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Synthesis of Novel Steroidal Inhibitors of HIV-1 Protease.[1]

James J. Harburn, Gabrielle C. Loftus and Brian A. Marples*

Department of Chemistry, Loughborough University, Loughborough, Leics., LE11 3TU

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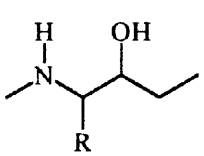
Abstract: The design and synthesis of potential steroidal HIV-1 protease inhibitors is described. Compounds derived from 11-amino-12-keto-cholanic acid derivatives show modest activity.

Introduction

Therapeutic potential has been established for specific inhibitors of the HIV-1 protease, which is a C_2 homodimer containing two aspartate residues in the catalytic site. Numerous examples of potent inhibitors of HIV-1 protease have been disclosed.[2]

The most active inhibitors which are competitive by nature, either contain the partial structure **(1)**[3] or the related carbonyl equivalent **(2)** which are believed to mimic the hydrolysis transition state leading to the tetrahedral *gem* diol **(3)**, thought to be involved in the aspartate catalysed proteolysis.



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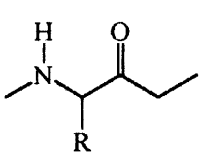

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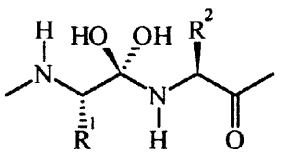

(3)

Other important structural features include the incorporation of lipophilic residues such as cyclohexylmethyl or benzyl and a bulky hydrophobic group or solubility increasing substituent on the nitrogen.[4] Typical of these are the symmetrical inhibitor A74704 **(4)**, a cyclohexyl relative[5] **(5)**, the difluoroketone derivative **(6)**[6] and the pyrrolidine based α-keto amide **(7)**.[7]

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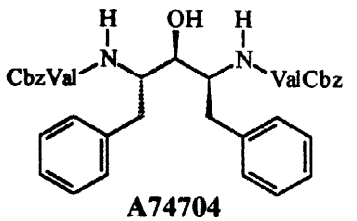

A74704
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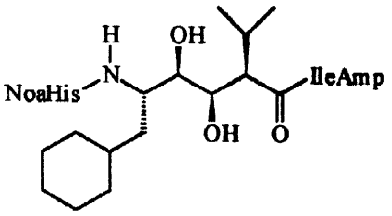

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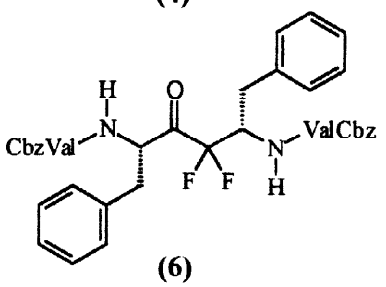

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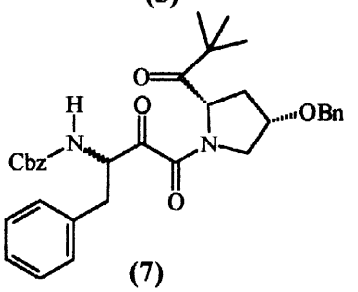
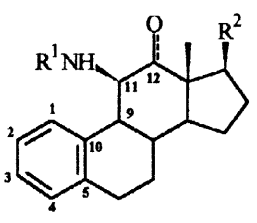


(7)

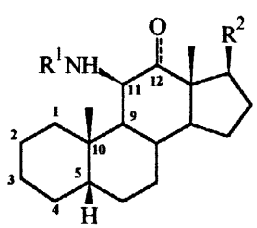
More recently inhibitors have been based upon sulfonamide-substituted cyclooctylpyranones,[8] *trans*-oxabicyclo[3,3,0]octane systems[9] and C_2 symmetric cyclic urea and sulfamide derivatives.[10]

Design of Inhibitors

The X-ray crystallographic structures of a number of the hydroxyethylamine and related HIV-1 protease/inhibitor complexes have been solved and all show similar hydrogen bonding and hydrophobic interactions within the active site.[11] We have used the conformation of inhibitor A74704 **(4)** in its HIV-1 protease complex[11] to explore best fit superimpositions of potential steroid targets using energy minimisation and modelling performed with SYBIL *ver.* 5.5 molecular modelling package.[12]



(8a)



(8b)

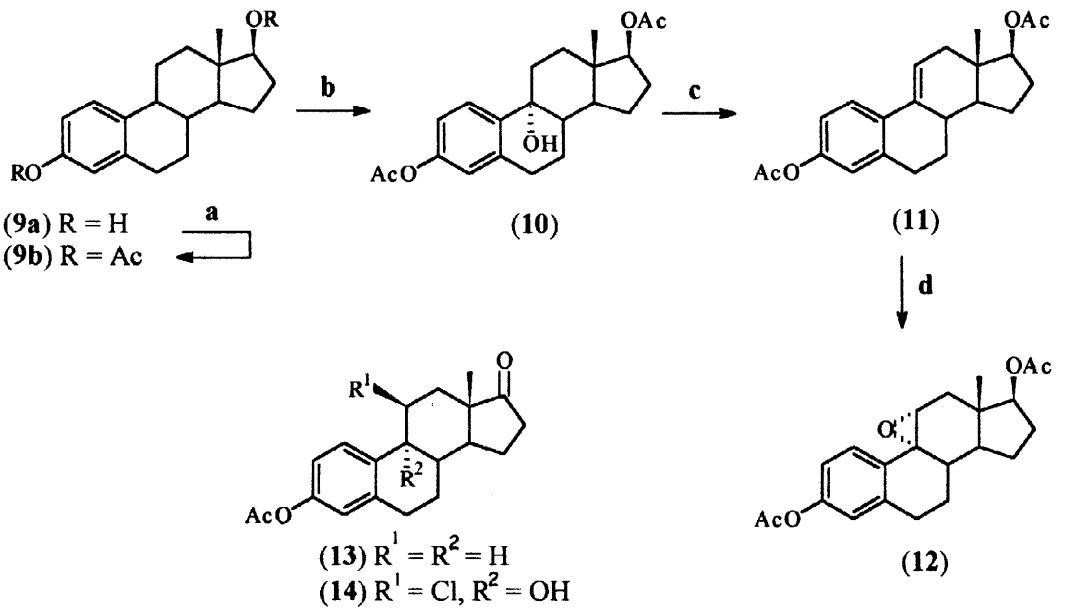
The primary targets are 11-amino-12-hydroxy/keto-steroids, based upon estra-1,3,5(10)-trienes **(8a)**/bile acids **(8b)** incorporating various C-17 side chains. In these structures the aromatic/saturated A ring and C-9 mimics the desirable benzyl/cyclohexylmethyl residue and the 11-amino-12-hydroxy/keto moiety mimics the hydroxy/ketoethylamine present in A74704 **(4)** and many other active inhibitors. It was

anticipated that variation of the C-17 side chain would afford the opportunity to provide alternatively lipophilic targets.

The increased lipophilicity of the target steroids over other inhibitors was thought to offer potentially improved *in vivo* activity as has been observed in some renin inhibitors.[13]

This work is complementary to the use of steroidal peptidomimetics as versatile scaffolds to bind to the fibrinogen receptor on blood platelets.[14]

Approaches from Estrogens.



Scheme 1 – a) Ac_2O, py, 86%. b) DMD, Me_2CO, 0 °C, 75%. c) Oxalic acid, PhH, 80 °C, 97% ($\Delta^{9,11}$: $\Delta^{8,9}$ = 95:3). d) *m*-CPBA, Na_2CO_3, $CHCl_3$, 59% (α:β = 7:1).

It was envisaged that 11-amino-12-oxygenated compounds would be available from 9α-hydroxy,11-azido-compounds and that these may be available *via* reaction of 9α,11α-epoxides with HN_3. The 3,17β-diacetoxy epoxide **(12)**[15] was prepared by a slight variation from the literature procedure in that the intermediate $\Delta^{9,11}$-compound **(11)** was prepared by the oxalic acid-catalysed dehydration of the 9α-hydroxy-compound **(10)** available from dimethyldioxirane oxidation of estradiol diacetate **(9b)**.[16]

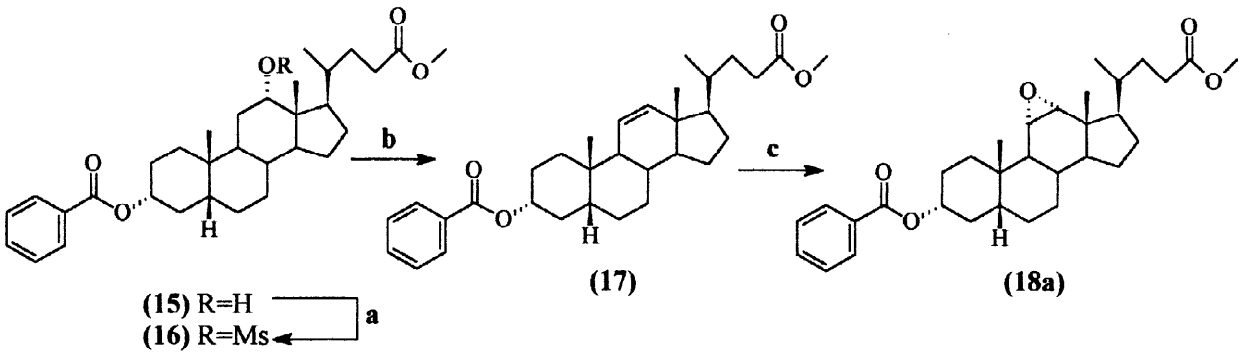
Reaction of the 3,17β-diacetoxy epoxide **(12)** with NaN_3/NH_4Cl in ethanol[17] gave intractable mixtures which were thought to be unpromising although there was evidence of azide formation (ν_{max} 2105 cm^{-1}).

A speculative approach to 9α-hydroxy-11-azido-compounds involving the reaction of estrone acetate **(13)**

with LiN_3/ceric ammonium nitrate also proved unsuccessful and it was not possible to satisfactorily purify the products. This reaction was based on the observed conversion of estrone acetate **(13)** to the 9α-hydroxy-11β-chloro-compound **(14)** with LiCl/CAN.[18]

Since the approach from aromatic A-ring steroids was unpromising, we turned our attention to the use of deoxycholic acid derivatives.

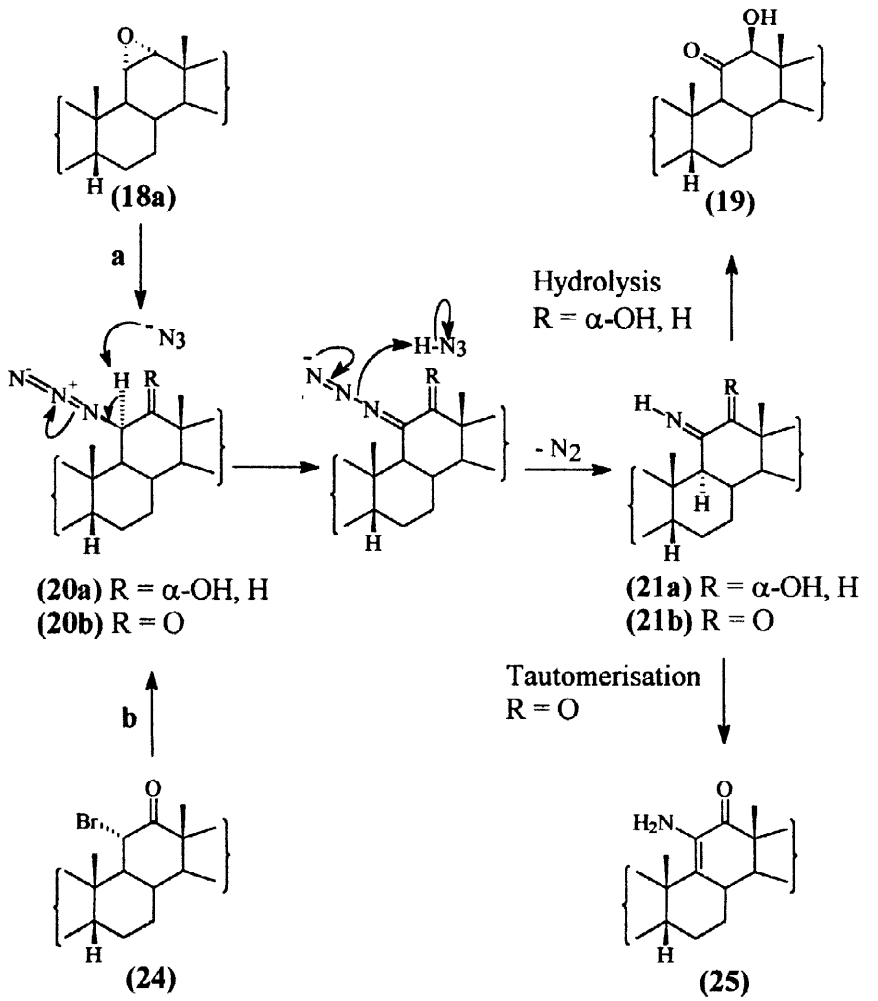
Approaches from the Bile Acid series.



Scheme 2 – a) MsCl, py, 0 °C, 100%. b) Δ (125 °C), KOAc, DMPU, 88%.
c) *m*-CPBA. $CHCl_3$, 0 °C, 67%.

Methyl 3α-benzyloxy-12α-hydroxycholan-24-oate **(15)**[19] was converted to the mesylate **(16)** and without purification, on heating (125 °C) with KOAc in 1,3-dimethyl-3,4,5,6-tetrahydro-2-pyrimidone (DMPU)[20] gave the $\Delta^{11,12}$-compound **(17)**. The ^{1}H NMR spectrum of **(17)** showed the alkene proton signals at δ_H 6.11 (dd, J = 10 and 3 Hz, CH-11) and 5.43 (d, J = 10 Hz, CH-12). Epoxidation of the $\Delta^{11,12}$-compound **(17)** with *m*-CPBA gave largely the 11α,12α-epoxide **(18a)** which was separated from the β-epoxide **(18b)** by chromatography and the observed ^{1}H NMR spectrum showed characteristic doublets (J = 4 Hz) for the CH-11 and CH-12 protons at δ_H 2.94 and 3.14 respectively. Interestingly, use of 2,2,2-trifluoroacetophenone/Oxone©[21] gave only the α-epoxide **(18a)** as observed by ^{1}H NMR spectroscopy. Reaction of 11α,12α-epoxide **(18a)** with NaN_3/DMSO in the presence of concentrated H_2SO_4[22] afforded the 12β-hydroxy-11-oxo-compound **(19)** rather than the expected 11β-azido-12α-hydroxy-compound **(20a)**. A similar reaction occurred with LiN_3. The assignment of structure was supported by the NMR spectra which showed peaks at δ_H 3.87 (s, CH-12α) and δ_C 213.12 (C-11 C=O). Furthermore, the positions of the angular methyl groups at δ_H 0.61 (s, C-13-Me) and 1.20 (C-10-Me) are consistent with the 12-hydroxy-11-oxo structure.

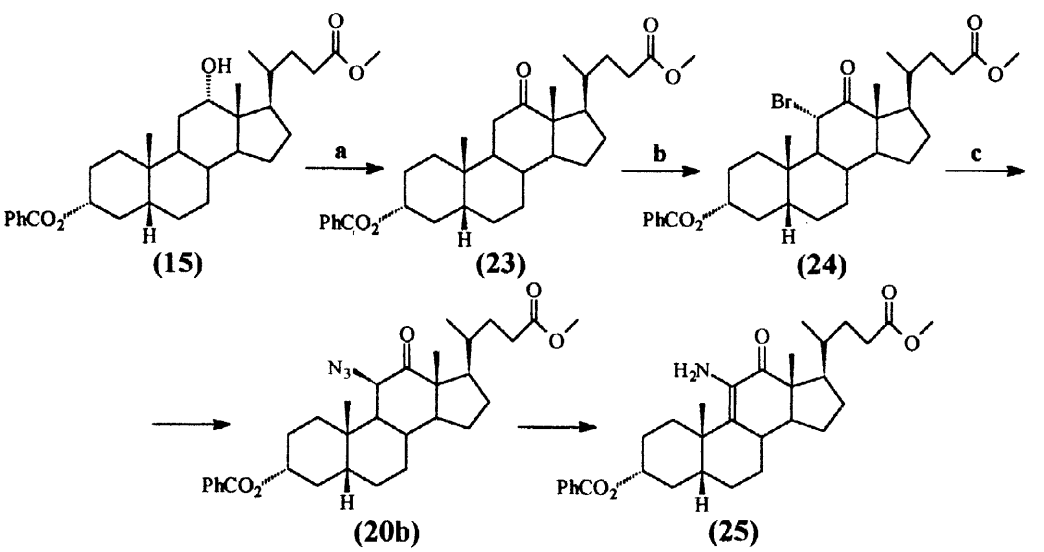
It is possible that the 12β-hydroxy-11-oxo-compound (**19**) arises from hydrolysis during chromatography of the imine (**21a**) which is formed from the unstable 11β-azido-12α-hydroxy-compound (**20a**). TLC evidence supports the intermediacy of an unstable product. Loss of nitrogen from α-azido-ketones (see below) has previously been reported[23,24,25] and is believed to be sterically driven, in part. The azido group of (**20a**), however, suffers significant interaction with the angular methyl groups and loss of nitrogen with formation of the imine (**21**) would release this (Scheme 3). It seems that this is sufficient to cause the observed decomposition. It would be anticipated that the thermodynamically stable 12β-hydroxy compound would be produced rather than its epimer.[26]



Scheme 3 - a) NaN_3/DMSO, H^+, 140 °C. b) NaN_3/DMSO, H^+, 100 °C.

Attempts to prepare the 11β-azido-12α-hydroxy compound (**20a**) using $TMSN_3/BF_3.OEt_2$ in DMF[27] appeared to give the 12α-hydroxy-$\Delta^{9,11}$-compound (**22**) presumably by Lewis acid-catalysed rearrangement of the epoxide moiety. The structure (**22**) was supported in particular by the appearance of important signals in the ^1H NMR spectrum at δ_H 5.66 (d, J = 6 Hz, CH-11) and 3.91 (t collapsing to d on addition of D_2O, J = 6 Hz, CH-12β) and the chemical shifts of the angular methyl groups at 0.57 (s, C13-Me) and 1.12 (s, C10-Me). No reaction was observed between the 11α,12α-epoxide (**18a**) and $TMSN_3/SmI_2$.[28]

Successful synthesis of the target molecules was achieved through the use of methyl 3α-benzoyloxy-12-oxo-5β-cholan-24-oate (**23**). Disappointing yields were obtained in the oxidation of compound (**15**) using Jones[29] (45%), Collins[30] (48%) and Swern[31] (17%) conditions. Use of a two-phase oxidation[32] in diethyl ether and water gave the 12-ketone (**23**)[33] (86%).

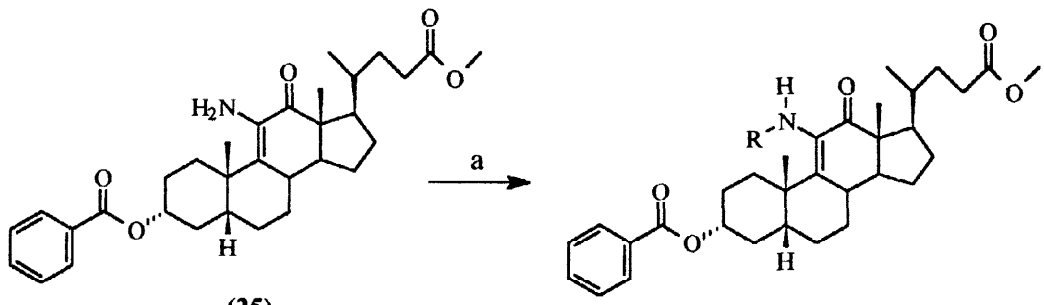


Scheme 4 – a) $Na_2Cr_2O_7$, H_2O, H_2SO_4, Et_2O, 90 min, 86%. b) Br_2, HBr, AcOH, 60 °C, 24 h, 73%. c) NaN_3, DMSO, 48 h, 100 °C, 53%.

Bromination of (**23**) in accordance with the method reported for the 3α-acetoxy analogue[34,35] gave the α-isomer (**24**) in 73% yield. The ^1H NMR spectrum of (**24**) shows a doublet at δ_H 4.94 (d, CH-11, J = 11 Hz) consistent with an α-bromine atom. Reaction of (**24**) with NaN_3 in DMSO at 100 °C failed to give the expected β-azido compound (**20b**). Instead, expulsion of nitrogen gas led to the formation of steroidal enamine (**25**) in 53% yield. This compound could be stored for limited periods in the freezer (-

15 °C). The 12-keto group would be expected to account for its reasonable stability compared to other simple enamines.

α-Keto azides are known to decompose relatively easily to form imino ketones or enamino ketones in the presence of azide ion on treatment with base (Scheme 3). The expected azido ketone **(20b)** would have an 11β-axial azide group which would be sterically congested as indicated for **(20a)** above. Attempts to increase the yield of enamine **(25)** or the azide **(20b)** using trimethylsilyl azide in dichloromethane,[36] sodium azide in DMF, methanol or ethylene glycol at various temperatures (50-150 °C), and lithium azide



(25)

(26a), R=Cbz, **27%**
(26b), R=Pivaloyl, **46%**
(26c), R=Ethoxycarbonyl, **27%**

Scheme 5 – a) RCl, DMAP, CH_2Cl_2, reflux, 18 h.

in DMF, methanol[23] or ethylene glycol at various temperatures (50-150 °C) all proved less successful than NaN_3 in DMSO. Derivatisation of enamine **(25)** with Boc using Boc_2O[37] and 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetone[38] proved unsuccessful. However, reactions with benzyl chloroformate, pivaloyl chloride and ethyl chloroformate in the presence of DMAP gave moderate yields of the derivatives **(26a)**, **(26b)** and **(26c)** respectively (Scheme 4) which unlike the enamine **(25)** are stable.

Compound **(26a)** showed line broadening in the ^1H NMR spectra at 25 °C, characteristic of certain bulky carbamates.[39] Increasing the temperature sharpened the spectra considerably and Figure 1 shows the angular methyl region; (a) in $CDCl_3$ (25-55 °C) and (b) in DMSO-d_6 (65-95 °C). A shift upfield for the C10 methyl can be seen from δ 1.00 ppm to 0.93 ppm when the solvent was changed from $CDCl_3$ to DMSO-d_6.

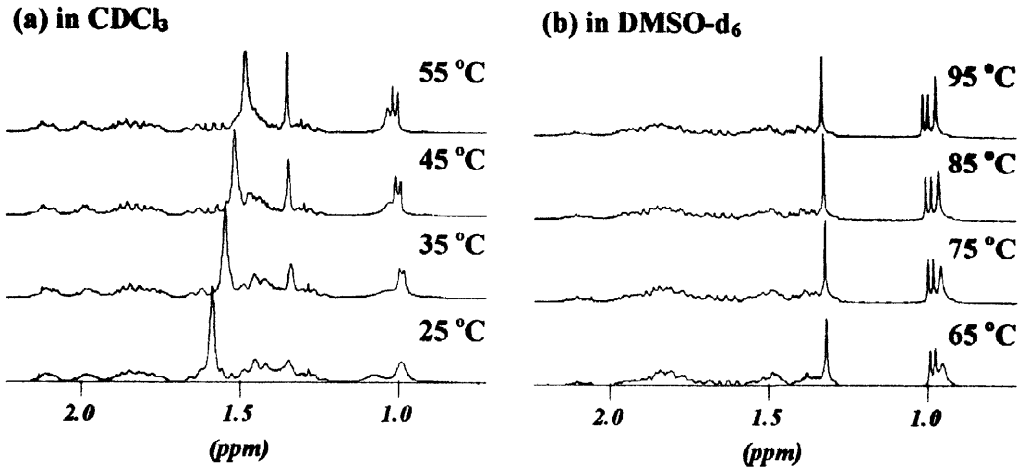


Figure 1 – Variable Temperature ^{1}H NMR of **(26a)**.

Attempted reductions of either the 12-ketone and/or the 9,11 double bond in enamine **(25)** and protected enamine **(26a)** using 9-BBN, $NaBH_4/CeCl_3$, $NaBH_3CN/HCl$[40] gave complex mixtures possibly because of the inherent instability of the enamine function in the absence of the 12-keto group. Attempted hydrogenation with Pt/C, Pd/C, PtO_2/C,[41] and Wilkinson's catalyst[42] gave only unreacted starting material perhaps not surprisingly in view of other observations on $\Delta^{9,11}$-12-ketones.[40]

Biological Testing

Table 1 below shows summary results for the biological testing of **(25)**, **(26a)**, **(26b)** and **(26c)** against HIV 111B C8166 infected cell lines[43] performed by Dr. Naheed Mahmood at MRC Collaborative Centre, Mill Hill, London.

Compound	$EC_{50}(\mu M)$	$TC_{50}(\mu M)$
25	20	50
26b	200	1000
26a	8	100
26c	20	100
Roche Sequinavir	0.001	20
AZT	0.016	>1000

Table 1 - Biological Activity against HIV.

EC_{50} represents the concentration which reduces Ag glycoprotein 120 by 50% in infected cell cultures. **TC_{50}** represents the concentration of drug which reduces cell growth by 50%. The pivaloyl derivative

(26a), showing the greatest potential, contains a bulky hydrophobic group upon C-11 nitrogen as previously predicted by molecular modelling studies.

If compared to Roche's HIV-1 protease inhibitor Sequinavir, the toxicity rating is comparable, however, it is not as active.

Experimental

Commercially available solvents and reagents were utilised throughout without further purification, unless detailed below.

'Light Petrol' refers to the fraction of petroleum ether boiling between 40 °C and 60 °C and was distilled over calcium chloride through a 36 cm Vigreux column before use. Dichloromethane was purified and dried by distillation from phosphorous pentoxide. Toluene was distilled from calcium hydride before being stored under nitrogen and over activated 4Å molecular sieves. Methanol was distilled from dried magnesium and iodine. Pyridine was distilled from potassium hydroxide pellets and stored over 4Å sieves. Analytical thin layer chromatography (TLC) was carried out using aluminium backed plates coated with Merck Kieselgel 60 GF254. All developed plates were visualised under ultra-violet light at 254 and 360 nm and/or by staining with molybdate and permanganate dips. Flash chromatography was carried out using Merck Kieselgel 60H silica and pressure was applied to the separation column. The samples were chromatographed either as pre-adsorbed sample on silica or applied to the silica as a saturated solution in an appropriate solvent.

Infrared spectra were recorded in the range 4000 - 600 cm^{-1} using a Nicolet FT-205 spectrometer with internal calibration. Spectra were recorded as either solutions in dichloromethane, as thin films or as nujol mulls. Thin film and nujol mull spectra were recorded using sodium chloride plates. ^{1}H NMR spectra were recorded using Bruker AC-250 (250 MHz) and Bruker-400 (400 MHz) instruments. ^{13}C NMR spectra were recorded on Bruker AC-250 (62.9 MHz) and Bruker-400 (100 MHz) instruments. ^{1}H NMR spectra are referenced against tetramethylsilane at 0.00 ppm. Chemical shift values δ_H and δ_C are accurate to ±0.01 ppm. Signals are described as being broad (br), singlets (s), doublets (d), triplets (t), quartets (q) and multiplets (m). High resolution mass spectra were recorded on a Kratos MS80 instrument or on a VAB-E instrument (Swansea EPSRC mass spectrometry service) by Electron Impact (EI) or Chemical Ionisation (CI). Melting points were recorded on a Koffler hotplate stage apparatus and are uncorrected. Optical rotations were recorded upon $_{POL}AA_R$ 2001 Polarimeter in an appropriate solvent.

3,17β-Acetoxyestra-1,3,5(10)9(11)-tetraene (11)

A solution of 9α-hydroxyestra-1,3,5(10)-triene 3,17β-diacetate (0.178 g, 0.5 mmol) in benzene (25 cm^3) was refluxed with a catalytic amount of oxalic acid for 30 minutes. The reaction mixture was evaporated and the residue chromatographed over silica eluting with diethyl ether / light petrol (2:3). The major product obtained was the title compound (0.168 g, 95%)

Mpt. 141-144 °C [lit. mpt.[15] 148-149 °C]; IR ν_{max} (cm^{-1}) 2924, 1749 (CH_3CO_2-C3), 1729 (CH_3CO_2-C17), 1602; ^1H NMR (250 MHz, $CDCl_3$) δ 0.83 (s, C$\underline{H}_3$-18, 3H), 2.07 (s, C$\underline{H}_3$CO, 3H), 2.28 (s, C$\underline{H}_3$CO, 3H), 4.77 (t, J = 9 Hz, CH-17α, 1H), 6.19 (m, CH-11, 1H), 6.80-6.89 (m, CH-2 & CH-4, 2H), 7.59 (d, J = 6 Hz, CH-1, 1H); ^{13}C NMR (62.9 MHz, $CDCl_3$) 12.00 (C-18), 21.04 (CH_3CO), 21.08 (CH_3CO), 23.04 (C-15), 27.51 (C-7), 27.91 (C-6), 29.67 (C-12), 38.35 (C-8), 39.27 (C-16), 41.23 (C-13), 47.20 (C-14), 82.62 (C-17), 119.24 (C-2), 119.72 (C-11), 121.61 (C-4), 124.99 (C-1), 132.21 (C-9), 134.52 (C-5), 137.30 (C-10), 149.15 (C-3), 169.56 (3-$CH_3\underline{C}O$), 171.03 (17-$CH_3\underline{C}O$). [Found M^+ 354.1818 (48 %), 312 (93), 252 (63) Calc. for $C_{22}H_{26}O_4$, 354.1831].

Methyl 3α-benzyloxy-12α-mesyloxy-5β-cholan-24-oate (16)

Methane sulphonyl chloride (2.2 cm^3, 30 mmol) in benzene (20 cm^3) was added dropwise to a stirred solution of methyl 3α-benzoyloxy-12α-hydroxy-5β-cholan-24-oate (2.02 g, 4.31 mmol) in anhydrous pyridine (20 cm^3) at 0 °C. Stirring was continued for 4 h, warming gradually to room temperature. The reaction mixture was diluted with dichloromethane (100 cm^3) and washed with water (2 x 100 cm^3), 20% aqueous copper sulphate (2 x 50 cm^3), water (50 cm^3) and dried over sodium sulphate. Removal of solvent *in vacuo* yielded a viscous orange oil which solidified after 24 h drying under high vacuum. This product (2.58 g, 100%) was used without further purification.

IR ν_{max} (cm^{-1}) 2947, 2870, 1740 (CO_2Me), 1714 ($PhCO_2$), 1377; ^1H NMR (250 MHz, $CDCl_3$) δ 0.78 (s, C$\underline{H}_3$-18, 3H), 0.97 (s, C$\underline{H}_3$-19, 3H), 1.01 (d, J = 6 Hz, C$\underline{H}_3$-21, 3H), 3.12 (s, $O_2SC\underline{H}_3$, 3H), 3.66 (s, OCH_3, 3H), 4.95 (m, CH-3β, 1H), 5.13 (s, CH-12β, 1H), 7.41-7.68 (m, *m*-Ar-H and *p*-Ar-H, 3H), 8.03-8.08 (m, *o*-Ar-H, 2H)

Methyl 3α-benzyloxy-5β-chol-11-en-24-oate (17)

Methyl 3α-benzyloxy-12α-mesyloxy-5β-cholan-24-oate (1.15 g, 1.95 mmol) was heated with potassium acetate (0.96 g, 9.78 mmol) in 1,3-dimethyl-3,4,5,6-tetrahydro-2-pyrimidone (DMPU, 11 cm^3) at 125 °C for 4 h. After cooling, the reaction mixture was diluted with water (100 cm^3) and extracted with dichloromethane (2 x 50 cm^3). The combined organic phases were further washed with water (50 cm^3)

and saturated aqueous sodium chloride (50 cm^3) and dried over Na_2SO_4. Removal of solvent *in vacuo* and chromatography over silica gel, eluting with diethyl ether / light petrol (1:3) afforded the *title compound* as colourless needles (0.88 g, 88%).

Mpt. 103-106 °C; IR ν_{max} (cm^{-1}) 1731 (CO_2Me), 1716 ($PhCO_2$); ^1H NMR (250 MHz, $CDCl_3$) δ 0.73 (s, $C\underline{H}_3$-18, 3H), 0.92 (s, $C\underline{H}_3$-19, 3H), 1.01 (d, J = 6 Hz, $C\underline{H}_3$-21, 3H), 3.65 (s, OCH_3, 3H), 4.99 (m, CH-3β, 1H), 5.43 (d, J = 10 Hz, CH-12, 1H), 6.11 (dd, J = 10 Hz and J = 3 Hz, CH-11, 1H), 7.41 (m, *m*-Ar-H, 2H), 7.52 (m, *p*-Ar-H, 1H), 8.03 (m, *o*-Ar-H, 2H); ^{13}C NMR (62.9 MHz, $CDCl_3$) 16.59 (C-18), 18.25 (C-21), 22.91 (C-15), 23.56 (C-19), 25.40 (C-16), 26.61 (C-7), 27.87 (C-8), 28.36 (C-2), 30.80 (C-23), 30.90 (C-22), 32.90 (C-4), 34.26 (C-8), 34.83 (C-1), 34.96 (C-10), 35.87 (C-20), 40.90 (C-5), 43.04 (C-9), 44.94 (C-13), 51.37 (OCH_3), 51.83 (C-14), 53.50 (C-17), 74.60 (C-3), 125.28 (C-12), 128.15 (*m*-Ar-C), 129.42 (*o*-Ar-C), 130.79 (*i*-Ar-C) 132.58 (*p*-Ar-C), 165.91 ($Ph\underline{C}O_2$), 174.47 (C-24). [Found MNH_4^+ 510.3580 (34 %), 371 (100), 255 (42) $C_{32}H_{48}NO_4$ requires MNH_4, 510.3583].

Methyl 3α-benzyloxy-11α,12α-epoxy-5β-cholan-24-oate (18a)

(a) A solution of methyl 3α-benzyloxy-5β-chol-11-en-24-oate (1.16 g, 2.35 mmol) in chloroform (50 cm^3) was cooled to 0 °C and treated with *meta*-chloroperbenzoic acid (1.60 g of 50% *m*-CPBA, 4.7 mmol) and sodium carbonate (0.50 g, 4.70 mmol). After stirring at room temperature for 24 h the reaction mixture was washed with water (2 x 50 cm^3) and dried over Na_2SO_4. Removal of the solvent *in vacuo* and chromatography over silica gel, eluting with diethyl ether / light petrol (1:2) afforded the *title compound* (0.80 g, 67%) as a gum.

(b) To methyl 3α-benzyloxy-5β-chol-11-en-24-oate (0.10 g, 0.200 mmol), acetonitrile (7.5 cm^3), 2,2,2-trifluoroacetophenone (0.167 g, 0.96 mmol) and $(EDTA)Na_2$ solution (4 x 10^{-4} M , 5 cm^3), was added a solid mixture of Oxone® (0.59 g, 0.96 mmol) and sodium hydrogen carbonate (0.33g, 3.10 mmol) over 30 minutes. This solution was stirred vigorously at room temperature for 24 h. After such time the reaction mixture was worked up by extraction with ethyl acetate (2 x 25 cm^3), washed with saturated sodium hydrogen carbonate solution (50 cm^3), dried over Na_2SO_4 and removal of solvent *in vacuo* to give the *title compound* as a colourless gum (0.099 g, 96%).

IR ν_{max} (cm^{-1}) 1746 (CO_2Me), 1722 ($PhCO_2$); ^1H NMR (250 MHz, $CDCl_3$) δ 0.79 (s, $C\underline{H}_3$-18, 3H), 1.03 (s, $C\underline{H}_3$-19, 3H), 1.05 (d, J = 6 Hz, $C\underline{H}_3$-21, 3H), 2.94 (d, J = 4 Hz, C11-H, 1H), 3.14 (d, J = 4 Hz, C12-H, 1H), 3.67 (s, OMe, 3H), 4.95 (m, CH-3β, 1H), 7.42 (m, *m*-Ar-H, 2H), 7.53 (m, *p*-Ar-H, 1H), 8.03 (m, *o*-Ar-H, 2H); ^{13}C NMR (62.9 MHz, $CDCl_3$) 11.80 (C-18), 18.25 (C-21), 22.21 (C-15), 23.76 (C-19), 25.19 (C-16), 26.67 (C-7), 27.20 (C-6), 30.60 (C-23), 30.80 (C-22), 32.60 (C-4), 32.70 (C-8), 34.80 (C-1), 35.00

(C-20 & C-10), 40.90 (C-5), 41.20 (C-9), 43.40 (C-13), 46.50 (C-17), 50.30 (C-14), 51.40 (OCH_3), 53.40 (C-12), 60.80 (C-11), 74.60 (C-3), 128.15 (*m*-Ar-C), 129.42 (*o*-Ar-C), 130.59 (*i*-Ar-C), 132.58 (*p*-Ar-C), 165.00 ($PhCO_2$), 174.57 (C-24). [Found MH^+ 509.3270 (8 %), 526 (MNH_4, 100), 508 (M, 8), 491 (M-OH, 33) $C_{32}H_{44}O_5$ requires MH, 509.3267].

Methyl 3α-benzyloxy-12β-hydroxy-11-oxo-5β-cholan-24-oate (19)

Methyl 3α-benzyloxy-11α,12α-epoxy-5β-cholan-24-oate (0.05 g, 0.10 mmol) was heated to 140 °C with excess sodium azide (0.032 g, 0.049 mmol) in anhydrous dimethyl sulphoxide (10 cm^3) containing two drops of 98% sulphuric acid. After 16 h the reaction mixture was cooled, diluted with dichloromethane (20 cm^3), washed with water (3 x 20 cm^3) and aqueous saturated sodium chloride (20 cm^3) and then dried over Na_2SO_4. TLC analysis indicated a single product which decomposed to a more polar second product when the crude mixture was chomatographed over silica gel, eluting with diethyl ether / light petrol (1:2) affording the *title compound* as a gum (0.030 g, 57%).

IR ν_{max} (cm^{-1}) 3450 (OH), 1738 (CO_2Me), 1712 ($PhCO_2$), 1693 (C11 C=O); ^1H NMR (250 MHz, $CDCl_3$) δ 0.61 (s, CH_3-18, 3H), 0.91-0.92 (d, J = 6 Hz, CH_3-21, 3H), 1.20 (s, CH_3-19, 3H), 2.74 (br, OH, 1H), 3.66 (s, OCH_3, 3H), 3.87 (m, CH-12α, 1H), 4.94-4.96 (m, CH-3β, 1H), 7.42-7.46 (m, *m*-Ar-H, 2H), 7.53-7.57 (m, *p*-Ar-H, 1H), 8.03-8.05 (m, *o*-Ar-H, 2H); ^{13}C NMR (62.9 MHz, $CDCl_3$) 10.70 (C-18), 17.12 (C-21), 22.88 (C-15), 23.93 (C-19), 26.33 (C-16), 26.73 (C-7), 27.28 (C-6), 27.67 (C-2), 30.62 (C-23), 30.85 (C-22), 32.34 (C-4), 33.34 (C-10), 34.10 (C-1), 34.79 (C-20), 37.05 (C-8), 42.67 (C-5), 45.28 (C-9), 45.73 (C-17), 47.08 (C-14), 48.96 (C-13), 51.48 (OCH_3), 74.56 (C-3), 82.83 (C-12), 128.29 (*m*-Ar-C), 129.55 (*o*-Ar-C), 130.76 (*i*-Ar-C), 132.76 (*p*-Ar-C), 166.07 ($PhCO_2$), 174.56 (C-24), 213.12 (C-11). [Found MNH_4^+ 542.3490 (76 %), 524 (M, 12), 507 (M-OH, 5), 491 (22) $C_{32}H_{48}NO_6$ requires MNH_4, 542.3482].

Methyl 3α-benzyloxy-12α-hydroxy-5β-chol-9(11)-en-24-oate (22)

Methyl 3α-benzyloxy-11α,12α-epoxy-5β-cholan-24-oate (0.01 g, 0.20 mmol) was treated with trimethylsilylazide (0.13 cm^3, 0.98 mmol) in anhydrous DMF (5 cm^3) for 10 minutes, then two drops of boron trifluoride etherate were added and the mixture heated for 16 h at 140 °C. The reaction mixture was then cooled, diluted with diethyl ether (50 cm^3), washed with water (3 x 25 cm^3) and aqueous saturated sodium chloride (25 cm^3) and dried over Na_2SO_4. Removal of solvent under reduced pressure and purification over silica gel, eluting with diethyl ether / petrol (1:1) afforded the rearranged *title compound* as a colourless gum (0.05 g, 50%).

IR ν_{max} (cm^{-1}) 3550 (OH), 2952, 2852, 1743 (CO_2Me); ^1H NMR (250 MHz, $CDC1_3$)

δ 0.57 (s, C<u>H</u>$_3$-18, 3H), 1.01 (d, J = 6 Hz, C<u>H</u>$_3$-21, 3H), 1.12 (s, C<u>H</u>$_3$-19, 3H), 3.67 (s, OCH_3, 3H), 3.91 (t, collapses to a doublet on addition of D_2O, J = 6 Hz, CH-12β, 1H), 4.99 (m, CH-3β, 1H), 5.66 (d, J = 6 Hz, CH-11), 7.42 (m, *m*-Ar-H, 2H), 7.53 (m, *p*-Ar-H, 1H), 8.03 (m, *o*-Ar-H, 2H); ^{13}C NMR (62.9 MHz, $CDCl_3$) 11.21 (C-18), 17.21 (C-21), 25.04 (C-15), 26.60 (C-16), 26.75 (C-7), 27.92 (C-6), 28.01 (C-2), 29.95 (C-19) 30.98 (C-23), 31.14 (C-22), 32.34 (C-4), 34.06 (C-1), 35.13 (C-20), 37.35 (C-8), 38.80 (C-10), 41.92 (C-5), 45.13 (C-13), 46.59 (C-14), 46.92 (C-17), 51.47 (OMe), 72.41 (C-3), 75.12 (C-12), 122.11 (C-11), 128.25 (*m*-Ar-C), 129.57 (*o*-Ar-C), 130.81 (*i*-Ar-C), 132.71 (*p*-Ar-C), 145.97 (C-9), 166.17 (Ph<u>C</u>O$_2$), 174.72 (C-24). [Found M^+ 508.3189 (5%), 386 (67), 271 (17), 253 (18) $C_{32}H_{44}O_5$ requires M, 508.3188].

Methyl 3α-benzyloxy-12-keto-5β-cholan-24-oate (23)

To a rapidly stirred solution of sodium dichromate (0.40 g, 1.709 mmol) in distilled water (5 cm^3) was added dropwise concentrated sulphuric acid (0.5 cm^3, 98%). The resulting chromic acid was added to methyl 3α-benzoyl-12α-hydroxy-5β-cholan-24-oate (1.00 g, 1.96 mmol) in diethyl ether (30 cm^3) at 0 °C and then stirred for 90 minutes. The two-phase mixture was separated, the aqueous layer extracted with diethyl ether (2 x 25 cm^3), the combined organic extracts washed with saturated $NaHCO_3$ (3 x 30 cm^3), dried over $MgSO_4$ and the solvent removed *in vacuo* to yield a colourless gum. Recrystallisation from methanol yielded the title compound as white prisms (0.86 g, 86%).

Mpt. 126-128 °C [lit. mpt.[33] 128-129 °C]; IR ν_{max} (cm^{-1}) 1735 (CO_2Me); ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.85-0.87 (d, J = 6Hz, C<u>H</u>$_3$-21, 3H), 1.03 (s, C<u>H</u>$_3$-18, 3H), 1.06 (s, C<u>H</u>$_3$-19, 3H), 3.67 (s, OCH_3, 3H), 4.82-5.23 (m, CH-3β, 1H), 7.39-7.55 (m, *m*-Ar-H, 2H), 7.52-7.55 (m, *p*-Ar-H, 1H), 8.01-8.04 (m, *o*-Ar-H, 2H); ^{13}C NMR (62.9 MHz, $CDCl_3$) 11.62 (C-18), 18.52 (C-21), 22.70 (C-19), 24.26 (C-15), 25.95 (C-16), 26.41 (C-4), 26.89 (C-7), 27.45 (C-6), 30.45 (C-11), 31.23 (C-2), 32.18 (C-22), 34.93 (C-23), 35.43 (C-14), 35.55 (C-10), 35.61 (C-20), 36.08 (C-1), 41.34 (C-5), 44.06 (C-8), 46.40 (C-17), 51.40 (OCH_3), 57.47 (C-13), 58.61 (C-9), 74.20 (C-3), 128.40 (*m*-Ar-C), 129.45 (*o*-Ar-C), 130.64 (*i*-Ar-C), 132.68 (*p*-Ar-C), 166.90 (Ph<u>C</u>O$_2$), 174.60 (C-24), 214.73 (C-12); $[\alpha_D]^{20}$= +90.8 (0.051 g cm^{-3}, EtOH). [Found M^+ 508.3188 (29%), 386 (54), 271 (20), 231 (100) Calc. for $C_{32}H_{44}O_5$ M, 508.3188].

Methyl 3α-benzyloxy-11α-bromo-12-keto-5β-cholan-24-oate (24)

To a solution of methyl 3α-benzyloxy-12-keto-5β-cholan-24-oate (0.15 g, 0.295 mmol), hydrogen bromide (0.2 cm^3, 4 M) and glacial acetic acid (10 cm^3) was added bromine (0.052 g, 0.32 mmol) in glacial acetic acid (5 cm^3) dropwise at 60 °C. After stirring for 24 h the reaction mixture was poured

over iced water (150 cm^3), extracted with diethyl ether (3 x 30 cm^3) and successively washed with distilled water (25 cm^3), saturated $NaHCO_3$ (4 x 25 cm^3) and distilled water (25 cm^3). Drying over $MgSO_4$ and removal of solvent *in vacuo* yielded a colourless solid (0.164 g). Chromatography (silica, 2:1 light petrol / diethyl ether) afforded a colourless solid as prisms (0.127 g, 73%).

Mpt. 138-139 °C; IR ν_{max} (cm^{-1}) 1738 (CO_2Me), 1706 (C12 C=O); ^1H NMR (400 MHz, $CDCl_3$) δ 0.79-0.80 (d, J = 6 Hz, C$\underline{H}_3$-21, 3H), 0.97 (s, C$\underline{H}_3$-18, 3H), 1.16 (s, C$\underline{H}_3$-19, 3H), 3.59 (s, OC$\underline{H}_3$, 3H), 4.92-5.23 (m, CH-3β, 1H), 4.93-4.97 (d, J = 11 Hz, CH-11, 1H), 7.33-7.38 (m, *m*-Ar-H, 2H), 7.45-7.49 (m, *p*-Ar-H, 1H), 7.95-7.97 (m, *o*-Ar-H 2H); ^{13}C NMR (100 MHz, $CDCl_3$) 11.41 (C-18), 18.52 (C-21), 23.14 (C-19), 24.98 (C-15), 26.61 (C-16), 27.34 (C-4), 27.67 (C-7), 27.84 (C-6), 30.36 (C-2), 31.19 (C-22), 33.23 (C-23), 35.38 (C-20), 37.20 (C-10), 37.21 (C-1), 38.08 (C-14), 43.79 (C-5), 48.19 (C-17), 51.49 (OCH_3), 51.69 (C-8), 56.29 (C-11), 57.15 (C-13), 58.63 (C-9), 74.35 (C-3), 128.27 (*m*-Ar-C), 129.55 (*o*-Ar-C), 130.78 (*i*-Ar-C), 132.74 (*p*-Ar-C), 166.13 (Ph$\underline{C}O_2$), 174.52 (C-24), 203.72 (C-12); $[\alpha_D]^{20}$ = +35.4 (0.05 g cm^{-3}, EtOH). [Found M-Br^+ 507.3109 (29%), 384 (38), 229 (81), 121 (100) $C_{32}H_{43}O_5$ requires M-Br, 507.3110].

Methyl 11-amino-3α-benzyloxy-12-keto-5β-chol-9,11-en-24-oate (25)

To a solution of sodium azide (0.10 g, 1.54 mmol) in anhydrous DMSO (10 cm^3) was added methyl 3α-benzyloxy-11β-bromo-12-keto-5β-cholan-24-oate (0.075 g, 0.128 mmol) at 100 °C. After stirring for 48 h the reaction mixture was poured over ice water (50 cm^3) and extracted with diethyl ether (3 x 25 cm^3). The combined organic extracts were washed successively with sodium nitrite solution (25 cm^3, 20%), saturated brine (25 cm^3), water (25 cm^3), dried over $MgSO_4$ and solvent removed *in vacuo* to yield a colourless gum (0.064 g). Chromatography (silica, 20:10:1 light petroleum / diethyl ether / triethylamine) afforded a colourless solid (0.036 g, 53%)

Mpt. 72-74 °C; IR ν_{max} (cm^{-1}) 3512 and 3367 (NH_2), 1737 (CO_2Me), 1715 (C-12 C=O), 1685 (α,β-unsat. ketone), 1600 (NH_2); ^1H NMR (400 MHz, $CDC1_3$) δ 0.88 (s, C$\underline{H}_3$-18, 1H), 0.95-0.96 (d, J = 6 Hz, C$\underline{H}_3$-21, 3H), 1.20 (s, C$\underline{H}_3$-19, 3H), 3.60 (s, OC$\underline{H}_3$, 3H), 3.80 (br, NH, 1H), 4.94-4.97 (m, CH-3β, 1H), 7.32-7.36 (m, *m*-Ar-H, 1H), 7.44-7.48 (m, *p*-Ar-H, 1H), 7.94-7.96 (m, *o*-Ar-H, 2H); ^{13}C NMR (100 MHz, $CDC1_3$) 9.37 (C-18), 18.49 (C-21), 23.61 (C-15), 26.03 (C-16), 26.25 (C-7), 26.35 (C-6), 26.51 (C-19), 29.70 (C-2), 29.88 (C-23), 30.52 (C-4), 30.52 (C-22), 33.89 (C-1), 34.35 (C-20), 36.82 (C-8), 40.72 (C-10), 42.64 (C-5), 47.25 (C-17), 50.42 (C-14), 50.72 (C-13), 51.18 (OCH_3), 72.16 (C-3), 125.76 (C-9), 127.27 (*m*-Ar-C), 128.57 (*o*-Ar-C), 129.81 (*i*-Ar-C), 133.11 (*p*-Ar-C), 131.77 (C-11), 165.09 (Ph$\underline{C}O_2$),

173.61 (C-24), 202.18 (C-12); $[\alpha_D]^{20}$= +55.2 (0.051 g 100 cm^{-3}, EtOH). [Found M^+ 521.3139 (100%), 506 (18), 384 (42), 317 (22) $C_{32}H_{43}O_5N$ requires M, 521.3141].

Methyl (N-benzyloxycarbonyl)-11-amino-3α-benzoyl-12-keto-5β-chol-9,11-en-24-oate (26a)

Methyl 11-amino-3α-benzoyl-12-keto-5β-chol-9,11-en-24-oate (0.075 g, 0.144 mmol), 4-dimethylaminopyridine (0.012 g, 0.098 mmol) and benzyl chloroformate (0.1 cm^3, 0.665 mmol) were refluxed under nitrogen for 48 h in dichloromethane (30 cm^3). The resulting mixture was allowed to cool, then successively washed with HCl (2 M, 20 cm^3), saturated $NaHCO_3$ (20 cm^3), dried over Na_2SO_4 and then concentrated *in vacuo* to yield a colourless gum (0.12 g). Chromatography (silica, 4:1:1 light petrol / dichloromethane / EtOAc) gave the *title compound* as a colourless crystalline solid (0.026 g, 27%).

Mpt. 66-67 °C; IR ν_{max} (cm^{-1}) 3309 (NH), 1715 ($PhCO_2$); ^{1}H NMR (400 MHz, $CDCl_3$, 55°C) δ 0.96-0.98 (d, J = 6 Hz, $C\underline{H}_3$-21, 3H), 1.00 (s, $C\underline{H}_3$-19, 3H), 1.31 (s, $C\underline{H}_3$-18, 3H), 3.65 (s, $OC\underline{H}_3$, 3H), 4.98-5.06 (m, CH-3β, 1H), 5.09-5.11 (d, J = 4 Hz, $C\underline{H}_2$-Ph, 2H), 5.96 (br, NH, 1H), 7.28-7.31 (m, Ar-H (Cbz)), 5H), 7.38-7.42 (m, *m*-Ar-H, 2H), 7.49-7.51 (m, *p*-Ar-H, 2H), 7.98-8.00 (m, *o*-Ar-H, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) 9.78 (C-18), 19.14 (C-21), 24.88 (C-15), 26.57 (C-16), 26.60 (C-7) , 27.62 (C-6), 28.02 (C-19), 30.60 (C-2), 30.66 (C-23), 31.42 (C-22), 34.93 (C-4), 35.46 (C-20), 36.73 (C-1), 38.63 (C-8), 42.55 (C-10), 43.66 (C-5), 47.60 (C-17), 51.49 (OCH_3), 52.49 (C-14), 52.76 (C-13), 67.10 (Ph-$C\underline{H}_2$-O), 73.42 (C-3), 127.86 (Cbz *i*-Ar-C), 128.31 (Cbz Ar-C), 128.46 (*m*-Ar-C), 129.55 (*o*-Ar-C), 130.61 (*i*-Ar-C), 132.84 (*p*-Ar-C), 136.33 (C-9), 154.67 ($\underline{C}$ONH), 155.90 (C-11), 166.07 ($Ph\underline{C}O_2$), 174.69 (C-24), 206.91 (C-12); $[\alpha_D]^{20}$ = +13° (0.028 g 100 cm^{-3}, methanol). [Found M^+ 655.3510 (4%), 547 (4), 520 (6), 490 (2) $C_{40}H_{49}O_7N$ requires M, 655.3509].

Methyl (N-pivaloyl)-11-amino-3α-benzoyl-12-keto-5β-chol-9,11-en-24-oate (26b)

Methyl 11-amino-3α-benzoyl-12-keto-5β-chol-9,11-en-24-oate (0.075 g, 0.144 mmol), 4-dimethylaminopyridine (0.012 g, 0.098 mmol) and pivaloyl chloride (0.1 cm^3, 0.812 mmol) were refluxed under nitrogen for 48 h in dichloromethane (30 cm^3). The resulting mixture was allowed to cool, then, successively washed with HCl (2 M, 20 cm^3), $NaHCO_3$ (20 cm^3), dried over Na_2SO_4 and then concentrated *in vacuo* to yield a colourless gum (0.15 g). Chromatography (silica, 5:1:1 light petroleum / dichloromethane / EtOAc) gave *the title compound* as a colourless microcrystalline solid (0.040 g, 46%).

Mpt. 74-75 °C; IR ν_{max} (cm^{-1}) 3375 (NH), 1731 (CO_2Me), 1716 ($PhCO_2$), 1671 (C-12 C=O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92-0.94 (d, J = 6 Hz, $C\underline{H}_3$-21, 3H), 1.08 (s, $C\underline{H}_3$-18, 3H), 1.22 (s, $C(C\underline{H}_3)_3$, 9H),

1.35 (s, C<u>H</u>$_3$-19, 3H), 3.66 (s, OC<u>H</u>$_3$, 3H), 4.83-5.23 (m, CH-3β, 1H), 6.90 (br, NH, 1H), 7.42-7.46 (m, *m*-Ar-H, 2H), 7.54-7.56 (m, *p*-Ar-H, 1H), 8.03-8.06 (m, *o*-Ar-H, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) 9.95 (C-18), 18.93 (C-21), 25.00 (C-15), 26.66 (C-4), 26.72 (C-7), 27.29 (C<u>C</u>H$_3$), 27.68 (C-6), 28.05 (C-19), 30.64 (C-22), 30.73 (C-1), 31.41 (C-2), 34.96 (C-23), 35.43 (C-20), 36.68 (C-16), 38.79 (C-14), 38.94 (<u>C</u>CH$_3$), 42.43 (C-10), 43.73 (C-5), 47.51 (C-17), 51.46 (OMe), 52.88 (C-8), 53.01 (C-13), 73.60 (C-3), 128.33 (*m*-Ar-C), 128.97 (C-9), 129.57 (*o*-Ar-C), 130.58 (*i*-Ar-C), 132.89 (*p*-Ar-C), 152.70 (C-11), 166.09 (Ph<u>C</u>O$_2$), 174.71 (C-24), 179.11 (<u>C</u>ONH), 203.74 (C-12); $[\alpha_D]^{20}$ = +12° (0.036 g 100 cm^{-3}, methanol). [Found MH^+ 606.3800 (100%), 588 (4), 524 (19), 507 (8) $C_{37}H_{52}O_6N$ requires MH, 606.3795].

Methyl (N-ethoxycarbonyl)-11-amino-3α-benzoyl-12-keto-5β-chol-9,11-en-24-oate (26c)

Methyl 11-amino-3α-benzoyl-12-keto-5β-chol-9,11-en-24-oate (0.75 g, 0.144 mmol), 4-dimethylaminopyridine (0.012 g, 0.098 mmol) and ethyl chlorochromate (0.1 cm^3, 1.046 mmol) were refluxed under nitrogen for 48 h in dichloromethane (30 cm^3). The resulting mixture was allowed to cool, then successively washed with HCl (2 M, 20 cm^3), saturated $NaHCO_3$ (20 cm^3), dried over Na_2SO_4 and then concentrated *in vacuo* to yield a colourless gum (0.15 g). Chromatography (silica, 5:1:1 light petroleum / dichloromethane / EtOAc) gave the title compound as a colourless microcrystalline solid (0.023 g, 27%).

Mpt. 61-62 °C; IR ν_{max} (cm^{-1}) 3315 (NH), 1731 (CO_2Me), 1717 ($PhCO_2$), 1703 (C12 C=O); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.97-0.98 (d, J = 6 Hz, CH_3-21, 3H), 1.05 (s, C<u>H</u>$_3$-18, 3H), 1.21-1.25 (t, J = 7 Hz, C<u>H</u>$_3$-CH_2, 3H), 1.34 (s, C<u>H</u>$_3$-19, 3H), 3.66 (s, OC<u>H</u>$_3$, 3H), 4.07-4.15 (m, C<u>H</u>$_3$-CH_2, 2H), 5.02-5.05 (m, CH-3β, 1H), 5.84 (b, NH, 1H), 7.41-7.44 (m, *m*-Ar-H, 2H), 7.52-7.56 (m, *p*-Ar-H, 1H), 8.01-8.03 (m, *o*-Ar-H, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) 9.83 (C-18), 14.55 (O-CH_2C<u>H</u>$_3$), 19.14 (C-21), 24.91 (C-15), 24.91 (C-16), 26.59 (C-4), 26.64 (C-7), 27.59 (C-6), 28.02 (C-19), 29.71 (C-8), 30.63 (C-22), 30.66 (C-1), 31.43 (C-2), 34.95 (C-23), 35.45 (C-20), 38.63 (C-14), 42.56 (C-10), 47.64 (C-17), 51.47 (OMe), 52.64 (C-13), 52.82 (C-5), 61.43 (O-C<u>H</u>$_2$$CH_3$), 73.47 (C-3), 128.47 (C-9), 129.56 (*m*-Ar-C), 130.65 (*o*-Ar-C), 132.84 (*i*-Ar-C), 154.17 (C-11), 156.06 (<u>C</u>ONH), 166.08 (*p*-Ar-C), 174.67 (C-24), 203.55 (C-12); $[\alpha_D]^{20}$ = +28° (0.017 g 100 cm^{-3}, methanol). [Found MNH_4^+ 611.3696 (38%), 594 (40), 569 (12), 567 (40) $C_{35}H_{51}N_2O_7$ requires MNH_4, 611.3696].

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