# Dynamic combinatorial chemistry with hydrazones: cholate-based building blocks and libraries

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## Supporting information

| | |
|---|---|
| General comments | S2 |
| Experimental section | S2-S5 |

## Experimental section

$^1$H NMR Spectra were recorded on Bruker DRX–400 MHz spectrometer unless otherwise stated. $^{13}$C NMR were obtained on a DRX–400 operating at 100 MHz. All NMR spectra were recorded using $CDCl_3$ which was de–acidified prior to use by standing over anhydrous $K_2CO_3$, or filtering through a plug of basic alumina, unless stated otherwise. Chemical shifts (δ) are expressed relative to TMS ($^1$H, $^{13}$C) and are quoted in ppm. Coupling constants are given in Hz and quoted to ± 0.05 Hz. The following abbreviations are used to indicate the multiplicity of the signals: s = singlet; d = doublet; dd = doublet of doublets; m = multiplet; t = triplet; dt = doublet of triplets; tt = triplet of triplets; q = quartet; brs = broad singlet. In the steroidal $^1$H NMR spectra overlapping backbone resonances are not quoted. COSY, NOESY spectra were obtained on a Bruker DRX–500 MHz spectrometer in $CDCl_3$. HPLC analysis was carried out using a Hewlett–Packard 1050 instrument, with UV analysis employing a HP 1050 DAD detector, and data was analysed using the HP ChemStation software. Reverse phase HPLC separations were carried out using a 15cm x 4.6mm i.d. 3Å particle size, Supelco ABZ$^+$ $C_{16}$ alkylamide column using acetonitrile and *iso*–propanol gradients.

Positive–ion Electrospray mass spectra (ESI–MS) were obtained on a VG BioQ triple quadrupole apparatus with a mass–to–charge (m/z) range up to 4000 (VG Bio Tech Ltd, Altrincham, UK). The electrospray source was heated to 100˚C and the sampling cone voltage varied between 40–65 V. The samples were introduced into the mass spectrometer source with an LC pump (Shimadzu LC–9A LC pump) at a flow rate of 4μLmin$^{-1}$ of acetonitrile/water (1:1). Calibration was performed using protonated horse myoglobin. The data system was operated as a multichannel analyser, and several scans were summed (>10) to obtain the final spectrum. A constant volume (5μL) of centrifuged solution was injected. Infrared spectra were recorded on a Perkin Elmer 1600 series FTIR spectrometer; the spectra of solids were run as solutions in deuterated chloroform. Fast atom bombardment (FAB) mass spectra were obtained using a *m*-nitrobenzyl alcohol matrix on a Kratos MS–50 instrument. Experimental peak (mode) masses are compared with calculated mean masses. Microanalyses were carried out by the University Chemical Laboratory Microanalysis Department in Cambridge.

**General Procedures:**

The synthetic sequence was modified depending upon the nature of the steroid to be synthesised. Monomers (**6**), (**8**), (**9**), (**10**) and (**12**) were synthesised from the parent bile acid using synthetic steps A, B, C, D and E (Scheme 2). Monomers (**14**)–(**19**) were synthesised using steps A, B, C and E (Scheme 3). Monomer (**21**) was synthesised using B, C and E, following coupling of glycine methyl ester to deoxycholic acid using B (Scheme 4). Monomer (**23**) was synthesised using B, C, D, following benzyl deoxycholate formation using B. Hydrogenation of the benzyl ester (**22**) using F was then followed by coupling to glycine methyl ester using B then E to give (**23**) (Scheme 5). Formation of the inverted methyl ester of deoxycholic acid, according to literature procedure, was followed by steps B, C, D and E (Scheme 6). The following sequence is illustrated with the synthesis of the dimethyl acetal hydrazide (**6**). The yields and procedures for this monomer are typical of all of the monomers described herein.

**(A) Steroidal Methyl Ester formation (2)**

Deoxycholic acid (**1**) (25 g, 63.7 mmol) was stirred vigorously in dry, freshly distilled MeOH (100 ml) and acetyl chloride (31.8 mmol, 2.07 ml) added dropwise over a period of 10 minutes. The mixture was then stirred at room temperature for 4 hours, after which time TLC (2:1 hexane/acetone) indicated that all of the acid had been converted to the ester. Distilled water was then added dropwise to the crude solution to initiate precipitation of methyl deoxycholate. The solid was filtered and dried under vacuum to yield the title compound as a white powder (24.5 g, 94.6%), $R_f$ 0.34 (2:1 hexane/acetone), $\nu_{max}$/cm$^{-1}$ ($CDCl_3$) 3612, 1730; $^1$H NMR ($CDCl_3$) 0.65 (s, 3H), 0.88 (s, 3H), 0.95 (d, J = 6.3 Hz, 3H), 3.59 (m, 1H), 3.65 (s, 3H), 3.95 (m, 1H); $^{13}$C NMR ($CDCl_3$) 12.7, 17.3, 23.2, 23.7, 26.1, 27.1, 27.5, 28.7, 30.5, 30.9, 31.1, 33.7, 34.1, 35.1, 35.2, 36.0, 36.4, 42.1, 46.5, 47.3, 48.3, 51.5, 71.8, 73.2, 174.7. ESI–MS: m/z 429 [M+Na]$^+$.

**(B) EDC coupling of 3 or 4–carboxybenzaldehyde to 3–position of steroid (3)**

Methyl deoxycholate **(2)** (2.0 g, 4.92 mmol) was dissolved in dry DCM (20 ml). 3–Carboxybenzaldehyde (5.91 mmol, 887 mg) and DMAP (0.49 mmol, 60 mg) were added and the reaction mixture cooled in an ice–bath (0˚C). EDC (7.4 mmol, 1.42 g) was added in portions and stirring under argon continued at ice–bath temperature for a further 30 minutes. The reaction mixture was then allowed to warm to room temperature, at which it was stirred for a further 3 hours. The solvent was removed under reduced pressure and the residue subjected to column chromatography (3:1 hexane/EtOAc). The pure compound was obtained as a white foam (2.2 g, 83%), $R_f$ = 0.32 (3:1 hexane/EtOAc); $^1$H NMR ($CDCl_3$) 0.69 (s, 3H), 0.89 (s, 3H), 0.97 (d, *J* = 6.3 Hz), 3.65 (s, 3H), 4.00 (m, 1H), 5.01 (m, 1H) 7.60 (t, *J* = 7.7 Hz), 8.07 (d, *J* = 7.7 Hz), 8.30 (d, *J* = 7.7 Hz), 8.51 (s, 1H), 10.08 (s, 1H); $^{13}$C NMR ($CDCl_3$) 12.8, 17.4, 23.1, 23.6, 26.0, 26.6, 27.0, 27.4, 28.8, 30.9, 31.1, 32.3, 33.8, 34.2, 34.9, 35.1, 36.0, 42.0, 46.6, 47.4, 48.4, 51.5, 73.2, 75.6, 129.2, 131.5, 132.0, 132.7, 135.2, 136.5, 165.0, 174.7, 191.5; ESI–MS: m/z 561 [M+Na]$^+$, found 561.3182, $C_{33}H_{46}O_6Na$ requires 561.3192.

**(C) Dimethyl acetal formation (4)**

Aldehyde **(3)** (1.0 g, 1.85 mmol), was dissolved in freshly distilled, dry MeOH. TsOH monohydrate (0.18 mmol, 36 mg) was added and the reaction mixture stirred under argon at room temperature for 2 hours, after which time TLC (3:1 hexane/EtOAc) indicated that all of the starting material had been consumed. Dilution of the reaction mixture with diethyl ether (200 ml) was followed by washing with saturated $NaHCO_3$ (100 ml) and distilled water (2 x 100 ml). After drying (anhydrous $Na_2SO_4$) and filtration, the solvent was removed and the acetal obtained as a pure white foam (1.08 g, 100%), $R_f$ = 0.41 (3:1 hexane/EtOAc); $^1$H NMR ($CDCl_3$) 0.68 (s, 3H), 0.93 (s, 3H), 0.96 (d, J = 6.0 Hz), 3.30 (s, 6H), 3.65 (s, 3H), 4.00 (m, 1H), 4.96 (m, 1H), 5.41 (s, 1H), 7.42 (t, J = 7.7 Hz), 7.62 (d, J = 7.7 Hz), 8.00 (d, J = 7.7 Hz, 1H), 8.08 (s, 1H); $^{13}$C NMR ($CDCl_3$) 12.7, 17.4, 23.1, 23.6, 26.0, 26.7, 27.0, 27.4, 28.8, 30.9, 31.0, 32.3, 33.8, 34.2, 34.9, 35.1, 36.0, 42.0, 46.5, 47.4, 48.3, 51.5, 52.7, 52.8, 52.8, 73.2, 75.0, 76.7, 77.3, 102.7, 128.0, 128.2, 129.7, 131.0, 138.5, 165.7, 166.0, 174.7; ESI–MS: m/z 607 [M+Na]$^+$, found 607.3622, $C_{35}H_{52}O_7Na$ requires 607.3723.

**(D) EDC coupling of pyridine–3 or 4–carboxylic acid to 7 or 12–position of steroid (5)**

Alcohol **(4)** (1.0 g, 1.71 mmol) was dissolved in dry DCM (20 ml). DMAP (3.42 mmol, 418 mg) and pyridine–4–carboxylic acid (2.56 mmol, 315 mg) were added and the mixture cooled to 0˚C (ice–bath). EDC (2.56 mmol, 490 mg) was added in portions and stirring continued at 0˚C for a further 30 minutes. The reaction was then left to stir at room temperature for 18 hours. The solvent was removed under vacuum and the pure compound obtained, following column chromatography (2:1 hexane/EtOAc) as a pure white foam (1.1 g, 93%), $R_f$ = 0.34 (2:1 hexane/EtOAc); $^1$H NMR ($CDCl_3$) 0.79 (d, J = 6.0 Hz, 3H), 0.81 (s, 3H), 1.09 (s, 3H), 3.31 (s, 6H), 3.60 (s, 3H), 4.88 (m, 1H), 5.38 (m, 1H), 5.40 (s, 1H), 7.41 (t, J = 7.7 Hz), 7.61 (d, J = 7.7 Hz), 7.80 (d, J = 7.7 Hz), 7.88 (d, J = 6.0 Hz, 2H), 7.98 (s, 1H), 8.82 (d, J = 6.0 Hz, 2H); $^{13}$C NMR ($CDCl_3$) 12.6, 17.6, 23.1, 23.5, 25.9, 26.0, 26.8, 27.4, 30.8, 31.0, 32.3, 34.1, 34.8, 35.8, 41.8, 45.5, 48.1, 50.1, 51.5, 52.8, 52.8, 74.6, 77.7, 102.6, 122.7, 127.8, 128.3, 129.5, 130.8, 131.1, 137.9, 138.6, 150.8, 164.4, 165.8, 174.5; ESI–MS: m/z 690 [M+H]$^+$, 712 [M+Na]$^+$, found 712.3802, $C_{41}H_{55}NO_8Na$ requires 712.3825.

**(E) Hydrazinolysis of steroidal methyl ester (6)**

Methyl ester **(5)** (1.1 g, 1.6 mmol), was dissolved in a mixture of distilled THF and MeOH (1:2 v/v, 10 ml). Hydrazine monohydrate (31.2 mmol, 1.6 g) was added and the reaction mixture stirred at room temperature for 48 hours, after which time LC–MS indicated all the starting material had been consumed. The reaction mixture was diluted with EtOAc (200 ml) and washed with distilled water (3 x 50 ml) and saturated brine solution (2 x 50 ml). After drying (anhydrous $Na_2SO_4$), filtering and removal of the solvent under vacuum the compound was purified by column chromatography (5%MeOH/$CHCl_3$) to give the hydrazide as a white foam (900 mg, 82%), $R_f$ = 0.34 (10%MeOH/$CHCl_3$), $\nu_{max}/cm^{-1}$ ($CDCl_3$) 3448, 1715, 1673; $^1H$ NMR ($CDCl_3$) 0.79 (d, J = 6.0 Hz, 3H), 0.80 (s, 3H), 0.94 (s, 3H), 3.31 (s, 6H), 3.83 (br, 2H), 4.87 (m, 1H), 5.38 (m, 1H), 5.40 (s, 1H), 6.72 (br, 1H) 7.41 (t, J = 7.7 Hz), 7.62 (d, J = 7.7 Hz), 7.80 (d, J = 7.7 Hz), 7.88 (d, J = 6.0 Hz), 7.98 (s, 1H), 8.82 (d, J = 6.0 Hz, 2H); $^{13}C$ NMR ($CDCl_3$) 12.6, 17.6, 23.1, 23.5, 25.6, 25.9, 26.0, 26.6, 26.8, 27.4, 31.3, 31.4, 32.3, 34.1, 34.7, 34.9, 35.7, 41.8, 45.6, 46.5, 48.2, 50.1, 52.8, 52.8, 74.6, 77.7, 102.6, 122.7, 127.8, 128.3, 129.5, 130.8, 131.1, 137.9, 138.6, 150.8, 164.4, 165.9, 174.0; ES–MI: m/z 712 $[M+Na]^+$, found 712.3955, $C_{40}H_{55}N_3O_7Na$ requires 712.3938. Anal. Calcd for $C_{40}H_{55}N_3O_7{\cdot}0.5H_2O$: C, 68.76; H, 8.02; N, 6.01. Found: C, 68.77; H, 7.98; N, 5.92,

**(F) Hydrogenation of (22)**

Benzyl ester **(22)** (1.5 g, 1.96 mmol) was dissolved in THF (50 ml) and palladium on carbon (10%) (300 mg) added. The flask was then evacuated and charged with $H_2$. This was repeated and stirring under the $H_2$ atmosphere continued for 3 hours, after which time TLC (2:1 hexane/EtOAc) indicated that all of the starting material had been consumed. The palladium on carbon was removed by filtration through a plug of pre–packed celite. Removal of the solvent under reduced pressure furnished the acid as a pure white foam (1.32g, 100%).

**3α–(3–dimethoxyformylbenzoyloxy)–12α–pyridine–3–carboxy–5β–deoxycholan–24–oic hydrazide (9)**

Methyl 3α–(3–dimethoxyformylbenzoyloxy)–12α–pyridine–3–carboxy–5β–deoxycholan–24–oate (1.2 g, 1.74 mmol) was dissolved in a mixture of MeOH and THF (9:1 v/v) (10 ml). Hydrazine monohydrate (34.8 mmol, 1.74 g) was added. Following aqueous work–up and column chromatography (5% MeOH/DCM) (**8**) was obtained as a white foam (834 mg, 69.5%) , $R_f$ = 0.25 (5%MeOH/DCM), $\nu_{max}/cm^{-1}$ ($CDCl_3$) 3449, 1711, 1673; $^1H$ NMR ($CDCl_3$) 0.81 (d, J = 10.7 Hz, 3H), 0.82 (s, 3H), 0.96 (s, 3H), 3.30 (s, 6H), 3.84 (brs, 2H), 4.87 (tt, J = 5, 11 Hz, 1H), 5.38 (brs, 1H), 5.39 (s, 1H), 6.72 (brs, 1H), 7.39 (t, J = 7.7 Hz, 1H), 7.43 (dd, J = 4.9, 7.9 Hz, 1H), 7.62 (d, J = 7.7 Hz, 1H), 7.84 (dt, J = 1.3, 7.7 Hz, 1H), 7.97 (s, 1H), 8.32 (dt, J = 1.9, 7.9 Hz, 1H), 8.77 (dd, J = 1.9, 4.9 Hz, 1H), 9.30 (d, J = 1.9 Hz, 1H); $^{13}C$ NMR ($CDCl_3$) 12.6, 17.6, 23.1, 23.5, 25.9, 26.0, 26.6, 26.9, 27.4, 31.3, 32.3, 34.1, 34.8, 34.9, 35.8, 41.8, 45.6, 48.1, 50.1, 52.8 (2), 74.7, 77.3, 102.7, 123.6, 126.6, 127.9, 128.1, 129.7, 130.8, 131.0, 137.0, 138.5, 150.8, 153.5, 164.5, 165.9, 174.1; ESI–MS: m/z 712 $[M+Na]^+$, found 712.3931, $C_{40}H_{55}N_3O_7Na$ requires 712.3938. Anal. Calcd for $C_{40}H_{55}N_3O_7{\cdot}0.5H_2O$: C, 68.76; H, 8.02; N, 6.01. Found: C, 68.79; H, 7.98; N, 6.11.

**3α–(3–dimethoxyformylbenzoyloxy)–7α–pyridine–4–carboxy–5β–chenodeoxycholan–24–oic hydrazide (10)**

Methyl 3α–(3–dimethoxyformylbenzoyloxy)–7α–pyridine–4–carboxy–5β–chenodeoxycholan–24–oate (1.2 g, 1.74 mmol) was dissolved in a mixture of MeOH and THF (9:1) (15 ml). Hydrazine monohydrate (34.8 mmol, 1.74 g) was added and the reaction mixture stirred at room temperature for 48 hours. Following aqueous work–up and column chromatography (5%MeOH/DCM) (9) was obtained as a pure white foam (900 mg, 75%), $R_f$ = 0.46 (10% MeOH/DCM), $\nu_{max}/cm^{-1}$ ($CDCl_3$) 3450, 1716, 1671; $^1H$ NMR ($CDCl_3$) 0.68 (s, 3H), 0.92 (d, J = 6.5 Hz, 3H), 1.02 (s, 3H), 3.33 (s, 6H), 3.87 (brs, 2H), 4.85 (tt, J = 4.5, 11.2 Hz, 1H), 5.23 (m, 1H), 5.41 (s, 1H), 6.68 (brs, 1H), 7.42 (t, J = 7.7 Hz, 1H), 7.62 (d, J = 7.7 Hz, 1H), 7.85 (dd, J = 1.5, 4.4 Hz, 2H), 7.87 (dt, J = 1.3, 7.7 Hz, 1H), 8.02 (s, 1H), 8.76 (dd, J = 1.5, 4.4 Hz, 2H); $^{13}C$ NMR ($CDCl_3$) 11.8, 18.3, 20.7, 22.7, 23.6, 26.9, 27.9, 31.3, 31.4, 31.5, 34.5, 34.8, 34.9, 35.3, 38.2, 39.5, 40.7, 42.9, 50.7, 52.7, 52.8, 55.7, 73.1, 74.4, 103.2, 122.8, 127.6, 128.2, 129.5, 130.8, 131.2, 137.9, 138.6, 150.7, 164.2, 165.8, 174.2; ESI–MS: m/z 712 $[M+Na]^+$, found 712.3941, $C_{40}H_{55}N_3O_7Na$ requires 712.3938. Anal. Calcd. for $C_{40}H_{55}N_3O_7{\cdot}0.5H_2O$: C, 68.76; H, 8.02; N, 6.01. Found: C, 68.90; H, 8.01; N, 5.78.

**3α–(3–dimethoxyformylbenzoyloxy)–7α–pyridine–3–carboxy–5β–chenodeoxycholan–24–oic hydrazide (11)**

Methyl 3α–(3–dimethoxyformylbenzoyloxy)–7α–pyridine–4–carboxy–5β–chenodeoxycholan–24–oate (1.4 g, 2.03 mmol) was dissolved in a mixture of MeOH and THF (9:1 v/v) (20 ml). Hydrazine monohydrate (40.6 mmol, 2.03 g) was added and the reaction mixture stirred at room temperature for 48 hours. Following aqueous work–up and column chromatography (5%MeOH/DCM) (**10**) was obtained as a pure white foam (1.1 g, 78%), $R_f$ = 0.26 (5%MeOH/DCM), $\nu_{max}/cm^{-1}$ ($CDCl_3$) 3450, 1713, 1673; $^1H$ NMR ($CDCl_3$) 0.67 (s, 3H), 0.91 (d, J = 6.4 Hz, 3H), 1.01 (s, 3H), 3.31 (s, 6H), 3.84 (brs, 2H), 4.83 (tt, J = 4.5, 11.2 Hz, 1H), 5.23 (m, 1H), 5.40 (s, 1H), 6.68 (brs, 1H), 7.37 (t, J = 4.1 Hz, 1H), 7.39 (t, J = 7.7 Hz, 1H), 7.62 (d, J = 7.7 Hz, 1H), 7.88 (d, J = 7.7 Hz, 2H), 7.98 (s, 1H), 8.30 (d, J = 7.9 Hz, 1H), 8.72 (d, J = 4.1 Hz, 1H), 9.27 (s, 1H); $^{13}C$ NMR ($CDCl_3$) 11.8, 18.4, 20.7, 22.8, 23.6, 26.9, 28.0, 31.2, 31.4, 31.5, 34.5, 34.9, 35.0, 35.3, 38.3, 39.5, 40.8, 42.9, 50.8, 52.8, 52.9, 55.7, 72.8, 74.5, 102.7, 123.4, 126.7, 127.9, 128.2, 129.7, 130.3, 130.8, 131.0, 137.1, 138.5, 150.9, 153.3, 164.4, 165.9, 174.3; ESI–MS: m/z 690 $[M+H]^+$, 712 $[M+Na]^+$, found 712.3903, $C_{40}H_{55}N_3O_7Na$ requires 712.3938.

**3α–(4–dimethoxyformylbenzoyloxy)–12α–pyridine–4–carboxy–5β–deoxycholan–24–oic hydrazide (12)**

Methyl 3α–(4–dimethoxyformylbenzoyloxy)–12α–pyridine–4–carboxy–5β–deoxycholan–24–oate, (1.17 g, 1.69 mmol) was dissolved in a mixture of distilled THF and MeOH (1:2 v/v, 10 ml). Hydrazine monohydrate (33.9 mmol, 1.70 g) was added and the mixture stirred at room temperature for 48 hours, Following aqueous work–up and column chromatography (5%MeOH/$CHCl_3$) (**11**) was obtained as a white foam (1.05 g, 90%), $R_f$ = 0.36 (10%MeOH/$CHCl_3$), $\nu_{max}/cm^{-1}$ ($CDCl_3$) 3449, 1712, 1672; $^1H$ NMR ($CDCl_3$) 0.78 (d, J = 6.0 Hz, 3H), 0.80 (s, 3H), 0.94 (s, 3H), 3.36 (s, 6H), 3.81 (br, 2H), 4.87 (m, 1H), 5.40 (m, 1H), 5.42 (s, 1H), 6.65 (br, 1H) 7.49 (d, J = 8.0 Hz, 2H), 7.85 (d, J = 8.0 Hz, 2H), 7.88 (d, J = 6.0 Hz, 2H), 8.84 (d, J = 6.0 Hz, 2H); $^{13}C$ NMR ($CDCl_3$) 12.6, 17.6, 23.0, 23.5, 25.8, 26.0, 26.5, 26.8, 27.4, 31.3, 31.4, 32.3, 34.0, 34.7, 34.9, 35.7, 41.7, 45.5, 48.2, 50.2, 52.6, 74.5, 77.7, 102.3, 122.7, 126.7, 129.3, 130.7, 137.9, 142.8, 150.8, 164.3, 165.7, 174.0; ESI–MS: m/z 690 $[M+H]^+$, 712 $[M+Na]^+$, found 712.3921, $C_{40}H_{55}N_3O_7Na$ requires 712.3938. Anal. Calcd. for $C_{40}H_{55}N_3O_7{\cdot}0.5H_2O$: C, 68.76; H, 8.02; N, 6.01, Found: C, 68.79; H, 8.08; N, 5.88.

**3α–(3–dimethoxyformylbenzoyloxy)–5β–lithocholan–24–oic hydrazide (14)**

Methyl 3α–(3–dimethoxyformylbenzoyloxy)–5β–lithocholan–24–oate (1.41 g, 2.48 mmol) was dissolved in a mixture of MeOH and THF (9:1 v/v) (20 ml). Hydrazine monohydrate (49.6 mmol, 2.48 g) was added and the reaction mixture stirred for 48 hours at room temperature. Following aqueous work up and flash chromatography (5%MeOH/DCM) (14) was obtained as a white solid (900 mg, 64%), $R_f$ = 0.41 (5%MeOH/DCM), $\nu_{max}/cm^{-1}$ ($CDCl_3$) 3450, 3336, 1708, 1672; $^1H$ NMR ($CDCl_3$) 0.64 (s, 3H), 0.91 (d, J = 6.3 Hz), 0.94 (s, 3H), 3.33 (s, 6H), 3.89 (brs, 2H), 4.97 (tt, J = 4.8, 11.3 Hz), 5.42 (s, 1H), 6.72 (brs, 1H), 7.43 (t, J = 7.7 Hz), 7.63 (d, J = 7.7 Hz), 8.01 (d, J = 7.7 Hz), 8.10 (s, 1H); $^{13}C$ NMR ($CDCl_3$) 12.1, 18.4, 20.9, 23.4, 24.2, 26.3, 26.8, 27.1, 28.3, 31.5, 31.6, 32.4, 34.7, 35.1, 35.5, 35.8, 40.2, 40.5, 42.0, 42.8, 52.8, 56.0, 56.5, 75.1, 102.7, 127.9, 128.3, 129.7, 131.0, 131.1, 138.5, 166.0, 174.4; ES–MI: m/z 591 $[M+Na]^+$, found 591.3774, $C_{34}H_{52}N_2O_5Na$ requires 591.3774. Anal. Calcd. for $C_{34}H_{52}N_2O_5{\cdot}H_2O$: C, 69.6; H, 9.2; N, 4.7. Found: C, 69.94; H,

8.93; N, 4.72.

**3α–(3–dimethoxyformylbenzoyloxy)–12α–hydroxy–5β–deoxycholan–24–oic hydrazide (15)**

Methyl 3α–(3–dimethoxyformylbenzoyloxy)–12α–hydroxy–5β–deoxycholan–24–oate (800 mg, 1.37 mmol) was dissolved in a mixture of EtOH and THF (9:1 v/v) (15 ml). Hydrazine monohydrate (27.3 mmol, 1.37 g) was added and the mixture stirred at room temperature. Aqueous work–up and column chromatography (5%MeOH/DCM) after 48 hours afforded (**15**) as a white foam (700 mg, 87.5%), $R_f$ = 0.4 (10%MeOH/$CHCl_3$), $\nu_{max}/cm^{-1}$ ($CDCl_3$) 3617, 3450, 1700, 1672; $^1H$ NMR ($CDCl_3$) 0.69 (s, 3H), 0.96 (s, 3H), 0.99 (d, J = 6.1 Hz, 3H), 3.34 (s, 6H), 3.90 (brs, 2H), 4.00 (brs, 1H), 4.97 (tt, J = 4.5, 11.3 Hz, 1H), 5.43 (s, 1H), 6.84 (brs, 1H), 7.43 (t, J = 7.7 Hz, 1H), 7.64 (d, J = 7.7 Hz, 1H), 8.00 (d, J = 7.7 Hz, 1H), 8.09 (s, 1H); $^{13}C$ NMR ($CDCl_3$) 12.8, 17.4, 23.2, 23.6, 26.0, 26.7, 27.0, 27.5, 28.9, 31.3, 31.5, 32.3, 33.8, 34.2, 35.0, 35.2, 36.0, 42.0, 46.5, 47.2, 48.4, 52.8, 52.8, 73.2, 75.0, 102.7, 128.0, 128.3, 129.8, 131.0, 131.0, 138.5, 166.0, 174.4; ESI–MS: m/z 607 $[M+Na]^+$, found 607.3742, $C_{34}H_{52}N_2O_6Na$ requires 607.3723. Anal. Calcd. for $C_{34}H_{52}N_2O_6{\cdot}H_2O$: C, 67.77; H, 8.97; N, 4.65. Found: C, 67.73; H, 8.74; N, 4.68.

**3α–(3–dimethoxyformylbenzoyloxy)–7α–hydroxy–5β–chenodeoxycholan–24–oic hydrazide (16)**

Methyl 3α–(3–dimethoxyformylbenzoyloxy)–7α–hydroxy–5β–chenodeoxycholan–24–oate (1.4 g, 2.4 mmol) was dissolved in a mixture of MeOH and THF (9:1 v/v) (20 ml). Hydrazine monohydrate (47.9 mmol, 2.40 g) was added. After 48 hours aqueous work–up, followed by column chromatography (5%MeOH/DCM) afforded (**16**) as a pure white foam (1.1 g, 78.5%), $R_f$ = 0.25 (5%MeOH/DCM), $\nu_{max}/cm^{-1}$ ($CDCl_3$) 3614, 3450, 1707, 1672; $^1H$ NMR ($CDCl_3$) 0.66 (s, 3H), 0.93 (d, J = 6.6 Hz, 3H), 0.94 (s, 3H), 3.32 (s, 6H), 3.87 (brs, 2H), 3.89 (brs, 1H), 4.82 (tt, J = 4.5, 11.3 Hz, 1H), 5.41 (s, 1H), 6.76 (brs, 1H), 7.41 (t, J = 7.7 Hz, 1H), 7.62 (d, J = 7.7 Hz, 1H), 8.00 (d, J = 7.7 Hz, 1H), 8.08 (s, 1H); $^1H$ NMR ($CDCl_3$) 11.8, 18.4, 20.7, 22.8, 23.7, 26.9, 28.2, 31.4, 31.6, 32.9, 34.6, 35.1, 35.2, 35.4, 35.5, 39.4, 39.6, 41.3, 42.7, 50.5, 52.8, 55.8, 68.5, 75.1, 102.7, 128.0, 128.2, 129.8, 130.9, 131.0, 138.4, 166.1, 174.4; ESI–MS: m/z 607 $[M+Na]^+$, found 607.3749, $C_{34}H_{52}N_2O_6Na$ requires 607.3723. Anal. Calcd. for $C_{34}H_{52}N_2O_6{\cdot}0.5H_2O$: C, 68.80; H, 8.93; N, 4.72. Found: C, 68.49; H, 8.92; N, 4.74.

**3α–(4–dimethoxyformylbenzoyloxy)–5β–lithocholan–24–oic hydrazide (17)**

As for **14**. 4–carboxybenzaldehyde was substituted for its regioisomer. Following column chromatography (5%MeOH/DCM) (**17**) was obtained as a white solid (900 mg, 64%), $R_f$ = 0.46 (5%MeOH/DCM), $\nu_{max}/cm^{-1}$ ($CDCl_3$) 3450, 1707, 1672; $^1H$ NMR ($CDCl_3$) 0.65 (s, 3H), 0.92 (d, J = 6.3 Hz, 3H), 0.94 (s, 3H), 3.31 (s, 6H), 3.88 (brs, 2H), 4.96 (tt, J = 4.8, 11.3 Hz, 1H), 5.43 (s, 1H), 6.73 (brs, 1H), 7.51 (d, J = 8.3 Hz, 2H), 8.04 (d, J = 8.3 Hz, 2H); $^{13}C$ NMR ($CDCl_3$) 12.1, 18.4, 20.9, 23.4, 24.2, 26.4, 26.8, 27.1, 28.3, 31.5, 31.6, 32.4, 34.7, 35.1, 35.5, 35.8, 40.2, 40.5, 42.0, 42.8, 52.6, 56.0, 56.5, 75.1, 102.4, 126.7, 129.5, 131.0, 142.7, 165.9, 174.4; ESI–MS: m/z 591 $[M+Na]^+$, found 591.3777, $C_{34}H_{52}N_2O_5Na$ requires 591.3774.

**3α–(4–dimethoxyformylbenzoyloxy)–12α–hydroxy–5β–deoxycholan–24–oic hydrazide (18)**

As for **15**. 4–Carboxybenzaldehyde was substituted for its regioisomer. (**18**) was obtained as a white foam (700 mg, 87.5%) following column chromatography (5%MeOH/DCM), $R_f$ = 0.4 (10%MeOH/$CHCl_3$), $\nu_{max}/cm^{-1}$ ($CDCl_3$) 3617, 3450, 1707, 1672; $^1H$ NMR ($CDCl_3$) 0.68 (s, 3H), 0.93 (s, 3H), 0.97 (d, J = 6.2 Hz, 3H), 3.31 (s, 6H), 3.89 (brs, 2H), 4.00 (brs, 1H), 4.95 (tt, J = 4.5, 11.3 Hz, 1H), 5.43 (s, 1H), 6.82 (brs, 1H), 7.50 (d, J = 8.3 Hz, 2H), 8.02 (d, J = 8.3 Hz, 2H); $^{13}C$ NMR ($CDCl_3$) 12.8, 17.4, 23.2, 23.6, 26.0, 26.7, 27.0, 27.5, 28.9, 31.3, 31.5, 32.3, 33.8, 34.2, 34.9, 35.2, 36.0, 41.9, 46.5, 47.2, 48.4, 52.6, 73.2, 74.9, 102.4, 126.7, 129.5, 130.9, 142.7, 165.9, 174.4; ESI–MS: m/z 607 $[M+Na]^+$, found 607.3719, $C_{34}H_{52}N_2O_6Na$ requires 607.3723. Anal. Calcd for $C_{34}H_{53}N_2O_6{\cdot}0.5\ H_2O$: C, 68.80; H, 8.93; N, 4.72, Found: C, 68.86; H, 8.86; N, 4.70.

**3α–(4–dimethoxyformylbenzoyloxy)–7α,12α–hydroxy–5β–cholan–24–oic hydrazide (19)**

Methyl 3α–(4–dimethoxyformylbenzoyloxy)–7α,12α–hydroxy–5β–cholan–24–oate (1.0 g, 1.66 mmol) was dissolved in a mixture of MeOH and THF (9:1 v/v) (15 ml) and hydrazine monohydrate added (33.3 mmol, 1.67 g). (**19**) was obtained as a pure white foam (860 mg, 86%) following aqueous work–up and column chromatography (5%MeOH/DCM), $R_f$ = 0.23 (5%MeOH/DCM), $\nu_{max}/cm^{-1}$ ($CDCl_3$) 3612, 3447, 1707, 1670; $^1H$ NMR ($CDCl_3$) 0.69 (s, 3H), 0.92 (s, 3H), 0.98 (d, J = 6.1 Hz, 3H), 3.31 (s, 6H), 3.86 (m, 1H), 3.89 (br, 2H), 3.99 (m, 1H), 4.81 (m, 1H), 5.43 (s, 1H), 6.99 (br, 1H), 7.50 (d, J = 8.3 Hz, 2H), 8.02 (d, J = 8.3 Hz, 2H); $^{13}C$ NMR ($CDCl_3$) 12.6, 17.4, 22.5, 23.2, 26.7, 26.8, 27.5, 28.34, 31.0, 31.4, 34.6, 34.8, 34.9, 35.3, 39.4, 41.3, 42.1, 45.1, 46.5, 46.8, 52.6, 68.3, 73.1, 75.0, 102.4, 126.7, 129.5, 131.0, 142.7, 165.9, 174.5; ESI–MS: m/z 623 $[M+Na]^+$, found 623.3643, $C_{34}H_{52}N_2O_7Na$ requires 623.3673. Anal. Calcd. for $C_{34}H_{52}N_2O_7{\cdot}H_2O$: C, 66.01; H, 8.73; N, 4.53, Found: C, 66.12; H, 8.62; N, 4.68.

**Glycine methyl 3α–(3–dimethoxyformylbenzoyloxy)–12α–hydroxy–5β–deoxycholan–24–oic hydrazide (21)**

Glycine methyl 3α–(3–dimethoxyformylbenzoyloxy)–12α–hydroxy–5β–deoxycholan–24–oate (1.0 g, 1.56 mmol) was dissolved in MeOH (10 ml). Hydrazine monohydrate (15.6 mmol, 780 mg) was added and stirred at room temperature for 2 hours. Following aqueous work–up (**21**) was obtained as a pure white foam (900 mg, 90%), $\nu_{max}/cm^{-1}$ ($CDCl_3$) 3616, 3440, 3336, 1707, 1666; $^1H$ NMR ($CDCl_3$) 0.68 (s, 3H), 0.93 (s, 3H), 0.97 (d, J = 6 Hz, 3H), 3.33 (s, 6H), 3.90 (d, J = 5.1 Hz, 2H), 4.0 (m, 1H), 4.95 (tt, J = 4.6, 11.3 Hz, 1H), 5.41 (s, 1H), 6.65 (t, J = 4.7 Hz, 1H), 7.43 (t, J = 7.7 Hz, 1H), 7.63 (d, J = 7.7 Hz, 1H), 7.98 (dd, J = 1.3, 7.7 Hz, 1H), 8.07 (brs, 1H), 8.09 (s, 1H); $^{13}C$ NMR ($CDCl_3$) 12.7, 17.5, 23.2, 23.7, 26.1, 26.7, 27.0, 27.6, 28.8, 31.4, 32.3, 32.9, 33.8, 34.2, 35.0, 35.3, 36.0, 42.0, 46.5, 47.0, 48.3, 52.9, 73.1, 75.0, 102.7, 127.9, 128.3, 129.7, 131.0, 131.0, 138.5, 166.0, 169.9, 174.6; ESI–MS: m/z 664 $[M+Na]^+$, found 664.3953, $C_{36}H_{55}N_3O_7Na$ requires 664.3938. Anal. Calcd. for $C_{36}H_{55}N_3O_7{\cdot}H_2O$: C, 65.5; H, 8.65; N, 6.37. Found: C, 65.13; H, 8.41; N, 6.29.

**Glycine methyl 3α–(3–dimethoxyformylbenzoyloxy)–12α–pyridine–4–carboxy–5β–deoxycholan–24–oic hydrazide (23)**

Glycine methyl 3α–(3–dimethoxyformylbenzoyloxy)–12α–pyridine–4–carboxy–5β–deoxycholan–24–oate (1.14 g, 1.53 mmol) was dissolved in MeOH (10 ml). Hydrazine monohydrate (15.3 mmol, 764 mg) was added and the reaction mixture stirred at room temperature for 2 hours. Following aqueous work–up and trituration with $Et_2O$ (**23**) was obtained as a pure beige solid (1.08 g, 95%), $\nu_{max}/cm^{-1}$ ($CDCl_3$) 3440, 3336, 1714, 1672; $^1H$ NMR ($CDCl_3$) 0.79 (d, J = 6.6 Hz, 3H), 0.81 (s, 3H), 0.96 (s, 3H), 3.31 (s, 6H), 3.85 (d, J = 5.4 Hz, 2H), 3.85 (brs, 2H), 4.88 (tt, J = 4.8, 10.9 Hz, 1H), 5.39 (m, 1H), 5.4 (s, 1H), 6.26 (brs, 1H), 7.41 (t, J = 7.7 Hz, 1H), 7.61 (d, J = 7.7 Hz, 1H), 7.61 (brs, 1H), 7.79 (dt, J = 1.3, 7.7 Hz, 1H), 7.88 (dd, J = 1.6, 4.4 Hz, 2H), 7.98 (s, 1H), 8.83 (dd, J = 1.6, 4.4 Hz, 2H); $^{13}C$ NMR ($CDCl_3$) 12.6, 15.3, 17.7, 23.1, 23.5, 25.9, 26.0, 26.6, 26.8, 27.4, 31.3, 32.3, 33.2, 34.1, 34.7, 34.9, 35.7, 41.8, 41.9, 45.6, 48.2, 50.1, 52.8, 65.9, 74.6, 77.7, 102.6, 122.8, 127.8, 128.3, 129.5, 130.8, 131.1, 137.9, 138.6, 150.8, 164.4, 165.9, 169.7, 174.0; ESI–MS: m/z 747 $[M+H]^+$, 769 $[M+Na]^+$, found 747.4318, $C_{42}H_{59}N_4O_8$ requires 747.4333. Anal. Calcd. for $C_{42}H_{58}N_4O_8{\cdot}0.5\ H_2O$: C, 66.75; H, 7.81; N, 7.41, Found: C, 66.51; H, 7.77; N, 7.25.

**3β–(3–dimethoxyformylbenzoyloxy)–12α–pyridine–4–carboxy–5β–deoxycholan–24–oic hydrazide (25)**

(**24**) was prepared according to the literature procedure. The general procedures were then employed to form the dimethyl acetal hydrazide. Methyl 3β–(3–dimethoxyformylbenzoyloxy)–12α–pyridine–4–carboxy–5β–deoxycholan–24–oate. (950 mg, 1.38 mmol)

was dissolved in distilled MeOH (10 ml). Hydrazine monohydrate (27.5 mmol, 1.38 g) was added and the reaction mixture stirred at room temperature for 48 hours, The white foam obtained following aqueous work–up was purified by column chromatography (7%MeOH/$CHCl_3$) to furnish (**25**) as a white foam (800 mg, 84.2%), $R_f$ = 0.34 (7%MeOH/$CHCl_3$), $\nu_{max}/cm^{-1}$ ($CDCl_3$) 3448, 1714, 1673; $^1H$ NMR ($CDCl_3$) 0.78 (d, J = 6.5 Hz, 3H), 0.81 (s, 3H), 1.01 (s, 3H), 3.31 (s, 6H), 5.18 (brs, 1H), 5.37 (brs, 1H), 5.40 (s, 1H), 6.69 (br, 1H), 7.42 (t, J = 7.7 Hz, 1H), 7.63 (d, J = 7.7 Hz, 1H), 7.84 (dd, J = 1.6, 6.0 Hz, 2H), 7.95 (dt, J = 1.3, 7.7 Hz, 1H), 8.06 (t, J = 1.3 Hz, 1H), 8.84 (dd, J = 1.6, 6.0 Hz, 2H); $^{13}C$ NMR ($CDCl_3$) 12.6, 17.6, 23.5, 23.7, 25.0, 25.9, 26.0, 26.3, 27.4, 30.6, 30.7, 31.3, 31.4, 34.1, 34.4, 34.9, 35.6, 37.5, 45.6, 48.3, 50.1, 52.8, 71.0, 77.7, 102.6, 122.6, 128.0, 128.3, 129.5, 131.0, 137.8, 138.6, 150.9, 164.3, 165.7, 174.0; ESI–MS: m/z 690 $[M+H]^+$, 712 $[M+Na]^+$, found 712.3921, $C_{40}H_{55}N_3O_7Na$ requires 712.3938. Anal. Calcd. for $C_{40}H_{55}N_3O_7 \cdot H_2O$: C, 67.89; H, 8.06; N, 5.94. Found: C, 67.97; H, 7.89; N, 5.95.

**3α–(3–dimethoxyformylbenzoyloxy)–12α–hydroxy–5β–deoxycholan–24–oic hydrazone dimer (26)**

(**15**) (20 mg, 0.034mmol) was dissolved in dry DCM (6.85 ml) to afford a 5mM solution. Trifluoroacetic acid (290 µL) was then added and stirring continued overnight after which time excess TEA was added to neutralise the acid. The volume of the solution was reduced to ~ 0.5 ml using a stream of $N_2$ and a few drops of MeOH added to prevent precipitation of product. The crude product was then purified using preparative TLC plates ($SiO_2$) with DCM/EtOH/$NH_3$ (200:8:1) as eluant to furnish the dimer as a glassy solid (15 mg, 84%), $^1H$ NMR ($CDCl_3$) 0.68 (s, 3H, 18–Me), 0.91 (s, 3H, 19–Me), 1.19 (d, *J* = 6.0 Hz, 3H, H–21), 2.57 (m, 1H, 23–H), 2.96 (m, 1H, 23–H), 3.90 (m, 1H, 12βH), 4.87 (tt, *J* = 5.2, 10.1 Hz, 1H, 3βH), 7.30 (d, *J* = 7.7 Hz, 1H, $H_\delta$), 7.42 (t, *J* = 7.7 Hz, 1H, $H_\varepsilon$), 7.55 (s, 1H, $H_\mu$), 8.08 (d, *J* = 7.7 Hz, 1H, $H_\eta$), 8.51 (s, 1H, $H_\gamma$), 9.34 (brs, 1H, $NH_{cis}$); $^{13}C$ NMR ($CDCl_3$) 12.9, 18.4, 23.3, 23.9, 26.1, 27.0, 27.7, 29.0, 29.6, 31.6, 32.3, 33.7, 34.3, 35.1, 35.5, 36.1, 42.0, 44.9, 45.9, 46.5, 48.2, 73.0, 76.0, 124.7, 128.7, 131.3, 131.4, 133.6, 133.9, 141.8, 165.6, 177.4; ESI–MS: m/z 1063 $[M+Na]^+$, found 1063.6417, $C_{64}H_{88}N_4O_8$ requires 1063.6494.

**3α–(3–dimethoxyformylbenzoyloxy)–12α–pyridine–4–carboxy–5β–deoxycholan–24–oic hydrazone dimer (27)**

As for **26**. (**6**) (20 mg, 0.029 mmol) was dissolved in dry DCM (5.80 ml) to afford a 5 mM solution. Preparative TLC furnished the product as a white solid (14 mg, 83%) after using (5%MeOH/DCM) as the eluant. $\nu_{max}/cm^{-1}$ ($CDCl_3$) 1717; $^1H$ NMR ($CDCl_3$) 0.87 (s, 3H, 18–Me), 0.95–0.97 (m, 6H, 21–Me, 19–Me), 4.91 (tt, *J* = 5.3, 10.8 Hz, 1H, 3βH), 5.52 (m, 1H, 12βH), 7.43 (t, *J* = 7.7 Hz, 1H, $H_\varepsilon$), 7.85 (s, 1H, $H_\gamma$), 7.87 (d, *J* = 7.7 Hz, 1H, $H_\eta$), 7.90 (d, *J* = 5.9 Hz, 2H, β–pyridyl), 8.01 (d, *J* = 7.7 Hz, 2H, $H_\delta$), 8.62 (s, 1H, $H_\theta$), 8.65 (d, *J* = 5.9 Hz, 2H, α–pyridyl), 10.85 (s, 1H, $NH_{trans}$); $^{13}C$ NMR ($CDCl_3$) 12.7, 18.0, 22.9, 23.3, 25.8, 25.9, 26.3, 26.4, 27.2, 30.6, 30.9, 32.1, 33.7, 34.3, 34.6, 35.6, 41.4, 45.3, 47.2, 50.6, 74.3, 78.1, 123.1, 127.6, 128.9, 130.9, 131.1, 131.7, 132.3, 134.7, 138.7, 148.2, 150.3, 163.6, 164.9, 171.2; ESI–MS: m/z 1251 $[M+H]^+$, 1273 $[M+Na]^+$, found 1251.7046, $C_{76}H_{95}N_6O_{10}$ requires 1251.7104.

**3α–(4–dimethoxformylbenzoyloxy)–12α–pyridine–4–carboxy–5β–deoxycholan–24–oic hydrazone dimer (28)**

As for **26**. (**11**) (20 mg, 0.029 mmol) was dissolved in dry DCM (5.80 ml) to afford a 5mM solution of the monomer. Preparative TLC furnished (**28**), (9.2 mg, 51%) and (**29**) (8.9 mg, 49%) using (200:10:1 DCM/EtOH/$NH_3$) as eluant; $\nu_{max}/cm^{-1}$ ($CDCl_3$) 1716, 1674; $^1H$ NMR ($CDCl_3$) 0.84 (m, 6H, 18, 21–Me), 1.02 (s, 3H, 19–Me), 2.33 (m, 1H, 23–H), 2.75 (m, 1H, 23–H), 4.75 (m, 1H, 3βH), 5.33 (m, 1H, 12βH), 7.19 (d, J = 8.2 Hz, 2H, $H_\varepsilon$), 7.61 (d, J = 8.2 Hz, 2H, $H_\delta$), 7.64 (s, 1H, $H_\mu$), 7.68 (d, J = 5.0 Hz, 2H, β–pyridyl), 8.30 (d, J = 5.0 Hz, 2H, α–pyridyl), 9.17 (br, 1H, $NH_{cis}$); $^{13}C$ NMR ($CDCl_3$) 12.5, 17.5, 23.2, 23.4, 25.7, 25.9, 26.7, 27.0, 27.3, 29.1, 30.6, 31.3, 32.4, 34.4, 34.5, 34.8, 35.9, 41.9, 45.6, 47.5, 49.9, 76.1, 78.4, 122.3, 126.3, 129.7, 130.9, 131.9, 132.3, 137.3, 142.0, 150.3, 164.3, 165.9, 176.6; ESI–MS: m/z 1251 $[M+H]^+$, 626 $[M+2H]^{2+}$.

**3α–(4–dimethoxyformylbenzoyloxy)–12α–pyridine–4–carboxy–5β–deoxycholan–24–oic hydrazone trimer (29)**

As for **26**. $\nu_{max}/cm^{-1}$ ($CDCl_3$) 1718, 1668; $^1H$ NMR ($CDCl_3$) 0.88 (s, 3H, 18–Me), 0.97 (s, 3H, 19–Me), 1.03 (d, J = 6.4 Hz, 3H, 21–Me), 2.55 (m, 1H, 23–H), 2.69 (m, 1H, 23–H), 4.81 (m, 1H, 3βH), 5.48 (m, 1H, 12βH), 7.69 (m, 4H, $H_{\delta,\varepsilon}$), 7.86 (s, 1H, $H_\mu$), 7.93 (d, J = 5.0 Hz, 2H, β–pyridyl), 8.75 (d, J = 5.0 Hz, 2H, α–pyridyl), 11.10 (br, 1H, $NH_{cis}$); $^{13}C$ NMR ($CDCl_3$) 12.6, 18.1, 22.7, 25.6, 25.9, 26.1, 26.3, 27.5, 29.3, 30.6, 31.8, 31.9, 31.9, 33.7, 34.3, 34.6, 35.2, 35.5, 41.2, 45.3, 47.3, 49.9, 74.5, 77.7, 122.8, 126.9, 128.8, 129.6, 130.9, 138.1, 138.2, 143.4, 150.8, 164.1, 165.3, 178.4 ; ESI–MS: m/z 1877 $[M+H]^+$, 939 $[M+2H]^{2+}$.

**3β–(3–dimethoxyformylbenzoyloxy)–12α–pyridine–4–carboxy–5β–deoxycholan–24–oic hydrazone dimer (30)**

As for **26**. (**25**) (20 mg, 0.029 mmol) was dissolved in dry DCM (5.80 ml) to afford a 5mM solution of the monomer. Trifluoroacetic acid (290 µL) was added and stirring continued for 24 hours after which time excess TEA was added to neutralise the acid. Preparative TLC furnished the product as a frosted solid (16.3 mg, 90%) using (200:10:1 DCM/EtOH/$NH_3$) as eluant; $^1H$ NMR ($CDCl_3$) 0.83 (s, 3H, 18–Me), 0.87 (d, J = 6.8 Hz, 2H, 21–Me), 1.07 (s, 3H, 19–Me), 3.16 (m, 1H), 5.22 (m, 1H, 3αH), 5.39 (m, 1H, 12βH), 7.50 (m, 2H, $H_{\varepsilon,\eta}$), 7.79 (s, 1H, $H_\mu$), 8.04 (dt, J = 1.9, 6.4 Hz, 1H, $H_\delta$), 8.16 (d, J = 5.9 Hz, 2H, β–pyridyl), 8.60 (d, J = 5.9 Hz, 2H, α–pyridyl), 8.69 (s, 1H, $H_\gamma$), 9.58 (s, 1H, $NH_{cis}$); $^{13}C$ NMR ($CDCl_3$) 12.4, 17.6, 23.5, 23.9, 25.3, 25.5, 25.6, 26.2, 26.6, 27.4, 31.0, 31.1, 31.4, 33.4, 34.1, 34.5, 35.7, 37.6, 45.1, 45.4, 49.0, 71.0, 123.2, 125.1, 128.8, 130.4, 132.2, 133.6, 134.4, 137.6, 142.0, 150.7, 164.5, 164.8, 177.8; ESI–MS: m/z 1251 $[M+H]^+$, 626 $[M+H]^{2+}$.