The Design and Synthesis of New Steroidal Compounds as Potential Mimics of Taxoids

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Eight new steroidal compounds bearing the phenylisoserine and benzoate side-chains of docetaxel were synthesised as potential mimics of antitumour taxoids. They were readily obtained either from cholic acid or from 4-androstene-3,17-dione by a reductive Tsuji–Trost/reduction one-pot reaction. Two compounds showed an unexpected inhibitory activity of tubulin assembly.

Introduction

Paclitaxel (Taxol®, **1a**)[1] and docetaxel (Taxotere®, **1b**)[2] are very potent anticancer drugs of the taxoid series (Figure 1). Paclitaxel has been shown to promote the polymerisation of tubulin to microtubule.[3] This interaction with microtubules has been strongly correlated with the antitumour properties of taxoids. Since their discovery, structure–activity relationships have been studied in order to determine the minimal structural requirements necessary for biological activity.[4] These studies highlighted the importance of the C-13 side-chain, the ester groups at C-2 and C-4 and the rigid core to which these patterns are connected. In addition, extensive studies have been devoted to the elucidation of the bioactive conformation of the taxoids, namely those they adopt when bound to microtubules and which induce the inhibition of depolymerisation in vitro. Recently, a conformation called "T-shaped"[5] has been proposed as the active one, using the 3.7-Å structure of the α,β-tubulin taxol complex obtained by electron crystallography of zinc-induced tubulin sheets[6] and an analysis of paclitaxel conformations with the NAMFIS methodology.[7] Since then, active constrained analogues mimicking the "T-shaped" conformation have been synthesised.[8] Despite the strong antitumour activity of paclitaxel and docetaxel, many tumours show significant resistance to taxoid derivatives[9,10] which are, in addition, very expensive treatments. Therefore, it would be highly useful to elaborate synthetic non-taxane compounds with the same tubulin activity but without the above-mentioned drawbacks. A survey of the literature shows that a few taxoid analogues, in which a simpler core replaces the taxane skeleton, have already been elaborated.[11] Some of them are of particular interest. Klar and co-workers,[11h] for example, have synthesised a new class of borneol esters **3** which stabilize microtubules much better than paclitaxel but show reduced cytotoxic activity. On the other hand, Ojima et al. have elaborated the indolizidinone-type compounds **4**,[11i] which mimic the bioactive conformation of taxoids (Figure 1). They show micromolar level IC_{50} values against two human breast-cancer cell-lines but no activity in promoting the formation of microtubules. Recently, Frejd et al. have designed, by computer modelling, spirobicyclooctane derivatives, whose first synthesised compound failed to stabilize microtubules.[11j]

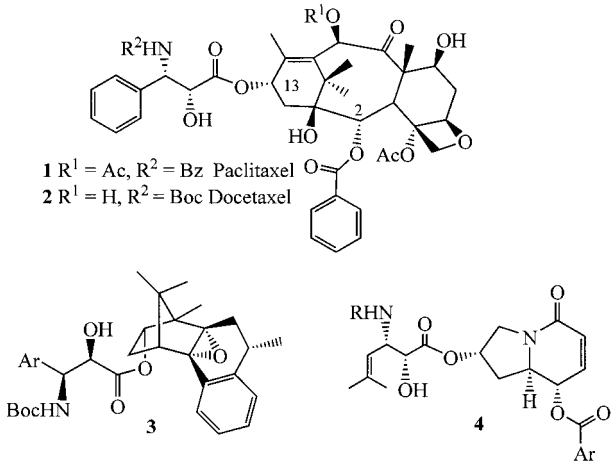

Figure 1. Examples of taxoid analogues.

Our group has been involved in taxoid chemistry for many years, and more recently in the search for new taxoids with a locked conformation.[12] We are also interested in finding compounds with a simpler scaffold that retains

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most of the three-dimensional features and mimics the active conformation of taxoids. We thus began searching for commercially available compounds that fit our requirements for a "T-shaped" conformation, in other words products with a rigid structure and two hydroxy groups at about 4 Å from each other on the same side of the molecule. Molecular dynamic modelling studies (Sybyl 6.9) were performed on pre-selected molecules with the phenylisoserine and benzoate side-chains of taxoids to determine their conformational behaviour. We thus found that cholic acid derivatives **7** (Figure 2) could be good potential mimics of taxoids as we noticed that these steroidal compounds greatly overlay T-shaped taxoids (Figure 3).

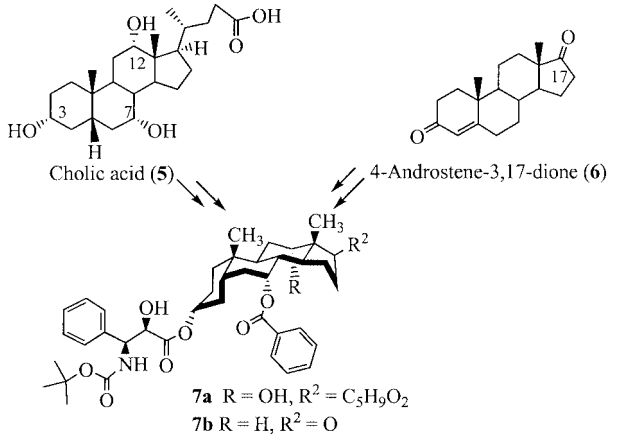


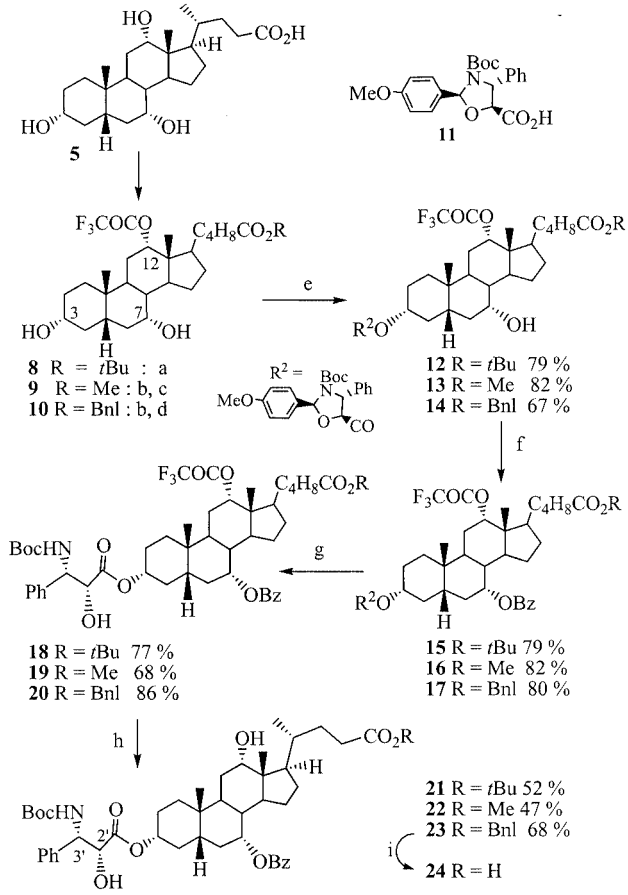
Figure 2. Potential mimics of taxoids from cholic acid and 4-androstene-3,17-dione.

Figure 3. Superposition of T-shaped docetaxel (yellow) with a conformer of cholic-type analogue **7a** (left; magenta) and with a conformer of simplified cholic-type analogue **7b** (right; cyan).

Indeed, the rigid cholic-type skeleton presents a *cis* A-B ring junction that gives it a U conformation. This curved conformation is similar to that of docetaxel and is increased by the presence of cycles C and D, for, in computational studies, simple decaline systems were found to be much flatter. Furthermore, the two α-hydroxy groups at C-3′ and C-7 in cholic acid **5** are at about 4 Å from each other and they can be esterified by the acids corresponding to the side-chains of docetaxel. Two examples of steroids with a *trans* A-B ring junction showing a moderate microtubule-stabilizing activity can also be found in the literature.[13–15] The synthesis of two sets of analogues was therefore considered, with and without the lateral chain at C-17, starting either from cholic acid (**5**) or from 4-androstene-3,17-dione (**6**).

Results and Discussion

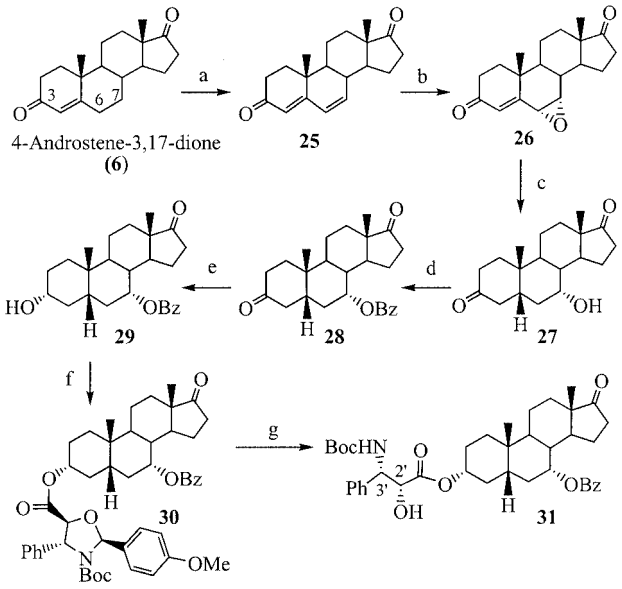
Cholic acid (**5**) is characterised by two axial hydroxy groups on C-7 and C-12, an equatorial OH on C-3 and a long carboxylic acid chain on C-17. The main problem for the synthesis of our first set of analogues was to functionalise positions 3 and 7 selectively. For that, we used Davis' methodology,[16] as shown in Scheme 1, to protect the 12α-OH as a trifluoroacetate ester as well as the carboxyl acid as a *tert*-butyl ester (compound **8**). In addition, we elaborated two other starting materials whose acid chain was protected as a methyl[17] or a benzyl ester (compounds **9** and **10**). From these different starting products **8**–**10**, we wanted to elaborate derivatives with different steric hindrance and/or polarity on their northern part, as the trifluoroacetate and the benzyl ester can be easily removed. From compounds **8**–**10**, the selective esterification of the more reactive equatorial 3α-OH was cleanly performed to give the expected compounds **12**–**14** in good yields. These reactions were carried out with one equivalent of the protected docetaxel side-chain **11** [2-(4-methoxyphenyl)-1,3-oxazolidine derivative of *N*-Boc-phenylisoserine],[18] under



Scheme 1. Reaction conditions: (a) 1. TFAA, THF, –35 to –40 °C, 2. *t*BuOH, 3. $NaHCO_3$, THF, MeOH, 72%; (b) 1. TFAA, THF, –35 to –40 °C, 2. $NaHCO_3$, THF, MeOH, 40%; (c) concd. HCl, MeOH, 88%; (d) benzyl alcohol, DCC, DMAP, toluene, 60%; (e) **11**, DCC, DMAP, toluene; (f) PhCOBr, DMAP, pyridine, 80 °C; (g) PTSA, MeOH; (h) NH4OH, THF, MeOH; (i) H_2, Pd/C, THF, 98%.

standard conditions (DCC, DMAP in toluene at room temperature). More drastic conditions were required for the functionalisation of the axial 7α-OH, which is not very reactive because of a congested environment. Thus, benzoyl bromide and DMAP were used in pyridine at 90 °C for 3 h to obtain the desired bis-functionalised compounds **15**–**17** in good yields (79–82%). Deprotection of the phenylisoserine side-chain was then performed in acidic conditions to give compounds **18**–**20** as the first analogues to be tested. The 12α-trifluoroacetates were deprotected[16] selectively and smoothly under basic conditions to provide compounds **21**–**23**. Finally, deprotection of the benzyl ester led to the formation of **24**. Seven new compounds **18**–**24** were thus easily elaborated from cholic acid as potential mimics of taxoids.

Furthermore, we investigated the synthesis of a simplified "cholic-type" steroid with a carbonyl function at position 17.[19,20] Indeed, unlike taxoids, the northern part of the former analogues (**18**–**24**) is rather lipophilic because of the long acid chain. This chain may cause a steric hindrance with amino acids of the tubulin active site.[21] In addition, the carbonyl group at C-17 could be useful for further functionalisation of cycle D. The proposed straightforward strategy started from commercially available 4-androstene-3,17-dione (**6**) and required no protection step (Scheme 2). The main difficulties were to attach a 7-α-OH group in a regio- and diastereoselective manner as well as a *cis* A-B ring junction and to allow the selective reduction of the ketone to a 3α-OH. The key step of this strategy was a reductive Tsuji–Trost[22] reaction, which was performed on the transient 6α,7α-oxirane **26** to form the desired the 7α-OH group in a regioselective manner.



Scheme 2. Reaction conditions: (a) PTSA, 4-chloranil, *t*BuOH/toluene, 105 °C, 73%; (b) *m*CPBA, $CHCl_3$, 55%; (c) $Pd_2(dba)_3$, PPh_3, HCOOH, Et_3N, dioxane, reflux, 49%; (d) benzoic acid, DCC, DMAP, toluene 83%; (e) $LiAlH(OtBu)_3$, THF, 52%; (f) **11**, DCC, DMAP, toluene, 88%; (g) PTSA, MeOH, 76%.

The sequence began with a regiospecific dehydrogenation reaction of compound **6** with tetrachloro-*p*-benzoquinone (4-chloranyl) under acidic conditions,[23] which provided the dienone **25** in one step and in good yield (73%). The diastereoselective epoxidation of the obtained dienone **25** to give 6α,7α-oxiranoandrost-4-ene-3,17-dione (**26**) was performed according to a classical procedure. As already observed,[24] we obtained the desired compound (55%) as well as its regioisomer (8%) as a secondary product. Both compounds could easily be separated. The reductive Tsuji–Trost reaction was performed on compound **26** under standard conditions, i.e. with $Pd_2(dba)_3$ [tris(dibenzylideneacetone) dipalladium], triphenylphosphane, formic acid, and triethylamine. In our case, the regioselectivity of this reaction was not important, as the double bond of the expected compounds (homoallylic alcohol **33** and allylic alcohol **34**) was to be reduced at the next stage. Nevertheless, although the expected homoallylic alcohol **33** could be isolated, the allylic alcohol **34** was never observed. The dienone **25**, which was probably formed by dehydration of the unstable allylic alcohol **34**, was isolated instead.

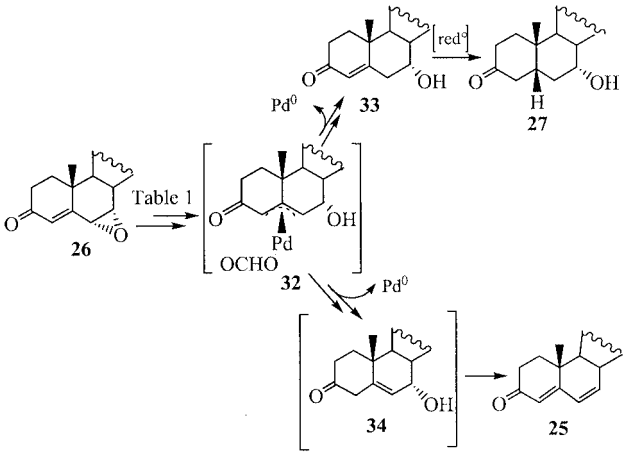
To avoid the formation of compound **25**, a survey of the reaction solvent was performed as it has been shown by Tsuji et al.[25] that it influences the selectivity of the reaction (Table 1 and Scheme 3). Dioxane and THF seemed to be the best solvents. In some cases, when the reaction was performed under anhydrous conditions, a new product **27** was formed by palladium-catalysed reduction of the α,β-unsaturated ketone **33**.[26] This reduction occurs at the β-face of the steroid by attack of the hydride on the same side of the molecule as the palladium. Thus, compound **27** possesses the desired C-5 configuration, which corresponds to a *cis* A-B ring junction. This was confirmed by comparison of its optical rotation with those described in the literature[20] of the same compound obtained by another way {$[\alpha]_D$ = +78 (*c* = 1.0, CH_2Cl_2); ref.[20] $[\alpha]_D$ = +79 (*c* = 1.07, CH_2Cl_2)}. In dry dioxane, the formation of **27** was even almost exclusively and selectively observed from epoxide **26**. This reaction can be performed at room temperature (it was completed in 16 h), but an increase of temperature allowed the reaction to be completed in five hours. The ^1H NMR spectrum of the crude mixture was very clean and showed **27** to be the only product formed. The overall yield of the reaction was moderate, however, perhaps because of the purification conditions. The lower regioselectivity of the Tsuji–Trost reaction in non-distilled solvents can be accounted for by the ability of water to disfavour the more stable σ-palladium complex by inhibiting the intramolecular coordination of the hydroxy group, as shown by Tsuji.[25] Nevertheless, the reasons why compound **27** needs dry conditions to be formed remain unclear.

After esterification of compound **27** with benzoic acid (83%), a diastereoselective reduction of the C-3 carbonyl group to its 3α-hydroxy group was performed with lithium tri-*tert*-butoxyaluminium hydride.[20] Some over-reduction at position 17 was observed (about 20% in the crude material), but the desired compound **29** could be easily separated by purification on a chromatography column (52%). This

Table 1. Effect of different solvents on the reaction selectivity.

Compound[a]	CH_2Cl_2	CH_2Cl_2[b]	Dioxane	Dioxane[b]	THF	THF[b]
25[c]	68	traces	9	traces	17	5
33[c]	32	35	91	traces	83	6
27[c]	–	64	–	>95	–	89

[a] All reactions were carried out with 0.17 mmol of **29**, 0.017 mmol each of $Pd_2(dba)_3$ and PPh_3, 0.88 mmol of HCOOH and 0.5 mmol of NEt_3 in 2 mL of solvent. [b] Solvents and reagents distilled prior to use. [c] Proportions calculated by integration of ^1H NMR peak intensities of the crude mixture.



Scheme 3.

four-step sequence provided an easy and efficient access to cholic-type compounds. The expected compound **31** was then obtained in two steps from **29** (esterification of the 3α-hydroxy group with docetaxel-protected lateral chain **11** followed by deprotection under acidic conditions) with good yields (Scheme 2). Finally, steroid **31** was synthesised in seven steps from commercial **6**.

For all final compounds **18**–**24** and **31**, the 2′-H/3′-H coupling constant is small in both apolar ($CDCl_3$) and polar ([D_6]DMSO) solvents. In addition, NMR conformational analysis (NOESY and ROESY) on compounds **18**–**24** and **31** in $CDCl_3$ showed an intense NOE between 2′-H and 3′-H, characteristic of a *gauche* interaction between these two protons. Furthermore, compound **31** is the only one to present weak NOEs between the benzoate and 3′-H and NH (Figure 4). This means that the conformation of compound **31** in apolar solvents is close to those obtained by molecular dynamic modelling studies and close to the "T-shaped" conformation of taxoids, as all these results account for a folded conformation of the phenylisoserine sidechain in the direction of the benzoyl group. Broadly speaking, because of their strong structural similarities, the other final compounds **18**–**24** should be able to adopt the same conformation.

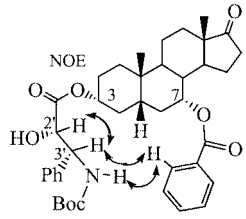


Figure 4. Observed NOEs for compound **31**.

The activity of compounds **18**–**24** and **31** in tubulin polymerisation and depolymerisation assays, as well as their cytotoxicity towards KB, MCF7 and MCF7R cell lines, were evaluated. The most interesting results are summarised in Table 2. None of these compounds show appreciable activity in inhibiting the disassembly of microtubules in the standard in vitro tubulin depolymerisation assay. However, two of them, **23** and **24**, show a significant microtubule assembly inhibitory activity (3.8 and 94 μM, respectively). None of the tested compounds were active against the MCF7R cell line and compound **22** was the only one to show a weak activity against the MCF7 cell line. Finally, compounds **22**, **24** and **31** show a significant cytotoxic activity on KB cells (IC_{50} of 2.0, 7.2 and 3.2 μM, respectively), whereas cholic acid is devoid of cytotoxicity on this cell line.

These results clearly show that the cytotoxic activity of these compounds results from a different mechanism of action than that of paclitaxel and docetaxel, and that a good structural similarity with taxoids is not sufficient to have the same tubulin activity.

Conclusions

In summary, we have elaborated new cholic-type steroidal compounds possessing a benzoate and a phenylisoserine

Table 2. Biological evaluation of steroidal compounds.

Compound	Microtubule disassembly inhibitory activity IC_{50}[a] [μM]	Microtubule assembly inhibitory activity IC_{50}[b,c] [μM]	Cytotoxicity against KB cell line IC_{50}[d] [M]	Cytotoxicity against MCF7 cell line IC_{50}[d] [M]
Docetaxel	0.078	inactive	$1.6\ 10^{-10}$	inactive
Cholic acid	inactive	inactive	inactive	inactive
22	inactive	inactive	$2.7\ 10^{-6}$	10^{-5}
23	inactive	3.8	inactive	inactive
24	inactive	94	$7.2\ 10^{-6}$	inactive
31	inactive	120	$3.2\ 10^{-6}$	inactive

[a] IC_{50} is the concentration that inhibits 50% of the rate of microtubule disassembly. [b] IC_{50} is the concentration that inhibits 50% of the rate of microtubule assembly. [c] Under the same conditions, the IC_{50} of vinblastine is 2 μM. [d] IC_{50} measures the drug concentration required for the inhibition of 50% cell proliferation after 72 h of incubation.

side-chain in order to mimic taxoid derivatives. They were readily synthesised in few steps either from cholic acid or from 4-androstene-3,17-dione. Although they show some structural similarity, according to molecular dynamic modelling studies, and have the correct conformation of the lateral chains in apolar solvents, these compounds show no microtubule disassembly inhibitory activity, although three of them show microtubule assembly inhibitory activity. These experimental results, as well as those previously published by other teams, show how challenging it is to find synthetic non-taxane compounds that possess a tubulin activity similar to that of paclitaxel and docetaxel.

Experimental Section

General Remarks: All chemicals were purchased from Aldrich or Acros Organics and were used without further purification unless mentioned otherwise. Solvents were purchased from SDS. Anhydrous THF and dioxane were obtained by distillation immediately prior to use from a dark blue-purple solution of sodium benzophenone ketyl. Merk silica gel 60 (230–400 mesh) was used for the flash chromatographic purification of some compounds. The acid chain of docetaxel **11** was a gift from Alain Commerçon (Sanofi-Aventis). Microtubular proteins were purified from mammalian brain as described previously.[27] Cytotoxicity and microtubule disassembly inhibition were carried out according to established literature protocols.[28,29] Molecular modelling studies were realized with the Sybyl software from Tripos with the MMFF94 force field.

***tert*-Butyl 3α,7α-Dihydroxy-12α-trifluoroacetoxy-5β-cholan-24-oate (8):** This compound, prepared as reported previously,[16] gave the expected spectral and analytical data.

Methyl 3α,7α-Dihydroxy-12α-trifluoroacetoxy-5β-cholan-24-oate (9): Four drops of concentrated HCl were added to a solution of 3α,7α-dihydroxy-12α-trifluoroacetoxy-5β-cholan-24-oic acid[16] (176 mg, 0.35 mmol) in refluxing MeOH (6 mL). The reaction mixture was then cooled to room temperature and neutralised with a saturated solution of Na_2CO_3. MeOH was evaporated under reduced pressure and the aqueous solution was extracted with EtOAc (3 × 10 mL). The combined organic layers were dried with $MgSO_4$, filtered and concentrated to give the desired product (160 mg, 88%) as a white powder which could be used without further purification. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.82 (s, 3 H, 18-Me), 0.84 (d, ^3J = 6.0 Hz, 3 H, 21-Me), 0.95 (s, 3 H, 19-Me), 0.95–2.37 (m, 24 H), 3.44 (m, 1 H, 3-H), 3.68 (s, 3 H, OMe), 3.89 (s, 1 H, 7-H), 5.34 (s, 1 H, 12-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.0 (C-18), 17. 3 (C-21), 22.4 (C-19), 22.5 (CH_2), 25.2 (CH_2), 27.0 (CH_2), 27.5 (CH), 30.3 (CH_2), 30.5 (CH_2), 34.4 (CH + 2 CH_2), 34.8 (CH_2), 37.5 (C), 38.9 (CH), 39.4 (CH_2), 41.2 (CH), 43.2 (CH), 44.9 (C), 47.2 (CH), 51.4 (CH_3), 67.5 (C-7), 71.6 (C-3), 80.8 (C-12), 157.2 (CO), 174. 3 (CO) ppm. IR (film): $\tilde{\nu}_{max}$ = 3401, 1776, 1735 cm^{-1}. $[\alpha]_D^{20}$ = +50 (c = 1.05, CH_2Cl_2). MS (ESI, MeOH): m/z = 541.3. $C_{27}H_{41}F_3O_6$ (518.61): calcd. C 62.53, H 7.97; found C 62.56, H 8.21.

Benzyl 3α,7α-Dihydroxy-12α-trifluoroacetoxy-5β-cholan-24-oate (10): 1,3-Dicyclohexylcarbodiimide (307 mg, 1.49 mmol) and 4-(dimethylamino)pyridine (121 mg, 0.99 mmol) were added to a solution of 3α,7α-dihydroxy-12α-trifluoroacetoxy-5β-cholan-24-oic acid[16] (500 mg, 0.99 mmol) and benzyl alcohol (103 μL, 1.49 mmol) in toluene (10 mL). The reaction mixture was stirred at room temperature for 1.5 h and filtered. Toluene was evaporated under reduced pressure and the residue was diluted with Et_2O (50 mL). The organic phase was washed with aqueous HCl (1 M, 10 mL), a saturated solution of $NaHCO_3$ (25 mL) and brine. The organic layer was dried with $MgSO_4$, filtered and concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel (EtOAc/heptane = 3:7) to afford compound **10** (588 mg, 60%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.65 (s, 3 H, 18-H), 0.70 (d, ^3J = 6.2 Hz, 3 H, 21-H), 0.79 (s, 3 H, 19-H), 0.83–2.34 (m, 24 H), 3.31 (m, 1 H, 3-H), 3.76 (s, 1 H, 7-H), 5.05 (d, ^2J = 2.0 Hz, 2 H, 24-H), 5.20 (s, 1 H, 12-H), 7.25 (m, 5 H, H_{ar}) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.0 (C-18), 17. 3 (C-21), 22.4 (C-19), 22.5 (CH_2), 25.3 (CH_2), 27.0 (CH_2), 27.5 (CH), 30.4 (C), 30.5 (CH_2), 30.9 (CH_2), 34.4 (CH + 2 CH_2), 34.9 (CH_2), 38.9 (CH), 39.4 (CH_2), 41.2 (CH), 43.3 (CH), 44.9 (C), 47.3 (CH), 66.0 (C-24), 67.5 (C-7), 71.6 (C-3), 80.8 (C-12), 128.1, 128.4, 135.9 (6 C_{ar}), 157.0 (CO), 173.7 (CO) ppm. IR (film): $\tilde{\nu}_{max}$ = 3397, 1777, 1737 cm^{-1}. $[\alpha]_D^{20}$ = +51 (c = 1.12, CH_2Cl_2). MS (ESI, MeOH): m/z (%) = 617.3 (100) [M + Na]$^+$. $C_{33}H_{45}F_3O_6$ (594.70): calcd. C 66.65, H 7.63; found C 66.81, H 7.84.

General Procedure for the Esterification Reaction of Cholic Esters with Protected Side-Chain 11: 1,3-Dicyclohexylcarbodiimide (1.1 equiv.), 4-(dimethylamino)pyridine (1.0 equiv.) and protected side-chain **11** (1.1 equiv.) were added to a solution of cholic esters in toluene. The reaction mixture was stirred at 80 °C for 16 h, cooled and filtered. Toluene was evaporated under reduced pressure and the residue was diluted with EtOAc. The organic layer was washed with aqueous HCl (1 M), a saturated solution of $NaHCO_3$ and brine, dried with $MgSO_4$, filtered and concentrated. The crude mixture was purified by column chromatography on silica gel (EtOAc/heptane = 3:7) to afford the desired compound.

***tert*-Butyl 3α-[*N*-Boc-*N*,*O*-(4-methoxybenzylidene)-β-phenylisoserinoyl]-7α-hydroxy-12α-trifluoroacetoxy-5β-cholan-24-oate (12):** The reaction was carried out with 0.71 mmol (396 mg) of compound **8** and 0.78 mmol (311 mg) of **11** in toluene (15 mL) to give, after purification, the expected compound **12** (527 mg, 79%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.78 (s, 3 H, 18-Me), 0.81 (d, ^3J = 6.0 Hz, 3 H, 21-Me), 0.89 (s, 3 H, 19-Me), 1.06 (s, 9 H, Boc), 1.44 (s, 9 H, *t*Bu), 0.97–2.35 (m, 24 H), 3.81 (s, 3 H, OMe), 3.87 (s, 1 H, 7-H), 4.50 (m, 1 H, 3-H), 4.52 (d, ^3J = 4.4 Hz, 1 H, 2′-H), 5.30 (m, 2 H, 12-H, 3′-H), 6.35 (bs, 1 H, 4′-H), 6.86–7.39 (m, 9 H, H_{ar}) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.2 (C-18), 17.5 (C-21), 22.4 (C-19), 22.8 (CH_2), 25.3 (CH_2), 26.0 (CH_2), 27.2 (CH_2), 27.5 (CH), 27.8 (3CH_3 of Boc), 28.1 (3CH_3 of *t*Bu), 30.7 (CH_2), 32.2 (CH_2), 34.3 (CH_2), 34.4 (CH + CH_2), 34.5 (C), 34.9 (CH_2), 39.1 (CH), 41.0 (CH), 43.3 (CH), 45.1 (C), 47.5 (CH), 55.3 (OCH_3), 63.4 (C-3′), 67.6 (C-7), 75.6 (C-3), 80.0 (C), 80.5 (C), 80.8 (C-12), 83.4 (C-2′), 92.0 (C-4′), 113.6, 126.3, 127.8, 128.5, 128.7 (12C_{ar}), 157.2 (CO), 160.1 (CO), 169.2 (CO), 173.4 (CO) ppm. IR (film): $\tilde{\nu}_{max}$ = 3530, 1777, 1721, 1709 cm^{-1}. $[\alpha]_D^{20}$ = +58 (c = 1.03, CH_2Cl_2). MS (ESI, MeOH): m/z (%) = 964.5 (100) [M + Na]$^+$. $C_{52}H_{70}F_3NO_{11}$ (942.11): calcd. C 66.29, H 7.49, N 1.49; found C 66.41, H 7.74, N 1.61.

Methyl 3α-[*N*-Boc-*N*,*O*-(4-methoxybenzylidene)-β-phenylisoserinoyl]-7α-hydroxy-12α-trifluoroacetoxy-5β-cholan-24-oate (13): The reaction was carried out with 0.64 mmol (332 mg) of compound **9** and 0.70 mmol (281 mg) of **11** in toluene (8 mL) to give, after purification, the desired compound **13** (471 mg, 82%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.80 (s, 3 H, 18-Me), 0.81 (d, ^3J = 6.5 Hz, 3 H, 21-Me), 0.85 (s, 3 H, 19-Me), 1.06 (s, 9 H, Boc), 0.92–1.82 (m, 14 H), 1.93–2.08 (m, 6 H), 2.09–2.39 (m, 4 H), 3.66 (s, 3 H, OMe), 3.80 (s, 3 H, OMe), 3.87 (s, 1 H, 7-H), 4.45 (m, 1 H, 3-H), 4.51 (d, ^3J = 4 Hz, 1 H, 2′-H), 5.30 (m, 2 H, 12-H, 3′-H), 6.34 (bs,

1 H, 4′-H), 6.87–7.38 (m, 9 H, H_{ar}) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.2 (C-18), 17.5 (C-21), 22.4 (C-19), 22.7 (CH_2), 25.3 (CH_2), 26.0 (CH_2), 27.1 (CH_2), 27.5 (CH), 27.8 (3 CH_3 of Boc), 30.6 (CH_2), 34.3 (CH_2), 34.4 (CH_2), 34.5 (CH + CH_2), 34.9 (CH_2), 38.9 (C), 39.1 (CH), 41.0 (CH), 43.2 (CH), 45.0 (C), 47.3 (CH), 51.4 (OCH_3), 55.3 (OCH_3), 63.4 (C-3′), 67.6 (C-7), 75.6 (C-3), 80.5 (C), 80.7 (C-12), 83.3 (C-2′), 92.0 (C-4′), 113.6, 126.3, 127.8, 128.6, 128.7 (12 C_{ar}), 157.2 (CO), 160.1 (CO), 169.2 (CO), 174.4 (CO) ppm. IR (film): $\tilde{\nu}_{max}$ = 3531, 1776, 1733, 1701 cm^{-1}. $[\alpha]_D^{20}$ = +63 (*c* = 1.23, CH_2Cl_2). MS (ESI, MeOH): *m*/*z* (%) = 923.4 (100) [M + Na]$^+$. $C_{49}H_{64}F_3NO_{10}$ (884.03): calcd. C 65.39, H 7.17; found C 65.68, H 7.41.

Benzyl 3α-[*N*-Boc-*N*,*O*-(4-methoxybenzylidene)-β-phenylisoserinoyl]-7α-hydroxy-12α-trifluoroacetoxy-5β-cholan-24-oate (14): The reaction was carried out with 0.75 mmol (445 mg) of compound **10** and 0.82 mmol (328 mg) of **11** in toluene (10 mL) to give, after purification, the expected compound **14** (490 mg, 67%) as white crystals. ^1H NMR (300 MHz, $CDCl_3$): δ = 0.74 (s, 3 H, 18-Me), 0.81 (d, ^3J = 6.0 Hz, 3 H, 21-Me), 0.85 (s, 3 H, 19-Me), 1.05 (s, 9 H, Boc), 0.88–2.40 (m, 24 H), 3.77 (s, 3 H, OMe), 3.82 (s, 1 H, 7-H), 4.48 (m, 2 H, 2′-H, 3-H), 5.09 (s, 2 H, 24-H), 5.29 (m, 2 H, 12-H, 3′-H), 6.36 (bs, 1 H, 4′-H), 6.86–7.39 (m, 14 H, H_{ar}) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 11.9 (C-18), 17.2 (C-21), 22.3 (C-19), 22.5 (CH_2), 25.2 (CH_2), 25.9 (CH_2), 27.0 (CH_2), 27.3 (CH), 27.8 (3 CH_3 of Boc), 30.4 (CH_2), 30.7 (CH_2), 34.3 (CH_2), 34.3 (CH_2 + C), 34.4 (CH), 34.7 (CH_2), 38.9 (CH), 40.9 (CH), 43.1 (CH), 44.9 (C), 47.3 (CH), 55.0 (OCH_3), 63.2 (C-3′), 66.0 (C-24), 67.2 (C-7), 75.5 (C-3), 80.3 (C), 80.7 (C-12), 83.2 (C-2′), 91.8 (C-4′), 113.4, 126.2, 127.7, 128.3, 128.5, 135.8 (18 C_{ar}), 157.2 (CO), 159.9 (CO), 169.0 (CO), 173.6 (CO) ppm. IR (film): $\tilde{\nu}_{max}$ = 3440, 1775, 1730, 1700 cm^{-1}. $[\alpha]_D^{20}$ = +54 (*c* = 1.0, CH_2Cl_2). MS (ESI, MeOH): *m*/*z* (%) = 998.5 (100) [M + Na]$^+$. $C_{55}H_{68}F_3NO_{11}$ (976.12): calcd. C 67.67, H 7.02, N 1.43; found C 67.83, H 7.07, N 1.26. M.p. 86–88 °C.

General Procedure for the Esterification Reaction of Cholic Esters with Benzoyl Bromide: Benzoyl bromide (10 equiv.) was added dropwise, under argon, at 0 °C, to a solution of ester and 4-(dimethylamino)pyridine (1 equiv.) in dry pyridine. The reaction mixture was stirred at 90 °C for 3 h. The cooled mixture was diluted with Et_2O. The organic layer was washed with aqueous HCl (1 M), a saturated solution of $NaHCO_3$ and brine, dried with $MgSO_4$, filtered and concentrated. The crude mixture was purified by column chromatography on silica gel (CH_2Cl_2, then CH_2Cl_2 with an increasing amount of 1–5% of MeOH) to afford the desired compound.

***tert*-Butyl 7α-Benzoyloxy-3α-[*N*-Boc-*N*,*O*-(4-methoxybenzylidene)-β-phenylisoserinoyl]-12α-trifluoroacetoxy-5β-cholan-24-oate (15):** The reaction was carried out with 0.25 mmol (236 mg) of compound **12** and 2.50 mmol (295 μL) of benzoyl bromide in dry pyridine (1 mL) to give, after purification, the expected compound **15** (205 mg, 79%). ^1H NMR (300 MHz, $CDCl_3$): δ = 0.80 (s, 3 H, 18-Me), 0.82 (d, ^3J = 6.0 Hz, 3 H, 21-Me), 0.98 (s, 3 H, 19-Me), 0.96–1.92 (m, 18 H), 1.06 (s, 9 H, Boc), 1.40 (s, 9 H, *t*Bu), 1.99–2.35 (m, 6 H), 3.79 (s, 3 H, OMe), 4.38 (d, ^3J = 4.4 Hz, 1 H, 2′-H), 4.41 (m, 1 H, 3-H), 5.18 (m, 2 H, 7-H, 3′-H), 5.39 (s, 1 H, 12-H), 6.31 (bs, 1 H, 4′-H), 6.20–8.05 (m, 14 H, H_{ar}) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.0 (C-18), 17.6 (C-21), 22.3 (C-19), 22.7 (CH_2), 25.1 (CH_2), 26.1 (CH_2), 27.0 (CH_2), 27.9 (3 CH_3 of Boc), 28.1 (3 CH_3 of *t*Bu), 28.5 (CH), 30.7 (CH_2), 31.3 (CH_2), 32.1 (CH_2), 34.3 (CH + C), 34.5 (2 CH_2), 38.2 (CH), 40.6 (CH), 42.9 (CH), 45.0 (C), 47.5 (CH), 55.3 (OCH_3), 63.1 (C-3′), 71.0 (C-7), 75.4 (C-3), 80.0 (C), 80.5 (C), 80.7 (C-12), 83.3 (C-2′), 92.0 (C-4′), 113.6, 126.2, 127.8, 128.3, 128.7, 129.4, 130.3, 133.2 (18 C_{ar}), 156.9 (CO), 160.1 (CO), 165.3 (CO), 168.9 (CO), 173.3 (CO) ppm. IR (film): $\tilde{\nu}_{max}$ = 1779, 1750, 1713 cm^{-1}. $[\alpha]_D^{20}$ = +61 (*c* = 0.9, CH_2Cl_2). MS (ESI, MeOH): *m*/*z* (%) = 1068.8 (100) [M + Na]$^+$. $C_{59}H_{74}F_3NO_{12}$ (1046.2): calcd. C 67.73, H 7.13, N 1.34; found C 67.91, H 7.49, N 1.39.

Methyl 7α-Benzoyloxy-3α-[*N*-Boc-*N*,*O*-(4-methoxybenzylidene)-β-phenylisoserinoyl]-12α-trifluoroacetoxy-5β-cholan-24-oate (16): The reaction was carried out with 0.52 mmol (471 mg) of compound **13** and 5.23 mmol (620 μL) of benzoyl bromide in dry pyridine (8 mL) to give, after purification, the expected compound **16** (426 mg, 82%). ^1H NMR (300 MHz, $CDCl_3$): δ = 0.81 (s, 3 H, 18-Me), 0.84 (d, ^3J = 6.0 Hz, 3 H, 21-Me), 0.90 (s, 3 H, 19-Me), 1.06 (s, 9 H, Boc), 0.90–2.50 (m, 24 H), 3.62 (s, 3 H, OMe), 3.79 (s, 3 H, OMe), 4.40 (d, ^3J = 2.5 Hz, 1 H, 2′-H), 4.43 (m, 1 H, 3-H), 5.19 (m, 2 H, 7-H, 3′-H), 5.40 (s, 1 H, 12-H), 6.32 (bs, 1 H, 4′-H), 6.76–8.06 (m, 14 H, H_{ar}) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 11.8 (C-18), 17.4 (C-21), 22.2 (C-19), 22.6 (CH_2), 25.0 (CH_2), 26.0 (CH_2), 26.8 (CH_2), 27.8 (3 CH_3 of Boc), 28.4 (CH), 30.4 (2 CH_2), 31.1 (CH_2), 34.2 (CH + CH_2), 34.4 (CH_2 + C), 38.0 (CH), 40.4 (CH), 42.8 (CH), 44.9 (C), 47.2 (CH), 51.4 (OCH_3), 55.1 (OCH_3), 63.0 (C-3′), 70.8 (C-7), 75.2 (C-3), 80.4 (C), 80.8 (C-12), 83.2 (C-2′), 91.6 (C-4′), 113.4, 126.0, 126.2, 127.7, 128.2, 128.5, 129.3, 130.0, 130.2, 133.2, 133.4, 133.5 (18 C_{ar}), 156.1 (CO), 160.0 (CO), 165.2 (CO), 168.7 (CO), 174.2 (CO) ppm. IR (film): $\tilde{\nu}_{max}$ = 1779, 1735, 1712 cm^{-1}. $[\alpha]_D^{20}$ = +64 (*c* = 1.04, CH_2Cl_2). MS (ESI, MeOH): *m*/*z* (%) = 1026.6 (100) [M + Na]$^+$. $C_{56}H_{68}F_3NO_{12}$ (1004.1): calcd. C 66.98, H 6.83, N 1.39; found C 67.24, H 6.67, N 1.56.

Benzyl 7α-Benzoyloxy-3α-[*N*-Boc-*N*,*O*-(4-methoxybenzylidene)-β-phenylisoserinoyl]-12α-trifluoroacetoxy-5β-cholan-24-oate (17): The reaction was carried out with 0.45 mmol (440 mg) of compound **14** and 4.50 mmol (530 μL) of benzoyl bromide in dry pyridine (6 mL) to give, after purification, the expected compound **17** (390 mg, 80%). ^1H NMR (300 MHz, $CDCl_3$): δ = 0.77 (s, 3 H, 18-Me), 0.82 (d, ^3J = 6.0 Hz, 3 H, 21-Me), 0.99 (s, 3 H, 19-Me), 1.06 (s, 9 H, Boc), 0.90–2.40 (m, 24 H), 3.80 (s, 3 H, OMe), 4.38 (d, ^3J = 4.0 Hz, 1 H, 2′-H), 4.42 (m, 1 H, 3-H), 5.08 (d, ^2J = 2.1 Hz, 2 H, 24-H), 5.18 (m, 2 H, 7-H, 3′-H), 5.38 (s, 1 H, 12-H), 6.31 (bs, 1 H, 4′-H), 6.79–8.05 (m, 19 H, H_{ar}) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 11.5 (C-18), 17.1 (C-21), 21.9 (C-19), 22.4 (CH_2), 24.7 (CH_2), 25.7 (CH_2), 26.6 (CH_2), 27.5 (3 CH_3 of Boc), 28.1 (CH), 30.1 (CH_2), 30.5 (CH_2), 30.9 (CH_2), 33.9 (CH + CH_2), 34.2 (CH_2 + C), 37.8 (CH), 40.2 (CH), 42.6 (CH), 44.6 (C), 47.1 (CH), 51.8 (OCH_3), 62.8 (C-3′), 65.7 (C-24), 70.6 (C-7), 75.0 (C-3), 80.0 (C), 80.3 (C-12), 83.0 (C-2′), 91.4 (C-4′), 113.4, 125.8, 127.4, 127.8, 127.9, 128.0, 128.1, 128.3, 129.0, 129.9, 132.9, 135.6 (24 C_{ar}), 156.1 (CO), 160.0 (CO), 164.8 (CO), 168.3 (CO), 173.1 (CO) ppm. IR (film): $\tilde{\nu}_{max}$ = 1778, 1709 cm^{-1}. $[\alpha]_D^{20}$ = +59 (*c* = 0.99, CH_2Cl_2). MS (ESI, MeOH): *m*/*z* (%) = 1102.5 (100) [M + Na]$^+$. $C_{62}H_{72}F_3NO_{12}$ (1080.2): calcd. C 68.94, H 6.72, N 1.30; found C 68.96, H 6.72, N 1.12.

General Procedure for the Deprotection of the Phenylisoserine Side-Chain: PTSA (3 equiv.) was added to an MeOH solution of the ester. The reaction mixture was stirred at room temperature for 3 h and then quenched with an excess of ethyl acetate. The organic layer was washed with a saturated solution of $NaHCO_3$ and brine, dried with $MgSO_4$, filtered and concentrated. The crude mixture was purified by column chromatography on silica gel (CH_2Cl_2, then CH_2Cl_2 with an increasing amount of 1–5% MeOH) to afford the desired compound.

***tert*-Butyl 7α-Benzoyloxy-3α-(*N*-Boc-β-phenylisoserinoyl)-12α-trifluoroacetoxy-5β-cholan-24-oate (18):** The reaction was carried out with 0.12 mmol (125 mg) of compound **15** in MeOH (3 mL) to give, after purification, the expected compound **18** (85 mg, 77%). ^1H

NMR (300 MHz, $CDCl_3$): δ = 0.82 (s, 3 H, 18-Me), 0.83 (d, ^3J = 6.0 Hz, 3 H, 21-Me), 0.90–1.95 (m, 16 H), 1.02 (s, 3 H, 19-Me), 1.37 (bs, 9 H, Boc), 1.41 (s, 9 H, *t*Bu), 2.03–2.37 (m, 8 H), 3.20 (s, 1 H, OH), 4.30 (s, 1 H, 2′-H), 4.60 (m, 1 H, 3-H), 4.84 (d, ^3J = 7.1 Hz, 1 H, 3′-H), 5.20 (d, ^3J = 7.1 Hz, 1 H, NH), 5.21 (s, 1 H, 7-H), 5.41 (m, 1 H, 12-H), 7.10–8.11 (m, 10 H, H_{ar}) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.0 (C-18), 17.6 (C-21), 22.3 (C-19), 22.7 (CH_2), 25.1 (CH_2), 26.4 (CH_2), 27.0 (CH_2), 27.2 (3 CH_3 of Boc), 28.0 (3 CH_3 of *t*Bu), 28.5 (CH), 30.6 (CH_2), 31.2 (CH_2), 32.1 (C), 34.2 (CH_2), 34.3 (CH), 34.4 (2 CH_2), 38.2 (CH), 40.7 (CH), 43.0 (CH), 45.0 (C), 47.5 (CH), 56.5 (C-3′), 70.9 (C-7), 73.8 (C-2′), 75.3 (C-3), 79.7 (C), 80.0 (C), 80.7 (C-12), 126.6, 127.6, 128.3, 128.5, 128.6, 129.5, 130.5, 133.1, 138.9, 129.4, 130.3, 133.2 (12 C_{ar}), 154.9 (CO), 156.8 (CO), 165.2 (CO), 172.1 (CO), 173.3 (CO) ppm. IR (film): $\tilde{\nu}_{max}$ = 3448, 1779, 1719 cm^{-1}. $[\alpha]_D^{20}$ = +38 (*c* = 1.1, CH_2Cl_2). MS (ESI, MeOH): *m*/*z* (%) = 950.4 (100) $[M + Na]^+$. HRMS: calcd. for $C_{51}H_{68}F_3NNaO_{11}$ 950.4642; found 950.4642.

Methyl 7α-Benzoyloxy-3α-(*N*-Boc-*N*,*O*-β-phenylisoserinoyl)-12α-trifluoroacetoxy-5β-cholan-24-oate (19): The reaction was carried out with 0.08 mmol (80 mg) of compound **16** in MeOH (2 mL) to give, after purification by preparative TLC (CH_2Cl_2/MeOH 1%), the expected compound **19** (48 mg, 68%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.75 (s, 3 H, 18-Me), 0.78 (d, ^3J = 6.0 Hz, 3 H, 21-Me), 0.94 (s, 3 H, 19-Me), 1.29 (bs, 9 H, Boc), 1.02–2.45 (m, 24 H), 3.56 (s, 3 H, OMe), 4.42 (s, 1 H, 2′-H), 4.52 (m, 1 H, 3-H), 4.76 (d, ^3J = 8.1 Hz, 1 H, 3′-H), 5.08 (m, 1 H, NH), 5.13 (s, 1 H, 7-H), 5.33 (m, 1 H, 12-H), 6.03–8.03 (m, 10 H, H_{ar}) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 11.9 (C-18), 17.5 (C-21), 22.2 (C-19), 22.7 (CH_2), 25.0 (CH_2), 26.3 (CH_2), 26.9 (CH_2), 28.1 (3 CH_3 of Boc), 28.4 (CH), 30.4 (CH_2), 30.5 (CH_2), 31.2 (CH_2), 34.1 (CH_2), 34.2 (C + CH_2), 34.6 (CH), 38.1 (CH), 40.6 (CH), 42.9 (CH), 44.9 (C), 47.3 (CH), 51.4 (OCH_3), 56.5 (C-3′), 70.8 (C-7), 73.7 (C-2′), 75.8 (C-3), 79.5 (C), 80.5 (C-12), 126.6, 127.5, 128.3, 128.5, 128.6, 129.5, 130.5, 132.9 (12 C_{ar}), 154.9 (CO), 156.7 (CO), 165.2 (CO), 172.0 (CO), 174.2 (CO) ppm. IR (film): $\tilde{\nu}_{max}$ = 3447, 2953, 1779, 1734, 1718 cm^{-1}. $[\alpha]_D^{20}$ = +48 (*c* = 1.0, CH_2Cl_2). MS (ESI, MeOH): *m*/*z* (%) = 908.4 (100) $[M + Na]^+$. $C_{48}H_{62}F_3NO_{11}$ (886.00): calcd. C 65.07, H 7.05, N 1.58; found C 65.07, H 7.33, N 1.52.

Benzyl 7α-Benzoyloxy-3α-(*N*-Boc-β-phenylisoserinoyl)-12α-trifluoroacetoxy-5β-cholan-24-oate (20): The reaction was carried out with 0.35 mmol (383 mg) of compound **17** in MeOH (12 mL) to give, after purification, the expected compound **20** (293 mg, 86%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.81 (m, 6 H, 18-Me, 21-Me), 1.0 (s, 3 H, 19-Me), 1.35 (bs, 9 H, Boc), 1.03–1.55 (m, 14 H), 1.67–1.91 (m, 4 H), 2.06–2.40 (m, 6 H), 3.28 (s, 1 H, OH), 4.28 (s, 1 H, 2′-H), 4.58 (m, 1 H, 3-H), 4.82 (d, ^3J = 8 Hz, 1 H, 3′-H), 5.08 (d, ^2J = 2.1 Hz, 2 H, 24-H), 5.20 (m, 2 H, 7-H + NH), 5.39 (m, 1 H, 12-H), 7.09–8.10 (m, 15 H, H_{ar}) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 11.9 (C-18), 17.5 (C-21), 22.3 (C-19), 22.7 (CH_2), 25.0 (CH_2), 26.4 (CH_2), 26.8 (CH_2), 28.1 (3 CH_3 of Boc), 28.4 (CH), 30.4 (CH_2), 30.8 (CH_2), 31.1 (C), 34.2 (2 CH_2), 34.3 (CH), 34.4 (CH_2), 38.1 (CH), 40.6 (CH), 42.8 (CH), 44.9 (C), 47.4 (CH), 56.4 (C-3′), 60.3 (C-24), 70.8 (C-7), 73.7 (C-2′), 75.8 (C-3), 79.6 (C), 80.6 (C-12), 126.4, 126.6, 127.5, 128.2, 128.3, 128.4, 128.6, 129.4, 129.6, 130.4, 133.1, 135.9, 138.9 (18 C_{ar}), 154.9 (CO), 156.8 (CO), 165.2 (CO), 171.2 (CO), 173.3 (CO) ppm. IR (film): $\tilde{\nu}_{max}$ = 3440, 1778, 1718 cm^{-1}. $[\alpha]_D^{20}$ = +47 (*c* = 1.0, CH_2Cl_2). MS (ESI, MeOH): *m*/*z* (%) = 984.5 (100) $[M + Na]^+$. $C_{54}H_{66}F_3NO_{11}$ (962.10): calcd. C 67.40, H 6.91, N 1.46; found C 67.03, H 6.98, N 1.15.

General Procedure for the Deprotection of the Trifluoroacetoxy Group: Aqueous ammonia (35%, w/w) was added to a stirred solution of the trifluoroacetate in a mixture of MeOH and THF (1:1, w/w). After 1 h, the reaction mixture was partitioned between Et_2O and aqueous phosphate buffer (pH = 7; 1 M). The organic layer was dried ($MgSO_4$) and concentrated in vacuo. The crude mixture was then purified to give the desired 12α-hydroxy compounds.

***tert*-Butyl 7α-Benzoyloxy-3α-(*N*-Boc-β-phenylisoserinoyl)-12α-hydroxy-5β-cholan-24-oate (21):** The reaction was carried out with 0.044 mmol (41 mg) of compound **18** and aqueous ammonia (600 μL) in a mixture of MeOH and THF (1.8 mL) to give, after purification by preparative TLC (CH_2Cl_2/3% MeOH), compound **21** (19 mg, 52%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.64 (s, 3 H, 18-Me), 0.91 (m, 6 H, 19-Me, 21-Me), 1.26 (bs, 9 H, Boc), 1.34 (s, 9 H, *t*Bu), 0.69–2.44 (m, 24 H), 3.42 (bs, 1 H, OH), 3.56 (s, 1 H, OH), 3.97 (s, 1 H, 12-H), 4.25 (s, 1 H, 2′-H), 4.56 (m, 1 H, 3-H), 4.82 (m, 1 H, 3′-H), 5.14 (m, 2 H, 7-H + NH), 7.10–8.03 (m, 10 H, H_{ar}) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.5 (C-18), 17.5 (C-21), 22.0 (C-19), 22.5 (CH_2), 23.9 (CH_2), 24.6 (CH_2), 25.6 (CH_2), 26.2 (CH_2), 27.0 (3 CH_3 of Boc), 27.2 (3 CH_3 of *t*Bu), 27.4 (CH_2), 30.3 (CH), 31.4 (CH_2), 32.9 (CH_2), 33.4 (CH_2), 33.7 (C), 33.9 (CH), 37.6 (CH), 39.9 (CH), 42.1 (CH), 45.7 (C), 47.3 (CH), 51.5 (C-3′), 71.4 (C-7), 72.7 (C-12), 73.9 (C-2′), 76.5 (C-3), 77.6 (C), 79.0 (C), 125.7, 126.6, 127.4, 127.5, 128.7, 129.8, 131.9 (12 C_{ar}), 153.9 (CO), 164.7 (CO), 171.0 (CO), 172.5 (CO) ppm. IR (film): $\tilde{\nu}_{max}$ = 3400, 2940, 1713 cm^{-1}. $[\alpha]_D^{20}$ = +11 (*c* = 0.95, $CHCl_3$). MS (ESI, MeOH): *m*/*z* (%) = 854.4 (100) $[M + Na]^+$. HRMS: calcd. for $C_{49}H_{69}NNaO_{10}$ 854.4819; found 854.4851.

Methyl 7α-Benzoyloxy-3α-(*N*-Boc-*N*,*O*-β-phenylisoserinoyl)-12α-hydroxy-5β-cholan-24-oate (22): The reaction was carried out with 0.12 mmol (104 mg) of compound **19** and aqueous ammonia (600 μL) in a mixture of MeOH and THF (3 mL) to give, after purification by preparative TLC (CH_2Cl_2/2% MeOH), compound **22** (44 mg, 47%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.71 (s, 3 H, 18-Me), 0.96 (d, ^3J = 6.1 Hz, 3 H, 21-Me), 0.98 (s, 3 H, 19-Me), 1.11–2.47 (m, 24 H), 1.33 (bs, 9 H, Boc), 3.26 (s, 1 H, OH), 3.62 (s, 3 H, OMe), 4.03 (s, 1 H, 12-H), 4.31 (s, 1 H, 2′-H), 4.61 (m, 1 H, 3-H), 4.88 (d, ^3J = 8.1 Hz, 1 H, 3′-H), 5.20 (s, 1 H, 7-H), 5.24 (m, 1 H, NH), 7.16–8.10 (m, 10 H, H_{ar}). IR (film): $\tilde{\nu}_{max}$ = 3433, 1714 cm^{-1}. $[\alpha]_D^{20}$ = +9 (*c* = 1.0, CH_2Cl_2). MS (ESI, MeOH): *m*/*z* (%) = 812.4 (100) $[M + Na]^+$. $C_{46}H_{63}NO_{10}$ (789.99): calcd. C 69.94, H 8.04, N 1.77; found C 70.03, H 8.21, N 1.54.

Benzyl 7α-Benzoyloxy-3α-(*N*-Boc-β-phenylisoserinoyl)-12α-hydroxy-5β-cholan-24-oate (23): The reaction was carried out with 0.14 mmol (134 mg) of compound **20** and aqueous ammonia (700 μL) in a mixture of MeOH and THF (3 mL) to give, after purification by column chromatography on silica gel (CH_2Cl_2, then CH_2Cl_2/1% MeOH), compound **23** (82 mg, 68%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.70 (s, 3 H, 18-Me), 0.97 (d, ^3J = 6.1 Hz, 3 H, 21-Me), 1.0 (s, 3 H, 19-Me), 1.36 (bs, 9 H, Boc), 0.75–2.50 (m, 24 H), 4.04 (s, 1 H, 12-H), 4.33 (s, 1 H, 2′-H), 4.63 (m, 1 H, 3-H), 4.90 (d, ^3J = 8.2 Hz, 1 H, 3′-H), 5.09 (d, ^2J = 2.0 Hz, 2 H, 24-H), 5.22 (s, 1 H, 7-H), 5.31 (m, 1 H, NH), 7.18–8.12 (m, 15 H, H_{ar}) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.4 (C-18), 17.2 (C-21), 22.4 (C-19), 22.9 (CH_2), 25.5 (CH_2), 26.5 (CH_2), 27.1 (CH_2), 28.1 (3 CH_3 of Boc), 28.4 (CH_2), 30.2 (CH), 30.7 (CH_2), 31.2 (C), 31.4 (CH_2), 34.2 (CH_2), 34.6 (CH_2), 34.8 (CH), 38.4 (CH), 40.8 (CH), 42.0 (CH), 46.5 (C), 47.2 (CH), 56.5 (C-3′),60.3 (C-24), 71.3 (C-7), 72.6 (C-12), 73.7 (C-3), 76.4 (C-2′), 79.6 (C), 125.4, 126.6, 127.4, 128.1, 128.4, 129.6, 130.6, 132.8, 135.9 (18 C_{ar}), 154.9 (CO), 165.7 (CO), 171.9 (CO), 173.8 (CO) ppm. IR (film): $\tilde{\nu}_{max}$ = 3509, 3432, 1777, 1714 cm^{-1}. $[\alpha]_D^{20}$ = +16 (*c* = 1.0, CH_2Cl_2). MS (ESI, MeOH): *m*/*z* (%) = 888.4 (100) $[M + Na]^+$. HRMS (APCI): calcd. for $C_{52}H_{67}NNaO_{10}$ 888.4663; found 888.4645.

7α-Benzoyloxy-3α-(*N*-Boc-β-phenylisoserinoyl)-12α-hydroxy-5β-cholan-24-oic Acid (24): A catalytic amount of 10% Pd/C (5 mg, 4.72

10^{-3} mmol) was added to a solution of compound **23** (34 mg, 0.039 mmol) in THF (1.5 mL). The resulting mixture was stirred under 1 atm of hydrogen for 1.5 h. The catalyst was removed by filtration through a pad of Celite and the filtrate was concentrated in vacuo to give the pure compound, which crystallised slowly under vacuum (30 mg, 98%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.69 (s, 3 H, 18-Me), 0.96 (d, ^3J = 6.4 Hz, 3 H, 21-Me), 0.97 (s, 3 H, 19-Me), 1.26 (bs, 9 H, Boc), 0.85–2.50 (m, 24 H), 3.39 (m, 1 H, OH), 4.03 (s, 1 H, 12-H), 4.30 (s, 1 H, 2′-H), 4.61 (m, 1 H, 3-H), 4.87 (m, 1 H, 3′-H), 5.19 (s, 1 H, 7-H), 5.26 (m, 1 H, NH), 7.17–8.10 (m, 10 H, H_{ar}) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 12.4 (C-18), 17.2 (C-21), 22.4 (C-19), 22.9 (CH_2), 25.5 (CH_2), 26.5 (CH_2), 27.1 (CH_2), 28.1 (3 CH_3 of Boc), 28.3 (CH), 30.7 (CH_2), 30.5 (CH_2), 30.8 (C), 31.4 (CH_2), 33.6 (CH), 34.2 (CH_2), 34.6 (CH_2), 38.3 (CH), 40.8 (CH), 42.0 (CH), 46.5 (C), 47.0 (CH), 56.5 (C-3′), 71.3 (C-7), 72.6 (C-12), 73.9 (C-2′), 76.3 (C-3), 77.4 (C), 126.6, 127.4, 129.5, 129.6, 130.6, 132.7 (12 C_{ar}), 154.9 (CO), 165.7 (CO), 171.0 (CO), 178.6 (CO) ppm. IR (film): $\tilde{\nu}_{max}$ = 3405, 1713 cm^{-1}. $[\alpha]_D^{20}$ = +13 (c = 1.0, CH_2Cl_2). MS (ESI, MeOH): m/z (%) = 798.4 (100) $[M + Na]^+$. $C_{45}H_{61}NO_{10}$ (775.97): calcd. C 69.65, H 7.92, N 1.81; found C 69.42, H 7.88, N 1.52.

$\Delta^{4,6}$-Androstene-3,17-dione (25): PTSA (0.26 g, 1.4 mmol) and 4-chloranil (3.43 g, 13.96 mmol) were added to a solution of 4-androstene-3,17-dione (**6**; 2.0 g, 6.98 mmol) in a 7:3 mixture of *tert*-butyl alcohol and toluene (300 mL). The reaction mixture was stirred at 105 °C for 16 h, cooled and filtered. The solvent was evaporated under reduced pressure and the residue was diluted with CH_2Cl_2 (100 mL). The organic layer was washed with water, a solution of 5% NaOH (4 × 50 mL) and brine (3 × 50 mL), dried with $MgSO_4$, filtered and concentrated. The crude mixture was purified by column chromatography on silica gel (EtOAc/heptane = 3:7) to afford the desired compound as a pale-yellow amorphous solid (1.45 g, 73%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.91 (s, 3 H, 18-Me), 1.08 (s, 3 H, 19-Me), 1.10–2.60 (m, 15 H), 5.63 (s, 1 H, 7-H), 6.12 (s, 2 H, 4-H + 6-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.1 (C-18), 16.3 (C-19), 19.9 (CH_2), 21.3 (CH_2), 29.2 (CH_2), 30.9 (CH_2), 33.8 (CH_2), 35.7 (CH_2), 36.0 (C), 36.9 (CH), 48.6 (CH), 50.6 (CH), 53.7 (C), 124.0 (C-7), 128.6 (C-4), 138.4 (C-6), 163.0 (C-5), 199.2 (C-17), 210.7 (C-3) ppm. IR (film): $\tilde{\nu}_{max}$ = 1739, 1663, 1618 cm^{-1}. $[\alpha]_D^{23}$ = +128 (c = 1.0, CH_2Cl_2). MS (ESI, MeOH): m/z = 307.2 $[MNa]^+$. $C_{19}H_{24}O_2$ (284.39): calcd. C 80.44, H 8.52; found C 80.24, H 8.51.

6α,7α-Dihydro-6,7-epoxyandrost-4-ene-3,17-dione (26): This compound, prepared as reported previously,[24] gave the expected spectral and analytical data.

7α-Hydroxy-5β-androstane-3,17-dione (27): Formic acid (596 μL, 5.30 mmol) and distilled triethylamine (878 μL, 2.10 mmol) were added, under argon, to a suspension of $Pd_2(dba)_3$ (77 mg, 0.025 mmol) and triphenylphosphane (20 mg, 0.025 mmol) in degassed, dry dioxane (22 mL). After 10 min, a solution of compound **26** (892 mg, 2.97 mmol) in dry dioxane (2 mL) was added. The reaction mixture was stirred at 80 °C for 5 h, then passed through a short pad of silica gel and the solvent was removed. The crude product was purified by flash chromatography on silica gel (CH_2Cl_2/2% MeOH) to give compound **27** (440 mg, 49%) as white crystals. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.90 (s, 3 H, 18-Me), 1.04 (s, 3 H, 19-Me), 1.22–1.88 (m, 10 H), 1.97–2.24 (m, 8 H), 2.34-2.52 (m, 2 H), 3.41 (t, ^3J = 14.5 Hz, 1 H, 4-H), 4.09 (s, 1 H, 7-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.5 (C-18), 20.3 (CH_2), 21.4 (CH_2), 21.8 (C-19), 31.3 (CH_2), 33.7 (CH), 34.3 (CH_2), 35.5 (C), 35.8 (CH_2), 36.8 (CH_2), 36.9 (CH_2), 38.9 (CH), 43.1 (CH), 45.6 (CH_2), 45.7 (CH), 47.6 (C), 67.2 (C-7), 212.0 (2 CO) ppm. IR (film): $\tilde{\nu}_{max}$ = 3449, 1738, 1694 cm^{-1}. $[\alpha]_D^{23}$ = +78 (c = 1.0, CH_2Cl_2). MS (ESI, MeOH): m/z = 327.5 $[MNa]^+$. $C_{19}H_{28}O_3$ (304.42): calcd. C 74.76, H 9.27; found C 74.81, H 9.05.

7α-Hydroxyandrost-4-ene-3,17-dione (33): Formic acid (85 μL, 2.20 mmol) and triethylamine (125 μL, 0.87 mmol) were added, under argon, to a suspension of $Pd_2(dba)_3$ (26 mg, 0.025 mmol) and triphenylphosphane (7 mg, 0.025 mmol) in degassed dioxane (2.5 mL). After 10 min, a solution of compound **26** (250 mg, 0.83 mmol) in dioxane (2 mL) was added. The reaction mixture was stirred at room temperature for 3 d, then passed through a short pad of silica gel and the solvent was removed. The crude product was purified by flash chromatography on silica gel (EtOAc/heptane = 1:1) to give compound **33** (130 mg, 52%) as white crystals. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.95 (s, 3 H, 18-Me), 1.13 (s, 3 H, 19-Me), 1.20–2.57 (m, 14 H), 2.34–2.52 (m, 2 H), 2.68 (dt, ^3J = 2.2/15 Hz, 1 H), 4.11 (m, 1 H, 7-H), 5.81 (s, 1 H, 4-H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.5 (C-18), 17.0 (C-19), 20.1 (CH_2), 21.3 (CH_2), 30.9 (CH), 31.0 (CH_2), 33.9 (CH_2), 35.4 (CH_2), 35.7 (CH_2), 38.5 (C), 39.4 (CH), 41.0 (CH_2), 45.6 (CH), 47.3 (C), 67.0 (C-7), 127.1 (C-4), 167.1 (C-5), 198.8 (CO), 207.0 (CO) ppm. IR (film): $\tilde{\nu}_{max}$ = 3435, 1735, 1659 cm^{-1}. $[\alpha]_D^{23}$ = +202 (c = 1.0, CH_2Cl_2). MS (ESI, MeOH): m/z = 325.2 $[MNa]^+$. HRMS (APCI): calcd. for $C_{19}H_{26}NaO_3$ 325.1780; found 325.1766.

7α-Benzoyloxy-5β-androstane-3,17-dione (28): A solution of benzoic acid (594 mg, 4.9 mmol) and dicyclohexylcarbodiimide (1003 mg, 4.9 mmol) in toluene (10 mL) was stirred at room temperature for 5 min. 4-(Dimethylamino)pyridine (237 mg, 1.93 mmol) and compound **27** (370 mg, 1.21 mmol) were then added. The resulting solution was stirred at 45 °C for 24 h. The reaction mixture was cooled and filtered. The solvent was removed under reduced pressure. The crude mixture was diluted with CH_2Cl_2 (100 mL), and the organic phase was washed with aqueous HCl (1 M, 50 mL), a saturated solution of $NaHCO_3$ (50 mL) and brine. The organic layer was dried with $MgSO_4$, filtered and evaporated under reduced pressure. The crude mixture was purified by column chromatography on silica gel (EtOAc/heptane = 3:7 then 1:1) to afford compound **28** (407 mg, 83%) as a white amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.94 (s, 3 H, 18-Me), 1.13 (s, 3 H, 19-Me), 1.36–2.21 (m, 17 H), 2.27–2.50 (m, 2 H), 3.01 (t, ^3J = 14.6 Hz, 1 H, 4-H), 5.38 (d, ^3J = 2.6 Hz, 1 H, 7-H), 7.47–7.95 (m, 5 H, H_{ar}) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.5 (C-18), 20.4 (CH_2), 21.4 (CH_2), 21.8 (C-19), 30.9 (CH_2), 31.4 (CH_2), 35.2 (C), 35.6 (CH_2), 35.9 (CH), 36.5 (CH_2), 36.8 (CH_2), 38.1 (CH), 42.3 (CH), 44.6 (CH_2), 46.1 (CH), 47.7 (C), 70.4 (C-7), 128.7, 129.3, 130.0, 133.3 (6 C_{ar}), 165.5 (CO), 211.7 (2 CO) ppm. IR (film): $\tilde{\nu}_{max}$ = 1737, 1711 cm^{-1}. $[\alpha]_D^{23}$ = +57 (c = 1.0, CH_2Cl_2). MS (ESI, MeOH): m/z = 431.2 $[MNa]^+$. HRMS (APCI): calcd. for $C_{26}H_{32}NaO_4$ 431.2198; found 431.2215.

7α-Benzoyloxy-3α-hydroxy-5β-androstane-3,17-dione (29): A suspension of lithium tri-*tert*-butoxyaluminium hydride (249 mg, 0.98 mmol) in anhydrous THF (12 mL) was cooled to 0 °C under argon. A solution of compound **28** (364 mg, 0.89 mmol) in anhydrous THF (3 mL) was then added slowly. The reaction mixture was stirred at 0 °C for 2 h, then quenched with aqueous HCl (1 M, 5 mL). The resulting milky suspension was stirred at room temperature for 5 min and diluted with CH_2Cl_2. The organic phase was washed with a saturated solution of $NaHCO_3$ (10 mL) and brine. The organic layer was dried with $MgSO_4$, filtered and concentrated under reduced pressure. The crude mixture was purified by column chromatography on silica gel (EtOAc/heptane = 3:7) to afford compound **29** (190 mg, 52%) as a white amorphous solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.88 (s, 3 H, 18-Me), 1.02 (s, 3 H, 19-Me),

1.16–2.19 (m, 19 H), 2.40 (dd, ^3J = 8.7, 19.3 Hz, 1 H, 4- H), 3.50 (m, 1 H, 3- H), 3.71 (s, 1 H, OH), 5.31 (s, 1 H, 7-H), 7.48–8.03 (m, 5 H, H_{ar}) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.4 (C-18), 20.4 (CH_2), 21.4 (CH_2), 22.7 (C-19), 30.41 (CH_2), 31.43 (CH_2), 31.4 (CH_2), 35.0 (CH + C), 35.2 (CH_2), 35.6 (CH_2), 38.1 (CH), 39.2 (CH_2), 41.0 (CH), 46.2 (CH), 47.7 (C), 70.8 (C-3), 71.4 (C-7), 128.6, 129.4, 130.4, 133.1 (6 C_{ar}), 165.8 (CO), 209.1 (CO) ppm. IR (film): $\tilde{\nu}_{max}$ = 3448, 1736, 1709 cm^{-1}. $[\alpha]_D^{23}$ = +61 (*c* = 1.0, CH_2Cl_2). MS (ESI, MeOH): *m*/*z* = 433.2 $[MNa]^+$. HRMS (APCI): calcd. for $C_{26}H_{34}NaO_4$ 433.2355; found 433.2379.

7α-Benzoyloxy-3α-[*N*-Boc-*N*,*O*-(2,4-dimethoxybenzylidene)-β-phenylisoserinoyl]-5β-androstan-17-one (30): 1,3-Dicyclohexylcarbodiimide (139 mg, 0.68 mmol), 4-(dimethylamino)pyridine (61 mg, 0.50 mmol) and protected side-chain **11** (270 mg, 0.68 mmol) were added to a solution of compound **29** (184 mg, 0.45 mmol) in toluene (8 mL). The reaction mixture was stirred at 80 °C for 4 h, cooled and filtered. The toluene was evaporated under reduced pressure and the residue was diluted with CH_2Cl_2. The organic layer was washed with aqueous HCl (1 M), a saturated solution of $NaHCO_3$ and brine, dried with $MgSO_4$, filtered and concentrated. The crude mixture was purified by column chromatography on silica gel (EtOAc/heptane = 3:7) to afford the desired compound **30** (315 mg, 88%) as a white amorphous solid. ^1H NMR (300 MHz, $CDCl_3$): δ = 0.80 (s, 3 H, 18-Me), 0.92 (s, 3 H, 19-Me), 1.00 (s, 9 H, Boc), 1.19–2.26 (m, 20 H), 3.73 (s, 3 H, OCH_3), 4.31 (d, ^3J = 3.75 Hz, 1 H, 2′-H), 4.38 (m, 1 H, 3-H), 5.22 (m, 2 H, 3′-H + 7-H), 6.28 (s, 1 H, 4′-H), 6.78–7.93 (m, 14 H, H_{ar}) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.5 (C-18), 20.0 (CH_2), 21.4 (CH_2), 22.6 (C-19), 25.0 (CH_2), 27.9 (3 CH_3 of Boc), 31. 3 (2 CH_2), 34.1 (CH_2), 34.8 (CH + C), 35.0 (CH_2), 35.2 (CH_2), 38.1 (CH), 41.8 (CH), 45.8 (C), 46.2 (CH), 55.3 (OCH_3), 63.0 (C-3′), 70.6 (C-7), 75.6 (C-3), 80.6 (C), 83.0 (C-2′), 91.8 (C-4′), 113.6, 127.8, 128.6, 129.4, 130.4, 133.2 (18 C_{ar}), 160.1 (CO), 163.0 (CO), 165.2 (CO), 209.2 (CO) ppm. IR (film): $\tilde{\nu}_{max}$ = 1737, 1709 cm^{-1}. $[\alpha]_D^{23}$ = +58 (*c* = 1.0, CH_2Cl_2). MS (ESI, MeOH): *m*/*z* = 814.5 $[MNa]^+$. HRMS (APCI): calcd. for $C_{48}H_{57}NNaO_9$ 814.3931; found 814.3929.

7α-Benzoyloxy-3α-(*N*-Boc-β-phenylisoserinoyl)-5β-androstan-17-one (31): Compound **30** (315 mg, 0.40 mmol) and PTSA (227 mg, 1.23 mmol) were dissolved in MeOH (10 mL). The resulting solution was stirred at room temperature for 3 h and then quenched with an excess of CH_2Cl_2 (25 mL). The organic layer was washed with a saturated solution of $NaHCO_3$ and brine, dried with $MgSO_4$, filtered and concentrated. The crude mixture was purified by column chromatography on silica gel (CH_2Cl_2/2% MeOH) to afford the desired compound **31** (205 mg, 76%) as a white amorphous solid. ^1H NMR (300 MHz, $CDCl_3$): δ = 0.81 (s, 3 H, 18-Me), 0.95 (s, 3 H, 19-Me), 1.00 (s, 9 H, Boc), 1.06–2.37 (m, 20 H), 3.00 (s, 1 H, OH), 4.25 (s, 1 H, 2′-H), 4.57 (m, 1 H, 3-H), 4.83 (m, 1 H, 3′-H), 5.12 (d, ^3J = 9.4 Hz, 1 H, NH), 5.26 (s, 1 H, 7-H), 7.09–7.98 (m, 10 H, H_{ar}) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.3 (C-18), 19.8 (CH_2), 21.2 (CH_2), 22.4 (C-19), 26.4 (CH_2), 28.0 (3 CH_3 of Boc), 31.3 (CH_2), 31.6 (C), 33.7 (CH_2), 34.2 (CH_2), 34.6 (CH_2), 35.8 (CH), 35.4 (CH_2), 37.8 (CH), 40.6 (CH), 45.9 (CH), 47.5 (C), 56.3 (C-3′), 70.2 (C-7), 73.8 (C-2′), 76.2 (C-3), 79.5 (C), 126.7, 127.6, 128.4, 128.6, 129.4, 130.1, 130.4, 133.1, 139.0 (12 C_{ar}), 154.9 (CO), 165.6 (CO), 171.1 (CO), 209.9 (CO) ppm. IR (film): $\tilde{\nu}_{max}$ = 3442, 1714 cm^{-1}. $[\alpha]_D^{23}$ = +54 (*c* = 1.0, CH_2Cl_2). MS (ESI, MeOH): *m*/*z* = 696.3 $[MNa]^+$. HRMS (APCI): calcd. for $C_{40}H_{51}NNaO_8$ 696.3512; found 696.3524.

Supporting Information: Characterisation data for compounds **18**, **21**, **23**, **28**, **30** and **31**.

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