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Synthesis and binding ability of bile acid-based receptors for recognition of flavin analogues

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Abstract—Novel cholaphanes **6a**,**b**, based on lithocholic and deoxycholic acids, were synthesised through **3a**,**b** by a sequence of reactions involving Cs-salt methodology of macrocyclisation. Cholaphanes **6a**,**b** and acyclic steroidal receptors **3a**,**b** bind flavin analogues via three hydrogen bonds in $CHCl_3$.

1. Introduction

Enzymes containing riboflavin as cofactor play very important roles in cellular metabolism such as dehydrogenation of NAD(P)H and D-amino acids, hydroxylation of aromatic substrates, activation of molecular oxidation, etc.[1] The major reason for this is the unique chemical property of the isoalloxazine hetero-aromatic system present in riboflavin, which undergoes reversible oxidation–reduction involving one- or two-electron transfer. The apoprotein of the enzyme acts as a modulator to change its reduction potential. Considerable efforts have been made to design model systems to understand the role of the hydrogen-bond interactions and their effect on the regulation of the flavin activity.[2]

Rotello and Cooke have studied the effect of hydrogen bond interaction on the redox potential of isoalloxazine derivatives in aprotic solvents using synthetic receptors based on 2,6-diaminopyridine to reproduce the specific hydrogen bond patterns present in flavoenzymes.[3] The results show that in addition to hydrogen bonding, the hydrophobicity of the system also plays a significant role in the stabilisation of the flavin radical anion. Yano and co-workers have utilised melamine derivatives bearing a guanidinium ion to show the effect of the hydrogen bond and electrostatic interactions of guanidinium ion on the redox potential of isoalloxazine derivatives.[4]

To investigate the effect of these factors in more detail, we have designed and synthesised various acyclic and cyclic bile acid-based 2,6-diaminopyridine systems and studied their binding behaviour with isoalloxazine derivatives. Although bile acids have been used for the design of supramolecular hosts for carbohydrates,[5] nucleic acid bases[6] and for anion recognition,[7] receptor molecules based on bile acid for flavin coenzyme have not been reported so far. These systems provide well-defined binding sites in terms of hydrogen bond and hydrophobic interactions for the regulation of the binding and reduction potential of isoalloxazine–receptor interactions.

2. Results and discussion

Cholaphanes **6a**,**b** involving head-to-head combination of bile acids **1a**,**b** were synthesised through a five-step synthetic route (Scheme 1). For the synthesis of **6b**, the 3α- and 12α-hydroxy groups of deoxycholic acid (3α,12α-dihydroxy-5β-cholan-24-oic acid) were first protected by formylation with formic acid (100%). Attempts to condense the formylated compound with 2,6-diaminopyridine in the presence of DCC proved futile. However, condensation of the acid chloride of 3α,12α-*O*-diformyldeoxycholic acid **2b** with 2,6-diaminopyridine in a molar ratio 2.2:1 in dry THF yielded *N*,*N*′-bis(3α,12α-*O*-diformyldeoxycholyl)-2,6-diaminopyridine **3b** in 77% yield. Hydrolysis of the diamide with LiOH in THF–H_2O resulted in *N*,*N*′-bisdeoxycholyl-pyridine-2,6-diamine **4b** in 80% yield. The selective bromoacetylation of both the equatorial 3-OH groups was achieved in 70% yield by stirring a mixture of the hydrolysed product (1 equiv), bromoacetyl bromide (2.0 equiv) and K_2CO_3 in dry $CHCl_3$ for 10 min. The crucial cyclisation step was accomplished by using the Cs-salt methodology.[8] The synthetic route leading to cholaphane **6a** is similar to the one described above except the bromoacetylation step, where no selective bromoacetylation was required.

Keywords: Cholaphanes; Flavoenzyme; Flavin mimic; Isoalloxazines; Molecular recognition.
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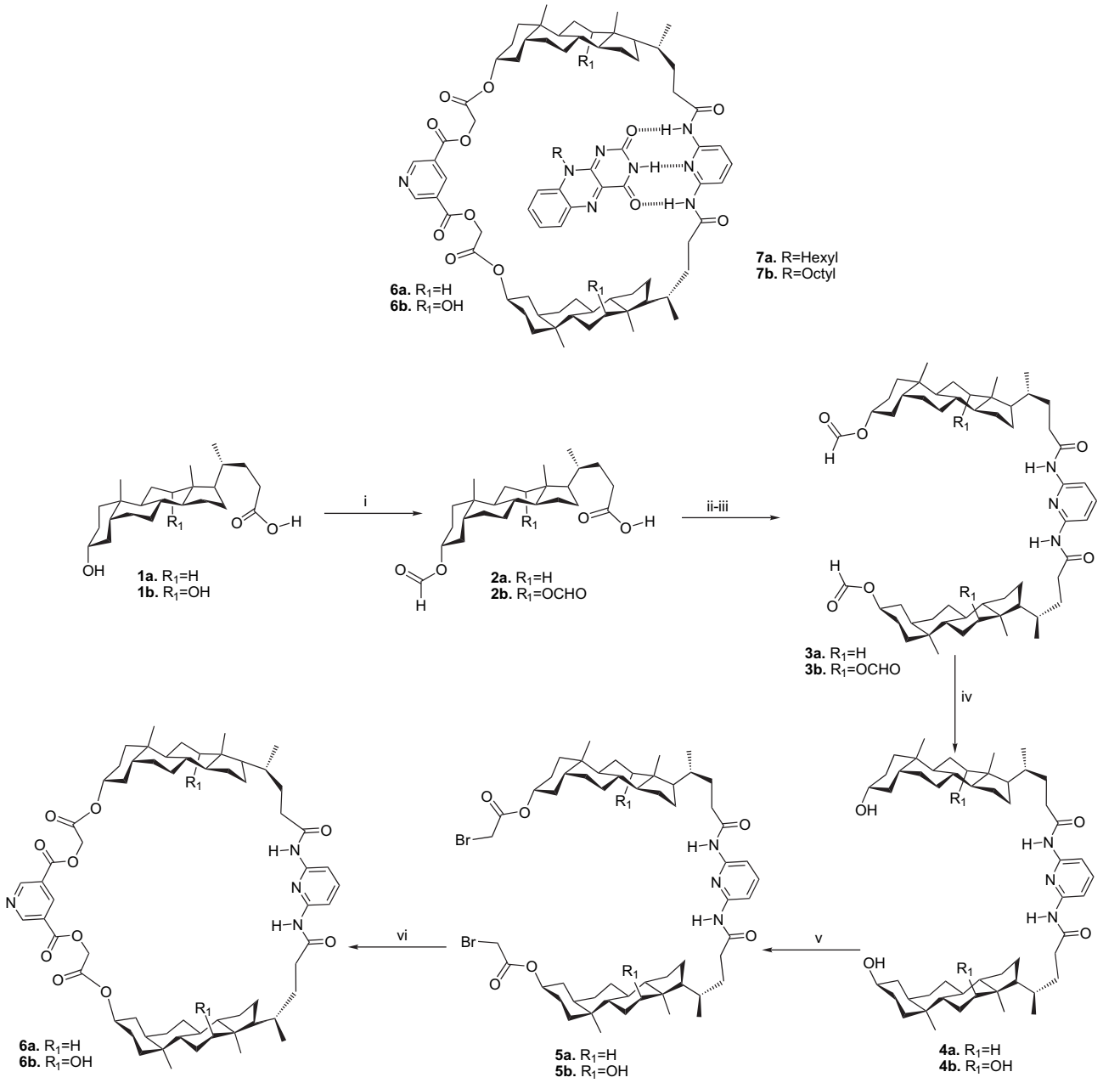

Scheme 1. Reagents and conditions (and yields): (i) HCOOH, 60 °C, 4 h, (98%); (ii) $SOCl_2$, benzene, 4 h, reflux, (~100%); (iii) 2,6-diaminopyridine, triethylamine, THF, 0–5 °C, 12 h, (80%) for **3a** and (77%) for **3b**; (iv) LiOH, THF–H_2O, rt, 24 h, (84%) for **4a** and (80%) for **4b**; (v) $BrCH_2COBr$, anhydrous K_2CO_3, $CHCl_3$, 55–60 °C, 10 min, (85%) for **5a** and (70%) for **5b**; (vi) bis-caesium pyridine 2,6-dicarboxylate, DMF, 12 h, rt, (69%) for **6a** and (68%) for **6b**.

Flavin analogues (Fig. 1) were prepared by the selective monoalkylation of 1,2-phenylenediamine followed by cyclisation of the 2-amino-*N*-alkylanilines with alloxan in acidic conditions resulting in 10-hexyl- and 10-octylisoalloxazines **7a**,**b**.[9] Binding behaviour of the receptors (**3a**,**b**, **6a**,**b**) with **7a**,**b** was examined by ^{1}H NMR spectroscopy. As a typical example, the complexation between receptor **3a** and flavin analogue **7a** was studied by ^{1}H NMR titration experiment in $CDCl_3$, with receptor **3a** (0.01 M) against various aliquots of 0.06 M solution of **7a**. The chemical shifts of the amide protons were recorded at each concentration until saturation of chemical shifts was observed. Each titration was carried out in duplicate. Analysis of the saturation data with WinEQNMR software, a nonlinear regression curve-fitting program,[10] revealed 1:1 complexation with a binding constant of 600 M^{-1}. Job's plot further confirmed the 1:1 binding with maximum complexation at 0.5 mole fraction.

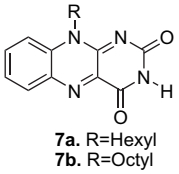

Figure 1. Flavin analogues.

The binding constants for the complexation of the receptors with flavin analogues are listed in Table 1. In case of acyclic receptors **3a**,**b**, **7a** shows better binding behaviour than **7b**.

Table 1. Binding constants K_a (M^{-1}) for complexation of flavin analogues with bile acid-based receptors[a]

Receptor	Flavin analogues, K_a	
	7a	**7b**
3a	600	250
3b	400	240
6a	60	50
6b	110	110

[a] Determined in $CDCl_3$ at 25 °C, errors estimated to be ≤10%.

This may be because of the better fit of **7a** with receptors **3a**,**b** due to the smaller size of the hexyl group. Also, cholaphanes **6a**,**b** have smaller binding constants than receptors **3a**,**b**. The smaller K_a values of the cholaphanes **6a**,**b** as compared to **3a**,**b** can be attributed to the steric hindrances faced by the flavin analogues towards cholaphanes during complexation. Moreover, cholaphane **6b** having two hydroxyl groups at 12α- and 12α′-positions shows larger binding constant than cholaphane **6a**, which may be due to the additional hydrogen bond interactions.

3. Conclusion

In conclusion, we have developed the synthesis of novel cholaphanes in view of mimicking the specific hydrogen bond patterns present in the flavoenzyme. The ability of the cholaphanes to bind to flavin analogues was overshadowed by the acyclic diamides because of steric hindrance; however, they showed a notable ability to bind flavin analogues. We feel that such mimicry has not been exploited before. The cholaphanes can also be converted into 1,4-dihydropyridine system by standard methods. The structural features of these macrocyclic steroidal dihydropyridine systems help to bind flavin analogues and may mimic the role played by oxidoreductases using the NADH–flavin coupled system. Work in this direction is underway and will be reported in due course.

4. Experimental

4.1. General

Melting points are uncorrected. IR spectra were recorded on a Nicolet Protégé 460 FTIR Spectrometer, using potassium bromide pellets. ^1H and ^{13}C NMR spectra were recorded on a Bruker Spectrospin DPX 300. Tetramethylsilane was used as internal reference and the chemical shifts are expressed as displacement (δ) in parts per million downfield from tetramethylsilane. High-resolution mass spectra (ES) were recorded on a VG-Fisons 'Autospec' spectrometer. Column chromatography was carried out using Spectrochem silica gel 230–400 mesh for flash chromatography. The solid compounds were dried under vacuum in the presence of P_2O_5.

4.1.1. 3α-*O*-Formyllithocholic acid 2a. Lithocholic acid (3α-hydroxy-5β-cholan-24-oic acid) **1a** (3 g, 7.96 mmol) was dissolved in 20 ml of 100% formic acid. The solution was stirred at 60 °C for 4 h. The reaction mixture was cooled to room temperature and was added dropwise to water (100 ml) with stirring to get white precipitate. The precipitate was filtered and vacuum dried to give 3.15 g of **2a** (98%). Mp 127–128 °C; IR ν_{max} (KBr)/cm^{-1} 3446, 2948, 1725, 1450, 1182; ^1H NMR (300 MHz, $CDCl_3$, TMS) δ 0.65 (s, 3H, 18-Me), 0.91 (br s, 6H, 21-Me and 19-Me), 1.08–2.40 (28H, steroidal H), 4.85 (m, 1H, 3β-H), 8.04 (s, 1H, –OCHO); ^{13}C NMR (75 MHz, $CDCl_3$, TMS) δ 12.02, 18.21, 20.81, 23.29, 24.14, 26.27, 26.60, 26.95, 28.12, 30.72, 31.00, 32.18, 34.55, 34.93, 35.27, 35.76, 40.09, 40.41, 41.88, 42.72, 55.92, 56.43, 74.41, 160.84, 180.39; ES-HRMS calcd for ($C_{25}H_{40}O_4$·Na) 427.2824, found 427.2823.

4.1.2. *N*,*N*′-Bis(3α-*O*-formyllithocholyl)-pyridine-2,6-diamine 3a. Freshly distilled thionyl chloride (1 ml) was added dropwise to a solution of **2a** (4 g, 9.9 mmol) in 20 ml of dry benzene and a drop of DMF at 0 °C. The reaction mixture was stirred at 60 °C for 4 h and then evaporated to dryness in vacuo. Dry benzene (10 ml) was added and the syrup evaporated twice to completely remove leftover thionyl chloride. The acid chloride was dissolved in dry THF (10 ml) and added dropwise to a solution of 2,6-diaminopyridine (0.44 g, 4.03 mmol) and triethylamine (1.4 ml) in dry THF (15 ml) at 0 °C. After the reaction was completed, the solution was concentrated, which was then extracted with chloroform, dried, and evaporated to dryness. The residue was purified by flash chromatography (elution with EtOAc–hexane 1:6) to give 2.84 g of **3a** (80%). Mp 105–107 °C; IR ν_{max} (KBr)/cm^{-1} 3326, 2937, 2863, 1720, 1588, 1507; ^1H NMR (300 MHz, $CDCl_3$, TMS) δ 0.65 (s, 6H, 18-Me), 0.93 (s, 6H, 19-Me), 0.96 (br s, 6H, 21-Me), 1.08–2.44 (56H, steroidal H), 4.85 (m, 2H, 3β-H), 7.67–7.72 (m, 3H, 2×–NHCO– and Py-4-H), 7.89 (d, 2H, *J*=8.1 Hz, Py-3,5-H), 8.04 (s, 2H, –OCHO); ^{13}C NMR (75 MHz, $CDCl_3$, TMS) δ 12.07, 18.40, 20.83, 23.30, 24.18, 26.29, 26.63, 26.96, 28.24, 30.88, 31.41, 32.20, 34.75, 34.94, 35.44, 35.77, 40.13, 40.43, 41.88, 42.76, 56.02, 56.46, 74.39, 109.37, 140.99, 149.43, 160.77, 171.97; ES-HRMS calcd for ($C_{55}H_{83}N_3O_6$·H)$^+$ 882.6360, found 882.6361.

4.1.3. *N*,*N*′-Bislithocholyl-pyridine-2,6-diamine 4a. To a solution of **3a** (2 g, 2.26 mmol) in THF–H_2O (10:1, 25 ml), was added LiOH (0.2 g, 4.65 mmol). The solution was stirred at room temperature for 12 h. The solution was evaporated and the residue was extracted with chloroform, dried over sodium sulfate and purified by flash chromatography (elution with EtOAc–hexane 1:5) to give 1.57 g of **4a** (84%). Mp 132–134 °C; IR ν_{max} (KBr)/cm^{-1} 3422, 2934, 1683, 1585; ^1H NMR (300 MHz, $CDCl_3$, TMS) δ 0.58 (s, 6H, 18-Me), 0.84 (s, 6H, 19-Me), 0.88 (d, 6H, *J*=6.1 Hz, 21-Me), 1.0–2.3 (56H, steroidal H), 3.56 (m, 2H, 3β-H), 7.53 (s, 2H, 2×–NHCO–), 7.62 (t, 1H, *J*=8.0 Hz, Py-4-H), 7.82 (d, 2H, *J*=8.0 Hz, Py-3,5-H); ^{13}C NMR (75 MHz, DMSO, TMS) δ 11.90, 18.31, 20.42, 23.28, 23.87, 26.17, 26.90, 27.76, 30.37, 31.24, 33.19, 34.21, 35.03, 35.39, 36.28, 38.85, 38.94, 40.12, 41.53, 42.28, 55.62, 56.10, 69.88, 108.89, 139.81, 150.37, 172.59; ES-HRMS calcd for ($C_{53}H_{83}N_3O_4$·H)$^+$ 826.6462, found 826.6434.

4.1.4. *N*,*N*′-Bis(3α-*O*-bromoacetyllithocholyl)-pyridine-2,6-diamine 5a. Compound **4a** (1.5 g, 1.81 mmol) was stirred at 55–60 °C in dry chloroform (20 ml) until it was

completely dissolved. Anhydrous K_2CO_3 was then added. To this, a solution of bromoacetyl bromide (1.09 g, 5.44 mmol) in dry chloroform (10 ml) was added. After 10 min, the heating was stopped, ice-cold water (20 ml) was added and the organic layer was separated, which was subsequently dried over Na_2SO_4 and evaporated. The crude product was purified by flash chromatography (elution with EtOAc–hexane 1:6) to give 1.65 g of **5a** (85%). Mp 152–154 °C; IR ν_{max} (KBr)/cm^{-1} 3337, 2936, 1734, 1702, 1586, 1284; ^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ 0.65 (s, 6H, 18-Me), 0.86 (s, 6H, 19-Me), 0.93 (d, 6H, J=6.2 Hz, 21-Me), 1.08–2.43 (56H, steroidal H), 3.8 (s, 4H, –$COCH_2$Br), 4.79 (m, 2H, 3β-H), 7.52 (s, 2H, 2×–NHCO–), 7.69 (t, 1H, J=7.9 Hz, Py-4-H), 7.88 (d, 2H, J=7.9 Hz, Py-3,5-H); ^{13}C NMR (75 MHz, $CDCl_3$, TMS) δ 12.04, 18.39, 20.81, 23.25, 24.15, 26.34, 26.94, 28.23, 29.66, 30.22, 31.38, 31.89, 31.96, 34.62, 34.87, 35.42, 35.73, 40.09, 40.38, 41.84, 42.73, 56.02, 56.42, 76.6, 109.31, 140.88, 149.40, 166.49, 171.91; ES-HRMS calcd for $(C_{57}H_{85}N_3O_6Br_2\cdot H)^+$ 1066.4883, found 1066.4932.

4.1.5. Bis(3α-*O*-hydroxyacetyllithocholyl)-pyridine-2,6-diamine cyclic 3,5-pyridine dicarboxylate (lithocholaphane) 6a. Compound **5a** (0.99 g, 0.92 mmol) was dissolved in 250 ml of dry DMF and to this was added an equivalent amount of bis-caesium 3,5-pyridine dicarboxylate (0.39 g, 0.92 mmol). The reaction mixture was stirred at room temperature for 12 h. Then, DMF was evaporated under vacuo. The crude product obtained was dissolved in chloroform (30 ml) and washed with brine (10 ml), dried (Na_2SO_4), and evaporated to dryness under vacuum. The impure cholaphane was then purified by flash chromatography (elution with EtOAc–hexane 1:3) to give 0.75 g of **3a** (69%). Mp 175–177 °C; IR ν_{max} (KBr)/cm^{-1} 3369, 2936, 1741, 1702, 1586, 1287, 1205; ^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ 0.59 (s, 6H, 18-Me), 0.85 (s, 6H, 19-Me), 0.88 (d, 6H, J=6.2 Hz, 21-Me), 1.01–2.29 (56H, steroidal H), 4.72 (m, 2H, 3β-H), 4.80 (s, 4H, –$COCH_2$–), 7.57 (s, 2H, 2×–NHCO–), 7.62 (t, 1H, J=8.0 Hz, Py-4-H), 7.81 (d, 2H, J=8.0 Hz, Py-3,5-H), 8.91 (s, 1H, Py′-4-H), 9.42 (s, 2H, Py′-2,6-H); ^{13}C NMR (75 MHz, $CDCl_3$, TMS) δ 11.99, 18.47, 20.84, 23.21, 24.07, 26.19, 26.35, 26.81, 28.20, 31.01, 31.86, 33.44, 34.47, 34.88, 35.65, 40.16, 40.53, 41.76, 42.69, 54.88, 56.52, 56.69, 62.30, 76.58, 109.24, 125.37, 138.66, 140.92, 149.43, 154.76, 163.76, 163.65, 172.04; ES-HRMS calcd for $(C_{64}H_{88}N_4O_{10}\cdot H)^+$ 1073.6579, found 1073.6625.

4.1.6. 3α,12α-*O*-Diformyldeoxycholic acid 2b. Deoxycholic acid (3α,12α-dihydroxy-5β-cholan-24-oic acid) **1b** (3 g, 7.64 mmol) was dissolved in 20 ml of 100% formic acid. The solution was stirred at 60 °C for 4 h. The reaction mixture was cooled to room temperature and was added dropwise to water (100 ml) with stirring to get white precipitate. The precipitate was filtered and vacuum dried to give 3.35 g of **2b** (98%). Mp 173–175 °C; IR ν_{max} (KBr)/cm^{-1} 3446, 2948, 1725, 1721, 1450, 1182; ^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ 0.75 (s, 3H, 18-Me), 0.84 (d, 3H, J=6.2 Hz, 21-Me), 0.92 (s, 3H, 19-Me), 1.08–2.39 (26H, steroidal H), 4.85 (m, 1H, 3β-H), 5.25 (m, 1H, 12β-H), 8.03 (s, 1H, –OCHO), 8.13 (s, 1H, –OCHO); ^{13}C NMR (75 MHz, $CDCl_3$, TMS) δ 12.34, 17.44, 22.93, 23.44, 25.74, 25.90, 26.47, 26.78, 27.34, 30.48, 30.95, 32.09, 34.03, 34.20, 34.67, 34.78, 35.97, 41.73, 45.01, 47.35, 49.25, 73.21, 76.00, 160.60, 160.86, 180.09; ES-HRMS calcd for $(C_{26}H_{40}O_6\cdot K)$ 487.2462, found 487.2464.

4.1.7. *N*,*N*′-Bis(3α,12α-*O*-diformyldeoxycholyl)-pyridine-2,6-diamine 3b. Acid chloride of **2b** (4 g, 8.91 mmol) was prepared following the method described for the synthesis of **3a**. The acid chloride was dissolved in dry THF (10 ml) and added dropwise to a solution of 2,6-diaminopyridine (0.44 g, 4.03 mmol) and triethylamine (1.4 ml) in dry THF (15 ml) at 0 °C. The reaction was worked up as described above for **2b** and the residue obtained was purified by flash chromatography (elution with EtOAc–hexane 1:6) to give 3.02 g of **3b** (77%). Mp 154–155 °C; IR ν_{max} (KBr)/cm^{-1} 3334, 2946, 2869, 1722, 1586, 1506; ^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ 0.75 (s, 6H, 18-Me), 0.87 (d, 6H, J=6.0 Hz, 21-Me), 0.93 (s, 6H, 19-Me), 1.04–2.42 (52H, steroidal H), 4.84 (m, 2H, 3β-H), 5.26 (s, 2H, 12β-H), 7.59 (s, 2H, 2×–NHCO–), 7.69 (t, 1H, J=8.0 Hz, Py-4-H), 7.88 (d, 2H, J=8.0 Hz, Py-3,5-H), 8.03 (s, 2H, –OCHO), 8.14 (s, 2H, –OCHO); ^{13}C NMR (75 MHz, $CDCl_3$, TMS) δ 12.36, 17.60, 22.91, 23.43, 25.75, 25.80, 26.46, 26.74, 27.40, 30.56, 31.02, 32.07, 33.96, 34.18, 34.62, 34.88, 35.58, 41.70, 45.02, 47.45, 49.24, 74.04, 75.98, 109.32, 141.08, 149.24, 160.54, 160.59, 171.79; ES-HRMS calcd for $(C_{57}H_{83}N_3O_{10}\cdot H)^+$ 970.6157, found 970.6161.

4.1.8. *N*,*N*′-Bisdeoxycholyl-pyridine-2,6-diamine 4b. To a solution of **3b** (2 g, 2.06 mmol) in THF–H_2O (10:1, 25 ml), was added LiOH (0.4 g, 9.3 mmol). The solution was stirred at room temperature for 24 h. The solution was evaporated and the residue was extracted with chloroform, dried over sodium sulfate and purified by flash chromatography (elution with EtOAc–hexane 1:3) to give 1.42 g of **4b** (80%). Mp 176–178 °C; IR ν_{max} (KBr)/cm^{-1} 3420, 2932, 1680, 1586; ^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ 0.63 (s, 6H, 18-Me), 0.83 (s, 6H, 19-Me), 0.94 (d, 6H, J=6.0 Hz, 21-Me), 1.04–2.5 (52H, steroidal H), 3.54 (m, 2H, 3β-H), 3.9 (s, 2H, 12β-H), 7.60 (t, 1H, J=7.8 Hz, Py-4-H), 7.72 (d, 2H, J=7.8 Hz, Py-3,5-H), 8.91 (s, 2H, 2×–NHCO–); ^{13}C NMR (75 MHz, DMSO, TMS) δ 13.27, 17.89, 23.24, 23.89, 24.33, 26.57, 27.55, 27.80, 29.42, 31.01, 32.15, 33.74, 34.13, 34.62, 35.94, 36.47, 37.07, 42.41, 46.81, 48.28, 70.78, 71.86, 79.29, 108.78, 139.07, 151.18, 173.22; ES-HRMS calcd for $(C_{53}H_{83}N_3O_6\cdot H)^+$ 858.6360, found 858.6346.

4.1.9. *N*,*N*′-Bis(3α-*O*-bromoacetyldeoxycholyl)-pyridine-2,6-diamine 5b. Compound **4b** (1.5 g, 1.74 mmol) was stirred at 55–60 °C in dry chloroform (30 ml) until it was completely dissolved. Anhydrous K_2CO_3 was then added dropwise. To this, a solution of bromoacetyl bromide (0.7 g, 3.48 mmol) in dry chloroform (15 ml) was added. After 10 min, the heating was stopped, ice-cold water (20 ml) was added and the organic layer was separated, which was subsequently dried over Na_2SO_4 and evaporated. The crude product was purified by flash chromatography (elution with EtOAc–hexane 1:4) to give 1.33 g of **5b** (70%). Mp 162–165 °C; IR ν_{max} (KBr)/cm^{-1} 3423, 2939, 1734, 1585, 1287; ^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ 0.69 (s, 6H, 18-Me), 0.93 (s, 6H, 19-Me), 1.01 (d, 6H, J=5.8 Hz, 21-Me), 1.08–2.44 (52H, steroidal H), 3.79 (s, 4H, –$COCH_2$Br), 4.00 (s, 2H, 12β-H), 4.78 (m, 2H, 3β-H), 7.61 (s, 2H, 2×–NHCO–),

7.69 (t, 1H, J=7.9 Hz, Py-4-H), 7.88 (d, 2H, J=7.9 Hz, Py-3,5-H); ^{13}C NMR (75 MHz, $CDCl_3$, TMS) δ 12.68, 17.41, 19.28, 22.97, 23.51, 25.90, 26.19, 26.80, 27.41, 28.69, 30.45, 31.11, 33.60, 34.02, 34.44, 34.66, 35.00, 35.86, 41.74, 46.42, 47.10, 48.20, 71.37, 76.48, 109.26, 140.98, 149.21, 166.74, 171.94; ES-HRMS calcd for $(C_{57}H_{85}N_3O_8Br_2\cdot H)^+$ 1098.4782, found 1098.4810.

4.1.10. Bis(3α-*O*-hydroxyacetyldeoxycholyl)-pyridine-2,6-diamine cyclic 3,5-pyridine dicarboxylate (deoxycholaphane) 6b. Compound **5b** (0.8 g, 0.72 mmol) was dissolved in 250 ml of dry DMF and to this was added an equivalent amount of bis-caesium 3,5-pyridine dicarboxylate (0.30 g, 0.72 mmol). The reaction mixture was stirred at room temperature for 12 h. The reaction was worked up following the method described above for **5a**. The impure cholaphane obtained was purified by flash chromatography (elution with EtOAc–hexane 1:2) to give 0.54 g of **6b** (68%). Mp 170–171 °C; IR ν_{max} (KBr)/cm^{-1} 3423, 2936, 1740, 1702, 1586, 1288, 1207; ^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ 0.69 (s, 6H, 18-Me), 0.91 (s, 6H, 19-Me), 1.01 (d, 6H, J=5.6 Hz, 21-Me), 1.08–2.40 (52H, steroidal H), 3.98 (s, 2H, 12β-H), 4.83 (m, 2H, 3β-H), 4.88 (s, 4H, –$COCH_2$–), 7.69 (t, 1H, J=7.9 Hz, Py-4-H), 7.79–7.84 (m, 4H, Py-3,5-H and 2×–NHCO–), 8.99 (s, 1H, Py′-4-H), 9.50 (s, 2H, Py′-2,6-H); ^{13}C NMR (75 MHz, $CDCl_3$, TMS) δ 11.67, 16.58, 21.98, 22.54, 24.94, 25.28, 25.74, 26.41, 27.93, 29.83, 30.81, 32.12, 32.63, 32.98, 33.53, 33.65, 34.81, 40.68, 44.75, 45.37, 47.60, 61.28, 71.99, 75.27, 108.46, 124.42, 137.69, 139.95, 148.51, 153.85, 162.91, 165.72, 171.37; ES-HRMS calcd for ($C_{64}H_{88}N_4O_{12}$) 1104.6399, found 1104.6387.

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Supplementary data

^{13}C and ^{1}H NMR spectra of bile acid derivatives, binding isotherms and mass spectra are provided in supplementary data. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2006.06.029.

References and notes

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