Synthesis and Evaluation of the Deoxycholic Acid Derivative with Two 4-Trifluoroacetylbenzoyl Groups as a Carbonate Ionophore

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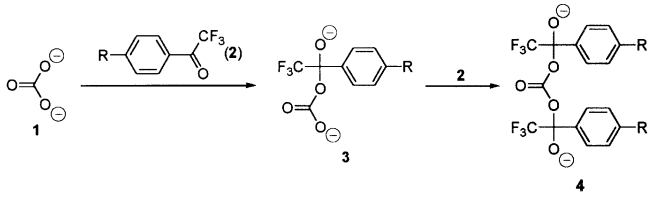
Uncharged receptors for anions and cations have many potential applications ranging from membrane transport carriers for ion-selective electrodes to reaction catalysts.[1] Thus, design and synthesis of neutral receptors for ions have been the focus of many research efforts to achieve high binding affinities and/or selectivity. Although many receptors for cations have been developed, there are only limited numbers of anion receptors reported. Among the anion receptors, several trifluoroacetophenone (TFAP) derivatives have been known to induce anion selectivity in membranes and act as electrically neutral carriers for carbonate ion with remarkable selectivity.[2]

According to the mechanism suggested by Meyerhoff *et al.*, one carbonate ion is covalently bound to the carbonyl groups of two TFAP moieties, as shown in scheme I, resulting in a 1:2 complex.[3] We expected that compounds with two TFAP moieties in a molecule would capture a carbonate ion more favorably, based on this mechanism, if their linker provides appropriate conformation. For this reason, we chose deoxycholic acid (**5**), one of bile acids, as a possible rigid linker. Deoxycholic acid has attracted much attention in recent years because it is readily available and possesses a unique disposition of two hydroxyl groups on one surface of the conformationally rigid steroidal skeleton.[4] The distance between 3α- and 12α-hydroxyl groups is about 6 Å and the C-O bonds of this molecule are almost parallel even though they diverge away slightly from the steroid.[5] We wish to report here the synthesis of **7** in which two 4-trifluoroacetylbenzoyl (TFAB) groups are attached to 3α- and 12α-hydroxyl groups of a deoxycholic acid derivative. Its characteristic as an ionophore of carbonate ion was evaluated and compared to the control compounds which had only one TFAB group at 12α (**8b**) and 3α (**9c**).

Experimental Section

General procedure. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury 300 MHz NMR spectrometer.



Scheme 1. The proposed mechanism of interaction between carbonate ion and TFAP derivatives.

Chemical shifts (δ) are reported as ppm down field from tetramethylsilane internal standards or using residual solvent peak as a standard. ^{19}F NMR spectra were recorded on a Varian UNITYplus-300 NMR spectrometer and chemical shifts (δ) are reported as ppm down field from fluorotrichloromethane internal standards. IR spectra were recorded on a Nicolet 205 FT-IR spectrophotometer. Mass spectra were obtained by Jeol HX110/HX110 mass spectrometer at the Korea Basic Science Institute, Daejon, Korea, by fast atom bombardment (FAB) ionization method. UV spectra were obtained on a Shimadzu UV-240 UV-Vis spectrophotometer.

All anhydrous reactions were carried out under nitrogen atmosphere. THF and ether were distilled from sodium ketyl of benzophenone. CH_2Cl_2 was distilled from CaH_2. Toluene was purified as described in a reference[6] and stored in Type 4A grade of molecular sieve. Absolute methanol was obtained from Aldrich Chemical Co. and used without further purification. For spectroscopic experiments, CH_2Cl_2 for UV spectroscopy and tetrabutylammonium chloride (Bu_4NCl) for ion pair chromatography from Fluka Chemical Co. were used. All the other reagents were purchased from either Aldrich or Fluka Chemical Co. unless noted otherwise. 4-Trifluoroacetylbenzoyl chloride (TFAB-Cl) and ETH 6024 were prepared by the procedure of Simon *et al.*[7]

***N,N*-Dioctyl-3α,12α-diformyloxy-5β-cholan-24-amide (6a)** To a solution of 500 mg (1.11 mmol) of **5b**[8] and 171 μL (1.23 mmol) of NEt_3 in 10 mL of CH_2Cl_2 was added 95 μL (1.23 mmol) of methyl chloroformate at 0 °C. After 1 h, 401 μL (1.33 mmol) of *N,N*-dioctylamine was added and the resulting solution was stirred at 0 for 2 h. The reaction mixture was diluted with CH_2Cl_2 (100 mL), washed with water (100 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by column chromatography on silica using ethyl acetate-hexane (1:4) as eluent to give 670 mg (90%) of the amide **6a** as a waxy solid. R_f = 0.57 (EA:Hex=3:7); IR (film) ν_{max} 2934, 2861, 2736, 1729, 1644, 1466, 1381, 1190, 762 cm^{-1}; ^{1}H NMR (300 MHz; $CDCl_3$) δ 0.74 (s, 3H, 18-CH_3), 0.92 (s, 3H, 19-CH_3), 0.83-2.37 (m, 59H), 3.16-3.35 (m, 4H, $N(CH_2R)_2$), 4.82 (m, 1H, 3β-H), 5.26 (s, 1H, 12β-H), 8.03 (s, 1H, CHO), 8.13 (s, 1H, CHO).

***N,N*-Dioctyl-3α,12α-dihydroxy-5β-cholan-24-amide (6b).** A solution of 1.13 g (1.68 mmol) of **6a** in 20 mL of THF and 15 mL of 3% K_2CO_3 in 80% aq. methanol was stirred at 60 °C for 3 days. After the solution was concentrated, the resi-

due was dissolved in CH_2Cl_2 (100 mL), washed with saturated NH_4Cl solution (100 mL) and water (100 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by column chromatography on silica using ethyl acetate-hexane (1:1) as eluent to give 827 mg (80 %) of the amide **6b** as a waxy solid. R_f=0.59 (EA:Hex=2:1); IR (film) ν_{max} 3401 (br), 2940, 2868, 1631, 1473, 1381, 1315, 1262, 1223, 1104, 1052, 762 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.68 (s, 3H, 18-CH_3), 0.88 (d, 3H, *J*=5.2 Hz, 21-CH_3), 0.91 (s, 3H, 19-CH_3), 0.82-2.39 (m, 58H), 3.18-3.35 (m, 4H, $N(CH_2R)_2$), 3.60 (m, 1H, 3β-H), 3.97 (s, 1H, 12β-H).

***N,N*-Dioctyl-3α,12α-bis(4-trifluoroacetylbenzyloxy)-5β-cholan-24-amide (7).** A suspension of 220 mg (0.36 mmol) of **6b**, 158 mg (3.57 mmol) of CaH_2, 29 mg (0.09 mmol) of Bu_4NBr, and 1.18 g (5.00 mmol) of TFAB-Cl in toluene (4 mL) was refluxed for 24 h and filtered through celite (5 g) pad after cooling. After the celite pad was washed with ethyl acetate (100 mL), the combined filtrates were concentrated, and the residue was dissolved in toluene (60 mL). To the solution was added silica gel (30 g) and water (0.1 mL) and the mixture was stirred for 2 h at rt. After the mixture was filtered and the filter cake was washed with ethyl acetate (150 mL), the combined filtrates were washed with saturated $NaHCO_3$ (2x50 mL) and water (50 mL), dried ($MgSO_4$), and concentrated. Purification of the residue by chromatography on silica using ethyl acetate-hexane (3:17) gave **7** (230 mg, 63%) as a waxy solid. R_f=0.22 (EA:Toluene=3:7); IR (film) ν_{max} 2933, 2861, 1723, 1618, 1473, 1282, 1190, 1117, 946, 736 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.85 (s, 3H, 18-CH_3), 0.88 (s, 3H, 19-CH_3), 0.85-2.36 (m, 59H), 3.10-3.32 (m, 4H, $N(CH_2R)_2$), 4.92 (m, 1H, 3β-H), 5.44 (s, 1H, 12β-H), 8.05-8.10 (m, 4H, C_6H_4), 8.18-8.30 (m, 4H, C_6H_4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.59, 14.08, 17.81, 22.60, 22.62, 23.04, 23.49, 25.88, 25.95, 26.48, 26.77, 26.85, 27.02, 27.42, 27.75, 29.08, 29.16, 29.22, 29.26, 29.37, 29.69, 30.08, 31.36, 31.72, 31.78, 32.20, 34.02, 34.63, 34.81, 35.02, 35.70, 41.74, 45.52, 45.92, 47.94, 48.43, 50.22, 75.64, 77.67, 116.34 (q, *J*=290 Hz, CF_3), 116.33 (q, *J*=291 Hz, CF_3), 129.70, 129.89, 129.90, 130.09, 132.77, 132.93, 136.36, 136.56, 164.23, 164.38, 172.62, 179.86 (q, *J*=36Hz, $\underline{C}OCF_3$), 179.98 (q, *J*=35Hz, $\underline{C}OCF_3$); ^{19}F NMR (282 MHz, $CDCl_3$) δ -72.31, -72.36; LRFABMS (NBA) m/z 1016.53 (M+H), 1034.54 ($M+H_2O$+H), 1052.54 ($M+2H_2O$+H), 1169.64 (M+NBA+H), 1187.61 (M+NBA+H_2O+H); HRFABMS (NBA) Calcd for $C_{55}H_{80}F_6NO_7$ (M+H): 1016.5839 Found: 1016.5834.

***N,N*-Dioctyl-3α-acetoxy-12α-hydroxy-5β-cholan-24-amide (8a).** A solution of 622 mg (1.01 mmol) of **6b**, 422 μL (3.03 mmol) of NEt_3, and 286 μL (3.03 mmol) of Ac_2O in 10 mL of CH_2Cl_2 was stirred at rt for 86 h. The reaction mixture was diluted with ether (50 mL), washed with 1 N HCl (3x50 mL) and water (50 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by column chromatography on silica using ethyl acetate-hexane (1:4) as eluent to afford 538 mg (81%) of the 3-monoacetate **8a** as a waxy solid. R_f=0.44 (EA:Hex=3:7); IR (film) ν_{max} 3454 (br), 2934, 2861, 1743, 1631, 1473, 1387, 1368, 1249, 1032 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.69 (s, 3H, 18-CH_3), 0.92 (s, 3H, 19-CH_3), 0.83-2.35 (m, 60H), 2.02 (s, 3H, OAc), 3.17-3.32 (m, 4H, $N(CH_2R)_2$), 4.00 (s, 1H, 12β-H), 4.71 (m, 1H, 3β-H).

***N,N*-Dioctyl-3α-acetoxy-12α-(4-trifluoroacetylbenoxy)-5β-cholan-24-amide (8b).** Compound **8b** was synthesized by the same procedure for the synthesis of **7** using 210 mg (0.32 mmol) of **8a**, 71 mg (1.60 mmol) of CaH_2, 26 mg (0.08 mmol) of Bu_4NBr, and 264 mg (1.12 mmol) of TFAB-Cl in toluene (3 mL). After the reaction, the crude residue was dissolved in toluene (30 mL) with water (0.1 mL) and silica gel (10 g), and the suspension was stirred for 2 h at rt. The suspension was filtered and the filter cake was washed with of ethyl acetate (150 mL). After the filtrate and the washing were combined and concentrated, the residue was dissolved in ether (50 mL), washed with saturated $NaHCO_3$ (2x50 mL) and water (50 mL), dried ($MgSO_4$), and concentrated. Purification of the crude product by chromatography on silica using ethyl acetate-hexane (1:4) gave 200 mg (73%) of **8b** as a waxy solid. R_f=0.54 (EA:Toluene=3:7); IR (film) ν_{max} 2934, 2861, 1723, 1624, 1473, 1387, 1275, 1249, 1183, 1117, 1071, 1032, 762 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.83 (s, 3H, 18-CH_3), 0.95 (s, 3H, 19-CH_3), 0.82-2.29 (m, 59H), 1.91 (s, 3H, OAc), 3.09-3.32 (m, 4H, $N(CH_2R)_2$), 4.64 (m, 1H, 3β-H), 5.42 (s, 1H, 12β-H), 8.28-8.20 (m, 4H, C_6H_4); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.56, 14.09, 17.80, 21.28, 22.61, 22.63, 23.05, 23.52, 25.86, 25.97, 26.47, 26.81, 26.86, 27.03, 27.43, 27.76, 29.09, 29.17, 29.24, 29.26, 29.38, 29.65, 30.05, 31.36, 31.73, 31.79, 32.20, 34.02, 34.66, 34.73, 35.00, 35.70, 41.71, 45.53, 45.91, 47.92, 48.41, 50.14, 73.93, 77.71, 116.41 (q, *J*=291 Hz, CF_3), 129.96, 130.19, 132.90, 136.57, 164.31, 170.41, 172.61, 180.00 (q, *J*=36Hz, $\underline{C}OCF_3$); ^{19}F NMR (282 MHz, $CDCl_3$) δ -72.21; LRFABMS (NBA) m/z 858.5 (M+H), 876.5 ($M+H_2O$+H), 1011.5 (M+NBA+H); HRFABMS (NBA) Calcd for $C_{51}H_{79}F_3NO_6$ (M+H): 858.5859 Found: 858.5839; HRFABMS (NBA) Calcd for $C_{51}H_{81}F_3NO_7$ ($M+H_2O$+H): 876.5964 Found: 876.5983.

***N,N*-Dioctyl-3α,12α-diacetoxy-5β-cholan-24-amide (9a).** A solution of 635 mg (1.03 mmol) of **6b**, 0.72 mL (5.15 mmol) of NEt_3, 490 μL (5.15 mmol) of Ac_2O, and 63 mg (0.52 mmol) of DMAP in 5 mL of CH_2Cl_2 was stirred at rt for 3.5 h and diluted with ether (100 mL). The solution was washed with 1 M HCl (3x50 mL) and water (50 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by column chromatography on silica using ethyl acetate-hexane (1:9) as eluent to afford 750 mg (100 %) of the diacetate **9a** as a waxy solid. R_f=0.59 (EA:Hex=3:7); IR (film) ν_{max} 2934, 2861, 1743, 1651, 1473, 1381, 1249, 1032 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.71 (s, 3H, 18-CH_3), 0.88 (s, 3H, 19-CH_3), 0.79-2.29 (m, 59H), 2.01 (s, 3H, OAc), 2.07 (s, 3H, OAc), 3.14-3.28 (m, 4H, $N(CH_2R)_2$), 4.67 (m, 1H, 3β-H), 5.07 (s, 1H, 12β-H).

***N,N*-Dioctyl-12α-acetoxy-3α-hydroxy-5β-cholan-24-amide (9b).** A solution of 553 mg (0.79 mmol) of **9a**, 218 mg (1.58 mmol) of K_2CO_3 in 5 mL of methanol was stirred

at rt for 1.5 h, and 3 mL of acetic acid was added to the solution. After 10 min, the reaction mixture was diluted with ether (50 mL), washed with brine (50 mL) and water (50 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was purified by column chromatography on silica using ethyl acetate-hexane (1:4) as eluent to afford 503 mg (97%) of the 12-monoacetate **9b** as a waxy solid. R_f −0.27 (EA:Hex−3:7); IR (film) ν_{max} 3421 (br), 2934, 2861, 1743, 1637, 1473, 1381, 1249, 1052, 762 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.73 (s, 3H, 18-CH_3), 0.90 (s, 3H, 19-CH_3), 0.77-2.31 (m, 60H), 2.08 (s, 3H, OAc), 3.31-3.16 (m, 4H, $N(CH_2R)_2$), 3.60 (m, 1H, 3β-H), 5.08 (s, 1H, 12β-H).

***N,N*-Dioctyl-12α-acetoxy-3α-(4-trifluoroacetylbenzoxy)-5β-cholan-24-amide (9c).** Compound **9c** was synthesized by the same procedure for the synthesis of 7 using 210 mg (0.32 mmol) of **9b**, 42 mg (0.96 mmol) of CaH_2, 26 mg (0.08 mmol) of Bu_4NBr, and 188 mg (0.80 mmol) of TFAB-Cl in toluene (3 mL). After the reaction, the crude residue was dissolved in toluene (30 mL) with water (0.1 mL) and silica gel (10 g), and the suspension was stirred for 2 h at rt. The suspension was filtered and the filter cake was washed with of ethyl acetate (150 mL). After the filtrate and the washing were combined and concentrated, the residue was dissolved in ether (50 mL), washed with saturated $NaHCO_3$ (2x50 mL) and water (50 mL), dried ($MgSO_4$), and concentrated. Purification of the crude product by chromatography on silica using ethyl acetate-hexane (1:4) gave 230 mg (84 %) of **9c** as a waxy solid. R_f−0.49 (EA:Toluene−3:7); IR (film) ν_{max} 2934, 2861, 1723, 1624, 1479, 1387, 1282, 1256, 1183, 1117, 1065, 1025, 762 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.75 (s, 3H, 18-CH_3), 0.85-2.36 (m, 62H), 2.11 (s, 3H, OAc), 3.32-3.18 (m, 4H, $N(CH_2R)_2$), 4.98 (m, 1H, 3β-H), 5.12 (s, 1H, 12β-H), 8.22-8.12 (m, 4H, C_6H_4); ^{13}C NMR (75 MHz, $CDCl_3$) 12.38, 14.03, 17.71, 21.30, 22.57, 23.01, 23.39, 25.64, 25.80, 26.59, 26.86, 26.99, 27.34, 27.71, 29.10, 29.14, 29.18, 29.25, 29.30, 29.60, 30.12, 31.45, 31.69, 31.74, 32.18, 34.00, 34.43, 34.62, 34.95, 35.61, 41.83, 44.97, 45.91, 47.87, 48.00, 49.39, 75.85, 75.91, 116.32 (q, *J*–291 Hz, CF_3), 129.82, 129.89, 132.66, 136.59, 164.42, 170.31, 172.84, 179.97 (q, *J*–35Hz, $\underline{C}OCF_3$); ^{19}F NMR (282 MHz, $CDCl_3$) δ -72.20; LRFABMS (NBA) m/z 858.4 (M+H), 876.5 (M+H_2O+H), 1011.5 (M+NBA+H); HRFABMS (NBA) Calcd for $C_{51}H_{79}F_3NO_6$ (M+H); 858.5859 Found; 858.5836; HRFABMS (NBA) Calcd for $C_{51}H_{81}F_3NO_7$ (M+H_2O+H); 876.5964 Found; 876.5918.

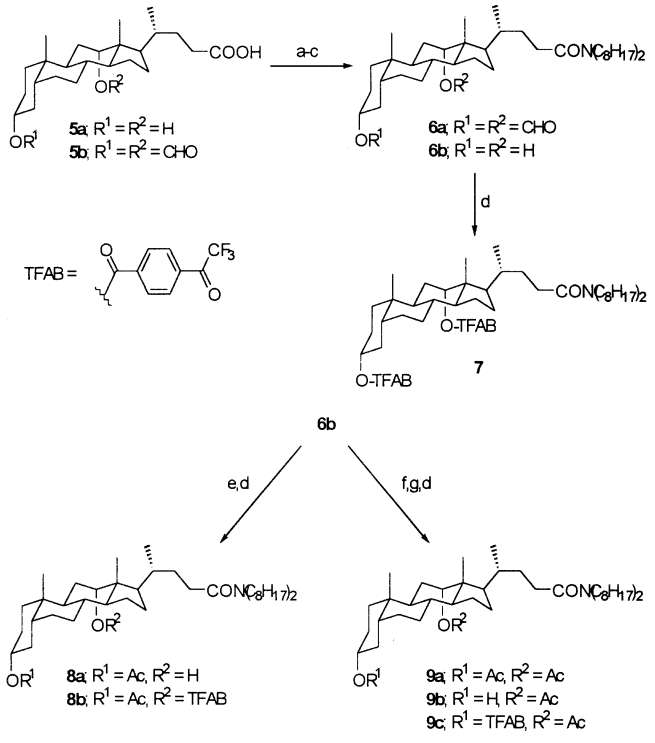
Method of solvent extraction and spectroscopic evaluation. Tris-H_2SO_4 buffer solution (0.10 M; pH 8.6) was prepared just before the experiment and Bu_4NCl ($2.0x10^{-3}$ M) and $NaHCO_3$ ($3.0x10^{-2}$ M) were dissolved in this buffer if necessary. The pH of the buffer solution containing 30 mM $NaHCO_3$ was maintained to be 8.6. The solutions of **7**, **8b**, and **9c** were prepared by dissolving each compound in CH_2Cl_2 for UV spectroscopy and the concentration of the solutions were $4.0x10^{-5}$ M (**7**) or $8.0x10^{-5}$ M (**8b** and **9c**), respectively.[9]

For extraction, 4 mL of CH_2Cl_2 solution of each compound and 4 mL of Tris-H_2SO_4 buffer containing Bu_4NCl, with or without $NaHCO_3$, were thoroughly mixed and the solution was centrifuged for 1 min. The lower organic layer was taken to obtain UV spectrum. The experiments were triplicated and averaged.[10]

Results and Discussion

The synthesis of the target molecule **7** was started with the protection of carboxylic acid group of **5a** followed by trifluoroacetylbenzoylation of the hydroxyl groups. (Scheme 2) The protecting group of the carboxylic acid moiety chosen was a long chain dialkylamide because ionophores should be relatively hydrophobic for solvent extraction and to be used as an additive in ion-selective membranes. Then TFAB groups were introduced on the hydroxyl groups of **6b** in 63% yield by adopting Oppenauer condition (CaH_2, toluene, Bu_4NBr) with excess amount of 4-trifluoroacetylbenzoyl chloride (TFAB-Cl).[11] TFAB-Cl was prepared from 1,4-dibromobenzene by the procedure of Simon *et al.*[7] For the comparison purpose, two control compounds **8b** and **9c** were also synthesized by selective acetylation and hydrolysis of **6b** followed by trifluoroacetylbenzoylations.[12]

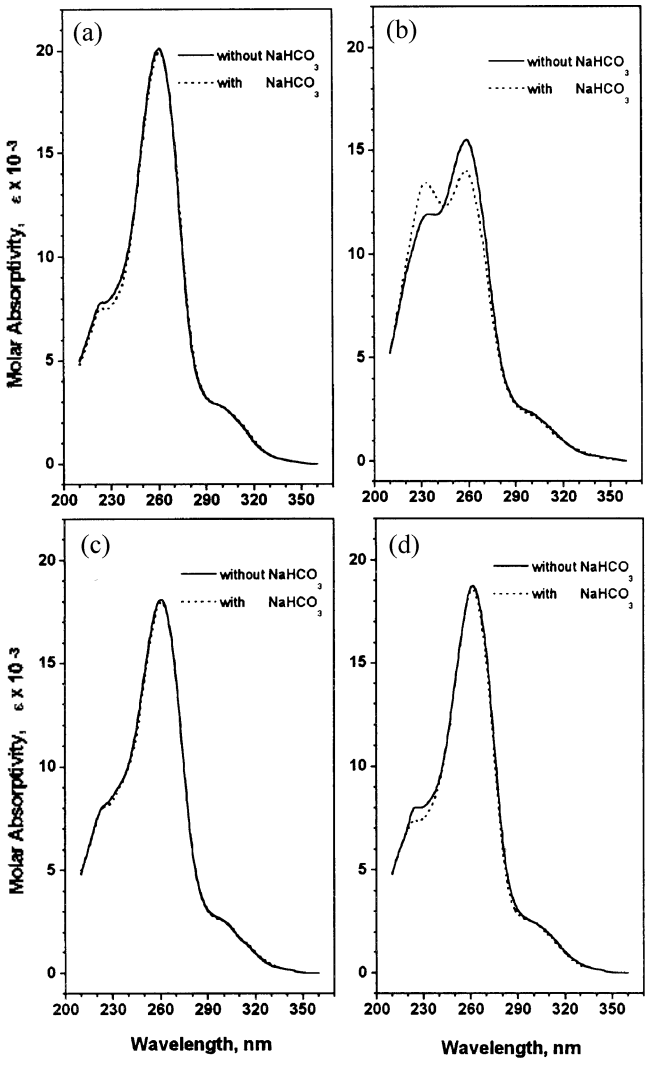
The differences of binding affinities of these compounds to carbonate ion were determined by solvent extraction followed by spectroscopic method. The UV absorption at 260 nm was compared after extracting dichloromethane solution of the these compounds ($8.0x10^{-5}$ M of TFAB groups regardless the number of TFAB groups in the molecule) with



Scheme 2. (a) HCOOH, cat. $HClO_4$, Ac_2O, 55 °C. (b) ClCOOMe, NEt_3, $HN(C_8H_{17})_2$, CH_2Cl_2, 0 °C. (c) K_2CO_3, aq. MeOH, 60 °C. (d) CaH_2, Bu_4NBr, ex. TFAB-Cl, Toluene, reflux. (e) Ac_2O, NEt_3, CH_2Cl_2, rt. (f) Ac_2O, NEt_3, DMAP, CH_2Cl_2, rt. (g) K_2CO_3, MeOH, rt.

buffer solutions (0.10 M Tris-H_2SO_4, pH 8.6) containing 2.0 mM tetrabutylammonium chloride, as a source of the hydrophobic counter cation, with or without 30 mM $NaHCO_3$.[13] The previous results of ion-selective membranes containing TFAP derivatives indicated that these compounds are poor ionophores for sulfate and chloride ions (log $K^{pot}_{CO_3^{2-},SO_4^{2-}}$ and log $K^{pot}_{CO_3^{2-},Cl^-}$ are less than 2.0).[7,14] In addition, it has also been previously reported that TFAP derivatives act as carbonate ionophores, not as bicarbonate ionophores, based on the ISE experiments.[15]

The UV absorption spectra of the control compounds (**8b** and **9c**), after extraction with buffer solution without $NaHCO_3$, showed main absorption (ε=1.85-1.91x10^4) at 260 nm with a small shoulder at 230 nm, which is believed to be the absorption of the hydrated species containing *gem*-diol. (Figures) That is not surprising because TFAP derivatives have been known to exist as *gem*-diol species depending on the substituents on the phenyl ring and the pH of the solution.[16] In addition, TFAP derivatives have been used as additives in solvent polymeric membranes for humidity[17] or ethanol[18] sensors. The differences in the size of the peak at 260 nm after extraction with buffer solution containing carbonate ion were within acceptable error limit for both **8b** and **9c**,[10] which indicated that binding of the carbonate ion to these control compounds is negligible in this condition. The same result was also observed with *n*-heptyl 4-trifluoroacetylbenzoate (ETH6010) and 4-trifluoroacetyldodecylbenzene, commercially available carbonate ionophores.



Figures Ultraviolet spectra of a known compound (ETH6010), 7, and controls after extraction with buffer solution (pH 8.6) with or without $NaHCO_3$. (a) ETH6010, (b) Compound 7, (c) Compound **8b**, (d) Compound **9c**.

On the other hand, the UV absorption spectrum of 7 showed a slight decrease of UV absorption at 260 nm (ε=1.58x10^4) even without carbonate ion. Thus, it seems that even small hydroxide ion can bind cooperatively by two TFAB moieties to some extent assuming TFAB groups on 3α-and 12α-position have almost the same absorption at 260 nm.[19] When the solution of 7 was extracted with a buffer containing carbonate ion, the UV absorption at 260 nm diminished further by 9%.[20]

These results demonstrated that the two TFAB groups in a molecule, where a rigid linker facilitated an appropriate conformation, were able to cooperatively bind a carbonate or bicarbonate ion. However, the reason that compound 7 has an ability to bind to a small hydroxide ion more favorably than the control compounds is not clear at this point. In addition, it is still an open question whether a carbonate ion actually bind to the two TFAB groups in compound 7 through covalent bonds simultaneously as proposed by Meyerhoff *et al.*[3] Utility of the compound 7 as a membrane additive for carbonate selective electrode will be tested and reported in due course.

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10. Error limit for the determination of absorbance at 260 nm was within 2 %.
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19. The absorption at 260 nm increased by 11% (ε=1.76x10^4) when the solution of compound **7** was extracted with a buffer of pH 7.6 (0.10 M Tris-H_2SO_4).
20. Although TFAP derivatives have been known to interact more strongly with carbonate ion than bicarbonate ion, the same experiment with a buffer of pH 7.6 (0.10 M Tris-H_2SO_4) also showed the similar decrease (7-8%) of the absorption. The selectivity between carbonate and bicarbonate ion will be tested using ion selective membrane technique and reported elsewhere.