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# Stereoselective synthesis of 3,7-diarylaminocholestanes by titanium-mediated reductive amination

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ABSTRACT

An efficient method for the synthesis of aryl aminocholestanes, using a chlorotriisopropoxytitanium (IV)-mediated reductive amination reaction of 5α-cholestane-3,7-dione, is reported. A series of 3, 7-diarylaminocholestane derivatives were prepared according to this methodology in up to 98% yield. These compounds were primarily characterized by $^{1}$H NMR, $^{13}$C NMR, and mass spectrometry.



## 1. Introduction

Steroids are biologically and pharmaceutically important compounds and are involved in many physiological activities. They are widely found in nature and display a variety of biological activities [1–4]. Owing to their diverse biological properties and wide applications, the synthesis of steroidal compounds has gained much attention. For many years, the preparation of aminosteroidal compounds has attracted considerable attention from medicinal and synthetic organic chemists [5–12]. Cholesterol is an essential metabolite required for major biological functions, such as formation the cell membrane structure where the steroid combines with phospholipid molecules, the integral part of the lipid bilayers [13]. However, cholesterol not only plays a role in the physical structure of the membrane (e.g., its viscosity and interference with the phospholipids), it also has specific functions within the membrane. For example, the caveolae (detergent-resistant domains) are important for transduction and ceramide-induced apoptosis. It is also essential in embryonic development, and cholesterol deficiency during embryogenesis and organogenesis cause severe abnormalities [14].

Reductive amination of aldehydes and ketones is an important reaction for the synthesis of amines. It is a useful tool for the introduction of a nitrogen atom to intermediates. Chiral amines are key features in a multitude of biologically active natural products and pharmaceuticals, and therefore their synthesis is of high importance in medicinal chemistry. Moreover, stereoselective reduction has been a major focus in synthetic chemistry for many decades and has been the subject of numerous and diverse studies. The introduction of amino groups to the cholesterol framework by reductive amination has been reported by us [5–7] and others [8–10], and the products were evaluated for biological potency and molecular recognition. These modified steroid molecules were found to have enhanced hydrophilicity and flexibility, which assists in intramolecular hydrogen bonding and solubility in polar solvents, and also could be manipulated for further transformations [15]. The preferred method to introduce the amine group was via a $Ti(O^{i}Pr)_4$–$NaBH_4$ mediated reaction, forming the 3β isomer as the major product [8–10,16]. We have also described a highly stereoselective procedure for the synthesis of 3α-alkylaminosteroid [5,6], 7α-alkylaminosteroid and a high-yielding, stereoselective sequential reductive amination procedure for the synthesis of 3α,7α-dialkylaminocholestane [7,15]. However, these methods are only applicable for the synthesis of aliphatic aminocholestanes. Recently, we developed a method for one-pot reductive amination of aromatic ketones with various aryl amines using a $TiCl(O^{i}Pr)_3$–$NaBH(OAc)_3$ mediated system [17]. This protocol allows for the synthesis of various secondary amines and 1,1′-bis(*N*-aryl-1-aminoethyl)ferrocene derivatives in yields up to 82%. With a reliable method for the reductive amination of 1,1-diacetylferrocene with aryl amines, we turned our attention to the synthesis of aryl aminocholestanes.

The 5α-skeleton of cholestane provides more space between the functionalized positions at C-3 and C-7. The introduction of 3- and

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7-axial amine groups provide two necessary inward-pointing hydrogen bonding sites [15,18–22], which is not possible for cholestanes with 5β-configuration [23]. We have synthesized various alkyl aminocholestanes with urea [15] and imidazole [18–22] as recognizing pendants at the 3- and 7-postions of 5α-cholestane, as well as various alkyl aminocholestanes, for biological activity studies [24–26]. Considering the importance of alkyl aminocholestanes, the design and synthesis of well-organized aryl aminocholestanes for use as molecular receptors in biological systems is highly desirable. The aryl amine pendant at C-3 and C-7 of 5α-cholestane provides a more rigid frame for molecular recognition compared to an alkyl amine pendant. Therefore, it would be interesting to introduce an aryl amine at the C-3 and C-7 position of the steroidal skeleton stereoselectively. Herein, we report for the first time, an efficient method for the introduction of aryl amine into the cholesterol framework stereoselectively through a one-pot $TiCl(O^iPr)_3$-mediated reductive amination reaction.

## 2. Experimental section

Melting points were determined using a Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR spectra were recorded on a Bruker AM-400 spectrometer in $CDCl_3$ using $Me_4Si$ as the internal standard. High-resolution mass spectrometry data were obtained from the Korean Basic Science Institute (Daegu Branch) on a Jeol JMS 700 high resolution mass spectrometer. TLC analyses were carried out on a 0.2-mm HPTLC silica gel 60 plate and the compounds were detected by spraying with 5% ammonium molybdate in 10% $H_2SO_4$ followed by heating. Flash column chromatography was performed using Merck silica gel 60 (70–230 mesh) and the two isomers were separated by MPLC. Reactions were carried out under an argon atmosphere, and the solutions were washed with brine and dried over anhydrous sodium sulfate. Reagents were purchased from Aldrich Chemical Co. 5α-Cholestane-3,7-dione 1 [7] and sodium tris(ethylhexanoic)borohydride were prepared according to literature procedures [5].

### 2.1. 3α-Phenylamino-5α-cholestan-7-one (**2a**) and 3β-phenylamino-5α-cholestan-7-one (**2b**)

A mixture of 5α-cholestane-3,7-dione (**1**, 200 mg, 0.5 mmol), 1.2 M $TiCl(O^iPr)_3$ in hexane (0.6 mL, 0.6 mmol) and aniline (70 mg, 0.75 mmol) in methanol (10 mL) was stirred at room temperature for 30 min. $NaBH(OAc)_3$ (106 mg, 0.5 mmol) was then added and the resulting mixture was stirred for an additional 2 h. After the reaction was completed, the solvent was removed. The residue was neutralized with $NaHCO_3$ solution, and extracted with ethyl acetate. The organic layer was dried and concentrated. The residue was purified by MPLC ($SiO_2$, elution with 1% EtOAc in hexane) to give a less polar isomer **2a** and more polar isomer **2b** with an 80% total yield.

**2a**: Yield 64%; amorphous; TLC $R_f$ 0.35 (10% EtOAc in hexane); $^1$H NMR $\delta$ 0.66 (3H, s, H-18), 0.86 (3H, d, $J$ = 6.6 Hz, H-26), 0.87 (3H, d, $J$ = 6.6 Hz, H-27), 0.91 (3H, d, $J$ = 6.6 Hz, H-21), 1.09 (3H, s, H-19), 3.71 (1H, br s, 3β-H), 6.69 (2H, d, $J$ = 7.8 Hz), 6.76 (1H, t, $J$ = 7.3 Hz), 7.18 (2H, dd, $J$ = 7.3, 7.8 Hz); $^{13}$C NMR $\delta$ 11.1, 12.2, 18.9, 21.3, 22.7, 22.9, 23.9, 25.1, 25.6, 28.1, 28.5, 32.3, 32.7, 35.7, 36.2, 36.6, 38.8, 39.6, 42.6, 42.7, 46.0, 47.7, 49.0, 50.3, 55.1, 55.8, 114.8, 129.4, 212.1; HR-FAB MS calcd. for $C_{33}H_{51}NO$ $(M+H)^+$: 478.4049, Found: $m/z$ 478.4053.

**2b**: Yield 16%; amorphous; TLC $R_f$ 0.19 (10% EtOAc in hexane); $^1$H NMR $\delta$ 0.66 (3H, s, H-18), 0.86 (3H, d, $J$ = 6.6 Hz, H-26), 0.87 (3H, d, $J$ = 6.6 Hz, H-27), 0.92 (3H, d, $J$ = 6.6 Hz, H-21), 1.09 (3H, s, H-19), 3.26 (1H, m, 3α-H), 6.61 (2H, d, $J$ = 7.6 Hz), 6.70 (1H, t, $J$ = 7.1 Hz), 7.16 (2H, t, $J$ = 7.6 Hz); $^{13}$C NMR $\delta$ 12.0, 12.2, 18.9, 21.8, 22.7, 22.9, 23.9, 25.1, 28.1, 28.5, 28.7, 35.2, 35.7, 36.2, 36.4, 36.9, 38.8, 39.6, 42.6, 42.7, 46.2, 47.7, 49.0, 50.2, 55.1, 55.5, 114.6, 129.5, 212.0; HR-FAB MS calcd. for $C_{33}H_{51}NO$ $(M+H)^+$: 478.4049, Found: $m/z$ 478.4047.

### 2.2. 3α,7α-Di(phenylamino)-5α-cholestane (**3a**) and 3β,7α-di(phenylamino)-5α-cholestane (**3b**)

A mixture of 5α-cholestane-3,7-dione (**1**, 200 mg, 0.5 mmol), 1.0 M $TiCl(O^iPr)_3$ in hexane (1.2 mL, 1.2 mmol) and aniline (140 mg, 1.5 mmol) in dry $CH_2Cl_2$ (10 mL) was stirred at room temperature for 30 min. $NaBH(OAc)_3$ (212 mg, 1.0 mmol) was then added and the resulting mixture was stirred for an additional 1 h. After the reaction was completed, the solvent was removed. The residue was neutralized with $NaHCO_3$ solution, and extracted with ethyl acetate. The organic layer was dried and concentrated. The residue was purified by MPLC ($SiO_2$, elution with 0.5% EtOAc in hexane) to give a less polar isomer **3a** and more polar isomer **3b** with a 90% total yield.

**3a**: Yield 72%; amorphous; TLC $R_f$ 0.56 (10% EtOAc in hexane); $^1$H NMR $\delta$ 0.72 (3H, s, H-18), 0.89 (3H, d, $J$ = 6.6 Hz, H-26), 0.90 (3H, d, $J$ = 6.6 Hz, H-27), 0.92 (3H, s, H-19), 0.95 (3H, d, $J$ = 6.6 Hz, H-21), 1.85 (1H, br, 7α-NH), 2.02 (1H, br t, 3α-NH), 3.54 (1H, br s, 7β-H), 3.66 (1H, br s, 3β-H), 6.62 (4H, d, $J$ = 7.6 Hz), 6.68 (2H, dd, $J$ = 7.6, 7.8 Hz), 7.15 (2H, d, $J$ = 7.1 Hz), 7.19 (2H, d, $J$ = 7.1 Hz); $^{13}$C NMR $\delta$ 10.9, 12.0, 18.8, 20.9, 22.7, 22.9, 23.5, 23.9, 25.2, 25.4, 28.0, 28.1, 32.8, 32.9, 33.9, 35.9, 36.2, 36.5, 38.2, 39.6, 42.6, 42.7, 46.0, 47.3, 49.8, 51.6, 56.4, 112.6, 113.4, 116.5, 129.3, 129.4, 148.0; HR-FAB MS calcd. for $C_{39}H_{58}N_2$ $(M+H)^+$: 555.4678, Found: $m/z$ 555.4675.

**3b**: Yield 18%; amorphous; TLC $R_f$ 0.43 (10% EtOAc in hexane); $^1$H NMR: $\delta$ 0.70 (3H, s, H-18), 0.86 (3H, d, $J$ = 6.6 Hz, H-26), 0.87 (3H, d, $J$ = 6.6 Hz, H-27), 0.88 (3H, s, H-19), 0.92 (3H, d, $J$ = 6.6 Hz, H-21), 1.99 (2H, br m, 3β-NH, 7α-NH), 3.23 (1H, br m, 3α-H), 3.50 (1H, br s, 7β-H), 6.57 (4H, d, $J$ = 7.8 Hz), 6.68 (2H, dd, $J$ = 7.6, 7.8 Hz), 7.15 (2H, d, $J$ = 7.6 Hz), 7.17 (2H, d, $J$ = 7.6 Hz); $^{13}$C NMR $\delta$ 11.8, 12.0, 18.8, 21.3, 22.7, 22.9, 23.5, 23.9, 28.0, 28.1, 28.9, 32.7, 34.9, 35.8, 36.1, 36.2, 37.6, 38.2, 39.0, 39.6, 39.6, 42.6, 42.7, 47.4, 49.6, 51.6, 56.4, 112.5, 114.8, 116.5, 129.4, 129.4, 147.8; HR-FAB MS calcd. for $C_{39}H_{58}N_2$ $(M+H)^+$: 555.4678, Found: $m/z$ 555.4681.

### 2.3. 3α,7α-Di[(4-methylphenyl)amino]-5α-cholestane (**3c**) and 3β,7α-di[(4-methylphenyl)-amino]-5α-cholestane (**3d**)

**3c**: Yield 82%; amorphous; TLC $R_f$ 0.54 (10% EtOAc in hexane); $^1$H NMR $\delta$ 0.72 (3H, s, H-18), 0.89 (3H, d, $J$ = 6.8 Hz, H-26), 0.90 (3H, d, $J$ = 6.6 Hz, H-27), 0.90 (3H, s, H-19), 0.95 (3H, d, $J$ = 6.6 Hz, H-21), 2.00 (1H, m, 7α-NH), 2.03 (1H, m, 3α-NH), 2.25 (3H, s, $CH_3$), 2.26 (3H, s, $CH_3$), 3.51 (1H, br s, 7β-H), 3.62 (1H, br s, 3β-H), 6.56 (4H, d, $J$ = 6.6 Hz), 6.97 (2H, d, $J$ = 7.8 Hz), 6.99 (2H, d, $J$ = 7.8 Hz); $^{13}$C NMR $\delta$ 11.2, 12.4, 19.1, 20.7, 20.8, 20.8, 21.2, 23.0, 23.2, 23.8, 24.2, 25.6, 28.1, 28.4, 32.7, 33.0, 34.0, 36.2, 36.5, 36.7, 38.5, 39.8, 39.9, 43.70, 47.5, 50.3, 51.9, 54.1, 56.7, 113.1, 125.8, 130.1, 130.2, 146.1; HR-FAB MS calcd. for $C_{41}H_{62}N_2$ $(M^+)$: 582.4913, Found: $m/z$ 582.4916.

**3d**: Yield 14%; amorphous, TLC $R_f$ 0.40 (10% EtOAc in hexane); $^1$H NMR $\delta$ 0.67 (3H, s, H-18), 0.85 (3H, d, $J$ = 6.6 Hz, H-26), 0.86 (3H, d, $J$ = 6.6 Hz, H-27), 0.86 (3H, s, H-19), 0.90 (3H, d, $J$ = 6.3 Hz, H-21), 1.96, (1H, m, 3β-NH), 1.99 (1H, m, 7α-NH), 2.22 (3H, s, $CH_3$), 2.24 (3H, s, $CH_3$), 3.15 (1H, br m, 3α-H), 3.44 (1H, br s, 7β-H), 6.48 (2H, d, $J$ = 8.4 Hz), 6.75 (2H, d, $J$ = 6.8 Hz), 6.96 (2H, d, $J$ = 8.1 Hz), 6.99 (2H, d, $J$ = 8.1 Hz); $^{13}$C NMR $\delta$ 12.1, 12.4, 19.1, 20.7, 21.0, 21.6, 23.0, 23.2, 23.8, 24.2, 25.6, 28.4, 28.5, 32.9, 34.0, 36.2, 36.5, 36.6, 37.8, 38.5, 39.1, 39.9, 40.0, 43.0, 47.6, 50.0, 51.9, 54.1, 56.7, 112.9, 125.9, 130.2, 130.3, 145.8; HR-FAB MS calcd. for $C_{41}H_{62}N_2$ $(M^+)$: 582.4913, Found: $m/z$ 582.4918.

*2.4. 3α,7α-Di[(4-methoxyphenyl)amino]-5α-cholestane (**3e**) and 3β,7α-[di(4-methoxy-phenyl)amino]-5α-cholestane (**3f**)*

**3e**: Yield 88%; amorphous; TLC $R_f$ 0.5 (25% EtOAc in hexane); $^1$H NMR $\delta$ 0.69 (3H, s, H-18), 0.87 (3H, d, $J$ = 6.6 Hz, H-26), 0.87 (3H, d, $J$ = 6.6 Hz, H-27), 0.87 (3H, s, H-19), 0.92 (3H, d, $J$ = 6.6 Hz, H-21), 1.99 (1H, d, $J$ = 12.6 Hz, 7α-NH), 3.44 (1H, br s, 7β-H), 3.55 (1H, br s, 3β-H), 3.73 (3H, s, $OCH_3$), 3.74 (3H, s, $OCH_3$), 6.58 (2H, d, $J$ = 7.6 Hz), 6.64 (2H, br s), 6.72 (2H, d, $J$ = 8.8 Hz), 6.76 (2H, d, $J$ = 9.1 Hz); $^{13}$C NMR $\delta$ 11.0, 12.0, 18.8, 20.9, 22.7, 22.9, 23.6, 23.9, 25.4, 28.1, 29.4, 29.9, 32.6, 32.7, 33.3, 35.9, 36.2, 36.4, 38.2, 38.3, 39.6, 39.6, 42.7, 47.2, 50.9, 51.5, 55.8, 56.0, 56.3, 114.2, 115.0, 115.1, 138.7, 142.4, 151.5; HR-FAB MS calcd. for $C_{41}H_{62}N_2O_2$ $(M+H)^+$: 615.4890, Found: $m/z$ 615.4886.

**3f**: Yield 10% amorphous; TLC $R_f$ 0.5 (25% EtOAc in hexane); $^1$H NMR $\delta$ 0.69 (3H, s, H-18), 0.86 (3H, d, $J$ = 6.3 Hz, H-26), 0.87 (3H, d, $J$ = 6.5 Hz, H-27), 0.87 (3H, s, H-19), 0.92 (3H, d, $J$ = 6.3 Hz, H-21), 3.15 (1H, br m, 3α-H), 3.35 (1H, br s, 7α-NH), 3.41 (1H, br s, 7β-H), 3.62 (1H, br t, 3β-NH), 3.73 (3H, s, $OCH_3$), 3.75 (3H, s, $OCH_3$), 6.54 (2H, d, $J$ = 9.0 Hz), 6.58 (2H, s), 6.75 (2H, d, $J$ = 8.8 Hz), 6.77 (2H, d, $J$ = 8.8 Hz); $^{13}$C NMR $\delta$ 11.8, 12.0, 18.8, 21.3, 22.6, 22.9, 23.5, 23.8, 28.0, 28.1, 29.5, 29.8, 32.8, 35.6, 35.8, 36.2, 37.7, 38.3, 38.9, 39.5, 39.6, 42.7, 47.3, 50.6, 51.5, 54.0, 55.8, 55.9, 56.3, 113.8, 115.0, 115.1, 115.5, 128.3, 130.1, 141.1, 142.3, 151.5, 152.3; HR-FAB MS calcd. for $C_{41}H_{62}N_2O_2$ $(M+H)^+$: 615.4890, Found: $m/z$ 615.4886.

*2.5. 3α,7α-Di[(2-aminophenyl)amino]-5α-cholestane (**3g**)*

**3g**: Yield 80% amorphous; TLC $R_f$ 0.5 (25% EtOAc in hexane); $^1$H NMR $\delta$ 0.70 (3H, s, H-18), 0.86 (3H, d, $J$ = 6.6 Hz, H-26), 0.87 (3H, d, $J$ = 6.8 Hz, H-27), 0.90 (3H, s, H-19), 0.92 (3H, d, $J$ = 6.5 Hz, H-21), 3.23 (1H, m, 7α-NH), 3.58 (1H, br s, 7β-H), 3.69 (1H, br s, 3β-H), 3.93 (4H, br s, $NH_2$), 2.03 (1H, m, 3α-NH), 6.56 (4H, d, $J$ = 6.6 Hz), 6.97 (2H, d, $J$ = 7.8 Hz), 6.99 (2H, d, $J$ = 7.8 Hz); $^{13}$C NMR $\delta$ 11.0, 11.8, 12.0, 12.1, 18.7, 20.9, 21.3, 22.6, 22.9, 23.5, 23.6, 23.9, 28.0, 28.1, 28.7, 32.1, 32.2, 35.8, 35.9, 36.2, 36.3, 36.4, 37.5, 38.3, 38.4, 38.9, 39.6, 42.8, 47.3, 49.0, 51.6, 51.7, 56.2, 56.3, 110.5, 117.1, 117.2, 117.3, 120.5, 121.3, 133.7, 137.7; HR-FAB MS calcd. for $C_{39}H_{60}N_4$ ($M^+$): 584.4820, Found: $m/z$ 584.4822.

*2.6. 3α,7α-Di[(4-pyridyl)amino]-5α-cholestane (**3h**)*

**3h**: Yield 80% amorphous; TLC $R_f$ 0.51 (30% EtOAc in hexane); $^1$H NMR $\delta$ 0.78 (3H, s, H-18), 0.82 (3H, d, $J$ = 6.6 Hz, H-26), 0.85 (3H, d, $J$ = 6.8 Hz, H-27), 0.88 (3H, s, H-19), 0.90 (3H, d, $J$ = 6.5 Hz, H-21), 2.01 (1H, br s, 7α-NH), 2.03 (1H, br s, 3α-NH), 3.56 (1H, br s, 7β-H), 3.69 (1H, br s, 3β-H), 6.45 (4H, d, $J$ = 7.0 Hz), 8.02 (4H, d, $J$ = 7.0 Hz); $^{13}$C NMR $\delta$ 11.1, 12.0, 18.6, 20.7, 21.4, 22.9, 23.0, 23.2, 23.6, 24.6, 28.0, 28.2, 28.7, 32.1, 32.3, 35.2, 35.7, 36.2, 36.4, 37.9, 38.4, 38.5, 38.9, 39.6, 42.9, 47.0, 51.6, 51.7, 56.2, 56.3, 112.5, 112.8, 148.6, 148.7, 155.6; HR-FAB MS calcd. for $C_{37}H_{56}N_4$ ($M^+$): 556.4521, Found: $m/z$ 556.4523.

*2.7. 3α,7α-Di[(4-bromophenyl)amino]-5α-cholestane (**3i**) and 3β,7α-di[(4-bromo-phenyl)amino]-5α-cholestane (**3j**)*

**3i**: Yield 68%; amorphous; TLC $R_f$ 0.51 (10% EtOAc in hexane); $^1$H NMR $\delta$ 0.69 (3H, s, H-18), 0.87 (3H, d, $J$ = 6.8 Hz, H-26), 0.88 (3H, d, $J$ = 6.8 Hz, H-27), 0.88 (3H, s, H-19), 0.92 (3H, d, $J$ = 6.6 Hz, H-21), 1.99 (1H, br s, 7α-NH), 2.02 (1H, br s, 3α-NH), 3.44 (1H, br s, 7β-H), 3.58 (1H, br s, 3β-H), 6.46 (4H, d, $J$ = 8.3 Hz), 7.21 (2H, d, $J$ = 8.8 Hz), 7.22 (2H, d, $J$ = 8.8 Hz); $^{13}$C NMR $\delta$ 10.9, 12.0, 18.5, 18.8, 20.9, 22.7, 23.0, 23.5, 24.0, 25.3, 28.0, 28.1, 32.6, 32.8, 33.9, 35.8, 36.2, 36.4, 38.1, 38.6, 39.6, 42.8, 47.3, 50.0, 51.6, 56.4, 107.8, 114.1, 114.4, 115.0, 125.5, 132.0, 132.1, 147.0; HR-FAB MS calcd. for $C_{39}H_{56}N_2Br_2$ $(M+H)^+$, $(M+2)^+$ and $(M+4)^+$: 711.2888, 712.2922 and 714.2902, Found: $m/z$ 711.2888, 712.2947 and 714.2902.

**3j**: Yield 22%; amorphous, TLC $R_f$ 0.35 (10% EtOAc in hexane); $^1$H NMR $\delta$ 0.68 (3H, s, H-18), 0.85 (3H, d, $J$ = 6.6 Hz, H-26), 0.86 (3H, d, $J$ = 6.6 Hz, H-27), 0.86 (3H, s, H-19), 0.91 (3H, d, $J$ = 6.3 Hz, H-21), 1.96, (1H, br d, 3β-NH), 2.00 (1H, br s, 7α-NH), 3.17 (1H, m, 3α-H), 3.42 (1H, br s, 7β-H), 6.44 (2H, d, $J$ = 8.8 Hz), 6.53 (2H, d, $J$ = 8.3 Hz), 7.22 (2H, d, $J$ = 8.6 Hz), 7.24 (2H, d, $J$ = 8.6 Hz); $^{13}$C NMR $\delta$ 11.8, 12.0, 18.4, 18.8, 21.3, 22.7, 23.0, 23.5, 24.0, 25.1, 28.0, 28.1, 28.7, 32.5, 34.8, 35.8, 36.1, 36.2, 37.5, 38.1, 39.0, 39.5, 42.7, 47.4, 49.8, 51.6, 56.3, 107.9, 114.1, 114.2, 116.3, 132.1, 132.2, 141.8, 146.7; HR-FAB MS calcd. for $C_{39}H_{56}N_2Br_2$ $(M+H)^+$, $(M+2)^+$ and $(M+4)^+$: 711.2888, 712.2922 and 714.2902, Found: $m/z$ 711.2892, 712.2941 and 714.2950.

*2.8. 3α,7α-Di[(3-bromophenyl)amino]-5α-cholestane (**3k**) and 3β, 7α-di[(3-bromophenyl)-amino]-5α-cholestane (**3l**)*

**3k**: Yield 63%; amorphous; TLC $R_f$ 0.59 (10% EtOAc in hexane); $^1$H NMR $\delta$ 0.69 (3H, s, H-18), 0.87 (3H, d, $J$ = 6.6 Hz, H-26), 0.87 (3H, d, $J$ = 6.8 Hz, H-27), 0.88 (3H, s, H-19), 0.92 (3H, d, $J$ = 6.3 Hz, H-21), 2.00 (1H, br s, 7α-NH), 3.47 (1H, br s, 7β-H), 3.60 (1H, br s, 3β-H), 3.97 (1H, br s, 3α-NH), 6.48 (2H d, $J$ = 6.8 Hz), 6.74 (4H, m), 6.99 (2H, dd, $J$ = 8.1, 8.1 Hz); $^{13}$C NMR $\delta$ 10.9, 12.0, 18.7, 22.7, 22.9, 23.4, 23.8, 25.3, 28.0, 28.1, 32.5, 32.8, 34.0, 35.4, 35.5, 36.1, 36.4, 37.9, 39.5, 39.6, 42.7, 47.3, 47.5, 49.7, 54.2, 51.6, 56.3, 111.1, 111.6, 114.4, 114.9, 119.2, 119.4, 123.4, 123.5, 130.5, 130.7, 148.7, 149.1; HR-FAB MS calcd. for $C_{39}H_{56}N_2Br_2$ $(M+H)^+$, $(M+2)^+$ and $(M+4)^+$: 711.2888, 712.2922 and 714.2902, Found: $m/z$ 711.2892, 712.2922 and 714.2950.

**3l**: Yield 21%; amorphous; TLC $R_f$ 0.32 (10% EtOAc in hexane); $^1$H NMR $\delta$ 0.68 (3H, s, H-18), 0.86 (3H, d, $J$ = 6.6 Hz, H-26), 0.87 (3H, d, $J$ = 6.8 Hz, H-27), 0.87 (3H, s, H-19), 0.91 (3H, d, $J$ = 6.3 Hz, H-21), 2.00 (1H, br s, 7α-NH), 2.03 (1H, br d, 3β-NH), 3.19 (1H, br m, 3α-H), 3.45 (1H, br s, 7β-H), 4.01 (1H, br s, 3α-NH), 6.49 (2H, t, $J$ = 8.6 Hz), 6.73 (4H, m), 6.99 (2H, dd, $J$ = 7.8, 7.8 Hz); $^{13}$C NMR $\delta$ 11.8, 12.0, 18.7, 21.2, 22.7, 22.9, 23.4, 23.8, 28.0, 28.1, 28.9, 32.4, 35.1, 35.8, 36.0, 36.1, 37.5, 38.0, 39.0, 39.5, 39.6, 42.7, 47.4, 49.5, 51.5, 52.9, 56.3, 111.1, 112.4, 115.0, 116.3, 119.2, 120.4, 123.3, 123.5, 130.6, 130.7, 147.9, 149.0; HR-FAB MS calcd. for $C_{39}H_{56}N_2Br_2$ $(M+H)^+$, $(M+2)^+$ and $(M+4)^+$: 711.2888, 712.2922 and 714.2902, Found: $m/z$ 711.2893, 712.2951 and 714.2942.

*2.9. 3α,7α-Di[(2-bromophenyl)amino]-5α-cholestane (**3m**) and 3α-(2-bromophenyl)amino-5α-cholestan-7-one (**2c**)*

A mixture of 5α-cholestane-3,7-dione (**1**, 200 mg, 0.5 mmol), 1.0 M $TiCl(O^iPr)_3$ in hexane (1.2 mL, 1.2 mmol) and 2-bromoaniline (140 mg, 1.5 mmol) in dry $CH_2Cl_2$ (10 mL) was stirred at room temperature for 30 min. $NaBH(OEh)_3$ (212 mg, 1.0 mmol) was then added and the resulting mixture was stirred for an additional 30 h. After the reaction was completed, the solvent was removed. The residue was neutralized with $NaHCO_3$ solution, and extracted with ethyl acetate. The organic layer was dried and concentrated. The residue was purified by MPLC ($SiO_2$, elution with 0.5% EtOAc in hexane) to give a less polar compound **3l** and more polar compound **2c**.

**3m**: Yield 65%; amorphous; TLC $R_f$ 0.67 (10% EtOAc in hexane); $^1$H NMR $\delta$ 0.71 (3H, s, H-18), 0.88 (3H, d, $J$ = 6.5 Hz, H-26), 0.89 (3H, d, $J$ = 6.5 Hz, H-27), 0.93 (3H, s, H-19), 0.94 (3H, d, $J$ = 6.3 Hz, H-21), 2.01 (1H, m, 7α-NH), 3.62 (1H, br s, 7β-H), 3.7 (1H, br s, 3β-H), 4.73 (1H, br s, 3α-NH), 6.49 (1H, dt, $J$ = 7.6, 1.8 Hz), 6.51 (1H, dt, $J$ = 7.6, 1.8 Hz), 6.58 (2H, d, $J$ = 8.3 Hz), 7.12 (1H, dt, $J$ = 7.6, 1.8 Hz), 7.14 (1H, dt, $J$ = 7.6, 1.8 Hz), 7.40 (1H, d, $J$ = 8.3 Hz), 7.41 (1H, d, $J$ = 8.3 Hz); $^{13}$C NMR $\delta$ 11.0, 12.1, 18.8, 20.9, 22.7, 22.9, 23.4, 23.5,

23.9, 25.1, 28.0, 28.1, 32.5, 32.6, 34.2, 35.9, 36.2, 36.5, 38.2, 39.5, 39.6, 42.7, 47.3, 47.9, 49.9, 51.6, 56.2, 110.5, 110.7, 111.0, 111.8, 116.9, 117.2, 128.4, 128.5, 132.6, 132.6, 143.9, 144.4; HR-FAB MS calcd. for $C_{39}H_{56}N_2Br_2$ $(M+H)^+$, $(M+2)^+$ and $(M+4)^+$: 711.2888, 712.2922 and 714.2902, Found: *m/z* 711.2881, 712.2930 and 714.2927.

**2c**: Yield 11%; white solid; mp 82–84 °C ($CH_2Cl_2$–$CH_3CN$); TLC $R_f$ 0.32 (10% EtOAc in hexane); $^1$H NMR $\delta$ 0.66 (3H, s, H-18), 0.85 (3H, d, $J$ = 6.5 Hz, H-26), 0.87 (3H, d, $J$ = 6.5 Hz, H-27), 0.91 (3H, d, $J$ = 6.5 Hz, H-21), 1.10 (3H, s, H-19), 3.77 (1H, br s, 3β-H), 4.77 (1H, br s, 3α-NH), 6.51–6.57 (1H, m), 6.63 (1H, d, $J$ = 8.0 Hz), 7.14 (1H, dt, $J$ = 8.0, 1.2 Hz), 7.40 (1H, d, $J$ = 8.0 Hz); $^{13}$C NMR $\delta$ 11.1, 12.2, 18.9, 21.3, 22.7, 22.9, 23.9, 25.1, 25.6, 28.1, 28.5, 32.3, 32.8, 35.8, 36.2, 36.6, 38.8, 39.6, 42.6, 42.8, 46.0, 47.8, 49.0, 50.3, 55.1, 55.8, 110.6, 112.3, 118.0, 128.5, 132.7, 143.2, 211.8; HR-FAB MS calcd. for $C_{33}H_{50}BrNO$ $(M+H)^+$ and $(M+2)^+$: 557.2808, 558.2808 Found: *m/z* 557.2871 and 558.2871.

### *2.10. 3α,7α-Di[(4-carbomethoxyphenyl)amino]-5α-cholestane (**3n**) and 3β,7α-di[(4-4-carbomethoxyphenyl)amino]-5α-cholestane (**3o**)*

**3n**: Yield 63%; amorphous; TLC $R_f$ 0.59 (10% EtOAc in hexane); $^1$H NMR $\delta$ 0.66 (3H, s, H-18), 0.84 (3H, d, $J$ = 6.6 Hz, H-26), 0.85 (3H, d, $J$ = 6.6 Hz, H-27), 0.87 (3H, s, H-19), 0.89 (3H, d, $J$ = 6.5 Hz, H-21), 1.97 (1H, d, $J$ = 12.6, 7α-NH), 3.55 (1H, br s, 7β-H), 3.68 (1H, br s, 3β-H), 3.82 (3H, s, $CH_3$), 3.83 (3H, s, $CH_3$), 4.52 (1H, br s, 3α-NH) 6.53 (4H, d, $J$ = 8.3 Hz), 7.80 (2H, d, $J$ = 8.8 Hz), 7.83 (2H, d, $J$ = 8.8 Hz); $^{13}$C NMR $\delta$ 10.8, 11.9, 18.7, 20.8, 22.6, 22.9, 23.3, 23.8, 25.2, 27.9, 28.0, 32.7, 34.1, 35.8, 36.1, 36.3, 37.9, 39.4, 39.5, 42.7, 47.2, 49.5, 51.5, 51.6, 56.2, 111.3, 111.5, 117.5, 131.6, 131.8, 151.0, 151.6, 167.4; HR-FAB MS calcd. for $C_{43}H_{62}N_2O_4$ $(M+H)^+$: 671.4788, Found: *m/z* 671.4790.

**3o**: Yield 21%; amorphous; TLC $R_f$ 0.32 (10% EtOAc in hexane); $^1$H NMR $\delta$ 0.66 (3H, s, H-18), 0.83 (3H, d, $J$ = 6.8 Hz, H-26), 0.84 (3H, d, $J$ = 6.6 Hz, H-27), 0.85 (3H, s, H-19), 0.89 (3H, d, $J$ = 6.3 Hz, H-21), 2.16 (1H, s, 7α-NH), 3.24 (1H, m, 3α-H), 3.54 (1H, br s, 7β-H), 3.83 (3H, s, $CH_3$), 3.84 (3H, s, $CH_3$), 4.45 (1H, br s, 3α-NH) 6.63 (4H, d, $J$ = 8.6 Hz), 7.84 (4H, d, $J$ = 8.5 Hz); $^{13}$C NMR $\delta$ 11.7, 12.0, 18.7, 21.2, 22.6, 22.9, 23.3, 23.8, 27.9, 28.1, 28.2, 29.8, 31.0, 32.5, 34.2, 35.7, 35.9, 36.1, 37.2, 37.8, 38.9, 39.4, 39.5, 42.7, 47.3, 49.3, 51.5, 51.6, 51.7, 51.8, 52.0, 56.2, 111.3, 113.9, 117.5, 117.9, 119.6, 130.9, 131.4, 131.6, 131.8, 150.9, 151.4, 167.3, 167.4; HR-FAB MS calcd. for $C_{43}H_{62}N_2O_4$ $(M+H)^+$: 671.4788, Found: *m/z* 671.4790.

### *2.11. 3α,7α-Di[(4-nitrophenyl)amino]-5α-cholestane (**3p**) and 3β,7α-di[(4-nitrophenyl)-amino]-5α-cholestane (**3q**)*

**3p**: Yield 64%; amorphous; TLC $R_f$ 0.80 (33% EtOAc in hexane); $^1$H NMR $\delta$ 0.66 (3H, s, H-18), 0.83 (3H, d, $J$ = 6.6 Hz, H-26), 0.84 (3H, d, $J$ = 6.6 Hz, H-27), 0.88 (3H, d, $J$ = 6.6 Hz, H-21), 0.90 (3H, s, H-19), 1.97 (1H, br s, 7α-NH), 3.62 (1H, br s, 7β-H), 3.77 (1H, br s, 3β-H), 5.20 (1H, br s, 3α-NH), 6.46 (4H, d, $J$ = 8.8 Hz), 7.91 (2H, d, $J$ = 9.1 Hz), 7.98 (2H, d, $J$ = 9.1 Hz); $^{13}$C NMR $\delta$ 10.8, 11.9, 18.7, 20.8, 22.6, 22.9, 23.4, 23.9, 25.0, 27.9, 28.1, 29.8, 32.3, 32.6, 34.2, 35.8, 36.1, 36.3, 37.8, 39.4, 39.5, 42.8, 47.0, 47.8, 49.9, 51.5, 56.3, 110.8, 111.3, 126.6, 126.8, 136.9, 137.1, 152.8, 153.3; HR-FAB MS calcd. for $C_{39}H_{56}N_4O_4$ $(M)^+$: 645.4380, Found: *m/z* 645.4383.

**3q**: Yield 21%; amorphous; TLC $R_f$ 0.68 (33% EtOAc in hexane); $^1$H NMR $\delta$ 0.68 (3H, s, H-18), 0.84 (3H, d, $J$ = 6.6 Hz, H-26), 0.85 (3H, d, $J$ = 6.8 Hz, H-27), 0.89 (3H, s, H-19), 0.90 (3H, d, $J$ = 6.6 Hz, H-21), 2.01 (1H, br s, 7α-NH), 3.34 (1H, m, 3α-H), 3.63 (1H, br s, 7β-H), 4.83 (1H, br s, 3α-NH), 6.48 (2H, d, $J$ = 8.1 Hz), 6.53 (2H, d, $J$ = 9.1 Hz), 8.02 (2H, d, $J$ = 9.1 Hz), 8.07 (2H, d, $J$ = 8.8 Hz); $^{13}$C NMR $\delta$ 11.8, 12.0, 18.7, 21.3, 22.6, 22.9, 23.4, 23.9, 27.9, 28.1, 28.8, 29.8, 32.6, 34.9, 35.8, 36.0, 36.1, 37.3, 37.8, 39.2, 39.4, 39.5, 42.8, 47.5, 49.8, 51.6, 56.3, 107.4, 111.0, 111.3, 126.6, 126.8, 137.7, 152.4, 152.7; HR-FAB MS calcd. for $C_{39}H_{56}N_4O_4$ $(M)^+$: 645.4380, Found: *m/z* 645.4377.

## 3. Results and discussion

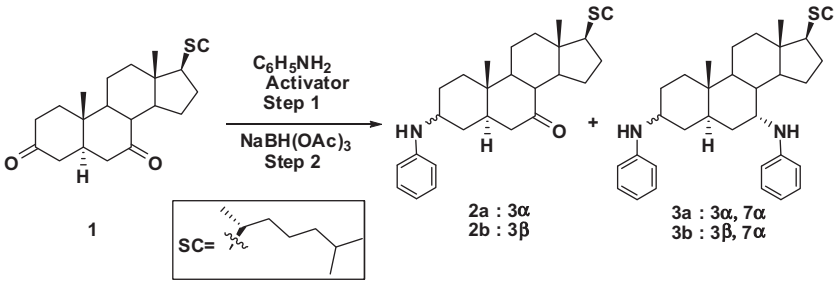
Recently, we reported a sequential stepwise procedure for the synthesis of highly stereoselective 3α,7α-dialkylamino-5α-cholestanes, starting from 5α-cholestane-3,7-dione (**1**), in good yields of up to 84% [15]. Unfortunately, this methodology was not applicable to the synthesis of 3,7-diarylamino-5α-cholestanes. Using an efficient $TiCl(O^iPr)_3$-mediated reductive amination [17,27,28], we have envisioned a one-step procedure for the preparation of new 3,7-diarylamino-5α-cholestane derivatives from 5α-cholestane-3,7-dione (**1**) according to Scheme 1. This study initially examined the reductive amination reaction using **1** and aniline, with the results presented in Table 1.

It was found that the yields of 3,7-diphenylamino-5α-cholestane (**3**) depended on the solvent and titanium source used. Dichloromethane faired best among the test solvents and desired product **3** was isolated in 90% yield using a $TiCl(O^iPr)_3$-mediated reaction at room temperature (Table 1, entry 4). When the same reaction was carried out in MeOH, 3-phenlyamino-5α-cholestan-7-one (**2**) was isolated with 80% yield (Table 1, entry 5). To evaluate the influence of the titanium source as a Lewis acid, the reaction was performed using acetic acid in $CH_2Cl_2$. A mixture of **2** (29–36%) and **3** (47–53%) was isolated from the direct and indirect reductive amination, respectively (Table 1, entries 1–2). Furthermore, $TiCl(O^iPr)_3$ and $Ti(O^iPr)_4$ were investigated as the titanium source in $CH_2Cl_2$ at room temperature (Table 1, entries 4 and 6), with the best results obtained using $TiCl(O^iPr)_3$. Interestingly, the $Ti(O^iPr)_4$-mediated reaction of **1** with aniline, using $NaBH_4$ in MeOH at −78 °C, afforded 3α-amino (**2a**) and 3β-amino (**2b**) in a 1:9 ratio and 70% overall yield (Table 1, entry 8) [8–10,16]. In an attempt to minimize the formation of undesired product **2**, the $TiCl(O^iPr)_3$-mediated reductive amination of **1** was carried out using aniline in $CH_2Cl_2$ and $NaBH(OAc)_3$ as the reducing agent at room temperature to afford 3α,7α (**3a**) and 3β,7α (**3b**) in a 4:1 ratio (Table 1, entry 4).

It was hypothesized that replacing one of the isopropoxide ligands from $Ti(O^iPr)_4$ with a chlorine atom would increase the Lewis acidity of the reagent and facilitate the condensation of the amine with the ketone [17,27,28]. A possible mechanism for the nucleophilic attack of the amino group on the Lewis acid activated carbonyl compound is shown in Scheme 2 [29,30]. In this case, the reaction may proceed through titanium complex **I**, which could be reduced directly or through transient imine species **II**.

The stereoselective introduction of an amino group at C-3, with configuration 3α and 3β, depended on the reducing agent selection. Smaller reagents like $NaBH_4$ and $NaBH_3CN$ yielded the 3β isomer as the major product, as it attacks from the axial side at C-3. Bulky reagents such as sodium triacetoxyborohydride [$NaBH(OAc)_3$] and sodium tris[2-(ethylhexanoic)]borohydride [$NaBH(OEh)_3$] [7,15,31] attack from the equatorial side at C-3 leading to the formation of the 3α isomer predominantly [32].

The stereochemistry of **3** was determined based on the $R_f$ value and the chemical shifts of the protons and carbons at the 3- and 7-positions in the $^1$H and $^{13}$C NMR, respectively. The $^1$H NMR spectrum of the 3α-amino isomer (**3c**), peaks representing the 3β-H and 7β-H protons appeared at 3.62 ppm (br s) and 3.51 ppm (br s), respectively. In the $^{13}$C NMR spectrum of **3c**, C-3 and C-7 carbons appeared at 47.5 ppm and 50.3 ppm, respectively. In comparison, peaks for the 3α-H and 7β-H of the 3β-isomer (**3d**) appeared at $\delta$ 3.15 (br m) and 3.44 (br s), respectively. For the $^{13}$C NMR spectrum of **3d**, the C-3 and C-7 carbon appeared at $\delta$ 47.6 and 50.0, respectively. The 2D-HETCO spectrum of **3c** revealed that 3β-1H




**Scheme 1.** Initial optimization for the synthesis of 3,7-diarylamino-5α-cholestane derivatives.

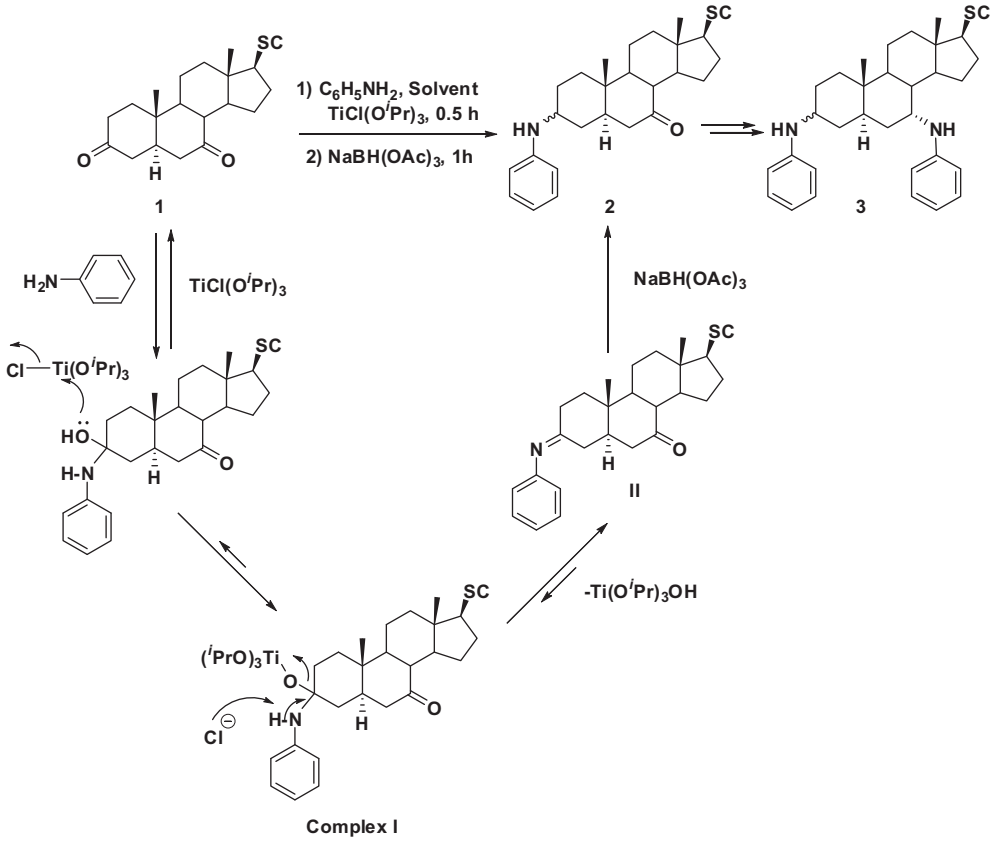
**Table 1**
Reductive amination of **1** with aniline using $NaBH(OAc)_3$ at room temperature.

| Entry | Activator[b] | Solvent | Time (h) Step 1 | Time (h) Step 2 | Yield[c] (%) **2a**:**2b** | Yield[c] (%) **3a**:**3b** |
|---|---|---|---|---|---|---|
| 1 | A | $CH_2Cl_2$ | 0 | 24 | 36 (2:1) | 47 (2:1) |
| 2 | A | $CH_2Cl_2$ | 14 | 24 | 29 (2:1) | 53 (2:1) |
| 3 | B | THF | 0.5 | 24 | – | 83 (4:1) |
| 4 | B | $CH_2Cl_2$ | 0.5 | 1 | – | 90 (4:1) |
| 5 | B | MeOH | 0.5 | 2 | 80 (4:1) | – |
| 6 | C | $CH_2Cl_2$ | 12 | 5 | 77 (4:1) | – |
| 7 | C | MeOH | 12 | 2 | 78 (4:1) | – |
| 8[a] | C | MeOH | 12 | 2 | 70 (1:9) | – |

[a] $NaBH_4$ at −78 °C.
[b] A: AcOH (5 drops), B: $TiCl(O^iPr)_3$, C: $Ti(O^iPr)_4$.
[c] Isolated yields.

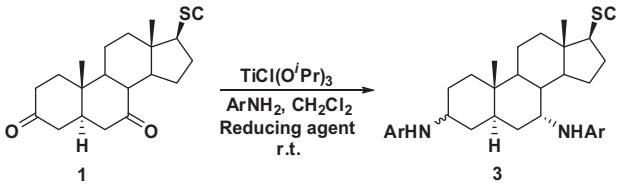
and 7β-1H were connected to C-3 and C-7, respectively. Further relative stereochemistry assignment was established by data obtained from the COSY and DEPT spectra (Supplementary information). The chemical shifts observed were in accordance with those found previously [10,15,32].

The general applicability of this procedure for the synthesis of a diverse range of aromatic amines was then studied and the results are presented in Table 2. The generality of this method was evaluated using 2-, 3- and 4-substituted aniline derivatives. Based on these results, it was observed that the presence of substituents on aniline improved the stereoselectivity without affecting the reactivity. The reaction of **1** with 4-methylaniline using $NaBH(OAc)_3$ gave the corresponding 3,7-di[(4-methylphenyl)amino]-5α-cholestanes as a mixture of **3c**:**3d** (6:1) in 96% yield (Table 2, entry 3). High diastereoselectivity was achieved by modifying $NaBH_4$ with 2-ethylhexanoic acid (Eh). $NaBH(OEh)_3$ afforded products **3c**:**3d** in a 36:1 ratio and 97% yield (Table 2, entry 4). The highest stereoselectivity and yield were achieved using 4-anisidine. Even electron-deficient anilines containing bromo, ester, and nitro groups (Table 2, entries 10–17) reacted with **1** to yield the corresponding secondary aryl amines. The




**Scheme 2.** Proposed mechanism.

**Table 2**
Stereoselective synthesis of 3,7-diarylaminocholestanes derivatives

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| Entry | Amine Ar | Reagent[a] | Time (h) | Yield[b] (%) | Ratio 3α,7α:3β,7α | Product |
|---|---|---|---|---|---|---|
| 1 | $C_6H_5$ | D | 1 | 90 | 4:1 | **3a–3b** |
| 2 | $C_6H_5$ | E | 1 | 95 | 10:1 | **3a–3b** |
| 3 | 4-$CH_3C_6H_4$ | D | 1 | 96 | 6:1 | **3c–3d** |
| 4 | 4-$CH_3C_6H_4$ | E | 1 | 97 | 36:1 | **3c–3**d |
| 5 | 4-$CH_3OC_6H_4$ | D | 1 | 98 | 8:1 | **3e–3f** |
| 6 | 4-$CH_3OC_6H_4$ | E | 1 | 98 | 100:0 | **3e** |
| 7 | 2-$NH_2C_6H_4$ | D | 24 | 80 | 100:0 | **3g** |
| 8 | 2-$NH_2C_6H_4$ | E | 20 | 83 | 100:0 | **3g** |
| 9 | 4-$NC_5H_4$ | E | 24 | 80 | 100:0 | **3h** |
| 10 | 4-$BrC_6H_4$ | D | 3 | 90 | 3:1 | **3i–3j** |
| 11 | 4-$BrC_6H_4$ | E | 3 | 92 | 14:1 | **3i–3j** |
| 12 | 3-$BrC_6H_4$ | D | 24 | 84 | 3:1 | **3k–3l** |
| 13 | 3-$BrC_6H_4$ | E | 24 | 89 | 9:1 | **3k–3l** |
| 14 | 2-$BrC_6H_4$ | E | 30 | 65[c] | 100:0 | **3m** |
| 15 | 4-$CH_3O_2CC_6H_4$ | D | 24 | 83 | 3:1 | **3n–3o** |
| 16 | 4-$CH_3O_2CC_6H_4$ | E | 20 | 85 | 6:1 | **3n–3o** |
| 17 | 4-$O_2NC_6H_4$ | D | 72 | 85 | 3:1 | **3p–3q** |

[a] D: $NaBH(OAc)_3$, E: $NaBH(OEh)_3$.
[b] Isolated yields.
[c] 3α-(2-Bromophenyl)amino-5α-cholestan-7-one (**2c**) was isolated in 11%.

reaction of **1** with 2-, 3-, 4-bromoaniline proceeded slowly and afforded the desired product in good to high yields. A marked stereoselective effect was observed with respect to the position of the bromo group of the substrate (Table 2, entries 11, 13 and 14). Unhindered 4-bromoaniline afforded the corresponding product in 92% yield (Table 2, entry 11). Reaction of more hindered 2-bromoaniline occurred at room temperature, affording the desired product in moderate yield (65%). Steric hindrance appeared to play a role in the outcome of the reaction as exemplified by the conversion of electron-rich 2-aminoaniline (Table 2, entry 7) which produced only a single isomer in good yield even with $NaBH(OAc)_3$. In comparison, the reaction of **1** with 2-bromoaniline (Table 2, entry 14) produced only 3α,7α-diaryl compound (**3m**) in a moderate yield of 65%, with 11% of the 3α-mono aminated product **2c** also being isolated. Likewise, the reaction also proceeded in good yields with deactivated heterocyclic amine such as 4-aminopyridine (Table 2, entry 9). The reaction was very slow with 4-nitroaniline, and required 72 h for completion, giving a good yield of 85% (Table 2, entry 17).

Four 3,7-diarylaminocholestane derivatives **3a**, **3b**, **3h** and **3i** were examined for their antimicrobial activities against nine of Gram-positive strains, such as *Staphylococcus aureus* ATCC6538P, *S. aureus* ATCC25923, *S. aureus* giorgio, *S. aureus* 77, *S. aureus* 241, *Staphylococcus epidermidis* 887E, *Micrococcus luteus* ATCC9341, *Bacillus subtilis* ATCC6633, *Bacillus licheniformis* EMR and three of Gram-negative strains, such as *Escherichia coli* ATCC25922, *Salmonella typhimurium* ATCC13311, *Klebsiella pneumoniae* 2011E as described previously [25,26]. Among the tested 3,7-diarylamino derivatives, the best antimicrobial result was observed for 3α,7α-dipyriylamino compound (**3h**) with the lowest minimum inhibitory concentrations (MIC) value of 8 μg/mL toward *S. aureus* ATCC6538P and *Micrococcus luteus* ATCC9341. Furthermore, the compound **3h** exhibited a moderate activity with the MIC value of 16 μg/mL toward *S. aureus* ATCC25923, *S. aureus* giorgio, *S. aureus* 77, *S. aureus* 241, *Staphylococcus epidermidis* 887E, *Bacillus subtilis* ATCC6633 and *Bacillus licheniformis* EMR. None of Gram-positive and Gram-negative bacteria were resistant to compounds **3a**, **3b** and **3i** having MIC value >32 μg/mL.

## 4. Conclusion

An efficient method for the one-pot synthesis of a new class of steroids has been developed. 3α,7α-Diarylamino-5α-cholestane derivatives were synthesized in high yields. This novel method could be useful for a wide range of synthetic applications as it is mild, efficient, and environmentally friendly. 3α,7α-Dipyridylamino compound (**3h**) showed moderate antimicrobial activity toward the Gram-positive bacterias, *S. aureus* ATCC6538P and *Micrococcus luteus* ATCC9341.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.steroids.2014.06.018.

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