Synthesis of Facial Amphiphile 3,7-Diamino-5α-cholestane Derivatives as a Molecular Receptor

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A series of facial amphiphiles 3,7-diaminocholestane were synthesized from 3,7-diketocholestane *via* 2 sequential reductive aminations and anion recognition was evaluated with acetate, chloride, bromide, fluoride and phosphate anions. The stereo-selective reductive amination protocol was utilized to synthesized facial amphiphiles afforded receptors in high yields. The molecular receptor **2** showed the highest binding constant with acetate in a 1:1 ratio.

Key Words: Facial amphiphile, Anion receptor, Reductive amination, Aminosteroids, Stereoselectivity

Introduction

Design of preorganized molecular receptor provides the advantage of rigid scaffolds that could be functionalized further with ligands for anion recognition. Steroid molecules are notorious for their preorganized structures provide a platform to constitute a molecular receptor and offer ease of functionalization. The ligands for the H-bonding e.g. amine group in the steroid scaffold of cholic acid were introduced through ether, ester or amide linkages.[1] The hydroxyl group could be transformed to ether linkages or ester linkages conveniently but were found to be unstable in higher pH values.[2] Hence introduction of the amine group directly to steroids were cynosure in steroid based molecular receptor syntheses.[3]

Cholic acid scaffolds having a *cis* AB ring or 5β configuration supports functionalization at C3, C7 and C12 while cholesterol-based receptors having an AB *trans* ring or 5α configuration supports modification at C3 and C7. The advantage of cholesterol-based receptors is that they offer exactly the same bond length attachment of ligands at C3 and C7 in an axial manner, while in the same position with 5β-configuration it is not possible. The introduction of an axial amino group at C3 and C7 could be derivatized rapidly in a quantitative yield. The preferred method by Davis *et al.* to introduce the NH_2 group was through inversion at stereogenic centers and azide formation or by oximation and metal-assisted reduction in a multi-step synthesis afforded overall low yield.[4] The amino group becomes highly hydrophilic, which gives an edge in intramolecular hydrogen bonding and solubility in non-polar solvents. Figure 1 showed cholesterol based facial amphiphilic anionic receptors, which was derivatized with urea at C3 and C7 in an axial fashion. A highly stereoselective synthesis of 3α,7α-diaminocholestane (**1**) from 3β-acetoxy-5α-cholest-7-one (**3**) by reductive amination methodology has been investigated, and was further elaborated on to synthesize anionic receptors.[5]

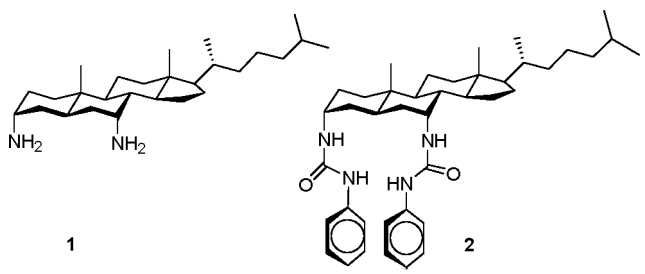


Figure 1. Perspective drawing of cholesterol-based molecular receptor.

To get the facial amphiphile **2**, one-step direct reductive amination of diketone **4** with NH_4OAc in the presence of $NaBH_3CN$ was carried out which resulted in the formation of **6a** (34%) along with a mixture of 3α/3β-isomers of 3,7-dihydroxycholestane. To improve the yield and stereo-selectivity of **6a**, the sequential procedure was investigated.

Experimental Section

Melting points were determined using a Thomas-Hoover capillary melting point apparatus and are uncorrected. The NMR spectra were recorded on a Bruker AM-400 spectrometer in $CDCl_3$ using Me_4Si as the internal standard. Elemental analyses were performed on a Calro Erba 1106 at the Center for Scientific Instruments, Kyungpook National University. HR-FAB Mass spectra were taken at KBSI Daegu branch. TLC analyses were carried out on a plate precoated with 0.2 mm of HPTLC silica gel 60; substances were visualized by spraying with 5% ammonium molybdate in 10% H_2SO_4 followed by heating. Flash column chromatography was performed with Merck silica gel 60 (70 - 230 mesh). Reactions were carried out under an argon atmosphere, and the solutions were washed with brine and dried over anhydrous sodium sulfate. Compound **4** was obtained by literature method.[5]

***3α-(tert*-Butyloxycarbonyl)amino-5α-cholestane (5a).** $NaBH(OEh)_3$[8] (4 mL, 2 eq) was added to a solution of **4** (200 mg, 0.50 mmol) and NH_4OTf (250 mg, 1.50 mmol) in a dry THF (10 mL) and stirred at room temperature for 1 h. After the solvent was removed, the residue was extracted with ethyl acetate. The organic layer was washed, dried, and concentrated. Without further purification, the residue was treated with $(Boc)_2O$ (164 mg, 0.75 mmol) in methanol (20 mL) for 3 h. After the solvent was removed, the residue was extracted with ethyl acetate. The organic layer was washed, dried, and con-

centrated. The residue was purified by a column chromatography (elution with 5% EtOAc-hexane) to give 190 mg of **5a** (76%) and 23 mg of 3β-isomer (9%). **5a:** TLC R_f 0.52 (EtOAc-hexane 1:4); mp 169 - 172 °C (CH_2Cl_2-hexane); ^{1}H NMR ($CDCl_3$) δ 0.59 (s, 3H, 18-CH_3), 0.80 (d, J = 6.5 Hz, 3H, 26-CH_3), 0.84 (d, J = 6.5 Hz, 3H, 27-CH_3), 0.86 (d, J = 6.5 Hz, 3H, 21-CH_3), 1.00 (s, 3H, 19-CH_3), 1.40 (s, 9H, -COC(CH_3)$_3$), 3.83 (s, 1H, 3β-H), 4.88 (s, 1H, 3α-NH); ^{13}C NMR ($CDCl_3$) δ 11.0, 12.2, 14.2, 18.9, 21.3, 22.6, 22.9, 23.8, 25.0, 26.2, 28.1, 28.5, 29.2, 30.2, 32.9, 33.5, 35.7, 36.2, 36.5, 38.8, 39.6, 42.6, 43.1, 46.0, 49.1, 50.3, 55.1, 55.9, 65.3, 79.2, 155.3, 212.1; FAB-MS Calcd for $C_{32}H_{55}NO_3Na$: 524.4080, Found: *m/z* 524.4080 $[M+Na]^+$.

3α-(*n*-Butyl)amino-5α-cholestan-7-one (5b). yield: 77%, TLC R_f 0.75 (CH_2Cl_2-MeOH-NH_4OH 20:1:0.2); ^{1}H NMR ($CDCl_3$) δ 0.63 (s, 3H, 18-CH_3), 0.78 (s, 3H, 19- CH_3), 0.84 (d, 3H, J = 6.6 Hz, 26-CH_3), 0.85 (d, 3H, J = 6.6 Hz, 27-CH_3), 0.87 (d, 3H, J = 6.3 Hz, 21-CH_3), 1.74-1.94 (m, 3H), 2.34 (dt, J = 11.2, 7.1 Hz, 1H), 2.53 (t, J = 7.3 Hz, 2H), 2.69 (m, 1H), 2.82 (bm, 1H, 3β-H), ^{13}C NMR ($CDCl_3$) δ 11.2, 12.2, 14.1, 18.9, 20.6, 21.3, 22.7, 22.9, 23.9, 25.1, 25.7, 28.1, 28.6, 32.3, 32.8, 35.8, 36.2, 36.7, 38.8, 39.6, 41.8, 42.6, 46.2, 47.0, 49.0, 50.3, 52.4, 55.1, 55.5, 212.3; FAB-MS Calcd for $C_{31}H_{56}NO$: 458.4362, Found: *m/z* 458.4342 $(M+H)^+$.

3α-*N*-[(3-*tert*-butyloxycarbonyl)propylamino]-5α-cholestan-7-one (5c). yield: 72%, TLC R_f 0.61 (CH_2Cl_2-MeOH-NH_4OH 20:1.5:0.5); ^{1}H NMR ($CDCl_3$) δ 0.60 (s, 3H, 18-CH_3), 0.81 (d, 3H, J = 6.6 Hz, 26-CH_3), 0.82 (d, 3H, J = 6.6 Hz, 27-CH_3), 0.86 (d, 3H, J = 6.3 Hz, 21-CH_3), 1.00 (s, 3H, 19-CH_3), 1.39 (s, 9H, -COC(CH_3)$_3$), 2.12 (bs, 1H, 3α-NH), 2.12 (t, J = 13.4 Hz, 1H), 2.39 (t, J = 11.4 Hz, 1H), 2.59 (bt, 2H, HNCH_2), 2.84 (bs, 1H, 3β-H), 3.16 (bm, 1H, CH_2NH-Boc), 5.41 (bs, 1H, N*H*-Boc); ^{13}C NMR ($CDCl_3$) δ 11.4, 12.4, 19.1, 21.5, 22.9, 23.1, 24.1, 25.3, 28.3, 28.5, 28.7, 28.8, 29.3, 32.3, 33.1, 36.0, 36.5, 36.9, 39.0, 39.5, 39.9, 42.0, 42.9, 45.7, 46.3, 49.2, 50.5, 53.2, 55.4, 55.5, 79.3, 156.7, 212.4; FAB-MS Calcd for $C_{35}H_{63}N_2O_3$: 559.4839, Found: *m/z* 559.4842 $(M+H)^+$.

3α-*N*-[(4-*tert*-butyloxycarbonyl)butylamino]-5α-cholestan-7-one (5d). yield 76%, TLC R_f 0.62 (CH_2Cl_2-MeOH-NH_4OH 15:2:0.5); ^{1}H NMR ($CDCl_3$) δ 0.59 (s, 3H, 18-CH_3), 0.80 (d, 3H, J = 7.0 Hz, 26-CH_3), 0.81 (d, 3H, J = 7.0 Hz, 27-CH_3), 0.85 (d, 3H, J = 7.5 Hz, 21-CH_3), 1.00 (s, 3H, 19-CH_3), 1.38 (s, 9H, -COC(CH_3)$_3$), 2.57 (m, 2H, HN(Boc)CH_2), 2.93 (bs, 1H, 3β-H), 3.06 (bs, 1H, NH-Boc), 4.93 (bs, 1H, 3α-NH); ^{13}C NMR ($CDCl_3$) δ 11.4, 12.4, 12.7, 14.5, 19.1, 21.5, 22.9, 23.1, 23.2, 24.1, 25.3, 26.2, 26.5, 28.3, 28.8, 30.4, 32.3, 32.8, 36.0, 36.4, 36.8, 39.0, 39.8, 40.5, 41.9, 42.8, 46.3, 46.7, 49.2, 50.5, 52.7, 55.3, 55.6, 79.2, 156.4, 212.3; FAB-MS Calcd for $C_{36}H_{65}N_2O_3$: 573.4995, Found: *m/z* 573.4998 $(M+H)^+$.

3α-*N*-[(4,9-Di-*tert*-butyloxycarbonyl)spermidinyl]-5α-cholestan-7-one (5e). yield 78%, TLC R_f 0.69 (CH_2Cl_2- MeOH-NH_4OH 15:2:0.5); ^{1}H NMR ($CDCl_3$) δ 0.59 (s, 3H, 18-CH_3), 0.80 (d, 3H, J = 6.5 Hz, 26-CH_3), 0.81 (d, 3H, J = 6.5 Hz, 27-CH_3), 0.85 (d, 3H, J = 6.5 Hz, 21-CH_3), 0.99 (s, 3H, 19-CH_3) 1.38 (s, 18H, -COC(CH_3)$_3$), 2.51 (bm, 2H, CH_2NH), 2.83 (s, 1H, 3α-H), 2.90 (bs, 1H, 3β-H), 3.00-3.15 (bm, 6H, CH_2), 4.62 (bs, 1H, N*H*Boc); ^{13}C NMR ($CDCl_3$) δ 11.2, 12.2, 14.3, 18.9, 21.3, 22.7, 22.9, 23.9, 25.1, 25.4, 25.7, 25.9, 27.5, 28.1, 28.5, 29.8, 32.2, 32.4, 32.6, 35.8, 36.2, 36.7, 38.8, 39.6, 40.3, 41.7, 42.0, 42.6, 44.5, 45.4, 46.1, 46.7, 47.0, 49.1, 50.3, 53.0, 55.1, 55.4, 55.8, 79.4, 79.7, 155.7, 156.1, 212.1; FAB Mass Calcd for $C_{44}H_{80}N_3O_5$: 730.6098, Found: *m/z* 730.6086 $(M+H)^+$.

3α-[3-(1-Imidazolyl)propyl]amino-5α-cholestan-7-one (5f). yield: 81%, TLC R_f 0.64 (CH_2Cl_2-MeOH-NH_4OH 15:2:0.5); ^{1}H NMR ($CDCl_3$) δ 0.60 (s, 3H, 18-CH_3), 0.81 (d, J = 6.6 Hz, 3H, 26-CH_3), 0.82 (d, J = 6.6 Hz, 3H, 27-CH_3), 0.86 (d, J = 6.6 Hz, 3H, 21-CH_3), 1.00 (s, 3H, 19-CH_3), 2.26 (m, 2H, HNCH_2 CH_2CH_2Im), 2.58 (bs, 2H, HNCH_2CH_2CH_2Imi), 2.94 (bs, 1H, 3β-H), 4.03 (bm, 2H, CH_2-Im), 5.69 (bs,1H, NH), 6.91 (s, 1H, Im H-5), 6.99 (s, 1H, Im H-4), 7.52 (s, 1H, Im H-2); ^{13}C NMR ($CDCl_3$) δ 10.1, 11.0, 17.8, 20.2, 21.5, 21.8, 22.8, 23.9, 26.9, 27.4, 28.7, 28.7, 30.9, 34.7, 35.1, 35.5, 37.6, 38.4, 40.7, 41.5, 42.7, 43.6, 44.9, 45.9, 47.9, 48.9, 49.2, 54.1, 54.4, 60.7, 71.3, 117.9, 128.2, 136.2, 211.4; FAB-MS Calcd for $C_{33}H_{56}N_3O$: 510.4424, Found: *m/z* 510.4421 $(M+H)^+$.

3α-[2-(2-Pyridyl)ethyl]amino-5α-cholestan-7-one (5g). yield: 84%, TLC R_f 0.47 (CH_2Cl_2-MeOH-NH_4OH 20:1.5:0.5); ^{1}H NMR ($CDCl_3$) δ 0.62 (s, 3H, 18-CH_3), 0.82 (d, J = 6.6 Hz, 3H, 26-CH_3), 0.83 (d, J = 6.6 Hz, 3H, 27-CH_3), 0.88 (d, J = 6.3 Hz, 3H, 21-CH_3), 1.04 (s, 3H, 19-CH_3), 3.15 (bs, 1H, 3β-H), 3.57 (m, 2H, NHCH_2CH_2Py), 3.71 (m, 2H, NHCH_2CH_2Py) 4.39 (bs, 1H, NH), 7.16 (d, 1H, J = 7.6 Hz, 1H), 7.17 (d, 1H, J = 7.8 Hz, 1H), 7.61 (ddd, 1H, J = 7.8, 7.6, 1.5 Hz, 1H), 8.37 (d, 1H, J = 4.8, Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 11.2, 12.1, 18.8, 21.2, 22.6, 22.8, 23.8, 24.8, 25.0, 28.0, 28.4, 35.7, 36.2, 36.5, 38.7, 39.5, 42.2, 42.5, 45.8, 46.0, 49.0, 50.3, 53.1, 55.0, 55.9, 61.6, 72.4, 122.1, 123.7, 137.1, 148.7, 159.4, 211.9; FAB-MS Calcd for $C_{34}H_{55}N_2O$: 507.4314, Found: *m/z* 507.4317 $(M+H)^+$.

3α-(2-Pyridylmethyl)amino-5α-cholestan-7-one (5h). yield: 78%, TLC R_f 0.60 (CH_2Cl_2-MeOH-NH_4OH 20:1.5:0.5); ^{1}H NMR ($CDCl_3$) δ 0.61 (s, 3H, 18-CH_3), 0.82 (d, J = 6.6 Hz, 3H, 26-CH_3), 0.83 (d, J = 6.6 Hz, 3H, 27-CH_3), 0.87 (d, J = 6.3 Hz, 3H, 21-CH_3), 1.02 (s, 3H, 19-CH_3), 3.00 (bs, 1H, 3β-H), 3.89 (d, 2H, J = 10.6 Hz, NHCH_2Py), 3.94 (bs, 1H, NH), 7.14 (dd, 1H, J = 7.8, 7.8 Hz, 1H), 7.31 (d, 1H, J = 7.8, Hz, 1H), 7.61 (ddd, 1H, J = 7.8, 7.5, 1.8 Hz, 1H), 8.50 (d, 1H, J = 4.5 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 11.1, 12.1, 18.8, 21.2, 22.6, 22.9, 23.8, 25.1, 25.7, 28.0, 28.5, 32.3, 32.9, 35.7, 36.2, 36.7, 38.8, 39.5, 41.8, 42.6, 46.1, 49.0, 50.3, 52.4, 52.5, 55.0, 55.6, 122.2, 122.7, 136.7, 149.1, 158.8, 212.2; FAB-MS Calcd for $C_{33}H_{55}N_2O$: 493.4158, Found: *m/z* 493.4158 $(M+H)^+$.

3α-[Di-(2-pyridylmethyl)amino]-5α-cholestan-7-one (5i). yield: 56%, TLC R_f 0.50 (CH_2Cl_2-MeOH-NH_4OH 20:1.5:0.5); H NMR ($CDCl_3$) δ 0.55 (s, 3H, 18-CH_3), 0.77 (d, J = 6.6 Hz, 3H, 26-CH_3), 0.78 (d, J = 6.6 Hz, 3H, 27-CH_3), 0.82 (d, J = 6.6 Hz, 3H, 21-CH_3), 0.95 (s, 3H, 19-CH_3), 2.99, (bs, 1H, 3β-H), 3.82 (d, J = 15.2 Hz, 2H, CH_2), 3.98 (d, J = 15.2 Hz, 2H, CH_2), 7.05 (dd, J = 7.3, 7.3 Hz, 2H), 7.29 (d, J = 7.8 Hz, 2H), 7.54 (ddd, J = 7.8, 7.6, 1.0 Hz, 2H), 8.40 (dd, J = 5.0, 1.0 Hz, 2H). ^{13}C NMR ($CDCl_3$) δ 11.8, 12.1, 18.8, 21.2, 22.6, 22.8, 23.8, 24.8, 25.0, 28.0, 28.4, 31.8, 33.2, 35.7, 36.1, 36.5, 38.8, 39.5, 42.4, 42.5, 45.8, 48.9, 50.2, 55.1, 56.2, 57.9, 58.0, 61.6, 72.4, 122.0, 123.4, 136.6, 148.6, 159.5, 212.3.

3α,7α-Bis(*tert*-butyloxycarbonylamino)-5α-cholestane (6a). $NaBH_3CN$ (79 mg, 1.20 mmol) was added to a mixture of **5a** (200 mg, 0.40 mmol), NH_4OAc (925 mg, 12.00 mmol), and

bromocresol green in THF-MeOH (v/v; 1:1, 20 mL) and stirred at room temperature for 2 h. At the same time, pH was adjusted to 6 by acetic acid. After the solvent was removed, the residue was neutralized with aqueous NaOH solution, and extracted with CH_2Cl_2. The organic layer was washed, dried and concentrated. The residue was treated with $(Boc)_2O$ (131 mg, 0.60 mmol) in methanol (10 mL). After 1 h, a few drops of 1N NaOH solution were added to the reaction mixture and stirred for 5 h. The solvent was removed and residue was extracted with CH_2Cl_2. The organic layer was washed, dried, and concentrated. The residue was purified by column chromatography (10% EtOAc-hexane) to give 175 mg of **6a** (71%). TLC R_f 0.33 (EtOAc-hexane 1:9, developed four times); mp 97 - 99 °C (CH_2Cl_2-hexane).[8]

3α,7α-Bis[(*n*-butyl)amino]-5α-cholestane (6b). yield: 80%. TLC R_f 0.56 (CH_2Cl_2-MeOH-NH_4OH 20:1.5:0.5); ^{1}H NMR ($CDCl_3$) δ 0.58 (s, 3H, 18-CH_3), 0.74 (s, 3H, 19-CH_3), 0.80 (d, 3H, J = 7.0 Hz, 26-CH_3), 0.81 (d, 3H, J = 7.0 Hz, 27-CH_3), 0.84 (d, 3H, J = 7.5 Hz, 21-CH_3), 0.85 (t, J = 7.5 Hz, CH_3), 1.70 - 1.88 (m, 2H, CH_2), 2.48 (bt, J = 7.5 Hz, 2H, CH_2), 2.54 (bm, 1H, 7β-H), 2.76 (bs, 1H, 3β-H); ^{13}C NMR ($CDCl_3$) δ 11.0, 11.9, 14.2, 14.2, 18.7, 20.8, 22.7, 23.0, 23.7, 23.9, 26.0, 28.1, 31.7, 31.8, 32.6, 32.7, 32.7, 32.9, 35.9, 36.3, 36.7, 39.2, 39.6, 39.6, 42.7, 46.4, 47.1, 47.6, 50.8, 52.3, 54.6, 56.2; Anal. Calcd for $C_{35}H_{68}Cl_2N_2$: C, 71.51, H, 11.66, N 4.77. Found: C, 71.20, H, 12.47, N, 4.86; FAB-MS Calcd for $C_{35}H_{67}N_2$: 515.5304. Found: *m/z* 515.5300 $(M+H)^+$.

3α,7α-Bis[*N*-(3-*tert*-butyloxycarbonyl)propylamino]-5α-cholestane (6c). yield: 71%. TLC R_f 0.33 (CH_2Cl_2-MeOH-NH_4OH 20:1.5:0.5); ^{1}H NMR ($CDCl_3$) δ 0.67 (s, 3H, 18-CH_3), 0.84 (d, 6H, J = 6.6 Hz, 26,27-CH_3), 0.86 (s, 3H, 19-CH_3), 0.89 (d, 3H, J = 6.6 Hz, 21-CH_3), 1.41 (s, 9H, -$COC(CH_3)_3$), 1.43 (s, 9H, -$COC(CH_3)_3$), 1.99 (bm, 2H, $CH_2CH_2CH_2$), 2.02 (bm, 2H, $CH_2CH_2CH_2$), 2.16 (bm, 2H, N*H*), 2.96 (bm, 2H, 3β, 7β-H), 3.59 (t, J = 4.5 Hz, 4H, $NHCH_2$), 3.74 (t, J = 4.5 Hz, 4H, CH_2NHBoc), 4.86 (bs, 1H, N*H*-Boc), 5.41(bs, 1H, N*H*-Boc); ^{13}C NMR ($CDCl_3$) δ 11.0, 11.9, 18.7, 20.8, 22.6, 22.9, 23.7, 24.1, 26.2, 28.1, 28.2, 28.6, 28.7, 29.4, 29.7, 29.9, 31.4, 31.7, 32.3, 32.7, 35.9, 36.2, 36.7, 39.2, 39.8, 40.6, 42.7, 45.6, 46.3, 46.6, 50.8, 52.7, 54.9, 56.3, 78.8, 78.9, 156.2, 156.3; FAB-MS Calcd for $C_{43}H_{81}N_4O_4$: 715.6083, Found: *m/z* 715.6092 $(M+H)^+$.

3α,7α-Bis[*N*-(4-*tert*-butyloxycarbonyl)butylamino]-5α-cholestane (6d). yield: 80%. TLC R_f 0.50 (CH_2Cl_2-MeOH-NH_4OH 15:2:0.5); ^{1}H NMR ($CDCl_3$) δ 0.57 (s, 3H, 18-CH_3), 0.74 (s, 3H, 19-CH_3), 0.78 (d, 3H, J = 6.5 Hz, 26-CH_3), 0.79 (d, 3H, J = 6.5 Hz, 27-CH_3), 0.82 (d, 3H, J = 7.0 Hz, 21-CH_3), 1.36 (s, 18H, $COC(CH_3)_3$), 2.02 (m, 4H), 2.55 (bs, 1H), 2.73 (bm, 4H), 3.06 (bs, 4H), 3.11 (m, 2H, 3β-H, 7β-H), 4.80 (bs, 1H), 5.24 (bs, 2H, N*H*-Boc); ^{13}C NMR ($CDCl_3$) δ 11.1, 11.9, 12.6, 14.3, 14.4, 18.8, 20.8, 22.8, 23.1, 23.1, 24.2, 26.3, 26.3, 27.6, 28.2, 28.3, 28.7, 30.3, 31.6, 31.9, 32.8, 32.9, 36.0, 36.3, 36.4, 38.9, 39.3, 39.7, 40.0, 40.2, 43.0, 45.7, 45.9, 49.9, 50.2, 53.5, 55.7, 56.2, 79.0, 156.4, 182.4; Anal. Calcd for $C_{35}H_{72}Cl_4N_4{\cdot}5H_2O$: C, 53.58, H, 10.58, N, 7.18; Found: C, 54.23, H, 10.74, N, 7.74; FAB-MS Calcd for $C_{45}H_{85}N_4O_4$: 745.6571, Found: *m/z* 745.6577 $(M+H)^+$.

3α,7α-Bis[*N*-(4,9-Di-*tert*-butyloxycarbonyl)spermidinyl]-5α-cholestane (6e). yield: 81%. TLC R_f 0.4 (CH_2Cl_2-MeOH-NH_4OH 15:2:0.5); ^{1}H NMR ($CDCl_3$) δ 0.58 (s, 3H, 18-CH_3), 0.72 (s, 3H, 19-CH_3), 0.79 (d, J = 6.5 Hz, 3H, 26-CH_3), 0.80 (d, J = 6.5 Hz, 3H, 27-CH_3), 0.83 (d, J = 6.0 Hz, 3H, 21-CH_3), 1.34 (s, 18H, -$COC(CH_3)_3$), 1.38 (s, 18H, -$COC(CH_3)_3$), 3.20 (bs, 1H, 7β-H), 3.73 (bs, 1H, 3β-H), 4.63 (bs, 2H, 3α-NH, 7α-NH); ^{13}C NMR ($CDCl_3$) δ 10.6, 11.1, 11.9, 12.0, 18.8, 20.7, 22.7, 23.0, 23.7, 23.8, 23.9, 25.7, 26.0, 27.5, 28.2, 28.3, 28.4, 28.5, 28.6, 30.9, 31.9, 32.0, 32.1, 32.4, 35.9, 36.3, 36.4, 36.6, 39.6, 39.7, 39.9, 40.4, 42.7, 42.8, 44.7, 45.4, 45.9, 46.3, 46.4, 46.9, 47.3, 49.3, 50.8, 55.7, 56.2, 68.1, 79.1, 79.3, 79.4, 155.7, 155.9, 156.2, 156.2; FAB-MS Calcd for $C_{61}H_{115}N_6O_8$: 1059.8838, Found: *m/z* 1059.8840 $(M+H)^+$.

3α,7α-Bis{3-(1-imidazolyl)propyl]amino}-5α-cholestane (6f). yield: 78%. TLC R_f 0.55 (CH_2Cl_2-MeOH-NH_4OH 15:2:0.5); ^{1}H NMR ($CDCl_3$) δ 0.57 (s, 3H, 18-CH_3), 0.72 (s, 3H, 19-CH_3), 0.79 (d, J = 6.6 Hz, 3H, 26-CH_3), 0.793 (d, J = 6.6 Hz, 3H, 27-CH_3), 0.83 (d, J = 6.6 Hz, 3H, 21-CH_3), 1.95 (m, 4H, $HNCH_2CH_2CH_2Imi$), 2.30 (dd, J = 11.6, 5.8 Hz, 7β-H), 2.51 (m, 4H, $HNCH_2CH_2CH_2Imi$), 2.61 (bs, 1H, 3β-H), 3.97 (t, 2H, J = 6.8 Hz, CH_2-Imi), 4.03 (dd, 2H, J = 13.6, 6.8 Hz, CH_2-Imi), 4.80 (bs, 1H, N-H)), 6.83 and 6.86 (s, 1H, Im H-5), 6.95 and 6.96 (s, 1H, Im H-4), 7.40 and 7.55 (s, 1H, Im H-2); ^{13}C NMR ($CDCl_3$) δ 10.5, 11.5, 18.3, 20.4, 22.2, 22.5, 23.3, 23.5, 24.7, 27.7, 27.8, 31.3, 31.6, 32.1, 32.3, 35.4, 35.8, 36.2, 38.6, 39.1, 42.3, 43.6, 44.1, 44.4, 44.6, 45.9, 50.5, 52.9, 54.6, 55.9, 118.6, 128.7, 129.0, 136.9, 137.4; FAB-MS Calcd for $C_{39}H_{67}N_6$: 619.5427, Found: *m/z* 619.5431 $(M+H)^+$.

3α,7α-Bis[2-(2-pyridyl)ethylamino]-5α-cholestane (6g). yield: 80%. TLC R_f 0.53 (CH_2Cl_2-MeOH-NH_4OH 15:2:0.5); ^{1}H NMR ($CDCl_3$) δ 0.55 (s, 3H, 18-CH_3), 0.73 (s, 3H, 19-CH_3), 0.81 (d, J = 6.8 Hz, 3H, 26-CH_3), 0.82 (d, J = 6.3 Hz, 3H, 27-CH_3), 0.83 (d, J = 7.1 Hz, 3H, 21-CH_3), 1.84 (m, 1H,), 1.96 (m, 1H,), 2.58 (bs, 1H,-NH), 3.05 (m, 4H, $HNCH_2CH_2Py$), 3.08 (m, 4H, $HNCH_2CH_2Py$), 3.69 (t, 1H, 3β-H), 3.54 (t, 1H, 7β-H), 7.04 (t, J = 7.4 Hz, 2H, PyH-2), 7.13 (t, J = 7.5 Hz, 2H, Py H-4), 7.53 (m, 2H, Py H-3), 8.42 and 8.50 (s, 2H, J = 4.8 Hz, Py H-5); ^{13}C NMR ($CDCl_3$) δ 11.5, 12.2, 19.1, 21.1, 22.9, 23.2, 23.7, 24.1, 28.4, 28.6, 31.6, 32.2, 32.8, 36.1, 36.5, 36.8, 39.4, 39.8, 39.9, 43.1, 46.4, 46.6, 48.1, 50.9, 53.2, 55.8, 56.4, 62.0, 72.9, 75.2, 121.7, 121.9, 123.8, 123.9, 136.8, 137.1, 149.3, 149.5, 160.2, 161.1; FAB-MS Calcd for $C_{41}H_{65}N_4$: 613.5209, Found: *m/z* 613.5212 $(M+H)^+$.

3α,7α-Bis[2-(2-pyridylmethyl)amino]-5α-cholestane (6h). yield: 84%. TLC R_f 0.70 (CH_2Cl_2-MeOH-NH_4OH 15:2:0.5); ^{1}H NMR ($CDCl_3$) δ 0.58 (s, 3H, 18-CH_3), 0.75 (s, 3H, 19-CH_3), 0.80 (d, 3H, J = 6.5 Hz, 26-CH_3), 0.81 (d, 3H, J = 7.0 Hz, 27-CH_3), 3.86 (d, J = 2.5 Hz, N-CH_2), 7.04-7.09 (m, 2H), 7.50-7.58 (m, 2H), 8.44-8.47 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 11.1, 11.9, 18.7, 20.8, 22.7, 23.0, 23.5, 23.9, 25.9, 28.1, 30.2, 31.8, 32.6, 32.9, 35.9, 36.2, 36.7, 39.3, 39.6, 42.0, 42.8, 46.2, 50.7, 52.4, 52.8, 53.3, 54.6, 56.1, 121.8, 122.7, 136.4, 149.1, 160.0, 160.9; FAB-MS Calcd for $C_{39}H_{61}N_4$: 585.4896. Found: *m/z* 585.4897 $(M+H)^+$.

3α,7α-Di(phenylureido)-5α-cholestane (2). Compound **6a** (200 mg, 0.33 mmol) was dissolved in THF (10 mL) and trifluoroacetic acid (0.26 mL, 3.30 mmol) was stirred at room temperature for 3 h. After the completion of reaction, THF was removed under *vacuo*, and the mixture was neutralized with

aqueous NaOH solution (25 mL), and extracted with ethyl acetate. The organic layer was washed, dried and concentrated to give crude product **1**. Without further purification crude product was dissolved in dry chloroform (10 mL) and phenyl isocyanate (0.09 mL, 0.86 mmol, 2.5 eq) was added at room temperature. After the completion of reaction, chloroform (15 mL) was added and the mixture was washed with $NaHCO_3$ solution (20 mL) followed by water (20 mL × 2) and brine (25 mL). The organic layer was dried and concentrated. The crude product was subjected to column chromatography (10% EtOAc-hexane) to give 158 mg of **2** (75%). TLC R_f 0.50 (EtOAc-hexane 1:1); mp 150 - 152 °C (MeOH-hexane); ^{1}H NMR (DMSO-d_6) δ 0.53 (s, 3H, 18-CH_3), 0.84 (d, 3H, J = 6.5 Hz, 26-CH_3), 0.85 (d, 3H, J = 6.5 Hz, 27-CH_3), 3.95 (bs, 2H, 3α-H, 7α-H), 6.97 (bs, 2H, NH), 7.52 (bs, 2H, NH); ^{13}C NMR (DMSO-d_6) δ 10.5, 12.0, 14.3, 18.8, 20.5, 21.3, 22.6, 22.7, 22.8, 23.0, 23.6, 24.0, 26.5, 28.1, 29.5, 29.8, 32.0, 33.7, 34.5, 35.8, 35.9, 36.2, 37.4, 38.9, 39.2, 39.6, 42.6, 46.9, 51.2, 56.1, 119.4, 122.7, 129.1, 139.3, 156.1; Anal. Calcd for $C_{41}H_{60}N_4O_2{\cdot}2H_2O$: C, 72.74, H, 9.53, N, 8.28, Found: C, 72.43, H, 9.68, N, 8.08.

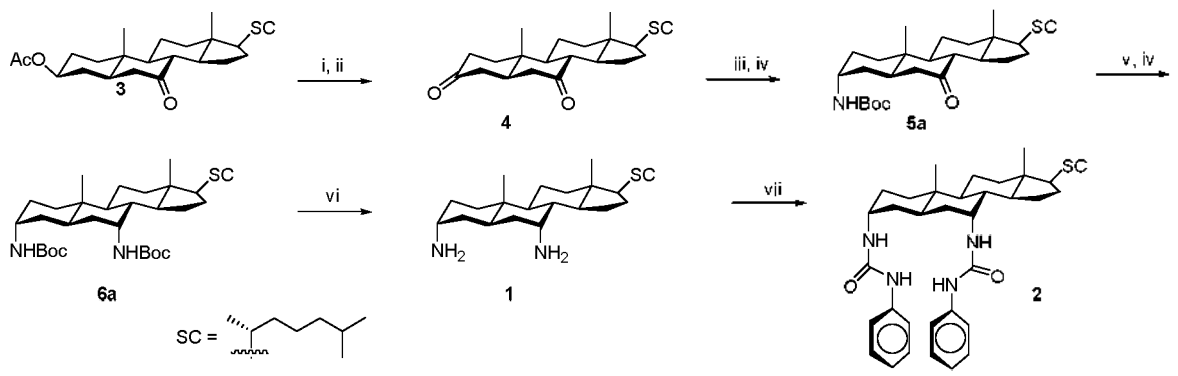
Results and Discussion

The requisite starting material **4** was prepared from commercially available 3β-acetoxy-cholest-5-ene.[5] The reductive amination of **4**, with $NaBH(OAc)_3$ and NH_4OTf, followed by protection with $(Boc)_2O$, gave **5a** as a mixture of a 3α/3β-isomer in a 69% yield. High diastereo-selectivity has been achieved by modifying $NaBH_4$ with different carboxylic acids, in the synthesis of squalamine analogues.[6] Thus, in order to improve selectivity toward the 3α isomer, various acyloxyborohydrides were prepared from $NaBH_4$ and the bulky carboxylic acids.[7] $NaBH(OEh)_3$ prepared from 2-ethylhexanoic acid (Eh) improved the stereo-selectivity of **5a**, and reaction time was shortened to 1 h: it provided 3α/3β with a ratio of 9:1 in an 85% yield. The stereochemistry of **5a** was determined based on the R_f value and the chemical shift of the 3α-NH proton in the ^{1}H, and those of C-3 and C-7 in the ^{13}C NMR spectrum. The ^{1}H NMR of **5a** showed a 3α-NH proton of an α isomer at δ 4.88. In the ^{13}C NMR spectrum of **5a**, C-3 carbamate and C-7 carbonyl carbons appeared at δ 155.3 and 212.1, and *tert*-butyloxy carbon at δ 79.2. Further relative stereochemistry was confirmed by data obtained from COSY, HETCO, DEPT, and comparisons were made with the similar published structure.[8]

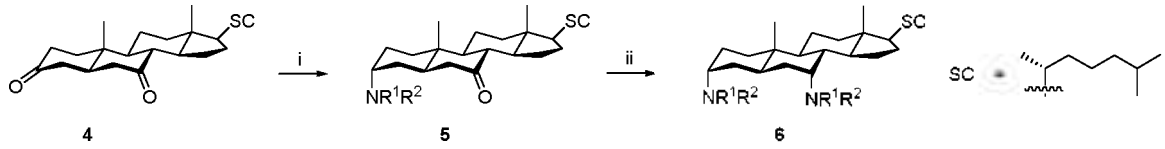
The reductive amination of **5a** at C-7 was accomplished by $NaBH_3CN$ and NH_4OAc in a co-solvent of THF and methanol in high yield. The ^{1}H NMR of **6a** showed the 7α-NH and 7β-H protons of the 7α isomer at δ 4.73 and 3.67, respectively. In the ^{13}C NMR, C-7 and C-3 of the α isomer appeared at δ 46.1 and 43.0, respectively. These values are in accordance with published values.[5] It is interesting to note that a bulky reducing reagent such as $NaBH(OEh)_3$ did not give any amino product. Hence it was concluded from the pattern of reducing reagent that bulkier reagents gave 3α product and smaller reagents furnish 7α products preferentially.

Compound **4** was further derivatized with *n*-$BuNH_2$, $BocNH(CH_2)_3NH_2$, $BocNH(CH_2)_4NH_2$ and $BocNH(CH_2)_3N(Boc)(CH_2)_4N(Boc)(CH_2)_3NH_2$ (Boc-spermidine) having various alkyl chain lengths in consistently high yield and stereoselectivity. 1-(3-Aminopropyl)imidazole and 2-(2-pyridyl)ethylamine were also used to prepare receptors. They reacted with **4** smoothly to give **5f** and **5g** in 92% and 93% yields, respectively. The latter could be converted to facial amphiphiles **6f** and **6g** in a high yield by reductive amination at C7 with $NaBH_3CN$. When 2-picolylamine reacted with **4** which gave **5h** in a 78%, and it undergo reductive amination at C7 with $NaBH_3CN$ to yield **6h** in an 84% yield (entry 8, Table 1). The reductive amination of **4** with secondary amine, di-(2-picolyl)amine, proceeded smoothly at C3 but failed to aminate at C7. It was evident from Table 1 that steric hindrance played an important role while synthesizing 3α-amines **5** by reductive amination from **4**.

Boc deprotection of **6a** with TFA gave diamine **1**, and subsequent reaction with phenyl isocyanate in a dry chloroform provided receptor **2** in good yield. The anion binding properties of **2** were initially investigated by ^{1}H NMR titrations in $CDCl_3$ with tetrabutylammonium salts of F^-, Cl^-, $CH_3CO_2^-$, and $H_2PO_4^-$. The highest association constant (K_a = 42,000 M^{-1}) was obtained for $CH_3CO_2^-$ anion as shown in Table 2. The complexation of Y-shaped $CH_3CO_2^-$ anion with **5** easily formed H-bonding with urea protons.[3a] The molecular calculations of complex between **2** and acetate ion showed acetate ion bind with two 3α-urea N-H and two 7α-urea N-H protons through hydrogen bonds



Scheme 1. Reagents and conditions: (i) KOH/EtOH, r.t.; (ii) PCC, CH_2Cl_2, r.t.; (iii) NH_4OTf, $NaBH(OEh)_3$, THF, r.t.; (iv) $(Boc)_2O$, MeOH; (v) NH_4OAc, $NaBH_3CN$, MeOH/THF = 1:1; (vi) TFA/THF = 3:1; (vii) PhNCO, $CHCl_3$, reflux.



Scheme 2. (i) Amine, $NaBH(OEh)_3$, THF, r.t.; (ii) Amine, $NaBH_3CN$, MeOH/THF = 1:1.

Table 1. The reductive amination of **4** with various amines

Entry	Amine[a]	R^1, R^2	Time (h) 1st RA	Yield[c] (%) 3α/3β	Product 3α	Time (h) 2nd RA	Yield[c] (%)	Product[d] 7α
1	NH_4OT_f	H, Boc	1	76/9	**5a**[b]	3	71	**6a**[b]
2	*n*-$BuNH_2$	H, *n*-Bu	1	77/9	**5b**	3	80	**6b**
3	$BocNH(CH_2)_3NH_2$	H, $BocNH(CH_2)_3$	2	72/8	**5c**	3	71	**6c**
4	$BocNH(CH_2)_4NH_2$	H, $BocNH(CH_2)_4$	1	76/9	**5d**	4	80	**6d**
5	Boc-spermidine	H, Boc-spermidine	1	78/9	**5e**	4	81	**6e**
6	3-$Im(CH_2)_3NH_2$	H, 3-$Im(CH_2)_3$	2	81/9	**5f**	3	78	**6f**
7	2-$Py(CH_2)_2NH_2$	H, 2-$Py(CH_2)_2$	2	84/8	**5g**	3	80	**6g**
8	2-$PyCH_2NH_2$	H, 2-$PyCH_2$	1	78/9	**5h**	3	84	**6h**
9	$(2\text{-}PyCH_2)_2NH$	$(2\text{-}PyCH_2)_2$	3	56/33	**5i**	72	No Rxn	**6i**

[a]See experimental section. [b]Both **5a** and **6a** are Boc-protected amines, see reagents and conditions in the Scheme 1. [c]Isolated yields. [d]The 7β isomer was obtained in negligible amount.

Table 2. Association constants (K_a) of receptor **2** with various anions[a]

OAc^-	$H_2PO_4^-$	F^-	Cl^-
42,100[b]	2,700[b]	1,300	1,500

[a]TBA salt of the anions were used in $CDCl_3$ at 298 K. [host] = 4.5×10^{-3} M. [b]Errors estimated to be ≤ 10%.

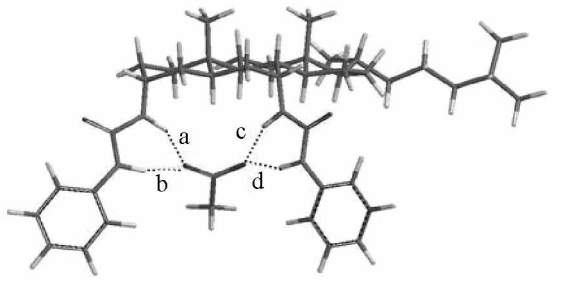


Figure 2. The H-bonding of **2** with acetate anion was shown by simulated molecular modeling.

(distances of N-H$\cdots$ O_2CCH_3: a = 1.79, b = 1.66, c = 1.78, d = 1.68 Å) as shown in Figure 2.[9]

In conclusion, 3α,7α-aminocholestane-based anionic receptors have been synthesized by reductive amination protocol with modified sodium acyloxyborohydride reagent in high yield and stereoselectivity. This procedure will be used to prepare various molecular receptors with compounds **6a-6g**. The anion-binding studies of the remainder of the receptors are currently under investigation.

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9. MP2 calculation at the AM1 mode level was performed SPARTAN'04 for Windows (Wavefunction, Inc.: Irvine, CA).