The Synthesis of Potential Steroid Receptor Antagonists.

by

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Contents.

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Abstract.

The first section of this thesis presents a review of the literature on endocrine hormones, synthetic steroids, and synthetic steroid receptor antagonists. Particular emphasis is placed upon the estrogen, progesterone and glucocorticoid activity of these compounds. The structure, activity and preparation of some progesterone/glucocorticoid receptor antagonists is also reviewed.

The second section of this thesis presents a route for the synthesis of 19-aryl substituted androstanes. These compounds have been designed to explore their potential antiprogestational and antiglucocorticoid activity. It is demonstrated that a bulky aromatic functionality may be introduced at the sterically hindered C-19 methyl group, by the nucleophilic attack of an organometallic nucleophile on a 10β-formyl-19-norandrostane, which is suitably protected at positions C-3 and C-17. The subsequent careful manipulation of the C-3 and C-17 functionalities has led to a succesful 11 step synthesis (scheme 4.19) of 17β-hydroxy-17α-(prop-1-yne)-19-(p-N,N-dimethylaminophenyl)androst-5-en-3-one (3.1), which should be of interest for biological evaluation.

Declaration.

The recommendations and observations described in this thesis are those of the author, except where acknowledgements have been made to previously published results and ideas. The research was carried out at the Department of Chemistry, City University and Department of Chemistry, University of Warwick between October 1989 and October 1993 and has not been previously been submitted for a degree at any institution.

Abbreviations.

ACTH	adrenocorticotrophin hormone
ADH	antidiuretic hormone
ATP	adenosinetrisphosphate
cAMP	cyclic adenosinemonophosphate
CRF	corticotrophin releasing factor
CI-MS	chemical ionisation mass spectra
^{13}C NMR	carbon-13 nuclear magnetic resonance spectra
δ	delta
DBD	DNA binding domain
DG	diacyl glycerol
EI-MS	electron impact mass spectra
FAB-MS	fast atom bombardment mass spectra
FSH	follicle stimulating hormone
g	gram
GnRH	gonadotrophin releasing hormone
HBD	hormone binding domain
HCG	human chorionic gonadotrophin
^{1}H NMR	proton nuclear magnetic resonance spectra
HRE	hormone response element
hsp	heat shock protein
Hz	hertz
IP_3	inositoltrisphosphate
I.R.	infrared
L	litre
LH	luteinizing hormone
m	multiplet
ml	millitres
mmol	millimoles
mol	moles
M^+	molecular ion
m.p.	melting point
m/z	mass to charge ratio
PIP_2	phosphoinositolbisphosphate
PLA_2	phospholipase A_2
ppm	parts per million
RBA	relative binding affinity
s	singet
Ser	serine
t	triplet
TAF	transcription activation factor
νmax	absorption frequency

Section 1: Literature Review

Chapter 1.

Hormones and the Endocrine System.

1.1 Structure and nomenclature of steroids.

Steroids are a class of widely occurring compounds. In the vast majority of steroids, the junctions of rings B, C and C, D are trans.[1] (fig. 1.1).

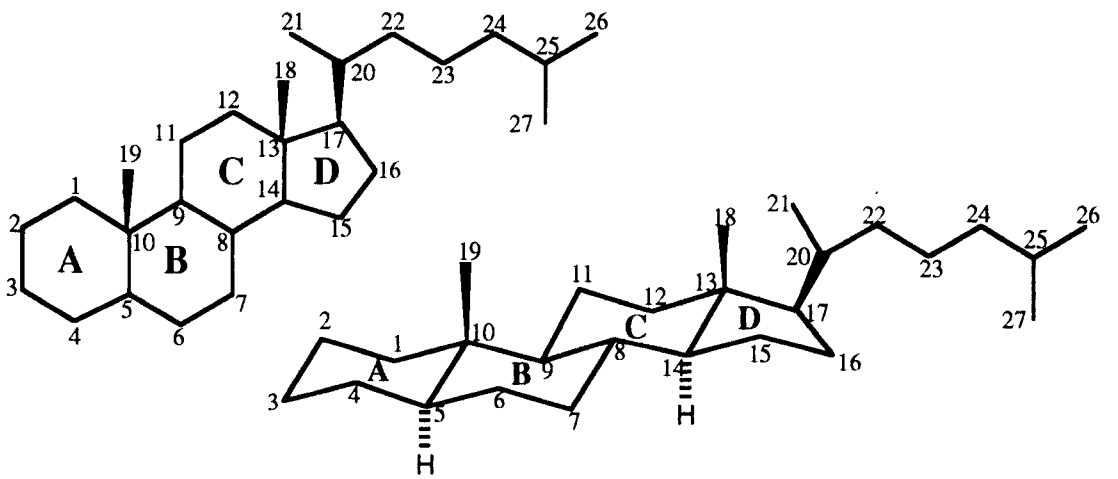

Figure 1.1 The basic ring system of steroids

The methyl groups attached at the positions C-10 and C-13 of the ring junctions are called angular methyl groups.[1] By convention, other groups that lie on the same side of the molecule as these two methyl groups, are designated as β-substituents, whilst groups on the opposite side are designated as α-substituents.[1] In systematic nomenclature, the nature of the alkyl group at position C-17 determines the base name of the individual steroid: e.g. androstanes have no C-17 alkyl group, whilst pregnanes carry a 2-carbon side chain.[1]

1.2 Nature of hormones.

Hormones are specialised chemical messengers which help to co-ordinate the activities of cells in a multicellular organism.[2,3] They are secreted by the cells of endocrine glands, usually directly into the blood stream, in response to some external stimuli (fig. 1.2).[2-4] They can then be carried by the circulatory system to most of the tissues in the body. Despite the wide distribution of the hormone, only certain cells are capable of responding to this chemical messenger, because they contain a specific receptor protein which will bind with the molecule.[2,3] In different target cells a hormone may give rise to different responses[4] and is effective at very low levels of blood concentrations.[2-4]

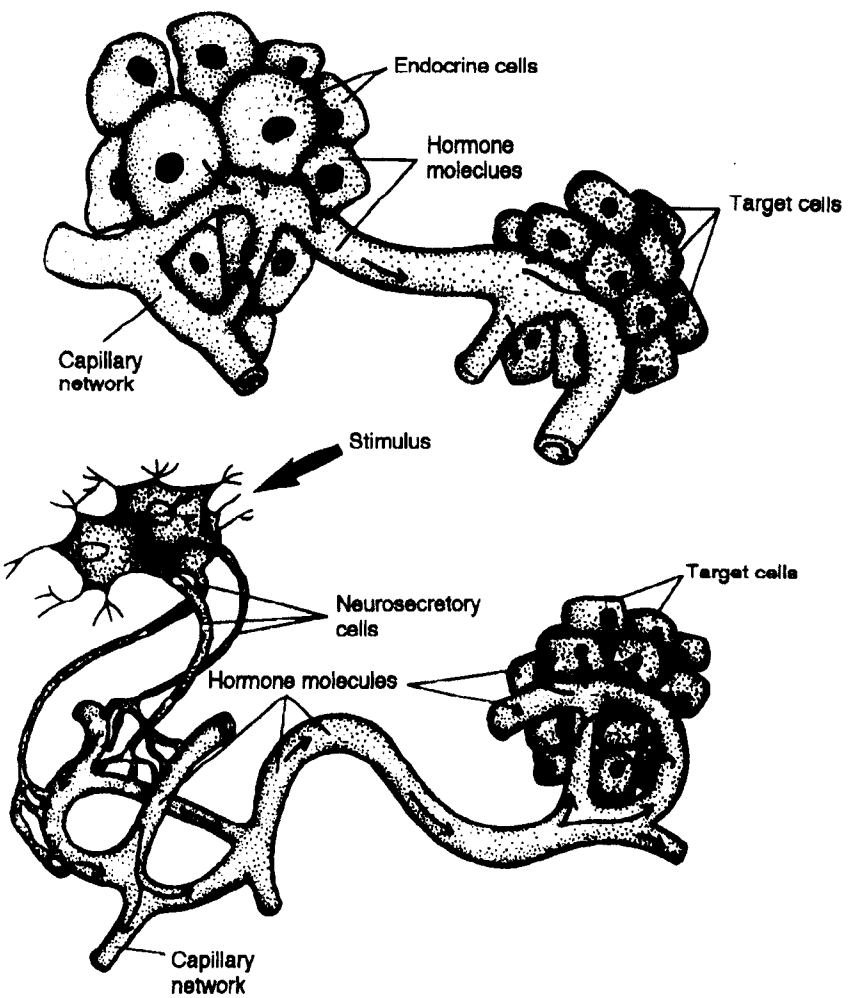

Figure 1.2 Hormones are carried through the circulatory system to target cells

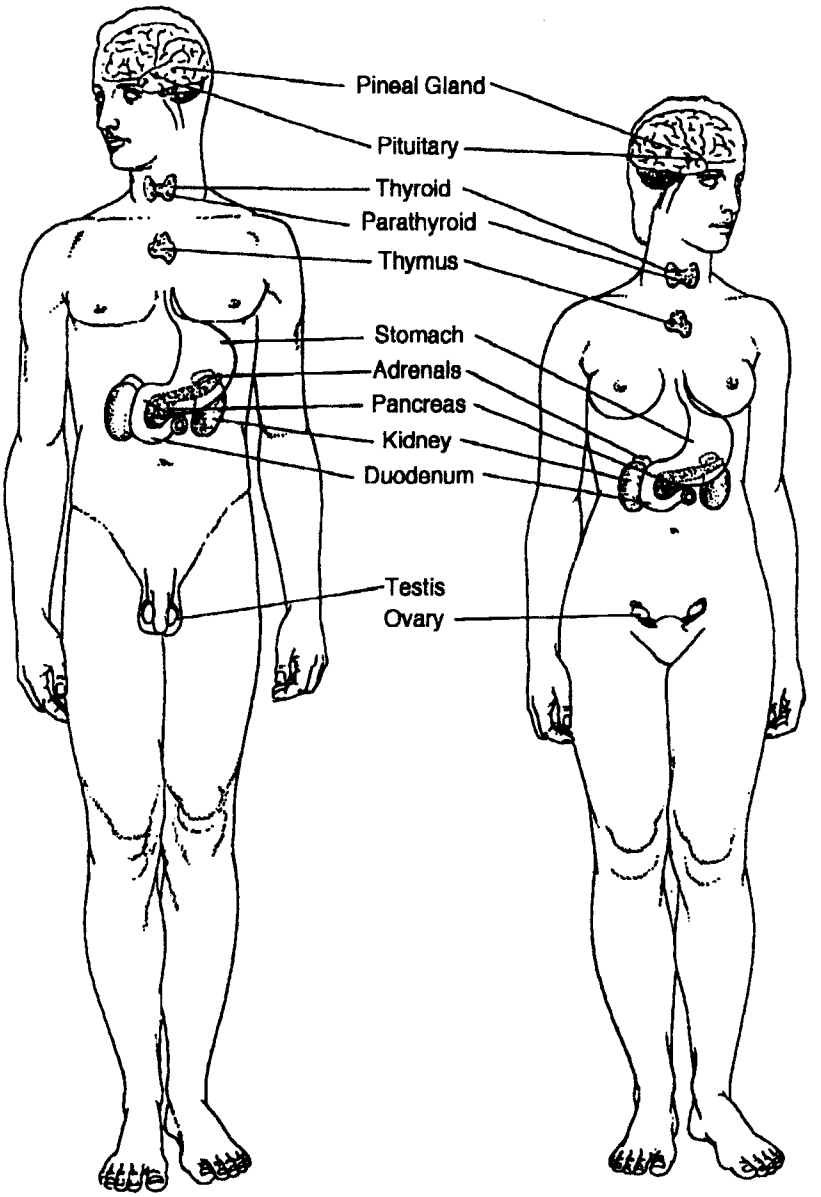

Figure 1.3 Locations of endocrine glands in the human body.

The endocrine cells, hormones and target cells integrate to form one of the most important communication systems of the body, known as the Endocrine System (fig. 1.3).[2-4] This system is responsible for the control and regulation of a diverse number of physiological processes; it regulates blood pressure, water and electrolyte balance, reproduction, growth, metabolic rate and responses to stress.[2-4]

At the highest level of control of the endocrine system is the hypothalamus, which is located at the base of the brain within a boney structure called the sella turcica (fig. 1.4).[3] For the most part the endocrine system is arranged in a hierarchical manner, consisting of 3 tiers (fig. 1.5). Immediately below the hypothalamus lies the pituitary gland (second tier), to which it is connected by a hypothalamic extension called the pituitary stalk.[3] At the third tier are the various endocrine glands.

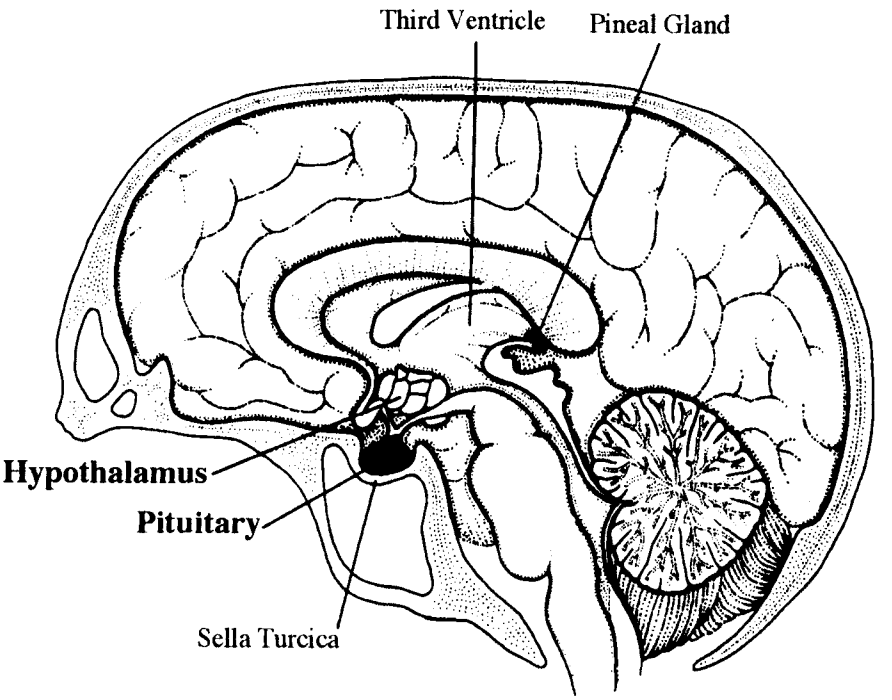

Figure 1.4 The locations of the pituitary gland and the hypothalamus at the base of the brain.

It is at the hypothalamus that both the endocrine system and nervous systems are linked physically and by function.[3] The bridging function is mediated by cells that have properties of both nerve cells and endocrine cells. They possess nerve processes that carry electrical impulses but release their signalling molecules into the blood (fig. 1.6).[3] For this reason the endocrine cells of the hypothalamus are sometimes called neurosecretary cells and the chemical messengers that they release are called neurohormones.[3]

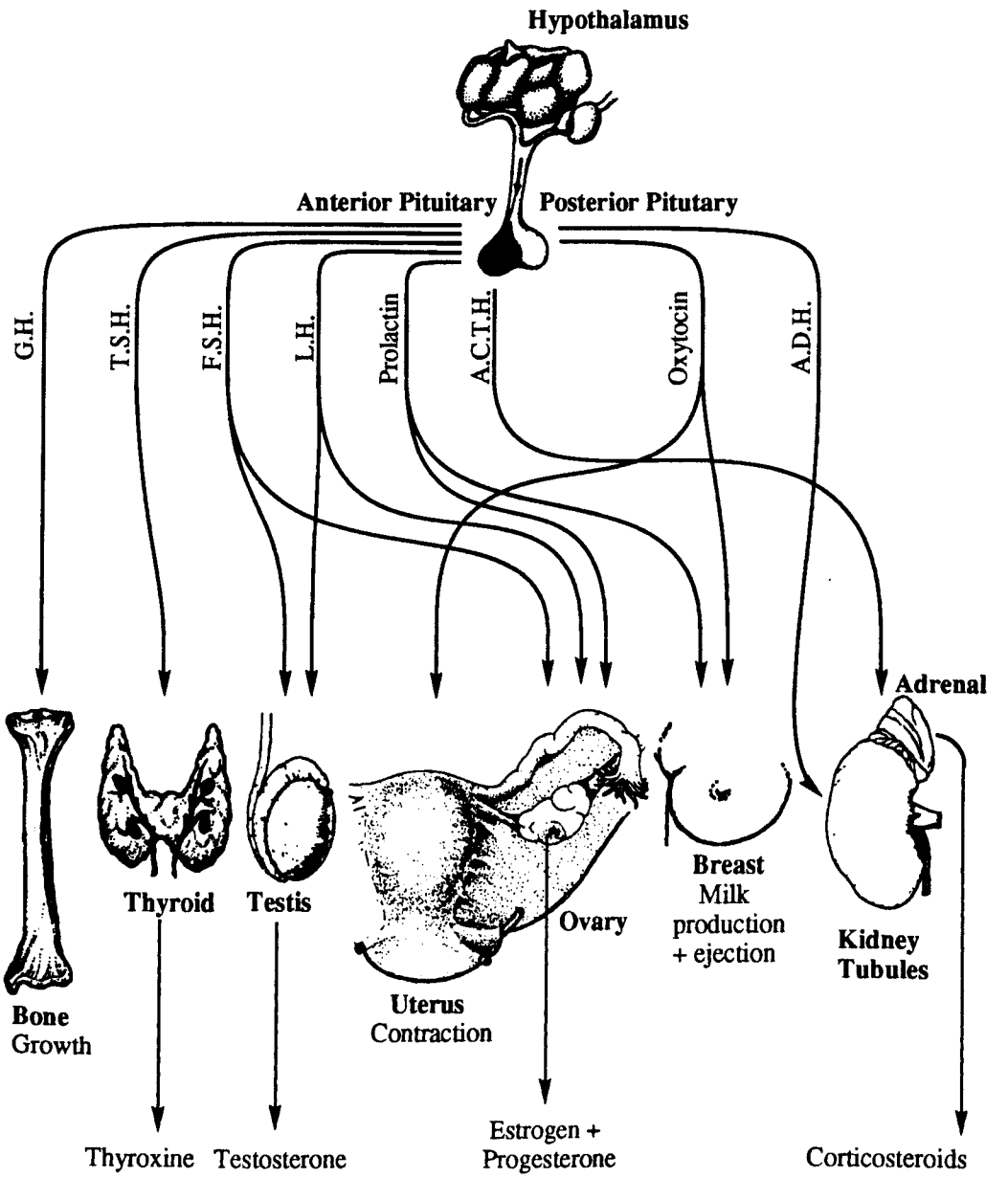

Figure 1.5 The three tier endocrine system.

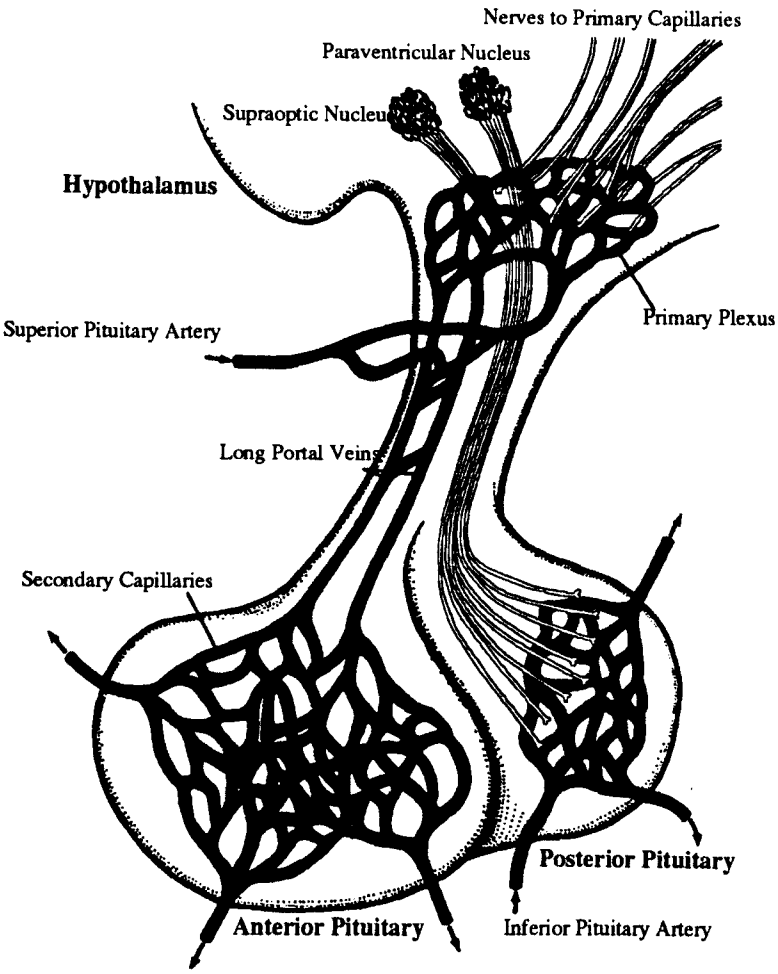

Figure 1.6 The anterior pituitary, posterior pituitary and hypothalamus.

When each of the hypothalamic neurosecretary cells (and its extension, the posterior pituitary) is stimulated by other nerve cells from higher regions of the brain, it begins to secrete specific peptide neurohormones into the blood vessels of the pituitary stalk.[2] The neurohormones are then carried to the anterior pituitary gland by the circulatory system, where they specifically stimulate or suppress the secretion of a second hormone from the pituitary gland.[2-4] The pituitary hormones then modify the secretary rates of the endocrine gland into the blood.[2-4] Consequently, the hypothalamus serves as the main regulator of the endocrine system.[2-4]

1.3 Structural diversity of hormones.

Chemically, hormones are diverse, but they generally fall into four different chemical groups: (1) amines, (2) peptides and proteins, (3) steroids and (4) prostaglandins.[2-4]

Noradrenaline (1.1) and adrenaline (1.2) are hormones derived from the amino acid residue tyrosine and produced by the medulla of the adrenal gland.[2-4] The thyroid hormones thyroxine (T_4) (1.3) and triiodothyronine (T_3) (1.4) are produced by the thyroid gland.[2-4]

The majority of hormones are peptides or proteins ranging in size from 3 to 200 amino acids. Oxytocin (1.5) and antidiuretic hormone (ADH) (1.6), produced by neurosecretary cells in the hypothalamus, are both nona-peptides. Seven of these amino acids are identical in the two hormones, but the actions of these hormones are quite different.[2] The hormones glucagon (produced in the islets of Langerhans), adrenocorticotrophic hormone (ACTH, produced in the anterior pituitary) and calcitonin (produced in the thyroid gland), are polypeptides with about 30 amino acid residues in the chain. Insulin (1.7) (secreted by the islets of the Langerhans in the pancreas) is a protein consisting of two peptide chains joined by disulphide bonds.[2]

The third class are the steroid hormones, derived from cholesterol (1.8), secreted by the testes, ovaries, placenta and adrenal cortex.[2,3] The fourth group, the prostaglandins, are closely related to lipids (fatty acids, each with a 5 membered ring in its structure) synthesised and released by many different tissues in the body.[2] Although they are present in very small quantities, prostaglandins exert a wide range of physiological effects on many tissues. They are often referred to as local hormones, because they act on cells in their immediate vicinity.

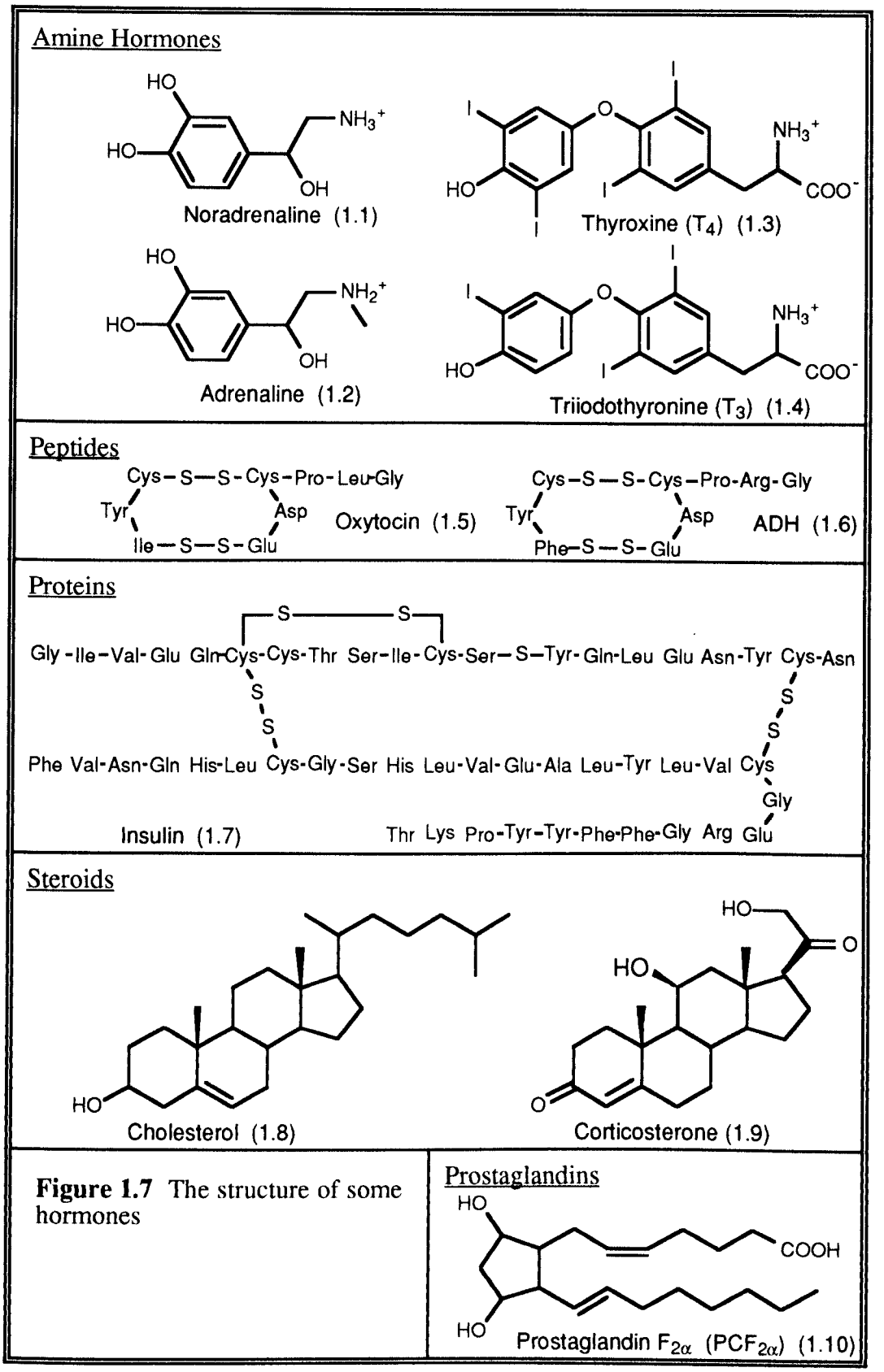

Figure 1.7 The structure of some hormones

1.4 Cellular Mechanisms of Action.

When a hormone is secreted by an endocrine gland into the blood stream, it will be carried throughout the body along with 30-40 other hormones.[4] It then diffuses through the walls of the capillaries and enters the interstitial spaces.[2]

The ability of a cell to respond to a hormone depends upon the presence of a specific receptor which will recognise the hormone and be able to activate a cascade of activities within the cell.[2] The receptor is a protein or glycoprotein molecule, which folds into a specific conformation. It is this shape and chemical composition of a receptor which allows it to bind strongly to the hormone (rather like a lock and key).[5] This binding usually occurs through H-bonding, electrostatic and Van der Waals interactions.[5]

When the hormone and receptor bind together, they both undergo a conformational change so that they may form an 'induced fit '. This gives rise to a tightly bound hormone-receptor complex. The change in conformation of the receptor protein in this adduct causes the activation of a cascade of reactions within the cell. This cascade mechanism gives rise to the amplification of the hormonal signal within the cell.[3]

The link between hormone receptor binding and physiological response is mediated by two alternative mechanisms of action,[1,2] depending on the types of hormones and the receptors to which the hormones bind to: 1) membrane bound receptors (fig. 1.8) and 2) intracellular receptors. Those hormones which are soluble in water or which have a high molecular weight (e.g. proteins, glycoproteins and polypeptides), will not easily be able to pass through the lipid plasma membrane of the cell and so must bind to membrane bound receptors. These receptors send a signal into the cell by altering the intracellular concentration of ions, or by increasing the intracellular concentration of chemical secondary messengers.

The opening of membrane-bound receptors which are gated ion channels, has the effect of altering the position of the electrochemical equilibrium that

exists across the membrane.[6] In nerve axons, this change may depolarise the membrane,[2] which may cause the opening and shutting of voltage dependant ion channels in the local vicinity of the membrane. In this manner an electrical signal may be transmitted along the length of a nerve cell axon and hence cause a physiological response.[2]

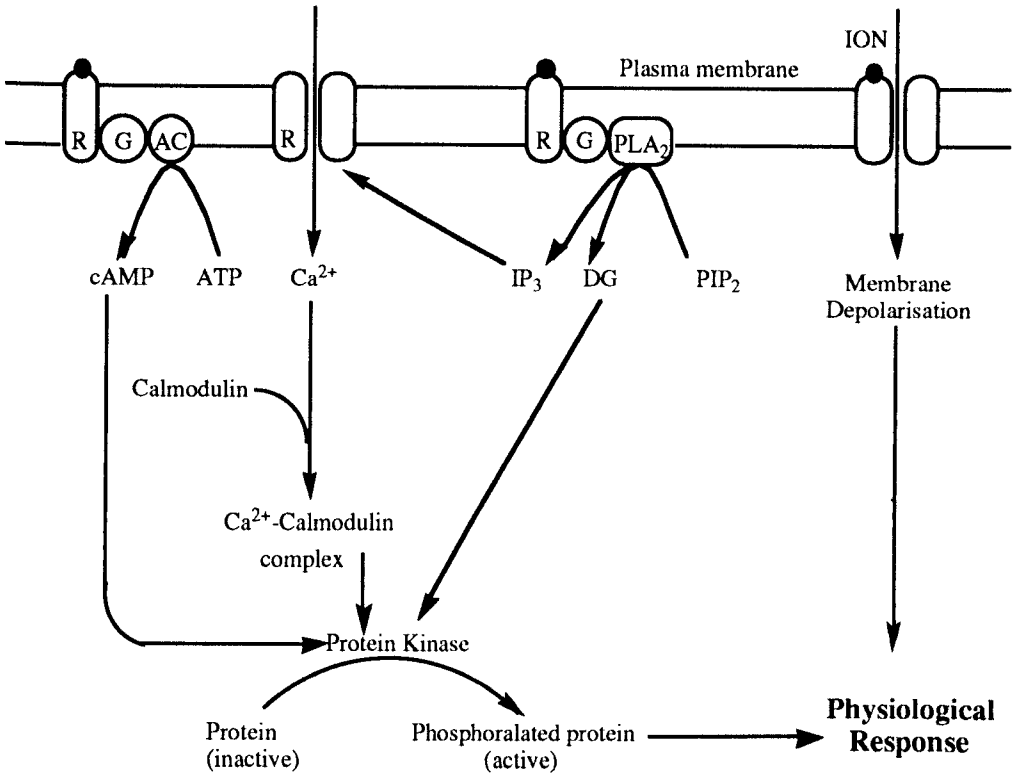

Figure 1.8 The cellular mechanisms of action of secondary messengers.

Alternatively, a membrane bound receptor may indirectly effect a physiological response through a secondary messenger. A number of species have been identified as secondary messengers; cyclic adenosine monophosphate (cAMP), calcium ions (Ca^{2+}), diacyl glycerol (DG) and inositol trisphosphate (IP_3)).[6] The secondary messengers directly (cAMP, DG) or indirectly (IP_3, Ca^{2+}), activate protein kinases. These activated protein kinases then activate inactive proteins by phosphorylation. The phosphorylated proteins give rise to a physiological response.[6]

Lipid soluble hormones (e.g. steroids, vitamin D_3 and thyroid hormones) are able to freely diffuse across the lipid soluble plasma membrane and so enter the cell.[2,6] Intracellular receptors are present in target cells, which exist as a

heterocomplex of a number of proteins[2] (receptor protein, heat shock proteins (hsp) and association proteins).[7] Each hormone binds with high specificity and affinity to a region of the receptor protein known as the hormone binding domain (HBD).[8] The binding of hormone and receptor, gives rise to a conformational change in the receptor protein which activates 'transcription activation factors' (TAFs) in the receptor protein and the dissociation of the heterocomplex. The dissociation of one of the heat shock proteins (hsp 90) from the receptor protein unmasks a region of the receptor protein, termed the DNA binding domain (DBD).[7,8] This region exists as two 'zinc fingers' each containing a zinc ion tetrahedrally surrounded by four cysteine amino acid residues from the primary amino acid sequence of the receptor protein.[7,8]

The DBD binds to a region of the DNA termed the hormone response element (HRE)[8] by insertion of the zinc fingers into the clefts of the DNA double helix. The binding of DBD to HRE is not sufficient for the activation of transcription: activated TAFs are also required.[8]

1.5 Intra cellular receptors.

There are a number of different types of intracellular receptors that exist in cell systems.[8] Each receptor is capable of specifically binding to one of the classes of hormonal thyroid hormones (fig. 1.7), steroids (fig. 1.10), or vitamin D_3 (1.16). All of these receptors belong to a 'super family' of ligand inducible transcription factors and show a high degree of molecular similarity.[8,9] It has been postulated that the members of this 'super family' have evolved (e.g. by point mutations) from a single ancestral receptor gene.[8]

Due to the evolutionary conservation of essential amino acid sequences, the primary amino acid sequence can be classified in seven regions; A/B to F. The receptor proteins all possess a variable N-terminal region (A/B), a centrally located DNA binding domain (C), a hinge region (D), a hormone binding domain (E) and a short C-terminal region (F).

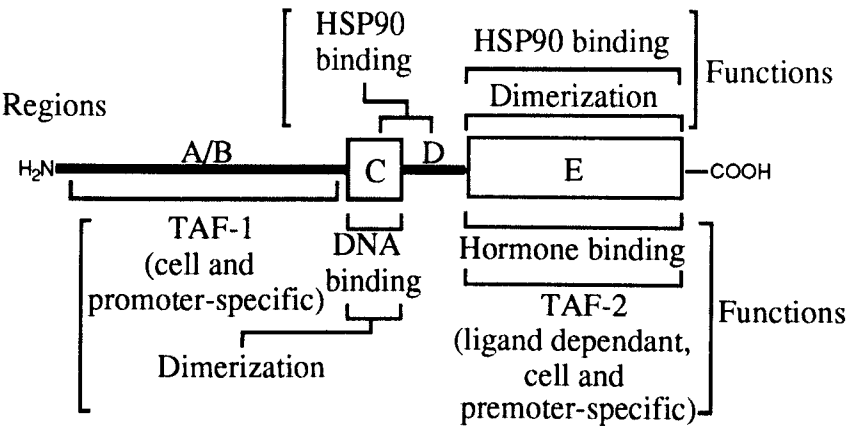

Figure 1.9 Regions within the primary amino acid sequence responsible for different functions in the receptor protein.

The HBD comprises approximately 250 amino acid residues.[8] Of the various domains, the DNA binding domain is the most highly conserved, comprising approximately 70 amino acid residues with a high content of basic amino acid residues due to the presence of the cysteine residues in the zinc fingers.[7-9]

1.6 Steroids and other intracellular hormones.

There are five classes of hormonal steroids that are currently recognised to exist in mammalian systems; androgens, glucocorticoids, mineralcorticoids, estrogens (1.14) and progestins.[9] Vitamin D (1.16), which is derived from cholesterol (1.8), binds to an intracellular receptor which belongs to the 'super family' of ligand inducible transcription factors.[9] Androgens (e.g. testosterone (1.11); synthesised in the testes) regulate male secondary sexual characteristics, Glucocorticoids (e.g. cortisol (1.12); synthesised in the adrenal cortex) promote gluconogenisis, formation of glycogen and degradation of fats and proteins.[2-4] Mineralcorticoids (e.g. aldosterone (1.13)) raise blood pressure and volume by increasing the net reabsorption of Na^+, Cl^- and HCO_3^- by the kidneys. Estrogens (e.g. estradiol (1.14); synthesised in the ovary), are responsible for the development of female secondary sexual characteristics. Progestins (e.g.

progesterone (1.15); synthesised in the corpus luteum), are responsible for the maintenance of pregnancy.[2-4]

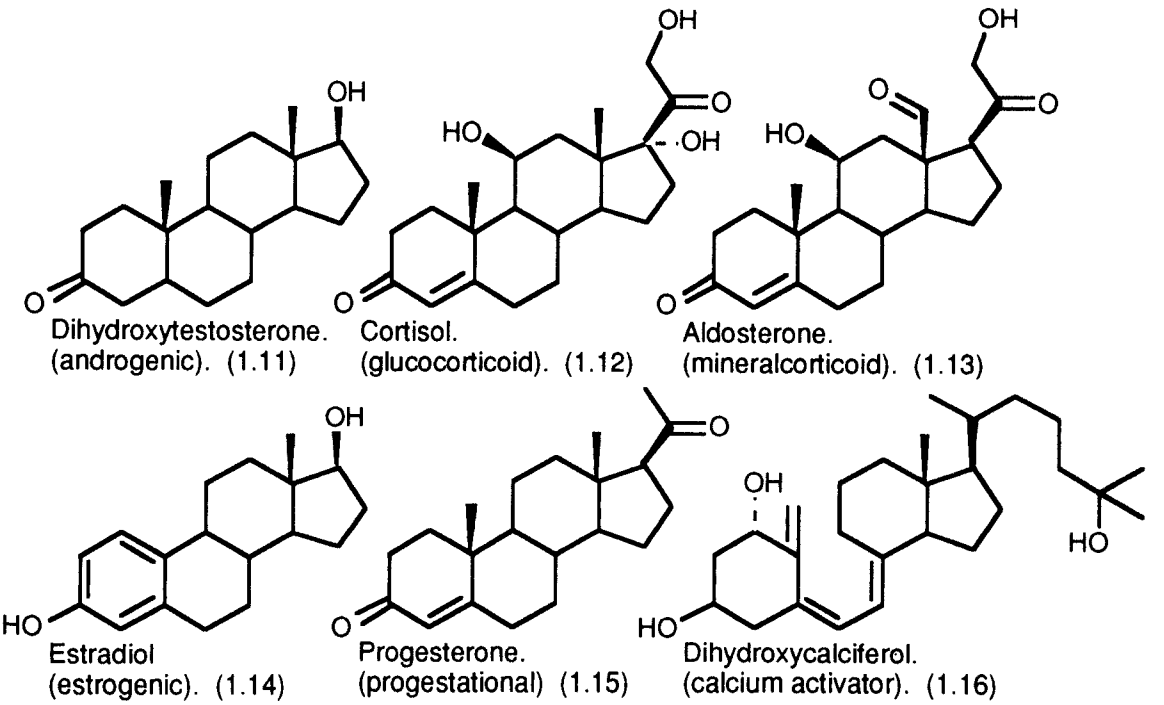

Figure 1.10 Some examples of naturally occurring ligands that bind to each class of intracellular receptor.

1.7 Sex hormones: regulation and secretion.

The sex steroids (testosterone (1.11), estrogen (1.14) and progesterone (1.15)) are responsible for the regulation of reproduction in the male and female.[2-4] Testosterone is responsible in the male for the control and development of the testes, spermatogenesis and the development of secondary male sexual characteristics.[2-4,6] Estrogens (1.14) and progesterone (1.15) are responsible for the development and control of the female genital tract, secondary female sexual characteristics and the development and function of the ovaries.[2-4]

There is a common mechanism by which the secretion of the male and female sex steroids is regulated.[2,3] The neurosecretary cells in the hypothalamus rhythmically release a small peptide hormone called gonadotrophin-releasing hormone (GnRH) into the hypothalamohypophyseal portal vein, which then

carries the hormone through the pituitary stalk directly to the pituitary gland, where it stimulates the pituitary gland to secrete two gonadotrophin hormones; luteinizing hormone (LH) and follicle stimulating hormone (FSH).[2-4,6] These gonadotrophins are then carried via the circulatory system to the gonads where they elicit the secretion of the sex steroids.[2-4]

1.8 Male reproductive function.

The male reproductive structures consist of the testes, scrotum, epididymis, vas deferens, seminal vesicles, prostate gland, urethra and the penis.[2-4] At the beginning of puberty, the hypothalamus becomes less sensitive to the inhibitory effects of testosterone and so levels of GnRH secreted in each periodic pulse are increased.[2,6] This then raises the levels of LH and FSH secreted by the anterior pituitary and therefore raises the level of testicular stimulation.[2]

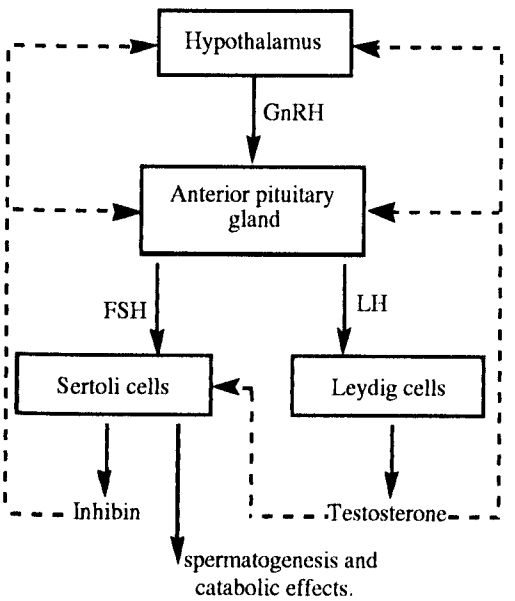

Figure 1.11 Hormonal control mechanism in male

Spermatogenesis (production of sperm) occurs in the seminiferous tubules of the testes,[2] these tubules contain germ cell and Sertoli cells.[2,4,6] Interspersed between the seminiferous tubules are groups of Leydig cells.[2,6] The germ cells, which are found close to the basement membrane of the tubules, divide and differentiate to produce immature sperm cells, each containing 23 chromosomes.[6] The Leydig cells are stimulated by periodic pulses of LH to secrete testosterone.[2-4] The Sertoli cells are stimulated by both testosterone and FSH to secrete a protein hormone called inhibin and most importantly to facilitate spermatogenisis by secreting a luminal fluid which nourishes the germ

cells and developing sperm attached to it.[2] The function of inhibin is to down-regulate the anterior pituitary's secretion of FSH, (it is also thought to down regulate the hypothalimic secretion of GnRH).[6] After ten weeks of development within the seminiferous tubules,[2] the sperm cells are released into the lumen of the seminiferous tubules. The immature sperm are then carried by the luminal fluid and pass through a network of highly coiled ducts which lead to the epididymis.[2-4] The final maturation of the sperm cells occurs during their twelve day passage through the epididymis.[2] The pressure of the luminal flow from the Sertoli cells carries the mature sperm cells into the vas deferens, were they are stored.[2] When ejaculation occurs the contents of the vas deferens, pass through the urethra and acquire the bulk of the ejaculatory fluid from the seminal vesicles and prostate gland.[2]

1.9 Female reproductive function.

The female reproductive structures consist of the ovaries, the uterinal tubes, the uterus, the vagina and the breasts.[2-4] The physiology of the female reproductive function is more complicated than that of the male due to the number of functions it has to perform.[2-4]

Oogenesis (production of ova) occurs in the ovaries, unlike spermatogenesis in the male, the production of ova in the female is not continuous, but occurs in two stages.[2-4] At the beginning of puberty, the level of GnRH secretion by the hypothalamus begins to increase.[4] This stimulates an increase in the levels of LH and FSH secreted by the anterior pituitary gland, so raising the levels of estrogens (1.14) and progesterone (1.15) being secreted by the ovaries.[4] With the alteration in the hormonal pattern of secretion comes the beginning of menstrual bleeding and the menstrual cycle.[2]

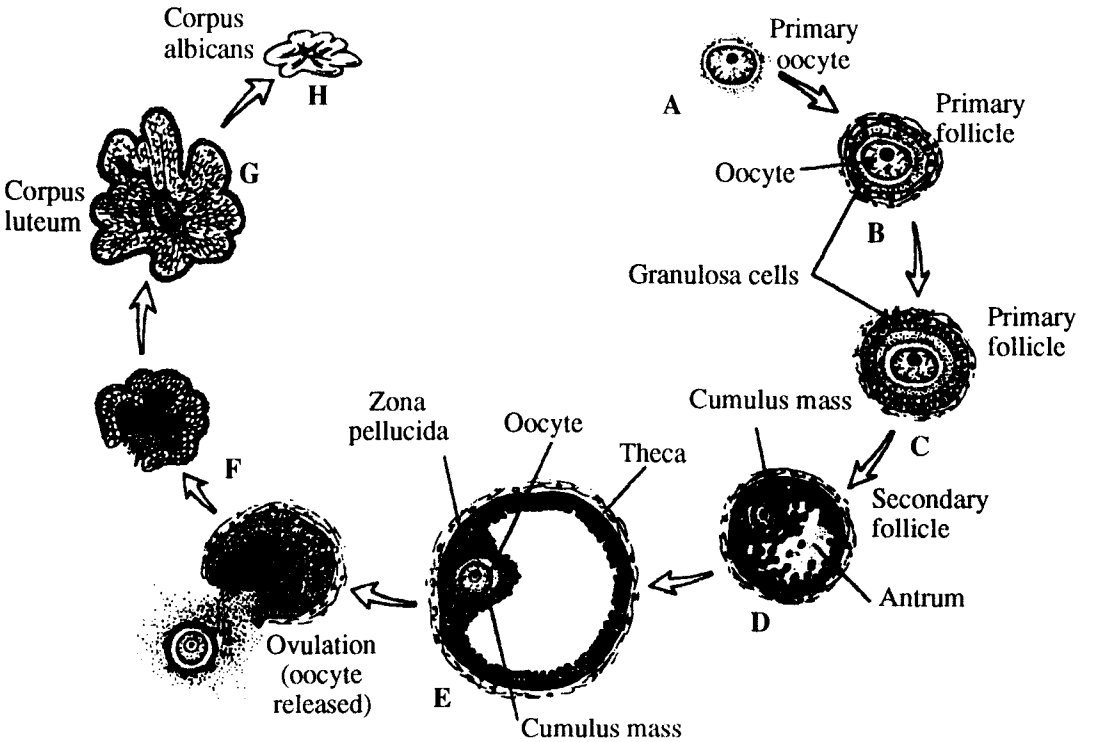

Figure 1.12 The developmental stages that occur during formation and release of ova.

The menstrual cycle (fig. 1.13) prepares the female reproductive structures for pregnancy.[6] The menstrual cycle is taken as beginning from the first day of menstruation, which lasts for 3-6 days.[2-4] During this time the superficial layer of the endometrium is shed.[2-4] When the menstrual flow stops, the endometrium is regenerated.[2] In the first part of the cycle, GnRH secreted by the hypothalamus stimulates the anterior pituitary gland to secrete increasing quantities of follicle stimulating hormone (FSH), along with smaller amounts of luteinizing hormone (LH).[6] Both of these hormones cause the development of small groups of primary follicles in the ovaries.[2] One of these follicles develops faster than the others and the remainder degenerate.[3] The increasing level of FSH stimulates the granulosa cells to multiply, to secrete a fluid into the centre of the follicle and to secrete estrogen (1.14).[2] The increasing numbers of granulosa cells form a thicker layer of cells around the oocyte.[2-4] A fluid-filled chamber develops within the follicle and a thin capsule of theca cells forms around it.[2-4]

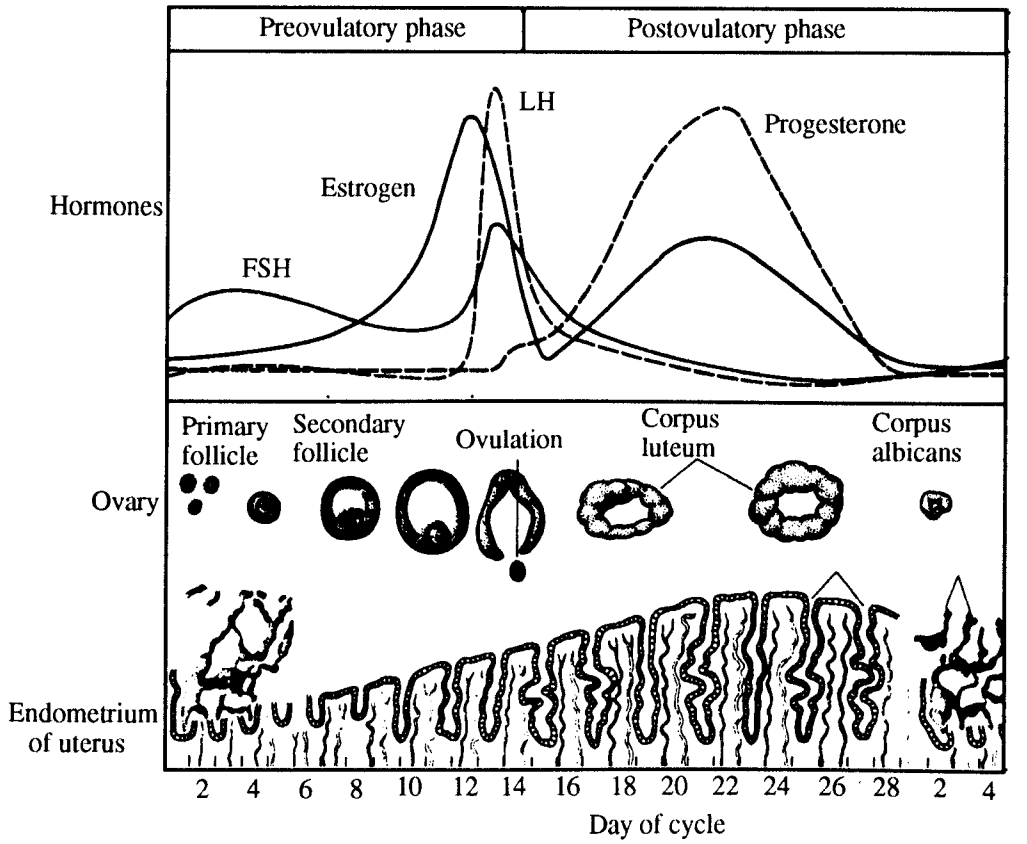

Figure 1.13 The hormonal changes that occur during the menstrual cycle stimulate follicular development and gives rise to endometrial changes.

The granulosa cells begin to secrete estrogens (1.14) under the influence of FSH.[2] This has a negative feedback effect on the hypothalamus and the anterior pituitary gland and so causes their secretary rates to fall to a low ebb at about the tenth day of the cycle.[2] During this phase, the endometrium, under the influence of estrogen, increases in thickness and vascularity.[2] Then, suddenly, the anterior pituitary begins to secrete large amount of both FSH and LH, but especially LH.[2-4,6] This surge of secretion is called the LH surge and it is believed to result from a transient second action of estrogens (1.14)[6]; a positive feedback effect. It is this surge that causes the very rapid swelling and rupture of the main follicle, resulting in ovulation.[3] The burst follicle then develops into the corpus luteum, which begins to secrete large amounts of progesterone (1.15) and continues to secrete estrogens (1.14).[2] Both of these hormones have a negative feedback effect on the hypothalamus and the anterior pituitary.[2-4,6]

Once again FSH and LH secretion diminish and this prevents the development of a new follicle or ovum.[2-4] Beside the negative feedback effect, progesterone (1.15) causes further thickening of the endometrium, initiates secretion of a nutrient fluid by the endometrial glands, promotes an increase in the blood flow to endometrial cells, inhibits contractility of uterine smooth muscle, firms the cervix and inhibits dilatation and prepares the mammary glands for secretion of milk.[4]

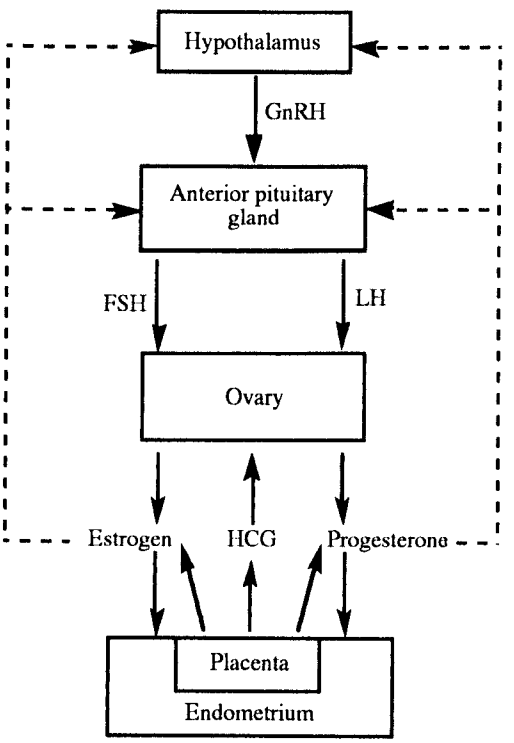

Figure 1.14 Hormonal control mechanism in male

If implantation of a fertilised ovum does not occur, then the corpus luteum will degenerate because the low levels of FSH and LH are not able to maintain it.[6] As a result, the negative feedback of progesterone (1.15) and estrogens (1.14) on the hypothalamus and anterior pituitary is removed and so the cycle begins again.[6] If a fertilised ovum does implant itself into the endometrium, then the early developing foetal tissue and placenta begin to secrete human chorionic gonadotrophin (HCG).[4] This substance is similar to LH and therefore maintains the secretion of progesterone (1.15) and estrogen by the corpus luteum. As the placenta develops, it begins to secrete large amounts of progesterone (1.15) and estrogens (1.14) and its secretion of HCG diminishes.[3,6] The placenta takes over from the corpus luteum in the secretion of these two hormones.[2-4]

1.10 Adrenal glands and the adrenal steroids.

The adrenal glands are situated directly above the kidneys.[2-4,6] Structurally each of the two glands consists of an inner medulla and an outer cortex.[4] The adrenal cortex secretes three types of corticosteroid hormones; mineralcorticoids, glucocorticoids and androgens.[2-4,6] Mineralcorticoids are secreted by zona glomerulosa cells situated in the outer region of the cortex[2] and are responsible for the regulation of water and electrolyte balance. The principal mineral corticoid secreted by the adrenal cortex is aldosterone.[2] The glucocorticoids are secreted by zona fasciculata cells situated in the middle region of the cortex.[2] This group of hormones are responsible for the regulation of increased glycogenesis, protein metabolism, fat metabolism, anti inflammatory and immunosupressive action.[26] These are involved in the body's response to stress.[6] Cortisol accounts for 95% of the glucocorticoid activity of the adrenal cortex.[3] Glucocorticoid secretion is regulated by the hypothalamus which secretes corticotrophin releasing hormone (CRF). This hypothalamic hormone stimulates the anterior pituitary to release adrenocorticotrophin hormone (ACTH), which has a negative feed back effect on the hypothalamus.[2] ACTH stimulates the secretion of glucocorticoids by the adrenal cortex.[2-4] The glucocorticoids have a negative feed back effect on the hypothalamus and pituitary glands.[2-4]

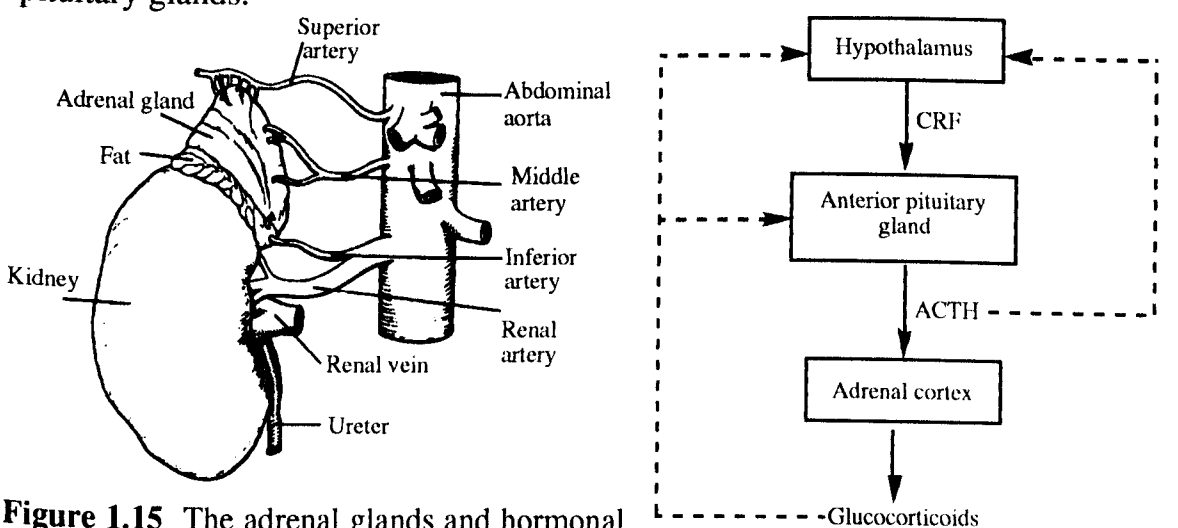

Figure 1.15 The adrenal glands and hormonal control mechanism of glucocorticoids.

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(8) H. Gronemeyer, B. Benhamou, M. Berry, M.T. Bocquel, D. Gofflo, T. Garcia, T. Lerouge, D. Metzger, M.E. Meyer, L. Tora, A Vergezac, P. Chambon, *J. Steroid Biochem. Molec. Biol.*, 1992, **41**, 217.

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Chapter 2

Steroid Receptor Antagonists

2.1 Introduction

Steroid receptor antagonists are those compounds which show a high binding affinity for steroid receptor proteins, without themselves triggering the normal physiological response controlled by those receptors.[1-9] The receptor antagonist will compete with the natural hormone agonist for binding to the steroid receptor site. Consequently, the level of physiological response that a given tissue will produce in response to a given concentration of a hormone will be reduced.[6-10]

There are a number of important clinical applications for steroid receptor antagonists. They are clinically used to control fertility [1-3,7-9.11]; for the treatment of testosterone (1.11),[1,4,5] progesterone (1.15)[9] and estrogen (1.14)[1,13] dependant cancers; for treatment of Cushings syndrome[9]; and to facilitate fertility.[1,3-5,13]

2.2 Cellular mechanism of antagonism.

The mechanism by which steroid receptor antagonists interfere with their intracellular receptors has not been fully elucidated. The availability of a number of synthetic steroid receptor antagonists (e.g. RU 38486 (2.40), tamoxifen (2.15)) with strong antagonist properties and little or no agonist action has made possible detailed studies of their receptor binding and of the subsequent effects on DNA activation and termination.[14] Such studies are complicated by the species specific nature of many of the effects,[15] e.g. RU 38486 (2.40) has been shown to bind to and antagonise the progesterone receptor in humans,[3] but not in the chick or hamster.[15]

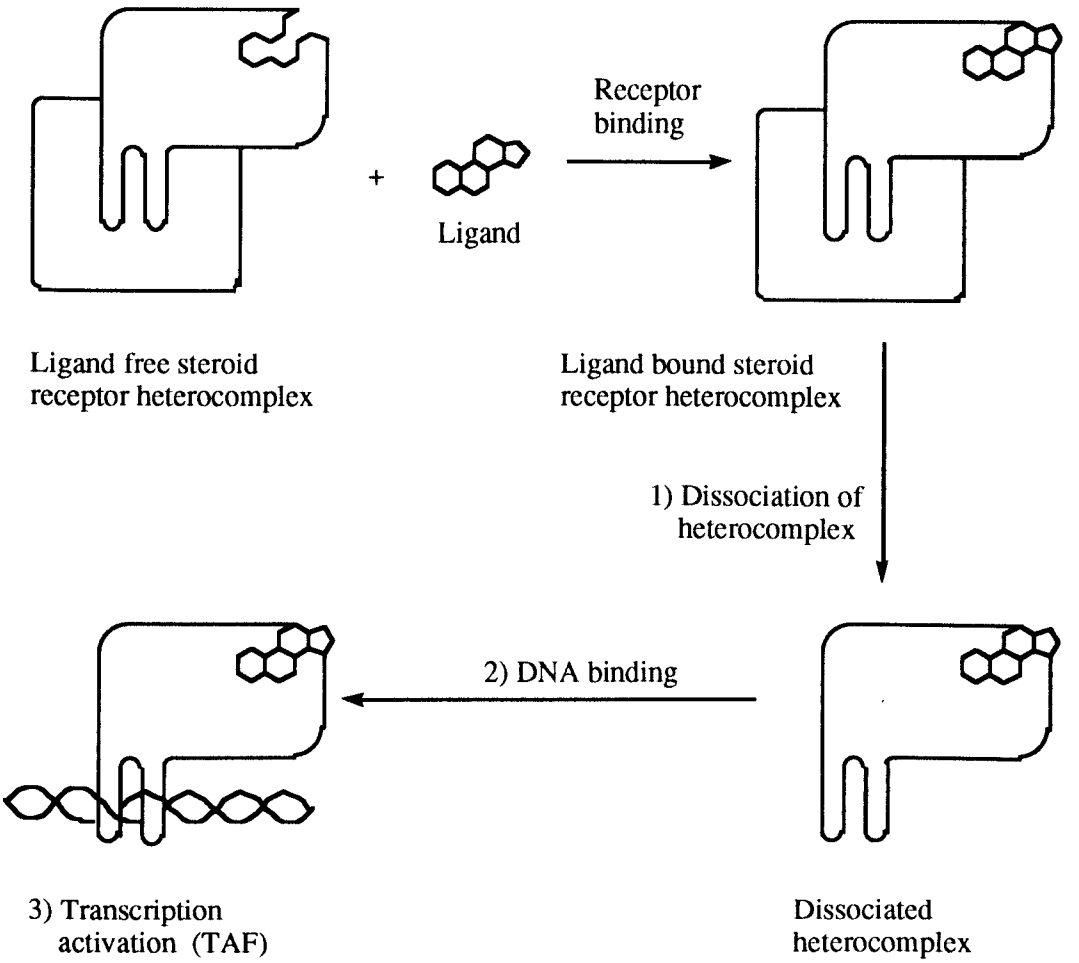


Figure 2.1

Steroid receptor antagonists may interrupt the process of transcription activation at three distinct steps (fig. 2.1).[14]

1) They may slow down or even inhibit the dissociation of the steroid receptor heterocomplex (composed of steroid receptor, heat shock proteins and association proteins).[14]

2) They may not induce the association of the steroid receptor to the corresponding response element in DNA.[14]

3) Interfere with the processes of TAFs of steroid receptors that normally activate the transcriptional machinery of DNA.[14]

The mechanism by which different steroid receptor antagonists interact and interfere with the transcription activation of DNA varies. The different steroid receptor antagonists may be classified on the basis of the mechanism of antagonism into two classes ; Type I and Type II antagonists.[14]

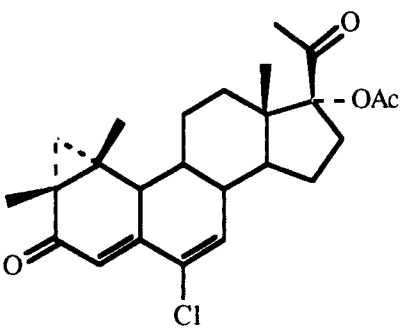
Hydroxytamoxifen (2.16), RU 38486 (2.40) and ICI 164,384 (2.20) are all Type I antagonists.[14] These compounds are able to bind to steroid receptors and induce the dissociation of the heterocomplex.[1] The DBD is then able to bind to the HRE of DNA.[1,14] The binding of the antagonist to the receptor does not activate TAF-2 which is essential for the activation of DNA transcription.[1,14] As a result the steroid receptor binding to DNA does not give rise to transcription.[14]

Although RU 38486 (2.40) binds with a high affinity to both the human progesterone and glucocorticoid receptors,[16-18] it does not bind to the corresponding chicken and hamster receptors.[15] Substitution of the cysteine amino acid residue at position 575 for a glycine residue in the HBD of the chicken progesterone receptor generates a mutated chicken progesterone receptor that possesses an affinity for the progesterone receptor antagonist RU 38486 (2.40) which has increased by more than 1000 fold, up to 20% of that for progesterone.[14] In fact all receptors that bind RU 38486 (2.40) posses a glycine at the corresponding position.[15]

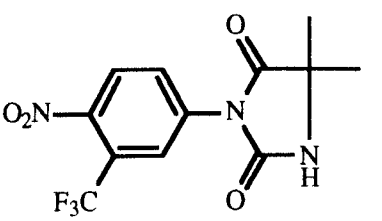
Type II antagonists (e.g. ZK 98,299 (2.41))[1] abolish or severely decrease the DNA binding capability of steroid receptors.[14] It is not yet entirely clear by what mechanism this type of antagonism occurs.[14]

2.3 Antiandrogens.

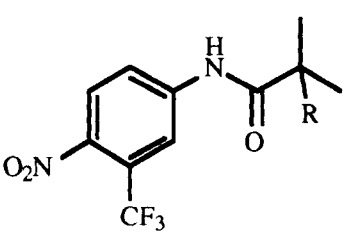
Cypristone acetate (2.1) was the first antiandrogen to be discovered.[1,2,4,5,13] It showed progestational activity and also suppressed the secretion of gonadotrophins.[1,2,4] Pure antiandrogens were subsequently developed that lacked the undesirable side effects.[1] Flutamide (2.3) was the first to be developed.[1,2] *In vivo*, flutamide (2.3) is metabolised by the liver to 4-hydroxy flutamide (2.4) which shows a dramatic 100 fold increase in the affinity for the androgen receptor.[1,3,13] Unfortunately this antiandrogen possesses a short half life of approximately 8 hrs.[2] Additional antiandrogens with longer half lives were subsequently developed e.g. Anandron (2.2) (RU 23,908) and Casodex (ICI 176324) (2.5).[1]



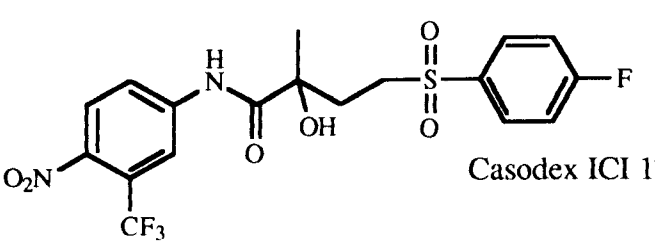
Cypristone acetate (2.1)



Anandron
RU 23,908 (2.2)



R		
H	Flutamide	(2.3)
OH	4-Hydroxyflutamide	(2.4)



Casodex ICI 176324 (2.5)

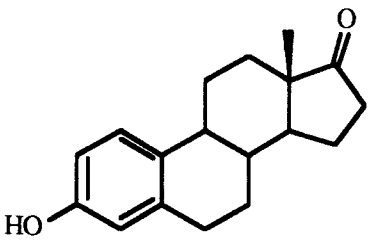
Figure 2.2 Some antiandrogens

2.4 Antiestrogens.

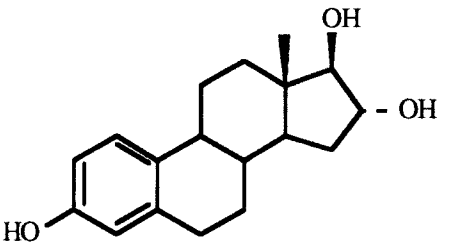
MER 25 (2.13) was the first triphenyl synthetic antiestrogen to be described.[1,2] Unfortunately this compound was not suitable as a drug due to its toxicity.[1,2] This compound did however lead to the synthesis of clomiphene (2.14).[1-3] Although these compounds were first developed as potential contraceptive agents, they were used to aid fertility.[1,2,13] The antagonist effectively removes the negative feedback effect of estrogen (1.14) on the hypothalamo-pituitary axis.[2] This has the effect of increasing the levels of LH and FSH being secreted by the anterior pituitary gland and so stimulating fertility.[2] Many estrogen receptor antagonists (fig. 2.4) were synthesised in an attempt to asses their contraceptive activity.[2] Unfortunately these compounds were not suitable for this purpose and have instead been used to treat advanced estrogen dependant breast cancers.[1,2,13]

To date, tamoxifen (2.15) is the most widely used antiestrogen which is used for the treatment of estrogen dependant breast cancers.[1,2,4] This compound possesses a triphenylethylene structure. The liver metabolises tamoxifen and converts it to 4-hydroxytamoxifen (2.16),[1,2] and this conversion increases its affinity for the estrogen receptor site by approximately 100 fold.[2] The antiestrogenic activity of tamoxifen is associated with the alkylamide side chain of the trans configuration of tamoxifen (2.15) (Z-isomer).[19] The cis configuration (E-isomer) has been reported to posses estrogenic activity.[19]

Natural

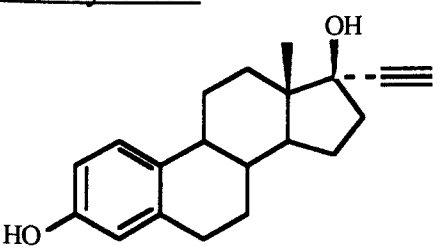


Estrone (2.6)

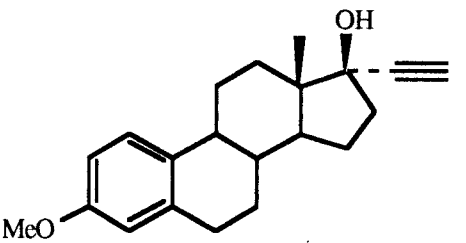


Estriol (2.7)

Semisynthetic

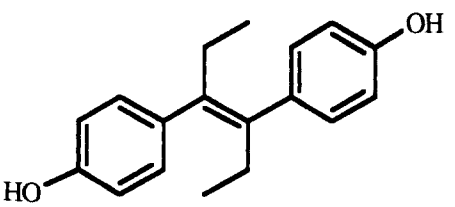


Ethynyl estradiol (2.8)

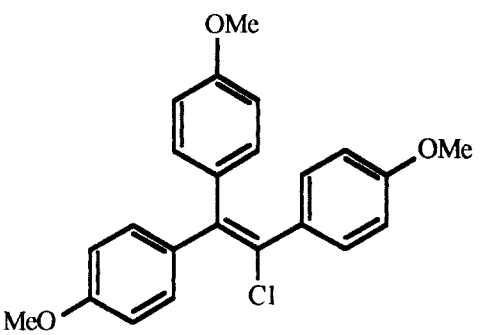


Mestranol (2.9)

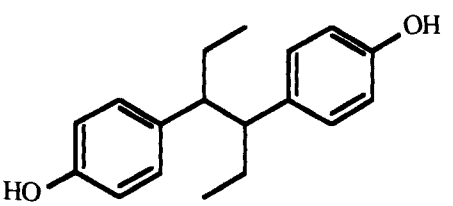
Nonsteroidal



Trans-diethylstilbestrol (2.10)



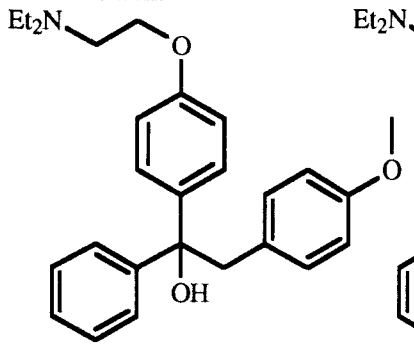
Chlorotrianisene. (2.11)



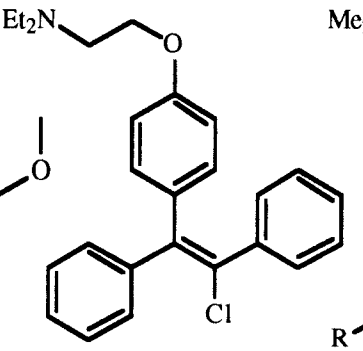
Hexestrol (2.12)

Figure 2.3 A number of estrogen agonists

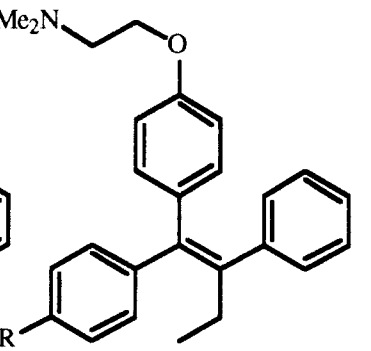
Nonsteroidal



MER 25 (2.13)



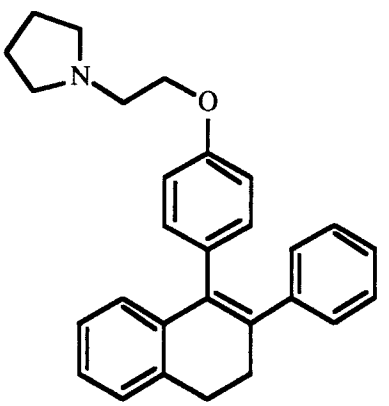
Clomiphene (2.14)



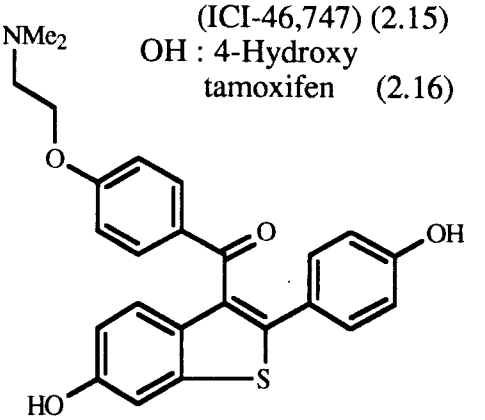
R

H : Tamoxifen (ICI-46,747) (2.15)

OH : 4-Hydroxy tamoxifen (2.16)

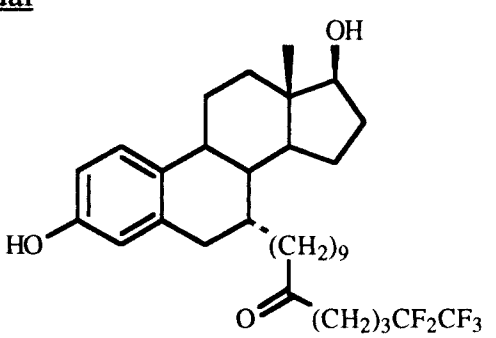


Nafoxidine (2.17)

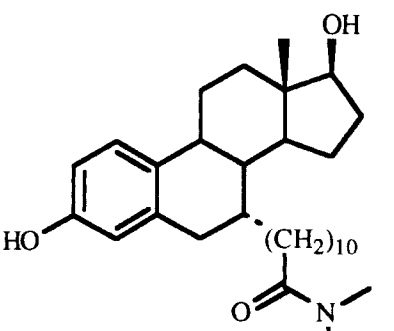


LY 117,018 (2.18)

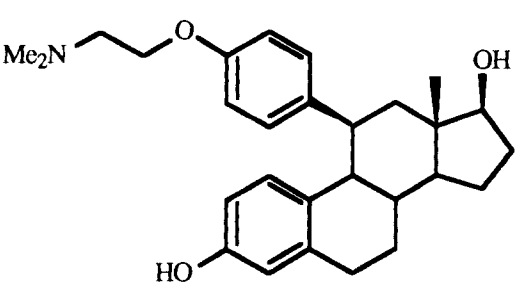
Steroidal



ICI 182780 (2.19)



ICI 164384 (2.20)



RU 39411 (2.21)

Figure 2.4 Antiestrogenic compounds

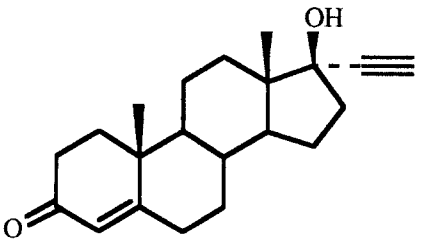
2.5 Progestins.

For many years progesterone has been known to inhibit the development and release of an ovum from the ovaries.[20] In this regard progesterone has been considered as a contraceptive agent.[7,20] Unfortunately progesterone is not orally active due to its rapid metabolism by the liver.[22-28] With this draw back came the pharmaceutical drive for the discovery of an orally active progestin.[20]

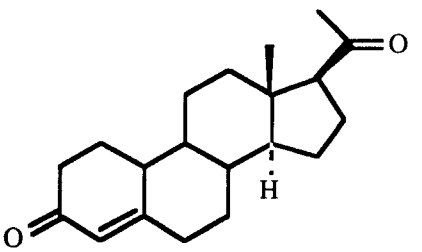
The first orally active progestin was synthesised by researchers at Schering who introduced the ethynyl functionality at position 17α in the testosterone series to afford 17α-ethynyltestosterone (2.22) (ethisterone).[21] Later literature reported the synthesis of 19-norprogesterone (2.23)[22,23] and 14-iso-17-iso-19-norprogesterone (2.24).[24,25] Surprisingly these synthetic steroids exhibited a pharmaceutical level of activity which was 4-8 times greater than that of the natural hormone progesterone (1.15).[23-25]

With the discovery that the removal of the 19-methyl functionality from progesterone augmented its biological activity,[23-25] the researchers at Syntex applied this methodology to the Schering compound ethisterone thus affording 19-nor-17α-ethynylethisterone (2.25) (norethisterone),[26] which exhibited a remarkable improvement in activity.[26]

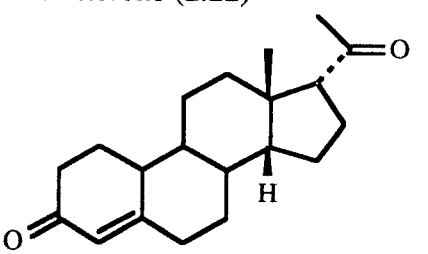
Since the discovery of norethisterone (2.25),[26] additional 19-norsteroids have been synthesised,[27] the first of these was norethindrone acetate (2.27) which was synthesised at Schering.[27] A more interesting variant is that of lynesterol (2.28) which is devoid of the 3-keto functionality and was synthesised by Organon.[27] Interestingly all of these synthetic steroids are metabolised by the body into norethisterone (2.25).[27] The compound levonorgestrel (2.26) exhibits a lower level of biological activity than norethisterone (2.25).[27]



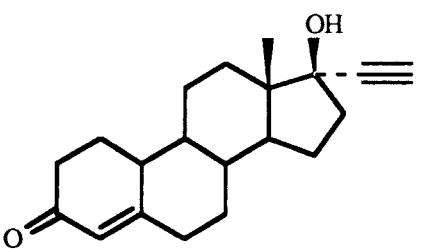
Ethisterone (2.22)



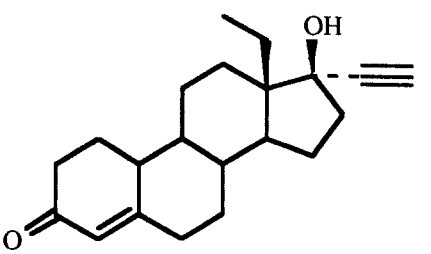
19-Norprogesterone (2.23)



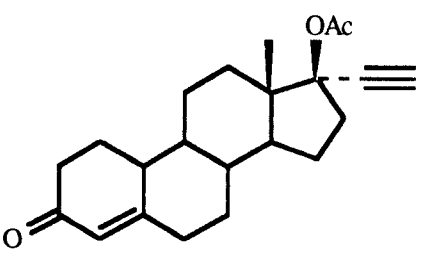
14-Iso-17-iso-
19-norprogesterone (2.24)



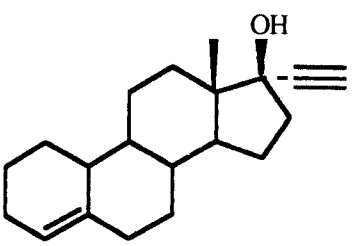
Norethisterone (2.25)



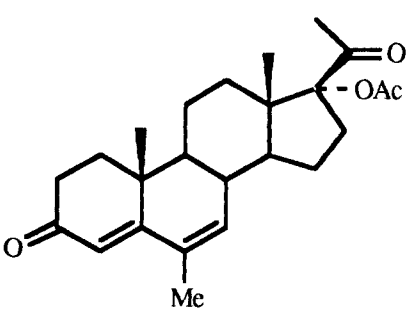
Levonorgestrel (2.26)



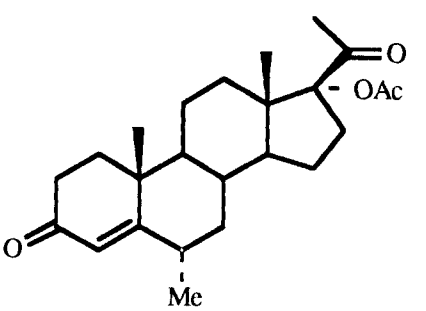
Norethindroneacetate (2.27)



Lynesterol (2.28)



Megestrol acetate (2.29)



Medroxyprogesterone
acetate (2.30)

Figure 2.5 Progesterone receptor agonists

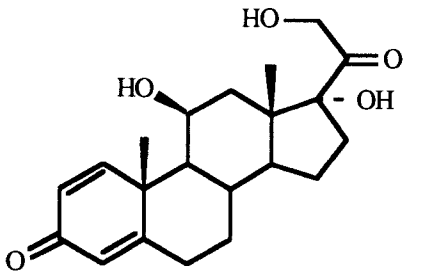
Other orally active progestins are megestrol acetate (2.29) and medroxyprogesterone acetate (2.30),[7] these compounds both possess a 6-methyl functionality.[7] The 17α-acetoxy group of these compound makes the C-17α ethan-1-one group on the pregnane skeleton more resistant to metabolism.[7]

2.6 Glucocorticoids.

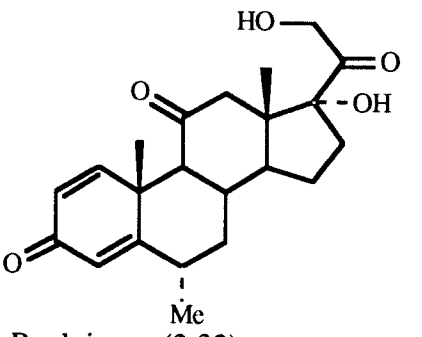
Corticosterone (1.9) and cortisol (1.12) are two hormones secreted by the adrenal cortex of the adrenal glands.[4,13] These naturally occurring steroids exhibit strong glucocorticoid activity and weak mineralcorticoid activity.[4,13] The most important pharmacological actions of glucocorticoids are their antiinflammatory and antirheumatic activity.[4]

Commercially a number of potent semisynthetic glucocorticoids have been developed by structural variation of the natural hormones.[4,13,28] Prednisolone (2.31) is an analogue of corticosterone (1.9) which possesses a double bond at C-1,[28] which alters the A-ring geometry and augments glucocorticoid activity.[4] The introduction of a 9α-fluoro group and a 16α or 16β methyl group gives dexamethasone (2.35) and betamethasone (2.36) which are amongst the most potent commercially available semisynthetic glucocorticoids.[28]

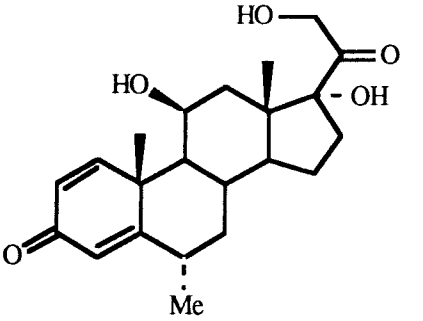
These synthetic glucocorticoids are clinically used to suppress inflammatory and allergic reactions.[4,28] They are also useful in replacement therapy where patients are suffering from Addisons disease (due to low level of circulating glucocorticoids).[28] In patients who suffer from Cushings syndrome with high levels of circulating glucocorticoids, an antagonist of the hormone (e.g. RU 38486 (2.40)) is useful for controlling the disease.[9]



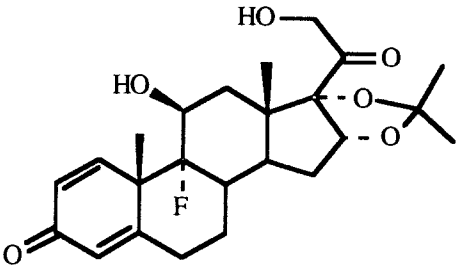
Prednisolone (2.31)



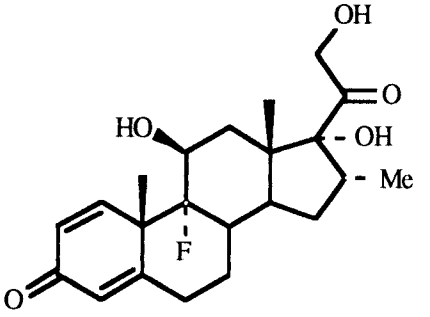
Prednisone (2.32)



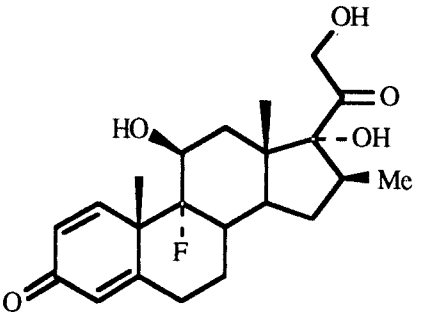
6α-Methylprednisolone (2.33)



Triamcinolone acetonide (2.34)



Dexamethasone (2.35)



Betamethasone (2.36)

Figure 2.6 Synthetic glucocorticoids.

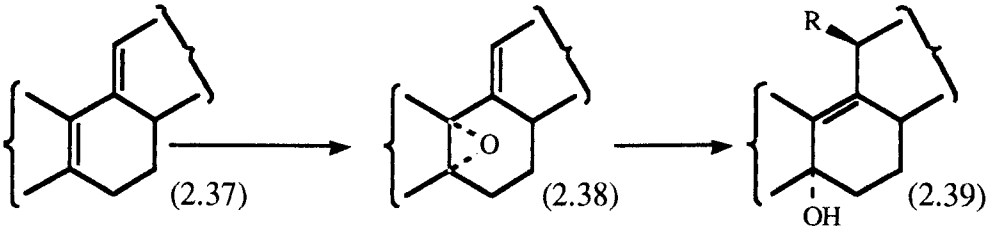
2.7 Antiprogestins + Antiglucocorticoids.

By the 1970s a number of structurally interesting non-steroidal estrogens (e.g. trans-diethylstilbestrol (2.10), hexestrol (2.12) and non-steroidal antiestrogens (MER25 (2.13), clomiphene (2.14), tamoxifen (2.15)) had been discovered.[1-3,19]

At that time researchers at Roussel-Uclaf were involved in the total synthesis of cortisone[29] and methods for the purification of the estradiol receptor.[30] In the course of studying how small structural alterations affect

the ability of novel corticosteroids[31-34] steroids to bind to and activate their receptors, the researchers developed a general synthetic strategy by which alkyl and aryl functionalities could stereospecifically be introduced at the 11β-position of 19-norsteroids.[16-18,35-37]

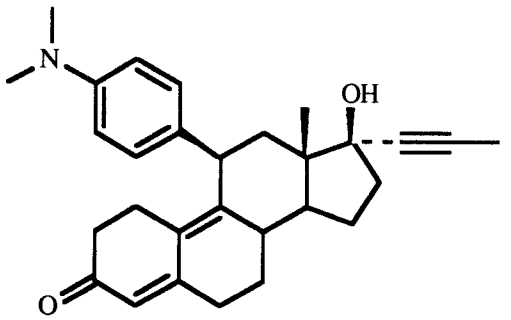
The strategy primarily relies on two key features, the first involved the stereospecific synthesis of 5α,10α-epoxy-9(11)-ene steroids (2.38).[16-18,35-37] The second key stage involved the treatment of the 5α,10α-epoxy-9(11)-ene steroids (2.39) with lithium organocuprates or copper catalysed Grignard reagents to give exclusive 11β-substituted-19-norsteroids with an excellent yield.[16-18,35-37] The researchers at Roussel-Uclaf synthesised a number of 11β-substituted steroids[16-18,35-37] which led to the discovery of RU 38486 (2.40) (mifepristone) possessing antiprogestational and antiglucocorticoid activity.[38,39] The 17α substitution of RU 38486 (2.40) imparts a higher binding affinity for the receptor and the 11β-aryl substitution is responsible for its antagonistic action.[16,17,37]



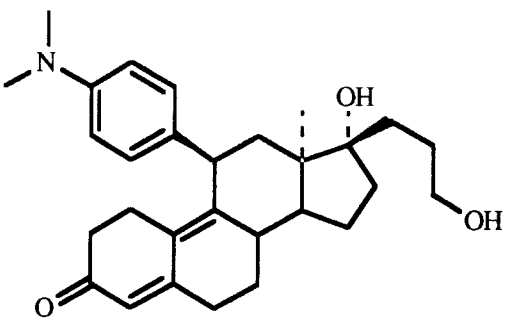
(2.37) (2.38) (2.39)

Scheme 2.1

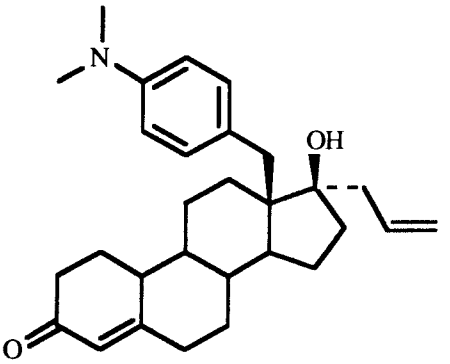
Following the discovery of RU 38486 (2.40), a great many analogues were synthesised by Roussel-Uclaf,[16-18,35-37] Schering,[40-44] and Organon[45] in order to improve their level of activity and receptor specificity. Although there is some structural variation between these analogues, the vast majority of antiprogestins, antiglucocorticoids and antiestrogen analogues[18] of RU 38486 (2.40) utilise the synthetic strategy that was originally developed at Roussel-Uclaf to stereospecifically introduce 11β substituents into the steroid skeleton.[16-18,35-37]



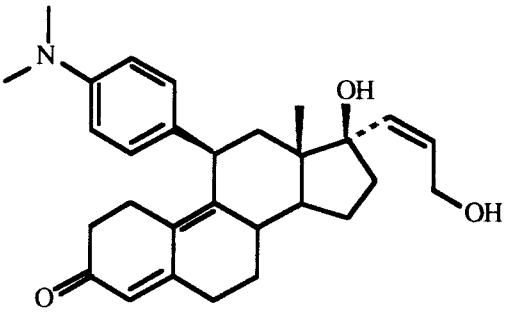
RU 38486 (Mifeprisone) (2.40)



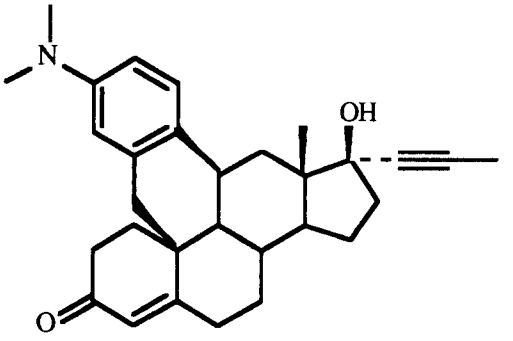
ZK 98.299 (Onapristone) (2.41)



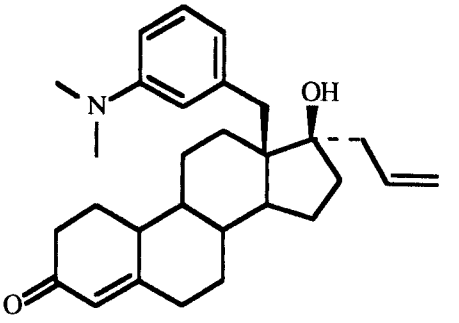
Org 31343 (2.42)



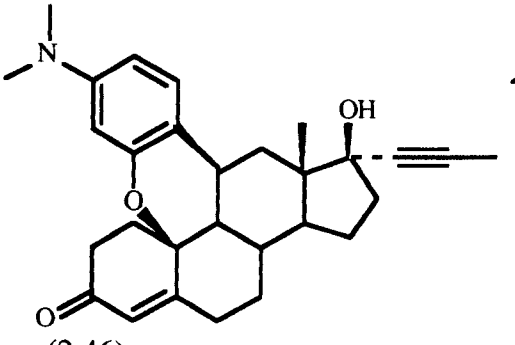
ZK 98.734 (Lilopristone) (2.43)



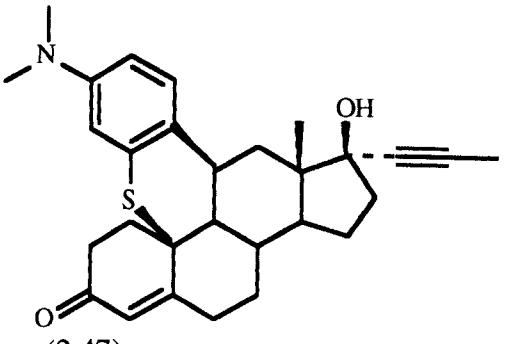
(2.44)



Org 31167 (2.45)



(2.46)

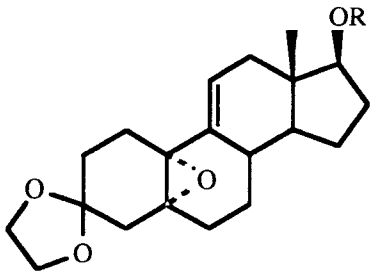


(2.47)

Figure 2.7 Progesterone and glucocorticoid receptor antagonists

2.8 The synthesis of 5α,10α-epoxides.

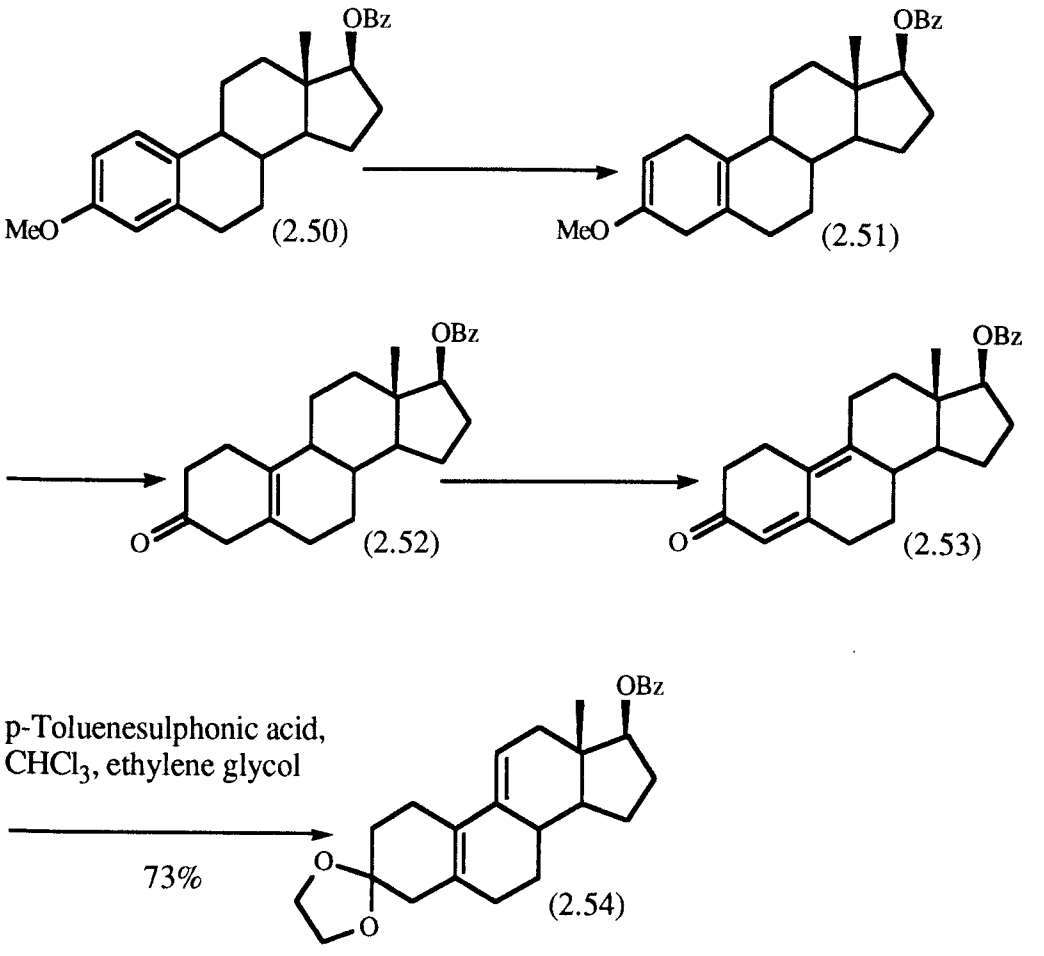
In the course of the synthesis of novel 19-norsteroids, L. Nédélec *et al* reported[45,46] the synthesis of 9(11)-en-5α,10α-epoxides of the general structural formulae (fig. 2.8).



R
H (2.48)
Bz (2.49)

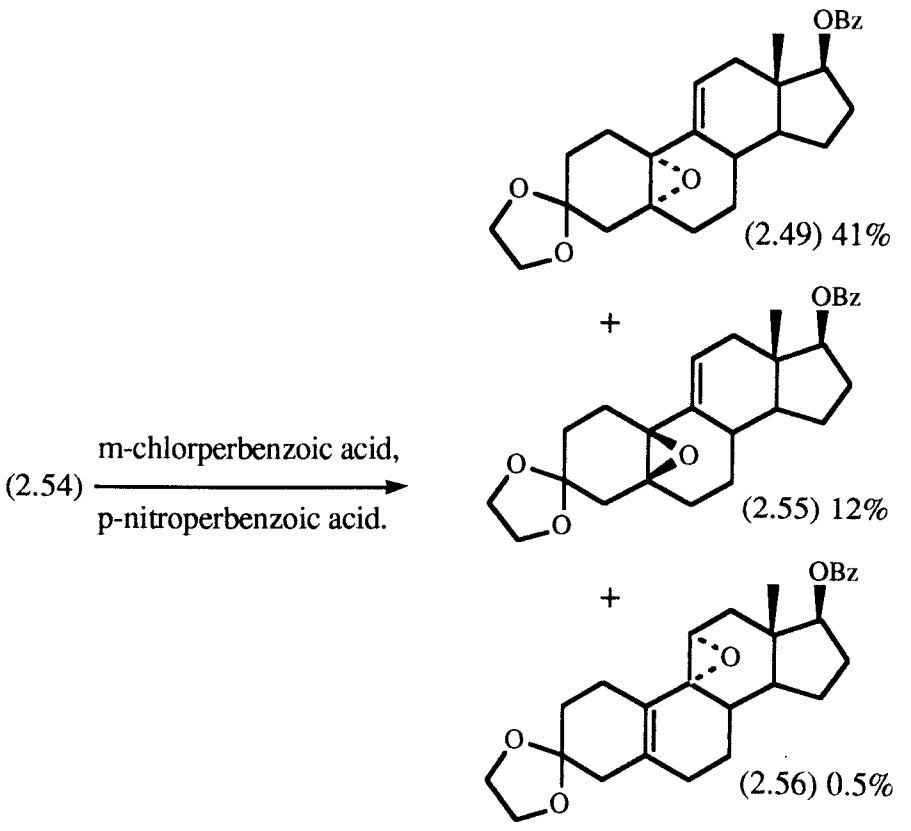
Figure 2.8 General structure of 9(11)en-5,10α-epoxides.

The epoxide (2.49) was synthesised in five steps via Birch reduction of the readily available estradiol methyl ether (2.48) (scheme 2.2, 2.3).[45] Hydrolysis of the methyl ether (2.51) led to the deconjugated enone (2.52), which was converted to the corresponding dieneone (2.53) by a bromination-dehydrobromination procedure.[45] Ketalization of the dienone (2.53) afforded the conjugated diene (2.54) with a double bond shift to 5(10),9(11)-diene in 73% yield.[46]



Scheme 2.2 Synthetic pathway to 5(10),9(11)-ketals.

The conjugated diene ketal (2.54) was oxidised with m-chloro perbenzoic acid in chloroform at 0°C (scheme 2.3).[46] Under these conditions the epoxidation occurred regiospecifically to afford a mixture of 3 epoxides which were purified by chromatography to give (2.49), (2.55) and (2.56) in 41%, 12% and 0.5% yields respectively.[46]

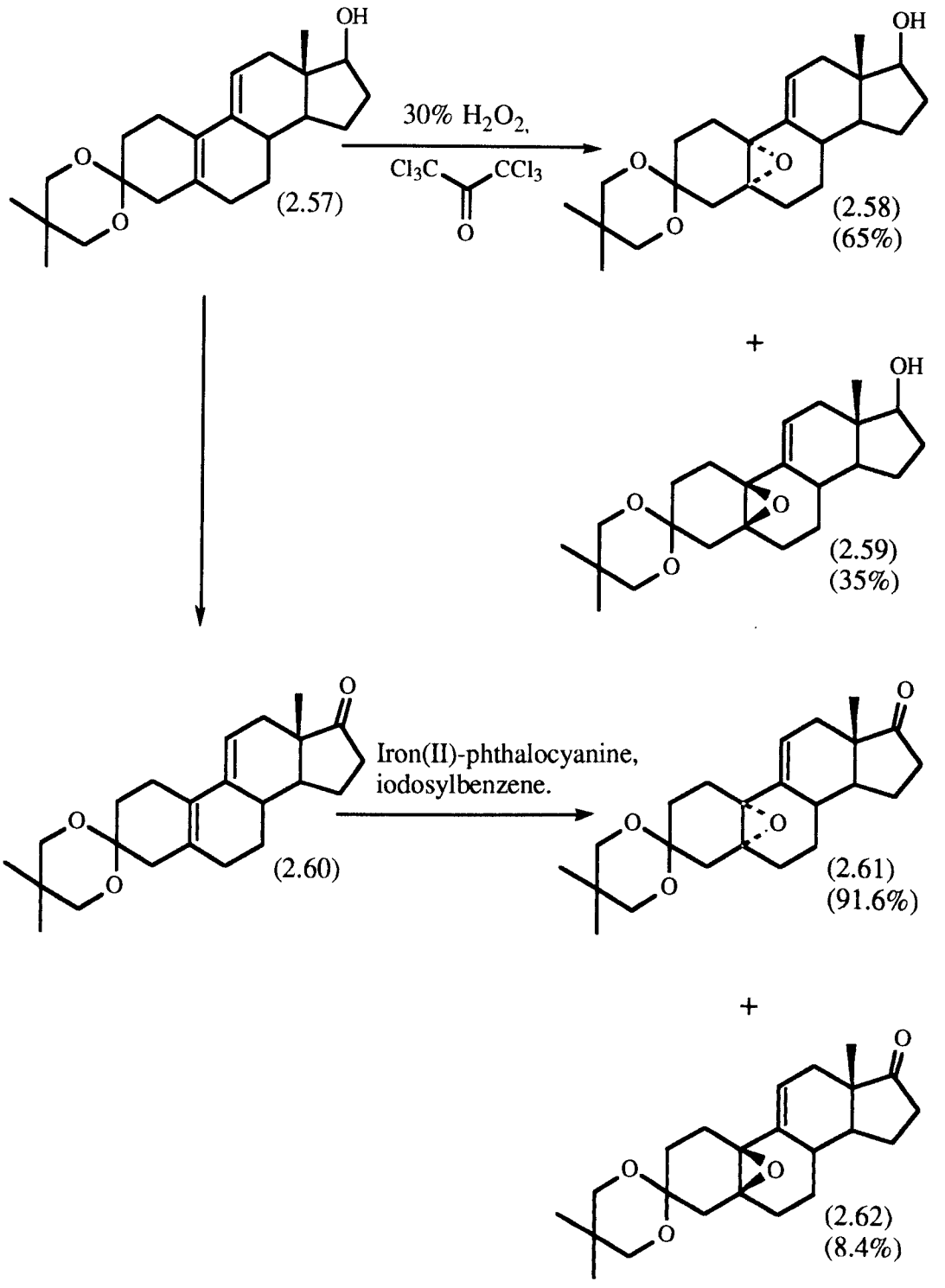


Scheme 2.3 Epoxidation of conjugated diene ketal

Although the epoxidation reaction occurred regiospecifically, it did not proceed in a stereospecific manner and afforded a low yield of the 5α,10α-epoxide (2.49).[46]

The researchers at Roussel-Uclaf were able to improve upon the stereospecificity of the epoxidation procedure when hydrogen peroxide and hexachloroacetone was used as the oxidant.[32,33] This improved the stereoselectivity and yield of the 5α,10α-epoxide (2.49) from 41% to 65% and that of the 5β,10β-epoxide (2.55) from 12% to 35%.[32,33]

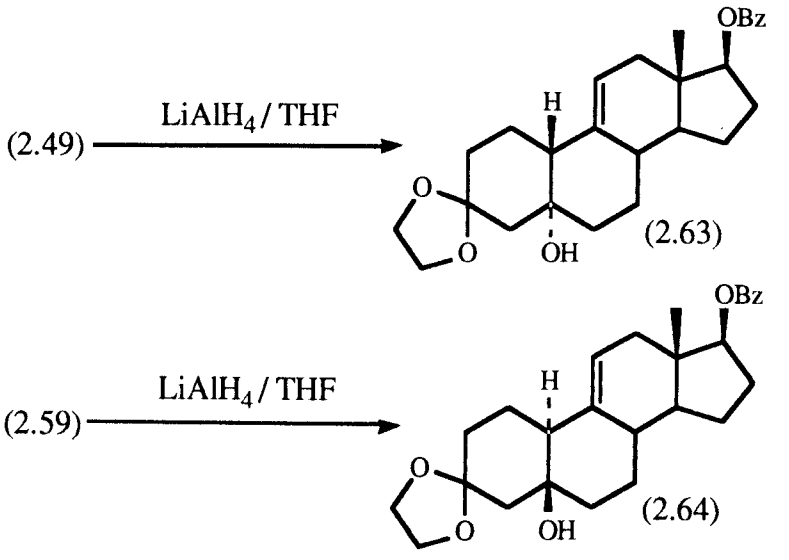
Workers at Schering were able to improve upon the stereoselectivity by employing iron(II)phthalocyanine as catalyst and iodosyl benzene as the oxygen source (scheme 2.4).[40] This gave a product mixture of 5α,10α-epoxide (2.61) (91.6%) and the 5β,10β-epoxide (2.62) (8.4%).


OH
30% H_2O_2,
Cl_3C CCl_3
O
(2.57)
OH
O
O
(2.58)
(65%)
+
OH
O
O
(2.59)
(35%)
O
Iron(II)-phthalocyanine,
iodosylbenzene.
O
O
(2.60)
O
O
(2.61)
(91.6%)
+
O
O
(2.62)
(8.4%)

Scheme 2.4

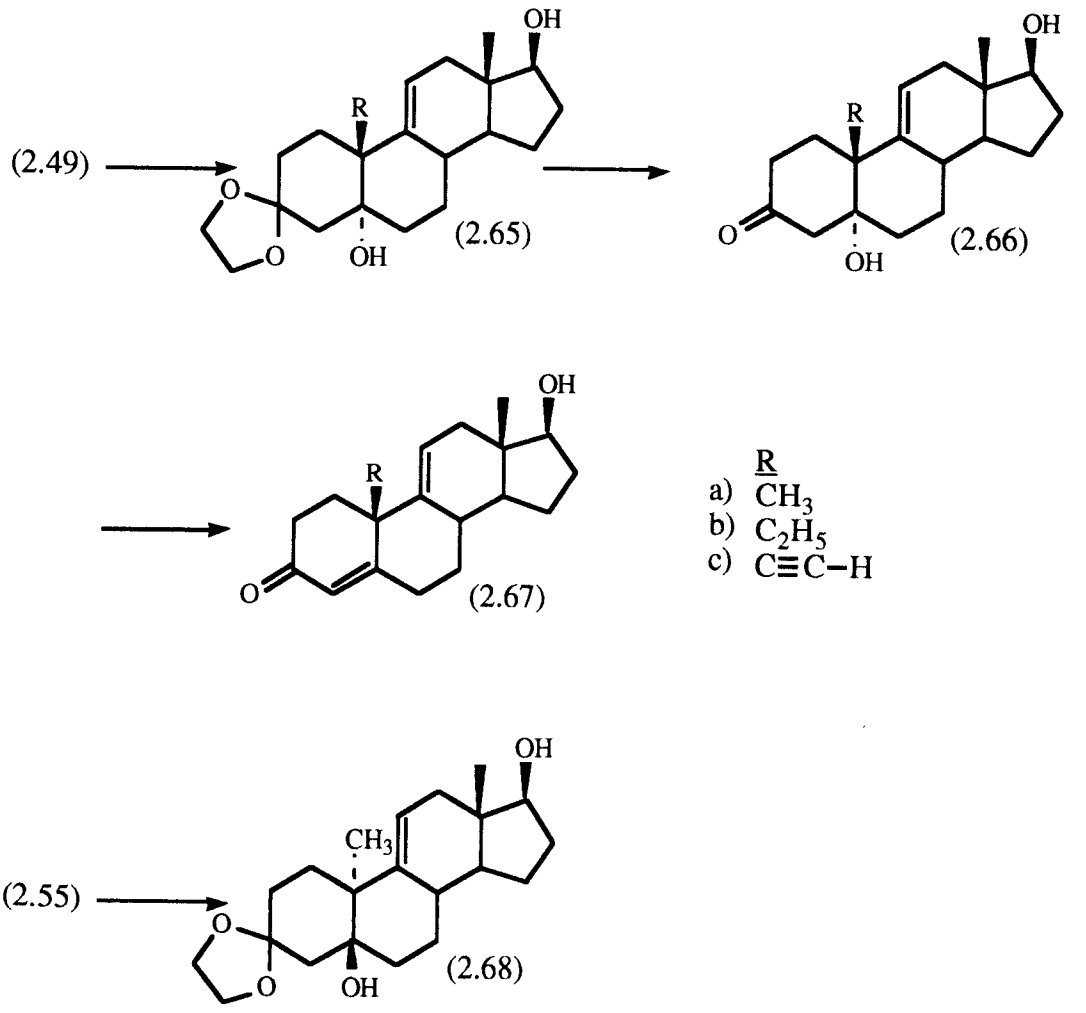
2.9 Ring opening of 5α,10α-epoxides in the synthesis of 10β substituted steroids.

At Roussel-Uclaf the epoxides (2.49, 2.55) were considered to be intermediates that could potentially lead to novel steroids.[18] Nédélec demonstrated that reduction the of 9(11)-en-5(10)-epoxides (2.49) and (2.55) with $LiAlH_4$ in THF occurred regiospecifically and stereospecifically.[46] The introduction of the hydride nucleophile at position C-10 occurred with an orientation that was opposite to that of the original oxirane (fig. 2.5).[46]



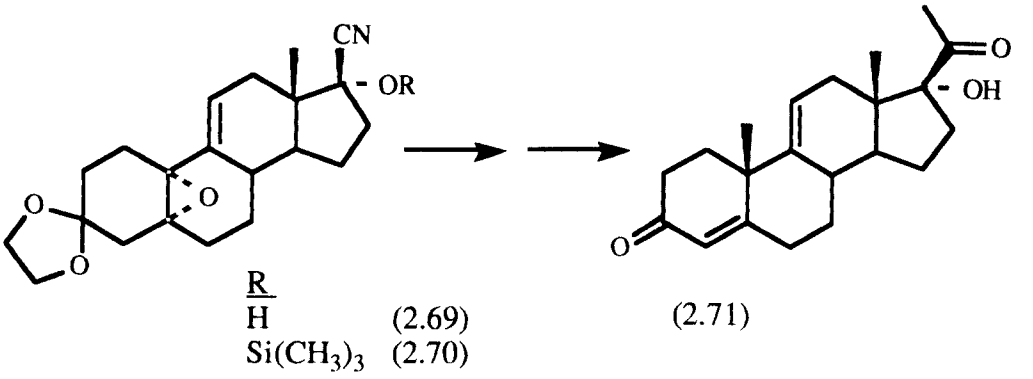
Scheme 2.5

Using this methodology the researchers at Roussel-Uclaf embarked upon the synthesis of novel 10α and 10β substituted steroids.[31-34,46] Nédélec and Gasc reported the introduction of methyl, ethyl and ethynyl substituents at the 10β position and methyl at the 10α position of steroids in the testosterone series (scheme 2.6).[46]

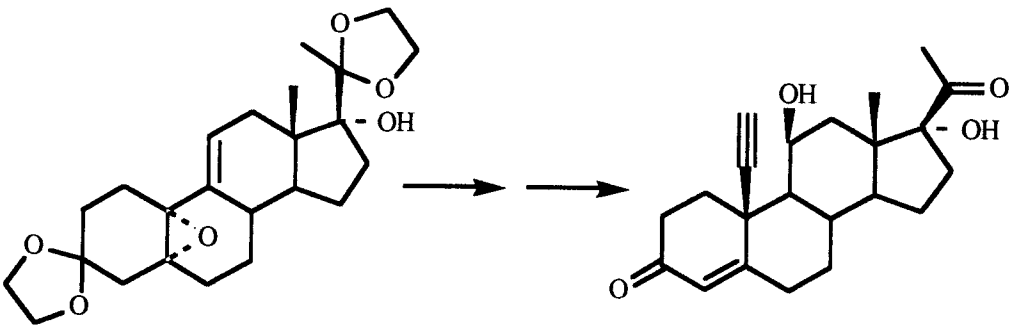


Scheme 2.6

The researchers at Roussel-Uclaf further reported the introduction of 10β-methyl group in the corticoid series[31] (scheme 2.7) and the 10β-ethynyl group in the hydrocortisone series [32,33] (scheme 2.8).



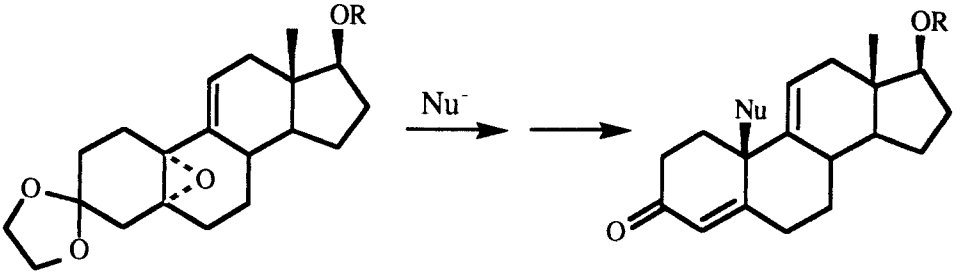
Scheme 2.7



(2.72) (2.73)

Scheme 2.8

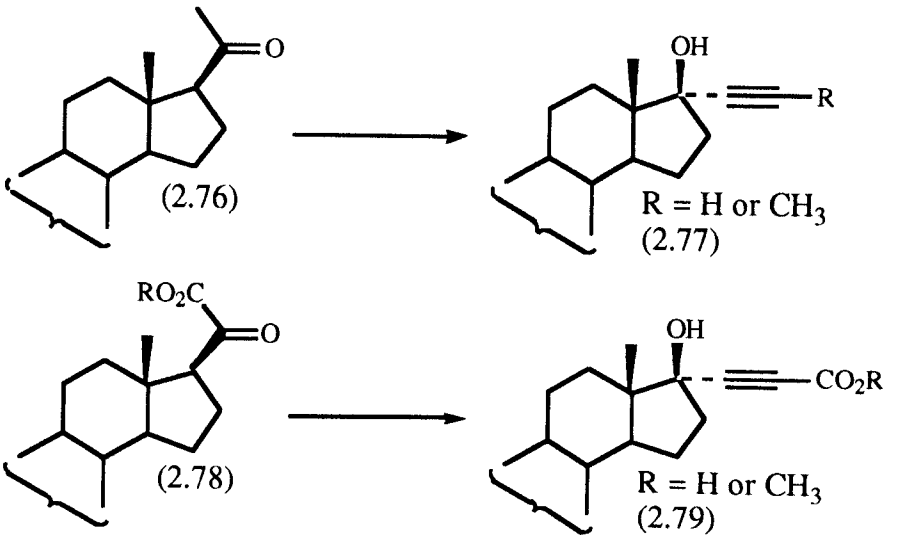
Unfortunately the 10β-ethynyl analogue (2.73) showed a reduced level of biological activity to that of hydrocortisone.[18] In spite of this result, the researchers continued to attempt ring openings of the 5α,10α-epoxide using various nucleophiles such as azide, thiocyanate and mercaptides (scheme 2.9).[18]



(2.74) (2.75)

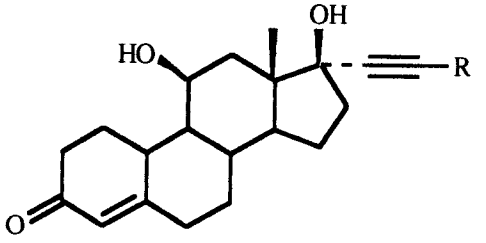
Scheme 2.9

In early 1975, a project had been initiated at Roussel-Uclaf on " New steroids with anti-allergic or anti-stress activities".[18] The main thrust for this research was to be directed towards agonists.[18] In the original project, the replacement of the 10β-methyl group by other substituents and the bioisosteric relationships of a number of chemical modifications at position C-17 were also to be investigated.[18]



Scheme 2.10

It was well known at the time that for progestational steroids, the 17β-acetyl side chain could be replaced by the 17α-ethynyl-17β-hydroxy system without altering the biological activity of the steroid (scheme 2.10).[21,26] Similarly it was postulated that 17β-hydroxy-17α-propionolates should be used in the corticoid series.[18] To test this hypothesis, a number of analogues were synthesised (fig. 2.9).[18]



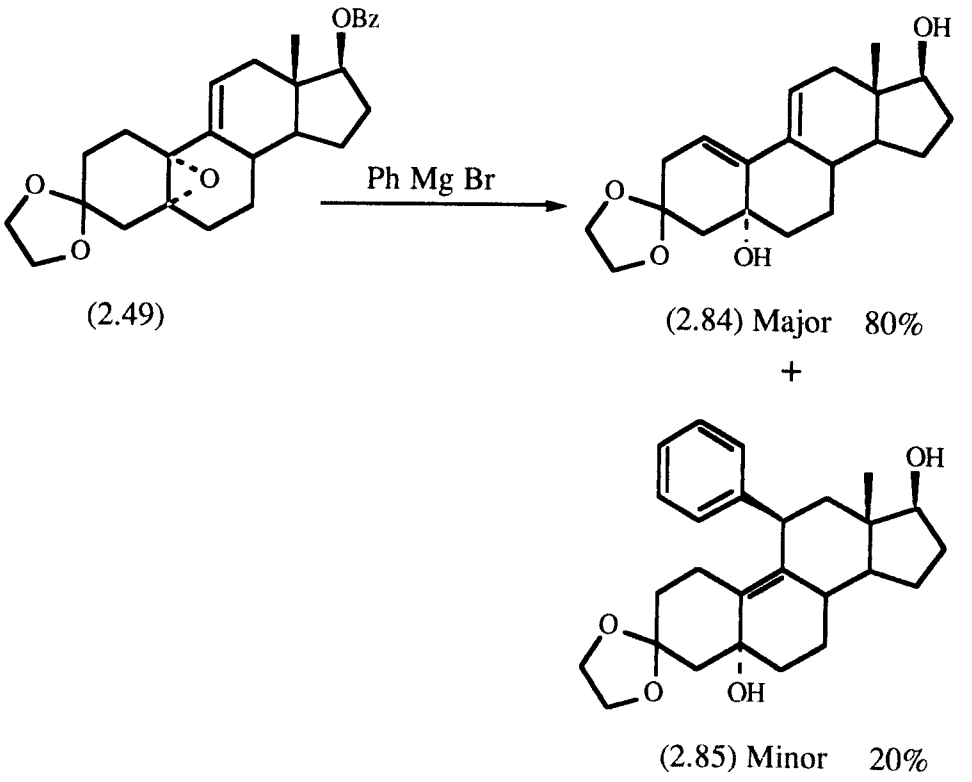
R		
CO_2Me	RU 24450	(2.80)
CO_2H	RU 25101	(2.81)
H	RU 200	(2.82)
Me	RU 25458	(2.83)

Figure 2.9

The carboxylic methyl ester (2.80) exhibited anti-inflammatory activity but its corresponding acid RU 25101 (2.81) was shown to be inactive.[18,48] To assess whether the ester significantly contributed to biological activity, the compounds RU 200 (2.82) and RU 25458 (2.83) were synthesised.[18,48] RU 200 (2.82) showed no activity but surprisingly RU 25458 (2.83) displayed an activity that was four times that of cortisol (1.12).[18,48]

2.10 The discovery of the stereospecific introduction at C-11β.

Meanwhile, Teutsch attempted to synthesise 10β-phenyl steroids, by cleavage of the oxirane (2.49) (scheme 2.11).[36] When the epoxide (2.49) was reacted with phenyl magnesium bromide, the product was found to be a mixture of two steroids in a ratio of 4:1; (2.84) and (2.85).[18,36]



(2.49)

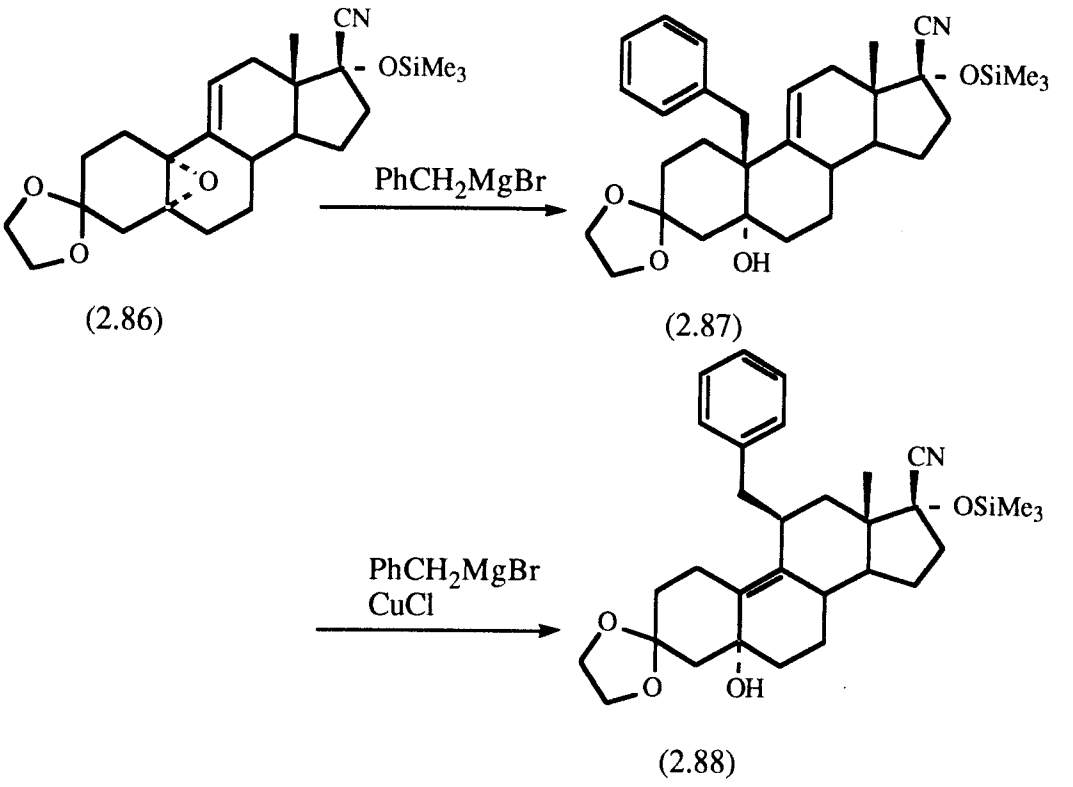
(2.84) Major 80%

+

(2.85) Minor 20%

Scheme 2.11

The epoxide (2.49) was reacted with diphenyl copper lithium, in the hope that it would shift the reaction pathway entirely to the 11β-substituted steroid (2.85), which it did (scheme 2.11).[18,36] Later it was demonstrated that Grignard reagents in the presence of catalytic amounts of copper salts could also achieve the same 11β-transformation.[36]



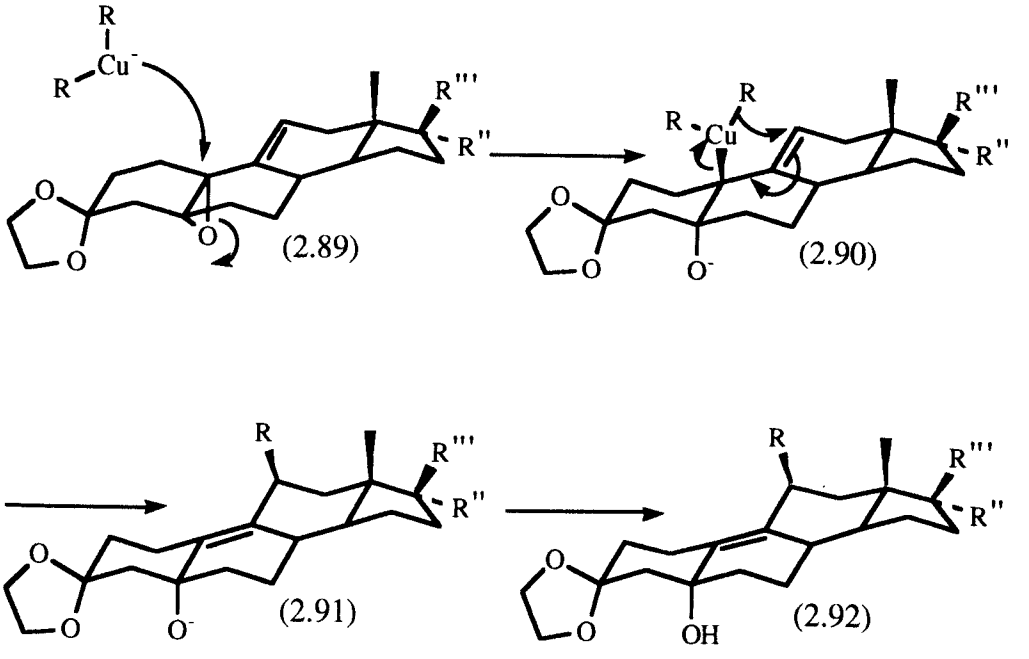
(2.86) (2.87) (2.88)

Scheme 2.12

When benzyl magnesium bromide was used, addition of the benzyl group went entirely to the 10β-position, but in the presence of cuprous chloride the group was introduced at the 11β position (2.88)[36] (scheme 2.12). This elegant stereospecific method for the introduction of 11β-substituents into a sterically hindered environment was the second key stage in the synthetic methodology.[35,36]

It was proposed that the mechanism involves the nucleophilic attack on the epoxide (2.89) from the β-face at position 10 by the organocuprate.[35] This causes the opening of the vinyl oxirane (2.89) to produce an unstable intermediate (2.90) which undergoes an intramolecular allylic rearrangement (scheme 2.13).[35] Since the organocopper substituent is attached to the β face at position 10, the nucleophilic attack by the alkyl

functionality upon the sp^2 hybridised position at C-11 in the allylic rearrangement may only occur stereospecifically from the β-face to give the 11β-steroid (2.91) (scheme 2.13).[38]

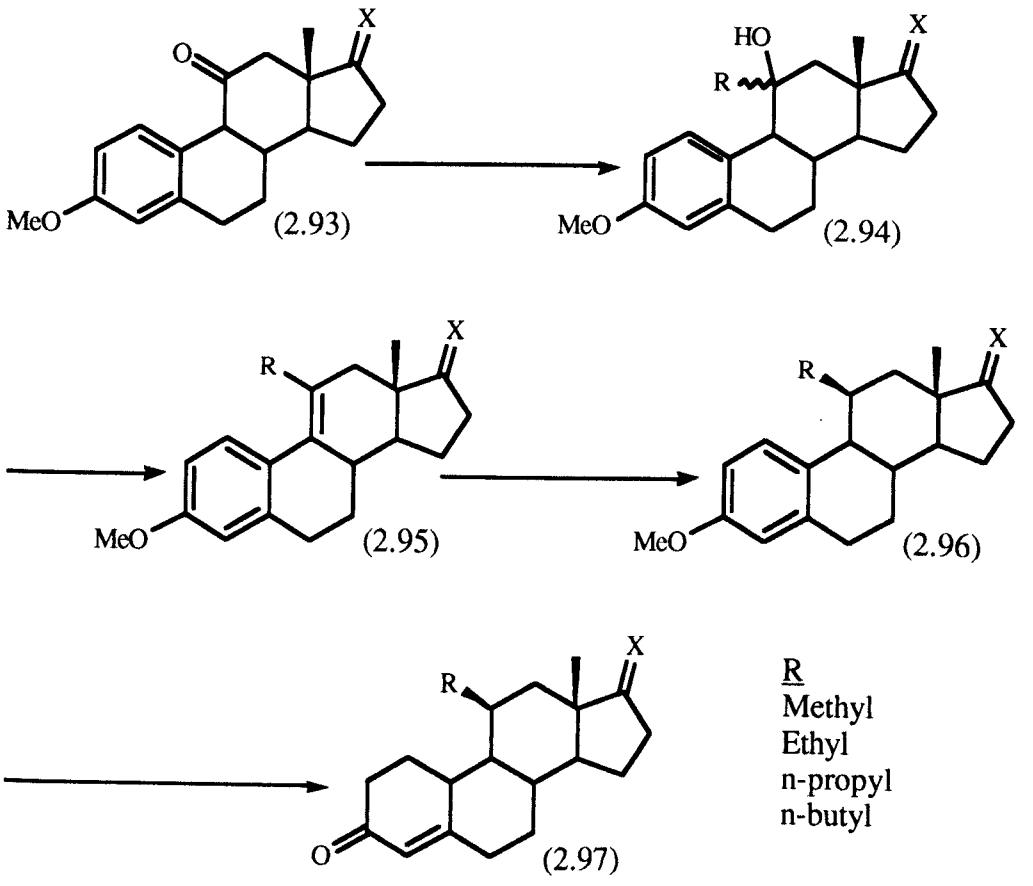


Scheme 2.13

Although conjugate opening of an unsaturated epoxide by copper(II) modified Grignard reagents or cuprates is not an unusual procedure,[49-52] it is surprising that a large number of bulky groups are able to overcome the unfavourable sterically hindered situation of position C-11β which is also in a 1,3-diaxial relationship with the angular C-13 methyl group.[36]

2.11 11-Keto Pathway.

Before the advent of the Roussel-Uclaf strategy, the synthesis of 11β,19-norsteroids had already been reported for 11β-methylestra-1,3,5(10)-trienes (2.96) which were further transformed to the estra-4-ene series (2.97).[53]

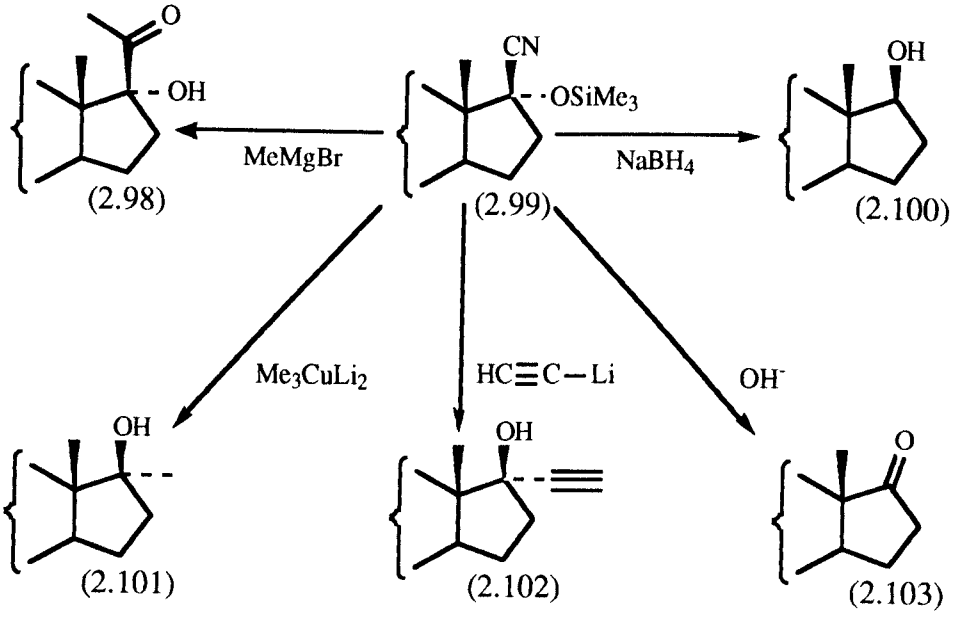


Scheme 2.14

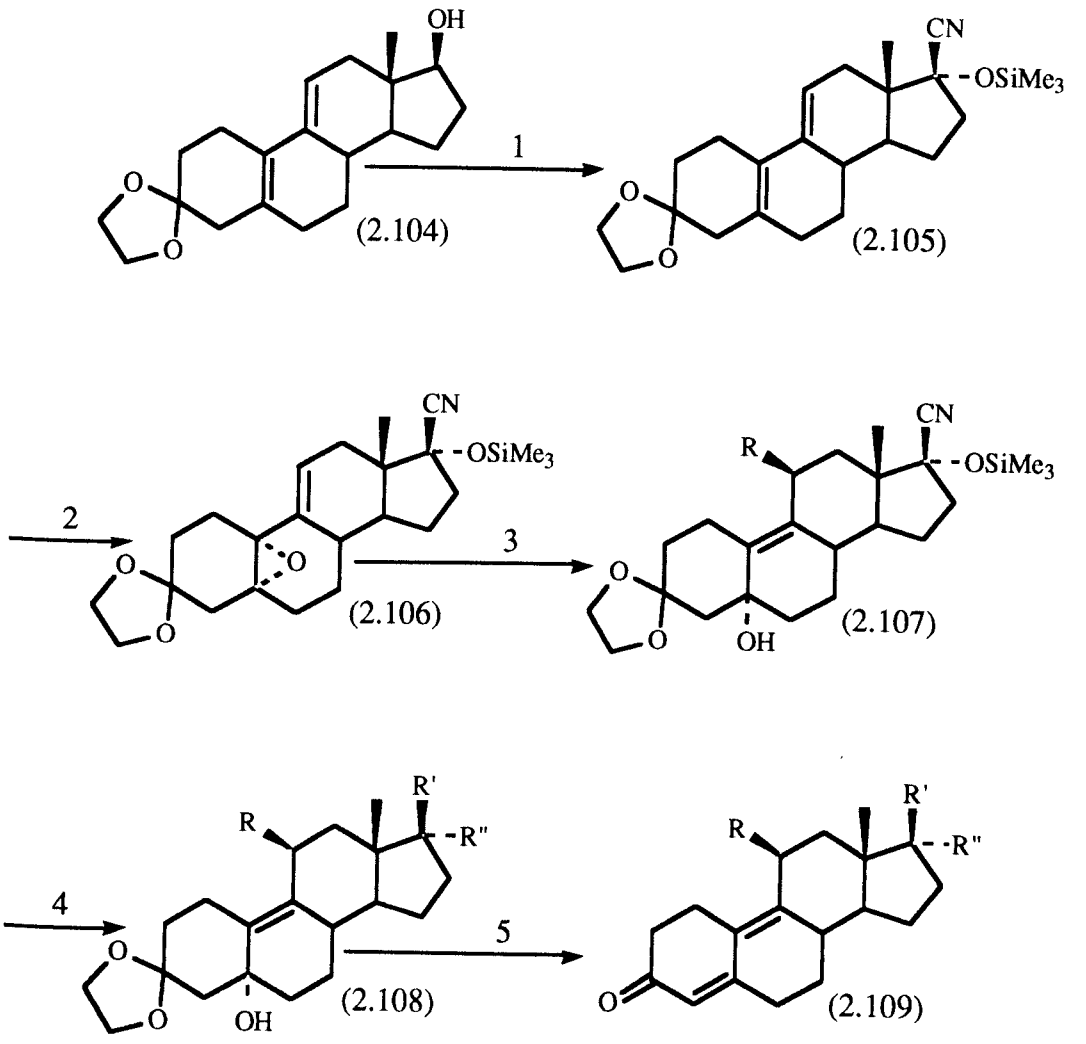
The strategy reported by Kirk and Petrow[54] involved the action of organometallic reagents on an 11-keto steroid followed by dehydration and a catalytic hydrogenation procedure. This methodology was further utilised in the synthesis of 11β-ethyl estrone and 11β-ethyl, n-propyl and n-butyl estrenes.[54] Unfortunately this scheme is impractical as a general synthetic strategy for 11β-substituted steroids as it gives a poor yield of the adduct (2.94) due to competing reduction of the ketone by Grignard reagent.[16,17,37]

2.12 The Discovery of RU 38486 (2.40).

Researchers at Roussel-Uclaf devised a general strategy that utilised the stereospecific method of introducing 11β-substituents, whilst affording a flexible choice of 17α substituents.[18] The 5(10), 9(11)-diene ketal (2.104) was oxidised and reacted with Me_3SiCN to form the 17β-cyano-17-α-trimethyl silyl ester (2.105).[36] This cyanohydrin side chain was chosen as a protecting group for position C-17 as it could easily be transformed into a variety of functionalities at an appropriate step (scheme 2.15). [36] Epoxidation of the cyanohydrin (2.105) followed by a nucleophilic addition with a copper-catalysed Grignard reagent gives the 11β-substituted 5α-hydroxy-9(10)-ene ketal (2.107).[36] Functionalisation of (2.107) at the position C-17 and reflux of the 17α-ethynyl product (2.108) in aqueous ethanol in the presence of sulphonic resin (Redex Cf.) gave the 11β-substituted dienone (2.109).[36]

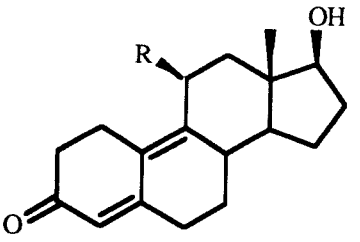


Scheme 2.15 The accessibility of a variety of 17 functionalities from 17-cyano-17-trimethylsilyl ether.

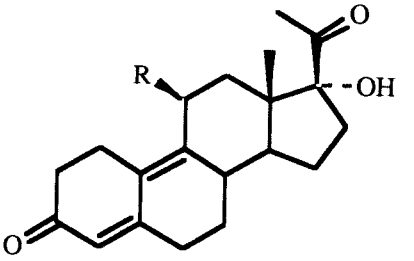


Scheme 2.16 1) CrO_3 /Me_3SiCN, 2) $H_2O_2/Cl_3CCOCCl_3$, 3) R_2CuLi or RMgX, CuCl 4) R^-, THF 5) H_3O^+

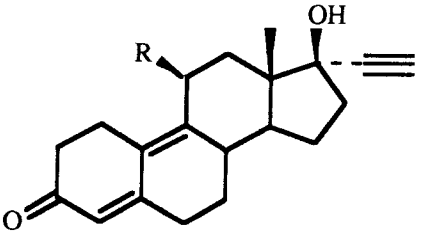
Having established this general strategy for the synthesis of 11β-substituted 19-norsteroids, the researchers investigated how chemical modifications of 11β-functionalities affect the relative binding affinity (RBA) of steroids in the 19-nortestosterone (2.110) and 19-norpregnane series (2.112).[16-18] Some were 17α-benzoates and others had 17α-hydroxy progesterone (2.111) type substituents which had been known to be damaging to progesterone receptor binding.[16-18,35-37] The binding affinities of the compounds synthesised were assessed by Philibert.[36] Further 11β-substituted 19-norsteroids were synthesised for the three classes of sex hormones: progesterone, estrogen and androgen.[18]



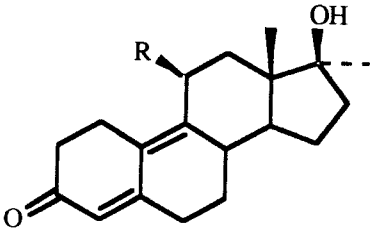
(2.110)



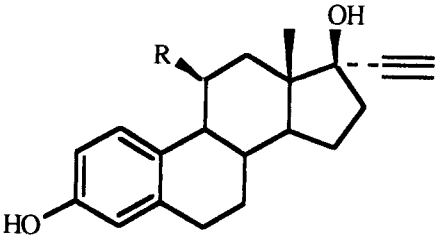
(2.111)



(2.112)



(2.113)



(2.114)

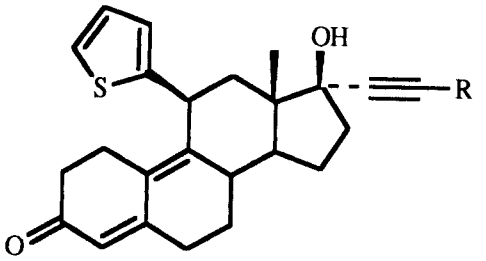
Figure 2.10

A wide range of analogues were synthesised with substituents including alkyl groups (ethyl, n-propyl, i-propyl, t-butyl, n-decyl),[36] alkenyls (vinyl, isopropenyl and allyl),[36] aromatics and hetero aromatics (phenyl, substituted phenyl, benzyl and thienyl).[36] Some nitrogen containing substituents (e.g. pyrazole, pyridine) could not be introduced.[18] It was found that unsaturated 11β-substituents (vinyl, isopropenyl, phenyl, p-substituted phenyl rings) of the 17α-ethynyl series (2.112) displayed a high affinity for the progesterone receptor.[36,55] This remarkable discovery indicated[18] :-

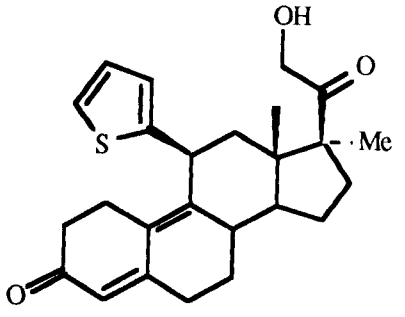
1) The presence of a hydrophobic pocket in the progesterone receptor, which was able to accommodate large unsaturated substituents.

2) The remarkable binding specificity of unsaturated substituents; (vinyl = 535) as opposed to saturated substituents (ethyl = 25) for the progesterone receptor.

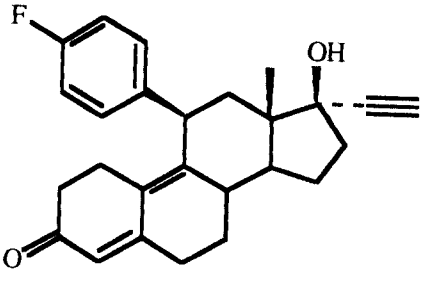
One of the compounds RU 25055 (2.115)[16-18,36] which exhibited the highest RBA for the glucocorticoid receptor was chosen to be tested for its antiinflammatory action in rat thymus tissue.[18] The researchers were working on the assumption that a high binding affinity for the steroid receptor would relate directly to the agonistic activity at that receptor.[16-18] Surprisingly the compound showed no antiinflammatory activity, which for the first time suggested the possibility of antagonism.[18]



	R	
(2.115)	H	RU25055
(2.116)	CO_2Me	RU25402
(2.117)	Me	RU28289



RU 25375 (2.118)



RU 25593
(2.119)

Figure 2.11

Since the researchers were also concerned with the bioisosteric relationships of the 17α-substituents, compounds RU 25402 (2.116) and RU 25375 (2.118) were synthesised.[18] Both molecules were tested for antiinflammatory activity in rat thymus tissue but were shown to be inactive and to have poor binding affinity.[18]

With the possibility of antagonism, some of the molecules including RU 25055 (2.115) and RU 25593 (2.119) were then tested for their ability to antagonise the effects of dexamethasone (2.35).[9,16-18,56] The results confirmed the antagonistic activity but the results were not clear cut, as there were problems with the low level of RBA for the glucocorticoid receptor.[18]

In order to increase the RBA, there was a switch from the 17α-ethynyl to the 17α-propynyl substituent, as it conferred an increase in the RBA for the 11β-hydroxy substituent steroid RU 200 (5 %) (2.82) to RU 25458 (68 %) (2.83) (table 2.1). This alteration had a profound effect as there was a significant improvement for RBA of the glucocorticoid receptor.[16-18,55]

Table 2.1 Relative binding affinity (RBA) for rabbit uterus progesterone receptor determined after 2h and 24h of incubation at 0°C in a rabbit uterus preparation. Value for progesterone is 100.

Code		4hrs	24hrs
RU 200	(2.82)	5	
RU 25458	(2.83)	68	
RU 25055	(2.115)	135	55
RU 28289	(2.117)	270	300
Cortisol	(1.12)	31	

Alongside the glucocorticoid research, an 11β-substituted estradiol bearing the tamoxifen side chain, as a potential antiestrogen RU 39411 (2.21) was synthesised.[18,57]

Since the 11β substituted p-fluorophenyl and p-methoxyphenyl derivatives of the 17α-ethynyl series showed high binding affinity for the progesterone receptor,[16-18,36] the researchers synthesised 17α-propynyl analogues bearing 11β-(p-fluorophenyl), 11β-(p-methoxyphenyl), 11β-(p-methylthiophenyl) and 11β-(p-dimethylaminophenyl) analogues (fig. 2.12).[16-18] In order to progress quickly, the researchers chose to synthesise these molecules from the 17α-hydroxy-17β-propynyl-9(11)-ene-5α,10α-epoxide (2.120).[16-18] The 17α-ethynyl analogue bearing the 11β-p-dimethylaminophenyl substituent RU 38473 (2.122) was also synthesised.

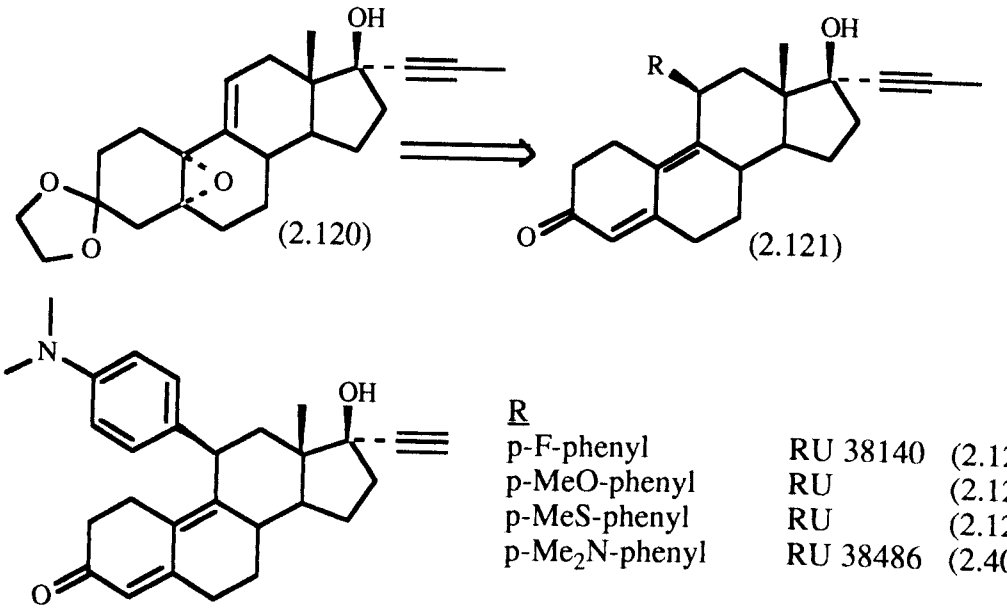
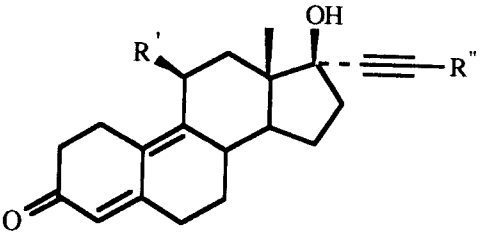


Figure 2.12

Table 2.2 Relative binding affinity (RBA) for rat thymic glucocorticoid receptor determined after 4h and 24h of incubation at 0°C in a rat thymus preparation. Value for dexamethasone (2.35) is 100

Code		R'	R''	RBA 4h	RBA 24h
RU 25055	(2.115)	2-thienyl	H	136	54
RU 28289	(2.117)	2-thienyl	Me	268	299
RU	(2.126)	p-MeO-phenyl	H	237	87
RU	(2.124)	p-MeO-phenyl	Me	279	299
RU 25593	(2.119)	p-F-phenyl	H	45	23
RU 38140	(2.123)	p-F-phenyl	Me	216	283
RU 38473	(2.122)	p-Me_2N-phenyl	H	279	235
RU 38486	(2.40)	p-Me_2N-phenyl	Me	283	302



When the RBA of these molecules was evaluated for rat thymus glucocorticoid receptor, it became clear that the propynyl functionality significantly enhanced the RBA for the glucocorticoid receptor (table 2.2).[16-18,55]. The RBA of RU 38486 (2.40) and RU 38473 (2.122) were most potent (table 2.2).[16-18,55]

Table 2.3 Antiglucocorticoid activity on hepatic trypsin pyrolase in adrenalectomysed rats.

Code	Dose (mg/kg)	% inhibition of the effect of dexamethasone (2.35)
RU 38473 (2.122)	10	100
RU 38486 (2.40)	1	18
"	2.5	65
"	10	94
"	100	100

The antiglucocorticoid activity was not the only outstanding feature of RU 38486 (2.40), as when the RBA of this compound was evaluated for the five classes of steroid receptors it exhibited an affinity for rabbit uterus progesterone receptor which was more than five times greater than that for the natural hormone progesterone (1.15) (table 2.4).[16-18,39,55]

In vivo, RU 38486 (2.40) was completely able to antagonise the ability of progesterone (1.15) to cause endometrial growth of estradiol (1.14) treated immature female rabbits[39] and interrupt pregnancy in both female rats and monkeys (*Macaca fascicularis*).[8,39] This *in vivo* result confirmed that RU 38486 (2.40) acts as a progesterone receptor antagonist.

Table 2.4 Relative binding affinity (RBA) for rabbit uterus progesterone receptor determined after 2h and 24h of incubation at 0°C in a rabbit uterus preparation . Value for progesterone is 100.

Code		R'	R"	RBA 4h	RBA 24h
RU 25055	(2.115)	2-thienyl	H	70	85
RU 28289	(2.117)	2-thienyl	Me	230	438
RU	(2.126)	p-MeO-phenyl	H	130	335
RU	(2.124)	p-MeO-phenyl	Me	136	506
RU 25593	(2.119)	p-F-phenyl	H	38	36
RU 38140	(2.123)	p-F-phenyl	Me	46	85
RU 38473	(2.122)	p-Me_2N-phenyl	H	81	350
RU 38486	(2.40)	p-Me_2N-phenyl	Me	78	530

Clinical trials of RU 38486 (2.40) in human volunteers showed that it was possible to bring about the termination of early pregnancy (up to a week after menstruation had been expected) in 80% of patients.[1-3,7-9,11] When a prostaglandin was administered 36-48 hrs after the administration of RU 38486 (2.40), the success rate dramatically improved to 96%.[9] This synergistic combination of RU 38486 (2.40) and prostaglandins was also shown to be successful in 96% of patients for up to three weeks passed the missed period.[2,3,9,11,18,39]

2.13 Potent analogues of RU 38486 (2.40).

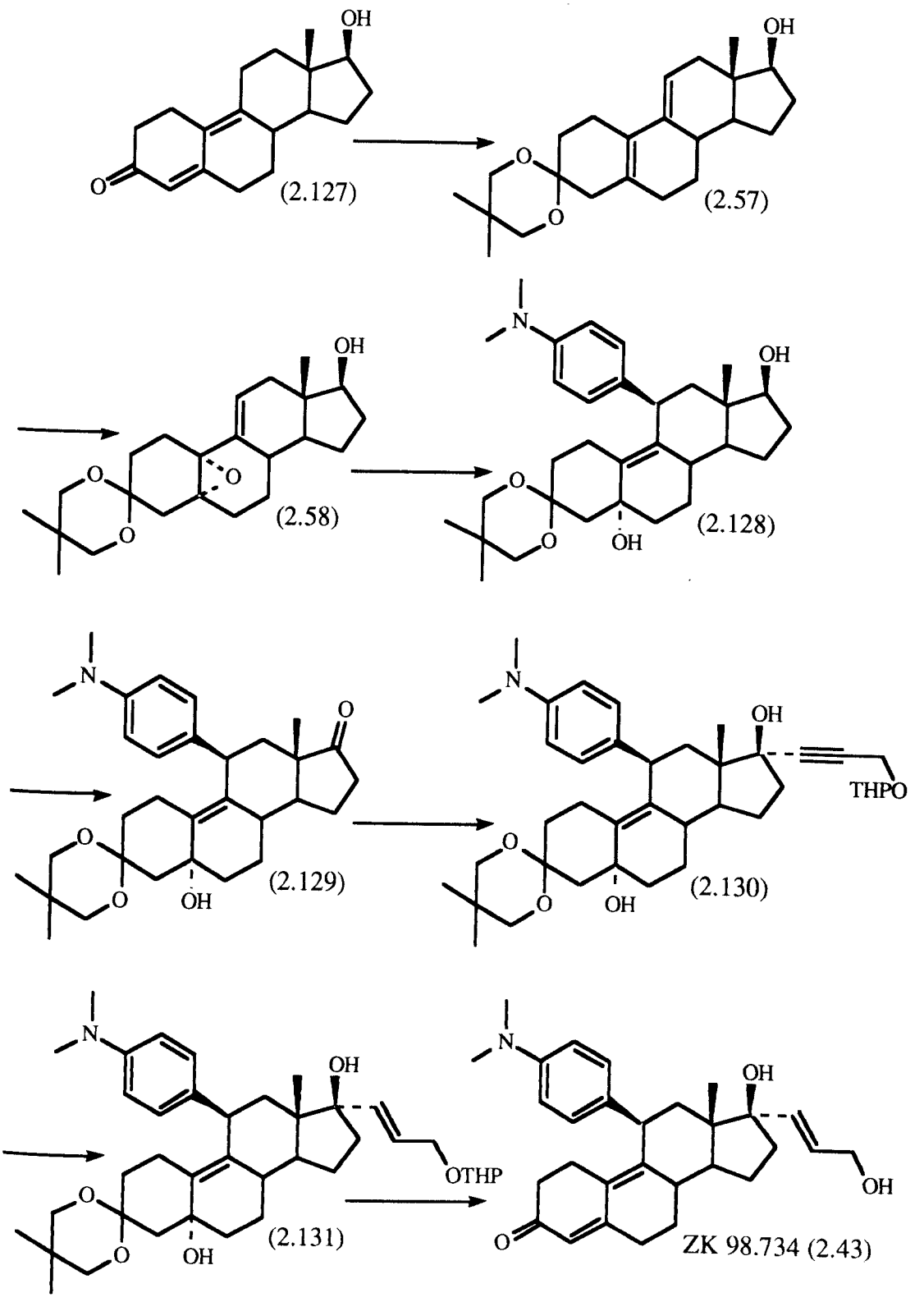
Researchers at Schering similarly discovered that ZK 98.299 (2.41) and ZK 98.734 (2.43) also displayed antiglucocorticoid effects.[1-3,40-42] These compounds were also tested for their ability to bind to other steroid receptors. As with RU 38486 (2.40), these compounds were shown to exhibit antiprogestational and antiglucocorticoid activity *in vitro*.[1-3]

Researchers at Organon (Oss, Netherlands) reported that some 18-dimethylaminophenyl-19-norsteroids are equipotent to RU 38486 (2.40) as regards to the termination of early pregnancy in rat after oral administration, but surprisingly display no glucocorticoid activity *in vitro*.[45] The most potent of their series of compounds were Org 31343 (2.42) and Org 31167 (2.45).[45] Organon reported that their compounds were far more active when administered orally as opposed to subcutaneously.[45] This was an interesting finding as it indicated that the compounds were being metabolised by the liver into biologically active intermediates. This idea was further supported by the fact that these synthetic compounds also showed a low binding affinity for the progesterone receptor *in vitro* and that no glucocorticoid activity was exhibited *in vivo* in a test system for termination of early pregnancy in rat.[45] Unfortunately little pharmacological data has been reported by Organon and their synthetic route to 18-dimethylaminophenyl 19-norsteroids has not been published at all.

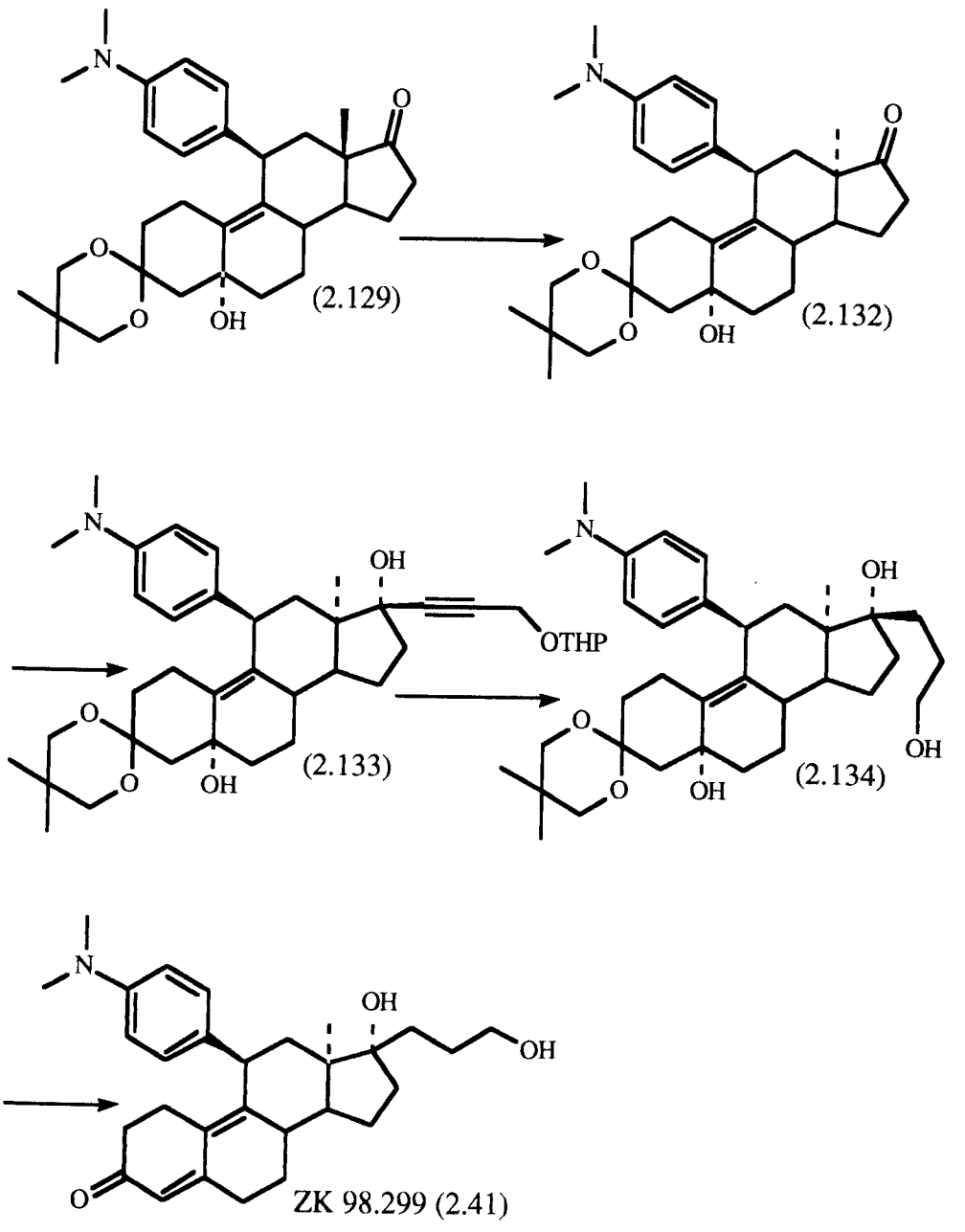
The Schering strategy (scheme 2.17, 2.18) for the synthesis of their related compounds ZK 98.299 (2.41) and ZK 98.734 (2.43),[40,42,58,59] is similar to that for RU 38486 (2.40) discussed above. The 17β-hydroxy dienone (2.127) was ketalised using 2,2-dimethyl-1,3-dihydroxypropane to give the 5(10),9(11)-diene ketal (2.57) product with shifted double bonds.[40] Epoxidation of (2.57) produced the vinyl epoxy ketal (2.58).[40] The 11β-

substituent was introduced using copper catalysed Grignard reagents to give the 11β-aryl-5α,17β-dihydroxy-9(10)-ene ketal (2.128).[40] Oppenauer oxidation of this intermediate gave the 9(10)-en-17-one (2.129), which was then treated with the litho derivative of propargyl tetrahydropyranyl ether to afford (2.130).[40] Hydrogenation and deprotective dehydration using acid-catalysed conditions led to the compound ZK 98.734 (2.43).[40]

In the preparation of ZK 98.299 (2.41),[40,42] following the Oppenauer oxidation, the next step involved the photolysis of the intermediate (2.129) in dioxane using a mercury high pressure lamp. Ring opening by a Norrish-type I cleavage and subsequent recombination of the radical sites afforded the 11α-methyl gonane (2.132) in good yield.[40] Treatment of (2.132) with the litho derivative of propargyl tetrahydropyranyl ether afforded the 17β-substituted product (2.133).[40] Hydrogenation of the 17β adduct (3.133) followed by deprotective dehydrogenation under acid catalysed conditions led to the desired conjugate dienone ZK 98.299 (2.41).[40]


OH
O
(2.127)
OH
O
O
(2.57)
OH
O
O
(2.58)
N
OH
O
O
OH
(2.128)
N
O
O
O
OH
(2.129)
N
OH
THPO
O
O
O
OH
(2.130)
N
OH
OTHP
O
O
O
OH
(2.131)
N
OH
OH
O
ZK 98.734 (2.43)

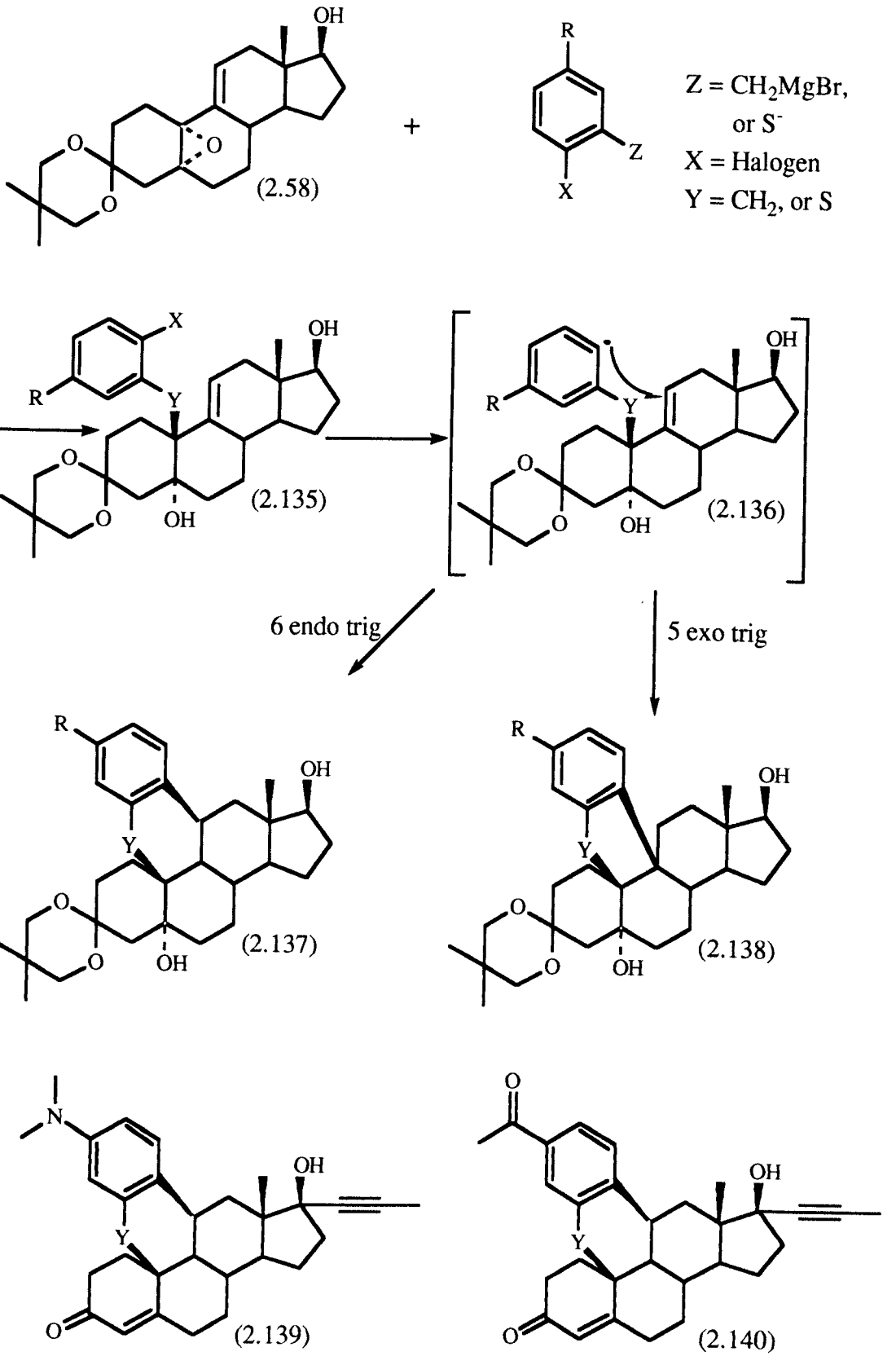
Scheme 2.17



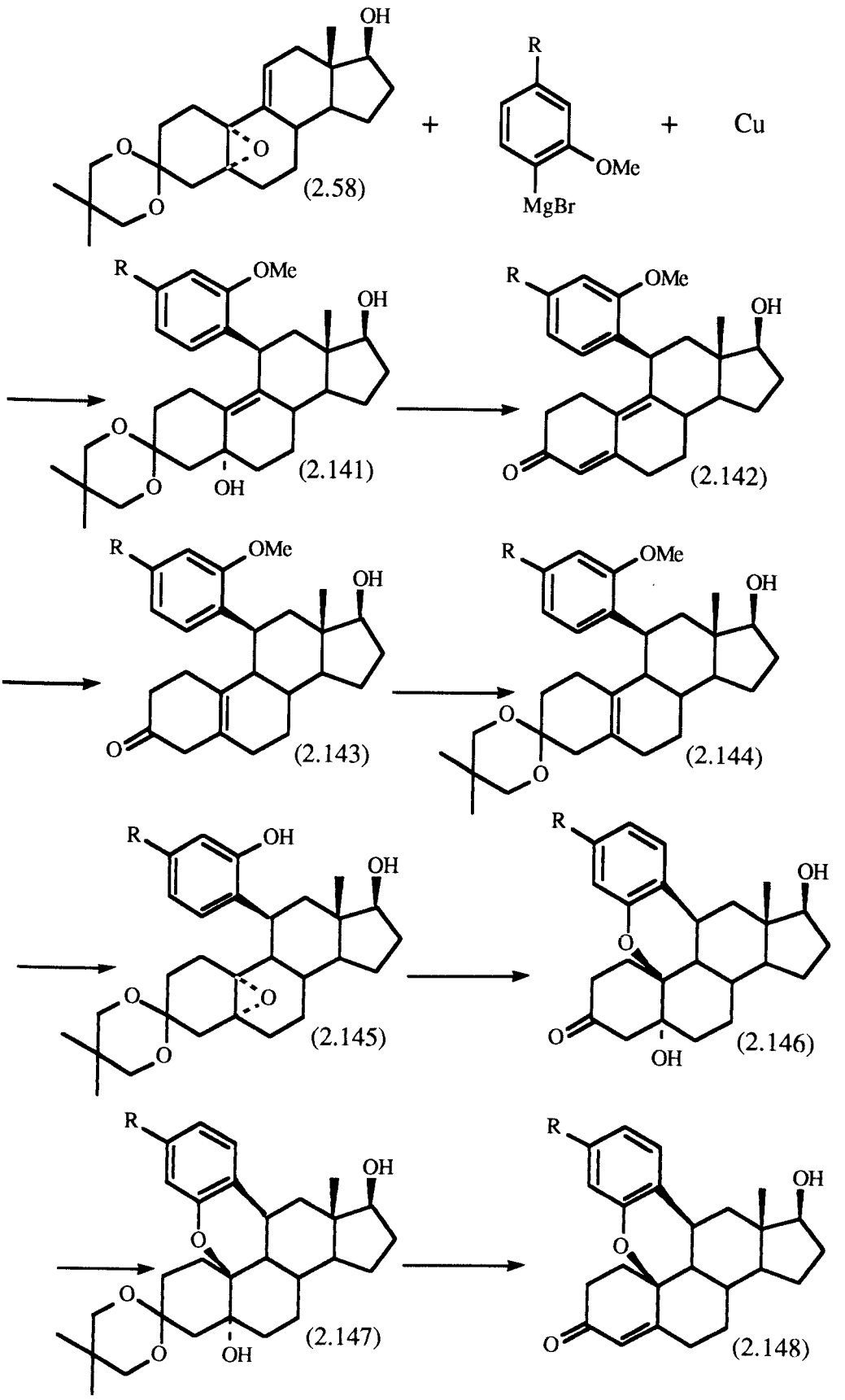
Scheme 2.18

Schering employed a stereospecific and regiospecific 6-endo-trig cyclisation of aryl radicals to gain entry to a series of 11β,19-bridged steroids (scheme 2.19).[41,43] The introduction of the 11β,19-bridged structural element was accomplished surprisingly easily in two steps from 5α,10α-epoxy-3,3-(2,2-dimethylpropylenedioxy)-9(11)-estren-17β-ol (2.58). The protocol leading to the methylene or sulphur bridged compounds (scheme 2.19),[41,43] involved S_N2 opening of the 5α,10α-epoxide (2.58) by (2-haloaryl)methyl magnesium halides, or by the corresponding thiophenolate, to furnish 10β-substituted steroids (2.135). The second step involved the regiospecific radical initiated intramolecular cyclisation of the 19-(2-haloaryl)-9(11)-androstene derivatives (2.135).[41,43] The phenyl radical was generated by the action of lithium or tributylstannane, on the aryl halide (2.135). Intramolecular ring closure of the aryl radical (2.136) with the 9(11) double bond can theoretically occur by a 6-endo trig or a 5-exo trig cyclisation.[40] Both of these processes are favoured according to Baldwin's rules, but investigations carried out by Beckwith *et al*[60,61] suggest that the 6-endo trig cyclisation has a lower relative energy for the transition state than does the 5-exo trig cyclisation transition state.

Schering later developed the synthesis of C-19 oxygen bridged steroids (scheme 2.20).[44] The intermolecular epoxide opening by the weak nucleophile phenolate/phenol was not succesful even when assisted by base or lewis acid conditions.[44] Instead the strategy of cyclisation was reversed, by utilising the procedure presented by Teutsch *et al*,[35] to introduce an (orthomethoxy)arene into the 11β-position (2.143). The formation of the bond between the aryl oxygen and C-10 carbon atom by the nucleophilic attack of a phenolate/phenol on a new 5α,10α-epoxide (2.145) would become an entropically favoured intramolecular process.[44]


OH
O
O
O
(2.58)
+
R
Z
X
Z = CH_2MgBr, or S^-
X = Halogen
Y = CH_2, or S
X
R
Y
OH
O
O
OH
(2.135)
R
Y
OH
O
O
OH
(2.136)
6 endo trig
5 exo trig
R
Y
OH
O
O
OH
(2.137)
R
Y
OH
O
O
OH
(2.138)
N
Y
OH
O
(2.139)
O
Y
OH
O
(2.140)

Scheme 2.19


OH
O
O
(2.58)
+
R
OMe
MgBr
+
Cu
R
OMe
OH
O
O
OH
(2.141)
R
OMe
OH
O
(2.142)
R
OMe
OH
O
(2.143)
R
OMe
OH
O
O
(2.144)
R
OH
OH
O
O
(2.145)
R
OH
O
O
OH
(2.146)
R
OH
O
O
OH
(2.147)
R
OH
O
O
(2.148)

Scheme 2.20

2.14 References.

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Section 2 Synthetic approach to 19-aryl steroids

Chapter 3

Strategy for Design and Synthesis of 19-aryl steroids.

3.1 Introduction.

Historically, natural chemical products have provided the oldest and richest source for new drugs[1] (e.g. penicillin, morphine). These natural chemicals have evolved for a specific function in biological systems.[1] The activity of such compounds can therefore be exploited for a pharmacological effect. Modern pharmacological random screening methods have meant that natural products have continued to be a rich source for drug leads.[2,3] This rather expensive random method does not make any assumption about structure activity relationships and may uncover biological activity in novel structures.[2] Alternatively in cases where lead compounds exist, a vast number of structural analogues may be synthesised for biological screening, in order to discover drugs which exhibit improved activity.[1] This method requires large chemical and financial resources.

3.2 Design by Receptor Fit.

The biological activity of a drug is related to its ability to bind to the active site of a receptor molecule. The nature of this binding is dependant on the topology and electronic architecture of the ligand and receptor site. A more rational approach to drug discovery is to design a ligand which best fits the atomic constraints of the active site in the receptor.[2,3] A variety of methods can be used to gain access to the structural and electronic detail of an active site. The most informative method is to obtain an X-ray crystallographic structure of the crystallised receptor protein.[3] This method is only useful for relatively small proteins as most can not be crystallised.

Alternatively a vague 3-dimensional map of the active site may be generated by the use of molecular probes to explore the shape, size and polar characteristics of the active site.[3]

3.3 Receptor Specificity.

Since the late 1970s, there has been intense research activity in the design and synthesis of novel antiprogestational and antiglucocorticoid agents.[4] This has culminated in the discovery of the potent steroid receptor antagonist RU 38486 (2.40).[5] Unfortunately no receptor specific antagonist has been reported in the literature for the progesterone or glucocorticoid receptors. In this work it is proposed to synthesise a steroid receptor antagonist which may possess an enhanced receptor specificity.

3.4 Receptor Fit and RBA.

In the absence of any crystallographic data for the crystallised pure progesterone, glucocorticoid receptor protein, or receptor steroid complex, the only way to determine the electronic and steric environment of the active site is through molecular comparisons of the steric and electronic features of molecular probes which exhibit a good RBA for the receptor. The RBA of a ligand is considered to be a good method of evaluating the degree to which a ligand may fit an active site.[6]

From the structures of molecules which exhibit a good RBA for the progesterone and glucocorticoid receptors (fig. 2.7),[6] it can be deduced that the features of a C-3 carbonyl functionality conjugated to a C-4 double bond, with a 17β-hydroxy functionality are condusive to the binding of steroids to the progesterone and glucocorticoid receptors. Notable exceptions to this general rule are the 17α-hydroxy functionality of ZK 98.299 (2.41), the 17β-(ethan-1-one) functionality in progesterone (1.15)

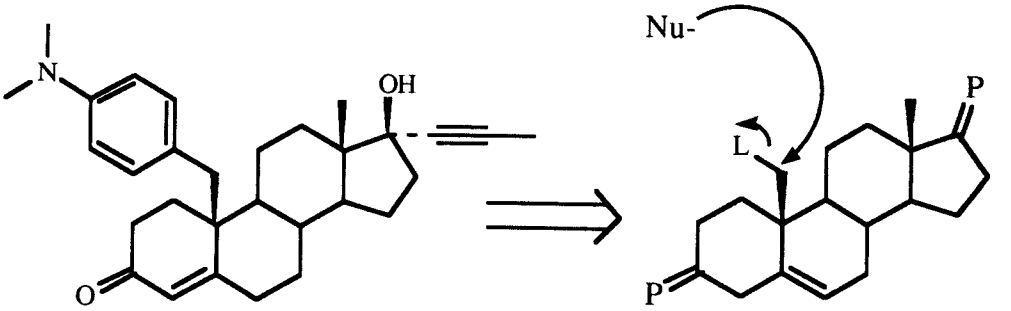
and natural occurring glucocorticoids (1.12). Most of the antagonists of these two receptors (fig. 2.7) possess an aryl functionality located on the β-face of a steroid. The aryl functionality is also observed in a number of receptor antagonists of estrogen, (e.g. tamoxifen (2.15), clomiphene (2.14)).

For the vast majority of the antagonists (fig 2.7) there is a high degree of structural similarity in the steroid skeleton with some difference of hybridisation states of carbons at positions C-1, C-2, C-9 and C-10. The polar functions at positions C-3 and C-17 are responsible for a good RBA and the substituted aryl functionalities are responsible for antagonistic activity. The aryl functionalities are located at a number of different position in the steroid skeleton

3.5 Strategies for the synthesis of 19 substituted steroids.

Steroids which possess a good RBA and a substituted aryl functionality on the β-face of the steroid (position 9, 10, 11, 18, 19) which are able to reach into the hydrophobic pocket of the receptor are of pharmaceutical interest. The objective of this work is the synthesis of 17α-propynyl-17β-hydroxy-19(p-dimethylaminophenyl)androst-4-en-3-one (3.1). Retro-synthetic analysis (scheme 3.1) suggests that this type of structure should be obtained by using a carbanion or organometallic reagent to displace a C-19 leaving group from a steroid whose A and D ring functionalities are suitably protected. This strategy requires the preparation of an intermediate steroid bearing a C-19 functionality which can be converted into a good leaving group.

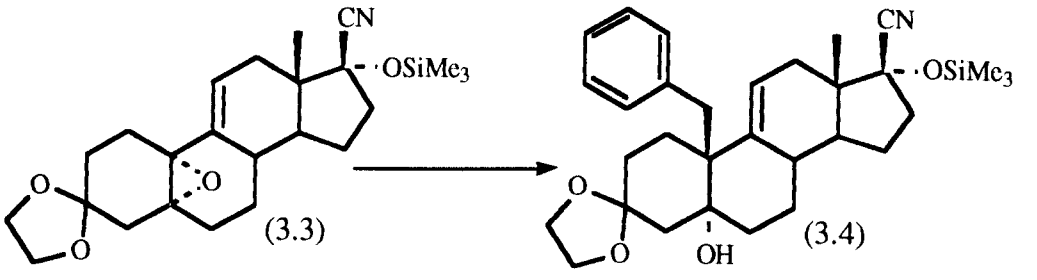
A number of 19-hydroxy steroids occur on the biosynthetic pathway to estradiol from testosterone,[7] unfortunately these naturally occurring steroids are not available in large quantities and are extremely expensive to purchase commercially. Naturally occurring 19-hydroxy steroids therefore do not appear to be suitable starting materials.



(3.1) P = Protecting group L = Leaving group (3.2)

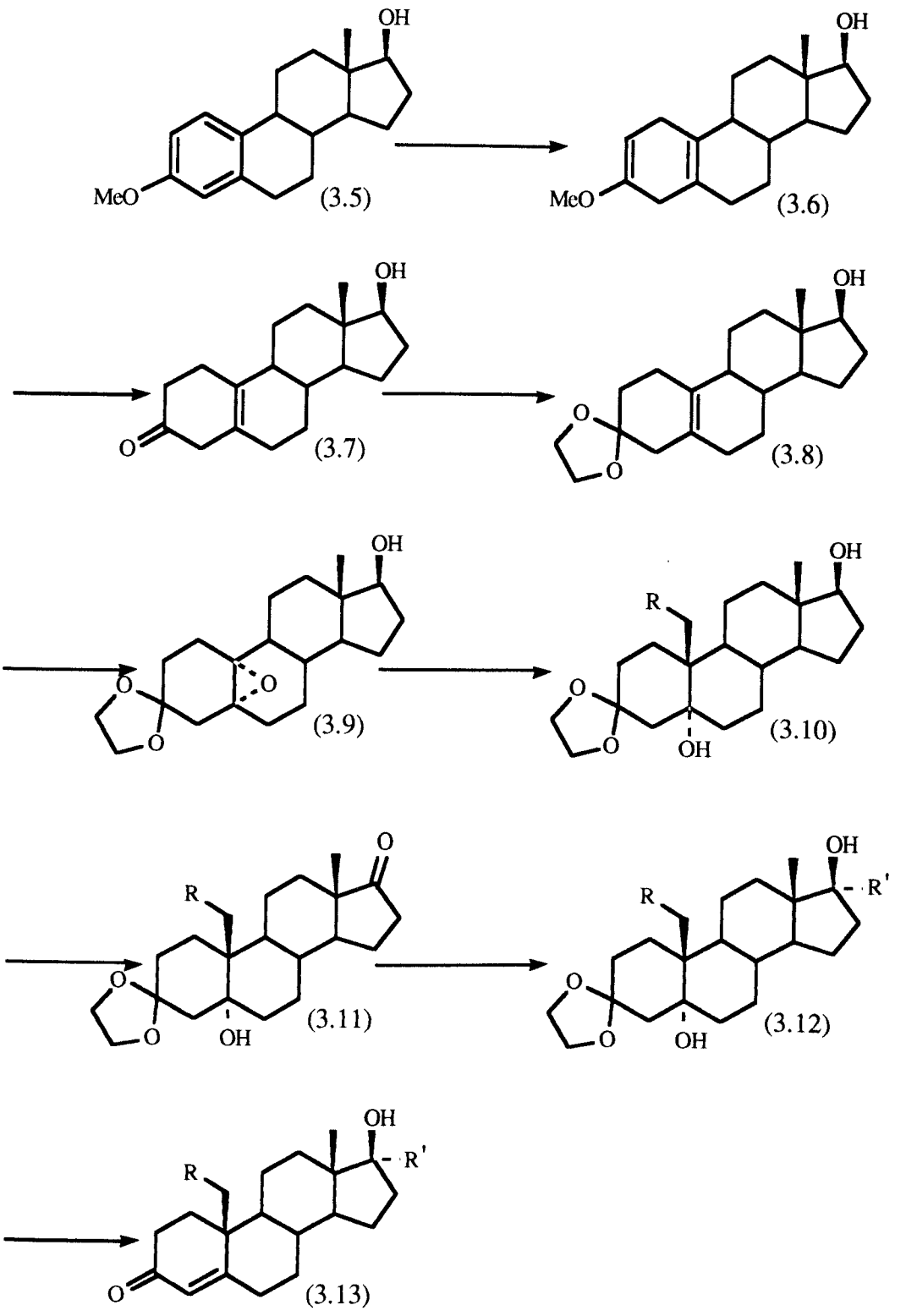
Scheme 3.1

In principle, enzymatic oxygenation methods offer a promising approach for the C-19 hydroxylation of non active C-19 methyl steroids (adrenal incubation and microbiological hydroxylation methods). However, such reactions have been reported to be characterised by low and unpredictable yields.[8] Fortunately, chemical methods have been developed and reported in the literature for the functionalisation of position C-19.[4,9,10] One of the strategies relies upon free radical intramolecular attack on a suitably located non-active group.[9,10] A second strategy was reported by researchers at Roussel-Uclaf,[4] they reported that a benzyl group could be introduced into the 10β position of a 19-norsteroid epoxide (3.3), by the action of benzyl magnesium bromide as the nucleophile (scheme 3.2).[4] This method could be used to synthesis the target molecule (3.1) (scheme 3.3).



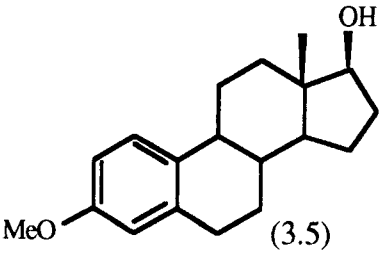
(3.3) (3.4)

Scheme 3.2



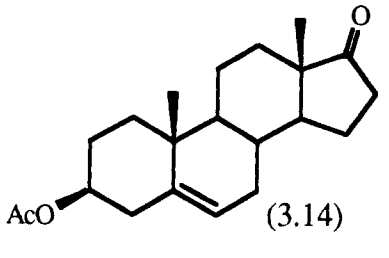
Scheme 3.3 A synthetic route to 19-substituted steroids via a 5α,10α-epoxide

In principle this route could be used in achieving our own objectives via the synthesis of the epoxide intermediate (3.9). In developing the strategy for a multistep synthesis, one must evaluate the choice of starting materials and number of stages involved as the overall yield exponentially diminishes during the course of such a synthesis. Commercially dehydroisoandrosterone is available at a fifth of the cost of the methyl ether of estradiol (3.5). With these considerations it was chosen to explore a route based on the funtionalisation of position C-19 in an androstane.



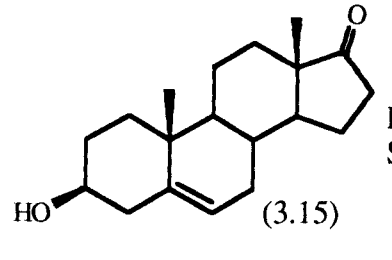
(3.5)

3,17β-estradiol 3-methyl ether
Sigma g = £62.70



(3.14)

Dehydroisoandrosterone-5-ene 3β-acetate.
Sigma 5g = £16.80
25g = £61.20



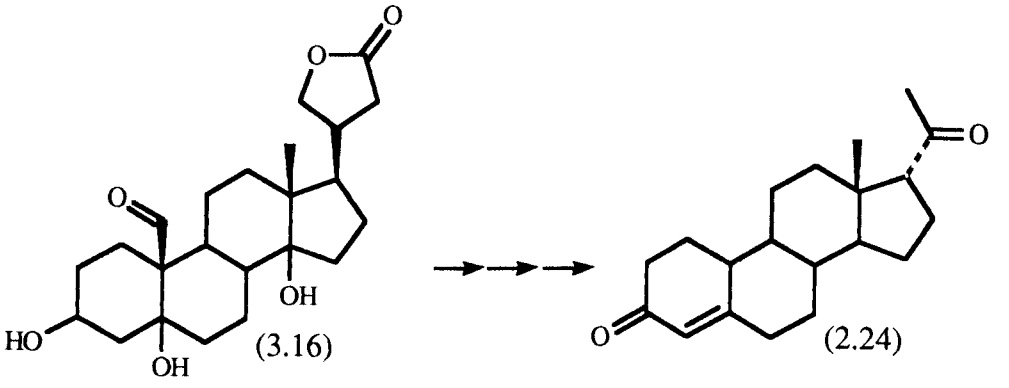
(3.15)

Dehydroisoandrosterone-5-ene.
Sigma 5g = £12.00
25g = £44.50
250g = £315.30

Figure 3.1 relative cost of proposed stating materials

3.6 19-Norsteroids from 19-functionalised steroids.

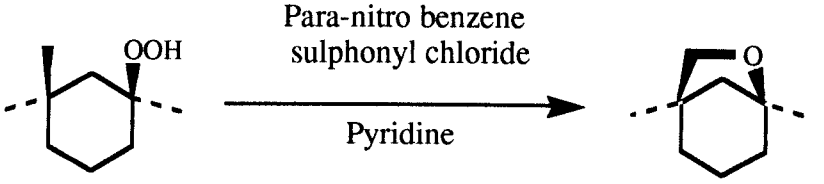
With the synthesis of 14-iso-17-iso-19-norprogeseterone came the discovery that the removal of the 19-methyl functionality did not reduce, but raised the level of biological activity of the steroid as a progestin.[7,12,13] Ehrenstein et al reported that 19-norsteroids could be synthesised from 19-oxygenated steroids since the 19-hydroxy, carbonyl and carboxylic functionalities underwent conversion with base to the 19-norsteroid.[13-15] With the realisation of the importance of 19-oxygenated steroids, a method to functionalise the 19-methyl position was developed.[9-11,16-18] A method of intra-molecular transannulation of the C-18 and C-19 methyl functionalities has been developed by researchers at Syntex[9,10] and by Barton et al.[16]



Scheme 3.4 The first reported synthesis of 19-norsteroids.

3.7 Transannular oxygenation of saturated carbon atoms via an intramolecular attack by a cationic or radical oxygenation species.

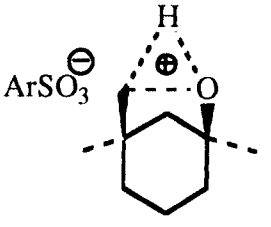
The earliest reported example of an intramolecular transannular oxygenation of a saturated carbon atom is for the conversion of 1,3,3-trimethylcyclohexylperoxide (3.17) into the corresponding bicyclic ether (3.18) by treatment with benzenesulphonyl chloride in pyridine.[17]



(3.17) (3.18)

Scheme 3.5

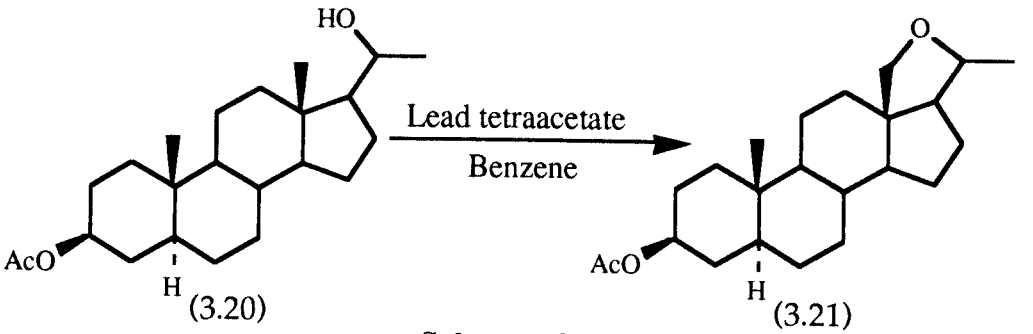
It was suggested in the literature[17] that since the oxygen-oxygen bond cleavage of the p-nitrobenzene sulphonyl derivative is unlikely to occur homolytically under these reaction conditions, the reaction must therefore proceed via a cationic transition state such as (3.19).



(3.19)

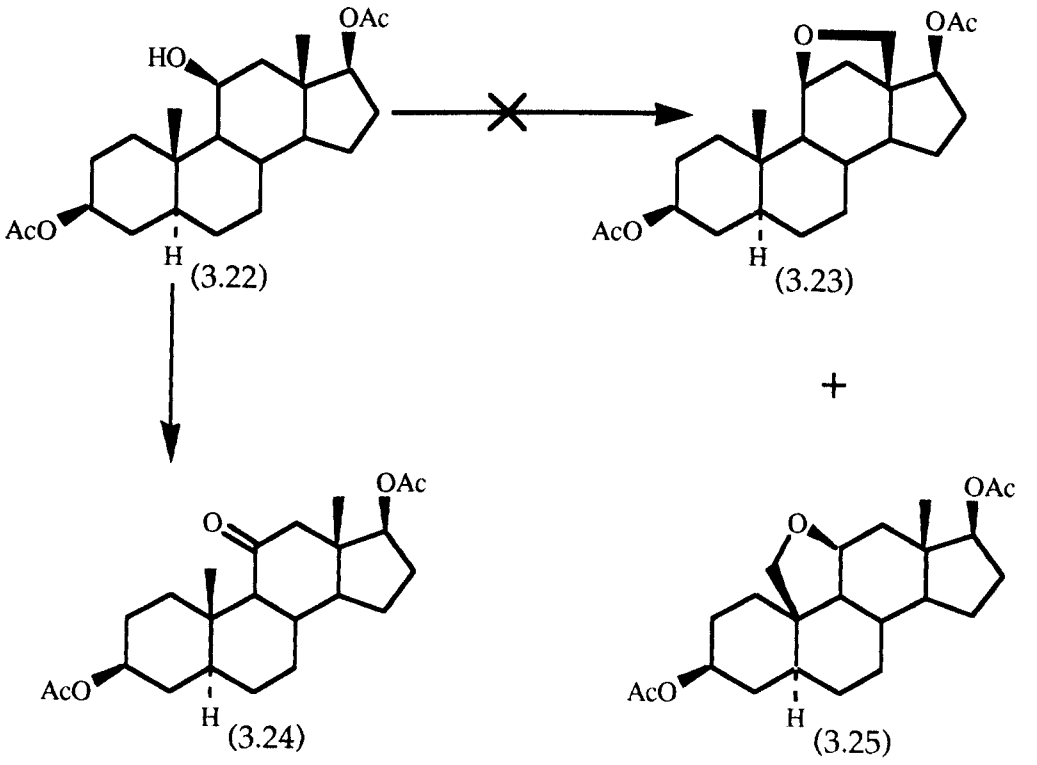
Figure 3.2

Shortly after this report, the first example of a transannular reaction in a steroid system was published.[18] 3β,20-Dihydroxypregnane 3-acetate (3.20) was treated with lead tetraacetate in benzene solution under reflux (scheme 3.6).[18]



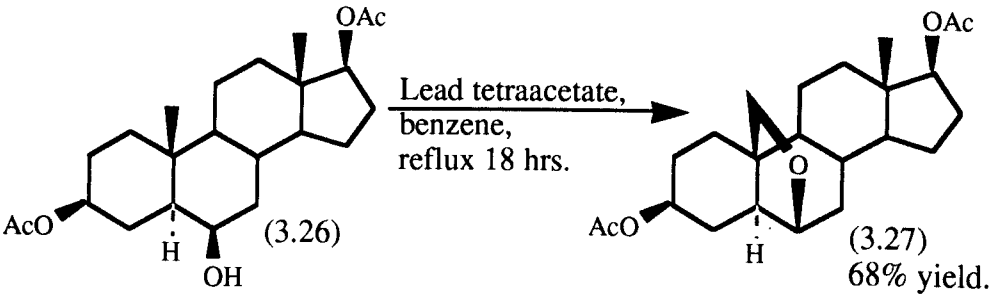
Scheme 3.6

Under these reaction conditions, a free radical intramolecular transannular reaction gave rise to 3β-hydroxy-18,20-oxido-5α-pregnane 3-acetate (3.21).[18] This breakthrough was seen as a potential method that could be utilised for a partial synthesis of aldosterone as it offered a solution to the C-18 oxygenation.[11] It was against this background that attempts were made to functionalise the C-19 and C-18 positions from an 11β-hydroxy group. A benzene solution of 3β,11β,17β-trihydroxyandrostane-3,17-diacetate (3.22) was refluxed with lead tetraacetate.[11]



Scheme 3.7

Although the 11β-hydroxy group is 1,3 diaxially oriented to both the C-18 and C-19 positions, it was reported that there was no trace of the expected 11β,18-oxidoandrostane (3.23) or 11β,19-oxidoandrostane (3.25). Instead the attempts at a transannular reaction from a 11β-hydroxy group afforded the 11-keto steroid (3.24). It was reported that the gain in energy associated with the ketone formation due to the release of steric compression in the C-ring was probably the governing factor.[18]

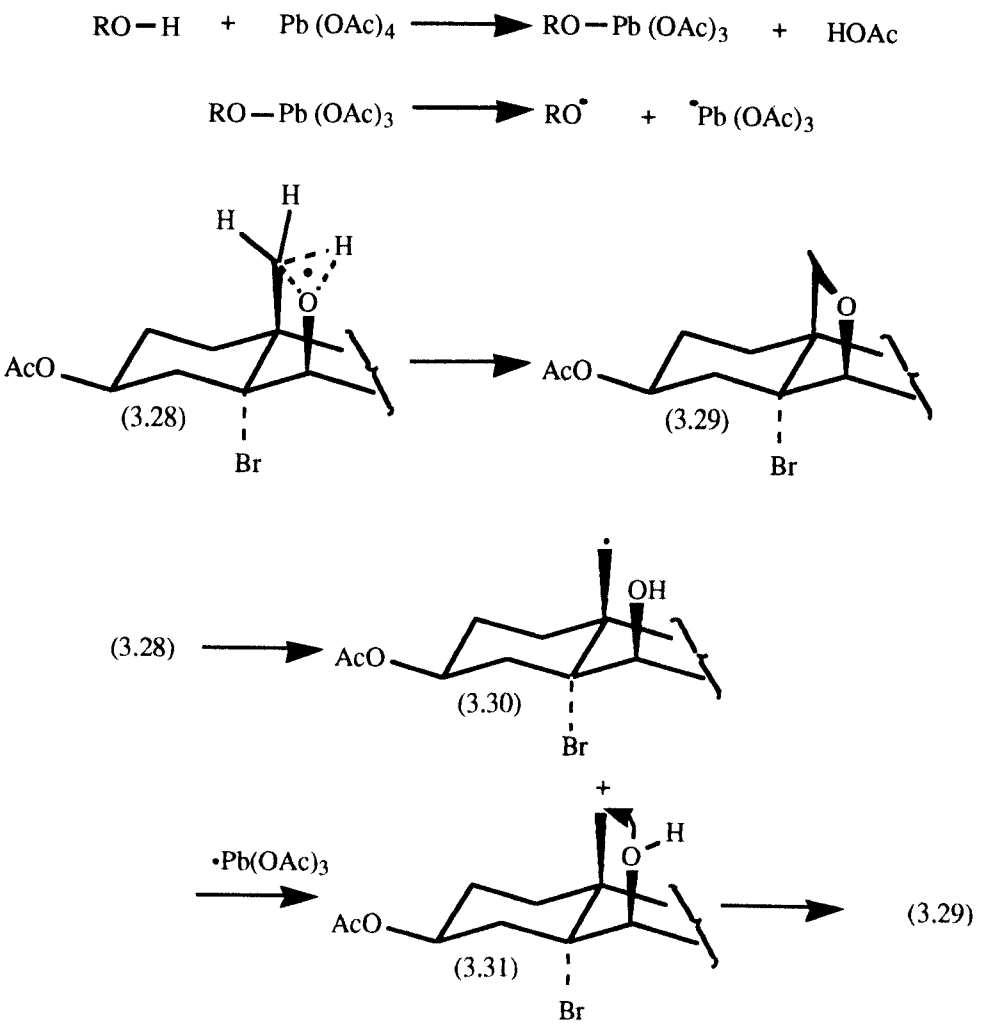


Scheme 3.8

With the failure to oxygenate the C-19 position via an intramolecular transannulation reaction from readily available C-11β hydroxy steroids, the literature methods then focused on oxygenation procedures of the methyl group from suitable 6β-functionalities, which are 1,3 diaxially oriented to the C-19 methyl group.[9-11,16,18]

The first reported intramolecular oxygenation of the C-19 position of a steroid system involved 3β,6β,17β-trihydroxyandrostane 3,17-diacetate (3.26).[10] This 6β-hydroxy steroid (3.26) was refluxed with lead tetraacetate in benzene and the reaction afforded 3β,17β-dihydroxy-6β,19-oxido androstane 3,17-diacetate (3.27).[10]

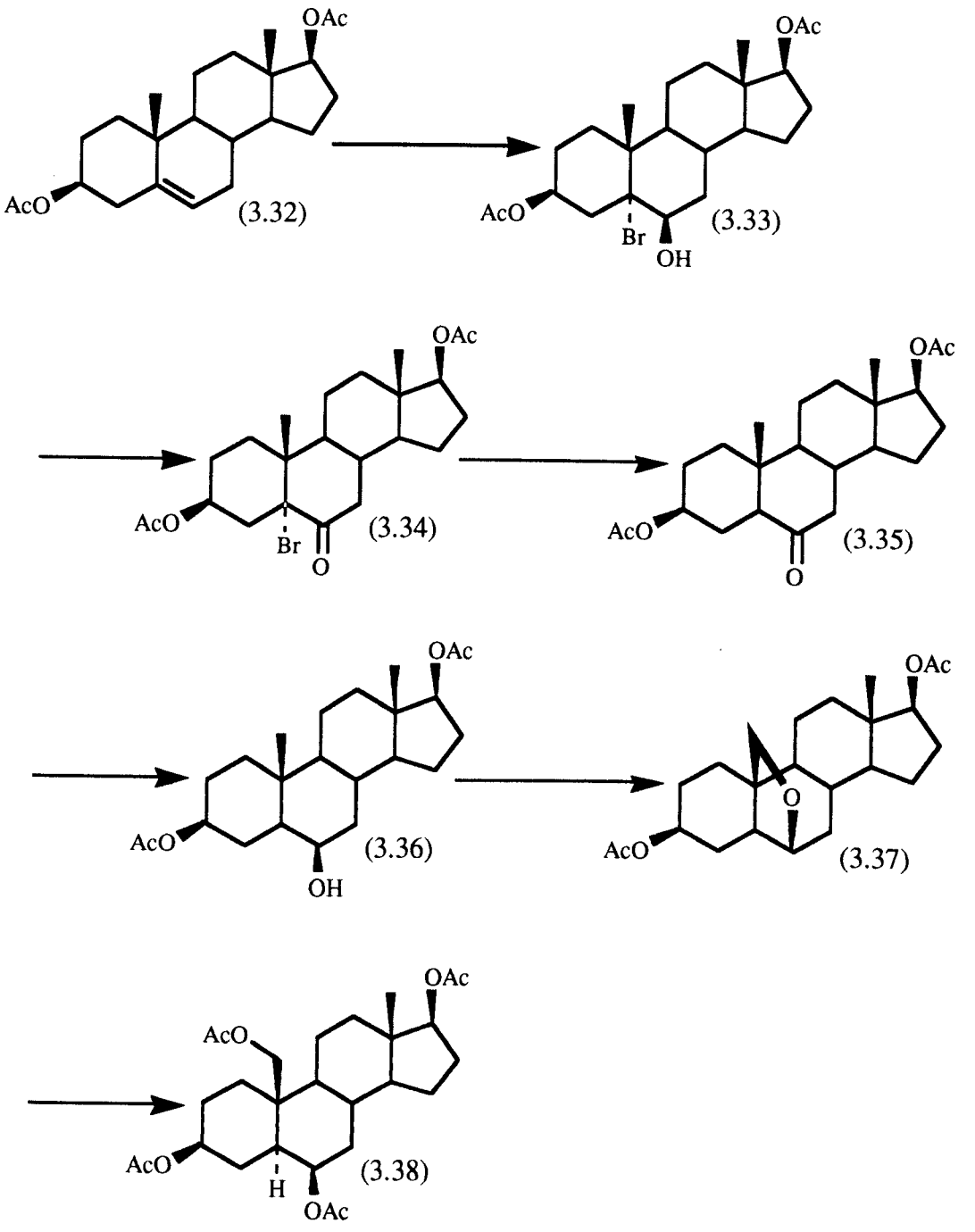
In this reaction the action of lead tetraacetate on a 6β-hydroxy steroid gives rise to an alkyl lead intermediate which homolytically cleaves to generated a 6β-alkoxy radical.[19] The 1,3 diaxial arrangement of the 6β-alkoxy radical and the C-19 methyl functionality enables the direct ring closure at C-19 methyl or an abstraction of a C-19 hydrogen generating a C-19 alkyl radical. Both of these processes occur through a cyclic 6-membered transition state. The C-19 alkyl radical is oxidised to a C-19 carbonium ion which may then ring close at C-19 (scheme 3.9).[19]



Scheme 3.9

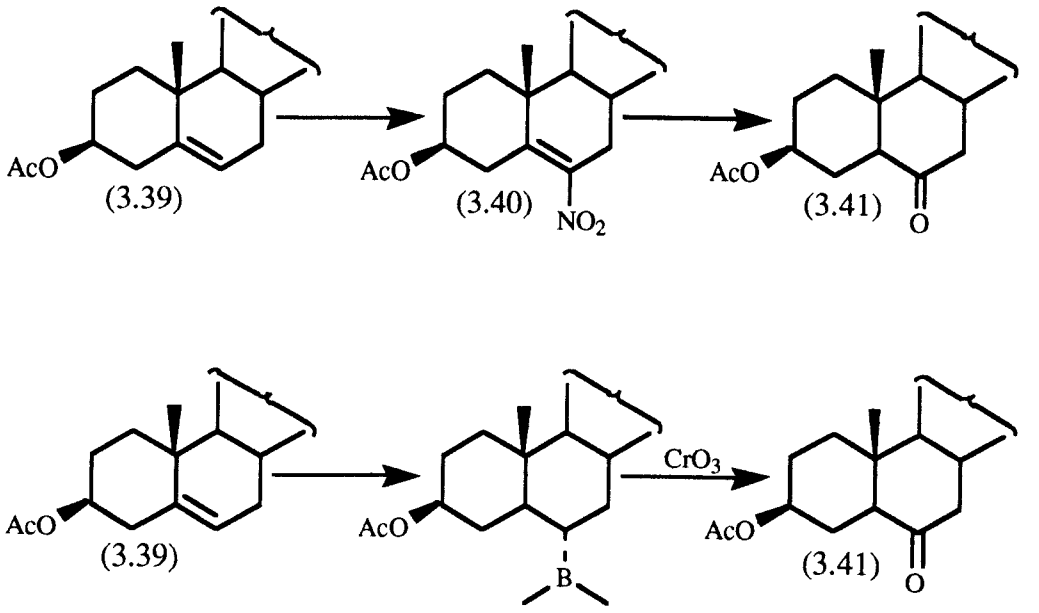
In the literature, the stereospecific introduction of a 6β-hydroxy functionality was reported to occur in four steps from 3β,17β-dihydroxyandrost-5-ene diacetate (3.32) (scheme 3.10).[10] The action of hypobromous acid on the androst-5-ene steroid (3.32) in dioxane, afforded 5α-bromo-6β-hydroxy androstane (3.33), oxidation of which gave the 6-keto steroid (3.34). The 5α-bromo functionality in the 6-keto steroid (3.34) was removed by reflux with zinc dust in acetic acid to give the 5α-H androstane (3.35), reduction of which gave the product 3β,6β,17β-trihydroxyandrostane diacetate (3.36). This compound could then undergo an intramolecular oxygenation of the C-19 methyl group by treatment with lead tetraacetate to give 3β,17β-dihydroxy-6β,19-oxidoandrostane diacetate (3.37) (scheme 3.9).

Treatment of the 6β,19-oxido steroid (3.37) with acetic anhydride and boron trifluoride led to the cleavage of the oxide bridge to give 3β,6β,17β,19-tetrahydroxyandrostane tetraacetate (3.38).[10] This demonstrated the feasibility of oxygenation of the C-19 position in the androstane series.



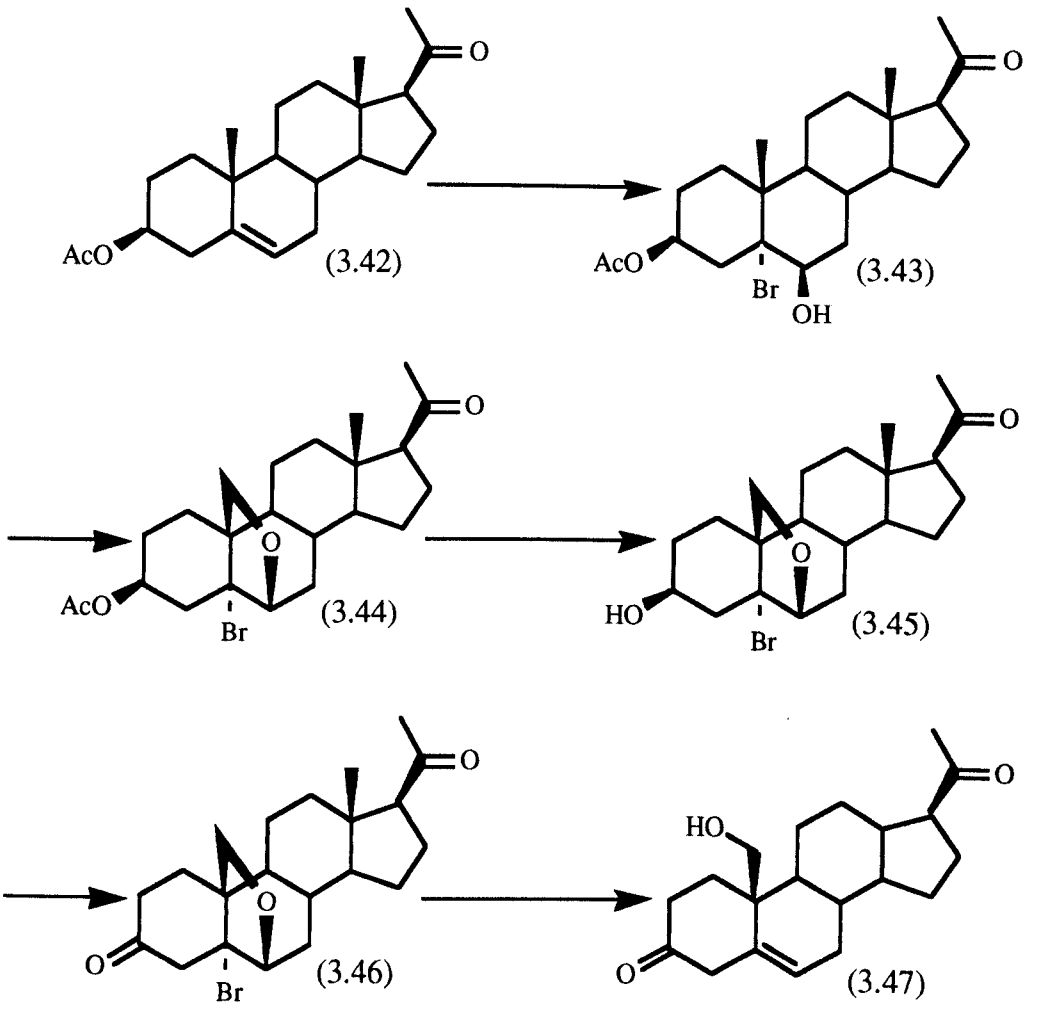
Scheme 3.10

A number of alternative methods have been reported for the synthesis of 6β-hydroxy steroids, via the reduction of 6-keto steroids. Amongst these methods is the nitration[20] of unsaturated steroids followed by reduction with zinc and acetic acid to afford the 6-keto steroid (3.41). Also the chromic acid oxidation of boranes has been used to synthesise 6-keto steroids (3.41) (scheme 3.11).[21]



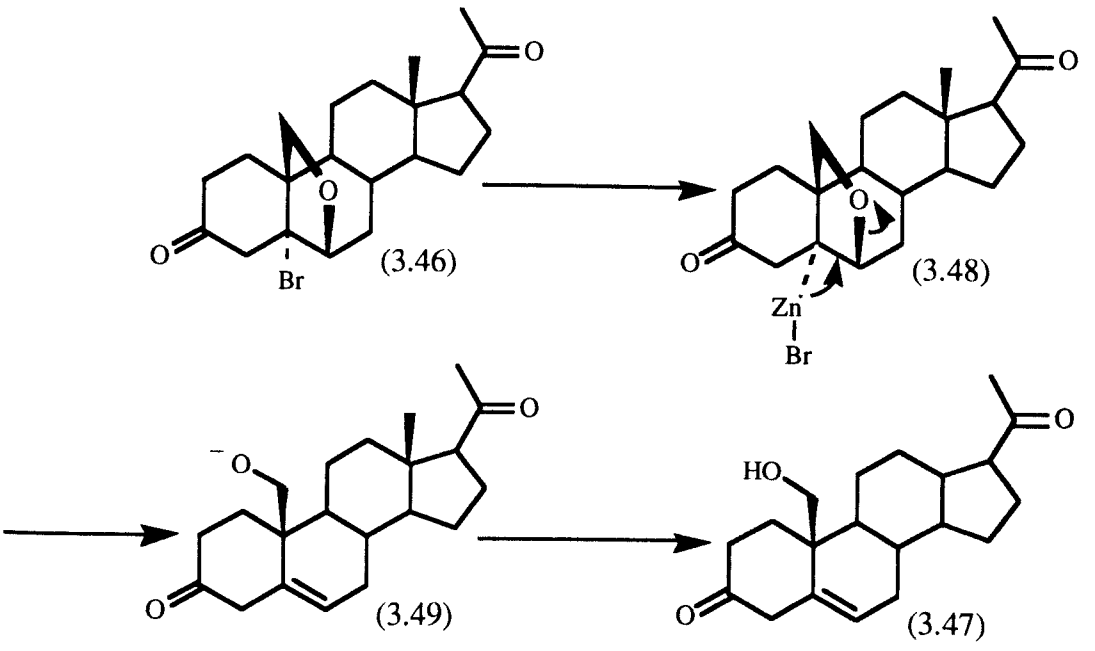
Scheme 3.11

A significant advance in the strategy (scheme 3.12) to oxygenate the C-19 methyl functionality was reported by researchers at Syntex, where pregnenolone acetate (3.42) was converted into the corresponding bromohydrin (3.43) by addition of hypobromous acid.[10] Treatment of the 5α-bromo-6β-hydroxy pregnane (3.43) with lead tetraacetate afforded the 6β,19-oxido steroid (3.44). The 3β-acetoxy substituent was hydrolysed and oxidised to the 3-keto functionality to give the 6β,19-oxido-3,20-diketo steroid (3.46). Reduction of this compound with zinc in isopropyl alcohol afforded the 19-hydroxypregna-5-ene steroid (3.47).[10] The insertion of zinc in to the carbon bromine bond of the 5α-bromo-6β,19-oxido pregnane (3.46) enabled an elimination to take place across the C-5, C-6 carbon bond, thus affording the 19-hydroxy-5-en-pregenolone (3.49) (scheme 3.13). The advantage of this methodology was that it was not necessary to remove the 5α-bromo functionality in three synthetic steps before ring cleavage of the 6β,19-oxido ring.



Scheme 3.12

A further advance in the strategy to oxygenate the C-19 methyl functionality is the use of the hypoiodite reaction in the intramolecular transannular step.[22] The reaction employs iodine alongside lead tetraacetate to enhance the formation of the 6β,19-oxido steroid under photolytic conditions. In this reaction the alkoxy radical is generated from the photolytic homolysis of the oxygen-iodine bond of the intermediate hypoiodite (scheme 3.14).



Scheme 3.13

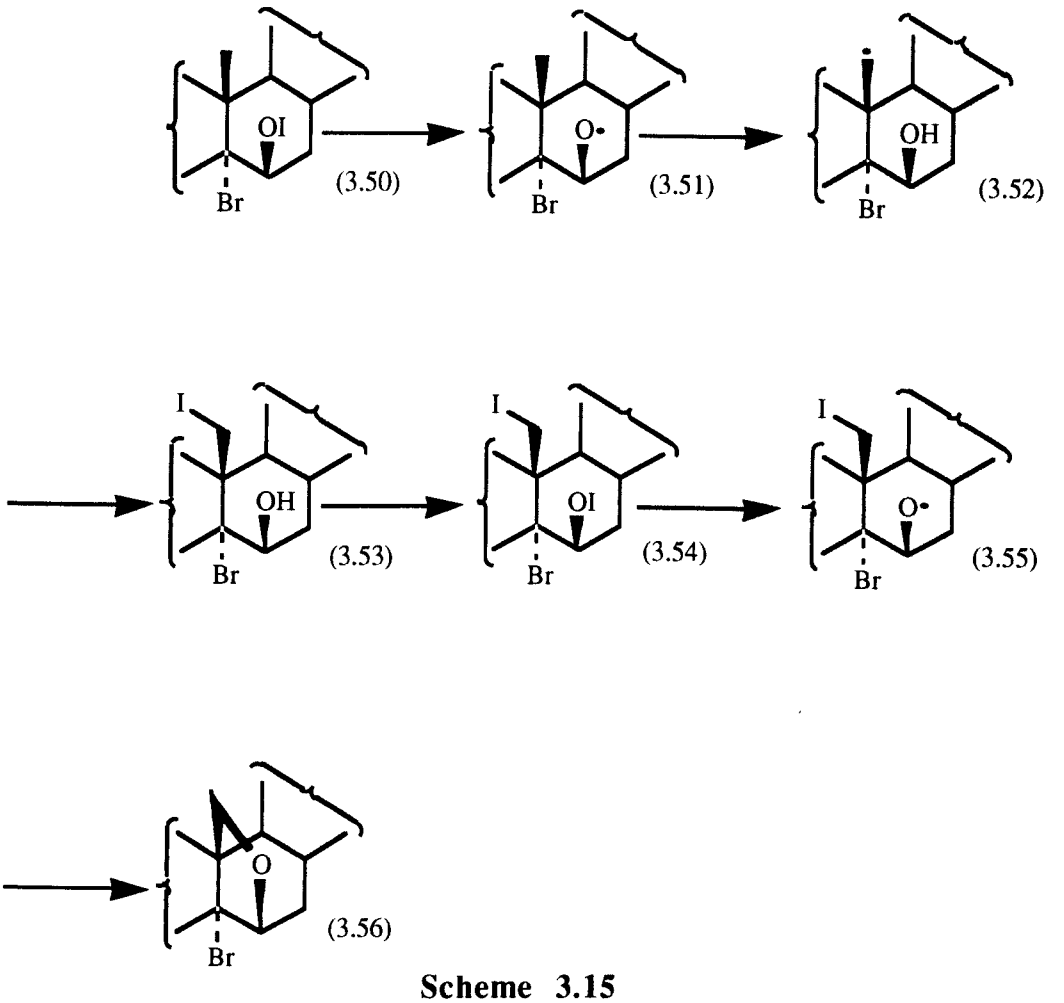
As before the alkoxy radical abstracts a hydrogen atom from the C-19 methyl group to form the 19-methyl radical. This intermediate then reacts with iodine to form the iodohydrin (3.53). The large iodine atom attached to the C-19 methyl group restricts the free rotation of the methyl group and locks it in a position where the halogen is directly in line with the C-19 and the 6β-oxygen atom.[23] As before, the 6β-hydroxy group is converted via a hypoiodite (3.54) into the corresponding 6β-alkoxy radical. The conformation of the 19-iodomethyl group and the 6β-alkoxy radical is conducive to cyclisation by homolytic substitution.[23]

$$I_2 + Pb(OAc)_4 \longrightarrow Pb(OAc)_2 + AcOI$$

$$AcOI + RO{-}H \longrightarrow ROI + HOAc$$

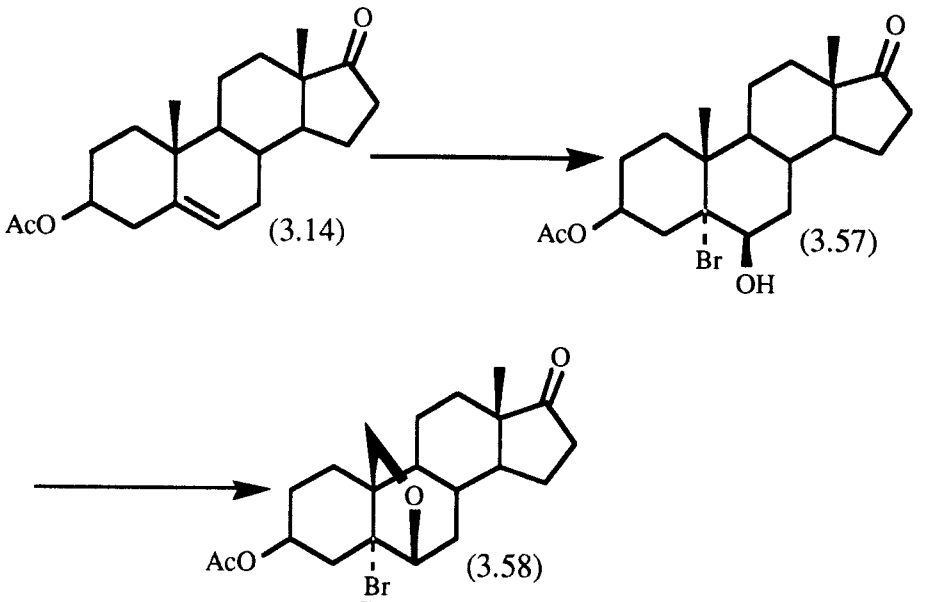
$$ROI \longrightarrow RO^{\bullet} + I^{\bullet}$$

Scheme 3.14



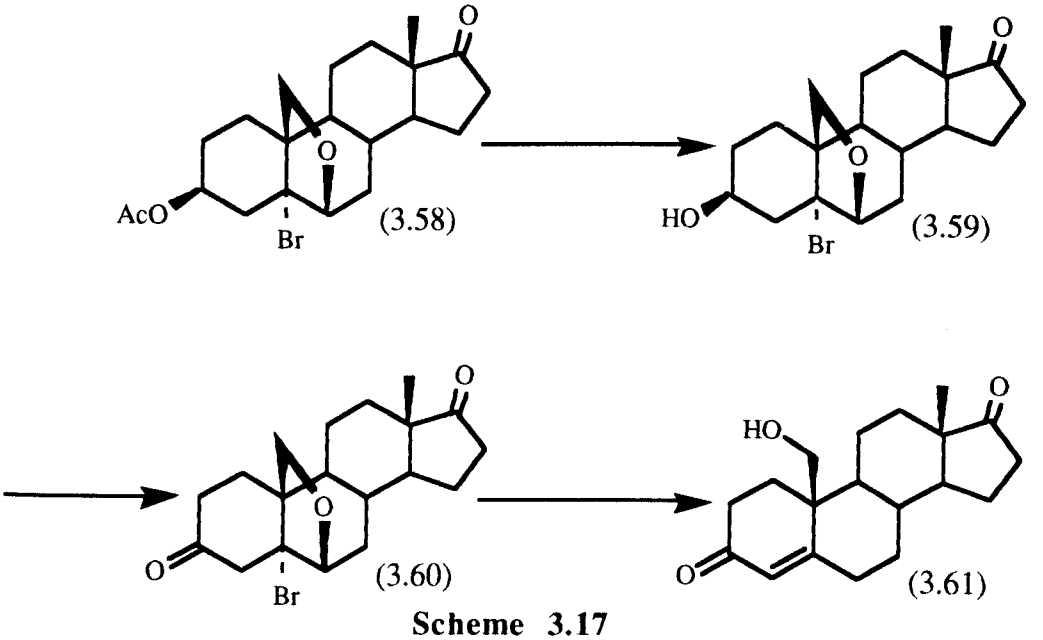
Scheme 3.15

Of interest for our research were publications relating to the synthesis of 19-hydroxyandrostanes.[19-26] In these reports 3β-hydroxy-6β,19-oxidoandrost-17-one 3-acetate (3.58) was a common intermediate, which could be synthesised from dehydroisoandrosterone 3β-acetate (3.14) via the bromohydrin followed by an intramolecular transannular oxygenation as previously described (scheme 3.16).



Scheme 3.16

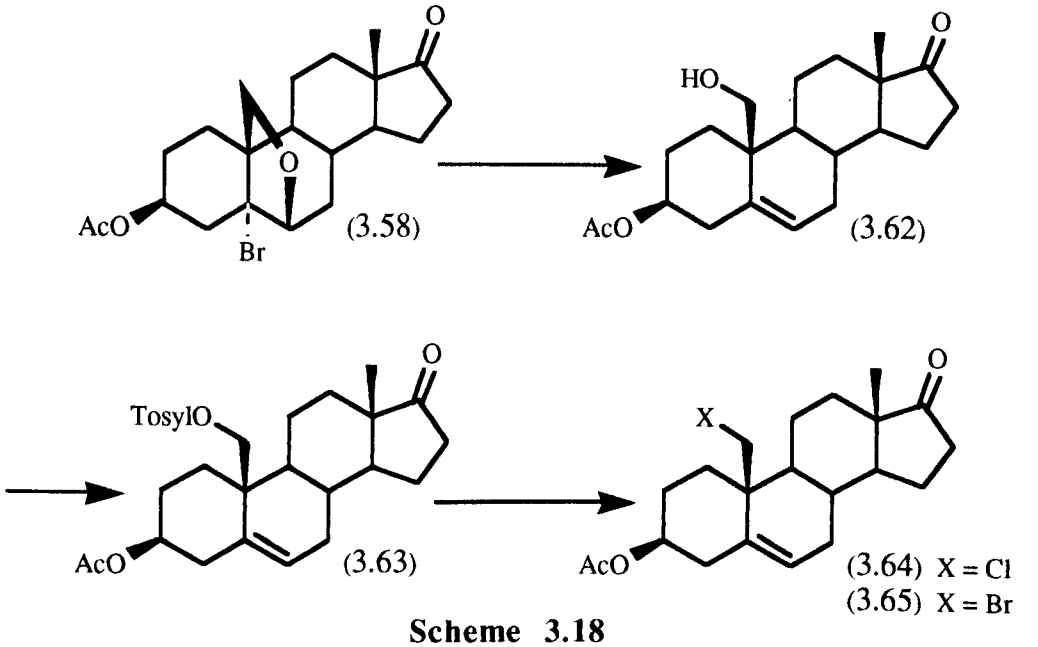
Researchers in Romania[24] were able to convert this intermediate (3.58) to 19-hydroxy-5-en-3,17-dione (3.61) by hydrolysis of the 3β-acetate (3.58), oxidation of the resulting alcohol (3.59), cleavage of the 6β,19-oxido ring followed by conjugation under acidic conditions (scheme 3.17).



Scheme 3.17

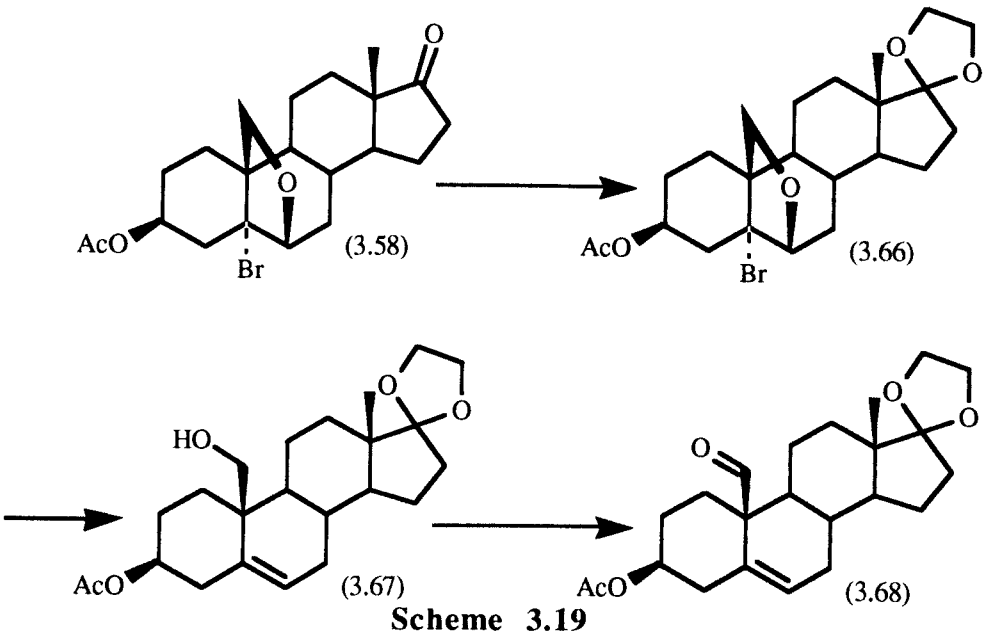
Protection of the C-3 and C-17 positions of 19-hydroxyandrostan-3,17-dione (3.61) with a suitable leaving group at position C-19 would afford a steroid which would fulfill all of the structural criteria required for the key intermediate (3.2) in the previously described strategy.

Researchers at Syntex (1966) described the preparation of 10β-angular hydroxymethyl, formyl, carboxyl, chloromethyl, vinyl, ethyl, N,N-dimethyl carboxamide and N,N-diethyl aminomethyl analogues of various 4-ene and 5-ene androstenes.[25] The synthesis began with 3β-hydroxy-6β,19-oxidoandrost-17-one (3.58).



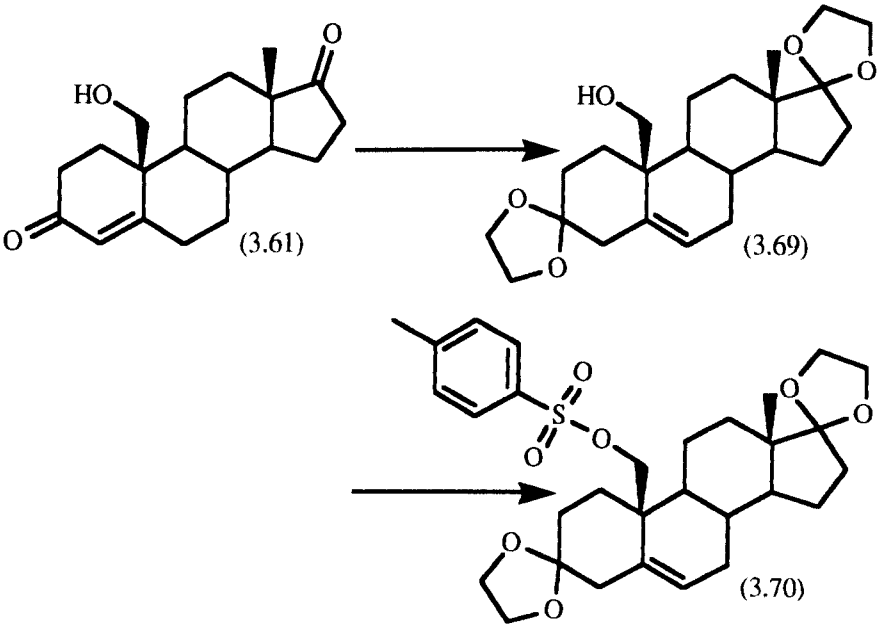
Scheme 3.18

The features in this report that were particularly interesting for our research are the synthesis of a 19-tosyl steroid (which subsequently underwent nucleophilic displacement with both LiCl and LiBr) and the synthesis of the 17-ketal-19-formyl-3β-acetate (3.68) which was prepared from the 6β,19-oxidosteroid (3.58) by ketalization of the 17-ketone to afford the corresponding ketal (3.66). The 6β,19-oxido bridge was then cleaved with zinc in ethanol to afford the 19-hydroxy ketal (3.67) which was oxidised with CrO_3 to afford the 19 formyl steroid (3.68).



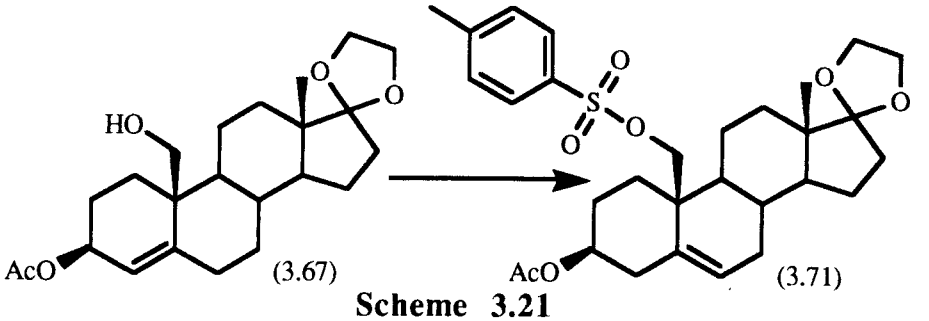
Scheme 3.19

Given the Syntex publication, it was considered that 19-tosyl and 19-mesyl functionalities would be ideally suited for nucleophilic substitution at the C-19 methyl group. Therefore an appropriately protected 19-tosylated steroid would fulfill all of the structural criteria considered to be essential for the key intermediate in the previously described strategy.



Scheme 3.20

It was proposed that 3,3:17,17-bisethylenedioxyandrost-5-ene 19-tosylate (3.70), or 3β,19-dihydroxy-17,17-ethylenedioxyandrost-5-ene 3-acetate 19-tosylate (3.71)[26] would be ideal candidates for the key intermediate of our synthetic strategy and could be synthesised from the steroids (3.61) and (3.67).



Scheme 3.21

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Chapter 4 Synthetic approach to C-19 aryl androstanes.

4.1 Introduction.

In line with the synthetic strategy (scheme 3.1) attempts were made to synthesise a differentially protected steroid at C-3 and C-17, bearing a leaving group at C-19 as the key intermediate for nucleophilic substitution. This was to be synthesised from 3β-hydroxyandrost-5-en-17-one 3-acetate (3.14). The ^{1}H NMR (table 4.1) and ^{13}C NMR (table 4.2) of (3.14) were run in order to follow the reaction.

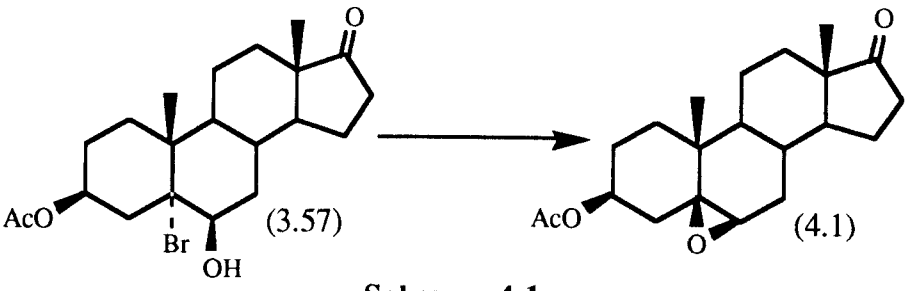
Table 4.1
400 MHz ^{1}H NMR of 3β-hydroxyandrost-5-en-17-one 3-acetate (3.14)

multiplicity	chemical shift	coupling constant	assignment
1 H (d)	5.35	5.0 Hz	C-6 H
1 H (t,t)	4.54	5.5 Hz, 11.0 Hz	C-3α H
3 H (s)	1.97		acetate methyl
3 H (s)	0.99		Me
3 H (s)	0.78		Me
methylene envelope	2.50-0.90		

Table 4.2
400 MHz ^{13}C NMR of 3β-hydroxyandrost-5-en-17-one 3-acetate (3.14)

chemical shift	assignment	dept
220.7	17	(C)
170.2	acetate carbonyl	(C)
139.7	5	(C)
121.6	6	CH
73.4	3	CH
51.4	9/14	CH
49.9	9/14	CH
47.3	13	C
37.9		CH_2
36.2		CH_2
36.0	10	C
35.6		CH_2
31.3	8	CH
31.2		CH_2
30.5		CH_2
27.4		CH_2
22.6	15(11)	CH_2
21.1	acetate methyl	CH_3
20.1	11(15)	CH_2
19.0	19	CH_3
13.5	18	CH_3

4.2 Results and Discussion



Scheme 4.1

In accordance with the literature,[1] 3β-hydroxyandrost-5-en-17-one 3-acetate (3.14) was stirred at room temperature for 30 minutes with N-bromoacetamide, perchloric acid and distilled water in dioxane to afford 3β,6β-dihydroxy-5α-bromoandrost-17-one 3-acetate (3.57). The product could not be purified by column chromatography as this facilitated the formation of the by-product, 3β-hydroxy-5β,6β-oxidoandrost-17-one 3-acetate (4.1).[2] The product was purified by suspension in chloroform at 0°C followed by filtration, this method improved the yield over that cited in the literature[1] which employed recrystallisation. The product was identified by its spectra. The ^{1}H NMR (table 4.3) indicated a shift in the position of the C-6 H doublet from 5.35δ to give a triplet at 4.22δ with a reduction in the coupling constant from 5.0 Hz to 2.94 Hz due to the electron withdrawing nature of the 5α-bromo and 6β-hydroxy functionalities. The ^{13}C NMR (table 4.4) indicated a shift in the position of C-5 signal from 139.7δ to 86.02δ and a shift in the position of C-6 signal from 121.6δ to 75.29δ. The infrared spectrum provided further evidence for the structure with the appearance of a free O-H stretch at 3479 cm^{-1} and C-O stretch at 1729 cm^{-1}. The mass spectra showed signals consistent with the structure, the EI-MS exhibited signals at 428, 426 both corresponding to the two molecular ions. The CI-MS(NH_4^+) exhibited signals at 446, 444 corresponding to the two (MNH_4^+) ions.

Table 4.3
400 MHz ^{1}H NMR of 3β,6β-dihydroxy-5α-bromoandrost-17-one 3-acetate (3.57)

multiplicity	chemical shift	coupling constant	assignment
1 H (t,t)	5.45	5.48 Hz, 10.96 Hz	C-3α H
1 H (t)	4.22	2.94 Hz	C-6α H
3 H (s)	2.02		acetate methyl
3 H (s)	1.34		C-19 Me
3 H (s)	0.87		C-18 Me
methylene envelope	1.00-2.55		

Table 4.4
400 MHz ^{13}C NMR of 3β,6β-dihydroxy-5α-bromoandrost-17-one 3-acetate (3.57)

chemical shift	assignment	Dept
220.68	17	(C)
170.36	acetate carbonyl	(C)
86.02	5	(C)
75.29	6	CH
71.85	3	CH
50.68	9/14	CH
47.72	13	C
47.49	9/14	CH
40.41	10	C
38.21		CH_2
35.70		CH_2
34.94		CH_2
33.35		CH_2
31.21		CH_2
30.21	8	CH
26.17		CH_2
21.56	15/(11)	CH_2
21.23	acetate methyl	CH_3
20.47	11/(15)	CH_2
17.75	19	CH_3
13.82	18	CH_3

Following the original method for oxygenation of the C-19 methyl groups,[3] 3β,6β-dihydroxy–5α-bromoandrostan-17-one 3-acetate (3.57) was refluxed with lead tetraacetate in benzene. The product was purified by silica gel column chromatography to give 3β-hydroxy–5α-bromo-6β,19-oxidoandrost-17-one 3-acetate (3.58). Unfortunately however the yield by this procedure was unsatisfactory (23%) for a long synthetic strategy.

In accordance with the literature,[4] 3β,6β-dihydroxy–5α-bromo androstan -17-one 3-acetate (3.57) was refluxed with lead tetraacetate and iodine in carbon tetrachloride and irradiated with light from halogen lamps, to afford 3β-hydroxy–5α-bromo-6β,19-oxidoandrost-17-one 3-acetate (3.58) in quantitative yield. The product was purified by silica gel column chromatography (acetonitrile/dichloromethane) and identified by its spectra. The ^1H NMR (table 4.5) indicated the loss of the C-19 methyl signal that was present at 1.34δ in the precursor (3.57). This was accompanied by the appearance of two doublets (each integrating to 1 H), one at 4.09δ and the other at 3.94δ with a geminal coupling constant of 8.50 Hz. This corresponding to the two chemically different C-19 hydrogens. The infrared spectrum provided further evidence for the structure with the absence of any free O-H stretch. The EI-MS exhibited signals at 426 and 424 both corresponding to the two molecular ions. The CI-MS(NH_4^+) exhibited signals at 444, 442 both corresponding to two (MNH_4^+) ions.

Table 4.5
400 MHz ^1H NMR of 3β-hydroxy–5α-bromo-6β,19-oxidoandrost-17-one 3-acetate (3.58)

multiplicity	chemical shift	coupling constant	assignment
1 H (t,t)	5.16	5.28 Hz, 10.56 Hz	C-3α H
1 H (d)	4.09	4.28 Hz	C-6α H
1 H (d)	3.94	8.52 Hz	C-19 H
1 H (d)	3.72	8.48 Hz	C-19 H
3 H (s)	2.01		acetate methyl
3 H (s)	0.88		C-18 Me
methylene envelope	1.01-2.47		

Table 4.6
400 MHz ^{13}C NMR of 3β-hydroxy–5α-bromo-6β,19-oxidoandrost-17-one 3-acetate (3.58)

chemical shift	assignment
219.70	17
170.00	acetate carbonyl
81.73	
74.06	
69.54	
67.35	
50.88	
49.18	
48.57	
48.00	
45.80	
41.04	
35.51	
32.84	
31.66	
31.16	
26.62	
23.09	
21.68	
21.13	
14.01	18

In accordance with the literature,[5] 3β-hydroxy–5α-bromo-6β,19-oxidoandrost-17-one 3-acetate (3.58) was hydrolysed with sodium hydroxide solution in methanol to give 3β-hydroxy–5α-bromo-6β,19-oxidoandrost-17-one (3.59). The product was purified by recrystallisation and identified by its spectra. The ^{1}H NMR (table 4.7) indicated a shift in the position of the C-3αH signal from 5.16δ to 4.14δ. The ^{13}C NMR (table 4.8) indicated the presence of 19 signals with the loss of the acetate signal from 170.00δ. The infrared spectrum showed the appearance of a free OH adsorption signal at 3439 cm^{-1}. EI-MS exhibited signals at 384 and 382 corresponding to the two molecular ions. CI-MS(NH_4^+) exhibited signals at 402 and 400 corresponding to the two (MNH_4^+) ions.

Table 4.7
400 MHz ^{1}H NMR of 3β-hydroxy–5α-bromo-6β,19-oxidoandrost-17-one (3.59)

multiplicity	chemical shift	coupling constant	assignment
1 H (t,t)	4.14	4.85 Hz, 10.49	C-3α H
1 H (d)	4.12	4.07 Hz	C-6 H
1 H (d)	3.96	8.45 Hz	C-19 H
1 H (d)	3.73	8.47 Hz	C-19 H
3 H (s)	0.91		C-18 Me
methylene envelope	1.14-2.50		

Table 4.8
400 MHz ^{13}C NMR of 3β-hydroxy–5α-bromo-6β,19-oxidoandrost-17-one (3.59)

chemical shift	assignment
220.38	17
81.89	
75.08	
67.55	
66.61	
49.29	
48.75	
48.16	
45.82	
44.81	
35.64	
32.92	
31.76	
31.32	
30.41	
23.46	
21.80	
21.22	
14.01	18

In accordance with the literature,[5] 3β-hydroxy–5α-bromo-6β,19-oxidoandrost-17-one (3.59) was oxidised with chromium trioxide in pyridine to give 5α-bromo-6β,19-oxidoandrost-3,17-dione (3.60), which was then reduced by reflux with zinc dust in ethanol and treated with dilute sulphuric acid to afford 19-hydroxyandrost-4-en-3,17-dione (3.61).[5] The product was purified by silica gel chromatography (acetonitrile/dichloromethane) and identified by its spectra. The ^{1}H NMR (table 4.9) exhibited a 1 hydrogen

singlet at 5.89δ corresponding to the C-4 olefinic hydrogen which shows no coupling. This was accompanied by a shift in the position of the two chemically different geminally coupled C-19 hydrogens to 4.03δ and 3.89δ with an increase in the ^2J coupling constant from 8.50 Hz to 11.75 Hz. The infrared spectrum provided further evidence for the structure with the reappearance of a free OH stretch at 3494 cm^{-1} and three double bond stretches at 1738 cm^{-1}, 1658 cm^{-1} and 1618 cm^{-1}. EI-MS exhibited a signal at 302 corresponding to the molecular ion. CI-MS(NH_4^+) exhibited signals at 320 and 303 corresponding to the (MNH_4^+) and (MH^+) ions.

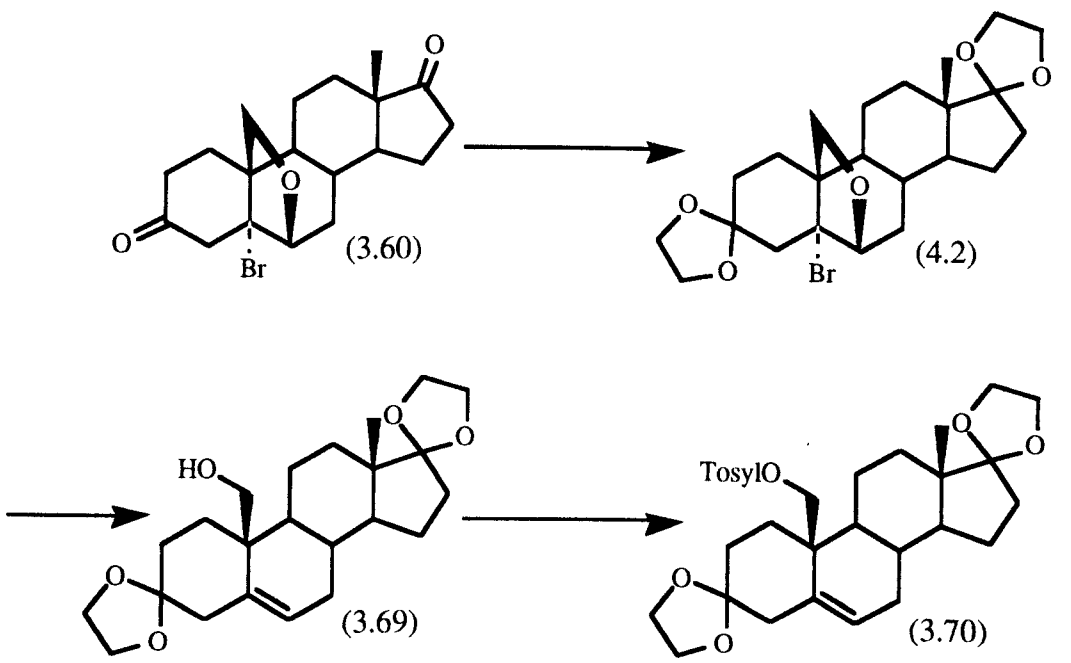
Table 4.9
400 MHz ^1H NMR of 19-hydroxyandrost-4-en-3,17-dione (3.61)

multiplicity	chemical shift	coupling constant	assignment
1 H (s)	5.89		C-4 H
1 H (d)	4.03	11.5 Hz	C-19 H
1 H (d)	3.89	12.0 Hz	C-19 H
3 H (s)	0.87		C-18 Me
methylene envelope	1.00-2.77		

Unfortunately, attempts to synthesise 3,3:17,17-bisethylenedioxy androst-5-ene (3.69) by ketalisation of 19-hydroxyandrost-3,17-dione (3.61) were unsuccessful due to the instability of the neopentyl 5-ene system. This problem could theoretically be overcome by altering the sequence of reactions in the synthetic strategy (scheme 4.2). Ketalisation of 5α-bromo-6β,19-oxidoandrostan-3,17-dione (3.60) would furnish 3,3:17,17-bisethylenedioxy-5α-bromo-6β,19-oxidoandrostane (4.2) which could be reduced by reflux with zinc in ethanol to afford 3,3:17,17-bisethylenedioxy-19-hydroxyandrost-5-ene (3.69). Tosylation of this product would afford the desired key intermediate 3,3:17,17-bisethylenedioxy-19-hydroxyandrost-5-ene 19-tosylate (3.70) (scheme 4.2).

Although this is a viable strategy, the route was not pursued as a

differentially protected intermediate (e.g. 3β,19-dihydroxy-17,17-ethylene dioxyandrost-5-ene 3-acetate 19-tosylate (3.71)) was considered to offer a synthetic advantage in the strategy as the C-3 and C-17 positions could be selectively chemically manipulated.

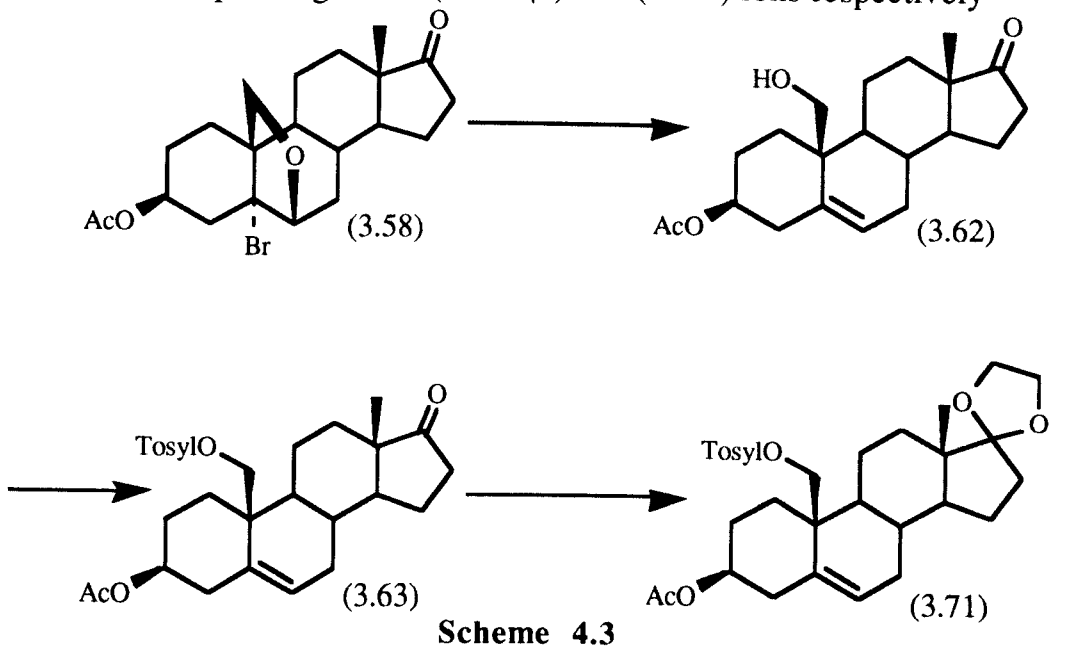


Scheme 4.2

At this point a route from 3β,19-dihydroxyandrostan-5-en-17-one 3-acetate (3.62) was explored. This compound could be synthesised by the reduction of 3β-hydroxy-5α-bromo-6β,19-oxidoandrostan-17-one 3-acetate (3.58).[6] The idea of this strategy being that a leaving group could be introduced at C-19 and the C-17 carbonyl functionality could be protected (scheme 4.3).

In accordance with the literature,[6] 3β-hydroxy-5α-bromo-6β,19-oxidoandrostan-17-one 3-acetate (3.58) was reduced by reflux with zinc dust in ethanol to afford 3β,19-dihydroxyandrostan-5-en-17-one 3-acetate (3.62). The product was purified by recrystallisation and identified by its spectra. The ^{1}H NMR (table 4.10) indicated the shift of the C-6αH from 4.09δ to the olefinic region at 5.77δ. The infrared spectrum exhibited a free OH absorption signal at 3463 cm^{-1}. CI-MS(NH_4^+) exhibited signals at 364

and 347 corresponding to the (MNH_4^+) and (MH^+) ions respectively



Scheme 4.3

Table 4.10
250 MHz ^1H NMR of 3β,19-dihydroxyandrostan-5-en-17-one 3-acetate (3.62).

multiplicity	chemical shift	coupling constant	assignment
1 H (m)	5.78-5.76		C-6 H
1 H (t,t)	4.61	4.80 Hz, 11.50 Hz	C-3α H
1 H (d)	3.86	11.52 Hz	C-19 H
1 H (d)	3.60	11.48 Hz	C-19 H
3 H (s)	2.00		acetate methyl
3 H (s)	0.90		C-18 Me
methylene envelope	2.46-1.06		

In accordance with the literature,[7] 3β,19-dihydroxyandrostan-17-one 3-acetate (3.62) was stirred with tosyl chloride in dry pyridine to afford 3β,19-dihydroxyandrostan-5-en-17-one 3-acetate 19-tosylate (3.63). The product was purified by silica gel column chromatography and identified by its spectra. The ^1H NMR (table 4.11) indicted the appearance of two pairs of doublets in the aromatic region at 7.40δ and 7.76δ with a vicinal coupling constant of 7.5 Hz. This was accompanied by a reduction in the geminal coupling constant between the two C-19 hydrogens from 11.50 Hz to 10.00 Hz. The ^{13}C NMR (table 4.12) exhibited 24 chemically different signals. The signal at 21.50δ resulted from the coincidence of 2 signals. The infrared

spectrum provided further evidence for the structure with a carbonyl stretch at 1733 cm^{-1}. The CI-MS(NH_4^+) exhibited a peak at 518 corresponding to the (MNH_4^+) ion.

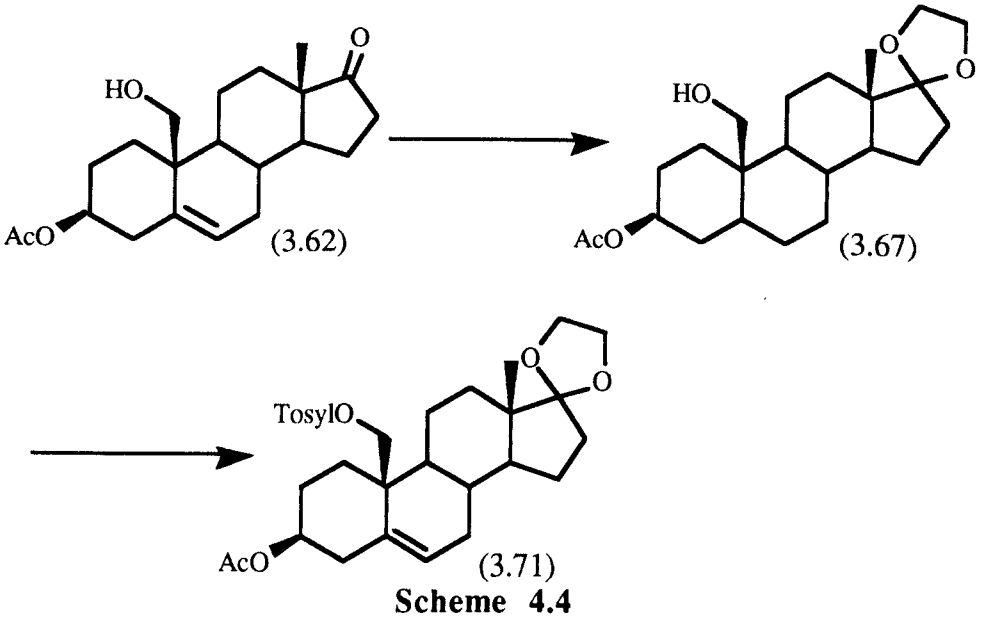
Table 4.11
250 MHz ^1H NMR of 3β,19-dihydroxyandrostan-5-en-17-one 3-acetate 19-tosylate (3.63).

multiplicity	chemical shift	coupling constant	assignment
2 H (d)	7.76	7.4	aromatic H
2 H (d)	7.40	7.6	aromatic H
1 H (m)	5.64-5.61		C-6 H
1 H (t,t)	4.56	5.0 Hz, 11.0 Hz	C-3α H
1 H (d)	4.15	10.0	C-19 H
1 H (d)	3.88	10.0 Hz	C-19 H
3 H (s)	2.44		acetate methyl
3 H (s)	0.75		C-18 Me
methylene envelope	2.2-0.75		

Table 4.12
400 MHz ^{13}C NMR of 3β,19-dihydroxyandrostan-5-en-17-one 3-acetate 19-tosylate (3.63).

chemical shift	assignment
220.46	17
170.25	acetate carbonyl
144.82	1'/4'
133.34	1'/4'/5
132.62	1'/4'/5
129.74	2'/3'
127.74	6
127.02	
72.60	
69.44	
51.91	
49.75	
47.36	
39.91	
37.65	
35.56	
32.61	
31.98	
31.33	
29.95	
27.46	
21.50	
21.16	
20.69	
13.42	18

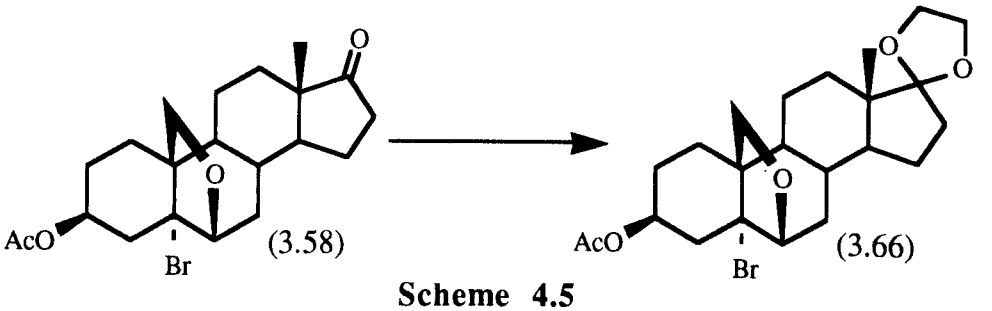
Attempts were made to synthesise 3β,19-dihydroxy-17,17-ethylene dioxyandrostan-5-ene 3-acetate 19-tosylate (3.71) by the reflux of 3β,19-dihydroxyandrostan-5-en-17-one 3-acetate 19-tosylate (3.63) with p-toluenesulphonic acid, ethylene glycol and dry benzene in a Dean and Stark apparatus (scheme 4.3). The steroid was not stable under these conditions and so the product could not be isolated.



Scheme 4.4

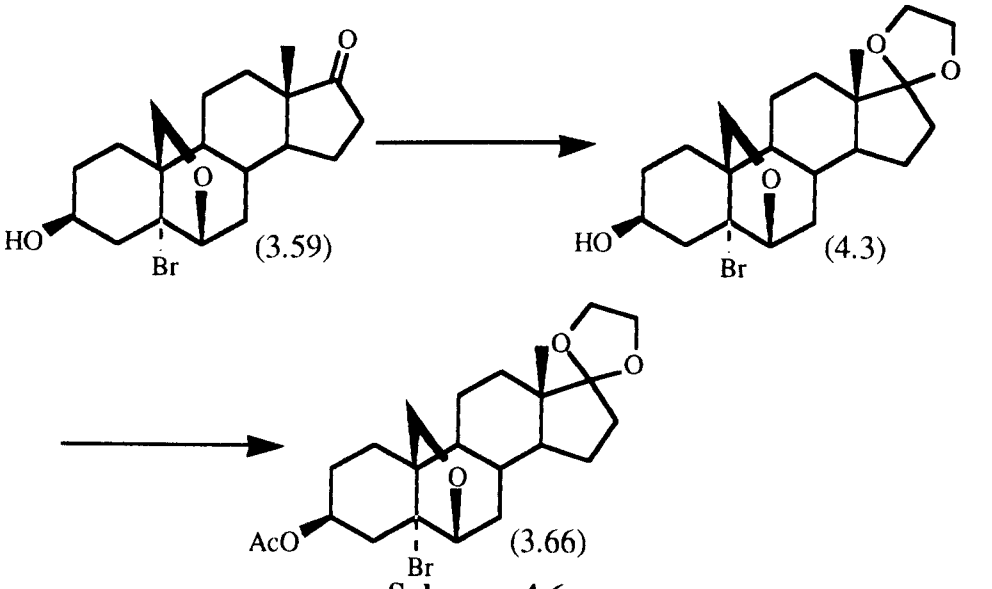
An alternative strategy (scheme 4.4) via the ketalisation of 3β,19-dihydroxyandrostan-5-en-17-one 3-acetate (3.62) and subsequent introduction of a leaving group was considered to offer a suitable route to 3β,19-dihydroxy-17,17-ethylenedioxyandrostan-5-ene 3-acetate 19-tosylate (3.71). Attempts were made to ketalise 3β,19-dihydroxyandrostan-5-en-17-one 3-acetate (3.62) by reflux with p-toluenesulphonic acid, ethylene glycol and dry benzene in a Dean and Stark apparatus. The product could not be isolated due to the instability of the neopentyl hydroxy 5-ene system under these reaction conditions. This meant that protection of the C-17 functionality was necessary prior to the reductive cleavage of the 6β,19-oxido ring.

A strategy was explored via the ketalisation of 3β-hydroxy–5α-bromo-6β,19-oxidoandrost-17-one 3-acetate (3.58) by refluxing for four hours with ethylene glycol, p-toluenesulphonic acid and dry benzene in a Dean and Stark apparatus to give 3β-hydroxy-5α-bromo-6β,19-oxido-17,17-ethylenedioxyandrostane 3-acetate (3.66). These reaction conditions also caused concomitant partial hydrolysis of the 3β-acetate and therefore a low yield. This would cause a serious problem for the overall yield in the synthetic strategy.



Scheme 4.5

We also explored an alternative strategy (scheme 4.6) which involved the ketalisation of 3β-hydroxy–5α-bromo-6β,19-oxidoandrost-17-one (3.59), followed by an acetylation of the 3β-alcohol (4.3) to assess whether this would provide a more suitable route to 3β-hydroxy–5α-bromo-6β,19-oxido-17,17-ethylenedioxyandrostane 3-acetate (3.66).



Scheme 4.6

3β-Hydroxy–5α-bromo-6β,19-oxidoandrost-17-one (3.59) was refluxed for 4 hours with ethylene glycol, p-toluenesulphonic acid and dry benzene in a Dean and Stark apparatus to afford 3β-hydroxy-5α-bromo-6β,19-oxido-17,17-ethylenedioxyandrostane (4.3). The product was purified by silica gel column chromatography (acetonitrile/dichloromethane) and identified by its spectra. The ^{1}H NMR (table 4.13) exhibited a 4 H multiplet in the region 3.73δ -3.88δ corresponding to the C-17 ketal functionality. The ^{13}C NMR (table 4.14) indicated the presence of 21 signals with the shift of the position of the C-17 signal from 220.38δ to 118.91δ and the appearance of two ketal signals at 65.06δ and 64.42δ. The infrared spectrum exhibited no carbonyl absorption signal. The CI-MS(NH_4^+) exhibited signals at 444 and 446 corresponding to the two (MNH_4^+) ions and signals at 426 and 428 corresponding to the (MH^+) ions. This was accompanied by a new signal at 99 which corresponds to the ketal ion fragment.

Table 4.13
250 MHz ^{1}H NMR of 3β-hydroxy-5α-bromo-6β,19-oxido-17,17-ethylenedioxyandrostane (4.3).

multiplicity	chemical shift	coupling constant	assignment
1 H (t,t)	4.06	4.54 Hz, 10.80 Hz	C-3α H
1 H (d)	3.09	4.53 Hz	C-6α H
4 H (m)	3.88 - 3.73		ketal H's
1 H (d)	3.83	8.45 Hz	C-19 H
1 H (d)	3.64	8.32 Hz	C-19 H
3 H (s)	0.81		C-18 Me
methylene envelope	2.30-1.02		

Table 4.14
400 MHz ^{13}C NMR of 3β-hydroxy-5α-bromo-6β,19-oxido-17,17-ethylenedioxyandrostane (4.3).

chemical shift	assignment
118.91	17
82.10	5
75.03	6
67.48	3
66.80	19
65.06	ketal
64.42	ketal
48.48	
48.25	
46.35	
45.72	
44.91	
34.15	
33.45	
32.14	
30.85	
30.44	
23.52	
22.00	15
21.84	11
14.53	18

3β-Hydroxy-5α-bromo-6β,19-oxido-17,17-ethylenedioxyandrostane (4.3) was re-acetylated by stirring with freshly distilled acetic anhydride in dry pyridine to give 3β-hydroxy-5α-bromo-6β,19-oxido-17,17-ethylene dioxyandrostane 3-acetate (3.66). An easier synthesis of 3β-hydroxy-5α-bromo-6β,19-oxido-17,17-ethylenedioxyandrostane 3-acetate (3.66) was by ketalisation of 3β-hydroxy–5α-bromo-6β,19-oxidoandrost-17-one 3-acetate (3.58) followed by acetylation. In accordance with the literature 3β-hydroxy–5α-bromo-6β,19-oxidoandrost-17-one 3-acetate (3.58) was refluxed for four hours with ethylene glycol, p-toluenesulphonic acid and dry benzene in a Dean and Stark apparatus to afford 3β-hydroxy-5α-bromo-6β,19-oxido-17,17-ethylene dioxyandrostane 3-acetate (3.66). These reaction conditions also caused concomitant partial hydrolysis of the 3β-acetate. In order to maximise the final yield, the crude mixture was re-

acetylated by stirring with freshly distilled acetic anhydride in dry pyridine. In both reactions the product was purified by silica gel column chromatography (acetonitrile/dichloromethane) and identified by its spectra. The ^{1}H NMR (table 4.15) exhibited a 4 H multiplet in the region 3.77δ - 3.90δ (beneath which was concealed one of the C-19 H doublets giving a total integral over this region of 5 H). The ^{13}C NMR (table 4.16) indicated the presence of 23 signals with the shift of the position of C-17 signal from 219.70δ to 118.83δ and the appearance of two ketal signals at 65.07δ and 64.42δ. The infrared spectrum exhibited an absorption signal at 1733 cm^{-1} corresponding to the 3β-acetate carbonyl group. The EI-MS exhibited signals at 470 and 468 corresponding to the two molecular ions. This was accompanied by a signal at 99 which corresponds to the ketal ion fragment.

Table 4.15
250 MHz ^{1}H NMR of 3β-hydroxy-5α-bromo-6β,19-oxido-17,17-ethylenedioxyandrostane 3-acetate (3.66).

multiplicity	chemical shift	coupling constant	assignment
1 H (t,t)	5.11	5.12 Hz, 10.24 Hz	C-3α H
1 H (d)	3.99	4.43 Hz	C-6α H
5 H (4 H (m) + 1 H (d))	3.90 - 3.77		ketal H's + C-19 H
1 H (d)	3.66	8.40 Hz	C-19 H
3 H (s)	1.95		acetate methyl
3 H (s)	0.81		C-18 Me
methylene envelope	2.30-0.99		

Table 4.16
400 MHz ^{13}C NMR of 3β-hydroxy-5α-bromo-6β,19-oxido-17,17-ethylenedioxyandrostane 3-acetate (3.66).

chemical shift	assignment
170.22	acetate carbonyl
118.83	17
80.03	5/6/19
74.05	3/5/6/19
69.81	3/5/6/19
67.37	5/6/19
65.07	Ketal
64.42	Ketal
48.32	
48.18	
46.32	
45.75	
41.15	
34.12	
33.43	
32.10	
30.37	
26.73	
23.18	
21.93	
21.82	
21.20	
14.51	18

In accordance with the literature[7] 3β-hydroxy-5α-bromo-6β,19-oxido-17,17-ethylenedioxyandrostane 3-acetate (3.66) was refluxed for 3 hours with zinc dust (freshly activated with acetic acid) in ethanol (with the apparent pH adjusted to 5.5 with glacial acetic acid) in order to cause elimination across the 5 - 6 C-C bond to give 3β,19-dihydroxy-17,17-ethylenedioxyandrost-5-ene 3-acetate (3.67). The careful adjustment of pH of the solvent was important as otherwise concomitant partial hydrolysis of the 3β-acetate occurred. The re-acetylation of the 3β-hydroxy functionality was not suitable at this stage as it would also bring about the acetylation of the newly formed 19-hydroxy functionality. The product was purified by recrystallisation from acetone/hexane and identified by its spectra. The ^{1}H NMR (table 4.17) indicated a shift of the C-6 H doublet from 3.99δ to the olefinic region at 5.74δ. The position of the two chemically different, geminally coupled C-19 hydrogens changed to 3.82δ and 3.58δ with an increase in the ^{2}J coupling constant from 8.40 Hz to 11.0 Hz. The ^{13}C NMR (table 4.18) indicated the presence of 23 signals with the disappearance of 3 signals from 80.03, 69.81, 67.37, the reappearance of a C-5 olefinic signal at 134.39δ, C-6 olefinic at 127.84δ and a new signal for C-19 at 62.59δ. The infrared spectrum provided further evidence for the structure with the reappearance of a free O-H stretch at 3494 cm^{-1} and two double bond stretches at 1732 and 1712 cm^{-1} corresponding to the acetate C=O double bond stretch and the C-5 C-C double bond stretch. The EI-MS exhibited signals at 390 and 99 corresponding to the molecular ion and ketal ion fragment.

Table 4.17
400 MHz ^{1}H NMR of 3β,19-dihydroxy-17,17-ethylenedioxyandrost-5-ene 3-acetate (3.67).

multiplicity	chemical shift	coupling constant	assignment
1 H (d)	5.74	4.96 Hz	C-6 H
1 H (t,t)	4.61	5.41 Hz, 10.42 Hz	C-3α H
4 H (m)	3.93-3.82		ketal H's
1 H (d)	3.82	10.6 Hz	C-19 H
1 H (d)	3.58	11.4 Hz	C-19 H
3 H (s)	2.00		acetate methyl
3 H (s)	0.88		C-18 Me
methylene envelope	0.99- 2.46		

Table 4.18
400 MHz ^{13}C NMR of 3β,19-dihydroxy-17,17-ethylenedioxyandrost-5-ene 3-acetate (3.67).

chemical shift	assignment
170.38	acetate carbonyl
134.39	5
127.84	6
119.25	17
73.21	3
65.03	ketal
64.39	ketal
62.59	19
51.23	
49.97	
45.76	
41.44	
38.03	
34.04	
33.41	
33.00	
30.67	
30.42	
27.91	
22.40	15
21.23	11/ acetate methyl
21.00	11/ acetate methyl
14.45	18

p-Toluenesulphonyl chloride was stirred with 3β,19-dihydroxy-17,17-ethylenedioxyandrost-5-ene 3-acetate (3.67) in dry pyridine, to afford 3β,19-dihydroxy-17,17-ethylenedioxyandrost-5-ene 3-acetate 19-tosylate (3.71).[8] The product was purified by silica gel chromatography (acetonitrile/

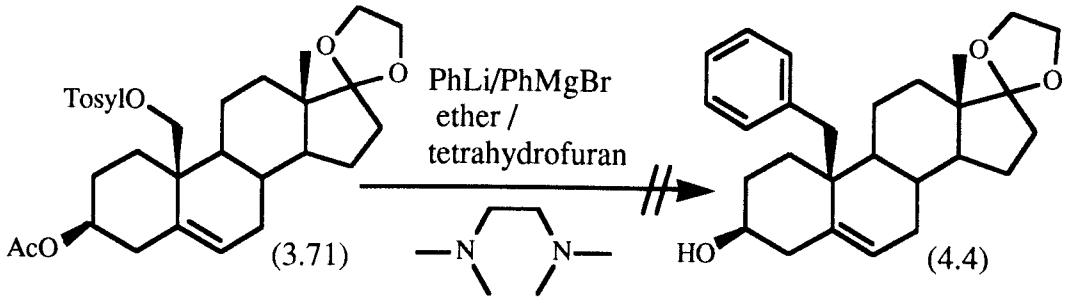
dichloromethane) and identified by its spectra. The ^1H NMR (table 4.19) exhibited two chemically different doublets in the aromatic region, each integrating to 2 hydrogens at 7.12δ and 7.55δ with a coupling constant of 7.88 Hz. This was accompanied by the appearance of a new 3 hydrogen singlet at 1.78δ corresponding to the aromatic methyl substituent. The infrared spectrum showed the disappearance of the free OH stretch signal. The CI-MS(NH_4^+) exhibited signals at 568, 545 and 99 corresponding to the (MNH_4^+), (MH^+) ion peaks and ketal ion fragment.

Table 4.19
250 MHz ^1H NMR of 3β,19-dihydroxy-17,17-ethylenedioxyandrost-5-ene 3-acetate 19-tosylate (3.71).

multiplicity	chemical shift	coupling constant	assignment
1 H (d)	7.55	7.98 Hz	aromatic H
1 H (d)	7.12	7.78 Hz	aromatic H
1 H (m)	5.37		C-6 H
1 H (t,t)	4.35	5.25 Hz, 12.01 Hz	C-3α H
1 H (d)	3.87	10.18 Hz	C-19 H
1 H (d)	3.76	10.08 Hz	C-19 H
4 H (m)	3.75-3.44		ketal H's
3 H (s)	2.22		acetate methyl
3 H (s)	1.78		tosyl methyl
3 H (s)	0.46		C-18 Me
methylene envelope	0.15-0.80		

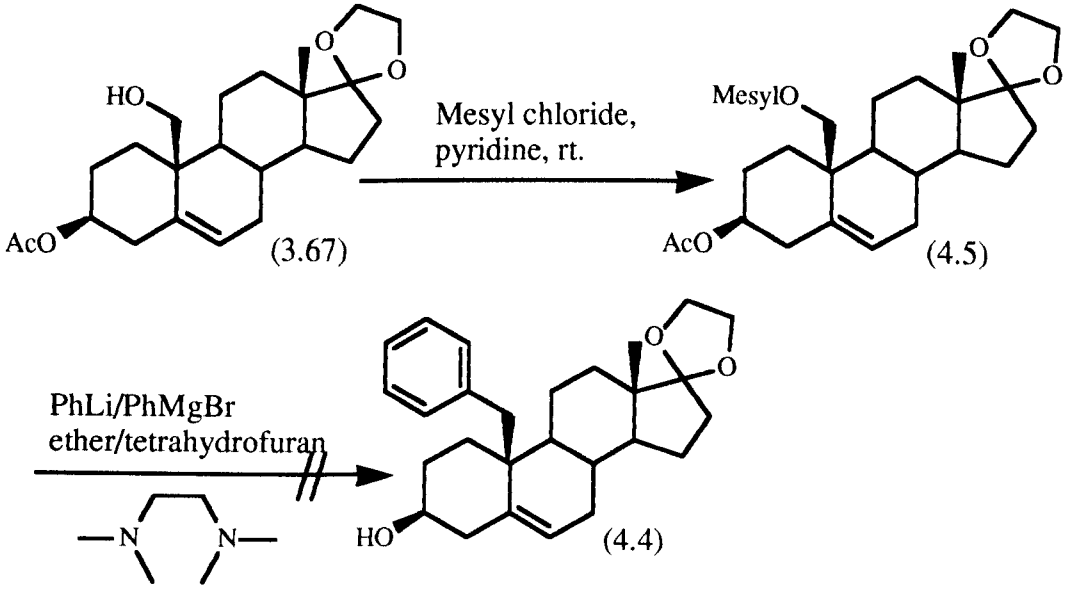
4.3 Nucleophilic substitution reaction at neopentyl sites.

Attempts were made to introduce a phenyl substituent at the C-19 neopentyl methyl group by nucleophilic displacement of the C-19 tosyl leaving group from 3β,19-dihydroxy-17,17-ethylenedioxyandrost-5-ene 3-acetate 19-tosylate (3.71), with phenyl lithium or phenyl magnesium bromide in ether or tetrahydrofuran.[9] The nucleophilic substitution did not proceed, even when the chelating agent N,N,N',N'-tetramethylethylenediamine was used to increase the nucleophilicity of the phenyl anion.



Scheme 4.7

Although nucleophilic substitution of the tosyl leaving group from the C-19 neopentylic site had been reported with lithium chloride and lithium bromide,[7] when we used phenyl lithium, the displacement of the tosyl leaving group did not occur. It is considered that the level of steric hindrance in the reaction is sufficient to prevent substitution from occurring. The effect of steric hindrance may be minimised by reducing the size of the leaving group and nucleophile. Since the aromatic functionality is an essential feature in the desired compound, only the leaving group was changed. In order to minimise the effects of steric hindrance we synthesised the corresponding C-19 mesyl steroid (4.5) (scheme 4.8).



Scheme 4.8

Methanesulphonyl chloride was stirred with 3β,19-dihydroxy-17,17-ethylenedioxyandrost-5-ene 3-acetate (3.67) in dry pyridine, to afford 3β,19-dihydroxy-17,17-ethylenedioxyandrost-5-ene 3-acetate 19-mesylate (4.5).[8] The product was purified by silica gel chromatography (acetonitrile/ dichloromethane) and identified by its spectra. The ^{1}H NMR (table 4.20) indicated the appearance of a new three hydrogen singlet at 1.37δ corresponding to the mesyl methyl group. The ^{13}C NMR (table 4.21) indicated the presence of 24 signals. The infrared spectrum exhibited a carbonyl stretch at 1734 cm^{-1}. EI-MS exhibited peaks at 468 and 99 corresponding to the molecular ion and ketal ion fragment.

Attempts to introduce a phenyl group at the C-19 position by the nucleophilic attack of the C-19 mesyl leaving group by phenyl lithium or phenyl magnesium bromide in ether or tetrahydrofuran failed. When a chelating agent was used, the substitution reaction still did not proceed.

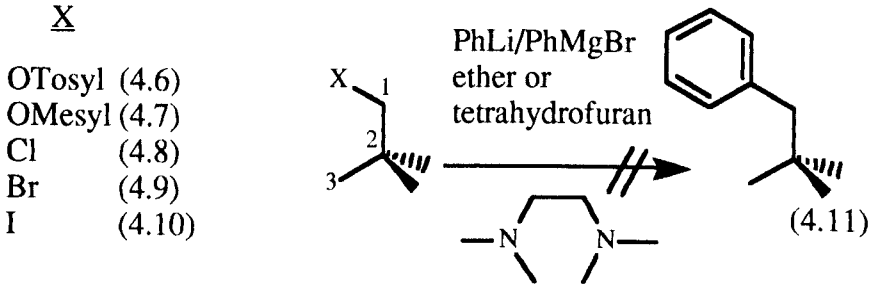
Interestingly the researchers at Roussel-Uclaf have also tried to develop a synthetic route to C-19 substituted steroids from C-19 hydroxyandrostanes,[10] but reported that they encountered extreme difficulty when working at the hindered C-19 neopentylic site.[10]

Table 4.20
400 MHz ^{1}H NMR of 3β,19-dihydroxy-17,17-ethylenedioxyandrost-5-ene 3-acetate 19-mesylate (4.5).

multiplicity	chemical shift	coupling constant	assignment
1 H (m)	5.71-5.69		C-6 H
1 H (t,t)	4.58	4.78 Hz, 11.44 Hz	C-3α H
5 H (m)	3.97 - 3.76		ketal H's +
(4 H (m)+1 H (d))			C-19 H
1 H (d)	3.56	11.48 Hz	C-19 H
3 H (s)	1.97		acetate methyl
3 H (s)	1.37		mesyl methyl
3 H (s)	0.85		C-18 Me
methylene envelope	1.01 - 2.49		

Table 4.21
400 MHz ^{13}C NMR of 3β,19-dihydroxy-17,17-ethylenedioxyandrost-5-ene 3-acetate 19-mesylate (4.5).

chemical shift	assignment
170.30	acetate carbonyl
134.43	6
127.57	5
119.17	17
73.18	3
64.94	ketal
64.30	ketal
62.50	19
51.14	
49.91	
45.68	
41.34	
37.97	
33.96	
33.30	
32.92	
30.60	
30.38	
27.84	
26.68	
22.34	15
21.14	11/ acetate methyl
20.93	11/ acetate methyl
14.37	18



Scheme 4.9

The problem of displacing C-19 leaving groups was further investigated using neopentyl derivatives as model systems. p-Toluene sulphonyl chloride was stirred with neopentyl alcohol in dry pyridine, to afford 2,2-dimethylpropan-1-ol 1-tosylate (4.6). The product was purified by silica gel chromatography (acetonitrile/ dichloromethane) and identified by its spectra. The ^{1}H NMR (table 4.22) exhibited two chemically different doublets in the aromatic region, each integrating to 2 hydrogens at 7.30δ and 7.73δ with a coupling constant of 8.00 Hz. This was accompanied by a new 3 hydrogen singlet at 2.41δ corresponding to the aromatic methyl substituent. The ^{13}C NMR (table 4.23) indicated the appearance of four signals in the aromatic region and a signal for the benzylic alcohol at 79.38δ. EI-MS exhibited a peak at 242 corresponding to the molecular ion.

Attempts to displace the tosyl leaving group from 2,2-dimethylpropan-1-ol 1-tosylate (4.6) by nucleophilic attack with phenyl lithium or phenyl magnesium bromide in ether or tetrahydrofuran failed. When N,N,N',N'-tetra methylethylenediamine was used as a chelating agent the C-19 tosyl group was not displaced.

Table 4.22
400 MHz ^{1}H NMR of 2,2-dimethylpropan-1-ol 1-tosylate (4.6).

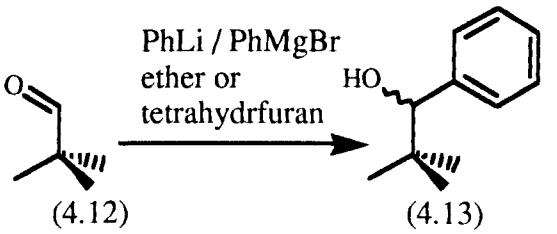
multiplicity	chemical shift	coupling	assignment
2 H (d)	7.75	13.24 Hz	C-2' H
2 H (d)	7.32	12.48 Hz	C-3' H
1 H (s)	3.62		C-1 H
3- H (s)	2.41		C-5' methyl
9 H (s)	0.86		C-3 methyl

Table 4.23
400 MHz ^{13}C NMR of 2,2-dimethylpropan-1-ol 1-tosylate (4.6).

chemical shift	assignment
144.57	1'/4'
132.83	1'/4'
129.71	2'/3'
127.74	2'/3'
79.38	1
31.48	2
25.86	3
21.48	5'

Neopentyl alcohol was stirred at room temperature with mesyl chloride in pyridine to afford 2,2-dimethylpropan-1-ol 1-mesylate (4.7). Nucleophilic displacement of the mesyl leaving group by phenyl lithium or phenyl magnesium bromide in ether or tetrahydrofuran from the 2,2-dimethylpropan-1-ol 1-mesylate (4.7) was attempted but also failed. When N,N,N',N'-tetra methylethylenediamine was used as a chelating agent the C-19 mesyl group was not displaced.

Following these unsuccessful attempts, displacements on neopentyl chloride, bromide and iodide were also tried using phenyl lithium and phenyl magnesium bromide. The desired nucleophilic substitution again did not proceed, even when the chelating agent N,N,N',N'-tetramethylethylene-1,2-diamine was used to increase the nucleophilicity of the phenyl anion. The nucleophilic displacement did not occur in any of the above sp^3 hybridised neopentyl systems.



(4.12) (4.13)

Scheme 4.10

A nucleophilic addition with phenyl lithium[4] was also tried on the neopentyl aldehyde 2,2-dimethylpropanal (4.12) (scheme 4.10). Phenyl lithium solution was slowly added to a solution of 2,2-dimethylpropanal (4.12) in dry tetrahydrofuran[9] to afford 1-phenyl-2,2-dimethylpropan-1-ol (4.13). The same product was also synthesised when the Grignard reagent of bromobenzene was used as the nucleophile. The product in both reactions was purified by silica gel preparative thin layer chromatography and identified by its spectra. The ^{1}H NMR (table 4.24) exhibited a 5 hydrogen multiplet in the aromatic region 7.26δ -7.65δ corresponding to 5 aromatic hydrogens. This was accompanied by the shift of the aldehydic proton to 4.41δ. The ^{13}C NMR (table 4.25) indicated the presence of 4 new chemically different signals at 142.11δ, 127.54δ, 127.47δ and 127.19δ corresponding to the aromatic carbon signals. There was a shift in the position of the aldehydic carbon singlet to 82.28δ. EI-MS exhibited signals at 164 and 107 corresponding to the molecular ion and a substituted tropylium ion fragment.

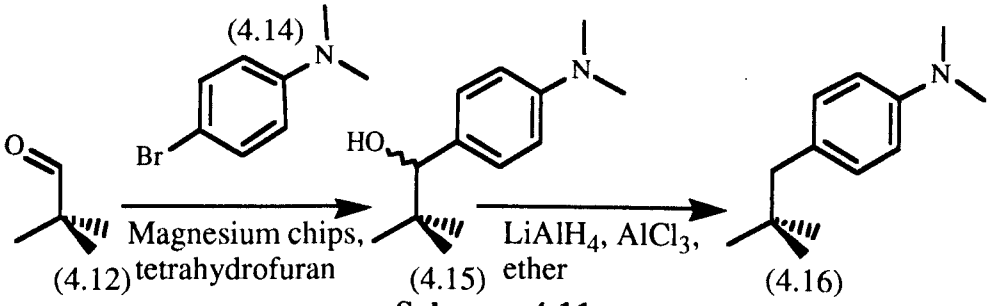
In contrast to the lack of nucleophilic substitution reactions occurring with the sp^3 hybridised neopentyl systems bearing the mesyl, tosyl, chloro, bromo, or iodo leaving groups, an sp^2 hybridised system, 2,2-dimethyl propanal (4.12), smoothly reacted with phenyl lithium or phenyl magnesium bromide to give the addition product of 1-phenyl-2,2-dimethylpropan-1-ol (4.13). The product was identified by its spectra.

Table 4.24
250 MHz ^{1}H NMR of 1-phenyl-2,2-dimethylpropan-1-ol (4.13).

multiplicity	chemical shift	assignment
5 H (m)	7.65 - 7.26	aromatic H
1 H (s)	4.41	C-1 H
9 H (s)	0.95	methyl groups

Table 4.25
250 MHz ^{13}C NMR of 1-phenyl-2,2-dimethylpropan-1-ol (4.13).

chemical shift	assignment
142.11	1'
127.54	2'/3'
127.47	2'/3'
127.19	4'
82.28	1
35.53	2
25.85	3



Scheme 4.11

In order to assess whether the amino substituent would interfere with the nucleophilic addition, the reaction was tried between p-N,N-dimethyl aminophenyl magnesium bromide[11] and 2,2-dimethylpropanal (4.12). To a stirred solution of p-N,N-dimethylaminophenyl magnesium bromide in dry terahydrofuan was added 2,2-dimethylpropanal (4.12), to afford 1-(p-N,N-dimethylaminophenyl)-2,2-dimethylpropan-1-ol (4.15), which was purified by silica gel preparative thin layer chromatography (acetonitrile/ dichloromethane) and identified by its spectra. During this nucleophilic addition reaction, a benzylic hydroxy functionality was also generated. The ^{1}H NMR (table 4.26) indicated the presence of two doublets, each integrating to two hydrogens at 7.17δ and 6.69δ with a coupling constant of 8.74 Hz corresponding to the p- substituted aromatic system. This was

accompanied by the shift of the aldehydic proton signal to 4.30δ. The ^{13}C NMR (table 4.27) indicated the presence of eight chemically different carbon signals, with a shift of the aldehydic carbon signal to 82.10δ. The infrared spectrum provided further evidence for the structure with a free O-H stretch at 3612 cm^{-1}. CI-MS(NH_4^+) exhibited peaks at 208 and 151 corresponding to the (MH^+) ion and a substituted tropylium ion. The nucleophilic addition at the sp^2 hybridised carbon atom had occurred, showing that the amino substituent did not interfere with the nucleophilic addition reaction.

Table 4.26
250 MHz ^1H NMR of 1-(p-N,N-dimethylaminophenyl)-2,2-dimethylpropan-1-ol (4.15).

multiplicity	chemical shift	coupling	assignment
2 H (d)	7.17	8.55 Hz	C-2' H
2 H (d)	6.69	8.93 Hz	C-3' H
1 H (s)	4.30		C-1 H
6 H (s)	2.93		C-5' methyl
9 H (s)	0.92		C-3 methyl

Table 4.27
250 MHz ^{13}C NMR of 1-(p-N,N-dimethylaminophenyl)-2,2-dimethylpropan-1-ol (4.15).

chemical shift	assignment
149.81	4'
130.39	1'
128.25	2'
111.71	3'
40.62	5'
82.10	1
35.71	2
25.92	3

In the previous two examples, a hydroxy functionality is also generated when 2,2-dimethylpropanal (4.12) is attacked by a nucleophile. If an aryl nucleophile were to attack a steroid bearing a C-10β formyl group, a C-19 hydroxy group would also be generated. When the structure of the target compound is considered, a strategy must be developed to remove the C-19 hydroxy functionality. In this regard a benzylic reduction[12] was

undertaken in the model neopentyl system. 1-(p-N,N-dimethylaminophenyl)-2,2-dimethylpropan-1-ol (4.15) was refluxed with lithium aluminium hydride and aluminium chloride in dry ether, to furnish 1-(p-N,N-dimethyl aminophenyl)-2,2-dimethylpropane (4.16), the product was identified by its spectra. The ^{1}H NMR (table 4.28) exhibited a 2 hydrogen singlet at 3.42δ. The infrared spectrum showed the disappearance of the free OH signal from 3612 cm^{-1}. The CI-MS(NH_4^+) exhibited a peak at 192 corresponding to the (MH^+) ion.

Table 4.28
250 MHz ^{1}H NMR of 1-(p-N,N-dimethylaminophenyl)-2,2-dimethylpropane (4.16).

multiplicity	chemical shift	coupling	assignment
2 H (d)	6.87	8.62 Hz	C-2' H
2 H (d)	6.66	8.65 Hz	C-3' H
2 H (s)	3.42		C-1 H
6 H (s)	2.89		C-5' methyl
9 H (s)	0.89		C-3 methyl

In view of the investigation of the nucleophilic substitution reactions of neopentyl systems, it was considered that 3β-hydroxy-17,17-ethylenedioxyandrost-5-en-19-one 3-acetate (3.68) would be a suitable candidate for the nucleophilic introduction of a C-19 aryl functionality. The 19-alcohol (3.67) needed to be oxidised to the corresponding aldehyde 3β-hydroxy-10β-formyl-17,17-ethylenedioxy-19-norandrost-5-ene 3-acetate (3.68).

In accordance with the literature,[7,13,14] chromium trioxide was stirred at room temperature with pyridine in dichloromethane for 15 minutes. To this solution was added 3β,19-dihydroxy-17,17-ethylenedioxyandrost-5-ene 3-acetate (3.67) and stirred for 15 minutes. The product was filtered through a bed of celite and purified by recrystallisation from acetone/hexane and identified by its spectra. The ^{1}H NMR (table 4.29) indicated the loss of the geminally coupled C-19 hydrogens from 3.82δ and 3.58δ and the appearance

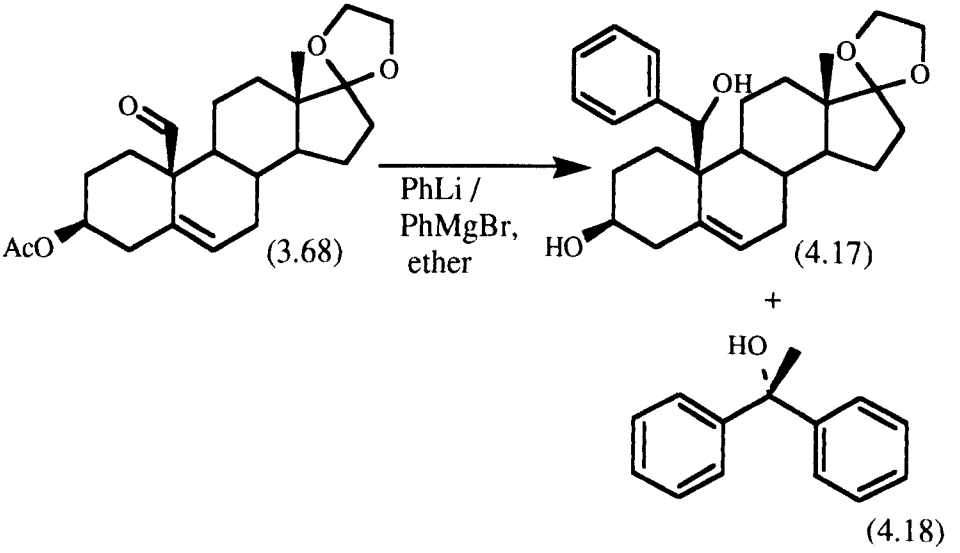
of an aldehydic doublet at 9.63δ with a ^4J coupling constant of 1.36 Hz (to the C-1α H or C-9α H). The ^{13}C NMR (table 4.30) indicated the presence of 23 signals, with a shift in position for the C-19 signal from 62.59δ to 204.75δ. The infrared spectrum provided further evidence for the structure with the absence of the OH stretch that was present in 3β,19-dihydroxy-17,17-ethylenedioxyandrost-5-ene 3-acetate (3.67) at 3494 cm^{-1}. Also two absorption signals were observed at 1730 cm^{-1} and 1712 cm^{-1} corresponding to the acetate carbon oxygen double bond stretch and the C-19 carbonyl stretch. EI-MS exhibited signals at 388 and 99 corresponding to the molecular ion and ketal fragment.

Table 4.29
400 MHz ^1H NMR 3β-hydroxy-17,17-ethylenedioxyandrost-5-en-19-one 3-acetate (3.67).

multiplicity	chemical shift	coupling constant	assignment
1 H (d)	9.63	1.36 Hz	C-19 aldehyde H (couples to 9α H)
1 H (d)	5.85	5.48 Hz	C-6 H
1 H (t,t)	4.57	4.50 Hz, 11.55 Hz	C-3α H
4 H (m)	3.94-3.76		ketal H's
1 H (d)	3.94	8.52 Hz	C-19 H
1 H (d)	3.72	8.48 Hz	C-19 H
3 H (s)	2.01		acetate methyl
3 H (s)	0.87		C-18 Me
methylene envelope	1.01-2.47		

Table 4.30
400 MHz ^{13}C NMR 3β-hydroxy-17,17-ethylenedioxyandrost-5-en-19-one 3-acetate (3.67).

chemical shift	assignment
204.75	19
170.35	acetate carbonyl
131.32	5/6
128.14	5/6
118.91	17
72.47	3
65.08	ketal
64.40	ketal
53.36	
50.14	
48.39	
45.38	
39.33	
33.88	
33.00	
30.43	
30.28	
30.06	
28.53	
22.26	15
21.53	11/acetate methyl
21.20	11/acetate methyl
13.98	18



Scheme 4.12

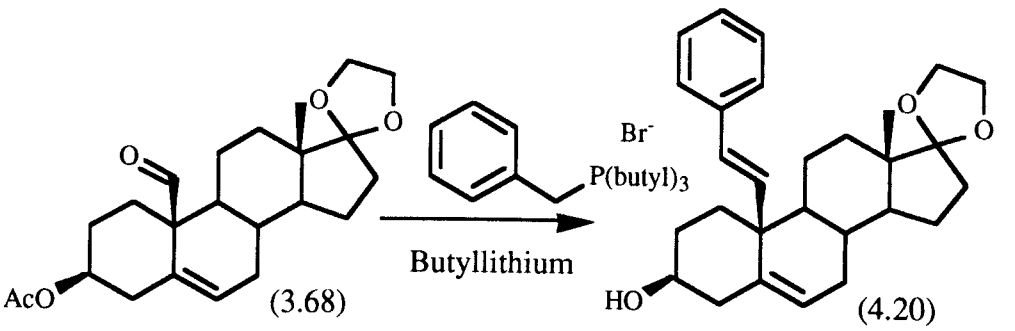
To a stirred solution of 3β-hydroxy-17,17-ethylenedioxyandrost-5-en-19-one 3-acetate (3.68), in dry tetrahydrofuran was slowly added excess phenyl lithium solution (in ether/hexane),[9] which reacted with both of the functionalities at position C-3 and C-19, to afford 3β,19-dihydroxy-17,17-ethylenedioxy-19-phenylandrost-5-ene (4.17). The same product was synthesised when the Grignard reagent of bromobenzene was used as the nucleophile.[11] During the course of the nucleophilic addition reaction at the 10β-formyl group, a C-19 benzylic hydroxy functionality was generated and the 3β-acetate functionality was removed by reaction with two equivalents of phenyl lithium or phenyl magnesium bromide to furnish a by-product of 1,1-diphenylethanol (4.18). In both reactions the mixtures were purified by silica gel preparative thin layer chromatography (acetonitrile/ dichloromethane) and identified by their spectra. The ^{1}H NMR (table 4.31) indicated a shift in the position of the C-19 hydrogen from the aldehydic position from 9.63δ to 4.95δ. This was accompanied by a 5 hydrogen multiplet in the region of 7.20δ -7.43δ corresponding to the aromatic hydrogens and the disappearance of the acetate methyl signal from 2.01δ. The ^{13}C NMR (table 4.32) indicated the presence of 25 chemically different signals and a shift of the C-19 aldehydic signal from 204.75δ to 75.75δ, with the appearance of 4 new signals in the aromatic region. The infrared spectrum provided further evidence for the structure with the appearance of a free O-H stretch at 3420 cm^{-1}. CI-MS(NH_4^+) exhibited signals at 425 and 99 corresponding to the (MH^+) ion and ketal ion fragment. This was accompanied by a peak at 107 corresponding to the fragmentation of the 10 benzyl functionality which forms a substituted tropylium ion fragment.

Table 4.31
250 MHz ^{1}H NMR of 3β,19-dihydroxy-17,17-ethylenedioxy-19-phenylandrost-5-ene (4.17).

multiplicity	chemical shift	coupling constant	assignment
5 H (m)	7.43 - 7.20	1.36 Hz	aromatic hydrogens
1 H (t)	5.55	2.00 Hz	C-6 H
1 H (s)	4.95		C-19 H
4 H (m)	3.90-3.71		ketal H's
1 H (t,t)	3.56	5.22 Hz, 10.44 Hz	C-3α H
3 H (s)	0.78		C-18 Me
methylene envelope	2.69 - 0.80		

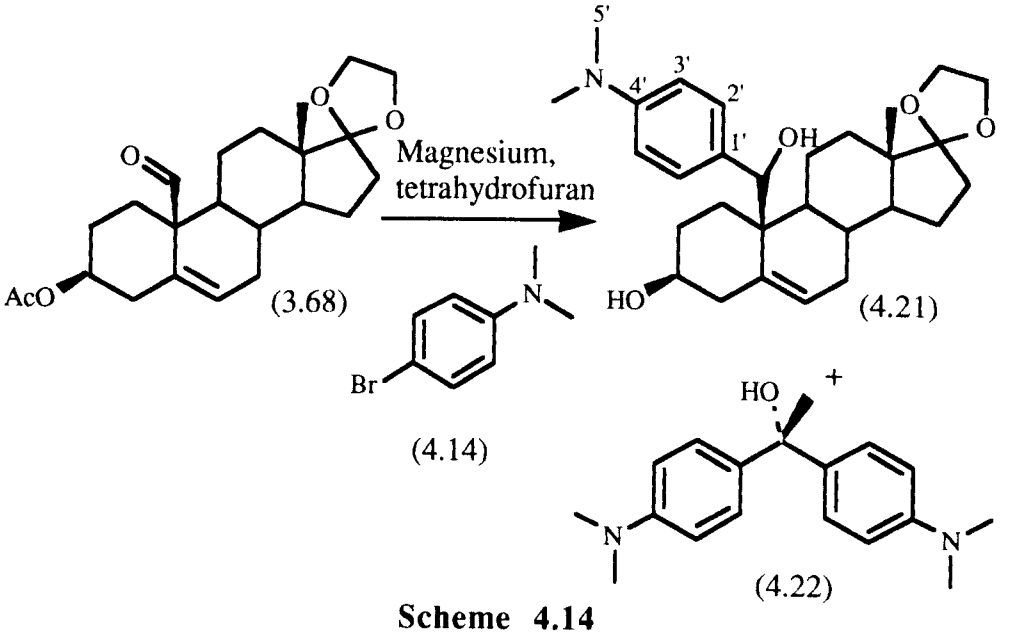
Table 4.32
250 MHz ^{13}C NMR of 3β,19-dihydroxy-17,17-ethylenedioxy-19-phenylandrost-5-ene (4.17).

chemical shift	assignment
143.45	4'/5
137.55	4'/5
127.60	2'/3'
127.43	2'/3'
127.11	1'/6
126.56	1'/6
119.23	17
75.75	(19)
71.00	(3)
65.03	Ketal
64.44	Ketal
51.96	9/14
51.83	9/14
45.73	
45.03	
43.52	
34.02	
33.48	
32.45	
32.01	
31.12	
31.01	
22.42	15(11)
20.98	11(15)
14.53	18



Scheme 4.13

The researchers at Syntex were able to introduce a C-19 methylene group by a Wittig reaction with 3β-hydroxy-17,17-ethylenedioxy androstan-5-ene-19-one 3-acetate (3.68).[7] In line with this strategy we attempted to introduce a benzyl group at position C-19 via a Wittig reaction between benzyltributylphosphonium bromide and 3β-hydroxy-17,17-ethylenedioxy androstan-5-en-19-one 3-acetate (3.68). Unfortunately the product 3β-hydroxy-17,17-ethylenedioxy-19-benzylandrostan-5,19-diene (4.20) could not be isolated from the reaction mixture. It is considered that steric hindrance between the large Wittig reagent and the hindered C-19 aldehydic site prevented the reaction from occurring.



Scheme 4.14

Having successfully introduced a phenyl group at the hindered C-19 neopentyl site attempts were made to introduce a substituted aryl functionality. p-Bromo-N,N-dimethylaniline (4.14) was converted by reaction with magnesium turnings in tetrahydrofuran to the corresponding Grignard reagent.[11] To this stirred solution was added 3β-hydroxy-17,17-ethylenedioxyandrost-5-en-19-one 3-acetate (3.68) in tetrahydrofuran, to give a diastereomeric mixture of 3β,19-dihydroxy-17,17-ethylenedioxy-19-(p-(N,N-dimethylaminophenyl))androst-5-ene (4.21). During the course of the reaction nucleophilic addition of the p-N,N-dimethylaminophenyl substituent occurred at the 10β-formyl group generating a C-19 benzylic hydroxy functionality and the 3β-acetate functionality was removed by nucleophilic attack by two equivalents of Grignard reagent, to give a by-product of 1,1-di(p-(N,N-dimethylaminophenyl)ethanol (4.22). The crude product was dissolved in a minimum of dichloromethane and treated with hexane, this reduction in polarity of solvent caused the steroid to come out of solution as an oil, leaving the by-product in solution. The product was further purified by silica gel preparative thin layer chromatography (acetonitrile/ dichloromethane) and the major and minor diastereomeric components were identified by their spectra. The ^{1}H NMR (table 4.33) indicated the presence of two doublets, each integrating to two hydrogens at 7.23δ and 6.65δ with a vicinal coupling constant of 8.84 Hz, corresponding to the p-substituted aromatic system. This was accompanied by the shift of the aldehydic proton signal to 4.30δ. The ^{13}C NMR (table 4.35) indicated the presence of 26 chemically different carbon signals, with a shift of the aldehydic carbon signal from 204.75δ to 75.75δ. The infrared spectrum provided further evidence for the structure with a free O-H stretch at 3605 cm^{-1}. FAB-MS exhibited signals at 468, 150 and 99 corresponding to the (MH^{+}) ion, substituted tropylium ion fragment and ketal ion fragment. The ratio of the

major to minor diastereomers synthesised in this reaction was estimated by comparison of the ^1H NMR signal integrals of the hydrogens at C-6, C-18 and C-19 and calculated to be 78% to 22%.

Table 4.33
400 MHz ^1H NMR 3β,19-dihydroxy-17,17-ethylenedioxy-19-(p-(N,N-dimethylaminophenyl))androst-5-ene (4.21).

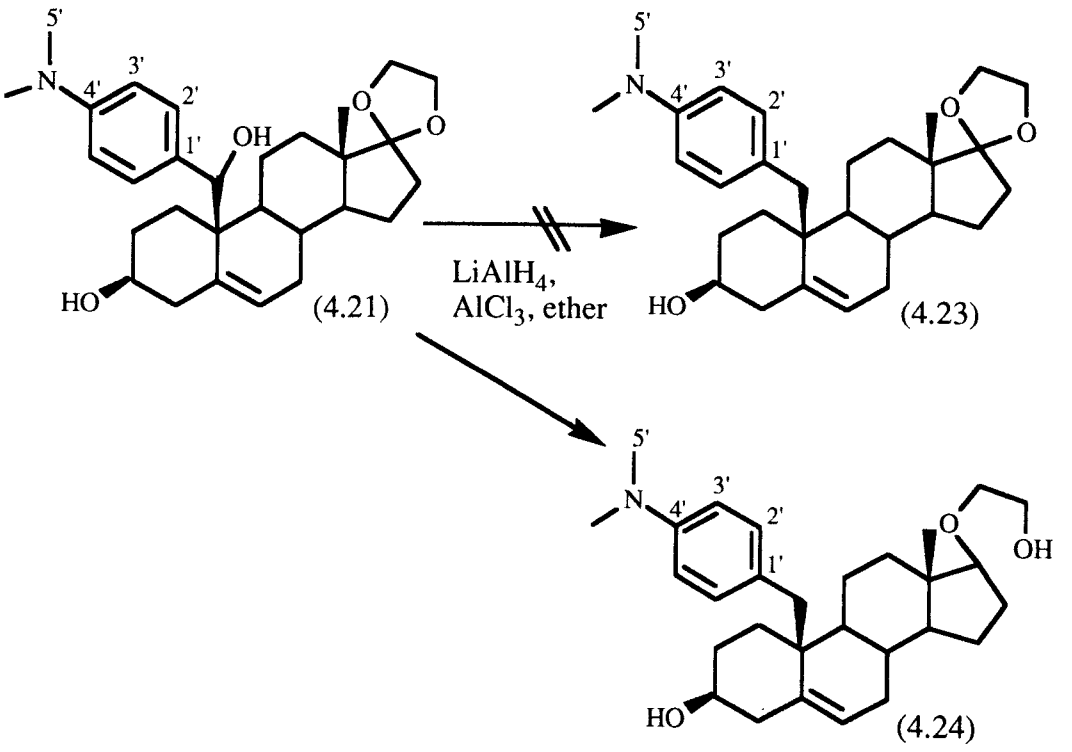
multiplicity	chemical shift	coupling constant	assignment
2 H (d) major	7.23	8.76 Hz	aromatic 2' H
(minor)	(7.20)	(8.68 Hz)	
2 H (d) major	6.65	8.88 Hz	aromatic 3' H
(minor)	(6.66)	(8.88 Hz)	
1 H (m)	5.52 - 5.56		C-6 H
1 H (s) major	4.89,		C 19 H
(minor)	(5.02)		
4 H (m)	4.00 - 3.75		ketal H's
1 H (t,t)	3.55	5.20 Hz, 10.40 Hz	C-3α H
6 H (s) major	2.91		5'
(minor)	(2.89)		
3 H (s)	0.76		C-18 Me
methylene envelope	0.80 - 2.60		

Table 4.34
400 MHz ^{13}C NMR signals in diastereomeric mixture attributed to the minor isomer of 3β,19-dihydroxy-17,17-ethylenedioxy-19-(p-(N,N-dimethyl aminophenyl))androst-5-ene (4.21).

chemical shift	assignment	dept
149.5	4'	(C)
136.6	5	(C)
127.5	2'	CH
126.7	6	CH
112.2	3'	CH
72.0	19	CH
71.3	3	CH
52.2		CH
45.1		
43.4		
40.4		
34.4		
31.5		
31.4		
31.3		
30.8		
22.2		
22.0		
14.6		

Table 4.35
400 MHz ^{13}C NMR 3β,19-dihydroxy-17,17-ethylenedioxy-19-(p-(N,N-dimethylaminophenyl))androst-5-ene (4.21). (major isomer)

chemical shift	assignment	dept
149.68	4'	(C)
137.93	5	(C)
131.65	1'	(C)
128.21	2'	CH
125.99	6	CH
119.30	17	C
111.97	3'	CH
75.97	19	CH
71.09	3	CH
64.99	Ketal	CH_2
64.41	Ketal	CH_2
52.13	9/14	CH
51.97	9/14	CH
45.78	10/13	C
44.77	10/13	C
43.74		CH_2
40.63	5'	CH_3
34.03		CH_2
33.96		CH_2
32.63		CH_2
31.90	8	CH
31.25		CH_2
31.03		CH_2
22.42	15(11)	CH_2
20.97	11(15)	CH_2
14.57	18	CH_3



Scheme 4.15

Following the successful introduction of the substituted and unsubstituted aryl functionalities into the C-19 position, it was necessary to find a suitable method to remove the C-19 alcohol.

Alternative methods to do this were considered.

a) Oxidation of the 19-hydroxy functionality to the ketone, followed by a Wolf Kishner (Huang Minlon modification) reduction to the alkane.

b) Benzylic reduction.[12]

The conditions for oxidation of the C-19 hydroxy group would also oxidise the 3β-alcohol. Protection of the 3β-hydroxy functionality and a later deprotection would increase the length of the synthetic sequence by two steps. It was therefore considered that the second method of benzylic reduction was most suitable.

The benzylic reduction[11] of 3β,19-dihydroxy-17,17-ethylenedioxy-19-(p-N,N-dimethylaminophenyl)androst-5-ene (4.21) was then examined, by reflux with lithium aluminium hydride and aluminium chloride in dry ether. The product was purified by silica gel preparative thin layer chromatography (acetonitrile/dichloromethane) and identified by its spectra. The reduction of the benzylic C-19 hydroxy functionality occurred smoothly, but unfortunately there was an unexpected cleavage of the C-17 ketal protecting group as well, affording the product of 3β-hydroxy-17-(2-hydroxyethoxy)-19-(p-N,N-dimethyl aminophenyl)androst-5-ene (4.24). The ^{1}H NMR spectra (table 4.36) indicated the loss of the C-19 1 hydrogen singlet from 4.89δ and the appearance of two geminally coupled C-19 1 hydrogen doublets at 2.90δ and 2.37δ with a ^{2}J coupling constant of 14.41 Hz. This was accompanied by a change in the pattern observed for the ketal multiplet with a shift in the position to 3.72-3.34δ. This was accompanied by a new 1 hydrogen triplet at 3.24δ. The ^{13}C NMR spectra (table 4.37) indicated the presence of 26 chemically different signals with a shift in the position of the C-19 signal from 75.97δ to 36.66δ. This was accompanied by the loss of the C-17 and ketal signals from 119.30δ, 64.99δ and 64.41δ. EI-MS exhibited a peak at 453 and 134 corresponding to the molecular ion and substituted tropylium ion fragment. CI-MS exhibited signals at 454 and 134 corresponding to the (MH^{+}) ion and substituted tropylium ion fragment. The tropylium ion fragment possess a mass that is 16 less than the corresponding fragment in the precursor due to the loss of an hydroxy functionality in the reaction. There was no evidence for the signal at 99 for the ketal ion fragment.

The ketal functionality is normally chemically resistant to lithium aluminium hydride or aluminium chloride. The combination of these two reagents was however able to bring about the partial cleavage of the C-17 ketal protecting group furnishing 3β-hydroxy-17-(2-hydroxyethoxy)-19-(p-

(N,N-dimethylaminophenyl))androst-5-ene (4.24). The cleavage can be explained in the following manor. During the course of the reaction, oxonium ions are formed when a lone pair of electrons from an oxygen atom is accepted by a vacant orbital in an aluminium species. In the ketal functionality this will raise the electrophilic nature of the C-17 carbon atom A new oxonium ion is formed when a lone pair of electrons from the second oxygen atom attacks the electrophilic C-17 carbon atom, which causes the heterolytic cleavage of the bond that exists between the C-17 carbon atom and the original oxonium ion. The new oxonium ion is then reduced by lithium aluminium hydride to afford 3β-hydroxy-17-(2-hydroxyethoxy)-19-(p-(N,N-dimethylaminophenyl))androst-5-ene (4.24).

Table 4.36
250 MHz ^{1}H NMR 3β-hydroxy-17-(2-hydroxyethoxy)-19-(p-(N,N-dimethylaminophenyl))androst-5-ene (4.24).

multiplicity	chemical shift	coupling constant	assignment
2 H (d)	7.01	8.65 Hz	aromatic 2' H
2 H (d)	6.60	8.70 Hz	aromatic 3' H
1 H (m)	5.53 - 5.27		C-6 H
5 H (m)	3.72 - 3.42		ethoxy H's + C-3α H
1 H (t)	3.24	8.35 Hz	C-17 H
1 H (d)	2.90	14.47 Hz	C-19 H
6 H (s)	2.85		C-5' Me
1 H (d)	2.37	14.35 Hz	C-19 H
3 H (s)	0.50		C-18 Me
methylene envelope	0.80 - 2.40		

Table 4.37
250 MHz ^{13}C NMR 3β,19-dihydroxy-17-(2-hydroxyethoxy)-19-(p-(N,N-dimethylaminophenyl))androst-5-ene (4.24).

chemical shift	assignment
148.56	4'
137.94	5
130.78	2'
129.23	1'
124.54	6
113.05	3'
89.23	
71.63	
70.85	
61.89	
52.86	
51.81	
42.84	
42.78	
41.23	5'
41.06	
38.70	
38.37	
36.66	19
31.64	
31.26	
30.93	
27.80	
22.84	15(11)
20.99	11(15)
11.74	18

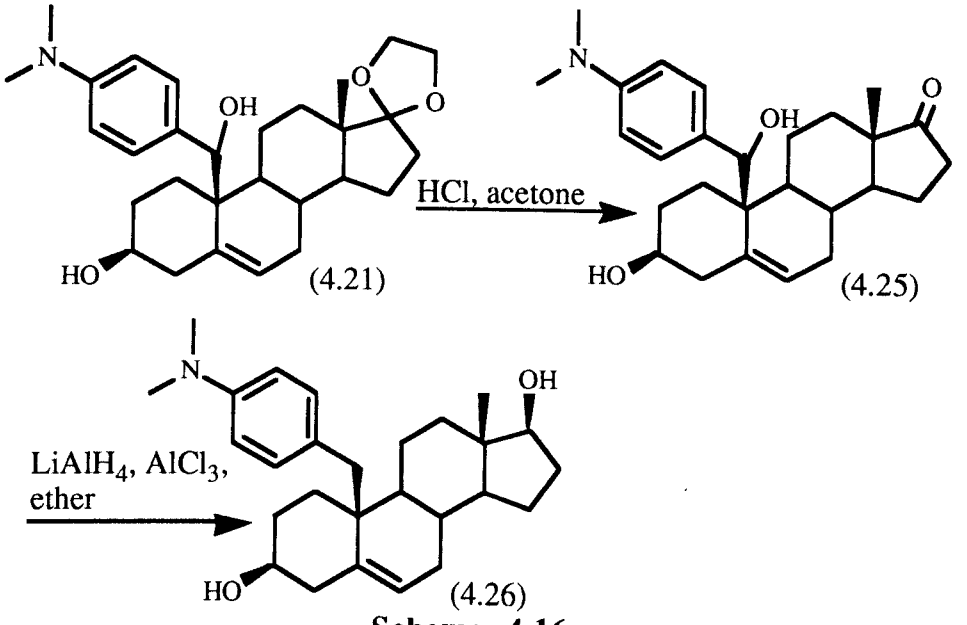
An alternative route had to be found to overcome this problem. The options at this point were:

a) The use of an alternative protecting group for the C-17 ketone functionality.

b) Alter the sequence of events of manipulation of the C-3, C-17 and C-19 positions.

The option to use a different protecting group was not considered to be an attractive proposition, as it would mean its introduction at a much earlier step in the synthetic sequence The alternative idea, of rearranging the sequence of chemical modifications of positions C-3, C-17, C-19 was

considered to be most suitable for our purpose. The next logical step was to deprotect the C-17 functionality of 3β-hydroxy-17,17-ethylenedioxy-19-(p-N,N-dimethylaminophenyl)androst-5-ene (4.21) before manipulating the C-19 group.



(4.21) (4.25) (4.26)

Scheme 4.16

To a stirred solution of 3β,19-dihydroxy-17,17-ethylenedioxy-19-(p-(N,N-dimethylaminophenyl))androst-5-ene (4.21) in acetone was added aqueous hydrochloric acid,[11] to cause deketalisation and to furnish 3β,19-dihydroxy-19-(p-(N,N-dimethylaminophenyl))androst-5-en-17-one (4.25), which was purified by silica gel preparative thin layer chromatography and identified by its spectra. The ^{1}H NMR (table 4.38) indicated the disappearance of the 4 H ketal multiplet from 4.00δ - 3.75δ. The ^{13}C NMR (table 4.39) indicated the shift of the C-17 signal from 119.30δ to the carbonyl position at 201.71δ and the loss of the ketal signals from 64.99δ and 64.41δ. The infrared spectrum exhibited an absorption band at 1600 cm^{-1} corresponding to the newly formed C-17 carbonyl group. The molecular ion, (MH^+) and (MNH_4^+) fragments were not stable in EI-MS, CI-MS(NH_4^+), or FAB-MS. EI-MS did however exhibited signals at 405 and 150 corresponding to molecular ion -18 and substituted tropylium ion fragments.

Table 4.38
400 MHz ^{1}H NMR of 3β,19-dihydroxy-19-(p-(N,N-dimethylamino phenyl))androst-5-en-17-one (4.25).

multiplicity	chemical shift	coupling constant	assignment
2 H (d) major	7.19	8.93 Hz	aromatic 2' H
2 H (d) major	6.62	8.93 Hz	aromatic 3' H
1 H (m)	5.60 -5.50		C-6 H
1 H (s) major	4.72		C 19
(minor)	(4.91)		
1 H (t,t)	3.60		C-3α H
6 H (s) major	2.87		5'
(minor)	(2.83)		
3 H (s) major	0.72		C-18 Me
(minor)	(0.60)		
methylene envelope	0.90 - 2.70		

Table 4.39
400 MHz ^{13}C NMR of 3β,19-dihydroxy-19-(p-(N,N-dimethylamino phenyl))androst-5-en-17-one (4.25). (major isomer)

chemical shift	assignment
201.71	17
150.42	4'
135.74	5
130.29	2'
129.62	1'
120.85	6
112.34	3'
81.78	19
66.77	3
53.34	
52.03	
47.82	
47.42	
40.79	5'
39.43	
35.71	
35.47	
31.82	
30.97	
29.59	
26.85	
21.43	15/(11)
19.90	11/(15)
13.17	18

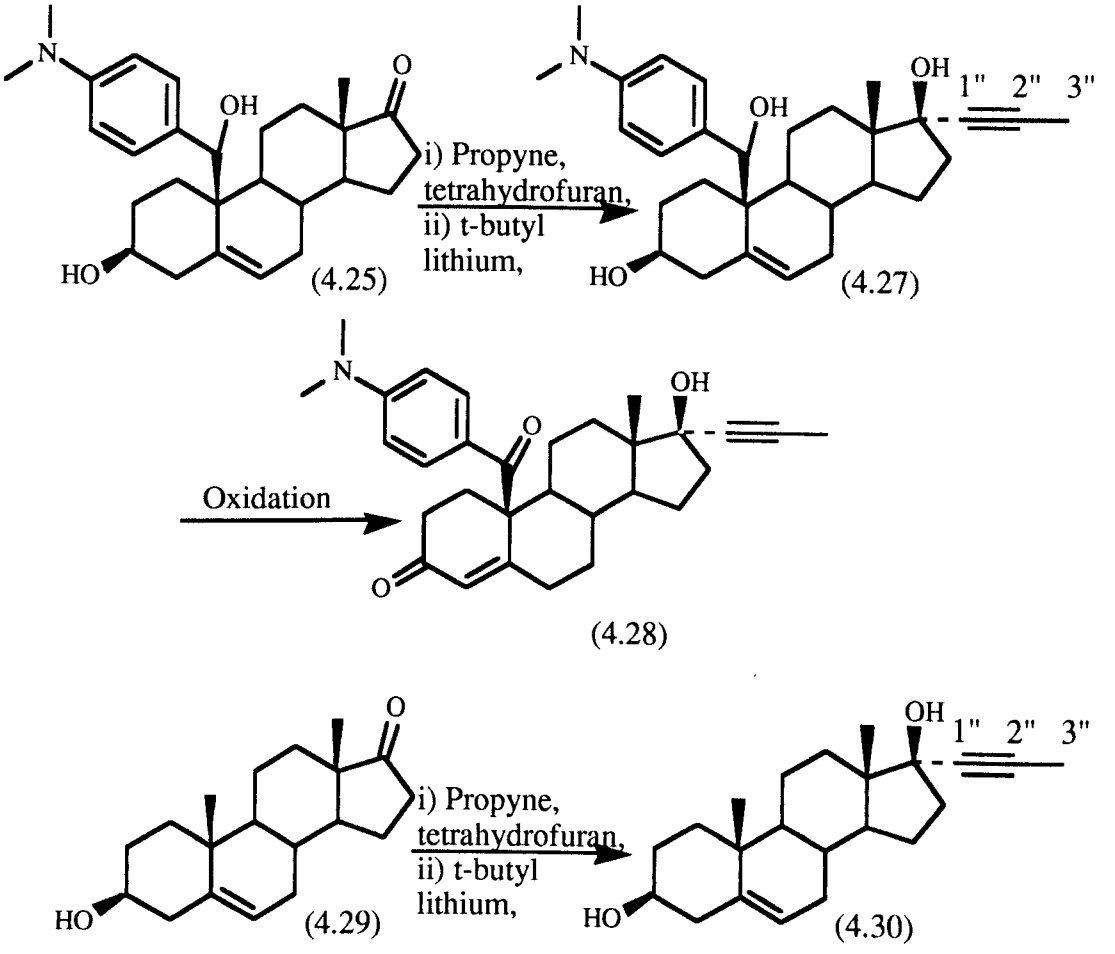
Since the conditions for benzylic[12] reduction of the C-19 benzylic alcohol involved reflux with lithium aluminium hydride and aluminium chloride in ether, the benzylic reduction would also reduce the C-17 keto group to a 17β-hydroxy functionality. In accordance with the literature[12] method reported for the benzylic reduction of alcohols, lithium aluminium hydride was refluxed with aluminium chloride in dry ether for 15 minutes. To this refluxing solution was slowly added 3β,19-dihydroxy-19-(p-(N,N-dimethylaminophenyl))androst-5-en-17-one (4.25) in dry ether solution, to afford 3β,17β-dihydroxy-19-(p-(N,N-dimethylaminophenyl))androst-5-ene (4.26), which was purified by recrystallisation from acetone and identified by its spectra. The ^{1}H NMR (table 4.40) indicated the loss of the C-19 H signal from 4.72δ and the appearance of the C-19 methylene group as two 1 hydrogen doublets at 3.12δ and 2.40δ with a geminal coupling constant of 14.38 Hz. The ^{13}C NMR (table 4.41) was completely assigned (except for the 2 quaternary carbons) by means of C-H correlation, COSY spectra and DEPT spectra. The ^{13}C NMR (table 4.41) indicated the shift of the C-19 signal from 81.78δ to 36.78δ. The infrared spectrum provided further evidence for the structure with two free O-H stretches at 3603 cm^{-1} and 3435 cm^{-1}. CI-MS(NH_4^+) showed signals at 427, 410 and 134 which correspond to the (MNH_4^+), (MH^+) ions and substituted tropylium ion fragment.

Table 4.40
400 MHz ^{1}H NMR of 3β,17β-dihydroxy-19-(p-(N,N-dimethylamino phenyl))androst-5-ene (4.26).

multiplicity	chemical shift	coupling constant	assignment
2 H (d)	7.03	8.64 Hz	aromatic 2' H
2 H (d)	6.62	8.64 Hz	aromatic 3' H
1 H (t)	5.54	2.40 Hz	C-6 H
1 H (t,t)	3.60	5.35 Hz, 10.70 Hz	C-3α H
1 H (t)	3.53	8.48 Hz,	C-17α H
1 H (d)	3.12	14.44 Hz	C-19 H
6 H (s)	2.87		C-5'
1 H (d)	2.40	14.32 Hz	C-19 H
3 H (s)	0.48		C-18 methyl
methylene envelope	2.08-0.53		

Table 4.41
400 MHz ^{13}C NMR of 3β,17β-dihydroxy-19-(p-(N,N-dimethylamino phenyl))androst-5-ene (4.26).

chemical shift	assignment	dept
148.83	4'	(C)
137.90	5	C
130.77	2'	CH
128.65	1'	C
124.65	6	CH
112.88	3'	CH
81.69	17	CH
71.82	3	CH
52.65	14	CH
51.96	9	CH
42.93	4	CH_2
42.64	10/13	C
41.08	10/13	C
40.94	5'	CH_3
38.79	1	CH_2
37.03	12	CH_2
36.78	19	CH_2
31.75	2	CH_2
31.49	8	CH
30.96	7	CH_2
30.31	16	CH_2
22.22	15	CH_2
20.95	11	CH_2
11.10	18	CH_3



Scheme 4.17

The next logical step in the reaction sequence was therefore the introduction of the 17α-alkynyl substituent (scheme 4.17). Initially this reaction was assessed in the model compound 3β-hydroxyandrost-5-en-17-one (4.29). To a solution of tetrahydrofuran saturated with propyne at 0°C under nitrogen, was added a solution of t-butyllithium in hexane and this was allowed to stir for 30 minutes.[11] A solution of 3β,17β-dihydroxyandrost-5-en-17-one (4.29) in tetrahydrofuran was added to this solution, to give 3β-dihydroxy-17α-(prop-1-yne)androst-5-ene (4.30), a portion of which was purified by silica gel thin layer chromatography (acetonitrile/ dichloromethane) and identified by its spectra. The ^{1}H NMR (table 4.42) exhibited a new 3 hydrogen singlet at 1.77δ corresponding to the propynyl

methyl group. The ^{13}C NMR (table 4.43) indicated the disappearance of the C-17 signal from 221.14δ. This was accompanied by the appearance of 4 new signals at 79.99δ, 71.67δ, 71.50δ and 3.60δ. The signal at 3.60δ corresponding to the propynyl methyl group and the remaining 3 signals for the C-17 and the two alkynyl carbon atoms. EI-MS exhibited a peak at 328 corresponding to the molecular ion.

Table 4.42
250 MHz ^1H NMR of 3β,17β-dihydroxy-17α-(prop-1-yne)androst-5-ene (4.30)

multiplicity	chemical shift	coupling constant	assignment
1 H (m)	5.30 - 5.24		C-6 H
1 H (t,t)	3.42	4.26 Hz, 11.50 Hz	C-3α H
3 H (s)	1.77		C-3" methyl
3 H (s)	0.93		C-19 methyl
3 H (s)	0.75		C-18 methyl
methylene envelope	0.80 - 2.50		

Table 4.43
250 MHz ^1H NMR of 3β,17β-dihydroxy-17α-(prop-1-yne)androst-5-ene (4.30)

chemical shift	assignment
140.69	5
121.29	6
82.67	3/1"/2"
81.55	3/1"/2"
79.99	3/1"/2"
71.67	
71.50	
50.53	
49.69	
46.54	
42.17	
38.91	
37.17	
36.61	
32.45	
31.54	
31.33	
23.11	15(11)
20.68	11(15)
19.32	19
12.65	18
3.60	3"

To a solution of tetrahydrofuran saturated with propyne at 0°C under nitrogen was added a solution of t-butyllithium in hexane and this was stirred for 30 minutes.[11] A solution of 3β,19-dihydroxy-19-(p-(N,N-dimethyl aminophenyl))androst-5-en-17-one (4.25) in tetrahydrofuran was added to this solution to give 3β,17β,19-trihydroxy-17α-(prop-1-yne)-19-(p-(N,N-dimethylaminophenyl))androst-5-ene (4.27) a portion of which was purified by silica gel thin layer chromatography (acetonitrile/ dichloromethane) and identified by its spectra. The ^{1}H NMR (table 4.44) indicated appearance of a new 3 hydrogen singlet at 1.85δ corresponding to the propynyl methyl group. The ^{13}C NMR (table 4.45) indicated the disappearance of the C-17 signal from 201.71δ. This was accompanied by the appearance of 4 new signals at 82.86δ, 81.23δ, 79.69δ and 3.59δ. The signal at 3.59δ corresponding to the propynyl methyl group and the remaining 3 signals for the C-17 and the two alkynyl carbon atoms. The infrared spectrum showed absorption bands at 3436 cm^{-1} and 3602 cm^{-1} corresponding to free OH stretches. EI-MS exhibited peaks at 463 and 150 corresponding to the molecular ion and a substituted tropylium ion. CI-MS(NH_4^+) exhibited peaks at 464 and 150 corresponding to (MH^+) ion and substituted tropylium ion fragment.

Table 4.44
250 MHz ^{1}H NMR of 3β,17β,19-trihydroxy-17α-(prop-1-yne)-19-(p-(N,N-dimethylaminophenyl))androst-5-ene (4.27)

multiplicity	chemical shift	coupling constant	assignment
2 H (d)	7.24	8.88 Hz	aromatic 2' H
2 H (d)	6.67	8.73 Hz	aromatic 3' H
1 H (m)	5.63 - 5.58		C-6 H
1 H (s)	4.85		C 19
1 H (m)	3.56 - 3.15		C-3α H
6 H (s)	2.93		C-5' H
3 H (s)	1.84		C-3" methyl
3 H (s)	0.75		C-18 methyl
methylene envelope	0.65 - 2.50		

Table 4.45
250 MHz ^{13}C NMR of 3β,17β,19-trihydroxy-17α-(prop-1-yne)-19-(p-(N,N-dimethylaminophenyl))androst-5-ene (4.27). (some of the peaks for major isomer)

chemical shift	assignment
149.68	4'
138.10	5
131.66	1'
128.25	2'
125.88	6
111.99	3'
82.86	17/1"/2"
81.23	17/1"/2"
79.69	17/1"/2"
76.11	19
71.14	
60.31	
52.17	9/14
51.85	9/14
22.83	15(11)
20.92	11(15)
14.07	18
3.59	3"

A number of attempts were made to oxidise 3β,17β,19-trihydroxy-17α-(prop-1-yne)-19-(p-(N,N-dimethylaminophenyl))androst-5-ene (4.27) using Jones reagent[5] and chromium trioxide/pyridine.[17,13,14] In none of these attempts were we able to isolate the desired product. CI-MS(NH_4^+) of the crude products showed no evidence for the (MH^+) or (MNH^{4+}) ions but peaks at 446 and 150 were observed. The ^1H NMR of the crude product exhibited a pair of doublets at 7.71δ and 6.75δ with a ^3J coupling constant of 8.74 Hz, which correlates with the calculated position of the aromatic signals expected for 3β,17β-dihydroxy-17α-(prop-1-yne)-19-(p-(N,N-dimethylaminophenyl))androst-4-en-3,19-dione (4.28).

The next logical step was to do a benzylic reduction on the 19-hydroxy steroid (4.27) (scheme 4.18). In accordance with the general literature method reported for benzylic reduction of alcohols,[12] lithium aluminium hydride was refluxed with aluminium chloride in dry ether for 15 minutes. To this refluxing solution was slowly added 3β,17β,19-trihydroxy-17α-(prop-1-yne)-19-(p-(N,N-dimethyl aminophenyl))androst-5-ene (4.27) in ether solution, to afford 3β,17β-dihydroxy-17α-(prop-1-yne)-19-(p-(N,N-dimethylamino phenyl))androst-5-ene (4.31), which was purified by silica gel thin layer chromatography (acetonitrile/dichloromethane) and identified by its spectra. The ^1H NMR (table 4.46) indicated the loss of the C-19 H signal from 4.85δ and the appearance of the C-19 methylene group as two 1 hydrogen doublets at 3.13δ and 2.41δ with a geminal coupling constant of 14.3 Hz. The ^{13}C NMR (table 4.47) indicated the disappearance of the C-19 signal from 76.11δ. The infrared spectrum provided further evidence for the structure with a free O-H stretch at 3604 cm^{-1} and 3444 cm^{-1}. EI-MS exhibited peaks at 447 and 134 corresponding to the molecular ion and substituted tropylium ion fragments. CI-MS(NH_4^+) exhibited peaks at 448 and 134 corresponding to (MH^+) ion and substituted tropylium ion

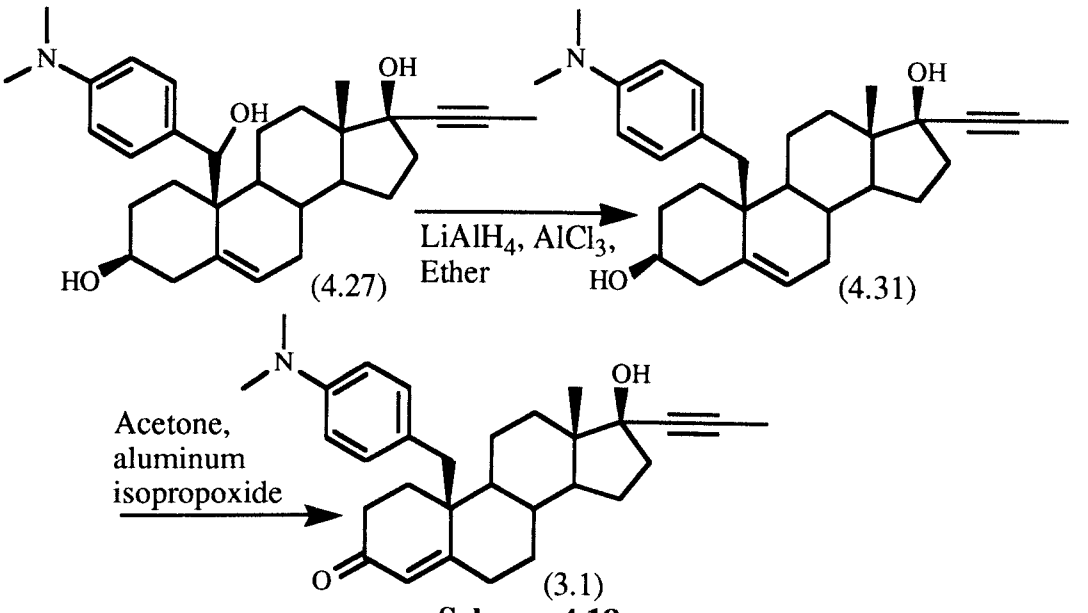
fragments.

Table 4.46
400 MHz ^{1}H NMR 3β,17β-dihydroxy-17α-(prop-1-yne)-19-(p-(N,N-dimethylaminophenyl))androst-5-ene (4.31).

multiplicity	chemical shift	coupling constant	assignment
2 H (d)	7.06	8.52 Hz	aromatic 2' H
2 H (d)	6.71	8.04 Hz	aromatic 3' H
1 H (t)	5.55	2.18 Hz	C-6 H
1 H (t,t)	3.59	5.20 Hz, 10.40 Hz	C-3α H
1 H (d)	3.13	14.4 Hz	C-19 H
6 H (s)	2.89		C-5' methyl
1 H (d)	2.41	14.2 Hz	C-19 H
3 H (s)	1.81		C-3" methyl
3 H (s)	0.54		C-18 methyl
methylene envelope	2.41 - 0.69		

Table 4.47
400 MHz ^{13}C NMR of 3β,17β-dihydroxy-17α-(prop-1-yne)-19-(p-(N,N-dimethylaminophenyl))androst-5-ene (4.31).

chemical shift	assignment	dept
148.71	4'	C
137.81	5	C
130.57	2'	CH
128.69	1'	C
124.35	6	CH
112.69	3'	CH
82.81	17/1"/2"	C
80.99	17/1"/2"	C
79.57	17/1"/2"	C
71.50	3	CH
51.79	9/14	CH
51.26	9/14	CH
46.45		C
42.66	4	CH_2
40.88	3"	CH_3
40.85		
40.67		
38.62		CH_2
36.47	(19)	CH_2
33.02		CH_2
31.82	8	CH
31.47		CH_2
30.72		CH_2
22.47	15(11)	CH_2
20.80	11(15)	CH_2
12.84	18	CH_3
3.44	3"	CH_3



Scheme 4.18

Oppenauer oxidation[15] of 3β,17β-dihydroxy-17α-(prop-1-yne)-19-(p-N,N-dimethylaminophenyl)androst-5-ene (4.31) by reflux with aluminium isopropoxide in dry acetone gave 17β-hydroxy-17α-(prop-1-yne)-19-(p-(N,N-dimethylaminophenyl))androst-4-ene-3-one (3.1), which was purified by silica gel preparative thin layer chromatography (acetonitrile/ dichloromethane) and identified by its spectra. The ^{1}H NMR (table 4.48) indicated the disappearance of the C-3 hydrogen triplet of triplets which was present in the precursor at 3.59δ. This was accompanied by the disappearance of the olefinic C-6 H triplet from 5.55δ and the appearance of the new olefinic C-4 H singlet at 5.85δ. The ^{13}C NMR (table 4.49) indicated the presence of 27 chemically different signals, with a shift in the C-3 signal from 71.50δ to 200.05δ, a shift in the C-5 signal from 137.81δ to 168.17δ, the loss of the C-6 signal from 124.35δ and the appearance of a new C-4 signal at ~ 125δ. EI-MS exhibited peaks at 445 and 134 which correspond to the molecular ion and substituted tropylium ion. CI-MS(NH_4^+) exhibited peaks at 446 and 134 which correspond to (MH^+) and substituted tropylium ion fragments.

Table 4.48
400 MHz ^{1}H NMR 17β-hydroxy-17α-(prop-1-yne)-19-(p-(N,N-dimethylaminophenyl))androst-4-en-3-one (3.1).

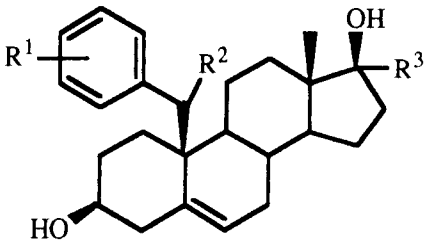
multiplicity	chemical shift	coupling constant	assignment
2 H (d)	6.93	8.64 Hz	aromatic 2' H
2 H (d)	6.55	8.64 Hz	aromatic 3' H
1 H (s)	5.85		C-4 H
1 H (d)	3.14	14.32 Hz	C-19 H
6 H (s)	2.87		C-5' methyl
1 H (d)	2.74	14.32 Hz	C-19 H
3 H (s)	1.85		C-3" methyl
3 H (s)	0.89		C-18 methyl
methylene envelope	2.5-1.0		

Table 4.49
400 MHz ^{13}C NMR 17β-hydroxy-17α-(prop-1-yne)-19-(p-(N,N-dimethyl aminophenyl))androst-4-en-3-one (3.1).

chemical shift	assignment
200.05	3
168.17	5
149.30	4'
129.93	2'
126.39	1'/4
125.57	1'/4
112.31	3'
81.82	17/1"/2"
79.68	17/1"/2"
77.10	17/1"/2"
56.29	9/14
50.21	9/14
46.57	10/13/16
43.30	10/13/16
40.40	5'
38.83	10/13/16
37.12	
36.00	
34.38	
33.29	
32.75	
31.96	
29.57	
22.96	15(11)
20.59	11(15)
12.76	18
3.58	3"

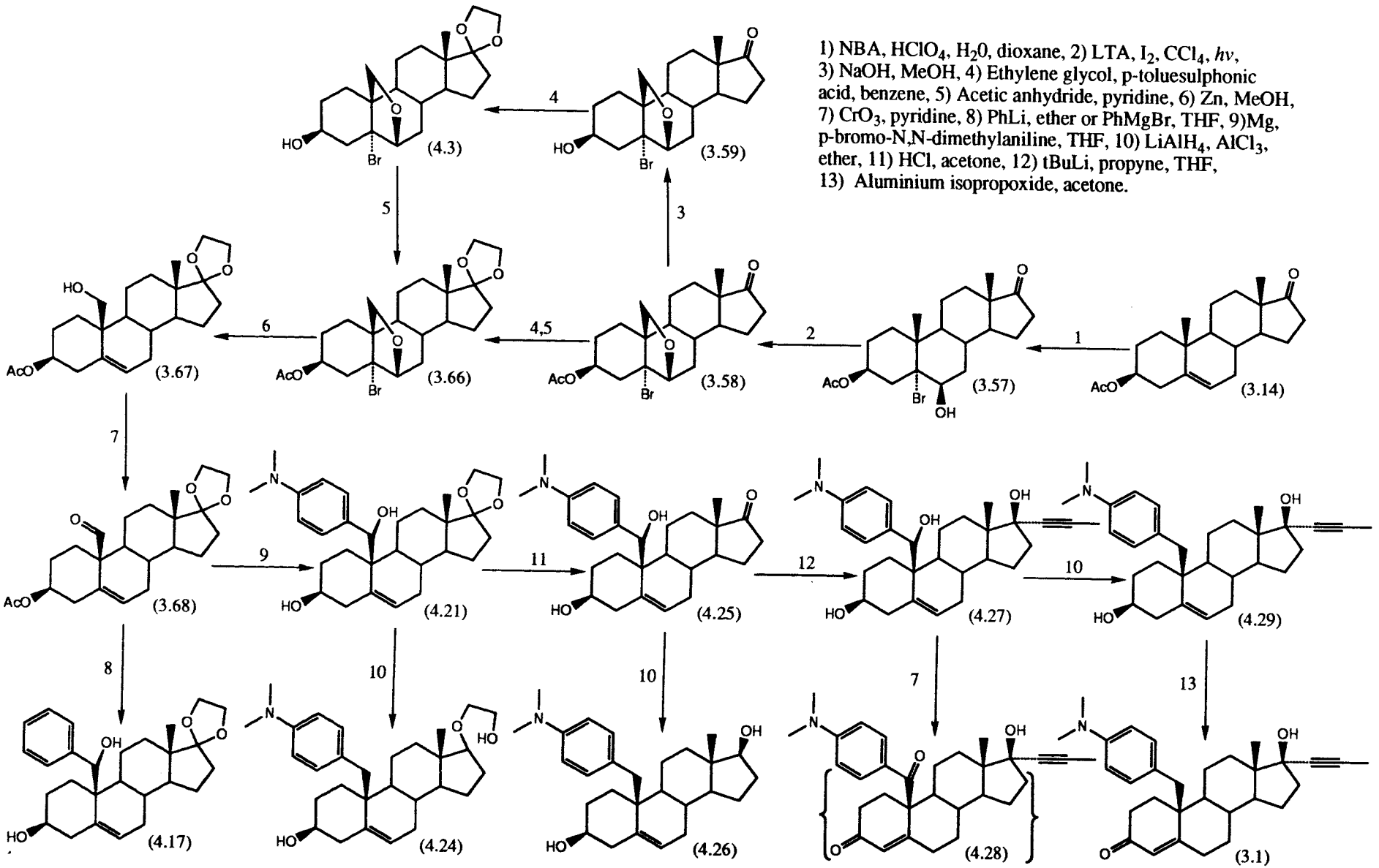
4.4 Conclusion and suggestions for further work.

The work described in this chapter has established a succesful 11 step route for the synthesis of 17β-hydroxy-17α-(prop-1-yne)-19-(p-(N,N-dimethylaminophenyl))androst-4-en-3-one (3.1) (scheme 4.19). It would be of interest to use the strategy established in this chapter to synthesise m-substituted and p-substituted analogues of 17β-hydroxy-17α-(prop-1-yne)-19-(p-(N,N-dimethylaminophenyl))androst-4-en-3-one (3.1) with a range of different substituents; (e.g. R^1= NMe_2, $COCH_3$); (. Also of interest would be the synthesis of analogues bearing different functionalities at C-19 (e.g. R^2= H, OH, =O) and at C-17 (e.g. R^3= H, Me, CCH, $CC\text{-}CH_3$)



R^1 = NMe2, COCH3
R^2 = H, OH, =O
R^3 = H, Me, Et,
C≡C–H,
C≡C–CH_3

Figure 4.1


1) NBA, HClO4, H20, dioxane, 2) LTA, I2, CCl4, hv,
3) NaOH, MeOH, 4) Ethylene glycol, p-toluesulphonic acid, benzene, 5) Acetic anhydride, pyridine, 6) Zn, MeOH,
7) CrO3, pyridine, 8) PhLi, ether or PhMgBr, THF, 9)Mg, p-bromo-N,N-dimethylaniline, THF, 10) LiAlH4, AlCl3, ether, 11) HCl, acetone, 12) tBuLi, propyne, THF,
13) Aluminium isopropoxide, acetone.
(4.3)
(3.59)
(3.67)
(3.66)
(3.58)
(3.57)
(3.14)
(3.68)
(4.21)
(4.25)
(4.27)
(4.29)
(4.17)
(4.24)
(4.26)
(4.28)
(3.1)

Scheme 4.19

4.5 References.

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Chapter 5

Molecular Modelling + Receptor Specificity.

5.1 Introduction

Receptor binding arises due to the complementary nature of the steric and electronic architecture of a ligand and its receptor.[1] Molecular modelling can be used as a tool to compare the similarities that exist between those molecules which exhibit a good relative binding affinity (RBA) for the progesterone and glucocorticoid receptors.[2,3] In this way the features of compounds that are conducive to receptor binding and antagonism can be assessed.[2,3]

5.2 Receptor Mapping.

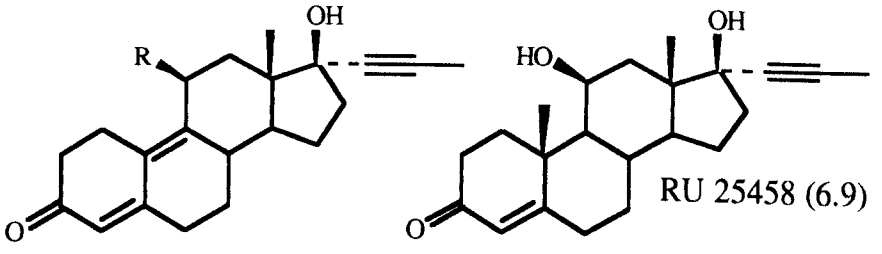
Whilst investigating the effects of various 11β substituents on the RBA of steroids for their receptors, the researchers at Roussel-Uclaf also embarked upon mapping of the progesterone and glucocorticoid receptor sites.[2-4] A number of 11β p-substituted phenyl steroids were synthesised (table 5.1), in order to probe the depth and width of the hydrophobic pocket.[2,3] The researchers used the information about compounds (5.1) - (5.7) (table 5.1) to generate a representation of the hydrophobic pocket by overlapping the 11β-functionalities of steroids using the 'Script' molecular modelling program.[2-4] The minimum size of the pocket above the C-ring is represented for the glucocorticoid receptor (fig. 5.3a) and the progesterone receptor (fig. 5.3b).[2-4]

The p-phenoxy steroid (5.8) exhibits a good RBA for the two receptors (table 5.1) and when free rotation is allowed for about the C-O in this compound, the 'Script' program was reported to generate a much larger pocket for the glucocorticoid (fig. 5.3c)[2] and progesterone (fig 5.3d)[2] receptor sites than that shown in the representations (fig. 5.3a and 5.3b),

although it is unlikely that all of the rotamers are accommodated by the hydrophobic pocket.

Table 5.1 Relative binding affinity (RBA) for rat thymic glucocorticoid receptor and rabbit uterus progesterone receptor determined after 24h of incubation at 0°C in rat thymus and rabbit uterus preparation. Values for dexamethasone and progesterone are 100 respectively. (See fig. 5.1 for structure).

Code		R	RBA GR	RBA PR
RU 38502	(5.1)	bu^t	59	0.7
RU	(5.2)	bu^t-phenyl	38	48
RU 39229	(5.3)	phenyl-phenyl	160	278
RU 43780	(5.4)	phenyl-CC-phenyl	7	8
RU	(5.5)	Me		234
RU 38486	(5.6)	p-Me_2N-phenyl	302	530
RU 40900	(5.7)	MeN$(Et)_2$N-phenyl	86	25
RU	(5.8)	phenyl-O-phenyl	100	177
RU 25458	(5.9)		68	0.1



RU 25458 (6.9)

Figure 5.1

When the researchers at Roussel Uclaf compared the RBA of the p-substituted biphenyl steroid RU 39299 (5.3) and the p-substituted diphenylacetylene steroid RU 43780 (5.4) (table 5.1), the depth of the pocket was estimated to be 10-12 Å for both receptors. A representation showing the estimated section of the hydrophobic cavity has been reported for the glucocorticoid (fig. 5.3e) and progesterone (fig 5.3f) receptors. The map of the progesterone receptor was further refined when the compound RU 40900 (5.8) (table 5.1) was taken into account in the 'Script' molecular modelling program (fig. 5.4 and 5.5).

On the basis of the RBA of the 11β-t-butyl substituted steroid RU 38502 (5.1) and the 10β-methyl steroid RU 25458 (5.9) (table 5.1), the researchers at Roussel-Uclaf suggested that the 10β-methyl and 11β-t-butyl groups might both protrude into the hydrophobic pocket of the glucocorticoid receptor.[2,3] It was postulated that the low affinity of these molecules for the progesterone receptor[2] (table 5.1), indicated that the opening of the pocket in the progesterone receptor (3.4 Å) was much narrower than that in the glucocorticoid receptor. When the compound RU 25458 (5.9) was taken into account in the 'Script' program for the glucocorticoid receptor, the topology of the hydrophobic pocket was refined (fig 5.6).[2,3] A 10β functionality will therefore reduce the affinity of a steroid for the progesterone receptor and increase the specificity for the glucocorticoid receptor. This is indeed the case for RU 39305 (5.10) and RU 43044 (5.11) (table 5.2),[5] which may explain why the removal of the C-19 methyl group from a progestational agent increases its affinity for the progesterone receptor.[6,7]

Table (5.2) Relative binding affinity (RBA) for rat thymic glucocorticoid receptor and rabbit uterus progesterone receptor determined after 24h of incubation at 0°C in rat thymus and rabbit uterus preparation. Values for dexamethasone and progesterone are 100 respectively. (See fig. 5.2 for structure)

Code		R	RBA GR	RBA PR
RU 39305	(5.10)	phenyl	57	0.1
RU 43044	(5.11)	pMephenyl	130	0.2
RU 45526	(5.12)	Me	26	14
RU 43230	(5.13)	vinyl	90	11

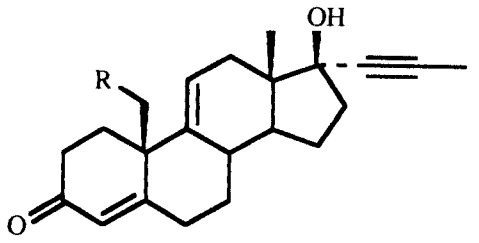


Figure 5.2

5.3 Molecular Modelling: Results and Discussion.

Alongside the synthetic work undertaken in this project, molecular modelling was also carried out. The purpose of these studies was:

(i) to compare the steric and electronic features of molecules which are known to have a high RBA for the progesterone and glucocorticoid receptors;

(ii) to examine the extent to which the steroid synthesised in our own work is compatible with these receptor features and thus to predict its possible spectrum of activity.

The crystal structure of RU 38486 or of any of the other active anti-hormonal steroids have not been published. However, the crystal structure of RU 25593 (2.119) has been reported. The crystallographic parameters for this steroid were obtained from the S.E.R.C Crystallographic Data Base, Daresbury Laboratory and imported into the 'Quanta' molecular modelling program on a Silicon Graphics Machine (Indigo + Elan). All molecular models of progesterone (1.15), ZK 98.734 (2.43), ZK 98.299 (2.41), Org 31343 (2.42) and the 11β,19-aryl steroid (2.44) were generated by changing the functionalities of the above crystal structure, followed by energy minimisation. Minimisations were carried out using Powell, steepest descent and Newton-Raphson calculations. The structural and electronic parameters of these molecular models were compared by overlapping the model of RU 38486 (2.40) with the models of progesterone (1.15) (fig. 5.7), ZK 98.734 (2.43) (fig. 5.8), ZK 98.299 (2.41) (fig. 5.9), Org 31343 (2.42) (fig. 5.10) and the 11β,19-aryl steroid (2.44) (fig. 5.11). In (fig. 5.10) the aryl functionality is oriented above the C-ring.

Structural comparisons of the molecular model for RU 38486 (2.40) against the models of 19-norandrost-4,9(11)-dien-3-one steroids (ZK 98.734 (2.43) (fig. 5.8) and ZK 98.299 (2.41) (fig. 5.9)) show that there exists an extremely good overlap of the steroid skeletons in ring A, B, C, the polar 3-keto, 17-hydroxy and 11-(p-N,N-dimethylaminophenyl) groups. The compound ZK 98.299 (2.41) is epimerized at positions C-13 and C-17. The structural comparison (fig. 5.9) shows little overlap between the D rings, however despite this difference the 17α-hydroxy group of ZK 98.299 (2.41) shows a surprisingly good overlap with the 17β-hydroxy group in RU 38486 (2.40).

Structural comparisons between the molecular model of RU 38486 (2.40) and the models of compounds which posses sp^3 hybridised carbon atoms at positions C-9 and C-10; progesterone (1.15) (fig. 5.7), Org 31343 (2.42) (fig. 5.10), and the 11β,19-aryl steroid (2.44) (fig. 5.11) all show a good correlation between the positions of C, D rings of the steroid skeletons, the polar 3-keto and 17β-hydroxy groups. There are however a number of differences that can be observed in these comparisons. The carbon atoms at positions C-1, C-2, C-9 and C-10 show some variation in position to that observed for RU 38486 (2.40).

Progesterone (1.15) is structurally different from RU 38486 (2.40) in that it possesses a C-19 methyl group and 17β-acetyl group but it does not have a 11β-(p-N,N-dimethylaminophenyl) group. In the molecular comparison (fig. 5.7), it can be seen that the polar 17β-hydroxy group of RU 38486 (2.40) shows a good correlation in position with the polar 17β-acetyl group of progesterone (1.15).

The compound Org 31343 (2.42) and the 11β,19-aryl steroid (2.44) both posses a p-N,N-dimethylaminophenyl substituent, which is attached at position C-18 in Org 31343 (2.42) and at position C-11β in (2.44) with the aryl group of (2.44) also attached at the ortho position to a C-19 methyl group. In the structural comparison of the molecular models of these molecules with that of RU 38486 (2.40) (fig. 5.10 and 5.11) it can be seen that the orientation of the aryl group is different to that observed for RU 38486 (2.40). Despite this difference the polar dimethyl substituents in Org 31343 (2.42) show a close correlation to their position in RU 38486 (2.40).

A much clearer picture of the topology of the progesterone and glucocorticoid receptor sites can be obtained by a molecular comparison between three representative examples of 11β-aryl, 18-aryl and 11β,19-aryl steroids (fig. 5.12). In this representation, the C-18 aryl functionality of Org 31343 (2.42) is oriented above the C-ring of the other steroids. It was postulated that two suitable amino acid residues may be located so that they form hydrogen bonds with the polar C-3 and C-17 functionalities, thus facilitating strong receptor binding.[4] On the basis of the close correlation that is observed between the polar dimethylamino substituents (fig. 5.12), it is postulated that within the hydrophobic pocket there exists an amino acid residue which may enhance receptor binding through the formation of a hydrogen bond with the polar amino group.

5.4 Predicted Specificity.

A molecular comparison between RU 38486 (2.40) and 17β-hydroxy-17α-(prop-1-yne)-19-(p-(N,N-dimethylaminophenyl))androst-5-ene (3.1) (fig. 5.13), shows that there is good overlap between the positions of the respective polar oxygen at 3-keto, 17β-hydroxy groups and amino-aryl nitrogens. In this representation (fig. 5.13), the C-19 aryl functionality

of (3.1) is oriented to give the best fit with that of the 11-aryl group in RU 38486 (2.40). The carbon atoms at positions C-1, C-2 and C-10 do not coincide with those in RU 38486 (2.40), but the polar aryl nitrogen shows a close correlation of position to that in RU 38486 (2.40). The main difference between the two molecules is the presence of an additional C-19 methylene group in 17β-hydroxy-17α-(prop-1-yne)-19-(p-(N,N-dimethyl aminophenyl)) androst-5-ene-3-one (3.1).

Taking into account the molecular comparison between RU 38486 (2.40) and 17β-hydroxy-17α-(prop-1-yne)-19-(p-(N,N-dimethylamino phenyl))androst-5-ene (3.1) (fig. 5.13) and the work reported by Roussel-Uclaf[2-5], it is suggested that 17β-hydroxy-17α-(prop-1-yne)-19-(p-(N,N-dimethylaminophenyl))androst-5-ene (3.1) will theoretically posses an increased specificity for the glucocorticoid receptor.

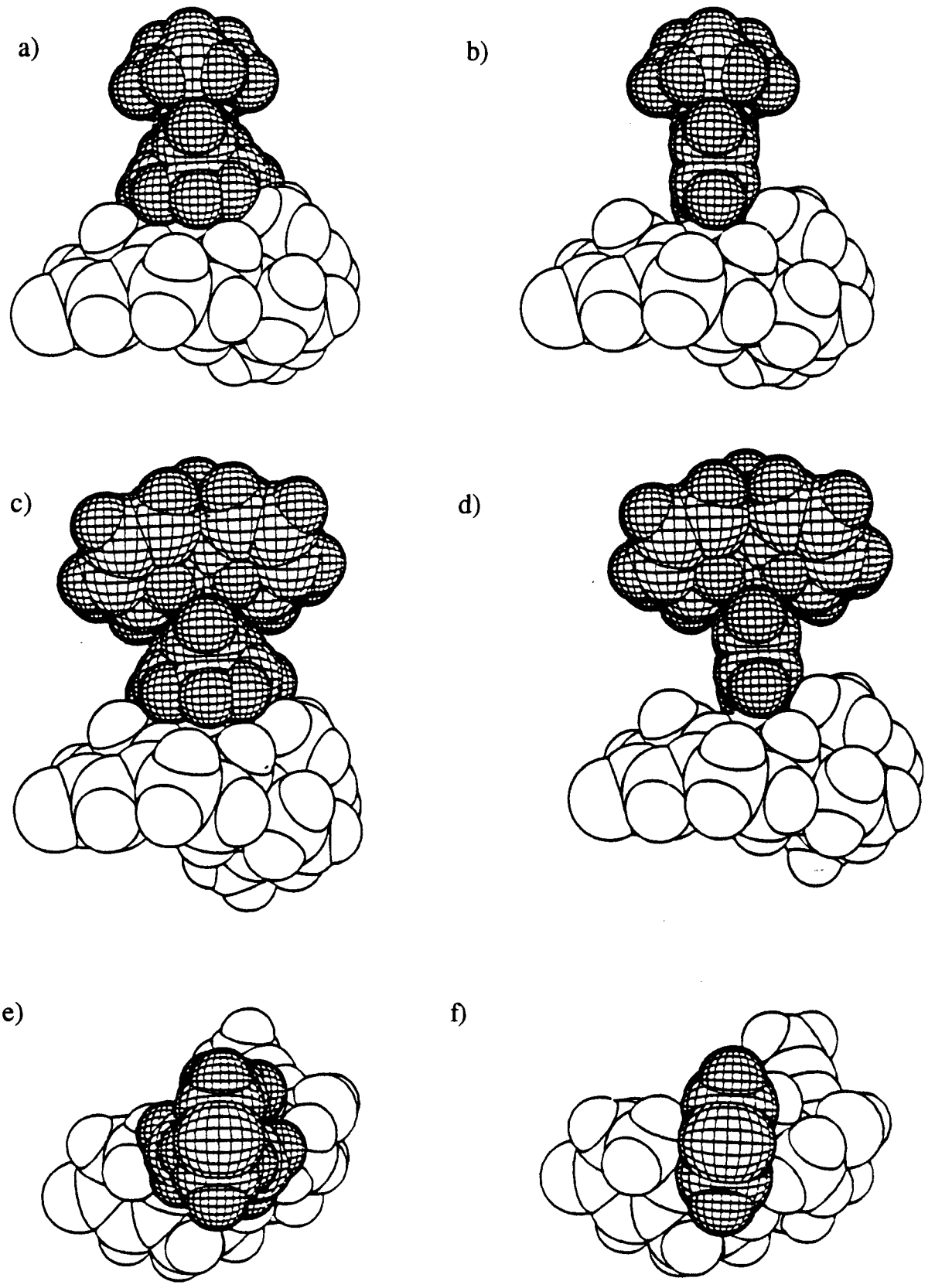


Figure 5.3

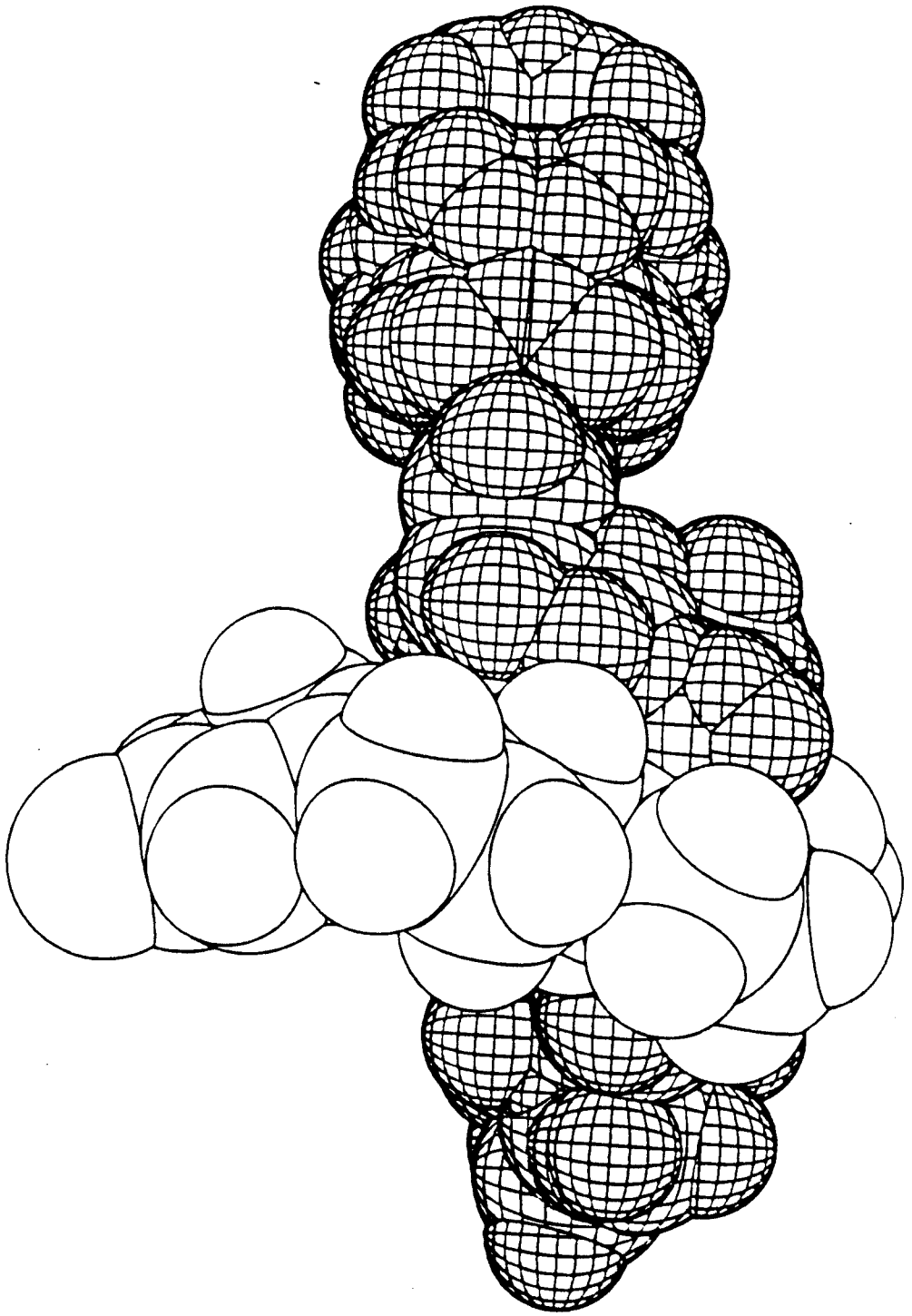


Figure 5.4 Computer generated map of the progesterone receptor showing the lipophilic pockets above and below the plane of the steroid; Front view.

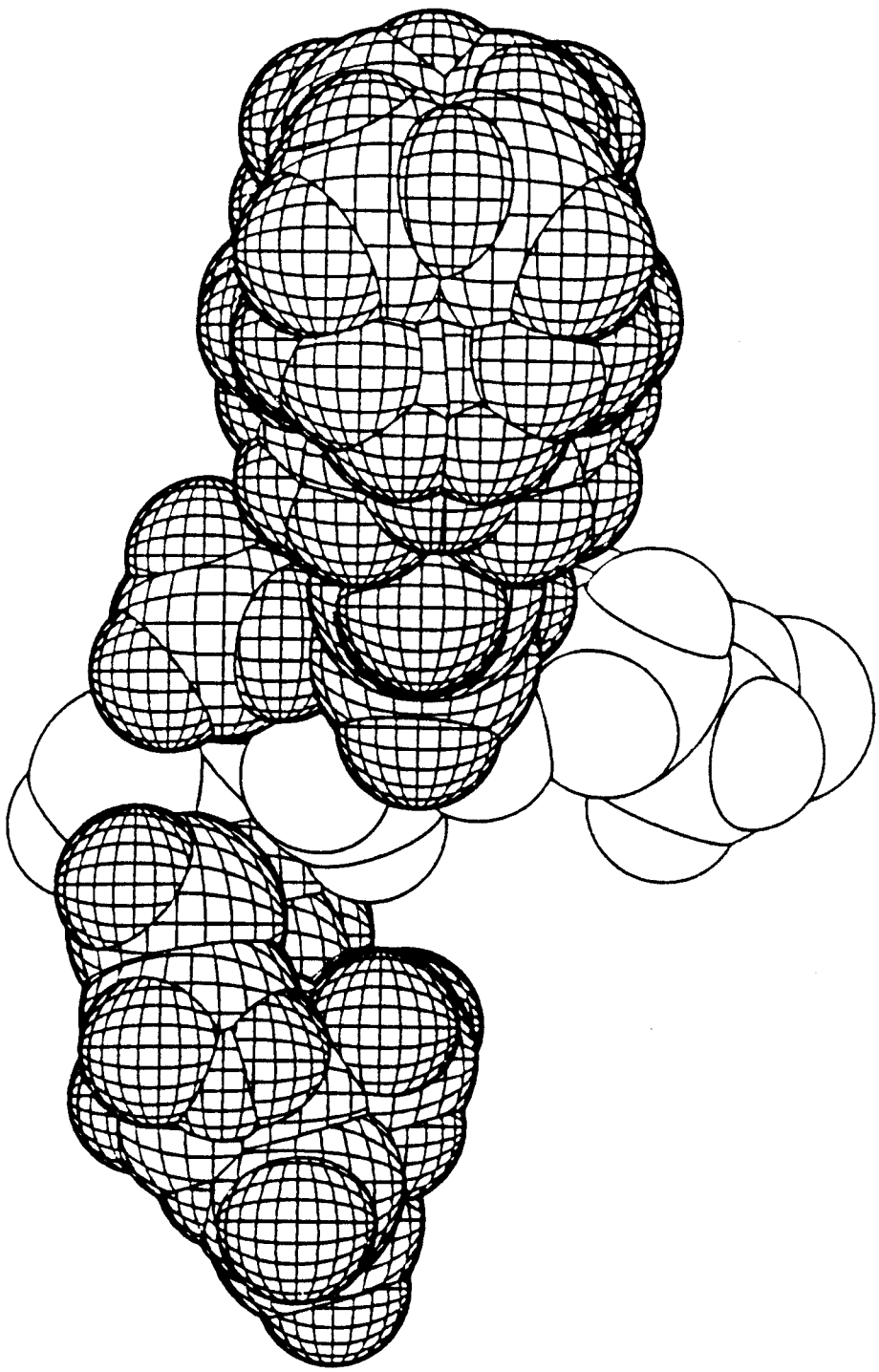
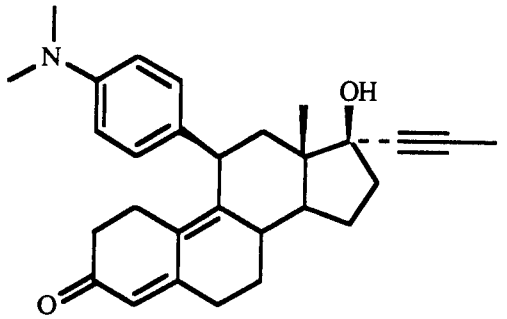
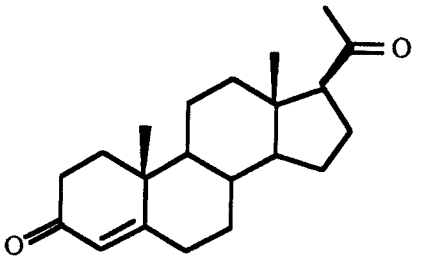


Figure 5.5 Computer generated map of the progesterone receptor showing the lipophilic pockets above and below the plane of the steroid; Rear view.



RU 38486 (Mifeprisone) (2.40)



Progesterone (1.15)

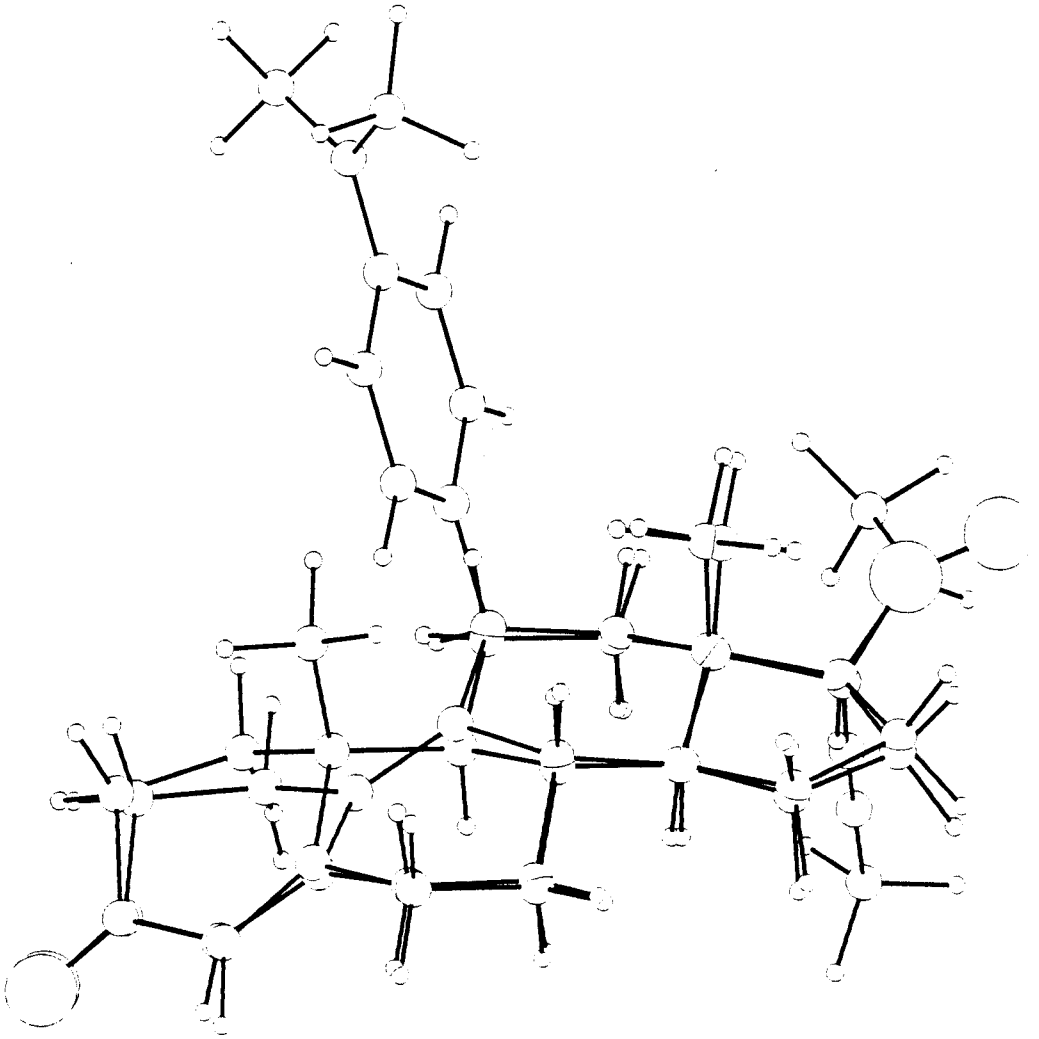
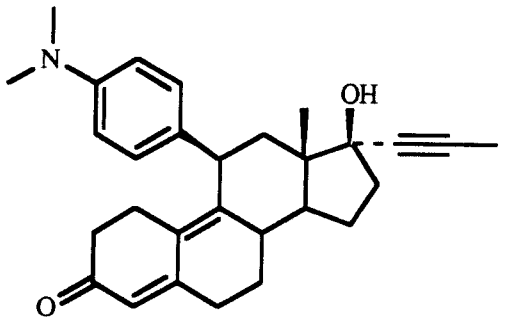
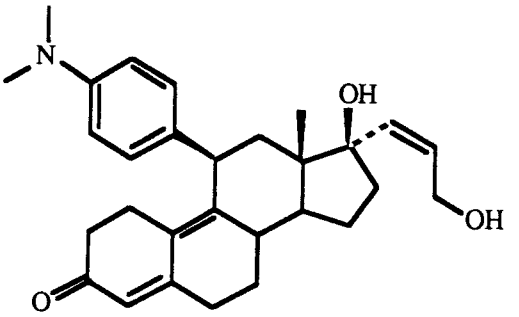


Figure 5.7



RU 38486 (Mifeprisone) (2.40)



ZK 98.734 (Lilopristone) (2.43)

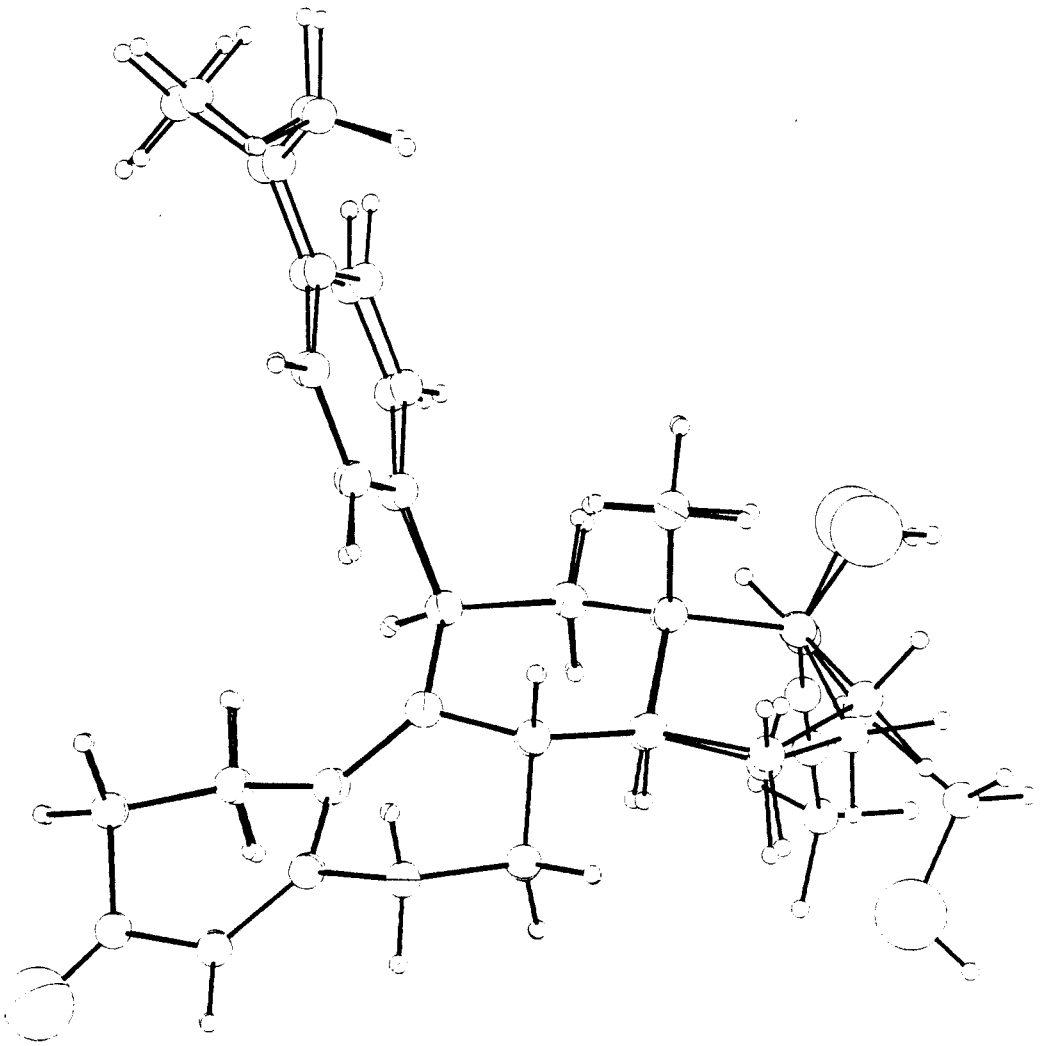
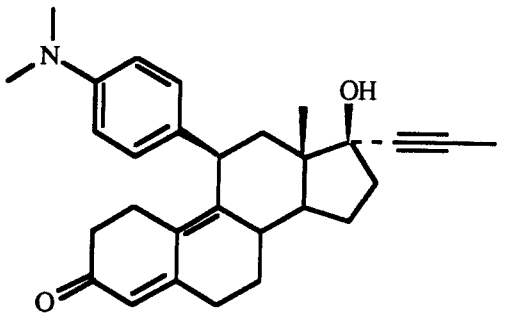
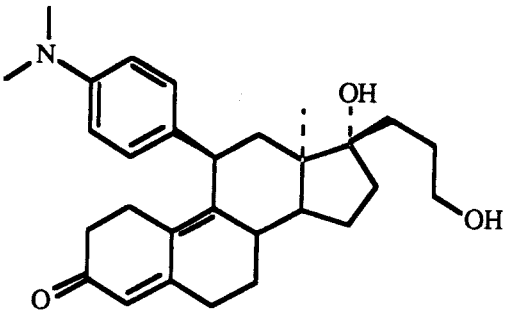


Figure 5.8



RU 38486 (Mifeprisone) (2.40)



ZK 98.299 (Onapristone) (2.41)

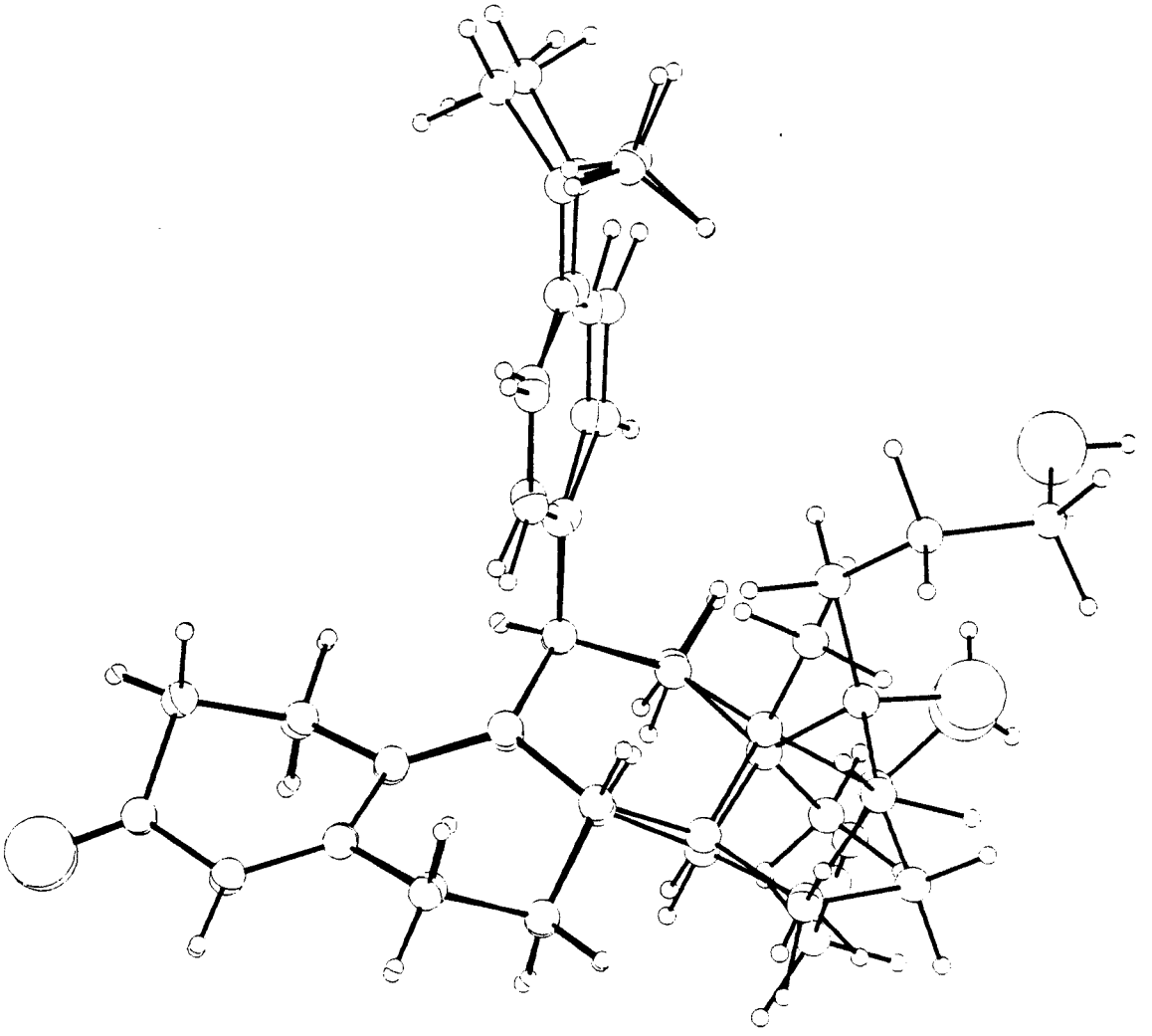
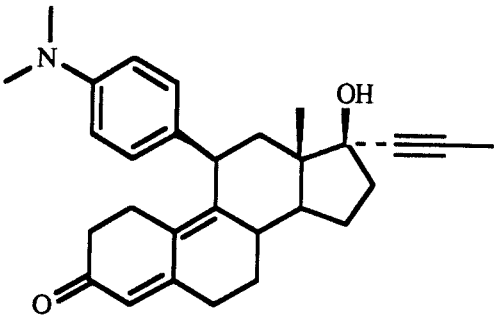
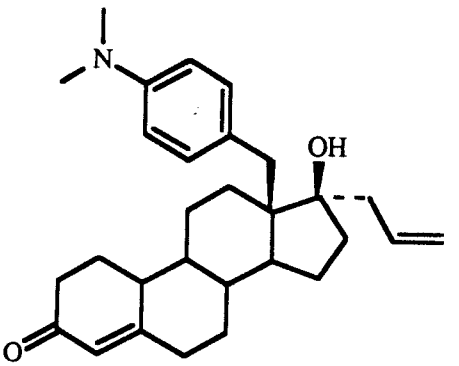


Figure 5.9



RU 38486 (Mifeprisone) (2.40)



Org 31343 (2.42)

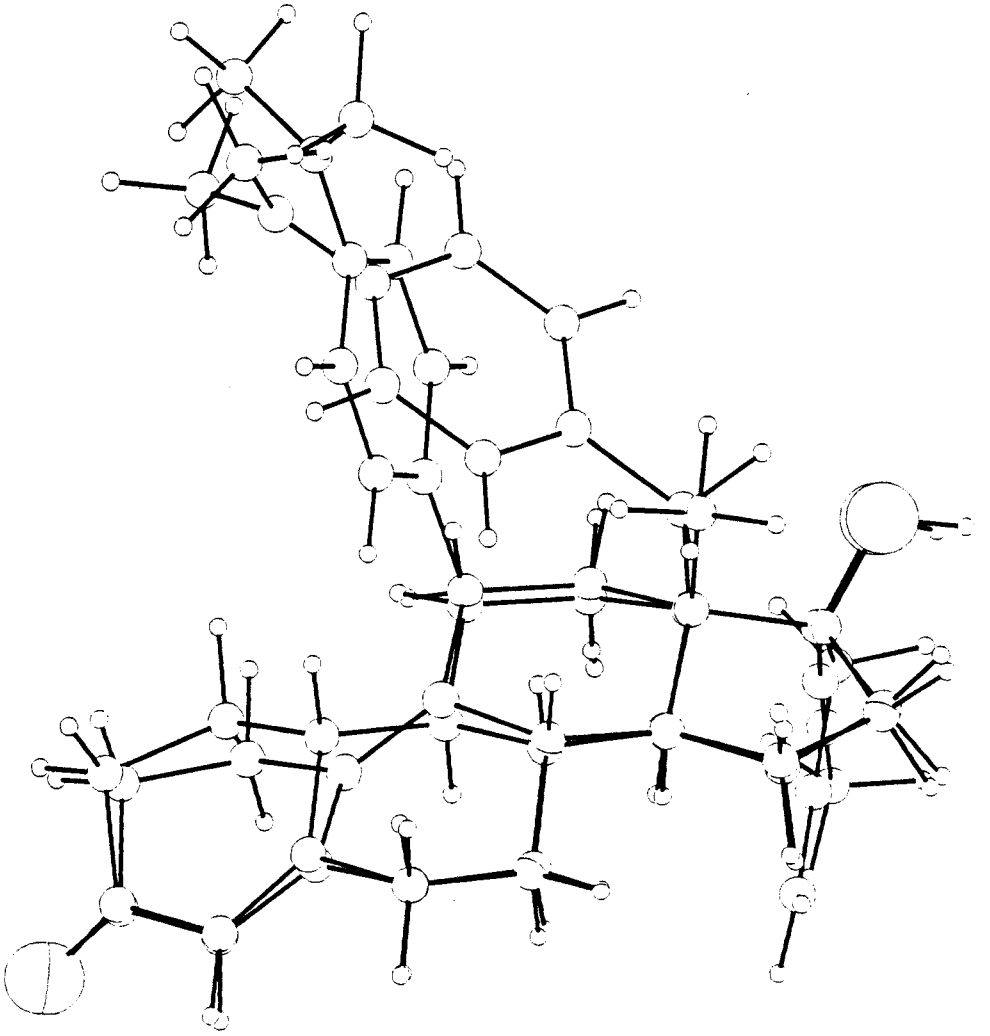
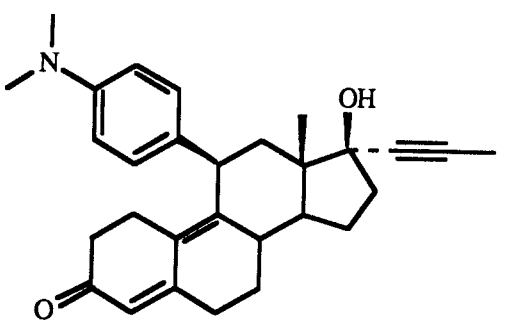
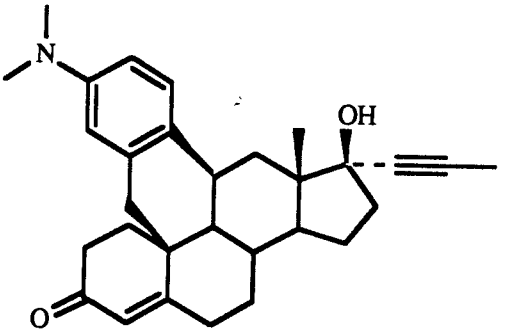


Figure 5.10



RU 38486 (Mifeprisone) (2.40)



(2.44)

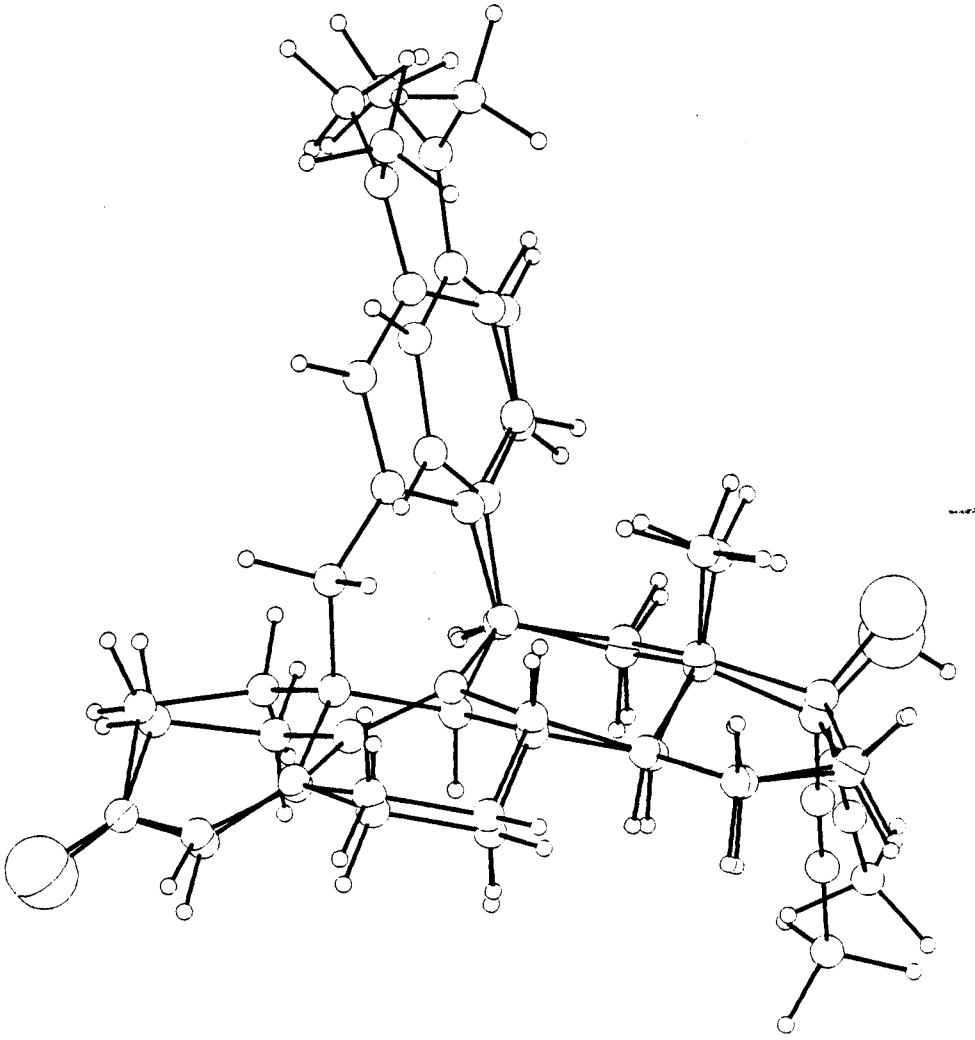
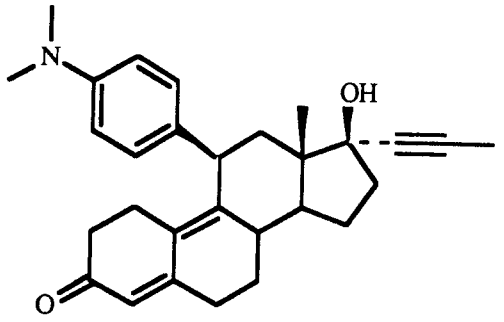
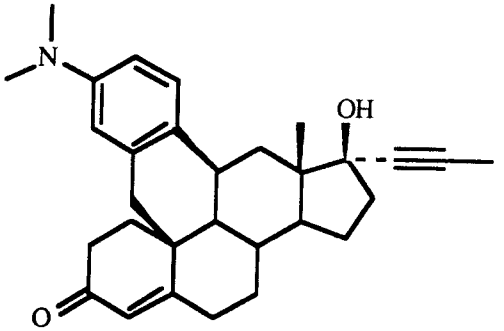


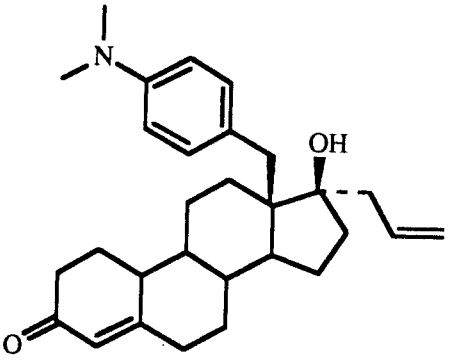
Figure 5.11



RU 38486 (Mifeprisone) (2.40)



(2.44)



Org 31343 (2.42)

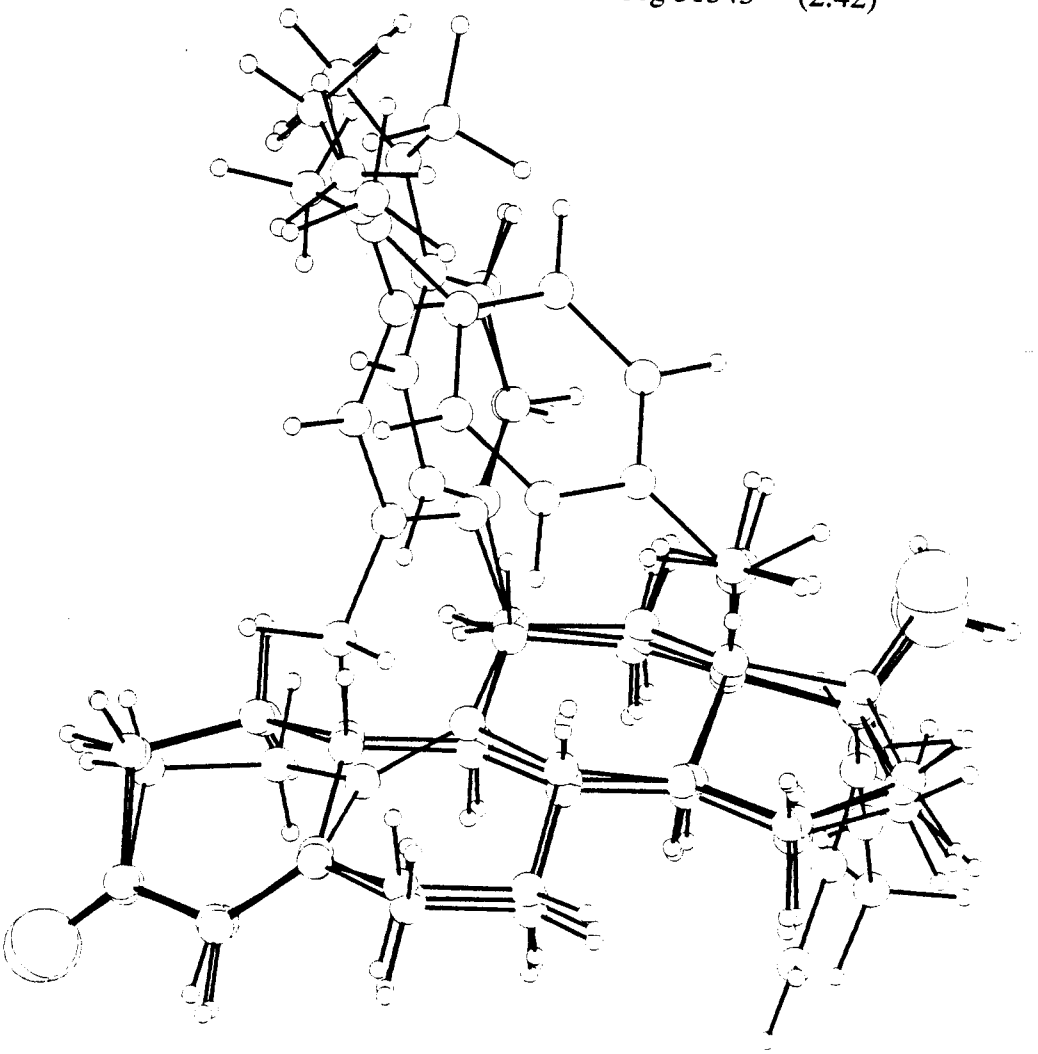
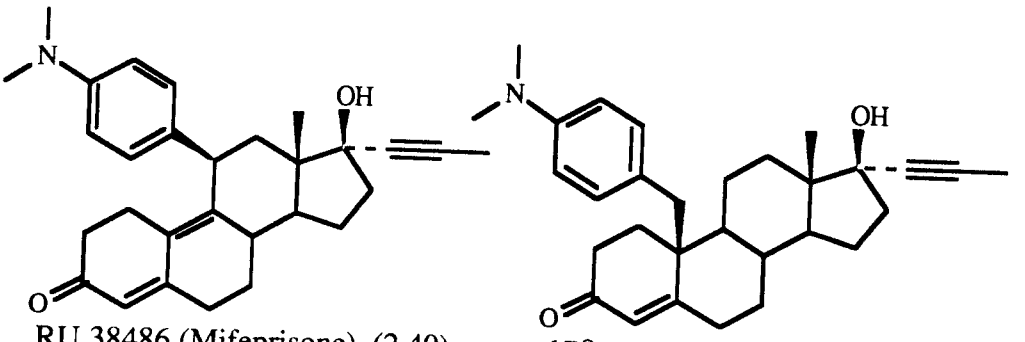


Figure 5.12



RU 38486 (Mifeprisone) (2.40)

17β-hydroxy-17α-propynyl -19-(p-(N,N-dimethylamino phenyl))androst-5-ene (3.1)

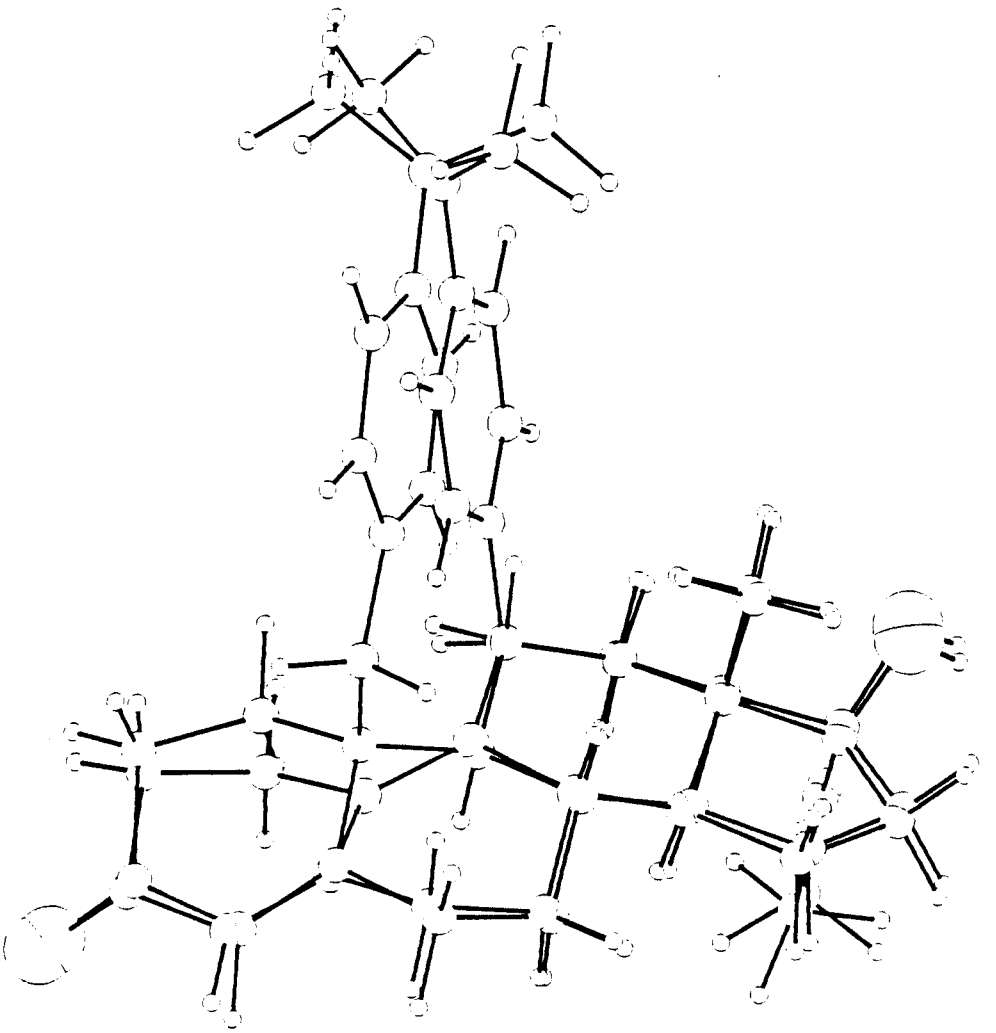


Figure 5.13

5.5 References.

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Chapter 6

6.1 Experimental.

All chemicals and solvents were purchased from Aldrich, Fluka, Jansens and Sigma. The solvents were of SLR grade and dried where necessary according to the procedure cited in the literature.[1] Melting points were determined on a Gallenkamp Melting point Apparatus and are uncorrected. Infrared spectroscopy was performed on samples in the individual methods on a Perkin Elmer instrument (FT-IR 1720X spectrometer) and recorded as wavenumber (cm^{-1}). ^{1}H NMR and ^{13}C NMR were performed on a Bruker AC 250 MHz or a Bruker AC 400 MHz spectrometers in $CDCl_3$ solutions. Chemical shifts are quoted in ppm (δ) and TMS was used as an internal standard. Assignments of NMR signal were made in accordance with the literature.[2,3] Low resolution EI-MS, CI-MS(NH_4^+) and FAB-MS were obtained on a Kratos MS-80 mass spectrometer. High resolution EI-MS and CI-MS(NH_4^+) were obtained from the SERC Mass Spectrometery Service Centre, University College of Swansea. Microanalysis was carried out on a Leeman Labs. CE440 Elemental Analyser or obtained from Medac, Brunel University. Thin layer chromatography was routinely used to follow reactions and measurements were carried out using DC-Alufolien Kieselgel 60 F_{254} (0.2 mm). Column chromatography was carried out using Kieselgel 60 (particle size 40-63 μm). Organic solvents were removed under reduced pressure on a Büchi rotary evaporator.

3β,6β-Dihydroxy-5α-bromoandrostan-17-one 3-acetate (3.57).

Dehydroisoandrosterone 3β-acetate (3.14) (250 g, 0.76 mols) and N-bromoacetamide (157g, 1.33 mols, 1.50 equiv.) were together dissolved in a solution of 1,4-dioxan (3.80 L), distilled water (450 ml), concentrated perchloric acid (37 ml, 0.61 mols, 0.81 equiv.) and stirred for 30 minutes at room temperature. During this period of time the reaction mixture slowly developed from a pale yellow to a translucent deep orange solution. The solution was decolourised with saturated sodium hydrogen sulphite solution, and diluted in excess distilled water (10 L), to cause precipitation of the steroid. The precipitate was filtered under reduced pressure, washed with water and dried at room temperature under reduced pressure over phosphorous pentoxide. The mixture (306.5g, 94%) was suspended in chloroform (700 ml) at 0-5^{0}C, stirred and filtered to afford (3.57) (269.8g, 0.63 mols, 83.4%) m.p. 170-171 ^{0}C (lit.[4-6] 173-175^{0}C), I.R. νmax (KBr cm^{-1}), 3479 (free OH), 1729 (>C=O); ^{1}H NMR and ^{13}C NMR data are shown in table 4.3 and 4.4; found C 58.95, H 7.33 found ($C_{21}H_{31}O_4Br$ requires C 59.2 H 7.31); EI-MS m/z; 428(M^+), 426(M^+), 346, 286; CI-MS(NH_4^+) m/z; 446(MNH_4^+), 444(MNH_4^+), 382, 364, 347, 287, 304.

A small quantity of this material was recrystallised from acetone/hexane furnishing needles m.p. 174 ^{0}C (lit.[4-6] 173-175^{0}C)

3β-Hydroxy-5α-bromo-6β,19-oxidoandrostan-17-one 3-acetate (3.58).

3β,6β-Dihydroxy-5α-bromoandrostan-17-one 3-acetate (3.57) (0.53 g, 1.24 mmols) was refluxed for 18 hrs with lead tetraacetate (2.1 g, 3.8 equiv.) in dry benzene (50 ml). The solution was allowed to cool, before being filtered and washed through with dichloromethane. The combined organic layers were washed with sodium thiosulphate solution (3 x 50 ml) and distilled water (50 ml). The organic phase was dried over anhydrous magnesium sulphate and solvent removed under reduced pressure. The crude product was purified by silica gel preparative thin layer chromatography (acetonitrile/dichloromethane) to furnish (3.58) (121 mg, 23 %).

m.p. 185 ^{0}C (lit.[7,8] 186-188^{0}C), I.R. νmax (nujol cm^{-1}), 1733 (>C=O); ^{1}H NMR and ^{13}C NMR data are shown in table 4.5 and 4.6; High resolution EI-MS m/z; 426.1182 ($C_{21}H_{29}O_4Br$ requires 426.1230), 424.1200 ($C_{21}H_{29}O_4Br$ requires 424.1250); EI-MS m/z; 426 (M^+), 424 (M^+), 285; CI-MS (NH_4^+) m/z ; 444 (M NH_4^+), 442 (M NH_4^+), 364, 304, 285.

3β-Hydroxy-5α-bromo-6β,19-oxidoandrostan-17-one 3-acetate (3.58).

To a refluxing solution of carbon tetrachloride (dry 1 L) was added lead tetraacetate (130.7g, 294.79 mmols, 5 equiv.), immediately followed by 3β,6β-dihydroxy-5α-bromoandrostan-17-one 3-acetate (3.57) (25.09g, 58.71 mmols) and iodine (29.76g, 117.25 mmols, 2 equiv.). The solution was allowed to reflux for 2 hrs during which time decolourisation of the deep red solution occurred. The solution was allowed to cool, before being filtered and the inorganic residue washed with dichloromethane. The combined organic layers were washed with sodium thiosulphate solution (3 x 400 ml) and distilled water (2 x 400 ml), dried

over anhydrous magnesium sulphate and concentrated under vacuum to afford a pale yellow solid (3.58) (25.04g, 58.71 mmols, 99.9%)
m.p. 183 ^{0}C (lit.[7,8] 186-188C), I.R. νmax (nujol cm^{-1}), 1733 (>C=O); ^{1}H NMR and ^{13}C NMR data are shown in table 4.5 and 4.6; EI-MS m/z; 426 (M^+), 424 (M^+), 285; CI-MS (NH_4^+) m/z ; 444 (M NH_4^+), 442 (M NH_4^+), 364, 304, 285.

3β-Hydroxy-5α-bromo-6β,19-oxidoandrostan-17-one (3.59).

3β-Hydroxy-5α-bromo-6β,19-oxidoandrostan-17-one 3-acetate. (3.58) (11.12g, 26.24 mmol) was stirred together with sodium hydroxide (1.13g, 3 equiv.), distilled water (7 ml), methanol (48 ml) and the temperature maintained between 10-13°C, until precipitation occurred. The precipitate was filtered, washed with distilled water and dried under reduced pressure over phosphorous pentoxide to furnish a white powder (3.59) (8.96 g, 2.4 mmols, 89.4 %) m.p. 201 °C (lit.[7] 199-201°C); I.R. νmax(nujol cm^{-1}); 3439 (free OH), 1721 (C=O);^{1}H NMR and ^{13}C NMR data are shown in table 4.7 and 4.8; EI-MS m/z; 384(M^+), 382(M^+), 285; CI-MS(NH_4^+) m/z; 402(MNH_4^+), 400(MNH_4^+), 302, 285.

19-Hydroxyandrost-4-ene-3,17-dione (3.61)

To a stirred solution of 3β-hydroxy-5α-bromo-6β,19-oxidoandrostan-17-one (3.59) (0.72g, 1.877 mmols) in acetone (14 ml), cooled to 0-2°C was added dropwise a solution of chromic anhydride (0.29g, 2.9 mmols, 1.5 equiv.), sulphuric acid (0.5g, 4.70 mmols, 2.5 equiv.) and distilled water (2.30 ml). After 30 minutes water was added to cause the precipitation of a crystalline solid, which was then filtered, washed with distilled water and air dried to give (3.60). 5α-Bromo-6β,19-oxidoandrostan-3,17-dione (3.60) was without previous purification refluxed with zinc powder (freshly activated, 1.21g) in ethanol (9 ml) for 3 hrs. The warm suspension was filtered and washed through with hot ethanol. The combined ethanolic extracts were reduced under vacuum. The solid was extracted with sulphuric acid 10% (1 ml) and chloroform (3 ml). The organic layer was washed with water (2 x 5 ml), saturated sodium bicarbonate solution (5 ml), distilled water (5 ml), dried over anhydrous magnesium sulphate, filtered and concentrated under vacuum. The mixture was purified by silica gel preparative thin layer chromatography (acetonitrile/dichloromethane), furnishing a white powder (120.4 mg, 0.40 mmols, 21.2% yield). m.p. 171°C (lit.[7] 168-170°C); I.R. νmax (nujol cm^{-1}) 3295 (free O-H), 1721, 1688, 1618 ; EI-MS m/z; 302(M^+), 272; CI-MS (NH_4^+) m/z; 320(MNH_4^+), 303(MH^+), 272.

3β,19-Dihydroxyandrostan-5-en-17-one 3-acetate (3.62).

3β-Hydroxy-5α-bromo-6β,19-oxidoandrostan-17-one 3-acetate (3.58) (12.8 g, 30.1 mmols), was allowed to reflux with zinc dust (25 g) in ethanol (130 ml), for 4 hrs. The hot mixture was filtered and washed through with hot ethanol. The combined ethanolic extracts were evaporated to dryness under reduced pressure, redissolved in dichloromethane (150 ml) and washed with distilled water (3 x 100 ml). The organic layer was dried over anhydrous magnesium sulphate, filtered and solvent removed under reduced pressure. The crude product was recrystallised from acetone-hexane furnishing needles (8.16 g, 78.3%) m.p. 167°C (lit.[9] 167-168°C); I.R. νmax (nujol cm^{-1}), 3463 (free OH), 1716 (>C=O) ; ^1H NMR data is shown in table 4.10; EI-MS m/z; 286, 255, 237; CI-MS(NH_4^+) m/z; 364(MNH_4^+), 347(MH^+), 304, 287, 255.

3β,19-Dihydroxyandrost-5-en-17-one 3-acetate 19-tosylate (3.63).

3β,19-Dihydroxyandrost-5-en-17-one 3-acetate (3.62) (9.68 g, 28.0 mmols) was stirred at room temperature for 18 hrs with tosyl chloride (10.7 g, 2 equiv.), in dry pyridine (100 ml). The reaction mixture was poured into distilled water (200 ml) and extracted with dichloromethane (3 x 150 ml). The combined organic extracts were washed with distilled water (3 x 300 ml), dilute hydrochloric acid (2 M, 300 ml), aqueous sodium hydrogen carbonate (300 ml) and water (300 ml). The organic phase was dried over anhydrous magnesium sulphate, filtered and evaporated to dryness under reduced pressure. The crude product was purified by silica gel preparative thin layer chromatography (acetonitrile/dichloromethane) furnishing a pale yellow solid (3.63) (11.61 g, 83%).

m.p. 154°C (lit[9] 155-157°C); I.R. νmax (nujol cm^{-1}), 1733 (>C=O); ^1H NMR and ^{13}C NMR data are shown in table 4.11 and 4.12 ; EI MS m/z; 368, 329, 268; CI-MS (NH_4^+) m/z; 518(MNH_4^+), 346, 329, 269.

3β-Hydroxy-5α-bromo-6β,19-oxido-17,17-ethylene dioxyandrostane (4.3).

3β-Hydroxy-5α-bromo-6β,19-oxidoandrostan-17-one (3.58) (8.3 g, 21.7 mmols), was allowed to reflux with ethyleneglycol (45 ml), p-toluenesulphonic acid (0.23 g, catalytic) and dry benzene (170 ml), for 4 hrs., in Dean and Stark apparatus. The mixture was washed with distilled water (3 x 100 ml), aqueous sodium bicarbonate (100 ml) and distilled water (100 ml). The organic phase was dried over anhydrous magnesium sulphate, filtered and evaporated to dryness under reduced pressure. The crude product was recrystallised from ethanol to furnish (4.3) (8.00g, 86.4%) m.p. 215 °C (lit.[9] 216-217°C), I.R. νmax (KBr cm^{-1}), 3495 (free OH); ^1H NMR and ^{13}C NMR data are shown in table 4.13 and 4.14; CI-MS(NH_4^+) m/z; 444 (MNH_4^+), 446 (MNH_4^+), 428 (MH^+), 426 (MH^+), 329, 99.

3β-Hydroxy-5α-bromo-6β,19-oxido-17,17-ethylene dioxyandrostane 3-acetate (3.66).

3β-Hydroxy-5α-bromo-6β,19-oxido-17,17-ethylenedioxy androstane (4.3) (12.43 g, 29.1 mmols), was stirred at room temperature for 20 hrs with freshly distilled acetic anhydride (30 ml) and dry pyridine (60 ml). The solution was poured into distilled water (100 ml) and extracted with dichloromethane (3 x 100 ml). The combined organic extracts were washed with distilled water (3 x 150 ml), aqueous sodium hydrogen carbonate (150 ml), distilled water (150 ml), dried over anhydrous magnesium sulphate and concentrated under vacuum to afford a pale

brown solid which was purified by silica gel column chromatography (acetonitrile/dichloromethane) (10.18 g, 74.6%) m.p. 166 ^{0}C (lit.[9] 164-165^{0}C), I.R. νmax (KBr cm^{-1}), 1733 (>C=O); ^{1}H NMR and ^{13}C NMR data are shown in table 4.15 and 4.16; EI-MS m/z; 470 (M^{+}), 468 (M^{+}), 329, 99.

3β-Hydroxy-5α-bromo-6β,19-oxido-17,17-ethylene dioxyandrostane 3-acetate (3.66).

3β-Hydroxy-5α-bromo-6β,19-oxidoandrostan-17-one 3-acetate (3.58) (48.5 g, 114 mmols), was allowed to reflux with ethylene glycol (240 ml), p-toluenesulphonic acid (1.2 g, 0.06 equiv., catalytic) and dry benzene (900 ml) for 4 hrs. in Dean and Stark apparatus. The reaction mixture was then allowed to cool before being washed with distilled water (2 x 300 ml), sodium bicarbonate solution (2 x 300 ml) and distilled water (300 ml). The organic layer was dried over anhydrous magnesium sulphate, filtered under reduced pressure and the solvent removed under reduced pressure to give a yellow oily residue (49.6g). The residue was dissolved in dry pyridine (100 ml) and stirred along with freshly distilled acetic anhydride (50 ml), at room temperature for 12 hrs. The solution was poured into distilled water (300 ml) and extracted with dichloromethane (2 x 200 ml). The combined organic extracts were washed with distilled water (3 x 250 ml), aqueous sodium hydrogen carbonate (250 ml), distilled water (300 ml), dried over anhydrous magnesium sulphate and concentrated under vacuum to give a pale brown solid which was purified by silica gel column chromatography (acetonitrile/dichloromethane) to afford (3.66) (25.04g, 58.71 mmols, 99.9%) m.p. 165 ^{0}C (lit.[9] 164-165^{0}C), I.R. νmax (KBr cm^{-1}), 1773 (>C=O); ^{1}H NMR and ^{13}C NMR data are shown in table 4.15 and 4.16; EI-MS m/z; 470 (M^{+}), 468 (M^{+}), 329, 237, 99.

3β,19-Dihydroxy-17,17-ethylenedioxyandrostan-5-ene 3-acetate (3.67).

3β-Hydroxy-5α-bromo-6β,19-oxido-17,17-ethylenedioxy androstane 3-acetate (3.66) (23.4 g, 49.8 mmols), was refluxed along with zinc powder (45 g) and ethanol (100 ml, pH adjusted to 5.5 with glacial acetic acid), for 3 hrs. The mixture whilst warm was filtered and washed with hot ethanol, the inorganic residue was further washed with dichloromethane. Solvent was removed from the combined organic extract under reduced pressure. The residue was redissolved in dichloromethane (200 ml) and washed with distilled water (3 x 100 ml). The organic layer was dried over anhydrous magnesium sulphate and solvent removed under reduced pressure to afford a pale yellow gum (19.4 g), which was recrystallised from acetone-hexane furnishing needles (14.6 g, 37.4 mmol, 75 %) m.p. 140°C (lit.[9]. 138-140°C); I.R. νmax (KBr cm^{-1}), 3494 (free OH), 1732 (C=O), 1712(C=O); ^{1}H NMR and ^{13}C NMR data are shown in table 4.17 and 4.18; found C 70.740 H 8.775 ($C_{23}H_{33}O_5$ requires C 70.59 H 8,69); EI MS m/z; 390 (M^+), 372, 331, 313, 99; CI -MS (NH_4^+) m/z 408(MNH_4^+), 391(MH^+), 331, 313, 99.

3β,19-Dihydroxy-17,17-ethylenedioxyandrost-5-ene 3-acetate 19-tosylate (3.71).[10]

3β,19-Dihydroxy-17,17-ethylenedioxyandrost-5-ene 3-acetate (3.67) (1.4g, 3.58 mmol) was stirred along with tosyl chloride (1.4g, 7.34 mmol, 2 equiv.), in dry pyridine (14 ml), at room temperature for 18 hrs. The reaction mixture was poured into water (20 ml) and extracted with ethyl acetate (3 x 30 ml). The combined organic extracts were washed with water (3 x 50 ml), dilute hydrochloric acid (50 ml), aqueous sodium hydrogen carbonate (50 ml) and water (50 ml). The organic phase was dried over magnesium sulphate, filtered and evaporated to dryness under

reduced pressure to afford a yellow oily residue (1.72 g, 88 %) which was purified by silica gel preparative thin layer chromatography (acetonitrile/dichloromethane) furnishing a pale yellow gum (1.34 g, 68.7%). I.R. νmax (KBr cm^{-1}), 1733 (>C=O); ^1H NMR data is shown in table 4.19; CI-MS(NH_4^+) m/z; 562(MNH_4^+), 545(MH^+), 407, 389, 99.

3β,19-Dihydroxy-17,17-ethylenedioxyandrost-5-ene 3-acetate 19-mesylate (4.5).[10]

3β,19-Dihydroxy-17,17-ethylenedioxyandrost-5-ene 3-acetate (3.67) (0.90 g, 2.30 mmol), was stirred along with mesyl chloride (0.60g, 1 equiv.), in dry pyridine (12 ml), at room temperature for 18 hrs. The reaction mixture was poured into water (20 ml) and extracted with ethyl acetate (3 x 15 ml). The combined organic extracts were washed with water (3 x 30 ml), dilute hydrochloric acid (30 ml), aqueous sodium hydrogen carbonate (30 ml) and water (30 ml). The organic phase was dried over magnesium sulphate, filtered and evaporated to dryness under reduced pressure to afford a yellow oily residue (1.02 g, 94%) which was purified by silica gel preparative thin layer chromatography furnishing a pale yellow gum (0.78 g, 72 %). I.R. νmax (KBr cm^{-1}), 1734 (>C=O); ^1H NMR and ^{13}C NMR data are shown in table 4.20 and 4.21; EI-MS m/z; 468(M^+), 408, 309, 99.

2,2-dimethylpropan-1-ol 1-tosylate (4.6).

2,2-dimethylpropan-1-ol (1.10 g, 12.5 mmol) was stirred at room temperature for 18 hrs along with tosyl chloride (2.85 g, 1.2 equiv.) in dry pyridine (30 ml). The reaction mixture was poured into distilled water (60 ml) and extracted with dichloromethane (3 x 40 ml). The combined organic extracts were washed with water (3 x 70 ml), dilute hydrochloric acid (2M, 80 ml), aqueous sodium hydrogen carbonate (70 ml) and

distilled water (70 ml). The organic phase was dried over anhydrous magnesium sulphate, filtered and evaporated to dryness under reduced pressure. The crude product was recrystallised from ether (1.66 g, 54.9%). m.p. 48^{0}C (lit.[11] 48-49^{0}C); I.R. νmax (KBr cm^{-1}), no free OH signal; the ^{1}H NMR and ^{13}C NMR data are shown in table 4.22 and 4.23; EI-MS m/z; 242, 92.

1-phenyl-2,2-dimethylpropan-1-ol (4.13).

2,2-Dimethylpropanal (1g, 11.6 mmol) was stirred with diethyl ether (dry, 20 ml) and cooled with dry ice. To this solution was added dropwise phenyl lithium solution in ether (10 ml, 1.8 M, 17.8 mmol, 1.5 equiv.) and was stirred for 1 hour. The solution was quenched with aqueous ammonia (10 ml) and extracted with dichloromethane (3 x 40 ml). The combined organic extracts were washed with distilled water (3 x 80 ml), aqueous sodium hydrogen carbonate (80 ml) and distilled water (80 ml). The organic phase was dried over anhydrous magnesium sulphate, filtered and evaporated under reduced pressure to afford a solid (1.23 g, 64.5 %). m.p. 44^{0}C (lit.[12] 43-45^{0}C), the ^{1}H NMR and ^{13}C NMR data are shown in table 4.24 and 4.25; EI-MS 164(M^+), 107.

1-phenyl-2,2-dimethylpropan-1-ol (4.13).

Dry magnesium turnings (0.34 g, 1.2 equiv.) were placed into a dry flask equipped with condenser and dropping funnel under nitrogen. Freshly dried and distilled tetrahydrofuran (15 ml) was added so as to the flask to just cover the magnesium turnings. A small iodine crystal was added to the mixture which was stirred and the flask was gently warmed with an air gun. To this stirred solution was slowly added a solution of freshly distilled bromobenzene (2.19 g, 1.2 equiv.) dissolved in dry tetrahydrofuran (40 ml) at a rate sufficient to maintain a steady reflux. At

the start of addition, the formation of the Grignard reagent was initiated by warming the flask such that the mixture just began to reflux. The solution was stirred for 30 minutes. To the stirred solution was added dropwise 2,2-dimethylpropanal (1.02 g) dissolved in dry tetrahydrofuran (20 ml) at a rate sufficient to cause the reaction mixture to gently reflux. The solution was stirred for 45 minutes, before being quenched with aqueous ammonia (30 ml). The reaction mixture was extracted with tetrahydrofuran (3 x 30 ml), the combined organic extracts were washed with distilled water, dried over anhydrous magnesium sulphate and evaporated to dryness under reduced pressure to give (1.72, 90.2 %). m.p. 42^{0}C (lit.[12] 43-45^{0}C) The ^{1}H NMR and ^{13}C NMR data are shown in table 4.24 and 4.25; EI-MS 164, 107.

1-(p-N,N-dimethylaminophenyl)-2,2-dimethyl propan-1-ol (4.15).

Dry magnesium turnings (0.34 g) were placed into a dry flask equipped with condenser and dropping funnel under nitrogen. Freshly dried and distilled tetrahydrofuran (20 ml) were added so as to the flask to just cover the magnesium turnings. A small iodine crystal was added to the mixture which was stirred and the flask was gently warmed with an air gun. To this stirred solution was slowly added a solution of p-bromo-N,N-dimethylaniline (2.79 g, 1.2 equiv.) dissolved in dry tetrahydrofuran (20 ml) at a rate sufficient to just maintain a reflux, the solution was stirred for 30 minutes. To the stirred solution was slowly added dropwise 2,2-dimethylpropal (1 g,) dissolved in dry tetrahydrofuran (10 ml) at such a rate as to only cause the reaction mixture to gently reflux. The solution was stirred for 45 minutes, before being quenched with aqueous ammonia (30 ml). The reaction mixture was extracted with tetrahydrofuran (3 x 30 ml). The combined organic extracts were washed with distilled water (3 x

70 ml), dried over anhydrous magnesium sulphate and evaporated to dryness under reduced pressure. The crude product was purified by silica gel column chromatography (acetonitrile/dichloromethane) to afford a grey solid (1.78g, 74.0 %). I.R. νmax(nujol cm^{-1}) 3612 (free OH); The ^{1}H NMR and ^{13}C NMR data are shown in table 4.26 and 4.27; CI-MS(NH_4^+) m/z; 208, 151.

1-(p-N,N-dimethylaminophenyl)-2,2-dimethyl propane (4.16).

Lithium aluminium hydride (310 mg, 3.3 equiv.) and aluminium chloride (1.00 g, 3 equiv.) were refluxed with dry diethyl ether (20 ml), for 15 minutes. To this refluxing solution was added 1-(p-N,N-dimethylaminophenyl)-2,2-dimethylpropan-1-ol (4.15) (0.52 g, 2.5 mmols) in dry diethyl ether (20 ml). The green mixture was allowed to reflux for 15 minutes and the reaction quenched with aqueous sodium hydrogen carbonate (20 ml). The mixture was extracted with dichloromethane (3 x 50 ml) and the combined organic extracts were washed with distilled water (2 x 70 ml). The organic solution was dried over anhydrous magnesium sulphate, filtered and evaporated to dryness under reduced pressure to give a gum. The residue was purified by silica gel preparative thin layer chromatography (acetonitrile/dichloromethane) to give a brown oil (0.21 mg, 43%). I.R. νmax (nujol cm^{-1}) (no free OH);^{1}H NMR and data is shown in table 4.28; CI-MS(NH_4^+) m/z; 192 (MH^+), 135, 91, 57.

3β-Hydroxy-17,17-ethylenedioxyandrostan-5-en-19-one 3-acetate (3.68).

Chromium trioxide (15.2 g, 38.9 mmol), was slowly added to a stirred solution of dry pyridine (40 ml) and dry dichloromethane. (820 ml). The solution was stirred for 15 minutes at room temperature. To this stirred solution was added 3β,19-dihydroxy-17,17-ethylenedioxy androstan-5-ene 3-acetate (3.68) (15.2 g), in dry dichloromethane (55 ml). The solution was stirred for a further 15 minutes after which the organic layer was decanted and the tarry residue leached with ether (4 x 100 ml) and dichloromethane (3 x 100 ml). Solvent was removed under reduced pressure from the combined organic extracts to afford a dark brown oily residue which was redissolved in dichloromethane and filtered through a bed of celite to remove any inorganic matter. Solvent was removed under reduced pressure from the organic filtrate affording a pale yellow gum (14.8 g, 38.1 mmol). The residue was recrystallised from acetone/hexane furnishing needles (12.2 g, 31.4 mmol, 84 %). m.p. 150 °C (lit.[9] 148°C); I.R. νmax (nujol cm^{-1}), 1730 (C=O), 1712 (C=O); ^{1}H NMR and ^{13}C NMR data are shown in table 4.29 and 4.30; found C 71.18 H 8.27 ($C_{24}H_{32}O_4$ requires C 71.11 H 8.30); EI-MS m/z; 388(MH^+), 328, 289, 229, 99.

3β,19-Dihydroxy-17,17-ethylenedioxy-19-phenyl-androst-5-ene (4.17).

To a stirred solution 3β-hydroxy-17,17-ethylenedioxyandrostan-5-en-19-one 3-acetate (3.68) (1.33 g, 3.42 mmols) dissolved in dry tetrahydrofuran was slowly added a solution of phenyl lithium (7.6 ml. 1.8 M, 4 equiv.). The solution was stirred for 3 hrs, before being quenched with aqueous ammonia. The reaction mixture was decanted and the inorganic residue was leached with tetrahydrofuran. The combined organic extracts were washed with distilled water, dried over anhydrous

magnesium sulphate and evaporated to dryness under reduced pressure. The crude product was purified by silica gel preparative thin layer chromatography (acetonitrile/dichloromethane) to give a gum (1.18g, 81 %). I.R νmax ($CHCl_3$), 3420 (free OH); ^1H NMR and ^{13}C NMR data are shown in table 4.31 and 4.32; high resolution CI-MS(NH_4^+) m/z; 425.2690(MH^+) ($C_{27}H_{37}O_4$ requires 425.2692); EI-MS m/z 318, 307, 300, 219, 201 105, 99; CI-MS(NH_4^+) m/z; 425(MH^+), 407, 319, 238, 107, 99.

3β,19-Dihydroxy-17,17-ethylenedioxy-19-phenyl-androst-5-ene (4.17).

Dry magnesium turnings (0.24 g, 5 equiv.) were placed into a dry flask equipped with condenser and dropping funnel under nitrogen. Minimum freshly dried and distilled tetrahydrofuran (45 ml) was added to the flask to just cover the magnesium turnings. A small iodine crystal was added to the stirred mixture and the flask was gently warmed with an air gun. To this stirred solution was slowly added freshly distilled bromobenzene (1.54g) dissolved in dry tetrahydrofuran (30 ml) at a rate sufficient to just maintain a reflux. The solution was stirred for 30 minutes. To the stirred solution was slowly added 3β-hydroxy-17,17-ethylenedioxyandrostan-5-en-19-one 3-acetate (0.76 g, 2.0 mmols) dissolved in dry tetrahydrofuran (20 ml) at such a rate as to only cause the reaction mixture to gently reflux. The solution was stirred for 2 hrs, before being quenched with aqueous ammonia (80 ml). The reaction mixture was extracted with dichloromethane (3 x 60 ml) and the combined organic extracts were washed with distilled water (3 x 100 ml), dried over anhydrous magnesium sulphate, filtered and evaporated to dryness under reduced pressure. The residue was purified by silica gel preparative thin layer chromatography to give a gum (0.57, 68.2 %).

I.R νmax ($CHCl_3$), 3420 (free OH); ^1H NMR and ^{13}C NMR data are shown in table 4.31 and 4.32; high resolution CI-MS(NH_4^+) m/z; 425.2690(MH^+) ($C_{27}H_{37}O_4$ requires 425.2692); EI-MS m/z 318, 307, 300, 219, 201 105, 99; CI-MS(NH_4^+) m/z; 425(MH^+), 407, 319, 238, 107, 99.

3β,19-Dihydroxy-17,17-ethylenedioxy-19-(p-N,N-dimethylaminophenyl)androst-5-ene (4.21).

Dry magnesium turnings (1.41 g) were placed into a dry flask equipped with condenser and dropping funnel under nitrogen. Minimum freshly dried and distilled tetrahydrofuran (45 ml) was added to the flask to just cover the magnesium turnings. A small iodine crystal was added to the mixture which was stirred and the flask was gently warmed with an air gun. To this stirred solution was slowly added a solution of p-bromo-N,N-dimethylaniline (11.59 g) dissolved in dry tetrahydrofuran (40 ml). At the start of addition the formation of the Grignard reagent was initiated by warming the flask such that the mixture just began to reflux. The refluxing of the solution was sustained by the exothermic nature of formation of the Grignard reagent. The solution was stirred for 30 minutes. To the stirred solution was slowly added 3β-hydroxy-17,17-ethylenedioxyandrostan-5-en-19-one 3-acetate (9.02 g, 19.3 mmols) dissolved in dry tetrahydrofuran (90 ml) at a rate sufficient to cause the reaction mixture to gently reflux. The solution was stirred for 3 hrs, before being quenched with aqueous ammonia. The reaction mixture was decanted and the inorganic residue leached with tetrahydrofuran. The combined organic extracts were washed with distilled water, dried over anhydrous magnesium sulphate and evaporated to dryness under reduced pressure affording a dark green oily residue (27.9 g). The oily residue was redissolved in minimum dichloromethane and treated with minimum hexane to cause the solution to become permanently cloudy and a green

residue separated from solution. The organic layer was decanted, the residue dissolved in warm dichloromethane and stirred with charcoal. The solution was filtered, evaporated to dryness under reduced pressure giving a green gum (7.37, 68.3 %).

I.R. νmax ($CHCl_3$ cm^{-1}) 3605 (free OH); ^1H NMR and ^{13}C NMR data are shown in table 4.33, 4.34 and 4.35; high resolution FAB-MS m/z; 468.3092 ($C_{29}H_{41}O_4N$ requires 468.3114); EI-MS m/z 449, 299, 200, 150, 99; CI-MS(NH_4^+) m/z; 468 (MH_4^+), 450, 329, 236, 150, 99; FAB-MS; 468 (MH^+), 450, 317, 150, 99.

3β-Hydroxy-17-(2-hydroxyethoxy)-19-(p-N,N-dimethylaminophenyl)androst-5-ene (4.24).

Lithium aluminium hydride (33.8 mg, 4 equiv.) and aluminium chloride (118.6 mg, 4 equiv.) were refluxed with dry diethyl ether (10 ml), for 15 minutes. To this solution was added 3β,19-dihydroxy-17,17-ethylenedioxy-19-(p-N,N-dimethylaminophenyl)androst-5-ene (4.21) (104 mg, 0.22 mmols) in dry diethyl ether (5 ml). The green mixture was allowed to reflux for 15 minutes and the reaction quenched with ethyl acetate followed by aqueous sodium hydrogen carbonate (20 ml). The solution was washed with ethyl acetate (3 x 30 ml) and the combined organic extracts were washed with distilled water (2 x 40 ml), aqueous sodium hydrogen carbonate (40 ml) and distilled water (40 ml). The organic solution was dried over anhydrous magnesium sulphate, filtered and evaporated to dryness under reduced pressure to give an oily residue. The residue was purified by silica gel preparative thin layer chromatography (acetonitrile/dichloromethane) to give (15 mg, 15%). ^1H NMR and ^{13}C NMR data are shown in table 4.36 and 4.37; high resolution CI-MS(NH_4^+) m/z; 454.3261 ($C_{29}H_{43}O_3N$ requires 454.3323); EI-MS m/z; 453 (M^+), 435, 419, 392, 374, 301, 258, 239, 134; CI-

MS(NH_4^+) m/z; 454 (MH^+), 436, 391, 239, 134; FAB-MS(NBA) m/z; 454 (MH^+), 436, 391, 239, 134.

3β,19-Dihydroxy-19(p-dimethylaminophenyl) androstan-5-en-17-one (4.25).

3β,19-Dihydroxy-17,17-ethylenedioxy-19-(p-N,N-dimethylamino phenyl)androst-5-ene (4.21) (7.66 g, 16.4 mmols) was dissolved in acetone (130 ml) and stirred with hydrochloric acid (4M, 35 ml). The solution was stirred for 10 minutes before being quenched with saturated sodium hydrogen carbonate solution (100 ml). The aqueous solution was extracted with dichloromethane (3 x 100 ml), the combined organic extracts were dried over anhydrous magnesium sulphate and solvent removed under reduced pressure to afford an oily residue (6.21g) which was purified by silica gel column chromatography to give a gum (2.93 g, 42.3%)

I.R. νmax ($CHCl_3$ cm^{-1}) 3691, 3606 (free OH), 1600 (>C=O); ^1H NMR and ^{13}C NMR data are shown in table 4.38 and 4.39; EI-MS m/z 405,150; CI-MS($NH4^+$) m/z; 406, 273, 256, 273, 256, 150; FAB-MS m/z; 406, 255.

3β,17-Dihydroxy-19-(p-N,N-dimethylaminophenyl) androstan-5-ene (4.26).

To a refluxing solution of aluminium chloride (0.94 g, 4 equiv.) and lithium aluminium hydride (0.27 g, 4 equiv.), in dry ether (30 ml) was added 3β,19-dihydroxy-19-(p-N,N-dimethylaminophenyl)androst-5-en-17-one (4.25) (0.748 g, 1.8 mmols) and refluxed for 15 minutes. The reaction mixture was slowly quenched with ethyl acetate followed by distilled water. The aqueous solution was treated with aqueous sodium hydrogen carbonate solution (40 ml) and extracted with dichloromethane (4 x 40 ml). The combined organic extracts were dried over anhydrous

magnesium sulphate and solvent removed under reduced pressure to give a solid. The solid was recrystallised from acetone furnishing needles (352 mg, 48.76 %). m.p. 234°C; I.R. νmax ($CHCl_3$ cm^{-1}) 3603, 3435 (free OH); ^1H NMR and ^{13}C NMR data are shown in table 4.40 and 4.41; found C 78.94, H 7.64 N 3.42 ($C_{27}H_{39}O_2N$ requires C 79.17 H 7.60 N 3.42); high resolution CI-MS(NH_4^+) m/z; 410.3059 ($C_{27}H_{40}O_2N$ requires 410.3060); EI-MS m/z; 409(M^+), 274, 256, 134; CI-MS(NH_4^+) m/z; 427, 410(MH^+), 392, 275, 257, 134.

3β,17β-Dihydroxy-17α-(prop-1-yne)androst-5-ene (4.30).

To a solution of tetrahydrofuran (150 ml) saturated with propyne at 0°C, was slowly added n-butyllithium solution (in hexane/ether 1.2 ml, 8.0 M), without raising the temperature. The solution was stirred for 30 minutes at 0°C, before a solution of 3β-hydroxyandrost-5-en-17-one (4.29) (1.2 g, 4.16 mmol) dissolved in dry tetrahydrofuran (50 ml) was slowly added. The reaction mixture was stirred for 30 minutes, before being poured into distilled water (300 ml) and treated with sodium hydrogen carbonate solution (50 ml). The aqueous solution was extracted with dichloromethane (3 x 200 ml) and the combined organic extracts were washed with distilled water (3 x 250 ml), dried over anhydrous magnesium sulphate, filtered under reduced pressure and evaporated to dryness under reduced pressure to afford an oily residue (1.28 g, 93%) which was purified by silica gel preparative thin layer chromatography (acetonitrile/dichloromethane) to furnish a solid (1.13 g, 82.7%). m.p. 177°C (lit.[13] 177-179°C), ^1H NMR and ^{13}C NMR data are shown in table 4.42 and 4.43; EI-MS m/z; 328 (M^+), 310; CI-MS(NH_4^+) m/z; 328, 311.

3β,17β,19-Trihydroxy-17α-(prop-1-yne)-19-(p-N,N-dimethylaminophenyl)androst-5-ene (4.27).

To a solution of tetrahydrofuran (150 ml) saturated with propyne at 0°C, was slowly added n-butyllithium solution (in hexane/ether 5 ml, 8.0M), without raising the temperature. The solution was stirred for 30 minutes at 0°C, before 3β,19-dihydroxy-19-(p-N,N-dimethylamino phenyl)androst-5-en-17-one (4.25) (2.44 g) dissolved in dry tetrahydrofuran (100 ml) was slowly added. The reaction mixture was stirred for 30 minutes, before being poured into distilled water (400 ml) and treated with sodium hydrogen carbonate solution (200 ml). The aqueous solution was extracted with dichloromethane (3 x 300 ml) and the combined organic extracts were washed with distilled water (3 x 250 ml), dried over anhydrous magnesium sulphate, filtered under reduced pressure and evaporated to dryness under reduced pressure to afford an oily residue (2.5 g) which was purified by silica gel preparative thin layer chromatography (acetonitrile/dichloromethane) to furnish a green gum (1.43 g, 53.5%).

I.R. νmax ($CHCl_3$ cm^{-1}) 3436, 3602 (free OH); ^1H NMR and ^{13}C NMR data are shown in table 4.44 and 4.45; EI-MS m/z; 463 (M^+), 445, 405, 295, 150; CI-MS(NH_4^+) m/z; 464 (MH^+), 446, 406, 337, 314, 313, 296, 274, 256, 150.

3β,17β-Dihydroxy-17α-(prop-1-yne)-19-(p-N,N-dimethylaminophenyl)androst-5-ene (4.29).

To a refluxing solution of aluminium chloride (1.43 g, 4 equiv.) and lithium aluminium hydride (0.41 g, 4 equiv.), in dry ether (30 ml) was added 3β,17β,19-trihydroxy-17α–(prop-1-yne)-19-(p-N,N-dimethylamino phenyl)androst-5-ene (4.27) (1.24 g, 2.7 mmols) and refluxed for 15 minutes. The reaction mixture was then slowly quenched with ethyl acetate followed by distilled water. The solution was treated with aqueous sodium hydrogen carbonate solution (100 ml) and extracted with dichloromethane (3 x 250). The combined organic extracts were dried over anhydrous magnesium sulphate and solvent removed under reduced pressure to afford a gum which was purified by silica gel column chromatography (acetonitrile/dichloromethane) to furnish (4.31) (220 mg, 17.8%).

I.R. νmax ($CHCl_3$ cm^{-1}) 3604, 3444 (free OH); ^1H NMR and ^{13}C NMR data are shown in 4.46 table and 4.47; high resolution EI-MS m/z; 447.3137 ($C_{30}H_{41}O_2N$ requires 447.3137); EI-MS m/z; 447 (MH^+), 429, 407, 389,134; CI-MS(NH_4^+) m/z; 448 (MH^+), 408, 134; FAB-MS m/z; 448 (MH^+), 409, 134.

3β,17β-Dihydroxy-17α-(prop-1-yne)-19-(p-N,N-dimethylaminophenyl)androst-4-ene-3-one (3.1).

3β,17β-Dihydroxy-17α-(prop-1-yne)-19-(p-N,N-dimethylamino phenyl)androst-5-ene (4.31) (200 g, 0.45 mmols) was refluxed for 3 hrs with aluminium isopropoxide (367 g, 4 eqiv) in dry acetone (50 ml). The solution was treated with aqueous sodium hydrogen carbonate solution (20 ml) and extracted with dichloromethane (3 x 50ml). The combined organic extracts were dried over anhydrous magnesium sulphate and solvent removed under reduced pressure. The crude product was purified by silica gel preparative thin layer chromatography (acetonitrile/ dichloromethane) to furnish (3.1) (11.2 mg, 5.6%).

^{1}H NMR and ^{13}C NMR data are shown in table 4.48 and 4.49; high resolution EI-MS m/z; 445.2981 ($C_{30}H_{39}O_2N$ requires 445.2981); EI-MS m/z; 445 (MH^+), 405, 311, 271, 134; CI-MS(NH_4^+) m/z; 446 (MH^+), 406, 312, 272, 134; FAB-MS m/z; 448 (MH^+), 409, 134.

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