E-Ring Modified Steroids as Novel Potent Inhibitors of 17β-Hydroxysteroid Dehydrogenase Type 1

Delphine S. Fischer,[†] Gillian M. Allan,[†] Christian Bubert,[†] Nigel Vicker,[†] Andrew Smith,[†] Helena J. Tutill,[‡] Atul Purohit,[‡] Lynn Wood,[#] Graham Packham,[#] Mary F. Mahon,[§] Michael J. Reed,[‡] and Barry V. L. Potter*[,†]

Medicinal Chemistry, Department of Pharmacy and Pharmacology and Sterix Ltd., University of Bath, Claverton Down BA2 7AY, U.K., Endocrinology and Metabolic Medicine and Sterix Ltd., Faculty of Medicine, Imperial College, St. Mary's Hospital, London W2 1NY, U.K., Cancer Research UK Oncology Unit, Cancer Sciences Division, University of Southampton School of Medicine, Southampton General Hospital, Southampton SO16 6YD, U.K., and Department of Chemistry, University of Bath, Bath BA2 7AY, U.K.

Received April 14, 2005

17β-Hydroxysteroid dehydrogenases (17β-HSDs) are an important class of steroidogenic enzymes that regulate the bioavailability of active estrogens and androgens and are as yet a relatively unexploited therapeutic target. Based on our investigations and those of others, E-ring modified steroids were identified as a useful template for the design of inhibitors of 17β-HSD type 1, an enzyme involved in the conversion of estrone into estradiol. The synthesis and biological evaluation of a new series of N- and C-substituted 1,3,5(10)-estratrien-[17,16-*c*]-pyrazoles and the corresponding SAR are discussed. Among the N-alkylated analogues, the most potent inhibitor was the 1′-methoxyethyl derivative, **41**, with an IC_{50} of 530 nM in T47-D human breast cancer cells. The X-ray crystal structure of the 1′-isobutyl derivative, **37**, was determined. Further optimization of the template using parallel synthesis resulted in a library of C5′-linked amides from which **73** emerged. This pyridylethyl amide had an IC_{50} of 300 nM and its activity, with that of **41**, suggests the importance of hydrogen bond acceptor groups in the pyrazole side chain. Both **41** and **73** displayed selectivity over 17β-HSD type 2, and preliminary investigations showed **41** to be nonestrogenic in vitro in a luciferase reporter gene assay in contrast to the parent pyrazole **25**. Molecular modeling studies, which support these findings, and a QSAR, the predictive power of which was demonstrated, are also presented.

Introduction

Hormones play a major role in the promotion and development of breast cancer because between one-third and two-thirds of all breast carcinomas rely on estrogens for their sustained growth. Steroidogenic enzyme inhibitors can potentially deplete circulating and tissue levels of active estrogens by blocking their biosynthetic pathways. Such compounds therefore represent an attractive treatment option for patients with hormone-dependent breast cancer (HDBC).[1−5]

In postmenopausal women, who account for nearly 80% of all cases of HDBC, almost all the estrogens are synthesized extraglandularly from inactive precursors. Aromatase inhibitors, which prevent the conversion of androgens into estrogens, have proven therapeutic and are currently used as adjuvant therapy to treat HDBC.[4] It was subsequently shown however that steroid sulfatase is likely to be the primary enzyme responsible for estrone production in hormone-dependent breast tumors: estrone production in such tumors is approximately 10-fold higher per gram of protein for the sulfatase (estrone-3-*O*-sulfate to estrone) pathway than for aromatase.[6,7] Estrone sulfate precursor may thus act as a reservoir for the peripheral and intratumoral synthesis of estrogens where the successive action of steroid sulfatase (STS) and 17β-hydroxysteroid dehydrogenase (17β-HSD) type 1 convert estrone-3-*O*-sulfate (E1S) into the biologically active estradiol (E2). Both of these enzymes therefore represent attractive targets for estrogen suppression strategies. While STS inhibitors are in clinical trials,[8] we decided to explore the inhibition of 17β-HSD type 1 as a therapeutic target.

The 17β-HSD type 1 enzyme is a 34.9 kDa protein with 327 amino acid residues that belongs to the class of 17β-hydroxysteroid dehydrogenases.[9] These enzymes catalyze the interconversion of the oxidized and reduced form of estrogenic and androgenic steroids at the 17-position. The conversion of E1 and E2 involving the 17β-HSDs is outlined in Figure 1. Although they are reversible, their activity is mainly unidirectional and thus can be classified as reductive or oxidative. Of the thirteen isoforms of the enzyme that have been identified to date,[10] eleven exist in humans where they regulate the bioavailability of active estrogens and androgens.[11] While they all require NAD(P)H or NAD(P) as a cofactor, each type has a selective substrate affinity, a directional activity, and a particular tissue distribution. The 17β-HSD type 1 isoform, which converts E1 to E2 and to a minor extent dehydroepiandrosterone (DHEA) to 5-androsten-3β,17β-diol (Δ[5]-diol), is found in the ovaries, endometrium, placenta, and in normal and cancerous breast tissue.[12] Since it preferentially reduces its substrate,[13] the activity of 17β-HSD type 1 directly

* Author to whom correspondence should be addressed. E-mail: B.V.L.Potter@Bath.ac.uk. Phone: +44 1225 386639. Fax: +44 1225 386114.
† University of Bath, Department of Pharmacy & Pharmacology.
‡ Imperial College London.
§ University of Bath, Department of Chemistry.
# University of Southampton.

10.1021/jm050348a CCC: $30.25
Published on Web 08/17/2005

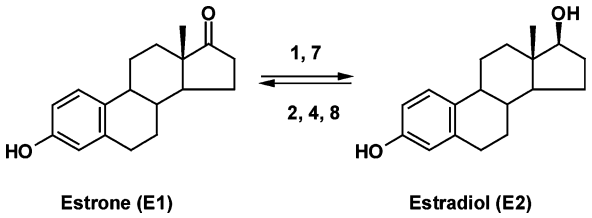


Figure 1. Conversion of E1 and E2 by 17β-HSD subtypes.

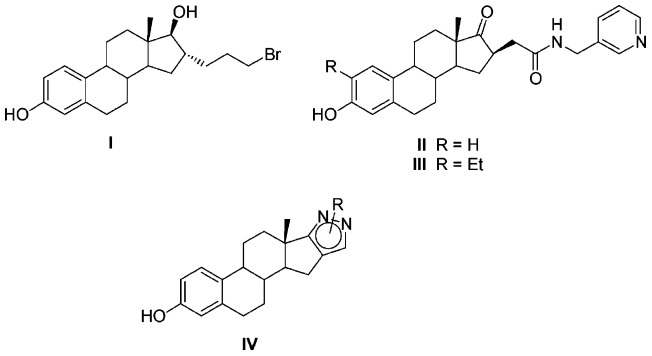


Figure 2. Inhibitors of 17β-HSD type 1 (**I**−**III**) and novel E-ring modified targets (**IV**).

supports the growth and development of estrogen-dependent tumors.[14−16]

Although the therapeutic value of inhibiting 17β-HSD type 1 has been known for many years, relatively few potent and selective inhibitors have been reported in the literature. Early work on affinity ligands identified 3-*O*-methyl-16-bromoacetoxyestradiol,[17] and acetylenic 16-seco-estradiol as alkylating agents.[18] More recently, Poirier et al. reported 16-(bromoalkyl)-estradiols to be active against human placental 17β-HSD type 1.[19] The best inhibitor in this series, 16α-bromopropyl-estradiol, **I** (Figure 2), had an IC_{50} of 0.46 μM and was shown to act via a competitive irreversible mechanism. It was a pure agonist on the estrogen-sensitive human breast tumor cell line ZR-75-1, which is undesirable in endocrine therapy. Attempts to suppress the intrinsic estrogenicity of such compounds using alkylamide side chains led to loss of activity.[20]

Interest in the inhibition of 17β-HSD type 1 was initiated after the identification of various 16-substituted steroids as moderately potent inhibitors of this enzyme. Prompted by these results, we decided to explore the potential of modification of the 16 position on the E1 nucleus, a strategy that has resulted in a recent patent application by our group.[21] Recently, we reported the discovery of the highly potent 17β-HSD type 1 inhibitors **II** and **III** (Figure 2), which showed selectivity over 17β-HSD type 2.[22] As an extension of this work, the 16 position was further explored alone and in combination with 17-substituents. This culminated in the discovery of novel E-ring pyrazole steroids (general formula **IV**, Figure 2) as potent, nonestrogenic inhibitors of 17β-HSD type 1 that did not inhibit 17β-HSD type 2. Herein, we report the chemical synthesis and the biological activity of these inhibitors and describe the SAR studies that led to the identification of active novel compounds. Furthermore, molecular modeling studies and QSAR give encouraging in silico results that support this work.

Chemistry

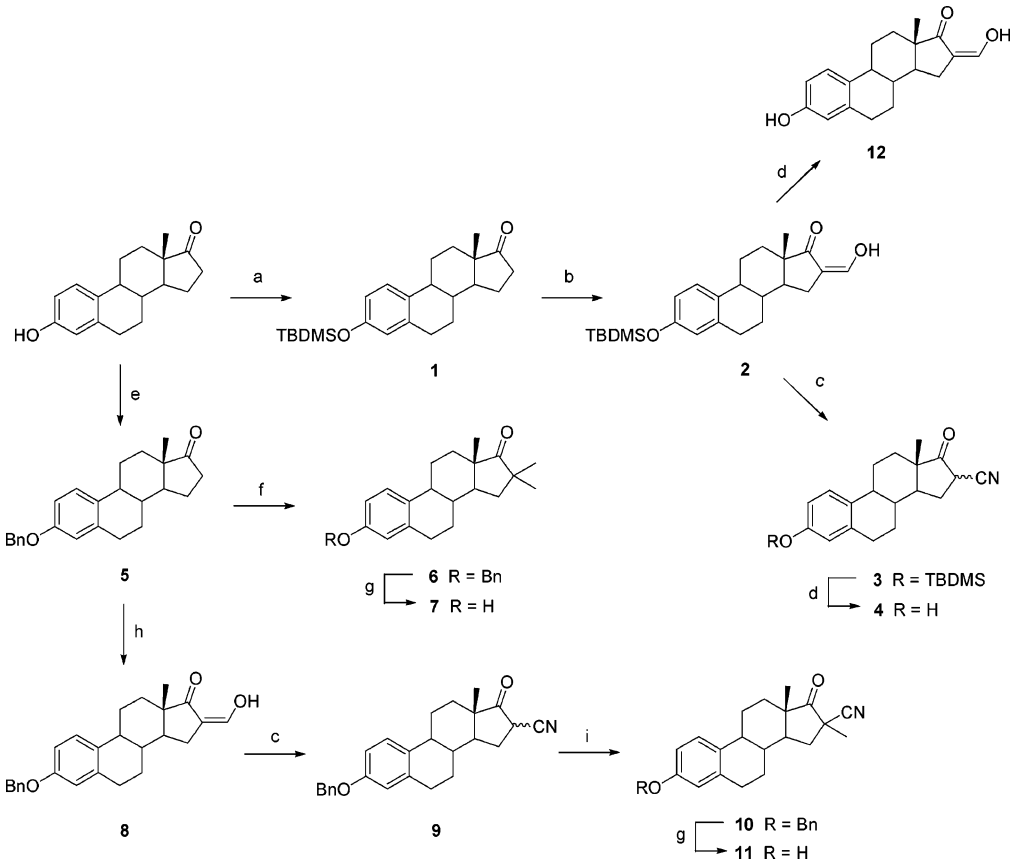
The syntheses of the various 17β-HSD type 1 inhibitors discovered are depicted in Schemes 1−8. Estrone was initially protected as a 3-*tert*-butyldimethylsilyl ether, **1**, and subsequently formylated at C16 as described previously (Scheme 1).[23−26] The reaction of **1** with sodium methoxide and ethyl formate afforded **2** in 98% yield. Conversion of the enol functionality of **2** into a cyano group was performed according to a literature procedure,[27] using *O*,*N*-bis-(trifluoroacetyl)-hydroxylamine in a mixture of toluene and pyridine at reflux. This gave **3** in 71% yield, and subsequent deprotection using a solution of tetrabutylammonium fluoride (TBAF) in THF afforded **4** in a good yield.

The final product **4** was found to exist as a mixture of diastereoisomers in solution in deuterated DMSO with a ratio of 1.6:1 for the two components. Structural assignment of the isomers was made on the basis of ^{1}H NMR−NOE difference experiments involving the signals for the proton at C16 in each isomer (δ 3.84 and δ 4.38) and C18 (δ 0.91 and δ 0.93). Significant enhancement of the C16−H signal at δ 4.38 was observed upon irradiation of a sample of **4** at the resonance frequency of the C18 methyl peak of the minor isomer (δ 0.91). The same experiment on the methyl peak of the major isomer (δ 0.93) did not produce any signal enhancement in the region of C16−H, suggesting a cis (18-methyl,-16-cyano) 16β-configuration for this product. The reciprocal experiments (irradiation at the resonance frequency of the C16−H signals) confirmed these observations. These findings were in agreement with the reported stereochemical ratio of isomers for the 3-methyl ether analogue of **4**, where the β product was identified as the major isomer formed.[28]

Further functionalization at C16 was performed using 3-*O*-benzyl precursors. The benzyl ether **5** was prepared by an adaptation of a reported procedure[29] by reacting E1 with sodium hydride and benzyl bromide (Scheme 1). Further treatment of **5** with sodium hydride and methyl iodide in DMSO afforded **6** in 49% yield. Deprotection of the latter by hydrogenolysis gave **7**. Compound **9** was then accessed following a similar route to that applied for the preparation of **3**, via the 16-hydroxymethylene intermediate **8**. The isomeric mixture of **9** was then subjected to alkylation with methyl iodide to yield **10** as a single isomer. Since we observed correlation with similar 3-methyl ether derivatives of E1, the orientation of the cyano group of **10** was assigned β in accordance with the report of Schaub and co-workers.[28] Subsequent cleavage of the benzyl protecting group of **10** afforded **11**.

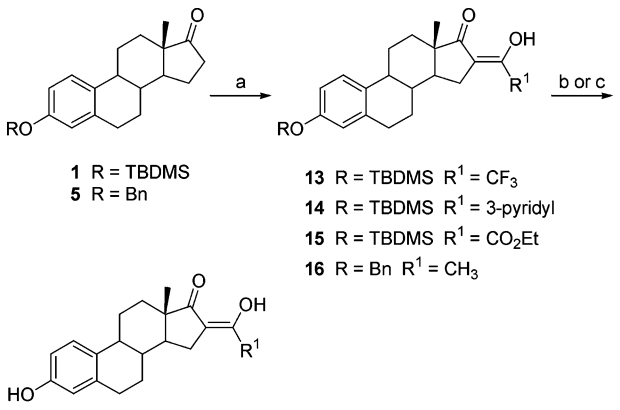
The investigation of compounds devoid of chirality at C16 was then initiated with the 16-hydroxymethylene derivative **12**, which was obtained after deprotection of the synthetic intermediate **2** using TBAF in THF (Scheme 1). Further homologation at the C1′ position was possible by reacting the precursors **1** or **5** with a series of ethyl esters (Scheme 2). Literature precedent for the synthesis of C1′-substituted 16-hydroxymethylene compounds include 16-acetylation and 16-trifluoroacetylation of 3-methoxyestrone.[30,31] The corresponding 3-hydroxy analogues were prepared, along with a pyridyl and an ethyl ester derivative, as part of our SAR investigation. To this end, a solution of **1** in toluene was

Scheme 1[a]



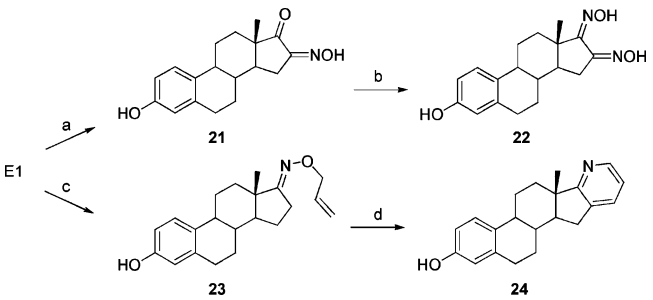
[a] Reagents and conditions: (a) imidazole, *tert*-butyldimethylsilyl chloride (TBDMSCl), DMF; (b) NaOMe/toluene, HCO_2Et; (c) *O*,*N*-bis-(trifluoroacetyl)-hydroxylamine, toluene/pyridine, reflux; (d) TBAF/THF, (e) NaH/DMF, BnBr; (f) NaH/DMSO, MeI; (g) Pd/C, H_2, MeOH/THF; (h) ^{t}BuOK/toluene, HCO_2Et; (i) NaH/DMF, MeI.

Scheme 2[a]



[a] Reagents and conditions: (a) ^{t}BuOK or NaOEt/toluene, R^1CO_2Et; (b) TBAF/THF; (c) Pd/C, H_2, MeOH/THF.

Scheme 3[a]

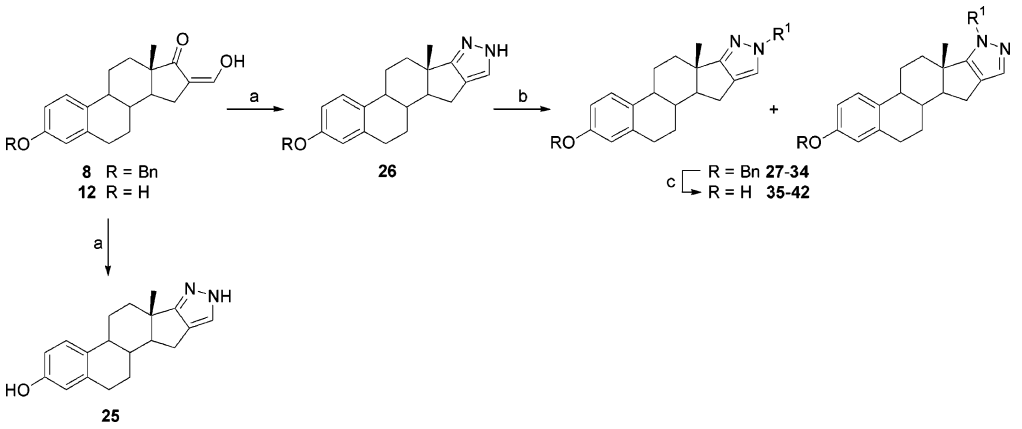


[a] Reagents and conditions: (a) ^{t}BuOK, $(CH_3)_2CH(CH_2)_2ONO$; (b) $H_2NOH.HCl$, NaOAc, MeOH/H_2O; (c) $H_2NOCH_2CHCH_2.HCl$, NaOAc, MeOH/H_2O; (d) 230 °C.

treated with potassium *tert*-butoxide and ethyl trifluoroacetate or ethyl nicotinate. The corresponding products **13** and **14**, obtained respectively in 100% and 77% yield, were then deprotected to afford **17** and **18**. Reaction of **1** with diethyl oxalate in the presence of sodium ethoxide gave **15**, which after deprotection afforded **19** in high yield. Similarly, **5** was reacted with potassium *tert*-butoxide and ethyl acetate at reflux in toluene to give **16** in 80% yield. Subsequent deprotection by hydrogenolysis under standard conditions afforded **20**.

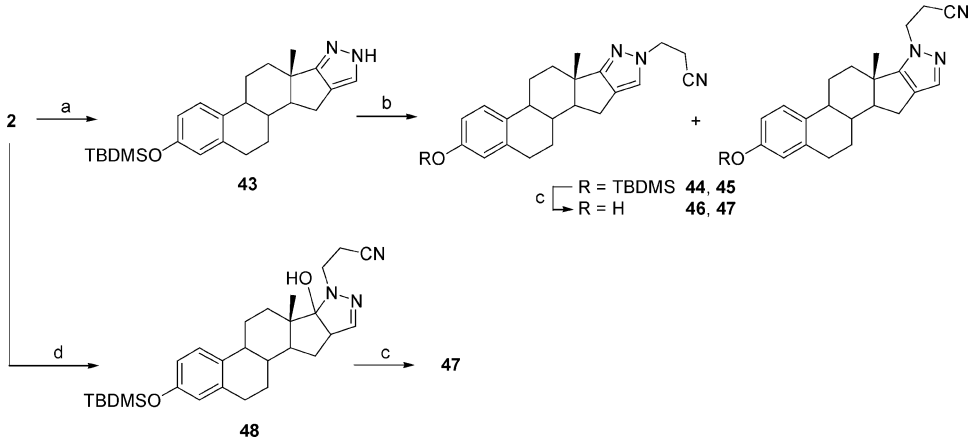
For some of these C1′-substituted derivatives, enol−ketone tautomerism was observed by ^{1}H NMR. Interestingly, while the methyl analogues **16** and **20** were a mixture of tautomers in a solution of $CDCl_3$, the trifluoro derivatives **13** and **17** were found to exist predominantly in the enol form. This is in accordance with previous reports, where it is believed that the electronegative character of the trifluoromethyl group in addition to the electronic properties of fluorine atoms are involved in the stabilization of the enolic form.[31,32] The pyridyl derivatives **14** and **18**, as well as their ethyl ester analogues **15** and **19**, with extended conjugated systems, were also found to be totally enolized in $CDCl_3$.

Scheme 4[a]



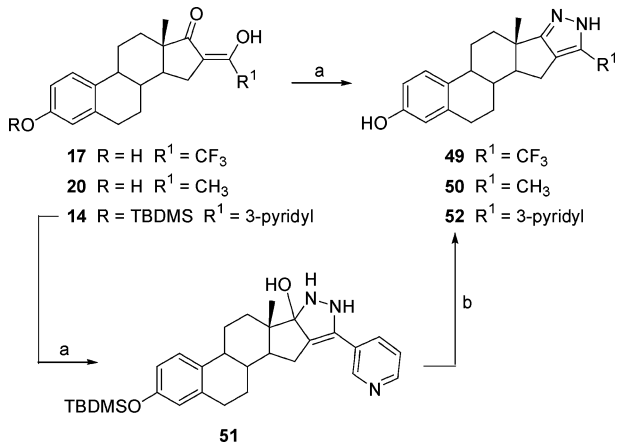
[a] Reagents and conditions: (a) $H_2NNH_2 \cdot H_2O$, EtOH, reflux; (b) NaH/DMF, R^1X; (c) Pd/C, H_2, MeOH/THF.

Scheme 5[a]



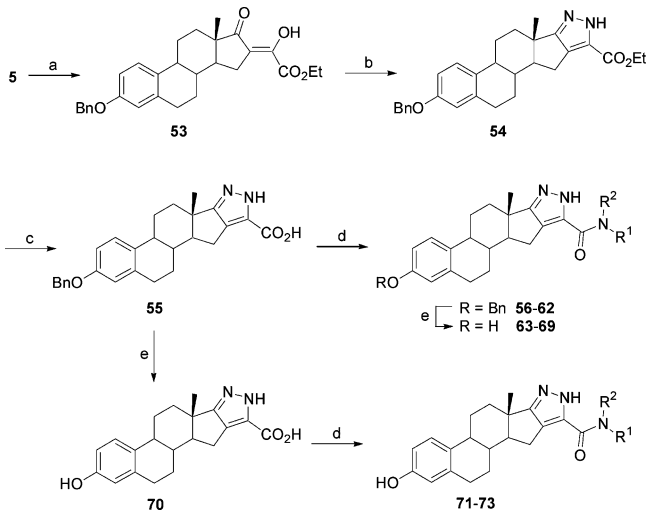
[a] Reagents and conditions: (a) $H_2NNH_2 \cdot H_2O$, EtOH, reflux; (b) ^{t}BuOK/toluene, CH_2CHCN; (c) TBAF/THF; (d) $H_2NNH(CH_2)_2CN$, EtOH.

Scheme 6[a]



[a] Reagents and conditions: (a) $H_2NNH_2 \cdot H_2O$, EtOH, reflux; (b) TBAF/THF.

Scheme 7[a]

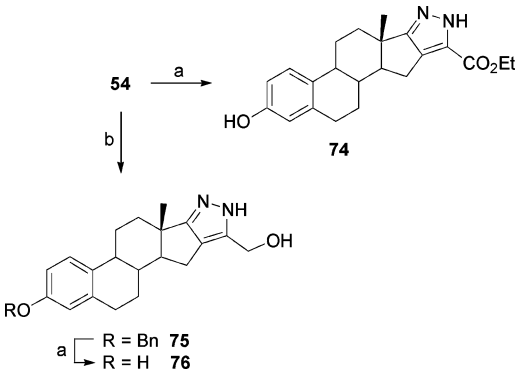


[a] Reagents and conditions: (a) ^{t}BuOK, $(CO_2Et)_2$, toluene; (b) $H_2NNH_2 \cdot H_2O$, EtOH/DCM, reflux, then rt; (c) NaOH/EtOH, reflux; (d) DMAP, EDC, NEt_3, DCM, R^1NHR^2; (e) H_2, Pd/C, EtOH/THF.

Preparation of the 16-oxime derivative **21** was performed as reported previously (Scheme 3).[33] This isosteric analogue of **12** was further reacted with hydroxylamine hydrochloride to give the bis-oximino derivative **22** in moderate yield. Because of the enolic character of **12** and that of some of its C1′ analogues, it was envisaged that internal hydrogen bonding might occur between the carbonyl at C17 and the C1′-OH group to create a pseudo-E-ring system on the steroidal backbone (Figure 3). Similarly, this was conceivable in the oximes **21** and **22**, which prompted us to investigate the synthesis of E-ring five- and six-membered heterocyclic derivatives of E1 as bioisoteric replacements for this intramolecular hydrogen bond network.

A [17,16-*b*]-pyridine derivative of E1 was initially synthesized as a putative six-membered ring mimic where the C=N bond is an isostere of the carbonyl at

Scheme 8[a]



[a] Reagents and conditions: (a) H_2, Pd/C, EtOH/THF; (b) $LiAlH_4$/THF.

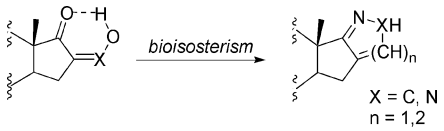
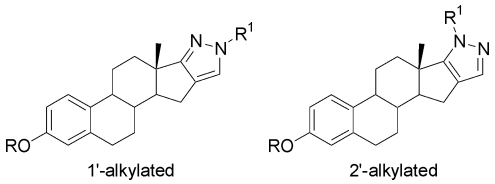


Figure 3. Intramolecular hydrogen bonding in C1′ derivatives of E1 and E-ring fused systems proposed as bioisosters.

C17. The synthesis was accomplished via the thermal rearrangement of an *O*-allyl oxime precursor, a method widely used to generate pyridine heterocycles,[34−37] which has however, to the best of our knowledge, not been applied to steroidal systems. Following the adaptation of a literature procedure,[38] the *O*-allyl oxime derivative **23** was easily obtained in 94% yield after treatment of E1 with *O*-allylhydroxylamine hydrochloride and sodium acetate in a mixture of MeOH and H_2O. The latter was then heated neat at 230 °C for 46 h to give a crude dark material, from which **24** was isolated in spectroscopically pure form after repeated column chromatography. The harsh conditions required for this reaction, which resulted in loss of material through decomposition and a consequent tedious separation of **24** from the crude material explains the poor yield of <10% for this final product. This is also consistent with moderate to low yields generally reported for this rearrangement.[34−37]

To probe the SAR for five-membered E-ring analogues, a series of N- and C-substituted 1,3,5(10)-estratrien-[17,16-*c*]-pyrazoles was synthesized (Schemes 4−8). Besides retaining the carbonyl isostere C=N at C17, the fused pyrazole ring system is also a convenient scaffold with three different attachment points for the rapid exploration of the three-dimensional space. Early reports describe the preparation of steroidal E-ring pyrazoles by reaction of hydrazine with an appropriate hydroxymethylene precursor.[39−41] This strategy was applied to the synthesis of our target compounds starting from **8**, while 3-hydroxy-1,3,5(10)-estratrien-[17,16-*c*]-pyrazole, **25**, was prepared from **12** as previously reported.[42] Condensation of **8** with hydrazine monohydrate in EtOH at reflux rapidly yielded the versatile precursor **26** in high yield. N-Alkylation was then performed under standard conditions, using sodium hydride in DMF, followed by treatment with the desired alkyl halide. As expected, each alkylation yielded two regioisomers corresponding to 1′- and 2′-alkylated products. Their difference in polarity allowed partial or total separation by flash chromatography, yielding the benzyl

Table 1. 1′- and 2′-Alkylated Pyrazole Derivatives of E1



	R			R		
R^1	Bn	TBDMS	H	Bn	TBDMS	H
CH_3	**27**		**35**	**28**		**36**
$CH_2CH(CH_3)_2$	**29**		**37**	**30**		**38**
$CH_2CO_2CH_3$	**31**		**39**	**32**		**40**
$(CH_2)_2OCH_3$	**33**		**41**	**34**		**42**
$(CH_2)_2CN$		**44**	**46**		**45**	**47**

derivatives **27**−**34** in yields ranging from 19% to 48%. Debenzylation by hydrogenolysis yielded the final products **35**−**42** (Table 1).

Structural assignment of the regioisomers was initially made on the basis of ^1H NMR−NOE difference experiments between compounds **27** and **28**. Irradiation of a sample of **27** (the less polar isomer) at the resonance frequency of the C18-methyl signal only yielded background noise. The same experiment performed on **28** (the more polar isomer) yielded a significant peak at δ 3.81, corresponding to the enhancement of the signal of the *N*-methyl group. Given that the NOE effect is only observed over short distances (2−4 Å), the *N*-methyl pyrazole derivative **28** was assigned the structure of a 2′-alkylated compound. Structural assignment of the other members of this series was made by analogy. For each alkyl motif, we also noticed that the chemical shift of the heterocyclic proton (C5′−H) showed a downfield shift of ca. 0.2 ppm in all of the more polar isomers. This can be used to predict the position of the N-substitution by comparing the ^1H NMR spectra of two regioisomers.

The conclusive evidence for assigning the structures of the regioisomers was provided by the X-ray crystal structure of the *N*-isobutyl derivative **37** (Figure 4). This compound was obtained after deprotection of the less polar regioisomer isolated after N-alkylation of **26** with isobutyl bromide. The experiment was performed on a crystal (approximate dimensions 0.50 × 0.50 × 0.30 mm^3) obtained by recrystallization from MeOH. As predicted from the ^1H NMR experiments on **27**/**28**, **37** was found to correspond to a 1′-alkylated product. The ORTEX[43] plot shows all four steroidal rings with the additional heterocyclic system fused to the 16 and 17 positions. The presence of a molecule of MeOH in the cell indicates that **37** cocrystallized with the solvent, to which it hydrogen bonds via O1 of the hydroxyl group at C3. Moreover, N2′ exhibits a hydrogen bond to the methanolic hydrogen, H2, of its closest lattice neighbor, affording hydrogen-bonded ribbons in the supramolecular array [H1···O2 1.847 Å, H2···N2′ 1.911(1) Å; O1−H1−O2 171.1(1)°, O2−H2−N2′ 172.6(1)°]. This is the first report of a crystal structure of a substituted pyrazole fused E-ring steroidal derivative.

While the *N*-methyl, *N*-isobutyl, *N*-methyl acetate and *N*-(2-methoxyethyl) pyrazole derivatives could easily be accessed following the reaction sequence described above, the synthesis of the *N*-cyanoethyl analogues

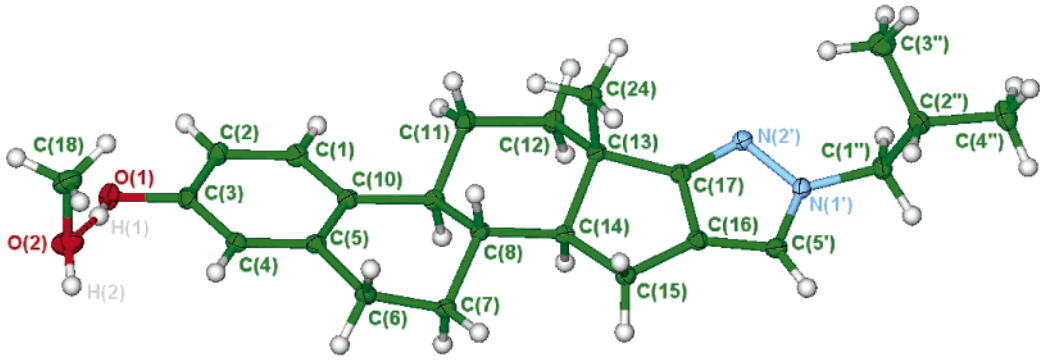


Figure 4. ORTEX[43] plot of the X-ray crystal structure of **37**. Ellipsoids are shown at the 30% probability level.

proved to be problematic. The use of bromopropionitrile to alkylate **26** under a variety of conditions only yielded mixtures in which the starting material was predominant. A 1,4-addition of the pyrazole anion on acrylonitrile was then envisaged to access the *N*-cyanoethyl derivative. For this pathway, the precursor **2** was reacted with hydrazine monohydrate to give **43** in 81% yield. Subsequent reaction with acrylonitrile in toluene yielded the regioisomers **44** and **45** in low yields. Deprotection using TBAF in THF afforded the phenolic derivatives **46** and **47**.

In an attempt to improve on the yields for the synthesis of *N*-cyanoethyl derivatives, **2** was directly reacted with *N*-cyanoethylhydrazine (Scheme 5). The partially condensed product **48** was obtained in 71% yield as the sole product of the reaction. Its structure was assigned on the basis of ^{1}H and ^{13}C NMR data. Reaction of **48** with TBAF afforded the aromatized and deprotected product **47** in good yield.

To further explore the SAR around the pyrazole nucleus, the synthesis of C5′-substituted pyrazole derivatives of E1 was also envisaged, starting from the C1′-substituted 16-hydroxymethylene derivatives previously prepared (Scheme 6). Both **17** and **20** were reacted with hydrazine monohydrate, yielding **49** and **50** in 60% and 35% yield, respectively. Because of the poor solubility of the 3-pyridyl precursor **18**, annulation to give the pyrazole was performed on the protected derivative **14**. The product of this reaction was however not the aromatized heterocyclic derivative but the hydrated analogue **51**, which was obtained in a yield of 89% as a single diastereoisomer. Removal of the TBDMS group using TBAF in THF afforded the dehydrated product **52** in good yield.

Additional C5′-linked pyrazole derivatives of E1 were prepared using solution-phase parallel synthesis (Scheme 7). The intermediate **55**, easily accessible in three steps from **5**, was chosen as the common intermediate for the preparation of a library of amides. Reaction of **5** with diethyloxalate gave **53**, which upon condensation with hydrazine monohydrate, afforded the pyrazole **54**. Hydrolysis of the ethyl ester released the free acid **55**, which was obtained in an overall yield of 77% from **5**. Diversity was then introduced by amide coupling to **55** (method A), after activation of the carboxylic acid moiety with *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC) in dichloromethane (DCM), followed by treatment with the required amine in the presence of 4-dimethylaminopyridine (DMAP) and triethylamine. These coupling reactions were carried out using a Radleys Greenhouse synthesizer followed by purification by flash chromatography, affording **56**−**62** in moderate to low yields. A diminished reactivity of the carboxylic acid at C5′ might explain the difficulties encountered in coupling **55** with amines in high yields. Subsequent deprotection of the benzyl precursors under a hydrogen blanket with Pd/C yielded the amides **63**−**69** (Table 2) in a high average purity of 96%. It is noteworthy that the tetrahydrofuran derivative **66** was obtained upon hydrogenation of the corresponding furan precursor **59** during the deprotection step.

A variation of this method was necessary for the preparation of **71**, since the pyridyl amide side chain was shown to be unstable under hydrogenation conditions. The key precursor **55** was therefore deprotected prior to coupling (method B) to give **70** in 98% yield. Reaction of the latter with 3-picolylamine afforded **71** in a poor 16% yield. The pyridyl-containing amides **72** and **73** were similarly prepared; however, as in the case of **71**, poor reactivity and solubility of the phenolic precursor **70** may have contributed to the low yields obtained.

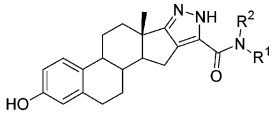
Compound **54** was used for the synthesis of additional final C5′-linked derivatives (Scheme 8). Debenzylation of **54** yielded **74** in 82% yield, while reduction of its ester moiety with $LiAlH_4$ followed by deprotection afforded **76** in good yield.

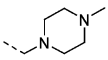
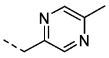
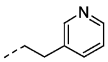
Results and Discussion

Inhibition of 17β-HSD Type 1 and Type 2. The compounds synthesized were tested for their ability to inhibit 17β-HSD type 1 activity in T47-D cells. As a measure of selectivity, inhibition of type 2 activity was also measured for each compound in MDA-MB-231 cells. The evaluation of the ability to inhibit these enzymes was performed by measuring the amount of labeled E1 or E2 formed from the labeled natural substrate. The percentage of inhibition achieved for a 10 μM concentration of the inhibitor, as well as the IC_{50} for some of the most potent compounds, is given in Tables 3−6.

Preliminary investigations around the 16-position of the E1 skeleton demonstrated that the active site of the enzyme was tolerant of small groups with varied steric and electronic properties. This was exemplified by the activity of compounds **4**, **7**, and **11**, where enzymatic activity was inhibited in excess of 77% for a 10 μM concentration of compound. Interestingly, the 16-cyano

Table 2. C5′-Linked Pyrazole Amide Derivatives[a]



compound	R^1	R^2	method	HPLC purity (%)
63		H	A	99
64		H	A	95
65			A	93
66		H	A	100
67		H	A	100
68		H	A	94
69		H	A	89
71		H	B	98
72		H	B	100
73		H	B	88

[a] - - - indicates the point of attachment to the amide nitrogen.

derivative **4**, which is a mixture of diastereoisomers (α/β 1:1.6), was a moderately potent inhibitor of 17β-HSD type 1 with an IC_{50} of 10 μM. Compound **11**, where the cyano group is fixed in β orientation, was found to be equipotent to **4** at 10 μM.

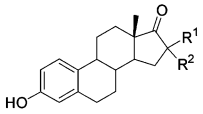
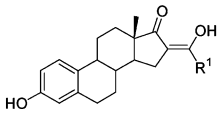
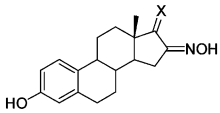
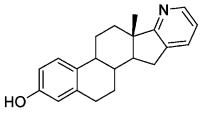
Removing the chirality at C16 led to the discovery of highly potent and selective inhibitors of 17β-HSD type 1. This was exemplified in the series of 16-hydroxymethylene analogues by the unsubstituted compound **12**. With an IC_{50} of 110 nM, it is among the most potent 17β-HSD type 1 inhibitors reported to date and is also selective over 17β-HSD type 2. Measurement of the selectivity of **12** and other compounds reported herein for other steroidogenic enzymes such as 17β-HSD type 3, 3β-HSD, and the 11β-HSDs is part of a planned program of work in this area. Probing for hydrophobic interactions, hydrogen bonding capabilities, or both in the active site gave the moderate inhibitors **17** and **19**. Although 10 times less potent than **12**, the isosteric 16-oximino-estrone **21** (IC_{50} = 1 μM) remained of interest for further investigations. Replacing the C17 carbonyl by an oxime moiety unfortunately further depleted the activity, **22** achieving only 55% inhibition of 17β-HSD type 1 at 10 μM.

Given the possibility of a hydroxy-ketone hydrogen bond network existing between the D-ring moieties in compounds such as **12** or **21**, we investigated E-ring heterocyclic derivatives of E1. The six-membered ring analogue **24** was moderately active against 17β-HSD type 1, which was in accordance with the lower activity of **22** when compared to **21**. This prompted us to probe the activity of compounds containing smaller ring systems, and the choice of a pyrazole E-ring system was driven by several considerations. First, 3-hydroxy-1,3,5-(10)-estratrien-[17,16-*c*]-pyrazole **25** had been reported as a 17β-HSD type 1 inhibitor by Sweet et al.,[42] having a K_i of 4.1 μM on human placental enzyme. Second, five-membered ring systems and in particular pyrazole heterocycles are synthetically accessible, the corresponding steroid derivatives being obtained from 16-hydroxymethylene precursors. In addition, the pyrazole heterocycle is amenable to rapid SAR exploration with three potential sites of diversification, namely N1′, N2′, and C5′, and the C=N bond in the heterocycle is a carbonyl mimic.

In the N-alkylated pyrazole series, all the 1′-substituted compounds significantly inhibited 17β-HSD type 1 activity; however, selectivity over the type 2 isozyme was modest for most of these derivatives. An inhibition of 94% or higher was observed at 10 μM for compounds bearing a side chain with directional electronics, **39**, **41**, and **46** showing IC_{50} values between 530 and 920 nM. A bulky hydrophobic group was not as well tolerated, as suggested by **37**, which was the weakest inhibitor of the series. The methoxyethyl derivative **41** was the most potent analogue (IC_{50} of 530 nM), suggesting the importance of hydrogen bond acceptor interactions of the ether oxygen atom in the side chain.

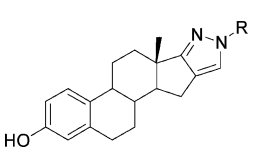
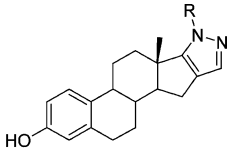
Interestingly, the activity of the 2′-alkylated derivatives was significantly lower than that of the corre-

Table 3. Inhibition of 17β-HSD Type 1 and Type 2 by C16 Derivatives of E1 and **24**

			Inhibition 17β-HSD (% at 10 μM)[a]		IC_{50} (μM)
General Structure	Substituents	Compound	Type 1	Type 2	Type 1
	R^1 = CN, R^2 = H	**4**	77	17	10
	R^1 = Me, R^2 = Me	**7**	95	35	*b*
	R^1 = CN, R^2 = Me	**11**	85	12	*b*
	R^1 = H	**12**	97	15	0.11
	R^1 = CF_3	**17**	78	-1.5	*b*
	R^1 = 3-pyridyl	**18**	*b*	*b*	*b*
	R^1 = CO_2Et	**19**	75	7	*b*
	R^1 = CH_3	**20**	*b*	*b*	*b*
	X = O	**21**	95	5	1.1
	X = NOH	**22**	55	-2	*b*
		**24**	78	34	*b*

[a] Mean of at least two measurements with typically a SD of ±5%. [b] Not determined.

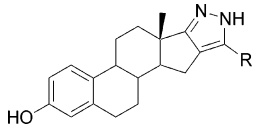
Table 4. Inhibition of 17β-HSD Type 1 and Type 2 by N-Alkylated Pyrazole Derivatives of E1

		% inhibition at 10 μM[a]		IC_{50} (μM)		% inhibition at 10 μM[a]	
R	compd	type 1	type 2	type 1	compd	type 1	type 2
H	**25**[b]	97	32	0.18			
CH_3	**35**	94	24	2.75	**36**	43	32
$CH_2CH(CH_3)_2$	**37**	77	61	*c*	**38**	70	44
$CH_2CO_2CH_3$	**39**	95	19	0.92	**40**	79	30
$(CH_2)_2OCH_3$	**41**	95	48	0.53	**42**	83	55
$(CH_2)_2CN$	**46**	95	44	0.73	**47**	*c*	*c*

[a] Mean of at least two measurements with typically a SD of ±5%. [b] May exist as a 1′-*H* or 2′-*H* tautomer. [c] Not determined.

Table 5. Inhibition of 17β-HSD Type 1 and Type 2 by C5′-substituted Pyrazole Derivatives of E1



		% inhibition at 10 μM[a]		IC_{50} (μM)
R	compd	type 1	type 2	type 1
CF_3	**49**	75	36	*b*
CH_3	**50**	*b*	*b*	*b*
3-pyridyl	**52**	*b*	*b*	*b*
CH_3 CO_2Et	**74**	94	7	1.85
CO_2H	**70**	32	−6	*b*
CH_2OH	**76**	94	−1	0.95

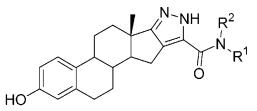
[a] Mean of at least two measurements with typically a SD of ±5%. [b] Not determined.

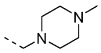
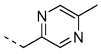
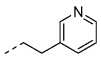
sponding 1′-alkylated compounds with inhibitory activities of 83% or below at 10 μM. The influence of the position of the substitution was especially noticeable in the case of **36**. While its N1′ analogue **35** achieved a level of inhibition close to 95%, compound **36** showed moderate inhibition of type 1 activity at 10 μM. However, for bulkier or longer side chains, the difference between 1′- and 2′-substitution was less marked. The selectivity for 17β-HSD type 1 over 17β-HSD type 2 was also poor for the 2′-alkylated compounds. These results clearly indicate that the active site has a limited tolerance for certain substituents in this area of space, where such moieties may prevent the steroid from binding in a high-affinity orientation.

In our assay, the unsubstituted analogue **25** was highly potent with an IC_{50} of 180 nM. Unexpectedly, the highest drop of activity was provoked by introducing a methyl side chain, **35** being 15 times less potent than **25**. The most potent novel N-alkylated derivative **41** was only 3 times less active than **25**, which indicates the importance of the interactions the side chain may have with the amino acid residues in the active site.

Most of the C5′-substituted pyrazoles prepared displayed activity against 17β-HSD type 1 with a high degree of selectivity over 17β-HSD type 2. In the first series of compounds (Table 5), the ethyl ester **74** and the hydroxymethyl analogue **76** inhibited enzymatic activity by 94% at a 10 μM concentration and had respective IC_{50} values of 1.85 and 0.95 μM. This may indicate the importance of hydrogen bond donor/acceptor interactions in the corresponding region of the active site. The carboxylic acid derivative **70** was however found inactive and probably has detrimental electronic effects in this region. The moderate activity observed

Table 6. Inhibition of 17β-HSD Type 1 and Type 2 by C5′-Amide Pyrazole Derivatives of E1[b]



			Inhibition 17β-HSD (% at 10 μM)[a]		IC_{50} (μM)
R^1	R^2	compound	Type 1	Type 2	Type 1
	H	**63**	62	14	c
	H	**64**	57	0	c
		65	88	6	c
	H	**66**	87	-4	c
	H	**67**	89	-4	2.3
	H	**68**	50	-1	c
	H	**69**	80	61	c
	H	**71**	92	28	0.78
	H	**72**	93	9	0.88
	H	**73**	99	3	0.30

[a] Mean of at least two measurements with typically a SD of ±5%. [b] - - - indicates the point of attachment to the amide nitrogen. [c] Not determined.

for the trifluoromethyl derivative **49** suggested that hydrophobic groups at C5′ were not well tolerated. These observations were also confirmed when the activity of the series of amides **63**−**69** and **71**−**73** (Table 6) was examined.

In this series, the methyl and isopropyl analogues **63** and **64** were among the least potent compounds with moderate inhibition (62% or below) achieved at a 10 μM concentration. In contrast, compounds possessing a heterocyclic group in the side chain displayed overall high activity against the enzyme (>80% inhibition at 10 μM), while remaining selective over 17β-HSD type 2. Weak activity was however observed for **68**, possibly due to the hydrophilic character and the bulk of the side chain. The best inhibitors were obtained with compounds containing a pyridyl side chain, namely, **71**−**73**. These analogues inhibited the enzymatic activity in excess of 92% at 10 μM. The position of the heteroatom was found not to be crucial for the activity, although the 3-pyridyl analogue **71** was slightly more potent than **72** (2-pyridyl). Homologation of the side chain of **71** by one carbon atom gave **73**, the most potent and selective inhibitor of this series, with an IC_{50} of 300 nM.

Such results are consistent with our previous findings that **II** (Figure 1) is a highly potent inhibitor of 17β-HSD type 1.[22] In the crystal structure of human 17β-HSD type 1 co-crystallized with E2 and cofactor, the nicotinamide carbonyl and the amide nitrogen form hydrogen bonds to the NH of Val 188 and the oxygen of Thr 140, respectively. The docked conformation of **II** shows that the carbonyl oxygen of the amide in the side chain is 3.16 Å from the closest nicotinamide NH. This observation indicates that there may be an interaction between the nicotinamide amide moiety and the amide carbonyl of the 16β side chain. In addition, a 16β side chain with sp^3 substitution may hinder the Pro(*S*) hydrogen being transferred to the 17 carbonyl 2.21 Å away. One of the hydrogen atoms in the methylene linker attached to the 16 position is only 2.63 Å from the Pro(*S*) hydrogen, and the methine hydrogen at the 16 position is 1.81 Å away. The pyridyl nitrogen of the 16β side chain is 3.16 Å from a phosphate oxygen and could form an interaction similar to Ile 14 with an alternative phosphate oxygen at the same phosphorus center. These additional interactions proposed for compound **II** with the cofactor may in part explain the high potency of this novel inhibitor and that of compounds such as **73**. Moreover, the fact that compounds bearing polar side chains display overall better activity indicates that they might interact with the cofactor binding domain, resulting in an enhanced affinity similar to **II**.

Molecular Modeling

Compounds **41** and **73** were identified as the most potent and selective inhibitors of 17β-HSD type 1 from our pyrazole library with IC_{50} values of 530 and 300 nM, respectively. The crystal structure of 17β-HSD1 in complex with E2 and NADP (PBD code 1FDT)[44] has previously been used by our group in docking studies of potent 17β-HSD1 inhibitors.[22] To compare the proposed binding modes of the pyrazoles **41** and **73** with **II**, they were docked in a similar manner into 1FDT with E2 removed using the flexible docking program GOLD (Figure 5).[45]

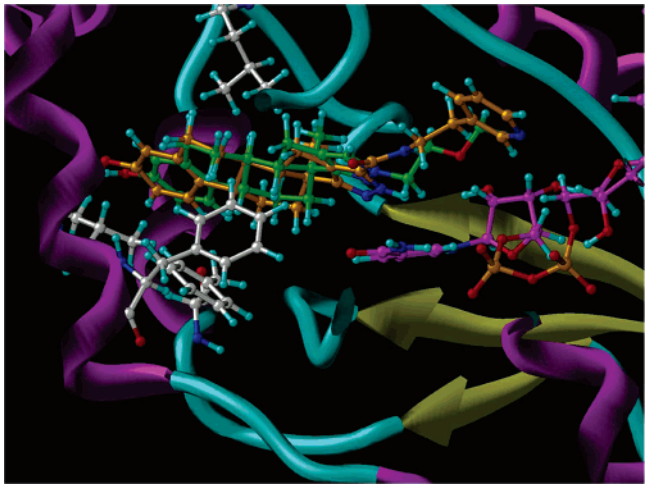


Figure 5. Compounds **41** (green) and **73** (brown) docked in 1FDT.

The top-ranked conformations of **41** and **73** gave high fitness scores of 64.0 and 78.6, respectively, and are depicted in Figure 5. The steroid backbones of **41** and **73** are essentially overlaid when docked into 1FDT forming the same hydrophobic interactions as the steroid substrate. The main common hydrophobic interactions involving the steroid backbone of both **41** and **73** are with amino acids Leu 149, Val 225, Phe 226, and Phe 259 (residues in gray); these hydrophobic interactions are also observed in the crystal structures reported wherein the steroid nucleus is cocrystallized.[44] The hydrogen bond acceptor groups in the side chains of **41** and **73** are in close proximity to the primary amide group of the nicotinamide group of the cofactor and may form favorable electrostatic interactions with this functionality. The ether oxygen atom in the side chain of **41** is 4.0 Å from the closest nicotinamide NH and the amide carbonyl of **73** is a similar distance away (3.8 Å) from the other nicotinamide NH. The side chain substitution distal to the hydrogen bond acceptor groups may form further interactions with the cofactor and protein backbone. These additional interactions of compounds **41** and **73** with the cofactor may in part explain the high potency of these inhibitors.

The novel 17β-HSD type 1 inhibitors described herein for which an IC_{50} had been determined were used to develop a QSAR using the three-dimensional method of comparative molecular field analysis (CoMFA), part of the SYBYL suite, where a set of aligned molecules is used in QSAR generation.[46] The set of molecules was aligned using FlexS;[47,48] CoMFA was then used to calculate the steric and electrostatic interaction energy of a probe atom with each molecule at points on a grid surrounding the molecules. The aligned molecules with the favored and disfavored steric interactions and electrostatic interaction regions are depicted in Figure 6a,b.

Using the IC_{50} values as continuous response data, the statistical tool in QSAR for CoMFA utilizes partial least squares (PLS) for regression analysis.[49] The results of the QSAR are shown in Figure 7, where the negative log of the observed IC_{50} is plotted against the negative log of the predicted IC_{50}. Compounds **41**, **67**, and **71** were used as the validation set and compounds **4**, **12**, **21**, **35**, **39**, **46**, **72**−**74**, and **76** as the training set to predict activity. The graph in Figure 7 shows good correlation of observed versus predicted activity with an r^2 value of 0.9, so the QSAR can be used in a predictive fashion to calculate activities in silico. This QSAR will be further refined as more data becomes available from other series with the same steroid template.

Compound **41** was docked into the crystal structure (PDB code 3ERD)[50] of the ligand binding domain of human estrogen receptor α (ERα) using GOLD. Poor docking scores indicated that **41** did not fit well in ERα: the maximum score observed was 28. This gives an in silico prediction that **41** would not be strongly estrogenic and similar docking studies examining the orientation of binding in ERα have previously proven predictive in identifying ERα selective agonists using the same crystal structure.[51]

Estrogenicity Measurements in Vitro. The N-alkylated compound **41** was evaluated for its estrogenic activity in vitro in a luciferase reporter gene assay. For the purpose of comparison, the nonalkylated pyrazole **25**, for which no estrogenicity data are available, was also tested in this assay, and the results are presented in Figure 8.

From the data collected, it is clear that the presence of a side chain at N1′ contributes to a reduced estrogenicity of the pyrazole nucleus. While **25** is estrogenic at the lowest concentration tested (100 pM), the estrogenic activity of the substituted derivative remains similar to that of the control over the range 100 pM to 100 nM.

These results suggest that the side chain at the N1′ position of the pyrazole template may prevent binding of **41** at the estrogen receptor. Alternatively, **41** may act as an antagonist, and this possibility needs to be further investigated since a compound displaying both inhibition of 17β-HSD type 1 and antiestrogenic properties would be a potential drug for the treatment of HDBC. A previous attempt to design "dual action" inhibitors by Poirier and co-workers had some success and the derivatives that emerged from such investigations were moderately potent against 17β-HSD type 1 but not fully antagonistic.[20]

Conclusions

The inhibition of 17β-HSD type 1 represents an important option in the search for novel endocrine agents to treat HDBC, yet this is a relatively unexplored area. There is a considerable need to identify lead structures and pharmacophores. Not only should the ideal 17β-HSD type 1 inhibitor be highly potent against its target, it also needs to be selective over other 17β-HSD isoforms and devoid of estrogenic activity. All these requirements have resulted in only a few candidates being identified, none of which has entered the clinic or even been shown to be efficacious in vivo. In an effort to develop new potent inhibitors of 17β-HSD type 1, we synthesized E-ring pyrazole derivatives of E1.

Preliminary work in our laboratory has identified the 16,17-fused pyrazole derivatives of E1 as useful templates for the design of novel 17β-HSD type 1 inhibitors. Compound **25** was functionalized at the 1′, 2′, and 5′ positions to build a comprehensive SAR which resulted in the discovery of several active compounds as new 17β-HSD type 1 inhibitors with selectivity over 17β-HSD type 2. The *N*1′-methoxyethyl analogue **41** and C5′-pyridylethyl amide derivative **73** emerged as novel and

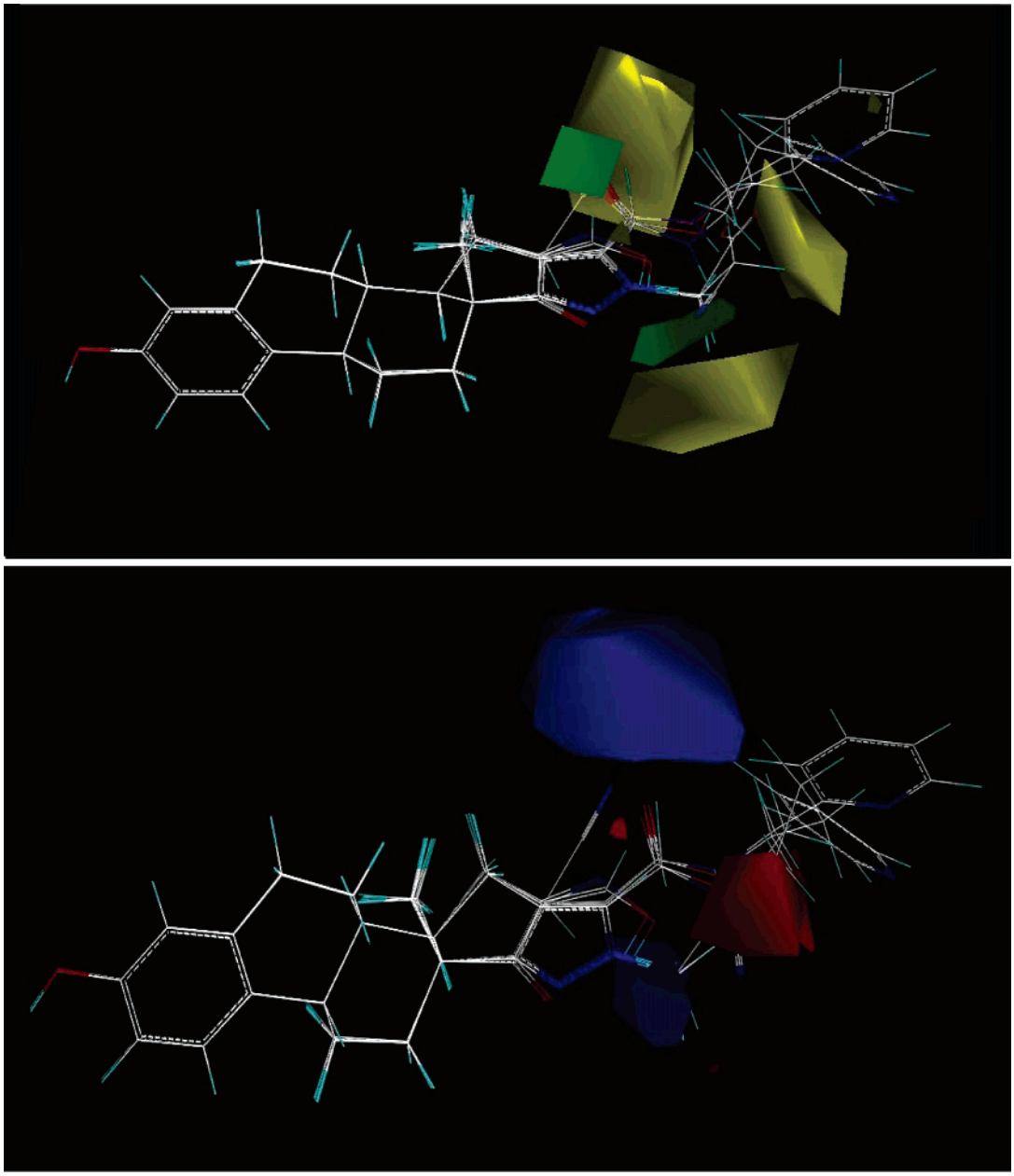


Figure 6. CoMFA alignment (a) showing regions of steric interactions with green indicating favored regions and yellow disfavored steric areas and (b) showing electrostatic interactions with blue areas indicating regions where hydrogen bond acceptor interactions are favored and red showing regions where hydrogen bond donor interactions are favored.

highly potent inhibitors of 17β-HSD type 1 with IC_{50} values of 530 and 300 nM, respectively. This has substantially extended the understanding of potential binding interactions in this area and suggests the importance of hydrogen bond acceptor interactions in the side chain at the N1′ position of the pyrazole template with the amino acid residues in the active site. This also strengthens the hypothesis that compounds with the appropriate side chain at C16 or at an equivalent position may also interact with the cofactor binding site.[22] Preliminary in vitro evaluation showed that N-alkylated compounds such as **41** are devoid of estrogenicity, which is in agreement with predictive in silico methods. New compounds of this type could be of importance to develop proof of concept models for evaluation of the therapeutic potential of in vivo inhibition of 17β-HSD1 in oncology.

Experimental Section

Chemistry. All chemicals were purchased from Aldrich Chemical Co. (Gillingham, U.K.) or Lancaster Synthesis (Morecambe, U.K.). All organic solvents of A. R. grade were supplied by Fisher Scientific (Loughborough, U.K.). E1 was purchased from Sequoia Research Products (Oxford, U.K.).

Thin-layer chromatography (TLC) was performed on precoated plates (Merck TLC aluminum sheets silica gel 60 F_{254}). Product(s) and starting material(s) were detected by either viewing under UV light or treating with an ethanolic solution of phosphomolybdic acid followed by heating, or both. Flash column chromatography was performed on silica gel (Sorbsil/Matrex C60) or using Argonaut prepacked columns with a Flashmaster. IR spectra were recorded on a Perkin-Elmer Spectrum RXI FT-IR as KBr disks, and peak positions are expressed in cm^{-1}. ^1H NMR (270 or 400 MHz) and distortionless enhancement by polarization transfer (DEPT)-edited ^{13}C NMR (100.4 MHz) spectra were recorded with a JEOL Delta 270 or a Varian Mercury VX 400 NMR spectrometer, and

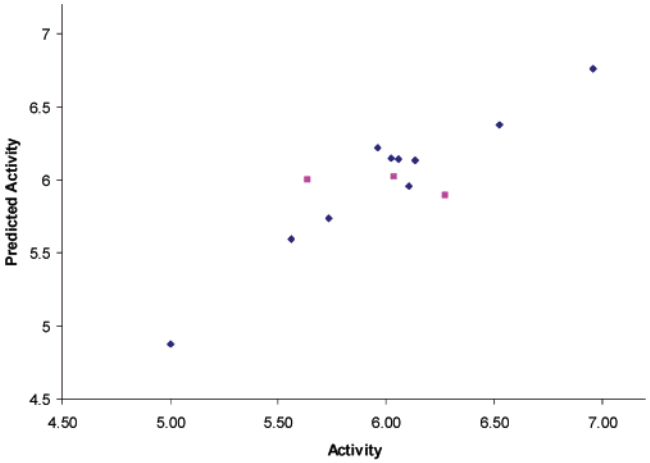


Figure 7. QSAR showing activity against predicted activity (units are -log IC_{50} values). Points in blue are the training set, and compounds in pink are the validation set.

chemical shifts are reported in parts per million (ppm, δ). HPLC analyses were performed on a Waters Millenium 32 instrument equipped with a Waters 996 PDA detector. A Waters Radialpack C18 reversed phase column (8 × 100 mm) was eluted with a MeOH/H_2O gradient at 2 mL/min. Fast atom bombardment (FAB) low- and high-resolution mass spectra were recorded at the Mass Spectrometry Service Center, University of Bath, using *m*-nitrobenzyl alcohol (NBA) as the matrix. Electrospray (ES) and atmospheric pressure chemical ionization (APCI) low-resolution mass spectra were obtained on a Waters Micromass ZQ. Elemental analyses were performed by the Microanalysis Service, University of Bath. Melting points were determined using a Reichert−Jung Thermo Galen Kofler block and are uncorrected. The X-ray crystallographic study of **37** was carried out by Dr. M. F. Mahon in the Department of Chemistry, University of Bath, on a κ CCD diffractometer with area detector.

Compounds **1**,[52] **5**,[29] **12**,[25] and **21**[33,53] have been described in the literature.

Molecular Modeling. For the docking studies of compounds **41** and **73**, the starting conformations used for receptor docking were generated from a random conformational search performed using the MMFF94S force field as implemented in Sybyl 7.0. The resulting lowest energy conformer was then used for docking studies. Charge calculations were determined using the MMFF94S method, and GOLD, version 2.1, was then used with default parameters to perform the docking studies. The active site was defined as a 12 Å radius around the C α atom of serine 142, and 30 attempts were computed for each compound and scored using Gold Score.

To develop a QSAR using CoMFA, the ligands were initially minimized using the MMFF94S force field as implemented within the Sybyl 7.0 package. The molecules were aligned using FlexS with a common core elucidated by DISTILL, which corresponded to the steroid backbone. Gastegier−Huckel charges were used for the charge descriptors in FlexS, and CoMFA was performed using the aligned compounds and the standard Sybyl 7.0 CoMFA fields.

Biology. Radiolabeled E1 and E2 (^{3}H and ^{14}C) were purchased from New England Nuclear (Boston, MA) or Amersham Biosciences UK Limited (Amersham, U.K.).

T47-D and MDA-MB-231 cells have previously been shown to possess predominantly reductive or oxidative 17β-HSD activity, respectively.[54]

Inhibition of 17β-HSD Type 1 Activity. Briefly T47-D human breast cancer cells were incubated with ^{3}H-E1 at a concentration of 2 nM per well in a 24-well tissue culture plate in the absence or presence of the inhibitor (0.1 nM to 10 μM). After incubation of the substrate with or without inhibitor for 30 min at 37 °C, the products were isolated from the mixture by extraction with Et_2O (4 mL) using ^{14}C-E2 (5000 dpm) to monitor procedural losses. Separation of ^{3}H-E2 from the mixture was achieved using TLC (DCM/EtOAc, 4:1 v/v), and the mass of ^{3}H-E2 produced was calculated from the ^{3}H counts detected and recovery of ^{14}C-E2.

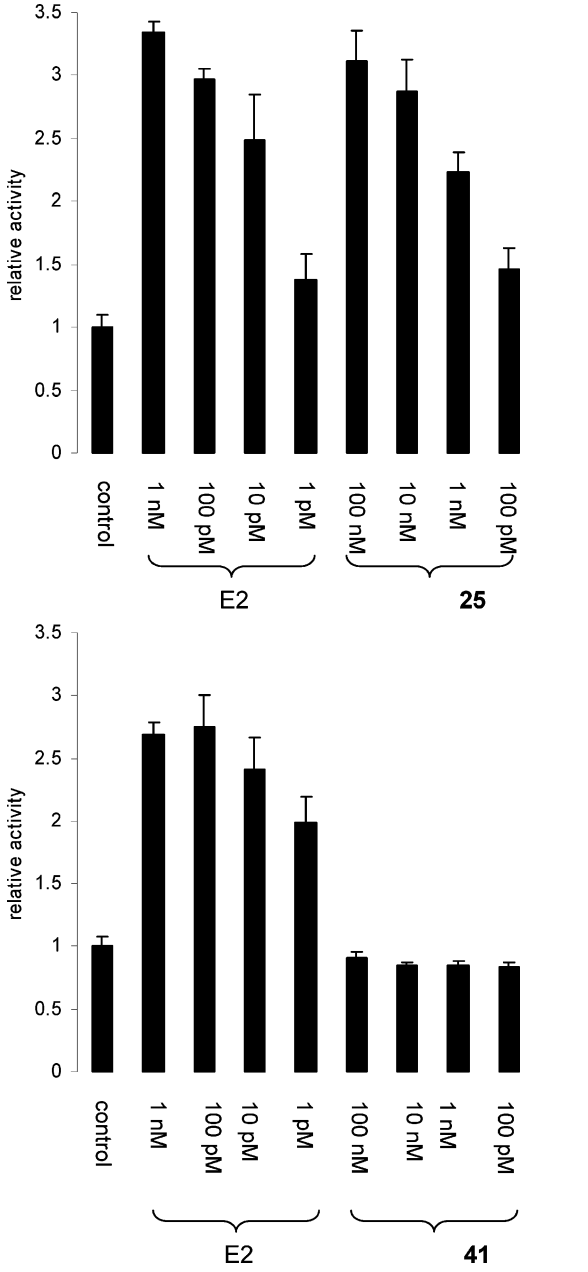


Figure 8. Estrogenic activity of 17β-estradiol (E2), **25**, and **41**. The results are luciferase activity (*y*-axis) relative to control cells (no added compound) in the presence of E2 and compounds **25** and **41** at different concentrations (*x*-axis). Data are means ± SD of triplicate determinations. Figure shows results from two separate experiments each with E2 as a positive control.

Inhibition of 17β-HSD Type 2 Activity. Briefly MDA-MB-231 human breast cancer cells were incubated with ^{3}H-E2 at a concentration of 2 nM per flask in the absence or presence of the inhibitor (0.1 nM to 10 μM). After incubation of the substrate with or without inhibitor for 3 h at 37 °C, the products were isolated from the mixture by extraction with Et_2O (4 mL) using ^{14}C-E1 (5000 dpm) to monitor procedural losses. Separation of ^{3}H-E1 from the mixture was achieved using TLC (DCM/EtOAc, 4:1 v/v), and the mass of ^{3}H-E1 produced was calculated from the ^{3}H counts detected and recovery of ^{14}C-E1.

Estrogenicity Assay. The ability of compounds to activate ERα was determined by transfecting cells with plasmid

pERET81luc, which contains ER binding sites cloned upstream of a minimal thymidine kinase (TK) promoter and the luciferase reporter gene. Cells were cotransfected with an ERα expression plasmid. Addition of estrogenic compounds stimulated the ER and increased the expression of luciferase. Cells were also transfected with a β-galactosidase expression plasmid to control for well-to-well variation in sample handling and potential nonspecific effects of compounds.

Human 293 HEK cells (ERα negative) were cultured for 3 days in growth medium depleted of estrogenic compounds (phenol-red free Dulbecco's modified Eagle's Medium (PRF-DMEM; Invitrogen) supplemented with 10% (v/v) charcoal-stripped serum, glutamine, and antibiotics). Cells (5×10^6) were then plated in a 150 mm tissue culture dish in the same medium. The following day, DNA for transfection was prepared by combining 6 μg of pERET81luc, 6 μg of an ERα expression plasmid (pSG5-HEGO), and 3 μg of a β-galactosidase expression plasmid (CMV-β-gal) in a total volume of 0.1 mL of sterile water. The DNA was added to a mixture of 995 μL of PRF-DMEM and 45 μL of Fugene 6 transfection reagent (Roche), and the solution was gently mixed and incubated at room temperature for 30 min. The DNA/Fugene mix was then added dropwise to the cells and incubated overnight. The following day, cells were recovered by trypsinization, and equal cell numbers were plated in individual wells of a 96-well plate (typically 5000−10 000 cells/well in a total volume of 50 μL). After 2 h, various concentrations of test compounds or 17β-estradiol as a positive control were added (each 50 μL, diluted at 2× final concentration in complete estrogen-depleted growth medium). For the negative control, 50 μL of complete estrogen-depleted growth medium lacking compound was added. Each determination was made in triplicate. The following day, 100 μL of SteadyLite luciferase reagent (Packard/Perkin-Elmer Life Sciences) was added to each well. Cell lysate (100 μL) was transferred to a white 96-well plate, and luciferase activity was determined using a TopCount scinitillation counter (Packard) in single photon counting mode. To measure β-galactosidase activity, 50 μL of cell lysate was transferred to a clear 96-well plate, and 50 μL of *O*-nitrophenyl-β-D-galactopyranoside (ONPG) reagent (Sigma) was added. The plate was incubated at 37 °C for approximately 2 h before measuring absorbance at 405 nm using a Dynatech MR-500 plate reader. Relative luciferase activity was calculated by dividing mean luciferase activity (of triplicate determinations) by mean β-galacotosidase activity, relative to untreated cells (value set at 1).

General Methods. Method 1. Hydrogenolysis. A suspension of Pd−C, 5% or 10% (catalytic), in THF (2 mL) was added to a solution of the starting material in MeOH/THF or EtOH/THF. The resulting suspension was hydrogenated at room temperature using a hydrogen-filled balloon until TLC indicated completion. After removal of the supported catalyst by filtration through Celite and concentration of the filtrate under reduced pressure, the product obtained was purified by flash chromatography, recrystallized, or both.

Method 2. Deprotection using TBAF. A 1.0 M solution of TBAF in anhydrous THF (1.5 or 2 equiv) was added to a stirred solution of the starting material in anhydrous THF under an atmosphere of N_2. The resulting solution was stirred at room temperature until TLC indicated completion, after which the solvent was removed under reduced pressure and H_2O added. For compounds **4**, **18**, and **19**, the final mixture was acidified with AcOH (0.5 mL). The organics were extracted with EtOAc, washed with H_2O and then brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography or recrystallized or triturated.

Method 3. Synthesis of Alkylated Derivatives 27−34. NaH (60% dispersion in mineral oil, 1.5 equiv) was added to a stirred solution of **26** in anhydrous DMF at 0 °C under an atmosphere of N_2. After 20 min of stirring, the parent alkylating agent (2 equiv) was added, and the resulting mixture was stirred at room temperature. The reaction was monitored by TLC and quenched with H_2O (50 mL) at completion. The organics were extracted with EtOAc (2 × 50 mL), washed with H_2O (2 × 30 mL) and brine (2 × 30 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography.

Method 4. Amide Coupling in a Radleys Greenhouse Synthesizer: Synthesis of 56−62. To a solution of **55** (107 mg, 250 μmol) in anhydrous DCM (2 mL) and NEt_3 (25 μL, 180 μmol) under N_2 was added 1 mL of a stock solution containing EDC (58 mg, 300 μmol), NEt_3 (17 μL, 160 μmol), and DMAP (catalytic) in anhydrous DCM. The reaction was stirred at room temperature for 30 min before addition of amine (1.1 equiv), and stirring was continued for 24 h. The mixture was then washed with saturated aqueous $NaHCO_3$ (2 mL) before separation and concentration of the organic layer. The product was semipurified by flash chromatography using EtOAc as eluent, except in the case of **62** for which 5% MeOH in DCM was used. Yields of products ranged from 9% to 35%. Products were then debenzylated without further purification.

Method 5. Hydrogenolysis in a Radleys Greenhouse Synthesizer: Synthesis of 63−69. A solution/suspension of the starting material in EtOH/THF (1:1, 2 mL) was degassed by bubbling N_2 through for 3 min before Pd−C 5% (catalytic) was added. Degassing was continued for 5 min more before hydrogen gas (balloon) was passed over the reaction, and stirring under a hydrogen blanket was continued until TLC indicated completion (up to 48 h). The mixture was then filtered through Celite, and the filtrate was concentrated under reduced pressure. The product was purified by flash chromatography using an elution gradient of hexane to EtOAc with the exception of **68** for which 10% MeOH in DCM was used.

16-Cyano-estrone (4). Following method 2, a solution of **3** (350 mg, 856 μmol) in anhydrous THF (20 mL) was treated with a 1.0 M solution of TBAF in anhydrous THF (1.0 mL, 1.0 mmol) for 30 min. The crude product was triturated in EtOAc/hexane to give **4** as a pale yellow solid (α/β = 1:1.6, 223 mg, 88%): δ_H (DMSO-d_6, 400 MHz) 0.91 (s, C-18-H_3, minor isomer), 0.93 (s, C-18-H_3, major isomer), 1.25−2.82 (13H, m), 3.84 (dd, J = 9.9 Hz, J = 8.8 Hz, C-16-H, major isomer), 4.38 (dd, J = 10.4 Hz, J = 2.0 Hz, C-16-H, minor isomer), 6.45 (1H, d, J = 2.4 Hz, C-4-H), 6.51 (1H, dd, J = 8.4 Hz, J = 2.4 Hz, C-2-H), 7.04 (1H, d, J = 8.4 Hz, C-1-H), 9.05 (s, OH, minor isomer), 9.06 (s, OH, major isomer); MS *m/z* (FAB+) 295.2 [100, $(M + H)^+$]; Acc MS *m/z* (FAB+) 295.1572, $C_{19}H_{21}NO_2$ requires 295.1572. Anal. ($C_{19}H_{21}NO_2$) C, H, N.

3-*O*-Benzyl-16,16-dimethyl-estrone (6). Methyl iodide (155 μL, 2.50 mmol) and NaH (60% dispersion in mineral oil, 146 mg, 3.66 mmol) were added to a stirred solution of **5** (300 mg, 832 μmol) in anhydrous DMSO (15 mL) at room temperature under an atmosphere of N_2. The resulting mixture was stirred at room temperature for 12 h, after which it was heated to 80 °C for 1 h. After the mixture cooled, H_2O (200 mL) was added, and the organics were extracted with EtOAc (2 × 200 mL), washed with H_2O (2 × 150 mL) and then brine (2 × 150 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was recrystallized from EtOH to give **6** as off-white crystals (157 mg, 49%): IR (KBr) 2955−2855, 1735, 1605, 1500, 1235 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 0.93 (3H, s, C-16-H_3), 1.08 (3H, s, C-16-H_3), 1.21 (3H, s, C-18-H_3), 1.18−2.91 (13H, m), 5.02 (2H, s, OCH_2Ar), 6.71 (1H, d, J = 2.7 Hz C-2-H), 6.77 (1H, dd, J = 8.6 Hz, J = 2.7 Hz, C-2-H), 7.18 (1H, d, J = 8.6 Hz, C-1-H), 7.27−7.42 (5H, m, C_6H_5); MS *m/z* (FAB+) 388.1 [47, M^+], 91.0 [100]; Acc MS *m/z* (FAB+) 388.2383, $C_{27}H_{32}O_2$ requires 388.2402.

16,16-Dimethyl-estrone (7). Following method 1, a suspension of **6** (90 mg, 0.225 mmol) and Pd−C 10% (40 mg) in MeOH/THF 2:1 (30 mL) was hydrogenated overnight to give **7** as a white solid (74 mg, 96%). This was recrystallized from MeOH/H_2O to give white crystals (42 mg, 54%): IR (KBr) 3300, 2960−2815, 1720, 1615, 1505−1450, 1235 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 0.95 (3H, s, C-16-H_3), 1.10 (3H, s, C-16-H_3), 1.22 (3H, s, C-18-H_3), 1.37−2.89 (13H, m), 4.75 (1H, s, OH), 6.58 (1H, d, J = 2.7 Hz, C-4-H), 6.64 (1H, dd, J = 8.2 Hz, J = 2.7 Hz, C-2-H), 7.14 (1H, d, J = 8.2 Hz, C-1-H); MS *m/z* (FAB+) 597.2 [61,

$(2M + H)^+$], 452.1 [39, $(M + H + NBA)^+$], 298.2 [100, M^+]; Acc MS *m/z* (FAB+) 298.1934, $C_{20}H_{26}O_2$ requires 298.1933. HPLC (MeOH/H_2O, 90:10) R_t = 3.26 min, 100%.

3-*O*-Benzyl-16-hydroxymethylene-estrone (8). ^{t}BuOK (9.4 g, 84.1 mmol) was added portionwise to a stirred solution of **5** (10 g, 27.7 mmol) in anhydrous toluene (250 mL) at room temperature under an atmosphere of N_2. After it was stirred for 20 min, ethyl formate (14.8 mL, 194 mmol) was added, and the resulting suspension was stirred for 2.5 h. The final mixture was poured into H_2O (300 mL) and acidified with 5 M HCl. The organics were extracted with EtOAc (2 × 200 mL), washed with H_2O (3 × 100 mL) and then brine (2 × 100 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was triturated with boiling EtOAc to give **8** as an off-white solid (9.25 g, 86%): mp 149−151°C; IR (KBr) 3235, 2930−2855, 1700, 1675−1500 cm^{-1}; δ_H (DMSO-d_6, 400 MHz) 0.81 (3H, s, C-18-H_3), 1.31−2.85 (13H, m), 5.05 (2H, s, OCH_2Ar), 6.71 (1H, d, J = 2.7 Hz, C-4-H), 6.75 (1H, dd, J = 8.4 Hz, J = 2.7 Hz, C-2-H), 7.16 (1H, d, J = 8.4 Hz, C-1-H), 7.28−7.33 (1H, m, =C*H*OH), 7.35−7.43 (5H, m, C_6H_5), 10.6−10.9 (1H, br s, exchanged with D_2O, =CHO*H*); δ_C (DMSO-d_6, 100.4 MHz) 15.3 (q, C-18), 24.9 (t), 26.5 (t), 27.1 (t), 30.0 (t), 32.3 (t), 38.2 (d), 44.3 (d), 48.6 (s, C-13), 49.1 (d), 69.7 (t, OCH_2Ar), 112.9 (d), 113.9 (s, C-16), 115.2 (d), 126.7 (d), 128.1 (2 × d), 128.3 (d), 129.0 (2 × d), 132.7 (s), 138.0 (2 × s), 150.8 (d, C-1′), 156.7 (s, C-3), 209.1 (s, C=O); MS *m/z* (FAB+) 389.3 [31, $(M + H)^+$], 91.1 [100, $(CH_2Ar)^+$]; Acc MS *m/z* (FAB+) 389.2099, $C_{26}H_{29}O_3$ requires 389.2117.

3-*O*-Benzyl-16-cyano-estrone (9). *O*,*N*-Bis-(trifluoroacetyl)-hydroxylamine[27] (486 mg, 2.16 mmol) was added to a solution of **8** (300 mg, 772 μmol) and pyridine (460 μL, 5.70 mmol) in toluene (10 mL), and the resulting mixture was heated to reflux for 3 h. After the mixture cooled, H_2O (20 mL) was added, and the organics were separated, washed with H_2O (10 mL) and then brine (10 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography (DCM) to give an off-white solid (200 mg, 67%). This was triturated in EtOAc/hexane to give **9** as light yellow crystals (α/β = 1:1.2, 84 mg, 28%): IR (KBr) 2930−2870, 2240, 1750, 1615, 1605, 1500, 1230 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 0.93 (s, C-18-H_3, minor isomer), 0.95 (s, C-18-H_3, major isomer), 1.32−2.85 (13H, m), 3.87 (dd, J = 10.1 Hz, J = 8.6 Hz, C-16-H, major isomer), 4.40 (dd, J = 10.1 Hz, J = 1.9 Hz, C-16-H, minor isomer), 5.07 (2H, s, OCH_2Ar), 6.74 (1H, d, J = 2.7 Hz, C-4-H), 6.78 (1H, dd, J = 8.4 Hz, J = 2.7 Hz, C-2-H), 7.18 (1H, d, J = 8.4 Hz, C-1-H), 7.31−7.45 (5H, m, C_6H_5); MS *m/z* (FAB+) 385.1 [40, M^+], 91.0 [100, $(CH_2Ar)^+$]; Acc MS *m/z* (FAB+) 385.2053, $C_{26}H_{27}NO_2$ requires 385.2042.

3-*O*-Benzyl-16-cyano,16-methyl-estrone (10). NaH (60% dispersion in mineral oil, 1.2 equiv, 0.248 mmol, 10 mg) was added to a stirred solution of **9** (0.207 mmol, 80 mg) in anhydrous DMF (5 mL) at 0 °C under an atmosphere of N_2. After 30 min of stirring, methyl iodide (0.310 mmol, 19 μL) was added to the white suspension, and the mixture was stirred for 3 h, in which time it was allowed to warm to room temperature. The resulting light yellow solution was then poured into H_2O (50 mL), and the organics were extracted with EtOAc (3 × 20 mL), washed with brine (2 × 20 mL), dried (Na_2SO_4), filtered, and evaporated under reduced pressure. The crude product was purified by flash chromatography (DCM/hexane, 3:2) to give **10** as a crystalline light yellow solid (60 mg, 72%): δ_H (DMSO-d_6, 270 MHz) 1.02 (3H, s, C-18-H_3), 1.25−2.83 (16H, m), 5.06 (2H, s, OCH_2Ar), 6.73−6.80 (2H, m, C-4-H and C-2-H), 7.18 (1H, d, J = 8.4 Hz, C-1-H), 7.29−7.46 (5H, m, C_6H_5); Anal. ($C_{27}H_{29}NO_2$) C, H, N.

16-Cyano,16-methyl-estrone (11). Following method 1, a suspension of **10** (90 mg, 0.225 mmol) and Pd−C 10% (40 mg) in MeOH/THF 1:1 (40 mL) was hydrogenated overnight to give a white foam (46 mg, 66%). A sample was then triturated in hexane to give **11** as a creamy powder: δ_H ($CDCl_3$, 270 MHz) 1.12 (3H, s, C-18-H_3), 1.32−2.92 (16H, m), 4.55 (1H, s, C-3-OH), 6.73−6.58 (1H, app d, C-4-H), 6.63 (1H, dd, J = 8.5 Hz, J = 2.5 Hz, C-2-H), 7.13 (1H, d, J = 8.5 Hz, C-1-H); MS *m/z* (FAB+) 309.1 [60, M^+], 97.1 [50], 83.0 [65]; Acc MS *m/z* (FAB+) 309.1276, $C_{20}H_{23}NO_2$ requires 309.1279. HPLC (MeOH/H_2O, 80:20) R_t = 2.53 min, 98%.

3-*O*-*tert*-Butyl-dimethylsilyl-16-(1′-hydroxy-2′,2′,2′-trifluoro-ethylidene)-estrone (13). ^{t}BuOK (875 mg, 7.80 mmol) was added to a stirred solution of **1** (1.0 g, 2.60 mmol) in anhydrous toluene (20 mL) at 0 °C under an atmosphere of N_2. After 20 min of stirring, ethyl trifluoroacetate (2.16 mL, 18.2 mmol) was added, and the mixture was heated to reflux for 2 h. After the mixture cooled, the resulting dark orange suspension was poured into H_2O (50 mL) and acidified with 5 M HCl. The organics were extracted with EtOAc (2 × 50 mL), washed with H_2O (2 × 30 mL) and then brine (2 × 30 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude orange oil that crystallized on standing was used without further purification (1.4 g, 100%). An analytical sample was recrystallized from EtOH to give **13** as light yellow crystals: mp 145−146 °C; IR (KBr) 2930−2860, 1690, 1640−1495 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 0.19 (6H, s, Si-$(CH_3)_2$), 0.98 (9H, s, C$(CH_3)_3$), 0.99 (3H, s, C-18-H_3), 1.42−2.90 (13H, m), 6.57 (1H, d, J = 2.6 Hz, C-4-H), 6.63 (1H, dd, J = 8.4 Hz, J = 2.6 Hz, C-2-H), 7.11 (1H, d, J = 8.4 Hz, C-1-H), C-1′-OH not seen; δ_C ($CDCl_3$, 100.4 MHz) −4.4 (2×q, Si$(CH_3)_2$), 14.8 (q, C-18), 18.1 (s, *C*$(CH_3)_3$), 25.2 (t), 25.6 (t), 25.7 (3×q, C(*C*$H_3)_3$), 26.7 (t), 29.3 (t), 31.0 (t), 37.5 (d), 43.9 (d), 48.5 (s, C-13), 49.5 (d), 110.9 (s, C-16), 117.4 (d), ∼119 (app d, J = 272 Hz, CF_3), 120.0 (d), 126.0 (d), 132.0 (s), 137.4 (s), 153.6 (s, C-3), ∼154 (app d, J = 38 Hz, C-1′), 216.0 (s, C=O); MS *m/z* (FAB+) 480.1 [47, M^+], 423.0 [38, $(M - C_4H_9)^+$], 73.0 [100]; Acc MS *m/z* (FAB+) 480.2296, $C_{26}H_{35}F_3O_3Si$ requires 480.2308. Anal. ($C_{26}H_{35}F_3O_3Si$) C, H.

3-*O*-*tert*-Butyl-dimethylsilyl-16-(1′-hydroxy-1″-pyridin-3″-ylmethylene)-estrone (14). ^{t}BuOK (875 mg, 7.80 mmol) was added to a stirred solution of **1** (1.0 g, 2.60 mmol) in anhydrous toluene (30 mL) at 0 °C under an atmosphere of N_2. After 20 min of stirring, ethyl nicotinate (2.48 mL, 18.2 mmol) was added, and the mixture was heated to reflux for 1.5 h. After it had cooled, the resulting dark red suspension was poured into H_2O (200 mL) and acidified with 5 M HCl. The organics were extracted with EtOAc (2 × 100 mL), washed with H_2O (100 mL) and then brine (2 × 100 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude yellow solid was recrystallized from EtOH to give **14** as white flaky crystals (985 mg, 77%): mp 169−170 °C; IR (KBr) 2930−2855, 1660, 1605, 1495 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 0.21 (6H, s, Si$(CH_3)_2$), 0.99 (9H, s, C$(CH_3)_3$), 1.11 (3H, s, C-18-H_3), 1.38−2.95 (13H, m), 6.58 (1H, d, J = 2.6 Hz, C-4-H), 6.63 (1H, dd, J = 8.5 Hz, J = 2.6 Hz, C-2-H), 7.12 (1H, d, J = 8.5 Hz, C-1-H), 7.41 (1H, ddd, J = 8.1 Hz, J = 4.9 Hz, J = 0.8 Hz, C-5″-H), 8.09 (1H, app dt, J = 8.1 Hz, J = 2.0 Hz, C-4″-H), 8.69 (1H, dd, J = 4.9 Hz, J = 1.9 Hz, C-6″-H), 8.97 (1H, app dd, J = 2.1 Hz, J = 0.8 Hz, C-2″-H), 13.63 (1H, s, exchanged with D_2O, C-1′-OH); MS *m/z* (FAB+) 490.1 [100, $(M + H)^+$]; Acc MS *m/z* (FAB+) 490.2775, $C_{30}H_{40}NO_3Si$ requires 490.2777.

3-*O*-*tert*-Butyl-dimethylsilyl-16-(1′-hydroxy-2′-carboxylic acid ethyl ester-ethylidene)-estrone (15). Sodium ethoxide (340 mg, 5.00 mmol) was added to a stirred solution of **1** (700 mg, 1.82 mmol) and diethyl oxalate (1.0 mL, 7.36 mmol) in toluene (20 mL). The resulting yellow solution was stirred for 2 h at room temperature after which it was poured into 6 M HCl (20 mL). The organics were extracted with EtOAc (50 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was recrystallized from EtOH to give **15** as colorless crystals (737 mg, 83%): mp 162−163 °C; δ_H ($CDCl_3$, 400 MHz) 0.19 (6H, s, Si$(CH_3)_2$), 0.98 (9H, s, C$(CH_3)_3$), 1.00 (3H, s, C-18-H_3), 1.40 (3H, t, J = 7.2 Hz), 1.43−3.08 (13H, m), 4.36 (2H, q, J = 7.2 Hz), 6.57 (1H, d, J = 2.3 Hz, C-4-H), 6.63 (1H, dd, J = 8.6 Hz, J = 2.3 Hz, C-2-H), 7.11 (1H, d, J = 8.6 Hz, C-1-H), 12.47 (1H, br s, C-1′-OH); δ_C ($CDCl_3$, 100.4 MHz) −4.2 (2×q, Si$(CH_3)_2$), 14.3 (q), 15.0 (q), 18.3 (t), 25.8 (q), 25.9 (t), 26.8 (t), 27.5 (t), 29.5 (t), 31.2 (t), 37.7 (d), 44.0 (d), 48.8 (s), 49.5 (d), 62.0 (t), 116.5 (s), 117.2 (d), 119.9 (d), 125.8 (d), 132.1 (s), 137.4 (s), 152.4 (s), 153.3 (s), 162.5 (s), 216.6 (s); MS *m/z* (FAB+) 485.3 [100, $(M + H)^+$],

73.1 [61]; Acc MS *m/z* (FAB+) 484.2638 $C_{28}H_{40}O_5Si$ requires 484.2645. Anal. ($C_{28}H_{40}O_5Si$) C, H.

3-Benzyloxy-16-(1′-hydroxy-ethylidene)-estra-1,3,5(10)-triene-17-one (16). ^{t}BuOK (438 mg, 4.14 mmol) was added to a stirred solution of **5** (500 mg, 1.38 mmol) in anhydrous toluene (6 mL) and DMSO (1.5 mL) at 0 °C under an atmosphere of N_2. EtOAc (1.27 mL, 17.94 mmol) was then added, and the mixture was heated to reflux for 1 h. After cooling, the resulting brown solution was poured into H_2O (200 mL) and acidified with 5 M HCl. The organics were extracted with EtOAc (2 × 150 mL), washed with H_2O (2 × 100 mL) and then brine (2 × 100 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography (DCM) to yield **16** as a light yellow crystalline solid (447 mg, 80%). This compound was recrystallized from EtOH to give yellow crystals (351 mg, 63%): mp 126−128 °C; IR (KBr) 2930−2860, 1660, 1615−1455 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 0.83, 0.95 (3H, 2 × s, C-18-H_3), 1.38−2.92 (16H, m), 3.43 (app dd, J = 9.4 Hz, J = 8.2 Hz, C-16-H, ketone tautomer), 5.02 (2H, s, OCH_2Ar), 6.71 (1H, d, J = 2.8 Hz, C-4-H), 6.77 (1H, dd, J = 8.3 Hz, J = 2.8 Hz, C-2-H), 7.17 (1H, d, J = 8.3 Hz, C-1-H), 7.28−7.42 (5H, m, C_6H_5), 12.91 (br s, exchanged with D_2O, C-1′-OH, enol tautomer); MS *m/z* (FAB+) 403.1 [68, $(M + H)^+$], 91.0 [100, $(CH_2Ar)^+$]; Acc MS *m/z* (FAB+) 403.2246, $C_{27}H_{31}O_3$ requires 403.2273. Anal. ($C_{27}H_{30}O_3$) C, H.

16-(1′-Hydroxy-2′,2′,2′-trifluoro-ethylidene)-estrone (17). Following method 2, a solution of **13** (1.18 g, 2.42 mmol) in anhydrous THF (50 mL) was treated with a 1.0 M solution of TBAF in anhydrous THF (4.90 mL, 4.90 mmol) for 4 h. The crude product was purified by flash chromatography (DCM) to give **17** as a light brown oil (535 mg, 60%). This compound was crystallized from cold DCM to give white crystals (249 mg, 28%): mp 91−93 °C; IR (KBr) 3430, 2930−2860, 1685, 1610, 1500 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 1.02 (3H, s, C-18-H_3), 1.43−2.91 (13H, m), 6.58 (1H, d, J = 2.7 Hz, C-4-H), 6.64 (1H, dd, J = 8.4 Hz, J = 2.7 Hz, C-2-H), 7.14 (1H, d, J = 8.4 Hz, C-1-H), C-3-OH and C-1′-OH not seen; MS *m/z* (FAB+) 366.1 [100, M^+]; Acc MS *m/z* (FAB+) 366.1476, $C_{20}H_{21}F_3O_3$ requires 366.1443. HPLC (MeOH/H_2O, 96:4) R_t = 1.66 min, 100%. Anal. [$C_{20}H_{21}F_3O_3.(CH_2Cl_2)_{3/4}$] C, H.

16-(1′-Hydroxy-1″-pyridin-3″-ylmethylene)-estrone (18). Following method 2, a solution of **14** (350 mg, 716 μmol) in anhydrous THF (10 mL) was treated with a 1.0 M solution of TBAF in anhydrous THF (1.43 mL, 1.43 mmol) for 3 h. The crude product was triturated with boiling EtOH to give **18** as a pale yellow solid (125 mg, 50%): mp 275−278 °C; IR (KBr) 2950−2855, 1645, 1610−1500 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 1.09 (3H, s, C-18-H_3), 1.39−2.91 (13H, m), 5.48 (1H, s, exchanged with D_2O, C-3-OH), 6.59 (1H, d, J = 2.7 Hz, C-4-H), 6.65 (1H, dd, J = 8.4 Hz, J = 2.7 Hz, C-2-H), 7.15 (1H, d, J = 8.4 Hz, C-1-H), 7.41 (1H, ddd, J = 8.1 Hz, J = 4.9 Hz, J = 0.8 Hz, C-5″-H), 8.09 (1H, dt, J = 8.1 Hz, J = 1.8 Hz, C-4″-H), 8.67 (1H, dd, J = 4.9 Hz, J = 1.8 Hz, C-6″-H), 8.94 (1H, app d, J = 1.8 Hz, C-2″-H), 13.58 (1H, s, exchanged with D_2O, C-1′-OH); MS *m/z* (FAB+) 376.0 [100, $(M + H)^+$], 242.1 [54]; Acc MS *m/z* (FAB+) 376.1903, $C_{24}H_{26}NO_3$ requires 376.1913. Anal. [$C_{24}H_{25}NO_3.(H_2O)_{1/2}$] C, H, N.

16-(1′-Hydroxy-2′-carboxylic acid ethyl ester-ethylidene)-estrone (19). Following method 2, a solution of **15** (485 mg, 1.00 mmol) in anhydrous THF (25 mL) was treated with a 1.0 M solution of TBAF in anhydrous THF (1.50 mL, 1.50 mmol) for 1 h. The crude product was recrystallized from EtOAc/hexane to give **19** as colorless crystals (303 mg, 82%): mp 200−202 °C; δ_H ($CDCl_3$, 400 MHz) 1.00 (3H, s, C-18-H_3), 1.40 (3H, t, J = 7.2 Hz), 1.42−3.09 (13H, m), 4.36 (2H, q, J = 7.2 Hz), 4.61 (1H, br s, OH), 6.59 (1H, d, J = 2.3 Hz, C-4-H), 6.64 (1H, dd, J = 8.2 Hz, J = 2.3 Hz, C-2-H), 7.14 (1H, d, J = 8.2 Hz, C-1-H), 12.47 (1H, br s, OH); δ_C (DMSO-d_6, 100.4 MHz) 14.2 (q), 15.0 (q), 25.9 (t), 26.7 (t), 27.5 (t), 29.4 (t), 31.1 (t), 37.7 (d), 43.9 (d), 48.8 (s), 49.4 (d), 62.2 (t), 112.8 (d), 115.2 (d), 116.5 (s), 126.2 (d), 131.6 (d), 137.7 (s), 152.4 (s), 153.4 (s), 162.6 (s), 216.7 (s); MS *m/z* (FAB+) 370.2 [100, $(M + H)^+$]. Anal. ($C_{22}H_{26}O_5$) C, H.

16-(1′-Hydroxy-ethylidene)-estrone (20). Following method 1, a suspension of **16** (200 mg, 497 μmol) and Pd−C 10% (80 mg) in MeOH/THF 2:1 (30 mL) was hydrogenated for 2 h to give **20** as a white solid (98 mg, 63%). This product was recrystallized from acetone/hexane to give white crystals (72 mg, 46%): mp 211−214 °C; IR (KBr) 3450, 2915−2860, 1705, 1655−1510 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 0.83, 0.96 (3H, 2 × s, C-18-H_3), 1.39−2.89 (16H, m), 3.43 (dd, J = 9.4 Hz, J = 8.2 Hz, C-16-H, ketone tautomer), 4.70 (1H, br s, exchanged with D_2O, C-3-OH), 6.55−6.57 (1H, m, C-4-H), 6.62 (1H, dd, J = 8.0 Hz, J = 2.7 Hz, C-2-H), 7.11 (1H, d, J = 8.0 Hz, C-1-H), C-1′-OH (enol tautomer) not seen; MS *m/z* (FAB+) 313.1 [100, $(M + H)^+$], 73.0 [19]; Acc MS *m/z* (FAB+) 313.1799, $C_{20}H_{25}O_3$ requires 313.1804. Anal. ($C_{20}H_{24}O_3$) C, H.

3-Hydroxy-16,17-bis-oximino-estra-1,3,5(10)-triene (22). NaOAc (556 mg, 6.79 mmol) followed by hydroxylamine hydrochloride (520 mg, 7.48 mmol) was added to a solution of **21** (200 mg, 668 μmol) in a mixture of MeOH/H_2O 5:1 (36 mL). The resulting pale yellow solution was stirred at room-temperature overnight, after which the solvent was removed under reduced pressure and brine was added (100 mL). The organics were extracted with EtOAc (100 mL), washed with brine (2 × 50 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was recrystallized from acetone to give **22** as off-white crystals (109 mg, 52%): mp 245−247 °C; IR (KBr) 3420−3200, 3020, 2935−2870, 1705, 1620, 1585−1500 cm^{-1}; δ_H (DMSO-d_6, 400 MHz) 0.97 (3H, s, C-18-H_3), 1.28−2.83 (13H, m), 6.44 (1H, d, J = 2.4 Hz, C-4-H), 6.51 (1H, dd, J = 8.5 Hz, J = 2.4 Hz, C-2-H), 7.04 (1H, d, J = 8.5 Hz, C-1-H), 9.02 (1H, s, exchanged with D_2O, OH), 10.81 (1H, s, exchanged with D_2O, NOH), 11.19 (1H, s, exchanged with D_2O, NOH); δ_C (DMSO-d_6, 100.4 MHz) 14.5 (q, C-18), 26.5 (t), 27.1 (t), 27.3 (t), 29.5 (t), 35.5 (t), 37.4 (d), 43.4 (d), 46.2 (s, C-13), 49.0 (d), 113.2 (d), 115.4 (d), 126.4 (d), 130.5 (s), 137.4 (s), 155.5 (s), 155.7 (s), 160.2 (s); MS *m/z* (FAB+) 315.2 [100, $(M + H)^+$]; MS *m/z* (FAB−) 466.2 [66, $(M - H + NBA)^-$], 313.2 [100, $(M - H)^-$], 276.1 [80]; Acc MS *m/z* (FAB+) 315.1711, $C_{18}H_{23}N_2O_3$ requires 315.1709. HPLC (MeOH/H_2O, 70:30) R_t = 2.71 min, 99%. Anal. [$C_{18}H_{22}N_2O_3.(CH_3)_2O$] C, H, N; C: calcd, 67.72; found 67.30.

3-Hydroxy-17-*O*-allyl-oximino-estra-1,3,5(10)-triene (23). NaOAc (3 g, 10.17 mmol) followed by *O*-allyl-hydroxylamine hydrochloride (4.5 g, 41.44 mmol) was added to a solution of E1 (1.0 g, 3.70 mmol) in a mixture of MeOH/H_2O 5:1 (180 mL). The resulting solution was stirred at room-temperature overnight, after which the solvent was removed under reduced pressure and H_2O was added (200 mL). The organics were extracted with EtOAc (300 mL), washed with H_2O (2 × 100 mL) and then brine (2 × 100 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give a white product (1.37 g). The crude material was recrystallized from MeOH/H_2O to give **23** as white crystals (1.13 g, 94%): mp 81−83 °C; IR (KBr) 3455, 3185, 2955−2845, 1640−1505, 1240 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 0.94 (3H, s, C-18-H_3), 1.35−2.87 (15H, m), 4.50−4.60 (2H, m, C-1′−H_2), 4.74 (1H, s, exchanged with D_2O, OH), 5.19 (1H, app dq, C-3′−H), 5.28 (1H, app dq, C-3′−H), 5.59−6.01 (1H, m, C-2′−H), 6.56 (1H, d, J = 2.7 Hz, C-4-H), 6.63 (1H, dd, J = 8.2 Hz, J = 2.7 Hz, C-2-H), 7.15 (1H, d, J = 8.2 Hz, C-1-H); δ_C ($CDCl_3$, 100.4 MHz) 17.7 (q, C-18), 23.4 (t), 26.5 (t), 26.6 (t), 27.6 (t), 30.0 (t), 34.5 (t), 38.5 (d), 44.3 (d), 44.8 (s, C-13), 53.3 (d), 74.6 (t, C-1′), 113.0 (d), 115.5 (d), 117.3 (t, C-3′), 126.7 (d), 132.4 (s), 134.7 (d, C-2′), 138.2 (s), 153.7 (s, C-3), 171.3 (s, C=N); MS *m/z* (FAB+) 326.1 [100, $(M + H)^+$], 268.1 [30]; Acc MS *m/z* (FAB+) 326.2116, $C_{21}H_{28}NO_2$ requires 326.2120. HPLC (MeOH/H_2O, 90:10) R_t = 3.01 min, 99%. Anal. ($C_{21}H_{27}NO_2$) C, H, N.

3-Hydroxy-estra-1,3,5(10)-triene-[17,16-*b*]-pyridine (24). Compound **23** (2.45 g, 7.53 mmol) was stirred and heated to 230 °C (using a sand bath) for 46 h. The resulting dark brown solid was allowed to cool, and EtOH was added until most of the solid was dissolved. The remaining undissolved material was crushed to a fine powder, silica was added, and the solvent was removed under reduced pressure. This was purified by flash chromatography ($CHCl_3$/EtOAc, 9:1) to give **24** as a dark

orange solid (200 mg, 9%). A second flash chromatography ($CHCl_3$/EtOAc, 95:5) yielded a light orange powder (146 mg, 6%). For analysis, a sample was triturated with $CHCl_3$ to give an off-white powder: mp 279−284 °C (dec); IR (KBr) 3415, 3115−3025, 2985−2855, 1615−1500 cm^{-1}; δ_H (DMSO-d_6, 400 MHz) 0.92 (3H, s, C-18-H_3), 1.36−2.83 (13H, m), 6.47 (1H, d, J = 2.4 Hz, C-4-H), 6.54 (1H, dd, J = 8.4 Hz, J = 2.4 Hz, C-2-H), 7.05−7.09 (2H, m, C-1-H and C-5′−H), 7.60 (1H, d, J = 7.4 Hz, C-4′−H), 8.25 (1H, app d, J = 4.3 Hz, C-6′−H), 9.05 (1H, s, exchanged with D_2O, OH); δ_H ($CDCl_3$, 400 MHz) 1.01 (3H, s, C-18-H_3), 1.43−2.96 (13H, m), 6.08 (1H, br s, OH), 6.61 (1H, d, J = 2.6 Hz, C-4-H), 6.67 (1H, dd, J = 8.4 Hz, J = 2.6 Hz, C-2-H), 7.04 (1H, dd, J = 7.4 Hz, J = 4.8 Hz, C-5′−H), 7.18 (1H, d, J = 8.4 Hz, C-1-H), 7.54 (1H, d, J = 7.4 Hz, C-4′−H), 8.33 (1H, d, J = 4.8 Hz, C-6′−H); δ_C (DMSO-d_6, 100.4 MHz) 18.5 (q, C-18), 26.9 (t), 27.9 (t), 29.9 (t), 30.4 (t), 34.4 (t), 38.2 (d), 44.7 (d), 46.3 (s, C-13), 55.4 (d), 113.5 (d), 115.7 (d), 121.7 (d), 126.5 (d), 130.9 (s), 133.2 (d), 136.4 (s), 137.7 (s), 147.2 (d), 155.6 (s, C-3), 173.1 (s, C═N); MS *m/z* (FAB+) 306.1 [85, $(M + H)^+$], 207.0 [95], 114.9 [100]; Acc MS *m/z* (FAB+) 306.1864, $C_{21}H_{24}NO$ requires 306.1858. HPLC ($MeOH/H_2O$, 80:20) R_t = 2.90 min, 98%. Anal. [$C_{21}H_{23}NO.(CHCl_3)_{1/5}$] C, H, N.

3-Benzyloxy-estra-1,3,5(10)-triene-[17,16-*c*]-pyrazole (26). Hydrazine hydrate (751 μL, 15.44 mmol) was added to a suspension of **8** (4 g, 10.3 mmol) in EtOH (200 mL) at room temperature under an atmosphere of N_2. The resulting yellow suspension was heated to reflux for 45 min and allowed to cool. After acidification with 5 M HCl, the solvent was removed under reduced pressure until precipitation of the product. H_2O (20 mL) was then added, and the precipitate was filtered and dried to give **26** as an off-white powder (3.73 g, 94%): mp 102−105°C; IR (KBr) 3410, 2930−2860, 1610−1500, 1255 cm^{-1}; δ_H (DMSO-d_6, 400 MHz) 0.91 (3H, s, C-18-H_3), 1.36−2.87 (13H, m), 5.05 (2H, s, OCH_2Ar), 6.72 (1H, d, J = 2.7 Hz, C-4-H), 6.76 (1H, dd, J = 8.6 Hz, J = 2.7 Hz, C-2-H), 7.18 (1H, d, J = 8.6 Hz, C-1-H), 7.27 (1H, s, C-5′−H), 7.29−7.44 (5H, m, C_6H_5), 12.00 (1H, s, exchanged with D_2O, NH); MS *m/z* (FAB+) 385.3 [75, $(M + H)^+$], 91.1 [100, $(CH_2Ar)^+$]; Acc MS *m/z* (FAB+) 385.2280, $C_{26}H_{29}N_2O$ requires 385.2280.

3-Benzyloxy-estra-1,3,5(10)-triene-[17,16-*c*]-(1′-methyl)-pyrazole (27) and 3-Benzyloxy-estra-1,3,5(10)-triene-[17,16-*c*]-(2′-methyl)-pyrazole (28). Following method 3, **26** (150 mg, 390 μmol) was treated with NaH (19 mg, 468 μmol) in DMF (6 mL), and the subsequent reaction with methyl iodide (57 μL, 780 μmol) was complete within 50 min. Purification of the crude mixture by flash chromatography (DCM/EtOAc, 98:2 to 95:5, gradient) gave two products.

The less polar fraction gave **27** as a light yellow oil that crystallized on standing (52 mg, 33%): mp 133−135 °C; IR (KBr) 2930−2860, 1606−1500, 1245 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 1.01 (3H, s, C-18-H_3), 1.42−2.99 (13H, m), 3.84 (3H, s, N−CH_3), 5.03 (2H, s, OCH_2Ar), 6.73 (1H, d, J = 2.5 Hz, C-4-H), 6.79 (1H, dd, J = 8.5 Hz, J = 2.5 Hz, C-2-H), 6.97 (1H, s, C-5′−H), 7.22 (1H, d, J = 8.5 Hz, C-1-H), 7.29−7.45 (5H, m, C_6H_5); δ_C ($CDCl_3$, 100.4 MHz) 18.4 (q, C-18), 24.0 (t), 26.2 (t), 27.4 (t), 29.6 (t), 34.1 (t), 37.5 (d), 38.5 (q, N−CH_3), 40.8 (s, C-13), 44.4 (d), 61.1 (d), 69.8 (t, OCH_2Ar), 111.9 (d), 114.6 (d), 121.5 (s), 124.0 (d), 125.9 (d), 127.1 (2 × d), 127.5 (d), 128.2 (2 × d), 132.6 (s), 136.9 (s), 137.5 (s), 156.4 (s), 168.6 (s); MS *m/z* (FAB+) 399.3 [74, $(M + H)^+$], 91.1 [100, $(CH_2Ar)^+$], 73.0 [24]; Acc MS *m/z* (FAB+) 399.2447, $C_{27}H_{31}N_2O$ requires 399.2436. Anal. ($C_{27}H_{30}N_2O$) C, H, N.

The more polar fraction gave **28** as an off-white solid (55 mg, 35%): mp 168−171°C; IR (KBr) 2930−2870, 1640−1495, 1255 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 1.01 (3H, s, C-18-H_3), 1.44−2.98 (13H, m), 3.82 (3H, s, N−CH_3), 5.04 (2H, s, OCH_2Ar), 6.74 (1H, d, J = 2.8 Hz, C-4-H), 6.80 (1H, dd, J = 8.6 Hz, J = 2.8 Hz, C-2-H), 7.15 (1H, s, C-5′−H), 7.20 (1H, d, J = 8.6 Hz, C-1-H), 7.30−7.45 (5H, m, C_6H_5); δ_C ($CDCl_3$, 100.4 MHz) 17.9 (q, C-18), 24.6 (t), 26.5 (t), 27.7 (t), 30.1 (t), 34.5 (t), 37.3 (q, N−CH_3), 37.8 (d), 42.0 (s, C-13), 44.6 (d), 62.3 (d), 70.3 (t, OCH_2Ar), 112.6 (d), 115.1 (d), 123.9 (s), 126.2 (d), 127.6 (2 × d), 128.1 (d), 128.7 (2 × d), 132.7 (s), 133.4 (d), 137.4 (s), 138.1 (s), 157.0 (s), 1 singlet not seen; MS *m/z* (FAB+) 399.3 [55, $(M + H)^+$], 91.1 [100, $(CH_2Ar)^+$]; Acc MS *m/z* (FAB+) 399.2434, $C_{27}H_{31}N_2O$ requires 399.2436. Anal. ($C_{27}H_{30}N_2O$) C, H, N.

3-Benzyloxy-estra-1,3,5(10)-triene-[17,16-*c*]-(1′-isobutyl)-pyrazole (29) and 3-Benzyloxy-estra-1,3,5(10)-triene-[17,16-*c*]-(2′-isobutyl)-pyrazole (30). Following method 3, **26** (250 mg, 650 μmol) was treated with NaH (31 mg, 780 μmol) in DMF (10 mL) and the subsequent reaction with 1-bromo-2-methyl-propane (124 μL, 1.30 mmol) was complete within 2 h. Purification of the crude mixture by flash chromatography (DCM/EtOAc, 95:5) gave two products.

The less polar fraction gave **29** as a pale yellow oil (121 mg, 42%): IR (KBr) 2960−2870, 1705, 1640−1495 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 0.87 (3H, d, J = 6.6 Hz, C-3″-H_3), 0.89 (3H, d, J = 6.6 Hz, C-4″-H_3), 1.01 (3H, s, C-18-H_3), 1.42−2.98 (14H, m), 3.81 (1H, dd, J_{BA} = 13.8 Hz, J = 7.5 Hz, N−C$H_A$$H_B$), 3.86 (1H, dd, J_{AB} = 13.8 Hz, J = 7.5 Hz, N−$CH_A$$H_B$), 5.04 (2H, s, OCH_2-Ar), 6.71−6.75 (1H, m, C-4-H), 6.79 (1H, dd, J = 8.6 Hz, J = 2.7 Hz, C-2-H), 6.94−6.99 (1H, m, C-5′−H), 7.22 (1H, d, J = 8.6 Hz, C-1-H), 7.31−7.46 (5H, m, C_6H_5); MS *m/z* (FAB+) 441.2 [100, $(M + H)^+$], 91.1 [93, $(CH_2Ar)^+$]; Acc MS *m/z* (FAB+) 441.2894, $C_{30}H_{37}N_2O$ requires 441.2906.

The more polar fraction gave **30** as a white crystalline solid (56 mg, 19%): mp 126−128 °C; IR (KBr) 2960−2870, 1635−1455, 1255 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 0.92 (3H, d, J = 6.6 Hz, C-3″-H_3), 0.95 (3H, d, J = 6.6 Hz, C-4″-H_3), 1.02 (3H, s, C-18-H_3), 1.46−2.94 (14H, m), 3.74 (1H, dd, J_{BA} = 13.3 Hz, J = 7.8 Hz, N−C$H_A$$H_B$), 3.85 (1H, dd, J_{AB} = 13.3 Hz, J = 7.4 Hz, N−$CH_A$$H_B$), 5.04 (2H, s, OCH_2Ar), 6.74 (1H, d, J = 2.6 Hz, C-4-H), 6.79 (1H, dd, J = 8.6 Hz, J = 2.6 Hz, C-2-H), 7.17−7.21 (2H, m, C-1-H and C-5′−H), 7.31−7.45 (5H, m, C_6H_5); MS *m/z* (FAB+) 441.1 [100, $(M + H)^+$], 91.1 [60, $(CH_2Ar)^+$]; Acc MS *m/z* (FAB+) 441.2898, $C_{30}H_{37}N_2O$ requires 441.2906.

3-Benzyloxy-estra-1,3,5(10)-triene-[17,16-*c*]-(1′-methyl acetate)-pyrazole (31) and 3-Benzyloxy-estra-1,3,5(10)-triene-[17,16-*c*]-(2′-methyl acetate)-pyrazole (32). Following method 3, **26** (300 mg, 78 μmol) was treated with NaH (47 mg, 1.17 mmol) in DMF (10 mL) and the subsequent reaction with methyl chloroacetate (136 μL, 1.56 mmol) was complete within 2.5 h. Purification of the crude mixture by flash chromatography (DCM to DCM/EtOAc, 8:2, gradient, Flashmaster) gave two products.

The less polar fraction gave **31** as a white crystalline solid (173 mg, 48%): mp 133−135°C; IR (KBr) 2960−2850, 1750, 1740, 1615−1500, 1265, 1255 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 1.03 (3H, s, C-18-H_3), 1.44−2.99 (13H, m), 3.76 (3H, s, OCH_3), 4.83 (1H, d, J_{BA} = 17.8 Hz, N−C$H_A$$CH_B$), 4.88 (1H, d, J_{AB} = 17.8 Hz, N−CH_ACH_B), 5.04 (2H, s, OCH_2Ar), 6.74 (1H, d, J = 2.6 Hz, C-4-H), 6.79 (1H, dd, J = 8.6 Hz, J = 2.6 Hz, C-2-H), 7.07 (1H, s, C-5′−H), 7.22 (1H, d, J = 8.6 Hz, C-1-H), 7.29−7.44 (5H, m, C_6H_5); δ_C ($CDCl_3$, 100.4 MHz) 18.9 (q, C-18), 24.5 (t), 26.7 (t), 27.9 (t), 30.1 (t), 34.4 (t), 38.0 (d), 41.4 (s, C-13), 44.8 (d), 52.9 (d or q), 53.2 (t, N−CH_2), 61.3 (d or q), 70.3 (t, OCH_2-Ar), 112.5 (d), 115.1 (d), 123.2 (s), 125.0 (d), 126.4 (d), 127.7 (2 × d), 128.1 (d), 128.7 (2 × d), 133.1 (s), 137.5 (s), 138.0 (s), 156.9 (s), 169.1 (s), 169.8 (s); MS *m/z* (FAB+) 457.3 [55, $(M + H)^+$], 91.1 [100, $(CH_2Ar)^+$], 73.0 [72]; Acc MS *m/z* (FAB+) 457.2495, $C_{29}H_{33}N_2O_3$ requires 457.2491.

The more polar fraction gave **32** as a pale yellow solid (70 mg, 20%): mp 78−82 °C; IR (KBr) 2930−2850, 1760, 1740, 1635−1500, 1260, 1210 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 1.02 (3H, s, C-18-H_3), 1.42−2.98 (13H, m), 3.78 (3H, s, OCH_3), 4.80 (1H, d, J_{BA} = 17.2 Hz, N−C$H_A$$CH_B$), 4.86 (1H, d, J_{AB} = 17.2 Hz, N−CH_ACH_B), 5.03 (2H, s, OCH_2Ar), 6.74 (1H, d, J = 2.6 Hz, C-4-H), 6.78 (1H, dd, J = 8.6 Hz, J = 2.6 Hz, C-2-H), 7.18 (1H, d, J = 8.6 Hz, C-1-H), 7.25 (1H, s, C-5′−H), 7.31−7.44 (5H, m, C_6H_5); MS *m/z* (FAB+) 457.3 [64, $(M + H)^+$], 91.1 [100, $(CH_2Ar)^+$], 73.0 [27]; Acc MS *m/z* (FAB+) 457.2507, $C_{29}H_{33}N_2O_3$ requires 457.2491.

3-Benzyloxy-estra-1,3,5(10)-triene-[17,16-*c*]-(1′-methoxyethyl)-pyrazole (33) and 3-Benzyloxy-estra-1,3,5(10)-triene-[17,16-*c*]-(2′-methoxyethyl)-pyrazole (34). Following method 3, **26** (300 mg, 780 μmol) was treated with NaH (47 mg, 1.17 mmol) in DMF (10 mL), and the subsequent reaction with 1-chloro-2-methoxy-ethane (142 μL, 1.56 mmol) was

complete within 4 h. Purification of the crude mixture by flash chromatography (DCM/EtOAc, 9:1) gave two products.

The less polar fraction gave **33** as a pale yellow solid (92 mg, 27%): mp 97−98 °C; IR (KBr) 2930−2870, 1615, 1500, 1255, 1105 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 1.01 (3H, s, C-18-H_3), 1.46−2.99 (13H, m), 3.33 (3H, s, OCH_3), 3.67−3.76 (2H, m, C-1″-H_2 or C-2″-H_2), 4.17−4.28 (2H, m, C-1″-H_2 or C-2″-H_2), 5.04 (2H, s, OCH_2Ar), 6.74 (1H, d, J = 2.8 Hz, C-4-H), 6.80 (1H, dd, J = 8.3 Hz, J = 2.8 Hz, C-2-H), 7.09 (1H, s, C-5′−H), 7.23 (1H, d, J = 8.3 Hz, C-1-H), 7.31−7.45 (5H, m, C_6H_5); MS *m/z* (FAB+) 443.3 [100, $(M + H)^+$], 91.1 [80, $(CH_2Ar)^+$]; Acc MS *m/z* (FAB+) 443.2696, $C_{29}H_{35}N_2O_2$ requires 443.2698.

The more polar fraction gave **34** as a pale yellow solid (80 mg, 23%): mp 86−88 °C; IR (KBr) 2930−2850, 1635−1495 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 1.04 (3H, s, C-18-H_3), 1.42−2.99 (13H, m), 3.32 (3H, s, OCH_3), 3.73−3.83 (2H, m, C-1″-H_2 or C-2″-H_2), 4.14−4.22 (2H, m, C-1″-H_2 or C-2″-H_2), 5.04 (2H, s, OCH_2Ar), 6.74 (1H, d, J = 2.7 Hz, C-4-H), 6.79 (1H, dd, J = 8.6 Hz, J = 2.7 Hz, C-2-H), 7.19−7.21 (2H, m, C-1-H and C-5′−H), 7.30−7.44 (5H, m, C_6H_5); MS *m/z* (FAB+) 443.3 [100, $(M + H)^+$], 91.1 [95, $(CH_2Ar)^+$], 73.1 [44]; Acc MS *m/z* (FAB+) 443.2712, $C_{29}H_{35}N_2O_2$ requires 443.2698.

3-Hydroxy-estra-1,3,5(10)-triene-[17,16-*c*]-(1′-methyl)-pyrazole (35). Following method 1, a suspension of **27** (50 mg, 125 μmol) and Pd−C 10% (20 mg) in MeOH/THF 2:1 (30 mL) was hydrogenated overnight to give a pale yellow solid (31 mg). The crude product was recrystallized from EtOH/H_2O to yield **35** as off-white crystals (26 mg, 68%): mp 287−289°C; IR (KBr) 3115, 2975−2855, 1605−1500 cm^{-1}; δ_H (DMSO-d_6, 400 MHz) 0.89 (3H, s, C-18-H_3), 1.32−2.83 (13H, m), 3.73 (3H, s, N−CH_3), 6.45 (1H, d, J = 2.4 Hz, C-4-H), 6.52 (1H, dd, J = 8.5 Hz, J = 2.4 Hz, C-2-H), 7.06 (1H, d, J = 8.5 Hz, C-1-H), 7.24 (1H, s, C-5′−H), 9.02 (1H, s, OH); MS *m/z* (FAB+) 309.2 [100, $(M + H)^+$], 219.2 [52]; Acc MS *m/z* (FAB+) 309.1975, $C_{20}H_{25}N_2O$ requires 309.1967. HPLC (MeOH/H_2O, 96:4) R_t = 1.90 min, 98%. Anal. ($C_{20}H_{24}N_2O$) C, H, N.

3-Hydroxy-estra-1,3,5(10)-triene-[17,16-*c*]-(2′-methyl)-pyrazole (36). Following method 1, a suspension of **28** (50 mg, 125 μmol) and Pd−C 10% (20 mg) in MeOH/THF 2:1 (30 mL) was hydrogenated overnight to give a white solid (40 mg). The crude product was recrystallized from EtOH to yield **36** as white crystals (20 mg, 53%): mp 321−323 °C (dec); IR (KBr) 3105−3010, 2935−2860, 1610−1500; δ_H (DMSO-d_6, 400 MHz) 0.95 (3H, s, C-18-H_3), 1.32−2.83 (13H, m), 3.73 (3H, s, N−CH_3), 6.45 (1H, d, J = 2.6 Hz, C-4-H), 6.52 (1H, dd, J = 8.4 Hz, J = 2.6 Hz, C-2-H), 7.04 (1H, s, C-5′−H), 7.06 (1H, d, J = 8.4 Hz, C-1-H), 9.03 (1H, s, OH); MS *m/z* (FAB+) 391.3 [28], 309.2 [100, $(M + H)^+$]; Acc MS *m/z* (FAB+) 309.1974, $C_{20}H_{25}N_2O$ requires 309.1967. HPLC (MeOH/H_2O, 96:4) R_t = 2.41 min, 99%.

3-Hydroxy-estra-1,3,5(10)-triene-[17,16-*c*]-(1′-isobutyl)-pyrazole (37). Following method 1, a suspension of **29** (110 mg, 250 μmol) and Pd−C 10% (40 mg) in MeOH/THF 2:1 (30 mL) was hydrogenated overnight to give a white solid (78 mg). The crude product was recrystallized from MeOH to yield **37** as colorless crystals (42 mg, 48%): mp 122−123 °C; IR (KBr) 3235, 2955−2853, 1635, 1610, 1575−1455, 1230 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 0.87 (3H, d, J = 6.4 Hz, C-3″-H_3), 0.89 (3H, d, J = 6.4 Hz, C-4″-H_3), 1.01 (3H, s, C-18-H_3), 1.42−2.95 (14H, m), 3.81 (1H, dd, J_{BA} = 13.7 Hz, J = 7.4 Hz, N−C$H_A$$H_B$), 3.86 (1H, dd, J_{AB} = 13.7 Hz, J = 7.4 Hz, N−C$H_A$$H_B$), 5.61 (1H, s, exchanged with D_2O, OH), 6.59 (1H, d, J = 2.6 Hz, C-4-H), 6.65 (1H, dd, J = 8.4 Hz, J = 2.6 Hz, C-2-H), 6.97 (1H, s, C-5′−H), 7.16 (1H, d, J = 8.4 Hz, C-1-H); δ_C ($CDCl_3$, 100.4 MHz) 18.5 (q, C-18), 20.0 (2 × t, C-3″ and C-4″), 24.1 (t), 26.4 (t), 27.5 (t), 29.6 (t), 30.0 (d), 34.2 (t), 37.6 (d), 41.0 (s, C-13), 44.5 (d), 59.6 (t, N−CH_2), 61.2 (d), 112.8 (d), 115.3 (d), 121.2 (s), 123.9 (d), 126.4 (d), 132.5 (s), 138.1 (s), 153.8 (s), 168.7 (s); MS *m/z* (FAB+) 351.3 [100, $(M + H)^+$]; Acc MS *m/z* (FAB+) 351.2448, $C_{23}H_{31}N_2O$ requires 351.2436. HPLC (MeOH/H_2O, 80:20) R_t = 3.27 min, 99%. Anal. ($C_{23}H_{30}N_2O$.MeOH) C, H, N.

3-Hydroxy-estra-1,3,5(10)-triene-[17,16-*c*]-(2′-isobutyl)-pyrazole (38). Following method 1, a suspension of **30** (45 mg, 102 μmol) and Pd−C 10% (20 mg) in MeOH/THF 2:1 (30 mL) was hydrogenated overnight to give a light yellow solid (103 mg). The crude product was recrystallized from EtOAc/hexane to yield **38** as off-white crystals (33 mg, 92%): mp 194−196 °C (dec); IR (KBr) 3195, 2960−2845, 1620, 1585, 1500 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 0.92 (3H, d, J = 6.6 Hz, C-3″-H_3), 0.95 (3H, d, J = 6.6 Hz, C-4″-H_3), 1.03 (3H, s, C-18-H_3), 1.41−2.91 (14H, m), 3.77 (1H, dd, J_{BA} = 13.4 Hz, J = 8.2 Hz, N−C$H_A$$H_B$), 3.89 (1H, dd, J_{AB} = 13.4 Hz, J = 7.6 Hz, N−C$H_A$$H_B$), ∼5.50 (∼1H, br s, exchanged with D_2O, OH), 6.60 (1H, d, J = 2.6 Hz, C-4-H), 6.66 (1H, dd, J = 8.4 Hz, J = 2.6 Hz, C-2-H), 7.14 (1H, d, J = 8.4 Hz, C-1-H), 7.22 (1H, s, C-5′−H); MS *m/z* (FAB+) 351.3 [100, $(M + H)^+$]; Acc MS *m/z* (FAB+) 351.2444, $C_{23}H_{31}N_2O$ requires 351.2436. HPLC (MeOH/H_2O, 80:20) R_t = 3.59 min, 100%.

3-Hydroxy-estra-1,3,5(10)-triene-[17,16-*c*]-(1′-methyl acetate)-pyrazole (39). Following method 1, a suspension of **31** (90 mg, 197 μmol) and Pd−C 10% (40 mg) in MeOH/THF 2:1 (30 mL) was hydrogenated for 6 h to give a white solid (66 mg). The crude product was recrystallized from MeOH to yield **39** as colorless crystals (49 mg, 68%): mp 236−239 °C; IR (KBr) 3210, 2955−2855, 1755, 1610−1455, 1215 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 1.03 (3H, s, C-18-H_3), 1.41−2.96 (13H, m), 3.75 (3H, s, OCH_3), 4.83 (1H, d, J_{BA} = 17.6 Hz, N−C$H_A$$H_B$), 4.88 (1H, d, J_{AB} = 17.6 Hz, N−C$H_A$$H_B$), 5.04 (1H, s, OH), 6.58 (1H, d, J = 2.7 Hz, C-4-H), 6.64 (1H, dd, J = 8.4 Hz, J = 2.7 Hz, C-2-H), 7.07 (1H, s, C-5′−H), 7.16 (1H, d, J = 8.4 Hz, C-1-H); δ_C ($CDCl_3$, 100.4 MHz) 18.8 (q, C-18), 24.5 (t), 26.7 (t), 27.9 (t), 29.9 (t), 34.3 (t), 38.0 (d), 41.4 (s, C-13), 44.8 (d), 52.9 (d or q), 53.1 (t, N−CH_2), 61.3 (d or q), 113.0 (d), 115.5 (d), 123.3 (s), 125.2 (d), 126.5 (d), 132.5 (s), 138.1 (s), 153.9 (s), 169.1 (s), 169.8 (s); MS *m/z* (FAB+) 367.3 [100, $(M + H)^+$], 73.1 [29]; Acc MS *m/z* (FAB+) 367.2038, $C_{22}H_{27}N_2O_3$ requires 367.2022. Anal. ($C_{22}H_{26}N_2O_3$.MeOH) C, H, N.

3-Hydroxy-estra-1,3,5(10)-triene-[17,16-*c*]-(2′-methyl acetate)-pyrazole (40). Following method 1, a suspension of **32** (60 mg, 131 μmol) and Pd−C 10% (25 mg) in MeOH/THF 2:1 (30 mL) was hydrogenated overnight to give a light brown foam (34 mg). The crude product was purified by flash chromatography (DCM/EtOAc, 8:2) to yield **40** as a light yellow solid (18 mg, 37%): mp 92−95°C; IR (KBr) 3250, 2925−2855, 1760, 1745, 1615−1455 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 1.02 (3H, s, C-18-H_3), 1.42−2.91 (14H, m), 3.77 (3H, s, OCH_3), 4.81 (1H, d, J_{BA} = 17.4 Hz, N−C$H_A$$H_B$), 4.87 (1H, d, J_{AB} = 17.4 Hz, N−C$H_A$$H_B$), 6.59 (1H, d, J = 2.7 Hz, C-4-H), 6.64 (1H, dd, J = 8.6 Hz, J = 2.7 Hz, C-2-H), 7.12 (1H, d, J = 8.6 Hz, C-1-H), ∼7.25 (∼1H, s, C-5′−H, under solvent peak); MS *m/z* (FAB+) 367.1 [37, $(M + H)^+$], 97.0 [39], 73.0 [55], 57.0 [100]; Acc MS *m/z* (FAB+) 367.2029, $C_{22}H_{27}N_2O_3$ requires 367.2022.

3-Hydroxy-estra-1,3,5(10)-triene-[17,16-*c*]-(1′-methoxy-ethyl)-pyrazole (41). Following method 1, a suspension of **33** (80 mg, 181 μmol) and Pd−C 10% (35 mg) in MeOH/THF 2:1 (30 mL) was hydrogenated for 6 h to give a white crystalline solid (58 mg). The crude product was recrystallized from MeOH to yield **41** as white crystals (32 mg, 50%): mp 123−125°C; IR (KBr) 3235, 2930−2850, 1640−1505 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 1.02 (3H, s, C-18-H_3), 1.43−2.93 (13H, m), 3.33 (3H, s, OCH_3), 3.67−3.75 (2H, m, C-1″-H_2 or C-2″-H_2), 4.17−4.28 (2H, m, C-1″-H_2 or C-2″-H_2), 5.57 (1H, br s, exchanged with D_2O, OH), 6.59 (1H, d, J = 2.7 Hz, C-4-H), 6.64 (1H, dd, J = 8.4 Hz, J = 2.7 Hz, C-2-H), 7.09 (1H, s, C-5′−H), 7.16 (1H, d, J = 8.4 Hz, C-1-H); MS *m/z* (FAB+) 353.3 [100, $(M + H)^+$]; Acc MS *m/z* (FAB+) 353.2232, $C_{22}H_{29}N_2O_2$ requires 353.2229. HPLC (MeOH/H_2O, 80:20) R_t = 2.47 min, 98%. Anal. [$C_{22}H_{28}N_2O_2$.$(MeOH)_{1/2}$] C, H, N.

3-Hydroxy-estra-1,3,5(10)-triene-[17,16-*c*]-(2′-methoxy-ethyl)-pyrazole (42). Following method 1, a suspension of **34** (70 mg, 158 μmol) and Pd−C 10% (30 mg) in MeOH/THF 2:1 (30 mL) was hydrogenated overnight to give an off-white foam (50 mg). The crude product was purified by flash chromatography (DCM/EtOAc, 8:2) to yield **42** as an off-white oil (25 mg, 45%): IR (KBr) 2920−2855, 1610−1500, 1255 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 1.05 (3H, s, C-18-H_3), 1.42−2.95 (13H, m), 3.31 (3H, s, OCH_3), 3.74−3.82 (2H, m, C-1″-H_2 or C-2″-H_2), 4.12−4.23 (2H, m, C-1″-H_2 or C-2″-H_2), 6.60 (1H, d, J =

2.6 Hz, C-4-H), 6.66 (1H, dd, J = 8.6 Hz, J = 2.6 Hz, C-2-H), 7.14 (1H, d, J = 8.6 Hz, C-1-H), 7.23 (1H, s, C-5′−H), OH not seen; MS m/z (FAB+) 353.3 [100, $(M + H)^+$]; Acc MS m/z (FAB+) 353.2236, $C_{22}H_{29}N_2O_2$ requires 353.2229.

3-*O*-*tert*-Butyl-dimethylsilyl-estra-1,3,5(10)-triene-[17,-16-*c*]-pyrazole (43). Hydrazine hydrate (440 μL, 9.1 mmol) was added to a suspension of **2** (2.5 g, 6.1 mmol) in EtOH (125 mL) at room temperature under an atmosphere of N_2. The resulting mixture was heated to reflux for 45 min. After the mixture cooled, the solvent was removed under reduced pressure, H_2O (200 mL) was added, and the mixture was acidified with 5 M HCl. The organics were extracted with EtOAc (2 × 10 mL), washed with H_2O (2 × 50 mL) and then brine (2 × 50 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give a yellow foam (2.9 g). The crude product was recrystallized from EtOH/H_2O to give **43** as yellow crystals (2.0 g, 81%): mp 122−124 °C; IR (KBr) 3190, 2930−2855, 1605−1495 cm^{-1}; δ_H (DMSO-d_6, 400 MHz) 0.18 (6H, s, $Si(CH_3)_2$), 0.95 (3H, s, C-18-H_3), 0.96 (9H, s, $C(CH_3)_3$), 1.36−2.87 (13H, m), 6.55 (1H, d, J = 2.3 Hz, C-4-H), 6.62 (1H, dd, J = 8.5 Hz, J = 2.3 Hz, C-2-H), 7.16 (1H, d, J = 8.5 Hz, C-1-H), 7.28 (1H, s, C-5′−H), 12.02 (1H, s, exchanged with D_2O, NH); δ_C ($CDCl_3$, 100.4 MHz) −3.8 (2×q, $Si(CH_3)_2$), 18.6 (s, $C(CH_3)_3$), 18.8 (q, C-18), 24.3 (t), 26.2 (3×q, $C(CH_3)_3$), 26.6 (t), 27.9 (t), 30.0 (t), 34.5 (t), 38.0 (d), 41.1 (s, C-13), 44.9 (d), 61.9 (d), 117.4 (d), 120.2 (d), 121.8 (s), 123.2 (s), 126.2 (d), 133.2 (s), 137.8 (s), 153.5 (s), 168.6 (s); MS m/z (FAB+) 409.2 [100, $(M + H)^+$], 73.0 [33]; Acc MS m/z (FAB+) 409.2662, $C_{25}H_{37}N_2OSi$ requires 409.2675. Anal. [$C_{25}H_{36}N_2OSi.(H_2O)_{2/3}$] C, H, N.

3-*O*-*tert*-Butyl-dimethylsilyl-estra-1,3,5(10)-triene-[17,-16-*c*]-(1′-propionitrile)-pyrazole (44) and 3-*O*-*tert*-Butyl-dimethylsilyl-estra-1,3,5(10)-triene-[17,16-*c*]-(2′-propionitrile)-pyrazole (45). ^{t}BuOK (91 mg, 807 μmol) was added to a stirred solution of **43** (300 mg, 734 μmol) in anhydrous THF (10 mL) at 0 °C under an atmosphere of N_2. After 20 min of stirring, acrylonitrile (58 μL, 881 μmol) was added, and the resulting bright orange solution was stirred for 5 h at room temperature. The resulting dark orange mixture was then concentrated under reduced pressure, and H_2O (50 mL) was added. The organics were extracted with EtOAc (2 × 50 mL), washed with H_2O (2 × 30 mL) and then brine (2 × 30 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography (DCM/EtOAc, 9:1) to give two products.

The less polar fraction gave **44** as a light yellow oil (29 mg, 8%): IR (KBr) 2930−2860, 2250, 1640−1495 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 0.20 (6H, s, $Si(CH_3)_2$), 0.98 (9H, s, $C(CH_3)_3$), 1.02 (3H, s, C-18-H_3), 1.42−3.01 (15H, m), 4.28−4.40 (2H, m, N−CH_2), 6.57 (1H, d, J = 2.6 Hz, C-4-H), 6.63 (1H, dd, J = 8.5 Hz, J = 2.6 Hz, C-2-H), 7.10 (1H, s, C-5′−H), 7.14 (1H, d, J = 8.5 Hz, C-1-H); MS m/z (FAB+) 462.2 [100, $(M + H)^+$], 73.0 [55]; Acc MS m/z (FAB+) 462.2914, $C_{28}H_{40}N_3OSi$ requires 462.2941.

The more polar fraction gave **45** as a light brown oil that crystallized on standing (42 mg, 12%): mp 80−82 °C; IR (KBr) 2930−2855, 2255, 1705−1445 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 0.20 (6H, s, $Si(CH_3)_2$), 0.98 (9H, s, $C(CH_3)_3$), 1.07 (3H, s, C-18-H_3), 1.41−3.06 (15H, m), 4.21−4.38 (2H, m, N−CH_2), 6.57 (1H, d, J = 2.4 Hz, C-4-H), 6.63 (1H, dd, J = 8.7 Hz, J = 2.4 Hz, C-2-H), 7.11 (1H, d, J = 8.7 Hz, C-1-H), 7.25 (1H, s, C-5′−H); MS m/z (FAB+) 462.1 [100, $(M + H)^+$], 73.0 [79]; Acc MS m/z (FAB+) 462.2922, $C_{28}H_{40}N_3OSi$ requires 462.2941.

3-Hydroxy-estra-1,3,5(10)-triene-[17,16-*c*]-(1′-propionitrile)-pyrazole (46). Following method 2, a solution of **44** (25 mg, 50 μmol) in anhydrous THF (5 mL) was treated with a 1.0 M solution of TBAF in anhydrous THF (110 μL, 110 μmol) for 3 h. The crude yellow oil was purified by flash chromatography (DCM/EtOAc, 8:2) to give **46** as a light yellow oil (12 mg, 63%). This product was then triturated with EtOAc/hexane to give a light yellow powder (8 mg): mp 92−94 °C; IR (KBr) 3230, 3095, 2925−2860, 2265, 1665−1500, 1245 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 0.94 (3H, s, C-18-H_3), 1.34−2.94 (15H, m), 4.19−4.31 (2H, m, N−CH_2), 4.92 (1H, br s, exchanged with D_2O, OH), 6.50 (1H, d, J = 2.7 Hz, C-4-H), 6.55 (1H, dd, J = 8.4 Hz, J = 2.7 Hz, C-2-H), 7.02 (1H, s, C-5′−H), 7.08 (1H, d, J = 8.4 Hz, C-1-H); MS m/z (FAB+) 348.1 [90, $(M + H)^+$], 147.1 [64], 73.0 [100]; Acc MS m/z (FAB+) 348.2072, $C_{22}H_{26}N_3O$ requires 348.2076. HPLC (MeOH/H_2O, 90:10) R_t = 2.27 min, 96%.

3-Hydroxy-estra-1,3,5(10)-triene-[17,16-*c*]-(2′-propionitrile)-pyrazole (47). Method A. Following method 2, a solution of **45** (35 mg, 76 μmol) in anhydrous THF (5 mL) was treated with a 1.0 M solution of TBAF in anhydrous THF (150 μL, 150 μmol) for 3 h. The crude brown oil was purified by flash chromatography (DCM/EtOAc, 8:2) to give **47** as a light brown oil (10 mg, 38%).

Method B. Following method 2, a solution of **48** (220 mg, 458 μmol) in anhydrous THF (20 mL) was treated overnight with a 1.0 M solution of TBAF in anhydrous THF (916 μL, 916 μmol). The crude product was purified by flash chromatography (EtOAc/$CHCl_3$, 9:1) to give **47** as a pale pink foam (136 mg, 85%). This compound was then triturated with EtOAc/hexane to give white crystals (99 mg, 62%): mp 198−200 °C; IR (KBr) 3160, 2925−2860, 2250, 1610−1500 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 1.06 (3H, s, C-18-H_3), 1.41−3.05 (15H, m), 4.22−4.34 (2H, m, N−CH_2), 5.42 (1H, br s, exchanged with D_2O, OH), 6.57 (1H, d, J = 2.4 Hz, C-4-H), 6.64 (1H, dd, J = 8.3 Hz, J = 2.4 Hz, C-2-H), 7.12 (1H, d, J = 8.3 Hz, C-1-H), 7.25 (1H, s, C-5′−H); δ_C ($CDCl_3$, 100.4 MHz) 18.6 (q, C-18), 19.7 (t, CH_2CN), 24.5 (t), 26.5 (t), 27.6 (t), 29.8 (t), 34.8 (t), 37.7 (d), 42.4 (s, C-13), 44.4 (d), 45.9 (t, N−CH_2), 62.2 (d), 113.1 (d), 115.7 (d), 117.4 (s), 124.3 (s), 126.4 (d), 132.0 (s), 135.2 (d), 138.2 (s), 154.1 (s), 157.98 (s); MS m/z (FAB+) 348.1 [100, $(M + H)^+$], 147.0 [50], 85.1 [75], 73.0 [94]; Acc MS m/z (FAB+) 348.2060, $C_{22}H_{26}N_3O$ requires 348.2076. Anal. ($C_{22}H_{25}N_3O$) C, H, N.

3-*O*-*tert*-Butyl-dimethylsilyl-estra-1,3,5(10)-triene-[17,-16-*c*]-(2′-propionitrile-3′,4′-dihydro)-pyrazol-3′-ol (48). Cyanoethylhydrazine (177 μL, 2.18 mmol) was added to a stirred solution of **2** (600 mg, 1.45 mmol) in EtOH (50 mL) under an atmosphere of N_2. After 4 h of stirring at room temperature, the resulting orange mixture was concentrated under reduced pressure, and H_2O (100 mL) was added, followed by 5 M HCl. The organics were extracted with EtOAc (2 × 100 mL), washed with H_2O (50 mL) and then brine (50 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography ($CHCl_3$/EtOAc, 7:3) to give **48** as a pale yellow solid (510 mg, 73%). This compound was recrystallized from EtOH to give white needles (240 mg, 34%): mp 145−147 °C; IR (KBr) 3380, 2950−2860, 2260, 1610, 1495 cm^{-1}; δ_H (DMSO-d_6, 400 MHz) 0.15 (6H, s, $Si(CH_3)_2$), 0.88 (3H, s, C-18-H_3), 0.93 (9H, s, $C(CH_3)_3$), 1.19−3.16 (18H, m), 5.97 (1H, s, exchanged with D_2O, OH), 6.48 (1H, d, J = 2.7 Hz, C-4-H), 6.56 (1H, dd, J = 8.2 Hz, J = 2.7 Hz, C-2-H), 6.74 (1H, d, J = 1.9 Hz, C-5′−H), 7.10 (1H, d, J = 8.2 Hz, C-1-H); δ_C ($CDCl_3$, 100.4 MHz) −4.4 (2×q, $Si(CH_3)_2$), 15.6 (q, C-18), 17.1 (t, CH_2CN), 18.0 (s, $C(CH_3)_3$), 25.6 (3×q, $C(CH_3)_3$), 25.8 (t), 27.0 (t), 29.1 (t), 29.4 (t), 31.4 (t), 38.6 (d), 43.2 (d), 43.9 (t, N−CH_2), 47.1 (s, C-13), 49.5 (d), 56.7 (d), 104.2 (s), 116.8 (d), 119.4 (d), 119.9 (s, CN), 126.0 (d), 132.5 (s), 137.3 (s), 144.6 (s), 152.4 (s); MS m/z (FAB+) 480.1 [100, $(M + H)^+$], 462.1 [63, $(M + H - H_2O)^+$], 72.9 [77]; MS m/z (FAB−) 632.3 [32, $(M + NBA)^-$]; Acc MS m/z (FAB+) 480.3038 $C_{28}H_{42}N_3O_2Si$ requires 480.3046.

3-Hydroxy-estra-1,3,5(10)-triene-[17,16-*c*]-(5′-methyl)-pyrazole (49). Hydrazine hydrate (56 μL, 1.15 mmol) was added to a refluxing solution of **17** (240 mg, 768 μmol) in EtOH (15 mL) under an atmosphere of N_2. The resulting pale yellow solution was heated to reflux for 45 min, after which the solvent was removed under reduced pressure, H_2O (50 mL) was added, and the mixture was acidified with 5 M HCl. The organics were extracted with EtOAc (2 × 50 mL), washed with H_2O (30 mL) and then brine (2 × 30 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography (DCM/EtOAc, 8:2) to give **49** as a pale brown solid (84 mg, 35%). This was triturated with acetone to give an off-white solid (32 mg, 13%): mp 234−236 °C; IR (KBr) 3295, 2930−2860, 1620−1500

cm^{-1}; δ_H (DMSO-d_6, 400 MHz) 1.01 (3H, s, C-18-H_3), 1.34−2.85 (13H, m), 2.28 (3H, s, C-1″-H_3), 6.45 (1H, d, J = 2.6 Hz, C-4-H), 6.52 (1H, dd, J = 8.6 Hz, J = 2.6 Hz, C-2-H), 7.04 (1H, d, J = 8.6 Hz, C-1-H), 9.04 (1H, s, exchanged with D_2O, NH or OH), NH or OH not seen; MS *m/z* (FAB+) 309.2 [100, $(M + H)^+$], 95.1 [33], 69.0 [39]; Acc MS *m/z* (FAB+) 309.1978, $C_{20}H_{25}N_2O$ requires 309.1967. HPLC (MeOH/H_2O, 96:4) R_t = 1.98 min, 99%.

3-Hydroxy-estra-1,3,5(10)-triene-[17,16-*c*]-(5′-trifluoromethyl)-pyrazole (50). Hydrazine hydrate (32 μL, 655 μmol) was added to a refluxing solution of **20** (160 mg, 437 μmol) in EtOH (15 mL) under an atmosphere of N_2. The resulting pale yellow solution was heated to reflux for 3 h, after which *p*-toluenesulfonic acid monohydrate (∼10 mg) was added. The mixture was then heated to reflux overnight, and after the mixture cooled, the solvent was removed under reduced pressure, and H_2O (50 mL) was added, followed by 5 M HCl. The organics were extracted with EtOAc (2 × 50 mL), washed with H_2O (30 mL) and then brine (2 × 30 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography (DCM/EtOAc, 8:2) to give **50** as a pale yellow solid (95 mg, 60%). This compound was precipitated from DCM/hexane (1:5) to give an off-white solid (51 mg, 32%): mp 152−155°C; IR (KBr) 3220, 2930−2860, 1610−1500 cm^{-1}; δ_H ($CDCl_3$, 400 MHz) 1.01 (3H, s, C-18-H_3), 1.40−2.89 (13H, m), 4.63 (1H, s, exchanged with D_2O, C-3-OH), 6.53 (1H, d, J = 2.7 Hz, C-4-H), 6.59 (1H, dd, J = 8.2 Hz, J = 2.7 Hz, C-2-H), 7.08 (1H, d, J = 8.2 Hz, C-1-H), NH not seen; MS *m/z* (FAB+) 363.1 [100, $(M + H)^+$]; Acc MS *m/z* (FAB+) 363.1689, $C_{20}H_{22}N_2F_3O$ requires 363.1684. HPLC (MeOH/H_2O, 85:15) R_t = 2.43 min, 98%.

3-*O*-*tert*-Butyl-dimethylsilyl-estra-1,3,5(10)-triene-[17,-16-*c*]-[5′-(1″-pyridin-3″-yl)-2′,3′-dihydro)]-pyrazol-3′-ol (51). Hydrazine hydrate (75 μL, 1.53 mmol) was added to a refluxing solution of **14** (500 mg, 1.02 mmol) in EtOH (20 mL) under an atmosphere of N_2. The resulting pale yellow solution was heated to reflux for 1 h, after which the solvent was partially removed under reduced pressure and H_2O (100 mL) was added. The resulting white precipitate was filtered and dried (460 mg, 89%), and an analytical sample was recrystallized from EtOH/H_2O to give **51** as white crystals: mp 144−145 °C; IR (KBr) 3480, 3330, 3190, 2940−2860, 1610−1495 cm^{-1}; δ_H (DMSO-d_6, 400 MHz) 0.14 (6H, s, $Si(CH_3)_2$), 0.92 (9H, s, $C(CH_3)_3$), 0.94 (3H, s, C-18-H_3), 1.12−2.71 (13H, m), 3.43 (1H, app d, J = 9.0 Hz, C-16-H), 5.97 (1H, s, exchanged with D_2O, C-17-OH), 6.46 (1H, d, J = 2.5 Hz, C-4-H), 6.55 (1H, dd, J = 8.4 Hz, J = 2.5 Hz, C-2-H), 7.10 (1H, d, J = 8.4 Hz, C-1-H), 7.26 (1H, s, exchanged with D_2O, NH), 7.35 (1H, app ddd, J = 8.1 Hz, J = 4.6 Hz, J = 0.8 Hz, C-5″-H), 7.91 (1H, app dt, J = 8.1 Hz, J = 1.7 Hz, C-4″-H), 8.43 (1H, dd, J = 4.6 Hz, J = 1.7 Hz, C-6″-H), 8.74 (1H, app d, J = 1.7 Hz, C-2″-H); MS *m/z* (FAB+) 504.1 [100, $(M + H)^+$], 486.1 [27, $(M + H - H_2O)^+$]; Acc MS *m/z* (FAB+) 504.3045, $C_{30}H_{42}N_3O_2Si$ requires 504.3046.

3-Hydroxy-estra-1,3,5(10)-triene-[17,16-*c*]-[5′-(1″-pyridin-3″-yl)]-pyrazole (52). Following method 2, a solution of **51** (110 mg, 218 μmol) in anhydrous THF (10 mL) was treated overnight with a 1.0 M solution of TBAF in anhydrous THF (436 μL, 436 μmol) for 3 h. The crude foam was triturated with boiling EtOAc/$CHCl_3$ (1:1) to give **52** as a white powder (61 mg, 75%): mp 298−300 °C (dec); IR (KBr) 2980−2840, 1610−1495 cm^{-1}; δ_H (DMSO-d_6, 400 MHz) 0.98 (3H, s, C-18-H_3), 1.37−2.87 (13H, m), 6.45 (1H, d, J = 2.6 Hz, C-4-H), 6.51 (1H, dd, J = 8.5 Hz, J = 2.6 Hz, C-2-H), 7.05 (1H, d, J = 8.5 Hz, C-1-H), 7.37−7.48 (1H, m, C-4″-H or C-5″-H), 7.97−8.06 (1H, m, C-4″-H or C-5″-H), 8.42−8.49 (1H, m, C-2″-H or C-6″-H), 8.84−8.89 (1H, m, C-2″-H or C-6″-H), 9.01 (1H, s, exchanged with D_2O, OH), 12.65 and 12.72 (1H, 2 × s, exchanged with D_2O, NH); MS *m/z* (FAB+) 372.0 [100, $(M + H)^+$]; Acc MS *m/z* (FAB+) 372.2080, $C_{24}H_{26}N_3O$ requires 372.2076.

3-*O*-Benzyl-16-(1′-hydroxy-2′-carboxylic acid ethyl ester-ethylidene)-estrone (53). ^{t}BuOK (2.24 g, 20.0.mmol) was added to a solution of **5** (5.77 g, 16.0 mmol) and diethyl oxalate (5 mL, 36.8 mmol) in toluene (100 mL). The resulting clear yellow solution was stirred overnight at room temperature after which AcOH (5 mL) and H_2O (100 mL) were added. The organics were extracted with EtOAc (50 mL), washed with H_2O (100 mL) and then brine (50 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The light yellow crude was recrystallized from EtOH to give **53** as a colorless crystalline solid (7.28 g, 99%): mp 176−178 °C; δ_H ($CDCl_3$, 400 MHz) 1.01 (3H, s, C-18-H_3), 1.41 (3H, t, J = 7.0 Hz), 1.44−3.10 (13 H, m), 4.37 (2H, q, J = 7.0 Hz), 5.05 (2H, s, OCH_2Ar), 6.75 (1H, d, J = 2.7 Hz, C-4-H), 6.80 (1H, dd, J = 8.6 Hz, J = 2.7 Hz, C-2-H), 7.20 (1H, d, J = 8.6 Hz, C-1-H), 7.30−7.46 (5H, m, C_6H_5), 12.48 (1H, s, OH); δ_C ($CDCl_3$, 100.4 MHz) 14.7 (q), 15.4 (q), 26.2 (t), 27.2 (t), 27.9 (t), 30.0 (t), 31.5 (t), 38.1 (d), 44.4 (d), 49.1 (s), 49.8 (d), 62.4 (t), 70.3 (t), 112.7 (d), 115.1 (d), 116.8 (s), 126.4 (d), 127.7 (d), 128.1 (d), 128.8 (d), 132.3 (s), 137.4 (s), 137.9 (s), 152.7 (s), 157.0 (s), 162.8 (s), 217.0 (s); MS *m/z* (FAB+) 461.2 [100, $(M + H)^+$]. Anal. ($C_{29}H_{32}O_5$) C, H.

3-*O*-Benzyl-estra-1,3,5(10)-triene-[17,16-*c*]-(5′-carboxylic acid ethyl ester)-pyrazole (54). Hydrazine hydrate (0.75 mL, 15 mmol) was added to a suspension of **53** (6.91 g, 15.0 mmol) in EtOH (100 mL) and DCM (20 mL) at room temperature. The resulting mixture was heated to reflux for 15 min and stirred overnight at room temperature. The solvents were removed under reduced pressure to give a white solid, which was dissolved in EtOH (50 mL). *p*-Toluenesulfonic acid monohydrate (200 mg, 1.05 mmol) was then added, and the mixture was heated to reflux for 5 min. After the mixture cooled, H_2O (50 mL) was added, and the organics were extracted with EtOAc (120 mL), washed with saturated aqueous $NaHCO_3$ (30 mL) and then brine (30 mL), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The crude product was recrystallized from EtOAc/hexane to give **54** as fine colorless crystals (5.43 g, 79%): mp 208−211 °C; δ_H ($CDCl_3$, 400 MHz) 1.05 (3H, s, C-18-H_3), 1.39 (3H, t, J = 7.0 Hz), 1.44−3.00 (13 H, m), 4.37 (2H, q, J = 7.0 Hz), 5.05 (2H, s, OCH_2Ar), 6.73 (1H, d, J = 2.3 Hz, C-4-H), 6.81 (1H, dd, J = 8.6 Hz, J = 2.3 Hz, C-2-H), 7.23 (1H, d, J = 8.6 Hz, C-1-H), 7.30−7.46 (5H, m, C_6H_5), NH not seen; δ_C ($CDCl_3$, 100.4 MHz) MS *m/z* (FAB+) 457.4 [100, $(M + H)^+$]; Acc MS *m/z* (FAB+) 457.2488, $C_{29}H_{33}N_2O_3$ requires 457.2491. Anal. ($C_{29}H_{32}N_2O_3$) C, H, N.

3-*O*-Benzyl-estra-1,3,5(10)-triene-[17,16-*c*]-(5′-carboxylic acid)-pyrazole (55). A 2 M NaOH solution (7.5 mL, 15 mmol) was added to a suspension of **54** (2.28 g, 5.0 mmol) in EtOH (40 mL). The resulting mixture was heated to reflux for 30 min and allowed to cool. AcOH (5 mL) was then added, and the mixture was stirred for an additional 2 h, while the product precipitated. This was filtered off, washed with H_2O (50 mL) and EtOH (20 mL), and dried under high vacuum for 2 days to give **55** as a white powder (2.13 g, 99%): mp > 290 °C (dec); δ_H (DMSO-d_6, 400 MHz) 0.95 (3H, s, C-18-H_3), 1.40−2.94 (13 H, m), 5.06 (2H, s, OCH_2Ar), 6.73 (1H, d, J = 2.3 Hz, C-4-H), 6.78 (1H, dd, J = 8.6 Hz, J = 2.3 Hz, C-2-H), 7.20 (1H, d, J = 8.6 Hz, C-1-H), 7.30−7.45 (5H, m, C_6H_5), 12.90 (2H, br s, OH, NH); MS *m/z* (FAB+) 429.2 [100, $(M + H)^+$].

Data for a Representative Benzylated Intermediate, 3-*O*-Benzyl-estra-1,3,5(10)-triene-[17,16-*c*]-[5′-(furan-2″-ylmethyl)carbamoyl]-pyrazole (59). Prepared according to method 4. 18%; δ_H ($CDCl_3$, 270 MHz) 1.02 (3H, s, C-18-H_3), 1.35−1.94 (5H, m), 2.10−2.48 (5H, m), 2.70−2.90 (3H, m), 4.61 (2H, d, J = 5.7 Hz), 5.03 (2H, s), 6.28−6.33 (2H, m), 6.72 (1H, d, J = 2.6 Hz, C-4-H), 6.78 (1H, dd, J = 8.4 Hz, J = 2.6 Hz, C-2-H), 7.20 (1H, d, J = 8.4 Hz, C-1-H), 7.24−7.44 (6H); MS *m/z* (ES+) 508.3 $[M + H]^+$. HPLC (MeOH/H_2O, 96:4) R_t = 2.77 min, 93%.

3-Hydroxy-estra-1,3,5(10)-triene-[17,16-*c*]-(5′-methylcarbamoyl)-pyrazole (63). Prepared according to method 5. 57%; δ_H (CD_3OD, 270 MHz) 1.03 (3H, s, C-18-H_3), 1.39−2.88 (13H, m), 2.89 (3H, s), 6.50 (1H, d, J = 2.5 Hz, C-4-H), 6.54 (1H, app dd, C-2-H), 7.09 (1H, d, J = 8.2 Hz, C-1-H); MS *m/z* (APCI+) 350.21 $[M - H]^+$. Acc MS *m/z* (FAB+) 353.2110, $C_{21}H_{27}N_3O_2$ requires 353.2103. HPLC (MeOH/H_2O, 96:4) R_t = 1.66 min, 99%.

3-Hydroxy-estra-1,3,5(10)-triene-[17,16-*c*]-(5′-isopropylcarbamoyl)-pyrazole (64). Prepared according to method 5. 63%; δ_H ($CDCl_3$, 270 MHz) 0.97 (3H, s, C-18-H_3), 1.25 (3H,

s), 1.27 (3H, s), 1.47−2.94 (13H, m), 4.19−4.30 (1H, m), 5.88 (1H, d, J = 8.1 Hz), 6.60 (1H, d, J = 2.3 Hz, C-4-H), 6.65 (1H, dd, J = 8.3 Hz, J = 2.9 Hz, C-2-H), 7.16 (1H, d, J = 8.3 Hz, C-1-H); MS *m/z* (AP+) 378.35 $[M - H]^+$; Acc MS *m/z* (FAB+) 380.2324, $C_{23}H_{30}N_3O_2$ requires 380.2338. HPLC (MeOH/H_2O, 96:4) R_t = 1.72 min, 95%.

3-Hydroxy-estra-1,3,5(10)-triene-[17,16-*c*]-(5′-ethylmethylcarbamoyl)-pyrazole (65). Prepared according to method 5. 76%; δ_H (CD_3OD, 270 MHz) 0.96 (3H, s, C-18-H_3), 1.12 (3H, t, J = 7.2 Hz), 1.25−2.79 (13H, m), 2.95 and 3.09 (total 3H, 2 × s), 3.44−3.54 (2H, m), 6.40 (1H, d, J = 2.6 Hz, C-4-H), 6.46 (1H, dd, J = 8.4 Hz, J = 2.6 Hz, C-2-H), 7.00 (1H, d, J = 8.4 Hz, C-1-H); MS *m/z* (FAB+) 380.0 $[M + H]^+$. Acc MS *m/z* (FAB+) 380.2330, $C_{23}H_{30}N_3O_2$ requires 380.2338. HPLC (MeOH/H_2O, 96:4) R_t = 1.69 min, 93%.

3-Hydroxy-estra-1,3,5(10)-triene-[17,16-*c*]-[5′-(tetrahydrofuran-2″-ylmethyl)carbamoyl]-pyrazole (66). Prepared according to method 5. 30%; δ_H (CD_3OD, 270 MHz) 0.92 (3H, s, C-18-H_3), 1.25−2.82 (17H, m), 3.25−3.45 (2H, m), 3.59−3.71 (1H, m), 3.74−3.85 (1H, m), 3.89−4.00 (1H, m), 6.40 (1H, d, J = 2.6 Hz, C-4-H), 6.45 (1H, dd, J = 8.4 Hz, J = 2.6 Hz, C-2-H), 6.99 (1H, d, J = 8.4 Hz, C-1-H); MS *m/z* (APCI+) 422.3 $[M + H]^+$. Acc MS *m/z* (FAB+) 422.2433, $C_{25}H_{32}N_3O_3$ requires 422.2443. HPLC (MeOH/H_2O, 96:4) R_t = 1.70 min, 100%.

3-Hydroxy-estra-1,3,5(10)-triene-[17,16-*c*]-[5′-(1″-methylpyrrol-2″-ylmethyl)carbamoyl]-pyrazole (67). Prepared according to method 5. 36%; δ_H (CD_3OD, 270 MHz) 0.94 (3H, s, C-18-H_3), 1.05−2.79 (13H, m), 3.52 (3H, s), 4.43 (2H, s), 5.85−5.86 (1H, m), 5.96−5.98 (1H, m), 6.40 (1H, d, J = 2.7 Hz, C-4-H), 6.45 (1H, dd, J = 8.4 Hz, J = 2.7 Hz, C-2-H), 6.51−6.53 (1H, m), 7.01 (1H, d, J = 8.4 Hz, C-1-H); MS *m/z* (FAB+) 431.0 $[M + H]^+$. Acc MS *m/z* (FAB+) 431.2442, $C_{26}H_{31}N_4O_2$ requires 431.2447. HPLC (MeOH/H_2O, 96:4) R_t = 2.11 min, 100%.

3-Hydroxy-estra-1,3,5(10)-triene-[17,16-*c*]-[5′-(1″-methylpiperazin-4″-ylmethyl)carbamoyl]-pyrazole (68). Prepared according to method 5. 96%; δ_H (CD_3OD, 270 MHz) 1.05 (3H, s, C-18-H_3), 1.28−2.50 (14H, m), 2.33 (3H, s), 2.67−2.94 (3H, m), 3.52−3.90 (4H, m), 6.50 (1H, d, J = 2.6 Hz, C-4-H), 6.55 (1H, dd, J = 8.5 Hz, J = 2.6 Hz, C-2-H), 7.10 (1H, d, J = 8.5 Hz, C-1-H); MS *m/z* (APCI+) 421.4 $[M + H]^+$. Acc MS *m/z* (FAB+) 421.2598, $C_{25}H_{33}N_4O_2$ requires 421.2603. HPLC (MeOH/H_2O, 96:4) R_t = 1.68 min, 94%.

3-Hydroxy-estra-1,3,5(10)-triene-[17,16-*c*]-[5′-(5″-methylpyrazin-2″-ylmethyl)carbamoyl]-pyrazole (69). Prepared according to method 5. 82%; δ_H (CD_3OD, 400 MHz) 1.04 (3H, s, C-18-H_3), 1.06−2.50 (10H, m), 2.54 (3H, s), 2.78−2.90 (3H, m), 4.66 (2H, s), 6.50 (1H, d, J = 2.5 Hz, C-4-H), 6.55 (1H, dd, J = 8.4 Hz, J = 2.5 Hz, C-2-H), 7.09 (1H, d, J = 8.4 Hz, C-1-H), 8.48 (1H, s), 8.49 (1H, s); MS *m/z* (APCI+) 442.3 $[M - H]^+$. Acc MS *m/z* (FAB+) 444.2385, $C_{26}H_{30}N_5O_2$ requires 444.2400. HPLC (MeOH/H_2O, 96:4) R_t = 1.67 min, 89%.

3-Hydroxy-1,3,5(10)-triene-[17,16-*c*]-(5′-carboxylic acid)-pyrazole (70). Following method 1, a suspension of **55** (214 mg, 0.50 mmol) and Pd−C 5% (50 mg) in EtOH/THF 1:1 (30 mL) was hydrogenated for 24 h to give **70** as a light gray solid (165 mg, 98%): mp > 300 °C; δ_H (DMSO-d_6, 400 MHz) 0.92 (3H, s, C-18-H_3), 1.30−2.86 (13H, m), 6.44 (1H, d, J = 2.3 Hz, C-4-H), 6.52 (1H, dd, J = 8.6 Hz, J = 2.3 Hz, C-2-H), 7.05 (1H, d, J = 8.6 Hz, C-1-H), 9.02 (1H, s, OH), 12.90 (2H, br s, OH, NH); MS *m/z* (FAB+) 329.1 [45, $(M + H)^+$], 176.0 [100]; Acc MS *m/z* (FAB+) 339.1711 $C_{20}H_{23}N_2O_3$ requires 339.1709.

3-Hydroxy-estra-1,3,5(10)-triene-[17,16-*c*]-[5′-(pyridin-3″-ylmethyl)carbamoyl]-pyrazole (71). DMAP (catalytic), EDC (116 mg, 0.6 mmol), and NEt_3 (77 μL, 0.55 mmol) were added to a suspension of **70** (170 mg, 0.5 mmol) in anhydrous DCM (10 mL) under N_2. After the suspension was stirred at room temperature for 20 min, 3-aminomethylpyridine (60 μL, 0.59 mmol) and anhydrous DMF (10 mL) were added, and the resulting solution was stirred at room temperature for 24 h. After the solution was washed with saturated aqueous $NaHCO_3$, the organic layer was separated and concentrated under reduced pressure. The crude product was precipitated from DCM/hexane to give an off-white precipitate, which was further purified by flash chromatography (DCM to DCM/MeOH, 90:10, gradient) to give **71** as a white crystalline solid (340 mg, 16%): mp > 240 °C (dec); δ_H (DMSO-d_6, 400 MHz) 0.92 (3H, s, C-18-H_3), 1.37−2.89 (13H, m), 4.41 (2H, app d), 6.44 (1H, d, J = 2.3 Hz, C-4-H), 6.51 (1H, dd, J = 8.2 Hz, J = 2.3 Hz, C-2-H), 7.04 (1H, d, J = 8.2 Hz, C-1-H), 7.33 (1H, app s), 7.69 (1H, app s), 8.29−8.63 (3H, m), 9.02 (1H, s), 12.65 and 12.92 (total 1H, 2 × s, NH); MS *m/z* (ES+) 429.2 $[M + H]^+$; Acc MS *m/z* (FAB+) 429.2279 $C_{26}H_{29}N_4O_2$ requires 429.2291. HPLC (MeOH/H_2O, 90:10) R_t = 2.04 min, 98%.

3-Hydroxy-estra-1,3,5(10)-triene-[17,16-*c*]-[5′-(pyridin-2″-ylmethyl)carbamoyl]-pyrazole (72). DMAP (catalytic), EDC (65 mg, 0.3 mmol), and NEt_3 (52 μL, 0.37 mmol) were added to a solution of **70** (950 mg, 0.28 mmol) in anhydrous DCM (6 mL) and anhydrous DMF (2 mL) under N_2. After the mixture was stirred at room temperature for 15 min, 2-aminomethylpyridine (40 μL, 0.39 mmol) was added, and the resulting solution was stirred at room temperature for 4 days. After the solution was washed with saturated aqueous $NaHCO_3$, the organic layer was separated and concentrated under reduced pressure. The crude product was purified by flash chromatography (DCM to DCM/MeOH, 90:10) followed by precipitation from hexane/DCM to give **72** as a white solid (10 mg, 8%): mp 187−190 °C; δ_H (DMSO-d_6, 270 MHz) 0.94 (3H, s, C-18-H_3), 1.72−2.97 (13H, m), 4.45−4.55 (2H, m), 6.45 (1H, d, J = 2.6 Hz, C-4-H), 6.51 (1H, dd, J = 8.6 Hz, J = 2.6 Hz, C-2-H), 7.05 (1H, d, J = 8.6 Hz, C-1-H), 7.22−7.33 (1H, m), 7.74 (1H, m), 8.28 (1H, m), 8.52 (1H, app s), 9.03 (1H, s); MS *m/z* (FAB+) 429.3 $[M + H]^+$; Acc MS *m/z* (FAB+) 429.2279 $C_{26}H_{29}N_4O_2$ requires 429.2291. HPLC (MeCN/H_2O, 96:4) R_t = 1.67 min, 100%.

3-Hydroxy-estra-1,3,5(10)-triene-[17,16-*c*]-[5′-(pyridin-3″-ylethyl)carbamoyl]-pyrazole (73). Following the same procedure as for the preparation of **72**, reaction of **70** (95 mg, 0.28 mmol) with 3-aminoethylpyridine (34 μL, 0.33 mmol) gave **73** as an off-white solid (12 mg, 10%): mp > 190 °C (dec); δ_H (DMSO-d_6, 270 MHz) 0.93 (3H, s, C-18-H_3), 1.36−3.50 (17H, m), 6.47 (1H, app d, C-4-H), 6.52 (1H, app dd, C-2-H), 7.07 (1H, d, J = 8.2 Hz, C-1-H), 7.31−7.36 (1H, m), 7.67 (1H, app d), 8.45 (2H, m), 9.07 (1H, s); MS *m/z* (APCI+) 441.5 $[M - H]^+$; Acc MS *m/z* (FAB+) 443.2442 $C_{27}H_{31}N_4O_2$ requires 443.2447. HPLC (MeCN/H_2O, 80:20) R_t = 2.15 min, 88%.

3-Hydroxy-1,3,5(10)-triene-[17,16-*c*]-[5′-(carboxylic acid ethyl ester)]-pyrazole (74). Following method 1, a suspension of **54** (913 mg, 2.00 mmol) and Pd−C 5% (100 mg) in EtOH/THF 1:1 (60 mL) was hydrogenated for 72 h. The crude product was crystallized from $CHCl_3$ to give **74** as pale yellow crystals (602 mg, 82%): mp 166−170 °C; δ_H (DMSO-d_6, 400 MHz) 0.92 (3H, s, C-18-H_3), 1.12−2.84 (16H, m), 4.14−4.40 (2H, m), 6.45 (1H, d, J = 2.3 Hz, C-4-H), 6.50 (1H, dd, J = 8.6 Hz, J = 2.3 Hz, C-2-H), 7.05 (1H, d, J = 8.6 Hz, C-1-H), 9.01 (1H, s, OH), 13.07 (1H, s, NH); MS *m/z* (FAB+) 367.2 [100, $(M + H)^+$]; Acc MS *m/z* (FAB+) 367.2038, $C_{22}H_{27}N_2O_3$ requires 367.2022.

3-*O*-Benzyl-1,3,5(10)-triene-[17,16-*c*]-(5′-hydroxymethyl)-pyrazole (75). Compound **54** (457 mg, 1.0 mmol) was added to a suspension of $LiAlH_4$ (100 mg, 2.64 mmol) in anhydrous THF (20 mL) under an atmosphere of N_2. The resulting mixture was stirred for 30 min at room temperature, after which H_2O (1 mL) was carefully added. Stirring was continued for 1 h, and AcOH (0.5 mL) was added. The suspension was filtered through a layer of Celite, and the resulting clear solution was diluted with EtOAc (50 mL), washed with brine (50 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was crystallized from EtOAc/Et_2O to give **75** as colorless crystals (215 mg, 52%): mp 210−213 °C; δ_H ($CDCl_3$, 400 MHz) 1.01 (3H, s, C-18-H_3), 1.40−3.00 (13 H, m), 4.69 (2H, s), 5.04 (2H, s, OCH_2Ar), 6.28 (2H, br s, OH, NH), 6.73 (1H, d, J = 2.3 Hz, C-4-H), 6.80 (1H, dd, J = 8.6 Hz, J = 2.3 Hz, C-2-H), 7.21 (1H, d, J = 8.6 Hz, C-1-H), 7.30−7.45 (5H, m, C_6H_5); δ_C ($CDCl_3$, 100.4 MHz) 18.8 (q), 23.9 (t), 26.6 (t), 27.9 (t), 30.1 (t), 34.3 (t), 38.0 (d), 41.5 (s), 44.8 (d), 56.5 (t), 62.0 (d), 70.3 (t), 109.3 (s), 112.5 (d), 115.1 (d), 119.5 (s), 126.4 (d), 127.7 (d), 128.1 (d), 128.7 (d), 132.9 (s), 137.4 (s), 138.0

(s), 156.9 (s), 168.0 (s); MS *m/z* (FAB+) 415.3 [60, $(M + H)^+$], 91.1 [100]; Acc MS *m/z* (FAB+) 415.2384, $C_{27}H_{31}N_2O_2$ requires 415.2385.

3-Hydroxy-1,3,5(10)-triene-[17,16-*c*]-(5′-hydroxymethyl)-pyrazole (76). Following method 1, a suspension of **75** (120 mg, 0.289 mmol) and Pd−C 5% (50 mg) in EtOH/THF 1:1 (40 mL) was hydrogenated for 24 h to give **76** as a white solid (93 mg, 99%): mp > 213 °C (dec); δ_H (DMSO-d_6, 270 MHz) 0.82 (3H, s, C-18-H_3), 1.20−2.80 (13H, m), 4.31 (2H, s), 4.95 (1H, br s, OH), 6.37 (1H, s), 6.44 (1H, d, J = 8.5 Hz), 6.97 (1H, d, J = 8.5 Hz), 8.92 (1H, s, OH), 11.78 (1H, br s, NH); MS *m/z* (FAB+) 325.2 [100, M^+]; Acc MS *m/z* (FAB+) 325.1925, $C_{20}H_{25}N_2O_2$ requires 325.1916.

X-ray Crystallography. Crystal Data for 37. $C_{24}H_{34}N_2O_2$, M = 382.53, λ = 0.710 73 Å, orthorhombic, space group $P2_12_12_1$, a = 8.3600(1), b = 13.2660(2), c = 19.1680(4) Å, U = 2125.80(6) Å^3, Z = 4, D_c = 1.195 mg/m^3, μ = 0.076 mm^{-1}, $F(000)$ = 832, crystal size 0.50 × 0.50 × 0.30 mm^3, 4846 unique reflections [R(int) = 0.0594], observed $I > 2\sigma(I)$ = 4002, data/restraints/parameters = 4846/0/261, $R1$ = 0.0414, w$R2$ = 0.0879 (obs. data), $R1$ = 0.0567, w$R2$ = 0.0939 (all data), max peak/hole 0.199 and −0.176 e Å^{-3}, software used, SHELXS,[55] SHELXL,[56] and ORTEX.[43] H1, H2 located in the penultimate difference Fourier map, but included at calculated positions in final least squares cycles.

Acknowledgment. We acknowledge Sterix Ltd, as a member of the Ipsen group for a studentship to D.S.F. We thank Dr. S. J. Black for useful discussions and Ms. A. C. Smith for technical assistance.

Supporting Information Available: Experimental and spectroscopic data for compounds **2**, **3**, and **25**, microanalysis data on selected compounds, and crystallographic data for compound **37** (which have also been deposited with the Cambridge Crystallographic Data Centre as CCDC263244; copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax (+44) 1223 336033, email: deposit@ccdc.cam.ac.uk]). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Labrie, F. Intracrinology. *Mol. Cell. Endocrinol.* **1991**, *78*, C113−C118.
(2) Smith, H. J.; Nicholls, P. J.; Simons, C.; Le Lain, R. Inhibitors of steroidogenesis as agents for the treatment of hormone-dependent cancers. *Expert Opin. Ther. Pat.* **2001**, *11*, 789−824.
(3) Lonning, P. E. Role of endocrine therapy in breast cancer − where are we going? *Best Pract. Res. Clin. Endocrinol. Metab.* **2004**, *18*, VII−VIX.
(4) Miller, W. R. Biological rationale for endocrine therapy in breast cancer. *Best Pract. Res. Clin. Endocrinol. Metab.* **2004**, *18*, 1−32.
(5) Lonning, P. E. Oestrogen suppression − lessons from clinical studies. *Best Pract. Res. Clin. Endocrinol. Metab.* **2004**, *18*, 33−45.
(6) Santner, S. J.; Feil, P. D.; Santen, R. J. In situ estrogen production via the estrone sulfatase pathway in breat tumors − relative importance versus the aromatase pathway. *J. Clin. Endocrinol. Metab.* **1984**, *59*, 29−33.
(7) James, V. H. T.; McNeill, J. M.; Lai, L. C.; Newton, C. J.; Ghilchik, M. W.; Reed, M. J. Aromatase activity in normal breast and breast tumor tissues: in vivo and in vitro studies. *Steroids* **1987**, *50*, 269−279.
(8) Purohit, A.; Woo, L. W. L.; Chander, S. K.; Newman, S. P.; Ireson, C.; Ho, Y.; Grasso, A.; Leese, M. P.; Potter, B. V. L.; Reed, M. J. Steroid sulphatase inhibitors for breast cancer therapy. *J. Steroid Biochem. Mol. Biol.* **2003**, *86*, 423−432.
(9) Peltoketo, H.; Isomaa, V.; Maentausta, O.; Vihko, R. Complete amino acid sequence of human placental 17-beta-hydroxysteroid dehydrogenase deduced from cDNA. *FEBS Lett.* **1988**, *239*, 73−77.
(10) Mindnich, R.; Moller, G.; Adamski, J. The role of 17β-hydroxysteroid dehydrogenases. *Mol. Cell. Endocrinol.* **2004**, *218*, 7−20. The 17β-HSD type 13 isoform was disclosed at a workshop on 11β- and 17β-hydroxysteroid dehydrogenases: role in human disease, Germany 2005. Here 17β-HSD type 13 was identified as human retinal shortchain dehydrogenase/reductase 3, PDB code 1YDE.
(11) Peltoketo, H.; Luu-The, V.; Simard, J.; Adamski, J. 17 beta-Hydroxysteroid dehydrogenase (HSD)/17-ketosteroid reductase (KSR) family; nomenclature and main characteristics of the 17HSD/KSR enzymes. *J. Mol. Endocrinol.* **1999**, *23*, 1−11.
(12) Vihko, P.; Isomaa, V.; Ghosh, D. Structure and function of 17 beta-hydroxysteroid dehydrogenase type 1 and type 2. *Mol. Cell. Endocrinol.* **2001**, *171*, 71−76.
(13) Jin, J. Z.; Lin, S. X. Human estrogenic 17 beta-hydroxysteroid dehydrogenase: Predominance of estrone reduction and its induction by NADPH. *Biochem. Biophys. Res. Commun.* **1999**, *259*, 489−493.
(14) Labrie, F.; Luu-The, V.; Lin, S. X.; Simard, J.; Labrie, C.; El Alfy, M.; Pelletier, G.; Belanger, A. Intracrinology: role of the family of 17 beta-hydroxysteroid dehydrogenases in human physiology and disease. *J. Mol. Endocrinol.* **2000**, *25*, 1−16.
(15) Labrie, F.; Luu-The, V.; Lin, S. X.; Simard, J.; Labrie, C. Role of 17 beta-hydroxysteroid dehydrogenases in sex steroid formation in peripheral intracrine tissues. *Trends Endocrinol. Metab.* **2000**, *11*, 421−427.
(16) Vihko, P.; Harkonen, P.; Oduwole, O.; Torn, S.; Kurkela, R.; Porvari, K.; Pulkka, A.; Isomaa, V. 17 beta-hydroxysteroid dehydrogenases and cancers. *J. Steroid Biochem. Mol. Biol.* **2003**, *83*, 119−122.
(17) Chin, C.-C.; Warren, J. C. Synthesis of 16α-bromoacetoxyestradiol 3-methyl ether and study of the steroid binding site of human placental estradiol 17β-dehydrogenase. *J. Biol. Chem.* **1975**, *250*, 7682−7686.
(18) Auchus, R. J.; Covey, D. F. Mechanism-based inactivation of 17-beta, 20-alpha-hydroxysteroid dehydrogenase by an acetylenic secoestradiol. *Biochemistry* **1986**, *25*, 7295−7300.
(19) Tremblay, M. R.; Auger, S.; Poirier, D. Synthesis of 16-(bromoalkyl)-estradiols having inhibitory effect on human placental estradiol 17beta-hydroxysteroid dehydrogenase (17beta-HSD type 1). *Bioorg. Med. Chem.* **1995**, *3*, 505−523.
(20) Tremblay, M. R.; Poirier, D. Overview of a rational approach to design type I 17 beta- hydroxysteroid dehydrogenase inhibitors without estrogenic activity: Chemical synthesis and biological evaluation. *J. Steroid Biochem. Mol. Biol.* **1998**, *66*, 179−191.
(21) Vicker, N.; Lawrence, H. R. R.; Allan, G. M.; Bubert, C.; Fischer, D. S. M.; Purohit, A.; Reed, M. J.; Potter, B. V. L. Preparation of estranes as 17β-hydroxysteroid dehydrogenase inhibitors. PCT, WO2004085457, 2004.
(22) Lawrence, H. R.; Vicker, N.; Allan, G. M.; Robinson, J. J.; Mahon, M. F.; Tutill, H. J.; Purohit, A.; Reed, M. J.; Potter, B. V. L. Novel and Potent 17β-Hydroxysteroid Dehydrogenase Type 1 Inhibitors. *J. Med. Chem.* **2005**, *48*, 2759−2762.
(23) Kissman, H. M.; Hoffman, A. S.; Weiss, M. J. Synthesis of certain steroidal α-cyano ketones. *J. Org. Chem.* **1961**, *26*, 2610−2611.
(24) Kissman, H. M.; Hoffman, A. S.; Weiss, M. J. The synthesis of certain α-cyano keto steroids. *J. Org. Chem.* **1962**, *27*, 3168−3175.
(25) Katzenellenbogen, J. A.; Myers, H. N.; Johnson, H. J. Reagents for photoaffinity labeling of estrogen binding proteins. Synthesis of some azide and diazo derivatives of estradiol, estrone, and hexestrol. *J. Org. Chem.* **1973**, *38*, 3525−3533.
(26) Akanni, O. A.; Marples, B. A. Preparation of 16-formylestradiol and the 16-(alpha-methylenebutanolide) derivative. *Steroids* **1993**, *58*, 234−238.
(27) Pomeroy, J. H.; Craig, C. A. Two new syntheses of nitriles from aldehydes, using *O*,*N*-bis-(trifluoroacetyl)-hydroxylamine or trifluoroacetohydroxamic acid. *J. Am. Chem. Soc.* **1959**, *81*, 6340−6341.
(28) Schaub, R. E.; Weiss, P.; Weiss, M. J.; Oradell, N. J. New 16-substituted 1,3,5(10)-estratrienes. US Patent US3091609, 1963.
(29) Tanabe, M.; Peters, R. H.; Chao, W.-R.; Shigeno, K. Estrone sulfamate inhibitors of estrone sulfatase, and associated pharmaceutical compositions and methods of use. PCT, WO 9933858, 1999.
(30) Yoshioka, K.; Goto, G.; Mabuchi, H.; Hiraga, K.; Miki, T. Studies on antiandrogenic agents. Synthesis of 16β-ethyl-19-nortestosterone. *Chem. Pharm. Bull.* **1975**, *23*, 3203−3207.
(31) Harnik, M.; Hürzeler, E.; Jensen, E. V. Acetylation and trifluoroacetylation of steroids at carbon 16. *Tetrahedron* **1967**, *23*, 335−340.
(32) Park, J. D.; Lacher, J. R. A study of some fluorine-containing β-diketones. *J. Am. Chem. Soc.* **1953**, *75*, 4753−4756.
(33) Fischer, D. S.; Woo, L. W. L.; Mahon, M. F.; Purohit, A.; Reed, M. J.; Potter, B. V. L. D-ring modified estrone derivatives as novel potent inhibitors of steroid sulfatase. *Bioorg. Med. Chem.* **2003**, *11*, 1685−1700.
(34) Kusumi, T.; Yoneda, K.; Kakisawa, H. A convenient synthesis of 5,6,7,8-tetrahydroquinoline. *Synthesis* **1979**, 221.
(35) Koyama, J.; Sugita, T.; Suzuta, Y.; Irie, H. Thermolysis of oxime *O*-allyl ethers − a new method for pyridine synthesis. *Chem. Pharm. Bull.* **1983**, *31*, 2601−2606.

(36) Wang, X. C.; Wong, H. N. C. The chemistry of novel C-2 diazabiaryl ligands − cycloocta[2,1-b/3,4-b′]dipyridine, cyclooctа-[2,1-b/3,4-b′]diquinoline and their related compounds. *Tetrahedron* **1995**, *51*, 6941−6960.
(37) Kelly, T. R.; Lebedev, R. L. Synthesis of some unsymmetrical bridged terpyridines. *J. Org. Chem.* **2002**, *67*, 2197−2205.
(38) Regan, B. M.; Hayes, F. N. 17- and 17a-Aza-D-homosteroids. *J. Am. Chem. Soc.* **1956**, *78*, 639−643.
(39) Robinson, C. H.; Bruce, N. F.; Oliveto, E. P. Steroidal[17,16-c]-pyrazoles. *J. Med. Chem.* **1963**, *6*, 793−795.
(40) de Ruggieri, P.; Gandolfi, C.; Chiaramonti, D. Steroidi. − Nota XVI. Eterociclici steroidali. [17,16-c]-pirazol-derivati dell'estrano. *Gazz. Chim. Ital.* **1963**, *93*, 269−288.
(41) de Ruggieri, P.; Gandolfi, C.; Chiaramonti, D. (17,16-c)-Steroidal pyrazoles and related intermediate products. US Patent, US3243432, 1966.
(42) Sweet, F.; Boyd, J.; Medina, O.; Konderski, L.; Murdock, G. L. Hydrogen bonding in steroidogenesis − studies on new heterocyclic analogues of estrone that inhibit human estradiol 17-beta-dehydrogenase. *Biochem. Biophys. Res. Commun.* **1991**, *180*, 1057−1063.
(43) McArdle, P. ORTEX2.1 − a 1677-atom veriosn of ORTEP with automatic cell outline and cell packing for use on a PC. *J. Appl. Crystallogr.* **1994**, *27*, 438−439.
(44) Breton, R.; Housset, D.; Mazza, C.; FontecillaCamps, J. C. The structure of a complex of human 17 beta-hydroxysteroid dehydrogenase with estradiol and NADP(+) identifies two principal targets for the design of inhibitors. *Structure* **1996**, *4*, 905−915.
(45) Jones, G.; Willett, P.; Glen, R. C.; Leach, A. R.; Taylor, R. Development and validation of a genetic algorithm for flexible docking. *J. Mol. Biol.* **1997**, *267*, 727−748.
(46) Cramer, R. D., III; Patterson, D. E.; Bunce, J. D. Comparative Molecular Field Analysis (CoMFACoMFA) 1. Effect of shape on binding of steroids to carrier proteins R. D. *J. Am. Chem. Soc.* **1988**, *110*, 5959−5967.
(47) Lemmen, C.; Lengauer, T. Time-efficient flexible superposition of medium-sized molecules. *J. Comput.-Aided Mol. Des.* **1997**, *11*, 357−368.
(48) Lemmen, C.; Lengauer, T.; Klebe, G. FlexS: A method for fast flexible ligand superposition. *J. Med. Chem.* **1998**, *41*, 4502−4520.
(49) Cramer, R. D., III; Bunce, J. D.; Patterson, D. E.; Frank, I. E. Cross-validation, bootstrapping, and partial least squares compared with multiple regression in conventional QSAR Studies. *Quant. Struct.-Act. Relat.* **1988**, *7*, 18−25.
(50) Shiau, A. K.; Barstad, D.; Loria, P. M.; Cheng, L.; Kushner, P. J.; Agard, D. A.; Greene, G. L. The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. *Cell* **1998**, *95*, 927−937.
(51) Stauffer, S. R.; Coletta, C. J.; Tedesco, R.; Sun, J.; Katzenellenbogen, B. S.; et al. Pyrazole ligands: structure-affinity/activity relationships of estrogen receptor-selective agonists. *J. Med. Chem.* **2000**, *43*, 4934−4947.
(52) Fevig, T. L.; Katzenellenbogen, J. A. A short, stereoselective route to 16-alpha-(substituted-alkyl)estradiol derivatives. *J. Org. Chem.* **1987**, *52*, 247−251.
(53) Gupta, R.; Jindal, D. P. Synthesis and biological activity of some D-ring modified estrone derivatives. *Ind. J. Chem* **1999**, *38B*, 563−571.
(54) Duncan, L. J.; Reed, M. J. The role and proposed mechanism by which oestradiol 17β-hydroxysteroid dehydrogenase regulates breast tumour oestrogen concentrations. *J. Steroid Biochem. Mol. Biol.* **1995**, *55*, 565−572.
(55) Sheldrick G. M. Phase annealing in SHELX-90: Direct methods for larger structures. *Acta Crystallogr.* **1990**, *A46* (6), 467−473.
(56) Sheldrick G. M. *SHELXL-97, a computer program for crystal structure refinement*; University of Göttingen: Göttingen, Germany, 1997.

JM050348A