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Bile acid-based receptors containing 2,6-bis(acylamino)pyridine for recognition of uracil derivatives

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Abstract—Hydrogen-bonding interactions of steroid-based cyclic and acyclic receptors containing 2,6-bis(acylamino)pyridine with uracil derivatives were studied in $CDCl_3$. Acyclic receptors show better binding behaviour as compared to cholaphanes with uracil derivatives.

The self-assembly of the two anti-parallel strands of DNA is mainly influenced by intermolecular forces which include aromatic π-stacking, hydrophobic forces, van der Waals forces and hydrogen-bonding interactions.[1] There has been considerable interest in recent years on the design of receptors for recognition of nucleobases with non-covalent interactions.[2,3] Rebek et al. introduced receptors having convergent functional groups based on Kemp's triacid for recognition of adenine derivatives.[4–9] His group also reported synthetic molecules capable of self-replication and autocatalysis.[10,11] Wilcox and Adrian prepared a receptor, having a carboxylic acid moiety with the Tröger's base spacer, capable of adenine binding with both Watson-Crick and Hoogsteen binding modes.[12]

Zimmerman and co-workers reported a molecular tweezer which complexes adenine through aromatic π-stacking and hydrogen bonding.[13]

Hamilton and Van Engen introduced a macrocycle, based on bis(acylamino)pyridine receptor, capable of binding thymine through hydrogen bonding and stacking forces.[14] Jorgenson and co-workers carried out Monte Carlo simulations with statistical perturbation theory to calculate free energy of binding in chloroform for 1-methyluracil with 2,6-diaminopyridine. The binding constant for these systems is small due to three alternate hydrogen bonds.[15,16] Sijbesma and Spek have extensively studied the complexation of diaminopyridine and diaminotriazine and their acylated derivatives with uracil/thymine derivatives through hydrogen bonding.[17] Interestingly, it was found that acylation of diaminopyridine and diaminotriazine had opposite effect on complex stabilities. High association constants were observed for complexes of 2,6-bis(acylamino)pyridines with uracil/thymine derivatives as equated to bis(acylamino)triazines. Moreover, the length of the alkyl chain of the 2,6-bis(acylamino)pyridines was found to affect the binding strength with uracil/thymine derivatives. Li et al. reported a fullerene-containing 2,6-bis(acylamino)pyridine receptor to bind uracil derivatives by hydrogen-bonding interaction with an association constant of 323 M^{-1} in $CDCl_3$.[18] It has been ascertained that 2,6-bis(acylamino)pyridine, having a donor–acceptor–donor (DAD) unit, complexes with an imide (e.g., uracil, succinimides, flavins, etc.) having acceptor–donor–acceptor unit (ADA) with association constants in $CDCl_3$ in the range of 50–500 M^{-1}, which is in accordance with the theoretical values predicted by Jorgenson et al.

The unique features of the bile acids in terms of their chiral, rigid framework and chemically different hydroxyl groups make them very appropriate compounds from the molecular engineering point of view.[19–21] Most recently, we introduced a new class of bile acid-based cyclic as well as acyclic receptors containing 2,6-bis(acylamino)pyridine for recognition of flavin analogues (Fig. 1).[22] A rigid and hydrophobic steroidal skeleton with flexible side chains for the acyclic receptor afforded ideal architecture for hydro-

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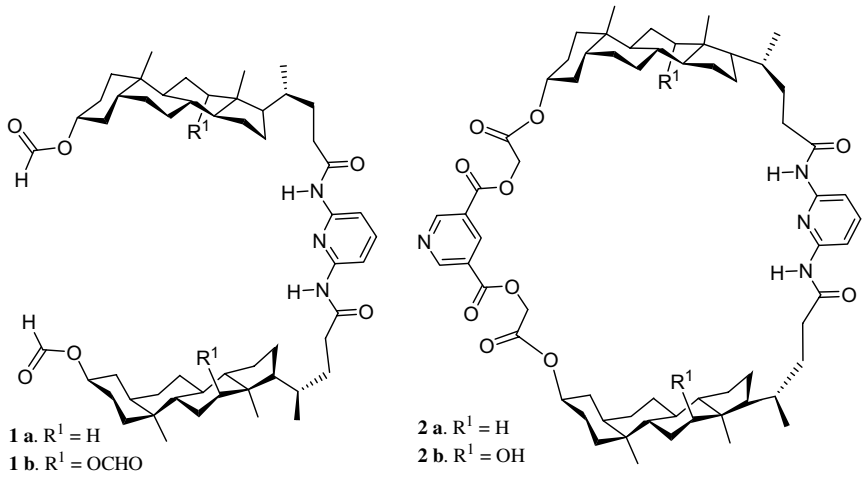
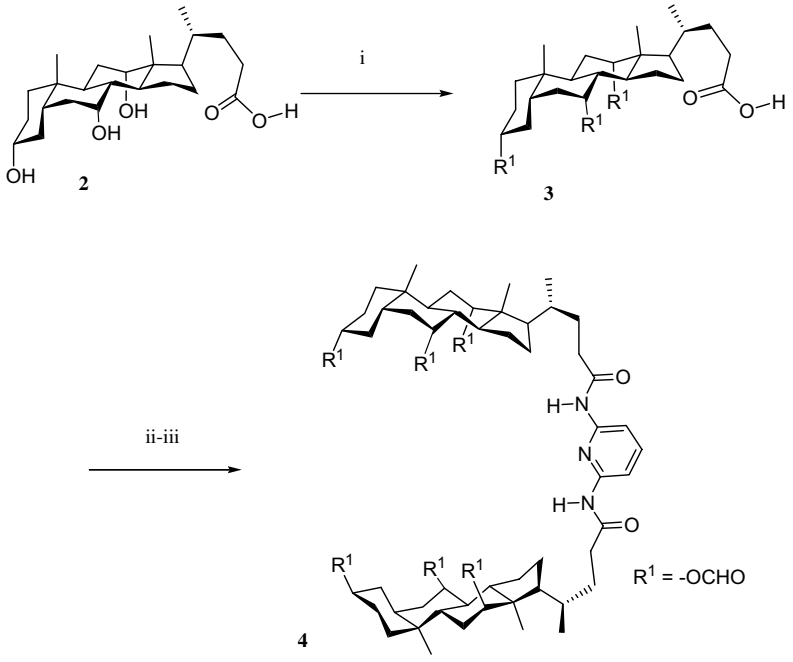


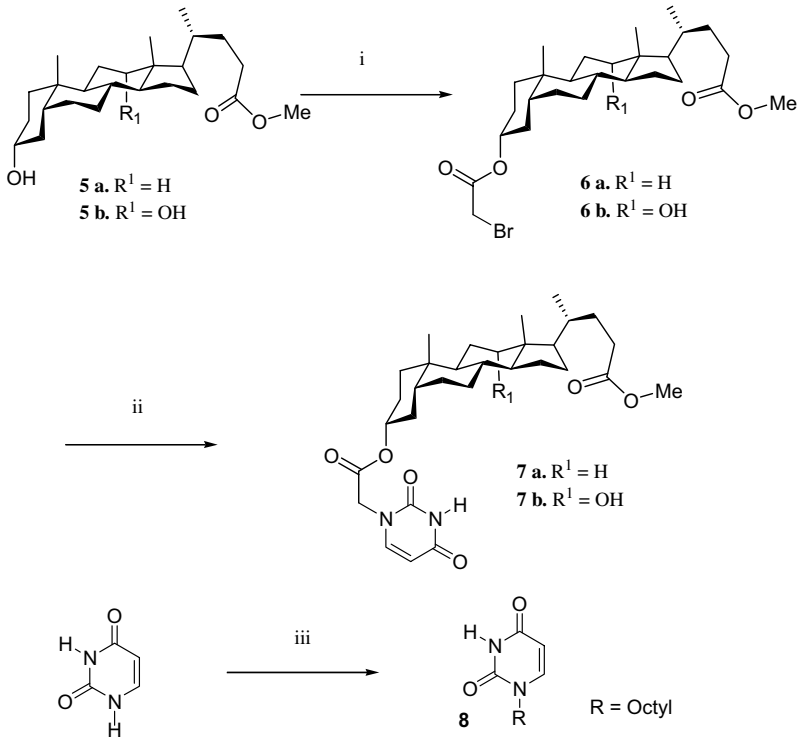
Figure 1. Steroid-based acyclic and cyclic receptors having 2,6-diaminopyridine unit.

gen-bond interactions with flavin analogues. Importantly, high association constants are observed for complexes of acyclic receptors with flavin analogues. However for the cholaphanes, due to steric hindrances, lower association constants were observed.

Nucleobases have been widely used as supramolecular motifs capable of forming strong hydrogen bonds to construct new biomaterials and complex nanostructures.[3,23–25] Earlier, we have reported the comparative binding ability of steroidal adenine with flavin and uracil derivatives.[26] In the present communication, we report the binding behaviour of acyclic and cyclic steroidal receptors containing 2,6-bis(acylamino)pyridine unit towards uracil derivatives in view of developing new steroidal materials involving DAP-uracil supramolecular motifs. The preparation of receptor **4** commenced with the formylation of cholic acid **1** with formic acid to give **3** in 98% yield (Scheme 1). Subsequent coupling of the acid chloride of **3** (2 equiv) with diaminopyridine in the presence of triethylamine gave the biscoupled product.[27] In general, nucleobases like uracil are scarcely soluble in chloroform. Attaching a long alkyl chain increases their solubility in non-polar solvents. Uracil derivatives were synthesized by selective *N*-1-alkylation of uracil with octyl bromide, methyl 3α-bromoacetyllithocholate and methyl 3α-bromoacetyldeoxycholate in the presence of potassium carbonate in dry DMF as described in (Scheme 2).[28]



Scheme 1. Reagents and conditions (and yields): (i), HCOOH, 60 °C, 4 h, (98%); (ii), $SOCl_2$, benzene, 60 °C, 4 h, (~100%); (iii), 2,6-diaminopyridine, triethylamine, THF, 0–5 °C, 12 h, (79%).



Scheme 2. Reagents and conditions (and yields): (i), $BrCH_2COBr$, anhydrous K_2CO_3, $CHCl_3$, rt for **6a** and 0 °C for **6b**, 12 h for **6a** and 10 min for **6b**, (90% for **6a**) and (70% for **6b**); (ii), uracil, anhydrous K_2CO_3, DMF, rt, 24 h, (72% for **7a**) and (70% for **7b**); (iii), octyl bromide, anhydrous K_2CO_3, DMF, rt, 24 h, (70%).

The binding efficacy of receptors (**1a**, **1b**, **4** and **2a**) for uracil derivatives (**7a**, **7b** and **8**) was evaluated by ^1H NMR titrations in $CDCl_3$. Addition of uracil derivatives (**7a**, **7b** and **8**) to solutions of bile acid-based acyclic and cyclic receptors (**1a**, **1b**, **4** and **2a**) in $CDCl_3$ resulted in a downfield shift in the resonances of amidic protons of the receptors. The change in the chemical shift of the amide protons was followed as a function of increasing guest concentration until saturation of the chemical shift values was reached. The ^1H NMR chemical shifts of the amidic protons for the receptors at zero concentration of the uracil derivatives and their saturation chemical shifts have been tabulated in Table 1. The titration was carried out in duplicate. Analysis of the saturation data with WinEQNMR software,[29] a non-linear regression curve fitting program, revealed a 1:1 complexation which was further confirmed by Job's plot.

The binding constants for the complexation of receptors with uracil analogues are listed in (Table 2).

Table 1. ^1H NMR chemical shifts of the amidic protons of receptors at zero concentration of the uracil guests and at their saturation respectively

Receptor	Uracil derivatives, δ		
	7a	**7b**	**8**
1a	7.70; 9.55	7.70; 9.56	7.73; 9.94
1b	7.51; 9.52	7.52; 9.52	7.54; 10.02
4	7.55; 9.54	7.58; 9.60	7.55; 9.96
2a	7.61; 9.21	7.59; 9.15	7.60; 9.73

Table 2. Binding constants *Ka* (M^{-1}) for complexation of uracil analogues with bile acid-based receptors[a]

Receptor	Uracil derivatives, *Ka*		
	7a	**7b**	**8**
1a	320	330	1100
1b	300	300	1150
4	350	370	1150
2a	225	220	550

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

The association constants of the complexes of acyclic receptors (**1a**, **1b** and **4**) with uracil derivative (**8**) are in the range of 1100–1150 M^{-1}. Upon introducing a bulky substituent on the uracil (**7a** and **7b**), the association constants decrease to 300–370 M^{-1}. This decrease is not unexpected because of the increased steric interactions among the bulky steroidal moieties of the host and guest. This is further substantiated by the weaker complexation of uracil derivatives (**7a** and **7b**) with cholaphane **2a** (220–225 M^{-1}) than with acyclic receptors (**1a**, **1b** and **4**). Cyclisation reduces the flexibility of the molecule and in the process increases the steric hindrance. However, when an octyl substituent on uracil was used, the association constant of the complex with cholaphanes **2a** increases to 550 M^{-1}.

In conclusion, bile acid-based 2,6-bis(acylamino)pyridine systems show better affinity for uracil derivatives as compared to flavin derivatives. The steroidal diami-

nopyridine systems can be used to design novel nanomaterials which may be utilized for drug delivery.

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References and notes

1. Sinden, R. R. *DNA Structure and Function*; Academic Press: New York, 1994.
2. Sessler, J. L.; Jayawickramarajah, J. *Chem. Commun.* **2005**, 1939.
3. Sivakova, S.; Rowan, S. *J. Chem. Soc. Rev.* **2005**, *34*, 9.
4. Rebek, J., Jr.; Askew, B.; Ballester, P.; Buhr, C.; Jones, S.; Nemeth, D.; Williams, K. *J. Am. Chem. Soc.* **1987**, *109*, 5033.
5. Askew, B.; Ballester, P.; Buhr, C.; Jeong, K. S.; Jones, S.; Parris, K.; Williams, K.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1989**, *111*, 1082.
6. Tjivikua, T.; Ballester, P.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1990**, *112*, 1249.
7. Rotello, V.; Hong, J.-I.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1991**, *113*, 9422.
8. Rotello, V.; Viani, E. A.; Deslongchamps, G.; Murray, B. A.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1993**, *115*, 797.
9. Conn, M. M.; Deslongchamps, G.; Mendoza, J. D.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1993**, *115*, 3548.
10. Hong, J.-I.; Feng, Q.; Rotello, V.; Rebek, J., Jr. *Science* **1992**, *255*, 848.
11. Wintner, E. A.; Conn, M. M.; Rebek, J., Jr. *Acc. Chem. Res.* **1994**, *27*, 198.
12. Adrian, J. C., Jr.; Wilcox, C. S. *J. Am. Chem. Soc.* **1989**, *111*, 8055.
13. Zimmerman, S. C.; Wu, W.; Zeng, Z. *J. Am. Chem. Soc.* **1991**, *113*, 196.
14. Hamilton, A.; Van Engen, D. *J. Am. Chem. Soc.* **1987**, *109*, 5035.
15. Jorgenson, W. L.; Pranata, J. *J. Am. Chem. Soc.* **1990**, *112*, 2008.
16. Pranata, J.; Wierschke, S. G.; Jorgenson, W. L. *J. Am. Chem. Soc.* **1991**, *113*, 2810.
17. Beiger, F. H.; Sijbesma, R. P.; Vekemans, J. A. J. M.; Meijer, E. M.; Kooijman, H.; Spek, A. L. *J. Org. Chem.* **1996**, *61*, 6371.
18. Shi, Z.; Li, Y.; Gong, H.; Liu, M.; Xiao, S.; Liu, H.; Li, H.; Xiao, S.; Zhu, D. *Org. Lett.* **2002**, *4*, 1179.
19. Davis, A. P.; Joos, Jean-Baptiste *Coord. Chem. Rev.* **2003**, *240*, 143.
20. Davis, A. P.; Wareham, R. S. *Angew. Chem., Int. Ed.* **1999**, *38*, 2978.
21. Davis, A. P. *Chem. Soc. Rev.* **1993**, *22*, 243.
22. Chattopadhyay, P.; Pandey, P. S. *Tetrahedron* **2006**, *62*, 8620.
23. Storhoff, J. J.; Mirkin, C. A. *Chem. Rev.* **1999**, *99*, 1849.
24. Seeman, N. C. *Chem. Biol.* **2003**, *10*, 1151.
25. Thibault, R. J.; Hotchkiss, P. J.; Gray, M.; Rotello, V. M. *J. Am. Chem. Soc.* **2003**, *125*, 11249.
26. Rai, R.; Pandey, P. S. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2923.
27. *N*,*N*′-Bis(3α-*O*-formylcholyl)-pyridine-2,6-diamine (**4**). Formylation of cholic acid with formic acid at 60 °C resulted in 3α-*O*-formylcholic acid **3**. Freshly distilled thionyl chloride (1 ml) was added dropwise to a solution of **3** (4 g, 8.12 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 1:4) to give 3.36 g of **4** (79%). Mp 130 °C (decomposed); IR υ_{max} (KBr)/cm^{-1} 3341, 2944, 2872, 1718, 1585, 1511; ^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ 0.76 (s, 6H, 18-Me), 0.88 (d, 6H, J = 6 Hz, 21-Me), 0.95 (s, 6H, 19-Me), 1.05–2.45 (48H, steroidal H), 4.72 (m, 2H, 3β-H), 5.08 (br s, 2H, 7β-H), 5.28 (br s, 2H, 12β-H), 7.55 (s, 2H, 2X–NHCO–), 7.68 (t, 1H, J = 8 Hz, Py-4-H), 7.87 (d, 2H, J = 7.9 Hz, Py-3,5-H), 8.02 (s, 2H, –OCHO), 8.10 (s, 2H, –OCHO) 8.17 (s, 2H, –OCHO); ^{13}C NMR (75 MHz, $CDCl_3$, TMS) δ 12.18, 17.62, 22.33, 22.79, 25.57, 26.58, 27.23, 28.56, 30.98, 31.34, 34.28, 34.44, 34.51, 34.82, 37.71, 40.78, 42.98, 45.05, 47.34, 70.68, 73.74, 75.29, 77.25, 109.43, 140.97, 149.40, 160.52, 160.59, 171.64; ES-HRMS calcd for $(C_{59}H_{83}N_3O_{14}\cdot H)^+$ 1058.5953, found 1058.5944.
28. General procedure for uracil derivatives (**7a**, **7b** and **8**): In a typical procedure, uracil (300 mg, 2.67 mmol) was treated with octyl bromide (0.44 mg, 2 mmol) in the presence of anhydrous K_2CO_3 (0.36 mg, 2.67 mmol) in dry DMF (10 ml) for 12 h at room temperature. 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 product was then purified by flash chromatography (elution with $CHCl_3$–MeOH 95:5) to give 0.31 g of **8** (70%).
N-1-Octyl uracil (**8**) mp 68–70 °C (Lit.[30] mp 69–70 °C); IR υ_{max} (KBr)/cm^{-1} 3510, 2921, 2866, 1728, 1691, 1463, 1241; ^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ 0.87–0.89 (br s, 3H, $-CH_3$), 1.28–1.73 (m, 12H, $-(CH_2)_6$), 3.73 (m, 2H, $-CH_2-$N), 5.71 (d, 1H, J = 7.8 Hz, H-5-uracil), 7.15 (d, 1H, J = 7.8 Hz, H-6-uracil), 8.90 (br s, 1H, NH-uracil ^{13}C NMR) (75 MHz, $CDCl_3$, TMS) δ 13.63, 22.72, 26.31, 29.01, 31.40, 48.26, 100.21, 144.85, 156.66, 164.14; ES-HRMS calcd for $(C_{12}H_{20}N_2O_2\cdot H)^+$ 225.1603, found 225.1608.
Methyl 3α-{*O*-(N_1-uracil)acetyl}lithocholate (**7a**) Yield 72%; mp 165–168 °C; IR υ_{max} (KBr)/cm^{-1} 3589, 3516, 2941, 2868, 1726, 1629, 1448, 1206; ^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ 0.64 (s, 3H, 18-Me), 0.90–0.93 (br s, 6H, 21-Me and 19-Me), 1.02–2.35 (28H, steroidal H), 3.66 (s, 3H, $-OCH_3$), 4.42 (s, 2H, $-CH_2-$N), 4.82 (m, 1H, 3β-H), 5.75 (d, 1H, J = 7.9 Hz, H-5-uracil), 7.10 (d, 1H, J = 7.9 Hz, H-6-uracil), 8.22 (br s, 1H, NH-uracil ^{13}C NMR) (75 MHz, $CDCl_3$, TMS) δ 12.04, 18.28, 20.85, 23.27, 24.17, 26.28, 26.56, 26.96, 28.18, 31.01, 31.07, 32.11, 34.56, 34.89, 35.36, 35.78, 40.07, 40.46, 41.91, 42.74, 48.99, 51.50, 55.96, 56.42, 76.95, 102.68, 144.40, 150.68, 163.47, 166.79, 174.81; ES-HRMS calcd for $(C_{31}H_{46}N_2O_6\cdot H)^+$ 543.3434, found 543.3439.
Methyl 3α-{*O*-(N_1-uracil)acetyl}deoxycholate (**7b**) Yield 70%; mp 235–238 °C; IR υ_{max} (KBr)/cm^{-1} 3487, 2940, 2866, 1730, 1691, 1458, 1207; ^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ 0.68 (s, 3H,18-Me), 0.92 (s, 3H, 19-Me), 0.97 (d, 3H, J = 5.2 Hz, 21-Me), 1.08–2.37 (26H, steroidal H), 3.66 (s, 3H, $-OCH_3$), 4.00 (m, 1H, 12β-H), 4.42 (s, 2H, $-CH_2-$N), 4.80 (br s, 1H, 3β-H), 5.74 (d, 1H,

J = 7.8 Hz, H-5-uracil), 7.11 (d, 1H, J = 7.8 Hz, H-6-uracil), 8.54 (br s, 1H, NH-uracil ^{13}C NMR) (75 MHz, $CDCl_3$, TMS) δ 12.74, 17.34, 23.03, 23.60, 25.98, 26.37, 26.89, 27.44, 28.69, 30.90, 31.07, 31.99, 33.67, 34.10, 34.74, 35.09, 35.96, 41.86, 46.50, 47.35, 48.25, 49.07, 51.53, 73.11, 76.83, 102.68, 144.44, 150.70, 163.39, 166.86, 174.73; ES-HRMS calcd for $(C_{31}H_{46}N_2O_7{\cdot}H)^+$ 559.3383, found 559.3387.

29. Hynes, M. *J. Chem. Soc. Dalton Trans.* **1993**, 311.
30. Takase, M.; Inouye, M. *J. Org. Chem.* **2003**, *68*, 1134.