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SYNTHESIS OF 5,5'-DIHYDROXYLEUCINE AND 4-FLUORO 5,5'-DIHYDROXYLEUCINE, THE REDUCTION PRODUCTS OF 4-CARBOXYGLUTAMIC AND 4-CARBOXY-4-FLUOROGLUTAMIC ACIDS

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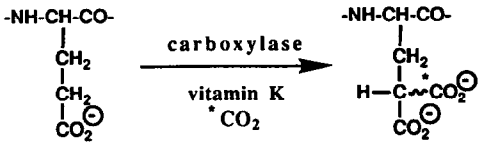
Abstract : Schemes for the synthesis of 5-5'-dihydroxyleucine **3** and its 4-fluoro analog **7** involving the condensation of a suitable "aminoacid moiety" with 2,2-dimethyl-5-iodomethyl-1,3-dioxane **15D** or its fluoro analog **27A** were tested. The anion of the ethyl N-diphenylmethylene-glycinate **25** gave better yields of **3** than the classical anion of diethyl acetamidomalonate. This strategy could not be successfully applied to the synthesis of **7**, which could be prepared by reduction of a suitably protected 4-fluoro-4-carboxyglutamate with BMS.

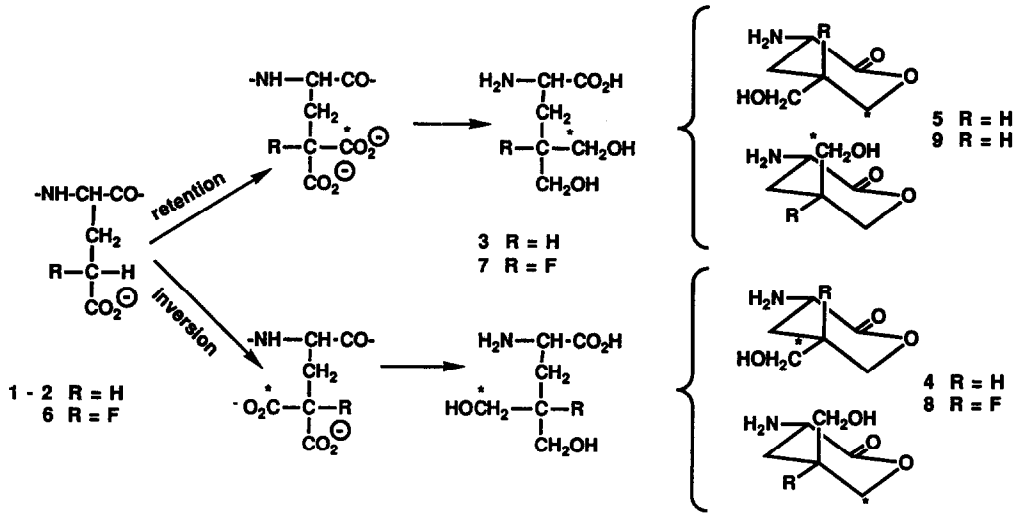
INTRODUCTION

To elucidate the stereochemistry of the Vitamin K dependent carboxylation of glutamic residues into 4-carboxy-glutamic (Gla) residues[1,2], it was necessary to establish the configuration at C-4 by conducting the enzymatic reaction with labelled CO_2 (Scheme I).



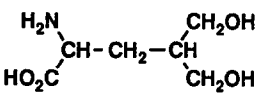
Scheme I : Vitamin K dependent carboxylation of glutamic residues

Due to the lability of the malonic hydrogen the configuration had first to be preserved by reduction. The following strategy was followed[3] : after carboxylation of the peptidic substrates Boc-Glu-Glu-Val **1** or Phe-Leu-Glu-Glu-Val **2**, the crude lyophilized product was reduced with borane-methyl sulfide in excess. The acidic hydrolysis of the crude reduction product yielded 5,5'-dihydroxyleucine **3** as a mixture of cis and trans lactones **4** and **5**. The determination of the configuration at C4 then relied on the location of the label in each isomeric lactone. This depended first on the assessment of the structure of each isomer and secondly on a method to locate the label (Scheme II).



Scheme II : Strategy for establishing the stereochemistry of the carboxylation

Since only a very small amount of 5,5'-dihydroxyleucine **3** could be isolated from the enzymatic experiments (< 1 µg), an independent synthesis of **3** was necessary, in order to carry out the preliminary experiments. The same strategy was also used to establish the absolute configuration of the carboxylation product of (2S,4R)-4-fluoroglutamate[1,4] **6** which implied the need to synthesize 4-fluoro-5,5'-dihydroxyleucine **7**.



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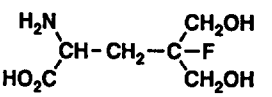


7

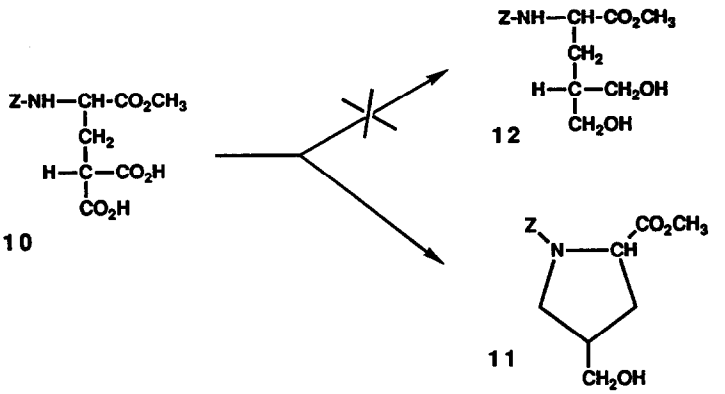
We have explored several pathways to compounds **3** and **7**. We present here an efficient synthesis of 5,5'-dihydroxyleucine **3** and we discuss the general reactivity problems encountered in this work : *reduction of malonic acids, alkylation of N-acetamidomalonate anions, influence of a fluorine substituent on the reactivity of alkylating moieties.*

RESULTS AND DISCUSSION

I - (DL) 5,5'-Dihydroxyleucine 3

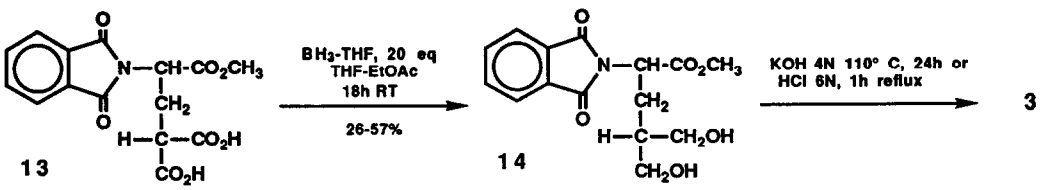
3 was identified for the first time by mass spectrometry, after derivatization, in the reduction product of Gla containing proteins[5]. The key step in the first synthesis of **3**, that we reported in a preliminary form in 1979[6], was the diborane reduction of a Gla derivative. We observed that the reduction of malonic acids with diborane was very slow and required a large excess of diborane, leading to medium yield. The same

observation has been made later by Choi et al.[7]* . Furthermore, in the case of Z-Gla α-methyl ester (prepared according to Schwyzer et al.[9]) we obtained a mixture in which the cyclized product **11** resulting from the participation of the nitrogen atom was the major component (Scheme III).



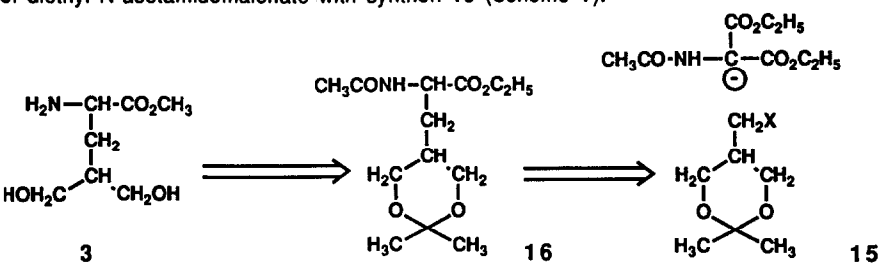
Scheme III : Reduction of Z-Gla α-methyl ester with BMS

To avoid this participation,the protective group was changed to phthaloyl (Scheme IV). The yield of the reduction step was not reproducible (from 26 to 57 %) and the phthaloyl group did not prove as easy to remove as anticipated**



Scheme IV : Synthesis of 5,5'-dihydroxyleucine 3[6]

Thus we tried to develop a more efficient and reproducible synthesis. This was first achieved by alkylation of diethyl N-acetamidomalonate with synthon **15** (Scheme V).



Scheme V : Retrosynthesis of 5,5'-dihydroxyleucine

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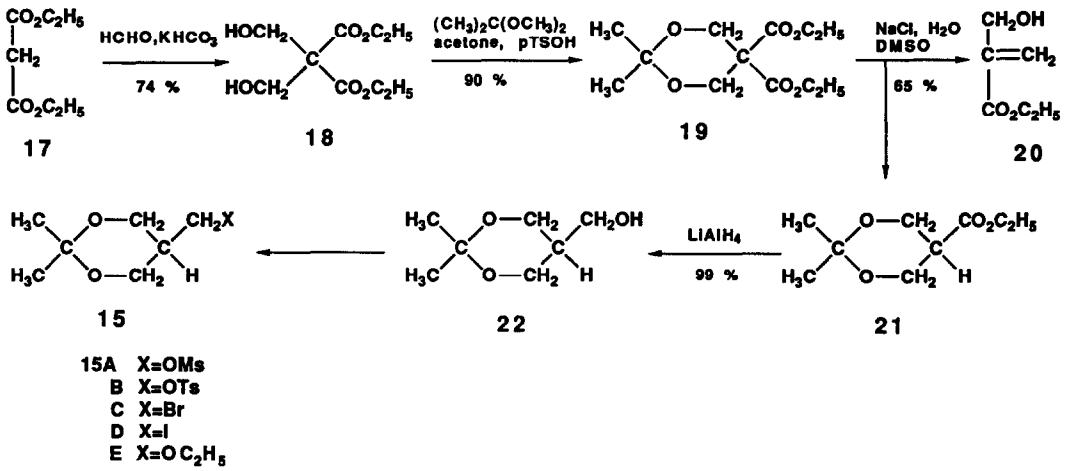
* However in preliminary assays we could achieve a complete reduction of phenylmalonic acid (10 BH3 per COOH) at room temperature in five hours[8], contrary to what Choi et al. observed[7].

** An uncomplete reaction was observed with hydrazine.

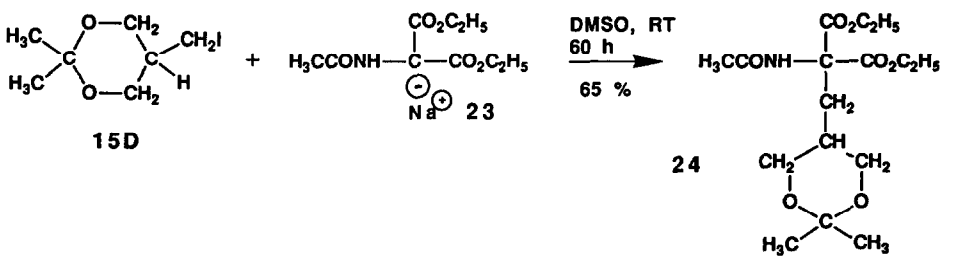
Different alkylating species **15** were prepared using classical reactions, according to Scheme VI, quite analogous to those used for the preparation of 2-hydroxymethyl-1,3-propanediol[10].



Scheme VI : Synthesis of **15A-D**

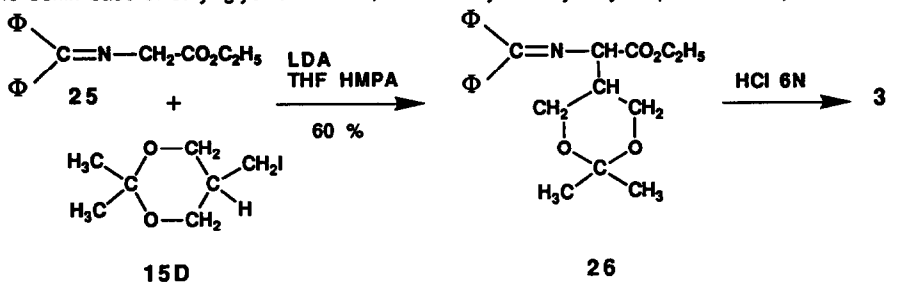
Some of these steps deserve comment. The ketalisation step **18** → **19** proceeded with a very poor yield by the azeotropic distillation method, but with a 90 % yield with dimethoxypropane in acetone[11]. The dealkoxycarbonylation step **19** → **21** did not reach completion and gave some elimination product **20** with NaCl (1 eq.) and H_2O (2 eq.) in DMSO. According to Krapcho[12], replacement of NaCl by LiCl should have improved the yield. However in our case, the amount of **20** was greatly increased, due to a better coordination of Li^+ to oxygen. The yield of conversion **15A** → **15C** was only 24 % with $Bu_4N^+Br^-$. After optimization, the yield of the **15A** → **15D** transformation reached 73 % with $Bu_4N^+I^-$ in benzene.

The alkylation step (Scheme VII) was not straightforward. When **15B** was treated with **23** in ethanol, no condensation was observed and the major compound was **15E**. The iodo derivative **15D** gave a complex mixture under the same conditions. The preformed sodium salt **23**[13] did not react with **15A** or **15B** in dimethylformamide and an incomplete reaction was observed with **15D**. A satisfactory yield of **24** could be obtained in DMSO. Hydrolysis with HCl 6N yielded **3**, in the lactone form. The open form was obtained after treatment with a base and ion exchange on AG1X2 resin.



Scheme VII : Alkylation of N-acetamido malonate with **15D**

Finally, the transformation of **15D** into **3** could be achieved in a better yield by condensation with the anion of the Schiff base of ethyl glycinate **25**[14], followed by HCl hydrolysis (Scheme VIII).

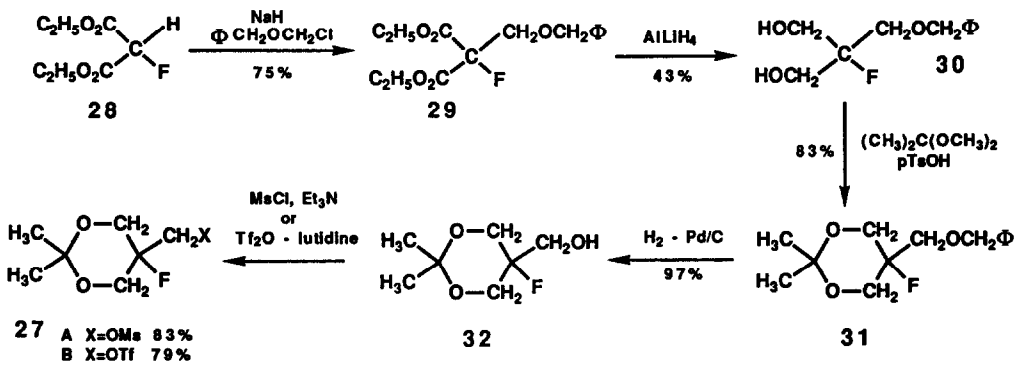


Scheme VIII : Alkylation of **25** with **15D**

II - (DL) 4-Fluoro-5,5'-dihydroxyleucine **7**

The first attempt to synthesize **7** started from **27**, the fluorinated analogue of **15**, prepared according to Scheme IX.

We observed that the reduction of **29** required a partially deactivated sample of $LiAlH_4$, fresh $LiAlH_4$ yielding the defluorinated product. The other steps leading to **32** do not require special comments. The mesylate **27A** was prepared easily, but could not be transformed into the corresponding iodide. Compound **27A** did not react with the sodium salt **23** in DMF.



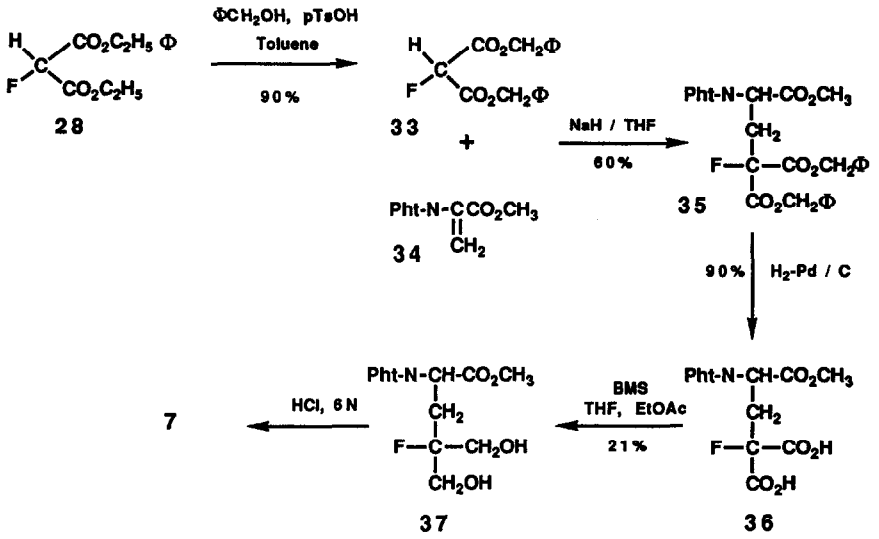
Scheme IX : Synthesis of compounds **27A-B**

The lack of reactivity of **27A** is likely due to the presence of the fluorine atom. A similar behaviour has been described in the case of carbohydrates. For instance, 6-sulphonates of galactopyranose derivatives reacted very slowly with anionic nucleophiles[15]. This lack of reactivity was attributed to the destabilization of the transition state of the SN_2 reaction* . This interpretation presumably holds in the case of our fluoro compound.

* Presumably as the result of the interaction between the negatively charged nucleophiles and the permanent dipole induced by the polar substituents α to the reaction center[15].

The triflate **27B** was then prepared, but it was very unstable in polar media (DMF, DMSO, HMPA) and did not react with **23** in CH_2Cl_2 or THF. Reaction of **27B** with the anion of ethyl N-phthalimidoglycinate **25** in THF did not take place, either.

Thus we had to return to the first synthesis of 5,5'-dihydroxyleucine **3**[6]. The phthaloyl derivative of 4-fluoro-4-carboxyglutamic acid α-methyl ester **36**, prepared according to Scheme X, was reduced with BMS in THF but again with a poor yield. Compound **37** was hydrolyzed with 6N HCl to give **7***.



Scheme X : Synthesis of **7**

EXPERIMENTAL SECTION

^1H NMR spectra were recorded on a Jeol FX90QX spectrometer in $CDCl_3$, otherwise stated, and chemical shifts are expressed in ppm relative to TMS as reference. IR spectra were recorded on a Perkin Elmer Infracord 237 spectrometer. Melting points were measured on a Kofler hot stage apparatus and are uncorrected. TLC were carried out on silica gel precoated plates from Merck. Microanalyses were carried out by the "Centre de Microanalyse de l'Université Paris VI".

Bis-(hydroxymethyl)-diethylmalonate **18**

To 81.1 g of a 37 % solution of formaldehyde (1 mol) and 4 g of $KHCO_3$, placed in a water-bath at 20°C, 80 g (0.5 mol) of diethylmalonate were added dropwise under stirring. Stirring was maintained for 1h after the end of the addition. A saturated solution of Na_2SO_4 was then added and the product was extracted with ether yielding 81 g of pure **18** (TLC) (Y : 74 %).

mp : 49-50°C (Litt 50-52°C)[16].

TLC (Hex.-EtOAc 1:1) R_F = 0.43.

IR (NaCl) 3640, 3140, 1715 cm^{-1}.

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* In contrast to 5,5'-dihydroxyleucine **3**, 4-fluoro-5,5'-dihydroxyleucine **7** did not lactonize under these acidic conditions.

2,2-Dimethyl-5,5-dicarbethoxy-1,3-dioxane **19**

75 g (0.34 mol) of **18**, 70.9 g (2 eq.) of 2,2-dimethoxypropane, 1.73 g (0.01 mol) of p-toluene sulfonic acid and 500 mL acetone were refluxed for 6h. After cooling, neutralization with solid Na_2CO_3, filtration and solvent evaporation, the residue was dissolved in $CHCl_3$ and washed with water. 80 g of **19** were obtained (Y : 90 %)
bp 110-113°C (2.5 mbar).
TLC (Hex.-EtOAc 1:1) R_F = 0.81.
NMR : 1.25 (t, J = 7, 6H, CH_2-CH_3) ; 1.40 (s, 6H $(CH_3)_2$-C) ; 4.21 (q, J = 7, 4H, CH_2-CH_3) ; 4.27 (s, 4H, -CH_2-O).
Anal. Calcd. for $C_{12}H_{20}O_6$: C, 55.37 ; H, 7.75. Found : C, 55.78 ; H, 7.91.

2,2-Dimethyl-5-carbethoxy-1,3-dioxane **21**

53.3 g (0.21 mol) of **19**, 7.4 mL (2 eq.) of water and 12 g (1eq.) of NaCl in 185 mL of anhydrous DMSO were refluxed for 6h. After cooling and addition of a saturated NaCl solution, the product was extracted with ether. The residual oil was distilled. Besides 10.9 g of unreacted **19**, 25.2 g of **21** (Y : 65 %) were obtained.
bp : 55-60°C (3.5 mbar).
TLC (Hex.-EtOAc 7:5) R_F = 0.70.
NMR : 1.25 (t, J = 7, 3H, CH_2-CH_3) ; 1.40 (s, 3H, CH_3) ; 1.43 (s, 3H, CH_3) ; 2.6-2.9 (m, 1H, CH-$COOC_2H_5$) ; 4.0 (t, J = 7, 2H, CH_2-CH_3) ; 3.95-4.20 (m, 4H, CH_2-O).
Anal. Calcd. for $C_9H_{16}O_4$: C, 57.43 ; H, 8.57. Found : C, 57.61 ; H, 8.72.
19 and **21** could not be separated by silica gel chromatography.
Compound **20** was separated by chromatography of the crude product (Hex.-EtOAc 9:1).
NMR : 1.27 (t, J = 7, 3H CH_2-CH_3) ; 4.08-4.4 (m, 4H, CH_2-OH and CH_2-CH_3) ; 5.78 (s, 1H, C=C-H) ; 6.20 (bs, 1H, C=C-H).
IR (NaCl) : 3600-3100, 1715, 1635 cm^{-1}.

2,2-Dimethyl-5-hydroxymethyl-1,3-dioxane **22**

A solution of 5 g (27 mmol) of **21** in 60 mL of anhydrous ether was added dropwise to a suspension of 1 g (27 mmol) of $LiAlH_4$ in 60 mL of ether under argon. After 2.5 h stirring at room temperature, 1 mL of water, then 1 mL of 15 % aqueous NaOH, then 3 mL of water were added cautiously. Alumina was filtered on a sintered-glass and washed abundantly with ether. 3.85 g (Y : 99 %) of pure **22** were recovered.
TLC (Hex.-EtOAc 2:3) R_F = 0.24.
NMR : 1.40 (s, 3H, CH_3) ; 1.44 (s, 3H, CH_3) ; 1.60-2.0 (m, 1H, CH-CH_2-OH) ; 3.66-4.12 (ABX 8 lines, J_{AB} = 12, 4H, $CH_2$0) ; 3.71 (d, J = 7, CH_2-OH).
Anal. Calcd for $C_7H_{14}O_3$: C, 57.51 ; H, 9.65. Found : C, 57.19 ; H, 9.84.

2,2-Dimethyl-5-methanesulfonyloxymethyl-1,3-dioxane **15A**

8.2 mL (2 eq.) of methanesulfonyl chloride were added dropwise to a solution of 7.77 g (53 mmol) of **22** and 15.6 mL (2.1 eq.) of distilled triethylamine at 0°C under argon. After 2.5 h stirring at 0°C, the mixture was warmed to room temperature. Icy water was added and pH adjusted at 7-8 with triethylamine. After extraction, pure (TLC) **15A** (oil) was obtained and used without further purification.
TLC (Hex.-EtOAc 1:4) R_F = 0.36.
NMR : 1.39 (s, 3H, C-CH_3) ; 1.45 (s, 3H, C-CH_3) ; 1.85-2.1 (m, 1H, CH-CH_2O) ; 3.04 (s, 3H, SO_2-CH_3) ; 4.41 (d, J = 7, 2H, CH_2-OSO_2) ; 3.66-4.16 (ABX, 8 lines, J_{AB} = 12, 4H, CH_2O-).
Anal. Calcd. for $C_8H_{16}0_5S$: C, 42.84 ; H, 7.19. Found : C, 42.69 ; H, 7.27

2,2-Dimethyl-5-tosyloxymethyl-1,3-dioxane **15B**

1.30 g (2 eq.) of p-toluenesulfonyl chloride were added to a solution of 504 mg (3.45 mmol) of **22** in 7.5 mL of anhydrous pyridine, at 0°C. When the mixture became homogeneous, stirring was prolonged for 2.5 h at 0°C. The mixture was left at 4°C overnight and poured on a mixture of water and ice. Crystals were filtered and dried by evaporation of anhydrous benzene at room temperature. 954 mg (Y : 93 %) of pure **15B** (TLC).
mp = 58-59°C.
TLC (Hex.-EtOAc 1:1) R_F = 0.59.

NMR : 1.28 (s, 3H, $C\underline{H}_3$) ; 1.38 (s, 3H, $C\underline{H}_3$) ; 1.7-2.05 (m, 1H, $C\underline{H}$-CH_2OTs) ; 2.69 (s, 3H, Ar-$C\underline{H}_3$) ; 3.54-4.06 (ABX, 8 lines, J_{AB} = 12, 4H, $C\underline{H}_2O$) ; 4.14 (d, J = 7, 2H, CH-$C\underline{H}_2OTs$) ; 7.34-7.78 (AB, 4H, J = 8.5).
Anal. Calcd. for $C_{14}H_{20}O_5S$: C, 56.35 ; H, 6.76. Found : C, 56.29 ; H, 6.69.

2.2-Dimethyl-5-bromomethyl-1.3-dioxane **15C**

2.9 g (6.6 eq.) of $nBu_4N^+Br^-$ in 8 mL acetone were added to a solution of 303 mg (1.4 mmol) of **15A** in 4 mL acetone. The mixture was heated at reflux for 1.5 h. After solvent evaporation the residue was extracted with anhydrous benzene. Silica gel chromatography with Hex.-EtOAc 7:3 yielded 67 mg of **15C** (Y : 24 %).
TLC (Hex.-EtOAc 7:3) R_F = 0.64.
NMR : 1.39 (s, 3H, $C\underline{H}_3$) ; 1.43 (s, 3H, $C\underline{H}_3$) ; 1.86-2.12 (m, 1H, $C\underline{H}$-CH_2Br) ; 3.46 (d, J = 7, 2H, $C\underline{H}_2Br$) ; 3.64-4.11 (ABX, 8 lines, J_{AB} = 12, 4H, $C\underline{H}_2$-O).

2.2-Dimethyl-5-iodomethyl-1.3-dioxane **15D**

5.96 g (27 mmol) of **15A**, 25 g (2.55 eq.) of $nBu_4N^+ I^-$ in 185 mL of anhydrous benzene (reaction in acetone instead of benzene was much slower and gave poorer yields) were refluxed for 1.5 h. After cooling, the solution was washed with $Na_2S_2O_3$, water, dried and evaporated. The crude product was chromatographed on silica gel (Hex.-EtOAc 3:2). 4.97 g of pure (TLC) **15D** (oil) were obtained.
TLC (Hex.-EtOAc 1:1) R_F = 0.75.
NMR : 1.38 (s, 6H, $C\underline{H}_3$) ; 1.8-2.1 (m, 1H, $C\underline{H}$-CH_2I) ; 3.23 (d, J = 7, 2H, $C\underline{H}_2I$) ; 3.6-4.1 (ABX, 8 lines, J_{AB} = 12, 4H, $C\underline{H}_2O$).
Anal. Calcd. for $C_7H_{13}O_2I$: C, 32.8 ; H, 5.2. Found : C, 33.3 ; H, 5.14.

2.2-Dimethyl-5-ethoxymethyl-1.3-dioxane **15E**

691 mg of **15B** in 9 mL of absolute ethanol and 673 mg (1.3 eq.) of **23** in 4.7 mL of absolute ethanol were added to a solution of 75 mg Na in 2.8 mL ethanol. After refluxing during 19h and cooling, the white solid was filtered and the residue extracted with ether and treated as usual.
Chromatography of the crude product on silica gel (Hex.-EtOAc 2:8) gave as main product 148 mg of **15E** (Y : 37 %).
TLC (Hex.-EtOAc 2:8) R_F = 0.70.
NMR : 1.18 (t, J = 7, 3H, CH_2-$C\underline{H}_3$) ; 1.39 (s, 3H, $C\underline{H}_3$) ; 1.42 (s, 3H, $C\underline{H}_3$) ; 1.76-2.1 (m, 1H, $C\underline{H}$-CH_2O) ; 3.45 (q, 4H, $C\underline{H}_2$-O-$C\underline{H}_2$-) ; 3.60-4.06 (ABX, 8 lines J_{AB} = 12, 4H, $C\underline{H}_2O$).

2.2-Dimethyl-5-[2-acetamido-2.2-dicarbethoxy-ethane]-1.3-dioxane **24**

a) Preparation of the sodium salt **23**[12]

1.40 g of sodium (1.1 eq.) were added under argon to 30 mL absolute ethanol. After dissolution of sodium, 12 g (0.55 mol) of diethyl acetamidomalonate were added dropwise to the solution warmed at 70°C and the reaction was kept at 70°C for 5.5 h. After evaporation of ethanol, the residue was treated with anhydrous acetonitrile and the crystals were filtered, washed with acetonitrile and ether and dried in a dessicator.

b) Alkylation

5 g (17.8 mmol) of the above sodium salt and 5 g (1.1 eq.) of **15D** in 30 mL of anhydrous DMSO were kept under argon at room temperature for 60h. The reaction was monitored by TLC. After evaporation of solvent, CH_2Cl_2 was added to the residue and the mineral part was filtered and washed with CH_2Cl_2. The organic phase was treated as usual and the crude product was chromatographed on silica gel (Hex.-AcOEt 2:3). In addition to the mixture of diethyl acetamidomalonate and **24**, 4.3 g of **15D** were recovered. Crystallization from hexane-diisopropyl ether yielded **24** (4 g, Y : 65 %).
mp = 115-116°C.
TLC ($CHCl_3$-CH_3OH 9:1) R_F = 0.82.
NMR : 1.25 (t, J = 7, 6H, CH_2-$C\underline{H}_3$) ; 1.36 (s, 3H, $C\underline{H}_3$) ; 1.41 (s, 3H, $C\underline{H}_3$) ; 1.64-1.94 (m, 1H, $C\underline{H}$-CH_2-) ; 2.03 (s, 3H, $COC\underline{H}_3$) ; 2.25 (d, J = 7, 2H, CH-$C\underline{H}_2$-) ; 3.42-3.86 (m, 4H, $C\underline{H}_2O$) ; 4.24 (q, J = 7, 4H, $C\underline{H}_2$-CH_3) ; 6.9 (bs, 1H, NH).
Anal. Calcd. for $C_{16}H_{27}O_7N$: C, 55.64 ; H, 7.88 ; N, 4.06. Found : C, 55.70 ; H, 7.87 ; N, 4.14.

2.2-Dimethyl-5-[2'-carbethoxy-2'-diphenylmethylimino-ethane]-1.3-dioxane **26**

To a solution of 2.58 mL of diisopropylamine in 100 mL of THF and 50 mL HMPA and a crystal of orthophenantroline, were added successively at -78°C : a solution of BuLi (1.6M in hexane) (10.3 mL after

apparition of the red colour), 5.07 g (20.9 mmol) of **25** in 30 mL THF and, after 15 min, 4.45 g (17.4 mmol) of **15D** in 20 ml THF. The mixture was allowed to warm to room temperature, under stirring, in 2h. After addition of a saturated brine solution, the product was extracted with ether. The crude product was chromatographed on silica gel (cyclohex.-EtOAc 8:2). 6.42 g of pure **26** were recovered (Y : 93 %). When running the reaction in the presence of 10 mL of HMPA, the yield of **26** was only 60 %.
TLC (cyclohex.-EtOAc 8:2) R_F = 0.28.
NMR : 1.3 (t, J = 7, 3H, CH_2-CH_3) ; 1.35 (s, 6H, CH_3) ; 1.7 (m, 1H, CH-CH_2) ; 1.85 (m, 2H, CH-CH_2) ; 3.3-3.9 (m, 4H, CH_2-O) ; 4.7 (q, 2H, CH_2-CH_3) ; 4.1 (t, 1H, N-CH-CO) ; 7.4 (m, 10H, aromatic).
M.S. (E.I 70ev) m/z ; 395(19)M^+, 380(38)M^+-CH_3, 336(7), 322(10)M^+-$CO_2C_2H_5$, 308(42), 267(69), 238(66)M^+-ϕ_2CH, 222(14), 206(17), 193(100), 182(61), 165(61). High resolution mass spectra : calcd : 395.2096 ; found : 395.2097.

5,5'-Dihydroxyleucine **3**
a) by hydrolysis of **24**
2.45 g (7 mmol) of **24** were dissolved in 25 mL of HCl 6N and heated at 110°C in a sealed tube during 15h. After evaporation under reduced pressure, water was evaporated (4 X 25 mL) and the residue was then dissolved in 25 mL water. After neutralisation of the solution with concentrated KOH, 7.1 mL (1 eq.) of KOH 1N was added and the mixture warmed at 40°C for 30 min. After cooling, the crude product was purified on a AG1X2 (OH^- form) column. After washing with H_2O, the product was eluted with CH_3COOH 0.3N. After lyophilisation, 1.16 g of **3** were obtained (Y : 73 %), identical to the product already described[6].
TLC (nBuOH-Pyridine-HOAc-H_2O. 6:6:1.2:4.8) R_F = 0.48.
NMR (Na salt in D_2O to avoid lactonisation).
^1H δ/DSS : 1.3-2 (m, 2H, CH_2-CH) ; 3-3.5 (m, 1H, N-CH-CO) ; 3.59 (d, J = 5, 4H, CH_2OH).
^{13}C δ/DSS : 37.25 (CH_2) ; 42.33 (CH) ; 56.88 (C-NH_2) ; 64.42 (CH_2-O-) ; 65 (CH_2-O).
b) by hydrolysis of **26**
6.4 g of **26** in 100 mL HCl were heated at reflux for 4h. The aqueous phase was washed three times with methylene chloride and evaporated. The residue was purified on an ion-exchange resin as above. After evaporation of the HOAc eluate, 2.2 g of the acetate of **3** were obtained (Y : 57 %).
TLC (nBuOH, Pyridine, HOAc, H_2O-6:6:1.2:4.8) 0.48.

Diethylbenzyloxymethylfluoromalonate **29**
5 mL of dry DMF were added to 135 mg (3.4 mmol) HNa suspension washed with hexane. 500 µl (3.2 mmol) of diethylfluoromalonate were added dropwise at 0°C. When the solution became clear, 900 µL (6.5 mmol) of benzyl chloromethyl ether were added slowly. The mixture was stirred at room temperature for 1.5 h. After evaporation of DMF the residue was extracted with ether and treated as usual. The crude product was purified on silica gel (Hex.-EtOAc 85:15) 724 mg of **29** were obtained (Y : 75 %) oil.
TLC (Hex.-EtOAc 85:15) R_F = 0.47.
NMR : 1.29 (t, 6H, O-CH_2-CH_3) ; 4.08 (d, 2H, J_{HF} = 24, -C-CH_2-O-) ; 4.29 (q, 4H, O-CH_2-CH_3) ; 4.63 (s, 2H, O-CH_2-ϕ) ; 7.31 (bs, 5H, arom.).
Anal. Cacld. for $C_{15}H_{19}O_5F$: C, 60.39 ; H, 6.42. Found : C, 60.22 ; H, 6.55.

2-Benzyloxymethyl-2-fluoro-1,3-propanediol **30**
To a suspension of 242 mg of $LiAlH_4$ partially deactivated (2H^-/mole) in 4 mL of ether, a solution of 453 mg (1.58 mmol) of **29** in 4 mL of ether was added dropwise at the rate which was necessary to maintain a gentle reflux. The mixture was then stirred for 1.5 h at room temperature and the reaction stopped by cautious addition of 100 µL H_2O, 300 µL of 15 % aqueous NaOH, 300 µL H_2O. Alumina was filtered on a sintered-glass and washed with ether until complete extraction of the product. The crude product (307 mg) was purified by recrystallisation in diisopropyl ether, giving 147 mg of pure **30** (Y : 43 %).
mp = 80-81°C.
TLC (Hex.-EtOAc 1:1) R_F = 0.18.
NMR : 3.69 (d, 2H, J_{HF} = 17.5, CH_2-O-CH_2 ϕ) ; 3.82 (d-d, 4H, J_{HH} = 6, J_{HF} = 18, CH_2-OH) : 4.56 (s, 2H, O-CH_2-ϕ) ; 7.32 (bs, 5H, arom.).
Anal. Calcd. for $C_{11}H_{15}O_3F$: C, 61.67 ; H, 7.06. Found : C, 61.82 ; H, 7.22.

2,2-Dimethyl-5-benzyloxymethyl-5-fluoro-1,3-dioxane **31**

A solution of 147 mg (0.73 mmol) of **30**, 180 mL of 2,2-dimethoxypropane and 15 mg of p-toluene sulfonic acid in 3 mL of acetone was heated at reflux overnight, with a few beads of molecular sieve. After cooling, and neutralisation with NaHCO3, the residue was extracted with ether and treated as usual. The crude product was purified on silica gel (Hex.-EtOAc 7:3), giving 137 mg of pure **31** (Y : 83 %) oil, and 9 mg of starting material.

TLC (Hex.-EtOAc 93:7) R_F = 0.35.

NMR : 1.38 (s, 3H, $C\underline{H}_3$) ; 1.43 (s, 3H, $C\underline{H}_3$) ; 3.60 (d, 2H, J_{HF} = 20, $C\underline{H}_2$-O-$C\underline{H}_2$-ϕ) ; 3.88 (d, 4H, J_{HF} = 18, C-$C\underline{H}_2$-O-) ; 4.56 (s, 2H, O-$C\underline{H}_2$-ϕ) ; 7.32 (bs, 5H, arom.).

Anal. Calcd. for $C_{14}H_{19}O_3F$: C, 66.12 ; H, 7.53. Found : C, 66.12 ; H, 7.61.

2,2-Dimethyl-5-fluoro-5-hydroxymethyl-1,3-dioxane **32**

137 mg (0.57 mmol) of **31** were hydrogenolyzed in 4 mL of ethyl acetate over 55 mg Pd, 10 % /C for 2.5 h. After addition of CH_2Cl_2, the solution was filtered and the catalyst washed with ethyl acetate until complete recovery of the product. 86 mg of pure **32** were obtained (Y : 97 %) oil.

TLC (Hex.-EtOAc) R_F = 0.49.

NMR : 1.40 (s, 3H, $C\underline{H}_3$) ; 1.45 (s, 3H, $C\underline{H}_3$) ; 3.72 (d-d, 2H, J_{HH} = 6, J_{HF} = 21, $C\underline{H}_2OH$) ; 3.88 (d, 4H, J_{HF} = 19, CF-$C\underline{H}_2$-O-).

Anal. Calcd. for $C_7H_{13}O_3F$: C, 51.20 ; H, 8.00. Found : C, 51.23 ; H, 8.05.

2,2-Dimethyl-5-fluoro-5-methanesulfonyloxymethyl-1,3-dioxane **27A**

To a solution of 70 mg (0.43 mmol) of **32** in 2 mL of dichloromethane at 0°C were added 125 µL (2.1 eq.) of triethylamine and then, dropwise 67 µL (2 eq.) of methane sulfonyl chloride. After stirring for 1.5 h at room temperature, 3 mL ether, 1 mL of icy water and triethylamine until pH 7-8 were added. The ethereal phase was treated as usual. 87 mg of pure **27A** were obtained (Y : 83 %) oil.

TLC (Hex.-EtOAc 3:7) R_F = 0.66.

NMR : 1.40 (s, 3H, $C\underline{H}_3$) ; 1.46 (s, 3H, $C\underline{H}_3$) ; 3,08 (s, 3H, -SO_2-$C\underline{H}_3$) ; 3.80 (d, 4H, J_{HF} = 14, C-$C\underline{H}_2$O) ; 4.47 (d, 2H, J_{HF} = 22, $C\underline{H}_2$-OSO_2-).

Anal. Calcd. for $C_8H_{15}O_5FS$: C, 39.67 ; H, 6.24. Found : C, 40.03 ; H, 6.29.

2,2-Dimethyl-5-fluoro-5-trifluomethanesulfonyloxymethyl-1,3-dioxane **27B**

To a solution of 30 mg (0.18 mmol) of **32** in 1 mL of dichloromethane at 0°C were added 97 µL (4.5 eq.) of freshly distilled lutidine, then, dropwise, 4 µL (1.5 eq.) of trifluoromethanesulfonic anhydride. After stirring for 30 min. at 0°C, the solution was poured on an alumina column and the product was eluted with dichloromethane. 43 mg of pure **27B** were obtained (Y : 79 %).

TLC (Hex.-EtOAc 1:1) R_F = 0.82.

NMR : 1.39 (s, 3H, $C\underline{H}_3$) ; 1.48 (s, 3H, $C\underline{H}_3$) ; 3.80 (d, 4H, J_{HF} = 11, C-$C\underline{H}_2$-O) ; 4.72 (d, 2H, J_{HF} = 23, $C\underline{H}_2$-OS).

Dibenzylfluoromalonate **33**

0,3 mL (1.9 mmol) of diethylfluoromalonate **28**, 1 mL of benzyl alcohol and 30 mg of p-toluene sulfonic acid in 4 mL of toluene were heated at reflux overnight. After solvent elimination and usual treatment, the residue was chromatographed on silica gel (Hex.-EtOAc 80:20). 530 mg of pure **33** were obtained (Y : 90 %).

mp : 52-53°C.

TLC (Hex.-EtOAc 7:3) R_F = 0.43.

NMR : 5.25 (s, 4H, O-$C\underline{H}_2$-ϕ) ; 5.38 (d, 1H, J_{HF} = 48, C$\underline{H}$F) ; 7.33 (bs, 10H, arom.).

N-phthalyl-4-fluoro-4-benzyloxycarbonyl-glutamic acid, α-methyl, γ-benzyl diester **35**

5 mL of THF were added to 96 mg (2.1 mmol) of oily NaH suspension washed with hexane. The solution was cooled to 0°C and 600 mg (2 mmol) of **33** in 5.5 mL of THF were added. After 5 min. of stirring at 0°C, dropwise addition of 440 mg of **34**, prepared according to Bory et al.[6], dissolved in 3.5 mL of THF, and further stirring at 0°C for 20 min., the reaction was stopped by addition of 1.4 mL of a 10 % citric acid solution. After solvent evaporation, the residue was dissolved in ethyl acetate, which was washed with brine. The crude product was purified on silica gel (Hex.-EtOAc 70:30) 647 mg of pure **35** were obtained (oil) (Y : 60 %).

TLC (Hex.-EtOAc 7:3) R_F = 0.27.

NMR : 3.24 (m, 2H, J_{HF} = 21, $C\underline{H}_2$-CHF) ; 3.72 (s, 3H, $OC\underline{H}_3$) ; 4.92 (s, 2H, O-$C\underline{H}_2$-ϕ) ; 5.20 (s, 2H, O-$C\underline{H}_2$-ϕ) ; 5.25 (t, 1H, -N-C$\underline{H}$-) ; 7.27 (bs, 10H, arom. benzyl.) ; 7.78 (m, 4H, arom. phthal.).
Anal. Calcd. for $C_{29}H_{24}O_8NF$: C, 65.29 ; H, 4.53 ; N, 2.62. Found : C, 65.46 ; H, 4.77 ; N, 2.45.

N-Phthalyl-4-fluoro-4-carboxyglutamic acid α-methyl ester **36**
647 mg of **35** were hydrogenolyzed in 10 mL of ethyl acetate over 200 mg of Pd 10 %/C for 1.5 h. After addition of CH_2Cl_2, the solution was filtered and the catalyst washed with ethyl acetate. After evaporation of the organic solvents, the residue was redissolved in ethyl acetate and washed with a 1M solution of $NaHCO_3$. The aqueous phase was acidified with HCl 1N and extracted with ethyl acetate. 327 mg of pure **36** were obtained (Y : 90 %).
NMR : 3.20 (m, 2H, J_{HF} = 22, -$C\underline{H}_2$-) ; 3.75 (s; 3H, $OC\underline{H}_3$) ; 5.19 (t, 1H, -N-C$\underline{H}$-) ; 7.78 (m, 4H, arom.) ; 9.15 (s, 2H, CO$\underline{O}$H).
Anal. Calcd. for $C_{15}H_{12}O_8NF$: C, 50.99 ; H, 3.42 ; N, 3.96. Found : C, 50.94 ; H, 3.44 ; N, 4.05.

N-Phthalyl-4-fluoro-5,5'-dihydroxy-leucine, methyl ester **37**
0.58 mL of borane-dimethyl sulfide were added dropwise to a solution of 307 mg (0.77 mmol) of **36** in 10 mL of ethyl acetate-THF 1:1. After 3h stirring at room temperature, the reaction was stopped by careful addition of methanol. After evaporation and three distillations of methanol on the residue it was dissolved in ethyl acetate, washed with 1M HCO_3Na and brine. After chromatography on silica gel (chloroform-methanol 95:5), 60 mg of pure **37** were obtained (oil) (Y : 21 %).
TLC (Hex.-EtOAc 1:1) R_F = 0.10.
(Hex.-$CHCl_3$ 5:95) R_F = 0.31.
NMR : 2.75 (m, 2H, J_{HF} = 24, -$C\underline{H}_2$-) ; 3.68 (d, 2H, J_{HF} = 30, -$C\underline{H}_2$OH) ; 3.74 (s, 3H, $COOC\underline{H}_3$) ; 3.78 (d, 2H, J_{HF} = 26, -$C\underline{H}_2$OH) ; 5.23 (t, 1H, J = 7, N-C$\underline{H}$-) ; 7.80 (m, 4H, arom.).
M.S. (E.I. 70 ev) m/ ; 325(10)M^+, 305(2.5)M^+-HF, 293(5)M^+-HF-H_2O, 266(21)M^+-CO_2CH_3, 228(17), 218(15), 187(17), 173(37)M^+-HF-$C_8H_4O_2$, 160(20)M^+-HF-$C_8H_5O_2N$, 148(100), 130(97), 119(17). High resolution mass spectra : calcd : 325.09615 ; found : 325.0966.

4-Fluoro-5,5'-dihydroxy-leucine **7**
60 mg (0.19 mmol) of **37** in 4 mL HCl 6N were heated at reflux for 1h. After evaporation, the residue was dissolved in water. The aqueous phase was washed 3 times with CH_2Cl_2 and poured on a Dowex 1X4 ($HCOO^-$ form) column. **7** was eluted with water. 25 mg of **7** were obtained after lyophilisation.
TLC (nButanol, pyridine, HOAc, H_2O-6:6:1.2:4.8) R_F = 0.58.
NMR (^2H_2O) : 2.05-2.85 (m, 2H, CH_2) ; 3.82 (m, 2H, J_{HF} = 21, $C\underline{H}_2$OH) ; 3.95 (m, 2H, J_{HF} = 24, $C\underline{H}_2$OH) ; 4.35 (m, 1H, -C$\underline{H}$-).
^{13}C NMR (D_2O, sodium salt) ^1H decoupled. δ : 44.0 (CH-$\underline{C}H_2$-CF), 55.5 ($\underline{C}H_2$OH), 55.8 ($\underline{C}H_2$OH), 57.3 (-$\underline{C}$H-CO), 174.8 ($\underline{C}OO^-$).

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