, , −30 °C; (*b*) $Pd(PPh_3)_4$, $(Me_3Sn)_2$, PhMe; (*c*) $FOSO_3Cs$, CH_2Cl_2, MeOH, N_2, 0 to 20 °C.

4-Fluoro-AD **109** is comparable with 4-OHA **91** in its inhibitory activity; however, its practical application is limited due to its low bioavailability.[94] The synthesis of 4-chloro-AD is given in a patent.[95]

Among other 4-substituted AD, special mention should be made of 4-azido-AD **111** and 4-amino-AD **112** prepared from 4-mesyloxy-AD **110** according to patent data.[96]

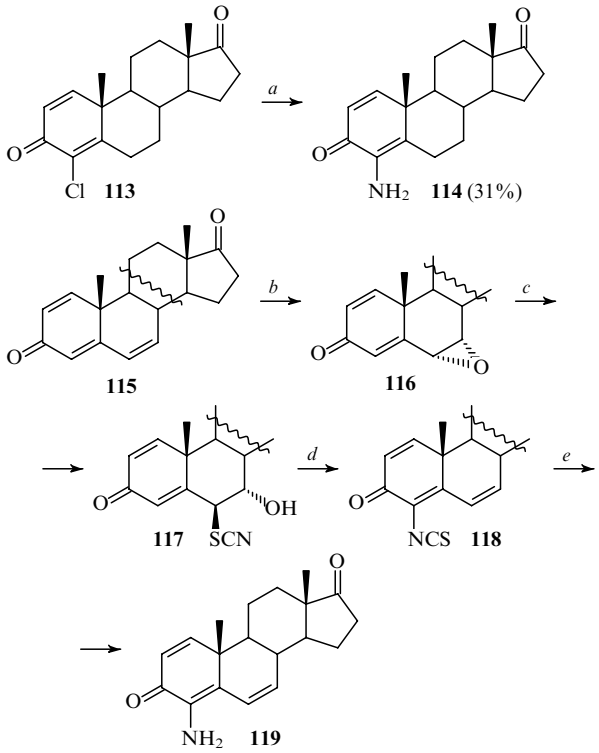


(*a*) NaN_3, H_2O, 60 °C, 1 h; (*b*) NaN_3, 100 °C, 1.5 h.

Highly active 4-amino-androstenediones **114** and **119** present special interest. One of them, 4-aminoandrosta-1,4,6-triene-3,17-dione **119**, appeared to be an efficient AI and was recommended for treatment of mammary tumours. This compound has passed successfully clinical trials under the trade name of 'minamestane'.[97–99]

4-Aminoandrosta-1,4-diene-3,17-dione (**114**) was obtained by ammonolysis of the corresponding 4-chloro derivative **113**[95] in dioxane (Scheme 5).

Scheme 5



(*a*) 30% NH_4OH, dioxane, 24 h; (*b*) $2\,KSO_5 \cdot KHSO_4 \cdot K_2SO_4$ – phosphate buffer, Me_2CO, 5–10 °C, 15 min; (*c*) KSCN; (*d*) DMF, 110–135 °C, 1.5 h; (*e*) dioxane – H_2O, 35% NaOH, 0.5 h.

The oxirane **116** obtained by selective epoxidation of the trienedione **115** was the key compound in the synthesis of minamestane **119**. Its reaction with potassium thiocyanate gave the 6-thiocyanate derivative **117**. Heating of the latter in DMF resulted in 4-isothionate **118** and its alkaline hydrolysis yielded the target compound, 4-aminotrienedione **119** (see Scheme 5).[100] Its 6- and 7-substituted analogues have been described in the patent.[101]

Studies of structure–activity relationships revealed the enhanced rate and selectivity of aromatase inhibition upon introduction of additional 1,2- and 6,7-double bonds into the molecule of 4-amino-AD **112**.[98] Steroids **114** and **119** are highly efficient and manifest *in vivo* antitumour activity both on subcutaneous and oral administration.[9,81,97–99]

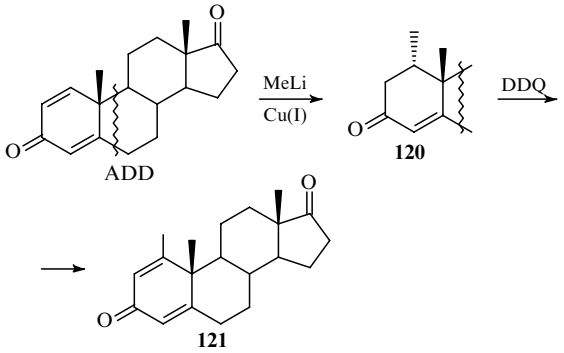
Thus, the majority of 4-substituted AD described above represent highly active irreversible AI with K_i ranging from 30 to 200 nM.[100]

3. 1-Substituted androstanes

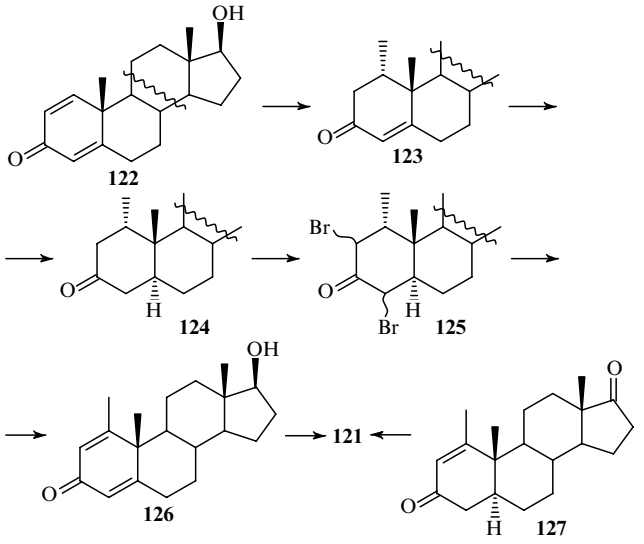
1-Methylandrosta-1,4-diene-3,17-dione (**121**) is the most valuable and perhaps the only steroid AI of this series. It was synthesised in 1983[102] and patented under the trade name atamestane in more than a dozen countries. Atamestane is an irreversible inhibitor of estrogen biosynthesis *in vitro* and *in vivo*.[102–105]

Androsta-1,4-diene-3,17-dione (ADD) is the starting compound in the synthesis of atamestane **121**. This synthesis required two principal problems to be solved, *viz*., introduction of a methyl group into position 1 of the steroid skeleton and regeneration of

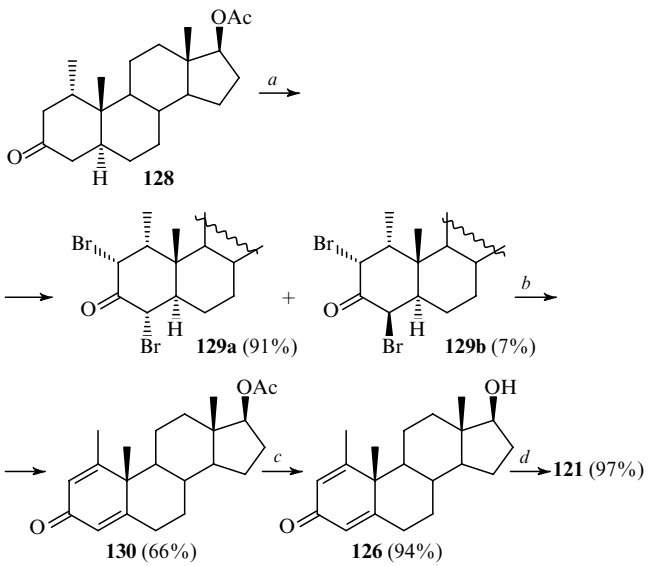
the 1,2-double bond in the resulting 1α-methyl-Δ^4-3-oxo derivative **120**. The first problem can be solved through selective 1,4-addition of organometallic compounds (*e.g.*, methyllithium cuprates) to ADD.[106] However, the high cost of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) employed in 1,2-dehydrogenation of Δ^4-4-ketosteroid **120** and the formation of a 4,6-dehydro derivative as a side product are the limiting factors.[107, 108]



Early patents applied by the 'Schering AG' company dealt with the synthesis of atamestane **121** by oxidation of its 17β-hydroxy derivative **126**[102] or by microbiological dehydrogenation of the 1-methyl-Δ^1-5α-derivative **127** in up to 65% yields.[109, 110] Compounds **126** and **127** were prepared in a multistep procedure (see below) and in unsatisfactory yields.



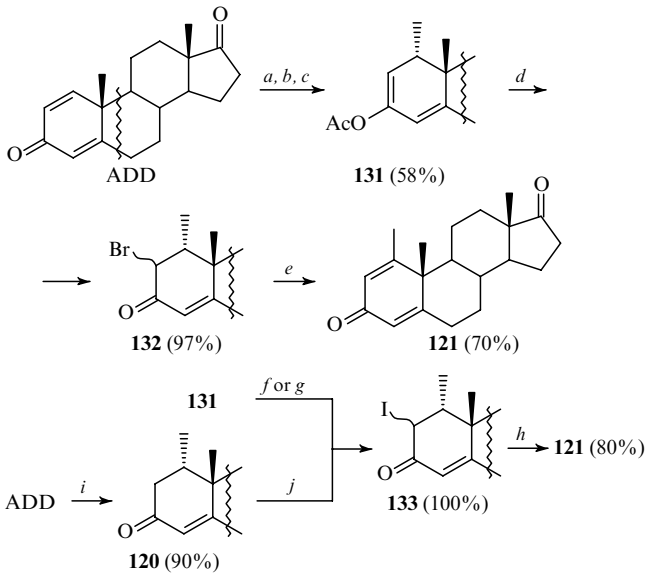
Thus the 4,5-double bond in the 1α-methyl derivative **123** obtained from the dienone **122**[106] was reduced and the resulting compound **124** was brominated to give the 2,4-dibromosteroid **125**. Elimination of two HBr molecules from the dibromide **125** gave the dienone **126**.[111, 112] Oxidation of the 17β-hydroxy group in compound **126** gave the target product **121**. The characteristic feature of this synthetic scheme is that the 4,5-double bond in the intermediate compounds is first reduced and then recovered together with the formation of the 1,2-double bond. Obvious disadvantages of this scheme consist in the formation of an undesirable side product, *viz.*, 17β-hydroxy-1α-methylandrostane-4,6-dien-3-one, and the necessity of tedious chromatographic purification following dehydrobromination. Nevertheless, this scheme has recently been applied[113] in the synthesis of atamestane **121** from 1α-methyldihydrotestosterone acetate (**128**)[114] with satisfactory yields at all steps.



(*a*) 2 equiv. Br_2, 4N HBr, AcOH, THF, 50 °C, 0.5 h; (*b*) LiBr, Li_2CO_3, DMF, 80 °C, 18 h; (*c*) MeOH, NaOH, Δ, 1.5 h; (*d*) TPAP – NMO, CH_2Cl_2, 20 °C.

Dibromination has led to the epimeric dibromides **129a**,**b** isolated in the individual state, and their configuration was established by ^{1}H NMR. However, dehydrobromination of compounds **129a**,**b** with lithium carbonate and lithium bromide occurred in only 66% yields. No formation of side products has been reported. The alcohol **126** prepared by deacetylation of the 17β-acetoxy derivative **130** was oxidised into atamestane **121** using a tetrapropylammonium perruthenate – *N*-methylmorpholine *N*-oxide system in quantitative yield. The overall yield was 60%.[113]

A shorter and more efficient method for the synthesis of atamestane **121** which does not require chromatographic procedures has been patented.[115]



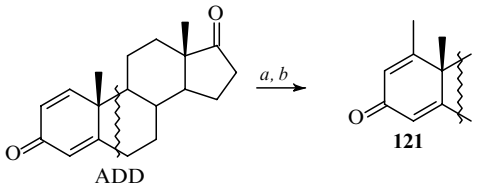
(*a*) MeLi, CuI, $Cl(CH_2)_2Cl$, −30 to 0 °C; (*b*) Ac_2O, $Cl(CH_2)_2Cl$, 20 °C; (*c*) NH_4Cl, H_2O, 20 °C; (*d*) 1,3-bromo-5,5-dimethylimidazolidin-2-one (dibromantin), dioxane, H_2O, 20 °C; (*e*) MgO, DMF, 110 °C, 2 h; (*f*) NaOAc, ICl, Me_2CO, −10 °C, 99% (2β-I-isomer); (*g*) $(CH_2CO)_2NI$, abs. MeOH, N_2, 20 °C, 55% (2α-I-isomer); (*h*) Li_2CO_3, DMF, 130 °C, 1.5 h; (*i*) Me_3Al, 1 mol.% – 5 mol.% CuBr, Me_3SiCl, THF – PhMe, 20 °C; (*j*) I_2, CuO, glacial AcOH, N_2, 60 °C, 24 h.

The intermediate 1α-methylenolate formed upon 1,4-addition of dimethyllithium cuprate to ADD in the presence of acetic anhydride is converted into the crystalline dienol acetate **131**. Bromination of the latter in aqueous dioxane gave a mixture of epimeric 2ξ-bromo-1α-methylandrost-4-en-17-ones (**132**) in which the 2β-isomer was predominant. Dehydrobromination of the bromo ketone **132** with magnesium oxide resulted in the target product **121** in 70% yield.

Dehydroiodination of the 2-iodo derivative **133** is a modification of the above-described procedure.[116] This compound is formed from dienol acetate **131** and monochloroiodine in acetone in quantitative yield. When *N*-iodosuccinimide was used, the yield of the iodide **133** dropped to 55%. The 2-iodosteroid **131** was also obtained by iodination of 1α-methyl-Δ^4-3-ketone **120** with elementary iodine in glacial acetic acid. Dehydroiodination proceeded smoothly on boiling of the iodo derivative **131** with lithium carbonate in DMF.

Yet another method for obtaining the intermediate **120** for the synthesis of atamestane **121** was the use of trimethylaluminium in the presence of catalytic amounts (1 mol.% – 5 mol.%) of monovalent copper salts for the introduction of the 1α-methyl group into ADD.[117] Various modifications of this approach, in particular, the use of Lewis acids for acceleration of the reaction and supression of competitive 1,2-addition and the mechanism of these reactions are discussed in detail with steroid 1,4-dien-3-ones and 1-en-3-ones as examples.[114] It was shown that selective 1,4-addition of trimethylaluminium to ADD occurs in the presence of equimolar amounts of chlorotrimethylsilane and catalytic amounts of copper(I) bromide, the yield of 1α-methylandrostat-4-ene-3,17-dione **120** being 90%.

Finally, a single-step procedure for the synthesis of atamestane **121** from ADD has been patented.[118] This method consists in successive treatment of ADD with trimethylaluminium in hexane with a stoichiometric amount of sterically hindered di-*tert*-butylphenol in the presence of catalytic amounts of nickel(II) acetylacetonate. The yield of steroid **121** was 42%.



(*a*) Me_3Al, n-C_6H_{14}; (*b*) 3,5-$(Bu^t)_2C_6H_3OH$, EtOAc, $Ni(acac)_2$, 58 °C, 2 h.

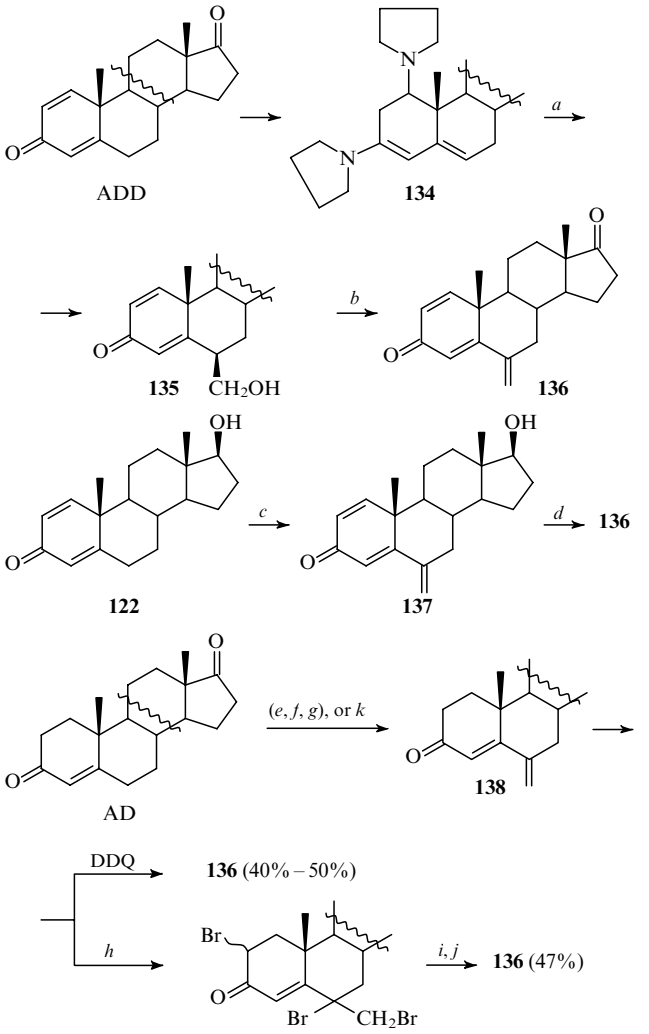
Syntheses of 15α-alkyl atamestane analogues[107, 119, 120] and 1β,2β-cyclopropa-4-OHA have been patented.[121]

4. 6-Substituted androstanes

6-Methyleneandrosta-1,4-diene-3,17-dione (**136**) synthesised in the late 80's as an anticancer drug known under the trade name 'exemestane' is the most well-known aromatase inhibitor of the 6-substituted androstane series. It represents an orally active irreversible selective AI.[9, 122 – 124] Exemestane **137** is shown to be 2.5 times more active than formestane **91**.[124]

Exemestane can be synthesised from both AD and ADD (Scheme 6). The key stage in this synthesis is methylenation of a steroid at position 6. When AD is used as the starting compound, the methylene group at position 6 is introduced by direct methylenation of 3-oxo-4,5-dehydrosteroid using the method of Annen *et al.* [125] The yields in this stage do not exceed 45%; the penultimate stage consists in 1,2-dehydrogenation of 6-methylene-AD **138** by boiling either with DDQ in anhydrous dioxane or with selenium dioxide in *tert*-butyl alcohol. The yields in the second stage are 40% – 50%.[117] Thus, the overall yield of exemestane was as low as 20% – 25%. This scheme involves tedious chromatographic purification of reaction products in both stages and the use of an expensive reagent (DDQ). An

Scheme 6



(*a*) HCHO, PhH, EtOH, 90%; (*b*) MeOH, HCl, 20 °C, 5 h (80%); (*c*) HCHO, $Me_2NH \cdot HCl$, Am^iOH, 131 °C (31%); (*d*) CrO_3, H_2SO_4 (79%); (*e*) $HC(OEt)_3$, *p*-TsOH, THF – EtOH, 40 °C, 2 h; (*f*) PhNHMe, HCHO, 40 °C, 2 h; (*g*) conc. HCl, 1 h (73%); (*h*) Br_2, Et_2O, HBr, AcOH, 2 – 3 °C (84%); (*i*) NaI, Me_2CO, Δ, 15 min; (*j*) Li_2CO_3, LiCl, DMF, 120 °C, 2.5 h; (*k*) $H_2C(OR)_2$, $POCl_3$, $CHCl_3$, NaOAc, Δ (40% – 50%).

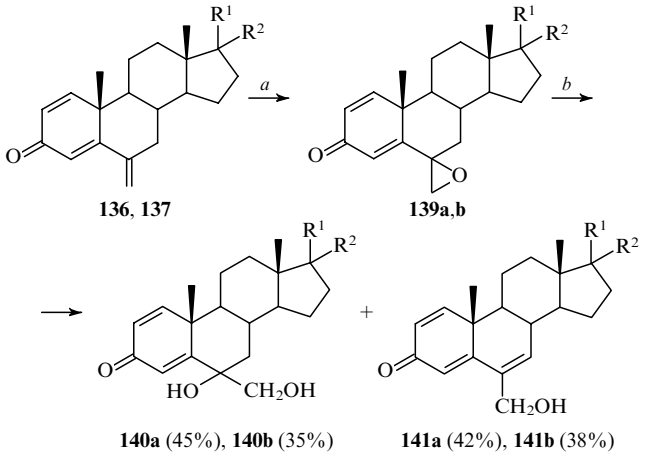
improved procedure for the synthesis of the steroid **136** has been patented. Although this requires a larger number of steps, it yields only crystalline intermediates and is suitable for large-scale syntheses. It includes the Mannich aminomethylation of a 3,5-dien-3-ol ester prepared from AD (see Scheme 6). The 6-methylene steroid **138** is obtained in > 70% yield. Compound **138** is then brominated with bromine in acetic acid. Dehydrobromination of the tribromide thus formed is carried out in two steps. At first, the 6-methylene group is regenerated by treatment of the tribromide with sodium iodide in acetone. The resulting 2-bromo-6-methylene steroid (not shown in Scheme 6) is further dehydrobrominated without purification by boiling with lithium salts in DMF to give exemestane **136** in 47% yield for the last two stages.[126]

Two approaches for the synthesis of exemestane **136** from 3-oxo-1,4-didehydrosteroids are shown in Scheme 6. Direct γ-methylenation, which is applicable for a wide range of substrates,[125] has failed in the case of ADD. However, the 17β-hydroxy derivative **122**, which is an accessible industrial raw material, can participate in the Mannich reaction. The resulting product **137** formed in 31% yield is oxidised to exemestane **136**.[127] And, finally, ADD was used directly as the starting material for introducing the 6-methylene group *via* the 1,3-bispyrrolidino derivative **134** which was converted into 6β-hydroxymethyl ste-

roid **135** by standard procedures.[128] Dehydration of the latter gave exemestane **136** in 80% yield.[129]

A series of exemestane analogues modified at position 6 and its 17β-hydroxy derivatives have been synthesised in order to investigate the structure – activity relationships.[124] The reason for these studies was the experimental evidence about a wide set of exemestane metabolites. The design of its analogues was based on the hypothesis according to which exemestane metabolism results in modification of not only the carbonyl group at C(17) but also the exocyclic double bond.[124]

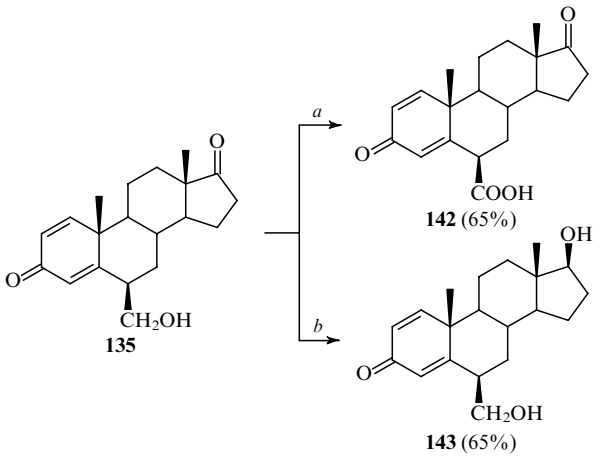
Synthesis of exemestane **136** analogues is considered below. Epoxidation of exemestane **136** or its 17β-hydroxy derivative **137** with a peroxy acid gave epimeric mixtures of spirooxiranes **139a**,**b**, respectively. Their subsequent hydrolysis with aqueous perchloric acid gave 6-hydroxymethyl-1,4,6-trienes **141a**,**b** along with epimeric 6-hydroxy-6-hydroxymethyl derivatives **140a**,**b**.[124]



136, **137** **139a**,**b** **140a** (45%), **140b** (35%) **141a** (42%), **141b** (38%)

R^1, R^2 = O (**136**, **139a**–**141a**); R^1 = β-OH, R^2 = H (**137**, **139b**–**141b**); (*a*) MCPBA, CH_2Cl_2, 20 °C, 48 h (80%); (*b*) $HClO_4$, THF, 20 °C, 3 h.

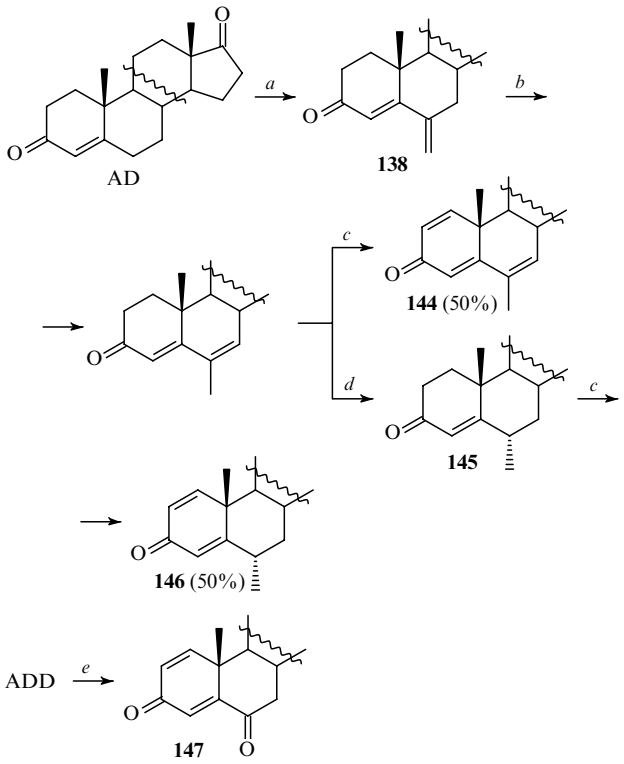
The Jones oxidation of 6β-hydroxymethyl-AD **135** gave 6β-carboxy-AD **142**, while selective reduction of the diketone **135** with sodium borohydride gave the 17β-hydroxy derivative **143**.[124]



135 **142** (65%) **143** (65%)

(*a*) CrO_3, H_2SO_4; (*b*) $NaBH_4$, MeOH.

A series of 6-methyl-substituted AD and ADD have been synthesised. Thus 6-methylene-AD **138** was isomerised over Pd/C into a 4,6-didehydro-6-methyl analogue. Its dehydrogenation with DDQ gave 1,4,6-triene **144** in 50% yield, while reduction over Pd/C in the presence of cyclohexene as a hydrogen donor yielded 6α-methyl-AD **145**. The latter was further dehydrogenated into 6α-methyl-1,4-diene steroid **146**. Finally, oxidation of ADD with oxygen in the presence of alkali gave androsta-1,4-diene-3,6,17-trione **147**.[124] Preparation of the 2-fluoro derivative of exemestane is patented.[130]



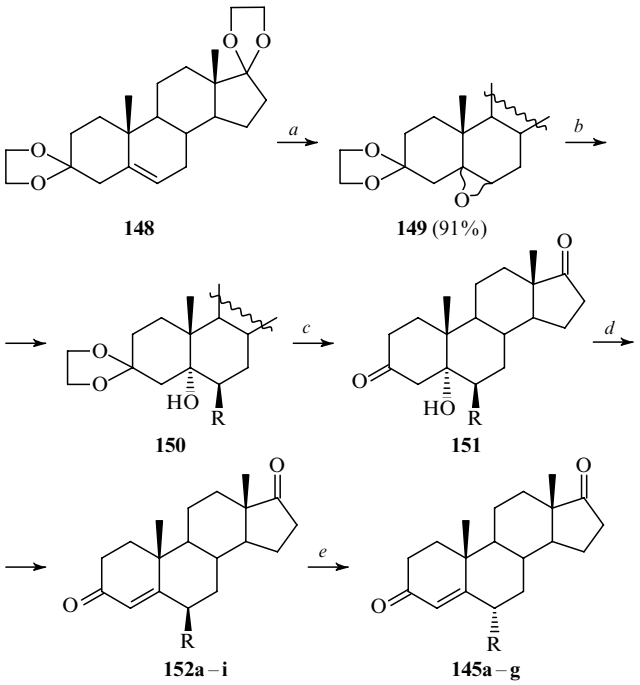
AD **138** **144** (50%) **145** **146** (50%) **147**

(*a*) $CH_2(OEt)_2$, $POCl_3$, $CHCl_3$ (60%); (*b*) 5% Pd/C, EtOH, Δ, $PhCH_2OH$ (90%); (*c*) DDQ, dioxane, Δ, 15 h; (*d*) Pd/C, cyclohexene (60%); (*e*) O_2, $KOBu^t$, Bu^tOH (15%).

Exemestane **136** is a highly selective irreversible AI. The irreversibility of its inhibitory action is assigned to the presence of a 1,2-double bond in the molecule;[123] the corresponding 1,2-hydrogenated analogues represent reversible AI. It is these properties rather than pharmacokinetic ones that account for the prolonged action of exemestane on the organism.[123]

None of the synthetic exemestane analogues displays *in vitro* activity which would be equal or greater than that of exemestane itself. Thus the 17β-hydroxy derivative **137** is active *in vitro*, but its activity is 2.6-fold less than that of compound **136**. According to their inhibitory activity, exemestane analogues modified at C(6) and having a 17-keto group can be arranged in the following order: 6-methylene (**136**) > 6-spirooxirane (**139a**) > 6β-hydroxymethyl (**135**) > 6-hydroxy-6-hydroxymethyl (**140a**) > 6β-carboxy derivative (**142**). 17β-Hydroxy analogues of some of these compounds, *e.g.*, **139b**, **140b** and **143**, manifest 3–8-fold lower activity than the corresponding 17-oxo compounds. Isomerisation or reduction of the double bond (leading to compounds **144** and **146**) decreases their inhibitory activity threefold in comparison with exemestane itself.[124]

A series of 6-alkyl- and 6-aryl-substituted AD has been described and their inhibitory activity has been studied with the view of determining the topography of the hydrophobic binding pocket in the active site of the enzyme.[131–134] The key compound in this synthesis was a mixture of isomers of 5,6-epoxide **149** obtained by epoxidation of androst-5-ene-3,17-dione diketal **148**. The opening of the oxirane ring with Grignard reagents gave 5α-hydroxy-6β-substituted androstanes **150**. Treatment of the latter with $HClO_4$ in THF gave the corresponding 3,17-diketones **151** in high yields. Dehydration of these hydroxy diketones with thionyl chloride in pyridine has led to a series of 6β-substituted AD **152a**–**i**. Epimerisation of the 6β-centre in 6β-substituted Δ^4-3-ketones **152** into thermodynamically more stable 6α-isomers **145a**–**g** occurred in 16%–56% yields.[131,132]

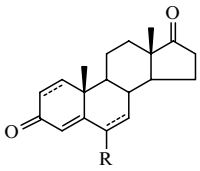


R = Me (**a**), Et (**b**), Pr^n (**c**), Bu (**d**), Pr^i (**e**), Ph (**f**), CH_2Ph (**g**), $CH=CH_2$ (**h**), $C\equiv CH$ (**i**).
(*a*) MCPBA, CH_2Cl_2, 20 °C, 4 h (a mixture of isomers ~ 1 : 1); (*b*) RMgBr, THF, Δ (86% – 98%); (*c*) 3M $HClO_4$, THF, 20 °C, 3 h; (*d*) $SOCl_2$, Py, 0 °C, 3 min; (*e*)1M HCl, EtOH, Δ (16% – 56%).

Biochemical assessment of both 6α-(**145**) and 6β-(**152**) substituted AD has been carried out (Table 3). These compounds appeared to be competitive aromatase inhibitors with K_i ranging from 1.4 to 63 nM. Introduction of a methyl group into 6α- and 6β-positions of AD increases its affinity for aromatase. Introduction of an additional methylene group into position 6 of the steroid molecule changes its inhibitory properties. Thus 6β-ethyl-AD **152b** had the highest K_i value (1.4 nM) in this series of compounds. Further elongation of the chain does not result in any significant alteration in the inhibitory activity. 6α-Benzyl-AD **145g** and 6β-vinyl-AD **152h** also manifest high activity. Bulky substituents, such as 6-isopropyl (**152e** and **145e**) or polar substituents, like 6β-ethynyl-(**152i**), decrease the inhibitory activity (K_i = 21 – 63 nM). The activity of 6β-substituted AD decreases in the following order: Et > Pr > $CH=CH_2$ > Bu > Me > Pr^i > Ph > $C\equiv CH$ > CH_2Ph, while that of 6α-substituted AD decreases in the order: Et > Me > Pr^n > CH_2Ph > Bu > Ph > Pr^i. The stereochemistry of the C(6) atom affects the inhibitory activity. Thus 6β-alkyl isomers possess greater affinity for the enzyme than the corresponding 6α-isomers with the exception of 6α- and 6β-methyl analogues **145a** and **152a**. Exchange of the 6β-ethyl substituent by the 6β-vinyl substituent decreases the affinity of compound **152h** compared to that of compound **152b**. It was shown that there is a hydrophobic binding pocket in the active site of the enzyme in the β-region of position 6 of the steroid substrate. Judging by the data on the crystal structure of cytochrome P-450 and the inhibitory activity of steroid substrates of this series, the following sizes for the pocket have been proposed: length 6,27 Å, width 5,25 Å, height 7,74 Å.[131] In 1995, Numazawa and Oshibe [131] synthesised and assayed 6α- and 6β-alkyl-substituted AD with 5 – 7 methylene groups in the side chains. Their K_i values varied from 2.8 to 80 nM. The earlier structure – activity regularities were also confirmed for compounds of this series. Thus 6β-pentyl-AD manifested the highest activity; further elongation of the chain sharply decreased the affinity.[133]

The analysis of effects of the chain length and configuration of the alkyl substituent at position 6 of ADD analogues [135] on their inhibitory activity is an extension of earlier studies.[131, 133]

Table 3. Data on *in vitro* inhibition of aromatase from human placenta by 6-substituted androstanes.

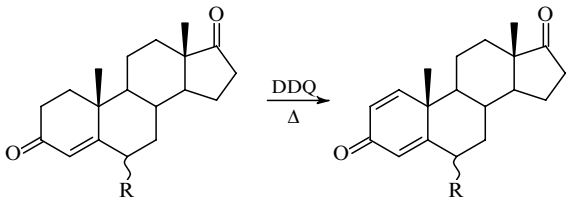


R	The position of double bond [a]		K_i /nM	IC_{50} /nM	Ref.
	Δ^1	Δ^6			
α-Me (**145a**)	–	–	5.6	350	131
β-Me (**152a**)	–	–	11	720	131
α-Et (**145b**)	–	–	4.7	260	131
β-Et (**152b**)	–	–	1.4	140	131
α-Pr^n (**145c**)	–	–	6.7	390	131
β-Pr^n (**152c**)	–	–	4.6	240	131
α-Bu^n (**145d**)	–	–	12	790	131
β-Bu^n (**152d**)	–	–	8.8	510	131
α-Pr^i (**145e**)	–	–	31	1400	131
β-Pr^i (**152e**)	–	–	22	1100	131
α-Ph (**145f**)	–	–	21	1100	131
β-Ph (**152f**)	–	–	37	1700	131
α-CH_2Ph (**145g**)	–	–	10	660	131
β-CH_2Ph (**152g**)	–	–	63	4000	131
β-$CH=CH_2$ (**152h**)	–	–	5.1	320	131
β-$C\equiv CH$ (**152i**)	–	–	62	1900	131
Pr^i	–	–	4.9		131
α-Br	–	–	3.4		173
β-Br	–	–	800		173
=O (**147**)	+	–		268	124
$=CH_2$ (**136**)	+	–	26 – 27	42.5	9, 97, 124
α-Me (**146a**)	+	–	54		135
β-Me (**153a**)	+	–	43		135
α-Et (**146b**)	+	–	19		135
β-Et (**153b**)	+	–	4.7		135
α-Pr^n (**146c**)	+	–	22		135
β-Pr^n (**153c**)	+	–	7.0		135
α-Bu^n (**146d**)	+	–	16		135
β-Bu^n (**153d**)	+	–	14		135
α-$(CH_2)_4Me$ (**146e**)	+	–	11		135
β-$(CH_2)_4Me$ (**153e**)	+	–	5.0		135
α-$(CH_2)_5Me$ (**146f**)	+	–	25		135
β-$(CH_2)_5Me$ (**153f**)	+	–	8.0		135
α-$(CH_2)_6Me$ (**146g**)	+	–	20		135
β-$(CH_2)_6Me$ (**153g**)	+	–	7.8		135
Me (**144**)	+	+		91	124

[a] Symbols + and – denote the presence and absence of the double bond in this position, respectively.

The 6α- and 6β-alkyl-substituted ADD **146a – g** and **153a – g** were obtained by dehydrogenation of their Δ^4-3-keto precursors.

Compounds **146a – g** and **153a – g** appeared to be competitive AI with K_i ranging from 4.7 to 54 nM (see Table 3). Among those, only 6α-ethyl-ADD **146b** is the suicide AI. 6β-Isomers manifest greater activity than 6α-analogues. However, there was no correlation between the length of the alkyl chain and the affinity for the enzyme. The ability of 6β-alkyl isomers to inhibit aromatase decreases in the following order: Et > $(CH_2)_4Me$ > Pr > $(CH_2)_6Me$ > $(CH_2)_5Me$ > Bu > Me, which is consistent with the results obtained for 6β-alkyl derivatives of AD.[133] The activity of 6α-isomers of alkyl-substituted ADD **146a – g**



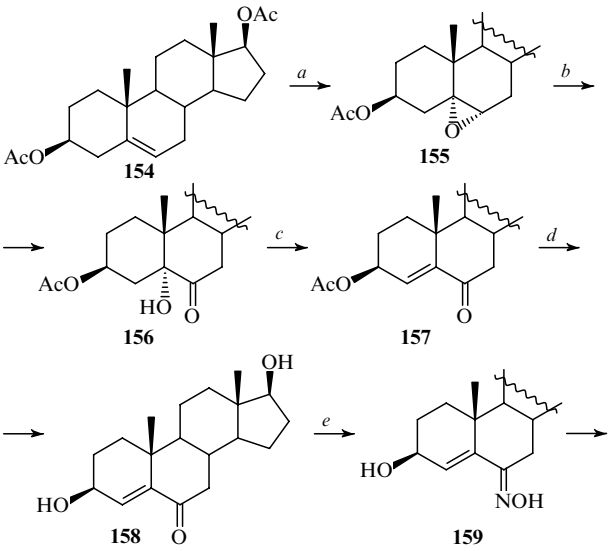
145a–d, i–l
152a–d, k–m

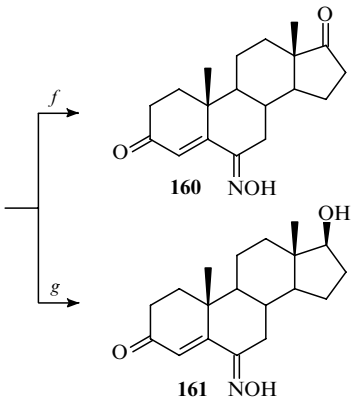
146a–g, 153a–g

R	α-Isomer		β-Isomer	
	original compound	product	original compound	product
Me	**145a**	**146a**	**152a**	**153a**
Et	**145b**	**146b**	**152b**	**153b**
Pr	**145c**	**146c**	**152c**	**153c**
Bu	**145d**	**146d**	**152d**	**153d**
Am	**145i**	**146e**	**152k**	**153e**
$(CH_2)_5Me$	**145k**	**146f**	**152l**	**153f**
$(CH_2)_6Me$	**145l**	**146g**	**152m**	**153g**

decreases in the order: $(CH_2)_4Me$ > Bu > Et > $(CH_2)_6Me$ > Pr > $(CH_2)_5Me$ > Me, which does not match that for 6α-alkyl-AD.[133] Based on the experimental data and the results of molecular modelling studies, Numazawa *et al.*[135] assumed that at least 6β-alkyl-ADD **153** can bind with the same hydrophobic pocket in the active centre of aromatase as does the corresponding 6β-alkyl-AD **152**.

6-Hydroxyimino-AD **160** and its 17β-hydroxy analogue **161** are competitive AI.[136] The key derivative **158** was obtained by standard methods from 3,17-diacetate **154** *via* 5α,6α-epoxide **155** with its subsequent oxidative conversion into 5α-hydroxy-6-ketone **156** with chromic anhydride. Dehydration of the latter and hydrolysis of 3,17-acetoxy groups in Δ^4-6-ketone **157** gave the dihydroxy derivative **158** in high yield. Treatment of the latter with hydroxylamine hydrochloride resulted in the (*E*)-oxime **159**; its oxidation with chromic anhydride in pyridine gave 6-hydroxyimino-AD **160** in 70% yield. Oxidation of oxime **159** with manganese dioxide under mild conditions resulted in the testosterone derivative **161**.

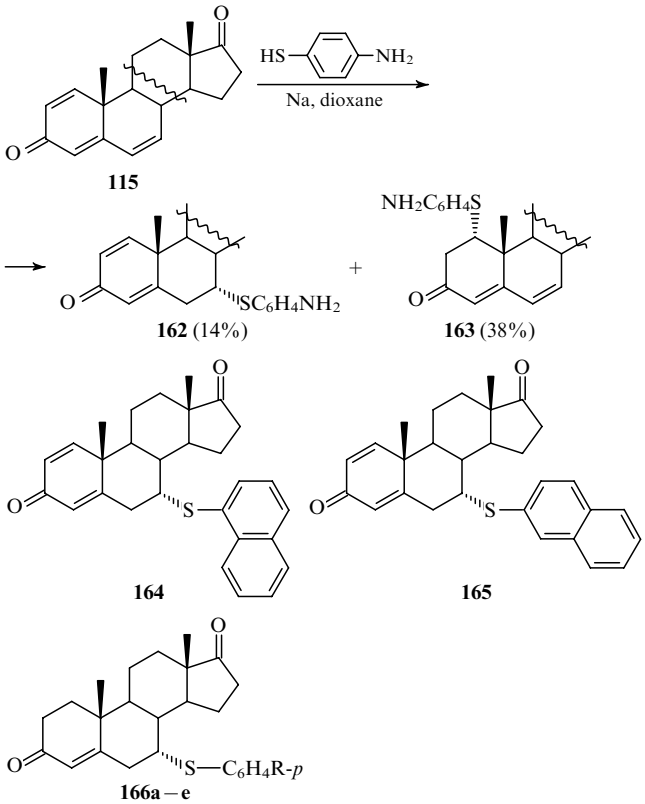




(*a*) MCPBA, CH_2Cl_2; (*b*) CrO_3, H_2O; (*c*) $SOCl_2$, Py (90%); (*d*) KOH, MeOH (82%); (*e*) $NH_2OH \cdot HCl$ (75%); (*f*) CrO_3–Py (70%); (*g*) MnO_2, $CHCl_3$, 20 °C (45%).

5. 7-Substituted and 6,7-disubstituted androstanes

The key stage in the synthesis of 7-substituted androstanes is 1,6-conjugate addition of the corresponding reagents to 6-dehydro derivatives of AD and ADD. Thus 7α-thio-substituted ADD **162**, one of the first highly active 7α-substituted androstanes, was obtained in 14% yield by addition of *p*-aminothiophenol to 6-dehydro-ADD **115**.[137]



R = H (**a**), NH_2 (**b**), I (**c**), OMe (**d**), N_3 (**e**).

This is accompanied by the formation of its inactive 2α-isomer **163** (yield ~ 40%). The attempts to improve the regioselectivity and to increase the yield of 7α-substituted compound **162** by changing the reaction conditions (solvent, temperature and reagent ratio) failed.[137]

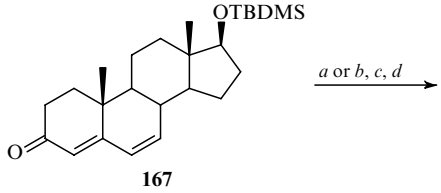
Further transformations of 7α-(*p*-aminophenylthio)-ADD **162** have led to its analogues with bromine or iodine atoms in the *para*-position of the aryl substituent. 7α-Naphthalene derivatives **164** and **165** are also described.[138] All these steroids are irreversible suicide AI (K_i = 12–27 nM).[138] According to the patent,[139]

7α-alkyl(acyl)thio- and 7α-mercapto-ADD are highly active preparations for *in vivo* treatment of mammary tumours.

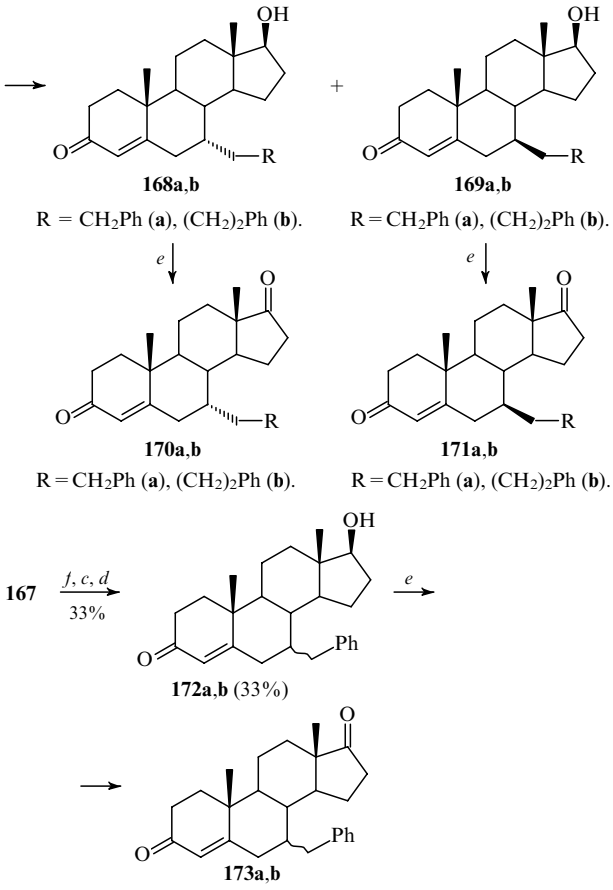
7α-Arylthio-substituted AD **166a**–**e** were obtained by analogous addition of substituted thiophenols to 6-dehydro-AD.[24] The activity of the steroid **166b** was twice as low as that of its ADD analogue **162**. The steroid **166e** (K_i = 1 nM) was the most efficient in this series (Table 4).[25, 140]

The compounds described above contain an aryl substituent which is linked to the steroid skeleton by the C–S bond. It was found, however, that the presence of a sulfur atom and the stereochemistry of the C(7) atom are not essential for the manifestation of activity, since 7α- and 7β-substituted AD **170a**,**b**, **171a**,**b** and **173a**,**b** with one or several methylene groups between the steroid skeleton and the phenyl substituent appear to be efficient AI.[141]

These androgens with arylaliphatic substituents were obtained by the same approach as that used for 7α-thio-substituted compounds described above. The silylated testosterone derivative **167** was used as the starting compound. Introduction of a phenylethyl or a phenylpropyl substituent was carried out by addition of the cuprate reagent prepared from the corresponding lithium reagent and 1 equiv. of copper(I) tetrakis(tributylphosphine) iodide. Subsequent addition of 0.2 equiv. of the steroid **167** and deprotection gave the desired 1,6-addition products as mixtures of α,β-diastereomers **168a** and **169a** (71%, α:β = 2:1) or **168b** and **169b** (50%, α:β = 1:1) isolated in the individual state by chromatography. The structure of the 7α-isomer **168a** was additionally confirmed by X-ray analysis. Oxidation of 17β-alcohols **168a**, **169a**, **168b** and **169b** gave 17-ketones **170a**, **171a** and **171b**, respectively. 7α- And 7β-benzyl derivatives **172** and **173** were obtained in a similar way.

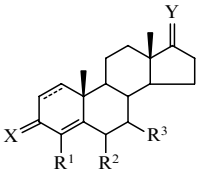


167



(*a*) $Ph(CH_2)_2I$, Bu^tLi, Et_2O, −78 °C; (*b*) $Ph(CH_2)_3I$, Bu^tLi, Et_2O, −78 °C; (*c*) $[CuI(Bu^n_3P)]_4$, Et_2O, −40 °C; (*d*) 6N HCl, CH_2Cl_2, 20 °C, (7α:7β = 5:1); (*e*) PCC, CH_2Cl_2, 20 °C; (*f*) $PhCH_2Br$, Mg, Et_2O, 20 °C.

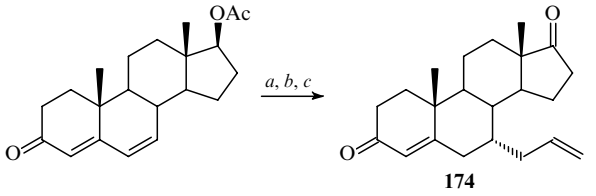
Table 4. Data on *in vitro* inhibition of aromatase from human placenta by 7-substituted and 6,7-disubstituted androstanes.



Compound	R^1	R^2	R^3	X	Y	The presence of a Δ^1 double bond	K_i /nM	IC_{50} /nM	Ref.
166a	H	H	α-SPh	O	O	–	212	650	80
166b	H	H	α-$SC_6H_4NH_2$	O	O	–	18		25, 141
166c	H	H	α-SC_6H_4I	O	O	–	12		25, 15
166d	H	H	α-SC_6H_4OMe	O	O	–	31		25
166e	H	H	α-$SC_6H_4N_3$	O	O	–	1		25
173a	H	H	α-CH_2Ph	O	O	–	18.9		141
173b	H	H	β-CH_2Ph	O	O	–	44.5		141
170a	H	H	α-$(CH_2)_2Ph$	O	O	–	13.1		141
170a	H	H	β-$(CH_2)_2Ph$	O	O	–	40.2		141
170b	H	H	α-$(CH_2)_3Ph$	O	O	–	16.5		141
172	H	H	α-CH_2Ph	O	β-OH	–	39.5		141
168a	H	H	α-$(CH_2)_2Ph$	O	β-OH	–	36.0		141
162	H	H	α-$SC_6H_4NH_2$	O	O	+	9.9		25
179	OPh	H	α-SPh	O	O	–	472	1650	80
187	H	α-CF_2		O	O	–	50		145
189	H	α-CH_2		H_2	O	–	5.0		145
190	H	α-CH_2		H_2	β-OH	–	120		145

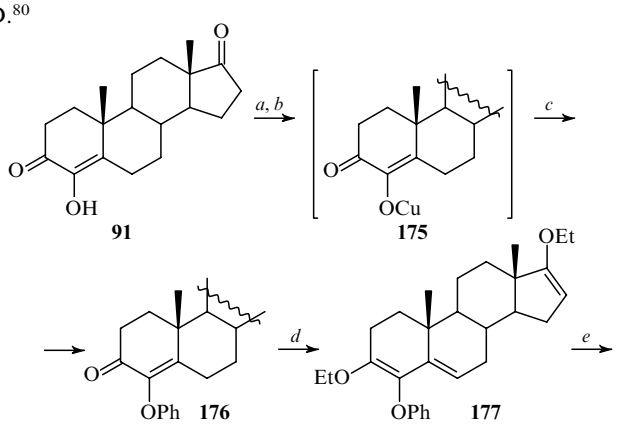
7α-Substituted AD **170a**,**b** and **173a** are efficient competitive AI with K_i 13–19 nM. The corresponding 17β-alcohols **168a**,**b** and **172** as well as 7β-substituted AD **171a**,**b** and **173b** are less active (K_i = 36–44 nM). Thus, introduction of bulky aryl-aliphatic substituents into position 7 of AD favours high inhibitory activity. It was found that the length of the carbon chain between the steroid and the aryl group does not significantly affect the activity, thus indicating the presence of a bulky pocket in the active site of the enzyme near position 7 of the steroid substrate.

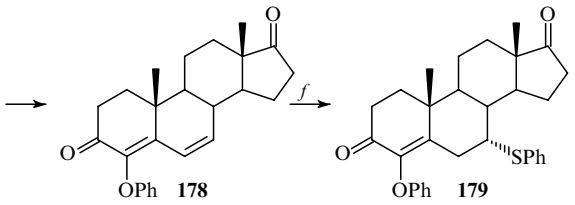
According to patent data,[142] 7α-allyl-AD **174** synthesised from the corresponding 17β-acetoxy-androsta-4,6-dien-3-one is a rather active AI.



(*a*) $H_2C=CHCH_2SiMe_3$, $TiCl_4$, CH_2Cl_2, −70 °C; (*b*) HC/MeOH; (*c*) PCC.

Disubstituted 4-phenoxy-7α-phenylthio-AD **179** has been synthesised in order to explore the nature of the binding site of aromatase in the vicinity of the C(4), C(6) and C(7) atoms of AD.[80]





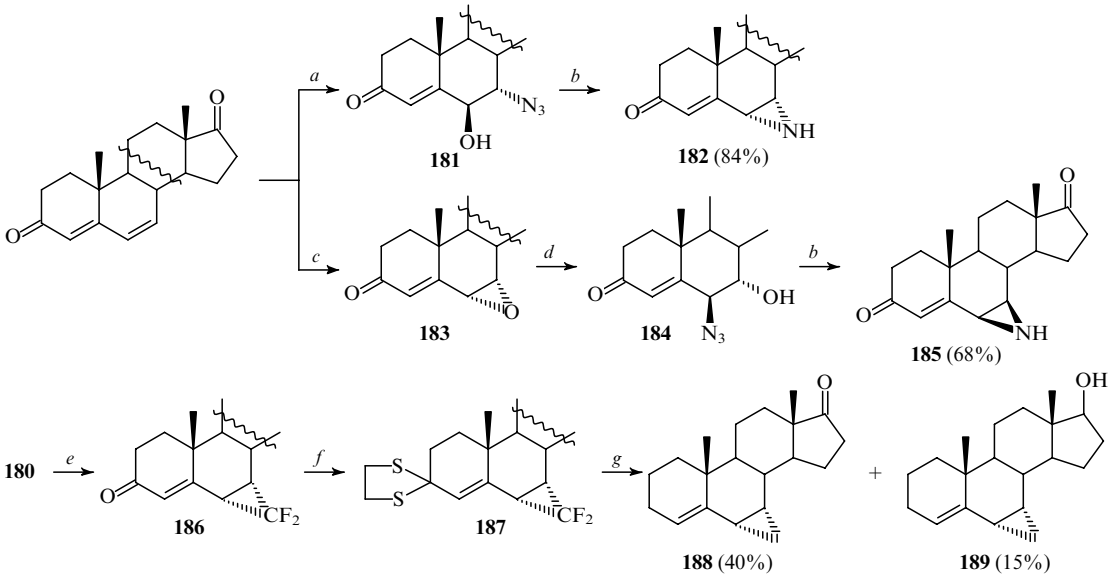
(*a*) CuI, Bu_2S, MeLi, Et_2O, −78 to 20 °C; (*b*) Et_2O, THF, Py, −78 °C; (*c*) PhI, Δ (55%); (*d*) $CH(OEt)_3$, *p*-TsOH, 20 °C, 24 h; (*e*) DDQ (5%) aqueous Me_2CO, 20 °C, 3 min (50%); (*f*) PhSH, Na, dioxane, 60 °C, 8 h (40%).

The starting compound in this synthesis, 4-OHA **91**, was converted into the 4-phenoxy derivative **176** *via* the intermediate copper enolate **175** (yield > 50%).[81] The formation of the necessary 6,7-double bond was achieved by treating the ether **177** with an equimolar amount of DDQ in aqueous acetone. Addition of thiophenol to $\Delta^{4,6}$-4-phenoxy-AD formed completes the synthesis of the 4-phenoxy-7-phenylthio-substituted compound **179**. However, its inhibitory activity was much lower than that of the monosubstituted analogues, *viz*., 4-phenylthio- (**98d**) and 7α-phenylthio-AD **166a**. These results together with molecular modelling data for all the three compounds have led Liu *et al*.[80,81] to infer the existence of a single hydrophobic pocket in the active site of aromatase in the vicinity of the C(4) and C(7) atoms of the steroid substrate.

In conclusion, it is appropriate to mention the synthesis of 6,7-disubstituted androstanes. Thus 6,7-epimino-AD [143] represent a practically novel class of derivatives. The key compounds in this synthesis are 7α-azido-6β-hydroxy- (**181**) and 6β-azido-7α-hydroxy-AD **184** (Scheme 7). Treatment of 4,6-dienedione **180** with sodium azide and chromic anhydride in acetic acid gives the azido alcohol **181**, which was converted into 6α,7α-aziridine **182** in 80% yield upon heating with 2 equiv. of triphenylphosphine in toluene. Epoxidation of 6-dehydro-AD **180** with *m*-chloroperbenzoic acid in boiling chloroform gave the 6α,7α-oxide **183**; its diaxial opening in 6β-azido-7α-hydroxy-AD **184** occurred in high yield and was performed by treatment of an equimolar mixture of sodium azide and concentrated sulfuric acid in DMSO. Reaction of the azide **184** with triphenylphosphine in toluene gives 6β,7β-epimino-AD **185**.

The β-configuration of its three-membered ring was confirmed by ^{1}H NMR spectral data and by independent synthesis,

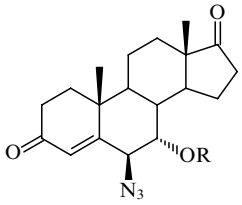
Scheme 7



(*a*) NaN_3, CrO_3, AcOH, 20 °C (42%); (*b*) Ph_3P, PhMe, Δ, 3 h; (*c*) MCPBA, Δ, 3 h (57%); (*d*) NaN_3, conc. H_2SO_4, DMSO, 20 °C (84%); (*e*) ClF_2CCO_2Na, diglyme Δ, 5 h; (*f*) $(CH_2)_2SH$, *p*-TsOH, AcOH, 20 °C, 24 h (77%); (*g*) Na–NH_3, THF, 0.5 h (40%).

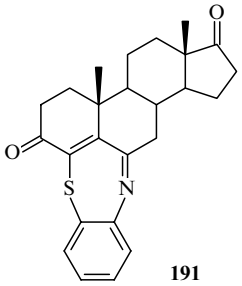
viz., by reduction of 7-mesylate **190a** with lithium aluminium hydride. The stereochemical aspect of the formation of steroids **182** and **185** deserves mention: in each case, the corresponding azido alcohol yields only one isomer with the aziridine ring configuration identical with that of the azido group.

The compounds obtained appeared to be weak aromatase inhibitors; only 6β-azido-7α-acetate **190b** manifested moderate inhibitory activity.[143, 144]



190a,**b**

R = Ms (**a**), Ac (**b**).



191

This section is finished with a description of a series of 6α,7α-cyclopropaandrost-4-en-17-ones.[145, 146] Addition of difluorocarbene generated from sodium difluorochloroacetate to 6-dehydro-AD **180** gave 6α,7α-difluorocyclopropane-AD **186**. Reduction of its 3-ethylenedithio derivative **187** with lithium in ammonia gave a mixture of 3-deoxy-6α,7α-cyclopropaandrost-4-en-17-one **188** and its 17β-hydroxy derivative **189**. These compounds proved to be efficient competitive AI,[145] the cyclopropane derivative **188** (K_i = 5 nM) being the most active one. However, introduction of a 6α,7α-difluorocyclopropane group into the AD molecule (compound **186**) did not affect considerably its affinity for the enzyme. This suggests that it is the keto group at position 17 of AD that plays the crucial role in the binding of the steroid with aromatase.

A recent study [147] devoted to the synthesis and biochemical assay of compound **191**, yet another representative of substituted androstanes obtained by the base-catalysed Michael addition of 2-aminothiophenol to 3,17-diacetoxyandrost-4-en-6-one with subsequent cyclisation, is also worth mentioning. Compound **191** manifested weak inhibitory activity of a competitive type (K_i = 42 300 nM).

IV. Structure – activity relationships in substituted androstanes as aromatase inhibitors

The data considered in Section III and those listed in Tables 1 – 4 allow one to make certain conclusions about the structural requirements for steroid substrates to act as aromatase inhibitors. Although the substituted androstanes described above are rather diverse in nature and mechanisms of their inhibitory action have hitherto been studied relatively little, one may note some general regularities concerning the relationships between their structure and inhibitory activity judging mainly by the affinity for the enzyme and inhibitory properties of a series of similarly substituted androstanes. According to Cole and Robinson,[15] efficient AI have two domains which play an important role in binding of the steroid substrate with the enzyme, namely, an iron-coordinating domain and a hydrophobic domain. The structural requirements for the latter are considered to be a crucial factor, although chemical details of its specificity are unknown.

The introduction into, or removal from, certain positions of the AD molecule of definite substituents and/or additional double bonds affect the inhibitory activity as indicated by arrows in Fig. 1.

The steroid molecule must contain at least one keto group, specifically at position 17 to manifest aromatase-inhibiting activity. All substituted androstanes sharply decrease their activity as AI upon reduction of the 17-keto group to the hydroxy group and especially following its esterification. Evidently, in some cases the carbonyl function at position 3 is not strictly required as a structural element, because, as has been shown in the preceding

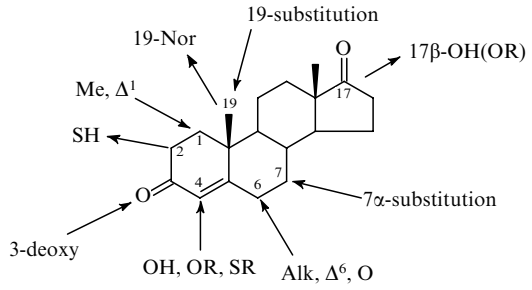


Figure 1. The functional groups affecting the inhibitory activity and their position in the AD molecule. The arrows directed towards a specific position denote enhancement of activity, those directed from this position denote decrease in activity.

section, some 3-deoxo analogues retain high inhibitory activity.[14, 33, 35, 36, 145] Moreover, replacement of the 3-keto group in AD or in 19-substituted AD by a 3-methylene group (or a halogenomethylene group) affords compounds possessing high competitive inhibitory activity.[148, 149] The study of inhibitory activity of androst-4-ene-3,6,17-trione and its 3-hydroxy analogue revealed that both of them can irreversibly bind with aromatase.[150, 151]

An important role in manifestation of biological activity is played by the conformation of the ring *A*. For example, androst-2-ene-3,17-dione binds with aromatase very weakly, whereas its 4-dehydro isomer is an efficient AI with K_i = 37 nM.[15,145,152] The presence of a substituent at position 10 of the steroid skeleton of AD is indispensable for activity. As has been shown in Section III, this substituent plays an essential role in the oxidation of the steroid substrate by the enzyme. Thus 19-nor-4-hydroxyandrost-4-ene-3,17-dione binds with aromatase but is devoid of inhibitory activity.[15, 64]

Introduction of additional double bonds into the molecule of AD and its substituted analogues enhances their inhibitory activity. It is particularly true for 1,2- and 6,7-double bonds. It is assumed [123] that the presence of a 1,2-double bond in a steroid molecule is responsible for the irreversible type of inhibition and, correspondingly, for the long-lasting effect of AI *in vivo*.

Introduction of various substituents into the rings *A* and *B* (positions 1, 4, 6, 7 and 19) as well as alkylation or arylation of 4-amino-, -mercapto- or -hydroxy-androstanes are the most common structural modifications. High affinity for the enzyme has been noted for steroid AI with small substituents at C(1), C(4) and C(19) and with bulky substituents at C(7). Introduction of hydrophobic substituents is a general trend in the design of efficient AI, although the optimal volume and position of functional groups are often difficult to predict.

2α-Substitution and involvement of the C(6) and C(7) atoms in the formation of a three-membered heterocycle have a negative effect.[14, 19]

Thus, studies of structure – activity relationships of steroid AI and their interactions with the active site of aromatase provides valuable information about the nature of the enzyme and allows one to gain better insight into the mechanism of action of this enzyme. Investigation of this mechanism is far beyond the scope of the present review; it is evident, however, that the synthesis and analysis of activity of various series of steroid AI represent a step on the way to its elucidation.

V. *In vivo* activities and applications of major steroid aromatase inhibitors

As can be seen from Tables 1 – 4, highly efficient AI usually have similar K_i which are close to the lower limits of sensitivity (0.5 – 1.0 nM). The possibility of their practical application will further be determined by their biological properties *in vivo,* pharmacological and pharmacokinetic characteristics, the pres-

ence or absence of side hormonal effects typical of some steroids of the androstane series, and, naturally, by the methods of their synthesis and the possibility of scaling.

This section considers pharmacological activities and clinical applications of several major substituted androstanes the properties of which have been studied in sufficiently great detail. Owing to their antitumour activity, some of these steroid compounds, *viz*., 4-hydroxy-AD **91**, 1-methyl-ADD **121** and 6-methylene-ADD **136**, have found wide use in the clinical practice. The former known under the trade name formestane is already used in the therapeutic practice, while the other two are undergoing clinical trials. Besides, there is ample literature evidence concerning the pharmacological activity of another two AI. The first of them, 19-ethynyl-AD **1** (promestane), manifests high activity in inhibiting ovarian aromatase on oral administration and efficiently reduces mammary and ovarian tumours in both experimental models and the clinic.[15, 36, 37] The synthetic 'Organon' product, 19-ethyldithio-AD, possesses very weak androgenic activity but very efficiently inhibits estrogen biosynthesis *in vivo*.[43, 44] Antitumour activities of these AI have been surveyed recently.[21, 153, 154]

4-Hydroxy-AD (**91**, formestane) was designed in 1984 by the 'Ciba-Geigy' company for treatment of post-menopausal mammary tumours in women and is now commercially available in Great Britain, Germany, Austria and some other countries.[71] Some patents issued by the 'Schering AG' company deal with their application in the therapy of benign prostatic hyperplasia.[65, 155]

Studies of formestane effects on human placenta aromatase revealed its high inhibitory activity.[62, 64] Formestane produced a significant antitumour effect in rats with experimental mammary tumours.[36, 70, 87] This inhibition is highly selective.[63] However, there is evidence that formestane also inhibits 5α-reductase.[69, 156]

One main disadvantage of formestane reported by some authors [48, 67, 88, 98, 157] is its low bioavailability and short-term effect. Since this drug is highly active on subcutaneous administration,[9] it is normally used in the form of injections. The results of clinical trials of this drug in patients with mammary tumours (500 – 1000 mg weekly, *i.m*.) testify to its high efficiency.[157 – 160] According to the experimental protocol elaborated for IInd phase of clinical trials, formestane injected once or twice a week causes partial or complete remission of the tumour.[66, 68, 158, 159] This drug is generally well tolerated and produces only nonspecific side effects. One should mention in this connection that replacement hydrocortisone therapy was necessary for patients receiving formestane for a prolonged period, since this drug inhibits pregnenolone biosynthesis and, as a consequence, biosynthesis of corticosteroids.[67, 161]

According to the literature data, atamestane and exemestane have no such shortcomings. Comparative characteristics of *in vivo* activities of formestane, atamestane and exemestane and analysis of mechanisms of their action in animals have been documented.[9, 69]

1-Methyl-ADD or atamestane was designed by the 'Schering AG' company in 1983 [102] and patented in more than 30 countries. The main objective of its clinical trials initiated in 1986 in Europe and Japan was to estimate its efficiency in the treatment of mammary tumours and endometriosis in women and of benign prostatic hyperplasia in men. As mentioned above, atamestane is a selective irreversible inhibitor of estrogen biosynthesis *in vitro* and *in vivo*. Studies in rats revealed that its activity exceeds that of formestane almost 1.5-fold;[102, 162] according to other data, its effect is weaker than those of formestane and examestane.[9] DMBA-induced mammary tumours in rats reduced appreciably after subcutaneous administration of atamestane (30 or 150 mg per kg per day).[103] Neither atamestane nor its main metabolites, *viz*., 5β- and 17β-reduced compounds, manifest any affinity for steroid receptors, which correlates with the endocrine-pharmacological profile of this drug.[104] *In vivo* studies failed to detect estrogen, anti-estrogen, androgen, progestane or antigonadotropic activities in atamestane.[105] As the affinity of atamestane for enzymes responsible for steroid biosynthesis in adrenal glands is negligibly small in comparison with its affinity for aromatase, it does not exert any appreciable influence on these glands even when used in high doses and upon prolonged administration. This finding has been confirmed experimentally.[105]

As has been mentioned above, atamestane has been recommended for treatment of benign prostatic hyperplasia.[154, 163] Along with other factors, estrogens play an important role in the etiology of this disease. Apart from the finging that the incidence of this disease correlates with a shift of the androgens/estrogens ratio towards estrogens upon ageing in males, the presence of the estrogen receptor meeting the classical criteria has also been demonstrated by Habenicht *et al*.[164, 165] The role of estrogens in the pathogenesis of this disease revealed in animals has been confirmed by clinical tests.[164] The results of successful clinical trials of atamestane administered in an oral dose of 200 mg 3 times daily for 3 months in the endocrine therapy of benign prostatic hyperplasia were published several years ago.[166] The use of atamestane in the form of tablets for increasing testosterone content in the organism has been patented.[162]

Exemestane **136** has also been recommended for clinical use. This preparation was designed in 1988 by the Italian company 'Farmitalia Carlo Erba' and tested in many countries of the world as an independent therapeutic agent for treating estrogen-dependent tumours, especially post-menopausal mammary tumours in women. The choice of exemestane is based on its pharmacological properties which characterise this drug as a highly active (on oral administration) selective AI.[9, 97] Studies in animals revealed long-term inhibition of ovarian aromatase on subcutaneous and oral administration.[9, 97, 167] The activity of exemestane exceeds that of formestane twofold on subcutaneous administration and 27-fold on oral administration.[123] This drug does not manifest any affinity for the majority of steroid receptors with the exception of its weak binding with the androgenic receptor; the androgenic activity of exemestane was as low as 10% of the testosterone activity.[98, 123] The results of endocrinological clinical trials of small doses of exemestane published in 1995 testify to a long-term selective decrease in estrogen levels and good tolerance of this drug.[168, 169] The antitumour effect of exemestane has been studied in a model of DMBA-induced rat mammary tumours.[167] Subcutaneous or oral administration of exemestane caused partial or complete remission of the tumour.[123, 124] Exemestane has a specific action on women: it does not influence the levels of hydrocortisone, aldosterone, 17-hydroxyprogesterone and luteinising and follicle-stimulating hormones even when used in very high doses.[123] The composition of tablets containing exemestane [170] and designed for the treatment of mammary tumours and the use of this AI as a component of a combined preparation for prophylaxis and treatment of benign prostatic hyperplasia have been patented.[155]

VI. Conclusion

Thus, substituted androstanes are aromatase inhibitors in their pharmacological effect and antitumour agents in the mode of action. They may compete successfully with other drugs and are sometimes indispensable in the treatment of hormone-dependent tumours. Special mention should be made of the relative simplicity of synthesis of these preparations from industrially available steroid sources.

Analysis of methods used for the synthesis of AI together with detailed studies of their activity *in vitro* and *in vivo* give a strong impetus to the design of new efficient drugs and the development of methods for their synthesis and offers a possibility to choose the most efficient drug in each concrete case.

References

1. A M H Brodie *Biochem. Pharmacol*. **34** 3213 (1985)
2. R J Santen *Breast Cancer Res. Treat. (Suppl.)* **7** 23 (1986)

3. A M H Brodie, R C Coombes, M Dowsett *J. Steroid Biochem.* **27** 899 (1987)
4. H V Bossche *J. Steroid Biochem., Mol. Biol.* **43** 1003 (1992)
5. P S Lonning, M Dowsett, T J Powles *J. Steroid Biochem.* **35** 355(1990)
6. J L Young *Breast Cancer* (Ed. A R Liss) (New York: Academic Press, 1989) p. 1
7. E A Thompson, P K Siiteri *J. Biol. Chem.* **249** 5373 (1974)
8. P L Bellino *J. Steroid Biochem.* **17** 261 (1982)
9. E DiSalle, D Giudici, G Briatico, G Ornati *Ann. N.Y. Acad. Sci.* **595** 357 (1990)
10. P K Siiteri, E A Thompson *J. Steroid Biochem.* **6** 317 (1975)
11. J P Burkhart, N P Peet, C L Wright, J O N Johnston *J. Med. Chem.* **34** 1748 (1991)
12. J Fishman, E F Hahn *Steroids* **50** 339 (1987)
13. J Mann, B Pietrzak *J. Chem. Soc., Perkin. Trans. 1* 2681 (1983)
14. P J Bednarski, S D Nelson *J. Med. Chem.* **32** 203 (1989)
15. P A Cole, C H Robinson *J. Med. Chem.* **33** 2933 (1990)
16. J N Wright, M Akhtar *Steroids* **55** 142 (1990)
17. J N Wright, G Slatcher, M Akhtar *Biochem. J.* **273** 533 (1991)
18. D F Covey, in *Sterol Biosynthesis Inhibitors* (Eds D Berg, M Plempel) (Chichester: Ellis Horwood, 1988) p. 535
19. P J Bednarski, S D Nelson *J. Steroid Biochem.* **32** 309 (1989)
20. F J Zeelen *Medicinal Chemistry of Steroids* (Amsterdam: Elsevier, 1990) p. 218
21. J M O'Reilly, R W Brueggemeier *Curr. Med. Chem.* **3** 11 (1996)
22. W C Schwarzel, W G Krugel, H J Brodie *Endocrinology* **92** 866 (1973)
23. E A Thompson, P K Siiteri *J. Biol. Chem.* **249** 5364 (1974)
24. D F Covey, W F Hood *Cancer Res.* (*Suppl. 8*) **42** 3327 (1982)
25. R W Brueggemeier, P K Li, C E Snider, M V Darby, N E Katlic *Steroids* **50** 163 (1987)
26. J O Johnston, C L Wright, G W Holbert *J. Steroid Biochem., Mol. Biol.* **52** 17 (1995)
27. D F Covey, V D Parikh, W W Chien *Tetrahedron. Lett.* 2105 (1979)
28. D F Covey, W F Hood, V D Parikh *J. Biol. Chem.* **256** 1076 (1981)
29. B W Metcalf, C L Wright, J P Burkhart, J O Johnston *J. Am. Chem. Soc.* **103** 3221 (1981)
30. P A Marcotte, C H Robinson *Steroids* **39** 325 (1982)
31. J O'N Johnston *Steroids* **50** 105 (1987)
32. BRD P. 3 644 358; *Chem. Abstr.* **108** 75 715 (1988)
33. BRD P. 3 644 301; *Chem. Abstr.* **108** 75 716 (1988)
34. Eur. P. 227 472; *Chem. Abstr.* **107** 21 7931 (1987)
35. BRD P. 3 706 647; *Chem. Abstr.* **109** 93 426 (1988)
36. E Perel, S P Davis, D F Covey, D W Killinger *Steroids* **38** 397 (1981)
37. S J Zimnitzki, M T Brandt, D F Covey, D Puett *Steroids* **50** 135 (1987)
38. J N Wright, P T van Leersum, S G Chamberlin, M Akhtar *J. Chem. Soc., Perkin Trans. 1* 1647 (1989)
39. P A Marcotte, C H Robinson *Cancer Res.* (*Suppl. 8*) **42** 3322 (1982)
40. M G B Drew, J Mann, E Pietrzak *J. Chem. Soc., Chem. Commun.* 1191 (1985)
41. J N Wright, M Calder, M Akhtar *J. Chem. Soc., Chem. Commun.* 1733 (1985)
42. Jpn. P. 62 155 295; *Chem. Abstr.* **107** 198 752 (1987)
43. P J Bednarski, D J Porubek, S D Nelson *J. Med. Chem.* **28** 775 (1985)
44. J A A Geelen, G H J Deckers, J T H van der Wardt, H J J Loozen, L J W Tax, H J Kloosterboer *J. Steroid Biochem., Mol. Biol.* **38** 181 (1991)
45. Eur. P. 149 499; *Chem. Abstr.* **104** 51 017 (1986)
46. D Lesuisse, J F Gourvest, Q Benslimane, F Canu, Ch Delaisi, B Doucet, Ch Hartmann, J M Lefrancois, B Tric, D Mansuy, D Philibert, G Teutsch *J. Med. Chem.* **39** 757 (1996)
47. D Lesuisse, F Canu, B Tric *Tetrahedron* **50** 8491 (1994)
48. D Lesuisse, J F Courvest, C Hartmann, B Tric, O Benslimane, D Philibert, J P Vevert *J. Med. Chem.* **35** 1588 (1992)
49. C Delaisi, B Doucet, C Hartmann, B Tric, J F Gournet, D Lesuisse *J. Steroid Biochem., Mol. Biol.* **41** 773 (1992)
50. M J Shih, M H Carrell, H L Carrell, C L Wright, J O N Johnston, G H Robinson *J. Chem. Soc., Chem. Commun.* 213 (1987)
51. W E Childers, M J Shih, P S Furth, C H Robinson *Steroids* **50** 121 (1987)
52. W E Childers, P S Furth, M J Shih, C H Robinson *J. Org. Chem.* **53** 5927 (1988)
53. W E Childers, C H Robinson *J. Chem. Soc., Chem. Commun.* 320 (1987)
54. J T Kellis, W E Childers, C H Robinson, L E Vickery *J. Biol. Chem.* **262** 4421 (1987)
55. W E Childers, J V Silverton, J T Kellis, L E Vickery, C H Robinson *J. Med. Chem.* **34** 1344 (1991)
56. V C O Njar, E Safi, J V Silverton, C H Robinson *J. Chem. Soc., Perkin. Trans. 1* 1161 (1993)
57. J A Lovett, M V Darby, R S Counsell *J. Med. Chem.* **27** 734 (1984)
58. V C O Njar, J Duerkop, R W Hartmann *Steroids* **61** 138 (1996)
59. Eur. P. 488 383; *Chem. Abstr.* **117** 131 436 (1992)
60. US P. 5 491 136 ; *Chem. Abstr.* **124** 289 995 (1996)
61. N P Peet, J P Burkhart, C L Wright, J O'N Johnston *J. Med. Chem.* **35** 3303 (1992)
62. A M H Brodie, W C Schwarzel, A A Shaikh, H J Brodie *Endocrinology* **100** 1684 (1977)
63. A M H Brodie, W M Garrett, J R Hendrickson, C H Tsai-Morris, P A Marcotte, C H Robinson *Steroids* **38** 693 (1981)
64. D F Covey, W F Hood *Mol. Pharmacol.* **21** 173 (1982)
65. Eur. P. 181 287; *Chem. Abstr.* **105** 102 601 (1986)
66. R C Coombes, P E Goss, M Dowsett, G Hutchinson, D Cunningham, M Jarman, A M H Brodie *Steroids* **50** 245 (1987)
67. A M H Brodie, L Y Wing *Steroids* **50** 89 (1987)
68. M J Reed, L C Lai, A M Owen, A Singh, N G Coldham, A Purohit, M W Ghilchik, N A Shaikh, W H T James *Cancer Res.* **50** 193 (1990)
69. A M H Brodie, P K Banks, S E Inkster, M Dowsett, R C Coombes *J. Steroid Biochem., Mol. Biol.* **37** 327 (1990)
70. P G Ciattini, E Morera, G Ortar *Synth. Commun.* **22** 1949 (1992)
71. A M H Brodie *J. Steroid Biochem.* **49** 281 (1994)
72. R H Bible, C Placek, R D Muir *J. Org. Chem.* **22** 607 (1957)
73. H B Henbest, W R Jackson *J. Chem. Soc., C* 2459 (1967)
74. D A March, H J Brodie, W M Garrett, C H Tsai-Morris, A M H Brodie *J. Med. Chem.* **28** 788 (1985)
75. S Hrycko, P Morand *J. Chem. Soc., Perkin. Trans. 1* 2899 (1990)
76. R D Burnett, D H Kirk *J. Chem. Soc., Perkin. Trans. 1* 1830 (1973)
77. B H Jennings, J M Bengtson *Steroids* **31** 49 (1978)
78. D P Jindal, M R Yadav *Ind. J. Chem.* **30B** 515 (1991)
79. M Numazawa, M Ogata *J. Chem. Soc., Chem. Commun.* 1092 (1986)
80. X P Liu, D M Lambert, Y J Abul-Hajj *J. Med. Chem.* **38** 4135 (1995)
81. Y J Abul-Hajj, X P Liu, M Hedge *J. Steroid Biochem., Mol. Biol.* **54** 111 (1995)
82. E J Tavares da Silva, M L Sa e Melo, A S Campos Neves *J. Chem. Soc., Perkin. Trans. 1* 1649 (1996)
83. J A R Salvador, M L Sa e Melo, A S Campos Neves *Tetrahedron. Lett.* **34** 357 (1993)
84. Br. P. 2 191 198; *Chem. Abstr.* **108** 204 885 (1988)
85. A M H Brodie, D A Marsh, H J Brodie *J. Steroid Biochem.* **10** 423 (1979)
86. J L Block, N L Block, B L Lokeshwar *Cancer Lett.* **101** 143 (1966)
87. A M H Brodie, W M Garrett, J P Hendrickson, C H Tsai-Morris *Cancer Res. (Suppl. 8)* **42** 3360 (1982)
88. A B Foster, M Jarman, J Mann, I B Parr *J. Steroid Biochem.* **24** 607 (1986)
89. BRD P. 3 213 328; *Chem. Abstr.* **100** 85 993 (1984)
90. Y J Abul-Hajj *J. Med. Chem.* **29** 582 (1986)
91. Y J Abul-Hajj *J. Steroid Biochem.* **34** 439 (1989)
92. Eur. P. 375 559; *Chem. Abstr.* **114** 24 321d (1991)
93. H F Hodson, D J Madge, D H Widdowson *J. Chem. Soc., Perkin. Trans. 1* 2965 (1995)
94. M G Rowlands, A B Foster, J Mann, B Pietrzak, J Wilkinson, R C Coombes *Steroids* **49** 371 (1987)
95. Eur. P. 291 290; *Chem. Abstr.* **110** 251 323 (1989)
96. BRD P. 3 604 179; *Chem. Abstr.* **105** 191 504 (1986)
97. D Giudici, G Ornati, G Briatico, F Buzzetti, P Lombardi, E DiSalle *J. Steroid Biochem.* **30** 391 (1988)
98. E Di Salle, D Giudici, G Ornati, G Briatico, R, D'Alessio, V Villa, P Lombardi *J. Steroid Biochem., Mol. Biol.* **37** 369 (1990)
99. T Zaccheo, E DiSalle *Cancer Chemother. Pharmacol.* **31** 308 (1993)

100. Br. P. 2 284 605; *Chem. Abstr*. **123** 228 634 (1995)
101. Jpn. P. 05 178 888; *Chem. Abstr*. **120** 192 083 (1994)
102. BRD P. 3 322 285; Eur. P. 129 500; *Chem. Abstr*. **102** 204 169 (1985)
103. Y Nishino, M R Schneider, H Michna, M F El Etreby *J. Steroid Biochem*. **34** 435 (1989)
104. D Herderson, in *Atamestane — a New Aromatase Inhibitor Symposium (Abstracts Reports of VIIIth International Congress on Hormones Steroids), The Hague, 1990* p. 1
105. M F El Etreby, in *Atamestane — a New Aromatase Inhibitor Symposium (Abstracts Reports of VIIIth International Congress on Hormones Steroids), The Hague, 1990* p. 6
106. BRD P. 2 046 640; *Chem. Abstr*. **76** 154 025 (1972)
107. BRD P. 3 720 234; *Chem. Abstr*. **111** 7673 (1989)
108. A B Turner, H J Ringold *J. Chem. Soc. C* 1720 (1967)
109. BRD P. 3 512 328; *Chem. Abstr*. **106** 48 639 (1987)
110. WO PCT 86 05 813 (1986)
111. BRD P. 3 338 212; *Chem. Abstr*. **103** 123 792 (1985)
112. BRD P. 3 539 244; *Chem. Abstr*. **108** 75 717 (1988)
113. M Lourdusamy, F Labrie, S M Singh *Synth. Commun*. **25** 3655 (1995)
114. J Westermann, K Nickisch *Angew. Chem., Int. Ed. Engl*. **32** 1368 (1993)
115. BRD P. 3 715 869; *Chem. Abstr*. **111** 7671 (1989)
116. BRD P. 4 015 247; *Chem. Abstr*. **116** 59 740 (1992)
117. Eur. P. 534 582; *Chem. Abstr*. **119** 117 611 (1993)
118. BRD P. 4 227 053; *Chem. Abstr*. **120** 270 960 (1994)
119. BRD P. 3 703 722; *Chem. Abstr*. **110** 95 625 (1989)
120. BRD P. 3 705 990; *Chem. Abstr*. **110** 95 626 (1989)
121. Eur. P. 265 119; *Chem. Abstr*. **109** 93 434 (1988)
122. E DiSalle, G Briatico, D Giudici, G Ornati, T Zaccheo *J. Steroid Biochem*. **34** 431 (1989)
123. E DiSalle, G Ornati, D Giudici, M Lassus, T R J Evans, R C Coombes *J. Steroid Biochem., Mol. Biol*. **43** 137 (1992)
124. F Buzzetti, E DiSalle, A Longo, G Briatico *Steroids* **58** 527 (1993)
125. K Annen, H Hofmeister, H Laurent, R Wiechert *Synthesis* 34 (1982)
126. Br. P. 8 801 697; *Chem. Abstr*. **112** 36 257 (1990)
127. Eur. P. 307 314; *Chem. Abstr*. **111** 134 643 (1989)
128. GDR P. 258 820; *Chem. Abstr*. **111** 7672 (1989)
129. GDR P. 264 220; *Chem. Abstr*. **111** 78 502 (1989)
130. WO PCT 95 01366; *Chem. Abstr*. **122** 291 310 (1995)
131. M Numazawa, M Oshibe *J. Med. Chem*. **37** 1312 (1994)
132. Jpn. P. 063 321 981; *Chem. Abstr*. **122** 265 773 (1995)
133. M Numazawa, M Oshibe *Steroids* **60** 506 (1995)
134. M Numazawa, T Kamijama, M Tachibana, M Oshibe *J. Med. Chem*. **39** 2245 (1996)
135. M Numazawa, M Oshibe, S Yamaguchi, M Tachibana *J. Med. Chem*. **39** 1033 (1996)
136. H L Holland, S Kumaresan, L Tan, V C O Njar *J. Chem. Soc., Perkin. Trans. 1* 585 (1992)
137. C E Snider, R W Brueggemeier *J. Biol. Chem*. **262** 8685 (1987)
138. S Ebrahimain, H H Chen, R W Brueggemeier *Steroids* **58** 414 (1993)
139. Jpn. P. 07 215 992; *Chem. Abstr*. **123** 340 545 (1995)
140. M V Darby, J A Lovett, R W Brueggemeier, M P Groziak, R E Counsell *J. Med. Chem*. **28** 803 (1985)
141. J M O'Reilly, N Li, R W Brueggemeier *J. Med. Chem*. **38** 2842 (1995)
142. WO PCT 91 12 206 ; *Chem. Abstr*. **115** 232 607 (1991)
143. V C O Njar, R W Hartmann, C H Robinson *J. Chem. Soc., Perkin. Trans. 1* 985 (1995)
144. V C O Njar, G Gruen, R W Hartmann *J. Enzyme Inhib*. **9** 195 (1995)
145. M Numazawa, A Mutsumi *Biochem. Biophys. Res. Commun.,* **177** 401 (1991)
146. WO PCT 94 10 190; *Chem. Abstr*. **121** 231 153 (1994)
147. H L Herbert, S Kumaresan, G Lakshmaiah *Can. J. Chem*. **73** 2185 (1995)
148. S Miyairi, J Fishman *J. Biol. Chem*. **261** 6772 (1986)
149. Eur. P. 289 451; *Chem. Abstr*. **110** 95 629 (1989)
150. M Numazawa, M Tsuji, A Mutsumi *J. Steroid Biochem*. **28** 337 (1987)
151. M Yoshihama, K Tamura, M Nakakoshi, J Nakamura, N Fujise, G Kawanishi *Chem. Pharm. Bull*. **38** 2834 (1990)
152. M Numazawa, A Mutsumi, K Hoshi, R Koike *Biochem. Biophys. Res. Commun*. **160** 1009 (1989)
153. K Hoeffken *Contrib. Oncol. (Hormone-Dependant Tumors)* **50** 324 (1995); *Chem. Abstr*. **124** 249 307 (1996)
154. U F Habenicht, U W Tunn, T Senge, F H Schroder, H U Schweikert, G Barsch, M F El Etreby *J. Steroid Biochem., Mol. Biol*. **44** 557 (1993)
155. WO PCT 91 00 731; *Chem. Abstr*. **115** 174 681 (1991)
156. A M H Brodie, C Son, D A King, K M Meyer, S E Inkster *Cancer Res*. **49** 6551 (1989)
157. R C Coombes, P Goss, M Dowsett, J C Gazet, A M H Brodie *The Lancet* **2** 1237 (1984)
158. A M H Brodie, L-Y Wing, P Goss, M Dowsett, R C Coombes *J. Steroid Biochem*. **24** 91 (1986)
159. P Goss, T J Powles, M Dowsett *Cancer Res*. **46** 4823 (1986)
160. P E Lonning, D C Johannessen *Drug Today* **27** 117 (1991)
161. P E Lonning, S Kvinnsland *Drugs* **35** 685 (1988)
162. BRD P. 4 435 368 FRG; *Chem. Abstr*. **124** 279 193 (1996)
163. M F El Etreby *J. Steroid Biochem., Mol. Biol*. **44** 565 (1993)
164. U F Habenicht, in *Atamestane — a New Aromatase Inhibitor Symposium (Abstracts Reports of VIIIth International Congress on Hormones Steroids), The Hague, 1990* p. 10
165. D Henderson, U F Habenicht, Y Nishino, M F El Etreby *Steroids* **50** 219 (1987)
166. H V Schweikert, U W Tunn, U F Habenicht *J. Steroid Biochem*. **44** 573 (1993)
167. T Zaccheo, D Giudici, P Lambardi, E Di Salle *Cancer Chemother. Pharmacol*. **23** 47 (1989)
168. T R J Evans, E Di Salle, G Ornati, M Lassus, M Benedetti, E Pianezzola, R C Coombes *Cancer Res*. **52** 5933 (1992)
169. N Zilembo, C Noberasco, E Bajetta, A Mortinetti, L Mariani, S Orefice, R Buzzoni *Br. J. Cancer* **72** 1007 (1995)
170. BRD P. 3 622 841; *Chem. Abstr*. **106** 162 586 (1987)
171. D F Covey, W F Wood *Endocrinology* **108** 1599 (1981)
172. P F Sherwin, P C McMullan, D F Covey *J. Med. Chem*. **32** 651 (1989)
173. Y Osawa, M J Coon *Endocrinology* **121** 1010 (1987)