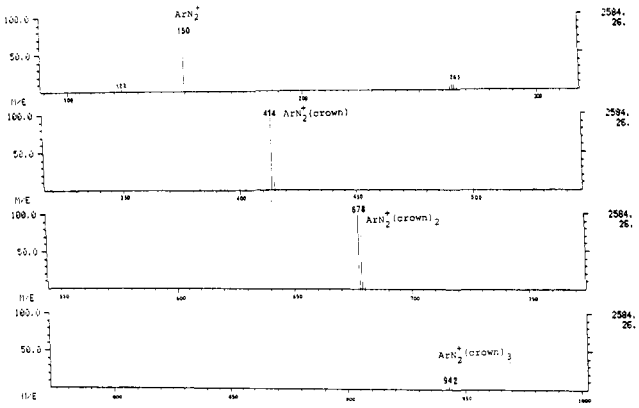


Figure 2. FD-mass spectrum of *p*-nitrobenzenediazonium ion/18-crown-6 showing the intact ArN_2^+ and 1:1, 1:2, and 1:3 complexes. Peaks at m/z 123 and 265 are due to $PhNO_2$ (homolytic dediazoniation product) and protonated crown, respectively.

In order to examine the effect of peri steric interaction on the complexation, we also studied anthracene-1-diazonium ion with crowns **2** and **3**. A 1:1 complex was detected in both cases (m/z 469 and 665, respectively). In addition, the intact ArN_2^+ (m/z 205 was observed in low abundance (FAB-MS) and anthracene (m/z 178) was detected, indicative of some homolytic dediazoniation.

We also examined the use of a sila-crown, namely, 1,1-dimethylsila-14-crown-5 for complexation with *p-tert*-butylbenzenediazonium ion and *p*-nitrobenzenediazonium ion; no complexation could be detected. Only the intact cations (ArN_2^+) and the uncomplexed crown were observed.

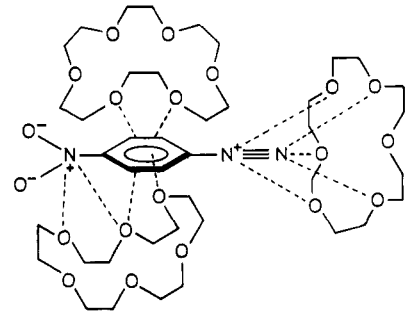
Comparison between Complexation in Solution and Gas Phase. The steric requirement for complex formation appears to be far less in the gas phase than in solution and complexation is observed both in the presence of ortho substituents and peri interactions. This suggests that charge transfer (CT) is the preferred complexation mode, which does not require close approach of the crown to diazonium ion. Nevertheless, the inability of a sila-crown-5 to form a charge-transfer complex indicates that the cavity size is still important.

Observation of diazonium/crown 1:2 complexes may be adequately explained by adopting the proposed solution structures[4] involving a π-acid complex. However, the σ-base to π-acid interaction must now involve a second crown molecule (Scheme Ic). The 1:3 complex observed with *p*-nitrobenzenediazonium ion indicates that a third crown may be complexed to the nitro group, via CT interactions with the nitrogen or via the "positive" oxygen in the extended conjugation form, creating an "encapsulated" diazonium ion with a hypothetical structure shown in Scheme II.

In conclusion, it should be pointed out that although a relationship between host–guest cluster ion detection in the gas phase and complexation in solution has not been established,[11] our study does raise the question as to the viability of "higher order complexation" in solution. Could the difficulty in obtaining a fully consistent picture based on combined spectroscopy and kinetics be, in part, due to intervention by higher order complexes, possible existence of which has not so far been considered in solution measurements? In drawing a parallel, it can be said that our gas-phase observations are adequately explained through

(11) We thank one of the reviewers for valuable comments and suggestions.

Scheme II. Hypothetical Representation of an "Encapsulated" Diazonium Ion within a 1:3 Complex



a charge-transfer model and by σ-base/π-acid interactions; both mechanisms have been recently proposed in order to explain complexation in solution in cases where the IC model is not satisfactory.[4,9]

Experimental Section

The crowns were highest purity commercially available (Aldrich, Fluka) samples and were used as received. The diazonium ions were prepared by conventional diazotization ($NaNO_2$ + HBF_4) of the anilines and were purified as previously described.[10]

Sample Preparation. To a slurry of the diazonium ion in dry CH_2Cl_2 was added a 1–2-fold molar excess of the corresponding crown and the mixture was vortexed until homogeneous. Turbid or heterogeneous samples were filtered to remove uncomplexed diazonium ion.

FAB and FD mass spectral data were acquired by use of a Finnigan MAT 311A/INCOS 2400 mass spectrometer system equipped with an EI/FD/FAB ion source. For FAB-MS, high energy xenon (8 kV) was used to effect sputtering. Spectra were recorded only in the positive ion mode. The samples were directly deposited on the stainless steel FAB probe. Standard high temperature activated carbon emitters were used for FD-MS. Samples were deposited onto the emitter by using the normal dipping technique.

Registry No. $PhN_2^+BF_4^-$/18-C-6 (1:1), 74879-30-4; $PhN_2^+BF_4^-$/18-C-6 (1:2), 117983-92-3; $PhN_2^+BF_4^-$/24-C-8 (1:1), 117983-93-4; o-$MeC_6H_4N_2^+BF_4^-$/18-C-6 (1:2), 117983-94-5; o-$MeC_6H_4N_2^+BF_4^-$/24-C-8 (1:1), 118017-02-0; 2,4,5-$Me_3C_6H_2N_2^+BF_4^-$/18-C-6 (1:1), 117983-96-7; 2,4,6-$Me_3C_6H_2N_2^+BF_4^-$/24-C-8 (1:1), 117983-97-8; p-$BrC_6H_4N_2^+BF_4^-$/18-C-6 (1:1), 85106-18-9; p-$BrC_6H_4N_2^+BF_4^-$/24-C-8 (1:1), 117983-98-9; p-$O_2NC_6H_4N_2^+BF_4^-$/18-C-6 (1:1), 74317-27-4; p-$O_2NC_6H_2^+BF_4^-$/18-C-6 (1:2), 76276-03-4; p-$O_2NC_6H_4N_2^+BF_4^-$/18-C-6 (1:3), 117983-99-0; m-$O_2NC_6H_4N_2^+BF_4^-$/24-C-8 (1:1), 117984-00-6; anthracene-1-$N_2^+BF_4^-$/18-C-6 (1:1), 117984-01-7; anthracene-1-$N_2^+BF_4^-$/24-C-8 (1:1), 117984-03-9.

Facile Synthesis of Optically Pure (2*R*,3*R*)- and (2*R*,3*S*)-3-Fluoroglutamic Acids Using Glutamate Dehydrogenase

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Received June 7, 1988

The development of syntheses of 3-fluoro amino acids[1,2] has been prompted by their interest as potential kcat inhibitors and tools for the elucidation of enzymatic reactions mechanisms.

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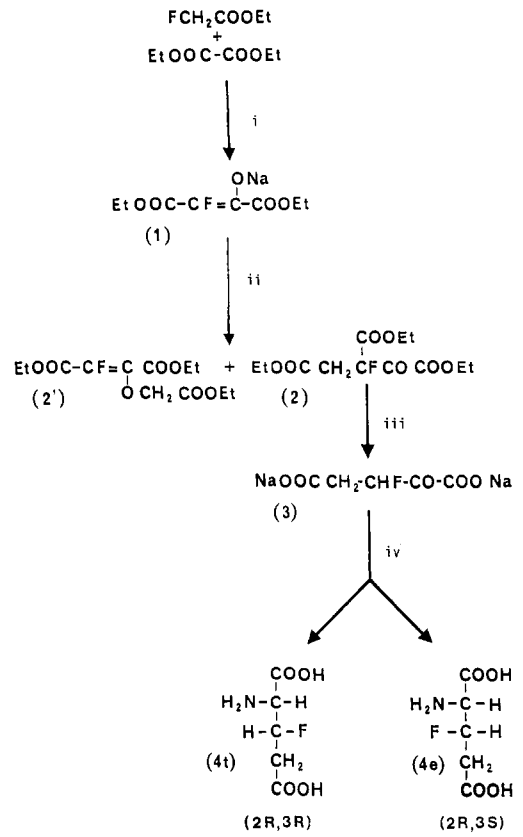
Scheme I.[a] Synthesis of (2*R*,3*R*)- and (2*R*,3*S*)-3-Fluoroglutamic Acids



[a] (i) EtO^-Na^+, Et_2O; (ii) $BrCH_2COOEt$; (iii) (a) CH_3COOH/HCl, (b) $NaHCO_3$; (iv) (a) GDH, YADH, NAD^+, ethanol, (b) Dowex 1×4.

However, most of these synthetic methods are restricted to cases having unfunctionalized side chains. Strikingly, no synthesis was described in the glutamate series until 1985 when we described the first synthesis of L threo and erythro 3-fluoroglutamic acids,[5] designed for the study of glutamate decarboxylase.[6] This synthesis, involving fluorodehydroxylation of 3-hydroxyglutamic acid derivatives by sulfur tetrafluoride in liquid hydrogen fluoride,[7] appeared rather tedious since this agent is relatively toxic and not easy to handle. Furthermore this synthesis implies a resolution either of the starting hydroxylated or of the fluorinated compounds, resulting in the loss of 50% of the material.

We present here a new and short synthesis of (2*R*,3*R*)- and (2*R*,3*S*)-3-fluoroglutamic acids **4t** and **4e** based on the enzymatic reductive amination of 3-fluoroketoglutarate **3** by glutamate dehydrogenase.

Results and Discussion

The sodium salt of diethyl fluorooxaloacetate **1** was prepared according to Bergmann[8] by Claisen condensation of diethyl oxalate with ethyl fluoroacetate (Scheme I). The proton NMR spectrum of the free ester, which could be recovered after acidification, exhibited two CHF signals. The high-field signal at 5.20 ppm (d,J_{HF} = 46.8 Hz) was ascribed to the gem-diol species and the low-field signal at 5.95 ppm (d,J_{HF} = 47.5 Hz) to the keto species, by analogy with fluoropyruvate.[9,10] According to the NMR data, the gem-diol appeared to be the predominent form, which is consistent with the well-known effect of fluorine.[11,12]

Condensation of **1** with ethyl bromoacetate afforded diethyl 2-oxo-3-carbethoxy-3-fluoroglutarate (**2**) along with the *O*-alkylation product **2'**, in a 1/1 ratio. Attempts to reduce the undesirable reaction by using the lithium salt failed, the same proportions of **2** and **2'** being observed. Here again, the ^{19}F NMR spectrum of **2** revealed the presence of two species, presumably the gem-diol and the keto forms.

Bergmann[8] reported that diethyl fluorooxaloacetate easily decomposes into oxalic and fluoroacetic acids on treatment with cold concentrated hydrochloric acid. However, Kun[13] and later Walsh[14] described a mild hydrolysis of this compound by a 2/1 mixture of hydrochloric and acetic acids. We likewise observed degradation of triester **2** during treatment in either acidic (3 N HCl) or basic (alcoholic 1 N KOH) conditions. This difficulty could be overcome by using the procedure described by Kun. Decarboxylation of the α-keto acid was performed by simply heating the acidic hydrolytic mixture at 50 °C. The 2-oxo-3-fluoroglutaric acid thus obtained was purified by precipitation of the disodium salt **3**. Boron tribromide hydrolysis[15] of triester **2** followed by decarboxylation yielded the desired 2-oxo-3-fluoroglutaric acid as well. Nevertheless, purification of the product appeared troublesome, certainly due to the presence of borate anions.

Keto acid **3** was specifically converted into (2*R*,3*R*)- and (2*R*,3*S*)-3-fluoroglutamic acids **4t** and **4e** by using glutamate dehydrogenase. This enzyme catalyzes the initial formation of an α-immunoglutarate followed by reduction of this imine.[16,17] Undesirable F^- elimination was very unlikely, in contrast with a PLP-dependent transaminase catalysis. The reducing agent, NADH, was generated in situ by using the NAD^+–yeast alcohol dehydrogenase–ethanol system.[18] The diastereoisomeric **4e** and **4t**, obtained in 95% yield, were separated by ion-exchange chromatography (Dowex 1×4).

It is worth noting that the enzymatic yield is highly dependent on the degree of purity of the starting material. Utilization of either the crude or even the ion-exchange purified diacid (Dowex 1×4 eluted with 7 N formic acid) led to variable yields, often as low as 10% and never exceeding 70%. Precipitation of the disodium salt thus appears to be essential to good enzymatic activity.

The optical purity of **4e** and **4t** was checked by gas-phase chromatography analysis of the *N*-acetyl diisopropyl esters on a chiral-phase column.[19] The acids were derivatized under mild conditions to avoid any degradation, especially

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during the esterification step. Both **4e** and **4t** proved to be optically pure.

The synthesis presented here is very convenient, owing to the simplicity of the different steps, avoiding in particular use of protection and deprotection operations, which are often a real problem with fluoro amino acids.[20,21] The enzymatic conversion leads directly to the desired optically pure products in very high yield.

Experimental Section

General Methods. Melting points were measured with a Kofler hot-stage apparatus and are uncorrected. ^1H NMR and ^{19}F NMR spectra were recorded at 90 MHz on a JEOL FX90Q spectrometer. Optical rotations were determined with a Perkin-Elmer Model 141 polarimeter with 10 cm path length cells.

Chemicals. Ethyl fluoroacetate was purchased from Fluka, boron tribromide was purchased from Merck, and ethyl bromoacetate and diethyl oxalate were purchased from Prolabo. Glutamate dehydrogenase (from beef liver) and NAD^+ (100%) were obtained from Boehringer; yeast alcohol dehydrogenase was from Sigma. Dowex resins were purchased from Fluka.

Diethyl Fluorooxaloacetate Sodium Salt (1). Compound 1 was obtained in 82% yield from ethyl fluoroacetate and diethyl oxalate according to Bergmann et al.,[8] mp 175–177 °C dec. The protonated form of 1 was recovered by acidification to pH 1 with sulfuric acid and extraction with diethyl ether: ^1H NMR ($CDCl_3$, TMS) δ 1.37 (6 H, m, 2 OCH_2CH_3), 4.37 (4 H, m, 2 OCH_2CH_3), 5.20 (0.75 H, d, J_{HF} = 46.8 Hz, CHF), 5.95 (0.25 H, d, J_{HF} = 47.5 Hz, CHF).

Diethyl 2-Oxo-3-carbethoxy-3-fluoroglutarate (2). Ethyl bromoacetate (25.5 mL, 0.23 mol) was added dropwise within 1 h to a solution of 1 (35 g, 0.153 mol) in dry dimethylformamide (125 mL) cooled at 0 °C. After the mixture was stirred at room temperature overnight, the sodium bromide was filtered, and the solvent was eliminated under vacuum, the product was taken up in ethyl acetate, washed with water, dried, and evaporated to dryness (47 g). Chromatography on silica gel with ethyl acetate–hexane (1:1) as solvent of 15 g of this crude product yielded **2** (7.5 g, 52%): ^1H NMR ($CDCl_3$, TMS) δ 1.30 (9 H, m, 3 OCH_2CH_3), 2.35–3.88 (2 H, m, CH_2), 4.25 (6 H, m, 3 OCH_2CH_3); ^{19}F NMR ($CDCl_3$, $CF_3C_6H_5$) δ −103.0 (X part of an ABX spectrum, $J_{AX} + J_{BX}$ = 49.6 Hz), −108.9 (X part of an ABX spectrum, $J_{AX} + J_{BX}$ = 47.3 Hz); mass spectrum (positive chemical ionization), m/z 310 ($(M + NH_4)^+$), 290 ($(M + NH_4 - HF)^+$ and **2'** (5.5 g, 48%); ^1H NMR ($CDCl_3$, TMS) δ 1.35 (9 H, m, 3 OCH_2CH_3), 4.35 (6 H, m, 3 OCH_2CH_3), 4.63 + 4.67 (2 H, CH_2); ^{19}F NMR ($CDCl_3$, $CF_3C_6H_5$) δ −88.0; mass spectrum (positive chemical ionization), m/z 310 ($(M + NH_4)^+$).

Disodium 2-Oxo-3-fluoroglutarate (3). Compound **2** (8 g; 25.8 mmol) was hydrolyzed at room temperature by a 2:1 solution of acetic acid–hydrochloric acid (60 mL) for 3 days and decarboxylated by heating at 50 °C for 5 h. After elimination of the solvent under reduced pressure, the crude product was stored in a dessicator on potassium hydroxide: ^1H NMR (D_2O with CF_3COOH, TMS) δ 2.7–3.3 (2 H, m, CH_2), 5.15 (1 H, dm, J_{HF} = 46.7 Hz, CHF); ^{19}F NMR (D_2O, CF_3COOH) δ −117.7 (eight lines). Anal. of the 2,4-dinitrophenylhydrazone of the dimethyl ester. Found: C, 41.97; H, 3.52; N, 14.86. Calcd for $C_{13}H_{13}FN_4O_8$: C, 41.93; H, 3.49; N, 15.05.

Neutralization with sodium bicarbonate and recrystallization from water–ethanol afforded **3** (3.55 g; 66%). Anal. Found: C, 26.59; H, 2.41. Calcd for $C_5H_3FO_5Na_2 \cdot 1H_2O$: C, 26.54; H, 2.21.

(2*R*,3*R*)- and (2*R*,3*S*)-3-Fluoroglutamic Acids (4t and 4e). A solution of **3** (1.04 g; 5 mmol) in 0.5 M ammonium phosphate buffer at pH 7 (100 mL) containing ethanol (2 mL), EDTA (5 mg), NAD^+ (400 mg), bovine serum albumin (100 mg), glutamate dehydrogenase (20 mg; 420 units) and yeast alcohol dehydrogenase (350 μL; 3000 units) was incubated at 30 °C. The same amounts of ethanol, NAD^+, GDH, and YADH were added after 24, 48, and 72 h. After precipitation of the proteins by addition of trichloroacetic acid (6 g) and centrifugation at 10 000 rpm for 10 min, the supernatant was applied to a Dowex 50W×2 column (100–200 mesh; 50 × 3.6 cm; H^+ form). After the mixture was washed with water, elution with 0.5 N acetic acid yielded a mixture of **4e** and **4t** (800 mg; 96%). The two diastereoisomers (500 mg) were separated on Dowex 1×4 (200–400 mesh; 92 × 3 cm; acetate form, 10 mL/fraction). After the mixture was washed successively with water and 0.1 and 0.2 N acetic acid, elution with 0.5 N acetic acid yielded **4e** [(0.72–1.03 L, 220 mg); recrystallized in water–acetone; mp 194–195 °C; $[\alpha]^{20}_D$ = +20° (*c* = 1, H_2O); $[\alpha]^{20}_D$ = +38° (*c* = 1, HCl, 1 N): ^1H NMR of the sodium salt (D_2O, TMS) δ 2.30–2.88 (2 H, m, CH_2), 4.06 (1 H, dd, J_{HH} = 2.6 Hz, J_{HF} = 20.5 Hz, CHN), 5.31 (1 H, dm, J_{HF} = 47.1 Hz, CHF); ^{19}F NMR of the sodium salt (D_2O, CF_3COOH) δ −156.0 (17 lines). Anal. Calcd for $C_5H_8FNO_4$: C, 36.36; H, 4.84; N, 8.48. Found: C, 36.24; H, 4.91; N, 8.32] and **4t** (1.13–1.49 L, 220 mg): recrystallized in water; mp 190–191 °C; $[\alpha]^{20}_D$ = +3° (*c* = 1, H_2O); $[\alpha]^{20}_D$ = +13.6° (*c* = 1, HCl, 1 N); ^1H NMR of the sodium salt (D_2O, TMS) δ 2.53–3.10 (2 H, m, CH_2), 3.91 (1 H, dd, J_{HH} = 3.9 Hz, J_{HF} = 26.3 Hz, CHN), 5.38 (1 H, dm, J_{HF} = 45.1 Hz, CHF); ^{19}F NMR of the sodium salt (D_2O, CF_3COOH) δ −154.0 (12 lines). Anal. Calcd for $C_5H_8FNO_4$: C, 36.36; H, 4.84; N, 8.48. Found: C, 36.23; H, 4.69; N, 8.38.

Optical Purity Determination. Compounds **4e** and **4t** were separately submitted to the following reactions:

(a) Acetylation. 3-Fluoroglutamic acid (7 mg) was treated with dry acetic anhydride (15 μL) in dry methanol (300 μL) at room temperature until complete dissolution (~2.1 h). After evaporation of the solvents, the crude product was applied to a Dowex 50W×2 column and eluted with water.

(b) Esterification. The *N*-acetyl-3-fluoroglutamic acid was treated for 15 min at 60 °C with dry 2-propanol–hydrogen chloride (1.5 mol/L, 300 μL). After elimination of the solvent under vacuum, the crude product was analyzed by gas chromatography on a Chrompack-fused silica capillary column (50 m × 0.25 mm) coated with XE-60-S-valine-(*S*)-phenylethylamide (175 °C, helium (1.5 bar)).

Registry No. 1, 7582-61-8; 1 (protonated), 55475-75-7; **2**, 1608-58-8; **2'**, 117860-26-1; **3**, 117860-25-0; **3** (dimethyl ester, 2,4-dinitrophenylhydrazone), 117860-27-2; **4e**, 97315-76-9; **4t**, 97315-77-0; FCH_2COOEt, 459-72-3; $(COOEt)_2$, 95-92-1; $BrCH_2COOEt$, 105-36-2; glutamate dehydrogenase, 9001-46-1.

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Synthesis of *all-cis*-1-Bromo-4,7,10,13-nonadecatetraene: A Precursor to C-1 Labeled Arachidonic Acid

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Received September 12, 1988

Arachidonic acid is a biosynthetic precursor to several biologically important compounds (e.g., prostaglandins, thromboxanes, prostacyclins, leukotrienes).[1] Progress in studying the enzymology of the arachidonic cascade is critically dependent on the availability of specifically labeled fatty acid precursors. The standard approach for the chemical synthesis of arachidonic acid is the preparation of the corresponding homoconjugated tetrayne followed by selective hydrogenation of the acetylenic bonds to cis olefins.[2] Because of the great instability[3] of these

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