Uncharged receptors for anions and cations have many potential applications ranging from membrane transport carriers for ion-selective electrodes to reaction catalysts. 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.

According to the mechanism suggested by Meyerhoff et al., one carbonate ion is covalently bound to the carbonyl group of two TFAP moieties, as shown in scheme I, resulting in a 1:2 complex. 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 deoxycorticosterone (5), one of bile acids, as a possible rigid linker. Deoxycorticosterone 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. 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. We wish to report here the synthesis of two TFAP moieties, as shown in scheme I, resulting in a 1:2 complex. 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 deoxycorticosterone (5), one of bile acids, as a possible rigid linker. Deoxycorticosterone 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. 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.

**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. 19F 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, Daejeon, 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 and benzophenone. CH2Cl2 was distilled from CaH2. Toluene was purified as described in a reference 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, CH2Cl2 for UV spectroscopy and tetrabutylammonium chloride (Bu4NCl) 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-Trifluoroacetophenyl benzyl chloride (TFAB-Cl) and ETH 6024 were prepared by the procedure of Simon et al.

**Experimental Section**

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

![Scheme 1](image)

A solution of 1.13 g (1.68 mmol) of 6a in 20 mL of THF and 15 mL of 3% K2CO3 in 80% aq. methanol was stirred at 60 °C for 3 days. After the solution was concentrated, the resi-
due was dissolved in CHCl₃ (100 mL), washed with saturated NH₄Cl solution (100 mL) and water (100 mL), dried (MgSO₄), 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. Rₑ=0.59 (EA:Hex=2:1); IR (film) νₚₐₓ 3454 (br), 2934, 2861, 1743, 1631, 1473, 1387, 1368, 1249, 1032 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 0.69 (s, 3H, 18-CH₃), 0.92 (s, 3H, 19-CH₃), 0.83-2.35 (m, 60H), 2.02 (s, 3H, OAc), 3.17-3.32 (m, 4H, N(CH₂R)₂), 4.00 (s, 1H, 1β-H), 4.71 (m, 1H, 3β-H).

**N,N-Diolyt-3α,12α-bis-(4-trifluoroacetylbenzyl)-5β-cholan-24-amide (7a, 8).** 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 CaH₂, 26 mg (0.08 mmol) of Bu₄NBr, 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 ethyl acetate (150 mL). After the filtrate and the washing solvent were combined and concentrated, 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 NH₄Cl (2x50 mL) and water (50 mL), dried (MgSO₄), and concentrated. Purification of the residue by chromatography on silica using ethyl acetate-hexane (3:1) gave 270 mg (63%) as a waxy solid. Rₑ=0.22 (EA:Toluene=3:7); IR (film) νₚₐₓ 3454 (br), 2934, 2861, 1723, 1624, 1473, 1387, 1275, 1249, 1183, 1117, 1071, 1032, 762 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 0.83 (s, 3H, 18-CH₃), 0.95 (s, 3H, 19-CH₃), 0.82-2.29 (m, 59H), 1.91 (s, 3H, OAc), 3.09-3.32 (m, 4H, N(CH₂R)₂), 4.64 (m, 1H, 3β-H), 5.42 (s, 1H, 12β-H), 8.28-8.20 (m, 4H, C₆H₄), 13C NMR (75 MHz, CDCl₃) δ 12.59, 14.08, 17.81, 22.60, 26.22, 23.04, 23.49, 25.88, 25.95, 26.48, 26.77, 28.65, 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, 34.62, 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, CF₃), 129.96, 130.19, 132.90, 136.41, 170.41, 172.61, 180.00 (q, J=36Hz, COOC₂F₅), 172.89 (q, J=35Hz, COCF₃); ¹F NMR (282 MHz, CDCl₃) δ -72.36; LRFABMS (NBA) m/z 858.5 (M+H), 876.5 (M+H₂O+H), 1011.5 (M+NBA+H); HRFABMS (NBA) Calcd for C₅₁H₇₉F₃NO₇ (M+H); 858.5859 Found; 858.5839; HRFABMS (NBA) Calcd for C₅₁H₇₉F₃NO₇ (M+H₂O+H), 876.5984 Found; 876.5983.

**N,N-Diolyt-3α,12α-diacteoyl-5β-cholan-24-amide (9a).** A solution of 635 mg (1.03 mmol) of 6b, 0.72 mL (5.15 mmol) of NEt₃, 490 μL (5.15 mmol) of Ac₂O, and 63 mg (0.52 mmol) of DMAP in 5 mL of CH₂Cl₂ 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 (MgSO₄), and concentrated under reduced pressure. The residue was purified by column chromatography on silica using ethyl acetate-hexane (1:1) as eluent to afford 750 mg (100 %) of the diacteoyl 9a as a waxy solid. Rₑ=0.59 (EA:Hex=3:7); IR (film) νₚₐₓ 3454, 2934, 2861, 1743, 1651, 1473, 1381, 1249, 1032 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 0.71 (s, 3H, 18-CH₃), 0.88 (s, 3H, 19-CH₃), 0.79-2.29 (m, 59H), 2.01 (s, 3H, OAc), 2.07 (s, 3H, OAc), 3.14-3.28 (m, 4H, N(CH₂R)₂), 4.67 (m, 1H, 3β-H), 5.07 (s, 1H, 12β-H).

**N,N-Diolyt-3α-acetoxy-3α-hydroxy-5β-cholan-24-amide (9b).** A solution of 553 mg (0.79 mmol) of 9a, 218 mg (1.58 mmol) of K₂CO₃ 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 (MgSO₄), and concentrated under reduced pressure. The residue was purified by column chromatography on silica using ethyl acetate-hexane (1:4) as an eluent to afford 503 mg (97%) of the 12-monoacetate 9b as a waxy solid. Rf =0.27 (EA:Hex=3:7); IR (film) νmax 3421 (br), 2934, 2861, 1743, 1637, 1473, 1381, 1249, 1052, 762 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 0.73 (s, 3H, 18-CH₃), 0.90 (s, 3H, 19-CH₃), 0.77-2.31 (m, 60H), 2.08 (s, 3H, OAc), 3.31-3.16 (m, 4H, N(CH₂R)₂), 5.12 (s, 1H, 12-H). ¹³C NMR (75 MHz, CDCl₃) 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, 31.65, 32.09, 34.46, 34.62, 34.79, 35.61, 41.83, 44.97, 45.91, 47.87, 48.00, 49.39, 75.85, 75.91, 116.32 (q, J=291 Hz, CF₃), 129.82, 129.89, 132.66, 136.59, 164.42, 170.31, 172.84, 179.97 (q, J=35Hz, COOCF₃); ¹⁹F NMR (282 MHz, CDCl₃) δ -72.20; LRFABMS (NBA) m/z 858.4 (M+H), 876.5 (M+H₂O+H), 1011.5 (M+NBA+H); HRFABMS (NBA) Caled for C₅₁H₇₆F₃NO₇ (M+H); 858.5859 Found; 858.5836; HRFABMS (NBA) Caled for C₅₁H₇₈F₃NO₇ (M+H₂O+H); 876.5964 Found; 876.5918.

Method of solvent extraction and spectroscopic evaluation. Tris-H₂SO₄ buffer solution (0.10 M; pH 8.6) was prepared just before the experiment and Bu₄NCl (2.0x10⁻³ M) and NaHCO₃ (3.0x10⁻² M) were dissolved in this buffer if necessary. The pH of the buffer solution containing 30 mM NaHCO₃ was maintained to be 8.6. The solutions of 7, 8b, and 9c were prepared by dissolving each compound in CH₂Cl₂ 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 CH₂Cl₂ solution of each compound and 4 mL of Tris-H₂SO₄ buffer containing Bu₄NCl, with or without NaHCO₃, 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 (CaH₂, toluene, Bu₄NBr) 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 trifluoroacetylbenzoylation.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. HClO₄, Ac₂O, 55 °C, (b) CI₂COOMe, NEts, HN(C₈H₁₇)₂, CH₂Cl₂, 0 °C, (c) K₂CO₃, aq. MeOH, 60 °C, (d) CaH₂, Bu₄NBr, ex. TFAB-Cl, Toluene, reflux, (e) Ac₂O, NEts, CH₂Cl₂, rt, (f) Ac₂O, NEts, DMAP, CH₂Cl₂, rt, (g) K₂CO₃, MeOH, rt.
buffer solutions (0.10 M Tris-H$_2$SO$_4$, pH 8.6) containing 2.0 mM tetrabutylammonium chloride, as a source of the hydrophobic counter cation, with or without 30 mM NaHCO$_3$. The previous results of ion-selective membranes containing TFAP derivatives indicated that these compounds are poor ionophores for sulfate and chloride ions (log $K^{\text{SO}_4^\text{2-}}$ and log $K^{\text{Cl}^-}$ are less than -2.0). In addition, it has also been previously reported that TFAP derivatives act as carbonate ionophores, not as bicarbonate ionophores, based on the ISE experiments.

The UV absorption spectra of the control compounds (8b and 9c), after extraction with buffer solution without NaHCO$_3$, showed main absorption ($\varepsilon=1.85\text{--}1.91\times10^4$) 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. In addition, TFAP derivatives have been used as additives in solvent polymeric membranes for humidity or ethanol 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, 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.

On the other hand, the UV absorption spectrum of 7 showed a slight decrease of UV absorption at 260 nm (equal to $1.58\times10^4$) 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 3$\alpha$- and 12$\alpha$-position have almost the same absorption at 260 nm. When the solution of 7 was extracted with a buffer containing carbonate ion, the UV absorption at 260 nm diminished further by 9%. 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. Utility of the compound 7 as a membrane additive for carbonate selective electrode will be tested and reported in due course.

Acknowledgement. We gratefully acknowledge the financial support from the Korean Ministry of Education through Basic Science Research Institute Program (BSRI-96-3448). Low and high resolution fast atom bombardment mass spectral data of the target molecule and controls were obtained by Korea Basic Science Institute, Daejon, Korea.

References

8. Tserng, K.-Y.; Klein, P. D. Steroids 1977, 29, 635.
9. Dichloromethane used for UV spectroscopic analyses was purchased from Fluka Chemical Co. The absorption by the solvent under 240 nm is fairly strong so that the peak at 230 nm does not seem to be meaningful for the quantitative analysis.
10. Error limit for the determination of absorbance at 260 nm was within 2 %.
14. The same experiment using 0.10 M Tris-HCl buffer (pH 8.6) gave the same result.
19. The absorption at 260 nm increased by 11% ($\varepsilon = 1.76 \times 10^4$) when the solution of compound 7 was extracted with a buffer of pH 7.6 (0.10 M Tris-H$_2$SO$_4$).
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$_2$SO$_4$) 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.