Molecular Tweezer Based on Chenodeoxycholic Acid: Synthesis and Anion Binding Properties[‡]

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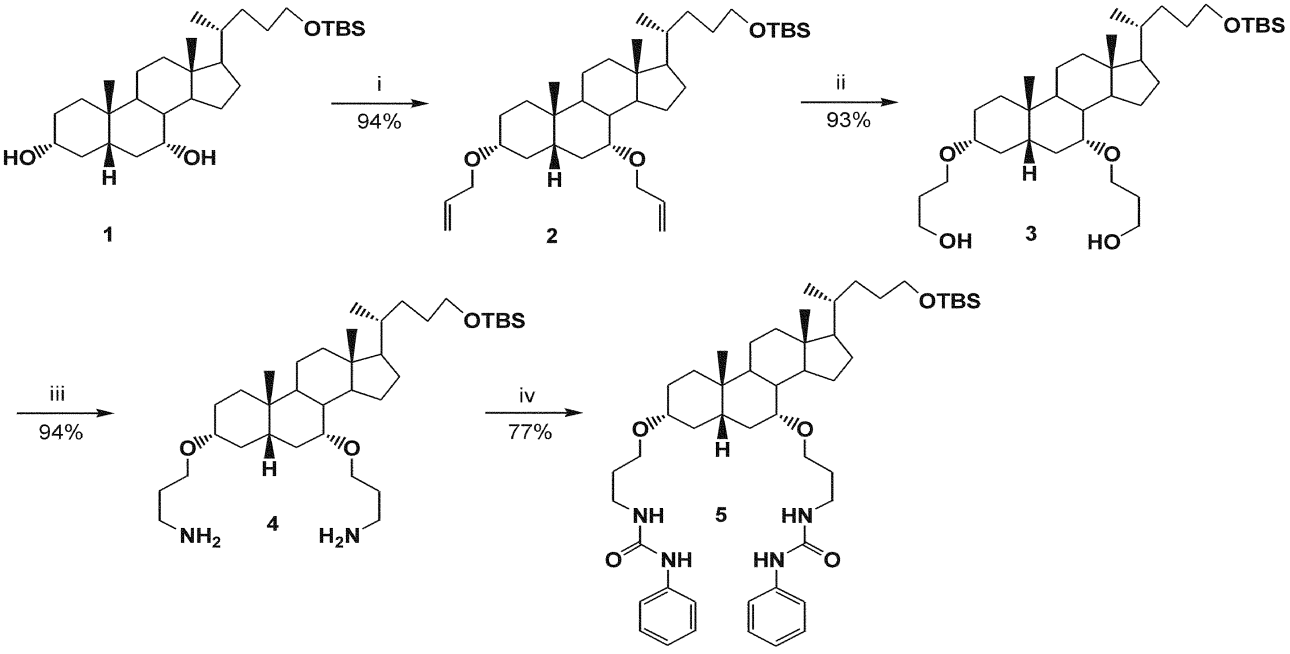
Received June 15, 2004

Key Words : Molecular tweezer, Chenodeoxycholic acid, Urea receptor, Anion recognition

The design and synthesis of anion receptors can interact with biologically relevant anions is prominent research field of supramolecular chemistry.[1] Steroid nucleus is one of the largest rigid and chiral units ubiquitous in nature. Due to the rigidity and preorganized structure of steroid, cholesterol and bile acid derivatives have been studied and shown novel recognition ability for cations, anions, and organic molecules.[2] Chenodeoxycholic acid is ideal for tweezer type receptor[3] design because it contains two hydroxyl groups at 3α and 7α that can be functionalized with a variety of recognition elements thus creating a binding pocket for target guests. Herein we report the first synthesis and anion binding study of molecular tweezer urea receptor based on chenodexoycholic acid.

The synthesis of the receptor **5** is outlined in Scheme 1. The 3α,7α-dihydroxyl groups of chenodeoxycholic acid were transformed to 3α,7α-diamines by three sequential steps. Allylation of 24-*tert*-butyldimethylsilyloxy-5β-cholane-3α,7α-diol **1** prepared from methyl chenodeoxycholate[4] in two steps ($LiAlH_4$ reduction, followed by protection with TBSCl) with allyl bromide in the presence of sodium hydride in THF resulted in 3α,7α-diallyl compound **2** in 94% yield. Hydroboration of the latter with 9-BBN provided the diol **3** in 93% yield. The 3α,7α-diamino steroid **4** was accessible via the corresponding phthalimide and subsequent hydrazinolysis in 94% yield. Compound **4** was immediately coupled with phenyl isocyanate in dry THF at room temperature provided cheno-bis(phenylurea) **5** in 77% yield.

The anion binding properties of **5** were investigated by ^1H NMR titrations in $CDCl_3$ solution in the presence of various anions such as Cl^-, Br^-, I^-, $CH_3CO_2^-$, $H_2PO_4^-$, NO_3^-, HSO_4^-, and SCN as their tetrabutylammonium (TBA) salt. The addition of equimolar TBACl to a solution of **5** ([TBACl]/[**5**] = 1.0) caused significant downfield shifts of both the phenyl and alkyl NH protons by up to $\Delta\delta$ = 1.79 and 1.08 ppm.



Scheme 1. Synthesis of Cheno-urea receptor **5**. *Reagents and conditions*: (i) NaH, CH_2=$CHCH_2Br$, THF; (ii) (a) 9-BBN, THF; (b) NaOH, H_2O_2; (iii) (a) DEAD, PPh_3, Phthalimide, THF; (b) $H_2NNH_2 \cdot H_2O$, EtOH; (iv) PhNCO, THF.

[‡]Dedicated to Professor Dong-Han Kim for his 70th birthday.

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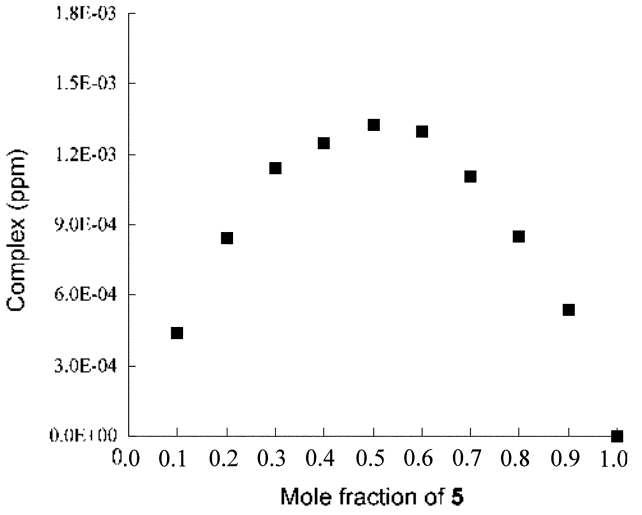

Figure 1. Job plots of cheno-urea receptor **5** with $TBA^+H_2PO_4^-$ in $CDCl_3$.

indicating anion binding taking place in the vicinity of urea. As shown in Figure 1, Job plot suggests a 1 : 1 stoichiometric complex between **5** and $H_2PO_4^-$ in $CDCl_3$.[5]

Association constants Ka of **5** presented in Table 1 were calculated by nonlinear curves fitting program EQ-NMR.[6] Cheno-urea **5** binds strongly oxyanions and halides, showing the highest binding affinity (K_a = 4,270) toward dihydrogen phosphate $H_2PO_4^-$. In addition, **5** binds Cl^- substantially more strongly than Br^-. The order of binding affinities in the series of halides ($Cl^- > Br^- > I^-$) is in accordance with that of the basicities.

The signals of the urea protons of **5** in the ^1H NMR spectra (on addition of 1 equiv. of salt) appeared at a very low field (δ = 9.63 for H_2PO_4, δ = 9.04 for Cl^-, δ = 8.66 for Br^-, and δ = 7.39 for I^-).

In summary, we have first synthesized and evaluated binding affinities towards anion of molecular tweezer urea receptor based on chenodeoxycholic acid. Further synthesis of other derivatives and binding studies are undergoing in this laboratory.

Experimental Section

General experimental procedures for melting points, FT-IR spectra, mass spectrometry, high resolution MS, elemental analyses, and TLC analysis have been described previously.[7] ^1H and ^{13}C NMR spectra were recorded on a Varian Unity Spectrometer (^1H, 300 MHz; ^{13}C, 75 MHz) with TMS as an internal standard. ^1H and ^{13}C NMR assignments were made by comparison with spectra of similar sterols.[8] Flash column chromatography was performed with silica gel Merck silica gel 60 (70-230 mesh). Reactions were carried out under argon atmosphere, and solution was dried over anhydrous sodium sulfate. Chenodeoxycholic acid and other reagents were purchased from either Aldrich or Fluka. Dichloromethane and chloroform were dried over CaH_2 and THF was dried over sodium and benzophenone and distilled prior to use. NMR titrations were run at 4.5 mM concentrations, with aliquots of a 0.25 M $(n\text{Bu})_4N^+X^-$ salts solution added. The non-linear curve fittings program (EQ-NMR) was used for curve fitting.[6]

Synthesis of 24-*tert*-Butyldimethylsilyloxy-3α,7α-dihydroxy-5β-cholane (1). A solution of methyl chenodeoxycholate (2.00 g, 4.92 mmol) in dry THF (50 mL) was added $LiAlH_4$ (2 eq, 370 mg) at 0 °C, and stirred for 16 h. The mixture was treated with 10% HCl and ethyl acetate. After the precipitated was removed, the filtrate was dried and evaporated to dryness. To a solution of the resulting residue, imidazole (500 mg, 7.40 mmol) and catalytic amount of 4-dimethylaminopyridine (10 mg) in dry dichloromethane (100 mL) and DMF (10 mL) was added *tert*-butyldimethylsilyl chloride (890 mg, 5.90 mmol) in dry dichloromethane (5 mL) at room temperature. After the reaction was completed, treated with 10% HCl and extracted with dichloromethane, dried, and evaporated to dryness. The residue was purified on silica gel chromatography (elution with EtOAc : hexane 1 : 3) to give **1**: yield 76%; TLC R_f 0.33 (EtOAc-hexane 1 : 1); mp 92-94 °C (CH_2Cl_2-hexane); IR (KBr) 3388, 2930, 2860, 1464, 1362, 1216, 1098, 979, 836, 751 cm^{-1}; ^1H NMR δ 3.79 (s, 1H, 3β-H), 3.52 (t, J = 14.7 Hz, 2H, 24-CH_2), 3.40 (m, 1H, 7β-H), 0.86 (d, J = 7.2 Hz, 3H, 21-CH_3), 0.85 (s, 9H, C(CH_3)$_3$), 0.77 (s, 3H, 19-CH_3), 0.61 (s, 3H, 18-CH_3), 0.03 (s, 6H, Si(CH_3)$_2$); ^{13}C NMR δ 71.9, 68.5, 63.8, 56.0, 50.4, 42.5, 41.4, 39.7, 39.6, 39.3, 35.5, 35.3, 35.0, 34.5, 32.8, 31.9, 30.6, 29.3, 28.2, 25.9, 23.6, 22.8, 20.5, 18.6, 18.4, 18.3, 11.7, -5.3.

Synthesis of 3,7-diallyl (2): To a solution of **1** (2.00 g, 4.06 mmol) in dry THF (100 mL) was added NaH (390 mg, 16.23 mmol), and heated at 60 °C for 30 min. Allyl bromide (1.34 mL, 16.23 mmol) was added to the resulting mixture and heated for 24 h, after that NaH (4 eq) and allyl bromide (4 eq) was added again and heated for another 24 h. Then the solvent was removed, and the residue was extracted with diethyl ether, washed with brine, dried and evaporated. The residue was purified on column chromatography (elution with 2% EtOAc-hexane) to give **2**: yield 94%: oil; TLC R_f 0.70 (5% EtOAc-hexane); IR (neat) 2933, 1466, 1379, 1254, 1096, 837, 775 cm^{-1}; ^1H NMR δ 5.86 (m, 2H), 5.24-5.02 (m, 4H), 4.08-3.94 (m, 3H), 3.64 (dd, J = 12.6, 5.7 Hz, 1H), 3.52 (t, J = 6.3 Hz, 2H, 24-CH_2), 3.28 (d, J = 2.1 Hz, 1H), 3.1 (m, 1H), 0.88 (d, J = 7.1 Hz, 3H, 21-CH_3), 0.85 (s, 9H, C(CH_3)$_3$), 0.82 (s, 3H, 19-CH_3), 0.58 (s, 3H, 18-CH_3), 0.03 (s, 6H,

Table 1. Association constants of Chen-urea receptor **5**[a]

Anion (X^-)	Cl^-	Br^-	I^-	$CH_3CO_2^-$	$H_2PO_4^-$	NO_3^-	HSO_4^-	SCN^-
Ka (M^{-1})[b]	2.750	1.200	260	690	4.270	1.160	1.160	270

[a]Determined in $CDCl_3$ by ^1H NMR titration at 298 K, [Host] = 4.5 × 10^{-3} M. [b]errors estimated to be ≤10%.

Si(CH_3)$_2$): ^{13}C NMR δ 135.8, 116.1, 115.6, 78.8, 75.0, 69.2, 68.5, 63.8, 55.9, 50.0, 42.4, 41.8, 39.5, 35.6, 35.3, 35.0, 33.5, 31.9, 29.4, 28.8, 28.2, 27.2, 26.0, 23.7, 22.9, 20.8, 18.6, 18.3, 11.7, -5.3; HRMS (EI) calcd for $C_{36}H_{64}O_3Si$ (M^+) 572.4625, found 572.4639.

Synthesis of 3,7-diol (3): To a solution of **2** (1.00 g, 1.75 mmol) in dry THF (100 mL) was added 9-BBN in THF (0.5 M, 14 mL) and stirred at room temperature for 12 h. After the reaction was completed, 20% NaOH (5 mL) and 30% H_2O_2 (5 mL) was added to the mixture and refluxed for 1 h. After the reaction was completed mixture was extracted with ethyl acetate, washed with brine, dried and concentrated to dryness. The residue was purified on column chromatography (elution with EtOAc : hexane 2 : 1) to give **3**: 93% yield; oil; TLC R_f 0.52 (EtOAc-hexane 2 : 1); IR (neat) 3362, 2934, 1468, 1366, 1253, 1096, 837, 781 cm^{-1}; ^{1}H NMR δ 3.71-3.59 (m, 8H), 3.52 (t, J = 6.3 Hz, 2H, 24-CH_2), 3.23 (s, 1H), 3.12 (m, 1H), 0.87 (m, 6H, 19- and 21-CH_3), 0.84 (s, 9H, C(CH_3)$_3$), 0.58 (s, 3H, 18-CH_3), 0.03 (s, 6H, Si(CH_3)$_2$); ^{13}C NMR δ 79.5, 76.3, 67.0, 66.3, 63.7, 62.1, 61.1, 56.0, 50.3, 42.4, 41.7, 39.5, 39.3, 35.5, 35.2, 35.0, 33.6, 32.6, 32.1, 31.8, 29.5, 28.5, 28.1, 27.2, 25.9, 23.7, 22.8, 20.7, 18.6, 18.3, 11.6, -5.3; FAB-MS calcd for $C_{36}H_{68}O_5Si$: 608.48, found: m/z 609 $(M+H)^+$.

Synthesis of 3,7-diamine (4): After a mixture of **3** (500 mg, 0.82 mmol), phthalimide (710 mg, 4.1 mmol) and triphenyl phosphine (1.00 g, 4.1 mmol) was stirred in dry THF (50 mL) at room temperature, diethyl azodicarboxylate (0.63 mL, 4.1 mmol) was added and continued to stir. After the solvent was removed, and the residue was extracted with ethyl acetate, washed with brine, dried, and concentrated. Without further purification, the residue and hydrazine monohydrate (410 mg, 8.2 mmol) was refluxed in ethanol (200 mL) for 24 h. Then the solvent was removed, extracted with diethyl ether, washed, dried, and concentrated. The residue was purified on column chromatography (elution with CH_2Cl_2 : MeOH : NH_4OH 16 : 3 : 0.5) to give **4**: 94% yield; oil; TLC R_f 0.39 (CH_2Cl_2-MeOH-NH_4OH 16 : 3 : 1); IR (neat) 2934, 2861, 1466, 1364, 1254, 1099, 837, 775, 737 cm^{-1}; ^{1}H NMR (400 MHz) δ 3.63-3.46 (m, 6H), 3.19 (s, 1H), 3.05 (m, 1H), 2.75 (m, 4H), 0.87 (d, J = 7.1 Hz, 3H, 21-CH_3), 0.87 (s, 3H, 19-CH_3), 0.85 (s, 9H, C(CH_3)$_3$), 0.58 (s, 3H, 18-CH_3), 0.03 (s, 6H, Si(CH_3)$_2$); ^{13}C NMR (125 MHz) δ 79.3, 75.9, 66.2, 65.8, 63.7, 56.0, 50.2, 42.3, 41.8, 39.7, 39.5, 39.4, 35.5, 35.3, 35.2, 35.0, 33.8, 33.6, 33.5, 31.8, 29.5, 28.6, 28.1, 27.3, 25.9, 23.7, 22.8, 20.7, 18.6, 18.3, 11.6, -4.9; MS (relative intensity, %) m/z 607 (M^+, 3).

Synthesis of cheno-urea (5): A solution of **4** (100 mg, 0.165 mmol) in dry THF (10 mL) was reacted with phenyl isocyanate (0.09 mL, 0.825 mmol) at room temperature for 2 h. Then the solvent was removed, extracted with ethyl acetate, washed, dried, and concentrated. The residue was purified on column chromatography (elution with EtOAc-hexane 1 : 3) to give **5**: yield 92%; TLC R_f 0.50 (EtOAc-hexane 1 : 1); mp 95-96 °C; IR (KBr) 3335, 2934, 2863, 1649, 1559, 1501, 1443, 1312, 1244, 1098, 756, 694 cm^{-1}; ^{1}H NMR δ 7.85 (s, 1H), 7.66 (s, 1H), 7.37-7.31 (dd, J = 15.3, 7.8 Hz, 4H), 7.26-7.15 (m, 6H), 6.7 (s, 1H), 6.43 (s, 1H), 3.72-3.39 (m, 10H), 3.16 (s, 1H), 3.04 (m, 1H), 0.86 (d, J = 7.2 Hz, 3H, 21-CH_3), 0.85 (s, 9H, C(CH_3)$_3$), 0.78 (s, 3H, 19-CH_3), 0.58 (s, 3H, 18-CH_3), 0.03 (s, 6H, Si(CH_3)$_2$); ^{13}C NMR δ 156.6, 156.4, 139.3, 129.0, 128.9, 123.0, 122.6, 120.1, 119.6, 79.3, 76.2, 66.6, 65.8, 63.8, 56.2, 50.6, 42.5, 41.6, 39.7, 39.4, 38.5, 35.6, 35.2, 33.9, 31.9, 30.5, 30.0, 29.5, 28.8, 28.2, 27.5, 26.0, 23.8, 22.9, 20.8, 18.7, 18.4, 11.7, -5.3; FAB-MS calcd for $C_{50}H_{80}N_4O_5Si$: 844.59, found: m/z 845 $(M+H)^+$. Anal. Calcd for $C_{50}H_{80}N_4O_5Si$: C, 71.05; H, 9.54; N, 6.63. Found C, 70.87; H, 9.57; N, 6.50.

Acknowledgements. This work was supported by grant No. R05-2001-000-00227-0 from Korea Science & Engineering Foundation and Brain Korea 21 Project.

References

1. (a) *Supramolecular Chemistry of Anions*; Bianchi, A.; Bowman-James, K.; García-España, E., Eds.; Wiley-VCH: New York; 1997. (b) Gale, P. A. *Coord. Chem. Rev.* **2003**, *240*, 191. (c) Beer, P. D.; Gale, P. A. *Angew. Chem. Int. Ed.* **2001**, *40*, 486. (d) Antonisse, M. M. G.; Reinhoudt, D. N. *Electroanalysis* **1999**, *11*, 1035. (e) Bühlmann, P.; Pretsch, E.; Bakker, E. *Chem. Rev.* **1998**, *98*, 1593. (f) Schmidtchen, F. P.; Berger, M. *Chem. Rev.* **1997**, *97*, 1609. (g) Kang, Y. S.; Ko, S. W.; Song, I. H.; Ryu, B. J.; Nam, K. C. *Bull. Korean Chem. Soc.* **2003**, *24*, 681.
2. For reviews, see: (a) Davis, A. P.; Joos, J.-B. *Coord. Chem. Rev.* **2003**, *240*, 143. (b) Wallimann, P.; Marti, T.; Fürer, A.; Diederich, F. *Chem. Rev.* **1997**, *97*, 1567. (c) Davis, A. P.; Gilmer, J. F.; Perry, J. J. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1312. (d) Davis, A. P. *Chem. Soc. Rev.* **1993**, *22*, 243.
3. For selected examples of steroid based tweezers, see: (a) Shim, J. H.; Jeong, I. S.; Lee, M. H.; Hong, H. P.; On, J. H.; Kim, K. S.; Kim, H.-S.; Kim, B. H.; Cha, G. S.; Nam, H. *Talanta* **2004**, *63*, 61. (b) Kim, B. H.; Lee, C. S.; Shim, J. H.; Hong, H. P.; Cha, G. S.; Jun, Y. M.; Nam, H. *Talanta* **2003**, *61*, 393. (c) Ayling, A. J.; Pérez-Payán, M. N.; Davis, A. P. *J. Am. Chem. Soc.* **2001**, *123*, 12716. (d) Potluri, V. K.; Maitra, U. *J. Org. Chem.* **2000**, *65*, 7764. (e) Pyun, H. J.; Chu, J.; Jun, Y. M.; Kim, D. J. *Bull. Korean Chem. Soc.* **1999**, *20*, 112.
4. Savage, P. B.; Allman, G. W.; Willardson, B. M.; Driscoll, C. D.; Budge, L. P.; Li, C. *J. Am. Chem. Soc.* **1999**, *121*, 931.
5. (a) Job, P. *Compt. Rend.* **1925**, *180*, 928. (b) Blanda, M. T.; Horner, J. H.; Newcomb, M. *J. Org. Chem.* **1989**, *54*, 4626.
6. Hynes, M. J. *J. Chem. Soc. Dalton Trans.* **1993**, 311.
7. Kim, H.-S.; Choi, B.-S.; Kwon, K.-C.; Lee, S.-O.; Kwak, H. J.; Lee, C. H. *Bioorg. Med. Chem.* **2000**, *8*, 2059.
8. Blunt, J. W.; Stothers, J. B. *Org. Mag. Reson.* **1977**, *9*, 439.