Synthesis of 3,3-Difluoro-DL-alanine and 3,3-Difluoro-DL-alanine Precursors

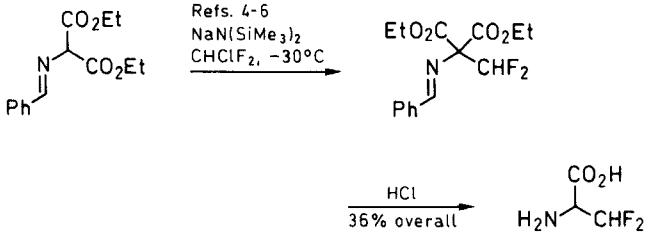
Hugues d'Orchymont

Marion Merrell Dow Research Institute, Strasbourg Research Center, 16, rue d'Ankara, B.P. 067, F-67046 Strasbourg Cedex, France

Received 1 March 1993

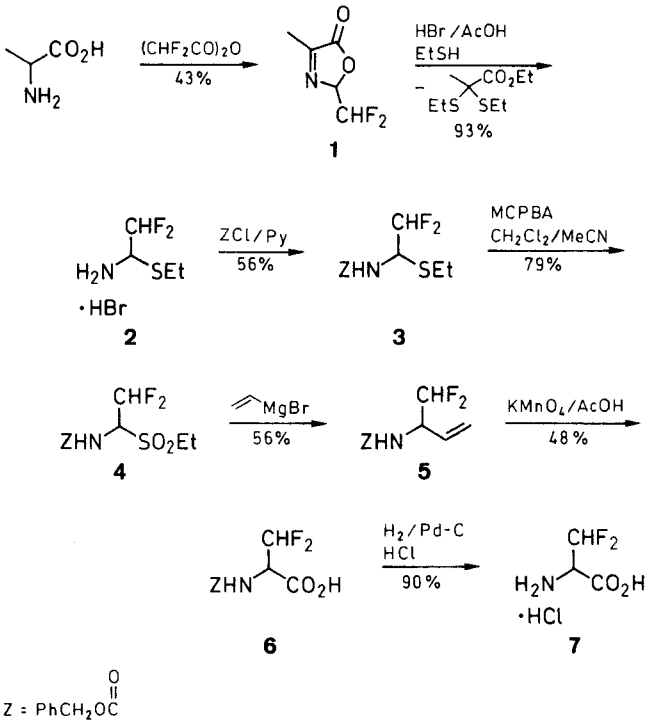
The key intermediate to 3,3-difluoroalanine, 2,2-difluoro-1-ethylthioethylamine hydrobromide **(2)**, was prepared by the Steglich and Weygand procedure. Its conversion to *N*-benzyloxycarbonyl-2,2-difluoro-1-ethenylethylamine **(5)** followed by oxidation and subsequent deprotection of the amine afforded the 3,3-difluoroalanine.

The interest in fluorine chemistry[1] relies on the fact that fluorine can have profound and unexpected consequences on biological activity. The discovery that β-fluoro amino acids could be useful inhibitors of pyridoxal phosphate (PLP) dependent enzymes stimulated synthetic efforts in this field. As an example, β-fluoroalanines were found to be suicide inhibitors of *Escherichia coli* Alanine Racemase.[2] The synthesis of fluorinated compounds often requires the use of molecular fluorine or toxic and hazardous reagents prepared from fluorine itself. Thus, a mixture of 3,3-difluoro-L-alanine with 3-fluoro-L-alanine was first obtained in very low yields (3%) by Kollonitsch et al. by fluorodesulfurization of L-cysteine using a fluorine/helium mixture.[3] At the time when we were developing our own methodology, Tsushima et al. described an elegant racemic synthesis via alkylation of the enolate of benzylideneaminomalonate by difluorocarbene and subsequent hydrolysis and decarboxylation, with a 36% overall yield as shown in Scheme 1.[4-6] A limitation we experienced for this attractive approach, was the preparation of diethyl benzylideneaminomalonate with an acceptable purity, probably owing to its propensity to dimerize into imidazolidine.[7-8]



Scheme 1

Our approach, depicted in Scheme 2, was adapted from the more general procedure described by Weygand and Steglich for the preparation of 3,3,3-trifluoroalanine and *C*-perfluoroalkylglycines.[9-13] The fluorine source was difluoroacetic acid which is devoid of highly hazardous properties. Most of the intermediates are crystalline and easily purified by crystallization of the crude mixtures. Therefore this synthesis, although less expeditious, appears to be a valuable alternative to the Tsushima approach. Furthermore, since we were interested in the preparation of 3,3-difluoroalanyl peptides analogous to 3,3,3-trifluoroalanyl peptides,[13] the intermediates **3** and **5** may have some synthetic utility as masked difluoroalanines.



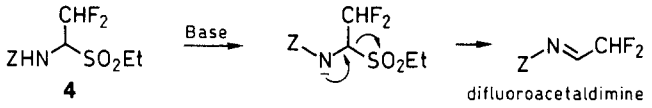
Scheme 2

In 1962 Weygand and Steglich discovered that 2-trifluoromethyl-4-methyl-5(2*H*)-oxazolone underwent an unusual fragmentation when treated with a solution of hydrogen bromide in acetic acid in the presence of ethanethiol to afford 2,2,2-trifluoro-1-ethylthioethylamine hydrobromide together with 2,2-bis(ethylthio)propanoic acid.[9] This reaction was extensively investigated and extended to the 2-pentafluoroethyl- and 2-heptafluoropropyloxazolones.[12] Its mechanism has been discussed: a nucleophilic attack by the thiol on the C–N double bond was proposed to initiate this fragmentation.[9]

Accordingly, 2-difluoromethyl-4-methyl-5(2*H*)-oxazolone **(1)** obtained by reaction of alanine with difluoroacetic anhydride was similarly treated to give 2,2-difluoro-1-ethylthioethylamine hydrobromide **(2)** which was easily obtained by crystallization in good yield (93%). The amino function was protected as the benzylcarbamate and the thioether **3** oxidized to the sulfone **4** under mild and effective conditions using 3-chloroperoxybenzoic acid (MCPBA) in an acetonitrile/dichloromethane mixture rather than in pure dichloromethane. Under these conditions, a reactive *m*-chlorobenzoylperoxyimidate formed by addition of MCPBA to acetonitrile, might be the active oxidizing species.[14] Another crucial step was the substitution of **4** by vinylmagnesium bromide, performed by adding **4** to an excess (5 equiv) of Grignard reagent. No side products arising from hydrogen fluoride elimination under the basic conditions were observed.

From a mechanistic point of view, vinylmagnesium bromide is not expected to give a direct substitution of the sulfonyl group, but rather to add onto a reactive difluoroacetaldimine intermediate formed by elimination of ethylsulfinic acid as shown in Scheme 3.[10,11] The penultimate step was the conversion of aminoalkene **5** to the amino acid **6** by oxidation with potassium permanganate in acetic acid. In a classical way the *Z*-protected amine was regenerated by hydrogenolysis over palladium on charcoal but these conditions repetitively failed to give analytically pure 3,3-difluoroalanine as free amino acid, suggesting a poor stability under neutral conditions.

Nevertheless by adding aqueous hydrochloric acid prior to hydrogenolysis, the chlorohydrate was obtained in a pure form. In contrast, Tsushima et al. isolated the pure free amino acid by adsorbing the hydrochloride on the ion exchanger AG 50W-X and eluting with aqueous 2N ammonia.[4]



Scheme 3

In conclusion, we have synthesized 3,3-difluoroalanine in an overall 10% yield based on the oxazolone **1** via the well established chemistry of Weygand and Steglich. By this method, intermediates of interest for the incorporation of difluoroalanine into peptides were also produced. All the intermediates are well characterized (see experimental part).

Melting points were recorded on a Büchi 535 melting point apparatus and are uncorrected. Elemental analyses were obtained on a Carlo Erba model 1106 analyzer. Mass spectra by the chemical ionization (CI) technique were obtained on Finnigan TSQ 46 or SSQ 70. ^{1}H and ^{19}F NMR spectra were recorded on a Bruker AC 200 spectrometer and ^{13}C NMR on a Bruker AM 360 spectrometer. IR spectra were obtained on a Bruker IFS 66 spectrophotometer. All new compounds gave satisfactory elemental analyses: C ± 0.50, H ± 0.16, N ± 0.35.

Anhydrous THF and CH_2Cl_2 were prepared by distillation from benzophenone/Na and P_2O_5, respectively. Pyridine was distilled over CaH_2. Other commercially available reagents were used without further purification. Column chromatography was performed on silica gel 60 (230–400 mesh) purchased from Merck.

2-Difluoromethyl-4-methyl-5(2*H*)-oxazolone (1):
Difluoroacetic anhydride[16] (49.1 g, 0.28 mol) was added slowly over 10 min to alanine (10.04 g, 0.11 mol) cooled at 0°C. After 0.5 h of additional stirring, the clear mixture was heated at 90°C for 2 h and distilled under reduced pressure to give crude oxazolone contaminated with difluoroacetic acid (bp 46°C/0.1 Torr). The acid was removed by washing a CH_2Cl_2 solution of the mixture with aq $NaHCO_3$ to afford, after solvent evaporation, **1** as an oil; yield: 7.08 g (43%).

^{1}H NMR ($CDCl_3$/TMS, 90 MHz): δ = 2.27 (d, 3 H, J = 3 Hz, CH_3), 5.94 (td, 1 H, $J_{H,F}$ = 54, J = 3 Hz, CHF_2), 6.0 (m, 1 H, $CHCHF_2$).[15]

2,2-Difluoro-1-ethylthioethylamine, Hydrobromide (2):
A mixture of HBr in AcOH (25%, 8 mL) and EtSH (9.3 mL, 0.125 mol) was added over a 15 min period to oxazolone **1** (4.0 g, 26.8 mmol) cooled at 0°C. The stirring was continued for 15 min, at 0°C and 1 h at r.t. Evaporation of the mixture under vacuum gave an oily residue which was crystallized from a mixed solvent of Et_2O/pentane to give **2** as a white solid; yield: 5.56 g (93%).

^{1}H NMR (CD_3OD/TMS, 90 MHz): δ = 1.20 (t, 3 H, J = 7.5 Hz, CH_2CH_3), 2.89 (q, 2 H, J = 7.5 Hz, CH_2CH_3), 4.62 (m, 1 H, $J_{H,F}$ = 18, J_{HF} = 9 Hz, J = 3 Hz, $CHCHF_2$), 6.10 (td, 1 H, $J_{H,F}$ = 55 Hz, J = 3 Hz, CHF_2).

^{13}C NMR (D_2O/TMS, 90 MHz): δ = 15.8, 27.7, 58.0 (t, $J_{C,F}$ = 23.5 Hz), 114.8 (t, $J_{C,F}$ = 246 Hz).

***N*-Benzyloxycarbonyl-2,2-difluoro-1-ethylthioethylamine (3):**
To a stirred suspension of **2** (2.18 g, 9.8 mmol) in anhydr. CH_2Cl_2 (12 mL) at −15°C) under N_2 was added dropwise anhydr. pyridine (2.0 mL, 24.7 mmol) and benzyl chloroformate (1.6 mL, 11.2 mmol). The mixture was stirred at −10°C for 40 min, allowed to warm to 0°C for 4 h and to r.t. overnight. The resulting mixture was diluted with CH_2Cl_2, washed successively with aq 0.1 N HCl and sat. aq $NaHCO_3$ and the organic layer was dried ($MgSO_4$). Evaporation of solvent yielded a solid which was recrystallized from hexane (50 mL) to afford **3**; yield: 1.51 g (56%); colorless needles; mp 79.5–80.5°C.

IR (KBr): ν = 3293, 1686, 1529, 1369, 1312, 1263, 1216, 1130, 1063, 1046, 957, 748, 696 cm^{-1}.

^{1}H NMR ($CDCl_3$/TMS, 200 MHz): δ = 1.24 (t, 3 H, J = 7.5 Hz, CH_2CH_3), 2.65 (q, 2 H, J = 7.5 Hz, CH_2CH_3), 5.15 (s, CH_2, $C_6H_5CH_2$), 5.15 (m, 2 H, $CHCHF_2$ + NH), 5.90 (t, 1 H, $J_{H,F}$ = 55 Hz, J < 2 Hz, CHF_2), 7.4 (s, 5 H, C_6H_5).

^{19}F NMR ($CDCl_3/C_6F_6$, 188 MHz) ($AB_{XX'}$ type spectrum): δ_A = 41.5, δ_B = 35.1 ($J_{F,F}$ = 281 Hz, $J_{A,X'} = J_{B,X'}$ = 55 Hz, $J_{A,X}$ = 11.5 Hz, $J_{B,X}$ = 15 Hz).

MS (CI, NH_3): m/z = 283 (MNH_4^+), 276 (MH^+), 153.

***N*-Benzyloxycarbonyl-2,2-difluoro-1-ethylsulfonylethylamine (4):**
To a stirred solution of sulfide **3** (1.4 g, 5.08 mmol) in CH_2Cl_2 (25 mL) cooled at −20°C, was added dropwise over 45 min a solution of MCPBA (2.35 g, 85%, 11.6 mmol) in MeCN (27 mL). The mixture was stirred for an additional 1 h at −20°C, then 1 h at 0°C and finally 16 h at r.t. The mixture was washed with aq 10% Na_2SO_3 and aq $NaHCO_3$ and the organic layer was dried ($MgSO_4$). Evaporation of the solvent and recrystallization of the residue from Et_2O/pentane gave **4**; yield: 1.23 g (79%); colorless solid; mp 95–96°C.

IR (KBr): ν = 3279, 1697, 1540, 1367, 1317, 1260, 1139, 1070, 756, 700, 603 cm^{-1}.

^{1}H NMR ($CDCl_3$/TMS, 200 MHz): δ = 1.35 (t, 3 H, J = 7.5 Hz, CH_2CH_3), 3.00 (q, 2 H, J = 7.5 Hz, CH_2CH_3), 5.17 (s, 2 H, $C_6H_5CH_2$), 5.2 (m, 1 H, $CHCHF_2$), 5.94 (d, 1 H, J = 9 Hz, NH), 6.40 (t, 1 H, $J_{H,F}$ = 54 Hz, J < 2 Hz, CHF_2), 7.34 (s, 5 H, C_6H_5).

^{13}C NMR ($CDCl_3$/TMS, 90 MHz): δ = 6.6, 47.7, 68.5 (t, $J_{C,F}$ = 22 Hz), 110.7 (t, $J_{C,F}$ = 248 Hz) (resonances of *Z*-protecting group not included).

MS (CI, NH_3): m/z = 325 (MNH_4^+), 308 (MH^+), 231.

***N*-Benzyloxycarbonyl-2,2-difluoro-1-ethenylethylamine (5):**
A solution of **4** (493 mg, 1.60 mmol) in anhydr. THF (8 mL) was added dropwise under N_2 to a stirred suspension of vinylmagnesium bromide in THF (1 M, 8 mL) cooled at 0°C. The stirring was maintained 0.5 h at 0°C and the mixture was quenched by addition of AcOH-water mixture (2:1, 1.5 mL). The mixture was extracted with CH_2Cl_2 (75 mL), the organic layer washed successively with aq 0.1 N HCl (50 mL) and sat. aq $NaHCO_3$ (25 mL) and dried ($MgSO_4$). Purification by chromatography on silica gel (50 g) with EtOAc/hexane (15:85) as eluent and crystallization of the residue from hexane gave **5**; yield: 216 mg (56%); colorless solid; mp 70–71°C.

IR (KBr): ν = 3312, 1685, 1543, 1256, 1130, 1063, 757, 698 cm^{-1}.

^{1}H NMR ($CDCl_3$/TMS, 200 MHz): δ = 4.6 (m, 1 H, $CHCHF_2$), 5.0 (br s, 1 H, NH), 5.10 (s, 2 H, $C_6H_5CH_2$), 5.4 (m, 2 H, $=CH_2$), 5.85 (m, 1 H, $-CH=$), 5.86 (t, 1 H, J_{HF} = 54 Hz, J < 3 Hz, CHF_2), 7.4 (s, 5 H, C_6H_5).

^{19}F NMR ($CDCl_3/C_6F_6$, 188 MHz) (ABXX' type spectrum): $\delta_A = 35.3$, $\delta_B = 32.3$ ($J_{F,F} = 284$ Hz, $J_{A,X'} = J_{B,X'} = 56$ Hz, $J_{A,X} = 11.5$ Hz, $J_{B,X} = 17.5$ Hz).

MS (CI, NH_3): $m/z = 258$ (MNH_4^+), 242 (MH^+), 152.

N-Benzyloxycarbonyl-3,3-difluoroalanine (6):

A solution of **5** (130 mg, 0.54 mmol) in AcOH (2.3 mL) was added to a stirred solution of $KMnO_4$ (270 mg, 1.70 mmol) in water (19 mL) cooled at 0 °C. The mixture was stirred for 2 h at 0 °C and then overnight at r.t. Following addition of aq Na_2SO_3, the mixture was acidified to pH 2 by aq 3 N HCl and extracted with EtOAc. The organic layer was washed with brine and dried ($MgSO_4$). Evaporation of solvents gave a residue which was taken up in EtOAc and extracted with aq sat. $NaHCO_3$. Acidification and extraction with EtOAc as previously, gave a residue which was crystallized from a mixed solvent of hexane/EtOAc to afford **6**; yield: 68 mg (48 %); colorless solid; mp 108.5–110 °C.

IR (KBr): ν = 3319, 1720, 1684, 1545, 1310, 1332, 1063 cm^{-1}.

^{1}H NMR ($CDCl_3$/TMS, 200 MHz): δ = 4.90 (m, 1 H, $CHCHF_2$), 5.17 (s, 2 H, $C_6H_5CH_2$), 5.51 (d, 1 H, J = 10 Hz, NH), 6.19 (td, 1 H, $J_{H,F}$ = 55 Hz, J = 2 Hz, CHF_2), 7.4 (s, 5 H, C_6H_5).

^{19}F NMR ($CDCl_3/C_6F_6$, 188 MHz) (ABXX' type spectrum): $\delta_A = 36.0$, $\delta_B = 33.9$ ($J_{F,F} =$ 284 Hz, $J_{AX'} = J_{B,X'} = 54$ Hz, $J_{A,X} = 11.5$ Hz, $J_{B,X} = 19$ Hz).

MS (CI, NH_3): $m/z = 277$ (MNH_4^+), 260 (MH^+), 240 (MH^+–HF), 216 (MH^+–CO_2), 108.

3,3-Difluoroalanine Hydrochloride (7):

1 N HCl (120 µL) and 10 % Pd/C (11 mg) were added to a solution of **6** (25 mg, 0.096 mmol) in EtOH (10 mL) and the resulting mixture was hydrogenated at atmospheric pressure and r.t. for 4 h. After removal of the catalyst by filtration and evaporation of solvent, **7** was isolated; yield: 14 mg (90 %); buff colored solid.

^{1}H NMR (D_2O/TMS, pH = 1.9, 360 MHz): δ = 4.40 (ddd, 1 H, $J_{H,F}$ = 26.2 Hz, $J_{H,F}$ = 5.4 Hz, J = 1.7 Hz, $CHCHF_2$), 6.51 (td, 1 H, $J_{H,F}$ = 52.9 Hz, J = 1.6 Hz, CHF_2).

^{19}F NMR (D_2O/CF_3CO_2H, 339 MHz) (ABXX' type spectrum): $\delta_A = -48.7$, $\delta_B = -54.6$ ($J_{F,F} = 283$ Hz, $J_{A,X'} = J_{B,X'} = 52.9$ Hz, $J_{A,X} = 5.4$ Hz, $J_{B,X} = 26.3$ Hz).

MS (CI, NH_3): $m/z = 160$ ($M + 2NH_4^+$), 143 (MNH_4^+), 126 (MH^+).

MS (CI, CH_4): $m/z = 126$ (MH^+).

Dr. F. Piriou is gratefully acknowledged for a mutual discussion on the NMR spectra.

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